Monitoring and managing mycotoxins on maize
Challenges and opportunities for resolving an emerging public health crisis in Kenya

Rebecca Nelson
ASM meeting
January 2014
Outline

• Overview of the problem
  – in the African context

• Assessment
  – Evidence of pervasive contamination of Kenyan maize

• Perception
  – Who knows; who needs to know?

• Management
  – Management options
  – The posho mill scheme

Photo: S. Mideros
Focus fungi/toxins for this study

<table>
<thead>
<tr>
<th>Feature</th>
<th>Aflatoxin</th>
<th>Fumonisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungus</td>
<td><em>Aspergillus flavus</em> and <em>A. parasiticus</em></td>
<td><em>Fusarium</em>, esp. <em>F. verticillioides</em></td>
</tr>
<tr>
<td>Health issue</td>
<td>Hepatitis and liver cancer; growth impairment; immunosuppression</td>
<td>Esophageal cancer; growth impairment; neural tube defects</td>
</tr>
<tr>
<td>Mechanisms</td>
<td>DNA damage; gut irritation</td>
<td>Sphingolipid interference; gut irrit’n</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>Generalist; weak ear-rot pathogen; pre- and post-harvest</td>
<td>Maize endophyte and stronger ear-rot pathogen; pre-harvest</td>
</tr>
<tr>
<td>Environmental drivers</td>
<td>Warmer temps (30 - 43°C); plant stress (drought)</td>
<td>Moderate temps (15 - 37°C)</td>
</tr>
<tr>
<td>Regulatory limit</td>
<td>10 ppb (Kenya)</td>
<td>1,000 ppb</td>
</tr>
</tbody>
</table>

Aflatoxin B1

Fumonisin B1

Why worry about mycotoxins on maize in Kenya?

- Maize as staple
  - 25% of calories; 25% of cropped area;
- Substantial self-provisioning
  - >70% by 3.5 M smallholders
- Climate and other stressors → high risk
- Most Kenyans HepB seropositive
- There are known problems...
  - Fatal aflatoxicosis in most years; highly toxigenic *Aspergillus* in E Kenya
  - Fumonisin also reported
- ... but there is limited data, awareness or management; methods are limiting
What is the extent of mycotoxin contamination of Kenyan maize?
2009/2010 study sites

Kenya
Grain mill survey

Samuel Mutiga, Vivian Hoffmann et al.
Mycotoxin measurements
Biosciences Eastern and Central Africa
ILRI, Nairobi

- Helica ELISA
- Total Aflatoxin Assay: Solid phase direct competitive immunoassay
- Range: 1-20 ppb

- VICAM Aflatest Immunocapture
- Fluorometer/monoclonal antibody based affinity chromatography
- Range: 0.1-300 ppb
Aflatoxin occurrence in eastern vs. western regions

Western
2009
26 mills
n=985
15% over legal limit

Eastern
2010
146 mills
n=1,500
39% over legal limit

S. Mutiga
Districts with sub-humid agroecologies at greatest risk (watch semi-humid and arid too)?

**Western**
- Rechungyo: 50%
- Homa Bay: 40%
- Kaleur: 30%
- Lush Geshu: 20%
- Trans-Nzoia: 10%
- Bungoma: 10%

**Eastern**
- Meru Central: 50%
- Mwala: 40%
- Meru North: 30%
- Meru South: 20%
- Mathi: 10%
- Kitui: 10%
- Makena: 5%
- Eribu: 5%
- Meshikos: 5%
- Kalabani: 5%

**Predominant AEZ**
- Semi Arid
- Semi-humid to Semi-arid
- Semi humid
- Sub humid
- Humid

S. Mutiga
Aflatoxin drivers in maize – E. Kenya

- 1,500 samples w/questionnaire on 31 management factors
- 60% samples home-grown
- 1/3 < 1 ppb

- Drivers of +/- (GLM)
  - Yield, land size, cropping system, AEZ
  - Home sorted, preservative

- Drivers of quantity (> 1 ppb; mixed model)
  - Quality, kernel integrity, AEZ

- 17% of variance explained by mills w/in AEZ

- Sub-humid most contaminated (post-harvest?)
- Rainfall during grain filling significant in semi-humid
- More land and yield → less toxin
  - Less crop stress
  - More ability to select

- Intercrops → less toxin than monocultures
- Kernel damage poor indicator

Mutiga and Vermeylen
Fumonisin occurrence in western and eastern Kenya

Western Kenyan
n = 270
31% over legal limit

Eastern Kenya
n = 569
38% over legal limit

S. Mutiga
Contamination with two mycotoxins across AEZs

Decreasing soil moisture availability
Do fumonisin levels affect child growth?

