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1975 ANNUAL REPORT

TITLE: Aflatoxin Research - Field and Storage
Section A - Field. Colonization of almond by Aspergillus flavus and other fungi.

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I. OBJECTIVES AND GOALS:

We are studying factors that contribute to or influence the occurrence of Aspergillus flavus and aflatoxins in almond hulls, shells and kernels. Factors that have been associated with Aspergillus flavus are then studied experimentally to examine the possibility of using these factors to reduce the potential hazard of fungi on almonds.

II. ABSTRACT:

Many fungi colonize the hull and kernel of the almond fruit while on the tree. Aspergillus flavus is one of these fungi. Aspergillus flavus and aflatoxin are more frequently associated with insect-damaged kernels than ~~in~~ sound kernels. However, under some conditions, sound kernels can be colonized by this fungus without insect damage.

A 1974 survey found Aspergillus flavus more frequently in the southern one-third than in the center one-third of the almond-growing area of California. The survey also suggested that the temperature of the almond while drying, and the presence or absence of other fungi on the hull or kernel, influenced the occurrence of Aspergillus flavus.

In 1975, field experiments evaluated the effect of other potentially antagonistic fungi on Aspergillus flavus colonization of the almond kernels. Groups of fungi that normally colonize the hull soon after hull split, were identified as successful competitors of Aspergillus flavus on the almond hull. These fungi reduced colonization of the kernel by Aspergillus flavus when placed on the hull with Aspergillus flavus.

A second 1975 plot tested the effect of high temperature or sunburn on A. flavus colonization. Although inconclusive, the results suggest that more colonization and toxin production occur in nuts that are dried under excessively high temperatures than in those dried at lower temperatures.

III. EXPERIMENTAL PROCEDURE:

Three almond cultivars, Milo, Nonpareil, and Neplus were utilized for 2 experimental field plots, in an orchard near Fresno, Calif.

Antagonism Studies:--Seven treatments were applied to fifteen trees of each cultivar. Each treatment was applied to two fruits on each tree. The treatment consisted of inoculating the hull with Aspergillus flavus alone, or A. flavus and other fungi that had been previously isolated from almond hulls. The treatments were: A. flavus (AF) alone, AF plus group A, fungi that colonized the hull early in the season; AF plus mid-season colonizers, group B; AF plus late colonizers, group C; and AF plus the latest fungal colonizer of the hull, group D. The treatments also included an untreated control and a washed only control.

Before inoculation, the fruit was washed with 100-50 ppm chlorine ($Cl\ O^-$). When dry, the fruit was inoculated with fungi other than A. flavus by taping to the fruit bits of straw colonized by the various fungi. The fruit was then inoculated with spores of 4 toxiogenic isolates of A. flavus by blowing spores onto the fruit after it was enclosed in a cloth-filter bag. The filter was used to prevent contamination of other fruit in the orchard.

The treated fruits were cut from the trees at harvest time and analyzed for A. flavus.

Analysis for Aspergillus flavus:--The hulls and kernels were separated in the laboratory, surface disinfested and tested for the presence of Aspergillus flavus. For surface disinfestation, samples were dipped in 70% ethanol for 10 seconds, and then immediately soaked in .5% sodium hypochlorite solution for 5 minutes. Without further washing, samples were then aseptically placed on plates of malt salt medium containing 7.5% sodium chloride, 2% malt extract and 2% agar. After one week at 30°C the area on each hull where A. flavus was found was recorded. Only the occurrence of A. flavus on each kernel was recorded.

Analysis for Aflatoxin:--Aflatoxins were analyzed by George Stanley in the Fresno Dried Fruit laboratory, using a milicolumn screening procedure and thin-layer chromotography.

Sunburn test:--A second experiment used four Neplus trees. Four 5-nut samples were selected from each tree, washed and covered with a bag. The nuts in two of the bags on each tree were inoculated as previously described with spores of Aspergillus flavus. At harvest time, the bagged nuts were taken from the tree, the bags opened and the nuts were placed on the soil for one week to dry. The samples were positioned so that inoculated and non-inoculated fruits were

placed in the sun and in the shade. Kernel temperatures were measured periodically with a thermocouple to determine the temperature of near-by kernels on the soil in sun or shade.

