BIULETYN INSTYTUTU HODOWLI I AKLIMATYZACJI ROŚLIN Nr 300 / 2023 : 67–75
E-ISSN: 2657–8913 DOI: 10.37317/biul-2023-0013

Oryginalny Artykuł Naukowy

Original Research Paper

Deoxynivalenol content in wheat kernels showing various levels of damage caused by *Fusarium culmorum*

Zawartość deoksyniwalenolu w ziarniakach pszenicy wykazujących różne poziomy uszkodzeń spowodowanych przez *Fusarium culmorum*

Piotr Ochodzki, Tomasz Góral✉

Instytut Hodowli i Aklimatyzacji Roślin – Państwowy Instytut Badawczy, Zakład Biologii Stosowanej, Radzików, 05-870 Błonie

✉t.goral@ihar.edu.pl

The content of *Fusarium* toxin deoxynivalenol (DON) was investigated in the grain of four winter wheat cultivars that differ in susceptibility to Fusarium head blight. Wheat heads were inoculated with *Fusarium culmorum* spores. The degree of damage to the kernels by *Fusarium* was assessed, and then the grain samples were divided into the following fractions: healthy kernels, healthy shriveled kernels, normal-sized discolored kernels (white), and shriveled discolored kernels (white, red). The highest content of DON was found in the fraction of discolored, shriveled kernels, i.e. the most severely damaged by *Fusarium*. Depending on the cultivar, the DON content was 16 to 47 times higher than in the healthy kernel fraction. This fraction contained from 54 to 91% of the DON contained in the total grain sample. A significant content of DON was also found in discolored normal-sized kernels, the weight of which was similar to that of healthy kernels. The healthy kernel fraction was divided into two parts based on fluorescence under ultraviolet (UV) light. Kernels exhibiting fluorescence had three times higher DON content.

Keywords: Fusarium head blight, mycotoxins, grain, *Fusarium*-damaged kernels

Badano zawartość toksyny fuzaryjnej deoksyniwalenolu (DON) w ziarnie czterech odmian pszenicy ozimej różniących się podatnością na fuzariozę kłosów. Kłosy pszenicy inokulowane były zarodnikami *Fusarium culmorum*. Oceniano stopień uszkodzenia ziarniaków przez *Fusarium*, a następnie próby ziarna dzielono na frakcje: ziarniaki zdrowe, ziarniaki zdrowe pomarszczone, ziarniaki normalnej wielkości przebarwione (białe), ziarniaki przebarwione (białe, różowe) pomarszczone. Najwyższą zawartość DON stwierdzono we frakcji ziarniaków przebarwionych pomarszczonych czyli najsilniej uszkodzonych przez *Fusarium*. Była ona od 16 do 47 razy wyższa niż we frakcji ziarniaków zdrowych, zależnie od odmiany. W tej frakcji znajdowało się od 54 do 91% DON zawartego w całej próbie ziarna. Znaczną zawartość DON stwierdzono również w przebarwionych ziarniakach normalnej wielkości, których masa tysiąca ziarniaków była zbliżona do masy ziarniaków zdrowych. Frakcję ziarniaków zdrowych podzielono na dwie części na podstawie obserwacji fluorescencji w świetle UV. We frakcji ziarniaków wykazujących fluorescencję zawartość DON był trzykrotnie wyższa.

Słowa kluczowe: fuzarioza kłosów, mykotoksyny, ziarno, ziarniaki uszkodzone przez *Fusarium*

Introduction

Fusarium head blight (FHB) is a disease posing serious problems for wheat and barley crops worldwide (Goswami and Kistler, 2004). The disease is caused by several species of *Fusarium* fungi, including *Fusarium culmorum* and *F. graminearum* (Bottalico and Perrone, 2002). These fungi infect developing heads of wheat and cause the grain to become discolored and shriveled, reducing the yield and quality of the crop. FHB can cause significant yield losses and reduce grain quality by contamination with mycotoxins. The presence of mycotoxins produced by *Fusarium* species in infected grain can also pose a risk to human and animal health (Bakker et al., 2018). The most important mycotoxins in small-grain cereals are deoxynivalenol (DON), its acetyl derivatives 15-AcDON and 3-AcDON, and zearalenone (ZEN) (Ji et al., 2019).

The accumulation of *Fusarium* toxins in cereal grains depends on different factors. Genetic resistance to FHB, which is very complex, includes different types - resistance to initial head infection (type I), resistance to spread within the head (II), resistance to kernel infection (III), tolerance against FHB and trichothecenes (IV), and resistance to mycotoxins itself (V) (Mesterhazy, 1995; Foroud and Eudes, 2009). The last type also covers different mechanisms (Boutigny et al., 2008). It is divided into two classes, V-1 and V-2. Plants with type V-1 resistance can chemically alter trichothecenes, which causes the toxins to be degraded or detoxified. The genotypes with type V-2 resistance are capable of preventing the invasive fungus from synthesizing trichothecenes. The influence of climatic conditions during kernel development is also an important factor in the amount of mycotoxins in grain (Miedaner, 1997). Many published results show that there is a corre-

Redaktor Wiodący / Leading Editor
Dariusz R. Mańkowski

lation between the severity of *Fusarium*-infection of head or kernels and mycotoxin content in grain (Liu et al., 1997). Fusarium head and kernel infection results in a decrease in kernel size and weight. The accumulation of mycotoxins in kernels of different sizes was studied. It was found that the smallest kernels have a considerable share in total mycotoxin content in cereal grain (Dowell et al., 1999; Perkowski et al., 2003)

This work aimed to detect the above mycotoxins in fractions of wheat kernels showing different levels of *Fusarium* damage. The results determined which fraction is the main source of mycotoxins in grain samples from FHB-infected wheat cultivars.