- Yellow- Demographic and Health Survey cluster location (n=73 clusters)
- Blue- mycotoxin sample location
- Orange- 20 km radius

Laura Smith, Becky Stoltzfus
Fumonisin associated with stunting

- Median fumonisin levels (controlling for FB variance) significantly predicted HAZ ($B=-0.00055; p=0.034$) and WAZ ($B=-0.0005; p=0.029$) of children.

- Controlled for known predictors of poor child growth: Gender, HH income, mother's education, mother's height, mother's weight, diet diversity.

- In a region with a median fumonisin level of 1000 ppb, the international legal limit for fumonisin, the average child is 0.5 Z scores shorter and lighter than a child in a region with no fumonisin.
What is the extent of mycotoxin contamination of Kenyan maize?

- **Aflatoxin**: lots where it is a known problem; present but less where not recognized.
- **Overall mean of [aflatoxin] = 3x legal limit**

**Fumonisin**: big and important

High rates of esophageal cancer in Kenya

**Perception**

**Response**
Assessment

Perception

Response

Do people know?
Figure 2
Aflatoxin Contamination by Stated Use of Grain

Notes: Excludes 5.4% of the sample with greater than 100 ppb aflatoxin contamination. Vertical lines indicate 10 ppb and 20 ppb aflatoxin.
Less care taken with maize for sale

Post-harvest: people take more care with maize they will eat

- 100 people surveyed; 38% sold maize
  - Keep small-grain local types; sell large-grain hybrid
  - 50% of sellers take less care in drying, storage
  - 50% of sellers use pesticides on maize to be sold

V. Hoffmann

Photo: James Gethi
Emerging concern about aflatoxin in Kenya

Acute aflatoxicosis

<table>
<thead>
<tr>
<th>Year</th>
<th>Fatalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 - 1999</td>
<td>15</td>
</tr>
<tr>
<td>2000 - 2010</td>
<td>207</td>
</tr>
</tbody>
</table>

Maize quality loss

- Inadequate surveillance
- No proper regulation

2010: 2.3 million bags = 20,700 t condemned
Assessment

Perception

Response

Increasing concern about aflatoxin in research, policy, & funding circles

Information asymmetry; lack of incentive for clean maize

Consumers need to know what they are feeding their families
What can people do to reduce mycotoxin accumulation and exposure?

- Pre-harvest resistance
- Post-harvest resistance
- Soil fertility management
- Grain sorting
Genetic resistance to mycotoxin accumulation?

Aspergillus ear rot evaluation trial, Mexico, July 2005.

Pre-harvest resistance  Post-harvest resistance  Soil fertility management  Grain sorting

CML 269 -derived hybrid
Pre-harvest resistance: QTL meta-analysis synthesis of 12 mapping studies

Heritability is low to moderate for aflatoxin resistance, moderate to high for fumonisin resistance.
Post-harvest resistance: mature kernel assay

26 diverse inbred maize lines; seed grown in five locations (7 sets)

<table>
<thead>
<tr>
<th>Location</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurora</td>
<td>2009</td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>2007</td>
<td>2008</td>
</tr>
<tr>
<td>Missouri</td>
<td>2007</td>
<td>2009</td>
</tr>
<tr>
<td>Florida</td>
<td>2007</td>
<td></td>
</tr>
<tr>
<td>Blacksburg</td>
<td>2009</td>
<td></td>
</tr>
</tbody>
</table>
Susceptibility of mature kernels to aflatoxin is influenced by grain production conditions

- Low heritability, $H^2 = 35\%$

Pre-harvest resistance

Post-harvest resistance

Soil fertility management

Grain sorting
Maize varieties in farmer’s storage conditions

Questions
Do the following influence mycotoxin accumulation in farmers’ stores?
• Variety/texture
• Moisture content at harvest
• Storage conditions
• Ear rot/integrity
• Preservative

Setup
• Humid AEZ in Bungoma, W. Kenya
• 182 farmers who grew major varieties:
  – H614
  – H513
  – WH505
  – Local # 8

