PROJECT 77-M4
MYCOTOXIN RESEARCH
PART 1 - FIELD

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The term "Aflatoxin" refers to four chemically different toxins, B_1, B_2, G_1 and G_2, which are produced by two fungi of very similar appearance, *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are dangerous chemicals capable of inducing cancer in some animals, and probably in man. The conditions for growth of the fungus are not identical to the conditions that stimulate toxin production and contamination of a commodity. Consequently, an isolate of *Aspergillus flavus* that is known to produce aflatoxins may sometimes grow on a commodity and not produce these toxins.

THE CONDITIONS REQUIRED FOR GROWTH OF *ASPERGILLUS FLAVUS*

Temperature--The fungus may grow between a high temperature of 60°C (140°F), and a low of 12°C (54°F).

Moisture--*Aspergillus flavus* will grow between 100% relative humidity (water activity 1) and about 85% relative humidity (a water activity of .85 = about 96% moisture in the almond kernel), Moisture enough for *A. flavus* growth on almonds occurs while the kernels are drying in the orchard.

Nutritional needs--The fungi *A. flavus* and *A. parasiticus* prefer, or may in fact require, dead or injured tissue as a food base and may grow on grains, seeds, and many kinds of plant materials. Damaged tissues in the almond kernel provide potential food for growth of *Aspergillus* sp. and many other fungi, which may be either beneficial or harmful. *A. flavus* and *A. parasiticus* grow best when there are few other fungi growing with them, or competing with them for the food base. We have identified some fungi that interfere with the growth of *Aspergillus* sp. and aflatoxin production. Our studies show *Ulocladium chartarum* is especially effective in reducing the aflatoxin problem experimentally (Table 1.).

Occurrence of the fungi--The fungi *A. flavus* and *A. parasiticus* are widespread throughout nature. These molds are, however, more prevalent in some areas than in other areas, or on some foods than on other foods. On almonds, *A. flavus* has been found in 1976 and 1977 about 10 times more frequently in hotter and drier of California's almond-growing areas than in the cooler areas. Earlier, we found 10 times more of these fungi on worm-damaged kernels (1 in 200) than on sound or undamaged kernels (1 in 2000). Our current research suggest that *A. flavus* occurs almost twice as frequently on almonds in the sun of the orchard than on almonds in the shaded areas (Table 2).

CONDITIONS FOR AFLATOXIN PRODUCTION

Toxin production can occur at a temperature as low as 12°C (54°F), is optimum at 27°C (81°F), and can occur as high as 42°C (108°F). Figures 1 and 2 show the day-time temperature of kernels in the orchard about a week before and after shaking. It requires only 24 hours for the toxin to be produced under optimum conditions and the level of toxin concentration increases for 10 days. Much more toxin has been found in worm-damaged almond kernels than in apparently sound kernels. So far, we have not found toxin in sound kernels outside our research plots, although our laboratory and field studies show that toxin production is possible in kernels not obviously worm-damaged.

