Occurrence of Trichothecene Mycotoxins in Cereals Harvested in the Czech Republic

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Abstract

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A large-scale survey of the natural occurrence of trichothecene mycotoxins in major cereals harvested in the Czech Republic was conducted during the years 1999–2001. In total, 198 cereal samples representing various wheat, barley, and rye cultivars were examined for deoxynivalenol (DON) using gas chromatography with electron capture detector (GC-ECD). Four years later, in 2005, the list of target analytes was fairly extended, 65 wheat and barley samples were analysed for seven trichothecene mycotoxins – deoxynivalenol (DON), nivalenol (NIV), fusarenon-X (Fus-X), 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol (ADONs), HT-2 toxin (HT-2). and T-2 toxin (T-2) by high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Trichothecenes represented mainly by DON were detected in almost all grain samples, its mean levels were the highest in the year 1999 which was characterised by very humid conditions during the growing season. The maximum concentration set in Commission Regulation EC (No. 856/2005) for DON (1250 μg/kg) in unprocessed cereals was exceeded only in two of all the samples analysed.

Keywords: trichothecene mycotoxins; deoxynivalenol; wheat; barley; rye; monitoring

The occurrence of mycotoxins in various crops is a food safety issue of a great concern worldwide. Trichothecenes, that are frequently found in cereals, are secondary metabolites produced mainly by *Fusarium* ear blight pathogens, which are common in the temperate climatic zone of Europe, America, and Asia (Creppy *et al*. 2002). The incidence of mycotoxins can vary from year to year depend-

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ing on many factors such as weather conditions and/or agricultural practices (Obst *et al.*, 2000). These toxins can never be completely removed from raw materials, therefore annual control of trichothecene contamination is necessary.

Depending on the dietary dose, trichothecene mycotoxins may cause both acute and chronic toxic syndromes in exposed humans and/or livestock. Toxic effects largely differ within the groups of organisms (Hussein & Brasel 2001). In experimental animals, type A compounds such as HT-2 toxin (HT-2) and T-2 toxin (T-2) were shown significantly more toxic as compared to type B trichothecenes, e.g. deoxynivalenol (DON), nivalenol (NIV), fusarenon-X (Fus-X), 15-acetyldeoxynivalenol (15ADON), 3-acetyldeoxynivalenol (3ADON) (Weidenbörner 2001). On the other hand, considering typical contamination patterns, the highest health risk due to the dietary intake of trichothecenes is associated probably with DON since this trichothecene is typically most abundant among other *Fusarium* secondary metabolites representing this group. Currently, DON is considered as contamination marker and is the only one representative of this groups for which regulation has been set in the European Union.

DON maximum level set by EC's Scientific Committee on Food on the basis of tolerable daily intakes is 1250 µg/kg for unprocessed cereals other than durum wheat, 1750 µg/kg for unprocessed durum wheat, oats, maize, and 750 µg/kg for cereal flours. It should be noted that the information on the presence of HT-2 and T-2 toxins in cereals is not sufficient enough for a comprehensive risk assessment, and therefore regulations for these toxins have not yet been established, nevertheless, they are in the preparation. Regarding NIV, 15ADON, and 3ADON, the human exposure to these trichothecenes is estimated to be relatively low and due to the co-occurrence with typically fairly more abundant DON, establishing maximum levels for these toxins is currently not considered (EC No. 856/2005).

The occurrence of *Fusarium* mycotoxins, mainly DON, in cereal grains as well as in various cereal-based foodstuffs has been reported in several studies (Tahala *et al.* 1990; Placenta *et al.* 1999). The recent report (Schothorst & Egmond 2004) provides the overview of *Fusarium* toxin levels in crops harvested in 12 European countries (Austria, Belgium, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Portugal, Sweden, the United Kingdom). DON, NIV, T-2, and HT-25 were found in 7, 16, 20, and 14% of cereal grains examined, respectively. Similarly, the survey presented by Joint FAO/WHO Expert Committee on Food Additives (JECFA 2001) showed that DON was the most abundant trichothecene in cereals, regardless of the country they were harvested in. In southwest Germany, the presence of DON, NIV, 3-ADON, HT-2, and T-2 in wheat flour was detected in 98, 12, 2, 3, and 7% samples, respectively. DON content was in the range from 15 to 965 µg/kg in white flour and from 15 to 1379 µg/kg in wholegrain flour (Schollenberger *et al.* 2002). These results are in good agreement with the earlier German study (Müller *et al.* 1997, 2001), in which the number of DON positive cereals was in the range from 83% to 96%. An extensive survey focused on trichothecene mycotoxins in cereal grains was recently conducted in Croatia (Sokolovic & Šimpraga 2005). In total, 465 samples were analysed for DON, T-2, and diacetoxyscirpenol (DAS), the target trichothecenes were detected in 41, 17, and 27% samples, respectively. Also the results obtained in other parts of the world are quite similar. For instance, the occurrence of DON in barley harvested in Uruguay was found in 30, 67, 26, 27, 90, 100, and 100% samples in particular crop years within the monitoring period of 1996–2002 (Dinorah *et al.* 2007).

