

FUNGI INFECTING ORNAMENTAL GRASSES AND THE PATHOGENICITY OF *Fusarium culmorum* (W.G.Sm.) Sacc. AND *Fusarium equiseti* (Corda) Sacc. TO SELECTED SPECIES

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Abstract. One of the reasons lowering the esthetic appearance of a lawn are diseases caused by fungi. The study on seed material of 11 ornamental grass species was carried out in 2009 and 2010. 681 fungi isolates belonging to 22 species and non-sporulating forms were obtained over the two year study period. Among fungi pathogenic to grasses, species of the genus *Fusarium* were obtained especially in 2010. In that year, isolates of these fungi constituted 29.94% of all isolated colonies. Colonies of *Alternaria alternata* were also frequently isolated from the analyzed seed material of the ornamental grasses; in 2009 and 2010 its isolates accounted for respectively 25.36 and 12.57% of all isolates. The study on the health of ornamental grasses conducted in field conditions revealed the occurrence of plants with necrosis of the roots and lower stem internodes. The percentage of such plants ranged from 12% for *Coix lacryma-jobi* to 69% in the case of *Festuca ovina*. The mean values of the diseases indices were from 2.6 in the case of *Coix lacryma-jobi* to 39.6 for *Lagurus ovatus* and they differed significantly. From the infected plants mainly species of genus *Fusarium* were obtained. Majority of isolates consisted *F. oxysporum* and also *F. culmorum*, *F. crookwellense*, *F. graminearum*, *F. solani*, *F. avenaceum*, *F. sporotrichioides* and species *Bipolaris sorokiniana* and *Exserohilum pedicellatum* were obtained. Studies on susceptibility of the seedlings of seven ornamental grass species to infection by *Fusarium culmorum* No. 34 and *F. equiseti* No. 62 were conducted in a growth chamber. The statistical analysis of disease indexes for plants that grew in the experimental combination with artificial infection of the subsoil with above-mentioned strains as compared to the control indicated significant differences in all studied species in the case of *F. culmorum* and in 5 species in the experimental combination with *F. equiseti*. Strain *Fusarium culmorum* No. 34 proved to be the most pathogenic towards *Festuca glauca* (98.25), and *F. equiseti* No. 62 towards *Phalaris canariensis* (89.5).

Key words: healthiness of seeds, pathogenicity, *Fusarium culmorum*, *Fusarium equiseti*, seedlings, necrosis of roots and lower stem internodes

INTRODUCTION

Ornamental grasses belong to the most resistant ornamental plants. They can be grown alone or complement tiered borders [Majtkowska and Majtkowski 2007]. Pathogens damaging the roots and stem base, which are transmitted through seed material or which inhabit the soil environment, decrease the decorative values of grasses.

Infected seeds are a very serious source of primary infection for plants derived from seed. Rich nutrients contained in seeds are a good nutritional substrate for the growth of various microorganisms, including grass pathogens. The existing research shows that grass seeds are predominantly colonized by fungi [Kutrzeba 1994, Wiewióra and Prończuk 2000, Prończuk 2000, Kiecana et al. 2012]. The most numerous group of microorganisms colonizing grass seeds comprises saprophytic fungi, representatives of the genera *Alternaria*, *Cladosporium*, *Rhizopus*, *Mucor*, *Aspergillus* and *Penicillium*. Saprophytic fungi of the genera *Alternaria* and *Cladosporium* are termed “field” fungi, since their colonization of seeds is determined by their occurrence on plants growing during the growing season. Necrotrophs, such as species of the genera *Fusarium*, *Bipolaris sorokiniana* and *Exserohilum pedicellatum*, deserve special attention among microorganisms colonizing seed material; in the case of these fungi, the course of the disease is rapid, because toxins and enzymes play a part in the pathogenesis [Wojciechowski et al. 1995, Packa 1997, Nakajima et al. 1998]. Necrotrophs are often polyphagous. These fungi can cause damage to the roots and stem base of plants of the family *Poaceae*, including ornamental grasses [Reed et al. 1983, Sivanesan 1987, Prończuk 2000, Gilbert 2003, Kiecana et al. 2012].

Due to the increasingly frequent use of ornamental grasses as an element of plant arrangements in designing and establishing gardens and parks as well as due to the sparse information on pathogens of ornamental grasses found in the phytopathological literature, a study was undertaken on the health of seed material, plant infection under field conditions, and the pathogenicity of *F. equiseti* and *F. culmorum* to seedlings.

