Mycotoxins
How important in Montana?

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Mycotoxins

- Mycotoxins are fungal metabolites that are toxic when consumed by animals
  - Major classes of mycotoxins
    - Aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin, ergot alkaloides
  - <50 different mycotoxins are well characterized
  - Estimated that there may be 20,000-30,000 unique mycotoxins
  - Routine analytical methods available for 20 mycotoxins
    - Analytical costs can be $75->150.00/ sample
- Produced by molds in grains, seeds, hay, silage, processed feeds, wallboard, wall paper, carpets, etc
  - Produced by fewer than 100 species of fungi of the several hundred thousand known fungal species
  - Aspergillus, Penicillium, Fusarium, Claviceps, Stachybotrys, etc
- Mycotoxicoses-disease in animals caused by mycotoxins
  - Non ruminants are generally most sensitive
  - Young animals most sensitive
  - Chronic vs Acute-"dose makes the poison"

Mycotoxins in Montana

- MT produced grains and feed stuffs
  - Ergot (Ergotamine and other ergot alkaloids)
  - Fusarium-DON, DAS, T-2, HT-2,Zearalenone
  - Penicillium-several
  - Alternaria- several
  - Aspergillus-aflatoxins (rare), sterigmatocystin
- Grains and feed stuffs produced out of state-watch weather conditions/news
  - Aspergillus- Aflatoxin-grain/cotton seed meal
  - Fusarium- DON and other trichothecenes, Fumonisin, Zearalenone

Many field molds-generally non-toxic, ear molds of corn

***Diplodia* ear rot**

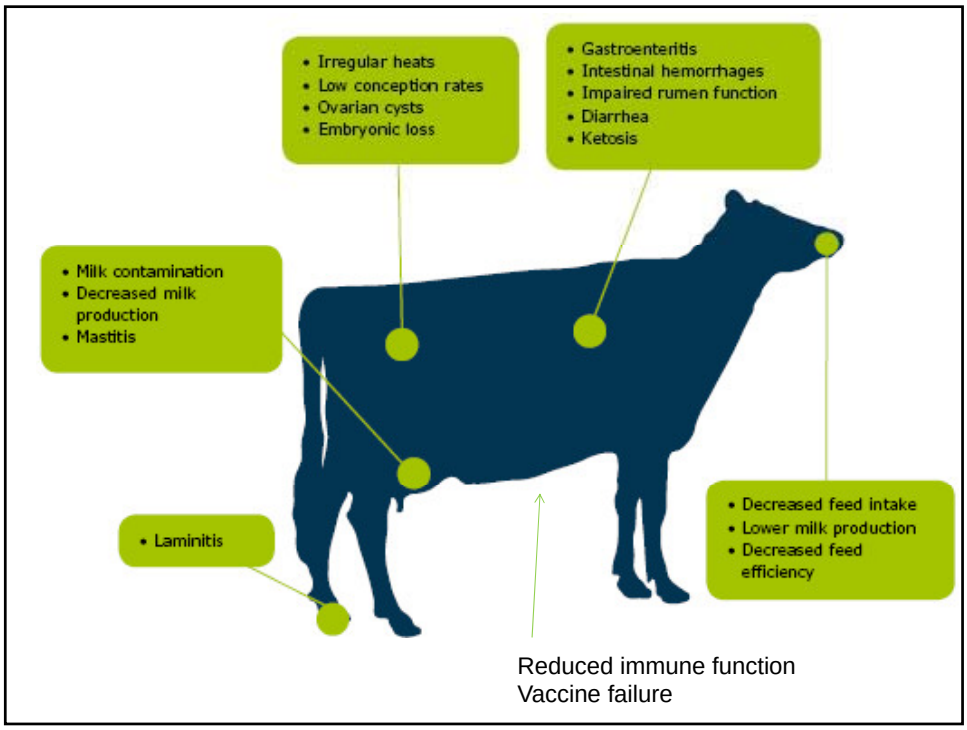
Most common molds-Alternaria, Cladosporium Rhizopus, Mucor, etc produce no known mycotoxins

Can be indicators of sound grain

***Cladosporium* ear rot**

Alternaria alternata-delayed harvested wheat
Several mycotoxins

Common Corn Smut = *Ustilago maydis*
Loose smut of Barley= Ustilago nuda
Black fungal spores inside a whitish membrane
Corn and other grain smuts are not a mycotoxin producers



