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Analysis of deoxynivalenol and deoxynivalenol-3-glucosides content in Canadian spring wheat cultivars inoculated with *Fusarium graminearum*

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ABSTRACT

Contamination of wheat grains with *Fusarium* mycotoxins and their modified forms is an important issue in wheat industry. The objective of this study was to analyse the deoxynivalenol (DON) and deoxynivalenol-3-glucosides (D3G) content in Canadian spring wheat cultivars grown in two locations, inoculated with a mixture of 3-acetyldeoxynivalenol (3-ADON)-producing *Fusarium graminearum* strains and a mixture of 15-acetlyldeoxynivalenol (15-ADON)-producing *F. graminearum* strains. According to the analysis of variance, significant differences were observed among the cultivars for *Fusarium* head blight (FHB) disease index, *Fusarium*-damaged kernel percentage (%FDK), DON content and D3G content. When the effect of chemotype was considered, significant differences were observed for FHB disease index, FDK percentage and DON content. The D3G content and D3G/DON ratio were not significantly different between the chemotypes, except for D3G content at the Winnipeg location. The Pearson correlation coefficient between DON and D3G was 0.84 and 0.77 at Winnipeg and Carman respectively. The highest D3G/DON ratio was observed in cultivars Carberry (44%) in Carman and CDC Kernen (63.8%) in Winnipeg. The susceptible cultivars showed lower D3G/DON ratio compared with the cultivars rated as moderately resistant and intermediate. The current study indicated that Canadian spring cultivars produce D3G upon *Fusarium* infection.

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Deoxynivalenol; deoxynivalenol-3-glucosides; masked mycotoxins; *Fusarium* head blight

Introduction

Fusarium head blight (FHB) continues to threaten the wheat industry in Canada and other major wheat-growing countries. The contamination of wheat products with *Fusarium* mycotoxins, mainly deoxynivalenol (DON), poses a major issue for global food and feed safety. DON acts as a virulence factor in disease development and facilitates the spread of the fungus from the point of invasion (Desjardins 2006). Upon production in the infected tissue these mycotoxins inhibit protein synthesis, alter cell signalling, membrane permeability and ultimately cause cell death (Bai et al. 2002; McCormick 2003). Plants have multifaceted detoxification systems to cope with a wide variety of xenobiotics such as mycotoxins (Boutigny et al. 2008; Audenaert et al. 2013; Berthiller et al. 2013). They are equipped with two main detoxification machineries: chemical modification and compartmentation (Coleman et al. 1997). Chemical modification consists of two main phase reactions. Phase I reaction involves hydrolysis or oxidation of xenobiotics; the resulting compounds are either more toxic than or as toxic as the parent compound. Phase II reaction involves conjugation of xenobiotics. In these reactions, residues such as glucose, malonic acid and glutathione are bound to the functional groups of xenobiotics. The final converted products from phase II reactions are either non-toxic or less phytotoxic than the parent compounds (Coleman et al. 1997; Cole & Edward 2000). These mycotoxin derivatives are not screened by routine analytical techniques as their structure has changed in the plants. But they can be reactivated or reconverted to the parent compound in the digestive tract of humans and animals. Therefore, these compounds are known as 'masked mycotoxins' (Berthiller et al. 2013). Recently Rychlik et al. (2014) proposed to restrict the usage of term 'masked mycotoxins' only to the fraction of biologically modified mycotoxins that were conjugated by plants. One major pathway of detoxifying DON is the conjugation of DON to a

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glucose moiety. This reaction gives rise to deoxynivalenol-3-glucoside (D3G), which exhibits a reduced ability to inhibit protein synthesis of wheat ribosomes *in vitro* compared with its parent compound, DON (Poppenberger et al. 2003). The occurrence of D3G in *Fusarium* infected wheat and maize was first reported by Berthiller et al. (2005). Then, the worldwide occurrence of D3G was confirmed after identification of D3G in Chinese wheat and maize samples in the same concentrations range as DON (Li et al. 2011; Streit et al. 2013). It has been shown that formation of D3G from DON is a detoxification reaction mediated by the UDP-glucosyl transferase enzyme (*AtUGT73C5*) in *Arabidopsis* (Poppenberger et al. 2003). This enzyme catalyses the transfer of glucose from UDP-glucose to the hydroxyl group at the carbon 3 of DON. Previous studies have indicated that *Fhb1* locus plays a role in resistance against DON, a major virulence factor for *F. graminearum*. In a study by Lemmens et al. (2005) the QTL for DON resistance has been mapped to the major QTL for FHB resistance on chromosome 3B. Therefore, it was hypothesised that *Qfhs.ndsu-3BS* QTL (*Fhb1*) may encode a DON-glucosyltransferase or regulates the expression of such an enzyme. Lemmens et al. also found that DON-resistant wheat lines are more efficient in converting DON to D3G than the DON-susceptible wheat lines. Therefore, it is expected that incorporation of glycosylation-based detoxification mechanism in *Fusarium*-susceptible cultivars will increase FHB resistance. Schweiger et al. (2010) have identified a highly DON-inducible barley uridine diphosphate-glycosyltransferase (*HvUGT13248*) capable of inactivating DON by transforming it to D3G. Recently a transgenic wheat line expressing the barley UDP-glucosyltransferase gene (*HvUGT13248*) has been developed; it demonstrated a high level of resistance to FHB (Li et al. 2015).

