SOUTH AFRICAN MEDICAL RESEARCH COUNCIL

Building a healthy nation through research

MYCOTOXINS IN GRAINS – *Causes, Prevention and Control*

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FOOD SAFETY

Food safety is an issue that is becoming increasingly important in national and international debates about agriculture, nutrition and health

"Food safety is not a luxury of the rich, but a right of all people"
Dr J Diouf (FAO Director General)

It is a complex and many-faceted issue

Aspects of pre-harvest, harvest and post-harvest mycotoxin contamination of grains

MYCOTOXINS

Mycotoxins

- natural toxic metabolites produced by fungi (mostly food-borne)
- potent carcinogens and mutagens

Mycotoxigenic fungi - fungi capable of producing mycotoxins

Mycotoxicoses

- diseases caused by ingestion of foods containing mycotoxins
- multiple factors: plant pathogenic fungus, host plant, insects, environmental factors, toxin, products, consumer
- fungi growing on the same substrate / toxin combinations: additive / synergistic / depressive effects
- long-term exposure to low /constant doses of mycotoxins

IMPORTANT MYCOTOXINS / MYCOTOXIGENIC FUNGI ASSOCIATED WITH GRAINS IN AFRICA

Aflatoxin	*Aspergillus flavus, A. parasiticus*
Fumonisins	*Fusarium verticillioides, F. proliferatum*
Deoxynivalenol	*Fusarium graminearum, F. culmorum*
Zearalenone	*Fusarium graminearum*
Ochratoxin	*A. ochraceus, A. carbonarius, Penicillium verrucosum*

Moniliformin	*Fusarium thapsinum, F. pseudonygamai, F. proliferatum, F. andiyazi* and other *Fusarium* species

EFFECTS OF FUNGAL GROWTH ON GRAIN

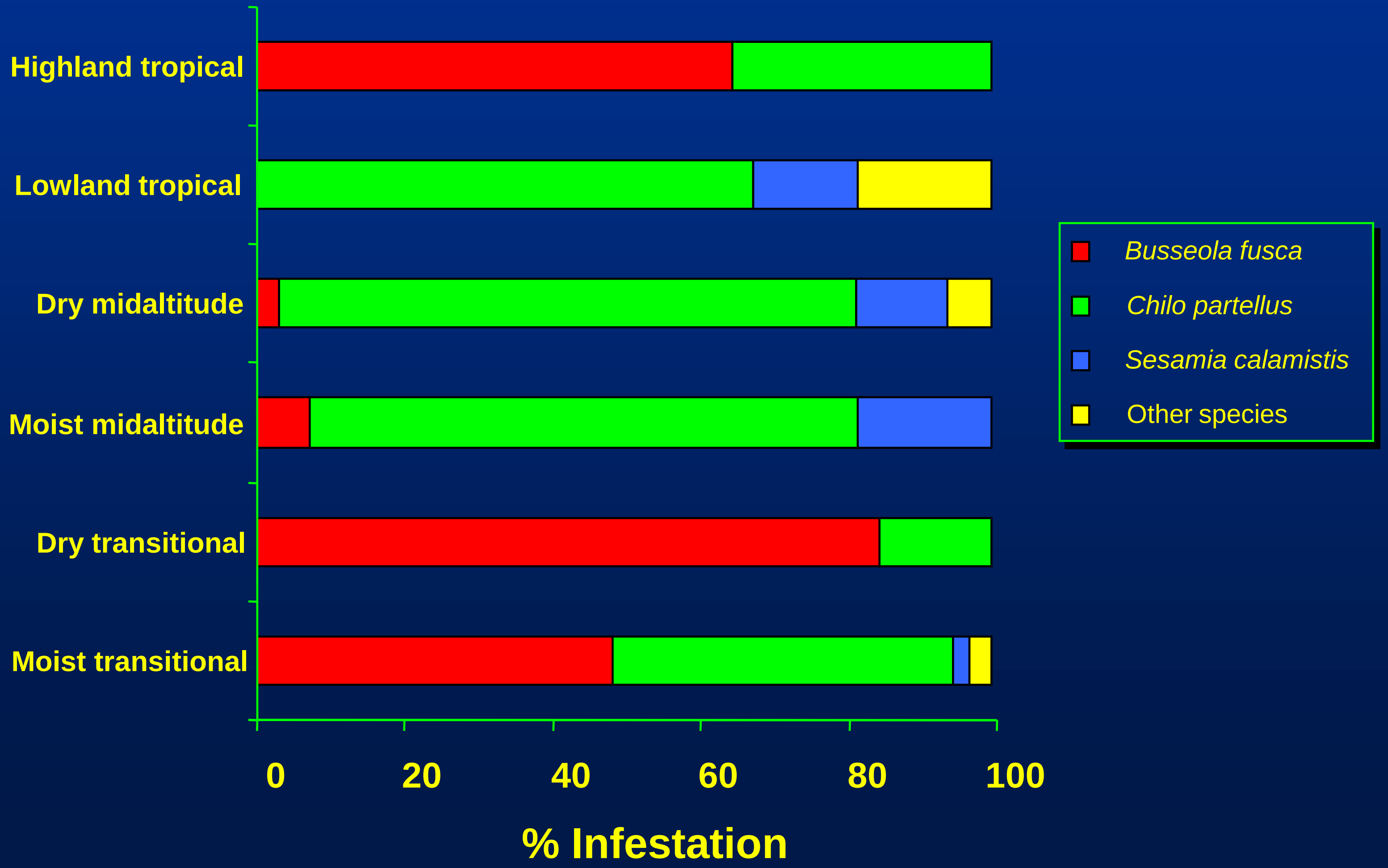
- Deterioration in and discoloration of grain quality due to fungal growth
- Reduction in nutritional value (energy and protein)
- Contaminated grain loses market value and is unfit for consumption
- Seed germination is affected leading to poor plant stand in the fields
- Occurrence of mycotoxins in food/feed affects the export potential of grain and grain products – economic implications

