Magnitude and strain composition of *Aspergillus flavus* soil surface populations in Yuma County commercial fields

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Abstract

Aflatoxin contamination of cottonseed occurs when cotton bolls are infected by certain strains of the fungus Aspergillus flavus. The risk of aflatoxin contamination in a field is partially dependent on both the quantity of A. flavus and the toxigenicity of A. flavus strains in that field. A. flavus can be easily divided into two major subdivisions known as strain S and strain L. Strain S isolates consistently produce large amounts of aflatoxin and, therefore, the percentage of strain S isolates in the population (percent S) is one indication of the aflatoxin producing potential of the population. Strain S isolates were found in every commercial field sampled at every sampling date in Yuma County, but percent S varied greatly among fields from 4% to 93%. Significant differences among fields located near each other suggest that locally important, but not yet identified, variables such as crop rotation histories or soil type are affecting A. flavus population magnitude and composition.

Introduction

When cotton bolls are infected by certain strains of the fungus, *Aspergillus flavus*, aflatoxin contamination of the cottonseed can occur. Because aflatoxin is one of the most potent carcinogens known, it is highly regulated in the food supply (Cotty et al. 1994). In addition to cottonseed, aflatoxin contamination is also a problem in peanuts, corn, and some tree nuts. Cottonseed produced in Arizona is routinely monitored for aflatoxin levels. Cottonseed must be less than 20 parts per billion aflatoxin to be used for dairy feed and less than 300 parts per billion aflatoxin for feedlot use. Thus, the aflatoxin content greatly influences the market value of cottonseed. *A. flavus* has been found to have a variety of genetic types which vary greatly in their ability to produce aflatoxin (Bayman and Cotty 1991) . Because some strains produce no aflatoxin at all and yet are competitive with aflatoxin producing strains in the crop environment, efforts are underway to use these naturally occurring atoxigenic strains as an aflatoxin control tool (Cotty 1989, Cotty 1992, and Cotty 1994a). This management possibility has greatly increased the interest in the population ecology of *A. flavus* both in terms of population magnitude and toxigenicity. The *A. flavus* population can be divided into two major subdivisions, known as strain S and strain L. The strain S isolates consistently (>98%) produce large amounts of aflatoxin in infected cotton bolls whereas the strain L isolates are more variable, with some producing no aflatoxin at all. Strain S and L isolates can be readily differentiated on culture media and therefore the percentage of isolates that are strain S can be used as one estimate of the toxigenicity of the *A. flavus* population. Because some strain L isolates are also highly toxigenic, a low percentage of strain S does not guarantee low toxigenic potential, but a high strain S percentage does indicate a highly toxigenic population. The epidemiology of aflatoxin contamination caused by *A. flavus* is best understood in terms of the fungus (various strains) , the host (cotton bolls - before and after opening, length of growing season), and the physical/biological environment (hot temperatures, rain, humidity, winds, irrigation practices, cropping patterns, pink bollworm populations). The fungus lives primarily on dead plant and insect tissue, but also has the ability to invade living tissue, particularly at wound sites caused by the pink bollworm in cotton (Ashworth et al. 1971, Cotty and Lee, 1989). Sampling from the soil surface provides one

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measure of the *A. flavus* population in the crop environment. Soil surface population magnitude and toxigenicity are important components of the risk of aflatoxin contamination in the cotton crop. In this study, therefore, we have focused not only on *A. flavus* soil surface population magnitude but also on its strain composition.

Materials and Methods

In the fall of 1993, sixteen 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. In 1994, fourteen fields were added to the study in a nested design. Nine groups of three or four fields were selected such that all fields in a group were within a circular area of radius 3 km (Fig. 1). Two clusters of three sample sites were selected at opposite corners of the field. The clusters were 150 to 300 m apart. The three sample sites were approximately 10 m apart within each cluster. The sampling design allows analysis of within field variation in terms of the distance between sample sites and to detect whether within-field variation is less than between-field variation and whether variation among fields within the groups is less than variation among groups. The purpose of this design is to understand whether factors specific to individual fields or factors related to local areas have a decisive impact on *A. flavus* population magnitude and composition. We were also looking for broad scale regional differences among groups across the county.

Between 50 and 100 g of soil were collected at each site, placed in a cotton bag and stored at room temperature in the laboratory (25-27C) until assayed. The soil in the bag was mixed and a subsample between 5 and 20 g was weighed and suspended in 50 ml of water. A 0.1 ml aliquot from the soil suspension was spread on each of six 10-cm petri plates containing a selective agar medium (Cotty 1994b). The plates were incubated for 3 to 4 days at 30 C and the number of *A. flavus* colonies were counted and recorded. When available on the dilution plates, for each site, as many as 30 *A. flavus* colonies were transferred from the dilution plates to 5/2 agar plates (5% V-8 juice, 2% Agar) for strain classification. Isolates were classified as strain S if many small sclerotia appeared on the 5/2 media within 3 or 4 days and classified as strain L if no sclerotia appear.

