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

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OBJECTIVES

The focus of this research is to provide background for obtaining an Experimental Use Permit (EUP) and ultimately an almond registration for the atoxigenic *Aspergillus flavus* strain AF 36 to use as a biocontrol agent to reduce aflatoxin potential in the orchard. AF 36 is currently registered and being used successfully in other crops.

- Identify risk factors and spatial patterns associated with aflatoxin development in almonds.
- Determine the spread and survival of the atoxigenic *Aspergillus flavus* strain AF36 previously applied to orchards.
- Obtain an EUP and registration for AF 36 in almonds.

BACKGROUND AND DISCUSSION

Aflatoxin is a carcinogenic contaminant produced by the fungi *Aspergillus flavus and A. parasiticus* **(Figure 1).**

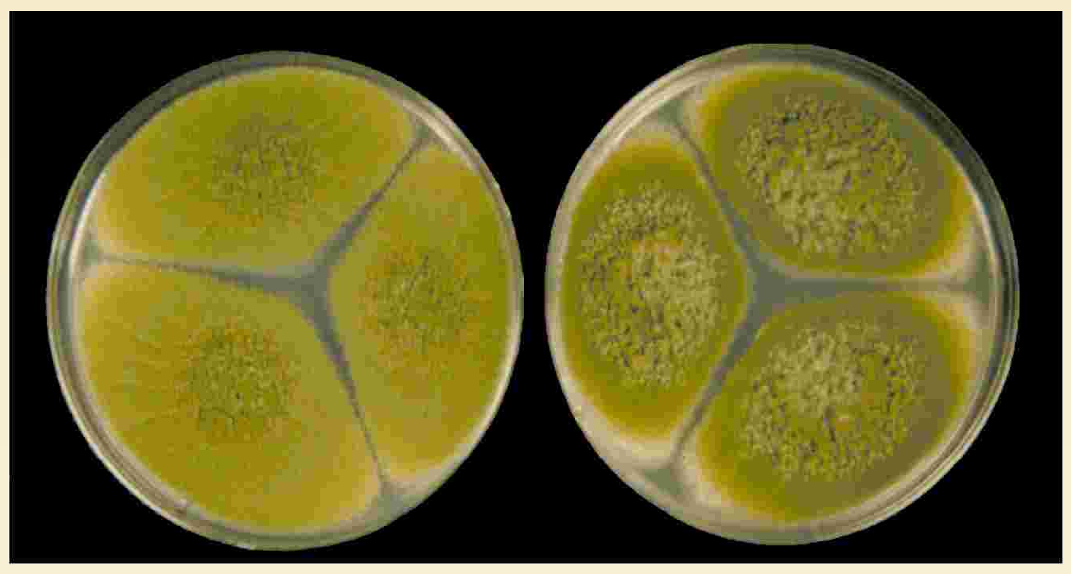

Figure 1. *Aspergillus flavus* (left) and *A. parasiticus* (right) occurring in Ca;lifornia almond orchards.

The almond industry has taken extensive measures to control aflatoxin. This project seeks to further this effort with the two pronged objectives outlined above.

Of note, the bio-control technique of "seeding" the atoxigenic (non-aflatoxin producing) AF36 strain of *A. flavus* is already showing promising results in almonds. The strain is applied in the field as infested wheat seed which after irrigation is covered by the spores of the activated strain **(Figure 2)**

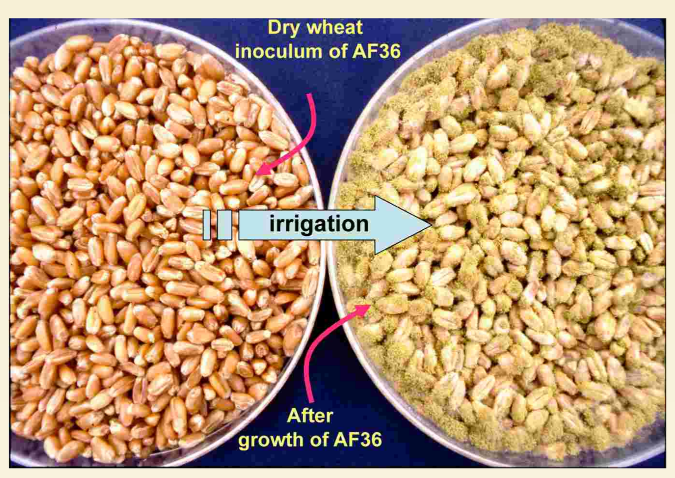

Figure 2. Wheat inoculum of AF36 atoxigenic strain dry (left) and after rehydration covered with spores of *Aspergillus flavus* (right).

