Relationship of Harvesting Methods and Laboratory Drying Procedures to Fungal Populations and Aflatoxins in Peanuts in Oklahoma

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ABSTRACT

Dominant fungi in harvested peanut pods in 1965 were *Fusarium* spp., *Penicillium* spp., and *Alternaria tenuis*. *Fusarium* spp., *Trichoderma viride*, and mucoraceous species (*Rhizopus* sp. and *Mucor* sp.) were dominant in 1966. *Aspergillus flavus* was rarely isolated either year. Populations from machine-combined pods were not significantly different from those of hand-picked pods except for mucoraceous species, which were greater in combine-harvested pods. Aflatoxins were found in all 1965 samples, but only in a few 1966 samples. Aflatoxins were not correlated with presence of *A. flavus*, but were correlated with isolations of *A. tenuis*. Phytopathology 61:1180-1184.

Many fungi invade developing pods of the peanut (*Arachis hypogaea* L.) in the soil (3, 9, 11, 17, 24). Smaller and regionally different populations occur in harvested pods (3, 5, 12, 14, 15, 16, 24, 26). Some of these fungi are known, or suspected, toxin producers (4, 10), and pose a potential health problem. Foremost of the toxin producers are *Aspergillus flavus* Link ex Fr. and *A. parasiticus* Speare, strains of which produce highly toxic metabolites (aflatoxins) (7, 25, 33) that are sometimes carcinogenic to domestic and experimental animals (4, 29, 33, 38). Invasion of peanut pods by *A. flavus* and production of aflatoxins have been associated with soil insects (32), nematodes (22, 23), growth cracks (31, 32), over-maturity (8, 14), and damage by machinery (20, 31). *Aspergillus flavus* is sometimes found in nondamaged pods, but nondamaged mature kernels are usually free of aflatoxins (9).

This report evaluates harvesting methods and drying procedures in relation to invasion of freshly harvested and field-dried peanut pods by fungi and subsequent aflatoxin production.

MATERIALS AND METHODS.—Mature Starr cultivar peanut pods were harvested at the Caddo Peanut Research Station, Ft. Cobb, Okla., during 1965 and 1966. Though rainfall and irrigation were approximately equivalent each season, most of the 1965 rains fell in September. Plants were dug with a 2-row digger and windrowed 145 days after planting. Twenty- to 25-lb. samples of pods were immediately hand-picked from plants in center sections of each of four replicated and randomized windrows. Four other windrows were immediately machine-combined, and similar samples obtained from the collecting bin at the end of each center section. Remaining rows were dug, windrowed, and left to dry until kernel moisture dropped to ca. 25%. Hand-picked and combined samples were obtained as described above. All samples were taken to Stillwater in an air-conditioned car, stored overnight at 5 C, and the following morning prepared for laboratory studies.

Each sample was washed free of soil; all immature and rotted pods were discarded. Each prepared sample was divided into two subgroups. One was dried in a forced draft oven at 40 C to 4-5% kernel moisture; the other was similarly dried at 25 C. After drying, 50 pods were randomly taken from each subgroup for mycofloral determination. The pods were surface-disinfected with 10% Clorox (0.525% NaOCl) for 3 min, and 25 were plated on a slightly modified Gilpatricks' RB-M2 modification (35) of Martin's rose bengal agar (only 0.02 g of dye/liter was used in our study). Kernels from the remaining 25 pods were aseptically removed, half were placed directly on our modified RB-M2, and half were surface-disinfected first, then plated. Single half shells from each pod were also plated. Our modified RB-M2 suppressed both bacterial and fungal growth; hence, even very slow-growing fungi were detected and isolated. All plates were held for 7 days at 25 C. Every fungus colony, regardless of similarity, growing on or from plated tissues was isolated, coded, and cultured on our RB-M2 and peptone-dextrose agar. After microscopic study, a culture of each apparent species was sent to R. T. Hanlin (Univ. Georgia, Athens) for final identification. Unused pods were sent to T. C. Campbell (Va. Polytechnic Inst., Blacksburg) for aflatoxin analyses. Only kernels were sent in 1966. Analyses of ca. 2 ppb sensitivity were done with a modified (6) method of Pons & Goldblatt (27). Necessary confirming tests were done according to the method of Andrellos & Reid (1).