Material and Methods

Field experiment

In 2020, four winter wheat cultivars were tested at PBAI-NRI Radzików for their resistance to Fusarium head blight (FHB). The cultivars Fregata and Turnia demonstrated a medium level of resistance, whereas Kampana and Muszelka were found to be susceptible (Góral and Walentyn-Góral, 2018). The field experiment was established as a randomized complete block design. Wheat cultivars were seeded in 1 m^2 plots in three replications and a control set. Until heading, all plots were treated with foliar fungicide Tilt Plus 400 EC.

Two isolates of *Fusarium culmorum* (KF 846, ZFR 112), producing deoxynivalenol in vitro (3ADON chemotype), were used for inoculum production (Góral et al., 2019; Ochodzki and Góral, 2006). These isolates were previously tested for aggressiveness to wheat and triticale and used for resistance screening under field conditions (Góral et al., 2013). Isolates were incubated with autoclaved wheat grain in glass flasks for about 4 weeks and next exposed to permanent UV for 4 to 7 days at 18°C. The mycelium-colonized grain was dried and stored in the refrigerator at 4°C until usage.

On the day of inoculation, the grain with *Fusarium* mycelium was suspended in tap water for 2 h and filtered to obtain conidial suspension. The suspensions of each of the isolates were combined and adjusted to 10^6 spores/ml using a hemocytometer.

Heads of wheat plants were sprayed with a spore suspension at anthesis at a rate of 100 ml/m^2. Inoculations were performed individually on each plot at the beginning of the anthesis (BBCH 61) and repeated about 3 days later at full anthesis (BBCH 65) (Buerstmayr et al., 1999). At this stage wheat is the most sensitive for *Fusarium* head infection (György et al., 2020). The inoculations were carried out in the evening when the humidity levels were higher.

The disease was first rated at about 10 days after the last inoculation. Three ratings were done at 7-d intervals. Fusarium head blight index was scored based on the mean percentage of blighted spikelets per infected head (disease severity) and the percentage of infected heads per plot (disease incidence).

After ripening, 30 heads were harvested manually from each plot and threshed with a laboratory thresher at a low wind speed to prevent loss of low -weight infected kernels. Relative to control reductions of yield components (yield per head, 1000-kernel weight, specific kernel weight) were determined. Next wheat kernels were divided into four fractions according to the level of *Fusarium* damage (Argyris et al., 2003): a) 'healthy-looking' kernels (HL), b) 'shriveled healthy-looking kernels (HLS), c) 'normal white' kernels (white discolored, normal size) (NW), d) 'white shriveled' kernels (pink or white discolored, shriveled, 'tombstone') (WS). Healthy-looking kernels were subsequently divided into two fractions emitting [UV(+)] or not emitting [UV(-)] light under ultraviolet (360 nm) lamp. Kernels from all fractions were counted and weighted.

Analysis of mycotoxins

Kernels from three replications of fractions were combined and ground in a coffee mill.

The content of the trichothecenes of B group (deoxynivalenol [DON], 3-acetyl deoxynivalenol [3Ac-DON], 15-acetyl deoxynivalenol, [15Ac-DON], nivalenol [NIV]) in the wheat grain was analyzed, using the technique of gas chromatography. Mycotoxins were extracted from 5 g of ground grains using 25 ml of an aqueous solution of acetonitrile (acetonitrile: water 84: 16). Samples were shaken on the laboratory shaker overnight, centrifuged (3000 rpm min^{-1}, 5 min.), and the extract was purified with MycoSep® 227 Trich+ columns (Romer Labs Inc., Union, MO). One microliter of the internal standard solution (chloralose) was added to 4 ml of purified extract. The solvent was evaporated to dryness in the air stream. Mycotoxins were derivatized to the trimethylsilyl derivatives using a derivatizing agent Sylon BTZ (BSA + TMCS + TMSI, 3: 2: 3, Supelco). After the dissolution of the sample in isooctane, the excess derivatizing agent was decomposed and removed with water. The organic layer was transferred to an autosampler vial and analyzed chromatographically with gas chromatograph SRI 8610C, with BGB-5MS column of 30 m in length, and an internal diameter of 0.25 mm.

Hydrogen was a carrier. Elution was carried out in the temperature gradient. Mycotoxin detection was carried out using an electron capture detector (ECD). Identification of individual compounds was made by comparing the retention

times of the pure standards of mycotoxins. The concentration of mycotoxins was established based on the calibration curve, using chloralose as the internal standard. The content of trichothecenes B was expressed as toxin weight (mg) per grain weight (kg).

The content of ZEN was determined using a quantitative direct and competitive enzyme-linked immunosorbent assay (ELISA) AgraQuant® Zearalenone 25-1000 (LOD 20 ppb, LOQ 25 ppb) (Romer Labs GmbH, Tulln, Austria). A 5 g ground sample was placed in a conical 50 mL Falcon centrifuge tube; then, 25 mL of the solvent (methanol –water 70:30 v/v) was added. The sample was extracted for 1 h on a shaker and then centrifuged (1620 g, 5 min). The obtained extract was analyzed with the ELISA method according to the procedure described by Romer Labs. The content of ZEN was expressed as toxin weight (mg) per grain weight (kg).

