Almond Stockpile Monitoring for Aflatoxin Potential

Project No.:	08-AFLA2-Lampinen
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Objectives:

The objectives of this study are to examine temperature and moisture conditions in stockpiled almonds in several production areas in California. The ultimate goal is to develop recommendations for stockpiling that minimize potential for growth of *Aspergillus* spp. (*A. flavus* and/or *A. parasiticus*) that result in aflatoxin contamination of nuts. In addition, monitoring of an ongoing trial with varying levels of canopy cover will be continued to provide orchard level environmental data as well.

Interpretive Summary:

Almond stockpiles in Kern, San Joaquin and Glenn Counties were monitored during the fall 2007 (Table 1). Seven stockpiles were outfitted with temperature and relative humidity sensors at four depths in the stockpiles. Dataloggers with relative humidity and temperature sensors (Onset, Pocasset, MA 02559), were installed in stockpiles in Glenn, San Joaquin and Kern County. Dataloggers were equipped to monitor temperature and relative humidity at four locations in each stockpile. Sensors were placed near the top of the pile, near the bottom of the pile, and at two intermediate distances. Piles were chosen with a north-south long axis since this is the most common based on the authors' observations. In addition, ambient temperature and relative humidity were monitored at each location. Data were recorded every three minutes. Conditions were monitored at 1 to 3 minute intervals in the stockpiles throughout the storage period ranging from 84 to 150 days.

Nut and hull samples were taken at the time sensors were installed at all locations. In 2007, additional samples were taken during the time the pile was left in place at the San Joaquin and Kern County sites but not at the Glenn County site.

When initial moisture content of nuts was low, mold growth in stockpiles was minimal and accordingly there was no aflatoxin detected. However, stockpiling of nuts with a water activity notably above the recommended 0.65 - 0.70 (= equilibrium relative humidity of 65 - 70%) resulted in significant mold growth near the pile surfaces. The two piles where this was observed had initial moisture contents of: 1) hulls 13.1% and kernels 5.2%; and 2) hulls 12.0% and kernels 7.3%. There was Aspergillus growth at the top and bottom edge of these stockpiles and analysis of one pile showed this was associated with aflatoxin production. This growth and aflatoxin production was associated with wetting of the nuts and hulls resulting from significant condensation of moisture on tarps that was observed in these areas. Significant mold growth and aflatoxin were only associated with these outer portions of the piles, likely because within the piles, the equilibrium relative humidity came to a steady state below the maximum limits recommended by current storage guidelines. The data generated in this first year of stockpile monitoring will make a substantial contribution to refining management guidelines for stockpiling that minimize potential for Aspergillus growth and result in aflatoxin contamination of nuts.

For detailed data from stockpile temperature and relative humidity dataloggers, please see the Annual Report included on the CD (07-AFLA2-Lampinen, Almond Orchard and Stockpile Monitoring for Aflatoxin Potential). To briefly summarize the results, temperatures at all locations inside the pile tended to be higher than ambient temperatures. Temperature at higher positions in the stockpiles tended to be greater and relative humidity lower compared to that in lower positions. Differences in temperature between high and low positions in the piles tended to get less through the storage period. As expected, temperatures in the stockpiles decreased as the season progressed. Since the air in the stockpile is at equilibrium with the nuts and hulls, the water activity in the pile should be equal to the (relative humidity)/100 as shown on the bottom axis of Fig. 1. These data agree well with published recommendations on almond storage in the UC Almond Production Manual, Page 275 (UC Division of Agriculture and Natural Resources, Publication 3364). The levels of relative humidity in the Kern County stockpiles were well below the 65 - 70% relative humidity recommended in the UC Almond Production Manual (Page 275) to balance the mold growth potential with optimal texture, color, flavor and stability. King et.al (1983) found that fungal growth occurred at a water activity greater than 0.75 which is equal to an equilibrium relative humidity greater than 75%.

Mold growth was generally low in the Kern County stockpiles. Mold data for the Kern County Nonpareil stockpile #1 (high water, high nitrogen treatment) is shown in Table 2. There was no aflatoxin detected in any samples from this stockpile. Mold data for San Joaquin County Nonpareil stockpile #1, which was the wettest of all of the stockpiles initially, is shown in Table 3. Substantially more mold growth of all types occurred in this stockpile. The top and bottom edge of this stockpile were sampled since significant condensation and mold growth was observed in these areas. In general, the incidence

of green mold was very low in all the samples from the stockpiles, except those from the top and bottom edge samples of the San Joaquin stockpiles #1 and #3. Analyses showed there was aflatoxin production in these two areas.

The results from the 2007 season suggest that a major concern of growers and handlers should be to pay attention to the initial moisture content when stockpiling nuts. In general, conditions at the top and bottom edge of the stockpiles tended to be most problematic. This is likely due to wetting of the nuts and hulls by condensation of moisture that has been observed in these areas. The data generated in this first year of stockpile monitoring will make a substantial contribution to refining management guidelines for stockpiling that minimize potential for *Aspergillus* growth and resulting aflatoxin contamination of nuts.

2008 Stockpile Monitoring

In 2008, almond stockpiles in Colusa, Kern and San Joaquin Counties are being monitored. Six stockpiles (two in each county) were outfitted with temperature and relative humidity sensors at two depths in the center of the stockiles (near the surface and near the bottom) and two positions on the west or north side of the pile near the outer surface. The sensor placement is 2008 was based on locations where most prolific mold growth was seen in 2007.

