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SHORT COMMUNICATION

Prevalence and levels of deoxynivalenol and zearalenone in commercial barley and wheat grain produced in Southern Brazil: an eight-year (2008 to 2015) summary

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Abstract Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, is one of the most important diseases of barley and wheat in Brazil. The disease causes yield losses and contaminates grain with mycotoxins produced by the fungus, mainly deoxynivalenol (DON) and zearalenone (ZEA). The objective of this study was to summarize the results of 16,487 analyses of DON and ZEA in barley and wheat commercial grain produced in Brazil from 2008 to 2015 using liquid chromatography tandem-mass spectrometry. For barley, DON and ZEA were detected in 67% and 41% of the samples, respectively, but 19% and 18% were above the maximum tolerated limits (MTL = 1250 μg/kg for DON and 100 μg/kg for ZEA). For wheat, DON and ZEA were detected in 73 and 38% with 30% and 9% of the samples above the MTL (1250 μg/kg for DON and 200 μg/kg for ZEA). The overall mean concentration of DON was 737 μg/kg in barley and 660 μg/kg in wheat. The mean yearly DON levels varied less in barley (446 μg/kg to 1114 μg/kg) compared to wheat (346 μg/kg to 1274 μg/kg). For the latter, a high peak of DON was found in 2014 when 58% of the samples were above the MTL and the toxin levels averaged 1274 μg/kg across all samples. The mean yearly concentration of ZEA was 138 and 111 μg/kg for barley and wheat, respectively, with the highest prevalence and concentration reported in 2008 and 2009, for both crops. To our knowledge, this is the most comprehensive summary of DON and ZEA contamination in barley and wheat in Brazil for almost a decade of monitoring. Continuous assessment and close inspection of highly contaminated batches are essential to ensure food safety and mitigate the risk that these mycotoxins can cause to human and animal health.

Keywords *Hordeum vulgare* · *Triticum aestivum* · Trichothecene · Mycotoxin · Fusarium head blight

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Introduction

Barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) are the most important commercial winter crops for southern Brazil. Although Brazil is not self-sufficient and produces generally around half of its need, wheat is a key crop to southern Brazilian farmers. Around 95% of wheat, and virtually all barley crops, are grown in the southern, subtropical, regions of the country (Raupp 2013).

Both wheat and barley are susceptible to fungal diseases that are favored by a subtropical web environment, including Fusarium head blight (FHB) caused by members of the *Fusarium graminearum* species complex (FGSC) (Del Ponte et al. 2015). These fungi infect the crop during flowering and grain filling stages and produce mycotoxins that accumulate in the developing kernels. The most common mycotoxins found in Brazilian wheat are the trichothecenes deoxynivalenol (DON) and nivalenol (NIV), and zearalenone (ZEA) (Calori-Domingues et al. 2016; Del Ponte et al. 2012; Duffeck et al. 2017). The presence of these toxins in grain and by-products is mainly influenced by warm and humid spring season that favor FHB epidemics (McMullen et al. 2012). Cropping practices for managing the disease in wheat and

barley include the use less susceptible cultivars, fungicides applied during flowering and harvesting operations (McMullen et al. 2012; Wegulo et al. 2015).

DON is the most common type B trichothecene produced by several *Fusarium* species, but mainly by FGSC members (Aoki et al. 2012). It is also known as vomitoxin due to triggering bouts of vomiting in pigs (Miller 1995). Other symptoms include acute temporary nausea, diarrhea, abdominal pain, headache, dizziness and fever (Sobrova et al. 2010). Zearalenone, a nonsteroidal estrogenic fungal metabolite, is also produced by members of the FGSC and other *Fusarium* species that infect cereals (Kuiper-Goodman et al. 1987). To mitigate the risk of food contamination with dangerous mycotoxins, the Brazilian health surveillance agency (ANVISA) established maximum tolerated limits (MTL) of several mycotoxins, including DON and ZEA, for raw cereals and foods for human consumption starting on 2011. The current MTLs are 1250 μg/kg for DON in barley and wheat grains, 100 μg/kg of ZEA in barley grains and 200 μg/kg in wheat grains (ANVISA 2011, 2017). The Ministry of Agriculture, Livestock and Supply (MAPA) established (in 1988) a limit of 50 μg/kg for aflatoxins in raw barley/wheat or feed intended for animal consumption, but no specific legislation has been set for DON and ZEA at this far (MAPA 1988).

