Risk Factors and Spatial Patterns Associated with Aflatoxin Development in California Almonds

Project No.: **08-AFLA1-Michailides**

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Objectives:

1. The main objective is to determine the levels of *Aspergillus flavus/A. parasiticus* in almond orchards and the risk factors in these orchards associated with aflatoxin contamination.
2. Determine the levels of navel orangeworm larvae and moths contaminated with *A. flavus /A. parasiticus* and other *Aspergillus* species. [This objective developed as we were involved in a cooperative project with Dr. Siegel (USDA, ARS, SJVERC, Parlier, CA) because the initial plating of navel orangeworm moths emerged from mummies on specific agar media developed high levels of *A. flavus*].

Interpretive Summary:

In 2007 - 2008, we studied the distribution and densities of *Aspergillus* section *Flavi* in the soil of a large number of commercial almond orchards located in three different regions of California, northern, central, and southern (Figure 1). All three species in the section *Flavi* group (*Aspergillus flavus, A. parasiticus,* and *A. tamarii*) were isolated from the majority of these almond orchard soils. Of these species, *A. flavus* and *A.*

parasiticus produce aflatoxins while *A. tamarii* does not. Contamination of some nut samples (kernels, hulls, and shells) with only B while other samples also had G aflatoxins suggests that both of these fungi (*A. flavus/A. parasiticus*) are involved in infection of almonds. *A. flavus* produces only B aflatoxins while *A. parasiticus* produces both B and G aflatoxins. Aflatoxin analyses of the 2007 samples revealed more aflatoxin positive samples collected from central and southern regions than the samples from the northern region. The higher frequency of positive samples from the central and southern regions can be partially explained by the lower rainfall in these areas, as it was shown in other crops (i.e., corn) and the higher incidence of damage by the navel orangeworm. We also detected a higher frequency of aflatoxin positive hull and shell samples than aflatoxin positive kernels, suggesting that some of the infections that started while the nuts were on the ground had not progressed enough to reach, infect, and contaminate the kernels with aflatoxins. Therefore, the length of time and the condition of the orchard floor when the nuts are lying on the ground to dry may also affect contamination with aflatoxins and be considered as risk factors. Another risk factor can be the density of aflatoxigenic fungi in the orchard soil, although the direct relationships between fungal density and aflatoxin contamination levels have not been yet defined.

Another important risk factor can be the kinds and strains of *A. flavus* and *A. parasiticus* present in almond orchards. For instance, it is important to know the ratio of the densities of S and L strains of *A. flavus* in the orchard. Our results showed that the densities of both the S strain of *A. flavus* and *A. parasiticus* are higher in northern region than the other regions (Figure 2). From other studies using isolates collected from pistachio orchards, we found that 57% of the L isolates did not produce aflatoxins while 100% of the S strains produced B aflatoxins. In addition, 100% of the *A. parasiticus* isolates produced both B and G aflatoxins. Although the risk for aflatoxin is high in northern orchards based on the densities and species of aflatoxigenic *Aspergillus* spp., apparently, due to other factors (most likely environmental and lower NOW damage) almond samples from northern region had the lowest frequency of aflatoxin positives.

Our 2007 - 2008 results also showed that there are a lot of atoxigenic isolates of *A. flavus* in almond orchard, and most importantly, the bio-pesticide AF36 (atoxigenic strain of *A. flavus*) ranged from 3.5 to 12.6% in almond orchard soils. The AF36 strain is registered on cotton to reduce aflatoxin contamination of cottonseed and it is conditionally registered in California to treat 3,000 acres of pistachio in 2008 (under an Experimental Use Permit) for the reduction of aflatoxin contamination. Furthermore, we initiated a study in 2007 by applying AF36 in an experimental almond orchard at Nickels Soil Laboratory in Colusa County to gather efficacy data and facilitate and expand the registration of AF36 to almonds.

In 2007 - 2008 seasons, we also showed that both larvae and moths of NOW can be heavily contaminated with *A. flavus, A. parasiticus*, and *A. tamarii*, as well as with other species of *Aspergillus* (*A. niger, A. ochraceous, A. terreus, A. melleus*, etc.) (Figures 3 and 4). This high incidence of these fungi carried by NOW moths suggests that NOW insect not only will create the wounds for infection but also can introduce the aflatoxigenic pathogens into the right site for infection of hulls, shells, and kernels. This

may explain why there is a very strong association between NOW damage of almond nuts and aflatoxin contamination for California nuts. We detected higher levels of contamination of NOW moths emerged from mummies collected from the ground and kept in closed buckets (Figure 3) than those trapped on sticky traps installed in the orchard (Figure 4), suggesting that this insect can get contaminated from the soil more so than the moths that emerged from mummies of infested nuts on the trees and trapped on sticky traps in commercial orchards.

