Spatial Relationships of Soil Texture and Crop Rotation to *Aspergillus flavus* Community Structure in South Texas

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ABSTRACT

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Aspergillus flavus, the causal agent of aflatoxin contamination of cottonseed, is a natural inhabitant of soils. *A. flavus* can be divided into the S and L strains, of which the S-strain isolates, on average, produce greater quantities of aflatoxins than the L-strain isolates. Aflatoxin contamination can be severe in several crops in South Texas. The structure of *A. flavus* communities residing in soils of South Texas was determined from 326 soil samples collected from 152 fields located from the Rio Grande Valley in the south to Fort Bend County in the north from 2001 through 2003. Analysis of variance indicated significant differences in the incidence of *A. flavus* isolates belonging to the S strain (percent S) among regions. The Coastal Bend (30.7%) and Upper Coast (25.5%) regions had

significantly higher percent S incidence than the Rio Grande Valley (4.8%). No significant differences in percent S among years were detected. The CFU per gram of soil were not significantly different among regions. Strain S incidence was positively correlated with clay content and negatively correlated with sand content. Fields cropped to cotton the previous year had a higher S-strain incidence, whereas fields cropped to corn had greater total quantities of *A. flavus* propagules. Maps of S-strain patterns show that the S strain constitutes >30% of the overall *A. flavus* community in the area extending from the central Coastal Bend region to the central Upper Coast region. The west Rio Grande Valley had the lowest S-strain incidence (<10%). Geographic variation in S-strain incidence may influence the distribution of aflatoxin contamination in South Texas.

Additional keywords: GIS, geostatistics, kriging, spatial analysis.

Throughout most of the world, concentrations of aflatoxins, potent toxic and carcinogenic fungal metabolites that frequently contaminate food and feed, are limited by regulation (28,37). Aflatoxins are produced by asexual fungi belonging to *Aspergillus* section *Flavi* (8). Soils in areas where contamination is common contain diverse communities of aflatoxin-producing fungi (6). Communities of section *Flavi* differ by region in both species composition and aflatoxin producing potential. *Aspergillus flavus*, the most common aflatoxin-producing species, can be divided into two morphotypes that commonly are called strains based on morphological, genetic, and physiologic criteria (2,4,14,15). The S strain produces numerous small sclerotia (average diameter <400 µm) and high levels of aflatoxins, whereas the L strain produces fewer, larger sclerotia (average diameter >400 µm) and variable levels of aflatoxin (4,6,17). Some isolates of the L strain produce no aflatoxin (6). The S strain of *A. flavus* has been reported as a natural soil inhabitant in several areas worldwide, including Thailand in Southeast Asia (32); Argentina in South America (24); and in Arizona (4,6,25), California (12,13), Texas (9,18), Louisiana, Mississippi, and Alabama (6) in the United States of America.

Cottonseed contributes 15 to 20% of farmer income from cotton in the United States. Aflatoxin contamination has long been a concern for the U.S. cottonseed industry because a small proportion of aflatoxin in feed is transferred to the milk of dairy cows in the slightly modified form aflatoxin M₁ (1,36). Cottonseed is a

preferred feed for dairy cows and regulations in the United States prohibit aflatoxin concentrations >0.5 ng/g in milk (29). Milk exceeding that limit may be dumped and the producing dairy placed on quarantine. Because dairies typically pay a premium for cottonseed, in areas where aflatoxin contamination of cottonseed is common, aflatoxin content is the most important factor determining seed value (7). Aflatoxin contamination of cottonseed costs millions of dollars annually. In the United States, contamination of cottonseed is most severe in the desert production regions of Arizona and Southern California, and in South Texas (7,30,33).

Spatial patterns of most variables can be described, analyzed, and displayed at any scale with geographic information systems (GIS) and geostatistics. The estimation of values at nonsampled locations is essential to describe variables spatially. Geostatistics adapt classical statistical regression techniques to estimate values at nonsampled locations (10,19) and describe spatial continuity, which can be used to assess uncertainty associated with estimated values (21). Kriging, a geostatistic tool, uses regression techniques for interpolation of spatially correlated data (22). Spatial patterns of the population density and strain composition of *A. flavus*, the primary causal agent of aflatoxin contamination, (25,26), and of aflatoxin contamination episodes (20) have been described with geostatistics. GIS can be used to capture, manipulate, process, and display spatially distributed data in computers (16). This allows description of objects from the real world in terms of (i) position with respect to a known coordinate system, (ii) relation to environmental variables (i.e., soil type, temperature, disease incidence, or insect density) unrelated to position, and (iii) spatial relationships with each other (topological relations) (16,34).

