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Mycotoxin contamination of Lithuanian-grown cereal grains and factors determining it

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During the period 2003–2005, spring and winter cereal grain samples were analysed for the presence of the mycotoxins deoxynivalenol (DON), zearalenone (ZEN), T-2 toxin, ochratoxin A and aflatoxin (total) by the ELISA (enzyme-linked immunosorbent assay) method at the Lithuanian Institute of Agriculture.

Mycotoxin DON produced by the fungi of the genus *Fusarium* was found to be most frequent in Lithuanian-grown cereal grains. DON-contaminated samples accounted for 84.0–98.0% of the total samples analysed. Grains of spring cereals were found to be more heavily contaminated by this toxin than those of winter cereals.

The level of ZEN contamination was higher in spring wheat, barley and oats as compared with winter wheat, rye and triticale grains.

T-2 toxin producers *Fusarium poae, F. sporotrichioides* prevailed in the Lithuanian-grown cereal grains. Analysis of T-2 toxin in oat grains showed the actual relevance of studies of this toxin, since during the period 2003–2005 the oat grains tested was 100% contaminated with this toxin. Elevated T-2 toxin concentrations were identified not only in oats but also in spring barley and winter rye grain samples.

Eighty per cent of spring barley grain samples were contaminated with ochratoxin; in 42.0% of samples its concentrations exceeded 3.0 μg kg^{-1}, and their contamination with aflatoxin (total) amounted to 67.9%, but the contents identified did not exceed the levels specified in the EU regulations.

Key words: cereal grain**,** micromycetes, mycotoxins, *Fusarium* spp.

INTRODUCTION

The main purpose of grain cultivation is the production of high – quality food-related raw materials for the processing industry. Natural toxins, such as mycotoxins, have emerged as a significant factor affecting the safety image of cereal grains as a raw material for the food and feed industry. Previous studies in Lithuania (Bakutis et al., 1997; Keblys et al., 2000; Baliukonienė et al., 2003; Gaurilčikienė et al., 2005, Semaškienė et al., 2005) and in other European countries (Muller et al., 1998; Tanaka et al., 1988; Bennett, 2003; Döll et al., 2002; Park, 1996; Schollenberger et al., 2002) as well as globally (Tanaka et al., 1988; Webley, Jackson, 1998) have shown that there is a reason to focus on *Fusarium* toxins and their appearance. A planned European Union directive will specify the maximum limits for trichothecenes such as deoxynivalenol (DON), T-2 toxin and HT-2 toxin and for zearalenone (ZEN) and fumonisins. DON is the most frequently found contaminant of barley, wheat, oats and corn throughout the world (Scott, 1989; Eskola, 2002). In addition to DON, T-2 toxin, HT-2 toxin and ZEN frequently occur in cereal crops cultivated in northern temperate regions (Hietaniemi et al., 2004; Grabarkiewicz-Szczesna, 2001; Edwards, 2004; Muller et al., 1998; Rasmussen, 2003; Thuvander et al., 2001). Fumonisins cause more extensive problems in the Southern than in the Northern Hemisphere (Shephard et al., 1996). The most frequently isolated *Fusarium* species are *F. graminearum, F. culmorum, F. moniliforme, F. poae, F. eqiseti* and *F. proliferatum* (Eriksen, Alexander, 1998; Eskola et al., 2001; Creppy, 2002).

The mycotoxin ochratoxin A (OTA) frequently contaminates cereal grains, coffee beans, nuts, olives (Fazekas et al., 2002; Wood et al., 1996). In Northern Europe, OTA is mainly produced by the moulds *Penicillium verrucosum* and *Aspergillus ochraceus* during storage of cereal grains (Creppy, 2002; Frisvad, Samson, 1991; Krogh, 1987; Kuiper-Goodman, 1989; Lund and Frisvad, 2003). OTA contamination has mainly been associated with post-harvest conditions (Abramson et al., 1990; Mills, 1990). Fungi of the genus *Penicillium* have been found to be responsible for

OTA-contaminated grain in colder areas (Scandinavia and Canada). *Aspergillus ochraceus* was isolated in warmer climatic zones (Yugoslavia, Australia) and in coffee bean producing countries (Frisvad and Samson, 1991). The experimental evidence on ochratoxin A occurrence in Lithuanian-grown cereals is rather limited; a more comprehensive research has been done into the fungi producing this mycotoxin (Lugauskas et al., 2004).