Previous surveys conducted during the last 5 years in Brazil have shown the presence of DON, NIV and ZEA in both barley and wheat samples (Del Ponte et al. 2012; Santos et al. 2013; Tibola et al. 2013; Boeira et al. 2015; Piacentini et al. 2015; Silva et al. 2015; Tralamazza et al. 2016; Duffeck et al. 2017). In general, these studies have shown that the contamination levels are usually higher for DON, followed by NIV and ZEA. Moreover, considerable variation is found across years with some years showing a significant proportion of the samples show contamination above the MTL. The different samples (origin, cultivar, etc.) and methods used by the laboratories are all sources of variation when analysis results from a same year are compared. In this study, we summarized eight years of results of over fifteen thousand chromatographic analyses of DON and ZEA in commercial Brazilian barley and wheat grain samples, which were conducted in our laboratory using ISO/IEC 17.025 accredited methodology.

A total of 16,487 analyses of DON and ZEA mycotoxins in wheat and barley samples received from 2008 to 2015 were performed. There were 6,280 and 2,714 results of DON analyses in barley and wheat samples, respectively, and 6,201 and 1,292 results of ZEA analysis in barley and wheat, respectively. All samples were originated from fields grown in the southern region of Brazil (Rio Grande do Sul, Santa Catarina and Paraná states). Information on the origin (state and municipality), cultivar and management practices were not provided but were assumed to represent fields in the southern region of the country. Samples were received in the laboratory each month during the year. For this study, each sample was assigned to a harvest year based on the following criteria: samples received from September/October of one year to August/September of the following year, were assigned to the first year. For example, a sample received in January 2014 was assigned to the 2013 harvest year.

At the laboratory, the samples were identified and ground in ultra-centrifugal mill model ZM200 (Retsch) with a 1 mm sieve, weighed into 50 mL Falcon type tube, and 24 mL of solution was added for mycotoxin extraction. For ZEA analysis, zearalanol was used as internal standard (Sigma-Aldrich). After agitation in a vortex mixer for 20 min at 2500 rpm, the content of the tube was filtered through filter paper and appropriately diluted in vial type flask. The DON and ZEA determination was carried out by a HPLC system consisting of an Agilent 1100 series (Agilent) equipped with an 1100 series degasser, binary pump and column oven (thermostated at 40 °C). Chromatographic separation was performed on a 150 mm 4.6 mm 5 um SB C-18 column (Agilent). A binary gradient at a flow rate of 0.8 mL/min was performed with solvent A (water) and solvent B (acetonitrile) both modified with 0.5% (*v*/v) amonium acetate (Sulyok et al. 2006).

DON and ZEA were identified using a triple quadrupole QtraP 4000 system (Applied Biosystems), equipped with an ion electrospray ionization (ESI) source in the negative ionization mode. The mass spectrometer was operated in MRM (multiple reaction monitoring) mode and the optimized MS/MS conditions respectively for DON and ZEA detection and quantification were as follows in Table 1.

The analytical method was developed in-house and validated in accordance with the recommendations of EC 2002/657. The accuracy of the technique used to measure DON and ZEA was evaluated using recovery experiments (purified analytes in blank wheat) and a certified reference material (CRM). The precision of the method was evaluated using the percent coefficient of variation (CV%). The inter-day precision results for DON and ZEA were 10.3 and 9.3 respectively. Results of CRM sample were reported as *z*-score. The CRM 22127 was tested and *z*-scores for DON (1.3) and ZEA (1.4) were within the acceptable range − 2 and +2. The established quantification limits for DON and ZEA analysis were 200 μg/kg and 20 μg/kg, respectively. The analytical method resulted in 80% and 85% recovery coefficients for DON and ZEA, respectively.

