Reaction of Peanut Genotypes under Drought Stress to *Aspergillus flavus* and *A. parasiticus*[1]

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ABSTRACT

Seven peanut genotypes (*Arachis hypogaea* L.) were tested in greenhouse and microplot experiments during two consecutive years to determine peanut peg colonization by *Aspergillus flavus* and to determine the effect of two drought stress treatments on the susceptibility of peanut shells and kernels to *Aspergillus* colonization and aflatoxin contamination. Moisture tensions within the soil and temperatures of the soil and air were monitored during these experiments. Colonization of peanut pegs was inconsistent among genotypes and between years. In general, low soil moisture tension enhanced colonization of peanut shells and kernels. Shells of most genotypes were highly colonized after harvest from each moisture regime. Kernels of all genotypes were more susceptible to *A. flavus* and *A. parasiticus* colonization under both long and short drought stress conditions compared to non-stressed conditions. However, no significant differences were obtained in the degree of Aspergillus infestation of kernels from PI 337409, Starr, and J-11 in the greenhouse experiments. Under microplot conditions, a comparison of several genotypes revealed that kernels from plants of genotypes TX811956 and TX798736 (short stress treatments) contained significantly lower Aspergillus infestation and kernels from the genotypes PI 337409 and TX811956 and TX798736 contained significantly less aflatoxin.

Key Words: Groundnut, mycotoxin, aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, and drought stress.

Colonization of peanut (*Arachis hypogaea* L.) shells and kernels by *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare and subsequent aflatoxin contamination, is a serious problem in subtropical and tropical regions. Aspergillus colonization can occur during flowering or during aerial peg formation (5) from viable air-borne propagules originating from peanut soils (4). In Texas, previous observations under field conditions have indicated that aerial pegs may become colonized and colonization increases to an average of 7% following soil penetration (Unpublished data). Colonization of shells and kernels may also occur before digging. Colonization is generally more extensive when plants are subjected to late season drought stress. Peanuts grown under drought stress frequently produce lower yields and higher percentages of aflatoxin contaminated kernels (3,6,13,16).

Several investigators have studied the threshold temperature range for the occurrence of aflatoxin contamination in drought-stressed peanuts (1,3,5,11,12). Blankenship *et al.* (1) imposed 5 soil temperature and moisture treatments on Florunner peanuts and found that in drought-stressed peanuts, *A. flavus* colonized fewer unshelled sound mature kernels as the mean temperature 5 cm deep decreased. The mean geocarposphere soil temperature required for aflatoxin development during the latter part of the peanut growth cycles ranged from 25.7 to 27.0 C. Hill *et al.* (5), and Sanders et al. (11) have demonstrated a relationship between drought conditions and concurrent elevated soil temperatures and also the necessity of a high threshold soil temperature for significant pre-harvest invasion of peanut by *A. flavus*. In general, moderate to high temperatures (25-38 C) favor Aspergillus growth, invasion of peanuts, and aflatoxin contamination (7). The severity of water and soil temperature stress and the length of the stress period together influence the extent of pre-harvest aflatoxin contamination. Davidson and coworkers (2) harvested sound mature kernels grown under low, moderate, and severe drought stress from three peanut fields in Georgia and recovered *A. flavus* from 34%, 55%, and 67% of the kernels, respectively. Aflatoxin contamination of comparable freshly-dug kernels from these fields averaged 5, 97, and 444 ppb aflatoxin, respectively. Additional evidence indicates that regardless of the severity of temperature and moisture stress, time periods longer than 20 days are required for pre-harvest aflatoxin contamination (14). Wilson and Stansell (16) found in 2 of 4 years that significantly more aflatoxin accumulated in peanut when stress was imposed at least 40 days preceding harvest.

