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 successful measures to control aflatoxin. This project seeks to further this effort with the two objectives outlined 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 *Aspergillus flavus* L-strain isolates were obtained from each orchard **(Figure 2)**. Using an HPLC, the ability of each isolate to produce aflatoxin was determined.

B. Physiological almond development associated with preharvest aflatoxin development

During the month of August 2010, shortly before and after the almond hull splits, weekly samplings of Nonpareil almonds were performed. Almond kernel moisture content was measured for each sampling time. In order to evaluate the most susceptible moisture stage for aflatoxin contamination of almonds, three highly toxigenic *Aspergillus* strains, *A. flavus* "S-strain", *A. flavus* "L-strain" and *A. parasiticus* were inoculated on the almond hull and the peeled almond kernel. After 30 days incubation at 30ºC, aflatoxin content was extracted with AflaTest® immunoaffinity columns and concentrations were quantified with High Pressure Liquid Chromatography (HPLC) analyses.

C. Damage by Navel Orangeworm, Infection by *Aspergillus flavus* and *A. parasiticus*, and potential of aflatoxin contamination in almond mummies.

Almond fruit (mummies) of the cultivars Nonpareil, Butte, and Padre were collected periodically during the winter of 2005 and 2006 in Madera County, California. The damage of almond kernels by the lepidopteron navel orangeworm (NOW; *Amyelois transitella*), the incidence, and strain distribution of *Aspergillus* section Flavi were recorded. In addition, the potential of the isolates from mummies in producing aflatoxins was determined and the amounts of aflatoxins were quantified.

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 June 28, 2007, July 2, 2008 and reapplied on June 6, 2011. 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 *Aspergillus flavus* as applied in the field **(left)** and after sporulation on the seed following rehydration after irrigation **(right)**.

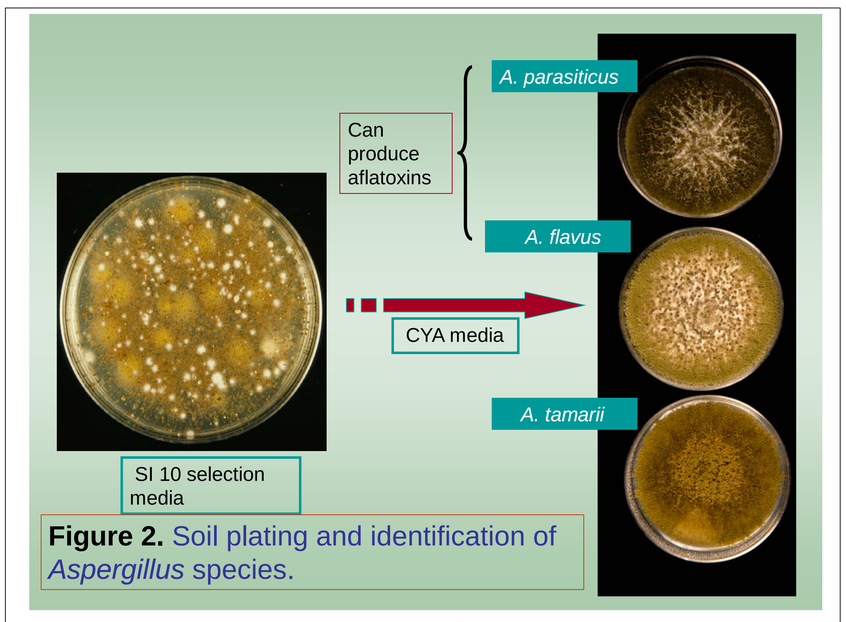

Figure 2. Soil plating and identification of *Aspergillus* species.