This strain is inoculated into the field and displaces the naturally present aflatoxin-producing fungal strains. This approach in other crops like cotton seed and corn has led to a substantial reduction in aflatoxin contamination. Application of the AF36 strain for 3 years in 3,000 acres of pistachios resulted in a 45% reduction of the aflatoxin contaminated samples by the third year in comparison with the samples taken from the untreated acreage **(Figure 3).** In 2012, the AF36 strain has been registered in pistachio and 73,000 acres of **pistachios** were treated.

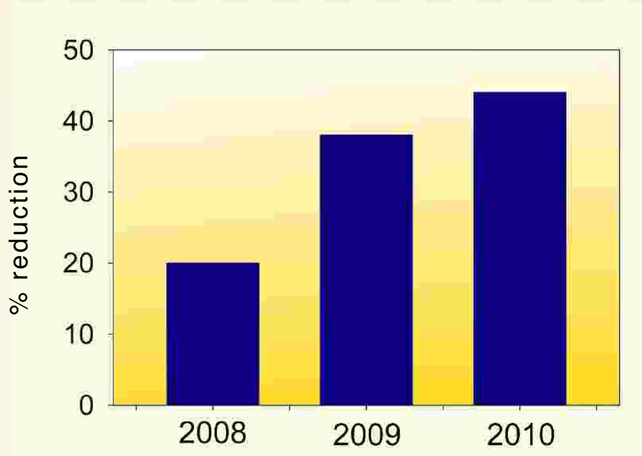

Figure 3. Percent reduction of aflatoxin contaminated **pistachio** samples after treating orchards with AF36 (all harvests).

Applying the wheat-AF36 product was very effective in increasing the population of the atoxigenic strain AF36 under the conditions present in an almond orchard. Although the frequency of the atoxigenic strain AF36 was very low in the soil before applying the wheat-AF36 product in 2007, after the applications in 2007 and 2008 almost all of the *A. flavus* isolates were AF36 (**Figure 4**). The frequency of AF36 remained high in the soil in treated areas from August 2007 to July 2008, which is evidence that AF36 survived the winter and spring well. In September 2009 (approximately 14 months since the last application) the level of AF36 in soil remained high in treated areas, indicating that the effect of application lasts more than a year and perhaps the wheat-AF36 product does not need to be applied every year. However, by September 2010 the level of AF36 decreased substantially (**Figure 4**), suggesting that an additional application of the wheat-AF36 product would be needed.

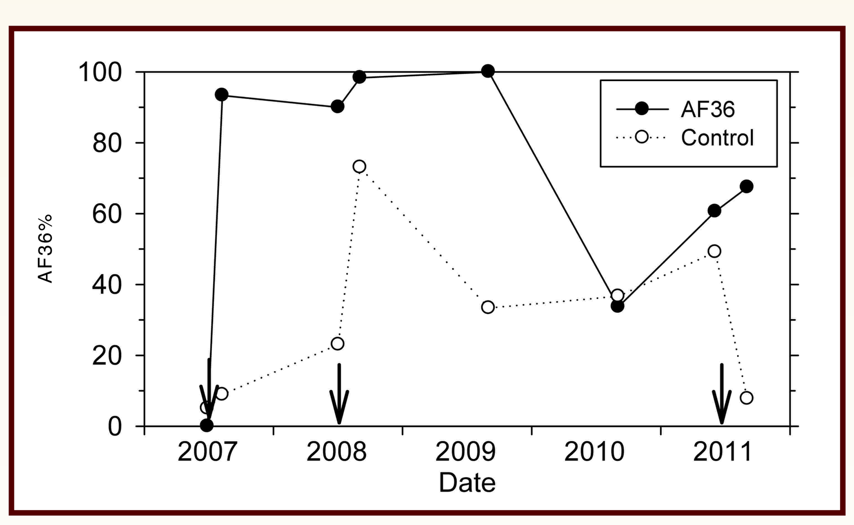

Figure 4. Percentage of *Aspergillus flavus* isolates belonging to the atoxigenic strain AF36 for isolates from soil collected from the areas treated with the wheat-AF36 product or from untreated areas in a research almond orchard at the Nickels Soil Laboratory. The wheat-AF36 product was applied on 28 June 2007, 2 July 2008, and 3 June 2011 (arrows).

