Spatial and Temporal Patterns of *Aspergillus flavus* Strain Composition and Propagule Density in Yuma County, Arizona, Soils

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ABSTRACT

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Aspergillus flavus isolates from Arizona can be divided into S and L strains on the basis of sclerotial morphology. These genetically distinct strains differ in aflatoxin production. To help understand factors influencing the aflatoxin producing potential of *A. flavus* communities, spatial and temporal patterns of strain incidence were compared with patterns of *A. flavus* propagule density in Yuma County soils. Strain S isolates were found in all sampled fields, but the percentage of strain S isolates ranged from 4 to 93%. A nested analysis of variance was used to determine the spatial scale at which most variability in strain composition and propagule density occurred. For both variables, the largest component of variance occurred among fields within areas at a spatial scale of 1 to 5 km. There was also spatial structure (12 to 21% of the variance) at the subregional level (> 20 km) in strain composition, but not in propagule density. Temporal patterns for both variables were similar. The sampling periods with the highest incidence of strain S isolates, August 1994 (60%) and July 1995 (62%), occurred during cotton boll formation. The regional average for *A. flavus* propagule density was near 1000 propagules/g in the summer, but less than 100 propagules/g in the spring. The results suggest that insights into factors influencing the toxigenicity and propagule density of *A. flavus* communities might be achieved most readily by contrasting fields in close spatial proximity.

Additional keywords: geographic information systems, geostatistics, GIS

Aspergillus flavus Link:Fr. is a soil-inhabiting fungus that saprophytically utilizes a wide range of organic substrates. From an agro-ecological perspective, it is an important degrader of crop debris contributing to nutrient recycling (11). *A. flavus* first attracted the attention of cotton researchers in the early 1950s, when it was associated with a fluorescent fiber condition of raw cotton (14). About a decade later, cottonseed meal was implicated in a widespread epidemic of trout liver cancer (18). The cause was subsequently found to be aflatoxins produced by *A. flavus* (12). Cottonseed is a preferred feed for dairy cows and, with the demonstration that aflatoxins were passed from feed to milk, aflatoxin content of cottonseed became regulated closely. An aflatoxin content greater than 20 µg/kg prevents dairy use and associated premiums. Thus, the aflatoxin content greatly influences the market value of cottonseed.

In Arizona, *A. flavus* isolates can be divided into two strains, S and L, on the basis of sclerotial morphology (S for small, L for large) (4). Each strain is composed of many vegetative compatibility groups (VCGs) (2). Gene flow is limited among VCGs and, as such, sites are occupied by communities of *A. flavus* VCGs rather than a single *A. flavus* population (2). Strain composition is determined by the percentage of isolates that are S strain or L strain. S strain isolates consistently (> 98%) produce large amounts of aflatoxin in infected cotton bolls, whereas L strain isolates on average produce much less aflatoxin, with some producing no aflatoxin at all (4). The two strains can be differentiated on culture media, and strain incidence can provide a rapid estimate of the toxigenicity of an *A. flavus* community. The toxigenicity of the *A. flavus* community in a field may be an important determinant of the extent of aflatoxin production within crops. The average toxigenicity of *A. flavus* communities differs significantly across the southern United States, as well as within Arizona, and is significantly correlated with S strain incidence (9).

Spatial and temporal analysis of plant pathogen communities may provide insights that are useful in plant disease management. Initial spatial analysis of a variable requires determination of the scale or

range of distance over which most variability occurs. Subsequent research may then be directed at the scale of greatest variance and emphasize interpretation of spatial patterns at the appropriate scale. Factors varying at a scale similar to the pathogen may then be sought and the putative influence of these factors on the pathogen empirically evaluated. Patterns caused by landscape, crop sequence, climate, and tillage may each differ in scale, and the influence of each factor may cause variance at different scales in a pathogen population. Determination of spatial scale can be efficiently done using a nested analysis of variance (15,17).

In the current study, our primary goal was to determine the spatial scales in which most variability occurs for both *A. flavus* strain composition and propagule density. In the process, we also assessed both the temporal stability of spatial scale and temporal variability in *A. flavus* propagule density and strain composition.

