Preharvest Infection of Corn Silks and Kernels by *Aspergillus flavus*

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ABSTRACT

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Field and greenhouse studies were used to determine the nature of preharvest infection of corn by *Aspergillus flavus*. Inoculation of external silks that were yellow-brown resulted in more extensive colonization of the silks and a greater number of infected kernels than inoculation of brown silks. In silk-inoculated and uninoculated ears, the pattern of tissue colonization was very similar: growth generally proceeded from the ear tip towards the base, colonizing the silks first, then the glumes and (by the late milk stage) the kernel surfaces but rarely penetrating the cob pith. Silk senescence and the subsequent growth of *A. flavus* down the silks was rapid in environment chambers at 30/34 C; the fungus reached the base of some ears in 4 days. Under field conditions, equivalent progress took between 4 and 13 days. The mycelium of *A. flavus* spread quickly from the silks onto the kernel surfaces, forming a clustered distribution. The percentage of kernels colonized within an ear half was correlated ($r \geq 0.67$ for six of seven harvest dates) with the extent of contamination of the associated silks by *A. flavus* but showed no relationship to the location or extent of visible insect damage. Internal infection of kernels did not appear until early dent stage.

Additional key words: infection process, *Zea mays*.

Early studies of the preharvest infection of corn by *Aspergillus flavus* were limited to observation of ears showing visible sporulation (2,3,26). Since these sporulating areas frequently coincided with areas of insect damage on the ears, investigators postulated that ears became infected by *A. flavus* only after they had been predisposed by insect injury.

The increased number of field studies brought on by the documentation, in 1975, of aflatoxin in corn before harvest (1,24) provided further evidence for a role of insects in the infection process. The results of these studies showed positive associations between insect damage and infection and sporulation by *A. flavus*, bright greenish yellow fluorescence (BGYF), and aflatoxin levels (4,6,11,13–15,29). However, there are notable exceptions to these associations in the literature. Data from a number of investigations have failed to show any relationship between insect damage and aflatoxin or the incidence of *A. flavus* (4,15–17, 19,20,28,29). Also, kernel and ear samples that were essentially free of insect damage yet contained moderate to high amounts of aflatoxin have been found (6,10,14,23,25). The exact relationship between insects, *A. flavus*, and aflatoxin in preharvest corn has not been delineated.

Data on the occurrence of *A. flavus* in corn ears before harvest have come mostly from the Midwest, where field-collected samples averaged 0–2% infection (5,22). In a study in the southeastern United States (6), where *A. flavus* and aflatoxin contamination in the field reach much greater levels, 276 of 297 kernel samples from freshly harvested corn were infested with *A. flavus*. There are few reported studies on the progress of colonization of corn ears by *A. flavus* in the field. In a study in Georgia, Wilson et al (29) sampled and plated damaged kernels from uninoculated ears. The incidence of *A. flavus* increased to nearly 100% of the ears by harvest in both 1975 and 1976. Importantly, <1% of these ears had visible sporulation when collected.

Jones et al (7) demonstrated that *A. flavus* is capable of colonizing silks, infecting kernels, and producing aflatoxin in developing ears under insect-free conditions. However, many questions remain on the colonization of corn silks and the infection of corn kernels by *A. flavus*. To study the nature of the preharvest infection of corn ears by *A. flavus*, we addressed three specific areas: the effect of the physiological age of the external silks (silks protruding from the tip of the ear) on the growth of *A. flavus* and the colonization and subsequent infection of the kernels; the path of infection from the external silks to the inside of the kernel and the rate of this process; and the infection of uninoculated corn ears via the silks under field conditions. Use of the term colonization in this study refers to growth of the fungus within and/or on plant tissue, while use of the term infection applies strictly to the establishment of the fungus within plant tissue.

