

# Mycotoxins in Feed Ingredients:

Why, After All These Years, Are We Still Dealing with This?

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Distillers Grains Technology Council  
Meeting

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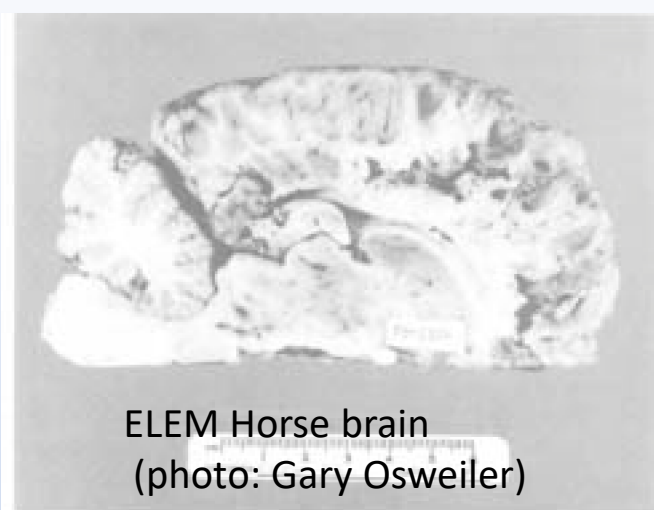


# Outline

- Mycotoxins: background
- Why we have this problem
- Major fungal diseases and their toxins
- How the problems are being addressed
- Monitoring
  - Different types of mycotoxin tests
  - 'Emerging' toxins
  - Regulations
- What does the future hold?
  - Fungi aren't going away
  - Impacts of climate change
  - Emerging Issues
  - New Technologies/ New Solutions
- ARS as *part of* the solution

# Mycotoxins: Why We Care:

- Potential for Human Disease: 1960s to date
  - Cancers (aflatoxins, fumonisins)
  - Kidney disease (ochratoxin A)
  - Estrogenic effects (zearalenone)
  - Sub-chronic effects (immune system)
- Effects on Animals
  - At low levels:
    - Decreased feed efficiency, weight gain
    - Increased susceptibility to infection
  - At high levels:
    - Death (Turkey-X)
    - Diseases: Porcine pulmonary edema, Leukoencephalomalacia (Horses),
- Because of Economic Losses
  - Direct (waste, lower yields, disposal costs, disease)
  - Indirect (feed efficiency, effects on fermentation, costs of monitoring)
- Why Does the GOV Care? ....because voters care



ELEM Horse brain  
(photo: Gary Osweiler)



# Mycotoxins: Why You (Should) Care:

- Most mycotoxins remain intact during ethanol production
- DDGS derived from dry-grind processing concentrate many of the toxins 3-fold over the original feedstock
- Potential for mycotoxins to stress yeasts during fermentation
- Multiple mycotoxins increase the odds of additive/synergistic effects
  
- Implications:
  - Potential for lower yields of ethanol
  - What may have been a healthy feed ingredient may now be unhealthy  
(Or, more commonly, reduce feed efficiency)
  - What may have passed regulatory levels now may not
  - Feedstock needs to be controlled at levels well below the regulations

# Why We Have This Problem

- Fungi like our food....and they are opportunistic
- They are, literally, everywhere
- Fungi Reproduce (and adapt) Rapidly



...and insects can spread the fungi...

# Fungal Diseases and their Toxins

**Aspergillus Ear Rot**



***Aspergillus flavus, A. parasiticus***

Toxins: **Aflatoxins, cyclopiazonic acid**

**Fusarium Ear Rot  
Fusarium Stalk Rot**



***Fusarium verticillioides, F. proliferatum***

Toxins: **Fumonisin**

**Gibberella Ear Rot  
Gibberella Stalk Rot  
Fusarium Head Blight**



***Fusarium graminearum, F. culmorum***

Toxins: **vomitoxin/deoxynivalenol (DON), zearalenone**

**Fusarium Head Blight**



***Fusarium langsethiae, F. poae***

Toxins: **T-2/HT-2, DON**

Any of these toxins can potentially be found in DDGS

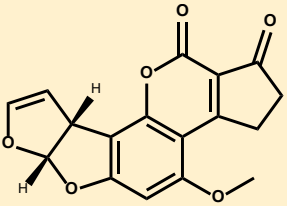
# Strategies to Reduce Mycotoxins

- Pre-Harvest
  - Resistant crops
  - Fungicides , monitoring
  - Insecticides
  - Biocontrol
- Post-Harvest
  - Proper dry-down and dry storage
  - Pest control
  - Shipping/Handling
  - Monitoring
  - Remediation/detoxification

Industry has done a good job of managing mycotoxins,  
And that requires monitoring...

