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Distribution of B type trichothecene producing *Fusarium* species in wheat grain and relation to mycotoxins DON and NIV concentrations

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Abstract

Fusarium head blight (FHB) is one of the most important cereal diseases causing yield losses and reducing its quality. B type trichothecenes (TRI) deoxynivalenol (DON) and nivalenol (NIV) are the main mycotoxins associated with FHB of wheat and other small-grain cereals. Usually, a particular *Fusarium* strain is able to produce only one type of B trichothecene. The detection of gene for NIV, DON and its acetylated derivatives 3-acetyldeoxynivalenol (3ADON) or 15-acetyldeoxynivalenol (15ADON) production is used for detection of FHB pathogens in plant material and their chemotyping.

The current study presents the distribution of DON and NIV mycotoxins and their potential producers in Lithuanian wheat grain grown in 2013 and 2014. Grain samples of spring wheat (114) and winter wheat (30) were collected from 49 farms situated in 12 administrative districts of Lithuania. *Fusarium* species were identified and quantified by the morphological and quantitative polymerase chain reaction (qPCR) techniques. DON concentrations were estimated by the enzyme-linked immunosorbent assay (ELISA) in all collected grain samples and NIV using ultra performance liquid chromatography coupled with mass spectrometry (UPLC/MS) in 17 arbitrarily selected samples. *F. graminearum*, *F. culmorum* and *F. poae* were identified as species capable of producing mycotoxins DON and NIV in wheat grain. The highest DON quantities were identified in the grain of spring wheat grown in 2013 and this was clearly linked to *F. graminearum* DNA quantities ($r = 0.783$, $p < 0.01$). *F. poae* stood out as a potential NIV producer in the grain of Lithuania-grown wheat, since a positive correlation ($r = 0.62$, $p < 0.01$) between the quantities of *F. poae* DNA and NIV concentrations was established. *F. culmorum* was detected in unexpectedly small quantities in wheat grain and was found to be the potential producer of DON, but not NIV.

Key words: chemotype, *Fusarium graminearum*, *Fusarium poae*, qPCR, UPLC/MS.

Introduction

Fusarium head blight (FHB) of small-grain cereals is an important issue in all cereal growing regions due to its relation to reduced yield quality and quantity (Foroud, Eudes, 2009; Wegulo, 2012; Becher et al., 2013; Gilbert, Haber, 2013; Matny, 2015). In Northern Europe, FHB is mostly associated with *Fusarium graminearum* infections, but species of *F. culmorum*, *F. avenaceum*, *F. cerealis*, *F. poae*, *Microdochium nivale* and some other less significant ones are also known to contribute to manifestations of this disease (Parry et al., 1995; Waalwijk et al., 2003; Yli-Mattila, 2010; Nielsen et al., 2011; Parikka et al., 2012). In Lithuania, FHB used to pose a minimal threat to cereals and *F. graminearum* was nearly absent until 2012 (Mačkinaite et al., 2006; Supronienė et al., 2010), when the disease reached epidemic scale and since then has persisted as the major problem in cereals, especially in spring wheat.

FHB is usually closely interlinked with the accumulation of mycotoxins DON and NIV (Becher et al., 2013). Not all the FHB-causing species produce B type trichothecenes (TRI) – this ability is restricted to *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. poae* and *F. equiseti*. Of these species *F. graminearum* and *F. culmorum* are known to produce both DON and NIV, while others are associated only with NIV contaminated grain (Bottalico, Perrone, 2002; Moss, Thrane, 2004; Yli-Mattila, 2010). Chemotype characterization of the *F. graminearum* and *F. culmorum* has been used for determination of their capacity to produce NIV, DON and its acetylated derivatives 3-acetyldeoxynivalenol (3ADON) or 15-acetyldeoxynivalenol (15ADON) (Desjardins, 2006).

DON is found in higher concentrations and therefore is seen as a bigger threat to food safety than NIV

(Bottalico, Perrone 2002; Desjardins, 2006). Naturally, DON contamination of grain and its producers have been receiving more attention than NIV and its producers. Several studies on this issue have revealed that DON contamination is common in Lithuania-grown wheat, but concentrations usually did not exceed critical $1250 \mu\text{g kg}^{-1}$ up until 2012, when 8 samples with DON concentrations with two to seven times ($2150\text{--}8845 \mu\text{g kg}^{-1}$) over the permissible limit were detected (Mankevičienė et al., 2007; 2011; 2014).

