Impact of Plant Disease on Malting

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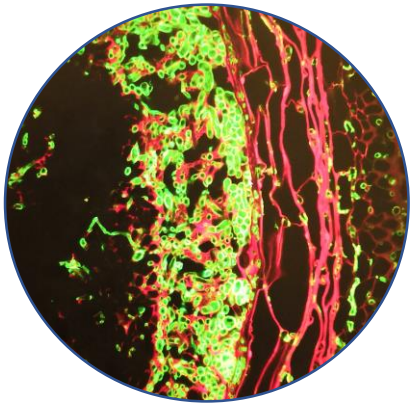


Diseases of Small Grains

- Disease can occur throughout the growing season, and damage can continue into storage and malting.
- Reductions in yield can occur from seed germination into grain fill and development.
- Reductions in grain quality occur after after pollination and into storage.

Discussion Topics

- General impact of disease on malting grain quality.
- Fungal Disease and Mycotoxins
  - Field Issues
    - ✓ Fusarium Head Blight
    - ✓ Ergot
  - Storage Issues
    - ✓ Aflatoxins
    - ✓ Ochratoxins

Diseases of Small Grains

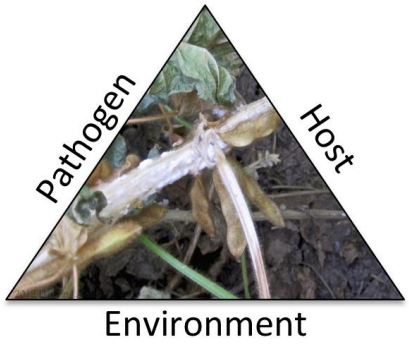
Disease Organism

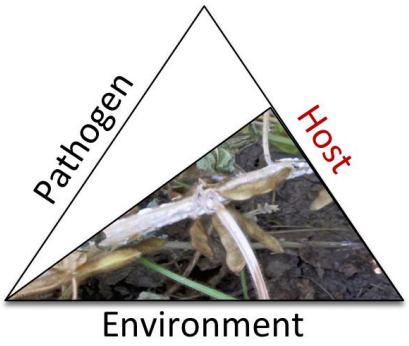
- Fungi
- Bacteria
- Virus
- Nematodes

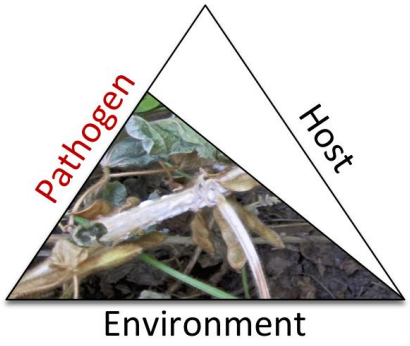
Disease Type

- Foliar Diseases
  - Leaf and crown rust
  - Powdery mildew
  - Spot and net Blotch
- Head disease
  - Fusarium Head Blight
  - Ergot
- Soilborne Diseases
  - Root rot
- Viral Diseases
  - Yellow dwarf
- Smut and Bunt Diseases

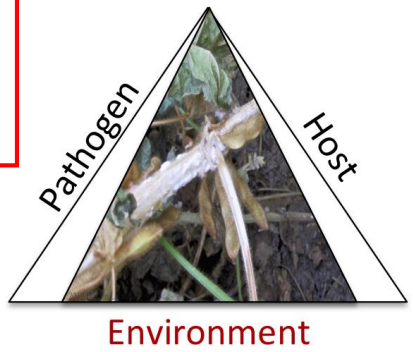
Plant Disease Triangle







- Disease only occurs if three things exist all at the same time
  - -Pathogen
  - -Host
  - -Environment
- Manipulating a component or combinations of triangle components influences the incidence and severity of disease



Field Crops Pathology

UW Extension University of Wisconsin–Extension

https://fyi.extension.wisc.edu/fieldcroppathology/field-crops-fungicide-information/

Impact of Disease on Small Grain Quality

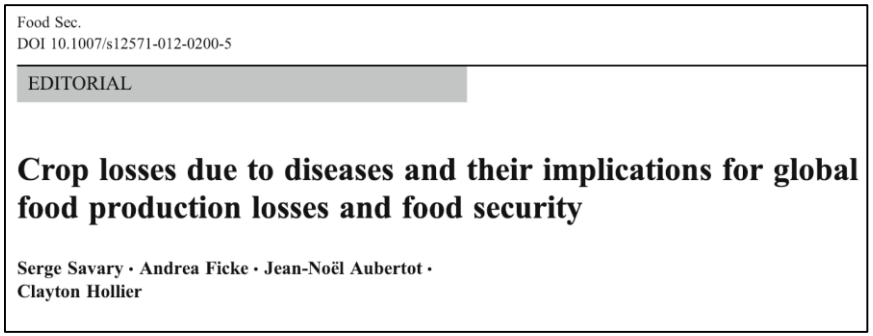
- In most cases it's a question of the disease "stressing" the plant
  - Yield impacts are common and direct
    - Can occur during throughout the season (planting to harvest)
  - Grain quality impacts are sometimes less direct:
    - Can occur from pollination to maturity

- Important are kernel/head diseases and infection in storage, where the quality and safety of otherwise normal grain can be directly impacted

How Plant Disease Causes Damage

1. Reduce plant number (stand reduction)
2. Reduce photosynthetic rate
3. Accelerates leaf senescence (death)
4. Compete for nutrients (assimilates)
5. Degrade and consume plant tissues
6. Reduce plant turgor

❖ also… Produce toxins



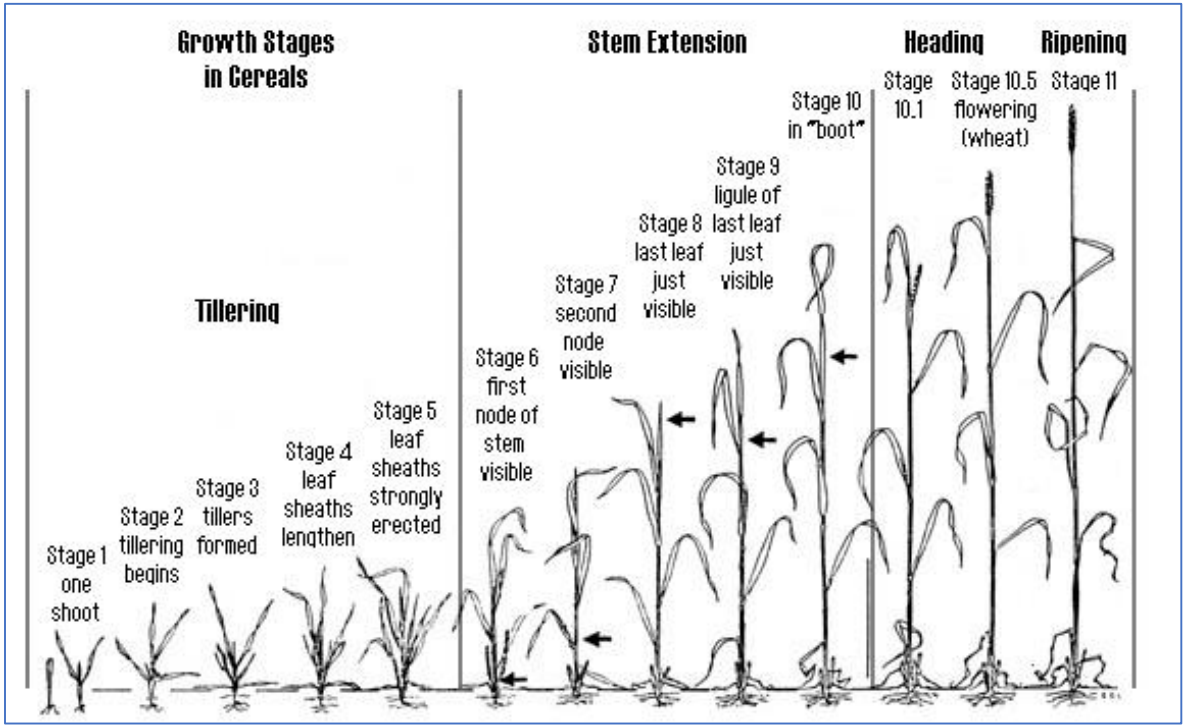
Food Sec.
DOI 10.1007/s12571-012-0200-5

EDITORIAL

Crop losses due to diseases and their implications for global food production losses and food security

Serge Savary · Andrea Ficke · Jean-Noël Aubertot · Clayton Hollier

Understanding the nature and timing of disease stress is important to understanding the outcome on yield and/or quality

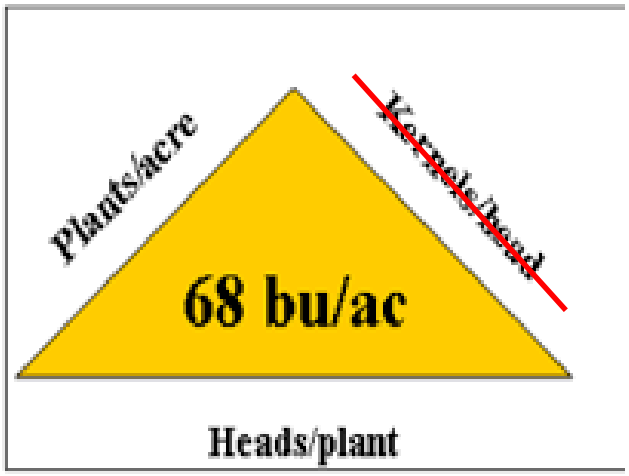


Cereal growth stages

U of Minnesota Spring Barley Guide: https://extension.umn.edu/growing-small-grains/spring-barley-growth-and-development-guide

Impact of Disease: Pre-Stem Elongation

- Stages: germination, seedling emergence, and tillering.
- Disease stress reduces number of plants and tillers.
- Impact at this time is on yield.