To analyze the data, we firstly summarized (frequency and means) for the month of year. Then, the 12 monthly values per year were summarized as mean prevalence (% of positive samples), mean concentrations (μg/kg) in all samples, mean concentration (μg/kg) in the positive samples, and prevalence (%) of samples above the MTL for each mycotoxin-crop combination each year (ANVISA 2011, 2017). Data were transformed to log base 10 prior to applying a bonferroni test at 5% of significance to compare years within each crop and

Table 1 Retention time and compound dependents parameters of mass spectrometer used to identified deoxynivalenol (DON) and zearalenone (ZEA) in commercial barley and wheat grain in southern Brazil from 2008 to 2015

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	DP[a]	CE[b]	CXP[c]
Deoxynivalenol	3.5	295	265	- 65	57	- 5
			138	- 65	43	- 9
Zearalenone	7.1	317	175	- 80	51	- 13
			131	- 80	41	- 7

[a] *DP* Declustering potential

[b] *CE* Collision energy

[c] *CXP* Collision exit potential

mycotoxin. The statistical analyses were performed in Statgraphics Centurion XV (Statgraphics Centurion 15.2.11, Manugistics Inc.).

The mean prevalence of DON in barley samples averaged 67% across all years (53 to 75% across years) (Fig. 1a, Table 2). The mean DON concentration in barley was 737 μg/kg (446 to 1114 μg/kg) (Fig. 1b). For the DON-positive barley samples, the mean concentration was 1101 μg/kg (706 to 1501 μg/kg) (Table 2). Of these samples, 8.1 to 37.3% were above MTL of 1250 μg/kg across year. The maximum DON level found in barley was 38,400 μg/kg (data not shown). The prevalence of DON in wheat averaged 73% and varied significantly across years (58 and 86%) (Fig. 1a, Table 2). The mean DON level in all samples was 660 μg/kg (346 to 1274 μg/kg) (Fig. 1b, Table 2). For the DON-positive wheat samples, the mean concentration averaged 855 μg/kg and ranged from 510 to 1524 μg/kg. The percentage of samples above the limit ranged from 3.6 to 58.2% (Table 2). One sample showed a peak of 12,950 μg/kg of DON (Data not shown).

The prevalence of ZEA in barley ranged from 19 to 84% ($P \leq 0.01$) and averaged 41% (Fig. 2a, Table 3). The overall mean of ZEA was 138 μg/kg (14 to 651 μg/kg) (Fig. 2b, Table 3). For the ZEA-positive samples, the mean concentration averaged 213 μg/kg (55 to 708 μg/kg). Of these samples, 3.3 to 62.5% were above the MTL (100 μg/kg) (Table 3). Maximum ZEA in barley was 19,800 μg/kg (data not shown). The prevalence of ZEA in wheat averaged 38% (8 and 89%) (Fig. 2a, Table 3). In all wheat samples, ZEA averaged 111 μg/kg (5 to 485 μg/kg) (Fig. 2b, Table 3). In the ZEA-positive samples, the mean was 180 μg/kg (58 to 503 μg/kg) and up to 46.2% of samples was above the MTL of 200 μg/kg (Table 3). Maximum ZEA in wheat was 16,100 μg/kg (Data not shown).

Variation in the mycotoxin levels in grain samples from different regions is explained mainly by spatial and temporal variability of weather conditions that affect inoculum build up and FHB development (Del Ponte et al. 2009; McMullen et al. 2012). In other regions of the world, such as North America and Europe, maize residues on the soil surface seem to increase the risk of FHB epidemics (Dill-Macky and Jones 2000; Landschoot et al. 2013). However, in Brazil, it has been suggested that seasonal weather is the main driver of FHB epidemics given the availability of inoculum in a predominantly no-till system where local residues seems to be of little importance to FHB risk (Spolti et al. 2015; Del Ponte et al. 2015). The use of fungicides, especially those of the triazole group, can help to combat the disease but the efficacy levels are relatively low compared to the control of foliar diseases, especially to reduce DON mycotoxin (Paul et al. 2008). Hence, our data