Statistical analysis

Statistical analysis was performed using the XLSTAT Life Science package (Version 2021.3.1.1177, Lumivero, Denver, CO, USA). An analysis of variance was performed on the FHB index, maximal FHB index, and reductions of GWH, TKW, and test weight (XLSTAT: ANOVA procedure). The factors were: cultivar and rating date. Fisher's NIR multiple comparison test was used to compare the means for cultivars. The significance of differences between fractions for TKW, DON, and 3Ac-DON content was tested using the Kruskal-Wallis nonparametric test (XLSTAT: Comparison of k-samples procedure).

Results

The weather conditions in Radzików in 2020 were favorable for the development of Fusarium head blight. During the inoculation in early June, the average temperature was 16°C (minimum 10.7°C, maximum 21.6°C). After the inoculation, the average temperature increased to 20.6°C (minimum 15.4°C, maximum 26.4°C). The total precipitation in the first two decades of June was 50 mm. In the third decade, the precipitation was low, but it occurred frequently. Overall, there were only 7 rainless days in June. In July, the rainfall was very low (total precipitation 11.6 mm). There were 11 days with rainfall. The temperature was lower than in June after the inoculation and averaged 19.2°C (minimum 12.2°C, maximum 26.4°C).

Wheat cultivars differ in their phenotype. Cultivars Muszelka and Kampana had short straws, Fregata . was average and Turnia was tall (Table 1). Cultivars Fregata and Muszelka were flowering 3-4 days later than Turnia and Kampana. The average FHB indexes for Fregata and Turnia were FHBi = 20.7 % and FHBi = 21.8 %, respectively, and did not differ significantly. Maximal FHBi was higher for Turnia cultivar. The average FHB indexes for cultivars Muszelka and Kampana were FHBi = 56.0 % and FHBi = 57.3 %, respectively, and did not differ significantly. Maximal FHBis did not differ significantly for these cultivars. Cultivars Muszelka and Kampana have about twofold higher head infected than Turnia and Fregata. Reductions of yield components were lowest for cultivar Fregata and somewhat higher for Turnia. Cultivar Muszelka exhibited the highest yield component reduction despite the head infection being similar to that for Kampana.

The TKW ranged from 17.0 g for white shriveled kernels of cultivar Muszelka at 53.4 g for normal white kernels of Turnia (Table 2). Normal white kernels of resistant cultivars Fregata and Turnia had higher weight than healthy-looking kernels. On average TKW was highest for cultivar Turnia (42.8 g) and the lowest for Muszelka (22.6 g).

The percentage of healthy-looking kernels (HL+HLS) was the highest for cultivar Fregata,

Table 1
Tabela 1

Characteristics of phenotype and reaction to FHB of four winter wheat cultivars
Charakterystyka fenotypu i reakcji na fuzarioza kłosów czterech odmian pszenicy ozimej

Cultivar Odmiana	Flowering date [1] Termin kwitnienia [1]	Height Wysokość (cm)	Disease incidence (%)	Disease severity (%)	FHB index Indeks FK (%)	Maximal FHBi [2] Maksymalny IFK (%)	GWH [3] reduction Redukcja MZK [3] (%)	TKW [4] reduction Redukcja MTZ [4] (%)	Test weight reduction Redukcja masy objętościowej (%)
Fregata	45.0	109.3	76.7	26.7	20.7 a	36.0 a	57.7 a	24.4 a	11.1 a
Turnia	42.0	114.7	76.7	27.5	21.8 a	40.0 a	58.2 a	29.7 a	16.1 a
Muszelka	42,0	89,3	80,0	70,0	56,0 b	72,0 b	80,2 b	50,6 b	33,1 b
Kampana	41,5	85,0	80,0	71,7	57,3 b	72,0 b	71,3 b	37,1 b	26,5 b

[1]- number of days from May 1; [2] – third rating; [3] - GWH – grain weight per head; [4] - TKW – thousand kernel weight; values marked with the same letter are not significantly different at p = 0.05 (analysis of variance, Fisher's LSD test)
[1]- liczba dni od 1 maja; [2] – trzecia ocena; [3] - MZK – masa ziarna w kłosie; [4] - MTZ – masa tysiąca ziarniaków; wartości oznaczone tą samą literą nie różnią się istotnie statystycznie dla $\alpha = 0,05$ (analiza wariancji, test NIR Fishera)

Table 2
Tabela 2

Thousand kernel weight of kernel fractions showing different levels of *Fusarium* damage and their contribution to whole samples of grain of four winter wheat cultivars
Masa tysiąca ziarniaków frakcji ziaren wykazujących różne stopnie uszkodzenia przez *Fusarium* i ich udział w całych próbach ziarna czterech odmian pszenicy ozimej

	Fregata	Turnia	Muszelka	Kampana
Fraction Frakcja	Thousand kernels weight TKW (g) Masa tysiąca ziarniaków (g)			
HL	38.8	48.9	36.5	45.9
HLS	23.1	22.5	26.6	24.9
NW	43.2	53.4	29.7	41.2
WS	21.5	25.2	17.0	20.2
Mean TKW Średnia MTZ	36.3	42.8	22.6	26.0

	Contribution of kernel fraction to the whole sample Udział frakcji w całej próbie							
Fraction Frakcja	% of kernels % ziarniaków	% weight % masy	% of kernels % ziarniaków	% weight % masy	% of kernels % ziarniaków	% weight % masy	% of kernels % ziarniaków	% weight % masy
HL	77.3	82.5	61.1	69.8	8.9	14.3	14.1	23.9
HLS	4.3	2.8	12.0	6.3	31.4	36.8	24.8	24.2
NW	6.4	7.7	12.3	15.3	7.2	9.3	5.1	8.0
WS	11.9	7.0	14.6	8.6	52.5	39.5	56.0	43.9

HL – healthy looking, HLS - healthy looking shriveled, NW – normal white, WS – white shriveled
HL – zdrowe, HLS – zdrowe pomarszczone, NW – normalne białe, WS – białe pomarszczone

and lower for Turnia which had a higher share of shriveled (HLS) kernels. For cultivars Muszelka and Kampana share of HL+HLS kernels was similar, however, Muszelka had more shriveled kernels than Kampana. Normal white kernels were found mainly in the grain sample of cultivar Turnia. Their number was lower in samples of resistant cultivar Fregata and susceptible cultivars Kampana and Muszelka.

