Mycotoxins: toxic compounds

Aspergillus  Penicillium  Fusarium

illnesses  deaths
In pistachios and almonds:

The most common mycotoxins are the aflatoxins

Aflatoxins: $B_1$, $B_2$, $G_1$, $G_2$, $M_1$
Aflatoxins: The #1 natural carcinogenic compounds

Effects:
- Acute
- Chronic

(aflatoxin B1; potential cause of liver cancer)
Commodities contaminated with aflatoxins:

Frequently contaminated with aflatoxins:
- Corn
- Peanuts
- Cottonseed

Occasionally contaminated:
- Tree nuts (almonds, pistachios, walnuts)
- Figs
- Sorghum
- Spices (paprika, etc…)
- Others
Incidence of aflatoxin contamination in California pistachio orchards

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

almonds: 1 in 25,000 nuts
figs: 1 in 5,000 fruit
Regulatory limits for aflatoxins

- **USA**
  - Aflatoxin B1 → 10 ppb
  - Total aflatoxins → 20 ppb
    (Pistachio Federal Marketing Order: → 15 ppb)

- **European Union**
  - Aflatoxin B1 → 2 ppb
  - Total aflatoxins → 4 ppb
    (pistachios for direct consumption)

(For pistachios subjected to sorting and processing:
  - B1: 5 ppb: & total aflatoxins: 10 ppb)

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

Preharvest? or Postharvest?

(if nuts are dried quickly after harvest, stored properly, kept, & transported dried)

Preharvest … starts in the field
Aflatoxin producing molds in pistachio orchards in California

Aspergillus flavus  
Aspergillus parasiticus
Aspergillus flavus strains producing sclerotia

L   M   S
CONTAMINATION RISK

(Host) pistachio

(Agent) Aspergillus (aflatoxins)

(Insect) navel orangeworm

(Environment) - Moisture
- Temperature
- Soil
Sources of spore inoculum of aflatoxin producing molds

male flowers (> 30%)
Aspergillus flavus on orchard debris
Life cycle of *Aspergillus flavus* in a pistachio orchard
Early split nuts: where most of *A. flavus* will grow

Early split nuts: main source of aflatoxin contamination in pistachio nuts
Early split nuts form in the orchard (in July and continuing until harvest)

Early splits = 2-5% of the crop
Aspergillus species in early splits

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>30.2 %</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>0.9 %</td>
</tr>
<tr>
<td><strong>A. flavus</strong></td>
<td><strong>0.7 %</strong></td>
</tr>
<tr>
<td>A. melleus</td>
<td>0.5 %</td>
</tr>
<tr>
<td>A. sydowii</td>
<td>0.2 %</td>
</tr>
<tr>
<td>A. tamarii</td>
<td>0.2 %</td>
</tr>
<tr>
<td>A. wentii</td>
<td>0.2 %</td>
</tr>
<tr>
<td><strong>A. parasiticus</strong></td>
<td><strong>0.1 %</strong></td>
</tr>
</tbody>
</table>
Problems with early split nuts

“Achilles heel” of pistachio

- Moldy kernels
- Navel orangeworm-infested kernels
- Stained shells
- Aflatoxin-contaminated
rough, shriveled hull

smooth hull

Early splits

Normal
Amounts of aflatoxins in Early Splits and normal pistachios
Reduce the amount of early splits

- Apply sufficient irrigation during spring (i.e., May) to avoid tree stress.
- Use care when applying dormancy-breaking chemicals (e.g., oil spray before bloom).
- Use a rootstock that minimizes early split nuts.
Percentage of early split nuts in rootstock trials

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Fresno County</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KAC</td>
</tr>
<tr>
<td>Atlantica</td>
<td>4.7 a</td>
</tr>
<tr>
<td>PGII</td>
<td>4.0 ab</td>
</tr>
<tr>
<td>PGI</td>
<td>4.0 ab</td>
</tr>
<tr>
<td>UCB-1</td>
<td>2.1 b</td>
</tr>
</tbody>
</table>
Navel orangeworm (NOW) moth on an early split pistachio

Adult of NOW

Places for laying eggs

UC IPM photo
<table>
<thead>
<tr>
<th>Rough hull; NOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough hull; no NOW</td>
</tr>
<tr>
<td>Smooth hull; NOW</td>
</tr>
<tr>
<td>Smooth hull; no NOW</td>
</tr>
</tbody>
</table>
**Amounts of aflatoxins in early splits with or without navel orangeworm (NOW)**

