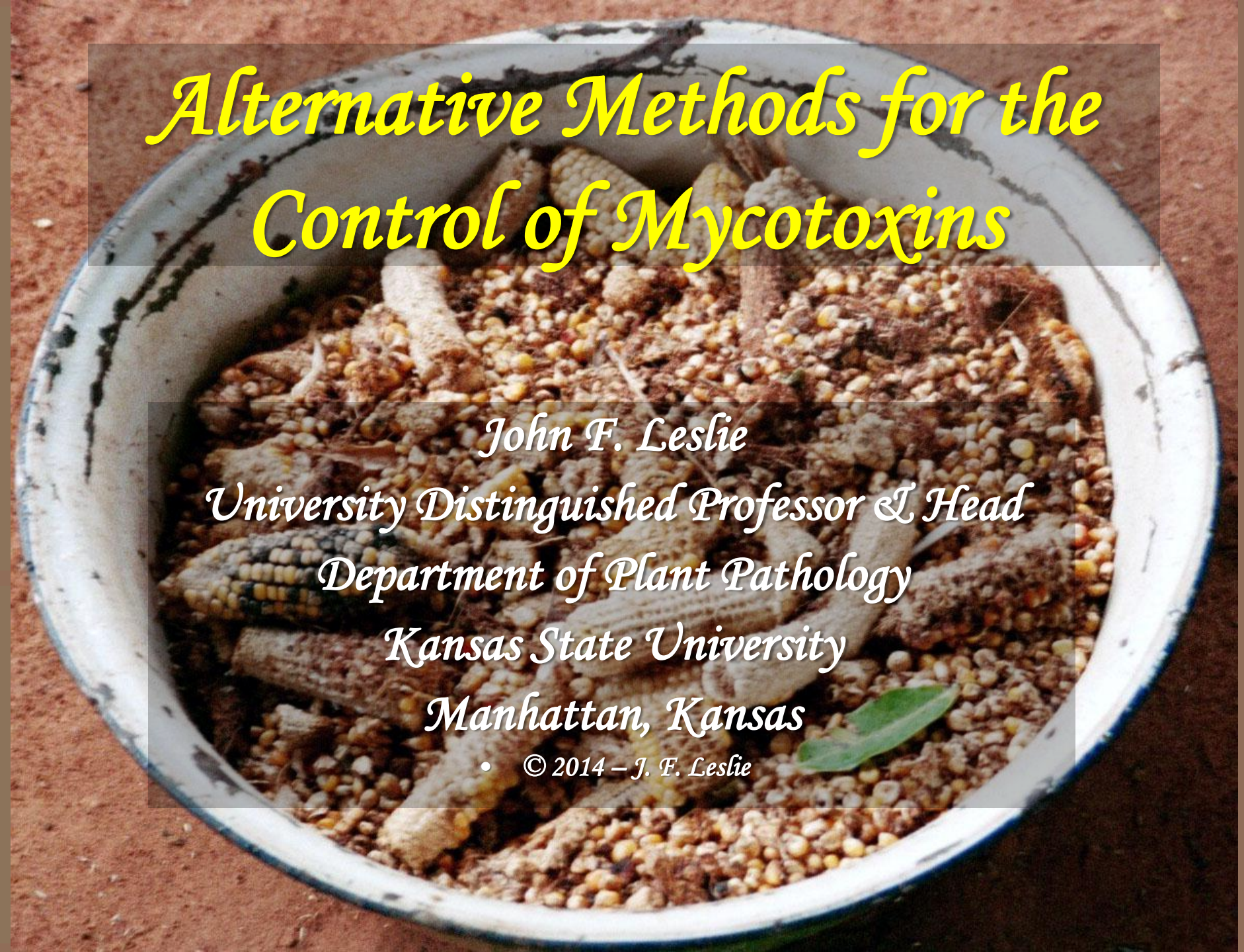
Alternative Methods for the Control of Mycotoxins



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What are Mycotoxins?