In Canada there is no routine testing done for D3G at grain mills or elevators. The fate of D3G after digestion by mammals has not yet been fully understood, and the concern is that this compound may be cleaved to DON and glucose by lactic acid bacteria in the digestive tract of mammals (Dall'Erta et al. 2013; Gratz et al. 2013; Nagl et al. 2014). Therefore, the knowledge about the natural occurrence of masked mycotoxins in *Fusarium*-infected wheat is important to evaluate the potential health risks associated with masked mycotoxin contamination. The objective of this study was to analyse the DON and D3G content in spring wheat cultivars in Canada after inoculating with *F. graminearum* 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) chemotypes. It also attempted to determine if there is a correlation between the DON, D3G, *Fusarium*-damaged kernel percentage (%FDK) and FHB disease index in spring wheat cultivars grown in Manitoba, Canada.

Materials and methods

Field experiment

Ten different spring wheat cultivars (Roblin-S, Harvest-S, CDC Teal-S, Glenn-I, CDC Kernen-I, AAC Elie-I, AAC Iceberg-I, Carberry-MR, 5602HR-MR and Waskada-MR) showing different levels of resistance to FHB were grown at the research field stations of University of Manitoba in Winnipeg and Carman in Manitoba, Canada. All cultivars belong to the Canada Western Red Spring (CWRS) class except for AAC Iceberg, which belongs to Canada Western Hard White Spring (CWHWS) class (Seed Manitoba 2015). Trial entries were replicated three times in a randomised complete block design. Plots at both locations consisted of 1 m rows with 17 cm row spacing. Sowing density was approximately 80 seeds per row. At the 50% flowering stage, the spikes of the entire row were spray-inoculated with a 50 ml inoculum mixture of 3-ADON-producing *F. graminearum* strains (M2-06-01, Q-06-11 and S3BS-06-01) and a mixture of 15-ADON-producing *F. graminearum* strains (S3AN-06-01, NB-06-18 and A1-06-01) using a CO_2 backpack sprayer calibrated at 30 psi. The *F. graminearum* inoculum concentration was adjusted to 5×10^4 spores/ml using water. Re-inoculation of the same rows was performed 3 days following the first inoculation. Control plots were sprayed with 50 ml of distilled water per row. The plots were mist irrigated after each inoculation using a sprinkler system for 5 min every hour for 10–12 h to increase the relative humidity.

FHB disease index and FDK percentage

FHB disease incidence and severity of each row were rated 21 days post-inoculation using the FHB disease scale described by Stack and McMullen (1995). At maturity rows were hand harvested and threshed using the Wintersteiger® Elite combine, the wind speed was set at 'very low' to retain as many FDK as possible. Harvested wheat samples were placed in paper bags and air dried for a week at 36°C. Threshed samples were again cleaned using a belt

thresher and a blower. *Fusarium*-damaged kernel percentage (%FDK) was calculated from a 10 g subsample from each row and recorded as a percentage of weight.

Total DON and D3G content

The same subsample taken for FDK assessment was ground, using a household coffee grinder and prepared for DON and D3G testing. DON and D3G testing was done using liquid chromatography-quadrupole time of flight mass spectrometry (LC-MS) according to the protocol described by Ovando-Martínez et al. (2013). The DON and D3G testing was done at the hard red spring wheat quality laboratory, North Dakota State University, Fargo, USA.

Statistical analysis

Analysis of variance (ANOVA) was performed individually for data from two locations using the PROC MIXED procedure in SAS 9.3 (SAS version 9.3, SAS Institute Inc., Cary, NC, USA). The wheat cultivars and treatments (3-ADON + 15-ADON + control) were considered as fixed effects. The main effects of cultivar, treatment and their interactions were tested for significance using the residual error terms. Treatment effects were partitioned into: between chemotypes (3-ADON versus 15-ADON) and inoculated versus water control lines. The reason for partitioning the sources of variation within treatment effects was to provide a comparison between the chemotypes and between the control and inoculated lines. The model statements used for analyzing variation between chemotypes and inoculated versus control were the same as in the complete analysis. The correlation between FHB response variables at each location was analysed using the SAS PROC CORR (SAS version 9.3) procedure.

Results

FHB disease index and FDK percentage

In Carman, significant differences were observed among the cultivars and between the two chemotypes (3-ADON versus 15-ADON). The two-way interaction, cultivar*treatment was also significantly different (Table 1). Similar results were observed in Winnipeg (Table 2). However, the two-way

Table 1. Analysis of variance (ANOVA) table for *Fusarium* head blight disease index, *Fusarium*-damaged kernel percentage, deoxynivalenol content, deoxynivalenol-3-glucoside content and the ratio between deoxynivalenol-3-glucoside to deoxynivalenol content in Carman, Manitoba.