At the Kern County site, samples for stockpiling were again taken from the Nonpareil rows in the high water/high nitrogen and moderate water/moderate nitrogen treatments in an irrigation/fertigation trial. However, the moderate water/moderate nitrogen treatment was converted to high water/high nitrogen in 2008, but canopy size differences still may make the differences significant. Stockpiles and sensors were installed at the Kern County site on 8/25/08. The piles were covered immediately and fumigated with phosphine gas on 8/27/08.

The San Joaquin County stockpile #1 was installed on 9/9/08 and San Joaquin County stockpile #2 was installed on 9/17/08. Both stockpiles were Nonpareil. San Joaquin County stockpile #2 has an east-west orientation for the long axis of the pile while all of the other stockpiles have a north south orientation for the long axis.

The Colusa County stockpile #1 was installed on 9/12/08 and Colusa County stockpile #2 was installed on 10/1/08. Both Colusa County stockpiles were also Nonpareil.

References:

- Kader, Adel A. 1996. "In-Plant Storage", pp. 274-277. In <u>Almond Production Manual</u>, Warren C. Micke, Technical Editor. University of California Division of Agriculture and Natural Resources, Publication 3364.
- King, A.D.Jr., W.U. Halbrook, G. Fuller, and L.C. Whitehand. 1983. Almond nutmeat moisture and water activity and it influence on fungal flora and seed composition. J. Food Sci. 48: 615-617.

		stockpile	hull	nut	stockpile	total days
Location	variety	start date	% moisture	% moisture	removal date	in stockpile
Glenn County #1	Nonpareil	9/9/2007	12.2	5.4	not sampled	
Glenn County #2	Nonpareil	9/9/2007	11.8	5.7	not sampled	
Kern County #1*	Nonpareil	8/20/2007	7.9	3.5	1/17/2008	150
Kern County #2**	Nonpareil	8/20/2007	5.7	3.1	1/17/2008	150
San Joaquin County #1	Nonpareil	9/6/2007	13.1	5.2	12/20/2007	104
San Joaquin County #2	Nonpareil	9/6/2007	9.2	5.6	not sampled	
San Joaquin County #3	Livingston	9/27/2007	12.0	7.3	12/19/2007	84

Summary of stockpile varieties, start dates, removal dates and initial moisture Table 1. content of hulls and nuts for all locations.

* High water/high nitrogen treatment.

** Moderate water/moderate nitrogen treatment.

Kern County stockpile #1 (cv. Nonpareil), high water/high nitrogen treatment, Table 2. sampled on January 17, 2008.

	Percentages of total inshell nuts sampled				
Location	Black mold ¹	White mold ¹	Green mold ^{1,2}	Mummies ¹	Aborted
Тор	3.7	1.4	0.0	2.1	4.8
Upper middle	2.2	0.3	0.1	2.4	5.4
Lower middle	1.5	0.0	0.0	1.6	5.7
Bottom	2.0	0.0	0.0	1.8	4.7

¹ Percentages include in shell nuts and hulls. ² No aflatoxin was detected from any samples from Kern County #1 in 2007.

Table 3. San Joa	quin County stockpile #1	(cv. Nonpareil)	il) sampled on December 20, 20)07
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	Percentages of total in shell nuts sampled					
Location	Black mold ¹	White mold ¹	Green mold ^{1,2}	Mummies ¹	Aborted	
Тор	10.3	3.1	31.8	13.7	9.2	
Upper middle	3.3	3.8	1.0	4.2	8.8	
Lower middle	5.4	7.7	0.0	11.7	6.7	
Bottom	6.4	9.9	2.1	12.4	3.6	
Bottom edge	7.5	11.3	14.4	43.2	3.2	

¹ Percentages include in shell nuts and hulls.

² Results from 120 isolations from kernels or shells with sporulation of green fungi revealed that 7.1% were Aspergillus flavus.

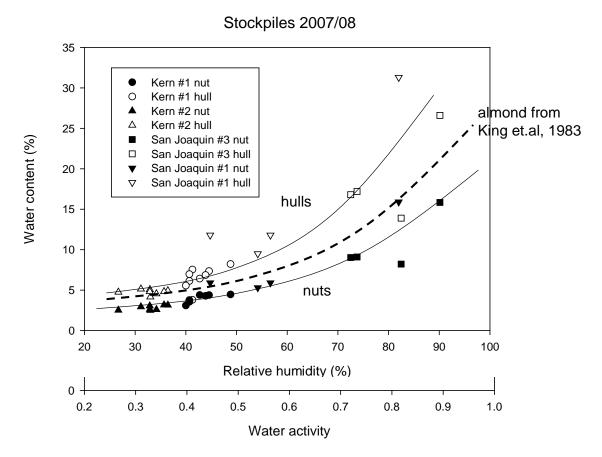


Fig. 1. Relative humidity and water activity versus water content for nuts (including shell) and hulls from the Kern and San Joaquin County stockpiles. Kern #1 nuts and hulls are from the high water, high nitrogen treatment and Kern #2 are from the moderate water, moderate nitrogen treatments. Data include cv. Nonpareil from Kern County as well as stockpile #1 and stockpile #3 from San Joaquin County. Dashed line is the approximate curve for almond kernels from King et. al, 1983.