MATERIALS AND METHODS

Thirty commercial fields were selected from a cross-section of Yuma County cultivated land, ranging from Texas Hill on the east to San Luis in the southwestern corner of the county. Soil samples were collected according to a nested sampling design to assess *A. flavus* communities over several spatial scales which were named "subregions" (20 to 100 km apart), "areas within subregions" (10 to 15 km), "fields within areas" (1 to 5 km), and "locations within fields" (150 to 300 m) (Fig. 1). The entire study area is considered a region in the context of Arizona agriculture. Four subregions contained two areas each and a fifth contained only one area. Each area consisted of three or four fields within a 3-km radius (Fig. 1). Within each field, locations at opposite corners were selected in order to maximize the estimate of within-field variance. Each location within a field contained three sites, 10 m apart. At each site, between 50 and 100 g of dry soil was collected from the soil surface (top 1 to 2 cm) of a 3 by 3 m square. Samples were stored dry in cotton bags (Hubco soil sample bags, Forestry Products Supply, Jackson, MS) at room temperature (23 to 27°C) in order to allow similar treatment of all samples and to maximize stability of propagules in the soil during the assay period.

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Crop sequences in Yuma County are variable (Table 1). Sixteen cotton fields (fields 1 to 16) were selected in the fall of 1993 based on grower plans to plant lettuce (5 fields), crucifers (5 fields), or remain fallow (6 fields) during the winter of 1994. Of those, only 7 were replanted in cotton the following season. Thus, the three crop sequences became seven sequences by the following cotton season. In the winter of 1995, 14 additional fields were added to the study in a nested design (Fig. 1). Crop sequence data were recorded, but comparisons based on farmer plans were not incorporated into the design. Market conditions favored different crops from one year to the next and influenced which rotations could be observed. Sudan grass was fa-

vored in the summer of 1994, cotton in the summer of 1995, and wheat in the winter of 1996. This provided an opportunity for comparisons of short crop sequences among subsets of the 30 fields selected for the nested spatial study.

Laboratory assays were performed within 100 days of sample collection. Repeat sampling from a subset of the bags indicated the *A. flavus* strain composition of the soil in the bags did not change within this time frame. The soil in each bag was mixed and a sub-sample (5 to 20 g) was suspended in 50 ml of sterile water. Smaller sub-samples (5 g) were used when previous experience suggested propagule densities would be high. The soil suspension (0.2 ml) was spread on each of six 10-

cm petri plates containing a modified rose bengal agar medium (7). Plates were incubated for 3 days at 30°C, after which *A. flavus* colonies were enumerated and transferred to 5/2 agar plates (5% V8 juice, 2% agar) for strain classification. Isolates were transferred by working outward from the center of selected dilution plates until 30 isolates were transferred per site. Isolates were classified as S or L strain on the basis of sclerotial formation on the 5/2 medium within 4 days. S strain isolates produced numerous small sclerotia.

The SAS statistical package (SAS Institute, Cary, NC) was used for analysis of variance (procedures GLM and NESTED) and for correlations (procedure CORR). PC ArcInfo and ArcView (version 1.0, ESRI, Redlands, CA) were used for geographical information system (GIS) analysis and display of the commercial field data. Propagule density (propagules per g of soil) at each location was determined by averaging propagule counts from three bags of soil collected from sites 10 m apart. To determine strain composition, all isolates characterized from the three sites at each location were pooled and the percentage of S strain isolates determined. For analyses where it was critical to measure within field variance, and for correlations between locations within fields, only those fields where at least 45 isolates were characterized from all sites at each of the two locations were used (10 fields in March 1995, 16 fields in July 1995, and 26 fields

Fig. 1. Illustration of the nested sampling design used for the collection of soil samples from agricultural fields in Yuma County, Arizona.