MATERIALS AND METHODS

Field inoculation studies. Commercial hybrid DeKalb XL394 was seeded in rows 1 m apart on 7 May 1981 at Research Unit 1, Raleigh, NC, at a population of 59,000 plants per hectare. Twenty plants per plot were randomly selected from the inner two rows of each four-row × 15-m plot, and the external silks were inoculated at the green-yellow (freshly pollinated, 19 July) and yellow-brown (27 July) silk stages. The inoculum consisted of a suspension of 10^6 spores of *A. flavus* (NRRL 3357) per milliliter, and 0.5 ml was applied per plant. These two treatments plus an uninoculated control constituted three plots within each of three blocks. Ears in the yellow-brown silk treatments were enclosed immediately after inoculation with a plastic bag inside a paper bag. The plastic bag was removed after 3 days of incubation. Ears in the green-yellow silk treatments were allowed natural pollination for 3 days after inoculation and then were bagged as above. Ears were harvested on 31 August, and kernels from all 20 ears of each plot were pooled. A random sample of 500 kernels, intact and insect-damaged, from each pooled plot was surface sterilized for 3 min in a solution of ethanol: 5.5% NaOCl:water (10:20:70, v:v) and plated on Czapek's agar containing 6% NaCl (CSA). Plates were incubated at 34 C for 3–5 days, and the number of infected kernels was recorded. This study was repeated the following summer with a tan-spored mutant of *A. flavus* isolate 5T (obtained from K. E. Papa, University of Georgia) to distinguish the applied isolate from the natural population of *A. flavus*. There were six replicates per treatment in a completely randomized design.

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Progress down silks and onto kernel surfaces. *Uninoculated plants.* Two hundred randomly chosen plants in a 1.2-ha field of Pioneer 3147 corn were numbered and tagged for one sampling of pollen and two samplings of external silks. On 17 July 1981 (4 days after 50% silk emergence) pollen was shaken from the tassels onto the surface of CSA in sterile petri plates. External silks were sampled on 22 July (yellow-brown) and 28 July (brown) by cutting off approximately half of the silk bundle and placing it on CSA plates. After each silk sampling, instruments were cleaned with a cloth soaked in 70% EtOH. Plates were enclosed in plastic and paper bags during transport to prevent contamination. After 3–5 days at 34 C, the plates were examined for aspergilli and other predominant fungi.

Three groups of 20 ears were harvested from the 200 randomly tagged plants. The first group was harvested 31 July (kernels still forming or in milk stage), the second group 9 August (dough stage), and the third group 14 August (early dent stage). Aseptic techniques were used to divide the ears transversely into three equal parts: tip, middle, and base. Each third was dissected into silks, 10 non-surface-sterilized kernels from around the circumference of the proximal end, and a fragment from the pith of the cob at the proximal end. The dissected parts were plated aseptically on CSA by the modified method of Koehler (8). The tissues were examined for the presence of *A. flavus*, insect damage, and the point of colony origin from the non-surface-sterilized kernels.

In 1982, silks and ears were sampled in a 1.2-ha field of hybrid Coker 56. Ears were removed from five randomly chosen plants at weekly intervals from 15 July to 27 August, placed in paper bags, and taken to a sterile hood. External silks were detached and placed on CSA. The remainder of the ear was dissected as described above.

Inoculated plants. On 27 July 1981, 20 plants at the yellow-brown silk stage within the previously described DeKalb XL394 plots were selected at random, and the external silks were inoculated with isolate 5T. Five ears were harvested at 4, 13, and 18 days after inoculation. Ears were dried in a forced-air oven at 55–60 C for 5 days after harvesting. After the outer husks and external silks were removed, each ear was divided transversely into three parts, dissected, and plated as described above for the uninoculated ears. An additional 20 intact kernels from each third of the ear were surface sterilized and plated. Scanning electron microscope observations were made on kernels adjacent to the kernels sampled for plating if incubation of these kernel samples indicated they came from areas of the ear heavily colonized by *A. flavus*. In 1982, silks were inoculated at the yellow-brown stage (5 July) and five ears from eight sample dates were processed as above.

