Aspergillus Species and Mycotoxins in Figs from California Orchards

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ABSTRACT

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Although 23 different *Aspergillus* spp. decayed figs in California orchards, only *A. niger* occurred in more than 0.2% of the figs. The black-spored *Aspergillus* isolates that caused the disease fig smut were classified as *A. niger* var. *niger*, *A. niger* var. *awamori*, *A. japonicus*, and *A. carbonarius*. Different fungi differed in their association with *Aspergillus* Section *Nigri* (causal agents of fig smut) infections in figs. For example, most figs infected with *Aspergillus* Section *Flavi* (potential aflatoxin producers) also had infections by Section *Nigri*. For other fungi, there was either no significant relationship between fig infections by these fungi and Section *Nigri* or simultaneous infections by Section *Nigri* were fewer than expected. Insect damage to the fig fruit, predominantly by navel orangeworm (*Amyelois transitella*), did not significantly increase the colonization of figs by *Aspergillus* spp. The incidences of infection by *Aspergillus* (Sections *Nigri*, *Aspergillus*, *Flavi*, and *Circumdati*) in figs differed little for different harvests. Figs naturally infected with *A. alliaceus*, *A. melleus*, *A. ochraceus*, and *A. sclerotiorum* of *Aspergillus* Section *Circumdati* contained ochratoxin up to 9,600 ng/g, although only 40% of the figs with these fungi had more than a trace amount of ochratoxin. Aflatoxin contamination in figs naturally infected with *Aspergillus* Section *Flavi* varied according to the species involved. No aflatoxins were detected in all figs infected with *A. tamarii* and in most figs infected with *A. flavus*. High levels of aflatoxin (>100 ng/g) were detected in 83% of the figs infected by *A. parasiticus*, but in only 32% of the figs infected by *A. flavus*. Section *Flavi* isolates from fig orchard soils were tested for their ability to produce the mycotoxins aflatoxin and cyclopiazonic acid. *Aspergillus parasiticus* isolates always produced aflatoxin but never cyclopiazonic acid; *A. flavus* strain S (producers of small sclerotia) isolates always produced both aflatoxin and cyclopiazonic acid, but strain L (producers of large sclerotia) isolates frequently did not produce aflatoxin or cyclopiazonic acid; and *A. tamarii* isolates never produced aflatoxin but always produced cyclopiazonic acid. *Aspergillus flavus* was recovered from the soil, at fewer than 6 CFU/g of dry soil of every fig orchard assayed in 1992 and 1993. Although *A. parasiticus* was rarer in fig fruit than was *A. flavus* for each year, in orchard soil *A. parasiticus* was more frequent than *A. flavus*. Isolates of *A. flavus* strain L were much more common in the orchard soil and fig fruit than those of strain S. Figs in commercial orchards seem to be a favorable substrate for infection by and growth of *Aspergillus* spp.

Additional keyword: *Eurotium* spp., *Ficus carica*

The approximately 6,000 hectares of commercial fig (*Ficus carica* L.) orchards in California provide more than 99% of the figs produced in the U.S. and two-thirds of the figs consumed in the U.S. (2). The fig fruit, an unusual type of multiple fruit known as a "syconium," has an opening, the ostiole, that allows access to the tiny flowers on the inside (21). The most popular fig cultivar, Calimyrna (60% of hectarage in California), requires the fig wasp (*Blastophaga psenes* L.) to pass through the ostiole and to pollinate the flowers. Unfortunately, because of the special fruit morphology, fungi and insects can also enter through the ostiole and develop in the interior cavity of fruit in the orchard. Other fig cultivars, such as Conadria or Black Mission (15 and 25% of hectarage, respectively), do not require the fig wasp to produce fruit, but these figs still have an open ostiole, permitting fungal colonization of the internal cavity.

Fungi in the genus *Aspergillus* grow in a wide range of substrates including many agricultural crops. *Aspergillus* spp. have been involved in nut decay of tree crops such as pistachios (20,39), almonds (47), and pecans (28). In addition, many *Aspergillus* spp. produce mycotoxins harmful to humans and animals (50). The mycotoxin that is most widely regulated by governments is aflatoxin, which is produced by *A. flavus* Link:Fr. and *A. parasiticus* Speare (61). Another mycotoxin of concern is ochratoxin, produced by *A. ochraceus* K. Wilh. and other species in *Aspergillus* Section *Circumdati* (45). In addition to the above well-known mycotoxins, many other toxic metabolites are produced by *Aspergillus* spp. (41).

