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Do black dots on wheat grains have an impact on deoxynivalenol accumulation?

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Abstract

A study, conducted at Lithuanian Research Centre of Agriculture and Forestry in 2016 and 2017, addressed the issue of black dots on common wheat (*Triticum aestivum* L.) grain, which had not been widely spread in Lithuania before 2016. This problem has provoked a lot of discussion among grain growers, purchasers, processors, scientists and consumers. According to the quality requirements for purchase and supply of wheat grain, visually estimated *Fusarium*-damaged grain (causal agents – fungi of *Fusarium* genus) in a sample must not exceed 1%. The objective of the study was to ascertain which of the *Fusarium* species cause black dots on wheat grain, to determine and compare deoxynivalenol (DON) contamination levels in the grain with different visually estimated *Fusarium* damage severity, and to verify whether black-dotted grain can be stored without posing the risk of increasing DON concentration. Black dots on grain were identified as the *Fusarium graminearum* teleomorph *Gibberella zeae* fruit bodies of ascospores producing perithecia. In our study, black dots were detected in 85% of the tested wheat grain samples collected from various commercial enterprises of Lithuania. The study showed the highest DON concentrations to be present in the samples with *Fusarium*-damaged grain exceeding 1%, while in the black-dotted grain samples DON concentrations were low. Grain samples for the storage experiment were selected according to the abundance of black-dotted and *Fusarium*-damaged grain with a moisture content ranging from 19.0% to 19.7%. Black dots on grain did not cause an increase in DON concentrations in grain samples, while the samples with more than 1% visually estimated *Fusarium*-damaged grain showed higher DON concentrations. The study findings suggest that grain damage by black dots, higher moisture content; storage temperatures (4, 16, 20 and 28 °C) had no significant effect on the increase in DON concentrations. However, it is risky to store grain of higher moisture when the amount of visually estimated *Fusarium*-damaged grain in the sample exceeds 1%. A significant increase in DON concentrations was recorded in such samples. Although DON concentrations in black-dotted grain samples were low, such grains are arguably a source of pathogens and need to be treated with a high degree of responsibility and thoughtfulness.

Keywords: black-dotted grain, *Fusarium*-damaged grain, deoxynivalenol, wheat grain.

Introduction

Cereals are a primary source of a human diet, with wheat being the third most produced grain worldwide. The cereal-infecting species *Fusarium graminearum* (teleomorph *Gibberella zeae*), *F. culmorum* and *F. pseudograminearum* produce a range of type B trichothecenes, including deoxynivalenol (DON) and its acetylated derivatives, which inhibit protein translation in eukaryotes that are known virulence factors on wheat (Kelly, Ward, 2018). *Fusarium graminearum* (sexual stage *Gibberella zeae*) is the causal agent of head blight of common wheat (*Triticum aestivum* L.). This disease can completely destroy potentially high-yielding crops within a few weeks of harvest (Keller et al., 2014; Paraschivu et al., 2014). The loss of billions of dollars in Asia, Europe, South and North America and Australia was inflicted by the disease due to reduced yield and price discounts from lowered grain quality (McMullen et al., 2012; Keller et al., 2014; Matny, 2015). In recent years, many *Fusarium* species have emerged, which now threaten the productivity and safety of small grain cereal crops worldwide. During floral infection and post-harvest on stored grains, *Fusarium* spp. produce various types of harmful mycotoxins, which subsequently contaminate food and feed products (Lowe et al., 2012). Although head blight causes low grain weight, the primary economic and health consequences of the disease are due to mycotoxin contamination, primarily DON (Trail, 2009). The European Community (European Commission, 2006) has established DON as a contamination marker. DON maximum level was set at 1250 μg kg^{-1} for unprocessed

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cereal grain and 750 μg kg^{-1} for cereals intended for direct human consumption, cereal flour, bran and germ as end products marketed for direct human consumption.

F. graminearum is a genetically diverse species, with eleven distinct lineages currently known as the FG complex (Qu et al., 2008). There is little gene flow within these lineages, and all are well suited to infect their hosts in warm and wet climates (Hope et al., 2005). According to Qu et al. (2008), one lineage in China can begin infection below 15°C, and all lineages can overwinter on crop debris in any climate wheat is grown. This leaves high risk of continual infection, especially with rotations of less than two years between host crops.

