Toxigenic fungi and their associated mycotoxins: a threat for crop production and food safety in Sub-Saharan Africa

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Introduction and background
What are toxigenic fungi?

- Filamentous fungi that produce toxic secondary metabolites or mycotoxins during infection and colonisation process
  - ‘Broad assortment’ of fungal species:
    - Are present in different environments: different crops, stored food and feed, silages, ...
    - Produce a wide variety of mycotoxins: diversity in chemical structure but also in (phyto)toxicity
    - Detection is challenging: different structures; matrices and modified forms
    - Biodiversity in genera and within species is high with implication in disease management and mycotoxin control
Biodiversity: genera and species level

**Fusarium species**
- F. graminearum
- F. culmorum
- F. Poae
- F. lansethiae
- F. sporotrichioides
- F. verticilliioides
- F. tricinctum
- F. equiseti
- F. acuminatum
- F. proliferatum
- F. sambucinum

**Aspergillus species**
- A. flavus
- A. niger
- A. parasiticus
- A. normius
- A. ochraceus
- A. Carbonerius
- A. aculeats
- A. versicolor

**Penicillium species**
- P. roqueforti
- P. paneum
- P. citrinum
- P. cuclopium
- P. verrucosum

**ZEN**
- DAS
- DON
- NIV
- ZEN
- TE/HT2
- Afatoxins
- citrinin
- Rafatoxins

**Penicillium species**
- P. roqueforti
- P. paneum
- P. citrinum
- P. cuclopium
- P. verrucosum

**Ochratoxin A**
- Roquefortin C
- patulin
- Sterigmatocystin

**Alternaria, Claviceps, Byssoschlamys**
• Other level of diversity: Chemotypes

*Fusarium poae*, a special case
• Mycotoxins are secondary metabolites!

Interaction host – environment and pathogen determines mycotoxin level

Winter wheat, Flanders
Are mycotoxins only an African problem?
But...big differences in food systems (1)

- **Developing countries**
  - Food systems are strongly regulated and large scale
  - Trade based
  - Advanced infrastructure e.g. storage, ...
  - Capital intensive
  - Diversity in diet and origin of food stuff

- **Developing countries**
  - Small scale and (less)(un)regulated
  - Informal (local) markets
  - No control on exceedance of MTL

- **Subsistence**
  - In case of contamination family members are exposed for several months to mycotoxin

- Diet exist of limited ingredients of local produced stable food
  - Maize provides 60% of dietary calories and 35% of the protein intake in Tanzania

- Food insecurity
But...big differences in food systems (2)

- Most staple foods in Africa are (very) susceptible for a plethora of toxigenic fungi!
- Growing - and storage conditions are in favorite for fungal growth in SSA countries
- Two examples:

**Mycotoxin content (median in µg/kg) of several staple foods in N-Uganda (2015-2016)**

<table>
<thead>
<tr>
<th></th>
<th>Sorghum</th>
<th>Maize</th>
<th>Millet</th>
<th>Groundnut</th>
<th>Simsim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>12,5</td>
<td>23,4</td>
<td>43,5</td>
<td>21</td>
<td>10,1</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>3,4</td>
<td>1,8</td>
<td>0,774</td>
<td>0</td>
<td>1,31</td>
</tr>
<tr>
<td>DON</td>
<td>456</td>
<td>344</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FUMs</td>
<td>158</td>
<td>244</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Echodu et al, 2018
Results of survey in Tanzania (2012): mycotoxin pattern (%) and range per Agro-ecological zone

**AEZ 1**
Northern highlands
Region: Manyara
District: Hanang’

- **FBs**: 11 – 197 µg/kg
- **AFBs**: 0.28 – 13 µg/kg

**AEZ 2**
Eastern lowlands
Region: Morogoro
District: Kilosa

- **FBs**: 35 - 6947 µg/kg
- **AFBs**: 71 – 1005 µg/kg

**AEZ 3**
Southern Highlands
Region: Mbeya
District: Rungwe

- **FBs**: 20 – 14366 µg/kg
- **AFBs**: 0.78 – 20 µg/kg

De Graeve et al., 2016
## Fumonisin contamination in maize from some African countries