For official food control, both gas chromatography (GC) and high performance liquid chromatography (HPLC) are commonly used. Various chemical derivatisations are carried out prior to gas chromatographic analysis to improve the analytes volatility and enable their detection by either electron-capture (ECD) or mass spectrometric detector (MS) (Tanaka *et al.* 2000; Mateo *et al.* 2001). Regarding *Fusarium* toxins control, enzyme-linked immunosorbant analysis (ELISA) and/or flow through immunoassays are widely employed for the screening purpose (Koch 2004; Schneider *et al.* 2002). In the recent decade, HPLC coupled with tandem mass spectrometry (MS/MS) has become a preferred method of choice in most laboratories focused on multi-mycotoxin analysis. Soft ionisation techniques represented by atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) are typically employed for this purpose (Rodrigues-Fo *et al.* 2002; Berthiller *et al.* 2004; Sulyok *et al.* 2006). Simplified sample preparation strategy

Table 1. Overview of cereal samples collected for analysis in the growing seasons

Harvest season	Cereal type	Total number of samples	Cultivar
1999	wheat	49	Alka, Astela, Boka, Brea, Bruta, Contra, Ebi, Elpa, Hana, Ilona, Mona, Munk, Nela, Samanta, Šárka, Saxana, Sida, Siria
2000	wheat	47	Alana, Alka, Apache, Athlet, Brea, Bruta, Contra, Ebi, Estica, Hana, Leguan, Mona, Nela, Niagara, Rialto, Samara, Samanta, Sulamit, Šárka, Saskia, Vlasta
2001	wheat	55	Alana, Alka, Brea, Bruta, Ebi, Hana, Nela, Niagara, Samanta, Šárka, Saskia, Saxana, Sepstra, Sulamit
	barley	32	Akcent, Amulet, Kompakt, Krona, Olbram, Tolar, Nordus
	rye	15	Apart, Locarno, Selgo
2005	wheat	41	Aranka, Axis, Banquet, Batis, Ebi, Ludwig, Nela, Samanta, Sandra, Saskia, Sulamit
	barley	24	Amulet, Bojos, Jersey, Malz, Prestige, Sebastian, Tolar, KM 1057, KM 1910, KM 2084, Merlin

together with low detection limits achievable by LC-MSMS technique are the main advantages of this approach.

The objective of the present study was to monitor the occurrence of the major regulated *Fusarium* mycotoxin – deoxynivalenol – in various cereals harvested in the Czech Republic. Following the three year monitoring survey (1999–2001), a pilot study aimed at a more detailed examination of *Fusarium* mycotoxins in wheats and barleys was conducted in 2005.

MATERIAL AND METHODS

Standards and chemicals

Mycotoxin standards – DON, NIV, Fus-X, 15ADON, 3ADON, HT-2, and T-2 – were purchased from Sigma-Aldrich (Germany) and Biopure (Austria). Certified reference materials (CRM), DON in wheat flour (< 0.05 mg/kg, BCR 396, Belgium) and DON in naturally contaminated wheat (0.7 ± 0.1 mg/kg, R-Biopharm, Rhone, UK) were used for the quality assurance in the mycotoxin analysis. Both analytical methods described below were accredited (ISO 17025) and as a part of external quality control, the trueness of the generated data was demonstrated through participation in Food Analysis Performance Assessment Scheme (FAPAS) organised by Central Science Laboratory (York, UK).

Samples

In total, 263 grain samples coming from various wheat, barley, and rye cultivars during four harvest years (1000 g of each) were collected in the main agricultural areas of the Czech Republic by the Crop Research Institute (Prague-Ruzyně), the Agricultural Research Institute, Ltd. (Kroměříž), the Research Institute for Fodder Crops, Ltd. (Troubsko), and Mendel University of Agriculture and Forestry (Brno). The samples overview is given in Table 1. Representative sub-samples (100 g of ground grains) were supplied for the determination of the selected *Fusarium* toxins to the Institute of Chemical Technology in Prague, where they were stored at –18°C prior to analyses.

Monthly data on the mean precipitations and air temperatures supplied by the Czech Hydrometeorological Institute are summarised to enable the comparison of particular years (Table 2).

Determination of DON

All the grain samples collected during the period of 1999–2001 were analysed for DON levels using gas chromatography coupled with electron

Table 2. Climatic conditions during growing season (mean values from the Czech Republic)

Month	Mean air temperature (°C)/total precipitation (mm) in particular year			
	1999	2000	2001	2005
May	13.6/50	14.9/61	14.4/61	13.2/75
June	15.4/98	17.4/52	12.7/79	16.2/60
July	18.8/80	15.6/123	18.0/119	18.1/130
August	17.0/46	18.6/48	18.3/91	16.0/99

capture detector (GC/ECD) for quantification of the target analyte (Radová *et al*. 1998). Briefly, the homogenised ground cereal sample (10 g) was extracted with 100 ml acetonitrile:water mixture (84:16, v/v) by shaking it for one hour, MycoSep TM 225 SPE cartridge was used for the purification of the crude extract, trifluoroacetanhydride (TFAA) was used for derivatisation of DON prior to the determination. The limit of detection (LOD) was 5 µg/kg, the limit of quantification (LOQ) was 15 µg/kg, and the average recovery was 86.3% (n = 5, DON level in spiked samples was 500 µg/kg).

Determination of trichothecene A and B type mycotoxin set

A new method employing high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used in the year 2005 for the determination of seven trichothecene mycotoxins: DON, NIV, Fus-X, ADONs, HT-2, and T-2. The instrument used for the measurements consisted of HP1100 Binary Series LC system (Agilent Technologies, USA) and mass spectrometer with ion trap mass analyser (Finnigan LCQ Deca, USA).

Extraction and clean-up procedure. The homogenised ground sample (12.5 g) was extracted with 50 ml acetonitrile:water mixture (84:16, v/v) for one hour using an automatic shaker (IKA Laboratortechnik, Germany). The crude extract was filtered (Filtrak No. 390, VEB Freiberger, Germany). 8 ml aliquots were transferred into sample tubes and to each 80 µl of acetic acid (99.99%, Sigma-Aldrich, Germany) were then added. Purification was achieved by solid phase extraction (SPE) employing the MycoSepTM#226 AflaZon+ cartridges (Romer, Austria). 4 ml of the purified extract was evaporated to dryness, dissolved in 1 ml of water:methanol mixture (80:20, v/v) and pressed through 0.2 µm microfilter (Alltech, USA) prior to further analysis.

Chromatographic conditions. Chromatographic separation of the sample components was carried out on a reverse phase column with polar endcapping (Synergi Hydro RP, 150 mm × 3 mm × 4 µm, Phenomenex, USA) at 40°C in a gradient of mobile phase A (10mM amonium acetate in distilled water) and mobile phase B (methanol), (Table 3). The flow rate was set to 0.5 ml/min and the injection volume was 20 µl.