Chemical analysis revealed the presence of DON and 3Ac-DON in analyzed samples. No 15AcDON or NIV were detected. The average content of DON in the grain of low-susceptible cultivars was 7.673 and 11.004 mg/kg (Table 3). It was 44.602 and 50.991 mg/kg in the grain of highly susceptible ones. Most mycotoxins (55-75%) were accumulated in kernels described as white shriveled (WS). The DON concentration in these kernels was 70.515-108.116 mg/kg (Figure 1). From 15 to 33% of the total DON amount was found in normal white (NW) kernels. The DON concentration in this fraction was 15.343-23.841 mg/kg. The rest of DON was detected in healthy-looking (HL) and shriveled healthy-looking (HLS)

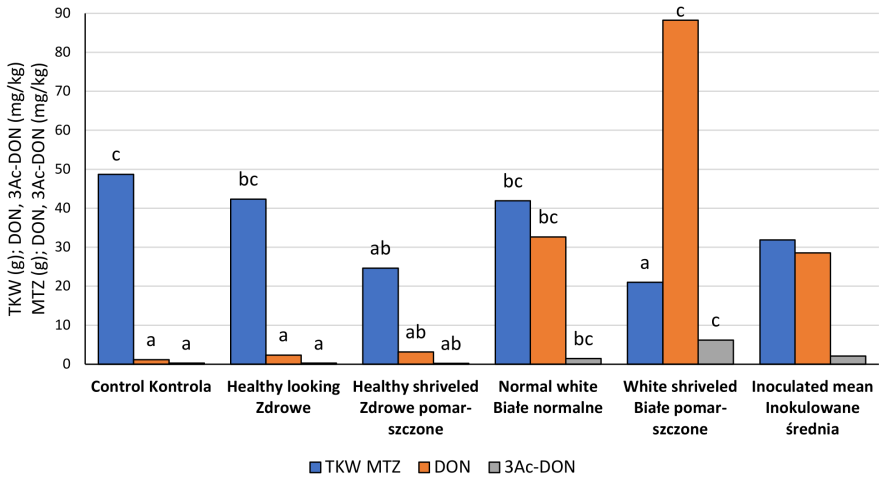

Figure 1. Average thousand kernel weight (TKW), DON, and 3Ac-DON content in the control grain and different fractions of the grain of four wheat cultivars inoculated with *F. culmorum*. Values marked with the same letter are not significantly different at p = 0.05 (Kruskal-Wallis test).
Rysunek 1. Średnia masa tysiąca ziaren (MTZ), zawartość DON i 3Ac-DON w ziarnie kontrolnym i różnych frakcjach ziarna czterech odmian pszenicy inokulowanych *F. culmorum*. *W*artości oznaczone tą samą literą nie różnią się istotnie statystycznie dla α = 0,05 (test Kruskala-Wallisa).

Table 3
Tabela 3

The concentration of DON and 3Ac-DON in kernel fractions of four winter wheat cultivars
Zawartość DON i 3Ac-DON we frakcjach ziarniaków czterech odmian pszenicy ozimej

Sample description Opis próby	DON (mg/kg)	3Ac-DON (mg/kg)	DON/3Ac-DON ratio Współczynnik DON/3Ac-DON
Fregata control / kontrola	0.681	0	-
Fregata HL and HLS	0.921	0.161	5.9
Fregata NW	15.343	1.020	15.0
Fregata WS	81.424	4.653	17.5
Fregata inoculated – mean / inokulowane - średnia	7.673	0.533	14.4
Turnia control / kontrola	0.737	0.771	0.9
Turnia HL	1.600	0.622	2.6
Turnia HLS	2.722	0.233	12.0
Turnia NW	23.841	0.981	24.4
Turnia WS	70.515	4.593	15.4
Turnia inoculated – mean / inokulowane - średnia	11.004	0.990	11.1
Muszelka control / kontrola	1.630	0	-
Muszelka HL	2.321	0.073	33.1
Muszelka HLS	3.464	0.171	20.4
Muszelka NW	62.423	2.502	25.0
Muszelka WS	108.116	9.324	11.6
Muszelka inoculated – mean / inokulowane - średnia	50.991	4.037	12.7
Kampana control / kontrola	1.635	0.241	6.8
Kampana HL	4.633	0.127	38.6
Kampana HLS	3.210	0.112	29.2
Kampana NW	29.021	1.132	25.7
Kampana WS	92.957	6.174	15.1
Kampana inoculated – mean / inokulowane - średnia	44.602	2.821	15.8

HL – healthy looking, HLS – healthy looking shriveled, NW – normal white, WS – white shriveled
HL – zdrowe, HLS – zdrowe pomarszczone, NW – normalne białe, WS – białe pomarszczone

kernels. The content of acetylated derivatives of DON increased along with the increase in the level of kernel infection, from about 0.073-0.622 mg/kg in healthy grain (HL, HLS) to 0.981-2.502 mg/kg in NW kernels and 4.593-9.324 mg/kg in WS kernels.

