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Influence of Fungicides and Irrigation Practice on Aflatoxin in Peanuts Before Digging[1]

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Peanuts grown under dryland conditions where drought stress occurred accumulated more aflatoxin before digging than peanuts grown under irrigation. Kernels became more susceptible to *Aspergillus flavus* and *A. parasiticus* invasion when the soil moisture in the pod zone approached levels at which moisture moved from the pod into the soil and the kernel moisture dropped below 31%. Isolation frequencies of these aspergilli from fresh-dug kernels were lowest in 1968 (maximum of 3%). In 1967 and 1969, maximum percentages of 100 and 74, respectively, were noted. Kernel infestation was correlated with degree of aflatoxin contamination. Dryland fresh-dug kernels contained a maximum of 35,800 parts per billion aflatoxin while a maximum of 50 parts per billion was detected in kernels from irrigated plots. In 1969 *A. flavus* infestation was as high as 59% in peanuts from irrigated plots; however, no aflatoxin was detected. Absence of aflatoxin in these samples is attributed to the higher kernel moisture content which reduced the aflatoxin-producing potential of *A. flavus*. Statistical analysis of the data revealed no significant differences in degree of fungal infestation, production levels, and grade factors between any fungicide treatments.

The fungi *Aspergillus flavus* Link and *A. parasiticus* Speare produce several structurally similar metabolites, called aflatoxins, which are among the most potent mycotoxins known (2, 9, 10, 16, 20). The ability of a specific *Aspergillus* isolate to produce and accumulate aflatoxin is controlled by such factors as genetic potential, environmental conditions, substrate moisture and composition, and duration of the fungus-substrate association (1, 3–8, 15).

A. flavus commonly invades edible seeds in the field just before harvest, during harvest and handling, or during storage (2, 8, 11–14, 20). Optimum temperatures for peanut kernel invasion range from 25 to 35 C (19). Optimum relative humidities range from 85 to 99% (6, 7), and optimum kernel moisture varies from 20 to 30% (1). Maximum production and accumulation generally occur after a 5- to 7-day incubation period (19).

Kernel moisture levels in the range most susceptible for *A. flavus* invasion commonly occur after digging during curing. Kernel moisture, which may be as high as 50% when dug (2), is reduced during curing and maintained below 10% for safe storage. When kernel moisture levels are above 30% they are commonly invaded by fungi other than the aspergilli. Invasion usually is preceded by physical damage to the pods by insects, other microorganisms, or growth cracks (18). Invasion of the pods by aspergilli may occur to a limited extent while they are in the soil (8, 14, 17), especially where the inoculum potential is high; however, penetration through the pod is generally very slow. However, once the pods have been damaged invasion of the kernels is much more rapid.

In Nigeria, McDonald and Harkness (13, 14) detected aflatoxin in peanuts immediately after digging. They attributed the presence of aflatoxin in fresh-dug kernels to overmaturity, excessive pod injury, and unfavorable weather conditions during the growing season.

The objectives of these experiments were to determine whether soil fungicides or soil moisture levels (as related to kernel moisture) or both influenced *A. flavus* infestation and aflatoxin accumulation before digging.

MATERIALS AND METHODS

Significant aflatoxin levels in fresh-dug peanuts were first detected in samples from Stephenville,

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Tex., plots in 1966. To verify this observation, replicated field plot experiments were set up for 3 years (1967, 1968, and 1969). These experimental plots were established in north central Texas at Stephenville and in south central Texas at Yoakum in soil known to have a high incidence of *A. flavus*. Each year both dryland (moisture from rainfall only) and irrigated (moisture from rain and supplemental irrigation) plots were planted to Starr variety, Spanish-type peanuts. Seeds were planted 2 to 3 inches deep on 3- to 4-inch beds treated with soil fungicides mixed in a 10- to 12-inch band above the seed. Six soil fungicides were tested for their ability to reduce *A. flavus* infestation. The soil fungicides tested were PCNB (pentachloronitrobenzene); Polyram [5.2 parts of ammoniates of ethylenebis(dithiocarbamato) zinc with 1 part ethylenebis(dithiocarbamic)acid]; bimolecular and trimolecular cyclic anhydrosulfides and disulfides; Difolatan [*N*-(1,1,2,2-tetrachlorethyl) sulfenyl cis-4-cyclohexene-1,2-dicarboximide]; Benomyl[(methyl-1-butylcarbamoyl)-2-benzimidazolecarbamate]; MBR 4635 (*p*-chlorobenzyl 1,1,5-trihydro-perfluroamyl sulfide); and carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide). In addition to these soil fungicides the following foliage fungicides were used: Benomyl; Dithane M-45 [zinc-manganese salt of ethylenebis(dithiocarbamate)]; chlorothalonil(tetrachloroisophthalonitrile); Fungi Sperse [sulfur-zinc ethylenebis(dithiocarbamate) + calcium]; and Kocide [86% copper hydroxide (56% Cu)]. Each treatment was replicated four to six times in dryland and irrigated blocks. In irrigated treatments five- to six-furrow irrigations were applied during the hot dry months at Yoakum and four to seven overhead sprinkler applications were made at Stephenville.

