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Species of the genus *Fusarium* and *Fusarium* toxins in the grain of winter and spring wheat in Poland

Gatunki z rodzaju *Fusarium* oraz toksyny fuzaryjne w ziarnie pszenicy ozimej i jarej w Polsce

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The aim of the study was to determine the presence of *Fusarium* species and mycotoxins in wheat grain from harvest in 2009 and 2010 in Poland. Samples from different locations were analyzed for the content of DNA of *Fusarium* species and mycotoxins. In 2009, DNA of *F. graminearum* and *F. poae* was present in all samples, *F. culmorum* in 82% of samples, and *F. avenaceum* in 55% of samples. In 2010, the highest content of DNA was found for *F. graminearum* followed by *F. avenaceum*, *F. poae* and *F. langsethiae*. The amount of *F. culmorum* DNA was very low. The most frequently occurring species were *F. poae* and *F. graminearum*, however, the amount of *F. poae* DNA was lower. In 2009, deoxynivalenol was detected in all samples. In 2010, the average content of deoxynivalenol was lower than in 2009. Nivalenol was detected at very low concentration in both years. Significant correlations between content of *F. graminearum* DNA and deoxynivalenol concentration in the grain and between content of *F. poae* DNA and nivalenol concentration in the grain in 2009 were found.

Keywords: deoxynivalenol, DNA, *Fusarium* head blight, nivalenol, real-time PCR, trichothecenes, zearalenone

Celem badania było określenie obecności gatunków *Fusarium* i mykotoksyn występujących w ziarnie pszenicy w Polsce w latach 2009 i 2010. Próby ziarna pochodzące z różnych regionów Polski zostały przeanalizowane pod kątem zawartości DNA grzybów z rodzaju *Fusarium* oraz mykotoksyn. W 2009 roku DNA *F. graminearum* i *F. poae* było obecne we wszystkich próbach, *F. culmorum* w 82% prób, *F. avenaceum* w 55% próbek. W 2010 r. najwyższą zawartość DNA stwierdzono dla *F. graminearum*, a następnie *F. avenaceum*, *F. poae* i *F. langsethiae*. Ilość DNA *F. culmorum* była bardzo niska. Najczęściej występującymi gatunkami były *F. poae* i *F. graminearum*. Jednakże ilość DNA *F. poae* była niższa. W 2009 r. we wszystkich próbach wykryto deoksyniwalenol. W 2010 r. średnia zawartość deoksyniwalenolu była niższa niż w 2009 r. Niwalenol wykryto w bardzo niskim stężeniu w obu latach. Stwierdzono istotność korelacji między zawartością DNA *F. graminearum* a stężeniem deoksyniwalenolu w ziarnie oraz między zawartością DNA *F. poae* a stężeniem niwalenolu w ziarnie w 2009 r.

Słowa kluczowe: deoksyniwalenol, DNA, fuzarioza kłosów, niwalenol, real-time PCR, trichoteceny, zearalenon

Introduction

Fusarium head blight (FHB) is a disease of wheat caused by a complex of toxicogenic fungi of the genus *Fusarium* (Parry *et al.* 1995). The main species of this complex in Europe are *F. graminearum* and *F. culmorum* identified as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN) producers. However, other *Fusarium* species producing mycotoxins are also prevalent: *F. avenaceum* – moniliformin, enniatins and beauvericin (BEA) producer; *F. poae* – NIV, BEA producer. *Fusarium langsethiae* and *F. sporotrichioides* – T-2 and HT-2 toxins producers are also prevalent (Bottalico and Perrone 2002; Jestoi *et al.* 2008; Vogelgsang *et al.* 2008; Somma *et al.* 2010; Imathiu *et al.* 2013). *Fusarium graminearum* and *F. culmorum* are highly pathogenic species, which can cause severe epidemics of FHB. The other species are medium or weakly pathogenic, however, due to the wide prevalence, they may also cause mycotoxin contamination of grain (Uhlig *et al.* 2007; Yli-Mattila *et al.* 2008; Nielsen *et al.* 2011; Dinolfio and Stenglein 2014).

Because of the diversity of *Fusarium* species causing FHB, monitoring of changes in the *Fusarium* population on wheat is important. Frequency of species infecting wheat

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is not stable and changes depending on the weather in particular year (Xue *et al.* 2019). Differences are also observed between regions of wheat production in Europe. For example, other species dominate in North-Eastern Europe (equal share of three species *F. avenaceum*, *F. culmorum*, *F. graminearum*) than in south-western part of the continent (mainly *F. graminearum*) (Bottalico and Perrone 2002). Species compositions changes over time, which is the results of climate warming, and changes in the acreage of major cereal crops –particularly increase of the maize area (Sundheim *et al.* 2013; Hofgaard *et al.* 2016; Maiorano *et al.* 2008; Vaughan *et al*. 2016). The main reported effect of the above factors is increase in *F. graminearum* occurrence and decrease in *F. culmorum* (Parikka *et al.* 2012; Miller 2008; Scherm *et al.* 2013; Hofgaard *et al.* 2016; Bilska *et al.* 2018). Chandelier *et al.* (2011) analysed winter wheat samples from Belgium over 2003–2009 period. They found that main species were *F. avenaceum* and *F. graminearum*; however, their frequency changed depending on year from 20 to 100%. The frequency of *F. poae* was relatively constant over the years (about 70%). The overall incidence of *F. culmorum* decreased during the study, from 80% in 2003 to 10% over the final three years. Similarly, Isebaert *et al.* (2009) observed that *F. graminearum* and *F. culmorum* were the most important species in Northern Belgium in 2002–2005. They found correlation between crop prevalence and both species frequency. *F. graminearum* dominated in areas of maize cultivation, *F. culmorum* in areas small grain cereals cultivation. In Luxemburg, the most common species isolated from wheat heads were *F. graminearum, F. avenaceum* and *F. poae*. Increase of frequency of *F. graminearum* and decrease in *F. culmorum* were observed (Giraud *et al.* 2010). Winter wheat cultivated in the Netherlands in 2009 was studied for *Fusarium* species and toxins (van der Fels-Klerx *et al.* 2012). In samples collected on harvest, authors found dominance of *F. graminearum. F. avenaceum* and *Microdochium nivale* were also frequent. However, in the pre-harvest samples, only *F. graminearum* and *M. nivale* were present. Waalwijk *et al.* (2004) analysed wheat heads and grain collected in the Netherlands in 2001 and 2002. In 2001, in samples collected at late milk stage, *F. graminearum* was predominant; however, some samples contained also *F. avenaceum* and/or *F. culmorum*. At harvest, *F. graminearum* dominated almost completely. In 2002 the weather conditions were more favorable for FHB, and they found relative dominance of *F. graminearum* in grain from the Netherlands and almost complete in samples from France. According to Birzele *et al.* (2002) in 1997 and 1998 the dominating species in Germany in wheat grain were *F. avenaceum*, *F. poae*, *F. culmorum* and *F. graminearum*. Frequencies of two last species were similar, however percentage of *F. graminearum* increased in 1998. In Germany in 2008, *F. graminearum* sensu stricto was the predominant species followed by *F. culmorum*. Other species (*F. poae*, *F. tricinctum*, *M. nivale* etc.) were identified in small amounts (Talas *et al.* 2011). Similar results were obtained by Birr *et al.* (2020) who analyzed winter wheat grain samples from seven locations in Germany from 2013 to 2017. In Hungary, in year 2010, which was very favorable for FHB development, predominantly *F. graminearum* was isolated from wheat grain (Laszlo *et al.* 2011).

Waalwijk *et al.* (2003) analyzed wheat ears with FHB symptoms collected in Netherlands in 2000 and 2001. They found that *F. graminearum* was the dominating *Fusarium* species in both years. As they stated, this was significant change comparing results from the 1980s and 1990s, which showed that *F. culmorum* was_the predominant species in the Netherlands. They presume that this shift could be connected with an increase in maize acreage. *F. graminearum,* unlike *F. culmorum,* is a major pathogen on maize and, can survive on maize debris (Xu and Nicholson 2009; Maiorano *et al.* 2008). The other factor could be climate warming which favors *F. graminearum* as it has higher optimal temperature of development (Vaughan *et al.* 2016)._The good example of this shift can be first detection of *F. graminearum* in wheat grain collected in 2017 in West Siberia, Russia (Gagkaeva *et al.* 2019) as well as absence of *F. graminearum* until 2012 in FHB infected cereal (Supronienė et al. 2010, 2016)

The prevalence of FHB pathogens differed significantly between studied countries in 2001 and 2002 (UK, Ireland, Italy and Hungary) (Xu *et al.* 2005). Overall, all pathogens (*F. graminearum, F. culmorum, F. avenaceum* and *F. poae*) were commonly detected in Ireland and to a lesser extent in the UK. In contrast, only two species, *F. graminearum* and *F. poae*, were regularly detected in Italy and Hungary. *Fusarium culmorum* was rarely detected except in Ireland. The latter country has the coolest summer weather among four studied countries. Authors stated that the increase in *F. graminearum*, especially in the UK, appears to have been at the expense of *F. culmorum*. The replacement of *F. culmorum*

by *F. graminearum* as the predominant FHB pathogen was also reported in Bavaria (Obst *et al.* 1997) where the change was linked with increased maize production in Poland in years 2016 and 2017 more than 80% of isolates collected from symptomatic wheat heads were *F. graminearum,* and less than 4% were *F. culmorum* (Bilska *et al.* 2018). *F. graminearum* dominated in wheat grain in 2012, and was replaced by *F. poae* in 2013 (Wolny-Koładka et al. 2015). It is worth to notice that in Poland grain maize acreage increased considerably from 1990 (59 000 ha) to 2017 (above 1 215 500 ha).

The *Fusarium* species can be isolated from cereal kernels and identified using classical and/or molecular methods (Wiśniewska *et al.* 2014). The molecular method widely used for identification and quantification of *Fusarium* DNA concentration in samples is real time PCR (Niessen 2007; Nicolaisen *et al.* 2009; Nielsen *et al.* 2011, 2013; Horevaj *et al.* 2011).

The aim of the present study was to determine the presence *Fusarium* species and content of mycotoxins in wheat grain in Poland, Samples were collected in 2009 and 2010. Results were compared with *Fusarium* species frequency reported earlier and the results obtained after 2010.

Material and methods

Cereal grain samples

Fifty samples of wheat grain were collected during the harvesting season 2010. They originated from 25 experimental stations of COBORU (the Research Centre for Cultivar Testing) located in different regions of Poland (Figure 1; marked with numbers). Two winter wheat cultivars 'Bogatka' (medium resistant to FHB) and 'Muszelka' (susceptible) were included. The winter wheat was grown with a moderate nitrogen input (avg. 90 kg/ha of N) and without chemical control of diseases. The grain was harvested using combine harvester.