<table>
<thead>
<tr>
<th>Country (Place)</th>
<th>Source</th>
<th>No of samples</th>
<th>Positive samples (%a)</th>
<th>Maximum contamination (µg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanzania</td>
<td>Market outlets</td>
<td>9</td>
<td>88</td>
<td>225*</td>
<td>Doko et al., 1996</td>
</tr>
<tr>
<td>HECA, South Africa</td>
<td>Households</td>
<td>141</td>
<td>100</td>
<td>10,140</td>
<td>Shephard et al., 2007a</td>
</tr>
<tr>
<td>Benin</td>
<td>Farms in villages</td>
<td>12</td>
<td>100</td>
<td>12,000</td>
<td>Fandohan et al., 2005</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>Silos</td>
<td>5</td>
<td>100</td>
<td>8,000</td>
<td>Gamaryya and Sibanda, 2001</td>
</tr>
<tr>
<td>LECA, South Africa</td>
<td>Home-grown</td>
<td>120</td>
<td>100</td>
<td>6,173</td>
<td>Shephard et al., 2007a</td>
</tr>
<tr>
<td>Ghana</td>
<td>Market outlets,</td>
<td>15</td>
<td>100</td>
<td>4,222*</td>
<td>Kpodo et al., 2000</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>Households</td>
<td>26</td>
<td>100</td>
<td>3,120</td>
<td>Nikiema et al., 2004</td>
</tr>
<tr>
<td>Botswana</td>
<td>Market outlets</td>
<td>8</td>
<td>100</td>
<td>370*</td>
<td>Doko et al., 1996</td>
</tr>
<tr>
<td>Malawi</td>
<td>Market outlets, human</td>
<td>8</td>
<td>100</td>
<td>135*</td>
<td>Doko et al., 1996</td>
</tr>
<tr>
<td></td>
<td>food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>Rural households</td>
<td>47</td>
<td>32</td>
<td>22,200**</td>
<td>Chelule et al., 2001</td>
</tr>
<tr>
<td>South Africa</td>
<td>Urban households</td>
<td>49</td>
<td>6</td>
<td>500**</td>
<td>Chelule et al., 2001</td>
</tr>
</tbody>
</table>

HECA, high incidence oesophageal cancer area; LECA, low incidence oesophageal cancer area; * Included FB3; ** FB1 alone.
## Wrong Emphasis on Aflatoxin and Human Disease

(Gong et al)

<table>
<thead>
<tr>
<th>Health effect</th>
<th>Possible deaths (No.)</th>
<th>Relative public attention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological weapon</td>
<td>0 (?)</td>
<td>Very high</td>
</tr>
<tr>
<td>Acute aflatoxicosis</td>
<td>100’s</td>
<td>High</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>10,000’s</td>
<td>Medium</td>
</tr>
<tr>
<td>Growth impairment/immunosuppression</td>
<td>100,000’s (?)</td>
<td>Low/None</td>
</tr>
</tbody>
</table>
Sub-lethal/chronic effects of mycotoxins are of major concern

- Kilimanjaro region:
  - Exposure to ‘FUMs’ is inversely correlated with growth of infants
  - Mean LAZ and WAZ scores at 6 and 12 months were significantly lower for the high exposure group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low exposure group (n=162)</th>
<th>High exposure group (n=26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAZ score</td>
<td>-1.56</td>
<td>-2.18</td>
<td>0.004</td>
</tr>
<tr>
<td>Change in LAZ score</td>
<td>-0.59</td>
<td>-0.58</td>
<td>0.929</td>
</tr>
<tr>
<td>WAZ score</td>
<td>-0.65</td>
<td>-1.08</td>
<td>0.035</td>
</tr>
<tr>
<td>Change in WAZ scores</td>
<td>-0.34</td>
<td>-0.33</td>
<td>0.918</td>
</tr>
<tr>
<td>WLZ score</td>
<td>0.18</td>
<td>0.05</td>
<td>0.496</td>
</tr>
<tr>
<td>Change in WLZ score</td>
<td>-0.28</td>
<td>-0.39</td>
<td>0.524</td>
</tr>
</tbody>
</table>

LAZ, length-for-age z score; WAZ, weight-for-age z score; WLZ, weight-for-length z score; Change in LAZ score is the mean difference between LAZ scores at 12 and 6 months of age; Change in WAZ score is the mean difference between WAZ scores at 12 and 6 months of age; Change in WLZ score is the mean difference between WLZ scores at 12 and 6 months of age

(source: PhD M.E. Kimanya, UGent)
Toxigenic fungi: direct effect on yield

• Fusarium ear rot (*Fusarium verticillioides*)/ Giberella ear rot in maize: till 80-90% and 32-50% yield losses
• *Fusarium* species cause also stalk rot and wide-spread germination problems
• Less yield effect of ‘storage’ fungal species as *Aspergillus* sp.
Pillars for controlling toxigenic fungi
1. Knowledge about pathogen biology ➔ know your enemy (1)

**F. graminearum** (Giberella ear rot)

**F. verticillioides** (Fusarium ear rot)

Often symptomless endophytic infections
Knowledge about pathogen biology (2)

Aflatoxigenic fungi
- Knowledge about pathogen biology (3)
  - Role of mycotoxins in infection/colonisation process?