Several researchers have reported that some peanut cultivars have resistance to *A. flavus* invasion and toxin contamination. Mehan and associates (9,10) reported that kernels from a commercial Spanish peanut cultivar (J-11) were resistant to invasion and colonizaiton by *A. flavus* in India. In North Carolina (6) fourteen peanut genotypes were evaluated during two years for resistance to *A. parasiticus* colonization. They were grown in rain-protected field microplots with imposed water stress and in unprotected rainfed microplots. Genotypes J-11 and Lampang were more resistant to *A. parasiticus* in both dry (1983) and moist (1984) years. Dry kernel resistance of 34 peanut genotypes to *A. parasiticus* colonization, including those tested in field microplots, was evaluated in the moist chambers. These results indicated that genotypes J-11 and PI 337409 had dry seed resistance.

The purpose of this study was to determine if aerial peanut pegs from different peanut genotypes differed in percentage of infection by *A. flavus* and *A. parasiticus*, and to determine if drought-stress conditions would be helpful in screening shells and kernels for resistance against *Aspergillus* invasion and subsequent aflatoxin contamination.

Materials and Methods

Greenhouse Experiments

A greenhouse experiment was established in sandy loan soil with good drainage in 23-cm-diameter plastic pots. An aqueous suspension (containing 2 drops Tween 20) of *A. flavus* (peanut isolate T7) and *A. parasiticus* (isolate NRRL 2999) conidia was mixed into the top 10 cm of soil in each pot to obtain a concentration of 5,000 - 8,000 viable propagules per g dry soil. Conidia for soil infestation were removed from cultures grown on ground peanut meal for 14 days at 35 C. The experiment was comprised of 15 replicates (pots with 3 plants per pot) of three genotypes (PI 337409, Starr, and J-11) and three moisture regimes arranged in a randomized block. The genotype PI 337409 is a Valencia germplasm line, and Starr and J-11 are Spanish cultivars from Texas and India respectively. Granular Bradyrhizobium inoculum was added to the seeds at planting time.

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Seedlings were thinned to one plant per pot 15 days after planting and 15 g of NPK fertilizer (10-20-20) was mixed into the potting mixture. All pots were harvested 120-130 days after planting.

Three water regimes were imposed as treatments within these experiments. The three water regimes imposed as treatments included: long stress (LS), during which watering was withheld 100 days after planting until harvest; short stress (SS), during which watering was withheld 100 days after planting for 15 days (or until severe wilting occurred) followed by normal irrigations as needed until harvest; and no stress (NS), during which sufficient irrigations were applied to maintain optimum plant growth. Soil temperature and moisture tension were measured at a depth of 5 cm using Campbell 107 temperature probes (Campbell Scientific 21X Micrologger, Campbell Scientific, Logan, UT. 84321) and Campbell 5201 soil moisture blocks. Air temperature and relative humidity were monitored with Campbell 207 probes. Each of these environmental parameters was recorded every hour with a Campbell Scientific micrologger throughout the experiments.

Microplot Experiments

Seven peanut cultivars and advanced breeding lines J-11, SN 55-437, Starr, PI 337409, TxAG-3, and two selections, TX798736 and TX811956 (from a cross of Toalson x UF 73-4022)] were grown in microplots (90 cm X 75 cm X 60-cm-deep open-bottom wooden boxes). Two rows of 10-15 plants each were grown in each microplot. Conidia produced by *A. flavus* (T7) and *A. parasiticus* (NRRL 2999) were incorporated into the top 10 cm of sandy-loam soil at a rate required to obtain 5,000-8,000 viable propagules per g dry soil. Three watering (with deionized water) regimes were imposed on the microplots 100 days after planting: the LS, SS, and NS treatments previously used in the greenhouse experiments. Each treatment was replicated 4-5 times in a randomized design.

