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Natural occurrence and distribution of *Fusarium* toxins in contaminated barley cultivars

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Abstract

Grain samples of 15 naturally contaminated barley cultivars, collected after harvest in southeastern Poland, were analysed for occurrence of *Fusarium* trichothecenes and zearalenone (ZEA). Barley kernels were contaminated with the following toxic metabolites: deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV), HT-2 toxin (HT-2), T-2 toxin (T-2), diacetoxyscirpenol (DAS), T-2 tetraol and ZEA. Significant correlations between concentrations of individual toxins and the dominant *Fusarium* species were found. Moreover, significant differences in toxin concentrations between cultivars were detected. Distribution of these mycotoxins was studied in two fractions of kernels (diameter >2.5 mm and <2.5 mm). A two-factor analysis of variance revealed significant differences between the two fractions, and between the analysed cultivars. Most of the interactions between fractions and cultivars were also significant. The highest concentration of the analysed toxins was in the fraction of small kernels. Kernel fraction <2.5 mm, although accounting for only 12.8% of sample weight, contained high proportions of the total toxin content: 80% of DON, 94% of NIV, 85% of ZEA, 83% of T-2 tetraol, 80% of DAS, 68% of HT-2 toxin and 81% of T-2 toxin. The results indicate that the level of contamination with *Fusarium* trichothecenes and ZEA, can be reduced by rejection of small kernels.

Introduction

Fusarium head blight is a severe disease of small grain, caused by several species of the genus *Fusarium.* In Poland and other countries *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides* are considered responsible for the disease and for occurrence of toxic metabolites (Mihuta-Grimm and Forster, 1989; Koizumi et al., 1991; Kiecana, 1994; Perkowski et al., 1997a). For about a decade, natural contamination of small grain has been reported in Europe and North America, and the following toxic metabolites of *Fusarium* have been identified: trichothecenes group B: deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON); trichothecenes group A: T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS), T-2 tetraol; and zearalenone (ZEA) (Tanaka et al., 1988; Gareis et al., 1989; Scott, 1990; Pohland, 1993; Goliński et al., 1996; Perkowski et al., 1997b).

In the case of barley in Europe, DON was present in 24% of grain samples, NIV in 24%, T-2 in 5% and HT-2 in 21% (Gareis et al., 1989). Mean concentrations (mg kg^{-1}) in grain amounted to 0.189 for DON, 0.028 for NIV, 0.050 for T-2 and 0.013 for HT-2 (Pettersson, 1996). Research conducted in Poland (Perkowski et al., 1997b) showed that *Fusarium* toxins were present in a higher percentage of barley grain samples, but their concentrations were similar to those reported in Scandinavia (Pettersson, 1996).

Higher amounts of some mycotoxins have been observed in small kernels (Scott et al., 1983; Lee et al., 1987; Tkachuk et al., 1991;

Perkowski and Miedaner, 1994). The aims of this study were: (1) to analyse the contamination of *Fusarium* toxins in kernels of 15 barley cultivars, (2) to assess correlations between concentrations of individual toxins found in grain and to relate these data with phytopathological observations, and (3) to compare the distribution of nine *Fusarium* toxins in two kernel size fractions.

Materials and methods

Barley samples

Grain samples were collected manually from mature naturally contaminated barley heads of 15 cultivars (Ars, Agat, Bielik, Dema, Diva, Grosso, Hockey, Klimek, Lot, Lotus, Nadgar, Roland, Rudzik, MOB-487, NAD-685) during the harvest of 1997. A two-factor experiment involving two fractions and 15 cultivars was carried out in southern Poland (Lublin region) in a three randomized complete block design. Each of the 15 cultivars (1000 g in each sample) × 2 fractions (30 factorial combinations) was assigned to the experimental plots in each of the three replications (90 samples).

Weather conditions

Differences between mean values for temperature (°C), rainfall (mm) and relative humidity (%) in the study period (May, June, July, August 1997) and mean values for the last two decades (100%) in the Lublin region are given in Table 1.

