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FUNGAL PATHOGENS OF THE GENUS *Fusarium* IN WINTER WHEAT *Triticum aestivum* L. PROTECTED WITH FUNGICIDES IN NORTH-EASTERN POLAND

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Abstract

Various diagnostic methods were used to evaluate the effect of fungicide protection on the prevalence of pathogenic fungi in wheat grain. Winter wheat cv. Nutka and Zyta was grown during a field experiment established in the Production and Experimental Station in Bałcyny in 2006–2007. The experimental factor was chemical crop protection: epoxiconazole, kresoxim-methyl and fenpropimorph applied at growth stages BBCH 33–35 as well as dimoxystrobin and epoxiconazole applied at BBCH 51–53. In this experiment, microscopic observations and conventional PCR assays were used as complementary methods. The quantification of *Fusarium poae* DNA by qPCR demonstrated the effectiveness of chemical protection against the analyzed fungal species. Lower monthly precipitation levels and higher daily temperatures intensified grain infections, in particular those caused by *F. poae*. A significant correlation was determined between the number of *F. poae* cultures isolated from winter wheat grain and the quantity of pathogenic DNA in grain identified by qPCR. Grain infections caused by *F. poae* lowered yield and thousand seed weight.

Key words: wheat, grain, *Fusarium*, PCR, real-time PCR (qPCR), conventional PCR.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is susceptible to fungal infections which lower productivity and deteriorate yield quality (Scheider et al. 2009). Fungi of the genus *Fusarium* attack open flowers of wheat plants during a critical growth stage which determines grain yield (Fischer, 1985). Plants are infected at different growth stages, and Fusarium head blight (FHB) is the most damaging disease which lowers grain yield, deteriorates yield quality and leads to the accumulation of various mycotoxins in crop plants (Goliński et al. 2010). FHB is caused by more than 20 species of the genus *Fusarium*, and the most commonly encountered taxa in Poland and in Europe are *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *F. sporotrichoides* (Kiecana et al. 2012, Leslie and Summerell, 2006; Pląskowska and Chrzanowska-Drożdż, 2009).

Chemical substances provide effective crop protection. Many fungicides eliminate pathogens, reduce their spread and inhibit the progression of crop diseases (Kryczyński, 2002). The pathogen has to be correctly identified for fungicide treatments to deliver optimal effects. Unambiguous identification of pathogens based on the observed symptoms may be difficult, in particular at early stages of disease when various pathogens may produce similar symptoms (Nicholson et al. 1998).

In microscopic analyses, pathogens are difficult to identify based on their morphological traits due to similarities in species description, the absence of unique morphological structures and the predominance of fast-growing species in fungal colonies. In conventional isolation methods, only a fraction of the total microbial population is revealed (Kwaśna and Bateman, 2009). Molecular methods based on polymerase chain reaction (PCR) offer superior detection capabilities (Schilling et al. 1996). The PCR technique is a sensitive and highly-specific tool for detecting and identifying pathogens in plant tissues (Nicholson et al. 2003). PCR and real-time PCR support fast identification of species (Mule et al. 2005). Molecular techniques have led to the reorganization of fungal taxonomy, and they support observations of disease progression (Koncz et al. 2008).

Numerous attempts to detect and identify fungi of the genus *Fusarium* in grain and plant tissue have accumulated into an extensive body of research. Spe-

cific primers and probes have been developed for identifying pathogens that attack crop plants at different stages of growth (Kulik, 2008; Kulik et al. 2011; Leišová et al. 2006; Nicolaisen et al. 2009; Yli-Mattila et al. 2008).

The objective of this study was to conduct microscopic observations and PCR assays of *Fusarium* pathogens infecting the grain of two winter wheat cultivars and to determine the effectiveness of the applied fungicide treatments.

MATERIALS AND METHODS

A two-factorial field experiment was performed in four replications in the Production and Experimental Station in Bałcyny during the growing seasons of 2005/2006 and 2006/2007. The experimental factors were two winter wheat cultivars: Zyta – a high grade wheat cultivar (A), and Nutka – a bread wheat cultivar (C), and fungicide treatments:

I. control (no fungicide treatment);

II. comprehensive fungicide treatment: Juwell TT 483 SE (epoxiconazole+kresoxim-methyl +fenpropimorph) – applied at stem elongation stages BBCH 33–35, and Swing Top 183 SC (dimoxystrobin+epoxiconazole) – applied at inflorescence emergence and heading stages BBCH 51–53.

Fungicides, dose rates and application dates were selected according to the recommendations of the Plant Protection Institute. The health status of wheat grain was evaluated under a microscope and by PCR after DNA isolation.

Mycological analyses of winter wheat grain were performed on 100 kernels in each replication. The grain of both wheat cultivars was surface disinfected with 70% ethanol and 1% sodium hypochlorite and placed on Petri plates containing solid PDA medium. After 14 days of incubation, fungal cultures were identified under a microscope based on the available reference standards (Ellis, 1971; Gerlach and Nirenberg, 1982; Kwaśna et al. 1991; Watanabe, 2002).

DNA was isolated by the method proposed by Kulik et al. (2007). Ground wheat grains were placed in a porcelain mortar, treated with liquid nitrogen and homogenized. The homogenized material was transferred to 1.5 ml Eppendorf tubes, incubated at 70°C for 40 minutes and centrifuged at 14000 rpm. The supernatant was loaded onto a minicolumn filled with silica and centrifuged. Silica-bound DNA was washed twice with 70% ethanol, rinsed in TE buffer and stored at 4°C until further analysis.