Research has shown high genetic variation of aggressiveness among isolates of *F. graminearum* (Walker et al., 2001; Zeller et al., 2003; Cumagun et al., 2006; Schmale et al., 2006). In some cases *F. graminearum* populations were found to be highly pathogenic and to produce high levels of DON indicating future threat of infection (Walker et al., 2001; Keller et al., 2014). *F. graminearum* ascospores were reported to lose the ability to germinate with exposure to low (50%) relative humidity suggesting limited infectivity after long-distance dispersal in these environmental conditions (Beyer, Verreet, 2005; Keller et al., 2014). However, Beyer et al. (2005) found ascospores to require 53% relative humidity while macroconidia required 80% relative humidity for germination. Light may be linked to perithecial formation and ascospore discharge (Trail et al., 2002). Several reports indicate that the ascospores may be more important for head blight epidemics than the macroconidia because the inoculum for head blight requires aerial dispersal to the wheat heads (Sutton, 1982) and the ascospores can be forcibly discharged into the air from perithecia. Beyer and Verreet (2005) demonstrated the importance of the ascospores for disease severity and mycotoxin level by deleting the mating type locus that controls the sexual reproduction of *G. zeae* and concluded that control strategies should target the ascospores. Airborne ascospores produced by *G. zeae*, the sexual stage of *F. graminearum*, fall on flowering spikelets of wheat, germinate and enter the plant through natural openings, such as degrading anther tissue or stomates (Bushnell et al., 2003; Trail, 2009). The fungus then grows, spreading through the xylem and pith of the wheat. As colonization continues, tissue becomes bleached and necrosis occurs.

The purpose of conservation tillage (no-till or reduced tillage) is to prevent soil erosion by leaving crop residue on the soil surface after harvest. The increasing adoption of conservation tillage practices (Dill-Macky, 2008; Keller et al., 2014) creates an increase in the amount of crop residue available for *F. graminearum* infection.

Although *F. graminearum* is arguably dangerous due to the production of DON and other mycotoxins, there is little information on how *G. zeae* produced perithecia (black dots) on grain affect grain quality indicators and variation of DON concentrations. Furthermore, there is a paucity of data on the storage of such grain, particularly if it has been harvested at a higher moisture level. Zhang et al. (2016) suggest that conversion of DON and its derivatives may occur during storage. This study proved that mycotoxin retention levels in wheat grain during storage are closely related to the state of grain and storage temperature.

It is known that DON can be degraded or detoxified into various derivatives by acetylation (Berthiller et al., 2005; Kushiro, 2008; Karlovsky, 2011; Warth et al., 2012), which can complicate DON detection during grain storage and processing. This is an interesting issue to be addressed in our future studies.

The principal objective of the present study was to identify, which plant pathogen is responsible for black dots on grain, to determine and compare DON contamination of grain differing in visually estimated black dot damage and to ascertain if black-dotted grain can be stored without causing the risk of increasing DON concentrations.

Materials and methods

Sample collection and stages of the study. Seventy nine 1-kg samples of common wheat (*Triticum aestivum* L.) grain were collected from various grain silos in Lithuania from 2016 and 2017 crop years, according to the standard methodology LST EN ISO 20333:2010 (Cereals and cereal products - Sampling). Mycotoxin analyses were conducted at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The samples were stored at −18°C until analyses.

The study was done in three stages: 1) assessment of black-dotted grain and *Fusarium*-damaged grain in wheat samples, 2) analyses of deoxynivalenol (DON) in BDG and FDG samples and 3) grain storage experiment. The moisture content of wheat grain samples, determined by an Infratec 1241 (Foss, Denmark), ranged between 12.9–13.2%.

Black-dotted grain (BDG) and Fusarium-damaged grain (FDG) in wheat samples. Wheat grain samples were divided into four groups: visually healthy grain, FDG, BDG and a composite sample. The FDG and BDG were analysed according to the standard methods LST 1524:2003/2K:2014 (Wheat - Requirements for purchase and supply), LST EN 15587:2008+A1:2014 (Cereals and cereal products - Determination of Besatz in wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), rye (*Secale cereale* L.) and feed barley (*Hordeum vulgare* L.)) and LST ISO 13690:2007 (Cereals, pulses and milled products - Sampling of static batches).

