Fusarium species and *Fusarium* toxins in wheat in Poland – a comparison with neighbour countries

Piotr Goliński [1,2], Juliusz Perkowski [1], Marian Kostecki [1], Jadwiga Grabarkiewicz-Szczęsna [1] & Jerzy Chełkowski [2]

[1] Department of Chemistry, University of Agriculture, ul. Wojska Polskiego 75, 60-625 Poznań, Poland
[2] Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszyńska; 60-479 Poznań, Poland

Goliński, P., J. Perkowski, M. Kostecki, J. Grabarkiewicz-Szczęsna & J. Chełkowski (1996). *Fusarium* species and *Fusarium* toxins in wheat in Poland – a comparison with neighbour countries. – Sydowia 48 (1): 12–22.

Fusarium species and their toxic metabolites were identified in 78 samples of wheat (6 cultivars), originating from 13 localities of various climatic areas of Poland. Ten percent of all fungal isolates were *Fusarium* spp. The most common were *F. poae* (64%), *F. tricinctum* (15%), *F. avenaceum* (8%) and *F. culmorum* (6%). Chemical analysis using HPLC and GC/MS revealed the presence of deoxynivalenol in 50% of the samples with the highest level of contamination (x_{max} = 102 μg/kg, mean toxin concentration for positive samples $\bar{x}$ = 21 μg/kg and distribution of positive results: < 10 μg/kg – 33%; ≥ 10, < 100 μg/kg – 64%; ≥ 100 μg/kg - 3%). Nivalenol was present in 30% of samples (x_{max} = 99 μg/kg; $\bar{x}$ = 34 μg/kg; < 10 μg/kg – 13%; ≥ 10, < 100 μg/kg – 87%). Moniliformin was identified in 62% of samples (x_{max} – 495 μg/kg; $\bar{x}$ = 182 μg/kg; < 100 μg/kg = 21%; ≥ 100, < 300 μg/kg – 62% and ≥ 300 μg/kg – 17%). The profile of toxic metabolites was similar and the concentration levels of the toxins were lower when compared to already publised data of the same climate zone. This is the first, complete report on the natural contamination of wheat with *Fusarium* toxins in Poland.

Keywords: cereals, contamination, *Fusarium*, trichothecenes, toxic secondary metabolites, toxins.

Fusarium spp. are common in agricultural environments. Species dominating in cereals vary from season to season depending on the geographical localization and climate conditions (Perkowski, 1993). The most aggressive and toxigenic species, *F. graminearum,* is cosmopolitan while *F. culmorum* is rather typical for temperate and cold climate. Both species are common in Central and North Europe.

In Poland, because of the significant variations in weather conditions, the frequency of occurrence in cereals of some *Fusarium* spp., among other fungi infesting heads of cereals, is variable (Chełkowski, 1989; Kiecana & al., 1988, 1994; Perkowski & Chełkowski, 1993; Perkowski, 1993). The occurrence of *F. graminearum* and *F. culmorum* in cereals, and especially in wheat, of Polish origin represents a high risk of cereals contamination with toxic secondary metabolites and consequently toxicity of food and feed products.

Reports over one decade on the world-wide contamination of cereals with *Fusarium* toxins have been examined. Inspection of the available, published data (Tab. 1) shows that in Central and North Europe deoxynivalenol (DON) and its acetylated derivatives (3-AcDON and 15-AcDON), nivalenol (NIV) and zearalenone (ZON) are the most common toxic metabolites present in ceral grain (Müller & Schwadorf, 1993; Müller & al., 1993; Lepschy-v. Gleissenthal & al., 1989; Tanaka & al., 1990; Sundheim & al., 1998; Hietaniemi & Kumpulainen, 1991; Tanaka & Ueno, 1989; Ueno & al., 1985).

The aim of this paper was to examine the occurrence of selected toxic metabolites in wheat grain produced in various climatic areas of Poland and to compare contamination of the same six cultivars of wheat in different localities.

