Survival of *Aspergillus flavus* Sclerotia and Conidia Buried in Soil in Illinois or Georgia

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ABSTRACT

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We examined the survival of sclerotia and conidia produced by *Aspergillus flavus* var. *flavus* and *A. flavus* var. *parasiticus* that were buried at a depth of 10–12 cm for up to 36 mo (October 1986 to October 1989) in sandy field soils near Kilbourne, Illinois, or Tifton, Georgia. Substantial losses of conidial inoculum were recorded after the first year of burial in Georgia and after the second year of burial in Illinois. Conidia of *A. f. parasiticus* survived for longer periods in soil than did conidia of *A. f. flavus*. Most sclerotia from eight *A. flavus* strains were viable at the conclusion of the experiment (Illinois, 77–99% viability; Georgia, 68–100% viability), as measured by colony growth of *A. flavus* on potato dextrose agar. None of these sclerotia, however, germinated sporogenically on sand in moist chambers. Production of large numbers of fungal propagules in the soil suggested that some of the sclerotia had previously germinated sporogenically. The number of *A. flavus* propagules generated from buried sclerotia varied according to strain and location; numbers were maximum after the first growing season. Fungal colonization of *A. flavus* sclerotia buried in Georgia was dominated by *Paecilomyces lilacinus;* in Illinois the sclerotia were colonized most frequently by *P. lilacinus* and *Periconia macrospinosa.*

Additional keywords: aflatoxin, *Bacillus megaterium,* mycoparasitism, population dynamics.

Aspergillus flavus var. *flavus* Link:Fr. and *A. flavus* var. *parasiticus* (Speare) Kurtzman et al. produce conidia that function in dispersal and as inoculum. Sclerotia, structures adapted for long-term soil survival, also may be produced. Both types of propagules produced by *A. f. flavus* are associated with damage to preharvest corn (*Zea mays* L.) kernels and are dispersed onto the soil surface during harvest (24,25). Sclerotia can also form on *A. flavus*-infested maize kernels incubated on unsterile soil (26). Corn-following-corn and corn-following-peanuts rotations are associated with increased inoculum densities of *A. flavus* in field soils (8). *A. flavus* sclerotia were recovered from the pith tissues of cobs that had overwintered on the ground in Mississippi (29). No *A. flavus* sclerotia were recovered from soil or corn debris collected from 40 Iowa maize fields in 1990, however; and development of sclerotia in kernels, cobs, and stalk pieces in the Midwest appears to be uncommon (18). Cool temperatures in Iowa fields during September and October may prevent sclerotial development on corn crop residues (18). However, sclerotia were produced in culture by 92% of the isolates recovered from soil samples or by 96% of the isolates recovered from pieces of corn debris collected from these same fields (18). We have observed that *A. flavus* sclerotia and aflatoxin are produced commonly on ears of commercial corn hybrids wound-inoculated with *A. flavus* grown near Bloomington, Illinois (*unpublished data*). *A. flavus* sclerotia may form naturally in association with damaged, aflatoxin-contaminated kernels on preharvest corn grown in the Midwest. *A. f. parasiticus* is commonly associated with aflatoxin-contaminated peanuts (5) and soil insects (12) but is reported infrequently from corn kernels (10,12,29). *A. f. parasiticus* is not necessarily excluded from corn fields, however. This variety constituted 57% of the *A. flavus*-group isolates from soils in long-term corn production plots at Columbia, Missouri, where peanuts have never been planted (1).

We wanted to determine whether a low survival rate for *A. flavus* inoculum (i.e., conidia, sclerotia) might explain why *A. flavus* is detected less frequently in cultivated field soils from temperate regions (14,18) as contrasted with similar soils from the near subtropics (22). Information on the survival of soilborne inoculum of *A. flavus* in the absence of a susceptible host crop (e.g., peanuts, corn) is necessary in the planning of crop rotation sequences as a control strategy. Results of an earlier study showed

TABLE 1. Inoculum pairings of strains of *Aspergillus flavus* var. *flavus* and *A. f. parasiticus* buried in teaballs[a]

Strain	Teaball code							
	1	2	3	4	5	6	7	8
A. f. flavus								
NRRL 6541	C	S	...	...	...	...	...	...
NRRL 6556	...	...	...	...	C	S	...	...
NRRL 13048	...	...	C	S	...	...	...	...
NRRL 13892	...	...	...	...	...	...	C	S
A. f. parasiticus								
NRRL 6433	...	...	S	C	...	...	...	...
NRRL 13005	S	C	...	...	...	...	...	...
NRRL 13006	...	...	...	...	...	...	S	C
NRRL 13539	...	...	...	...	S	C	...	...

[a]NRRL = Northern Regional Research Laboratory; C = conidial inoculum; S = sclerotial inoculum.

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that sclerotia of the *A. f. flavus* strain NRRL (Northern Regional Research Laboratory) 6541 survived overwintering (7 mo) in field soils both in Illinois and in Georgia (21).