The research on NIV in Lithuania is in quite a different situation: until now, direct detection and quantification of NIV in grain have not been attempted and the study on the presence of potential NIV producer in wheat has indicated that a very minor part (<1%) of overall *F. graminearum* and no part of *F. culmorum* / *F. cerealis* could contribute to possible wheat contamination with this mycotoxin (Suproniene et al., 2016). Of the other *Fusarium* species known to produce NIV, *F. poae* is annually recovered from Lithuania-grown wheat grain in significant quantities (Mačkinitė et al., 2006; Sakalauskas et al., 2014). The potential of *F. poae* to produce NIV cannot be determined by qPCR methods developed for quantification of potential B-type TRI producing *F. graminearum*, *F. culmorum*, *F. cerealis* and *F. pseudograminearum* (Ward et al., 2002; Nielsen et al., 2012). Therefore in our study possible correlational ties between the presence of *F. poae* DNA and NIV in wheat grain had to be performed by separate analyses.

With the ongoing FHB epidemic in the fields, monitoring of mycotoxins and their sources in grain has become as important as never before. The aim of this study was to evaluate the distribution of B type trichothecenes (TRI) producing *Fusarium* species in wheat grain and their relation to mycotoxins DON and NIV contamination.

Material and methods

Collection of grain samples. Grain samples, including 114 of spring wheat and 30 of winter wheat were collected in 49 commercial fields all over Lithuania in 2013 and 2014 (Fig. 1). Grain samples of 1.0 kg were taken after harvesting and sub-samples were stored in plastic jars in a freezer at -20°C until analysis.

Grain infection with *Fusarium* fungi. *Fusarium* species capable of producing B type trichothecenes (TRI) mycotoxins in grain were detected by plating surface sterilized wheat grain on potato dextrose agar (PDA) (“Merck”, Germany) and subsequent visual and microscopic analysis of morphological features according to Leslie et al. (2006).

Deoxynivalenol (DON) analysis. DON content in grain was estimated by a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) method (Wilkinson et al., 1992). Quantitative test kits Veratox® (“Neogen”, UK) were used for the analysis. The optical densities of the samples and controls from the standard curve were estimated by a photometer Multiskan Ascent (Thermo Electron Corp., Finland) using a filter of 650 nm.

Measured absorbances were automatically converted into mycotoxin concentration $\mu\text{g kg}^{-1}$. Since the method’s limit of detection is $100 \mu\text{g kg}^{-1}$ DON, samples with less than $100 \mu\text{g kg}^{-1}$ DON were regarded as negative. All the analyses were duplicated.

Nivalenol (NIV) detection and quantification by ultra performance liquid chromatography coupled with mass spectrometry (UPLC/MS). A total of 17 samples were arbitrarily selected for NIV analyses (Fig. 1). The samples were divided into four groups: group 1 – no NIV chemotype *F. graminearum* DNA, no internal *F. poae* infection (negative control group); group 2 – positive internal *F. poae* infection, but no NIV chemotype *F. graminearum* DNA; group 3 – positive NIV chemotype *F. graminearum* DNA, but no internal *F. poae* infection; group 4 – positive both NIV chemotype *F. graminearum* DNA and internal *F. poae* infection. Chemotype incidence in wheat grain samples has been previously presented by Suproniene et al. (2016).

For NIV extraction 3 g of homogenized grain was mixed with 12 mL acetonitrile – water (84:16) and shaken on an orbital shaker at 225 rpm for 90 min at room temperature. 6 mL of the extract was filtered through paper filter (Whatmann No. 1), extracted through MycoSep® 230 Niv push through clean-up column (Rommer Labs Incorp., Austria), 3 mL transferred into two 2 mL Eppendorf tubes (1.5 mL each) and evaporated to dryness in a centrifugal evaporator at 30°C 6000 rpm. The residue was reconstituted in 1 mL (2×0.5 mL combined into single sample) ddH₂O, filtered through $0.22 \mu\text{m}$ nylon syringe filter and subjected to UPLC/MS analysis. NIV separation was carried out using ultra high performance liquid chromatography ACQUITY UPLC H-Class CM Core System (“Waters”, USA) coupled with a mass spectrometry detector Xevo G2-XS QToF (“Waters”, USA). Chromatographic separations were performed on a reversed-phase C18 column (50×2.1 mm inner diameter, particle size $1.7 \mu\text{m}$). For chromatographic separation of NIV two mobile phase components were used: A – water with 0.1% formic acid and B – pure LC/MS grade acetonitrile. The flow rate

