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Fusarium Damage in Small Cereal Grains from Western Canada. 2. Occurrence of *Fusarium* Toxins and Their Source Organisms in Durum Wheat Harvested in 2010[†]

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Supporting Information

ABSTRACT: Samples of Canadian western amber durum harvested in 2010 were obtained as part of the Canadian Grain Commission Harvest Sample Program, inspected, and graded according to Canadian guidelines. A subset of *Fusarium*-damaged samples were analyzed for *Fusarium* species as well as mycotoxins associated with these species, including deoxynivalenol and other trichothecenes, moniliformin, enniatins, and beauvericin. Overall, *Fusarium avenaceum* and *F. graminearum* were the top two most frequently recovered species. *Phaeosphaeria nodorum* (a.k.a. *Septoria nodorum*), *F. culmorum*, *F. poae*, *F. acuminatum*, and *F. sporotrichioides* were observed in samples as well. All samples analyzed for mycotoxins contained quantifiable concentrations of enniatins, whereas beauvericin, deoxynivalenol, and moniliformin were measured in approximately 75% of the samples. Concentrations in *Fusarium*-damaged samples ranged from 0.011 to 34.2 mg/kg of enniatins plus beauvericin, up to 4.7 mg/kg of deoxynivalenol, and up to 6.36 mg/kg of moniliformin. Comparisons of enniatins, beauvericin, and moniliformin concentrations to the occurrence of various *Fusarium* species suggest the existence of an infection threshold above which these emerging mycotoxins are present at higher concentrations. The current grading factor of *Fusarium*-damaged kernels manages concentrations of these emerging mycotoxins in grain; lower provisional grades were assigned to samples that contained the highest concentrations of enniatins, beauvericin, and moniliformin.

KEYWORDS: *grain, emerging mycotoxins, Fusarium avenaceum, occurrence, distribution, grading*

INTRODUCTION

Durum is an important cereal crop in Canada. During the 2010 growing season, 5.4 Mt of durum was produced in Canada.[1] This comprised approximately 13% of the world durum production for that year.[2] Canada western amber durum (CWAD) is grown on the western prairies, primarily in the drier regions of the provinces of Alberta and Saskatchewan (Figure 1). Canadian durum is predominantly used in the production of pasta, couscous, and durum bread.

Various *Fusarium* species have been detected on durum in Canada. The predominant species are *Fusarium graminearum*, *F. avenaceum*, and *F. poae*.[3] Infection of durum by *Fusarium* affects crop yield and can degrade the milling and processing qualities, such as semolina yield, color, and gluten strength.[4] In contrast, the primary effects of *Fusarium*-damaged kernels (FDK) caused by *F. avenaceum* (FaDK) on durum wheat quality are limited to appearance, i.e., an increase in semolina speck count and higher spaghetti redness. These and other effects (e.g., on gluten strength and spaghetti texture), however, were insignificant when FaDK addition was below 2%, the limit for No. 3 and No. 4 CWAD.[5]

In addition, infection of grain by *Fusarium* species can produce a variety of mycotoxins that are potentially hazardous to humans and livestock who consume the contaminated grain. *F. graminearum*[6] and *F. culmorum*[7] produce the well-studied mycotoxin deoxynivalenol (DON), in addition to a variety of other compounds, including zearalenone, nivalenol, and fusarenone x. On small cereals such as wheat, beauvericin (BEAU), enniatins, and moniliformin (MON) are produced mainly by *F. acuminatum*, *F. avenaceum*, and *F. tricinctum*.[8,9]

Many durum cultivars are more susceptible to *Fusarium* infection and subsequent mycotoxin production than common wheat and other small cereal grains.[10] This is suggested by higher DON and MON concentrations in durum samples grown in the same crop districts as common wheat in western Canada.[3]

It has been established that environmental conditions, including temperature and precipitation around the time of anthesis, are important factors in *Fusarium* infections of cereals such as wheat.[11] In the 2010 growing year, the precipitation on much of the Canadian Prairies was greater than average.[12] In particular, in south central Saskatchewan the amount of precipitation received between the end of June and early July, the typical time of anthesis of durum in the province,[13] ranged from 115% to more than 200% of the 30-year average.

This excessive precipitation resulted in conditions that promoted fusarium head blight (FHB) and resulted in a higher

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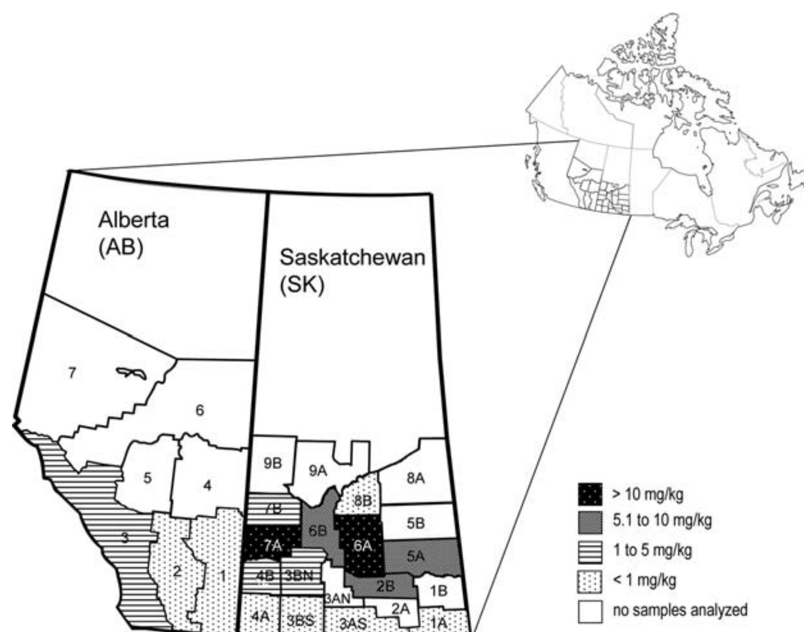

Figure 1. Geographical distribution of depsipeptides in 2010 Canadian durum samples from crop districts in Alberta and Saskatchewan.

proportion of durum harvested in 2010 receiving lower grades due to *Fusarium* damage than in previous years and 2011 (Twylla McKendry, personal communication). It was also hypothesized to contribute to the widespread occurrence of *F. avenaceum* and MON in small cereal grains such as oats and wheat.[14]

