Populations of *Aspergillus flavus* in the Iowa Cornfield Ecosystem in Years Not Favorable for Aflatoxin Contamination of Corn Grain

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ABSTRACT

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Populations of *Aspergillus flavus* were measured at monthly intervals from June to September in corn crop residues, corn leaf tissues, soil, and air over two periods, 1979 to 1980 and 1991 to 1993, in permanent tillage and rotation plots (64 × 64 m) established at Nashua, IA, in 1977. *A. flavus* was detected in soil each year of the study. Tillage and rotation practices had little impact on soilborne populations of *A. flavus*, but significantly greater populations occurred in July in 1991 to 1993 than occurred in June, August, or September. The fungus was recovered at greater frequencies from corn residues of continuous corn plots than from corn grown in the soybean-corn rotation. Populations of *A. flavus* on leaves were unaffected by tillage or rotational practices. Airborne spores of *A. flavus* were detected in all plots in each year of the study, but tillage and rotation treatments had no significant effect on airborne populations. A survey of 40 Iowa cornfields over the period 1991 to 1993 that had aflatoxin-contaminated corn in 1988 showed widespread occurrence of *A. flavus* in soil and corn residues. Populations in these fields were of the same magnitude as those found at the Nashua plots. Assays for *A. flavus* from soil from these fields showed greater soilborne populations in July of 1993. This study indicates that *A. flavus* is widely distributed in the corn ecosystem in Iowa at very low population levels in years that are not favorable for extensive aflatoxin contamination of corn grain.

Additional keyword: *Zea mays*

Sporadic outbreaks of preharvest contamination of corn (*Zea mays* L.) by aflatoxin in the midwestern U.S. can cause disruption and economic loss to the grain trade (7). Despite extensive research into many aspects of the biology of the causal agent, *Aspergillus flavus* Link:Fr., no economical and effective control practices have been developed. Progress on control has been hampered by a lack of knowledge of where *A. flavus* exists in the corn agroecosystem and of the cultural and environmental factors that influence population dynamics. Shearer et al. (11) found extensive colonization of corn crop residues and soil in fields with severe aflatoxin contamination in 1988, indicating that epidemics are associated with large increases in populations of the fungus. The high populations of the fungus occur in years favorable for aflatoxin development. Favorable years are hot and dry, with high maximum, minimum, and average ambient temperatures (especially in July and August), very low precipitation, and drought stress on corn plants (7). To understand the conditions that might result in these population increases, it is essential to understand the characteristics of the population in the corn ecosystem in years not favorable for aflatoxin contamination of corn grain.

The purpose of this study was to provide quantitative data on the distribution of *A. flavus* in the soil, corn crop residues, leaves, and air in permanent tillage and rotation plots at Nashua, IA. The study was carried out in 1979 to 1980, immediately after establishment of the plots in 1977, and again 11 years later (1991 to 1993). A broader geographic examination of the distribution of the fungus in soil and crop residues was made over the period 1991 to 1993 in commercial cornfields across Iowa in which extensive aflatoxin contamination was recorded in 1988.

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MATERIALS AND METHODS

Sampling permanent tillage and rotation plots. Corn crop residues, corn leaves, soil, and air were sampled over two periods, 1979 to 1980 and 1991 to 1993, at a permanent tillage and rotation experimental site at Nashua, IA, where crop rotation and tillage studies have been conducted since 1977. Samples were collected from two tillage treatments, identified as plow and no-till, and two rotational treatments, continuous corn and alternating corn and soybean. Treatments were randomized in a complete block design with four replicates of each treatment. The tillage treatment consisted of primary tillage with a moldboard plow in the fall and disking before planting in the spring. The no-till treatment consisted of slot-planting seed into undisturbed corn stubble (12). The dimensions of each plot (one replicate) were 64 × 64 m. Corn and soybean were planted in rows 76 cm apart.

