Distribution of *Aspergillus flavus* and Other Fungi in Several Almond-Growing Areas of California

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ABSTRACT

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Fungi were isolated from almond fruits grown in various parts of the Central Valley of California and were identified as members of the following groups: *Aspergillus flavus* (AF), *A. niger* (AN), *A. wentii* (AW), *A. glaucus* (AG), *A. ochraceus* (AO), *Penicillium* (PN), *Alternaria* (ALT), *Rhizopus* (RN). Samples were collected 1 wk before normal harvest, 1 wk after normal harvest, and after 2.5 mo of storage. *A. flavus* (AF) and AN were found most frequently and ALT least frequently from fruits in the warmer region (Bakersfield) in contrast to the cooler areas (Chowchilla and Snelling). The ALT and PN groups occurred more frequently and AF, AN, and AG less frequently before harvest than after harvest. The AF was found more frequently on samples taken from sunny locations in the orchard than from samples taken from shaded locations.

Additional key words: Prunus dulcis, aflatoxin, mycotoxin.

Almond orchards in the Central Valley of California produce most of the almonds grown in the United States. The Central Valley extends from about 40° N to 35° N latitude, and includes a range of temperature and moisture regimes (18). Within this valley *Aspergillus flavus* Link and *A. parasiticus* Speare, which produce highly carcinogenic aflatoxins, are found among the fungi isolated from the almond hull and kernel (7,9,10,14). Aflatoxins have been found in 36 of 557 almond samples taken from 1970 to 1974 (16).

Many factors, including temperature and moisture, influence the occurrence and distribution of *A. flavus* and *A. parasiticus* on crops in the field and in storage (3,4). *A. flavus* and *A. parasiticus* are relatively heat tolerant. Their cardinal growth temperatures are: optimum 36–38 C, maximum 44–46 C, and minimum 6–8 C (15). A laboratory study showed that *A. flavus* infected cotton bolls most rapidly at 35 C (1). Field studies showed that the high mean temperatures of a geographic area correlated with a high occurrence of cotton boll rot caused by *A. flavus* in California (6,8). The results of other studies also have generally demonstrated the association of *A. flavus* with high temperatures (13).

Isolates of *A. flavus* and *A. parasiticus* grow at relative humidities ranging from 78 to 100% (2), conditions which occur in or around the kernel while the almond fruit dries on the tree (10). Optimum growth occurs at relative humidities greater than 98% (2). Other fungi, such as *Ulocladium chartarum* (Pr.) Simmons, may interfere with the colonization of the almond fruit by *A. flavus* and *A. parasiticus* (11), but it is not clear whether this is primarily due to antagonism or to the physical or chemical environment of the hull.

In this study, we relate the occurrence of *A. flavus* and *A. parasiticus* and other fungi colonizing almond fruit in relation to orchard location within the Central Valley of California, the effect of exposure to sun while drying, and time of sampling after harvest.

MATERIALS AND METHODS

Isolation of fungi. Surface-disinfested and nondisinfested kernels and hulls were tested for the presence of *A. flavus, A. parasiticus,* and other fungi colonizing the almond fruit. Samples were surface-disinfested by dipping them in 70% (v/v) ethanol/water for 10 sec, then placing them in 0.5% sodium hypochlorite solution for 5 min. Samples were transferred to plates of malt salt medium containing 2% malt extract, 7.5% NaCl, 2% agar and 13 μg/ml 2, 6-dichloro-4-nitroaniline; the latter was added to inhibit growth of *Rhizopus.* Sections (one-fourth of the hull) from five hulls with each section including a ventral edge at the suture, or five whole kernels were placed on each plate. The fungal colonies that grew from the samples were counted after the samples had been incubated for 7 days at 30 C. Because of the large number of samples, we subdivided the fungi into "groups" based on colony color, morphology, and typical growth characteristics. The *A. flavus* group (AF) included *A. flavus* and *A. parasiticus,* whereas other *Aspergillus* species were classified into groups as proposed by Raper and Fennel (12). These groups were *A. niger* (AN), *A. glaucus* (AG), *A. wentii* (AW) and *A. ochraceus* (AO). Other groups included *Penicillium* spp. (PN), *Rhizopus* spp. (RN), and *Alternaria* spp. and *Ulocladium* spp. both in ALT.

