Development of *Aspergillus* Molds in Litter from Pistachio Trees

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ABSTRACT

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Aspergillus molds frequently infested, infected, and sporulated on pistachio litter such as fallen pistachio fruit and male inflorescences throughout summer in commercial pistachio orchards in California. *A. niger* was isolated much more frequently from pistachio litter than any other species, but the aflatoxin producers, *A. flavus* and *A. parasiticus*, also developed in pistachio litter. Two distinct strains of *A. flavus* were isolated: strain L (a few large sclerotia) and strain S (abundant small sclerotia). Strain L occurred substantially more often than strain S. All isolates of strain S and *A. parasiticus* were aflatoxin producers, but only 43% of the isolates of strain L produced aflatoxins. Other species isolated in the subgenus *Circumdati* were *A. tamarii, A. melleus, A. ochraceus, A. japonicus*, and *A. wentii*. The development of *Aspergillus* molds in pistachio litter could increase the amount of *Aspergillus* inoculum in the orchard, resulting in greater numbers of moldy and mycotoxin-contaminated pistachio nuts.

Additional keywords: debris, ochratoxin, *Pistacia vera*

Many molds in the genus *Aspergillus* infect and decay the kernels of pistachio (*Pistacia vera* L.) nuts (8,19). Although most of these molds occur infrequently, their presence can still be a serious problem because many of them produce mycotoxins that are harmful to humans and animals. Aflatoxins—potent toxins and carcinogens produced by *Aspergillus flavus* Link:Fr. and *A. parasiticus* Speare in section *Flavi*—have been found in nuts from pistachio orchards (24). Aflatoxins are the most widely regulated mycotoxins, with none or very little tolerated in foodstuffs (28). Another important group of mycotoxins are the ochratoxins, produced by *A. ochraceus* K. Wilh. and closely related species in section *Circumdati* (20). The amount of California pistachio nuts exported has increased until 30% of the crop for 1991–1992 was exported (3). Because so many countries have very low tolerances for aflatoxins (28), there is a strong desire by the pistachio industry in California to eliminate aflatoxins from pistachio nuts.

The kernels of pistachio nuts are not colonized by *Aspergillus* molds until late summer (8), although temperatures are favorable for growth of these molds throughout summer (1). If suitable substrates are available in the orchard, *Aspergillus* molds could develop and produce abundant spore inoculum, which could increase kernel infections. *Aspergillus* molds are able to decay a broad range of substrates, including plant debris (16). Possible substrates for *Aspergillus* molds in pistachio orchards are the litter from pistachio trees, such as the inflorescences from male pistachio trees that fall to the ground in late spring and throughout summer. In addition, some pistachio fruit drop to the ground throughout summer and may be colonized by *Aspergillus* molds. Pistachio litter is the predominant plant debris on the ground in many commercial orchards because weeds are controlled entirely. The objectives of this research were to determine how frequently pistachio litter was colonized by *Aspergillus* molds, to identify which *Aspergillus* spp. were involved, and to measure the density of *Aspergillus* molds in the soil of pistachio orchards.

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MATERIALS AND METHODS

Litter. Male inflorescences and pistachio fruit on the ground in commercial pistachio orchards (eight in 1991 and nine in 1992) were collected in late spring and summer and evaluated for colonization by *Aspergillus* molds. Commercial orchards were selected so that the wide range of cultural practices currently used by growers could be represented, e.g. methods of irrigation (flood, sprinkler, and microjet) and ways of managing vegetation on the orchard floor. The orchards were located in Madera County, California, unless stated otherwise. Pistachio litter was evaluated for the presence of *Aspergillus* in one of three ways that helped estimate infestation, infection, and spore production. First, while still in the orchard, litter was placed on sterile salt agar (6% NaCl, 0.5% agar) in 60-mm-diameter plastic petri dishes using forceps dipped in 95% ethanol; the dishes were then incubated at 30 C in the laboratory and examined for sporulation with a dissecting microscope after 5–14 days. Second, litter was surface-disinfested with NaOCl in the laboratory, placed on sterile salt agar, incubated at 30 C, and examined for sporulation with a dissecting microscope after 10 days. Third, litter was examined for sporulation with a dissecting microscope (10–60×) as soon as it arrived in the laboratory.