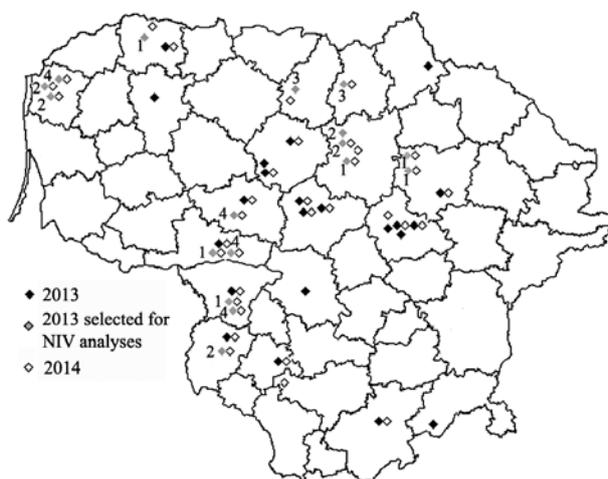


Figure 1. Grain sample collection sites in 2013 and 2014

was set to 0.5 ml min⁻¹, the injection volume was 10 µl. Binary gradient was as follows: 0 min 1% B, 3.0 min 50% B, 3.1 min 95% B and hold 95% B to 3.6 min, 3.7 min back to beginning at 1% B and hold to 5 min.

Mass spectrometer was used with negative electro-spray ionization (ESI)-mode with the following settings: source temperature – 120°C, desolvation temperature – 650°C, desolvation gas flow – 1000 Lh⁻¹, cone gas flow – 100 Lh⁻¹, capillary voltage – 1 kV, cone voltage – 40 V, collision energy – 6 V. System control was carried out using software *Masslynx V4.1*. Quantitative analysis of NIV was applied in MS resolution ESI mode. NIV was identified by retention time and m:z ratio. NIV concentration in wheat grain was calculated using NIV standard solution calibration curve ($R^2 = 0.996$), in which tenfold NIV dilutions with concentrations 0.01 to 100 ng mL⁻¹ were used.

DNA extraction. 10 g of each grain sample was ground to a fine powder in liquid nitrogen using a ball mill. DNA was extracted from 0.1 g of homogenized grain using FastDNA Kit (MP Biomedicals, Germany), DNA concentration was measured by Biophotometer (Eppendorf, Germany) and by electrophoresis in 1% agarose gel with MassRuller DNA Ladder Mix (Thermo Fisher Scientific, Lithuania) as a standard.

Quantification of *Fusarium poae* DNA. Primers FpoeA51 fwd (ACCGAATCTCAACTCCGCTTT) and FpoeA98 rev (GTCTGTCAAGCATGTTAGCACAAGT) were used for quantification of *F. poae* DNA in grain samples (Nicolaisen et al., 2009). DNA of pure culture *F. poae*

fungus (verified by qPCR with the same primers) was used for standard curve dilution series ($R^2 = 0.987$). Wheat leaf DNA was used for adjustment of fungal DNA quantity in each grain sample (Nicolaisen et al., 2009). qPCR was done in 12.5 µL total volume: 6.25 µL 2 × SYBR Green PCR Master Mix (Applied Biosystems, USA), 300 nM of forward and reverse primers, 0.4 µg µL⁻¹ bovine serum albumin (BSA) (Thermo Fisher Scientific, Lithuania) and 2.5 µL template DNA. Samples were duplicated. Reactions were carried out on the 7900HT Sequence Detection System (Applied Biosystems, USA). Cycling protocol: 2 min at 50°C and 95°C for 10 min; followed by 40 cycles with 95°C for 15 s and 62°C for 1 min followed by dissociation curve analysis at 60°C to 95°C.

Methods of statistical data analysis. Basic statistics and correlation analysis (Pearson correlation, 2-tailed significance test) were performed using the software *IBM SPSS Statistics 20*.

