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IK: idea of the study; IK, MC, AP: writing of the manuscript; MW: statistical analysis; IK, AP, MC: mycological analysis; IK, MC, AP: analysis of research results; LR: field research; IK, AP, MC: experiment in the growth chamber; MP: breeder of the genotypes used in the growth chamber experiment

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No competing interests have been declared.

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ORIGINAL RESEARCH PAPER

The occurrence of fungi on roots and stem bases of *Triticum aestivum* ssp. *spelta* L. Thell. grown under two levels of chemical protection and harmfulness of *Fusarium graminearum* Schwabe to seedlings of selected genotypes

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Abstract
Investigations were carried out in 2007–2009 on the plots of the Felin Experimental Station belonging to the University of Life Sciences in Lublin, Poland. The studies comprised two breeding lines of spelt wheat (*Triticum aestivum* ssp. *spelta* L. Thell.) – STH 3 and STH 715. Two levels of chemical protection were applied in the cultivation with minimal and complex protection. Infection of winter spelt wheat roots and stem bases was recorded in each growing season at hard dough stage (87 in Zadok's scale). After 3 years of the study, the mean values of disease indexes for the analyzed spelt wheat lines in the experimental treatment with minimal protection were 28.53 and 40.30 respectively for STH 3 and STH 715. In the experimental combination with complex protection, after 3 years of the study the mean values of disease indexes ranged from 25.96 (STH 3) to 26.90 (STH 715). The mycological analysis showed that *Fusarium* spp., especially *F. culmorum*, caused root rot and necrosis of stem bases of spelt wheat in the experimental combination with minimal and complex protection. Moreover, *Fusarium avenaceum* and *Bipolaris sorokiniana* caused root rot and necrosis of stem bases of spelt wheat. Investigation carried out in a growth chamber on susceptibility of seedlings of three lines of spelt wheat (LO 2/09/n/2, LO 5/09/13/3, LO 5/09/5/4) to infection with *Fusarium graminearum* No. 8 and *F. graminearum* No. 45 showed that the genotypes did not differ in their susceptibility. All of them were susceptible, as indicated by high values of the disease indexes. No interaction was found between genotypes and strains of the fungus. This indicates the differential pathogenicity of *Fusarium graminearum* species.

Keywords
spelt wheat; *Fusarium culmorum*; *Bipolaris sorokiniana*; *F. avenaceum*; root rot; stem base rot; seedlings; damping-off; susceptibility; *F. graminearum*

Introduction

Increased spelt wheat cultivation has been observed in recent years. In many countries of Europe and North America this is associated with the development of organic farming and results from paying attention to the nutritional qualities of this species, which are higher compared to common wheat (*Triticum aestivum* ssp. *vulgare* L.). Spelt wheat grain contains more mineral nutrients, vitamins, lipids, and unsaturated fatty acids. It is characterized by a higher content of protein with higher digestibility and biological quality compared to the protein of common wheat grain. Spelt wheat has lower agronomic requirements than common wheat [1–5]. In Europe, winter forms of spelt wheat are grown most frequently; these forms are characterized by higher yield compared to spring forms, but greater susceptibility to lodging [6].

There are many reports on fungi causing root and stem rot diseases of common wheat (*Triticum aestivum* ssp. *vulgare* L.) [7–13], but there is less information on pathogens infecting roots and stem base of spelt wheat (*Triticum aestivum* ssp. *spelta* L. Thell.).

The aim of the present study was to determine the contribution of various fungal species to the damage of roots and stem bases in two breeding lines of spelt wheat at two chemical protection levels and to investigate the pathogenicity of *Fusarium graminearum* to seedlings of selected breeding lines of spelt wheat under growth chamber conditions.

Material and methods

The field experiment was conducted during the period 2007–2009 at the Felin Experimental Station belonging to the University of Life Sciences in Lublin (51°22′ N, 22°64′ E), Poland. Experimental plants were grown on grey brown podzolic soil derived from loess deposits, with the granulometric composition of medium loam classified as good wheat soil complex, soil class II. The soil is characterized by high availability of nutrients: P – 76.0; K – 119.0; Mg – 55.5 (mg 100 g^{-1} soil), and has a pH of 6.3 in KCl solution.

The study comprised two breeding lines of winter spelt wheat (*T. aestivum* ssp. *spelta* L. Thell.): STH 3 and STH 715. Two levels of chemical protection were used in crops. The first level included minimal protection where grains were dressed with Oxafun T 75 DS/WS (200 g 100 kg^{-1} grains and Chwastox Trio 540 SL at a rate of 2 dm^3 ha^{-1}). The other level of complex chemical protection included seed dressing with Oxafun T 75 DS/WS at a rate of 200 g 100 kg^{-1} grains, and two herbicides: Puma Uniwersal 069 EW at a rate of 1.2 dm^3 ha^{-1} and Chwastox Trio 540 SL at a rate of 2 dm^3 ha^{-1}. At the tillering stage (20 in Zadok's scale [14]), the fungicide Alert 375 SC at a rate of 1.8 dm^3 ha^{-1} as well as the insecticide Decis 2,5 EC at a rate of 250 cm^3 ha^{-1} and Stabilan 750 SL at a rate of 1.8 dm^3 ha^{-1} were also applied. At both protection levels, mineral fertilization was applied before sowing at the rates of 26 kg P ha^{-1} and 66 kg K ha^{-1}. Besides, top dressing was applied twice: nitrogen at a rate of 70 kg ha^{-1} at the beginning of plant growth and 30 kg N ha^{-1} at the third node stage. Conventional tillage was used. Spelt wheat was sown in the third 10-day period of September each year. The sowing density was 500 grains per m^2.