The identification and quantification was performed using a tandem mass spectrometry with the following MS/MS parameters (ion source type – APCI in both negative and positive ion modes, capillary temperature – 150°C, vaporiser temperature – 450°C, nitrogen sheath gas flow – 1.2 l/min, nitrogen auxiliary gas flow – 3 l/min, source voltage – 6 kV, collision gas – helium, scan type – selected reaction monitoring). Ionisation modes and monitored ions (m/z) for particular analytes are shown in Table 4.

Table 3. LC gradient program

Time (min)	10mM ammonium acetate: methanol (%)
0	80:20
0–8	linear change from 80:20 to 30:70
8–15	30:70
15.0–15.1	linear change from 30:70 to 80:20
15–22	80:20

Performance characteristics. The limits of detection (LOD) and quantification (LOQ), recoveries and repeatabilities (RSD), that were obtained within the validation process, are reported in Table 5. Calibration curves for all analytes were linear within the working range from 5 µg/kg to 10 000 µg/kg. Squared correlation coefficients (R^2) were in the range of 0.9991–0.9999 for 11 point calibration curves.

RESULTS AND DISCUSSION

Various factors, including the resistance of cereal cultivars against *Fusarium* head blight infection, play a role in the contamination of harvested grains by trichothecenes. A four-year monitoring study, aimed at mapping the occurrence of *Fusarium* toxins in various cereals, was initiated in the Czech Republic in 1999. The follow-up study was then conducted in 2005 when a wide range of

Table 4. Ion mode and monitored ions (m/z)

Analytes	Ion mode	Parent ion > daughter ion (confirmation daughter ion)
NIV	–	371 > 311 (281)
DON	–	355 > 295 (265)
Fus-X	–	413 > 353 (263)
ADONs	–	397 > 337 (307)
HT-2	+	442 > 425 (263)
T-2	+	317 > 273 (299)

Table 5. Summary of validation data of LC-MS/MS method

	Sample type	DON	NIV	Fus-X	ADONs	HT-2	T-2
LOD (µg/kg)	wheat, barley	0.5	1	1	1	1	0.5
LOQ (µg/kg)	wheat, barley	5	10	10	10	10	5
Recovery (%)*	wheat	70.6	53.3	83.1	82.8	95.4	83.5
	barley	71.1	42.8	80.8	84.0	85.9	91.2
Repeatibility (%)*	wheat	3.6	3.5	1.4	1.5	3.9	3.9
	barley	3.1	9.7	7.1	6.5	6.0	4.0

*analytes levels in spiked samples were 500 µg/kg

Table 6. Natural occurrence of DON* in samples harvested in the Czech Republic within the current study

Sample type	Harvest season	Number of positive/ total samples	Mean (µg/kg)	Median (µg/kg)	Concentration range (µg/kg)
Wheat	1999	48/48	252.3	79.8	17.0–2265.2
	2000	43/47	74.1	48.0	22.8–804.9
	2001	44/55	77.4	32.6	18.6–721.9
	2005	40/41	99.3	32.4	6.8–702.0
Barley	2001	32/32	156.5	27.8	19.6–2021.8
	2005	24/24	37.3	27.3	8.4–170.2
Rye	2001	15/15	60.4	40.3	22.6–190.5

*DON levels were corrected on its recovery

wheat, barley, and rye cultivars grown under well characterised conditions were examined only for DON (marker of trichothecenes) using GC/ECD method in the first pilot survey. In the later study, LC-MS/MS procedure was employed allowing to determine also low levels of other trichothecenes including T-2 and HT-2 toxins considered for future regulation by EFSA (EC No. 856/2005).

The overview of DON content found in cereal samples is shown in Table 6. In total, 246 out of 261 analysed samples were DON positive with percentage incidences 100, 92, 89, and 98% in the particular years of 1999, 2000, 2001, and 2005, respectively. The proportions of DON in individual concentration ranges in all samples analysed are illustrated in Figure 1. Considering the Commis-

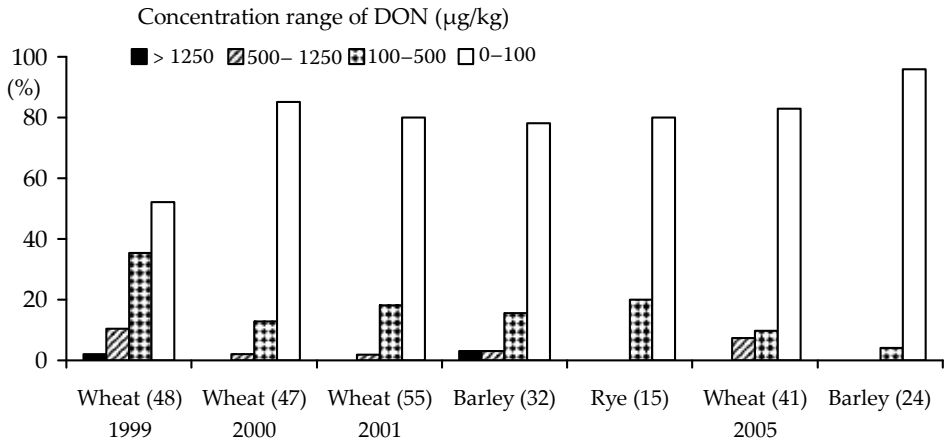

Figure 1. Percentage abundance of DON in cereal samples collected in the Czech Republic within the current study (the number of samples analysed in particular year is in parenthesis)

sion Regulation (EC No. 856/2005), the maximum level for DON (1250 µg/kg) was exceeded only in two samples (wheat cultivar Bruta harvested in 1999 in the locality Vyškov, and barley cultivar Amulet from the locality Svitavy harvested in 2001). From all the years monitored, the most severe contamination by DON, a major *Fusarium* toxin, was found in wheats from the harvest season of 1999. Favourable conditions – high precipitations and relatively high temperatures during the period when the wheat is most susceptible to infection (i.e. from flowering to the soft dough stage of kernel development) (Edwards 2004) were obviously factors responsible for the high mycotoxin levels.

