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Monitoring of *Fusarium* Trichothecenes in Canadian Cereal Grain Shipments from 2010 to 2012

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Supporting Information

ABSTRACT: A method involving dry grinding, rotary sample dividing, and gas chromatography−mass spectrometry was evaluated for the analysis of eight *Fusarium* trichothecenes in cereal grains. Processing of whole cereal grains by the method produced representative test portions for the analysis of deoxynivalenol (DON). Method validation data, as well as the successful participation in various international proficiency tests, demonstrated the analytical method produced accurate and precise results. The evaluated method was used to monitor DON, 3- and 15-acetyldeoxynivalenol, nivalenol (NIV), T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and fusarenon-X in shipments of Canadian wheat, durum, barley, corn, rye, and oats transported between August 1, 2010, and July 31, 2012. DON was the most frequently measured trichothecene, found in 231 of the 303 samples at concentrations up to 2.34 mg/kg; NIV was the next most frequently observed trichothecene, but its occurrence was limited to barley. Concentrations of DON were significantly associated with wheat class and grade. The median DON concentration in durum (0.09 mg/kg) was lower than that for hard red spring (0.21 mg/kg). Lower grades of wheat also contained higher median concentrations of DON than higher grades, supporting the current use of *Fusarium* damaged kernels as a grading factor to manage DON.

KEYWORDS: *mycotoxins, monitoring, stability, Canada, grading, sample preparation, GC-MS*

INTRODUCTION

The production of wheat, durum, oats, rye, barley, and corn in Canada is an important part of the country's agriculture and agri-food system and economy. In the 2011−2012 growing season, major cereal grains produced included wheat (21,116,000 t), corn (11,359,000 t), barley (7,892,000), and durum (4,172,000 t).[1] Bulk cereal grain is sold and transported to many different countries, as well as to domestic users, but Canada is a net exporter of most cereal grains. The percentages of these cereal crops that were exported ranged from 75% of wheat to 5% of corn produced.

Buyers and consumers of the exported cereal grains value safety as well as quality. Many jurisdictions have maximum limits set for mycotoxins in cereal grains that are used in the production of food. These regulations mainly apply to the *Fusarium*-produced mycotoxin deoxynivalenol (DON); existing maximum limits for DON in cereal grains range from 1.0 to 2.0 mg/kg.[2−5]

In Canada, *Fusarium graminearum* is one of the most common fungal pathogens to occur on cereal grains. Other *Fusarium* species routinely present include *F. avenaceum*, *F. poae*, and, to a lesser extent, *F. sporotrichoides*.[6−8] These species are associated with the production of a number of different mycotoxins such as DON, as well as other trichothecene and nontrichothecene compounds including moniliformin, nivalenol (NIV), and HT-2 and T-2 toxins.[9,10]

The Canadian Grain Commission (CGC) routinely analyzes grain shipments for *Fusarium* trichothecenes to ensure grain is meeting regulations and to monitor for trends in the occurrence of these mycotoxins. Due to the importance of sampling in obtaining representative samples of large bulk lots, sampling of shipments is performed during grain loading using automated diverters at various ports across Canada and employing defined sampling procedures and equipment.

This work describes the validation of a gas chromatographic−mass spectrometric method for the analysis of eight *Fusarium* trichothecenes in cereal grains and its use in the analysis of bulk export and domestic shipments of Canadian cereals, including common wheat, durum wheat, and barley.

MATERIAL AND METHODS

Samples. Samples of various cereal grains grown in western Canada were provided by CGC Industry Services inspectors. Grains sampled included wheat (n = 197), durum (CWAD; n = 68), barley (n = 27), corn (n = 9), rye (n = 1), and oats (n = 1). The majority of the wheat shipments were Canada Western Red Spring (CWRS; n = 167), but other classes were represented as well, including Canada Western Red Winter (CWRW; n = 13), Canada Prairie Spring Red (n = 3), Canada Western Extra Strong (n = 2), and Canada Western Hard White Spring (n = 2). An additional eight samples were designated as feed wheat, and the classes of the remaining two samples were not specified. These samples represented randomly selected domestic and export shipments of cereal grains that occurred between August 1, 2010, and July 31, 2012. It should be noted that the year of transport does not necessarily correspond to the year in which grain was grown and harvested.