The experiment was carried out in a randomized block design in four replicates, in the field after winter rape. The plot area was 10 m^2.

The evaluation of disease symptoms, the amount of plant material used for mycological analysis, and the experimental design were the same as in the study on fungi of roots and stem bases of common wheat and durum wheat grown under two levels of chemical protection [15].

In the laboratory, the proportion of stems with necrotic streaks on the roots and lower internodes was determined and the degree of infection was established according to a 5-point scale [16]. The disease index was calculated using McKinney's formula [17]. The obtained results were statistically analyzed using Tukey's half-intervals [18].

The mycological analysis methods were the same as in the previous study [19]. The experiment on the susceptibility of seedlings of three spelt wheat genotypes (LO 2/09/n/2, LO 5/09/13/3, LO 5/09/5/4) to infection with *Fusarium graminearum* No. 8 obtained from stem bases of rye and with *F. graminearum* No. 45 isolated from oat seedlings was conducted in a growth chamber at a temperature of 23–24°C and a relative air humidity of 85%. Strains whose pathogenicity had been earlier tested in the laboratory using Mishra's and Behr's method [20] were used in the investigations. The protocol of the growth chamber experiment was the same as in the study by Kiecana et al. [21].

The infection rates of spelt wheat seedlings derived from the growth chamber experiment were statistically analyzed using Statgraphics 4.1 for Windows (Statistical Graphics Corp. 1999, Statpoint Technologies, Inc. Warrenton, Virginia, USA). The significance of mean differences was evaluated by one-way and multi-way analysis of variance. A detailed comparison of the means was made by Fisher's LSD test. The statistical hypotheses were tested with an error rate of $\alpha = 0.05$. The study compared the pathogenicity of *F. graminearum* No. 8 and 45 used to infect culture medium and the susceptibility of spelt wheat genotypes to infection with the analyzed strains of *F. graminearum* as well as the interaction of the spelt wheat genotypes to infection with *F. graminearum* No. 8 and 45 was characterized.

Mycological analysis for pathogenic fungi was also conducted. The number of seedlings and the number of plant fragments taken for the mycological analysis and the analysis method were the same as in the previous study [21]. Fungi of the genus *Fusarium* were identified according to Nelson et al. [22] as well as Leslie and Summerell [23]. Other fungal species were identified according to the keys and monographs described in Kiecana et al. [21].

Results

The field observations showed that plants with disease symptoms on the roots and stem bases occurred in each growing season in the experimental treatment with minimal protection. The percentage of such plants ranged from 42.0 (STH 3) in 2007 to 94.0 (STH 3) in 2009. On average for the 3-year study period, the proportion of stems with disease symptoms in the treatment with minimal protection was from 63.8 (STH 3) to 79.5 (STH 715) for the spelt wheat lines analyzed (Fig. 1).

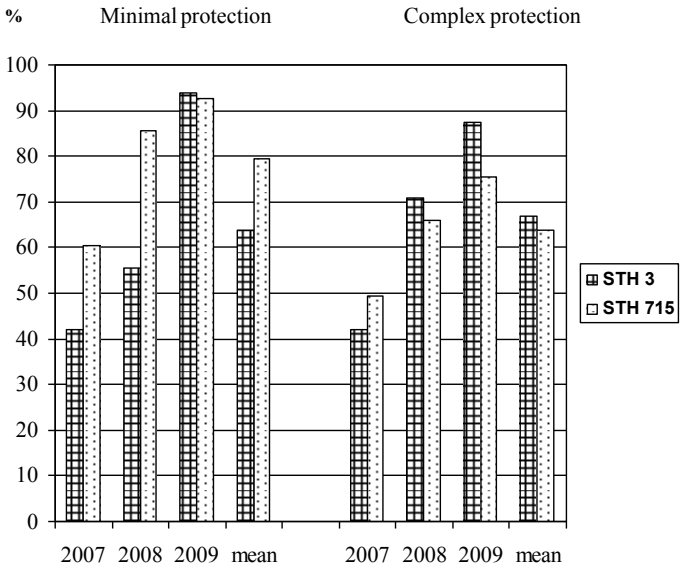

Fig. 1 Percentage of plants with necrosis of roots and lower internodes in spelt wheat (*T. aestivum* ssp. *spelta*) cultivated under minimal and complex plant protection conditions in 2007–2009.

