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Effect of Irrigation Regimes on Aflatoxin Contamination of Peanut Pods[1]

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ABSTRACT

Florunner and Florigiant peanuts were grown and foliar inoculated with an aflatoxin producing isolate of *Aspergillus parasiticus* (NRRL 2999) 30 days after planting. Four replicates were grown in plots under rainfall controlled shelters with six irrigation treatments: (1) wet from day 0-145, (2) dry from days 36-70, (3) dry from days 71-105, (4) dry from days 106-145, (5) dry from days 36-105, (6) dry from days 71-145. Aflatoxin concentrations from Florunner peanuts were significantly different between treatments ($P = 0.01$) in 1974 and 1976 but not in 1975 or 1977. In 1974 and 1976, sound mature kernels of Florunner from treatments 4 and 6 contained significantly more aflatoxin when compared to other treatments. Aflatoxin contamination of Florigiant sound mature kernels from treatments 4 and 6 in 1975 and treatment 6 in 1974 was significantly greater ($P = 0.01$) than other treatments, but not in 1977. No data were taken in 1976 for Florigiant peanuts. Water stress during the last 40 to 75 days of the season contributed to aflatoxin contamination of sound mature kernels three of the four years on one or both cultivars. Because of year to year variation, drought stress alone does not consistently affect field aflatoxin contamination. In some years other environmental factors must have interacted with drought stress to promote or inhibit preharvest aflatoxin contamination. However, in all treatments where irrigation was applied during the last 40 days of the season, no significant aflatoxin contamination was detected in any cultivar any year of the test.

Key Words: aflatoxin, peanuts, *Aspergillus flavus, Aspergillus parasiticus,* irrigation, and water stress.

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Segregation 3 peanuts are a chronic problem for peanut farmers. These peanuts have visible *Aspergillus flavus* growth on kernels in official grade samples (3). The assumption that visual detection of moldy peanuts will divert all aflatoxin contaminated lots to nonedible uses is dangerous since not all contaminated lots will be detected. Aflatoxin may be present in lots with no visible *A. flavus,* or there may be no aflatoxin when *A. flavus* is visible. There are several ways that contamination of farmers' stock peanuts may occur; these include contamination before digging, in the windrow, during the combining and drying operations (2), or in deficient warehouse storage (1)).

Pettit et al. (9) demonstrated that peanuts grown under dry land conditions, where drought stress occurred, contained more aflatoxin before digging than peanuts grown under irrigation. Their observations are supported by work of McDonald and Harkness (7) and Dickens, Satterwhite and Sneed (4).

The purpose of this research was to determine the effects of preharvest drought stress on aflatoxin contamination of peanuts.

Materials and Methods

The experimental procedures have been described previously in detail (8); therefore, only a summary of the methods will be given here. Florunner and Florigiant peanuts were grown from 1974 through 1977 in 1.52 x 1.83 m rainfall protected plots of Tifton loamy sand. Protection from rainfall was provided by large roof-like structures which automatically covered the plots at initiation of rainfall and uncovered the

plots when rainfall ceased. Each plot was inoculated by sprinkling 500 mL of a 1 x 10^6 conidia/mL spore suspension of *Aspergillus parasiticus* Speare (NRRL 2999), an aflatoxin producing isolate of the *A. flavus* group, over the plants 30 days after planting.

Six irrigation treatments (Table 1) were imposed in a randomized complete block design replicated four times. Plots were harvested after 145 days by loosening the soil with pitchforks and lifting the plants by hand. The vines and attached pods were dried at 38 C to a kernel moisture of approximately 8%. After drying, the pods were hand picked, shelled, and graded. Pods left in the soil after harvesting were recovered by screening. Separate aflatoxin analyses were done on sound mature kernels (SMK) from both harvested and screened peanuts using the CB method, an official AOAC peanut method (1).

Table 1. Irrigation regimes to induce drought stress at differing times during the growing season.

Days After Planting	Treatments 1	2	3	4	5	6
0-35	Irrig*	Irrig	Irrig	Irrig	Irrig	Irrig
36-70	Irrig	Dry**	Irrig	Irrig	Dry	Irrig
71-105	Irrig	Irrig	Dry	Irrig	Dry	Dry
106-145	Irrig	Irrig	Irrig	Dry	Irrig	Dry

*When under irrigation, plots watered to charge 61 cm depth to field capacity whenever soil-water tension in surface 31 cm reached 0.2 bar.

**Soil profile allowed to dry until the end of a 35 or 70-day interval, then rewatered to field capacity to 61 cm depth.

Six one inch diamter probe soil samples were taken from the top two inches of each plot when the peanuts were harvested. Soil dilution plates were made by slurrying 5 g of thoroughly mixed soil with 100 mL 0.38% w/v water agar. A 1/10, 1/100, and 1/1000 (v/v) dilution series from each plot was made and the resulting suspensions were plated on M3S1B, a selective *A. flavus* group - *A. niger* group isolation medium (6). Plates were incubated at 30 C for 7 days and colony counts of all members of the *A. flavus* group were recorded (6). Results were recorded as propagules of *A. flavus* group fungi per gram of soil.

Plot means were analyzed by standard analysis of variance for a randomized complete block design (12). Comparisons were made for total aflatoxins and propagules of the *A. flavus* group.