Ergot
affects all grasses/cereals

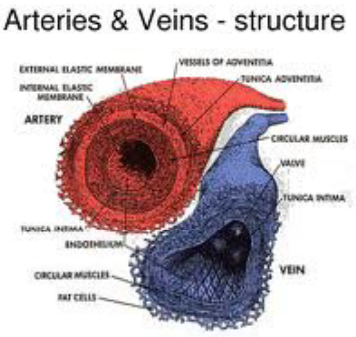
Barley ergot

Claviceps purpurea

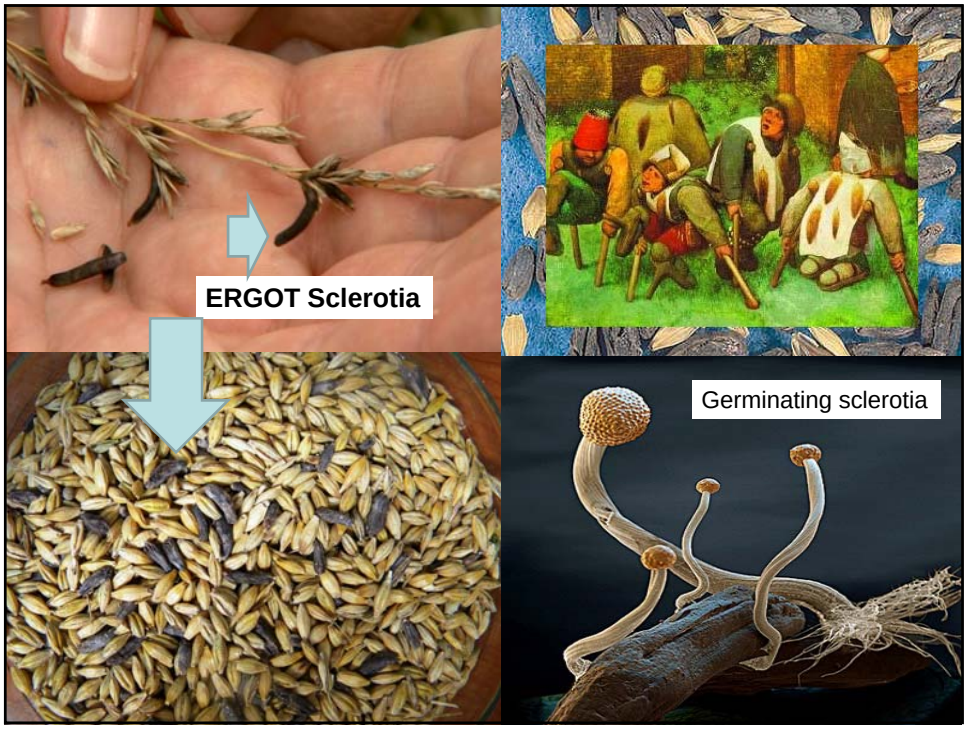
Sclerotia-mycotoxin-ergot alkaloids in sclerotia

Rye/Triticale most susceptible

Common in MT in years with cool, wet springs



Ergot- vasoconstriction, convulsions, staggers, lameness, poor hair condition, loss of extremities, abortion



Ergot

Barley ergot

Claviceps purpurea

Honeydew and sclerotia-animals love this stuff

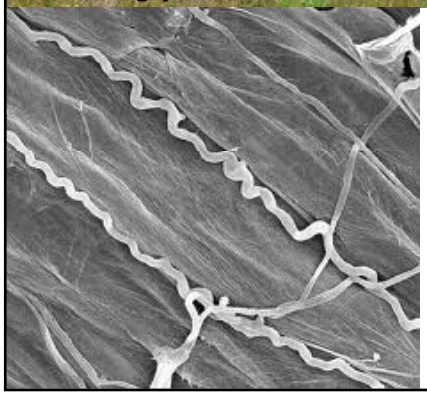
Source: V. Pederson

Ergot

- Common in almost all grasses/cereals if cool, wet weather predominates during anthesis- allows for infection of heads by ascospores from overwintering ergot sclerotia
- Grain >0.05% ergot is ergoty and cannot be sold- must clean-gravity table cleaner
- All warm blooded animals are sensitive
- Common in rye, triticale, some wheat (Waldron) and barley varieties
- Watch pastures where grasses have mature seeds. Animals often attracted to honey-dew stage

Breeding problems/Abortion

Rough hair coat



Fescue Toxicosis

Most common in south pure stands of tall fescue
dose make the poison- rarely a problem in mixedstands

Fungus growing inside of plant (endophyte) produces ergot alkaloids

Fescue endophyte/choke diseases

- Fescue toxicosis
- Ergopeptides
- Clavene alkeloids
- Ecological adaptation to prevent feeding by insects and animals
- Fungi identified in many pasture grasses in MT but no problems observed. Most common in mid south and southern USA
- Lack of MT problems due to mixed stands