Both *A. flavus* community structure and aflatoxin contamination present spatial autocorrelation (20,25,26). However, relationships of contamination to fungal community structure in soils

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have not been described. The objectives of the current study were to (i) spatially analyze the magnitude and strain composition of *A. flavus* communities in soils of South Texas at different geographic scales, (ii) evaluate influences of crop rotation on community size and structure of *A. flavus*, and (iii) determine relationships of *A. flavus* community size and structure to soil texture.

MATERIALS AND METHODS

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 spring 2001, 2002, and 2003. One to four samples were taken from each field. Soil samples from single-sample fields (88 fields) were taken from a transect of approximately 50 m. Each sample consisted of >50 subsamples taken from the soil surface (no deeper than 1 cm). In all, 5 to 10 of these subsamples were taken every 10 paces of the transect. Multiple-sample fields (64 fields) were sampled by walking zig-zag 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. In all, 50 to 70 subsamples were taken in a 10-pace-radius in 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.

Sample processing and isolation of fungi. Soil samples were dried in a forced air oven at 48 to 50°C for 48 h before processing. Dry samples then were placed inside a plastic bag, hammered to break clumps, and passed through a no. 12 sieve (1.7-mm opening). The sieve was vacuumed to remove loose contaminants and washed with ethanol to kill contaminating fungal spores between each sample. Soil texture (percent clay, percent silt, and percent sand) was determined for each sample using the hydrometer method (35). *A. flavus* was isolated from soil by dilution plating onto a modified Rose Bengal agar (4). For most samples, 10 g of soil was suspended in 50 ml of autoclaved water by stirring for 20 min. Adjustments in the soil dilution for samples that had either too many or too few CFU in the first isolation were made for subsequent isolations. Isolation 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) for 5 to 7 days at 31°C and assigned to either the *A. flavus* S or L strains, *A. tamarii*, or *A. parasiticus* on the basis of colony characteristics and isolate morphology (4). Isolations were made only from plates having ≤ 12 *A. flavus* colonies. Quantities of *A. flavus* in soil were calculated as the number of CFU of *A. flavus* per gram. The percentage 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.

Data analysis. The study area was ≈ 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 (20). The design was unbalanced, with a different number of samples (replicates) per field; therefore, analysis of variance (ANOVA) was performed, using the General Linear Model (GLM) procedure of SAS (version 8.0; SAS Institute, Cary, NC), to assess differences among both regions and areas in the incidence of isolates belonging to the S strain of *A. flavus* (percent S) and the CFU/g of soil. ANOVA also was used to assess the effect of the previous crop on percent S and CFU/g. Fields (30%) that previously were planted to crops other than cotton, corn, or sorghum, or for which there were no previous crop data, were discarded from the previous crop ANOVA analysis. To determine influences of geographic

scale (among regions, among areas, among fields, and within fields) on percent S, CFU/g, and soil texture (percent clay, percent silt, and percent sand content) variables, a variance components analysis (VCA) in GLM using the VARCOMP procedure of SAS 8.0 with a nested model was performed. The VARCOMP procedure of GLM was used because it best fit the unbalanced design. Pearson's correlation analysis using the CORR procedure of SAS 8.0 was used to determine relationships of both percent S and CFU to soil texture. All the statistical analyses for percent S and CFU/g also were performed with transformed data, using square root for percent S and natural logarithm (log) for CFU/g. However, only the log transformation of CFU/g for mean separation was used in the reported results because there was no improvement in the significance levels for other transformations. Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) was used for means separation on the original data for percent S and on log-transformed data for CFU/g.