In this work, samples of durum obtained as part of the Canadian Grain Commission's (CGC) Harvest Sample Program during the 2010 harvest were analyzed for *Fusarium* species as well as mycotoxins produced by these organisms, including DON and other trichothecenes, and the "emerging" mycotoxins of MON, enniatins, and BEAU. These mycotoxins are considered to be "emerging", as they have been less studied in the past and are only recently being monitored more extensively.[15] There are very limited existing data on MON, enniatins, and BEAU for Canadian durum and many other grains. These emerging mycotoxins are also of interest in other areas; for example, the European Food Safety Authority recently issued a call for scientific data on these compounds.[16]

■ MATERIALS AND METHODS

Samples. Samples of Canada western amber durum harvested in 2010 from various crop districts in the Canadian provinces of Saskatchewan and Alberta (Figure 1) were obtained from the CGC Harvest Sample Program. The CGC Harvest Sample Program samples consisted of CWAD that was voluntarily sampled at harvest by producers and submitted to the CGC. Sampling instructions were provided to producers prior to harvest.[14]

The CWAD samples (n = 54) selected for mycotoxin and fungal analysis were a subset of the total CWAD samples submitted to the CGC Harvest Sample Program. Samples that contained *Fusarium*-damaged kernels identified by CGC grain inspectors, and were available for use, were selected for mycotoxin and fungal analysis.

Once received, samples were examined by CGC inspectors and given an unofficial grade. They were then stored at room temperature in paper envelopes until analyses were performed. Before sample preparation, each Harvest Sample Program sample was passed through a centrifugal seed divider (Pascall Engineering Co. Ltd., England, UK) several times to ensure a good mixing and then split in halves. One half was retained for fungal analysis, and the other half was used for chemical analysis.

Visible *Fusarium* Damage and Fungal Analysis. *Fusarium*-damaged kernels were estimated and weighed by CGC grain inspectors according to grading specifications published in Canada's official grain grading guide for wheat.[17] Seeds of durum wheat moderately affected by FHB were identified visually based on mycelial growth at the germ and crease, occasionally including a wrinkled seed coat, broad crease, and bronzy color. FDK with severe symptoms showed abundant mycelial growth visible on both seed surfaces, the wrinkled seed coat, broadened crease, and a bronzy color.[18,19]

Up to 30 FDK were removed from each of 47 durum samples and surface disinfected before plating onto potato dextrose agar (PDA; Difco, VWR). Prior to plating, FDK were soaked in a 0.3% sodium hypochlorite solution for 1 min and then dried in a laminar-flow hood. Ten seeds per 9 cm plastic Petri dish were placed onto PDA and subsequently incubated for 5 days at room temperature under a 12 h on/off cycle of near-ultraviolet and fluorescent light or darkness. Fungal identifications were done according to the methods described previously.[20] The fusaria developing from the seed were each transferred to a new 6 cm plastic Petri dish and grown on PDA for 7 days at room temperature. Numerous pure cultures of *F. avenaceum* and *F. acuminatum* were isolated from incubated seeds based on macro- and micromorphological criteria and used for phylogenetic study.[14]

Mycotoxin Analysis. All samples were analyzed for a number of *Fusarium*-related mycotoxins. The specific mycotoxins, their method of analysis, and the method limits of quantitation are listed in Table 1. Prior to analysis the entire mass of grain available for chemical analysis (between 200 and 300 g) was ground using a commercial coffee grinder (model KR 804, Ditting Maschinen AG, Switzerland). The sample was ground fine enough that ≥85% of the sample mass passed through a US 50 sieve (nominal sieve openings of 300 μm).

Sample preparation and mycotoxin analysis were performed using three different instrumental-based analytical methods. MON was analyzed using high-performance liquid chromatography with photodiode array detection (HPLC-PDA) using the method and instrumentation as described in Gräfenhan et al.[14] Ground CWAD was extracted with a solution of acetonitrile and water. The extract was exchanged into methanol and cleaned using strong anion exchange solid phase extraction. The eluate was chromatographed using ion pairing reagent in the mobile phase and was analyzed using PDA scanning from 200 to 350 nm.

Table 1. Mycotoxin Methods Used in the Analysis of Canadian Durum Harvested in 2010

mycotoxin	mycotoxin group	abbreviation	method of analysis	method LOQ (mg/kg)
moniliformin	moniliformin	MON	HPLC-PDA	0.03
deoxynivalenol	trichothecene	DON	GC-MS	0.05
3-acetyl-deoxynivalenol	trichothecene	3-ADON	GC-MS	0.05
15-acetyl-deoxynivalenol	trichothecene	15-ADON	GC-MS	0.05
nivalenol	trichothecene	NIV	GC-MS	0.05
HT-2 toxin	trichothecene	HT2	GC-MS	0.05
T-2 toxin	trichothecene	T2	GC-MS	0.05
fusarenon-x	trichothecene	FUS-X	GC-MS	0.05
diacetoxyscirpenol	trichothecene	DAS	GC-MS	0.05
enniatin A	depsipeptide	ENN A	HPLC-MS/MS	0.00003
enniatin A1	depsipeptide	ENN A1	HPLC-MS/MS	0.00005
enniatin B	depsipeptide	ENN B	HPLC-MS/MS	0.00005
enniatin B1	depsipeptide	ENN B1	HPLC-MS/MS	0.0001
enniatin B2	depsipeptide	ENN B2	HPLC-MS/MS	0.00002
enniatin B3	depsipeptide	ENN B3	HPLC-MS/MS	0.00003
beauvericin	depsipeptide	BEAU	HPLC-MS/MS	0.00005

MON was considered to be detected if the retention time of the peak was within 0.1 min of the average retention time in the calibration standards, the peak signal-to-noise ratio was greater than 9, and acceptable peaks were observed at the quantitation wavelength of 229 nm, as well as the confirmation wavelengths of 249 and 260 nm. A six-point calibration curve of MON peak area versus mass of MON injected with an $R^2 \geq 0.99$ was constructed for quantitation using standards prepared in dilute ion pairing reagent.

