

Establishing LC-MS/MS based mycotoxin/deoxynivalenol (DON) platform to support FHB research and breeding programs

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Fusarium Head Blight (FHB)

- Fungal disease of small grain cereals such as wheat, barley, oats, rye, and canary seed.
- *Fusarium graminearum* is the predominant species in North America.
- Premature bleaching, Fusarium damaged kernels
- Losses in yield, grade and end-use quality



Fusarium Head blight of Wheat
Photo Dr. Gary Bergstrom, Cornell University



Bleached and shriveled tombstone kernels (left) compared to healthy wheat kernels.
Photos by Kiersten Wise and Greg Shaner

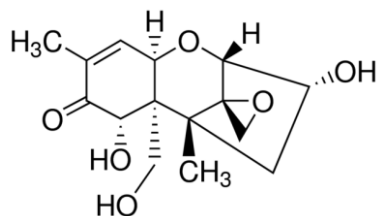
Fusarium produces mycotoxins

- *Fusarium* species produce trichothecene mycotoxins
- Contribute to plant pathogenesis.
- toxic to humans and livestock.
- Mycotoxin contamination significantly reduces grain grade and price.

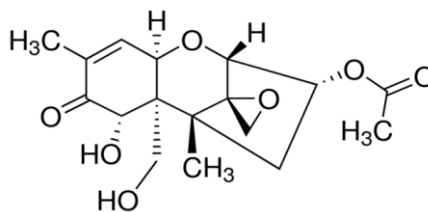


Trichothecene mycotoxins found in western Canada

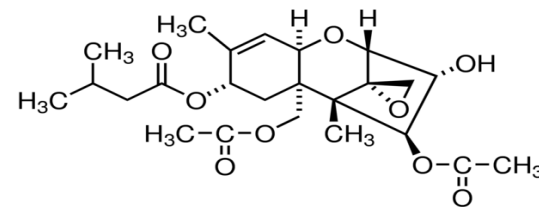
- **DON** is the predominant trichothecene in North America.
- **NIV** chemotypes prevail in Asia, but was detected in western Canada in 2015.
- Significant shift from the **15-ADON** to the more aggressive **3-ADON** chemotype in Western Canada.
- **T-2** and **HT-2** Toxin are more toxic than DON and found in Western Canada



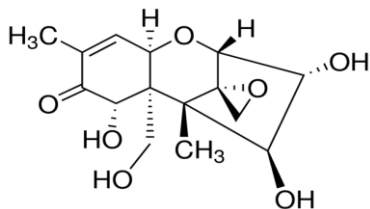
Deoxynivalenol
(DON)



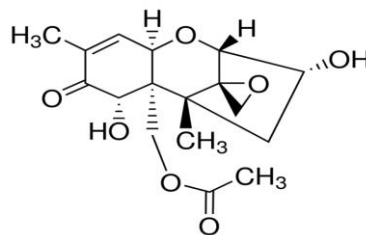
3-acetyldeoxynivalenol
(3-ADON)



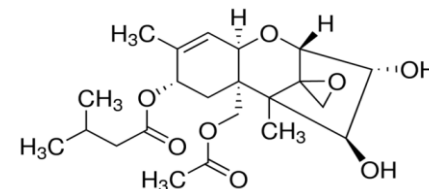
T-2 Toxin



Nivalenol
(NIV)



15-acetyldeoxynivalenol
(15-ADON)

