Aflatoxin Contamination of Peanuts from Plants Drought Stressed In Pod or Root Zones[1]

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ABSTRACT

Studies were conducted to investigate the separate roles of root stress and pod stress in pre-harvest aflatoxin contamination of peanuts. Pod and root zones were separated by a polystyrene barrier in a unique design and drought type conditions were applied either above or below the barriers. In the three year study, aflatoxin was consistently found in peanuts when pods were exposed to drought stress although roots of those plants were well watered. Generally, aflatoxin was not found in peanuts when pods were well watered although roots were subjected to drought stress conditions. Moisture content of Pod Maturity Profile classes was generally lower in root zone stress conditions especially in the immature classes. Moisture contents of mustard-colored pods in all classes were extremely low (< 26%).

Key Words: Drought stress, aflatoxin, peanut, seed moisture, pod stress, plant stress

The association of high aflatoxin contamination and drought stress was reported as early as 1965 in studies conducted in South Africa (15). The same relationship was observed in Nigeria (10) and in peanut producing areas of the United States (6, 12). Extensive studies have been conducted to define the environmental conditions associated with pre-harvest aflatoxin contamination of peanuts (3, 4, 5, 8, 13, 17). Wilson and Stansell (17) found aflatoxin in 2 of 4 years in studies using rainout shelters to create artificial drought conditions. Larger facilities subsequently constructed at the USDA, ARS, National Peanut Research Laboratory included subsurface heating cables to create high soil temperatures usually associated with natural drought conditions (2). Cole *et al.* (5) reported that visibly undamaged peanuts which were grown under drought the latter 4-6 weeks of the growing season with 5.08 cm depth soil

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temperatures of ca. 26-30 C were highly contaminated with aflatoxin. Sanders *et al.* (13) reported occurrence of aflatoxin contamination between 20 and 30 days of drought stress with soil temperatures between 28.0 and 30.5 C. Cole *et al.* (5) and Sanders *et al.* (13) reported that high *Aspergillus flavus* invasion percentages may be found without the presence of aflatoxin, suggesting that invasion and subsequent growth and aflatoxin contamination may be separate processes or at least regulated in different ways. Further, invasion (14) and aflatoxin contamination in peanuts grown under drought conditions (4, 8, 13) usually occur first and to a greater degree in small, immature peanuts. This information led to the suggestion that some seed resistance mechanisms preventing growth and aflatoxin production by the fungus fail first in immature peanuts in response to water and temperature stress (5).

Dorner *et al.* (7) subsequently demonstrated an association between the timing of *in vivo* loss of the capacity of seed to produce phytoalexins (a previously identified fungal resistance mechanism in stored peanuts) and the pre-harvest appearance of aflatoxin contamination. Seed water activity appeared to be the most important factor controlling the capacity of seed to produce phytoalexins. Dorner *et al.* (7) reported that the moisture contents of seed within a maturity class from drought stress conditions were not uniform and some seed within a maturity class possibly became contaminated before others. Moisture and temperature stress thus appeared to serve as the mechanism causing moisture loss from seed associated with pre-harvest aflatoxin contamination.

Studies on pre-harvest aflatoxin contamination in response to drought stress have logically included treatment of the whole plant (i.e., roots ad pods were subjected to the same treatments). In peanut growth studies Ono *et al.* (11) varied soil temperature in the pod zone separate from that in which the roots were growing and found that the soil temperature in which the pods were grown had a marked effect on the rate of development and final size. The studies reported here compare the effect of drought (moisture and temperature stress) in the root zone versus similar stress only in the pod zone on pre-harvest aflatoxin contamination of peanuts. The studies were conducted to determine whether or not plant (root) drought stress was essential for aflatoxin production in peanuts and thus to determine if the mechanism, or control of the mechanism, for resistance was located solely in the pods. If drought stressed plants are essential for contamination, then the mechanism for resistance could potentially involve some biochemical or physiological function of the plant. This manuscript reports techniques used to study differential stressing of roots and pods and the result of these stresses on aflatoxin and moisture variability within seed.