The samples used in this work were obtained from a variety of points throughout the Canadian grain handling system, including transfer and terminal grain elevators. During the loading of vessels for export and domestic shipments, moving grain was sampled according to standardized procedures at a constant interval using CGC approved

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Table 1. Mean ± Standard Deviation Percent Recoveries of *Fusarium*-Produced Mycotoxins from Fortified Grains[a]

matrix	DON	3-ADON	15-ADON	NIV	T-2	HT-2	DAS	FUS-X
wheat[b]	90 ± 13	93 ± 14	86 ± 9	60 ± 9	133 ± 26	131 ± 18	108 ± 11	81 ± 8
barley[c]	89 ± 10	100 ± 12	87 ± 6	96 ± 28	112 ± 13	114 ± 10	108 ± 14	97 ± 9
oats[b]	104 ± 15	119 ± 27	117 ± 31	106 ± 29	125 ± 30	141 ± 37	114 ± 25	119 ± 18
corn[c]	84 ± 7	87 ± 9	81 ± 5	62 ± 4	123 ± 10	132 ± 16	110 ± 17	77 ± 3

[a]Values presented are a summary of results from multiple days over three fortification levels (0.05, 0.5, and 2 mg/kg). [b]3 days, $n = 21$ per day. [c]1 day, $n = 21$.

automated cross-stream sample diverters and dividers.[11] The increments obtained during loading of the entire lots were combined into a composite sample that was considered to be representative of the entire lot. A Boerner divider (Seedburo Equipment Co.) was used to divide the composite sample into a 10 kg laboratory sample. The 10 kg laboratory samples were stored at room temperature in canvas bags for up to 5 weeks until further preparation.

Test portions for trichothecene analysis were prepared from the 10 kg laboratory sample of whole grain according to the process described in Tittlemier et al.[12] The 10 kg laboratory sample of whole grain was comminuted using a Retsch SR 300 rotor beater mill fitted with a 750 μm screen and coupled with a Retsch DR 100 vibratory feeder. After comminution, the entire mass of ground grain was homogenized and divided into 10 × 1 kg portions on a rotary sample divider (Materials Sampling Solutions, Southport, Australia). After the first division into 10 × 1 kg, all 10 portions were recombined in the RSD hopper and divided into 10 × 1 kg again. A 1 kg portion of ground grain was randomly selected and further divided on another RSD into 10 subsamples of 100 g of ground grain. Subsamples of 100 g were stored at room temperature in closed high-density polyethylene or polypropylene containers. The containers were not airtight, but were sealed to prevent the possibility of insects entering the samples. Most samples were analyzed within 30 days of receiving the laboratory samples.

The intensive grinding and dividing sample preparation procedure was also examined with respect to its ability to produce homogeneous samples for DON analysis. Four in-house wheat reference materials previously analyzed and found to contain between 0.58 and 5.30 mg/kg DON were ground and divided using the established protocol described above. A 1 kg portion from each of the four in-house reference materials was randomly selected and further divided. Test portions of 10 g were taken from the 100 g subsamples prepared from each 1 kg portion, extracted, and analyzed in duplicate. The 100 g subsamples were also tested for homogeneity. Five test portions of 10 g were analyzed in duplicate from one 100 g subsample taken from each of the four in-house reference materials.

Stability of Analytes during Sample Storage. Ground wheat ($n = 4$) and barley ($n = 3$) samples previously analyzed and found to contain quantifiable levels of DON and NIV were stored for up to 25 months in the laboratory. During this period samples were analyzed at least eight times to examine the stability of DON and NIV in ground grain during storage under laboratory conditions. Samples were kept in closed high-density polypropylene containers at room temperature. No attempt was made to shield samples from fluorescent light in the laboratory.

A separate ground wheat sample was also analyzed approximately every 2 weeks over a 90 week period to monitor the change in moisture content of ground grain during storage. Moisture content was determined gravimetrically. A measured amount of ground wheat was dried in an oven for 1 h at 130 ± 1 °C and cooled to room temperature in a desiccator containing anhydrous calcium sulfate. The mass of the cooled wheat was determined, and the moisture content was calculated from the mass lost during heating.