Samples of commercial almonds. During 1974, samples (200 shelled almonds each) of cultivar Nonpareil were obtained from 89

orchards located throughout the Central Valley of California. Half of each sample was disinfested, and both disinfested and nondisinfested almonds were analyzed for the presence of AF. For statistical purposes, the sampling area was divided into six regions, region 1 being the most northerly (Fig. 1). The frequencies of AF in these regions were compared by analysis of variance.

Samples from regional field plots. Mean summer temperatures gradually increase toward the northern and southern ends of the Central Valley of California. Because differences in source area significantly affected the occurrence of fungi on almonds in 1974, the following three specific regions were studied in 1976 and 1977: Bakersfield, located in the southern, hotter end of the valley; Chowchilla, intermediate in location and temperature; and Snelling, located in the Sierra Nevada foothills. Conditions in the Snelling area are similar to those in the cooler growing areas of the Central Valley. During July and August, the average maximum temperatures for the Bakersfield, Chowchilla, and Snelling regions are 38, 36, and 34 C, respectively; minimum temperatures vary correspondingly with about a 5 C difference between regions (18).

Three orchards were selected in each region and a plot of 8 – 10 Nonpareil trees were sampled within each orchard. Samples of fruit for isolating fungi were taken from sites where fruit was exposed to the sun much of the day and from sites where fruit was shaded most of the day. One hundred hulls and kernels each from the sun and shade from each of the three orchards within each region were analyzed for fungi at three times: after hull-split (about 1 wk before normal commercial harvest), after a normal harvest and holding 1 wk on the ground in the sun or shade, and after a normal harvest with holding 1 wk on the ground and 2.5 mo storage in open plastic cans in an enclosed building at ambient temperatures. Almond fruits were fumigated 48 hr with 0.02 g of hydrogen phosphide per cubic meter prior to storage.

Data were subjected to analysis of variance, and significance ($P = 0.05$) level is reported.

RESULTS

Samples from commercial almonds. The area in which the almonds were grown significantly influenced the occurrence of AF. In 1974, AF was found less frequently in samples in the central area 3, and most frequently in the southern area 6. Generally, the incidence of AF was lowest in the northern four areas of the Central Valley and increased at the warmer south end (Fresno and Bakersfield). There was a significant correlation (r = .90) between the average temperature of an area and the incidence of AF (Table 1).

Samples from orchard plots. *Influence of region.* Overall, AF was isolated with a significantly higher frequency in the Bakersfield region than in the Snelling region in both 1976 and 1977 (Fig. 2 A, B).

AN, like AF, had the lowest incidence in the Snelling region in one or both years. In contrast, the incidence of ALT and RN was least frequent on samples from the Bakersfield region.

The highest frequency of AO and AW occurred in the Chowchilla region, less frequently in Bakersfield and Snelling. The PN and AG showed less consistent trends.

Influence of sampling time. With each subsequent sampling, AF generally increased. The means for non-surface disinfested hulls for 1976 and 1977 was 12% for samples taken from the tree after hull split, 21% for samples taken after drying on the ground, and 26% for dry samples taken after 2.5 mo in storage. Similarily, AF on non-surface disinfested kernels averaged 7% from tree samples, 16% from ground samples, and 26% from stored samples. The incidence of AF on surface disinfested hulls and kernels was less than 1% at