Samples of soil, corn residues, fresh corn leaves, and air were collected from within each plot in the second or third week of June, July, August, and September of each year and assayed for *A. flavus* in the laboratory. Five subsampling sites were located 5 m apart across the center rows of each plot. All corn residues on the soil surface were removed from 0.25 m^2 quadrants at each site. Soil samples were taken from the top 3 cm of soil at one location in a quadrant. Corn crop residues and soil subsamples were combined to make single samples per plot. Leaves on five plants at each of the five subsampling sites in each plot were sampled by cutting a section (2 × 3 cm) from a single leaf on each plant and placing it in a sterilized paper bag. Airborne propagules of *A. flavus* were collected with an Andersen sampler (1) (Andersen Samplers Incorporated, Atlanta, GA), located 5 m from the edge of the plot within the canopy of the center rows. The instrument was set to sample 0.42 m^3 of air over a period of 15 min. The six Andersen sampler plates on which sampled spores were collected were then removed from the sampler and returned to the laboratory for incubation and enumeration.

Sampling commercial Iowa cornfields. Soil and corn crop residues were sampled from 40 fields throughout Iowa that had significant aflatoxin contamination in the corn crop of 1988 (11). Samples were collected in May and October of 1991 and 1992 and in May, July, and October of 1993 from 0.25 m^2 quadrants at 10 locations within each field by using a "W"-shaped sampling pattern.

Laboratory assays for *A. flavus*. Corn residue samples from each plot were air dried for 48 h in the laboratory. Five pieces

of cob, stalk, and leaf residues were taken arbitrarily from the bulked sample from each plot. Fifty sections (1 cm^2) each of stalk rind and stalk pith were excised from the stalk residues, 50 similar sections were cut from leaf residues, and 50 kernels were removed from the cob residues. Each section of tissue was incubated on moistened blotters at 37°C for 5 days. The percentage of kernels, stalk rind, stalk pith, or leaf sections on which at least one colony of *A. flavus* formed was then recorded and the data averaged across the types of residue to give a percentage of crop residue sections contaminated with *A. flavus*.

In 1991, 1992, and 1993, corn leaf sections from growing plants were placed in sterile petri plates, overlaid with molten cool *A. flavus*–selective medium M3S10B (5), and incubated at 37°C for 5 days. The number of leaf sections on which *A. flavus* colonies developed was determined. In 1979 and 1980, leaf sections were assayed by the blotter method as used for crop residues.

Soil samples were air dried in the laboratory. Suspensions containing 3 g of soil in 7 ml of sterile distilled water were prepared and 1-ml portions from each of five 10-fold serial dilutions were plated onto M3S10B agar. A total of five plates were used for each dilution series/sample. All plates were incubated at 37°C for 5 days, and the total number of *A. flavus* colonies counted. The less selective malt-salt agar (3,5) was used for soil population analysis in 1979 to 1980.

Populations of airborne spores of *A. flavus* were determined by incubating Andersen sampler plates containing M3S10B agar at 37°C for 5 days and counting the total number of *A. flavus* CFU in the six plates from each air collection. The data were expressed as CFU/0.42 m^3 of air. In 1979 and 1980, malt-salt agar was used for soil and air assays.

Composite samples of corn residue and of soils obtained from the Iowa fields were assayed for *A. flavus* as described. In addition, 0.5 g of soil was sprinkled directly onto M3S10B medium in a petri dish (10,11). All colonies of *A. flavus* detected from crop residues and soil were isolated and tested for putative aflatoxin production by incubation on coconut agar at 28°C for 5 days, then examined for visible blue-green fluorescence under long wave (365 nm) UV light (4,9).