IV. RESULTS:

Antagonism Studies:--(Results from Nonpareil only). The hull was colonized by all groups of test fungi. Alone, A. flavus colonized 74% of the inoculated hull where it was placed. When other fungi were present on the hull A. flavus colonization was reduced significantly (table 1). Fungus groups D (latest), A (early), C (late) and B (mid-season) reduced A. flavus to 28%, 39%, 44% and 47%, respectively.

Twenty-three percent of the kernels were colonized by A. flavus, when no other fungus was inoculated onto the hull. Group D (latest) did not affect kernel colonization but groups A and B reduced colonization to 7% of the kernels and group C to 10% (table 1).

Sunburn test:--Hulls inoculated with A. flavus contained aflatoxin whether dried in the sun or shade. More toxin was found in the 5 samples of sun-dried hulls than in shade-dried hulls; and aflatoxin was found in the kernels of sun-dried almonds (table 2).

V. DISCUSSION:

Antagonism Studies:--We have verified that other groups of fungi found on the almond hull affect the growth of A. flavus on almonds. Some fungi antagonize A. flavus and reduce its development on the kernel, others appear to have no effect or may encourage A. flavus. Natural colonization of the almond hull by other fungi minimizes the occurrence of A. flavus. Techniques that may alter this colonization in the orchard, such as fungicide sprays, may increase or decrease the occurrence of A. flavus. Controlled inoculation of almond hulls with fungi antagonistic to A. flavus and other undesirable fungi, may offer a new avenue for disease control. The work with antagonistic fungi should be expanded to identify single fungi or groups of fungi that can effectively colonize almond hulls safely. The feasibility of applying antagonistic fungi to large plots should be explored.

Sunburn tests:--High temperature may result in damage leading to 1) colonization of nuts by A. flavus and aflatoxin production; and 2) a general deterioration of kernel quality. Tests should compare the quality of kernels that are dried in the shade with those dried in the sun. These tests should include the occurrence of aflatoxin, the development of rancidity, and general viability of the kernel.

Table 1.--*Aspergillus flavus* colonization of Nonpareil almond hulls and kernels when other potentially antagonistic fungi are present on the hull.[1]

Fungus inoculated on the hull →	A. flavus plus fungal group				
	A	B	C	D	No other Fungus
Percent of each hull colonized by *A. flavus*	39ab[2]	47b	44ab	28a	74c
Percent of kernels in the sample colonized by *A. flavus*	7a	7a	10ab	27c	23bc

1. *A. flavus* was not found in non-inoculated controls.

2. Hull or kernel means within a row without a letter in common differ at the 90% confidence level.

Table 2.--Aflatoxins in Nonpareil Almond kernels (in hull) after drying on soil in the sun or shade.

Drying and inoculation treatments	Aflatoxin (ppb) Hull	Kernel	Total aflatoxin (µg) in hulls and kernels	Highest temperatur recorded (°F)
In sun (control)	0	0	0	127
In shade (control)	0	0	0	88
In sun + *A. flavus*	126	32	155	127
In shade + *A. flavus*	107	0	89	88

Some citations on the occurrence of microorganisms on almonds.

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JMS 1853

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Colonization of Almond by *Aspergillus flavus*[1]

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Abstract. The *Aspergillus flavus* group was associated with both sound and insect damaged kernels of almond *Prunus dulcis* (Mill.) D.A. Webb during 1972 and 1973. About 1 of 2,000 sound kernels and 1 of 200 insect damaged kernels were colonized. Surface contamination was common on sound and damaged kernels. In orchard plots, spores inoculated on the fruit colonized hulls, shells, and kernels of maturing almonds. Aflatoxins were detected in harvested kernels and hulls. Almond fruits were susceptible to colonization from the time of hull-split, when rapid drying of the fruit began, until after harvest when moisture of the kernel dropped below about 5% based on the fresh weight of the kernel. Infestations by the navel orangeworm, *Paramyelois transitella* (Walker), increased colonization of the kernels by *A. flavus* from experimental plots.

