The Relationship Between Date of Hull Splitting and Decay of Pistachio Nuts by *Aspergillus* Species

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ABSTRACT

Doster, M. A., and Michailides, T. J. 1995. The relationship between date of hull splitting and decay of pistachio nuts by *Aspergillus* species. Plant Dis. 79:766-769.

Atypical pistachio (*Pistacia vera*) nuts that have split hulls, known as "early splits," frequently have kernels decayed by *Aspergillus* spp. In 1992 and 1993 pistachio nuts that had split hulls were marked in commercial orchards at approximately 2-week intervals. Depending on the orchard, 15 to 48% of the early splits had formed more than 4 weeks before harvest and 10 to 30% formed within 2 weeks before harvest. At harvest these nuts were also evaluated for kernel decay, insect infestation, and several physical characteristics. The incidence of decay caused by *Aspergillus* spp. (predominantly *Aspergillus niger*) was three times greater in the oldest early splits than in early splits formed in the period before harvest (no decay was found in normal nuts with nonsplit hulls). The oldest early splits also had the most shriveled hulls, which is a feature associated with aflatoxin contamination. At harvest the oldest early splits had less fruit fresh weight, lower kernel moisture, smaller shells, and more shell discoloration compared with early splits formed in the period before harvest and with normal nuts. These characteristics could be used to remove decayed nuts during processing.

Additional keywords: *Aspergillus flavus, Aspergillus parasiticus*

The development of a pistachio (*Pistacia vera* L.) nut in the orchard involves a complicated interaction between hull and shell. In early summer the hull surrounds and is attached to the shell. Normally, as the nut matures the hull separates from the shell and then the shell splits. The shell splitting of pistachio nuts is a very desirable feature because it allows the consumer to open the nut easily. The hull typically does not rupture or split before harvest and protects the kernel from fungal infection and insect infestation. However, a small number of atypical nuts, known as "early splits," have the hulls split along the shell suture where the shell has split because the hull has not separated sufficiently before the shell split. The kernels of early splits are frequently moldy and insect-infested (7). Of special concern is kernel decay by *Aspergillus* spp. because many of these produce mycotoxins harmful to humans and animals (16). The most important group of mycotoxins are the aflatoxins produced by *Aspergillus flavus* Link:Fr. and *Aspergillus parasiticus* Speare (19). Many different *Aspergillus* spp. have caused decay in pistachio nuts in California (7), Iran (14), and Turkey (5). In California 14 *Aspergillus* spp. were isolated from pistachio kernels, although *Aspergillus niger* Tiegh. was the only species that occurred frequently (7). Although both *A. flavus* and *A. parasiticus* were isolated from pistachio nuts from California orchards (7), most isolates of *A. flavus* from pistachio orchards did not produce aflatoxin (6). However, aflatoxin has been detected in early split pistachio nuts from California orchards (7,18). High levels of aflatoxin in early split pistachio nuts were associated with navel orangeworm (*Amyelois transitella* (Walker)) infestation (7, 18) and the characteristic of shriveled hulls (7).

Although the importance of early splits for mold, aflatoxin, and insect contamination is well established, very little is known concerning when early splits form and when they become contaminated. It would be particularly useful to know when most early splits form because this knowledge could aid in timing the application(s) of either chemical or biological control treatments for decreasing the decay of early splits. In addition, it is important for processors to remove early splits in order to minimize mold and aflatoxin in pistachio nuts sold to the consumer. Because the kernel cannot be examined for fungal decay (since the pistachio nuts are usually sold in shell), processors need to know nut characteristics that are associated with kernel decay. Early splits with shriveled hulls had both a high incidence of kernel decay by *Aspergillus* spp. and almost all of the aflatoxin in contaminated pistachio nut samples (7). These hulls, however, are removed early in processing before any sorting so other characteristics are needed to identify the early split nuts. The objectives of this research were (i) to determine when during summer that hulls split resulting in early split pistachio nuts, (ii) to determine whether there is a relationship between date of hull split for early splits and kernel decay by *Aspergillus* spp., and (iii) to evaluate the physical characteristics of early splits formed at different periods.