Table 1. Crop sequences for sampled fields

Subregion	Area ²	Field	Crop				
			Winter 1994	Summer 1994	Winter 1995	Summer 1995	Winter 1996
San Luis	1	2	Lettuce	Sudan		Cotton	Lettuce
		3	Lettuce	Fallow	Lettuce	Cotton	Lettuce
		10	Crucifer	Fallow		Cotton	
		17			Lettuce	Cotton	Wheat
North Gila	2	4	Lettuce	Sudan		Cotton	Corn
		29	Alfalfa	Alfalfa	Fallow	Cotton	Wheat
		30	Melon	Fallow	Lettuce/wheat	Fallow	Wheat
	3	5	Lettuce	Cotton	Lettuce	Cotton	Wheat
		27	Lettuce	Cotton	Fallow	Cotton	Wheat
		28	Crucifer	Cotton	Fallow	Cotton	Wheat
Dome	4	9	Crucifer	Sudan		Cotton	Lettuce
		25	Wheat		Crucifer	Corn	Corn
		26	Fallow	Cotton	Fallow	Cotton	Lettuce
	5	7	Crucifer	Sudan	Fallow	Cotton	Lettuce
		8	Crucifer	Sudan	Fallow	Cotton	Lettuce
		24			Crucifer	Fallow	Lettuce
Tacna-West	6	16	Fallow	Cotton	Fallow	Cotton	Fallow
		22	Lettuce	Cotton	Fallow	Cotton	Lettuce
		23	Fallow	Cotton	Fallow	Cotton	
	7	15	Fallow	Cotton	Fallow	Cotton	Fallow
		20	Alfalfa	Alfalfa	Alfalfa	Alfalfa	Wheat
		21	Wheat	Sudan	Fallow	Cotton	Wheat
Texas Hill	8	13	Fallow	Cotton	Wheat	Sudan	Fallow
		14	Fallow	Cotton	Wheat	Fallow	
		18	Alfalfa	Alfalfa	Alfalfa	Cotton	
		19			Fallow	Cotton	Wheat
	9	1	Lettuce/wheat	Fallow	Lettuce	Cotton	Wheat
		6	Crucifer	Cotton	Fallow	Cotton	Lettuce
		11	Wheat	Fallow	Crucifer	Cotton	Crucifer
		12	Fallow	Cotton	Fallow	Cotton	Lettuce

² Areas were located throughout the agricultural part of Yuma County, from Texas Hill to San Luis.

in October 1995). Using locations with fewer than 45 isolates characterized introduced too much variability into the estimate of strain composition. For broader scale analyses where within-field variability was not an issue (Table 2), data from all sites at both locations within a field were pooled, and incidence of S strain was calculated without regard to within-field location. The number of isolates classified for each field is shown in Table 2.

RESULTS

A. flavus S strain was found in every field at each sampling period. The percentage of *A. flavus* isolates characterized as S strain (percent S) varied from 4% to 93% (Table 2). Using the variance between locations within fields for the error term, an analysis of variance of all areas indicated significant differences in percent S strain among fields within areas in March 1995 ($P = 0.0195$), July 1995 ($P = 0.0028$), October 1995 ($P = 0.0009$), and March 1996 ($P = 0.0013$). Within two areas, there were pairs of fields that consistently differed in percent S strain over the past four to five sampling periods: fields 5 and 28 in area 3, and fields 1 and 11 in area 9 (Table 2). Consistency among fields within areas over time was tested statistically by comparing means across all sampling dates using the interaction of sampling date and

field as the error term in an analysis of variance; significant ($P = 0.05$) differences occurred in areas 3 and 9 (Table 2). The spatial relationships among fields in area 9 are illustrated in Figure 2. On each sampling date, field 1 had the greatest S strain incidence (Fig. 2).

The basic spatial structure of *A. flavus* strain composition (as measured by percent S) was similar for all four sampling periods in 1995 and 1996 (Table 3). Most variance was observed among fields within areas (47.4 to 59.9%), which was successively greater than variance within fields (26.3 to 32.0%), among subregions (12.0 to 20.8%), and among areas within subregions (0.0 to 5.6%). The spatial scale among fields within areas is 1 to 5 km. This was also the spatial scale of greatest variance in propagule density (Table 4). Variance among fields within areas (38.9 to 78.3%) was greater than within fields (21.7 to 43.6%). Variance among areas within subregions (0.0 to 16.6%) and among subregions (0.0 to 5.6%) was similar and less than the finer spatial scales.

By requiring characterization of at least 45 isolates per location prior to inclusion in the analysis of spatial structure, random error was kept low. This is demonstrated by the significant correlations among percent S values from different locations within fields (March 95, 10 fields, $r =$

0.70, $P = 0.024$; July 95, 16 fields $r = 0.67$, $P = 0.005$; October 95, 26 fields $r = 0.68$, $P = 0.0001$; March 96, 23 fields, $r = 0.67$, $P = 0.0004$). The correlation coefficient for the relationship for all sampling dates was 0.69. There was no correlation between strain composition and propagule density among fields for any of the five sampling dates ($r = -0.26$ to 0.30 ; $P = 0.11$ to 0.69).