The drupe of the edible sweet almond has a distinct pericarp enclosing the kernel. This pericarp consists of an outer fleshy hull and inner hard shell. Fruits usually mature on the tree, and a longitudinal suture on one side of the hull splits exposing the shell, allowing rapid drying of the fruit. Cultivars vary in shell thickness and dehiscense (20).

Drying fruits are shaken onto the ground, picked up mechanically, transported to a location where the hulls are removed, and sent tot a plant for shelling, storing, and processing.

Many kinds of micro-organisms are found on almonds (12, 13, 14, 18) and may colonize the hull while the fruit is on the tree (14) or on the ground (12, 13).

Toxigenic species of *Aspergillus* are widespread on seed and other crops (8, 9, 10). *Aspergillus flavus* is a "group" species (16) containing 11 species. *A. flavus* Link, and *A. parasiticus* Speare both are included in this group and may produce toxic metabolites called aflatoxins. In the Central Valley of California, where most almonds are produced in the U.S., fungi in the *A. flavus* group occurs sporadically on cotton (2). Of 345 objective samples of almonds taken in the period 1970-1974, 8% had detectable aflatoxins at the average level of 20 μg/kg total aflatoxins and a range of 2-84 μg/kg (Dr. L. Stoloff, Food and Drug Admin., personal communication). These fungi may colonize almonds while they are drying on the tree or soil (6) and might lead to a pre- or postharvest invasion of kernels by *A. flavus* and contamination by aflatoxins. Preliminary information (Harry W. Schroeder, USDA, College Station, Texas, unpublished report) indicated *A. flavus* occurred on insect-damaged almonds more frequently than on sound nuts. We studied the susceptibility of the almond fruit to *A. flavus* colonization with emphasis on the role of the navel orangeworm (NOW) that commonly damages both kernel and hull.

Materials and Methods

Analysis for Aspergillus flavus. Surface disinfested and non-disinfested almond kernels, shells, or hulls were tested for presence of the *A. flavus group*. For surface disinfestation, samples were dipped in 70% (v/v) ethanol/H_2O for 10 sec, then immediately soaked in 0.5% sodium hypochlorite solution for 5 min. Without further washing, samples were then aseptically placed on plates of malt-salt medium (MSM) containing 7.5% NaCl, 2% malt extract and 2% agar. Non-disinfested samples were plated directly on MSM plus 13 μg/ml 2,6-dichloro-4-nitroaniline to inhibit growth of some *Rhizopus* spp. Five almond kernels or 5 half-shells, or 5 half-hulls were placed on each plate. After incubation for 1 week at 30°C, colonies of *A. flavus* were

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[3]We acknowledge the advice of L. Stoloff, Food and Drug Administration; the help of Mary Nelson, Agricultural Research Technician; and the cooperation of L. Todd Browne, Fresno County Farm Advisor.

counted. Most cultures were identified as *A. flavus* group by color. Representative cultures were compared to known isolates of *A. flavus* growing on MSM and usually were identified as either *A. flavus* Link or *A. parasiticus* Speare (16).

Analysis of aflatoxins. Aflatoxins were determined in hulls by the method of Pons et al. (15) with slight modification, and in kernels by the method of Robertson et al. (17) with a slight modification (M. Uota, unpublished). The amount of aflatoxin B_1 was estimated visually on the thin layer chromatogram.

Sampling of commercial almonds. During 1972 and 1973, 215 samples of almond kernels were obtained from commercial growers and handlers in California. Sampling began about July 15 and continued until the end of the storage season in May or June. In the laboratory, kernels were sorted into insect damaged and sound kernels. The samples, from various geographic locations in California, ranged in size from 50 to 400 sound kernels and from 5 to 50 insect-damaged kernels.

Inoculation of almond fruit with A. flavus before harvest. By the technique of Ashworth et al. (5), we introduced eggs of NOW and conidia of *A. flavus* into paper or cloth bags that enclosed 2 almond fruits attached to the tree. Five eggs of NOW were introduced onto each fruit on small pieces of paper. Dry conidia were blown toward the surface of the fruit with a powder blower. Conidia were from five 1-week old cultures of the *A. flavus* group isolated from almond kernels and shown to be toxigenic, and the NOW eggs (3-4 days old) were from laboratory-reared insects. The eggs generally hatched within 24 hr after being placed on the fruit. Specific techniques used in 1972 or 1973 orchard tests are presented in the results.

