

Mycotoxins

Tim Herrman

Professor, State Chemist and Director
Office of the Texas State Chemist



Outline

- ❑ Mycotoxins, a chemical hazard
- ❑ Mycotoxin development in the field
- ❑ Mycotoxin development in storage
- ❑ Sampling and detection of mycotoxins
- ❑ Best Management Practices to prevent or reduce mycotoxin contamination
- ❑ Regulatory compliance with action levels and advisory limits

Fungal Toxins

Fungus	Toxin
<i>Aspergillus flavus, A. parasiticus</i>	aflatoxin
<i>Fusarium</i> species	tricothecenes
<i>F. Verticillioides</i>	fumonisin
<i>F. Graminearum</i>	zearalenone
<i>Penicillium verrucosum</i>	ochratoxins
<i>Claviceps</i>	ergot

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Mycotoxinoses of Animals

Toxin	General Effect	Susceptible Species
aflatoxin	Liver damage including cancer, decreased milk and egg product	Young animals, dogs, fish, turkeys and ducks, dairy cattle
fumonisin	Equine leukoencephalomalacia, porcine pulmonary edema	Horses, rabbits, swine, breeding animals
tricothecenes	Necrosis and hemorrhage in digestive tract, depressed blood regeneration	Monogastric animals pigs, chickens and turkeys, then ruminants
ochratoxin	Kidney damage, liver damage, and intestinal necrosis and hemorrhage	Swine, turkeys and chickens decreased productivity

Mycotoxin: CAST report No 139 url cast@cast-science.org

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Aflatoxin Action Levels

ppb	Product Description
20	Corn, peanut products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known
20	Corn, peanut products, and other animal feeds and feed ingredients, but excluding cottonseed meal, intended for immature animals
100	Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry
200	Corn or peanut products intended for finishing swine of 100 pounds or greater
300	Corn and peanut products intended for finishing (i.e. feedlot) beef cattle

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Fumonisin Advisory Levels – Animal Feeds

ppm	Product Description
5	Equids and rabbits (no more than 20% of diet)**
10	All other species or classes of livestock and pet animals (no more than 50% of diet)**
20	Swine and catfish (no more than 50% of diet)**
30	Breeding ruminants, breeding poultry and breeding mink* (no more than 50% of diet)
60	Ruminants >3 months old being raised for slaughter and mink being raised for pelt production (no more than 50% of diet)**
100	Poultry being raised for slaughter (no more than 50% of diet)**

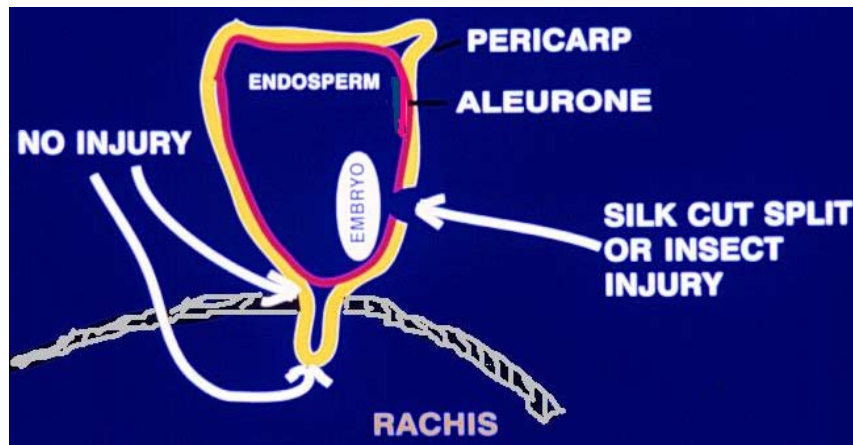
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DON Advisory Levels for Animals