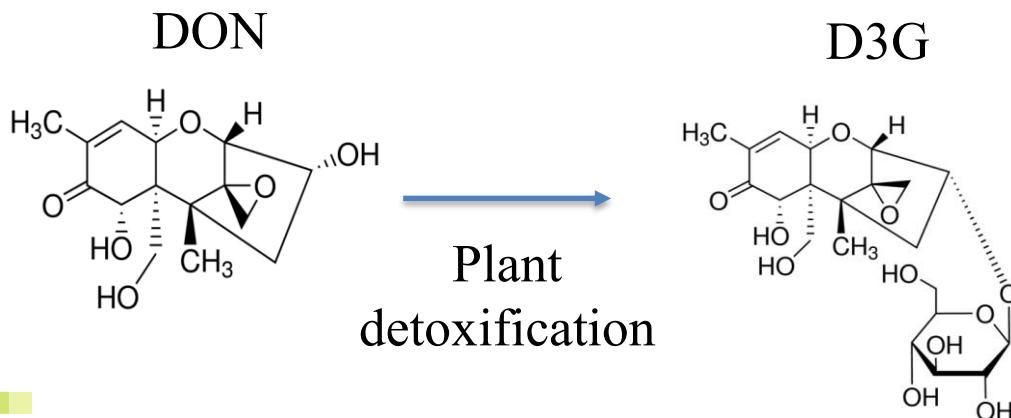


HT-2 Toxin



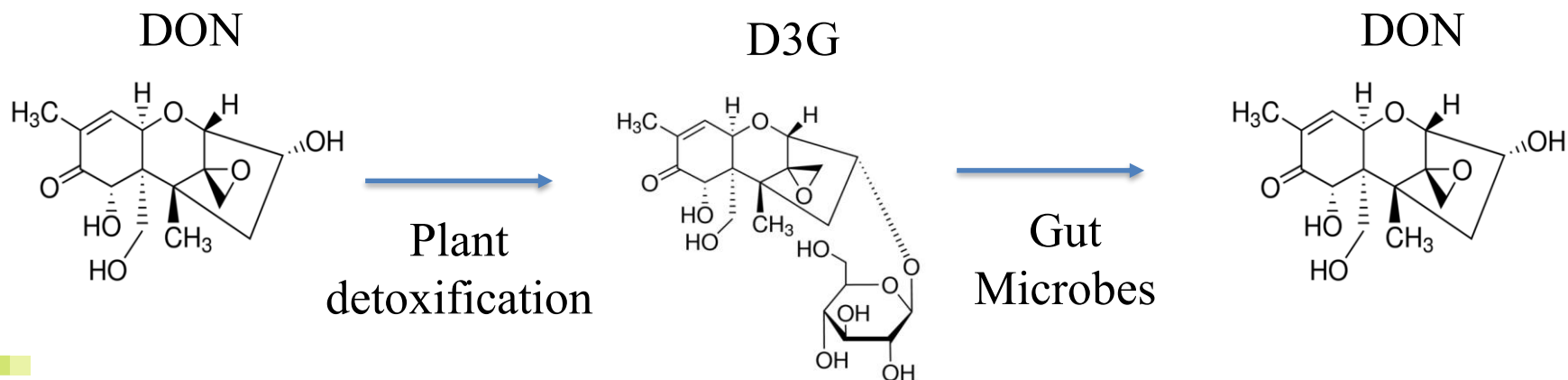
Masked/modified mycotoxins in grain

- Toxins that are masked from conventional chemical analysis because their structure has been changed in plant.
- DON is modified through plant detoxification system to form less toxic form, Deoxynivalenol-3-glucoside (**D3G**) in plant.



Masked/modified mycotoxins in grain

- Conjugated Mycotoxins might be reversed by gut microbes in mammalian digestive systems (Gratz et al., 2013).
- The resistant lines with low DON show a high relative level of D3G to DON (Amarashinghe et al., 2016).
- Are we breeding variety with low DON due to converting to D3G?



