Concentrations of Ergosterol and Trichothecenes in the Grains of Three *Triticum* Species

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Abstract

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The concentrations of ergosterol, type A trichothecenes (HT-2 toxin, T-2 tetraol and scirpentriol), and type B trichothecenes (deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenone X) were determined in the grains of three wheat winter cultivars of *Triticum aestivum, T. spelta*, and *T. durum*. The highest concentrations of ergosterol (3.3×10^4 μg/kg) and deoxynivalenol (654.67 μg/kg) were noted in the grain of *T. durum*. Ergosterol concentrations did not decrease following the fungicide application. The results of the principal component analysis showed that the quantitative and qualitative profiles of toxic metabolites in *T. durum* differed significantly from those obtained for the remaining two wheat species. A strong correlation between the concentrations of ergosterol and deoxynivalenol ($r = 0.920$) indicated the predominance of pathogenic fungi of the genus *Fusarium* in wheat grain.

Keywords: ergosterol; principal component analysis; trichothecenes; *Triticum aestivum*; *Triticum durum*; *Triticum spelta*

Wheat species of the genus *Triticum* other than the common wheat (*Triticum aestivum* L.) enjoy growing popularity due to the high nutritive value of their grains and products, high processing suitability, and resistance to unsupportive environmental factors (Abdel-Aal *et al*. 1995; Stallknecht *et al*. 1996). Spelt (*Triticum spelta* L.) occupies a special place in this group of wheat species, and a revival of spelt farming is observed not only in Europe, but also in North America (http://www.agmrc.org/commodities__products/grains__oilseeds/spelt.cfm). Spelt greatest disadvantage, namely that its grain cannot be threshed due to glume adherence and brittle rachis, is compensated for by a relatively high grain yield with high contents of minerals, protein, and components with health-promoting properties (Rozenberg *et al.* 2003; Ruibal-Mendieta *et al.* 2005). This wheat species is characterised by low soil and climatic requirements as well as a good health status (Mielke & Rodemann 2007). Spelt is processed into bread flour, cereals, and groats, and is also used to produce pasta whose quality comes only second to durum wheat (*Triticum durum* L.) pasta (Marconi *et al*. 1999).

In 2009, the global output of *T. durum* accounted for around 4.9% of the production of common wheat (31 919 mio. MT vs. 646 504 mio. MT). The leading suppliers of durum wheat are Canada, Italy, US,

and the European Union which produce around 30% of durum wheat grain worldwide (www.pecad.fas.usda.gov, www.fas.usda.gov/psdonline/). The processing suitability of spring cultivars of durum wheat is significantly higher than that of winter cultivars, which is why the spring cultivars play a predominant part in the wheat crop structure in Europe. In the early 1980s, winter durum wheat was introduced on a broad scale into Hungary (Beke & Barabas 1981) where the yield of winter varieties is only 15–30% lower in comparison with the most productive winter varieties of the common wheat, while the output of spring varieties of *T. durum* is roughly half that of the common wheat (Bojarczuk 2006). The shift of farming regions of winter durum wheat in the northern direction requires varieties that produce high and stable yields and are resistant to adverse environmental factors, including pathogenic fungi. The program promoting the cultivation of winter *T. durum* varieties, initiated in Poland in the 1990s, gave rise to high-yielding cultivars that are well adapted to the soil regime and the climate of Central-Eastern Europe and show a high degree of resistance to freezing temperatures.

The nutritive value and processing suitability of wheat grain often deteriorates due to the accumulation of toxic fungal metabolites produced mainly by fungi of the genus *Fusarium*, the causative agent of Fusarium head blight (FHB). Fusariotoxins are responsible for various metabolic disorders in humans and animals, and high toxin levels in grain cause diseases known as mycotoxicoses (Richard 2007). The largest group of fusariotoxins is trichothecenes, including type A trichothecenes (mainly T-2 toxin, HT-2 toxin, diacetoxyscirpenol) and type B trichothecenes (mostly deoxynivalenol and acetyl derivatives of this toxin and nivalenol), produced by the most toxigenic pathogens of the genus *Fusarium*, such as *Fusarium culmorum* (W.G.Smith) Sacc., *Fusarium graminearum* Schwabe, *Fusarium poae* (Peck) Wollenw. and *Fusarium sporotrichioides* Sherb., which are encountered in nearly all climate zones and all grain cultivation regions across Europe and the rest of the world as well (Ueno 1977; Bottalico & Perrone 2002). The growing share of grain crops in the cropping system intensifies the level of pathogen infestation responsible for FHB, thus increasing the risk posed by their toxic metabolites. Fungicides do not deliver the desired level of protection as these pathogens are resistant to the active substance, and they affect plants at the full flowering stage (Parry *et al.* 1995; Champeil *et al*. 2004). The significant risk posed by grain mycotoxins can be minimised only through the cultivation of resistant varieties. Wheat resistance to FHB is polygenic (Buerstmayr *et al*. 2009), and various types of resistance are manifested at different stages of pathogenesis and kernel development (Mesterházy *et al*. 1999). In comparison with the common wheat, *T. durum* is more susceptible to infestation with pathogens of the genus *Fusarium*, therefore, its grain is more exposed to higher mycotoxin concentrations (Stack *et al*. 2002). EU regulations (Commission Recommendation 2006/583/EC) allow higher DON concentrations in raw grain of durum wheat (1500 µg/kg) than in the grain of the common wheat (1250 µg/kg). The most reliable indicator of fungal biomass content in plant tissue is ergosterol (ERG). Ergosterol is found in fungal cell walls and is not a product of plant metabolism (Ekblad *et al*. 1998). Its concentrations in the plant tissues and organs, including cereal grain, are strongly correlated with the number of fungal cells.

