Factors Affecting **Aspergillus flavus** Lk. ex Fr. Colonization of Resistant and Susceptible Genotypes of **Arachis hypogaea** L.[1]

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ABSTRACT

Two **Arachis hypogaea** L. genotypes (P. I. 337-394F and P. I. 337409) resistant to seed colonization by aflatoxin-producing strains of **Aspergillus flavus** Lk ex. Fr., were used to study the effects of initial adjusted seed moisture, incubation and storage time, seed maturity, harvest time and seed handling on seed colonization by the fungus. Under conditions highly favorable to the growth of the fungus, seed colonization (P.I. 337394F) was greater at 20% adjusted seed moisture than at 25% seed moisture. Colonization was least at 15 and 30% adjusted moisture. **Aspergillus flavus** colonized a low percentage (17%) of the cotyledons of P.I. 337409 genotype after 48 hours of incubation, whereas, 100% of the cotyledons of P.I. 331326 (a susceptible genotype) were colonized. Colonization of P.I. 337394F seed with intact seed coats increased with each increase in storage time from 0 to 6 and 12 weeks, and for each increase in temperature from 5 to 20 and 35C. Immature and overmature seed of both resistant genotypes with intact seed coats were more susceptible to colonization than sound-mature seed. However, seed of the resistant genotypes were colonized at a low level, with no difference for seed harvested at 4 successive 2-week intervals, whereas P.I. 331326 had greater colonization for each successive harvest date. Seed coat abrasion, soaking for 5 min. in a H_2SO_4 solution, machine picking or machine-shelling increased colonization of seed over check treatments.

Additional key words: **A. Flavus** infection. Seed contamination, Toxins, Peanuts.

In two *Arachis hypogaea* L. peanut genotypes sound, mature seed with intact seed coats were reported to be resistant to seed colonization by toxin-producing strains of *Aspergillus flavus* Lk. ex Fr. (14) under laboratory procedures that were highly conducive to the growth of the fungus. Other workers have reported varietal resistance to *Aspergillus* spp. or to aflatoxin contamination by the fungus (1, 15). Optimum growth of the fungus is reported to be at 14 to 43C at 97-99 percent relative humidity (RH) (7). Invasion incidence and aflatoxin content was found to be greater in overmature than in sound-mature seed (3, 5, 6, 13). McDonald and Harkness (11) found seed from broken pods were more likely to be contaminated with *A. flavus* and aflatoxin than those from undamaged pods. Other research indicated pod damage and/or testa damage at harvest time increased the fungal contamination levels (1, 10, 11, 12). Sellschop (16) found aflatoxin contamination was concentrated in the lower grades of harvested peanuts, which contained a preponderance of immature seed.

In peanut genotypes reported to be resistant to seed invasion by *A. flavus,* La Prade and Bartz (8) suggested seed coat thickness or permeability was involved. Taber et al. (17) observed resistant genotypes had smaller hila, more compact arrangement of the palisade-like layer of the seed coat, and thicker more uniform waxy surface of the seed than susceptible genotypes. La Prade et al. (9) also reported thicker cuticular wax accumulations on seed of tolerant peanut lines.

Studies reported herein were conducted to determine the influence of several factors affecting *A. flavus* colonization of seed incubated under conditions highly conducive to development of the fungus.

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[3]**Aspergillus flavus** cultures obtained from the Northern Region Research Laboratory, ARS. USDA, Peoria, Illinois.

SEED MOISTURE EFFECT ON SEED COLONIZATION

METHODS. On August 9, green pods from P.I. 337-409 (resistant to seed penetration by **A. flavus**) plants from plots at the Wiregress Substation, Headland, Alabama, were placed in a 38C forced-draft drying oven for 5 days, then stored at 15C and 63% relative humidity (RH). On January 22, 20 g of hand-shelled seed samples with intact seed coats were placed in 250-ml breakers containing 100 ml of sterile demineralized water and 0.005% surfactant soaked for 6 minutes, drained and then soaked in water with no surfactant. Excess water was drained, and each sample was inoculated with a 1-ml suspension of **A. flavus** strain NRRL 29993 spores (ca. 4.0 x 10^6 spores/ml) from 2- to 3-weeks old Czapek's agar plates. The 20 g seed samples (8 replications) were placed in petri plates. sterile water was added in amounts to equal seed moistures (seed-weight basis) of 15, 20, 25, and 30%, and wrapped in plastic film. After 7 days of incubation at 26C in 98 ± 2% RH incubator, the percentage of seed colonized by **A. flavus** was recorded. Infection in these studies was indicated by the development of conidiospore after fungal colonization of the seed.

