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Effects of Soil Moisture and Temperature on Preharvest Invasion of Peanuts by the *Aspergillus flavus* Group and Subsequent Aflatoxin Development

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Four soil temperature and moisture treatment regimens were imposed on Florunner peanuts 94 days after planting in experimental plots in 1980. At harvest (145 days after planting), the incidence of the *Aspergillus flavus* group and the aflatoxin concentration were greatest in damaged kernels. Extensive colonization of sound mature kernels (SMK) by the *A. flavus* group occurred with the drought stress treatment (56% kernels colonized); colonization was less in the irrigated plot (7%) and the drought stress plot with cooled soil (11%) and was intermediate in the irrigated plot with heated soil (26%). Aflatoxin was virtually absent from SMK with the last three treatments, but it was found at an average concentration of 244 ppb (ng/g) in drought-stressed SMK. Colonization of SMK by the *A. flavus* group and aflatoxin production were greater with hot dry conditions. Neither elevated temperature alone nor drought stress alone caused aflatoxin contamination in SMK. When the ratio of SMK colonized by *A. flavus* compared with *A. niger* was > 19:1, there was aflatoxin contamination, but there was none if this ratio was < 9:1. Irrigation caused a higher incidence of *A. niger* than drought did. This may have prevented the aflatoxin contamination of undamaged peanuts.

Aspergillus flavus Link and the closely related fungus *A. parasiticus* Speare are two species of fungi that are capable of invading peanut plants and fruit (14, 17). Hereafter, *A. flavus* refers to both species. They cause extensive economic losses either by destroying the plant or by contaminating peanut kernels with the aflatoxins. After aerial fertilization of the peanut flower and extension of the gynophore into the soil, the peanut fruit develops in a subterranean environment and may be invaded by many species of soil microorganisms during its subsequent growth and development. Environmental conditions and management practices during production, harvest, handling, and storage may affect the nature and degree of mycofloral contamination (6, 8). Despite the presence of *A. flavus* in the soil during the development of the peanut fruit, extensive invasion by *A. flavus* and contamination with the aflatoxins rarely occur unless the peanut plant is subjected to environmental stress or the fruit is damaged. Two major environmental factors that affect *A. flavus* invasion and aflatoxin contamination of the peanut fruit during growth and development are drought stress and insect damage. Several studies have associated extensive preharvest invasion of peanuts by *A. flavus* and subsequent aflatoxin contamination with severe drought stress and insect damage during the latter part of the growing season (4, 10, 13). Under such conditions, damaged and sound peanuts were invaded by *A. flavus* and contaminated with aflatoxins in the soil before harvest. Additionally, in years in which severe droughts occurred in peanut-producing areas in the southeastern United States (1972, 1978, and 1980), there were also severe economic losses owing to aflatoxin contamination in peanuts. Peanuts grown under drought stress may also be predisposed to subsequent aflatoxin in contamination during harvest, handling, or storage (5).

Studies to delineate the specific environmental factors responsible for preharvest contamination of peanuts by *A. flavus* and aflatoxin were conducted with six unique research plots designed to control and monitor soil temperature and moisture (2). Temperature and the availability of water are the most important factors controlling the growth of microorganisms and plants (12). Independent control of soil temperature and moisture was a major advantage of the experimental plots. This paper presents the results of our first experiments.

MATERIALS AND METHODS

Experimental plot facility. The research plots contained soil of the Tifton loamy sand type obtained from the top 15 cm of a local peanut field. Soil from each plot was analyzed for major and minor plant nutrients by Waters Agricultural Laboratory and Consulting Company, Camilla, Ga. Adjustments in fertility were made as needed. Florunner cultivar peanuts were planted on May 10 1980 in a 92-cm row pattern. Fungicides, herbicides, and insecticides were applied as necessary at the rates recommended by the manufacturers. Spray applications of Bravo (chlorothalonil) to control leafspot were made on May 30, June 13 and 26, July 9 and 21, August 5, 12, and 19, and September 3. Terrachlor (pentachloronitrobenzene) for white mold control was applied on July 21. On May 9, the preplant herbicides Vernam (vernolate) and Balan (benefin) were applied, followed by a preemergence application of Lasso (alachlor) and Dyanap (naptalam and dinoseb) on May 20. Insecticides applications were: Azodrin (monocrotophos) to control spider mites on July 30, August 5, 12, and 19; Dasanit (fensulfothion) for wire worm control on July 21; Nudrin (methomyl) to control corn earworm on July 28; Omite (propargite) for spider mite control on August 22 and September 3; parathion to control the lesser corn-stalk borer on July 2 and 24 and September 12 and 15; Sevin (carbonyl) for corn earworm control on June 17 and 26; and toxaphene to control thrips on May 30. Sulfur, at the rate of 3.2 kg per hectare (10,000 m^2) was applied with each Bravo treatment.