PCR analyses were carried out with the use of species-specific primers for *Fusarium* fungi isolated from winter wheat grain in the growing seasons of 2005/2006 and 2006/2007: *Fusarium* spp. P58SL 5′-AGT ATT CTG GCG GGC ATG CCT GT-3′, P28SL 5′-ACA AAT TAC AAC TCG GGC CCG AGA-3′ (Hue et al. 1999); *Fusarium avenaceum* FaR 5′-CAA GCA TTG TCG CCA CTC TC-3′, FaF 5′-GTT TGG CTC TAC CGG GAC TG-3′ (Doohan et al. 1998); *Fusarium culmorum* Fc01F 5′-ATG GTG AAC TCG TCG TGG C-3′, Fc01R 5′-CCC TTC TTA CGC CAA TCT TCT CG-5′(Nicholson et al. 1998); *Fusarium graminearum* Fg16NF 5′-ACA GAT GAC AAG ATT CAG GCA CA-3′, Fg16NR 5′-TTC TTT GAC ATC TGT TCA ACC CA-3′ (Nicholson et al. 1998); *Fusarium poae* Poa1 5′-CTT GGT AGG GGG GAC AGA CAC GC-3′, Poa2 5′-CCA TTC CAC GCT CGA CAG ACC TG-3′ (Kulik, 2008); *Fusarium sporotrichioides* FspITS2K 5′-CTT GGT GTT GGG ATC TGT GTG CAA-3′ (Kulik in. 2004); P28SL 5′-ACA CAA CGG GCT ATA ACA CTC CCC-3′ (Hue et al. 1999).

The PCR assay was carried out in an Eppendorf thermocycler (Mastercykler Gradient) on 25 µl of the reaction mixture composed of: reaction buffer, $MgCl_2$ magnesium ions (2.5 mM), dNTPs free nucleotides (200 µM), 10 pM of each primer, MasterAmpTM Tfl DNA Polymerase (0.25 U) (Epicentre Biotechnologies, USA) and matrix DNA (5 µl).

The reaction product was subjected to electrophoretic separation in a Sigma-Aldrich system in 1.5% agarose gel with the addition of ethidium bromide. The size of PCR products was evaluated in a UV transilluminator (Fotodyne) by comparison with the Step Ladder 50bp molecular weight standard.

Real-time PCR was performed for quantitative DNA detection of *Fusarium poae* isolated from winter wheat grain. The reaction mixture had a volume of 25 µl, comprising: 12.5 µl of TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 10 pM of TMpoaef 5′-GCT GAG GGT AAG CCG TCC TT-3′, 10 pM of TMpoaer 5′-TCT GTC CCC CCT ACC AAG CT -3′ and 10 pM of 5′-ATT TCC CCA ACT TCG ACT CTC CGA GGA-3′ (Yli-Matilla et al. 2008) probe with 6-FAM (6-carboxyfluorescein) at the 5′-end and TAMRA (5-carboxytetramethylrhodamine) at the 3′-end as the quencher, 4.5 µl deoionized water and 5µl DNA. The following dilutions of standard reagents were used to make the standard curve: 25 $pg*\mu l^{-1}$, 2.5$*pg \times \mu l^{-1}$, 0.25$*pg \times \mu l^{-1}$, 0.025$*pg \times \mu l^{-1}$, 0.0125 $pg*\mu l^{-1}$ in 2006 and 250 $pg*\mu l^{-1}$, 25$*pg \times \mu l^{-1}$, 2.5$*pg \times \mu l^{-1}$, 0.25$*pg \times \mu l^{-1}$, 0.025$*pg \times \mu l^{-1}$ in 2007 (the quantity of DNA was determined in a Hoefer DynaQuant 200 fluorometer (Pharmacia, USA). A real-time PCR assay was carried out in a ABI Prism 7500 system (Applied Biosystems). The efficiency of real-time PCR was determined based on normalized transcript levels (Ct). A standard curve was plotted against the logarithmic values of initial matrix DNA concen-

trations. The efficiency of real-time PCR amplification [*E*] was determined based on the following formula: $E = 10^{(-1/slope)} - 1$ (Pfaffl, 2001) (Fig. 1).

The results were processed in the Statistica v. 10.0 application (www.statsoft.com) with the use of Duncan's test (p=0.01). Standard deviation and homogenous groups (A, B, C) identifying significant differences between means were determined (means denoted by the same letters did not differ significantly at p=0.01). Pearson's linear correlation coefficient was calculated to determine the strength of relationships between variables.

