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Preharvest Aflatoxin Contamination in Drought-Tolerant and Drought-Intolerant Peanut Genotypes[1]

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ABSTRACT

Peanuts become contaminated with aflatoxins when subjected to prolonged periods of heat and drought stress. The effect of drought tolerance on aflatoxin contamination is not known. The objectives of this research were to evaluate preharvest aflatoxin contamination in peanut genotypes known to have drought tolerance and to determine the correlation of drought tolerance characteristics with aflatoxin contamination. Twenty genotypes with different levels of drought tolerance were grown in Yuma, AZ (a desert environment) and under rain-protected shelters in Tifton, GA. Two drought-tolerant genotypes (PI 145681 and Tifton 8) and an intolerant genotype (PI 196754) were selected for further examination in a second experiment with two planting dates in 1997 at Tifton. Drought and heat stress conditions were imposed for the 40 d preceding harvest. The drought-intolerant genotype had greater preharvest aflatoxin contamination than Florunner (the check cultivar) in the tests conducted in 1997. Both drought-tolerant genotypes had less preharvest aflatoxin contamination than Florunner in these tests. Significant positive correlations were observed between aflatoxin contamination and leaf temperature and between aflatoxin contamination and visual stress ratings. Leaf temperature and visual stress ratings are less variable and less expensive to measure than aflatoxin contamination. Leaf temperature and visual stress ratings may be useful in indirectly selecting for reduced aflatoxin contamination in breeding populations.

Key Words: *Arachis hypogaea*, *Aspergillus flavus*, *Aspergillus parasiticus*, plant stress.

Preharvest aflatoxin contamination (PAC) of peanut (*Arachis hypogaea* L.) is closely associated with severe late-season drought stress (McDonald and Harkness, 1967; Pettit *et al.*, 1971; Dickens *et al.*, 1973; Wilson and Stansell, 1983). Less aflatoxin contamination may be associated with drought tolerance; however, the correlation between drought tolerance and aflatoxin contamination has not been clearly documented. Kisyombe *et al.* (1985) examined the colonization of kernels by *Aspergillus parasiticus* Speare in drought-stressed and nondrought-stressed plots. They examined 14 genotypes including three which had been reported to have some drought tolerance. Although the drought-tolerant lines were susceptible to *A. parasiticus*, infection of two of these genotypes was not enhanced by drought stress. Mehan *et al.* (1987) and Mehan (1989) also observed that several drought-tolerant genotypes were susceptible to colonization and subsequent contamination by aflatoxin. However, Mehan (1989) observed relatively low levels of seed infection in one drought-tolerant genotype and concluded that more research is needed to determine if drought tolerance can reduce stress on pod and seed to a level which would reduce aflatoxin contamination.

Using 16 genotypes which had been observed as having relatively large root systems—plus the cultivars Florunner, and Southern Runner, and the germplasm line Tifton 8 as checks—Rucker *et al.* (1995) conducted several studies to evaluate these genotypes for drought avoidance characteristics. They evaluated root characteristics of these genotypes in a pot study. Under drought-stressed-field conditions, they evaluated these genotypes using canopy temperature measurements and vi-

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sual stress ratings, two potential measures of drought tolerance. Differences were observed among these characteristics for this set of germplasm. The objectives of this study were to evaluate this set of germplasm for resistance to PAC and to estimate the correlations of PAC with canopy temperature measurements and with visual stress ratings.

Materials and Methods

Experiment I

The first experiment was conducted at one location (Tifton, GA) in 1992 and two locations (Tifton, GA and Yuma, AZ) in 1993 using the 19 genotypes examined by Rucker *et al.* (1995) and PI 298836. The 20 genotypes were planted in Tifton, GA on 13 May 1992 and 21 June 1993 in a randomized complete block design with five replications. Seeds were planted in single-row plots, 1.5 m long at four seeds/30 cm linear row. The same 20 genotypes also were planted in Yuma, AZ on 24 May 1993 in a randomized complete block design with five replications. Seeds were planted in two-row plots (1.5 × 1.8 m) at four seeds/30 cm linear row.

