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THE OCCURRENCE OF *Fusarium* spp. ON OAT (*Avena sativa* L.) AND SUSCEPTIBILITY OF SEEDLINGS OF SELECTED GENOTYPES TO INFECTION WITH *Fusarium graminearum* SCHWABE

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Abstract

The present study was carried out in the years 2010–2012 in the fields of the Strzelce Plant Breeding Company Ltd., belonging to the Plant Breeding and Acclimatization Institute in Radzików, and it included 39 oat genotypes. At the six-week seedling stage, the percentage of plants with root and leaf sheath necrosis symptoms was evaluated. In 2010 the percentage of seedlings with disease symptoms ranged from 6.5% to 25%, in 2011 it ranged from 17% to 34.5%, whereas in 2012 from 10% to 25%. In 2010 the disease index ranged from 1.4 to 5.7, in 2011 from 4.5 to 8.8, while in 2012 it was between 2.0 and 5.4.

Mycological analysis showed that large numbers of *Fusarium* spp. colonies were obtained both from the roots and leaf sheaths. Isolates of these fungi accounted for 63.48% of the total fungi isolated from seedlings. Seedlings grown under the conditions of central Poland were damaged by the species *F. culmorum*, *F. avenaceum,* and *F. solani*. The investigation of the susceptibility of 15 oat genotypes to infection with two *Fusarium graminearum* strains – Tz 56 and Tk 235 – was carried out under growth chamber conditions at a temperature of 22–23°C and relative air humidity of 85%. The *F. graminearum* strain Tz 56 proved to be the most pathogenic to seedlings of the breeding lines STH 0.9403 and POB 1316/08, for which the disease index was 80.5 and 75.5, respectively. The lowest pathogenicity of the a.m. strain was recorded in the case of the genotype DC 1832/05, for which the disease index was 26.5. The *F. graminearum* strain Tk 235 proved to be the most pathogenic to the genotypes STH 0.9403 and STH 0.9423, for which the disease index was 70.5 and 70.0, respectively, whereas this strain was least pathogenic to the breeding line DC 2112/05, in the case of which the disease index was 25.5.

Key words: *Avena sativa***,** genotypes, seedling damping-off, susceptibility, *Fusarium graminearum*

INTRODUCTION

Due to the introduction of reduced tillage systems, the problem of infection of seedlings as well as of the roots and stem base of cereals with fungi of the genus *Fusarium* is gaining special importance [1, 2, 3, 4, 5]. The oat is considered to be a species that produces good yields in crop rotations with a large proportion of cereals, but it is infected by fungi of the genus *Fusarium,* one of the components causing root and stem rot diseases [6, 1]. *F. culmorum* and *F. avenaceum* have been recognized as the main cause of these diseases in oat crops [7, 1, 2]. These fungi are distinguished by abundant conidial sporulation. Macroconidia of these fungi are formed in sporodochia developing in crop residue on the soil surface [8]. The above-mentioned *Fusarium* species are also characterized by high tolerance to temperature and humidity [1, 9]. Moreover, seedlings and the stem base of cereals are damaged by the species *F. graminearum*; seed material is thought to be the main source of primary infection of cereal seedlings with this fungus [10, 11, 12, 13]. The species *F. avenaceum* (teleomorph: *Gibberella avenacea*) and *F. graminearum* (teleomorph: *G. zeae*) form on cereal crop residue and on the lower nodes of the stem base perithecia containing ascospores, which are carried by the wind for long distances [12, 14, 15].

The aim of the present study was to determine the contribution of fungi of the genus *Fusarium* to the infection of oat seedlings grown under the conditions of central Poland as well as to determine the pathogenicity of *F. graminearum* to seedlings of several oat genotypes under controlled temperature and humidity conditions.

MATERIALS AND METHODS

The study on the occurrence of *Fusarium* spp. on oat was conducted in the years 2010–2012 in the fields of the Strzelce Plant Breeding Company Ltd., belonging to the Plant Breeding and Acclimatization Institute in Radzików. 15 oat genotypes were included in the study each year and a total of 39 genotypes were investigated (a list of cultivars and breeding lines is shown in Table 1). At the six-week seedling stage, 200 (4x50) seedlings of each genotype were sampled. The sampling method was the same as the one used in the study of oat conducted by Kiecana et al. [1].

