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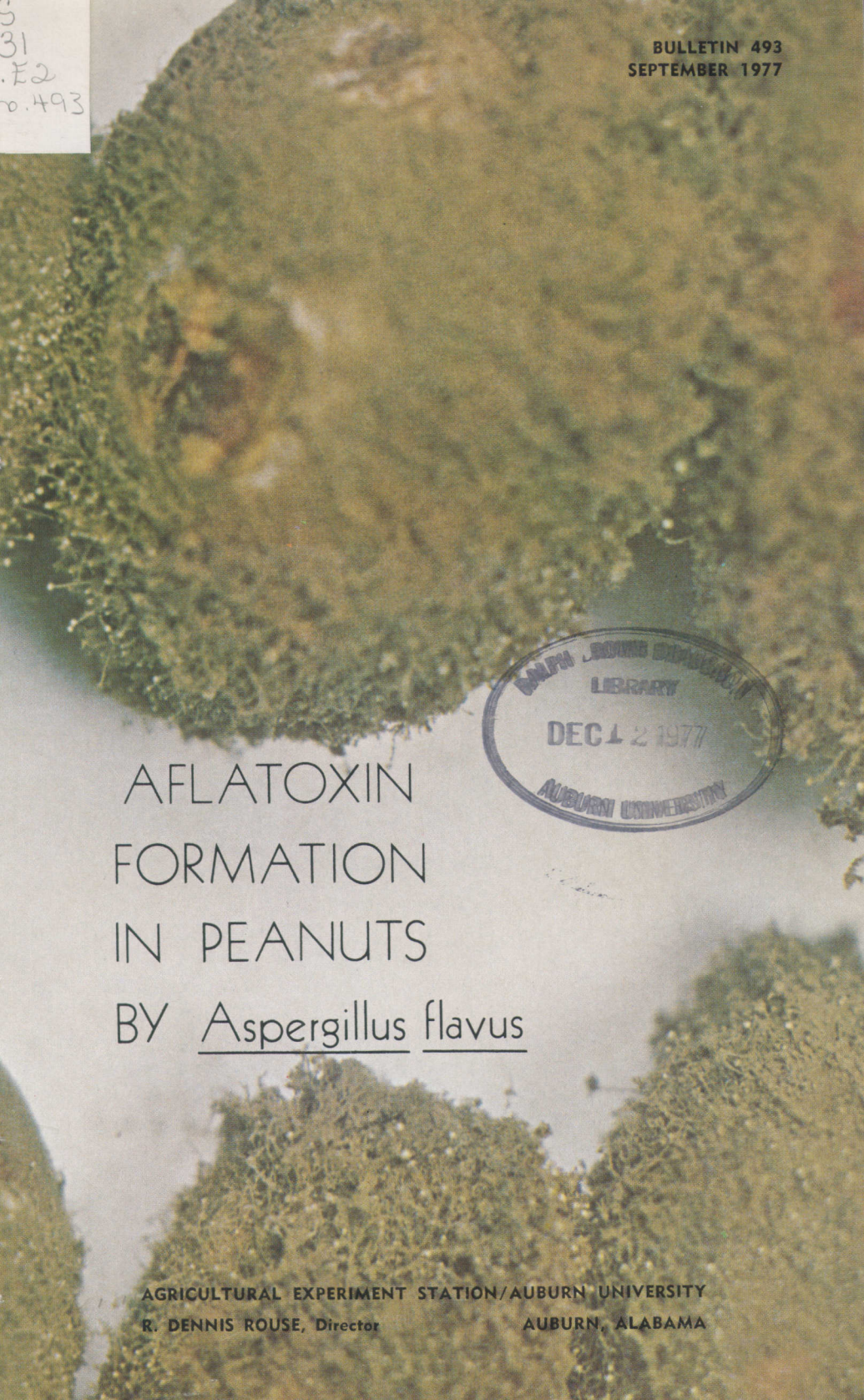

AFLATOXIN FORMATION IN PEANUTS BY *Aspergillus flavus*

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AFLATOXIN FORMATION in PEANUTS by *Aspergillus Flavus*

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INTRODUCTION

THE FACT THAT FUNGI or molds produce unpleasant flavors or other undesirable changes in foods has been known for a long time. In addition, some molds also have the capacity to form chemical substances that are poisonous or produce toxic symptoms when food containing them is ingested by man or animals. These chemicals are known as mycotoxins and the diseases caused by consumption of these fungal-contaminated foods or feeds are known as mycotoxicoses. Interest in mycotoxins was stimulated by the discovery that the death of 100,000 turkey poults at 500 locations in England in 1960 was due to a toxic, carcinogenic fungal metabolite produced by the fungus *Aspergillus flavus* being present in the protein supplement (Brazilian peanut meal) in the feed (47,77). In the next 10 years, more than 1,000 scientific papers were published on aflatoxin research estimated to have cost over 15 million dollars (12). Numerous book length reviews and symposia on aflatoxin (31) and other mycotoxins are available (8, 13, 29, 33, 45, 46, 49, 51, 72, 73, 75, 84, 86). In 1963, the first direct evidence that aflatoxin-producing isolates of *A. flavus* were present in American peanuts (Arachis hypogaea L.) and peanut meals was published by Auburn researchers (26).

Aspergillus flavus is used in this bulletin in a collective sense for both *A. flavus* and *A. parasiticus,* but not for the whole *A. flavus* group of species. The majority of isolates of these two species are known to produce aflatoxin. Most workers agree that only these two species of fungi produce aflatoxin despite several reports attributing aflatoxin-producing ability to other fungal genera and species (24).

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FACTORS INFLUENCING PRODUCTION OF AFLATOXIN IN PEANUTS

The Fungus

The *A. flavus* group of species is a normal constituent of the microflora in air and soil, and is found on or in living or dead plants and animals throughout the world (74). It has been found associated with peanut soils and peanuts wherever they are grown (20). *Aspergillus flavus* is an important storage fungus associated with the deterioration of wheat, corn, rice, barley, bran, flour, soybeans, and other seeds (11, 12, 81). It has also been reported as a pathogen of man and animals (4) and as a plant pathogen (14).

The *Aspergillus flavus* group of species (74) includes *A. flavus* and *A. parasiticus,* which produce aflatoxin, as well as *A. oryzae* and *A. tamarii,* which do not (35). A summary of data from investigators in England, Holland, India, Israel, South Africa, and the U.S. indicated that about 60 percent of about 1,400 isolates of the *A. flavus* group were aflatoxin producers (20). Isolates of *A. flavus* and *A. parasiticus* vary widely in the amount of aflatoxin produced on peanuts, and some isolates (strains) produced none (21). Aflatoxin production by 26 isolates of the *A. flavus* group (21) is shown in Table 1. The amount of aflatoxin B_1 varied from 0 to 17,000 parts per billion (ppb) of peanuts.

TABLE 1. AFLATOXIN PRODUCTION BY ISOLATES OF THE *Aspergillus flavus* GROUP ISOLATED FROM AND GROWN ON PEANUTS

State or country	No. of isolates	Aflatoxin B_1 Peanuts
		ppb
Alabama	7	2,870-17,000
Florida	1	6,900
Georgia	2	70- 2,450
New Mexico	1	0
North Carolina	1	2,760
Texas	2	280- 610
Uganda	1	2,400
Virginia	11	180- 9,200

Aflatoxin production by 11 isolates of the *A. flavus* group collected from four states and six different crops other than peanuts (21) is shown in Table 2. The amount of aflatoxin B_1 varied from 10 to 16,500 ppb of peanut substrate.

Aflatoxin production by the same strain of *A. flavus* will vary with the substrate. Thus, Hesseltine *et al.* (34) found that NRRL 2999

TABLE 2. AFLATOXIN PRODUCTION BY ISOLATES OF THE *Aspergillus flavus* GROUP FROM CROPS OTHER THAN PEANUTS

Isolate source	State	No. of isolates	Aflatoxin B_1 Peanuts
			ppb
Chestnut	Alabama	1	2,300
Corn	Georgia	1	30
Poultry litter	Alabama	1	16,560
Rice	Texas	1	2,300
Soybean	Minnesota	3	10-70
Wheat	Minnesota	3	30-120

produced the most aflatoxin B_1 on sorghum, peanuts, soybeans, and rice, whereas NRRL 3000 produced the most on wheat and corn. Loss in aflatoxin-producing ability with repeated transfers on synthetic media has been experienced by investigators in many laboratories. Isolates also vary in their ability to produce the several different types of aflatoxin. Naturally contaminated lots of corn associated with losses of swine in Alabama in 1965 contained only aflatoxins B_1 and B_2, while other naturally contaminated lots of peanuts, soybeans, corn, and cottonseed contain aflatoxins B_1, B_2, G_1, and G_2.

The Substrate

Aflatoxin production on substrates has been reviewed by several workers (24, 34). Aflatoxin has been produced by *A. flavus* when grown on every major cereal, grain, and oil-seed crop. *Aspergillus flavus* produced aflatoxin experimentally on over 25 fruit and vegetable juices and other foods (85). Aflatoxin production on any given substrate will vary quantitatively with the strain of the fungus, temperature, moisture in the substrate and/or surrounding relative humidity, aeration, length of the incubation period, and the method of aflatoxin analysis.

Relative Humidity and Moisture

It is widely recognized that the most important factor in growth and aflatoxin production by *A. flavus* is the moisture of or relative humidity (RH) surrounding a natural substrate such as a peanut (5). Moisture content for safe storage (no mold growth) of seeds and other natural substrates generally has been established at substrate moistures in equilibrium with 70 percent RH, at which few fungi grow. In pure culture studies in the laboratory (5), vegetative growth of *A. flavus* was limited by 80 percent RH, which is equivalent to 9 percent kernel moisture content (KMC) in peanuts; the growth rate on cul-

ture media maintained between 80 to 85 percent RH (9 to 11 percent KMC) was very slow (0.004 to .04 inch per day).

A quantitative determination was made of fungi inhabiting farmers' stock peanuts that had been stored from 8 to 56 months in 26 farm-size bins of several types of construction at the Wiregrass Substation of the Agricultural Experiment Station of Auburn University, Headland, Alabama (19). Peanuts differed in initial moisture, damage, sound mature kernels, type of bin, and length of time in storage. High mold counts were correlated more often with high initial moistures of peanut kernels than with any other factor, Table 3. Mold counts were consistently high in peanuts placed in storage with kernel moisture contents 12½ percent. After 3 to 4 weeks of storage, kernel moisture contents of peanuts in all bins were about 7 percent and remained there for 8 to 56 months.

Research has indicated that certain conditions may result in either preharvest or post harvest infection and aflatoxin formation by *A. flavus* in peanuts. Ten years ago, most experimental data (3, 5-7, 27, 40, 53, 56, 69) indicated that there was little invasion of kernels in intact and undamaged, immature and mature pods in the ground by *A. flavus,* and little or no aflatoxin present in kernels before lifting at the normal harvest time, although there was some contradictory data (42, 61). It appeared that invasion of peanut pods and kernels by *A. flavus* and other fungi usually occurred during curing when the variety was dug near maturity (5, 27, 56). When dehydration (drying) of the peanuts lowers the kernel moisture content rapidly and steadily downward within 4 or 5 days to safe storage moisture, little opportunity for fungal invasion occurs. After lifting, peanuts were most rapidly invaded by *A. flavus* during drying or dehydration in windrow and stack at 14 to 30 percent KMC (17, 56, 59); apparently *A. flavus* does not become established rapidly in peanuts at higher or lower moisture contents (5, 56, 57). Whether this is a moisture-based response or is based on the fact that moisture levels of peanuts 2 days after lifting are usually in this range and this is also coincident with the time required for spore deposition, germination, penetration, and development of the fungus is a matter of conjecture. Where kernel moisture was high there was some resistance to invasion by *A. flavus* (58). Data from field studies at Mokwa, Nigeria, showed that the 1963 crop had matured and was lifted well before the rains had ended; toxicity at lifting was rare (52). However, at Kano where the rains ceased before harvest, toxicity increased as the moisture content of the kernel at lifting decreased.

TABLE 3. MOLD COUNT, MOISTURE CONTENT, DAMAGE, AND SOUND MATURE KERNELS OF FARMER STOCK PEANUTS STORED IN SEVERAL TYPES OF BINS AT HEADLAND, ALABAMA, FROM 1952 TO 1957

Bin No.	Storage			% moisture		% damaged kernels		% sound mature kernels		Mold Count [a]
	Year	Months	Type of bin	In	Out	In	Out	In	Out	(col/g) [b]
1	1952	56	Steel-tube	9.0	5.0	0	7	69	58	4,743
2		56	Steel-tight	10.9	5.4	0	2	62	58	557
3		56	Wood-tight	6.0	5.8	1	2	66	63	33,209
4		56	Steel-tube	6.0	5.6	0	2	66	62	725
5	1953	45	Steel-tube	11.1	5.4	0	2	70	68	165
6		45	Steel-tight	9.8	5.1	0	3	69	61	1,557
7		45	Steel-tube	9.0	5.6	6	10	61	55	20,634
8		45	Wood-tight	8.6	5.7	0	1	68	66	4,480
9	1954	33	Steel-tube	7.6	5.5	0	3	69	66	270
10		33	Steel-tight	9.6	5.5	1	3	67	60	472
11		33	Wood-tight	11.2	5.9	3	3	65	62	117
12		33	Steel-tight	12.4	5.4	1	4	69	63	513
13	1955	21	Steel-tight	15.4	5.9	2	8	70	62	140,167
14		21	Steel-vent	10.0	6.0	1	2	70	67	1,949
15		21	Steel-tight	13.1	5.6	0	2	72	67	73,100
16		21	Wood-vent	12.9	6.3	0	3	70	68	29,683
17		21	Wood-vent	13.4	7.0	1	2	70	69	149,417
18	1956	8	Steel-tight	11.0	5.2	0	2	69	71	29,993
19		8	Wood-vent	6.1	5.8	0	0	70	72	1,282
20		8	Wood-vent	5.8	5.6	1	0	66	73	762
21		8	Home-made	5.8	5.8	1	0	64	70	945
22		8	Steel-tube	5.8	5.0	1	0	64	69	4,033
23		8	Steel-tube	6.0	4.9	0	0	61	66	4,250
24		8	Wood-vent	6.0	6.0	0	1	66	69	1,717
25		8	Steel-tight	6.1	6.1	0	0	67	70	1,387
26		8	Wood-vent	5.6	5.7	1	0	65	72	2,680

[a] Data are means of 4 replications (positional samples).
[b] Mold colonies per gram of peanut kernels.