Grain infection with Fusarium spp. fungi. An agar plate method was used for the estimation of internal wheat grain infection (n = 33). *S*urface-sterilized (for 3 minutes in 1% NaOCl solution) grains were plated in Petri dishes with a potato dextrose agar (PDA) and incubated for 7–14 days at 26 ± 2°C in the dark (Mathur, Kongsdal, 2003). The overgrown *Fusarium* colonies were isolated, purified and identified according to the manuals of Nelson et al. (1983) and Leslie and Summerell (2006). The morphological identification of *Fusarium* spp. fungi was carried out using an optical microscope Nicon Eclipse E 200 (Japan). Fungal colonies were identified and the contamination percentage was estimated according to the number of contaminated grain. Perithecium formation and ascospore discharge in *Fusarium graminearum* were estimated using an optical microscope Nicon Eclipse E 200 with an image documentation system and Olympus SZX10 (Japan) with Camera Imaging (Canada).

Deoxynivalenol (DON) analysis in wheat samples. The samples were analysed for DON contamination by enzyme linked immunosorbent assays

(ELISA). The concentration of DON was determined using competitive test kits as instructed by the kit manufacturer. Test kits Ridascreen No. R5901 were provided (R-Biopharm, Germany). The analytical methods were validated by the kit's manufacturer with the sample matrices for wheat, oats, and triticale. The limit of detection (LOD) for DON is 100 μg kg^{-1}. The method has been approved by the AOAC Research Institute (Certificate No. 950702). Mycotoxin analyses were done in duplicate. The optical densities of samples and controls from a standard curve were estimated by a multichannel photometer Multiskan Ascent (Thermo Electron Corp., Finland), supplied with internal software, using a 450 nm filter.

Storage experiment. The grain samples for analyses were selected according to the abundance of BDG and FDG and similar DON concentrations with a moisture content ranging from 19.0% to 19.7%. The storage samples were kept in the thermostats Binder (Germany) for 28 days at different temperatures 4, 16, 20 and 28°C. DON analyses were done in duplicate after 14 days and 28 days of storage. The storage experiment is detailed in Table 1.

Table 1. The distribution of wheat grain samples in the storage experiment

Grain group	DON concentration μg kg^{-1}	Grain moisture %	Contamination % (estimated visually)	Storage temperature °C	Storage time, days
Fusarium-damaged grain + black-dotted grain	595 ± 36	19.7	>1	4, 16; 20, 28	14 28
Black-dotted grain	522 ± 4	19.0	>1		
Meeting the quality requirements grain	560 ± 79	19.5	<1		

Grain quality indicators: sedimentation (ml), starch (%), gluten content (%), protein (%) and hectolitre weight (kg hL^{-1}) of grain were measured by a grain analyser Infratec 1241 Foss with a calibration package IM 9200 (Foss, Denmark).

Weather conditions. In 2016, the summer period was windy and warm. The beginning of the summer was dry, later it was wet. The average air temperature in June was 1.8°C higher than the long term average. The amount of rainfall was very close to the long-term average. The air temperature in July was 0.8°C higher than the long-term average with a significant amount of rain. The total amount of rainfall was 40.9% higher than the long-term average. In August, the average air temperature was 17.1°C, which was 0.3°C higher than the long-term average. The rainfall of August was 148% of the long-term average.

In 2017, the summer period was wet and cool. Average air temperature in June was 0.3°C lower and the amount of rainfall was 16% higher than the long-term average. July's temperature was 1.0°C lower than the long-term average. In July there were 13 days with rain and the total monthly rainfall amounted to 153.8 mm. This amount was 100% higher than the long-term average. In August, the average air temperature was 17.3°C, which was 0.5°C higher than the long-term mean. The amount of rainfall in August was 72% of the long-term average. September was warm and wet. The average air temperature in September was 1.2°C higher and the amount of rainfall 138% higher than the long-term average.

Statistical analysis was conducted using the software *SAS*, version 9.4 (SAS Institute Inc., USA). Significant differences between the samples (Duncan's test) were calculated according to one-way analysis of variance (*ANOVA*). The results with $P \leq 0.05$ were considered significant. The correlation and regression type of analysis was performed to examine the quantitative relationship between the investigated compounds. The strength of the correlation was estimated according to the value of determination coefficient *R*-square (R^2). The data significantly correlated when $R^2 > 0.2500$, at a significance level of $P \leq 0.05$. The data of mycotoxins were expressed as mean ± standard deviation (SD) using the software *MS Office Excel* (2010).