Spatial analysis. Soil samples were geo-referenced in the Universal Transverse Mercator (UTM) projected coordinate system. The UTM system gives positions in meters and facilitates correct computation of distances between sample locations in two dimensions. Geostatistical analyses (23) 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 also were obtained using geostatistics, by subdividing the complete data set based on the previous season's crop. Model variograms, a mathematical description of the spatial autocorrelation, then were developed for percent S, CFU, and soil texture variables. Omidirectional experimental variograms were obtained using VARIOWIN 2.21 (27) for percent S and CFU/g. Variogram equations and procedures to develop experimental variograms and fit model variograms followed previously described procedures (10,11,19,21,22). The maximum distance between sample locations was ≈ 450 km and the minimum distance was 2.5 km.

Kriging and map display. ArcGIS (version 9.0 for Windows; ESRI, Redlands, CA) was used to interpolate values in non-sampled areas by block kriging using model variograms on a 2-by-2-km grid with a search neighborhood of 60,000 m and a maximum of 40 sample locations. Previously digitized features were used as background for views and layouts of the kriged values.

RESULTS

***A. flavus* community structure in soils of South Texas.** *A. flavus* communities in soils of South Texas differed significantly among regions in community density (CFU/g) and S-strain incidence (percent S) (Table 1). Quantities of *A. flavus* were greater in the Upper Coast (882 CFU/g) than in the Coastal Bend (497 CFU/g) (Table 1). On the other hand, average S-strain incidence was lower in the Rio Grande Valley (4.8%) than in either the Coastal Bend (25.5%) or Upper Coast (30.8%). The Rio Grande Valley and Coastal Bend regions both had significant differences in percent S among years (Table 1). The Rio Grande Valley had higher percent S in 2002 compared with 2001 and the Coastal Bend had higher percent S in 2003 compared with either 2001 or 2002. Significant differences in percent S also occurred among the 11 areas into which the three regions were divided (Table 2). Areas in the east and north of the Coastal Bend and south and central of the Upper Coast had the highest percent S.

Variance at different geographic scales. Most variance (93.9%) for CFU/g is explained by the small geographic scales within and among fields, with among fields explaining 48.4% and within fields 45.5% (Table 3). Within fields also explained 53.8% of percent S variation whereas among fields explained only 13.5%. Among regions explained none of the CFU/g variance and 23.7% of the percent S variance. Most of the variation

(67.3 to 90.7%) for the soil texture variables clay, silt, and sand occurred among fields (47.4 to 57.4%) and among areas (19.9 to 34.1%).

Effect of crop rotation on *A. flavus* communities in soil.

A. flavus communities in soils were studied to determine whether crop rotation influenced 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 with either cotton (566 CFU/g) or sorghum (157 CFU/g) (Table 4). 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 significant only in the Coastal Bend.

Soil texture and *A. flavus* relationship. According to Pearson's correlation analyses, there were significant positive correlations between percent clay and both percent S ($r = 0.28$, $P < 0.0001$) and CFU/g ($r = 0.16$, $P = 0.008$) and a significant negative correlation between percent sand and both percent S ($r = -0.28$, $P < 0.0001$) and CFU/g ($r = -0.13$, $P = 0.04$) (Table 5). Analyses

using data averaged by area greatly improved correlation coefficients for percent S with clay and sand ($r = 0.82$, $P = 0.003$ for clay and $r = -0.85$, $P = 0.002$ for sand). Correlation coefficients between CFU/g and soil type content also were improved, but not appreciably ($r = 0.34$, $P = 0.34$ for percent clay and $r = 0.20$, $P = 0.58$ for percent sand) (Table 5).

Spatial patterns of *A. flavus* community structure in soils of South Texas. Geostatistical analyses, including variogram analyses and surface maps obtained by kriging, indicated patchy distribution of both *A. flavus* community structure (percent S and CFU/g) and soil texture (percent clay, sand, and silt).

Variogram analyses. Parameters of modeled variograms for soil and fungal characteristics of fields previously cropped with corn, cotton, sorghum, and other crops are presented in Table 6. Spatial continuity of both percent S and CFU/g was best described by covariograms. All the variograms and covariograms adjusted to the spherical type with an indicative goodness of fit (IGF) ranging from 0.002 to 0.046 based on a weighted mean square standardized. The range of CFU/g variograms (covariograms) was much shorter (30,000 to 44,000 m) than those given by percent S (36,500 to 75,000 m). Soil samples obtained from fields previously cropped with corn presented the shortest

TABLE 1. CFU of *Aspergillus flavus* and percentage of isolates of *A. flavus* belonging to the S strain (percent S) from soil of different regions in South Texas from 2001 to 2003^z