Trait	Source	d.f.	SS	MS	*F*-value	Pr > *F*
FHB disease index[a]	Cultivar	9	25345.00	2816.08	49.72	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	26532.00	13266.00	234.22	< 0.0001
	15-ADON versus 3-ADON	1	4420.41	4420.41	62.93	< 0.0001
	DON versus control	1	22111.00	22111.00	172.05	< 0.0001
	Cultivar*treatment	18	6824.44	379.41	6.70	< 0.0001
	Error	58	3285.00	56.63		
% FDK[b]	Cultivar	9	741.54	82.39	26.00	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	663.10	331.55	104.64	< 0.0001
	15-ADON versus 3-ADON	1	99.71	99.71	21.86	< 0.0001
	DON versus control	1	563.39	563.39	110.45	< 0.0001
	Cultivar*treatment	18	216.91	12.05	3.80	< 0.0001
	Error	58	183.77	3.16		
DON (ppm)[c]	Cultivar	9	1976.36	219.59	17.85	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	1948.68	974.34	79.22	< 0.0001
	15-ADON versus 3-ADON	1	80.75	80.75	5.51	0.0242
	DON versus control	1	1867.92	1867.92	144.41	< 0.0001
	Cultivar*treatment	18	570.78	31.71	2.58	0.0034
	Error	58	713.38	12.29		
D3G (ppm)[d]	Cultivar	9	68.25	7.58	6.42	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	163.45	81.72	69.15	< 0.0001
	15-ADON versus 3-ADON	1	1.86	1.86	2.27	0.1404
	DON versus control	1	161.59	161.59	136.54	< 0.0001
	Cultivar*treatment	18	33.76	1.87	1.59	0.0943
	Error	58	68.55	1.18		
D3G/DON ratio	Cultivar	9	3714.62	412.73	2.00	0.0500
	Treatment (15-ADON + 3-ADON + control)	2	3146.51	1573.25	7.62	0.0012
	15-ADON versus 3-ADON	1	55.75	55.75	0.72	0.4025
	DON versus control	1	3090.76	3090.76	16.61	0.0001
	Cultivar*treatment	18	3342.46	185.69	0.90	0.5817
	Error	58	11976	206.48		

Notes: [a]FHB disease index = *Fusarium* head blight disease index.
[b]% FDK = *Fusarium*-damaged kernel percentage.
[c]DON (ppm) = deoxynivalenol content in parts per million.
[d]D3G (ppm) = deoxynivalenol-3-glucosides content in parts per million.

Table 2. Analysis of variance (ANOVA) table for *Fusarium* head blight disease index, *Fusarium*-damaged kernel percentage, deoxynivalenol content, deoxynivalenol-3-glucoside content and the ratio between deoxynivalenol-3-glucoside to deoxynivalenol content in Winnipeg, Manitoba.

Trait	Source	d.f.	SS	MS	*F*-value	Pr > *F*
FHB disease index[a]	Cultivar	9	26254.00	2917.16	88.35	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	14712.00	7355.83	222.79	< 0.0001
	15-ADON versus 3-ADON	1	1706.66	1706.66	37.58	< 0.0001
	DON versus control	1	13005.00	13005.00	226.85	< 0.0001
	Cultivar*treatment	18	9377.22	520.95	15.78	< 0.0001
	Error	58	1915.00	33.01		
% FDK[b]	Cultivar	9	281.66	31.29	15.06	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	532.88	266.44	128.23	< 0.0001
	15-ADON versus 3-ADON	1	81.92	81.92	27.26	< 0.0001
	DON versus control	1	450.96	450.96	131.11	< 0.0001
	Cultivar*treatment	18	125.67	6.98	3.36	0.0002
	Error	58	120.51	2.07		
DON (ppm)[c]	Cultivar	9	592.98	65.88	14.62	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	1631.27	815.63	180.93	< 0.0001
	15-ADON versus 3-ADON	1	65.00	65.00	11.91	0.0014
	DON versus control	1	1566.27	1566.27	300.26	< 0.0001
	Cultivar*treatment	18	150.26	8.34	5.97	0.0401
	Error	58	261.46	4.50		
D3G (ppm)[d]	Cultivar	9	73.96	8.21	8.13	< 0.0001
	Treatment (15-ADON + 3-ADON + control)	2	231.90	115.93	114.70	< 0.0001
	15-ADON versus 3-ADON	1	8.05	8.05	6.30	0.0165
	DON versus control	1	222.84	222.84	187.37	< 0.0001
	Cultivar*treatment	18	27.21	1.51	1.50	0.1252
	Error	58	58.63	1.01		
D3G/DON ratio	Cultivar	9	5775.32	641.70	3.38	0.0022
	Treatment (15-ADON + 3-ADON + control)	2	25083.00	12542	66.06	< 0.0001
	15-ADON versus 3-ADON	1	87.74	87.74	0.42	0.5196
	DON versus control	1	24995.00	24995.00	127.66	< 0.0001
	Cultivar*treatment	18	7244.35	402.46	2.12	0.0163
	Error	58	11011.00	189.84		

Notes: [a]FHB disease index = *Fusarium* head blight disease index.
[b]% FDK = *Fusarium*-damaged kernel percentage.
[c]DON (ppm) = deoxynivalenol content in parts per million.
[d]D3G (ppm) = deoxynivalenol-3-glucosides content in parts per million.

interaction, cultivar*chemotype was not significantly different for FHB index in Carman and Winnipeg (Table 3). In both locations, cultivars Roblin and AAC Iceberg inoculated with a mixture of 3-ADON strains showed higher FHB disease index (96.7% in Carman and 80% in Winnipeg) than the other cultivars and chemotype combinations. The lowest FHB disease index was shown by cultivars Carberry, 5602HR and CDC Kernen (23.3%) inoculated with a mixture of 15-ADON strains in Carman (Figure 1). In Winnipeg, the lowest FHB disease index was observed in cultivars 5602HR, CDC Kernen and AAC Elie (5%) inoculated with a mixture of 15-ADON strains (Figure 2).

When FDK percentage is considered, significant differences were observed among the cultivars and the

Table 3. Mean square values of cultivar, chemotype and their interaction for *Fusarium* head blight disease index, *Fusarium*-damaged kernel percentage, deoxynivalenol content, deoxynivalenol-3-glucoside content and the ratio between deoxynivalenol-3-glucoside to deoxynivalenol content in Carman and Winnipeg locations.

