Aflatoxin Contamination of Peanuts Resistant to Seed Invasion by Aspergillus flavus

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ABSTRACT

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Harvested nonshelled and shelled peanuts of accessions, P. I. 337409 and P. I. 337394 F, previously reported to be resistant to penetration and colonization by *Aspergillus* spp. of the *A. flavus* group, were held under high humidity conditions and the aflatoxin levels that developed were compared with those reached in similarly stored peanuts of an easily colonized genotype, P. I. 343360, and a commercially grown cultivar, Florunner. The peanuts were not artifically inoculated, but each genotype had a natural seed infection of 2-3% by *Aspergillus* spp. of the *A. flavus* group. All genotypes had appreciable levels of aflatoxins after 9-10 days of storage in relative humidities of 87-95% at 23-26 C. Accession P. I. 337394 F accumulated aflatoxins at 80±2% RH after 9 days at 23 C. Peanut genotypes that are penetrated with difficulty by *Aspergillus* spp. of the *A. flavus* group may have advantages in the field, but not when stored in high relative humidities at temperatures favorable for fungal deterioration.

Additional key words: *Arachis hypogaea*, *ochratoxin*.

Species of *Aspergillus* in the *Aspergillus flavus* group (14) frequently cause mold of peanuts, *Arachis hypogaea* L., and contamination with aflatoxins. Aflatoxin contamination can occur before digging, between digging and combining, after combining but before drying, and in storage. Two fungi in this group *A. flavus* Lk. ex Fr. and *A. parasiticus* Speare that produce aflatoxins have been isolated from aflatoxin-contaminated peanuts (1, 2, 8, 12). Peanut cultivars that inhibit aflatoxin production have been reported (7, 13); however, analysis of United States' introductions with the same accession numbers did not confirm those results (3, 10). The development of genotypes of *Arachis hypogaea* L. not readily penetrated and colonized by *Aspergillus* spp. of the *A. flavus* group is desirable because the potential contamination of peanuts with aflatoxin may be minimized.

Mixon and Rogers (9, 10, 11) reported that two plant introduction (P. I.) accessions, P. I. 337409 and P. I. 337394 F, were poorly colonized by *A. parasiticus* (NRRL 2999) when seeds with sound intact testae (hand-picked and hand-shelled) were rehydrated, inoculated, and incubated for 7 days at 25 C and evaluated by observation for sporulation of *A. parasiticus*. This screening method is useful for evaluating differences in large numbers of genotypes, but should be supplemented by aflatoxin determinations. The purpose of this study was to compare aflatoxin production of P. I. 337409, P. I. 337394 F, with a readily colonized genotype, P. I. 343360, and a commercial cultivar, Florunner when stored at low and high relative humidities.

MATERIALS AND METHODS

Peanuts (P. I. 337409, P. I. 337394 F, P. I. 343360, and Florunner) grown at Tifton, Georgia, in 1974 and 1975 under normal cultural conditions were mechanically harvested. The genotypes were sorted into two categories: (i) sound mature pods with no visible damage and (ii) immature and damaged pods. One-half of each of these lots was hand-shelled and further sorted into two categories of shelled seed: (i) mature with no visible damage; and (ii) immature and visibly damaged.

Seeds from each genotype were surface-disinfested with 0.05% sodium hypochlorite, incubated on petri plates of M3S1B medium (100 seeds, 10/plate) for 5 days at 30 C, and then examined for conidial heads of *Aspergillus* spp. of the *A. flavus* group to determine the percentage of natural internal infection (5). This method is useful for identification of the *A. flavus* group by color, but does not distinquish species of the group.

The four categories of each genotype tested (inshell, visibly sound; inshell, visibly damaged; shelled, visibly sound; and shelled, visibly damaged) were separated into nine 100-g lots for exposure in controlled chambers to three different humidities (RH). In 1974 the temperatures and RH were: No. 1 - 26±1%, 95±2%; No. 2 - 25±1 C, 87±2%; and No. 3 - 23±1 C, 80±2%. In 1975 the three humidity chambers had the following temperatures and RH: No. 1 - 26±1 C, 94±4%; No. 2 - 26±1 C, 85±6%; and

No. 3 - 26±1 C, 73±2%. Three 100-g lots of each genotype were placed in each humidity chamber for 9 days in 1974 and 10 days in 1975. Temperature and RH were recorded daily and the values given are averages (15). The peanuts then were dried in a forced-air oven at 60 C for 24 hr and stored at 0 C until analyzed. Aflatoxins were determined by use of the Association of Official Analytical Chemists Method I (6) for chambers 2 and 3 in 1974 and 1975. Peanuts from chamber 1 were analyzed for aflatoxin, zearalenone, and ochratoxin by use of Eppley's method (4) in 1974; and for aflatoxin, zearalenone, ochratoxin, penicillic acid, and citrinin, by use of the method of Wilson et al. (16) in 1975.

