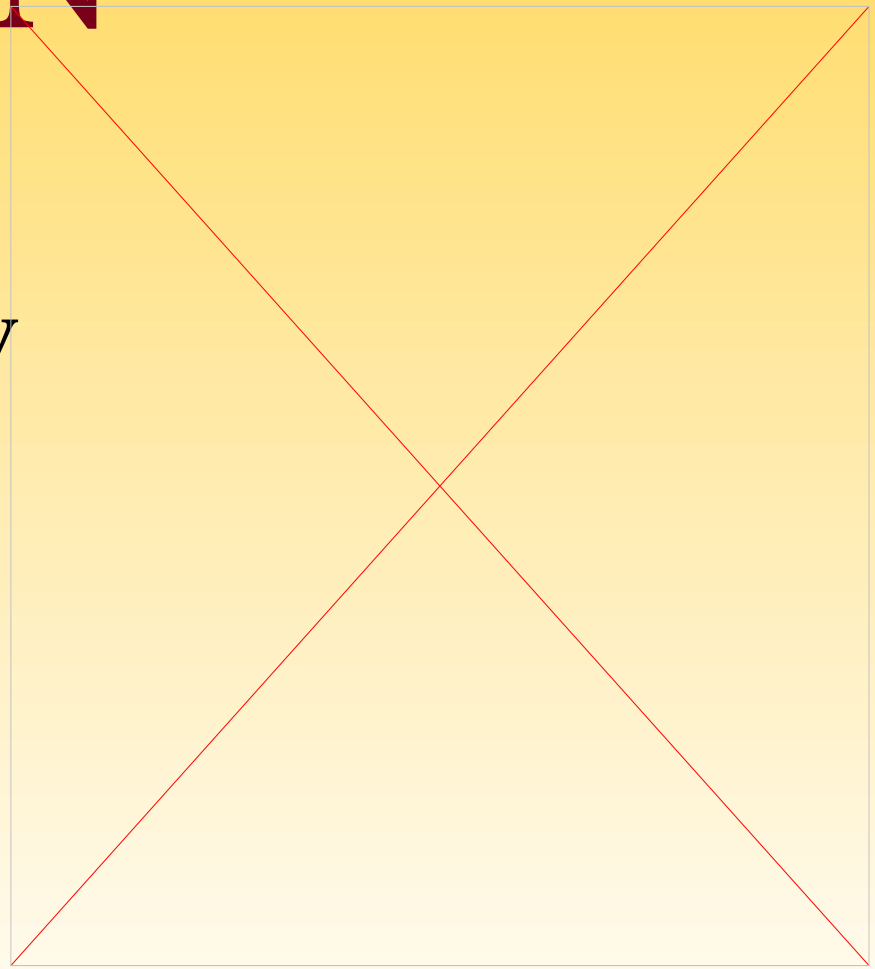
2024 NUTS & BOLTS SESSION

Welcome!

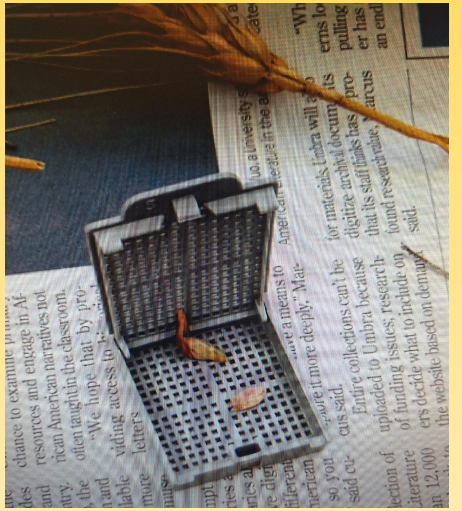
Beheshteh Zargaran
Lab Manager, Small Grains Pathology
University of Minnesota

Yanhong Dong
Research Associate Professor
Mycotoxin Diagnostic Lab
University of Minnesota



Fusarium graminearum Isolates

Develop a collection of the isolates:



- Collect infected heads
- Surface sterilizing the infected kernels
- Medias: KOMADA, Potato Dextrose Agar and
  Carnation Leaf Agar
- Storage of single spore isolates on soil or silica gel

Inoculum Production

Macroconidial Suspension Inoculum

- Select the isolates(s)
- Grow isolates on Mung Bean Agar
- Wash the plates
- Count the spore concentration using a hemocytometer

Inoculum Production

- Dilute inoculum to get desired spore concentration
- Inoculum can be stored in the freezer in 1 L plastic bottles for future use or in small vials for greenhouse inoculation

Inoculum Production

Colonized Corn Inoculum

- Grow the isolates on Mung Bean Agar
- Mix 1 kg of corn with 1 L of water in a pan
- Autoclave the pan one hour, 2 times
- Inoculate the sterile grain media with 4-5 plates of *Fusarium graminearum* cultures using sterile techni
- Check the pans after 2-3 weeks of incubation
- Use immediately or store the pans in a cold room until used for inoculation or you can dry the colonized grain down

Inoculation Methods

1- Spray Inoculation

- Head emergence/anthesis
- apply 1 -2 applications
- Use 50,000 to 200,000 spor

Inoculation Methods

2- Applying infected corn kernels

- Apply infected corn kernels from tillering through boot
- Inoculation Rate varies by location, dependent on environmental variables.
- You will likely need to adjust the inoculation rate according to your experience over time .
- Dry inoculum will take longer to produce mature spores compared to fresh inoculum.

Mist Irrigation System

- **Misting design**
  Pumps, pipes, nozzles
- **Misting schedule**
  Frequency, duration

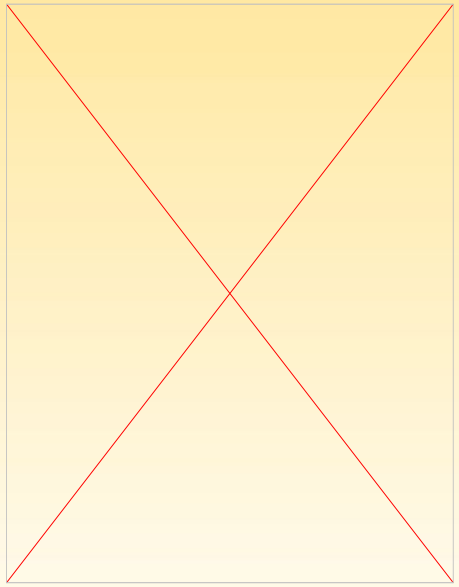
Stay vigilant!