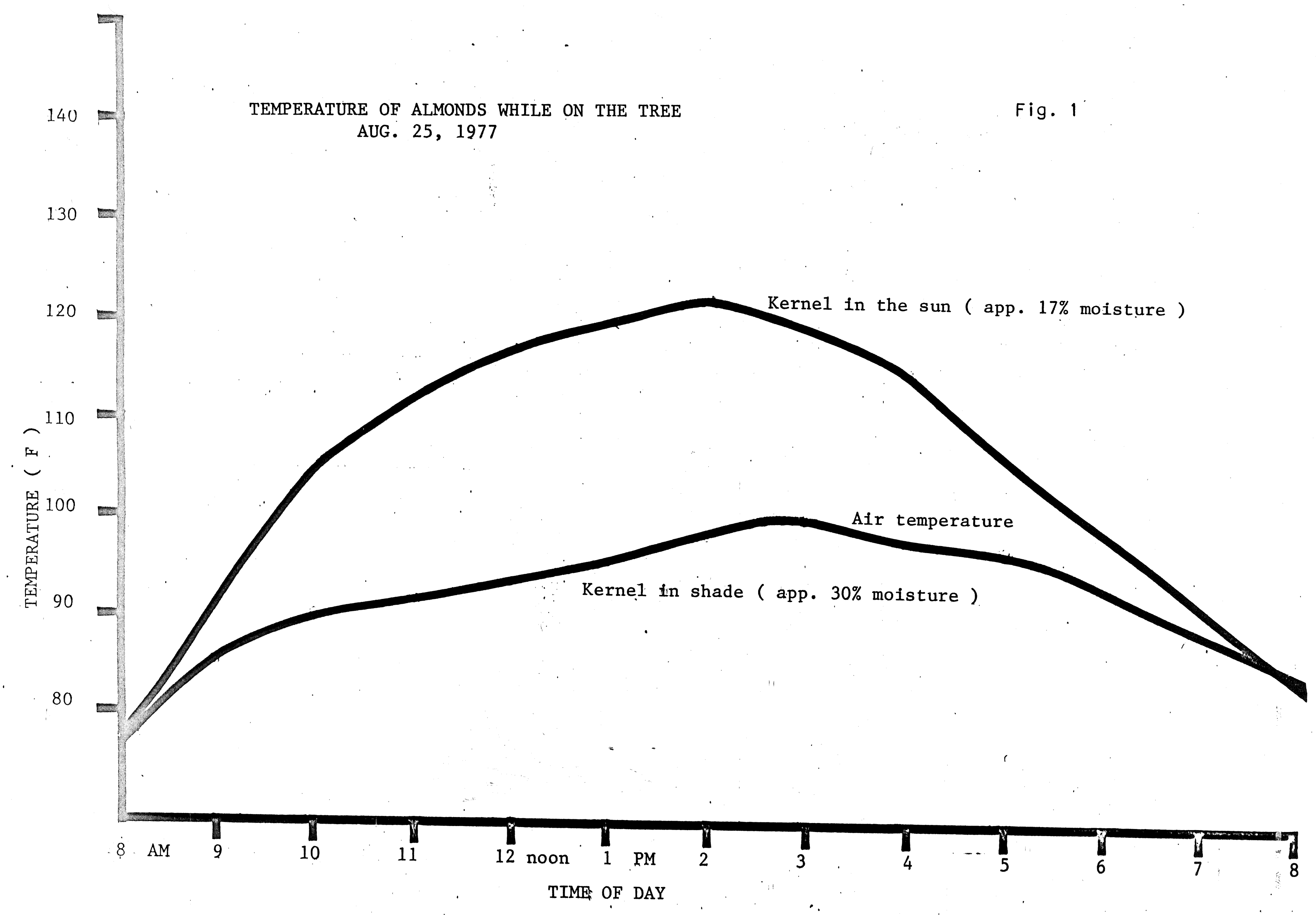
A combination of factors is required for aflatoxin contamination: (1) The presence of aflatoxin-producing strains of Aspergillus, and perhaps the lack of competing fungi; (2) A suitable substrate, which may mean some kind of damage to the almond kernels; (3) A moisture activity above .85 and a temperature above 12°C (54°F). This combination of factors may occur at times in the field, during the harvesting operation, or after harvest while hulling, shelling, and storing the almond crop.

REFERENCES

1. Mirocha, C. J. and C. M. Christensen. 1974. Fungus metabolites toxic to animals. Ann. Rev. Phytopath. 12: 303-330.

2. 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.

3. Schade, J. E., K. McGreevy, A. D. King, Jr., B. Mackey, and G. Fuller. 1975. Incidence of aflatoxin in California almonds. Applied Microbiology 29(1): 48-53.



TEMPERATURE OF ALMONDS WHILE ON THE TREE
AUG. 25, 1977
Fig. 1
140
130
120
110
100
90
80
TEMPERATURE (F)
Kernel in the sun (app. 17% moisture)
Air temperature
Kernel in shade (app. 30% moisture)
8 AM
9
10
11
12 noon
1 PM
2
3
4
5
6
7
8
TIME OF DAY