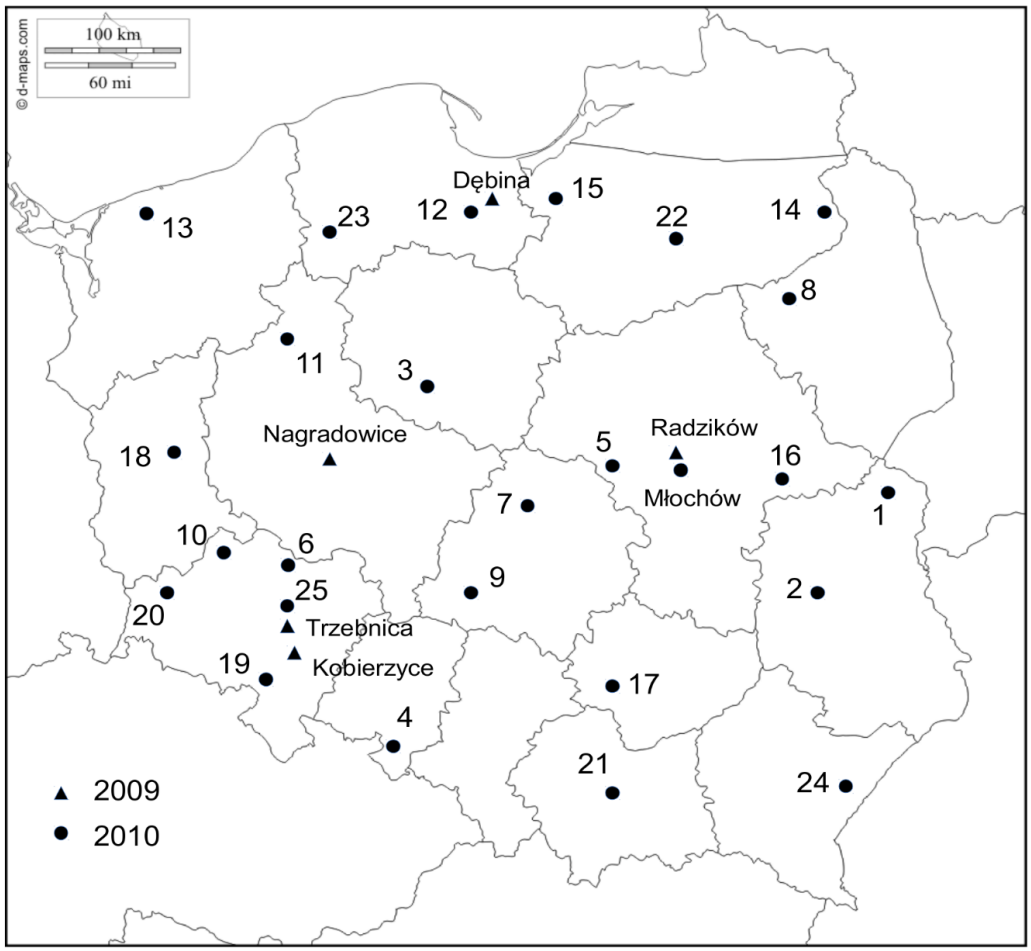

Figure 1. Map of Poland showing locations of sample collection of winter and spring wheat grain in 2009 (triangles) and 2010 (circles). Location numbers correspond to these in Table 2

Rysunek 1. Mapa Polski pokazująca miejsca pochodzenia prób ziarna pszenicy ozimej i jarej w latach 2009 (trójkąty) i 2010 (kółka). Numery miejscowości odpowiadają numerom w Tabeli 2

Additionally, 11 samples of wheat grain from 2009 (5 locations) and 8 samples from 2010 (2 locations) were analyzed. Samples were collected from different locations/fields and cultivars of spring and winter wheat (Figure 1, Table 1, Table 4).

Wheat grain samples were stored in a freezer at –20°C before DNA and mycotoxins extraction.

DNA extraction and analysis

Grain samples of 300g were initially ground with a laboratory grinder and 5 g was powdered in liquid N_2 with eight steel balls using Geno/Grinder 2000 (OPS Diagnostics, Bridgewater, NJ). DNA was extracted from 100 mg of that powdered sample using a modified CTAB method (http://gmo-crl.jrc.ec.europa.eu/summaries/NK603-WEB-Protocol-Validation.pdf) as described by Nicolaisen *et al.* (2009). DNA extracted from the wheat samples was further purified using a DNeasy kit (Qiagen) according to the manufacturer's instructions.

The *Fusarium* isolates: *F. avenaceum* 9605, *F. culmorum* 9560, *F. equiseti* 8752, *F. graminearum* 1955, *F. langsethiae* 8051, *F. poae* 8452, *F. sporotrichioides* 1926, and *F. tricinctum* 8048 were grown and extracted as described in Nielsen *et al.* (2011). They were grown on potato dextrose agar (PDA) medium at 22°C under 12 h of light and 12 h of darkness for 1–2 weeks prior to DNA extraction. PDA plates before inoculation were covered with sterile cellophane membranes (Horevaj *et al.* 2011). Mycelium was scraped off the cellophane membrane using a spatula and ground in liquid N_2 with eight steel balls using a Geno/Grinder 2000 (OPS Diagnostics, Bridgewater, NJ). Powdered mycelium (100 mg) was used for DNA extraction, using the same method as for grain samples. The concentration of DNA from *Fusarium* isolates was determined using NanoDrop 1000 (Thermo Fisher Scientific, MA).

Qualitative and quantitative determinations of eight *Fusarium* species in grain were performed by real time-PCR. Primers used were based on fungal TEF-1α gene sequences, designed by Nicolaisen *et al.* (2009), specific for the different *Fusarium* species: *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. langsethiae*, *F. poae*, *F. sporotrichioide*s, and *F. tricinctum*.

Real-time PCR was carried out in 12.5 µl consisting of 6.25 µl of 2× SYBR Green PCR Master Mix (Applied Biosystems), 250 nM each primer, bovine serum albumin at 0.5 µg/µl, and 2.5 µl of template DNA. PCR reactions were performed in duplicate on all samples. Genomic DNA from grain samples and pure cultures was diluted 1:10 before PCR.

PCR was performed on a 7900HT Sequence Detection System (Applied Biosystems) using the following cycling protocol: 2 min at 50°C; 95°C for 10 min; 40 cycles of 95°C for 15 s and 62°C for 1 min; followed by dissociation analysis at 60 to 95°C. For the plant assay annealing and extension was performed at 60 °C. Standard curves for *Fusarium* species and wheat were made of five-fold dilution series using pure fungal DNA and wheat DNA. The amount of fungal DNA was calculated from the cycle threshold (Ct) values using the standard curve. The result of each individual sample from each species-specific assay were evaluated by studying the dissociation curve and Ct value, as SYBR Green binds to all double stranded DNA and might create false positives. The plant EF1α assay was used to provide a normalized measurement for *Fusarium* DNA in each sample, which was calculated as picograms of fungal DNA per micrograms of plant DNA according to Nicolaisen *et al.* (2009).

Analysis of Fusarium toxins

The type B trichothecenes – deoxynivalenol (DON), nivalenol (NIV) were quantified using gas chromatography with electron capture detection (GC-ECD) technique. Mycotoxins were extracted from 5 g of ground grains using 25 ml of an aqueous solution of acetonitrile (acetonitrile: water 84:16) in a shaker 90 min, 300 r.p.m.. Samples were centrifuged (3000 rpm min^{-1}, 5 min.), and the extract was purified with MycoSep® 227 Trich+ columns (Romer Labs Inc., Union, MO). One milliliter of the internal standard solution (chloralose) was added to 4 ml of purified extract. The solvent was evaporated to dryness in the stream of air. Mycotoxins were derivatized to the trimethylsilyl derivatives using a derivatizing agent Sylon BTZ (BSA + TMCS + TMSI, 3: 2: 3, Supelco). After dissolution of sample in isooctane, excess of derivatizing agent was decomposed and removed with water. The organic layer was transferred to 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.

The carrier gas was hydrogen, adjusted to pressure 12 psi, with nitrogen as a make-up gas at 60 mL/min. Elution was carried out in the temperature gradient: Initial temperature was 170 °C, increased to 250 °C at 5 °C/min., and increased from 250 °C to 300 °C at 10 °C/min., followed by a holding time of 5 min., and decreased to 170 °C. Mycotoxin detection was carried out using 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. Results were corrected for recoveries, ranged from 73% (NIV) to 85% (DON). The limits of detection (LOD) was on average 5 µg/kg, and limit of quantification 10 µg/kg.

The content of zearalenone (ZEN) was determined using a quantitative direct competitive enzyme-linked immunosorbent assay (ELISA) AgraQuant® ZON 25/1000 (Romer Laboratories). (LOD 10 µg/kg, LOQ 25 µg/kg). Based on results of reference sample (Quality Control Material, Biopure, Austria), correction for recovery was not applied.

Statistical analysis

The original *Fusarium* DNA and toxin concentrations were transformed to logarithmic values to obtain a normal distribution for the variables. The relationships between the results for *Fusarium* DNA and *Fusarium* toxins were investigated by Pearson correlation tests. Principal component analysis was used to analyze relationship between concentrations of DNA of *F. avenaceum, F. culmorum*, *F. graminearum, F. langsethiae* and *F. poae* in grain samples from 25 locations. Next, PCA was applied to analyze relationship between concentrations of *Fusarium* toxins (DON, NIV, ZEN) and DNA of producing species *F. culmorum*, *F. graminearum* and *F. poae* in grain samples from 25 locations. The correlation and PCA analyses were performed using Microsoft® Excel 2010/ XLSTAT©-Pro (Version 2013.4.07, Addinsoft, Inc., Brooklyn, NY, USA).

Results

Samples from 2009

In 2009, the highest amount of *Fusarium* DNA was found in the grain of spring wheat 'Griwa' (Radzików 1) and winter wheat 'Muszelka' (Dębina 2), which is highly susceptible to FHB (Table 1). The lowest amounts were detected in the grain of winter wheat cultivars 'Zawisza' (Radzików 6) and 'Tonacja' (Radzików 5) and in spring wheat 'Raweta' (Radzików 3).

Table 1
Tabela 1

Concentration of *Fusarium* species DNA and DON, NIV and ZEN mycotoxins levels in samples of grain of spring and winter wheat collected in 2009

Zawartość DNA gatunków *Fusarium* oraz mykotoksyn DON, NIV i ZEN w próbach ziarna pszenicy jarej i ozimej zebranych w 2009 r.