Material and methods

Samples of wheat grain of the same six cultivars (Alba, Almari, Begra, Gama, Kamila and Parada) of the 1993 harvest were collected at experimental stations in 13 localities of different climatic zones of Poland. The sites were representative of regions of the country with various level of economy/agriculture. The samples did not show any indication of fusariosis (scab). Average data indicating weather conditions (temperature, precipitation and humidity) were collected.

Fusarium spp. were isolated using to the method described by Nirenberg (1985) and identified according to Nelson & al. (1983).

Trichothecenes (DON, 3-AcDON, NIV) were extracted and purified according to the method described by Tanaka & al. (1985). Before HPLC analysis extracts were additionally purified on type 213 charcoal columns (# 930907, Romer Labs, Inc., Union, MO 63084, USA). Columns were preconditioned with acetonitrile-water (84:16 v/v), the same solvent which was used to elute the metabolites off the column.

DON, 3-AcDON and NIV were analysed using HPLC and a Shimadzu LC-6A Liquid Chromatograph with 20 µl loop; the C-R4A Chromatopac Bondapac C 18, 3.9 x 300 mm HPLC Column and a Schimadzu SPD-6A Spectrophotometric Detector at $\lambda = 229$ nm were used. DON and NIV were eluted off the HPLC column with 20% water solution of methanol after retention time 5.88 and 9.88 min respectively, while 3-AcDON, in separate analysis, was detected after 5.35 min when 35% MeOH (aq) was used as a solvent.

Tab. 1. – Natural occurrence of ZON, DON and NIV in wheat in European countries in climate zone similar to those found in Poland (selected data since 1985).

	ZON			DON			NIV		
Country	%	$\bar{x}$[a] (µg/kg)	x_{max}[a] (µg/kg)	%	$\bar{x}$ (µg/kg)	x_{max} (µg/kg)	%	$\bar{x}$ (µg/kg)	x_{max} (µg/kg)
Germany	11–80	3.2–179	6–8036	69–96	152–3960	1187–43800	26–42	9–43	3–290
Holland	54	45	174	100	115	231	92	38	203
Norway	15	3	4	50–92	307–420	1571–3193	100	58–60	150–887
Finland	5–29	22–39	32–43	93–100	81–170	356–700	ND[b]		
Sweden				0–33	ND–161	35–390	0–24	ND–85	ND–140
World Wide	34	25		45	438		55	118	
Poland				32	116	390	88	50	350
Poland (Own Results)				50	21	102	30	34	99

[a] $\bar{x}$ and x_{max} – average and the highest concentration of metabolite
[b] ND – not detected

The presence of DON and NIV were confirmed by GC-MS according to Schwardorf & Müller (1991) on a HP 5972 benchtop mass spectrometer linked to a 5890 Series II gas chromatograph.

Extraction, purification and analysis of MON.

Samples of wheat (ca 5 g) were extracted with 25 ml of acetonitrile-methanol-water (16:3:1 v/v/v), the filtrate was evaporated, dissolved in 1 ml of methanol-water (1:1 v/v), applied on a column containing 1.5 g of previously activated (2h, 110 C) Florisil and conditioned with acetonitrile (5 ml) followed by chloroform (5 ml). Impurities were washed off the column with 5 ml of chloroform followed by 5 ml of acetonitrile. MON was eluted with 5 ml of water and analysed by HPLC with Waters 501 chromatograph; 20 μl loop; C 18 Nova Pak HPLC column (3.9 x 159 mm) coupled with a UV Waters 486 detector at λ = 229 nm and solvent [water-acetonitrile (85:15 v/v)] – [1M KH_2PO_4/ H_2O - tetrabutylammoniumhydroxide/H_2O (2:1 v/v)] (100:1 v/v). MON was eluted off the HPLC column and detected after retention time 5.5 min (flow rate 0.7 ml/min).

Results and discussion

Species of *Fusarium* in the examined wheat samples represented 10% of all fungi infesting the heads of the cereals. *F. poae* (64%), *F. tricinctum* (15%), *F. avenaceum* (8%), *F. culmorum* (6%) and *F. graminearum* (4%) were the most common.