Aflatoxins can contaminate agricultural commodities including corn, wheat, rice, peanuts and many other crops (Aly, 2002, Sinha and Sinha, 1991). Aflatoxins, each of which is a group of closely related mycotoxins, may be produced by *A. flavus*, *A. parasiticus*. Four different aflatoxins, B1, B2, G1 and G2, have been identified, B1 being the most toxic, carcinogenic and prevalent. Monitoring of aflatoxins and their producers in Lithuania is mainly done for imported commodities, while data on Lithuanian-grown grain contamination with this toxin are insufficient.

Temperature and moisture conditions during the growing season and insect infestations are critical factors affecting fungal infection and toxin synthesis (Cromey, 2001). More mycotoxins were produced during the warm, dry summers than in rainy and cool summers (Grabarkiewicz-Szczesna, 2001).

The aim of the present study was to investigate the occurrence of mycotoxins DON, T-2, ZEN, ochratoxin A, aflatoxin (total) in grains of different cereal species grown for food and feed in Lithuania during 2003–2005.

MATERIALS AND METHODS

Samples. Grain samples of winter and spring wheat (*Triticum aestivum* L.), spring barley (*Hordeum distichon* L.), winter triticale (x*Triticosecale* Wittm.), winter rye (*Secale cereale* L.) and spring oats (*Avena sativa* L.) were collected at harvest in 2003–2005 from the Lithuanian Institute of Agriculture in Dotnuva and analysed for contamination by DON, ZEN, T-2 toxin, aflatoxin (total) and ochratoxin A. The number of samples analysed is given in Table 1. Part of each sample was subjected to mycological contamination and the other part (about 50 g) was air-dried, milled in a IKA A11 Basic mill and kept at –20 ^{0}C until analysis.

Analysis of mycotoxins. The wheat, rye, oats and barley samples were analysed by the ELISA (enzyme-linked immunosorbent assay) method (Bennet et al., 1994; Wilkinson et al., 1992). The method is based on the antibody–antigen interaction, provides a sensitive, rapid and accurate monitoring of mycotoxins and is suitable for screening large numbers of samples. The Veratox test kits (Neogen Corporation, USA) approved by the AOAC Research Institute (Certificate N 950702) were used for the analysis. Mycotoxin extraction and tests were performed according to manufacturer's instructions. The optical densities of samples and controls from standard curve were estimated with a multichannel programmable Multiskan MS photometer (Labsystems, Finland) using a 650 nm filter and the Point to Point calculation mode. The measured absorbances were automatically converted to the mycotoxin concentration units μg kg^{-1}.

While assessing our data with regard to food and forage safety we referred to the EU document No 856/2005 for deoxynivalenol and zearalenone, No 123/2005 for ochratoxin, No 466/2001 for aflatoxins and global research recommendations for T-2 toxin (Eriksen, Alexander, 1998).

Mycological analysis. To determine grain internal contamination by mould fungi the agar plate method (Наумова, 1970; Mathur and Kongsdal, 2003) was applied. One hundred grains were tested for each sample. The grains were sterilized with 3% sodium hypochlorite for 2 min and rinsed three times with sterile water. After that the grains were drained with sterile filter paper and placed onto Petri dishes containing MEA with addition of streptomycin (250 mg l^{-1}). The dishes were incubated for seven days at 26 ^{o}C. After incubation, the number of micromycete species detected in each grain was calculated. The species frequency of occurrence (FO) as the ratio of the number of grains where the species was detected to the total number of grains tested, expressed in percentage, were calculated (Booth, 1971; González et al., 1995). The species were identified on the basis of their morphological and cultural characteristics according to Ellis (1971, 1976), Билай (1977), Arx (1981), Gerlach, Nirenberg (1982), Nelson et al. (1983).

RESULTS AND DISCUSSION

Results of mycotoxins in grain samples of winter and spring cereals in 2003–2005 are reported in Tables 2–5.

