Project Number 76-M3

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1976 ANNUAL REPORT

Project No: Continuation of Project No. 76-M3

Title: Almond Diseases
Mycotoxin Research - Field and Storage
Section A - Field

Personnel: Dr. Douglas J. Phillips
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1. Objectives:

To study factors that contribute to or influence the occurrence of *Aspergillus flavus* and aflatoxins in almond hulls, shells and kernels and to examine the possibility of using these factors to reduce the potential hazard of fungi on almonds.

2. Interpretive Summary:

High temperatures favor the development of *A. flavus* on almonds, as evidenced by increased incidence of diseased nuts in the warmest growing areas and in nuts dried in the sun vs. those dried in the shade. When *A. flavus* occurred alone on the nuts it caused more damage than when it had to compete with other molds, i.e. *Alternaria*. Cultural practices that favor competing organisms may provide a method of reducing damage from *A. flavus*. The navel orange worm (NOW) is capable of carrying spores of *A. flavus* on its surface or in its gut from diseased to sound nuts under laboratory conditions, but we have rarely observed this phenomenon under field conditions. However, the high correlation between worm damage and the occurrence of aflatoxins suggests that NOW either disseminates spores more widely than we can detect, or provides infection ports for the fungus, or alters some other environmental factor that favors infection.

High temperature effects on A. flavus.--Temperatures of the kernels and hulls of almonds were measured in 3 different orchards in each of the growing areas tested--Bakersfield, Chowchilla, and Snelling. Temperatures were taken between Noon and 4 p.m. over a two-week period coinciding with the almond harvest in each area. Temperatures of nuts on the tree and on the ground, and in the shade and in the sun were taken. Colonization of the nuts by various fungi was evaluated by plating kernels and hulls on malt-salt agar after hull split, after the samples were on the ground for one week, and after the samples were in storage for about 3 months. Heat damage was evaluated by staining the kernels with tetrazolium chloride, and will be further evaluated after 3 months' storage, by roasting (concealed damage) and by tests for rancidity.

Reduction of A. flavus colonies by competing fungi.--During the growing season, fruit of three almond cultivars--Milo, Nonpareil, and Neplus--were inoculated with A. flavus (AF) alone, or AF in combination with a wine yeast, a yeast isolated from almond hulls, a Stemphylium sp., an Alternaria sp., a Fusarium roseum, a Cladosporium sp., or a mixture of all these fungi. Three appropriate controls also were maintained. The inoculation was made on the tree after first washing the fruit with 50-100 ppm chlorine ($Cl\ O^-$) and, when dry, by taping bits of straw colonized by the various organisms other than A. flavus. The fruit was then enclosed in a cloth filter bag and spores of A. flavus were blown into the bag and onto the fruit. The filter prevented contamination of other fruit in the orchard.

Treated fruits were cut from the trees at harvest time and the hulls and kernels were analyzed for A. flavus by disinfesting the surface (10 sec. dip in 70% ethanol + 5 min soak in 0.5% sodium hypochlorite) and plating on malt-salt agar. After incubation for 1 week at 30°C (86°F) A. flavus colonies were counted.

Dissemination of A. Flavus by NOW.--Clean NOW larvae reared from surface sterilized eggs were allowed to feed on almond kernels that had been either inoculated or not inoculated with A. flavus. The (1) frass, (2) surface washings, and (3) homogenate of surface disinfested NOW larvae were plated on Bell and Crawford's A. flavus isolation medium (with 50 mg/L Botran added.) The inoculated plates were incubated 7 days at 30°C (86°F) and the A. flavus colonies counted. The same procedure was used for NOW larvae collected from almonds still on the trees or from those in bins awaiting hulling.

4. Results:

High temperature effects on A. flavus.--A. flavus occurred more frequently in field plots in the southernmost almond orchards than in the two centrally located orchards (Table 1.) Of the two latter orchards, Chowchilla had a higher incidence than Snelling. A. flavus generally occurred more frequently on almonds in the sun than on those in the shade. Differences in temperature in the sun and shade this season were not great enough to affect heat injury. Concealed damage and rancidity tests are not yet complete.

