Metabolism, Toxicity and Occurrence of Deoxynivalenol-3-glucoside

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Who am I?

- Associate professor at North Dakota State University (NDSU)
- PhD, Purdue University, 2006
- Cereal Chemist
- Wheat DON Analysis (replaced Michelle Mostrum from NDSU)

Bound Mycotoxins

Deoxynivalenol-3-glucoside (D3G)

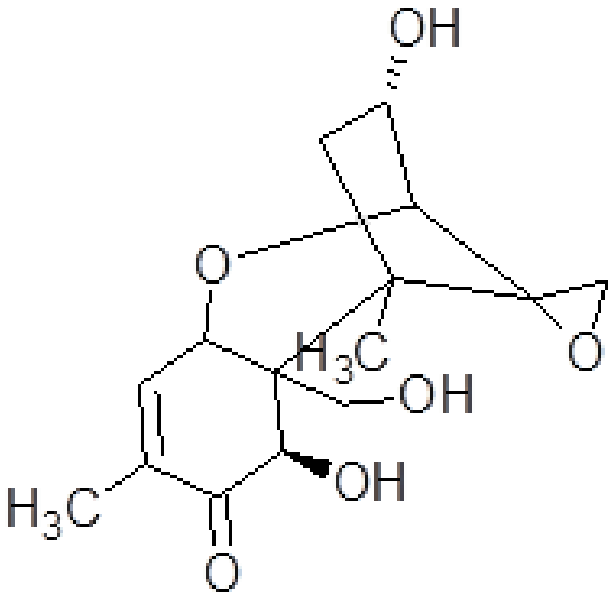
- **3-β-D-glucopyranosil-4-deoxynivalenol**

Produced by the plant as a detoxification method

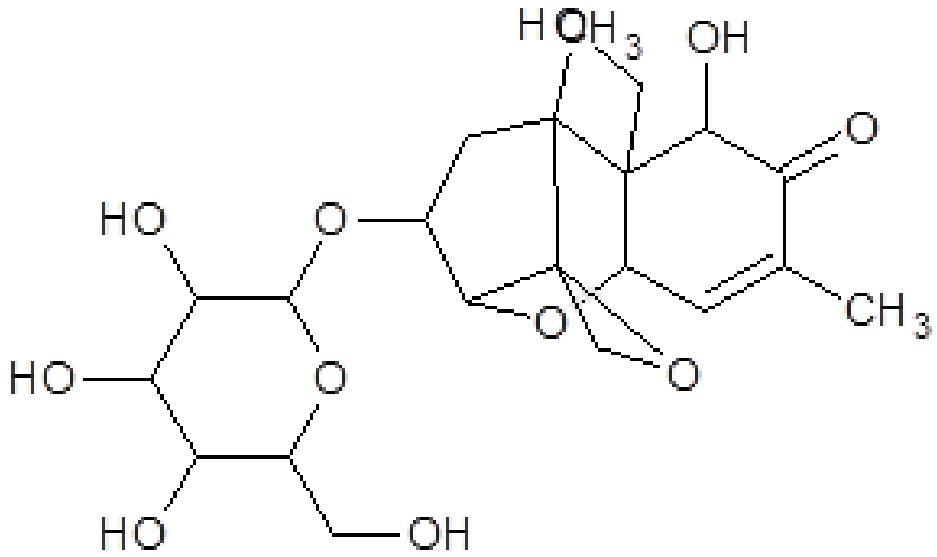
Samples with DON may have DON-3-glucoside

D3G/DON ≈ 10-30%.

Structures of DON and DON-3-glucoside



Deoxynivalenol
MW: 296 amu



Deoxynivalenol-3-glucoside
MW: 458 amu

Modification of Mycotoxins

- Biological and chemical changes can modify mycotoxin structure
- “Modified mycotoxins”
  - Different forms of mycotoxins
- “Masked mycotoxins”
  - Only toxins conjugated by plants

(Payros, 2016)

