toxins
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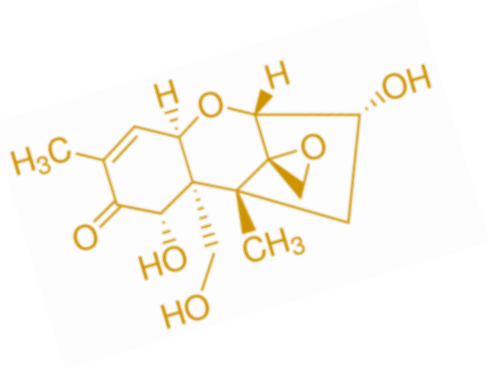

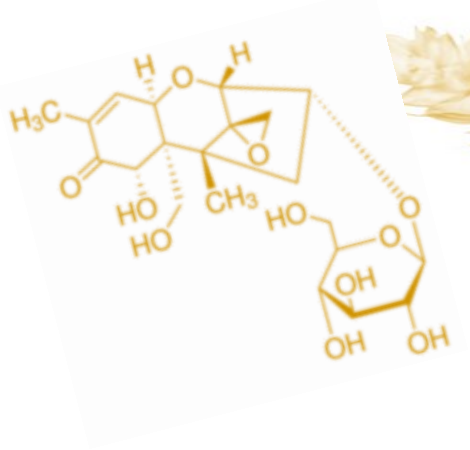

Analysis of Deoxynivalenol and Deoxynivalenol-3-glucoside in Hard Red Spring Wheat Inoculated with *Fusarium graminearum*.

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Introduction

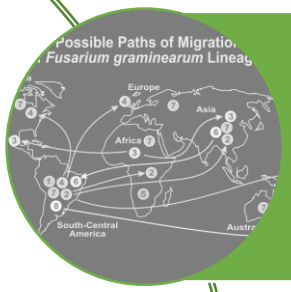
Fusarium Head Blight

Fungal patoghen cause the Fusarium Head Blight (FHB) disease in wheat and other cereal crops

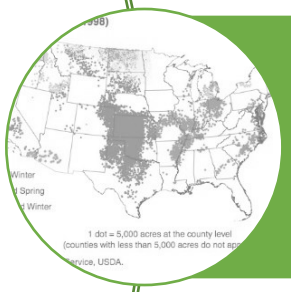
Couges et al., 2005

FHB as a worldwide problem

Fusarium



Global problem with outbreaks in the USA, Canada, Europe and Central America

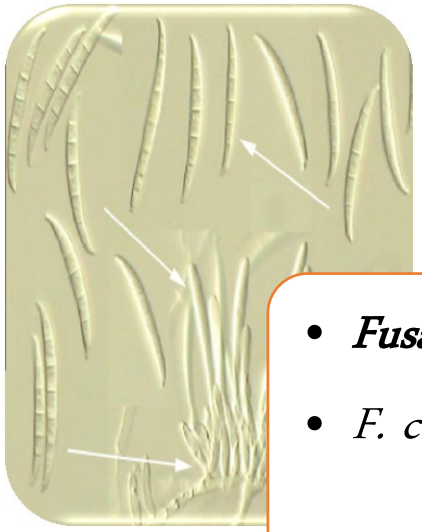


In the **USA it is causing economic losses** to the agricultural production and marketing system

Rising cost and risks for growers, inducing them to use more costly management practices or shifting to other crops