FACTORS CONDUCIVE FOR FUNGAL GROWTH AND MYCOTOXIN PRODUCTION

Pre-harvest conditions

- Climatic / soil / plant varieties / other conditions
  - soil fertility / poor seed / susceptible plant varieties
    eg. short and medium duration cultivars that mature during rains
  - over crowded plant population in field
  - plants suffering from other diseases
  - crop rotation / geographical regions
  - planting time in season / temperature / rain, humidity
  - drought stress / warm, wet conditions between flowering to harvest
  - insect infestation - damage to developing grain in the panicle / ear
  - crop damage, eg. birds / other animals

Distribution of stalk borer species in maize by agroecological zone in Kenya



De Grootte *et al.* (Int Agric Conference, Durban 2003)

FACTORS CONDUCIVE FOR FUNGAL GROWTH AND MYCOTOXIN PRODUCTION

❑ Harvest and post-harvest conditions

- Climatic conditions
  - optimal/maximum temperatures and
  - moisture for fungal growth and toxin production
- Substrates
  - grains, nuts, etc. – higher level of contamination, eg. carbohydrate, protein, fat, oil content, etc.
- Crop damage pre- and post harvest
  - insect damage of kernels/seeds of grains in storage
  - mechanical and other damage harvest

FACTORS CONDUCIVE FOR FUNGAL GROWTH AND MYCOTOXIN PRODUCTION

❑ Harvest and post-harvest conditions (contd.)

- Storage conditions
  - timeliness of harvest-ready crops
  - clean-up, temperature, humidity, eg. not storing grain with >12% moisture content and at temperature >25°C
  - drying & keep grain dry, eg. not rewetting of grains in storage due to improper storage conditions, eg. moist ground or roof leakage / open air exposure to wet conditions / stacking the harvested panicles for long periods

FACTORS AFFECTING MYCOTOXIN LEVELS IN COMMERCIAL PRODUCTS

- **Food processing techniques**
  - Wet and dry milling processes
    - concentrates/reduces mycotoxins, eg. *Fumonisins*: removal of hulls of maize kernels (90% ↓)
  - Thermal processing - cooking and boiling
    - generally reduces mycotoxins, eg. *Aflatoxin*: boiling (28% ↓), frying (34-53% ↓) and alkaline cooking (20-90% ↓)
  - Sorting / screening / washing techniques
    - *Aflatoxin*: electronic sorting and hand-picking
    - *Fumonisins*: separation of fungal-damaged maize

FACTORS AFFECTING MYCOTOXIN LEVELS IN COMMERCIAL PRODUCTS

❑ Food processing techniques (contd.)

- Fermentation - reduces mycotoxins, eg. Ochratoxin A
- Chemical inactivation - ammoniation, ozonation, nixtamalization - addition of hydrogen peroxide and sodium bicarbonate
- Adsorbtion techniques – addition of clays (HSCAS…)

❑ Mycotoxin residues

- "Carry-over" / accumulation effects
  - Animal meat, milk & eggs, eg. Aflatoxin (AFM_1), Ochratoxin A
  - Human breast milk (AFM_1), eg. Sierra Leone, Ghana, Nigeria, Sudan, Kenya

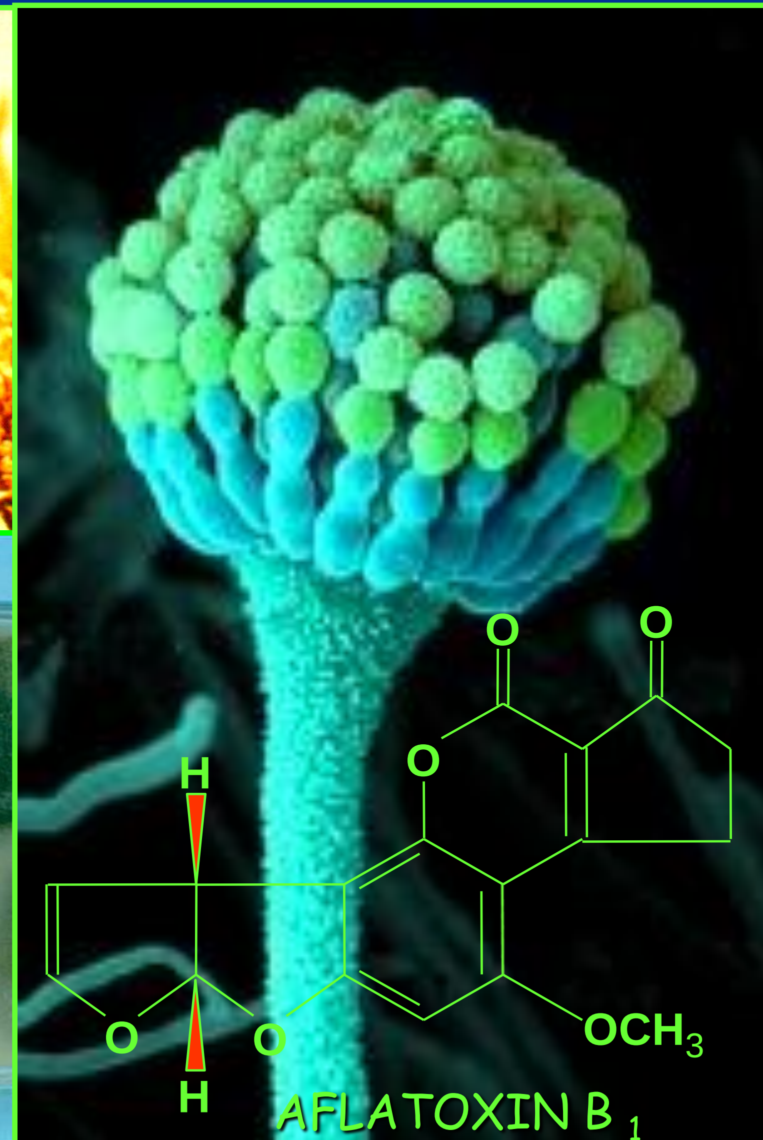
CRITERIA FOR EVALUATING MYCOTOXIN REDUCTION OR DECONTAMINATION MYCOTOXIN IN COMMERCIAL PRODUCTS

