Evaluation of Preharvest Aflatoxin Contamination in Several Potentially Resistant Peanut Genotypes[1]

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ABSTRACT

Peanut (*Arachis hypogaea* L.) is susceptible to aflatoxin contamination when pods are developing under drought conditions in the field. The development of cultivars which resist preharvest aflatoxin contamination would be advantageous, but has been limited by the lack of genes for resistance. Several genotypes have been suggested as potential sources of resistance. Conflicting results have been reported on how useful this resistance may be, and some of these sources have never been specifically examined for resistance to preharvest aflatoxin contamination. The objective of this study was to evaluate aflatoxin contamination under drought stressed conditions in potentially resistant peanut genotypes. Twelve peanut genotypes were planted in a randomized complete-block design in field plots in Yuma, AZ in 1991 and 1992. Ten of these genotypes were also planted in a randomized complete-block design in field plots in Tifton, GA in 1992. All plots were inoculated with *Aspergillus* inoculum and were subjected to 40 to 50 d of drought stress immediately prior to harvest. After harvest, aflatoxin contamination (ppb) of seed was measured. None of the genotypes included in this study were more resistant ($P \leq 0.05$) to preharvest aflatoxin contamination than Florunner. The results of this study indicate that it would be desirable to identify higher levels of resistance to preharvest aflatoxin contamination in peanut.

Key Words: Peanut, aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*.

Peanut (*Arachis hypogaea* L.) is susceptible to aflatoxin accumulation when pods are developing under drought conditions in the field. *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare invade the seed and, under suitable conditions, produce aflatoxin. Concerns over toxic and carcinogenic effects of aflatoxin in peanut food products have stimulated much effort to reduce preharvest aflatoxin contamination. The development of cultivars which resist preharvest aflatoxin contamination would be advantageous, but genes for resistance have not been fully documented.

Various methods have been used to screen peanut genotypes for aflatoxin resistance, but the most prevalent has been evaluation of *in vitro* seed colonization by *A. flavus* (IVSCAF) in the laboratory. This procedure was developed by Mixon and Rogers (1973) who identified two peanut plant introductions (PI 337394F and PI 337409) with high levels of resistance to *in vitro* seed colonization by *A. flavus*. Several breeding lines with this type of resistance were developed by Mixon (1986).

Conflicting results have been reported on the correlation of resistance to IVSCAF and seed infection under natural field conditions. Kisyombe *et al.* (1985) tested 14 genotypes for resistance to *A. parasiticus* infection and only J 11 had similar rankings for resistance to dry seed infection and resistance under field conditions. In contrast, Mehan *et al.* (1986) observed a significant reduction in the level of seed infection by *A. flavus* and other soil fungi under field conditions in genotypes reported as resistant to *in vitro* colonization in comparison to genotypes reported as susceptible to *in vitro* colonization. In

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separate studies, Mehan *et al.* (1987) and Mehan *et al.* (1988) did not observe a perfect correlation between resistance to IVSCAF and natural field colonization and warned that it should not be assumed that all genotypes resistant to *in vitro* seed colonization will also be resistant to colonization under field conditions. Nevertheless, 83% (Mehan *et al.*, 1987) and 67% (Mehan *et al.*, 1988) of the lines with resistance to IVSCAF did show resistance to field colonization.

Conflicting results have also been reported on the correlation of resistance to IVSCAF and aflatoxin contamination under field conditions. Blankenship *et al.* (1985) examined four peanut genotypes that were selected as IVSCAF-resistant and found that all contained high levels of aflatoxin when subjected to an extended period of heat and drought stress. In contrast, Mixon (1986), Mehan *et al.* (1986, 1987), and Waliyar *et al.* (1994) observed reduced levels of aflatoxin contamination in IVSCAF-resistant genotypes.

The objective of this study was to evaluate 12 genotypes for preharvest aflatoxin contamination under drought-stressed field conditions. Ten of these genotypes have been reported to have either IVSCAF resistance or resistance to preharvest aflatoxin contamination in the field (Mixon and Rogers, 1973; Mehan *et al.*, 1981; Mixon, 1986; Wilson *et al.*, 1990).

Methods and Materials

Inoculum. Inoculum of *A. flavus* (NRRL 3357) and *A. parasiticus* (NRRL 2999) was prepared and introduced into test plots to insure the presence of sufficient aflatoxin producing fungi in the peanut pod zone. *Aspergillus* inoculum was prepared using the organic-matrix method (Will *et al.*, 1994). Ten-day-old light green conidia of *A. flavus* or *A. parasiticus* were suspended in sterile distilled water (10 mL/114 g of corn) and were used to inoculate sterile moisture-equilibrated cracked corn (25% moisture). The corn was then incubated 3 d at room temperature (25-30 C). Fungi did not sporulate during the 3-d incubation, diminishing workers' exposure to airborne conidia.

