



The prevalence of deoxynivalenol and its derivatives in the spring wheat grain from different agricultural production systems in Lithuania

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ABSTRACT

Deoxynivalenol (DON) together with two acetylated derivatives, 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) occurs in cereal grains and their products. Co-occurrence of DON and acetylated derivatives in cereal grain is detected worldwide. Until now, DON and its derivatives have been considered equally toxic by health authorities. In this study, we analysed 103 samples of spring wheat grain, originating from the fields of different production systems in Lithuania, for the co-occurrence of type-B trichothecenes (DON, 3-ADON, 15-ADON). The samples were classified according to the production system—organic, sustainable and intensive. Mycotoxin levels in the spring wheat grain samples were determined by the HPLC method with UV detection. The type-B trichothecenes were found to be present at higher concentrations in the grain from the intensive production system. Eighty-one percent of the spring wheat grain samples from the intensive production system were co-contaminated with a combination of DON +3-ADON+15-ADON, 1% with DON+3-ADON. Additionally, DON+15-ADON and DON were found in 5% and 10% of the tested samples, respectively. Two percent of the samples were free from mycotoxins. In the grain samples from the sustainable production system, DON and a combination of DON+3-ADON showed a higher incidence – 47% and 23%, respectively. The samples with a combination of DON+3-ADON+15-ADON accounted for 18%. Completely different results were obtained from the analyses of organic grain samples. A large number of the organic spring wheat grain samples were contaminated with DON+3-ADON (55%) or DON (36%). The combination of DON+3-ADON+15-ADON was not present, while DON+15-ADON was present in 9% of the samples tested. The production systems did not lead to significant differences in mycotoxin levels, although a trend toward higher incidence and higher contamination was observed for the samples from the intensive and sustainable production systems.

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Introduction

Mycotoxins are produced by fungi as secondary metabolites in cereals and food. Trichothecene mycotoxins are a large group of chemically related toxic fungal compounds produced mainly by *Fusarium* spp. in cereal grains (Aureli et al. 2015). Grain contaminated by fungi and mycotoxins is a risk to human and animal health. As a natural contaminant of grain, *Fusarium*, a genus of ubiquitous fungi, is known to contain important plant pathogens and to produce a variety of mycotoxins that are toxic to both animals and humans (Birzele et al. 2000). Deoxynivalenol (DON) is the most common mycotoxin, which belongs to type B trichothecene group (Aureli et al. 2015), and is produced by a variety of *Fusarium* fungi commonly found in cereals (Sun and Wu 2016).

DON, detected most frequently and at the highest concentrations, is predominantly produced by *F. graminearum* and *F. culmorum* (Birzele et al. 2000). DON is one of the most important mycotoxins in wheat and wheat-based foods and feeds (Kushiro 2008). Wheat is one of the most important staple grains in the world and is mainly used in human food (Tibola et al. 2016). Mycotoxin presence depends on several factors, including fungal strain, climatic and geographical conditions, cultivation technique and crop protection, particularly during storage (Lo Curto et al. 2004; Matič Jovana et al. 2011). Mycotoxin composition in cereal grains is affected by environmental factors, such as water activity, temperature, substrate and competition (Vogelgsang et al. 2008; Yli-Mattila 2010). Agronomic and climatic factors

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explained 10–30% of the variation in *Fusarium* species and mycotoxins. Significantly lower *Fusarium* infestation and concentrations of important mycotoxins were found in the organic cereals (Bernhoft et al. 2012).

DON contamination in cereal grains and processed grain products is a major problem. DON was found in more than 90% of all mycotoxin-contaminated samples and its presence is usually a good indicator that other mycotoxins are also present (Sobrova et al. 2010).

Organically cultivated grains may have lower concentrations of mycotoxins, because the crop rotation routinely used reduces the transmission of plant diseases (Jestoi et al., 2004; Bernhoft et al. 2010). In January 2017, the European Food Safety Authority (EFSA) accepted the mandate for a Scientific Opinion on the risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. Overall, the EFSA data showed that the concentrations of DON in food and feed grains tended to be higher in conventionally cultivated cereal grains than in grains from organic farming (EFSA 2017). Several studies have reported that increased *Fusarium* infestation and mycotoxin contamination in cereals are associated with an increased level of nitrogen fertilisation, and that mineral fertilisers seem to stimulate *Fusarium* infestation and infection more than organic fertilisers (Elen et al. 2000; Lemmens et al. 2004; Heier et al. 2005; Bernhoft et al. 2010). Pesticides, insecticides and herbicides are commonly used in agriculture to protect plants from insects, pests and pathogens that may produce mycotoxins, and to improve the amount and quality of crop yield (Attallah et al. 2012; Reinholds et al. 2017)