Pistachio fruit on the ground used for infestation studies were collected throughout eight orchards (50 fruit per orchard) on 3 September 1991 and throughout five orchards (200 fruit per orchard) on 9 July 1992. The fruit collected in 1992 were also examined for sporulation before incubation. For infection studies, fruit on the ground were collected from eight orchards (100 fruit per orchard) on 11 September 1991 and from five orchards (100 fruit per orchard) on 5 August 1992. Surface disinfestation was performed in a laminar flow hood by placing fruit in 70% ethanol for 15 sec, followed by 1 min in 0.5% NaOCl solution, and rinsing them with sterile distilled water. Three hundred pistachio fruit from an orchard in Tulare County were examined for sporulation on 2 June 1992.

Male inflorescences were collected from the ground in commercial orchards periodically from spring throughout summer. Male inflorescences, which are panicles, were not surface-disinfested because of their complex and convoluted surfaces but were immediately placed on sterile medium while still in the orchard. For the most extensive studies, inflorescences were collected from several male trees throughout eight orchards (50 inflorescences per orchard) on 3 June 1991 and from five orchards (100 inflorescences per orchard) on 19 August 1992. Inflorescences were also collected from the orchard in Tulare County, 150 on 15 August 1991 and 148, 200, and 160 on 9 April, 27 May, and 2 July 1992, respectively. In addition, inflorescences were collected on 5 August 1991 from three orchards (between 58 and 208 inflorescences from each) and on 11 September 1991 from two orchards (50 inflorescences per orchard).

Pistachio nuts left in the orchard after harvest were evaluated for *Aspergillus* infection. On 4 December 1990, approximately 150 nuts of the previous crop were collected from the trees or ground in each of seven orchards. Hulls and shells were removed by hand. The kernels were soaked in 95% ethanol for 30 sec, rinsed

in sterile distilled water, soaked in 5% NaOCl solution for 1 min, rinsed in sterile distilled water, plated on sterile salt agar, incubated for 12 days at 30 C, and examined for sporulation. All *Aspergillus* isolates were identified to species.

Identification of molds. The *Aspergillus* molds observed were assigned to the sections of subgenus *Circumdati* on the basis of color of conidia and general appearance as observed with a dissecting microscope (10–60×). The sections, which were described by Gams et al (11) and used by Klich and Pitt (15), correspond roughly to groups used by Raper and Fennell (22). For example, sections *Nigri, Flavi, Circumdati*, and *Wentii* correspond to *A. niger*, *A. flavus*, *A. ochraceus*, and *A. wentii* groups, respectively. More than 120 *Aspergillus* isolates from pistachio litter were identified to species for each of the sections *Nigri, Flavi*, and *Circumdati* using the method of Klich and Pitt (15). The microscopic characteristics of these isolates were examined with a compound microscope (400–1,000×). All colonies observed in the litter in section *Flavi* or *Wentii* were isolated into pure culture and identified to species. Since section *Nigri* was so common, only certain colonies were isolated and identified to species (10 isolates from each of five orchards for male inflorescences and seven to 20 isolates from each of six orchards for pistachio fruit). Certain isolates were tentatively classified as *A. melleus* Yukawa in section *Circumdati* using Raper and Fennell (22) and Christensen (4). These isolates best fit the description of *A. quercinus* (Bainier) Thom & Church given in Thom and Raper (26) because of the abundant production of orange-yellow to rufous sclerotia. However, Raper and Fennell (22) considered *A. quercinus* a synonym of *A. melleus*. In addition, all isolates of *A. flavus* were classified as strain L or strain S according to whether a few large sclerotia or abundant small ones were produced on media (5).