- **Procedures should**
  - inactivate, destroy or remove the toxin
  - not produce or leave toxic residues in the food or feed
  - retain nutritive value and food/feed acceptability of the product
  - not alter significantly the technological properties of the product
  - destroy fungal spores, if possible and
  - be cost effective, i.e. decontamination process should cost less than the value of the contaminated commodity

Jemmali, 1979; Park et al., 1988; Jemmali, 1989 and others

AFLATOXIN

Aspergillus flavus, A. parasiticus



TOXICOLOGICAL ASPECTS OF AFLATOXINS

Potent human hepatocarcinogen

Dose effects

High doses: lethal if consumed - lung, myocardial & kidney tissues

Sub-lethal doses: causes chronic toxicity, eg. liver cirrhosis

Low level exposure: human hepatocellular carcinoma (liver cancer)

Mutagenicity

AFB_1 covalently binds to DNA – induces G to T transversions

Teratogenicity

Embryonic abnormalities

TOXICOLOGICAL ASPECTS OF AFLATOXINS

Hepatitis B (HB) Virus infections/carriers

Potency of aflatoxins in $HBsAg^+$ is significantly higher than in $HBsAg^-$ individuals – influence on liver cancer rates

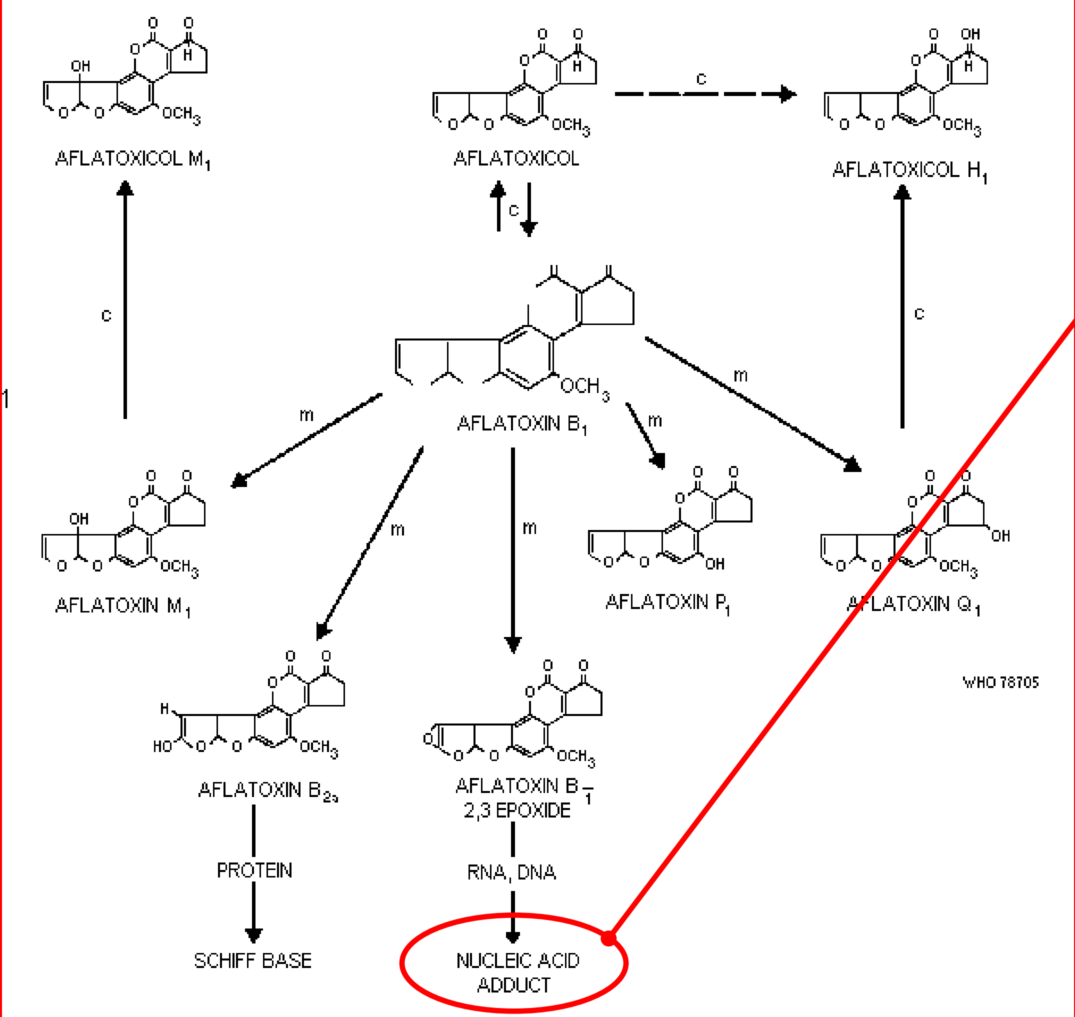
Impaired growth in children

Children from Benin & Togo (Gong *et al.*, 2002)

Immunosuppression

Immunodiluting agent – cell mediated immunity and Phagocytic cell function (Bondy & Pestka, 2000)

Gambian children: Continuous low level exposure to dietary aflatoxins – may enhance susceptibility to infection (Turner *et al.*, 2003)



Aflatoxin B_1 metabolism in the liver

IARC classification (1998)

AFB_1 classified as a human carcinogen

ALARA Principle

Not technically possible to completely eliminate aflatoxins in food – maximum levels are set

As **L**ow **A**s **R**easonably **A**chievable

Levels of aflatoxins in foods

10µg/kg of which $AFB_1 \leq 5$µg/kg

ACUTE AFLATOXICOSIS

"According to the official news agency, the Kenya Broadcasting Corporation, the death toll by 16 May 2004 had reached 40 in Makueni and Kitui Districts of Kenya. There were others who were admitted to the district hospital with jaundice, leg edema and hepatomegaly. Maize samples showed high levels of aflatoxin. There were deaths reported in animals and poultry as well (maize meal is a component of animal and poultry feeds)".