For the analyzed *T. aestivum* ssp. *spelta* lines STH 3 and STH 715, in 2007 the values of the disease indexes for plants from the experimental treatment with minimal protection were, respectively, 15.3 and 25.9 and they differed significantly (Tab. 1). In 2008, an increase was found in the disease indexes for the analyzed spelt wheat lines, which were, respectively, 27.9 (*T. aestivum* ssp. *spelta* STH 3) and 47.7 (*T. aestivum* ssp. *spelta* STH 715) and they differed significantly. In 2009, a significantly higher value of the disease index was recorded in the case of *T. aestivum* ssp. *spelta* STH 715 – 47.3. After the 3-year study period, the mean values of the disease indexes for the analyzed spelt wheat genotypes STH 3 and STH 715 were, respectively, 28.53 and 40.3 and they did not differ significantly (Tab. 1).

In the field investigations carried out in the individual growing seasons in the treatment with complex protection, spelt wheat plants with necrosis of roots and stem bases were also observed to occur. The percentage of such plants was from 42.0 (*T. aestivum* ssp. *spelta* STH 3) in 2007 to 87.5 (*T. aestivum* ssp. *spelta* STH 3) in 2009 (Fig. 1).

Tab. 1 Mean values of the disease index for *Triticum aestivum* ssp. *spelta* genotypes grown in experimental plots in Felin in 2007–2009.

Genotypes	Study year / protection level							
	minimal protection				complex protection			
	2007	2008	2009	mean	2007	2008	2009	mean
T. aestivum ssp. *spelta* STH 3	15.30 [a]	27.90 [a]	42.40* [a]	28.53 [a]	15.10 [a]	29.40 [a]	33.40 [a]	25.96 [a]
T. aestivum ssp. *spelta* STH 715	25.90* [b]	47.70* [b]	47.30* [b]	40.30 [a]	15.60 [a]	32.10 [a]	33.00 [a]	26.90 [a]
NIR	2.27	3.56	1.76	29.56	2.13	2.83	1.49	22.03

Means in columns differ significantly ($p \leq 0.05$), if they are not marked with the same letter. Means in lines, in particular years for each genotype, marked with "*" differ significantly ($p \leq 0.05$).

On average over the 3-year study period, the percentage of plants with disease symptoms in the treatment with complex protection for the analyzed spelt wheat lines was from 63.7 (STH 715) to 66.8 (STH 3; Fig. 1).

In the experimental treatment with complex protection, in 2007 the disease index for spelt wheat STH 3 and STH 715 were, respectively, 15.1 and 15.6. In 2008 the disease indexes of the analyzed spelt wheat lines were 29.4 (STH 3) and 32.1 (STH 715), whereas in 2009 the values of the disease index for the analyzed spelt wheat lines were, respectively, 33.4 (*T. aestivum* ssp. *spelta* STH 3) and 33.0 (*T. aestivum* ssp. *spelta* STH 715). In all years analyzed, the values of the disease index did not differ significantly (Tab. 1).

The statistical analysis of the disease index showed the protection level to significantly affect the health of plants in the years 2007 and 2008 in the case of *T. aestivum* ssp. *spelta* breeding line STH 715. In 2009, the level of chemical protection was found to have a significant effect on the health of plants of both genotypes analyzed (Tab. 1).

Mycological analysis of plants of both *T. aestivum* ssp. *spelta* genotypes grown in the treatment with minimal protection, revealed 866 isolates of various fungal species and non-sporulating forms were isolated, including 338 isolates from infected roots and 528 from stem bases (Tab. 2). Fungi of the genus *Fusarium*, whose isolates (667) accounted for 77% of total isolations (Tab. 2), were most frequently isolated from the analyzed organs of spelt wheat plants over the 3-year study period. In 2007, isolates of these fungi accounted for 86.5% (327 of total isolates), in 2008 they made up 75.6% (164 isolates), whereas in 2009 64.94% (176 isolates) of all fungal colonies isolated over the investigated growing season (Tab. 2). Among the fungi isolated from infected roots and stem bases of spelt wheat in all study years, the dominant species was *Fusarium culmorum* whose isolates accounted for 42.84% (371 isolates) of the total number of fungal colonies isolated in the treatment with minimal protection (Tab. 2). Moreover, in the case of this experimental treatment the following fungal species were isolated from the infected organs of plants in each growing season: *Fusarium avenaceum* 11.1% (96 isolates) and *Fusarium oxysporum*, 13.4% (116 isolates) of total isolates (Tab. 2). The following fungal species of the genus *Fusarium* were also isolated from the roots and stem bases of spelt wheat, but not in each growing season: *F. equiseti* 2.7% (23 isolates), *F. poae* 1.7% (15 isolates), *F. solani* 2.3% (20 isolates), and *F. sporotrichioides* 3.0% (26 isolates) of all fungal colonies isolated in this experimental treatment (Tab. 2).