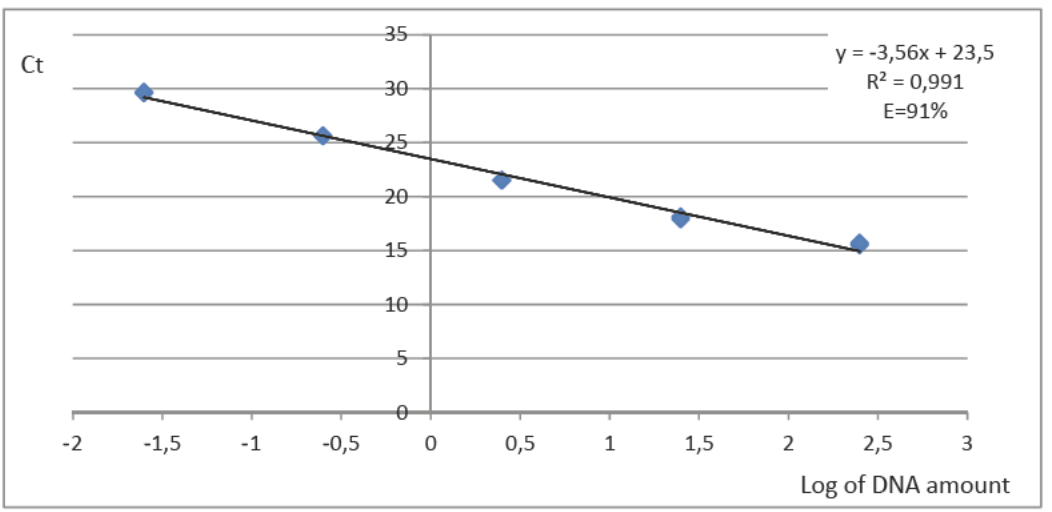

Fig. 1 Standard curve with 10-fold serial dilutions of *F. poae* DNA indicating the efficiency of real-time PCR assay.

RESULTS

The grain of winter wheat cultivars Nutka and Zyta was infected by fungi of the genus *Fusarium* in both years of the study (Table 1). The fungal species of the genus *Fusarium* identified by PCR were largely consistent with those observed in microscopic analysis. *F. poae* was identified by PCR with the use of specific primers (Poa1, Poa2) and in microscopic observations of fungal cultures. When universal primers (P58SL/P28SL) and specific primers for *F. graminearum* (Fg16NF, Fg16NR) were applied, PCR proved to be more effective – the analyzed fungi were identified by genus-specific primers in three out of eight experimental treatments (2006–2007) and by species-specific primers in five treatments (Table 1). The primers Fc01F, Fc01R (*F. culmorum*) and Faf, Far (*F. avenaceum*) failed to identify the examined fungi, and microscopic evaluations produced more accurate results (Table 1).

Microscopic analysis revealed the presence of fungi of the genus *Fusarium* in all experimental treatments (Table 1). In both years of the study, *Fusarium poae* was the predominant and most abundant pathogen in the analyzed grain, and it was subjected to qPCR analysis. *Fusarium culmorum* and *F. graminearum*, fungal species which produce trichothecenes, were rarely observed. The analyzed grain was also colonized by other pathogenic species, mainly *Drechslera biseptata* and *Bipolaris sorokiniana* as well as *Alternaria alternata* which was the most abundant of all identified fungal species and genera in all treatments (Table 2).

The use of real-time PCR for the quantification of *F. poae* DNA revealed that Juwell TT 483 SE (epoxiconazole+kresoxim-methyl+fenpropimorph) and Swing Top 183 SC (dimoxystrobin+epoxiconazole) significantly reduced the amount of *F. poae* DNA in the grain of winter wheat cv. Zyta (Table 3). The above correlation was observed in the grain of winter wheat cv. Nutka in the second year of the experiment, whereas in the first year of the study the analyzed fungicides led to an insignificant increase in the amount of pathogenic DNA in comparison with the control (Table 3). Real-time PCR revealed a higher quantity of *F. poae* DNA in 2007, which suggests that the analyzed species is affected by weather conditions. In May and June 2007, the average daily temperatures were determined at 13.7°C and 17.5°C, respectively, and they were higher than the temperatures recorded in the respective periods of 2006 and than the long-term mean, which probably contributed to higher infection rates in 2007 (Fig. 2). The temperatures in May and June 2007 were higher, while precipitation levels were lower by 13.2 mm and 22.7 mm in comparison with the corresponding months of 2006 (Fig. 3).

In 2006, significantly fewer *F. poae* cultures were isolated from wheat grain and smaller amounts of pathogenic DNA were observed. The number of isolated cultures increased in 2007, and an increase in the amount of pathogenic DNA was reported in a qPCR assay.

Mathematical analysis revealed that the results of qPCR assays investigating winter wheat cv. Zyta corresponded with the results of microscopic evaluations of pathogen cultures. A positive correlation (R=0.61) was observed between the amount of *F. poae* DNA and the number of *F. poae* colonies (Fig. 4a). No significant correlations were reported in winter wheat cv. Nutka (Fig. 4b).

The effect of *F. poae* on the yield and thousand grain weight (TGW) of winter wheat was also determined statistically (TGW data were presented by Jarmołkowicz and Olszewski, 2010). The studied wheat cultivars responded differently to the analyzed pathogen. Zyta, the more affected cultivar, was characterized by lower yield and lower TGW. The observed results validate the correlation coefficients between the amount of pathogenic DNA vs. yield (R=-0.76) (Fig. 5a) and TGW (R=-0.83) (Fig. 5b), and between the number of isolated cultures vs. yield (R=-0.68) (Fig. 6a) and TGW (R=-0.41) (Fig. 6b).