Arizona Field Plots. Nine peanut genotypes were selected for evaluation of preharvest aflatoxin contamination based on reported resistance to *Aspergillus* and/or aflatoxin contamination (Table 1). The cultivars Sunbelt Runner, Florunner, and Southern Runner were included as checks. Seed were planted in Yuma, AZ according to time of maturity between 3 June and 24 June 1991 and 11 May and 30 May 1992. Two-row plots (1.5 x 1.8 m) were seeded at four seed per 30 cm linear row. Genotypes in each trial were arranged in a randomized complete-block design (RCBD) with 10 replications. All plants were maintained with adequate moisture using flood irrigation. Each two-row plot was infested with 57 g of *A. flavus* and 57 g of *A. parasiticus* inoculum 22 July 1991 and 10 July 1992. Drought stress was induced by terminating irrigation 23 August 1991 and 10 August 1992. Irrigations after these dates were applied using subsurface perforated tubing when plants showed significant drought stress (leaf flagging). Air and soil temperatures and soil moisture were monitored in the same field in adjacent tests (Holbrook *et al.*, 1994). Pods were harvested 28 October 1991 and 14 October 1992, dried and sent to the Coastal Plain Experiment Station, Tifton, GA for processing.

Table 1. Peanut genotypes included in evaluations for preharvest aflatoxin in Tifton, GA and Yuma, AZ, 1991 and 1992.

Genotype	Maturity[a]	Reference to resistance
	d	
J 11	-30	Mehan *et al.*, 1981
AR-1	-20	Mixon, 1986
AR-2	-20	"
AR-4	-20	"
GFA-1	-7	"
GFA-2	-7	"
PI 337409[b]	-10	Mixon & Rogers, 1973
PI 337394F[b]	-10	"
Tifton 8	0	Wilson *et al.*, 1990
Sunbelt Runner	-15	
Southern Runner	0	
Florunner	-10	

[a]In relation to Southern Runner.

[b]Excluded from Tifton test.

Georgia Field Plots. Ten of the genotypes (excluding PIs 337409 and 337394F) were evaluated under field conditions at the Coastal Plain Experiment Station. Genotypes were planted according to time of maturity between 23 June and 15 July 1992. Seed were planted in single-row plots, 1.5 m long at the rate of four seed/30 cm linear row in a RCBD with 10 replications. Plants were maintained with standard cultural practices and irrigation. Single-row plots were inoculated with 28 g of corn infested with *A. flavus* and 28 g of corn infested with *A. parasiticus* on 25 August. Drought and heat stress was induced by covering the entire test with a mobile greenhouse (Atlas Greenhouse Systems, Inc., Route 1, Box 339, Alapaha, GA) on 23 September 1992. Pods were dug 9 November, hand-picked from the plant, and then dried.

Aflatoxin Determination. Seed from undamaged pods were stored at -20 C until processing to prevent post-harvest accumulation of aflatoxin. Peanuts were shelled using a Peerless peanut sheller and ground in a household food processor for about 1 min. Aflatoxin contamination was measured on a 100-g subsample with the immunoaffinity column fluorometer method (Trucksess *et al.*, 1991). The fluorometer was calibrated from 0 to 400 ppb. If the initial sample analysis indicated contamination above 400 ppb, then a 1:10 dilution of the extract was made and the sample was reanalyzed. If the reanalyzed sample indicated contamination above 4000 ppb, then an additional 1:10 dilution and analysis were performed. Concentrations exceeding 50,000 ppb were truncated at that amount. Aflatoxin concentrations were analyzed by the GLM procedure of SAS (1985).

Results and Discussion

Highly significant environmental, genotype and genotype-by-environment (G x E) interaction effects on the production of aflatoxin (Table 2) were evident from analysis of aflatoxin concentrations. The mean concentrations of aflatoxin for samples from Arizona in 1992 (11,418 ppb) was much higher than for Arizona in 1991

Table 2. Combined analysis (GLM) for aflatoxin contamination over three environments (Arizona 1991 and 1992, Georgia 1992).

Source	DF	Mean square ($x10^8$)	F value
Environment (E)	2	26.6	19.3**
Error 1	18	1.4	
Genotype (G)	11	3.4	2.9**
G x E	20	2.8	2.4**
Error	236	1.1	

**Significant at the 0.01 probability level.

(2070 ppb) and Georgia in 1992 (1139 ppb). The environmental differences may have been affected in part by the duration of drought stress placed on the peanuts. Stress was induced earlier and was more extreme in Arizona during 1992, while the stress period was shorter and less extreme in Arizona during 1991 and in Georgia (under shelters) in 1992. Soil temperature and moisture regimes varied with the different environmental conditions, and these variables have been reported to affect the invasion of seed by *A. flavus* and subsequent aflatoxin contamination (Blankenship *et al.*, 1984).

Mixon and Rogers (1973) developed the laboratory inoculation method for screening peanut genotypes for resistance to *A. flavus* invasion and colonization of rehydrated, mature, sound stored seed. Using this technique, they identified two accessions, PI 337394F and PI 337409, that showed high levels of resistance to IVSCAF. Both genotypes were found susceptible to preharvest aflatoxin contamination in the present study (Table 3). This is the first reported evaluation of aflatoxin contamination in PI 337409. Other researchers (Zambettakis *et al.*, 1981; Mehan *et al.*, 1987; Waliyar *et al.*, 1994) have observed a reduced level of preharvest aflatoxin contamination in PI 337394F.