Soil. Soil samples were collected from eight commercial pistachio orchards on 4 December 1990 and from six orchards on 14 November 1991 for quantification of *Aspergillus* molds. Half the orchards had cover crops between rows and the other half disked regularly to limit vegetation. Each orchard was divided into three approximately equal areas for sampling the soils. Samples of 10 soil cores 5 cm deep were taken randomly (with an average of 20 m apart) from each area and placed in thick, sterile plastic bags. The soil samples in the bags were kneaded by hand to remove any clods and then thoroughly shaken to blend the soil. A soil dilution method was used to quantify the fungi present: 1 g of soil from each sample was placed in 100 ml of sterile distilled water and shaken for 15 min in a mechanical shaker. The soil suspension was further diluted, and 0.1 ml of the suspension was spread evenly with a glass stick on petri dishes containing potato-dextrose agar (PDA) to which NaCl (60 g/L) was added in 1991 and *A. flavus* and *A. parasiticus* agar (AFPA) (21) in 1992. Addition of NaCl to PDA inhibited many microorganisms, but the *Aspergillus* spp. present grew as well as or better than those on PDA without NaCl. AFPA is a selective medium for *A. flavus* and *A. parasiticus* but is also useful for quantifying *A. niger* and *A. ochraceus*. The dishes were incubated at 30 C for 4–7 days to allow the *Aspergillus* colonies present to develop sufficiently for observation. All molds in sections *Flavi, Circumdati*, and *Wentii* were isolated into pure cultures and identified to species using the method of Klich and Pitt (15). Dry weights for soils were obtained by placing 10–20 g of soil in an oven at 105 C until the weight became constant. Results are presented as colony-forming units per gram of dry soil.

Aflatoxins. Isolates of *A. flavus* and *A. parasiticus* from pistachio litter were tested for aflatoxin production by the method of Filtenborg and Frisvad (9) plus an extraction solvent (10). Soon after being obtained, isolates were stored on silica gel at 6 C until needed (27). Conidia on silica gel were used to inoculate glucose yeast agar (20 g glucose, 5 g yeast extract, 20 g agar, and 1 L distilled water). Two culture dishes per isolate were incubated at 30 C for 7 days, and then 4-mm-diameter agar plugs were removed from the colony and placed at the origin on TLC plates (silica gel G). After 20 µl of extraction solvent (chloroform/methanol, 2:1) was placed on each plug, the TLC plates were developed in the solvent mixture of diethyl ether/methanol/water (96:3:1). Aflatoxins were visualized by means of long-wave UV light (365 nm), and their appearance and R_f were compared with standards. To determine the detection limit of this method, known amounts of aflatoxins B_1 and G_1 were added to melted medium, poured into petri dishes, allowed to cool, and then tested for the presence of aflatoxins. The minimum concentration of aflatoxins B_1 and G_1 detected was 25 ppb.

Data analysis. In general, statistical analysis of data was descriptive. The standard deviations were calculated and presented rather than the standard errors of the mean because the dispersion in the data was of interest (2). Analysis of variance (release 6.04, SAS Institute, Cary, NC) was used to compare disked orchards and orchards with cover crops, and logarithms (17) were used to transform soil data before analysis.

Table 1. Incidence of *Aspergillus* molds in pistachio fruit on the ground in commercial orchards

Fruit preparation[a]	Year	Percentage of pistachio fruit with molds per section[b]			
		Nigri	*Flavi*	*Circumdati*	*Wentii*
None	1992	5.5 ± 2.8	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
Surface disinfestation	1991	30.6 ± 17.4	0.3 ± 0.5	1.6 ± 2.1	0.0 ± 0.0
	1992	48.2 ± 19.7	0.8 ± 1.1	1.8 ± 2.5	0.0 ± 0.0
Incubation	1991	87.5 ± 14.6	7.3 ± 7.7	16.3 ± 12.6	0.3 ± 0.7
	1992	91.4 ± 4.6	4.7 ± 2.0	13.2 ± 7.4	0.7 ± 0.4

[a] Fruit for surface disinfestation were gathered from eight orchards on 11 September 1991 and from five orchards on 5 August 1992. Fruit for incubation were gathered from eight orchards on 3 September 1991 and from five orchards on 9 July 1992. Incubated fruit were not surface-disinfested but were placed in sterile salt agar in the orchard, then incubated at 30 C for 5–7 days. Fruit with no preparation were examined for sporulation as soon as they arrived in the laboratory.
[b] Values are means ± standard deviations.

