Correct Project Number 78-M5

78-M5

UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION

FEDERAL RESEARCH
WESTERN REGION
CALIFORNIA-HAWAII-NEVADA AREA
P.O. BOX 8143
FRESNO, CALIFORNIA 93727

January 12, 1979

1978 ANNUAL REPORT

Project No: 79-M6

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 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:

Summary (1978): We compared the aflatoxin content of worm-damaged kernels (Nonpareil) that were fumigated (a) before normal harvest, (b) after normal harvest and drying on the soil, and (c) just prior to hulling. Aflatoxin was found in the worm-damaged kernels of all three fumigation groups and did not occur at significantly different levels among the groups. Overall, 39% of the samples of worm-damaged kernels contained aflatoxin with an average of 111 ppb total aflatoxin. Because the aflatoxin was found in similar quantities in the worm-damaged kernels of the three fumigation groups, and because neither the fungus, A. flavus, nor the navel orange worm is commonly found colonizing the almond before hull split, it appears most likely that the aflatoxin in these samples was produced after hull split, but before harvest from the tree. Heat did not damage kernels in the laboratory when the heat exposure was comparable to the highest temperature observed in kernels on the tree in the orchards studied in 1976 and 1977.

3. Experimental Procedure:

Fumigation tests: The growth and development of Aspergillus flavus and the production of aflatoxin is favored in: (1) orchards located in the hot, southern region of the Central Valley of California,

(2) in orchards that are open and allow exposure of the almonds to sun, and (3) in orchards that have a history of damage caused by the navel orange worm. Samples of almonds from an orchard with all these characteristics were selected on three different dates--(1) August 9 (before normal harvest), (2) August 21 (after harvest and drying on the soil), and (3) on September 1 just prior to hulling. The three groups of samples were fumigated with phostoxin (0.06 g Hydrogen phosphide per 100 cubic feet) according to the following schedules: Group I was fumigated three times, 8-9-78, 8-21-78, and 9-1-78; Group II was fumigated twice, 8-21-78 and 9-1-78; and Group III was fumigated only once on 9-1-78. Each group contained six replicates. The first fumigation date for each group corresponded to the date navel orange worm activity should have stopped.

After hulling, the almonds were shelled (yield 15 - 25 pounds of kernels per replicated sample) and worm-damaged kernels were removed by hand sorting. The worm-damaged kernels (1-2% of the samples) were then ground and analyzed for aflatoxin at the Dried Fruit Association laboratory in Fresno.

Heat damage: Almond kernels (Nonpareil) were collected after hull split, but before they were dry enough for normal harvest onto the soil. Their moisture content was 35-43%. Kernels were held in eight temperature regimes which represented the highest temperature-time combinations (95% confidence) observed in our 1976 and 1977 studies of kernel temperatures in the field. The temperature regimes were: 40-45°C for 65 min., 45-50° for 55 min., 45-50° for 225 min., 50-55° for 15 min. and 205 min., 55-60° for 3 and 17 min., 60-65° for 1 min.

After exposure the kernels were soaked in water over night, their seed coats were removed, and they were stained 3 hours in 0.1% tetrazolium chloride. Seed with white spots after staining were considered damaged.

4. Results:

Fumigation plots: Aflatoxins were found in the worm-damaged kernels of all three fumigation groups, but did not occur at significantly different levels within the groups (Table 1). In the seven (out of the 18) samples that contained aflatoxin, the concentration ranged from 2.9 ppb to 1346 ppb.

During sorting, visible growth of the fungus, Aspergillus flavus, was observed on 10 kernels. If we consider the total number of kernels, about 1 in 20,000 unsorted kernels showed visible A. flavus, but it was observed only on worm-damaged kernels. If the total number of worm-damaged kernels is considered, about 1 in 300 worm-damaged kernels showed visible A. flavus.

Heat damage: Twenty percent of the kernels were injured after exposure to 50-55°C for 205 min., 28% were injured after 55-60°C for 17 min. and 65% of the kernels were injured after 60-65°C for 1 min.

The injurious regimes were those observed in orchards in 1977-78 to occur on the soil, but not while the kernels were on the tree.

5. Discussion:

Worm-damaged almond kernels may contain dangerous levels of aflatoxin, and it is important to control worm damage in the orchard before harvest. Early fumigation in the field did not stop or reduce the occurrence of aflatoxin. Thus, to reduce aflatoxin contamination of almonds in the orchard, the control treatment must be applied before harvesting the almonds from the tree.

It is unlikely that heat damage would normally occur while the almonds are on the tree. The possibility remains that moist almonds harvested abnormally early could be exposed to damaging temperatures.

Our earlier work (1977) indicated that dry kernels were not susceptible to heat damage at normal exposures. Therefore, it is unlikely that heat-damage to the kernel plays an important role in the occurrence of aflatoxin, or conditions that lead to the formation of aflatoxins.

Table 1.--The aflatoxin content of worm-damaged Nonpareil almonds fumigated at three dates, 1978

Groups dates selected, and fumigation dates	Kernels with worm damage[1]	Number of wormy kernels with *A. flavus* visible		Aflatoxin content of worm-damaged kernels
	(%)	(moldy kernels/ total kernels)		(ppb)[2]
Group I [3]				
Aug. 9	1.3	0/ 150		n.t
" "	1.0	0/ 125		51.3
" "	1.2	0/ 125		n.t.
" "	1.1	0/ 150		n.t.
" "	1.2	1/ 150		n.t
" "	1.3	1/ 200		254.0
		Total 2/ 900	(1 in 450)	
Group II [4]				
Aug. 21	2.5	0/ 300		2.9
" "	2.1	2/ 250		198.7
" "	1.4	1/ 200		63.9
" "	1.6	0/ 175		n.t.
" "	1.2	1/ 150		n.t.
" "	1.1	0/ 150		n.t
		Total 4/1225	(1 in 306)	
Group III				
Sep. 1	1.1	0/ 100		n.t.
" "	1.1	0/ 150		n.t.
" "	2.2	1/ 200		n.t.
" "	2.4	1/ 200		82.9
" "	0.9	1/ 100		1346.0
" "	0.9	1/ 75		n.t.
		Total 4/ 825	(1 in 206)	

1/ From 15-25 lb. samples.

2/ n.t. = less than 2 ppb

3/ Also fumigated on 8-21 and 9-1.

4/ Also fumigated on 9-1.