Atoxigenic Strains of *Aspergillus flavus*: What are they and how do they work?

*Aspergillus flavus* is the primary causal agent. Many other species produce aflatoxins and some may be locally significant (i.e. *A. parasiticus*, *A. nomius*, unnamed taxon in west Africa).
Aspergillus flavus occurs throughout warm regions. However, this fungus varies widely in several important traits.
Frequencies of Vegetative Compatibility Groups among 200 L Strain Isolates of *Aspergillus flavus* isolated from Cotton in South Texas During 1999

Vegetative Compatibility Groups (VCGs) are clonal groups genetically isolated from each other.

Many traits vary among VCGs.

Fields are occupied by mixtures of many VCGs.

84 Single Member VCGs

108 Vegetative Compatibility Groups
Aflatoxin Production by Fungal Isolates in Liquid Fermentation

54 Isolates from One Agricultural Field
On average, S strain isolates produce much more aflatoxin than L strain isolates.
Crops are infected by communities genetically distinct individuals.
Aflatoxin content of cotton bolls infected with *Aspergillus flavus* strains alone and in combination

<table>
<thead>
<tr>
<th>Strain</th>
<th>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt; Content of Cottonseed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
</tr>
<tr>
<td>Toxigenic A</td>
<td>72 w</td>
</tr>
<tr>
<td>Toxigenic B</td>
<td>17 x</td>
</tr>
<tr>
<td>Atoxigenic C</td>
<td>0 z</td>
</tr>
</tbody>
</table>

Values are PPB X 1,000. Those followed by the same letter are not significantly different by Fisher's least significant difference test.
## Effects of Five Atoxigenic A. flavus Isolates on Contamination of Developing Cotton bolls by A. flavus AF13

<table>
<thead>
<tr>
<th>Atoxigenic isolate</th>
<th>Aflatoxin B$_1$ (mg/kg)</th>
<th>Aflatoxin reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF36</td>
<td>45  c</td>
<td>94%</td>
</tr>
<tr>
<td>NRRL-5565</td>
<td>220 bc</td>
<td>71%</td>
</tr>
<tr>
<td>NRRL-5918</td>
<td>327 bc</td>
<td>57%</td>
</tr>
<tr>
<td>NRRL-5917</td>
<td>427 abc</td>
<td>NS</td>
</tr>
<tr>
<td>NRRL-1957</td>
<td>553 ab</td>
<td>NS</td>
</tr>
<tr>
<td>None</td>
<td>769 a</td>
<td></td>
</tr>
</tbody>
</table>
Fungi Vary Across Areas in Aflatoxin-Producing Ability

Farmers apply atoxigenic strains to reduce the average aflatoxin-producing potential of fungi on farms & thus the vulnerability of crops to aflatoxin contamination.

Aflatoxin Production by A. flavus from Two Fields

Aflatoxin-Producing Potential

Field 1 = Low, 3,400 ppb
Field 2 = High, 54,000 ppb
Aflatoxin Contamination Occurs in Two Phases

**Phase I: Before Crop Maturity**
- Developing crops become infected.
- Associated with crop damage (insect, bird, stress).
- Crop may exhibit BGYF (bright-green-yellow fluorescence).
- Favored by high temperature (night) and dry conditions.

**Phase II: After Crop Maturity**
- Aflatoxin increases in mature crop.
- May occur before or after harvest.
- Seed is vulnerable until consumed.
- Associated with high humidity in the field, & improper crop storage or transportation.
- Rain on the mature crop increases contamination.
As Applied

After Fungal Growth
Cotton Crop Biomass and Aspergillus flavus Dynamics Under Conditions Favorable to Aflatoxin Contamination

- soil A. flavus
- total A. flavus
- total crop biomass
- cotton bolls present

Cotton Crop Biomass and Aspergillus flavus Dynamics Under Conditions Favorable to Aflatoxin Contamination

Month

- J F M A M J J A S O N D

Total Crop Biomass

Propagules of A. flavus

Month

- J F M A M J J A S O N D
Aflatoxin in Crop versus Strain Incidence

Dots Represent Values for Replicate Plots

\[ r = 0.71, \ P = 0.0001 \]
Influences of Field Application of Atoxigenic *A. flavus* on:
Aflatoxin, Infection, & Total *A. flavus* on the Crop at Harvest

