Relation of Early Splitting and Tattering of Pistachio Nuts to Aflatoxin in the Orchard

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ABSTRACT

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Pistachio nuts that split abnormally to expose the kernels were prone to infection by *Aspergillus flavus* or *A. parasiticus* and contamination with aflatoxin. The incidence of aflatoxin was more frequent if the nuts were also infested by the navel orangeworm (*Amyelois transitella*). Nuts with tattered hulls found at the end of harvest as the hull tissues became senescent were subject to infestation by navel orangeworm moths, and aflatoxin developed. Aflatoxin accumulated in tattered nuts with or without insect infestation but with a higher frequency in the former.

Aflatoxin, a secondary metabolite of *Aspergillus flavus* Link and *A. parasiticus* Speare, contaminates many commodities. Especially common is the contamination of various air- or sun-dried seeds (cereals, cotton, peanuts, and tree nuts) and fruits (figs and dates) (4,12). Aflatoxin has commonly been found in pistachio (*Pistachia vera* L.) nuts from countries in the Middle East (1,2,5–8). The frequency of aflatoxin in pistachio nuts from foreign countries has created concern about its possible presence in California-grown pistachios.

In California, aflatoxin contamination has been minimized by rapid dehydration of the nuts and by avoiding delays between harvest and dehydration. Rapid cooling and storage at low temperature (<5 C) can be employed if delays before drying are unavoidable.

Several studies have suggested that some nuts contained aflatoxin before harvest. Although they failed to find aflatoxin, Thomson and Mehdy (13) isolated *A. flavus* from freshly harvested nuts. They suspected that the lack of aflatoxin contamination was the result of an inadequate number of samples. Also, Mojtahedi et al (7) reported aflatoxin-contaminated nuts in an orchard in Iran.

The Kerman pistachio nuts grown in California characteristically split at the stylar end, a feature that permits them to be easily hand-shelled and consumed as a snack. Normally, the hull (mesocarp + epicarp) loosens from the shell (endocarp) and covers the kernel (seed) before the shell splits at the distal end. Although the shell splits, the hull remains intact to completely cover and protect the kernel. Occasionally, the shell splits before it has loosened from the hull and the shell and adhering hull split simultaneously. This "early splitting" exposes the kernel to airborne fungal spores and infesting or foraging insects or other small animals that might carry fungal spores on their bodies. In particular, *Amyelois transitella* (Wik.), the navel orangeworm moth, preferentially infests nuts with exposed kernels (9).

Some shells never split; in such nuts, the kernels have not developed fully and are small. They do not appear to be a likely locus for infection by aflatoxin-producing fungi because they are protected by both the intact hull and the unsplit shell.

The purpose of these studies was to determine if the kernels of early-split nuts contained aflatoxin at harvest and, if so, to what extent the aflatoxin was associated with navel orangeworm infestations.

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MATERIALS AND METHODS

All pistachio nuts were harvested from two locations in California's San Joaquin Valley: one near Madera (in both 1980 and 1981) and the other near Kettleman City (1981 only). Plots from which nuts were selected consisted of about 1,200 trees at each location. Nuts were selected and picked by hand to a height not exceeding about 2.5 m. Early-split and normally split nuts were collected separately and taken to the Department of Pomology postharvest laboratory at Davis within 12 hr of harvest. Nuts were spread out in a layer in a refrigerated room at 0 C overnight to facilitate rapid removal of field heat, then stored at 0 C.

Samples were prepared by removing the hulls and shells. Kernels of early-split nuts were divided according to the presence or absence of larvae of the navel orangeworm moth. Normally split nuts were treated similarly, but none were infested with the navel orangeworm. Nuts that had not split at the stylar end were discarded. Kernels of normally and early-split nuts with and without navel orangeworm infestations were divided into samples of 50 (1980) or 100 nuts (1981). They were stored at about −10 C until removed for analyses.

The hulls of some nuts become tattered and torn while still on the tree and after the normal harvest season no longer appear to provide adequate protection for the kernel. Samples of tattered nuts were gleaned from the Kettleman City plot after commercial harvest (8 October 1981). Care was exercised to not include tattered early-split nuts. The tattered nuts were handled in the same manner as the early-split nuts.

Aflatoxin consisting of B_1 and G_1, with no more than traces of other aflatoxins, were combined for reporting. Analyses were done essentially as described by Buchanan et al (3). Briefly, extraction was done with a methanol-4% potassium chloride solution. Protein precipitation with lead acetate was followed by defatting with hexane and extracting the aflatoxin into chloroform. The aflatoxins were evaporated to dryness under N_2 before dissolving in chloroform. They were analyzed with a high-pressure liquid chromatograph, and absorbance was read at 360 nm.

RESULTS

Nut splitting. Early-split nuts were readily detected visually in the orchard or at any time after harvest until the hulls were removed (Fig. 1). The obvious split in the hull was made more conspicuous by the darkening of hull tissue along each side of the split. Generally, the hulls of early-split nuts eventually loosened, and hulls were removed normally after harvest. After hull removal, it was impossible to identify early-split nuts (Fig. 2). The amount of early splitting was highly variable from orchard to orchard, from

tree to tree, and from limb to limb. We estimated that in our plots, the early-split nuts appoximated 2% of the total during the years of testing.