			Mean square value and significance (Type III)				
Location	Source	d.f.	FHB index (%)[a]	FDK %[b]	DON (ppm)[c]	D3G (ppm)[d]	D3G/DON Ratio (%)
Carman	Cultivar	9	3295.60*	95.26*	262.63*	8.02*	294.52*
	Chemotype (3-ADON versus 15-ADON)	1	4420.41*	99.71*	80.75*	1.86 n.s.	55.75 n.s.
	Cultivar*chemotype	9	114.86 n.s.	7.04 n.s.	9.49 n.s.	1.11 n.s.	68.81 n.s.
	Residual	38	70.24	4.56	14.65	0.8	77.80
Winnipeg	Cultivar	9	3752.03*	38.36*	73.15*	8.80*	920.38*
	Chemotype (3-ADON versus 15-ADON)	1	1706.66*	81.92*	65.00*	8.05*	87.74 n.s.
	Cultivar*chemotype	9	30.74 n.s.	3.49 n.s.	3.13 n.s.	1.61 n.s.	246.10 n.s.
	Residual	38	45.41	3.00	5.45	1.27	207.64

Notes: [a]FHB disease index = *Fusarium* head blight disease index.
[b]% FDK = *Fusarium*-damaged kernel percentage.
[c]DON (ppm) = deoxynivalenol content in parts per million.
[d]D3G (ppm) = deoxynivalenol-3-glucosides content in parts per million.
n.s., Not significant.

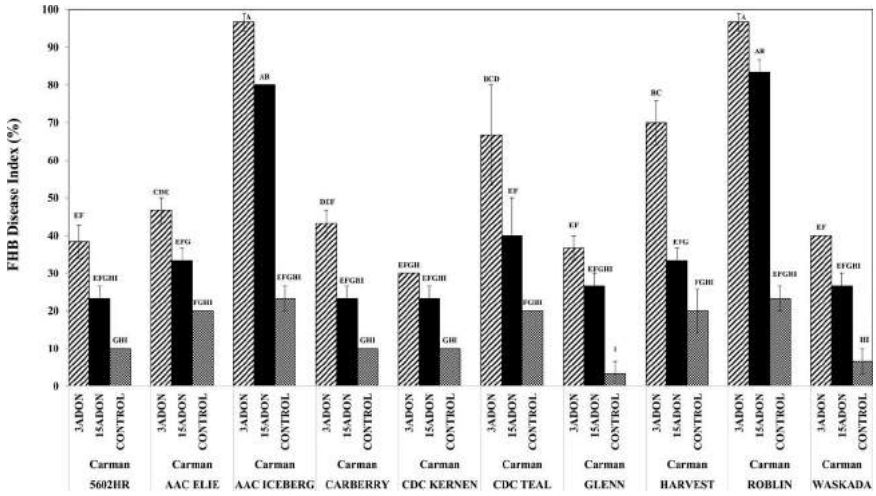

Figure 1. *Fusarium* head blight disease index in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Carman.

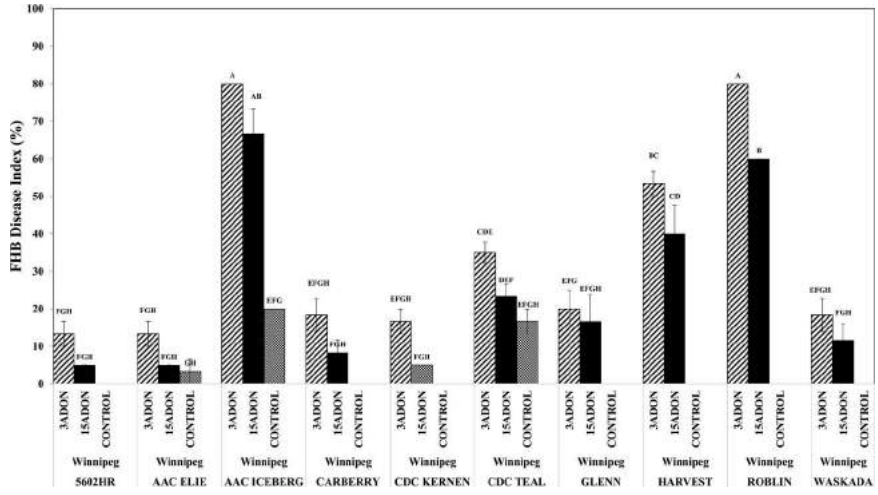

Figure 2. *Fusarium* head blight disease index in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Winnipeg.

chemotypes (Table 1). The two-way interaction, cultivar*treatment was significantly different at both locations. Similar to FHB index, the two-way interaction, cultivar*chemotype was not significantly different at both locations (Table 3). The highest percentage of FDK was observed in cultivar Roblin inoculated with a mixture of 3-ADON strains at both locations (18% in Carman and 11.5% in Winnipeg) (Figures 3 and 4). The lowest FDK percentage was observed in cultivar 5602HR inoculated with a mixture of 15-ADON strains in Carman (2.3%) and cultivar Glenn inoculated with a mixture of 15-ADON strains in Winnipeg (2.2%) (Figures 3 and 4).