Preparation of samples

Before the chemical analysis, all grain samples of 15 cultivars in three replications were transported to the laboratory in paper bags, then dried, manually threshed and separated into two groups: *Fusarium*-damaged kernals (shrivelled, light coloured, with mycelium on surface and/or pink to carmin red discolouration) (FDK); kernels looking healthy, without symptoms such as those above (HLK). The percentage of FDK was calculated in the whole sample. Samples were mixed, and divided by sieving (Vogel Sieve model FE-870, Spółdzielnia Pracy Mechaniki Precyzyjnej, Warsaw, Poland) into two size fractions: >2.5 mm (# I) and <2.5 mm (# II) in diameter.

Table 1. Mean values for temperature (°C), rainfall (mm) and relative humidity (%) of the season 1997, in comparison with mean values for the last two decades (in brackets) in southeastern Poland (Lublin region)

Month	Temperature (°C)	Rainfall (mm)	Relative humidity (%)
May	13.8 (95%)*	61 (106%)	56 (83%)
June	16.2 (101%)	50 (70%)	63 (89%)
July	17.2 (99%)	168 (221%)	74 (103%)
August	18.0 (94%)	29 (40%)	57 (77%)

*Compared to mean value for the last 20 years (100%).

Chemical analysis; trichothecenes

Sub-samples of 20 g were used for analysis of each toxin. They were ground in a WŻ–1 laboratory mill (Research Institute of Baking Industry Ltd. Bydgoszcz, Poland), designed especially for grinding cereal samples. Samples were extracted overnight with 100 ml of solvent (acetonitrile–water 82 : 18 (v/v)), and filtered (Whatman no. 5 filter paper), then purified on a column (5 ml) of mixed alumina (neutral activated, 70–230 mesh), Darco G 60-charcoal (100 mesh), Celite 545 4 : 3 : 4 (w/w/w). The extracts were evaporated to dryness using a rotary evaporator. The residue was dissolved using two aliquots of 2 ml ethyl acetate and 2 ml of chloroform/acetonitrile 4 : 1 (v/v) and divided into two portions.

The trichothecenes group A (H-2 toxin, T-2 toxin, T-2 tetraol, DAS) were analysed as TFAA derivatives. To the dried sample, 100 µl of trifluoroacetic acid anhydride was added. After 20 min, the reacting substance was evaporated to dryness under nitrogen. The residue was dissolved in 500 µl of isooctane, and 1 or 2 µl was injected onto a gas chromatograph-mass spectrometer (Hewlett Packard GC 6890, MS 5972 A, Waldbronn, Germany).

The trichothecenes group B (DON, NIV, 3-AcDON, 15-AcDON) were analysed as TMS (trimethylsilyl silyl ethers) derivatives. To the dried extract 100 µl of TMSI/TMCS (trimethylsilyl imidazole/ trimethylchlorosilane v/v 100/1) mixture was added. After 10 min, 500 µl of isooctane was added, and the reaction was quenched with 1 ml of water. Isooctane layer was used for the analysis and 1 or 2 µl of sample was injected on a GC/MS system.

The column used was the HP-5MS (30 m) column. The injection port temperature was 280 °C, the transfer line temperature was 280 °C, and the analysis was performed with the programmed temperature (from 80 °C (1 min) to 280 °C at 25 °C min^{-1}), the final

temperature being kept for 10 min. The helium flow rate was constant at 0.7 ml min^{-1}. Trichothecenes were quantified by comparison of retention times and ion intensities with the standard compounds. To confirm the identities of the toxins, fullscan analysis in the range of 100–600 amu was performed. The following ions were used for trichothecene detection: DON-m/z 235, 422, 3-AcDON-117, 482, 15-AcDON-193, 482, NIV-585, 289, HT-2-180, 455, 532, 327, T-2-180, 205, 401, T-2 tetraol, 455, 568, DAS-402, 374, 329. The first ion in each set was used for quantification. The detection limit was 0.01 mg kg^{-1}. Average recoveries of the toxins were: trichothecenes group A – 86% ± 3.8 for T-2, 88% ± 4.0 for T-2 tetraol, 91% ± 3.2 for HT-2, 84%±4.6 for DAS; trichothecenes group B – 84%±3.8 for DON, 81%±4.4 for NIV, 74%±2.2 for 15-AcDON, 78% ± 4.8 for 3-AcDON.