To more closely monitor progress of *A. flavus* in the ear, external silks of nine greenhouse-grown Gaspe × W103 plants were inoculated with the 5T isolate, and silks of three plants were left as uninoculated controls. Ears were enclosed in plastic and paper bags, and the plants were transferred to an environment chamber maintained at 34/30 C day/night temperatures. Three ears were harvested at 2, 4, and 6 days after inoculation, dissected, and plated. Silks were removed as individual bundles within each third of the ear, and kernels were removed in blocks of 20 to 30 kernels corresponding to each silk bundle. Incubated ear segments were viewed with a dissecting microscope and photographed.

Distribution within the ears. In 1982, five ears per week were randomly sampled from Coker 56 plants inoculated with 5T at the yellow-brown silk stage (5 July). External silks were trimmed off, ears were divided transversely into two approximately equal pieces, and the silk bundles from each ear half were plated on CSA. All kernels (non-surface-sterilized) were aseptically plated in order from tip to base. This permitted mapping of surface colonization by isolate 5T for each of the ears. Growth of 5T from the plated silks was rated on a 0–3 scale: 0 = no growth, 1 = 1–33%, 2 = 34–66%, and 3 = 67–100% of the silks colonized. The origin of colony growth on the kernels and the presence or absence of insect damage to individual kernels were noted. Kernel moisture was determined at each sampling.

RESULTS

Field inoculation studies. In 1981, heavy sporulation on the external silks of both inoculated treatments was evident when the plastic incubation bags were removed. The number of kernel infections in the inoculated treatments was significantly greater than in the control; however, no significant differences existed between the two inoculation treatments (Table 1). In 1982, only the inoculation of yellow-brown silks produced significantly greater kernel infection.

Progress down silks and onto kernel surfaces. *Uninoculated plants.* Pollen and external silks sampled in 1981 and 1982 were infested primarily with species of *Fusarium, Penicillium, Alternaria,* and *Aspergillus* (Table 2). *Fusarium* spp. were the most common and were present in all samples from all but two collection dates. *A. flavus* was present on corn silks in both 1981 and 1982. In 1982, *A. flavus* was readily isolated from silks throughout the season, with the greatest recovery occurring at the milk stage.

Table 3 summarizes the frequencies of ears with internal silks, kernels, or pith colonized by *A. flavus* at each of three sampling dates in 1981. *A. flavus* was isolated from the silks of 30% of the ears harvested on 31 July. These ears were in the early stages of development with most just reaching milk stage, and in three ears the kernels had not formed. Among these ears with colonized silks, 100% had *A. flavus* in silks from the tip section, 67% in silks from the middle, and 33% in silks from the base. Two ears (10%) had *A. flavus* throughout the entire length of the silks. The percent ears with silks colonized by *A. flavus* approximately doubled to 72% of the ears by dough stage (9 August), and growth on the kernels was present in 47% of the ears. The first isolation of *A. flavus* from the

TABLE 1. Percentage of mature corn kernels infected with *Aspergillus flavus* when silks at three stages were inoculated in the field[x]

Treatment[z]	Mean infection (%)[y] 1981	1982
Uninoculated, ears not covered	2.3 a	4.3 a
Uninoculated, ears covered		4.0 a
Inoculated, green-yellow silks	21.0 b	5.2 a
Inoculated, yellow-brown silks	29.5 b	27.2 b
Inoculated, brown silks		9.7 a

[x]Silks were inoculated with isolate NRRL 3357 in 1981, and with isolate 5T, a color mutant, in 1982.

[y]Means within columns with the same letter are not significantly different at $P = 0.05$.

[z]Ears of plants in all treatments except the first were enclosed in a plastic bag and a paper bag after treatment. Three days later the plastic bag was removed.