The *Fusarium* trichothecenes were extracted, derivatized, and analyzed using gas chromatography−mass spectrometry (GC-MS) based on a method developed by Tacke and Casper[21] and performed as described in Clear et al.[3]

The depsipeptide compounds—the enniatins and beauvericin—were analyzed using modified versions of existing methods.[22,23] The depsipeptides were extracted from ground CWAD in a single step using an acidified acetonitrile/water solution. MON and DON were also included in this method and thus provided a confirmation of the results generated by the HPLC-PDA and GC-MS methods. Crude extract was then diluted and analyzed using liquid chromatography electrospray ionization−tandem mass spectrometry (HPLC-MS/MS). The chromatographic and mass spectrometric parameters used in the analyses are as described in Vishwanath et al.;[23] however an Agilent 1290 U-HPLC coupled to an ABSciex 5500Qtrap was used instead of the instrumentation described in the older publication. The tandem mass spectrometric analysis was performed in multiple reaction monitoring mode. Each sample was analyzed twice, once using positive and once using negative polarities. Two fragmentation reactions per analyte were monitored (with the exception of MON, which exhibited only one fragment ion). Confirmation of analyte identification was obtained by the acquisition of two transitions per analyte, which yielded 4.0 identification points.[24] In addition, the chromatographic retention time and the intensity ratio of the two transitions agreed with the corresponding standard values within 0.1 min and 30% relative abundance, respectively.

Fortified blank samples, certified reference materials, and in-house reference materials were all used during the analysis of the CWAD samples as quality control measures.

RESULTS

Visible *Fusarium* Damage and Fungal Analysis. Canada western amber durum wheat harvested in 2010 was affected by *Fusarium*. In total, 14.5% of the 820 samples submitted and inspected as part of the Harvest Sample Program contained FDK. In comparison to Harvest Sample Program results from 2003 to 2012, in 2010 a larger proportion of samples was affected by *Fusarium* and the degree of damage was more severe (Table 2). This was especially apparent in samples from central Alberta and Saskatchewan. Crop districts (CDs) 1 and 2 in Alberta and CDs 2, 3B, 4B, 5A, 6, and 7A in Saskatchewan were most affected in 2010, with the percentage of samples damaged by *Fusarium* ranging from 9.3% to 60%. In these central crop districts, the mean percentage (by weight) of damage ranged from 1.25% to 3.86% FDK in the samples with *Fusarium* damage. Damage reached a maximum of 14% in individual samples (data not shown). In previous years, neither frequency nor severity of *Fusarium* damage was of much concern in most parts of the durum growing area in western Canada. Since 2009−10 however, *Fusarium* damage has gained importance as a degrading factor with up to 60% of the samples containing FDK in central Saskatchewan in 2012. In 2010, *Fusarium* damage was not listed among the top three degrading factors in the Harvest Sample Program, but it was in the 2012 harvest, for CWAD harvested in Alberta and Saskatchewan.

From 47 durum samples, six identified *Fusarium* species were recovered from a total of 800 FDK with occasionally more than one species isolated from an individual seed. By far, *F. avenaceum* was the predominant species, which was most frequently recovered from more than 70% of FDK (i.e., 533 seeds). The FDK from only three samples did not contain *F. avenaceum*. *F. graminearum* was detected in 147 FDK from eight CWAD samples (Supporting Information). The other *Fusarium* species, including *F. acuminatum*, *F. culmorum*, *F. poae*, and *F. sporotrichioides*, were recovered only at low frequencies (<1.5%). In addition, *Phaeosphaeria nodorum* (a.k.a. *Septoria nodorum*) was detected in a total of 64 FDK, being the third most frequently recovered fungal species from durum wheat (~8% of all FDK plated).

Mycotoxins in CWAD. During the sample analysis, acceptable recoveries and precision were obtained from the fortified blanks and reference materials used as quality control samples. The mean ± standard deviation percentage recovery of MON from fortified blank samples (n = 14) was 79 ± 12%; for DON it was 94 ± 11% (n = 8). As well, certified ground wheat DON reference material mean ± standard deviation concentration was 1.2 ± 0.1 mg/kg (n = 8 replicates), which compared very well with the certified concentration of 1.1 ± 0.1 mg/kg. The mean percentage recovery of the seven depsipeptides from a fortified sample was 100%; individual recoveries ranged from 97% to 108%.

As mentioned in the previous section, MON and DON data were generated by the HPLC-PDA and GC-MS methods as well as the HPLC-MS/MS method. The relationships between the results obtained by the various methods are displayed in Figure 2. There is very good agreement between the DON results from the two methods. For the MON results, the correlation appears to be affected by the five results that are greater than 3 mg/kg as determined by HPLC-PDA. When focusing on results that were below 3 mg/kg, the linear regression equation is $y = 1.123x - 0.0343$, with an R^2 of 0.97.

Table 2. Frequency of Detection of *Fusarium*-Damaged Kernels and Severity of *Fusarium* Damage Observed in Durum Grown in Saskatchewan (SK) and Alberta (AB) Crop Districts and Submitted to the Canadian Grain Commission Harvest Sample Program from 2003 through 2012[a]