Early-split nuts and aflatoxin. Preliminary data taken in 1980 (Table 1) showed that among 50-nut samples collected in the Madera plot, all detected aflatoxin was in early-split nuts. Incidence of aflatoxin was higher if the nuts had also been navel orangeworm-infested. No aflatoxin was found in nuts covered by intact hulls.

More extensive sampling was done in 1981 at the same location near Madera and at Kettleman City (Table 2). The results from both locations were similar to those from the preliminary study made the preceding year. At Madera, two samples of nuts protected by intact hulls showed aflatoxin but at a concentration near the limits of detectability. Similar Kettleman City samples were completely free of detectable aflatoxin. In 100-nut samples, 55% of the 61 samples of navel orangeworm-infested early-split nuts from Madera and 76% of the 59 similar samples from Kettleman City contained aflatoxin. In contrast, 20% of the navel orangeworm-free early-split nut samples from Madera and 23% of those from Kettleman City contained aflatoxin.

Tattered hulls. By 6–8 wk after the start of harvest, the hulls of many nuts remaining on the tree that were originally intact had started to tear and no longer provided the original protection. Navel orangeworm-infested nuts contained aflatoxin in 25 of 34 samples of 100 nuts each (Table 3). Among these insect-infested nuts, nine samples contained aflatoxin at 20 μg/kg or more. Only 10 of 46 samples of insect-free nuts had aflatoxin, and only three contained aflatoxin at 20 μg/kg or more.

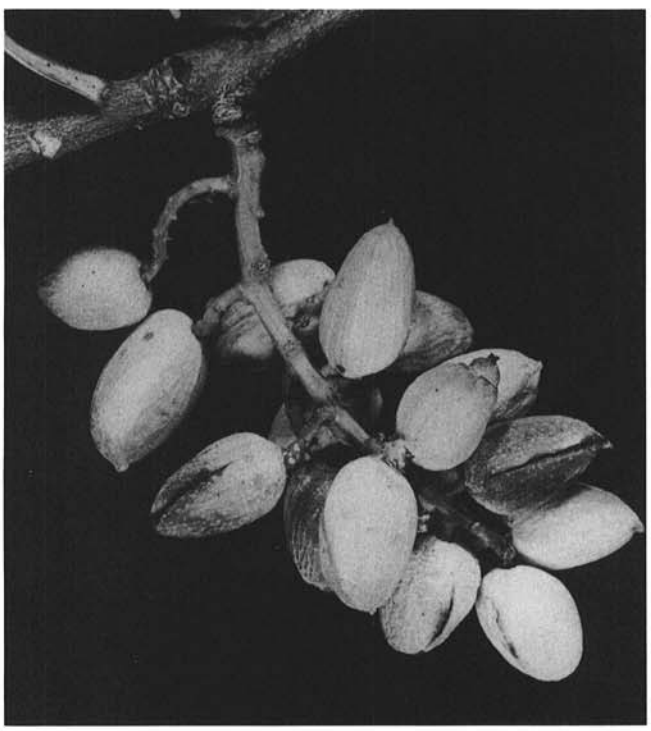

Fig. 1. Pistachio inflorescence before harvest showing several early split nuts.

Fig. 2. (Top row) Pistachio nuts with hulls and (bottom row) similar nuts after hulling. (Left) Desired normal nuts with the split shell protected by an intact hull, (middle) various types of early-split nuts, and (right) undesirable unsplit nut.

DISCUSSION

It is clear that some nuts are infected by *A. flavus* or *A. parasiticus* in the orchard before harvest, and early-split nuts are especially subject to infection and aflatoxin contamination. Normally split nuts, which are protected by intact hulls during the

TABLE 1. Aflatoxin in early-split pistachio nuts collected near Madera in 1980 with or without navel orangeworm (*Amyelois transitella*) infestations and in nuts protected by intact hulls

Type of nuts	Total samples (no.)	With aflatoxin		Aflatoxin[a] (μg/kg)			
		No.	%	2–15	16–25	26–1,000	>1,000
Early-split nuts							
Insect-infested	8	6[b]	75	5	0	1	0
Insect-free	25	4	16	3	0	0	1
With intact hulls	18[c]	0	0	0	0	0	0

[a] Aflatoxins $B_1 + G_1$ in 50-nut samples.
[b] Aflatoxin in insect-infested early-split nut samples was significantly more frequent ($P = 0.01$) than in insect-free early-split nuts as determined by the chi-square test.
[c] Nuts with intact hulls were insect-free.