Statistical analysis. Data on *A. flavus* populations in soil, crop residue, and air, and on corn leaves, were summarized by

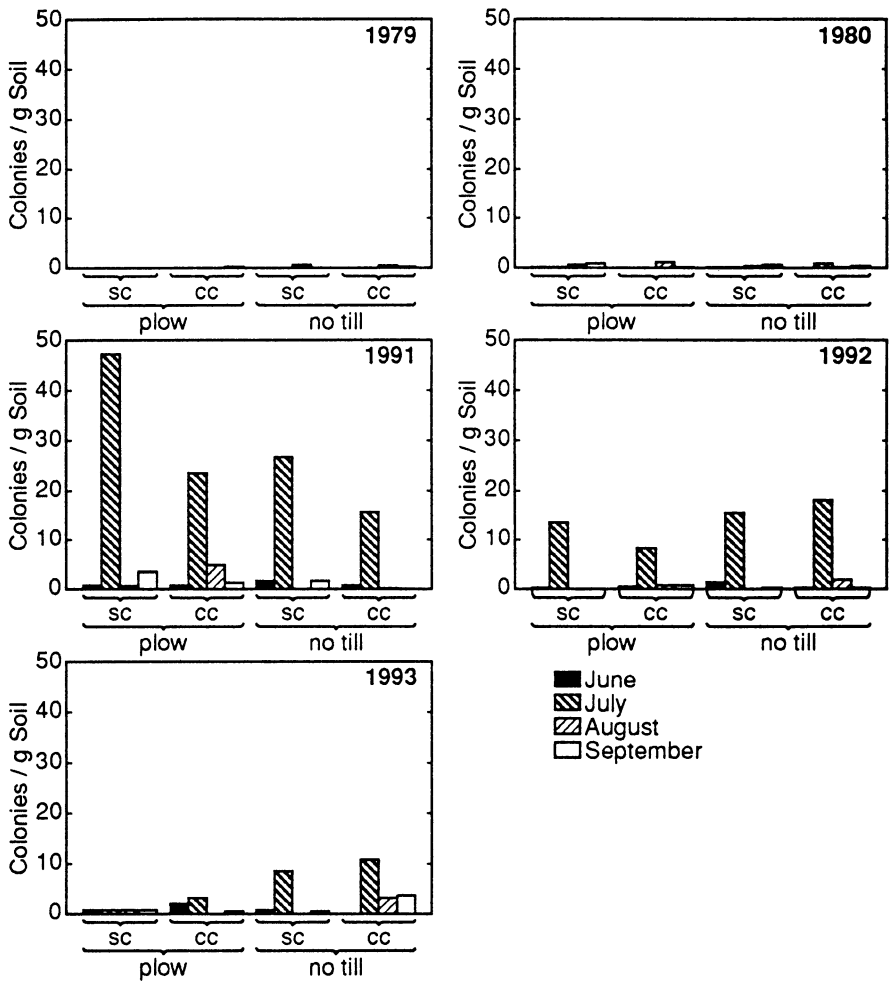

Fig. 1. Populations of *Aspergillus flavus* (colonies per g of soil) in permanently established corn-soybean rotation study at Nashua, IA. Corn was grown with plow or no-tillage systems, and rotations were alternating soybean and corn or continous corn. Samples were collected monthly and values are means of four replicates.

Table 1. Statistical analysis of variance of *Aspergillus flavus* populations in soil, crop residues, leaves, and air in permanent tillage and rotation plots during 5 years[a]

	Soil		Crop residues		Fresh leaves		Air	
Source	***F* Value**	**$P_r > F$**	***F* Value**	**$P_r > F$**	***F* Value**	**$P_r > F$**	***F* Value**	**$P_r > F$**
Rotation (R)[b]	4.40	0.0369	7.38	0.0071	0.09	NS	0.86	NS
Tillage (T)[c]	0.09	NS[f]	0.00	NS	1.33	NS	2.04	NS
Month (M)[d]	21.83	0.0001	1.60	NS	3.50	0.0163	0.48	NS
Year (Y)[e]	16.95	0.0001	1.88	NS	15.05	0.0001	2.69	0.0319
R × T	0.01	NS	0.17	NS	2.07	NS	0.28	NS
R × M	2.97	0.0324	1.31	NS	0.77	NS	1.16	NS
R × Y	2.92	0.0219	0.43	NS	2.11	NS	1.07	NS
T × M	0.11	NS	2.52	NS	1.99	NS	0.60	NS
T × Y	0.82	NS	3.03	0.0185	3.38	0.0105	0.74	NS
M × Y	6.98	0.0001	1.47	NS	6.95	0.0001	1.27	NS