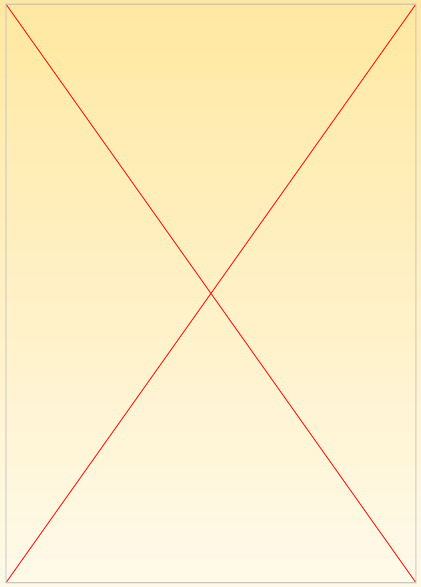
FHB Rating

Types of resistance

Type I: Resistance to initial infection

Type II: Resistance to disease spread within a spike



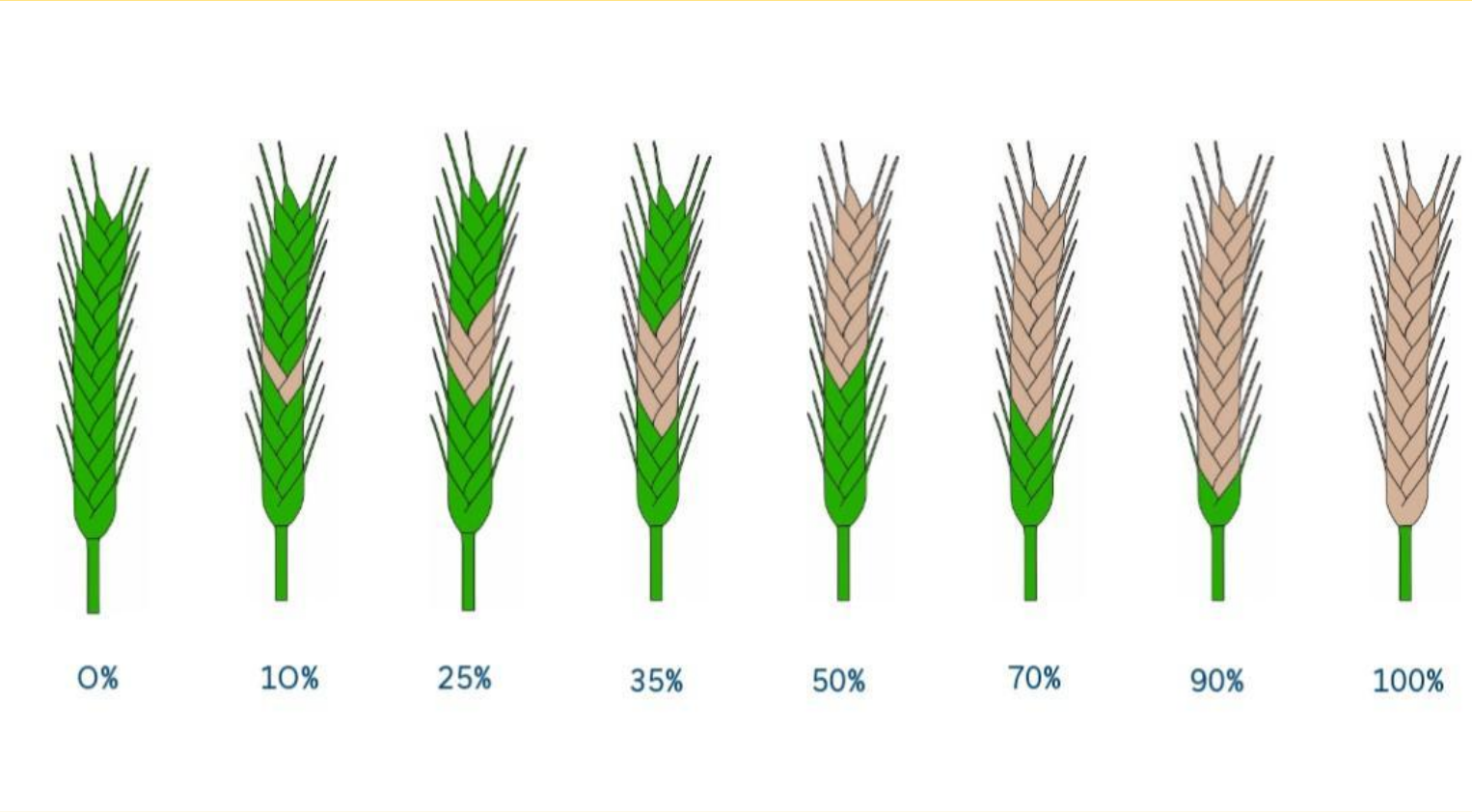


Incidence/ Severity/ FHB Index

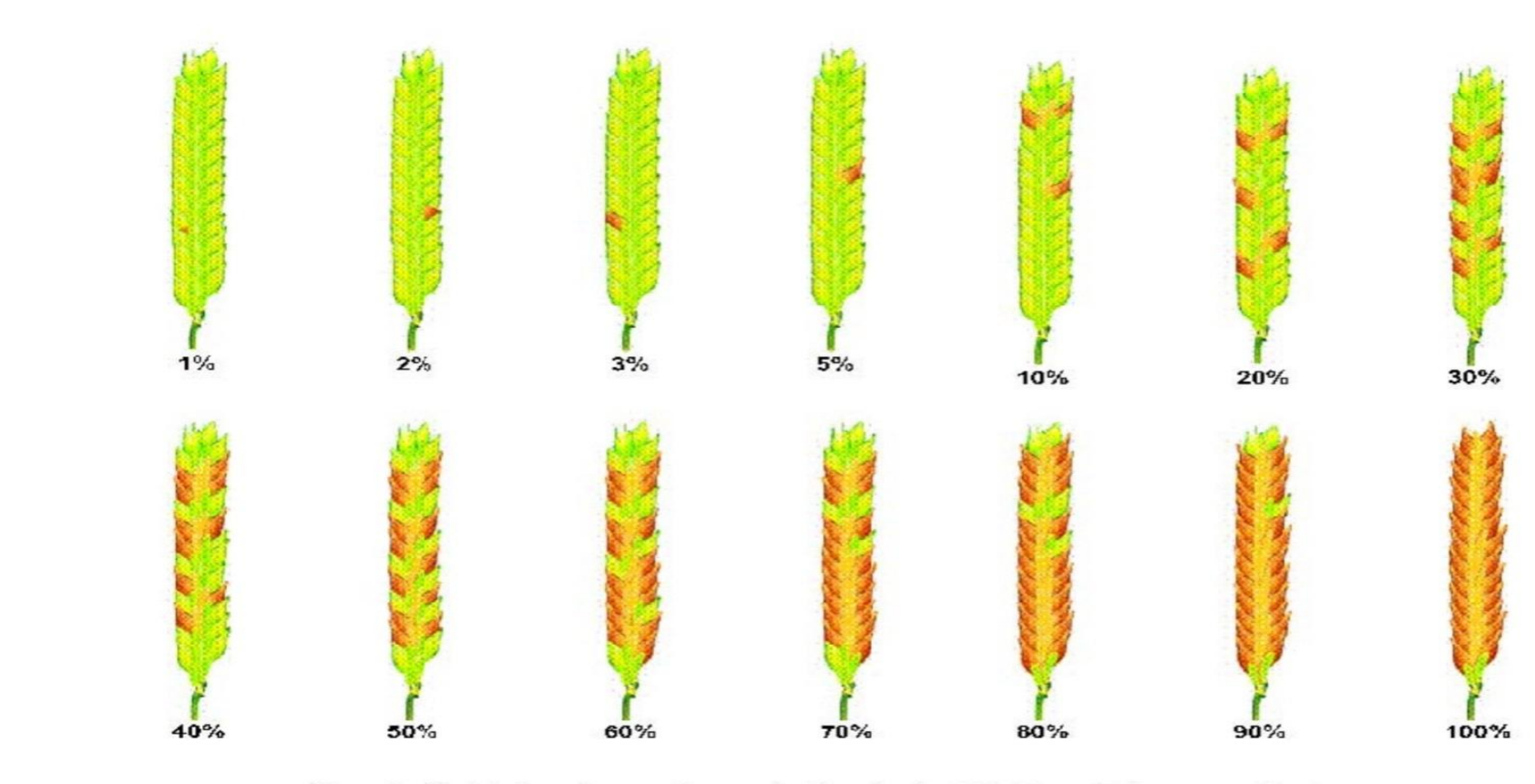
- **Incidence -** proportion of diseased spikes (number of spikes with

  nonzero severity divided by the total number of spikes sampled).
- **Severity -** average proportion of diseased spikelets per spike on ***diseased spikes***.
- **FHB Index -** (Scale of 0 to 100 or 0 to 9) - average proportion of diseased spikelets per spike (sum of the proportion of diseased spikelets per spike divided by the total number of spikes sampled, ***including those with zero severity***).