The objective of this study was to compare fusariotoxin and ergosterol concentrations in the grains of the spelt and durum wheat cultivars and in the grain of the high-yielding common wheat cultivar at different fungicide application levels.

MATERIALS AND METHODS

Samples. The experimental materials comprised three cultivars of three different winter wheat taxa: cv. Olivin (common wheat – *T. aestivum*, the seed material was supplied by RAGT Semences Poland Ltd., a business representative of R2n SAS, France), cv. Schwabenkorn (spelt – *T. spelta*, the seed material was reproduced at the Department of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, Poland), and cv. Komnata (durum wheat – *T. durum*, the seed material was supplied by the Plant Breeding Station in Smolice, Poland, where maintenance breeding is carried out). Cv. Olivin has very good bread-making quality ("A" quality group according to the classification of the Research Centre for Cultivar Testing, List of Agricultural Cultivars 2009).

Field experiment. The analysed grain samples, each weighing 250 g, were obtained in a precise factorial experiment conducted in the growing

season of 2008/2009 at the Production and Experimental Station in Bałcyny (53°36'N, 19°51'E), University of Warmia and Mazury in Olsztyn (Poland). Wheat grain, naturally infected by fungal pathogens, was harvested following the treatments differing with respect to the level of fungal disease control (Table 1). To limit lodging, Moddus 250 EC (trinexapac-ethyl, 250 g/l, Syngenta Crop Protection, Warsaw, Poland) was applied in the treatments with full fungicide control (level 2) at BBCH 37/39 (Growth Stages..., 2001).

The forecrop for wheat was winter rapeseed. A total of 450 germinating kernels were sown per m^2 of the experimental plots with a surface area of 15 m^2. Wheat growing and harvesting was performed in accordance with good agricultural practice standards.

Ergosterol determination. The samples were analysed for the presence of ERG according to Perkowski *et al*. (2008). Briefly, the samples of 100 mg were placed into 17 ml culture tubes, suspended in 1 ml of methanol, treated with 0.1 ml of 2M aqueous NaOH, and tightly sealed. Then the culture tubes were placed within 250 ml plastic bottles, tightly sealed, and placed inside a microwave oven (Whirpool model AVM 401/WH) operating at 2450 MHz and 900 W maximum output. The samples were irradiated (370 W) for 20 s, after 5 min for additional 20 s, and were then extracted with pentane (HPLC grade; Sigma-Aldrich, Steinheim, Germany) (3 × 4 ml) within the culture tubes. The combined pentane extracts were evaporated to dryness in a gentle stream of high purity nitrogen. Prior to analysis, the samples were dissolved in 4 ml of methanol, filtered through 13 mm syringe filters with a 0.5 µm pore diameter (Fluoropore Membrane Filters), evaporated to dryness in a stream of nitrogen and dissolved in 1 ml of methanol. The prepared samples were analysed by HPLC. The separation was run on a 150 mm length × 3.9 mm diameter Nova Pak C-18, 4 µm particle size column, and the samples were eluted with methanol/acetonitrile (90:10) at a flow rate of 0.6 ml/min. ERG was detected with a Waters 486 Tunable Absorbance Detector set at 282 nm. The estimation of ERG concentrations was performed by comparing the obtained peak areas with those of the external standard (> 95%; Aldrich, Milwaukee, USA) or by co-injection with a standard. The detection limit was 0.01 mg/kg.