[a] Treatments replicated four times.
[b] Continuous corn or corn alternating with soybeans.
[c] Plow or no-till.
[d] June, July, August, September.
[e] 1979 to 1980 and 1991 to 1993.
[f] Not significant at $P = 0.05$.

Proc Means of the Statistical Analysis System (SAS Institute, Cary, NC) and presented as mean CFU per g of soil, percent corn residues infested, CFU/0.42 m^3 air, and incidence of contaminated leaves. To compare the independent variables tillage and crop rotation with dependent variables *A. flavus* populations in soil, corn crop residues, and air, and on leaf samples, data were analyzed by Proc ANOVA of the Statistical Analysis System. Means separation and comparison were performed by using the Waller-Duncan multiple range test.

RESULTS

***A. flavus* populations in tillage and rotation plots.** *A. flavus* was detected in soils in tillage and rotation plots at Nashua, IA, in each year of the study, and the average populations across tillage and rotational treatments were greater in 1991 to 1993 than in 1979 to 1980 (Fig. 1; Table 1). This could be accounted for by the less selective malt-salt agar used for soil population assays in 1979 to 1980. Significantly greater populations were observed in July than in June, August, or September. This pattern was consistent across tillage and rotational treatments from 1991 to 1993 (Table 1). A significant interaction indicated a higher soilborne population in the soybean-corn rotation compared with continuous corn in 1991 than was indicated in other years. A rotation-by-year interaction was accounted for by the lack of difference in populations under different rotations in 1979 and 1980 compared with populations under different rotation in other years (Table 1).

A. flavus was recovered from corn crop residues at greater frequencies in continuous corn than in the soybean-corn rotation. This was indicated by the significant effect of rotation on *A. flavus* populations on crop residues. The significant tillage-by-year interactions indicate that lower populations on crop residues were evident in the plowed compared with no-till plots in 1991 and 1992 (Fig. 2; Table 1).

A. flavus was detected on leaves of growing corn plants in all treatments in each year of the study (Fig. 3; Table 1). Percent contaminated foliage was unaffected by rotational practices. Tillage practice had little influence on leaf population except for a higher population under no-till than in plowed plots in 1992. A significant month-by-year interaction could be explained by detection of the pathogen in June in 1979 and 1980, while it was not found on plants in subsequent years (Table 1).

Airborne spores of *A. flavus* were detected in all plots in each year of the study (Fig. 4; Table 1). The airborne population was significantly smaller in 1991 than in other years. Tillage and rotation treatments had no significant effect on airborne populations (Table 1). A barrier of 84 corn rows and 64 m long was established on either side of the sampling area (plots) to minimize the effects of interplot interference.

***A. flavus* populations in 40 commercial cornfields.** *A. flavus* was isolated from the soil of almost all fields sampled in 1991, but was detected in fewer fields in 1992 and 1993 (Table 2). *A. flavus* was

