Minnesota Wheat Research and Promotion Council
CROP YEAR 2013 RESEARCH REPORTING FORM
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1. PROJECT TITLE

Analysis of Bound DON in Hard Red Spring Wheat

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9: EXECUTIVE SUMMARY

Research Question:

Fusarium head blight (FHB) is a major fungal disease affecting several gramineous hosts, including wheat (Triticum aestivum L.). Hard Red Spring (HRS) wheats of the Northern Great Plains of the United States and Western Provinces of Canada are susceptible to scab, especially in years that have wetter than average growing seasons.

FHB infection often results in the production of several trichothecene mycotoxins including deoxynivalenol (DON) and nivalenol (NIV), as well as, zearalenone (ZEA) and moniliformin (MON), all of which have a range of toxicity to animals. DON is the most common mycotoxin produced by Fusarium. Plants are able to "detoxify" mycotoxins such as DON by chemically modifying and/or including them in the plant matrix. These modified versions of the toxins are called "bound mycotoxins", also known as masked deoxynivalenol. One of the most common forms of bound DON is DON- 3- glucoside. In this form, a glucose molecule has been attached to the DON molecule at carbon 3. Recent studies have shown that masked DON in wheat is a cause for concern, and escapes detection by routine analytical methods. The evidence that suggests masked DON may be released into the free form under some food processing conditions, through enzymolysis in dough processing or in digestion, raises concerns that the potential toxicity of samples is being under estimated.
This research is aimed at: 1) determining the prevalence of bound DON in commercial samples of wheat and 2) determining bound DON and native DON in Fusarium graminearum infected wheat samples.

Results:

Objective 1:
In this study, DON and D3G were measured using gas chromatographic (GC) and liquid chromatography-mass spectrometry (LC/MS) in wheat samples collected during 2011 and 2012 in USA. Results indicate that the growing region had a significant effect on the DON and D3G ($P<0.0001$). There was a positive correlation between both methods (GC and LC-MS) used for determination of DON content. DON showed a significant and positive correlation with D3G during 2011. Overall, DON production had an effect on D3G content and kernel damage, and was dependent on environmental conditions during Fusarium infection. The HRS wheat kernel quality of the 2011 and 2012 crop surveys is presented in Table 1.1. The values of percent damage and percent total defects in 2011 crop survey were higher that 2012 crop survey. According to the HRS wheat 2012 Regional Quality Report, there were differences in the environmental factors during planting, growing and harvest of crops of both years.

Least square means values of DON analyzed with GC and LC-MS, D3G determined with LC-MS, and percent damage kernels are given in Table 1.2. These variables showed significant differences between state and region mean values, indicating that HRS wheat samples collected from different state or regions might have different levels of DON and D3G. Samples collected from ND presented higher values of DON (both GC and LC-MS methods), D3G, and percent damaged kernels than other states in both years. Samples from MT presented the opposite trend.

The analysis of variance (ANOVA) indicates that state and region had significant effects on variation in DON and D3G content and percent damaged kernels in 2011 survey samples (Table 1.3). The ANOVA for 2012 survey samples showed slightly different results (Table 1.3). During 2012 the percent damaged kernels was not affected by the state, region or their interaction. The ANOVA on DON and D3G for both years indicates that the wheat growing environment affects greatly the variations in DON and D3G content.

The correlation between GC and LC-MS methods used to analyze DON during 2011 and 2012 is shown in Figure 1.2a, b. The high coefficient of determination (R^2) indicates strong, positive and significant correlation between both methods in both years (Figure 1.2). When the GC and LC-MS methods for both survey 2011 and 2012 were compared (Figure 1.2c), an R^2 of 0.947 and mean square error (MSE) of 0.90 were found. To identify relationships between mycotoxin contents and percent damaged kernels, linear and rank correlation coefficients were estimated and given in Table 1.4. The DON values determined GC and LC methods also showed very high and positive correlation for 2011 and 2012 data, individually (Table 1.4). These results mean that the LC method is as precise as or better in evaluating DON concentration of HRS wheat lines when compared to the GC method.

The correlation between DON and D3G (Figure 1.3) in survey samples from 2011 and 2012 was significant with a moderate R^2=0.521. This means that the D3G production is related positively to the DON content and increasing DON levels also increase the D3G level in wheat. The correlation among DON and D3G with damage kernel during 2011 and 2012 is given in Table 1.4 and Figure1. 4. The percent damaged kernels had very highly significant correlation with GC-DON and LC-DON (P<0.001) in 2011; damage also had a very highly significant correlation at P<0.01 with DON (GC and LC) in 2012.