Samples for *A. flavus* and aflatoxin determination were dug with a hand shovel on typical harvest dates of 120, 130, and 140 days after planting. Peanut fruits were pulled from the vines immediately and taken to the field laboratory where 50 g of kernels was hand-shelled from each sample. Each kernel sample was placed in a flask and covered with acetone to terminate microbial activity. An additional 200 unshelled peanuts from each sample were placed in plastic bags, packed in ice, and transported to the central laboratories for determination of degree of kernel infestation by *A. flavus*. One hundred kernels were surface-disinfested (successive 1-min submersions in 70% ethyl alcohol, aqueous 1:10 5.25% active NaOCL, and sterile distilled water) and plated on modified Martins rose bengal agar medium (15).

Aflatoxin determinations were made with aqueous acetone extractions quantified by thin-layer chromatography using the method of Pons and Goldblatt as adapted by Schroeder (19).

RESULTS AND DISCUSSION

Observations in 1967. *A. flavus* infestation and aflatoxin accumulation were highest in peanut kernels harvested from dryland plots during 1967 and 1969. *A. flavus* incidence in fresh-dug peanuts harvested in 1967 (Table 1) was highest in kernels collected 140 days after planting at Yoakum. Infestation ranged from 36 to 100%, an abnormally high level seldom detected in peanut samples. In comparison, infestation of peanuts from adjacent irrigated plots ranged from 1 to 18%. The high incidence of *A. flavus* in the dryland kernels is attributed, in part, to the lack of moisture available to the plant late in the growing season. Rainfall records at Yoakum during the growing season showed: May, 4.20 inches; June, 0.01 inch; July, 1.08 inch; and during the first 18 days of August, 0.00 inch. Between 18 and 28 August (140-day harvest date) 4.85 inches of rain was recorded. Adequate early moisture gave the peanuts good initial growing conditions; however, dryland peanuts lacked moisture just after pods began to set and as a result were stunted throughout the latter part of the growing season. Early formed kernels matured and dried in the soil to a moisture level as low as 24% (wet weight basis). Most of these kernels germinated while still attached to the parent plant in the soil when excess moisture became available after 130 days. As a result, only approximately 150

TABLE 1. *Isolation frequency of Aspergillus flavus from fungicide-treated dryland and irrigated plots (1967)*

Soil fungicide treatments	Yoakum						Stephenville					
	Dryland			Irrigated			Dryland			Irrigated		
	120[a]	130	140	120	130	140	120	130	140	120	130	140
None	10[b]	14	36	1	4	0	8	8	0	0	0	0
PCNB	14	20	100	0	6	0	0	0	0	0	0	0
Polyram	3	2	60	0	2	18	1	0	4	6	2	0
Difolatan	18	34	96	0	6	8	4	0	4	0	2	6
Avg	11	12	73	0	4	6	3	2	2	1	1	1

[a] Number of days after planting.

[b] Values expressed as percentages of kernels from which *A. flavus* grew out onto agar.

ungerminated kernels were found within each dryland plot by 140 days. These kernels were used to determine fungal isolation frequency; thus no samples were available for aflatoxin analysis. Germinating kernels split the pods and allowed *A. flavus* to enter, accounting for the high infestation levels.

A. flavus infestation of kernels harvested at Stephenville in 1967 averaged less than 4% with a maximum of 8%. These levels are considered typical of peanuts grown in infested soil when normal rainfall patterns occur. Rainfall midway through the growing season was lower than normal and resulted in yield reductions for all dryland plots.