It should be noted that, besides weather conditions, pathogen virulence, and agricultural practices such as chemical treatment, crop rotation, soil cultivation, fertiliser, the development of *Fusarium* head blight also depends on the resistance of the particular cultivar. Unfortunately, their spectrum within the monitored experimental harvest seasons rather varied from year to year, depending on farmers' decisions and market needs and, therefore, longer term assessment of the cultivar resistance involved in our study was practically impossible; moreover, the cultivation conditions have to be specified which was not the case with data monitoring. DON levels in wheat cultivars examined for more than 2 years are shown in Figure 2. Considering the aggregated data (mean values of DON levels over monitoring years), wheat cultivars Alka and Ebi might be classified as susceptible to *Fusarium* infection. However, these results do not fully comply with the data reported in another Czech study concerned with the accumulation of DON in various artificially *F. culmorum* infected wheat cultivars (Šíp *et al.* 2007). For instance, cultivar Ebi was (under experimental conditions) identified by the authors as medium resistant/medium susceptible to *Fusarium* head blight. To draw general conclusions on the cultivar resistance is obviously a complicated task since many factors influence DON levels in food crops. This should be borne in mind when considering the reporting value of monitoring data shown in Figures 3–6 (DON contamination levels in 18, 21, and 14 various wheat cultivars collected in years 1999, 2000, and 2001, respectively).

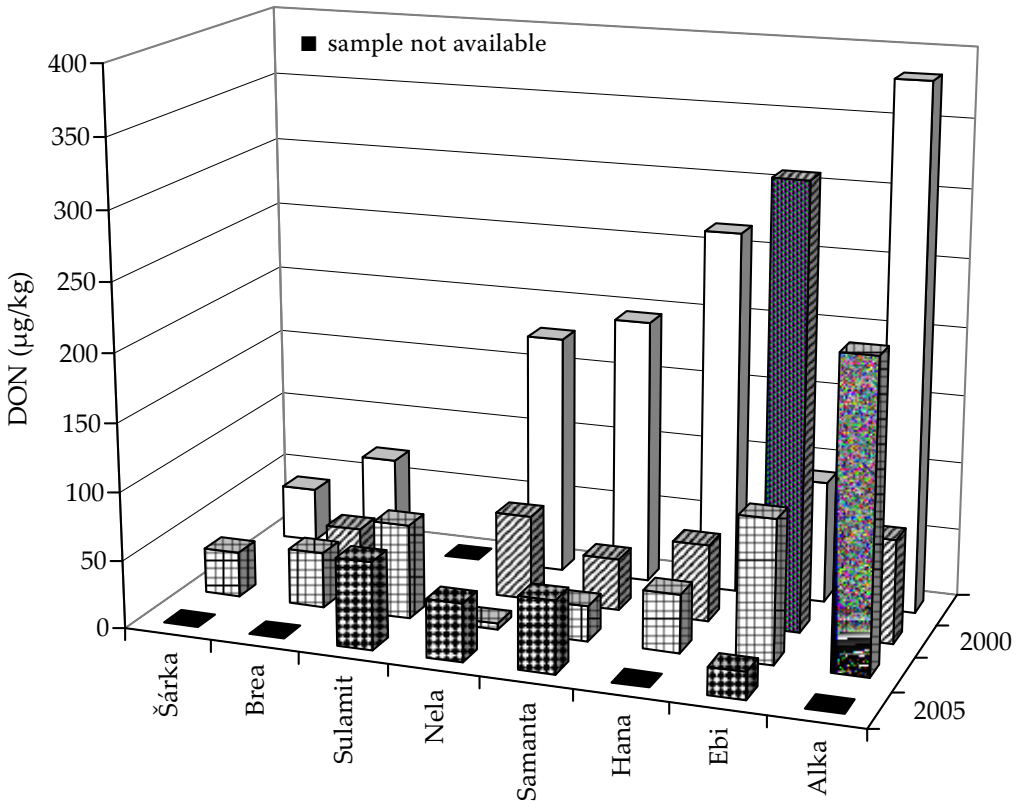

Figure 2. Natural occurrence of DON in wheat cultivars collected in monitored harvest years