- *Natural toxic metabolites produced by fungi*
- *Known since Ancient Greece*
- *Five agriculturally most important mycotoxins:*
  - *Aflatoxins*
  - *Fumonisins*
  - *Deoxynivalenol*
  - *Zearalenone*
  - *Ochratoxin*

Some also are potent carcinogens and mutagens

Several toxins are 3-5 orders of magnitude more toxic than fungicides that control them and break down more slowly

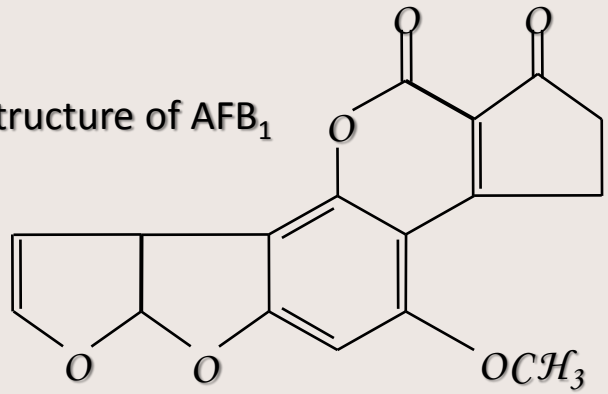
Mycotoxicoses

- *Mycotoxicoses*
- *Diseases caused by ingestion of foods containing mycotoxins*
- *Multiple factors: plant pathogenic fungus, host plant, insects, environmental factors, toxin, products, consumer*
- *Acute or long-term exposure to low doses of mycotoxins*

Aflatoxins

Aspergillus flavus / A. parasiticus

Structure of AFB_1



Cause:

Liver failure

Liver cancer

Growth stunting

Immune deficiency or suppression

Grains – especially maize

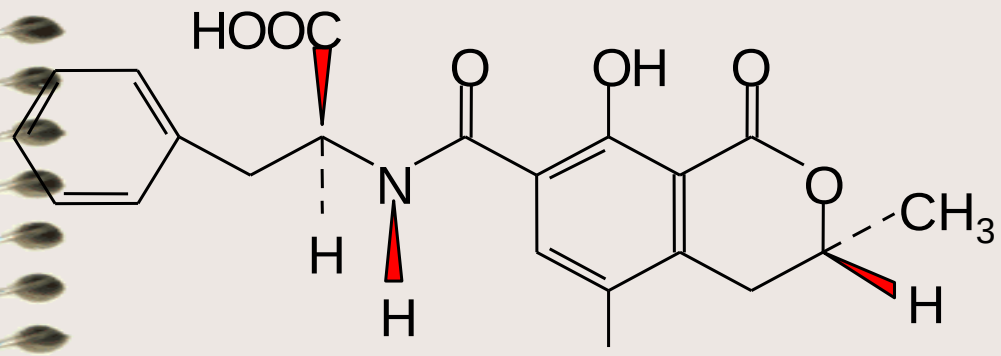
Peanuts

Nuts

Ochratoxins

Aspergillus ochraceus

Kidney failure
Cacao
Nuts
Grapes
Coffee
Wheat



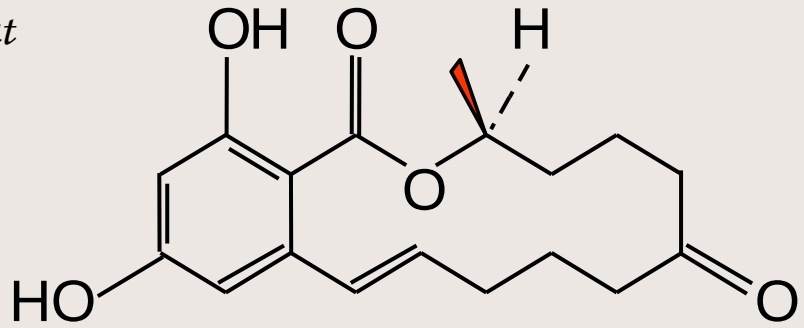
Structure of Ochratoxin A

Zearalenone

Fusarium graminearum

F. culmorum

Hyperestrogenism
Pseudoestrogen
Maize
Wheat



Structure of zearalenone

Fumonisins

Fusarium verticillioides

F. proliferatum

Esophageal cancer
Neural tube defects
Leukoencephalomalacia
Pulmonary edema
Grains – especially maize

Mycotoxin Regulations

- *Main risk in developed countries is economic, as current commercial processes catch most problems*
- *In LDCs regulations may exist, but be unenforced or unenforcable*
- *Food insecurity problems all but guarantee everything edible will be eaten*

Health Effect	Possible number of deaths	Relative Attention
Chemical weapon	0 (?)	Very high
Acute aflatoxicosis	100s	High
Hepatocellular carcinoma	10,000s	Medium
Growth impairment and immunosuppression	100,000s (?)	Low/None