MATERIAL AND METHODS

The study on seed material of 11 ornamental grass species was carried out in 2009 and 2010 (tabs 1, 2). This seed material was marketed in the domestic market by the seed production companies – Breeding and Seed Company W. Legutko and Seed and Reproduction of Ornamental Plants “Rekwiat” Piaski. For each species, 100 seeds randomly selected from a 500 seed sample were analyzed each year. The Petri dish method was employed to isolate fungi colonizing the grass seed material, using a mineral medium with the composition given by Mielniczuk et al. [2010] as culture medium.

The study on the health of ornamental grasses was conducted in experimental fields near the city of Zamość, on leached soil derived from loess deposits, where root crops had been the previous crop. Eight grass species were used for the investigations (tab. 3). Seeds of the grass species analyzed were sown in 2010 according to the seed producers’ recommendations. Each species was grown in four 1 m² plots. The plots were hand weeded. Observations of plant health were made in the first half of September (on the 8th)

Table 1. Fungi isolated from grains of selected ornamental grass species on mineral medium in 2009

Fungi species	Number of isolates										Total	%
	ornamental grass species											
	<i>L. o.</i>	<i>B. m.</i>	<i>F. g.</i>	<i>S. t.</i>	<i>P. m. V.</i>	<i>C. s.</i>	<i>S. m.</i>	Total	Total	%		
<i>Alternaria alternata</i> (Fr.) Keissler	(24)40	(0)3	(2)2	(0)0	(12)2	(0)1	(2)0	(56)32	88	25.36		
<i>Bipolaris sorokiniana</i> (Sacc.) Shoem.	(1)2	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(2)1	3	0.86		
<i>Botrytis cinerea</i> Pers.	(7)3	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(7)3	10	2.88		
<i>Chaetomium indicum</i> Corda	(0)0	(0)0	(6)8	(0)0	(0)0	(0)0	(0)0	(6)8	14	4.03		
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	(0)0	(0)2	(0)2	(0)3	(1)5	(0)0	(0)0	(1)12	13	3.75		
<i>Epicoccum nigrum</i> Link ex Link	(2)2	(0)0	(0)2	(0)1	(2)1	(0)0	(0)0	(4)6	10	2.88		
<i>Fusarium equiseti</i> (Corda) Sacc.	(1)1	(0)0	(0)0	(0)0	(1)0	(0)0	(3)0	(5)1	6	1.73		
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclospium</i> (Westling.) Samson et al.	(0)12	(12)32	(3)3	(0)4	(50)19	(0)41	(7)0	(84)99	183	52.74		
<i>Talaromyces flavus</i> (Klöcker) Stolk et Samson	(2)2	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(2)2	4	1.15		
<i>Trichoderma aureoviride</i> Rifai	(0)0	(0)1	(0)0	(0)0	(0)0	(0)0	(0)0	(0)1	1	0.29		
<i>Trichothecium roseum</i> Link.	(0)0	(0)0	(0)0	(0)0	(7)1	(0)0	(0)0	(7)1	8	2.31		
<i>Stemphylium botryosum</i> Walther	(0)0	(0)5	(0)0	(0)0	(0)0	(0)0	(0)0	(5)0	5	1.44		
<i>Mycelia sterilia</i>	(0)0	(0)0	(0)0	(0)0	(0)0	(0)2	(0)0	(0)2	2	0.58		
Total	(37)62	(17)38	(11)17	(0)8	(73)28	(0)44	(12)0	(179)168	347	100		

L. o. – *Lagurus ovatus* L., *B. m.* – *Briza maxima* L., *F. g.* – *Festuca glauca* Vill., *S. t.* – *Stipa tenuissima* Trin., *P. m. V.* – *Panicum miliaceum* *Violaceum* L., *C. s.* – *Cortaderia selloana* Aschers et Graebn., *S. m.* – *Setaria macrostachya* Kurth

Values in brackets determine number of isolates obtained from germinating seeds, before brackets from non germinating seeds

Table 2. Fungi isolated from grains of selected ornamental grass species on mineral medium in 2010