The DON/3Ac-DON ratio was the lowest for WS kernels (14.9) where considerable amounts of 3Ac-DON were detected (Table 3). For the other fractions (HL, HLS, NW) ratios were similar amounting to 20.0, 20.5, and 22.5. respectively. However, for HL kernels the ratio was high for susceptible cultivars (35.9) and low for resistant (4.2). In the other fractions, the variability of the ratio was low.

ZEN was only detected in normal white kernels (NW) and white shriveled (WS) in inoculated samples, at levels of 0.070 mg/kg and 0.341-0.721 mg/kg, respectively. The level of ZEN in healthy-looking kernels (HL, HLS) was below the limit of detection.

In HL fractions of Fregata and Turnia, about 25% of kernels emitted light when exposed to a UV lamp (Table 4, Figure 2). The amount of DON in the UV(+) fraction was three times higher (3.148 mg/kg) than in the UV(-) fraction (0.706 mg/kg). In the UV(+) fraction of Fregata grain, the amount of 3Ac-DON was low. However, in the UV(+) fraction of Turnia, it was significantly higher.

Table 4
Tabela 4

The concentration of DON and 3Ac-DON in two fractions of healthy-looking kernels (HL) of Fregata and Turnia cultivars
Zawartość DON i 3Ac-DON w dwóch frakcjach zdrowych ziarniaków (HL) odmian Fregata i Turnia

Sample description Opis próby	Contribution to the whole sample (% weight) Udział w całej próbie (% masy)	DON (mg/kg)	3Ac-DON (mg/kg)	DON/3Ac-DON
Fregata HL UV(-)	79.1	0.371	0.144	2.6
Fregata HL UV(+)	20.9	3.014	0.241	12.5
Turnia HL UV(-)	75.0	1.040	0.522	2.0
Turnia HL UV(+)	25.0	3.282	0.915	3.6

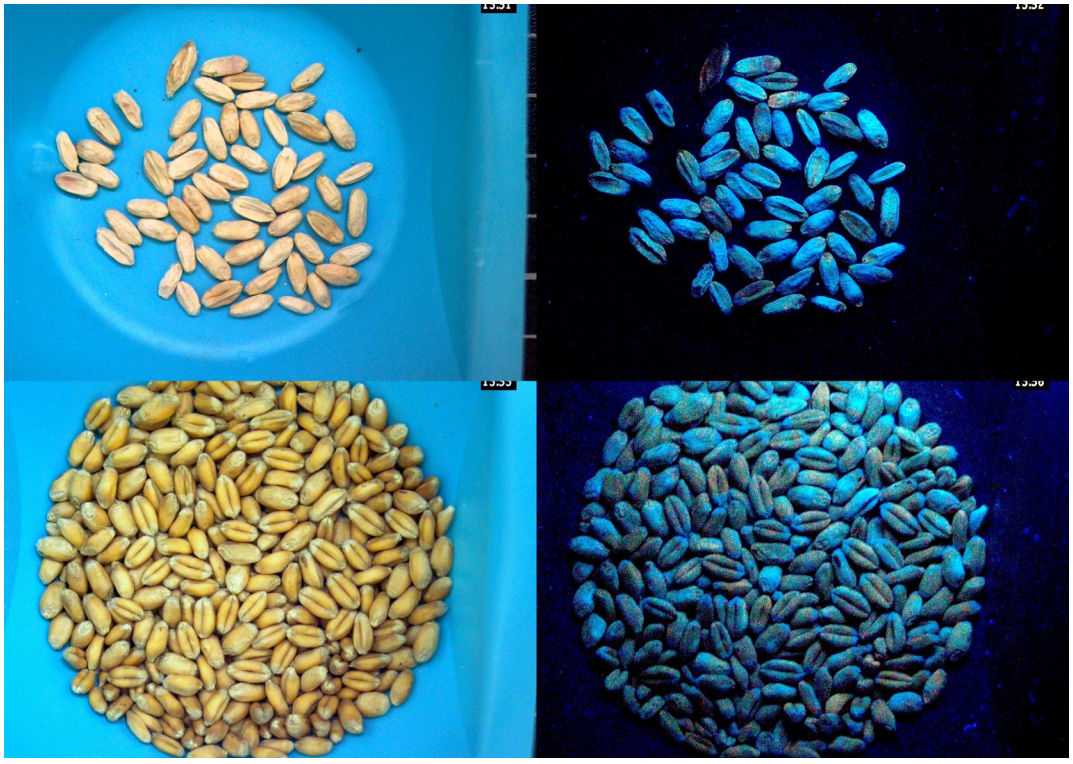

Fig. 2. Normal white kernels (NW) (top) and healthy-looking kernels (HL) (bottom) of the Turnia cultivar in visible and UV light
Rys. 2. Ziarniaki normalnej wielkości przebarwione (NW) (góra) i zdrowe ziarniaki (HL) (dół) odmiany Turnia w świetle widzialnym i świetle UV

Discussion

There was a significant variation in the severity of Fusarium head blight (FHB) between the two cultivar groups, leading to a marked difference in the level of kernel damage. This finally resulted in significant differences in the levels of DON and 3Ac-DON present. The data from the literature showed a correlation between the degree of infection of the heads and grains of triticale and wheat and the content of mycotoxins (Buerstmayr and Lemmens, 2015; Paul et al., 2006, 2005). This was confirmed by the author's research on winter wheat and triticale, in which higher correlations were obtained between the degree of kernel damage than the degree of head infection and the content of DON (Góral et al., 2019). For both cereals, the strength of the correlations varied depending on the environment. However, this variability was more pronounced in triticale (Góral et al., 2021; Mesterházy et al., 1999).