Black Head Molds on Wheat/Barley

Alternaria and Cladosporium molds

Common where premature plant death or rain delayed harvest

Mycotoxins: Alternariol, altenuene, alterntoxin,Tenuazonic acid

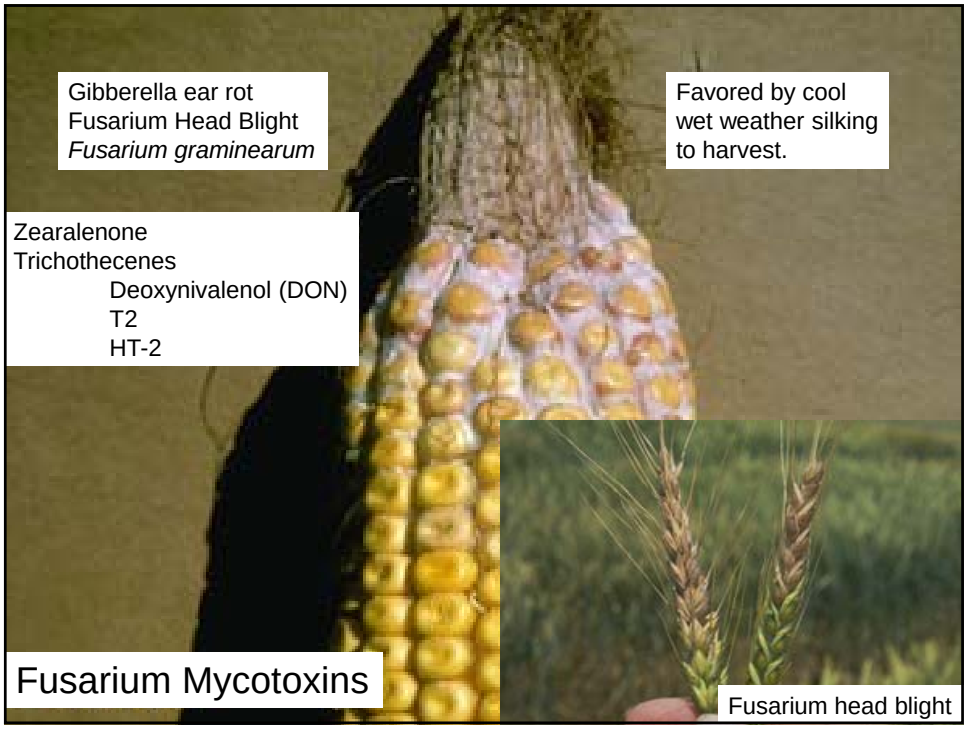
Can be found in whole wheat bread products

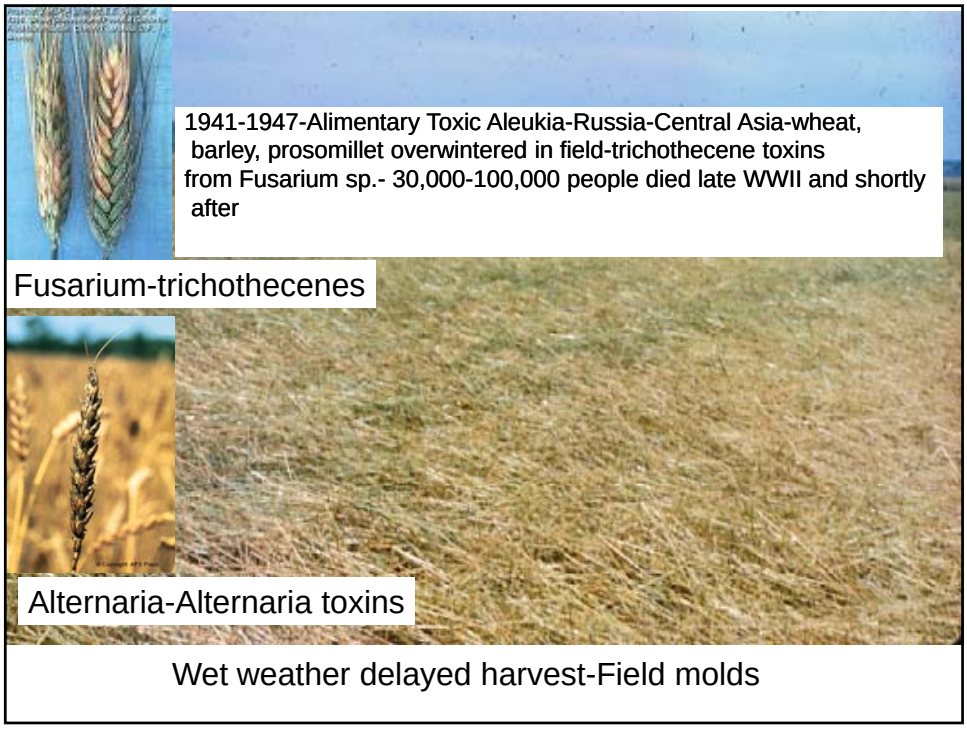
Chronic not acute poisoning

Moldy Hay- mycotoxins?-immune suppression-vaccine failure
Fungi: Cladosporium, Alternaria, Fusarium, Aspergillus, Penicillium
Avoid feeding to horses
Ruminants can detoxify many mycotoxins
No good tests for Alternaria and Penicillium mycotoxins

Fusarium mycotoxins

- Deoxynivalenol (DON) and acetylated derivatives
- Diacetoxyscripenol (DAS)
- T-2 and HT 2
- Fumonisins
- Zearalenone





Wet weather delayed harvest-Field molds

Fusarium graminearum after hail injury

Field molds such as Fusarium produce several mycotoxins

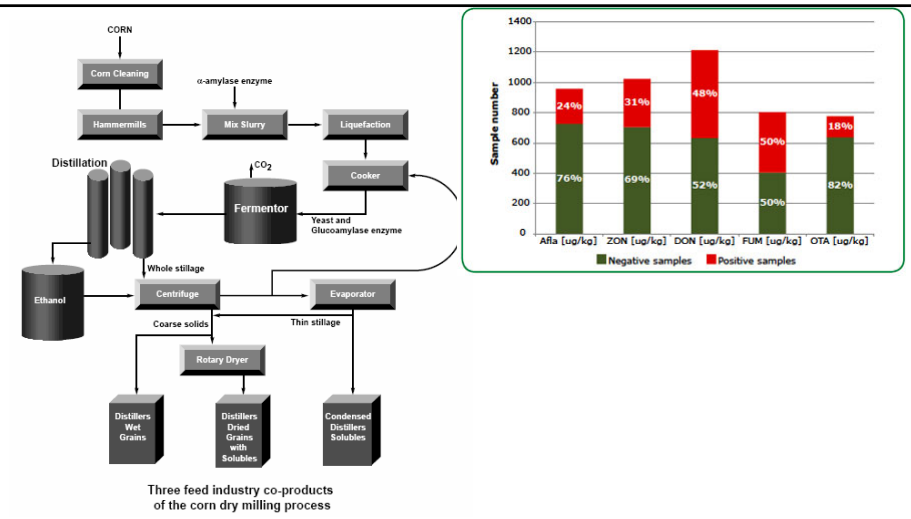
Fusarium mold growth with DON, DAS and T-2,Zearalenone toxin contamination

Mycotoxigenic Fusarium sp.