Analysis of *Fusarium* Trichothecenes. Eight *Fusarium* trichothecenes (Supporting Information, Supplementary Table 1) were included in the analytical method: deoxynivalenol (DON) and the related 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), T-2 toxin, and its conversion product HT-2 toxin, diacetoxyscirpenol (DAS), and fusarenon-X (FUS-X). Telodrin (isobenzan) was used as an internal standard for the analytical method. Standards of all analytes (purity > 98.0%) were obtained from Romer Laboratories Diagnostic GmbH, and telodrin (purity > 99.0%) was obtained from Shell Canada.

Compounds were extracted, derivatized, and analyzed using a method based on that of Tacke and Casper;[13] some method details have also been provided in Clear et al.[6] Briefly, 10 g of ground sample was extracted with an 84:16 (v/v) solution of acetonitrile/water for 1 h on a mechanical shaker. The sample mixture was centrifuged, and an aliquot of the supernatant was passed through a cleanup column containing a 1:1 (m/m) mixture of C_{18} reverse phase bonded silica gel and aluminum oxide. An aliquot of the eluate was evaporated to dryness and then derivatized using a 100:3 (v/v) solution of *N*-trimethylsilylimidazole and trimethylchlorosilane. A solution of telodrin in isooctane was added to the derivatized extract, followed by 1 mL of purified water to neutralize any remaining derivatizing agent. After mixing, the organic and aqueous layers were allowed to separate. A portion of the organic layer was then removed and analyzed by gas chromatography–mass spectrometry (GC-MS).

The prepared samples were chromatographed on an Agilent capillary 6890 GC equipped with a Hewlett-Packard 5973 mass selective detector and a Hewlett-Packard 7673A autosampler. The GC contained a DB35-MS 30 m × 0.25 mm × 0.25 μm column (Agilent). Sample was injected in pulsed splitless mode using a pulse pressure of 40.0 psi. Four ions were monitored in electron impact ionization mode for each analyte (Table 1).

Analytes were considered to be positively identified and quantified if their retention times were within 0.1 min of the average retention time of the corresponding analyte in the external calibration standards, the peak had a signal-to-noise ratio greater than 9:1, and the ratio of qualification to quantitation ions was within acceptable tolerances.[14] The analyte responses were normalized to the telodrin responses during calculation of concentrations. Finally, the analyte concentrations were recovery corrected using the percent recovery of analyte from a fortified blank run with every sample batch.

Blank samples fortified with a solution of standards, certified reference materials, and in-house reference materials were all used during the analyses for quality control. In addition, duplicate portions of every 20th sample were extracted and analyzed to monitor method performance.

RESULTS

Validation and Method Performance. Fortified blank samples of wheat, barley, oats, and corn were used in the validation of the method because of a lack of certified reference materials (CRMs) or naturally incurred samples containing all eight analytes. Blank samples ($n = 7$) were fortified at three different concentrations (approximately 0.05, 0.5, and 2 mg/kg), extracted, and analyzed on three separate days for wheat and oats. There were no significant differences in the recovery of fortified analyte across days or fortification levels (data not shown). Validation of the method for barley and corn was performed on only one day instead of three because no interday effects were observed for the other cereal matrices.

Table 1 aggregates and summarizes the results from the method validation. Mean recoveries of analytes ranged from 60% (NIV) to 141% (HT-2). Subsequent quantitation using

standards prepared in solvent and matrix demonstrated that the elevated recoveries for T-2 and HT-2 were due to signal enhancement by matrix effects. The ratios of peak areas for postextraction fortified matrix matched standards to peak areas for standards prepared in solvent were 1.37 and 1.48 for T-2 and HT-2, respectively. The ratios for the other six analytes ranged from 1.01 to 1.19. However, recovery correction using matrix-specific fortified blanks in each batch mitigates the signal enhancement due to matrix effects.

