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Colonization and Biochemical Changes in Peanut Seeds Infected with *Aspergillus flavus*[1]

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ABSTRACT

Seeds from three commercially grown peanut cultivars and two "resistant" genotypes had varying degrees of resistance to colonization by the storage fungus, *Aspergillus flavus*. Peanut genotypes PI337409 and PI337394F had significantly higher resistance to colonization than other tested cultivars. Some of the biochemical changes in peanuts resulting from *A. flavus* infestation included: Reduction of oil and protein content, rapid increase in free fatty acids and changes in the amino acid composition.

Key Words: *Arachis hypogaea*, seed contamination, mycotoxins, storage fungi.

Peanut seed composition is rapidly altered due to colonization by the storage fungus *Aspergillus flavus* under favorable conditions of temperature and high moisture (18). Induced biochemical changes of this nature result in reduction of dry matter and oil, increase in free fatty acids and in eventual deterioration of seed quality and nutritional value of infested peanuts (11, 13). Studies conducted by Cherry *et al.* (4, 6, 7, 8, 9, 10) on the effect of *Aspergilli (A flavus, A. parasiticus,* and *A. oryzae)* and other fungi *(Neurospora sitophila* and *Rhizopus oligosporus)* have shown the following changes that were distinguishable from "standard" profiles of uninoculated peanuts: Decomposition of the major storage proteins to small molecular weight components and quantitative depletion of the small protein components (4, 6, 7); changes in enzyme activity (10); changes in total amino acids (8); and changes in quantity of ether-soluble oil (9). Other reports (2, 5, 12) have shown the production of aflatoxin when peanuts are invaded by fungi. However, varying degrees of resistance to *A. flavus* invasion of peanut seeds has been reported. Recently, Bartz *et al.* (3) observed a high degree of resistance in cultivars such as 'Florunner' and 'Altika' to colonization by *A. flavus*. Two peanut genotypes PI337409 and PI337394F have been reported by Mixon and Rogers (14) to be highly resistant to *A. flavus* invasion under varying conditions of temperature and humidity. The so called "resistant" cultivars and genotypes have potential value in a peanut breeding program to develop cultivars with seeds that have low incidence of colonization by fungi. However, these peanut cultivars and genotypes need to be evaluated for the biochemical changes induced because of A. *flavus* infestation. The purpose of this study was to monitor some specific biochemical changes of peanut seeds colonized by *A. flavus*.

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Materials and Methods

Peanut (*Arachis hypogaea* L.) cultivars and genotypes used in this study were: Two Virginia-type cultivars ('Altika' and 'Early Bunch'), one Runner-type cultivar ('Florunner'), and two resistant genotypes (PI337409 and PI337394F). Twenty grams of hand shelled seed samples with intact seed coats, representing each cultivar or genotype, were placed in a petri dish and surface sterilized by soaking for 5 minutes in 2.6 percent sodium hypochlorite. Soaking was followed by washing until the smell of chlorine disappeared (5-6 washings). The seeds were then transferred to a sterile petri dish and sterile glass distilled water was added to approimate 20 percent moisture on a seed weight basis. At least two replicates of each sample were inoculated with a one ml suspension of *A. flavus* strain NRRL 3251 (ca. 5 x 10^6 spores/ml) from 2 to 3 weeks old cultures growing on Czapek's agar. The control plates received one ml of sterile glass distilled water instead of A. *flavus* inoculum. The plates were closed, swirled gently to distribute inoculum, sealed using parafilm, and incubated at 30°C for 4, 7, and 14 days except in the case of the two resistant genotypes where lack of adequate seeds allowed incubation only for the 14-day period. The percentage of seeds colonized by *A. flavus* was recorded by visual inspection of the plates. After the desired incubation period, seeds were air dried and placed in an oven at 60°C for 48 hours to remove moisture. The seeds were then used for various chemical analyses. Total oil was extracted with acetone from three gram samples of ground peanut meal by Soxhlet extraction (1). Oil obtained by Soxhlet extraction was slowly heated to 60°C to remove acetone and the free fattty acid content of the oil was determined by the AOAC method (1). Total nitrogen in the defatted peanut meal was determined by micro-Kjeldahl analysis (1). Percent nitrogen was multiplied by 5.46 to obtain the total protein.

Amino acid composition of the defatted peanut meal was obtained by hydrolyzing the samples for 18 hours at 110°C (16), followed by analysis on a JEOL 6-AH automated amino acid analyzer. Statistical analysis of the data was done by the LSD method of comparing paired mean values (17).

