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THE FUNGAL COMMUNITIES ON SPRING WHEAT SEEDS
FROM DIFFERENT ENVIRONMENTAL CONDITIONS

ABSTRACT

Seeds of ten cultivars of spring wheat from five different localized breeding stations were tested for seed health. The same seeds were sown in Radzików in field experiment and its seed health after harvest was evaluated also. The basic material of wheat was infected by numerous fungi: 36 fungal species belonging to 24 genera and the most often occurred: *Alternaria alternata*, *Epicoccum purpurascens*, *Fusarium* spp., *Drechslera* spp. and *B. sorokiniana*. It was found significant differences between cultivars for total fungi occurred on seeds, for seed infection by main pathogens (*B. sorokiniana*, *Drechslera* spp. and *Fusarium* spp.). Also seeds from breeders were strongly infected by these pathogens in comparison to seeds harvested in field experiment in Radzików. .

Key words: fungi, pathogens, seed health, sowing material.

INTRODUCTION

Sown area of spring wheat in Poland in 2013 according to Central Statistical Office (GUS, 2014) was 270 thousand ha and change in the coming years, depending on the losses that occurred in the winter crops. Growing this species is of the greatest importance in Podlasie, West Pomerania and Lower Silesia.

Seeds on its surface or floral tissues can transmit different fungal species, both saprotrophes and pathogens, therefore they provide an important source of plant infection. Infected seeds are the most important carrier of pathogens for trans-regional and long-distance dissemination (Dipali *et al.*, 2013).

The most important and serious concern of wheat to both growers and researchers in the world are seed pathogens *Bipolaris sorokiniana* (Sacc.) (Dubin and Ginkel, 1991), the causal agent of common root rot, leaf spot disease, seedling blight and black point and *Fusarium* fungi cause fusarium head blight (Goswami and Kistler, 2004). The first is one of the most serious foliar disease constraints for both crops in warmer growing areas and causes significant yield losses (Wiewióra, 2006a). The effects of fusarium head blight as well as *B. sorokiniana* infection, is the yield reduction and lower kernel quality, due to different mycotoxins production during infection, which contaminate grain and processed wheat products (Edwards, 2009; Edwards *et al.*, 2009).

The purpose of this research was to determine the environmental factors influencing the fungal communities on wheat seeds.

MATERIALS AND METHOD

Seeds of ten cultivars of spring wheat (Raweta, Hewilla, Radunia, Łagwa, Ostka Smolnicka, Zadra, Cytra, Waluta, Bombona i Katoda), originated from five different breeding stations (Radzików, Kobierzyce, Smolice, Kończewice, Choryń) were investigated as basic material. These seeds were sown in field experiment in Radzików (the same climatic and soil conditions) and after harvest the occurrence of seedborne fungi were determined. Seed health of wheat harvested in field experiment in Radzików was compared to seed health of basic material. Research were conducted in the years 2011-2013.

The mycological assays were carried out on 50 seeds in four replications taken from seed sample of each cultivar. Seeds were disinfected with 2% sodium hypochlorite for 1 minute and then washed with sterile water three times. Disinfected seeds were placed on potato-agar medium with 0.003% streptomycin sulphate. Fungal colonies were grown at 18°C in alternating cycle of 12 h NUV radiation (360 nm) and 12 h darkness. Developed colonies were transferred to potato-agar plates and incubated in above-mentioned conditions to stimulate sporulation. Fungi were identified after 15-20 days of incubation according to the descriptions of Chidambaram *et al.* (1974), Malone and Muskett (1997) and Kwaśna *et al.* (1991). Data were presented as mean number of colonies of 100 seeds in 4 replications (50 seeds per replication were tested). Statistical analysis of total fungi was performed using the Statistica 12.5 software (StatSoft, Inc. 2014). Two-way and three-way analyses of variance (ANOVA) for main effects with Tukey-Kramer contrasts were made.