The positive correlations indicate that samples which were rated to have higher percent damaged kernels had higher levels of DON in the sample. This was also shown in Figure 1.4a-b, where the scatter plot between GC-DON and damage was depicted. D3G also had a significant (P<0.05) correlation with percent damaged kernels in 2011 and 2012. This indicates that the D3G contributes to the damage in wheat although this "masked" mycotoxin is a product of the plant defense mechanisms during the infection with *Fusarium*.

The results indicated that DON levels varied with the survey crop year and they have a relationship with the kernel quality and D3G detected in wheat. Also, it was found that the growing state cause a larger effect on DON and D3G but not on percent damaged kernels. The D3G levels were significantly correlated with the percent damaged kernels, but at lower levels than the DON content. DON infection in wheat caused more effect on the kernel quality between years analyzed. Otherwise, the ANOVA and correlation coefficient indicate that both GC and LC-MS can be used to determine DON in HRS. However, due to the ease of the method (sample can be extracted and analyzed without derivitization) and simultaneous determination of the D3G, LC-MS is more advantageous.

Objective 2:
The aim of this work was to analyze DON and D3G content in different inoculated near-isogenic wheat lines grown at two locations in Minnesota, USA during three different years. Regression analysis showed positive correlation between DON content measured with LC and GC among wheat lines, locality and year.

The data obtained showed correlation between GC-DON, LC-DON and D3G. The analysis showed that there was a significant correlation (R2=0.956, P<0.001) between both methods used to determine DON content in wheat (Figure 2.1). The same trend was observed in Hard Red Spring wheat of 2011 and 2012 Crop survey samples from Montana, North Dakota, South Dakota and Minnesota, USA. So, this result indicates that it could be possible to use both LC and GC, and get accurate results between them. However, the use of LC-MS is more convenient because of the lack of derivitization step in sample preparation. Another advantage of the LC-MS is that it was possible to determine the D3G content in wheat simultaneously with the DON determination. The correlation between these parameters is shown in Figure 2. The coefficient of determination was moderate and significant (R2= 0.872). The equation model obtained with this R2 value was a second-order curve. The D3G content rose as the DON content increased in samples with DON content between 0-30 ppm.

Table 2.1 shows the means of DON (GC and LC) and D3G content of wheat lines grown in Minnesota collected during 2008, 2009 and 2010. The values for 2008 ranged from 0.7-33.1, 0.1-33.9 ppm and 0.1-1.9 ppm for GC-DON, LC-DON and D3G, respectively. Overall, the mycotoxin contents for 2009 were lower and ranged from 0.5-26.5 ppm, 0.0-23.6 ppm and 0.0-3.0 ppm, for GC-DON, LC-DON and D3G, respectively. The analysis of variance (ANOVA) for DON and D3G in the samples for individual years is showed in Table 2.2. During 2008, DON (GC and LC) and D3G contents were not statistically related to the main effects (Line and Location (Loc)) or their interaction (Line x Loc). In 2009, it was observed that Loc and the interaction between Line x Loc on GC-DON showed significant differences; while the Line and Loc on LC-DON were statistically significant. This means that between both methods of DON determination, growing location has the main effect on DON content among samples. Concerning the D3G, during 2009 only the Loc had a significant effect. On the other hand, during 2010, the main effects were significantly related to the DON (both GC and LC) and D3G content in the samples, whereas the interaction between factors was not significant. These results indicated that genetic and environmental conditions play an important role in the DON and D3G production in 2010.

The Pearson and Spearman's correlations were used to determine the correlation between DON and D3G in wheat grown at two localities of Minnesota, and are shown in Table 2.3. During 2008 the ANOVA did not

show any significant effect of the Loc between these two parameters. However, the Spearman's correlation

showed positive correlation coefficients with significant levels (P< 0.05, 0.01 and 0.001) among DON (GC and LC) and D3G from Crookston and St. Paul (Table 2.3). In 2008 for samples grown at Crookston and St. Paul, the GC-DON showed a very highly significant (P<0.001) correlation with LC-DON and D3G. In 2009, a significant correlation among GC-DON and LC-DON between localities was seen (Table 2.3). With respect to D3G, the Pearson correlation indicated that there were no significant correlation with GC-DON and LC-DON from Saint Paul. However, the Spearman correlation found a coefficient correlation of 0.57 (P<0.001) and 0.44 (P<0.01) for GC-DON and LC-DON, respectively.