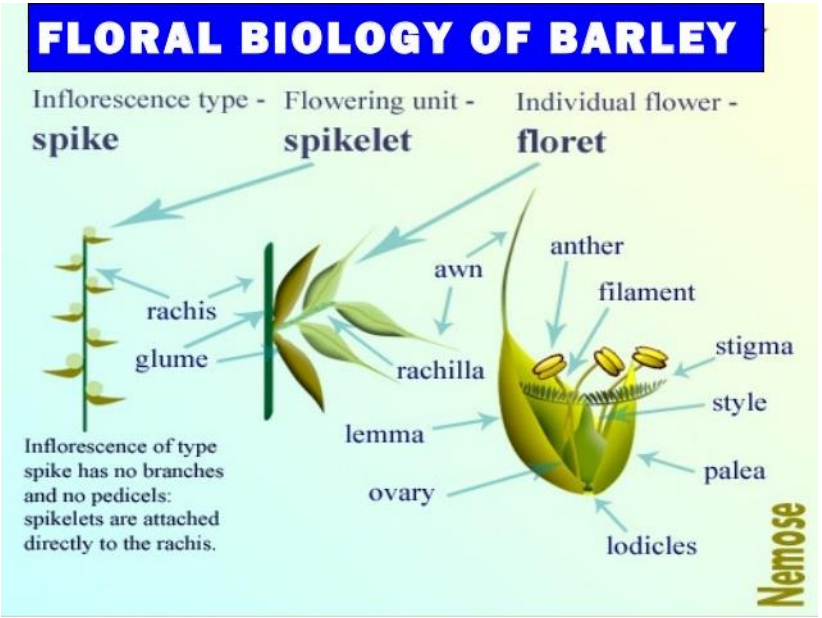
Impact of Stress: Stem Extension (jointing) to Pollination

- Disease impact is mainly on yield.
  - Plant death,
  - Reduced photosynthesis,
  - Reduced plant development.

http://www.ag.ndsu.nodak.edu/cropdisease/wheat/images/leaf5.jpg

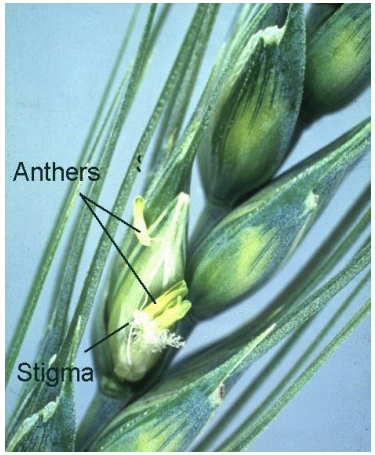
Pollination

- Pollination occurs 6-7 weeks following emergence.
  - Barley: just before or during emergence of head from the ”boot”
  - Wheat and rye: after head emergence

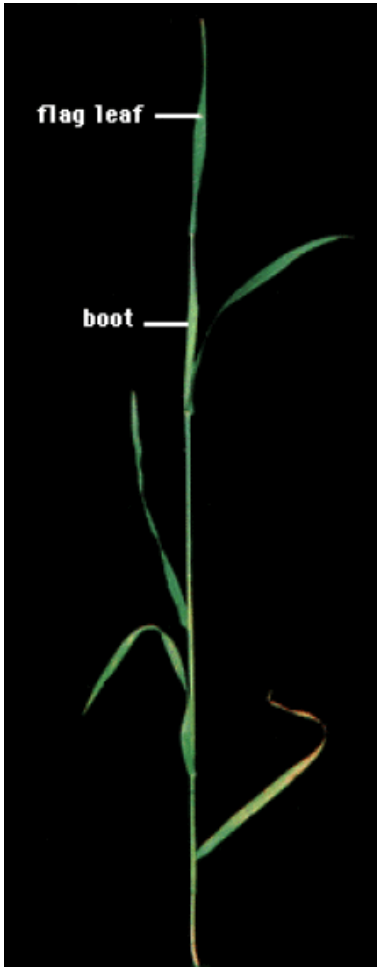


Impact of Stress: Pollination

- Disease at pollination can reduce:
  - kernel number through floret death.
  - Yield is reduced.



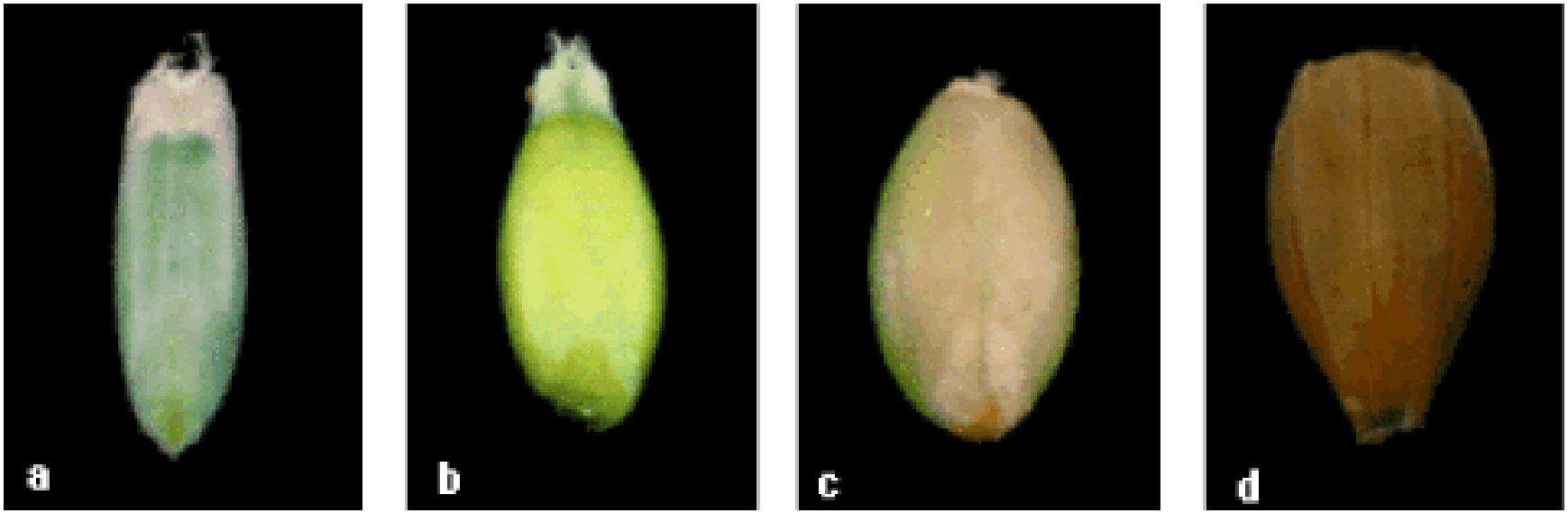
Flowering in wheat, after head emergence



Barley flowers in the boot, Before head emergence

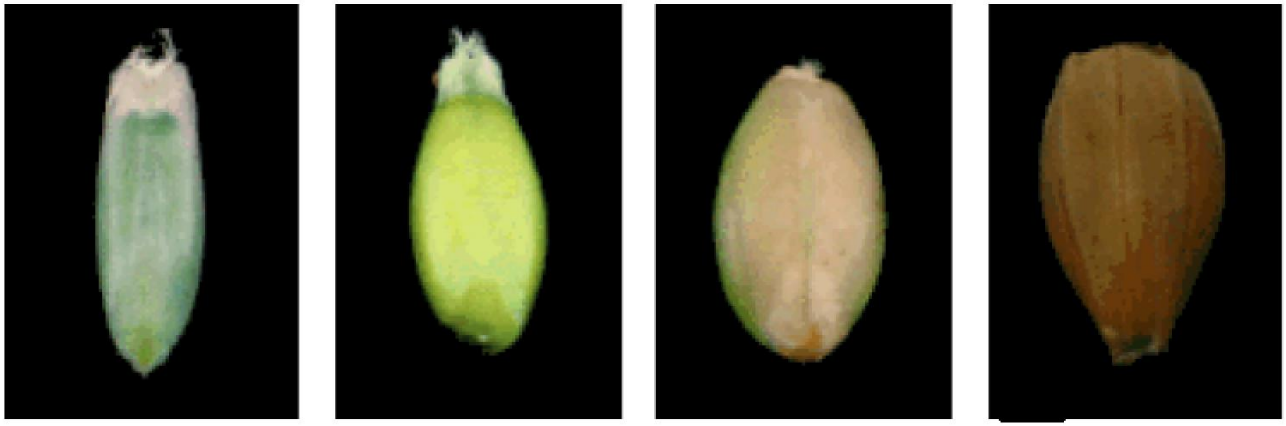
Kernel Development and Maturation

- Barley kernels begin to develop after head emergence and pollination.



https://extension.umn.edu/growing-small-grains/spring-barley-growth-and-development-guide#kernel-development-and-maturity-792765

Kernel Development Stages



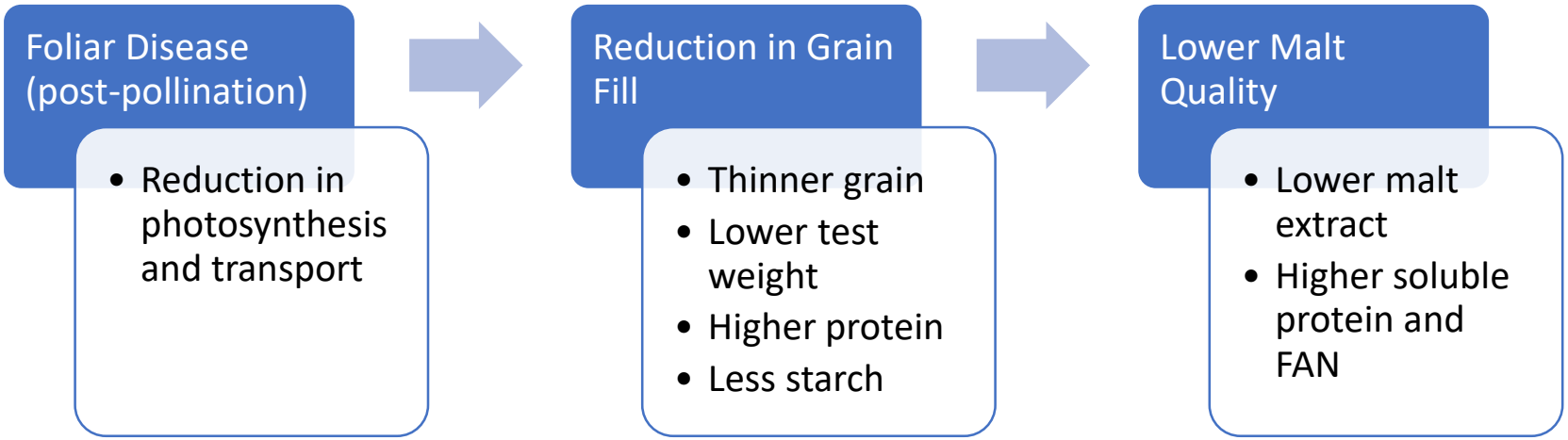
Watery ripe Late milk Hard dough Ripe

- **Watery ripe**: 10 days. Determines the number of cells formed in which starch is deposited.
- **Late-milk to soft dough**: 10 days. Rapid period of starch deposition.
- **Hard dough**: rapid loss of water.
- **Maturity (ripe):** 30-40% moisture: Loss of green color