Monitoring for aflatoxin: the FDA limit is 20 ppb for human foods & dairy feed

## How Much is 20 Parts per Billion (20 ppb)?



A Pellet of Pure Aflatoxin, the size of a corn kernel,  
in 625 bushels of corn



or a semi-trailer a little over 3/4 full



About 3.4 tablespoons in an Olympic-size pool



1 second in 19 months (1.58 years)



1 penny in \$500,000 dollars



**Because of this,  
statistically based  
sampling is key**

# Thousands of different Mycotoxin tests....

- Presumptive Tests (Predictive Tests)

Results are based on markers correlated with toxin content (NIR, Black light, hyperspectral imaging, etc.)

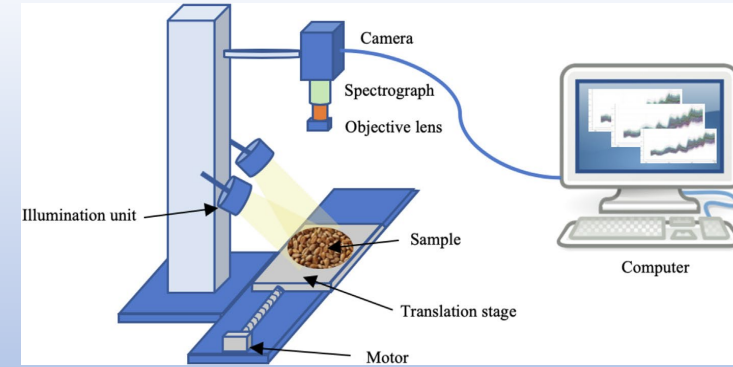
*but the presence of mycotoxins is only moderately correlated with the level of fungal contamination*

- Indirect Tests

The toxin *interacts with something else* that is detected (immunoassays, enzyme activity assays)

- Direct Tests

The toxin itself is detected by measuring some aspect of its physical properties (absorbance, fluorescence, mass spectrum, etc.)