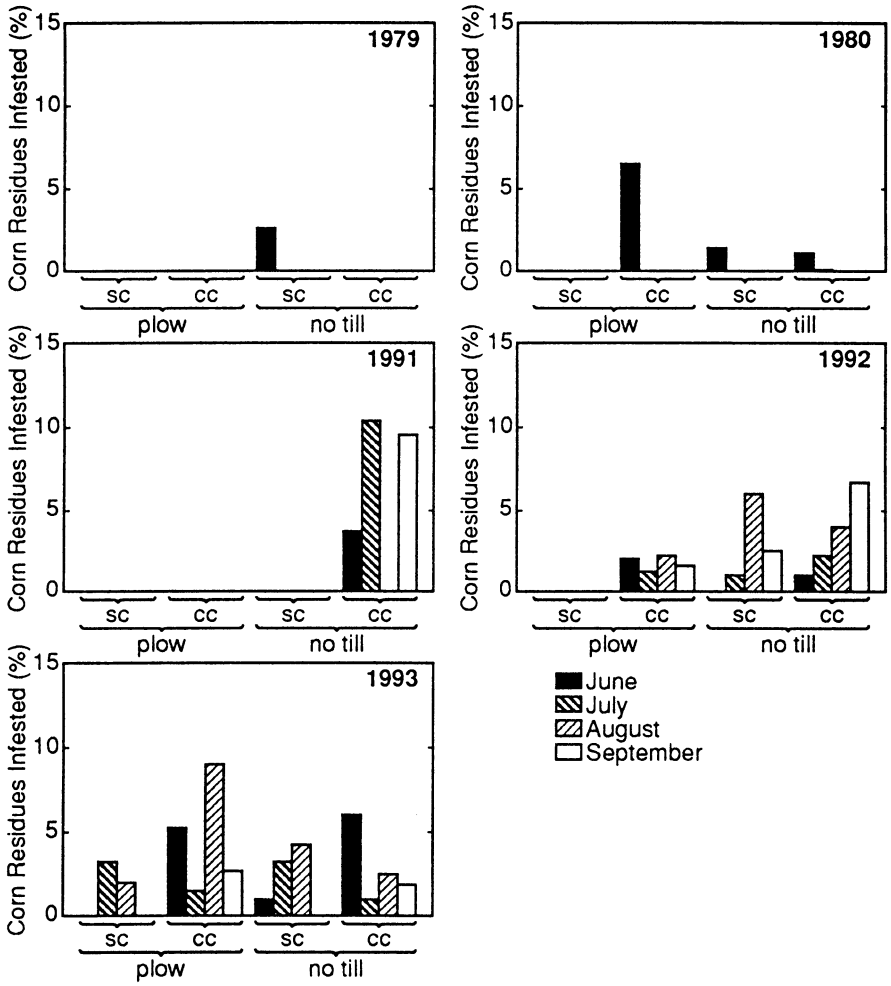

Fig. 2. *Aspergillus flavus* (percentage of corn residue sections infested) in permanently established corn-soybean rotation study at Nashua, IA. Corn was grown with plow or no-tillage systems, and rotations were alternating soybean and corn or continous corn. Samples were collected monthly and values are means of four replicates.

Table 2. *Aspergillus flavus* population in soils and crop residues sampled from Iowa cornfields during different months of 1991 to 1993

			Soil analysis			Crop residue analysis			
				Colonies / g of soil			Crop residue part infested (%)		
Sampling time		No. fields sampled	No. fields infested[b]	Mean	S.E.[a]	No. fields infested[b]	Kernels	Stalk pith	Stalk rind
1991	May	40	39	14.00	3.61	7	43.00	1.20	5.20
	October	40	33	1.20	0.59	14	16.00	8.30	8.60
1992	May	41	6	7.50	3.51	5	8.40	14.50	12.30
	October	38	8	2.00	1.19	8	7.00	3.60	4.60
1993	May	41	6	0.34	0.20	4	2.30	2.90	2.50
	July	36	10	2.25	0.22	3	1.00	1.30	2.10
	October	36	3	0.33	0.30	3	0.75	0.83	1.37

[a] Standard error based on all fields including those found noninfested.
[b] Soil populations were determined by dilution plating and crop residues were assayed by blotter tests.

recovered in crop residues from fields in each year, but the incidence of infested crop residues was higher in 1991 and 1992 than in 1993. No consistent population patterns emerged in relation to month of sampling; however, a small but significant increase in the soilborne population of *A. flavus* (CFU per g of soil) occurred in July 1993 compared with those found in June or October. This pattern is in agreement with the July increase in soilborne populations observed at Nashua. The average soilborne populations and percentages of infested crop residues were of the same magnitude as those detected in the permanent tillage and rotation plots at Nashua (Fig. 1). The proportion of putative aflatoxin-producing isolates tended to decline over the period 1991 to 1993 (Table 3).