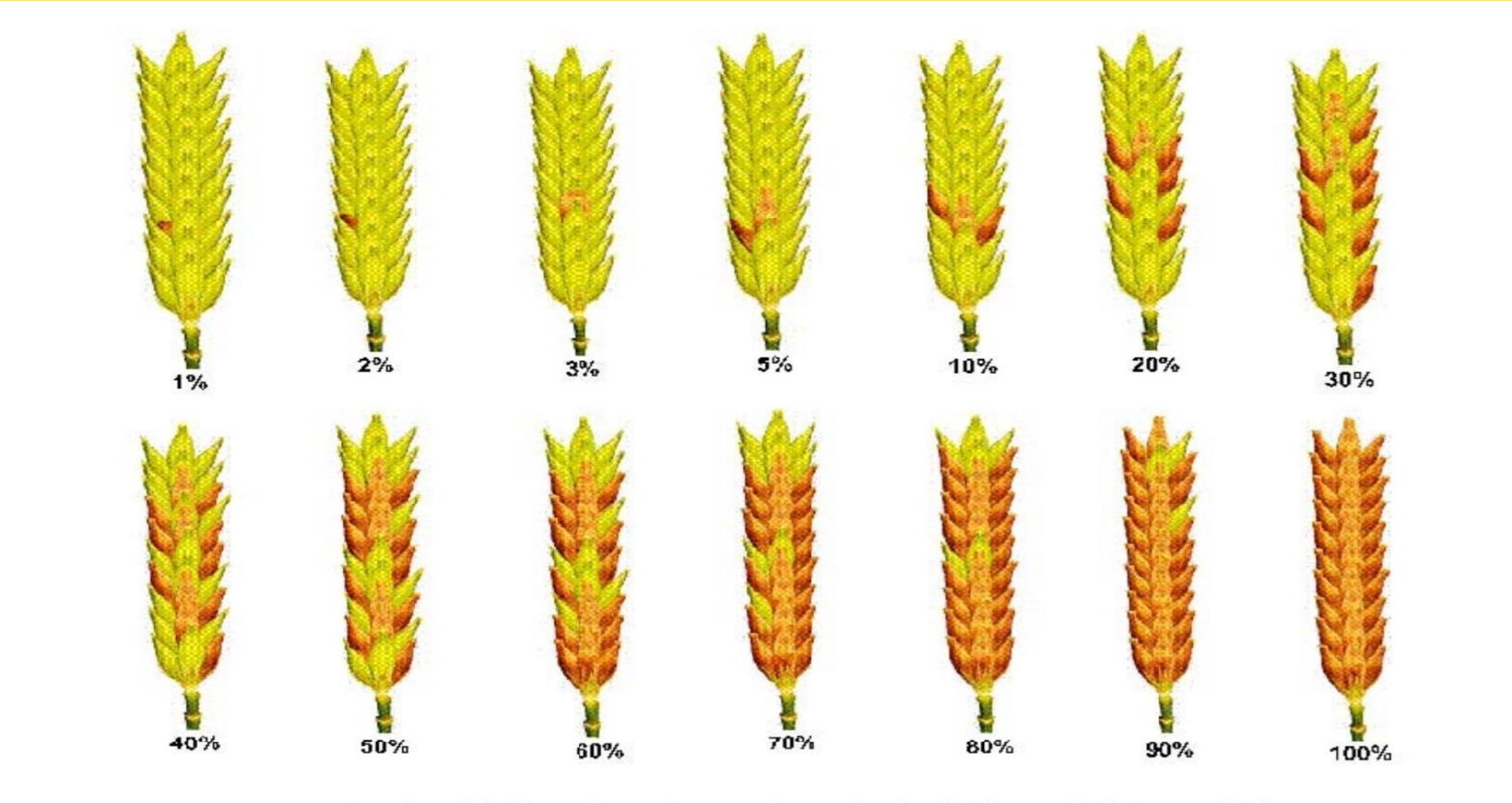
Rating FHB- Severity (%) Wheat



Rating FHB- Severity (%)
2-row Barley



Rating FHB- Severity (%)
6-row Barley

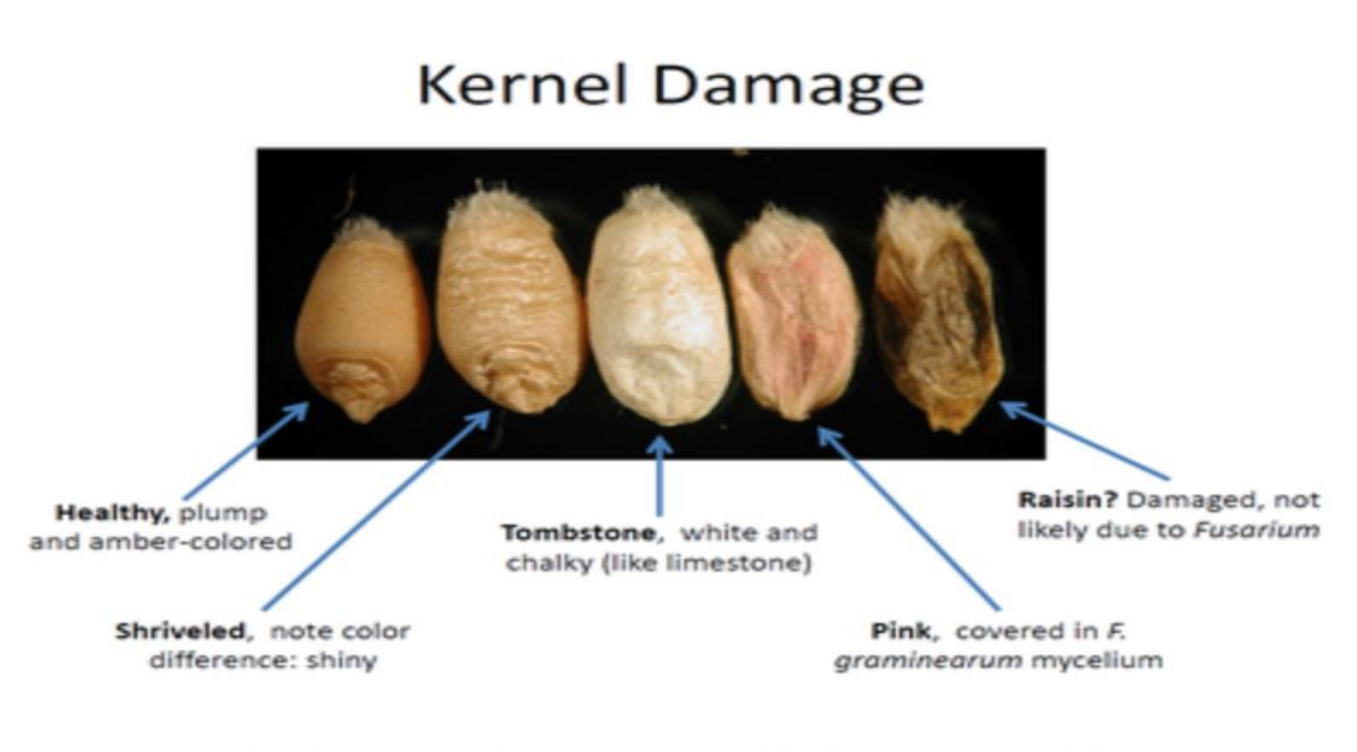


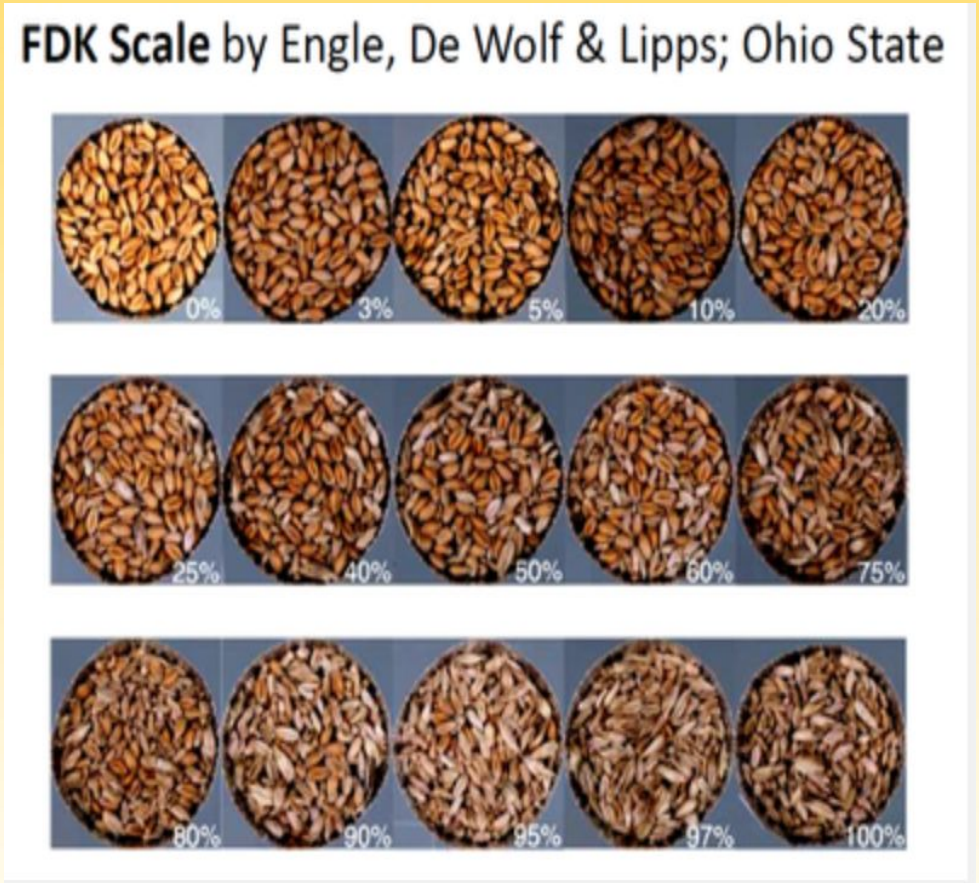
VSK Assessment

- Wait one week after harvest before scoring (allows grain to dry)
- Mix the grain before subsampling as damaged kernels tend to rise to the top
- Scoop subsample into a petri dish or weighing boat and fill till top is level
- 2 or 3 people should do the assessment and compare the results

VSK Assessment

Scoring FDK
(Fusarium Damaged Kernels)





Greenhouse inoculation of *Fusarium graminearum*

- Point Inoculation
- Spray Inoculation

Toxin levels generally higher in the greenhouse than field.

Harvesting Samples

- Harvest 1-2 feet of row from center o
- Thresh harvested heads on belt thres
- Clean the sample using a seed cleane
- Obtain a 100g sample using a divider
- Send samples to a testing lab

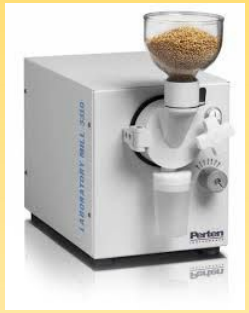
Cleaning Samples

Belt
Thresher

Kornservice seed cleaner

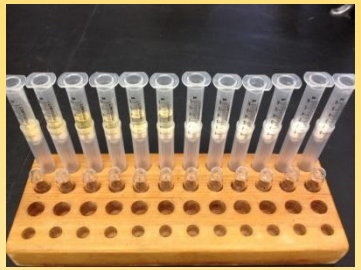
Analysis of DON in Cereal Grains

sample

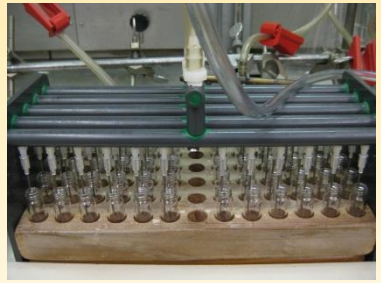


grinding

extraction



purification



drying

Derivatization

GC-MS
(Gas Chromatography–Mass Spectrometry)