No. Lp.	Sample name Próba	*Fusarium* DNA (pg/µg) [a]				DON (µg/kg)	NIV (µg/kg)	ZEN (µg/kg)
		Fa	*Fc*	*Fg*	*Fp*			
1	Radzików 1[b]	1300	153	60248	70	5719	43	63
2	Radzików 2[b]	89	41	21804	0	2020	0	25
3	Radzików 3[b]	53	31	911	9	104	0	0
4	Dębina 1	0	316	18966	63	2937	45	78
5	Dębina 2[c]	533	8862	46102	287	7170	281	29
6	Kobierzyce	0	387	26384	949	n/a	n/a	n/a
7	Nagradowice[c]	366	22949	5462	67	9239	33	230
8	Radzików 4	0	34	4277	137	658	177	0
9	Radzików 5	63	38	2115	187	213	36	12
10	Radzików 6	0	0	207	33	47	0	17
11	Trzebnica	1753	252	2044	285	123	61	0
	Mean Średnia	378	3006	17138	190	2823	68	45

[a] – *F. langsethiae, F. sporotrichioides* and *F. tricinctum* were excluded; [b] – spring wheat; [c] – grain from collected symptomatic spikes; *Fa = F. avenaceum, Fc = F. culmorum, Fg = F. graminearum, Fp = F. poae;* n/a – not analysed

[a] – *F. langsethiae, F. sporotrichioides* i *F. tricinctum* nie zostały pokazane; [b] – pszenica jara; [c] – ziarno z kłosów z objawami fuzariozy; *Fa = F. avenaceum, Fc = F. culmorum, Fg = F. graminearum, Fp = F. poae;* n/a – nie analizowane

Of the eight *Fusarium* species tested, seven were detected in wheat grain, except for *F. langsethiae*. *Fusarium graminearum* was present in all samples, *F. poae* and *F. culmorum* in ten samples (91%), *F. avenaceum* in seven samples (64%). *Fusarium sporotrichioides* and *F. tricinctum* were found in two individual samples: first species in sample 'Radzików 1' at 69 pg/µg, and the second in wheat grain from Dębina ('Dębina 2') at 428 pg/µg. Traces of *F. equiseti* were found in two samples ('Dębina 2', 'Nagradowice').

Despite large differences in *Fusarium* DNA content in the grain samples, amount of *F. graminearum* DNA was the highest in nine samples (Figure 2). *F. culmorum* dominated only in a sample from Nagradowice and in sample from Trzebnica concentrations of *F. avenaceum* and *F. graminearum* DNA were similar.

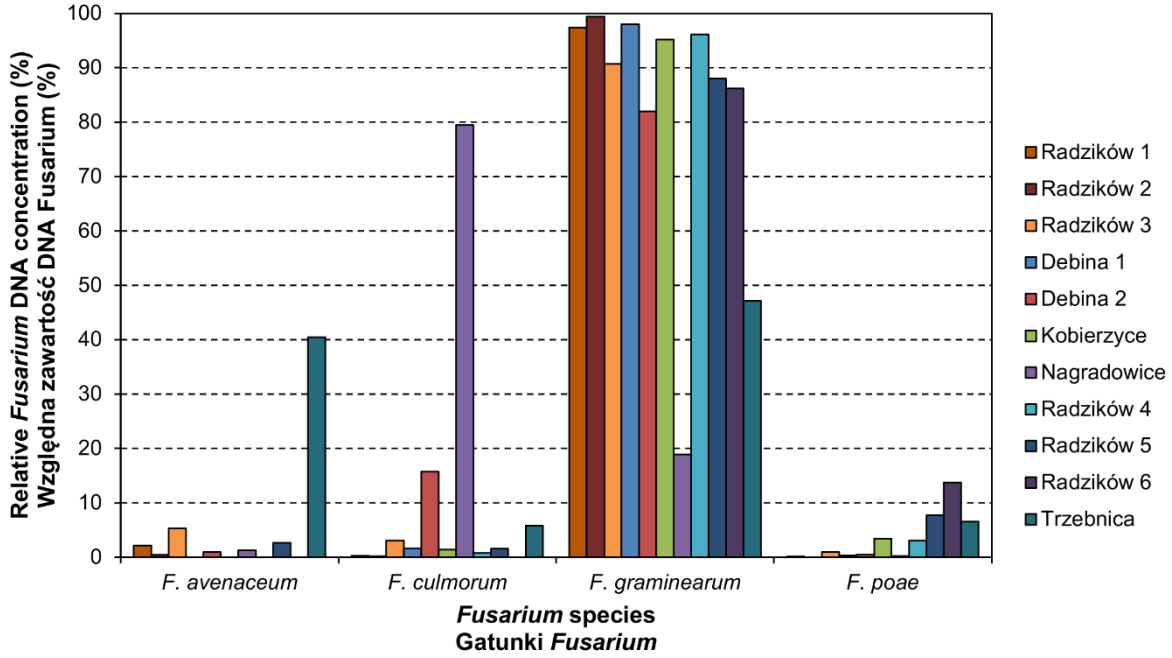

Figure 2. Relative concentration of DNA of four *Fusarium* species in 11 samples of spring and winter wheat collected in 2009. *F. equiseti*, *F. sporotrichioides* and *F. tricinctum* were excluded

Rysunek 2. Względna zawartość DNA czterech gatunków *Fusarium* w 11 próbach pszenicy jarej i ozimej zebranych w 2009 r. *F. equiseti*, *F. sporotrichioides* i *F. tricinctum* nie zostały włączone

DON was detected in all analysed samples at the average level of 2823 µg/kg (Table 1). The most contaminated were the grain samples of winter wheat 'Nagradowice' and 'Debina 2' and spring wheat 'Radzików 1'. Levels of NIV were much lower. On average, it was 68 µg/kg. NIV was detected in seven samples. The highest concentration was in the grain of winter wheat from 'Debina 2' and winter wheat 'Radzików 4'. ZEN was detected in six samples at the average level of 45 µg/kg. Considerable amounts of ZEN were found in samples from Nagradowice and in samples of spring wheat 'Radzików 1' and winter wheat 'Dębina1'.

DON concentration correlated significantly with total *Fusarium* DNA ($r = 0.947$, $p < 0.001$), for NIV and ZEN coefficients were insignificant ($r = 0.537$, $p = 0.109$ and $r = 0.561$, $p = 0.092$, respectively). When looking at individual species, high correlation between DON and DNA of *F. graminearum* and *F. culmorum* were evident ($r = 0.885$, $p = 0.001$ and $r = 0.740$, $p = 0.014$, respectively). As regards NIV, significant correlation was observed with *F. poae* DNA ($r = 0.875$, $p = 0.001$).

Samples from 2010

In 2010, average concentration of *Fusarium* DNA was 1970 pg/µg (1430 pg/µg in 'Bogatka' grain and 3770 pg/µg in 'Muszelka' grain) (Table 2). The difference in *Fusarium* DNA concentration between cultivars was statistically significant according to paired samples t-test. The highest concentration of DNA was detected in the grain from Zadąbrowie, South-Eastern Poland (Figure 1). The DNA amount was five-six times lower in the grain from Czesławice (South-Eastern Poland), Rychliki, Radostowo (Northern PL) and Głubczyce (Southern PL). Very low concentration of DNA was

found in the grain from Naroczyce, Nowa Wieś Ujska (Western Poland), Kawęczyn (Central Poland), and Rarwino (North-Western Poland). At a regional scale, the highest *Fusarium* DNA concentration was observed in the grain from South-Eastern and North-Eastern Poland and the lowest concentrations was observed in the grain from Western, North-Western and Central Poland (Figure 1).

Table 2
Tabela 2

Concentration of total *Fusarium* DNA, and DON, NIV and ZEN mycotoxins levels in grain of winter wheat cultivars 'Bogatka' and 'Muszelka' from 2010 harvest

Sumaryczne stężenie DNA *Fusarium* oraz zawartość mykotoksyn DON, NIV i ZEN w ziarnie odmian pszenicy ozimej Bogatka i Muszelka ze zbiorów w 2010 r.

No. Lp.	Location Miejscowość	***Fusarium* DNA (pg/µg) [a]**		**DON (µg/kg)**		**NIV (µg/kg)**		**ZEN (µg/kg)**	
		Mean Średnia	**SD**	**Mean Średnia**	**SD**	**Mean Średnia**	**SD**	Mean Średnia	SD
1	Cicibór	2295	620	76.3	7.6	53.7	0.4	17.9	17.9
2	Czesławice	4126	1319	181.4	37.4	63.7	0.0	93.1	35.2
3	Głębokie	842	203	63.5	7.4	59.5	1.1	18.1	18.1
4	Głubczyce	2919	969	127.2	35.5	60.7	5.6	26.8	26.8
5	Kawęczyn	55	54	61.1	2.9	51.8	1.6	0	0
6	Krościna Mała	1327	1276	61.0	7.1	52.2	0.5	0	0
7	Lućmierz	315	36	110.7	54.3	61.5	8.0	0	0
8	Marianowo	1743	948	53.3	1.4	52.1	0.1	0	0
9	Masłowice	650	203	65.3	9.4	51.7	1.7	10.6	10.6
10	Naroczyce	38	38	63.0	5.3	50.8	1.9	9.9	9.9
11	Nowa Wieś Ujska	65	65	51.9	1.7	50.3	1.1	10,1	10.1
12	Radostowo	3466	1428	58.6	1.9	53.0	0.2	0	0
13	Rarwino	108	108	53.5	3.3	52.8	1.1	0	0
14	Ruska Wieś	951	73	55.4	2.7	51.8	0.7	13.5	13.5
15	Rychliki	3230	2138	87.9	9.2	54.3	0.6	42.0	21.5
16	Seroczyn	731	731	78.1	15.0	54.6	3.4	27.6	27.6
17	Słupia	1116	474	107.3	27.7	53.6	3.7	36.9	36.9
18	Świebodzin	303	165	51.2	1.8	51.9	0.2	13.8	13.8
19	Tarnów	1213	14	89.1	28.5	54.8	4.5	0	0
20	Tomaszów Boles.	231	148	76.3	3.5	55.1	0.2	0	0
21	Węgrzce	927	451	86.9	7.2	56.5	3.3	20.1	20.1
22	Wrócikowo	1679	723	165.8	96.7	61.6	9.2	0	0
23	Wyczechy	645	288	83.0	24.0	56.7	5.4	0	0
24	Zadąbrowie	19269	6931	420.3	131.7	57.7	1.3	227.0	21.3
25	Zybiszów	1017	97	76.9	12.4	56.6	0.6	29.3	29.3
Mean Średnia		1970	-	96.2	-	55.2	-	23.9	-
Mean Średnia 'Bogatka'		1430	-	78.2	-	53.4	-	11.4	-
Mean Średnia 'Muszelka'		3770	-	114.2	-	56.9	-	36.3	-

[a] – sum of DNA of detected *Fusarium* species
[a] – suma DNA wykrytych gatunków *Fusarium*

Of the eight *Fusarium* species tested, five were detected in wheat grain. DNA of *F. equiseti*, *F. sporotrichioides* and *F. tricinctum* was not detected in any sample. The highest was the content of *F. graminearum* DNA (1252 pg/µg), then *F. avenaceum* (259 pg/µg), *F. langsethiae* (237 pg/µg) and *F. poae* (168 pg/µg) (Figure 3). The content of *F. culmorum* DNA (55 pg/µg) was very low.

The most frequently occurring species were *F. poae* (detected in 74% of samples) and *F. graminearum* (detected in 52% of samples) (Figure 3). In 18% of samples *F. poae* was the only species found. *F. langsethiae* was detected in six samples (five from three locations in Northern Poland – Wyczechy, Radostowo, Rychliki). The concentration of *F. langsethiae* in these samples was relatively high (1972 pg/µg) as compared with an average for samples containing *F. graminearum* DNA (2235 pg/µg).