Trichothecene analysis. Subsamples of 10 g each were used for the analysis of each toxin. All the subsamples were prepared in the same way. They were ground in a WŻ-1 laboratory mill (Research Institute of the Baking Industry Ltd., Bydgoszcz, Poland), designed for cereal samples grinding.

Determination of type A trichothecenes. The samples were extracted overnight with 100 ml acetonitrile-water solvent (82:18, v/v) and filtered (Whatman No. 5 filter paper). The extracts were then purified on a 5 ml column of mixed alumina (neutral activated, 70–230 mesh; Merck, Darmstadt, Germany), Darco G 60 – charcoal (100 mesh; Aldrich, Steinheim, Germany), and Celite 545 (Serva, Heidelberg, Germany) 4:3:4 (w/w/w). The extracts were evaporated to dryness in a rotary evaporator. The residue was dissolved using two aliquots of 2 ml ethyl acetate and 2 ml chloroform-acetonitrile (4:1, v/v).

Type A trichothecenes (HT-2 toxin, T-2 tetraol, and scirpentriol – STO) were analysed as trifluoroacetic anhydride (TFAA) derivatives, 100 µl

Table 1. Chemical disease control applied in the experiment. Due to the high severity of yellow rust, durum wheat was treated with Tilt 400 EC[4] + Amistar 250 EC[5]

Disease control level	Chemicals applied in the experiment (doses recommended by the Institute of Plant Protection, National Research Institute, Poznań, Poland)
0 (low)	seeds were dressed with Funaben T[1] (applied pre-sowing)
1 (medium)	seeds were dressed with Funaben T (applied pre-sowing) Alert 375 SC[2] (BBCH 30/35)
2 (high)	seeds were dressed with Funaben T (applied pre-sowing) Alert 375 SC (BBCH 30/35); Artea 330 EC[3] (BBCH 70)

[1]thiuram – 29.6%, carbendazim 13.2% (Zakłady Chemiczne „Organika –Azot" S.A., Jaworzno, Poland)

[2]flusilazol – 125 g/l, *carbendazim* 250 g/l (DuPont, Warsaw, Poland)

[3]cyproconazole – 7.1%, propiconazole – 22.3% (Syngenta Crop Protection, Warsaw, Poland)

[4]propiconazole – 13.1%, fenpropidin – 28.9% (Syngenta Crop Protection, Warsaw, Poland)

[5]azoxystrobin – 22.9% (Syngenta Crop Protection, Warsaw, Poland)

of TFAA was added to the dried sample. After 20 min, the reacting substance was evaporated to dryness under nitrogen. The residue was dissolved in 500 µl isooctane, and 1–2 µl was injected onto a gas chromatograph-mass spectrometer (Hewlett Packard GC 6890, MS 5972 A, Waldbronn, Germany).

The HP-5MS capillary column (0.25 mm × 30 m) was used. The injection port temperature was 280°C, the transfer line temperature was 280°C, and the analysis was performed at the programmed temperature (from 80°C at 1 min to 280°C at 25°C/min), the final temperature being kept for 10 minutes. The helium flow rate was constant at 0.7 ml/minutes. Each sample was run twice: in full scan mode (*m/z* 100–600) for the identification, and in selected ion-monitoring (SIM) mode for quantification in comparison with type A trichothecene standards supplied by Sigma (St. Louis, USA). The following ions were used for trichothecene detection: HT-2 toxin – *m/z* 455, 327; T-2 tetraol – *m/z* 455, T-2 toxin – *m/z* 327, 401, 568; STO – *m/z* 456, 555, DAS – *m/z* 402, 374. The first ion in each set was used for quantification. The detection limit was 1 µg/kg. The average recoveries ($n = 9$) of the toxins determined by the above method were: 91 ± 3.2% for HT-2, 88 ± 4.0% for T-2 tetraol, 86 ± 3.8% for T-2 toxin, 84 ± 4.6% for DAS, and 82.3 ± 3.8% for STO, respectively.

Determination of type B trichothecenes. The samples placed in 200 ml Erlenmeyer flasks were extracted using a 100 ml acetonitrile-water mixture (82:18 v/v) by shaking for 15 minutes. Then they were left for 12 h and the shaking was repeated for 15 minutes. The extracts obtained were filtered through Whatman No. 5 cellulose filter on Büchner funnel under reduced pressure.

The extracts were purified by extraction to the solid phase using columns packed with a (5 ml) mixture of active carbon (Draco G 60, 100 mesh), Celite 545, and neutral aluminum oxide (70–230 mesh) at a weight ratio of 1:1:1.