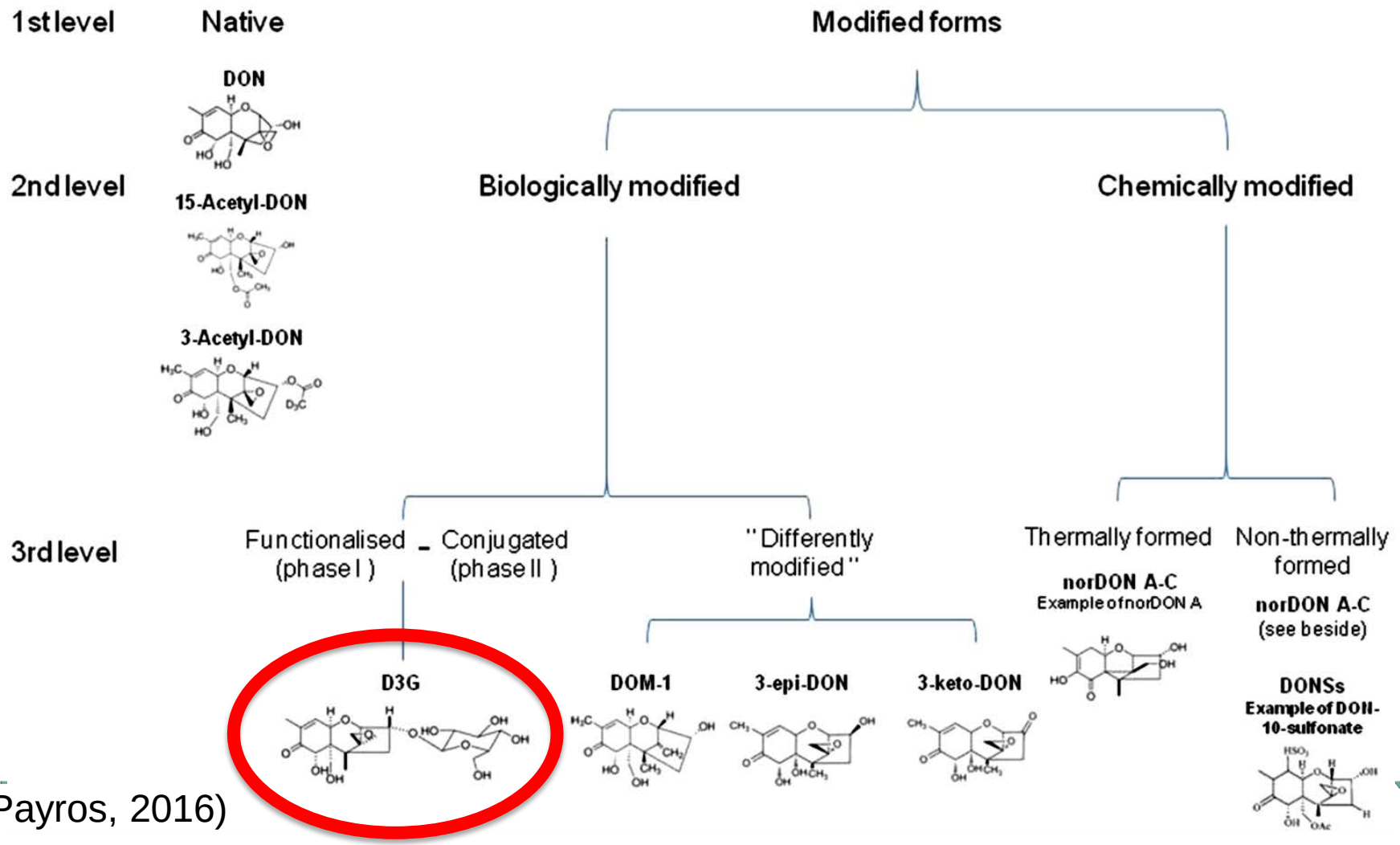
Hierarchic levels of modification

1. Differentiates the native (free and unmodified forms) mycotoxins from the “modified” forms and matrix-associated mycotoxins;
2. Differentiates the “modified” forms further in detail: the biologically modified forms from the chemically modified forms;
3. Differentiates more precisely the single modified forms, discriminating the functionalized from conjugated forms in biologically modified forms and thermally from nonthermally forms in chemically modified forms

Hierarchic levels of modification

- Detoxification mechanisms include several chemical modifications,
  - phase I transformation (hydrolysis, reduction or oxidation),
  - phase II solubilization (conjugation) and
  - phase III compartmentalization.
- D3G is issued from phase II metabolisation, glucosylation, of DON by the action of UDP-glycosyl-transferase enzymes naturally presents in the plant.

Modification of D3G

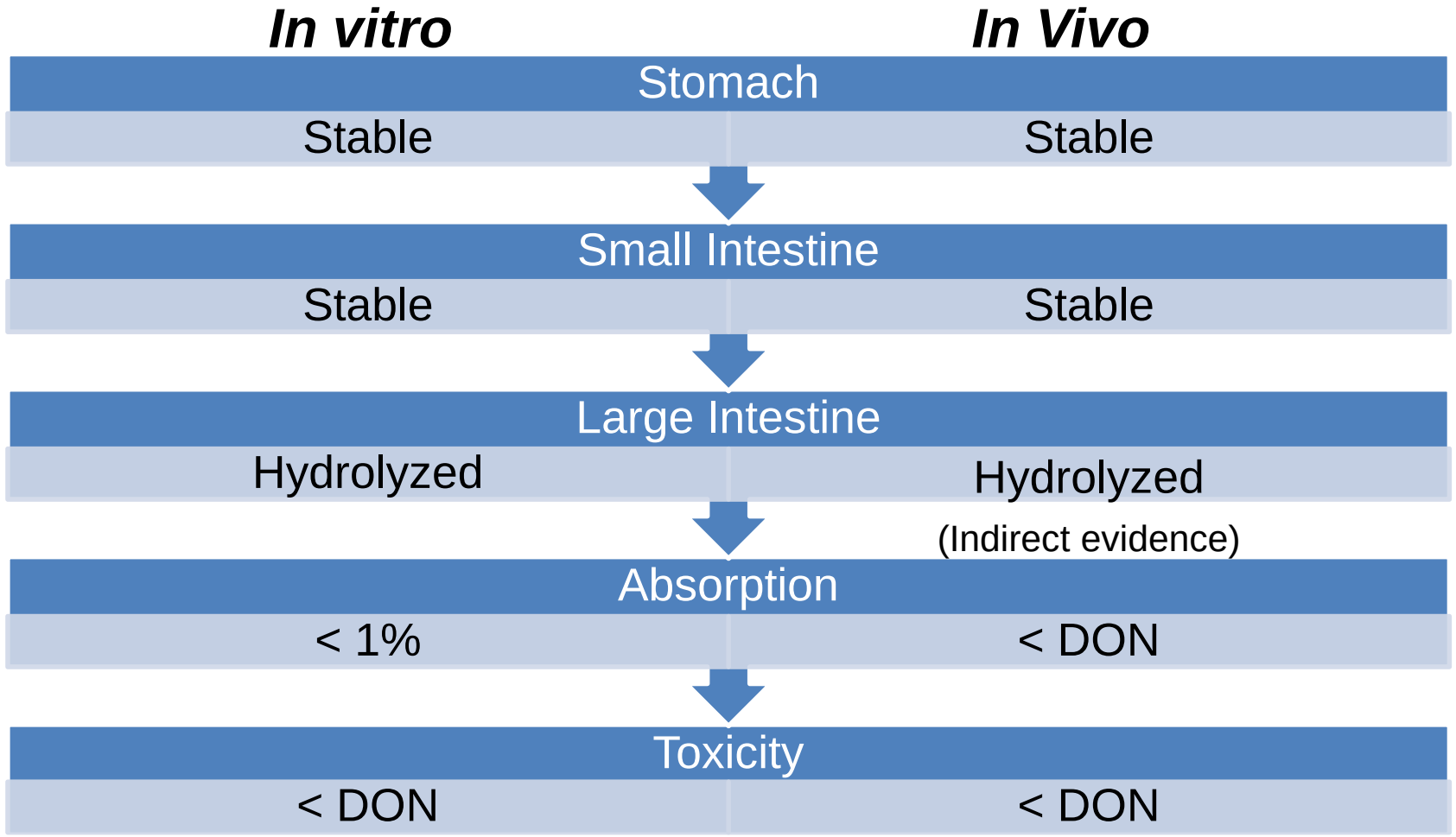


(Payros, 2016)

