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Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts

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Abstract

Samples of Florunner peanuts were collected throughout a period of late-season drought stress with mean geocarposphere temperatures of 29 and 25 °C, and determinations of maturity, kernel water activity (a_w), percent moisture, capacity for phytoalexin production, and aflatoxin contamination were made. Results showed an association between the loss of the capacity of kernels to produce phytoalexins and the appearance of aflatoxin contamination. Kernel a_w appeared to be the most important factor controlling the capacity of kernels to produce phytoalexins. Mature peanuts possessed additional resistance to contamination that could not be attributed solely to phytoalexin production. Kernel moisture loss was accelerated in the 29 °C treatment compared to the 25 °C treatment, and data indicated that the higher soil temperature also favored growth and aflatoxin production by *Aspergillus flavus* in peanuts susceptible to contamination.

Introduction

Preharvest aflatoxin contamination of peanuts (*Arachis hypogaea* L.) is a major economic problem of the peanut industry. *Aspergillus flavus* Link and *A. parasiticus* Speare are the two closely related species of fungi that invade peanuts and subsequently lead to their contamination with aflatoxins B_1, B_2, G_1, and G_2. These fungi hereafter are collectively termed *A. flavus* and the toxins collectively termed aflatoxin.

Preharvest aflatoxin contamination does not occur when peanuts are provided with adequate moisture throughout the growing season; however, a close association exists between late season drought and aflatoxin occurrence in peanuts before digging [4, 8, 14, 18–20, 26]. Studies have indicated that elevated soil temperatures in addition to drought are necessary for aflatoxin contamination to occur [4, 7]. Blankenship *et al.* [4] reported that peanuts subjected to drought stress with a mean soil temperature of 23.6 °C or lower were not contaminated, except in damaged and loose shelled kernel categories. Cole *et al.* [7] reported a lower soil temperature limit of between 25.7 and 26.3 °C for aflatoxin contamination of peanuts subjected to drought stress in the last 4–6 weeks of the growing season.

Preharvest contamination appears first, and is more concentrated in smaller, immature kernels. Commercial seed size categories, in order of decreasing size, include: jumbo, medium, number 1,

other edible, and oil stock, and the smaller sizes (other edible and oil stock) correspond to the more immature peanuts. Cole *et al.* [7] reported that oil stock peanuts contained 2600 ppb aflatoxin after 30 days of stress, whereas 40–50 days of stress were required for significant contamination of jumbos. Several studies have shown consistently that levels of both *A. flavus* invasion and aflatoxin contamination are higher in smaller, more immature kernels than in mature kernels [6, 7, 14, 21, 22].

Aflatoxin contamination is not always directly correlated with incidence of invasion by *A. flavus*. Sanders *et al.* [21] found that jumbo peanuts from a 40-day drought treatment had a 56% colonization rate by *A. flavus* with no aflatoxin contamination. In contrast, number 1 peanuts from a 30-day drought treatment had a colonization rate by *A. flavus* of only 32.7% but were aflatoxin-contaminated. Peanuts from irrigated plots were colonized by *A. flavus* at rates as high as 25% without aflatoxin contamination. Cole *et al.* [7] showed that 89% of kernels from a drought treatment were colonized with *A. flavus* with no aflatoxin contamination, whereas a drought treatment with 35% of kernels colonized had 45 ppb aflatoxin.

Since colonization or invasion of peanuts by *A. flavus* does not always result in aflatoxin contamination, Cole *et al.* [7] suggested that after invasion occurred, growth of the fungus and aflatoxin production could not occur until a natural resistance mechanism broke down as a result of environmental (water and temperature) stress. It was suggested as early as 1972 that resistance of immature peanut pods to fungi was due to phytoalexins produced in high quantities in response to fungal infection [23]. Although the chemical nature of the phytoalexins was not determined, it was shown that peanuts produced phytoalexins when challenged by several species of fungi, including *A. flavus*. Several stilbene phytoalexins subsequently were isolated from inbibed peanut seeds that were sliced and incubated in the dark [1, 15–17]. Wotton and Strange [27] showed that three of these compounds inhibited spore germination and hyphal extension of *A. flavus*.

If peanuts are protected from fungal proliferation by their capability of producing phytoalexins under ideal growing conditions (ample moisture), such a capability may be decreased or eliminated when severe drought occurs. However, there is no data to support this hypothesis. Peanut phytoalexins have been investigated with cured peanuts that were rehydrated before slicing and then incubated in a moist environment for several days before measuring phytoalexin production. Although large quantities of phytoalexins are produced in this manner, the capacity of kernels to produce phytoalexins under various field conditions remains unknown.