This may be related to the trend (second order curve) observed among the DON and D3G content among localities and years of study obtained in Figure 2.2. The low significance level could be due to the different kind of inoculum used to infect the wheat lines, differences in the growth stage development of the plant when the inoculum was applied and the differences in the weather conditions between Crookston and St. Paul during the three years of study.

In conclusion, there was a positive and high correlation between GC and LC methods for DON determination among year, locality and wheat line. The relationship between DON and D3G fit a second order curve, indicating that the tolerance of the wheat lines to the Fusarium infection has a relationship to the ability of the wheat line to convert the DON to D3G during the detoxification process. Also, the most important factor affecting the DON and D3G formation is the 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 stage of the wheat during the inoculation process.

Application/Use:

There is a very limited research on analysis of masked DON in HRS wheat in our region. The proposed research is the first of its kind in linking the masked DON level in FHB-infected HRS wheat and the quality defects in wheat kernels and end-products. As such, the research results will be helpful in quantifying the extent of FHB damage to wheat quality and hence its marketability. Thus, our research results will be directly helpful to wheat growers and wheat processors in both understanding the actual impact of FHB in terms of the severity of the damage and its direct economic consequence. Furthermore, state and federal regulatory agencies will be able to use the research results in establishing guidelines for both domestic and international marketing of the HRS wheat.

Materials and Methods:

For objective 1, the hard red spring (HRS) wheat from 2011 and 2012 HRS wheat crop survey samples were used as raw material. A total of 441 and 436 samples were selected as wheat grader samples from the 2011 and 2012 HRS wheat crop surveys, respectively, and used in this study. The samples were collected based on production data obtained from the National Agricultural Statistics Service for the 16 regions in the 4 state HRS wheat growing regions (Figure 1.1). The Montana (MT), North Dakota (ND), South Dakota (SD) and Minnesota (MN) state office of the National Agricultural Statistics Service obtained wheat samples during harvest directly from growers either in the fields or farm bins and local elevators. Samples from the 2011 Crop Survey represented a high FHB infection and incidence of DON, while 2012 Crop Survey samples represented wheat with low FHB infection.

For objective 2, Different wheat lines ranging from moderately susceptible to susceptible to Fusarium Head Blight (FHB) were analyzed. All lines were grown under two field screening during 2008, 2009 and 2010 in two locations of Minnesota, USA. At the St. Paul location (StP), F. graminearun 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. At the Crookston location (Crk), grain spawn inoculum was spread at the rate of 56 kg/ha at the jointing stage and a second application one week later. Both nurseries were misted periodically overnight to maintain high humidity environments. The samples were ground and conserved under refrigeration until their analysis.

The kernel quality based on non-grading factors consisted of the determination of the protein content (expressed in 12% moisture basis, Method 39.10.01), falling number expressed in seconds (Method 56.81.03), both approved methods of the AACC [19] and thousand kernel weight determined on a 10 g sample of cleaned wheat (free of foreign material and broken kernels) counted by electronic seed counter. The wheat grade and class of the samples was determined by a licensed grain inspector for the Official

United States Standards for Grain. North Dakota Grain Inspection Service, Fargo, ND, provided grades for

composite wheat samples. The final grade of the samples was based on dockage (elimination of all material other than wheat), shrunken and broken kernels and percent damaged kernels, test weight measured as pounds per bushel (lb/bu) (Method 55-10, AACC) and percent vitreous kernels (percentage of kernels having vitreous endosperm), as well as the summation of these defects referred to as total defects using an official procedure of USDA (United Stated Department of Agriculture).

Free DON was determined using the procedure of Tacke and Casper (1996) with some modifications (Simsek et al 2012) to extract and derivatize the DON for analysis by GC-ECD, which is available in Dr. Simsek's laboratories. Free DON and bound DON will be determined using a liquid chromatrography – quadrapol-time-of-flight (LC-QTOF) system, which is available in Dr. Simsek's laboratories. The extraction of DON and bound DON for LC-QTOF analysis will also be done according to the method of Tacke and Casper (1996). However, after extraction the samples will be directly analyzed with the LC-QTOF, without further sample preparation. Samples for objective 1 were obtained from a regional wheat quality survey, while those for objective 2 were obtained from the North American Wheat Scab Evaluation Nursery (collaboration with Dr. Jim Anderson and Mohamed Mergoum).