Fungi species	Number of isolates										Isolates	
	ornamental grass species										Total	%
	L. o.	B. m.	F. o.	C. l.	P. c.	P. m. V.	S. s.	S. m.	Total			
<i>Alternaria alternata</i> (Fr.) Keissler	(0)0	(0)0	(0)0	(14)2	(19)2	(2)0	(0)3	(0)0	(35)7	42	12.57	
<i>Chaetomium indicum</i> Corda	(0)0	(5)5	(0)0	(0)0	(1)0	(0)0	(0)0	(0)0	(6)5	11	3.29	
<i>Epicoccum nigrum</i> Link ex Link	(0)0	(0)0	(0)0	(0)0	(0)0	(3)0	(0)0	(0)0	(3)0	3	0.90	
<i>Fusarium equiseti</i> (Corda) Sacc.	(0)0	(14)2	(0)0	(0)0	(0)0	(2)0	(0)0	(0)0	(16)2	18	5.39	
<i>Fusarium graminearum</i> Schwabe	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)67	(0)0	(0)67	67	20.06	
<i>Fusarium oxysporum</i> Schlecht.	(0)0	(0)0	(0)0	(0)0	(0)1	(2)0	(0)0	(0)0	(2)1	3	0.90	
<i>Fusarium semitectum</i> Berk. & Ravenel	(0)0	(0)0	(0)0	(5)0	(0)0	(0)0	(0)0	(0)0	(5)0	5	1.50	
<i>Fusarium sporotrichioides</i> Scherb.	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)7	(0)0	(0)7	7	2.09	
<i>Mucor hiemalis</i> Wehmer	(0)0	(0)0	(0)0	(0)0	(0)0	(0)1	(0)0	(0)0	(0)1	1	0.30	
<i>Penicillium funiculosum</i> Thom.	(0)0	(0)0	(1)0	(0)0	(0)0	(0)0	(0)0	(3)1	(4)1	5	1.50	
<i>Penicillium nigricans</i> (Bainier) Thom	(0)0	(0)0	(0)0	(0)0	(0)3	(16)0	(0)0	(0)0	(16)3	19	5.70	
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling.) Samson et al.	(0)1	(0)0	(0)0	(1)1	(0)0	(18)13	(0)0	(1)0	(20)14	34	10.18	
<i>Rhizopus nigricans</i> Ehrenberg	(0)0	(0)0	(0)0	(0)0	(1)0	(0)0	(0)0	(0)0	(1)0	1	0.30	
<i>Trichoderma koningii</i> Oudem.	(2)8	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(2)8	10	3.0	
<i>Trichoderma polysporum</i> Rifai	(18)16	(9)10	(4)7	(6)4	(0)0	(0)0	(0)0	(0)0	(37)37	74	22.15	
<i>Mycelia sterilia</i>	(0)3	(6)4	(0)1	(0)0	(0)0	(2)0	(0)5	(10)3	(18)16	34	10.17	
Total	(20)28	(34)21	(5)	(26)7	(21)6	(45)14	(0)82	(14)4	(165)169	334	100	

L. o. – *Lagurus ovatus* L., B. m. – *Briza media* L., F. o. – *Festuca ovina* L., C. l. – *Coix lacryma-jobi* L., P. c. – *Phalaris canariensis* L., P. m. V. – *Panicum miliaceum* L., S. s. – *Sorghum sudanense* Stapf, S. m. – *Setaria macrostachya* Kutrh
 Values in brackets determine number of isolates obtained from germinating seeds, before brackets from non germinating seeds

in 2010. 100 stems were analyzed for each species (25 from each plot). The percentage of plants with necrosis symptoms on the roots and lower internodes was estimated in a laboratory and subsequently the level of stem base infection was determined using a 5-point scale [Eng-Chong Pua et al. 1985]. A mycological analysis of infected plants was also performed in the laboratory. The Petri dish method was applied to isolate the fungi colonizing infected plants, using a mineral medium as culture medium [Mielniczuk et al. 2010]. 100 root fragments and 100 stem base fragments were analyzed for each species.

Table 3. Percentage participation of culms with necrosis of roots and lower internodes and mean values of disease index for ornamental grass species growth on experimental plots in Zamość region in 2010

Ornamental grass species	Mean values of disease index	% of infected culms
<i>Lagurus ovatus</i> L.	39.6 b	51
<i>Briza media</i> L.	39.2 b	51
<i>Coix lacryma-jobi</i> L.	2.6 a	12
<i>Festuca ovina</i> L.	28.8 ab	69
<i>Phalaris canariensis</i> L.,	9.2 a	55
<i>Panicum miliaceum</i> Violaceum L.	32.4 ab	52
<i>Sorghum sudanense</i> Stapf	20.2 ab	43
<i>Setaria macrostachya</i> Kutrh	24.8 ab	23

Mean values in columns followed by the same letter do not differ significantly at $p \leq 0.05$ differ significantly

The investigation of the susceptibility of seedlings of seven ornamental grasses (tab. 5) to infection by *Fusarium culmorum* No. 34 and *Fusarium equiseti* No. 62 – both obtained from grass roots – was conducted in a growth chamber at a temperature of 22–23°C and a relative air humidity of 85%. The study used strains whose pathogenicity had been earlier tested in the laboratory by the method of Mishra and Behr [1976]. The method of inoculum preparation for the analyzed strains and of plant material preparation as well as the experimental design and the type of culture medium used for the experiment were the same as in the study of Kiecana et al. [2012].

