Relation of Environmental Stress Duration to *Aspergillus flavus* Invasion and Aflatoxin Production in Preharvest Peanuts

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ABSTRACT

Previous experiments have established that late-season water deficit conditions and 28-30.5 C in the geocarposphere are optimum for preharvest aflatoxin contamination of Florunner peanuts. Visibly-undamaged peanuts from plants exposed to these stress conditions during the last 45-50 days before harvest were highly contaminated with aflatoxin at harvest. The objective of this study was to determine the duration of water and soil temperature stress required for extensive preharvest invasion by *Aspergillus flavus* and contamination of peanuts with aflatoxin. Stress conditions were imposed 20, 30, 40 and 50 days before harvest. Incidence of *A. flavus* was greatest in edible peanuts from the 30, 40, and 50 day stress treatments. A stress period of 20 days before harvest did not result in aflatoxin contamination of edible peanuts by *A. flavus*. Peanuts subjected to defined temperature and water deficit stress conditions for 30, 40, and 50 days became contaminated, therefore, a threshold stress period for preharvest aflatoxin contamination of peanuts by *A. flavus* was more than 20, and possibly less than 30 days before harvest.

Key Words: *Arachis hypogaea*, soil water stress, soil temperature stress, aflatoxin, *Aspergillus flavus*, drought, groundnut.

The relation of severe late season water stress and the presence of *Aspergillus flavus* and aflatoxin in peanuts was documented as early as 1965 and has been confirmed by researchers in various locations (5,10,13). Collectively, these studies demonstrated that water stress alone was not responsible for *A. flavus* invasion or aflatoxin production since stressed peanuts were not always contaminated. Further, Dickens *et al.* (5) demonstrated that the lesser cornstalk borer, commonly associated with water stress and often contributing to the aflatoxin problem, was not always involved since kernels from pods without damage often contained aflatoxin.

Wilson and Stansell (15) conducted studies on the relation of water stress and aflatoxin formation in preharvest peanuts including timing of the stress period as a variable. In 2 of 4 years they found significantly more aflatoxin in peanuts when stress was imposed at least 40 days immediately preceeding harvest. The reason for aflatoxin in only 2 of 4 years was not readily understood until the relationship of water stressed soil temperatures to invasion and aflatoxin production in peanuts was reported (3,7,8,11). Since those first reports, a series of experiments has been conducted to determine that the optimum mean geocarposphere temperature range for aflatoxin production in water stressed soil is ca. 28 to 30.5 C during the last part of the growing season (4,12). In 1981, maximum aflatoxin in peanuts occurred at a mean of 30.5 C, the highest temperature used in that study (2). In 1982 maximum aflatoxin occurred at a mean of 29.6 C, but at the next highest mean temperature, 31.3 C, very little aflatoxin was detected (4,12). This demonstrates, as in *in vitro* environmental chamber studies (6), that a small mean temperature change may result in significantly different growth and aflatoxin production by *A. flavus* on peanuts.

The study conducted in 1983 and reported here was designed to accurately determine the duration of end-of-season water stress necessary for *A. flavus* invasion and aflatoxin production in preharvest peanuts when mean soil temperatures were in the optimum range (28-30.5 C) for aflatoxin development.

Materials and Methods

The 5.5 x 12.3 m environmental control plots used in this study have been previously described (1). The plots were situated within a gravel drainage bed and equipped with automatic, mechanized roof systems for water control. Soil temperature modification was accomplished with thermostatically controlled lead-shielded heating cables placed approximately 10 cm below the soil surface. Each plot was equipped with thermocouples and gypsum blocks to measure soil temperature and soil water. Data from these sensors at 5, 30, and 60 cm below the soil surface were collected automatically every 2 hr. Each plot contained at least 20 sensors of each type. Only the data collected at the 5 cm level, corresponding to the fruiting zone of peanuts, are presented. Mean temperatures (5 cm) were monitored daily and optimum mean temperatures were regulated with thermostats controlling the heating cables.

Soil fertility adjustments based on soil analysis were accomplished as recommended by the Georgia Extension Service. Florunner peanuts were planted on May 3, 1983 in a 91-cm row pattern. Fungicides, herbicides, and insecticides were applied as necessary at the rates recommended by the manufacturers. Temik (aldicarb) was aplied in the planting furrows to control thrips. On May 4, Dual (metolachlor) was applied as a preemergence herbicide followed on May 13 by Lasso (alachlor) and Dynap (naptalam and dinoseb) and on May 20 Premerge 3 (dinoseb). For leafspot control, spray applications of Bravo (chlorothalonil) were made on June 2, 14, 23, and 28; July 11 and 25; August 4 and 23, and September 6 and 9. Sulfur was applied with Bravo except on June 23, August 4 and 23, and September 6 and 9. Sevin (carbaryl) was applied with Bravo on August 4 and September 9. Comite (propargite) was applied on August 17 for spider mite control. Cultural practices were identical for all plots until treatments were initiated.