Emerging issues in mycotoxin research

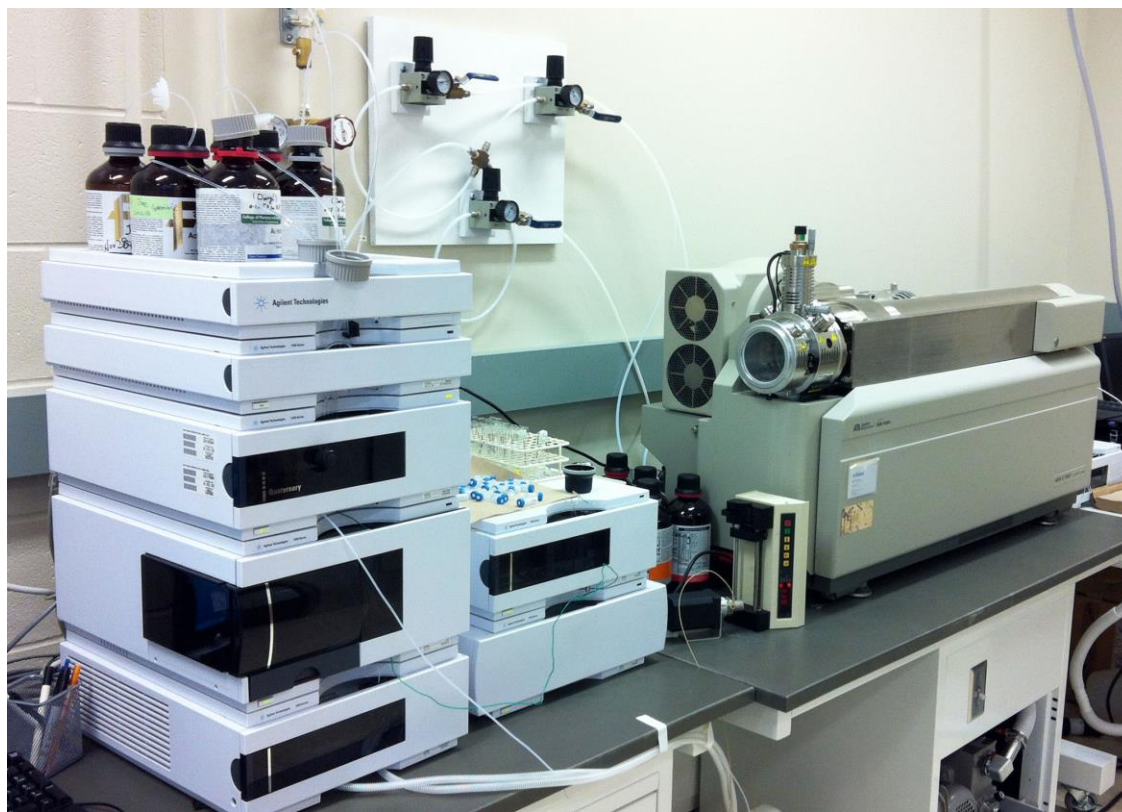
- Monitoring chemotypes shift in western Canada
- Identifying types of mycotoxins
- Investigating masked toxins in varieties

requires an analytic tool able to determine these mycotoxins qualitatively and quantitatively



LC-/MS/MS (MS²)