<table>
<thead>
<tr>
<th></th>
<th>Aflatoxin B$_1$ (mg kg$^{-1}$)</th>
<th>Infection (%)</th>
<th>A. <em>flavus</em> on crop (spores/g)</th>
<th>Applied strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>0.3 b</td>
<td>1.03 a</td>
<td>23,949 a</td>
<td>100 a</td>
</tr>
<tr>
<td>Control</td>
<td>81.8 a</td>
<td>0.85 a</td>
<td>28,949 a</td>
<td>7 b</td>
</tr>
</tbody>
</table>

Values followed by a common letter do not differ significantly.
Mohawk Valley, Arizona: First Commercial Field Test of the Use of an Atoxigenic Strain to Prevent Aflatoxin Contamination

A Single 40 Acre Field was Treated at 10 lb./acre

1996 Crop Harvest Dates are Indicated

- Nov. 1: 312 ppb
- Nov. 3-5: 200 to 312 ppb
- Nov. 6: 341 ppb
- Oct. 28: 19 ppb
- Sept. 28: 92 ppb

Legend:
- Green = crop not cotton
- Red = seed over 20 ppb
- Blue = seed under 20 ppb
Crops from Different Fields Often Vary in Aflatoxin Content

HIGH AFLATOXIN LEVELS MAY BE MORE COMMON IN CERTAIN AREAS

If we mapped the distribution of aflatoxin in cottonseed among fields - it may resemble this:

- = greater than 20 ppb but less than 300 ppb.
- = less than 20 ppb.
- = greater than 300 ppb.
Vegetative Compatibility Groups (VCGs) are identified with complementation tests. In these tests, a mutant of an isolate is paired with tester mutants. If the isolate mutant receives genetic material from a tester to repair its mutation, a zone of thick growth occurs and the mutant belongs to the same VCG as the tester.
Minimal Requirements for a Single Sample

- Isolation Medium
- V-8 Juice Agar
- Selection Agar
- Mutant Clean-up
- Complementation agar

Each Circle is 1 Petri Dish

Crop or Soil Sample

Storage Vial

= Isolation Medium
= V-8 Juice Agar
= Selection Agar
= Mutant Clean-up
= Complementation agar
Application of Atoxigenic Strain AF36 in Commercial Cotton Influences the Composition of Fungi on Crops in both Treated and Nearby Fields

Soil Community Before Treatment

Treated Field

4% AF36

1% AF36

Application Rate = 10 lb/acre

0% AF36

Treated Field

Community on Crop After Ginning

57% AF36

60% AF36

92% AF36

42% AF36

Data from 564 vegetative compatibility analyses.

Blue dots represent the percent of the A. flavus community composed of AF36.

0.5 miles
Incidence of AF36 within Aspergillus flavus Communities

Prior to and One Year After Application

- **Treated**
- **Adjacent**
- **Diagonal**
- **Other**

Incidence of AF36

Fungi Resident in the Soil

- Incidence of AF36 = 1997
- = 1996
- * = 1997 & 1996 differ

Treated

Adjacent

Diagonal

Other

MV: 0.3 mi-SE
YV: 0.6 mi-NE
MV: 0.7 mi-E
MV: 1.2 mi-W
Percent of the *A. flavus* Soil Community Composed of the Applied Atoxigenic Strain & the Highly Toxigenic S Strain

Long-term Influences Suggest Area-wide Programs may be Effective

Application

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>J</td>
<td>Cotton</td>
</tr>
<tr>
<td>1997</td>
<td>J</td>
<td>Wheat</td>
</tr>
<tr>
<td>1998</td>
<td>J</td>
<td>Lettuce</td>
</tr>
<tr>
<td>1999</td>
<td>J</td>
<td>Broccoli</td>
</tr>
</tbody>
</table>

= % Atoxigenic Strain  = % S strain
Texas Hill: *A. flavus* in Soil Before 2001 Treatment

- **S** strain;  
- **AF36**;  
- Other *A. flavus* strains.

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**Texas Hill:**

* = Cotton Treated 2001.  
= Cotton Not Treated 2001.  
= Cotton Treated 2002.  
= Not Cotton in either 2001 or 2002.
Texas Hill: *A. flavus* on 2001 Crop

- **= S strain;**
- **= AF36;**
- **= Other *A. flavus* strains.**

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Texas Hill: *A. flavus* on 2001 Crop

- **= Cotton Treated 2001.**
- **= Cotton Not Treated 2001.**
- **= Cotton Treated 2002.**
- **= Not Cotton in either 2001 or 2002.**
Texas Hill: A. flavus in Soil Before 2002 Treatment

- S strain;
- AF36;
- Other A. flavus strains.