TEMPERATURE OF ALMONDS WHILE ON THE GROUND
SEPT. 7, 1977

Fig. 2

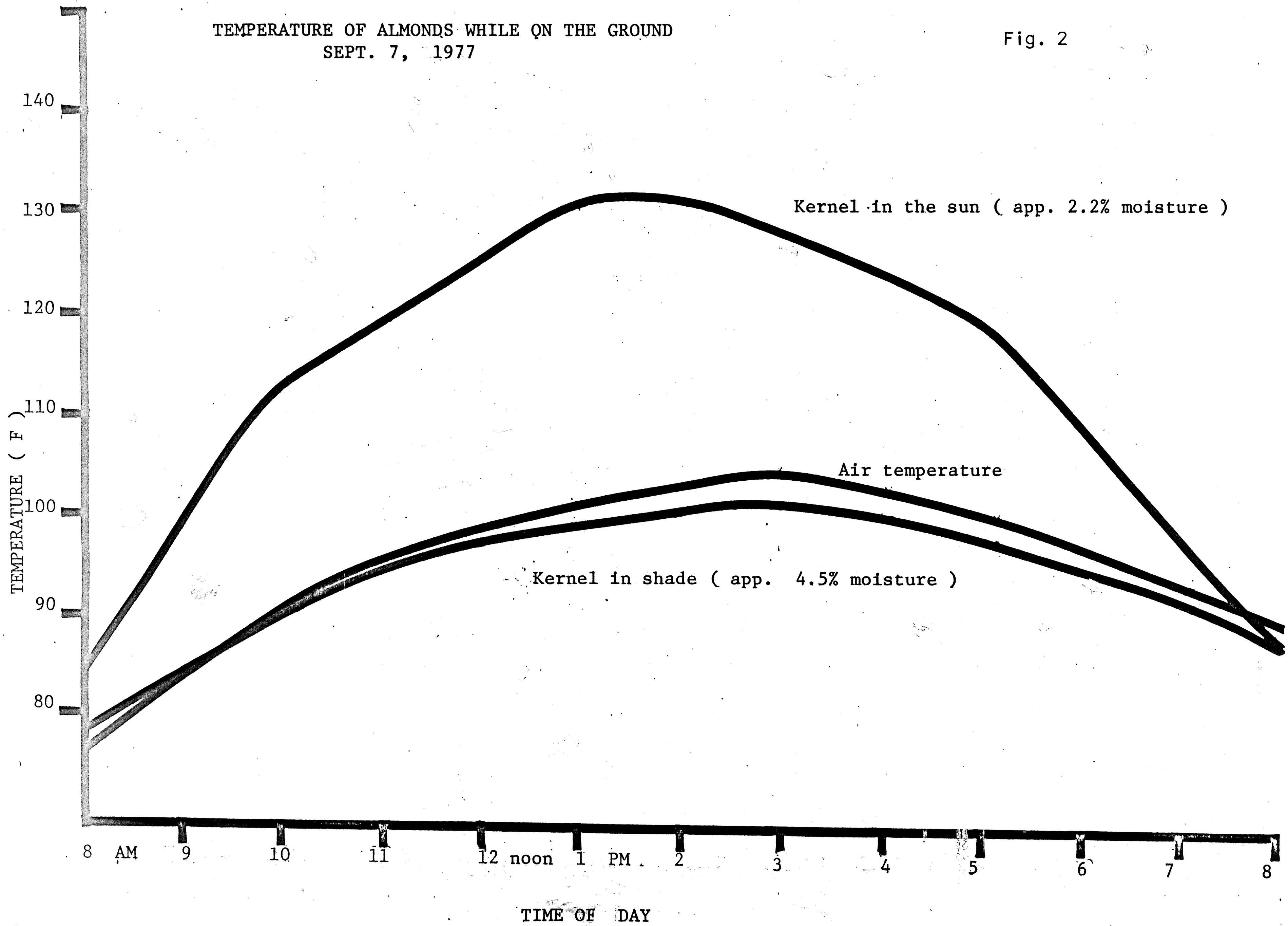


Table 1. Aflatoxin in undamaged almond hulls and kernels. The hulls were inoculated before hull split with dry spores or mycelium of 4 toxicogenic isolates of *Aspergillus flavus* or *A. parasiticus* with or without *Ulocladium chartarum*. The samples were analyzed for aflatoxin after 2 months of dry storage.