Reduction of A. flavus colonies by competing fungi.--All of the test fungi successfully colonized the hulls. When alone, A. flavus colonized about 74% of the surface of inoculated hulls (the same value was found in 1975); but when other fungi were present fewer A. flavus colonies occurred. Alternaria sp. was particularly effective, as was the mixture of fungi (Table 3.)

Twenty-five percent of the kernels were colonized by A. flavus when alone (vs. 23% in 1975.) The presence of Alternaria sp. and the mixture of fungi reduced A. flavus colonization to 4 and 11%, respectively (Table 3.)

Dissemination of A. flavus by NOW.--Larvae of NOW grown in the laboratory on almonds naturally contaminated with spores of A. flavus, or on almonds inoculated with A. flavus were found to have spores of A. flavus in their frass, on their surface, and in their gut. Larvae collected in the orchard or in bins at the huller were not found to carry spores, when collected in 1975 and 1976.

5. Discussion:

High temperature effects on A. flavus.--Higher temperatures in plots in the southern end of the San Joaquin Valley than in plots in the central part of the Valley, and higher temperatures of almonds in the sun than in the shade correlate with the increased occurrence of A. flavus. High temperatures may also contribute to concealed damage and early development of rancidity in nuts. These factors will be studied further.

Reduction of A. flavus colonies by competing fungi.--Earlier work, in which we found that the presence of competing organisms on the surface of almond inhibit the development of A. flavus, has been verified in this year's studies. Alternaria sp. was particularly effective. Cultural techniques in the orchard, i.e. fungicide sprays, may alter the occurrence of such competitors for nutrients, and therefore, may also affect the occurrence of A. flavus.

Dissemination of A. Flavus by NOW.--Although NOW larvae are capable of carrying spores of *A. flavus* in their guts or on their surface, under laboratory conditions, we have not found this phenomenon to be generally common in field samples collected in the orchard or in bins awaiting hulling. We have not ruled out the possibility of dissemination of spores by worms infesting nuts after hulling or during storage prior to shelling. NOW may alter the environment in other ways that favor establishment of *A. flavus* on almonds.

6. Publications:

Phillips, D. J., M. Uota, D. Monticelli, and C. Curtis. 1976. Colonization of Almond by *Aspergillus flavus*. J. Am. Soc. Hort. Sci. 101(1): 19-23.

three growing areas during the week before and after harvest.

Area and sample location	Avg. high	Avg. low	Mean	Highest temp. recorded	Lowest temp. recorded
	°C	°C	°C	°C	°C
Bakersfield:					
Almond on tree in shade	36.8	28.4	31.8	41.7	23.8
Almond on tree in sun	44.7	30.8	37.2	49.4	26.6
Air in tree	35.5	30.2	33.8	38.3	25.6
Almond on ground in shade	39.9	25.2	30.2	41.7	22.8
Almond on ground in sun	50.2	33.9	42.2	56.7	28.9
Air at ground surface	36.4	28.2	30.7	40.0	26.7
Chowchilla:					
Almond on tree in shade	37.0	30.0	32.3	39.4	27.8
Almond on tree in sun	44.8	31.8	38.0	50.0	29.4
Air in tree	34.1	30.7	32.1	36.6	27.8
Almond on ground in shade	38.5	34.3	34.8	43.9	28.9
Almond on ground in sun	56.8	40.7	49.2	61.1	30.0
Air at ground surface	38.6	33.2	36.2	41.7	28.3
Snelling:					
Almond on tree in shade	37.2	31.1	34.3	37.3	30.0
Almond on tree in sun	44.6	31.7	37.4	48.9	30.6
Air in tree	36.4	32.6	34.8	38.3	30.6
Almond on ground in shade	31.1	23.1	27.2	37.2	21.1
Almond on ground in sun	46.3	30.0	38.4	50.5	22.2
Air at ground surface	29.8	25.3	27.6	33.3	21.6

Temperatures for seven days (spanning header over Avg. high through Lowest temp. recorded) and in almonds that had been surface disinfested or not disinfested.