Data were analyzed by analysis of variance using procedures GLM and NESTED in the SAS statistical package (The SAS Institute, Cary, N. C.). 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.

Results and Discussion

The highly toxigenic *A. flavus* strain S isolates were found in every commercial field sampled at every sampling date in Yuma County, but the percentage of *A. flavus* isolates that were strain S (percent S) varied greatly among fields from 4% to 93% (Table 1). Percent S, rather than mere presence or absence of strain S, is an important measure of aflatoxin risk because S and L strains compete at entry sites on the cotton bolls (Cotty 1990, Cotty and Bayman 1993, Cotty 1994a). Many L strain isolates produce little or no toxin, whereas almost all S strain isolates produce toxin (Cotty 1989). Thus, the incidence of strain S influences the toxigenicity of *A. flavus* populations. Nested analysis of variance of *A. flavus* soil surface populations indicates that percent S differs among fields and sometimes among groups of fields (Table 1). This suggests that there are locally important factors - including possibly crop rotation history - that influence the *A. flavus* population. In March 1995, but not in July 1995, there were significant differences among groups of fields across Yuma County. Fields in group 3, located in the North Gila Valley, were significantly lower in percent S than fields in groups 2, 7, and 9 (Fig. 2). Ongoing research will help determine whether these regional scale patterns are recurring and predictable or whether they are random. A temporal comparison shows that population magnitude was much lower in March 1995 than either in August 1994 or in July 1995 (Fig 3A). The *A. flavus* population increases rapidly as the cotton crop develops during the hot summer and crashes during the winter months. This cycle in the population magnitude might also provide an opportunity for the population composition to shift rapidly between years. In March 1995, percent S was significantly lower than in August 1994 or July 1995 (Fig 3B.) In general, percent S throughout Yuma County was found to be much higher than percent S in fields along Maricopa Road and at the Maricopa Agricultural Center (MAC) in Pinal County. Percent S was frequently greater than 50% and sometimes greater than 90% in Yuma County, but was often less than 20% at MAC and along Maricopa Road in sampling done over several years. Interpreting the spatial and temporal dynamics of

the composition of *A. flavus* populations is important because the composition of the population can be changed by adding naturally occurring atoxigenic strains (Cotty 1994a). Soil surface *A. flavus* population data, such as reported here, will be helpful in deciding what practices might encourage atoxigenic strain success. Many landscape scale variables, such as soil type, field size, crop sequence history, irrigation water quality, soil temperature etc. can influence the way in which the *A. flavus* population expands during the summer growing season. Increased knowledge of environmental and crop management influences on *A. flavus* populations may lead to improved management practices that reduce the risk of aflatoxin contamination of cottonseed.

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Tables

Table 1. Magnitude (propagules per gram) and strain composition (Percent S[y]) of *A. flavus* soil surface populations in thirty commercial fields in Yuma County. All fields within a group were located within a circular area of radius 3 km. Groups of fields were located throughout the agricultural areas of Yuma County from Texas Hill to San Luis (Fig. 1 and 2). Nested analysis of variance indicates that population magnitude and percent S differs among fields and, in March 1995, percent S also differs among groups.

Group	Field	Propagules per gram			Percent S[y]		
		August 1994	March 1995	July 1995	August 1994	March 1995	July 1995
1	2	1163	75	150	nd[z]	32	61
	3	3335	126	2249	36	46	65
	10	493	61	738	52	34	77
	17	nd[z]	85	786	nd	39	46
2	4	771	204	790	63	40	68
	29	nd	357	328	nd	45	66
	30	nd	16	412	nd	34	nd
3	5	604	298	3054	44	6	12
	27	nd	70	24	nd	4	nd
	28	nd	176	176	nd	36	70
4	9	244	20	552	nd	49	60
	25	nd	126	893	nd	30	54
	26	nd	956	1570	nd	21	61
5	7	629	22	297	79	24	75
	8	2237	63	722	71	43	47
	24	nd	27	1195	nd	29	77
6	16	1021	10	107	59	25	77
	22	nd	12	358	nd	36	55
	23	nd	78	540	nd	49	32
7	15	1711	327	593	32	25	35
	20	nd	1067	258	nd	74	38
	21	nd	5	285	nd	nd	nd
8	13	411	18	68	44	30	90
	14	355	87	72	82	39	34
	18	nd	29	622	nd	31	85
	19	nd	15	1733	nd	nd	nd
9	1	1741	263	16561	83	91	93
	6	3572	29	3862	66	35	87
	11	868	16	252	nd	nd	nd
	12	1522	32	1726	65	56	86

[y]The percentage of *A.flavus* isolates characterized as strain S.

