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AF36 in Almonds – Another Grower Tool for Aflatoxin Control in the Orchard

Themis Michailides

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AF36 Registration in Feb 2012
- Acreage: 330,000 acres
- In production: 250,000 acres
- Production: 690 million lbs. (= 313,500 tons of in shell pistachios)

AF36 Registration in Aug 2017
- Acreage: 1,110,000 acres
- In production: 900,000 acres
- Production: 2,050 million lbs. (= 930,000 tons of kernels)

AF36 Registration in Aug 2017
- Acreage: 7,500 acres
- In production: 7,000 acres
- Production: 21,000 lbs (= 9,560 tons of dried figs)
Molds that can produce aflatoxin in almond (also in pistachio and fig) orchards in California

Aspergillus flavus                  Aspergillus parasiticus
*Aspergillus flavus* and *A. parasiticus* produce:

Aflatoxins $B_1$, $B_2$, $G_1$, $G_2$, $M_1$

$B_1$ is the most potent aflatoxin; it can cause liver cancer.
Frequency and tolerance limits of aflatoxin contamination in California almonds

**Frequency:** 1 nut in 32,000 nuts

**Regulatory limits for aflatoxins**

- **USA**
  - Aflatoxin B1: 10 ppb
  - Total aflatoxins: 15 ppb

- **European Union**
  - Aflatoxin B1: 8 ppb
  - Total aflatoxins: 10 ppb

... in milk: 0.5 ppb

In almonds “further processed”: 12 ppb B1; & 15 total aflatoxin
Nut samples contaminated with aflatoxins
(data of Dried Fruit Association in Fresno from 1985-1989 aflatoxin analyses)
Aflatoxin contamination of almonds

Preharvest vs. Postharvest?
(if almond nuts are dried quickly after harvest, stored properly, and kept dried).

▪ Preharvest problem!

▪ Leaky stockpiles; no proper storage, etc… ➔ Postharvest
Life cycle of *Aspergillus flavus* in almond orchards
Relationship of navel orangeworm infestation and aflatoxin levels

![Graph showing the relationship between navel orangeworm infestation and aflatoxin levels.](image-url)
In general, the damage of NOW you have after the first week of harvest will be doubled following the third week of harvest (according to Dr. Joel Siegel).
Association of NOW with aflatoxigenic fungi

Sticky traps with NOW moths

A. flavus

Si10
Effect of feeding sites (wounds) in almond on levels of aflatoxin contamination
Sanitation
Sanitation
Sanitation
Aspergillus flavus in almond orchards

S strain (small sclerotia): almost all toxigenic

L strain (large sclerotia): 50%:50% toxigenic:atoxigenic
Delivery of AF36 inoculum for treatment of pistachio orchards in 2012
Application rate: 10 lbs. per acre
AF36 inoculum

Irrigation is needed for spore production

After growth of AF36

As applied

Sporulation
Sorghum is now used as a carrier of AF36 atoxigenic strain
After irrigation, the wet seeds will produce spores of AF36
Non-treated orchard
Treated Orchard
Samples taken at processing plant as nuts are being unloaded.
Reduction in aflatoxin-contaminated pistachio samples (1\textsuperscript{st} and 2\textsuperscript{nd} harvests)

2008 - 2011

Reduction of contaminated samples (%)

20.4\% 38.6\% 44.9\% 36.7\% 39.9\%

\(P\) value = 0.0033

(Doster et al. (2014), Plant Disease 98:948-956)
Reduction in aflatoxin-contaminated pistachio samples (2nd harvest)

2008 2009 2010 2011 2009-2011

Reduction of contaminated samples (%)

- 2008: No samples
- 2009: 23.6%
- 2010: 85.4%
- 2011: 58.1%
- 2009-2011: 54.6%

(3 years average)
Since 2013, about 150,000 to 200,000 acres were treated yearly!