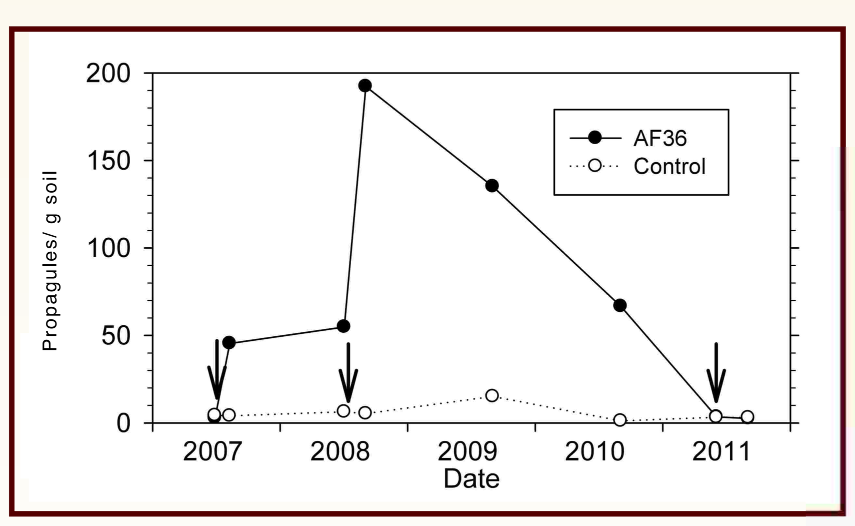

Figure 5. Density of *Aspergillus flavus*/*A. parasiticus* in soil collected from areas treated with the wheat-AF36 product or from untreated areas in a research almond orchard at the Nickels Soil Laboratory. The wheat-AF36 product was applied on 28 June 2007, 2 July 2008, and 3 June 2011 (arrows).

Application of the wheat-AF36 product to the orchard floor resulted in a moderate increase in *A. flavus*/*A. parasiticus* initially, but by September of 2008 a major increased had occurred. Most of these propagules were the AF36 strain (**Figure 5**). In 2011 the density remained low even though the wheat-AF36 product had been applied (**Figure 5**). Results for the 2012 are being currently evaluated.

Before applying the wheat-AF36 product in 2007, the most common aflatoxin-producing fungus present was *A. parasiticus* (**Figure 6**), which consistently produces aflatoxins at a high level. In addition, the S strain of *A. flavus* (which also tends to produce high levels of aflatoxins) was at approximately the same percentage of isolates as the L strain of *A. flavus* (which includes AF36 and many other atoxigenic strains). However, after applying the wheat-AF36 product, the percentage of isolates that were the L strain of *A. flavus* increased until almost none of the *A. flavus*/*A. parasiticus* isolates in the treated areas belonged to the aflatoxin-producing *A. parasiticus* or *A. flavus* S strain (**Figure 6**).

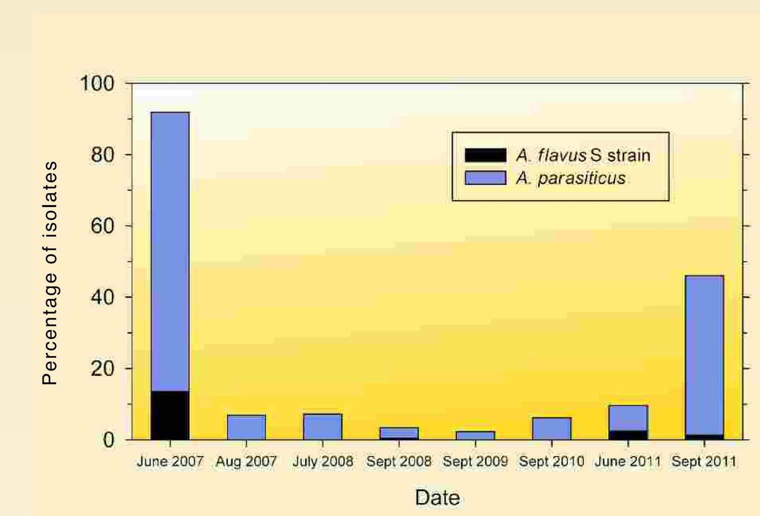

Figure 6. Percentage of *Aspergillus flavus*/*A. parasiticus* isolates that are the aflatoxin-producers *A. parasiticus* and *A. flavus* S strain for isolates from soil collected from areas treated with the wheat-AF36 product in a research almond orchard at the Nickels Soil Laboratory.