Cv. Nutka was less infected by *F. poae*, and it was characterized by a smaller reduction in yield and TGW than cv. Zyta, as demonstrated by the correlation coefficients between the amount of pathogenic DNA vs. yield (R=-0.24) (Fig. 5c) and TGW (R=-0.32) (Fig. 5d), and between the number of isolated cultures vs. yield (R=-0.04) (Fig. 6c) and TGW (R=-0.26) (Fig. 6d).

Table 1
Fungi of the genus *Fusarium* isolated from winter wheat grain and identified with PCR assay in 2006–2007

Startery Primers	Fungal genus/species	Nutka		Zyta	
		control	fungicide	control	fungicide
(2006) – 1st year of study					
P58SL, P28SL (Hue et al. 1999)	*Fusarium* spp.	+*/2**	+/2	+/4	+/0
Faf, Far (Doohan et al. 1998)	*Fusarium avenaceum* (Fr.) Sacc.	-/2	-/0	-/1	-/2
Fc01F, Fc01R (Nicholson et al. 1998)	*Fusarium culmorum* (W.G. Sm.) Sacc.	-/0	-/0	-/1	-/0
Fg16NF, Fg16NR (Nicholson et al. 1998)	*Fusarium graminearum* Schwabe	+/0	+/0	-/0	+/2
Poa1, Poa2 (Kulik, 2008);	*Fusarium poae* (Peck) Wollenw.	+/1	+/6	+/9	+/4
FspITS2K (Kulik, 2008), P28SL (Hue et al. 1999)	*Fusarium sporotrichioides* Sherb.	+/0	+/0	+/0	+/0
-	*Fusarium dimerum* Penz.	0	0	2	0
-	*Fusarium equiseti* (Corda) Sacc.	1	0	1	0
-	*Fusarium oxysporum* Schltdl.	2	1	1	1
Total		**8**	**9**	**20**	**9**
(2007) – 2nd year of study					
P58SL, P28SL (Hue et al. 1999)	*Fusarium* spp.	+/1	+/1	+/0	+/0
Faf, Far (Doohan et al. 1998)	*Fusarium avenaceum* (Fr.) Sacc.	+/5	+/5	+/7	+/7
Fc01F, Fc01R (Nicholson et al. 1998)	*Fusarium culmorum* (W.G. Sm.) Sacc.	-/0	-/3	-/0	+/1
Fg16NF, Fg16NR (Nicholson et al. 1998)	*Fusarium graminearum* Schwabe	+/1	+/0	+/0	+/0
Poa1, Poa2 (Kulik, 2008)	*Fusarium poae* (Peck) Wollenw.	+/8	+/12	+/26	+/18
FspITS2K (Kulik, 2004); P28SL (Hue et al. 1999)	*Fusarium sporotrichioides* Sherb.	+/1	+/3	+/5	+/7
-	*Fusarium equiseti* (Corda) Sacc.	0	0	3	0
-	*Fusarium tricinctum* (Corda) Sacc.	3	3	6	8
-	*Fusarium verticillioides* (Sacc.) Nirenberg	1	0	0	1
Total		**19**	**27**	**47**	**42**

*-/+ – negative / positive (classic PCR)
** – number of isolates

Table 2
Other fungi isolated from winter wheat grain in 2006–2007

Fungal genus/species	Nutka		Zyta	
	control	fungicide	control	fungicide
(2006) – 1st year of study				
Alternaria alternata (Fr.) Keissler	328	299	243	262
Aureobasidium pullulans (De Bary) Arnaud		2	2	2
Bipolaris sorokiniana (Sacc.) Shoemaker	5	8	4	5
Botrytis cinerea Pers.	3	1	0	0
Cladosporium cladospirioides (Fries.) de Vries	1	3	11	2
Drechslera biseptata (Sacc. & Roum.) M.J. Richardson & E.M. Fraser	11	10	4	5
Epicoccum nigrum Link	26	28	8	12
Oidiodendron griseum Robak	0	0	0	1
Papularia spp.	1	1	3	2
Penicillium spp.	3		1	2
Rhizoctonia spp	1	1	0	1
Stemphylium spp.	0	0	0	2
Non-sporulating colonies	3	1	6	3
Total	**382**	**354**	**282**	**299**
(2007) – 2nd year of study				
Alternaria alternata (Fr.) Keissler	268	296	264	252
Aureobasidium pullulans (De Bary) Arnaud	5	0	0	0
Bipolaris sorokiniana (Sacc.) Shoemaker	40	37	27	29
Botrytis cinerea Pers.	1	1	0	2
Cladosporium cladospirioides (Fries.) de Vries	13	2	9	13
Drechslera biseptata (Sacc ex Roum) Richardson et Fraser	16	2	13	4
Epicoccum nigrum Link	29	35	25	43
Nigropora spp.	1	1	1	
Papularia spp.	2	0	3	2
Penicillium spp.	3	0	0	1
Phoma medicaginis Malbr. & Roum.	0	0	1	0
Stemphylium spp.	1	1	0	0
Total	**379**	**375**	**343**	**346**

Table 3
Amount of *F. poae* DNA in winter wheat grain treated with fungicides Juwell TT 483 SE and Swing Top 183 SC in 2006–2007 (pg of DNA)