RESULTS AND DISCUSSION

The mycological assay showed that the basic material of wheat was infected by numerous fungi: 36 fungal species belonging to 24 genera. Simi-

lar results were obtained by Pusz *et al.* (2016) in paper about relationships between wheat cultivars, *Fusarium* head blight and mycoflora grains. The most often fungi occurring on the seed of cultivars Raweta and Zadra (mean for both 114,5 colonies/100 seeds), while the lowest amount was determined on the seed of variety Waluta (mean 59,2 colonies/100 seeds). Among the isolated fungi most frequently were observed: *Alternaria alternata*, *Epicoccum purpurascens*, *Fusarium* spp., *Drechslera* spp. and *B. sorokiniana* (Tab. 1). The importance of these species are often highlighted because of their genetic diversity, which may be affected by environmental conditions (Pusz, 2009; Walker *et al.* 2001), or because of the mycotoxins production (Aufhammer *et al.*, 2000; Kumar *et al.*, 2008).

Table 1
Fungal composition on seeds of spring wheat (number of colonies/100 seeds mean for examined cultivars)

Fungi	Cultivars									
	Raweta	Hewilla	Radunia	Łagwa	Ostka Smolicka	Zadra	Cytra	Waluta	Bombona	Katoda
<i>Acremoniella atra</i>	-	-	-	-	-	1.0	0.7	-	-	-
<i>Acremonium</i> spp.	-	-	0.5	0.2	-	-	2.0	0.2	-	0.2
<i>Alternaria alternata</i>	45.2	60.5	55.5	52.2	57.5	46.5	41.2	37.2	30.2	54.0
<i>Arthrobotryx superba</i>	0.5	0.7	1.2	1.0	1.5	5.5	3.0	-	1.5	1.0
<i>Aspergillus</i> spp.	-	0.5	-	-	-	-	-	-	0.5	-
<i>Aureobasidium pullulans</i>	-	-	0.2	0.2	0.5	0.7	-	0.2	1.8	1.5
<i>Bipolaris sorokiniana</i>	15.5	0.5	3.2	0.2	-	8.2	5.0	-	-	0.5
<i>Botrytis cinerea</i>	-	-	-	1.0	-	0.2	0.5	-	-	-
<i>Cladosporium herbarum</i>	1.0	3.0	2.0	0.5	2.0	-	0.2	0.2	0.5	1.0
<i>Drechslera dematioidea</i>	-	-	1.0	-	1.0	-	0.5	-	-	0.5
<i>Drechslera siccans</i>	3.0	3.0	5.0	3.5	8.5	0.7	1.8	-	4.5	3.0
<i>Razem Drechslera</i> spp.	3.0	3.0	6.0	3.5	9.5	0.7	2.3	-	4.5	3.5
<i>Epicoccum purpurascens</i>	27.8	15.0	19.2	22.8	27.5	28.2	14.5	6.0	7.8	13.2
<i>Fusarium avenaceum</i>	-	-	-	1.5	2.5	0.5	3.0	1.0	-	2.5