ppm	Product Description
1	Finished wheat products, e.g. flour, bran, and germ, that may potentially be consumed by humans. FDA is not stating an advisory level for wheat intended for milling because normal manufacturing practices and additional technology available to millers can substantially reduce DON levels in the finished wheat product from those found in the original raw wheat. Because there is significant variability in manufacturing processes, an advisory level for raw wheat is not practical.
10	Grains and grain by-products (on an 88% dry matter basis) and 30 ppm in distillers grains and brewers grains (on an 88% dry matter basis) destined for ruminating beef and feedlot cattle older than 4 months and ruminating dairy cattle older than 4 months, with the added recommendations that the total ration ¹ for ruminating beef and feedlot cattle older than 4 months not exceed 10 ppm DON, and the total ration for ruminating dairy cattle older than 4 months not exceed 5 ppm DON. For chickens, 10 ppm DON with the added recommendation that these ingredients not exceed 50% of the diet of chickens.
5	Grains and grain by-products destined for swine with the added recommendation that these ingredients not exceed 20% of their diet.
5	Grains and grain by-products destined for all other animals with the added recommendation that these ingredients not exceed 40% of their diet.

<http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/NaturalToxins/UCM217558.pdf> (Nonbinding Recommendations. June 29, 2010.) 7

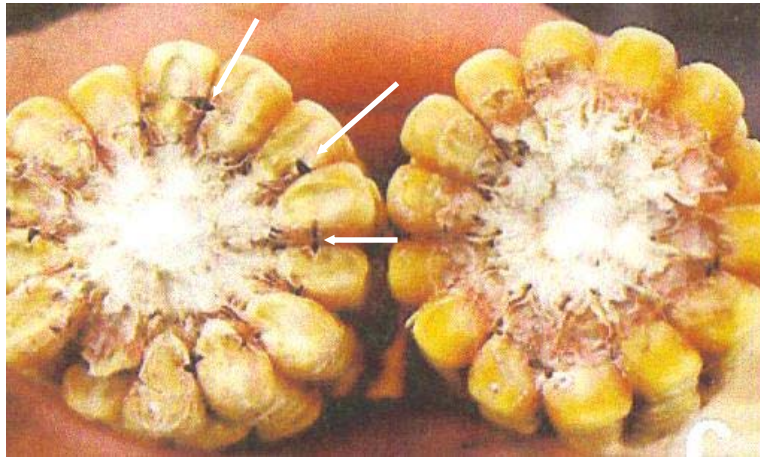
Field Development: How the fungus enters the seed



A wound is usually needed

(Photo: Tom Isakeit) 8

Silk Cut



Stress-related loss of kernel integrity

(Photo: Gary Odvody) 9

Aspergillus



(Photo: Tom Isakeit) 10

Environmental Conditions Determining Mycotoxin Production in the Field

Fungus/Toxin	Environmental Conditions
Aspergillus flavus/aflatoxin	In corn, high temperature and drought favor toxin production; kernel moisture < 32% to 15%; optimum temperature 25-30°C.
Fusarium verticillioides/ Fumonisin	In corn, not well understood. Ear rot associated with dry growing condition followed by wet weather during silking and maturation.
F. graminearum/ DON and zearalenone	In corn, cool wet weather
Ochratoxin	More common in south east

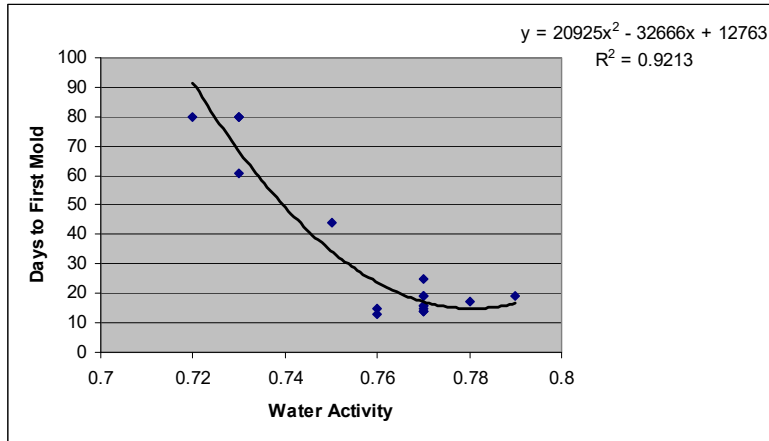
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Storage Environmental Conditions Favorable for Mycotoxin Production

Fungus/Toxin	Storage Conditions
Aspergillus flavus/aflatoxin	Germination and growth of require a_w greater than 0.85 and temperatures greater than 10°C
Fusarium verticillioides/ Fumonisin	No reports of fumonisin accumulation during storage
F. graminearum/ DON and zearalenone	Not common to accumulate in storage
Penicillium/ Ochratoxin	Wider range of moisture/water activity ranges