This paper describes the survival of sclerotia and conidia produced by four strains of *A. f. flavus* and four strains of *A. f. parasiticus* that were buried for up to 36 mo (October 1986 to October 1989) in sandy soils at the University of Illinois River Valley Sand Field, Kilbourne, Illinois, or at Tifton, Georgia. There are substantial biological differences in the population densities of *A. flavus* in field soils cultivated to corn between the locations in Illinois and Georgia. The University of Illinois River Valley Sand Field was chosen as our Midwest field site to minimize differences in the physical properties of the soil. Center pivot irrigation is commonly used for corn cultivation at both field locations. Survival of these propagules in temperate and near-subtropical latitudes was tested on a similar soil type in fields that have not been cultivated for approximately 15 yr.

MATERIALS AND METHODS

To produce sclerotia and conidia, *A. flavus* strains were cultured on autoclaved corn kernels at 50% (w/w) moisture for 28 days at 28 C (21). The strains included *A. f. flavus* NRRL 6541, from corn, North Carolina; NRRL 13048 and NRRL 13892, from corn, Tifton, Georgia; NRRL 6556, from soil, Canton, Illinois; *A. f. parasiticus* NRRL 6433, from corn, North Carolina; NRRL 13005, from microarthropod, beech forest, Michigan; NRRL 13006, from corn, South Africa; and NRRL 13539, from peanuts, Georgia. Three of these strains (NRRL 6541, NRRL 6556, and NRRL 13539) do not produce aflatoxins (22,27; *unpublished data*). Strains identified as *A. flavus* or *A. parasiticus* have 70% nuclear DNA complementarity and may represent varieties of a single species (11).

Conidia were first washed from the mold-fermented corn kernels by irrigation of the culture surface with 250 ml of distilled water containing 0.01% Triton X-100 (Sigma Chemical Co., St. Louis, MO). The conidial suspension was filtered through glass wool and diluted to approximately 1×10^6 spores per milliliter. Sclerotia were harvested from each culture flask (21). Air-dried sclerotia were blended into 2,000-g lots of air-dried field soil collected from each of the two field sites to give approximately 18 sclerotia per gram of soil. Dilution platings (soil diluted by 10^{-2}) of field-moist soil samples collected before sample burial revealed no *A.*

TABLE 2. Colony-forming units of *Aspergillus flavus* conidia per gram of air-dried soil after burial for up to 36 mo in Illinois or Georgia[a,b]

Location Strain	Start Oct. 1986	Recovery date Apr. 1987	Oct. 1987	Apr. 1988	Oct. 1988	Apr. 1989	Oct. 1989
Illinois							
A. f. flavus							
NRRL 6541	1,600	2,000	2,300	100	0	0	0
NRRL 13048	2,300	1,500	1,800*	800	0	0	0*
NRRL 6556	7,300	31,300	5,000	2,300*	0*	0	0
NRRL 13892	600	800	800	300	0**	0	0
Mean[c]	...	9,600	2,500[d]	750[d]	0[e]	0	0[d]
SE	...	5,300	900	250	0	0	0
A. f. parasiticus							
NRRL 13005	2,000	4,000	2,300	800*	200	300*	400*
NRRL 6433	1,000	2,100	400	2,500	1,100	100	400
NRRL 13539	600	700	500	200*	0*	100	300*
NRRL 13006	1,300	700	2,200	1,200	300	0*	0
Mean[c]	...	1,900	1,400	1,300[f]	450[d]	120[f]	280[f]
SE	...	550	350	650	240	70	110
All other fungi							
Mean[g]	20,000	29,200	10,900[h]	7,500[i]	29,500[j]	11,800[k]	19,200[i]
SE	...	2,700	2,700	1,600	8,200	1,500	3,600
Georgia							
A. f. flavus							
NRRL 6541	8,600	8,600	0	4,600	0	0	0
NRRL 13048	13,600	11,800	0	300	200	0	0
NRRL 6556	37,600	9,400	300	1,800	100	0	0
NRRL 13892	1,600	2,200	200	200	0	0	0
Mean[c]	...	7,900	130	1,700	80	0	0
SE	...	1,200	60	700	60	0	0
A. f. parasiticus							
NRRL 13005	2,000	2,900	0	500	100	0	0
NRRL 6433	1,600	1,000	100	800	200	0	0
NRRL 13539	600	1,700	0	500	300	0	0
NRRL 13006	3,300	2,600	100	600	500	0*	100
Mean[c]	...	2,000	50	600	270	0[d]	20
SE	...	340	30	100	70	0	10
All other fungi[g]							
Mean	33,700	46,400	2,400	4,500	2,900	4,400[h]	1,900
SE	...	4,600	650	650	450	950	200