Impact of Stress: Post-pollination

- Stress following pollination can reduce kernel development and grain fill.
  - Low test weight and 1000 kernel weight
  - Thin grain (low plump)
  - Often higher protein
- Impacts are on both:
  - Grain yield (lower kernel weight)
  - Grain quality

Example of Disease Impact on Malt Quality



Spike and Kernel Diseases:

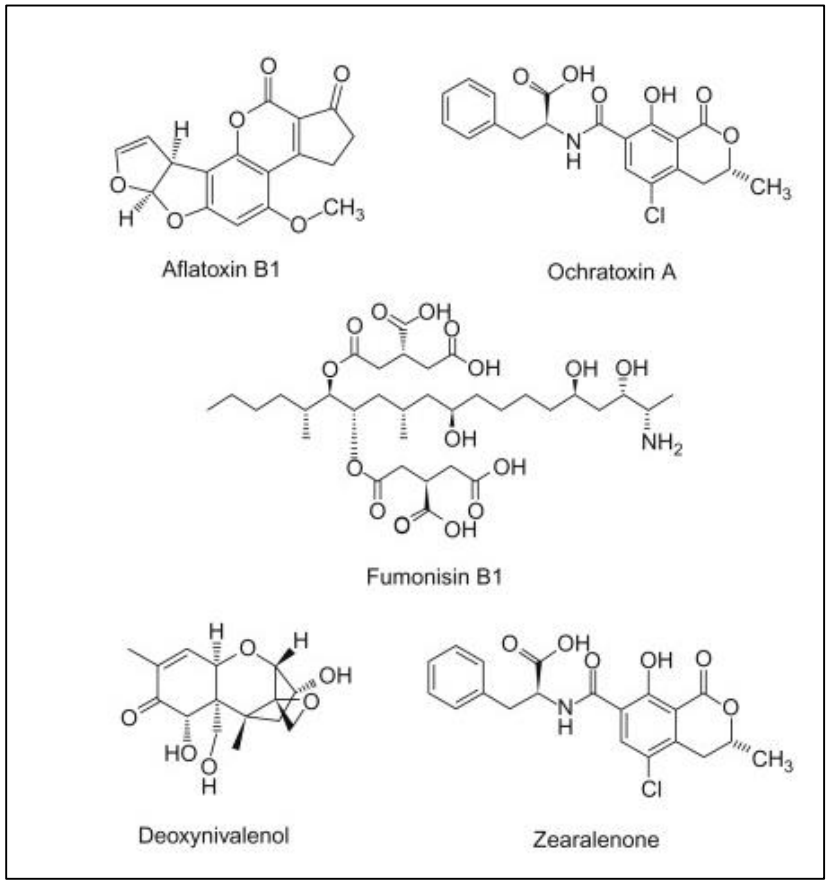
Potential impacts on processing quality and safety

- Diseases prior to harvest:
  - Fusarium Head Blight (Scab)
  - Black Point
  - Ergot
  - Smut
- Post-harvest
  - Growth of toxigenic fungi in improperly stored grain

Loose smut
https://www.agric.wa.gov.au/mycrop/diagnosing-barley-loose-smut

Fungal Pathogens and Mycotoxins

- Mycotoxins are secondary metabolites produced by a variety of fungal genera, which are capable of causing disease and death in humans and livestock.
- They can be produced in the field, storage, and in some cases, during malting



https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/mycotoxins

Fungal Pathogens and Mycotoxins

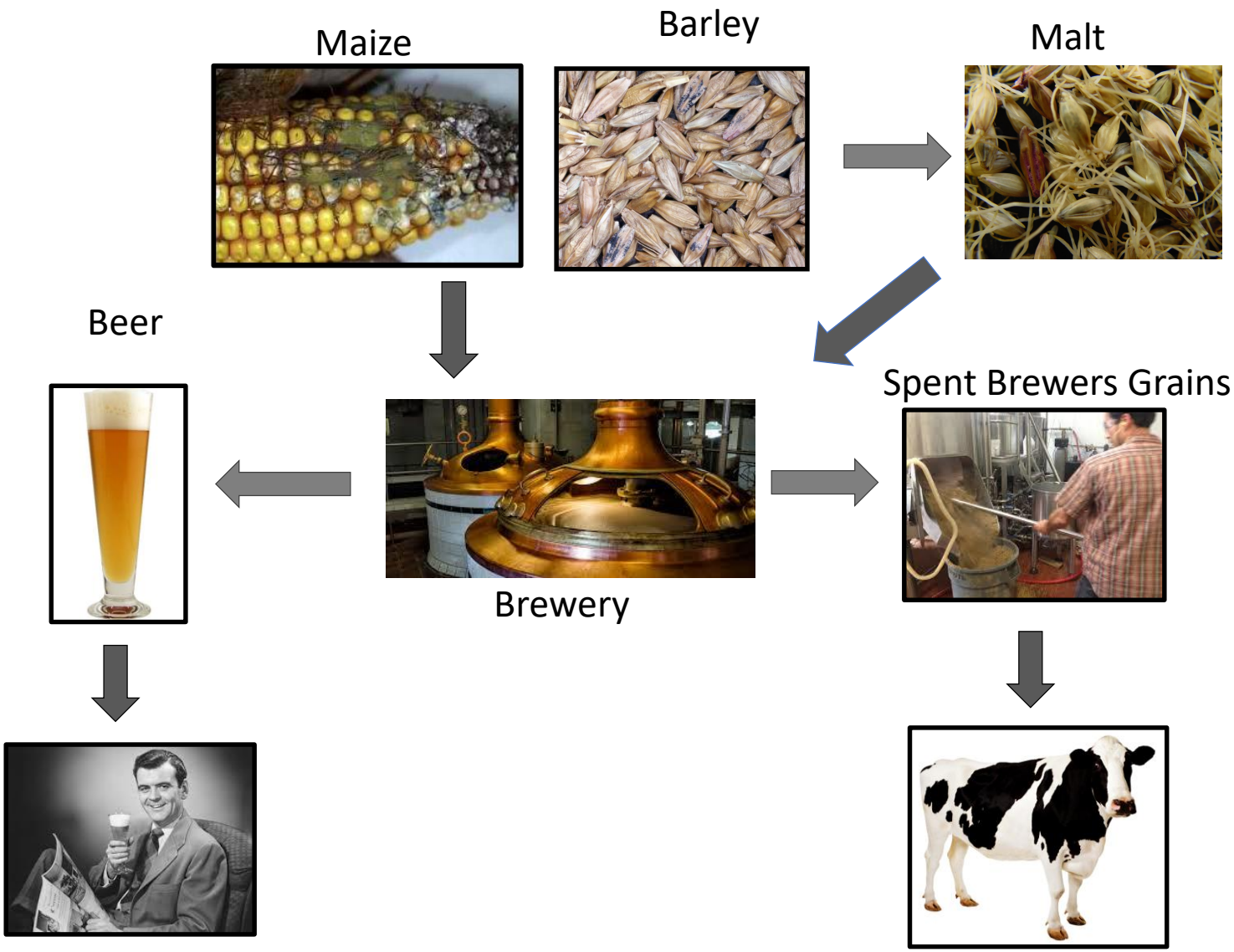
- DON, zearalenone, ergot alkaloids, alternariol, fumonisins and ochratoxin have all been detected in beer.
- Mycotoxins pose a “food safety” risk, and the levels of several are regulated.
  - Chronic exposure
- Fungal infection can also damage, grain, malt and beer quality

Beer gushing caused by infection with *Fusarium*

Summary of the major mycotoxins, associated fungi, and their physiological effects

Mycotoxin	Fungi	Toxicity	Presence on Malting Grain
Aflatoxin	*Aspergillus flavus* and *A. parasiticus*	Carcinogenic, hepatotoxic, immune suppression	Uncommon with barley and wheat. Common on maize
Fumonisins	*Fusarium verticillioides* and *F. proliferatum*	Carcinogenic, hepatotoxic	Uncommon with barley. Common on maize
Ochratoxins	*Aspergillus ochraceus, A. carbonarius* and *Penicillium verrucosum*	Carcinogenic, nephrotoxic, hepatotoxic, teratogenic	Does occur on barley and wheat, but has not been frequently tested in USA
Trichothecenes (DON)	*Fusarium. sporotrichioides, F. graminearum, F. culmorum, F. roseum, F. tricinctum, F. acuminatum*	Gastrointestinal hemorrhaging, immunodepressant	Very Common
Zearalenone	*Fusarium graminearum*	Estrogenic activity	Commonly seen with DON

Possible Flow of Mycotoxins in Malting and Brewing



Fusarium Head Blight (Scab)

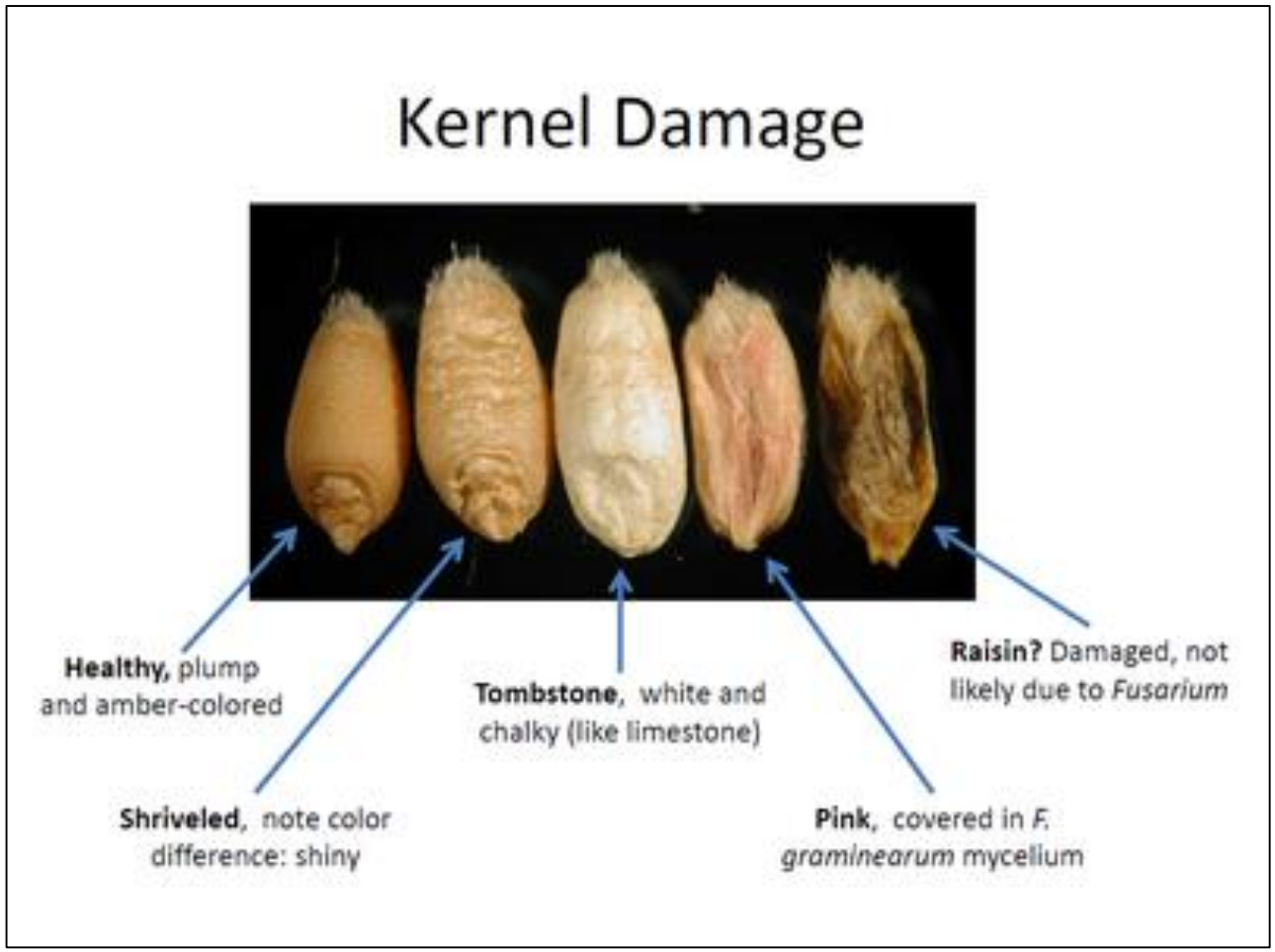
- FHB is the most problematic disease in terms of actual malt quality of the grain on a worldwide basis.
- Barley, wheat, rye
- Potential impacts on:
  - Grain yield
  - Grain quality
  - Malting performance
  - Beer quality
  - Food safety