To prevent lateral movement of soil moisture, each of the six research plots (5.5 by 12.3 m in area by 2.5 m deep) was completely encased in a drainage bed of gravel. Moisture from precipitation was excluded from the plots by moisture-sensor-equipped mechanized roofs that closed automatically to cover the plots when precipitation began. Irrigation was provided to the plots as needed when moisture tension reached 0.6 bar (60 kPa), as measured with a tensiometer (Irrometer Company, Riverside, Calif.) at a soil depth of 30 cm. All plots were provided with adequate soil moisture for 94 days after planting, when the different regimens were imposed. Soil moisture tension under and between the rows at 5, 30, and 60 cm below the surface was measured with Delmhorst gypsum blocks (Delmhorst Instrument Co., Boonton, N.J.) throughout the growing season. In each plot, there were at least 10 moisture sensors at each depth.

One plot was equipped with thermostatically controlled lead-shielded heating cables placed at a depth of approximately 10 cm to increase soil temperatures in the geocarposphere. Another plot was equipped with 6.35-mm copper tubing coated with chemical-resistant epoxy paint, and water was circulated through the coils periodically to reduce soil temperatures in the geocarposphere. The soil temperatures of the other four plots remained at ambient temperatures. Soil temperatures under and between the rows at 5, 30, and 60 cm below the surface were measured with copper constantan thermocouples. Soil temperature and moisture data were automatically recorded on cassette tapes every 2 h throughout the growing season with a 500-channel data collection system (model 9302, Monitor Laboratory, San Diego, Calif.).

Treatment regimens. Cultural practices were identical for all plots with ample moisture provided by irrigation until 94 days after planting, when four different regimens were imposed. Treatments were irrigated (I; 2 plots); irrigated with increased soil temperature (IH; 1 plot); drought stressed (D; 2 plots); and drought stressed with reduced soil temperatures (DC; 1 plot).

Assessment of the microflora. Numbers and kinds of fungi on and within peanut kernels at harvest (145 days after planting) were estimated by plating out both untreated and surface-sterilized (0.5% sodium hypochlorite solution [Clorox], 5 min) material on 2% malt extract agar with and without 10% NaCl added and incubating at 25 and 37°C. Fungi were identified by genus and species with emphasis on the genera *Aspergillus* and *Penicillium*. Actinomycetes and bacteria were recorded but not classified in most cases. In addition to kernels, the microfloras of peanut flowers, pegs, leaves, roots, stems, pods, and developing fruits were assessed at intervals during the growing season. Soil, rhizosphere, and geocarposphere microfloras were also studied. Results, presented here, are for *A. flavus* and *A. niger* from harvested kernels only.

Harvesting, shelling, and grading the peanuts. Peanuts were dug by hand 143 days after planting and were placed in a windrow to dry for two days. The windrow-dried peanuts (approximately 20 to 25% moisture) were fed manually into a commercial peanut combine. Those peanuts that were shelled during combining (loose shelled kernels [LSK]) for each treatment were separated from unshelled peanuts before shelling and were analyzed as a distinct category.

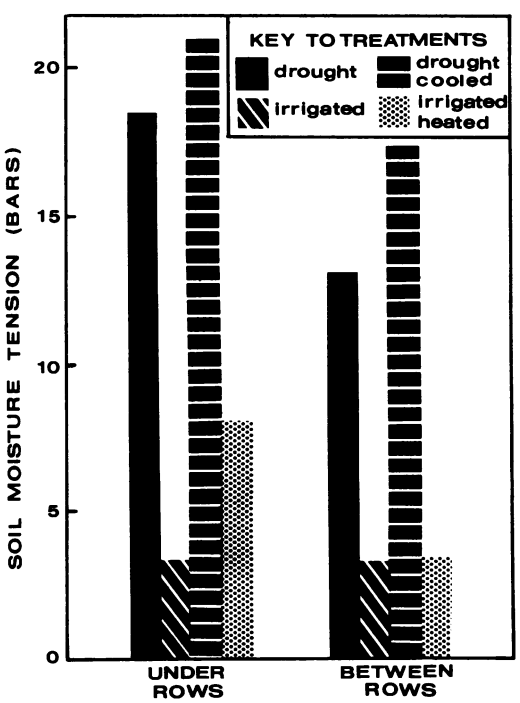

FIG. 1. Mean soil moisture tension 5 cm below soil surface for treatment period.

