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Organic farming does not allow the use of conventional mineral fertilizers and crop protection products. As a result, in our experiments we chose to grow different species of cereals and to see how cereal species affect mycotoxin accumulation. This study describes the occurrence of deoxynivalenol (DON), zearalenone (ZEA) and T-2/HT-2 toxin in a survey of spelt and common wheat and their bran as well as flour. The analysis was conducted using an enzyme-linked immunosorbent assay (ELISA) method. The concentrations of DON, ZEA and T-2/HT-2 in *Triticum spelta* and *T. aestivum* were influenced by species, cereal type and year interaction. The highest concentrations of these mycotoxins were found in spelt grain with glumes, in spelt glumes and in spring wheat. These results show significantly higher concentrations of *Fusarium* toxins in glumes than in dehulled grain, which indicates the possible protective effect of spelt wheat glumes. The lowest DON, ZEA and T-2/HT-2 concentrations were determined in spelt grain without glumes. The research shows that it is potentially risky to produce bran from grain in which mycotoxin concentrations are below limits by European Union Regulation No. 1881/2006, since the concentration of mycotoxins in bran can be several times higher than that in grain. As a result, although bran is a dietary product characterised by good digestive properties, it can become a harmful product that can cause unpredictable health damage.

Keywords: *Triticum spelta*; *Triticum aestivum*; *Fusarium* mycotoxins; organic farming

Introduction

In Lithuania, like in many other European countries, agricultural policy is oriented towards organic farming, whose primary objective is to solve environmental and food safety problems. Organic producers give their preference to competitive crops and cultivars, with high disease resistance (Ostergard & Jensen 2004).

Wheat is the most consumed cereal worldwide. The common wheat (*Triticum aestivum* L.) is the most common species of the major sources of food in many countries, including Lithuania. Spelt (*Triticum spelta* L.), an old wheat species cultivated in ancient Egypt and Italy, is becoming increasingly popular in Lithuania. The main centres of spelt cultivation in Europe are southern Germany, Switzerland, Belgium, Austria, Slovenia and parts of northern Italy (Bonafaccia et al. 2000; Mielke & Rodemann 2007). For many years, the cultivation of spelt declined, but recent interest in the use of spelt for ecologically grown foods has led to a resurgence in its cultivation (Kohajdová et al. 2009). Spelt is an alternative crop, growing without any special demands for soil as well as climate (Rüegger et al. 1990; Schober et al. 2006; Sliesaravičius et al. 2006). The nutritive value of spelt wheat is high as it contains all the basic components necessary for humans (Bojňanská & Frančáková 2002). In favourable conditions it can produce yields similar to those of common wheat (*Triticum aestivum* L.) (Laghetti et al. 1999; Burgos et al. 2001). Thanks to a higher stalk and hard adherent glumes, spelt has resistance to fungal infestation (Solarska, Kuzdralinski, et al. 2012).

Cereal products are major ingredients of the human diet but they are also an important source of mycotoxins (Solarska, Marzec, et al. 2012). They are secondary metabolites of fungi and harmful to human and animal health. It is a priority for many countries to minimise the risk of contamination of food and feed with mycotoxins. Maximum limits have been established in the European Union (European Commission Recommendation 2006). The contamination of mycotoxins in cereals is known to be affected by the local climate (rainfall, temperature or relative humidity), agricultural practices, harvest logistics, transport and storage conditions, and processing of products (Bakan et al. 2002).

The Fusarium genus is probably the most prevalent toxin-producing fungi of the Northern temperate region. The occurrence of mycotoxin-producing moulds is a worldwide problem, but the nature and quantity of the produced mycotoxins depend on the interaction of several factors (Bhat et al. 2010). These fungi are responsible for the production of trichothecenes, which are divided into four groups, of which type A (e.g. deoxynivalenol) and type B (e.g. T-2 toxin) are the most important. Other mycotoxins like zearalenone (ZEA) are also produced by these *Fusarium* moulds (Devegowda & Murthy 2005).