Field Molds in grains, silage and hay

- F. acuminatum-DAS, T-2 HT-2
- F. culmorum-DON, nivalenol, zearalenone
- F. equiseti-DAS, nivalenol, zearalenone
- F. graminearum-DON, nivalenol, zearalenone
- F. poae-DAS, nivalenol, T-2, HT-2
- F. sporotrichioides-DAS, T-2, (HT-2)
- F. verticillioides (moniliformae), F. proliferatum-fumonisins

Dog food and other processed feeds make contain mycotoxins
-aflatoxins and trichothecenes are most common



Distillers grains-
mycotoxins are concentrated ~3X compared to parent grain
Aflatoxin, DON and other Trichothecenes, Fumonisin, Zearalenone, Ochratoxins

Fusarium head blight
Scab
Infection-warm wet weather at anthesis

DON concentrations up to 30-40 ppm found in scabby wheat in MT

Grain "Tombstone Kernels"

- **Chalky appearance from the fungal mycelium.**
- **Mycotoxins, including "Deoxynivalenol (DON) found in infected kernels**
- **FDA advisory level, 1.0 ppm 'DON'**

Symptoms at harvest

- **Diagnostic tan to brown discoloration of stem below the head.**
- **Infected plant residue serves as in-field inoculum and contains DON.**

Fusarium Head Blight of Wheat

- *F. graminearum, F. culmorum, F. crookwellense, and F. avenaceum*
- Dominant species is determined by temperature more than any other factor
- Disease incidence is most affected by moisture at anthesis (Cook, 1981)

Fusarium head blight in Montana

Map http://www.peninsulaflyfishers.org/Fishing_Tales/montanaSpring02/montana_map.gif

Reducing DON in harvested grain

- Can blow light weight scabby seed out of combine with higher fan speed at harvest, **but** this **WILL** serve as a nice source of inoculum the following year
- Clean grain after harvest- typically removal of light weight scabby grain (tombstones) will reduce DON levels by > 50%

Advisory levels

- 1ppm of vomitoxin for bran, flour, and germ intended for human consumption
- 10 ppm of vomitoxin in grain and grain by products destined for ruminating beef and feedlot cattle older than 4 months and for chickens –not exceed 50% diet
- 5ppm of vomitoxin in grain or grain products destined for swine and all other animals-not exceed 20% diet for swine, 40% other animals

A Self-Learning Resource From MSU Extension

MONTANA STATE UNIVERSITY EXTENSION

MontGuide

Fusarium Head Blight (scab) of Wheat and Barley

by Mary Burrows, Extension Plant Pathologist, William Grey, Extension Cereal Agronomist, and Alan Dyer, Cereal Plant Pathologist

Fusarium head blight is a disease of wheat and barley. The pathogen reduces yield, seed quality and produces a vomitoxin called DON.

MT200806AG New 6/08

FUSARIUM HEAD BLIGHT (FHB) (SCAB) IS A destructive disease of wheat and barley in Montana and in most wheat-growing regions around the world. The primary symptom of the disease is bleaching of some of the florets in the head before maturity. Severe infections can cause premature blight or bleaching of the entire spike or head (Figure 1). Other symptoms include tan to brown discoloration at the base of the head (Figure 2), a pink or orange colored mold at the base of the florets under moist conditions, and kernels that are shriveled, white, and chalky in appearance ('tombstones' in Figure 3). Visible expression of FHB is observed in heads during the soft to hard dough growth stage as premature ripening accompanied by dark brown discoloration of the peduncle and shriveled kernels with a chalky (tombstone) appearance (Figure 3). The disease is caused by the fungi in the genus *Fusarium* with the following species being most commonly involved: *F. graminearum*, *F. pseudograminearum*, *F. avenaceum* and *F. culmorum*.

The disease causes yield loss, low test weights, low seed germination and contamination of grain with mycotoxins. A vomitoxin called deoxynivalenol (DON) is considered the primary mycotoxin associated with FHB and is subject to regulatory limits by the U.S. Food and Drug Administration (FDA). There is a 1 ppm limit for DON in all finished wheat products (flour, bran and germ) that may be consumed by humans. Limits of 5-10 ppm have been set for animals. Animals with simple stomachs such as dogs or swine are more sensitive than ruminant animals. There is a zero tolerance for DON in malt barley. All these toxins are highly stable and levels will remain unchanged for years in storage. Producers should not mix clean grain with grain identified to have greater than 1 ppm of DON to achieve a lot that will be accepted by the elevator. This is considered to be adulteration of grain by the FDA and is subject to criminal penalties.