The percentage of plants with root and leaf sheath necrosis symptoms was evaluated in a laboratory using a five-point scale and the disease index was calculated in the same way as in the study of oat conducted by Kiecana et al. [2]. The obtained results were statistically analyzed using T-Tukey's confidence half-intervals [16].

Subsequently, a mycological analysis of the diseased plants was performed. The number of pieces of seedlings collected for analysis and the analysis method were the same as in the study by Kiecana and Mielniczuk [7]. The cultures of *Fusarium* spp. were identified to the species level using monographs and keys by Kwaśna et al. [17] and Leslie and Summerell [18].

The infection experiment to evaluate the susceptibility of seedlings of 15 oat genotypes to infection by *Fusarium graminearum* No. Tz 56 obtained from kernels and with *F. graminearum* No. Tk 235 isolated from the roots was conducted in a growth chamber at a temperature of 23–24ºC and relative air humidity of 85%. The strains whose pathogenicity had been earlier tested in the laboratory by the method of Mishra and Behr [19] were used in the investigation. 14-day-old cultures of *F. graminearum* grown on PDA medium in Petri dishes at 22ºC were used as fungal inoculum.

Kernels of the analyzed oat genotypes whose sprouts had reached a length of 10 mm and were normally developed were selected for the investigation of susceptibility. The selected material was placed on a PDA layer with the studied fungal strains, which were in plastic pots with a diameter of 10 cm and a height of 15 cm filled with all-purpose growing medium with an addition of sand at a 2:1 ratio and a pH 6.5, previously sterilized twice in an autoclave for two hours at 121˚C and then covered with the medium [20]. Pots in which sprouted kernels were placed on layers of PDA medium without fungus were the control. The experiment was established on 4 May 2012 and was performed in four replicates with 25 plants per replicate. Plants grew for 24 days and then the degree of infection of seedlings was determined using a four-point scale and the disease index was calculated in the same way as in the study by Kiecana and Kocyłak [21]. The results were statistically analyzed in the same way as in the case of plants collected from plantations.

Ten seedlings with disease symptoms from each treatment of the growth chamber experiment were designated for mycological analysis, which was carried out in the same way as in the case of plants grown under field conditions. The cultures of fungi were identified to the species using monographs and keys [22, 23, 24, 25, 26, 27].

RESULTS

Spring observations revealed the occurrence of oat seedlings with root and leaf sheath necrosis. In 2010 the percentage of plants with disease symptoms varied from 6.5% (POB 648/06) to 25% (CHD 2316/03) (Fig. 1), in 2011 from 17% (STH 9110) to 34.5% (STH 9410) (Fig. 2), whereas in 2012 from 10% (POB 4129-4416/11) to 25% (DC 3674/02) (Fig. 3). In 2010 the significantly highest disease index was observed in the case of the breeding line CHD 2316/03 (5.7), whereas the significantly lowest value was found in the breeding line POB 648/06 (1.4) (Table 1). In 2011 the significantly highest disease index was recorded in the case of the genotype DC 1329/05 (8.8), while the significantly lowest index was found for the genotypes Nagus (4.5) and POB 1882/09 (4.7). In 2012 the significantly highest disease index was found in the case of the following genotypes: DC 3674/02 (5.7), DC 2112/05 (5.4), STH 0.8124 (5.3), and POB 2885/08 (5.3), whereas the significantly lowest value was found in the breeding line POB 4129-4416/11 (2.0) (Table 1).

The mycological analysis showed that large numbers of *Fusarium* species were obtained both from the roots and leaf sheaths. On average for the three-year study period, isolates of these fungi accounted for 63.48% of total isolates from oat seedlings. Among pathogenic species, the following were isolated from seedlings: *F. culmorum,* whose isolates accounted for 19.84% of total isolations, *F. solani* (6.70% of all fungi obtained from seedlings), *F. avenaceum* (7.04%), *F. sporotrichioides* (2.22%), *F. equiseti* (2.30%), *F. graminearum* (1.39%), and *F. crookwellense* (0.08%). Moreover, *F. oxysporum* (21.76% of all fungi) and *F. poae* (2.15 % of total isolates) were isolated from oat seedlings (Table 2).