[a] Values represent the mean numbers of *A. flavus* colony-forming units per gram of soil sampled from each of three teaballs, except where noted. * = two and ** = one teaball, respectively, because of lost teaballs.
[b] Significant ($P < 0.05$) sources of variation from the analysis of variance were state (S), year (Y), S × Y, month (M), S × M, Y × M, S × Y × M, variety (V), S × V, Y × V, M × V, and strain (Northern Regional Research Laboratory [NRRL]) within V.
[c] Values represent the means of 12 replicates (three replicates each of four strains), except where noted because of lost teaballs.
[d] $n = 11$.
[e] $n = 9$.
[f] $n = 10$.
[g] Values represent the means of all non-*A. flavus* fungal colony-forming units per gram of soil sampled from each of 24 teaballs, except where noted because of lost teaballs.
[h] $n = 23$.
[i] $n = 21$.
[j] $n = 20$.
[k] $n = 22$.

flavus colonies at the Illinois field site (Bloomfield fine sand). Fewer than 100 colony-forming units (cfu) of *A. f. flavus* per gram of soil were detected in five of 10 soil samples collected from the Georgia site (Tifton loamy sand). Because colonies of *A. f. flavus* are readily distinguished from those of *A. f. parasiticus* on dextrose peptone yeast extract agar (17), the conidia of one variety could be paired with the sclerotia from another variety in the same lot of soil (Table 1). We paired individual strains of *A. f. flavus* with individual strains of *A. f. parasiticus* to reduce the number of experimental units needed for this field experiment. To pair specific strains at random, 10 ml of the conidial suspension was pipetted into the 2,000-g lots of soil that contained sclerotia, which resulted in 600–37,000 cfu/g of soil, depending on the conidial strain (Table 2). The soil was mixed by hand for 7–10 min to evenly distribute the conidial inoculum. Each of 18 stainless steel teaballs (50 × 30 mm) (Fox Run Craftsman, Ivyland, PA) was filled with 60 g of soil containing sclerotia and conidia from paired isolates of *A. f. flavus* with *A. f. parasiticus* (Table 1). Teaballs were fastened together with nylon fishing line, labeled, and buried (October 1986) at a depth of 10–12 cm (Fig. 1). Teaballs have numerous tiny holes that permit migration of moisture, soil microarthropods, and fine roots. The teaballs were filled with inoculated sandy soil while they were partially buried in sterile damp sand in paper cups. The damp sand served to plug the holes and prevent the dry sandy soil from draining out until we could bury each teaball. Teaballs filled with sandy soil from a particular field site were buried only at that field site.

At 6-mo intervals beginning in April 1987, three teaballs were recovered for each of the eight strain pairings in both Georgia and Illinois. Soil from teaballs was transferred to paper containers and air-dried overnight at room temperature. Soils were stored at room temperature and processed within 30 days. Sclerotia were recovered from the soil inside each teaball by wet-sieving and flotation (25). To test for viability, 30 randomly selected sclerotia were surface-sterilized for 2 min in 0.25% sodium hypochlorite, rinsed twice with 5 ml of sterile distilled water, and transferred to petri dishes that contained potato dextrose agar (PDA). After incubation for 5–10 days at 25 C, plates were examined for *A. flavus* colony growth as evidence of sclerotium viability. In addition, fungi other than *A. flavus* that had colonized sclerotia were isolated and identified.

To measure sporogenic germination of sclerotia recovered after 36 mo of burial (October 1989), 30–50 sclerotia from each teaball were transferred onto the surface of moist sand in 30-ml plastic medicine cups and incubated at 25 C and 100% RH for 7 days. The number of sclerotia that developed yellow-green conidial heads was recorded (23).

Numbers of *A. flavus* and all other fungal colony-forming units in the soil were quantified on dilution plates. Two grams of soil from each teaball was added to 198 ml of 0.2% sterile distilled water containing 0.01% Triton X-100 and gently shaken by hand for 10–15 s. Mechanical blending of sclerotia mixed with sand produces large numbers of sclerotial fragments that are impossible to distinguish from conidium-derived colony-forming units formed by germinated sclerotia. One milliliter of a final dilution, yielding 20–40 total fungal colonies per plate, was distributed over the surface of each of three plates of dextrose peptone yeast extract agar containing streptomycin (25 mg/L) and tetracycline (1.25 mg/L). Plates were incubated in the dark at 25 C for 10 days.

Analysis was conducted primarily by least squares analysis of variance. Responses (Y variables) studied were survival of *A. flavus* conidia (natural log of colony-forming units per gram of soil; Table 2), viability of *A. flavus* sclerotia (percentages; Table 3), and inoculum densities that followed germination of *A. flavus* sclerotia (natural log of colony-forming units per gram of soil; Table 4). Sources of variation (X variables) were state, year, month, and variety, each of which was examined individually and in all possible two-, three-, and four-way interactions. Strain within variety (NRRL strain designation) was included as a random effect. Comparisons of means within interactions were made by t tests of least squares means at $P < 0.01$.