Two of the analysed trichothecenes (DON, NIV) and the mycotoxin MON were found in 50%, 30% and 62% of the samples (Tab. 2). We did not found 3-AcDON in the extracts of the wheat samples. Taking into consideration the rather dry weather conditions experienced during the 1993 vegetation period, the frequency of positive samples can be considered as high. In contrast, the average

Table 2. – Natural contamination with DON, NIV and MON of 6 wheat cultivars collected in 13 localities in Poland (1993 crop).

Toxin	Samples positive/analyzed (%)	Level of contamination (μg/kg) Range	Average[a]
DON	39/78 (50%)	ND[b] - 102	21
NIV	23/78 (30%)	ND - 99	34
MON	48/78 (62%)	ND - 495	182

[a] Average of positive samples
[b] ND – not detected

level of positive samples contamination (21, 34 and 182 µg/kg with maximum concentrations of 102, 99 and 495 µg/kg, respectively) for DON, NIV and MON is low and from a toxicological point of view not significant. Distribution of positive results (Tab. 3) indicates that most wheat samples (64% for DON and 87% for NIV) were contaminated in the range of concentration ≥ 10, < 100 µg/kg while

Tab. 3. – Distribution of positive results in naturally contaminated wheat samples.

Toxin	Concentration range (µg/kg)	Percent of samples	
DON	< 10	33 %	(13/39)
	≥10, <100	64 %	(25/39)
	≥ 100	3 %	(1/39)
NIV	< 10	13 %	(3/23)
	≥ 10, < 100	87 %	(20/23)
MON	< 100	21 %	(10/48)
	≥ 100, < 300	62 %	(30/48)
	≥ 300	17 %	(8/48)

62% of the positive samples contained MON in the range of contamination ≥ 100, < 300 µg/kg. On the base of the present results we tried to answer which wheat cultivars exhibited the lowest contamination with toxic metabolites and which one the highest. Grain of cultivar Gama, followed by Begra, contained the lowest amounts of toxins, while Parada cultivar had the highest contamination (Tab. 4). In 1994, however, as a result of an inoculation experiment with *F. avenaceum*, Begra was one of the cultivars most

Table 4. – Comparison of the average[a)] results with wheat cultivars exhibiting the lowest and the highest natural contamination with DON, NIV and MON.

Toxin	Cultivar	Gama	Average[a]	Parada
DON	%[b)]	31	50	77
	$\bar{x}$ (µg/kg)[c]	18	21	20
	x_{max} (µg/kg)[c]	48	102	41
NIV	%	23	30	39
	$\bar{x}$ (µg/kg)	23	34	40
	x_{max} (µg/kg)	33	99	90
MON	%	39	62	77
	$\bar{x}$ (µg/kg)	129	182	226
	x_{max} (µg/kg)	264	495	390

[a] Average results represent data of all analysed samples
[b] % – percentage of positive samples
[c] $\bar{x}$ and x_{max} - average and the highest concentration of metabolite

resistant to infection and MON formation, while Gama was the most susceptible one, with the highest level of MON concentration. In contrast the cultivar Parada, with the highest level of moniliformin in this screening, was rather resistant to scab and MON accumulation after inoculation with *F. avenaceum* (Kostecki & al., 1995).

We did not found clear relationships between locality and frequency of samples contamination, but most localities with lower contamination rates were in the north-central part of Poland (Białogard) while the highest contamination with MON was seen in samples from the south-central part of the country (Przecław) (Tab. 5).

The results confirm our earlier findings indicating that *F. culmorum* and *F. graminearum* belong to the most common microorganisms in Poland. The country-wide occurrence of *F. poae* is

Table 5. – Comparison of the average[a] results with localities exhibiting the lowest and the highest natural contamination of wheat samples.