The experiment was set up on 25 May 2010 and each experimental treatment had four replicates, with 25 plants in each. The plants grew for 25 days after which the number of healthy and infected plants and the number of plants that died before emergence were determined. The level of infection of plants with disease symptoms was determined according to a 4-point scale [Kiecana et al. 2012]. The disease indices for plants from the field experiment and for seedlings obtained in the growth chamber experiment were calculated using McKinney's formula [Łacicowa 1969]. The obtained results were statistically analyzed using Tukey's confidence half-intervals [Żuk 1989].

Ten seedlings with disease symptoms from each treatment in the growth chamber experiment were taken for mycological analysis. Fifty 3 mm-long fragments of the roots and leaf sheaths of diseased seedlings from each species were analyzed. The monographs and keys used to identify fungal species isolated from diseased rye plants were used to identify the fungi isolated from the grass seed material as well as from infected plants growing under field conditions and obtained from the growth chamber experiment [Kiecana et al. 2009].

RESULTS

As a result of the mycological analysis of seeds of 11 ornamental grass species, 681 fungal isolates belonging to 22 species and non-sporulating forms were obtained over the two-year study period. Out of this number, 344 colonies were derived from germinating seeds and 337 from non-germinating seeds (tabs 1, 2). Colonies of *Alternaria alternata* were frequently isolated from the analyzed seed material of the ornamental grasses; in 2009 and 2010 its isolates accounted for respectively 25.36 and 12.57% of all isolates (tabs 1, 2). Among fungi pathogenic to grasses, species of the genus *Fusarium* were obtained especially in 2010. In that year, isolates of these fungi constituted 29.94% of all isolated colonies. In 2010 the species *F. graminearum* was predominant and its isolates accounted for 20.06% of total isolates. On the other hand, *F. equiseti* was obtained in both years of the study and its isolates made up respectively 1.73 and 5.39% of all fungal isolates (tabs 1, 2). Moreover, in 2010 the following species were isolated from seeds of the ornamental grass: *F. sporotrichioides*, *F. semitectum* and *F. oxysporum*, and their isolates accounted in total for 4.49% of all isolates in that year. In 2009 pathogens of *Bipolaris sorokiniana* and *Botrytis cinerea* were derived and their isolates constituted respectively 0.86 and 2.88% of all isolates (tab. 1). In both years of the study, the grass seed material was colonized in large numbers by the species *Penicillium verrucosum* var. *cyclopium*, whose isolates made up respectively 52.74 and 10.18% of all isolates (tabs 1, 2). The other species, *P. funiculosum* and *P. notatum*, were obtained in 2010 and their isolates constituted respectively 1.50 and 5.70% of all isolates. The genus *Trichoderma* was represented by the species *T. aureoviride*, *T. koningii* and *T. polysporum* (tabs 1, 2). The remaining fungal species colonizing the seed material of the ornamental grasses during the study period in question were represented by *Chaetomium indicum*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Mucor hiemalis*, *Rhizopus nigricans*, *Trichothecium roseum* and *Stemphylium botryosum* (tabs 1, 2).

In 2009 the seed material of panic grass and hare's-tail grass was most colonized by fungi. *Penicillium verrucosum* var. *cyclopium* and *A. alternata* were predominantly isolated from their seeds. Moreover, hare's-tail grass seeds were colonized by the following pathogenic species: *B. sorokiniana*, *F. equiseti* and *B. cinerea*. On the other hand, in 2010 the species sudangrass was characterized by frequent seed infection with *F. graminearum* (tabs 1, 2).

The field observations revealed the occurrence of plants with necrosis of the roots and lower stem internodes. The percentage of such plants ranged from 12% for *Coix*

Table 4. Fungi isolated from plants with necrosis of roots and lower internodes in 2010