	Fungus present (+) or absent (-)			
Aspergillus flavus and *A. parasiticus*	+	+	-	-
Ulocladium chartarum	-	+	+	-
	Total aflatoxins (ng/g)[1]			
Hulls[2]				
I	395	143	0	0
II	420	0	0	0
III	20	15	0	0
IV	255	0	0	0
V	91	138	0	0
Mean	236.2	59.2	0	0
Kernels[2]				
I	6.4	0	0	0
II	0	0	0	0
III	0	0	0	0
IV	4.0	0	0	0
V	0	0	0	0
Mean	~~5.2~~ 2.1	0	0	0

[1]Aflatoxin analysis by G. Stanley, Dried Fruit Assoc. Laboratory, Fresno. 0 = less than 2 ng/g.

[2]Each sample contained 90 kernels or hulls.

Table 2.--The occurrence of *Aspergillus flavus* on almonds in sun or shade, and in almonds that had been surface disinfested or not disinfested.

Area and sample location	Percent *A. flavus* on Kernels[1]											
	Almonds in Shade						Almonds in Sun					
	Disinfested			Not Disinfested			Disinfested			Not Disinfested		
	Pct.			Pct.			Pct.			Pct.		
Bakersfield	1976	1977	Avg.	1976	1977	Avg.	1976	1977	Avg.	1976	1977	Avg.
On tree	.7	0	.4	7.3	18.0	12.7	1.3	0	.7	31.3	3.3	17.3
On ground	.7	0	.4	23.3	25.3	24.3	1.3	0	.7	25.3	41.3	33.3
Chowchilla												
On tree	0	0	0	2.7	3.3	3.0	0	0	0	3.3	10.0	6.7
On ground	0	0	0	0	11.3	5.7	0	0.6	0.3	7.3	46.6	27.0
Snelling												
On tree	0	1.3	.7	1.3	0.6	1.9	0	0.6	0.3	0.7	6.6	3.7
On ground	0	0	0	0	2.6	1.3	0	1.3	.7	2.0	4.0	3.0

[1] Each datum represents 3 samples of 50 kernels.

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WESTERN REGION

OF UNITED STATES DEPARTMENT OF AGRICULTURE

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Market Quality & Transportation Research Laboratory
Phone (209) 487-5334

December 31, 1977

1977 ANNUAL REPORT

Project No: 77-M4

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

Personnel: Dr. Douglas J. Phillips
Mr. Steven Purcell
Mr. Thor Hansen

1. Objectives:

To study factors that contirubte to or influence the occurrence of *Aspergillus flavus* and aflatoxins in almons 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 occurrence of *A. flavus* on almonds, as shown by increased incidence of *A. flavus* on hulls and kernels in the warmest growing areas and on almonds dried in the sun vs. those dried in the shade. When *A. flavus* was placed alone on the nuts it grew more and produced more aflatoxin than when it had to compete with other molds, i.e. *Ulocladium*. Cultural practices that favor competing organisms may provide a method of reducing damage from *A. flavus*.

3. Experimental Procedure:

Temperature of almonds.--Temperatures of almond kernels near Bakersfield were measured between 8 a.m. and 8 p.m. for a 30-day period beginning at the end of August through early September, 1977. Temperatures were taken of nuts on the tree and on the ground, and in the shade and in the sun.

Competing fungi to reduce aflatoxin production by A. flavus.--During the growing season, fruits of Nonpareil almond were inoculated with A. flavus (AF) alone or in combination with Ulocladium chartarum (referred to as Alternaria sp. in earlier reports). Two appropriate controls were maintained. The inoculation was made on the tree after first washing the green fruit with 50-100 ppm chlorine ($Cl\ O^-$) and, when dry, by taping bits of straw colonized by U. chartarum onto the fruit. The fruit was then enclosed in a cloth filter bag and spores of AF 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 aflatoxins after two months of dry storage by George Stanley of the Fresno Dried Fruit Laboratory.

The occurrence of A. flavus.--Almonds, hulls, and kernels were analyzed for Aspergillus spp. in 3 different orchards in each of 3 growing areas--Bakersfield, Chowchilla, and Snelling. Colonization of the nuts by Aspergillus spp. 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.

4. Results:

Temperature of almonds.--Figures 1 and 2 show the day-time temperature of kernels on the tree about a week before shaking and on the ground about a week after shaking. Although analysis of the 30 days of temperature date is incomplete, we generally observed that temperatures of kernels in the sun were 15-20°F higher than those in the shade or air while on the tree, and that temperatures of kernels in the sun were 20-25° higher than those in the shade or air while on the ground. The highest kernel temperature noted to date was 150°F for a kernel in the sun.