= Cotton Treated 2001.
= Cotton Not Treated 2001.
= Cotton Treated 2002.
= Not Cotton in either 2001 or 2002.
Each bar represents a separate crop sample from a treated cotton field. Bar heights indicate the percent of the *Aspergillus flavus* on each crop sample that is AF36. Samples from 3 farms were obtained. No AF36 was detected in field soils prior to treatment. Excellent displacement of aflatoxin producers by AF36 occurred on all 3 farms with JM averaging 91% displacement, AAK averaging 95% displacement, and LLM averaging 98% displacement. It is unusual to get such uniform and high levels of displacement across a treatment area.
Afla-guard®
Manufacturer: Circle One Global
Registered for use on Peanuts and Corn

Aspergillus flavus AF36
Manufacturer: Arizona Cotton Research and Protection Council
Registered for use on Cotton.
EUP Pistachios (3,000 acres - California)
EUP Corn (5,000 acres - Texas and Arizona)
Crops are infected by complex communities of diverse fungi
We can influence aflatoxin-producing ability of fungal communities resident in production areas through crop rotations, agronomic practice, and by applying atoxigenic strains

There are many atoxigenic strains
Select strains best adapted to rotations, ecosystems, & climates

Atoxigenics are Already Present on the Crop
Just increasing the frequency of endemic strains & natural interference with contamination

Treatments May have Long-Term Influence & Cumulative Benefits

More than One Crop May Benefit From the Same Strain

Atoxigenic Strains can be Applied Without Increasing Infection
and without increasing the overall quantity of *A. flavus* on the crop & throughout the environment
On average, S strain isolates produce much more aflatoxin than L strain isolates.
Cross-section of a Seed Filled with Sclerotia of the S Strain

Comparison of Aflatoxin Production the S & L Strains

Percent of crop infection & percent of aflatoxin contamination caused by two Morphotypes of *A. flavus*

The S Strain Caused only 11% of Crop Infections but 81% of Aflatoxins in the Crop

Kenya steps up humanitarian measures for toxic grain victims

NAIROBI, Jun 10, 2004 (Xinhua via COMTEX) -- Kenyan authorities have stepped up humanitarian aid in two districts where over 100 people died after eating aflatoxin-contaminated maize, officials said Thursday.

While launching the Save a Life Fund for the victims of food poisoning, which has spread countrywide, Kenyan Vice President Moody Awori said his government was mobilizing the resources to help those affected.

The death toll from eating contaminated maize, beans and vegetable oil in exchange for contaminated cooking oil in urban slums and rural areas.

Awori sent a passionate plea to individuals and organizations to come forward and assist needy Kenyans.

Meanwhile, Kenyan Director of Medical Services James Nyika said 1,000 people have died and another 5,000 were reported to have been taken ill.

The victims who are mostly children are said to have eaten maize and beans for more than seven days.

The patients suffering from the food poisoning have been treated by the Ministry of Health.

The two areas are the ones that had been attacked by "Mambo" food, which was sold for a song.

Outbreak of Aflatoxin Poisoning -- Kenya, January--July 2004

In May 2004, CDC Kenya, together with the CDC-supported Field Epidemiology and Training Organization, and CDC were invited by the Kenya Ministry of Health (KMOH) to assist the Ministry of Health (KMOH) in the districts of Makhuen and Kitui, Eastern Province. Preliminary data suggested that the outbreak was caused by aflatoxin poisoning, the level of which was extremely high.

September 3, 2004

Claudia Probst

The patients have symptoms of liver failure, they are coming in with yellow eyes, swollen legs, vomiting and bleeding from nose.

Killer maize sparks Kenya alarm

Several Kenyan politicians and officials have urged the government to declare a national emergency, warning that the death toll could rise to 500 people from eating aflatoxin-contaminated maize.

In total, 11 people were reported to have died while another 14 people were hospitalised at Makindu (Makindu) Hospital in Kegano.

"The patients have symptoms of liver failure, they are coming in with yellow eyes, swollen legs, vomiting and bleeding from nose," said Kenyan Health Minister Dr Jared Omollo.

