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# Almond Orchard and Stockpile Monitoring for Aflatoxin Potential

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**Project No.:** 07-AFLA2-Lampinen

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## **Interpretive Summary:**

Almond stockpiles in Kern, San Joaquin and Glenn Counties were monitored during the fall 2007. Seven stockpiles were outfitted with temperature and relative humidity sensors at four depths in the stockpiles. Conditions were monitored at 1 to 3 minute intervals in the stockpiles throughout the storage period. Mold growth was minimal at all locations in stockpiles when initial moisture content of nuts was low. However, stockpiling of nuts with a water activity above the recommended 0.65 resulted in substantial mold growth. Sample analysis has not yet been completed to show whether or not aflatoxin was produced under these conditions. 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 resulting from condensation of moisture on tarps that was observed in these areas.

## **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* and resulting aflatoxin contamination of nuts. In addition, monitoring of an ongoing trial with varying levels of irrigation and canopy cover will be continued to provide orchard level environmental data as well.

## Field Data Collection

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.

## **Materials and Methods:**

### Samples- all sites

Nut and hull samples were taken at the time sensors were installed at all locations. 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. Samples were taken from the pile using an approximately 2 inch diameter metal tube with a perforation near the end. The tube was inserted into the pile and hooked to a vacuum cleaner to suck the samples out. A subsample (approximately 2.2 lbs) was taken from each location, weighed and separated in the field into hulls and in-shell kernels. These subsamples were then weighed in the field, brought to Davis, dried in an oven to constant weight, and reweighed. The stockpiles were removed at the Glenn County site before samples could be taken at the end of the storage period.

Samples were refrigerated until used. Each sample was split into 3 sub-samples. From each of the upper, upper middle, lower middle, and bottom of each stockpile for each sampling date.

Nuts of each sample were hulled, and examined macroscopically for obvious mold mycelia and sporulation. When mold was observed in the hull, shell, or in the nuts (viewed through the cracked shell), they were classified as black, white or green mold. In general, black mold included *Rhizopus stolonifer* (the hull rot fungus), *Aspergillus niger*, and *Alternaria* species as well as other black fungi. The white mold included mainly species of *Fusarium*. The green mold included *Aspergillus flavus*, green *Penicillium* species, and bluish-greenish species of *Emericella* and *Eurotium*.

Mummies (black nuts from the previous crop year), found in the samples were counted and separated for separate analysis. It is important to know if these nuts harbor aflatoxins to determine how much they may contribute to the aflatoxin contamination of a sample since some of many of these nuts were intact and will be hulled, cracked, and intermingled with the kernels of the 2007 crop.

## **Preliminary Results**

### Stockpile monitoring results

#### Glenn County Site

Dataloggers were installed as the two stockpiles were formed on September 9, 2007. The stockpiles were both in a north/south orientation and were approximately 14' wide and 38' long. The stockpiles were tarped (0.025" polyethylene tarp with white surface exposed and

black surface underneath) and fumigated on September 19, 2007. The stockpiles were taken down before samples could be taken.

### San Joaquin County Site

The initial installation was done on September 6, 2007 in two north/south oriented stockpiles that were approximately 32' wide, 600' long and 12' tall. The stockpiles were covered with 0.025" clear polyethylene tarps. Monitoring continued through September 18, 2007 when stockpile #2 was removed. The sensors from stockpile #2 were placed in a stockpile of the variety Livingston on September 27, 2007. Samples were taken from near all four sensors on December 5, 2007. The Livingston stockpile was removed on December 20, 2007 and samples were taken at all four sensor locations before stockpiles were dismantled.

### Kern County Site

At the Kern County site, samples were taken from the Nonpareil rows in the high water/high nitrogen and moderate water/moderate nitrogen treatments in an irrigation/fertigation trial for stockpiling. Stockpiles and sensors were installed at the Kern County site on 8/20/07 and removed on 1/17/08. The stockpiles were covered with 0.025" clear polyethylene tarps. Initial moisture content of nuts and hulls from the high water/high nitrogen treatment were 3.5 and 7.9% respectively. Initial moisture content of nuts and hulls from the moderate water/moderate nitrogen treatment were 3.2 and 5.7% respectively.

