Proliferation of Aspergillus flavus in Artificially Infested Windrow-Dried Peanut Fruit in Virginia

D. Morris Porter and F. Scott Wright

Plant Pathologist, Plant Science Research Division, and Agricultural Engineer, Agricultural Engineering Research Division, ARS, USDA, Holland, Virginia 23391; and Adjunct Professor, Department of Plant Pathology and Plant Physiology and Department of Agricultural Engineering, respectively, Virginia Polytechnic Institute and State University.

Cooperative investigations of Plant Science Research Division and Agricultural Engineering Research Division, ARS, USDA, and Virginia Polytechnic Institute and State University. Contribution No. 208, Department of Plant Pathology and Plant Physiology, Virginia Polytechnic Institute and State University, Blacksburg.

Accepted for publication 7 May 1971.

ABSTRACT

Artificially infested fruit, field-dried in random windrows (fruit covered with foliage and not exposed to sunlight), yielded more isolates of *Aspergillus flavus* than fruit dried in inverted windrows (fruit resting on top of the foliage and exposed to direct sunlight). Windrow-dried, small (ca. 15 mm long), whole immature peanut fruits were invaded more often by *A. flavus* after artificial infestation than were pieces of shell and seed of larger (ca. 30 mm long) immature and mature fruit. The isolation frequency of *A. flavus* from artificially infested mature fruit was about that of untreated mature fruit. Isolates of *A. flavus* were obtained more readily from shell pieces and seed from infested fruit after a 4-day exposure period in either windrow than after a 12-day exposure period. Periods of adverse drying conditions enhanced aflatoxin production in seed of mature windrow-dried fruit. Phytopathology 61:1194-1197.

Additional key words: soil microflora, aflatoxins, fungi.

Aspergillus flavus Lk. ex Fr., a common companion of the peanut fruit (*Arachis hypogaea* L.), has received much attention since the discovery in 1961 that this fungus can produce aflatoxins which are highly toxic to certain species of warm-blooded animals (18). Seed from unblemished peanut fruit are often infested with *A. flavus* when removed from the soil (4, 5, 10, 11, 16). The behavior of *A. flavus* after fruit are removed from the soil and placed in windrows to partially air-dry is uncertain. Isolates of *A. flavus* were obtained at low frequencies from windrowed fruit in Georgia (9) and in Virginia (17). *Aspergillus flavus* was isolated frequently but never abundantly from windrowed peanut fruit in Nigeria (12). *Aspergillus flavus* was isolated more frequently from fruit dried in random windrows than from fruit dried in inverted windrows (9, 12).

Peanut fruit are generally free of aflatoxin contamination, provided fruit are subjected to prompt and steady drying immediately after removal from the soil (2, 9). In Texas, aflatoxin content was greatest in windrowed samples subjected to high humidities and high temperatures (14). Under favorable drying conditions, aflatoxin production was greater in fruit dried in random windrows than in inverted windrows (2).

In Virginia and North Carolina, peanut plants are removed from the soil with fruit intact and placed in either random or inverted windrows in the field to partially air-dry. In the random windrow, much of the fruit is shaded by the foliage and in contact with, or in close proximity, to the soil. In the inverted windrow, most of the fruit rest on top of the foliage well above the soil surface, and are exposed to direct sunlight. The drying rate of fruit is thus accelerated in the inverted windrow (2, 9, 17). Fruit remain in the windrows until the moisture content is reduced to 25 to 35%, at which time fruit are picked and placed in forced air dryers to complete the drying process.

We attempted in this study to determine the relationship of windrow type and length of field exposure on the proliferation of *A. flavus* and the production of aflatoxins in immature and mature peanut fruit during the field-drying process.

MATERIALS AND METHODS.—Virginia-type peanuts (cultivar Virginia 61-R) planted between 10 and 16 May were grown near Holland, Va., during 1967, 1968, and 1969, according to approved agronomic practices. Plots were two rows wide and 18 m long, with two guard rows between plots arranged in a randomized block design with four replications.