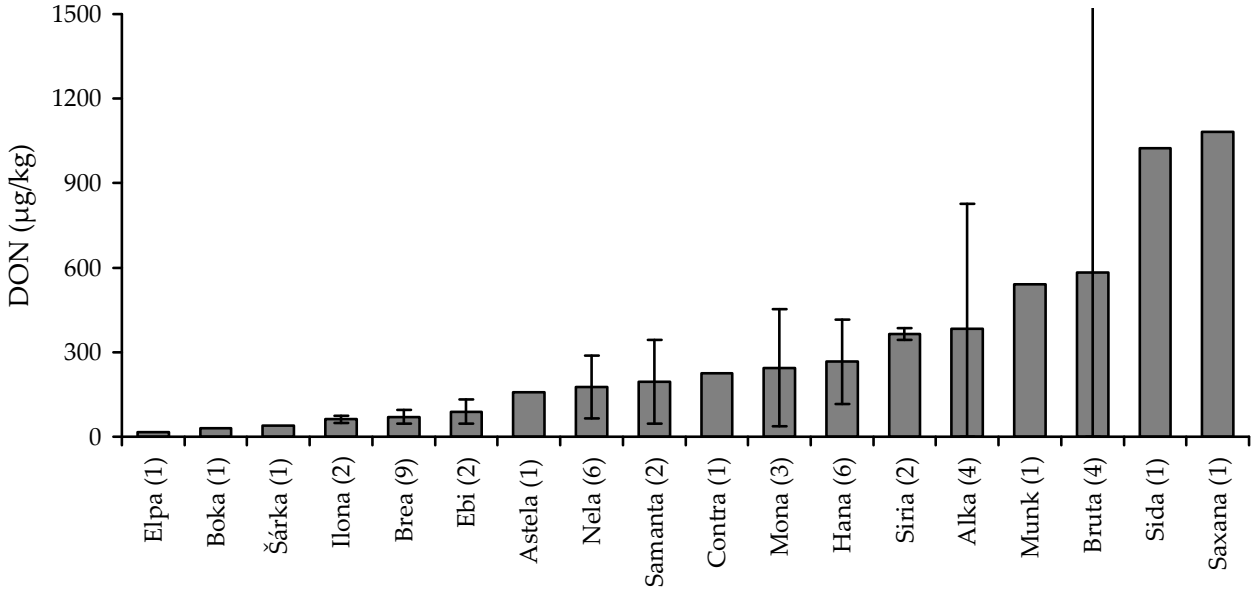

Figure 3. DON levels in various wheat cultivars harvested in the year 1999 (the number of samples analysed of each cultivar is in parenthesis)

In addition to the wheat samples, DON was also detected in 32 barleys represented by 7 various cultivars (Figure 8). Comparing this mycotoxin levels in 3 different cereals examined in the year 2001, barley seems to be the most susceptible while rye the most resistant to *Fusarium* infection. It should be borne in mind that the infection cycles are different with various cereals. For instance (contrary to wheat), with barley, which flowers when the head is in the boot, the infection is most common after the flowering period, once the head breaks through the leaf sheath (Edwards 2004).

The samples of cereals collected during the harvest of 2005 were analysed for the B type (DON, NIV, Fus-X, ADONs) as well as A-type (HT-2 and T-2) trichothecene mycotoxins, the results are summarised in Table 7. In total, 40 of 41 wheat samples were positive for at least one of the target toxins, DON, NIV, ADONs, HT-2, and T-2 with their incidences 98, 78, 7, 15, and 39%, respectively.

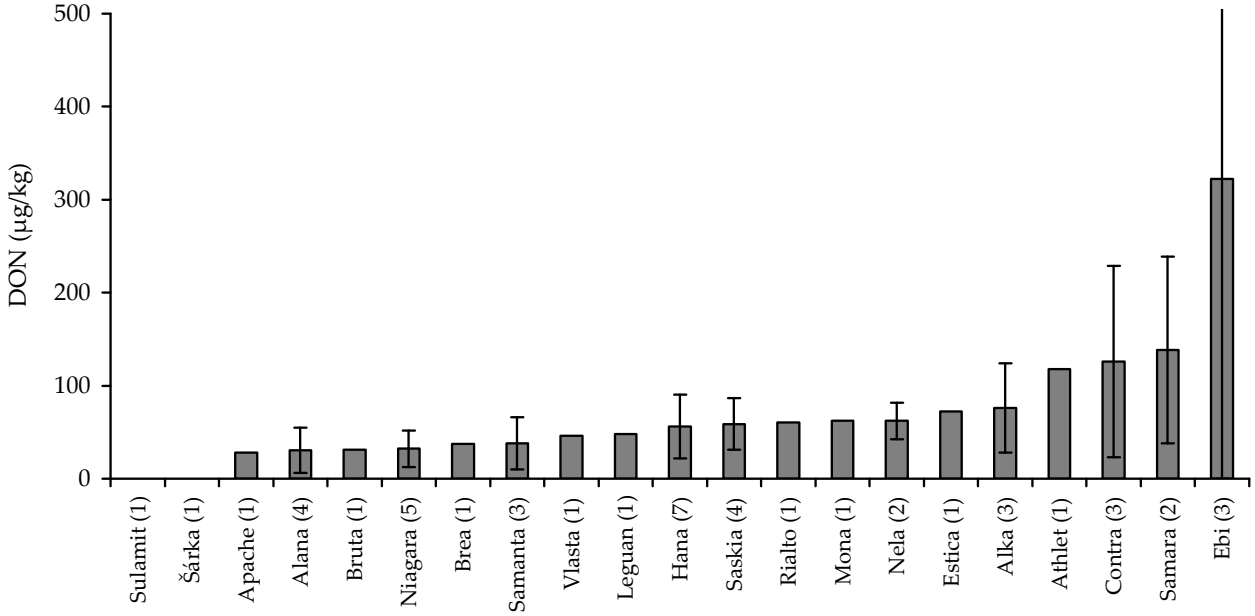

Figure 4. DON levels in various wheat cultivars harvested in the year 2000 (the number of samples analysed of each cultivar is in parenthesis)