Dr S K Sharif (Ministry of Health, Kenya)

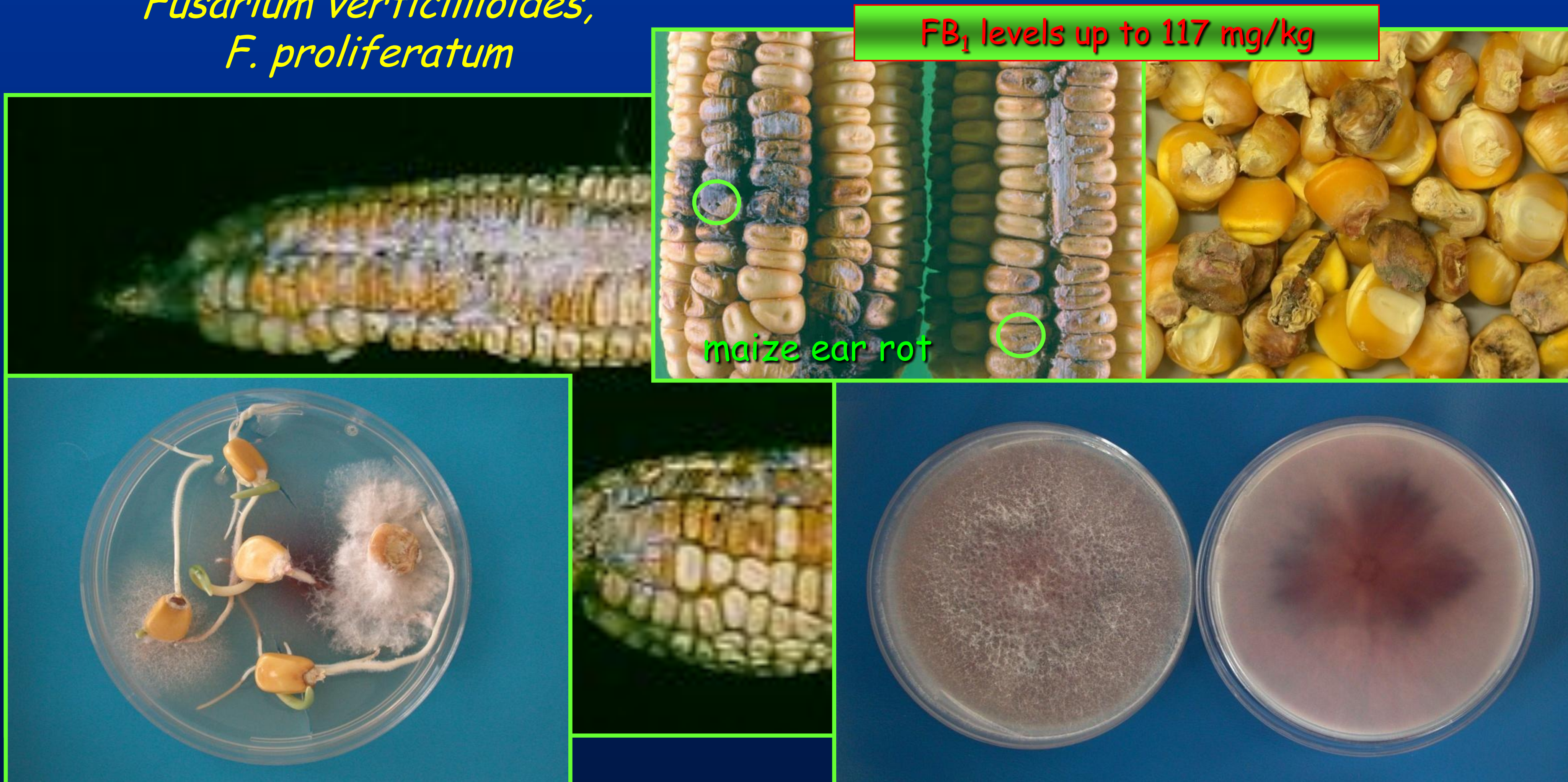
sksharif@africaonline.co.ke

…by September 2004, 317 cases reported and 125 deaths. A less severe outbreak occurred in 2005, resulting in 16 deaths. Food samples collected from households in the affected areas contained high levels of aflatoxin B_1 (20 to > 1000 μg/kg), suggesting that the outbreak was caused by acute aflatoxin poisoning. The outbreak resulted from aflatoxin contamination of locally grown maize that was stored under damp conditions….

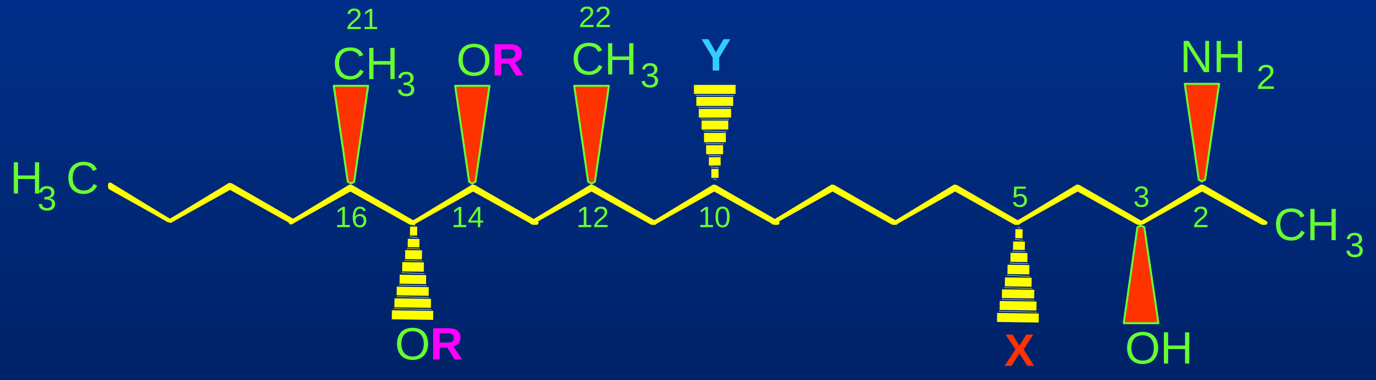
Mwancha N Okioma (2008). Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade, Ed JF Leslie, CABI, p129-133.