RESULTS. Seed colonization of P.I. 337409 with intact seed coats was greater at 20% (Fig. 1) than at 25% seed moisture (21.0 and 16.6% colonization, respectively). However, seed colonization at 25% seed moisture was greater than at 15 (8.3%) or 30% (7.9%).

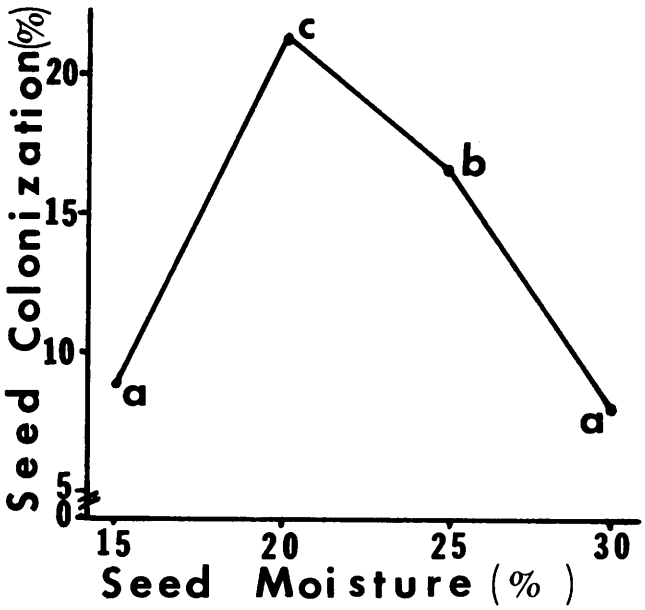

Fig. 1. Comparison of adjusted moisture content on Aspergillus flavus colonization of seed of P.I. 337409 with intact seedcoats. (Data with similar letters not significantly different, P < 0.05).

STORAGE TIME AND TEMPERATURE EFFECT ON SEED COLONIZATION

METHODS. P.I. 337394F (resistant to seed penetration by **A. flavus**) pods were harvested and dried as those in Seed Moisture Study. Seed were hand-shelled and stored at OC. To allow all treatments to be inoculated and rated at the same time, 20 g seed samples (4 replications) were removed from storage and placed in 5, 20, and 35C incubators 12, 6, and 0 weeks before laboratory evaluation. Except for adjusting seed moisture to 25%. laboratory procedures were identical to those previously described.

RESULTS. Seed colonization by *A. flavus* increased for each increase in storage temperature above 5C (Fig. 2). Average seed colonization was greater at 20 and 25C storage temperature for seed stored for 12 weeks than for 6 weks. Differences between colonization were not evident between storage times for seed held at 0 or 5C. The colonization differences resulted in temperature x time interaction. Results indicated that as storage time increased at temperatures of 20C or above, the seed coat colonization by the fungus increased.