**DON/NIV = pathogenicity factors**
Stress induces DON: sub-lethal fungicide concentrations

Audenaert et al, 2010
• Knowledge about pathogen biology (4)
  • Role of mycotoxins in infection/colonisation process?
  • Other mycotoxins: less clear
    - Antibiotic activity? Increase competitiveness?
    - N source? Roquefortin C?
    - Certain mycotoxins are able to hijack and neutralize the defence mechanisms of host plant
    - Fumonsins: different opinions but in most studies no relation between virulence and Fumonisins
      BUT: FB1 interact with several pathways (ET, Jasmonates, Salicylic acid), cause depletion of ATP reservoir, cause PCD by ubiquitination, ...
2. Detection and monitoring (1)

Survey Tanzania in different agriclimatic zones (2012)

Seasonal effect on the percentage of maize stalk borer ear injury (EI), kernel injury (KI), *Fusarium* ear rot (FER) and *Fusarium* kernel rot (FKR), fungal biomass and FUMs during 2013 and 2014 (Tanzania)

Different letters indicated significant differences between treatments according to Dunnett T3 test
Detection and monitoring (2)

Penicillium species in samples from bins

- P. oxalicum: 8%
- P. funicolurum: 9%
- P. brevicompactum: 50%
- Unidentified: 33%

Field: 100%: P. citrinum

Tazania 2012
3. Cultural strategies

Crop residues: inoculum sources

Lack of crop rotation

Crop rotation: 15.0% (Northern Highlands), 10.0% (Eastern lowlands)
Mulching: 22.5% (Northern Highlands), 7.5% (Eastern lowlands)
Shifting cultivation: 27.5% (Northern Highlands), 75% (Eastern lowlands)
Plant green manure: 37.5% (Northern Highlands), 85.0% (Eastern lowlands)
None: 0% (Northern Highlands), 0% (Eastern lowlands)

Lack of incorporating crop residues

Tillage method:
- Zero tillage: 2.5% (Northern Highlands), 0.0% (Eastern lowlands)
- Minimum tillage: 30.0% (Northern Highlands), 2.5% (Eastern lowlands)
- Primary and secundary tillage: 65.0% (Northern Highlands), 95.0% (Eastern lowlands)
- No answer: 2.5% (Northern Highlands), 2.5% (Eastern lowlands)

Questionnaire Tanzania, 2012
Crop rotation experiment since 2002 (UGent Experimental farm)

Sum = sum of 23 mycotoxins
Effect of fertilization (unfertilized control, nitrogen (N), phosphorus (P) and N+P) on the percentage of maize stalk borer ear injury (EI), kernel injury (KI), *Fusarium* ear rot (FER) and *Fusarium* kernel rot (FKR) during 2013 and 2014 (Tanzania)

Different letters indicated significant differences between treatments according to Dunnett T3 test

*Madege et al., 2018*
4. Antifungals

Effect of crop protection (control, endosulfan (END), triadimenol+tebucunazole (T TBZ) and endosulfan combined with triadimenol+tebucunazole (END+T TBZ)) on percentage of Maize Stalk borer ear injury (EI), kernel injury (KI), Fusarium ear rot (FER) and Fusarium kernel rot (FKR) during 2013 and 2014, Tanzania.

Different letters indicated significant differences between treatments according to Dunnett T3 test.

Madege et al., 2018
Effect of crop protection (control, endosulfan (END), triadimenol+tebucunazole (T TBZ) and endosulfan combined with triadimenol+tebucunazole (END+T TBZ)) on the level of fumonisin B1 (FB1), fumonisin B2 (FB2) and the total fumonisin level (FBtot) in 2013 and 2014, Tanzania.

![Graph showing the effect of crop protection on fumonisin levels in 2013 and 2014 in Tanzania.]

Different letters indicated significant differences between treatments according to Dunnett T3 test.
Fungicides are not the hoy grail...

Winter wheat, Flanders (2002-2017)
Fungicides are not the holy grail...
Effect of different Bt-event on *Fusarium ear rot* and Fumonisin content of maize

Munkvold et al, 1999

Linear correlation coefficients among insect feeding damage, Fusarium ear rot, and fumonisin B1 concentrations. All coefficients were highly significant ($P < 0.0001$).