The research strategy required water stress (S) and optimum aflatoxin occurrence temperatures (28.0-30.5 C) in the geocarposphere for 50, 40, 30, and 20 days before harvest (S50, S40, S30, and S20, respectively). Other treatments were optimum aflatoxin temperature with adequate irrigation (IH) and an irrigated control (I) at ambient temperature. At 87 days after planting (DAP) final irrigation was applied to the S50 treatments and 7 days later (94 DAP) soil temperature modification began in the S50 and IH treatments. At 10 day intervals from 87 DAP, the same procedure was followed in other plots to produce water stress and elevated temperatures for 40, 30, and 20 days before harvest.

Peanut plants in all plots were dug by hand 143 DAP and placed in windrows for 3 days. Immediately after digging and after windrow drying, duplicated small samples were collected and analyzed to establish the presence or absence of aflatoxin at those times. Peanuts were picked with a small stationary combine, placed in ventilated bags, and

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air dried at ambient air temperature until moisture was less than 10%. Peanuts were shelled and screened into commercial grade categories before the degree of fungus invasion and aflatoxin concentration were determined. The grade categories, primarily based on size, were jumbo, medium, number 1, splits, other edible, and oil stock. Those peanuts that were shelled during combining (loose shelled kernels = LSK) for each treatment were separated from unshelled peanuts prior to shelling and analyzed as a distinct category. Damaged kernels were hand-picked and separated from all grade categories, mixed, and also analyzed as an additional category.

The quantity of peanuts available for analysis from each plot was inversely proportional to the duration of stress; therefore, fewer peanuts were available in the most severely stressed plots. All kernels in each category were analyzed for aflatoxin. Before aflatoxin analysis, categories in which total kernel weight exceeded 200 g were divided into 1-5 subsamples of 100-200 g to facilitate analysis of all kernels. All samples were screened for aflatoxin using the minicolumn method of Holaday and Lansden (9). Samples with high concentrations were diluted before all extracts from the minicolumn analyses were analyzed by high-pressure liquid chromatography (4). Data, reported as total aflatoxin, were statistically analyzed with the Waller-Duncan K-Ratio T-Test (14) based on log transformations.

Numbers and kinds of fungi within peanut kernels in various categories were estimated by plating surface-sterilized (0.5% sodium hypochlorite solution 5 min) kernels on 2% malt extract agar with and without 10% NaCl followed by incubation at 25 C and 37 C. Quantity of peanuts in each category from each plot varied depending on the severity of the treatment. The mean number of kernels plated per category was 165 but ranged from 25 to 534. Results presented are for the *A. flavus* group fungi, *A. flavus* and *A. parasiticus*, only.

Results and Discussion

The conditions for optimum *A. flavus* invasion and aflatoxin contamination in preharvest peanuts have been defined as a mean geocarposphere temperature of 28-30.5 C in water stress conditions during the 45-50 days preceeding harvest (2,4,12). The treatments in this study were designed to 1) produce those stress conditions for varying periods of time preceeding harvest and 2) produce optimum temperature conditions for *A. flavus* invasion and aflatoxin production in adequate moisture conditions for 50 days preceding harvest. Geocarposphere temperature and soil moisture data during the treatment periods are presented in Table 1. Geocarposphere temperature was 29.4 C in the IH treatment compared to 25.9 C for the I treatment. Soil moisture in the I (-3.7 bars) and IH (-2.9 bars) treatments were not conducive to preharvest aflatoxin formation in peanuts. Mean soil moisture tensions of -2.9 or -3.7 bars at 5 cm below the soil surface seem to indicate relatively dry conditions; however, normally moist soil conditions changed to very dry conditions just before irrigation and the resulting mean moisture tension was disproportionately low. The lowest soil moisture tension measurement considered in the drought treatments was -15 bars because gypsum blocks decrease in usefulness at very negative soil moisture tensions.

The IH treatment was included in this study to determine the response of *A. flavus* when soil temperatures were optimum for invasion and aflatoxin production but adequate soil water was available. In 1980 (8), a heated-soil, irrigated treatment with a mean geocarposphere temperature of 34.5 C, resulted in aflatoxin (12 ppb) only in the damaged kernel category, while 26.1% of the edible kernels and 42.5% of other kernels (damaged and oil stock) were colonized by *A. flavus*. These data were interpreted to mean that adequate irrigation effectively

Table 1 Mean[a] geocarposphere temperature and moisture tension of soils in stress treatments of varying duration.

