

Establishing a mycotoxin quantification platform to support FHB research and breeding programs

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Fusarium head blight (FHB)

- Fungal disease of small grain cereals
- bleaching spikes and Fusarium damaged kernels

wheat



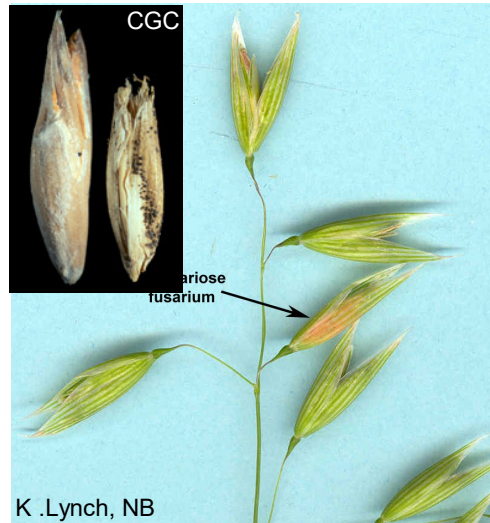
Erick DeWolf, K-State

barley



Janet Lewis, CIMMYT

oat



K. Lynch, NB

rye



Carl Bradley, UK

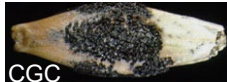
canary seed



Paulina Cholango Martinez, CDC/UofS



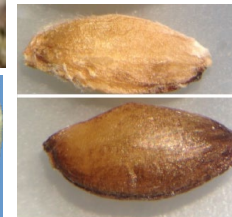
Canadian Grain Commission (CGC)



CGC



CGC



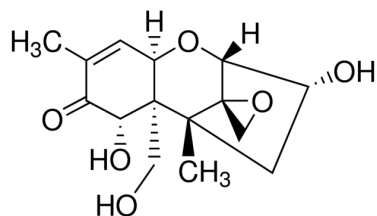
Fusarium produced Mycotoxins

- Secondary metabolites and virulent factors
- harmful to humans and livestock.
- Significantly reduced grain quality and price.
- Poor-quality food products (e.g., bread and beer)
- Regulations on the maximum acceptable mycotoxin level

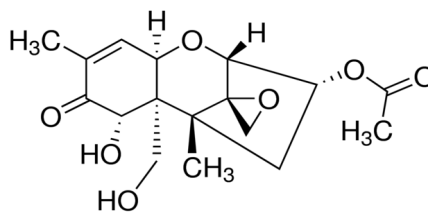


Fusarium mycotoxins found in western Canada

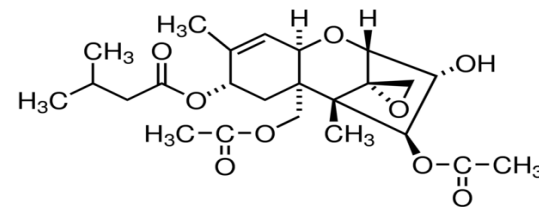
- **Deoxynivalenol (DON, or Vomitoxin)**, is predominant in North America.
- **Nivalenol (NIV)** prevail in Asia, but was detected in western Canada in 2015.
- Significant shift from the **15-ADON** to the more aggressive **3-ADON** chemotype in western Canada.
- **T-2** and **HT-2** Toxin are more toxic than DON.



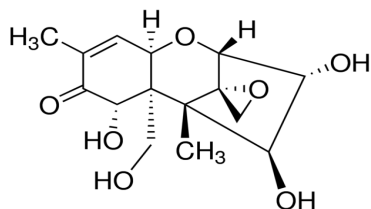
Deoxynivalenol
(DON)



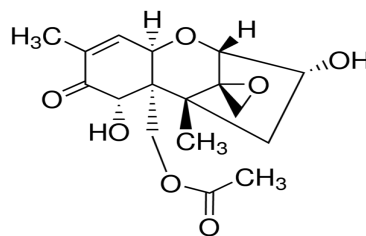
3-acetyldeoxynivalenol
(3-ADON)