Interaction between Fumonisin Contamination and Maize Intake

FB (µ/g)	Maize intake (g/60kg person/day)						PDI (µg/kg bw/day)
	10	50	100	150	200	400	500
0.2	0	0.2	0.3	0.5	0.7	1.4	1.7
0.5	0.1	0.4	0.8	1.3	1.7	3.4	4.2
1	0.21	0.8	1.7	2.5	3.3	6.6	8.3
2	0.3	1.7	3.3	5.0	6.7	13.4	16.7
3	0.5	2.5	5.0	7.5	10.0	20.0	25.0
4	0.7	3.3	6.7	10.0	13.3	26.6	33.3

PMTDI = 2 µg/kg bw/day (JECFA, 2002)

The Phytobiome

- *Plants are naturally infected with fungi & bacteria*
- *May be different microbial communities for different parts of the plant, different environments, cropping regimes, etc.*
- *Mycotoxin producing fungi usually are part of these communities and may not be causing disease or producing toxins*

AflaSafe – Scientific basis

- Two strain types of A. flavus – "L" and "S"
- S strains make high levels of aflatoxin, while L strains produce little or none
- Co-culturing L and S strains synergistically reduces the amount of aflatoxin produced
- L and S strains commonly coexist under field conditions
- Pioneered by Peter Cotty 0f USDA-ARS and Ranajit Bandyopadhyay of IITA

AflaSafe – The Product

- Biological control through competitive exclusion
- Contains A. flavus L strains that do not produce any aflatoxin
- Strains used vary by location
- Strains are grown on sorghum seed until the seed is colonized by hyphae, but there is no sporulation, and then dried
- Sorghum seed is distributed in the field and provides large number of L strain propagules that effectively swamp out S strain propagules for places in the host plant

AflaSafe - Results

- Reduces aflatoxin contamination 60-95%
- Commercially successful in Arizona
- Being implemented in a number of African countries on an experimental basis. Formal registration and commercialization are in progress
- Not a silver bullet, as crop management in other areas required
- May not reduce plant disease observed

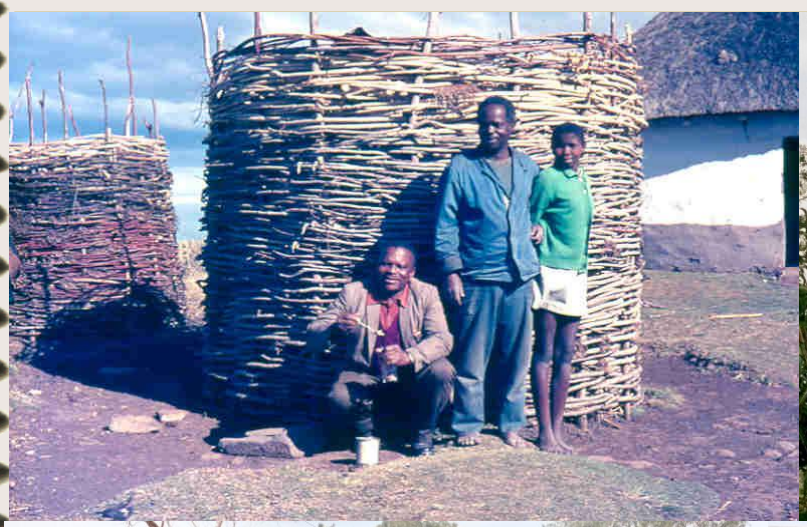
AflaSafe Encores?

- Non-toxin producing A. flavus strains are fit
  - Ochratoxin nonproducing strains – frequency and fitness are unknown
  - Fusarium toxin producing strains – non-toxin producing strains generally are rare, and in some cases (deoxynivalenol) are known to be less fit
- A. flavus asexuality enables release of stable strains
  - Major Fusarium toxin producers all have potential for sexual reproduction which could disrupt co-adapted gene complexes
  - No data on impact of co-culture on toxin production

Some Things that Might be Done

- *Ammoniation*
- *Ozonation*
- *Blending*
- *Food preparation*
  - *Extrusion*
  - *Nixtamalization*
- *Clays and other additives that prevent uptake of toxins when consumed with contaminated food*
- *Probiotics that can degrade toxins prior to uptake*

Storage is a Problem!