Results

Occurrence of A. flavus in commercial almond samples. One hundred seventeen samples consisting of 12,580 sound kernels and 2,960 insect-damaged kernels in 98 samples were analyzed for *A. flavus*. *A. flavus* was found 10 times as often in insect-damaged kernels as in sound kernels when both were surface disinfested (Table 1). Surface contamination occurred in both insect-damaged and sound kernels, but again, more frequently on insect-damaged kernels (Table 1).

Twenty-seven isolates of *A. flavus* from the surface-disinfested kernels were screened for aflatoxin production. Twenty-one of the isolates produced detectable amounts of aflatoxin on a medium of sterile rice or almonds when incubated for 5-7 days at 30°C.

Table 1. Amount of *Aspergillus flavus* associated with almond kernels in samples collected from growers and handlers in 1972 and 1973.

Sample type	Surface-disinfested[z]	Total kernels examined	Total samples examined[y]	*A. flavus* colonization (%)	
				Kernels	Sample
Insect-damaged	No	540	45	58	100
	Yes	2,420	53	0.6	17
Sound	No	1,880	34	47	100
	Yes	10,700	83	0.06	7

[z]Kernels dipped in 70% ethanol for 10 sec followed by 0.5% sodium hypochlorite solution for 5 min.

[y]Samples contained 50-400 sound kernels, or 5-50 insect damaged kernels.

Inoculation of almond fruit with A. flavus in the orchard. In 1972, 'Kapareil' soft shelled almond trees, located near Clovis, California were selected for a test plot. Treatments were arranged in a split plot design of 4 random blocks of 4 treatments applied at 3 stages of growth of the fruit (20 fruit/sample). Representative fruit was selected for all treatments on May 18. At this time fruits to be bagged were washed with 0.01% sodium hypochlorite solution and covered with paper bags. The treatments were applied: 1) before hull split, June 1; 2) at the time 50% of the hulls had split, June 29; and, 3) 2 weeks before harvest, July 20. Treatments of fruit were: 1) bagged fruit infested with NOW and *A. flavus*, 2) bagged fruit infested with *A. flavus* alone, or 3) non-infested bagged fruit, and 4) non-infested, non-bagged fruit.

Surface-disinfested hulls, shells and kernels were analyzed for *A. flavus* 2 weeks after each inoculation and again after 2 months of storage. After harvest, all samples were fumigated with 2 g of methyl bromide/m^3 at 18°C for 24 hr to eliminate insects and then held in dry storage until the final analysis. The methyl bromide did not eradicate spores of *A. flavus* on paper strips fumigated with the almonds and did not appear to affect the final analysis.

Almond fruits were not colonized by *A. flavus* before the hulls split open on the tree. Although part of the sample inoculated before hull split was lost due to laboratory contamination,

Table 2. Analysis of *Aspergillus flavus* in 'Kapereil' almond fruits from a 1972 experimental field plot, 2 weeks after inoculation.

Time of inoculation	Part of fruit	% colonization of fruit after indicated field treatments[z]				
		Uncovered control	Covered[y] control	Fungus[x] introduced and covered	Fungus and worm[w] introduced and covered	Overall mean for inoculation time
At 50% hull split (June 29, 1972)	Kernels	0	0	0	0	0
	Shells	8	0	15	20	11
	Hulls	0	0	35	53	22a
2 weeks before harvest (July 20, 1972)	Kernels	0	0	3	10	3
	Shells	0	8	3	8	4
	Hulls	0	8	65	65	34b
Overall Means						
	Kernels	0	0	1.3	5.0	
	Shells	3.3	3.3	8.8	13.7	
	Hulls	0b	3.3b	50.0a	58.8a	

[z]Statistical analysis indicated significant differences only in hulls. Overall hull means within a row or column without a letter in common differ at the 95% confidence level. Each datum represents *A. flavus* isolated from 10 surface disinfested fruit parts replicated 4 times.

[y]Fruits of 'Kapereil' were covered with a paper bag to allow controlled inoculation and to reduce contamination.