Processing Plant Parts

Peanut pegs, shells, and kernels from pot and microplot experiments were processed to determine the extent of *A. flavus* and *A. parasiticus* invasion. Kernels were also analyzed for aflatoxin content. Twenty-five aerial pegs (5-10 days old) were collected at random from each replicate plot 70-75 days after planting. Within 8 hours after collection, pegs were washed for 2 min in running tap water and then surface-disinfested by submersion for 1 min in an aqueous solution of 1% NaOCl followed by 3 rinses in sterile distilled water. Whole pegs were plated on modified Griffins medium (4) (peptone, 5 g; glucose, 10 g; KH_2PO_4 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 75 g; agar, 20 g; rose bengal 33 mg/L distilled water; after sterilization, 50 mg of streptomycin sulfate and 50 mg of chlorotetracycline) in 9-cm-diameter petri dishes. The pegs were incubated at 35 C and the percentage of pegs colonized by Aspergillus was recorded after 3-4 days.

Mature pods were removed by hand from 110-130 day old plants immediately after digging, placed in paper bags, dried with forced air (7-10 days) to prevent further *Aspergillus* activity, and hand-shelled. Half-shells from 25 random pods per replication were surface disinfested for 2 min in 1.5% NaOCl and plated on Griffins medium. Thirty-five sound mature kernels were disinfested (successive 1-min submersions in 70% ethyl alcohol, aqueous 5.25% active NaOCl and sterile distilled water) and plated on Griffins medium (5 kernels per plate).

Replicate kernel samples from each pot and microplot were also collected at digging (three 15 g samples per treatment) and immediately analyzed for aflatoxin contamination using high pressure liquid chromatography (HPLC) (11,15). Kernels were homogenized in a Waring blender, the lipids removed with hexane, and the aflatoxin extracted with chloroform. The aflatoxin residue was dissolved in Pons solution and injected into the HPLC (silica gel column) for analysis of aflatoxins B_1, B_2, G_1, and G_2. The flow rate was adjusted to 2.0 ml/min and the wavelength set at 365 nm.

Results and Discussion

The recovery of *A. flavus* and *A. parasiticus* from aerial peanut pegs 75 days after planting from plants grown in the greenhouse (Table 1) and microplots (Table 2) revealed that there were significant differences in peg colonization between cultivars. Under greenhouse and microplot conditions pegs of genotype J-11 contained significantly higher levels (38-81%) of these Aspergilli compared to the pegs of genotype PI 337409 with 12-51% (Table 1). Recovery of *A. flavus* and *A. parasiticus* from pegs harvested in the covered microplots was relatively low (less than 8%) in 1985, possibly because conditions were less than optimum for *Aspergillus* activity. The following year *Aspergillus* was more active and peg isolation frequency was greater than 47% (Table 2). The amount of peg colonization in 1986 was significantly lower in pegs from cultivars Starr, PI 337409, and TX811956 (selection from a Toalson X UF 73-4022 cross) compared to cultivars J-11, TxAG-3, and SN 55-437. Other researchers have reported that peg colonization levels differ from year to year. Kisyombe, *et al.* (6) reported that the mean isolation frequency of *A. parasiticus* from pegs was 67% in 1983 and 36% in 1984. They did not detect significant differences between genotypes in terms of peg colonization rates. These experiments verified previous observations in Texas that peanut pegs are readily invaded by these Aspergillus spp. These experiments were unable to establish a relationship between peg, pod, and kernel colonization levels.

Table 1. Mean percentage of peanut pegs colonized by *Aspergillus flavus* and *A. parasiticus* from greenhouse experiments.

	Isolation frequency (%)	
Genotype	1986	1987
J-11	38 a*	81 a
Starr	12 b	74 a
PI 337409	12 b	51 b

* Values followed by a different letter are significantly different at the p = 0.05 level (Duncan-Waller test).