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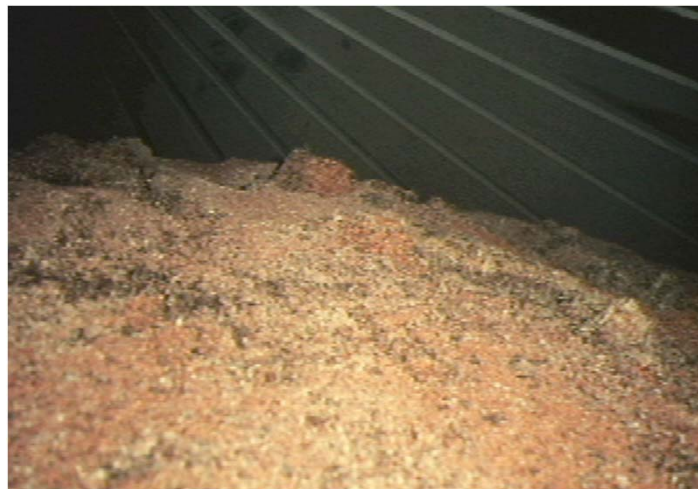
Relationship Between Water Activity and Mold Development



Herrman, T.J. and T.M. Loughin. 2003. Processing and shelf-life performance of feed manufactured from high moisture corn. Transactions ASAE 46(3)697-703.

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Storage Management



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Sampling – Office of the Texas State Chemist Field Procedures

- ❑ Samples must be obtained by a procedure which yields a representative sample using procedures of AOAC International or procedures that are determined dependable through research and/or investigation
- ❑ Refer to Section 965.16 of the 15th Edition of AOAC “Sampling of Animal Feed – Procedure”

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Sampling while Loading or Unloading

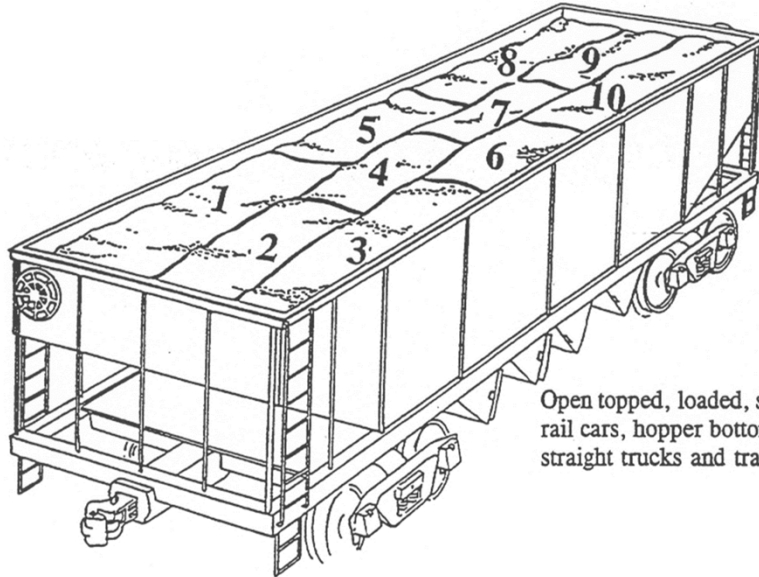
- ❑ Railcars, hopper bottom trucks, straight trucks and trailers
- ❑ Use a stream cutter
- ❑ Minimum of 10 cuts at equal intervals to provide approximately 10 pounds

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Probe Sampling of Bulk Containers