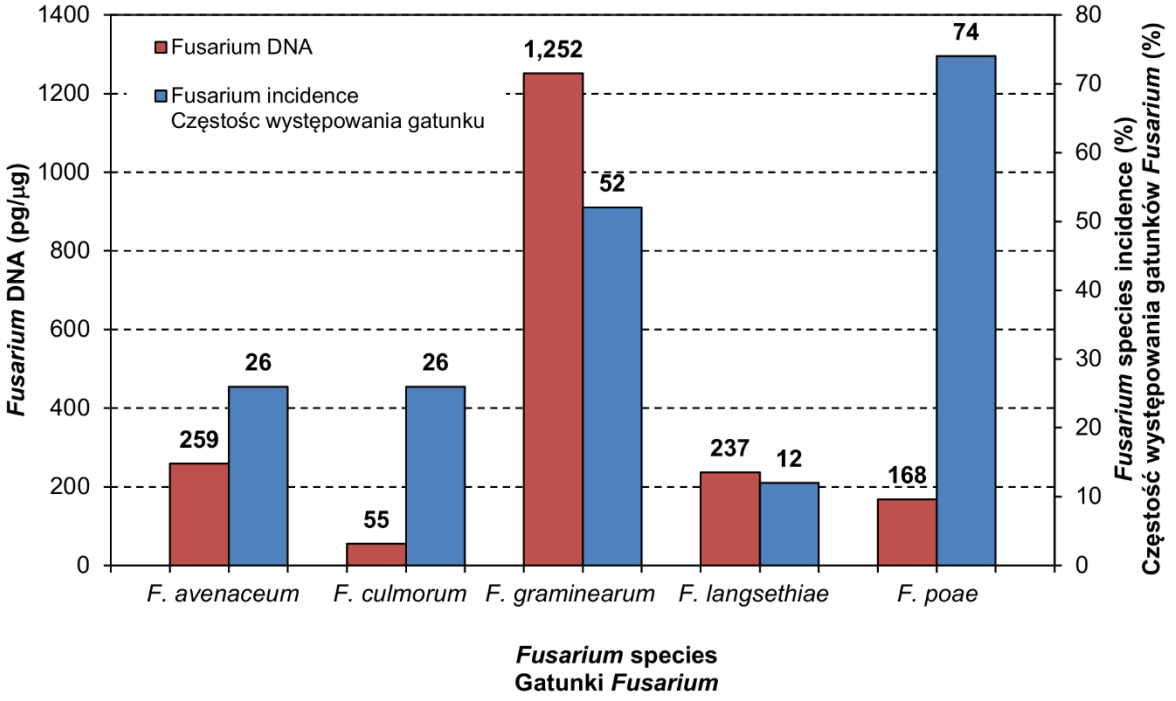

Figure 3. Average concentration of DNA (pg of fungal DNA/mg of wheat DNA) and incidence (percentages) of five *Fusarium* species in 50 samples of winter wheat collected in Poland in 2010

Rysunek 3. Średnie stężenie DNA (pg DNA *Fusarium*/µg DNA pszenicy) i częstość występowania (procent) pięciu gatunków *Fusarium* w 50 próbach pszenicy ozimej zebranych w Polsce w 2010 r.

F. poae was detected in all samples of medium resistant cultivar 'Bogatka' but only in 48% of samples of susceptible 'Muszelka'. Another species *F. avenaceum* was also found more frequently in the grain of 'Bogatka' (32%) than 'Muszelka' (20%). Three other species (*F. culmorum*, *F. graminearum*, *F. langsethiae*) were detected in the grain of both cultivars with similar frequency.

Amounts of DNA of *Fusarium* species weakly correlated with each other. Only coefficient of correlation of *F. graminearum* with. *F. culmorum* was statistically significant (r = 0.461, p = 0.02). Positive relationship was found between *F. avenaceum* and *F. culmorum* or *F. graminearum* (r = 0.306, r = 0.162) as DNA of the first species was mostly detected in the same locations as the other two species – 1, 4, 14, 19, 22 (only *F. graminearum*), and 24. DNA of *F. langsethiae* did not correlate with other species, as it was found only in six samples. Otherwise, *F. poae* DNA did not correlate with other species because the species was present in the most of samples (74%) and in the most samples (except two) amounts of *F. poae* DNA were similar.

Biplot produced by PCA analysis on DNA concentration of five *Fusarium* species showed uneven distribution of these species in different locations (Figure 4). *F. culmorum* was present mostly in the same locations as *F. graminearum* (except 12). *F. avenaceum* was present in the same six locations as *F. culmorum* and *F. graminearum* (except 22, where only the second species was detected). In three locations (7, 8, 13) this species was

accompanied only by *F. poae*. As it was mentioned earlier, *F. langsethiae* was found in four locations (12, 15, 17, 23). In Słupia (17) it was accompanied by *F. culmorum* and *F. graminearum*, in Radostowo (12) and Rychliki (15) by *F. culmorum* or *F. graminearum*, respectively.

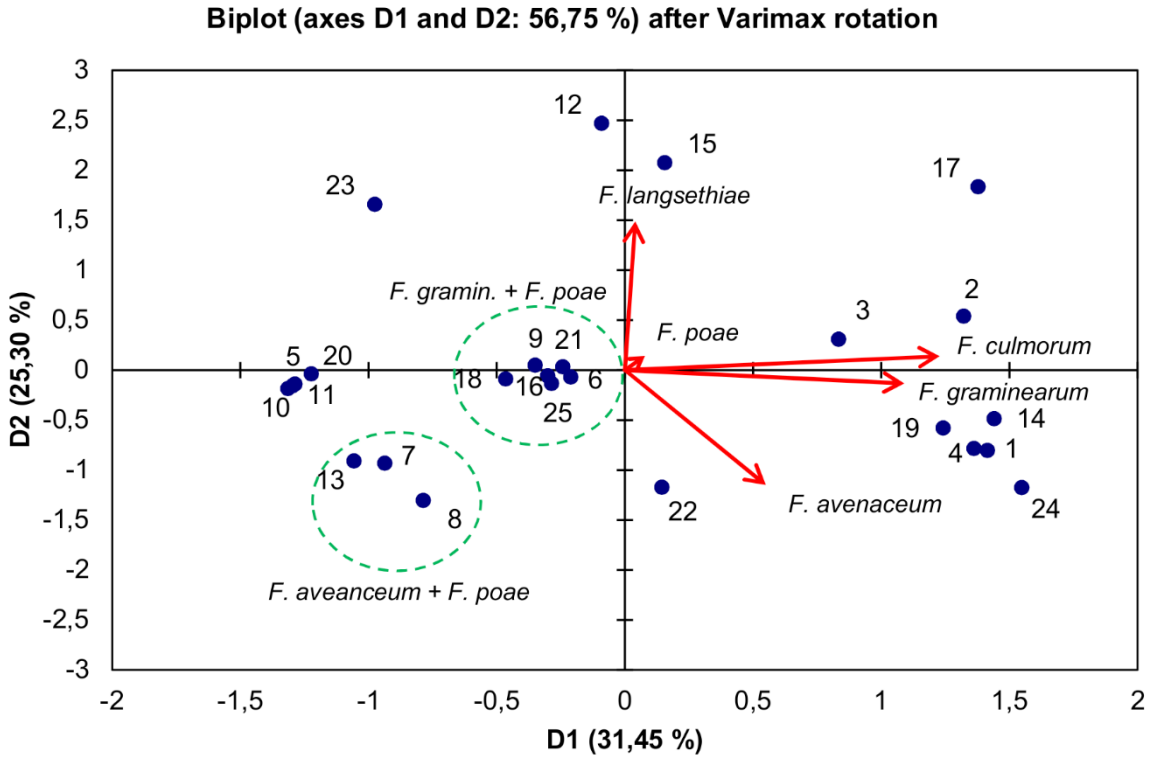

Figure 4. Principal Component Analysis based on DNA of *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. langsethiae* and *F. poae* in grain samples of winter wheat collected from 25 locations in Poland in 2010. Location numbers correspond to those in table 3. Variables were log transformed prior to the analysis

Rysunek 4. Analiza składowych głównych dla zawartości DNA *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. langsethiae* i *F. poae* w próbach ziarna pszenicy ozimej zebranych z 25 lokalizacji w Polsce w 2010 roku. Numery lokalizacji odpowiadają numerom w Tabeli 3. Zmienne zostały przekształcone logarytmicznie przed analizą

Average content of DON was low and amounted to 96.2 µg/kg, at a range from.49.3 to 552.0 µg/kg (Table 3). The content of NIV was very low – 55.2 µg/kg, at a range 49.2 – 70.8 µg/kg. The average content of DON for 'Bogatka' was 78.2 µg/kg, and 114.2 µg/kg for 'Muszelka'. Difference of DON content between cultivars was not statistically significant. The highest concentration of DON was found in samples of both cultivars from Zadąbrowie and Czesławice, South-Eastern Poland (Figure 1). High concentration of this toxin was also found in the samples of 'Muszelka' from Wrócikowo, Lućmierz and Głubczyce.

ZEN was detected in 12% of samples of 'Bogatka' and in 60% of samples of 'Muszelka' cultivars. Average content was 23.9 µg/kg and was 3 times higher in the grain of 'Muszelka' than in 'Bogatka'. The difference in ZEN content between cultivars was statistically significant according to paired samples t-test. High concentration of ZEN was present in samples of 'Muszelka' and 'Bogatka' grain from Zadąbrowie (248 and 206 µg/kg, respectively) and in 'Muszelka' sample from Czesławice (128 µg/kg).

Six samples of the grain containing DNA of *F. langsethiae* were analyzed for T-2/HT-2 toxins. In all the samples, the total concentration of both mycotoxins was below detection limit of 35 µg/kg.

Amount of *Fusarium* DNA in grain correlated significantly with concentration of *Fusarium* toxins (DON, NIV, ZEN) (Table 3). *F. graminearum* DNA correlated significantly with DON and ZEN concentrations, whereas *F. culmorum* DNA with ZEN concentration only. DNA of *F. poae* did not correlate with DON and ZEN – toxins not produced by this species. There was some positive relationship between *F. poae* and NIV concentration. Summarized amount of *F. culmorum* and *F. graminearum* DNA did not improve the strength of correlation with the toxins. Correlation of NIV with *F. graminearum* + *F. poae* DNA (possible NIV producers) was statistically significant (r = 0.511).

Table 3
Tabela 3

Coefficients of correlation between concentration of DNA (pg/µg) of three *Fusarium* species and concentration (µg/kg) of mycotoxins DON, NIV and ZEN in grain of winter wheat cultivars 'Bogatka' and 'Muszelka' from 2010 harvest in 25 locations

Współczynniki korelacji między stężeniem DNA (pg/µg) trzech gatunków *Fusarium* a stężeniem (µg/kg) mykotoksyn DON, NIV i ZEN w ziarnach odmian pszenicy ozimej Bogatka i Muszelka ze zbiorów w 2010 r. w 25 lokalizacjach

n = 25	*Fusarium*	*Fg*	*Fc*	*Fg + Fc*	*F. poae*	DON	NIV	ZEN
DON	0.622	0.534	0.320	0.509	-			
NIV	0.467	0.381	0.242	0.354	0.300	0.695		
ZEN	0.400	0.672	0.406	0.658	-	0.438	0.186	
Toxins Toksyny	0.649	0.609	0.365	0.587	-0.035	0.974	0.643	0.612

Values in bold are different from 0 with a significance level of P≤0.05; all variables were log transformed; *Fg – F. graminearum, Fc – F. culmorum,* toxins – sum of DON, NIV and ZEN.
Wartości pogrubione różnią się od 0 na poziomie istotności P≤0,05. Wszystkie zmienne zostały przekształcone logarytmicznie. *Fg – F. graminearum, Fc – F. culmorum,* toksyny – suma DON, NIV i ZEN.