DISCUSSION

The consistent recovery of *A. flavus* from soil, crop residues, air, and leaves of growing corn plants under different tillage and rotation practices over several years and locations clearly indicates that *A. flavus* is distributed widely in the agroecosystem of cornfields in Iowa. Although differences occurred in the size of populations of *A. flavus*, soilborne populations values were at least 50 times lower, and crop residue contamination five times lower, than those detected in the epidemic year of 1988. In the fall of that year, Shearer et al. (11) detected an average soilborne population of *A. flavus* of 1,200 CFU/g and isolated the pathogen from 56% of the crop residues in the same fields surveyed in the present study. Surveys by the same authors in the fall of 1989 and 1990 showed a drop in average soilborne counts to 700 and 396 CFU/g, respectively (11). This decline continued to the low levels (14.00 to 0.33 CFU/g) recorded between 1991 and 1993 in this study (10).

The intensive sampling made over periods separated by 11 years in the permanent tillage and rotation plots at Nashua, IA, provided a unique opportunity to study the long-term effects of tillage and rotation practices on the population of *A. flavus* in non-aflatoxin-epidemic years. These data showed that the pathogen was constantly present at very low levels in each component of the ecosystem studied, compared with levels present in an epidemic year. Population levels of *A. flavus* in these plots were of the same magnitude as those in soil and on corn crop residues in the commercial cornfields sampled from 1991 to 1993.

Although *A. flavus* infestation of corn crop residues was greater in continuous corn than in the soybean-corn rotation at the Nashua plots and populations on crop residues were lower in the plowed than in the no-till plots in 1991 and 1992, no corresponding population changes were detected in soil or air, or on corn leaves, of the same plots. It is likely that the differences in populations on the residues were too small to affect populations in the other components of the ecosystem. That situation could conceivably change under weather conditions that favor *A. flavus* infection and aflatoxin development, such as the 2 to 3°C increase in average temperature in July and August associated with the aflatoxin epidemic in Iowa in 1983 (7).

Other studies have shown significant differences in populations of *A. flavus* in