Grain Sampling

- DON doesn't distribute evenly in grain seeds. A representative sample is a "**key**" to obtain accurate DON results.

- Grain sampling for deoxynivalenol (DON) analysis: A researchers guide (USWBSI website).

  - *Sampling Recommendations for Small Research Plots* -

  (1) Mechanically harvested plots: Harvest the plot □ Obtain a 100g sample using a divider □ Clean the sample.

  (2) Manually harvested plots: Harvest 1-2 feet of row from the center of the plot & Select 30 heads from primary tillers □ Thresh these heads & Clean the sample. If 100 g samples are not available, consider bulking sample.

Harvesting Samples

- Harvest 1-2 feet of row from center o
- Thresh harvested heads on belt thres
- Clean the sample using a seed cleane
- Obtain a 100g sample using a divider
- Send samples to a testing lab

Examples of Grinders and Ground Samples

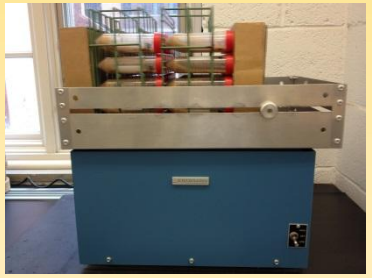
- A good ground sample resembles whole wheat flour

A. Expensive Grinder: $x,xxx

B. Economic Grinder: Cuisinart (DCG-20BKN), $20

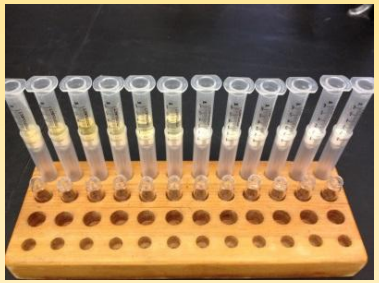
Sample Preparation in UMN DON Testing Lab

Extraction



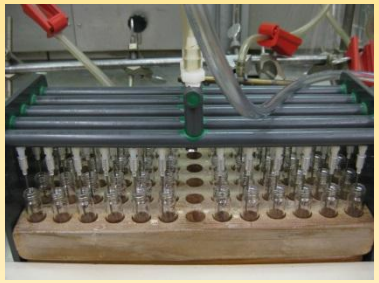
4 g + 16 mL ACN/H_2O (84/16), Shake 1 hr

Purification



4 mL, pass column (C18/Al_2O_3, 1/3)