Zearalenone

Each sample was extracted with a method similar to that described by Tanaka et al. (1985). For clean-up of the extracts, a chromatographic column (10 ml) was filled with 2 g anhydrous sodium sulphate, 5 g portion of Florisil and 2 g of sodium sulphate (in n-hexane). The extracts were dissolved in 3 ml methanol and a 2 ml portion was charged to the column. After draining the excess of methanol and washing the column with 50 ml of *n*-hexane, ZEA was eluted with 100 ml of chloroform–methanol (9 : 1).

A portion of the Florisil column eluate was subjected to analysis of ZEA by high-performance liquid chromatography with fluorescence detection. The chromatographic separation was performed using a silica gel column (LiChrosorb Si 60). The mobile phase was water saturated dichloromethane and 1-propanol (98.5 : 1.5 v/v). The column temperature and solvent flow rate were set at 25 °C and 2.0 ml min^{-1}, respectively.

The fluorescence programmable detector (HP 1046 A) was set at an emission wavelength of 450 nm, and the excitation wavelength was 236 nm. On the LiChrosorb Si 60 column, retention time for ZEA was 4.9 min. The detection limit was 0.001 mg kg^{-1}. Average recovery of the ZEA was: 96% ± 2.8.

Statistical analyses

Two-factor analysis of variance (Gomez and Gomez, 1984) for size fractions (F), and cultivars (C) was performed for each *Fusarium* toxin (except 3-AcDON, where one-factor analysis of variance was made for fraction <2.5 mm). The hypotheses of no difference between both fractions, and between all cultivars and the hypotheses of no interaction fractions × cultivars were tested by the F statistic. The least significant difference (LSD) test was used for making mean comparisons. Two means are declared significantly different at the significance level α (0.05 or 0.01) if their difference exceeds the computed LSD value; otherwise they are not significantly different. Coefficients of correlation between the analysed toxins within the fractions were also calculated and tested at the significance level $\alpha = 0.05$ and $\alpha = 0.01$.

Results

Weather conditions

The analysed samples were collected in 1997, when unusually high precipitation (221% of the mean value for the last two decades) was observed in July in the Lublin region (Table 1).

Concentration of Fusarium *toxins in 15 barley cultivars*

For the 15 barley cultivars, the mean concentrations of seven *Fusarium* toxins were calculated: DON 0.085 mg kg^{-1}, NIV 0.061 mg kg^{-1}, ZEA 0.063 mg kg^{-1}, DAS 0.012 mg kg^{-1}, HT-2 toxin 0.055 mg kg^{-1}, T-2 toxin 0.047 mg kg^{-1} and T-2 tetraol 0.027 mg kg^{-1} (Table 2). Mean concentrations of 3-AcDON and 15-AcDON were below the detectable level (<0.01 mg kg^{-1}). Table 2 shows the mean percentage of FDK for each cultivar. No significant correlation between FDK values and concentrations of individual toxins in grain was found.

The analyses revealed considerable differences in mean toxin concentrations between the analysed cultivars (Table 2). The highest mean concentrations were recorded in kernels of cv. Lotus (DON 0.37 mg kg^{-1}, NIV 0.13 mg kg^{-1}, ZEA 0.07 mg kg^{-1}, T-2 tetraol 0.03 mg kg^{-1}, DAS 0.01 mg kg^{-1}, HT-2 0.05 mg kg^{-1}, T-2 0.06 mg kg^{-1}), followed by cvs NAD-687, Agat, Grosso, Ars, MOB-487, Klimek, Bielik, Dema, Roland, Nadgar, Rudzik, Hockey and Diva. The lowest mean concentrations were observed in cv. Lot (DON 0.03 mg kg^{-1}, NIV 0.04 mg kg^{-1}, ZEA 0.04 mg kg^{-1}, T-2 tetraol <0.01 mg kg^{-1}, DAS 0.01 mg kg^{-1}, HT-2 <0.01 mg kg^{-1}, T-2 0.04 mg kg^{-1}).