Digestion of D3G

- Limited studies on
  - Fate of D3G and other masked mycotoxins in the gut
  - Contribution to toxicity

Digestion and Absorption



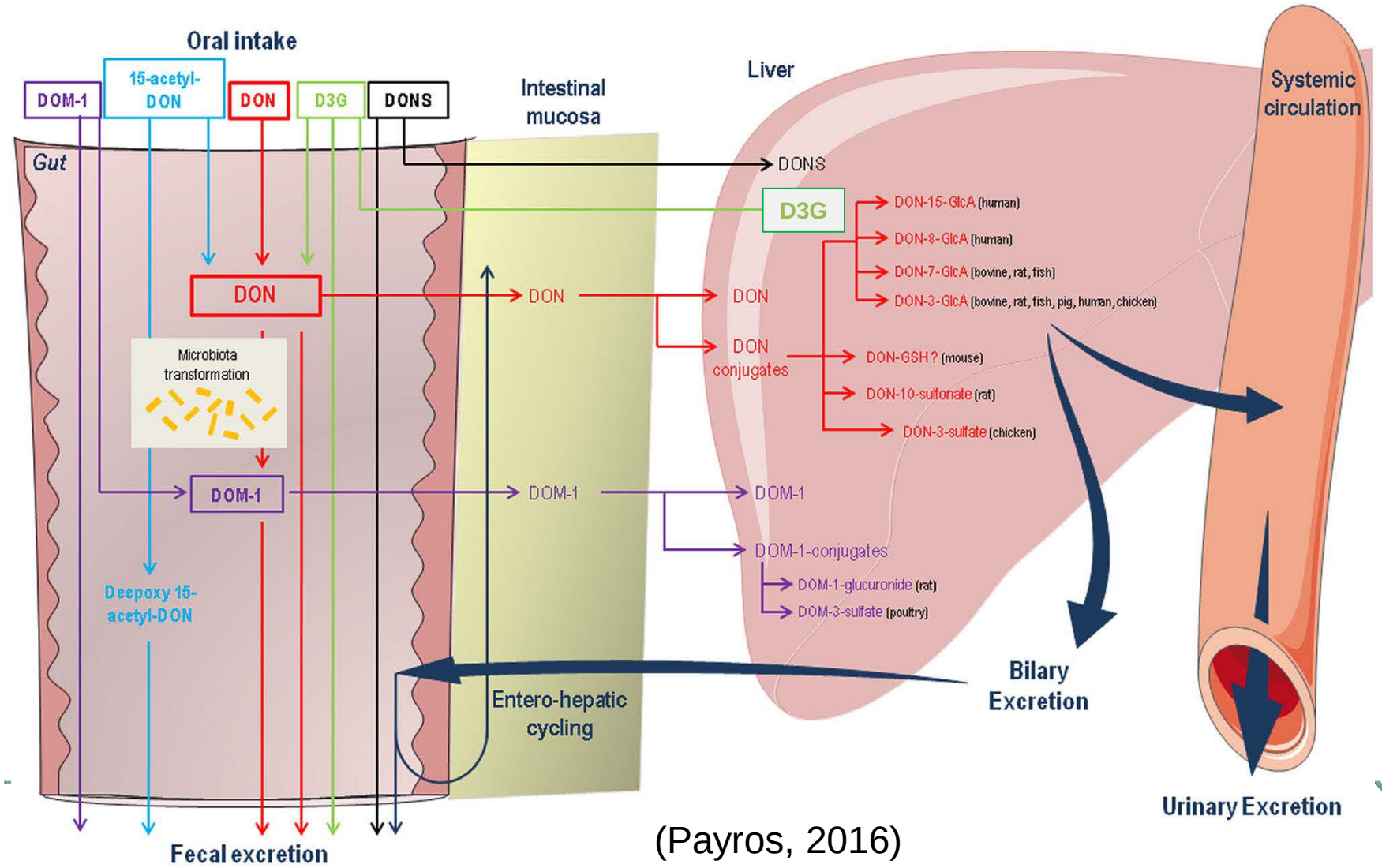
Gratz 2017

Toxicity of D3G

- Thought to have limited bioavailability in the gut
  - Due to covalent binding
- D3G cannot sterically bind to ribosomal 60S subunit A-site pocket
  - Target for DON-induced ribosomal toxicity

Gratz 2017

Metabolism of DON and D3G



(Payros, 2016)

Metabolism of D3G

- Oral bioavailability of D3G
  - 2-5 times lower than DON
- No evidence of transfer from apical to baso-lateral side in human intestinal epithelial cells
- D3G is hydrolyzed to DON by intestinal microbiota

(Payros, 2016)