Past research on decay of figs caused by *Aspergillus* spp. has focused either on the disease fig smut caused by *Aspergillus niger* Tiegh. or on aflatoxin. Fig smut has long been recognized as a problem for the California fig industry (21). Some early researchers in the 1920s and 1930s investigated fig smut but ignored *Aspergillus* spp. other than *A. niger* (16, 44, 52). Because fig smut continues to be a major problem, recent researchers have further investigated decay of figs caused by *A. niger* (38,57). Research on aflatoxin in figs has shown that aflatoxin is produced in figs inoculated with *A. flavus* (9,40), and aflatoxins have been detected in naturally infected figs from Turkey (8,51,54). In addition, in northern Africa *A. flavus* grew best after figs became ripe but before they were dried completely (7). Ochratoxin has been detected in samples of Turkish figs (43). The objectives of this research were to determine the incidences of *Aspergillus* spp. in figs from commercial California orchards, to investigate the production of aflatoxin and other mycotoxins in figs, and to detect fungi of *Aspergillus* Section *Flavi* (potential aflatoxin producers) in soil from fig orchards.

MATERIALS AND METHODS

Harvests from commercial orchards. Figs mature on the trees, dry slowly, drop onto the ground, and periodically are swept up (commercial fig orchards have multiple harvests). Dried figs were collected from the ground during harvests in commercial orchards in Fresno, Madera, and Merced counties in California. Nine and eight orchards of the fig cultivar Calimyrna were sampled in 1991 and 1992 to 1993, respectively, whereas in 1994 three orchards of the cultivar Conadria were sampled. The dates for collecting figs were 10 September and 4 October 1991, 25 August 1992, 30 August and 10 September 1993, and 19 August, 8 September, and 22 September 1994. Each collection date represented a different commercial harvest. For all collection dates, 100 figs were arbitrarily selected from each of 10 rows in each orchard, except for the second collection date in 1991 when only 50 figs were collected from each row. The figs were sliced open in the laboratory and examined with a dissecting microscope (magnification 10×) for the presence of damage by insect larvae, predominantly navel orangeworm (*Amyelois transitella*

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(Walker)), and of fungal sporulation, which was used to classify the fungus. Figs with *Aspergillus* sporulation were put aside, and the fungus present was isolated into culture for identification. Furthermore, in 1993 and 1994 every fig with a blue-green fungal colony was kept in order to avoid confusing *Eurotium* spp. with *Penicillium* spp. Each isolate was identified to *Aspergillus* section and species using a taxonomic key and species descriptions (32). Because a large number of figs were infected with Section *Nigri*, only 279 and 195 isolates in Section *Nigri* were identified to species in 1993 and 1994, respectively. Certain isolates that produced abundant orange-yellow to rufous sclerotia were tentatively classified as *A. melleus* Yukawa in Section *Circumdati* by the keys and descriptions of Raper and Fennell (48) and Christensen (10). Figs infected with Sections *Circumdati* or *Flavi* were stored at −19°C for mycotoxin analysis.

Isolations from soil. Three composite soil samples were gathered from each of eight and nine commercial Calimyrna fig orchards on 28 August 1992 and 30 August 1993, respectively. Each soil sample consisted of 30 cores (2 cm in diameter and 2 cm in depth). The soil samples were air dried, ground, passed through a 2-mm sieve, and thoroughly mixed. For each sample, 0.5 g of soil was sprinkled on the medium of each of 10 and four petri dishes for 1992 and 1993, respectively. A special soil isolation medium was developed and used (10 g of sucrose, 1 g of yeast extract, 60 g of NaCl, 10 ml of dichloran solution [0.2% dichloran in ethanol], 0.1 g of chloramphenicol, 1 ml of a 0.05% copper [$CuSO_4 \cdot 5H_2O$] and 0.1% zinc [$ZnSO_4 \cdot 7H_2O$] solution, 15 g of agar, and 1 liter of distilled water). Dichloran at this concentration was necessary to inhibit growth by *A. niger* (6). After incubation at 37°C for 4 to 5 days, the plates were examined for the presence of fungi in Section *Flavi*. Under these conditions fungal colonies of Section *Flavi* were clearly visible. Each colony of *Aspergillus* Section *Flavi* was isolated into pure culture and identified to species and strain. Isolates were stored on silica gel at 6°C for future use (59).

Mycotoxin analyses. Individual figs naturally infected by fungi in the Section *Circumdati* were analyzed for ochratoxin A by the Dried Fruit Association in Fresno, CA. Ochratoxin A was extracted from the fig using MycoSep No. 212 cleanup column (Romer Labs, Inc., Union, MO) and quantified by high-pressure liquid chromatography with a C_{18} reversed-phase column. The detection limit for ochratoxin A was 10 ng/g. In addition, two perpendicular diameters of each visible fungal colony were measured, and the mean was calculated.