Table 2. Incidence of *Aspergillus* molds in pistachio male inflorescences on the ground in commercial orchards[a]

Year	Percentage of inflorescences that developed molds per section[b]			
	Nigri	*Flavi*	*Circumdati*	*Wentii*
1991	51.1 ± 28.8	13.4 ± 9.5	28.8 ± 19.6	1.5 ± 1.4
1992	65.8 ± 25.2	26.4 ± 15.8	14.6 ± 19.1	...

[a] Male inflorescences were gathered from eight orchards on 3 June 1991 and from five orchards on 19 August 1992. Inflorescences were placed in sterile salt agar in the orchard, then incubated at 30 C for 2 wk.
[b] Values are means ± standard deviations.

RESULTS

Litter. *Aspergillus* spp. were commonly associated with litter from pistachio trees on the orchard floor (Tables 1 and 2), and sporulation by *Aspergillus* molds was found occasionally on pistachio litter in commercial orchards. For example, in 1991, 5% of male inflorescences in an orchard in Madera County had sporulation by *Aspergillus* molds in section *Nigri*, and in 1992, 11 and 20% of male inflorescences in an orchard in Tulare County that had been dampened by irrigation had sporulation by fungi in sections *Nigri* and *Circumdati*, respectively. In five commercial orchards in 1992, 6% of pistachio fruit on the ground

had *Aspergillus* sporulation (Table 1). In the orchard in Tulare County, 54 and 1% of the fallen fruit had sporulation by fungi in sections *Nigri* and *Circumdati*, respectively. Sporulating fungi on fallen pistachio fruit were most commonly in section *Nigri*, occasionally in section *Circumdati*, rarely in section *Flavi* (*A. parasiticus* sporulated on one fruit), and never in section *Wentii*.

Substantially more pistachio litter was infected with *Aspergillus* molds than had visible sporulation. Over 30% of the pistachio fruit on the ground were found to be infected by *Aspergillus* spp. after surface disinfestation and incubation (Table 1). In an orchard with 5% of the male inflorescences showing section *Nigri* sporulation, 32% of the inflorescences had sporulation after 24 hr of incubation, indicating that many of these inflorescences had infections without visible sporulation.

Both pistachio fruit and male inflorescences on the ground were heavily infested with *Aspergillus* (Tables 1 and 2). When these types of litter were incubated under favorable conditions, abundant sporulation by *Aspergillus* occurred. Almost all of the pistachio fruit were also infested with *Aspergillus*, mainly section *Nigri* but also sections *Flavi, Circumdati*, and *Wentii* (Table 1). Most of the male inflorescences were also infested with *Aspergillus*, mainly section *Nigri* but with substantial numbers in section *Flavi* or *Circumdati* (Table 2).

Aspergillus molds were associated with male inflorescences during spring and summer. Sporulation was observed on inflorescences by both sections *Nigri* and *Circumdati* in the Tulare County orchard on 31 July and 15 August 1991 and 27 May and 2 July 1992 and by just section *Nigri* in an orchard in Madera County on 5 and 12 August 1991 (observations were not conducted in other months). Inflorescences were infested by *Aspergillus* from April through September. On 9 April 1992, 80, 13, and 68% of the male inflorescences from the orchard in Tulare County were infested with *Aspergillus* molds in sections *Nigri, Flavi*, and *Circumdati*, respectively. Infestations of male inflorescences were 83, 81, and 76% for section *Nigri*; 22, 28, and 27% for section *Flavi*; and 46, 37, and 66% for section *Circumdati* on 3 June, 5 August, and 11 September 1991, respectively, for two commercial orchards.

Aspergillus molds colonized nuts left in the orchard after harvest. On 4 December 1990 (approximately 2–3 mo after harvest), 6.7% of the kernels of nuts left in seven orchards were infected with molds in section *Nigri* (96 and 4% were *A. niger* and *A. japonicus* Saito, respectively). There was no significant ($P = 0.65$) difference in kernel infection by section *Nigri* between nuts on the ground (7.1%) and those on the tree (5.6%). Three of the seven orchards had more infected nuts on the ground, two had more infected nuts on the tree, and two had no infections by section *Nigri*. Molds in sections *Flavi* (*A. flavus*) and *Wentii* (*A. wentii*) were found in 0.1% of the kernels. However, no infection by *Aspergillus* molds in section *Circumdati* was found. Infections of kernels after harvest were also observed in 1991, although no thorough study was completed.