The species *Rhizoctonia solani* with a percentage contribution of 3.2% (28 isolates) was isolated from infected organs of spelt wheat plants grown in the experimental treatment with minimal plant protection in all study years, whereas in the years 2007 and 2009 *Bipolaris sorokiniana*, whose percentage was, respectively, 2.4% (eight isolates) and 3.3% (nine isolates) of total isolates in the above-mentioned growing seasons (Tab. 2). Moreover, in 2007 10 isolates of *Aureobasidium pullulans* were obtained from infected roots of spelt wheat STH 715, which constituted 4.8% of total fungi isolated.

Tab. 2 Fungi isolated from the diseased roots and stem bases of *T. aestivum* ssp. *spelta* cultivated under minimal and complex plant protection conditions in 2007–2009.

Fungal species	Number of isolates in 2007–2009																								total number of isolates
	2007						2008						2009						total after 3 years						
	1			2			1			2			1			2			1			2			
	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	
Acremonium roseum (Oud.) W. Gams	-	-		-	-	-	-	-	-	-	-	-	34	7	41	2	-	2	34	7	41	2	-	2	43
Alternaria alternata (Fr.) Keissler	3	7	10	3	2	5	17	24	41	5	9	14	3	-	3	-	-	-	23	31	54	8	11	19	73
Aureobasidium pullulans (de Bary) Arnaud.	10	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	10	-	-	-	10
Aspergillus flavus Link	-	-	-	5	1	6	1	-	1	-	-	-	-	-	-	-	-	-	1	-	1	5	1	6	7
Bipolaris sorokiniana (Sacc.) Shoemaker	4	4	8	-	5	5	-	-	-	-	-	-	4	5	9	7	-	7	8	9	17	7	5	12	29
Botrytis cinerea Pers.	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1
Botryotrichum piluliferum Sacc. et Marchal	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	1	-	1	-	-	-	1	1	2	2
Chaetomium globosum Kunze	-	-	-	2	3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3	5	5
Epicoccum nigrum Link	-	-	-	-	-	-	-	2	2	1	-	1	2	2	4	-	-	-	2	4	6	1	-	1	7
Fusarium avenaceum (Fr.) Sacc.	55	25	80	10	2	12	2	6	8	-	7	7	3	5	8	-	-	-	60	36	96	10	9	19	115

Tab. 2 Continued

Fungal species	Number of isolates in 2007–2009																								total number of isolates
	2007						2008						2009						total after 3 years						
	1			2			1			2			1			2			1			2			
	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	
Fusarium culmorum (W. G. Sm.) Gams	27	155	182	48	101	149	15	94	109	6	27	33	39	41	80	16	30	46	81	290	371	70	158	228	599
Fusarium equiseti (Corda) Sacc.	-	-	-	-	-	-	8	-	8	-	-	-	4	11	15	-	-	-	12	11	23	-	-	-	23
Fusarium oxysporum Schl.	12	21	33	4	10	14	10	20	30	10	7	17	18	35	53	18	29	47	40	76	116	32	46	78	194
Fusarium poae (Peck.) Wollenw.	15	-	15	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	15	-	15	-	2	2	17
Fusarium solani (Mart.) Sacc.	-	-	-	-	-	-	-	-	-	-	-	-	9	11	20	-	-	-	9	11	20	-	-	-	20
Fusarium sporotrichioides Sherb.	7	10	17	-	9	9	3	6	9	2	5	7	-	-	-	-	-	-	10	16	26	2	14	16	42
Gliocladium catenulatum Gilman Abbott	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	-	1	-	-	-	1
Mucor hiemalis Wehmer	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1
Penicillium verrucosum Dierckx var. *cyclopium* (Westling.) Samson et al.	1	3	4	16	1	17	5	-	5	-	-	-	-	3	3	-	-	-	6	6	12	16	1	17	29

Tab. 2 Continued

Fungal species	Number of isolates in 2007–2009																								total number of isolates
	2007						2008						2009						total after 3 years						
	1			2			1			2			1			2			1			2			
	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	r	s	r+s	
Periconia macrospinosa Lefebvre & Johnson	-	-	-	-	-	-	-	-	-	-	-	-	5	-	5	-	-	-	5	-	5	-	-	-	5
Rhizoctonia solani Kühn	3	11	14	-	1	1	2	-	2	-	-	-	2	10	12	-	-	-	7	21	28	-	1	1	29
Rhizopus nigricans Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	10	7	17	-	-	-	10	7	17	-	-	-	17
Trichoderma aureoviride Rifai	-	-	-	-	-	-	-	-	-	22	-	22	-	-	-	-	-	-	-	-	-	22	-	22	22
Trichoderma viride Pers. ex S. F. Gray	-	3	3	4	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3	4	1	5	8
Non sporulating fungi	1	-	1	1	7	8	2	-	2	14	2	16	-	-	-	-	-	-	3	-	3	15	9	24	27
Total	139	239	378	93	146	239	65	152	217	60	58	118	134	137	271	44	59	103	338	528	866	197	263	460	1326

1 – minimal chemical protection; 2 – complex chemical protection; r – root; s – stem base.