The levels of aflatoxins detected in our 2007 samples collected from windrows were very low, and all were below the EU tolerance. However, the multiple rapid alerts for California almonds exceeding the EU tolerance suggests that higher levels of aflatoxins may develop after the nuts are removed form the orchard, perhaps when the nuts are stockpiled and/or during transport to the foreign destination markets.

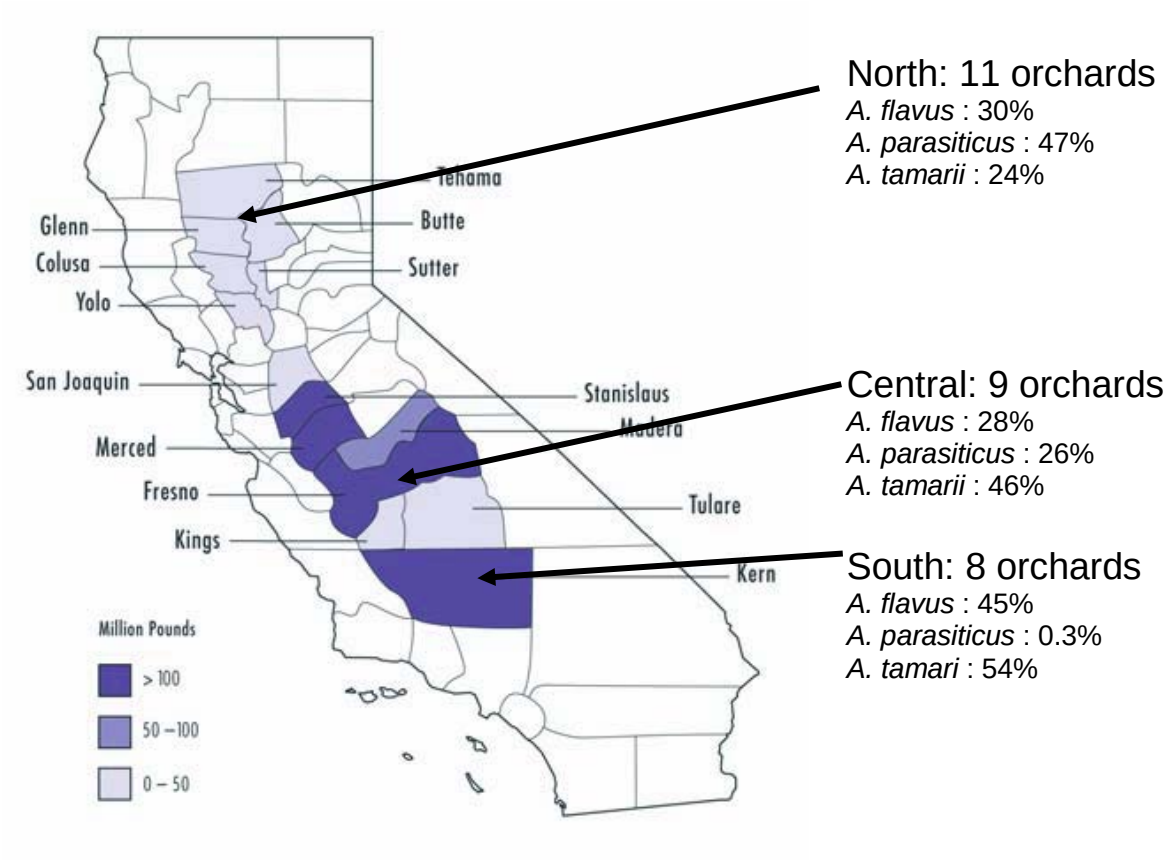

Figure 1. Locations of almond orchards in California where soil samples were collected in 2007 and composition of *Aspergillus* isolates in the section *Flavi.* (Isolates of *A. flavus* and *A. parasiticus* produce aflatoxins while none of the isolates of *A. tamari* produce any aflatoxin.)