Results and discussion

The analysis of grain from the 2016 and 2017 crop years, collected from different grain silos of Lithuania, showed the presence of black dotted-grain (BDG) in 85% of the samples (Fig. 1), while the samples with *Fusarium*-damaged grain (FDG) accounted for 5%. Only 10% of the samples met the quality standards. Out of 85% of the contaminated samples, in 37% of samples BDG accounted for >1%, in 30% of the samples BDG + FDG accounted for >1%. However, there were samples in which BDG accounted for 5% to 10%. This is likely to have been caused by the difficult weather conditions prevalent during the 2016 and 2017 harvesting periods, which resulted in a delay in harvesting due to excess rainfall. These conditions were highly favourable for the occurrence of various pathogens, including *Fusarium* spp., which due to their long presence on mature grain, moved to the next stage of development (teleomorph *Gibberella zeae*) and spread on the grain in the form of black dots. Many researchers have linked such grain contamination with the ambient temperature and relative air humidity (Beyer, Verreet, 2005; Keller et al., 2014). It was important for us to establish the grain contamination level with *Fusarium* spp. fungi (particularly *F. graminearum*) and to quantify DON concentrations. Foreign researchers have reported a direct relationship between *F. graminearum* presence on grains and the occurrence of DON, zearalenone (ZEA), T2/HT2 toxins in them (Cumagun et al., 2006; Lowe et al., 2012; Keller et al., 2014); however, there are opinions that the toxicity of the mycotoxin-producing fungus is related to its genetic properties (Cumagun et al., 2006; Schmale et al., 2006; Kelly, Ward, 2018).

It was found that some black dots (Fig. 2A) formed on grain hull while others under it (Fig. 2 B and C). The dots formed on the surface of hulls are easily removed with them, but those present under hulls cause not only aesthetic problems but also raise questions about the further use of such grain in food production.

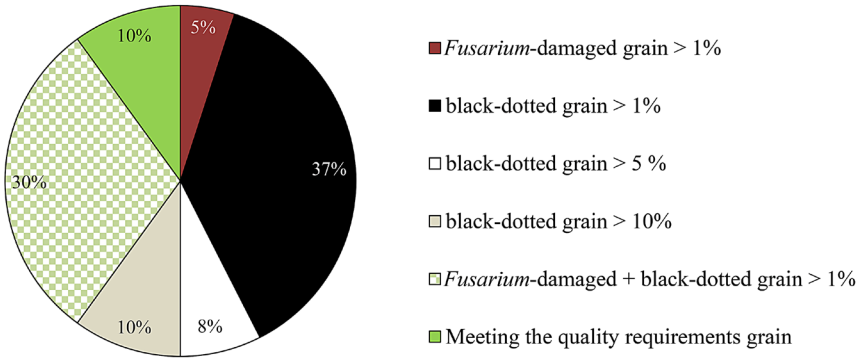


Figure 1. Contamination of visually assessed wheat grain samples (2016 and 2017)

Figure 2. Black-dotted wheat grain (A), black dots on the grain surface (B) and black dots under the hulls (C)

The microscopic visualization showed these dots to be perithecia with ascospores of the *F. graminearum* fungus (Fig. 3 A, B and C). In Figure 3C one can clearly see the eight-spored ascus characteristic of *G. zeae* (*F. graminearum*), which has also been described by other researchers (Leslie, Summerell, 2006; Son et al., 2011). To ascertain whether BDG was contaminated with DON, the test samples were divided into four groups: composite sample, FDG, BDG and visually healthy grain.