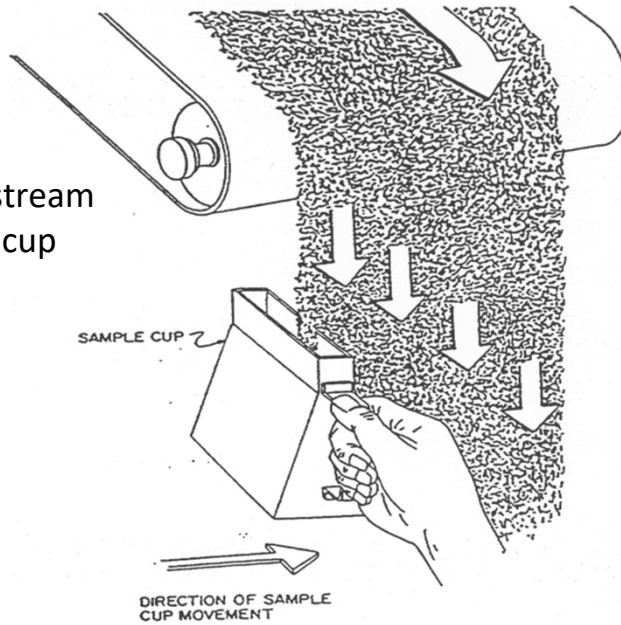
- ❑ Railcars, hopper bottom trucks, straight trucks and trailers that are open-topped. 10 probes.
- ❑ Limited-access vehicles, with one compartment. 10 probes.
- ❑ For vehicles with multiple compartments. Minimum of 10 probes.
- ❑ Collect and retain a sample size of about 10 lbs.

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Open topped, loaded, stationary rail cars, hopper bottom trucks, straight trucks and trailers

- Use of a stream sampling cup



Sampling Statistics

$s^2_{c(s)} = aC^b$, where a and b are constants and C is the estimated concentration.

Using regression analysis, the relationship between sampling variance and aflatoxin concentration is:

$$s^2_{c(s)} = 11.361C^{0.98}$$

100 ppb aflatoxin $s^2_{c(s)} = 11.361 \times 91.2011 = 1036$

Standard deviation sqrt of the variance = 32

Coefficient of variance = $s \div \mu \times 100 = 32$

(Johansson et al. 2000. Testing shelled corn for aflatoxin, Part I: Estimation of variance components, AOACI 83(5)1264-1269.)

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Sampling Statistics – Sample Size

$s^2_{c(s)} = (1.13/ns) \times 11.361C^{0.98}$, where ns is the sample size in kg.

Example 1: 1.13 kg (2.5 lbs)

100 ppb aflatoxin $s^2_{c(s)} = 11.361 \times 91.2011 = 1036$

cv = 32

Example 2: 4.54 kg (10 lbs)

$s^2_{c(s)} = (1.13/4.54) \times 11.361 \times 91.2011 = 256.859$

cv = 16

Example 3: 9.08 kg (20 lbs)

cv = 11.35

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Sampling and Sample Preparation

- ❑ 10 pound sample collected
- ❑ Entire sample ground and subdivided by Romer Mill
- ❑ 5 pound portion further ground by Retsch Mill with 1.5 mm screen
- ❑ Sample passes through a number 20 sieve

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Sample Grinding



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Confirmation Analysis (HPLC)

- ❑ 50 grams extracted with 70% methanol
- ❑ Aflatoxins cleaned, concentrated, and eluted through Vicam Aflatest column
- ❑ Aflatoxins quantitated by PHRED (Photochemical Reactor for Enhanced Detection) fluorescence after separation on high performance liquid chromatography

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OTSC Aflatoxin Analyses



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BEST MANAGEMENT PRACTICES TO Prevent or Reduce Mycotoxin Contamination in Texas

Pre-Harvest

- Aflatoxin management in the field
 - Host resistance and insect control
 - Planting cultural practices
 - Biological treatment

Host Resistance

- ❑ To insect, conventional and transgenic (Bt): not enough
- ❑ To fungus: not easy to introduce into hybrids
- ❑ Improved host tolerance to stress

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Husk Cover



PHOTO: G. ODVODY

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Cultural Practices

- ❑ Planting date
- ❑ Plant populations
- ❑ Weed and pest control
- ❑ Irrigation
- ❑ Tillage to break hard pan
- ❑ Adequate fertility

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Harvest Practices

- ❑ Segregate harvest of “good” and “bad” fields
- ❑ Change combine settings to remove damaged seed
- ❑ Early harvest, followed by artificial drying
- ❑ Cleaning seed after harvest

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Post Harvest BMPs

- ❑ Store grain over the action level separately
- ❑ Test incoming unit trains
- ❑ Test incoming grain from farm storage
- ❑ Do not commingle grain exceeding the action level with grain below the action level during reclaim
- ❑ Correctly label grain exceeding the action level