Total DON and D3G content

Similar to other FHB variables represented thus far, the total DON content was significantly different among cultivars and between the two chemotypes (Tables 1 and 2). The two-way interaction, cultivar*treatment was significantly different for total DON content at both locations. The total DON content in different cultivars inoculated with *F. graminearum* strains ranged from 5.6 to 34.4 ppm (Figures 5 and 6). The highest DON content was observed in cultivar Roblin inoculated with a mixture of 3-ADON strains in Carman (34.4 ppm) (Figure 5). In Winnipeg, the highest total DON content was found in cultivar AAC

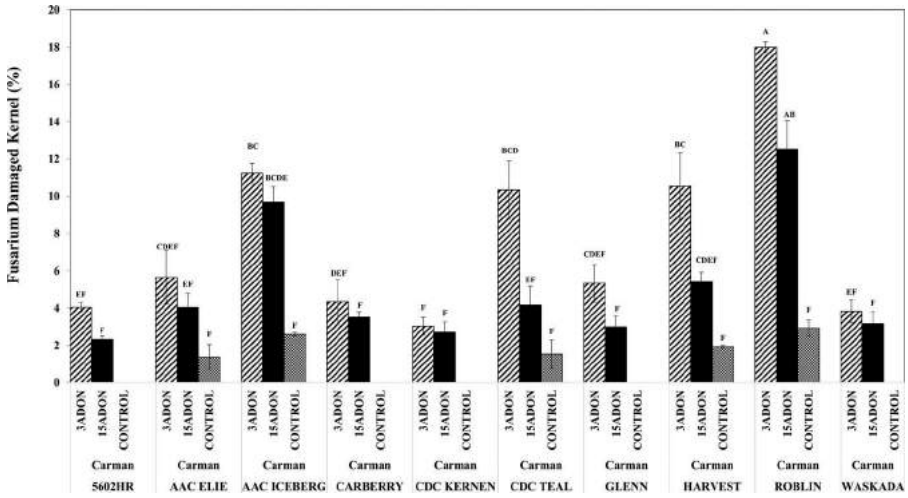

Figure 3. *Fusarium*-damaged kernel percentage in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Carman.

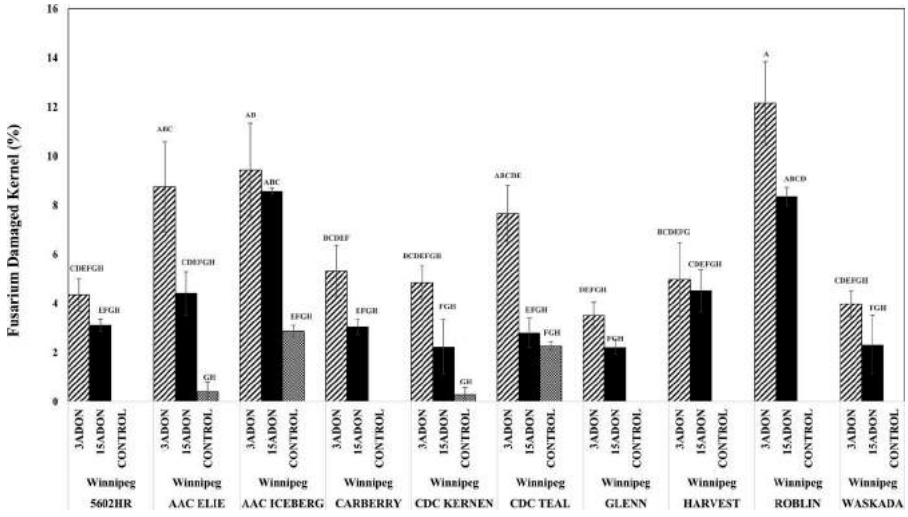

Figure 4. *Fusarium*-damaged kernel percentage in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Winnipeg.

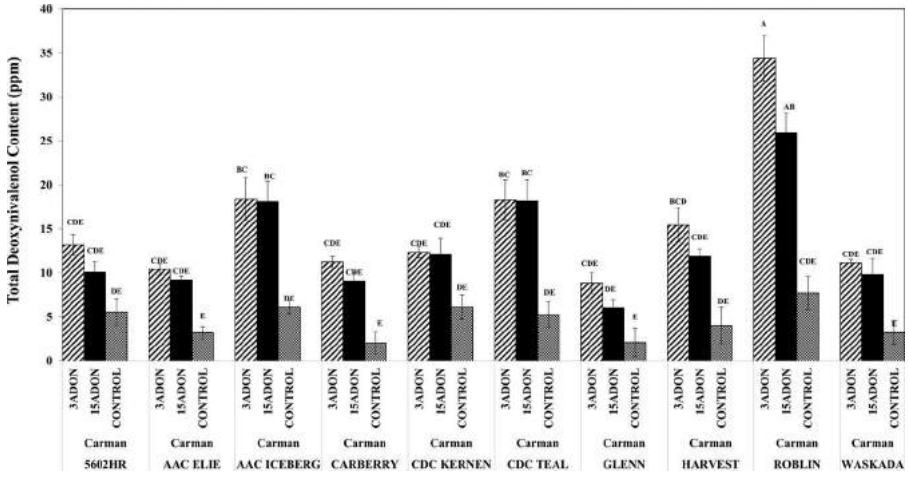

Figure 5. Total deoxynivalenol (DON) content in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Carman.