TABLE 1. Mean geocarposphere temperatures during the treatment period (94 to 145 days after planting) for the four treatment regimens

Treatment	Mean temp (°C)					
	Maximum		Overall		Minimum	
	Under rows	Between rows	Under rows	Between rows	Under rows	Between rows
I	30.5	32.1	25.1	25.3	20.3	20.6
D	34.9	44.6	28.4	29.6	22.5	20.8
IH	38.7	40.3	34.1	34.8	30.3	30.3
DC	33.6	37.0	24.4	25.2	19.6	19.5

Peanuts were placed in ventilated bags and dried at ambient air temperature (21 to 35°C) until the moisture level was less than 10% (5 days). Cured peanuts were then shelled with a model 4 National Peanut Research Laboratory sheller described by Davidson et al. (3) and screened into commercial grade categories before the degree of mold invasion was determined and the aflatoxin was analyzed. These grade categories were LSK, jumbo, medium, number 1, other edible, and oil stock.

Analysis for aflatoxins. Aflatoxin analyses were performed on samples by means of the minicolumn method of Holaday and Lansden (7) followed by high-pressure liquid chromatography (R. J. Cole and J. W. Kirksey, unpublished). High-pressure liquid chromatography was performed with a Waters Associates chromatography system equipped with two M-6000A pumps, a WISP 710 B autoinjector, a data module, and a systems controller. Aflatoxins were separated with a Waters radial compression module containing a Radial-Pak Silica Gel column and a solvent system consisting of water-saturated chloroform supplemented with 0.6% methanol. A flow rate of approximately 2.0 ml/min or a flow rate sufficient to elute all four aflatoxins within 7 min was used. The aflatoxins were detected with a Varian Flurochrom fluorescence detector. Standard aflatoxin mixtures were obtained from Applied Science Laboratories, State College, Pa., for qualitative and quantitative analyses.

Data presented for the drought and irrigated treatments are the average values of two replications each. All statistical analyses of data were made by the least squares analysis of variance (Statistical Analysis System 74 program).

RESULTS AND DISCUSSION

Soil temperature and moisture. The treatments imposed were designed to isolate the effects of soil temperature and soil moisture on *A. flavus* invasion of peanuts and subsequent aflatoxin contamination. Soil moisture and temperature data during the treatment period are reported in Fig. 1 and Table 1, respectively. Data collected 5 cm below the soil surface are reported herein as this depth corresponds to the fruiting zone of peanuts. Figure 1 shows the average soil moisture tension under and between the rows for each of the four treatments. Plant uptake of water and transpiration probably account for the consistently higher soil moisture tensions under the rows as compared with those between the rows.

Soil moisture tension in the I-treated plots was similar between and under the rows. A mean soil moisture tension of 3.3 bars at 5 cm below the soil surface in the I-treated plots seems to indicate relatively dry conditions; however, normally low (0.3 bars) soil moisture tension increased to very high levels just before irrigation, and the resulting mean value was disproportionately high. This phenomenon was accelerated with the IH treatment. The I- and IH-treated plots received water when tensiometers in the plots indicated 0.6 bars tension at ca. 30 cm below the surface.

Microflora and aflatoxin. At harvest, the incidence of the *A. flavus* group (88% of isolates *A.*