S. Mutiga

Grain moisture at harvest associated with fumonisin

Varietal differences in toxin levels in storage
Mycotoxin levels much higher in posho mills than in grain stores

Aflatoxin

Fumonisin

In storage

At mill
CIMMYT IMAS goal: develop varieties tolerant to low N

403 genotypes

KARI Embu
Low N
Long Rains

KARI Kiboko
Low N
Long Rains

Bulked

KARI Kiboko
Optimal N
Long Rains

KARI Kiboko
Optimal N
Short Rains

Rep 1
Rep 2
Bulked
Rep 1
Rep 1

109 genotypes common to all environments

Pre-harvest resistance
Post-harvest resistance
Soil fertility management
Grain sorting
Nitrogen management v. mycotoxins

N treatment associated with lower aflatoxin but not lower fumonisin

Collaboration with CIMMYT’s Improve Maize for African Soils Project
Grain from high-nitrogen fields:

- Larger kernel size
- Higher nitrogen content
- Higher bulk density

Aflatoxin was negatively correlated with kernel density ($r=-0.35$, $p<0.0001$) and percent protein ($r=-0.3$, $p<0.0001$)

Collaboration with CIMMYT’s Improve Maize for African Soils Project
Mycotoxin management by grain sorting?

Contamination highly skewed. Can consumers sort maize to reduce exposure?

Pre-harvest resistance  Post-harvest resistance  Soil fertility management  Grain sorting
Pair-wise testing (E. Kenya)

- Moldiness correlated with fumonisin ($r=0.5^{**}$)
Mechanized spectral grain sorting

- Low-cost, limited spectrum sorter
- Calibrated on n=378 single kernels from open markets and field trials
- 77% sensitivity and 83% specificity to reject kernels over legal limits.
- Reject rates:
  - Toxic samples: 0 - 25%
  - Clean samples: 0 - 1%.
- Accepted maize had lower toxin levels than the rejected maize (14/16 samples lower observed aflatoxin and 16/17 samples with lower fumonisin).
- Sorting → retain food supply while reducing mycotoxin exposure.

Tom Pearson, USDA-KSU
Matt Stasiewicz, Cornell
Murithi Mutuma, U of Nairobi
Samuel Mutiga, Cornell
Jagger Harvey, IRLI-BecA
What can people do to reduce mycotoxin accumulation and exposure?

- Less susceptible varieties
- Pre- and post-harvest management
- Visual sorting for fumonisin; spectral sorting for both toxins
- ? Decortication? Nixtamalization?
- Sorptive clays?
Food safety as added value at posho mill?

Cheap diagnostics and sorting needed

NovaSil, micronutrient sachets

Information on mycotoxins/management
Ways forward by stakeholder

**Policy, R&D**
- Raise awareness across value chain
- Less susceptible varieties
- Support millers, farmers
- Surveillance

**Consumers**
- Access to information
- Reject toxic product
- Sort to keep food
- Sorbtive clay (NovaSil)
- Learn to reduce problem in self-provisioned maize

**Farmers**
- Less susceptible varieties
- Manage crop, soil, water
- AflaSafe = biocontrol
- Good storage

**Traders and millers**
- Analyze and pay more for clean product
- Sort grain
- Contribute to awareness by producers and consumers

**Affordable testing, Increased awareness**
Summary and conclusions

• Too much mycotoxin contamination
  – Fumonisine needs attention too
• Some variation explicable
• Spectral but not visual sorting works for aflatoxin contamination
• Needed: systems approach
  – Varieties and practices that reduce infection, colonization and toxin accumulation
  – Rapid diagnostics (non-destructive) and sorting at posho mills
Team and context

• Atkinson Center for a Sustainable Future/StART: Cornell
  – Samuel Mutiga, Rebecca Nelson, Michael Milgroom, Cornell U.
  – Vivian Hoffman, U. of Maryland
  – Vincent Were, Jagger Harvey and Patrick Kariuki et al., Biosciences E & C Africa-ILRI, Kenya

• NSF-IGERT at Cornell: Laura Morales; Laura Smith; Matt Staciewicz

• Capacity and Action for Aflatoxin Reduction in Eastern Africa project (CAAREA): Australian Govt.
  – BecA, KARI, ARI-Tz, Cornell, as above
  – CSIRO: Ross Darnell, others