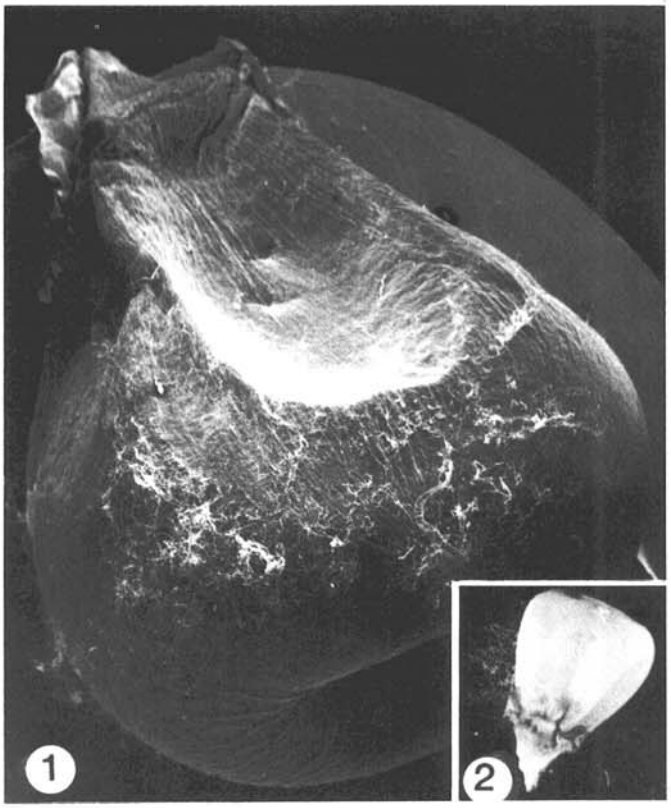
TABLE 2. Predominant fungal species on corn pollen and silks in the field in 1981 and 1982[x]

Collection date	Kernel stage	Part sampled[y]	Percentage of samples with: *Fusarium* spp.	*Alternaria* spp.	*Penicillium* spp.	*Aspergillus flavus*
1981						
17 July		pollen	100	100	10	4
22 July		Y-B silk	100	86	18	16
28 July	milk	B-silk	100	97	10	4
1982						
15 July		B-silk	20	60	80	80
19 July	milk	B-silk	80	20	20	60
23 July	milk	B-silk	100	40	20	100
30 July	dough	B-silk	100	100	40	20
6 August	dough	B-silk	100	20	0	40
13 August	dent	B-silk	100	80	0	40
20 August	dent	B-silk	100	100	20	20
27 August	dent	B-silk	100	40	0	60

[x]Two hundred samples were taken on each 1981 collection date and five samples were taken on each date in 1982.

[y]Pollen was sampled from tassels at anthesis and external silks were sampled at two stages, yellow-brown (Y-B) and brown (B).

pith tissue was made from the tip third of one ear. The silks and kernels of this ear were heavily colonized and all 10 kernels sampled from the tip showed colonization by *A. flavus*. Insect damage also was present in the tip and middle sections. By early dent stage (14 August), about the same percentage of the ears (75%) had colonization of silks by *A. flavus* and 50% revealed colonization of kernels by *A. flavus*. Surface sterilization of kernels dramatically decreased the presence of *A. flavus*. Just one ear had kernels that were internally infected with *A. flavus* and the silks of this ear were extensively colonized by the fungus along with the pith samples from all three ear sections. Infection of the pith by *A. flavus* generally was uncommon, occurring in only two of the 57 ears examined.



Figs. 1 and 2. Location of *Aspergillus flavus* growing on non-surface-sterilized kernels from inoculated ears of corn. **1,** Scanning electron micrograph of mycelium on kernel shoulder (×30). **2,** Colony originating from the side of a plated kernel (×2).

TABLE 3. Frequency of colonization of uninoculated corn ears by *Aspergillus flavus* in 1981

Collection date[z]	Kernel stage	Silks			Kernels			Pith		
		Tip	Mid	Base	Tip	Mid	Base	Tip	Mid	Base
31 July	Early milk	5	4	2	0	1	0	0	0	0
9 August	Dough	11	11	8	3	5	6	1	0	0
14 August	Early dent	10	10	8	2	2	8	1	1	1

[z] Twenty ears were sampled on 31 July. On 9 August, silks were sampled from 18 ears; kernels and pith were sampled from 17 ears. On 14 August, 20 ears were sampled.

Presence of insect damage (European corn borer and corn ear worm) over the three sampling dates progressed from 20% of the tip, 10% middle, and 10% base on 31 July; 100% tip, 25% middle, and 35% base on 9 August; to 100% tip, 85% middle, and 60% base on 14 August.