Identification of molds. Eight different species were identified in isolates in *Aspergillus* subgenus *Circumdati* from pistachio litter (Table 3). *A. niger* was the most common species, although another black-spored *Aspergillus, A. japonicus*, was found occasionally. In section *Flavi, A. flavus* was much more common than *A. parasiticus* and *A. tamarii* Kita (Table 3). Among isolates of *A. flavus*, strain L (which produces a few large sclerotia on media) was more than seven times more frequent than strain S (many small sclerotia). In section *Circumdati, A. melleus* was found twice as often as *A. ochraceus* (Table 3). In addition to species in the subgenus *Circumdati*, other *Aspergillus* spp., including *A. sydowii* (Banier & Sartory) Thom & Church, *A. terreus* Thom in Thom & Church, *A. ustus* (Banier) Thom & Church, and *A. versicolor* (Vuill.) Tiraboschi, and *Eurotium* spp. were frequently found associated with pistachio litter.

Soil. *Aspergillus* fungi were very common in soil from commercial pistachio orchards (Table 4). Section *Nigri* was much more common in soil than any other section of subgenus *Circumdati*. For example, populations of section *Nigri* were 45 and 160 times greater than those of section *Flavi* in 1990 and 1991, respectively (Table 4). Sections *Flavi, Circumdati*, and *Wentii* were present in soil at approximately the same low population density (Table 4). Most of the isolates in section *Flavi* from soil were *A. flavus*, although *A. parasiticus* and *A. tamarii* were also present. *Eurotium* spp. were common in the soil in 1990 (44 cfu/g dry soil).

Disked orchards had more section *Nigri* in the soil than orchards with cover crops. In 1990, orchards that were disked regularly had 6,800 cfu/g of dry soil, whereas orchards with cover crops had only 1,200 cfu ($P = 0.001$). Similarly, in 1991, disked orchards had 2,510 cfu/g of dry soil, whereas orchards with cover crops had only 660 cfu ($P = 0.003$). There were no significant differences among sections *Flavi, Circumdati*, and *Wentii* in the density in soil from disked orchards compared with orchards with cover crops for either 1990 or 1991. Similarly, there were no significant differences in total fungal counts in soil from disked orchards and orchards with cover crops—both had 58,000 cfu/g of dry soil. Pistachio litter from disked orchards did have slightly more contamination by fungi in section *Nigri* than litter from orchards with cover crops, although these differences were never statistically significant. For example, in 1991, 35% of the fallen pistachio fruit from disked orchards were infected with

Table 3. Identification to species for isolates in *Aspergillus* subgenus *Circumdati* from pistachio litter

Section[a]	Species	Percentage of isolates[b] 1991	Percentage of isolates[b] 1992	Orchards[c] (%)
Nigri (0,123)	*A. niger*	...	97	100
	A. japonicus	...	3	60
Flavi (111,227)	*A. flavus*, strain L	66	59	100
	A. flavus, strain S	6	8	67
	A. parasiticus	17	25	100
	A. tamarii	11	8	78
Circumdati (13,144)	*A. melleus*	69	62	78
	A. ochraceus	31	38	78
Wentii (7,9)	*A. wentii*	10	100	100

[a] Numbers of isolates from 1991 and 1992, respectively, identified to species are shown in parentheses.
[b] Each section was considered separately for calculation of percentages, and data for all orchards and for both male inflorescences and pistachio fruit were grouped together for the calculations. Years refer to when isolates were obtained from pistachio debris.
[c] Percentage of commercial orchards in which species were isolated in either 1991 or 1992. Isolates were from nine commercial orchards, except those for section *Nigri*, which were from only five orchards.

Table 4. Density of *Aspergillus* fungi in soil from commercial pistachio orchards[a]

Year	*Aspergillus* (cfu/g dry soil) per section[b] *Nigri*	*Flavi*	*Circumdati*	*Wentii*
1990	4,260 ± 3,160	95 ± 156	50 ± 47	86 ± 201
1991	1,921 ± 1,146	12 ± 16	14 ± 28	...