The content of mycotoxins, especially DON, in the most severely damaged kernels, was similar to the values obtained during the growth of isolates of *F. culmorum* and *F. graminearum* used for inoculation in wheat as a solid medium (Ochodzki and Góral, 2006). This is in line with other reports, showing that the smallest kernels are the most contaminated with mycotoxins (Beyer et al., 2010). However, it is surprising that the 'normal white' kernels (NW) that also contained a high concentration of DON were also the heaviest among the fractions examined.

The kernels defined as healthy also contained mycotoxins at or above the maximum permitted level by the European Union (Anonymous, 2006).

It was the effect of a severe infection of the heads and kernels after inoculation with *Fusarium*. It was possible to separate this fraction into two subsamples according to the light emittance in UV light. About 20-25% of kernels emitted light and this subsample contained more than 3 mg/kg of DON. The remaining kernels contained much lower DON comparable to the DON amount in control kernels.

Our results showed that in a sample of grain obtained from FHB-infected cereal the most infected kernels are the main source of *Fusarium* toxins. In our experiment concentration of total DON and 3Ac-DON in healthy (or healthy-looking) kernels was 16-47 times lower than in visible damaged kernels. This fact can be used to reduce the contamination of the grain sample by removing infected kernels with the highest mycotoxin content. Brodal et al. (2020) investigated the possibility of reducing oat grain contamination with T-2 and HT-2 toxins, DON, and enniatins. Grain sorting consisted of removing the smallest grains. This resulted in a reduction in T-2/HT-2 content by 44%, DON by 24%, and enniatin by 44%. At the same time, it should be noted that the small reduction in the content of DON resulted from the fact that the fraction of large kernels contained significant amounts of this toxin. On average, in small cocci, the concentration of DON was twice as high. For T-2/HT-2 toxins, it was from 4 to 8, depending on the year of testing.

In their review paper, Cheli et al. (2013) investigated the effectiveness of different sorting methods in removing mycotoxins from wheat grains. For DON it was from 7 to 63%, for T-2 and HT-2 above 50%, and for ZEN from 7 to 40%. Manual sorting, size-based sorting, and gravity sorting were used. A paper published in 2018 by Peng et al. (2018) also reviewed the results of various studies and cleaning of wheat grain samples. In the case of DON, the mycotoxin removal efficiency was up to 89% using gravity separation (Tibola et al., 2016). For T-2/HT-2 toxins, this efficacy was up to 100, but scouring and polishing were also used in addition to sorting (Lancova et al., 2008). Interestingly, in this study, the efficiency of removing DON was lower (about 50%) than in the work cited above.

Schaarschmidt and Fauhl-Hassek (2018) presented another review. They summarized several papers on reducing the content of mycotoxins (DON, NIV, ZEN, T-2/HT2). A very wide variation in efficiency was observed for DON, ranging from 5% to almost 90%. The authors draw attention to the possibility of sorting kernels not only based on size and density but also based on discoloration of kernels damaged by *Fusarium*. For example, the cited paper (Neuhof et al., 2008) shows that most of the toxins (DON, NIV, ZEN) were found in the kernels defined as "red", which corresponds approximately to the WS fraction in this work. The authors also distinguished the "shrunken" (=HSW) and "white" (= NW) fractions. In both of these fractions, the toxin content was slightly higher than in the unsorted sample. Contrary to the results of our work, no high content of toxins was observed in the "white" fraction. It should be added, however, that the content of DON in the unsorted sample was 0.16 mg/kg (much below the limit of 1.25 mg/kg), while in our study it was 25.57 mg/kg.

The results suggest that sorting grain solely based on grain size and density may not effectively remove kernels contaminated with Fusarium mycotoxins. Some kernels may contain significant amounts of mycotoxins, despite no reduction in size or density according to this study's results. A possible solution to this problem involves utilizing infrared, visible light, and UV spectroscopy, as well as hyperspectral imaging (Alisaac et al., 2019; Kautzman et al., 2015; Nadimi et al., 2023; Ollier et al., 2018; Peiris et al., 2010; Siuda et al., 2006). These methods use variations in the coloration of kernels under the influence of *Fusarium* infection or differences in light reflectance between damaged (or toxin-contaminated) kernels and sound kernels. They can be used in the construction of real-time sorters of cereal kernels (Chavez et al., 2023; Delwiche, 2008; Pascale et al., 2022; Tatzer et al., 2005).

Conclusions

1) Different levels of DON were detected in grain fractions with varying degrees of *Fusarium* damage.
2) The highest concentration of DON was found in the most severely damaged (white shriveled kernels). This fraction contains 54% to 91% of the total DON present in the grain sample.
3) A significant amount of DON was found in discolored normal-sized kernels of similar thousand kernel weight to that of healthy kernels.
4) In a subset of healthy kernels, those emitting light under UV light had three times higher levels of DON compared to those not emitting.

References

Alisaac, E., Behmann, J., Rathgeb, A., Karlovsky, P., Dehne, H.-W., Mahlein, A.-K., 2019. Assessment of *Fusarium* infection and mycotoxin contamination of wheat kernels and flour using hyperspectral imaging. Toxins 11, 556. https://doi.org/10.3390/toxins11100556

Anonymous, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union L364, 5–24.