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Deoxynivalenol (DON) is the most commonly detected mycotoxin in cereal grains. This mycotoxin is produced by several *Fusarium* species, mainly *F. graminearum* and *F. culmorum* (Bottalico & Perrone 2002; Perkowski et al. 2008; Cortinovis et al. 2012; Gagkaeva et al. 2013).

ZEA is a mycotoxin synthesised by many species of fungi of the genus *Fusarium*: *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. crookwellense* and *F. semitectum* (Bennett & Klich 2003).

Organic farming does not allow the use of conventional mineral fertilizers and crop protection products. As a result, in our experiments we chose to grow different species of cereals and to investigate how cereal species might affect mycotoxin accumulation. Information from the literature about mycotoxin contamination in spelt is scarce. 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 ability to accumulate fusariotoxins has been poorly investigated (Wiwart et al. 2004). This study describes the occurrence of DON, ZEA and T-2/HT-2 toxin in a survey of spelt and common wheat grain, bran and flour.

Materials and methods

Field experiments and samples

Field experiments – winter spelt (*T. spelta* L.) and common wheat (*T. aestivum* L.) – were carried out in a certified ecological field at the Joniškėlis Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry during 2010–2012. A total of 148 grain and grain product samples were collected to quantify mycotoxins. Spring common wheat (*T. aestivum* L.) samples were collected at harvesting in 2011–2012 from Central Lithuania's commercial entities. All samples were analysed for contamination with deoxynivalenol (DON), zearalenone (ZEA) and T-2/HT-2 toxin (T-2/HT-2). The number of samples analysed is given in Table 1.

Method

Quantitative analysis of DON, ZEA and T-2/HT-2 was carried out using an ELISA commercial kit (Neogen Corporation, Food Safety Diagnostics, Ayr, UK: Veratox® for DON 5/5 – 8331NE; Veratox® for DON HS (for flour analyses), Veratox® for zearalenone – 8110; and Veratox® for T-2/HT-2 – 8210). The method is based on the antibody–antigen interaction and approved by the AOAC Research Institute (Certificate No. 950702).

Reagents

Most of the reagents used were the Neogen test kit products. Standard solutions of DON, DON HS, ZEA and T-2/HT-2 used for the calibration curves were at the following levels: DON – 0, 250, 500, 1000 and 2000 ppb (μg kg^{-1}); DON HS – 0, 25, 50, 100 and 250 μg kg^{-1} (ppb); ZEA – 0, 25, 75, 150 and 500 ppb (μg kg^{-1}); and T-2/HT-2 – 0, 25, 50, 100 and 250 ppb (μg kg^{-1}); all were included in the ELISA test kit.

Sample preparation and test procedure

Part of each sample was subjected to mycotoxicological contamination, and the other part (about 50 g) was air dried; grain was milled in a IKA A11 Basic mill (Staufen, Germany), flour and bran were milled in a mill Quadrumat Junior (Brabender, Duisburg, Germany). Flour and bran samples were stored at –20°C until analysis. Mycotoxin extraction and tests were performed according to the manufacturer's instructions. Analysis of mycotoxin was repeated twice. The optical densities of samples and controls from a standard curve were estimated by a multichannel photometer Multiskan Ascent (Thermo Electron Corp., Vantaa, Finland), supplied with internal software, using a 650 nm filter. Calibration curves of the standards were plotted for each toxin dilution (reagents) using a standard concentration against the percentage inhibition of the respective standard. For quantification, each mycotoxin concentration was automatically calculated from the calibration curves, obtained by plotting absorbance intensity against the logarithm of analyte concentration. The determination coefficients r^2 ranged as follows: for DON, 0.988–0.998; for ZEA, 0.982–0.998; and for T-2/HT-2, 0.980–0.996. The measured absorbance was automatically converted to the mycotoxin concentration units (μg kg^{-1}). The results were estimated by taking into account the

Table 1. Number of samples analysed for grain and grain product contamination with DON, ZEA and T-2/HT-2.