RESULTS.—In 1965, regardless of harvesting methods and subsequent drying procedures, dominant fungi in whole pods and half shells were *Fusarium* spp, *Penicillium* spp, and *Alternaria tenuis* Auct. (Tables 1, 2). Dominants in 1966 were *Fusarium* spp., mucoraceous

TABLE 1. Per cent fungi isolated from oven-dried whole pods of Starr cultivar peanuts sampled without preliminary field-drying in Oklahoma

	Machine combined at digging							Hand picked at digging							
	1965			1966			Combining avg	1965			1966			Hand picking avg	Grand avg
Fungi	25 C	40 C	Avg	25 C	40 C	Avg		25 C	40 C	Avg	25 C	40 C	Avg		
Fusarium spp.	62	53	58	63	38	51	55	45	42	44	74	59	67	56	56
Penicillium spp.	2	11	7	0	0	0	3	20	8	14	0	0	0	7	6
Alternaria tenuis Auct.	10	20	15	2	5	4	10	17	30	24	1	8	5	15	13
Mucoraceous spp.	2	8	5	8	32	20	13	1	2	2	<1	7	4	3	8
Sclerotium bataticola Taub.	6	4	5	<1	2	1	3	6	6	6	1	7	4	5	4
Rhizoctonia solani Kuehn	5	3	4	4	3	4	4	2	1	2	1	5	3	3	4
Aspergillus niger v. Tiegh.	2	1	2	<1	<1	<1	1	2	2	2	0	<1	<1	1	1
Trichoderma viride Pers. ex Fr.	0	<1	<1	17	8	13	7	0	0	0	17	5	11	6	7
Aspergillus flavus Link ex Fr.	0	0	0	0	0	0	0	0	<1	<1	0	0	0	<1	<1

TABLE 2. Per cent fungi isolated from oven-dried whole pods of Starr cultivar peanuts sampled after field-drying in Oklahoma

	Machine combined after field drying							Hand picked after field drying							
	1965			1966			Combining avg	1965			1966			Hand picking avg	Grand avg
Fungi	25 C	40 C	Avg	25 C	40 C	Avg		25 C	40 C	Avg	25 C	40 C	Avg		
Fusarium spp.	39	42	41	49	45	47	44				44	33	39	39	42
Penicillium spp.	4	7	6	<1	3	2	4				<1	<1	<1	<1	2
Alternaria tenuis Auct.	41	36	39	4	2	3	21				7	5	6	6	14
Mucoraceous spp.	<1	0	<1	14	4	9	5				9	6	8	8	7
Sclerotium bataticola Taub.	0	0	0	4	5	5	3				4	8	6	6	5
Rhizoctonia solani Kuehn	0	9	5	4	4	4	5				3	8	6	6	6
Aspergillus niger v. Tiegh.	0	0	0	5	8	7	4				5	10	8	8	6
Trichoderma viride Pers. ex Fr.	0	0	0	10	9	10	5				19	19	19	19	12
Aspergillus flavus Link ex Fr.	0	0	0	0	0	0	0				0	0	0	0	0

TABLE 3. Parts per billion (ppb) of aflatoxins found in oven-dried whole pods of Starr cultivar peanuts sampled without preliminary field-drying in Oklahoma[a]

Aflatoxins	Machine combined at digging			Hand picked at digging			Grand avg
	25 C	40 C	Avg	25 C	40 C	Avg	
Aflatoxin B_1	21	15	18	92	73	83	50.5
Aflatoxin B_2	0	0	0	0	0	0	0.0
Aflatoxin G_1	0	2	1	27	0	14	7.5
Aflatoxin G_2	0	0	0	0	0	0	0.0
Total	21	17	19	119	73	96	58.0

[a] Data of 1965.

species, and *Trichoderma viride* Pers. ex Fr. (Tables 1, 2). *Aspergillus flavus* was rarely isolated in 1965, and not at all in 1966. *Aspergillus parasiticus* was not isolated. Fungi were rarely isolated from kernels. When results of both seasons were averaged, no significant differences in populations within freshly harvested pods were found, regardless of harvesting and drying methods (Table 1). In field-dried pods, more *A. tenuis* was found in combine-harvested pods than in hand-picked pods, whereas more *T. viride* was found in hand-picked pods (Table 2). More *Fusarium* spp. were isolated from 25 C-dried pods than from 40 C-dried pods in the freshly harvested samples. There were no significant differences in populations between pods dried at both temperatures in the pods previously field-dried (Table 2).

Samples of freshly harvested whole pods in 1965 contained 10-168 ppb aflatoxins (Table 3). Statistically greater amounts of aflatoxin B_1 occurred in hand-pulled pods than in machine-combined pods (Table 3). More B_1 occurred in 25 C-dried pods than in 40 C-dried pods. Only traces (< 3 ppb) of aflatoxins were found in kernels from freshly harvested pods in 1966. Slightly greater amounts (< 25 ppb) occurred in kernels from field-dried pods (Table 4).