Aflatoxin accumulation in 1967 in fresh-dug kernels was highest in dryland samples at both locations (Table 2). Over 2,000 parts per billion (ppb) was detected in Yoakum dryland samples beginning at 120 days. Comparable samples from irrigated plots contained only traces of aflatoxin. Stephenville samples also contained aflatoxin, and dryland kernels were more contaminated compared to kernels from irrigated plots. Aflatoxin accumulation was detected later in the season (130 and 140 days after planting) at Stephenville. These results suggest that maturity levels are only partially responsible for the increased susceptibility of peanuts to *A. flavus* in the field. The interaction of temperature and moisture also plays an important role. Mature peanuts contain less moisture than immature peanuts. Moisture loss associated with maturity is attributed to water used in metabolic reactions and movement of moisture from the kernels into the atmosphere. The rate of kernel moisture loss was reduced at Stephenville where the soil temperatures were lower (a range of 20 to 32 C occurred at Stephenville compared to a range of 24 to 45 C at Yoakum). Temperature influenced not only fungal growth rate but also water removal rate and kernel susceptibility to *A. flavus* penetration.

Aflatoxin accumulation and fungal infestation were unaffected by test fungicidal treatments. Some fungicidal activity was apparent early in the growing season, especially in Polyram-treated plots which were less severely damaged by typical pod-rotting fungi. At harvest, however, pod rot differences were negligible. Environmental factors and microbial activity apparently exerted a greater influence than the fungicides on plant-fungal interactions.

Fungicide treatments failed to exert any statistically significant influence on peanut production or grade factors in plot samples harvested at Yoakum in 1967 (Table 3). However, significant differences in yield and grade determinations were detected between dryland and irrigated samples. Dryland peanuts averaged 375 lb/acre while irrigated peanuts averaged 2,853 lb/acre. Higher grades were noted in all irrigated samples.

Peanut quality was also influenced by soilborne fungi other than *A. flavus*. Kernel samples from Yoakum contained a high incidence of *Fusarium* (maximum 9%), *Chaetomium* (maximum 38%), *Penicillium* (maximum 44%), and *Rhizopus* (maximum 6%).

Observations in 1968. Differences between fungal infestation of dryland and irrigated fresh-dug kernels in 1968 (Tables 4 and 5) were less pronounced than in 1967. *A. flavus* incidence was low even in dryland samples. These findings are attributed to the fact that adequate rainfall during the growing season helped maintain vigorous plant growth. However, damp, warm soil conditions at Yoakum stimulated the activity of sev-

TABLE 2. *Aflatoxin detected in fresh-dug peanuts harvested from fungicide-treated dryland and irrigated plots (1967)*

Soil fungicide treatments	Yoakum						Stephenville					
	Dryland			Irrigated			Dryland			Irrigated		
	120[a]	130	140[c]	120	130	140	120	130	140	120	130	140
None	30[b]	400		0	0	0	0	68	27	0	0	22
PCNB	598	2,275		Tr	0	0	0	0	35	0	0	0
Polyram	2,147	303		Tr	0	0	0	0	17	0	0	30
Difolatan	Tr[d]	862		0	0	0	0	30	100	0	Tr	Tr
Avg	694	960		Tr	0	0	0	24	45	0	Tr	13

[a] Number of days after planting.
[b] Aflatoxin concentration expressed in parts per billion, average of two replications.
[c] Peanut kernels germinated in the soil, after a 4-inch rain.
[d] Trace.

TABLE 3. *Production and grade factors of peanuts from fungicide-treated dryland and irrigated plots at Yoakum, Texas (1967)*

Soil fungicide treatments	Production (lb/acre)		Sound[a] mature kernels (%)		Other[b] kernels (%)		Damaged[c] kernels (%)		Hulls (%)	
	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated
None	360	3,016	66	71	6.0	4.5	0.5	0.5	27.5	24.0
PCNB	414	3,006	64	69	6.5	6.0	0.5	0.0	29.0	25.0
Polyram	447	2,690	63	71	7.0	4.0	1.0	0.5	29.0	24.5
Difolatan	207	2,701	60	69	7.5	5.5	1.0	0.5	31.5	25.0
Avg	357	2,853	63	70	6.8	5.0	0.8	0.4	29.2	24.6

[a] Sound mature kernels were obtained by passing the kernels over a $^{15}/_{64}$ by $^{3}/_{4}$-inch slotted screen with a $^{1}/_{4}$-inch stroke and a 20-sec vibration period.
[b] Other kernels pass through the screen described in footnote *a*.
[c] Damaged kernels included mold infested or discolored kernels which ride the screen.