Area and sample location	Percent *A. Flavus* on Kernels					
	Almonds in Shade			Almonds in Sun		
	Dis-infested	Not disinfested		Dis-infested	Not disinfested	
	Pct.	Pct.		Pct.	Pct.	
Bakersfield:						
On tree	.7	7.3	(37.6)*	1.3	31.3	(33.3)
On ground	.7	23.3	(14.1)	1.3	25.3	(2.7)
Chowchilla:						
On tree	0	2.7	(37.5)	0	3.3	(26.8)
On ground	0	0.0	(3.0)	0	7.3	(2.2)
Snelling:						
On tree	0	1.3	(3.6)	0	.7	(3.2)
On ground	0	0.0	(5.9)	0	2.0	(4.1)

* ( ) = % Moisture of kernels.

Table 3.--*Aspergillus flavus* colonization of Nonpareil almond hulls and kernels when other potentially competitive fungi are present on the hull. [1/]

Colonization by *A. flavus*	Inoculation with indicated organisms [1/] and *A. flavus*										
	A	B	C	D	E	F	G	H	I	J	K
Percent of each hull colonized	85	77	70	53	61	64	58	71	74	4	6
Percent of kernels colonized	10	9	13	4	14	18	11	26	25	0	1

1/ A--wine yeast, B--wild yeast, C--*Stemphylyum* sp., D--*Alternaria* sp., E--*Fusarium roseum*, F--*Cladosporium* sp., G--Mixture, H--*Aspergillus flavus* (+ tape,) I--*A. flavus*, J--Control (Bagged,) K--No treatment.

Test Site	Date	*A. flavus* colonies developing from indicated source		
		Frass	Surface	Gut
Laboratory:				
Natural almonds	4/23/75	0	0	0
" "	5/23/75	1,000	100	100
" "	6/20/75	14,000	6,000	5,000
Inoculated almonds	6/ 4/75	3,000	100	1,500
" "	6/13/75	900,000	18,000	30,000
Field Collections:				
Natural almonds	8/20/75	0	0	0
" "	8/27/75	"	"	"
" "	8/28/75	"	"	"
" "	9/27/75	"	"	"
" "	10/ 7/75	"	"	"
" "	10/16/75	"	"	"
" "	12/16/75	"	"	"
" "	8/12/76	"	"	"
" "	8/30/76	"	"	"
" "	10/15/76	"	"	"

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1976 ANNUAL REPORT

Project No: Continuation of No. 76-M3

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

Personnel: Dr. M. Uota, Horticulturist
Miss Elizabeth Elliston
Miss Klazina Ryken

1. Objectives:

(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) at various storage temperatures on the quality of shelled almonds;

(b) and to determine effects of various controlled atmospheres on insect infestation (cooperative with Stored-Product Insects Research Laboratory.)

2. Interpretive Summary:

Storage of almonds in controlled atmospheres with reduced levels of oxygen (O_2) and increased levels of carbon dioxide (CO_2) was effective in retaining the quality of the meats at relatively high temperatures. Such atmospheres could be maintained with only slight change during storage by holding the nuts in sealed plastic bags.

Almonds with 6 percent moisture were found to have a water activity (W_a) of 0.7. Since this W_a has been proposed by the FDA as the level at which fungal growth will cease, almonds should be dried to at least 6 percent before storage.