The study in the growth chamber showed that plants with disease symptoms occurred in the experimental treatments with oat kernels infected with both *Fusarium graminearum* Tz 56 and *F. graminearum* Tk 235. Plant losses caused by pre- and post-emergence damping-off were recorded in the case of both

investigated *F. graminearum* strains. Infected plants were characterized by root and hypocotyl necrosis and quite often by hypocotyl contraction and root reduction. Furthermore, infected oat seedlings showed inhibited growth and yellowing leaves. Control seedlings of the tested genotypes did not exhibit distinct disease symptoms; they had a well-developed root system and root necrosis was observed sporadically.

Table 1
Mean values of the disease index for seedlings of the tested oat genotypes grown under field conditions in 2010–2012

No.	Oat genotypes	2010	2011	2012
1.	ARDEN	2.8 ab	8.4cde	
2.	NAGUS n	3.5 bc	4.5a	
3.	DC 1193/04	3.0 b	5.7abcd	
4.	DC 1329/05		8.8e	
5.	CHD 2316/03	5.7 d		
6.	CHD 3047/03	3.5 bc		
7.	DC 1776/04		7.9bcde	
8.	DC 1832/05			3,6ab
9.	DC 2112/05			5,4b
10.	DC 2359/03			4,4ab
11.	DC 239/06			2,8ab
12.	DC 3674/02 n			5,7b
13.	POB 648/06	1.4 a		
14.	POB 1316/08	3.5 bc		3,5ab
15.	POB 1813/08	2.9 ab		
16.	POB 2053/06	3.4 bc		
17.	POB 4703-99/08	1.7 ab		
18.	POB 342/09		7.9bcde	
19.	POB 1882/09		4.7a	
20.	POB 2085/09		5.5abc	
21.	POB 2149/09		8.2bcde	
22.	POB 158/09		6.5abcde	
23.	POB 2842/08			2,9ab
24.	POB 2885/08			5,3b
25.	POB 3034 /08			3,1ab
26.	POB 4129-4416/11			2,0a
27.	BINGO	3.7 bc	8.6de	
28.	MACZO n	3.2 bc	5.2ab	
29.	Haker	4.4 cd		
30.	STH 8608	3.8 bc		
31.	STH 8809	2.8 ab		
32.	STH 9110		5.3ab	
33.	STH 9210		7.1abcde	
34.	STH 9410 n		8.6de	
35.	STH 0.8124			5,3b
36.	STH 9322			3,2ab
37.	STH 0.9403 n			4,1ab
38.	STH 0.9423 n			2,9ab
39.	STH 0.9770			4,8ab
	Mean	3.29	8.86	3.93

Means in columns followed by the same letter are not significantly different at P≤0.05
n – naked oat

Table 2
Fungi isolated from oat seedlings grown under field conditions in 2010–2012

Fungal species	Number of isolates (%)											Total number of isolates
	2010			2011			2012			Total		
	r	ls	r+ls	r	ls	r+ls	r	ls	r+ls	r	ls	
Fusarium avenaceum (Fr.) Sacc.	-	2	2 (0.38)	39	86	125 (11.92)	7	53	60 (5.57)	46	141	187 (7.04)
Fusarium crookwellense Burgess, Nelson & Tousson	-	-	-	-	-	-	-	2	2 (0.19)	-	2	2 (0.08)
Fusarium culmorum (W. G. Sm.) Sacc.	4	9	13 (2.45)	128	129	257 (24.5)	76	181	257 (23.86)	208	319	527 (19.84)
Fusarium equiseti (Corda) Sacc.	4	21	25 (4.72)	7	24	31 (2.96)	-	5	5 (0.46)	11	50	61 (2.30)
Fusarium graminearum Schwabe	-	1	1 (0.19)	15	17	32 (3.05)	1	3	4 (0.37)	16	21	37 (1.39)
Fusarium oxysporum Schl.	46	129	175 (33.02)	102	73	175 (16.68)	95	133	228 (21.17)	243	335	578 (21.76)
Fusarium poae (Peck.) Wollenw.	1	-	1(0.19)	-	13	13 (1.24)	15	28	43 (3.99)	16	41	57 (2.15)
Fusarium solani (Mart.) Sacc.	2	2	4 (0.75)	76	29	105 (10.01)	42	27	69 (6.41)	120	58	178 (6.70)
Fusarium sporotrichioides Sherb.	9	12	21 (3.96)	8	-	8 (0.76)	11	19	30 (2.79)	28	31	59 (2.22)
Other colonies	174	114	288 (54.34)	117	186	303 (28.88)	188	191	379 (35.19)	479	491	970 (36.52)
Total	240	290	530	492	557	1049	435	642	1077	1167	1489	2656