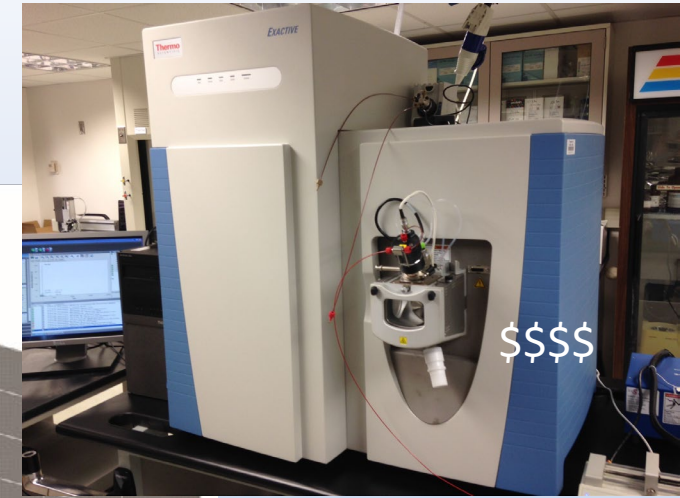
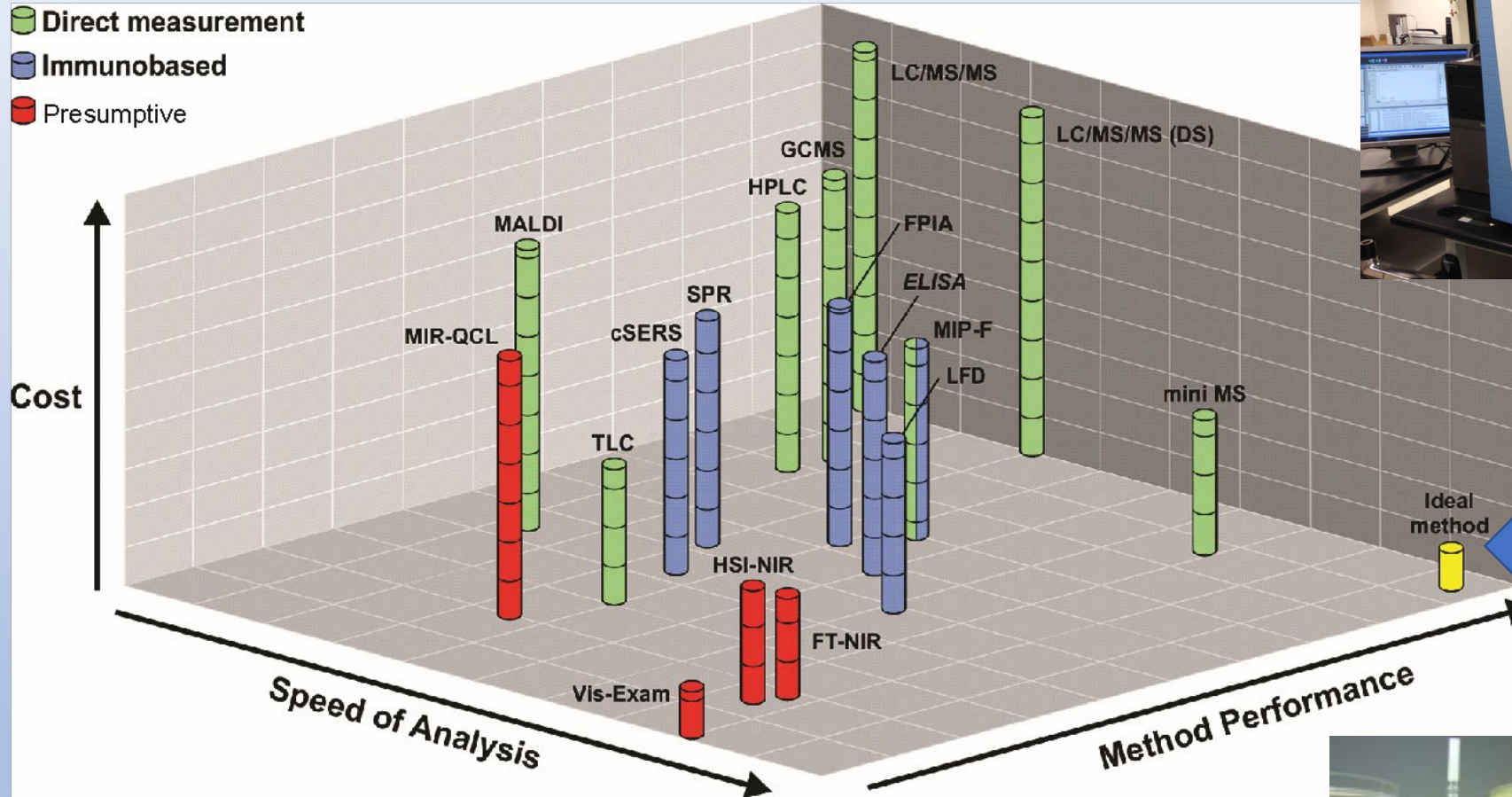


A. Fremenias et al. *Food Control* 108:106819



# Mycotoxin Testing: Tests Compared by Type

Each bar on this graph represents a different group of mycotoxin testing technologies



## The Ideal:

- Low Cost
- Fast
- Accurate



# Current & Upcoming Technologies:

## • Direct (i.e. “chemical”) Tests

- LC-MS/MS systems have become **more affordable**, widely used
  - Greater number of toxins can be tested, in shorter times than ever before
  - Non-targeted analyses & the metabolome
- **Portable** MS instruments have recently been introduced
  - Potentially taking the accuracy of MS out of the laboratory



TLC-MS



## • Indirect Tests

- Improvements to dipstick tests
  - **Multiplexing** more widely available
- Aptamer based tests (?)
- Biosensors- perhaps becoming better established & accepted
  - Automation to improve ease-of-use