Region	2001	2002	2003	Mean
CFU/g				
Rio Grande Valley	786 (42) a, d	712 (29) a, d	92 (19) b, e	616 (90) ab
Coastal Bend	257 (88) b, e	1,061 (50) a, d	148 (20) b, e	497 (158) b
Upper Coast	902 (44) a, d	569 (16) a, d	1,110 (18) a, d	882 (78) a
Mean	548 (174) e	872 (95) d	433 (57) e	...
Percent S				
Rio Grande Valley	1.9 (42) b, e	10.1 (29) c, d	3.0 (18) b, e	4.8 (89) b
Coastal Bend	22.5 (88) a, e	23.9 (50) b, e	42.9 (20) a, d	25.5 (158) a
Upper Coast	29.5 (44) a, d	36.8 (16) a, d	28.4 (18) a, d	30.8 (78) a
Mean	19.3 (174) d	21.9 (95) d	25.4 (56) d	...

^z Numbers in parentheses = number of samples analyzed. Averages with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$). Letters before the comma indicate differences among regions (rows) and letters following the comma indicate differences among years (columns).

TABLE 2. CFU of *Aspergillus flavus* and percentage of isolates of *A. flavus* belonging to the S strain (percent S) from soil of different areas in South Texas from 2001 to 2003^z

Area	2001	2002	2003	Mean
CFU				
Rio Grande South	946 (34) a, e	950 (15) ab, e	52 (15) b, e	738 (64) a
Rio Grande North	102 (8) a, e	457 (14) ab, e	243 (4) ab, e	315 (26) a
Coastal Bend South	100 (4) a, e	326 (9) ab, e	...	256 (13) a
Coastal Bend Southeast	92 (12) a, e	...	111 (6) b, e	98 (18) a
Coastal Bend Southwest	331 (5) a, e	434 (14) ab, e	85 (1) b, e	365 (35) a
Coastal Bend Northwest	373 (24) a, e	990 (9) ab, e	...	541 (33) a
Coastal Bend Northeast	181 (16) a, e	116 (1) b, e	169 (13) b, e	174 (30) a
Coastal Bend North	218 (12) a, f	2,107 (15) a, e	...	1268 (27) a
Upper Coast South	1,207 (32) a, e	827 (8) ab, e	1923 (9) a, e	1276 (49) a
Upper Coast Central	91 (12) a, f	1,022 (5) ab, e	297 (9) ab, f	341 (26) a
Upper Coast North	...	156 (5) b	...	156 (5) a
Percent S				
Rio Grande South	0.8 (34) d, e	4.8 (15) b, e	3.0 (14) c, e	2.2 (63) c
Rio Grande North	6.2 (8) cd, e	15.9 (14) ab, e	3.1 (4) bc, e	10.9 (26) bc
Coastal Bend South	1.6 (4) d, e	15.0 (9) ab, e	...	10.9 (13) bc
Coastal Bend Southeast	26.3 (12) bc, e	...	41.9 (6) ab, e	31.5 (18) a
Coastal Bend Southwest	16.3 (20) bcd, e	25.4 (14) ab, e	22.2 (1) abc, e	20.1 (35) abc
Coastal Bend Northwest	15.5 (24) bcd, e	26.2 (9) ab, e	...	18.4 (33) abc
Coastal Bend Northeast	21.6 (16) bcd, e	38.5 (1) ab, e	44.9 (13) a, e	32.2 (30) a
Coastal Bend North	51.4 (12) a, e	26.1 (15) ab, e	...	37.3 (27) a
Upper Coast South	27.7 (32) bc, e	35.5 (8) a, e	30.2 (9) abc, e	29.4 (49) ab
Upper Coast Central	34.5 (12) ab, e	41.2 (5) a, e	26.6 (9) abc, e	33.0 (26) a
Upper Coast North	...	27.4 (5) ab	...	27.4 (5) ab

^z Numbers in parentheses = number of samples analyzed. Averages with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$). Letters before the comma indicate differences among regions (rows) and letters following the comma indicate differences among years (columns).