During September and October of each year, plants were lifted from the soil and windrowed in one operation with a tractor-mounted digger-inverter. Plants dug in this manner are oriented with fruit exposed and on top of the foliage. Immediately after windrowing, a spore suspension of a strain of *A. flavus* known to produce aflatoxins was sprayed on the fruits. This suspension was prepared from the mycelium and spores of 7-day-old cultures of *A. flavus* grown on potato-dextrose agar at 25 C. The mycelium and spores scraped from the agar surface with glass slides were macerated for 1 min in a blender containing distilled water, then filtered through four layers of cheesecloth. The final spore concentration contained ca. 50,000 spores/ml. Each plot was sprayed with a hand-operated stainless steel sprayer containing 4 liters of the spore suspension.

After spraying, plants in one-half the plots were positioned by hand to simulate a random windrow. The other half of the sprayed plants were not disturbed, and remained in the inverted position to simulate an inverted windrow. Fruit from simulated random and inverted windrows which were not sprayed served as checks. After a 4-day and 12-day exposure period in

each windrow type, fruit from individual plots were harvested with a conventional peanut combine.

The proliferation of *A. flavus* in windrow-dried, artificially infested fruit was determined after havesting by plating on Rose Bengal-streptomycin agar 3,520 whole (ca. 15 mm long) immature fruit, 2,944 seed, and 3,036 pieces of shell (1 cm^2) from large (ca. 30 mm long) immature fruit and 6,400 seed and 6,400 pieces of shell (1 cm^2) from mature fruit after surface-disinfestation in 0.5% NaOCl for 3 min. Immature whole fruit and pieces of shell and seed from both immature and mature fruits from noninfested (check) windrows were plated also. After incubation for 10 days at 25 C, most of the thalli which grew on to the medium from the fruit parts could be identified. At each reading, the percentage of shell pieces and seed from which at least one thallus of *A. flavus* grew was determined. This percentage was recorded as the isolation frequency of *A. flavus*.

The aflatoxin content was determined from samples of hand-shelled, mature fruit dried for 3 days at 72 C by J. W. Dickens (USDA, ARS, MQRD, Raleigh, N.C.) who used the method described by Pons & Goldblatt (15).

The moisture content (wet weight basis) of mature fruit was determined by drying the fruit at 84 C for 60 to 70 hr in a forced-air oven.

Data in per cent were subjected to analysis of variance by using the arc sine transformation, and comparisons were made at the 5% level of significance.

RESULTS.—*Effects of windrow type; mature fruit.*—Isolation frequency of *A. flavus* from seed of artificially infested fruit, dried in random windrows was significantly greater than that of inverted windrows (fruit exposed above foliage after harvest) at all harvest dates. Means for the 4-year period were 8.2 and 3.4%, respectively. Moreover, the fungus was also usually obtained more frequently from shell pieces of mature fruit dried in random windrows, but means were significantly greater only for the September 1967 harvest (Table 1).

TABLE 1. Effect of windrow type on the isolation frequency of *Aspergillus flavus* associated with shell pieces and seed of mature peanut fruit artificially infested with this fungus

	Isolation frequency of *Aspergillus flavus*			
	Shell pieces		Seed	
Harvest dates	Inverted windrow	Random windrow	Inverted windrow	Random windrow
Sept. 67	0.4 a[a]	1.7 b	3.4 a	7.8 b
Oct. 67	0.6 a	1.6 a	3.9 a	5.2 b
Sept. 68	1.0 a	1.9 a	2.2 a	7.7 b
Oct. 68	2.7 a	2.6 a	4.4 a	12.1 b
Avg, infested	1.2	1.9	3.4	8.2
Avg, not infested	0.7	1.2	3.1	4.8

[a] Treatments having the same letters are not statistically different at the 5% level of significance. Data were analyzed between windrow types (horizontally) for each harvest date.

The isolation frequency of *A. flavus* from artificially infested fruit was relatively low when combined data for the 4-year period (shell pieces, 1.5%; seed, 5.8%) were considered. Damage to mature fruit was similar, regardless of whether they were artificially infested at harvest or not, but recovery of the fungus from those artificially infested was consistently greater (Table 1).