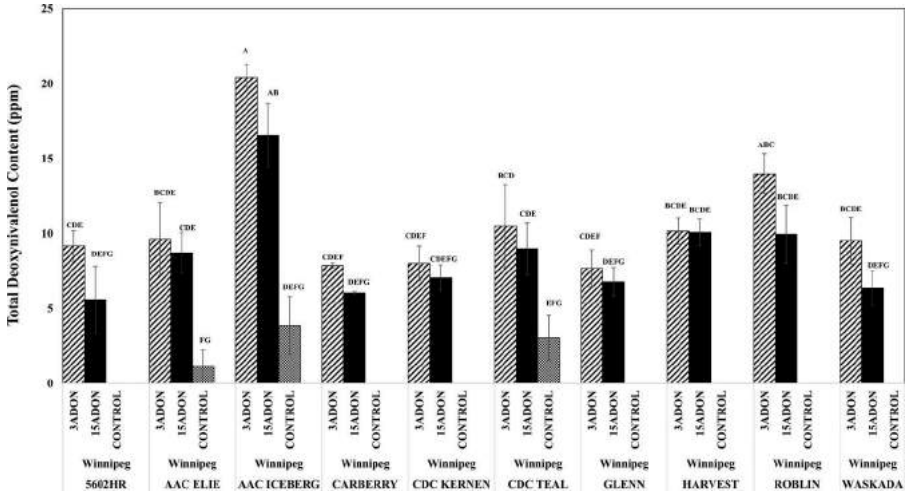

Figure 6. Total deoxynivalenol (DON) content in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Winnipeg.

Iceberg inoculated with a mixture of 3-ADON strains (20.4 ppm) (Figure 6). The lowest total DON content was observed in cultivar Glenn inoculated with a mixture of 15-ADON in Carman (6.0 ppm) and cultivar 5602HR (5.6 ppm) in Winnipeg. In Winnipeg location, cultivars AAC Elie, AAC Iceberg, CDC Teal and Harvest showed DON contaminations in water control lines.

As with other variables presented, and again for D3G content, significant differences were observed among the cultivars. In contrast to other variables, there was no significant difference between the chemotypes for D3G content at Carman location. Also the two-way interaction of cultivar*treatment was not significant (Table 1). But in Winnipeg location, the two chemotypes were significantly different for D3G content (Table 2). The D3G content ranged between 0 and 6.9 ppm in different wheat cultivars (Figures 7 and 8). The highest D3G content was observed in cultivar AAC Iceberg inoculated with a mixture of 3-ADON strains at both locations (6.9 ppm in Winnipeg and 6.3 ppm in Carman) (Figures 7 and 8). The D3G/DON ratio was significantly different among the cultivars and between the chemotypes at both locations (Tables 1 and 2). However, the two-way interaction cultivar*chemotype was not significantly different at both locations (Table 3). The highest D3G/DON ratio was observed in cultivar Carberry inoculated with a mixture of 15-ADON strains in Carman (44%) (Figure 9(a)). In Winnipeg, the highest D3G/DON ratio was observed in cultivar CDC Kernen inoculated with a mixture of 3-ADON

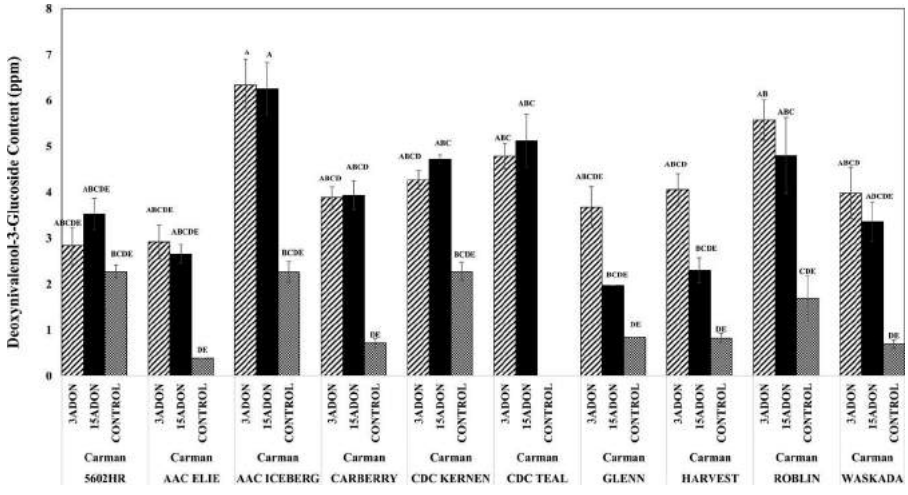

Figure 7. Deoxynivalenol-3-glucoside (D3G) content in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Carman.

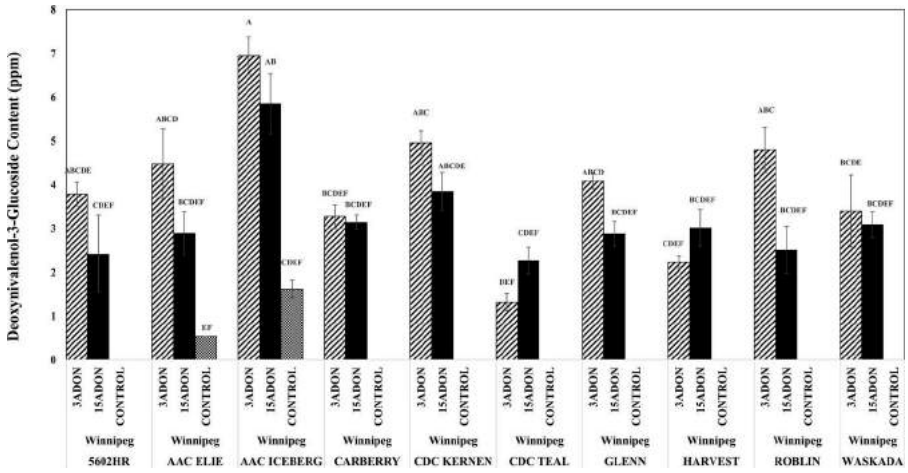

Figure 8. Deoxynivalenol-3-glucoside (D3G) content in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Winnipeg.