TABLE 3. Variation among regions, areas, and fields and within fields in *Aspergillus flavus* (CFU/g), percentage of isolates belonging to the S strain of *A. flavus*, (percent S), and of soil texture as described by the percent of clay, silt, and sand in soils of South Texas^z

Variance component	df	Estimates	Variance (%)	<i>P</i> > <i>F</i>
Percent S				
Among regions	2	131.5	23.7	<0.0001
Among areas	9	49.8	9.0	<0.0001
Among fields	140	75.1	13.5	0.007
Within fields (error)	170	298.4	53.8	...
CFU/g				
Among regions	2	0	0	0.0016
Among areas	9	73,815	6.1	<0.0001
Among fields	140	581,545	48.4	<0.0001
Within fields (error)	171	546,137	45.5	...
Clay				
Among regions	2	0	0	<0.0001
Among areas	9	105.3	34.1	<0.0001
Among fields	139	166.2	53.7	<0.0001
Within fields (error)	170	37.8	12.2	...
Silt				
Among regions	2	5.2	5.2	<0.0001
Among areas	9	20.0	19.9	<0.0001
Among fields	139	47.6	47.5	<0.0001
Within fields (error)	170	27.5	27.4	...
Sand				
Among regions	2	0.3	0.1	<0.0001
Among areas	9	160.1	33.3	<0.0001
Among fields	139	275.7	57.4	<0.0001
Within fields (error)	170	44.3	9.2	...

^z df = degrees of freedom and *P* > *F* = probability of the calculated *F* value.

covariogram range for both CFU/g and percent S compared with fields cropped to cotton or sorghum. Fields previously cropped with corn presented the highest spatial variation for CFU/g with a sill (partial sill + nugget) = 3,708,000, whereas fields previously cropped with cotton presented the highest spatial variation for percent S with a sill (partial sill + nugget) = 610.

Ordinary kriging maps. The S strain constituted >30% of the overall *A. flavus* community in the area extending from the central Coastal Bend to the central Upper Coast regions (Fig. 1A). The west Rio Grande Valley had the lowest S strain incidence (<10%). When spatial patterns were obtained individually by previous crop, fields that were cropped with cotton or sorghum the previous year had higher percentages of the S strain (Fig. 1B to E) than fields cropped with corn. Most of the areas in Coastal Bend and Upper Coast had percentages of the S strain >30% when only samples from fields previously cropped with cotton or sorghum were considered in the analysis, whereas most of the areas throughout South Texas had a percentage of S strain <30% when only samples from corn fields were included.

Average quantities of *A. flavus* propagules were greatest, averaging >500 CFU/g, from the north Coastal Bend to the central Upper Coast. Areas within 50 km of the coast averaged >1,000 CFU/g (Fig. 2A). Maps obtained from fields previously cropped to corn had the highest quantity of *A. flavus*, averaging >500 CFU/g over most of South Texas and >2,000 CFU/g in areas of the Rio Grande Valley and the Port Lavaca peninsula (Fig. 2C). Maps of fields previously cropped with sorghum (Fig. 1D) had populations averaging <500 CFU/g throughout South Texas. Maps generated from fields cropped to cotton the previous season

TABLE 4. CFU/g and percentage of *Aspergillus flavus* isolates belonging to the S strain in soil planted the previous year to either cotton, corn, or sorghum from 2001 to 2003^z

Previous crop	South Texas regions			
	Rio Grande	Coastal Bend	Upper Coast	South Texas
CFU/g				
Cotton	516 (24) b, d	531 (46) b, de	644 (36) ab, e	566 (106) b
Corn	3,911 (8) a, e	938 (44) a, d	1,687 (23) a, d	1,485 (75) a
Sorghum	198 (24) b, e	138 (52) b, e	158 (10) b, e	157 (86) c
Mean	865 (56) e	513 (142) d	921 (69) e	...
Percent S				
Cotton	7.3 (24) a, d	34.0 (46) a, e	35.8 (36) a, e	28.6 (106) a
Corn	1.6 (8) a, d	16.6 (44) b, e	23.2 (23) a, e	17.0 (75) b
Sorghum	4.0 (24) a, d	27.1 (52) ab, e	33.1 (10) a, e	21.3 (86) ab
Mean	5.1 (56) d	26.1 (142) e	31.2 (69) e	...

^z Numbers in parentheses = number of samples analyzed. Averages with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$). Letters before the comma indicate differences among regions (rows) and letters following the comma indicate differences among years (columns).