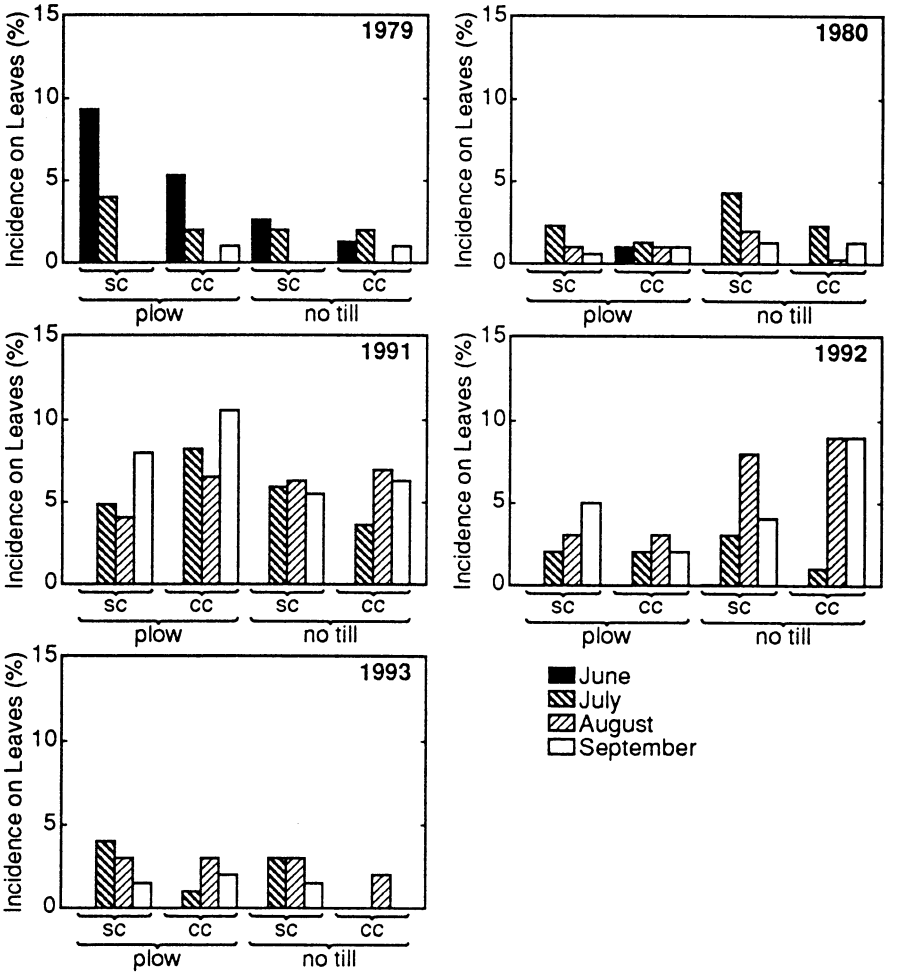

Fig. 3. *Aspergillus flavus* (percent incidence of leaves colonized) on leaf tissue of growing corn plants in permanently established corn-soybean rotation study at Nashua, IA. Corn was grown with plow or no-tillage systems, and rotations were alternating soybean and corn or continous corn. Samples were collected monthly and values are means of four replicates.

Table 3. Proportion of *Aspergillus flavus* isolates from soils and crop residues putatively producing aflatoxin, sampled during different months of 1991 to 1993

Sampling time		Soil		Kernels		Stalk pith		Stalk rind	
		Isolates (No.)	Putative aflatoxin producer (%)	Isolates (No.)	Putative aflatoxin producer (%)	Isolates (No.)	Putative aflatoxin producer (%)	Isolates (No.)	Putative aflatoxin producer (%)
1991	May	111	1,001	211	66	6	100	44	43
	October	42	45	56	50	33	31	39	26
1992	May	74	43	50	4	42	29	41	14
	October	62	55	13	75	24	24	32	36
1993	May	30	43	10	30	32	16	43	12
	July	77	35	4	25	12	17	57	25
	October	10	10	6	17	29	14	17	6

