Distribution of AF36 in Pistachio and Almond Orchards within the San Joaquin Valley

Arizona Cotton Research & Protection Council, Phoenix, Arizona
David Edmunds, AF36 Program Specialist

The biological control *Aspergillus flavus* AF36 Prevail has established itself as an effective tool in the management of aflatoxins by using a native strain of the fungus incapable of producing aflatoxins.

The beneficial strain, AF36, is coated onto a carrier grain that is sterilized, meaning it will not germinate, and serves as a food source for the fungus. By applying *Aspergillus flavus* AF36 Prevail to crops at the appropriate time, the fungus grows on the carrier grain in the field, and its spores move to the desired crop by wind and insects before the aflatoxin-producing strains (*A. flavus* S-morphotype and *A. parasiticus*) which are present in the environment have an opportunity to infect the crops.

Using AF36 actually changes the composition of the fungi in the environment, which shifts the majority of the population from potential aflatoxin producers to the safe non-toxin producing strain of *Aspergillus flavus* in AF36 Prevail.

The pistachio industry broadly adopted this technology across the San Joaquin Valley in California starting in 2012.

AF36 in the San Joaquin Valley soil

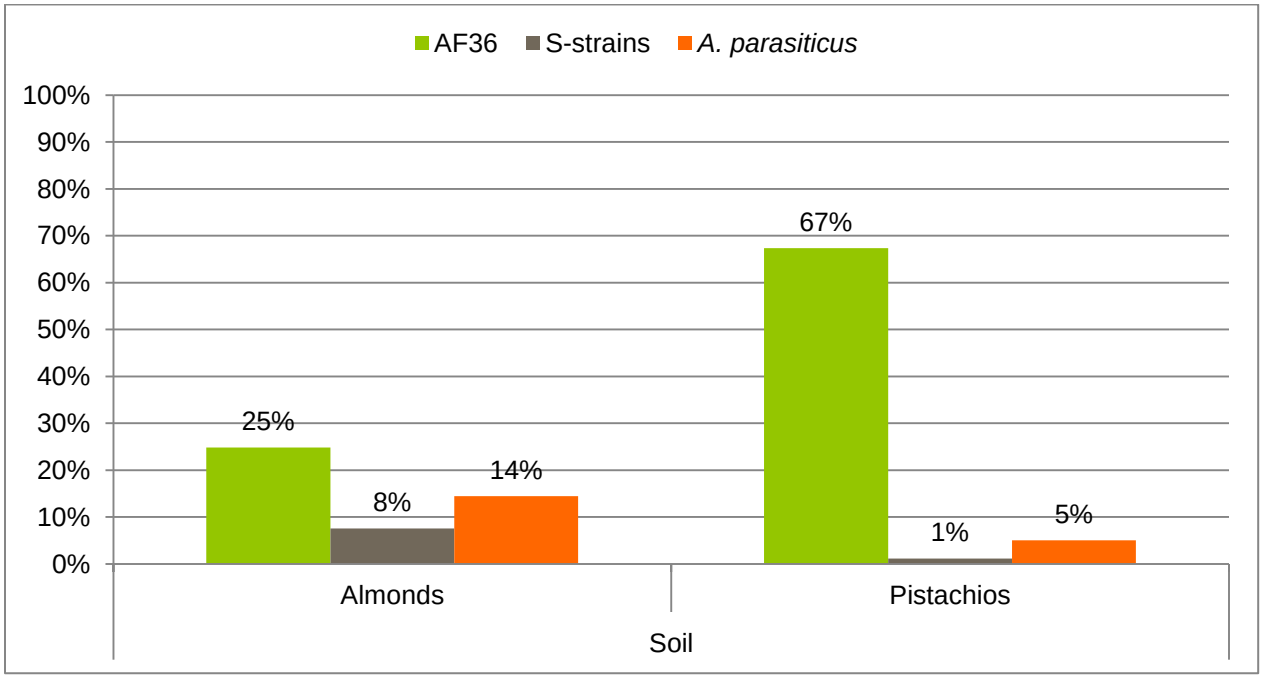

Figure 1 Soil data from 2018-2020 showing proportions of atoxigenic biological control (AF36) and known toxin-producers (S-strain, AP) separated by crop. Sample size: Almonds (n=237), Pistachios (n=390).

Beginning in 2018, the Arizona Cotton Research and Protection Council (ACRPC) has taken samples across the region to characterize the population of *Aspergillus flavus* and determine composition of the fungal community since the widespread adoption of *Aspergillus flavus* AF36 Prevail in the pistachio industry.