Colonies of other fungi were represented by the following species: *Alternaria alternata*, *Acremonium roseum*, *Aspergillus flavus*, *Botrytis cinerea*, *Epicoccum nigrum*, *Gliocladium catenulatum*, *Penicillium verrucosum* var. *cyclopium*, *Periconia macrospinosa*, *Rhizopus nigricans*, *Trichoderma viride*, and non-sporulating forms (Tab. 2).

The mycological analysis of infected spelt wheat plants, sampled from the plots with full protection, revealed 460 colonies of various fungal species isolated over the period 2007–2009, including 197 isolates from infected roots and 263 from stem bases (Tab. 2). Fungi of the genus *Fusarium* were isolated most frequently, as their percentage was 74.6% (343 isolates, including 114 isolates from roots and 229 from stem bases) of all isolates (Tab. 2). Among the genus *Fusarium*, in the years 2007 and 2008 the dominant species was *F. culmorum*, whose isolates accounted for 62.34% (149 isolates) and 27.97% (33 isolates) of total fungal isolates in a given growing season in the above-mentioned treatment (Tab. 2).

Among *Fusarium* spp., *F. oxysporum*, whose isolates accounted for 17% (78 isolates) of fungal colonies obtained (Tab. 2), was isolated from infected roots and stem bases of spelt wheat in each growing season. In 2007 and 2008, the species *F. avenaceum* and *F. sporotrichioides* were isolated and their percentages were, respectively, 5% (12 isolates) and 3.8% (nine isolates) in 2007, while in 2008 5.9% (seven isolates) and 5.9% (seven isolates) of all fungi isolated in a given growing season (Tab. 2).

Moreover, from fungi of the genus *Fusarium* two isolates of *F. poae* were isolated from stem bases in 2007 and its percentage was 0.4% of all colonies isolated (Tab. 2).

In 2007, *B. sorokiniana* (five isolates) and *R. solani* were also isolated from the studied organs of spelt wheat (one isolate; Tab. 2). In 2007 and 2008, *A. alternata* was isolated and its isolates accounted, respectively, for 3.8% (five isolates) and 11.9% (14 isolates) of all fungi isolated in these study years. These colonies belonged to the following species: *A. roseum*, *A. flavus*, *Botryotrichum piluliferum*, *Chaetomium globosum*, *E. nigrum*, *Mucor hiemalis*, *Penicillium verrucosum* var. *cyclopium*, *Trichoderma aureoviride*, *T. viride*, and non-sporulating forms (Tab. 2).

In the growth chamber, necrosis symptoms on the roots and leaf sheaths of spelt wheat seedlings occurred in the experimental treatment with culture medium infected with both *F. graminearum* No. 8 and *F. graminearum* No. 45. In these experimental treatments, completely necrotized seedlings were also found, while in some cases the sprouts died even before they reached the medium surface. Control spelt wheat seedlings did not generally exhibit disease symptoms, in particular on the roots. Seedlings with small necrotic spots on the leaf sheaths were found in some control treatment replicates of the spelt wheat genotypes.

In the experimental treatment with *F. graminearum* No. 8, the mean values of the seedling infection rates for the studied spelt genotypes were from 76.0 (LO5/09/13/3) to 86.25 (LO5/09/5/4), whereas in the experimental treatment with *F. graminearum* No. 45 they ranged between 80.25 (LO5/09/5/4) and 90.25 (LO5/09/13/3). The mean values of the disease indexes for control seedlings were from 3.25 (LO5/09/13/3) to 17.75 (LO5/09/5/4; Tab. 3).

No significant differences were found between the virulence of *F. graminearum* No. 8 and No. 45 (Fig. 2) as well as between the susceptibility of the spelt wheat genotypes to infection with the analyzed strains of *F. graminearum* (Fig. 3). The variance analysis showed a significant interaction between the studied factors (*F. graminearum* strain

Tab. 3 Mean values of the disease index for seedlings of selected *T. aestivum* ssp. *spelta* genotypes infected by *Fusarium graminearum* No. 8 and No. 45 under growth chamber conditions.

	Experimental combination		
Genotypes	***F. graminearum* No. 8**	***F. graminearum* No. 45**	**control**
LO2/09/n/2	77.50	84.75	11.75
LO5/09/13/3	76.00	90.25	3.25
LO5/09/5/4	86.25	80.25	17.75

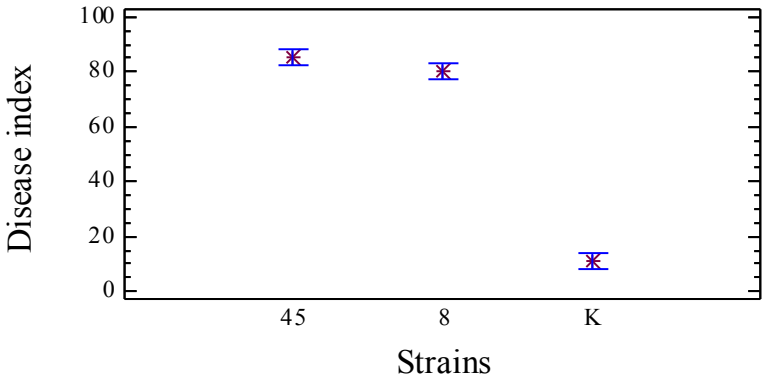

Fig. 2 Comparison of pathogenicity of *Fusarium graminearum* No. 8 and 45 based on the disease index value.