Things that Can be Done Now

- *Reduce plant stress – fertilizer, pesticides & H_2O*
- *Dry quickly*
- *Do not dry on the ground*
- *Sort out visibly moldy kernels*
- *Do not store in plastic bags, use fiber bags instead*
- *Place storage bags on pallets, not the ground*
- *Limit insect and rodent access during storage*

What to Do with Maize?

- Becomes more susceptible to mycotoxin contamination when plants are stressed by drought, heat and/or disease/insect pressure
- Is relatively easy to prepare
- Is the 'modern" food
- Has a huge technical resource base with large time and financial inputs

Sorghum & Millets as Alternatives

- Indigenous African cereals
- Extraordinarily well-adapted to drought and heat stresses
- More difficult to prepare than maize
- An "old-fashioned" food
- Important for beer & celiacs
- Variable technical resource base

A Nigerian Experiment

- 14 farmers' fields in Northern and Southern Guinea Savanna
- Maize, sorghum and pearl millet grown by farmers in adjacent plots
- Harvested at maturity by farmers
- Samples brought to lab in Ibadan, divided with portions sent to PROMEC in South Africa
- Analysis: aflatoxin by ELISA, frequency of S (more toxic) and L (less toxic) strains of A. flavus and A. parasiticus; fumonisins via HPLC
- Exposure calculated based on historic cereal consumption data

Fungal Genus Present on Grains

Crop	Sample size	Aspergillus	Fusarium
Maize	23	18 a	47 a
Pearl millet	7	1.9 b	26 b
Sorghum	40	4.2 b	26 b

- Maize 4 & 9 fold more likely to be contaminated with *Aspergillus* than sorghum & pearl millet
- Maize 1.8 fold more likely to be contaminated with *Fusarium* than sorghum & pearl millet

Aflatoxin Exposure

Crop	Aflatoxin (ng/g) Mean ± SD	Median	Range	Samples > 20 ppb aflatoxin (%)	Expo-sure (ng/kg bw/day)
Maize	36 ± 100	4.2	1 – 480	17	207.1
Sorghum	9 ± 14	5.0	1 – 90	5	50.6
Pearl millet	4.6 ± 1.8	4.4	2 – 8	0	26.5

Risk from sorghum is 4-fold less, and pearl millet 8-fold less than maize (consumption: 147 kg/year; BW: 70 Kg)

Fumonisin Exposure

Crop	Fumonisin (ng/g) Mean ± SD	Median	Range	Samples > 1 ppm fumonisin (%)	Exposure (µg/kg bw/day)
Maize	229 ± 551	52	5 – 2856	5	1.3
Sorghum	132 ± 276	15	5 – 1345	3	0.76
Pearl millet	19 ± 7.2	18	8 – 29	0	0.11

Risk from sorghum is 1.7-fold less, and pearl millet 12-fold less than maize (consumption: 147 kg/year; BW: 70 Kg)

Co-Contamination?

Crop	*Aspergillus/ Fusarium*	Aflatoxin/ Fumonisin
Maize	-0.36/-0.31	-0.07
Pearl millet	0.17/0.07	-0.51
Sorghum	0.17/-0.03	-0.04