TABLE 5. Pearson's correlation coefficients of the relationship of the quantity (CFU) and composition (percentage of *Aspergillus flavus* composed of the S strain = percent S) of *A. flavus* to soil texture variables (clay, silt and sand) from fields of South Texas^z

	Percent S	CFU/g	Log CFU	Clay (%)	Silt (%)	Sand (%)
All data						
Percent S, <i>n</i> = 262	1.0	...	...	...	...	...
CFU/g, <i>n</i> = 262	-0.09, 0.13	1.0	...	...	...	...
Log CFU, <i>n</i> = 262	-0.15, 0.01	0.75, <0.0001	1.0	...	...	...
Clay (%), <i>n</i> = 260	0.28, <0.0001	0.16, 0.008	0.12, 0.05	1.0	...	...
Silt (%), <i>n</i> = 260	0.10, 0.12	-0.03, 0.67	0.01, 0.83	0.1, 0.12	1.0	...
Sand (%), <i>n</i> = 260	-0.28, <0.0001	-0.13, 0.04	-0.11, 0.08	-0.89, <0.0001	-0.53, <0.0001	1.0
Averaged by areas						
Percent S, <i>n</i> = 10	1.0	...	...	...	...	...
CFU/g, <i>n</i> = 10	0.33, 0.35	1.0	...	...	...	...
Log CFU, <i>n</i> = 10	0.15, 0.68	0.92, 0.0001	1.0	...	...	...
Clay (%), <i>n</i> = 10	0.82, 0.003	0.34, 0.34	0.20, 0.58	1.0	...	...
Silt (%), <i>n</i> = 10	0.48, 0.16	-0.15, 0.68	-0.15, 0.69	0.28, 0.44	1.0	...
Sand (%), <i>n</i> = 10	-0.85, 0.002	-0.20, 0.58	-0.09, 0.81	-0.90, 0.0004	-0.67, 0.04	1.0

^z Samples from the South Rio Grande area (area 1) were excluded from the Pearson's correlation analysis because the incidence of S-strain isolates in this area was very low (2.2%), causing insufficient variation for this variable in area 1. Normal font represents coefficient of correlation and italics represents probability of significance.

were more variable among the areas in the magnitude of *A. flavus* population, with areas averaging >500 CFU/g in the three regions (Fig. 1B).

Soil texture patterns varied across South Texas, with heavier soils (percent clay >50%) mainly in the eastern and northern part of the Coastal Bend to the south-central part of the Upper Coast and the lower part (southeast) of the Rio Grande Valley (Fig. 3A). Percentages of silt were <30% across most of South Texas, with only the northern part of the Upper Coast and a small area in the eastern part of the Rio Grande Valley having between 30 and 40% silt (Fig. 3B). Lighter soils (percent sand >50%) were found from the Rio Grande Valley (except the lower part of the valley) to the southern and western part of the Coastal Bend area (Fig. 3C).

DISCUSSION

Variation in both *A. flavus* population density and strain composition in soils of South Texas may be influenced by various factors. Population density (CFU/g) varies mainly temporally, probably resulting from variation in weather conditions. Overall, in 2002, there was significantly more CFU/g than in either 2001 or 2003. Although, in our data, there were significant differences among regions for CFU/g, spatial variation at regional scales in CFU/g might not play an important role in aflatoxin contamination in South Texas. The Coastal Bend region displayed significantly lower population densities than the Upper Coast region; however, this difference might be due to the previous crop. A greater proportion of sampled fields in the Coastal Bend region had been cropped with sorghum than sampled fields in the Upper Coast (Table 4). Sorghum supports relatively lower *A. flavus* densities compared with corn or cotton. Furthermore, VCA in both the current study and a previous study in Arizona (26) agree with geostatistical analyses that population density (CFU/g) of *A. flavus* varies spatially, but mainly at small geographic scales among and within fields. This is consistent with a major component of variation in population density attributed to variation in crop types.