Crop sequences varied greatly among the fields (Table 1), but in 1995, 24 of the 30 fields were planted in cotton. A nested analysis of variance of S strain incidence, based only on the cotton fields, partitions the variance in almost exactly the same way as when all fields were included (plus or minus 1% of the values shown in Table 3 for July 95). Three pairs of crop sequences occurred at adequate frequency for statistical evaluation of crop sequence influence on *A. flavus* communities (Table 5). In most cases, no significant differences among crop sequences were detected. However, in 1995, propagule density was greater and the increase in S strain incidence lower in the "lettuce to cotton" than in the "fallow to cotton" sequence. Note that the "lettuce to cotton" sequence in 1995 included both extremes in S strain incidence: field 1 (March 91%, July 93%) and field 5 (March 6%, July 12%; Tables 1 and 2). Cotton was not planted in either 1994 or 1995 in three fields (fields 20, 25,

Table 2. Strain composition of *Aspergillus flavus* soil communities in 30 commercial fields in Yuma County

Subregion	Area ^w	Field	<i>A. flavus</i> isolates characterized as strain S (%)					Mean ^x
			August 1994	March 1995	July 1995	October 1995	March 1996	
San Luis	1	2	40 (43) ^y	32 (75)	61 (61)	25 (184)	24 (144)	36 a
		3	36 (77)	46 (84)	65 (139)	59 (167)	63 (140)	54 a
		10	52 (50)	34 (71)	77 (126)	87 (194)	19 (154)	54 a
		17	nd ^z	39 (64)	46 (100)	59 (126)	53 (80)	49 a
North Gila	2	4	63 (54)	40 (113)	68 (176)	38 (132)	50 (106)	52 a
		29	nd	45 (104)	66 (130)	61 (211)	50 (26)	56 a
		30	nd	53 (32)	34 (177)	58 (43)	37 (27)	46 a
	3	5	44 (48)	6 (138)	12 (232)	11 (180)	6 (151)	16 b
		27	nd	4 (89)	59 (39)	34 (132)	14 (108)	28 b
		28	nd	36 (128)	70 (175)	61 (147)	31 (108)	50 a
Dome	4	9	87 (39)	49 (67)	60 (116)	72 (158)	26 (99)	59 a
		25	nd	30 (205)	54 (224)	45 (213)	28 (139)	39 a
		26	nd	21 (252)	61 (254)	33 (175)	32 (125)	37 a
	5	7	79 (53)	24 (66)	75 (151)	35 (154)	19 (118)	46 a
		8	71 (55)	43 (60)	47 (144)	11 (173)	8 (145)	36 a
		24	nd	29 (56)	77 (90)	40 (98)	50 (105)	49 a
Tacna-West	6	16	59 (64)	25 (52)	77 (81)	35 (200)	46 (139)	44 a
		22	nd	36 (99)	55 (152)	15 (172)	9 (114)	29 a
		23	nd	49 (117)	32 (173)	32 (173)	35 (148)	37 a
	7	15	32 (56)	25 (218)	35 (256)	13 (154)	26 (141)	24 a
		20	nd	74 (153)	38 (123)	39 (171)	21 (110)	43 a
		21	nd	46 (39)	76 (107)	63 (198)	29 (117)	54 a
Texas Hill	8	13	44 (52)	30 (50)	90 (51)	74 (81)	28 (89)	53 a
		14	82 (50)	39 (97)	34 (67)	36 (73)	24 (88)	43 a
		18	nd	31 (81)	85 (164)	59 (171)	36 (152)	53 a
		19	nd	37 (40)	79 (182)	83 (185)	67 (186)	67 a
	9	1	83 (81)	91 (151)	93 (150)	76 (165)	86 (162)	86 a
		6	66 (65)	35 (80)	87 (150)	63 (155)	78 (126)	66 b
		11	44 (41)	50 (36)	52 (113)	51 (145)	29 (133)	45 c
		12	65 (55)	56 (88)	86 (118)	68 (136)	66 (122)	68 b

^w Areas were located throughout the agricultural part of Yuma County from Texas Hill to San Luis.

^x Means followed by the same letter are not significantly different from means of other fields within the same area by the Duncan's multiple range test ($P = 0.05$) using the interaction of sampling date and field as the error term.

^y Number of isolates characterized are in parentheses following the percent Strain S.

^z No data. Data were collected from 16 fields in 1994 and 30 fields in 1995 and 1996.

30). S strain incidence in these fields ranged from 21 to 74% and did not appear to differ from other fields in their respective areas (Table 2).