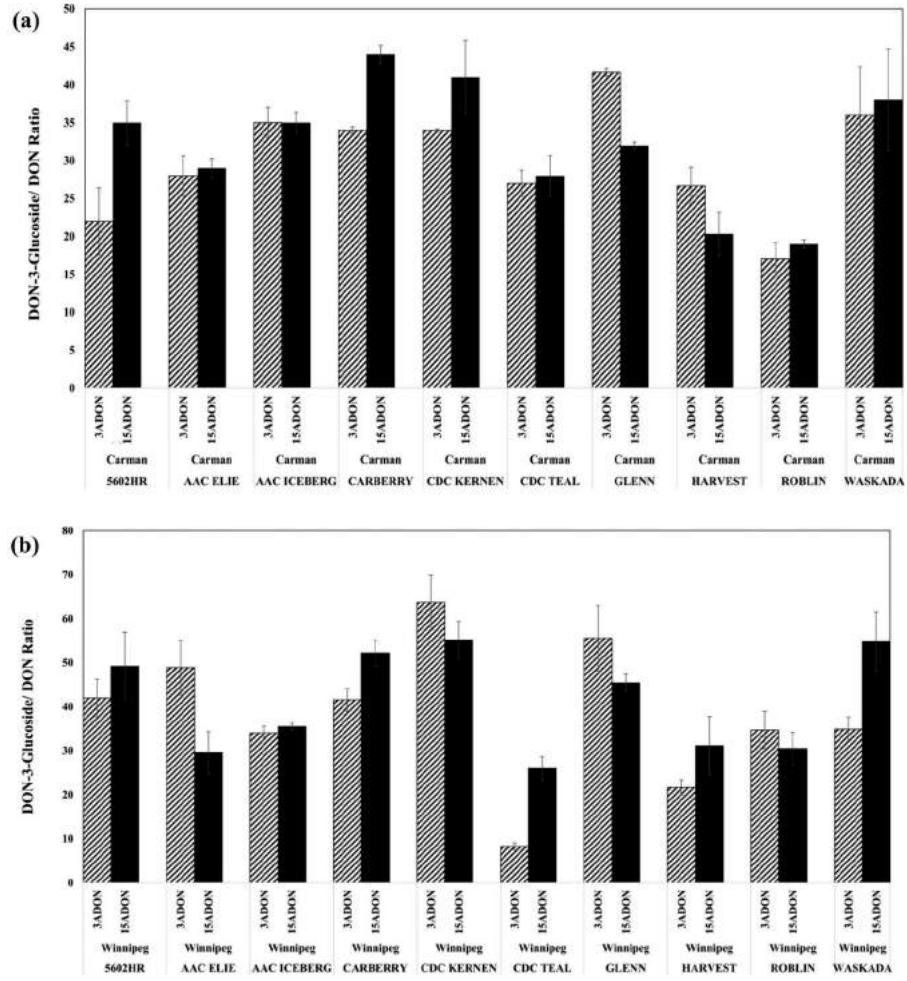

Figure 9. (a) Deoxynivalenol-3-glucoside/deoxynivalenol ratio in spring wheat cultivars inoculated with a mixture of 3-acetyldeoxynivalenol- and 15-acetlyldeoxynivalenol-producing *F. graminearum* strains in Carman (b) in Winnipeg.

strains (63.8%) (Figure 9(b)). The D3G/DON ratio was low in susceptible cultivars such as CDC Teal, Harvest and Roblin.

Correlation between FHB disease variables

Significant positive correlations were observed for all FHB disease variables analysed (Tables 4 and 5). A strong positive correlation was observed between DON and D3G content at both locations. When two locations were analysed separately, the highest correlation between DON and D3G was observed at the Winnipeg location (Table 5). The correlation between DON and D3G was 0.84. The correlation between FDK percentage and D3G percentage was 0.66. Also the correlation between DON and FDK percentage was 0.81. In Carman, the correlation between DON and D3G was 0.77 and the correlation between FDK percentage and D3G was 0.61. Further a strong positive correlation was observed between the FDK percentage and DON content (0.78) at Carman location (Table 4).

Table 4. Pearson correlation coefficients between *Fusarium* head blight disease index, *Fusarium*-damaged kernel percentage, total deoxynivalenol content and deoxynivalenol-3-glucoside content in Carman, Manitoba.

	FHB index[a]	% FDK[b]	DON (ppm)[c]	D3G (ppm)[d]
FHB index	1.00	0.92*	0.79*	0.69*
% FDK		1.00	0.78*	0.61*
DON (ppm)			1.00	0.77*
D3G (ppm)				1.00

Notes: [a]FHB disease index = *Fusarium* head blight disease index.
[b]% FDK = *Fusarium*-damaged kernel percentage.
[c]DON (ppm) = deoxynivalenol content in parts per million.
[d]D3G (ppm) = deoxynivalenol-3-glucosides content in parts per million.
*Correlation coefficient is significant at $p < 0.0001$.
Number of samples (n) = 90.

Table 5. Pearson correlation coefficients between *Fusarium* head blight disease index, *Fusarium*-damaged kernel percentage, total deoxynivalenol content and deoxynivalenol-3-glucoside content in Winnipeg, Manitoba.