Results

Fusarium infection level in spring wheat grain was twice or more as high as that in winter wheat (Table 1). In wheat grain of both seasonal types *F. graminearum* and *F. avenaceum* were dominant species, followed by *F. poae*, *F. culmorum* and *F. sporotrichioides*, *F. tricinctum*, *F. equiseti* and some other species were rarely found (only data of potential TRI producers are presented in this paper).

Table 1. *Fusarium* infection level and deoxynivalenol (DON) concentrations in wheat grain samples in 2013 and 2014

Description	<i>Fusarium</i> infected grain %				DON concentrations µg kg ⁻¹
	<i>Fusarium</i> spp.	<i>F. graminearum</i>	<i>F. culmorum</i>	<i>F. poae</i>	
Spring wheat, 2013, n = 64					n = 64
Mean	45.3	12.3	0.2	4.3	1163.8
Sx	3.4	1.6	0.1	0.7	246.8
Min (>0)	0.8	0.8	0.8	0.8	<LOD
Max	96.7	49.2	1.7	25.8	10644.0
Spring wheat, 2014, n = 50					n = 49
Mean	40.7	15.9	0.6	12.2	322.0
Sx	3.6	3.1	0.2	2.5	63.7
Min (>0)	2.5	0.8	0.8	0.8	108.0
Max	100.0	95.0	5.8	79.2	2401.0
Winter wheat, 2013, n = 15					n = 11
Mean	23.4	2.6	0.2	3.9	391.9
Sx	6.4	0.6	0.1	0.9	72.6
Min (>0)	2.5	0.8	0.8	1.6	224.0
Max	90.0	6.7	2.0	13.3	784.6
Winter wheat, 2014, n = 15					n = 9
Mean	8.7	1.8	0.1	1.8	374.3
Sx	2.7	0.7	0.1	1.0	152.2
Min (>0)	0.0	0.8	0.8	0.8	<LOD
Max	39.2	8.3	0.8	14.2	1393.0

LOD – limit of detection (100 µg kg⁻¹)

Of the species capable of producing B type TRI (DON and/or NIV), *F. graminearum*, *F. culmorum* and *F. poae* were detected in wheat grain. *F. graminearum* and *F. poae* were found in 89% and *F. culmorum* – in 35% of the total spring wheat samples tested. In 2013, infection level of *F. poae* was much lower than that of *F. graminearum*; however, in 2014 it became comparable. In winter wheat *F. graminearum* was detected in 60%

of the grain samples tested, *F. poae* – in 57% and *F. culmorum* – in 17%. In 2013, *F. poae* infection level in winter wheat grain was slightly higher than that of *F. graminearum* (Table 1).

DON was found in 96.7% of spring wheat and 91.0% of winter wheat samples tested in 2013 and in 67.3% of spring wheat and 88.9% of winter wheat samples in 2014. The average DON concentration

Table 2. Correlations between *Fusarium graminearum* and *F. culmorum* infection level, DNA quantities (ng μg^{-1} plant DNA) and deoxynivalenol (DON) concentrations in spring wheat grain in 2013 and 2014 (n = 114)

Target	<i>F. graminearum</i> %	<i>F. culmorum</i> %	<i>F. graminearum</i> DNA	<i>F. culmorum</i> DNA
<i>F. culmorum</i> %	0.476**			
<i>F. graminearum</i> DNA	0.294**	0.017		
<i>F. culmorum</i> DNA	0.094	0.004	0.414**	
DON $\mu\text{g kg}^{-1}$	0.351**	-0.006	0.783**	0.401**

** – significant at the 0.01 level

in spring wheat in 2013 was 1163.8 $\mu\text{g kg}^{-1}$ (0 to 10644.0 $\mu\text{g kg}^{-1}$), about three times higher than in 2014 (average of 322.0 $\mu\text{g kg}^{-1}$) or in winter wheat (average of 391.9 $\mu\text{g kg}^{-1}$ in 2013 and 374.3 $\mu\text{g kg}^{-1}$ in 2014). The DNA quantities of *F. graminearum* and *F. culmorum* are presented in Suproniene et al. (2016), where large variation between and within spring and winter grain samples is shown. Correlations between DNA quantity of these two species and DON are stronger than between internal grain infection determined by plating and DON (Table 2).

F. poae DNA quantities in positive samples ranged from 1.9 to 148.1 $\mu\text{g kg}^{-1}$ wheat DNA. Five of the 17 tested grain samples were found to be free of *F. poae*. Variable quantities of *F. poae* DNA were detected in all samples from which *F. poae* was isolated during internal grain mycobiota survey. Five samples that were deemed free of *F. poae* in internal grain infection contained low quantities of *F. poae* DNA (Fig. 2 A, B).