Table 1

Fungi	Cultivars									
	Raweta	Hewilla	Radunia	Łagwa	Ostka Smolicka	Zadra	Cytra	Waluta	Bombona	Katoda
<i>Fusarium culmorum</i>	1.5	0.5	3.0	1.5	-	2.5	7.0	1.0	1.2	3.0
<i>Fusarium equiseti</i>	2.0	1.0	1.0	-	-	1.2	3.5	-	1.0	0.2
<i>Fusarium graminearum</i>	4.0	4.4	2.5	1.5	5.0	3.2	12.5	2.0	-	2.0
<i>Fusarium heterosporum</i>	-	-	-	-	0.5	-	-	-	-	-
<i>Fusarium oxysporum</i>	0.5	1.0	0.5	1.0	1.0	2.0	2.5	-	0.5	0.7
<i>Fusarium poae</i>	7.5	11.0	11.0	17.2	2.5	7.2	6.8	2.0	1.3	1.5
<i>Fusarium semitectum</i>	-	-	-	-	-	-	-	0.5	-	0.5
<i>Fusarium solani</i>	1.0	1.0	0.5	1.5	1.5	0.2	0.2	3.0	0.2	2.2
<i>Fusarium sporotrichioides</i>	1.0	-	1.5	-	-	0.2	1.8	2.2	-	0.5
<i>Fusarium tricinctum</i>	0.5	2.5	-	3.6	1.0	1.8	2.5	1.0	0.2	0.5
Razem <i>Fusarium</i> spp.	18.0	21.3	20.0	27.8	14.0	18.8	39.8	12.7	4.4	13.6
<i>Microdochium nivale</i>	-	1.0	-	-	-	2.8	-	0.5	-	0.5
<i>Nigrospora</i> sp.	-	0.2	0.8	-	-	-	1.3	-	9.0	6.2
<i>Papularia arundinis</i>	1.5	3.2	5.0	1.2	1.0	0.2	2.0	-	0.2	-
<i>Penicillium</i> spp.	-	-	0.2	0.2	-	0.2	-	1.0	0.8	-
<i>Phoma</i> spp.	-	-	-	-	-	-	-	-	0.2	-
<i>Rhizoctonia solani</i>	0.8	-	-	-	-	-	-	-	-	0.5
<i>Rhizopus</i> spp.	-	-	-	-	0.5	0.2	-	-	-	-
<i>Septoria</i> sp.	-	-	0.2	-	-	0.5	-	-	-	-
<i>Sordaria fimicola</i>	0.5	-	-	-	-	-	-	-	0.2	-
<i>Stemphylium botryosum</i>	0.5	-	-	-	-	-	-	1.0	0.5	1.5
<i>Stemphylium consortiale</i>	-	-	-	-	-	0.8	-	-	0.2	3.8
<i>Trichoderma viride</i>	-	-	-	-	-	-	0.3	-	-	-
<i>Torula herbarum</i>	-	-	-	-	-	-	-	0.2	-	-
non sporulating mycelium	0.2	-	-	-	-	-	-	-	0.5	-
Total fungi/ 100 seeds	114.5	109.0	114.0	110.8	114.0	114.5	112.8	59.2	62.8	101.0

The largest part of all the fungi isolated from seeds of examined cultivars of spring wheat were *Alternaria* species, which occurred on the level 36,7 – 62,8%. Pathogens occurring on wheat seeds were represented by *Fusarium* spp., *Drechslera* spp., *B. sorokiniana*, *Microdochium nivale*, *Rhizoctonia solani* and *Septoria* sp. Fungi of the genus *Fusarium* belonged to 11 species, among them *F. poae*, *F. graminearum* and *F. culmorum* were the most frequent ones (Table 1).

This confirms the observations of Bottalico and Perrone (2002) and Xu *et al.* (2008) that in Europe these fungi are the most prevalent species of the *Fusarium* head blight. These fungi most often occur on the seeds of cultivars: Cytra, Łagwa and Hewilla (means 39.8, 27.8 and 21.3 colonies/100 seeds respectively), which accounted for 35.2, 25.0 and 19.5% of all isolated fungi. Similar results were obtained by Hampton (1980), who observed *Fusarium* spp. the most commonly on the certified seeds of wheat .

The study showed, that presence of fungi from genus *Drechslera* was determined at the level of 0.0% all isolated fungi for cultivar Waluta to 8.3% for Ostka Smolicka. *Bipolaris sorokiniana* species was also isolated quite often. It infected more than 5% seeds of two out of all tested samples (13.5% for cultivar Raweta and 7.2% for Zadra. On the seeds of other cultivars this fungus was found less frequently and it represented from 0 to 4.4% of all isolated fungi (Fig. 1). Similar differences in susceptibility to *B. sorokiniana* were also observed for cultivars of spring barley (Wiewióra, 2006b; Wiewióra, 2009). Agarwal and Sinclair (1997) found that cultivars react differently to seed infection due to incompatibility to infection, restricted pathogen invasion of the embryo or ovary wall due to inhibitors, or impermeability to aqueous solutions, because of a waxy coating on the seed surface, compact and uniform arrangement of cells, or reduced amounts of amino acids or phenolic compounds. .