Table 3. Concentration of aflatoxin in peanut genotypes grown in three drought-stressed environments.

	Aflatoxin concentration						
	Arizona				Georgia		Overall
Genotype	1991		1992		1992		mean
	ppb						
J 11	7,504	(1)[a]	34,833	(1)	9	(10)	15,737
Tifton 8	689	(10)	21,728	(2)	6,260	(1)	9,353
PI 337394F	1,468	(7)	12,157	(4)	.		8,594
PI 337409	1,522	(6)	10,055	(6)	.		6,073
AR-4	3,334	(3)	11,416	(5)	2,005	(3)	6,035
Southern Run.	1,265	(8)	12,892	(3)	900	(5)	5,006
Sunbelt Run.	2,081	(4)	8,396	(7)	1,132	(4)	3,714
AR-1	5,349	(2)	2,086	(12)	214	(8)	2,381
GFA-2	465	(12)	6,137	(8)	130	(9)	2,305
Florunner	1,685	(5)	4,633	(10)	898	(6)	2,267
AR-2	1,206	(9)	4,831	(9)	3,437	(2)	2,129
GFA-1	474	(11)	2,991	(11)	320	(7)	1,262
$LSD_{(0.05)}$	3,751		22,811		5,304		
MEAN	2,070		11,418		1,139		
CV	160		146		501		

[a]Numbers in parentheses indicate ranking. Data are means of 10 replications.

Mixon (1983a,b) also developed six breeding lines (AR-1, AR-2, AR-3, AR-4, GFA-1, and GFA-2) which exhibited significant resistance to *in vitro* seed colonization over 4 yr of testing (Mixon, 1986). Mixon (1986) observed numerically lower levels of aflatoxin contamination in field-grown plots of these breeding lines in comparison to the check cultivars Florunner and Sunbelt Runner. However, the field plots had not been subjected to extensive drought stress and the levels of contamination were relatively low. Blankenship *et al.* (1985) examined the aflatoxin contamination of two of these breeding lines (AR-3 = A7404 and GFA-1 = A72118) under severe drought stress and found both to be susceptible. This is the first reported evaluation of aflatoxin contamination under drought-stressed conditions for the other four breeding lines (AR-1, AR-2, AR-4, and GFA-2). All were observed to be susceptible to preharvest aflatoxin contamination (Table 3). GFA-1 was also included in the present study and was not found to have a significant level of resistance, supporting the results of Blankenship *et al.* (1985). Although not significantly different, GFA-1 had the lowest mean concentration of aflatoxin and was consistently lower than for Florunner in each environment. No similar trend was evident in the tables presented by Blankenship *et al.* (1985).

The Indian cultivar J 11 is the most extensively evaluated peanut genotype with regards to *Aspergillus* colonization and aflatoxin contamination under field conditions. Kisyombe *et al.* (1985) observed a reduction in colonization in J 11, but they did not measure aflatoxin contamination. Mehan *et al.* (1987, 1988) and Waliyar (1994) observed a reduction in colonization and aflatoxin contamination in J 11 in comparison to susceptible checks. Although J 11 exhibited a low level of aflatoxin contamination in the 1992 test at Tifton, it was very susceptible to contamination during both years of testing at Yuma (Table 3).

Tifton 8 and Southern Runner have been reported to have resistance to preharvest aflatoxin contamination (Wilson *et al.*, 1990; Cole *et al.*, 1993) under drought stress conditions in Georgia. In the present study, neither genotype exhibited resistance to preharvest aflatoxin contamination in testing in Georgia or Arizona. These results support the observation of Wilson *et al.* (1991) that Southern Runner did not have less contamination than Florunnner under drought stressed conditions. However, in another study (Holbrook, unpubl. data, 1993) we have observed a reduction in contamination of Southern Runner in comparison to Florunner. We also have observed conflicting results in other studies (Holbrook, unpubl. data, 1993) involving Tifton 8.

None of the genotypes included in this study exhibited a significant level of resistance to preharvest aflatoxin contamination. These results are in conflict with several published reports of resistance in peanut (Mehan *et al.*, 1986; Mixon, 1986; Wilson *et al.*, 1990; Cole *et al.*, 1993; Waliyar *et al.*, 1994). There also are several conflicts among the various reports dealing with resistance to

aflatoxin contamination in peanut (Blankenship *et al.*, 1985; Kisyombe *et al.*, 1985; Wilson *et al.*, 1991). One possible explanation for these differences is that the reported resistance in these sources is an artifact from the extreme variability inherent in data on aflatoxin contamination of peanut. We believe that a more likely explanation is that real sources of resistance have been reported in the literature. However, the level of resistance is low and can be overwhelmed under certain environmental conditions.

The results of this study indicate that it would be desirable to identify higher levels of resistance to preharvest aflatoxin contamination in peanut germplasm. These results also suggest the need for multiple replications in multiple environments when studying resistance to preharvest aflatoxin contamination.

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