Symptoms of FHB-Wheat

- The first symptoms of FHB occur after flowering.
- Infected spikelets generally show premature bleaching .
- This premature bleaching may progress (spread) throughout the entire spike.
- Kernels that are infected during later grain development may not appear to be affected, but can still be contaminated with mycotoxins.

www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Fusarium.aspx

Symptoms of FHB-Wheat



http://agcrops.osu.edu/corn/newsletters/2015/2015-21/rating-fusarium-damages-kernels-fdk-in-scabby-wheat

Symptoms of FHB-Barley

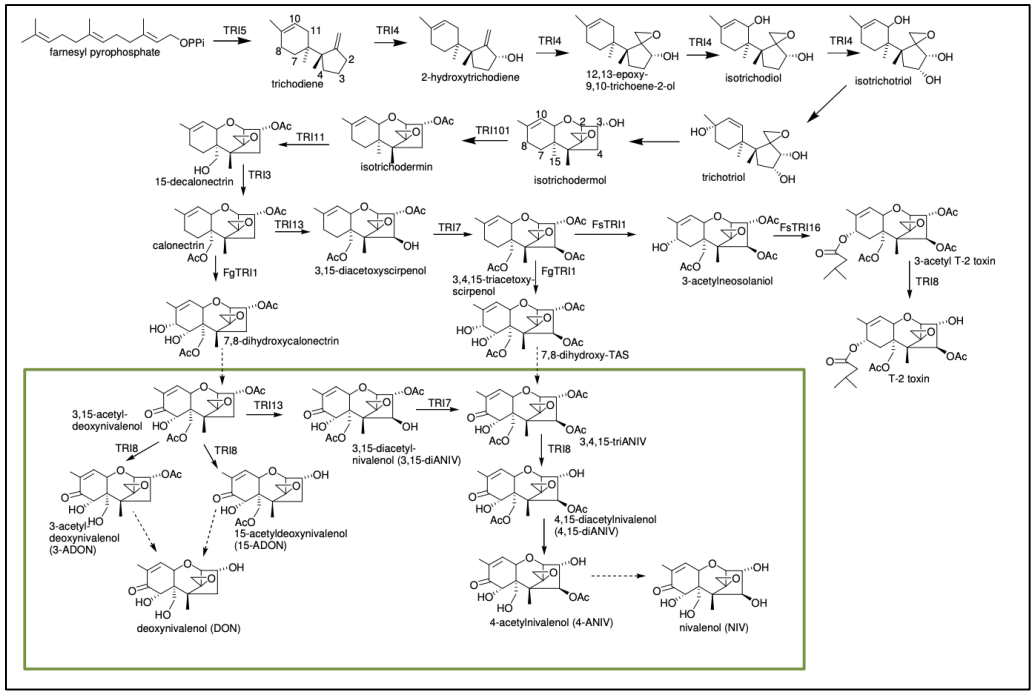
Barley is different than wheat!

- Barley flowers while the spike is still in the "boot" and is not as impacted by FHB at this growth stage
  - Infection of barley is not the same as it is for wheat or rye.
  - However, barley can be infected by FHB anytime from spike emergence to harvest.
- Shriveled, low test-weight kernels in barley are not as common as for wheat.
- Symptoms are not always visible.
- Unlike wheat, infection does not spread (⬇⬆ in the spike).

Symptoms of FHB-Barley

Fusarium Mycotoxins

Trichothecene Mycotoxins

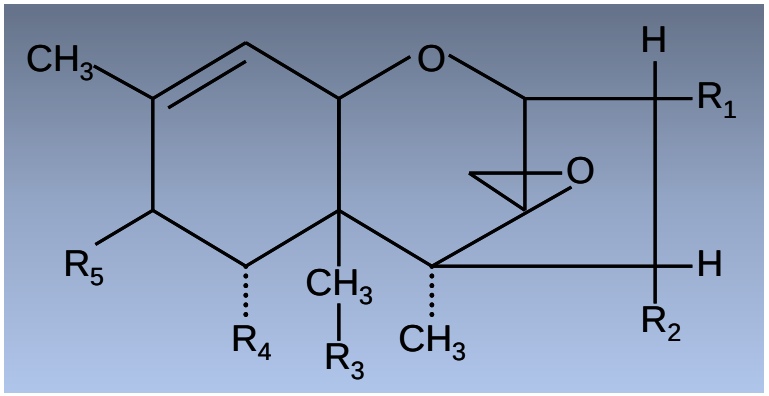


- Toxic secondary metabolites (>200)
- Protein synthesis inhibitors
- Human and animal toxicoses
  - Gut
  - Immune system

Toxins 2011, 3, 802-814; doi:10.3390/toxins3070802

FHB Produces Several Trichothecenes

Specific toxins depend on pathogen



		R1	R2	R 3	R4	R5
Deoxynivalenol	DON	OH	H	OH	OH	=O
3-Acetyldeoxynivalenol	3-ADON	OAc	H	OH	OH	=O
15-Acetyldeoxynivalenol	15-ADON	OH	H	OAc	OH	=O
Nivalenol	NIV	OH	OH	OH	OH	=O
Fusarenon X	FX	OH	OAc	OH	OH	=O

Tolerable Daily Intake (DON) is Based on Growth Suppression in Mice

FDA	EFSA
Guidance Limit	Maximum Level
1 ppm Processed Products	0.2- 1.25 ppm Processed to Unprocessed Grain Products
No Tolerable Daily Intake	TDI = NOEL x Uncertainty Factor TDI = 100 μg/kg bw x (1/10) x (1/10) = 1 μg/kg bw
No Published Risk Assessment	Based on Iverson et. al (1995) and JECFA TDI with X Uncertainty Factor

Canady et. al. 2001. JECFA47 Report.
FDA. 2010. http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/NaturalToxins/UCM217558.pdf

FHB and Malting and Brewing: Historical Perspective

- Brewers have long recognized it is best to “avoid moldy grain”
- FHB recognized as a disease by the late 1800’s to early 1900’s.
- Moldy barley recognized as a cause of “beer gushing” as early as 1938.
- Chemical identification of DON in 1973.
- Regulations: 1990s

Fusarium and Mycotoxins in Malting and Brewing

Key Concerns:

- DON is transferred from grain to malt to beer
  - Worldwide surveys show DON is commonly present in beer, albeit at low (ppb) levels
- *Fusarium* can grow during malting and damage grain components and reduce malt quality.
- *Fusarium* produces metabolites that can cause gushing in bottled beer.

Fusarium and Mycotoxins in Brewing

Key Concerns:

- DON in beer
  - Safety concern
  - Public perception
- **Beer Gushing**
  - Spontaneous over-foaming of bottled beer upon opening (loss of a few milliliters to half the bottle)
  - Caused by *Fusarium*, as well as species of *Rhizopus, Aspergillus, Alternaria* and *Penicillium*

Fusarium and Mycotoxins in Brewing

DON in Beer

- There have been many surveys of beer for DON.
  - Varga et al (2013) analyzed 374 beer samples from 38 countries, and detected DON and DON3G in 77% and 93% of all beers.
  - The average concentrations of DON and DON-3-G were 8.4 ppb and 6.9 ppb, respectively.
  - Maximum levels were < 90 ppb.
  - The above levels in beer suggest malts with <0.1 ppm DON were generally used. Below the LOQ for most commercial test labs.