On the other hand, maximum permitted limits for DON in food have been established in many countries. The European Commission has set maximum tolerated levels for several mycotoxins in cereals and also the methods of sampling and analysis of mycotoxins: Regulation (EC) 1881/2006 consolidated version and Regulation (EC) 1126/2007. The maximum level for DON is 1250 $\mu\text{g kg}^{-1}$ in unprocessed cereals other than durum wheat, oats and maize. The exposure assessments conducted to date at national or European level concluded that consumers and young children were exposed to DON at levels close to or even higher than the TDI (EFSA 2013; Lacko-Bartošova et al. 2017).

It is known that DON can be degraded or detoxified into various derivatives by acetylation, oxidation, deep oxidation or glycosylation (Kushiro 2008; Karlovsky 2011; Warth et al. 2012; Zhang et al. 2016). The derivatives like the acetylated forms 3- and 15-acetyldeoxynivalenol (3-ADON and 15-ADON) are of particular importance (Maul et al. 2012; Zhang et al. 2016). In addition to DON, the most abundant trichothecenes, 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON) are naturally occurring fungal secondary metabolites and contribute to the total DON-induced toxicity (JECFA 2010a; Sun and Wu 2016).

Combinations of trichothecenes are often present in grain samples, and 10–20% of DON exists as an acetylated form such as 3- and 15-ADON. However, to date, health authorities consider DON and its acetylated derivatives as a unique toxic contributor. For example, the EU considers that the regulation established for DON also protects the human population against exposure to 3-ADON and 15-ADON (EC 2005). Pinton et al. (2012a) compared the intestinal toxicity of DON and its acetylated derivatives and observed a different effect of DON and its acetylated derivatives.

In the last few years, the problem of these modified forms has become increasingly prominent since their presence could increase the total amount of toxins in food (Dall'Erta et al. 2013; De Boevre et al. 2015; Righetti et al. 2017)

Fusarium head blight (FHB) of wheat in Lithuania reached an epidemic scale in 2012 and persisted as a major problem in wheat growing areas in 2013 and 2014, especially affecting spring wheat (Suproniene et al. 2016). FHB is usually closely interlinked with the accumulation of mycotoxin DON (Becher et al. 2013; Suproniene et al. 2016a).

The aim of the current study was to ascertain which production systems—organic, sustainable and intensive are most closely related to the mycotoxin concentrations in spring wheat grain.

Material and methods

Sample collection and conditions

A total of 103 spring wheat samples (about 1 kg) were collected from commercial fields of spring wheat (crop years 2013 and 2014). The samples

were collected considering the different agricultural production systems (organic, sustainable and intensive) and according to the distribution of areas of agricultural production systems in Lithuania. Agronomic factors for organic, sustainable and intensive production systems are different. In the intensive production system, farmers are allowed to use synthetic fertilisers, pesticides, growth regulators and other chemical products. In the intensive production farms that we chose for the current study fungicides were applied against foliar diseases not less than three times and an additional spray-application against head blight was performed at the BBCH 65 growth stage. In the sustainable production system, the application of chemical inputs is limited. In the farms chosen for our study, fungicides were not used against head blight, and only one or two spray applications against foliar diseases were made.

In the organic production system, the use of synthetic chemical products is banned. They are replaced by natural organic and mineral substances. The farms included in our study hold official certificates proving the system of production. The largest number of samples, about 73% ($n = 75$), was collected in the farms of intensive production system. Samples collected in the farms of sustainable and organic production systems accounted for about 16% ($n = 17$) and 11% ($n = 11$), respectively.

In this study, 103 spring wheat grain samples were analysed for the co-occurrence of type-B trichothecenes (DON, 3-ADON, 15-ADON). Analysis of mycotoxins was done at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The samples were stored at -18°C until analyses.

The weather conditions of the experimental period markedly differed between the growing seasons. The 2013 season was characterised by a very late, dry and warm spring and changeable summer with heat waves, while the 2014 season was characterised by a warm summer (particularly July) and a long and rainy autumn.