r-roots
ls-leaf sheaths

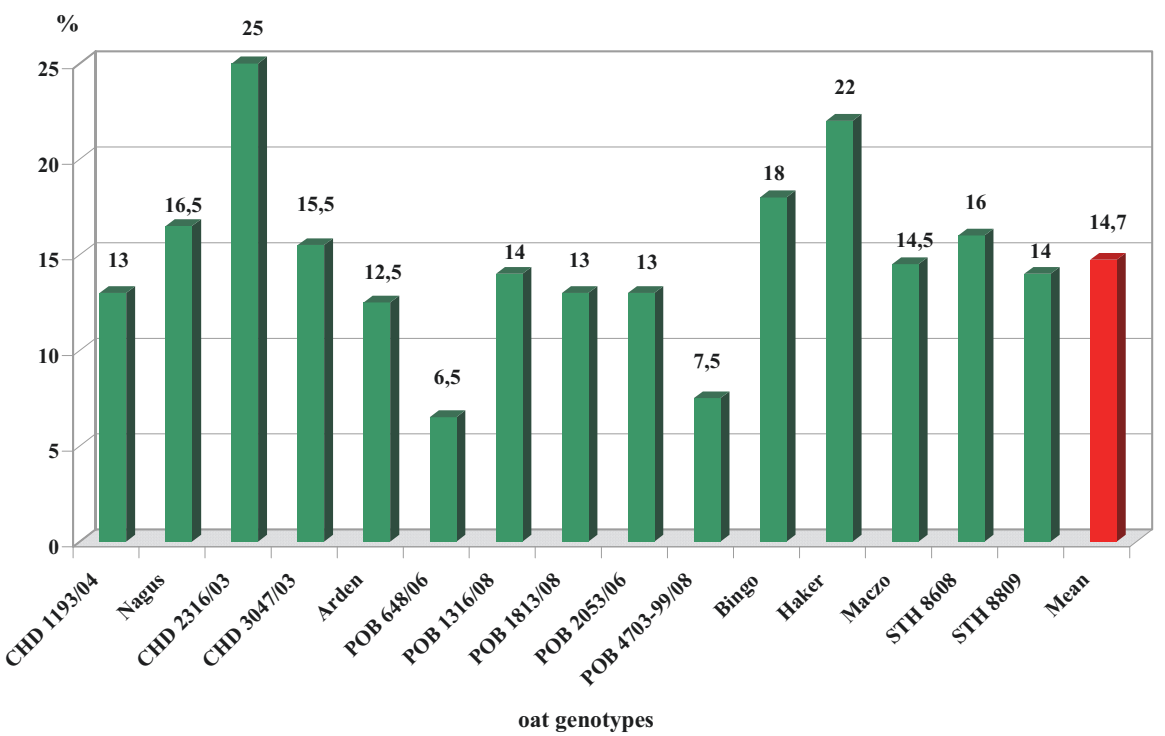

Fig. 1. Percentage of plants with root and leaf sheath necrosis symptoms in 2010.