Dahl et al., 2018

FHB & *Fusarium graminearum*



- ***Fusarium graminearum***
- *F. culmorum*

FHB

Severity

- Depends on precipitation during flowering

- Deoxynivalenol (DON)
- ↑ levels during frequent rainfall & high humidity

Produce a mycotoxin

Quantitative trait loci (QTL)-FhBb1 governs resistance towards FHB

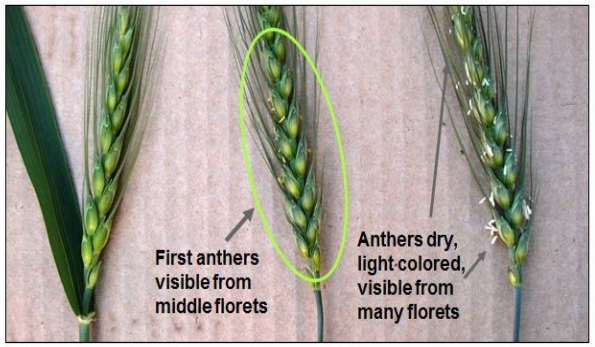
Wegulo, 2012

Factors influence germination of wheat *& Fusarium*

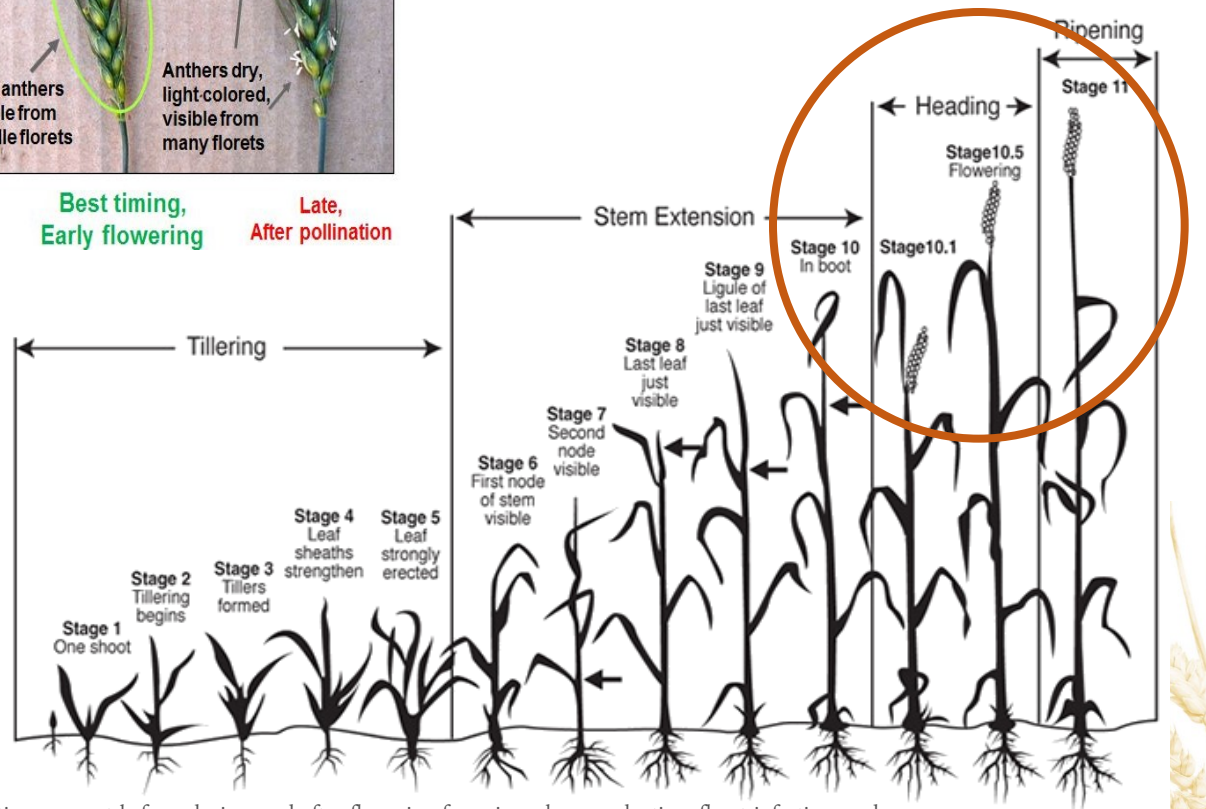
DON production

High moisture or relative humidity (>90%) and duration

Warm temperatures (5-30 ºC/59-86 ºF)



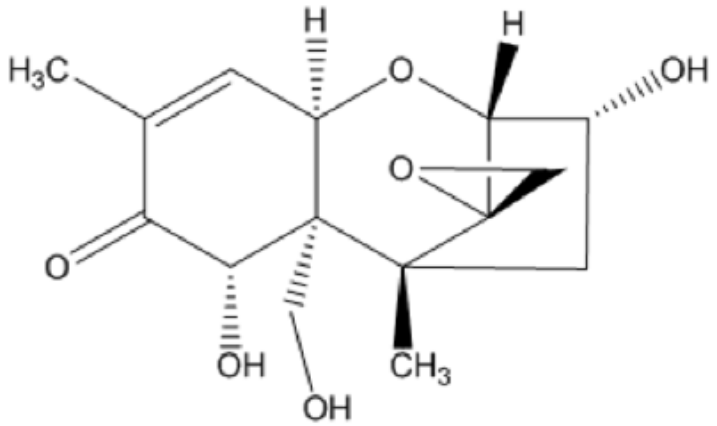
Early, Not yet flowering | Best timing, Early flowering | Late, After pollination



These conditions present before, during, and after flowering favor inoculum production, floret infection, and colonization of developing grains.

Wegulo, 2012; Schmidt-Heydt et al., 2011

Deoxynivalenol (DON)



deoxynivalenol

Most prevalent mycotoxin found in small grains grown in temperate regions around the world

The European and Food Safety Authority:

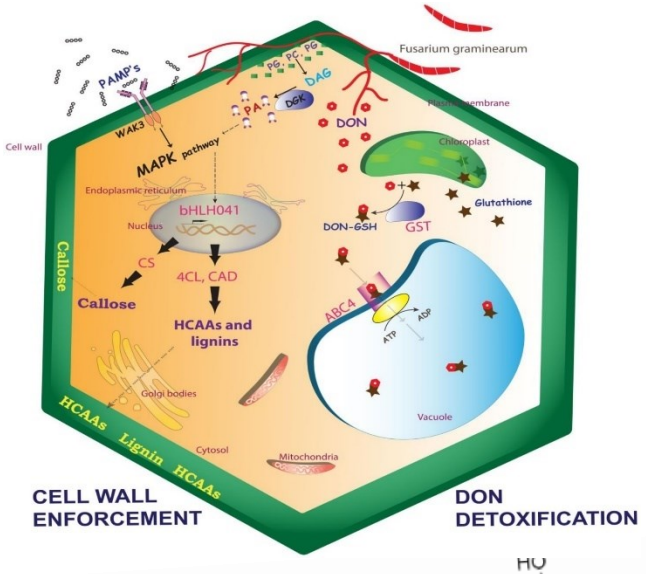
DON levels: **44.6%, 43.5% & 75.2% in unprocessed grains of undefined end-use, food and feed samples** (maize, wheat and oat)

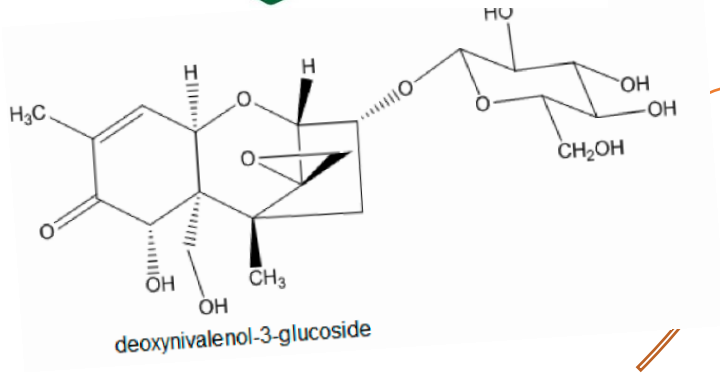
DON forms a "masked" mycotoxin named Deoxynivalenol-3-glucoside (D3G)

Mycotoxins analysis represents a major challenge in the control and inspection of foodstuffs: cereal based foods

European Food Safety Authority, 2013; Maul et al., 2012

Deoxynivalenol-3-glucoside (D3G)





D3G is one the **most common forms of masked DON**

It is **formed as part of a detoxification process in the plant** through **the glycosylation of DON** and is stored in the plant vacuoles

D3G formation is connected with glycosyltransferases

UDP-glucosyltransferase AtUGT73C5 transferred glucose to the OH group at carbon 3 of DON forming D3G in *Arabidopsis* (Poppenger et al. 2003)

It has been **found in wheat lines with low FHB susceptibility**: **D3G is converted by the quantitative trait locus QTL-Fhb1** (encodes a glycosyltransferase or regulates the expression of such enzyme)

D3G was found in approximately 5% of the food, feed, and unprocessed grains of undefined end-use by European countries. D3G should be measured because it is unknown how this can be affect the human and animals health

Rasmussen et al., 2012; Maul et al., 2012; Shin et al., 2012; Lemmens et al., 2005; European Food Safety Authority, 2013

Objectives

To analyze the DON and D3G content and **determine** if there is a **correlation between the DON and D3G production** in **samples with variation in susceptibility to FHB grown in Minnesota**, USA at **two locations and three years** of study using liquid chromatography-quadrupole time of flight mass spectrometry (**LC-MS**)

Wheat samples

- Wheat lines ranging from moderately susceptible to susceptible to FHB
- Samples collected when latest maturing lines were at harvest ripeness (14% or less moisture content)
- Experimental spring wheat lines (wheat breeding program) ranging from 1st year to 3rd year field trial lines