Results and Discussion

All sound mature kernels were split for observation of internal damage. Kernels with external or internal damage were removed before aflatoxin determinations were made. The removal of all visible damage from the samples eliminated all physically damaged kernels as well as those with obvious insect or fungal damage. Mean total aflatoxin values for SMK peanuts from plots grown under the rainfall shelters are presented in Table 2. In 1974 and 1976, Florunner peanuts from treatments 4 and 6 subjected to drought during the last 40 to 75 days of the season contained significantly more aflatoxin (403 to 3582 ppb) when compared to peanuts from plots that received water for the last 40 days (0 to 10 ppb). Florigiant peanuts contained significantly more aflatoxin in 1975 when drought conditions were imposed during the last 40 to 75 days (729 to 1355 ppb) than in those peanuts from treatments with irrigation for the last 40 days (0 ppb). In 1974 Florigiant peanuts from the 75 day drought (treatment 6) contained statistically significant ($p = 0.01$) more aflatoxin (7208 ppb) than the other treatments. Peanuts from the 40 day drought (treatment 4) contained more aflatoxin (570 ppb) than peanuts collected from plots with irrigation the last 40 days (1-176 ppb) and these treatments were significantly different at the 5% level. These data from sound mature kernels suggest that there are complex interactions between drought during the last 40 or 75 days of the growing season and aflatoxin contamination of visibly sound peanuts.

Table 2. Mean total aflatoxins (ppb)* in sound mature kernels from pods obtained from peanut plots grown under rainfall shelters.

Irrigation Treatments	Days of Drought Period	Florunner 1974	1975	1976	1977	Florigiant 1974	1975	1977
1	none	1 b**	0 a	0 c	0 a	13 b	0 b	0 a
2	36-70	7 b	0 a	8 c	0 a	1 b	0 b	1 a
3	71-105	4 b	0 a	10 c	65 a	16 b	0 b	0 a
4	106-145	3582 a	0 a	403 b	0 a	570 b	729 a	0 a
5	36-105	1 b	0 a	6 c	0 a	176 b	0 b	0 a
6	71-145	3420 a	0 a	2407 a	0 a	7209 a	1355 a	0 a

* Total aflatoxins are the sum of $B_1 + B_2 + G_1 + G_2$ in ppb (ng/g) of peanuts.

** Means followed by the same letter are not significantly different (P=0.01). Analyses from four replicate plots were used to obtain the means.

Peanuts that were left in the ground at harvest were recovered by screening the soil. These screened peanuts were generally contaminated with more than 100 ppb total aflatoxins and would not have been marketable (Table 3). Treatment 6 always contained kernels with significantly more aflatoxin than treatments with irrigation during the last 40 days.

Table 3. Mean total aflatoxins (ppb)* from peanuts left in the ground at harvest and subsequently recovered by screening the soil one day after harvest. The peanuts were grown under rainfall shelters.

Irrigation Treatment	Florigiant 1975	1977	Florunner 1976	1977
1	5875 b**	167 b	418 b	11 b
2	6363 b	377 b	98 b	0 b
3	15750 b	3117 b	1768 b	763 b
4	22786 b	251 b	52750 a	12 b
5	1113 b	656 b	95 b	***
6	73616 a	13219 a	80200 a	111686 a

*Total aflatoxins are the sum of $B_1 + B_2 + G_1 + G_2$ in ppb (ng/g) peanuts.

**Means followed by the same letters are not significantly different (P=0.01). Analyses from four replicate plots were used to obtain the means.

***No peanuts were recovered by screening this treatment in 1977. Peanuts were not recovered from all plots in either cultivar in 1974 and from Florunner in 1975. Therefore data was omitted from analyses.

Propagules of *A. flavus* group per gram of soil at harvest is presented in Table 4. Soil from the 75 day drought treatment (treatment 6) contained a high number of propagules. Populations of the *A. flavus* group were variable over years in the other treatments. Thus, no consistent conclusions could be made about populations of the *A. flavus* group. It appeared that the heavy inoculation of plots with a member of the *A. flavus* group (*A. parasiticus*) produced large populations that persisted under both irrigated and drought conditions.

These results are substantially in agreement with those reported by Sanders et al. (10) and Hill et al. (5).

Table 4. ***Aspergillus flavus*** **group (propagules/g soil) from soil samples obtained at harvest from peanut plots maintained under rainfall shelters.**

Irrigation Treatment	Year 1975	1976	1977
1	783 b	844 b	1516 cd
2	881 b	365 b	1176 d
3	2692 ab	786 b	2493 bcd
4	4000 a	1066 b	2986 ab
5	2124 ab	386 b	2816 abc
6	2616 ab	2056 a	4000 a

*Means followed by the same letter are not significantly different (P=0.05). Analyses from four replicate plots were used to obtain the means.

These results are substantially in agreemnt with those reported by Sanders et al. (10) and Hill et al. (5). The temperature relationships with drought that Hill et al. (5) reported may help explain our year to year variation in contamination levels of sound mature kernels. Aflatoxin is probably produced in the field in a restricted temperature range and if the soil temperature is too high or too low, aflatoxins may not be produced. The large populations of the *A. flavus* group in our experiments were influenced by inoculation of the peanuts with a member of this group. This inoculation technique could have influenced the aflatoxin levels in sound mature kernels.

The data reported in this paper indicate that irrigation during the last 40 or 75 days of the growing season will help prevent aflatoxin contamination of sound mature kernels. However, drought stress alone will not consistently induce aflatoxin contamination. The relationship between drought stress and aflatoxin contamination is not simple. The large year to year variations in aflatoxin contamination probably result from several complex environmental interactions including water, temperature and biological factors.

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