Symptoms can be confused with various root and crown diseases which cause entire heads to bleach. Premature blight symptoms can also be confused with black chaff and glume blotch which cause discoloration of the glumes and seed. These diseases will not cause tombstone grain or stem (peduncle) discoloration.

Currently no barley or durum varieties are available with tolerance to the disease. Head blight symptoms and scabby grain in durum are similar to those found in bread spring and winter wheats. Barley with severe scab will have a pre-mature blight or bleaching of the head. Initial infection is characterized as a discolored lesion on the base of the glume and rachis that then spreads in both

FIGURE 1. Partial bleaching of the wheat head due to Fusarium head blight.

US Wheat and Barley Scab Initiative

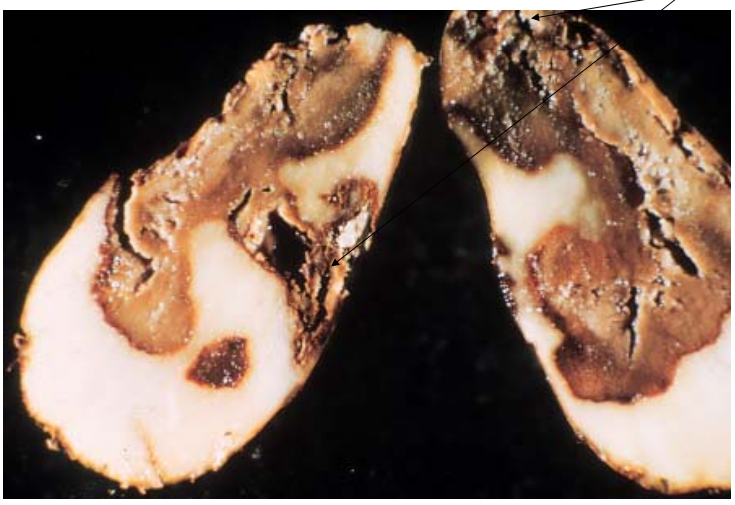
Montana Wheat & Barley Committee

Fusarium dry rot

Fusarium sambucinum

Infections at injuries

Trichothecene toxins in decayed tissues



Trichothecene Mycotoxins (DON, T-2, DAS)

- Produced primarily by field fungi – *Fusarium* spp.
- Mold growth and toxin production initiated by alternating cool and warm temperatures (41 to 59 °F), humid conditions, optimal toxin production 81 °F.
- May occur in late-harvested or over-wintered grain.
- Common substrates are corn, milo, wheat, barley, rye (can occur in hay and straw).

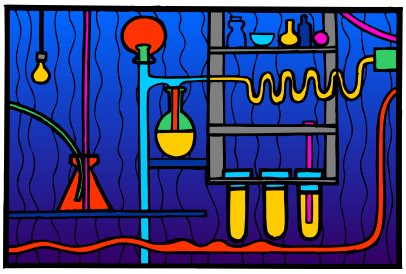
Trichothecene Clinical Effects

- Feed refusal and reduced feed consumption from feed palatability or neurochemical changes in brain.
- Weight loss.
- Ulcers on mucous membranes.
- Diarrhea to bloody diarhhea.
- Skin ulcerations, rough hair coat.
- Immune suppression.
- Possible abortions if dam affected.
- ? Death (T-2 toxin, DAS)

Trichothecene Mycotoxins in Ruminants

- Cause feed refusal and emesis and thus can self-limit ingestion of moldy feed.
- Some trichothecenes (T-2, DAS, and vomitoxin), zearalenone, and ochratoxin thought to be broken down by rumen microbes.
- Low rumen pH (< 5.2) may block metabolism of mycotoxins.

- **Trichothecenes** inhibit protein synthesis and membrane transfer of glucose, calcium, some amino acids.
- Contact cell poison.
- Affect immune function.
- Voluntary **feed refusal** often prevents full expression of toxicosis in animals from contaminated feeds.



Trichothecenes and the Immune System

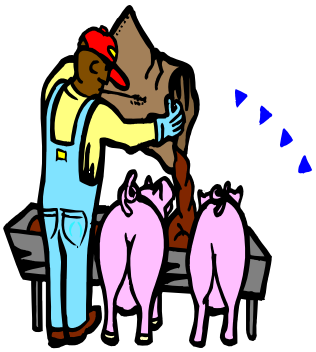
- A reduction or inhibition in the proliferation of T- and B- cells *in vitro*
- T-2 toxin caused inhibition at the lowest levels followed by DON
- Increases susceptibility to pathogens such as *Salmonella* and *Mycobacterium*
- Increased production of IgA leading to glomerulonephritis
- Superinduction of cytokines

DAS,HT-2, T-2 Toxins

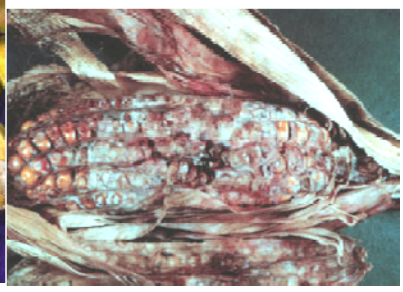
- Chemically related to DON
- Causes irritation, hemorrhage, and necrosis throughout the digestive tract
- Depresses the regenerative process in the spleen and bone marrow
- Causes changes in the reproductive organs
- Affected animals show weight loss, lack of appetite, vomiting, bloody diarrhea, abortion

