Breeding Peanuts Resistant to Preharvest Aflatoxin Contamination
What is Aflatoxin?

• “Aflatoxins are a family of toxins produced by certain fungi that are found on agricultural crops such as maize (corn), peanuts, cottonseed, and tree nuts.”

• “The main fungi that produce aflatoxins are *Aspergillus flavus* and *Aspergillus parasiticus*, which are abundant in warm and humid regions of the world.”

• “Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage.”
Where Is Aflatoxin a Problem?
What are the Consequences of Aflatoxin Exposure?

- 4.5 billion people in developing countries consume foods contaminated with aflatoxin
- Consumption leads to chronic and acute hepatocellular injury and child stunting
- Liver cancer is a frequent outcome of aflatoxin exposure, particularly in immunocompromised individuals
- In Kenya 2004, 317 cases of aflatoxin poisoning were reported with 125 deaths due to contaminated maize
What are the Consequences of Aflatoxin Exposure?

[Diagram showing the consequences of aflatoxin exposure through various steps including aflatoxin consumption, liver biotransformation, and resultant health effects such as acute aflatoxicosis, hepatocellular carcinoma, and chronic hepatitis B infection.]
# FDA Action Levels for Aflatoxin

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Action Level (ppb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Feeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn and peanut products intended for finishing (i.e., feedlot) beef cattle</td>
<td>300</td>
<td>CPG 683.100</td>
</tr>
<tr>
<td>Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)</td>
<td>300</td>
<td>CPG 683.100</td>
</tr>
<tr>
<td>Corn and peanut products intended for finishing swine of 100 pounds or greater</td>
<td>200</td>
<td>CPG 683.100</td>
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<tr>
<td>Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry</td>
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<td>CPG 683.100</td>
</tr>
<tr>
<td>Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals</td>
<td>20</td>
<td>CPG 683.100</td>
</tr>
<tr>
<td>Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known</td>
<td>20</td>
<td>CPG 683.100</td>
</tr>
<tr>
<td>Brazil nuts</td>
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<td>CPG 570.200</td>
</tr>
<tr>
<td>Foods</td>
<td>20</td>
<td>CPG 555.400</td>
</tr>
<tr>
<td>Milk</td>
<td>0.5 (aflatoxin M1)</td>
<td>CPG 527.400</td>
</tr>
<tr>
<td>Peanuts and Peanut products</td>
<td>20</td>
<td>CPG 570.375</td>
</tr>
<tr>
<td>Pistachio nuts</td>
<td>20</td>
<td>CPG 570.500</td>
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</table>

Peanut Reproduction and Evolution
Peanut Reproduction and Evolution
Peanut Reproduction and Evolution

**Arachis hypogaea**

2n=4x=40; allotetraploid, inbred, homozygous

**A genome progenitor**

- *A. duranensis* (2n=2x=20)

- *A. ipaensis* (2n=2x=20)

**B genome progenitor**

- homoeologs

- homologs homologs

- A

- A

- B

- B
Peanut Reproduction and Evolution

Arachis duranensis AA
controlled crosses
chromosome doubling
Arachis ipaensis BB
evolutionary history
Synthetic tetraploid AABB
Introgressed genetic diversity from wild AA and BB species
Selected changes within linkage blocks

Arachis hypogaea AABB
Where Does Aflatoxin Accumulate in Peanut?
How Can Pre-harvest Aflatoxin Contamination Be Mitigated?

Diagram:
- Host
- Environment
- Aflatoxin
- Fungus
- Invasion
- Competitiveness
- Stress
How Can Pre-harvest Aflatoxin Contamination Be Mitigated?

- insect resistance
- fungal resistance
- aflatoxin biosynthesis resistance

- insecticides
- atoxigenic strains

- Host
- Stress
- Environment

- Invasion
- Competitiveness
Aflatoxin Mitigation Through Insect Control

- Lesser cornstalk borer damages pods during drought stress
- LCB damage is associated with increased aflatoxin
- Drought management (irrigation) can reduce risk
- Insecticides (Lorsban) can reduce risk but irrigation also needed for pesticide to be effective
- Lorsban is detrimental to predatory insects (beneficials) resulting in outbreaks of other insect pests such as spider mites
Insects and Aflatoxin

- Lesser cornstalk borer is difficult to monitor and control with chemicals because the larvae feed on the underground pods
- Lesser cornstalk borer – limited genetic resistance in cultivated peanut gene pool
- Alternative sources of genetic resistance
  - Transgenes (Bt)
  - Wild relatives
Aflatoxin Mitigation Through LCB Control

Bt Toxin Efficacy Against Lesser Cornstalk Borer

Percent Survival

Conc (ug/ml)