Gushing

Fusarium Head Blight and Beer Gushing

- Toxin does not cause gushing
  - Cyclic peptides
  - Hydrophobins (Sarlin et al 2012)
- Gushing propensity increases with degree of FHB infection
- Gushing factors are very sensitive to dilution:
  - Gushing is less of a problem in adjunct beers
  - Gushing will be more of a concern in 100% malt beers
  - Malt from infected wheat seems to be most problematic

Fusarium and Mycotoxins in Malting

Fusarium on barley: symptoms of FHB are not always visible

Fusarium and Mycotoxins in Malting

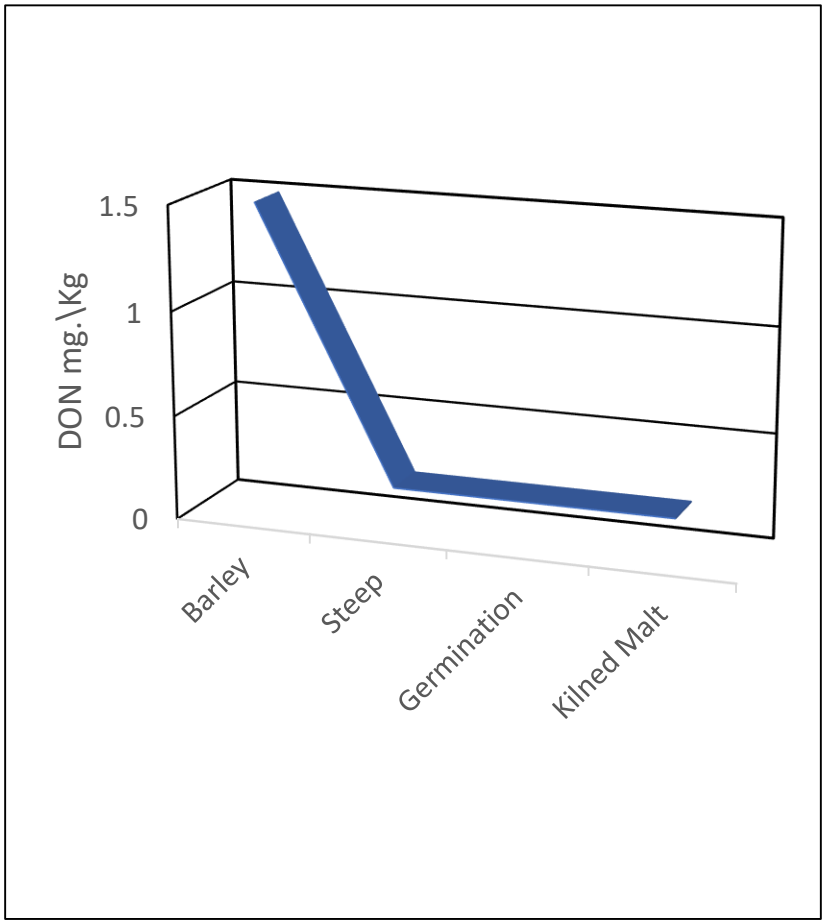
- Malting conditions provide an ideal environment for the growth of fungi.
- Growth of *Fusarium* and DON production are possible (Schwarz et al 1995)
  - DON on malt is largely transferred to beer
- Viability of *Fusarium* declines with grain storage (Beattie 1998).

Fusarium and Mycotoxins in Malting

Fusarium on germinating barley

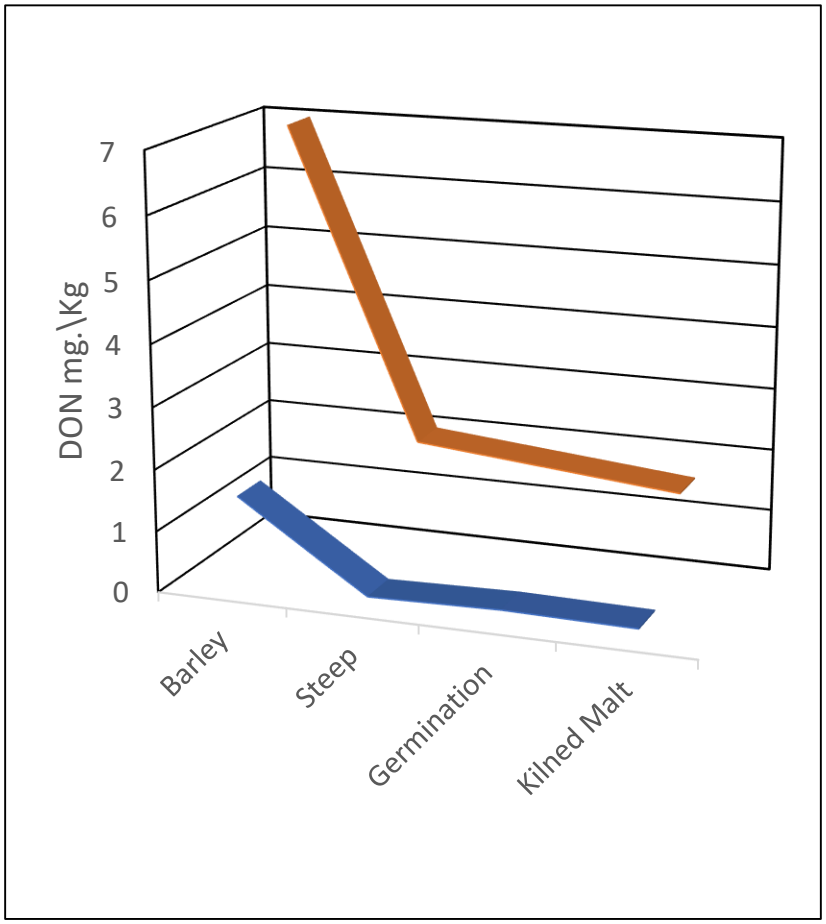
Fusarium and Mycotoxins in Malting

- The normal pattern is to see DON decrease in the steep, and remain low on the finished malt.
- DON is solubilized or DON/Fusarium are rinsed off with dust, etc.
- Fusarium is no longer viable.
- Malt DON<Barley DON



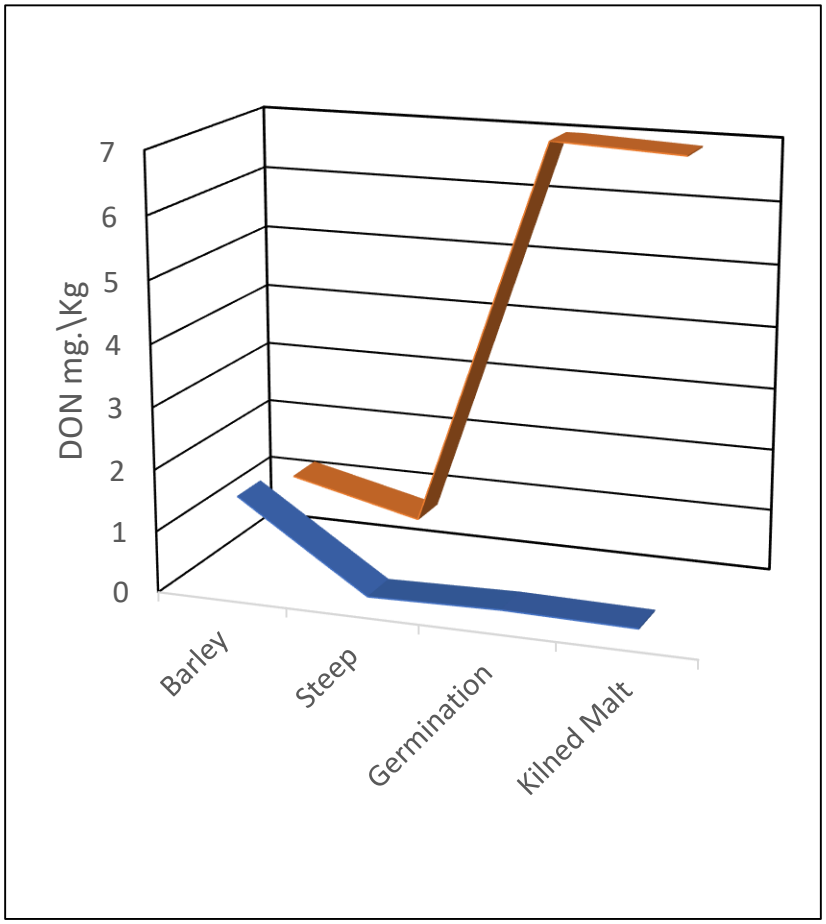
Fusarium and Mycotoxins in Malting

- However, this only works to a point.
- DON levels in more heavily infected samples generally will not be reduced to satisfactory levels by steeping.



Fusarium and Mycotoxins in Malting

- In some cases, the Fusarium will grow during malting.
  - Additional DON is produced
- The finished malt can contain significant levels of DON Malt DON>Barley DON
- This is a problem



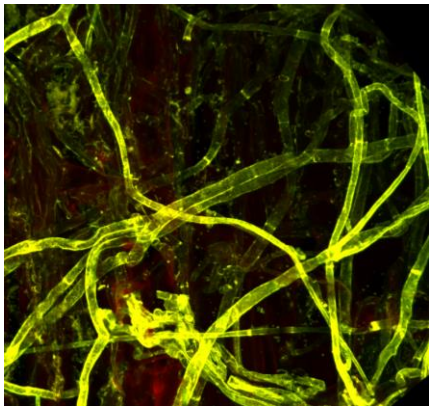
Summary: Fusarium and Mycotoxins in Malting

- Exact behavior in malting is difficult to predict
- DON production seems to be more pronounced in wheat, rye, and triticale
- Barley DON is a poor predictor of malt DON
- In general, *Fusarium viability* will decline with grain storage. *But not always……why?*

Fusarium infected wheat malt

Why do we see differences in DON production in malting?

- Pathogen
- Environment
- Grain type and structure
- Timing of infection
  - Early (pollination-heading)
  - Late (grain maturation)
- Other?



Dr. Zhao Jin, NDSU Plant Sciences
Characterization of Trichothecene Mycotoxin Development During the Malting of Fusarium Infected Barley.

Funded by American Society of Brewing Chemists

Why do we see differences in DON production in malting?

Observations

- Pronounced increases in DON are seen more often with wheat and rye
  - Husk threshes free
    - Barley retains husk
  - Flower after heading
    - Barley is before heading
  - Wheat is more prone to internal infection, while barley is external?

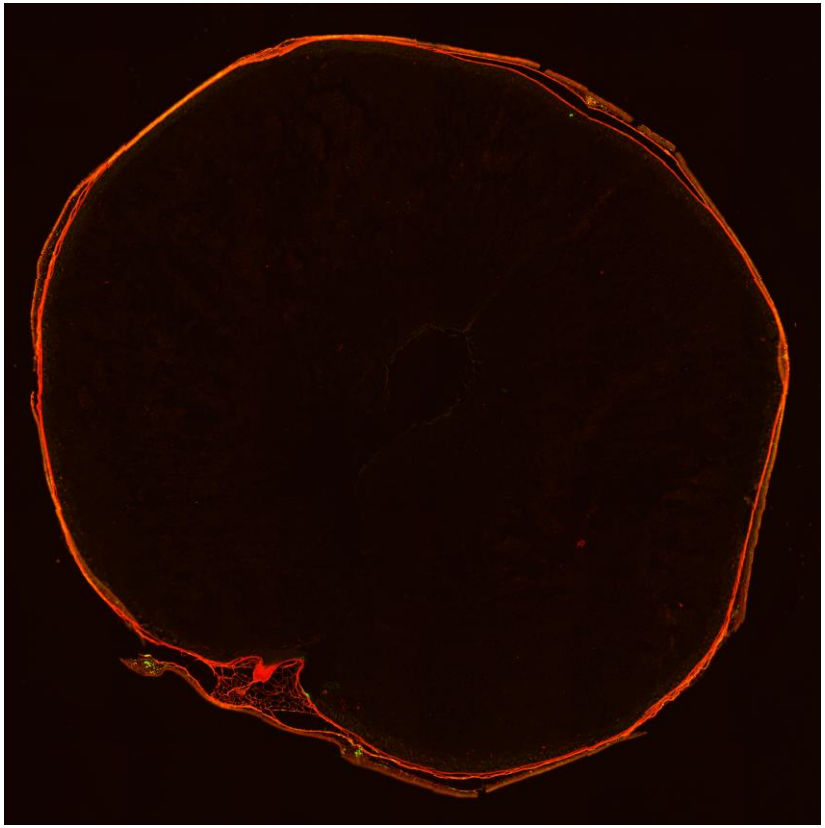
Methods

- confocal laser scanning microscopy
  - Fluorescent dye for fungi
- Scanning electron microscopy (SEM)

