The Use of an Atoxigenic *Aspergillus flavus* Strain AF36 to Reduce Aflatoxin Contamination in Nut Crops and Figs in California

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Los Angeles

= region with nut crops and figs
- **Acreage:** 330,000 acres
- **In production:** 250,000 acres
- **Production:** 690 million lbs. (= 313,500 tons of in shell pistachios)

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

- **Acreage:** 7,500 acres
- **In production:** 7,000 acres
- **Production:** 21,000 lbs (= 9,560 tons of dried figs)
Commodities contaminated with aflatoxin:

Highest risk of aflatoxin contamination:
- Corn
- Peanuts
- Cottonseed

Occasionally contaminated:
- Tree nuts (*almonds, pistachios, walnuts*)
- Figs
- Sorghum
- Spices
- Others
Incidence of aflatoxin contamination in California tree nut and fig orchards

**Pistachio**
1 in 5,000 nuts (off years) to 1 in 20,000 nuts (on years)

**Almond**
1 in 25,000 to 35,000 nuts

**Figs**
1 in 5,000 figs
Regulatory limits for aflatoxins

- **USA**
  - Aflatoxin B1 $\Rightarrow$ 10 ppb
  - Total aflatoxins $\Rightarrow$ 20 ppb
  - (FDA)
  - (Pistachio Federal Marketing Order): 15 ppb

- **European Union**
  - Aflatoxin B1 $\Rightarrow$ 8 ppb
  - Total aflatoxins $\Rightarrow$ 10 ppb
  - (pistachios/almonds for direct consumption)

1 ppb = 0.000 000 001 g per g of product
Aflatoxin contamination of nuts

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

➡️ Preharvest problem!

➡️ Leaky silos; no proper storage, etc… ➡️ Postharvest
Molds that can produce aflatoxin in pistachio, almond, and fig orchards in California

Aspergillus flavus                  Aspergillus parasiticus
Sources of spore inoculum of alfatoxigenic fungi

- male flowers (> 30% with *Aspergillus flavus*)
The life cycle of *Aspergillus flavus* in a pistachio orchard

- **SPRING / SUMMER**
  - Infection of nuts on trees
  - Conidia in the air
  - Sporulation on male flower
  - Sclerotia in or on soil
  - Survival on orchard debris
  - Navel orangeworm

- **AUTUMN / WINTER**
  - Mummies
  - Conidia
  - Sclerotia

**Sporulation on debris**
Early split nuts develop in the orchard in July and continuing until harvest; (2-5% of the crop)
Suture staining of ES
Navel orangeworm (NOW; *Amyelois transitella*) moth on an early split pistachio

Places for laying eggs

(Moth (adult) of NOW)
Relationship of navel orangeworm infestation and aflatoxin levels
Reduce early split nut formation

- Apply sufficient irrigation during spring to avoid tree stress.
- Use a rootstock that minimizes early split nuts.

Control navel orangeworm.

Do no delay harvest.
Use of atoxigenic strains of *Aspergillus flavus* as biopesticides to reduce aflatoxins

Atoxigenic strains = those strains that do not produce aflatoxins

**Rationale:** The atoxigenic strains when applied in the field, increase in numbers, and displace the toxigenic strains
Strains of *Aspergillus flavus*

- **L - strain**: about 50:50 toxigenic: atoxigenic
- **M - strain**: undescribed
- **S - strain**: most toxigenic
Natural occurrence of the *atoxigenic strain AF36* among *A. flavus* isolates from orchards in California

<table>
<thead>
<tr>
<th>County</th>
<th>Pistachio</th>
<th>Almond</th>
<th>Fig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butte</td>
<td>...</td>
<td>6.5</td>
<td>...</td>
</tr>
<tr>
<td>Colusa</td>
<td>...</td>
<td>3.0</td>
<td>...</td>
</tr>
<tr>
<td>Fresno</td>
<td>3.1</td>
<td>...</td>
<td>6.1</td>
</tr>
<tr>
<td>Glenn</td>
<td>...</td>
<td>4.4</td>
<td>...</td>
</tr>
<tr>
<td>Kern</td>
<td>12.7</td>
<td>8.5</td>
<td>...</td>
</tr>
<tr>
<td>Madera</td>
<td>7.2</td>
<td>5.0</td>
<td>7.2</td>
</tr>
<tr>
<td>Merced</td>
<td>15.0</td>
<td>...</td>
<td>5.8</td>
</tr>
<tr>
<td>Tulare</td>
<td>2.9</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
- Flood-irrigated orchard \((A564; A815)\) (2001) and \textbf{AF36} (2002)

AF36 inoculum

Irrigation is needed for spore production

As applied

Sporulation

After growth of AF36
Application rate: 10 lbs. per acre
After irrigation, the wet wheat seeds will produce spores of AF36
Burkard spore trap in a pistachio orchard
Concentration of fungal spores in the air in two commercial pistachio orchard (2008)