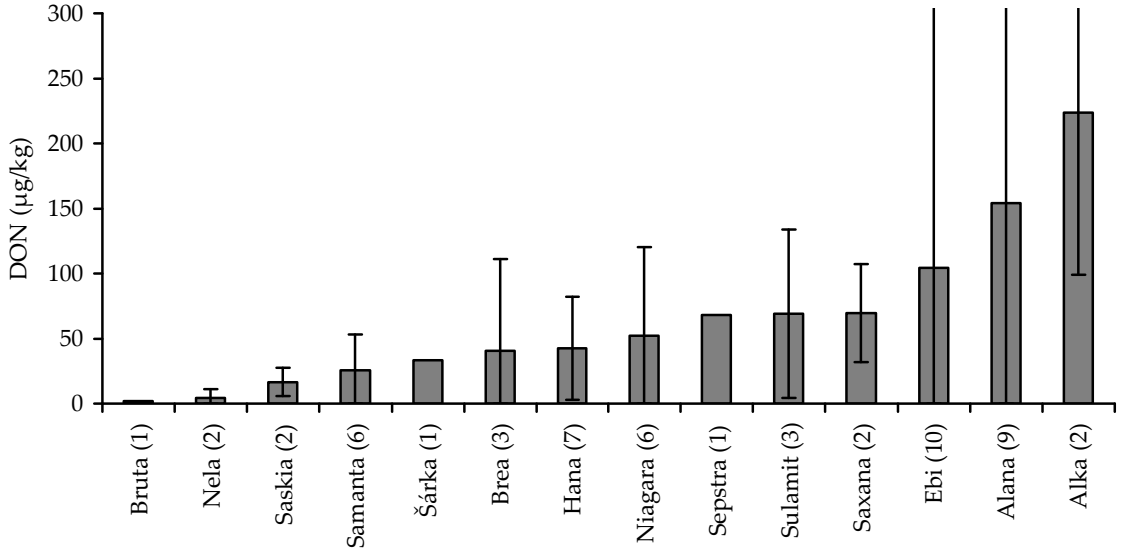

Figure 5. DON levels in various wheat cultivars harvested in the year 2001 (the number of samples analysed of each cultivar is in parenthesis)

Fus-X was not found in any sample. Similarly to previous monitoring years, DON was the prevalent *Fusarium* toxin in 2005 with concentrations ranging from 5.8 to 596.8 µg/kg (median was 27.5 µg/kg). Other trichothecenes of type B, NIV and ADONs, were, compared to DON, present at relatively low levels ranging from 15.4 to 25.9 µg/kg and 12.6 to 26.6 µg/kg, respectively. The contamination of the wheat samples with trichothecenes of type A, HT-2, and T-2, was in the concentration range from 12.7 to 18.3 µg/kg and from 5.7 to 8.2 µg/kg, respectively. The occurrence of trichothecenes in wheat harvested in 2005 was relatively low, only in 2 of 11 cultivars (Batis, Axis), the level of sum trichothecenes was above 500 µg/kg, Figure 7. In addition, 24 barley samples collected from two localities in the Czech Republic, Žabčice and Kroměříž, were analysed for 7 above mentioned mycotoxins. All the barley samples analysed were positive for at least one of the toxins DON, NIV, HT-2, and T-2 with incidences 100, 21, 87, and 13%, respectively. Fus-X and ADONs were not detected in any of the barley samples. The overview of the contamination patterns in the barley

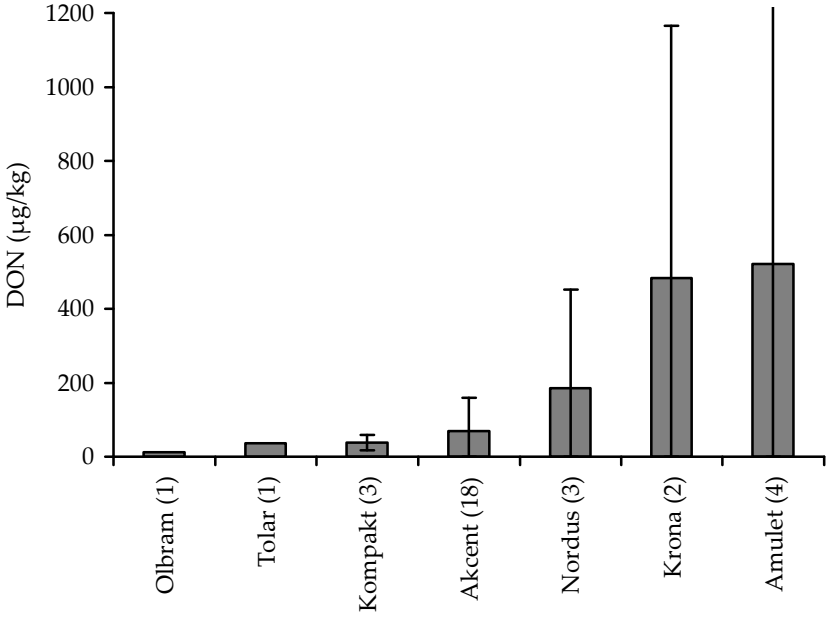

Figure 6. DON levels in various barley cultivars harvested in the year 2001 (the number of samples analysed of each cultivar is in parenthesis)

Table 7. Trichothecene contamination of wheats and barleys harvested in 2005*

Sample type	Trichothecene type	Target toxin	Positive/ total samples	Mean (µg/kg)	Median (µg/kg)	Concentration range** (µg/kg)
Wheat	B	DON	40/41	84.3	27.5	5.8–596.7
		NIV	32/41	< LOQ	< LOQ	15.4–25.9
		ADONs	3/41	< LOQ	< LOD	12.6–26.6
		Fus-X	0/41	< LOD	< LOD	< LOD
	A	HT-2	6/41	< LOQ	< LOD	12.7–18.3
		T-2	16/41	< LOQ	< LOD	5.7–8.2
Barley	B	DON	24/24	37.3	27.3	8.4–170.2
		NIV	5/24	< LOQ	< LOD	9.4–15.4
		ADONs	0/24	< LOD	< LOD	< LOD
		Fus-X	0/24	< LOD	< LOD	< LOD
	A	HT-2	21/24	20.8	17.9	13.0–72.2
		T-2	3/24	< LOQ	< LOD	5.9–7.7

*trichothecene levels were corrected on their recoveries; **in positive samples

samples is summarised in Table 8. In total, 19 samples were both DON and HT-2 positive. The assessment of trichothecene contents in 24 barley samples harvested in two localities, Žabčice and Kroměříž, is presented in Figure 8 (identical agricultural practices i.e. chemical treatment, crop rotation, soil cultivation, fertiliser, were used). Rather higher DON contamination was found in the samples from the latter locality, however, the sum of trichothecenes was higher in the samples from Žabčice. In any case, the comparison of these two localities indicates differences in the incidence of toxinogenic *Fusarium* fungi responsible for barley infection. This assumption was confirmed in the study focused on the determination of *Fusarium* sp. occurring in all barley samples harvested in