Pistachios, which have been treated with AF36 for a number of years, have responded well to biocontrol overall throughout the region. Soil samples taken between 2018 and 2020 have resulted in 67% overall recovery of the biological control strain AF36 in those samples, indicating that the biological control is contributing to the considerably lower proportions of toxin producers in the regularly treated orchards through displacement of aflatoxin producing fungi.

Almonds, which throughout the region have not historically been treated, or treated regularly with AF36, have not enjoyed the same success typically found with the widespread use of the technology. Both S-strains and *A. parasiticus* have been recovered at a higher incidence in almond orchards than in pistachio orchards. The higher proportions of toxin-producers in these orchards indicate that there is a significant opportunity for the biological control to succeed in the crop if widespread adoption is to take place.

County/Crop/Substrate	Sample Size	AF36	S-strains	*A. parasiticus*
Fresno	**194**	**51%**	**5%**	**4%**
Almonds	**22**	**24%**	**17%**	**17%**
Leaf				
Soil	22	24%	17%	17%
Pistachios	**172**	**60%**	**1%**	**0%**
Leaf	73	39%	0%	0%
Soil	99	83%	2%	0%
Kern	**95**	**42%**	**3%**	**0%**
Almonds	**26**	**19%**	**2%**	**0%**
Leaf	4	50%	0%	0%
Soil	22	3%	3%	0%
Pistachios	**69**	**49%**	**3%**	**0%**
Leaf	20	33%	0%	0%
Soil	49	70%	6%	0%
Kings	**170**	**40%**	**6%**	**4%**
Almonds	**121**	**16%**	**12%**	**7%**
Leaf	68	5%	15%	0%
Soil	53	32%	6%	18%
Pistachios	**49**	**69%**	**0%**	**0%**
Leaf	9	88%	0%	0%
Soil	40	59%	0%	0%
Madera	**240**	**33%**	**0%**	**7%**
Almonds				
Soil				
Pistachios	**240**	**33%**	**0%**	**7%**
Leaf	94	14%	0%	0%
Soil	146	55%	0%	14%
Merced	**34**	**2%**	**9%**	**0%**
Almonds	**34**	**2%**	**9%**	**0%**
Leaf	6	0%	0%	0%
Soil	28	3%	19%	0%
Modesto	**37**	**8%**	**0%**	**20%**
Almonds	**37**	**8%**	**0%**	**20%**
Leaf	12	8%	0%	0%
Soil	25	5%	0%	80%
Tulare	**303**	**30%**	**5%**	**0%**
Almonds	**203**	**21%**	**6%**	**0%**
Leaf	116	11%	8%	0%
Soil	87	39%	1%	0%
Pistachios	**100**	**40%**	**5%**	**0%**
Leaf	44	24%	6%	0%
Soil	56	83%	0%	1%
Grand Total	**1073**	**35%**	**4%**	**4%**

Table 1. Percentage of isolates that belong to *Aspergillus flavus* (strain AF36 or strain S) or *A. parasiticus* for isolates obtained from soil and leaf tissue from commercial orchards 2018 to 2020 broken down by county.

AF36 in the Tree Canopy

One of ACRPC's objectives is to determine the degree in which the biological control AF36 moves from the ground into the canopy of the orchards. To study this movement, leaf tissue samples in the canopies of pistachio and almond orchards were taken.