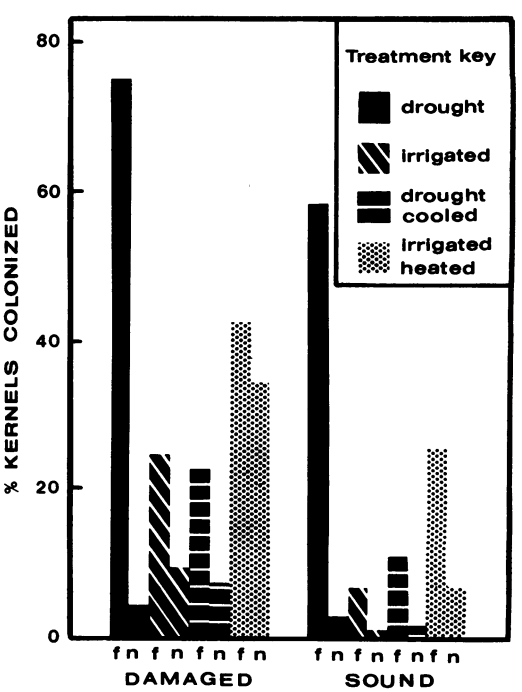

FIG. 2. Incidence of *A. flavus* group (f) and *A. niger* group (n) on peanuts at harvest.

TABLE 2. *Aspergillus* colonization and aflatoxin contamination of peanuts, Dawson, Ga.[a]

Peanuts (treatment/grade)[b]	*A. flavus* (% kernels colonized)	Total aflatoxin concn (ppb)	*A. flavus:A niger* (ratio of kernels colonized)
I/Edible	6.7	0	6:1
I/Other	24.9	122	3:1
D/Edible	55.9	243	29:1
D/Other	75.1	9,234	19:1
IH/Edible	26.1	0	4:1
IH/Other	42.5	4	1:1
DC/Edible	10.5	0	8:1
DC/Other	23.1	214	3:1

[a] Data are shown for time of harvest, 145 days after planting.
[b] Grades: Edible comprises jumbo, medium, number 1, and other edible; other comprises LSK, oil stock, and damaged.

flavus, 12% of *A. parasiticus*) inside kernels was increased by any kind of damage in all treatments (Fig. 2). In sound mature kernels (SMK), colonization by the *A. flavus* group was greatest in peanuts receiving the D treatment (56% kernels colonized), least with I (7%) and DC (11%) treatment, and intermediate with the IH treatment (26%). Extensive colonization of peanut fruit by the *A. flavus* group, a prerequisite for aflatoxin production, was favored by hot, dry conditions when most associated microorganisms failed to grow (because temperature or water activity or both became limiting) or grew only weakly. When the ratio of SMK colonized by *A. flavus* compared with *A. niger* was > 19:1, there was aflatoxin contamination, but there was none if this ratio was < 9:1 (Fig. 2, Tables 2 and 3). An antagonistic interaction between *A. flavus* and *A. niger* has been reported by Joffe for peanuts (9) and by Wicklow et al. for corn (18).

Peanut plants that have grown for approximately 100 days with adequate moisture overlap the row middles in such a fashion that the soil surface under and between the rows is shaded from direct sunlight. Peanut plants that are grown under severe and prolonged drought conditions during the last 4 to 6 weeks of the growing season recede and also lose their erect posture, exposing soil near the base of the plant, which is close to the geocarposphere. Exposure of the soil surface to direct sunlight causes an increase in temperature in the geocarposphere. The temperature differences in the geocarposphere between and under the rows (no shade versus shade) are evident in all treatments (Table 2).

The mean temperatures under and between the rows with the DC treatment were equal to or slightly below those with the I treatment; temperatures with the IH treatment were consistently higher than those with the D treatment. Figure 3 presents the data as percentages of observations of >25, >30, and >35°C. The percentages of observations at >30°C with the I and DC treatments were relatively low when compared with the D and IH treatments. This relationship was especially true of the observations of >35°C.

Data on aflatoxin contamination in the various commercial categories of peanuts are presented in Tables 2 and 3. The D treatment without soil temperature modification was the only treatment that contained aflatoxin in the edible grade peanuts (jumbo, medium, and number 1) (Tables 2 and 3). Only damaged peanuts from the I and IH treatments contained more than a trace of aflatoxin. This was also true, but to a lesser extent, with the DC treatment. All categories of peanuts from the D treatment contained unacceptable levels of aflatoxin, with between 2,000 and 23,000 ppb (ng/g) in edible, oil stock, and LSK categories (Table 3). Based on the data presented in Tables 2 and 3, several conclusions can be drawn. SMK of all grade categories grown with adequate moisture or cool soil temperature were free of aflatoxin contamination.