Competing fungi to reduce aflatoxin production by A. flavus.--The fungus U. chartarum, was antagonistic to AF and reduced aflatoxin to an undetectable level (less than 2 ng/g) in all the kernels. Aflatoxin in the hulls was reduced by 62% (from 161.1 to 61.2 ng/g) (Table 1). These results confirm our 1975 and 1976 tests, which provided evidence that the antagonistic fungi normally present on the hulls and kernels reduce the growth of AF. Although aflatoxin was found in sound kernels, the low amounts (average 1.9 ng/g) produced when the fruits were inoculated with 4 toxogenic isolates of Aspergillus sp, emphasize the low aflatoxin risk associated with sound kernels.

The occurrence of A. flavus.--AF occurred more frequently on almonds from field plots in the southernmost almond orchards than in those from the two centrally located orchards (Table 2). Of the two latter orchards, almonds from Chowchilla had a higher incidence of AF than those from Snelling. The 1977 results agree well with those of 1975 and 1976. AF generally occurred more frequently on almonds in the sun than on those in the shade.

5. Discussion:

Temperature of almonds.--With the complete analysis of data collected in the 1977 season we will have a good basis for estimating the intensity and duration of heat reaching almond kernels while drying on the tree or on the ground. Temperatures in excess of 120°F are potentially damaging to the kernels. The specific effects of these high temperatures on almonds while drying are not known, but other work suggests that such high temperatures may reduce the population of competing saprophytic fungi on the kernels, while not reducing the *Aspergillus* sp. Heat-damaged tissues also may increase the potential for invasion by AF and aflatoxin production.

Competing fungi to reduce aflatoxin production by *A. flavus*.--The reduction of aflatoxin by naturally occurring field fungi is now well documented in results from 4 years of experiments. As noted above, the competing population of saprophytic organisms on the surface of almonds are reduced during harvesting and drying. Consequently, the potentially dangerous condition of high AF populations and low competitive fungal populations may occur, and the re-wetting of thoroughly dry kernels triggers AF growth uninhibited by normally active competitors.

The occurrence of *A. flavus*.--The distribution of AF and aflatoxin is influenced by both the macro-climates and micro-climates found within the individual orchards. High temperature, moisture, and competitive fungi have been shown to influence the distribution of AF, while aflatoxin production is primarily influenced by insect damage. Thus the rate of drying and Navel orange worm activity are important in the production of aflatoxin in almonds. If the activity of the NOW is stopped by fumigation during the drying process, the production of aflatoxin also may be affected. The occurrence of AF on the hulls suggest factors that influence the rate of drying, such as irrigation and tillage practices at the time of hull split, are especially important to the establishment of high AF populations on the hull and to aflatoxin production.

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.

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Market Quality & Transportation Research Laboratory
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December 31, 1977

1977 ANNUAL REPORT

Project No: 77-M4

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

Personnel: Dr. M. Uota (Report prepared by Dr. Harvey)
Miss Elizabeth Elliston

1. Objectives:

(1) To determine the effects of controlled atmospheres (lowered oxygen and/or increased carbon dioxide levels) at various storage temperatures on the quality of shelled almonds;

(2) To develop systems for maintaining controlled or modified atmospheres in storage, transport, and distribution;

(3) To relate moisture content to the water activity of various forms of almonds (in shell, natural meats, blanched).

2. Interpretive Summary:

Almonds stored for a year at 80°F in sealed plastic bags with low oxygen or high carbon dioxide atmospheres had as good flavor and quality as almonds stored in normal air at 37°F. Use of the liners to maintain controlled atmospheres would reduce the need for refrigerated storage, and would improve quality maintenance during transport and distribution when almonds may be exposed to undesirably high temperatures. The liners would be particularly beneficial for export shipments.

Blanched, sliced, and diced almonds must be brought to a lower moisture content (4.5 to 5.0%) than whole natural kernels (5.5%) to reach the same level of water activity (0.65). The FDA has proposed that water activity must be below 0.70 to stop the growth of molds.

3. Experimental Procedure:

Storage.--Shelled almonds were held in sealed, laminated plastic bags with initial atmospheres of 0.1 or 21% oxygen (O_2) in combination with various levels of carbon dioxide (CO_2) for 14 months at 80°F (27°C). A check lot was held in air at 37°F (3°C). Quality of the nuts was evaluated by a taste panel (cooperative with D. Gaudagni, WRRC).