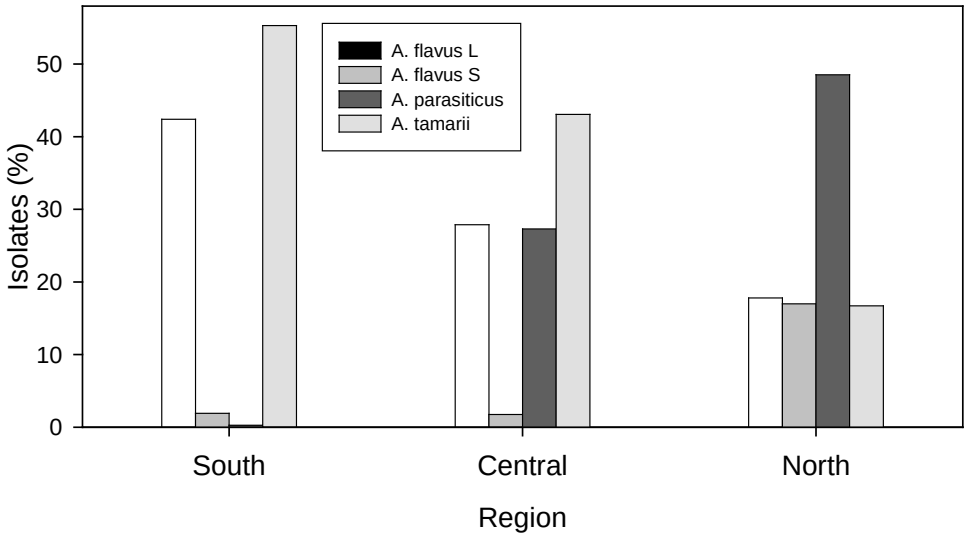

Figure 2. Percentage of *Aspergillus* section *Flavi* isolates belonging to the various species and strains from soil collected from commercial almond orchards in California.

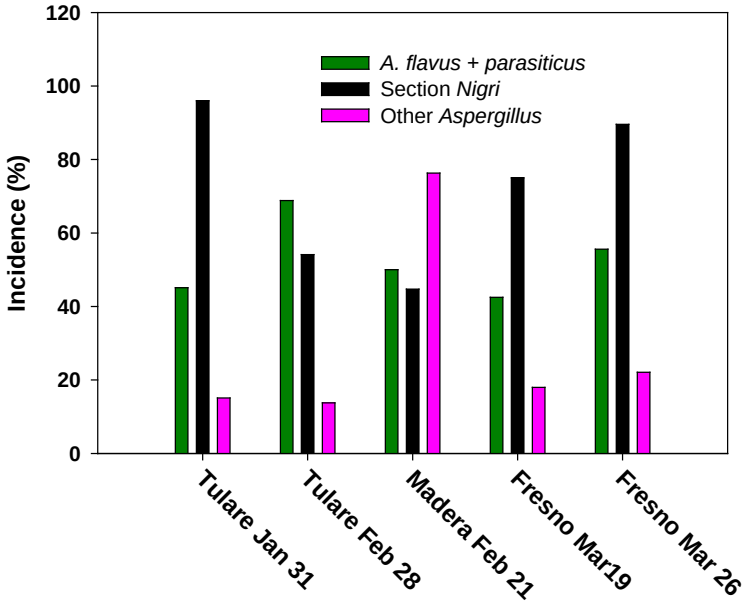

Figure 3. Incidence of *Aspergillus flavus* and *A. parasiticus* and other *Aspergillus* spp. on navel orangeworm moths emerged from almond mummies collected from commercial orchards in 2007 and kept in plastic buckets. Incidence was determined after plating NOW moths on Si10 agar media that were incubated at 35°C for 1 to 2 weeks and then recorded.