All but four samples were found to be NIV positive (Fig. 2 C). In two of these negative samples no *F. poae* DNA was detected, the other two contained very small amounts of the *F. poae* DNA. Three of the four NIV negative samples were found to be *F. poae*-free in internal grain mycobiota survey. NIV concentrations were in the range between 0.5 and 63.5 $\mu\text{g kg}^{-1}$ wheat grain. The highest NIV concentrations were detected in samples 29 and 45, which contained both *F. poae* and NIV chemotype *F. graminearum* DNA. Samples 21, 47 and 57, that contained somewhat larger quantities of NIV are associated with *F. poae* but not NIV chemotype *F. graminearum*. Only two samples of the supposedly control group 1, namely 20 and 61, were found to be free of *F. poae* DNA and NIV. The other four samples of this group contained either NIV (sample 9), or *F. poae* DNA (sample 12), or both (samples 41 and 47).

Positive correlation ($r = 0.81, p < 0.01$) between *F. poae* DNA quantity and NIV concentration in grain was observed. No significant correlation was identified

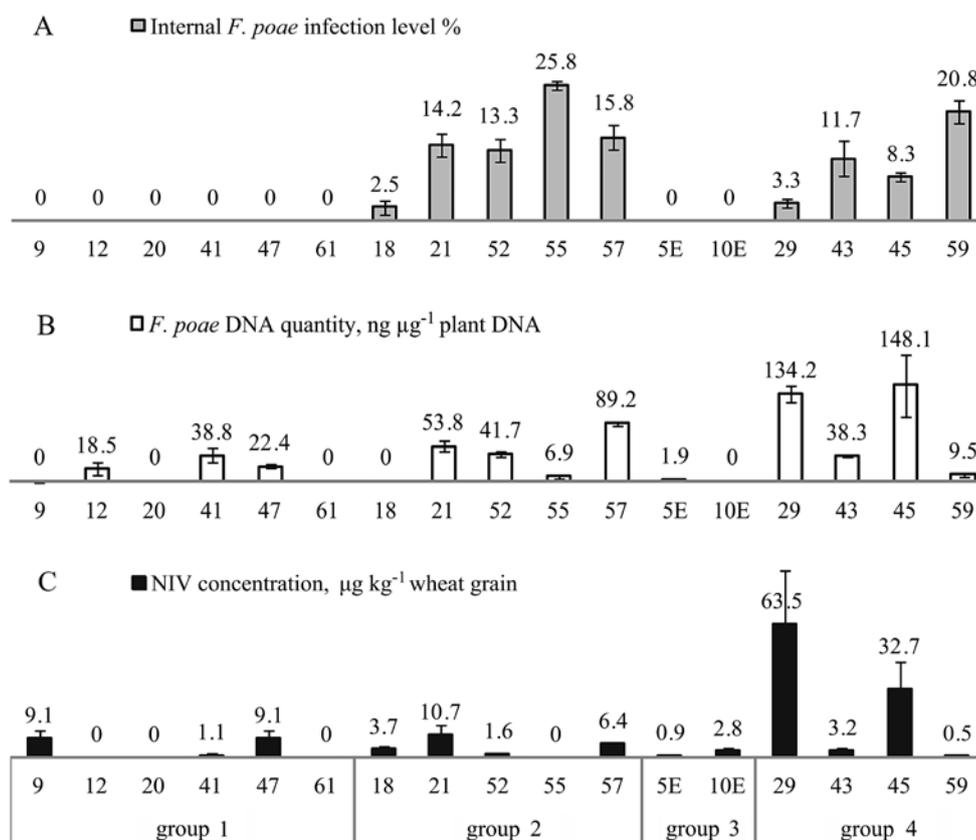
between internal *F. poae* grain infection determined by classic microbiology methods and neither *F. poae* DNA quantity ($r = 0.18$) nor NIV concentration ($r = -0.08$) was detected (Table 4).

Discussion

This study, supported by the results from Suproniene et al. (2016), concluded that two *Fusarium* species, namely *F. graminearum* and *F. poae*, are the key producers of DON and NIV mycotoxins in Lithuania-grown wheat. The third potential TRI producer *F. culmorum* was found to be a rather occasional or secondary pathogen to *F. graminearum* rather than the main cause of FHB epidemics in the fields.