Data from 1982 samplings are not presented because levels of *A. flavus* within these ears were relatively low. *Fusarium* spp., *Penicillium* spp., and other aspergilli (*A. niger, A. ochraceus, A. versicolor,* and *A. glaucus*) were isolated. Yeasts were present although not nearly at the high frequencies reported by Hesseltine and Bothast (5). A species of *Mortierella* was also isolated.

Inoculated plants. Sporulation of isolate 5T on the external silks was equal to that on the silks inoculated with NRRL 3357 in all field inoculation studies. Progress of subsequent ear colonization in 1981 is shown in Table 4. The overall frequency of colonization was found to be greatest in the silks, followed by the kernels, and then the pith. None of the surface-sterilized kernels showed internal infection at any of the three dates, even though colonization of the silks and kernel surfaces was quite extensive at the last two collection dates.

Yeasts, *Fusarium* spp., Mucorales, *Penicillium* spp., *A. flavus, A. niger,* and bacteria also were isolated. The yeasts and bacteria were the dominant organisms within the ear at the first sampling date, but their frequency later declined. These fungi and bacteria were uncommon in the pith tissue and were only found in ear sections where the associated silks and kernel surfaces also were colonized. Insect damage became significant at the second sampling date, when six of the 15 ear sections contained injured kernels. By this time, isolate 5T was present in the silks of all 15 ear sections and on the kernel surfaces of 12 of the 15 ear sections. The same situation existed at the third sampling date, when five of the 15 ear sections were insect damaged.

Observation of non-surface-sterilized kernels by both dissecting and scanning electron microscopy showed that colonies of isolate 5T originated from the pericarp on the silk scar end and/or sides of the kernel where the glumes were in contact with the kernel (Figs. 1 and 2). Scanning electron microscope observations also revealed surface colonization of intact kernels to be mycelial, with no conidiophores.

Data from the 1982 sampling of ears inoculated with isolate 5T are shown in Fig. 3. The early stages of ear colonization observed in plants inoculated in 1981 (Table 4) were not seen since the 15 July 1982 ear samples were in milk stage, and the majority of mycelial spread within the ear had already taken place.

In the ears inoculated with isolate 5T and transferred to the 34/30 C environment chamber, the mycelium advanced more quickly down the silks than it did under field conditions. Mycelium of the 5T mutant was recovered from the silks of the tip sections of all three sample ears 2 days after inoculation and from the silks in the base of all three sample ears just 4 days after inoculation. No growth was present on the pericarp surfaces at 2, 4, or 6 days after inoculation; however, sporulation from the glumes of kernels and

TABLE 4. Frequency of colonization of inoculated corn ears by *Aspergillus flavus* isolate 5T in 1981[v]

Collection date	Silks[w]			Ears[x]			Kernels[y]			Pith		
	Tip	Mid	Base	Tip	Mid	Base	Tip	Mid	Base	Tip	Mid	Base
31 July[z]	5	0	0	...	...	...	...	...	...	1	0	0
9 August	5	5	5	4	4	4	38	27	27	1	0	1
14 August	5	5	4	5	4	5	29	19	11	0	0	1

[v] External silks were inoculated 27 July and five ears were collected at each date.
[w] Number of ears out of five with silks colonized in the tip, middle, or base of the ear.
[x] Number of ears out of five with ≥1 kernel colonized in the tip, middle, or base of the ear.
[y] Number of kernels colonized by isolate 5T out of 50 non-surface-sterilized kernels (10 kernels × five ears) in the tip, middle, or base of the ear.
[z] Kernels not yet formed.