<table>
<thead>
<tr>
<th>Bt event</th>
<th>Trademark</th>
<th>Cry protein</th>
<th>Promoter(s)</th>
<th>Expression</th>
<th>Approved in EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>176</td>
<td>KnockOut, NatureGard</td>
<td>Cry1A(b)</td>
<td>PEPC + pollen</td>
<td>Green tissue + pollen</td>
<td>Yes</td>
</tr>
<tr>
<td>BT11</td>
<td>Yieldgard</td>
<td>Cry1A(b)</td>
<td>CaMV 35S</td>
<td>All tissue</td>
<td>Yes</td>
</tr>
<tr>
<td>CBH351</td>
<td>StarLink</td>
<td>Cry9C</td>
<td>CaMV 35S</td>
<td>All tissue</td>
<td>Yes</td>
</tr>
<tr>
<td>DBT418</td>
<td>BTXtra</td>
<td>Cry1A(c)</td>
<td>CaMV 35S</td>
<td>All tissue</td>
<td>No</td>
</tr>
<tr>
<td>MON810</td>
<td>Yieldgard</td>
<td>Cry1A(b)</td>
<td>CaMV 35S</td>
<td>All tissue</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Fusarium ear rot severity</th>
<th>Fumonisin B1 (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>0.66</td>
<td>0.50</td>
</tr>
<tr>
<td>1997</td>
<td>0.86</td>
<td>0.69</td>
</tr>
<tr>
<td>1998</td>
<td>0.81</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Insect feeding severity (kernels/ear): 0.66, 0.86, 0.81
Fusarium ear rot severity (kernels/ear): 0.69, 0.76, 0.73

Fumonisin B1 (µg/g) average: 0.69, 0.76, 0.73
Effect of Bt-maize on Fumonisin, DON and ZEA content of maïze

Munkvold et al, 1999
Alternative antifungal strategies
Antitoxigenic strains

- Strong reduction of aflatoxin (80-100%)
- 10kg per ha 2-3 weeks before crop flowering
- Region specific strains are needed
- What with mutations in AFLA gene cluster?

https://aflasafe.com/aflasafe/
Priming with green leaf volatiles in FHB ears

Ameye et al., New Phytologist., 2018
Increased defense against *Fusarium graminearum*?
Experimental design

A  Detached leaf assay

- **H₂O**
- Z-3-HAC
- MeSA
- MeJA

Purified air from pump
Filter paper

15 hbi

Prime with Z-3-HAC
Overnight Exposure

Challenge

0 hai

Detached leaf

Disease Scoring
X hai
Priming against FHB

• Delay in disease progression

• Lower active fungal biomass

Ameye et al., Plant Physiology, 2015
Metabolomics Workflow
Metabolomics - treatments

Control

Z-3-HAC
+ Fusarium graminearum

Z-3-HAC

Fusarium graminearum
METABOLOMICS: PCA score plot

- One circle represents 1 sample
- 4310 metabolites
- Samples that cluster together share same metabolite pattern
Metabolomics: plant hormones

- SA
- JA
- ABA
- IAA
- ...

![Graph showing changes in Jasmonic acid levels over time.](image)
Metabolomics results

• Z-3-HAC and Z-3-HAC + Fg treatment

• MS/MS analysis revealed presence of glucose group

Hexenyl-diglucoside
Glycosylation: physiological significance

• Glucose donor + metabolite $\rightarrow$ gly(u)cosylated-R
• Inactivation of metabolites
  • Storage of defensive compounds (Priming)

Storage of inactive plant defensive compounds

Activation in times of attack
Screening for endophytes with bio-control activities
Isolation and characterization of *Piriformospora*

1. Sampling in Kisangani, DRCongo

2. Trapping *Piriformospora* by the use of a soil-based system with *Sorghum*

3. Isolation

4. Morphological and molecular characterization of 51 isolates obtained from 6 different locations in the Kisangani region

Venneman Jolien – 17/11/2017

Venneman et al, 2017
In vitro testing *Piriformospora*

![Image of petri dishes and wheat plants with fungal growth]

*F. graminearum + endophyte* // *F. graminearum*
Bio-control effects – greenhouse trials

Inoculation with *Fusarium*-infected rice kernels

- **FUS control (without autoclaved mycelium)**
- **FUS + mycelium isolate 30**

*Graph showing plant survival rates for different treatments:*
- F-cont
- F-Pl myc
- F-RW myc
- F-Ia. 30 myc
- non-infected cont

Surviving plants (%) vs. Treatment
Biofumigation
Bio-fumigation: best known system is with Brassicaceae

- Glucosinolates are converted to isothiocyanate (ITC) after cells are mechanically damaged
- ITC = volatile
- Myrosinase activity is temperature and pH depended!
Screening of Brassica species and varieties

- Inverted petri dish method: volatiles

Reduction of mycelium growth: *F. culmorum*

![Diagram showing reduction of mycelium growth for different species and varieties]
Biofumigation

Reduction of mycelium growth: *F. graminearum*

![Graph showing reduction in mycelium growth](image)
5. Breeding for resistance

Fusarium ear rot
- Polygenetic
- GCA and SCA are significant for Fusarium ear rot resistance
- $H^2$ varied from 0.4 to 0.8 for ear rot resistance and from 0.75-0.86 for fumonisin contamination
- Many QTLs are known

Lanubile et al. 2017
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