Table 2. Mean percentage of FDK and mean concentration of *Fusarium* toxins in 15 naturally contaminated barley cultivars

Cultivar	FDK (%)	Concentration of *Fusarium* toxins (mg kg^{-1})						
		DON	NIV	ZEA	T-2 tetraol	DAS	HT-2	T-2
Ars	4.0	0.14	0.08	0.04	0.02	0.01	0.03	0.06
Agat	1.0	0.10	0.07	0.08	0.04	0.01	0.12	0.05
Bielik	3.0	0.05	0.08	0.06	n.d.*	n.d.	0.04	0.04
Dema	0.5	0.02	0.07	0.06	0.03	n.d.	0.05	0.04
Diva	0.5	0.01	0.04	0.06	0.02	n.d.	0.03	0.03
Grosso	0.5	0.16	0.12	0.04	0.02	0.01	0.05	0.04
Hockey	0.5	0.05	0.05	0.05	0.01	n.d.	0.02	0.05
Klimek	1.0	0.10	0.02	0.08	n.d.	0.01	0.03	0.04
Lot	3.0	0.03	0.04	0.04	n.d.	0.01	n.d.	0.04
Lotus	1.0	0.37	0.13	0.07	0.03	0.01	0.05	0.06
Nadgar	2.0	0.05	0.01	0.08	n.d.	0.01	0.05	0.06
Roland	3.0	0.01	0.02	0.05	0.03	0.01	0.10	0.04
Rudzik	3.0	0.01	0.05	0.05	0.02	0.01	0.04	0.05
MOB-487	2.5	0.03	0.06	0.06	0.04	0.03	0.08	0.05
NAD-687	2.0	0.14	0.07	0.12	0.04	0.01	0.08	0.05
$LSD_{0.05}$		0.142	0.056	0.048	0.022	0.025	0.038	0.030
$LSD_{0.01}$		0.192	0.076	0.056	0.030	0.034	0.052	0.041
Mean	1.8	0.085	0.061	0.063	0.027	0.012	0.055	0.047

*n.d. = not detected (detection limit = 0.01 mg kg^{-1}).

Occurrence of Fusarium *toxins in different kernel fractions*

The results on occurrence of *Fusarium* toxins in two kernel size fractions are presented in Table 3. On average, kernels >2.5 mm accounted for 87.2% of sample weight, and kernels <2.5 mm accounted for the remaining 12.8%. Concentrations of *Fusarium* toxins were significantly lower in the fraction of regular kernels (>2.5 mm), namely for trichothecenes of group B: DON 0.06 mg kg^{-1} and NIV 0.02 mg kg^{-1}; and for group A from 0.01 mg kg^{-1} (DAS) to 0.05 mg kg^{-1} (HT-2 toxin). Kernels of this fraction (>2.5 mm) were generally classified as healthy looking (HLK).

A two-factor (fractions and cultivars) analysis of variance revealed highly significant differences ($\alpha = 0.01$) between fractions, between the analysed cultivars and for the interaction fractions × cultivars (Table 4). Taking the interaction into consideration the differences in toxin concentrations between cultivars within fractions was analysed. In fraction >2.5 mm no significant differences were found, while in fraction <2.5 mm significant differences in toxin occurrence were detected between cultivars.

Mean concentrations of all toxins in the analysed fractions were calculated. On average, fraction <2.5 mm accounted for only 12.8% of sample weight, but accumulated very high toxin concentrations, and contained from 68% (HT-2 toxin) up to 94% (NIV) of the total toxin content of the whole sample (Table 5). Correlation coefficients for individual toxins, both between the size fractions, and between toxins within the size fractions are given in Table 6.