When peanuts that are being cured are in the general range of 14 to 24 percent KMC, interruption and retardation of the field drying cycle by showers, overcast humid weather, or a regain of moisture after picking and storage usually result in the development of *A. flavus* with subsequent toxin formation (5, 6). In other experiments (54), artificial drying after 4 to 6 days in the field gave toxin-free kernels, but samples left for 8 to 12 days or sun-dried for 10 to 16 days gave low to medium toxin yields of 25 to 500 parts per billion. Under tropical conditions, peanuts that were free of toxin at digging contained detectable toxin in 48 hours (6). Also, kernels with testae damaged during shelling showed sporulating *A. flavus* in 4 days. In northern Nigeria, no aflatoxin was formed in peanuts left windrowed for 6 days after lifting, whereas those left for longer periods were found to contain toxic kernels (54). The longer the crop was in the field before artificial drying, the greater was the amount of aflatoxin. Other field studies showed that contamination of kernels with aflatoxin occurred at least 5 to 6 days after lifting (56). More *A. flavus* and other fungi were isolated from kernels from slowly-dried pods (attached to plant) than from rapidly-dried pods (57). In Georgia, high levels of aflatoxin as well as high percentages of kernel invasion by *A. flavus* occurred experimentally in slowly-dried pods as compared to rapidly-dried pods (39). In North Carolina, peanut samples harvested from random and inverted windrows contained little or no aflatoxin after being dried to 15 percent KMC at 32 C and 50 percent RH, while the majority of 128 samples dried at 32 C and 85 percent RH contained aflatoxin B_1 in quantities of 6 to 960 ppb (17). Other research has demonstrated that moisture percentage decreased more rapidly in inverted windrows than in random windrows; lower *A. flavus* mycofloral invasion also occurred in inverted windrows (17, 62, 65, 70, 71).

Data demonstrating that invasion by *A. flavus* and aflatoxin formation in peanuts frequently occurs in the field before harvest has been steadily accumulating. As early as 1956, *A. flavus* was reported to be the dominant fungus in unblemished Spanish peanuts, having been isolated from 16 percent of the kernels and shells over a 6-week period before and after harvest in Texas (61). Fungi grew from 22.2 percent of the nuts plated out; with *A. flavus* occurring 79.3 percent of the time. However, the preponderance of data has associated *A. flavus* and aflatoxin in peanuts in the field before harvest with overmaturity (6, 27, 56, 58, 59, 79), drought stress (6, 18, 52, 67, 79), and physical

and biological damage to the pod by fungi and insects (2, 6, 54, 55, 56, 59, 64, 78, 79, 80).

In 1963, Nigerian peanuts left in the ground 4 weeks after maturity contained aflatoxin (58). It has been suggested that in overmaturity there is a drop in moisture content to a more susceptible state associated with the physiological change from active growth to one of a low metabolic state (56). Data from Alabama, Table 4, in the same year show that a much higher percentage of *A flavus* invasion occurred in overmature kernels and pods than in immature and mature kernels and pods from the same plants at harvest. There was also a notable increase in the number of other fungi isolated from kernels (27). In Nigeria, aflatoxin was associated with overmature pods and kernels and with pods from dead plants with kernels of low moisture content (59). In 1963 and 1964, peanut crops in northern Nigeria harvested at or earlier than normal were free from aflatoxin, whereas late harvesting usually resulted in some toxic peanuts (58). Experimentally, both pods and kernels from peanuts stored for 1 year were more rapidly invaded by *A. flavus* than freshly-dug immature and mature pod and kernels in the laboratory (56). In Israel, the high mycofloral counts from fresh and stored peanut kernels were attributed to the (a) advanced maturity of the crop at harvest, (b) peanut after peanut rotation, (c) a 3-day time lag from field to laboratory, and a long incubation period (3 weeks vs. 1 week) on a rich medium (42, 43).

Drought stress has been closely associated with aflatoxin occurrence in peanuts before digging (6, 18, 52, 56, 67, 69). Drought stress probably increases susceptibility to fungal invasion, since it decreases the moisture content of the pod and kernels and greatly lowers the physiological activity of the peanut. In Nigeria, aflatoxin was detected in a late planted crop in sandy soils in a semi-arid region, although the plants had been in the ground for only the normal length of time (6). Metabolic activity of these peanuts probably had been reduced by a 9-week drought. In South Africa, the pods and kernels of peanuts

TABLE 4. OCCURRENCE OF *Aspergillus flavus* AND OTHER FUNGI IN KERNELS AND SHELLS OF FRESHLY DUG EARLY RUNNER PEANUTS FROM HENRY COUNTY, ALABAMA, IN 1963

Pod category	Kernel moisture %	No. of fungal cultures per 100 tissues					
		Kernel			Shell		
		A. flavus	Storage fungi	Field fungi	*A. flavus*	Storage fungi	Field fungi
Immature	48.0	0	19	52	1	62	87
Mature	29.5	2	13	28	9	50	74
Overmature	27.1	8	25	72	55	39	57

that had not recovered from drought were conspicuously invaded by *A. flavus* (79).

A survey of 282 lots of segregation-3 peanuts in North Carolina in 1968 showed a good correlation between drought stress occurring after peanuts are formed but before they are dug and *A. flavus* infection before digging (18). Data from an irrigation experiment also indicated that the incidence of aflatoxic kernels, insect damage, and concentrations of aflatoxin in farmers stock peanuts were related to drought conditions before digging (18). In Texas, it was found that peanuts grown under dryland conditions, where drought stress occurred, accumulated more aflatoxin before digging than peanuts grown under irrigation (67). It appeared that when KMC was above 30 percent or below 10 percent, *A. flavus* activity was restricted as previously noted by other investigators (5, 56, 57, 59). Kernels became more susceptible to *A. flavus* invasion when the soil moisture in the pod zone approached levels at which moisture moved from the pod into the soil and KMC dropped below 31 percent (17).

Overmaturity, drought stress, KMC, and the level of physiological activity in the peanut are interrelated and moisture-related, although other factors may also be involved. The high level of pod invasion by *A. flavus* in the soil has been associated both with overmaturity and with low KMC due to a lack of rain. Decreased vigor of the plant and reduced physiological activity in seed coincided with the drop in KMC and overmaturity, resulting in increased susceptibility of pod and kernel to *A. flavus* invasion and aflatoxin formation (56, 58). Pods collected from dead plants of "Samaru 61" at Kano, Nigeria contained toxic kernels of 5 to 14 percent KMC, whereas living plants at harvest had no toxic kernels and 24 to 32 percent KMC (52). It was concluded that delayed lifting could have undesirable results. Apparently decreased physiological activity associated with maturity or from low moisture in the soil environment favors invasion of kernels and pods by *A. flavus* and the production of aflatoxin in the kernels of such peanuts.

Thus, *A. flavus* propagules in the soil may invade an intact developing peanut pod and kernel under certain conditions of KMC and maturity in the field favorable for the fungus. Infection in the field is increased by drought stress, overmaturity, and other factors that decrease plant vigor and physiological activity of the seed. If pod and kernel are not infected in the field, invasion by the fungus may occur after digging during curing in the windrow or stack, or after picking during storage, if the KMC or relative humidity around

the pod is high enough. Little opportunity for fungal invasion of pod and kernel occurs after digging when drying (dehydration) proceeds rapidly, lowering the KMC steadily downward within 4 or 5 days to safe storage moisture. Once below 30 percent KMC, interruptions in field drying rate may result in *A. flavus* infection and subsequent toxin formation. Thus, *A. flavus* invasion and subsequent aflatoxin elaboration may occur either in the field before harvest or after digging during curing and storage.

Temperature and Time

It is difficult and probably arbitrary to separate the interrelationships of temperature and time from that of RH or moisture. *Aspergillus flavus* has cardinal growth temperatures as follows: minimum 6 to 8 C, optimum 36 to 38 C, and maximum 44 to 46 C (81). Minimal and maximal temperatures for growth are affected by moisture, oxygen concentration, availability of nutrients, and other factors. *Aspergillus flavus* has a higher maximal temperature for growth on natural substrates than on synthetic media (11).

The optimal temperature and time for aflatoxin production by *A. flavus* on sterilized peanuts in culture flasks was 25 C and 7 to 9 days (21). At 30 C, the optimum was reached in 5 to 7 days, while at 20 C the maximal amount of aflatoxin was produced in 11 to 13 days. With *A. parasiticus,* maximal aflatoxin B_1 was produced at 30 to 35 C and maximal G_1 at 25 to 30 C. Large amounts of total aflatoxins were produced at 25 to 30 C during incubation periods of 7 to 15 days. Experimentally, it was determined that a constant temperature of 45 C inhibited growth of *A. flavus* in peanuts and an exposure of 2 to 4 hours at 50 C prevented further growth for about 24 hours (10). Other workers (17) found that in 10 days aflatoxin developed rapidly in peanut samples of 15 to 30 percent moisture held at 90 F (32 C), but developed in relatively few samples held at 70 F (21 C). After inoculation, aflatoxin developed within 2 days at KMC between 15 to 30 percent at 32 C and within 4 days at KMC between 20 to 31 percent at 21 C in freshly-dug peanuts.

The relation of aeration, physical and biological damage to the peanut pod and kernel, and microbial interaction to *A. flavus* invasion or infection and subsequent aflatoxin elaboration will be taken up after the presentation of data on the precise limiting and optimal moisture and temperature requirements for aflatoxin production by *A. flavus.*

Research in Precisely Controlled Environment

A series of studies was conducted to determine the optimal and limiting environmental conditions necessary for production of aflatoxins in peanuts, thus providing scientific knowledge to eventually assure that processed peanuts are of the highest quality (22, 23, 25).

A. Aflatoxin Production in Heat-treated Peanuts

The initial study investigated the effects of temperature and relative humidity (RH) on growth and production of aflatoxins and free fatty acids (FFA) by *A. flavus* in heat-treated Early Runner peanuts (22). *Aspergillus flavus,* strain Ala-6, used in these experiments was isolated from Alabama peanuts in 1964.

One ton of peanuts of the 1964 crop obtained from the Wiregrass Substation, Headland, Alabama, was shelled, cleaned, and segregated into lots that consisted of four categories of peanuts: (1) sound mature kernels (SMK) large enough to be retained on a 16/64-inch

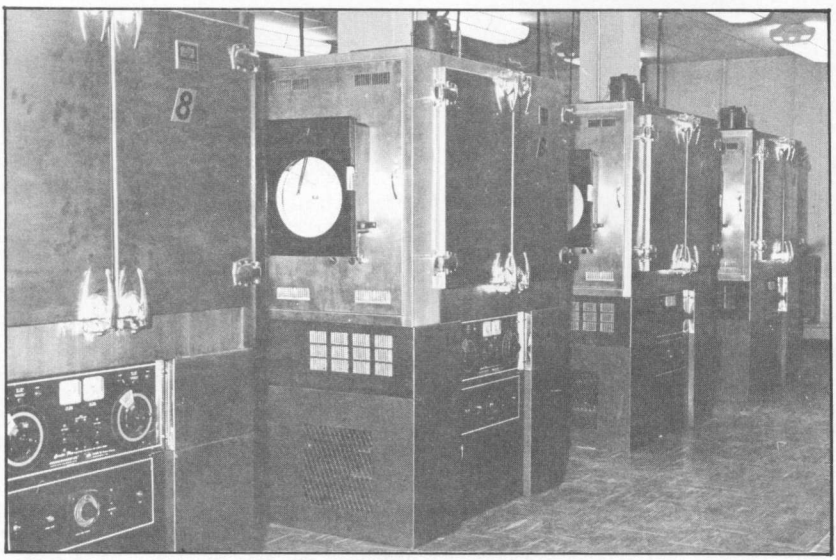

Fig. 1. Eight temperature/humidity cabinets were used to study the mold. Each cabinet had working dimensions of 37″ wide by 19″ deep x 25″ high and was controlled by Duel Wet and Dry Bulb POWER-O-MATIC 60 Saturable Reactor Control Systems.

screen during shelling; (2) broken mature kernels (BMK) that were deliberately split to simulate 'damaged' or 'loose shelled kernels'; (3) immature kernels (IMK), small undeveloped kernels (frequently referred to as 'pegs' or 'shrivels') that dropped through the 16/64-inch screen during shelling; and (4) unshelled peanuts with visibly undamaged shells.

Environmental studies were conducted in Blue-M Power-O-Matic 60 (model CFR-7752C) refrigerated humidity cabinets with two shelves each supporting two perforated stainless steel trays (16 inches square by 1 inch deep), as illustrated by figures 1 and 2.

Sound mature, broken mature, and immature kernels in lots of 900 grams were distributed in each of 3 trays in a layer one to two peanuts deep. Unshelled peanuts (1,300 grams) were placed two to three pods deep in the fourth tray. The four treatments were placed randomly in different shelf locations in each of the eight cabinets. Peanuts were heat-treated by exposure to 12 to 14 hours of wet heat (95 to 99 percent RH) at 80 to 85 C. After cooling, cabinets were adjusted to RH ranging from 70 to 99 percent at a constant temperature of 30 ± 0.5 C in one experiment and to temperatures from 10 to 45 C at a constant RH of 98 ± 1 percent in a succeeding experiment. The

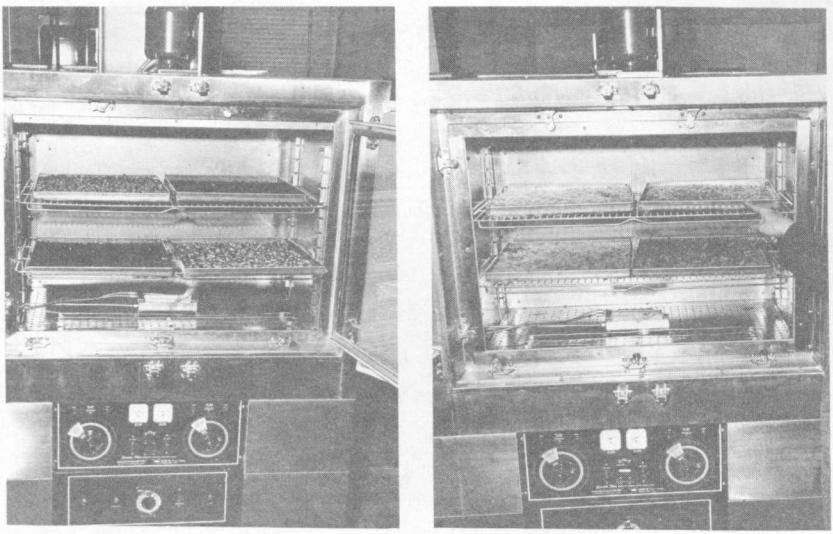

Fig. 2. On the left is interior of chamber with stainless trays loaded with peanuts ready for sterilization, subsequent inoculation, and incubation for mold development. *Aspergillus flavus* developed (right) after 7 days at 30 C and 92.5 RH.

moist peanuts were then inoculated by spraying with a suspension of *A. flavus* spores calculated to give 12 to 15 million spores per tray. Control lots (400 to 500 grams) were taken randomly from the supply while loading the trays and immediately after the wet heat treatment.