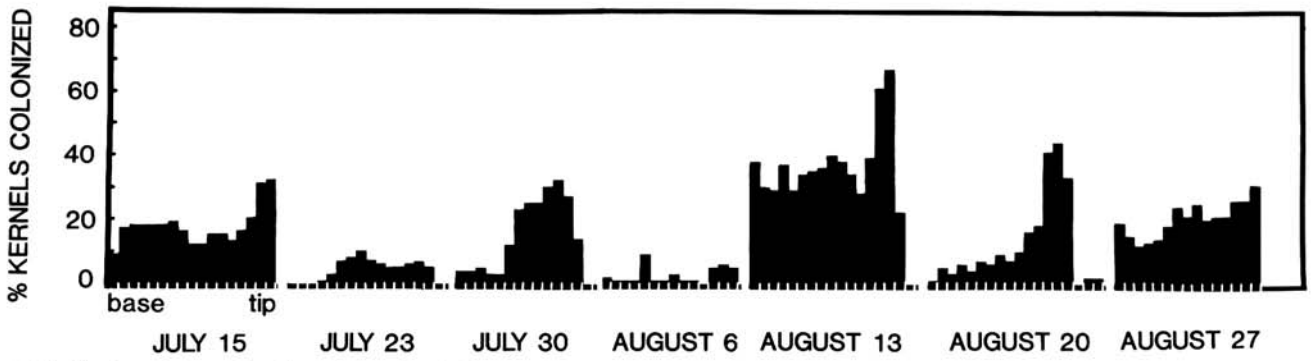


Fig. 3. Distribution of *Aspergillus flavus* isolate 5T within corn ears at seven sample dates. Percent kernels colonized at each date is the average of five ears. (Each abscissa unit represents a cylinder of three circumferential kernel rows progressing from the base of the ear to the tip).

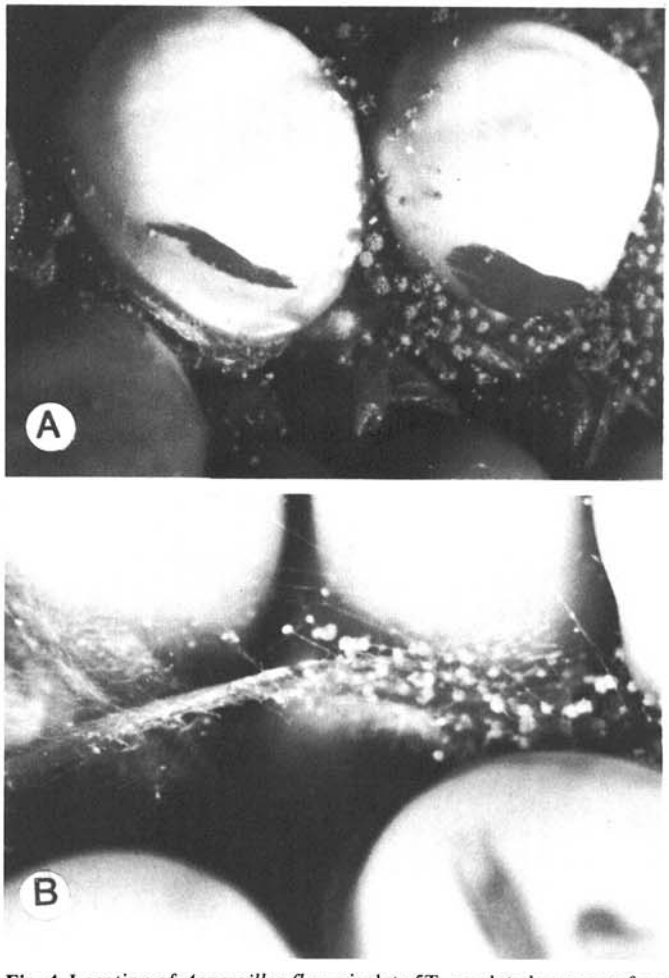


Fig. 4. Location of *Aspergillus flavus* isolate 5T, on plated segments from silk-inoculated corn ears: **A,** sporulation on glumes (×15) and **B,** sporulation on adjacent silk (×20).