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 (A_w/100). After A_w was calculated for nuts with different moisture contents, the values were plotted against one another on a chart.

4. Results:

Storage.--Almonds stored in laminated plastic bags with either low oxygen or high carbon dioxide atmospheres had acceptable flavor and texture after 14 months at 80°F. During the long storage period, the oxygen levels in the sealed bags increased gradually,and the carbon dioxide levels decreased. Nevertheless, the quality of the nuts was better in the sealed bags than in a check lot held in air at 37°F.

Water activity (A_w).--Previous work showed that at 5.5% moisture, whole almond kernels have a water activity of about 0.65. An A_w of 0.70 has been proposed as the maximum level for almonds in packages or in storage. In blanched, whole almonds, the moisture content had to be reduced to 4.5% in order to achieve an A_w of 0.65; and in diced, or sliced, blanched almonds, a moisture content of 5.0% was equivalent to an A_w of 0.65.

5. Discussion:

Almond storage.--Plastic bin liners or carton liners potentially provide a means of maintaining desirable controlled or modified atmospheres during storage, transport, and distribution. Low oxygen or high carbon dioxide atmospheres can maintain almond quality as well at 80°F as regular air storage at 37°.

The liners tested to date are not completely impermeable to O_2 and CO_2, and consequently the concentrations of these gases change during lengthy storage. Various laminations of two or more kinds of film may retain these gases more effectively.

Use of an effective liner for maintaining desirable controlled atmospheres would reduce the need for refrigerated storage, and would maintain quality during transport or distribution when almonds are exposed to undesirably high temperatures. Controlled atmospheres would be particularly beneficial in export shipments.

Water activity (A_w).--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). 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 on other forms of almonds (blanched) shows that their moisture content must be lower than that of regular kernels to achieve the same A_w.

6. Publications:

None.