[x]Inoculated with conidia from 5 toxigenic isolates of *A. flavus* from kernels.

[w]Eggs of the navel orangeworm were placed on the fruit at the time of inoculation.

Table 3. Analysis of the *Aspergillus flavus* in 'Kapareil' almond fruits from a 1972 experimental field plot 2 months after harvest.

Time of inoculation	Part of fruit	% colonization of the fruit after indicated field treatments[z]				
		Uncovered control	Covered[y] control	Fungus[x] introduced and covered	Fungus and worm[vw] introduced and covered	Overall mean for inoculation time
2 weeks before hull split (June 1, 1972)	Kernels	5	5	65	57	33a
	Shells	0	0	40	47	44a
	Hulls	0	5	100	93	49a
At 50% hull split (June 29, 1972)	Kernels	0	5	35	85	31a
	Shells	0	0	12	50	31a
	Hulls	12	0	95	98	51a
2 weeks before harvest (July 20, 1972)	Kernels	3	0	30	40	18b
	Shells	3	0	8	20	10a
	Hulls	3	0	98	85	46a
Overall Means						
	Kernels	3c	3c	43b	61a	
	Shells	1a	0a	20b	39c	
	Hulls	5a	2a	98b	92b	

[z]Statistical analysis indicated significant differences as shown for the overall means. Overall means from a fruit part within a row or column without a letter in common differ at the 95% confidence level. Each datum represents the *A. flavus* isolated from 10 surface disinfested fruit parts replicated 4 times.

[y]Fruits of 'Kapareil' were covered with a paper bag to allow controlled inoculation and to reduce contamination.

[x]Inoculated with conidia from 5 toxigenic isolates of *A. flavus* from kernels.

[w]Eggs of the navel orangeworm were placed on the fruit at the time of inoculation.

[v]Samples were fumigated after harvest with 24 g of methyl bromide/m^3 for worm control.

samples were taken from adjacent trees as an attempt to evaluate the colonization before hull-split; these yielded no *Aspergillus* when surface-disinfested. After the hulls had split, the hulls, and to a lesser degree the shells and kernels, were rapidly invaded by many *Aspergillus* spp., including *A. flavus*. Two weeks after inoculation, a high percentage of the hulls were colonized by *A. flavus* (Table 2). Two months after harvest, the percentage of *A. flavus* had increased in hulls, shells and kernels, indicating that activity of the fungus continued after harvest (Table 3).

After foraging on the fruit for 2 weeks, NOW only slightly increased colonization by *A. flavus* (Table 2), however, 2 months after harvest NOW had significantly increased the incidence of *A. flavus* in shells and kernels (Table 3). The fruits were susceptible to colonization with or without worm damage from the period of hull split until some time after harvest.

Fruit became susceptible to infection when the hulls split and the fruit moisture content was about 80% (Fig. 1). At harvest, the kernels, shells, and hulls had 7, 15, and 23% moisture, respectively. These represent moisture levels in equilibrium with 80% RH at 24°C as determined by 4 tests in which kernels were held over saturated salt solutions for 1-2 months (19) (Fig. 2). Fungal activity probably would continue until a moisture level in equilibrium with 70% RH at 24° was reached or about 5% moisture in the kernels (11).

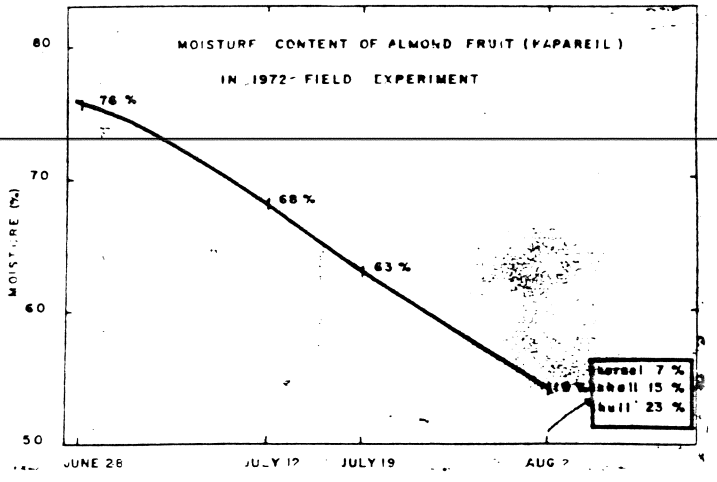

Fig. 1. Moisture content based on the fresh-fruit wt of whole almond fruit on the tree from the time of hull-split until the fruit was removed from the tree at normal harvest time. The data are from an orchard used in a field test in 1972, and each point represents a sample of 10 fruits taken from untreated control trees.