	2012	2011	2010	2009	2008	2007	2006	2005	2004	2003
					Saskatchewan					
1A	40.0	0.0	0.0	0.0	0.0	0.0	0.0	23.8	0.0	14.7
	0.95	0.00	0.00	0.00	0.00	0.00	0.00	1.14	0.00	0.29
	(5)	(1)	(5)	(41)	(13)	(15)	(12)	(42)	(15)	(34)
1B	33.3	0.0	100.0	0.0	33.3	0.0	0.0	20.0	0.0	16.0
	1.10	0.00	3.00	0.00	1.00	0.00	0.00	2.80	0.00	0.10
	(3)	(2)	(1)	(1)	(3)	(7)	(9)	(10)	(5)	(25)
2A	45.5	14.3	9.3	6.3	0.0	0.0	0.0	12.9	0.0	4.9
	1.20	0.97	1.55	0.85	0.00	0.00	0.00	0.86	0.00	0.08
	(55)	(35)	(43)	(63)	(58)	(66)	(68)	(85)	(58)	(143)
2B	63.2	10.6	29.0	21.2	8.7	2.7	2.6	8.5	0.8	6.1
	1.78	0.84	2.14	1.10	0.67	1.83	0.76	1.44	1.20	0.08
	(87)	(94)	(62)	(137)	(138)	(149)	(156)	(200)	(130)	(246)
3AN	11.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.0
	0.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.00
	(26)	(25)	(16)	(17)	(14)	(25)	(19)	(31)	(17)	(31)
3AS	8.1	3.3	1.1	0.0	0.0	0.0	0.0	0.7	1.0	2.2
	1.08	0.74	1.60	0.00	0.00	0.00	0.00	0.05	1.20	0.03
	(99)	(92)	(88)	(142)	(130)	(116)	(108)	(149)	(104)	(182)
3BN	8.7	3.8	15.1	1.3	0.0	0.0	0.0	0.5	2.5	0.4
	1.08	0.74	1.92	0.80	0.00	0.00	0.00	0.20	0.80	0.09
	(127)	(132)	(126)	(157)	(158)	(167)	(197)	(200)	(161)	(271)
3BS	1.3	0.0	10.8	0.0	1.4	0.0	0.0	0.0	1.9	2.2
	0.20	0.00	1.39	0.00	0.60	0.00	0.00	0.00	0.70	0.05
	(79)	(54)	(65)	(65)	(72)	(67)	(73)	(80)	(53)	(90)
4A	0.0	0.0	2.4	0.0	0.0	0.0	0.0	4.4	4.2	0.0
	0.0	0.00	1.25	0.00	0.00	0.00	0.00	0.15	0.70	0.00
	(53)	(42)	(42)	(35)	(50)	(43)	(33)	(45)	(24)	(40)
4B	7.0	0.0	17.1	2.0	0.0	0.0	0.0	0.0	0.8	1.3
	0.49	0.00	2.30	0.56	0.00	0.00	0.00	0.00	0.60	0.03
	(128)	(101)	(105)	(101)	(105)	(81)	(153)	(166)	(123)	(152)
5A	81.8	50.0	60.0	35.3	14.8	14.3	0.0	27.8	0.0	16.7
	2.44	0.55	3.43	1.58	0.83	0.72	0.00	1.83	0.00	0.04
	(11)	(4)	(15)	(17)	(27)	(21)	(24)	(36)	(19)	(48)
5B	0.0		0.0	0.0	33.3	0.0	0.0	0.0	0.0	37.5
	0.00		0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.07
	(1)	(0)	(1)	(1)	(3)	(3)	(3)	(3)	(1)	(8)
6A	60.0	30.0	41.2	14.8	2.9	0.0	2.9	3.0	0.0	17.9
	1.55	0.78	3.77	1.62	0.50	0.00	0.65	1.10	0.00	0.12
	(25)	(20)	(17)	(27)	(34)	(30)	(34)	(67)	(41)	(78)
6B	50.0	38.9	44.4	11.1	7.7	0.0	0.0	6.1	0.0	7.3
	1.48	1.08	3.12	1.19	1.48	0.00	0.00	0.80	0.00	0.02
	(16)	(18)	(18)	(18)	(26)	(19)	(25)	(33)	(29)	(55)
7A	46.2	13.2	54.6	5.1	0.7	0.0	0.0	1.9	0.9	0.7
	1.17	2.11	3.86	1.22	0.80	0.00	0.00	0.90	1.20	0.02
	(91)	(121)	(97)	(118)	(140)	(127)	(133)	(162)	(114)	(138)
7B	50.0	8.3	0.0	6.7	0.0	0.0	0.0	10.0	0.0	3.8
	0.90	2.98	0.00	0.73	0.00	0.00	0.00	2.65	0.00	0.02
	(4)	(12)	(8)	(15)	(14)	(24)	(12)	(20)	(22)	(26)
8A		0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	25.0
		0.00	0.00	1.10	0.00	0.00	0.00	0.00	0.00	0.14
	(0)	(1)	(2)	(6)	(4)	(1)	(1)	(4)	(2)	(8)
8B	85.7	33.3	50.0	50.0	0.0	10.0	0.0	23.1	0.0	21.4
	4.36	0.98	1.30	1.11	0.00	5.00	0.00	2.13	0.00	0.17
	(7)	(6)	(4)	(6)	(11)	(10)	(10)	(13)	(4)	(14)
9A			0.0	0.0		0.0	0.0			33.3
			0.00	0.00		0.00	0.00			0.03
	(0)	(0)	(1)	(1)	(0)	(2)	(1)	(0)	(0)	(3)
9B	0.0							100.0		0.0

Table 2. continued

	2012	2011	2010	2009	2008	2007	2006	2005	2004	2003
					Saskatchewan					
9B	0.00							8.50		0.00
	(4)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(1)
					Alberta					
1	11.1	1.8	19.6	7.7	0.0	1.5	0.0	1.6	0.8	4.2
	0.91	0.87	1.77	1.65	0.00	0.90	0.00	1.50	0.55	0.06
	(63)	(55)	(46)	(52)	(93)	(66)	(69)	(124)	(132)	(166)
2	16.1	2.9	11.3	17.6	2.5	0.0	1.9	0.7	1.0	11.5
	1.19	2.44	1.81	2.10	1.55	0.00	1.20	2.00	1.60	0.05
	(93)	(69)	(53)	(108)	(80)	(86)	(103)	(143)	(97)	(243)
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
	(10)	(11)	(6)	(7)	(11)	(25)	(15)	(17)	(18)	(29)
4						0.0	0.0	0.0		0.0
						0.00	0.00	0.00		0.00
	(0)	(0)	(0)	(0)	(0)	(2)	(1)	(4)	(0)	(3)

[a]From top to bottom in each cell, data are provided for mean percentage of samples containing *Fusarium*-damaged kernels, mean % (mass basis) *Fusarium*-damaged kernels in samples containing damaged kernels, and number of samples inspected (in parentheses).