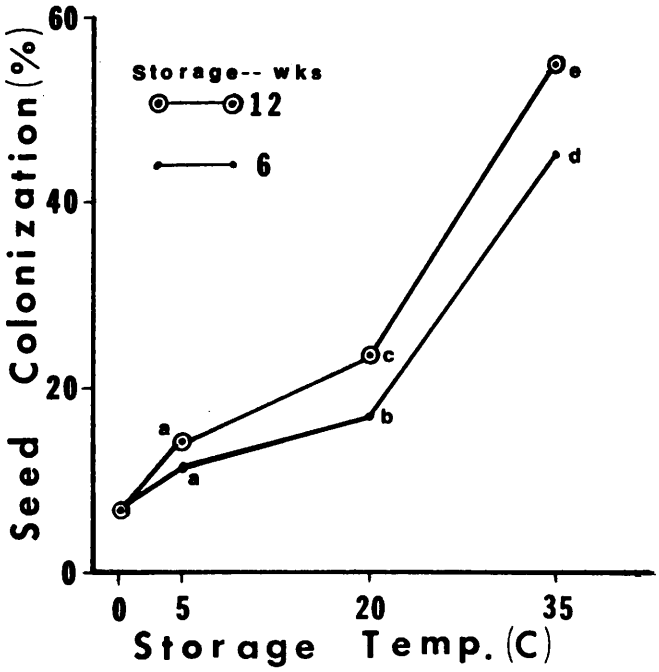

Fig. 2. Comparison of storage temperature and time on Aspergillus flavus colonization of P.I. 337394F with intact seedcoats. (Significant differences for colonization levels followed by different letters, and for average colonization of different storage times; significant interaction of temperature X time, P < 0.05).

INTACT SEED INCUBATION EFFECT ON COTYLEDON COLONIZATION

METHOD. On March 27, pods from P.I. 337409 and P.I. 331326 (the latter is susceptible to seed penetration by **A. flavus**) were handpicked at or near optimum maturity from green plants on plots in the USDA Winter Peanut Nursery in Puerto Rico$_4$. Peanut pods were dried for about one week with forced-draft ambient air or alternating forced air heated to 32C with non-forced ambient air. Pod samples were shipped immediately to Auburn, Alabama and placed in previously indicated storage on April 10. On June 11, peanut samples were hand shelled, seed adjusted to 25% moisture, and inoculated as previously described. Atfer 0, 24, 48, 72, and 96 hours of incubation, seed (8 replications of 5 seed) were removed from the plates, and testa were removed. Seed were then surface sterilizd in a 2.0% sodium hypochlorit solution for 3 minutes, rinsed in two changes of 100 ml of sterile water, drained, and transferred onto malt-salt agar (2). After incubating for 4 days at 26C and 98 ±2% RH, percentage re-colonization of the cotyledons was recorded.

RESULTS. Resistant genotype P.I. 337409 had less cotyledon colonization (Fig. 3) than the susceptible genotype after each of the four time periods tested (24, 48, 72, and 96 hours). P.I. 337409 did not differ in cotyledon colonization between the incubation times. Apparently, any penetration

of the seed coats of the resistant genotype occurred during the first 24 hurs. The maximum seed coat invasion (100%) for the susceptible genotype was reached after 48 hours of prior incubation. The Colonization differences for the two genotypes at the four incubation periods resulted in a genotype x incubation time interaction.

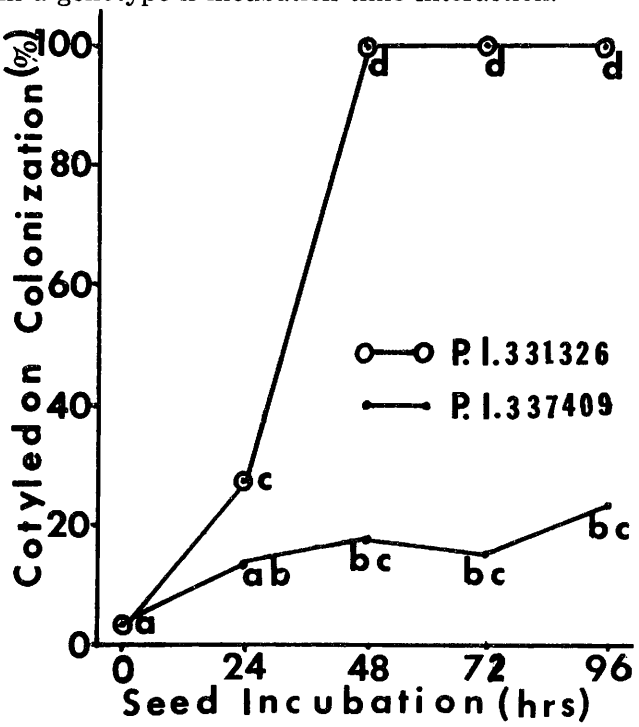

Fig. 3. Comparison of incubation time of seed with intact seedcoats on the invasion of cotyledons by Aspergillus flavus. (Significant differences for colonization levels followed by different letters, and for average colonization of different genotypes, significant interaction of genotypes X incubation time, $P < 0.05$).