RESULTS

Most of the *A. flavus* conidial inoculum survived the first winter (October 1986 to April 1987) after burial in Illinois and Georgia (Table 2). *A. f. flavus* was not observed in dilution platings of teaball soil samples after the second growing season in Illinois (October 1988), whereas three of the *A. f. parasiticus* strains were detected even after 36 mo (in October 1989). In Georgia, substantial losses of *A. flavus* conidial inoculum occurred during the first crop growing season (April to October 1987). *A. f. flavus* was isolated from only two of 36 teaballs sampled after the 1988 crop growing season. Numbers of fungal colony-forming units for taxa other than *A. flavus* declined substantially (10- to 20-fold) after the first growing season in Georgia but not in Illinois (Table 2).

A large majority of the *A. flavus* sclerotia remained viable throughout the 36-mo burial experiment, as determined by *A. flavus* colony growth on PDA (Table 3). Over all sites and dates, *A. f. parasiticus* had a lower survival rate than did *A. f. flavus* (84 and 93%, respectively). Over all dates, the rates for sclerotium survival in Illinois (88.4%) were similar to the overall rates in Georgia (89%). Within *A. f. parasiticus*, strain NRRL 13539, with an overall mean (all sites and dates) of 74%, had a lower rate of sclerotium survival than did strains NRRL 6433, NRRL 13005, and NRRL 13006, with overall means of 80%, 89%, and 94%, respectively. *A. f. flavus* strain NRRL 6541, with an overall mean of 89%, had a lower rate of sclerotium survival than did strain NRRL 13892, which had an overall mean of 97%. Sclerotia from *A. f. parasiticus* NRRL 13539 exhibited a pattern of reduced viability after the first growing season at each field site. None of the sclerotia recovered after 36 mo at either field site germinated sporogenically on moist sand.

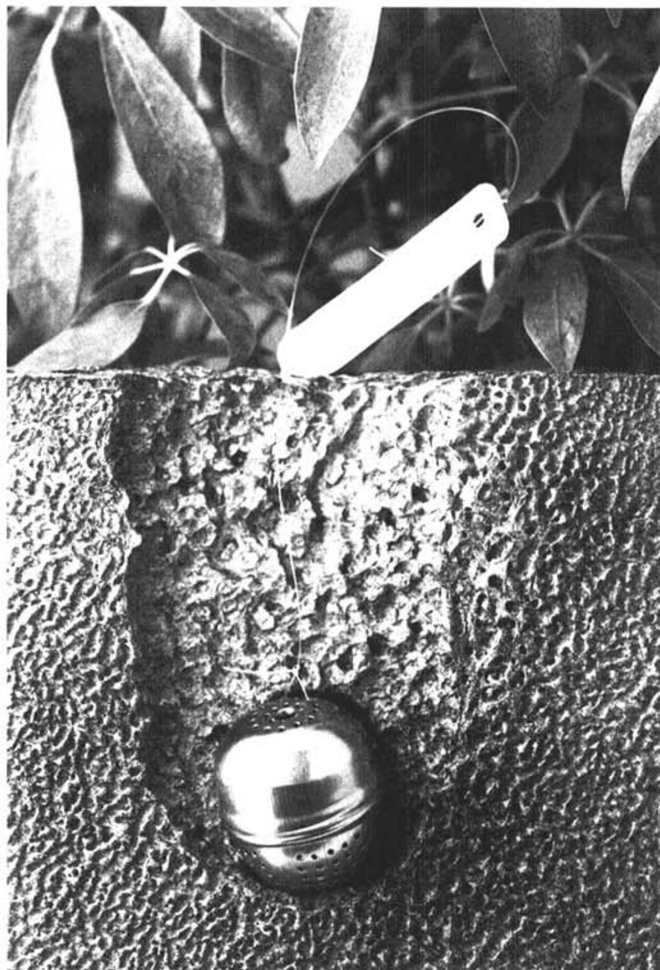

Fig. 1. Teaball, with label attached, shown in simulated soil burial.