Effects of windrow type; immature fruit.—Colonization of immature fruit by *A. flavus* was also reduced by the inverted windrow technique. The mean percentage isolation frequency for the three harvest dates was: random windrow, 29.6%; inverted windrow, 12.7%. Frequency of isolation was always lower, but significantly so only on two of the three harvest dates tested. Similarly, isolation frequencies from shell pieces and seed were less in samples from inverted windrows; however, differences were significant at only one harvest date (October 1969) for shell pieces, and were not significant at any harvest date for seed.

The ratio of recovery of *A. flavus* from whole, immature, artificially infested fruit at harvest compared to those not infested was 29.6% to 16.1% for samples from random windrows, and 12.7% to 6.3% for those from inverted windrows.

Effects of field exposure; mature fruit.—Prolonged drying of artificially infested fruit under field conditions reduced the amount of colonization by *A. flavus* regardless of the windrow type used. Isolation frequencies after 4 days were significantly greater than after 12 days for three of four harvest dates for shell pieces and for two harvest dates of seed (Table 2).

A similar trend was noted in isolations from shell pieces which were not artificially infested at harvest; but longer field exposure had no detectable effect on the recovery of the fungus from seed in the noninfested plots (Table 2).

Effect of field exposure; immature fruits.—The effect of prolonged drying in the windrow was even more pronounced when isolations were made from immature fruits. Isolation frequencies of *A. flavus* from whole

TABLE 2. Effect of field exposure on the isolation frequency of *Aspergillus flavus* associated with shell pieces and seed of mature peanut fruit artificially infested with this fungus

	Isolation frequency of *Aspergillus flavus*			
	Shell pieces		Seed	
Harvest dates	4-Day exposure	12-Day exposure	4-Day exposure	12-Day exposure
Sept. 67	1.5 a[a]	0.6 a	5.9 a	5.3 a
Oct. 67	1.9 b	0.4 a	7.9 b	1.1 a
Sept. 68	2.9 b	0.0 a	6.0 b	3.8 a
Oct. 68	4.4 b	0.9 a	9.1 a	7.4 a
Avg, infested	2.7	0.5	7.2	4.4
Avg, not infested	2.3	1.2	3.2	3.1

[a] Each value is the mean isolation frequency from both random and inverted windrows. Treatments having the same letters are not statistically different at the 5% level of significance. Data were analyzed between exposure periods (horizontally) for each harvest date.

fruits after 4 days in the windrow were significantly greater than after 12 days on all harvest dates. Isolation means from whole fruits after 4-day and 12-day field exposure periods were 30% and 12%, respectively. Similarly, on all harvest dates the isolation frequencies of *A. flavus* from seed of immature fruits after 4 days (11.3%) in the windrow were significantly greater than after 12 days (3.5%). *Aspergillus flavus* was also isolated more consistently from shell pieces following a 4-day exposure than after a 12-day exposure period; however, differences were significant at only one harvest date (October 1969) (Table 3).

Aspergillus flavus was isolated less frequently from whole fruits and shell pieces and seed from immature fruits not artificially infested than from fruits artificially infested at harvest. However, isolation frequencies were similar in that isolates were obtained more readily from fruits exposed for 4 days than fruits exposed for 12 days (Table 3).

Effects of windrow type and exposure period on aflatoxin production.—Aflatoxin was detected in samples of seed from artificially infested fruit only in 1968 (Table 4). Fruit drying in inverted windrows contained less aflatoxin after a 12-day windrow exposure than after a 4-day exposure. These differences were not apparent in fruits drying in random windrows. Aflatoxin was not detected in fruit not artificially infested with *A. flavus*.

Effects of windrow type and field exposure on moisture content of peanut fruit.—The average moisture content of fruit was influenced by windrow type and exposure period. The average moisture content of fruit dried in inverted and random windrows was 25.8% and 32.6%, respectively. Fruit moisture content was considerably less after a 12-day windrow exposure (23.9%) than after a 4-day exposure period (34.5%).