Individual figs naturally infected by fungi in the Section *Flavi* were analyzed for aflatoxins according to the Romer method, as specified by the Association of Official Analytical Chemists (49,56). The extracts were derivatized with trifluoroacetic acid, and aflatoxins were quantified by high-pressure liquid chromatography with a C_{18} reversed-phase column and fluorescence detector (20). The detection limit for aflatoxins was less than 1 ng/g. Amounts of aflatoxins were calculated by summing the amounts of B_1, B_2, G_1, and G_2 aflatoxins.

Isolates in Section *Flavi* from fig orchard soil were tested for aflatoxin and cyclopiazonic acid production by growing the fungi in media and detecting the mycotoxins using thin-layer chromatography (22,23). A single piece of silica gel covered with conidia was used to inoculate glucose-yeast agar (20 g of glucose, 5 g of yeast extract, 20 g of agar, and 1 liter of distilled water) for aflatoxins and Czapek yeast agar (32) for cyclopiazonic acid. For each medium, 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 thin-layer chromatography plates (silica gel G). A more detailed description of these methods has been previously provided for aflatoxins (19) and for cyclopiazonic acid (23). The minimum concentration of aflatoxins B_1 and G_1 detected was 25 ng per g of sample.

Data analysis. In general, statistical analysis of data was descriptive. For counts, Fisher's exact test was used. In some cases, repeated measures analysis of variance was used with least significant difference (LSD) for mean separation and for an indication of the confidence interval for differences. All analyses were done with SAS (SAS Institute Inc., Cary, NC, release 6.04).

RESULTS

Harvests from commercial orchards. Although 23 different *Aspergillus* spp. had infected and sporulated in California figs, only *A. niger* occurred in more than 0.2% of the figs (Tables 1 and 2). Nine species occurred in fewer than 0.008% of the figs. The species isolated represented eleven *Aspergillus* Sections, but only Sections *Nigri* (5.05% of the figs), *Aspergillus* (*Eurotium* spp.) (0.20%), *Flavi* (0.09%), *Circumdati* (0.08%), and *Terrei* (0.06%) occurred in more than 0.02% of the figs for the years 1991 to 1994. Fungi in Section *Nigri* were isolated from figs collected from every orchard in 1991 to 1993, while fungi in Section *Flavi* occurred in figs from two, four, and six of the orchards and in Section *Circumdati* from four, two, and seven orchards in 1991, 1992, and 1993, respectively. In 1994, Sections *Nigri*, *Flavi*, and *Circumdati* occurred in figs from all three orchards. The incidence for any *Aspergillus* species was similar for each of the years with a few exceptions. In general, *Aspergillus* colonies occurred in the internal cavity of figs. In 1994, external lesions occurred with only 9.8, 11.8, and 5.6% of the figs infected with Sections

Table 1. Incidence of *Aspergillus* species in figs from commercial orchards for harvests in 1991 to 1994[a]

Aspergillus species[b]	Percentage of figs with specified fungus sporulating				
	1991	1992	1993	1994	Mean
Section *Nigri* ("smut")	4.27	6.71	3.46	5.77	5.05
Eurotium amstelodami	0.04	0.06	0.19	0.11	0.10
Aspergillus terreus	0.08	0.09	0.04	0.04	0.06
Eurotium chevalieri	0.04	0.05	0.10	0.06	0.06
Aspergillus flavus	0.02	0.05	0.04	0.06	0.04
Aspergillus ochraceus	0.05	0.03	0.05	0.01	0.04
Aspergillus tamarii	0.00	0.01	0.01	0.12	0.04
Aspergillus melleus	0.04	0.03	0.03	0.04	0.03
Eurotium repens	0.01	0.01	0.06	0.00	0.02
Eurotium rubrum	0.00	0.00	0.04	0.02	0.02
Aspergillus fumigatus	0.00	0.00	0.01	0.04	0.01
Aspergillus parasiticus	0.00	0.01	0.02	0.02	0.01

[a] The numbers of figs examined were 1,500 from each of nine orchards, 1,000 from each of eight orchards, 2,000 from each of eight orchards, and 3,000 from each of three orchards for 1991, 1992, 1993, and 1994, respectively. The fig cultivar was Calimyrna for 1991 to 1993 and Conadria for 1994.