The Core Mass Spectrometry Facility
College of Pharmacy and Nutrition



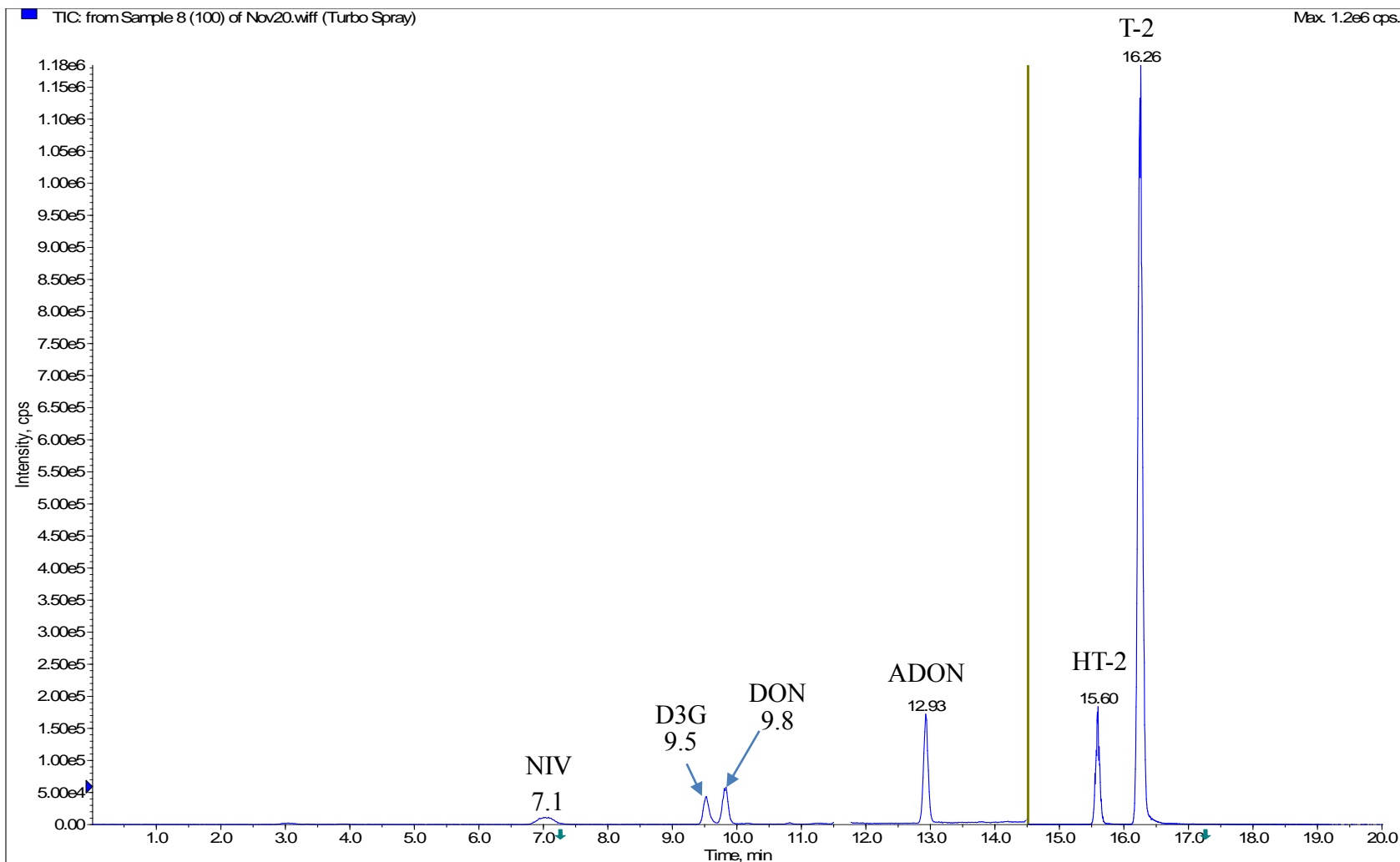
The 4000 QTRAP[®] LC/MS/MS System is a hybrid triple quadrupole/linear ion trap mass spectrometer coupled to an Agilent 1200 quaternary HPLC



PLATFORM I: a state-of-the-art analytical platform to quantify multiple mycotoxins simultaneously

Analyte	Retention time RT (min)	Precursor ion	Product ions	CE (eV)
NIV	7.1	371.0 [M+OAc] ⁻	59.0/280.9	42/22
D3G	9.5	517.2 [M+OAc] ⁻	457.2/426.9	20/30
DON	9.8	355.0 [M+OAc] ⁻	295.1/264.9	16/22
3-ADON	12.9	397.0 [M+OAc] ⁻	337.1/307.1	14/22
15-ADON	12.9	397.0 [M+OAc] ⁻	337.0/276.9	12/16
HT-2	15.6	442.2 [M+NH ₄] ⁺	263.2/215.5	17/31
T-2	16.3	484.2 [M+NH ₄] ⁺	215.2/185.3	29/27





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



PLATFORM I: a state-of-the-art analytical platform to quantify multiple mycotoxins simultaneously