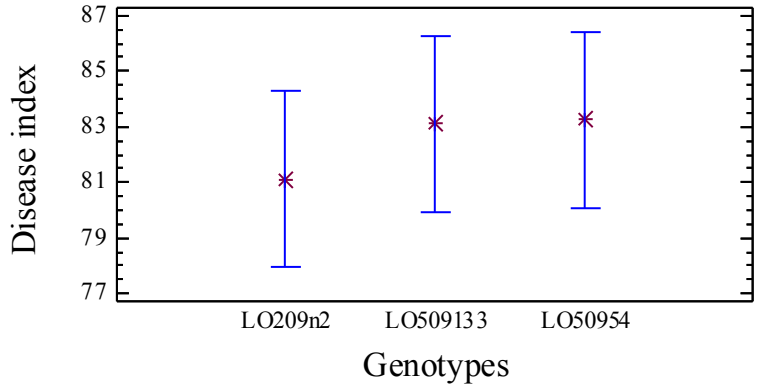

Fig. 3 Comparison of the susceptibility of the spelt wheat (*T. aestivum* ssp. *spelta*) genotypes to infection with *Fusarium graminearum* No. 8 and 45 based on the disease index value.

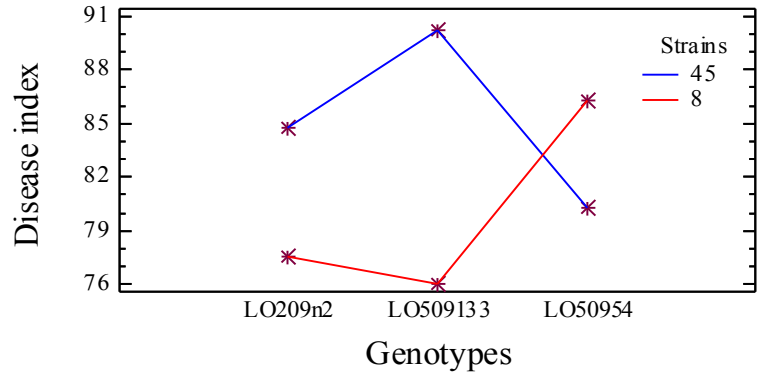

Fig. 4 Interaction of spelt wheat (*T. aestivum* ssp. *spelta*) genotypes and infection with *Fusarium graminearum* No. 8 and 45 based on the disease index value.

× spelt wheat genotype; Fig. 4). This phenomenon may significantly indicate a varied structure of *F. graminearum* in terms of pathogenicity within this species. However, at the specific level of the factor (*F. graminearum* strain) Tukey's test did not reveal a clear division into homogeneous groups for the three spelt wheat genotypes studied.

The mycological analysis of infected spelt wheat seedlings indicates that *F. graminearum* was the cause of pre- and post-emergence damping-off (Tab. 4).

In total, 288 isolates belonging to five species were isolated from infected seedlings of all the spelt wheat genotypes, cultivated in the growth chamber experiment with medium inoculated with *F. graminearum* No. 8 (Tab. 4). In this experimental treatment, the species that were most frequently isolated from infected organs of the seedlings included *F. graminearum*, whose isolates constituted 92.4% of fungal isolates (Tab. 4), and in small amounts *F. culmorum*, whose isolates accounted for 3.5% of fungi isolated from the examined organs of spelt wheat seedlings grown in medium artificially infected with *F. graminearum* No. 8. Moreover, *A. alternata* (1.0%), *E. nigrum* (1.0%), and *M. hiemalis* (2.1%) were isolated among all fungi found in the above-mentioned treatment (Tab. 4).

The mycological analysis of infected spelt wheat seedlings grown in the experimental treatment with medium artificially infected with *F. graminearum* No. 45 revealed 292 isolates of fungi belonging to six species (Tab. 4). *Fusarium graminearum*, whose isolates accounted for 96.2%, also proved to be the dominant fungal species isolated from seedlings derived from the above-mentioned experimental treatment and besides *F. culmorum* (0.7%) and *F. oxysporum* (0.7%) were also found (Tab. 4). Moreover, *A. alternata*, *Penicillium verrucosum* var. *cyclopium* and *R. nigricans* were isolated (Tab. 4). Seventy-four fungal isolates were obtained from seedlings grown in the growth chamber experiment in the control treatment. *Chaetomium globusom* (24 isolates) and *A. alternata* (23 isolates) proved to be the most frequently isolated species among all colonies obtained in this treatment (Tab. 4).