Chemicals, reagent, standards and instruments

HPLC grade acetonitrile, methanol and polyethylene glycol PEG 8000 were obtained from Merck (Darmstadt, Germany). Certified mycotoxin

standards for DON, 3-ADON, 15-ADON were purchased from LGC Standards (Wesel, Germany). Wheat reference material was purchased from Trilogy Analytical Laboratory (Washington, MO, USA). The working standard solutions ranging from $0.1 \mu\text{g ml}^{-1}$ to $50 \mu\text{g ml}^{-1}$ were prepared in acetonitrile and stored at -20°C . The identification of mycotoxins was performed on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of an LC-20 AT pump equipped with a FCV-10AL quaternary valve, an SIL-20A autosampler, a DCU-20A5 degasser, a CTO-20A column oven maintained at 30°C , a UV detector (wavelength of 218 nm), equipped with Inertsil ODS-3 $5 \mu\text{m}$ ($4.6 \times 150 \text{ mm}$) column. Data collection and evaluation were performed by using an operating system, LC solution LC/GC, 5.42 (Shimadzu, Kyoto, Japan). Deionised water with resistivity of $18.2 \text{ M}\Omega$ was generated by a Milli-Q plus system (Millipore, Temecula, CA). For the sample clean-up step, an immunoaffinity column (IAC) of DON-Test (Vicom, Milford, MA) was used according to the manufacturer's procedures. DON-Test IAC antibodies cross-react with 3-ADON and 15-ADON, only DON-Test IAC succeeded in retaining DON and the two conjugates with good recoveries (Gonsalves and Storka 2016).

Sample preparation

All grain samples were ground in an IKA A11 Basic mill (Staufen, Germany) and stored at $+4^{\circ}\text{C}$ until analysis. The ground spring wheat samples were analysed according to the method previously validated for the determination of DON in cereals (Kotal and Radova 2002). For extraction, 10 g of ground sample was placed into 100 ml glass bottle and then 40 ml deionised water and 2 g polyethylene glycol were added. The mixture was stirred for 1 min. The extract was filtered through a fluted filter and then through a microfibre filter.

1 ml of the final extract was placed into the DON test column. 10 ml of redistilled water was used for column washing. The elution of DON was done by 1 ml of methanol. The elution solvent was evaporated to dryness and redissolved in 1000 μl mobile phase (H_2O : acetonitrile (90:10)). The extracts were placed in a chromatographic system (Kotal and Radova 2002).

Chromatographic conditions

HPLC conditions of DON: the mobile phase consisted of H₂O: methanol:acetonitrile (450:25:25, v/v/v). All separations were carried out at 40 °C, applying a flow rate of 1 ml·min⁻¹. DON was determined at a wavelength of 218 nm using a UV detector. The injection volume was 100 µl. The linear gradient 0–15 min 100% A-acetonitrile, 15–25 min 100% B-methanol, 25–35 min 100% A-acetonitrile were applied. Retention time was 25 min.

HPLC conditions for 3-ADON: the mobile phase consisted of H₂O: acetonitrile (90:10, v/v). All separations were carried out at 30 °C, applying a flow rate of 0.8 ml·min⁻¹. 3-ADON was determined at a wavelength of 218 nm using a UV detector. The injection volume was 50 µl. The linear gradient conditions were 0.01 min-0% -C-acetonitrile, 0.01 min-100% -D- H₂O:acetonitrile (90:10), 2.00 min-100%-D, 5.00 min-20%-C, 5.00 min-80%-D, 13.00 min-20%-C, 13.00 min-80%-D, 15.00 min-100%-D, 20.00 min- 0%-C, 20.00 min-100%-D, 20.00 min-stop. Retention time was 35 min.

HPLC conditions for 15-ADON: the mobile phase consisted of H₂O: acetonitrile (90:10, v/v). All separations were carried out at 30 °C, applying a flow rate of 0.8 ml·min⁻¹. 15-ADON was determined at a wavelength of 218 nm using a UV detector. The injection volume was 50 µl. The linear gradient conditions were 0.01 min-20% -C-acetonitrile, 0.01 min-80% -D-H₂O, 2.00 min-20%-C, 2.00 min-80%-D, 5.00 min-30%-C, 5.00 min-70%-D, 13.00 min-30%-C, 13.00 min-70%-D, 15.00 min- 20%-C, 15.00 min-80%-D, 20.00 min- 20%-C, 20.00 min-80%-D, 20.00 min-stop. Retention time was 35 min.