Fungi species	Number of isolates										Isolates	
	ornamental grass species										Total	%
	<i>L. o.</i>	<i>B. m.</i>	<i>F. o.</i>	<i>C. l.</i>	<i>P. c.</i>	<i>P. m.</i>	<i>S. s.</i>	<i>S. m.</i>	Total	Total	%	
<i>Bipolaris sorokiniana</i> (Sacc.) Shoem.	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)1	(0)1	1	0.12	
<i>Epicoccum nigrum</i> Link ex Link	(0)0	(16)0	(0)0	(13)0	(0)0	(8)0	(8)0	(0)0	(45)0	45	5.36	
<i>Exserohilum pedicellatum</i> (Henry) Leonard & Suggs	(0)0	(0)0	(0)0	(0)0	(0)0	(1)0	(0)0	(0)1	(1)1	2	0.24	
<i>Fusarium avenaceum</i> (Fr.) Sacc	(0)0	(0)0	(0)0	(9)0	(0)0	(0)0	(0)4	(0)0	(9)4	13	1.55	
<i>Fusarium crookwellense</i> Burgess, Nelson & Toussoun	(0)45	(9)0	(0)0	(0)3	(0)0	(0)4	(16)4	(0)1	(25)57	82	9.77	
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	(0)0	(9)25	(14)48	(2)11	(26)50	(10)3	(4)10	(0)16	(65)163	228	27.18	
<i>Fusarium equiseti</i> (Corda) Sacc.	(0)0	(0)0	(6)0	(0)0	(0)0	(3)14	(15)14	(8)24	(32)52	84	10.01	
<i>Fusarium graminearum</i> Schwabe	(16)0	(0)0	(0)0	(0)4	(0)0	(0)2	(1)0	(0)1	(17)7	24	2.86	
<i>Fusarium oxysporum</i> Schlecht.	(34)5	(14)25	(12)2	(24)14	(24)0	(22)24	(0)14	(28)3	(158)87	245	29.2	
<i>Fusarium solani</i> (Mart.) Sacc.	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(33)1	(0)0	(33)1	34	4.05	
<i>Fusarium sporotrichioides</i> Sherb.	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)16	(0)0	(0)16	16	1.91	
<i>Fusarium verticillioides</i> (Sacc.) Nirenberg	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)4	(0)4	4	0.48	
<i>Hemicola grisea</i> Traaen	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)4	(0)0	(0)4	4	0.48	
<i>Penicillium fellutanum</i> Biourge	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)1	(0)0	(0)1	1	0.12	
<i>Penicillium islandicum</i> Sopp	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(11)0	(11)0	11	1.31	
<i>Trichoderma aureoviride</i> Rifai	(0)0	(0)0	(2)0	(0)5	(0)0	(0)0	(0)0	(0)0	(2)5	7	0.83	
<i>Trichoderma harzianum</i> Rifai	(0)0	(0)0	(0)0	(0)0	(0)0	(0)0	(6)0	(0)0	(6)0	6	0.71	
<i>Trichoderma koningii</i> Oudem.	(0)0	(0)0	(0)0	(5)0	(0)0	(0)0	(0)0	(0)0	(5)0	5	0.60	
<i>Mycelia sterilia</i>	(0)0	(0)0	(5)0	(2)0	(0)0	(6)1	(3)10	(0)0	(16)11	27	3.22	
Total	(50)50	(48)50	(39)50	(55)37	(50)50	(50)48	(86)78	(47)51	(425)414	839	100	

L. o. – *Lagurus ovatus* L., *B. m.* – *Briza media* L., *F. o.* – *Festuca ovina* L., *C. l.* – *Coix lacryma-jobi* L., *P. c.* – *Phalaris canariensis* L., *P. m.* *V.* – *Panicum miliaceum* L., *S. s.* – *Sorghum sudanense* Stapf, *S. m.* – *Setaria macrostachya* Kutth
Values in brackets determine number of isolates obtained from roots, before brackets from stem base

Table 5. Mean values of disease index for seedlings of selected ornamental grass species infected by *F. culmorum* No. 34 i *F. equiseti* No. 62 in a growth chamber conditions

Ornamental grass species	Experimental combination		
	<i>F. culmorum</i> No. 34	<i>F. equiseti</i> No. 62	control
<i>Lagurus ovatus</i> L.	44.00*a	75.00*c	5.75a
<i>Festuca glauca</i> Vill.	98.25*d	77.00*c	15.75b
<i>Phalaris canariensis</i> L.	95.00*c	89.50*d	3.50a
<i>Panicum miliaceum</i> <i>Violaceum</i> L.	96.00*cd	36.25*a	28.00d
<i>Sorghum nigrum</i> L.	90.00*b	42.25*a	22.00c
<i>Cortaderia selloana</i> Aschers et Graebn	89.5*b	89.00*d	5.00a
<i>Setaria macrostachya</i> Kutrh	96.25*cd	64.00*b	22.00c

* Mean values differ significantly as compared to the control at $p \leq 0.05$

Mean values in columns followed by the same letter do not differ significantly at $p \leq 0.05$

Table 6. Fungi isolated from roots and leaf sheaths of selected ornamental grass species obtained from experiment with artificial infection of subsoil