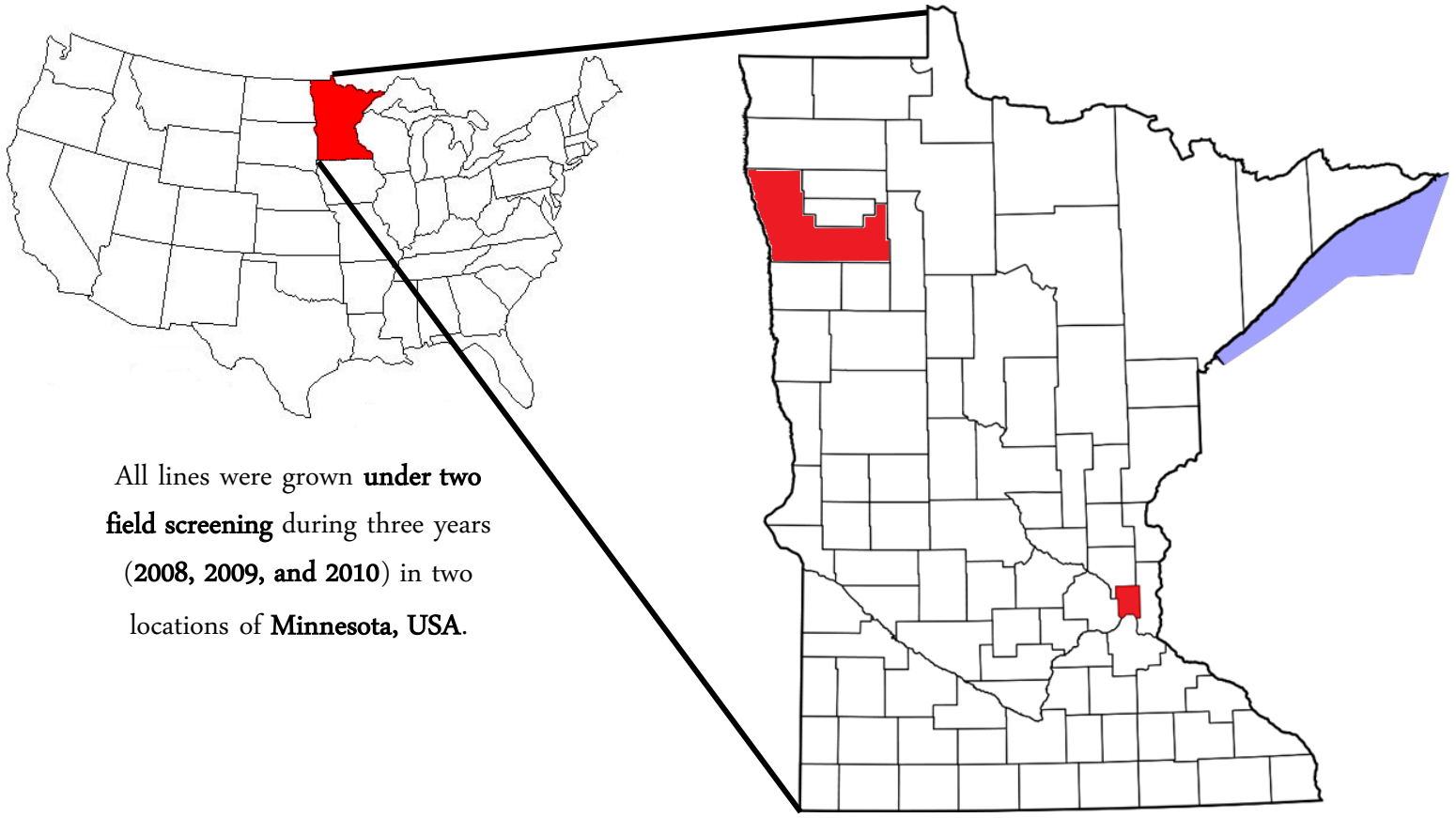
Alsen | BacUp | Roblin | Wheaton | MN00269

University of Minnesota
Driven to Discover℠

The checks are in each nursery and are represented 53-55 times in each d
The experimental lines represented 1-4 times (totaled 287 samples)

Growing locations

St. Paul (44.9441° N, 93.0852° W)



Crookson (47.7742° N, 96.6081° W)

All lines were grown **under two field screening** during three years (**2008, 2009, and 2010**) in two locations of **Minnesota, USA**.

Year	Weather conditions
2008	**During Planting:** cool and wet wet **Growing conditions:** hot and slight dry. Adequate soil slight dry. Adequate soil moisture moisture
2009	**Growing conditions:** Cooler than average with adequate than average with adequate precipitation precipitation
2010	**Growing conditions:** Cooler growing temperatures and growing temperatures and adequate precipitation adequate precipitation

Fusarium graminearum inoculation

St. Paul (44.9441° N, 93.0852° W)

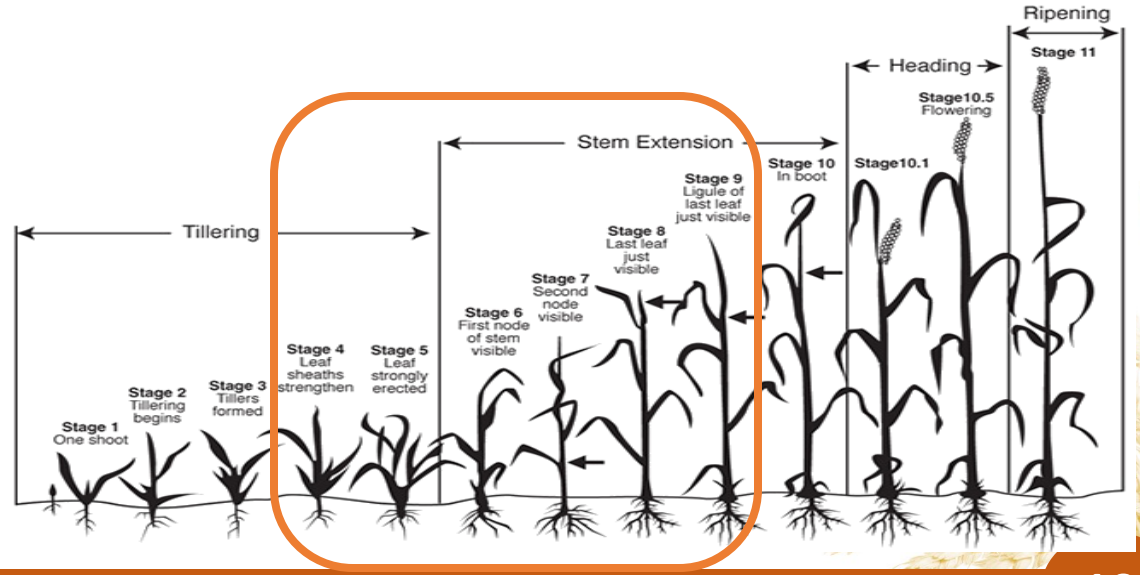
Backpack sprayer: control timing and inoculum dose