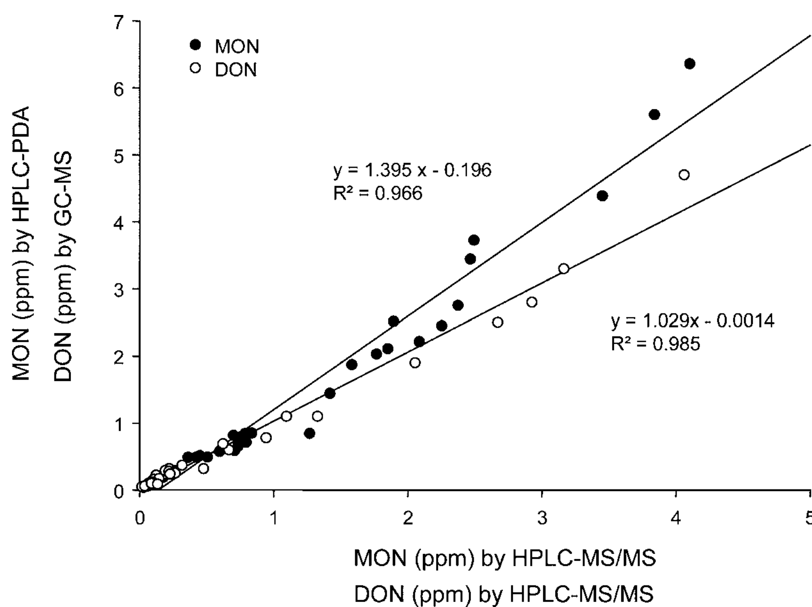

Figure 2. Correlation of results from HPLC-PDA, GC-MS, and HPLC-MS/MS methods for moniliformin (MON) and deoxynivalenol (DON).

Table 3 summarizes the results from the analysis of the CWAD samples for the mycotoxins detected the most frequently: MON, DON, and the depsipeptides. Aside from one sample containing 0.07 mg/kg of HT-2, the remaining seven analytes in the GC-MS method were not detected in any of the samples. All samples contained quantifiable concentrations of enniatins, whereas BEAU, DON, and MON were measured in approximately 75% of the samples.

A greater proportion of CWAD samples contained MON as compared to wheat, oats, and rye that were also sampled during the 2010 harvest.[14] Only 56% of wheat samples and less rye (33%) and oats (16%) contained measurable amounts of MON, as compared to 75% of CWAD samples. When MON was detected in CWAD, it was often observed at concentrations greater than those reported in the other small cereal grains. The overall mean and median MON concentrations in CWAD (1.32 and 0.68 mg/kg, respectively) were approximately 3 to 10 times greater than the mean and median concentrations measured in wheat (0.29 and 0.17 mg/kg), oats (0.070 and 0.057 mg/kg), and rye (0.07 and 0.06 mg/kg).

A similar phenomenon occurred for DON in CWAD: a greater proportion of CWAD contained DON (75%) as compared to wheat (48%), oats (57%), and rye (33%). However, mean and median concentrations of DON in CWAD were approximately half of those measured in wheat samples from the 2010 harvest.[14] Mean and median DON concentrations in CWAD were comparable to those observed in oats and rye from the 2010 harvest.

The total concentration of depsipeptides, reported as $\sum$Depsi in Table 3, was generally greater than concentrations of MON and DON. Concentrations of $\sum$Depsi were up to 10 times the concentrations reported for MON or DON, particularly in samples from crop districts in the southwestern area of Saskatchewan (Figure 1). Only the CWAD samples from the 2010 harvest were analyzed for depsipeptides; there

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Table 3. Occurrence and Concentrations (mg/kg) of Mycotoxins in Canadian Durum Samples Harvested in 2010 from Crop Districts in Alberta and Saskatchewan

crop district	*n* samples	MON mean[a]	MON median[a]	MON range	DON mean	DON median	DON range	∑Depsi[b] mean	∑Depsi median	∑Depsi range
					Alberta					
1	3	0.01	0	0.04	0.14	0.09	nd−0.32	0.10	0.17	0.078−0.20
2	3			nd[c]	0.14	0.17	nd−0.25	0.20	0.24	0.066−0.28
3	1	0.11	0.11	0.11			nd	2.4	2.4	2.4
					Saskatchewan					
1A	1			nd			nd	0.035	0.035	0.035
2B	6	0.82	0.70	0.04−2.51	1.3	0.48	0.10−4.7	7.3	7.4	0.74−17.2
3A	2			nd	0.025	0.025	nd−0.05	0.013	0.013	0.011−0.015
3BN	3	0.41	0.49	0.08−0.65	1.1	0.06	nd−3.3	4.1	5.7	0.83−5.77
3BS	2			nd			nd	0.014	0.014	0.012−0.016
4A	1			nd			nd	0.012	0.012	0.012
4B	3	0.30	0.064	nd−0.84	0.017	0	nd−0.05	1.9	0.52	0.17−4.9
5A	3	0.65	0.59	0.51−0.85	0.83	0.78	0.60−1.1	7.7	5.7	3.8−13.4
6A	2	1.4	1.4	0.04−2.75	1.6	1.6	0.37−2.8	11.0	11.0	3.35−18.7
6B	5	0.99	0.67	0.19−2.45	0.34	0.11	0.06−3.3	7.7	6.6	1.82−15.9
7A	16	2.1	1.7	nd−6.36	0.30	0.14	nd−2.7	14.9	13.2	1.23−34.2
7B	2	0.29	0.29	nd−0.58			nd	2.1	2.1	0.015−4.26
8B	1	0.10	0.10	0.10	0.06	0.06	0.06	0.87	0.87	0.87

[a]Results less than the limit of quantitation were set to 0 for the calculation of means and medians. [b]Sum of enniatin A, enniatin A1, enniatin B, enniatin B1, enniatin B2, enniatin B3, and beauvericin concentrations. [c]Not detected above limit of quantitation.

are no data from other cereals to compare to the CWAD results.

The predominant depsipeptide was enniatin B, which averaged 56% of ∑Depsi, followed by enniatin B1 at 35% and enniatin A1 at 7%. These three compounds accounted for approximately 98% of the depsipeptides present in the CWAD samples analyzed. The remaining depsipeptides were still frequently detected, but at lower concentrations than enniatins B, B1, and A1. Enniatin B2 averaged 2% of ∑Depsi, followed by enniatin A at 0.4%, beauvericin at 0.14%, and enniatin B3 at 0.01%.