The *F. graminearum* strains were the main cause of damage to seedlings of the spelt wheat genotypes grown in the growth chamber experiment (Tab. 4).

Tab. 4 Fungi isolated from seedlings of *T. aestivum* ssp. *spelta* genotypes obtained from the experiment with soil inoculation.

Fungal species	Experimental combination			
	Fusarium graminearum No. 8	*Fusarium graminearum* No. 45	control	total number of isolates
Alternaria alternata (Fr.) Keissler	3	2	23	28
Chaetomium globosum Kunze	-	-	24	24
Epicoccum nigrum Link	3	-	8	11
Fusarium culmorum (W. G. Sm.) Sacc.	10	2	-	12
Fusarium graminearum Schwabe	266	281	5	552
Fusarium oxysporum Schl.	-	2	2	4
Mucor hiemalis Wehmer	6	-	8	14
Penicillium verrucosum Dierckx var. *cyclopium* (Westling.) Samson et al.	-	2	-	2
Rhizopus nigricans Ehrenberg	-	3	4	7
Total	288	292	74	654

Discussion

Our field experiment conducted in Poland revealed that spelt wheat plants, grown at two levels of protection, with root and stem base necrosis symptoms occurred at a higher percentage than in the case of spelt wheat grown in western Canada [24], but at a lower percentage than common wheat 'Tonacja' grown under the above-mentioned conditions with complex protection [15]. Species of the genus *Fusarium* proved to be the cause of damage to spelt wheat plants grown at the two levels of protection. The species composition and number of *Fusarium* spp. isolates differed between years. Presumably, weather conditions and crop protection level greatly affected the occurrence of these fungi.

Regardless of the protection level applied, *F. culmorum* was the species that was most frequently isolated from infected roots and stems of spelt wheat. This species has a high contribution to various root and stem rot diseases, including in spelt wheat [6,15,21,25–28]. The highest number of isolates of this species was isolated both from roots and stem bases of spelt wheat in the case of both protection levels in 2007, which could be attributed to the weather conditions favorable for the growth of this fungus, i.e., the high temperature and humidity from May to July [15].

According to Łacicowa and Pięta [29] as well as Kiecana et al. [19], the harmfulness of *F. culmorum* to cereals is greater at higher temperature. The study conducted by Wiwart et al. [6] shows that *F. culmorum* contributes to the damage of spelt wheat seedlings. A reduction in the weight of spelt wheat seedlings after inoculation with *F. culmorum* can reach as much as 90% [30].

Fusarium culmorum was detected in 36% of the 91% wheat fields analyzed in the years 2008–2009 in Montana and was the dominant *Fusarium* crown root pathogen in Glacier, Toole, and Blaine counties [28].

The harmfulness of *F. culmorum* to cereals results, among others, from its capacity to produce toxic metabolites, primary deoxynivalenol. This toxin reduces the content of chlorophyll *a* and *b* and carotenoids in leaf tissues [31].

Fusarium oxysporum was the fungus isolated in each year of the study from roots and stem bases of spelt wheat cultivated at both levels of chemical protection.

This fungus is known to affect flex [32] and leguminous plants [33,34], red clover [35], sweet pepper [36], but *F. oxysporum* has not been described as infecting cereals [28,37].

The presence of *Fusarium avenaceum* on *Triticum aestivum* ssp. *spelta* in growing seasons with different weather conditions is confirmed by the reports on great

tolerance of this fungus to temperature and humidity [38,39]. This pathogen is also one of main *Fusarium* spp. causing Fusarium head blight in different cereals in Poland [39–41]. *Fusarium avenaceum* was the dominant species on ears of wheat crops in Saskatchewan, Canada, in 2011 in the dark brown soil zone [42].

The occurrence of the species *F. avenaceum* and *F. culmorum* on the roots and stem bases of spelt wheat in the minimal chemical protection treatments and in all study years confirms the competitive abilities of these fungi, enabling them to live in the soil and infect plant roots [15,21,25]. Fernandez et al. [43] indicated a differential effect of the input level on the most common *Fusarium* spp. in a wheat crop. The species *F. avenaceum* and *F. culmorum* were most associated with the nonorganic input system – RED, which used conservation tillage together with integrated pest and nutrient management and higher seeding rate practices to enhance soil protection and reduce the use of pesticides and fuel.

The fungus *F. sporotrichioides* occurred on the roots and stem bases of *T. aestivum* ssp. *spelta* grown at both levels of chemical protection applied. The species is commonly noted on the roots and stem bases of cereals and ornamental grasses as well as on the roots and leaves of lawn grasses [15,21,44,45].

Fusarium sporotrichioides is a polyphagous species with group A trichothecene toxin-producing properties [46]. The harmfulness of *F. sporotrichioides* to cereals is associated with infection of panicles and ears and accumulation of toxic metabolites in grain [47–49].

The species *F. equiseti* known to be weakly pathogenic to cereal roots but pathogenic to turfgrasses and ornamental grasses [43–45] was isolated especially from the roots and stem bases of *T. aestivum* ssp. *spelta* in 2009 in the treatment with minimal protection. The fungus may develop root and stem base infection of wheat cultivated in the northeastern regions of Poland [50].