FDA Guidance Levels for Vomitoxin in Livestock Feed		
Animal	Portion of Diet	Maximum DON Level
Ruminating beef & feedlot cattle older than 4 months	Grain & grain by-products not to exceed 50% of the diet	10 ppm
Chickens	Grain & grain by-products not to exceed 50% of the diet	10 ppm
Swine	Grain & grain by-products not to exceed 20% of the diet	5 ppm
All other animals	Grain & grain by-products not to exceed 40% of the diet	5 ppm

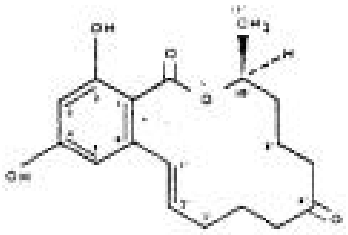
Vomitoxin (DON)



***Gibberella zeae* ear rot**
Reddish-pink to white mold on ears
***Fusarium* molds may produce vomitoxin or DON, zearalenone, T-2 toxin**



Zearalenone



Mimics the effect of the female hormone estrogen

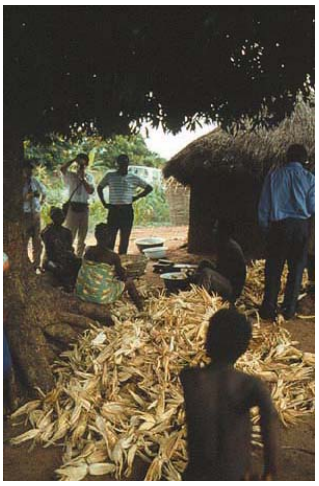
Low doses increase the size or early maturity of mammary glands and reproductive organs

High doses interfere with conception, ovulation, implantation and fetal development

Zearalenone Advisory Levels

- Swine-0.5 ppm
- Breeding cattle-12.5 ppm

Fumonisins



- 1988 Gelderblom et. al
- South Africa, esophageal cancer in humans
- Intense interest because fumonisisns are found in measurable concentrations in corn from all over the world

Infection typically observed with drought and insect damage-Common in MT but no Fumonosin toxicosis noted to date

Fusarium verticilliodes, F. subglutinana, F, proliferatum-kernal rot

Fumonomsins

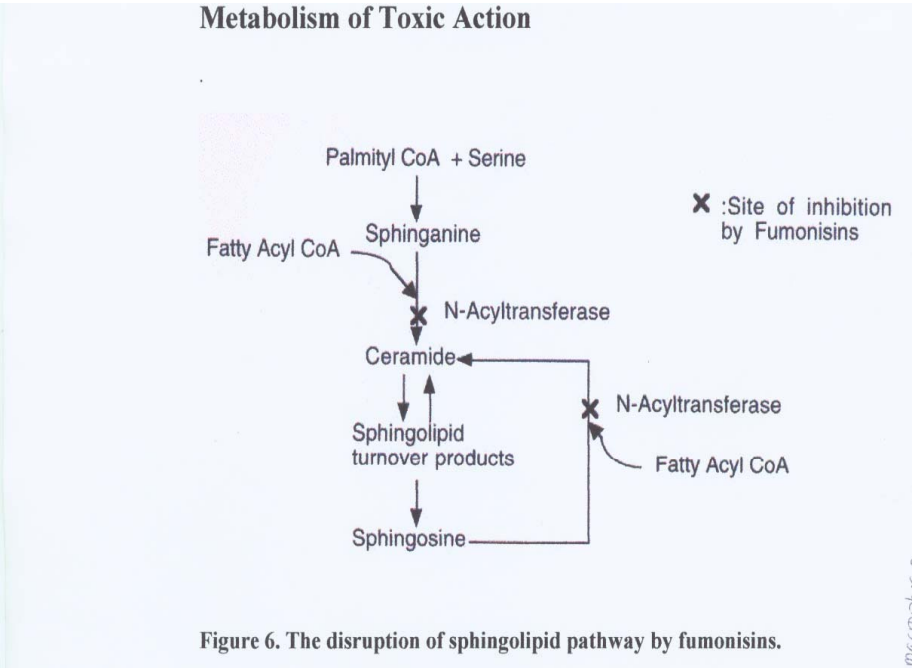


Figure 6. The disruption of sphingolipid pathway by fumonisins.

Functions of Sphingolipids

- Sphingomyelins: important in the nervous tissue of higher animals
- Gangliosides:present in nerve endings and appear to be important in nerve pulse transmission
- Glycosphingolipids: present at cell surface and determine in part certain elements of tissue and organ specificity, cell to cell recognition, and tissue immunity