- ***F. graminearum*** **macrodinia applied at the rate of 60 mL**
- **100,000 conidia/mL per 2.4 m row at anthesis and 3-4 days later**

Both nurseries were misted periodically overnight to maintain high humidity environments

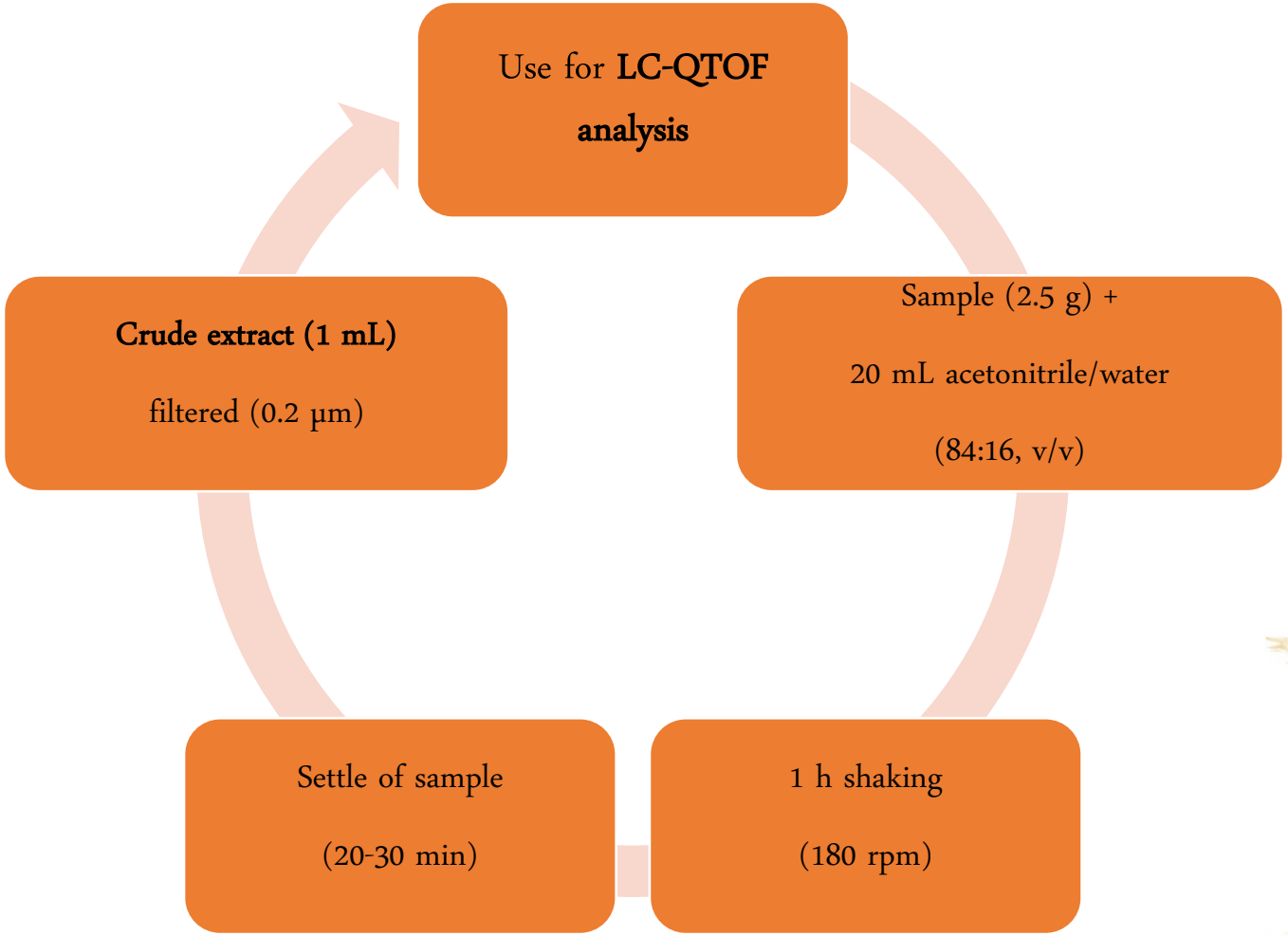
Crookson (47.7742° N, 96.6081° W)