Six ml polypropylene columns were packed successively with glass wool, 0.25 g Celite 545, a 3 g mixture of active carbon, Celite 545, and neutral aluminum oxide, followed by glass wool. The prepared columns were activated by rinsing with 15 ml of an acetonitrile-water mixture (82:18 v/v) under reduced pressure at a flow rate of 1 ml/minute. The flasks were emptied onto the columns, and the filtered extracts were introduced. Next, the columns were washed with 30 ml of an acetonitrile-water mixture (82:18 v/v). Both combined filtrates were collected and evaporated to dryness by means of a vacuum evaporator (Büchi R-205). The residue was transferred quantitatively using ethyl acetate (2.5 ml) and two portions of 2.5 ml each of a chloroform-acetonitrile mixture (4:1 v/v) to 8 ml vials by evaporating on the on-going basis in a stream of nitrogen.

Type B trichothecenes (deoxynivalenol – DON, 3-acetyldeoxynivalenol – 3-AcDON, 15-acetyldeoxynivalenol – 15-AcDON, nivalenol – NIV, fusarenone X – FX) were analysed as trimethylsilyl derivatives using an external standard. Trimethylsilyl derivatives were obtained through reaction with a mixture of trimethylsilyl imidazole and trimethylchlorosilane (100:1 v/v, 100 µl) run in a 8 ml vial at room temperature for 20 minutes. The reaction was terminated by adding 3 ml distilled water and 0.5 ml isooctane to the reaction mixture. The mixture was shaken in a shaker (MS 1; Ika-Works, Inc., Wilmington, USA) and the isooctane layer was transferred into a tightly sealed vial. Chromatographic separation and the analysis of type B trichothecenes were run using a gas chromatograph (Hewlett Packard 6890) coupled with a mass detector (Hewlett Packard 5972 A). The apparatus was equipped with an autosampler (HP 18593B) and a capillary column (HP-5MS, 0.25mm × 30 m). Samples of 1 µl were injected into the injector chamber at 280°C without stream division, at the separator temperature of 280°C. In the assay of type B trichothecenes, the initial oven temperature was 80°C and it was maintained for 1 minute. Further, the temperature was increased by 15°C/min to 200°C and it was maintained at that for 6 minutes. After that time, the temperature was increased by 10°C/min to 280°C and it remained at that level for 5 minutes. The total time of analysis was 28 minutes. Selective ion monitoring (SIM) was carried out for: DON ions 103 and 512, 3-AcDON 117 and 482, 15-AcDON 193 and 482, FX 103 and 570, NIV 191 and 600. The retention times for the above toxins were 19.53, 20.88, 21.07, 21.01 and 21.25 min, respectively. The flow rate for helium was 0.7 ml/minutes. In order to confirm the presence of the assayed toxins in the sample, an analysis was conducted over the entire range of weights (100–700 amu) yielding the mass spectrum which was compared with the analogously obtained spectrum for the standard. This spectrum together with the comparisons of the retention times for the analysed

compounds with the standard constituted the basis for the toxin identification. Apart from qualitative analysis, the concentrations of the analysed toxins were determined by comparing the relative heights of the selected ions. The results were subjected to processing in the ChemStation program. The recovery rates for the analysed toxins were as follows: DON 84 ± 3.8%, 3AcDON 78 ± 4.8%, 15 AcDON 74 ± 2.2% and NIV 81 ± 3.8%, respectively. Detection limit for each of the analysed toxins was 1 µg/kg.

Statistical analysis. The results of laboratory analyses were processed statistically using STATISTICA software (Statsoft Inc. 2008). The significance of differences between the mean values was estimated by analysis of variance, and these were compared by the Student-Newman-Keuls (SNK) test. The data were subjected to the principal components analysis (PCA) whose results are presented in graphic form. A simple correlation analysis was performed for ERG and the mycotoxins found in the grains of all tested wheat species.

RESULTS

Growing season

The temperature and moisture conditions in the fall and winter promoted the growth of winter wheat and plant tillering before the winter dormancy, thus supporting the winter survival. An acute drought was observed in April (monthly precipitation of 6.7 mm) and May (total precipitation in the first two weeks of May – 12.3 mm) which weakened the stand density in all crops and visibly shortened the shooting and milk-dough stages. Intense precipitation was reported in June (monthly total of 144.1 mm) and July (monthly total of 82.2 mm), i.e. the months covering the flowering stage (BBCH 65), the milk-dough stage (BBCH 75), and the early dough stage (BBCH 83). The mean temperatures reached 14.7°C in June and 18.9°C in July. The mean temperatures for June and July between 1961 and 2005 were 15.8°C and 17.2°C, respectively. The noted weather conditions supported the development of the head diseases, in particular *Fusarium* head blight (FHB). Durum wheat was also significantly affected by yellow rust.