Substantial increases in the number of colony-forming units in teaball soils were recorded for all of the *A. flavus* strains that were buried as sclerotia (Table 4). In Illinois and Georgia, an increase in inoculum density was recorded for each of the four *A. f. flavus* strains, followed by a progressive decline through the last recovery date. Final inoculum densities for *A. flavus* exceeded 5,000 cfu/g of soil for six of the eight sclerotial strains buried in Illinois, but only one of the strains buried in Georgia attained this level. The increase in *A. flavus* inoculum apparently occurred in the April 1988 measurement. The effect of time on the inoculum densities across sites was evaluated by comparison of the means in the significant ($P < 0.01$) year × month × variety interaction. *A. f. parasiticus* showed a higher inoculum density ($P < 0.05$) than did *A. f. flavus* in April 1987 (ln cfu = 6.66 vs. 1.25) and in October 1989 (ln cfu = 8.63 vs. 7.32). The densities of the two varieties were similar in October 1987 (*A. f. parasiticus* = 8.45 and *A. f. flavus* = 7.92), in April 1988 (9.57 and 9.59), in October 1988 (9.18 and 8.92), and in April 1989 (9.07 and 8.61). Both varieties increased ($P < 0.01$) in density from April 1987 to October 1987, and *A. f. flavus* increased ($P < 0.01$) again from October 1987 to April 1988. Neither variety had a significant ($P < 0.01$) change in density from April 1988 through April 1989, but *A. f. flavus* did decrease ($P < 0.05$) in density from April 1989 to October 1989. In the state × year interaction ($P < 0.01$), Georgia showed a higher inoculum density than did Illinois in 1987, but Illinois had higher inoculum densities in 1988 and 1989. At both sites there was an increase in inoculum density from 1987 to 1988, but Georgia then had a decrease from 1988 to 1989.

More fungal taxa colonized sclerotia buried in Illinois than in Georgia, but only two species (*Paecilomyces lilacinus* (Thom) R. A. Samson and *Periconia macrospinosa* Lefebvre & A. G. Johnson) were isolated from ≥10% of the sclerotia representing any *A. flavus* strain or exposure interval. *P. lilacinus* was the fungus isolated most frequently from sclerotia buried in Georgia (Table 5). Sclerotia from *A. f. flavus* NRRL 13892 was the least-colonized group at either field site, but this was within the realm of normal variation. *Bacillus megaterium* was an important colonist of sclerotia in samples recovered after 36 mo of burial in Georgia (Table 6). This bacterium produced visible colonies on the surface of PDA and typically intermingled with *A. flavus* mycelium from a germinated sclerotium.

DISCUSSION

The inoculum density of *A. flavus* in any habitat increases with production of new propagules (i.e., hyphal cells, conidia, and sclerotia) and immigration (e.g., conidia vectored by insects). The fungal population cannot continue to exist in a given place if the outputs resulting from the death of propagules and emigration exceed inputs. From a demographic perspective, the *A. flavus* colony or clone can be viewed in terms of a population of parts, such as the leaves or stems produced from the same root system of bamboo (20). Thus, the hyphae, conidia, and sclerotia that comprise a single *A. flavus* clone can be examined as separate populations; each has its own survivorship curve. *A. flavus* sclerotia survived burial in sandy soils for up to 36 mo in Illinois and Georgia. Substantial losses of *A. flavus* conidial

TABLE 3. Germination (%) of *Aspergillus flavus* sclerotia recovered after burial for up to 36 mo in Georgia or Illinois[a,b]

Location Strain	Recovery date					
	Apr. 1987	Oct. 1987	Apr. 1988	Oct. 1988	Apr. 1989	Oct. 1989
Illinois						
A. f. flavus						
NRRL 6541	89	96	82*	85	85*	90*
NRRL 13048	94	94	66	91	92	95
NRRL 6556	99	99	90*	92*	90	92*
NRRL 13892	98	98	94	92	98*	97
Mean[c]	95	97	82[d]	90[e]	91[e]	94[d]
SE	6.0	4.5	18.0	7.9	7.4	5.1
A. f. parasiticus						
NRRL 13005	87	85	81	96	92	93
NRRL 6433	89	88*	51	88	88	77*
NRRL 13539	93	75	55*	69*	78	80
NRRL 13006	98	95	83	90**	99	99
Mean[c]	92	86[e]	69[e]	86[f]	89	88[e]
SE	6.4	11.9	16.1	12.3	12.0	14.0
Georgia						
A. f. flavus						
NRRL 6541	99	100	97	93	86	68
NRRL 13048	100	100	97	93	92	90
NRRL 6556	100	97	92	97	89	90
NRRL 13892	100	100	94	95	98*	99
Mean[c]	100	99	95	95	91[e]	87
SE	0.9	1.4	5.7	4.2	8.5	16.3
A. f. parasiticus						
NRRL 13005	100	80	82	91	80	100
NRRL 6433	99	80	56	88	81	72
NRRL 13539	98	75	51	62	61	83
NRRL 13006	100	94	84	94	93	92
Mean[c]	99	83	68	84	79	87
SE	2.1	10.9	18.7	13.5	16.4	23.3

[a]Viability (%) of 90 sclerotia (30 sclerotia from each of three buried teaballs) as determined by *A. flavus* colony growth from surface-disinfested sclerotia on potato dextrose agar except where noted. * = 60 sclerotia and ** = 30 sclerotia because of lost teaballs. Teaballs were originally buried in October 1986.

[b]Significant ($P < 0.05$) sources of variation from the analysis of variance were year (Y), state (S), S × Y, month (M), Y × M, variety (V), S × V, Y × V, Y × M × V, and strain (Northern Regional Research Laboratory [NRRL]) within V.