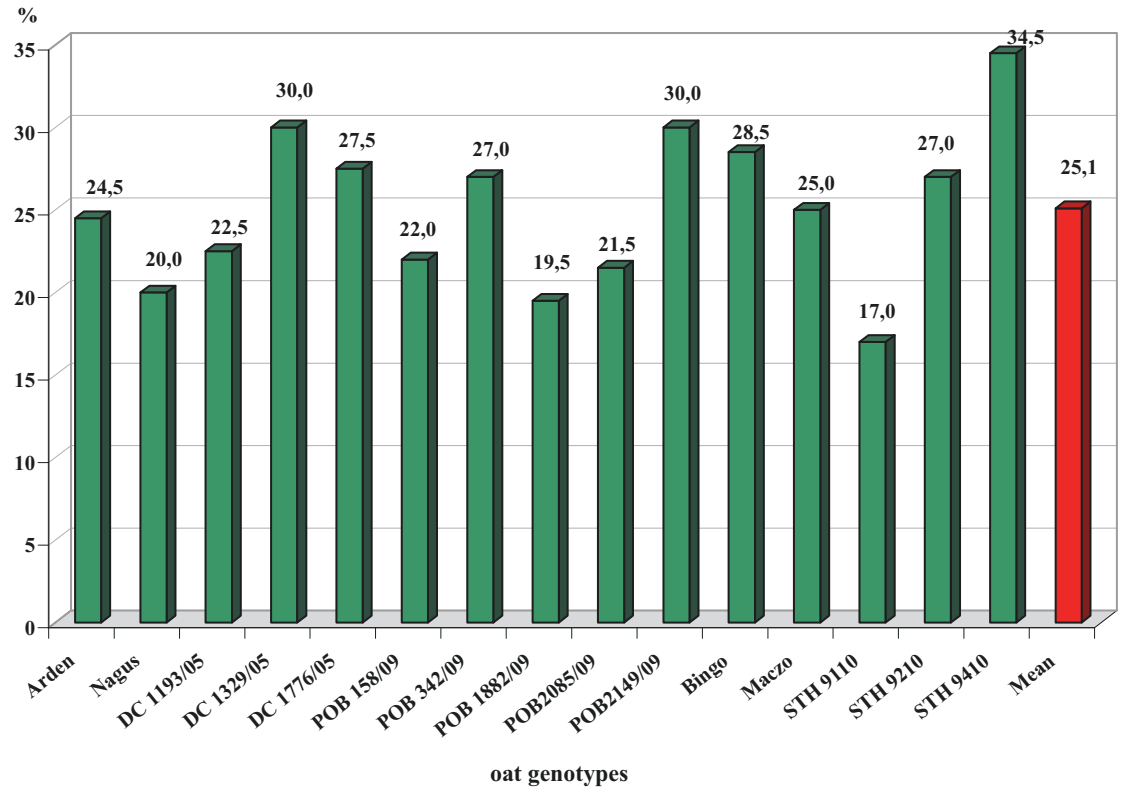

Fig. 2. Percentage of plants with root and leaf sheath necrosis symptoms in 2011.