MATERIALS AND METHODS

Three commercial pistachio (cv. Kerman) orchards in Madera County, California, were used. In 1992 three trees were used in orchard A and one tree in orchard C (orchard B was not used in 1992) and in 1993 two blocks of three trees were used in orchards A and B, and two blocks of one tree in orchard C. (Trees in orchard C had so many more early splits than normally observed that fewer trees were needed than in orchards A and B.) For each selected tree, all pistachio nuts less than 2 m from the ground were carefully examined for hull splitting on 5, 19, and 31 August 1992, and 12 and 26 August and 7 September 1993. In order to keep track of the nuts that had split hulls, pieces of colored yarn were wrapped around the stems of the early splits. Harvests occurred on 31 August 1992 and 7 September 1993 (during the period of commercial harvests in the area) when all early splits were removed from the selected trees and the total number of nuts on these trees was counted. For comparison, normal nuts with nonsplit hulls and a developed kernel were also collected from the same trees and evaluated. In 1992, the numbers of nuts examined per tree were 1,557 for orchard A and 1,038 for orchard C, whereas in 1993 the numbers per tree were 1,514, 783, and 2,380 for orchards A, B, and C, respectively. The developmental status (hull separation and shell split) of normal nuts was determined for approximately 35 and

This research was supported by USDA Cooperative Research Agreement No. 58-5325-1-194.

Accepted for publication 18 April 1995.

90 filled nuts per orchard for each preharvest date for 1992 and 1993, respectively, and at harvest for 56 and approximately 500 nuts per orchard for 1992 and 1993, respectively. Pistachio nuts were transported to the laboratory in ice chests and stored at 0 to 2°C until evaluated. In 1992, pistachios matured earlier than normal so the harvest was earlier than in 1993.

The characteristics and condition of the pistachio fruit at harvest were determined for orchard A in 1992 and orchards A and B in 1993 as follows: Each fruit was weighed. The hull was evaluated as being shriveled or smooth, removed by hand, and put aside for drying. The shell was measured, evaluated for external discoloration, and removed by hand. Two types of external shell discoloration were distinguished: a staining around the shell suture where the shell split and a general staining that was not restricted to any specific location (7). A pretransformed scale (12) was used to rate the amount of general shell discoloration: 0 for 0% of shell surface stained; 1 for 1 to 10%; 2 for 11 to 35%; 3 for 36 to 64%; 4 for 65 to 89%; 5 for 90 to 99%; and 6 for 100%. Using a dissecting microscope (10 to 60×), each kernel was carefully examined for sporulation by *Aspergillus* species and navel orangeworm infestation. The fungi were assigned to the sections within the genus *Aspergillus* (10). The hulls and kernels were dried in an oven at 90°C until no further weight loss occurred and the moisture content was calculated from the fresh and dry weights.

All statistical analyses were done with SAS (SAS Institute Inc., Cary, N.C., release 6.04). In general, analysis of variance was used with the least significant difference for mean separation. Data in the form of percentages were transformed with the standard arcsine transformation (12), although percentages were not transformed if the range of values was small (11).

RESULTS AND DISCUSSION

In early summer all pistachio nuts have the hull attached firmly to the shell but as the nuts develop normally the hull detaches from the shell before the shell splits open (3,4). Early splits, however, have the hull attached when the shell splits, causing the hull to rupture. The hull splitting that characterizes early splits began more than 4 weeks before harvest and continued to within 2 weeks of harvest (Table 1). In 1992, a few scattered early splits were observed in orchard C and nearby orchards as early as 22 July. Most early splits formed earlier than 2 weeks before harvest, exposing the kernel to colonization by molds and insects for several weeks (Table 1). Although the pistachio crop matured earlier in 1992 than in 1993, the general pattern of development of early splits was similar for both years (Table 1). Even though the splitting of shell and hull

Table 1. Formation of early split pistachio nuts and the status of normal nuts with nonsplit hulls in commercial orchards A, B, and C