TEMPERATURE OF ALMONDS WHILE ON THE TREE
AUG. 25, 1977

Fig. 1

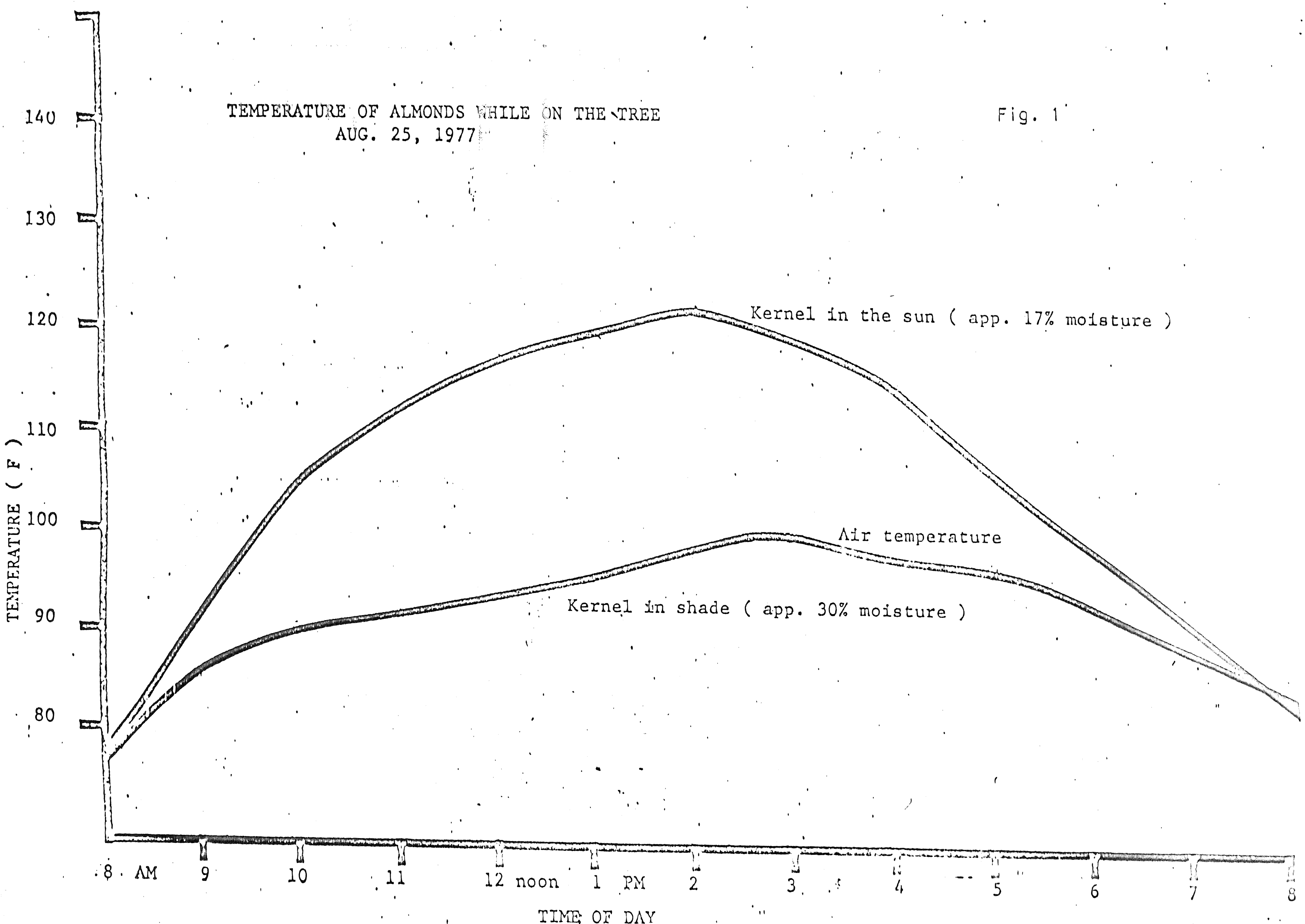

Fig. 2

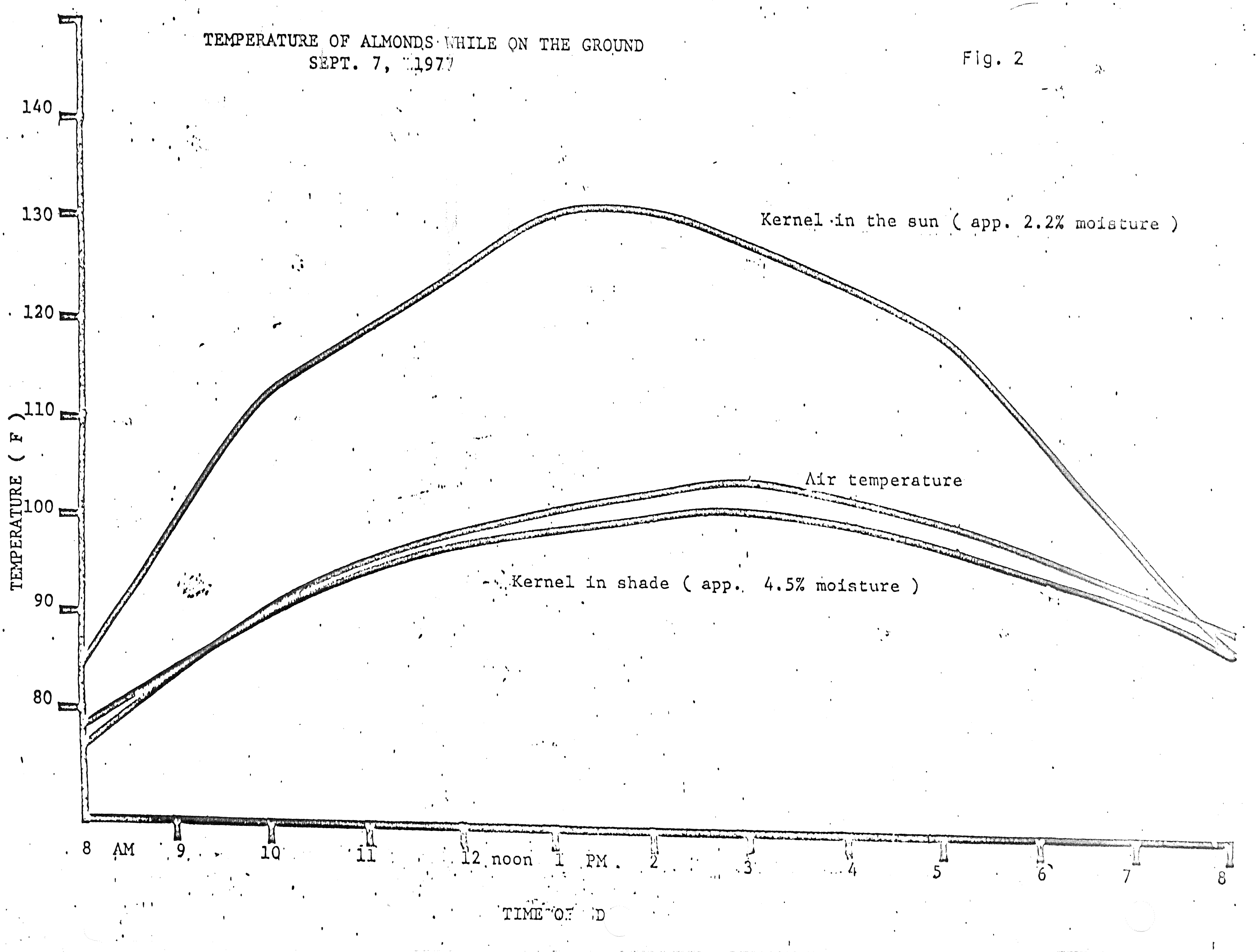
TEMPERATURE OF ALMONDS WHILE ON THE GROUND
SEPT. 7, 1977
Kernel in the sun (app. 2.2% moisture)
Air temperature
Kernel in shade (app. 4.5% moisture)
TEMPERATURE (F)
140
130
120
110
100
90
80
8 AM
9
10
11
12 noon
1 PM
2
3
4
5
6
7
8
TIME OF D