With the evidence suggesting phytoalexin production as a mechanism for natural resistance to contamination, a hypothesis was formulated to more fully explain the phenomenon of preharvest aflatoxin contamination of peanuts. The hypothesis is comprised of a central hypothesis and three sub-hypotheses. The central hypothesis is that phytoalexin production by peanut kernels is the natural resistance mechanism that breaks down under drought stress, allowing aflatoxin contamination to occur. The following subhypotheses serve as the basis for our experiments: (i) In preharvest peanuts, kernel water activity (a_w) is the primary factor controlling the phytoalexin-producing capacity of peanut kernels. As kernel a_w decreases, as a result of prolonged drought, the capacity of those kernels to produce phytoalexins also decreases and eventually is lost. (ii) Immature peanuts lose the capacity to produce phytoalexins sooner than mature peanuts, leading to earlier contamination of the smaller, more immature kernels. (iii) Soil temperature controls the rate at which a_w of kernels decreases and, therefore, the length of time natural resistance via phytoalexin production persists.

This paper reports the results of experiments designed to test the hypothesis by monitoring kernel a_w, moisture, capacity for phytoalexin production, and aflatoxin contamination in peanuts of different stages of maturity during a period of drought stress with two different mean soil temperatures.

Materials and methods

Culture and treatment of peanuts

Florunner peanuts were grown in the environmental control plot facility at the National Peanut Research Laboratory, Dawson, GA [3]. Conventional cultural practices were used in growing peanuts through 96 days after planting (DAP) at which time drought peanuts received a final irrigation. A control plot continued to receive optimal moisture and other recommended cultural practices. Two plots were equipped with thermostatically-controlled, lead-shielded heating cables to provide a mean soil temperature of ca. 29 °C (optimal for aflatoxin contamination [5]). The other three plots were equipped with heating cables as well as epoxy-coated copper tubing through which cool water was circulated as necessary to maintain a mean soil temperature of ca. 25 °C (not conducive for aflatoxin contamination [5]). Drought treatments were considered to begin with control of soil temperature at 103 DAP (7 days after final irrigation).

Sampling of peanuts

Samples were collected by hand-digging ca. 18 m of row beginning at 114 DAP in the irrigated control plot, and five other samples were taken periodically with a final sampling at 184 DAP. Samples from the two drought treatments were taken at ca. weekly intervals with the initial sample from the 29 °C treatment taken at 120 DAP (17 treatment days [TD]) and the final sampling at 162 DAP (59 TD). The initial sample from the 25 °C treatment was taken at 121 DAP (18 TD) and the final sampling at 183 DAP (80 TD). Immediately after digging, pods were hand-picked from the plants and damaged pods were separated from sound pods.

Categorizing maturity stages

Sound pods were placed in a wet impact blaster [25] to remove the exocarp and expose the color of the middle layer of the hull (mesocarp). Color and structural differences in the mesocarp are the basis for the hull-scrape maturity classification method [13, 24], and, on this basis, each pod was placed in one of five maturity stages. The stages in order of increasing maturity were yellow 1, yellow 2, orange, brown, and black.

Water activity and moisture determinations

Immediately after maturity classification, samples from each maturity stage were taken to determine a_w and percent kernel moisture. For a_w measurements, ca. 10 pods per maturity stage were hand-shelled, kernels split, and placed in sealed sample dishes at 25 °C until measurements were made with a model E2BFA Beckman hygroline sensor attached to a model VFB2 Beckman hygroline flat-bed recorder (Beckman Industrial Corp., Cedar Grove, NJ). The instrument was calibrated using saturated salt slurries according to instrument instructions. Plug-in units with a_w range of 0.50–1.00 were used, and measurements were made in an incubator adjusted to 25 $\pm$ 0.2 °C. For moisture determinations, triplicate 50-pod samples from each maturity stage were hand-shelled, kernels weighed, oven dried for 6 h at 130 °C, and reweighed to determine moisture loss [2]. Percent moisture was calculated as: (initial wet weight – final weight)/initial weight $\times$ 100.

Evaluation of phytoalexin-producing potential

Standards of known stilbene phytoalexins from peanuts were not available for quantitative purposes. Therefore, phytoalexins were elicited from Florunner peanut kernels, extracted, and purified as previously described by Aquamah *et al.* [1]. Liquid chromatographic (LC) analysis of the purified extract showed seven compounds presumed to be phytoalexins, because they were present only when peanuts were wounded and challenged with fungi [1, 15]. Proton nuclear magnetic resonance analyses of the three major compounds showed that they were stilbenes but chemically different

from those stilbene phytoalexins previously reported from peanuts [1, 15–17]. The chemical structures and biological properties of these phytoalexins currently are under investigation.