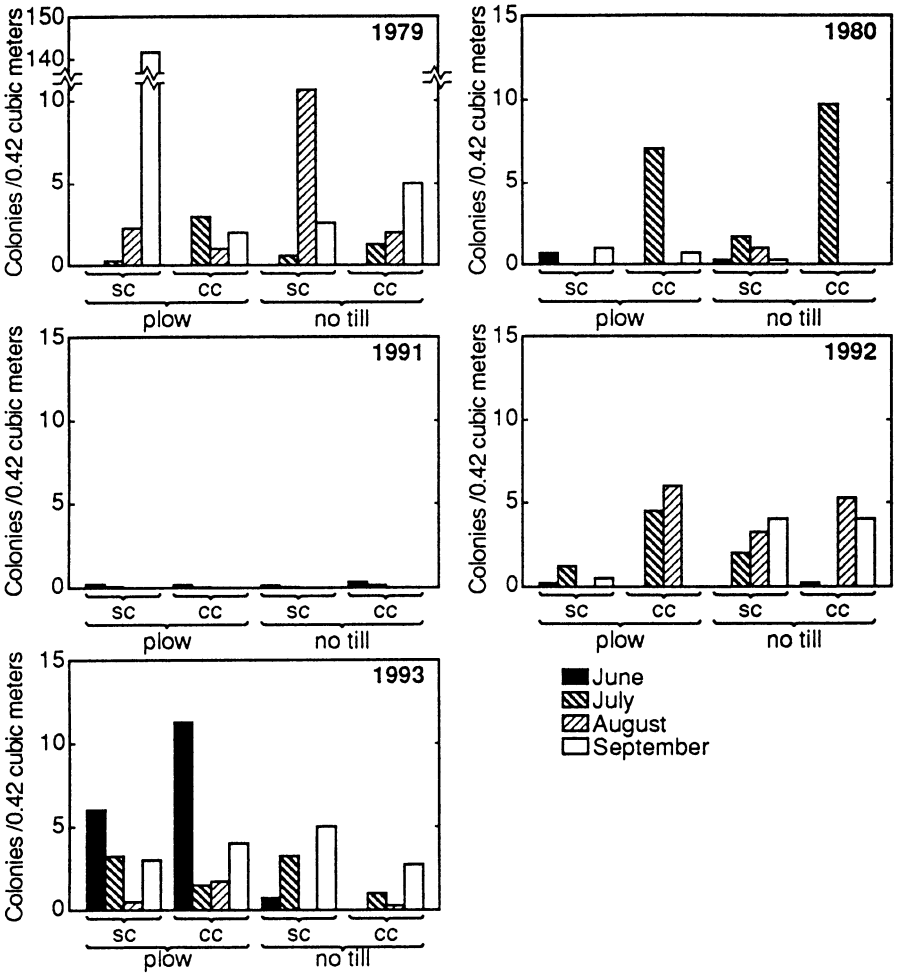

Fig. 4. Populations of *Aspergillus flavus* (CFU/0.42 m^3 of air) in permanently established corn-soybean rotation study at Nashua, IA. Corn was grown with plow or no-tillage systems, and rotations were alternating soybean and corn or continous corn. Samples were collected monthly and values are means of four replicates.

soils under various tillage or rotation practices (2,6). However, there is no evidence for any particular cultural practice strongly influencing soilborne *A. flavus* populations except a finding that *A. flavus* was not recovered from virgin prairie soil (2).

A decrease in the percentage of isolates that contained putative aflatoxin-producing *A. flavus,* recovered from soil and crop residues in the field survey, was observed over the period 1991 to 1993. This is similar to the trend reported by Shearer et al. (11) of a gradual decline in aflatoxin-producing isolates in the same fields with increasing time from the 1988 epidemic.

No consistent patterns of *A. flavus* population were evident in corn crop residues, air, or leaves in the permanent rotation and tillage plots during the months of the growing season. The soilborne populations, however, were remarkably greater in July than in June, August, or September from 1991 to 1993 at the Nashua location. Further studies (D. C. McGee, O. M. Olanya, G. M. Hoyos, and L. H. Tiffany, unpublished) have demonstrated that populations of *A. flavus* in field soils can be dramatically increased by exposure to temperatures in the 37 to 40°C range. Average temperatures in the Nashua, IA, area were 21.1 and 20.1°C in July and August, respectively, in 1991 to 1993. These are well below the temperatures during this experiment or those reached in July and August in the epidemic year of 1983 (7). Also, no aflatoxin epidemics were present in those years. The increase in July populations, therefore, would seem to be typical of what would occur in non-epidemic years.

It is possible that, during the hot, dry years that are related to *A. flavus* and aflatoxin epidemics, rapid increases in the soilborne population in July could significantly influence aflatoxin development by increasing available inoculum for infection of corn silks. The optimum time for *A. flavus* infection of silks occurs in July (8). If a threshold level for the soilborne population was proven critical for *A. flavus* infection and aflatoxin epidemic, control of the disease might be enhanced either by minimizing the build-up of the soilborne population before July or by protecting plants from infection during silking (8).

ACKNOWLEDGMENTS

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