Multi-strip readers



MycoFoss,  
Automated sample  
prep & testing

## • Presumptive/Predictive Tests

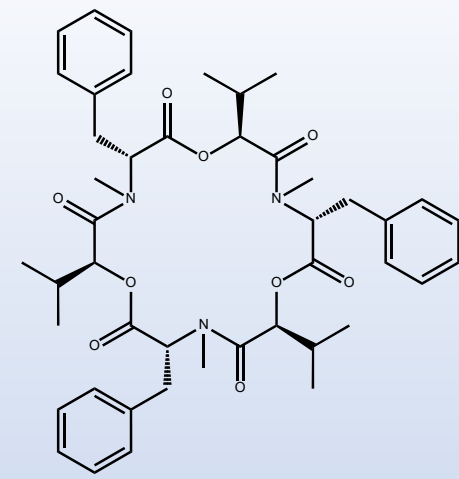
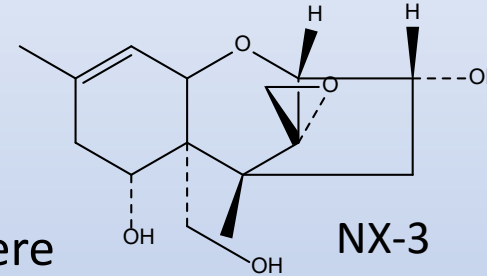
- Applications of Machine Learning and **Artificial Intelligence**
- **Hand-held** hyperspectral imaging systems



# Emerging Issues

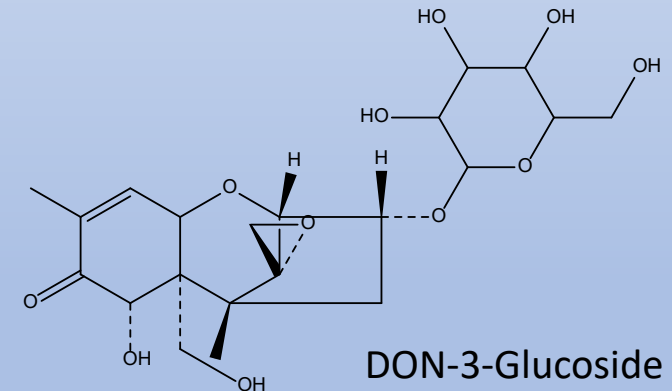
- Perceived Mycotoxin Food Safety Issues (*Consumer Driven*)

- “Emerging” mycotoxins
  - Most are not really new, just being rediscovered
- “New” derivatives of known mycotoxins
  - Intermediates in biosynthesis...have always been there
  - Plant defenses: storing mycotoxins in forms that can be regenerated after consumption?
    - Mycotoxin glucosides



- Regulatory Barriers (*Government Driven*)

- As a response to consumer’s demands
- As deliberate trade barriers
- Adding more toxins



How will we address this?:

detection methods must measure more things, but with no added costs...

# Regulations

## U.S. FDA

- **Stringently Regulated: Aflatoxins**  
Action levels: FDA has the power to condemn contaminated products (20 to 300 ppb depending on species)
- Less Stringently Controlled:
  - Deoxynivalenol/vomitoxin  
Advisory levels: 1-5 ppm, finished diet
  - Fumonisin  
Guidance levels: 1-50 ppm, finished diet

VS

## European Commission

(EC 1881/2006 and amendments, i.e. **stringently regulated**)

- Aflatoxins
- Deoxynivalenol
- Fumonisin
- Ochratoxin A
- Zearalenone
- Patulin
- T-2 & HT-2
- Citrinin
- Ergot Alkaloids

## How New (“Emerging”) are Handled:

### **EU** Regulatory Process:

1. Toxic material is discovered
2. *Call for Data* on occurrence & toxicity
3. Data collected, given to EFSA panel
4. *EFSA Scientific Opinion* is generated
5. Discussion & imposition of regulation
6. Further data collection to ‘fine tune’

### A. EFSA Scientific Opinions

Alternaria Toxins & Tenuazonic acid  
Phomopsins  
Sterigmatocystin  
Beauvericin and Enniatins  
Nivalenol

### B. “On the Radar”

(EU “Call for Data”)  
-Diacetoxyscirpenol  
-Fusaric Acid  
-Moniliformin

# What Does The Future Hold?

- Fungi aren't going away
- Industry will need to keep managing
- Impacts of Climate Change
  - Models of European data predict that the area where aflatoxin is an issue will expand northward
  - Effects of higher CO<sub>2</sub> on plant diseases
- Emerging Issues:
  - Higher levels of well-known mycotoxins
  - 'new' mycotoxins....regulations
- New Technologies/New Solutions
  - Multi-pronged approaches
  - Improved monitoring