- **Grain spawn inoculum was spread at the rate of 56 kg/ha (jointing stage). Second application one week later.**
- **Grain-spawn** inoculum **method mimics** more closely what happens in **nature**



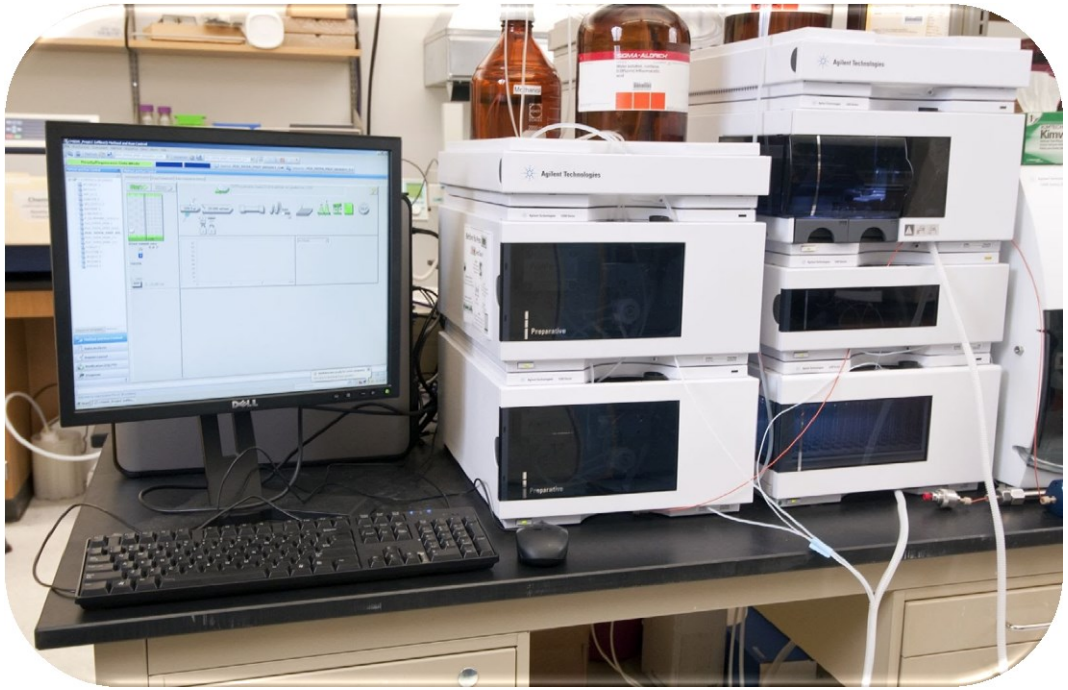
Sample preparation

0.8 mm screen



Tacke et al., 1996; Simsek et al., 2012

DON and D3G analysis with LC-QTOF



1200 series HPLC system (Agilent Technologies)

Eclipse Plus C18 column (2.1 x 100 mm, 1.8-Micron, Agilent Technologies)

Volume injection: 5 µL

LC conditions:

Column temperature: 40 ºC

Solvent system:

0.1 % formic acid/water (Solvent A)

0.1% formic acid/acetonitrile (Solvent B)

Gradient:

Purge: 100% A, flow rate: 4 mL/min, 15 s

***Flow rate:* 0.4 mL/min**

97% A and 3% B, 0.75 min

Linear increase to 100% B, 4 min

Hold time 100% B, 6 min

Re-equilibration at 97% A, 10 min

Washout with 100% A, 2 min

DON and D3G analysis with LC-QTOF

Mass spectrometer quadruple time of flight (Agilent 6500 series TOF LC/MS)

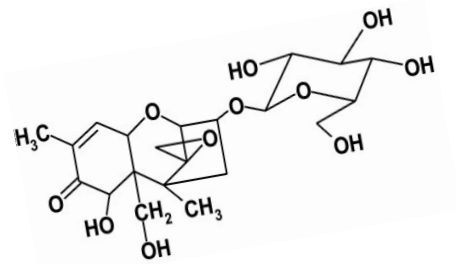
ESI interface in positive ionization mode at 300 °C

Settings:

Gas flow: 7 L/min

Nebulizer gas: 30psig

225 sheath gas temperature and 12 of sheath gas flow



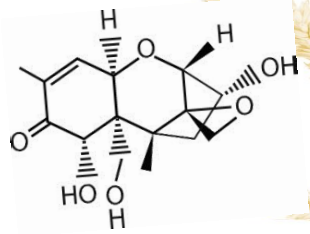
Mode MSI parameters

Minimal range(*m/z*): 100

Maximum range(*m/z*): 1700

Scan range: 2 spectra/s

Data analysis: MassHunter Qualitative analysis B.05.00 program (Agilent Technologies)



Statistical analysis

ANOVA was performed individually for three year data in SAS (V 9.2, SAS Institute, Inc., Cary, NC, USA).