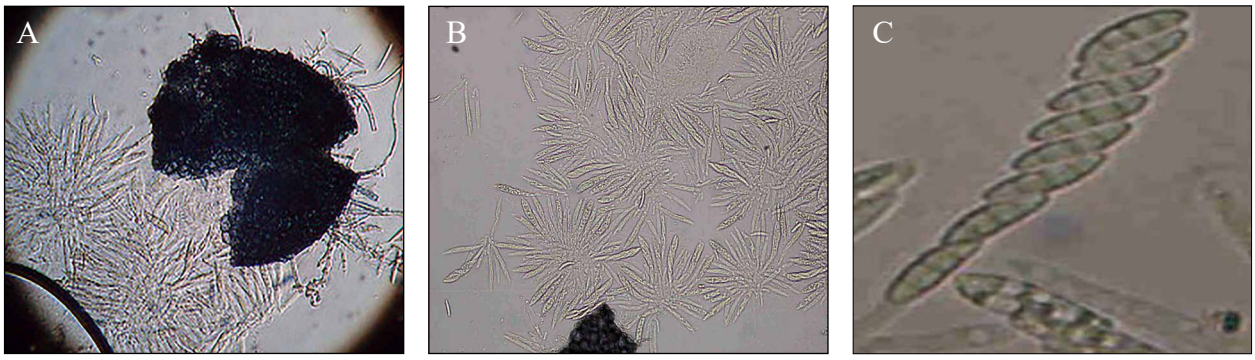


Figure 3. *Fusarium graminearum* perithecia with ascus (A), ascus accumulation (B) and eight ascospores in ascus (C)

The findings of the study showed that DON contamination in the composite sample was low (below the limit of detection) despite the fact that BDG accounted for more than 1% (Table 2). Similar DON concentrations were detected in the BDG samples and in visually healthy grain samples. Desjardins (2006) has showed that there exists trichothecene-nonproducing mutant of *F. graminearum*; however, we found that in the FDG samples DON concentrations averaged 7620 µg kg^{-1}. This suggests that FDG is much more dangerous due to possible higher concentrations of DON compared to BDG (Lowe et al., 2012; Kelly, Ward, 2018). In the well-dried wheat grain samples with BDG, the concentrations of DON were below the limit of detection.

There were found no significant correlations between the abundance of BDG and DON concentrations (Fig. 4A) and between contamination with *Fusarium* fungi of grain cultured on a PDA and BDG (Fig. 4B). This indicates that black dots on grain cultured on the PDA medium did not exhibit viability for 14 days and did not have influence on the abundance of *Fusarium* spp. contamination although 26°C temperature and approx. 80% relative ambient humidity were optimal for spore germination (Beyer et al., 2005).

Since 30% of the samples contained FDG and BDG (Fig. 1), for further analyses of DON concentrations, the samples were divided into three groups: FDG + BDG, BDG and wheat grain samples meeting the quality requirements.

Table 2. Deoxynivalenol (DON) concentrations in wheat grain samples (n = 33), 2016

Grain group	DON-contaminated samples %	Average DON concentration µg kg^{-1}	Minimum – maximum µg kg^{-1}	Standard deviation
Fusarium-damaged grain (n = 6)	100	7620	1880–14460	863
Black-dotted grain (n = 9)	44	<LOD	0–538	8
Visually healthy grain (n = 9)	22	<LOD	0–512	6
Composite sample (n = 9)	33	<LOD	0–465	3

LOD – limit of detection 100 µg kg^{-1}

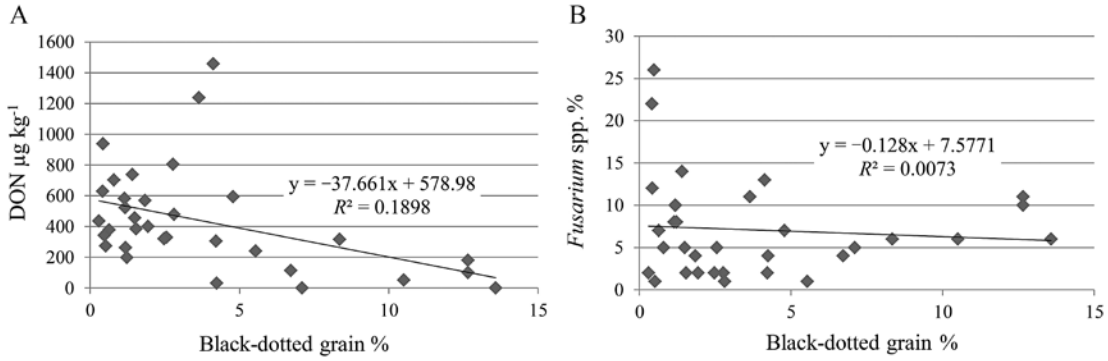


Figure 4. The correlation between wheat grain contamination with deoxynivalenol (DON) (A) and *Fusarium* spp. (B) and black-dotted grain

The analyses showed that higher concentrations of DON were detected in the grain samples with FDG accounting for >1% (Table 3). Although averaged data indicate that DON concentrations did not exceed the allowable limit set forth in the EC regulation No. 1881/2006, the concentrations in the individual samples ranged from 800 to 1458 µg kg^{-1}. Such samples accounted for 86%. DON was found in 73% of the tested BDG samples at an average concentration of 379 µg kg^{-1}. DON was detected in 75% of the samples meeting the quality requirements at concentrations very similar to those in BDG.