[c]Values represent the means of 12 replicates (three replicates each of four strains), except where noted because of lost teaballs.

[d]$n = 9$.

[e]$n = 10$.

[f]$n = 11$.

inoculum were recorded after the first year of burial in Georgia and after the second year of burial in Illinois. Therefore, in the absence of sclerotia, inoculum density of *A. f. flavus* should return to preinfestation densities (<100/g of soil) within 24 mo in noncultivated soil. Conidia of *A. f. parasiticus* apparently can survive for longer periods in Illinois than can conidia of *A. f. flavus*. Numbers of fungal propagules other than those of *A. flavus* declined substantially in teaball soil samples buried in Georgia but not in those buried in Illinois.

A large majority of the *A. flavus* sclerotia survived this interval of soil burial and germinated while buried to produce a population "bloom." There are several potential explanations for these fluctuations in viability-germinability of sclerotia over time. There is the potential for genetic or phenotypic variation in the resistance of the individual sclerotia added to each teaball. There may be some variation among microhabitats in which the teaballs were buried. Sclerotia that were present but nonviable at 18 mo may have subsequently rotted. If sclerotia from this "susceptible" subset of the sclerotial population can no longer be recovered after 24, 30, or 36 mo, the percentage of recovered sclerotia that are viable would appear to increase. Sporogenic germination of buried sclerotia (19) could account for high inoculum densities of *A. flavus*. Fluctuating levels of *A. flavus* inoculum may have occurred because sporogenic germination occurred more than once; *A. flavus* sclerotia can produce more than one crop of conidial heads (23).

P. lilacinus, the most frequently isolated fungus from *A. flavus* sclerotia in both locations, was first isolated from *A. flavus* sclerotia in samples recovered from Georgia 1 yr after burial. Numbers of sclerotia that were colonized by *P. lilacinus* during the first 18 mo of field burial (28) and after 36 mo were similar. We had expected that during the 18- to 36-mo interval of field exposure, *P. lilacinus* would colonize larger numbers of sclerotia than were colonized during the initial 18-mo interval. These results suggest that individual sclerotia produced by the same fungal strain may vary in their resistance-susceptibility to *P. lilacinus* or to other mycoparasites. A larger number of fungal species were recorded from *A. flavus* sclerotia buried in Illinois than

TABLE 4. Inoculum densities (colony-forming units per gram of air-dried soil) for *Aspergillus flavus* strains buried as sclerotia for up to 36 mo in Illinois or Georgia[a,b]

Location Strain	Start Oct. 1986	Recovery date Apr. 1987	Oct. 1987	Apr. 1988	Oct. 1988	Apr. 1989	Oct. 1989
Illinois							
A. f. flavus							
NRRL 6541	<100	<100	39,300	11,900*	7,100	4,100*	3,100*
NRRL 13048	<100	<100	37,700	41,000	31,200	8,800	6,400
NRRL 6556	<100	<100	<100	74,100*	43,800*	9,500	13,700*
NRRL 13892	<100	<100	64,900	27,900	27,700	13,600*	12,800
Mean[c]	...	0	35,400	37,900[d]	25,900[e]	9,400[d]	9,100[d]
SE	...	0	7,300	8,600	4,900	1,500	3,100
A. f. parasiticus							
NRRL 13005	<100	7,900	61,000	76,000	84,200	46,400	47,100
NRRL 6433	<100	5,100	10,000*	5,100	4,500	8,000	2,500*
NRRL 13539	<100	<100	<100	1,800*	2,900*	5,800	15,800
NRRL 13006	<100	32,600	38,300	69,600	24,200**	77,000	83,600
Mean[c]	...	11,100	27,200[e]	41,400[e]	32,800[f]	34,200	37,200[e]
SE	...	4,300	9,600	11,600	16,900	9,100	10,000
All other fungi							
Mean[g]	20,000	29,200	10,900[h]	7,500[i]	29,500[j]	11,800[k]	19,200[i]
SE	...	2,700	2,700	1,600	8,200	1,500	3,600
Georgia							
A. f. flavus							
NRRL 6541	<100	25,600	2,000	5,700	2,700	2,200	300
NRRL 13048	<100	<100	1,000	15,000	5,300	3,400	1,000
NRRL 6556	<100	<100	4,400	8,000	3,300	3,800	600
NRRL 13892	<100	<100	7,100	14,900	3,800	3,700*	500
Mean[c]	...	6,300	3,600	11,100	3,700	4,200[e]	600
SE	...	3,800	920	2,300	770	780	170
A. f. parasiticus							
NRRL 13005	<100	8,200	33,000	12,200	7,200	4,300	1,900
NRRL 6433	<100	<100	14,000	14,500	32,600	19,000	2,500
NRRL 13539	<100	1,600	6,200	14,000	5,200	12,500	1,400
NRRL 13006	<100	25,000	23,700	47,000	11,000	26,500	5,700
Mean[c]	...	8,800	19,200	22,000	14,000	14,800	2,600
SE	...	3,100	4,600	5,700	5,000	4,200	670
All other fungi							
Mean[g]	33,700	46,400	2,400	4,500	2,900	4,400[h]	1,900
SE	...	4,600	650	650	450	950	200