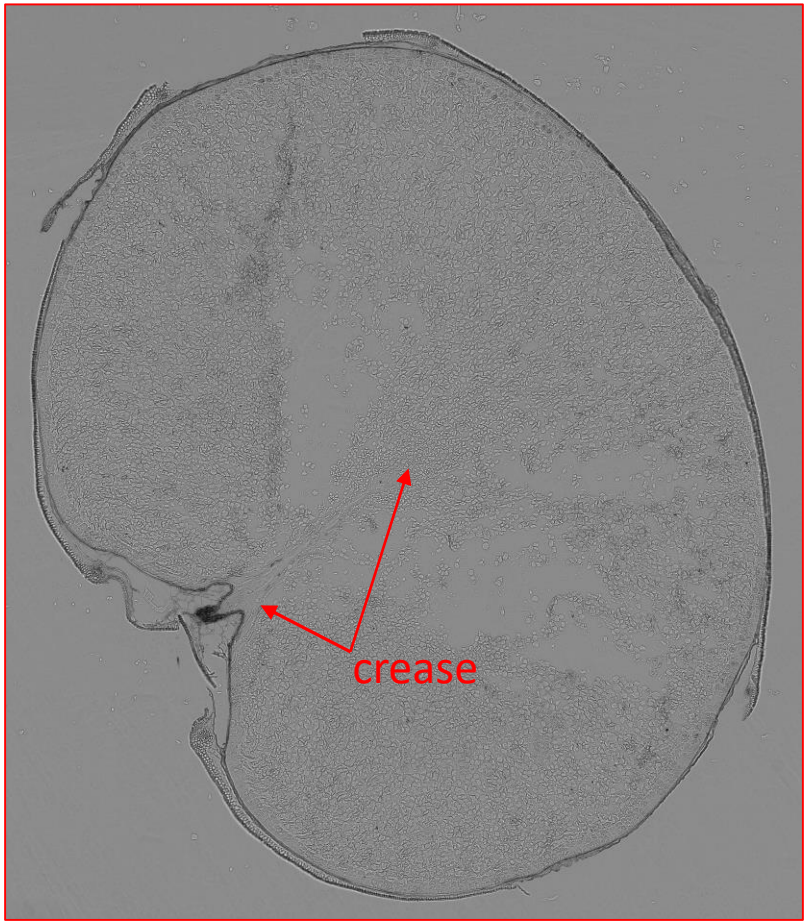
Materials

- Wheat, rye and triticale samples showing large increases in DON following malting (e.g. 1 to 40 ppm)
- Barley from 2016 Canada crops with abnormal DON production in malting

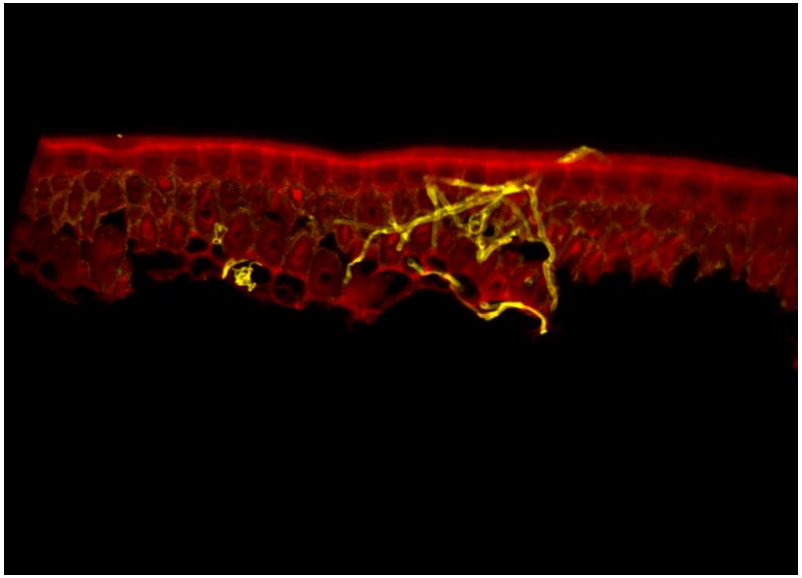
Barley (cross section)

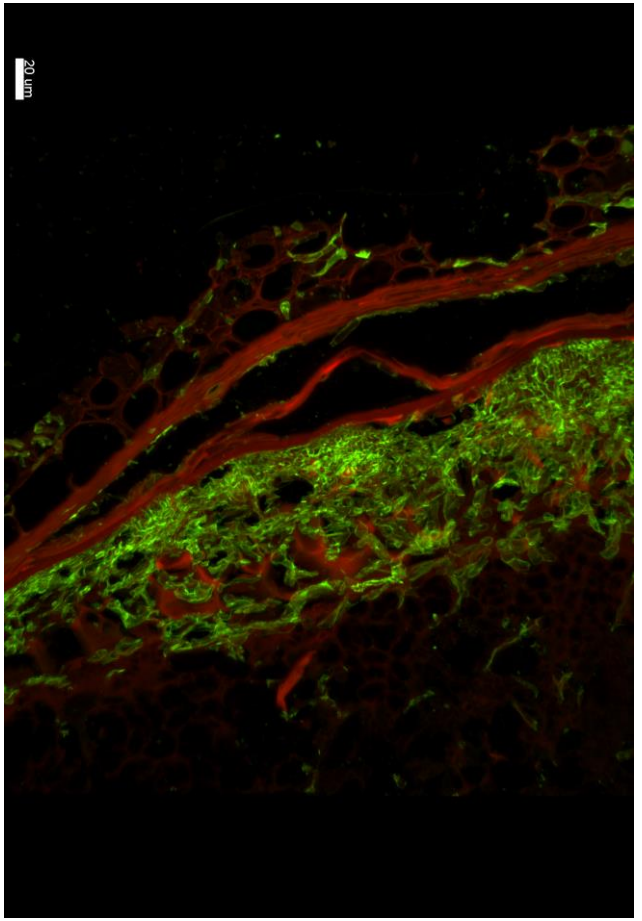


Confocal Microscopy. Husk and pericarp tissues are red. Fungi:green



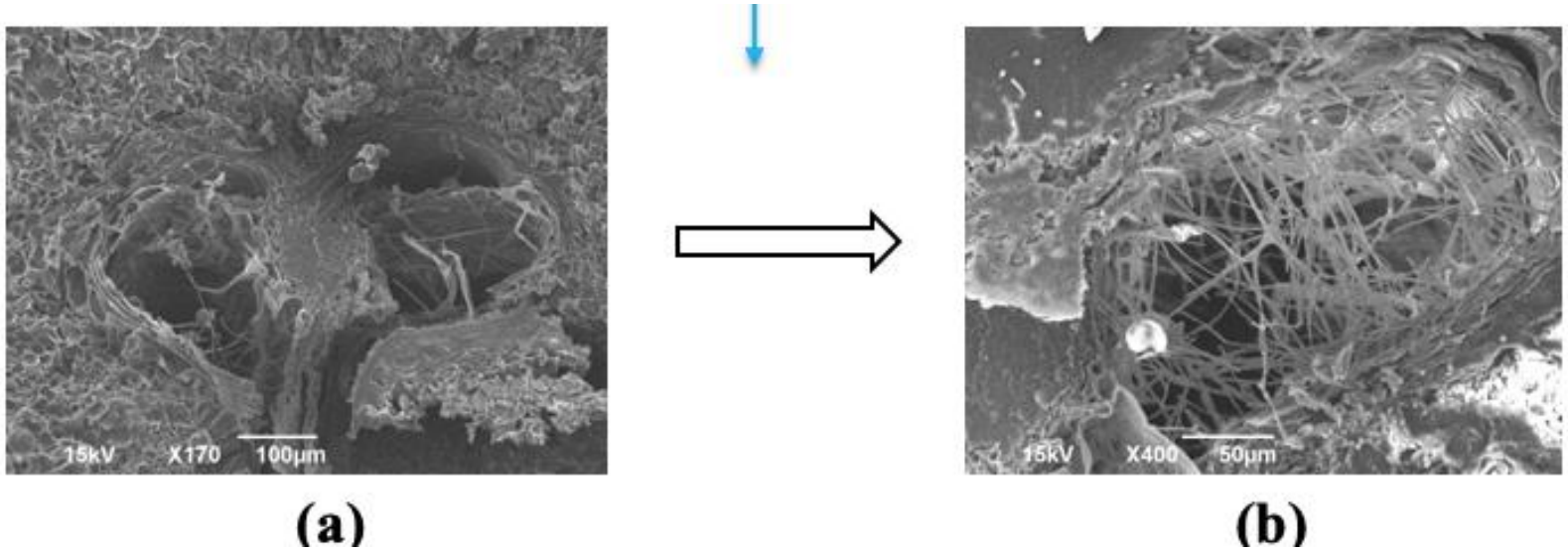
Fungi in barley and malt





Fungal infection is present in barley husk (left), but increases greatly following malting (right).

Mold in the Crease??