Samples of peanuts (400 to 500 grams) were removed at random from each treatment tray after 7 and 21 days incubation for chemical analyses. Safety precautions for personnel during sampling included surgical masks, rubber gloves, and long laboratory coats. Sample baskets were covered until placed in the drying oven. Air was exhausted from the room for several hours and the work areas mopped with 5 percent NaOCl (82).

Samples (400 to 500 grams) were weighed in wire baskets, placed in a forced draft oven at 135 C for 20 minutes, dried to near constant weight at 70 C, and then ground in a Universal model No. 71 food chopper with a 16-point cutter. The ground peanuts were again dried to near constant weight at 70 C and stored in screw-cap amber bottles in a cold room at 1 to 2 C until analyzed.

Peanut KMC was determined by the oven-dry method (1) on duplicate 25-gram samples of seed taken directly from the environmental cabinets. The following modification was required because of the high moisture content of some of the samples. After weighing the samples in aluminum moisture dishes, peanuts and fungus were exposed in a forced draft oven at 135 C for 20 minutes, brought to near constant weight at 70 C, and finally dried for 3 hours at 130 C.

Aflatoxin analyses were made of duplicate 50-gram samples by the aqueous-acetone method of Pons and Goldblatt (68). FFA were determined on duplicate samples (1) except that samples were first ground with a Universal model No. 71 food chopper rather than a Henry nut slicer.

Relative Humidity.—The relation of RH to aflatoxin production in heat-treated SMK, BMK, and IMK is shown in Figure 3. Total aflatoxins of 50 to 82,000 ppb formed at 30 C in 21 days between 89 to 99 percent RH in SMK and IMK. Aflatoxins of 71 to 115,000 ppb formed under the same condition in BMK. Control raw and sterilized peanut samples averaged about 15 ppb. Little aflatoxin was formed at RH of 85 percent or lower.

The relation of RH to aflatoxin in the kernels of unshelled peanuts is shown in Figure 4. Aflatoxins of 40 to 92,000 ppb formed at 89

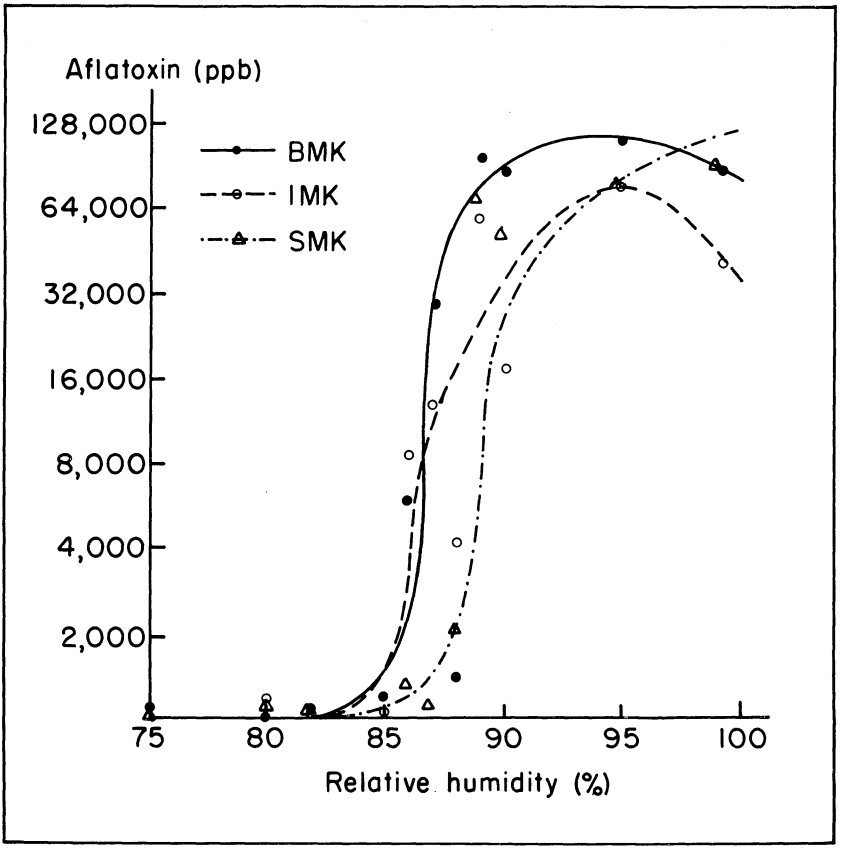

Fig. 3. Effect of relative humidity on aflatoxin production in broken mature kernels, immature kernels, and sound mature kernels of heat-treated peanuts incubated at 30 C for 21 days.

to 99 percent RH. Kernels of unshelled peanuts contained little aflatoxin at 86 percent RH as compared to 1,100 to 8,500 ppb for SMK, IMK, and BMK, Figure 3. Little or no aflatoxin was formed in any treatment at 85 percent RH except for BMK which formed 77 ppb.

Analyses of 7-day samples (Diener and Davis, unpublished data) show aflatoxin levels of 8 to 10 percent of those found after 21 days incubation in SMK, IMK, and BMK. Kernels of unshelled peanuts (7-day samples) showed less than 0.5 percent the amount of aflatoxin present in 21-day samples. Thus, the shell appeared to delay *A. flavus* invasion and aflatoxin production in the kernel. The limiting RH for aflatoxin production in sterile peanuts appeared to be between 82 and 84 percent RH at 30 C with a 21-day incubation period.

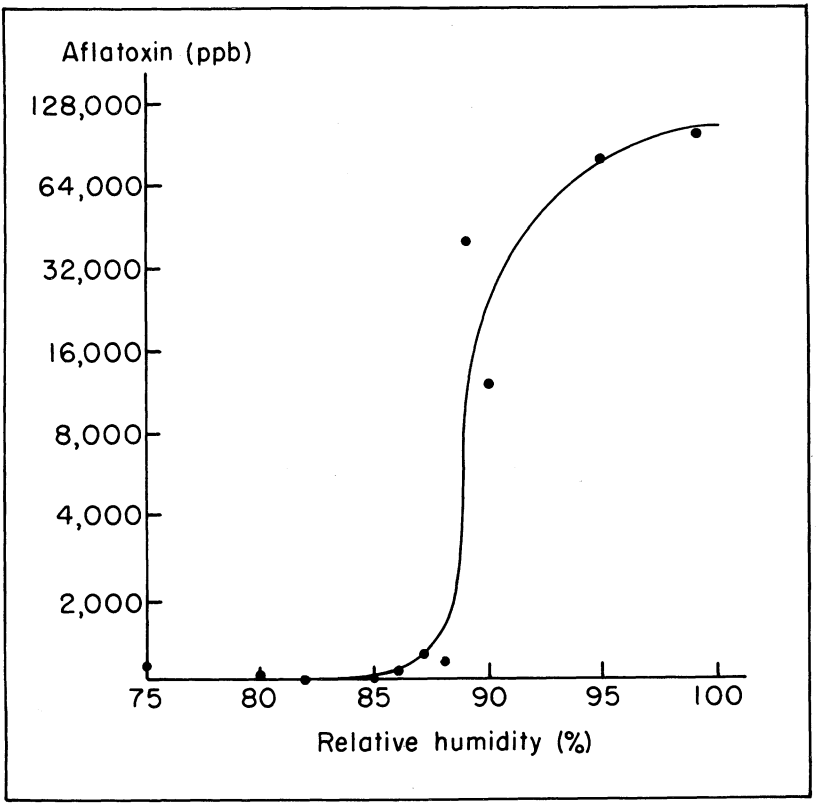

Fig. 4. Effect of relative humidity on aflatoxin production in kernels of unshelled peanuts (heat-treated) incubated at 30 C for 21 days.

Temperature.—The relation of temperature to aflatoxin production in SMK, IMK, and BMK is presented in Figure 5. The optimal temperature range for aflatoxin production of 80 to 360,000 ppb was 20 to 35 C for a 21-day incubation period at 99 percent RH. Some aflatoxin formed at 14 to 15 C and 40 C, but none formed at 12 C and 43 C, except in BMK (95 ppb) at 12 C with none at 10 C. Only a trace (less than 2 ppb) of aflatoxin was found in raw and sterilized control samples.

The relation of temperature to aflatoxin production in the kernels of unshelled peanuts is presented in Figure 6. The optimal range for aflatoxin production of 127 to 320,000 ppb was similar to that for SMK, IMK, and BMK. Again, some aflatoxin formed at 14 and 40 C, but none at 12 and 43 C at 99 percent RH and 21 days incubation. Only traces of aflatoxin were found in control samples.

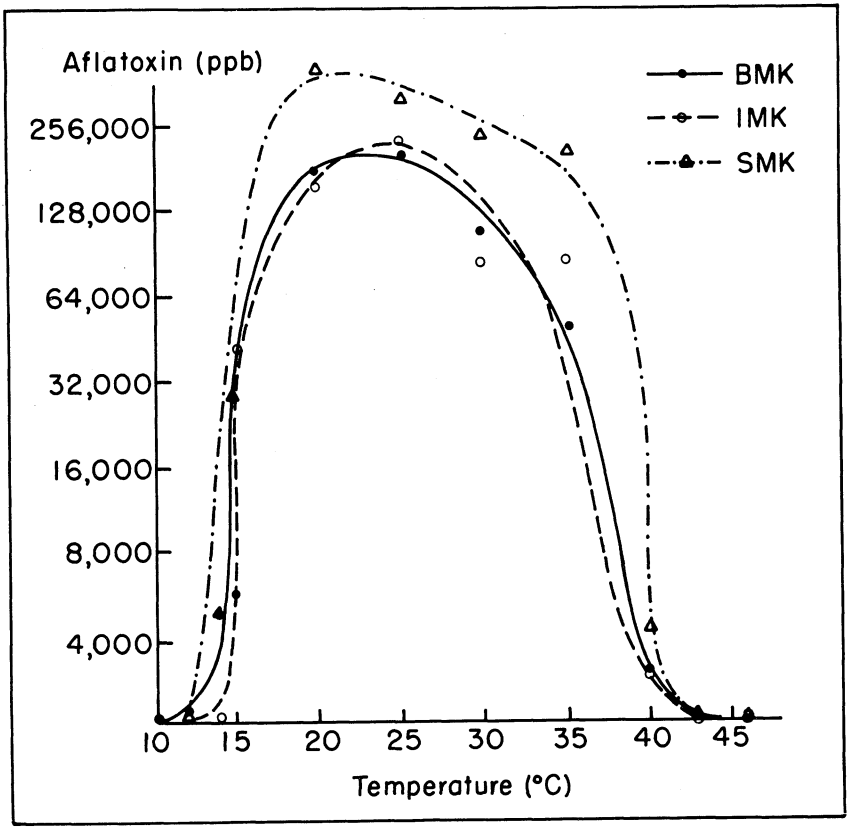

Fig. 5. Effect of temperature on aflatoxin production in broken mature kernels, immature kernels, and sound mature kernels of heat-treated peanuts incubated at 99 percent relative humidity for 21 days.

Analyses of 7-day data (Diener and Davis, unpublished) show the relationship of time with temperature at 99 percent RH to aflatoxin formation. Little or no aflatoxin formed in 7 days in SMK, BMK, and kernels of unshelled peanuts at 15 C. No aflatoxin was found in IMK at either 14 C or 15 C in 7 days or 14 C in 21 days incubation at 99 percent RH. Thus, the limiting temperatures for aflatoxin production by *A. flavus* were 12 and 43 C when grown at 99 percent RH for 21 days on a sterile peanut substrate. The only exception was the production of aflatoxin (95 ppb) at 12 C in 21 days on BMK.

Growth and Sporulation by *A. flavus*.—Vegetative growth of *A. flavus* was observed on BMK and IMK in 24 to 30 hours after inoculation at favorable temperatures and RH. Heavy sporulation was typically

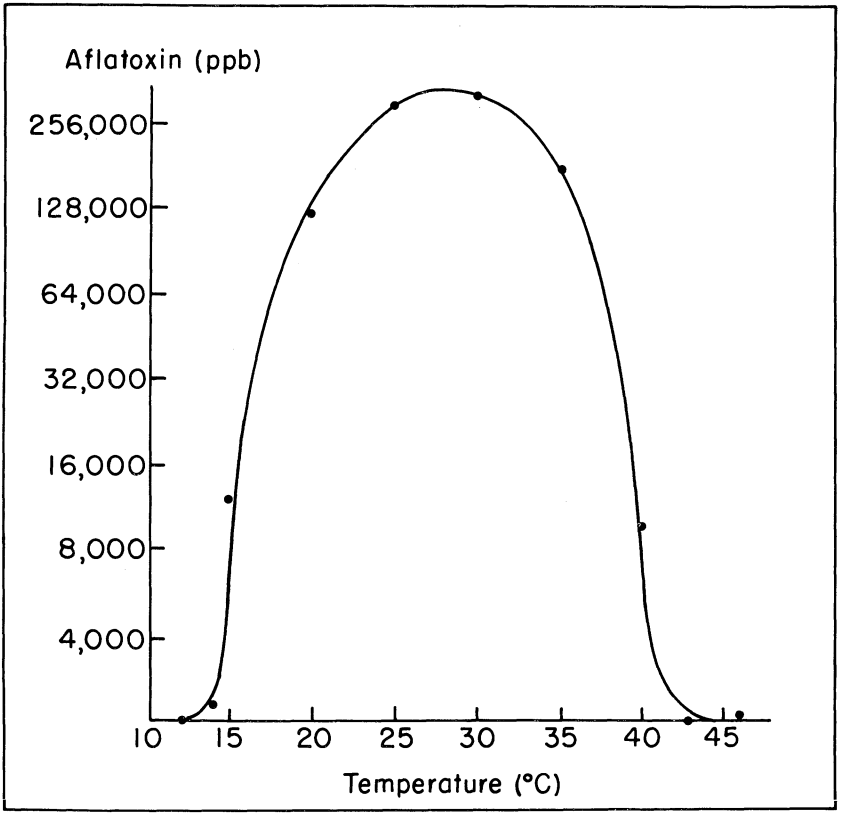

Fig. 6. Effect of temperature on aflatoxin production in kernels of unshelled peanuts (heat-treated) incubated at 99 percent relative humidity for 21 days.

noted in 48 to 72 hours. Growth and sporulation were temporarily inhibited by the seedcoats of unblemished SMK, but by 21 days the level of aflatoxin in SMK was similar to that in BMK and IMK. Except at temperatures above 40 C, visible growth and sporulation by *A flavus* was associated with aflatoxin production. Sporulation occurred at 43 C but not at 46 C. *A. flavus* could still be isolated at the end of the incubation period at either temperature. Fungus growth was restricted at temperatures below 14 C and at RH below 86 percent during the 21-day storage period, correlating with toxin production.

