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# Bridging the Use of Biocontrol Products with the Active Ingredient *Aspergillus Flavus* AF36 from Pistachio to Almonds

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**Project No.:** 15-AFLA1-Michailides

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**Objectives:**

Evaluate formulations of the AF36 product in almond and pistachio orchards.

1. Compare the sporulation of AF36 in pistachio, which has an AF36 registration to sporulation in almonds. This bridging from pistachio to almond is needed as a step toward registration of AF36 in almond.
2. Compare the sporulation of the traditional wheat AF36 formulation to the newer sorghum formulation.

**Interpretive Summary:**

The use of the atoxigenic *Aspergillus flavus* strain AF36 (a strain not able to produce aflatoxins) as a biopesticide has been successful in reducing aflatoxin contamination of pistachio nuts in commercial pistachio orchards in California in addition to reducing contamination of cottonseed and corn. The AF36 product was registered by the US Environmental Protection Agency (EPA) in 2012 for application in commercial pistachio orchards in California. We are attempting to obtain registration with the EPA of the AF36 product for application in almond orchards. To accomplish this, we compared the AF36 product in an almond orchard with that in a pistachio orchard. In our study, we examined spore production on the AF product in both types of orchard, since the spores produced would spread the biocontrol agent throughout the orchard contributing to its effectiveness. We found that the spore production on the AF36 product was similar in almond and pistachio orchards.

The manufacturer who produces the AF36 product has now started using sorghum instead of wheat as a carrier of the AF36 inoculum. Furthermore, a new process is being used to produce

the AF36 product. This new process coats the seeds with spores of the AF36 fungus (instead of inoculating and incubating the seeds), resulting cheaper and faster production. In our study, the new seed-coated sorghum-AF36 product performed similarly to the wheat-AF36 product in the almond orchard, suggesting that the new AF36 product is an adequate replacement for the original product.

## Materials and Methods:

Sorghum as a carrier for the AF36 fungus was compared with the currently used wheat-AF36 product in a research almond orchard and in a research pistachio orchard at the Kearney Agricultural and Extension Center. The experimental design was a randomized block design with four replications, each of which consisted of the area around one tree. Both AF36 products were applied to the soil around the same trees on the same day and at the rate of 10 pounds per acre (label rate). Seeds (25 per replicate) were examined periodically (starting 7 days until 40 days after application) using a dissecting microscope (magnification  $\times 10$ ) for the presence of sporulation. The same experiment was performed in the almond and pistachio orchard in order to compare the performance of the AF36 products in both types of orchard.

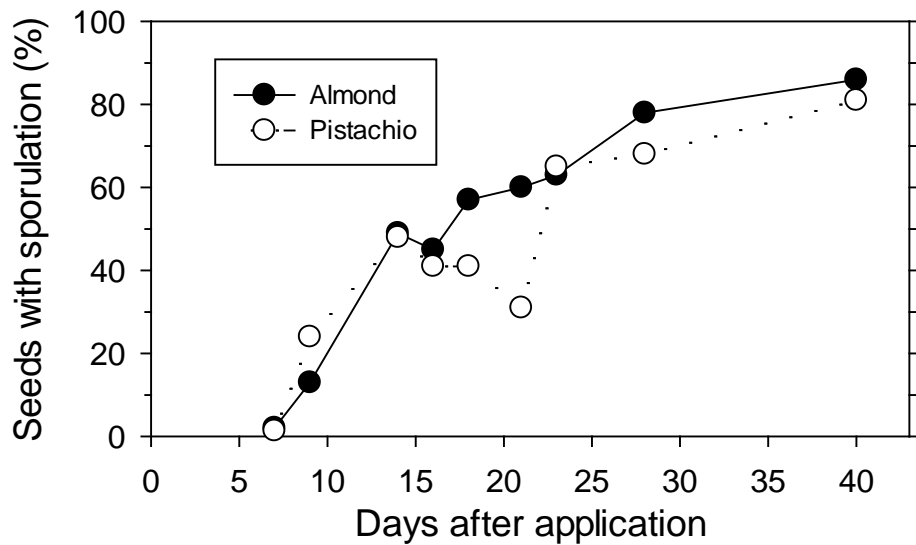
## Results and Discussion:

**Sporulation on the AF36 product is similar in almond and pistachio orchards.** The amount of visible sporulation by the atoxigenic strain on the AF36 product was very similar over time in a research almond orchard and a nearby research pistachio orchard (**Figure 1**).