Results showed significant correlations between concentrations of all toxins except NIV within the size fractions. All correlations between trichothecenes of group B were significant at $\alpha \leq 0.01$. Correlations for group A trichothecenes except DAS were also significant, but in some cases at $\alpha \leq 0.05$. ZEA, which is not a trichothecene, proved to be significantly ($\alpha \leq 0.01$) correlated with trichothecenes of both groups, except DAS.

Discussion

Infestation of cereal heads with *Fusarium* species results in a reduced number of kernels per head and/or in an increased number of small kernels. Each year in southeastern Poland (Lublin region) 0.1–4.0% of barley heads are usually infected. The analysed samples were collected in 1997, when unusually high precipitation was observed in July in the Lublin region. Grain samples of fifteen naturally contaminated barley cultivars were contaminated with the following *Fusarium* toxins: DON, 15-AcDON, 3-AcDON, NIV, T-2 toxin, HT-2 toxin, T-2 tetraol.

Table 3. Mean concentration of trichothecenes (mg kg^{-1}) in two kernel fractions (>2.5 mm and <2.5 mm) and distribution (%) of fractions in 15 naturally contaminated barley cultivars

Cultivars	Percentage fraction (%)		Concentration of *Fusarium* toxins (mg kg^{-1}) in kernel fractions # I (>2.5 mm) and # II (<2.5 mm)																	
	>2.5 mm	<2.5 mm	DON		NIV		3-AcDON		15-AcDON		ZEA		T-2 tetraol		DAS		HT-2 toxin		T-2 toxin	
			I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Ars	94.4	5.5	0.17	0.03	0.04	0.65	n.d.[a]	0.01	0.01	0.01	0.02	0.39	0.01	0.14	n.d.	0.07	0.03	0.29	0.05	0.22
Agat	90.9	9.1	0.09	0.27	0.01	0.60	n.d.	0.01	0.01	0.01	0.06	0.28	0.03	0.10	n.d.	0.04	0.12	0.16	0.04	0.16
Bielik	90.1	9.1	0.02	0.02	0.02	0.22	n.d.	0.01	n.d.	n.d.	0.03	0.33	n.d.	n.d.	n.d.	0.03	0.04	0.07	0.02	0.22
Dema	88.8	11.2	0.02	0.07	0.01	0.57	n.d.	0.01	n.d.	n.d.	0.02	0.38	0.02	0.07	n.d.	0.03	0.04	0.16	0.02	0.16
Diva	83.6	16.4	0.01	0.30	n.d.	0.20	n.d.	0.01	n.d.	n.d.	0.05	0.13	n.d.	0.10	n.d.	n.d.	0.02	0.01	0.01	0.12
Grosso	85.7	14.3	0.01	1.05	0.01	0.80	n.d.	0.03	n.d.	0.03	0.01	0.25	0.01	0.09	0.01	0.02	0.05	0.06	0.04	0.03
Hockey	87.8	12.2	0.05	0.08	0.04	0.09	n.d.	n.d.	n.d.	n.d.	0.04	0.13	0.01	0.04	n.d.	n.d.	0.01	0.06	0.04	0.12
Klimek	83.6	16.4	0.06	0.31	0.02	0.03	n.d.	0.01	n.d.	0.01	0.07	0.12	n.d.	0.03	0.01	0.01	0.02	0.07	0.04	0.05
Lot	88.9	11.1	0.02	0.10	0.01	0.27	n.d.	0.01	n.d.	n.d.	0.03	0.14	n.d.	n.d.	n.d.	0.04	n.d.	0.04	0.03	0.11
Lotus	93.4	6.6	0.34	0.77	0.08	0.88	n.d.	0.01	0.01	0.02	0.06	0.25	0.01	0.12	0.01	0.05	0.04	0.13	0.04	0.29
Nadgar	92.3	7.7	0.05	0.02	0.01	0.02	n.d.	0.01	n.d.	0.01	0.06	0.26	n.d.	n.d.	0.01	0.06	0.08	0.09	0.05	0.22
Roland	80.0	20.0	0.01	0.03	0.01	0.05	n.d.	n.d.	n.d.	0.01	0.02	0.17	0.01	0.14	0.01	0.01	0.07	0.19	0.03	0.09
Rudzik	75.4	24.6	0.01	0.03	0.01	0.18	n.d.	n.d.	n.d.	n.d.	0.03	0.13	0.01	0.04	0.01	0.02	0.04	0.05	0.04	0.08
MOB-487	83.6	16.4	0.02	0.08	0.02	0.26	n.d.	n.d.	n.d.	n.d.	0.04	0.17	0.03	0.07	0.01	0.10	0.07	0.12	0.03	0.12
NAD-685	88.6	12.8	0.11	0.44	0.05	0.20	n.d.	0.01	n.d.	0.01	0.09	0.36	0.04	0.07	n.d.	n.d.	0.09	0.04	0.03	0.18
$LSD_{0.05}$			0.229		0.207		0.016[b]		0.007		0.099		0.062		0.088		0.069		0.074	
$LSD_{0.01}$			0.304		0.276		0.021[b]		0.010		0.131		0.082		0.117		0.092		0.098	
Mean	87.2	12.8	0.06	0.24	0.02	0.34	n.d.	0.01	<0.01	0.01	0.04	0.23	0.02	0.08	0.01	0.04	0.05	0.10	0.04	0.15