Method validation

According to the linear regression analysis, the calibration curves for DON were linear from 0.9 to 59.8 µg/ml, while those for 3-ADON and 15-ADON were linear from 1 to 50 µg/ml, which showed correlation R^2 of 0.9999 to DON, 0.9999 and 0.9999 to 3-ADON and 15-ADON, respectively. The LODs (s/n 3) of DON, 3-ADON and 15-ADON were 84, 65 and 60 µg kg⁻¹, respectively, and the LOQs (s/n 10) were 282, 219 and 230 µg kg⁻¹, respectively. The mean recoveries were 95 ± 5%, 99 ± 3% and 89 ± 11% for DON, 3-ADON and 15-

ADON, respectively. These results suggest that the chosen analytical method exhibited good accuracy and precision for the detection of DON, 3-ADON and 15-ADON in spring wheat samples.

Quantitation and calculation

Quantitation of DON and its derivatives in the test solution should be performed by measuring the peak area at DON (3-ADON, 15-ADON) retention time and comparing it with the standard curves. One should plot peak area (response, y -axis) of DON (3-ADON, 15-ADON) standard against concentration (µg/ml, x -axis) and determine slope (S), setting y -intercept to zero (Trucksess et al. 2010). The following formula should be used to calculate the level of mycotoxins in test sample:

$$W_{\text{DON}} = P_{\text{DON}} \times V_1 \times V_2 / V_3 \times m_s$$

where P_{DON} is the concentration of DON (3-ADON, 15-ADON), in micrograms per millilitre, in the aliquot of test solution derived from the calibration curve and V_1 is the volume, in millilitres, of the solvent used for extraction in wheat. V_2 is the final volume, in millilitres, of the injected test solution. V_3 is the volume, in millilitres, of the extract aliquot used for immunoaffinity clean up and m_s is the mass, in grams, of the sample material taken for analysis (European Standard LST EN 15891:2010).

Statistical analysis

Descriptive statistics of all data were expressed as mean ± standard deviation using MS Office Excel software (2010). Relationships between mycotoxin concentrations were determined using regression analysis. Coefficients of variation were calculated using additional extensions of the Excel programme.

Results and discussion

The results of the current study showed that DON was detected in 97%, 3-ADON in 84% and 15-ADON in 85% of the spring wheat grain samples from the intensive production system. The levels of DON in positive samples are shown in Table 1.

DON contamination in the grain from sustainable and intensive production systems was similar (94%). However, fewer samples were contaminated with 3-

Table 1. Prevalence of DON, 3-ADON, 15-ADON (% of samples analysed), the lowest and highest contamination level and the mean of the positives samples.

Mycotoxin	Production system	Samples analysed	Positive samples (incidence) %	% occurrence >1250 ($\mu\text{g kg}^{-1}$) of the positive samples	Lowest contamination level ($\mu\text{g kg}^{-1}$)	Highest contamination level ($\mu\text{g kg}^{-1}$)	Mean of the positive samples ($\mu\text{g kg}^{-1}$) (SD)	CV, %
DON	Intensive	75	97	11.65	43	2493	524 ± 581	111
	Sustainable	17	94	1.94	44	6804	835 ± 1591	191
	Organic	11	100	0	52	316	188 ± 81	93
3ADON	Intensive	75	84	0	21	1149	256 ± 198	78
	Sustainable	17	38	0	49	363	202 ± 104	52
	Organic	11	50	0	130	577	332 ± 202	147
15ADON	Intensive	75	85	0.97	21	1319	270 ± 252	93
	Sustainable	17	25	0	8,5	594	169 ± 248	61
	Organic	11	10	0	n.d.	98	98 ± 0	0

n.d.: not detected; CV: coefficient of variation.