Summarizing the research results of grain collected in 2017, it can be concluded that DON concentrations in BDG were not higher than those in grain meeting the quality requirements. This suggests that in the BDG dried to 12.9–13.2% moisture the concentrations of DON were low.

Table 3. Deoxynivalenol (DON) concentrations in wheat grain samples (n = 40), 2017

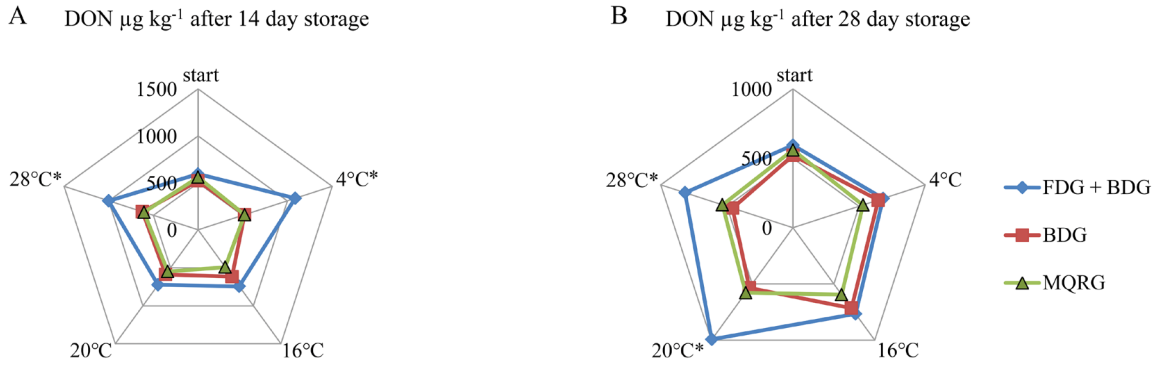
Grain group	DON-contaminated samples %	Minimum – maximum µg kg^{-1}	Average DON concentration µg kg^{-1}	Average DON concentration in positive samples µg kg^{-1}
Fusarium-damaged grain + black-dotted grain (n = 14)	86	0–1458	479 ± 440	556 ± 428
Black-dotted grain (n = 22)	73	0–738	276 ± 230	379 ± 179
Meeting the quality requirements grain (n = 4)	75	0–437	290 ± 197	387 ± 47

Note. According to EC regulation No. 1881/2006, DON maximum level was set at 1250 µg kg^{-1} for unprocessed cereal grain and 750 µg kg^{-1} for cereals intended for direct human consumption.

When storing grain of different moisture contents (19.0–19.7%) and different contamination levels, it was noted that in the samples with FDG exceeding 1%, DON concentrations increased, although the initial concentrations before storage for all analysed sample groups were very similar (522–595 µg kg^{-1}). After 14 days of storage, significantly higher ($p < 0.05$) DON concentrations were detected in the samples with FDG > 1% stored at 4°C and 28°C (Fig. 5A). Other researchers have documented that grain moisture and ambient humidity are one of the key risk factors for the occurrence of mycotoxins and increase in their concentrations, particularly if the samples contain FDG (Beyer et al., 2005; Beyer, Verreet, 2005; Keller et al., 2014). However, DON contamination is often associated with the species of mycotoxin producing fungi, their physiological state and storage peculiarities (Trail et al., 2002; Son et al., 2011; Zhang et al., 2016), especially when *F. graminearum* is a prevalent species among other *Fusarium* species on grain (Trail, 2009; Turkington et al., 2014). In the BDG and meeting the quality requirements samples DON concentrations practically did not change compared with those before storage. The analysis carried out after 28 days of storage showed a similar situation,

significantly ($p < 0.05$) higher DON concentrations were revealed only in the FDG > 1% samples stored at 20°C and 28°C (Fig. 5B). The BDG had no significant effect on the increase in DON concentrations when storing grain of higher moisture.

It has been reported in literature that FDG impair the quality of grain (Bergamini et al., 2010; Tibola et al., 2015; 2016) and cause changes in flour colour, rheological properties of dough and deteriorate bread quality (Wang et al., 2005; Lancova et al., 2008).