During the course of the current study, *A. flavus* strain structure in soils of South Texas presented significant spatial variation but not significant temporal variation. The Rio Grande Valley had a significantly lower percentage of the S strain than the Coastal Bend and Upper Coast. Overall percentages of the S strain did not differ significantly among the 3 years of study, although there were significant differences for the percent S among seasons when analyses were performed individually by region. These differences might be attributed to spatial variation rather than to temporal variation. The Rio Grande Valley had a greater percent S

in 2002 and the Coastal Bend had significantly more percent S in 2003. However, these differences could be explained by increased sample numbers from areas within these regions where the S strain is more prevalent. In the case of the Rio Grande Valley, a higher proportion of samples from the northern part of the Valley were taken in the 2002 season whereas, in the Coastal Bend area, a higher proportion of samples from the southeast and northeast areas were taken in the 2003 season.

In this study, both VCA and geostatistics partitioned variation among scales in a similar manner, if one considers that the sampling error and small-scale variability (replications within fields in this study) are represented by the nugget in geostatistics (19) and by the residual (error) in VCA, whereas the variability explained by larger scales will be defined by the variogram (covariogram) range in geostatistics and by the proportion of variance explained by each of the larger scales in VCA. In this study, all the variables that have a large residual (i.e., percent S and CFU/g) also have a large nugget effect, whereas those that have a smaller residual (i.e., percent clay, percent silt, and percent sand) present a smaller nugget effect. On the other hand, for variables that present long variogram ranges (i.e., percent S), an important proportion of the variance is explained by large (among regions) scales in VCA, whereas the variables with short variogram ranges (i.e., CFU/g and percent silt) have none or a very small proportion of the variance explained by the among-regions and among-areas scales in VCA.

Both VCA and geostatistics indicate that approximately half of the variance for S strain incidence occur at the very small scale, which might be explained by both the within-fields spatial variation and the sampling error. An important proportion of the variation occurs at the large scale of among regions. These data are similar to those reported by Orum et al. (26) for Yuma, AZ, where most of the variance for S-strain incidence was explained by the small scales (<5 km) among fields and within fields, with some proportion explained by subregions. However, in that study, larger scales were not explored. In the present study, among fields has a larger scale (up to 25 km) than the Arizona study (26), and this might contribute to the lower variation. Considering that among regions explains an important proportion of the variation in percent S, then some regions might be more conducive to the S strain than others. Factors influencing S strain incidence are not yet understood although, based on our study, we suggest that soil type and crop rotation may play roles.

There were significantly higher quantities of *A. flavus* propagules in soils from fields previously cropped with corn (1,485 CFU/g) than with cotton (566 CFU/g) and sorghum (157 CFU/g), and

TABLE 6. Variogram models for *Aspergillus flavus* (CFU/g), percentage of isolates belonging to the S strain (percent S), and soil texture in South Texas during 2001 to 2003

Variable	Variography	Type	Lag distance (m)	Nugget	Partial sill ^x	Range (m)	IGF ^y
CFU/g							
All crops ^z	Covariogram	Spherical	16,000	896,000	532,000	35,100	0.009
Corn	Covariogram	Spherical	18,000	2,124,000	1,584,000	30,000	0.005
Cotton	Covariogram	Spherical	17,500	607,000	334,000	37,500	0.007
Sorghum	Covariogram	Spherical	16,000	18,200	12,200	35,000	0.025
Others	Covariogram	Spherical	15,700	131,000	37,400	44,000	0.003
Percent S							
All crops	Covariogram	Spherical	18,500	350	102	75,000	0.004
Corn	Covariogram	Spherical	16,300	254	92	56,000	0.005
Cotton	Covariogram	Spherical	17,800	420	190	69,000	0.002
Sorghum	Covariogram	Spherical	18,300	312	208	75,000	0.017
Others	Variogram	Spherical	22,000	58	190	36,500	0.014
Texture							
Clay (%)	Covariogram	Spherical	14,000	90	182	54,000	0.012
Silt (%)	Variogram	Spherical	13,500	37	48	33,000	0.046
Sand (%)	Variogram	Spherical	12,000	128	308	50,000	0.012

^x Partial sill is the sill minus the nugget. It represents the spatially dependent variance.

^y IGF = indicative goodness of fit based on a weighted mean square standardized, where values close to zero indicate good fit.