RESULTS AND DISCUSSION

Storage of peanuts under high humidities favors the growth of the fungus and this increases the potential for aflatoxin production. However, storage in high RH is not uncommon, and extremely high aflatoxin levels sometimes develop. All hand-shelled kernels in the susceptible genotype P. I. 343360 were colonized when inoculated in the laboratory with *A. parasiticus* by Mixon and Rogers' (9) method. Colonization of Florunner was intermediate and P. I. 337409 and P. I. 337394 F were colonized least (9, 10). None of the genotypes was free of aflatoxin contamination after storage for 9 days at high

TABLE 1. Mean and range of total aflatoxins found in peanuts stored under various relative humidities for 9 days in 1974

Peanut line and condition[a]	Humidity chamber[b]		
	1	2	3
P. I. 337409 inshell, sound	ND[c]	2(0-11)[d]	ND
P. I. 337409 inshell, damaged	797(298-1,238)	484(332-1,087)	2(0-4)
P. I. 337409 shelled, sound	365(0-1,326)	353(0-1,465)	ND
P. I. 337409 shelled, damaged	1,368(172-2,502)	68(56-139)	ND
P. I. 337394 F inshell, sound	18(0-50)	26(0-143)	ND
P. I. 337394 F inshell, damaged	657(0-986)	1,283(1,162-1,424)	8(0-20)
P. I. 337394 F shelled, sound	570(0-1,819)	616(0-1,788)	25(0-74)
P. I. 337394 F shelled, damaged	1,546(332-2,981)	1,228(161-2,475)	145(37-254)
P. I. 343360 inshell, sound	303(0-910)	ND	ND
P. I. 343360 inshell, damaged	1,253(20-2,502)	219(0-658)	ND
P. I. 343360 shelled, sound	581(199-808)	9(0-26)	ND
P. I. 343360 shelled, damaged	3,460(2,600-3,600)	1,112(505-1,667)	ND

[a]Nuts of the indicated condition categories were obtained by hand-shelling and hand-sorting.
[b]Chamber 1 - 26±1 C, 95±2% RH; Chamber 2 - 25±1 C, 87±2% RH; Chamber 3 - 23±1 C, 80±2% RH.
[c]ND = no aflatoxins detected.
[d]Total aflatoxins ($B_1 + B_2 + G_1 + G_2$) μg/kg dry wt. Mean of three replicates followed by the range in parentheses.

TABLE 2. Mean and range of total aflatoxins found in noninoculated peanuts stored under various relative humidities for 10 days in 1975

Peanut line and condition[a]	Humidity chamber[a]		
	1	2	3
P. I. 337409 inshell, sound	797(122-2,122)	141(0-420)[b]	ND[c]
P. I. 337409 inshell, damaged	5,900(5,694-6,108)	647(93-1,362)	ND
P. I. 337409 shelled, sound	7,577(749-17,501)	122(34-292)	ND
P. I. 337409 shelled, damaged	12,909(4,965-17,056)	1,080(26-2,910)	ND
P. I. 337394 F inshell, sound	271-(0-812)	4(0-11)	ND
P. I. 337394 F inshell, damaged	4,725(2,878-5,881)	378(34-560)	ND
P. I. 337394 F shelled, sound	3,756(107-5,064)	26(0-78)	ND
P. I. 337394 shelled, damaged	12,073(3,244-17,825)	1,925(72-4,641)	ND
Florunner inshell, sound	52(0-156)	ND	ND
Florunner inshell, damaged	44(0-144)	1(0-3)	ND
Florunner shelled, sound	3,368(182-1,151)	46(6-117)	ND
Florunner shelled, damaged	4,931(1,063-11,762)	4(0-12)	ND

[a]Nuts of the indicated condition categories were obtained by hand-shelling and hand-sorting.
[b]Chamber 1 - 26±1 C, 94±4% RH; Chamber 2 - 26±1 C, 85±6% RH; Chamber 3 - 26±1 C, 73±2% RH.
[c]Total aflatoxins ($B_1 + B_2 + G_1 + G_2$) μg/kg dry weight.
[d]ND = no aflatoxin detected.

RH, even though they were not intentionally inoculated. For each genotype, the background internal infection with *Aspergillus* spp. of the *A. flavus* group before storage averaged 2% of the seeds in 1974 and 3% in 1975; no aflatoxins were found in the prestorage samples. After storage at the high RH, aflatoxins were found in 1974 or 1975 in both the sound and damaged lots of all genotypes (Tables 1 and 2). For example, in those stored in the highest humidity chamber, inshell sound kernels of P. I. 337409 had an average of 0 and 797 μg/kg of aflatoxins and P. I. 337394 F had 18 and 271 μg/kg aflatoxins in 1974 and 1975, respectively. Seed from sound pods stored in the shell had less aflatoxins than did the sound-shelled seeds in all genotypes. The condition of the pods and kernels of the resistant genotypes, influenced the incidence of colonization by species of the *A. flavus* group in the same manner as for the commonly grown susceptible cultivars (2, 11). Low levels of ochratoxin A were found in three samples of damaged, shelled nuts of P. I. 337409 and P. I. 337394 F in chamber 2 in 1974.

Nuts of genotypes not readily penetrated when the seeds are sound are readily penetrated when they are damaged (10, 11). Seed of genotypes that are not readily colonized are of potential advantage to the peanut industry since defective kernels may be more easily sorted out and removed. The working hypothesis has been that damaged and defective seed can be removed and the total aflatoxin contamination lowered if genotypes suitable for commercial production can be developed that are not readily penetrated and colonized under normal production practices. However, we question whether, in practice, such genotypes will result in low levels of aflatoxin contamination under field, post-digging, and pre-storage curing and drying conditions favorable for fungal deterioration. The development of such genotypes may be of more benefit in areas where high levels (above 100 μg/kg) of aflatoxins are encountered in the field. Whether use of these cultivars can reduce aflatoxin contamination can be determined only by the gathering of data for several years from large production areas and from different environmental conditions. Storage conditions that minimize deterioration in stored peanuts can be maintained. However, our results indicate that mechanically-harvested genotypes that are not readily colonized will accumulate high levels of aflatoxin if stored under high RH at temperatures of 23 C to 26 C.

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