[a] Soils were collected from eight orchards on 4 December 1990 and from six orchards on 14 November 1991. Total fungal count was 58,000 cfu/g dry soil.
[b] Values are means ± standard deviations.

section *Nigri*, whereas 27% of the fruit from orchards with cover crops were infected ($P = 0.59$). For male inflorescences in 1991, 59% from disked orchards and 44% from orchards with cover crops were infested with section *Nigri* ($P = 0.20$). In addition, there were no significant differences for sections *Flavi* and *Circumdati* in the colonization of litter of disked orchards compared with orchards with cover crops.

Aflatoxin producers. Not all of the isolates of *A. flavus* from pistachio litter produced aflatoxins, although all isolates of *A. parasiticus* did (Table 5). The two strains of *A. flavus* differed; all isolates of strain S produced aflatoxins whereas fewer than one-half of the isolates of strain L did. The different species produced different types of aflatoxins, with *A. flavus* producing only B aflatoxins and *A. parasiticus* producing both B and G aflatoxins.

DISCUSSION

Various *Aspergillus* molds heavily infested, infected, and sporulated on litter from pistachio trees, such as fallen pistachio fruit and male inflorescences, in commercial orchards (Tables 1 and 2). This pistachio litter was colonized by *Aspergillus* from late spring throughout summer. The kernels of pistachio nuts are exposed to infection by *Aspergillus* when some of the hulls and shells split in late July until harvest in September (8). Sporulation by *Aspergillus* on pistachio litter was observed before and during this period when kernels of pistachio nuts become infected in late summer. In addition, all of the *Aspergillus* spp. found in pistachio litter (Table 3) were also found in decayed pistachio kernels in commercial orchards (*unpublished*). Pistachio litter may play an important role in the infection of nuts by increasing the amount of *Aspergillus* inoculum in pistachio orchards. Although male inflorescences are produced only on the relatively few male trees present in a pistachio orchard, they can be important in increasing kernel infections by *Aspergillus* because the male trees are found uniformly throughout the orchard and the numerous inflorescences produced fall to the ground throughout late spring and summer. Since *Aspergillus* spp. are able to decay many different substrates, including plant debris (16), it is not surprising to find these molds developing in pistachio litter. However, no research has previously investigated the incidence of *Aspergillus* in litter in nut orchards. In other crops, *A. flavus* colonized decomposing peanut fruit and rye green manure in peanut fields (13) and infected corn debris such as cob and stalk pieces in corn fields (23). In general, litter in fields and orchards may be important for increasing levels of disease caused by *Aspergillus* spp. The litter from pistachio trees may be especially important because *Aspergillus* was sporulating on this litter during the period that kernels were susceptible in late summer.

Aspergillus molds infected nuts on trees and on the ground that were left in the pistachio orchards after harvest. These infected nuts along with infected litter may help the molds to survive during winter and to produce inoculum during the following spring. At present, little is known about the overwintering of *Aspergillus* in nut orchards.

Eight different *Aspergillus* spp. in the subgenus *Circumdati* were identified from isolates obtained from pistachio litter (Table 3). These species probably represent part of the typical mycobiota of pistachio orchards in California, since they were present in most orchards (Table 3). In addition, all of these species have been isolated from pistachio kernels from orchards in California (*unpublished*). *A. niger*, the most common *Aspergillus* species infecting pistachio kernels (*unpublished*), was also the most common in pistachio litter. Although other *Aspergillus* spp. occurred much less frequently, their presence is a serious problem because of their ability to produce mycotoxins, especially aflatoxins produced by *A. flavus* and *A. parasiticus* (20). In addition, *A. flavus* and *A. tamarii* can produce the toxin cyclopiazonic acid (7,20). All three of these species in section *Flavi* that produced mycotoxins were associated with pistachio litter (Tables 1, 2, and 3). *A. melleus* and *A. ochraceus* in section *Circumdati* can produce the important group of toxins and carcinogens called ochratoxins and the minor mycotoxin penicillic acid (20) and were found in pistachio litter (Tables 1, 2, and 3). If development of these toxigenic molds can be restricted in pistachio litter, for example, by burying the litter, then possibly fewer pistachio nuts will become contaminated with mycotoxins.