The subregions were an important source of variance for strain composition (Table 3), but not for propagule density (Table 4). The Texas Hill subregion had a significantly higher incidence ($P = 0.05$) of

the S strain (averaged over the five sampling times) than any of the other subregions (Fig. 3A). However, there was no evidence for a subregional pattern in *A. flavus* propagule density (Fig. 3B). Both S strain incidence and *A. flavus* propagule density were greater in August 1994 and July 1995 than in March 1995 and March 1996 (Fig. 4A and 4B).

DISCUSSION

Many studies of the spatial structure of soil population magnitude have examined within-field variability for various fungal genera and species (3), but few have characterized spatial structure across orders of magnitude of spatial scale, and none have compared the spatial scale of variability in propagule density with the spatial scale of variability in fungal strain composition. In the current study, partitioning of variance indicated that most variability in both *A. flavus* propagule density and strain composition of communities occurred among fields within areas. This corresponds to the spatial scale of 1 to 5 km. Cropping sequences are complex in this region, and fields typically have multiple crops in a single year. Crop rotation, in this study, explained relatively little of the observed variability in strain composition. The extremes in S strain incidence, field 1 (averaging 86% S strain) and field 5 (averaging 16% S strain), had identical sequences in 1995 and 1996 (lettuce to cotton to wheat).

Fig. 2. Spatial and temporal pattern of *Aspergillus flavus* strain S incidence. (A) Fields in area 9 are located in a 3-km-wide strip of agriculture between the railroad tracks on the north and the Gila River on the south, with desert beyond. (B) Within each collection date, bars with the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

Fig. 3. (A) *Aspergillus flavus* strain composition and (B) propagule density among subregions in Yuma County, Arizona, averaged over five collection dates from August 1994 through March 1996. Bars with the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

Although sampling in the current study extended over less than 2 years, the composition of several fields (for example, fields 10 and 13) varied widely over time. This indicates *A. flavus* community composition may shift rapidly. The observed increased propagule density of *A. flavus* in the hot dry summer months in Arizona is consistent with previous reports (11), but a seasonal shift in strain composition at the regional scale has not been reported previously. Despite the similarity in temporal patterns of the strain composition and propagule density variables when averaged over the entire region, there was no correlation between propagule density and strain composition at the field level. This suggests that the preferential increase of the S strain in soil may occur both in soils that are highly favorable to *A. flavus* increases, and in those that are only marginally favorable. The most obvious difference in the sampling times is temperature, but many variables, such as soil condition, day length, crop sequence history, insect levels,

fungicide application history, irrigation water quality, and irrigation frequency may influence *A. flavus* communities. However, selective forces which favor differential strain success are not known. Rapid changes in the composition of *A. flavus* vegetative compatibility groups in a Yuma County cotton field have been attributed to founder effects caused by a few VCGs establishing early in the crop (1).

Fields within certain areas (for example, areas 3 and 9) retained significant differences in strain composition over time. Apparently, some factor other than crop rotation, with variability at the 1 to 5 km scale, is influencing strain composition. Potential factors include soil type, irrigation source and frequency, tillage practices, and micro-climatic factors. Identification of the putative factor may permit development of techniques to reduce S strain incidence and, in so doing, reduce aflatoxin producing potential of *A. flavus* communities. None of the fields within the present study were adjacent to each other. Future

efforts to understand the influence of landscape variables on the *A. flavus* community in Yuma County soils should concentrate on a scale of about 5 km and include the sampling of adjacent fields in order to partition short range spatial structure within and between fields.

Patterns in *A. flavus* strain composition were contrasted with patterns in *A. flavus* propagule density in order to better understand factors influencing the aflatoxin contamination of cottonseed. The expectation that the strain composition of the *A. flavus* community can affect the aflatoxin level in the cotton crop is based on field plot studies (8). In plots where the soil surface was treated with wheat kernels inoculated with atoxigenic strains of *A. flavus*, infected cotton bolls had significantly lower aflatoxin levels than infected cotton bolls in untreated control plots (8). These field plot studies are supported by greenhouse studies that show that atoxigenic strains have the capacity to competitively exclude toxigenic strains from wound sites on cotton bolls (5,10). It is much more difficult to develop evidence of the effect of strain toxigenicity on aflatoxin levels in commercial fields for at least two reasons. First, many factors other than strain toxigenicity influence aflatoxin levels (for example, high variation in pink bollworm infestation might mask effects of strain composition). Second, special coordination with farmers and gins is required to associate aflatoxin levels at the gins with particular fields. Studies such as the one reported here help build a foundation for commercial field studies which, in the future, may be able to relate strain composition to aflatoxin levels in the crop.