0 0.09 0.18 0.37 0.75 1.5

Institute of Plant Breeding, Genetics and Genomics
College of Agricultural & Environmental Sciences
UNIVERSITY OF GEORGIA
Aflatoxin Mitigation Through LCB Control

- Introduced into Marcl in 1995
- Lines with resistance to lesser cornstalk borer (LCB) selected
- Field tests for insect resistance conducted in ‘97, ‘98, ‘99
- Significant reduction in LCB damage and aflatoxin contamination
Peanut Cultivation With Temperature and Water Stress

Imposing stress with rainout shelters
# Peanut Cultivation With Temperature and Water Stress


<table>
<thead>
<tr>
<th>Measurement date</th>
<th>Aug.</th>
<th>Sept.</th>
</tr>
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<tr>
<td></td>
<td>20</td>
<td>24</td>
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<tr>
<td>Aflatoxin &amp; leaf temp.</td>
<td>.19*</td>
<td>.25**</td>
</tr>
<tr>
<td>Aflatoxin &amp; visual rating</td>
<td>-.01</td>
<td>.04</td>
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</table>

*, ** = significant at P = 0.05 and 0.01, respectively.
Peanut Cultivation With Temperature and Water Stress

Aflatoxin set visual drought stress rating _year 2014

Daily area under drought stress curve

Worst six genotypes

IGV 94431
IGV 6750
IGV 93305

IGV 91324
IGV 2266

Best six genotypes

CS1-2-3
Florida-07
A116

CS1-1-11
CS1-1-46
CS1-1-22
**Peanut Cultivation With Temperature and Water Stress**

**Aflatoxin set visual drought stress rating_year 2014**

<table>
<thead>
<tr>
<th>Entry</th>
<th>N</th>
<th>Mean Relative Toxin</th>
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<tbody>
<tr>
<td>ICGV2184</td>
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<td>ICGV93280</td>
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<td>A116</td>
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<td>ICG1448</td>
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<td>ICGV2207</td>
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<td>ICG2800</td>
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<td>A104-12</td>
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<td>0.2</td>
</tr>
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<td>ICGV87110</td>
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<tr>
<td>A91</td>
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<td>0.3</td>
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<tr>
<td>ICG10094</td>
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<td>0.3</td>
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<tr>
<td>*C321-2-3</td>
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</tr>
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<td>*C321-2-2</td>
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<td>A103</td>
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<td><strong>C431-1-4</strong></td>
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<td>0.4</td>
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Peanut Cultivation With Temperature and Water Stress

Aflatoxin set visual drought stress rating_year 2014

<table>
<thead>
<tr>
<th>Entry</th>
<th>N</th>
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<tr>
<td>ICGV93305</td>
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<td>ICGV4750</td>
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<td>A47</td>
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Peanut Cultivation With Temperature and Water Stress
## Peanut Cultivation With Temperature and Water Stress

### Table 3. Correlation between aflatoxin contamination, yield and drought stress evaluation methods of the peanut genotypes planted at the Gibbs Farm shelter.

<table>
<thead>
<tr>
<th></th>
<th>Aflatoxin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pod Yield&lt;sup&gt;b&lt;/sup&gt;</th>
<th>- Chlorophyll fluorescence&lt;sup&gt;c&lt;/sup&gt;</th>
<th>- Visual rating&lt;sup&gt;d&lt;/sup&gt;</th>
<th>SCMR&lt;sup&gt;e&lt;/sup&gt;</th>
<th>CT&lt;sup&gt;f&lt;/sup&gt;</th>
<th>CTD&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pod yield</td>
<td>-0.44**&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-0.27</td>
<td>-0.62**</td>
<td>-0.26</td>
<td>0.26</td>
<td>0.85**</td>
<td>0.85**</td>
</tr>
<tr>
<td>PI&lt;sub&gt;ABS&lt;/sub&gt;</td>
<td></td>
<td></td>
<td>-0.26</td>
<td>0.25</td>
<td>0.98**</td>
<td>0.59**</td>
<td>0.98**</td>
</tr>
<tr>
<td>F&lt;sub&gt;v&lt;/sub&gt;/F&lt;sub&gt;m&lt;/sub&gt;</td>
<td>-0.62**</td>
<td>-0.12</td>
<td>0.56**</td>
<td></td>
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<tr>
<td>φ&lt;sub&gt;EO&lt;/sub&gt;</td>
<td>-0.26</td>
<td>0.25</td>
<td>0.98**</td>
<td>-0.60**</td>
<td>-0.62**</td>
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<tr>
<td>Visual rating AM</td>
<td>0.85**</td>
<td>-0.40</td>
<td>-0.29</td>
<td>-0.41*</td>
<td>-0.25</td>
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<tr>
<td>Visual rating PM</td>
<td>0.85**</td>
<td>-0.26</td>
<td>-0.24</td>
<td>-0.50**</td>
<td>-0.21</td>
<td>-0.60**</td>
<td>-0.62**</td>
</tr>
<tr>
<td>SCMR</td>
<td>-0.57**</td>
<td>0.32</td>
<td>0.22</td>
<td>0.43*</td>
<td>0.21</td>
<td>-0.60**</td>
<td>-0.62**</td>
</tr>
<tr>
<td>CT</td>
<td>0.73**</td>
<td>0.01</td>
<td>-0.09</td>
<td>-0.64**</td>
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<td>0.73**</td>
<td>0.84**</td>
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<td>CTD</td>
<td>-0.81**</td>
<td>0.19</td>
<td>0.15</td>
<td>0.42*</td>
<td>0.15</td>
<td>-0.79**</td>
<td>-0.85**</td>
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<tr>
<td>NDVI</td>
<td>-0.79**</td>
<td>0.17</td>
<td>0.25</td>
<td>0.62**</td>
<td>0.24</td>
<td>-0.84**</td>
<td>-0.87**</td>
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</tbody>
</table>