Treatment	Temperature (C)	Moisture Tension[b] (bars)
I	25.9	-3.7
IH	29.4	-2.9
S20	28.9	<-15.0
S30	29.6	<-15.0
S40	30.2	<-15.0
S50	30.5	<-15.0

I = irrigated, IH = irrigated/heated 50 days before harvest

S20, S30, etc. = no water and elevated soil temperature for 20, 30, etc., days before harvest.

[a] Based on data collected at 2 hr intervals throughout treatment periods.

[b] Due to the inaccuracy of gypsum blocks at very low moisture tension measurements below -15 bars were not considered.

prevented aflatoxin contamination even though invasion percentages were somewhat greater than in the control samples. However, in 1982, studies (4,12) demonstrated that a water stressed soil temperature mean of 31.3 C was approaching an upper limit for aflatoxin production even with a high incidence of kernel invasion (56.8% and 88.9% in edible and other kernels, respectively). Such a phenomenon had been demonstrated *in vitro* (6) and decreased levels of aflatoxin in some areas of the Southwestern United States in 1980, when temperatures were extremely high in connection with severe water stress conditions, suggested a similar natural occurrence. Therefore, in retrospect, the lack of aflatoxin formation by *A. flavus* in 1980 may have been due to the effect of the high mean temperature on fungus metabolism and not to adequate irrigation. The *A. flavus* colonization and aflatoxin data for the IH treatment in Tables 2 and 3 demonstrate that adequate irrigation prevents preharvest aflatoxin formation even when soil temperatures are optimal for aflatoxin production. The incidence of *A. flavus* group fungi in peanuts from the I (control) and IH treatments was relatively uniform over the various kernel categories exept for split kernels (Table 2). The IH damaged category contained almost 3:1 more contaminated kernels than any other category from irrigated plots although damaged kernels from the I treatment were not evaluated. Results from irrigated plots in several previous studies (2,3,8,11) showed that 6-43% of edible and 24-52% of other kernels were contaminated with *A. flavus*, but aflatoxin developed only in kernels subjected to water and temperature stress. Since many kernels that have been invaded don't have detectable aflatoxin (Table 3), apparently only invasion or limited growth occurred without aflatoxin production. Cole et al. (4) indicated that *A. flavus* invasion and aflatoxin production were separate events and suggested that some inherent mechanism preventing aflatoxin formation broke down under stress in response to increased growth of the fungus after invasion. It is possible that such a resistance mechanism operates, in fact, at the level of fungus invasion/growth and thus indirectly regulates aflatoxin production. This idea is supported by the fact that there was an increase in the percent of kernels colonized under stress conditions.

Table 2. Incidence of *A. flavus* group fungi in peanut grade categories from plants grown in water and soil temperature stress treatments of varying duration.

Category	Treatment					
	I	IH	S20	S30	S40	S50
	percent kernels colonized					
Jumbo[a]	10.3	23.2	5.4	32.2	56.0	40.0
Medium[a]	12.0	16.7	7.1	32.4	68.9	56.9
Number 1[a]	25.5	21.5	15.0	32.7	67.0	60.0
Splits[a]	51.7	72.3	59.2	90.5	96.2	95.7
Other edible[a]	14.2	22.8	10.7	59.2	50.5	55.8
Oil stock[b]	19.1	20.8	54.4	64.2	75.4	76.9
Damaged[b]	-[c]	56.0	95.0	96.3	89.6	92.9
Edible	22.7A	31.3A	19.5A	49.4AB	67.7B	61.7B
Other	19.1A	38.4A	74.7A	80.3A	82.5A	84.9A

I = irrigated, IH = irrigated/heated 50 days before harvest

S20, S30, etc. = no water and elevated soil temperature for 20, 30, etc., days before harvest.

[a] Edible category.

[b] Other category.

[c] All damaged kernels utilized in aflatoxin analysis.

For edible and other categories, means in a row followed by the same letter are not significantly different (5% level, Duncan's New Multiple Range Test).

Table 3. Total aflatoxin concentration (ppb) in peanut grade categories from plants grown in water and soil temperature stress treatments of varying duration.

