Occurrence of Aspergillus flavus in Pistachio Nuts Prior to Harvest

S. V. Thomson and M. C. Mehdy

Assistant Professor and Laboratory Assistant, respectively, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Accepted for publication 27 February 1978.

ABSTRACT

THOMSON, S. V., and M. C. MEHDY. 1978. Occurrence of Aspergillus flavus in pistachio nuts prior to harvest. Phytopathology 68:1112-1114.

Pistachio nuts were sampled periodically from California orchards prior to harvest to determine when *Aspergillus flavus* infected the developing nuts and if aflatoxins were present. *Aspergillus flavus* was not isolated from nuts in July and from only 0.3% of the nuts in August. However, 11.8% of the nuts were infected at harvest time (15 September). The increased infection was correlated with the exposure of the nutmeat as the endocarp (shell) and often the mesocarp (hull) were split during maturation. At harvest, 28% of the insect-damaged nuts were infected with *A. flavus* compared to only 6% of the nondamaged nuts. When tested for synthesis of aflatoxin, 70% of the *A. flavus* isolates produced aflatoxin B_1. However, aflatoxins were not detected in 29 samples of pistachio nuts.

Species of the *Aspergillus flavus* group produce secondary metabolites called aflatoxins that can cause hepatomas in laboratory animals (3, 11). Aflatoxins occur in several nut crops including almonds (8), peanuts (6), pecans (7), and pistachios (5, 9, 10). Pistachios are a relatively new crop in California and have been extensively planted during the last 15 yr. With greater annual production of nuts, there is a need to evaluate the aflatoxin potential prior to harvest. In Iran, aflatoxins have been detected in pistachio nuts sampled from trees prior to harvest indicating that aflatoxin production in pistachios may not result solely from improper shipping and storage (5, 10). A recent study in California showed that aflatoxins and another mycotoxin, sterigmatocystin, were produced in artifically inoculated pistachio nuts while on the tree (9). Although this earlier study indicated that environmental conditions in California permit growth of the fungus and subsequent elaboration of aflatoxin in pistachio nuts, the methods of inoculation did not simulate natural infection and the level of inoculum used was very high.

Our objectives over the past 3 yr, 1974-1976, were to determine: (i) the natural occurrence of the *Aspergillus flavus* group of fungi in California-grown pistachio nuts, (ii) whether isolates of *A. flavus* from pistachio nuts produce aflatoxin, and (iii) through limited analyses, if aflatoxin commonly is present in nuts prior to harvest.

MATERIALS AND METHODS

Pistachio nuts were obtained from four different orchards in the vicinity of Wasco, California, and assessed for the presence of *A. flavus* and aflatoxin. The samples weighed 2-3 kg and the nuts were randomly collected from about 2.5-hectare blocks. In 1975 and 1976, the maturing nuts were sampled periodically from late July until harvest in mid-September. The samples were collected in paper bags (polyethylene bags in early 1975), transported to Berkeley, and refrigerated at 7 C upon arrival. Approximately 4-8 hr elapsed between collection and refrigeration.

Isolation of the fungus.—In 1976, nuts were processed within 2-3 days of collection. The hull and shell were removed and the seeds were split between the cotyledons. The two pieces were surface sterilized in 0.5% sodium hypochlorite for 1 min. Then the halves were drained briefly and plated split side down. The medium was a Botran-amended medium prepared as described (2) except that streptomycin was added at 50 mg/liter. Generally, 100 nuts were plated from each orchard sample with the maturity and condition of each nut noted. The plates were incubated for 10 days in the dark at 30 C and then inspected for *A. flavus*.

The 1974-1975 isolations were made as above except that surface sterilization ranged from 15 sec to 2 min; the temperatures of incubation ranged from 25-30 C, and usually only 75 nuts were plated from each orchard.

Assay of Aspergillus flavus isolates for aflatoxin production.—The isolates of *A. flavus* were grown on Czapek's medium for 2 wk at 27 C. Ten grams of the agar and mycelium were triturated in 30 ml of chloroform and centrifuged. The chloroform phase was evaporated and the residue was assayed for the presence of aflatoxin by thin-layer chromatography on a silica-gel G plate. Plates were developed with a chloroform:acetone:*n*-hexane (85:15:20, v/v) solvent system. Aflatoxin standards were spotted on the same plates as references and aflatoxin spots were identified using 310-390 nm UV (1).