The two strains of *A. flavus* found in pistachio litter are quite distinct in aflatoxin production and possibly their life cycle. Strain S produces abundant small (<400 μm in diameter) sclerotia on media, whereas strain L produces a few large sclerotia (5). Among isolates from soils in Arizona, strain S produced aflatoxins more frequently and in larger quantities than strain L (5). Similarly, the isolates from pistachio litter belonging in strain S always produced aflatoxins, whereas 57% of those in strain L did not (Table 5). In addition, the life cycle of strain S may differ from that of strain L because strain S can produce abundant sclerotia under conditions that strain L cannot. For example, strain S produced abundant sclerotia on inoculated pistachio litter, whereas strain L produced no sclerotia (*unpublished*). Sclerotia could be important for overwintering of the fungus (30) and may play an important role for crops such as corn (31). Because these two strains are so distinct, they should perhaps be regarded separately in ecological studies on *A. flavus*.

Most isolates of *A. flavus* from pistachio litter did not produce detectable amounts of aflatoxins in media (detection limit of 25 ppb), although all isolates of *A. parasiticus* produced aflatoxins (Table 5). Although there are many reports that some *A. flavus* isolates do not produce aflatoxins, the exact percentage of these atoxigenic *A. flavus* isolates varies substantially. In the American Type Culture Collection, only 34–41% (depending on substrate) of the *A. flavus* and 85% of the *A. parasiticus* produced aflatoxins (29). Other studies (6,14,25) have found that 41–95% of isolates studied produced aflatoxins. In addition, different types of aflatoxins are produced by *A. flavus* and *A. parasiticus*. For isolates from pistachio litter, the G aflatoxins were produced by all *A. parasiticus* isolates but by no *A. flavus* isolates (Table 5). In other studies, none of the *A. flavus* isolates produced the G aflatoxins (5,25), although in the American Type Culture Collection, 26% of the aflatoxigenic *A. flavus* isolates produced the G aflatoxins (29).

Aspergillus fungi were frequently isolated from soils in commercial pistachio orchards in California (Table 4). Similar to the situation with pistachio litter, section *Nigri* was much more common in soils than were the other sections in subgenus *Circumdati*. In Virginia, however, populations of *A. flavus* were about the same or only slightly less dense than those of the *A. niger* group in soils from peanut, soybean, and corn fields (12). *A. flavus* and other closely related fungi in section *Flavi* occurred at low densities in the soils of commercial pistachio orchards (Table 4). Similar low levels of *A. flavus* in soil were found in Virginia

Table 5. Production of aflatoxins by isolates of *Aspergillus* species in section *Flavi* from pistachio litter[a]

Species	No. of isolates	Percentage of isolates producing: No aflatoxins	Only B aflatoxins	Both B and G aflatoxins
A. flavus strain L	173	57	43	0
A. flavus strain S	25	0	100	0
A. parasiticus	71	0	0	100

[a]Isolates were grown on glucose-yeast agar, and the presence of aflatoxins was determined by thin-layer chromatography.

in peanut, soybean, and corn fields (12). In Iowa, somewhat higher levels of *A. flavus* (396 colonies per gram of dry soil) were found in corn field soils (although these fields had been selected because of the higher levels of aflatoxins detected in the corn harvested from them) (23). Although the importance of *Aspergillus* propagules in soil in pistachio orchards is not clear, the spores in the soil could be important because they would be dispersed in dust to aerial parts of trees by such practices as disking. In fig orchards, for example, the frequency of *A. niger* in the fruit was strongly correlated with the amount of dust on the leaves (18).

Certain practices such as burying or removing litter may decrease colonization by *Aspergillus* molds of pistachio litter in the orchard. Another possible treatment for pistachio litter would be application of microorganisms that limit the development of *Aspergillus* molds. Because rains usually do not fall in California pistachio orchards during summer, irrigation may play an important role in wetting pistachio litter enough for colonization by *Aspergillus* molds. Perhaps manipulation of irrigation could limit colonization of litter by *Aspergillus*. Certainly, growers should take care to avoid creating an environment favorable for the growth and sporulation of *Aspergillus* molds on the pistachio litter because of the heavy infestation (Tables 1 and 2). Further research is needed to determine if any of these practices would reduce *Aspergillus* colonization of litter enough to decrease the numbers of moldy and mycotoxin-contaminated nuts harvested.

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