To measure phytoalexin-producing capacity of sampled peanuts, ca. 6 g of kernels (three replications) from each maturity stage were sliced in 1–2 mm thicknesses, distributed in open 60 mm-diameter tissue culture dishes, and dusted with spores of a non-aflatoxin-producing strain of *A. parasiticus* (CP461; SRRC 2043). The open dishes were incubated in the dark at 25 ± 1.0 °C for 4 days in sealed dessicators over unsaturated NaCl solutions of a_w corresponding to that determined for each maturity stage at digging, in order to maintain a_w during incubation comparable to that in the field.

Each sample was extracted by homogenization for 1 min in a Waring Blendor with a volume of 95% ethanol equalling 10× the weight of the sample. Extracts were filtered by gravity flow through Whatman #4 filter paper, and 10 ml of filtrate was evaporated to dryness with a rotary evaporator at 45 °C. Samples were redissolved in 2 ml of benzene, applied to a silica Sep-PAK (Waters Assoc., Milford, MA), and eluted with 5 ml of benzene followed by 5 ml of benzene-ethyl acetate (1:1, v/v). Twenty-five µl of the benzene-ethyl acetate eluate was analyzed by LC with a µ-porasil Radial-PAK cartridge (Waters Assoc.), a mobile phase of hexane: ethyl acetate: methanol (67:32:1, v/v/v), and UV detection at 335 nm. Comparative analysis of all samples for phytoalexins was achieved by summing area counts for peaks corresponding to the phytoalexins previously purified.

Aflatoxin analyses

The remaining peanuts in each maturity stage were dried, shelled, and analyzed for aflatoxin by the method of Dorner and Cole [12].

Results

Relationship between soil temperature and kernel moisture

Peanut kernels within the five maturity stages from the irrigated control plot maintained a high, steady moisture content throughout the experiment (Fig. 1). Although differences in moisture among the five maturity stages appear great, there was no difference in their a_w, which remained constant at 1.00. These peanuts consistently produced relatively high quantities of phytoalexins, and no sample contained significant aflatoxin contamination (> 5 ppb).

With time, the percent moisture of drought-stressed peanuts decreased, with earlier moisture loss occurring in the 29 °C treatment compared with the 25 °C treatment, particularly in the more immature stages (yellow 1, yellow 2, orange). However, the moisture content of kernels within a given maturity stage at any given time was not uniform. For example, in the 141 DAP (38 TD) sampling from the 29 °C treatment, the moisture content of kernels in the yellow 2 maturity stage ranged from 40.3% to 17.0% (a_w range of 1.00 to 0.92). The yellow 1 and orange stages had similar variations, whereas moistures in the mature stages (brown, black) were more uniform. This phenomenon was observed in both drought treatments, but the quantity of low-moisture kernels within a maturity stage at a given time was greater

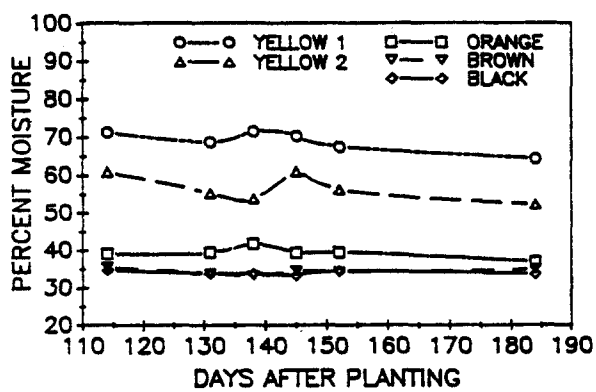

Fig. 1. Percent moisture of peanut kernels of five maturity stages from an irrigated control plot determined over the course of the experiment.

in the 29 °C treatment. In addition, in the yellow 2 and orange categories, pods containing very low moisture kernels (which had released from the pod) had a characteristic mustard-colored appearance after exocarp removal, which proved to be a consistently sound indicator of low kernel moisture. Kernels in the yellow 1, yellow 2, and orange maturity stages were, thus, further segregated based on visual assessment of kernel moisture. All kernels from mustard-colored pods were placed in one group, and kernels from normally-colored pods were separated into groups of high, medium, and low moisture before determinations of moisture, a_w, phytoalexin-producing potential, and aflatoxin contamination were made. These segregations were essential in testing the hypothesis that kernel a_w is the primary factor controlling phytoalexin-producing capacity. Any determinations made on a mix of peanuts over a wide range of a_w, obviously, would fail to reveal the relationship of a_w to the parameters of interest. Since a_w and moisture of peanuts in the brown and black maturity stages were generally quite uniform, it was not necessary to further segregate those kernels.