RESULTS:

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

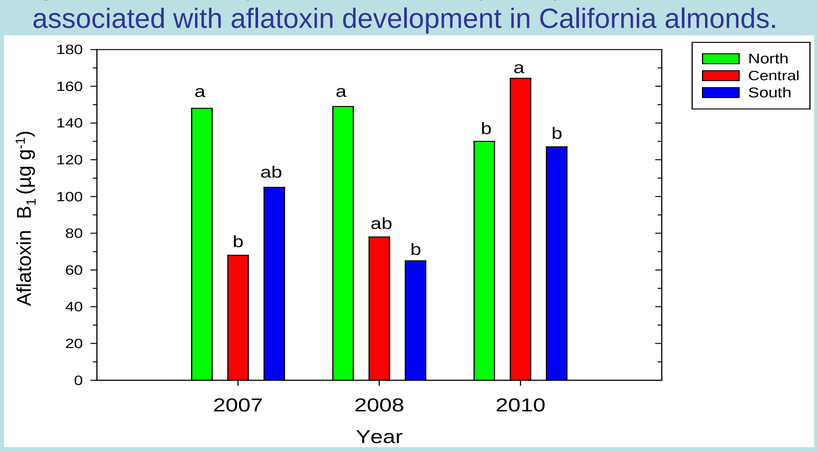

Figure 3. Aflatoxin quantities of *Aspergillus flavus* L-strain isolates collected from different regions for three years.

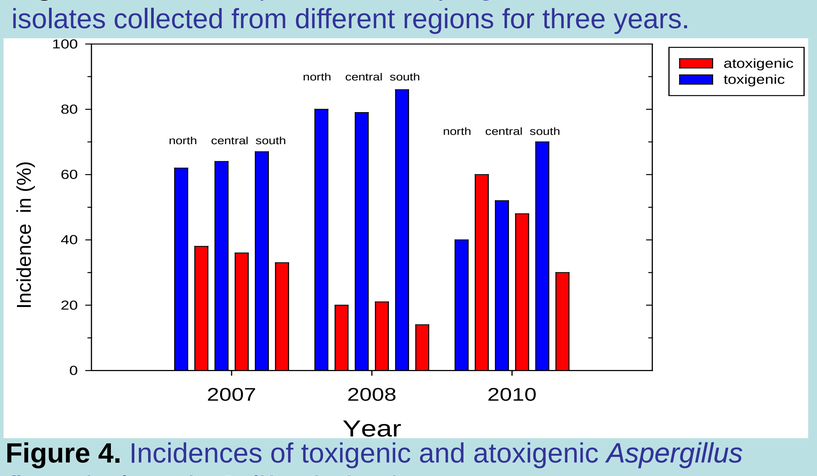

Figure 4. Incidences of toxigenic and atoxigenic *Aspergillus flavus* isolates in California for three years.

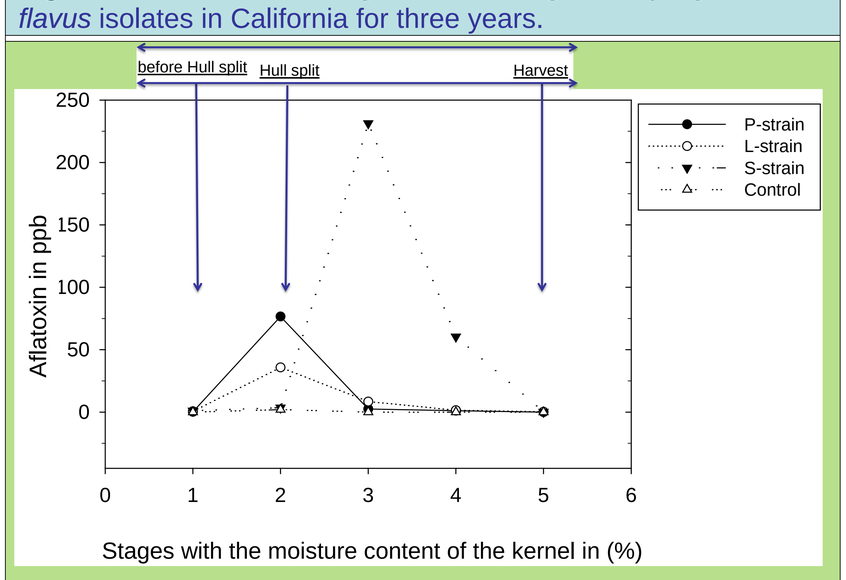

Figure 5. Aflatoxin concentrations of almonds inoculated on the **shells** with different *Aspergillus* isolates