Year	Cultivar	Treatments	Amount of DNA [pg]	SD	Homogenous groups
2006	Nutka	control	0,0032	0,0002	BC
		fungicide	0,0041	0,0002	B
	Zyta	control	0,0090	0,0088	A
		fungicide	0,0025	0,0019	C
2007	Nutka	control	0,0062	0,0011	B
		fungicide	0,0029	0,0003	C
	Zyta	control	0,0237	0,0003	A
		fungicide	0,0055	0,0013	B

p= 0,01

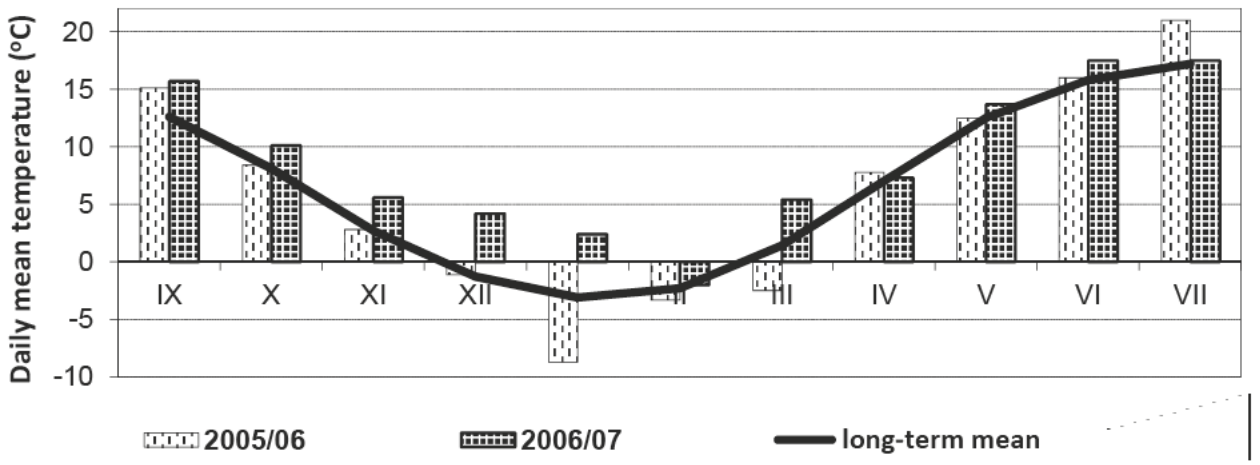

Fig. 2 Mean daily air temperature during the growing season of winter wheat in 2006–2007 compared with the long-term mean (1961–2000).

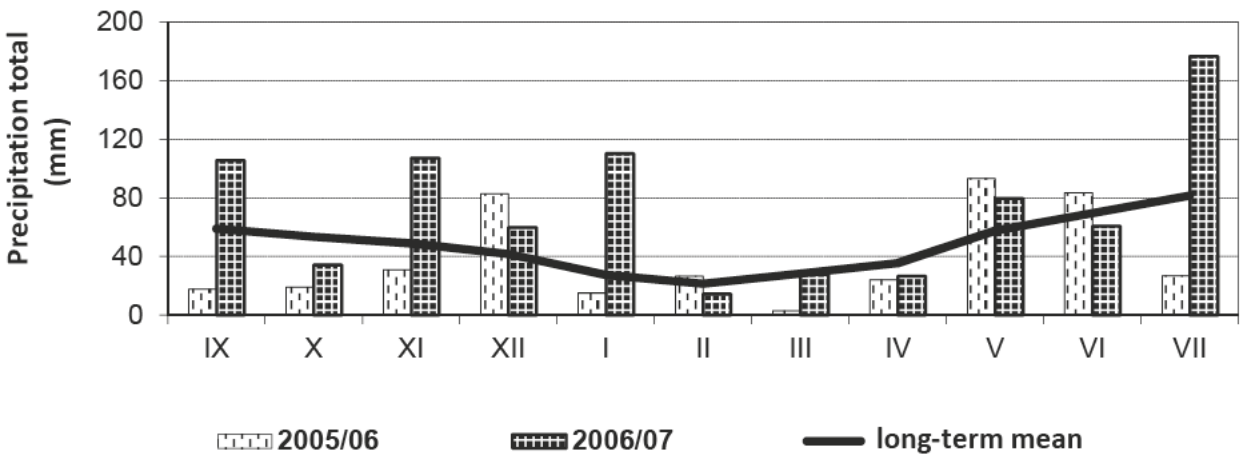

Fig. 3 Mean total precipitation during the growing season of winter wheat in 2003–2007 compared with the long-term mean (1961–2000).