D3G Hydrolysis by Fecal Microbiota

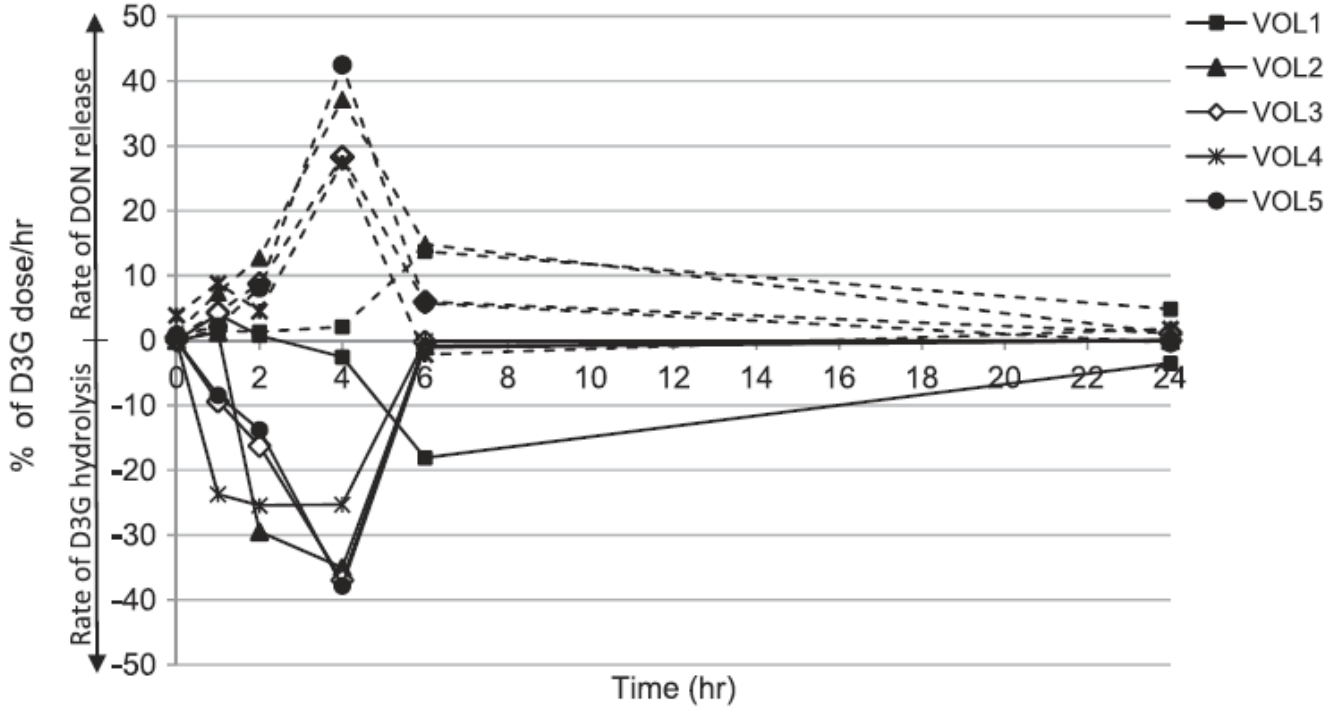


FIG 1 Rate of hydrolysis of D3G and release of DON by the fecal microbiota of 5 volunteers (VOL). Data are presented as D3G hydrolyzed per hour (% of D3G dose [solid lines]) and DON released per hour (% of D3G dose [dashed lines]).

Gratz et al., 2013

D3G Hydrolysis by Fecal Microbiota

- Fecal microbiota cleave D3G and release DON
- Hydrolysis rates range from 25-38% per hour after 4 hour incubation
- 100% D3G is degraded after 6 hours

Gratz et al., 2013

D3G Metabolism by Human Microbiota

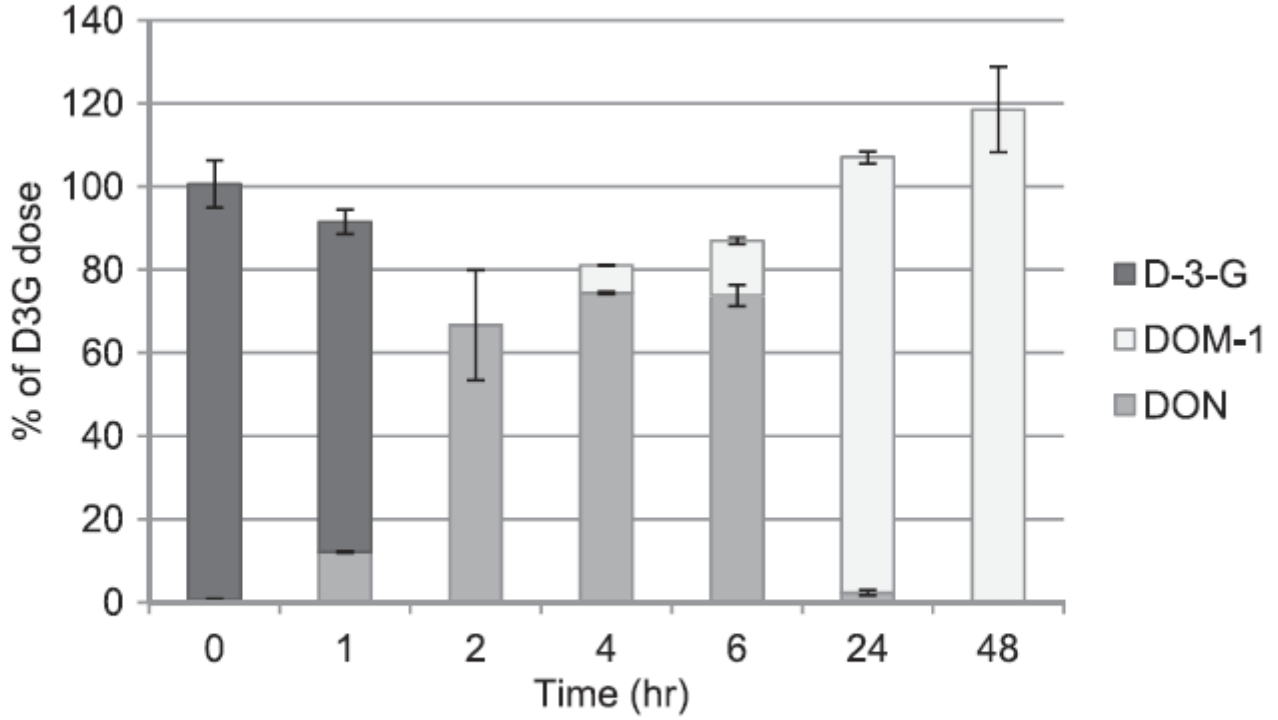


FIG 2 Metabolism of D3G by the human microbiota of volunteer 5. Results are presented as means of duplicate incubations for each time point.