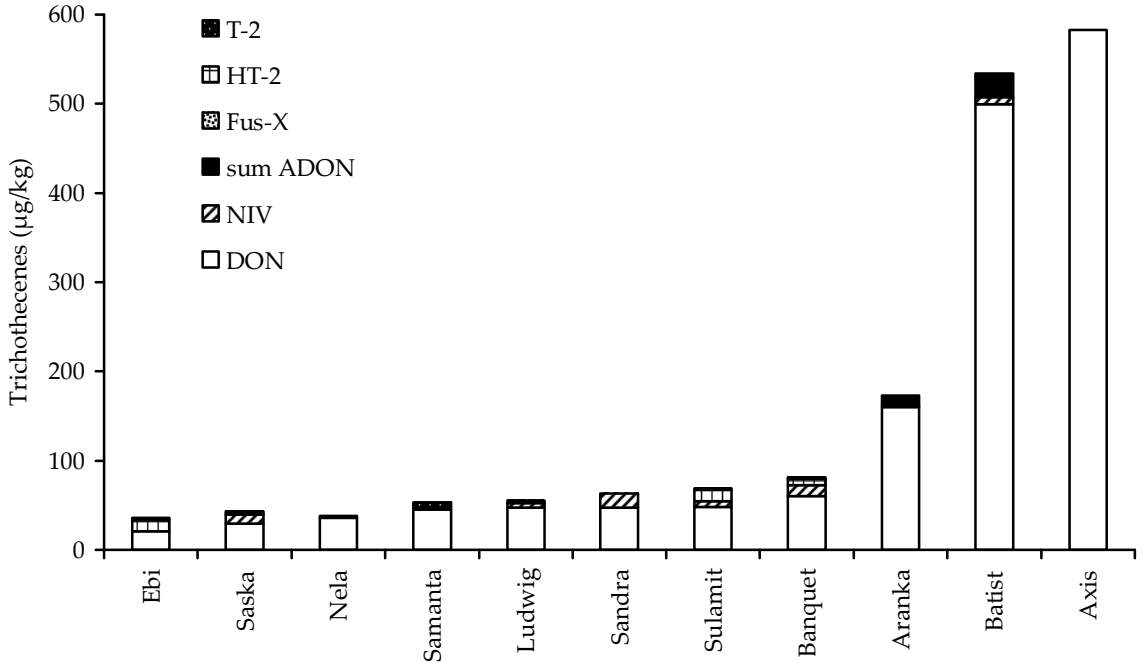

Figure 7. Trichothecenes in various wheat cultivars harvested in the Czech Republic in 2005

Table 8. Spectrum of trichothecene mycotoxins determined in cereal samples harvested in 2005

Sample type (number)	Mycotoxin	Positive samples (%)
Wheat (41)	DON	3 (7)
	DON + NIV	21 (51)
	DON + T-2	1 (2)
	DON + ADONs	1 (2)
	DON – HT-2 + T-2	1 (2)
	DON + NIV + T-2	8 (20)
	DON + NIV + ADONs	1 (2)
	DON + NIV + T-2 + HT-2	4 (10)
	DON + ADONs + NIV + HT-2 + T-2	1 (2)
Barley (24)	DON	2 (8)
	DON + NIV	1 (4)
	DON + HT-2	14 (58)
	DON + HT-2 + T-2	2 (8)
	DON + NIV + HT-2	4 (17)
	DON + NIV + HT-2 + T-2	1 (4)

the above mentioned localities. Not only higher number of *F. graminearum* damaged grains, but also the presence of *F. poae* (HT-2 producer) was detected in the samples from Žabčice. In overall, the levels of trichothecene mycotoxins in 65 cereal samples collected during the harvest 2005 were relatively low, all DON concentrations were much lower than the established maximum level in the EU for unprocessed cereals (1250 µg/kg).

CONCLUCIONS

Trichothecenes are ubiquitous natural contaminants occurring unavoidably in almost all cereals

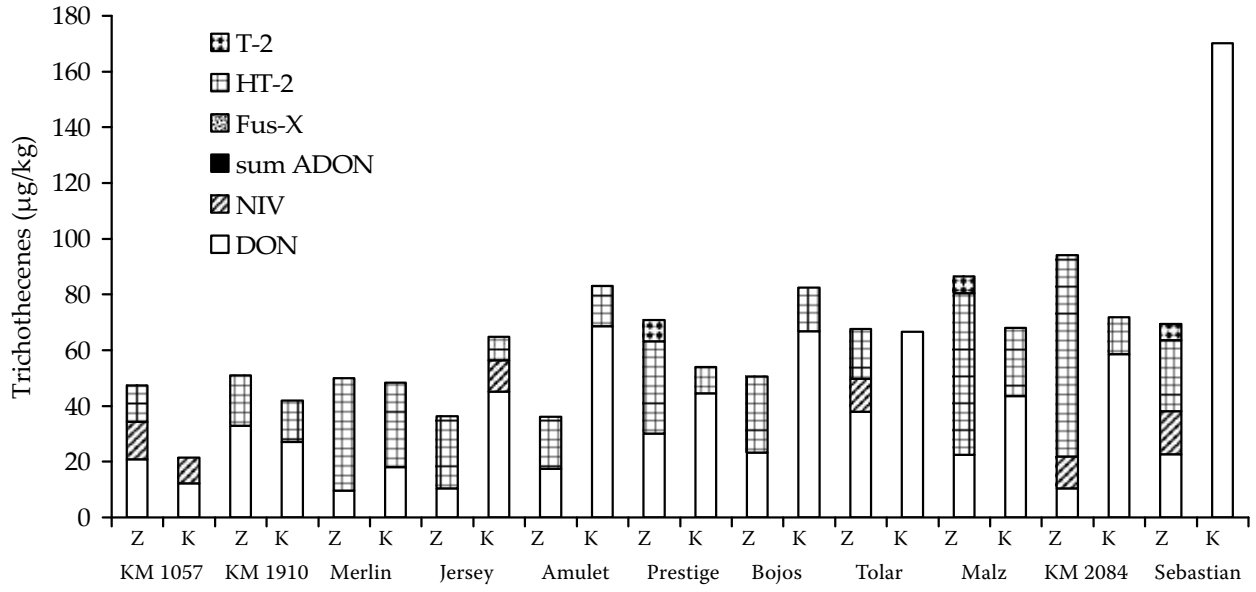

Figure 8. Trichothecenes in various barley cultivars harvested from two localities in the Czech Republic in 2005 (K – Kroměříž, Z – Žabčice)