<http://mycotoxinbmps.tamu.edu>

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Mycotoxin Mitigation

Successful in Texas

- ❑ Atoxigenic fungi
- ❑ Sequestering agents

Less successful in Texas

- ❑ Ozonation
- ❑ Anhydrous ammonia

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Contact Us

Mary Sasser
Technology Manager
Office of the Texas State Chemist
Phone: (979) 845-1121
Email: msasser@otsc.tamu.edu

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Texas Laws Regulating Aflatoxin

Texas Commercial Feed Control Act

- Texas Agriculture Code, Chapter 141

141.002 Commercial Feed

- c) The following are not commercial feeds subject to this Chapter:
 - 2) Whole grain or whole seeds not containing toxins or chemical adulterants;

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Texas Laws Regulating Aflatoxin and Fumonisin

Texas Commercial Feed Control Act

- Texas Agriculture Code, Chapter 141

141.148 Distribution of Adulterated Feed

- a) A person commits an offense if the person distributes, conspires to distribute, or causes another person to distribute commercial feed:
 - 2) That is moldy, sour, heated, or otherwise damaged, because of which it is injurious to animals;
 - 6) That contains or bears poisonous or deleterious substance that may render it injurious to animals under ordinary conditions of use
- b) An offense under this section is a Class C misdemeanor

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Texas Rules for Aflatoxin and Fumonisin Align with FDA Guidance Documents

- ❑ Except, Texas has action levels for fumonisin
- ❑ Texas has a 100 ppb action level for aflatoxin in grain fed to sheep and non-lactating goats
- ❑ Texas allows blending for grain between 301 to 500 ppb aflatoxin
- ❑ Texas approves the use of aflatoxin binders

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OTSC Aflatoxin and Fumonisin Policies

- ❑ Feed Industry Memorandum 5-12
- ❑ Feed Industry Memorandum 5-17
- ❑ Feed Industry Memorandum 5-20
- ❑ Feed Industry Memorandum 5-23

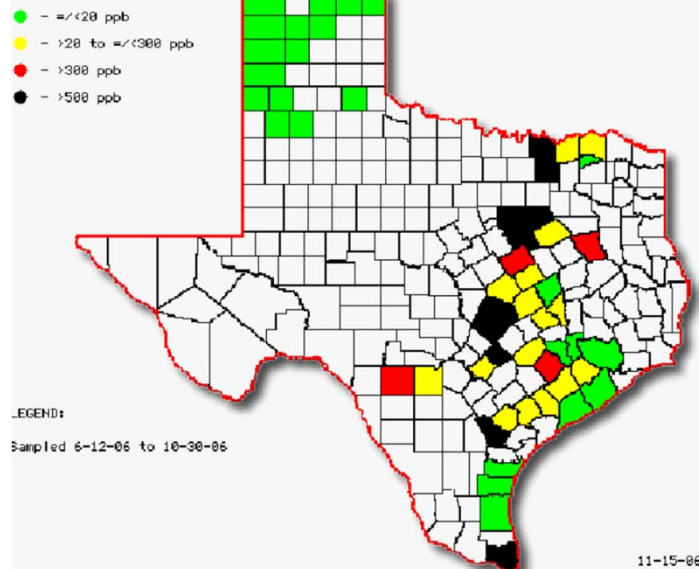
<http://otscweb.tamu.edu/>

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OTSC Plan of Work

- ❑ Active surveillance (500 harvest samples)
- ❑ OTSC reports results to trade associations
- ❑ Follow up on all milk dumping cases
- ❑ New measurement methodology (HPLC)
- ❑ Grain elevator census by visiting all facilities (250 commercial warehouses) in top 40 corn producing counties in 2005 and all commercial warehouses in 2006

Aflatoxin - 2006 Crop Year



Summary

- ❑ Mycotoxin present a serious chemical hazard to the feed industry
- ❑ Adoption of BMPs for field and storage management will help reduce the risk
- ❑ Sampling, sample preparation, and analysis are science-based
- ❑ In Texas, the Office of the Texas State Chemist is the designated state agency that provides regulatory oversight for mycotoxins in grain
- ❑ OTSC works closely with state and federal agencies in the U.S. and Mexico to manage the risk and problems posed by mycotoxins

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Contact Information:
tjh@otsc.tamu.edu

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Professor, State Chemist and Director
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