For objective 1, analysis of variance (ANOVA) was performed for individual years using the 'MIXED' procedure in SAS (V 9.2, SAS Institute Inc., Cary, NC). The model for ANOVA was a nested fixed model in which region was nested in state and city was nested in region. Least square mean values were estimated using the 'LSMEAN' option. Correlation and regression was performed using 'CORR' and 'GLM' procedures in SAS, respectively.

For objective 2, analysis of variance (ANOVA) was performed individually for three year data. The 'GLM' procedure in SAS (V 9.2, SAS Institute Inc., Cary, NC) 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' procedures in SAS, respectively.

Economic Benefit to a Typical 500 Acre Wheat Enterprise:

According to the Council for Agricultural Science and Technology the annual cost to the United States because of the DON corruption of food crops is $637 million in 2003. In another research, direct losses to wheat producers in United States owing to Fusarium Head Blight is approximated as about $260 million in a year and total economic losses for all small grains in period of 1998-2000 is $ 2.7 billion.

10: RELATED RESEARCH

Previously Dr. Simsek has published a study on the determination of DON and bound DON in samples taken during milling and baking processing of wheat.

11: RECOMMENDED FUTURE RESEARCH

Future research includes refining the LC-QTOF method for more sensitive and accurate determination of the DON and bound DON. Also, additional research is needed to develop methods for simultaneous measurement of other bound forms of DON and other mycotoxins and their conjugates.

12: APPENDIX

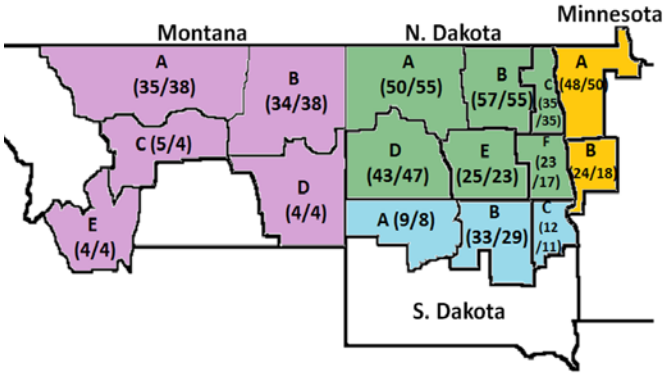

Fig 1.1. Distribution of hard red spring (HRS) wheat samples from the 2011 and 2012 Crop Surveys from Montana, North Dakota, Minnesota and South Dakota. A, B, C, D, E and F: regions in which the samples were collected from each state. The numbers inside the parenthesis represent the number of samples taken, from left to right: 2011 Crop Survey and 2012 Crop Survey.

Table 1.1. Mean, standard deviation (SD), minimum (MIN) and maximum (MAX) values for wheat kernel quality characteristics for 2011 and 2012 crop survey samples.

Factors	2011				2012			
	Mean	SD	MIN	MAX	Mean	SD	MIN	MAX
Dockage (%)[a]	1.1	1.4	0.0	13.4	1.1	1.2	0.0	13.3
Shrunken & broken[a]	1.6	1.4	0.0	10.0	1.2	1.1	0.0	10.1
Damage (%)[a]	0.5	0.8	0.0	10.6	0.1	0.2	0.0	2.8
DHV (%)[a]	75.8	21.9	5.0	99.0	74.5	28.4	2.0	99.0
Test weight (lbs/bu)[a]	60.0	2.4	52.5	65.7	60.9	1.9	53.9	65.1
Total defects (%)[a]	2.1	1.7	0.1	13.1	1.3	1.1	0.0	10.1
Protein (12% mb)[b]	14.8	1.3	10.2	18.6	14.6	1.4	10.3	20.1
Falling # (sec)[b]	386.3	44.1	226.0	621.0	429.3	48.8	238.0	654.0
1000 KWT (g)[b]	26.7	4.1	16.8	40.0	29.1	4.1	16.6	44.1

a) Grading factors used to evaluate the kernel quality to ensure general standards of acceptance in flour or semolina production. b) Non-grade factors used to evaluate the kernel quality. mb= moisture basis.