Inoculum of *A. flavus* Link ex Fries (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-d-old light green conidia of *A. flavus* or *A. parasiticus* were suspended in sterile distilled water (10 mL/114 g of corn) and used to inoculate sterile moisture-equilibrated cracked corn (25% moisture). The corn was incubated 3 d at room temperature (25-30 C) and then frozen. Plots were inoculated approximately 60 d after planting (DAP). Each plot received 28 g of corn infested with *A. flavus* and 28 g of corn infested with *A. parasiticus* per 1.5-m linear row.

Drought stress was induced in Tifton by covering the entire test plots with a mobile greenhouse (Atlas Greenhouse Systems, Alapaha, GA) on 13 Aug. 1992 (97 DAP) and 20 Sept. 1993 (91 DAP). Flood irrigation was used in Yuma until 20 Aug. (88 DAP) when drought stress was imposed in the pod zone using the subsurface irrigation system described by Holbrook *et al.* (1994). Peanut plants were dug and picked on the same day. Pods were hand picked in Tifton on 24 Sept. 1992 (134 DAP) and 15 Nov. 1993 (147 DAP) and in Yuma on 30 Sept. 1993 (129 DAP). Harvested pods were dried to 7% moisture and hand sorted to remove and discard visibly damaged pods.

Experiment II

Two drought-tolerant genotypes, PI 145681 and Tifton 8, and an intolerant genotype, PI 196754, were selected for further examination based on observations in Experiment I. This test also included the check genotype Florunner and an accession (PI 158839) from the peanut core collection (Holbrook *et al.*, 1993) that had exhibited low levels of preharvest aflatoxin concentration in other studies (C. C. Holbrook, unpubl. data, 1996). These five genotypes were examined using two planting dates to simulate two environments in Tifton in 1997. Seeds were planted (four seeds/30-cm linear row) in single-row plots, 1.5 m long in a randomized complete block design with 20 replications.

The two planting dates for this experiment were 2 and 24 April. The tests were inoculated on 18 June and 1 July, respectively, using the previously described inoculation procedure. Drought and heat stress was imposed by covering the test plots with a mobile greenhouse on 25 June and 30 July for planting dates one and two, respectively. Peanut plants were dug and pods were hand picked on 9 Aug. and 16 Sept. for planting dates one and two, respectively. Harvested pods were dried to 7% moisture and hand-sorted to remove and discard visibly damaged pods.

Aflatoxin Analysis. Peanuts were shelled using a Penco peanut sheller (Peerless Engineering Company, Chula, GA) and ground in a household food processor for about 1 min. Aflatoxin concentration 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 ng/g. If the initial sample analysis indicated contamination above 400 ng/g, then a 1:10 dilution of the extract was made and the sample was reanalyzed. If the reanalyzed sample indicated contamination above 4000 ng/g, then an additional 1:10 dilution and analysis were performed. The maximum aflatoxin contamination recorded was 40,000 ng/g.

Statistical Analysis. Aflatoxin data were analyzed using PROC GLM from SAS (SAS, 1990). Differences between means were determined using the Duncan-Waller multiple range test. The Tifton field test for Experiment I in 1992 is the same test that Rucker *et al.* (1995) used to take biweekly measurements of canopy temperature and visual stress ratings. These data were used to calculate correlations between final aflatoxin contamination and the biweekly measurements of visual stress ratings on a genotype mean basis.

Results and Discussion

Experiment I

There was a significant genotype × environment interaction when data from all three environments were analyzed. Separating the Arizona environment from the two Georgia environments eliminated this interaction.