Table 1.--Aflatoxin in undamaged almonds inoculated before hull split with dry spores or mycelium of 4 toxicogenic isolates of <u>Aspergillus flavus</u> or <u>A. parasiticus</u> (AF), with or without the competitor, <u>Ulocladium chartarum</u> (UC), and held 2 months in dry storage.

Hull or Kernel	Sample No.	Total aflatoxins in almonds with indicated fungus			
		AF -	AF UC	- UC	- -
		ng/g	ng/g	ng/g	ng/g
Hulls[2/]	I	272.0[1/]	146.5	0	0
"	II	294.5	34.0	0	0
"	III	17.5	17.5	0	0
"	IV	145.0	0.8	0	0
"	V	76.5	107.0	0	0
"	Mean	161.1	61.2[3/]	0	0
Kernels[2/]	I	6.6	0	0	0
"	II	0	0	0	0
"	III	0	0	0	0
"	IV	2.8	0	0	0
"	V	0	0	0	0
"	Mean	1.9	0	0	0

[1/] Aflatoxin analysis by G. Stanley, Dried Fruit Assoc. Laboratory, Fresno. 0 = less than 2 ng/g.

[2/] Each sample contained 90 kernels or hulls.

[3/] Significantly lower than <u>Aspergillus</u> spp. alone at 80% confidence level.

Table 2.--The occurrence of *Aspergillus flavus* on almonds in sun or shade, and in almonds that had been surface disinfested or not disinfested.

Area and sample location	Percentage of kernels[1] with *A. flavus*											
	Almonds in Shade						Almonds in Sun					
	Disinfested			Not Disinfested			Disinfested			Not Disinfested		
	%	%	%	%	%	%	%	%	%	%	%	%
Bakersfield	1976	1977	Avg.	1976	1977	Avg.	1976	1977	Avg.	1976	1977	Avg.
On tree	0.7	0	0.4	7.3	18.0	12.7	1.3	0	0.7	31.3	3.3	17.3
On ground	0.7	0	0.4	23.3	25.3	24.3	1.3	0	0.7	25.3	41.3	33.3
Chowchilla												
On tree	0	0	0	2.7	3.3	3.0	0	0	0	3.3	10.0	6.7
On ground	0	0	0	0	11.3	5.7	0	0.6	0.3	7.3	46.6	27.0
Snelling												
On tree	0	1.3	0.7	1.3	0.6	1.9	0	0.6	0.3	0.7	6.6	3.7
On ground	0	0	0	0	2.6	1.3	0	1.3	0.7	2.0	4.0	3.0

[1] Each datum represents 3 samples of 50 kernels.