Data from the high water, high nitrogen and moderate water, moderate nitrogen stockpiles are shown in Fig. 1. Data are shown for one day near each date on which nut samples were taken from the piles. Generally, temperatures at all locations inside the pile tended to be higher than ambient temperatures (Fig. 1). Temperature at higher positions in the stockpiles tended to be greater and relative humidity lower compared to 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 (Fig. 1). Temperatures were similar in both of the Kern County stockpiles throughout the storage period (Fig. 1). Relative humidity was generally higher in all positions in the high water, high nitrogen treatment stockpile compared to the moderate water, moderate nitrogen stockpile (Fig. 1). Moisture content in the samples from the Kern County stockpiles is shown in the top of Fig. 2. The relationship between relative humidity in the stockpile versus water content of nuts and hulls for Kern (Nonpareil) and San Joaquin (Livingston) County stockpiles are shown in Fig. 3. 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. 3. These data agree well with published data (Almond Production Manual, Page 275). The levels of relative humidity in the Kern County stockpiles were well below the 65% relative humidity recommended in the 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 of 0.75 and above.

Mold growth was generally low in the Kern County stockpiles (Table 2-5). Black mold growth tended to be slightly higher at the end of the stockpiling period in the moderate water, moderate nitrogen treatment stockpile (Table 5) compared to the high water, high nitrogen treatment (Table 4). Growth of white and green mold was similar in these stockpiles. Only the samples from the high water, high nitrogen treatment stockpile have been analyzed for aflatoxins and there were no positives detected (Table 2a).

### San Joaquin County Site

The initial water content for the nuts and hulls from the San Joaquin County stockpile #1 was 5.2% and 13.1% respectively. These were the wettest values for any of the stockpiled nuts. During the storage period, relative humidity (and hence water activity) increased to above 80% near the top of the San Joaquin Nonpareil stockpile #1 (Fig. 5). Mold growth tended to be high in this stockpile (Table 6). Stockpiling nuts at this high initial moisture content could be problematic and should be avoided, if possible.

Relative humidity and temperature from the stockpile #3 in San Joaquin County (Livingston variety) are shown in Fig. 4. Water content at the time of stockpiling was 12% and 7.2% for the hulls and nuts respectively for the Livingston variety nuts at the San Joaquin County site. This is considerably wetter than the stockpiled hulls (7.9%) and nuts (3.5%) from the high water, high nitrogen treatments in Kern County. As expected, this resulted in considerably higher relative humidity in the San Joaquin County Livingston stockpile #3 (Fig. 4) compared to the Kern County stockpiles (Fig. 1).

The water activity at the bottom and top of the San Joaquin County Livingston stockpile was above 0.75 suggesting problems might occur. Growth of white and green mold was higher in the San Joaquin County Livingston stockpile (Table 7) compared to the Kern County Nonpareil stockpiles (Table 2 and 3).

In general, the incidence of green mold was very low in all the samples from the stockpiles, except those of the top samples of the San Joaquin stockpiles #1 and #3. For instance, preliminary analysis of samples from the San Joaquin County Nonpareil stockpile #1 on December 20 showed that 7.1% of isolations from 120 nuts were positive for *Aspergillus flavus*. The bottom edge of the pile was also sampled since condensed water running down the inside of the tarp resulted in wetting of nuts and hulls in this location. Considerable mold growth was observed in this location (Table 8) suggesting this is an area of concern.

### 2007 Summary

Thus far, only the samples from the high water, high nitrogen treatment from the Kern County stockpile have been analyzed for aflatoxin (Table 2). No trace of aflatoxin was detected in any of the samples from the top, upper middle, lower middle, bottom, nor in the mummies from these samples (Table 2A). However, looking at the results in Tables 6, 7 and 8, the nuts from the San Joaquin stockpiles should be analyzed for aflatoxin in order to make definite conclusions about the contribution, if any, of the stockpile conditions to aflatoxin

contamination. The presence of a relatively high incidence of green mold in these samples suggests that the conditions should have been favorable for growth of all of these fungi.

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.

### References

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.

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

Location	variety	stockpile	hull	nut	stockpile	total days
		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	12/19/2007	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	12/20/2007	84

**Table 2.** Kern County Nonpareil stockpile, high water/high nitrogen treatment sampled on November 17, 2007

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1</sup>	Mummies <sup>1</sup>	Aborted
Top	5.0	1.2	0.2	1.4	0.0
Upper middle	13.8	1.4	1.0	1.7	0.0
Lower middle	13.2	5.5	0.1	1.0	0.0
Bottom	12.3	2.2	0.4	1.6	0.0

<sup>1</sup> Percentages include in shell nuts and hulls.