TABLE 4. *Aflatoxin detected in fresh-dug peanuts from fungicide-treated dryland and irrigated plots at Stephenville and Yoakum (1968)*

Fungicide treatments		Yoakum					Stephenville			
		Dryland			Irrigated		Dryland		Irrigated	
Soil	Foliage	120[a]	130	140	120	130	130	140	130	140
None	Kocide	Tr[b]			17		7	6		Tr
MBR	Kocide	5[c]		13		50				
Polyram	Polyram	Tr				12				
PCNB	Chloro-thalonil			Tr					12	Tr
PCNB	Benomyl		Tr	Tr		12			Tr	Tr
PCNB	Kocide	Tr		7						8

[a] Number of days after planting.
[b] Trace.
[c] Aflatoxin concentration expressed as parts per billion, average of two replications.

TABLE 5. *Production and grade factors related to peanuts from fungicide-treated dryland and irrigated plots at Yoakum, Texas (1968)*

Fungicide treatments		Production (lb/acre)		Sound[a] mature kernels (%)		Other[b] kernels (%)		Damaged[c] kernels (%)	
Soil	Foliage	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated
None	Kocide	1,629	1,799	67.8	67.5	4.8	5.5	2.5	1.8
MBR	Kocide	1,472	1,516	67.0	66.2	4.5	6.8	3.2	1.8
Polyram	Polyram	1,943	2,078	68.8	67.2	4.8	6.5	1.5	1.2
PCNB	Chlorothalonil	1,838	1,768	69.0	68.8	4.5	5.8	1.5	0.8
PCNB	Benomyl	1,847	1,590	71.8	67.2	3.5	6.2	0.8	0.8
PCNB	Kocide	1,664	1,764	71.0	68.5	4.2	6.2	0.5	0.8
Average		1,732	1,752	69.2	67.6	4.4	6.2	1.7	1.2

[a] Sound mature kernels were obtained by passing the peanuts over a $^{15}/_{64}$ by $^{3}/_{4}$-inch slotted screen with a $^{1}/_{4}$-inch stroke and a 20-sec vibration period.
[b] Other kernels included those sound immature kernels which pass through the screen.
[c] Damaged kernels included those mold-infested or discolored kernels removed from the sound mature kernels.

eral other fungi such as *Alternaria*, *Chaetomium*, *Diaporthe*, *Fusarium*, *Penicillium*, *Rhizoctonia*, and *Thielavia*. As a result, visible kernel damage was noted in the 140-day Yoakum samples. In comparison, Stephenville kernels generally were free of visible mold damage; however, some species of *Alternaria*, *Fusarium*, *Penicillium*, and *S. rolfsii* were isolated. Such infestation may cause visible kernel damage, but more frequently it is superficial, either on the cotyledon surface, below the testa, or between the cotyledons.

Since *A. flavus* activity appeared to be restricted in 1968, one would also expect aflatoxin contamination to be low. This was borne out by the observation that the highest aflatoxin (50 ppb) was detected in one irrigated treatment from Yoakum (Table 4). The dryland peanuts were superior in grade (Table 5) with an average of 69.2% sound mature kernels (SMK) compared to 67.6% SMK for irrigated samples. The lower grade for irrigated samples is primarily attributed to a high percentage of immature kernels (6.2%).

Observations in 1969. *A. flavus* infestation in fresh-dug peanuts was much higher in 1969 than in 1968 (Table 6). Infestation at Yoakum was highest in 120- and 130-day dryland samples and lowest in the 140-day samples. Many pods in the 130-day dryland plots were covered with spores of *A. flavus*. These high infestations occurred during a drought period with an apparent reduction in infestation following a period of showers. The reduction is attributed to the increased soil

TABLE 6. *Isolation frequency of Aspergillus flavus from peanut kernels harvested from fungicide-treated dryland and irrigated plots at Stephenville and Yoakum, Texas (1969)*