[a]Values represent the mean numbers of *A. flavus* propagules per gram of soil sampled from each of three teaballs except where noted. * = two and ** = one teaball, respectively, because of lost teaballs.
[b]Significant ($P < 0.05$) sources of variation from the analysis of variance were year (Y), state (S), S × Y, month (M), Y × M, variety (V), Y × V, M × V, Y × M × V, and strain (Northern Regional Research Laboratory [NRRL]) within V.
[c]Values represent the means of 12 replicates (three replicates each of four strains), except where noted because of lost teaballs.
[d]$n = 10$.
[e]$n = 11$.
[f]$n = 9$.
[g]Values represent the averages of all non-*A. flavus* group fungal propagules per gram of soil sampled from each of 24 teaballs, except where noted because of lost teaballs.
[h]$n = 23$.
[i]$n = 21$.
[j]$n = 20$.
[k]$n = 22$.

TABLE 5. Percentage of *Aspergillus flavus* sclerotia colonized after burial in soil for up to 36 mo in Illinois or Georgia[a]

Location	*A. f. flavus*				*A. f. parasiticus*			
Taxa	NRRL 6541	NRRL 6556	NRRL 13048	NRRL 13892	NRRL 6433	NRRL 13005	NRRL 13006	NRRL 13539
Illinois								
Acremonium sp.	...	...	3	...	1	...	...	2
Apiospora montagnei (*Arthrinium* state)	2	...	1	...	1	3	...	...
Curvularia inaequalis	...	...	1	...	1	...	...	1
Fusarium oxysporum	3	3	3	3	2	1	3	5
F. compactum	5	1	4		1	3	2	1
Mycelia sterilia No. 1	2	1	1	...	...	...	...	1
M. sterilia No. 2	...	2	2	1	1		1	...
Paecilomyces lilacinus	8	5	3	4	4	4	1	10
Periconia macrospinosa	...	...	...	...	...	...	...	10
Phoma sp.	4	3	1	...	1	1	...	8
Thielavia terricola	...	...	...	...	1	1	1	
Torula herbarum		1	1	...	1	...	...	3
Trichoderma harzianum	1	...	...	...	2	1	1	2
Bacillus megaterium	2	1	0	0	0	0	0	0
Georgia								
P. lilacinus	13	16	17	7	16	23	10	22
P. macrospinosa	2	1	1	...	4	1	3	1
T. harzianum	...	1	2	...	...	3	...	...
B. megaterium	12	31	42	18	17	21	0	22

[a]Values represent the highest frequency (%) of sclerotial colonization (30 sclerotia × three teaballs) recorded for any recovery date. Teaballs were originally buried in October 1986. NRRL = Northern Regional Research Laboratory.

TABLE 6. Isolation frequency (percentage of sclerotia recovered) of *Paecilomyces lilacinus* and *Bacillus megaterium* from *Aspergillus flavus* sclerotia buried for up to 36 mo in Illinois or Georgia[a]

Location	Recovery date					
Isolate	Apr. 1987	Oct. 1987	Apr. 1987	Oct. 1988	Apr. 1989	Oct. 1989
Illinois						
A. f. flavus						
NRRL 6541	0	0	2*	7	8*	3 (2)[b]*
NRRL 6556	0	0	5	0 (2)	1	3
NRRL 13048	0	0	3*	2*	1	1 (1)*
NRRL 13892	0	0	4	0	0*	1
A. f. parasiticus						
NRRL 6433	0	0	4	1	0 (2)	0
NRRL 13005	0	0*	4	2	1 (1)	0*
NRRL 13006	0	0	1*	0*	0	0
NRRL 13539	0	0	10	2**	2 (1)	0
Georgia						
A. f. flavus						
NRRL 6541	0	0	3	6	9 (9)	3 (12)
NRRL 6556	0	2	7	2	16 (8)	4 (31)
NRRL 13048	0	1	17	2	12 (5)	7 (42)
NRRL 13892	0	1	1	3 (1)	7 (5)*	0 (18)
A. f. parasiticus						
NRRL 6433	0	1	2	0	13 (5)	16 (17)
NRRL 13005	0	20	11	0	23	0 (21)
NRRL 13006	0	2	10	4	4 (3)	0
NRRL 13539	0	11	22	17	20 (1)	8 (22)

[a]Isolation frequency (%) of 90 sclerotia (30 sclerotia × three teaballs) except where noted. * = two and ** = one teaball, respectively, because of lost teaballs. Teaballs were originally buried in October 1986. NRRL = Northern Regional Research Laboratory.