TABLE 5. Colonization of corn ears by *Aspergillus flavus* isolate 5T at seven sampling dates in 1982

Date	Kernel moisture (%)	Kernels colonized (%)[x]		Silk colonization rating[y]		S/K[z] (*r*)
		Tip	Base	Tip	Base	
15 July		15.0	18.1	1.6	1.0	0.69
23 July	60	18.6	2.2	2.0	0.6	0.67
30 July	51	23.3	4.4	1.2	1.4	0.80
6 August	40	1.9	3.1	1.2	0.0	0.04
13 August	38	40.5	32.1	2.8	1.4	0.86
20 August	32	20.2	4.9	2.0	1.0	0.75
27 August	28	24.4	14.2	1.4	0.6	0.78
Overall mean		20.5	11.3	1.7	0.8	0.65

[x] Means of kernel colonization percentages for five ear tips and five ear bases per sample date.

[y] Means of silk colonization ratings for five ear tips and five ear bases per sample date; ratings based on 5T growth from plated silks: 0 = no growth, 1 = 1–33%, 2 = 34–66%, and 3 = 67–100% of the silks colonized.

[z] Coefficient of correlation (*r*) of silk colonization rating(s) with percent kernels colonized (*k*) for 10 ear halves per sample date.

TABLE 6. Colonization of corn kernels by *Aspergillus flavus* isolate 5T on seven insect-damaged ears

Date	Insect damaged kernels		Intact kernels	
	Colonized	Not colonized	Colonized	Not colonized
15 July	0	32	6	530
23 July	5	20	44	267
30 July	0	42	4	488
	0	2	9	474
6 August	0	9	8	579
13 August	0	9	228	166
27 August	1	27	6	439
Total	6	141	305	2,943

adjacent silks was noted in some of the incubated ear segments (Fig. 4A and B). Initial growth of the 5T mutant from incubated silk segments began from the cut ends, indicating that growth down the internal silks (silks enclosed by the husks) may be confined to the interior cells of the silk tissue. Controls were free of any mycelium of isolate 5T.

Distribution within the ears. Fig. 3 depicts the 5T colonization pattern from a compilation of the data from five ear maps at each 1982 sampling date. Kernel colonization percentages were generally greater in the ear tips than in the ear bases. Table 5 shows that the silk colonization ratings also were generally greater in the ear tips than in the ear bases. Also listed are the correlation coefficients for the 10 kernel colonization percentages and the 10 silk ratings at each sample date. The percentage of kernels colonized per ear varied greatly among ears, the variance equaling 521.47 over all 35 sample ears. Data on the relationship between insect damage and isolate 5T colonization of kernels is shown in Table 6. The total number of kernels with both insect damage and isolate 5T colonization is only a small fraction of the total number of kernels with isolate 5T colonization.

After *A. flavus* is established in the internal silks of the ear, colonization of kernel surfaces occurs. Although the source of *A. flavus* for kernel colonization appears to be the silks, colonization of a particular kernel does not require that *A. flavus* be present in the silk or silk bundle adjacent to this kernel. Apparently, *A. flavus* can spread from kernel to kernel by mycelial growth on the pericarps or glumes. In the earlier stages of ear development silks

are in closer contact with the glumes and bases of the kernels. Fungal hyphae bridging the tissues at these contact points could serve to initiate the spread of *A. flavus* on kernel surfaces. Rambo et al (23) reported some superficial spreading of mycelium of *A. flavus* among kernels, especially near the base (tip) of the kernels. Growth of *C. acremonium* down the ear by way of the glumes has been observed by Koehler (8). Evidence of the growth of *A. flavus* on glumes, as detected by bright greenish-yellow fluorescence (BGYF) of the glumes was reported by Lillehoj et al (12). Also, sporulation of *A. flavus* on glumes of plated, non-surface-sterilized kernels was noted in our study and by Payne (21). Observations that colonies of *A. flavus* usually originate from the sides (glume region) and shoulders of plated non-surface-sterilized kernels further corroborate these areas as the first points of contact of the fungus with the kernels.

Penetration and internal infection of the kernels by *A. flavus*, exclusive of insect wounds, appears to occur largely in the later stages of kernel development when the kernels are denting. Koehler (8) found that internal infection by *F. moniliforme* started about 5–7 October, approximately 2 mo after pollination or when grain moisture was 29.5%, and continued to increase until harvest. Results from our study and the study by Payne (21) showed that some internal infection was beginning at the time kernels were in the early dent stage.