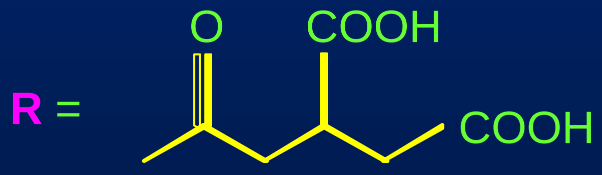
FUMONISINS

Fusarium verticillioides,
F. proliferatum



STEREOCHEMISTRY OF B SERIES FUMONISINS





tricarballylic acid (TCA)

FB_1 : X = OH, Y = OH

FB_2 : X = OH, Y = H

FB_3 : X = H, Y = OH

TOXICOLOGICAL ASPECTS OF FUMONISINS

Farm Animals

Leukoencephalomalacia in horses
Pulmonary oedema syndrome in pigs

Experimental Animals

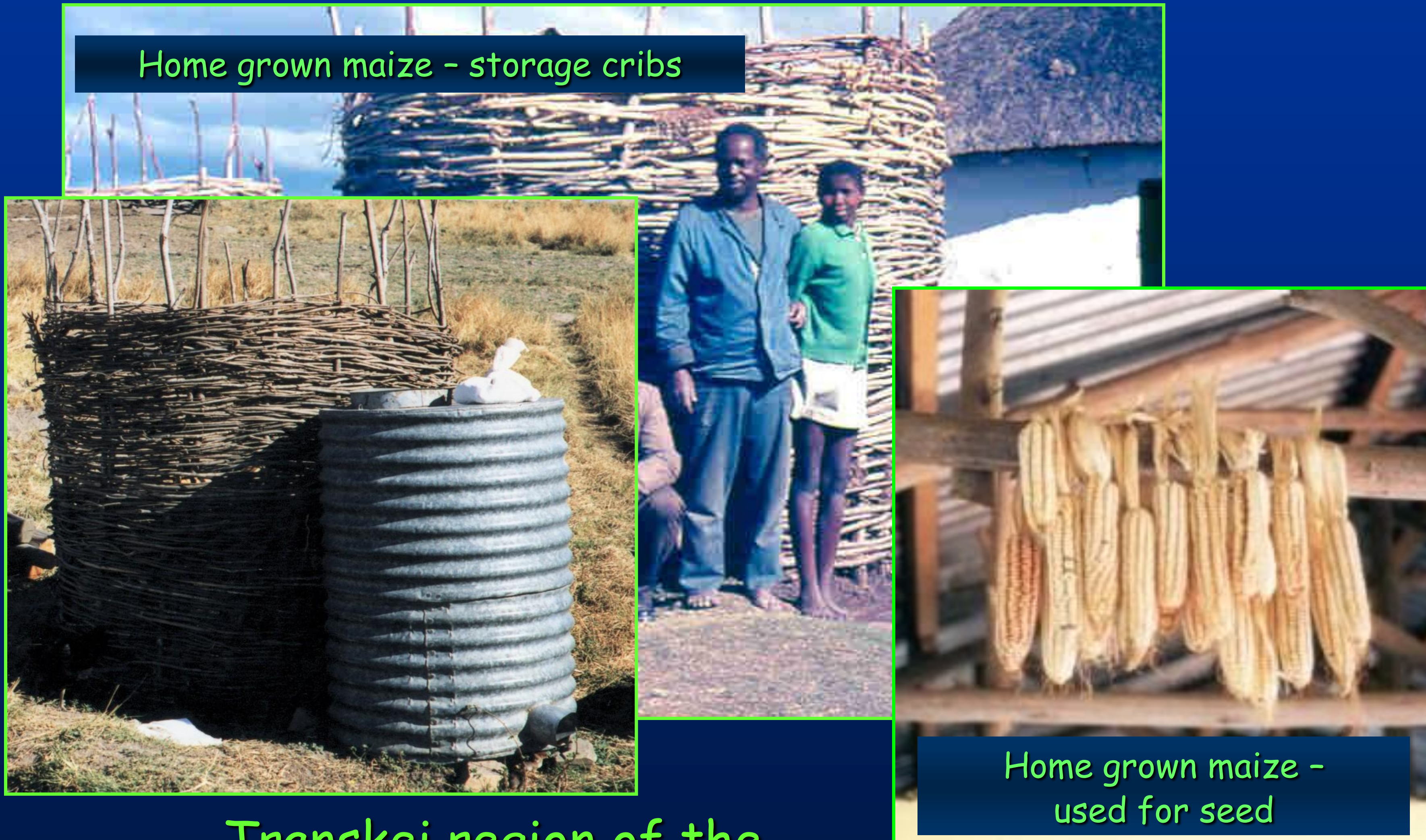
Hepato-, nephro- and cardiotoxic in rats and mice
Hepato- and nephrocarcinogenic in rats and mice
Neural tube defects (NTD) in mouse embryos

IARC Classification

Classified fumonisin B_1 as a Group 2B carcinogen,
ie possibly carcinogenic to humans

In Humans - associated with:

Oesophageal cancer in Transkei and China;
Birth defects (anencephaly & spina bifida) – Texas-Mexico border, Guatemala, Transkei


Home grown maize – storage cribs

Home grown maize – used for seed

Transkei region of the Eastern Cape

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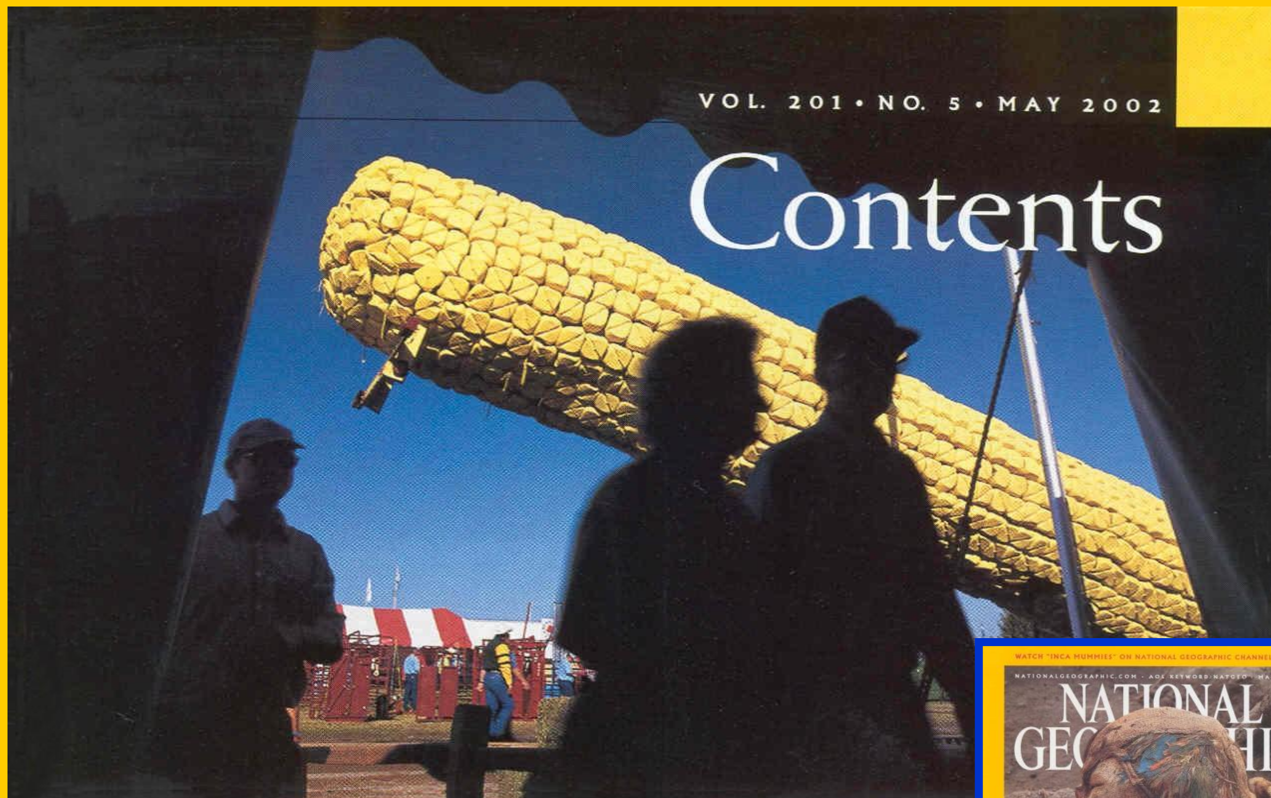

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FEATURES

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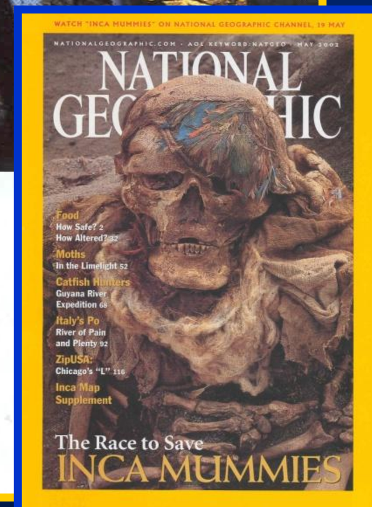
BY JENNIFER ACKERMAN   PHOTOGRAPHS BY JIM RICHARDSON



In Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade,
Ed: JF Leslie, CABI, p 29-40