Analyte	LOQ (ng/mL)	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



PLATFORM II: a high throughput DON screening for breeding programs

Rapid and Easy to operate

High sensitivity

High accuracy

High throughput

Low cost

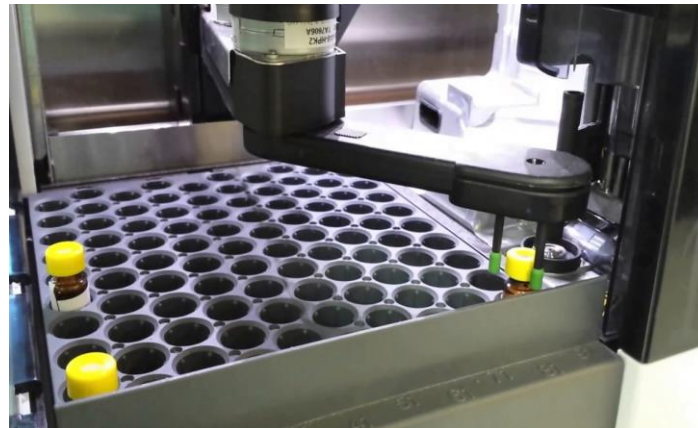


Rapid

LOQ

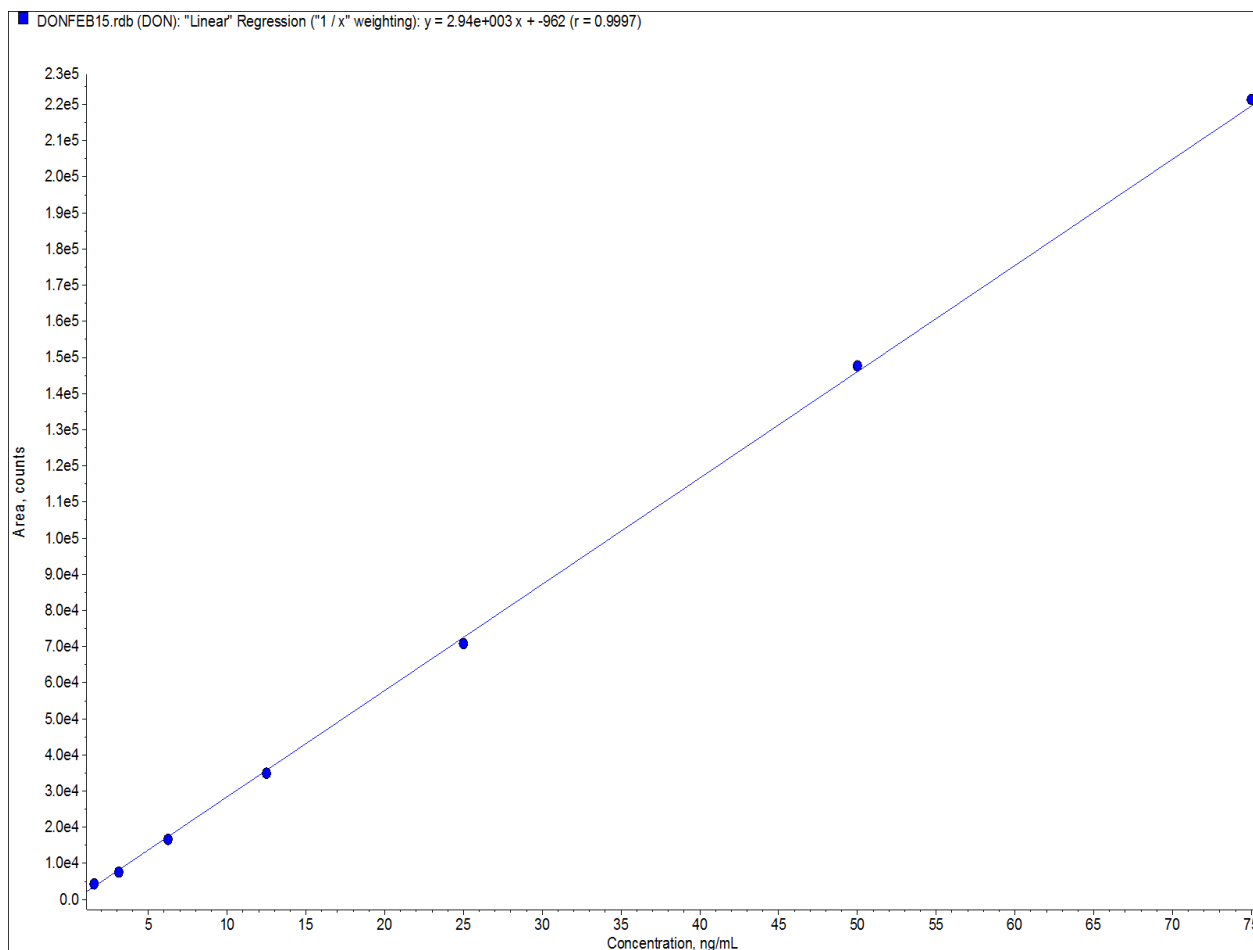
High-throughput

Low-cost



HPLC autosamplers: Large numbers of samples can be automatically injected onto an HPLC system.





Standard curve of DON from 1.5 ng/mL to 75 ng/mL with an r^2 value of 0.9997 representing good linearity.



Future direction

- Expand mycotoxin diagnostic library, NX
- Support research studies
- Support breeding programs
- Expand HTP DON assay into other cereal crops such as barley and canary seed



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