Materials and Methods

Two plots 2.4 m wide by 12.2 m long were added to existing environmental control plots (2) at the National Peanut Research Laboratory at Dawson, GA and fitted with polystyrene barriers and heating cables to provide either pod or root stress (Fig. 1). Soil in the two plots was replaced with 0.9 m of Tifton sandy loam. Sheets of polystyrene 2.54 cm thick by 1.2 m wide by 2.4 m long were laminated in three rows with similar material that was 7.62 cm wide. These strips of material were glued to the sheets of polystyrene 30.48 cm from each end and down the center to accommodate eventual planting of three rows in a conventional 91.4 cm (36 in) row pattern. Holes for planting peanut seed were bored at 5.08 cm intervals

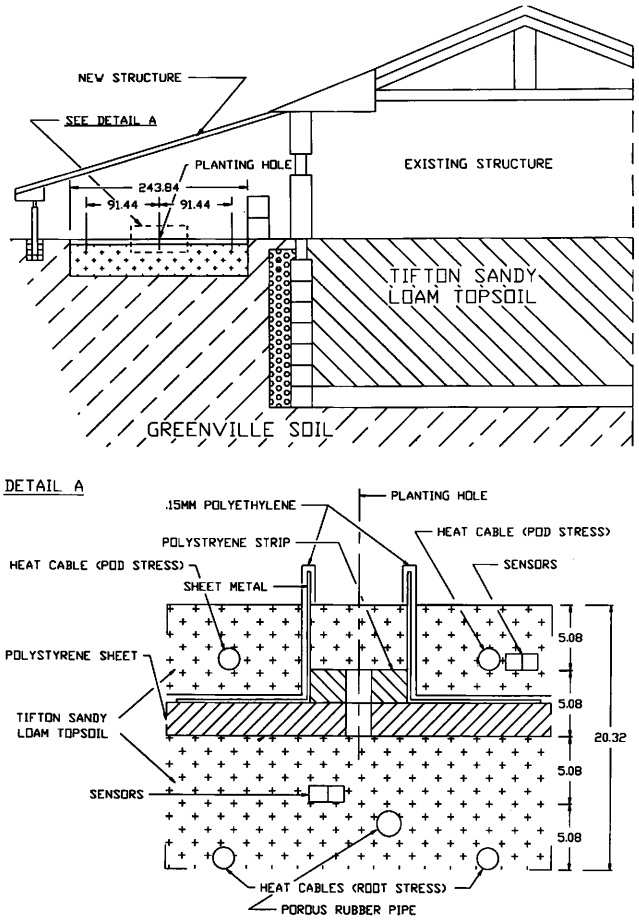

Fig. 1. Cross sectional view of plots used to separate peanut pod zone and root zone drought stress. Dimensions are cm unless otherwise indicated.

along the length of the strips. Soil was removed from the plots to a depth of 20.32 cm and thermostatically-controlled, lead shielded heating cables (General Electric, 73.2 m long, 240v, 1600 W) were placed on the soil 20.32 cm apart and 2.54 cm of topsoil was placed over the cables in the plant stress treatment. These cables were placed 2.54 cm above the polystyrene in the pod stress treatment. Porous rubber tubing was placed near planned row locations and an additional 2.54 cm of topsoil was added. Copper-constantan thermocouples and Delmhorst gypsum blocks were placed between and under the plant rows across the plot at this level and at 15.24 cm ca. 4 m from each end of the plot. After an additional 5.08 cm of topsoil was added, Temik (aldicarb) was placed in a band near the row locations and 10 laminated polystyrene sheets were placed side by side to make a total plot length of 12.2 m. Sheet metal bent into 90 degree angles was placed on both sides of each 7.62 cm polystyrene strip and 0.15 mm thick polyethylene was used to cover the entire polystyrene layer. Another 2.54 cm of topsoil was added, additional sensors were put into place and an additional 5.08 cm of topsoil was used to give the finished plot a total of 7.62 cm of topsoil above the polystyrene. Before planting, the polyethylene covering the row area was split and clipped to the sheet metal.

Dry topsoil was placed in the bored planting holes in the polystyrene sheets and Florunner peanuts (*Arachis hypogaea* L.) were hand-planted and watered. Water was applied beneath the polystyrene through the porous rubber tubing and above the polystyrene by a handheld spray nozzle to grow plants until the treatments began. Drought treatments (water and temperature stress) were applied above the polystyrene barrier for pod stress and below the barrier for plant stress. Water was applied either above or below the polystyrene as appropriate for the particular treatment when data from the gypsum blocks began to approach 0.3 bars tension.

Planting dates were May 8, 1985, April 28, 1986 and April 24, 1987. Treatment dates and harvest dates varied for the three years. In 1985, treatments began 104 days after planting (DAP) and peanuts were hand-

harvested at 145 DAP. In 1986 treatments began 105 DAP and peanuts were hand-harvested 150 DAP. In 1987 the treatments were started at 101 DAP and the pod stress treatment harvest occurred at 146 DAP while the plant stress treatment was harvested at 154 DAP.

Data from all sensors were collected at 2-hr intervals using a 500 channel, model 9302 Monitor Labs datalogger. During 1985 and 1986 plot facilities and soil temperatures were monitored and controlled manually but in 1987 a microcomputer based system was developed to monitor and control the facilities (1).