[z]No data. Data were collected from sixteen fields in 1994 and thirty fields in 1995. Percent S is reported from only those fields where at least 45 isolates were characterized.

Figures

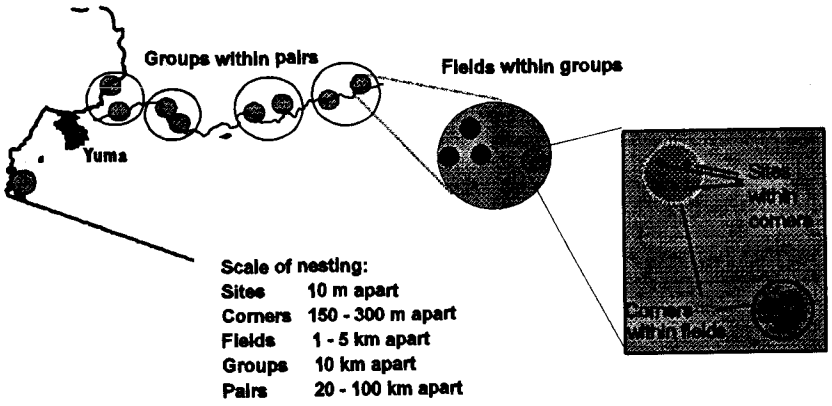

Figure 1. Nested sampling design for commercial fields in Yuma County. Samples were collected from three sites 10 m apart at two locations within each field. Groups consisting of three or four fields selected within a 3-km radius circular area were paired along the Gila River between Texas Hill and Yuma.

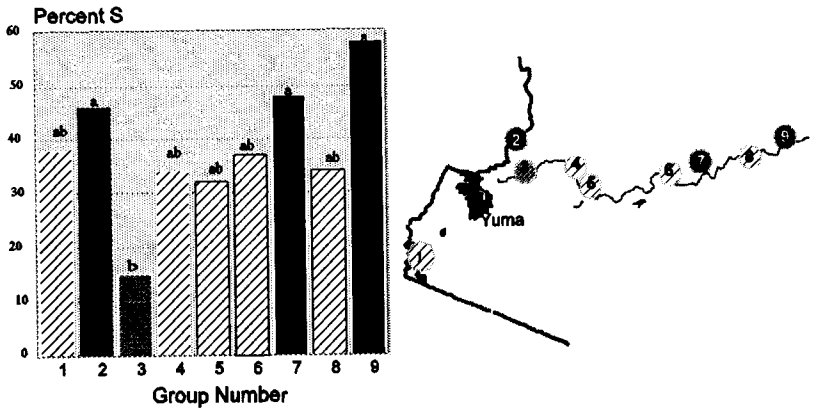

Figure 2. Differences in *A. flavus* population composition among groups of fields in Yuma County in March 1995. Fields in group 3, located in the North Gila Valley, were significantly lower in percentage of strain S isolates than fields in groups 2, 7, and 9. Differences in percent S among groups were not significant in July 1995 (not shown).

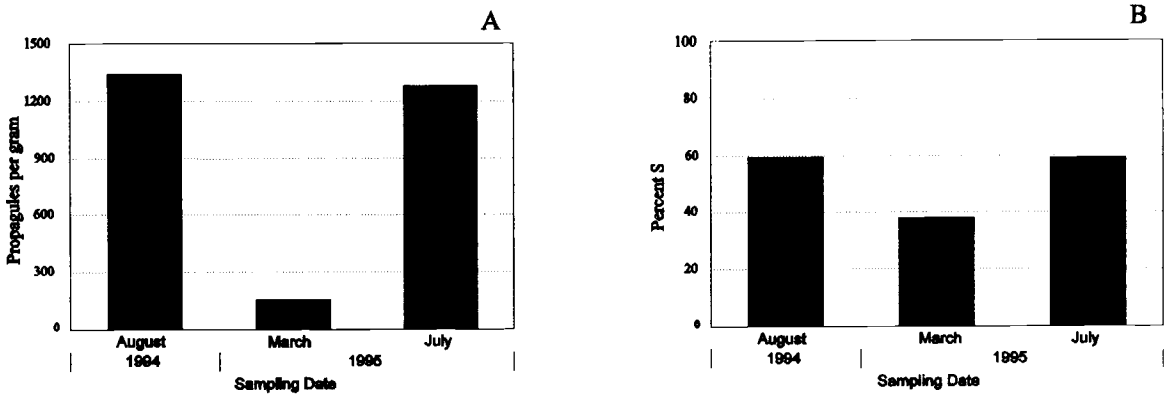

Figure 3. Temporal patterns in *A. flavus* soil surface population magnitude (propagules per gram) and strain composition (percent S) in Yuma County commercial fields. Population magnitude (**A**) and percentage of strain S isolates (**B**) were lower in March 1995 than either in August 1994 or July 1995.