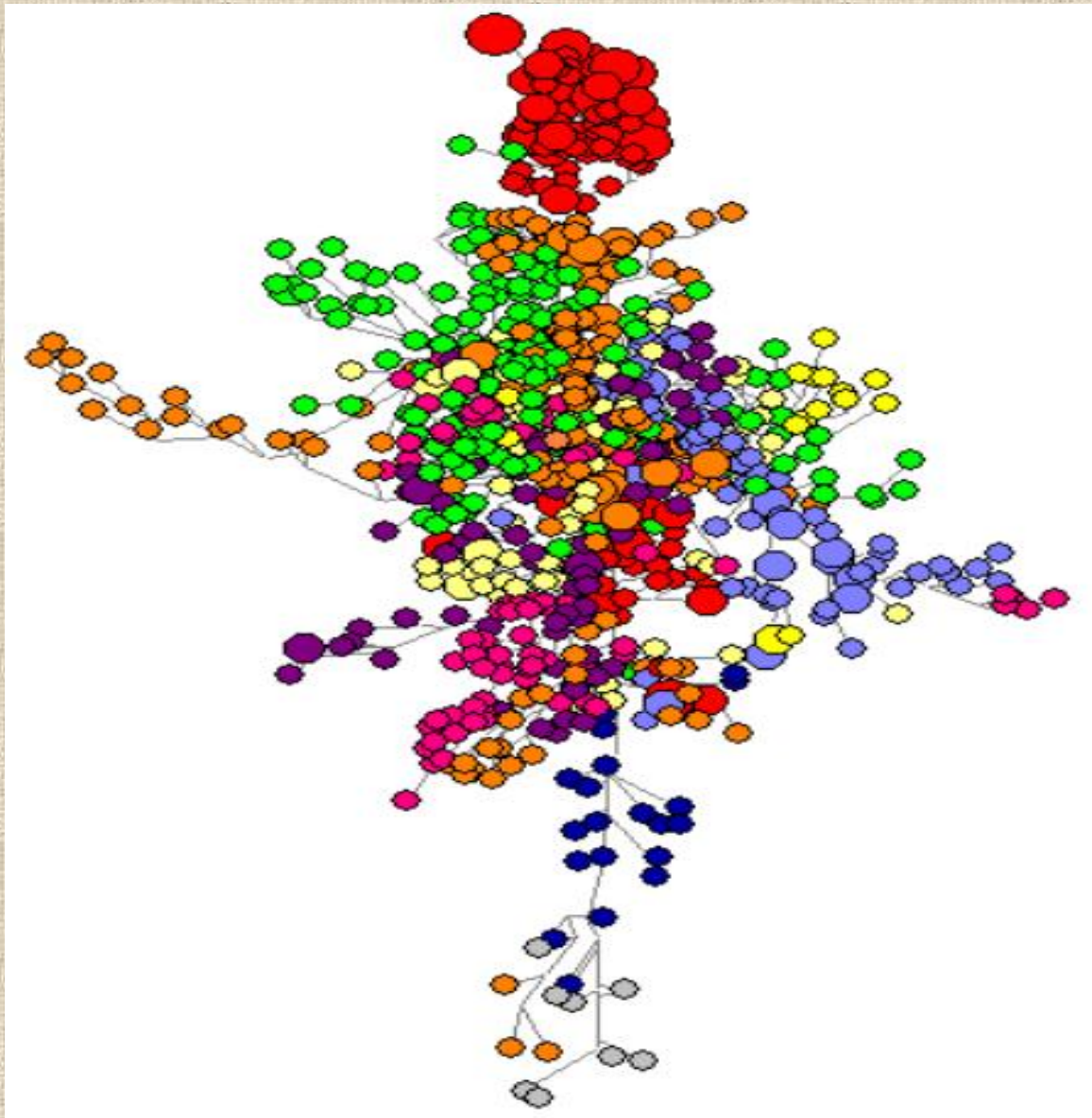
- Correlations between *Aspergillus* strains and total/fumonisin producing *Fusarium* strains. Most strains from sorghum and pearl millet were not fumonisin producers.
- No significant correlation between aflatoxin and fumonisin accumulations except for pearl millet where all numbers are small.

The Phytobiome Revisited

- *How to keep endophytes from turning into pathogens or producing toxins?*
- *Breeding for lower levels of mycotoxin accumulation is possible. What is really being changed?*
- *What is the role of GMO traits?*
- *Can stable changes be made to microbial populations to maintain desirable traits?*

AFLP Variation in South African F. graminearum

Can we keep ourselves from over-simplifying the problem?



Agricultural Policy & Climate Change

- Corn has more toxin contamination when grown under stressed conditions
- Expansion of area planted to corn in Africa is into areas that are hotter, drier and have less fertile soils
- Climate change generally will increase heat and drought stresses on corn
- Investing in sorghum and millet may be a more cost-effective way of providing food in these regions

A Trickle-up Story

- Traders in developing countries purchase the "best" grain from the farmers who end up with a little cash and the most heavily contaminated grain
- Developing countries sell developed countries their best quality agricultural products to get hard currency
- Consumers in developed countries eat the most diversified diets and have regulatory systems that usually allow the lowest level of mycotoxins in their foods

Special thanks to Wally Marasas

As well as to all of my collaborators and my wife!

Questions?


HAPPY BIRTHDAY
Charles Darwin, Fusarium and Abraham Lincoln
200
1809 was a good year!
Fusarium Laboratory Workshop 2009
Kansas State University - Manhattan, Kansas