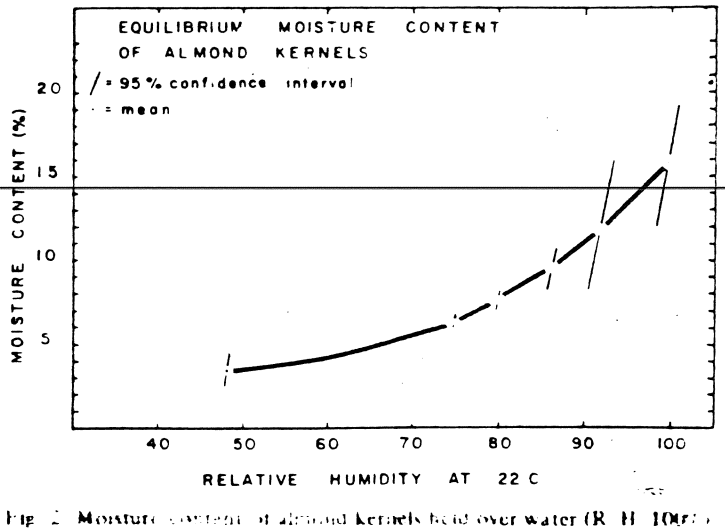

Fig. 2. Moisture content of almond kernels held over water (R.H. 100%) or saturated solutions of $NH_4H_2PO_4$, K_2CrO_4, $(NH_4)_2SO_4$, NaCl, or KNO_2 respectively 92, 86, 80, 75, 48% RH at 22°C for 1-2 months. Each point represents the average of 4 tests with 50 g of whole almond kernels. Moisture content is the oven dry wt (96 hr at 86°C)/wet x 100.

Table 4. Aflatoxins in 'Kapareil' almond fruits from an experimental field plot 2 months after harvest.

Part of fruit	% of samples containing aflatoxin and range of the concn in fruits after indicated field treatments[z]					
	Uncovered control		Covered[y] control		Fungus[x] introduced and covered	Fungus and worm[vw] introduced and covered
	%	μg/kg	%	μg/kg	% μg/kg	% μg/kg
Kernels	0	0	0	0	17 (0-50)	17 (0-335)
Hulls	0	0	8	(0-51)	92 (0-1290)	92 (22-520)

[z]Each datum represents 12 assays of the hull or kernels from 10 fruits.

[y]Fruits of 'Kapareil' were covered with a paper bag to allow controlled inoculation and to reduce contamination.

[x]Inoculated with conidia from 5 toxigenic isolates of *A. flavus* obtained from kernels.

[w]Eggs of the navel orangeworm were placed on the fruit at the time of inoculation.

[v]Samples were fumigated after harvest with 24 g of methyl bromide/m^3 for worm control.

Aflatoxin was found in the inoculated kernels and hulls 2 months after harvest. NOW infestations did not increase the occurrence or quantity of aflatoxin in this test (Table 4).

In 1973, unwashed almond fruits from 'Nonpareil', 'NePlus', 'Davey', 'Mission' (TX), 'Vesta', and 2 unnamed trees, grown at Fresno State University or at the Univ. of California, Kearney Field Station were enclosed in muslin bags and inoculated with *A. flavus* at the time of 10-50% hull split. No NOW were placed on the fruit and worm damage was not found in the test fruit. 'Mission' apparently was less susceptible to invasion by *A. flavus* than other cultivars tested. Some kernels of all the cultivars were infested (Table 5) and these tests confirmed the 1972 test in 'Kapareil', indicating that kernels can be colonized without insect damage to the fruit.

