As applied

# Risk Factors, Spatial Patterns, and Biocontrol of Aflatoxin Contamination in California Almonds

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## INTRODUCTION:

**Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*, are the most potent liver carcinogens and are widely regulated by governments who have set very low tolerances for aflatoxins in food and feed. The almond industry has taken extensive measures to control aflatoxin. This project seeks to further this effort with the two objectives outline below.**

## OBJECTIVES:

1. **Identify risk factors and spatial patterns associated with aflatoxin development in California almonds.**
2. **Determine the spread and survival of the atoxigenic (non-aflatoxin) *Aspergillus flavus* strain AF36 previously applied to an orchard.**

## MATERIALS AND METHODS:

**Objective 1.** Risk factors identified include:

A.Toxigenicity of *Aspergillus* populations

Soil samples were collected in 2007, 2008, and 2010 from 28 almond orchards in southern, central, and northern regions. Ten isolates of *Aspergillus* section Flavi were obtained from each orchard. Using an HPLC, the ability of each isolate to produce aflatoxin was determined; only results of the 2007 & 2008 isolates are presented.

B. Aflatoxin contamination of almond nuts in the field.

Samples of Nonpareil almond nuts were collected in August to September in both 2008 and 2009 from one orchard each in Kern, Stanislaus, and Glenn Counties immediately after trees were shaken and when the nuts were swept on the day they were removed from the orchard. The samples were analyzed for aflatoxins using a HPLC at Kearney Agricultural Center.

C. Navel orangeworm (NOW) studies.

In 2009 and 2010, we continued sampling periodically NOW moths from an almond orchard in Madera County to follow on the population levels of moths carrying viable propagules of *Aspergillus* section Flavi fungi. A total of 50 moths were plated per trap when there were >50 moths per trap and all the moths were plated onto Si10 salt medium, when there were <50 moths per trap. *Aspergillus* spp. were identified after incubating the plates at 30ºC (86ºF) for a week.

D. *Aspergillus* in almond mummies.

To determine the incidence of *Aspergillus* section Flavi on mummies, more than 500 mummies were collected from a Nonpareil almond orchard in Madera County. Isolations from the mummies were classified in the sections Flavi (*A. flavus, A. parasiticus,* and *A. tamarii*) and section Nigri (*A. niger, A. japonicus,* and *A. carbonarius*).

**Objective 2.** Bio-control technique of "seeding" the non-aflatoxin producing AF36 *A. flavus*.

The atoxigenic strain AF36 was applied as colonized wheat seed **(Figure 1)** to the soil at a rate of 10 pounds of seed per acre in a Nonpareil orchard at the Nickels Soil Laboratory on 28 June 2007 and 2 July 2008. After applying the wheat-AF36 product, the orchard was irrigated with micro-sprinklers and soil samples were collected just before applying the AF36 and at harvest in 2007, 2008, and again in 2009 and 2010. However, no AF36 was applied in July 2009 and 2010. Soil (0.02 to 0.2 g per sample) was plated on Si10 media and the *Aspergillus* spp. recovered were identified **(Figure 2).** Strain determination was done using Vegetative Compatibility Group (VCG) assays.


**Figure 1.** Wheat inoculum of non-aflatoxin producing strain AF36 of *A. flavus* as applied in the field **(left)** and after sporulation on the seed following rehydration after irrigation **(right)**.