Free Fatty Acids.—The development of high percentages of FFA at 30 C was generally correlated with high RH and high total aflatoxin (22). FFA were relatively low at RH below 86 percent as was total

aflatoxin. At high RH and moderate temperatures (15 to 40 C) that permitted good fungus growth, percentage FFA was correlated with total aflatoxin. However, aflatoxin and FFA levels were not proportionally correlated at temperature extremes, e.g., percentage FFA was high at 40 C, but aflatoxin level was relatively low. Likewise, percentage FFA was high and aflatoxin low at 14 C. Also, percentage FFA was high at 43 C, although aflatoxin was not produced during the 21-day storage period. FFA production, growth, and sporulation of *A flavus* were extensive at 43 C, but little or no toxin was present. Thus, percentage FFA was closely related to fungus growth in all cases, but was not directly proportional to aflatoxin production.

Discussion.—From data (not presented here) on KMC in these experiments (22), it appears that RH is a better criterion for safe storage levels than KMC. Aflatoxin was not produced in either SMK or IMK stored at 85 percent RH, even though IMK contained an average KMC of 13 percent as compared with 9 to 10 percent in SMK. It appeared that a minimal kernel moisture in SMK of 11 to 12 percent was required for aflatoxin to form, whereas approximately 15 percent was required in IMK.

RH data agree with those of Austwick and Ayerst (5), who reported that *A. flavus* grew at a rate of 1 mm/day at 85 percent RH and 30 C in pure culture. However, at 80 percent RH the growth rate was only 0.1 mm/day. In our experiments, no significant amount of aflatoxin formed in 21 days at 85 percent RH and lower. Thus, the limiting RH for aflatoxin production by *A. flavus* in *nonliving* peanuts for a 21-day storage period was 85 ± 1 percent RH.

The apparent optimal temperature range for aflatoxin production in SMK, BMK, and IMK was 20 to 25 C in 21 days, whereas it was 25 to 30 C for unshelled peanuts. The optimal temperature was 25 C for all lots for a 7-day storage period (unpublished data), although more aflatoxin was present at 30 C than at 20 C regardless of the kernel type. After 21 days, there was more aflatoxin present at 20 C than 30 C in all lots except unshelled peanuts. Peak aflatoxin content was found at 25 C in all lots except SMK in which maximal production was at 20 C. Thus, the optimal temperature for aflatoxin production was approximately 25 C.

In 7 days BMK contained 50 percent more aflatoxin than the other three lots at 20, 25, and 30 C. At the end of 21 days, BMK contained 30 to 50 percent less aflatoxin than SMK and kernels of unshelled peanuts at all temperatures from 15 to 40 C. Some aflatoxin may have been degraded in the BMK after it was formed since

the source and nature of peanut kernels were the same. This effect was more apparent at the more extreme temperatures of 15, 35, and 40 C. No measurable aflatoxin was found in peanuts stored at temperatures of 43 C and higher. Thus, the lower temperature limit for aflatoxin development was 13 ± 1 C for a 21-day incubation period (except for damaged kernels that had some toxin at 12 C), and the maximal temperature for aflatoxin production was 41.5 ± 1.5 C.

Growth and sporulation of *A. flavus* was correlated with time at RH above 85 percent and temperatures of 14 to 43 C. Mycelial growth was abundant at 46 C, but no sporulation occurred in 21 days. However, in environments of 97 to 99 percent RH and 43 C, fungus growth did not result in aflatoxin production. Thus, temperature, not fungal growth, was the limiting factor in aflatoxin production at high temperatures, since at 43 C growth and sporulation by *A. flavus* was equal to that at 40 C, but no aflatoxin was found.

Formation of free fatty acids was directly related to fungal growth. However, percentage of FFA was not necessarily correlated with aflatoxin production (22).

Aflatoxin production by *A. flavus* varied considerably with the nature of the four peanut substrates. The testae of SMK were a barrier primarily to the visible aerial development of the fungus rather than to the invasion of peanut kernel itself. BMK appeared to be more rapidly invaded by *A. flavus* than the other peanut lots, since aflatoxin levels were higher in BMK than in the other three treatments after 7 days; after 21 days, aflatoxin levels in BMK were similar to other treatments. IMK averaged 3 to 4 percent higher kernel moistures than the other lots, but aflatoxin did not develop to greater concentrations than in SMK, BMK, and kernels in unshelled peanuts. In general, IMK had the same RH and temperature requirements for aflatoxin production as the other lots. Kernels of unshelled peanuts produced less aflatoxin under suboptimal conditions only for short periods of time (unpublished data), since aflatoxin levels were similar in all lots after 21 days. Presence of the intact shell was apparently only a temporary physical barrier to invasion by *A. flavus.*

By sterilizing the substrate, competition, antagonism, and interaction of other microorganisms in the normal mycoflora of the peanut pod and kernel with *A. flavus* were eliminated. Thus, these results revealed the potential of the peanut substrate to support aflatoxin production and defined the minimal, optimal, and maximal RH and temperatures for the growth of *A. flavus* in a sterilized substrate.

B. Aflatoxin Production in Freshly Dug Peanuts

In this study, the effects of RH and temperature on growth and production of aflatoxin and free fatty acids by *A. flavus* in freshly dug Early Runner and Florigiant peanuts were investigated (23). Freshly dug, high moisture peanut pods were dehydrated under controlled conditions that stimulated constant curing environments to determine their effect on fungus growth and subsequent toxin formation.

Early Runner and Florigiant variety peanuts were hand-picked from plants immediately after digging during the 1965 and 1966 seasons at the Wiregrass Substation, Headland, Alabama. Peanuts were planted April 30th and dug August 23rd and September 22nd in 1965; 1966 peanuts were planted April 21st and dug August 31st and September 22nd. Pods were stored overnight in closed containers at 2 C, and mechanically damaged pods and pods visibly damaged by insects, nematodes, and fungi were discarded. Pods were surface-sterilized for 10 minutes with 1.25 percent sodium hypochlorite solution, aired for 2 hours, and inoculated immediately after placing in environmental cabinets (previously described) with a spore suspension of *A. flavus,* strain Ala-6. Samples were removed after 7 and 21 days incubation in a series of RH from 70 to 98 ± 1 percent RH at a temperature of 30 ± 0.5 C. Additional plants were dug and pods were picked from adjacent rows 3 to 4 weeks later in both years. These peanuts were processed in a similar manner except that incubation was at a series of temperatures from 10 to 45 ± 0.5 C at 98 ± 1 percent RH. Samples were dried to constant weight, shelled, kernels ground, and KMC, aflatoxin, and FFA were determined by standard methods. Only data for 1966 is presented because 1965 data was similar.

Relative Humidity.—The relation of RH to aflatoxin production by *A. flavus* in kernels of unshelled 'Early Runner' and 'Florigiant' peanuts incubated for 21 days at 30 C is shown in Figure 7. Aflatoxin production was correlated with increasing RH from 85 to 99 percent and ranged from 300 to 168,000 ppb and 7 to 34,000 ppb in Early Runner and Florigiant peanuts, respectively. No significant amount of aflatoxin occurred at 83 percent RH in Early Runner and at 85 percent RH in the Florigiant variety, nor in the 7-day samples (data not published). Only trace amounts (less than 1 ppb) were found in control samples of both varieties.

Temperature.—The relation of temperature to aflatoxin production by *A. flavus* in kernels of unshelled Early Runner and Florigiant

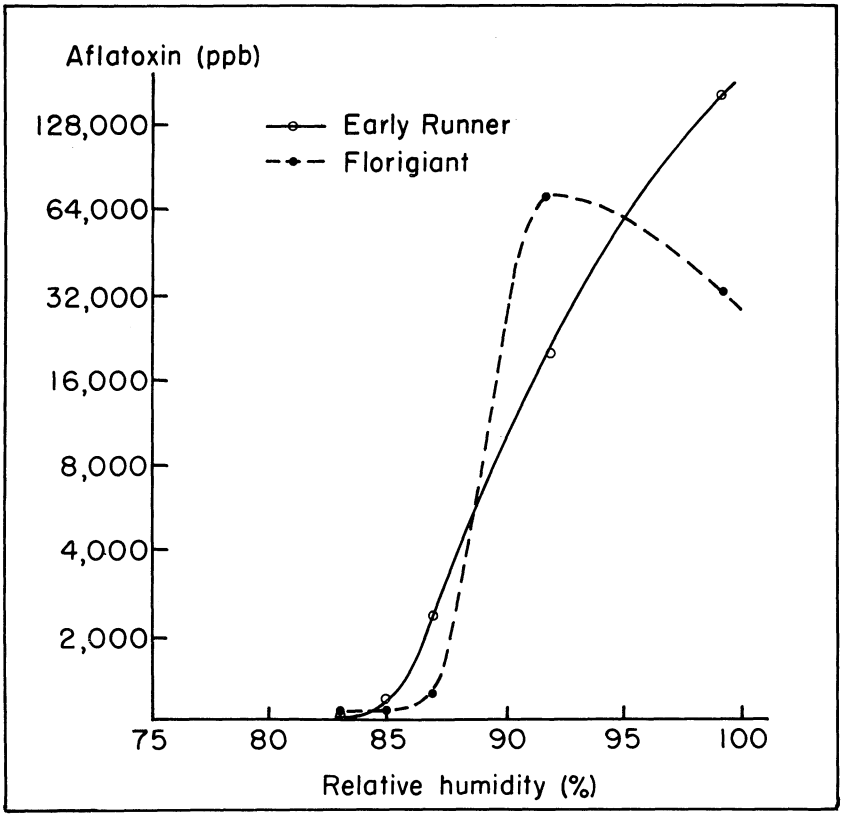

Fig. 7. Effect of relative humidity on aflatoxin production in kernels of freshly dug pods of Early Runner and Florigiant peanuts incubated at 30 C for 21 days.

peanuts incubated for 21 days at 99 percent RH is shown in Figure 8. Significant levels of aflatoxin 1,300 to 9,700 ppb were produced at 20 to 35 C in both varieties with a trace to 61 ppb being recorded at 15 C and a trace to 53 ppb at 40 C. Small amounts of aflatoxin (86 to 1,020 ppb) were formed at 25, 30, and 35 C in the first 7 days (data not presented). Only traces were recorded at 45 C and in the control samples.

Relation of Free Fatty Acids.—Increases in percentages of FFA were correlated with temperatures and RH favorable for the growth of *A. flavus* and other fungi. Percentages FFA were high when aflatoxin was formed and also at high temperatures of 40 and 45 C, when little or no aflatoxin was produced. Other fungi developed at these high temperatures and at RH unfavorable for *A. flavus*.

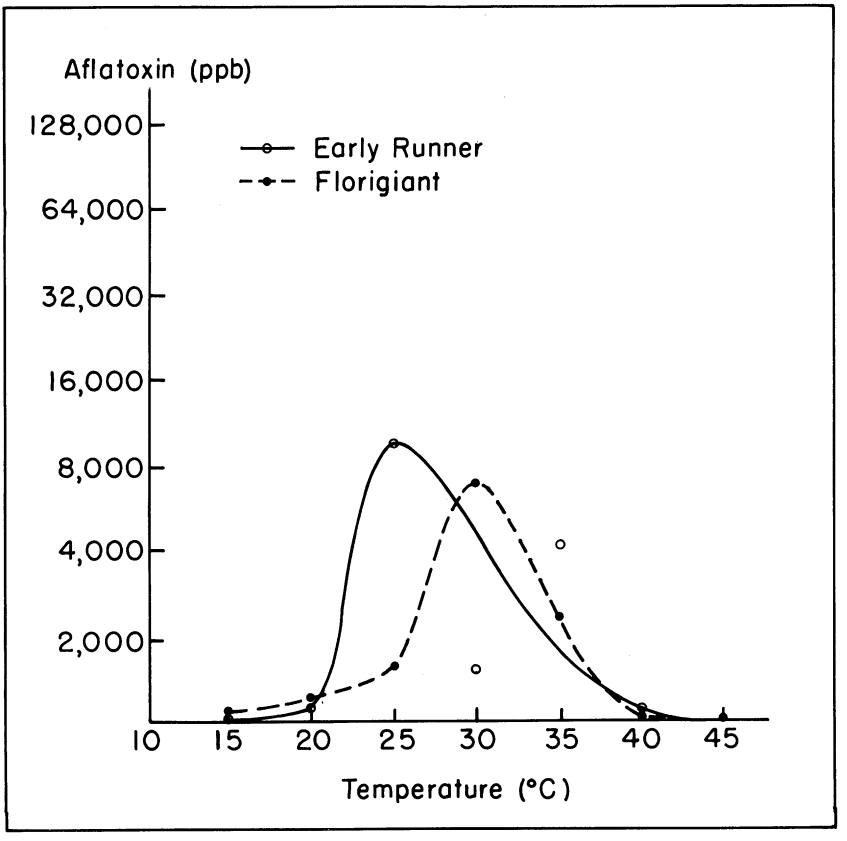

Fig. 8. Effect of temperature on aflatoxin production in kernels of freshly dug pods of Early Runner and Florigiant peanuts incubated at 99 percent relative humidity for 21 days.