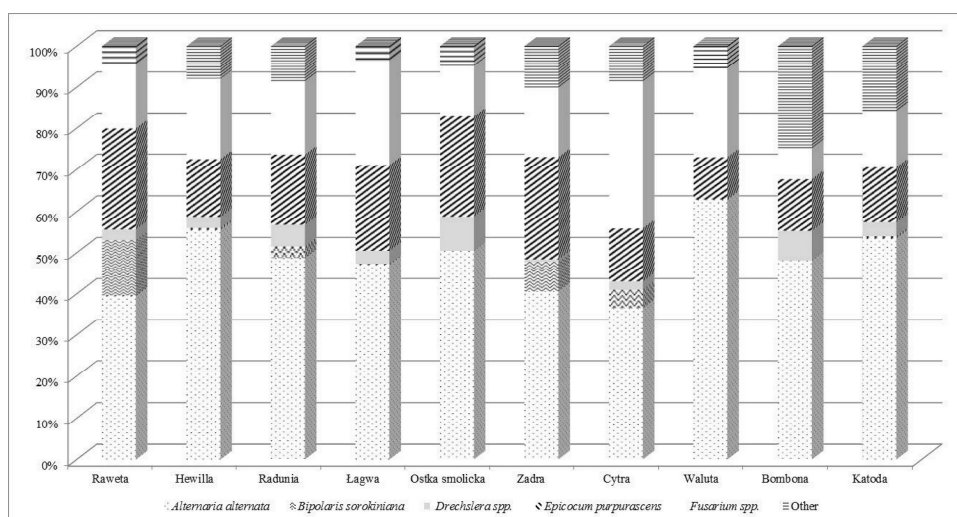


Fig. 1. Share of selected species of fungi infecting seeds of examined cultivars of spring wheat

The statistical analyses revealed significant differences between cultivars for total fungi occurred on seeds, for seed infection by *B. sorokiniana* and *Drechslera* spp. (together) and for infection by *Fusarium* spp. No differences were found for total fungi depending on the year of vegetation, but for *B. sorokiniana*+*Drechslera* spp. and *Fusarium* spp. differences for these years were found (Table 2).

Table 2
Analysis of variance and homogeneous groups for some fungi infected seeds of spring wheat

Factor	Total fungi		<i>Drechslera</i> & <i>Bipolaris sorokiniana</i>		<i>Fusarium</i>	
	Mean	Group	Mean	Group	Mean	Group
Year						
2012	100.7	a	8.4	a	14.55	b
2013	101.8	a	5.3	b	24	a
<i>F</i> -statistic	0.01		19.69		12.46	
<i>P</i> -value	0.9110		0.0001		0.0014	
Cultivar						
Bombona	62.75	c	4.5	bc	4.25	d
Cytra	112.75	a	7.25	bc	39.5	a
Hewilla	109.0	ab	3.5	bc	22.25	bc
Katoda	101.0	b	4	bc	13.75	bcd
Łagwa	110.75	a	3.75	bc	27.5	ab
Ostka Smol.	114	a	9.25	b	14	bcd
Radunia	114	a	8.75	b	19.25	bc
Raweta	114.5	a	18.5	a	18	bcd
Waluta	59.25	c	0	c	13.25	cd
Zadra	114.5	a	9	b	21	bc
<i>F</i> -statistic	169.90		9.79		11.50	
<i>P</i> -value	<0.0001		0.0067		<0.0001	

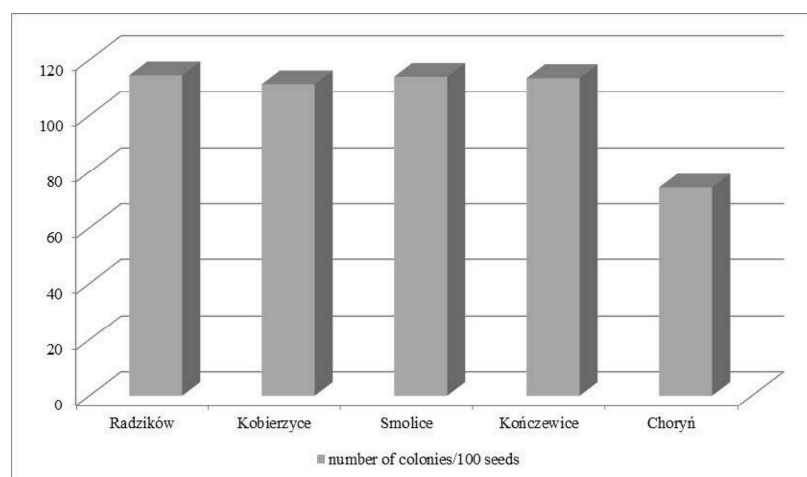


Fig. 2. Total number of fungi isolated from sowing material of spring wheat depending on seeds origin

The studies have shown that the most often fungi occurred on seed from Radzików and Smolice (mean 114.5 colonies/100 seeds and 114.0 colonies/100 seeds respectively), while the lowest amount was determined on the seeds received from Choryń (mean 74.4 colonies/100 seeds) (Fig. 2).