Discussion

Fungi, including *Aspergillus* may invade the split almond hull. We found *A. flavus* on drying hulls on non-inoculated almond fruits on the tree in commercial orchards. The low frequency of the fungus found in surface-sterilized commercial samples of sound nuts contrasts with the high frequency of kernel colonization of inoculated fruits. The low frequency of *A. flavus* in commercial samples may be due to low inoculum densities in California almond orchards or to the effect of other micro-organisms that are antagonistic to the *A. flavus* group, or to environmental conditions not found in our inoculated field tests.

The moisture content of the fruit influences growth of the fungus (2, 4, 9). We found an initially high moisture content at the time of hull split; the moisture then fell to a level that might restrict growth. This point may be at or below 5% moisture by weight in the kernel, but the exact moisture content at which fungal growth ceases may vary with temperature (7) or oil content of the kernel (8) and has not been established by this study. Because drying might be delayed or bagged or covered fruit, the time of susceptibility might have been prolonged.

The soft shelled commercial cultivars do not appear to provide a marked barrier to invasion by *A. flavus*. The increase of *A. flavus* associated with insect-damaged nuts in commercial samples, and in worm-infested test samples may not only result from opening the shell and exposing the kernel to air-borne inoculum, but NOW larvae foraging in the hulls might encounter *flavus* colonies and carry the fungus to the kernel. In addition, NOW might increase the period of susceptibility of the kernels by increasing the available moisture, through respiration, in the areas of the kernel where the larva is burrowing.

Table 5. The colonization of kernels from the fruit of 7 almond cultivars inoculated with conidia of *Aspergillus flavus* at a time when 10 to 50% of the hulls had split open.

Almond cultivar	% kernels colonized by *A. flavus*[z]	No. of trees sampled[y]
Seedling #1	31	1
Seedling #2	57	1
Vesta	63	1
Davey	53	1
Nonpareil	22	5
NePlus	23	2
Mission	2*	5

*Considered to be different from other data in the column.

[z]Almond fruit were inoculated at hull split while on the tree and covered with muslin bags to protect the fruit from contamination and worm infestation.

[y]20 inoculated kernels and 20 non-inoculated kernels from each tree were surface disinfested and plated on malt-salt agar medium. *A. flavus* was not found in isolations from non-inoculated fruits.

The colonization of hulls and kernels of almonds by *A. flavus* and the subsequent production of aflatoxin suggest a potential hazard. The low frequency of the fungus in sound kernels, as compared with insect damaged kernels, suggests that the number of contaminated nuts could be reduced by effective control of navel orangeworm and other insects.

Moisture content and insect damage are important factors in preharvest colonization of almond hulls and cotton bolls (1, 2, 3, 4). Consequently, cultural practices that promote rapid drying of the almond fruit on the tree may inhibit mold development and toxin production.

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1975 ANNUAL REPORT

TITLE: Aflatoxin Research - Field and Storage
Section B - Storage

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I. OBJECTIVES AND GOALS:

1. To determine the incidence of aflatoxins in almond samples taken at various points in the harvesting, handling, and storage system.

2. (a) To determine the effects of controlled atmospheres (lowered oxygen levels, increased carbon dioxide levels) on the quality of stored shelled almonds; (b) to relate atmosphere composition and temperature during storage to almond quality; (c) and to determine effects of various controlled atmospheres on insect infestation (cooperative with Stored Product Insects Laboratory).

II. ABSTRACT:

Although aflatoxin analyses made in prior seasons indicated a close correlation between insect infestation and the presence of toxin, no toxins were found in 535 samples of commercially harvested and hulled nuts in 1975, even though some samples had some insect damage.

Almonds stored in controlled atmospheres (1% oxygen + elevated CO_2 levels) for six months at 59° or 68°F were of superior quality to those stored in normal air. Almonds stored at 75% relative humidity deteriorated more than those stored at 55%. Potentially, controlled atmospheres may partially compensate for low temperature storage, may improve quality maintenance, and prevent insect infestation.