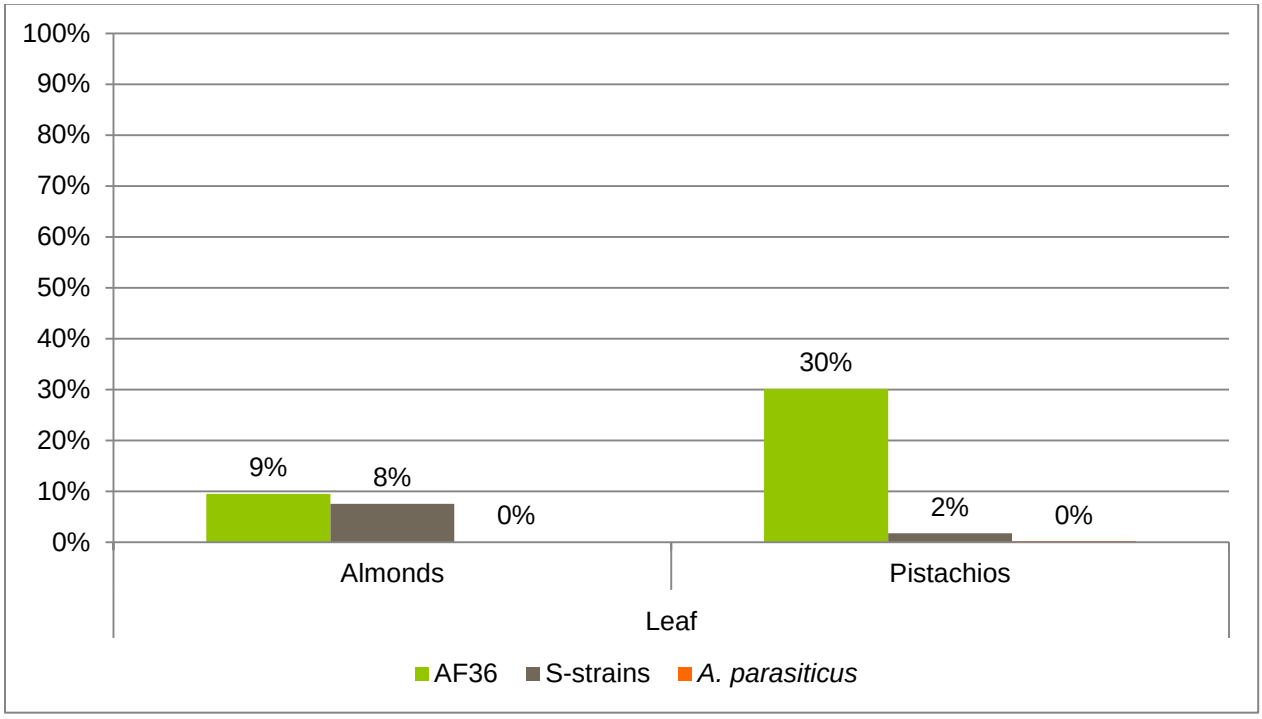

Figure 2. Leaf canopy sample data from 2018-2020 showing proportions of atoxigenic biological control (AF36) and known toxin-producers (S-strain, AP) separated by crop and sample type. Almonds (n=206). Pistachios (n=240).

AF36 is moving into the canopy of the orchards that are regularly treated with AF36 and effectively displacing toxin-producing fungi. If we consider pistachios as being regularly treated with the biological control and almonds as infrequently treated, we see much higher proportions (30%) of AF36 being recovered in the regularly treated orchards (compared to 9%). Additionally, we see low levels of aflatoxin-producing fungi (2%) making it into the canopy compared to the infrequently treated crop (8%).

While we see comparatively lower levels of AF36 in the tree canopy than in the soil, we were still able to recover AF36 from the canopy of 83% of the almond orchards and 70% of the pistachio orchards. We detected AF36 in the soil of every orchard we sampled regardless of crop.

These results indicate that despite AF36 treatment being a ground-application, the beneficial fungi move into the canopy and effectively displace aflatoxin-producing fungi. Regular treatment with the biological control only serves to increase efficacy and displacement.

AF36 over the Years

One of the long established benefits of applying AF36 annually is the carry-over and additive effect from the AF36 strain persisting from one year to another and shifting the fungal community towards an increasing population of atoxigenic isolates.

Prior to EPA registration of AF36 use in Tree Nuts, the natural occurrence of AF36 in pistachio orchards across the region was between 2.9% to 15% with an average of 7.4% (Doster, et. al, 2014). Now, occurrence of AF36 is well over 60%.