Other key findings include the following. The incidence of *A. flavus* and *A. parasiticus* in orchards of all growing regions presents a risk of aflatoxin contamination **(Figure 7).**

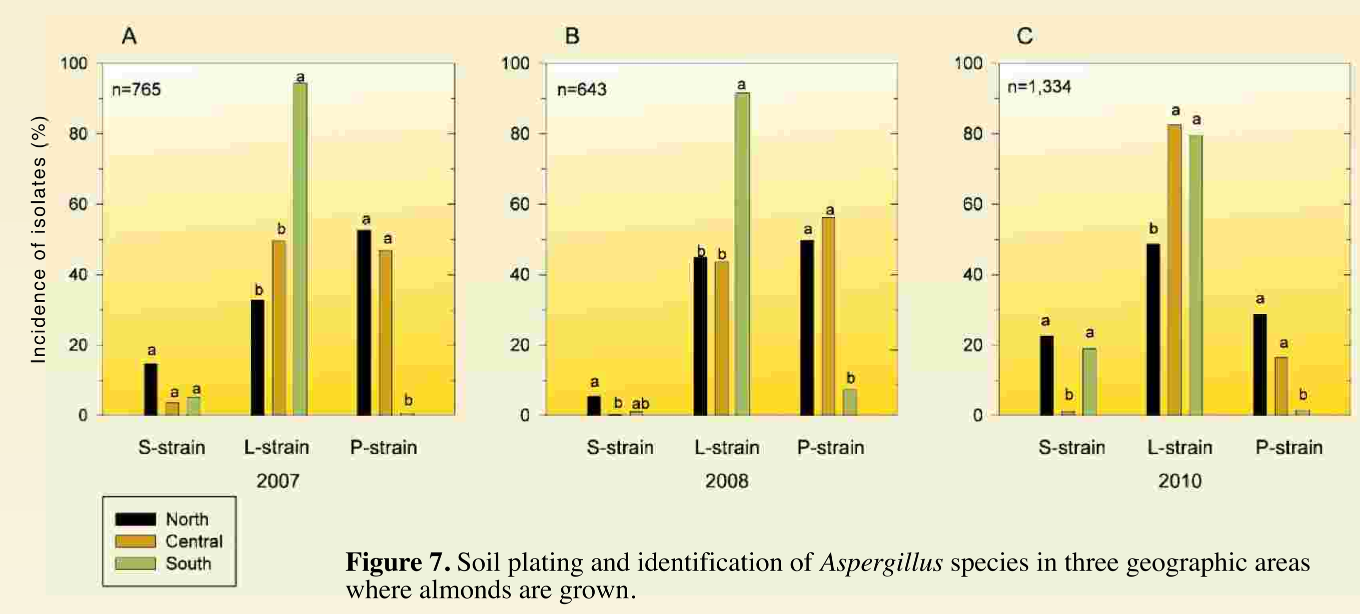

Figure 7. Soil plating and identification of *Aspergillus* species in three geographic areas where almonds are grown.

This work continues to document insect feeding and damage, particularly by the navel orangeworm (NOW), which contributes to the carrying spores of aflatoxigenic fungi, invasion and development of *Aspergillus* fungi and the production of aflatoxin (**Figure 8**).

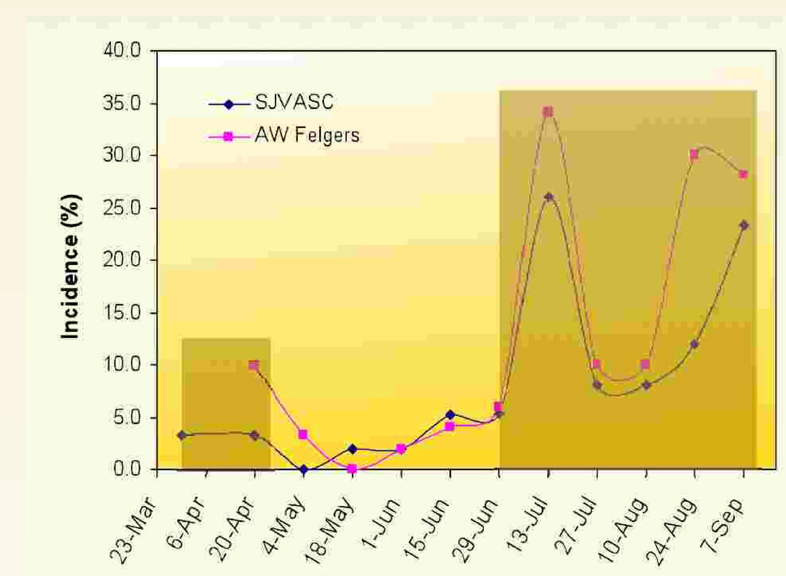

Figure 8. *Aspergillus flavus* and *A. parasiticus* from NOW in almond orchards (SJVASC, Fresno Co. and AW, Madera Co. –'d0 2012)

The high levels of incidence of *Aspergillus* section Flavi during late March and April (**Figure 8**) may be due to the fact that mummies show high levels of infection by *Aspergillus flavus* and *A. parasiticus* (previous year's report). The high levels of these fungi occurring during July through September are due primarily to the fact that propagules of this fungus increase over time as it was shown in pistachio research.