Comparisons of moisture content of particular maturity stages from the 29 and 25 °C treatments were, therefore, based on up to four moisture contents. For example, in the yellow 2 group from the 29 °C treatment sampled at 148 DAP (45 TD), the moisture content of the different segregations within the group were: mustard-colored, 11.7%; normally-colored low moisture, 15.0%; medium moisture, 29.3%; high moisture, 37.3%. Therefore, to arrive at a single moisture content for each maturity stage, the weight of kernels in a given moisture range was taken into account and used in the following formula to produce a weighted kernel moisture content (WKMC) for each maturity stage:

$$\text{WKMC} = \frac{\Sigma\,(\text{dry wt of each segregation} \times \text{its KMC})}{\text{Sum of segregations dry wt}}.$$

The moisture loss from kernels of each maturity from the two drought treatments using the weighted kernel moisture content is illustrated in Fig. 2, indicating that moisture loss, particularly in the more immature peanuts, was accelerated in the 29 °C soil compared with the 25 °C soil. Generally, it took about a week longer for kernels in the cooler treatment to reach lower moistures previously attained in the warmer treatment.

Relationship between phytoalexin production and kernel a_w

The relationship between phytoalexin production and a_w is plotted in Fig. 3, with maturities and drought treatments combined. Results showed that kernels of high a_w produced abundant phytoalexins. As the a_w of kernels decreased, however, the capacity for phytoalexin production also decreased. Regardless of maturity or treatment, essentially no phytoalexin production occurred when the a_w of kernels was below 0.95.

Generally, the more immature peanuts (yellow 1, yellow 2, orange) produced more total phytoalexins than mature peanuts (brown, black) at high a_w. However, the trend toward reduced phytoalexin production as a_w decreased was the same for all maturities. Mature peanuts did not retain the capacity for phytoalexin production longer than immature peanuts. In this study, the capacity to produce phytoalexins was not dependent on maturity or treatment, but it was highly associated with a_w.

Effect of a_w, *treatment, and maturity on aflatoxin contamination*

There was little or no aflatoxin detected in peanuts of high a_w, whereas considerable variation in aflatoxin contamination was found in peanuts of low a_w. To evaluate the relationship between a_w and aflatoxin contamination, the percentages of samples contaminated with aflatoxin when a_w was both above and below 0.95 (the a_w below which the capacity to produce phytoalexins was lost) were calculated. Table 1 shows the per-