harvested in the temperate climatic zone, the Czech Republic included. Deoxynivalenol (DON) representing trichothecene B group was the dominating *Fusarium* mycotoxin in most cereal samples examined in the current study, nevertheless, its levels were fairly below maximum limit established in the EU for this toxin in cereals. The presence of *Fusarium* producing trichothecenes A, HT-2 toxin and T-2 toxin, was documented in some localities. Although the current data indicate differences in resistance of various wheat and barley cultivars against *Fusarium* infection, drawing a general conclusion on this issue has to be based on the results obtained through a more comprehensive, long-term monitoring program.

References

Berthiller F., Schuhmacher R., Buttinger G., Krska R. (2004): Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A, **1062**: 209–216.

Campbell H., Choo T.M., Vigier B., Underhill L. (2002): Comparison of mycotoxin profiles among cereal samples from eastern Canada. Canadian Journal of Chemistry, **80**: 526–532.

Commission regulation (EC) No. 856/2005 of 6 June 2005. Official Journal of the European Union L 143: 3–5.

Creepy E.E. (2002): Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters, **127**: 19–28.

Dinorah P., Bonsignore F., Rivas F., Perera G., Bettucci L. (2007): Deoxynivalenol in barley samples from Uruguay. International Journal of Food Microbiology, **114**: 149–152.

Edwards S.G. (2004): Influence of agricultural practices on fusarium infestation of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicology Letters, **153**: 29–35.

Hussein H.S., Brasel J.M. (2001): Toxicity, metabolism, and impact of mycotoxins on human and animals. Toxicology Letters, **167**: 101–134.

JECFA (2001): Evaluation of certain mycotoxins in food, WHO Technical Report Series 906. Joint FAO/WHO Expert Committtee in Food Additives. 56th Report. Geneva.

Koch P. (2004): State of the art of trichothecenes analysis. Toxicology Letters **153**: 109–112.

Mateo J.J., Llorens A., Mateo R., Jimenez M. (2001): Critical study of and improvements in chromatographic methods for the analysis of type B trichothecenes. Journal of Chromatography A, **918**: 99–112.

Müller H.-M., Reimann J., Schumacher U., Schwadorf K. (1997): *Fusarium* toxins in wheat harvested during six years in an area of southwest Germany. Natural Toxins, **5**: 24–30.

Müller H.-M., Reimann J., Schumacher U., Schwadorf K. (2001): Further survey of the occurrence of *Fusarium* toxins in wheat grown in southwest Germany. Archives of Animal Nutrition, **54**: 173–182.

Obst A., Lepschy J., Beck R., Bauer G., Bechtel A. (2000): The risk of toxins by *Fusarium graminearum* in wheat – interactions between weather and agronomic factors. Mycotoxin Research, **16A**: 16–20.

Placinta C.M., D'Mello J.P.F., Macdonald A.M.C. (1999): A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Animal Feed Science and Technology, **78**: 21–37.

Radová Z., Holadová K., Hajšlová J. (1998): Comparison of two clean-up principles for determination of trichothecenes in grain extract. Journal of Chromatography A, **829**: 259–267.

Rodrigues-Fo.E., Mirocha C.J., Xie W., Krick T.P., Martinelli J.A. (2002): Electron ionization mass spectral fragmentation of deoxynivalenol and related tricothecenes**.** Rapid Communications in Mass Spectrometry, **16**: 1827–1835.

Schneider E., Curtui V., Seidler C., Dietrich R., Usleber E., Martlbauer E. (2004): Rapid methods for deoxynivalenol and other trichothecenes. Toxicology Letters, **153**: 113–121.

Schollenberger M., Jara H.-T., Suchy S., Drochner W., Müller H.-M. (2002): *Fusarium* toxin in wheat flour collected in an area in southwest Germany. International Journal of Food Microbiology, **72**: 85–89.

Schothorst R.C., Egmond H.P. (2004): Report from SCOOP task 3.2.10 "Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states", Subtask: trichothecenes. Toxicology Letters, **153**: 133–143.

Sokolovic M., Šimpraga B. (2005): Survey of trichothecene mycotoxins in grains and animal feed in Croatia by thin layer chromatography. Food Control, **17**: 733–740.

Sulyok M., Berthiller F., Krska R., Schuhmacher R. (2006): Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. Rapid Communications in Mass Spectrometry, **20**: 2649–2659.

Šíp V., Chrpová J., Leišová L., Sýkorová S., Kučera L., Ovesná J. (2007): Effects of genotype, environment

and fungicide treatment on development of Fusarium head blight and accumulation of DON in winter wheat grain. Czech Journal of Genetics and Plant Breeding, **43**: 16–31.

Tanaka T., Yamamoto S., Hasegawa A., Aoki N., Besling J.R., Suguira Y., Ueno Y. (1990): A survey of the natural occurrence of *Fusarium* mycotoxins, deoxynivalenol, nivalenol and zearalenone, in cereals harvested in the Netherlands. Mycopathologia, **110**: 19–22.

Tanaka T., Yoneda A., Inoue S., Suguira Y., Ueno Y. (2000): Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography–mass spectrometry. Journal of Chromatography A, **882**: 23–28.

Weidenbörner M. (2001): Encyklopedia of Food Mycotoxins. Springer-Verlag, Berlin, Heidelberg.

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