In September 2012, the Almond Board of California submitted a letter of support to the IR-4 program for the preparation of a package to request an Experimental Use Permit to treat with the AF36 biopesticide large acreage of almonds and obtain efficacy data to support registration of the biopesticide in almonds.

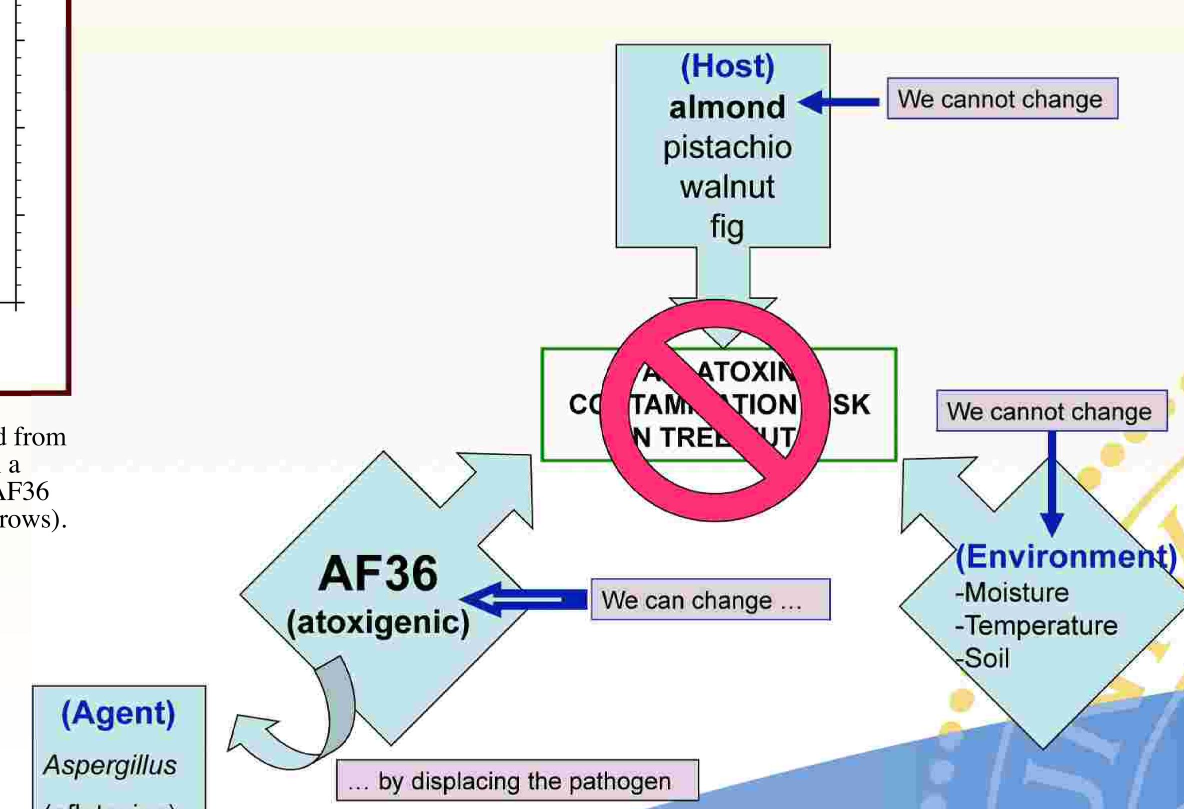

Figure 9. General diagram depicting displacement of the aflatoxigenic *Aspergillus* species to prevent aflatoxin contamination in almonds, pistachios, walnuts, and figs.

CONCLUSIONS

The almond industry has taken a number of measures pre- and post-harvest to assure control and compliance with aflatoxin standards. These measures include:

- Good agricultural practices like insect pest management and product handling.
- Sorting of insect damaged kernels.
- The bio-control technique of "seeding" the atoxigenic (non-aflatoxin) producing AF36 strain of A. flavus is showing alot of promise in almonds.
- This strain is inoculated into the field and displaces the naturally present aflatoxin-producing fungal strains for up to 2 years.
- An application for an Experimental use Permit is being prepared with the help of the IR-4 Project and the University of California.

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