**Table 2a.** Kern County Nonpareil stockpile almond samples from the high water/high nitrogen treatment (Table 1) analyzed for aflatoxins.

Location in Stockpile	Sample	Rep 1	Rep 2	Rep 3
Top	Inshell Nuts	None Detected	None Detected	None Detected
	Hulls	None Detected	None Detected	None Detected
	Mummies	None Detected	None Detected	None Detected
Upper Middle	Inshell Nuts	None Detected	None Detected	None Detected
	Hulls	None Detected	None Detected	None Detected
	Mummies	None Detected	None Detected	None Detected
Lower Middle	Inshell Nuts	None Detected	None Detected	None Detected
	Hulls	None Detected	None Detected	None Detected
	Mummies	None Detected	None Detected	None Detected
Bottom	Inshell Nuts	None Detected	None Detected	None Detected
	Hulls	None Detected	None Detected	None Detected
	Mummies	None Detected	None Detected	None Detected

**Table 3.** Kern County Nonpareil stockpile, moderate water/moderate nitrogen treatment sampled on November 17, 2007

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1</sup>	Mummies <sup>1</sup>	Aborted
Top	7.3	5.4	0.5	1.6	0.0
Upper middle	4.1	3.9	0.6	2.0	0.0
Lower middle	4.6	0.7	0.1	2.0	0.0
Bottom	4.4	0.9	0.3	1.4	0.0

<sup>1</sup> Percentages include in shell nuts and hulls.

**Table 4.** Kern County Nonpareil stockpile, high water/high nitrogen treatment sampled on January 17, 2008.

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1</sup>	Mummies <sup>1</sup>	Aborted
Top	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

<sup>1</sup> Percentages include in shell nuts and hulls.

**Table 5.** Kern County Nonpareil stockpile, moderate water/moderate nitrogen treatment sampled on January 17, 2008.

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1</sup>	Mummies <sup>1</sup>	Aborted
Top	7.8	0.5	0.0	2.5	6.9
Upper middle	5.7	1.3	0.0	2.4	5.6
Lower middle	8.3	0.7	0.0	2.0	9.6
Bottom	9.6	1.6	0.2	2.0	4.1

<sup>1</sup> Percentages include in shell nuts and hulls.

**Table 6.** San Joaquin County Nonpareil stockpile #1 sampled on December 3, 2007

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1,2</sup>	Mummies <sup>1</sup>	Aborted
Top	9.5	3.8	10.4	1.9	10.0
Upper middle	4.8	3.8	1.8	3.7	16.9
Lower middle	6.9	5.5	1.5	3.5	3.9
Bottom	5.6	5.9	1.3	2.8	1.9

<sup>1</sup> Percentages include in shell nuts and hulls.

<sup>2</sup> Isolations from 50 kernels or shells with sporulation of green fungi did not recover any *Aspergillus flavus*.

**Table 7.** San Joaquin County Livingston variety stockpile #3 sampled on December 19, 2007

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1</sup>	Mummies <sup>1</sup>	Aborted
Top	16.7	52.6	40.2	9.9	2.1
Upper middle	36.1	2.9	1.2	2.5	1.1
Lower middle	23.6	11.3	2.0	2.3	0.9
Bottom	34.4	9.4	4.2	1.4	0.0

<sup>1</sup> Percentages include in shell nuts and hulls.

<sup>2</sup> Results from 50 isolations from kernels or shells with sporulation of green fungi revealed that 8.0% were *Aspergillus flavus*.