**A**

Aspergillus flavus/parasiticus

**B**

Aspergillus niger group
Percentage of *A. flavus* isolates from soil belonging to AF36

![Graph showing the percentage of A. flavus isolates from soil belonging to AF36.](image-url)
Library samples for aflatoxin analysis

Samples taken at processing plant as nuts are being unloaded.
Reduction in aflatoxin-contaminated pistachio samples (1st and 2nd harvests)

Reduction in contaminated samples (%)

- 2008: 20.4%
- 2009: 38.6%
- 2010: 44.9%
- 2011: 36.7%
- 2008-2011: 39.9%

P value = 0.0033

(Doster et al. (2014), Plant Disease 98:948-956)
About 73,000 acres of pistachios have been treated in 2012, and 150,000 to 200,000 in 2013, 2014, 2015, & 2016.
Substrates (carriers) of biocontrol strain AF36
Aflatoxin reduction ability of AF36 when co-inoculated with highly toxigenic isolates of *Aspergillus flavus* and *A. parasiticus* on viable almond

2A1L-11 is a toxigenic isolate of *A. flavus*
4C1P-11 is a toxigenic isolate of *A. parasiticus*
Biocontrol of aflatoxins in almonds using the atoxigenic AF36 strain

Almond orchard at Nickels Soil Laboratory Estate
Incidence of *Aspergillus flavus* isolates from soil collected from an almond orchard

(arrows indicate application dates)

<table>
<thead>
<tr>
<th>Date</th>
<th>AF36 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>no application</td>
</tr>
<tr>
<td>2008</td>
<td>20</td>
</tr>
<tr>
<td>2009</td>
<td>40</td>
</tr>
<tr>
<td>2010</td>
<td>60</td>
</tr>
<tr>
<td>2011</td>
<td>80</td>
</tr>
<tr>
<td>2012</td>
<td>100</td>
</tr>
</tbody>
</table>
Incidence of aflatoxin-producing fungi among *A. flavus/A. parasiticus* isolates (from the soil of areas in an almond orchard treated with the AF36 product)
Research Calimyrna fig orchard (Kearney) used for atoxigenic experiments in 2002
Percentage of *Aspergillus flavus* isolates from soil belonging to AF36 in drip-irrigated orchard.

![Graph showing survival study of AF36 application over years](image-url)
Aflatoxin reduction ability of AF36 when co-inoculated with highly toxigenic isolates of *Aspergillus flavus* and *A. parasiticus* on viable figs (G aflatoxins were not detected)
Aspergillus flavus AF36 Prevail
For displacing aflatoxin-producing fungi
Arizona Cotton Research and Protection Council
"for growers by growers"

COTTON: FOR USE ONLY IN THE STATES OF ARIZONA, TEXAS AND CALIFORNIA (Imperial, Riverside and San Bernardino counties only)

CORN: FOR USE ONLY IN THE STATES OF ARIZONA AND TEXAS

PISTACHIO, ALMOND AND FIG: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, TEXAS AND NEW MEXICO

Aspergillus flavus AF36 is a strain of Aspergillus flavus that was isolated in Arizona and is native and endemic to many states. Apply to cotton just prior to first bloom, to corn from the 7 leaf stage (V7) until silking, to pistachio from late May through early July, or to fig from early May through late June. Aspergillus flavus AF36 competes with strains of Aspergillus flavus that produce large amounts of aflatoxin and, in doing so, limits the amount of these high aflatoxin producers that become associated with the crop.

Active ingredient: Aspergillus flavus strain AF36* .......................................................... 0.0008%
Other ingredients: .......................................................... 99.9992%
Total: ...................................................................... 100.0000%

* Contains a minimum of 3,000 CFU/gram in the End-Use Product

KEEP OUT OF REACH OF CHILDREN

CAUTION

In case of an accident, immediately call a physician or your health care provider.
Conclusions from the AF36 studies in pistachios, almonds, and figs

✓ The AF36 strain of *A. flavus* is widespread and is the most common atoxigenic strain in Ca. orchards.

✓ The AF36 strain became the dominant strain in the soil after application.

✓ The AF36 strain persisted well in the soil.

✓ It did not cause any increase in decay of pistachio, almond, and figs.

✓ In general, results were similar in these 3 tree crops.

✓ Aflatoxins were reduced substantially when AF36 was co-inoculated with highly-toxigenic strains in pistachios, almonds, and figs (lab studies).
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... and a multitude of pistachio growers

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Life cycle of *Aspergillus flavus* in almond orchards

**SPRING / SUMMER**

- conidia in the air
- Infection of nuts on trees
- sclerotia in or on soil

**AUTUMN / WINTER**

- mummies
- Survival on orchard debris
- sclerotia

- navel orangeworm

Extra figure