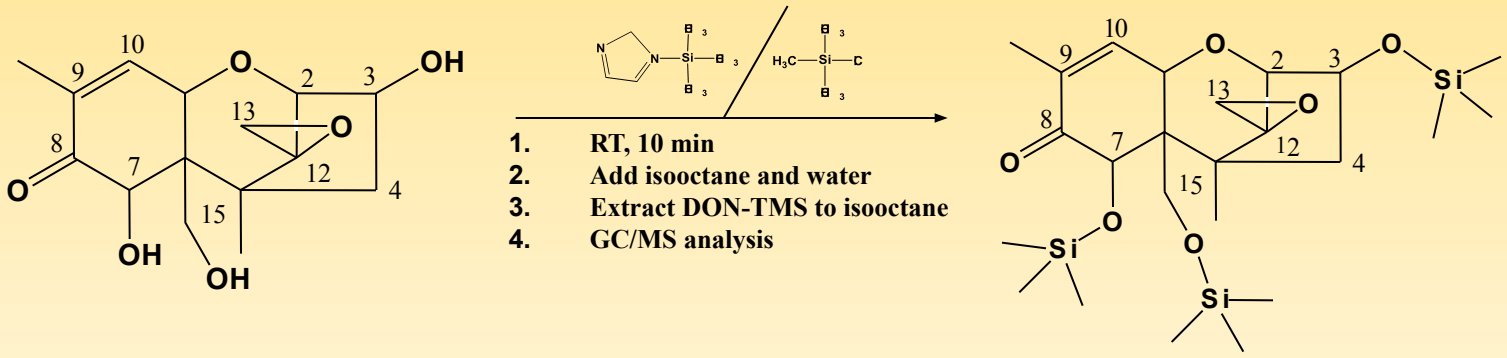
Drying



1 mL, dry under N_2 at RT

GC requirements: volatile & thermally stable

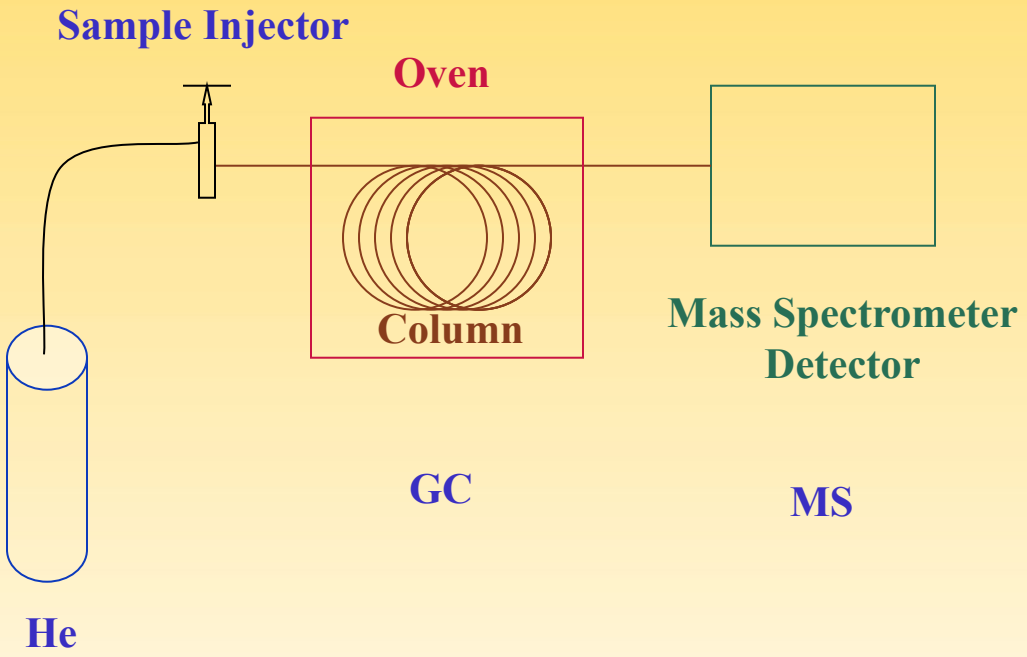
- Silylating reagent: TMS {TMSI/TMCS (100/1, v/v) }
- TMS derivatization: Increases volatility and thermal stability of DON.