The species *B. sorokiniana*, isolated from the roots and stem bases of *T. aestivum* ssp. *spelta* in the years 2007–2009, is recognized as a wheat pathogen [15,24,43,51–53]. In western Canada, root and crown rot is mostly caused by *B. sorokiniana* together with *Fusarium* spp. [53]. Fernandez et al. [43] reported that the frequencies of *B. sorokiniana* were higher in subcrown internodes and crowns under organic than nonorganic management, especially under reduced tillage. Infection of host tissues by *B. sorokiniana* includes several phases: germination of conidiospores on the host surface and formation of an appressorium from the germ tube that supports direct penetration of the host surface by an infection hypha and colonization of host tissues [54]. Studying the early preinfectional interactions between *B. sorokiniana* and roots of barley, chemotropic growth of germ tubes towards roots and root exudates has been shown [54].

At the minimal chemical protection level, *R. solani* should be considered an additional infection factor causing root and stem base diseases of spelt wheat, which is in accordance with the study by Kiecana et al. [15] on *Triticum aestivum* and *Triticum durum*.

To investigate the harmfulness of *F. graminearum* isolates to seedlings of the selected spelt wheat genotypes, an inoculum was used in the form of 14-day cultures of the tested strains *F. graminearum* No. 8 and 45, grown on PDA medium, following the study of Mańka [55]. With this method used, which allowed direct contact of *F. graminearum* with pregerminated grains, *F. graminearum* proved to show high and different virulence to all spelt wheat genotypes analyzed. This is explained by the toxic properties of this fungus. The pathogenicity of *F. graminearum* is associated with production of phytotoxic metabolites, especially trichothecenes from group B such as deoxynivalenol (DON) and nivalenol (NIV). Phytotoxicity assays indicate that DON is at least sevenfold more active than NIV in inhibiting the growth of wheat seedlings and the elongation of wheat coleoptiles [56,57].

Tamburic-Ilincic et al. [58] reported that a single QTL (quantitative trait loci) on chromosome 5B that controlled FSB (Fusarium seedling blight) resistance of wheat lines to *F. graminearum* was identified in the mapping population. The marker WMC 75 explained 13.8% of the phenotypic variation for FSB and is most closely linked to the QTL peak. This implies that there may be other QTL with minor effects present in the population.

Conclusions

- The species *Fusarium culmorum* is the main threat to spelt wheat cultivated in the southeastern part of Poland
- Due to considerable harmfulness of *Fusarium graminearum* to seedlings of spelt wheat breeding lines, the pathogen should be taken into consideration while cultivating new cultivars of spelt wheat in Poland.

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Grzyby występujące na korzeniach i podstawie źdźbła *Triticum aestivum* ssp. *spelta* L. Thell. wzrastającej w dwóch poziomach ochrony chemicznej i szkodliwość *Fusarium graminearum* Schwabe dla siewek wybranych genotypów

Streszczenie

Badania przeprowadzono w latach 2007–2009 na polach Gospodarstwa Doświadczalnego Felin należącego do Uniwersytetu Przyrodniczego w Lublinie. Objęto nimi dwa rody hodowlane orkiszu ozimego (*Triticum aestivum* ssp. *spelta* L. Tell.): STH 3 i STH 715. W uprawie zastosowano dwa poziomy ochrony chemicznej: minimalną i kompleksową. W każdym sezonie wegetacyjnym oceniano porażenie korzeni oraz podstawy źdźbła orkiszu, w fazie dojrzałości woskowej twardej ziarna (87 w skali Zadoksa). Średnie wskaźniki chorobowe dla badanych genotypów pszenicy orkiszowej z minimalną ochroną po trzech latach badań wynosiły odpowiednio: 28.53 (STH 3) i 40.30 (STH 715). W kombinacji doświadczenia z ochroną kompleksową średnie wartości wskaźników chorobowych wynosiły od 25.96 (STH 3) do 26.90 (STH 715).

Analiza mikologiczna porażonych roślin wykazała, że przyczyną zgnilizny korzeni i nekrozy podstawy źdźbła orkiszu były *Fusarium* spp., a w szczególności *F. culmorum*. Za przyczynę w/w schorzeń można uznać również *F. avenaceum* i *Bipolaris sorokiniana*. Badania podatności siewek trzech genotypów orkiszu (LO 2/09/n/2, LO 5/09/13/3, LO 5/09/5/4) na porażenie przez *F. graminearum* Nr 8 i *F. graminearum* Nr 45 przeprowadzone w fitotronie wykazały, że analizowane genotypy nie różniły się podatnością. Wszystkie trzy genotypy były podatne i charakteryzowały się wysokimi wskaźnikami chorobowymi. Wykazano interakcję pomiędzy analizowanymi genotypami i szczepami *F. graminearum*, co wskazuje na zróżnicowaną patogeniczność wewnątrz tego gatunku.