Discussion.—In general, the limiting temperature and RH data for aflatoxin production closely parallel that previously reported for sterile peanuts. The amounts of aflatoxin produced in 7 days (data not detailed) indicated that shell and testa of the peanut were at least temporary barriers to invasion and utilization of the living peanut by *A. flavus* in comparison with the amount formed in sterilized peanuts. Other workers have postulated and stressed that undamaged shell and testa gave protection in the soil and at digging as long as moisture and physiological activity were high (6, 56). Aflatoxin and FFA in 21-day incubation periods did not attain the high concentrations found in sterile peanuts. This might be attributed to the natural resistance of living tissue, but the sterilizing treatment (wet heat)

probably also increased nutrient availability in the killed peanut substrate.

Another factor involved is the stage of maturity of the peanut substrate. The sterile Early Runner peanuts of the previous investigation were harvested and stored for several months at air temperature and then placed in cold storage (2 C) for another 3 to 4 months before experimentation. The increased susceptibility of old, overmature or moribund peanut tissues to *A. flavus* invasion and aflatoxin formation has been previously reported from laboratory and field (6, 18, 27, 56, 58, 59, 79).

Differences in this study with living peanuts between the 2 years, as well as the two experiments in the same year, can be attributed not only to the effect of differences in environmental conditions of one growing season and another, but also to the difference in the length of time the peanuts were in the soil (stage of maturity). The range from planting to maturity for Early Runner and Florigiant peanut varieties is 135 ± 5 days. Peanuts for the RH experiment in 1965 (data not presented) were dug early (115 days), while those for the temperature experiment were overmature (145 days). In 1966, peanuts for the RH experiment were dug at maturity (132 days), while those for the temperature experiment were relatively overmature (155 days). Differences in maturity can be associated with the KMC of raw checks of early dug peanuts (tables 1 and 3) and those dug later (tables 2 and 4). The KMC from early dug pods averaged 41 percent for the 2 years, whereas kernels from late dug pods averaged 30 percent KMC.

In conclusion, the lower limiting RH for aflatoxin production in living peanut kernels was 84 ± 1 percent RH at 30 C in Early Runner variety and 86 ± 1 percent RH in Florigiant for a 21-day incubation period. These RH are equivalent to KMC of 10 to 11 percent. The lower limiting temperature for aflatoxin production in living peanuts was not closely defined, but was 12.5 ± 2.5 C. The higher limiting temperature was 42.5 ± 2.5 C. Growth of *A. flavus* was generally correlated with aflatoxin production except at high temperatures of 43 to 45 C. FFA formation generally was correlated with the growth of *A. flavus* or other fungi, but was not correlated with aflatoxin production.

C. Aflatoxin Production in Stored Peanuts

In this culminating study, the effects of RH, temperature, and time on growth and production of aflatoxin and FFA by *A. flavus* in living, cured Early Runner peanuts were investigated (25). Peanuts

of the 1966 crop obtained from the Wiregrass Substation, Headland, Alabama, were prepared in a similar manner as the study with heat-treated peanuts.

Equipment and procedures were similar to those previously reported (22) except that tray lots were increased to 1,000 g of SMK, BMK, and IMK and 1,400 g of unshelled peanuts. Peanuts were field-run, farmers stock peanuts placed in cold storage at 2 C about 30 days after harvest. The peanuts were inoculated with spore suspensions of *A. flavus*, strain Ala-6, immediately after the trays were placed in environmental cabinets that had been adjusted to specific temperatures or relative humidities (RH) or both. An additional experiment with varying RH at 20 C was conducted to evaluate the effect of reduced temperature on aflatoxin production. Random samples were removed after incubation periods of 7, 21, 42, and 84 days. The occurrence of species of fungi other than *A. flavus* was noted. Determinations of KMC, aflatoxin, and FFA were made by methods reported in the previous two sections.

Relative Humidity.—The relation of RH to aflatoxin production by *A. flavus* in SMK, IMK, and BMK of Early Runner peanuts incubated at 30 C is shown in Figure 9. Aflatoxin production increased with each rise in RH from 85 to 99 percent. No aflatoxin was produced at 84 percent RH in 21 and 42 days, and less than 50 ppb of aflatoxin were produced at 84 percent RH after 84 days incubation. No aflatoxin was produced at 83 percent RH in 84 days in SMK, IMK, or BMK. In SMK and BMK, only about 25 percent of the large amount of aflatoxin present in 42-day samples was noted in 84-day samples from the 85 and 87 percent RH treatments.

The relation of RH to aflatoxin production in the kernels of stored unshelled peanuts is presented in Figure 10. Aflatoxin production in kernels of unshelled peanuts was less than 3 percent of the amount present in SMK at most RH levels and less than 3 and 25 percent of the amount present in IMK and BMK, respectively. A small amount (300 ppb) formed at 87 percent in 21 days, but no aflatoxin was found in 42- and 84-day samples. Less than 50 ppb aflatoxin formed at 84 percent RH after 84 days and at 85 percent RH after 21, 42, and 84 days incubation; none was found at 83 percent RH.

Temperature.—The relation of temperature to aflatoxin production in SMK, IMK, and BMK of Early Runner peanuts held at 99 percent RH is shown in Figure 11. High total aflatoxin production occurred at 25 to 35 C in 21 days. At 16, 18, and 20 C, there was a large increase in aflatoxin production between 21 and 42 days incubation

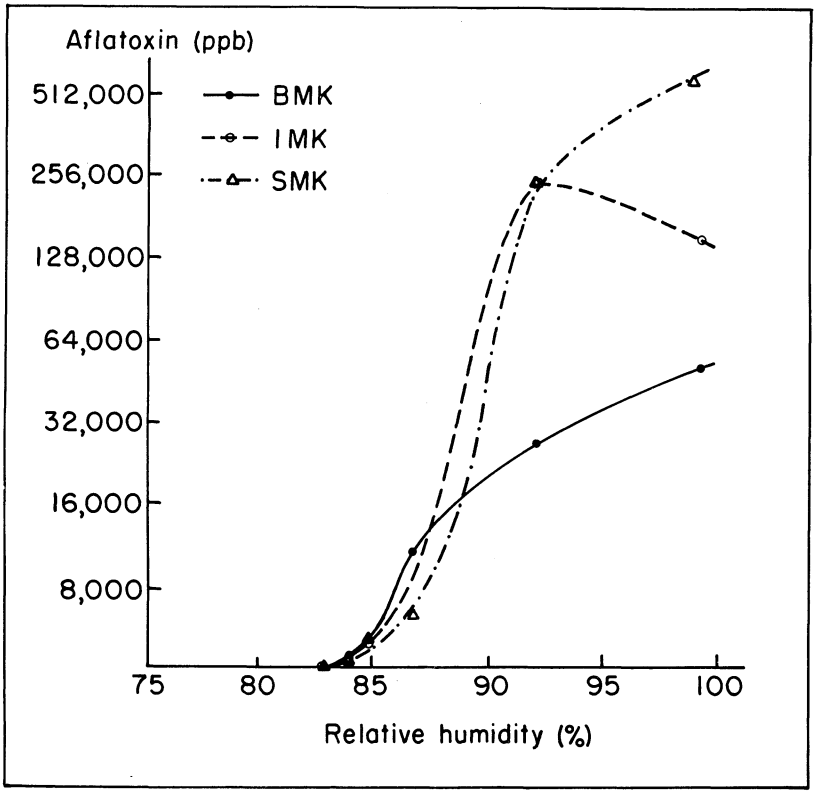

Fig. 9. Effect of relative humidity on aflatoxin production in broken mature kernels, immature kernels, and sound mature kernels of stored peanuts incubated at 30 C for 21 days.

followed by a noticeable drop after 84 days. Small amounts of 300 ppb or less were formed at 13 and 40 C in SMK, at 15 and 40 C in IMK, and 40 C in BMK. No aflatoxin was produced at 12 C or lower in SMK and BMK and at 14 C or lower in IMK. The upper limiting temperature was 41 C in SMK and BMK and 43 C in IMK.

The relation of temperature to aflatoxin production in kernels of unshelled Early Runner pods held at 99 percent RH is shown in Figure 12. Moderate amounts of aflatoxin formed at 25 to 35 C in 21 days. Aflatoxin was much higher in 42-day samples than in 21 days at 18 and 20 C, but decreased greatly after 84 days. No aflatoxin formed at 16 C in 84 days nor at 41 C in 21 days.

Growth and Sporulation by *A. flavus* and Other Fungi.—Growth and sporulation by *A. flavus*, as observed in environmental cabinets, were

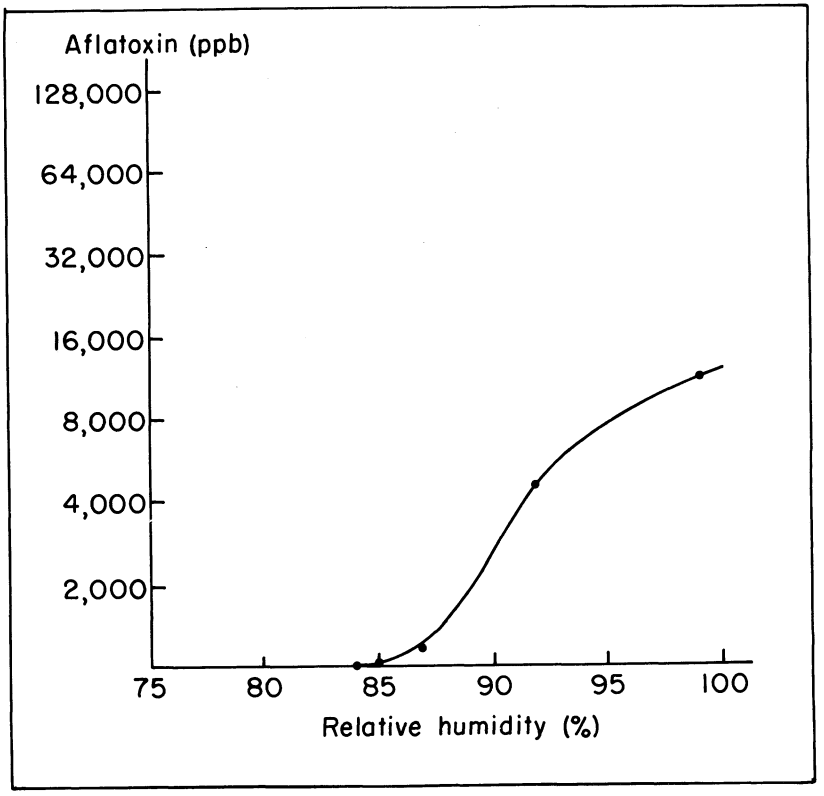

Fig. 10. Effect of relative humidity on aflatoxin production in kernels of unshelled stored peanuts incubated at 30 C for 21 days.

correlated with temperature and RH combinations at which aflatoxin was produced. Growth and sporulation by *A. flavus* were profuse at temperatures of 41 to 45 C, at which no aflatoxin was formed. Other fungi grew at RH and temperatures favorable and unfavorable for the development of *A. flavus*. Most of the fungi growing at RH below 86 percent were members of the *A. glaucus* group (*A. amstelodami, A. ruber, A. chevalieri,* and *A. repens*). Several fungi (*A. niger* and *A. wentii*) competed with *A. flavus* at high RH of 92 to 99 percent and temperatures of 30 to 40 C, whereas *A. ochraceus, A. tamarii,* and several species of *Penicillium* were prominent at high RH and temperatures of 15 to 25 C.

Relation of RH to Aflatoxin Production at 20 C.—Supplementary experiments were conducted to determine the limiting RH for aflatoxin production at the lowered constant temperature of 20 C with incuba-

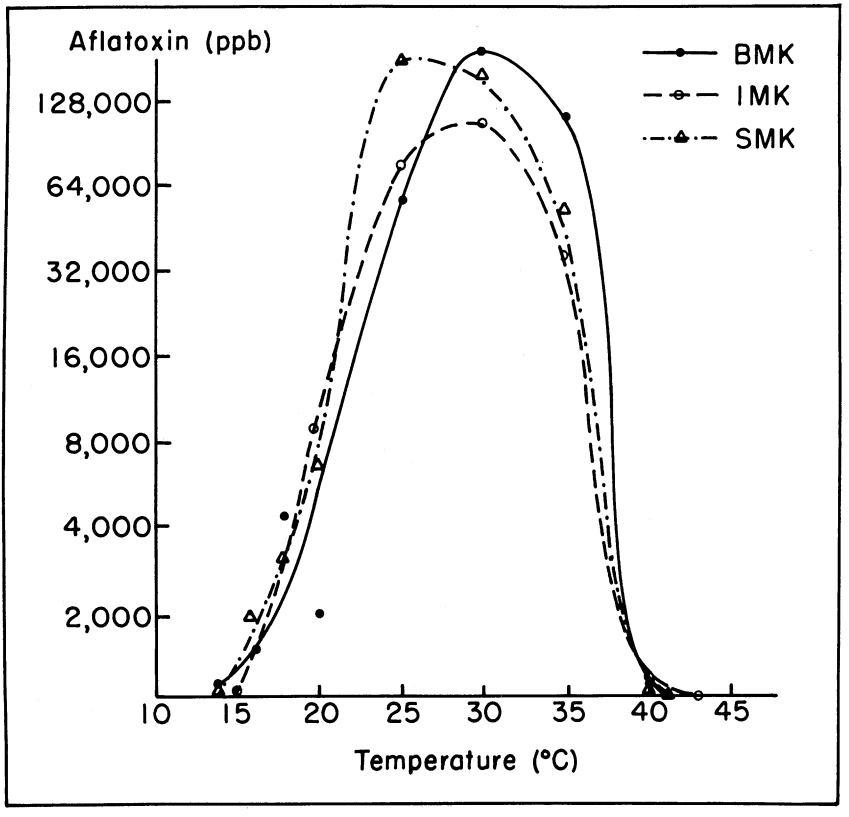

Fig. 11. Effect of temperature on aflatoxin production in broken mature kernels, immature kernels, and sound mature kernels of stored peanuts incubated at 99 percent relative humidity for 21 days.

tion (storage) for as long as 84 days. Data on aflatoxin production in SMK, IMK, and BMK for 21 days at 20 C is presented in Figure 13. Moderate amounts of aflatoxin developed at 92 percent and 99 percent RH in 21 and 42 days in SMK, IMK, and BMK; aflatoxin decreased to about 15 to 18 percent of that amount in 84 days. No aflatoxin was produced at 83 percent RH, and only low levels at 86 percent RH after 84 days incubation of SMK and BMK. No aflatoxin formed at 86 percent in IMK nor at 92 percent RH after 21 days, but only at 92 percent after 42 and 84 days storage.