Table 1. **The number of cereal grain samples tested for mycotoxins contamination, 2003–2005**

Mycotoxins	Number of samples						Total
	Winter cereals			Spring cereals			
	Wheat	Rye	Triticale	Wheat	Barley	Oats	
DON	220	23	21	53	116	14	447
ZEN	176	17	21	33	112	7	366
T-2	91	8	7	12	47	10	175
Ochratoxin A	35	1	2	2	15	-	55
Aflatoxin	-	-	-	-	56	-	56

Table 2. **DON contents in grains of different cereal species and seasonal types, 2003–2005**

Species	Total number of samples	Positive		Distribution of positive samples according to content of DON (μg kg^{-1})			Average concentration μg kg^{-1}	Max concentration μg kg^{-1}
		Samples	%	<100	100–300	>300		
Winter cereals								
Wheat	220	185	84.0	134	49	2	64.3	987.0
Rye	23	21	91.3	12	7	2	138.0	919.0
Triticale	21	19	90.5	10	6	3	135.0	427.0
Spring cereals								
Wheat	53	52	98.0	15	28	9	204.0	847.0
Barley	116	113	97.4	66	45	2	95.5	375.0
Oats	14	13	92.8	7	6	0	94.6	204.0

Table 3. **ZEN contents in grains of different cereal species and seasonal types, 2003–2005**

Species	Total number of samples	Positive		Distribution of positive samples according to content of ZEN (μg kg^{-1})			Average concentration μg kg^{-1}	Max concentration μg kg^{-1}
		Samples	%	<100	100–300	>300		
Winter cereals								
Wheat	176	80	45.5	54	20	6	4.8	76.0
Rye	17	6	35.3	5	1	0	3.6	28.8
Triticale	19	4	21.0	4	0	0	1.3	7.5
Spring cereals								
Wheat	33	21	63.6	9	10	2	11.3	95.6
Barley	112	63	56.3	37	17	9	9.5	193.4
Oats	7	4	57.0	1	3	0	7.1	16.3

Table 4. **T-2 toxin contents in grains of different cereal species and seasonal types, 2003–2005**

Species	Total number of samples	Positive		Distribution of positive samples according to content of T-2 toxins (μg kg^{-1})			Average concentration μg kg^{-1}	Max concentration μg kg^{-1}
		Samples	%	<100	100–300	>300		
Winter cereals								
Wheat	91	53	58.2	29	23	1	4.7	32.9
Rye	8	8	100	2	3	3	40.9	153.6
Triticale	7	4	57.1	1	0	3	21.5	52.5
Spring cereals								
Wheat	12	5	41.7	3	2	0	2.8	11.9
Barley	47	36	76.6	6	24	6	19.7	319.0
Oats	10	10	100	0	5	5	44.1	122.0

DON was found to be the most widely spread mycotoxin in Lithuanian-grown cereal grain. It was present in 84.0–98.0% of the grain samples tested (Table 2). DON was more prevalent in spring than in winter cereals (92.8–98.0% and 84.0–91.3%). The greatest content of contaminated grains with higher DON concentrations were determined in spring wheat samples. In most spring wheat samples, DON concentration was higher than in winter cereal samples, however, the highest DON contents were identified in separate samples of winter wheat (987.0 μg kg^{-1}) and winter rye (919.0 μg kg^{-1}) grains. Although DON was the most prevalent of all mycotoxins tested in Lithuania, its contents identified at harvesting were not high and did not exceed the allowable levels. According to the Commission of the European Communities regulation for mycotoxins, which will come into effect on July 2006,

Table 5. **Ochratoxin A and aflatoxin (total) contents in different cereal species and seasonal types, 2003–2005**

Species	Total number of samples	Positive		Distribution of positive samples according to content of toxins (μg kg^{-1})			Average concentration μg kg^{-1}	Max concentration μg kg^{-1}
		Samples	%	<1	1–3	>3		
Ochratoxin A in winter cereals								
Wheat	27	5	18.5	1	4	0	0.3	2.9
Rye	1	1	100	1	0	0	0.9	0.9
Triticale	2	0	0	0	0	0	0	0
Ochratoxin A in spring cereals								
Wheat	2	1	50.0	0	0	1	13.0	26.1
Barley	15	12	80.0	3	4	5	8.3	49.6
Aflatoxin (total)								
Barley	56	38	67.9	13	25	0	0.9	2.8

the maximum level of DON in unprocessed cereals is 1250 μg kg^{-1}. In other countries also DON contamination at grain harvesting ranges from 60 to 100%, but the levels identified are very dependent on the weather conditions at cereal flowering and harvesting (Schollenberger, 2002; Scott, 1997; Tutelyan, 2004).