Advisory Levels of Fumonisins

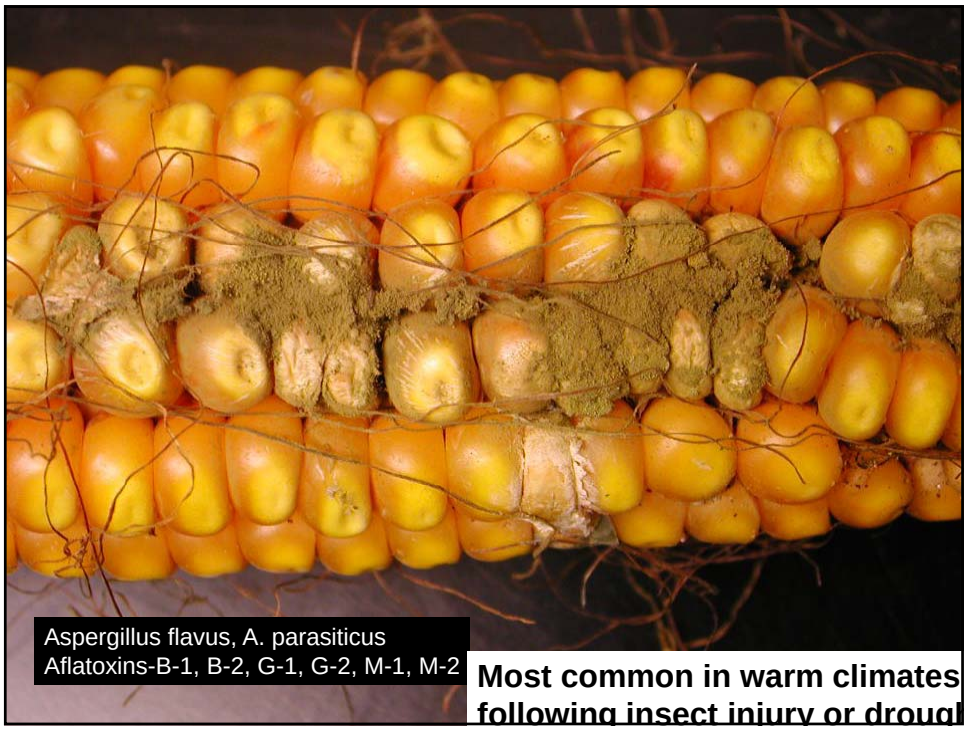
Commodity	FDA Advisory Level
Human foods <2.25% fat	2 ppm
Human foods>2.25% fat	4 ppm
Popcorn	3 ppm
Horse feeds	5 ppm <20% of diet
Swine feeds	20 ppm <50% of diet
Breeding ruminants, poultry	30 ppm< 50% of diet
Ruminants for kill> 3 months old	60 ppm<50% diet
Poultry for slaughter	100 ppm <50% diet
All other species of livestock	10 ppm< 50% of diet

Effects on Animals

- Equine leukoencephalomalacia
- Porcine pulmonary edema
- Hepatotoxic and hepatocarcinogenic in rats
- Associated with esophageal cancer in humans and neural tube defects

Aspergillus/ Penicillium

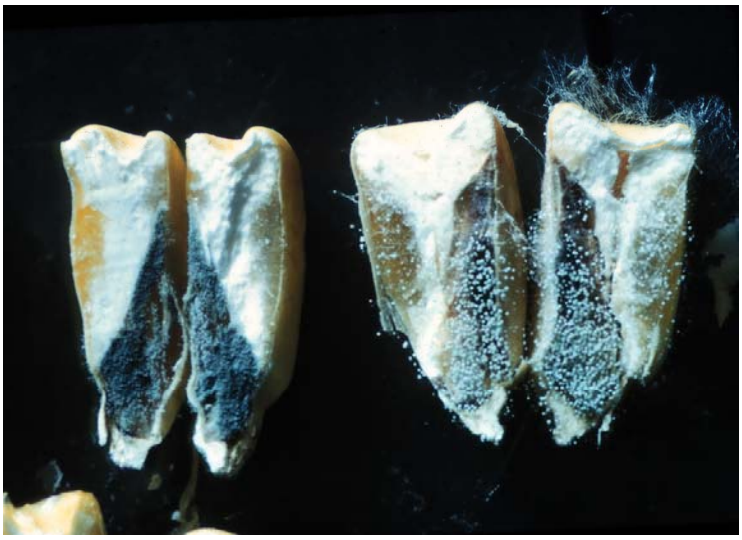
- Cosmopolitan- found in all soils and are associated with decay of organic materials
- Can grow at very low water availability < 20% moisture
- Aspergilli favored by warm to hot conditions
- Penicillium favored by cooler conditions



Grain storage molds

Penicillium ear rot/ Penicllium mold on Pelleted feed

Mycotoxins: Ochratoxins, sterimatocyctin, Tremogenic toxins, Luteoskyrin, Patulin, Rubratoxin,Cyclopiazonic acid, Citrinin, Citreoviridin, etc



Penicillium sp.-many mycotoxins-most poorly characterized and few analytical procedures available

Aflatoxins-first IDed mycotoxin

- Carcinogens-dose makes the poison-allow 20ppb in human foods-(peanut butter)
- Most common where drought damage, high temperatures occur-e.g. southern US, hail damage, bird, insect damage- **not in MT except on imported feeds: corn, cottonseed meal**
  - **Major human health problem in Africa**
- **Can develop in** field or **storage**-cereals, cottonseed, processed feeds.
- Poultry, swine, horses more susceptible than ruminants-aflatoxin into milk @ 1% of feed dose
- **Sterigmatocystin**- precursor to aflatoxin-common in improperly stored wheat/barley~20-50% as toxic