In kernels from unshelled peanuts with intact pods, aflatoxin formed only at 99 percent RH, and failed to form at 83, 86, and 92 percent RH for up to 84 days storage, Figure 14. Reducing the storage temperature to 20 C reduced the maximal level of aflatoxin

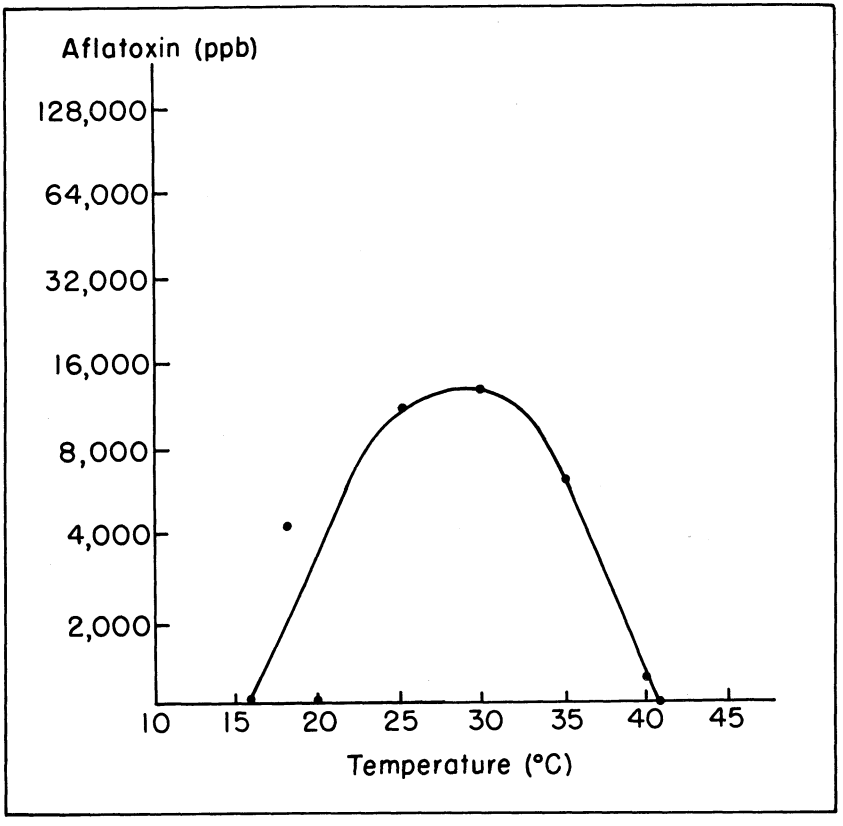

Fig. 12. Effect of temperature on aflatoxin production in kernels of unshelled stored peanuts incubated at 99 percent relative humidity for 21 days.

formed at all combinations of RH and length of incubation. Some aflatoxin was formed at 86 percent RH in SMK and BMK; none was formed at 86 percent RH in IMK and none at 92 percent in kernels of unshelled peanuts.

Discussion.—Estimation of aflatoxin by the methods used in this investigation has been reported to be sensitive to about ± 1 to 2 ppb. The limiting RH data in living cured peanuts closely paralleled that obtained with sterile peanuts (22) in that 85 percent was the lowest RH at which aflatoxin formed in 21 days. When the storage period was extended to 84 days in these experiments, aflatoxin formed in IMK and in BMK at 84 percent RH. However, no aflatoxin formed in IMK and in BMK at 84 percent RH. However, no aflatoxin was present at 84 percent RH in SMK after 84 days incubation. In kernels

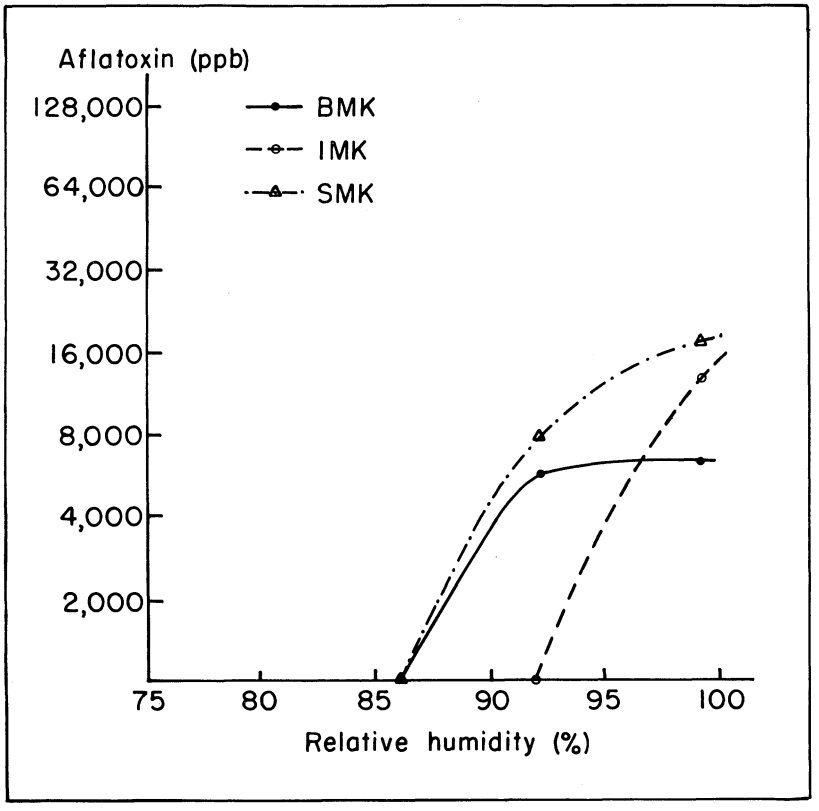

Fig. 13. Effect of relative humidity on aflatoxin production in broken mature kernels, immature kernels, and sound mature kernels of stored peanuts incubated at 20 C for 21 days.

of unshelled, living, cured peanuts, aflatoxin was present at 87 percent RH in 21 days, which is similar to that reported for sterile peanuts (22) and for freshly dug peanuts (23).

The limiting temperature data for living stored peanuts closely paralleled those of previous studies using sterile mature and immature peanuts (22) in that aflatoxin was formed at 14 C in SMK, but not at 12 C. Likewise, aflatoxin formed in immature kernels at 15 C, but not at 14 C. However, in damaged or BMK, some aflatoxin formed at 12 C in sterile peanuts, whereas in cured, living peanuts BMK aflatoxin formed at 14 C, but not at 12 C. The greatest contrast between results obtained here and those previously reported was that living kernels of unshelled peanuts showed no aflatoxin at 16 C (even after 84 days), whereas aflatoxin formed in heat-treated kernels

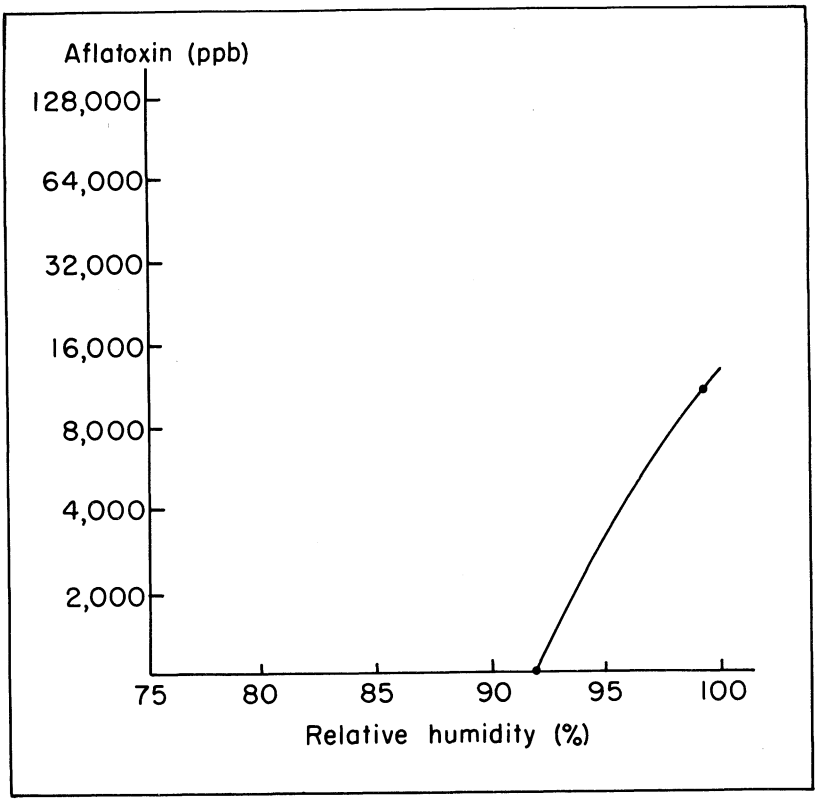

Fig. 14. Effect of relative humidity on aflatoxin production in kernels of unshelled stored peanuts incubated at 20 C for 21 days.

of unshelled peanuts at 14 C in 21 days. This difference in the limiting temperature is probably associated with the barrier or resistance, or both, of the intact shell and the testa of the living kernel to penetration and colonization by *A. flavus* at high RH (99 percent). Physiological activity of the living peanut at high RH and 16 C must have afforded resistance adequate to prevent invasion by the fungus, which readily colonized heat-treated kernels with intact shells under those conditions.

FFA formation was generally correlated with growth of the fungi composing the mycoflora of stored, living peanuts and not to the growth of *A. flavus* alone nor to aflatoxin production.

Substrate moisture was correlated with fungal growth rather than with aflatoxin or FFA production. Although aflatoxin accumulation was largely a measure of the growth of *A. flavus* as influenced by RH,

temperature, and time, it was also probably reduced by the competition of other fungi in the mycoflora. Many of these fungi grew profusely at RH and temperatures unfavorable for *A. flavus* e.g., the *A. glaucus* group of species visually dominated the mycoflora at RH of 70 to 80 percent and temperatures of 20 to 25 C. There were probably more species of fungi in the peanut mycoflora with growth optima near 25 C than at 20, 30, or 35 C (63). *A. flavus* grew profusely at 25 C, although competitively it was more dominant in our experiments at 30 and 35 C, since its optimum is 35 to 37 C.

In experiments at 20 C, aflatoxin formed in SMK and BMK at 86 percent RH only after 84 days. In IMK, no aflatoxin was found below 92 percent RH. Aflatoxin formed only at the highest RH (99 percent) in kernels from unshelled peanuts with intact shells. Thus reducing the temperature had a notable effect on the limiting RH for aflatoxin formation in kernels of unshelled peanuts as compared to SMK.

Aeration

The influence of the atmospheric gases oxygen (0_2), carbon dioxide (CO_2) and nitrogen (N_2) on growth, sporulation, and aflatoxin formation by *A. flavus* in peanuts was investigated by Auburn researchers (48, 76). *A. flavus,* isolate Ala-6, obtained from Alabama peanuts in 1964 was used in both investigations. Sound mature kernels of the 1964 and 1965 crops of Early Runner were obtained from the Wiregrass Substation, Headland, Alabama. Compressed air and varying gas mixtures were metered into water filled flasks and then passed to the bottom of a 1.25 liter culture vessel containing 150 grams of peanut kernels dispersed in nine layers on wire baskets, Figure 15. Peanut kernels that had been previously surface-disinfected with 20 percent Clorox (1 percent sodium hypochlorite) were inoculated with *A. flavus* spores.

The gas was released in 125 ml of solution in the bottom of the culture vessel, through the inoculated peanuts, and into a second culture vessel where the cycle was repeated. The gas passed from the second culture vessel to an outlet seal and then into the atmosphere of the laboratory. At the beginning of each experiment, the culture vessels were flushed with the gas at 2 to 4 times the normal rate for 30 minutes; then the gas flow was reduced for the duration of the 14-day incubation period.

Gas mixtures were certified to be within a tolerance of ± 0.5 percent by volume. O_2 and CO_2 were measured during the experiments

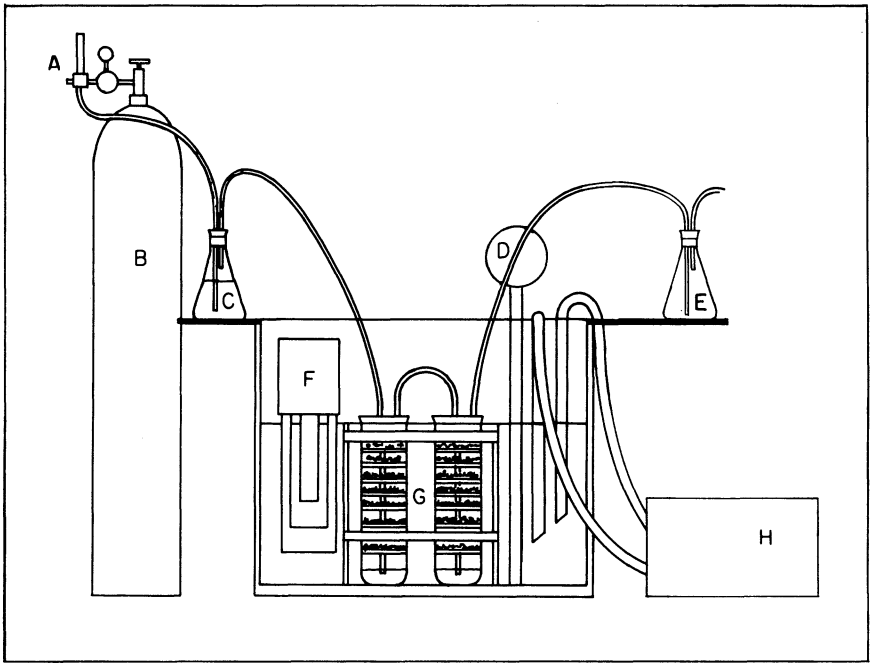

Fig. 15. Diagram of apparatus for studying the influence of atmospheric gases on aflatoxin production in peanuts.

with an appropriate oxygen meter and a gas analyzer. Moisture content and free fatty acid analyses of peanut kernels were determined by official methods of the American Oil Chemists' Society (1). Aflatoxin determinations were made on duplicate 30 g samples of dried, ground kernels from each culture vessel by the aqueous acetone method of Pons and Goldblatt (68).