Aflatoxin contamination is extremely variable in peanut. The mean aflatoxin contamination for the check cv. Florunner in the two Georgia environments was 1167 ppb (Table 1). Because of the extreme variability, it was not possible to have a contamination level which was significantly lower than Florunner. However, seven of the genotypes exhibited at least a 92% reduction in mean aflatoxin contamination in comparison to Florunner. Six of these were the same genotypes identified by Rucker *et al.* (1995) as having improved drought avoidance traits.

Results from Arizona (Table 2) differed from the Georgia trials. Environmental conditions are hot and extremely dry in Yuma. These conditions require the use of subsurface irrigation to prevent rapid plant death and to insure aflatoxin contamination in susceptible genotypes (Holbrook *et al.*, 1994). The use of subsurface irrigation may have masked any advantage that a larger root system might provide in maintaining adequate moisture in developing pods.

Rucker *et al.* (1995) took biweekly measurements of canopy temperature and visual stress ratings in the Tifton field test for Experiment I in 1992. When these data were compared to the aflatoxin results from that environment, there were significant positive correlations be-

Table 1. Aflatoxin contamination in 20 peanut genoytpes grown under drought-stressed conditions in 1992 and 1993 at Tifton, GA[a].

Entry	Aflatoxin contamination			Visual stress rating[b]
	1992	1993	Mean	
	----------ng/g----------			
PI 315622	24206 a	70 ab	12138 a	2.7 bcd
PI 196754	14604 ab	37 b	7320 ab	3.9 a
PI 319736	12209 ab	12 b	6788 ab	3.0 b
PI 268885	11884 ab	10 b	5947 ab	2.6 bcd
PI 318740	11790 ab	32 b	5911 ab	2.8 bc
PI 315634	10760 ab	26 b	5393 ab	3.0 b
PI 161869	10188 ab	19 b	5103 ab	3.0 b
PI 314893	9473 ab	29 b	4751 ab	2.5 bcd
PI 315631	5653 b	14 b	2833 b	3.6 a
PI 196744	2838 b	7 b	1580 b	3.8 a
Florunner	2309 b	25 b	1167 b	2.8 bc
PI 315626	190 b	165 a	177 b	3.0 b
PI 145681	290 b	18 b	154 b	2.4 cd
Tifton 8	149 b	19 b	84 b	2.2 d
PI 298836	14 b	112 ab	63 b	-
PI 259639	24 b	77 ab	50 b	2.6 bcd
PI 295722	36 b	12 b	24 b	2.5 bcd
PI 269106	13 b	25 b	19 b	2.5 bcd
PI 315628	7 b	27 b	17 b	2.3 cd
South. Runner	8 b	15 b	12 b	2.3 cd

[a]Means followed by the same letter are not significantly different (P = 0.05) according to Duncan-Waller multiple range test.

[b]Mean drought stress ratings from three environments reported by Rucker *et al.* (1995). Ratings are visual ratings on a 1-5 scale where 1 = no stress and 5 = most stressed.

tween aflatoxin contamination and leaf temperature for all measurement dates (Table 3). There also were significant positive correlations between aflatoxin contamination and visual stress ratings for the measurements made on 30 Aug. and 10, 15, and 21 Sept.

Aflatoxin contamination is expensive to measure. The use of leaf temperature data and/or visual stress ratings for preliminary screening of germplasm for resistance to PAC would greatly reduce the expense of developing resistant cultivars. PAC is extremely variable also. The CV for PAC in the 1992 Tifton test was 232%. Leaf temperature data and visual stress ratings were much less variable with CVs of 7 and 36%, respectively, in the 1992 Tifton test.

Experiment II

PI 196754 had the greatest amount of preharvest aflatoxin contamination in the two tests conducted in 1997 (Table 4). This genotype is highly sensitive to dry soil conditions (Rucker *et al.*, 1995). In Experiment I, this genotype exhibited an aflatoxin contamination 6 × that of Florunner, although the difference was not significant.