Biplot produced by PCA analysis distinguished some locations based on concentrations of DNA of three *Fusarium* species and *Fusarium* toxins (Figure 5). In Zadąbrowie (24), we found the highest amount of DON and ZEN as well as amount of *F. graminearum* DNA. Grain from Czesławice (2) were characterized by the highest amounts of *F. poae* DNA and NIV but also have high concentrations of the others toxins/DNA. On the other hand, in Słupia (17) concentration of *F. poae* DNA and NIV was low, but analysis showed high concentration of *F. culmorum* accompanied by moderate concentration of *F. graminearum* and DON.

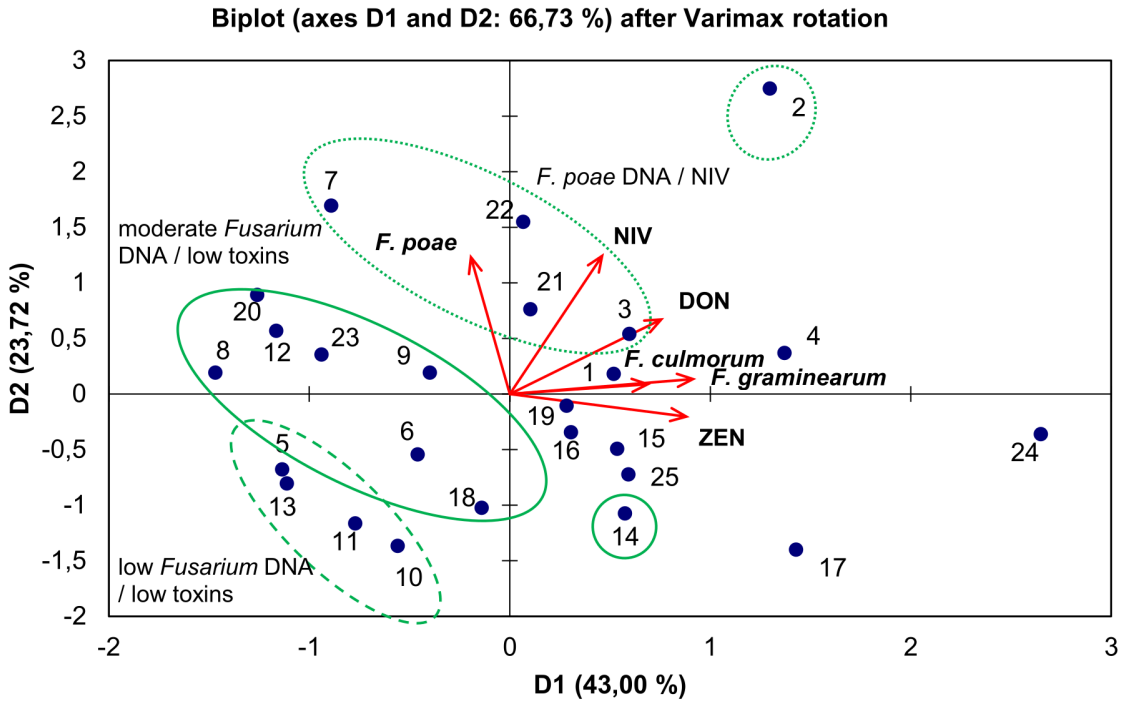

Figure 5. Principal Component Analysis based on DNA of *Fusarium culmorum*, *F. graminearum* and *F. poae*, and concentration of *Fusarium* toxins (DON, NIV, ZEN) in grain samples of winter wheat collected from 25 locations in Poland in 2010. Location numbers correspond to these in table 3. Variables were log transformed prior to the analysis.

Rysunek 5. Analiza składowych głównych dla zawartości DNA *Fusarium culmorum*, *F. graminearum* i *F. poae* oraz stężenia toksyn fuzaryjnych (DON, NIV, ZEN) w próbach pszenicy ozimej pobranych z 25 miejsc w Polsce w 2010 r. Numery lokalizacji odpowiadają numerom w Tabeli 3. Zmienne zostały przekształcone logarytmicznie przed analizą.

In the four locations (5, 10, 11, 13) the concentration of DNA of three *Fusarium* species as well as the concentration of toxins were low. In another eight locations (Figure 5, solid line), concentration of toxins was low, but amount of *Fusarium* DNA varied from low (23) to high (6). In Ruska Wieś (14) we found the highest concentration of *F. culmorum* DNA (511.8 pg/µg). Five locations (2, 3, 7, 21, and 22) could be characterized by above average concentration of NIV and moderate to high concentration of *F. poae* DNA. This species was present at considerable amounts also in samples from other locations (1, 8, 9, 12, 20) but NIV concentration was low.

In samples of the grain of spring and winter wheat collected from Radzików and neighboring Młochów we found more *Fusarium* DNA than in most samples of 'Bogatka' and 'Muszelka' (Table 4). The highest amount of DNA was present in samples of winter wheat 'Tonacja' and 'Zawisza' (6998 pg/µg and 5738 pg/µg, respectively). In spring wheat, it was lower, except for the sample of 'Raweta' from Radzików (Raweta R1) (5513 pg/µg).

Table 4
Tabela 4

Concentration of DNA of four *Fusarium* species, and DON and NIV mycotoxins levels in grain of spring and winter wheat from 2010 harvest in Radzików (R) and Młochów (M)

Zawartość DNA czterech gatunków *Fusarium* oraz toksyn fuzaryjnych DON i NIV w ziarnie pszenicy jarej i ozimej z 2010 r. w Radzikowie (R) i Młochowie (M)

No. Lp.	Cultivar (location) Odmiana (lokalizacja)	*Fusarium* DNA (pg/µg)				*Fusarium* toxins (µg/kg) Toksyny fuzaryjne (µg/kg)	
		Fa	*Fc*	*Fg*	*Fp*	DON	NIV
1	Griwa (R) [a]	587	70	1146	262	60,8	50,4
2	Parabola (R) [a]	874	65	257	59	58,1	50,9
3	Raweta (R) [a]	0	57	379	58	61,6	50,0
4	Raweta (R1) [a]	3522	159	1671	161	92,1	51,1
5	Raweta (M) [a]	0	0	0	244	53,0	49,7
6	Tonacja (R)	3223	0	2566	434	64,6	52,3
7	Tonacja (M)	6169	0	0	829	54,5	50,9
8	Zawisza (R)	0	181	5441	116	135,3	52,6
	Mean Średnia	1797	67	1432	270	72,5	51,0

Fa = F. avenaceum, Fc = F. culmorum, Fg = F. graminearum, Fp = F. poae; [a] – spring wheat
Fa = F. avenaceum, Fc = F. culmorum, Fg = F. graminearum, Fp = F. poae; [a] – pszenica jara

Four *Fusarium* species were detected in grain. *F. langsethiae* and *F. sporotrichioides* were not present. *F. avenaceum* dominated in three samples (on average 1797 pg/µg of DNA) and *F. graminearum* in three (1432 pg/µg). In one sample (Tonacja R) amounts of DNA of these species were similar. *F. poae* was present in all samples of winter wheat (270 pg/µg). In the grain of spring, wheat 'Raweta' from Młochów only this species was present. The concentration of *F. culmorum* DNA was generally the lowest of all species (67 pg/µg).

The concentration of trichothecene toxins was low (Table 4). ZEN amount was below limit of detection. The highest concentration of DON was found in the samples with high concentration of *F. graminearum* and *F. culmorum* DNA – Zawisza R and Raweta R1. The same was true for NIV concentration in grain. No relation was found between *F. poae* and NIV; however, total concentration of *F. graminearum* and *F. poae* correlated the best with NIV amount.

Discussion

Presence and concentration of *Fusarium* DNA in naturally infected wheat in two years of the study was generally in accordance with data on occurrence of *Fusarium* species on wheat in Poland. According to the published data, dominant species on wheat spikes and kernels were *F. culmorum, F. graminearum, F avenaceum* and *F. poae* (Perkowski *et al.* 1990; Goliński e*t al.* 1996; Bottalico and Perrone 2002; Stępień *et al*. 2008; Chełkowski *et al.* 2012; Wiśniewska *et al.* 2014; Kuzdraliński *et al*. 2017; Bilska *et al*. 2018; Iwaniuk et al. 2018). Proportions

of these four species changed depending on year and study as well as region of sampling. Other species were also detected were not present in all published results, for example *F. langsethiae* (Łukanowski & Sadowski 2008), *F. sporotrichioides* (Kuzdraliński *et al*. 2017), *F. tricinctum* (Wiśniewska *et al*. 2014).

Weather in 2009 was more favorable for FHB development than in 2010, which is also reflected in the difference in amount of *Fusarium* DNA and mycotoxins (Table 5).

Table 5
Tabela 5

Air temperature (°C) and sum of rainfall (mm) in May, June and July of 2009 and 2010 in Radzików and in 2010 in 25 locations

Temperatura powietrza (°C) i suma opadów (mm) w maju, czerwcu i lipcu 2009 i 2010 w Radzikowie i w 2010 w 25 miejscowościach

Month Miesiąc	25 locations (mean; range) 25 miejscowości (średnia; zakres)		Radzików	
	Rainfall Suma opadów	Temperature Temperatura	Rainfall Suma opadów	Temperature Temperatura
		2009		
May maj			71.8	13.7
June czerwiec			84.0	16.3
July lipiec			138.6	20.0
		2010		
May maj	134.1 (74.5 — 227.8)	12.3 (8.7 —14.7)	149.6	13.7
June czerwiec	59.0 (13.1 — 166.6)	16.8 (14.1 —17.9)	64.6	17.8
July lipiec	110.4 (31.6 — 238.2)	20.9 (20.0 —21.8)	131.6	21.7

In some regions (e.g., Radzików) in 2010, the drought conditions occurred in June and July with high temperatures and infrequent, heavy rainfalls. Despite differences in weather and limited number of samples in 2009*, F. graminearum* was occurring more frequently than *F. culmorum*. Amount of DNA of the first species was also higher in both years. While *F. culmorum* DNA was very low in 2010, we can conclude that dry weather is affecting to a large extent occurrence of this species (Scherm *et al.* 2013). In the Netherlands in 2009 incidence and amount of *F. culmorum* DNA was similarly low as in our study (van der Fels-Klerx *et al.* 2012). Authors found this species only in 2% of samples and DNA concentration was 80-times lower than for *F. graminearum*.