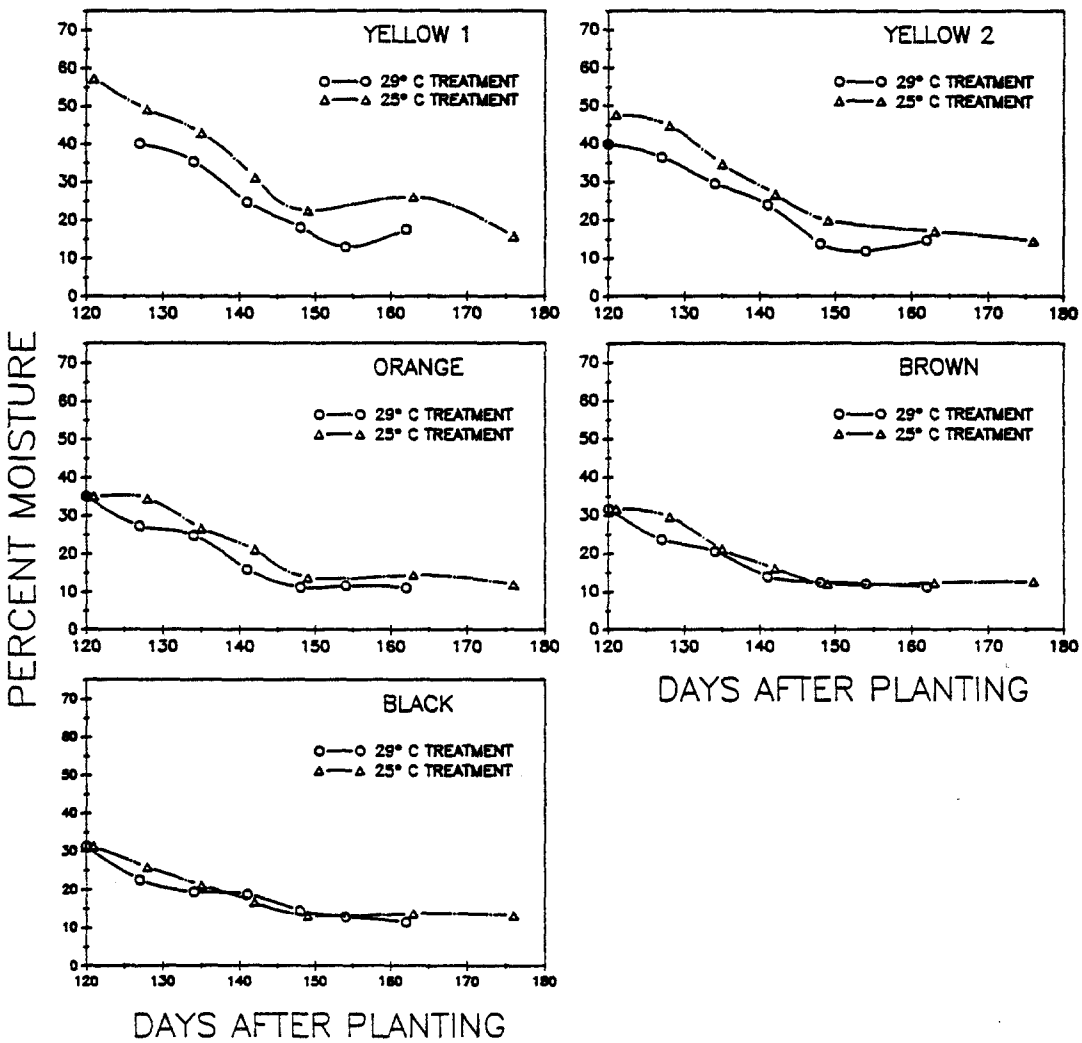

Fig. 2. Comparison of moisture loss of peanut kernels of five maturity stages from drought treatments at 25 and 29 °C over the course of the experiment.

centages of all samples that contained >5 and >20 ppb aflatoxin when a_w was > or <0.95. When data from all maturities and both treatments were combined, only 6% of all samples analyzed that were >0.95 a_w contained >5 ppb aflatoxin compared with 35% of the samples that were <0.95 a_w. When 20 ppb was used as the cut-off, the percentages were 2% and 27% for a_w > 0.95 and <0.95, respectively. Therefore, disregarding the effects of treatment and maturity, a significantly higher percentage of samples were contaminated at a_w's below 0.95 compared to samples with a_w above 0.95.

The effect of soil temperature on aflatoxin contamination can be viewed similarly (Table 1). For samples of a_w < 0.95 with maturities combined, 50% contained >5 ppb aflatoxin in the 29 °C treatment compared with 26% from the 25 °C treatment. Forty percent of all samples from the 29 °C treatment contained >20 ppb compared with 19% of samples from the 25 °C treatment when a_w was <0.95. In addition to the effect soil temperature had on accelerating loss of kernel moisture, the higher soil temperature also resulted in twice as many contaminated samples.