<sup>a</sup> Aflatoxin contamination

<sup>b</sup> Pod Yield

<sup>c</sup> Chlorophyll fluorescence

<sup>d</sup> Visual rating

<sup>e</sup> SCMR

<sup>f</sup> CT

<sup>g</sup> CTD

<sup>h</sup> Correlation coefficient
Pre- and Post-harvest Resistance

<table>
<thead>
<tr>
<th>Entry</th>
<th>Relative Yield (wd/ww)</th>
<th>Relative Toxin (log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICG 1471 (55-437)</td>
<td>0.48</td>
<td>1.18</td>
</tr>
<tr>
<td>ICGV 88145</td>
<td>0.43</td>
<td>2.71</td>
</tr>
<tr>
<td>ICG 862</td>
<td>0.74</td>
<td>1.06</td>
</tr>
<tr>
<td>ICG 8285</td>
<td>0.65</td>
<td>1.25</td>
</tr>
<tr>
<td>ICG 1703</td>
<td>0.41</td>
<td>1.12</td>
</tr>
<tr>
<td>ICG 4729</td>
<td>0.58</td>
<td>0.97</td>
</tr>
<tr>
<td>ICG 6667</td>
<td>0.32</td>
<td>2.34</td>
</tr>
<tr>
<td>ICG 6766</td>
<td>0.43</td>
<td>2.71</td>
</tr>
<tr>
<td>ICG 1471</td>
<td>0.48</td>
<td>1.18</td>
</tr>
<tr>
<td>Fleur 11</td>
<td>0.41</td>
<td>1.42</td>
</tr>
</tbody>
</table>
Post-harvest Resistance

RFU / 1 g protein /1mm² of seed surface area

Aflatoxin B1 in ppb
Post-harvest Resistance

Aflatoxin B1 in ppb

RFU / 1 g protein /1mm² of seed surface area
Genetic Factors in ICG 1471 (55-437) for Aflatoxin Reduction

• Improvement of aflatoxin resistance in peanut by identifying genomic regions controlling post-harvest aflatoxin production that may also contribute to pre-harvest reduction in contamination
Combine existing host resistance with transgene resistance
- Insect resistance
- Defensins
- Reactive oxygen scavenging enzymes
- Host-induced gene silencing

Aflatoxin biosynthesis gene cluster

Yu. 2012. Toxins
Sharma. 2018. Plant Biotech J
Biological Control Through Competition

Increased proportion of applied atoxigenic strains in soil and grains translates into reduced aflatoxin concentration in grain.

Atoxigenic strains in soil and grain

Applied atoxigenic VCGs (%)

Maize

Peanut

Aflatoxin in grain at harvest

Applied atoxigenic VCGs (%)

0 20 40 60 80 100

Soil before application

Soil at harvest

Grain at harvest

Control

Treated

Aflatoxin (ppb)

Control

Treated

Log Aflatoxin (ppb)

% Nontoxigenic A. flavus and A. parasiticus

y = -0.011x + 3.71

R² = 0.703

y = -0.023x + 3.13

R² = 0.675

Atehnkeng et al. 2014. Biol Control

Dorner and Horn 2007. Mycopathologia
Conclusion – All Technologies Must Be Made Available To Combat Aflatoxin Contamination

- insect resistance
- fungal resistance
- aflatoxin biosynthesis resistance
- insecticides
- atoxigenic strains
- irrigation

Diagram:
- Host
- Stress
- Environment
- Invasion
- Competitiveness
- Fungus