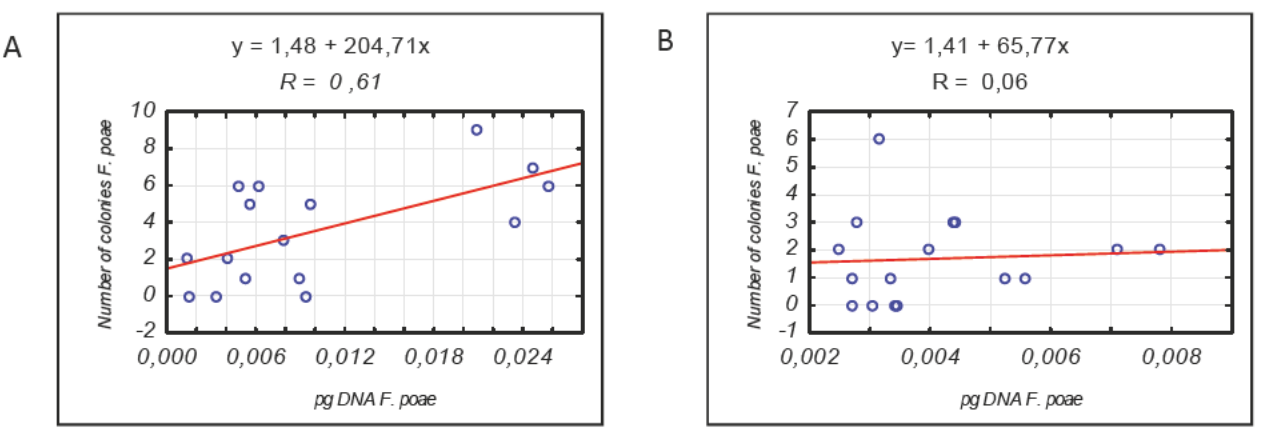

Fig. 4 Linear correlation between the number of *F. poae* colonies isolated from winter wheat grain and amount of DNA [A] – Zyta, [B] – Nutka.

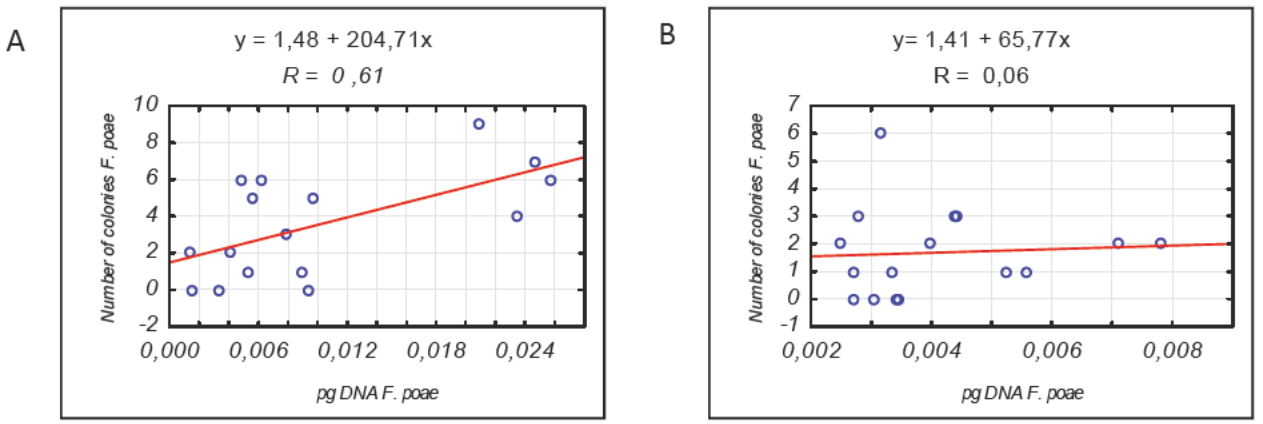

Fig. 5 Linear correlation between amount of *F. poae* DNA and yield; thousand grain weight (MTZ) of winter wheat cultivars [A, B] – Zyta, [C, D] – Nutka.