Fungicide treatments		Yoakum						Stephenville					
		Dryland			Irrigated			Dryland			Irrigated		
Soil	Foliage	120[a]	130	140	120	130	140	120	130	140	120	130	140
None	M45	33[b]	37	16	48	16	0	0	1	0	0	0	0
PCNB	Fungi Sperse	6	19	6	10	10	1	2	3	2	0	0	0
PCNB	Chlorothalonil	7	19	7	48	59	2	10	1	2	0	0	0
Polyram	Benomyl	20	10	3	0	3	4	2	2	18	0	1	0
Polyram carboxin	Chlorothalonil	32	74	13	4	2	0	16	3	10	0	1	0
Benlate	M45	30	9	3	11	5	0	2	0	6	0	0	0
Average		21	28	8	20	16	1	5	2	6	0	0	0

[a] Number of days after planting.

[b] Isolation frequency expressed as percentage of kernels from which *Aspergillus flavus* grew on nutrient agar surface.

TABLE 7. *Aflatoxin detected in fresh-dug peanuts harvested from fungicide-treated dryland and irrigated plots at Stephenville and Yoakum, Texas (1969)*

Fungicide treatments		Yoakum						Stephenville					
		Dryland			Irrigated			Dryland			Irrigated		
Soil	Foliage	120[a]	130	140	120	130	140	120	130	140	120	130	140
None	M45	80[b]	0	2,240	0	0	0	0	Tr	0	0	Tr	0
PCNB	Fungi Sperse	0	234	0	0	0	0	0	Tr	0	0	0	0
PCNB	Chloro-thalonil	0	1,251	0	0	0	0	0	0	0	0	0	0
Polyram	Benomyl	35,800	11,700	0	0	Tr[c]	0	0	0	0	0	0	Tr
Polyram carboxin	Chloro-thalonil	0	7,020	0	0	Tr	0	0	0	0	0	0	0
Benlate	M45	35,000	0	0	0	0	0	0	0	0	0	0	0
PCNB	M45	0	12,000	2,240	0	Tr	0						

[a] Number of days after planting.

[b] Aflatoxin concentrations expressed as parts per billion.

[c] Trace.

moisture which stimulated the activity of several peg- and pod-rotting organisms. As a result, overmature pegs and pods were damaged and lost during the digging process. Many kernels in such pods were undoubtedly invaded by *A. flavus*. Several irrigated kernel samples were also infested (maximum 59%) with *A. flavus*.

Significant aflatoxin levels were detected in fresh-dug Yoakum dryland samples in 1969 (Table 7). The highest levels were detected in the 120-day dryland samples; however, a larger percentage of the 130-day samples contained aflatoxin. Six kernels selected from the highly infested 130-day dryland plants contained 725,000 ppb aflatoxin. In general, those kernels found to be highly infested with *A. flavus* were more mature and had dried while in the soil. Aflatoxin was not detected in the irrigated kernels, although many kernels were infested. Kernels from the irrigated plots had reached maturity levels somewhat similar to those of the dryland plants; therefore, higher aflatoxin levels in dryland samples is attributed to moisture differences in such kernels.

Environmental conditions influenced the degree of *A. flavus* infestation and amount of aflatoxin in samples harvested at Stephenville in 1969. Only traces of aflatoxin were detected in these samples; however, up to 18% of the kernels were infested with *A. flavus* (Table 6). A lack of aflatoxin accumulation in these kernels is attributed to low soil temperatures (10 to 21 C) and to slightly higher kernel moisture levels. It should be noted that kernel moisture levels vary considerably in peanuts harvested from a single plant. Observations reported in this paper are based on average moisture levels.

From our results it would appear that where the kernel moisture averages are above 30% or below 10% *A. flavus* activity is restricted. Also, for extensive invasion of the peanuts to take place before digging, there must be a fairly high level of *A. flavus* inoculum present within the pegging zone. After invasion, aflatoxin accumulation is dependent on the physical and chemical characteristics of the kernels and environmental conditions which regulate *A. flavus* activity. Overmature or damaged peanuts are most likely to become invaded and aflatoxin-contaminated. Such contamination is in part determined by moisture content of the kernels. However, if moisture contents remain within the optimum for *A. flavus* growth and temperatures fall below optimum, little aflatoxin accumulates. These observations indicate that moisture and temperature levels, combined with damaged pods, are primary factors in aflatoxin contamination.

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