PI 145681 had less preharvest aflatoxin contamination than Florunner in Experiment II (Table 4). Rucker

Table 2. Aflatoxin contamination of 20 peanut genotypes grown under drought-stressed conditions in 1993 at Yuma, AZ[a].

Entry	Aflatoxin contamination
	ng/g
PI 298836	36,240 a
PI 259639	32,930 ab
PI 314893	29,010 abc
PI 315626	25,660 abc
PI 315628	25,392 abc
PI 315631	20,663 abc
PI 196754	16,001 abc
PI 319736	13,453 abc
PI 315622	12,659 bc
PI 315634	12,439 abc
Southern Runner	11,768 abc
PI 268885	10,268 abc
PI 318740	9264 abc
PI 295722	8784 abc
PI 196744	5508 abc
Tifton 8	4264 bc
PI 269106	1446 bc
PI 145681	1368 bc
Florunner	1233 bc
PI 161869	727 c

[a]Means followed by the same letter are not significantly different (P = 0.05) according to Duncan-Waller multiple range test.

Table 3. Correlation coefficients of aflatoxin contamination with leaf temperature and visual drought stress ratings from plots in Tifton, GA in 1992.

	Measurement date								
	Aug.				Sept.				
	20	24	28	30	4	8	10	15	21
Aflatoxin & leaf temp.	.19*	.25**	.26**	.21*	.21*	.22*	.25**	.23*	.22*
Aflatoxin & visual rating	-.01	.04	.04	.35**	.09	.16	.21*	.19*	.21*

*,** = significant at P = 0.05 and 0.01, respectively.

et al. (1995) reported that this genotype had relatively low visual stress ratings under dry soil conditions. They observed numerically lower visual stress ratings for this accession in comparison to Florunner in three environments with dry soil conditions. In one of these environments the difference was statistically significant. In Experiment I tests in Tifton, this genotype exhibited a greater than 90% reduction in aflatoxin contamination in comparison to Florunner, although the difference was not significant.

The accession PI 158839 from the peanut core collection did not exhibit a difference in aflatoxin contamina-

Table 4. Aflatoxin contamination in a drought-tolerant, a drought-intolerant, and three check genoytpes grown in two tests at Tifton, GA in 1997[a].

Entry	Aflatoxin contamination	Visual stress rating[b]
	ng/g	
PI 196754	18,693 a	3.9 a
Florunner	10,872 b	2.8 bc
PI 158839	8370 bc	-
PI 145681	4370 c	2.4 cd
Tifton 8	3771 c	2.2 d

[a]Means followed by the same letter are not different (P = 0.05) according to Duncan-Waller multiple range test.

[b]Mean drought stress ratings from three environments reported by Rucker *et al.* (1995). Ratings are visual ratings on a 1-5 scale where 1 = no stress and 5 = most stressed.

tion in comparison to Florunner in Experiment II (Table 4). This genotype was not included in Experiment I or in the studies by Rucker *et al.* (1995); therefore, the drought sensitivity of this accession is not known.

The germplasm line Tifton 8 exhibited a significant reduction in aflatoxin contamination in comparison to Florunner in the two tests conducted in 1997 (Table 4). Wilson *et al.* (1990) reported that this line was resistant to PAC; however, in other studies it was as susceptible as Florunner (Anderson *et al.*,1995; Holbrook *et al.*, 2000). Tifton 8 had relatively low visual stress ratings under dry soil conditions (Rucker *et al.*, 1995). In Experiment I, this genotype exhibited a greater than 90% reduction in aflatoxin contamination in comparison to Florunner, although the difference was not significant.

The differences in susceptibility to aflatoxin were not stable over all environments. However, improvements in aflatoxin susceptibility over existing cultivars could save the peanut industry millions of dollars in losses due to this toxin. This study documents genetic differences in susceptibility to preharvest aflatoxin contamination in peanut. These differences may be related to differences in drought tolerance.

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