Despite somewhat similar incidence levels in grain, *F. graminearum* should be viewed as far greater threat to wheat than *F. poae*. This is partly due to limitations of internal grain mycobiota surveys, which do not represent the actual amounts of fungus in grain. As a result, *F. graminearum* DNA quantities (Suproniene et al., 2016) and subsequently DON concentrations in grain tend to be times higher than those of *F. poae* and NIV, even when percentage of infected grain is very similar. Differences in toxin production in grain could be related to certain fungal species or strain capacity and their special need for environmental conditions (Leslie et al., 2006; Stenglein, 2009). At least partly this should be due to host susceptibility. Nielsen et al. (2011) indicated clear differences in the ratio between the NIV producers and the crop species: the *F. graminearum* or *F. culmorum*: *F. poae* ratio in wheat varies from 10:1 to 100:1, whereas in barley – from 2:1 to 1:3.

Meteorological conditions are another important issue for host colonization and mycotoxin production. *F. graminearum* incidence in grain was comparable in 2013 and 2014, but DON concentrations in grain were significantly lower in a cooler summer of 2014. NIV quantities have not been estimated in grain samples



Note. Group 1 – no NIV chemotype *F. graminearum* DNA and no internal *F. poae* infection; group 2 – positive internal *F. poae* infection, but no NIV chemotype *F. graminearum* DNA; group 3 – positive NIV chemotype *F. graminearum* DNA, but no internal *F. poae* infection; group 4 – positive both NIV chemotype *F. graminearum* DNA and internal *F. poae* infection

Figure 2. Results of internal *Fusarium poae* infection, *F. poae* DNA quantity and nivalenol (NIV) concentrations in the tested samples

Table 4. Correlations between *Fusarium poae* DNA quantities, internal *F. poae* grain infection and nivalenol (NIV) concentrations

Target	<i>F. poae</i> DNA quantity	NIV concentration
NIV concentration	0.81**	
Internal <i>F. poae</i> grain infection	0.18	-0.08

** – significant at the 0.01 level

harvested in 2014. Since most of NIV is clearly produced by *F. poae*, mycotoxin quantities in grain could have been higher, as *F. poae* is a well-established pathogen in Nordic countries and is able to thrive in relatively cool summers (Stenglein, 2009).

In the present study we focused on *F. poae* as one of the most frequently isolated FHB pathogens (Xu et al., 2005; Mankevičienė et al., 2007; Yli-Mattila et al., 2008; Stenglein, 2009; Sakalauskas et al., 2014). Previously we have determined that the other supposedly major wheat pathogens *F. culmorum* / *F. cerealis* play an insignificant role in production of DON and are not a producer of NIV in wheat (Supronienė et al., 2016). This effectively leaves only *F. poae* as an alternative source of NIV – the other FHB causing *Fusarium* species either do not produce B-type TRI or are found

in negligible numbers (Mankevičienė et al., 2007; Supronienė et al., 2012; Sakalauskas et al., 2014). *F. poae* is more widespread in cereals in Northern Europe and some studies show correlation between *F. poae* and NIV presence in these crops (Bottalico, Perrone, 2002; Nielsen et al., 2011). Our study indicates that *F. poae* infection and NIV accumulation do not pose an immediate threat to Lithuanian wheat. NIV quantities in grain were times lower than those of DON and went well under the acceptable limit of 1250 µg kg⁻¹. According to manufacturer description, the output of MycoSep® 230 Niv push through clean-up column should be no less than 80% of the initial mycotoxin concentration. Considering results obtained, even if 20% of mycotoxin is lost during sample preparation, the NIV concentrations remain quite low (Fig. 2).

Only one NIV chemotype *F. graminearum* positive sample 10E (group 3) did not contain *F. poae* DNA. This indicates at least two potential NIV contamination sources in grain. In addition, sample 9 (group 1) contained NIV (mycotoxin) but no *F. poae* or NIV chemotype *F. graminearum*. Since the primers used for the detection of NIV chemotype DNA should also detect NIV chemotype of species *F. culmorum*, *F. cerealis* and *F. graminearum* (Nielsen et al., 2012), the NIV in sample 9 must be associated with yet another species, capable of producing NIV. The highest NIV concentration and the highest *F. poae* DNA quantity coincided to be in the same samples 29 and 45 of group 4. This means that the main actual source of NIV in those samples cannot be pointed out accurately as it is NIV chemotype *F. graminearum* DNA positive as well. Other samples with the highest NIV concentrations 21, 47 and 57 were found to be free of NIV chemotype *F. graminearum* DNA and also had some of the highest *F. poae* DNA quantities (Fig. 2).