A visual inspection of the head infestation levels revealed significant differences in disease intensity between the wheat cultivars studied. None of the applied fungicide regimes had a significant preventive effect on FHB (Table 2).

ERG and trichothecene toxin concentrations in the grain of the investigated wheat cultivars are presented in Table 3. Durum wheat was marked by the highest ERG levels (3.3×10^4 µg/kg) which were more than 20-fold higher than in spelt and nearly seven-fold higher than in common wheat. The above points to the lowest fungal biomass levels in the grain of *T. spelta*, and the highest levels in the grain of *T. durum*. The analysis revealed the presence of three type A trichothecene toxins (HT-2 toxin, T-2 tetraol, STO – T-2 toxin and DAS were not detected) and five type B trichothecene toxins (DON, 3Ac-DON, 15Ac-DON, NIV, FX) in the wheat cultivars studied. The highest metabolite concentrations were determined in durum wheat grain. DON levels exceeded 888 µg/kg in durum wheat, and they were nearly 73-fold higher in comparison with those in spelt (9 µg/kg). NIV levels were marked by a similar trend – the concentrations of this toxin in *T. spelta* grain (3.25 µg/kg on average) were 34-fold lower than in *T. durum* grain (112.4 µg/kg). Although noted in the grain of *T. durum*, the presence of highly toxic HT-2 and FX metabolites was not observed in spelt and common wheat.

Table 2. Average levels of head infection (FHB) by pathogens of the genus *Fusarium* in the examined wheat species at three levels of chemical disease control

Disease control level	*T. spelta* (cv. Schwabenkorn)	*T. aestivum* (cv. Olivin)	*T. durum* (cv. Komnata)
0	0.8	4.3	49.7
1	2.0	5.7	48.0
2	0.8	5.1	48.4
Mean	1.2[c]	5.0[b]	48.7[a]

The values correspond to the percentage of diseased spikelets per head
[a,b,c]mean values followed by different superscript letters differ significantly at $P \leq 0.01$

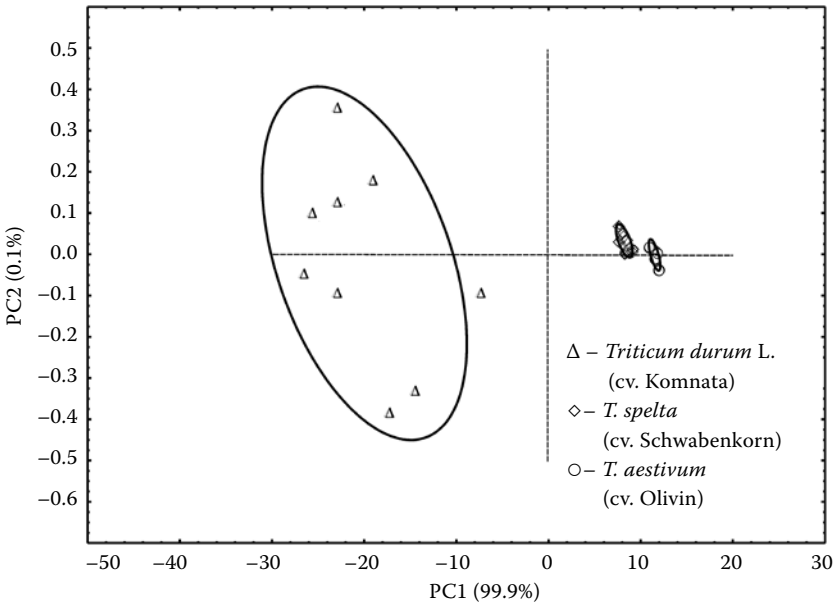

Figure 1. Scatter-plot of the principal component analysis performed for ERG and eight trichothecenes (STO, T-2 Tetraol, HT-2, DON, FX, 3-Ac DON, 15-Ac DON and NIV)

Chemical control did not significantly affect the concentrations of the analysed metabolites (Table 4), although all of the investigated toxins levels reached the lowest value at level 2 of chemical protection. In comparison with level 0 (61.83 μg/kg), the average NIV concentrations were reduced nearly three-fold (19.26 μg/kg), DON levels decreased nearly 1.8-fold, and 3-AcDON concentra-

Table 3. Concentrations (in μ/kg) of *Fusarium* metabolites in the grain of the examined cultivars of *Triticum spelta* (cv. Schwabenkorn), *T. aestivum* (cv. Olivin), and *T. durum* (cv. Komnata)