The limit of quantitation (LOQ) was estimated as the concentration required to produce a signal-to-noise ratio of 9:1 for all ions monitored, whereas the proper ratio of quantitation to qualification ions is maintained. For all eight analytes the LOQ was estimated to be 0.05 mg/kg.

The method has been successfully used in a number of international proficiency tests and check sample programs. Over the past decade, 93 such samples from external programs have been analyzed for DON, with only two instances of unacceptable *Z* scores. Four proficiency test samples have been analyzed for T-2 and HT-2; one has been analyzed for NIV. All results obtained from these tests were acceptable.

Over the period of the study (August 1, 2010, through July 31, 2012) 38 sets of duplicates were analyzed. Results from the duplicate analyses confirm the good precision of the method. For the 28 pairs with quantifiable levels of DON, the relative standard deviation of the duplicate results ranged from 0 to 32%, with a median of 5.1%. Both duplicates had results <LOQ for the remaining 10 samples. Only three sets of duplicates contained quantifiable levels of NIV; the relative standard deviation of these results ranged from 4.6 to 5.7%. Finally, one pair of duplicates contained HT-2. The relative standard deviation of the duplicate results was 24%.

Multiple analyses of CRMs (Trilogy Analytical Laboratory, Washington, MO, USA) also demonstrated the good performance of the method during this study. The mean ± standard deviation of replicate analyses [(n = 17) 0.69 ± 0.07 and (n = 122) 1.1 ± 0.1 mg/kg] matched the certified values of two different CRMs of DON in wheat (0.7 ± 0.1 and 1.1 ± 0.1 mg/kg).

The method uncertainty was estimated using a "top-down" approach to determine method bias and precision separately.[15,16] These two values were then combined to obtain an overall expanded method uncertainty. Values were only estimated for DON, NIV, T-2, and HT-2, because data from proficiency tests were available for only these four analytes. The expanded relative uncertainty was estimated to be 47, 65, 61, and 67% for DON, NIV, T-2, and HT-2, respectively. The estimated method uncertainty for DON is similar to those reported for other mycotoxins.[12,17]

The analytical method begins with an intensive grinding and dividing procedure. This was originally developed to mitigate effects of sample heterogeneity on the analysis of ochratoxin A.[12] The procedure was also shown to produce 1 kg and 100 g portions from whole grain that were all considered to be sufficiently homogeneous with respect to concentrations of DON according to the method outlined in Fearn and Thompson.[18] The coefficient of variation of DON ranged from 3.05 to 6.44% (Supporting Information, Supplementary Table 2) in test portions taken from the 1 kg aliquots and from 1.37 to 9.73% in test portions taken from the 100 g subsamples (Supporting Information, Supplementary Table 3).

Stability of Analytes during Sample Storage. Results of the reoccurring analyses to monitor the stability of DON and NIV are shown in Figure 1. There was no indication of decreasing or increasing trends in concentration of the two

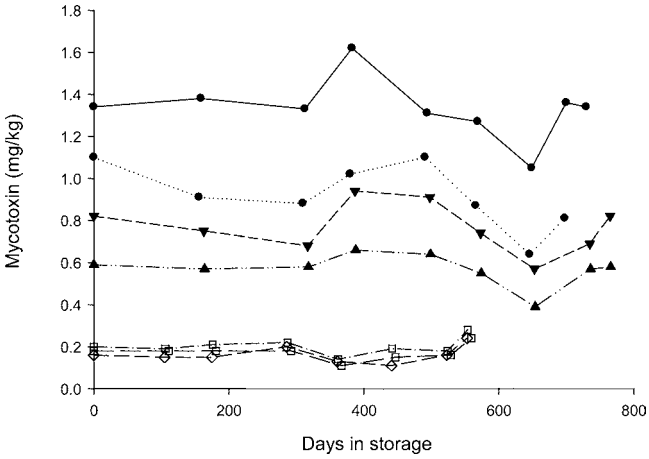

Figure 1. Stability of deoxynivalenol in four ground wheat samples (solid symbols) and nivalenol in three ground barley samples (open symbols) during long-term laboratory storage.

mycotoxins in samples during storage, demonstrating that storage of ground sample under the laboratory conditions used is adequate to preserve concentrations of DON and NIV. There was some variability in the results from the multiple analyses of the wheat and barley samples, but the variability appeared consistent with the variability observed for multiple CRM analyses and duplicate samples.