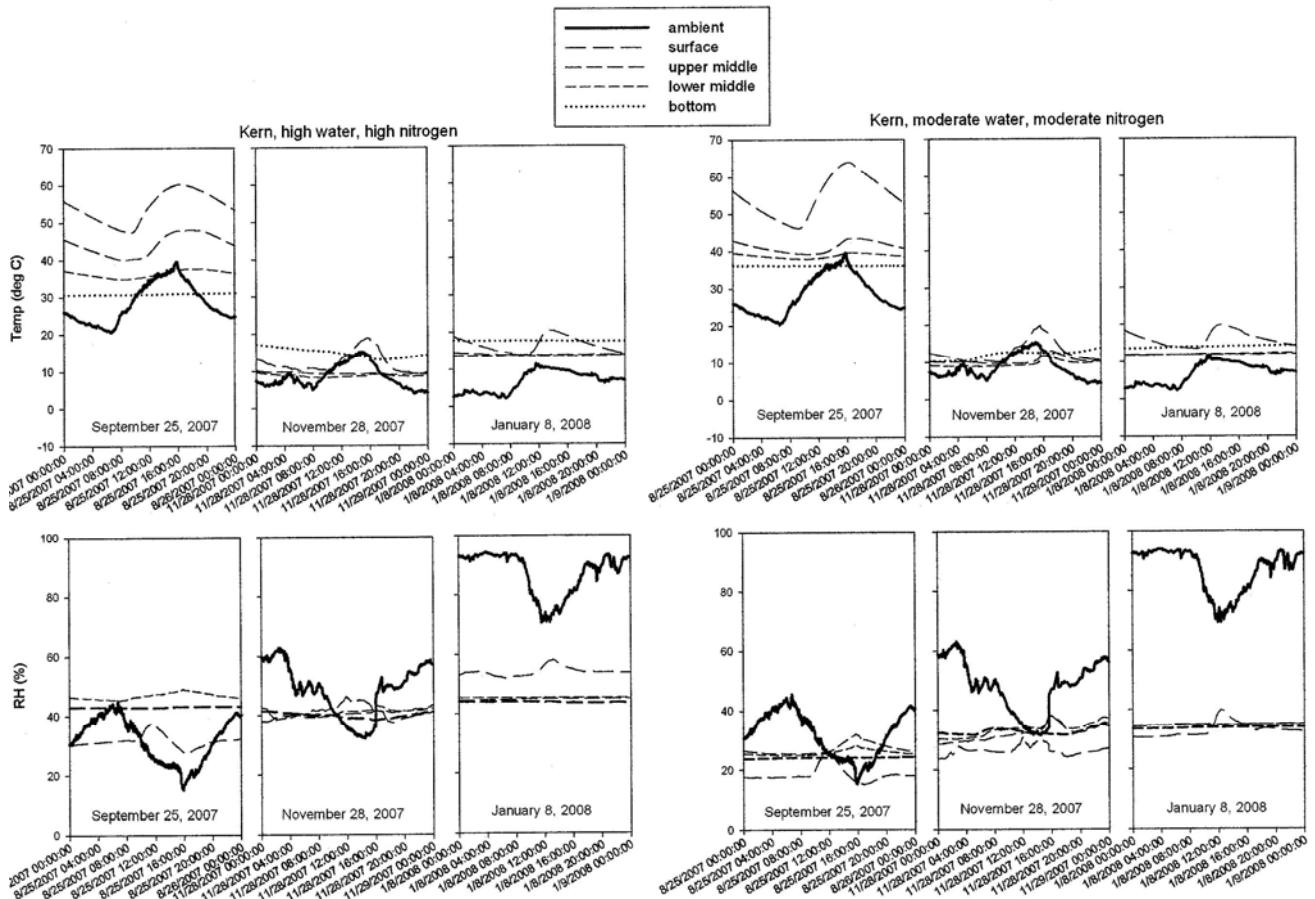
**Table 8.** San Joaquin County Nonpareil stockpile #1 sampled on December 20, 2007

Location	Percentages of total in shell nuts sampled				
	Black mold <sup>1</sup>	White mold <sup>1</sup>	Green mold <sup>1,2</sup>	Mummies <sup>1</sup>	Aborted
Top	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