Tomczak *et al.* (2002) analyzed *Fusarium* species causing FHB epidemics in 1998 and 1999 in two regions of Poland. In 1998 in northern and central regions *F. avenaceum* dominated, being followed by *Fusarium graminearum* and *F. culmorum* with similar frequency. In 1999, ranking of species was the same; however, frequency of *F. graminearum* was 3–5 times higher than *F. culmorum*. Authors reminds that no *F. graminearum* was detected in the previous decade (1980's) in wheat grown in Northern Poland. Kuzdraliński *et al*. (2018) found dominance of *F. graminearum* in samples of wheat grain from South-Eastern Poland collected in 2013. *F. culmorum* was fifth species as regards frequency. Wiśniewska *et al.* (2014) found that *F. culmorum* was the most common species on strong infected heads of wheat in 2009. They analyzed samples from six locations, and only in two from Southern Poland *F. graminearum* prevailed over *F. culmorum*. Iwaniuk *et al.* (2018) observed variability in *F. culmorum* and *F. graminearum* frequency in grain of spring wheat collected in 2017 in North-Eastern Poland. First species dominated in two cultivars and the second in two others. Stępień and Chełkowski (2010) summarized frequencies of *Fusarium* species infecting wheat heads in Poland from 1985 to 2009. In 1985 *F. avenaceum* and *Microdochium nivale* dominated, *F. culmorum* being the third species. In 2009, *F. graminearum* dominated and *F. culmorum* was the second species with about half frequency of first species. Increase in *F. graminearum* was obvious;

however, differences between years were substantial. *F. culmorum* predominated in some localities in several studies. It may be explained by the influence of local weather conditions on the frequency of species. Variability of *Fusarium* species can be high even at the single field level (Xu *et al*. 2008b)

Sexual stage of *Fusarium graminearum* is *Gibberella zeae*, which produces sexual spores (ascospores) in perithecia (Desjardins 2003). For *F. culmorum* perfect stage is not known and fungus produces only asexual spores – macroconidia (Scherm *et al.* 2013). Thus *F. graminearum* can disperse and infect host plants with ascospores and macroconidia, whereas *F. culmorum* only with macroconidia. Nature of *F. graminearum* is the homothallic which allows the production of large masses of ascospores and effectively compete against *F. culmorum* (Waalwijk *et al.* 2003). In a German study, the important contribution of ascospores to inoculum pressure was emphasized (Obst *et al.* 2002). Ascospores required a relative humidity below 53%, whereas macroconidia required relative humidity of above 80% for germination, as was observed by Beyer *et al.* (Beyer *et al.* 2005). It can be another factor favoring *F. graminearum* over *F. culmorum* under dry conditions.

Fusarium poae was the most frequently species detected in grain (100% of samples in 2009 and 74% of samples in 2010). In 2010 in 9 samples out of 50 it was the only *Fusarium* species present. However, amount of *F. poae* DNA was about 10-times lower than *F. graminearum* DNA in dry 2010 year and up to 200 times lower in year 2009 of weather favorable for FHB. According to other reports *F. poae* was frequently isolated from wheat spikes and kernels in Poland (Goliński *et al.* 1996; Lenc et al. 2015; Kuzdraliński *et al*. 2017; Iwaniuk *et al*. 2018). This is a weak pathogen of cereals, however, is widespread on wheat in Europe (Vogelgsang *et al*. 2008, 2019; Isebaert *et al.* 2009; Xu *et al.* 2003; Lindblad *et al*. 2013; Polišenská *et al*. 2021). Vogelgsang *et al.* (2019) in eight-year survey found similar pattern. The highest frequency of *F. graminearum* and *F. poae* in winter wheat, but 3-times higher amount of *F. graminearum*. Audenaert *et al.* (2009) observed dominance of *F. poae* in Flanders in 2007 and in 2008 it was isolated with lower frequency. In 2007, the infection pressure was very high as compared with 2008. The authors suggested that this is because *F. poae* was a secondary pathogen infecting the weakened heads. Additionally, high frequency of occurrence of *F. poae* was explained by its sporulation strategy. This species produces very large amounts of microconidia in a dry powdery form that can easily invade cereal heads. It could be true for dry conditions and wind dispersal, because for splash dispersal Hörberg (2002) did not find any difference in patterns between *F. poae* microconidia and much larger macroconidia of *F. culmorum*. It is to add that *F. poae* as a weak pathogen was rarely isolated when only FHB symptomatic wheat kernels were analyzed (Bilska *et al.* 2018).

Xu *et al.* (2008a) associated *F. poae* with dry and warm weather conditions, whereas *F. graminearum* with warm/humid conditions. *F. avenaceum* and *F. culmorum* were both associated with niches of cooler/wet/humid conditions. This was confirmed for *F. poae by* Covarelli *et al.* (2013) but they observed that in dry season of 2009 *F. graminearum* was replaced by *F. poae* and also by *F. avenaceum*. Parikka *et al.* (2012) who expected increase of importance of *F. poae* (accompanied by *F. langsethiae*) in more dry conditions of Scandinavia stated the similar. Similarly, the results obtained by Chrpova *et al.* (2016) showed increase in *F. poae* occurrence in 2012 in Czech Republic. The weather in 2012 was warmer and drier than in the other studied years (2011, 2013). The weather conditions in the most regions of Poland in 2010 were dry and warm during and after flowering. Results showed that this favored *F. poae* spread on wheat. Only in the South/South-Eastern Poland weather was warm and humid, and *F. graminearum* dominated in the grain samples from this region.

Low *F. poae* DNA in the grain observed in our study could be explained by lower aggressiveness of this species as compared to *F. graminearum* (Vogelgsang *et al.* 2008; Stenglein 2009). It was also found that *F. poae* that predominated in wheat glumes was not detected in grain, which was infected by *F. culmorum*, *F. avenaceum* and *M. nivale* (Doohan *et al.* 1998). Authors did not detect *F. graminearum* in wheat samples (collected in England, UK in 1994) which is good example of later *Fusarium* species shift in Europe. Polley and Turner (Polley and Turner 1995) found that *F. poae* was associated with distinct glume spot lesions and was the most frequently isolated from glumes. Doohan (1998) supposed that the infection process and colonization by *F. poae* differs from that of other *Fusarium* species causing FHB.

Fusarium poae is known as NIV producer (Thrane *et al.* 2004; Schollenberger *et al.* 2006). Consequently, we detected NIV in most samples but at very low quantities. In Poland, NIV was found primarily in oats infected by *F. poae* (Perkowski *et al.* 1997). Edwards *et al.* (Edwards *et al.* 2012) found that correlation of nivalenol concentration

in oat grain and *F. poae* DNA was highly significant but only accounted for 9% of the variance. It showed that other species such as *F. graminearum* and *F. culmorum* were involved in NIV production. NIV chemotypes of these species are not frequent in Poland. Stępień *et al.* (2008) found that only 12% of *F. graminearum* isolates in Poland displayed the NIV chemotype.

Besides NIV, *F. poae* isolates were found to produce wide range of toxins including type A and B trichothecenes, beauvericin, enniatins, moniliformin, and others (Bottalico and Perrone 2002; Thrane *et al.* 2004; Uhlig *et al.* 2006; Stenglein 2009; Somma *et al.* 2010). The surveys of wheat harvested in Poland in 2006 and 2007 as well as in 2013 showed that increased importance of *F. poae* in the FHB complex in Poland (Kulik and Jestoi 2009; Wolny-Koładka *et al.* 2015).

In 1994 Norwegian researchers found "powdery *F. poae*" strains which were the most abundant potential producer of HT-2 and T-2 toxins in cereals (Kosiak *et al.* 2003). In 1999 these *F. poae* strains were proved to produce T-2 toxin (Torp and Langseth 1999). Strains originated mainly from Norwegian oats but were found also on wheat in Austria and the Netherlands. Further these strains were described as a new species *F. langsethiae* by Torp and Nierenberg (2004). The species was being found primarily in Northern Europe on oats and barley (Yli-Mattila *et al.* 2008; Edwards *et al.* 2012).

Occurrence of *F. langsethiae* on wheat in Poland was confirmed in 2008 (Lukanowski *et al.* 2008). This species was found mainly in Northern Poland (including Radostowo mentioned in present study), however it was present in some samples of wheat grain from Central Poland (Lukanowski and Sadowski 2008). In 2009 *F. langsethiae* was found on wheat grain in the Netherlands but at low level (8% of samples) (van der Fels-Klerx *et al.* 2012). Presence of *F. langsethiae* was detected by Czaban *et al.* (2015) in years 2008 – 2010 in South-Eastern Poland. Percentages of winter wheat kernels colonized by this species was low. It ranged from 0 to 2.9% in susceptible cultivar 'Kris' in 2010. In our research, we did not detect *F. langsethiae* in 2009, however limited number of samples was analyzed. In 2010, DNA of this species was found mainly in samples from Northern Poland and in only one from southern region at low concentration.

F. langsethiae and *F. poae* are favored by dry conditions (Supronienė *et al.* 2010; Parikka *et al.* 2012; Czaban *et al*. 2015), however it seems that the first species prefer lower temperatures than the former. Kokkonen *et al.* (2012) found that *F. langsethiae* produced the highest amount of the type A trichothecenes at 15°C, whereas *F. poae* could produce beauvericin at both cool and warm conditions.

Deoxynivalenol (DON) was the toxin which amount was the highest in the analysed grain samples. In their review, Perkowski et al. (2004) summarized results of several papers on mycotoxins in cereal grain in Poland. Amounts of DON detected in wheat grain were similar to these in present work in 2010, but lower than in 2009. DON concentration in 2009 and 2010 was similar to that detected by Czaban et al. (2015) in four winter wheat cultivars in the same years. Authors found DON mainly in the grain from 2009 and in 2010, DON was present only in small concentrations. In 2017 (moist season) and 2018 (dry season), Bryła et al. (2019) observed similar pattern of DON concentration: low in 2018 and high in 2017. Lindblad *et al.* (2013) found similar amounts of DON in grain of winter wheat collected in Sweden in 2009 and 2011. We detected higher amounts of DON, especially in 2009. In 2010, it was also higher, however did not exceeded the legislative limit of 1250 μg/kg like in Swedish samples in 2011.

DON accumulation was closely associated with the presence of *F. graminearum* (Bryła *et al.* 2015; Lindblad *et al.* 2013). Coefficient was very high in 2009, because of high DON accumulation and high *F. graminearum* DNA amount in grain. In this year DON concentration correlated strongly also with *F. culmorum* DNA despite its low concentration in the most of samples. In 2010, coefficients were lower and significant only for *F. graminearum*.

Nivalenol (NIV) accumulation was much lower than DON and amounts was comparable to detected by Bryła *et al.* (2015) in 2017 and 2018. Its concentration was significantly associated with the presence of *F. graminearum* and *F. poae* in 2009. In 2010, coefficients were insignificant but positive for all three possible NIV producers: *F. culmorum*, *F. graminearum* and *F. poae*. Xu *et al.* (2003) studied wheat grain samples harvested in 2001 from UK, Ireland, Italy, and Hungary. They did not find quantitative relationships between amount of *Fusarium* DNA and the concentration of the mycotoxins in the grain. However, for total *F. graminearum* and *F. culmorum* DNA and DON concentration linear model was nearly significant. In the next survey (Xu *et al.* 2008a) they studied *Fusarium* species frequency and mycotoxin content in wheat samples from the same countries over two years (2003–2004). They found DON being

the most frequently detected toxin. DON amount correlated strongly with *F. graminearum* DNA. NIV was related significantly only to the amount of *F. culmorum* DNA. As regards ZEN, authors found strong association with both *F. culmorum* and *F. graminearum*. In 2005 in Poland, the highest amount of ZEN was found in wheat grain infected by *F. graminearum* (Gromadzka *et al.* 2008). In grain were *F. culmorum* was the main pathogen, ZEN content was 10-times lower. We found higher amounts of ZEN in both 2009 and 2010 comparing with results obtained by Czaban et al. (2015) for the same years (all values below LOD = 10 µg/kg). However, ZEN content was very diverse and high in individual samples (above 100 µg/kg).