Figure 2 shows that the moisture content of stored ground wheat decreased over time. There was decrease of approx-

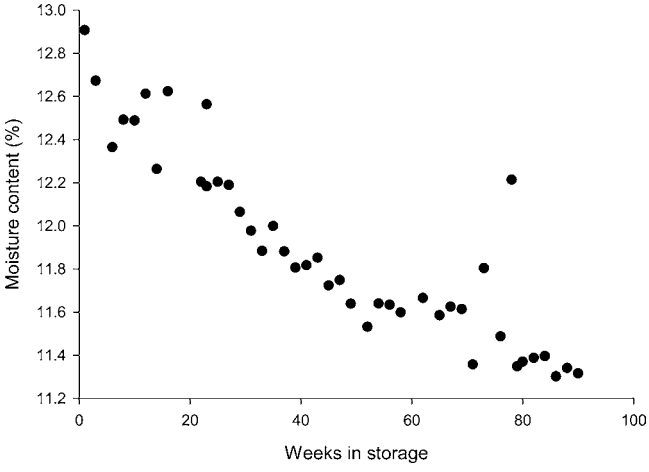

Figure 2. Change in moisture content of ground wheat during laboratory storage.

imately 1.5% over the storage period. This decrease would result in a small change in mass of ground wheat over a year and a half of storage and was not expected to have altered mycotoxin concentrations during the study period.

***Fusarium* Trichothecenes in Cereal Grain Shipments.** Table 2 contains a summary of the results from the analysis of the 303 cereal grain shipments. DON was the most frequently measured trichothecene and was quantified in samples of all types of cereal grains studied. NIV was the next most frequently observed trichothecene, but its occurrence was limited to

Table 2. Deoxynivalenol (DON) and Nivalenol (NIV) Measured in Canadian Cereal Samples Obtained from Shipments Occurring between August 1, 2010, and July 31, 2012[a]

grain	no. of samples analyzed	no. of positive DON samples	range of DON detected (mg/kg)	no. of positive NIV samples	range of NIV detected (mg/kg)
barley	27	4	0.05−0.07	4	0.09−0.20
corn	9	9	0.32−2.34	0	
oats	1	1	0.08	0	
rye	1	1	0.12	0	
durum	68	46	0.05−0.40	0	
wheat	197	170	0.05−1.25	0	

[a]Concentrations of DON and NIV are recovery corrected.

barley. There was no apparent correlation between NIV and DON in barley; only one of the four samples that contained NIV also contained DON. The only other compound detected was 15-ADON. It was present at 0.43 mg/kg in one corn sample that also contained 2.34 mg/kg DON. The remaining five analytes were not detected in any sample.

There was no significant difference between median DON concentrations in export versus domestic shipments of CWRS (0.18 vs 0.23 mg/kg) and CWAD (0.09 vs 0.07 mg/kg) (Mann−Whitney rank sum test). Therefore, data from export and domestic shipments were combined for further analyses.

Histograms displaying the distribution of DON in CWAD and CWRS, the two major classes of wheat studied, are shown in Figure 3. Higher concentrations of DON occurred more often in CWRS than in CWAD. The median concentration of DON in CWRS (0.21 mg/kg) was significantly greater than that for CWAD (0.09 mg/kg) (Mann−Whitney rank sum test, $p < 0.001$). For the purpose of all calculations and figures, results of <LOQ were set to 0.05 mg/kg. Figure 4 shows the DON concentrations measured in individual samples with respect to the date that the shipments were loaded. The grades for the shipments of CWAD and wheat are also provided on a monthly mean basis.