The GLM was used for ANOVA in which wheat line and location were considered as fixed effects.

The main effects of wheat line and location and their interaction were tested for significance using the residual error terms.

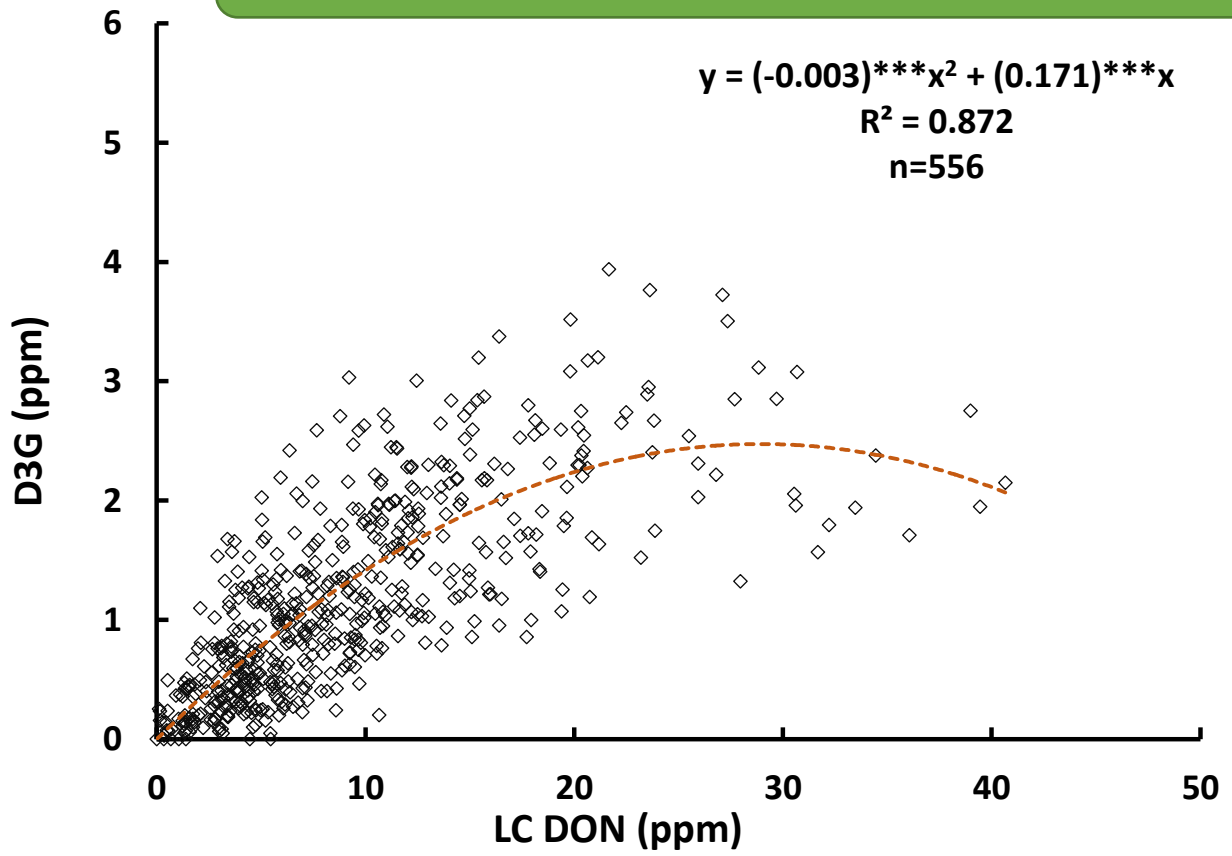
Correlation and regression was performed using "CORR" and "GLM" procedure in SAS.

Results and discussions

Relationship between DON and D3G

Samples with **lower FHB susceptibility** (↓ **DON**)→↑ **D3G**

Samples with **higher FHB susceptibility**(↑**DON**)→ ↓ **D3G**



*** significantly different from 1 at $p<0.001$

There was a correlation between DON and D3G (**second order curve**)

D3G content rose as DON content increased in samples with DON content: **0-30 ppm**

But at **higher DON** concentration, a **decrease in the D3G** content was observed

The **type of inoculum** used between locations **and inoculation at slightly different growth stages** could be causing this behavior

Effect of the line, location and the

DON: 0.1 to 33.9 ppm
D3G: 0.1 to 1.9 ppm

DON: 0.0 to 23.6 ppm
D3G: 0.0 to 3.0 ppm

DON: 0.2 to 17.7 ppm
D3G: 0.0 to 2.2 ppm
Lowest mycotoxin contents

Year	Location	Range	DON	D3G
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
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
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

ppm, n=number of lines in each set.