Meanwhile, Makueni, Kitui, Mwingi and Machakos districts have been put on "food poisoning alert" unless all poisoned maize was seized.

"We are seeking donations to set up a fund to help the victims," Dr Omollo said.

But unless the government helped, more cases would die.

"The lives of our children, the lives of our children, the lives of our children, the lives of our children, are at stake," David Njoroge, a mother of a seven-year-old girl at a girls' school in Makueni, told a BBC reporter last week.

Njoroge was quoted last week as saying that the available maize stocks were affected.

The government was said to be planning to buy and sell the maize in Kenya, is milled into flour to make a porridge known as ugali.
The S Strain of *A. flavus* Caused the Kenyan Maize Contamination Episodes that Led to Hundreds of Deaths through Acute Aflatoxicosis

History of Acute Aflatoxicoses Outbreaks

Kenya
1982
2002
2004-
2008

Taiwan
1967

Uganda
1970

Western India
1974

Malaysia
1988

Kenya
1982
2002
2004-
2008
Kenyan Sample sites in 2005 and 2006

Figure legend
- Sample sites in 2005, aflatoxicosis districts
- Sample sites in 2006, aflatoxicosis districts
- Sample sites in 2006, non-aflatoxicosis provinces
Table 1. Incidences of *Aspergillus* Section Flavi isolates in Kenyan maize.

<table>
<thead>
<tr>
<th>Sampling Year</th>
<th>Kenyan Province</th>
<th>Kenyan District</th>
<th>Aflatoxicosis Outbreaks</th>
<th>No. samples</th>
<th>No. isolates</th>
<th>Total aflatoxin (ppb)</th>
<th>A. flavus</th>
<th>A. parasiticus</th>
<th>A. tamarii</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Eastern</td>
<td>Kitui</td>
<td>yes</td>
<td>38</td>
<td>435</td>
<td>189.6</td>
<td>75a</td>
<td>24a</td>
<td>1a</td>
<td>0a</td>
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<tr>
<td>2004</td>
<td>Eastern</td>
<td>Makueni</td>
<td>yes</td>
<td>37</td>
<td>448</td>
<td>330.1</td>
<td>73a</td>
<td>27a</td>
<td>1a</td>
<td>0a</td>
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<tr>
<td>2004</td>
<td>Eastern</td>
<td>Machakos</td>
<td>yes</td>
<td>21</td>
<td>249</td>
<td>180.2</td>
<td>76a</td>
<td>18a</td>
<td>7a</td>
<td>0a</td>
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<tr>
<td>2005</td>
<td>Eastern</td>
<td>Kitui</td>
<td>yes</td>
<td>39</td>
<td>585</td>
<td>426.3</td>
<td>83a</td>
<td>15a</td>
<td>2a</td>
<td>0a</td>
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<td>2006</td>
<td>Eastern</td>
<td>Kitui</td>
<td>yes</td>
<td>45</td>
<td>540</td>
<td>219.6</td>
<td>75a</td>
<td>25a</td>
<td>0a</td>
<td>0a</td>
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<td>2006</td>
<td>Eastern</td>
<td>Makueni</td>
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<td>60</td>
<td>791</td>
<td>375.9</td>
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<td>4a</td>
<td>1a</td>
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<td>Coast</td>
<td>Taita taveta</td>
<td>no</td>
<td>2</td>
<td>37</td>
<td>0.1</td>
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<td>2</td>
<td>32</td>
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<td>0a</td>
<td>10a</td>
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<td>100b</td>
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<td>2006</td>
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<td>Marakwet</td>
<td>no</td>
<td>2</td>
<td>32</td>
<td>0</td>
<td>13b</td>
<td>84b</td>
<td>0a</td>
<td>0a</td>
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<td>2006</td>
<td>Rift Val</td>
<td>Baringo</td>
<td>no</td>
<td>3</td>
<td>47</td>
<td>0</td>
<td>11b</td>
<td>85b</td>
<td>0a</td>
<td>2a</td>
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<td>2006</td>
<td>Rift Val</td>
<td>Keiyo (ii)</td>
<td>no</td>
<td>2</td>
<td>30</td>
<td>13.4</td>
<td>10b</td>
<td>87b</td>
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<td>0s</td>
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<td>2</td>
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