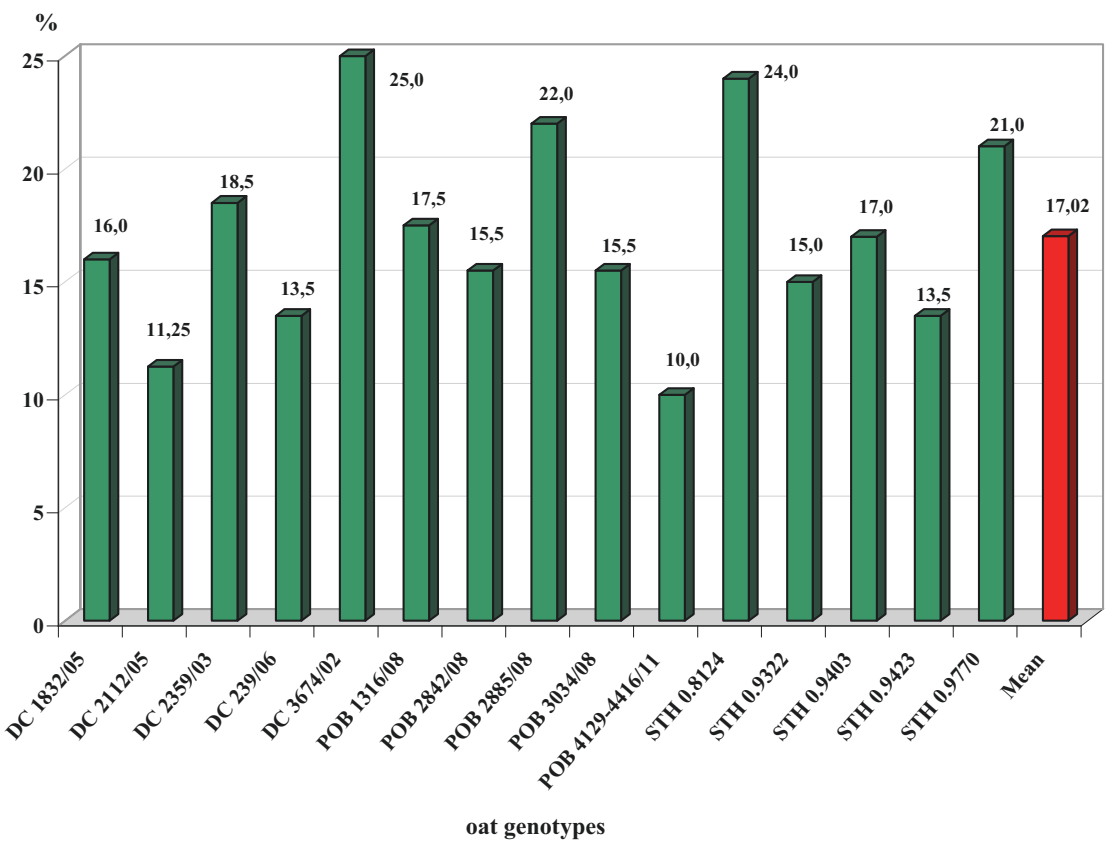

Fig. 3. Percentage of plants with root and leaf sheath necrosis symptoms in 2012.

On the basis of the statistical analysis of the disease indices, seedlings of the oat genotypes in question were found to show varying susceptibility to infection by the studied strains of *Fusarium graminearum*. The statistical analysis of the disease indices demonstrated that artificial inoculation of the medium with the *F. graminearum* strain Tz 56 had a significant effect on the health of investigated seedlings compared to the control treatment in the case of all studied oat genotypes (Table 3).

The *F. graminearum* strain Tz 56 proved to be most pathogenic to seedlings of the breeding lines STH 0.9403 and POB 1316/08, for which the disease index was 80.5 and 75.5, respectively. The lowest pathogenicity of the a.m. strain was found in the case of the genotypes DC 1832/05 and STH 0.9322, for which the disease index was 26.5 and 32.0, respectively (Table 3).

The present study showed that inoculation of the medium with the *F. graminearum* strain Tk 235 also had a significant effect on the infection of oat seedlings in all tested genotypes compared to the control (Table 3).

The *F. graminearum* strain Tk 235 proved to be most pathogenic to the genotypes STH 0.9403 and STH 0.9423, for which the disease index was 70.5 and 70.0, respectively, while it was least pathogenic to the breeding lines DC 2112/05, POB 4129-4416/11, DC 1832/05 and DC 239/06, in the case of which the disease index was, respectively, 25.5, 26.75, 28.25, and 29.25 (Table 3).

The disease index for seedlings in the control treatment ranged from 0.5 to 2.25 (Table 3).

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

Table 3
Mean values of the disease index for seedlings of the tested oat genotypes in the experimental treatment with *Fusarium graminearum* No. Tz 56, *F. graminearum,* No. Tk 235 and in the control treatment under growth chamber conditions.

Oat genotypes	Experimental treatment		
	Fusarium graminearum No. Tz 56	*Fusarium graminearum* No. Tk 235	Control
DC 1832/05	26.5*a	28.25*a	1.25
DC 2112/05	55.75*bcd	25.5*a	1.25
DC 2359/03	49.0*bc	51.0*cd	1.75
DC 239/06	54.75*bcd	29.25*a	0.5
DC 3674/02**n**	65.25*de	68.5*ef	1.25
POB 1316/08	75.5*ef	59.25*def	1.25
POB 2842/08	47.25*b	56.75*cde	1.0
POB 2885/08	57.0*bcd	50.75*cd	1.5
POB 3034 /08	49.0*bc	35.0*ab	1.0
POB 4129-4416/11	49.0*bc	26.75*a	1.75
STH 0.8124	54.5*bcd	48.0*c	2.25
STH 0.9322	32.0*a	46.0*bc	1.5
STH 0.9403**n**	80.5*f	70.5*f	0.5
STH 0.9423**n**	60.0*cd	70.0*f	1.0
STH 0.9770	51.25*bc	53.75*cd	0.75
Mean	54.32	47.95	1.23

*Means in lines are significantly different compared to the control at $0 \leq 0.05$
– Means in columns followed by the same letter are not significantly different at $0 \leq 0.05$
n – naked oat

Table 4
Fungi isolated from oat seedlings grown in the growth chamber experiment with inoculation of the medium with the *Fusarium graminearum* strain No. Tz 56 and the *F. graminearum* strain No. Tk 235 and in the control treatment.