Cultivar	Metabolite	ERG	STO	T-2 Tetraol	HT-2	DON	FX	3-Ac DON	15-Ac DON	NIV
Schwabenkorn	mean	1.6×10^{3a}	0.40^{a}	9.77	< LOD	9.00^{a}	< LOD	< LOD	0.60^{a}	3.25^{a}
	max	2.2×10^{3}	2.74	19.77	–	38.71	–	–	1.88	10.88
	min (≥ LOD)	1.3×10^{3}	–	4.57	–	2.71	–	–	1.38	–
	SD	2.5×10^{2}	0.88	5.07	–	12.78	–	–	0.60	2.86
	No. of samples < LOD	–	8	–	9	4	9	9	7	8
Oliwin	mean	4.8×10^{3a}	2.12^{a}	12.30	< LOD	44.17^{a}	< LOD	1.27^{a}	< LOD	5.56^{a}
	max	5.4×10^{3}	5.82	42.55	–	69.58	–	4.24	–	16.00
	min (≥ LOD)	4.0×10^{3}	3.52	5.33	–	17.09	–	2.30	–	6.75
	SD	5.1×10^{2}	2.50	12.27	–	16.45	–	1.23	–	4.68
	No. of samples < LOD	–	5	1	9	–	9	7	9	5
Komnata	mean	3.3×10^{4b}	12.32^{b}	12.42	2.31	654.67^{b}	4.94	20.25	6.35^{b}	112.40^{b}
	max	4.0×10^{4}	24.73	39.77	7.29	888.97	12.24	35.88	10.55	366.18
	min (≥ LOD)	2.0×10^{4}	6.38	4.49	2.06	352.24	5.09	5.87	3.53	20.00
	SD	6.2×10^{3}	6.49	11.70	2.59	203.94	3.62	10.95	2.27	109.56
	No. of samples < LOD	–	–	–	4	–	5	–	–	–

Limit of detection (LOD) for each metabolite is 1 μg/kg

[a,b]mean values followed by different superscript letters differ significantly at $P \leq 0.01$

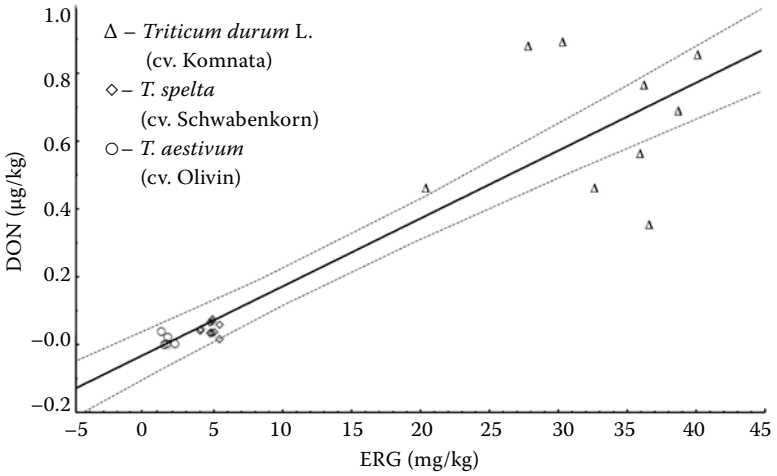

Figure 2. Scatter-plot of ERG vs. DON concentrations in wheat grain throughout the experiment. Dotted lines correspond of 0.95 confidence level

tions were marked by more than a two-fold drop. Chemical protection had no significant effect on ERG, suggesting that the applied fungicides did not inhibit the development of fungal microflora in the grain of the wheat cultivars studied.

The results of chemical analyses were subjected to the principle component analysis (PCA). A multivariate analysis, accounting for all variables (ERG and eight trichothecene toxins), led to a very clear discrimination of the three species (Figure 1). Whereas the distance between the areas mapped for *T. aestivum* and *T. spelta* proved to be insignificant, *T. durum* was separated by a long distance from the areas corresponding to the remaining wheat species. The above suggests that the analysed metabolites had a completely different profile in the

Table 4. Concentrations (in µg/kg) of *Fusarium* metabolites in the grain of the examined wheat species at three levels of chemical disease control

