Population Levels of Aspergillus flavus and the A. niger Group in Virginia Peanut Field Soils

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ABSTRACT

The populations of *Aspergillus flavus* and the *A. niger* group were determined in the fruiting zone of Virginia peanut field soils during the middle of the fruit-forming period with a selective medium (M3S1B) containing NaCl and 2,6-dichloro-4-nitroaniline. The mean populations of *A. flavus* were much lower than reported previously for peanut field soils. In five fields sampled in 1971, and in six fields sampled in 1972, *A. flavus* populations ranged from 0.8 to 12.8, and from 0.5 to 57.3 propagules per g soil, respectively. *A. niger* group populations were mostly low also, and there was little relation between *A. flavus* and the *A. niger*-group populations or between *A. flavus* populations and soil properties. Population determinations of soil from two fields for three years suggested that planting peanuts did not result in an increased *A. flavus* population. *A. flavus* may be able to colonize peanut fruits from low inoculum density levels.

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Determinations of the population of *Aspergillus flavus* Link ex Fries in the fruiting zone of peanut (*Arachis hypogaea* L.) fields are needed to gain insight about the inoculum potential that exists for peanut fruit colonization by this toxigenic fungus; this information is also needed for spore germination studies concerned with the propagule density at which *A. flavus* spore germination may occur in soil in nature (6, 12). In Georgia, Bell and Crawford (2) have indicated the populations of *A. flavus* in soil collected from each of two fields was greater than 10^4 propagules/g soil. Populations greater than 10^4 propagules/g soil have been reported also for field soils in Israel (8), and populations of 10^2 to 10^3 propagules/g soil have been reported for Nigerian field soils (10). Using the selective medium of Bell and Crawford (2) we were unable to detect *A. flavus* on soil dilution plates of Virginia peanut field soils at 10^{-2} or greater dilutions (6). In contrast, the data of Garren and Porter (4, 5, 15, 16) indicated that *A. flavus* may be a dominant member of the endocarpic community of peanut fruits harvested from these Holland, Virginia fields. This study was undertaken to determine the population of *A. flavus* in the fruiting zone of several peanut field soils in Virginia, near the middle of the fruit-forming period. The population of the *A. niger* group was also determined in light of the reported antagonism of this group to *A. flavus* (1, 8).

MATERIALS AND METHODS. – Five of the six peanut fields (A-E) studied were located in the vicinity of Holland, Virginia. The sixth field (F) studied one year only (1972) for comparison, was located near Boykins. The properties of these soils are indicated in Table 1. All of these fields were deep-plowed as recommended for control of stem rot caused by *Sclerotium rolfsii* (3).

Soil samples (about 500 g each) were collected from six locations in each field at a depth of 0-10 cm. One sample each was collected underneath peanut plants from two adjacent rows, and at each of three 9-m intervals down the rows. In 1971, samples were collected on 30 August, and 1972 samples were collected on 4 September. Approximately the same areas of the fields were sampled both yr. Samples were placed in plastic bags with pin holes for gas exchange, taken to the laboratory and stored frozen at −10 C until assayed. Preliminary tests showed frozen samples and samples processed immediately yielded similar *A. flavus* and *A. niger*-group recoveries.

Soils were assayed by the dilution plate technique. One random 11.0-g subsample was removed from each bag and added to a sterile Waring Blendor containing 95 ml of water. The soil suspension was blended at high speed for 2 min. Preliminary tests indicated that recoveries of *A. flavus* from soil samples processed by this method usually were similar to those obtained by shaking soil suspensions on a wrist-action shaker. After blending, the soil suspension was transferred to a sterile 250-ml beaker containing a magnetic stirring bar. Samples (1.0 ml) were removed from the continuously agitated soil suspension and plated on M3S1B *A. flavus - A. niger* group selective medium (7). The medium was modified from the 2,6-dichloro-4-nitroaniline-amended medium (10 mg/l) developed by Bell and Crawford (2). M3S1B medium has the following composition: 5.0 g peptone (Fisher No. J-2007C), 10.0 g glucose, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$,30.0 g NaCl, 20.0 g agar, 50.0 mg streptomycin sulfate, 50.0 mg chlorotetracycline, 1.0 mg 2,6-dichloro-4-nitroaniline (added in 3 ml of acetone), and 1 liter distilled water (pH 5.2). Antibiotics and fungicide were added to the cooled (45 C) medium after autoclaving. Both fungi formed large colonies from naturally infested soil on this medium, in contrast to the inhibition of *A. niger* on the Bell and Crawford medium (2). Sixteen 9-cm diam dilution plates were prepared for each sample and incubated at 35 C for three days when colony counts of *A. flavus* or *A. niger*-group colonies did not develop on any M3S1B check plates exposed to laboratory air for each soil processing period. Several colonies of *A. flavus* developing on dilution plates from each soil were transferred to Czapek-Dox agar for confirmation of identity according to the criteria of Raper and Fennell (17).