* – $p < 0.05$

Figure 5. Deoxynivalenol (DON) concentrations in *Fusarium*-damaged + black-dotted (FDG + BDG) grains, BDG and meeting the quality requirements (MQRG) wheat grain samples stored at different temperatures for 14 (A) and 28 (B) days

Analyses of starch, protein and sedimentation values conducted in our study (Fig. 6 A, B and C) suggest that BDG did not have significant effect on them, as the correlations were insignificant. The analyses also indicated that according to protein and sedimentation values, the BDG met the requirements for wheat grain grade 1 (LST 1524:2003/2K:2014).

Analysis of grain hectolitre weight and gluten content showed a weak correlation between these indicators and grain contamination with black dots (Fig. 7 A and B).

According to the hectolitre weight, only 27% of the grain samples met the requirements for wheat grain grade 1 and 2, 73% of the samples met the requirements for wheat grain grade 3. Assessment of gluten content (%) showed that only 11.5% of the samples met the requirements for wheat grain grade 1; 50% of the samples

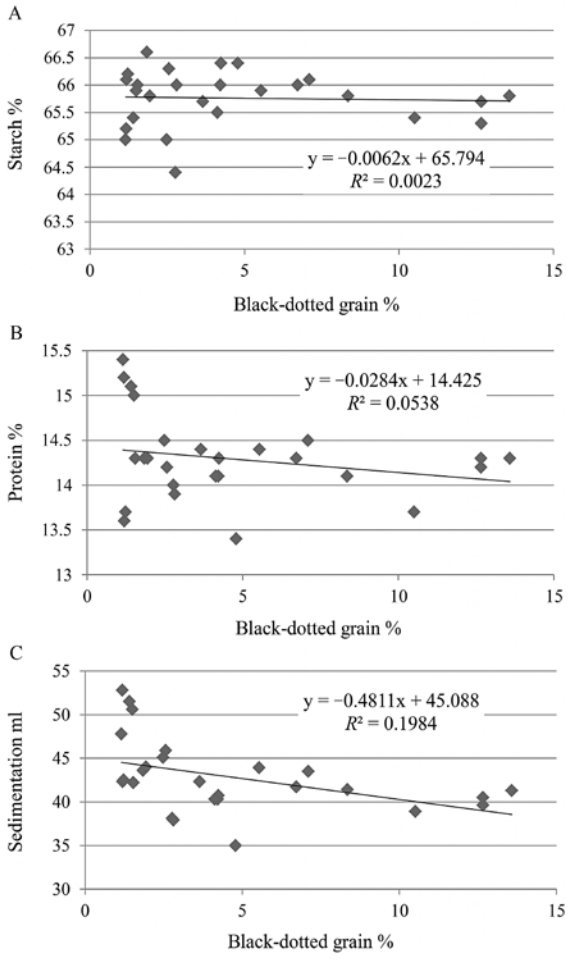


Figure 6. The influence of black-dotted grain on the wheat grain quality indicators: starch (A), protein (B) and sedimentation (C)

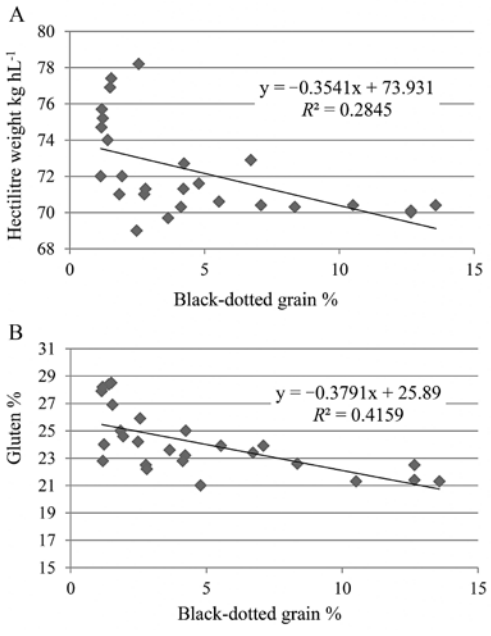


Figure 7. The influence of black dots on the wheat grain quality indicators: hectolitre weight (A) and gluten content (B)

met the requirements for grade 2 and 38.5% for grade 3. Despite the weak correlation between the hectolitre weight and gluten content, BDG might have had a negative impact. Malaker et al. (2009) have noticed that black dot infection of grains exerted marked influence on protein, fat, dry matter and ash content of wheat grains. Significant variations in these parameters were found among the different grades of black dot-affected grains.