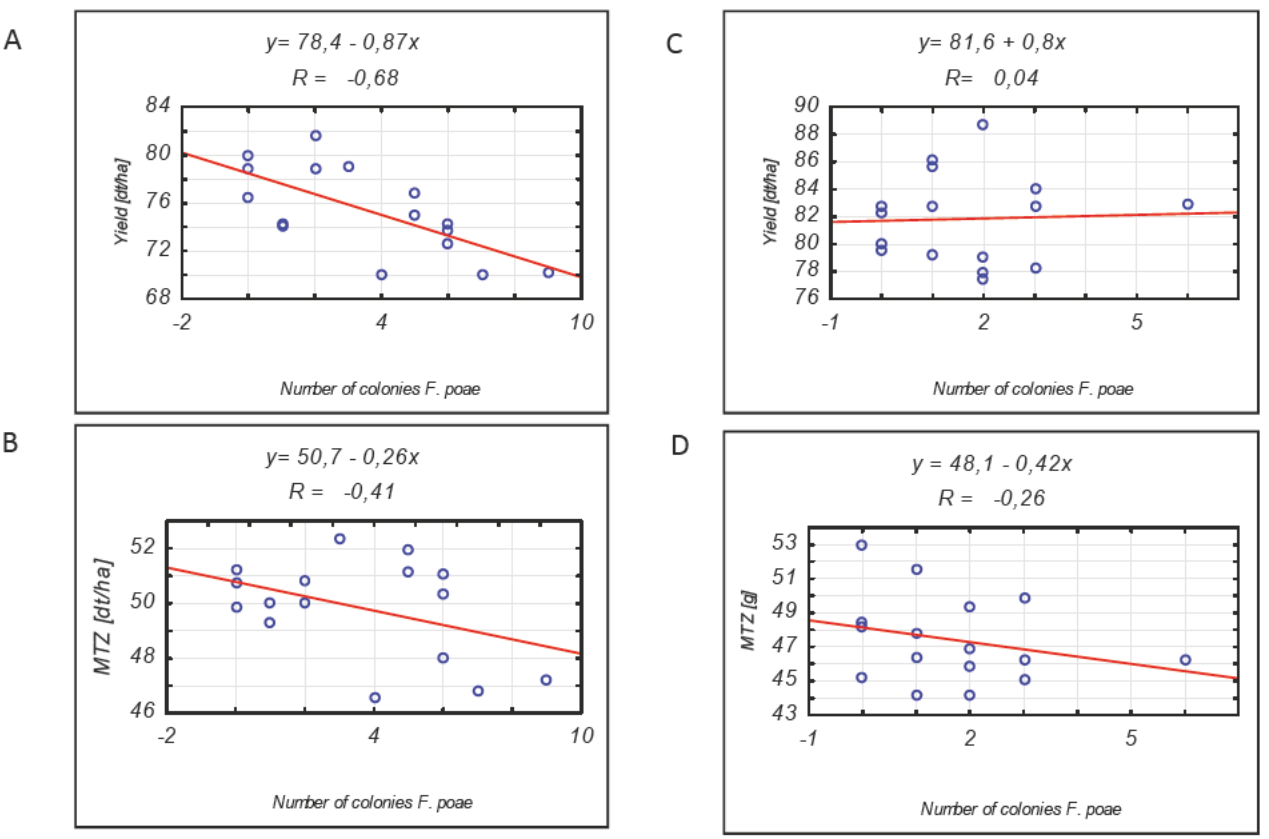

Fig. 6 Linear correlation between the number of *F. poae* colonies and yield; thousand grain weight (MTZ) of winter wheat cultivars [A, B] – Zyta, [C, D] – Nutka.

DISCUSSION

The grain of winter wheat cv. Nutka and Zyta was infected by fungi of the genus *Fusarium*. The applied pair of universal primers (P58SL/P28SL) generated a product with the length of 339 bp in all treatments and all years of the study. Other primer sets also supported the identification of selected species in the experimental treatments. Similar results were reported by Pszczółkowska (2008) who investigated the health status of wheat grain treated with various fungicides and confirmed the usefulness of molecular diagnostic methods.

PCR results were largely consistent with microscopic observations of the analyzed fungi. However, the species-specific primers for the detection of *F. culmorum* (Fc01F, Fc01R) and *F. avenaceum* (Faf, Far) were less effective than the microscopic method.

The above could be attributed to small quantities of pathogenic fungi in wheat grain as well as difficulties in DNA isolation. According to Quarta et al. (2006), the PCR detection threshold for primers amplifying *Tri* gene fragments was 4 pg of DNA, which corresponds to approximately 88 fungal spores of the genus *Fusarium*. Brennan et al. (2007) isolated *F. poae* characterized by similar aggressiveness to *F. culmorum* and *F. graminearum*, but the evaluated grain contained less *F. poae* DNA because this species colonizes glumes rather than deeper layers of the grain.

The results of microscopic observations and qPCR assays confirmed that *F. poae* was the predominant pathogen of the genus *Fusarium* in both years of the study. The predominance of *F. poae* in the species composition of fungal communities colonizing cereal crops in various regions of Europe was also observed by other authors (Bottalico and Perrone, 2002; Łukanowski and Sadowski, 2002; Kiecana et al. 2005; Pszczółkowska, 2008; Kulik, 2008,; Yli-Mattila et al. 2008; Xu et al. 2005).

Fungicidal treatments applied in this experiment failed to fully eliminate fungal pathogens of the genus *Fusarium*. In both years of the study, the fungicides decreased the number of *F. poae* colonies and isolates of the genus *Fusarium* in winter wheat cv. Zyta. A reverse correlation was observed in wheat cv. Nutka. The results of real-time PCR revealed a significant decrease in the amount of *F. poae* DNA in winter wheat cv. Zyta in both experimental years. In wheat cv. Nutka, the above correlation was noted only in the second year of the study. Our results are consistent with the findings of other authors who demonstrated that triazole-based fungicides were most effective in controlling FHB (Mesterhazy et al. 2003; Paul et al. 2008; Simpson et al. 2001). In a study by Edwards and Godley (2010), the application of prothioconazole significantly alleviated FHB symptoms and lowered DON concentrations in winter wheat grain. Chala et al. (2003) demonstrated that fungicides significantly reduced mycotoxin concentrations and increased yield. Mesterhazy et al. (2003) observed that wheat plants treated with various fungicides were characterized by lower levels of *Fusarium* spp. infection, lower yield loss and reduced DON concentrations in comparison with the control. In the above study, the best results were reported for tebuconazole. In a field experiment carried out by Simpson et al. (2001), the application of azoxystrobin led to changes in pathogen populations colonizing wheat spikes. The population of *Microdochium nivale* was reduced, but a higher incidence of infections caused by *Fusarium* spp. was observed. Tebuconazole was significantly more effective in eliminating *Fusarium* spp. than *Microdochium* spp. In the work of Pszczółkowska (2008), a higher incidence of infections caused by *Fusarium* spp. was observed in wheat plots where Amistar 250 SC (azoxystrobin) was applied as the second fungicide treatment.

Šip et al. (2010) investigated the effectiveness of the Swing Top fungicide in reducing *F. graminearum* populations, DON concentrations and yield loss in wheat plants artificially inoculated with this fungal species. The analyzed fungicide reduced DON concentrations by 49.5% and minimized yield loss by 63.9% on average, but the observed results varied throughout different years and experimental sites. The combined effect of cultivar resistance and fungicide treatment was responsible for an 86.5% decrease in DON concentrations and a 95.4% reduction in yield loss.