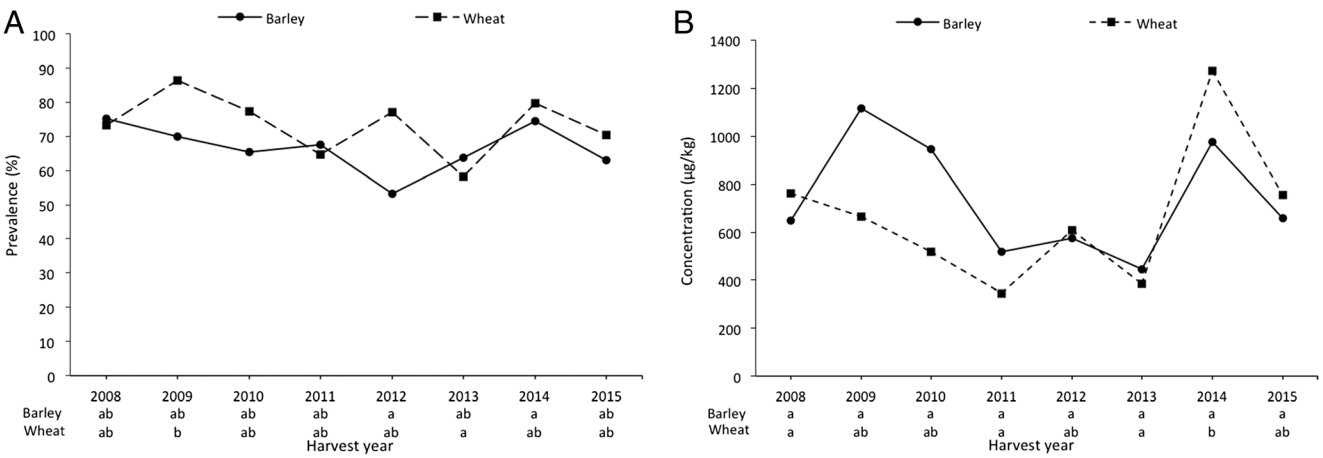

Fig. 1 Mean prevalence (**a**) and concentration (**b**) of deoxynivalenol in barley and wheat grain samples from southern Brazil during the 2008–2015 period. The same letters below the harvest year do not differ by Bonferroni test ($P \leq 0.05$)

Table 2 Summary statistics for deoxynivalenol (DON) in commercial barley and wheat grain in southern Brazil from 2008 to 2015

Crop	Year[1]	N[2]	Mean[3] (μg/kg)	Mean P[4] (μg/kg)	Prevalence[5] (%)	Above MTL[6] (%)
Barley	2008	742	648	1146ab[7]	75ab	13.7
	2009	844	1114	1501ab	70ab	37.3
	2010	972	945	1440b	65ab	29.1
	2011	1201	518	720a	67ab	11.8
	2012	916	573	973ab	53ab	16.4
	2013	755	446	706a	64ab	8.1
	2014	439	977	1303ab	74b	18.0
	2015	411	658	990ab	63ab	21.9
	Total	6280	737	1101	67	19.5
	P-value[8]		0.01	0.03	0.08	
Wheat	2008	176	761a	954ab	73ab	30.7
	2009	292	664ab	730ab	86b	16.4
	2010	105	517ab	664ab	77ab	10.5
	2011	225	346a	510a	65ab	3.6
	2012	469	608ab	779abc	77ab	12.4
	2013	228	385a	664ab	58a	7.9
	2014	947	1274b	1524c	80ab	58.2
	2015	272	756ab	1084bc	70ab	26.1
	Total	2714	660	855	73	30.2
	P-value		≤0.01	≤0.01	0.02	

[1] Year: Barley harvest year includes the months of September and October of the following year. Wheat harvest year covers the months from October to September of the following year