[a]n.d. = not detected (detection limit = 0.01 mg kg^{-1}). [b]For comparing cultivars in fraction # II (<2.5 mm) only.

Table 4. Two-factor analysis of variance for individual toxins (DON, NIV, 15-AcDON, ZEA, T-2 tetraol, DAS, HT-2 toxin, T-2 toxin)

Source of variation	Degrees of freedom	Mean square							
		DON	NIV	15-AcDON	ZEA	T-2 tetraol	DAS	HT-2 toxin	T-2 toxin
Blocks	2	0.1887**	0.0726*	0.00061**	0.0797**	0.0184**	0.0122*	0.0422**	0.0566**
Fractions (F)	1	0.6502**	1.8720**	0.00007	0.8199**	0.0689**	0.0352**	0.0678**	0.2756**
Cultivars (C)	14	0.1871**	0.0996**	0.00027**	0.0152**	0.0043**	0.0049	0.0113**	0.0083**
F × C	14	0.1235**	0.0944**	0.00010**	0.0155**	0.0031*	0.0043	0.0080**	0.0075**
Error	58	0.0196	0.0161	0.00004	0.0037	0.0014	0.0029	0.0018	0.0020

*Significant at $P < 0.05$. **Significant at $P < 0.01$.

Table 5. Mean percentage distribution of *Fusarium* toxins in kernel fractions # I (>2.5 mm) and # II (<2.5 mm) in samples of 15 naturally contaminated barley cultivars

Toxin	Fraction # I (>2.5 mm) (%)	Fraction # II (<2.5 mm) (%)
DON	20	80
NIV	6	94
ZEA	15	85
T-2 tetraol	17	83
DAS	20	80
HT-2	32	68
T-2	19	81

Investigation of trichothecene occurrence in barley has been carried out in other European countries. Data presented by Gareis et al. (1989) showed that over 20% of analysed samples contained DON, NIV and HT-2, while 2–7% contained DAS, T-2 and acetylated DON derivatives. By contrast, Pettersson (1996) reported that in Scandinavia as many as 58% of barley samples contained DON; the mean concentration of DON in 1687 European samples of barley grain was 0.189 mg kg^{-1}, in 1267 Scandinavian samples 0.229 mg kg^{-1} and in 234 North American samples 2.690 mg kg^{-1}. NIV concentrations amounted to 0.028 mg kg^{-1} in Europe and 0.030 mg kg^{-1} in Scandinavia. The most frequently analysed among group A trichothecenes are T-2 and HT-2. Their mean concentrations in European samples were 0.050 and 0.013 mg kg^{-1}, and in Scandinavia 0.012 and 0.013 mg kg^{-1}, respectively.