Conclusions

1. The most common species detected in wheat grain in 2009 was *F. graminearum* and *F. poae* in 2010.The highest DNA content in wheat grain in both years was found for *F. graminearum*.
2. *F. graminearum* DNA was detected in 100% of the grain samples in 2009 and in 50% of the samples in 2010.
3. In 2010 *F. culmorum* DNA was detected only in 25% of the grain samples and content of DNA of this species was low.
4. DNA of *F. langsethiae* was detected in 2010 mainly in the grain samples originating from Northern Poland.
5. Deoxynivalenol (DON) was detected in the most grain samples.
6. DON amount in the grain was higher in 2009 than in 2010.
7. DON accumulation in the grain was significantly correlated with the presence of *F. graminearum* DNA.

References

Audenaert, K., van Broeck, R., van Bekaert, B., de Witte, F., Heremans, B., Messens, K., Höfte, M., & Haesaert, G. (2009). Fusarium head blight (FHB) in Flanders: population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. *European Journal of Plant Pathology*, *125* (3), 445–458.

Beyer, M., Verreet, J.-A., & Ragab, W. S. M. (2005). Effect of relative humidity on germination of ascospores and macroconidia of *Gibberella zeae* and deoxynivalenol production. *International Journal of Food Microbiology*, *98* (3), 233–240.

Bilska, K., Jurczak, S., Kulik, T., Ropelewska, E., Olszewski, J., Żelechowski, M., & Zapotoczny, P. (2018). Species composition and trichothecene genotype profiling of *Fusarium* field isolates recovered from wheat in Poland. *Toxins*, 10 (8), 325.

Birr, T., Hasler, M., Verreet, J.-A. & Klink, H. (2020) Composition and predominance of *Fusarium* species causing Fusarium head blight in winter wheat grain depending on cultivar susceptibility and meteorological factors. *Microorganisms, 8*, 617.

Birzele, B., Meier, A., Hindorf, H., Krämer, J., & Dehne, H. W. (2002). Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. *European Journal of Plant Pathology*, *108*, 667–673.

Bottalico, A., & Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology*, 108, 611–624.

Bryła, M., Ksieniewicz-Woźniak, E., Yoshinari, T., Waśkiewicz, A., & Szymczyk, K. (2019). Contamination of wheat cultivated in various regions of Poland during 2017 and 2018 agricultural seasons with selected trichothecenes and their modified forms. *Toxins*, *11* (2), 88.

Chandelier, A., Nimal, C., André, F., Planchon, V., & Oger, R. (2011). *Fusarium* species and DON contamination associated with head blight in winter wheat over a 7-year period (2003–2009) in Belgium. *European Journal of Plant Pathology*, *130* (3), 403–414.

Chełkowski, J., Gromadzka, K., Stępień, Ł., Lenc, L., Kostecki, M., & Berthiller, F. (2012). *Fusarium* species, zearalenone and deoxynivalenol content in preharvest scabby wheat heads from Poland. *World Mycotoxin Journal*, *5* (2), 133–141.

Chrpová, J., Šíp, V., Sumíková, T., Salava, J., Palicová, J., Štočková, L., Džuman, Z., & Hajšlová, J. (2016). Occurrence of *Fusarium* species and mycotoxins in wheat grain collected in the Czech Republic. *World Mycotoxin Journal*, *9* (2), 317–327.

Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C., Ferrer, E., & Mañes, J. (2015). *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *Journal of the Science of Food and Agriculture*, *95* (3), 540–551.

Czaban, J., Wróblewska, B., Sułek, A., Mikos, M., Boguszewska, E., Podolska, G., & Nieróbca, A. (2015). Colonisation of winter wheat grain by *Fusarium* spp. and mycotoxin content as dependent on a wheat variety, crop rotation, a crop management system and weather conditions. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *32* (March), 799–807.

Desjardins, A. E. (2003). *Gibberella* from *A* (*venaceae*) to *Z* (*eae). Annual Review of Phytopathology*, *41*, 177–198.

Dinolfo, M. I., & Stenglein, S. A. (2014). *Fusarium poae* and mycotoxins: Potential risk for consumers. *Boletin de La Sociedad Argentina de Botanica*, *49* (1), 5–20.

Doohan, F. M., Parry, D. W., Jenkinson, P., & Nicholson, P. (1998). The use of species-specific PCR-based assays to analyse Fusarium ear blight of wheat. *Plant Pathology*, *47* (2), 197–205.

Edwards, S. G., Imathiu, S. M., Ray, R. V., Back, M., & Hare, M. C. (2012). Molecular studies to identify the *Fusarium* species responsible for HT-2 and T-2 mycotoxins in UK oats. *International Journal of Food Microbiology*, *156* (2), 168–175.

Gagkaeva, T., Gavrilova, O., Orina, A., Lebedin, Y., Shanin, I., Petukhov, P., & Eremin, S. (2019). Analysis of toxigenic *Fusarium* species associated with wheat grain from three regions of Russia: Volga, Ural, and West Siberia. *Toxins*, *11* (5).

Giraud, F., Pasquali, M., El Jarroudi, M., Vrancken, C., Brochot, C., Cocco, E., Hoffmann, L., Delfosse, P., & Bohn, T. (2010). Fusarium head blight and associated mycotoxin occurrence on winter wheat in Luxembourg in 2007/2008. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *27* (6), 825–835.

Goliński, P., Perkowski, J., Kostecki, M., Grabarkiewicz-Szczęsna, J., & Chełkowski, J. (1996). *Fusarium* species and *Fusarium* toxins in wheat in Poland – a comparison with neighbour countries. *Sydowia*, *48* (1), 12–22.

Gromadzka, K., Chełkowski, J., Stępień, Ł., & Goliński, P. (2008). Occurrence of zearalenone in wheat and maize grain in Poland. *Cereal Research Communications*, *36* (Supplement 6), 361–363.

Hofgaard, I. S., Aamot, H. U., Torp, T., Jestoi, M., Lattanzio, V. M. T., Klemsdal, S. S., Waalwijk, C., van der Lee, T., & Brodal, G. (2016). Associations between *Fusarium* species and mycotoxins in oats and spring wheat from farmers' fields in Norway over a six-year period. *World Mycotoxin Journal*, *9* (3), 365–378.

Hörberg, H. M. (2002). Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum*. *European Journal of Plant Pathology*, *108*, 73–80.

Horevaj, P., Milus, E. A., & Bluhm, B. H. (2011). A real-time qPCR assay to quantify *Fusarium graminearum* biomass in wheat kernels. *Journal of Applied Microbiology*, *111* (2), 396–406.

Imathiu, S. M., Edwards, S. G., Ray, R. V., & Back, M. A. (2013). *Fusarium langsethiae* – a HT-2 and T-2 toxins producer that needs more attention. *Journal of Phytopathology*, *161* (1), 1–10.

Isebaert, S., De Saeger, S., Devreese, R., Verhoeven, R., Maene, P., Heremans, B., & Haesaert, G. (2009). Mycotoxin-producing *Fusarium* species occurring in winter wheat in Belgium (Flanders) during 2002–2005. *Journal of Phytopathology*, *157* (2), 108–116.

Iwaniuk, P., Konecki, R., Snarska, K., & Łozowicka, B. (2018). Quantitative evaluation of *Fusarium* species and crop quality traits in wheat varieties of northeastern Poland. *Journal of Plant Protection Research*, 58 (4), 413–419.

Jestoi, M. N., Paavanen-Huhtala, S., Parikka, P., & Yli-Mattila, T. (2008). In vitro and in vivo mycotoxin production of *Fusarium* species isolated from Finnish grains. *Archives Of Phytopathology And Plant Protection*, *41* (8), 545–558.

Kokkonen, M., Jestoi, M., & Laitila, A. (2012). Mycotoxin production of *Fusarium langsethiae* and *Fusarium sporotrichioides* on cereal-based substrates. *Mycotoxin Research*, *28* (1), 25–35.

Kosiak, B., Torp, M., Skjerve, E., & Thrane, U. (2003). The prevalence and distribution of *Fusarium* species in Norwegian cereals: a survey. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, *53* (4), 168–176.

Kulik, T., & Jestoi, M. (2009). Quantification of *Fusarium poae* DNA and associated mycotoxins in asymptomatically contaminated wheat. *International Journal of Food Microbiology*, *130* (3), 233–237.

Kuzdraliński, A., Nowak, M., Szczerba, H., Dudziak, K., Muszyńska, M., & Leśniowska-Nowak, J. (2017). The composition of *Fusarium* species in wheat husks and grains in south-eastern Poland. *Journal of Integrative Agriculture*, *16* (7), 1530–1536.

Laszlo, E., Varga, B., & Veisz, O. (2011). Composition of *Fusarium* species causing natural spike infection in wheat. *Acta Agronomica Hungarica*, *59* (3), 255–260.

Lenc, L., Czecholiński, G., Wyczling, D., Turów, T., & Kaźmierczak, A. (2015). Fusarium head blight (FHB) and *Fusarium* spp. on grain of spring wheat cultivars grown in Poland. *Journal of Plant Protection Research*, 55 (3), 266–277.

Lindblad, M., Gidlund, A., Sulyok, M., Börjesson, T., Krska, R., Olsen, M., & Fredlund, E. (2013). Deoxynivalenol and other selected *Fusarium* toxins in Swedish wheat — Occurrence and correlation to specific *Fusarium* species. *International Journal of Food Microbiology*, *167* (2), 284–291.

Lukanowski, A., Lenc, L., & Sadowski, C. (2008). First Report on the occurrence of *Fusarium langsethiae* isolated from wheat kernels in Poland. *Plant Disease*, *92* (3), 488–488.

Lukanowski, A., & Sadowski, C. (2008). *Fusarium langsethiae* on kernels of winter wheat in Poland — Occurrence and mycotoxigenic abilities. *Cereal Research Communications*, *36*, 453–457. https://doi.org/10.1556/CRC.36.2008.Suppl.B.40

Maiorano, A., Blandino, M., Reyneri, A., & Vanara, F. (2008). Effects of maize residues on the *Fusarium* spp. infection and deoxynivalenol (DON) contamination of wheat grain. *Crop Protection*, *27* (2), 182–188.

Miller, J. D. (2008). Mycotoxins in small grains and maize:

old problems, new challenges. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *25* (2), 219–230.

Nicolaisen, M., Suproniene, S., Nielsen, L. K., Lazzaro, I., Spliid, N. H., & Justesen, A. F. (2009). Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. *Journal of Microbiological Methods*, *76* (3), 234–240.