RESULTS. – The population of *A. flavus* in the fruiting zone of peanut fields in 1971 was generally low (Table 2). Individual assays ranged from 0 to 48.7 propagules/g soil, but the mean population in all soils was less than 15 propagules/g soil. The mean *A. niger*-group populations in three soils were also low ($<10^2$ propagules/g soil) but typically greater than

TABLE 1. Properties of different peanut field soils used for *Aspergillus flavus* population studies

Field	Textural class	Sand (%)	Silt (%)	Clay (%)	Salts (Conductivity) (mmhos/cm)	$NO_3^- - N$ µg/g	pH[a]	Organic matter (%)	Water content (%) at −0.1 bars	Water content (%) at −15.2 bars
A	Sandy loam	57.6	34.1	0.3	0.160	6.0	5.7	2.6	21.2	4.7
B	Loamy sand	83.9	12.6	3.5	0.081	3.0	6.2	2.6	10.2	2.5
C	Loamy sand	77.8	16.2	6.0	0.092	3.5	5.2	1.8	13.0	3.5
D	Sandy loam	75.5	17.9	6.6	0.069	4.1	5.2	2.7	14.8	3.8
E	Loamy sand	81.6	15.3	3.1	0.080	1.4	5.8	0.5	9.1	1.3
F	Sand	86.7	10.8	2.5	0.130	13.8	5.4	1.2	6.9	1.1

[a]Determined by the water-saturation percentage method.

for *A. flavus*, and only one soil (A) had a mean population less than the mean *A. flavus* population. However, there appeared to be no consistent relation of the *A. niger*-group populations to the *A. flavus* populations. For all soils, two colony types of *A. flavus* developed on Czapek-Dox agar plates. One produced abundant sclerotia, but the other produced none. On the M3S1B selective medium, all *A. flavus* colonies appeared somewhat similar and no sclerotia were produced. Analysis of soils A and B in the 1970 growing season (peanuts were planted in these fields) with M3S1B medium and a slightly modified but equally effective selective medium, M3SR (7), indicated that the *A. flavus* populations were also lower than 15 propagules/g soil. With M3S1B medium, means of 12.2 and 2.2 propagules per g soil were obtained for *A. flavus*, and 8.9 and 3.0 for *A. niger* group, in soils A and B, respectively.

TABLE 2. Populations of *Aspergillus flavus* and the *A. niger* group in the fruiting zone of five Virginia peanut fields in 1971

	Propagules/g soil			
	A. flavus		*A. niger* group	
Field	Range[a]	Mean[a]	Range[a]	Mean[a]
A	1.9 – 33.0	11.8	1.3 – 6.5	4.0
B	0.0 – 1.9	0.8	1.9 – 17.1	7.9
C	1.9 – 48.7	12.8	3.8 – 71.2	20.2
D	0.0 – 19.1	3.7	4.4 –122.9	58.3
E	0.0 – 9.8	4.3	4.3 – 9.1	7.2

[a]Based on analysis of samples from the fruiting zone (0- to 10-cm depth) for six locations in each field .