The effect of maturity on aflatoxin contami-

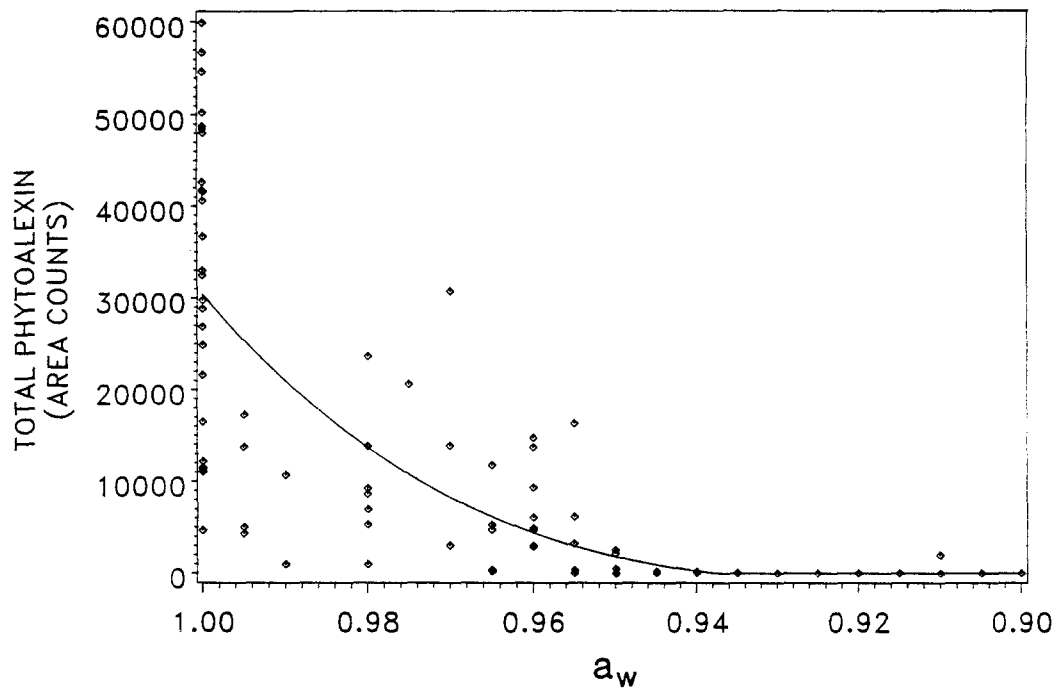

Fig. 3. Relationship of phytoalexin production to kernel water activity (a_w). Total phytoalexins were determined by combining areas under phytoalexin peaks (area counts).

nation can be seen with treatments both separated and combined (Table 1). For samples of <0.95 a_w and with treatments combined, the percentage of samples >5 ppb decreased with increasing maturity accordingly: yellow 1, 67%; yellow 2, 50%; orange, 24%; brown, 17%; black, 17%. The percentage of samples >20 ppb followed the same trend: yellow 1, 58%; yellow 2, 35%; orange, 24%; brown, 8%; black, 8%. These results showed that immature peanuts were more likely to be contaminated than mature peanuts.

Relationship between phytoalexin production and aflatoxin contamination

Table 2 compares phytoalexin production to aflatoxin contamination from each sampling and each maturity stage with the drought treatments separated. In samplings where yellow 1, yellow 2, and orange peanuts were further segregated based on visual moisture assessment, the data are from the

Table 1. Percentages of peanut samples contaminated with aflatoxin at >5 and >20 ppb when water activity (a_w) was $>$ and <0.95.

Maturity/treatment	$a_w > 0.95$		$a_w < 0.95$	
	>5 ppb	>20 ppb	>5 ppb	>20 ppb
Maturities and treatments combined	6	2	35	27
All maturities/25 °C	7	0	26	19
All maturities/29 °C	5	5	50	40
Yellow 1/25 °C	18	0	57	43
Yellow 1/29 °C	0	0	80	80
All yellow 1	13	0	67	58
Yellow 2/25 °C	0	0	50	33
Yellow 2/29 °C	17	17	50	37
All yellow 2	5	5	50	35
Orange/25 °C	8	0	8	8
Orange/29 °C	0	0	50	50
All orange	6	0	24	24
Brown/25 °C	0	0	0	0
Brown/29 °C	0	0	50	25
All brown	0	0	17	8
Black/25 °C	0	0	14	14
Black/29 °C	0	0	20	0
All black	0	0	17	8

Table 2. Phytoalexin production and aflatoxin contamination in five maturities of peanuts grown under drought stress at two soil temperatures.

	Total phytoalexins (Combined peak area counts)[a]					Total aflatoxins (ppb)				
	Yellow 1	Yellow 2	Orange	Brown	Black	Yellow 1	Yellow 2	Orange	Brown	Black
Treatment										
days					29 °C Treatment					
17	17000	10700	8600	4800	9200	0	0	0	0	0
24	0	13700	11800	2900	3200	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	861	15	0	0	6
45	0	0	0	0	0	1190	624	0	12	0
51	0	0	0	0	0	2419	118	329	0	0
59	0	0	0	0	0	785	1915	33	291	0
					25 °C Treatment					
18	11000	4600	5000	7000	1000	0	0	0	0	0
25	11500	11300	4400	5300	6100	0	0	0	0	0
32	35000	500	0	4700	6100	0	0	0	0	0
39	200	200	0	0	0	12	0	0	0	0
46	0	0	0	0	0	784	62	0	0	0
52	0	0	0	0	0	0	447	0	0	0
60	0	0	0	0	0	0	484	0	0	0
66	0	0	0	0	0	5	8	13	0	0
73	0	0	0	0	0	1189	234	173	0	0
80	0	0	0	0	0	0	291	0	0	82

[a] Data are the mean of three determinations.

driest (lowest a_w) kernels from each group. For example, in the 38 TD sampling from the 29 °C treatment there were yellow 1, yellow 2, and orange maturity stage kernels of high a_w that produced abundant phytoalexins and had no aflatoxin contamination. However, the driest kernels in each of those categories produced no phytoalexins, and for comparative purposes data from those kernels are presented.