		Cumulative percentage of early splits formed[x]			Cumulative percentage of normal nuts[y]					
					Shell split			Hull separated		
Year	Date of observation	A	B	C	A	B	C	A	B	C
1992	5 August	16	NM[z]	48	4	NM	0	7	NM	0
	19 August	70	NM	90	72	NM	NM	49	NM	NM
	31 August	100	NM	100	100	NM	NM	100	NM	NM
1993	12 August	15	23	48	7	15	19	3	0	3
	26 August	74	72	87	54	73	36	62	73	52
	7 September	100	100	100	100	100	100	100	100	100

[x] In 1992 the numbers of early splits observed were 123 for orchard A and 366 for orchard C, whereas in 1993 the numbers were 243, 165, and 201 for orchards A, B, and C, respectively. In 1992 the total numbers of nuts examined were 4,670 for orchard A and 1,038 for orchard C, whereas in 1993 the numbers were 9,085, 4,700, and 4,670 for orchards A, B, and C, respectively.

[y] For this table, only normal nuts that had shells split or hulls separated on 31 August 1992 or 7 September 1993 were considered. As the pistachio fruit matures, the hull detaches from the shell. On 31 August 1992, 84% of the shells were split and 91% of the hulls separated for normal nuts in orchard A. On 7 September 1993, shell split of normal nuts was 73, 72, and 80% and hull separation was 90, 96, and 99% for orchards A, B, and C, respectively.

[z] Not measured.

Table 2. The relationships between period of hull split for early split pistachio nuts to kernel contamination by fungi and insects and to hull characteristics[w]

Year	Period of hull split	*Aspergillus* infected (%)[x]	Navel orangeworm infested (%)	Shriveled hull (%)
1992	Before 5 August	20.6 a[y]	17.7 a	73 a
	5 to 19 August	14.8 ab	20.6 a	26 b
	19 to 31 August	6.8 b	14.8 a	19 b
	Normal nuts[z]	0.0 c	0.0 a	0 c
1993	Before 12 August	7.4 a	13.7 a	79 a
	12 to 26 August	5.0 a	5.4 ab	59 a
	26 Aug. to 7 Sept.	0.5 b	1.8 bc	14 b
	Normal nuts	0.0 b	0.0 c	0 c

[w] The data are from commercial orchards A and C for 1992 and orchards A, B, and C for 1993.

[x] The following *Aspergillus* sections were observed: *Nigri* (89% of colonies), *Circumdati* (5%), *Terrei* (2%), and *Flavi* (1%).

[y] Data were transformed by the standard arcsine transformation for statistical analysis. Values presented were back transformed from the means of the transformed data. Numbers in columns with same letters are not significantly different ($P = 0.05$) by pairwise comparisons using Fisher's LSD test. Each year was analyzed separately.

[z] Normal nuts have nonsplit hulls prior to harvest.

Table 3. The characteristics at harvest of early split pistachio nuts that form during different periods[u]

		Fruit fresh weight (g)	Kernel moisture (%)	Shell		
				Length (mm)	Suture stained (%)[v]	Rating for general stain[w]
Year	Period of hull split					
1992	Before 5 August	1.3	20	16.5	100 (1.57)[x]	2.2
	5 to 19 August	2.4	35	19.9	71 (1.00)	1.3
	19 to 31 August	2.7	44	20.3	69 (0.98)	1.1
	Normal nuts[y]	4.1	51	22.6	0 (0.06)	0.0
	LSD 0.05[z]	0.4	9	1.7	. . . (0.22)	0.4
1993	Before 12 August	1.2	20	16.8	94 (1.32)	2.2
	12 to 26 August	1.9	31	18.6	74 (1.04)	1.4
	26 Aug. to 7 Sept.	2.7	44	20.0	38 (0.67)	0.7
	Normal nuts	3.8	49	22.4	0 (0.03)	0.1
	LSD 0.05	0.3	7	0.9	. . . (0.18)	0.4

[u] The data are from one and two commercial pistachio orchards for 1992 and 1993, respectively. Each year was statistically analyzed separately.

[v] Percentage of nuts that have dark brown discoloration along the shell suture.