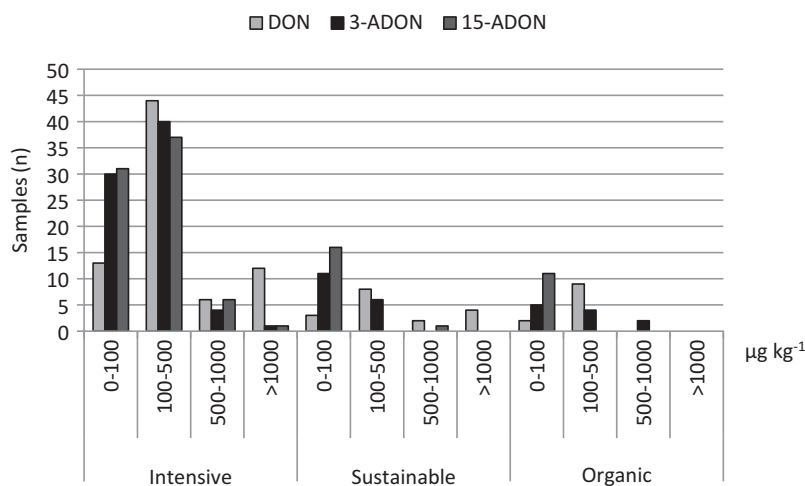
ADON and 15-ADON – 38% and 25%, respectively. One hundred percent of the organically grown grain samples were positive for DON, 50% were positive for 3-ADON and 10% were positive for 15-ADON. In Polish wheat grain samples, DON was detected in 80%, 3-ADON in 80%, and 15-ADON in 53% of the samples tested (Perkowski et al. 2012). According to Maresca (2013), next to DON, there are other masked forms of DON (deoxynivalenol-3-glucoside, 3,15-diacetyldeoxynivalenol) which have been little investigated so far.

From the viewpoint of food safety it is essential to be aware of which trichothecene predominates in grain samples, as sample toxicity depends on this. It is thought that 15-ADON is more toxic than DON and 3-ADON (Pinton et al. 2012). Pazderu et al. (2016) suggest that it is highly important to evaluate not only DON, but all its derivatives simultaneously. Our results in this study indicated that in the grain

samples from all three production systems tested, 15-ADON co-occurred with DON and 3-ADON. Samples contaminated with 15-ADON alone were not detected.

A trend was revealed showing that DON, 3-ADON and 15-ADON predominated at higher or lower concentrations in the grain samples from the intensive production system (Figure 1). In 14.7% of the samples DON concentration exceeded the requirements of the EU Commission's Regulation No. 1881/2006. The highest DON concentration amounted to 2493 $\mu\text{g kg}^{-1}$, 3-ADON—1149 $\mu\text{g kg}^{-1}$ and 15-ADON—1319 $\mu\text{g kg}^{-1}$.

Most of the grain samples (94%) grown in the sustainable production system were DON-positive and its concentration in one sample was as high as 6804 $\mu\text{g kg}^{-1}$ (Table 1). This concentration exceeded the requirements of the EU Commission's Regulation No. 1881/2006 more than 5-fold (EC 2006).

**Figure 1.** Prevalence of DON, 3-ADON and 15-ADON in spring wheat grain from different agricultural production systems: number of samples containing mycotoxins in a given concentration range ($\mu\text{g kg}^{-1}$).

The largest 3-ADON ($363 \mu\text{g kg}^{-1}$) and 15-ADON ($594 \mu\text{g kg}^{-1}$) concentrations were quantified in the sample with a DON concentration of $6804 \mu\text{g kg}^{-1}$.

Although 100% of the spring wheat grain samples from the organic production system were found to be contaminated with DON, its concentrations were lower than in the samples from the sustainable and intensive production systems and ranged from <100 to $316 \mu\text{g kg}^{-1}$. Only DON and 3-ADON predominated in the organic wheat grain samples, and only in one sample were traces of 15-ADON were detected. Compared with the grain samples from the intensive and sustainable production systems, organic grain samples differed not only in the mycotoxin contamination level but also in their composition. As the diversity of compositions of DON and its derivatives was lesser in the organic grain samples, this allows us to presume that DON toxicity and contamination level is generally lower in the grain from this production system. The published studies in which the occurrence and concentrations of DON in organic and conventionally produced grains and foods were compared showed a slight trend of more frequent occurrence and higher concentrations of DON in conventional products as compared to organic products. Nevertheless, relevant conclusions cannot be drawn about the differences in the occurrence and concentrations of 3-ADON, 15-ADON due to the lack of the data (EFSA 2017). Researchers in other countries warn that the level of contamination with DON and its derivatives, particularly 3-ADON and 15-ADON, may differ between years. It has been noticed that grain samples with higher DON concentrations contained higher concentrations of 3-ADON and 15-ADON as well (Ibanez-Vea et al. 2012).