Analysis of nuts for aflatoxin.—In 1976, each orchard sample was assayed for aflatoxin within 7 days of collection. Two to 3 kg of fresh whole nuts were ground to a paste in a Wiley mill. Hulled and dried nuts were obtained from several processors. Six 7-kg samples of nuts with discolored shells and two 5-kg samples of premium nuts were ground separately in a Hobart vertical cutter-mixer. Small portions were taken from the mixed batch and combined to make two 100-g subsamples from

00032-949X/78/000 197$03.00/0

TABLE 1. Incidence of *Aspergillus flavus* in pistachio nuts prior to harvest, 1976

Date of collection	Location								Total	
	1		2		3		4			
	(No.)[a]	(%)[b]	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
21 July	0/100	0	...	...	0/100	0	0/100	0	0/300	0
19 August	0/160	0	...	...	...	...	1/150	.7	0/310	0.3
2 September	9/100	9	2/100	2	0/100	0	17/100	17	28/400	7
14 September	10/100	10	1/100	1	33/100	33	3/100	3	47/400	11.8

[a]Represents the number of nuts infected with *A. flavus*/number of nuts sampled.
[b]Percent of sampled nuts infected with *A. flavus*.

TABLE 2. Incidence of *Aspergillus flavus* in insect-damaged and not insect-damaged pistachio nuts (1976)

Date of collection	Not insect-damaged		Insect-damaged	
	(No.)[a]	(%)[b]	(No.)	(%)
19 August	1/308	0.3	0/2	0
2 September	25/389	6.4	3/11	27.3
14 September	40/375	10.7	7/25	28

[a]Represents the number of nuts infected with *A. flavus*/number of nuts sampled.
[b]Percent of sampled nuts infected with *A. flavus*.

each nut sample. The minicolumn method of analysis (1) was employed for aflatoxin detection. Aflatoxins adsorb onto a florisil band in the column and fluoresce blue or green when irradiated with long wave (320-390 nm) ultraviolet. The sensitivity of the analysis was determined by adding 5, 10, and 20 μg/kg of aflatoxin B_1 to 100 g of aflatoxin-free ground pistachio nuts.

RESULTS

At harvest in mid-September in 1974 and 1975, *A. flavus* was isolated from 12% (30/257) and 1.3% (3/225) of the nuts, respectively. In 1976 *A. flavus* was not isolated from nuts sampled in July and was isolated only once from those sampled in August. By 2 September seed expansion had split the endocarp (shell) of 90% of the nuts, but the mesocarp (hull) was intact on about 85% of the nuts; the fungus was present in 7% of all nuts sampled on this date. At harvest in mid-September, the seed was fully exposed in about half the nuts; the mesocarp usually split at the stylar end where the expanding seed had split the endocarp. Infection averaged 11.8% (range 1-33%) (Table 1).

The distribution of the fungus in insect-damaged nuts and in those not damaged by insects is compared in Table 2. On 2 September, *A. flavus* was present in 6.4% of the nuts not damaged by insects in contrast to 27.3% infection of insect-damaged nuts. The data for 14 September similarly reflected a higher incidence of *A. flavus* in insect-damaged nuts; 10.7% of the undamaged and 28% of the insect-damaged nuts. Most of the insect damage was caused by navel orange worm larvae (*Paramyelois transitella*).

Isolates of *A. flavus* were tested in culture for the synthesis of aflatoxin and 70% (16/23) produced aflatoxin B_1. Aflatoxin, however, was not detected in any of the 29 samples of pistachio nuts. Preliminary studies with spiked samples demonstrated the method of analysis was sensitive to at least 10μg/kg, aflatoxin B_1.

DISCUSSION

The occurrence of *A. flavus* in Calfironia-grown pistachio nuts prior to harvest is demonstrated by this study. The studies in 1976 revealed that nuts sampled prior to the occurrence of appreciable hull splitting were relatively free of *A. flavus,* and that at harvest the fungus was present in 11.8% of the nuts. Presumably the difference is due to greater physical exposure of the seed.