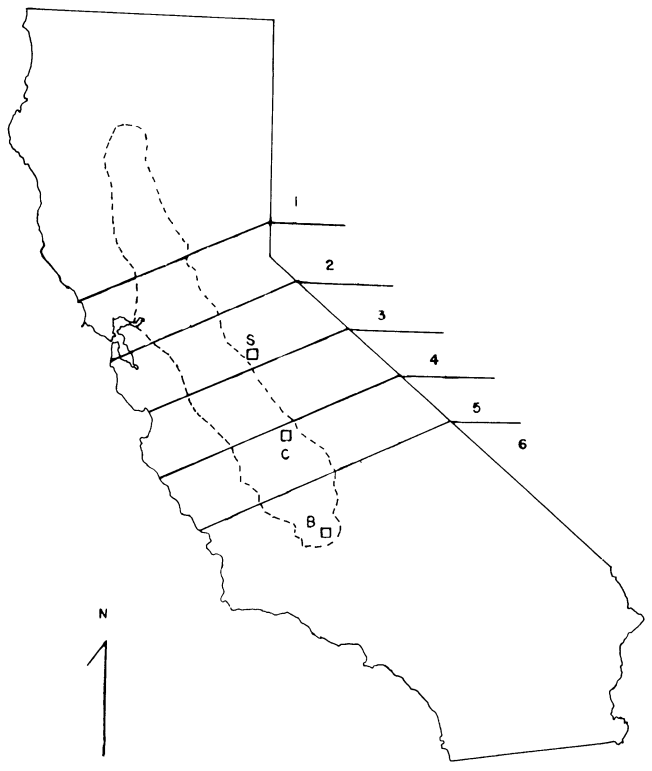

Fig. 1. A map of California showing the zones used in 1974 for the determination of area effects on the occurrence of *Aspergillus flavus* on almonds. The Central Valley of California (dotted line) was divided into six areas as shown on the map: 1, Colusa; 2, Sacramento; 3, Madera; 4, Chowchilla; 5, Fresno; and 6, Bakersfield. The squares (□) indicate the location of the regional field plots studied in 1976 and 1977: Snelling (S), Chowchilla (C), and Bakersfield (B).

TABLE 1. The incidence of *Aspergillus flavus* group (AF) fungi in commercial lots of almond kernels taken from 89 orchards throughout the Central Valley of California

Zone location[a]	Orchards sampled (no.)	Kernels with *Aspergillus flavus*[b]		Least squares mean[d] of all samples	Standard error	Temperature for area[e]
		Surface[c] disinfested (%)	Non-disinfested (%)			
Colusa	15	0.3	53	25	2.0	24.9
Sacramento	7	0.2	46	21	2.9	24.4
Madera	15	0.1	42	22	2.2	25.0
Chowchilla	27	0.3	54	25	1.7	25.8
Fresno	16	0.4	69	32	2.2	26.7
Bakersfield	9	1.2	71	36	3.5	28.6

[a] Zone locations given in Fig. 1.
[b] Each datum represents the average for all the total orchards sampled from within the location. Isolations were made from 100 nondisinfested kernels from each orchard.
[c] Kernels were dipped in 70% ethanol for 10 sec and then into 0.5% sodium hypochlorite solution for 5 min.
[d] Data transformed by arc sin $\sqrt{x}$, in which x = proportion of sample with *A. flavus*.
[e] Average daily temperatures from representative locations within area for July and August 1974.

all sampling times.

AN and AG were less frequently found on nuts collected from the tree than on those from the ground or from storage (Fig. 2C, D).

The incidence of ALT and PN groups, unlike the AF, AN, and AG, occurred least frequently in stored samples. The incidence of PN was significantly lower on non-surface disinfested kernels in storage, than in samples taken after drying on the soil. The ALT group decreased significantly in storage compared to samples from the tree.

AO, AW, and RN groups occurred with only slightly different or inconsistent frequencies at each sampling.

Influence of exposure to the sun while drying. AF was isolated more frequently from almonds taken from sunny sites than from those taken from shaded sites (Fig. 3). Of the other fungi studied ALT showed a preference for sun, and RN and AO a preference for shade.