	Number of wheat grain and grain product samples					
Harvest year	*T. spelta* grain/grain with glumes	*T. aestivum* grain winter/spring	*T. spelta* glumes	Flour of spelt/winter common wheat	Bran of spelt/winter common wheat	Total
2010	8/8	8/–	–	8/8	8	48
2011	8/–	8/12	8	8/8	8/8	68
2012	8/–	8/8	8	–	–	32
Overall	32	44	16	32	24	148

lowest calibration curve's mycotoxin concentration value (LOD), which according to the manufacturer's methodical guidelines is: for DON, 100.0 µg kg^{-1} (ppb); for DON HS, 25.0 µg kg^{-1} (ppb); for ZEA, 5.0 µg kg^{-1} (ppb); and for T-2/HT-2, 10.0 µg kg^{-1} (ppb). While assessing the data with regard to food and forage safety, we referred to European Union Document No. 1881/2006 for DON and ZEA (European Commission Regulation 2006), and global research recommendations for T-2 (Eriksen & Alexander 1998).

Weather conditions

A description of the weather conditions was based on the data from the meteorological site of Joniškėlis Experimental Station. The mean daily air temperature and amount of precipitation are presented for more important experimental periods in Figure 1. In 2010, the winter was cold; moreover, a lengthy drought occurred in spring that aggravated the state of the crops and caused a dramatic thinning of both species. At the end of June a heatwave replaced the rainy weather. The weather conditions in 2010 were absolutely conducive to *Fusarium* fungi spread. On the contrary, in 2011, hot and dry weather during the cereal flowering period inhibited the spread of the disease. In 2012, mid-July was warm and dry. High temperatures and humidity in late July triggered the spread of fungus. Rain delayed cereal harvesting.

The research evidence suggests that the mycotoxin accumulation in spring wheat harvested in Central Lithuania was significantly influenced by the rainy weather during the wheat flowering period.

Statistical analysis

Statistical analysis was performed using the software Stat Eng (Tarakanovas & Raudonius 2003).

Results and discussion

This study compared two Lithuania-registered winter species of *T. aestivum*, *T. spelta* and spring species of *T. aestivum* to determine the concentrations of trichothecene toxins (DON, T-2/HT-2) and ZEA in wheat grain. All samples from 2010 and 2011 were found to contain ZEA and T-2/HT-2 (Table 2). The highest DON contamination (100%) in 2010 was found in the samples of spelt with glumes. In spelt grain without glumes this toxin occurred in 25% of the samples. In the common wheat grain samples DON was not detected. In all examined samples, the safe level of mycotoxins was not exceeded (European Commission Regulation 2006). Solarska et al. (2009) found up to 0.31 mg kg^{-1} as the average concentration of DON in spelt wheat. Researchers from other countries also indicate that only slight amounts of DON, T-2/HT-2 toxin and ZEA were found on spelt; because of its tough glumes it is one of the cereals that is least infected by fungi (Wiwart et al. 2011). In the samples from 2011 mostly DON was found in spelt glumes (the range of contamination was 237.1–1027.0 µg kg^{-1}). Wiwart et al. (2009) indicated that the average concentration of DON was up to 486.50 µg kg^{-1} in spelt kernels and up to 1508.55 µg kg^{-1} on glumes. Other authors consistently found lower contamination levels of DON in spelt (Castoria et al. 2005; Wiwart et al. 2011).