Disease control level	Metabolite	ERG	STO	T-2 Tetraol	HT-2	DON	FX	3-Ac DON	15-Ac DON	NIV
0	mean	1.3×10^4	4.82	12.62	0.38	307.02	3.57	10.45	3.14	61.83
	max	4.0×10^4	16.71	42.55	3.41	888.97	9.58	35.88	10.55	366.18
	min (≥ LOD)	1.5×10^3	2.74	5.00	–	5.63	5.06	2.30	1.38	8.61
	SD	1.5×10^4	5.31	12.46	–	424.31	2.41	15.43	3.76	118.92
	No. of samples < LOD	–	3	1	8	1	7	5	5	4
1	mean	1.3×10^4	6.31	10.86	1.04	231.59	3.58	6.44	2.58	40.12
	max	3.9×10^4	24.73	39.76	7.29	759.00	12.24	25.50	8.50	190.50
	min (≥ LOD)	1.4×10^3	12.62	4.48	2.06	2.71	–	5.76	3.53	6.75
	SD	1.5×10^4	9.79	11.50	2.44	313.44	–	10.67	3.11	69.93
	No. of samples < LOD	–	6	–	7	1	8	6	6	5
2	mean	1.4×10^4	3.70	11.28	0.89	169.24	2.79	4.67	2.22	19.26
	max	3.7×10^4	10.24	19.76	4.48	560.71	5.09	15.13	5.76	84.47
	min (≥ LOD)	1.3×10^3	3.52	5.45	3.50	33.41	–	4.24	1.88	7.15
	SD	1.6×10^4	3.82	4.70	1.78	222.71	–	6.21	2.07	27.06
	No. of samples < LOD	–	4	–	7	2	8	5	5	4

Limit of detection (LOD) for each metabolite is 1 µg kg

grain of durum wheat. High discrimination results were affected mostly by PC1 which explained 99.9% of total variance. The following toxins contributed most to the variance explained by PC1: DON (16.74%), 15-AcDON (16.37%), 3-AcDON (15.79%), and ERG (15.60%), and as regards PC2: T-2 Tetraol (51.57%) and HT-2 toxin (38.71%). The variance was least affected by NIV (10.42% for PC1 and only 2.47% for PC2).

A very strong positive correlation between ERG and DON levels (r = 0.920, significant at P = 0.01) was determined throughout the experiment (Figure 2). The above suggests that predominant quantities of fungal biomass differentiating the studied *Triticum* species (in particular *T. durum*) were observed in *Fusarium* sp. species producing DON. These findings were validated by the results presented in Table 3. A strong correlation was also reported between ERG and STO (r = 0.756, significant at P = 0.01).

NIV concentrations were 34-fold higher in the grain of cv. Komanta than in the grain of cv. Schwabenkorn, and 20-fold higher than in the grain of cv. Olivin, suggesting that the isolates of *Fusarium* species producing this toxin may develop dynamically in the grain of *T. durum*. Nevertheless, the correlation between NIV and ERG levels proved to be lower (r = 0.576, significant at P = 0.05) than between DON and ERG.

DISCUSSION

This study compared three cultivars of *Triticum aestivum, T. spelta* and *T. durum,* registered in several Central European countries (http://ec.europa.eu/food/plant/propagation/ catalogues/comcat_agri_2009/index_en.htm; List Of Agricultural Cultivars 2009), to determine the concentrations of ERG and trichothecene toxins in wheat grain. Following the introduction of winter *T. durum* varieties, attempts were made to expand durum wheat growing areas to reduce the costs of grain acquisition in pasta production. The problem of common wheat infestation with toxic metabolites produced by fungi of the genus *Fusarium* has been well researched as regards toxin profiles in naturally infected grain and in artificially inoculated heads (Stępień & Chełkowski 2010). Various studies have been initiated to investigate the genetic factors of resistance to FHB (Bai & Shaner 2004) and fungicide prevention options (Edwards & Godley 2010). A vast body of research has also been dedicated to the above issues in reference to durum wheat (Visconti & Pascale 2010). Fewer studies focused on spelt whose resistance to FHB and ability to accumulate fusariotoxins has been poorly investigated (Wiwart *et al.* 2004). The results of our previous studies indicated that the concentrations of trichothecenes, in particular type B trichothecenes, are lower in the grain of *T. spelta* than in common wheat in both naturally infested grain (Wiwart *et al*. 2009) and in artificially inoculated grain (Suchowilska *et al.* 2010). These findings are yet another argument promoting spelt farming, in particularly in organic and integrated crop systems. The factors which contribute to lower fusariotoxin levels in spelt grain, in comparison with common wheat grain, include longer stems, a lower spike density, and a lower stand density which decreases air humidity around the spikes and creates a less supportive environment for the growth of pathogenic fungi (Lammerts van Bueren *et al.* 2002).