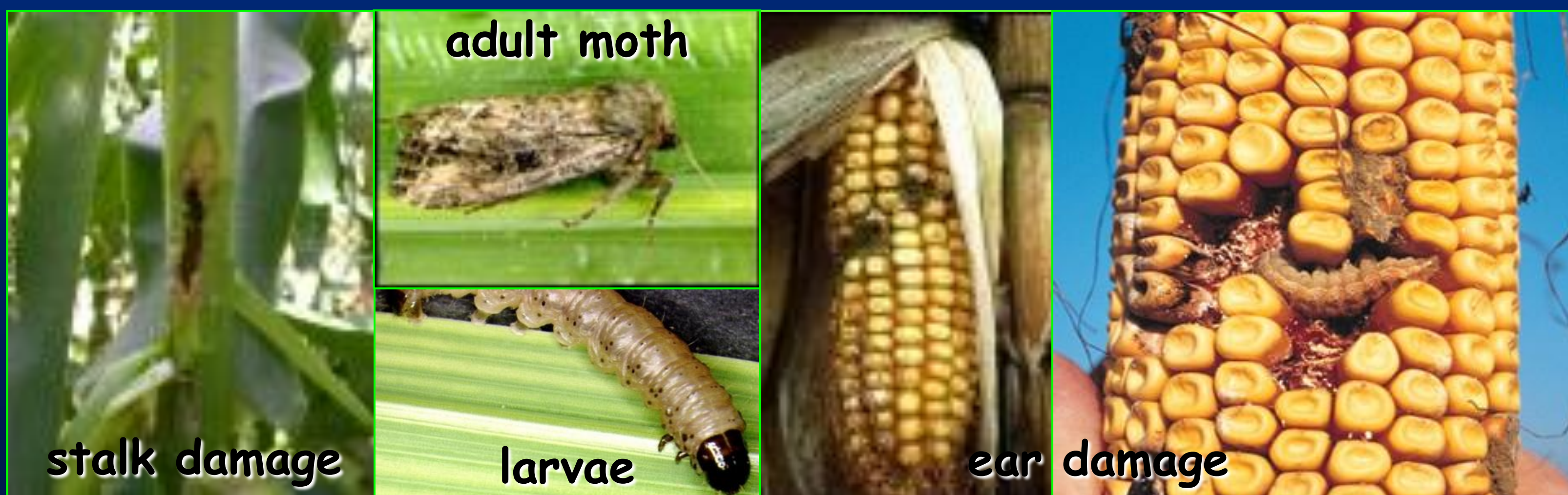
FB (ppm)	MAIZE INTAKE g/person (60kg)/day					
	10	50	100	150	200	400
0.2	0	0.2	0.3	0.5	0.7	1.4
0.5	0.1	0.4	0.8	1.3	1.7	3.4
1	0.2	0.8	1.7	2.5	3.3	6.6
2	0.3	1.7	3.3	5.0	6.7	13.4
3	0.5	2.5	5.0	7.5	10.0	20.0
4	0.7	3.3	6.7	10.0	13.3	26.6
5	0.8	4.2	8.3	12.5	16.7	33.4
10	1.7	8.3	16.7	25.0	33.3	66.6
12	2.0	10	20	30	40	80

FB INTAKE (µg/kg bw/day)

Provisional Maximum Tolerable Daily Intake (PMTDI) = 0.8 µg/kg bw/day (carcinogenicity)
PMTDI = 2 µg/kg bw/day (nephrotoxicity) [JECFA 2001]

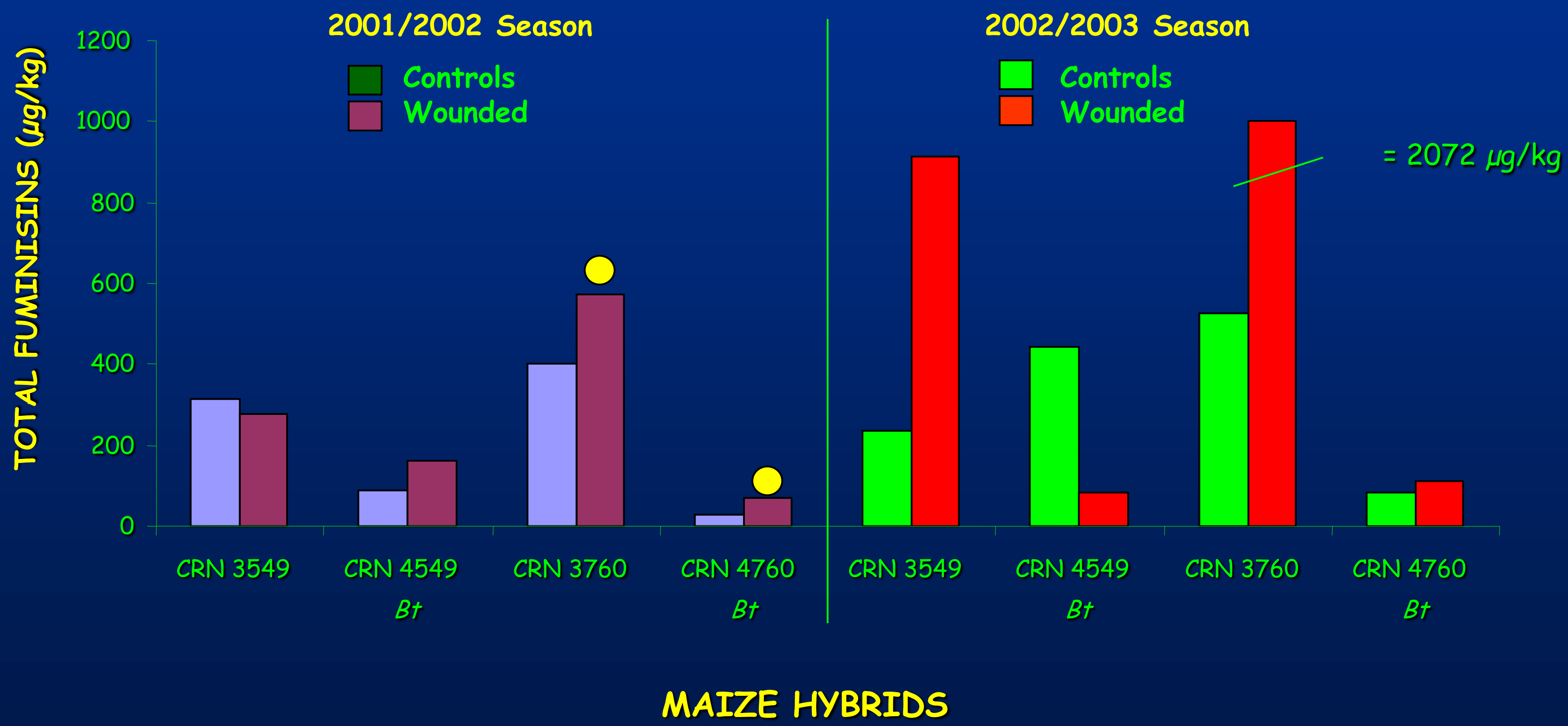
FUMONISINS IN *BT*-MAIZE

- Field and Harvest conditions
  - Naturally high incidence *Fusarium* areas
  - Good soil & quality seed / monitored relative humidity & temp /locality
  - Monthly rainfall over planting and growing period
  - Yield and moisture content of maize at harvest
  - Insect load over growing period / wounded & control ears