Toxin	Locality	Białogard	Average[a]	Pawłowice
	%[b]	50	50	83
DON	$\bar{x}$ (µg/kg)[c]	10	21	21
	x_{max} (µg/kg)[c]	14	102	29
	%	50	30	33
NIV	$\bar{x}$ (µg/kg)	19	34	41
	x_{max} (µg/kg)	30	99	57
	%	0	62	100
MON	$\bar{x}$ (µg/kg)	ND[d]	182	280
	x_{max} (µg/kg)	ND	495	390

[a, b, c] see footnotes Table 4
[d] ND – not detected

noteworthy and can be an explanation for the high frequency of NIV positive wheat samples in this screening. The potential for production of NIV by *F. poae* has been described by Petterson (1993). Results of chemical analysis confirm earlier data on the presence of *Fusarium* toxins in cereals in Poland. Since 1985 the following cereal samples were collected and screened for toxin presence: scabby cereal samples from farms; field samples of cereals naturally contaminated but with visible symptoms of fusariosis (field scabby samples); samples artificially inoculated with *F. culmorum* and/or *F. graminearum*. Until now the following toxic metabolites were determined in the samples: ZON, DON, 3-AcDON, 15-AcDON, NIV, 4,7-dideoxyNIV, fusarenone-X, MON, T–2 toxin, HT–2 toxin and fumonisins (B_1, B_2, B_3) (Chełkowski & al., 1989, 1990, 1992; Lew & al., 1993; Logrieco & al., 1993; Perkowski & al., 1988, 1990a, 1990b, 1990c, 1991, 1992,

Table 6. – Occurrence of DON in cereals in Poland since 1985.

	Selected natural samples (scabby samples)			Inoculated samples		
Cereal	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)
Wheat	100	2.7–38	14.5	90	ND[b]–38	7.0
Triticale	100	1.6–16.4	8.1	100	1.5–53	18.2
Rye				100	0.7–28	10.8
Barley				100	1.4–178	40.5
Corn	100	5.7–109	50			

[a] Average of positive samples
[b] ND - not detected

Tab. 7. – Occurrence of 3-AcDON and 15-AcDON in cereals in Poland (since 1985).

Cereal	Selected natural samples (scabby samples) 3-AcDON			15-AcDON			Inoculated samples 3-AcDON			15-AcDON		
	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)
Wheat	88	ND[b]–5.6	1.05	100	0.1–2.0	0.73						
Triticale	100	0.6–2.4	1.40				80	ND–16	4.00			
Rye							100	0.2–3.4	1.18			
Barley							96	ND–29	5.20	17	ND–2.5	1.3
Corn	20	ND–0.3	0.30	80	ND–3.5	2.70						

[a] Average of positive samples
[b] ND – not detected

Tab. 8. – Occurrence of NIV and ZON in cereals in Poland (since 1985).

Cereal	Selected natural samples (scabby samples)						Inoculated samples					
	NIV			ZEA			NIV			ZEA		
	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)	%	Range (mg/kg)	Average[a] (mg/kg)
Wheat	33	ND[b]–0.01	0.01	47	ND–2.0	0.60						
Triticale										40	ND–5.0	2.400
Rye										100	0.02–0.15	0.068
Barley							46	ND–0.6	0.27	100	0.1–0.9	0.330
Corn				60	ND–0.9	0.73						

[a] Average of positive samples
[b] ND – not detected

1993, 1995). Taking into consideration the prevalence of pathogenic/toxigenic microorganisms and the frequency of positive samples with average and maximum levels of contamination DON, 3-AcDON, 15-AcDON, NIV, ZON and MON were found to be the most significant *Fusarium* toxins in cereals of Polish origin (Tab. 6–8).

Comparison with neighbouring countries reveals that the frequency of cereals contamination with *Fusarium* toxins as well as their average and maximum levels of concentration are lower in Poland than in other countries (Tab. 1). On the other hand, it should be pointed out that the contamination of cereals (including wheat) was investigated in different crop years, under different weather conditions.

Acknowledgments

We thank Anna Matysiak, Katarzyna Pauszek, Iwona Rissmann and Ewa Rymaniak for skillful technical assistance. The authors appreciate very much the significant contributions by Ewa Rymaniak and Iwona Rissmann during the technical preparation of this manuscript.

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(Manuscript accepted 14th October 1995)