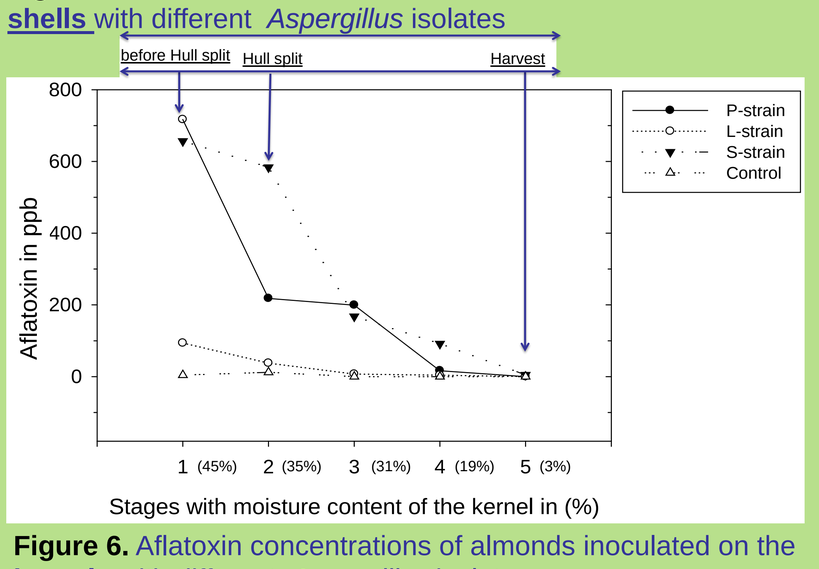

Figure 6. Aflatoxin concentrations of almonds inoculated on the **kernels** with different *Aspergillus* isolates

Aspergillus in almond mummies:

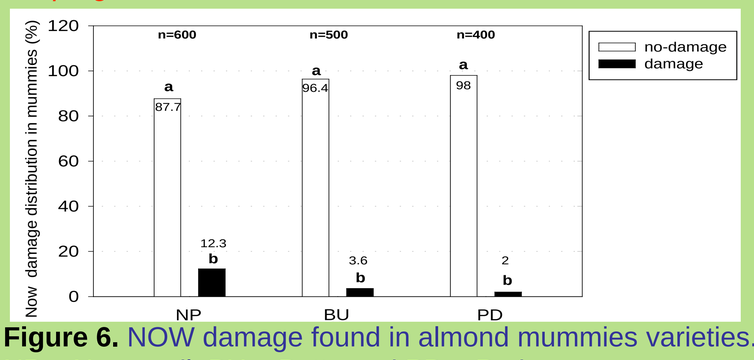

Figure 6. NOW damage found in almond mummies varieties. NP = Nonpareil, BU = Butte and PD = Padre.

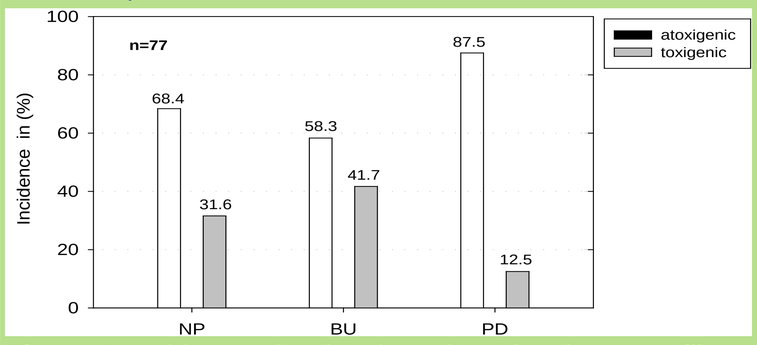

Figure 7. Incidence of toxigenic and atoxigenic *Aspergillus section* Flavi isolates from almond mummies.NP = Nonpareil, BU = Butte and PD = Padre.