Registration of *Aspergillus flavus* AF36 strain for use in pistachio in 2012
Occurrence of *A. flavus* atoxigenic strains in almond-growing counties of California

Each colored circle represents a different atoxigenic strain; 

AF36 incidence: 3.0% to 8.5%
Reduction of aflatoxigenic *Aspergillus flavus/A. parasiticus* in areas of an almond orchard treated with the AF36

![Graph showing reduction of aflatoxigenic Aspergillus species over time with AF36 applications](image)

**Nickels Soil Laboratory**

**Nut samples**
Ability of AF36 to reduce aflatoxins when co-inoculated with highly toxigenic isolates

Greater than 94% reduction in aflatoxins in comparison to levels in kernels inoculated with the toxigenic isolate alone

2A1L-11: toxigenic isolate of *A. flavus*

4C1P-11: toxigenic isolate of *A. parasiticus*
Burkard spore trap in a pistachio orchard
PISTACHIO, ALMOND, AND FIG: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, NEW MEXICO, AND TEXAS

Registration of A. flavus AF36 Prevail

Aspergillus flavus AF36 Prevail
For displacing aflatoxin-producing fungi
Arizona Cotton Research and Protection
“for growers by growers”

PRODUCT INFORMATION
Aspergillus flavus AF36 Prevail is for application to cotton, corn, pistachio, almond and fig for the control of aflatoxin producing fungi. Aspergillus flavus AF36 Prevail is a strain of Aspergillus flavus that produces a proteinase that destroys aflatoxin. Application of Aspergillus flavus AF36 Prevail will not increase the yield of crops.

USE PRECAUTIONS
Do not apply as a tank mixture with fertilizers, insecticides, or other pesticides that are toxic to beneficial insects. Apply at the rate recommended for the crop being treated. Do not exceed the rate of use recommended for the crop being treated. Do not exceed the rate of use recommended for the crop being treated.

APPLICATION
1. Apply Aspergillus flavus AF36 Prevail to the soil in the planting rows or as a broadcast application before planting. Do not apply directly to the seed. Do not apply directly to the seed. Do not apply directly to the seed. Do not apply directly to the seed.
2. Adjust the application rate to ensure delivery of Aspergillus flavus AF36 Prevail to the soil. Do not apply Aspergillus flavus AF36 Prevail to the soil. Do not apply Aspergillus flavus AF36 Prevail to the soil. Do not apply Aspergillus flavus AF36 Prevail to the soil.
3. Apply Aspergillus flavus AF36 Prevail to the soil over the plant canopy. Do not apply Aspergillus flavus AF36 Prevail to the soil over the plant canopy. Do not apply Aspergillus flavus AF36 Prevail to the soil over the plant canopy. Do not apply Aspergillus flavus AF36 Prevail to the soil over the plant canopy.
4. Follow labels for proper use.
5. Use a minimum of 3,000 CFU/gram in the End Use Product. Do not exceed the rate of use recommended for the crop being treated.

KEEP OUT OF REACH OF CHILDREN
CAUTION
First Aid
If inhaled: Move person to fresh air. If person is not breathing, call 911 or an ambulance. Move person to fresh air. If person is not breathing, call 911 or an ambulance. Move person to fresh air. If person is not breathing, call 911 or an ambulance.

Date: 08/07/2017
Reg. No. 71693-2-AA

LABELING ACCEPTABLE STATE OF CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
Conclusions of studies from the AF36 application in almonds

✓ The AF36 persists well in the soil.

✓ It does not cause any increase in nut decay.

✓ Results on almond were similar to pistachio results.

✓ The atoxigenic strain AF36 Prevail® is registered now and can be applied in 2018.

Rate: 10 lbs per acre
Application timing: Late May to early/mid-July
40 lbs capacity
Some Challenges…

✓ Soil moisture (and temperature)
✓ Timing of application
✓ Harvest time
✓ Insect seed pests
✓ Predators
Effect of the soil moisture on sporulation of *Aspergillus flavus* (AF36 Prevail®) on grains of product.
Inoculum dropped into a very wet soil will not produce any spores of AF36; it will decay.
Warmer temperatures favor sporulation of the AF36 fungal strain on the AF36 product.
Effect of date of application on sporulation of *Aspergillus flavus* strain AF36

Best results with the 15 July application
Harvest and stockpiling

Hulls moisture ≤12%; kernel moisture 6%
Predation of grain by insects

Ants
Predation of grain and decay by other fungi

Roly polies

Fusarium spp.
Suggestions for best AF36 application

✓ The application method and product rate (10 lbs per acre) are the same as in other crops.

✓ Apply product in late May to early / mid-July.

✓ Irrigate within 3 days after application.

✓ Make sure that most of the inoculum will be spread in the areas wetted by irrigation.

✓ Avoid covering the inoculum by plowing or with too much water.

✓ Do not spray herbicides 1 to 2 weeks after application.

✓ Control the ants, other arthropods, and birds in the orchard.

<< This is a novel approach to reduce aflatoxin contamination >>
Link of video on application of AF36:

www.calpistachioresearch.org/training-videos/
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