[w] A pretransformed scale was used to rate the amount of discoloration on external shell surfaces: 0 for 0% of shell surface discolored; 1 for 1 to 10%; 2 for 11 to 35%; 3 for 36 to 64%; 4 for 65 to 89%; 5 for 90 to 99%; and 6 for 100%.

[x] Data were transformed using the standard arcsine transformation. In the parentheses are the means for the arcsine transformed values. Percentage values for the means were calculated from the means of the transformed data.

[y] Normal nuts have nonsplit hulls prior to harvest.

[z] Least significant difference.

for early splits tended to precede the shell splitting of normal nuts, a number of early splits formed after many normal nuts had split their shells (Table 1). Even normal pistachio nuts matured unevenly over a period of several weeks during which shells split (Table 1).

Early splits that ruptured their hulls earliest were of the poorest quality with kernels frequently contaminated with molds and insects. The early splits formed before the first observation period (5 and 12 August for 1992 and 1993, respectively) had the highest percentage of kernels infected with *Aspergillus* spp. (Table 2). The earlier the hull ruptures the more time there is for fungal infection and growth. If the harvest is delayed it would be expected that later-formed early splits would increase in infections by *Aspergillus* fungi, in which case it would be important that growers do not delay harvest. Other factors could also influence the fungal colonization of kernels of early splits. For example, because *A. niger* inoculum increases in pistachio orchards during the summer (13), it would be expected that the highest level of *Aspergillus* inoculum would be close to harvest. In addition, the kernels of early splits lose moisture after hull split (Table 3), a condition that might favor the development of *Aspergillus* species, because *Aspergillus* species can grow at lower moisture contents than most fungi (1). The growth rate of the toxigenic species *A. flavus* is similar to that of the common species *A. niger* at the same temperature and water activity (1). Although 89% of the *Aspergillus* colonies observed in our study were in section *Nigri*, sections *Circumdati* (potential ochratoxin producers), *Terrei*, and *Flavi* (potential aflatoxin producers) were also present. These results are similar to those found in a more thorough study on *Aspergillus* species in early split pistachio nuts (7).

Navel orangeworm causes a serious problem in pistachio nuts in California orchards (17). The larvae invade and damage pistachio kernels, resulting in substantially higher levels of infection of early split pistachio kernels by fungi in each of the sections *Nigri*, *Flavi* (potential aflatoxin producers), and *Circumdati* (potential ochratoxin producers) (7). In 1993, the older early splits had more navel orangeworm than the later-formed early splits (Table 2), probably because kernels were exposed to infestation for a longer period. In 1992 there were no significant differences in infestation between the different aged early splits. Some variation from year to year would be expected because navel orangeworm eggs are laid during several peak periods that vary from year to year (17). No navel orangeworm was observed in the kernels of normal nuts with nonsplit hulls.

Aflatoxins, which are carcinogens produced by *A. flavus* and *A. parasiticus*, contaminate pistachio nuts prior to harvest (7,18) and are a concern for the industry. Early splits are probably the most important source of preharvest aflatoxin contamination for pistachio nuts in California (7,18). Although we did not measure aflatoxin, we did evaluate nuts for shriveling of the hull, which is a feature associated with aflatoxin. In a previous study, early splits with shriveled hulls had more than 99% of the aflatoxin detected (7). The oldest early splits usually had shriveled hulls (>70%), while the early splits formed close to harvest did not (<20%) (Table 2). In 1993, the oldest early splits had the highest level of navel orangeworm infestation, which is also a factor associated with aflatoxin contamination (7,18). Because the early splits formed close to harvest had less kernel decay by *Aspergillus*, less navel orangeworm infestation, and fewer nuts with shriveled hulls (Table 2), these nuts would be expected to have less aflatoxin than older early splits.

Another reason that early splits result in poor quality nuts is the discoloration of the shell exterior (7). Since pistachio nuts are usually sold to consumers in shell, the appearance of the shell is very important. The older early splits had substantially more external shell discoloration than the early splits formed closer to harvest (Fig. 1, Table 3). For example, in 1993 early splits formed before 12 August had a mean rating of 2.2 while early splits formed after 26 August had a rating of only 0.7. Although it is not clear why older early splits have more shell discoloration, one possibility is that the intensity of the wound response decreased as pistachio nuts matured (2).