III. EXPERIMENTAL PROCEDURE:

Aflatoxin determinations.--Almond samples obtained at packing plants as growers delivered the hulled nuts were segregated into sound, damaged by navel orange worm (NOW), and damaged by twig borer (TB) lots, which were analyzed separately for aflatoxin. Thin-layer chromotography methods detected and quantified aflatoxins B_1, B_2, G_1, and G_2.

Controlled atmosphere storage.--Almond samples were stored for 6 months in controlled atmospheres at 59° or 68°F and at 55 or 75% relative humidity. Atmospheres of 21 or 1% oxygen and 0, 10, 20, 40, or 80% carbon dioxide were maintained at the above temperatures, as well as a check lot in air at 36°F.

Almond quality was determined in a sensory evaluation performed by a trained taste panel supervised by Dr. D. Guadagni at the Western Regional Research Laboratory. The panel used a hedonic rating system in which 1 equaled disliked extremely and 9 equaled liked extremely.

IV. RESULTS:

Aflatoxin determinations.--Of the 535 samples analyzed, 253 were sound, 224 with NOW damage, and 58 with TB damage. On a location basis, 312 samples were from The Ballico area, 193 from Fresno, and 30 from Chico. No aflatoxins were found in these lots in 1975, regardless of insect infestation or growing area.

Controlled atmosphere storage.--After six months, the poorest quality almonds were those stored in normal air at 59 or 68°F and those stored in several of the atmosphere combinations at 75% RH at 68° (table 1). The best lot was that stored in 1% O_2 and 10% CO_2 at 55% RH at 59°. This lot was almost equalled by a high CO_2 lot (80%) stored at the same temperature and humidity. Hedonic acceptance ratings are shown in table 2. The nuts with highest ratings were all stored at 55% RH.

V. DISCUSSION:

Aflatoxin determinations.--Since the aflatoxin level was very low in the 1975 crop, differences in incidence of toxin due to various insect control programs or to geographical factors could not be demonstrated, as in prior seasons. Some lots from "clean" orchard plots in the Ballico area remain to be analyzed.

Controlled atmosphere storage.--Although additional work must be done, preliminary results suggest that high levels of carbon dioxide in the atmosphere are beneficial in maintaining almond quality. Low oxygen levels (1%) also show some benefit, but not as consistently as high CO_2. High relative humidity levels (75%) were consistent in contributing to quality loss, particularly at the higher temperatures used in this study. When lots that have been in storage for 10 months have been evaluated, we should have a better determination of CA effects on quality.

Table 1.--Sensory evaluation of off-flavors in almond kernels stored for 6 months in controlled atmospheres, in comparison with a control stored at 36°F in air

Storage conditions				Percentage of judgments indicating least off-flavor compared to control
Atmosphere		Temp.	R.H.	
% O_2	% CO_2	(°F)	(%)	
1	10	59	55	34 *
21	80	59	55	44
21	0	36	55 (control)	--
21	10	59	55	49
21	40	59	55	51
1	0	59	55	54
1	40	59	55	54
1	20	59	55	54
1	0	68	55	55
1	80	59	55	57
1	80	68	75	58
21	20	59	55	59
1	20	68	55	62 *
1	80	68	55	65 *
1	0	68	75	68 *
21	0	59	55	68 *
21	0	68	55	68 *
1	20	68	75	72 *
21	0	68	75	83 *

* Indicates significant difference

Table 2.--Effect of storage conditions on hedonic acceptance ratings of almonds after 6 months of storage

Storage conditions				Mean hedonic acceptance rating *
Atmosphere		Temp.,	R.H.	
% O_2	% CO_2	(°F)	(%)	
1	0	59	55	6.6
1	10	59	55	6.3
1	20	59	55	6.6
1	40	59	55	6.0
1	80	59	55	6.6
21	0	59	55	6.5
21	10	59	55	6.8
21	20	59	55	6.5
21	40	59	55	6.9
21	80	59	55	7.0
21	0	68	55	6.4
1	0	68	55	6.8
1	20	68	55	7.0
1	80	68	55	6.6
21	0	36	55	7.0 d
21	0	68	75	4.9 a
1	0	68	75	5.3 ab
1	20	68	75	5.7 bc
1	80	68	75	6.2 c

* Numbers followed by different letters are significantly different at 1% level.