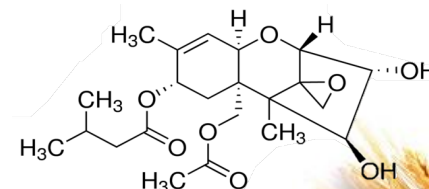
T-2 Toxin



Nivalenol
(NIV)



15-acetyldeoxynivalenol
(15-ADON)

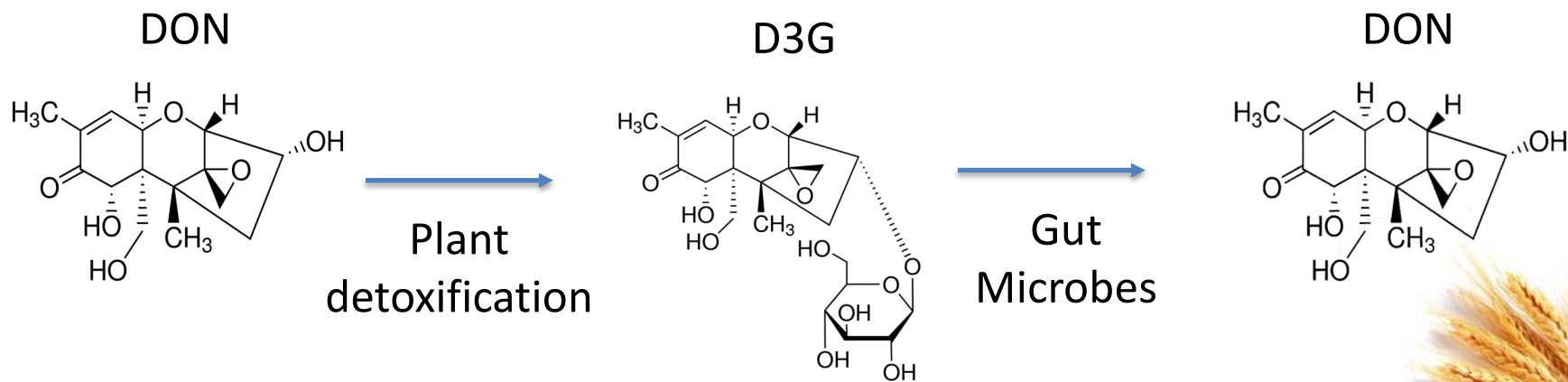


HT-2 Toxin



Masked mycotoxin in grain

- DON is modified through plant detoxification system to form less toxic form, Deoxynivalenol-3-glucoside (**D3G**) in plant.
- Unlike DON, D3G are not routinely monitored.
- D3G can be reversed to toxic form in mammalian digestive systems (Gratz et al., 2013).
- The high level of D3G has been detected in the resistant wheat lines (Amarashinghe et al., 2016).



High demand on mycotoxin measurement

Plant pathologist Understand the disease mechanism and surveil the mycotoxins in western Canada



Liquid Chromatography- Tandem Mass Spectrometry (LC-MS/MS)



The Core Mass Spectrometry Facility, College of Pharmacy and Nutrition, U of Saskatchewan
The 4000 QTRAP® LC/MS/MS System is a hybrid triple quadrupole/linear ion trap mass spectrometer coupled to an Agilent 1260 Binary HPLC