ANOVA table for DON and D3G of wheat samples for 2008-2010

Year	Traits	Source	DF	Sum of Squares	Mean square	F value	Pr > F
2008	DON	Line	21	3369.6	160.5	2.9	0.0095
		Loc	1	418.7	418.7	7.5	0.0122
		Line*Loc	**21**	**1169.0**	**55.7**	**3.5**	**0.015**
		Error	12	191.7	16.0		
	D3G	Line	21	12.7	0.60	3.8	0.0016
		Loc	1	0.9	0.88	5.6	0.0275
		Line*Loc	**21**	**3.3**	**0.16**	**4.7**	**0.004**
		Error	12	0.4	0.03		
2009	DON	**Line**	**34**	**5314.4**	**156.3**	**5.3**	**< 0.001**
		Loc	**1**	**1013.4**	**1013.4**	**34.3**	**< 0.001**
		Line*Loc	34	1004.5	29.5	1.3	0.177
		Error	72	1639.7	22.8		
	D3G	Line	34	27.9	0.82	2.5	0.0048
		Loc	**1**	**49.3**	**49.28**	**149.0**	**< 0.0001**
		Line*Loc	34	11.2	0.33	2.6	0.000
		Error	72	9.0	0.13		
2010	DON	**Line**	**89**	**3479.0**	**39.1**	**8.0**	**< 0.0001**
		Loc	**1**	**650.1**	**650.1**	**132.5**	**< 0.0001**
		Line*Loc	87	426.8	4.9	1.0	0.497
		Error	88	419.0	4.8		
	D3G	**Line**	**89**	**48.1**	**0.54**	**4.0**	**< 0.0001**
		Loc	**1**	**33.1**	**33.09**	**247.4**	**< 0.0001**
		Line*Loc	87	11.6	0.13	1.0	0.497
		Error	88	11.8	0.13		

DON and D3G content not statistically related to the main effects (Line*Loc)

The effects of **Line and Loc on DON** were **statistically significant** →**growing location** is the main **influence of DON** content among samples

The effects of **Line and Loc were significantly related to the DON and D3G** content in the samples, but there was not interaction between factors→ **genetic and environmental conditions play an important role in the DON and D3G production**

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DON:

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

D3G:

The **D3G production will depend** on the **tolerance/susceptibility** level of the wheat line **to FHB** and its **response to environmental conditions**

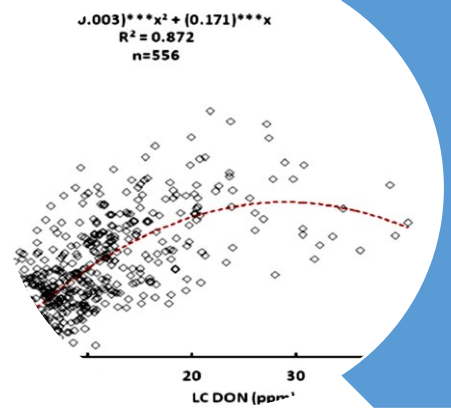
The results on **D3G content could help to increase the data about its occurrence in hard red spring wheat** in the USA, in terms of **food safety** because this masked mycotoxin **might be converted back to DON**

Pearson and Spearman's correlation coefficients between DON and D3G of two localities of Minnesota for 2008-2010

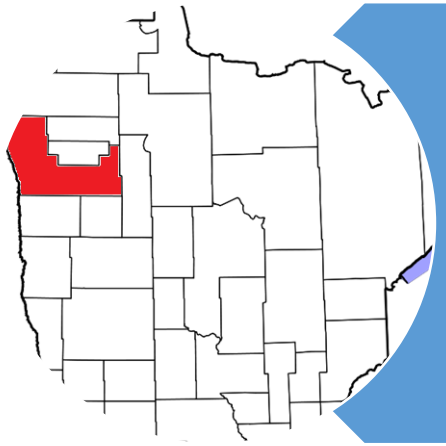
Year	Crk DON	Stp DON	Crk D3G	Stp D3G
			Pearson correlation	
2008	-	0.59 **	0.56 **	0.56 **
	0.51 *	-	0.47 *	**0.90 *****
	0.68 ***	**0.46 ***	-	0.59 **
	**0.58 ** **	**0.87 *****	**0.51 ***	-
		Spearman correlation		
			Pearson correlation	
2009	-	0.52 **	0.66 ***	0.50 **
	0.45 **	-	0.24 ^{NS}	**0.75 *****
	0.56 ***	**0.44 ** **	-	0.41 *
	0.49 **	0.69 ***	0.56 ***	-
		Spearman correlation		
			Pearson correlation	
2010	-	0.63 ***	0.69 ***	0.41 ***
	0.57 ***	-	0.51 ***	**0.67 *****
	0.55 ***	0.45 ***	-	0.48 ***
	0.34 **	0.61 ***	0.44 ***	-
		Spearman correlation		

Crk: Crookston, Stp: Saint Paul, NS: no significant. *,**,***: means correlation coefficient is significant at *p<0.05, 0.01, 0.001.*

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



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

Acknowledgments

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Thank you for listening!