■ DISCUSSION

Mycotoxins in Durum Wheat. Even though 2010 excessive precipitation resulted in conditions that promoted FHB 2010, concentrations of DON observed in the *Fusarium*-damaged CWAD samples harvested in 2010 were generally similar to those seen in the past in Canadian durum samples, as well as durum grown in the United States, Italy, and Argentina. In a study by Clear et al.[3] DON concentrations in crop district composites prepared in 2000 through 2002 ranged from <0.1 to 2.2 mg/kg, which is similar to the range of DON means observed for the 2010 samples. Mean DON concentrations ranged from 0.4 to 9.9 mg/kg in durum grown in the state of North Dakota.[25] Maximum concentrations of 1.8 mg/kg were reported for samples from Italy in 2008, a year with a particularly warm and rainy spring,[26] and mean concentrations of 0 to 2.6 mg/kg DON were observed in Argentinian durum.[27]

However, the DON concentrations in the 2010 CWAD samples were well below the concentrations reported in Tunisian durum harvested in 2007.[28] Average concentrations of DON in samples from the five major durum-growing regions in Tunisia ranged from 12.8 to 30.5 mg/kg. This year was characterized by lengthy rains during the harvest, which was noted by the authors as a major factor that contributed to the high DON concentrations observed.

There are fewer data in the literature regarding MON in durum. Concentrations of MON observed in CWAD from the 2010 harvest are generally greater than concentrations reported for other years or other small cereals. MON was observed at a lower frequency and at lower concentrations in CWAD crop district composites prepared in 2000 through 2002.[3] Only 47% of samples contained measurable MON, at concentrations from <0.1 to 0.13 mg/kg in the early 2000s. In samples that represented "ordinary Finnish grain", MON was observed at a similar frequency to the current study in small cereals at 74%, but the maximum concentration was only 0.81 mg/kg in wheat.[29] In other samples of European small cereal grains, maximum MON concentrations reported are in the range of 0.224 to 0.95 mg/kg.[30−32] Samples used in the European studies were not biased toward *Fusarium*-damaged samples, and thus the higher MON concentrations observed in the current study are likely due to a focus on studying *Fusarium*-damaged samples.

As with MON, there are fewer data in the literature describing the occurrence of enniatins and beauvericin in small cereal grains. No data on these emerging mycotoxins have been previously published for Canadian grains, but three European studies analyzed depsipeptide compounds in wheat grown in the early 2000s in northern Europe. Mean and median concentrations of the sum of BEAU, ENN A, ENN A1, ENN B, and ENN B1 were 4.15 and 0.215 mg/kg for wheat harvested in 2001 in Finland.[29] Wet conditions were experienced during the 2001 growing season, and the maximum sum of depsipeptide compounds observed was 24.8 mg/kg from a sample obtained in 2001. In Norwegian wheat harvested in 2000 through 2002, the highest concentrations of depsipeptides were about 7 mg/kg in a sample from 2001.[33] As with the Canadian results, ENN B was the major depsipeptide compound, followed by ENN B1. Finally, the sum of BEAU, ENN A, ENN A1, ENN B, and ENN B1 in wheat grown in Lichtenhagen, Germany, in 2004 peaked at 1.22 mg/kg.[31]

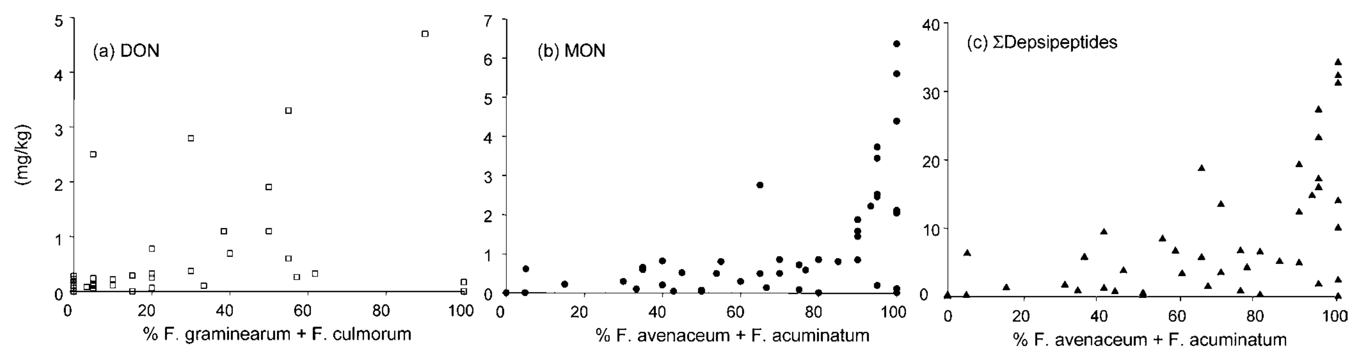

Figure 3. Relationship between mycotoxin concentrations (mg/kg) and presence of *Fusarium* species on Canadian durum harvested in 2010.

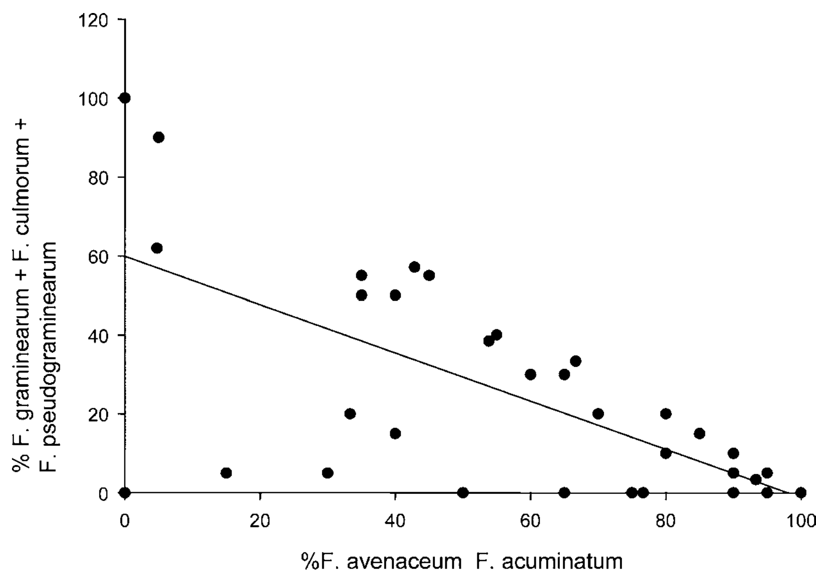

Figure 4. Relationship between the occurrences of *Fusarium* species on single kernels of Canadian durum harvested in central Saskatchewan in 2010.