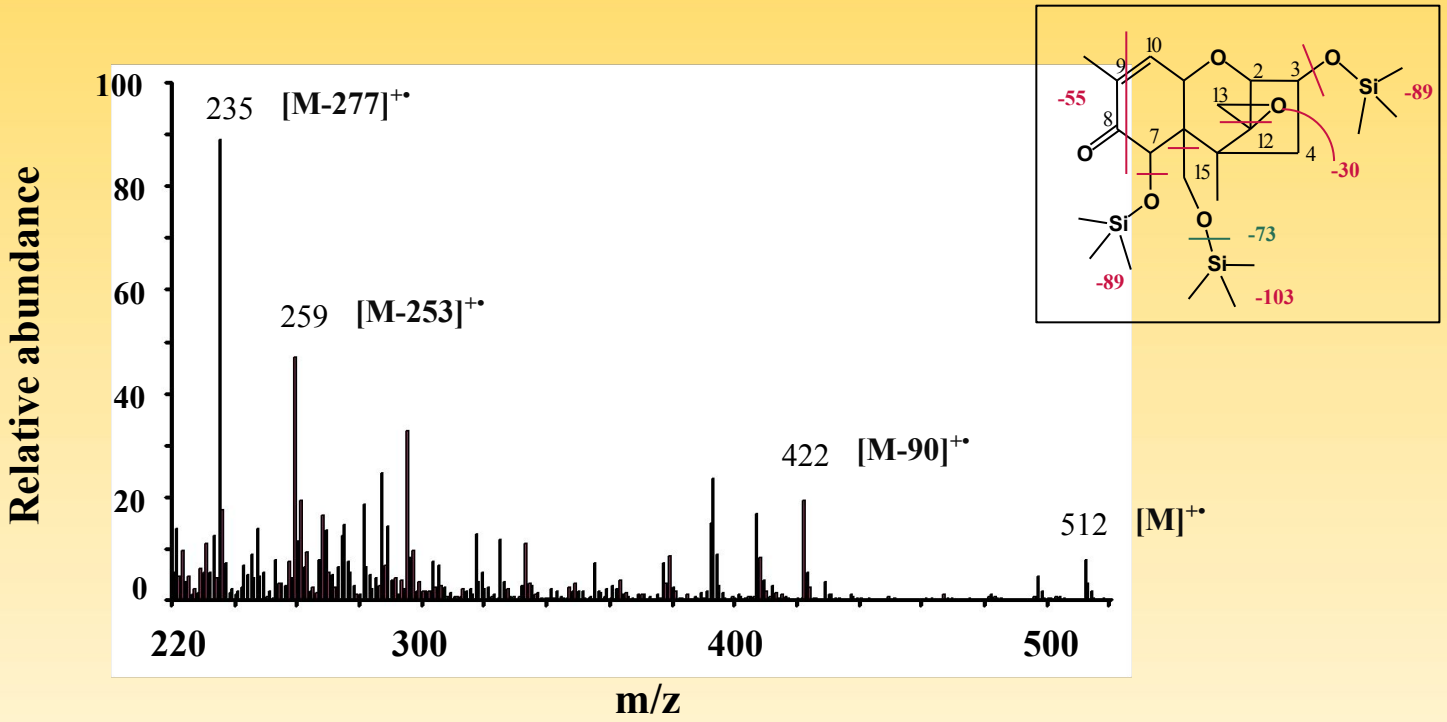
DON

DON-TMS

Gas Chromatography-Mass Spectrometry (GC-MS)