Eighty-one percent of the spring wheat grain samples from the intensive production system were co-contaminated with a combination of DON+3-ADON+15-ADON, 1% with DON+3-ADON, while DON+15-ADON and DON were found in 5% and 10% of the tested samples, respectively. Two percent of the samples were free from mycotoxins (Figure 2 (a)). In the grain samples from the sustainable production system, DON and a combination of DON+3-ADON showed a higher incidence—47% and 23%, respectively. The samples with a combination of DON+3-ADON+15-ADON accounted for 18% (Figure 2(b)). Completely different results were

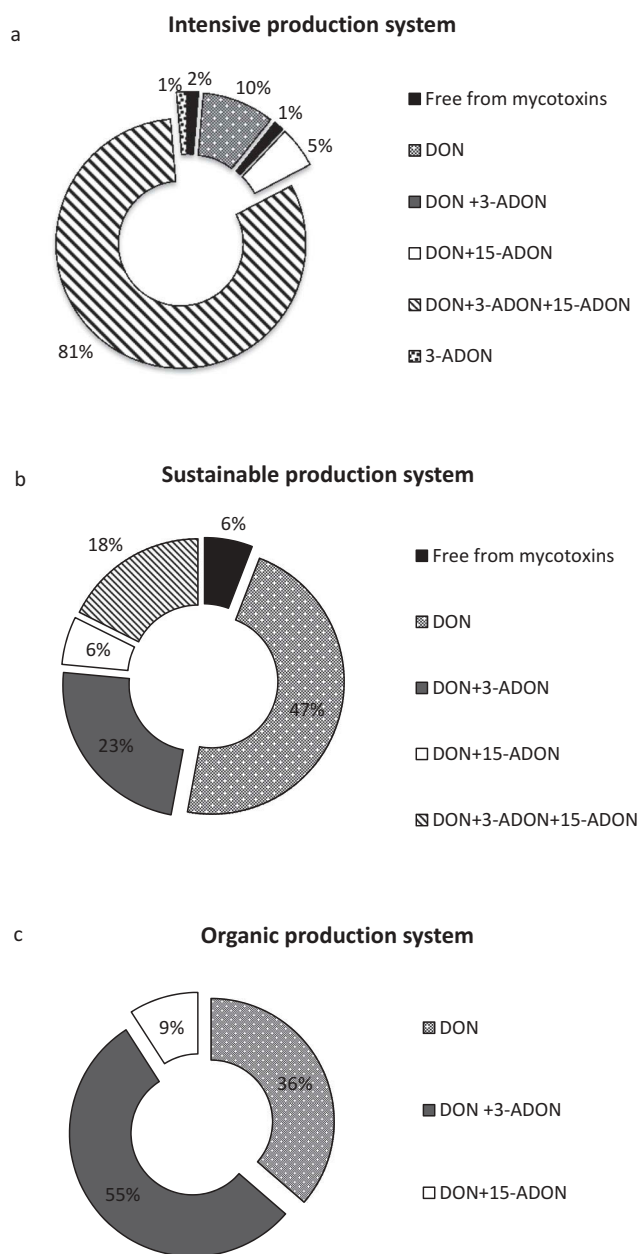


Figure 2. The distribution of spring wheat grain samples according to the composition of mycotoxins in the intensive (a) sustainable (b) and organic (c) production systems.

obtained for the organic grain samples (Figure 2 (c)). A large number of organic spring wheat grain samples (55%) were contaminated with DON+3-ADON, and 36% of the samples with DON alone. The combination of DON+3-ADON+15-ADON was not present, while DON+15-ADON was found in 9% of the samples tested.

In the present study, in several grain samples from the intensive and sustainable production systems DON co-occurred with 15-ADON and 3-ADON. However, we did not find any grain samples

containing 3-ADON alone in the sustainable and organic production systems. The samples from the intensive production system, contaminated by 3-ADON alone, constituted only 1%.

Most mycotoxins can act alone, but some also act synergistically with others causing more severe effects on human health. Ellner (2001) has reported that mycotoxin co-occurrence is quite common, especially in highly contaminated grain samples. Over 23% of the samples in the above study were contaminated with at least two mycotoxins.