ZEN contamination in grains of spring cereals was higher than in grains of winter cereals (Table 3). Spring wheat, barley and oat contamination ranged from 56.3 to 63.6%. In one spring barley grain sample the concentration of ZEN exceeded the allowable level almost twice (193.4 μg kg^{-1}). According to the Commission of the European Communities regulation for mycotoxins, which will come into effect on July 2006, the maximum level of ZEN in unprocessed cereals is 100 μg kg^{-1}. Experimental evidence suggests that the incidence of *Fusarium* spp. is generally higher on spring than on winter cereal grains (Semaškienė et al., 2005), therefore mycotoxin concentrations may be higher. Winter wheat, triticale, and rye were less contaminated (21.0–45.5%). Similar results were obtained by other countries' researchers investigating ZEN in winter wheat grain (Lepschy-v Gleissenthal, 1989).

T-2 toxin concentration in grain samples of various cereals varied within 2.8–44.1 μg kg^{-1} (Table 4.), however, there were samples that exceeded the allowable level (Eriksen, Alexander, 1998). Lower concentrations of this mycotoxin were found in spring and winter wheat samples, although contamination ranged from 41.7 to 58.2%. Spring barley, winter rye and oats were contaminated more heavily. In one spring barley grain sample the content of T-2 toxin exceeded the recommended level thrice (Eriksen, Alexander, 1998) and was as high as 319.0 μg kg^{-1}. Higher than allowable concentrations were found in winter rye (153.6 μg kg^{-1}) and oat grain samples (122.0 μg kg^{-1}). Data on T-2 toxin occurrence are limited, therefore it is necessary to conduct comprehensive research into the factors that determine its concentration in grains and grain products, since under Lithuania's conditions T-2 toxin producers *Fusarium poae, F. sporotrichioides* (Rasmussen et al., 2003) are rather common on grains, especially on oats (Figure). The frequency of identification of these fungi in oats was 43.0% and 5.0%, respectively. *F. graminearum*,

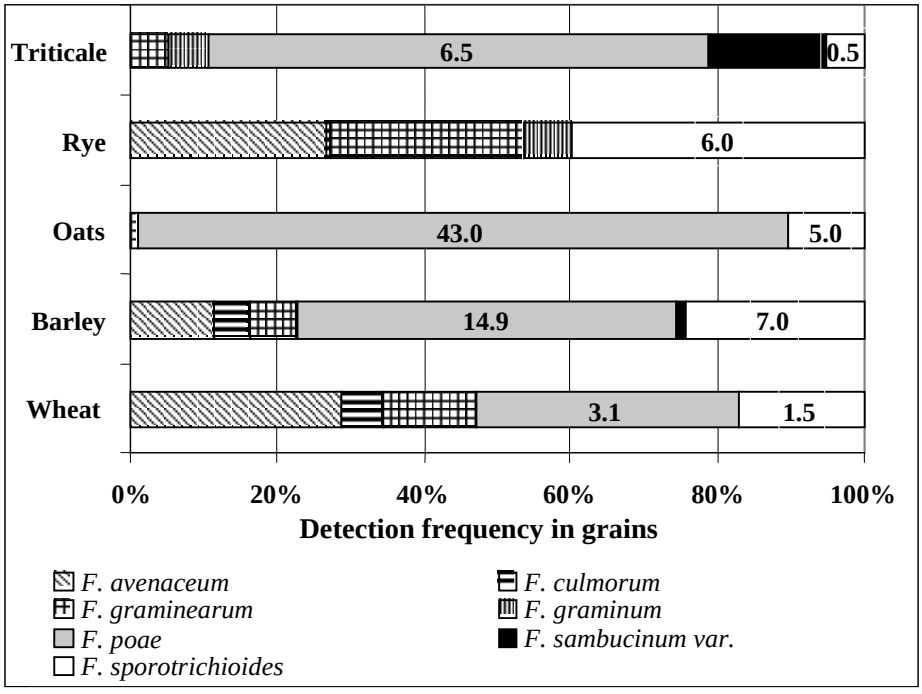

Figure. Predominant *Fusarium* species on cereal grains

which is the main producer of DON and ZEN in oats, was identified in low concentrations (FO 0.5%).

In spring barley grains, *F. poae* identification frequency was 14.9% and of *F. sporotrichioides* 7.0%. This also might have determined higher concentrations of this toxin in the samples tested. A greater *Fusarium* species diversity was identified in grain samples of wheat, barley, triticale (9 species). Oats and triticale were characterised by a lower diversity of *Fusarium* fungi (4–5 species).

The occurrence of *Fusarium* species in Lithuania in samples of various cereal species suggests that in wheat and barley grains more frequent are *F. poae, F. avenaceum, F. sporotrichioides* which are not producers of DON and ZEN, therefore the concentrations of these toxins were low in the samples tested.