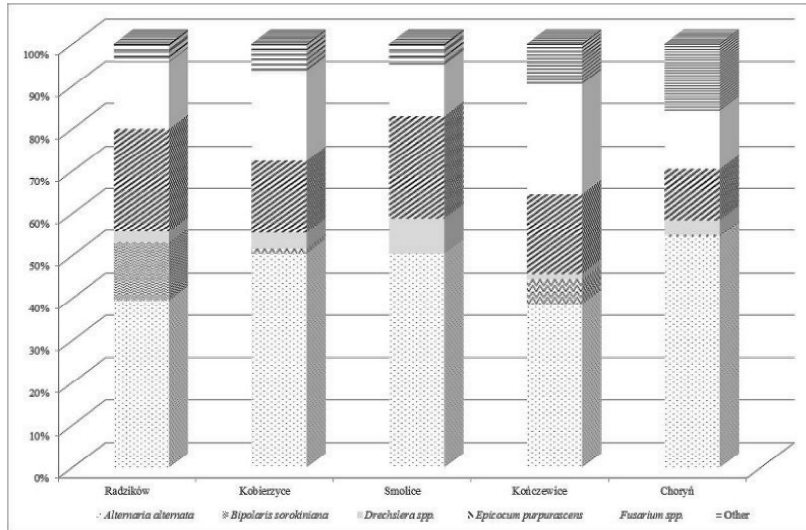


Fig. 3. Share of selected species of fungi infecting seeds of spring wheat depending on seeds origin

Among the isolated fungi the most frequently observed were: *A. alternata*, *E. purpurascens*, *Fusarium* spp., *B. sorokiniana* and *Drechslera* spp. Isolates of *A. alternata* were isolated in range from 40.5 colonies (54.4% of all fungi) to 57.5 colonies (50.4%). *Fusarium* fungi occurred quite often, ranged from 10.2 colonies (13.7%) on seeds from Choryń to 29.3 colonies (25.8%) on sowing material originating from Kończewice (Fig. 3).

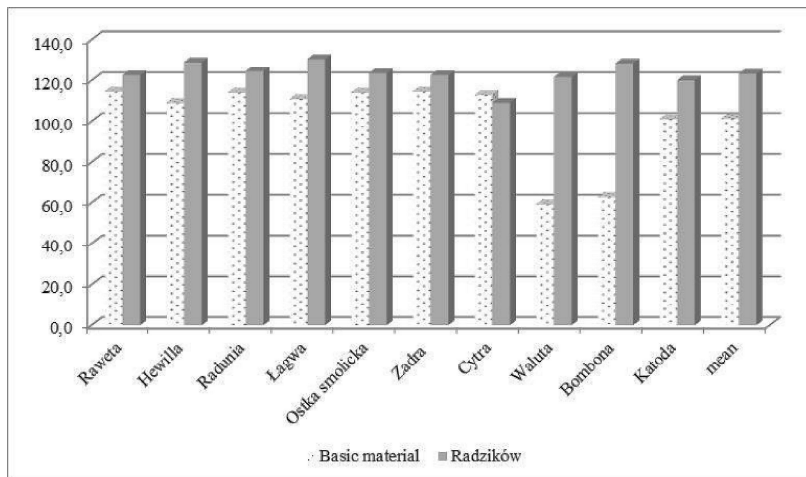


Fig. 4. Comparison of colonization by fungi seeds from breeders with seeds collected in a field experiment

In the case of fungi of *Drechslera* genus their existence was determined at 1.3% for seed from Kończewice to 8.3% of all isolated fungi of seed from Smolice. Quite often, the isolates of *B. sorokiniana*, that most infected sowing material was from Radzików (mean 13.5%) and Kończewice (mean 5.8%) were isolated. Infection of seeds from other growing regions does not exceed 2% and ranged from 0.0 to 1.2% (Fig. 3).