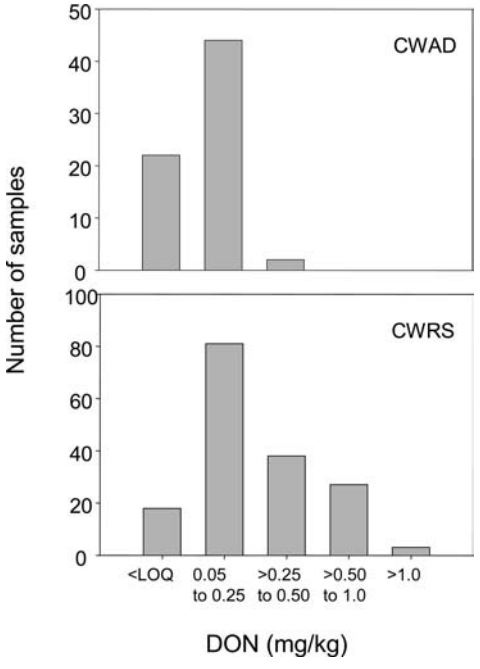

Figure 3. Histograms of deoxynivalenol (DON) in shipments of Canada Western Amber Durum (CWAD) and Canada Western Red Spring (CWRS) wheat transported between August 1, 2010, and July 31, 2012.

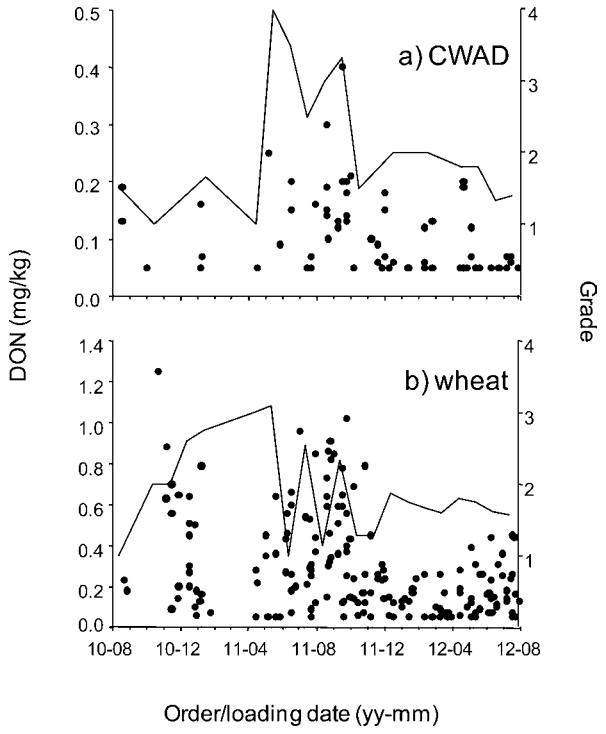

Figure 4. Variation of deoxynivalenol (DON) and grade of Canada Western Amber Durum (CWAD) and wheat shipments transported between August 1, 2010, and July 31, 2012. Circles represent concentrations of DON in shipments; the line graphs plot the mean monthly numerical grade.

The relationship between DON concentration and grade of a shipment is more closely examined in Figure 5 for three classes of wheat: CWAD, CWRS, and CWRW. There were not enough samples of other grains or other wheat classes to examine the effect of grade on DON concentrations. The median DON concentrations in the top grades (i.e., number 1) of CWAD and CWRS were significantly lower than those of the lower grades (Kruskal−Wallis one-way analysis of variance on ranks, $p \leq 0.001$). There was no statistically significant difference in median DON concentrations among different grades of CWRW shipments. It should be noted there were only 13 CWRW available for analysis, so a low sample number may have affected the examination of a grade and DON concentration relationship for this class.

DISCUSSION

The samples analyzed represent approximately 10% of Canadian cereal grains exported and 7% of grain shipped domestically over the course of the study. The major *Fusarium* trichothecene present in samples was DON. It was quantified in 76% of the samples analyzed; the next most frequently measured trichothecene was NIV at 1%. The high frequency of occurrence for DON is consistent with the predominance of DON-producing *Fusarium* species such as *F. graminearum* in western Canadian cereal grains, particularly those grown in the eastern prairies.[7,8,19]