The trends of DON, ZEN, T-2 toxin variation in cereal grains might have been determined not only by the weather conditions, but also by other factors such as soil peculiarities in different districts, application of plant protection products, choice of varieties, etc. (Cromey et al., 2001, Edwards, 2004; Heier et al., 2005; Hietaniemi et al., 2004).

In Lithuanian-grown cereals, higher ochratoxin contents were identified in spring wheat and barley samples (Table 5). All the barley samples tested were 80.0% contaminated with ochratoxin; in 42.0% of them the concentrations exceeded 3.0 μg kg^{-1}. These results indicate that the problem of ochratoxin in Lithuanian-grown barley grains is undoubtedly relevant. This is corroborated by previously conducted tests that barley grains, especially during storage, are heavily infested with fungi of the genus *Penicillium* capable of producing ochratoxin (Lugauskas et al., 2004).

The total aflatoxin content in Lithuanian-grown barley grain is not high (Table 5), however, of the 56 samples tested 67.9% were found to be contaminated with this mycotoxin. In most of the samples (25) aflatoxin concentration ranged from 1.0 to 3.0 μg kg^{-1}, the highest allowable concentration being 4.0 μg kg^{-1}. Regardless of the low aflatoxin contamination level, the previous research has shown that *Aspergillus flavus*, which is the chief producer of aflatoxin, is quite frequent in barley grains (Lugauskas et al., 2004).

CONCLUSIONS

1. The DON mycotoxin is the most frequent toxin produced by the genus *Fusarium* found in Lithuanian-grown cereal grains. It was identified in 84.0–98.0% of cereal grain samples assayed in 2003–2005. Spring cereals were found to be more heavily infested with this toxin than winter cereals.

2. ZEN was more prevalent in grains of spring wheat, barley and oats (63.6, 56.3 and 57.0%, respectively), and T-2 toxin was identified in all oat samples tested in 2003–2005.

3. Eighty percent of spring barley grain samples were contaminated with ochratoxin in 42.0% of them the concentrations exceeded 3.0 μg kg^{-1}, and their contamination with aflatoxin (total) amounted to 67.9%, but the contents identified did not exceed the levels specified in the EU regulations.

4. The most frequent T-2 toxin producers in Lithuanian-grown grains of various cereal species were found to be *F. poae* ir *F. sporotrichioides*. These fungi, as well as the rather frequent *F. avenaceum*, are not producers of DON and ZEN, therefore the concentrations of these toxins in the samples tested were not high and did not exceed the levels specified in the EU regulations.

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LIETUVOJE IŠAUGINTŲ VARPINIŲ JAVŲ GRŪDŲ UŽTERŠTUMAS MIKOTOKSINAIS BEI JUOS LEMIANTYS VEIKSNIAI

S a n t r a u k a

Lietuvos žemdirbystės institute 2003–2005 m. vasariniuose ir žieminiuose javų grūdų mėginiuose imunofermentiniu metodu ištirta mikotoksinų (deoksinivalenolio (DON), zearalenono (ZEN), T-2 toksino, ochratoksino A, aflatoksino) gausa.

Nustatyta, kad Lietuvoje išaugintuose javų grūduose dažniausiai aptinkamas *Fusarium* genties grybų produkuojamas mikotoksinas DON. Juo užteršta 84,0–98,0% analizuotų grūdų mėginių. Vasarinių javų grūdai buvo gausiau užteršti šiuo toksinu nei žieminių. Vasarinių kviečių, miežių ir avižų grūdai ZEN buvo užteršti labiau nei žieminių kviečių, rugių ir kvietrugių.

Lietuvoje išaugintuose javų grūduose vyravo *Fusarium poae, F. sporotrichioides* – T-2 toksino producentai. T-2 toksino analizės avižų grūduose parodė šio toksino tyrimo aktualumą, nes 2003–2005 m. tirti grūdai 100% buvo užteršti šiuo toksinu. Didesnės T-2 toksino koncentracijos nustatytos ir vasarinių miežių bei žieminių rugių grūdų mėginiuose.

Vasarinių miežių grūdų mėginiai 80,0% buvo užteršti ochratoksinu ir 42,0% iš jų nustatytos koncentracijos buvo didesnės nei 3,0 μg kg^{-1}, tuo tarpu jų užterštumas aflatoksinu (total) siekė 67,9%, tačiau nustatyti kiekiai neviršijo ES reglamento nustatytų ribų.

Raktažodžiai: javų grūdai**,** mikromicetai, mikotoksinai, *Fusarium* spp.