# Solutions on the Horizon

- Expect advances in monitoring
- How will improvements come?
  - Applications of **Artificial Intelligence** (ex: hyperspectral imaging)
  - Greater **portability** of instruments
  - Better toxin binding/**recognition elements** (antibodies, aptamers, MIPs)
  - Changing how **extractions** are done, or eliminating them altogether
- All of this will allow detection to move into locations and situations where it hasn't been done before
  - On the farm? Incorporated into harvesting equipment?
  - In-line during processing?
  - Directly by consumers?

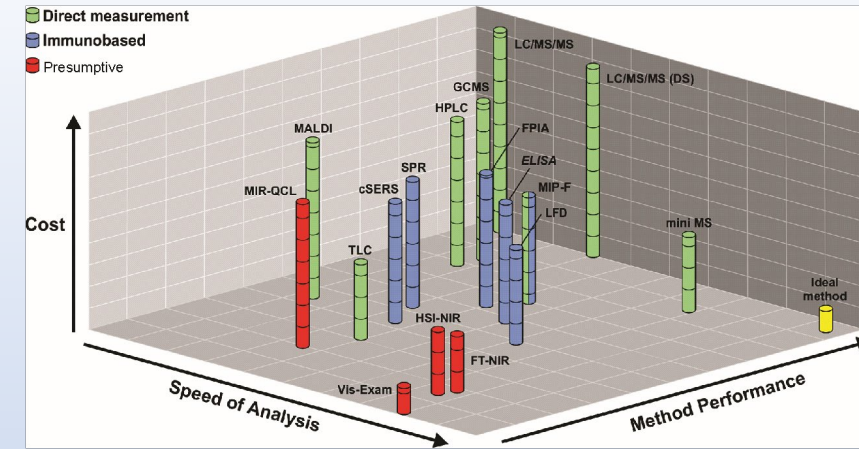
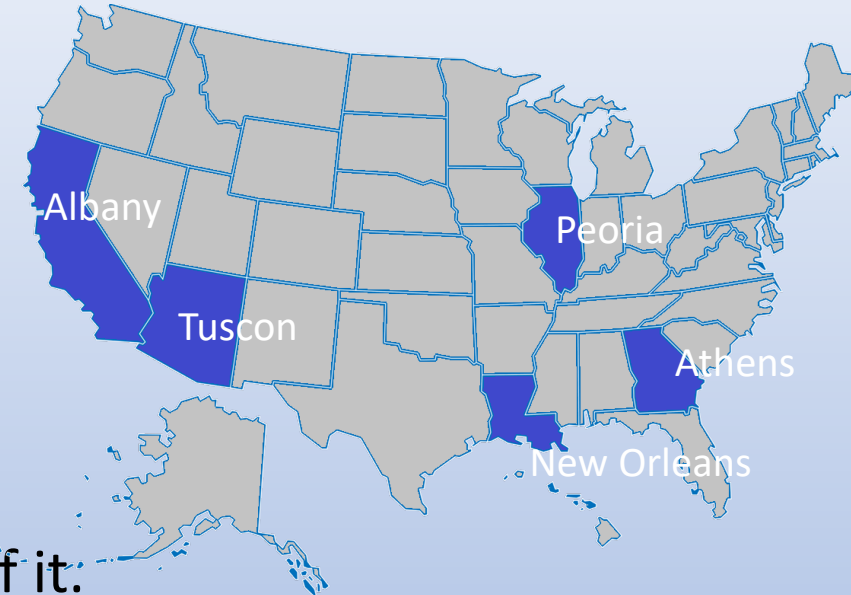


Image: Georgetown University

# The Role of USDA-ARS Research as *Part Of* the Solution

- Research within ARS, at multiple locations
- Where we ‘fit’ in the research enterprise
- Our funding
- How you can influence ARS research
  - Your Federal tax dollars pay for us...take advantage of it.
  - Tell us how we can help (yes, we’re the government...)
  - Tell your federal representatives what you need
  - Directly participate with ARS as collaborators in projects



# Thanks for Your Time

- If you have suggestions, have an idea for a project, or would like to collaborate, please contact me, either here or at:

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