DISCUSSION

We found that the incidence of AF and other fungi on almonds changes as the almonds dry on the tree after hull split, dry further on the ground, and as they are held in storage. Generally, as the nuts dried, the incidence of AF increased. Our study with almond, and studies with cotton bolls (1,6,8,11), peanuts, and corn (13) have shown a positive association between high temperatures and a high incidence of AF. This is supported by our observations that: AF occurred more frequently on almonds taken from the soil surface than on those taken from the tree; AF occurred more frequently on almonds from orchards in an area with high average day and night temperatures than on those from orchards in areas with lower average day and night temperatures, and AF occurred more frequently in those samples taken from sites in the sun than in samples taken from sites in the shade.

We could not separate the effects of heat from the effects of moisture on the occurrence of fungi in the field. Rapid drying of a commodity is generally considered to limit the growth of AF and other fungi (3). However, rapid drying of a commodity at high temperature may, in fact, favor for a time the growth of thermophilic fungi (5).

The presence of certain microorganisms, particularly at cool temperatures, may tend to reduce the occurrence of *A. parasiticus* (AF) (17). More specifically it has been demonstrated that *U. chartarum* may compete, or in some way act as an antagonist to AF on almonds (11). In our study we found that the incidence of certain organisms corresponds with that of AF. The AN especially showed a regional and sampling similarity with AF. By contrast, certain