In samples analysed earlier in Poland (Perkowski et al., 1997b) DON was detected in 91% of barley samples, and the mean concentration was 0.120 mg kg^{-1}. The corresponding values for other toxins were: 72% and 0.053 mg kg^{-1} for NIV, 20% and 0.018 mg kg^{-1} for AcDON, 62% and 0.038 mg kg^{-1} for T-2, 50% and 0.049 mg kg^{-1} for HT-2 (Perkowski et al., 1997b).

A comparison of the above data with results presented in Table 2 shows that the samples analysed in 1997 were highly contaminated with *Fusarium* toxins. DON, NIV and HT-2 were detected in 100% of samples, HT-2 in 93%, while DAS and T-2 tetraol in 73%. Concentrations of these toxins varied greatly, but were generally higher than in barley samples analysed in Poland in 1994 and 1995 (Perkowski et al., 1997b), and in Germany (Müller et al., 1993). This may be due to the unusually high precipitation in the summer of 1997. Although the percentage of contaminated samples was higher, no significant increase in concentrations of these toxins was observed in the analysed cultivars. Thus it is likely that the concentrations and profiles of the toxins in the tested samples are similar to other European countries, because high concentrations of trichothecenes of group A in oat and barley kernels have been observed by other authors (Gareis et al., 1989; Pettersson and Olvang, 1995; Pettersson, 1996). The data presented that the mean concentrations of *Fusarium* toxins are lower than the current and the postulated norms (Smith et al., 1994; Eriksen and Alexander, 1998). Nevertheless, great caution is necessary because of synergistic effects between these metabolites (Forster et al., 1986; Dowd, 1989; Friend et al., 1992; Koshinsky and Khachatourians, 1992).

A two-factor (fractions and cultivars) analysis of variance revealed highly significant differences (mostly at $\alpha = 0.01$ level) between fractions, between the analysed cultivars and for the interaction fractions × cultivars. For example, differences between barley cultivars for DON, $F = 9.55$; NIV, $F = 6.19$; 15-AcDON, $F = 6.75$; ZEA, $F = 4.11$; T-2 tetraol, $F = 3.07$; HT-2 toxin, $F = 6.28$; T-2 toxin, $F = 4.15$ at critical value F-statistic equal to $F_{0.05} = 1.86$ and $F_{0.01} = 2.29$. For other sources of variation similar results were observed.

Within certain ranges of temperature, humidity and other environmental conditions, contamination of cereals with *Fusarium* and mycotoxins may be unavoidable. From the practical point of view, it is essential for grain producers to decontaminate the

Table 6. Correlation coefficients between *Fusarium* toxins for kernel fractions # I (>2.5 mm) and # II (<2.5 mm)

	Fraction # I (>2.5 mm)							
Fraction # II (<2.5 mm)	DON	NIV	15-AcDON	ZEA	T-2 tetraol	DAS	HT-2 toxin	T-2 toxin
DON	*0.41***	0.77**	0.68**	0.39**	0.24	0.20	0.17	0.37*
NIV	0.43**	*0.13*	0.62**	0.39**	0.37**	0.25	0.10	0.37*
15-AcDON	0.70**	0.47**	*0.53***	0.45**	0.12	0.30*	0.22	0.36*
ZEA	0.18	0.41**	0.37*	*0.30**	0.42**	0.15	0.46**	0.38*
T-2 tetraol	0.46**	0.37**	*0.56***	0.32*	*0.46***	0.16	0.70**	0.31*
DAS	0.09	0.33*	0.24	0.16	0.28	*0.32**	0.31*	0.44**
HT-2 toxin	−0.07	0.45**	0.37*	0.45**	0.55**	0.28	*0.41***	0.37*
T-2 toxin	0.06	0.18	0.15	0.60**	0.32*	0.41**	0.41**	*0.54***

*Significant at $P < 0.05$. **Significant at $P < 0.01$.

mycotoxin-contaminated grain. One of the best methods is to divide grain into size fractions and to exclude the smaller kernels (Scott et al., 1983; Lee et al., 1987; Tkachuk et al., 1991; Perkowski and Miedaner, 1994). Our results showed that small kernels (<2.5 mm) were frequently light, shrivelled and/or discoloured. Concentrations of *Fusarium* metabolites in kernels of this fraction were much higher than in the >2.5 mm fraction. In the <2.5 mm fraction, 68% HT-2 toxin, 80% DAS and DON, 81% T-2 toxin, 83% T-2 tetraol, 85% ZEA and 94% NIV were found. These results are comparable with earlier data obtained for barley kernels infected with *F. graminearum* or *F. culmorum* where 80% of DON was present in fraction <2.5 mm (Perkowski, 1998).