Gratz et al., 2013

D3G Metabolism by Human Microbiota

During Incubation

- D3G is degraded
- DON is released
- DON is detoxified to deepoxy-deoxynivalenol (DOM-1)

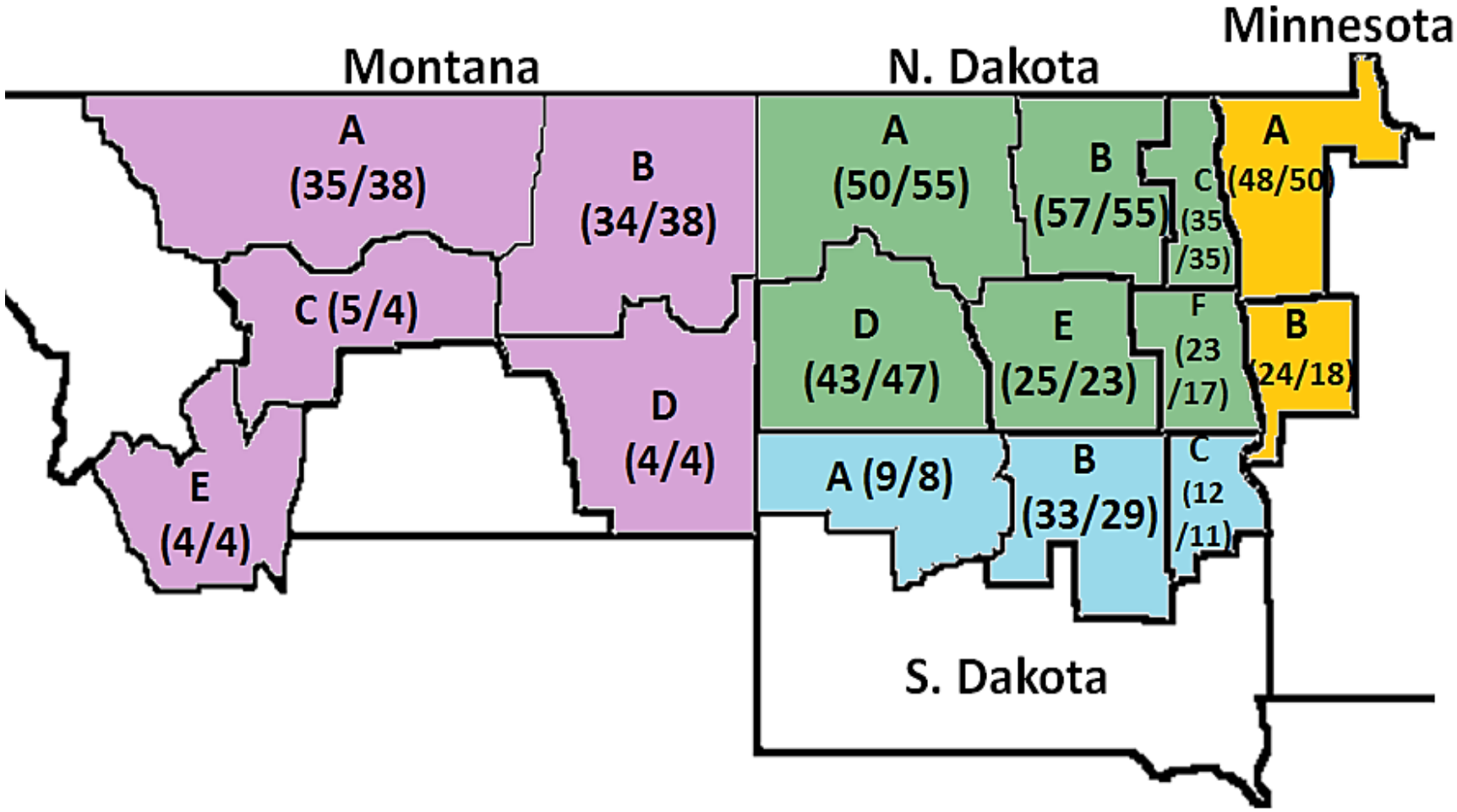
Gratz et al., 2013

OCCURRENCE OF D3G

Methods

- Deoxynivalenol: GC-ECD (Tacke and Casper, 1996)
  - LOD: 0.05 mg/kg LOQ: 0.20 mg/kg
- Deoxynivalenol and D3G: LC-MS
  - Extraction with Acetonirile:Water (Tacke and Casper, 1996)
  - LC-MS (Simsek et al., 2012 and Vendl et al., 2009)
- Statistics:
  - ANOVA for individual years using “MIXED” procedure in SAS (V 9.2)
  - Nested fixed model (region nested in state, city was nested in region)
  - Correlation and regression were preformed using “CORR” and “GLM” in SAS, respectively