Table 2. Mean percentage of peanut pegs colonized with *Aspergillus flavus* or *A. parasiticus* from covered microplots

	Isolation frequency (%)	
Peanut Genotype	1985	1986
J-11 (ICRISAT)	--*	78 a
TxAG-3	7.0 a**	--
SN 55-437	4.0 b***	75 a
TX798736	0.0 c	74 a
Starr	0.0 c	51 b
PI 337409	0.0 c	48 b
TX811956	--	59 b

* Genotype not tested

** Values followed by a different letter are significantly different at the p = 0.05 level (Duncan-Waller test).

*** Mean of 3 replications

The effects of soil moisture regimes on the recovery of A. flavus and A. parasiticus from shells and kernels of greenhouse-grown plants in 1986 and 1987 are summarized in Table 3. Shells and kernels from plants subjected to prolonged water stress (LS) were the most highly colonized, with mean levels of 98% and 68%, respectively, in 1986 and 80% and 54% respectively, in 1987. In contrast, the shells and kernels from plants subjected to short moisture stress (SS) had mean colonization levels of 33% and 15%, respectively, in 1986 and 65% and 29%, respectively, in 1987. The lowest mean level of kernel colonization was detected in kernels from the non-stress treatments, with mean colonization levels of 3% in 1986 and 19% in 1987.

Table 3. Effect of moisture regimes on shell and kernel colonization with Aspergillus flavus and A. parasiticus from plants grown in the greenhouse.

Treatment*	Genotypes	Isolation frequency (%)**			
		1986		1987	
		Shells	Kernels	Shells	Kernels
LS	Starr	100a***	40 b	75a	55ab
LS	PI 337409	99a	69ab	80a	51ab
LS	J-11	95a	95a	85a	55ab
	Mean	98	68	80	54
SS	Starr	36 b	17 c	60 b	20 bc
SS	PI 337409	25 bc	14 c	83a	22 bc
SS	J-11	37 b	13 c	53 b	45ab
	Mean	33	15	65	29
NS	Starr	50 b	7 c	67ab	17 c
NS	PI 337409	69a	2 c	75a	20 bc
NS	J-11	26 bc	1 c	59 b	19 bc
	Mean	48	3	67	19

* Moisture regimes: LS, Drought stress initiated 100 days after planting until harvest; SS, Fifteen days of drought stress initiated 100 days after planting followed by optimum irrigations; NS, No drought stress (optimum irrigations).

** Mean of five replications.

*** Values followed by a different letter are significantly different at the p = 0.05 level (Duncan-Waller test).

Table 4. Mean geocarposphere temperatures and moisture tensions of soil in drought stress treatments in greenhouse experiment.

Treatment*	Temperature (C)**			Moisture tension bars**		
	Max.	Min.	Avg.	Max.	Min.	Avg.
LS	29.0	25.7	27.5	-15	- 7	-9.0
SS	27.1	25.0	26.3	-10	- 3	-6.5
NS	27.2	24.9	25.1	- 3	- 2	-2.5

* Moisture regimes: LS, Drought stress initiated 100 days after planting until harvest; SS, Fifteen days of drought stress initiated 100 days after planting followed by optimum irrigations; NS, No drought stress (optimum irrigations).

** Summary of data collected at one hour intervals during the least 25 days of the experiment.

The temperature of the geocarposphere has also been reported to influence Aspergillus activity within the soil. In these experiments, geocarposphere conditions (temperature and moisture tension) for each of the three treatments (LS, SS, and NS) differed (Table 4). The air temperature in the greenhouse ranged from 24-40 C and the highest mean soil temperature (27.5C) occurred in the LS treatment. These environmental conditions (soil temperature and a maximum soil moisture tension of -15 bars) caused considerable plant stress and death of many plants within the LS treatment. Of the kernels harvested from plants grown in the greenhouse only those from the LS and SS treatments contained low levels of aflatoxin.