LC-MS/MS

Crude
Extraction

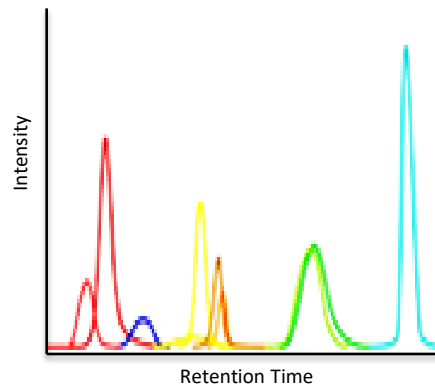


LC
separation

LC Column



MS/MS
separation

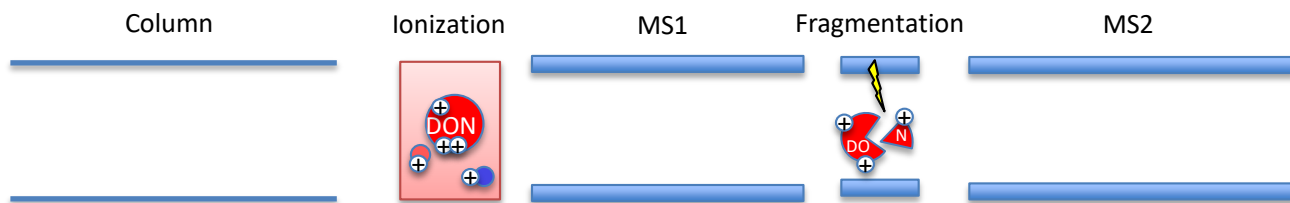
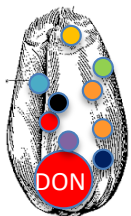


LC-MS/MS

Crude
Extraction

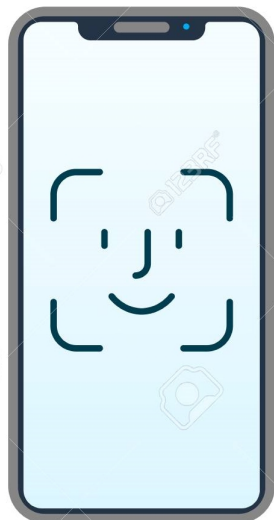
HPLC
separation

MS/MS
separation



Precursor Ion:
355.0 m/z

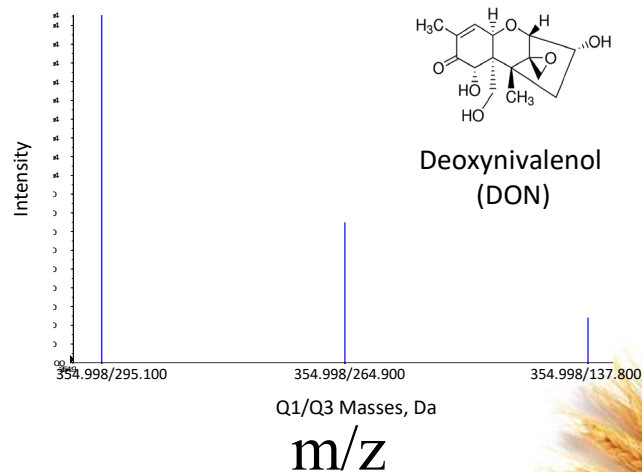
Product Ion:
296.1 m/z and 264.9 m/z



FACE ID



TOUCH ID



m/z: mass-to-charge ratio



I: high-throughput DON phenotyping assay

- Breeding programs dealing with a large number of samples

II: multiple mycotoxins quantification assay

- Mycotoxin surveillance in Western Canada
- Masked toxins determination in grain



Method validation

the US Food and Drug Administration (FDA)
Guidelines for Bioanalytical Method Validation