[2] N: Number of samples analyzed

[3] Mean: Mean concentration of the samples

[4] Mean P: Mean concentration of positive samples

[5] Mean prevalence: Mean prevalence

[6] Above MTL: Percentage of samples with results above the maximum tolerable limits in effect established by ANVISA of 1250 μg/kg for deoxynivalenol in wheat and barley (ANVISA 2011, 2017)

[7] Means followed by different letters in columns differ by Bonferroni test ($P \leq 0.05$)

[8] P–value: probability of the statistical model

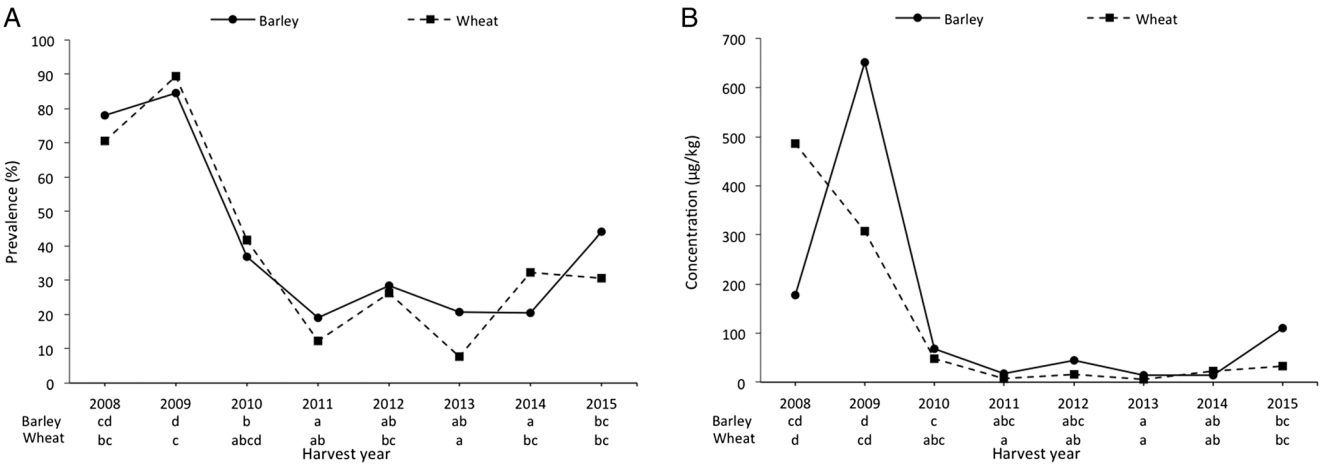

Fig. 2 Mean prevalence (**a**) and concentration (**b**) of zearalenone in barley and wheat grain samples from southern Brazil during the 2008–2015 period. The same letters in the lines below the harvest year do not differ by Bonferroni test ($P \leq 0.05$)

Table 3 Summary statistics for zearalenone (ZEA) in commercial barley and wheat grain in southern Brazil from 2008 to 2015

Crop	Year[1]	N[2]	Mean[3] (μg/kg)	Mean P[4] (μg/kg)	Prevalence[5] (%)	Above MTL[6] (%)
Barley	2008	695	178 cd[7]	223cd	78cd	35.8
	2009	837	651d	708d	84d	62.5
	2010	882	069c	179c	37b	9.2
	2011	1199	18abc	103abc	19a	3.3
	2012	915	44abc	157bc	28ab	8.9
	2013	895	014a	55a	21ab	3.4
	2014	413	014ab	73ab	21a	4.8
	2015	365	110bc	207bc	44bc	27.4
	Total	6201	138	213	41	18.1
	P-value[8]		≤0.01	≤0.01	≤0.01	
Wheat	2008	88	485d	503b	71bc	38.6
	2009	158	308cd	369ab	89c	46.2
	2010	36	48abc	107ab	42abcd	8.3
	2011	182	7a	60a	12ab	0.5
	2012	318	16ab	58a	26bc	0.6
	2013	177	5a	72a	8a	0.0
	2014	204	23ab	65a	32bc	0.5
	2015	129	33bc	109a	31bc	4.7
	Total	1292	111	180	38	9.3
	P-value		≤0.01	≤0.01	≤0.01	