Fungal species	Experimental treatment			Total number of isolates
	F. graminearum No. Tz 56	*F. graminearum* No. Tk 235	Control	
Acremonium roseum (Oud.) W. Gams	-	-	19	19
Alternaria alternata (Fr.) Keissler	6	8	43	57
Aspergillus niger van Tiegh.	3	-	-	3
Bipolaris sorokiniana (Sacc.) Shoemaker	3	-	-	3
Epicoccum nigrum Link	27	2	41	70
Fusarium culmorum (W.G. Sm.) Sacc.	-	2	15	17
Fusarium equiseti (Corda) Sacc.	-	3	6	9
Fusarium graminearum Schwabe	738	786	7	1531
Fusarium oxysporum Schl.	6	6	20	32
Fusarium poae (Peck.) Wollenw.	-	2	25	27
Fusarium sporotrichioides Sherb.	-	-	24	24
Mucor hiemalis Wehmer	42	3	10	55
Mucor mucedo Fres.	9	6	-	15
Penicillium puberulum Bainier	6	-	29	35
Penicillium verrucosum Dierckx var. *cyclopium* (Westling.) Samson et al.	18	4	35	57
Rhizopus nigricans Ehrenberg	6	-	20	26
Trichoderma aureoviride Rifai	-	-	11	11
Trichoderma harzianum Rifai	-	-	23	23
Trichoderma koningii Oud.	15	8	47	70
Trichoderma viride Persoon ex S.F. Gray	23	3	-	26
Total	902	833	375	2110

DISCUSSION

Field observations carried out in central Poland revealed that oat plants with root and leaf sheath necrosis symptoms occurred at a higher percentage than in the case of oat grown in the eastern area of the Lublin region [1].

The species *Fusarium culmorum*, *F. avenaceum* and *F. solani* proved to be the cause of damage of oat seedlings in the spring. The pathogenicity of *F. avenaceum* and *F.culmorum* to oat seedlings has also been confirmed by the research of Kiecana and Kocyłak [21] as well as of Mańka [20]. *Fusarium culmorum* has been found to show high pathogenicity to seedlings of various cereal species [20, 28, 29].

Secondary metabolites, including deoxynivalenol (DON), participate in the disease process caused by *Fusarium culmorum*. This metabolite exhibits phytotoxic activity in particular against the coleoptile, shoots and callus tissue of wheat [30]. Moreover, DON reduces the germination capacity of seeds and causes a reduction in the number and length of radicles of wheat seedlings. Reduced germination capacity and growth inhibition in seedlings occur already at a DON concentration of 4 μg/ml, whereas the use of a concentration of 25 μg/ml completely inhibits root growth and mitotic activity in the cells of the apical meristems of DON-treated roots. This mycotoxin leads to disturbances in the mitotic division of the meristematic cells of wheat roots [31]. In wheat roots subjected to the effect of DON, these authors observed the occurrence of chromatin bridges in the chromosomes and they also found the occurrence of lagging chromosomes in the anaphase. According to Packa [32], DON leads to excessive condensation of chromosomes in the metaphase and anaphase and disturbs the synthesis of proteins making up the microtubules of the karyokinetic spindle. Other secondary metabolites can also participate in the pathogenesis of diseases caused by *F. culmorum*, including culmorin, which can inhibit wheat coleoptile elongation at a concentration from 100 μM to 1 mM [33].

Fusarium avenaceum is considered to be a serious pathogen of cereals, including oat [1, 34, 35]. Moniliformin participates in the pathogenesis of diseases caused by *F. avenaceum*; this mycotoxin affects the permeability of the cytoplasmic membranes and causes disturbances in mitotic cell division [32].

Fusarium solani has contributed to root infection in single cropped oat and in oat mixtures with spring barley and spring triticale as well as in triticale grown with oat. This fungus was the main cause of root damage in winter wheat grown after previous crops such as single cropped oat and oat grown in mixtures [35]. This species is considered to be an important producer of fusaric acid (5-(butyl)-2-pyridinecarboxylic acid) [36], a phytotoxin that destroys the vascular bundles in plants. The strong phytotoxicity of this metabolite to rice seedlings has been proved [33].