Results and Discussion

Colonization of peanut samples by the fungus *A. flavus*, was visible after 4 days of incubation and progressed rapidly thereafter. During this period, the extent of colonization varied from three percent for Altika and Florunner to 66 percent for Early Bunch; no infestation was observed in the control plates (Fig. 1). After 7 days of incubation, Altika and Florunner were 50 percent infested and Early Bunch was completely colonized by *A. flavus*. Peanut seeds in the uninoculated plates showed no infestation. Commercially grown cultivars such as Altika and Early Bunch were completely infested by the fungus after 14 days of incubation, however, only 91% of the seeds of Florunner were colonized (Fig. 1). The two resistant gento-

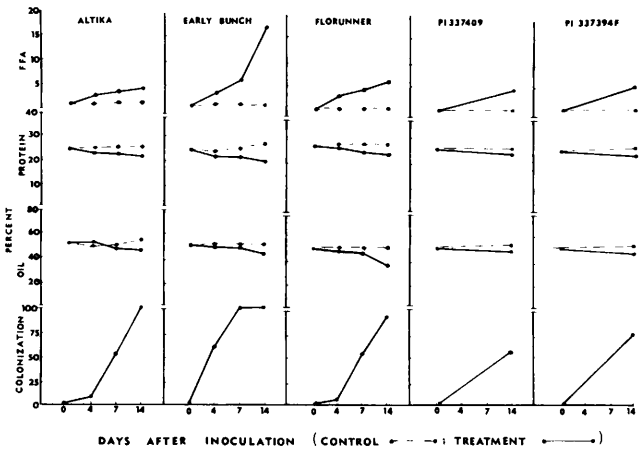

Fig. 1. Colonization and biochemical changes in control and *Aspergillus flavus* infested peanuts. LSD (P = 0.05) was 30.8, 2.13, 1.90, and 1.90 for percent colonization, oil, protein, and FFA, respectively.

types PI337409 and PI337394F showed considerable resistance to *A. flavus* and had only 55 and 75 percent infestation respectively (Fig. 1).

The data obtained in this study show that among the three commercial cultivars, Florunner had higher resistance to *A. flavus* than Altika and Early Bunch. However, the two genotypes (PI337409 and PI337394F) showed the highest resistance, especially PI337409 which was infested only 55 percent even after 14 days of incubation. Similar results have been reported by Mixon and Rogers (14) who found genotypes PI337409 and PI337394F to be resistant to *A. flavus* under varying environmental conditions. Recently, Bartz *et al.* (3) reported resistance to *A. flavus* in Florunner, Altika and genotypes PI337409, PI337394F, and UF71513. These authors further indicated that resistance in peanut seeds to fungus infestation seemed to be an extremely variable varietal characteristic.

There was no significant change in oil content of any of the inoculated peanut seed samples after 4 days of incubation (Fig. 1). However, after 7 days, all three commercially grown cultivars had reductions in the amount of oil. The uninoculated peanut seeds either had an increase or no change in the amount of oil. Reduction in oil content of *A. flavus* infested cultivars was associated with the production of an acrid-burnt odor and darkening of oil color. This odor was detected in all *A. flavus* colonized peanut samples regardless of the degree of infestation but not in control samples. After 14 days, significant reductions in oil content were noted in Early Bunch, Florunner and PI337394F However, Altika and PI337409 had no significant change in oil content (Fig. 1). On the basis of reduced oil content, the peanut seed samples can be arranged as follows:

Florunner (most reduction) > Early Bunch > PI337394F > Altika > PI337409 (least reduction)

It was reported earlier by Ward and Diener (18) that fungi belonging to the *Aspergillus* group can substantially reduce the oil content of peanuts during infestation. Cherry and Beuchat (19) have also observed a decline in the percentage of ether-soluble material obtained from peanut seeds that had been inoculated with various fungi. The results of our study show that Altika and PI337409 had the least change in the oil content when they were infested with *A. flavus*.

The three commercially grown cultivars and two resistant genotypes had a moderate to significant reduction in total protein due to A. *flavus* colonization (Fig. 1). The changes in protein content were evident after 7 days of incubation and continued through 14 days, however, very little changes occurred in uninoculated peanut seed samples. Reduction in total protein can be summarized as follows:

Early Bunch (most reduction) > Florunner > Altika PI337409 > PI337394F (Least reduction)

The results show that there was an insignificant change in the protein content of Florunner, Altika and two resistant genotypes. Cherry *et al.* (7) have reported a quantitative reduction in the amount of protein in peanuts 5 days after inoculation with *A. parasiticus*. Furthermore, they ovserved a significant decrease in the high molecular weight proteins and a corresponding increase in the low molecular weight proteins. It must be pointed out that our results refer only to the total protein fraction and not to the individual protein components.