Fungi species	Experimental combination			Total of isolates
	<i>F. culmorum</i> No. 34	<i>F. equiseti</i> No. 62	control	
<i>Alternaria alternata</i> (Fr.) Keissler	(0)0	(2)16	(10)7	35
<i>Aspergillus flavus</i> Link	(0)1	(0)2	(0)0	3
<i>Chaetomium globosum</i> Kunze	(1)14	(17)18	(7)15	72
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	(0)0	(1)0	(0)0	1
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	(128)132	(20)25	(16)31	352
<i>Fusarium equiseti</i> (Corda) Sacc.	(0)0	(105)106	(10)6	227
<i>Fusarium oxysporum</i> Schlecht.	(0)1	(17)8	(0)2	28
<i>Mucor racemosus</i> Fres.	(0)0	(0)16	(0)0	16
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopi-um</i> (Westling.) Samson et al.	(2)2	(4)0	(6)4	18
<i>Rhizopus nigricans</i> Ehrenberg	(0)0	(0)0	(0)7	7
<i>Trichoderma aureoviride</i> Rifai	(6)0	(0)0	(21)0	27
<i>Trichothecium roseum</i> Link	(0)0	(0)0	(0)2	2
<i>Mycelia sterilia</i>	(0)0	(5)0	(11)0	16
Total	(137)150	(171)191	(81)74	804

Values in brackets determine number of isolates obtained from roots, before brackets from leaf sheaths

lacryma-jobi to 69% in the case of *Festuca ovina* (tab. 3). The mean values of the disease index varied respectively from 2.6 in the case of *Coix lacryma-jobi* to 39.6 for *Lagurus ovatus* and they differed significantly (tab. 3). As a result of the mycological analysis of plants showing disease symptoms, species of the genus *Fusarium* were ob-

tained and their isolates accounted for 87.01% of all isolates. *F. oxysporum* was derived in greatest numbers – 33.56% (245 isolates) of all *Fusarium* spp. isolates. The species *Fusarium culmorum* was isolated both from the roots and stem base of the examined plants. Colonies of this fungus constituted 27.18% of all isolates. *F. crookwellense* (9.77% – 82 isolates) and *F. equiseti* (10.01% – 84 isolates) (tab. 4) were also isolated in large numbers. Furthermore, the following species were obtained from infected plants: *F. graminearum* – 2.86% (24 isolates), *F. solani* – 4.05% (34 isolates), *F. avenaceum* – 1.55% (13 isolates), *F. sporotrichioides* – 1.91% (16 isolates), and *F. verticillioides* – 0.48% (4 isolates) of all isolates (tab. 4). Colonies of other fungi belonged to 9 species and non-sporulating forms. *Exserohilum pedicellatum*, the isolates of which accounted for 0.24% of total isolates, and one isolate of *B. sorokiniana* were found among pathogens of plants of the family *Poaceae* (tab. 4).

In the experiment conducted in the growth chamber root and leaf sheath necrosis occurred on seedlings in both experimental treatments with inoculation of seeds and subsoil with *F. culmorum* No. 34 and *F. equiseti* No. 62. In the experimental treatment with inoculation of subsoil with *F. equiseti*, a reduction in the root system as well as yellowing and necrosis of leaf tips were also visible in the case of *Festuca glauca* and *Sorghum nigrum*. In the experimental treatment with *F. culmorum*, on the other hand, in addition to root and leaf sheath necrosis, drying of leaf tips and progressive necrosis covering the entire leaf occurred in the case of *Sorghum nigrum*, *Setaria macrostachya* and *Panicum miliaceum Violaceum*. Control seedlings usually had three healthy leaves and a well-developed root system, with no visible necrosis. However, in some replicates of the control treatment for the ornamental grass species analyzed, seedlings with necrotic spots on their leaf sheaths were observed. The statistical analysis of the disease indices revealed varying susceptibility of the ornamental grass species to the analyzed species of *F. culmorum* and *F. equiseti*. Such statistical analysis of the disease indices for seedlings grown in the experimental treatment with inoculation of subsoil with *F. culmorum* and *F. equiseti* demonstrated significant differences in all the studied grass species compared to the control (tab. 5). The strain *F. culmorum* No. 34 showed the highest pathogenicity towards *Festuca glauca*, *Setaria macrostachya* and *Panicum miliaceum Violaceum*, for which the disease indices were respectively 98.25, 96.25 and 96.00. *Lagurus ovatus*, whose disease index was 44.00, was the least susceptible to *F. culmorum* (tab. 5). The strain *F. equiseti* No. 62 proved to be the most pathogenic to *Phalaris canariensis* (89.50) and *Cortaderia selloana* (89.00). *Panicum miliaceum Violaceum* was the least susceptible to infection by *F. equiseti* No. 62 (36.25) (tab. 5). The values of the disease indices in control plants ranged from 3.50 in the case of *Phalaris canariensis* to 28.00 for *Panicum miliaceum Violaceum* (tab. 5). The mycological analysis of seedlings with disease symptoms allowed us to conclude that the studied strains of *F. culmorum* and *F. equiseti* were the cause of injuries (tab. 6).