Fusarium equiseti belonged to the species isolated from infected oat roots and leaf sheaths. In the study by Majchrzak et al. [37], this fungus proved to be the main causal agent of 190 infections of wheat roots and had a significant contribution to stem base damage in this plant grown in the conditions of the north-eastern regions of Poland. *Fusarium oxysporum*, which is not considered to be a pathogen of cereal plants [38], was a species that was isolated in each year of the study.

The results obtained in the growth chamber experiment confirmed the high phytotoxicity of *Fusarium graminearum* to oat seedlings. Under the studied conditions in which 14-day cultures of the tested *F. graminearum* strains grown on dextrose-potato medium were used as an inoculum, this method proved effective, since there were seedlings with pre- and post-emergence damping-off in the studied oat genotypes in the experimental treatments with both *F. graminearum* Tz 56 obtained from grass seed and with *F. graminearum* Tk 235 obtained from grass roots. The present growth chamber study and previous information in the literature indicate the contribution of *F. graminearum* to the damage of cereal seedlings [10, 11].

CONCLUSIONS

1. The species *F. culmorum* and *F. avenaceum* are a threat to oat seedlings grown in central Poland.
2. Due to the high pathogenicity of these species, these pathogens should be taken into account in the breeding of new oat cultivars.
3. The breeding line DC 1832/05 seems to be useful in breeding oat for resistance to *F. graminearum*.

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Author's contributions

Concept of the study: IK; writing of the manuscript: IK, EM, MC, AP; statistical analysis: MC, EM; mycological analysis: IK, AP, MC, EM; analysis of research results: IK, MC, EM, field research: IK, EM, MC, AP; experiment in the growth chamber: AP, MC, EM.

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Występowanie *Fusarium* spp. na owsie (*Avena sativa* L.) i podatność siewek wybranych genotypów na porażenie przez *Fusarium graminearum* Schwabe

Streszczenie

Badania przeprowadzono w latach 2010–2012 na polach Hodowli Roślin Strzelce Sp. z o. o., Grupa IHAR, objęto nimi 39 genotypów owsa. W fazie 6-tygodniowych siewek oceniano udział roślin z objawami nekrozy korzeni oraz pochew liściowych. Udział siewek z objawami chorobowymi wynosił w 2010 roku od 6,5% do 25%, w 2011 roku od 17% do 34,5%, zaś w 2012 roku od 10,0% do 25,0%. Wartości wskaźnika chorobowego wahały się w 2010 roku od 1,4 do 5,7, w 2011 roku od 4,5 do 8,8, zaś w 2012 roku od 2.0 do 5,4. Analiza mykologiczna wykazała, że zarówno z korzeni jak i z pochew liściowych licznie uzyskiwano kolonie *Fusarium* spp. Izolaty tych grzybów stanowiły 63,48% ogółu grzybów wyosobnionych z siewek. Przyczyną uszkodzenia siewek owsa uprawianego w warunkach centralnej Polski były gatunki: *F. culmorum*, *F. avenaceum* i *F. solani*. Badania podatności siewek 15 genotypów owsa na porażenie przez dwa szczepy *Fusarium graminearum* - Tz 56 i Tk 235 przeprowadzono w warunkach fitotronowych, w temperaturze 22–23°C i wilgotności względnej powietrza 85%. Szczep *F. graminearum* Tz 56 okazał się najbardziej patogeniczny w stosunku do siewek rodów hodowlanych STH 0.9403 oraz POB 1316/08, dla których wartości wskaźnika chorobowego wynosiły odpowiednio 80,5 oraz 75,5. Najmniejszą szkodliwość w/w szczepu zanotowano w przypadku genotypu DC 1832/05, dla którego wartość wskaźnika chorobowego wynosiła 26,5. Szczep *F. graminearum* Tk 235 okazał się najbardziej patogeniczny dla genotypów STH 0.9403 i STH 0.9423, dla których wartości wskaźnika chorobowego wynosiły odpowiednio: 70,5 i 70,0, zaś w/w szczep był najmniej patogeniczny dla rodu hodowlanego DC 2112/05, w przypadku którego wartość wskaźnika chorobowego wynosiła 25,5.

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