ERG is the most popular indicator of fungal biomass in various plant parts, and its concentrations in cereal grain are usually correlated with fusariotoxin levels, mostly DON (Perkowski *et al.* 1995). The above has been validated by the results of this study where the coefficient of linear correlation between ERG and DON levels (r = 0.92) was nearly identical to that (r = 0.91) reported by Wiśniewska and Buśko (2005) for spring wheat. A similarly high correlation between ERG and DON concentrations (r = 0.83) was noted earlier by Abramson *et al.* (1998), thus justifying the rationale behind the analyses of ergosterol concentrations in the studies investigating fusariotoxin levels in cereal grain. To date, the allowable norms for ERG concentrations in cereal grain have not been introduced. According to Müller and Schwadorf (1993), ERG levels in wheat grain naturally colonised by fungi are in the range of 4.0 mg/kg to 35.8 mg/kg. Perkowski *et al.* (2008) have argued that the studied metabolite concentrations in grain samples of the type may reach 3.4 mg/kg. Schnürer and Jonsson (1992) claim that ERG concentrations in grain intended for consumption should not exceed 3.0 mg/kg. Ergosterol concentrations in common wheat, spelt, and durum wheat samples investigated in this experiment proved to be much higher than in the referenced studies, reaching 40 mg/kg in the grain of *T. durum* cv. Komnata and 5.4 mg/kg in the grain of *T. aestivum* cv. Olivin. The above

suggests that the grain of winter durum wheat is much more susceptible to fungal infections than the grains of common wheat and spelt, posing a high risk for consumers of durum wheat products, in particular pasta.

The guideline concentrations of DON, set at 1250 µg/kg for common wheat and 1500 µg/kg for durum wheat under The Commission Recommendation 2006/583/EC, were not exceeded in any of the grain samples tested. NIV levels were very high in excess of 366 µg/kg. This metabolite is characterised by higher cytotoxicity than DON (Fornelli *et al*. 2004; Sundstøl Eriksen *et al*. 2004), therefore, high NIV concentrations in the grain of cv. Komnata pose a high risk, especially if accompanied by the presence of FX and type A trichothecenes which are marked by high zootoxicity (Ueno 1977). The identified toxins may also exert a synergistic effect. FX, HT-2 toxin, and 3-AcDON concentrations below LOD values (1 µg/kg) in spelt grain indicate that *T. spelta* wheat ensures a higher level of consumer safety.

The applied fungicides did not produce a significant drop in the analysed metabolite levels, thus validating other authors' claims regarding the absence of effective chemical protection against FHB. The above forces the wheat producers to search for sources of genetic resistance (Mesterházy *et al.* 1999; Champeil *et al.* 2004). The reported results clearly indicate that fusariotoxin infestation could pose a serious problem in the near future as Central-European countries expand the area sown with winter durum wheat.

As one of the methods in multivariate analyses, the principal component analysis (PCA) permits a comparison of the treatments based on selected attributes. Those attributes are included in the analysis as independent variables, and they enable the discrimination of the treatments which cannot be achieved by way of the standard one-dimensional techniques. PCA reduces all variables to two principal components for which the percentage share of the explained variance is determined. Unlike multidimensional techniques such as cluster analysis, PCA identifies the contribution made by each variable separately for both components. The results indicating that type B trichothecenes and ERG made the greatest contribution to the variance explained by PC1, and type A trichothecenes – to PC-2 variance, are interesting because the above toxin groups are produced by different *Fusarium* species. Significant disproportions in variance explained by PC1 and PC2 (99.9% vs. 0.1%) constitute an indirect proof that the grain of the analysed cultivars was nearly entirely colonised by species producing type B trichothecenes. In the studies of the type, PCA not only discriminates the varieties of different wheat taxa, but it also contributes to our knowledge about the share of grain-colonising *Fusarium* species characterised by different toxin production profiles.

CONCLUSIONS

The results of this study suggest that the cultivation of winter durum wheat in Poland faces a significant risk of contamination with toxic metabolites produced by fungi of the genus *Fusarium*. In the grain of the tested *T. durum* cultivar, DON concentrations (654. 7 µg/kg) were 73-fold higher, and NIV levels (112.4 µg/kg) – 34-fold higher than in *T. spelta*. The ERG content of *T. durum* grain (3.3×10^4 µg/kg) was more than 20-fold higher than that of spelt and nearly seven-fold higher in comparison with common wheat. PCA results demonstrate that the grains of the studied wheat species were colonised predominantly by fungi producing type B trichothecenes. Significant differences were observed in trichothecene profiles in the grains of the three analysed wheat cultivars belonging to three different taxa. The present results validate the previous findings regarding spring spelt, showing that the concentrations of fungal metabolites are lower in spelt than in common wheat. The above constitutes an additional argument supporting the cultivation of *T. spelta* not only in Central Europe, but also in other regions of our continent.

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