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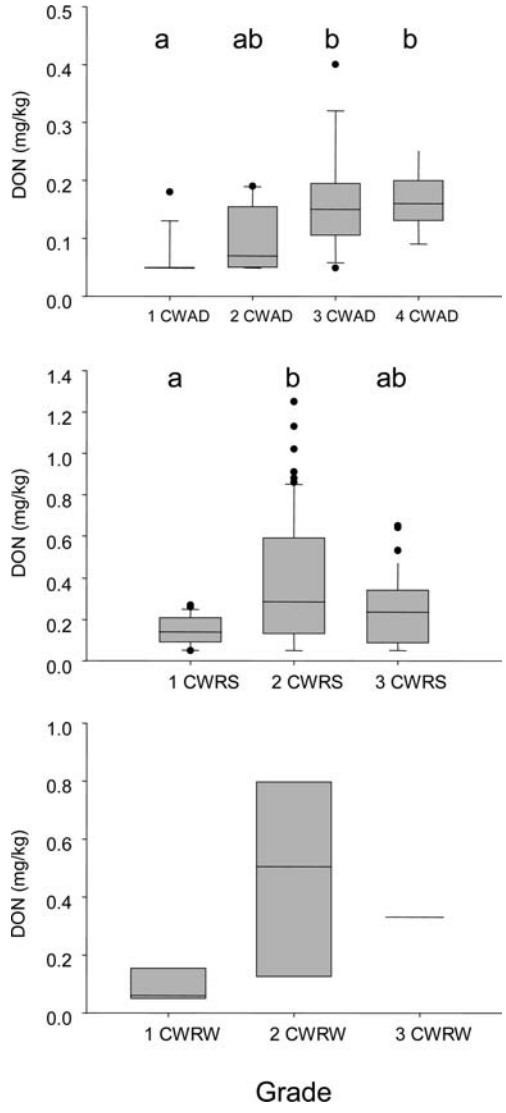

Figure 5. Variation of deoxynivalenol (DON) with grade of Canada Western Amber Durum (CWAD), Canada Western Red Spring (CWRS), and Canada Western Red Winter (CWRW) wheat in shipments occurring between August 1, 2010, and July 31, 2012. Median DON concentrations are indicated by horizontal lines in the boxes encompassing the 25th−75th percentiles. The whiskers above and below the boxes indicate the 90th and 10th percentiles. Unique letters indicate medians are significantly different.

The presence of NIV was limited to barley samples; no other types of grain analyzed in this study contained NIV. *F. graminearum*, *F. culmorum*, and *F. poae* are the main species known to produce NIV.[10] Historically, *F. graminearum* and *F. poae* combined were detected more frequently than other *Fusarium* species on barley grown in western Canada.[20] In the provinces of Manitoba and Saskatchewan, *F. poae* was prevalent on barley (at 34 and 57% of the kernels analyzed) from 2003 to 2007.[21]

Concentrations of DON in all but one shipment complied with existing and proposed maximum limits. No samples exceeded the Codex proposed draft maximum limit of 2 mg/kg for raw wheat, maize, and barley grains[22] or the Canadian maximum limits of 1.0 and 2.0 mg/kg for soft wheat for use in baby foods and nonstaple foods, respectively. Two of the 197 wheat samples contained DON at 1.1 and 1.3 mg/kg.[2] However, it should be noted that the Canadian limits are specified for soft wheat. The shipments sampled and analyzed were all hard wheat. In addition, these Canadian maximum limits are currently under review. Of the 303 samples analyzed, one corn sample at 2.34 mg/kg exceeded the European Union DON maximum level of 1.75 mg/kg.[5] The wheat, rye, and barley shipments, as well as those for durum and oats, were all compliant with the European maximum levels of 1.25 and 1.75 mg/kg, respectively.

The median and range of concentrations of DON in CWAD shipments were similar to those reported for CWAD in samples of the same grade from the 2010 western Canadian harvest[19] and for CWAD imported into Puglia, Italy, in 2010.[23] A valid comparison cannot be made between DON concentrations in CWRS from the current study and harvest samples from 2010 though, because >90% of the harvest samples were graded lower than 3 CWRS,[8] whereas only about 20% of the shipments analyzed in the current study were graded lower than 3. As will be discussed, grade is a significant factor in the concentration of DON in wheat and durum.