The data reflect considerable variability in the distribution of the fungus both among different orchards at the same sampling date and within a given orchard. In particular, infection by *A. flavus* was unusually high in one sample (33%, Location 3, 14 September). Nevertheless, the prevalence of other species of fungi in this sample was comparable to the incidence in other samples. This comparison diminishes the likelihood of contamination as the cause of the anomalous degree of infection by *A. flavus*. The reasons for the variability in infection are unknown; there was no apparent correlation with the method of irrigation or age of the trees (5 to 13 yr). Perhaps areas exist in a given orchard where *A. flavus* has become established more successfully and conversely, there may be areas where the fungus has not yet become established.

The higher percentage of *A. flavus* in insect-damaged nuts may be due to spread of the fungus by the insect, or to better conditions for infection following physical damage of the nuts.

In view of our limited number of analyses, the absence of detectable amounts of aflatoxin in the nuts is not surprising. Schade et al. (8) conducted studies on California almonds and estimated that only one nut in 26,500 nuts was contaminated with aflatoxin. Cucullu et al. (4) reported that only 12 out of 4,920 peanuts contained detectable amounts of aflatoxin in a sample from a mold-damaged lot of peanuts. Clearly, a more extensive investigation of possible aflatoxin contamination in California-grown pistachios is needed, especially as the trees mature. Most California orchards are still young with very little canopy. This situation may be restrictive to the growth of fungi since the humidity is generally lower and air movement and exposure to the sun is greater than in mature orchards.

The occurrence of *A. flavus* in pistachio nuts, the finding that most isolates from nuts produce aflatoxin in

culture, and the demonstration that aflatoxin is produced under California conditions by inoculating developing nuts (9) indicates a potential for aflatoxin production prior to harvest. Previous studies have shown that aflatoxin is not always produced when *A. flavus* grows on a substrate (6). Our studies demonstrated that even in samples where as many as 33% of the nuts were infected there was no detectable aflatoxin. Therefore, the conditions necessary for production of aflatoxin in pistachio nuts prior to harvest are fairly restrictive.

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL ANALYTIC CHEMISTS. 1975. Natural poisons 26. Official methods of analysis. Association of Official Analytic Chemists, Washington, D.C. 24 p.
2. BELL, D. K., and J. L. CRAWFORD. 1967. A botran-amended medium for isolating Aspergillus flavus from peanuts and soil. Phytopathology 57:939-941.
3. BUTLER, W. H., and J. M. BARNES. 1963. Toxic effects of groundnut meal containing aflatoxin to rats and guinea pigs. Br. J. Cancer 17:699-710.
4. CUCULLU, A. F., L. S. LEE, R. Y. MAYNE, and L. A. GOLDBLATT. 1966. Determination of aflatoxins in individual peanuts and peanut sections. J. Am. Oil Chem. Soc. 43:89-92.
5. DICKENS, J. W., and R. E. WELTY. 1975. Fluorescence in pistachio nuts contaminated with aflatoxin. J. Am. Oil Chem. Soc. 52:448-450.
6. GOLDBLATT, L. A. (ed.). 1969. Aflatoxin. Academic Press, New York. 472 p.
7. KOEHLER, P. E., R. T. HANLIN, and L. BERAHA. 1975. Production of aflatoxins B_1 and G_1 by Aspergillus flavus and Aspergillus parasiticus isolated from market pecans. Appl. Microbiol. 30:581-583.
8. SCHADE, J. E., K. MC GREEVY, A. D. KING, JR., B. MACKEY, and G. FULLER. 1975. Incidence of aflatoxin in California almonds. Appl. Microbiol. 29:48-53.
9. SOMMER, N. F., J. R. BUCHANAN, and R. J. FORTLAGE. 1976. Aflatoxin and sterigmatocystin contamination of pistachio nuts in orchards. Appl. Environ. Microbiol. 32:64-67.
10. SUZANGER, M. A. EMANI, and R. BARNETT. 1974. Contamination of pistachio nuts with aflatoxin while on the trees and in storage. Proc. 2nd International IUPAC Symposium on Mycotoxins in Food. July 23-25, Pulaway, Poland. 237 p.
11. WOGAN, G. N. 1966. Chemical nature and biological effects of the aflatoxins. Bacteriol. Rev. 30:460-470.