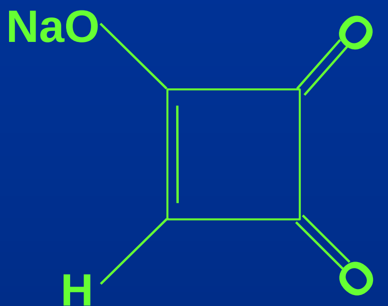
Busseola fusca – stalk and ear damage of maize

Results - Fumonisin levels



POTCHEFSTROOM

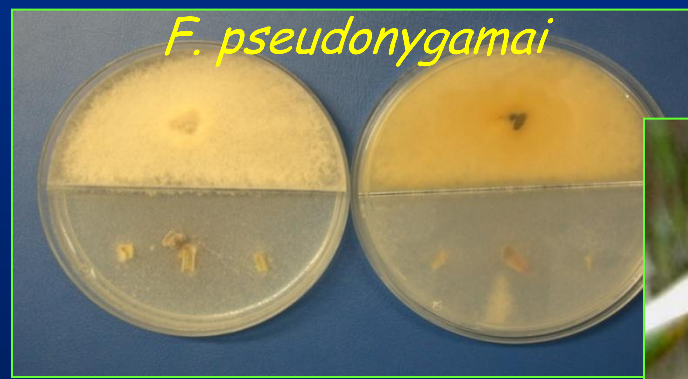
● = Significantly different ($P < 0.05$)

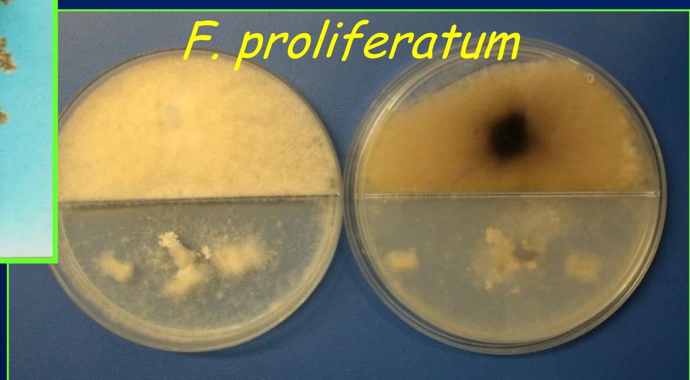


MONILIFORMIN

MONILIFORMIN

Fusarium thapsinum, F. pseudonygamai,
F. proliferatum, F. andiyazi and other *Fusarium* species





TOXICOLOGICAL ASPECTS OF MONILIFORMIN

Dose effects

High doses: Can be lethal to chickens, ducklings, and turkeys
– heart (cardiotoxic) & kidney tissues affected

The toxic dose of moniliformin in chickens reported to be 5mg/kg bw

Low level exposure: information on human health effects outstanding

NATURAL OCCURANCE OF FUMONISIN AND MONILIFORMIN IN SORGHUM AND PEARL MILLET FROM MALI AND NIGERIA

Sample Origin	Sample type	Total fumonisin (mg/kg)	Moniliformin range (mg/kg)
Mali	Sorghum	10 - 1025	ND[1]
	Millet	5 - 70	ND – 524
Nigeria	Sorghum	ND - 1345	-
	Millet	8 - 29	-

[1]ND = not detected at a detection limit of 1 mg/kg

Production of fumonisin and moniliformin by *Fusarium* isolates from **sorghum and pearl millet samples** from Mali in maize cultures

Fusarium species	Original sample type	Number of isolates	Fumonisin (mg/kg)	Moniliformin range (mg/kg)
F. thapsinum	Sorghum	7	ND[1]	2214 – 8734
F. pseudonygamai	Sorghum	2	ND	5675 – 8907
Unique species [2]	Sorghum	2	ND	9947 – 14010
New species 2 & 3[3]	Sorghum	4	ND	10280 – 33080
F. pseudonygamai	Millet	8	1 – 13	5886 – 15470
"*F. pseudoandiyazi* "[2]	Millet	1	10	1997
New species 4 & 5[3]	Millet	5	1 – 5290	18 – 17240

[1]Not detected, detection limit 1 mg/kg; [2]Distinct from all other strains based on AFLP (undescribed); [3]New species 2 and 3 from sorghum and 4 and 5 from millet are clusters based on AFLP;

Production of total fumonisin and moniliformin by *Fusarium proliferatum* and two unknown *Fusarium* isolates from **maize, sorghum and pearl millet** samples from Nigeria grown in grain cultures

Fusarium species	Original sample type	Number of isolates	Fumonisin (mg/kg)	Moniliformin range (mg/kg)
F. proliferatum	Maize	9	ND[1]-3617	6 – 1963
F. proliferatum	Sorghum	7	1 – 6478	7 – 8892
Fusarium sp.[2]	Sorghum	1	3 – 14	2 – 31
Fusarium sp.[2]	Millet	1	3 – 43	124400 – 222400

[1]Not detected, detection limit 1 mg/kg;
[2]*Fusarium* species originally isolated from sorghum and millet, yet to be identified

DISCUSSION

Mycotoxins

Are a diverse group of food-borne, naturally occurring toxins

Regulatory applications

Are appropriate for export crops, but
has little relevance in developing countries' agriculture –
particularly in the case of small, subsistence and emerging farmers

Outbreak of aflatoxicosis in India (1974)

"Starving today by not consuming contaminated food in order to live a better life tomorrow is not a practical option."

Still holds true today

MYCOTOXIN CONTROL

Integrated management system

Prevention through pre-harvest management is the best method for controlling mycotoxin contamination

Education of small-scale, emerging farmers and commercial grain producers

Mycotoxin contamination should be minimized in every phase of grain production
- harvesting, processing and distribution

Long-term objective should be to reduce natural contamination of cereal grains and development / exploitation of disease-resistant cultivars

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