Aflatoxin Guidelines

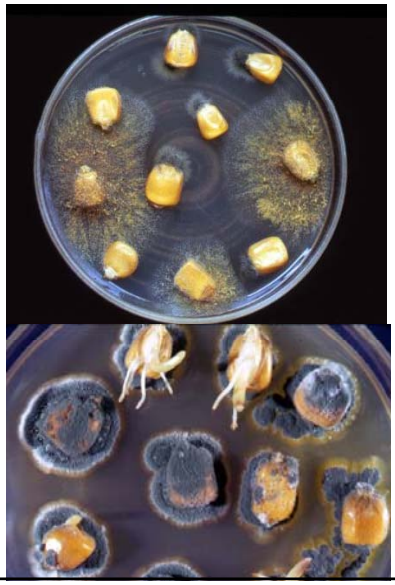
Commodity	Regulatory level
milk	0.5 ppb (limit of detection)
Food for human consumption	20 ppb
Feed for beef	300 ppb
Feed for swine> 100 Lb.	200 ppb
Feed for breeding cattle, swine, poultry	100 ppb
Feed for other animals	20 ppb
Feed for dairy animals	20 ppb

Dust at harvest full of Aspergillus, Penicillium and Cladosporium spores

Dust can contain aflatoxins, ochratoxins, toxins from Stachybotrys

Dust arising when handling grain is primarily Aspergillus and Penicillium mold spores

Grain storage molds
Aspergillus and *Penicillium* spp.



These fungi decay all seeds given the right commodity moisture and temperature-some species produce mycotoxins

Bin Burned Corn-no fire but biological heating from molds

Mycotoxigenic Aspergillus and Penicillium sp.

- *A. flavus, A. parasiticus* -aflatoxins
- *A. versicolor, A. nidulellus(nidulans*), *A. terreus*, some members of the *A. glaucus* group (Eurotium)-sterigmatocystin-most important mycotoxin in stored wheat and other cereals in Canada and N. USA
- *A. alutaceus var alutaceus (ochraceous*), *A. melleus* -ochratoxins-warm to hot climates
- *A. fumigatus* -gliotoxin
- *P. verrucosum, P. viridicatum* -ochratoxin-cooler climates
- *P. islandicum* -islanditoxin, luteoskyrin
- *P. rubrum* -rubratoxins
- Penicillium sp. –citrinin, Penicillic acid, cyclopiazonic acid, penitrem A, patulin, citreoviridin, many others

Moldy clumps: usually starts in areas of high moisture seeds (weed, immature kernels or where transferred moisture condenses) or where broken kernels and fines block air movement- mycotoxigenic molds can be involved

Equilibrium Moisture/Mold Growth

%RH	Starchy grains	Soybean, pea, bean, lentils	Peanut, canola, camelina, safflower	Fungi
65-70	12.5	12.0	5.0	A. halophilcus/ A. restrictus
70-75	14.0	13.0	6.0	A. glaucus
75-80	15.0	14.0	7.0	A. candidus
80-85	16.0	15.0	8.0	A. flavus Penicillium sp
85-90	18.0	18.0	10.0	Above +Penicillium
>95	22.0	20.0	13.0	Yeasts/ bacteria /most field molds

Conditions that Contribute to Spoilage

- Moisture content of the grain-individual seeds
- Temperature of the grain-higher= faster mold growth
- Amount of broken seeds and foreign material
  - Remember weed seeds are often higher moisture than grain
  - Pulse crops –harvest moisture
- Degree which the grain is invaded by insects and molds- pre-harvest-post harvest
- When molds and insects grow they produce metabolic heat and water-succession of organisms

Mycotoxin Effects in Animals

Sometimes hard to evaluate with mycotoxins because

- the presence of Mycotoxins may be very uneven in feeds (i.e., hotspots),
- clinical signs in animals from mycotoxin exposure can be vague and may appear long after the feed has been consumed.
- Generally reduced feed efficiency is first sign
- Diarrhea-sometimes bloody
- Vaccine failure
- Reproduction failure

Analysis

- Find out what mold is involved-target mycotoxin analysis- Schutter Plant Diagnostic lab
- Get a good representative sample
- Determine what part of ration will use questionable feed
- North Dakota State University-aflatoxin, Fumonisins, DON, 14 other trichothecenes, zeralenone, zeralenol $25-75/sample
- Romer Labs-Aflatoxins, full range of trichothecenes, zeralenone, zeralenol, ochratoxin A, sterimatocyctin, patulin, cyclopiazonic acid-mold counts and ID
- ELISA based kits-Arrow Scientific, SDI, etc-Aflatoxin, DON, Fumonisin, Ochratoxin A, T-2, zeralenone

Managing Mycotoxins

- Anticipate based on weather-Fusarium head blight,Ergot, Aflatoxin,Fusarium toxins from distant grain or oil seed sources
- Use resistant varieties where appropriate-corn, wheat
- Fungicides where appropriate-wheat/barley scab
- Clean grain (can often reduce DON, aflatoxin by ~50%)
- Store at proper moisture
- Watch moisture in storage-remember not average but individual seeds
- Avoid feeding to reproductive animals/young animals
- Sample-Sample-more the better
  - 12 truck loads-10 subsamples each: 0-230ppb aflatoxin
- Test when appropriate
  - Test kits
  - Analytical labs

Thank You & Happy Trails