^z Crop that was cultivated the previous year.

significantly higher percent S in soils from fields previously cropped with cotton (28.6%) than with corn (17.0%). Geostatistical analyses confirmed these trends with higher sill values in corn for CFU/g and in cotton for percent S and a higher average *A. flavus* density (CFU/g) for corn and higher average percent S for cotton. Thus, in South Texas, crop rotation influenced both population density and incidence of the S strain, with corn favoring a high population density of *A. flavus* and cotton and sorghum favoring a high S strain incidence. Cotton previously was suggested to favor the S strain based on greenhouse studies (17). However, this is contrary to what Orum et al. (26) found in Yuma, AZ, where they observed that S-strain incidence was not

dependent on crop sequence and concluded that S-strain incidence was dependent on factors other than crop rotation which extended beyond field boundaries. However, corn rotations were not included in the previous study (26). This rotation is shown in the current study to favor the common L strain in South Texas. Based on data presented in the current study, crop rotation influenced S-strain incidence at the among-fields scale in South Texas.

The *A. flavus* S strain had patchy distribution on large scales, with some areas or regions with higher incidences of the S strain (25,26). However, factors favoring strain S over regional scales have not been characterized. Orum et al. (26) mentioned soil type as a potential influence on strain composition at larger scales than

Fig. 1. Estimated spatial patterns of the incidence of *Aspergillus flavus* S strain in South Texas soils sorted by the previous year crop. Soils were sampled March 2001 through March 2003. Estimations of percent S are based upon Block Kriging (2-by-2-km blocks), which only included fields where the previous year crop was A, all crops, B, cotton, C, corn, D, sorghum, or E, other crops. A search neighborhood of 60 km and a maximum of 40 sample points (fields) were used to generate the Kriging estimates.

among fields. Correlation and geostatistical analyses in the present study indicate that soil type might be a factor affecting *A. flavus* strain composition at regional (among-areas) scales, but not among regions (in the current study, areas extend >25 km and regions >100 km). Percent S correlated positively with percent clay and negatively with percent sand, but only when the Rio Grande Valley region, with low S strain incidence, was not included in the correlation analysis. Likewise, maps of both percent S strain and soil type variables, obtained by kriging, show that those areas with heavy (clay) soils, except for the Rio Grande Valley region, also are the areas with the highest S strain incidence. This indicates that heavy soils might favor the S strain

within regions but not among regions. Factors influencing S-strain incidence among regions have not been described. However, crop rotation may be influencing *A. flavus* strain composition at regional levels. Crop rotations in the Coastal Bend and Upper Coast regions are mainly cotton, sorghum, and corn, whereas crop rotation in the Rio Grande Valley region is more diverse, including sugarcane, citrus, and many vegetable crops such as cucurbits, onion, 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, whereas influences on *A. flavus* by many crops in the Rio Grande Valley are not known.

Fig. 2. Estimated spatial patterns of CFU of *Aspergillus flavus* per gram of soil (CFU/g) in South Texas fields sorted by the previous year crop. Soils were sampled March 2001 through March 2003. Estimations of CFU/g are based upon Block Kriging (2-by-2-km blocks), which only included fields where the previous year crop was A, all crops, B, cotton, C, corn, D, sorghum, or E, other crops. A search neighborhood of 60 km and a maximum of 40 sample points (fields) were used to generate the Kriging estimates.

Fig. 3. Estimated spatial patterns of the soil texture variables **A**, percent clay, **B**, percent silt, and **C**, percent sand in South Texas. Estimations of percentages of clay, silt, and sand are based upon Block Kriging (2-by-2-km blocks). A search neighborhood of 60 km and a maximum of 40 sample points (fields) were used to generate the Kriging estimates.

The S strain of *A. flavus* is an important cause of aflatoxin contamination in Arizona (4,6,17). Based on maps of aflatoxin contamination (20) and the incidence of the S strain in South Texas, areas with high aflatoxin levels also have high incidences of the S strain. Interestingly, on average, soils of all of the high-aflatoxin areas studied contain high percent clay and low percent sand. Although there are some high-clay soils adjacent to the Rio Grande River, they are in an area with neither high levels of aflatoxin (20) nor high incidences of the S strain (Fig. 1). Taken together, these observations suggest that soil type influences S-strain incidence within regions but not among regions. Heavy soils might favor aflatoxin contamination in South Texas by both favoring the S strain, which possesses high aflatoxin-producing potential (6), and favoring growth of *A. flavus* on the crop by retaining high humidity and, thus, favoring the second phase of aflatoxin contamination (3,5,7,31).

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