Table 1.2. Least square mean values for DON, D3G and damage kernel in 2011 and 2012 survey.

State	2011				2012			
	GC-DON	LC-DON	D3G	Damage (%)	GC-DON	LC-DON	D3G	Damage (%)
MN	**1.35 ****	**1.74****	0.04	**0.35****	0.89	0.78	**0.128****	0.05
MT	0.03	0.03	0.00	0.03	0.18	0.18	0.090	0.00
ND	**2.80*****	**3.15*****	**0.24*****	**0.63*****	**1.93*****	**1.71*****	**0.142*****	**0.06*****
SD	**1.35****	**1.72***	**0.12**	**0.36***	0.37	0.33	0.085	0.03
Region								
MN-A	1.07	1.21	0.01	0.23	0.82	0.72	**0.124***	**0.08***
MN-B	**1.63***	**2.27****	0.06	**0.47***	0.96	0.84	0.133	0.02
MT-B	0.00	0.00	0.00	0.05	0.04	0.05	**0.176****	0.00
ND-A	**3.89*****	**4.26*****	**0.31*****	**0.75*****	**6.91*****	**5.97*****	**0.295*****	**0.15*****
ND-B	**3.77*****	**4.32*****	**0.30*****	**0.69*****	**2.48*****	**2.22*****	**0.174*****	**0.12*****
ND-C	**2.91*****	**3.36*****	**0.30*****	**0.61*****	0.66	0.60	0.060	0.06
ND-D	1.08	1.10	0.02	**0.61*****	0.34	0.35	0.094	0.01
ND-E	**2.19****	**2.54****	0.09	**0.66*****	0.71	0.72	0.096	0.02
ND-F	**3.00****	**3.34*****	**0.39*****	**0.49****	0.47	0.38	0.133	0.01
SD-B	**2.41*****	**3.20*****	**0.29****	**0.57****	0.22	0.23	0.097	0.00

*, **, and *** means significant differences (H0: least square mean=0) at P<0.05, 0.01, and 0.001, respectively. Regions with no significant correlation are not shown. Values of DON and D3G are in ppm (parts per million).

Table 1.3. Mean square values of state (ST), region (Rga) and county (CTY) on DON, D3G and damaged kernel in 2011 and 2012 survey.

Source	Degrees of Freedom	Mean Square			
		GC-DON	LC-DON	D3G	Damage
Year 2011					
ST	3	221.3***	282.8***	2.15***	10.19***
Rga (ST)	12	28.0**	40.4***	0.43*	0.45
CTY (Rga*ST)	100	8.9	13.2	0.19	0.44
Residual	320	9.7	14.0	0.24	0.70
Year 2012					
ST	3	151.2***	111.2***	0.15***	0.22**
Rga (ST)	12	104.3***	72.8***	0.12	0.12***
CTY (Rga*ST)	100	14.5	11.3	0.07	0.04
Residual	320	12.0	10.9	0.07	0.04

*, **, and *** means F values are significant at P<0.05, 0.01, and 0.001, respectively.