SEED MATURITY EFFECT ON SEED COLONIZATION

METHODS. P.I. 337394F and P.I. 337409 pods were harvested, dried, and stored as in Incubation Time Study. On May 12, seed were hand-shelled and visually separated into immature, mature, and over-mature classes. These seed (six 20-g replications) were evaluated as in Storage Time Study.

RESULTS. Immature and over-mature seed with intact seed coats of P.I. 337394F and P.I. 337409 were more susceptible to colonization than mature seed (Fig. 4). Average colonization of P.I. 337409 seed for the 3 seed classes was less than that for P.I. 337394F. There was no difference in colonization of the immature seed of the two genotypes, but seed colonization was greater on P.I. 337394F mature and over-mature seed than for the same classes of P.I. 337409 seed. This was indicated by a genotype x maturity class interaction. Obviously, the mature seed in this study were less susceptible to seed colonization by the fungus than were immature or over-mature seed.

HARVEST DATE EFFECT ON SEED COLONIZATION

METHODS. Green pods of P.I. 337394F, P.I. 337409, and P.I. 331326 were hand-picked from plots at Headland, Alabama on 4 harvest dates at 2-week intervals beginning July 26, dried and stored as in the Seed Moisture Study. On September 22, seed were hand-shelled

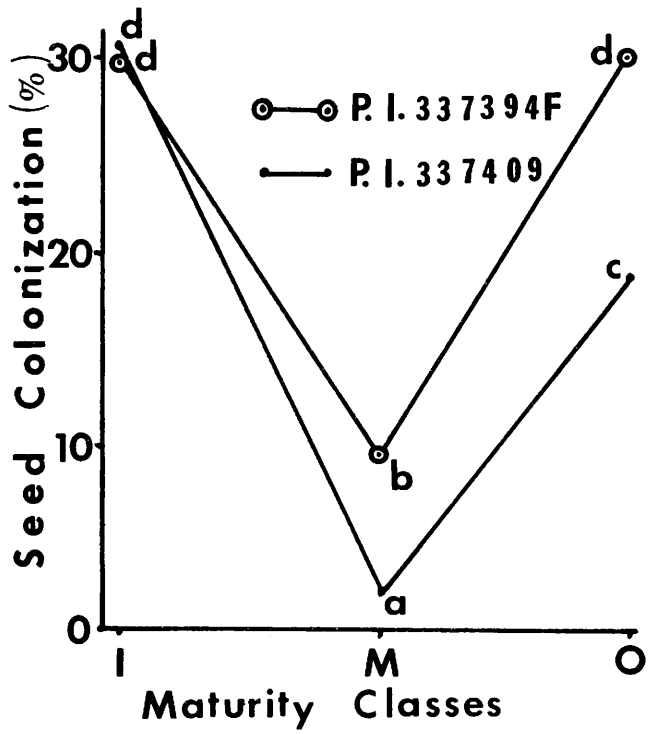

Fig. 4. Comparison of Aspergillus flavus colonization of seed of tolerant genotypes with intact seedcoats separated into maturity classes (I = immature, M = mature, O = immature; signfficant differences for colonization levels followed by different letters, and for average colonization of different genotypes; significant interaction of genotype X maturity classes, $P < 0.05$).

and evaluated by laboratory procedures described for Seed Maturity Study.

RESULTS. Sound and mature seed of the 2 resistant genotypes (P.I. 337394F and P.I. 337409) were colonized at a low level with no difference in colonization between the two genotypes or between seed harvested at 4 different dates (Table 1). Seed colonization on the susceptible genotype (P.I. 331326) increased with time as harvest was delayed (16.4, 61.0, 85.0%), with average coloniza-

Table 1. Effect of harvest dates on Aspergillus flavus colonization of tolerant and susceptible peanut seed after laboratory incubation.