DISCUSSION.—Dominant and subdominant fungus populations from harvested peanut pods in Oklahoma (see RESULTS) were similar to those reported from other states and Israel (5, 12, 14, 15, 16, 24, 26). *Aspergillus flavus* was dominant or subdominant in Texas (24, 25), but it was rarely isolated in Oklahoma. The culture medium was not specified in one Texas report (24), but was the same in the other report as that used in Oklahoma and Georgia (16). *Aspergillus flavus* is considered ubiquitous in soils long planted to peanuts. Our plots were planted to peanuts only 1 year previously; hence, a typical *A. flavus* population may not have developed. Subdominants and minor components vary between states and regions. Whereas *T. viride* was a dominant in Oklahoma in 1966, it was a minor component in 1965 and in Texas (24, 25). *Chaetomium* spp. and *Sclerotium bataticola* (*Macrophomina phaseoli*) were subdominants in Georgia (16) and Texas (24, 25), but were of minor importance in Oklahoma. *Alternaria* spp. were isolated more often in Oklahoma (3) and Texas (24, 25) than in Georgia (16). Differences in soils, climate, weather, cultural practices, and peanut cultivars may account for the differences. *Fusarium* spp. and mucoraceous fungi were isolated frequently in all three states. They are probably ubiquitous in mature pods. The near absence of *A. flavus* from pods, and absence from kernels, in Oklahoma suggests that it was a minor component of the soil fungal population or that it does not readily invade nondamaged shells and kernels. Similar results are reported (2, 21). Thus, instances of high *A. flavus* invasion may occur after field-drying, perhaps when pods are held in bulk prior to final drying to a safe moisture content.

The occurrence of aflatoxins in all of the 1965 samples as opposed to occurrence in only a few samples in 1966 is startling, but there is a likely explanation Whole pods were analyzed in 1965, whereas only kernels were analyzed in 1966. Perhaps aflatoxins accumulate more readily in shells of whole pods than in kernels because the shells are invaded first. The reported aflatoxins may also have been false positives due to fluorescing materials in the shells.

Greater amounts of aflatoxins in pods dried at 25 C

TABLE 4. Parts per billion (ppb) of aflatoxins found in oven-dried kernels from Starr cultivar peanut pods sampled after field-drying in Oklahoma[a]

Aflatoxins	Machine combined after field-drying			Hand picked after field-drying			Grand avg
	25 C	40 C	Avg	25 C	40 C	Avg	
Aflatoxin B_1	13	7	10	0	2	1	6.0
Aflatoxin B_2	2	0	1	0	0	0	0.5
Aflatoxin G_1	8	0	4	0	0	0	2.0
Aflatoxin G_2	2	0	1	0	0	0	0.5
Total	25	7	16	0	2	1	9.0

[a] Data of 1966.

than in those dried at 40 C were apparently the result of more favorable conditions for mold growth at the lower temperature.

The occurrence of more aflatoxins in hand-picked pods than in machine-combined pods is contrary to reports from Georgia (15). Apparently, our combining operation resulted in very little damage. These data illustrate the importance of proper combine adjustment during harvesting.

There was no correlation between the presence of aflatoxins and *A. flavus,* which was isolated only rarely in very few samples. Aflatoxins may have been produced by fungi other than *A. flavus.* Additional species of *Aspergillus,* several *Penicillium* species, and a *Rhizopus* sp. are reported to produce aflatoxins (7, 13, 18, 25, 36), though this claim has been challenged (37). It is possible that *A. flavus* was present to a greater extent in the samples but was not detected. This seems unlikely, as the same isolation technique has detected *A. flavus* elsewhere (11, 12, 15, 16, 25). The reported aflatoxins may have been false positives caused by other fungal or bacterial metabolites that also fluoresce blue-green under ultraviolet light. It is possible to mistake fluorescence of various materials for aflatoxins unless chemical or biological confirmation tests are made. *Alternaria tenuis* was a dominant fungus in the 1965 samples which provided many positive aflatoxin results. *Alternaria tenuis* produces alternariol and related compounds that fluoresce blue-green under ultraviolet light (28, 34). The correlation of a high population of *A. tenuis* with such a fluorescence suggests a causal relationship. Such a relationship has been found with *M. phaseoli* (*S. bataticola*) (30). Metabolites of *A. tenuis* in peanuts should be investigated, because alternariol and related compounds are isocoumarins. Some compounds containing an isocoumarin structure are carcinogenic (19), and pose a health threat. *Alternaria* spp. from peanuts and other foods have been demonstrated to be lethal to rats (10).

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