Number of HRS Samples Collected in 2011/2012



DON and D3G Occurrence

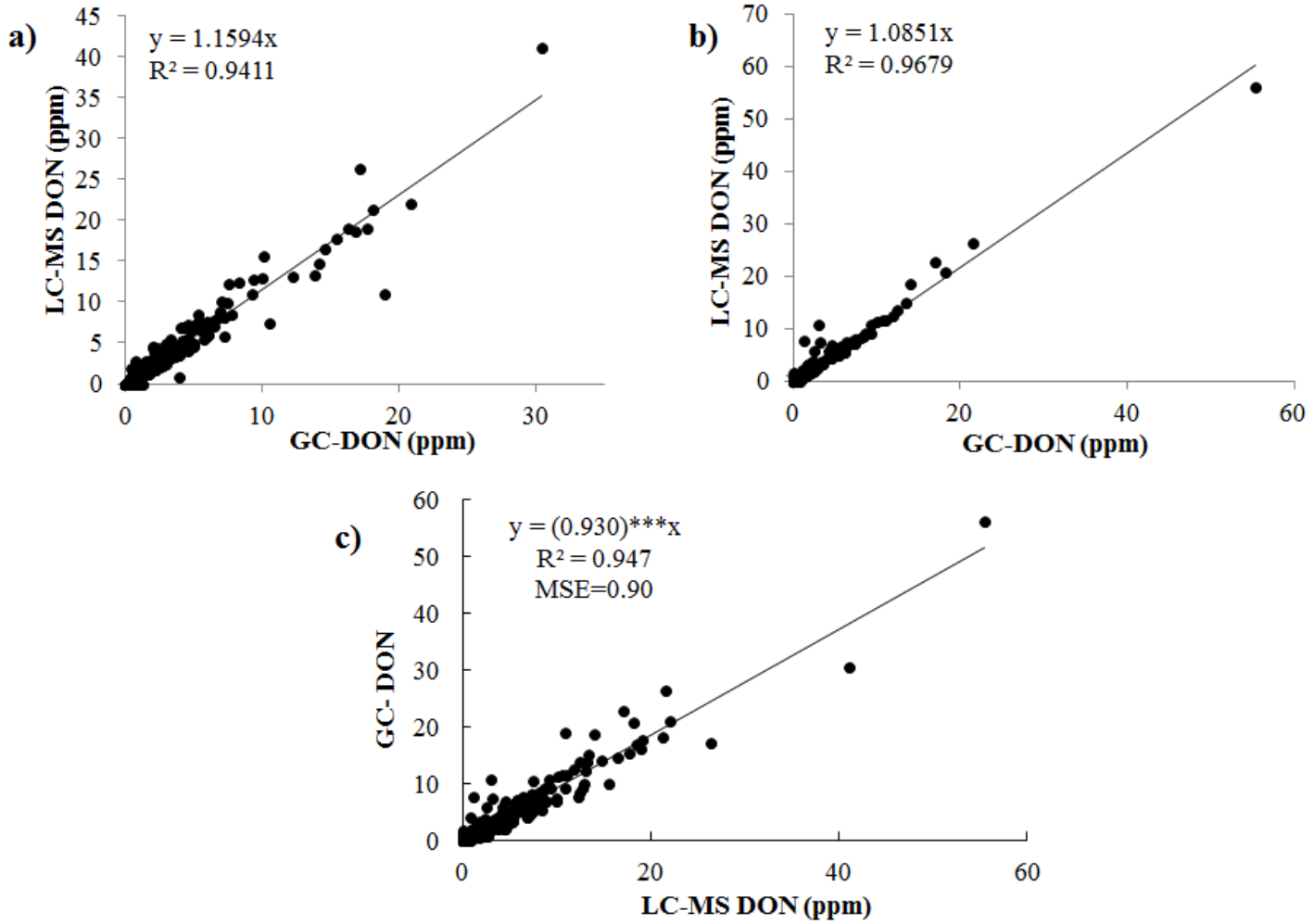
- In 2011 and 2012
  - DON: 0 mg/kg to 55 mg/kg
  - D3G: 0 mg/kg to 4.5 mg/kg

DON and D3G Occurrence

Least square mean values for deoxynivalenol (DON), deoxynivalenol-3-glucoside (D3G) and damage kernel in 2011 and 2012 survey.

State	2011				2012			
	GC-DON (mg/kg)	LC-DON (mg/kg)	D3G (mg/kg)	Damage (%)	GC-DON (mg/kg)	LC-DON (mg/kg)	D3G (mg/kg)	Damage (%)
MN	1.35**	1.74**	0.04	0.35**	0.89	0.78	0.13**	0.05
MT	0.03	0.03	0.00	0.03	0.18	0.18	0.09	0.00
ND	2.80***	3.15***	0.24***	0.63***	1.93***	1.71***	0.14***	0.06***
SD	1.35**	1.72*	0.12	0.36*	0.37	0.33	0.09	0.03

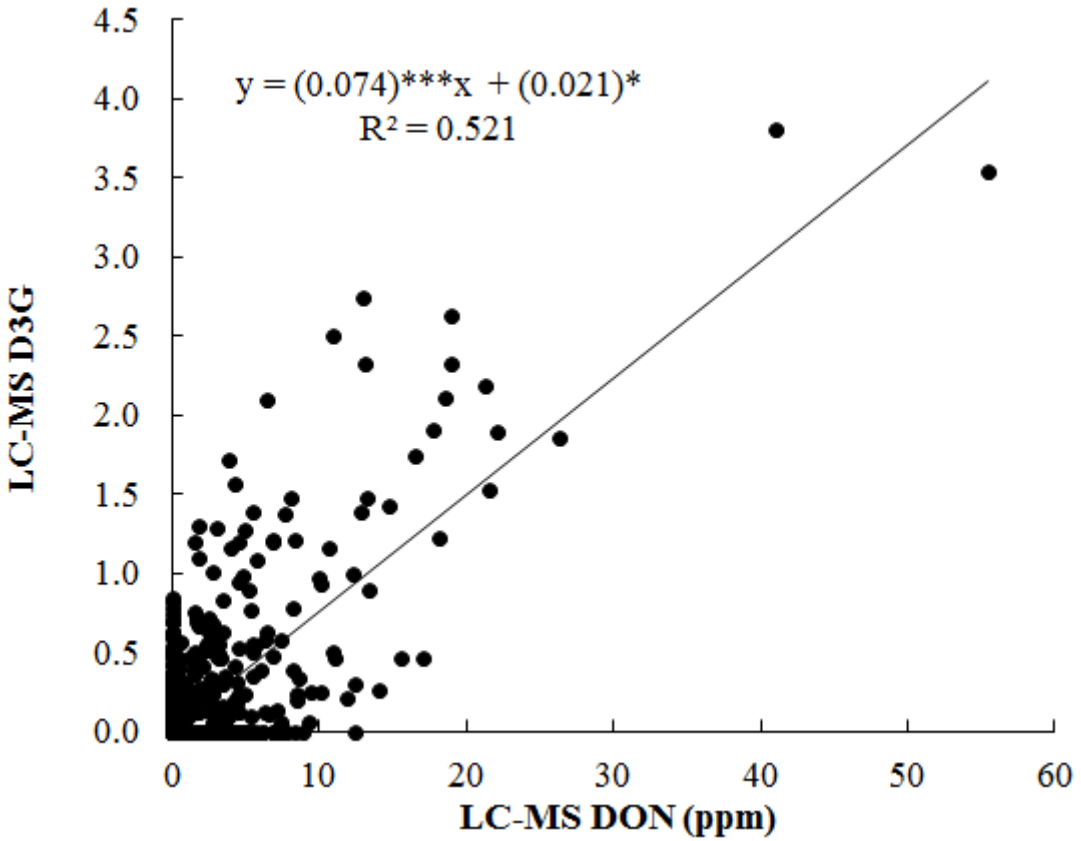
Notes: *, **, and *** means significant differences (H0: least square mean = 0) at $p < 0.05$, 0.01, and 0.001, respectively.