<table>
<thead>
<tr>
<th>Characteristics of early split fruit</th>
<th>positive samples (%)</th>
<th>aflatoxins per nut (ppb)</th>
<th>% of total aflatoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough hull; NOW</td>
<td>60</td>
<td>2998</td>
<td>83.7</td>
</tr>
<tr>
<td>Rough hull; no NOW</td>
<td>20</td>
<td>141</td>
<td>16.2</td>
</tr>
<tr>
<td>Smooth hull; NOW</td>
<td>20</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Smooth hull; no NOW</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Normal nuts had no aflatoxins.
Pea size, BB nuts, DBOM, C-shape nuts

Dr. Joel Siegel, USDA
DBOM ... C-nuts from a processing plant (surface sterilized - 2007)
very high in aflatoxins
One or few of these nuts may end in a retailed package...
Nut length, dark stain, and navel orangeworm (NOW) infestation

No dark stain

Dark stain 1-10%
Relationship of navel orangeworm infestation and amounts of aflatoxins

- Green bars: > 0.0 ppb
- Blue bars: > 10 ppb
- Purple bars: > 100 ppb

Navel orangeworm (%)

- 0-0.5
- 0.5-1.0
- 1.0-2.0
- >2.0
NOW larvae
Aspergillus species from larvae and pupae of NOW in *early split pistachios*

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Date</th>
<th>A. <em>flavus</em> + <em>parasiticus</em> (%)</th>
<th>Section <em>Nigri</em> (%)</th>
<th>Other Aspergillus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tulare</td>
<td>July 10</td>
<td>0.0</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Madera</td>
<td>August 7</td>
<td>2.7</td>
<td>49</td>
<td>14</td>
</tr>
<tr>
<td>Madera</td>
<td>Sept 21</td>
<td>41.7</td>
<td>77</td>
<td>12</td>
</tr>
</tbody>
</table>

2007
Sticky traps with NOW moths
Navel orangeworm with *A. flavus*
Navel orangeworm moths from traps in pistachios (Madera Co.) - 2007

15%
The life cycle of *Aspergillus flavus* in a pistachio orchard
Reduce navel orangeworm infestation

✔ Follow an aggressive insecticide program (sprays in summer/ sprays postharvest).

✔ Decrease number of nuts left after harvest.

✔ Winter sanitation should be an annual practice.
Winter sanitation
Avoid late harvests.

<table>
<thead>
<tr>
<th>Year 1</th>
<th></th>
<th>Year 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest date</td>
<td>NOW(%)</td>
<td>Harvest date</td>
<td>NOW(%)</td>
</tr>
<tr>
<td>30 Sept.</td>
<td>12.1</td>
<td>12 Oct.</td>
<td>14.2</td>
</tr>
<tr>
<td>20 Sept.</td>
<td>5.2</td>
<td>4 Oct.</td>
<td>9.1</td>
</tr>
<tr>
<td>10 Sept.</td>
<td>1.8</td>
<td>28 Sept.</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 Sept.</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 Sept.</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Harvest dates, incidence, and amounts of aflatoxins

- > 0.0 ppb
- > 10 ppb
- > 100 ppb
Late harvest also mean more stains ...
Suture staining = early split nut
Summary: Ways to reduce aflatoxins in pistachio nuts

For growers:

1. Reduce amounts of early splits (irrigation, dormancy-breaking chemicals, rootstock).
2. Follow an aggressive NOW program (insecticides in summer; and postharvest).
3. Winter sanitation / destroy mummies.
4. Avoid late harvests/ caution with re-shakes.
5. Reduce humidity (north – south planting?)
6. Transfer harvested nuts to dehydrators ASAP.
Ways to reduce aflatoxins in pistachio nuts

For processors:

1. Dry nuts ASAP.
2. Remove contaminated kernels.
3. Remove contaminated in-shell nuts using shell discoloration (brown/dark stain, suture stain, & other stains).
4. Sort out NOW infested, oily, and nuts showing signs of insect frass.
5. Keep/store nuts at right moisture levels.
6. Avoid postharvest rehydration (leaks in silos, etc…).
Various levels of NOW damage in pistachio
Use an atoxigenic strain AF36 to displace the toxigenic *A. flavus* and *A. parasiticus*
Irrigation is needed for spore production

As applied

Atoxigenic strain, AF36

Sporulation

After growth of AF36
Aflatoxin in cottonseed versus strain AF36 incidence (Peter Cotty’s data)

Dots represent values for replicate plots

$r = 0.71, P = 0.0001$
AF36 full registration in 2003

Arizonans to meet with EPA soon

Growers await antoxigenic fungi

ARIZONA COTTON Growers Association president Roger Hooper, left of Casa Grande, Ariz.; C.L. "Bill" Scott, center, Stanfield, Ariz., producer and chairman of the association's seed development committee, and Kevin Rogers of Mesa, Ariz., ACGA vice president, visit during a break at the ACGA annual meeting in Mesa, Ariz.