[b]Colonization frequencies for *Bacillus megaterium* are in parentheses.

from those buried in Georgia, but there was no evidence that sclerotium survival was negatively affected. *B. megaterium* was a principal colonist of *A. flavus* sclerotia that were buried for 36 mo in Georgia. Aflavinines are major components of *A. flavus* sclerotia and exhibit mild antibiotic activity against *B. subtilis* in standard disk assays (6). Moreover, aflavinines leak from sclerotia (J. B. Gloer, *personal communication*) and may interfere with *B. megaterium* colonization of these structures in field soils. This could explain why *B. megaterium* was recorded so infrequently from *A. flavus* sclerotia for field exposure intervals of less than 36 mo. If the aflavinines leach out over time, by the third year the sclerotial aflavinine content may be insufficient to prevent colonization by *B. megaterium*.

We speculated that low inoculum densities of *A. flavus* in soils from the Midwest's corn belt would reflect a low survival rate for *A. flavus* inoculum. Our results show that *A. flavus* conidia and sclerotia survived burial as well in Illinois as in Georgia. We need to explain the larger numbers of *A. flavus* propagules recorded from Georgia soils. Bell and Crawford (2) found 5,000–20,000 *A. flavus* propagules per gram of soil in a peanut field, whereas soil sampled from five corn fields near Tifton had 300–3,000 cfu/g of soil (22). *A. flavus* has been reported to occur with "very low incidence" in Iowa crop soils (18) and in cultivated soils at the University of Illinois River Valley Sand Field (<1–4 cfu/g of soil, *unpublished data*). The more frequent occurrence of aflatoxin outbreaks in Georgia corn fields, as contrasted with Illinois, should result in more frequent dispersal of *A. flavus* inoculum onto the soil at harvest. Increased inoculum densities of *A. flavus* occurred immediately following picker-sheller combine harvesting of corn in Georgia (25). High levels of *A. flavus* inoculum in field plots under conventional tillage may be promoted by *A. flavus* colonization of buried crop residues (1). For example, low densities of *A. flavus* in the soil of peanut fields increased approximately nine times in soil around buried ryegrass (7). Furthermore, *A. f. flavus* was a predominant fungal colonist of corn cobs collected from the field in Mississippi (29) and exhibited a greater colonizing ability at low moisture potentials and elevated soil temperatures than did other common soil-inhabiting fungi (9). The ability of *A. flavus* to competitively colonize corn residues increases its exposure to detrivorous corn insects (e.g., sap beetles, Nitidulidae) that transmit this fungus to ripening corn (13).

These results should be of immediate interest to scientists who are investigating the use of nonaflatoxigenic strains of *A. flavus* to control aflatoxin contamination of crops before harvest (3). We have shown that some sclerotia of some strains are superior to others in resisting colonization by other fungi and in supporting a massive population "bloom." This conidial inoculum contributes to the competitive saprophytic ability of *A. flavus* to colonize crop debris while increasing the probability of both horizontal and vertical dispersal by arthropods to maturing corn, cotton, or peanuts. These strain characteristics are important, particularly if sclerotia are to serve as the source of primary inoculum. For example, *A. f. parasiticus* NRRL 13539 (=CP 461), which

exhibited a pattern of reduced sclerotial viability after the first growing season, was isolated from soil in which peanuts were grown at Dawson, Georgia, and did not produce aflatoxin. Aflatoxin-producing ability and sclerotial viability may be unrelated, however.

Population growth and mortality are inconsistent from year to year because of yearly variations in the physical conditions of the environment. We anticipated that during the 3 yr of soil burial, different abiotic and biotic environmental factors (i.e., precipitation, soil temperature, intervals of drought, freezing and thawing events, soil biota, etc.) would result in differential propagule survival between locations. We also expected that there would be year-to-year differences at a single location. Our results show significant sources of variation between field locations (states) and years. Sclerotia produced by individual strains of *A. flavus* apparently differed in their resistance to mycoparasitism. *B. megaterium* frequently colonized *A. flavus* sclerotia buried for 36 mo in Georgia. The ability of *A. flavus* sclerotia to initiate a rapid increase in inoculum density in field soil and the resistance of these sclerotia to colonization by other fungi should be considered when the importance of sclerotia as a source of primary inoculum is investigated.

In fields planted with different crops and in adjacent woodlands, the biotic factors affecting local populations of *A. flavus* may be qualitatively different. *A. flavus* occurred in 10–27% of the isolation plates inoculated with soil from forest and cultivated soils from Georgia (15). If one of the local populations of *A. flavus* were to become extinct, the habitat may be recolonized from other local populations, a process that is a selective advantage against widespread extinction (4). *A. flavus* sclerotia, such as those reported to form on corn kernels (24), could provide the means by which genetically diverse populations, or different vegetative compatibility groups, accumulate in a single field (16).

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