TABLE 3. Populations of *Aspergillus flavus* and the *A. niger* group in six Virginia fields in 1972

	Propagules/g soil				
	A. flavus		*A. niger* group		
Field	Range[a]	Mean[a]	Range[a]	Mean[a]	Crop
A	30.0 –104.5	57.3	2.5 – 13.1	7.4	Soybeans
B	0.0 – 3.0	1.3	3.6 – 12.0	6.5	Peanuts
C	2.5 – 21.0	8.3	3.1 – 13.4	5.5	Corn
D	0.0 – 1.8	0.5	9.2 – 63.1	30.5	Soybeans
E	0.0 – 5.8	1.9	4.8 – 26.7	12.8	Corn
F	3.0 – 18.7	6.7	14.1 –175.8	112.0	Peanuts

[a]Based on analyses of samples from 0- to 10-cm depth for six locations in each field.

In 1972 the populations of *A. flavus* were generally low again, except in one field (Table 3). Although the mean population in this field was still less than 10^2 propagules/g soil, it was much greater than the population in the other fields, or in this field in 1970 and 1971. There appeared to be no relation between the *A. flavus* population and the crops planted for the preceding yr or yrs. The mean *A. niger*-group populations were greater than the mean *A. flavus* population in all soils except A and C. These soils also had the highest mean *A. flavus* populations of all soils tested for both 1971 and 1972 (and in 1970 for soil A). The mean *A. niger*-group population was greater than 10^2 propagules/g soil only in soil F collected near Boykins.

DISCUSSION. – The results of this study indicate that the population of *A. flavus* in the fruiting zone of Virginia peanut fields is much lower than that reported in soils of other peanut-growing areas (2, 8, 10). This, together with the results of Garren and Porter (4, 5, 15, 16) obtained for some of these same fields, suggests that *A. flavus* is able to colonize peanut fruits from low inoculum density levels and establish itself in the endocarpic community. Based on the data obtained for soil C in 1972 (as an example), and our previous data on G/S (geocarposphere population/soil population) values for *A. flavus*, and the amounts of geocarposphere soil obtained from pegs and mature peanut fruits (6), an average of only 2.0 *A. flavus* propagules may have been present in the inner 0.5-mm layer of geocarposphere soil of each peanut fruit (0.0 - 26.1 = the range for all soil samples); and an average of 0.3 *A. flavus* propagules may have been present in the inner 0.5-mm layer of geocarposphere soil of each peanut peg (0.0 - 3.1 = the range for all soil samples). Much higher *A. flavus* populations occur in Israel soils (8) than in Virginia soils, but not greater seed colonization by *A. flavus* (4, 5, 15, 16). *A. flavus* colonization of peanut seed in other areas of the United States in some instances may be quite high (2, 14).

It is not clear why the *A. flavus* populations are generally low. *A. flavus* activity in soil (6, 7) and axenic culture (11) is favored by high temp (30 - 35 C). Possibly, low soil temp in Virginia, northern-most state in the United States commercial peanut growing area, are responsible, in part, for the low *A. flavus* population. Separate tests conducted by us indicate that the *A. flavus* population may be relatively constant in the fruiting zone, and that the increase in soil A in 1972 may be associated with the saprophytic colonization of the rye cover crop in soil of this field by *A. flavus* (6; and G. J. Griffin, and K. H. Garren, *unpublished*).

Although peanuts were planted in field B for all three yr of sampling, the mean *A. flavus* population was less than three propagules per g soil for all years. Pettit and Taber (13) reported that planting peanuts the previous year resulted in higher infestation of peanut fruits by fungi and higher aflatoxin content in peanuts than planting of rye, oats, melons, or potatoes the previous year. Joffe and Lisker (9) found no influence of crop sequence on *A. flavus* colonization of peanut seed.

Soil A is characterized by having the lowest sand content, and the highest average *A. flavus* population of all soils tested. Joffe (8) reported that *A. flavus* occurred more commonly in soil of medium and heavy types, while Bell and Crawford (2) reported similar populations of *A. flavus* in a clay loam soil

and a sandy loam soil. There appears to be no correlation between soil water properties and *A. flavus* population in the soils.

The soil in field A also was the only soil to have a consistently higher mean *A. flavus* than *A. niger*-group population (for 3 yr). However, there appeared to be no other general relationship between the populations of these fungi. The data of Garren and Porter (4, 5, 15, 16) indicate that *A. niger* is not a dominant colonizer of peanuts in Virginia, and that *A. flavus* may be dominant. This, and the present data, suggest that antagonism of *A. flavus* by *A. niger* may not occur to a great extent under Virginia conditions.

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