Table 2 presents a comparison of winter wheat spelt and common wheat grain contamination data, which indicate that spring wheat was much more severely contaminated with mycotoxins. The differences were especially vivid in 2012 since all spring wheat grain samples were contaminated with DON, whose concentrations in some samples exceeded the safe level by two to seven times (European Commission Regulation 2006). As a result, it was noted that spelt grain has a unique, nature-shaped protection system: glumes that partly protect the grain from the harmful environmental effect, phytoptahogen occurrence and mycotoxin synthesis. This becomes

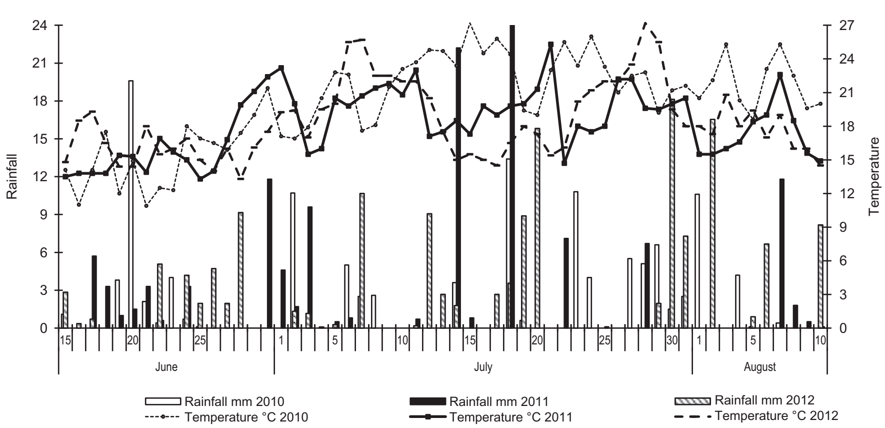

Figure 1. Meteorological conditions in 2010–2012 from cereal anthesis (the second half of June) till harvest (the end of July to the first 10 days of August).

Table 2. DON, ZEA and T-2/HT-2 toxin occurrence in winter *T. spelta*, *T. aestivum* and spring *T. aestivum* grain samples from the 2010, 2011 and 2012 harvest years.

	DON		ZEA		T-2/HT-2	
Sample item	Number of positive samples/%	Range of contamination (μg kg^{-1})	Number of positive samples/%	Range of contamination (μg kg^{-1})	Number of positive samples/%	Range of contamination (μg kg^{-1})
2010						
T. spelta	2/25	0–103.0	8/100	11.1–23.1	8/100	10.2–12.8
T. spelta with glumes	8/100	104.0–114.0	8/100	10.8–12.9	8/100	10.6–25.1
T. aestivum (winter)	n.d.[a]	n.d.	8/100	11.0–12.7	6/75	10.2–24.6
T. aestivum (spring)	n.a[b]	n.a.	n.a	n.a.	n.a	n.a.
2011						
T. spelta	8/100	164.7–200.2	8/100	10.7–11.9	8/100	10.7–22.6
T. spelta glumes	8/100	237.1–1027.0	8/100	50.4–66.7	8/100	50.2–217.9[c]
T. aestivum (winter)	8/100	183.8–470.9	8/100	10.2–20.0	8/100	10.0–115.0[c]
T. aestivum (spring)	11/91.7	206.1–990.9	1/8.3	0–11.6	n.d.	n.d.
2012						
T. spelta	8/100	106.0–311.4	4/50	0–18.5	n.d.	n.d.
T. spelta glumes	8/100	319.1–951.7	8/100	21.3–60.4	8/100	39.0–48.8
T. aestivum (winter)	8/100	115.5–195.7	8/100	17.2–20.5	2/25	0–23.7
T. aestivum (spring)	8/100	2150.0[c]–8845.1[c]	8/100	23.6–302.5[c]	n.a	n.a.

Notes: [a]Not detected.
[b]Not analysed.
[c]Exceeded safe level.

especially obvious when comparing the contamination results for winter spelt and common wheat mycotoxin.