	Seed colonization		
Harvest	Tolerant genotypes		Susceptible genotypes
dates	P.I. 337409	P.I. 337394F	P.I. 331326
	%	%	%
7-26	1.6a	0.0a	6.4a
8-9	0.8a	0.0a	16.4b
8-23	1.6a	2.4a	61.0c
9-6	2.4a	6.8a	85.0d
Average	1.6A	2.3A	42.2B

Numbers followed by the same letter are not significantly different ($P < 0.05$).

tion greater than that for either P.I. 337394F or P.I. 337409. There was a genotype x harvest time interaction. The resistant genotypes resisted laboratory colonization by *A. flavus* throughout the harvest periods, whereas susceptible genotype did not.

POD AND SEED HANDLING EFFECTS ON SEED COLONIZATION

METHODS. Two separate experiments were conducted. In Experiment 1. on August 16, plants of P.I. 337394F. P.I. 337409, Starr (**A. flavus**-susceptible), and P.I. 331326 were dug at or near optimum maturity from field plots at Headland, Alabama. Plants were dug and inverted with a mechanical peanut digger-shaker and ambient air-dried in the row. On August 22, part of the dry pods were hand-picked (Lot A), part were machine-pcked (Lot B), and both were stored at room temperature. On January 9, part of each lot was hand-shelled (Lots A-1 and B-1), and another part was machine-shelled (Lots A-2 and B-2) on a peanut sheller used by Federal-State grading station. Six 20-g samples of sound seed from each of the 4 lots were evaluated in the laboratory as above.

In Experiment 2. P.I. 337394F peanuts were harvested, dried, and stored as in Seed Incubation Study. On June 21, pods were hand-shelled and, 20-g samples (6 replications) were subjected to six laboratory treatments. Treatments were: (1) seed gently rolled for 30 seconds in medium coarse flint abrasive paper (9x11 inches) which was rolled lengthwise and capped on each end with 250 ml beakers, (2) same as first treatment, but rolled for 1 minute, (3) seed soaked for 1 minute in 100 ml of 3.6N H_2SO_4, (4) same as third treatment, but continued for 5 minutes, (5) inoculated and (6) uninoculated checks. These conditioned seed were washed for 1 minute in running tap water, drained, inoculated (except uninoculated checks), adjusted to 25% moisture, incubated, and rated as above.

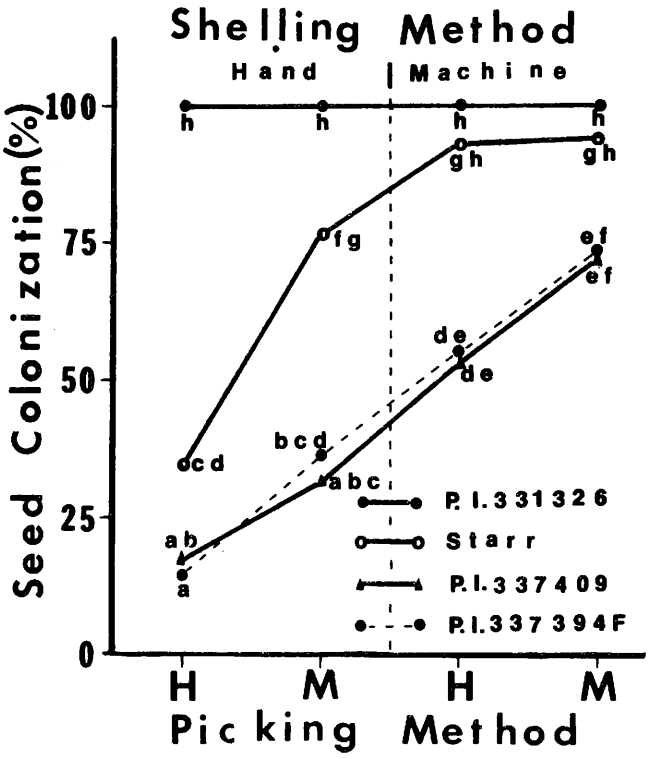

Fig. 5. Comparison of pod and seed handling procedures on Aspergillus flavus colonization of seed from tolerant and susceptible peanut gnetotypes with intact seed-coats (Picking method (Pods). H = hand, M = machine; significant differences for colonization of tolerant vs susceptible genotypes, hand shelling vs machine shelling, hand picking vs machine picking; significant interaction of genotypes X shelling method, P < 0.05).