Seed material collected from a field experiment in Radzików were numerously inhabited by fungi compared to seeds obtained from breeders - the basic material (mean for cultivars respectively 123.2 and 101.3 colonies/100 seeds). The biggest difference in the seed infection from field experiment in Radzików and originating from breeder was found for cultivars Waluta and Bombona (62.3 and 65.7 colonies respectively) (Fig. 4).

There were observed also differences in colonization by selected species of fungi between the seed received from breeders and harvested in a field experiment located in Radzików. Seeds of all tested cultivars collected from a field experiment were numerously inhabited by *Alternaria* genus than seeds obtained from breeders. Likewise it was for infestation by *A. alternata*, where the biggest difference between seed from field experiment and breeder was found for cultivars Waluta and Bombona (the differences respectively 36.3 and 51.73 colonies) (Fig. 5).

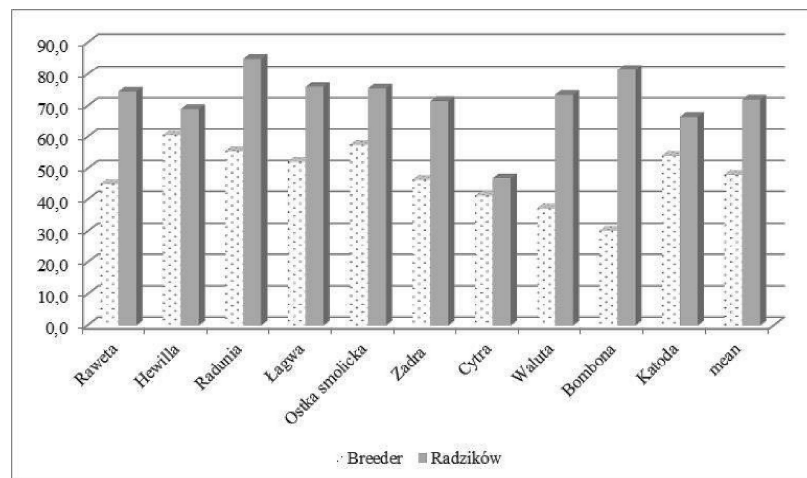


Fig. 5. Comparison of colonization by *Alternaria alternata* seeds from breeders with seeds collected in a field experiment

There were found also differences in seed infection by pathogenic fungi (*Drechslera* spp.+*B. sorokiniana* and *Fusarium* spp.) between the seed from breeders and harvested in a field experiment located in Radzików. In both cases, seeds from breeders were strongly infected by these pathogens in comparison to seeds harvested in field experiment in Radzików (Fig. 6).

The statistical analyses revealed significant differences for seed infection by all identified fungi and selected pathogens in dependence on origin and tested cultivars (Table 3).