The results of the study indicate T-2/HT-2 toxin as the most often occurring mycotoxin in common wheat grain and products in 2011 (Table 3). T-2/HT-2 toxin is one of the most toxic mycotoxins belonging to the trichotecenes group. This toxin has been shown to induce haematological and immunological toxicity and to impair synthesis of DNA and cellular proteins. To date, in the European Union there is no legislative maximum tolerated level for that mycotoxin in food. The TDI for the T-2/HT-2 toxins is 0.06 μg kg^{-1} of body weight day^{-1} (Hussein & Brasel 2001). Common wheat grain products are a base element of the daily human diet. T-2/HT-2 toxin is the most dangerous for children because of their high daily intake of grain products and their lower body weight. Solarska et al. (2009) found in winter wheat one sample of ancient wheat cultivated in Poland with a high T-2 toxin content of 179.6 μg kg^{-1}. We established that T-2/HT-2 concentrations in common wheat grain were up to 115.0 μg kg^{-1}; and in bran produced from common wheat grain the concentrations were even higher at 120.6–286.8 μg kg^{-1}. Other authors maintain that in whole-grain products the concentration of T-2 toxin is higher than in white flours (Schollenberger et al. 2002). Our study showed that mycotoxin contamination of both spelt and common wheat flour samples was low; however, in 2010–2011 samples were found in which ZEA and DON concentrations were higher than 20.0 and 200.0 μg kg^{-1}, respectively. Flour and brand with such a contamination level cannot be used for children's nutrition (European Commission Regulation 2006). The tolerated DON daily intake is 1 μg kg^{-1} of body weight day^{-1} according to European Union determinations (Hussein & Brasel 2001). In spelt flours, the maximum concentration is 165.5 μg kg^{-1}; in common flours it is 283.1 μg kg^{-1}. Our research evidence agrees with that obtained by other researchers (Schollenberger et al. 2002; González-Osnaya et al. 2011; Wiwart et al. 2011).

Figure 2 shows which *Fusarium* mycotoxin compositions predominated in spelt and common wheat grain, bran and flour in 2010 and 2011. The study showed that in 2010 in spelt grain two mycotoxin compositions (ZEA + T-2/HT-2) were prevalent; however, bran was 100% contaminated with all three mycotoxins (DON + ZEA + T-2/HT-2). Traces of all three mycotoxins were detected in flour. In 2011, spelt wheat was 100% contaminated with DON + ZEA + T-2/HT-2, and bran and flour 100% contaminated with DON + T-2/HT-2 toxins. Common wheat grain in 2010 was 100% contaminated with ZEA + T-2/HT-2, while in 2011 DON + ZEA + T-2/HT-2 predominated. In 2010, flour and bran produced from common wheat grain were 100% contaminated with the three mycotoxins assayed. In 2011, two mycotoxin compositions prevailed in common wheat flour and bran. It is thought that the mycotoxin spectrum and composition depended on the weather

Table 3. DON, ZEA and T-2/HT-2 toxin occurrence in winter *T. spelta*, *T. aestivum* wheat grain, bran and flour samples from the 2010 and 2011 harvest years.

	DON		ZEA		T-2/HT-2	
Cereals and product	Number of positive samples/%	Range of contamination (μg kg^{-1})	Number of positive samples/%	Range of contamination (μg kg^{-1})	Number of positive samples/%	Range of contamination (μg kg^{-1})
2010						
T. spelta grain	2/25	0–103.0	8/100	11.1–23.1	8/100	10.2–12.8
T. spelta bran	8/100	21.6–45.3	8/100	10.5–13.4	0[d]	< LOD[b]
T. spelta flour	8/100	23.9–26.0	8/100	11.7–13.7	0	0 to < LOD
T. aestivum grain	n.d.[a]	n.d.	8/100	11.0–12.7	6/75	< LOD–24.6
T. aestivum bran	8/100	27.9–90.8	8/100	12.8–14.4	8/100	47.2–69.2
T. aestivum flour	8/100	24.9–51.8	8/100	11.2–14.3	2/25	< LOD–34.0
2011						
T. spelta grain	8/100	164.7–200.2	8/100	11.0–11.4	8/100	10.7–22.6
T. spelta bran	8/100	210.3–247.8	n.d.	n.d.	8/100	61.7–90.8
T. spelta flour	8/100	59.6–165.5	n.d.	n.d.	8/100	10.3–22.9
T. aestivum grain	8/100	183.8–470.9	6/75	0–11.6	8/100	10.0–115.0[c]
T. aestivum bran	8/100	239.0–548.2	2/25	0–23.2	8/100	120.6[c]–286.8[c]
T. aestivum flour	8/100	136.6–283.1	n.d.	n.d.	6/75	0–44.9

Notes: [a]Not detected.
[b]Limit of detection.
[c]Exceeded safe level.
[d]Less than the LOD = negative samples.