[1] Year: Barley harvest includes the months of September and October of the following year. Wheat harvest covers the months from October to September of the following year

[2] N: Number of samples analyzed

[3] Mean: Mean concentration of the samples

[4] Mean P: Mean concentration of positive samples

[5] Mean prevalence: Mean prevalence

[6] Above MTL: Percentage of samples with results above the maximum tolerable limits in effect established by ANVISA of 100 and 200 μg/kg for zearalenone for barley and wheat, respectively (ANVISA 2011, 2017)

[7] Means followed by different letters in columns differ by Bonferroni test ($P \leq 0.05$)

[8] P–value: probability of the statistical model

corroborates previous studies, which showed that, in general, the weather in southern Brazil is favorable for FHB development almost every year (Del Ponte et al. 2009), thus explaining the relatively high prevalence of these mycotoxins in some years within a decade of monitoring. Knowledge of the occurrence of DON and ZEA over several years and regions are critical for risk assessment studies. In general our results corroborates previous findings in the region for these same mycotoxins in these crops, but there are differences likely due to different samples, methodology. For example, Boeira and collaborators (Boeira et al. 2015) analyzed 673 barley samples and reported 54% of those above MTL (2000 μg/kg). In our study, only 10.7% of the samples were above 2000 μg/kg during the same period. Recently, Piacentini et al. (2015) evaluated 50 barley samples harvested in 2013 and detected DON in 18% of the samples, which averaged 3400 μg/kg (200 to 15,100 μg/kg). In here, 64% of the samples from the same period were found contaminated with DON, but at much lower concentration on average (446 μg/kg). Although 73% of all wheat samples were contaminated with DON (averaging 660 μg/kg), 30.2% were above MTL, which are in general agreement with previous studies (Del Ponte et al. 2012; Santos et al. 2013; Duffeck et al. 2017). The prevalence levels varied across years, with the highest prevalence (>58%) and mean concentration occurring in 2014, which was likely due to excessive rains before harvesting which led to low yields and quality of wheat in that season (Conab 2015).

We report for the first time the most comprehensive data for ZEA contamination in barley in Brazil, which averaged 41% prevalence (138 μg/kg on average). The peak of ZEA contamination occurred in 2008 and 2009 for both wheat and barley, which are in general agreement with previous studies. For example, Tibola et al. (2013) detected ZEA in 31% (317 μg/kg on average) of 396 wheat samples produced from 2009 to 2012 in southern Brazil and also showed a peak of ZEA prevalence in 2009 (67%) and mean concentrations of 20 to

2960 μg/kg. Calori-Domingues et al. (2016) quantified ZEA in wheat and found a higher prevalence in the 2009 samples (85%) than in the 2010 samples (27%). It will be important to further investigate on factors that may have favored ZEA production in the 2008 and 2009 in comparison to the following years when the frequency of samples above the MTL remained below 10% and at concentration levels in agreement with previous reports of ZEA in wheat (Tibola et al. 2015; Tibola et al. 2016; Duffeck et al. 2017). The presence of both DON and ZEA in cereals is expected since the main pathogen in Brazil, *F. graminearum*, produces both toxins (Geraldo et al. 2006), but specific requirements for the strains to produce these two toxins are not entirely know. In conclusion, DON and ZEA are common contaminants of barley and wheat grain produced in southern Brazil at concentration levels of concern (above MTL) in up to two-thirds of the samples. The use of contaminated barley and wheat and their by-products in animal feed may have a negative effect on health and productivity. To ensure the availability of safe food and feed, it is essential to continuously monitor these mycotoxins in order to provide information for decision-making regarding segregation or disposal of highly contaminated batches. It is also essential that control methods are available in order to reduce the risk of contamination, therefore minimizing the risks to human and animal health.

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