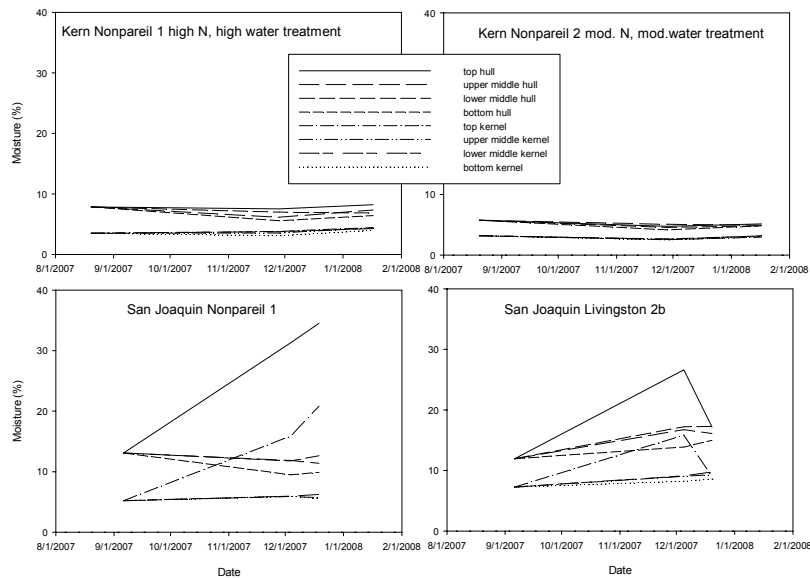
<sup>1</sup> Percentages include in shell nuts and hulls