Correlation GC-DON and LC-MS DON content values. a) 2011 survey samples; b) 2012 survey samples and c) 2011 and 2012 survey samples combined.
*** Significantly different from 1 at P<0.001.

GC-DON and LC-MS DON Correlation

- Strong positive and significant ($P<0.001$) correlation between DON measured with GC and with LC-MS
- LC-MS method is suitable to measure DON in wheat



Correlation between DON and D3G levels in survey samples from 2011 and 2012; where ***, and * indicate that regression coefficients are significant at P<0.001 and P<0.05, respectively

Correlation between DON and D3G

- The correlation between DON and D3G in survey samples between 2011 and 2012
  - Significant ($p<0.001$) with a Moderate $R^2 = 0.521$.
- This means that the D3G production is related positively to the DON content
  - Increasing DON levels also increase the D3G level in wheat.

Correlation between DON and D3G

- The moderate R^2 value indicates that DON concentration was only partially responsible for D3G variation in this sample set
- This is due to the low correlation between DON and D3G for 2012 samples

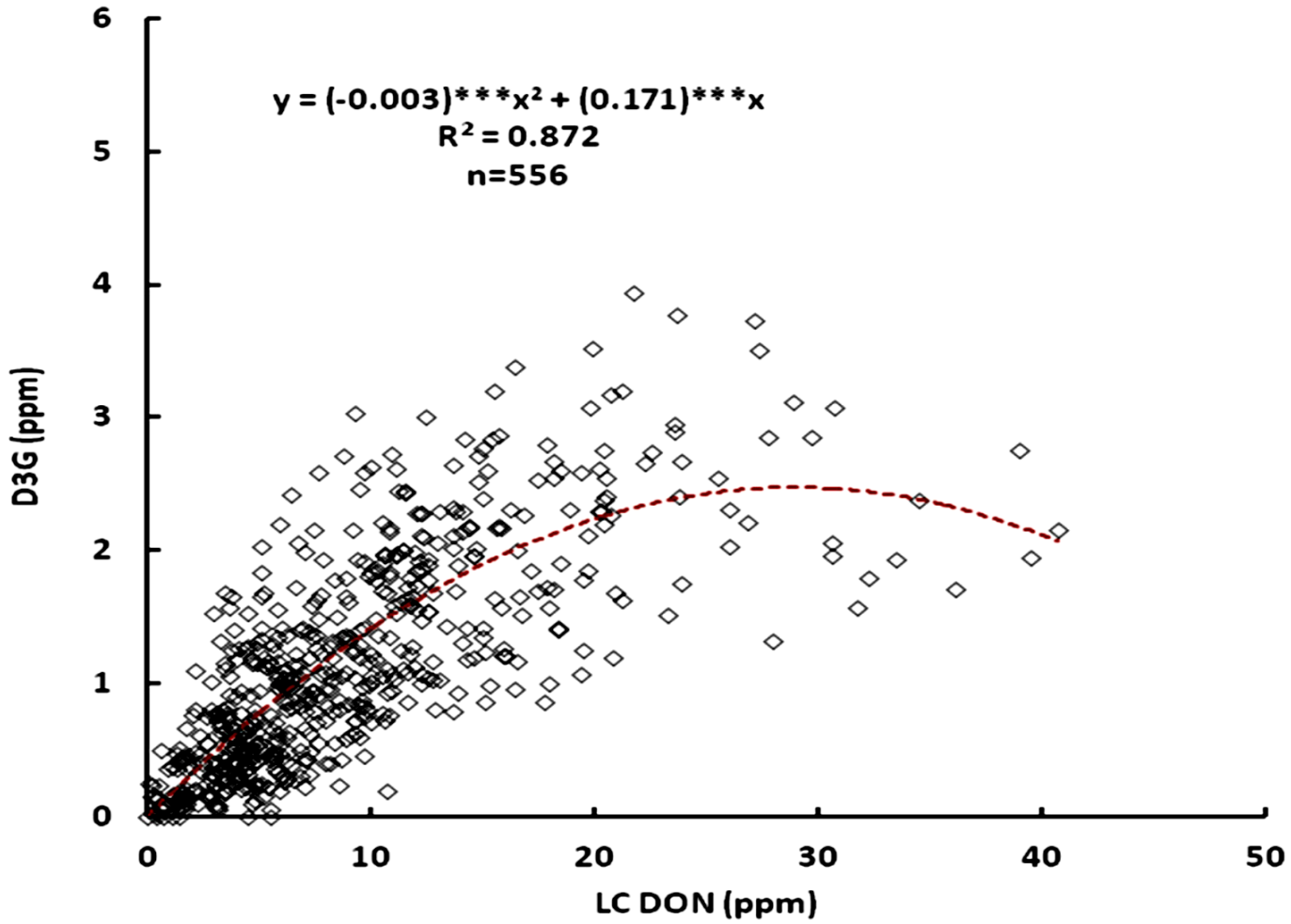
Conclusions

- DON and D3G levels varied with the survey crop year
- Growing state cause a larger effect on DON and D3G than year
- ANOVA and correlation coefficient indicate that both GC and LC-MS can be used to determine DON in HRS
- Due to the ease of the method LC-MS is more advantageous
  - Sample can be extracted and analyzed without derivatization and simultaneous determination of the D3G