Discussion.—The windrow types utilized represented two extremes in field-drying. The nature of the many environmental factors prevailing in each windrow type was complex, with some factors favoring fungal growth while others were deleterious. Isolates of *A. flavus* were obtained more readily from shell pieces and seed of mature and larger immature fruit, and from smaller whole immature fruit dried in random windrows, than from similar material taken from fruit dried in inverted windrows. Others (9, 17) have reported similar results. Perhaps this increased isolation frequency of *A. flavus* in random windrowed fruit (Table 1) was partly due to the higher moisture content of the fruit. Ashworth et al. (1) reported that *A. flavus* grew best at a fruit moisture content of 23 to 34%. In Nigeria (13), *A. flavus* was isolated more frequently from slowly dried fruit than from fruit that were dried rapidly. In this study, the average moisture content of fruit dried in random windrows was more than 30% greater than the moisture content of fruit dried in inverted windrows.

Shell pieces and seed of mature and immature fruit and whole immature fruit exposed for 4 days in either windrow type yielded a greater number of isolates of *A. flavus* than did similar material from plants windrowed for 12 days (Tables 2, 3). The size of shell pieces used was small (1 cm^2); and this may possibly account for the greater isolation frequency of *A. flavus* from seed than from shell pieces (Tables 1, 2, 3). However, lack of nutrients in mature shells may reduce the number of colonizations and thus account for greater isolation frequencies from seed than shells. Garren & Porter (7) theorized that competition with the quiescent fungi already established within the peanut fruit limited the proliferation of *A. flavus*. It is noteworthy that the isolation frequency of some other known toxin producers (e.g., *Fusarium* and *Alternaria*) increased manyfold during longer windrow exposure periods (6).

Griffin (8) recently showed that spores of *A. flavus* germinate readily when placed in the immediate vicinity of superficial wounds on the surface of developing peanut fruit. Spore germination was sparse in areas not adjacent to wounded tissue. Secretions emanating from the wounded areas apparently contain substances that stimulate germination. Due to the higher moisture content and the texture of the shell, immature fruit are more prone to mechanical damage that occurs at digging than are mature fruit which contain less water and are covered by a fairly hard shell. This wounded tissue, coupled with the availability of moisture from within the immature fruit, could serve as a reservoir of extensive fungal activity, particularly *A. flavus*. This may explain why we found that shell and seed of mature,

Table 3. Effect of field exposure on the isolation frequency of *Aspergillus flavus* associated with small, whole immature fruit and shell pieces and seed of larger immature peanut fruit artificially infested with this fungus

	Isolation frequency of *Aspergillus flavus*					
	Whole fruit		Shell pieces		Seed	
Harvest dates	4 Day exposure	12 Day exposure	4 Day exposure	12 Day exposure	4 Day exposure	12 Day exposure
Oct. 68	24.4 b[a]	15.3 a	3.6 b	1.9 a	11.6 b	4.5 a
Sept. 69	30.3 b	4.4 a	2.2 a	2.2 a	11.9 b	2.5 a
Oct. 69	36.3 b	16.3 a	7.2 a	2.8 a	10.3 b	3.5 a
Avg, infested	30.0	12.0	4.3	2.3	11 3	3.5
Avg, not infested	14.3	7.2	2.2	0.2	4.4	3.1

[a] Each value is the mean isolation frequency from both random and inverted windrows. Treatments having the same letters are not statistically different at the 5% level of significance. Data were analyzed between exposure periods (horizontally) for each harvest date.

TABLE 4. The effect of windrow type and field exposure on aflatoxin production in mature peanut fruit after artificial infestation with *Aspergillus flavus*

Digging dates	Exposure periods	Inches of rainfall	Days of rainfall	Aflatoxin (ppb) Windrow types	
				Inverted	Random
Sept. 67	4 days	0	0	0[a]	0
	12 days	0.2	2	0	0
Oct. 67	4 days	0	0	0	0
	12 days	0.5	1	0	0
Sept. 68	4 days	1.0	1	7.5	6.0
	12 days	1.0	4	1.5	7.5
Oct. 68	4 days	0.5	3	16.0	15.7
	12 days	1.1	7	11.5	17.0

[a] Expressed as an average of four replications.

larger fruit were invaded less frequently by *A. flavus* than were whole immature fruit.