By Harry Cline
Farm Press Editorial Staff

Arizona cotton growers now have their own cotton variety, and they are looking for federal registration soon for use of an antoxigenic fungi they developed to reduce aflatoxin in cottonseed and hopefully increase their income.

Larry Antilla, staff director of the Arizona Cotton Research and Protection Council (ACRPC), told the annual meeting of Arizona Cotton Growers Association (ACGA) that discussions will be held with the Environmental Protection Agency this spring he hopes will lead to full registration of AF3, which has proven in tests to dramatically reduce aflatoxin to below the 20 ppb level needed to sell untreated cottonseed commercially for livestock feed.

Reducing aflatoxin can increase yields for producers and improve cottonseed quality. Cottonseed with aflatoxin levels above 20 ppb are considered to be "non-marketable."
### Percentage of AF36 strain from soil in 2005 and 2006

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Jun 05</th>
<th>Sept. 05</th>
<th>Sept. 06</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF36 (2003 &amp; 2004)</td>
<td>83.9 a</td>
<td>84.8 ns</td>
<td>46.5</td>
</tr>
<tr>
<td>AF36 (2004 &amp; 2005)</td>
<td>56.9 a</td>
<td>82.0</td>
<td>88.4</td>
</tr>
<tr>
<td>Control</td>
<td>9.1 b</td>
<td>18.3</td>
<td>10.2</td>
</tr>
</tbody>
</table>

- Natural occurrence of AF36 is ~5% in CA pistachio orchards;
Experimental Use Permit (EUP) to apply the AF36 in 3,000 acres of commercial pistachio orchard

Granted May 2007; approved Jan. 8, 2008 in California
Aspergillus flavus propagules per m$^3$ air of pistachio orchards – summer 2007

Sampling date
Library sample analyses to develop spatial patterns of aflatoxins

2001 - 2006 samples

Labels have orchard location, date, and growers' names.
### Results from aflatoxin analyses of library samples for 2001 - 2005

<table>
<thead>
<tr>
<th>Year</th>
<th>&gt;0.0 ppb</th>
<th>&gt;10.0 ppb</th>
<th>&gt;100.0 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001 (off)</td>
<td>27.4</td>
<td>13.9</td>
<td>3.8</td>
</tr>
<tr>
<td>2002 (on)</td>
<td>9.8</td>
<td>5.6</td>
<td>0.7</td>
</tr>
<tr>
<td>2003 (off)</td>
<td>34.7</td>
<td>19.7</td>
<td>2.7</td>
</tr>
<tr>
<td>2004 (on)</td>
<td>12.2</td>
<td>3.5</td>
<td>0.0</td>
</tr>
<tr>
<td>2005 (off)</td>
<td>16.0</td>
<td>4.8</td>
<td>0.9</td>
</tr>
<tr>
<td>2006 (on)</td>
<td>(analyzed)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“on year” = high yield; “off year” = low yield
## Highly contaminated library samples by County

<table>
<thead>
<tr>
<th>County</th>
<th>% samples with $&gt;100.0$ ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merced</td>
<td>12.5 (2\textsuperscript{nd})</td>
</tr>
<tr>
<td>Tulare</td>
<td>3.6</td>
</tr>
<tr>
<td>Madera</td>
<td>5.8 (3\textsuperscript{rd})</td>
</tr>
<tr>
<td>Fresno</td>
<td>15.4 (1\textsuperscript{st})</td>
</tr>
<tr>
<td>Kern</td>
<td>1.4</td>
</tr>
<tr>
<td>Kings</td>
<td>0.0</td>
</tr>
<tr>
<td>Northern Calif.</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Distribution of aflatoxins
Aflatoxin control in pistachios (BIOCONTROL)

1. Biocontrol using the atoxigenic strain AF36

Displace the toxigenic strains and reduce aflatoxins

2. GIS study by analyzing 300 to 570 library samples

Determine the “aflatoxin-hot” locations (treat these first)

Full registration of AF36

EUP
Acknowledgments

Thank you

David Morgan
Mark Doster
Lorene Boeckler
Yong Luo
Heraclio Reyes
Jessica Windh
Ryan Puckett
Acknowledgments:

California Pistachio Industry
California Pistachio Commission
California Pistachio Research Board
USDA Aflatoxin Working Group
& Paramount Farming Company

Andy Azaldo, Brenda Hansen, & Andrew Craven
Steps to reduce aflatoxins

Field

Irrigation Management
Avoid water stress in spring (May)

Rootstock

NOW

Early Splits (ES)

Reduce incidence

Eliminate ES

Processing plant

Lower damage

Special Characteristics

Electronic sorting; hand sorting; insect damaged nuts; oily; suture stain

Reduce or eliminate aflatoxin