Argyris, J., Van Sanford, D., TeKrony, D., 2003. *Fusarium graminearum* infection during wheat seed development and its effect on seed quality. Crop Sci. 43, 1782–1788. https://doi.org/10.2135/cropsci2003.1782

Bakker, M.G., Brown, D.W., Kelly, A.C., Kim, H.S., Kurtzman, C.P., Mccormick, S.P., O'Donnell, K.L., Proctor, R.H., Vaughan, M.M., Ward, T.J., 2018. *Fusarium* mycotoxins: a trans-disciplinary overview. Can. J. Plant Pathol. 40, 161–171. https://doi.org/10.1080/07060661.2018.1433720

Beyer, M., Pogoda, F., Ronellenfitsch, F.K., Hoffmann, L., Udelhoven, T., 2010. Estimating deoxynivalenol contents of wheat samples containing different levels of *Fusarium*-damaged kernels by diffuse reflectance spectrometry and partial least square regression. Int. J. Food Microbiol. 142, 370–4. https://doi.org/10.1016/j.ijfoodmicro.2010.07.016

Bottalico, A., Perrone, G., 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108, 611–624. https://doi.org/10.1023/a:1020635214971

Boutigny, A.-L., Richard-Forget, F., Barreau, C., 2008. Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. Eur. J. Plant Pathol. 121, 411–423. https://doi.org/10.1007/s10658-007-9266-x

Brodal, G., Aamot, H.U., Almvik, M., Hofgaard, I.S., 2020. Removal of small kernels reduces the content of *Fusarium* mycotoxins in oat grain. Toxins 12. https://doi.org/10.3390/toxins12050346

Buerstmayr, H., Lemmens, M., 2015. Breeding healthy cereals: genetic improvement of *Fusarium* resistance and consequences for mycotoxins. World Mycotoxin J. 8, 591–602. https://doi.org/10.3920/wmj2015.1889

Buerstmayr, H., Lemmens, M., Berlakovich, S., Ruckenbauer, P., 1999. Combining ability of resistance to head blight caused by *Fusarium culmorum* (W.G. Smith) in the F_1 of a seven parent diallel of winter wheat (*Triticum aestivum* L.). Euphytica 110, 199–206. https://doi.org/10.1023/a:1003757002052

Chavez, R.A., Opit, G., Opoku, B., Stasiewicz, M.J., 2023. Spectral kernel sorting based on high-risk visual features associated with mycotoxin contamination reduces aflatoxin and fumonisin contamination in maize from Ghana. Food Control 151, 109788. https://doi.org/10.1016/j.foodcont.2023.109788

Cheli, F., Pinotti, L., Rossi, L., Dell'Orto, V., 2013. Effect of milling procedures on mycotoxin distribution in wheat fractions: A review. LWT - Food Sci. Technol. 54, 307–314. https://doi.org/10.1016/j.lwt.2013.05.040

Delwiche, S.R., 2008. High-speed bichromatic inspection of wheat kernels for mold and color class using high-power pulsed LEDs. Sens. Instrum. Food Qual. Saf. 2, 103–110. https://doi.org/10.1007/s11694-008-9037-1

Dowell, F.E., Ram, M.S., Seitz, L.M., 1999. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem. 76, 573–576. https://doi.org/10.1094/cchem.1999.76.4.573

Foroud, N.A., Eudes, F., 2009. Trichothecenes in cereal grains. Int. J. Mol. Sci. 10, 147–173. https://doi.org/10.3390/ijms10010147

Góral, T., Walentyn-Góral, D., 2018. Zróżnicowanie podatności odmian pszenicy ozimej i jarej na fuzariozę kłosów badanych w latach 2009–2016. Biul. Inst. Hod. i Aklim. Roślin 284, 3–11. https://doi.org/10.37317/biul-2018-0001

Góral, T., Wiśniewska, H., Ochodzki, P., Nielsen, L.K., Walentyn-Góral, D., Stępień, Ł., 2019. Relationship between Fusarium head blight, kernel damage, concentration of *Fusarium* biomass, and *Fusarium* toxins in grain of winter wheat inoculated with *Fusarium culmorum*. Toxins 11, 2. https://doi.org/10.3390/toxins11010002

Góral, T., Wiśniewska, H., Ochodzki, P., Twardawska, A., Walentyn-Góral, D., 2021. Resistance to Fusarium head blight, kernel damage, and concentration of *Fusarium* mycotoxins in grain of winter triticale (x *Triticosecale* Wittmack) lines. Agronomy 11, 16. https://doi.org/10.3390/agronomy11010016

Góral, T., Wiśniewska, H., Ochodzki, P., Walentyn-Góral, D., Kwiatek, M., 2013. Reaction of winter triticale breeding lines to Fusarium head blight and accumulation of *Fusarium* metabolites in grain in two environments under drought conditions. Cereal Res. Commun. 41, 106–115. https://doi.org/10.1556/crc.2012.0028

Goswami, R.S., Kistler, H.C., 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5, 515–525. https://doi.org/10.1111/j.1364-3703.2004.00252.X

György, A., Tóth, B., Varga, M., Mesterhazy, A., 2020. Methodical considerations and resistance evaluation against *Fusarium graminearum* and *F. culmorum* head blight in wheat. part 3. Susceptibility window and resistance expression. Microorganisms 8. https://doi.org/10.3390/microorganisms8050627

Ji, F., He, D., Olaniran, A.O., Mokoena, M.P., Xu, J., Shi, J., 2019. Occurrence , toxicity , production and detection of *Fusarium* mycotoxin : a review. Food Prod. Process. Nutr. 1, 1–14. https://doi.org/10.1186/s43014-019-0007-2