Data referring to significant correlations between toxins in barley grain have been achieved usually after artificial inoculation of heads with different *Fusarium* species (Mirocha et al., 1994; Perkowski et al., 1995; Perkowski and Kiecana, 1998; Tekauz, 2002). Studies using naturally contaminated barley cultivars indicated significant correlations between concentrations of most *Fusarium* toxins and were related to phytopathological observations.

In southeastern Poland the main *Fusarium* species infecting barley heads every year are: *F. avenaceum*, *F. culmorum* as the dominant species, *F. graminearum*, *F. sporotrichioides* and *F. poae* and sporadically *F. crokwellesse*, *F. equiseti* and *F. oxysporum* (Perkowski et al., 1997a). DON and its acetylated derivatives could have been produced by *F. graminearum* and/or *F. culmorum*, NIV by *F. poae* and/or *F. culmorum*, T-2, HT-2, T-2 tetraol by *F. sporotrichioides* and/or *F. poae*. ZEA is produced by many *Fusarium* species (Bottalico, 1998; Chełkowski, 1998).

We detected toxins produced by the *Fusarium* species mentioned above in the analysed grain samples. DON, NIV and T-2 toxin were present in all of the analysed samples, HT-2 in 14, while DAS and T-2 tetraol in 11 of the 15 analysed samples. It is also noteworthy that some toxins tended to occur together. Correlations between toxins formed by the same *Fusarium* species were significant. Correlation coefficients for all of the analysed group B trichothecenes (DON, NIV, 15-AcDON) were significant at a level of $\alpha = 0.01$ ($r = 0.41$–0.77). For DON (characterized by the highest concentration in samples) most probably produced by the dominating species in southeastern Poland, *F. culmorum* and *F. graminearum,* no significant correlations with the trichothecenes group A (T-2, HT-2) produced by *F. sporotrichioides* and *F. poae* were found. In the case of NIV, produced by *F. culmorum*, *F. graminearum* and *F. poae* the situation was not so clear. In case of trichothecenes group A (T-2, HT-2, T-2 tetraol) produced mainly by *F. sporotrichioides*, higher correlation coefficients between members of this group were observed. This confirms the observations of Langseth and Rundberget (1999), that T-2 and HT-2 were produced in Norway by the same fungi and were often found together. Similar observations for *F. culmorum* and *F. graminearum* and DON and Ac-DON production were made in Poland (Perkowski et al., 1988). For ZEA produced by different *Fusarium* species, significant correlation coefficients were found for trichothecenes of two groups. This suggests that at low concentrations (<1 mg kg^{-1}), more characteristic of natural contamination, the mechanism of toxin formation by each of the studied *Fusarium* species is similar in different cultivars. The simultaneous production of various toxins by several *Fusarium* species competing with

one another, was probably due to favourable climatic conditions.

These results confirm earlier findings obtained after inoculation of different barley cultivars with different *Fusarium* species. Perkowski et al. (1995) and Perkowski and Kiecana (1998), suggested that, in general, correlations between toxins (within cultivars) are significant, and that they are most significant for the toxins which have the highest concentrations in kernels.

In conclusion, our results indicate that there are significant differences in toxin concentrations between naturally infected barley cultivars, and significant correlations between the formation of different *Fusarium* toxins in grain by the dominant *Fusarium* species found in a region. Significant differences in toxin concentrations were also observed within the two kernel size fractions, with fraction <2.5 mm containing as much as 82% of the total toxin content of the whole sample.

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