[b] The following *Aspergillus* species were isolated from figs but occurred in fewer than 0.008% of the figs for the 4 years: *A. alliaceus*, *A. carneus*, *A. penicilloides*, *A. sclerotiorum*, *A. ustus*, *A. wentii*, *Emericella nidulans*, unidentified *Emericella* sp., and *Neosartorya fischeri*.

Table 2. Identification of black-spored *Aspergillus* fungi causing fig smut in Calimyrna and Conadria figs from commercial fig orchards[a]

Species	Smutted figs (%)	
	Calimyrna (1993)	Conadria (1994)
A. niger var. *niger*	79.9	79.0
A. niger var. *awamori*	13.3	20.5
A. japonicus var. *aculeatus*	3.6	0.5
A. japonicus var. *japonicus*	0.3	0.0
A. carbonarius	3.2	0.0

[a] The fungi from 279 smutted figs (eight orchards) and from 195 smutted figs (three orchards) were identified for 1993 and 1994, respectively.

Nigri, *Aspergillus* (*Eurotium* spp.), and *Flavi*, respectively.

The black-spored *Aspergillus* isolates from figs represented several taxa. Although *A. niger* was the most common species, two other black-spored *Aspergillus* species, *A. japonicus* and *A. carbonarius*, also decayed figs (Table 2). In addition, isolates that matched the description for *A. niger* varied in their morphology, particularly the amount of conidium ornamentation. The conidia of *A. niger* isolates ranged from having no or little ornamentation (variety *awamori*) to very prominent bars on the conidia (variety *niger*). Even within *A. niger* var. *niger*, isolates greatly varied in the size of the bars on the conidium. Both varieties of *A. niger* decayed figs in every orchard in 1993, while *A. japonicus* decayed figs in only five out of eight orchards, and *A. carbonarius* decayed figs in only three orchards. Approximately the same percentage of smutted figs were decayed by *A. niger* var. *niger* in both 1993 and 1994; however, no *A. japonicus* var. *japonicus* or *A. carbonarius* were isolated in 1994 (Table 2).

The type of association of *Aspergillus* Section *Nigri* with other taxa in figs depended on the taxa involved (Table 3). Most figs (58 to 100%, depending on the orchard) infected with *Aspergillus* Section *Flavi* also had infections by Section *Nigri*. Fig infections by *Eurotium* spp. and by dematiaceous fungi were not significantly related to infections by *Aspergillus* Section *Nigri*. For the group classified as "other fungi" (including *Cladosporium* and *Penicillium* spp.), significantly fewer simultaneous infections occurred by *Aspergillus* Section *Nigri* than expected (Table 3).

Insect damage to the fig fruit did not significantly increase the colonization of figs by *Aspergillus* spp. The percentages of figs infected by Section *Nigri* were less (not significant, $P > 0.05$) for insect-damaged figs (2.1 and 5.0% for 1993 and 1994, respectively) than for nondamaged figs (3.7 and 5.8%). For all other *Aspergillus* spp., the incidences were not significantly higher ($P > 0.05$) in insect-damaged figs (0.22 and 0.91% in 1993 and 1994, respectively) than in nondamaged ones (0.68 and 0.46%).

The incidences of infection of figs by fungi in Sections *Nigri*, *Aspergillus*, *Flavi*, and *Circumdati* differed little for the different harvests (Table 4). The only exception was the incidence of infection of Conadria figs by *Aspergillus* Section *Nigri* in 1994 when the earliest harvest had fewer infected figs than the two later harvests. In spite of infecting very few figs overall, fungi in Sections *Aspergillus*, *Flavi*, and *Circumdati* did decay figs in each harvest (Table 4).

Isolation of *Aspergillus* Section *Flavi* from soil. *Aspergillus flavus* was recovered from the soil of every fig orchard assayed in 1992 and 1993 (Table 5). Fungal densities in soil varied greatly for the various orchards. For example, *A. flavus* ranged for the different orchards from 0.2 to 2.0 and from 0.5 to 30.8 CFUs per gram dry soil in 1992 and 1993, respectively, and *A. parasiticus* ranged from 0.1 to 5.2 and from 0.0 to 9.7 in 1992 and 1993, respectively. *Aspergillus parasiticus* was rarer in fig fruit than *A. flavus* for each year (Table 1), but in orchard soil *A. parasiticus* was more frequent than *A. flavus* in 1992 and only slightly less frequent

Table 3. The expected (exp.) and observed (obs.) numbers of Conadria figs simultaneously colonized by *Aspergillus* Section *Nigri* and various groups of specified fungi in commercial orchards in 1994