In this study, the prevalence of wheat grain infections caused by *F. poae* was affected by weather conditions. Higher quantities of *F. poae* DNA were reported in 2007 which was characterized by higher temperatures and lower precipitation levels in May and June in comparison with the respective periods of the previous year and the long-term mean. In a study by Wagacha et al. (2012), higher temperatures contributed to the germination of *F. poae* spores – 10% of spores incubated at 22°C germinated after four hours, and 50% of spores after six hours. Spore germination was inhibited at 10°C when the percentage of germinated spores reached 10% after 10 hours and exceeded 30% after 12 hours of incubation (Wagacha et al. 2012).

The results of other research studies indicate that higher temperatures contribute to the proliferation of *F. graminearum*, whereas *F. culmorum*, *F. poae* and *F. avenaceum* thrive in colder climates (Doohan et al. 2003). According to Rohácik and Hudec (2005), in colder regions the prevalence of *F. poae* infections is higher, but the concentrations of the analyzed pathogen in grain tend to be lower. Kriss et al. (2012) observed a negative correlation between precipitation levels and *F. poae* concentrations in winter wheat grain, which implies that the examined pathogen thrives in periods characterized by lower rainfall. In our study, the lower levels of fungal infection in 2006 could be attributed to weather conditions which were less conducive to the growth of *F. poae*.

In winter wheat 'Zyta', a positive correlation was noted between the number of isolated *F. poae* colonies and the amount of pathogenic DNA in grain. The higher incidence of *F. poae* infections in Zyta decreased yield and TGW, which was validated by the results of mathematical analysis. Similar results were reported by Brennan et al. (2007) who observed a correlation between the DNA amount and TGW values in various wheat cultivars – the decrease in TGW values of the examined cultivars ranged from 3% to more than 20% in correlation analysis.

In our study, winter wheat was also colonized by other species of pathogenic fungi. *Bipolaris sorokiniana* and *Drechslera biseptata* were relatively abundant, whereas *Botrytis cinerea* and fungi of the genus *Rhizoctonia* were less frequently isolated. A much higher number of pathogenic species was observed in the second year of the experiment which was characterized by an abundance of *Bipolaris sorokiniana* isolates; they accounted for approximately 10% of all fungal taxa isolated from the grain of winter wheat cv. Nutka in 2007 and had an estimated 8% share in the taxa isolated from the grain of winter wheat cv. Zyta. *B. sorokiniana* causes spot blotch, common root rot and cereal grain infections (Kumar et al. 2002). The species contributes to a significant loss of spring grain crops, and it reduces the germination capacity of seeds (Wiewióra, 2006). The source of infection is contaminated seed material, which is why seed dressing is the most effective method of controlling the spread of *B. sorokiniana* (Acharya et al. 2011).

The fungicides used in this experiment did not reduce the abundance of *B. sorokiniana* in winter wheat. In the work of Baturo (2008), the discussed fungal species had a more damaging effect on organically-grown barley which was not chemically protected. According to Duveiller et al. (2005), triazole-based fungicides – tebuconazole, propiconazole and epoxiconazole – were the most effective in controlling the growth of *B. sorokiniana*.

In this experiment, we studied naturally infected winter wheat plants, which contributed to a better understanding of the relationships between host plants and pathogens. The observed results are, however, not exhaustive, and further work is needed to investigate the pathogenesis of *F. poae* and its susceptibility to weather conditions.

Author's contributions

The following declarations about authors' contributions to the research have been made: designing the experiments: AP, JO; field research: JO, JJ; laboratory research: AP, AO, JJ; determination of the fungal pathogens: AP, AO; data analyses: AO, AP; writing the manuscript: AP, AO, JO; drawings: AO.

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Diagnostyka patogenów grzybowych z rodzaju *Fusarium* z ziarna pszenicy ozimej uprawianej w warunkach ochrony fungicydowej w rejonie Polski północno-wschodniej

Streszczenie

Celem badań było zastosowanie różnych metod diagnostycznych do oceny wpływu ochrony fungicydowej na występowanie patogenicznych grzybów w ziarnie pszenicy. Doświadczenie polowe, w którym uprawiano pszenicę ozimą (odmiany Nutka i Zyta) przeprowadzono w latach 2006–2007 w Zakładzie Produkcyjno–Doświadczalnym w Bałcynach. Czynnikiem doświadczenia było zastosowanie ochrony chemicznej: epoksykonazolu, krezoksymu metylowego, fenpropimorfu w fazie BBCH 33–35 oraz dimoksystrobiny i epoksykonazolu w fazie BBCH 51–53. W badaniach wykazano, że metody mikroskopowa oraz klasyczny PCR uzupełniają się. Kwantyfikacja DNA grzyba *Fusarium poae* metodą qPCR wykazała skuteczność ochrony chemicznej w zwalczaniu tego gatunku. Niższe miesięczne sumy opadów oraz wyższe dobowe temperatury sprzyjały przede wszystkim, infekcji ziarna przez *F. poae*. Stwierdzono istotną korelację pomiędzy liczbą kultur *F. poae* wyizolowanych z ziarna pszenicy ozimej a ilością patogennego DNA określoną w ziarnie metodą qPCR. Wykazano także, że porażenia ziarna przez *F. poae* powoduje obniżenie wielkości plonu oraz MTZ.