Results from the greenhouse experiments revealed a lack of significant differences among genotypes in kernel colonization, but differences were observed among moisture treatments. The relationship of severe late-season water stress to increased incidence of Aspergillus invasion and aflatoxin contamination is well known (3, 6, 12, 13, 14, 16). The stress treatments utilized in the greenhouse test failed to result in significant aflatoxin production and thus we were unable to screen among peanut genotypes for Aspergillus resistance. The maximum levels detected were less than 15 ppb in the kernels. The low mean soil temperature of 25.1-27.5 C appeared to have been below the threshold level for aflatoxin production. The stress period (LS treatment) ranged from 24-32 days, a period considered sufficient for toxin production as Sanders and coworkers (14) indicated that 20-30 days stress was sufficient for pre-harvest aflatoxin contamination. In an earlier study, Wilson and Stansel (16) reported that higher levels of aflatoxin contamination occurred when the stress period preceding harvest was at least 40 days.

The highest levels of colonization in 1985 occurred in shells and kernels from the LS treatment, with mean levels of 49% and 21% respectively (Table 5). Shells from all cultivars contained relatively high levels of Aspergillus colonization (means 30%-49%). There were significant differences in the isolation frequency of the Aspergilli from kernels of genotypes harvested from each moisture regime; however, these differences were inconsistent between treatments. Overall the least amount of kernel colonization occurred in PI 337409. The coefficient of correlation between pod infestation and kernel infestation on samples from the LS, SS, and NS treatments were 0.62, 0.58, and 0.28 respectively. The maximum level of aflatoxin detected in kernels from the microplots was 25 ppb; with 12 kernel samples out of 45 samples (from all three treatments) analyzed containing aflatoxin.

Table 5. Influence of moisture regimes on shell and kernel colonization by *Aspergillus flavus* and *A. parasiticus* in covered microplots during 1985.

Treatments*	Cultivar	Isolation frequency (%)**	
		Shell	Kernels
LS	Starr	65a***	26ab
LS	TxAG-3	53ab	32a
LS	PI 337409	52ab	12 c
LS	TX798736	48ab	21 b
LS	SN 55-437	29 bc	13 c
	Mean	49	21
SS	Starr	37ab	17 b
SS	TxAG-3	13 c	11 cd
SS	PI 337409	34ab	18 b
SS	TX798736	30 bc	12 c
SS	SN 55-437	42ab	34a
	Mean	31	15
NS	Starr	38ab	23ab
NS	TxAG-3	22 bc	19 b
NS	PI 337409	22 bc	6 d
NS	TX798736	38ab	13 c
NS	SN 55-437	28 b	6 d
	Mean	30	15

* Moisture regimes: LS, Drought stress initiated 100 days after planting until harvest; SS, Fifteen days of drought stress initiated 100 days after planting followed by optimum irrigations: NS, No drought stress (optimum irrigations).

** Mean of four replications.

*** Values followed by a different letter are significantly different at the p = 0.05 level (Duncan-Waller test).

Shells from the microplots (1986) contained a higher incidence of Aspergillus (mean 79%-82%) compared to 1985 samples, with no significant difference among genotypes (Table 6). In contrast, isolation frequency from the kernel samples in 1986 was less than in 1985, with significant differences detected. Kernels from the genotypes TX811956 and TX798736, harvested from the SS treatment, contained significantly less Aspergillus and aflatoxin than some of the other genotypes (e.g. Starr and J-11). However, within the NS treatment, kernel samples of genotype SN 55-437 and TX 798736 contained significantly more Aspergillus and aflatoxin compared to all other cultivars. Kernel samples from the genotype TX811956 (SS and NS treatments)

Table 6. Influence of moisture regimes on shell and kernel colonization with *Aspergillus flavus* and *A. parasiticus* in covered microplots in 1986.