	Number of figs[a]					
	Orchard A		Orchard B		Orchard C	
Fungi infecting fig	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Aspergillus Section *Flavi*	0.3	7***[b]	0.2	3***	0.2	2*
Eurotium spp.	0.1	0 NS	0.6	1 NS	0.4	3**
Dematiaceous fungi[c]	0.7	0 NS	10.0	11 NS	7.3	3 NS
Other fungi[d]	2.4	1 NS	12.3	5*	12.0	3**

[a] For each orchard, 3,000 figs were examined. The incidences of *Aspergillus* Section *Nigri* in all figs were 2.8, 7.3, and 7.2% for orchards A, B, and C, respectively. The expected values for the simultaneous colonization assumes that the colonizations by the two types of fungi are independent.
[b] NS = not significantly dependent according to Fisher's exact test ($P > 0.05$); * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$.
[c] Hyphomycetes having dark-colored spores such as *Alternaria*, *Stemphylium*, and *Ulocladium*.
[d] Any fungus that is not dematiaceous and not *Aspergillus*; for example, *Fusarium*, *Penicillium*, and *Rhizopus*.

Table 4. The effect of harvest date on colonization of figs by *Aspergillus* fungi in commercial orchards with multiple harvests[a]

		Percentage of figs infected by *Aspergillus*			
Year	Collection date	Section *Nigri*	Section *Aspergillus*	Section *Flavi*	Section *Circumdati*
1991	10 September	4.23	–[b]	–	–
	4 October	4.33	–	–	–
	LSD[c]	1.18	–	–	–
1993	2 September	3.10	0.33	0.08	0.08
	10 September	3.83	0.44	0.06	0.11
	LSD	0.78	0.31	0.12	0.11
1994	19 August	3.00	0.13	0.20	0.07
	8 September	7.53	0.13	0.20	0.10
	22 September	6.77	0.30	0.20	0.03
	LSD	3.53[d]	0.20	0.28	0.24

[a] For each orchard, 1,000 figs were examined for each harvest, except only 500 figs were examined for the second harvest in 1991. There were 9, 8, and 3 orchards for 1991, 1993, and 1994, respectively. The cultivar was Calimyrna in 1991 and 1993 and Conadria in 1994.
[b] For this collection date, data were recorded only for Section *Nigri*.
[c] Least significant difference at $P = 0.05$.
[d] Significant ($P = 0.047$). All other comparisons were not significant ($P > 0.05$). The LSD allows calculation of the confidence interval for the differences.

Table 5. Incidences, densities, and mycotoxin production for *Aspergillus* Section *Flavi* from soils collected from commercial fig orchards

	Orchards with specified fungus / orchards evaluated		Colonies per g of soil		Number of isolates producing mycotoxin / number tested[a]			
					Aflatoxin		Cyclopiazonic acid	
Species	1992	1993	1992	1993	1992	1993	1992	1993
A. flavus strain L	8/8	9/9	0.7 (0.5)[b]	5.2 (9.9)	11/22	9/21	20/22	15/21
A. flavus strain S	6/8	4/9	0.2 (0.3)	0.5 (0.8)	8/8	7/7	8/8	7/7
A. parasiticus	8/8	8/9	1.2 (1.7)	3.1 (4.1)	15/15	13/14	0/15	0/14
A. tamarii	7/8	9/9	0.4 (0.4)	1.5 (1.6)	0/12	–	12/12	–

[a] Production of mycotoxin in sterile media as determined by thin-layer chromatography. One isolate for each species and strain was randomly selected for each soil sample that had the specified fungus. The total number of samples were 27 and 24 for 1992 and 1993, respectively. Of the 22 and 21 strain L isolates tested, 11 and 9 produced both aflatoxin and cyclopiazonic acid, 9 and 6 produced only cyclopiazonic acid, and 2 and 6 did not produce these toxins for 1992 and 1993, respectively. – , isolates were not tested.
[b] Numbers in parentheses are standard deviations.

in 1993 (Table 5). *Aspergillus flavus* was more frequent in soil than *A. parasiticus* in five out of eight orchards in 1992 and in four out of nine orchards in 1993. For *A. flavus*, strain L was much more common in the orchard soil and fig fruit than strain S, which was isolated from only one fig fruit.

Mycotoxin production. Isolates in *Aspergillus* Section *Flavi* obtained from fig orchard soil differed in their ability to produce aflatoxins and cyclopiazonic acid (Table 5). *Aspergillus parasiticus* isolates almost always produced aflatoxins (both B_1 and G_1), but never produced cyclopiazonic acid (Table 5). Although *A. flavus* strain S isolates always produced both aflatoxin (never G_1) and cyclopiazonic acid, only some of the strain L isolates produced aflatoxin (never G_1) and many but not all produced cyclopiazonic acid (Table 5). *Aspergillus tamarii* isolates never produced aflatoxin but always produced cyclopiazonic acid (Table 5).