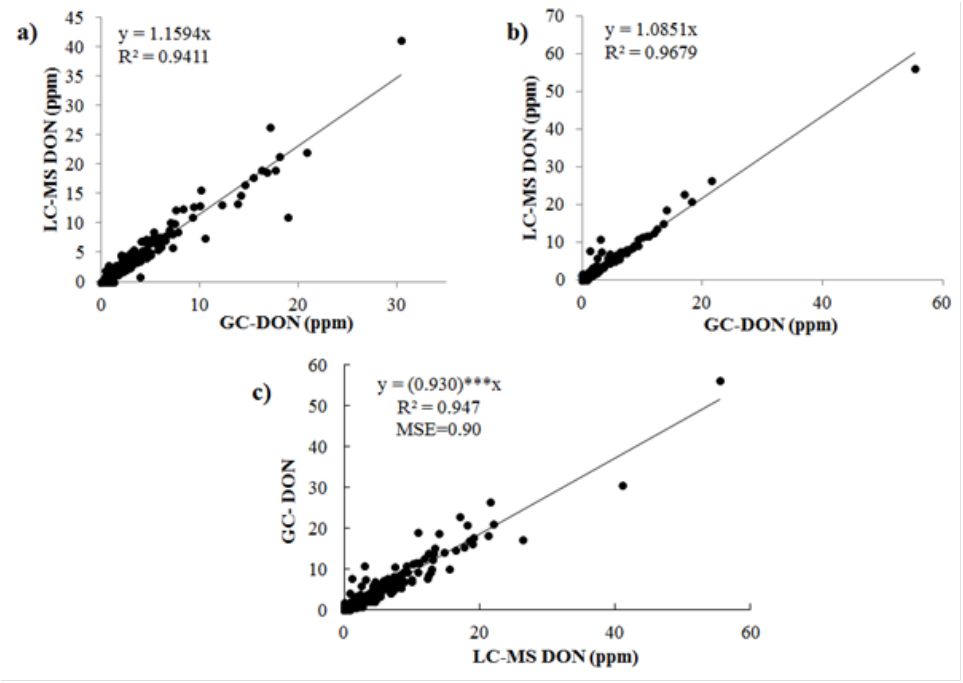

Fig 1.2. Correlation GC-DON and LC-MS DON content values. a) 2011 survey samples; b) 2012 survey samples and c) 2011 and 2012 survey samples combined. *** Significantly different from 1 at P<0.001.

Table 1-4. Pearson linear and Spearman rank correlation coefficients between DON, D3G and damage kernel for regions

	Variables	2011				2012			
		GC-DON	LC-DON	D3G	Damage	GC-DON	LC-DON	D3G	Damage
			Linear correlation						
2011	GC-DON	-	**0.99*** **	**0.91*** **	**0.91*** **	0.63**	0.64**	0.53*	0.68**
	LC-DON	**0.99*** **	-	**0.91*** **	**0.91*** **	0.59*	0.59*	0.50^{NS}	0.65**
	D3G	**0.94*** **	**0.92*** **	-	0.74**	0.47^{NS}	0.47^{NS}	0.41^{NS}	0.47^{NS}
	Damage	**0.89*** **	**0.89*** **	**0.87*** **	-	0.52*	0.53*	0.40^{NS}	0.54*
2012	GC-DON	0.71**	0.73**	0.53*	0.62*	-	**1.00*** **	**0.83*** **	**0.79*** **
	LC-DON	0.72**	0.74**	0.53*	0.66**	**0.99*** **	-	**0.83*** **	**0.79*** **
	D3G	0.42^{NS}	0.39^{NS}	0.42^{NS}	0.34^{NS}	0.41^{NS}	0.37^{NS}	-	0.60*
	Damage	0.61*	0.62*	0.58*	0.57*	0.72**	0.69**	0.28^{NS}	-
		Rank correlation							

*, **, and *** means correlation coefficient is significant at P<0.05, 0.01, and 0.001, respectively. NS: Not significant (P≥0.05).

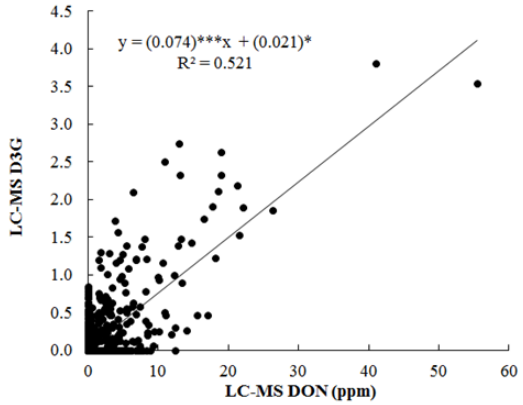

Fig 1.3. Correlation between DON and D3G levels in survey samples from 2011 and 2012; where ***, and * indicate that regression coefficients are significant at P<0.001 and P<0.05, respectively

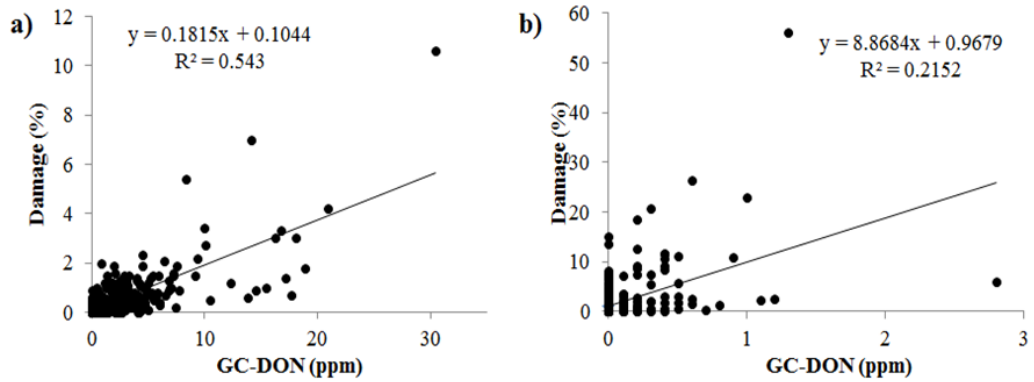

Fig 1.4. Correlation between GC-DON and damage levels in survey samples from a) 2011 and b) 2012.