As long as peanuts had the capacity for phytoalexin production, they were not contaminated with aflatoxin (Table 2). At both temperatures, there were 1–2 weeks between the loss of phytoalexin-producing capacity and significant aflatoxin contamination. Significantly, however, this was true only for the more immature peanuts. Kernels in the mature brown and black categories exhibited sustained resistance to aflatoxin contamination well after the disappearance of phytoalexin production, indicating that mature peanuts have additional resistance that cannot be attributed solely to these phytoalexins.

In the 29 °C treatment, the capacity to produce phytoalexins was lost in all maturity stages between 24 and 31 TD. However, for the comparable period (25–32 treatment days) in the 25 °C treatment, the yellow 1, brown, and black categories retained the capacity for significant phytoalexin production. These data support the results presented concerning the effect of soil temperature on the rate of moisture loss from kernels. Just as it took longer for peanuts to dry down in the cooler soil, likewise phytoalexin-producing capacity lasted longer in the cooler soil.

Temperature also appeared to affect aflatoxin contamination independent of phytoalexin pro-

duction and a_w. The contamination pattern was much more consistent in the 29 °C treatment than in the 25 °C treatment, indicating that not only was more time required at the lower soil temperature to reduce the kernel a_w to a range in which peanuts become susceptible to contamination, but lower temperature also was less favorable for growth and aflatoxin-producing potential of *A. flavus*.

Discussion

Experiments were conducted to monitor kernel a_w, kernel moisture, capacity for phytoalexin production, and aflatoxin contamination in preharvest peanuts during late-season drought with two different mean soil temperatures. The results validated certain aspects of the hypothesis, invalidated others, and shed new light on the interrelationship of the factors involved in preharvest aflatoxin contamination of peanuts. Data supported the central hypothesis that phytoalexin production by peanut kernels is the natural resistance mechanism that breaks down under drought stress allowing aflatoxin contamination to occur, since significant aflatoxin contamination did not occur until after the capacity to produce phytoalexins was lost. This was particularly true for immature peanuts; however, mature peanuts seemed to possess additional resistance beyond that which could be attributed to phytoalexins.

Data also supported the first sub-hypothesis that the capacity for phytoalexin production is controlled primarily by kernel a_w. A clear relationship between the capacity for phytoalexin production and a_w was seen. Data indicated that Florunner peanuts produce sufficient phytoalexins at high a_w (> 0.97) to inhibit growth of *A. flavus* and subsequent aflatoxin contamination. However, regardless of soil temperature or maturity, peanuts could not synthesize phytoalexins when a_w dropped below 0.95. Observations not reported in the results, concerning the growth of *A. parasiticus* on sliced peanuts incubated for 4 days, also support this subhypothesis. A consistent observation was that growth of *A. parasiticus* on sliced kernels, which were near or at a a_w of 1.00, was severely restricted even though inoculation with abundant *A. parasiticus* spores had taken place. Growth of other fungi (that may have been present) also was restricted at these high a_w's. This observation is consistent with data reported by Wotton and Strange [28] from a time-course study of colonization of intact peanut kernels by *A. flavus*. They found that after 2 days of incubation, virtual cessation of fungal growth was correlated with increased phytoalexin concentration. However, in our study, sliced kernels near a a_w of 0.95 (limit for phytoalexin production) supported abundant growth and sporulation of *A. parasiticus*. As the a_w of sliced kernels approached 0.92–0.90, growth of *A. parasiticus* was very slow. Diener and Davis [9, 10] reported that the limiting relative humidity for aflatoxin production after 21 days of incubation at 30 °C was ca. 85% in both sterile and freshly dug peanuts. However, aflatoxin production was much lower as the relative humidity dropped below 89–90%. Diener and Davis [11] speculated that, since drought stress decreases the moisture content of the pod and kernels, they become more susceptible to fungal invasion because of reduced physiological activity of the peanut. Results of our study indicate that this reduced physiological activity may in fact be reduced phytoalexin production, but the effect is on the growth of *A. flavus* in the peanuts rather than on invasion by *A. flavus*. Therefore, it seems that peanuts are most susceptible to aflatoxin contamination when their a_w is between 0.95 and 0.90. Above a_w of 0.95, phytoalexins seem to inhibit growth of *A. flavus* and aflatoxin production. As a_w drops below 0.90, growth and aflatoxin production again become restricted by the reduced availability of water in the peanuts.