Production of aflatoxin in windrow-dried peanut fruit seems to be dependent upon the existing environmental conditions (Table 4). Shower activity was negligible during periods of windrow-drying in 1967, and aflatoxin was not present in any of the samples analyzed. In 1968, showers occurred on 3 days of the 4-day windrow-exposure period, and on 7 days of the 12-day windrow exposure period during the last digging of 1968. Although present in relatively small amounts, aflatoxin was detected in fruit dried under these conditions. Similar results were reported previously (17). Pettit & Tabor (14) also noted an increase in aflatoxin content whenever fruit, drying in the windrow, was subjected to periods of high humidity. Diener & Davis (3) noted that the optimum relative humidity requirements for development and accumulation of aflatoxin on peanut fruit ranged between 89 and 95%. Aflatoxin was not detected in windrow-dried peanut fruit in Georgia (9) during favorable drying conditions. Thus, it appears that the production of aflatoxin in windrowed fruits is minimized under favorable field-drying conditions.

LITERATURE CITED

1. ASHWORTH, L. J., JR., H. W. SCHROEDER, & B. C. LANGLEY. 1965. Aflatoxins: environmental factors governing occurrence in Spanish peanuts. Science 148:1228-1229.
2. DICKENS, J. W., & H. E. PATTEE. 1966. The effects of time, temperature and moisture on aflatoxin production in peanuts inoculated with a toxic strain of Aspergillus flavus. Trop. Sci. 8:11-22.
3. DIENER, U. L., & N. D. DAVIS. 1967. Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by Aspergillus flavus in sterile peanuts. J. Amer. Oil Chem. Soc. 44:259-263.
4. DIENER, U. L., C. R. JACKSON, W. E. COOPER, R. J. STIPES, & N. D. DAVIS. 1965. Invasion of peanut pods in the soil by Aspergillus flavus. Plant Dis. Reptr. 49:931-935.
5. GARREN, K. H. 1966. Peanut (groundnut) microfloras and pathogenesis in peanut pod rot. Phytopathol. Z. 55:359-367.
6. GARREN, K. H., C. M. CHRISTENSEN, & D. M. PORTER. 1969. The mycotoxin potential of peanuts (groundnuts), the U.S.A. viewpoint. J. Stored Prod. Res. 5:265-274.
7. GARREN, K. H., & D. M. PORTER. 1970. Quiescent endocarpic floral communities in cured mature peanuts from Virginia and Puerto Rico. Phytopathology 60:1635-1638.
8. GRIFFIN, G. J. 1970. Conidial germination and population of Aspergillus flavus in the geocarposphere of peanut. Phytopathology 60:1293 (Abstr.).
9. JACKSON, C. R. 1965. Growth of Aspergillus flavus and other fungi in windrowed peanuts in Georgia. Trop. Sci. 7:27-34.
10. JACKSON, C. R. 1965. Peanut pod mycoflora and kernel infection. Plant Soil 23:203-212.
11. MCDONALD, D. 1970. Fungal infection of groundnut fruit before harvest. Brit. Mycol. Soc. Trans. 54: 453-460.
12. MCDONALD, D. 1970. Fungal infection of groundnut fruit after maturity and during drying. Brit. Mycol. Soc. Trans. 54:461-472.
13. MCDONALD, D., & C. HARKNESS. 1965. Growth of Aspergillus flavus and production of aflatoxin in groundnuts. Trop. Sci. 6:122-137.
14. PETTIT, R. E., & RUTH A. TABOR. 1968. Factors influencing aflatoxin accumulation in peanut kernels and the associated mycoflora. Appl. Microbiol. 16: 1230-1234.
15. PONS, W. A., JR., & L. A. GOLDBLATT. 1965. The determination of aflatoxins in cottonseed products. J. Amer. Oil Chem. Soc. 42:471-475.
16. PORTER, D. M., & K. H. GARREN. 1968. An analysis of the endogeocarpic microflora of peanuts in Virginia. Trop. Sci. 10:100-106.
17. PORTER, D. M., & K. H. GARREN. 1970. Endocarpic microorganisms of two types of windrow-dried peanut fruit (Arachis hypogaea L.). Appl. Microbiol. 20:133-138.
18. SARGEANT, K., A. SHERIDAN, J. O'KELLY, & R. B. A. CARNAGHAN. 1961. Toxicity associated with certain samples of groundnuts. Nature 192:1096-1097.