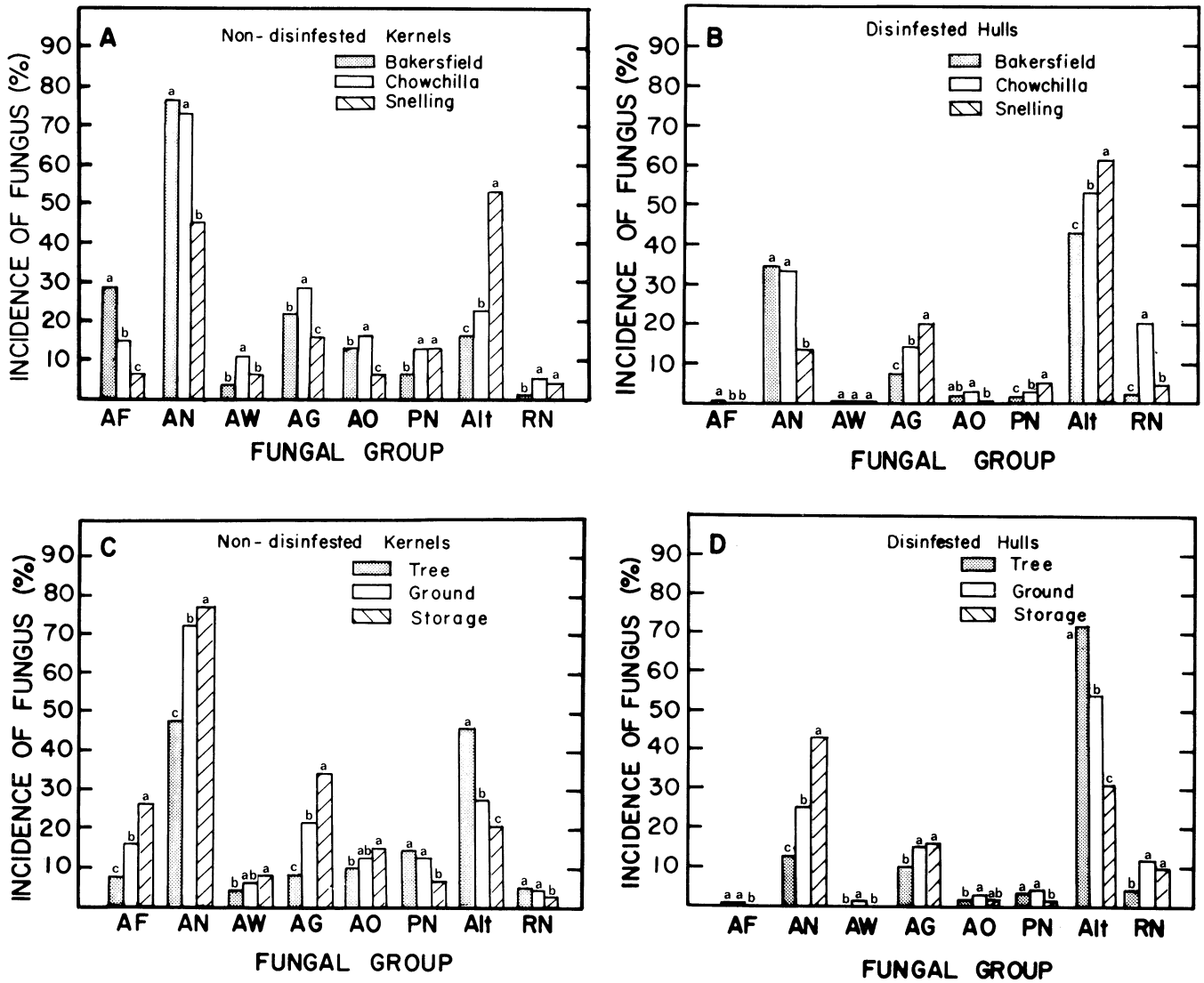

Fig. 2. The incidence of fungi detected on surface-disinfested or nondisinfested almond hulls and kernels in 1976 and 1977. **A, B:** graphs representing typical results from plots in the vicinity of Bakersfield, Chowchilla, or Snelling, California. **C, D:** graphs representing samples taken 1 wk prior to usual commercial harvest (tree); after a normal harvest, holding 1 wk on the ground (ground); and after a normal harvest, holding 1 wk on the ground, and for 2.5 mo of dry storage (storage). Abbreviations for fungal group are: AF = *Aspergillus flavus,* AN = *A. niger,* AW = *A. wentii,* AG = *A. glaucus,* AO = *A. ochraceus,* PN = *Penicillium,* Alt = *Alternaria,* and RN = *Rhizopus.* Mean shown for a fungal group with a letter in common are not statistically different, $P = 0.05$.

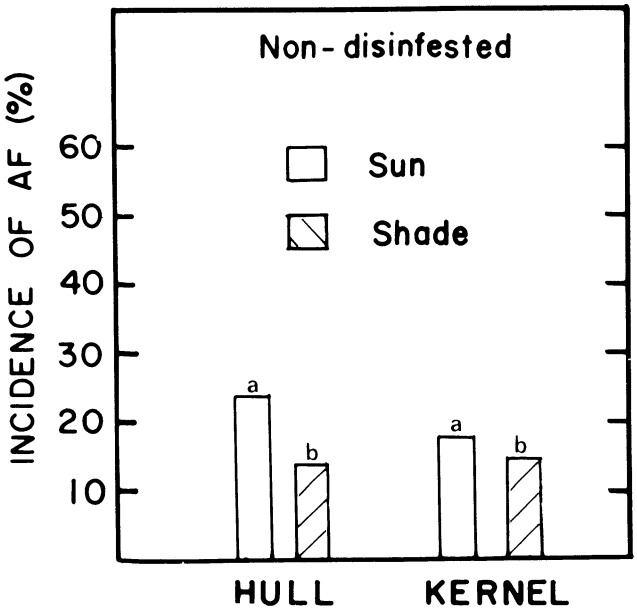

Fig. 3. The incidence of *Aspergillus flavus* or *A. parasiticus* (AF) on nondisinfested almond hulls and kernels taken from sunny or shaded sites within the orchard. Mean shown for a hull or kernel with a letter in common are not statistically different, $P = 0.05$.

organisms were found to correspond negatively with AF. The ALT group showed a negative association with AF regionally and along with PN had a negative association at the various sampling times.

Results of our studies showed that during or after the drying of almonds, AF has a high probability of being one of the most prevalent fungi, particularly on almonds from the warmer growing areas. Thus, wetting of the almonds after drying, when few antagonists are present could greatly stimulate growth of AF and result in substantial aflatoxin production. We suggest that avoidance of high drying temperatures and re-wetting of almonds will reduce the danger of aflatoxin contamination.

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