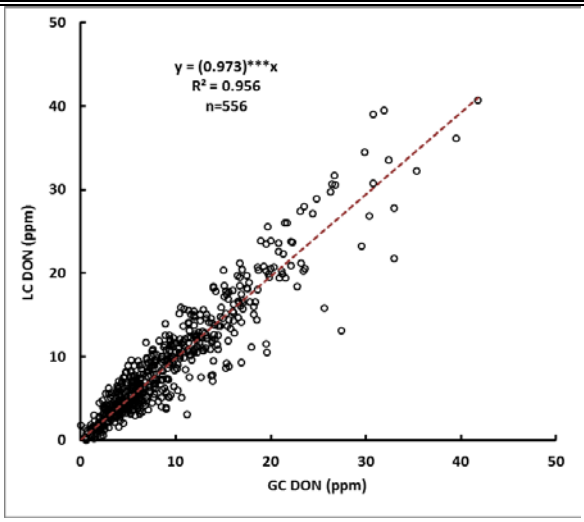

Figure 2.1. Correlation between GC-DON and LC-DON (combined 2008.2009 and 2010). *** Significantly different from 1 at P<0.001.

Table 2.1. Means of GC-DON, LC-DON and D3G of wheat samples collected during 2008-2010 in Crookston, St. Paul and Minnesota (MN).

Year	Location		GC-DON[a]	LC-DON[a]	D3G[a]
2008	**Crookston**	**Min (n=22)**	0.7	0.1	0.3
		Max (n=22)	21.4	24.2	1.8
		Average (n=22)	7.2	5.7	1.1
	St. Paul	**Min (n=22)**	0.7	0.7	0.1
		Max (n=22)	33.1	39.5	1.9
		Average (n=22)	9.8	11.1	0.9
	MN	**Min (n=44)**	0.7	0.1	0.1
		Max (n=44)	33.1	39.5	1.9
		Average (n=44)	8.5	8.4	1.0
2009	**Crookston**	**Min (n=35)**	5.0	0.0	0.0
		Max (n=35)	25.4	25.7	3.8
		Average (n=35)	13.7	11.9	2.1
	St. Paul	**Min (n=35)**	0.5	0.2	0.0
		Max (n=35)	26.5	21.0	1.5
		Average (n=35)	5.4	4.8	0.5
	MN	**Min (n=70)**	0.5	0.0	0.0
		Max (n=70)	26.5	25.7	3.8
		Average (n=70)	9.5	8.3	1.3
2010	**Crookston**	**Min (n=88)**	1.9	1.7	0.4
		Max (n=88)	19.5	20.2	2.6
		Average (n=88)	7.4	7.9	1.3
	St. Paul	**Min (n=90)**	0.1	0.2	0.0
		Max (n=90)	12.6	11.5	1.5
		Average (n=90)	4.3	4.2	0.5
	MN	**Min (n=178)**	0.1	0.2	0.0
		Max (n=178)	19.5	20.2	2.6
		Average (n=178)	5.9	6.1	0.9

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

Table 2.2. 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	**GC-DON**	Line	21	2787.7	132.7	2.8	0.0111
		Loc	1	96.6	96.6	2.0	0.1678
		Line*Loc	21	994.0	47.3	2.4	0.059
		Error	12	235.6	19.6		
	LC-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	**GC-DON**	Line	34	5749.3	169.1	3.6	0.0002
		Loc	1	1374.3	1374.3	29.0	**< 0.0001**
		Line*Loc	34	1612.9	1612.9	3.2	**< 0.0001**
		Error	72	1068.5	1068.5		
	LC-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	**GC-DON**	Line	89	3472.5	39.0	10.0	**< 0.0001**
		Loc	1	441.7	441.7	113.6	**< 0.001**
		Line*Loc	87	338.4	3.9	1.2	0.242
		Error	88	294.6	3.3		
	LC-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		

Table 2.3. Pearson and Spearman's correlation coefficients between DON and D3G of two localities of Minnesota for

2008-2010.