All four species in *Aspergillus* Section *Circumdati* that decayed figs produced ochratoxin in naturally infected figs from commercial orchards (Table 6). The amount of ochratoxin A detected varied greatly from none to 9,600 ng/g. Only 40% of the naturally infected figs had more than a trace amount of ochratoxin. Some figs infected with *A. melleus* or *A. ochraceus* had no ochratoxin even though large fungal colonies were present (for example, figs #5 and 13 in Table 6).

Aflatoxin contamination in figs naturally infected with *Aspergillus* Section *Flavi* varied according to the species involved (Table 7). No aflatoxins were detected in any of the figs infected with *A. tamarii* nor in most figs infected with *A. flavus*. High levels of aflatoxin (>100 ng/g) were detected in only 6 of 19 figs infected by *A. flavus*, but in 5 of 6 figs infected by *A. parasiticus*. All figs infected with *A. parasiticus* had both B and G aflatoxins, whereas the G aflatoxins were never detected in figs infected with *A. flavus*. None of the figs infected with *A. flavus* had more than 10,000 ng/g aflatoxins, whereas 3 of the 6 figs infected with *A. parasiticus* did (one had more than 77,000 ng/g).

Table 6. Production of ochratoxin A in Calimyrna figs naturally infected with *Aspergillus* Section *Circumdati* from commercial orchards

Fig No.	Aspergillus species present	Colony diameter (mm)[a]	Ochratoxin (ng/g)
1	*A. alliaceus*	10	9,600
2	*A. melleus*	10	130
3	*A. melleus*	18	Trace[b]
4	*A. melleus*	8	Trace
5	*A. melleus*	15	None
6	*A. melleus*	8	None
7	*A. ochraceus*	21	1,380
8	*A. ochraceus*	5	40
9	*A. ochraceus*	1	19
10	*A. ochraceus*	9	Trace
11	*A. ochraceus*	6	Trace
12	*A. ochraceus*	6	Trace
13	*A. ochraceus*	22	None
14	*A. ochraceus*	2	None
15	*A. sclerotiorum*	11	108

[a] The colony diameter was calculated as the mean of two perpendicular diameters of the fungal colony in the fig.
[b] Less than 10 ng/g.

DISCUSSION

The isolation of 23 *Aspergillus* species from decayed figs in California orchards (Tables 1 and 2) was not unexpected, because many *Aspergillus* species have been associated with other tree crops. For example, 14 *Aspergillus* species decayed pistachio nuts from California orchards (20), while 17 species contaminated almond kernels from a California processing plant (31). In addition, more than 12 *Aspergillus* species were found in pecans in Georgia (28) and in pistachio nuts from Iran (39).

Aspergillus niger was the most common *Aspergillus* species decaying figs in California (Tables 1 and 2). This was expected because *A. niger* causes fig smut (44), which has long been a problem for the California fig industry (21). In California, two other species of black-spored *Aspergillus* spp. decayed figs and could be considered causal agents of fig smut, even though both species were relatively rare (Table 2). This is the first record of *A. japonicus* and *A. carbonarius* decaying figs, although both also decayed pistachio nuts in California (20). *Aspergillus niger* might infect more figs during summer than *A. carbonarius* and *A. japonicus* because *A. niger* grows faster at high temperatures (37°C) than *A. carbonarius* and *A. japonicus* (32). Unlike the rarely found *A. carbonarius* and *A. japonicus*, both varieties of *A. niger* commonly decayed figs (Table 2). Previously, these two varieties, *A. niger* var. *niger* and *A. niger* var. *awamori*, were considered distinct species (48). It is not clear how these two varieties of *A. niger* differ in their epidemiology in fig orchards, but differences in conidium wall type and thickness that distinguish these two varieties should affect spore germination, survival, and dispersal.

Different groups of fungi differed in their association with *Aspergillus* Section *Nigri* in figs (Table 3). Substantially more fig infections by *Aspergillus* Section *Flavi* occurred with infections by Section *Nigri* than expected (Table 3). Similarly, more corn kernels were infected by both *A. flavus* and *A. niger* than would be expected if colonization were independent (26). Section *Flavi* could be associated with Section *Nigri* because these fungi develop similarly under similar conditions (4). Fewer figs than expected were infected with both *Aspergillus* Section *Nigri* and the category of "other fungi" (Table 3), which could result from competition between the two groups of fungi.