The concentrations of DON in the cereal grain shipments were generally lower than those reported in the literature for cereals grown in other locations, as is the frequency of occurrence of other *Fusarium* trichothecenes. For example, the median concentration of DON in shipments of western Canadian wheat (0.21 mg/kg) was half the median reported for DON in U.K. wheat harvested from 2001 to 2005 (0.42 mg/kg),[24] approximately one-sixth of the median for eastern Canadian wheat,[25] and about an order of magnitude lower than the median reported for Brazilian wheat.[26] Within the data set of western Canadian grain shipments, DON occurred more frequently and at higher concentrations in CWRS as compared to CWAD. This is consistent with the occurrence of *F. graminearum*, the main DON producer in Canada. *F. graminearum* is not as prevalent in the western prairies, where CWAD is mainly grown, as compared to the eastern prairies.[6,19]

Median concentrations of DON in corn (0.94 mg/kg) and barley (1.3 mg/kg) grown in eastern Canada[25] were also higher than those measured in the shipments of western Canadian corn (0.6 mg/kg) and barley (0.06 mg/kg). In addition, both 15- and 3-ADON were detected in U.K.[24] and eastern Canadian samples,[25] as well as NIV, HT-2, and T-2. Other monitoring of cereals grown in northern Europe also reported the presence of HT-2 and T-2.[27] These trichothecenes were not detected in the shipments of western Canadian grain analyzed.

Whereas growing conditions, species, and chemotype of *Fusarium*, as well as grain varieties, will contribute to the differences in concentrations and frequency of occurrence of trichothecenes, the type of sample analyzed will also be a factor in the differences discussed above. Samples for the current study were obtained near the end of the grain handling chain, after grading, sorting, cleaning, etc., had occurred, so the results characterize Canadian grain as it is sold, not as it is harvested.

As alluded to above, there is a correlation between the grade of a sample and the concentration of DON in wheat and durum. The amount of *Fusarium*-damaged kernels (FDK) present in a sample is a grading factor for Canadian wheat and durum. This grading factor is based on the relationship between DON concentrations and the amount of FDK due to *F. graminearum* in wheat.[28,29] Limits are set for the maximum amount of FDK allowed in various wheat classes. The maximum amounts of FDK permitted on a mass basis for the

top grades of CWRW, CWAD, and CWRS are 0.8, 0.5, and 0.25%, respectively.[30] Lower grades have higher tolerances. When FDK tolerances are exceeded, grain is downgraded due to *Fusarium* damage.

Even though *Fusarium* damage is just one of many grading factors, there is a relationship between grade and DON concentration in CWAD, CWRS, and CWRW shipments analyzed (Figure 4). Samples of better grades (e.g., one CWAD, one CWRS, and one CWRW) tended to contain lower concentrations of DON. However, there are exceptions with samples of poorer grade containing low concentrations of DON as well. These instances likely reflect downgrading due to factors other than *Fusarium* damage. Overall, these monitoring results demonstrate the current *Fusarium* damage grading factor is effectively managing DON in shipments of wheat and durum.

A further example of the relationship between grade and DON concentration can be seen in Figure 4. The increased number of shipments with higher DON concentrations observed through 2011 for CWAD and wheat appears to be accompanied by lower grades (i.e., higher numerical grades) for the shipments. This bump may be due to the movement of grain harvested in 2010. This was a growing year with increased incidence of *Fusarium* infection and downgrading due to *Fusarium* damage.[8]

In conclusion, the sample preparation and validated analytical method used for monitoring Canadian cereal grain shipments for *Fusarium* trichothecenes performed well over the two year study period. The storage conditions for samples were also shown to be appropriate for monitoring activities that may rely on medium- to long-term (i.e., from months to a few years) of storage.

Of the eight *Fusarium* trichothecenes monitored, DON was quantitated the most often and was measured in shipments of all types of grain analyzed. There was no difference in DON concentrations measured in export and domestic shipments. NIV was observed only in barley. Monitoring of shipments of wheat and durum transported over a two year period showed variation in DON over time, which appeared to be related to the grade of the grain being shipped and the quality of growing conditions. However, the current *Fusarium* damage grading factor is effectively managing DON in shipments of wheat and durum.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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