TABLE 3. Aflatoxin content of various commercial size categories

Seed size	Total aflatoxin concn (ppb)			
	I	D	IH	DC
Jumbo	0	29	0	0
Medium	0	127	0	0
Number 1	0	188	0	0
Other edible	0	629	Tr	0
Oil stock	0	2,109	0	11
LSK	0	2,692	0	38
Damaged	365	22,900	12	594

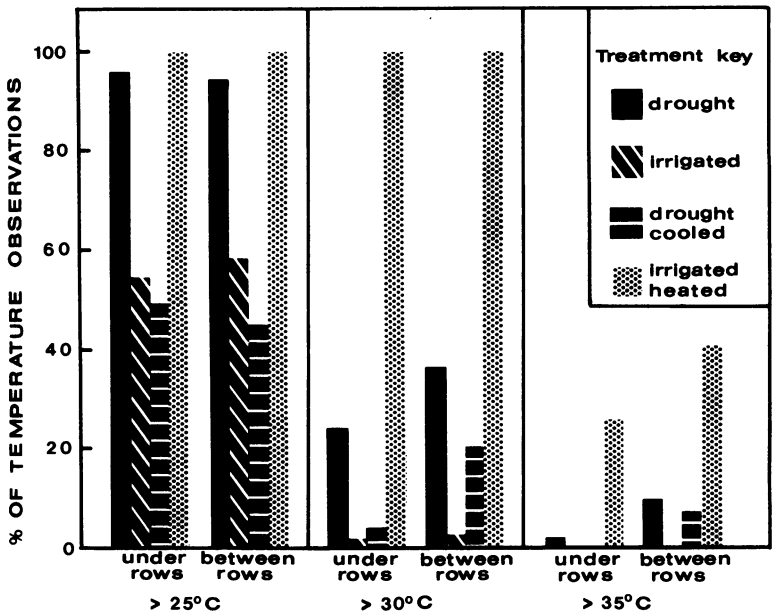

FIG. 3. Percentages of temperature observations of >25, >30, and >35°C 5 cm below soil surface for treatment period.

Therefore, the data strongly indicate that irrigation does effectively prevent aflatoxin contamination in SMK. Furthermore, under drought conditions, a reduction in mean geocarposphere temperature from 29.6 to 25.2°C resulted in no aflatoxin formation in the edible-grade peanuts and reduced levels in the oil stock and LSK categories. Therefore, under low-moisture conditions, the critical threshold temperature for aflatoxin contamination in the geocarposphere is between 25 and 28°C.

It is not known whether, under dry conditions, the elevated geocarposphere temperature predisposes the peanut fruit to contamination or whether other microbiological factors are involved. Cardinal values (maximal, optimal, and minimal) for growth and aflatoxin production, for water activity (a_w), and for temperature have been determined for *A. flavus*, and cardinal values for growth of *A. niger* and many associated species have been determined in laboratory studies (1, 6, 11, 12, 15). Whereas *A. flavus* and *A. niger* have very similar cardinal values for temperature, *A. flavus* will grow (minimum a_w, 0.78) when the a_w is too low to permit growth of *A. niger* (minimum a_w, 0.88) (12; M. D. Northolt, Ph. D. thesis, Landbouwhogeschool te Wageningen, Wageningen, The Netherlands). Thus, under hot, dry geocarposphere conditions, *A. flavus* is able to grow when growth of *A. niger* (and most other associated microorganisms) has ceased. When the a_w approaches the minimum for growth of *A. flavus*, such growth is only possible with temperatures at or close to the optimum (35°C). It is under these conditions, we suggest, that extensive invasion of immature peanuts by *A. flavus* and subsequent aflatoxin contamination occurs. By contrast, in hot, moist soil *A. niger* grows vigorously and either inhibits aflatoxin formation by *A. flavus* (18) or degrades any aflatoxin that is produced (16).

With cool dry conditions, both a_w and temperature are suboptimal for *A. flavus*, and neither extensive colonization of peanut kernels nor aflatoxin production occurs in SMK.

There appear to be two distinctly different types of aflatoxin contamination of peanuts grown under drought stress. One type of contamination is associated with damage, primarily that caused by soil inhabiting insects. This type is characterized by extremely high levels of aflatoxin (on the level of parts per million), and presumably the route of invasion is from the soil. The second type of contamination is characterized by moderate levels of aflatoxin (up to 1,000 ppb), more uniform contamination (present in all classes of peanuts), and no obvious damage; the mode of invasion is not known. The latter contamination is difficult to detect visually and difficult to remove by physical methods. Therefore, it is the type of contamination of primary concern.

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