Section *Aspergillus* (*Eurotium* spp.) was the second most common decayer of figs in California (Table 1). These fungi are "universally distributed in nature" and decay many substrates, particularly those with high osmotic pressure (48). Besides frequently decaying figs (Table 1), *Eurotium* spp. were associated with almonds (31,47), pecans (28), pistachios (20), prunes (46,58), and various kinds of other nuts (29,62). However, *Eurotium* spp. were much more common in figs in California orchards (Table 1) than in pistachio nuts in nearby pistachio orchards (20). *Eurotium* spp. can grow in substrates at lower moisture activities than almost all other *Aspergillus* spp. including *A. niger* and *A. flavus* (4), which might explain why these fungi are very common on dried fruits such as figs (Table 1) or dried prunes (46, 58). Figs in California might be an especially favorable substrate for *Eurotium* spp. because the figs are allowed to dry slowly in the orchard prior to harvest. *Eurotium amstelodami* might be the most common *Eurotium* species occurring in figs during the summer in California (Table 1) because it can grow at higher temperatures than the other *Eurotium* spp. present (4,32).

Insect damage did not predispose fig fruit to infection by either *A. niger* or other *Aspergillus* fungi. This is contrary to what was found for pistachios (20), corn (37), and cotton (3). Insect damage might not increase colonization of figs by *Aspergillus* spp. because figs lack a physical barrier that needs to be penetrated before fungal infection occurs. However, insects could still play an important role in transmitting spores leading to *Aspergillus* colonization of figs. Mites and thrips

Table 7. Amount of aflatoxins in figs naturally infected with *Aspergillus* Section *Flavi* from commercial orchards in 1991 to 1994

		Number of figs with specified amount of aflatoxins (ng/g)			
Aspergillus species present	No. of figs analyzed	0	1 to 100	101 to 1,000	>1,000[a]
A. flavus	19	13	0	4	2
A. parasiticus	6	0	1	2	3
A. tamarii	6	6	0	0	0

[a] Two figs infected by *A. flavus* had 9,600 and 1,800 ng/g aflatoxin, while three figs infected by *A. parasiticus* had 77,200, 24,100, and 17,900 ng/g, respectively.

might act as vectors for *A. niger* (25), but insects such as dried-fruit beetles (*Carpophilus* spp.) or ants do not (16).

Colonization of figs by *Aspergillus* spp. differed little for the different harvests (Table 4). However, for other crops such as corn, cottonseed, pecans, and pistachios, late harvests resulted in substantially higher incidences of *A. flavus* and in higher levels of aflatoxin contamination (13,30, 53,63). Because figs dry down in the orchard to moisture contents below which *Aspergillus* spp. can grow, delaying harvest should not increase colonization of figs by *Aspergillus* spp.

Four species in *Aspergillus* Section *Circumdati* (*A. ochraceus*, *A. melleus*, *A. alliaceus*, and *A. sclerotiorum*) decayed figs in California, but at very low incidences (<0.05% of the figs) (Table 1). However, even at low incidences the presence of these fungi can be important, because many species in *Aspergillus* Section *Circumdati* produce two mycotoxins, ochratoxin A and penicillic acid (11). Ochratoxin A, primarily a nephrotoxin (55), has been regulated by some countries (61). Natural infections of figs from California orchards by all four species in Section *Circumdati* resulted in ochratoxin contamination (Table 6). However, in some figs these fungi grew but did not produce ochratoxin (Table 6), which could be because of the inability of the strain involved to produce ochratoxin. In several studies, most of the *A. ochraceus* isolates did not produce ochratoxin (11,15,29). Another explanation for fungal growth without ochratoxin production is that at certain temperatures and moisture contents *A. ochraceus* can grow without producing ochratoxin (42). Table 6 gives some indication of the amount of ochratoxin contamination of figs at harvest. However, more ochratoxin might be present in figs than our study indicated because *Penicillium* spp. can also produce ochratoxin (55). Ochratoxin was detected in samples of Turkish figs, but less frequently and at lower levels than aflatoxin (43).

Fungi in *Aspergillus* Section *Flavi* are very important because of the mycotoxins they produce. Aflatoxins, the most widely regulated mycotoxins (61), are produced by three members of Section *Flavi*, *A. flavus*, *A. parasiticus*, and *A. nomius* (14). Both *A. flavus* and *A. parasiticus* decayed figs in California, although at very low incidences (Table 1). In our study, *A. nomius* was never isolated from figs or from soil of fig orchards. *Aspergillus flavus* decayed more figs from California orchards than *A. parasiticus* (Table 1), which was also true for decay of pistachio nuts in California (20).