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

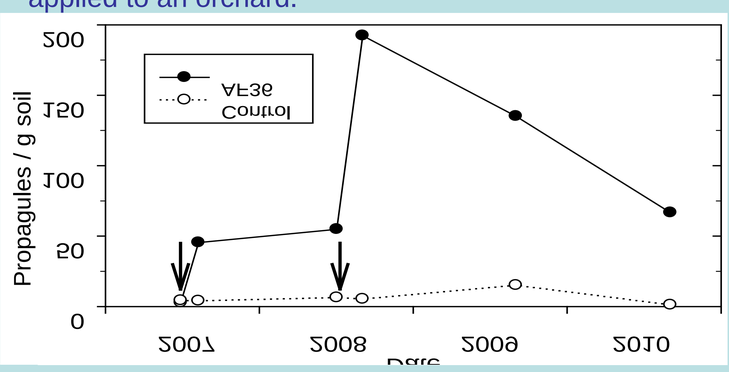

Figure 8. Density of *Aspergillus flavus/A. parasiticus* in soil collected from areas treated with the wheat-AF36 product or from untreated areas in a research almond orchard at the Nickels Soil Laboratory. The wheat-AF36 product was applied on June 28, 2007 and July 2, 2008 (arrows).

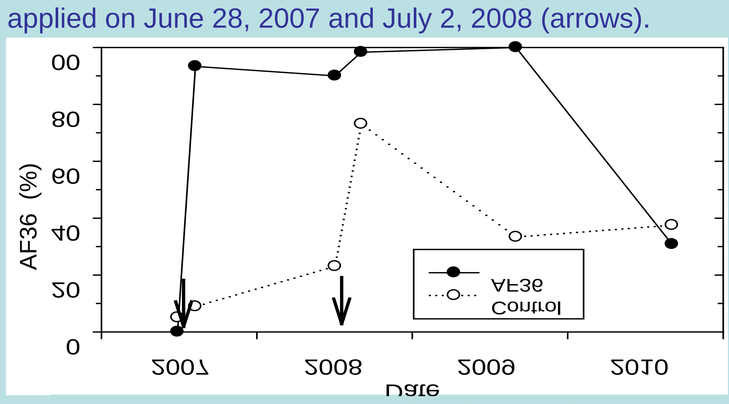

Figure 9. Percentage of *Aspergillus flavus* isolates belonging to the atoxigenic strain AF36 for isolates from soil collected from the areas treated with the wheat-AF36 product or from untreated areas in a research almond orchard at the Nickels Soil Laboratory. The wheat-AF36 product was applied on June 28, 2007 and July 2, 2008 (arrows).

CONCLUSIONS:

1. The highest average aflatoxin concentrations were found in the north in 2007 and 2008 and the central region in 2010.
2. Aflatoxin-producing strains of *Aspergillus* ranged from 60 to 65% in 2007 and about 80% in 2008, whereas in 2010 it varied widely from 40% in the north to 70% in the southern regions.
3. Susceptibility for aflatoxin contamination decreased with a decrease in the moisture content of the kernels.
4. Immediately after the hull split of almonds, their kernels are most susceptible.
5. The incidence of NOW damage was significantly higher in Nonpareil (12.3%) than in Butte (3.6%) or Padre (2%) almond mummies.
6. The bio-control technique of "seeding" the atoxigenic (non-aflatoxin) producing AF36 strain of *A. flavus* is showing a lot of promise in almonds.
7. This strain is inoculated into the field and displaces the naturally present aflatoxin-producing fungal strains for up to 2 years.

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