Landers et al. (48) observed that no visible change in *A. flavus* growth and sporulation occurred when CO_2 concentration was increased from 0.03 percent (air) to 20 percent; however, aflatoxin formation was reduced 75 percent, Table 5. Further reduction in aflatoxin production occurred with each increase in CO_2 from 20 to 80 percent, and no aflatoxin was produced at 100 percent CO_2. Similarly, Stotzky, and Goos (83) reported that *A. flavus* did not develop when stored 14 to 17 days under 100 percent CO_2, although the fungus was not killed. Fungus growth was observed after 24 hours in

TABLE 5. INFLUENCE OF VARIOUS CONCENTRATIONS OF CO_2 AND N_2 WITH 20 PERCENT O_2 ON KERNEL MOISTURE CONTENT AND FORMATION OF FREE FATTY ACIDS AND AFLATOXIN BY *Aspergillus flavus* IN PEANUTS INCUBATED AT 30 C AND 99 PERCENT RELATIVE HUMIDITY FOR 14 DAYS

Concentration of gases			Kernel moisture content	Free fatty acids	Aflatoxin
CO_2	O_2	N_2			Total
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*ppb*
0.03	21	79	21.84	56.78	299,381
20	20	60	25.26	47.83	74,550
40	20	40	27.27	36.95	35,420
60	20	20	30.27	25.78	19,815
80	20	0	28.33	12.58	103
100	0	0	28.04	0.08	6
Untreated check			6.03	0.15	0

culture vessels containing air. After 2 weeks, no difference in growth and sporulation of *A. flavus* was visible between peanuts incubated in air and in 20 percent CO_2. Growth and sporulation decreased with increasing concentrations of CO_2, closely paralleling the decrease in fatty acids. Thus, in gas mixtures with an O_2 concentration near that of air (20 percent), increasing the CO_2 concentration reduced growth, sporulation, free fatty acid formation, and aflatoxin production by *A. flavus* in peanuts.

The influence of various concentrations of O_2 and N_2 in the absence of CO_2 on production of aflatoxin and free fatty acids is shown in Table 6. The highest amounts of aflatoxin were present in peanuts incubated in air and at 15 percent O_2. Reductions in aflatoxin occurred when the O_2 concentration was decreased to 10 and 5 percent. Large decreases occurred with a reduction from 5 to 1 percent O_2 and from 1 to 0.1 percent O_2. Growth and heavy sporulation of *A. flavus* occurred on peanuts at O_2 concentration of 5 percent and

TABLE 6. INFLUENCE OF VARIOUS CONCENTRATIONS OF O_2 AND N_2 ON KERNEL MOISTURE CONTENT FORMATION OF FREE FATTY ACIDS AND AFLATOXIN BY *Aspergillus flavus* IN PEANUTS INCUBATED AT 30 C AND 99 PERCENT RELATIVE HUMIDITY FOR 14 DAYS

Concentration of gases			Kernel moisture content	Free fatty acids	Aflatoxin
CO_2	O_2	N_2			Total
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*ppb*
0.03	21	79	30.27	57.03	511,888
0	15	85	28.73	56.58	519,293
0	10	90	25.85	53.95	316,122
0	5	95	26.51	52.95	154,118
0	1	99	28.91	17.45	5,929
0	0.1	99.9	28.17	2.50	70
Untreated check			6.51	0.23	0

higher. Sporulation was noticeably less at 1 percent O_2. Peanuts in the vessels containing only a trace (0.1 percent) of O_2 showed fungal growth 6 days after inoculation and only a slight amount of sporulation after 14 days. Free fatty acids decreased significantly only with decreases in O_2 from 5 to 1 percent and from 1 to 0.1 percent.

Most fungi are aerobic organisms and the inhibitory effect of low O_2 on *A. flavus* growth has been shown to be in proportion to oxygen solubility in the medium or mycelium (60). Reduction and restriction of growth, sporulation, aflatoxin, and free fatty acid formation in this research were most striking when O_2 concentrations were dropped from 5 to 1 percent and thence to a trace (0.1 percent).

The growth of fungi in seeds is dependent not only on the availability of favorable moisture and temperature but also on atmospheric conditions surrounding the seed. Favorable moisture (99 percent RH) and temperature (30 C) were maintained in these experiments, with the varying concentrations of CO_2, O_2, and N_2 being the major variables. The concentration of N_2 probably had no effect on *A. flavus,* since N_2 is considered to be an inert gas as far as most fungi are concerned (15). Concentrations of N_2 as high as 99 percent—1 percent O_2 did not inhibit fungus growth, as evidenced by production of free fatty acids and aflatoxin.

The effect of lowered temperature (15 C) for an extended incubation period (6 weeks) on the influence of atmospheric gas mixtures on KMC and formation of aflatoxin and FFA was also investigated, Table 7. There was a decrease in aflatoxin production in peanuts stored under 20 percent CO_2 for 6 weeks when O_2 was reduced from 20 to 5 percent. Aflatoxin was negligible in peanuts stored under

TABLE 7. INFLUENCE OF VARIOUS CONCENTRATIONS OF ATMOSPHERIC GASES ON KERNEL MOISTURE CONTENT AND FORMATION OF FREE FATTY ACIDS AND AFLATOXIN BY *Aspergillus flavus* IN PEANUTS INCUBATED AT 15 C AND 99 PERCENT RELATIVE HUMIDITY FOR 42 DAYS

Concentration of gases			Kernel moisture content	Free fatty acids	Aflatoxin
CO_2	O_2	N_2			Total
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*ppb*
0.03	21	79	31.50	31.03	120,315
20	20	60	30.07	35.28	13,128
20	5	75	27.03	12.30	754
40	5	55	26.86	1.68	T[a]
60	5	35	26.43	0.20	13
80	5	15	25.91	0.18	O[b]
Untreated check			6.58	0.13	T

[a] T = trace; less than 1 ppb aflatoxins.
[b] One replication only.

the higher concentrations of CO_2 (40 to 80 percent) at 15 C for 6 weeks. Profuse fungus growth and heavy sporulation by *A. flavus* occurred in peanuts in air and at 20 percent CO_2 and 20 percent O_2. However, mycelial growth was restricted and sporulation light at 20 percent CO_2 and 5 percent O_2. No growth or sporulation of *A. flavus* occurred at concentrations of CO_2 from 40 to 80 percent in combination with 5 percent O_2. Concentration of free fatty acids dropped sharply with an O_2 decrease from 20 to 5 percent and a subsequent increase in CO_2 from 20 to 40 percent. Thus at 15 C, growth, sporulation, and production of aflatoxin and free fatty acids by *A. flavus* was suppressed for 6 weeks by concentrations of CO_2 of 40 to 80 percent and 5 percent O_2.

Discussion: Decreases in aflatoxin production by *A. flavus* were noted with 20 to 80 percent CO_2 regardless of O_2 concentration, and no aflatoxin was produced at 100 percent CO_2. The effect of CO_2 concentrations from 20 to 80 percent in reducing growth by *A. flavus* was similar to that reported by Golding (32), who found that the growth of *A. flavus* was restricted at all temperatures at concentrations of 20 to 70 percent CO_2 with inhibition being minimized at higher temperatures (75 to 95 F) as in this investigation (86 F). The inhibitory effect of CO_2 on the growth of several fruit-rotting fungi was also demonstrated by Brown (9), who showed that the rate of increase in diameter of fungal colonies on agar was retarded with 10 percent and 20 percent CO_2 at 15 to 18 C, and growth was further reduced at 3 C. Similarly, the results of this study showed that concentrations of CO_2 of 20 percent and above were far more inhibitory to growth, sporulation, and production of free fatty acids and aflatoxin by *A. flavus* at 15 C than at 30 C.

At 20 percent CO_2 or in the absence of CO_2, sharp reductions in growth, sporulation, and production of aflatoxin and free fatty acids occurred when O_2 dropped below 5 percent to 1 percent or lower. A similar significant reduction was reported in the growth of *Penicillium roqueforti* Thom with 2 and 4 percent O_2 (32). Oxygen concentrations below 1 percent were required to inhibit the growth of several common fruit-decay fungi (28). In this investigation, growth and aflatoxin production by *A. flavus* occurred at 1 percent O_2—99 percent N_2, and at 1 percent O_2—19 percent N_2—80 percent CO_2. The conclusion that soil-inhabiting microorganisms are generally tolerant to conditions of high CO_2 and low O_2 applies to *A. flavus*, which is a soil-inhabiting organism as well as a storage fungus (11, 83). Thus, our findings corroborate previous reports that high con-

centrations of CO_2 were the primary source of inhibition of growth and aflatoxin production by *A. flavus* on peanuts rather than low O_2 concentrations under optimal temperature and moisture conditions for fungal development.

The influence of relative humidity was incorporated into the same experimental design by using flasks of saturated water solutions of ammonium dihydrogen phosphate and potassium chromate to maintain RH levels of approximately 92 and 86 percent, respectively. The RH around kernels is closely correlated with KMC of the peanuts. The influence of a gas mixture of 60 percent CO_2, 20 percent O_2, and 20 percent N_2 on production of aflatoxin and FFA by *A. flavus* on peanuts stored for 14 days at various RH levels at 25 C is shown in Table 8. Aflatoxin production decreased approximately 10 percent and 65 percent in air as RH was lowered from 99 percent to 92 percent and 86 percent, respectively. Treatment with 60 percent CO_2 reduced aflatoxin production by more than 99.6 percent (from 250 ppb at 99 percent RH to 10 ppb at 86 percent RH); an amount no greater than that sometimes detected in untreated kernels assayed immediately when removed from cold storage. Visible growth and sporulation decreased in 60 percent CO_2 and air as RH decreased. No fungal growth or sporulation was observed in 60 percent CO_2 at 86 percent RH, but both were abundant in air at 86 percent RH. Percentage FFA decreased with decreased RH in both 60 percent CO_2 and air. The percentage FFA produced in peanuts in the 60 percent CO_2 and 86 percent RH treatment was only 0.05 percent more than that found in the untreated kernels.

The influence of a gas mixture of 40 percent CO_2, 20 percent O_2, and 40 percent N_2 on the production of aflatoxin and FFA by *A. flavus* on peanuts stored in various RH at 25 C is shown in Table 9.

TABLE 8. INFLUENCE OF 60 PERCENT CO_2 AND THREE RELATIVE HUMIDITIES ON KERNEL MOISTURE CONTENT AND FORMATION OF FREE FATTY ACIDS AND AFLATOXIN BY *Aspergillus flavus* IN PEANUTS INCUBATED AT 25 C FOR 14 DAYS

Gas conc.			RH	KMC	FFA	Aflatoxin
CO_2	O_2	N_2				Total
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*ppb*
0.03	21	79	99	22.4	69.2	206,300
0.03	21	79	92	26.5	58.5	185,200
0.03	21	79	86	15.0	44.1	72,100
60	20	20	99	24.5	8.1	200
60	20	20	92	20.0	3.3	+[a]
60	20	20	86	11.8	.6	0
Untreated check				6.0	.5	0

[a] + = 10-49 ppb aflatoxin.

TABLE 9. INFLUENCE OF 40 PERCENT CO_2 AND THREE RELATIVE HUMIDITIES ON KERNEL MOISTURE CONTENT AND FORMATION OF FREE FATTY ACIDS AND AFLATOXIN BY *Aspergillus flavus* IN PEANUTS INCUBATED AT 25 C FOR 14 DAYS

Gas conc.			RH	KMC	FFA	Aflatoxin
CO_2	O_2	N_2				Total
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*ppb*
0.03	21	79	99	26.8	19.4	196,600
0.03	21	79	92	21.7	6.4	37,400
0.03	21	79	86	17.1	3.0	11,800
40	20	40	99	29.2	7.0	3,800
40	20	40	92	24.8	1.5	300
40	20	40	86	17.4	.3	0
Untreated check				4.4	.3	0

Aflatoxin production in air decreased approximately 80 percent and 95 percent when RH was lowered from 99 percent to 92 percent and 86 percent RH, respectively. In 40 percent CO_2, aflatoxin production was decreased approximately 85 percent when RH was lowered from 99 to 92 percent. When RH was further lowered to 86 percent RH, aflatoxin production was prevented. In both air and 40 percent CO_2, growth and sporulation decreased as RH was lowered. In 40 percent CO_2 at 86 percent RH, fungus growth was negligible and percentage FFA approximated that of uninoculated checks.

The influence of lowered temperature with a gas mixture of 20 percent CO_2, 20 percent O_2, and 60 percent N_2 on the production of aflatoxin and FFA by *A. flavus* on peanuts stored in various RH at 17 C is shown in Table 10. In air at 17 C, aflatoxin production decreased approximately 80 percent when RH was lowered from 99 percent to 92 percent. A further decrease in RH to 86 percent resulted in the production of 210 ppb of aflatoxin in air. The largest amount of aflatoxin produced in 20 percent CO_2 was 174 ppb at 99 percent RH with a negligible amount at 92 percent and 86 per-

TABLE 10. INFLUENCE OF 20 PERCENT CO_2 AND THREE RELATIVE HUMIDITIES ON KERNEL MOISTURE CONTENT AND FORMATION OF FREE FATTY ACIDS AND AFLATOXIN BY *Aspergillus flavus* IN PEANUTS INCUBATED AT 17 C FOR 14 DAYS

Gas conc.			RH	KMC	FFA	Aflatoxin
CO_2	O_2	N_2				Total
Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*ppb*
0.03	21	79	99	24.3	10.7	57,100
0.03	21	79	92	17.3	3.3	2,500
0.03	21	79	86	15.5	.6	200
20	20	60	99	21.6	1.0	200
20	20	60	92	17.6	.3	+[a]
20	20	60	86	15.8	.5	+
Untreated check				6.1	.3	+

[a] + = 1-49 ppb aflatoxin.

cent RH. Thus at 17 C, 20 percent CO_2 with a slight reduction in RH virtually eliminated aflatoxin production in peanuts for 14 days, whereas at 25 C, this concentration of CO_2 gave little or no control over toxin production. Growth and sporulation were noted in all air treatments and in 20 percent CO_2 at 99 percent RH; however, little or no visible growth occurred in 20 percent CO_2 at 92 percent and 86 percent RH at 17 C. In 20 percent CO_2 at 92 percent and 86 percent RH, percentage FFA was approximately the same as that of the untreated check.