Year	Crk GC-DON	Stp GC-DON	Crk LC-DON	Stp LC-DON	Crk D3G	Stp D3G
2008	**Pearson correlation**					
Crk GC-DON	-	0.61 **	**0.90 *****	0.56 **	**0.73 *****	0.58 **
Stp GC-DON	0.58 **	-	0.65 **	**0.96 *****	0.45 *	**0.88 *****
Crk LC-DON	**0.93 *****	0.60 **	-	0.59 **	0.56 **	0.56 **
Stp LC-DON	0.49 *	**0.91 *****	0.51 *	-	0.47 *	**0.90 *****
Crk D3G	**0.71 *****	0.48 *	**0.68 *****	0.46 *	-	0.59 **
Stp D3G	0.55 **	**0.92 *****	0.58 **	**0.87 *****	0.51 *	-
	Spearman correlation					
Year	**Crk GC-DON**	**Stp GC-DON**	**Crk LC-DON**	**Stp LC-DON**	**Crk D3G**	**Stp D3G**
2009	**Pearson correlation**					
Crk GC-DON	-	**0.63 *****	**0.71 *****	**0.62 *****	0.47 **	**0.57 *****
Stp GC-DON	**0.74 *****	-	0.53 **	**0.96 *****	0.30 NS	**0.76 *****
Crk LC-DON	**0.71 *****	0.50 **	-	0.52 **	**0.66 *****	0.50 **
Stp LC-DON	**0.65 *****	**0.90 *****	0.45 **	-	0.24 NS	**0.75 *****
Crk D3G	0.52 **	**0.57 *****	**0.56 *****	0.44 **	-	0.41 *
Stp D3G	**0.57 *****	**0.77 *****	0.49 **	**0.69 *****	**0.56 *****	-
	Spearman correlation					
Year	**Crk GC-DON**	**Stp GC-DON**	**Crk LC-DON**	**Stp LC-DON**	**Crk D3G**	**Stp D3G**
2010	**Pearson correlation**					
Crk GC-DON	-	**0.73 *****	**0.85 *****	**0.67 *****	**0.71 *****	**0.45 *****
Stp GC-DON	**0.67 *****	-	**0.62 *****	**0.89 *****	**0.56 *****	**0.72 *****
Crk LC-DON	**0.77 *****	**0.54 *****	-	**0.63 *****	**0.69 *****	**0.41 *****
Stp LC-DON	**0.63 *****	**0.87 *****	**0.57 *****	-	**0.51 *****	**0.67 *****
Crk D3G	**0.65 *****	**0.46 *****	**0.55 *****	**0.45 *****	-	**0.48 *****
Stp D3G	**0.42 *****	**0.69 *****	0.34 **	**0.61 *****	**0.44 *****	-
	Spearman correlation					

Crk: Crookston, Stp: Saint Paul, DON: deoxynivalenol, D3G: deoxynivalenol-3-glucoside
NS: No significant. *, **, and *** means correlation coefficient is significant at P<0.05, 0.01, and 0.001, respectively

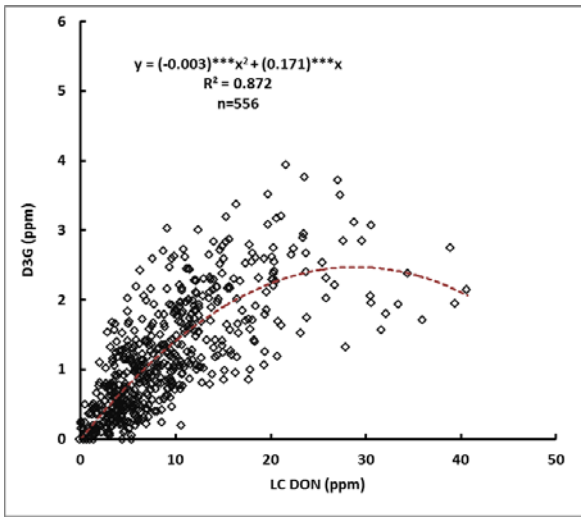

Figure 2.2. Correlation between LC-DON and D3G values (combined 2008, 2009 and 2010). *** Significantly different from 1 at P<0.001.