The growth of *A. flavus* on various peanut seed samples resulted in an increase in the amount of detectable free fatty acids (FFA) (Fig. 1). Early Bunch had the highest increase in FFA. After 14 days of incubation, Florunner and the two resistant genotypes showed a moderate elevation in FFA levels. Increases in FFA can be summarized as follows:

Early Bunch (most increase) > Florunner > PI337394F > PI337409 > Altika (least increase)

An increase in FFA indicates the breakdown of triglycerides in peanut oil leading to an eventual deterioration of the seed quality and production of hydrolytic rancidity. Altika, PI337409 and PI337394F were quite resistant to changes in FFA content. Average FFA content of non-infested peanuts varies between 0.02 to 0.06 percent, but *A. flavus* infestation causes a rapid increase in the amount of FFA. The relationship between the *A. flavus* infestation and increase in FFA has been shown by Pattee and Sessoms (15) and Ward and Diener (18).

The amino acid composition of control and *A. flavus* inoculated peanut samples after 14 days of incubation are shown in Table 1. *A. flavus* infestation resulted in decreased content of the basic amino acids (lysine, histidine, and arginine). The aspartic acid levels of the *A. flavus* infested peanut

Table 1. Amino Acid Composition of Various Peanut Cultivars and Genotypes.

	Altika		Early Bunch		Florunner		PI 337409		PI 337394F		LSD (P=0.05)
Amino Acid	C	T	C	T	C	T	C	T	C	T	
Lysine	3.0	2.4*	3.5	3.4	3.8	2.0*	3.0	2.3*	3.6	1.9*	0.46
Histidine	2.3	1.6*	2.5	1.6*	2.4	0.7*	1.9	1.9	2.0	1.1*	0.42
Ammonia	3.5	1.8*	2.4	1.6	2.3	1.7	2.4	0.4*	2.1	3.4*	0.96
Arginine	11.7	7.8*	12.4	6.4*	12.6	5.8*	11.2	1.1*	11.8	5.3*	2.06
Aspartic (acid)	11.5	14.9*	12.0	13.1	12.2	16.0*	11.3	9.4	12.5	17.6*	2.60
Theronine	4.5	7.0*	4.3	4.2	4.2	6.7*	4.2	3.3*	4.4	5.7*	0.90
Serine	5.0	3.8*	4.8	3.6*	4.9	2.3*	5.6	2.6*	5.4	n.d.**	0.40
Glutamic (acid)	19.2	28.7*	18.8	19.2	19.3	29.9*	20.9	14.4*	20.0	17.7	6.10
Proline	4.0	1.9*	5.2	4.3	4.7	1.3*	4.8	1.8*	5.3	0.6*	1.16
Glycine	5.8	7.9*	6.0	6.4	6.6	9.0*	5.3	4.9	6.6	7.9	1.34
Alanine	3.8	4.0	4.6	4.3	3.6	3.5	3.8	3.2	3.5	5.5	2.46
Valine	4.3	4.4	4.2	4.8	4.0	4.6	4.2	5.3*	3.7	6.6*	0.80
Methionine	1.0	1.9*	0.7	0.9	0.7	2.2*	0.8	1.1*	0.7	1.5*	0.20
Isoleucine	3.4	2.0*	3.2	3.9*	3.2	2.3*	3.2	3.7	3.2	n.d.	0.70
Leucine	5.8	3.7	6.4	12.3*	6.4	4.3	6.5	6.8	6.5	12.4*	3.36
Tryosine	3.6	2.7*	3.9	3.4	3.9	2.6*	4.0	2.8*	3.9	5.1*	0.88
Phenylalanine	7.0	3.6*	5.2	5.6	5.0	4.0	5.2	3.9*	5.3	7.2*	1.38

*Significant differences at 0.05 level; values expressed as percent of protein.
**Not determined
C=Control; T=Treatment (14 days after inoculation with A. flavus)

samples either remained unchanged or increased slightly. This observation was especially evident in the samples of PI337394F, a resistant genotype, where aspartic acid increased from 12.5 to 17.6 percent. A decrease in levels of proline and serine was observed in the infested samples. However, the amino acid methionine increased. Amino acids leucine and isoleucine, and the aromatic amino acids tyrosine and phenylalanine, showed no significant changes in there levels when compared to control (Table 1). The changes in the levels of amino acids may reflect the metabolic degradation of peanut globular proteins by *A. flavus* (10). Cherry *et al.* (7) have reported a continuous change in the nature of the protein preparations from infected seeds leading to an increase in quantities of free amino acids.

The present study shows that peanut cultivars and genotypes used in this investigation had varying degrees of chemical changes when inoculated with *A. flavus*. Two genotypes, PI337409 and PI337394F, had considerable resistance to *A. flavus* invasion and lower degree of biochemical changes when inoculated with the fungus. Commercially grown cultivar Florunner also had moderate resistance to the fungus. The two resistant genotypes have potential use in breeding *A. flavus* resistant cultivars.

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