Treatments[+]	Cultivar	Isolation frequency (%)[*]		Aflatoxin[**]
		Shells	Kernels	ppb
SS	SN 55-437	87a[***]	15a	16a
SS	PI 337409	79a	14ab	4 c
SS	J-11	68a	12abc	8 b
SS	Starr	77a	11 bc	8 b
SS	TX811956	91a	9 cd	4 c
SS	TX798736	73a	7 d	4 c
	Mean	79	10	7
NS	SN 55-437	70a	10 bcd	17a
NS	PI 337409	78a	4 e	6 c
NS	J-11	81a	3 e	4 c
NS	Starr	90a	6 de	0 d
NS	TX811956	84a	2 e	5 c
NS	TX798736	91a	11 bc	13 b
	Mean	82	6	8

[+] Moisture regimes: SS, Fifteen days of drought stress 100 days after planting followed by optimum irrigations: NS, No drought stress (optimum irrigations).

[*] Mean of 5 replications.

[**] Mean of 3 replications.

[***] Values followed by a different letter are significantly different at the p = 0.05 level (Duncan-Waller test).

contained significantly less Aspergillus and aflatoxin. The coefficient of correlation between kernel colonization and aflatoxin contamination for all cultivars was 0.61.

These observations indicate that drought stress conditions and the associated higher temperatures in the LS and SS treatments favored Aspergillus colonization of shells and kernels. Both geocarposphere temperatures and moisture tensions differed between treatments (Table 7 and 8). The mean 1985 geocarposphere temperatures in the LS, SS, and NS treatments were 24.4, 23.0, and 22.7 C respectively (Table 7). These temperatures, according to Blankenship (1) were not highly conducive to aflatoxin contamination. The higher 1986 temperatures of the microplot soils for the SS and NS treatments (31.4 and 30.7 C respectively) (Table 8) were more conducive to aflatoxin contamination (Table 6). Thirty-two of the 36 kernel samples analyzed contained aflatoxin (Table 6). Under the microplot conditions in 1986; there were significant differences in kernel infestation and aflatoxin contamination levels in samples from both the SS and NS treatments. Genotypes TX811956 and TX798736 appeared more resistant within the SS treatment, whereas genotypes SN 55-437 and TX798736 appeared more susceptible within the NS treatment. Kisyombe *et al.* (8) demonstrated that kernels of the genotype J-11 are resistant to *A. parasiticus* infection under moderate moisture and dry conditions. Results of our experiments differ from other reports since the kernels of genotype J-11 were more susceptible (compared to other genotypes tested) under short duration moisture stress. It is apparent that environmental conditions influence the severity of the aflatoxin problem. The moisture regimes used in our experiments were conducive to shell and kernel colonizaiton, but not for aflatoxin production. Various levels and combinations of moisture stress, temperature, and time intervals together influence the severity of aflatoxin contamination. These experiments suggest that screening of peanuts for resistance to the aflatoxin producing Aspergilli will require the use of drought stress conditions to gain a complete understanding of the genotypes potential defenses against invasion.

Table 7. Mean geocarposhpere temperature and moisture tensions of soils in drought stress treatments in microplots during 1985.

Treatments[*]	Temperature (C)[**]			Moisture tension (bars)[**]		
	Max.	Min.	Avg.	Max.	Min.	Avg.
LS	28.0	21.4	24.4	-15	-6.0	-9.0
SS	26.6	20.4	23.0	- 8	-2.5	-5.0
NS	25.9	19.8	22.7	- 2	-1.0	-1.5

[*] Moisture regimes: SS, Fifteen days of drought stress 100 days after planting followed by optimum irrigations; NS, No drought stress (optimum irrigations).

[**] Data collected at one hour intervals during the last 25 days of the experiments.

Table 8. Mean geocarposphere and moisture tension of soil in drought stress treatments in microplots during 1986.

Treatment[*]	Temperature (C)[**]			Moisture tension (bars)[**]		
	Max.	Min.	Avg.	Max.	Min.	Avg.
SS	34.2	27.7	31.4	- 8	- 5	- 7
NS	32.5	28.0	30.7	- 4	- 2	- 3

[*] Moisture regimes: SS, Fifteen days of drought stress 100 days after planting followed by optimum irrigations: NS, No drought stress (optimum irrigations).

[**] Based on data collected at one hour intervals during the last 25 days of the experiment.

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