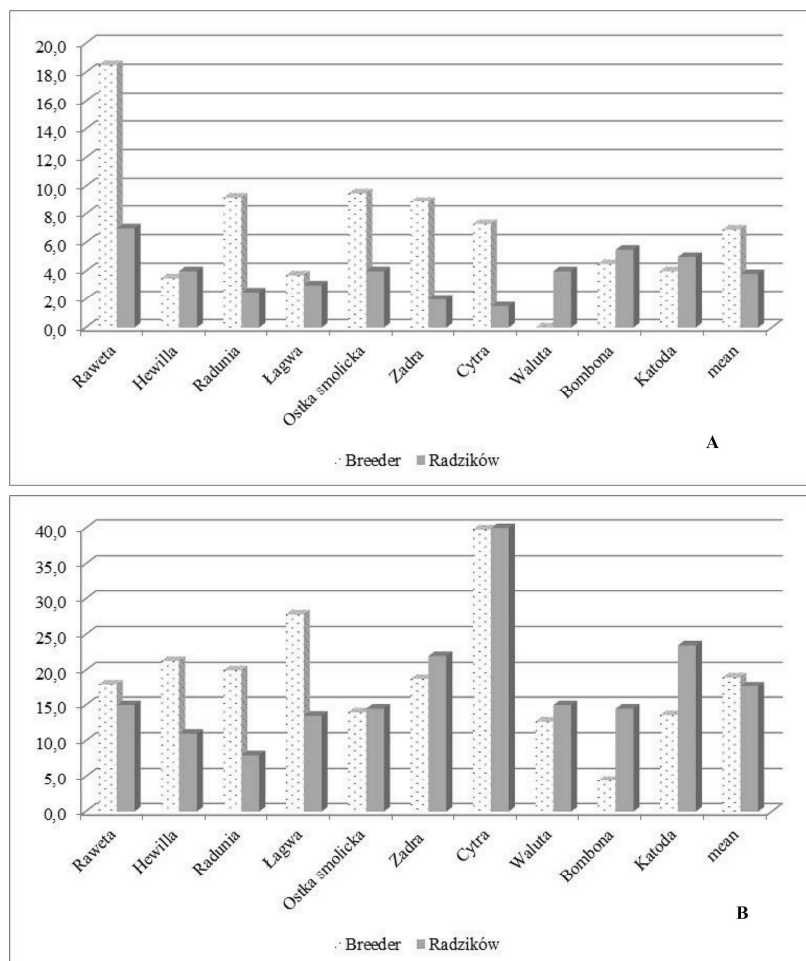


Fig. 6. Comparison of seed infection by *Drechslera* spp. + *Bipolaris sorokiniana* (A) and *Fusarium* spp. (B) from breeders and collected in a field experiment

These differences are largely due to the environmental and weather conditions prevailing during the growing season. Time of flowering is very much susceptible period for primary infection and also environmental conditions at flowering and during seed development influence seed infection and inoculum localization in seeds (Dipali *et al.* 2013). More seeds infection by *A. alternata* was found from plants grown at above 80% relative humidity than from those produced in dry areas (Harman *et al.*, 1973). Wheat infection by *F. avenaceum*, *F. culmorum* and *Microdochium nivale* was favored by high rainfall, especially during ear emergence and maturation (Mishra, 1973).

Table 3

Analysis of variance and homogeneous groups for some fungi infected seeds of spring wheat originating from breeders and field experiment in Radzików

Factor	Total fungi		<i>Drechslera & Bipolaris sorokiniana</i>		<i>Fusarium</i>	
	Mean	Group	Mean	Group	Mean	Group
Origin						
Breeder	101.25	b	6.85	a	19.275	a
Radzików	123.15	a	3.85	b	17.7	a
<i>F-statistic</i>	395.55		19.57		0.98	
<i>P-value</i>	<0.0001		<0.0001		0.3249	
Cultivar						
Bombona	62.75	c	4.5	bc	4.25	d
Cytra	112.75	a	7.25	bc	39.5	a
Hewilla	109.0	ab	3.5	bc	22.25	bc
Katoda	101.0	b	4	bc	13.75	bcd
Łągwa	110.75	a	3.75	bc	27.5	ab
Ostka Smol.	114	a	9.25	b	14	bcd
Radunia	114	a	8.75	b	19.25	bc
Raweta	114.5	a	18.5	a	18	bcd
Waluta	59.25	c	0	c	13.25	cd
Zadra	114.5	a	9	b	21	bc
<i>F-statistic</i>	169.90		9.79		11.50	
<i>P-value</i>	<0.0001		0.0067		<0.0001	

CONCLUSIONS

The basic material of wheat was infected by numerous fungi: 36 fungal species belonging to 24 genera and the most often occurred: *Alternaria alternata*, *Epicoccum purpurascens*, *Fusarium* spp., *Drechslera* spp. and *B. sorokiniana*.

The statistical analyses showed significant differences between cultivars for total fungi occurred on seeds and seed infection by pathogens (*B. sorokiniana*, *Drechslera* spp. and *Fusarium* spp.). No differences were found for total fungi depending on the year of vegetation, but for isolated pathogens differences for these years were found.

Seed material collected from a field experiment in Radzików were numerously inhabited by fungi compared to seeds obtained from breeders. However, seeds from breeders were strongly infected by these pathogens in comparison to seeds harvested in field experiment in Radzików .

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