We found that many characteristics can be used to distinguish and remove the older early splits that are most likely to be contaminated with *Aspergillus* and navel orangeworm. Although previous research found that early splits differed from normal nuts in many characteristics (7,15), the present study found that the characteristics of early splits were influenced by the date of formation. The older early splits had lighter fruit weights and smaller shells than later-formed early splits and normal nuts (Table 3). Although it is not mentioned in Table 3, the oldest early splits had hulls of less weight (fresh and dry) and lower moisture content, shells with smaller widths, and kernels of less weight (fresh and dry) than normal nuts in both 1992 and 1993; and the later-formed early splits had values intermediate to those for the oldest early splits and normal nuts. Although we did not measure density, it would be expected that the older early splits would be less dense than normal nuts because of the large difference in fresh weight and only a slight difference in size. The use of density to remove mycotoxin-contaminated products has been successfully tested for peanuts (9), corn, and wheat (8). In addition, the staining of the shell was greater the earlier the early splits formed. Two types of external shell discoloration were distinguished: first was the staining along the suture that is characteristic of early splits (7) and second was a general type of staining not localized in any one part of the shell. Older early splits had more of both types of staining than the later-formed early splits and normal nuts (Fig. 1, Table 3). Shell discoloration is the easiest characteristic for processors to use

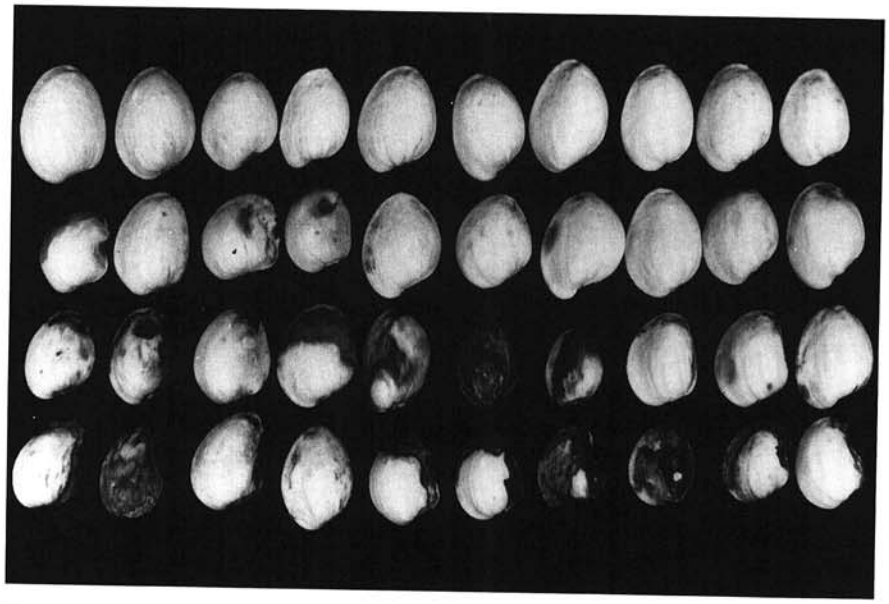

Fig. 1. Discoloration of shells from normal pistachio nuts that had nonsplit hulls (top row); from early split pistachio nuts that ruptured their hulls between 26 August and 7 September (harvest) (second row from top); from early splits that ruptured their hulls between 12 and 26 August (third row from top); and from early splits that ruptured their hulls before 12 August (bottom row). All nuts were gathered from orchard A in 1993.

to remove early splits because they already remove heavily stained nuts. Through adjustments of electronic color sorters and instructions to hand sorters most of these early-formed early splits can be removed.

ACKNOWLEDGMENTS

We thank L. D. Boeckler and D. P. Morgan for technical assistance. We also thank K. Munger and H. Zermeno (Munger Poonian Farms), K. Herman (Specialty Crop Company), and C. Wylie (First Associate Ranch Management Co.) for their cooperation.

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