I: high-throughput DON phenotyping assay

small amount of sample request 100 mg of any tissue from plant



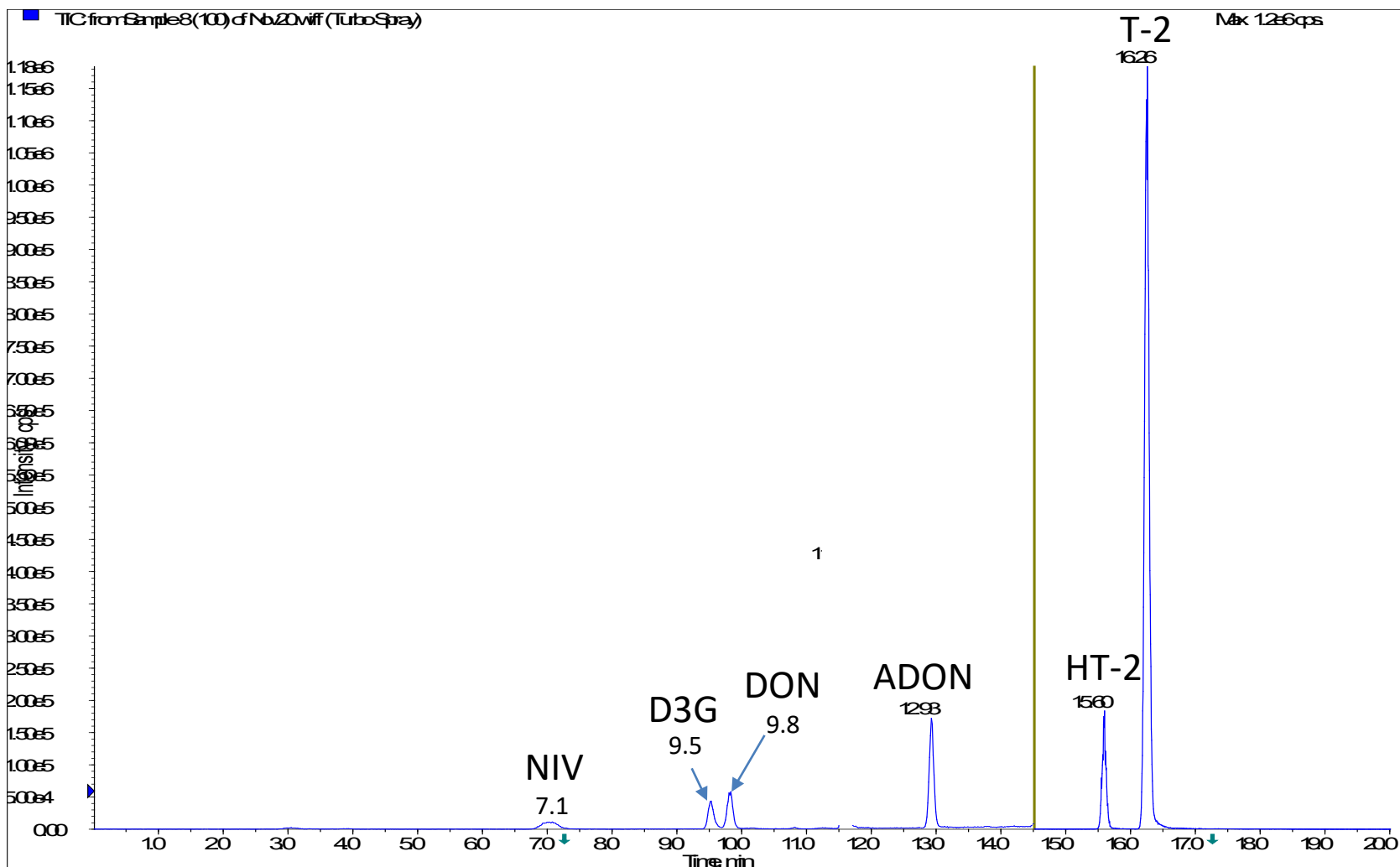
I: high-throughput DON phenotyping assay

Crop	LOQ (ng/mL or ppb)	r^2
durum	3.13	0.9990
barley	6.25	0.9993
oats	6.25	0.9973
canary seed	6.25	0.9953
rye	6.25	0.9987

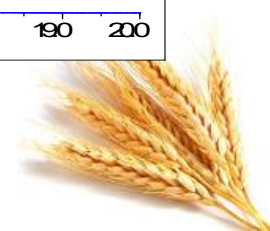
Limit of quantification (LOQ) and regression coefficient (r^2) for each crop



II: multiple mycotoxins quantification assay



LC-ESI-MS/MS TIC of a wheat sample spiked with 7 mycotoxins at a concentration level of 100 ng/mL.



II: multiple mycotoxins quantification assay

Analyte	LOQ (ng/mL or ppb)	r^2
NIV	6.25	0.9999
D-3-G	3.13	0.9984
DON	1.56	0.9973
3AcDON	1.56	0.9973
15AcDON	3.13	0.9996
HT-2	0.16	0.9997
T-2	0.16	0.9995

Limit of quantification (LOQ) and regression coefficient (r^2) for each mycotoxin



Collaboration opportunity:

- Mycotoxin diagnose service
- DON phenotyping service

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