Nielsen, L. K., Jensen, J. D., Nielsen, G. C., Jensen, J. E., Spliid, N. H., Thomsen, I. K., Justesen, A. F., Collinge, D. B., & Jørgensen, L. N. (2011). Fusarium head blight of cereals in Denmark: species complex and related mycotoxins. *Phytopathology*, *101* (8), 960–969.

Nielsen, L. K., Justesen, A. F., Jensen, J. D., & Jørgensen, L. N. (2013). *Microdochium nivale* and *Microdochium majus* in seed samples of Danish small grain cereals. *Crop Protection*, *43*, 192–200.

Niessen, L. (2007). PCR-based diagnosis and quantification of mycotoxin producing fungi. *International Journal of Food Microbiology*, *119*, 38–46.

Obst, A., Gunther, B., Beck, R., Lepschy-von Gleissenthall, J., & Tischner, H. (2002). Weather conditions conducive to *Gibberella zeae* and *Fusarium graminearum* head blight of wheat. *Journal of Applied Genetics*, *43A*, 185–192.

Obst, A., Lepschy-von Gleissenthall, J., & Beck, R. (1997). On the etiology of Fusarium head blight of wheat in south Germany – preceding crops, weather conditions for inoculum production and head infection, proneness of the crop to infection and mycotoxin production. *Cereal Research Communications*, *25* (3), 699–703.

Parikka, P., Hakala, K., & Tiilikkala, K. (2012). Expected shifts in *Fusarium* species' composition on cereal grain in Northern Europe due to climatic change. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *29* (10), 1543–1555.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a review. *Plant Pathology*, *44* (2), 207–238.

Perkowski J., Chełkowski J., Goliński P. (2004) Occurrence of mycotoxins in cereals, plants, foods and feeds in Poland. In: Logrieco A., Visconti A. (eds) An Overview on Toxigenic Fungi and Mycotoxins in Europe. Springer, Dordrecht.

Perkowski, J., Plattner, R. D., Golinski, P., Vesonder, R. F., & Chelkowski, J. (1990). Natural occurrence of deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, nivalenol, 4,7-dideoxynivalenol, and zearalenone in Polish wheat. *Mycotoxin Research*, *6*, 7–12.

Perkowski, J., Stachowiak, J., Kiecana, I., Golinski, P., & Chelkowski, J. (1997). Natural occurrence of *Fusarium* mycotoxins in Polish cereals. *Cereal Research Communications*, *25* (3), 379–380.

Polišenská, I., Jirsa, O., Salava, J., Sedláčková, I., & Frydrych, J. (2021). *Fusarium* mycotoxin content and *Fusarium* species presence in Czech organic and conventional wheat. *World Mycotoxin Journal*, *14* (2), 201–211.

Polley, R. W., & Turner, J. A. (1995). Surveys of stem base diseases and fusarium ear diseases in winter wheat in England, Wales and Scotland, 1989–1990. *Annals of Applied Biology*, *126* (1), 45–59.

Scherm, B., Balmas, V., Spanu, F., Pani, G., Delogu, G., Pasquali, M., & Migheli, Q. (2013). *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. *Molecular Plant Pathology*, *14* (4), 323–341.

Schollenberger, M., Müller, H. M., Rüfle, M., Suchy, S., Plank, S., & Drochner, W. (2006). Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. *Mycopathologia*, *161* (1), 43–52.

Somma, S., Alvarez, C., Ricci, V., Ferracane, L., Ritieni, A., Logrieco, A., & Moretti, A. (2010). Trichothecene and beauvericin mycotoxin production and genetic variability in *Fusarium poae* isolated from wheat kernels from northern Italy. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *27* (5), 729–737.

Stenglein, S. A. (2009). *Fusarium poae*: A pathogen that needs more attention. *Journal of Plant Pathology*, *91* (1), 25–36.

Stępień, Ł., & Chełkowski, J. (2010). Fusarium head blight of wheat: pathogenic species and their mycotoxins. *World Mycotoxin Journal*, *3* (2), 107–119.

Stępień, Ł., Popiel, D., Koczyk, G., & Chełkowski, J. (2008). Wheat-infecting *Fusarium* species in Poland – Their chemotypes and frequencies revealed by PCR assay. *Journal of Applied Genetics*, *49* (4), 433–441.

Sundheim, L., Brodal, G., Hofgaard, I. S., & Rafoss, T. (2013). Temporal variation of mycotoxin producing fungi in Norwegian cereals. *Microorganisms*, *1* (1), 188–198.

Supronienė, S., Justesen, A. F., Nicolaisen, M., Mankevičienė, A., Dabkevicius, Z., Semaskiene, R., & Leistrumaite, A. (2010). Distribution of trichothecene and zearalenone producing *Fusarium* species in grain of different cereal species and cultivars grown under organic farming conditions in Lithuania. *Annals of Agricultural and Environmental Medicine*, *17* (1), 79–86.

Supronienė, S., Sakalauskas, S., Mankevičienė, A., Barčauskaitė, K., & Jonavičienė, A. (2016). Distribution of B type trichothecene producing *Fusarium* species in wheat grain and relation to mycotoxins DON and NIV concentrations. *Zemdirbyste-Agriculture*, *103* (3), 281–288.

Talas, F., Parzies, H. K., & Miedaner, T. (2011). Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany. *European Journal of Plant Pathology*, *131* (1), 39–48.

Thrane, U., Adler, A., Clasen, P.-E., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielsen, K. F., & Ritieni, A.

(2004). Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. *International Journal of Food Microbiology*, *95* (3), 257–266.

Tomczak, M., Wiśniewska, H., Stępień, Ł., Kostecki, M., Chełkowski, J., & Goliński, P. (2002). Deoxynivalenol, nivalenol and moniliformin in wheat samples with head blight (scab) symptoms in Poland (1998–2000). *European Journal of Plant Pathology*, *108* (7), 625–630.

Torp, M., & Langseth, W. (1999). Production of T-2 toxin by a *Fusarium* resembling *Fusarium poae*. *Mycopathologia*, *147* (2), 89–96.

Torp, M., & Nirenberg, H. I. (2004). *Fusarium langsethiae* sp. nov. on cereals in Europe. *International Journal of Food Microbiology*, *95*, 247–256.

Uhlig, S., Jestoi, M., & Parikka, P. (2007). *Fusarium avenaceum* – the North European situation. *International Journal of Food Microbiology*, *119* (1–2), 17–24.

Uhlig, S., Torp, M., & Heier, B. T. (2006). Beauvericin and enniatins A, A1, B and B1 in Norwegian grain: A survey. *Food Chemistry*, *94* (2), 193–201.

Vaughan, M., Backhouse, D., & Del Ponte, E. M. (2016). Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to Fusarium head blight: A review. *World Mycotoxin Journal*, *9* (5), 685–700.

van der Fels-Klerx, H. J., de Rijk, T. C., Booij, C. J. H., Goedhart, P. W., Boers, E. A. M., Zhao, C., Waalwijk, C., Mol, H. G. J., & van der Lee, T. A. J. (2012). Occurrence of Fusarium head blight species and *Fusarium* mycotoxins in winter wheat in the Netherlands in 2009. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *29* (11), 1716–1726.

Vogelgsang, S., Beyer, M., Pasquali, M., Jenny, E., Musa, T., Bucheli, T. D., Wettstein, F. E., & Forrer, H. R. (2019). An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins. European *Journal of Agronomy*, *105*, 62–77.

Vogelgsang, S., Sulyok, M., Hecker, A., Jenny, E., Krska, R., Schuhmacher, R., & Forrer, H. R. (2008). Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on wheat. *European Journal of Plant Pathology*, *122* (2), 265–276.

Waalwijk, C., Kastelein, P., De Vries, I., Kerényi, Z., Van Der Lee, T., Hesselink, T., Köhl, J., & Kema, G. (2003). Major changes in *Fusarium* spp. in wheat in the Netherlands. *European Journal of Plant Pathology*, *109* (7), 743–754.

Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-de Corainville, G., Häuser-Hahn, I., Kastelein, P., Köhl, J., Lonnet, P., Demarquet, T., & Kema, G. H. J. (2004). Quantitative detection of *Fusarium* species in wheat using TaqMan. *European Journal of Plant Pathology*, *110* (5–6), 481–494.

Wiśniewska, H., Stępień, Ł., Waśkiewicz, A., Beszterda, M., Góral, T., & Belter, J. (2014). Toxigenic *Fusarium* species infecting wheat heads in Poland. *Central European Journal of Biology*, *9* (2), 163–172.

Wolny-Koladka, K., Lenart-Boroń, A., & Boroń, P. (2015). Species composition and molecular assessment of the toxigenic potential in the population of *Fusarium* spp. isolated from ears of winter wheat in southern Poland. *Journal of Applied Botany and Food Quality*, 144, 139–144.

Xu, X.-M., Nicholson, P., Thomsett, M. A., Simpson, D., Cooke, B. M., Doohan, F. M., Brennan, J., Monaghan, S., Moretti, A., Mule, G., Hornok, L., Beki, E., Tatnell, J., Ritieni, A., & Edwards, S. G. (2008). Relationship between the fungal complex causing Fusarium head blight of wheat and environmental conditions. *Phytopathology*, *98* (1), 69–78.

Xu, X. M., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G., Cooke, B. M., Doohan, F. M., Brennan, J. M., Moretti, a., Tocco, G., Mule, G., Hornok, L., Giczey, G., & Tatnell, J. (2005). Predominance and association of pathogenic fungi causing Fusarium ear blightin wheat in four European countries. *European Journal of Plant Pathology*, *112* (2), 143–154.

Xu, X. M., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G., Cooke, B. M., Doohan, F. M., Monaghan, S., Moretti, A., Tocco, G., Mule, G., Hornok, L., Béki, E., Tatnell, J., & Ritieni, A. (2008). Within-field variability of Fusarium head blight pathogens and their associated mycotoxins. *European Journal of Plant Pathology*, *120* (1), 21–34.

Xu, X., & Nicholson, P. (2009). Community ecology of fungal pathogens causing wheat head blight. *Annual Review of Phytopathology*, *47* (1), 83–103.

Xu, X., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G., Cooke, B. M., Doohan, F. M., Van Maanen, A., Moretti, A., Tocco, G., Mule, G., Hornok, L., Giczey, G., Tatnell, J., & Ritieni, A. (2003). Is the amount of mycotoxins in cereal grains related to the quantity of *Fusarium* DNA? *Aspects of Applied Biology*, *68*, 101–108.

Xue, A. G., Chen, Y., Seifert, K., Guo, W., Blackwell, B. A., Harris, L. J., & Overy, D. P. (2019). Prevalence of *Fusarium* species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. *Canadian Journal of Plant Pathology*, 41 (3), 392–402.

Yli-Mattila, T., Paavanen-Huhtala, S., Jestoi, M., Parikka, P., Hietaniemi, V., Gagkaeva, T., Sarlin, T., Haikara, A., Laaksonen, S., & Rizzo, A. (2008). Real-time PCR detection and quantification of *Fusarium poae*, *F. graminearum*, *F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. *Archives of Phytopathology and Plant Protection*, *41* (4), 243–260.