DON AND D3G IN WHEAT INOCULATED WITH *FUSARIUM GRAMINEARUM*

Occurrence in Inoculated Wheat

- Different wheat lines ranging from moderately susceptible to susceptible to Fusarium head blight (FHB) were analyzed
- Experimental spring wheat lines from the University of Minnesota wheat breeding program, ranging from first year to third year yield trial lines.
- Checks: Alsen, BacUp, Roblin, Wheaton, and MN00269 (53–55 times each)
- Total of 287 Samples for each year from each location for three years
- Grown in 2008, 2009 and 2010 in St. Paul and Crookston, MN
- St. Paul: *F. graminearum* macronidia was applied by backpack sprayer at the rate of 60 mL of a 100,000 conidia/mL per 2.4 m row at anthesis and 3–4 days later
- Crookston: grain spawn inoculum was spread at the rate of 56 kg/ha at the jointing stage and with a second application one week later



Correlation between liquid chromatography deoxynivalenol (LC-DON) and deoxynivalenol-3-glucoside (D3G) values (combined 2008, 2009 and 2010). *** Significantly different from 1 at $p < 0.001$.

Relationship between DON and D3G in Inoculated Wheat

- The coefficient of determination was moderate and significant (R^2 = 0.872).
- The equation model obtained with this R^2 value was a second-order curve.
- The D3G content rose as the DON content increased in samples with DON content between 0 and 30 ppm.
- However, at higher DON concentration, a decrease in the D3G content was seen.

Means of GC-DON, LC-DON and D3G of wheat samples collected during 2008–2010 in Crookston, Saint Paul and Minnesota (MN).

Year	Location	Range	DON [a]	D3G [a]
2008	Crookston	Min ($n = 22$)	0.1	0.3
		Max ($n = 22$)	24.2	1.8
		Average ($n = 22$)	5.7	1.1
	St. Paul	Min ($n = 22$)	0.7	0.1
		Max ($n = 22$)	39.5	1.9
		Average ($n = 22$)	11.1	0.9
	MN	Min ($n = 44$)	0.1	0.1
		Max ($n = 44$)	39.5	1.9
		Average ($n = 44$)	8.4	1.0

Notes: a in ppm (parts per million); n = number of lines in each set.

Means of GC-DON, LC-DON and D3G of wheat samples collected during 2008–2010 in Crookston, Saint Paul and Minnesota (MN).

Year	Location	Range	DON [a]	D3G [a]
2009	Crookston	Min ($n = 35$)	0.0	0.0
		Max ($n = 35$)	25.7	3.8
		Average ($n = 35$)	11.9	2.1
	St. Paul	Min ($n = 35$)	0.2	0.0
		Max ($n = 35$)	21.0	1.5
		Average ($n = 35$)	4.8	0.5
	MN	Min ($n = 70$)	0.0	0.0
		Max ($n = 70$)	25.7	3.8
		Average ($n = 70$)	8.3	1.3

Notes: a in ppm (parts per million); n = number of lines in each set.

Means of GC-DON, LC-DON and D3G of wheat samples collected during 2008–2010 in Crookston, Saint Paul and Minnesota (MN).

Year	Location	Range	DON [a]	D3G [a]
2010	Crookston	Min ($n = 88$)	1.7	0.4
		Max ($n = 88$)	20.2	2.6
		Average ($n = 88$)	7.9	1.3
	St. Paul	Min ($n = 90$)	0.2	0.0
		Max ($n = 90$)	11.5	1.5
		Average ($n = 90$)	4.2	0.5
	MN	Min ($n = 178$)	0.2	0.0
		Max ($n = 178$)	20.2	2.6
		Average ($n = 178$)	6.1	0.9

Notes: a in ppm (parts per million); n = number of lines in each set.

Occurrence of DON and D3G in Inoculated Wheat

- 2008 had the highest DON but lowest D3G
- The highest D3G was seen in 2009 in Crookston
- Genetic and environmental conditions play an important role in the DON and D3G production

Occurrence of DON and D3G in Inoculated Wheat

- DON content, dependent on the growing conditions in a particular season, is affected by the wheat line
- However, the main influence on DON production is the location where the wheat line is planted, which also showed the highest influence on the D3G content

Conclusions

- The relationship between DON and D3G fit a second order curve
- Indicating that the tolerance of the wheat lines to the fusarium infection is related to the ability of the wheat line to convert the DON to D3G during the detoxification process

Conclusions

- Also, the most important factor affecting the DON and D3G formation is locality
- Which may be due to differences in gene expression of the wheat line in different environmental conditions and its response to different inoculum and development stages of the wheat during the inoculation process.

Take Home Message??

Question??

- The results obtained in this study lead us to think that the samples which presented lower FHB susceptibility (lower DON), will produce high levels of DON-3-glucoside; whilst the samples with higher FHB susceptibility will have lower levels of this ‘masked’ mycotoxin.

Does breeding for enhanced FHB resistance result in more deoxynivalenol-3-glucoside in new wheat varieties?

1. All investigated wheat cultivars can convert DON to DON-3-glucoside.
   - Hence, detoxification of DON to DON-3-glucoside is not a new trait introduced by recent resistance breeding against FHB.
2. The amount of DON-3-glucoside relative to DON contamination can be substantial (up to 35%) and is among other things dependent on genetic and environmental factors.
3. Correlation analyses showed a highly significant relationship between the amount of FHB symptoms and DON contamination:
   - breeding for FHB resistance reduces DON contamination.

Does breeding for enhanced FHB resistance result in more deoxynivalenol-3-glucoside in new wheat varieties?

4. DON contamination data are highly correlated with DON-3-glucoside concentration data
   – in other words, reduction of DON content through resistance breeding results in a concomitant reduction in DON-3-glucoside content.
5. The DON-3-glucoside/DON ratio increases with decreasing DON contamination
   – the most resistant lines with the lowest DON contamination show the highest relative level of DON-3-glucoside to DON.

References

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