DISCUSSION

The seed material of the ornamental grass species studied was often colonized by fungi of the genus *Fusarium*. *F. graminearum* proved to be a species that reduced the

germination of *Sorghum sudanense* seeds. Little et al. [2012] report the occurrence of this fungus on sorghum seed. In the study of Kiecana et al. [2012], this fungus reduced the germination capacity of *F. rubra* in particular. The harmfulness of *F. graminearum* to grasses is manifested, among others, in causing pre and post-emergence damping-off [Gołębniak 2001, Clarke and Engling 1994]. Under the field conditions investigated, this fungus also played a part in damaging the roots and stem base of the ornamental grasses. *Fusarium graminearum* is known for its harmfulness to grasses, including *Panicum* and *Setaria* [Pereira and Dill-Macky 2008].

In the present study, the species *F. equiseti* was found on the seeds, roots and stem base of *P. miliaceum* and *Setaria macrostachya*, on the roots and lower stem internodes of *Sorghum sudanense*, on the roots of *F. ovina* as well as on the seeds of *L. ovatus* and *B. maxima*. This fungus colonizes seed material of various plants, including cereals and grasses [Machowicz-Stefaniak and Zimowska 2000, Mielniczuk et al. 2010, Kiecana et al. 2012, Little et al. 2012]. *Fusarium equiseti* is a common colonizer of aging or dying plant tissues. It is also considered to be a primary colonizer of sorghum roots [Reed et al. 1983]. This species produces group A and B trichothecenes as well as metabolites such as fusarochromanone, beauvericin and equisetin. Equisetin is a phytotoxic compound that can take part in the pathogenesis. It inhibits root and coleoptile development in seedlings of various plant species at a concentration of 7–27 μM [Wheeler et al. 1999, Desjardins 2006].

The fungus *F. sporotrichioides*, which occurred on non-germinating seeds in 2010 and on the stem base of *Sorghum sudanense*, is a species that is commonly found on seeds of lawn grasses and cereals [Kutrzeba 1994, Kiecana 1994, Mielniczuk 2001, Kiecana et al. 2012]. *Fusarium sporotrichioides* is a polyphagous species with strong toxin-producing properties due to its production of group A trichothecenes. According to Chelkowski et al. [1989], the pathogenicity of *F. sporotrichioides* to plants appears to depend on its capacity to synthesize these compounds.

In the community of fungi isolated from seeds of the ornamental grasses in both years of the study, worth noting is *A. alternata* which was the cause of reduced germination capacity especially of hare's-tail grass in 2009 and which was frequently isolated from the seed of *Coix lacryma-jobi* and *Phalaris canariensis* in 2010. This fungus has often been observed on grass seeds [Kućmierz and Gorajczyk 1991, Kutrzeba 1994, Wiewióra and Prończuk 2000, Kiecana et al. 2012, Little et al. 2012]. The growth of *A. alternata* is promoted by high humidity. It is a species that infects weakened plants, but in some cases it can also be a pathogen of plants in good condition [Rotem 1994]. Tentoxin and tenuazonic acid, among others, take part in the pathogenesis of *A. alternata*. Tentoxin mostly acts as a phosphorylation inhibitor. This compound combines with the protein important for building chloroplasts and blocks the phosphorylation of ADP to ATP [Thomma 2003]. The present study shows that the seed material of *Lagurus ovatus* can be colonized by *B. sorokiniana*, which is known for its harmfulness to lawn grasses [Kutrzeba 1994, Wiewióra and Prończuk 2000, Kiecana et al. 2012]. The occurrence of this fungus on the stem base of *S. macrostachya* confirms its relationship with grasses. The pathogenicity of *B. sorokiniana* is associated with the production of secondary metabolites, prehelminthosporol and sorokinianin [Nakajima et al. 1998, Apoga et al. 2002].

Penicillium verrucosum var. *cyclopium* predominated among species of the genus *Penicillium* isolated from the seed material of the ornamental grasses; in 2009 it reduced the germination capacity of most of the studied species. *Penicillium verrucosum* var. *cyclopium* is encountered on seed material of grasses [Kutrzeba 1994, Wiewióra and Prończuk 2000, Kiecana et al. 2012]. This fungus shows a destructive effect on seed material under conditions favorable for its growth [Christensen 1972]. *P. verrucosum* var. *cyclopium* is a producer of ochratoxin A [Benett and Klich 2003]. In 2009 the investigated seed material of *L. ovatus* was colonized by *B. cinerea*. This fungus produces phytotoxic metabolites – botrynodiol and botrydiol; the latter compound is accumulated in infected plant tissues [Clomenares et al. 2002 according to Elad et al. 2007]. The presented results also show the colonization of the seed material of *P. milliaceum*, *Stipa tenuissima*, *Lagurus ovatus* and *Festuca glauca* by *Epicoccum nigrum*. The occurrence of this fungus on species of the genera *Panicum*, *Stipa* and *Setaria* is reported by Mel'nik and Braun [1999]. In both years of the study, *Chaetomium indicum* was found on the seed material of some of the ornamental grasses. This fungus produces alkaloids termed chaetoinidines A–C [Guo-You et al. 2006 according to Marawh et al. 2007]. Species of the genus *Chaetomium* produce different metabolites, including among others musanahol, 3-epi aureonitol and lineleic acid. The two latter compounds exhibit antibiotic activity [Marawh et al. 2007].