Each year at the termination of each treatment, 6-8 random plants were hand-harvested from each plot and the peanuts were hand-picked and classified into Pod Maturity Profile (PMP) classes according to Williams and Drexler (16). In 1986 and 1987 the maturity determinations were made on hand-scraped pods to assure that no moisture would be gained in the classification process. After classification, pods that had an underlying dull, mustard color were removed. Dorner *et al.* (7) previously indicated that immature pods containing very low moisture seed that had released from the pod had a characteristic mustard-colored appearance. Mustard-colored pods and three replicate samples from each pod maturity class were hand-shelled and moisture contents of hulls and kernels were determined by drying at 130 C for 6 hrs. Remaining plants in the three rows in each of the two plots were hand-harvested, placed in windrows for 2-3 days, combined, and the peanuts were cured on ambient air dryers until moisture level reached ca. 7%. Peanuts were hand-cleaned, shelled, screened into commercial grade sizes, and picked to remove visually damaged seed. The total sample weight in each size and in the damaged category was used for total aflatoxin quantitation by high-pressure liquid chromatography (7) after screening by the mini-column method of Holaday and Lansden (9). Data from all replicated samples were subjected to analysis of variance (ANOVA) and significant differences among means were determined by Duncan's New Multiple Range Test.

Results and Discussion

Average soil temperature and moisture conditions for treatment periods in the three years of the study indicate that the plot design and materials adequately separated the stress and non-stress conditions in each plot. Temperature differentials were most evident in the root stress treatment (Table 1). Soil moisture data were not reported because gypsum blocks do not provide accurate data in extremely dry conditions; however, measurements from the gypsum sensors did show that there was no moisture migration between irrigated and drought areas of the plots (data not shown). In 1986 and 1987 the pod zone temperatures in both treatments were well within the pod zone temperature range for aflatoxin production (5). In 1985 the 5-cm soil temperature in the root stress treatment of 24.9 C was slightly lower than the low limit drought temperature (25.7 C) for aflatoxin previously indicated by Cole *et al.* (5). However, aflatoxin has been reported in non-edible peanut categories at drought temperatures as low as 19.8 C (3). Moisture control was appropriate to achieve stress, as desired, both above and below the polystyrene barrier. Plant moisture status was not measured but plants with root zone stress wilted rapidly under stress conditions. Plants with only pod stress were never visibly stressed at any time during the treatment period.

Table 1. Mean temperature and soil moisture condition above (5cm) and below (15 cm) the polystyrene barrier in root zone and pod zone drought stress treatments in 1985-1987.

		Root Zone Stress		
Depth	Moisture	1985	1986	1987
			C	
5 cm	Irrigated	24.9	27.6	27.6
15 cm	Dry	28.9	29.8	29.6
		Pod Zone Stress		
Depth	Moisture	1985	1986	1987
			C	
5 cm	Dry	29.1	29.6	29.3
15 cm	Irrigated	27.8	29.9	29.3

In each year, aflatoxin was consistently found in all peanuts from the pod stress treatment (Table 2). Aflatoxin was not generally found when pods were maintained in adequate moisture conditions (root zone stress). The notable exception in edible categories was in the No. 1 size in 1986 which contained 1122 ppb aflatoxin. It is possible that seed damaged internally were overlooked when visually damaged seed were picked from all size categories. The high level of aflatoxin in the damaged category in 1986 suggests that misidentifying a single seed could produce such results. The 1986 season in the facility was marked by extensive insect pressure and plots adjacent to these treatment plots were drought stressed. Hill *et al.* (8) previously reported aflatoxin contamination in damaged category peanuts from an irrigated plot when adjacent plots were in drought stress conditions and had high insect infestations.

Table 2. Total aflatoxins in peanut grade size categories from root zone and pod zone drought stress treatments in 1985-1987.

	Root Zone Stress			Pod Zone Stress		
	1985	1986	1987	1985	1986	1987
Size	Aflatoxin concentration (ppb)					
Jumbo	0	0	0	98	83	1
Medium	0	1	0	522	708	97
Number 1	0	1,122	0	1,780	439	1,232
Other Ed.	0	1	0	1,833	691	3,421
Oil Stock	0	4	4	727	409	4,608
Damaged	0	21,199	-	25,233	32,788	198,734

These data indicate that, within limits, drought stress level of peanut plants (root zone stress) is not the controlling factor in pre-harvest aflatoxin contamination of peanuts. The data further indicate the very important role that drought stress of pods plays in the aflatoxin contamination process.