Absence of correlation between *F. poae* DNA or NIV quantities in grain and the incidence of *F. poae* in grain obtained by agar plating methods demonstrate the limits of these methods in detection of potential mycotoxin producers. But there was one exception in our study: sample 18 was found to be free of *F. poae* DNA, but not of NIV. Sample group 2 to which it belongs contains grain samples with internal *F. poae* infection. The determination of *F. poae* infection by qPCR assay by default should be much more accurate than by plating on agar medium, but peculiarly it was not in this case. Misidentification of the fungal cultures growing from the plated seeds is probable, but then *F. poae* should have been confused with *Fusarium* species capable of producing NIV, of which none morphologically is too similar to *F. poae* (Moss, Thrane, 2004; Jestoi et al., 2008).

Conclusions

1. In 2013 and 2014, the main *Fusarium* species capable of producing B type trichothecenes (TRI) in Lithuania-grown wheat were *F. graminearum*, *F. poae* and *F. culmorum*, infecting on average 13.7, 8.2 and 0.4 % of spring wheat and 2.2, 2.9 and 0.2 % of winter wheat grain, respectively.

2. The DNA quantity of *F. graminearum* and *F. culmorum* in grain had stronger correlations with deoxynivalenol (DON) quantity ($p < 0.01$) than the internal grain infection; this demonstrates the advantages of qPCR technique over the morphological plating method.

The positive correlation determined between mycotoxin nivalenol (NIV) and *F. poae* DNA quantities in grain ($r = 0.81^{**}$) indicates that Lithuania-grown wheat is the source of at least two NIV producers – *F. poae* and *F. graminearum*.

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B tipo trichotecenų produkuojančių *Fusarium* genties grybų pasiskirstymas kviečių grūduose ir ryšys su mikotoksinų DON bei NIV koncentracijomis

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Santrauka

Varpų fuzariozė yra viena pagrindinių varpinių javų ligų, mažinančių grūdų derlių ir kokybę. Trichotecenai (TRI) deoksivalenolis (DON) ir nivalenolis (NIV) yra pagrindiniai mikotoksinai, susiję su kviečių ir kitų smulkiagrūdžių javų varpų fuzarioze. Straipsnyje pateiktas mikotoksinų DON bei NIV ir jų potencialių producentų pasiskirstymas Lietuvoje užaugintų kviečių grūduose 2013 ir 2014 m. Tyrimų laikotarpiu surinkta ir ištirta 114 vasarinių kviečių ir 30 žieminių kviečių grūdų mėginių iš 49-ių ūkių, esančių 12-oje Lietuvos administracinių rajonų. *Fusarium* rūšys identifikuotos ir kiekybiškai įvertintos morfologiškai ir taikant kiekybinį PGR metodą. DON koncentracijos įvertintos imunofermenčiu (ELISA) metodu, NIV – taikant efektyviają skysčių chromatografiją ir masių spektrometriją (UPLC/MS).

Kviečių grūduose kaip potencialios mikotoksinų DON ir NIV producentės identifikuotos *F. graminearum*, *F. culmorum* ir *F. poae* rūšys. Mikotoksino DON kiekis stipriai koreliavo su *F. graminearum* DNR kiekiu ($r = 0,783$, $p < 0,01$) ir buvo didžiausi 2013 m. užaugintų vasarinių kviečių grūduose. *F. poae* išryškėjo kaip potenciali mikotoksino NIV producentė Lietuvoje užaugintuose kviečiuose, nes nustatyti esminiai koreliaciniai ryšiai ($r = 0,62$, $p < 0,01$) tarp *F. poae* DNR kiekių ir NIV koncentracijų. *F. culmorum* rūšies kviečių grūduose aptikta labai mažas kiekis, ir ji pasižymėjo kaip potenciali DON, o ne NIV producentė.

Reikšminiai žodžiai: chemotipas, *F. graminearum*, *F. poae*, kiekybinė PGR, UPLC/MS.

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