The data presented in Table 2 indicated that the relative amounts of phytoalexins that could be produced by peanut kernels was not as important as simply having the capacity for significant production. Only one exception to this statement was found, that being in yellow 1 peanuts from the 37 TD sample from the 25 °C treatment. But here, the very low phytoalexin production

observed indicated that the majority of kernels already had lost the capacity to produce phytoalexins, and the low aflatoxin concentration indicated that most kernels still were not contaminated at that time. Wotton and Strange [27, 28] concluded that resistance of peanuts to aflatoxin contamination might depend on a rapid and efficient phytoalexin response. However, our studies indicate that as long as a_w is high enough to support phytoalexin production, sufficient growth of *A. flavus* for aflatoxin production to occur does not take place. It was only after the capacity for phytoalexin production was lost that preharvest aflatoxin contamination of peanuts took place. Therefore, in selecting genotypes for resistance to aflatoxin contamination, it might be worthwhile to look for the capacity to produce phytoalexins at lower a_w.

The second sub-hypothesis tested in this study related to the increased resistance that large, presumably mature peanuts have been shown to possess compared with small, presumably immature peanuts. We hypothesized that if phytoalexin production was the mechanism of resistance, then immature peanuts must lose that resistance earlier than mature peanuts, leading to earlier and higher levels of contamination. This point was invalidated by this study. It was shown that the capacity for phytoalexin production in both mature and immature peanuts was most influenced by kernel a_w, and that peanuts of all maturity stages lost the capacity for phytoalexin production at about the same time (ca. 24–31 treatment days in the 29 °C treatment and ca. 30–37 treatment days in the 25 °C treatment). However, because of the delay in or absence of aflatoxin contamination in mature peanuts, it seems apparent that mature peanuts possess additional resistance to contamination that cannot be attributed solely to phytoalexins.

The final sub-hypothesis stated that the role of elevated soil temperature in preharvest aflatoxin contamination of peanuts was in controlling the rate at which a_w of kernels decreases. Results showed that, particularly in the more immature stages, the 29 °C soil temperature did accelerate moisture loss from kernels compared with the 25 °C treatment. However, an additional effect of high temperature on aflatoxin contamination also was observed. At 29 °C 50% of all samples below 0.95 a_w were contaminated above 5 ppb, whereas only 26% of samples below 0.95 a_w had similar contamination at 25 °C. This indicated that the higher soil temperature also promoted growth and aflatoxin production by *A. flavus* after phytoalexin-producing capacity was lost.

In conclusion, the interrelationship of several factors seems to be involved in preharvest aflatoxin contamination of peanuts. The scheme in Fig. 4 is an illustration of this interrelationship based on data from the current study as well as prior studies. Simply stated, for a peanut kernel to become contaminated with aflatoxin, it must first be colonized or invaded by *A. flavus*, and the fungus then must grow and produce toxin. Data indicate, however, that under adequate moisture conditions invasion of the fungus elicits phytoalexin production by the peanut that is inhibitory to fungal growth and precludes subsequent aflatoxin contamination. However, when peanuts are subjected to drought stress, two factors are directly affected. First is the soil temperature, which rises dramatically as the peanut canopy recedes [7]. Second is the kernel a_w, which drops as a result of being in dry soil for an extended period. Elevated soil temperature, in turn, directly affects kernel a_w, growth of *A. flavus*, and aflatoxin production. The drop in kernel a_w is accelerated by the higher soil temperature. Also,

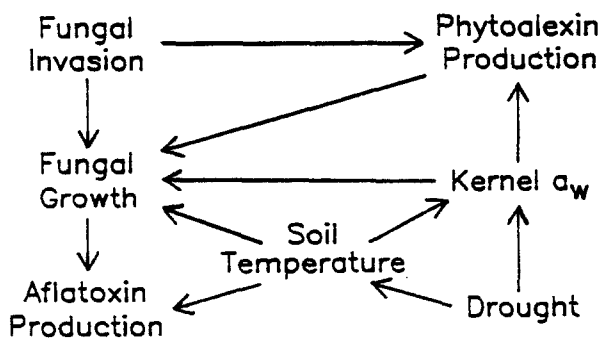

Fig. 4. Scheme showing the interrelationship of several factors involved in preharvest aflatoxin contamination of peanuts. Arrows indicate an influence of one factor upon another.

growth of *A. flavus* and aflatoxin production are favored by high soil temperature, normally associated with drought stress. Ultimately, however, it seems that the factor most important in the contamination process, particularly in the more susceptible immature kernels, is kernel a_w because of its affect on both phytoalexin production and growth of *A. flavus*. As long as kernel a_w remains high, peanuts retain the capacity for phytoalexin production and resistance to contamination. When the a_w becomes too low for phytoalexin production, however, it is in a range that is quite favorable for growth of *A. flavus* and aflatoxin production.

The one unknown factor not included in the scheme (Fig. 4) is the apparent additional resistance to contamination possessed by the more mature peanuts. Studies currently are underway in an attempt to identify this mechanism of resistance.

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Note

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