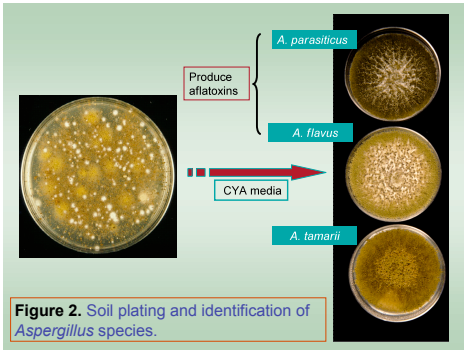


**Figure 2.** Soil plating and identification of *Aspergillus* species.

## RESULTS:

**Objective 1.** Identify risk factors and spatial patterns associated with aflatoxin development in California almonds.

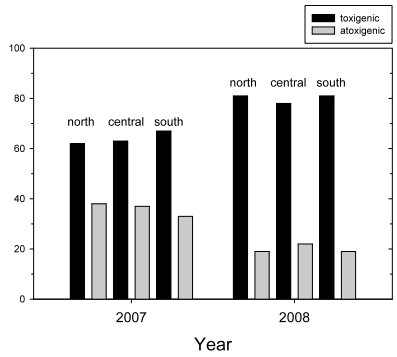


**Figure 3.** Incidences of toxigenic and atoxigenic *Aspergillus flavus* L-strain isolates across California.

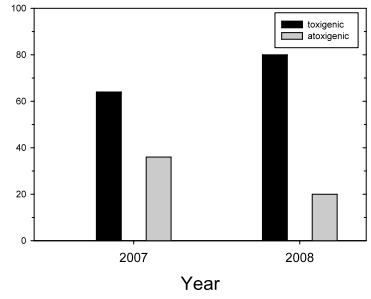


**Figure 4.** Incidences of toxigenic and atoxigenic *Aspergillus flavus* isolates in California.

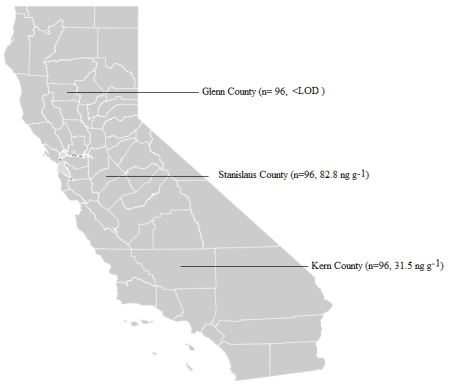


**Figure 5.** A California map showing the orchard locations where almond samples were collected and analyzed for aflatoxins. In parentheses, number of analyzed samples and the mean aflatoxin level in ng g-1 of positive samples.

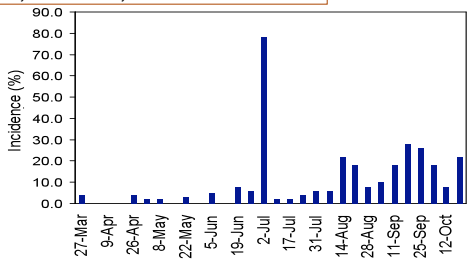


**Figure 6.** Incidence of *Aspergillus* section *Flavi* recovered from NOW moths trapped on sticky traps in an almond orchard in Madera County during March to October 2009.

*Aspergillus* in almond mummies:

In mummies collected in winter 2009 from a Madera orchard, 96% had *Aspergillus* section *Flavi.* Even mummies with now NOW damage were highly infected (84%) with *Aspergillus* section Flavi. This explains the high incidence of *Aspergillus* on NOW moths that emerge for mummies.

**Objective 2.** Determine the spread and survival of the atoxigenic *Aspergillus flavus* strain AF36 previously applied to an orchard.

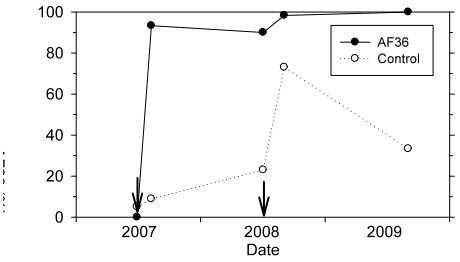


**Figure 7.** Percentage of *Aspergillus flavus* isolates belonging to the atoxigenic strain AF36 for isolates from soil collected from the areas treated with the wheat-AF36 product or from untreated areas in a research almond orchard at the Nickels Soil Laboratory. The wheat-AF36 product was applied on 28 June 2007 and 2 July 2008 (arrows).

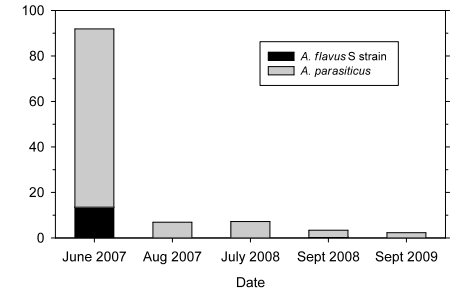


**Figure 8.** Percentage of *Aspergillus flavus*/*A. parasiticus* isolates that are the aflatoxin-producers *A. parasiticus* and *A. flavus* S strain for isolates from soil collected from areas treated with the wheat-AF36 product in a research almond orchard at the Nickels Soil Laboratory.

## CONCLUSIONS:

1. Aflatoxin-producing strains of *Aspergillus* ranged from 60 to 65% in 2007 and about 80% in 2008, in orchards of all south, central, and north regions.
2. Toxigenic strains of *Aspergillus* increased in 2008, following a year (2007) of high NOW damage.
3. No differences in aflatoxins were found in nuts collected immediately after shaking the trees and nuts taken after sweeping them in wind rows 7 to 10 days later.
4. Aflatoxin was found more in samples from southern orchards than northern.
5. The bio-control technique of "seeding" the atoxigenic (non-aflatoxin) producing AF36 strain of *A. flavus* is showing promise in almonds.
6. This strain is inoculated into the field and displaces the naturally present aflatoxin-producing fungal strains.