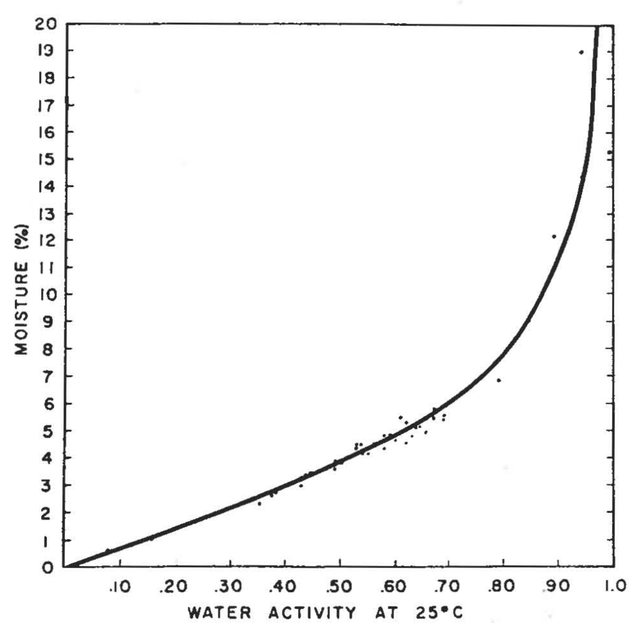
Almond storage.--Shelled almonds were held in sealed, laminated plastic bags with initial atmospheres of 0.1, or 21 percent O_2 in combination with 100, 80, 40, 20, 10, or 0 percent CO_2 for 5 months at 30°C (86°F.) A check lot was held at 2.5°C (36°F.) At the end of storage changes from the initial atmosphere in each bag was measured and the nuts were evaluated for quality by determining the free fatty acids (rancidity) and by tasting by a trained panel. Additional samples will be held for a full year before evaluation.

Water activity.--Nuts with various levels of moisture were placed in sealed chambers in which the dewpoint could be measured when the nuts came to equilibrium with the air around them. The equilibrium relative humidity at 25°C (77°F) was calculated from the dewpoint. Equilibrium relative humidity is equivalent to water activity (W_a.) After W_a was calculated for nuts with different moisture contents, the values were plotted against one another on a chart.

4. Results:

Almond storage.--After 5 months' storage of nuts in sealed bags, those with initial concentrations of 0 or 1 percent oxygen had about 2 to 4 percent oxygen; those with initial concentrations of 100, 80, 40, or 20 percent CO_2, had about 89, 59, 20, or 10 percent CO_2, respectively. A trained taste panel could not detect any significant difference in quality among nuts held in the different atmospheres for 5 months. Also, free fatty acids were about the same in all treatments. Differences may be greater after the full year's storage.

Water activity.--The relation of moisture content of almonds to water activity at 25°C is shown in Figure 1.



W_a has been proposed by FDA as the maximum level for nuts in packages or in storage.

5. Discussion:

Almond storage.--Controlled atmospheres potentially provide a means of maintaining the quality of almonds during storage without the expense and energy consumption required by refrigerated storage. Nuts stored in certain high carbon dioxide or low oxygen atmospheres at 20°C (68°F) have been found to equal those stored in normal air at 2.5°C (36°F.)

An evaluation of laminated plastic bags is being made to find bin liners or other container enclosures capable of retaining the desired atmospheres. Such atmospheres retard the development of rancidity and also control insect infestations. During 5 months' storage, the plastic bags retained oxygen better than CO_2. No off-flavors were detected in the bagged nuts. Although the quality of the nuts from the various storage treatments did not differ after 5 months, we must await the final examination to determine possible differences during a full year's storage. Additional work is needed on laminated plastic materials with less permeability to O_2 and CO_2.

Water activity.--The water activity or equilibrium relative humidity of the atmosphere in which almonds are held is one of the main factors that controls the biological activity of the nuts and the micro-organisms that may grow on them. Since water activity can be correlated with the moisture content of the nuts, understanding the relationship between the two factors is desirable.

When almonds harvested at different moisture contents are placed in closed chambers, they gradually come to equilibrium with the relative humidity in the air around them. Nuts with different moisture contents produce different equilibrium relative humidities (expressed as percent,) also called water activities (expressed as a decimal.) Figure 1 shows the relationship of moisture content to water activity. From the figure, one can determine that almonds dried to 6% or less moisture content would meet the proposed FDA standard of 0.70 water activity at 25°C (77°F.) Almonds with this water activity would not support mold growth during storage. Research is needed on other forms of almonds (blanched) to determine if their W_a differs from natural meats.

6. Publications:

None.