Kautzman, M.E., Wickstrom, M.L., Scott, T.A., 2015. The use of near-infrared transmittance kernel sorting technology to salvage high-quality grain from grain downgraded due to *Fusarium* damage. Anim. Nutr. 1, 41–46. https://doi.org/10.1016/j.aninu.2015.02.007

Lancova, K., Hajslova, J., Kostelanska, M., Kohoutkova, J., Nedelnik, J., Moravcova, H., Vanova, M., 2008. Fate of trichothecene mycotoxins during the processing: milling and baking. Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 25, 650–659. https://doi.org/10.1080/02652030701660536

Liu, W., Langseth, W., Skinnes, H., Elen, O.N., Sundheim, L., 1997. Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. Eur. J. Plant Pathol. https://doi.org/10.1023/A:1008693213656

Mesterhazy, A., 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breed. 114, 377–386. https://doi.org/10.1111/j.1439-0523.1995.tb00816.x

Mesterházy, Á., Bartók, T., Mirocha, C.G., Komoróczy, R., 1999. Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed. 118, 97–110. https://doi.org/10.1046/j.1439-0523.1999.118002097.x

Miedaner, T., 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breed. 116, 201–220. https://doi.org/10.1111/j.1439-0523.1997.tb00985.x

Nadimi, M., Saccon, F.A.M., Elrewainy, A., Parcey, D., Sherif, S.S., Paliwal, J., 2023. Investigation of *Fusarium* damage in wheat using hyperspectral imaging: An independent component analysis approach. J. Near Infrared Spectrosc. https://doi.org/10.1177/09670335231202258

Neuhof, T., Koch, M., Rasenko, T., Nehls, I., 2008. Distribution of trichothecenes, zearalenone, and ergosterol in a fractionated wheat harvest lot. J. Agric. Food Chem. 56, 7566–71. https://doi.org/10.1021/jf800971q

Ochodzki, P., Góral, T., 2006. Production of mycotoxins by selected *Fusarium graminearum* and *F. culmorum* isolates cultured on rice and wheat, in: Conference Papers of 28. Mykotoxin-Workshop. Bydgoszcz, Poland, 29-31 May, p. 73.

Ollier, M., Talle, V., Brisset, A.-L., Le Bihan, Z., Duerr, S., Lemmens, M., Goudemand, E., Robert, O., Hilbert, J.-L., Buerstmayr, H., 2018. Whitened kernel surface: A fast and reliable method for assessing *Fusarium* severity on cereal grains by digital picture analysis. Plant Breed. https://doi.org/10.1111/pbr.12667

Pascale, M., Logrieco, A.F., Lippolis, V., De Girolamo, A., Cervellieri, S., Lattanzio, V.M.T., Ciasca, B., Vega, A., Reichel, M., Graeber, M., Slettengren, K., 2022. Industrial-scale cleaning solutions for the reduction of *Fusarium* toxins in maize. https://doi.org/10.3390/toxins14110728

Paul, P.A., Lipps, P.E., Madden, L. V., 2006. Meta-analysis of regression coefficients for the relationship between Fusarium head blight and deoxynivalenol content of wheat. Phytopathology 96, 951–961. https://doi.org/10.1094/phyto-96-0951

Paul, P.A., Lipps, P.E., Madden, L. V., 2005. Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: A meta-analysis. Phytopathology 95, 1225–1236. https://doi.org/10.1094/phyto-95-1225

Peiris, K.H.S., Pumphrey, M.O., Dong, Y., Maghirang, E.B., Berzonsky, W., Dowell, F.E., 2010. Near-infrared spectroscopic method for identification of Fusarium head blight damage and prediction of deoxynivalenol in single wheat kernels. Cereal Chem. 87, 511–517. https://doi.org/10.1094/cchem-01-10-0006

Peng, W.X., Marchal, J.L.M., van der Poel, A.F.B., 2018. Strategies to prevent and reduce mycotoxins for compound feed manufacturing. Anim. Feed Sci. Technol. 237, 129–153. https://doi.org/10.1016/j.anifeedsci.2018.01.017

Perkowski, J., Kiecana, I., Kaczmarek, Z., 2003. Natural occurrence and distribution of *Fusarium* toxins in contaminated barley cultivars. Eur. J. Plant Pathol. 109, 331–339. https://doi.org/10.1023/A:1023547210060

Schaarschmidt, S., Fauhl-Hassek, C., 2018. The fate of mycotoxins during the processing of wheat for human consumption. Compr. Rev. Food Sci. Food Saf. 17, 556–593. https://doi.org/10.1111/1541-4337.12338

Siuda, R., Balcerowska, G., Sadowski, C., 2006. Comparison of the usability of different spectral ranges within the near ultraviolet, visible and near-infrared ranges (UV-VIS-NIR) region for the determination of the content of scab-damaged component in blended samples of ground wheat. Food Addit. Contam. 23, 1201–1207. https://doi.org/10.1080/02652030600699304

Tatzer, P., Wolf, M., Panner, T., 2005. Industrial application for inline material sorting using hyperspectral imaging in the NIR range. Real-Time Imaging 11, 99–107. https://doi.org/10.1016/j.rti.2005.04.003

Tibola, C.S., Fernandes, J.M.C., Guarienti, E.M., 2016. Effect of cleaning, sorting and milling processes in wheat mycotoxin content. Food Control 60, 174–179. https://doi.org/10.1016/j.foodcont.2015.07.031