Co-contaminations of cereals with DON and acetylated derivatives has been detected all over the world. Perkowski et al. (2012) detected co-occurrence of DON and 3-ADON in 63% of barley grain samples and DON and 15-ADON in 31% of samples. However, Tamburic-Illincic et al. (2015) did not find any grain samples containing 3-ADON and DON. Therefore it is recommended to monitor the occurrence of mycotoxins and changes in their composition.

In our study, grain samples contaminated with 15-ADON alone were not detected in any of the production systems studied; however, its presence in combination with DON and 3-ADON was detected in the grain from all production systems. Until now, DON and its derivatives have been considered equally toxic by health authorities. The high toxicity of 15-ADON should be taken into account when determining the maximum safe levels of the different trichothecenes in food/feed. From the toxicological point of view, the co-occurrence of several toxins from the same family may have additive effects (Speijers and Speijers 2004).

In our study, we calculated the correlation between DON and metabolites from different production systems. A highly significant positive correlation was found between DON and 15-ADON in sustainable production system ($r = 0.96$, $p \leq 0.05$). A significant positive correlation was also identified between DON and 3-ADON ($r = 0.567$, $p \leq 0.05$) and between 3-ADON and 15-ADON ($r = 0.577$, $p \leq 0.05$). A significant positive relationship was also identified between the 3-ADON concentration and 15-ADON concentration in the intensive production system ($r = 0.462$, $p \leq 0.05$). However, in the organic production system, no significant correlation was identified among the mycotoxins studied (Table 2).

There is still no clear consensus on the relationship between agricultural practices and mycotoxin

Table 2. Correlation between DON and its derivatives in different production systems.

	DON	3-ADON	15-ADON
Intensive production system			
DON	1.000	-0.353*	-0.132
3-ADON	-0.353*	1.000	0.462*
15-ADON	-0.132	0.462*	1.000
Sustainable production system			
DON	1.000	0.567*	0.960*
3-ADON	0.567*	1.000	0.577*
15-ADON	0.960*	0.577*	1.000
Organic production system			
DON	1.000	0.312	-0.149
3-ADON	0.312	1.000	-0.257
15-ADON	-0.149	-0.257	1.000

*- correlation is significant at the 0.05 level.

contamination. When a susceptible wheat cultivar is used, FHB severity increases and so do DON contents in grain (Abedi-Tizaki and Zafari 2015). Several researchers have reported higher trichothecene occurrence in traditionally grown grain samples, while others have shown higher contamination levels in organically grown samples or in sustainably grown samples (Ibanez-Vea et al. 2012). Sneller et al. (2012) indicate that agronomic, climate and several other factors affect the occurrence of FHB and DON accumulation in cereal grains. Based on the DON White Paper-Deoxynivalenol: Known Facts and Research Questions [online]), 15-ADON and 3-ADON were found only in the samples with high levels of DON. 3-ADON was found only in FHB-susceptible wheat cultivars. 15-ADON was detected only in the grain samples collected from the fields that had not been treated with fungicides or treated too early (before heading) for the fungicide to have been effective.

Conclusions

The analysis of spring wheat grain samples collected from the three production systems – organic, sustainable, and intensive revealed the co-occurrence of type-B trichothecenes. It is likely that the concentrations of trichothecenes DON, 3-ADON and 15-ADON in wheat grain were also influenced by the different growing conditions. The grain samples from the intensive and sustainable production systems contained higher concentrations of DON than those from the organic production system. Moreover, mycotoxin compositions differed between the production systems. Of the total grain samples tested, 78.6% were co-contaminated with two or more mycotoxins. The values

below the LOD or LOQ for DON, 3-ADON and 15-ADON in the intensive production system accounted for 52%, 60% and 60%, respectively, in the sustainable production system for 52.9%, 82.3% and 94.1% respectively, and in the organic production system for 81.8%, 72.7% and 100%, respectively. The largest diversity of DON, its derivatives and their compositions was identified in the grain samples from the intensive production system, in which 6 combinations of B-type trichothecenes were detected. The current study provided some new insights into the occurrence and co-occurrence of DON and its derivatives in relation to different grain production systems and validated the findings obtained by other researchers. From this study it could be concluded that further research needs to be done on the occurrence of mycotoxins in spring wheat grain in Lithuania as influenced by the production system in order to validate the obtained findings and to get new insights into the factors contributing to DON, 3-ADON and 15-ADON prevalence.

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