RESULTS. In Experiment 1, machine harvest contributed more to fungal colonization than hand harvest (Figure 5). However, of the resistant genotypes hand-picked, hand-shelled P.I. 337394F peanut had significantly less colonization than the machine-picked, hand-shelled peanuts. Machine-shelled P.I. 337394F peanuts did not differ in fungal colonization between hand-picked and machine-picked samples.

Average colonization was greater for seed of the two resistant genotypes and the Starr variety when machine-shelled than when hand-shelled. However, almost total seed colonization of the susceptible genotype occurred, regardless of the shelling or harvesting method. Also, the seed colonization of Starr averaged less than that of P.I. 331326 (susceptible). Interactions for genotype x shelling method occurred.

In Experiment 2, P.I. 337394F seed with intact seed coats that were scarified with abrasive paper for 30 seconds or for 1 min. were colonized equally (Table 2) by *A. flavus* (98.3 and 97.0%, respectively). More colonization was evident on seed from this treatment than on those soaked for 5 or 1 minute in 3.6N H_2SO_4 (19% and 10% colonization, respectively), or the inoculated check (11.0%). Colonization of uninoculated seed was less (1.7%) than that of all other treatments. This small amount of *A. flavus* contamination in the uninoculated check was apparently from natural field infection. It is thought that the abrasive and H_2SO_4 treatments damaged the seed coats of the resistant genotype or made it more permeable to infection by the fungus. Other workers (1, 10, 11, 12) have reported that seed coat damage increased *A. flavus* contamination of peanuts.

Table 2. Comparison of seed treatments on Aspergillus flavus colonization of P.I. 337394F seed.

Treatment	Seed colonization
	%
Abrasive paper (1 min)	98.3d
Abrasive paper (30 sec)	97.0d
3.6N H_2SO_4 (5 min)	19.0c
Inoculated (CK)	11.0b
3.6N H_2SO_4 (1 min)	10.2b
Uninoculated (CK)	1.7a

Numbers followed by the same letter not significantly different (P < 0.05).

Discussion

Laboratory evaluation of P.I. 337409 seed with intact seed coats revealed an increase in colonization by *A. flavus* at 20% adjusted seed moisture

in comparison with limited colonization at 15 or 30%% adjusted seed moisture (Fig. 1). The 20% seed moisture was more suitable for the growth of the fungus and colonization of the resistant genotype.

Storage temperature of 20 and 35C for 6 or 12 weeks greatly reduced the *A. flavus* resistance of P.I. 337394F (Fig. 2). At these temperatures the longer storage time increased the susceptibility of the genotype than at 0 or 5C.

The resistance to invasion of cotyledons of P.I. 337409 obtained from seed with intact seed coats inoculated and incubated in the laboratory was very pronounced in comparison to the susceptible genotype (P.I. 331326) (Fig. 3). *Aspergillus flavus* colonized 100% of the cotyledons of the susceptible genotype incubated 48, 72, or 96 hours. Only 17.5, 15.0, and 22.5% of the cotyledons of P.I. 337409 were colonized for these respective incubation times. Preliminary studies had previously revealed that removal or damaging seed coats of resistant genotypes made them highly susceptible to *A. flavus* colonization.

Immature and over-mature seed of the two resistant genotypes were more susceptible to colonization by *A. flavus* than mature seed (Fig. 4). It is known that over-mature seed usually has a greater incidence of field contamination by *A. flavus* and greater aflatoxin contamination (6, 12). P.I. 337394F had somewhat more seed colonization of the mature and over-mature seed classes than P.I. 337409. Even though selected over-mature seed classes had greater seed colonization, another experiment revealed that delayed harvest of both P.I. 337394F and P.I. 337409 (Table 1) produced no increase in *A. flavus* colonization of seed. However, delayed harvest of the susceptible genotype greatly increased *A. flavus* colonization.