This investigation showed that concentrations of 20 percent and 40 percent CO_2 in combination with reduced temperatures, reduced RH, or both, prevented aflatoxin formation in peanuts. In earlier experiments, Table 7, it was noted that growth and aflatoxin production of *A. flavus* was inhibited by 40 percent, 60 percent, and 80 percent CO_2 at 15 C and 99 percent RH. It was also found that at 99 percent RH, aflatoxin was formed at 20 percent CO_2 and 15 C, as was the case of 17 C in this study; it was further noted here that with a reduction of RH to 92 percent or less, no growth or aflatoxin production occurred. At higher temperatures (25 C) aflatoxin was formed at 40 percent CO_2 and 92 percent RH, but not at 86 percent RH. Also, at 25 C a concentration of 60 percent CO_2 inhibited aflatoxin at 92 percent RH. As in previous investigations (22, 23, 25, 48), percentage FFA was used as an index of fungus growth, since mycelial growth and sporulation are difficult to measure in a natural substrate. Carbon dioxide reduced the growth of *A. flavus* as evidenced by decreases in percentage FFA and in aflatoxin production in comparison to that in air. At a given temperature in air or at a given CO_2 concentration, lowering the relative humidity or the KMC of the substrate also reduced fungus growth and indirectly reduced FFA and aflatoxin. Thus, prevention of fungus growth and aflatoxin production was accomplished with several different concentrations of CO_2 in combination with certain temperatures and RH. Aflatoxin elaboration was prevented in simulated storage conditions with 20 percent CO_2, 17 C, and 86 percent RH.

Pod and Kernel Damage

Factors other than peanut kernel moisture content, relative humidity surrounding pod and/or kernel, moisture-related drought stress, time-related kernel maturity and drying in the field, and temperature-time have been related to the invasion of peanuts by *Aspergillus flavus* and subsequent aflatoxin formation. Rapid invasion of pods

by *A. flavus* in the soil frequently has been associated with physical and biological damage to the shell and kernels. In Nigeria, kernels of broken pods showed extensive fungal contamination in comparison with that of kernels from undamaged pods (55, 56, 59) with preharvest development of aflatoxin occurring only in kernels of broken pods (56). In Texas, aflatoxins were found in kernels of mechanically damaged pods, with much larger quantities developing when pod openings resulted from growth cracks (78). Similarly, visible fungal invasion was observed in growth cracks of pods in the soil of South Africa (79). *A. flavus* invasion and aflatoxin formation in kernels have been widely associated with pod damage caused by termites in the ground in South Africa (79, 80) and Nigeria (54, 59), and by lesser cornstalk borer in Texas (2). Pod openings caused by pathogenic fungi such as *Rhizoctonia solani* and *Sclerotium rolfsii* also provide entrance for *A. flavus* and subsequent aflatoxin formation (2, 78).

Bampton (6) concluded that damage to the shell is a major factor in *A. flavus* invasion and aflatoxin development in the kernel during drying. He observed that shelling (decortication) of peanuts at harvest resulted in testa damage and usually increased fungal invasion and the possibilities of toxin development in the kernel, especially before the seed were dried to safe moisture levels. The intact shell serves as a barrier to fungus invasion and some protection may also be afforded by the testa. In Georgia, it was found that the mycoflora of mature peanut kernels from unblemished intact pods was usually in or beneath the dead cells of the testa (36). Nigerian scientists showed that kernels from damaged pods were more frequently contaminated with *A. flavus* and aflatoxin than kernels from undamaged pods during sun- and artificial-drying (54, 56, 59). Under gnotobiotic conditions, it was demonstrated that *A. flavus* readily penetrated the shell, but was limited in its invasion of the kernel by the testa. Colonization of the embryos by *A. flavus* was relatively limited (50). Damage to shell or kernel affords increased opportunities for rapid and direct invasion of the kernel, which increases the possibility of aflatoxin formation. Pattee and Dickens found that "analysis of the damage segment in peanuts may be used as a sensitive indicator for detecting the presence or absence of aflatoxin in farmers' stock peanuts" (64). Damage also increases nutrient availability. Thus, the extent of fungus growth and toxin formation at minimal temperatures and RH usually will be determined by time and the availability of nutrients resulting from damage.

Microbial Interaction

Aspergillus flavus is frequently found associated with numerous other microorganisms in stored grains and seeds. Thus, the possibility arises that microbial competition between fungi for the substrate under favorable environmental conditions will restrict or reduce the amount of aflatoxin formed. *A. flavus* or competing fungi might absorb or degrade aflatoxin following its formation in the substrate. Research suggests that microbial competition or microbial breakdown may be responsible for smaller amounts of aflatoxin in the kernels of parasite-damaged pods than in kernels from broken pods (78). Later, Ashworth *et al.* (2) demonstrated that several fungi could break down aflatoxin in peanuts and in an aflatoxin-containing liquid medium. They also found that *A. niger* and *Rhizoctonia solani* limited the development of *A. flavus* and aflatoxin production in the substrate.

In Israel, it was noted that a large number of colonies of *A. niger* occurred in the geocarposphere (soil around the pods), while penetration into kernels was proportionally moderate in light and medium soil and more common in heavy soil (44). There was very little penetration into kernels by *A. flavus, Fusarium solani, Penicillium funiculosum* and *P. rubrum.* A positive relationship between prevalence in geocarposphere and kernels was noted only for *A. niger.* Ready penetration into kernels by this fungus may have prevented penetration by other fungi. The prevalence and dominance of *A. niger* in peanut shells and kernels may also explain the relatively low rate of seed transmission of other pathogens, particularly *Fusarium* spp. and *Sclerotium bataticola* (42). In other studies, the relationship between the dominant fungi *A. flavus, A. niger, P. funiculosum, P. rubrum,* and *F. solani* was studied in 234 samples and 5,850 culture plates made with fresh and stored peanuts kernels from 2 groundnut crop years in Israel (41). The number of colonies developing in individual plates showed pronounced antagonism between *A. flavus* and *A. niger* and slightly less but still marked antagonism between each of these species and *P. funiculosum, P. rubrum,* and *F. solani.* In Georgia, it was noted that *A. flavus* invaded pods infested by *S. bataticola,* but that the reverse did not occur (37). *A. flavus* had a pronounced depressing effect on the rate and extent of kernel infection by *S. batticola* (38). On malt-extract agar, growth of *S. bataticola* was strongly inhibited by *A. flavus,* but lysis was not observed.

Bacillus subtilis was frequently isolated from peanut kernels from New Mexico, Oklahoma, and Texas (66). The growth of the bacte-

rium in a peanut kernel prevented the development of fungal growth in that kernel. Species of *Macrophomina, Chaetomium, Choanephora,* and *Penicillium* growing from adjacent, bacterium-free kernels on the same plate were inhibited by an antifungal substance diffusing into the agar. This inhibition suggested that *B. subtilis* may play a role in the inhibition of potential pathogenic and toxigenic fungi in the peanut kernel.

Under gnotobiotic (aseptic) conditions, *A. flavus* penetrated and colonized a high percentage (9 to 77 percent) of shell tissues of living, attached, immature, and mature pods (50). Under natural conditions, *A. flavus* was found in only a small percentage of shells (30, 40) and less *A. flavus* was isolated from the shell portion of freshly-dug pods than from the kernels contained in those shells (69). Either the normal endogeocarpic mycoflora of the shells was antagonistic to *A. flavus,* which limits its colonization of shell tissue, or the faster growing components of the mycoflora masked *A. flavus* when shells were plated on agar media. Using surface-disinfested attached pods, Jackson (37) obtained similar results in that *A. flavus,* as well as *A. niger, S. bataticola,* and *R. stolonifer,* readily penetrated and colonized a large percentage of pods, especially at high temperatures (26 to 38° C). In the gnotobiotic study (50) there was no evidence of natural resistance to kernel invasion in living attached pods as suggested by Austwick and Ayerst (5). Thus, the presence of normal endogeocarpic mycoflora appears to provide a barrier to the invasion of peanut kernels by *A. flavus.*

CONCLUSIONS

This research at Auburn University was conducted to control the aflatoxin problem in peanuts based on the prevention of contamination. To approach a practical solution, the basic research presented here has determined rather precisely the limiting and optimal relative humidities (kernel moisture contents) and temperatures for aflatoxin production by *A. flavus* in shelled sound mature, broken mature, and immature kernels, and kernels from intact pods. In addition, data on the aflatoxin-producing ability of isolates of *A. flavus* from several countries and commodities, the relation of initial moisture prior to storage on fungal development in peanuts, the relation of overmaturity to invasion by *A. flavus* in the field, and the influence of varying concentrations of atmospheric gases on the growth and formation of aflatoxin by *A. flavus* in surface-sterilized sound mature kernels have been presented, and the significance of these results discussed in relation to that of other investigators.

From these data and the literature, it can be noted that *A. flavus* is present on a worldwide basis, particularly in areas of peanut production. A high percentage of isolates are aflatoxin-producers. *A. flavus* grows and produces aflatoxin on almost any raw or processed food or organic substrate if the environmental conditions are favorable. Some of the optimal conditions are temperatures from 25 to 35 C and relative humidities of 85 to 90 percent or higher, or peanut kernel moisture contents in excess of 10 percent, Table 11. Limiting conditions that prevent aflatoxin production by the fungus were demonstrated to be 83 percent relative humidity or lower (peanut KMC of 10 percent or lower) and temperatures of 12 C or lower and 41 C and higher. The effect of drought stress, overmaturity, and physical and biological damage to pod and kernel by *A. flavus* invasion and aflatoxin formation in the field was described and related to the findings of several investigators. Weather conditions at harvest, amount of damage to pod and kernel during digging and subsequent picking (threshing), and environmental conditions and the rate of dehydration during field and/or artificial drying are important factors in the degree of *A. flavus* invasion, deterioration, and aflatoxin production in peanut kernels. Microbial interaction and competition of soil and pod microflora appear to reduce growth and aflatoxin production by *A. flavus*. Microbial breakdown of aflatoxin by other fungi has been demonstrated (2) and may be a factor in field, windrow, and/or storage. The potential use of 20 to 60 percent CO_2 for control of fungi during commercial storage was indicated by research data utilizing relative humidities of 90 percent or lower and temperatures below 20 C. Possible effects on peanut flavor and quality were not evaluated.

The prevention of aflatoxin contamination is considered the ideal way to resolve the aflatoxin problem and is based primarily on intelligent, timely, good management practices to reduce the time pea-

TABLE 11. SUMMARY OF LIMITING RELATIVE HUMIDITIES AND TEMPERATURES FOR AFLATOXIN PRODUCTION BY *Aspergillus flavus* IN KERNELS OF UNSHELLED PEANUT PODS

Environmental variable	Limiting value		
	Heat-killed	freshly dug	Stored
RH at 30 C, 21 days	86.5 ± 0.5%	84.0 ± 1.0%	86.5 ± 0.5%
84 days			85.5 ± 0.5%
RH at 20 C, 84 days			95.0 ± 3.0%
Temperature at 98 ± 1.0% RH, 21 days	13.0 ± 1.0 C	17.5 ± 2.5 C	22.5 ± 2.5 C
84 days			17.0 ± 1.0 C
	40.0 ± 1.5 C	37.5 ± 2.5 C	40.5 ± 0.5 C

nuts are in the temperature-moisture regime favorable for aflatoxin formation (16). Harvesting at maturity with minimal damage to pods followed by rapid drying and storage in a low moisture environment will reduce aflatoxin development to a minimum. Cool dry weather is beneficial during the harvesting and drying of peanuts. Cold air can be used to aerate farmers stock peanuts in storage. The use of refrigerated storage for shelled peanuts is common. Several approaches for controlling the aflatoxin problem in peanuts have been thoroughly summarized by Dickens (16).

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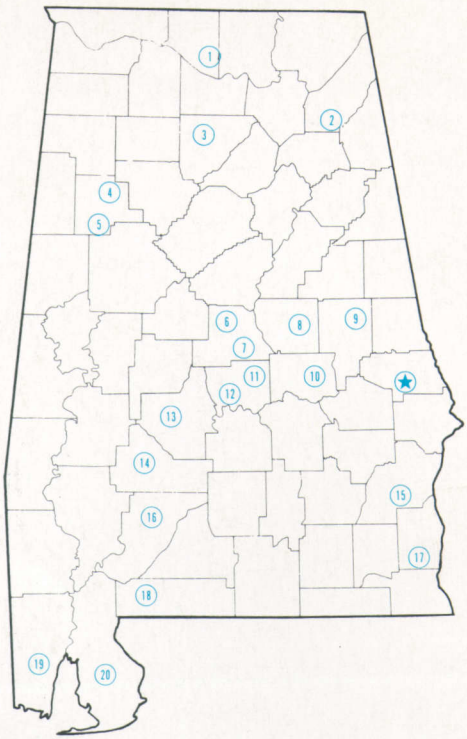
Alabama's Agricultural Experiment Station System
AUBURN UNIVERSITY

With an agricultural research unit in every major soil area, Auburn University serves the needs of field crop, livestock, forestry, and horticultural producers in each region in Alabama. Every citizen of the State has a stake in this research program, since any advantage from new and more economical ways of producing and handling farm products directly benefits the consuming public.



Research Unit Identification

★ Main Agricultural Experiment Station, Auburn.

1. Tennessee Valley Substation, Belle Mina.
2. Sand Mountain Substation, Crossville.
3. North Alabama Horticulture Substation, Cullman.
4. Upper Coastal Plain Substation, Winfield.
5. Forestry Unit, Fayette County.
6. Thorsby Foundation Seed Stocks Farm, Thorsby.
7. Chilton Area Horticulture Substation, Clanton.
8. Forestry Unit, Coosa County.
9. Piedmont Substation, Camp Hill.
10. Plant Breeding Unit, Tallassee.
11. Forestry Unit, Autauga County.
12. Prattville Experiment Field, Prattville.
13. Black Belt Substation, Marion Junction.
14. Lower Coastal Plain Substation, Camden.
15. Forestry Unit, Barbour County.
16. Monroeville Experiment Field, Monroeville.
17. Wiregrass Substation, Headland.
18. Brewton Experiment Field, Brewton.
19. Ornamental Horticulture Field Station, Spring Hill.
20. Gulf Coast Substation, Fairhope.