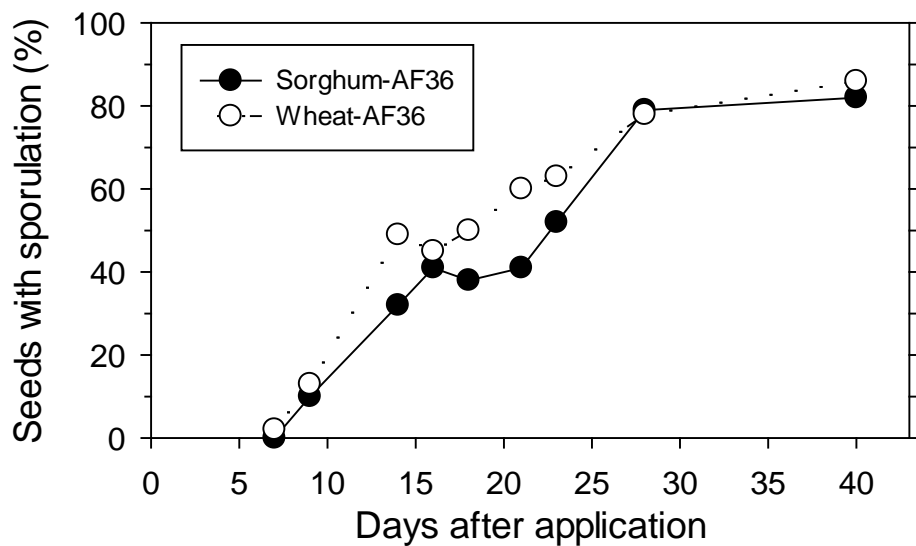
**The new seed-coated sorghum-AF36 product performed similarly to the wheat-AF36 product in the almond orchard.** In order to improve efficiency and increase the amounts of inoculum produced, the manufacturer who produces the wheat-AF36 inoculum has now started using sorghum as carrier of the AF36 inoculum in addition to the wheat-AF36 product, and coating sorghum seeds with spore of the AF36 fungus (instead of inoculating and incubating the seeds). The percentages of seeds of the AF36 products with visible sporulation of *A. flavus* was similar over time between the sorghum-AF36 product and the wheat-AF36 product after application of the products to the soil surface in a research almond orchard (**Figure 2**). Although the sporulation on sorghum was slightly delayed during the first 3 weeks after application in the orchard, after 4 weeks the amount of sporulation was very similar for the sorghum and the wheat product (**Figure 2**). Approximately 80% of the seeds of either wheat or sorghum had sporulation 4 weeks after the application. The abundant and similar sporulation on the sorghum and wheat products support the changes in the production of the AF36 inoculum.

## Summary:

The results of this study support bridging the use of biocontrol products with the active ingredient *Aspergillus flavus* AF36 from pistachio to almonds. These two nut crops, pistachio and almond, have significant contiguous acreage in California resulting in natural and unavoidable movement of fungi from one to the other. The registration of the AF36 product will facilitate the industry's efforts to achieve area-wide reductions in the vulnerability of both crops to aflatoxin contamination.



**Figure 1.** Percentage of seeds with visible sporulation of *Aspergillus flavus* on seeds of the AF36 product after application in an almond orchard and a pistachio orchard.



**Figure 2.** Percentage of seeds with visible sporulation of *Aspergillus flavus* on seeds of the AF36 products after application of the sorghum-AF36 product and the wheat-AF36 product to the soil surface in a research almond orchard.