Size	Treatment					
	I	IH	S20	S30	S40	S50
Jumbo	ND Ac	ND Ac	ND Ac	19 Cb	ND Fc	900 Ba
Medium	ND Ac	ND Ac	ND Ac	24 Cb	312 Da	438 Ba
Number 1	ND Ac	ND Ac	ND Ac	209 Bb	522 CDab	604 Ba
Splits	ND Ac	ND Ac	ND Ac	2 Dc	66 Eb	408 Ba
Other edible	ND Ac	ND Ac	ND Ac	263 Bb	1673 BCa	1123 Ba
Oil stock	ND Ac	ND Ac	ND Ac	3247 Aa	2665 Bab	747 Bb
LSK	ND Ab	ND Ab	ND Ab	2207 Aa	2021 Ba	4953 Aa
Damaged	ND Ac	ND Ac	784 Bb	2245 Ab	29876 Aa	17426 Aa

Waller-Duncan K-Ratio T Test performed based on log transformations and data reported are the antilogs of the mean log values. Means in each column followed by the same uppercase letter are not significantly different at the 5% level. Means in each row followed by the same lowercase letter are not significantly different at the 5% level.

I = Irrigated, IH = irrigated/heated 50 days before harvest.

S20, S30, etc., = no water and elevated soil temperatures for 20, 30, etc. days before harvest.

ND = none detected.

Increased duration of water and soil temperature stress generally resulted in increased percentages of peanuts colonized by *A. flavus*. Peanuts in the S40 and S50 treatments had similar levels of colonization (Table 2). Approximately 64% of edible kernels and 83% of other kernels contained viable *A. flavus* propagules. Colonization percentages in jumbo, medium, and No. 1 categories in the S30 treatment were all about 32%, substantially less than corresponding categories in the S40 and S50 treatments. However, the percentage in the S30 other edible category, which is composed of small whole kernels, was similar to percentages in the S40 and S50 treatments. In the S20 treatment, the oil stock category, which is composed of very small kernels and broken peanut pieces, contained 54.4% infected kernels while edible categories overall contained <20%. Data from both the S20 and S30 treatments indicated that smaller, generally more immature kernels were more easily colonized, or were invaded in a shorter period of time than kernels in more mature pods. The data indicate that in these environments, conditions in immature kernels are more conducive to growth of *A. flavus* than conditions in more mature, larger kernels and suggest that a resistance mechanism, if present, breaks down sooner in immature kernels. Overall, the data show that more than 20 days of environmental stress are needed for significant increases in preharvest invasion of edible peanut kernels by *A. flavus*. These data are in relative agreement with results of a previous study (11) in which differences between invasion percentage of peanuts in water and temperature stress and irrigation were apparent after 17 days of stress. In that study after 34 days of stress, ca. 60% of the stressed peanuts and ca. 12% of the irrigated peanuts contained *A. flavus* or *A. parasiticus*. Split kernels from all treatments had relatively high infection percentages. Whether or not split kernels are predisposed to split by fungus invasion or are more heavily invaded because they split is yet to be determined.

Aflatoxin content of peanut grade categories from various stress duration treatments is presented in Table 3. The small peanut samples taken at digging and after windrow drying contained aflatoxin concentrations similar to those presented in Table 3. More than 20 days but probably less than 30 days of water stress at soil temperatures optimum for aflatoxin development were necessary for preharvest aflatoxin contamination in all categories except damaged. Generally, kernel categories that were contaminated with aflatoxin had significantly higher levels of *A. flavus* colonization. The fact that the jumbo category in the S40 and S30 treatments and medium in the S30 treatment had either 0 or low aflatoxin levels suggests that peanuts beyond a certain developmental stage at the inception of stress are less likely to become contaminated. It further suggests that *A. flavus* invasion and/or the impetus for aflatoxin production occurs soon after pegs enter the soil and pods begin to form. This confirms earlier reports (3,4,8,11,12), of high levels of *A. flavus* and aflatoxin in smaller, generally more immature kernels. Wilson and Stansell (15) found that growing season water stress treatments (36-70, 71-105, and 36-105 days after planting) followed by irrigation did not result in high concentrations of aflatoxin. High concentrations occurred only 2 of 4 years with end

of season stress longer than 40 days. Soil temperatures were not determined by Wilson and Stansell (15); however, extrapolation of our data (2,4,12) suggests that mean geocarposphere temperatures were either too high or too low during treatment periods to result in high concentrations of aflatoxin. Further extrapolation of data from this and previous reports (3,4,8,11) suggests that less than optimum soil temperatures coupled with water stress for longer periods of time (>50 days) at the end of the growing season may result in *A. flavus* invasion and aflatoxin production in preharvest peanuts.

Acknowledgements

The contribution and technical support of R. L. Greene, R. A. Tennille, J. W. Kirksey, E. C. Gill, and K. H. Ashley are gratefully acknowledged.

This research was supported in part by the Georgia Agricultural Commodity Commission for Peanuts and the Southeastern Peanut Association.

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Accepted November 12, 1985