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

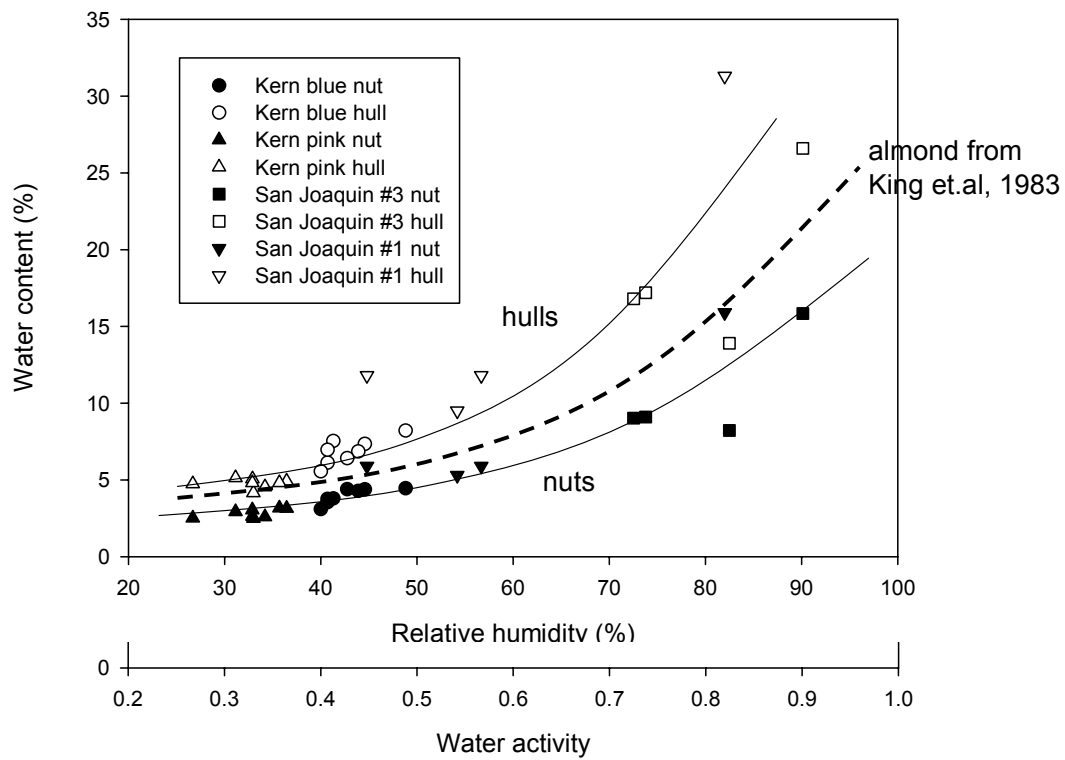




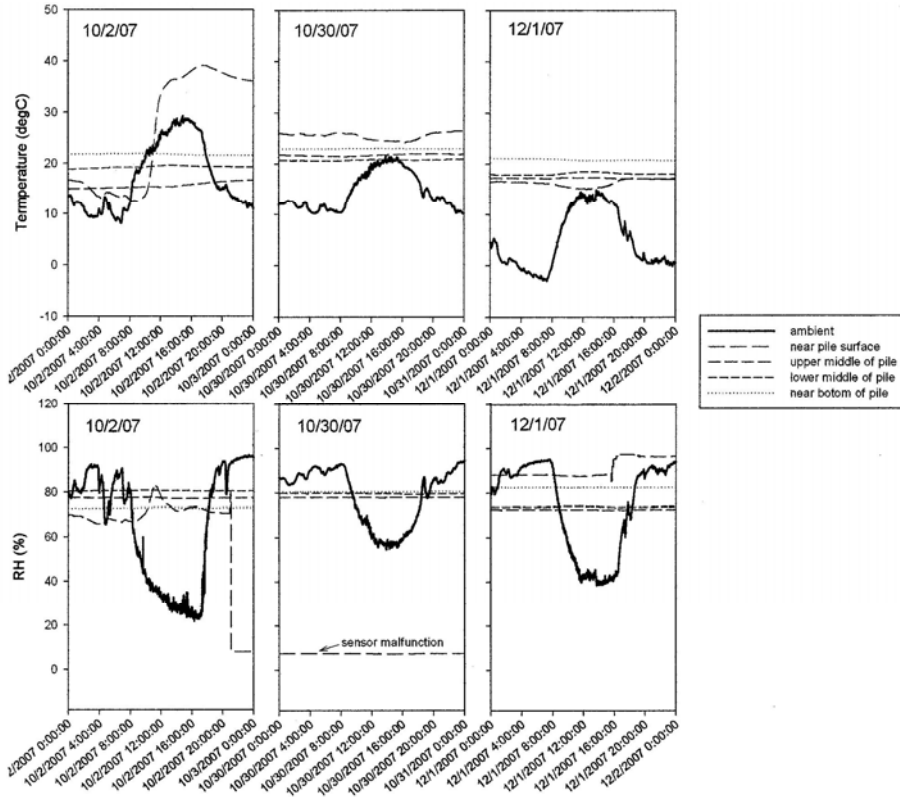
**Fig. 1.** Temperature and relative humidity on three dates at various location in stockpiles from the high water, high nitrogen treatment nuts and the moderate water, moderate nitrogen treatments in Kern County.



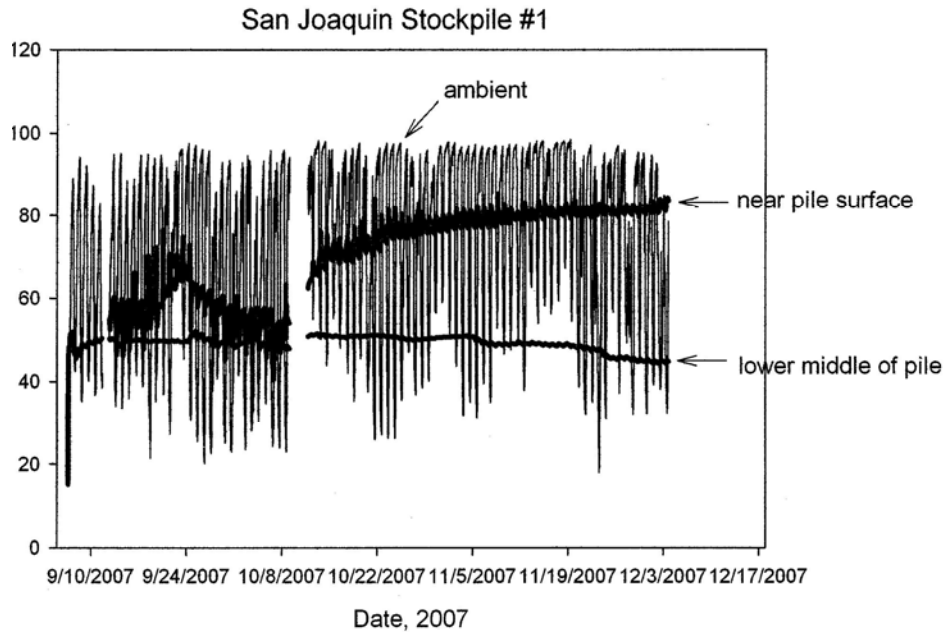
**Fig. 2.** Moisture content of nuts sampled from Kern and San Joaquin County stockpiles at three sampling dates.



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



**Fig. 4.** Temperature and relative humidity on three dates at various locations in stockpiles from the San Joaquin County stockpile #3 with the variety Livingston.



**Fig. 5.** Relative humidity inside radiation shelter next to stockpile (ambient), near top of stockpile and at middle bottom of stockpile. Bottom sensor malfunctioned partway through storage period and hence is not shown but values were similar to those for middle lower sensor shown.