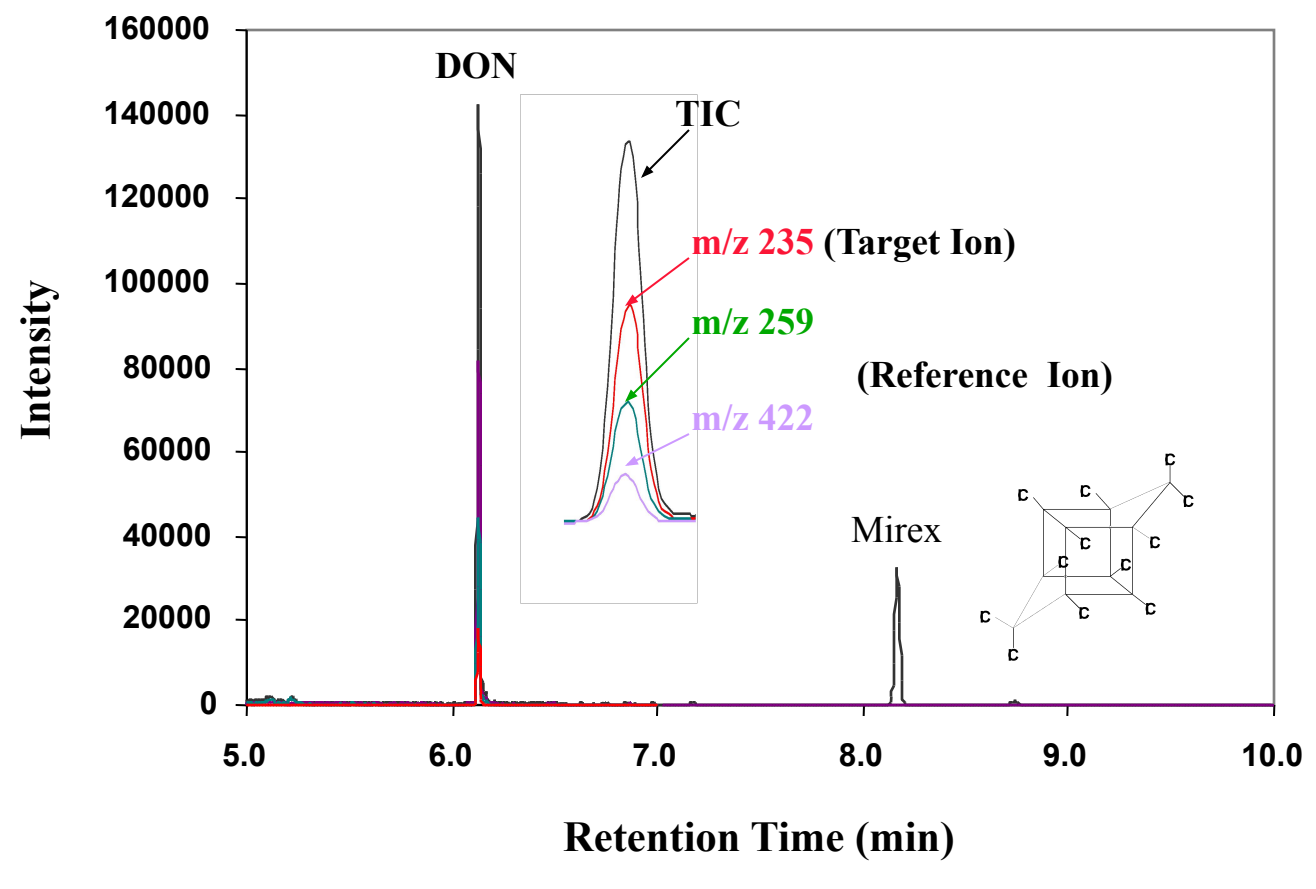


Mass Spectrum of DON-TMS

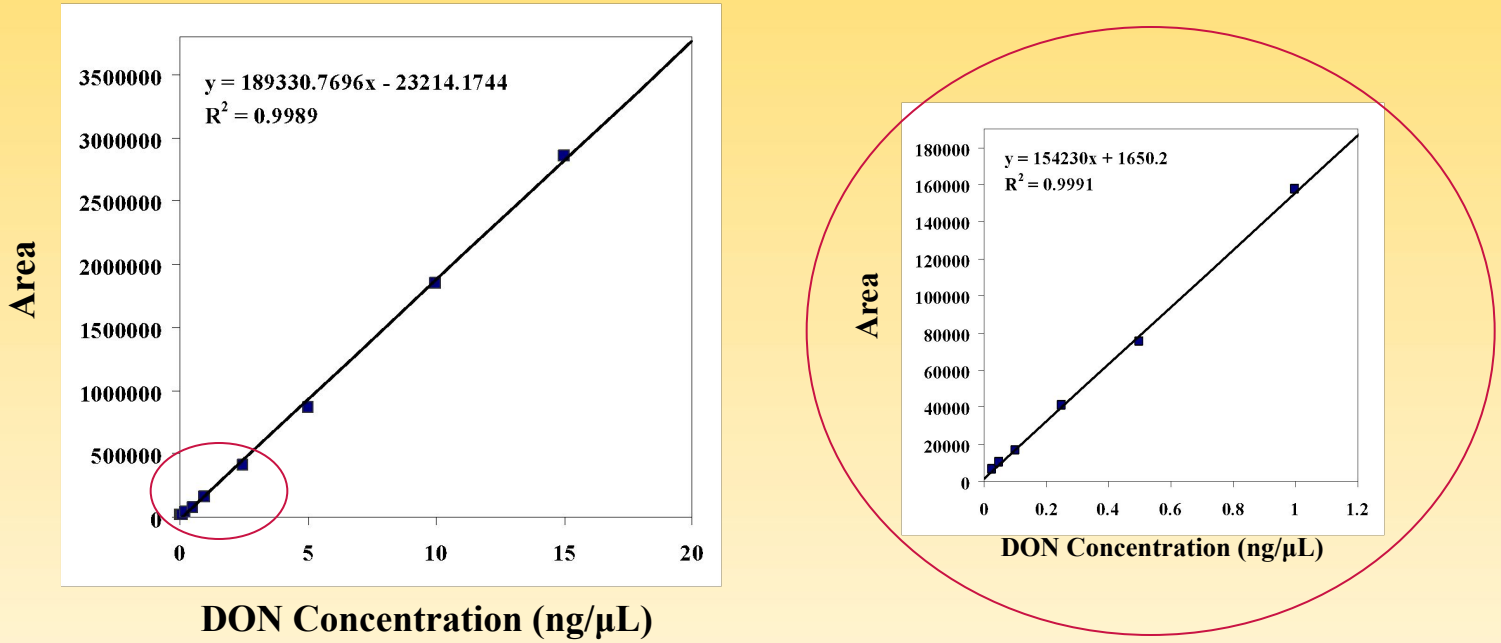


- Characteristic mass fragmentation pattern.
- Diagnostic fragment ions (m/z): 235, 259, 422 & 512.
- Ratios of diagnostic fragment ions are unique to the analyte.

Total Ion Chromatogram of DON in Grain Matrix