Results of the current study highlight variation among fields within areas, but subregional patterns were also observed. Fields in the Texas Hill subregion were, on average, consistently greater in S strain incidence than fields in other subregions. Thus, the Texas Hill subregion may have some broad characteristic that gives a competitive advantage to the S strain. Although significantly lower than Texas Hill, the percent S averages for the other three subregions in Yuma County (40 to 50%) are high compared with those observed in other cotton producing areas where regional averages are frequently below 20% S strain (6). S strain incidence along Mari-

Table 3. Description of the spatial scale of *Aspergillus flavus* strain composition using components of variance from a nested analysis of variance^z

Variance source	Spatial scale (km)	Percent total variance			
		1995			1996
		March	July	October	March
Among subregions	> 20	12.0	19.9	20.8	13.8
Among areas within subregions	10-15	0.0	0.0	0.0	5.6
Among fields within areas	1-5	59.9	53.8	47.4	48.6
Among locations within fields	0.2-0.4	28.1	26.3	31.8	32.0
Total		100.0	100.0	100.0	100.0

^z The nested sampling design is illustrated in Figure 1. Strain composition (the percentage of isolates that are strain S) was determined by characterizing at least 45 isolates representing three sites located 10 m apart at each location.

Table 4. Description of the spatial scale of *Aspergillus flavus* propagule density using the components of variance from a nested analysis of variance^z

Variance source	Spatial scale (km)	Percent total variance			
		1995			1996
		March	July	October	March
Among subregions	> 20	0.0	0.0	0.9	5.6
Among areas within subregions	10-15	0.0	14.0	16.6	0.0
Among fields within areas	1-5	78.3	62.2	38.9	70.8
Among locations within fields	0.2-0.4	21.7	23.8	43.6	23.6
Total		100.0	100.0	100.0	100.0

^z The nested sampling design is illustrated in Figure 1. Propagule density (propagules per g of soil) at each location was determined by averaging propagule counts from three bags of soil collected from sites 10 m apart.

Table 5. Comparison of changes in *Aspergillus flavus* propagule density and strain S incidence for selected crop sequences

Temporal sequence		Crop sequence		No. of fields (n)	Propagule density (prop/g)			Strain S incidence (%)		
Time 1	Time 2	Time 1	Time 2		Time 1	Time 2	Change	Time 1	Time 2	Change
Summer 94	Summer 95	Sudan	Cotton	5	1,008 ^y	502	-506	68	62	-6
		Cotton	Cotton	5	1,685	1591	-94	53	59	6
Winter 95	Summer 95	Lettuce	Cotton	4	193	5662 ^{z*}	5,469*	46	54	8*
		Fallow	Cotton	13	178	814*	636*	34	64	30*
Summer 95	Winter 96	Cotton	Lettuce	9	1,275	53	1,222	66	36	-30
		Cotton	Wheat	8	2,868	85	2,783	63	42	-21

^y The values for propagule density and strain S incidence are means.

^z * indicates crop sequences are different statistically by Wilcoxon's rank-sum test ($P < 0.05$).

Fig. 4. (A) *Aspergillus flavus* strain composition and (B) propagule density averaged over all fields in all subregions at five collection dates from August 1994 through March 1996. Bars with the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

copa Road in Pinal County, Arizona, was less than 10% in 1989 and 1993 (9).

References to spatial and temporal patterns in aflatoxin studies have been more

general than the present study. Manabe and Tsuruta (13) found that the proportion of aflatoxin-producing fungi increased as one approached the tropics in Southeast Asia, but they compared species rather than strains, and at the continental scale rather than the regional scale. Marsh and Taylor's geographic study (14) of *A. flavus*-caused fluorescent spots on cotton fiber was also framed at the continental scale. Russell et al (16) described, in broad terms, temporal and spatial patterns in aflatoxin levels on harvested cotton, and remarked on a high level of "field to field within an area" variation. Understanding patterns of *A. flavus* strain composition and propagule density may lead to a better understanding of variability in aflatoxin contamination. Spatial and temporal dynamics of *A. flavus* communities at the subregional scale will influence the success of efforts to modify toxigenicity of *A. flavus* communities through application of atoxigenic L strain isolates. Whether interpreting the results of large scale atoxigenic strain field trials or assessing the impact of cultural management practices, current results suggest variability at the 1 to 5 km scale will be important in the Yuma County soils.

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