Data reported by Dorner *et al.* (7) indicated that phytoalexin production was a seed-controlled resistance mechanism related to water activity. One concept tested in these experiments was that root zone stressed plants would not be able to supply sufficient moisture to pods and that the pods would lose enough moisture to move into the susceptible water activity range for the aflatoxin contamination as indicated by Dorner *et al.* (7). Conversely, in pod stress the concept tested was that pods would lose moisture at a rate faster than plants were able to supply it. Moisture content of PMP classes for the two treatments in 1986 and 1987 (Table 3) shows that, with the exception of mustard-colored pods, peanuts from the pod stress treatment had higher moisture contents than peanuts from the root stress treatment. As shown in Table 2, most of the aflatoxin was associated with peanuts from the pod stress treatment. Pods with a mustard-colored appearance contained seed with considerably lower moisture contents than normal pods.

Table 3. Moisture contents of peanut maturity classes from plants stressed in either pod or root zones.

Maturity Class	1986			1987		
	Zone of Stress					
	Root	Pod		Root	Pod	
	Percent Moisture [a,b]					
Yellow 1	49.8 C	71.0 E		36.3 C	67.8 D	
Yellow 2	34.6 B	54.5 D	25.9*	33.7 B	46.0 C	14.7*
Orange	31.2 A	41.9 C	19.0*	31.8 A B	35.7 B	
Brown	30.2 A	35.2 B	17.9*	31.7 A B	31.8 A	
Black	29.1 A	31.4 A	16.2*	30.6 A	29.2 A	

* Mustard colored pods, single bulk samples.

[a] Means for each maturity class within a treatment and year not followed by the same capital letter are signifcantly different ($p \leq 0.05$).

[b] Root and pod zone stress treatments resulted in significantly different ($p \leq 0.05$) moisture contents within each maturity class in 1986; yellow 1, yellow 2, and orange were significantly different in 1987.

Pods with mustard-colored appearance were found in all maturity classes from the pod stress treatment in 1986 but only in the yellow maturity stage in 1987. Dorner *et al.* (7) previously reported that mustard-colored pods contained low moisture seed that had released from the pod. Unpublished data (J. W. Dorner) indicate that water activity associated with the moisture contents reported in Table 3 (except mustard appearance) are in the range at which phytoalexins may still be produced.

In 1986 seed (5-25 g) from single samples of normal and mustard-colored pods from PMP classes from the pod stress treatment were analyzed for aflatoxin. Aflatoxin was found predominantly in seed from the mustard-colored pods. (Normal colored: yellow 2- 4 ppb, all other normal classes- 0; mustard colored: yellow 2- 12ppb, orange- 0, brown- 159 ppb, black- 88ppb) This very limited data is presented only as an indication of the potential relationship between low preharvest seed moisture and preharvest aflatoxin contamination. Dorner *et al.* (7) combined seed from mustard-colored pods with other visibly low moisture seed in tests for phytoalexin production and aflatoxin. The low moisture, immature categories consistently lost phytoalexin producing ability *in vitro* before the *in vivo* pre-harvest occurrence of aflatoxin. Our data confirm and extend the suggestion of Dorner *et al.* (7) that only a few seed become contaminated and that these seed for some reason have low moisture contents. The data suggest the need for additional research to identify the reason(s) that only certain pods/seed lose moisture and others in the same field or even on the same plant do not. The very high levels of aflatoxin found in the pod stress treatment may have occurred because moisture loss progressed over an extended period of time due to the fact that the plant was supplying water to the pod. Thus, water activity below that at which phytoalexins are produced but at which the fungus grows well were present for an extended period of time. Additionally, the fungus may have begun active growth after some water loss (phytoalexins not produced) and the moisture contents of 16-25% at harvest (Table 3) provided ample moisture for continued growth of the fungus until moisture content was reduced below 10% during curing. Dorner *et al.* (7) did not find aflatoxin contamination in drought-grown peanuts of any maturity class as long as high moisture was present.

Additional study is needed to determine if, in fact, the only seed contaminated in the pod stress situation are those of reduced moisture content or water activity. If high-moisture seed from pod stress treatments are found to contain high levels of aflatoxin then phytoalexin production as a resistance mechanism would be subject to reconsideration. However, the wide variability in moisture content of seed produced in separate pod and root zone drought stress environments and aflatoxin content associated with low moisture content adds to the body of information supporting the probability that phytoalexin production *in vivo* is centrally involved in the pre-harvest resistance of peanuts to aflatoxin contamination.

Acknowledgments

The authors acknowledge the outstanding technical support and insight of Larry Greene, Edwina Gill, Bobby Tennille and Jerry Kirksey.

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Accepted January 16, 1993