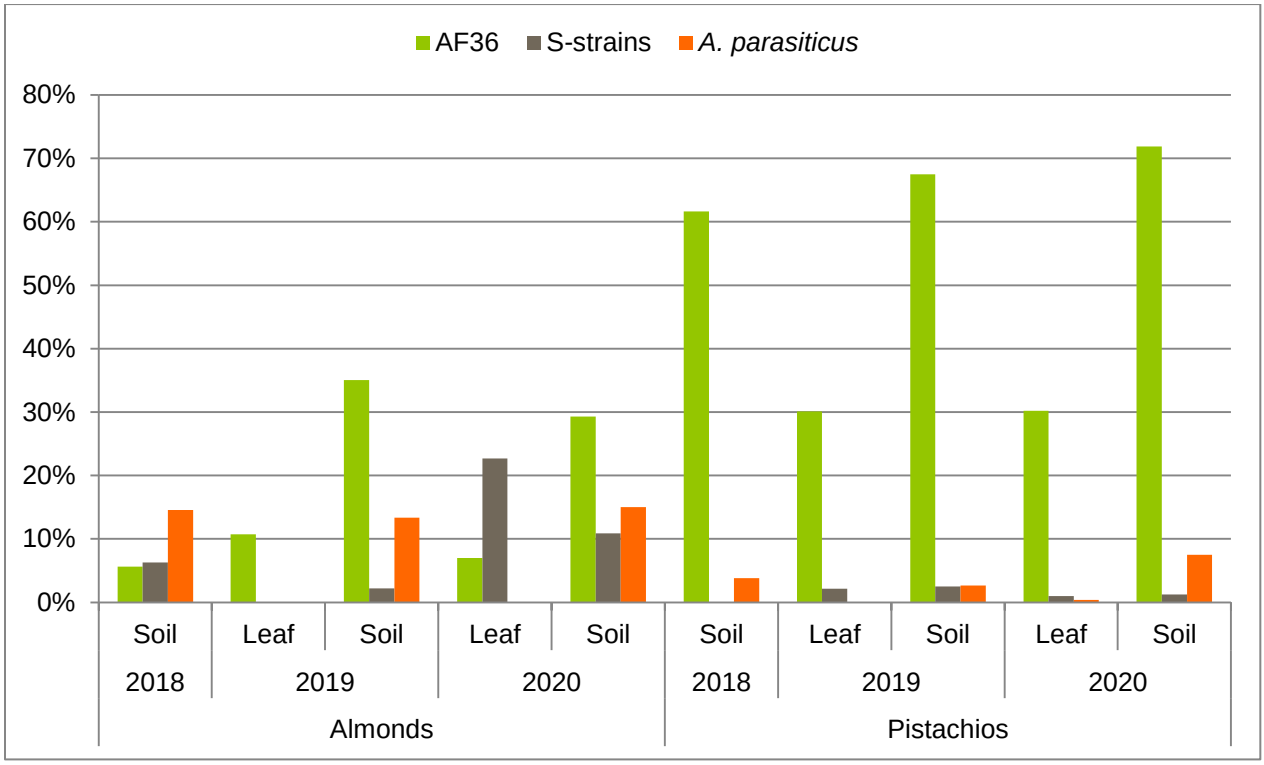

Figure 3. Fungal community by year and substrate. Almonds (soil '18 n=64, leaf '19 n=69, soil '19 n=93, leaf '20 n=137, soil '20 n=80). Pistachios (soil '18 n=117, leaf '19 n=117, soil '19 n=154, leaf '20 n=123, soil '20 n=119).

In pistachios, a crop that is treated annually, we see an additive effect from regular treatment in the orchards. Recovery of AF36 went up from 62% in 2018 to 68% in 2019 and culminated in 72% recovery in 2020 in the soil samples taken. While leaf tissue samples are only from 2019 and 2020, the recovery of AF36 in those samples held steady at 30%.

In almonds, a less frequently treated crop, results were quite different. Overall, there is more aflatoxin producing fungi in the samples and variability in the amount of AF36 that was able to be recovered from the samples over the years. Results suggest that a continued, area-wide approach that takes advantage of the additive effect created by nearby applications is the most effective method in displacing aflatoxin producing fungi in orchards.

Final Thoughts

Aflatoxin contamination levels in crops are closely tied to the amount of aflatoxin-producing fungi in the environment. Therefore, by increasing the population of atoxigenic biological control strain AF36 while decreasing the proportion of aflatoxin producing fungi is an effective strategy in aflatoxin mitigation. As evidenced throughout the Tree Nut growing region in California, when used recurrently, AF36 is a successful tool in displacing aflatoxin producing fungi.

We also have established considerable evidence that AF36 successfully moves into the canopy of orchards. The movement of AF36 into the canopy suggests that the biocontrol can move onto the desired crops and prevent the aflatoxin producing fungi from taking hold.

An expanded area-wide approach to aflatoxin mitigation in the region is critical for the pistachio and almond industries. An area-wide approach insures that there is minimal risk of harvests exceeding the regulatory limits that prevent the crops from entering premium markets. Additionally, because the fungi move via wind and insects, increased populations of AF36 in the environment not only protect the grower's orchard, but also produce additive effects in neighboring treated orchards as well by increasing the range of AF36 and limiting the dispersal of aflatoxin producers.