TABLE 2. Aflatoxin in early-split pistachio nuts collected near Madera and Kettleman City in 1981 with or without navel orangeworm (*Amyelois transitella*) infestations and in nuts protected by intact hulls

Type of nuts	Total samples (no.)	With aflatoxin		Aflatoxin[a] (μg/kg)			
		No.	%	2–15	16–25	26–1,000	>1,000
Madera							
Early-split nuts							
Insect-infested	61	34[b]	55	15	4	10	5
Insect-free	110	22	20	18	1	0	3
With intact hulls[c]	37	2	6	2	0	0	0
Kettleman City							
Early-split nuts							
Insect-infested	59	45[b]	76	31	6	3	5
Insect-free	130	30	23	24	2	3	1
With intact hulls[c]	37	0	0	0	0	0	0

[a] Aflatoxins $B_1 + G_1$ in 100-nut samples.
[b] Aflatoxin in insect-infested early-split nut samples was significantly more frequent ($P = 0.001$) than in insect-free early-split nuts as determined by the chi-square test.
[c] Nuts with intact hulls were insect-free.

TABLE 3. Aflatoxin in nuts collected near Kettleman City in 1981 with tattered hulls at the end of the harvest period as influenced by navel orangeworm (*Amyelois transitella*) infestation

Type of nuts	Total samples (no.)	With aflatoxin		Aflatoxin[a] (μg/kg)			
		No.	%	2–19	20–99	100–1,999	>2,000
Insect-infested	34	25[b]	74	16	5	2	2
Insect-free	46	10	22	7	1	2	0

[a] Aflatoxins $B_1 + G_1$ in 100-nut samples.
[b] Aflatoxin in insect-infested nut samples was significantly more frequent ($P = 0.001$) than in insect-free nuts with tattered hulls as determined by the chi-square test.

normal harvest season, were essentially free of aflatoxin. Aflatoxin was detected in only two of 74 100-nut samples of normally split nuts, and these were at the limits of detectability.

The early-split nuts are also preferentially infested by the navel orangeworm. Because of the very high incidence of aflatoxin in navel orangeworm-infested nuts, a relationship between navel orangeworm activity and aflatoxin-producing aspergilli, as shown earlier in almonds (10), appears indisputable. Nevertheless, aflatoxin also was found in early-split nuts showing no evidence of insect infestation, though at a lower incidence.

The relationship of the navel orangeworm with aflatoxin might lead to the conclusion that the primary health danger to humans is associated with insect-infested nuts. We believe that conclusion would be erroneous because nuts with navel orangeworm infestations are eliminated by rigorous sorting to remove nuts with obvious damage or insect frass. Furthermore, consumers are likely to reject any nut with evidence of insect activity. In contrast, aflatoxin-contaminated nuts without insect infestation are usually indistinguishable from aflatoxin-free nuts after hulling.

The incidence of aflatoxin is low among insect-free nuts. Data in Table 2 showed that at Madera, only 22 of 110 samples each containing 100 insect-free early-split nuts had aflatoxin. At Kettleman City, 30 of 130 similar samples contained detectable aflatoxin. If one assumed that each positive sample resulted from one aflatoxin-contaminated nut (the lowest possible frequency), the incidence of aflatoxin-contaminated early-split nuts would be about one in 500 at Madera and one in 433 at Kettleman City. Early-split nuts are usually present in orchards in a frequency of 1–5%. If the incidence of early splitting was 2% (as in our plots) and all the aflatoxin was in early-split nuts, the incidence of aflatoxin among all the harvested nuts in the orchard would be about one aflatoxin-contaminated nut per 25,000 nuts in the general population at Madera and one per 21,650 at Kettleman City. Similarly, the incidence of navel orangeworm-infested nuts containing aflatoxin in the general population would be about one in 8,500 at Madera and one in 6,550 at Kettleman City.

Aflatoxin in the orchard is probably much more important than the low incidence and generally low concentration of aflatoxins found in the orchard might suggest. Any aflatoxin represents an established infection by *A. flavus* or *A. parasiticus*. Aflatoxin can increase rapidly under highly favorable conditions. We believe the 100% relative humidity and warm temperatures commonly found in bins of harvested nuts provide the conditions conducive to rapid aflatoxin accumulation. New infections probably occur as the hulls tear during harvesting and handling and the kernels become exposed. However, the time required for spore germination and the establishment of infections suggests that those infections would be unimportant unless delays occurred between harvesting and dehydration.

Tattered hulls late in the harvest season are clearly another means by which infections can occur. Fortunately, harvesting is usually completed before hulls have seriously deteriorated.

In tests involving inoculations (11), we demonstrated that *A. flavus* is a wound pathogen of the hulls of developing pistachio nuts. From colonized hulls, the fungus grew through the shell into the kernel, preferentially through vascular tissue at the stem or through shell sutures. However, we were unable to demonstrate in these studies that aflatoxin in kernels commonly resulted from prior colonization of the hulls.

Clearly, the avoidance of aflatoxin in pistachio nuts centers on protecting early-split nuts from infection by toxin-producing fungi or by eliminating aflatoxin-containing nuts by sorting. The use of an effective fungicide against *A. flavus* and *A. parasiticus* might lead to control through sprays timed to coincide with early splitting.

The elimination of early-split nuts after harvest would require extensive sorting before hulling, because they cannot be recognized visually after they have been hulled. Such a sorting operation, if done by humans, would probably be very costly. Furthermore, the sorting might result in significant delays during the period between harvest and drying. The possibility that electronic color sorters might detect early-split nuts by the distinctive dark surfaces of early-split hulls has not been explored.

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