The maximum concentrations of depsipeptides reported in the European studies were lower than the maximum ∑Depsi measured in the 2010 CWAD, but even though CWAD analysis focused on those with *Fusarium* damage, many CWAD results fell within the range of total depsipeptide concentrations observed in the European studies. Even though ENN B3 was also quantified in this study, it represented only on average 0.01% of the total depsipeptides measured and is thus not expected to invalidate a comparison with concentrations observed in the European studies.

Visible *Fusarium* Damage and Distribution of *Fusarium* Species. Especially in the past few years of the last decade, environmental conditions seem to have favored *Fusarium* infections on durum wheat even in traditionally drier areas such as southern Alberta and central Saskatchewan. Due to prolonged periods of summer rains in 2010, excessive moisture in the fields may have increased the severity of FHB through more frequent "secondary" infections later in the season. As a result, more durum samples from areas affected by FHB were downgraded for *Fusarium* damage to much lower grades (No. 5 CWAD, sample account *Fusarium* damage, commercial salvage) than previously reported. Annual *Fusarium* surveys done on harvest samples between 1995 and 2008 revealed that *F. avenaceum* was the main causal agent of FDK on durum wheat in CDs 2–4, 6, and 7 of Saskatchewan (data not shown). However, over the same period of time in CDs 1 and 2 of Alberta, *F. graminearum* was most often associated with FHB on durum wheat. In 2010, only two durum samples from southern Alberta and six samples from eastern Saskatchewan had the majority of selected FDK caused by *F. graminearum*.

In 17 samples *Septoria nodorum* was associated with FDK. This fungus is commonly known to cause *Septoria* leaf and glume blotch in wheat and other small cereals, but especially in wet years it can also infect heads and cause similar symptoms to *Fusarium*. In more than 70% of the durum samples examined, *F. avenaceum* was identified as the major causal agent of FDK, supporting observations made in previous surveys.[34] Another *Fusarium* species known to produce MON and ENNs, *F. acuminatum*, was found to cause FDK only in one sample at low frequency (5%). The potent DON producer *F. culmorum* was recovered from 10 durum samples; however, species frequency did not exceed 10% in a sample. *F. culmorum* was recovered more often from FDK of samples, which were not co-infected with *F. graminearum*. Interestingly, these samples all came from CDs 6B and 7A in Saskatchewan, where *F. graminearum* was less commonly found than in CDs 2B and 5A of Saskatchewan or in Alberta.

In western Canada, two additional species have periodically been reported to be associated with FHB on barley, oat, and wheat.[35−37] *F. poae* and *F. sporotrichioides* are considered relatively weak pathogens compared to *F. graminearum* or *F. culmorum*, often unable to produce disease symptoms such as FDK on wheat. These observations were confirmed by our

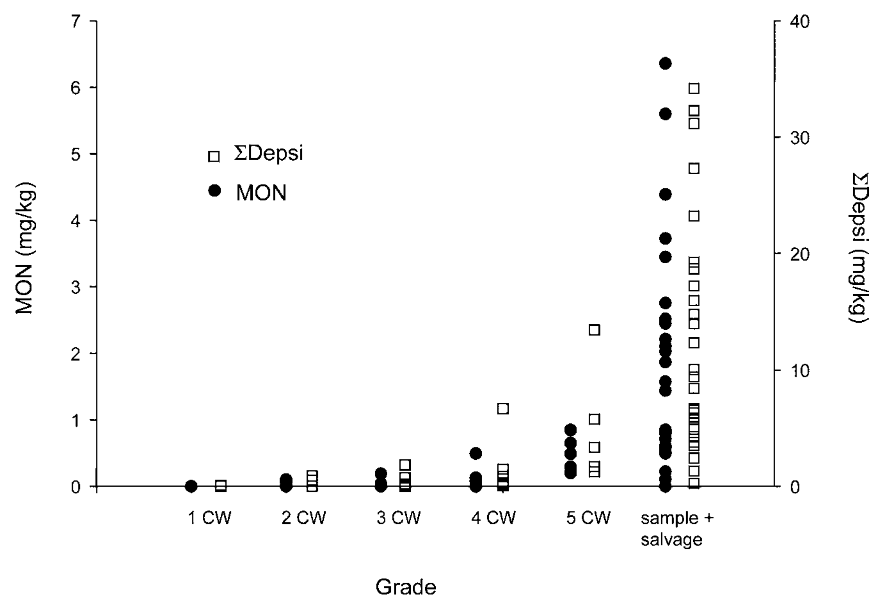

Figure 5. Moniliformin and sum depsipeptide concentrations in Canadian durum samples harvested in 2010 and segregated by grade.

analysis, with only four out of 800 FDK infected with *F. poae* or *F. sporotrichioides*. In cooler climates including Canada, *F. poae* is known to be the most important producer of nivalenol (NIV) in barley, oat, and wheat.[38,39] Yet, no durum samples contained NIV above the limit of quantitation of 0.05 ppm. In culture, *F. sporotrichioides* is known to produce a number of *Fusarium* toxins, e.g., trichothecenes of type A (T-2, HT-2) and beauvericin.[40] In this study, only one out of 53 samples analyzed contained a quantifiable amount of T-2 and/or HT-2 present (0.07 mg/kg HT-2), which is consistent with the results of the fungal analysis.

Relationships between Mycotoxins and Presence of *Fusarium* Species in CWAD. Comparisons of mycotoxin concentration and the occurrence of various *Fusarium* species suggest the existence of an infection threshold above which the emerging mycotoxins are present at higher concentrations. The percentage of CWAD infected with *Fusarium* in FDK is compared to the concentrations of relevant mycotoxins in Figure 3. There is a wide scattering of DON concentrations at lower percentages of infection by the DON-producing *Fusarium* species (Figure 3a). This is a different pattern than what is observed with the emerging mycotoxins (MON and depsipeptides) and *F. avenaceum* and *F. acuminatum* (Figure 3b and c). In this case higher concentrations of MON and ∑Depsi are seen at rates of infection greater than 90%.