We found that *A. flavus* is often present on the surface of a high percentage of kernels within ears showing no visible sporulation by the fungus. This must be taken into consideration in studies on the role of insects in spreading *A. flavus* within the ear. We believe that the rapid ear colonization exhibited by *A. flavus* in this study indicates that insects may be less important in spreading *A. flavus* within the ear. The amount of aflatoxin production resulting from the growth of *A. flavus* on kernel surfaces is unknown and may be insignificant in comparison to the amount of aflatoxin resulting from internal seed infections. However, surface colonization of kernels by *A. flavus* becomes important when insects damage the seed coat of a colonized kernel. The *A. flavus* already established on the kernel surface can readily enter the injured kernel and produce high levels of aflatoxin.

DISCUSSION

A. flavus can colonize silks, grow down the silks into the ear, and colonize the surface of developing kernels under suitable environmental conditions. Colonization of ears via silks is not unique to *A. flavus*. Results of an extensive study by Koehler (8) demonstrated that *Fusarium moniliforme, Gibberella zeae, Cephalosporium acremonium, Monilia* spp., and *Penicillium* spp. colonized the ear predominantly via the silks. Only *Diplodia zeae* and *Nigrospora* spp. entered the ears through the butt and/or the silks. Hesseltine and Bothast (5) sampled silks, kernels, and cob pith at weekly intervals from ears in an Illinois corn field and concluded that as the silks senesce after pollination, they become a suitable growth medium for the resident microflora and thereby provide an access route into the ear.

The rate at which *A. flavus* will colonize external silk tissue is affected by the physiological age of the silks as well as temperature, relative humidity, and moisture. Jones et al (7) reported extensive colonization of detached silks; however, photographs taken in our study show further that green-yellow silks will not support vigorous growth or sporulation, while reduced levels of growth and sporulation occur on brown silks (18). Characterization of silk susceptibility based on color is subjective; however, it seems to be a better indicator than either the stage of kernel development or the number of days after silk emergence, since the rate at which silks senesce to yellow-brown and then brown will vary with both environmental conditions and the cultivar of corn. The presence of inhibitors or the lack of available nutrients are two factors that could account for the unpollinated and green-yellow silks being such poor substrates for growth. Studies incorporating silks of each of the silk stages into water agar (S. F. Marsh, *unpublished*) indicate that sufficient nutrients are present in unpollinated silks. Possibly, autoclaving the silks made the nutrients more accessible or destroyed compounds inhibitory to *A. flavus*.

Kernel infection rates were not statistically different between ears inoculated at the green-yellow and yellow-brown silk stages in 1981. Apparently, conidia of *A. flavus* deposited on silks before pollination can remain viable for colonization once pollination does begin, although some loss in viability may occur.

Silks in this stage are just beginning to senesce yet they retain succulence. Growth of *A. flavus* in other plants also appears to be associated with maturing or senescing tissue. Lee et al (9) reported that the amount of a bright greenish-yellow fluorescent compound, produced as a byproduct of *A. flavus* growth within cotton bolls, was related to the maturity of the boll at the time it was inoculated. Reduced colonization of brown silks may be due to a lack of moisture, or lower nutrient levels. Jones et al (7) were able to obtain infection of ears by inoculating 4-wk-old brown silks and enclosing the ears in plastic bags that increased relative humidity and moisture levels.

Growth of *A. flavus* from the external silks into the kernel region can be rapid. In the field, inoculated ears showed extensive colonization of the silks and kernel surfaces 13 days after inoculation. The rate and path of colonization in naturally contaminated ears was similar, although the overall levels of *A. flavus* were lower. The progress of colonization by *A. flavus* in uninoculated ears also agrees with Hesseltine and Bothast's (5) findings on the development of other fungi in corn ears. The ability of *A. flavus* to colonize senescing silks and grow down them into the ear may depend on temperature. This hypothesis was supported by the increased colonization rate found in silks of ears maintained at 34/30 C in environment chambers in our study and by others (7,21,27). This fast growth rate might be due to a unidirectional nature of hyphal growth by *A. flavus* down into the ear within the confines of the silk tissue (18).

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