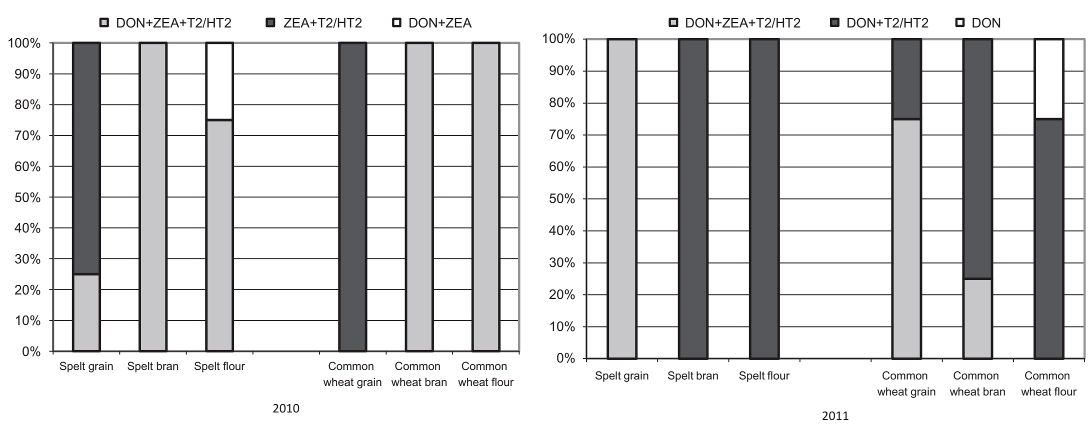

Figure 2. DON, ZEA and T-2/HT-2 toxin co-contamination in winter *T. spelta*, *T. aestivum* wheat grain, bran and flour samples from the 2010 and 2011 harvest years.

conditions during the vegetative season (Solarska, Marzec, et al. 2012).

The occurrence of harmful substances especially in health foods indicates the need for constant monitoring of these products.

Conclusions

The concentrations of DON, ZEA and T-2/HT-2 in *T. spelta* and *T. aestivum* were influenced by winter and spring wheat species and by year. The lowest DON, ZEA and T-2/HT-2 concentrations were found in spelt grain without glumes. The highest concentration of mycotoxins was found in spelt grain with glumes, in glumes and in spring wheat grain. The levels of DON and ZEA in spelt grain with glumes and glumes did not exceed the maximum allowable levels according to European Union Regulation No. 1881/2006. However, the DON level in spring wheat grain in 2012 exceeded the safe concentration by two- to seven-fold.

In 2011, T-2/HT-2 toxin concentrations detected in common wheat grain were higher than those in spelt grain. This was especially evident having analysed bran. Although bran was found to contain higher concentrations of T-2/HT-2 toxin than grain and spelt samples, in common wheat bran the concentrations of this mycotoxin in all samples tested exceeded the global research recommendations for T-2/HT-2 (Eriksen & Alexander 1998).

The results indicate the significantly higher concentrations of *Fusarium* toxins in glumes than in dehulled kernels, which indicates a possible protective effect of spelt wheat glumes.

It is risky to produce bran from grain in which mycotoxin concentrations do not exceed the safe level, since the concentrations of mycotoxins in bran can be several times as high as those in grain. As a result, for a dietary product characterised by good digestive properties, bran can become a harmful product that can cause unpredictable health problems.

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