The amount of aflatoxin in individual figs infected by *Aspergillus* Section *Flavi* varied greatly for different figs (Table 7). Most of the figs naturally infected with *A. flavus* did not contain aflatoxin (Table 7), probably because many *A. flavus* isolates in fig orchards could not produce aflatoxins (Table 5). There are several reports that many *A. flavus* isolates do not produce aflatoxins (12,19). Even though *A. parasiticus* is much less frequent in figs than *A. flavus* (Table 1), *A. parasiticus* is still very important for the aflatoxin problem in figs, because of the frequent occurrence and high level of aflatoxin produced (Table 7). Another factor affecting the amount of aflatoxin is that simultaneous infection by aflatoxigenic fungi and other fungi such as *A. niger* can result in decreased aflatoxin levels (64). Most figs colonized by *Aspergillus* Section *Flavi* were also infected with Section *Nigri* (Table 3). Yet another cause of variability in aflatoxin contamination of figs is that at certain temperatures and moisture contents *A. flavus* and *A. parasiticus* can grow without producing aflatoxins (42).

Strains L and S of *A. flavus*, which are morphologically and genetically distinct (5), differ in their aflatoxin production (12). For isolates from fig orchards (Table 5) and from pistachio orchards (19), the S strain, characterized by the production of many small sclerotia, always produced aflatoxins, whereas many isolates of strain L did not produce aflatoxin. Similar to the case in cotton fields (12) and pistachio orchards (19), the L strain is more common in fig orchards than the S strain (Table 5).

Cyclopiazonic acid is a toxin produced by *A. flavus* (24) and *A. tamarii* (17) but not *A. parasiticus* (18). Most of the isolates of *A. flavus* strain L from fig orchards and all those of strain S produced cyclopiazonic acid (Table 5). Previous studies using isolates from other crops and regions have found that not all *A. flavus* isolates produced cyclopiazonic acid, but the studies differ in the actual percentage of isolates producing the toxin from 7 to 74% (15,24,35,36,60). None of the strain L isolates in our study produced aflatoxin without producing cyclopiazonic acid, which agreed with the results of one study (36), but in another study 7% of the isolates produced only aflatoxin (24). Although many *A. flavus* isolates in our study produced both aflatoxins and cyclopiazonic acid, many other isolates produced only cyclopiazonic acid, a result similar to that found in other studies (24, 36). All of the *A. tamarii* isolates from fig orchards produced cyclopiazonic acid (Table 5). In a previous study, 96% of the *A. tamarii* isolates produced cyclopiazonic acid (17).

Although *Aspergillus* Section *Flavi* occurred in the soil of every fig orchard tested, the actual densities were quite low (Table 5). Slightly higher densities were observed in pistachio orchard soil in California (19). Very high densities of *A. flavus* occurred in cotton field soil in Arizona (34) and in peanut and corn fields in Georgia (27). The levels for *A. parasiticus* in fig orchard soil were very similar to those for *A. flavus* (Table 5), which was unexpected because *A. parasiticus* was much less common in fig fruit than *A. flavus* (Table 1). Similarly, the level of *A. parasiticus* was higher in soil for corn fields than would be expected from levels present in the crop (1,27). Although the importance of *Aspergillus* Section *Flavi* propagules in the soil of fig orchards is not clear, the conidia might play a role by being dispersed to aerial parts of the trees. The frequency of *A. niger* in the fig fruit was strongly correlated with the amount of soil dust accumulated on leaves (38).

Even though toxigenic fungi in Sections *Flavi* and *Circumdati* decayed figs in California, their frequency was extremely low (Table 1). Because these species frequently grew in figs without producing mycotoxins (Tables 5, 6, and 7), the frequency of mycotoxin contamination was substantially lower than that for fungal infections. Nevertheless, occasionally an individual infected fig had very high toxin levels. For example, one fig infected with *A. alliaceus* had 9,600 ng/g ochratoxin A (Table 6) and several figs had greater than 1,000 ng/g aflatoxin (Table 7). This is a serious problem because if just one of these figs with high mycotoxin levels contaminated a sample, detectable levels of mycotoxin would be found in that sample. Thus, even though the frequency of mycotoxin contamination is very low, there is still a desire to reduce mycotoxin contamination. Many strategies have been suggested for decreasing aflatoxin contamination of dried figs (33), although it is not clear which of these strategies could be useful in California. Our study investigated mycotoxin contamination in the fig orchards at harvest. Because many contaminated figs might be removed during processing, the mycotoxin levels in processed figs from California should be less. Current research is focusing on improving methods for removing contaminated figs during processing.

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