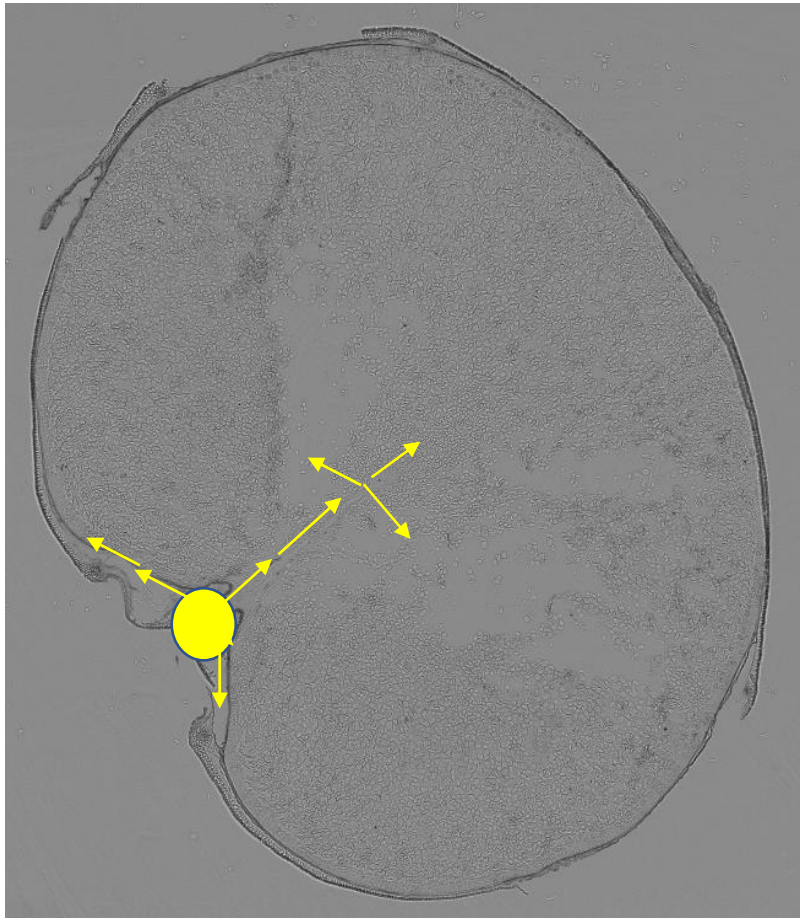


- SEM of Fusarium infected triticale. Fungal hyphae are visible in the pocket surrounding the pigment strand. Following malting this area was full of hyphae

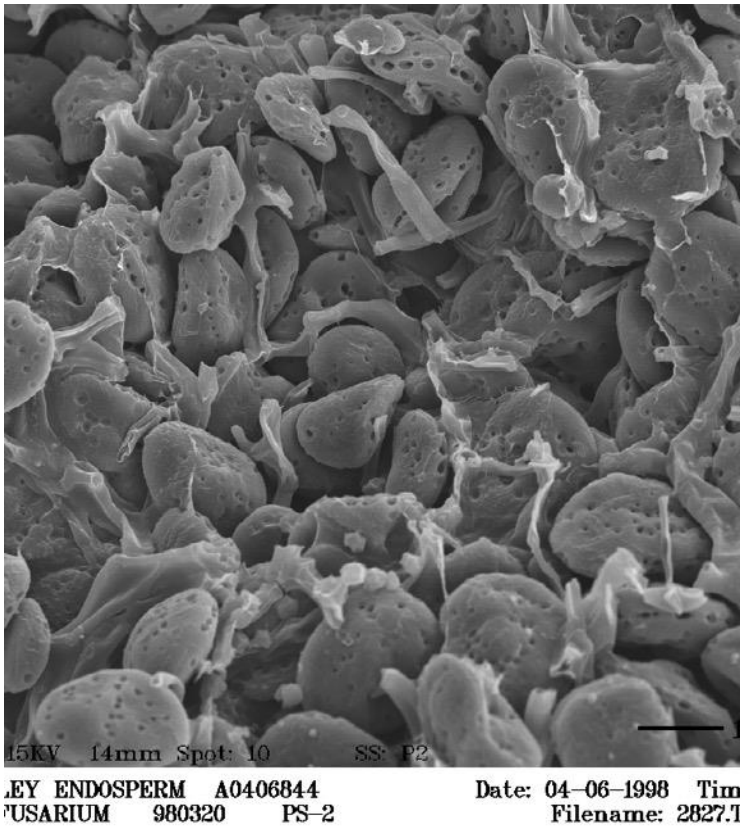
Fusarium Growth in “Problematic” samples

We propose:

- Fusarium can occur within husk/pericarp tissues.
- During malting the fungi continues to colonize the husk, but also grow inward into the crease
- It may grow outward into the endosperm near the pigment strand



FHB and Grain Quality

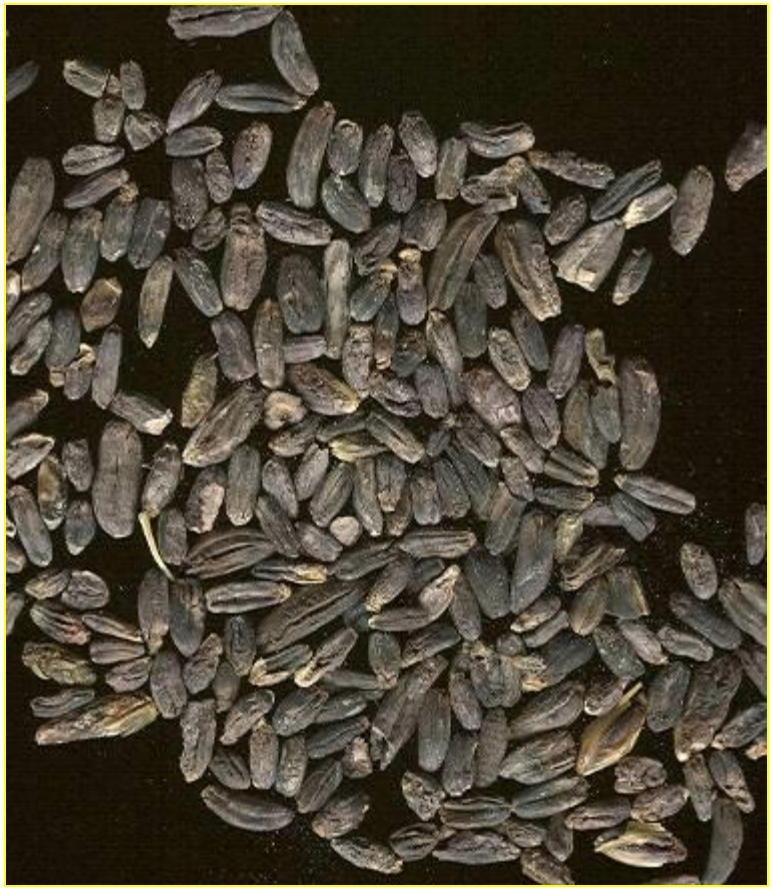


FHB Infected barley endosperm. Protein has been degraded. Starch shows extensive damage

- Heavy infection/colonization
- Extensive changes in malt protein properties
- Fusarium enzymes
  - Proteases
  - Xylanase
- Other metabolites
  - Beer gushing

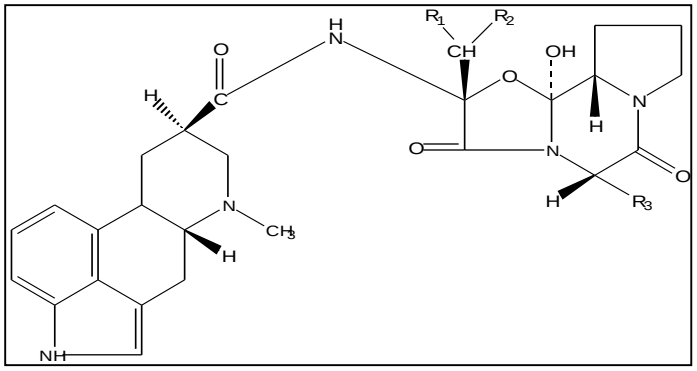
Ergot of Cereal Grains

Ergot in barley

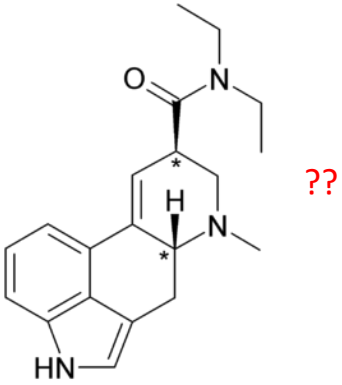


Ergot sclerotia from HRS wheat

Ergot of Cereal Grains



An ergot alkaloid



??

- Caused by *Claviceps purpurea*
- Infects developing floret and replaces kernel with a sclerotium
- Occurs in barley, wheat and rye
- Sclerotia contain amide derivatives of lysergic acid and ergot alkaloids
  - Alkaloids restrict blood flow and cause gangrene.
  - Simple amides cause convulsions and hallucinations.

Ergot of Cereal Grains

The Beggars, Pieter Bruegel, 1568

Believed to depict the results of gangrenous ergotism, resulting from consumption of contaminated grain

- Ergot was the the cause of mass mycotoxicosis in Europe in the middle ages.
  - Mainly a livestock problem in current times.
- Ergot has been associated with “witchcraft and witch trials”
  - Beer was cited as a cause in Northern Norway (17th century).
- Today, ergot is strictly regulated in the grain trade.
  - Believed to be removed by cleaning?
- Limited transfer to beer.
  - Spent grain?

Storage Issues

Grain Storage and Quality

- Poor grain storage can result in:
  - Rapid loss of germination
  - Insect and rodent problems
  - Develop of some fungi and the production of mycotoxins.
- Controlling moisture and temperature (humidity) are key considerations

Corn stored in outdoor piles and exposed to precipitation, may result in the development of grain molds and mycotoxins. Univ. of Nebraska: https://cropwatch.unl.edu/monitor-stored-standing-corn-grain-mold-diseases

Avoid Poor Storage Conditions!!!

Photo: Alan Salter, Busch Ag. Resources, LLC

Grain Storage and Germination

- Grain needs to be maintained at proper moisture (<12%) or germination can be lost.
- Grain bin must be monitored (aerated) to maintain safe conditions.
  - For example: germination can be lost in less than 2 months when storing at 16% moisture and 70°F.
- Problems are exacerbated in situations with no aeration (e.g. super sacks and grain bags).

Estimated Allowable Storage Time for Malting Barley (days)

(Criterion: Germinability)

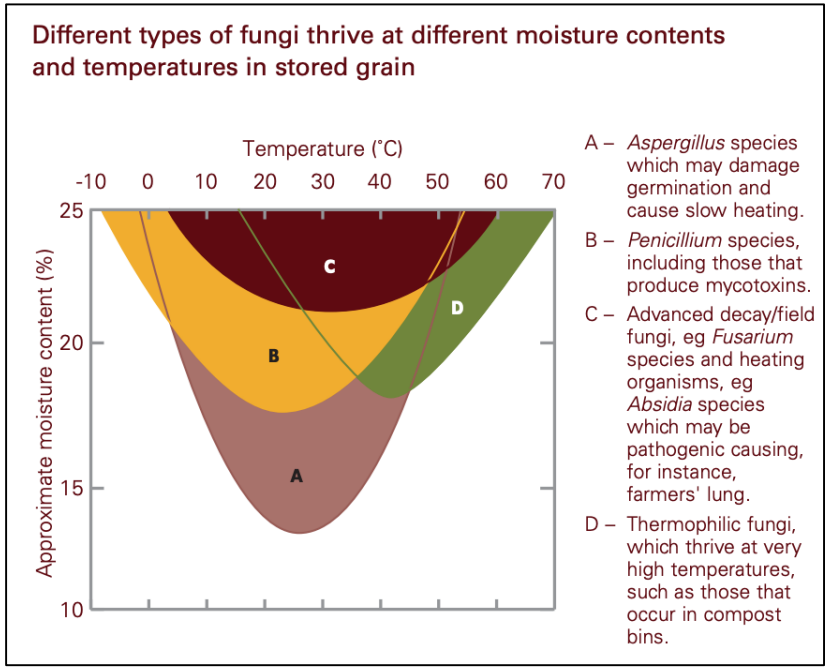
	11%	12%	13%	14%	15%	16%	17%	18%	19%
80 F	230	175	115	70	40	20	11	9	6
70 F	560	420	270	175	100	50	30	20	15
60 F	*	*	660	430	260	130	65	45	25
50 F	*	*	*	*	630	350	140	100	60

* Allowable storage time exceeds 700 days.

https://www.ag.ndsu.edu/graindrying/documents/BarleyAST.pdf

Mycotoxins in Storage

- Fungal growth in stored grain is optimal around 80ºF, 70% relative humidity and grain moistures above 14.5%
- Fungal growth can result in formation of several very serious toxins
- Dry grain to safe moisture after harvest, and maintain storage (e.g. aeration) throughout the seasons.