Conclusions

1. Visual estimation of the wheat grain samples of 2016 and 2017 harvest years, collected from the commercial enterprises showed that 85% of the samples tested were contaminated with black dots, 5% of the samples had *Fusarium*-damaged grain (FDG), and only 10% of the grain samples met the quality requirements for purchase and supply.

2. Deoxynivalenol (DON) was detected in 44% of the black-dotted grain (BDG) samples at concentrations below the detection level. DON was detected in 100% of the FDG samples at concentrations averaging 7620 µg kg^{-1}.

3. BDG dried to 12.9–13.2% moisture content did not pose any risk of increasing DON concentrations.

4. Higher grain moisture content (19.0–19.5%) and storage temperatures (4, 16, 20 and 28 °C), did not have significant influence on the variation of DON concentrations in the samples with BDG > 1%. However, it is dangerous to store wheat grain with FDG > 1% in a sample. In such samples, DON concentrations after 28 days of storage at 20°C and 28°C were found to be 34% ($p < 0.05$) higher compared with those before storage.

5. No significant effects of BDG > 1% were identified on starch, protein, and sedimentation values; however, significant correlations were determined between the hectolitre weight and BDG and between gluten content and BDG.

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Ar juodi taškeliai ant kviečių grūdų turi įtakos deoksinivalenolio kaupimuisi?

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Santrauka

Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institute 2016–2017 m. buvo atliktas tyrimas paprastojo kviečio (*Triticum aestivum* L.) grūdų pažeidimų juodais taškeliais, kurie iki 2016 m. Lietuvoje nebuvo reikšmingai paplitę. Šis pažeidimas sukėlė daug diskusijų tarp grūdų augintojų, supirkėjų, perdirbėjų, mokslininkų ir produkcijos vartotojų. Pagal kokybės reikalavimus, taikomus parduodamiems kviečių grūdams, juose neturi būti daugiau kaip 1 % vizualiai matomų fuzariozės (sukėlėjai – *Fusarium* genties grybai) pažeistų grūdų. Tyrimo tikslas – išsiaiškinti, kuris augalų patogenas ant grūdų formuoja juodus taškelius, nustatyti ir palyginti skirtingo pagal vizualų vertinimą kviečių grūdų pažeidimo užterštumą deoksinivalenoliu (DON), įsitikinti, ar juodų taškelių pažeisti grūdai gali būti sandėliuojami nesukeldami DON koncentracijos didėjimo rizikos.
Nustatyta, kad juodi taškeliai ant kviečių grūdų yra patogeno *F. graminearum* lytinės stadijos *Gibberella zeae* periteciai su grybo askosporomis, kurie pažeidė 85 % grūdų mėginių, surinktų iš įvairių komercinių įmonių. Tyrimo duomenys parodė, kad didžiausios DON koncentracijos aptiktos mėginiuose, kuriuose *Fusarium* pažeistų grūdų buvo daugiau nei 1 %, o mėginiuose su juodais taškeliais DON koncentracijos buvo nedidelės. Grūdų mėginiai laikymo eksperimentui buvo atrinkti pagal užterštumą juodais taškeliais ir *Fusarium* grybais, kurių drėgnis buvo nuo 19,0 iki 19,7 %. Juodi taškeliai neturėjo įtakos DON koncentracijos padidėjimui, o mėginiuose, kuriuose buvo daugiau kaip 1 % vizualiai *Fusarium* pažeistų grūdų, aptiktos didesnės DON koncentracijos. Nustatyta, kad grūdų mėginiuose su juodais taškeliais, esant didesniam drėgniui ir skirtingoms laikymo temperatūroms (4, 16, 20 ir 28 °C), DON koncentracijos reikšmingai nepadidėjo. Tačiau pavojinga laikyti didesnio drėgnio grūdus, kai vizualiai *Fusarium* grybų pažeistų grūdų kiekis mėginyje viršija 1 %. Tokiuose grūdų mėginiuose DON kiekis reikšmingai padidėjo. Nepaisant to, kad juodų taškelių pažeistuose grūduose buvo aptiktos nedidelės DON koncentracijos, tokie grūdai yra patogenų šaltinis ir juos vartoti reikia labai atsakingai.

Reikšminiai žodžiai: deoksinivalenolis, grūdai su juodais taškeliais, *Fusarium* pažeisti grūdai, kviečių grūdai.