	FHB index[a]	% FDK[b]	DON (ppm)[c]	D3G (ppm)[d]
FHB index	1.00	0.79*	0.79*	0.56*
% FDK		1.00	0.81*	0.66*
DON (ppm)			1.00	0.84*
D3G (ppm)				1.00

Notes: [a]FHB disease index = *Fusarium* head blight disease index.
[b]% FDK = *Fusarium*-damaged kernel percentage.
[c]DON (ppm) = deoxynivalenol content in parts per million.
[d]D3G (ppm) = deoxynivalenol-3-glucosides content in parts per million.
*Correlation coefficient is significant at $p < 0.0001$.
Number of samples (n) = 90.

Discussion

In this study, significant differences were observed among the cultivars for all the FHB response variables measured. This indicates that there were differences in the FHB disease progression for the cultivars. This was to be expected since the cultivars used in this study ranged in susceptible, intermediate and moderately resistant to FHB. The relative rankings of cultivars remained the same for all FHB disease variables except for cultivar AAC Iceberg. The highest FHB disease index, FDK percentage, DON and D3G content was shown by cultivars Roblin and AAC Iceberg inoculated with a mixture of 3-ADON strains. The cultivar Roblin is rated as highly FHB susceptible and usually included as a check in disease nursery trials. Although AAC Iceberg rated as intermediate resistance to FHB, we could observe higher levels of disease at both locations. The moderately resistant cultivars such as Carberry, Waskada and 5602HR showed lower FHB index, FDK percentage and DON content compared with the susceptible cultivars. The intermediate resistant cultivars, Glenn, CDC Kernen and AAC Elie also showed lower FHB index, FDK percentage and DON content compared with susceptible cultivars. Therefore, these findings further confirm that, the use of resistant cultivars is one of the major management strategies to combat FHB in wheat. When chemotype origin of strains is considered, significant differences were observed between 3-ADON and 15-ADON strains for FHB index, FDK percentage and total DON content. But there were no significant differences between the two chemotypes for D3G content and D3G/DON ratio. Although the cultivar*treatment interaction was significantly different for all FHB disease variables analysed (except for D3G at Carman and Winnipeg and D3G/DON ratio at Carman location); the two-way interaction cultivar*chemotype was not significantly different. Therefore, these results indicate that wheat cultivars used in this study may ranked similarly for both 3-ADON strains and 15-ADON strains under field environmental conditions. However, the magnitude of the difference in all measured FHB disease variables among cultivars differed with the chemotype used. The wheat cultivars inoculated with a mixture of 3-ADON strains always showed higher FHB disease index, FDK percentage and DON content. Similar results have reported in other studies, in which 3-ADON-producing strains showed higher disease severity and DON accumulation compared with the 15-ADON-producing strains (Ward et al. 2008; Puri & Zhong 2010).

Both DON and D3G contents were measured simultaneously using LC-MS. There was a strong positive

correlation between the DON and D3G content. Our results are in agreement with results by Ovando-Martínez et al. (2013). They have reported a positive correlation between DON and D3G in hard red spring wheat inoculated with *F. graminearum* strains. The ratios of D3G/DON were higher in cultivars CDC Kernen (I) and Carberry (MR). The cultivars CDC Kernen and Carberry used in this study, have been bred by introgressing the *Qfhs.ndsu-3BS* QTL. Lemmens et al. (2005) have also shown that DON resistant lines with *Qfhs.ndsu-3BS* QTL (*Fhb1* gene) have higher D3G to DON ratio. They hypothesised that *Qfhs.ndsu-3BS* QTL encodes a DON-glucosyltransferase or regulates the expression of such an enzyme. However, very recently a study done by Siegwart et al. (2015) have found no UDP-glucosyltransferase within the *Fhb1* interval of the sequenced susceptible cultivar Chinese Spring. Therefore, more studies are needed to explain the reasons for presence of higher D3G content in lines with *Fhb1* gene compared with other lines.

In the statistical analysis the D3G content in Carman location and D3G/DON ratio at both locations, were not significantly different between the chemotypes used in the study. The amount of D3G content in the infected wheat kernels were maintained by the resistance mechanisms within the wheat cultivars, not by the chemotypic origin of the *F. graminearum* strain. Therefore, these results clearly demonstrate that level of resistance in the wheat cultivar may plays a key role in regulating detoxification of DON into less toxic D3G during *Fusarium* infection. Deoxynivalenol acts as a virulence factor in FHB disease development; hence the detoxification of DON to less toxic D3G can reduce the virulence of the pathogen (Mesterházy 2002). This may lead to lower FHB symptoms and lower DON contamination in resistant wheat lines compared with susceptible lines.

According to our knowledge, this is the first study done in Canada to determine the amount of D3G in Canadian spring wheat cultivars after inoculating with different chemotypes of *F. graminearum*. The findings from this study help to understand the occurrence of D3G in commonly grown spring wheat cultivars in Canada. So far, D3G content is not assessed in routine food and feed safety protocols in many countries. Therefore, this study shows the importance of testing D3G in food and feed safety assessments in Canada, as these masked mycotoxins might be converted back to the toxic forms inside human/animal body.

In conclusion, this study shows that Canadian spring wheat cultivars produce D3G upon *F. graminearum* infection and there is a positive correlation between the total DON content and the D3G content. The moderately resistant and intermediate wheat cultivars such as Carberry and CDC kernen have shown a higher D3G/DON ratio suggesting that detoxification of DON by conjugating with glucose molecules is one of the major mechanisms to reduce DON content in moderately resistant and intermediate wheat cultivars. Further, this study shows the importance of a more detailed analysis of D3G in Canadian spring wheat cultivars using more locations and years. Finally, it is important to attain a more complete understanding of how plant's defence system is able to convert DON into other non or less toxic compounds in order to develop cultivars with improved FHB resistance.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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