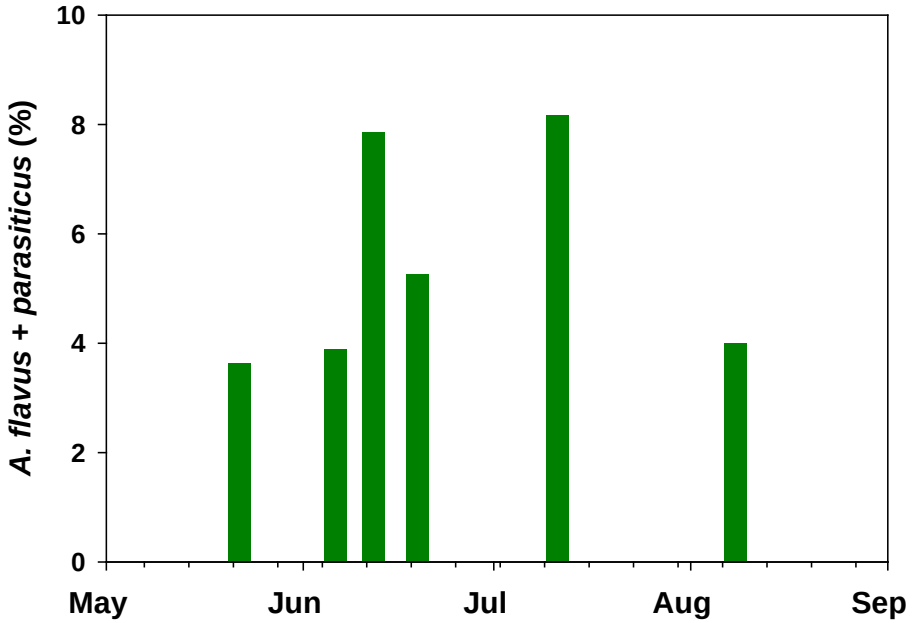

Figure 4. Incidence of *Aspergillus flavus* and *A. parasiticus* on navel orangeworm (NOW) moths trapped periodically on sticky traps placed in an almond orchard in Madera County in 2007. Incidence was determined after plating NOW moths on Si10 agar media that were incubated at 35°C for 1-2 weeks and then recorded.

Discussion and Conclusions:

1. All three species in the section *Flavi* group (*Aspergillus flavus*, *A. parasiticus,* and *A. tamarii*) were isolated from the majority of these almond orchard soils. Of these species *A. flavus* and *A. parasiticus* produce aflatoxins while *A. tamarii* does not.
2. Contamination of almond nut samples (kernels, hulls, and shells) with B and some also with G aflatoxins suggests that both *A. flavus* and *A. parasiticus* are involved in infection of almonds and contamination with aflatoxins. This is similar to infection and contamination of pistachios with aflatoxins and different from other crops where either *A. flavus* (i.e., in cotton) or *A. parasiticus* (i.e., peanuts) are involved.
3. Aflatoxin analyses of the 2007 samples revealed more aflatoxin contamination in the central and southern regions than in the northern regions. The higher frequency of positive samples from the central and southern regions can be partially explained by the lower rainfall in these areas as it was shown for other aflatoxin susceptible crops. Another explanation could be the deficit irrigation practices that some almond growers on the west side of the San Joaquin Valley were forced to carry out because their surface water deliveries were reduced in 2007.
4. The more frequent aflatoxin contamination of the hull and shell samples than of the kernels suggests that some of the infections that started while the nuts were on the ground had not progressed enough to reach and infect the kernels and produce aflatoxins. Therefore, the time and conditions the nuts are lying on the ground to dry may be considered as a risk factor.

5. Another important factor can be the kinds and strains of *A. flavus* and *A. parasiticus* present in almond orchards. For instance, it is important to know the ratio of the densities of S and L strains of *A. flavus* in the orchard. After checking isolates of *A. flavus* from pistachio orchards, we found that 57% of the L isolates did not produce aflatoxins while 100% of the S strains produced B aflatoxins. In addition, 100% of the *A. parasiticus* isolates produced both B and G aflatoxins. Our results showed that the densities of the S strain of *A. flavus* and *A. parasiticus* are higher in northern region than the other regions. Although the risk for aflatoxin is high in northern orchards based on the densities and species of aflatoxigenic *Aspergillus* spp., apparently, due to other factors (most likely environmental and lower NOW damage) almond samples from northern region had the lowest frequency of aflatoxin positives.
6. The presence of atoxigenic isolates in almond orchards (most of L strain of *A. flavus* and all the *A. tamarii* isolates) suggests that it could be possible to use biocontrol of aflatoxins in almonds as it is pursued with pistachios. (Interestingly, the bio-pesticide atoxigenic *A. flavus* strain AF36 ranged from 3.5% to 12.6% in almond orchard soils. Of the 637 isolates of *A. flavus* tested thus far, an average of 7.5% belonged to the AF36 strain. This level is higher than that of AF36 recorded in pistachio and fig orchards. We were granted in 2008 an Experimental Use Permit (EUP CA REG. #71693-550001-EX conditional registration on 01/23/08 (ID#220986)) and treated with AF36 3,000 acres of pistachio to study its effects in displacing the toxigenic *Aspergillus* isolates and reduce aflatoxins in pistachios.) Natural occurrence of the AF36 strain in soils of almond orchards will facilitate the registration of this strain for reducing aflatoxins in nut crops after the completion of the EUP period. We initiated a study in 2007 and continued in 2008 by applying AF36 in an experimental almond orchard at Nickels Soil Laboratory in Colusa County to gather efficacy data and facilitate and expand the registration of AF36 to almonds.
7. Our results also showed that both larvae and moths of NOW can be contaminated with *A. flavus, A. parasiticus*, and other species of *Aspergillus* (*A. niger, A. tamarii, A. ochraceus, A. terreus, A. melleus,* etc.). This high incidence of these fungi carried by NOW moths suggests that NOW insect not only will create the wounds for infection but also will introduce the aflatoxigenic pathogens into the right site for infection of almond kernels.