Peanuts with resistance to *A. flavus* colonization may have this resistance impaired by early or late maturity, improper harvesting and handling, and improper storage conditions.

Literature Cited

1. Carter, J. B. H. 1973. The influence of the testa, damage and seed dressing on the emergence of groundnut (**Arachis hypogaea**). Ann. Appl. Biol. 74: 315-323.
2. Christensen, C. M. 1946. The quantitative determination of molds in flour. Cereal Chem. 23: 322-329.
3. Dickens, J. W., and H. E. Pattee. 1966. The effects of time, temperature, and moisture on aflatoxin production in peanuts nioculated with toxic strains of **Aspergillus flavus.** Trop Sci. 8: 11-12.
4. Diener, U. L., and N. D. Davis. 1965. Effect of aflatoxin production by **Aspergillus flavus** in sterile peanuts. Proc. of 4th Nat. Peanut Conf. (Tifton, Ga.) p. 94-95.
5. Diener, U. L. 1965. Relation of **Aspergillus flavus** invasion to maturity of peanuts at harvest. J. Alabama Acad. Sci. 36: 21.
6. Diener, U. L., C. R. Jackson, W. E. Cooper, R. J. Stipes, and N. D. Davis. 1965. Invasion of peanut pods in the soil by **Aspergillus flavus.** Plant Disease Reptr. 49: 931-935.
7. Diener, U. L., and N. D. Davis. 1969. Aflatoxin formation by **Aspergillus flavus.** Pp. 13-54, **In** Leo A. Goldblatt (ed.) Aflatoxin scienitfic background, control and implications. Academic Press, New York.
8. La Prade, J. C., and J. A. Bartz. 1972. Mechanical resistance of selected genotypes of dried peanuts to colonization by strains of aflatoxin producing **Aspergillus** spp. Phytopathology 62: 771 (Abstr).
9. La Prade, J. C., J. A. Bartz, A. J. Norden, and T. J. Demuynk. 1973. Correlation of peanut seed coat surface wax accumulations with tolerance to colonization by **Aspergillus flavus.** J. Amer. Peanut Res. and Educ. Ass. 5: 89-94.
10. McDonald, D., and J. A'Brook. 1963. Growth of **Aspergillus flavus** and production of aflatoxin in groundnuts. (Part III). Trop. Sci. 5: 208-214.
11. McDonald, D., and C. Harkness. 1964. Growth of **Aspergillus flavus** and production of aflatoxin in groundnuts. (Part II). Trop Sci. 5: 143-154.
12. McDonald, D., and C. Harkness. 1964. Growth of **Aspergillus flavus** and production of aflatoxin in groundnuts. (Part IV). Trop. Sci. 6: 12-27.
13. McDonald, D., and C. Harkness. 1968. Aflatoxin in the groundnut crop at harvest in northern Nigeria. Trop. Sci. 9: 148-161.
14. Mixon, A. C., and K. M. Rogers. 1973. Peanut accessions resistant to seed infection by **Aspergillus flavus.** Agron. J. 65: 560-562.
15. Nagarajan, V., and R. V. Bhat. 1973. Aflatoxin production in peanut varieties by **Aspergillus parasiticus** Speare. Appl. Biol. 25: 319-321.
16. Sellschop, J. P. F. 1965. Field observations on conditions conducive to the contamination of groundnuts with the mold **Aspergillus flavus** Link ex Fr. Symp. Mycotoxins Foodstuffs, Agri. Aspects, Pretoria, South Africa pp. 47-52.
17. Taber, R. A., R. E. Pettit, C. R. Benedict, J. W. Deickert. and D. L. Ketring. 1973. Comparison of **Aspergillus flavus** tolerant and susceptible peanut lines. J. Amer. Peanut Res. and Educ. Ass. 5: 206 (Abstr.).