One possible explanation for the apparent threshold is competition between the DON-producing species of *F. graminearum* and *F. culmorum* and the producers of the emerging mycotoxins, *F. avenaceum* and *F. acuminatum*. Figure 4 shows an inverse linear relationship between the percentages of kernels infected by these two groups of *Fusarium* species. This is consistent with the general ranking of *F. graminearum* and *F. culmorum* as having high to moderate pathogenicity as compared to the low pathogenicity of *F. avenaceum*.[41] Kang et al.[42] examined the penetration and colonization of *F. avenaceum* in wheat spikelets. Results showed that its behavior resembled that of *F. culmorum* and *F. graminearum*, although mycotoxins produced by *F. avenaceum* differed from those of the latter two species. *In planta*, DON-producing *Fusarium* species appear to have an advantage when transitioning from the biotrophic to the necrotrophic stage. DON and related trichothecenes can be regarded as virulence factors during pathogenesis and play a significant role in the spread of FHB within a spike.[43,44] In contrast, little is known about the role of MON and other mycotoxins produced by *F. avenaceum* during infection and colonization of wheat spikes.[45,46]

Another potential explanation is that MON and ∑Depsi pathways are upregulated when the fungus has exploited most of the nutrients from the endosperm such as proteins and starch. In species of *Fusarium* section *Liseola*, starvation stress can induce or enhance fumonisin production.[47,48] In contrast, production of other mycotoxins, such as T-2 by *F. sporotrichioides*,[49] was reported as positively correlated with sugar concentration as lowering reduced toxin production. Although the biosynthesis of MON has been shown to follow the polyketide pathway,[50] the biosynthetic and regulatory genes involved in the production of MON have not been identified to date.[51] Moreover, the propensity to form MON is known to be a highly variable trait in the phenotype of numerous *Fusarium* species.[52,53] None of the *Fusarium* strains isolated from CWAD, however, grouped with a phylogenetic species of the *F. avenaceum* species complex found to produce >100 mg/kg MON *in vitro* and *in planta*.[14]

Management of Emerging Mycotoxins in CWAD. From a safety perspective, there is a need to manage mycotoxins in grains. The Canadian grain grading system uses the percentage of *Fusarium*-damaged kernels as a grading factor to manage DON in wheat and durum.[17] This grading factor is based on the positive correlation between the amount of FDK and DON concentration in these grains.[54]

Figure 5 demonstrates that use of FDK as a grading factor can also manage MON and depsipeptides in durum. Samples containing the highest concentrations of MON and depsipeptides were graded lower, particularly as 4 CW, 5 CW, sample, or commercial salvage. In addition, mean concentrations of MON and ΣDepsi were significantly lower for 1 CW, 2 CW, and 3 CW as compared to sample and commercial salvage grades (Kruskal−Wallis one-way ANOVA on ranks, $p < 0.001$ for both tests).

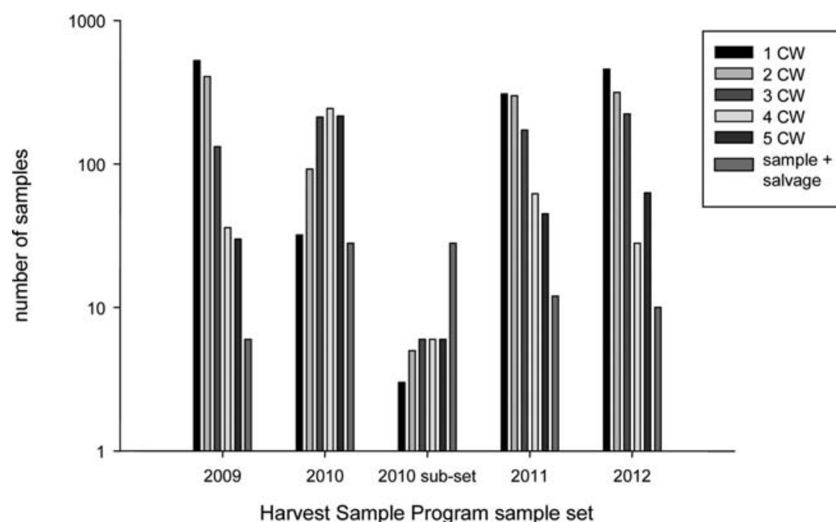

Figure 6. Distribution of Canadian durum sample grades (degraded for various reasons) in 2009−2012 Harvest Sample Program submitted samples and the subset of durum samples selected from 2010 for mycotoxin and fungal analysis.

The emerging mycotoxins of MON and depsipeptides do occur on CWAD. However, when considering whether or not the mycotoxin concentrations observed in the CWAD are abnormal, it must be kept in mind that the occurrence of *Fusarium* mycotoxins is affected by environmental conditions that vary from year to year. Factors such as rainfall and temperature can facilitate infection and mycotoxin production by fusaria between flowering and harvest.[55] Therefore, comparisons of concentrations observed in various studies must take into account the quality of the samples—including the degree of *Fusarium* damage—as well as the conditions of the growing year before drawing broader conclusions regarding the occurrence of *Fusarium* toxins in grain. As well, the limitations of single-year studies should be highlighted because DON,[3,26,27] MON, and depsipeptide[29,33] concentrations in grain grown in the same area over multiple years have been shown to vary.

Data from the CGC Harvest Sample Program demonstrate that 2010 was a particularly poor year for the quality of CWAD (Table 2). Over 80% of samples submitted to the CGC Harvest Sample Program in 2010 were graded as a 3 or lower, whereas in 2009 and 2011 only up to 30% of the samples submitted were assigned to these lower grades (Figure 6). In addition, the distribution of grades in the subset of samples from the 2010 harvest analyzed in this study is different than the total set of samples submitted in 2010, highlighting how *Fusarium*-damaged samples were the focus of this study.

As a result, the results from this study do not represent the whole 2010 CWAD harvest. However, these results do demonstrate that *F. avenaceum*, MON, and depsipeptides can occur on CWAD grown in the western Canadian Prairies and that the current Canadian grain grading system does manage concentrations of these emerging mycotoxins in durum. Additional data from subsequent harvests are needed before assessing implications of the mycotoxin concentrations and *F. avenaceum* occurrence reported in this study.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

This information 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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