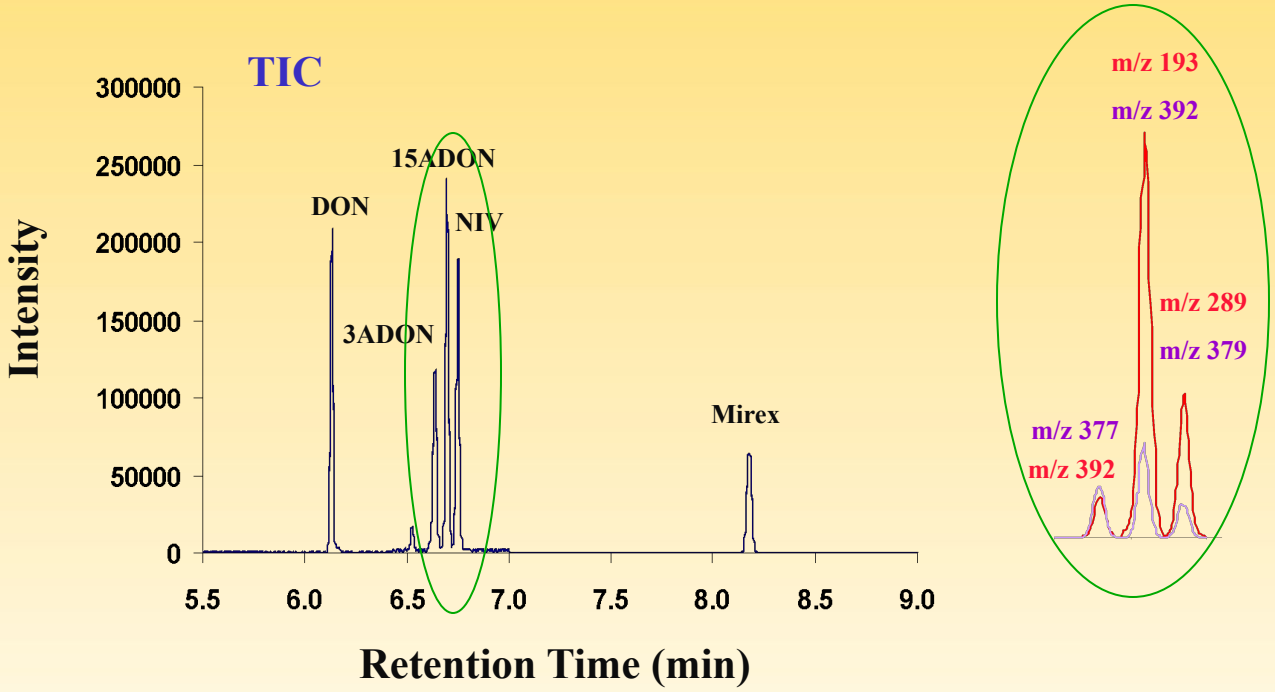


Standard Curve in Barley/Wheat Matrix



Good linearity

Chromatogram of A Mixture



Thank you!

Questions?

For follow-up questions, please contact:

Beheshteh Zargaran
zarga001@umn.edu

Yanhong Dong
dongx001@umn.edu

U.S. Wheat & Barley Scab Initiative