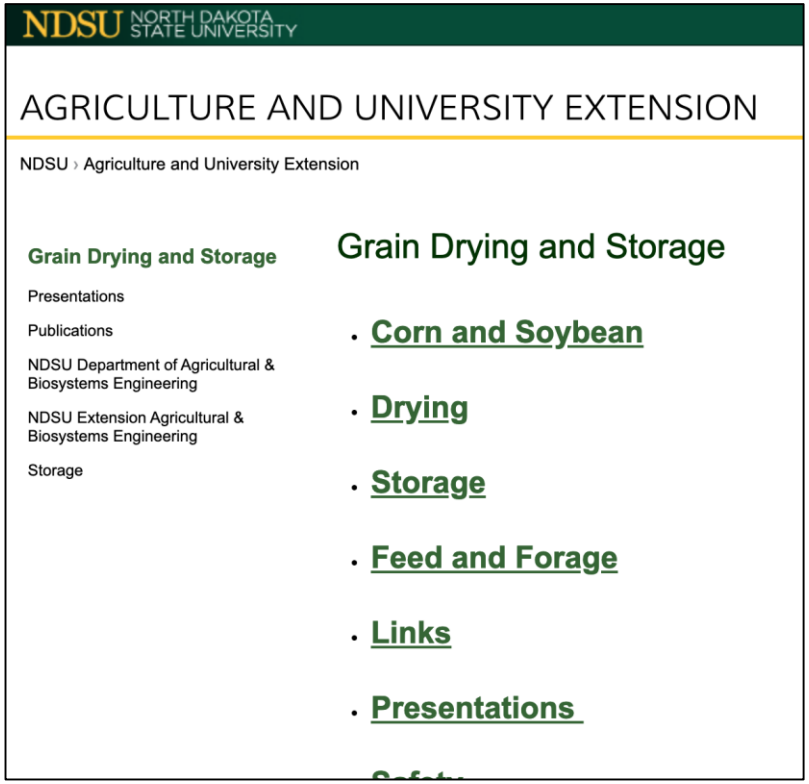


https://ahdb.org.uk/knowledge-library/grain-storage-guide

Grain Drying and Storage Resources

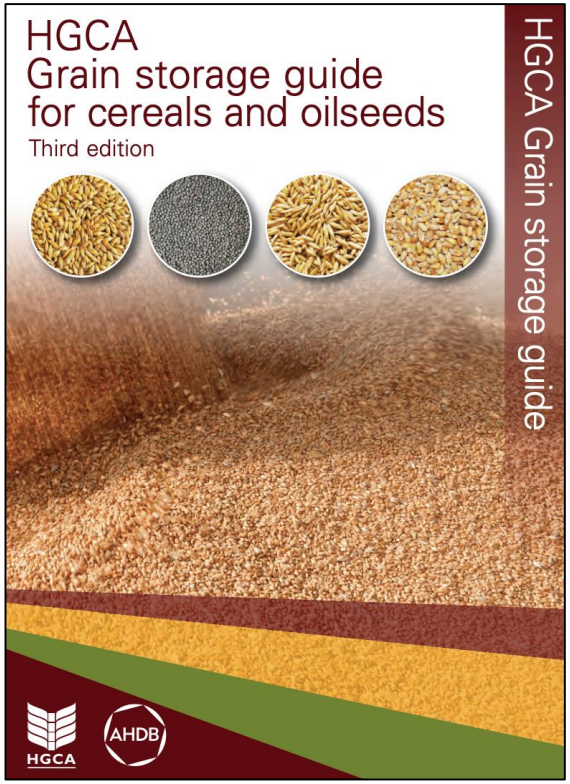
NDSU and Dr. Ken Hellevang

https://www.ag.ndsu.edu/graindrying



Home Grown Cereals Authority (UK) HACCP approach

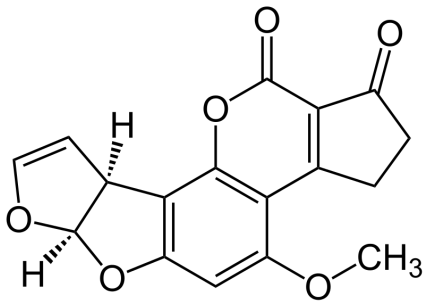
https://ahdb.org.uk/knowledge-library/grain-storage-guide



Aflatoxins

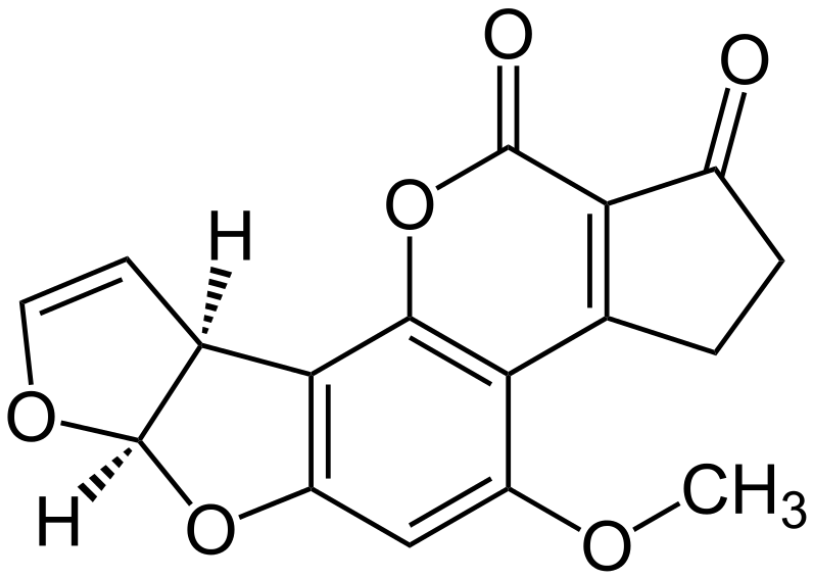
- Produced by *Aspergillus flavus* and *A. parasiticus.*
- Fungal contamination occurs in field, but mycotoxin production is generally in storage.
- More common with maize, groundnuts.
- Uncoomon, but has been reported on barley in Spain and South Africa
  - Detected in indigenous African beers

http://www.pkdiet.com/diet_aflatoxin.php



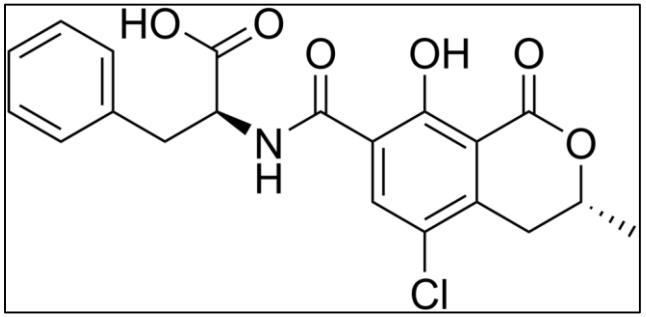
Aflatoxins

- Aflatoxin B1 (most toxic), B2, G1 and G2.
- Exposure has been linked to liver cancer.
- Maximum legal level in food (USA): 20 ppb.
- High thermal stability
- Limited water solubility
  - Spent grain, DDGS?



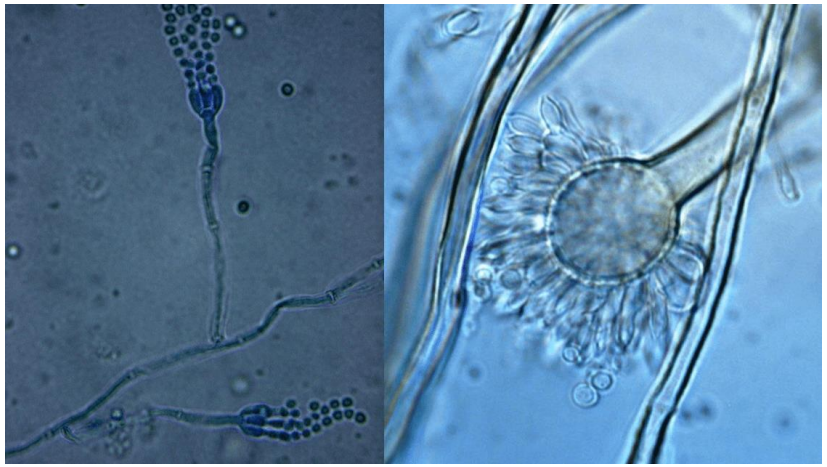
Ochratoxins

- Mycotoxins produced by *Aspergillus ochraceus* and *Penicillium verrucosum* and *P. carbonarius* .
- Carcinogen, immunosuppression, immunotoxicity and neurotoxicity
- Ochratoxin A is the most prevalent and relevant fungal toxin of this group (also B and C)
  - EU limit is 5 ppb in unprocessed grain
  - Main Concern with stored grain in the UK



Ochratoxins

- *P. verrucosum* is the main organism associated with cereal grains.
- Has been reported in barley, maize and and wheat.
  - Has been detected in beer
- Normally becomes problematic in storage, and generally doesn't occur before harvest.



https://i2.wp.com/www.fungusfactfriday.com/wp-content/uploads/2017/12/OTA-molds.png

Take-home message on DON and other mycotoxins in malting

- Behavior in malting is difficult to predict.
- Problems with wheat and rye can be more pronounced.
- Avoid highly contaminated grain!
- Don’t malt too soon after harvest
- When dealing with FHB infected grain, be sure to have the malt tested for DON.
- More serious problems can develop with poorly stored grain.