F. culmorum, known for its harmfulness to plants of the family *Poaceae*, proved to be the main cause of necrosis of *L. ovatus* roots and lower internodes under the studied conditions in 2010 [Kiecana 1994, Prończuk 2000, Płaskowska et al. 2006, Kiecana et al. 2006, 2009, 2012]. The harmfulness of *F. culmorum* to plants of the family *Poaceae* results, among others, from its capacity to produce toxic metabolites, primarily deoxynivalenol. This toxin may affect the functioning of the cytoplasmic membranes and causes mitotic cell division disturbances, induces chromosome aberrations, also inhibits the growth of cereal seedlings and reduces the germination capacity of seeds [Wiśniewska and Chełkowski 1994, Dahleen and McCormic 2001]. The species *Fusarium crookwellense* was obtained from both the roots and stem base of the ornamental grasses. Smiley et al. [1992] attributed to this species a significant role in causing Fusarium rot of grasses. Similarly as in the study of Sivanesan [1978 according to Gilbert 2003], the species *E. pedicellatum* was isolated from *Setaria macrostachya*. This author obtained this fungus from the roots of this species with rot symptoms, whereas in the present study it was isolated from the stem base and moreover from the roots of *Panicum milliaceum* *Violaceum*. Hosts of *E. pedicellatum* can also include the following genera: *Echinochloa*, *Oryza*, *Paspalum*, *Sorghum*, *Triticum*, *Hordeum* and *Zea*; in the case of the latter genus *E. pedicellatum* is the cause of brown root rot [Sivanesan 1987, Łacicowa 1993, Gilbert 2003, Isakeit et al. 2007]. *F. oxysporum*, which is not considered to be a pathogen of plants of the family *Poaceae*, was a species that was frequently isolated from the roots and lower internodes of the ornamental grasses. According to Truszkowska et al. [1983] in the case of oats, *F. oxysporum* seems to perform the role of secondary host that facilitates the survival of the fungus during the period without a host plant. Pathogenicity tests made by Fedel-Moen and Harris [1987] on barley and oats showed that *F. oxysporum* was capable of causing damage to roots, but there were differences between isolates and differences in their ability to damage barley and oats.

To investigate the harmfulness of *F. culmorum* and *F. equiseti* to ornamental grass seedlings, an inoculum was used in the form of 14-day cultures of the tested strains *F. culmorum* No. 34 and *F. equiseti* No. 42, grown on PDA medium, following the study of Mańka [1989]. Taking into account the varying virulence of strains in fungal populations of the genus *Fusarium* [Kiecana 1994, Kiecana et al. 2002, Mielniczuk 2008], strains of proven pathogenicity were used for the phytotron trial. With this method used, which allowed direct contact of *F. culmorum* and *F. equiseti* with pre-germinated seeds, *F. culmorum* proved to show high pathogenicity to *F. glauca*, *P. canariensis*, *P. miliaceum Violaceum*, *S. nigrum* and *S. macrostachya*. This is explained by the toxic properties of this species [Packa 1997]. The results obtained in the phytotron experiment also reveal the high harmfulness of *F. equiseti* to seedlings of the ornamental grass species investigated, in particular *P. canariensis* and *C. selloana*. *L. ovatus* proved to be a species of low susceptibility to seedling infection by *F. culmorum*, but it was susceptible to infection by *F. equiseti* *P. miliaceum Violaceum* and *S. nigrum*.

CONCLUSIONS

1. The large number of *Fusarium graminearum* isolates obtained from *Sorghum sudanense* seeds examined entitles us to conclude that under our growing conditions the seed of this plant is an important primary source of inoculum of this fungus.
2. The presence of *Exserohilum pedicellatum* on *Setaria macrostachya* and *Panicum miliaceum Violaceum* and the recognized harmfulness of this pathogen to other plants of the family *Poaceae* suggest that the mentioned ornamental grass species may also be infected under Polish conditions.
3. The species *Fusarium culmorum* and *F. equiseti* are a threat to ornamental grasses.

ACKNOWLEDGEMENTS

Research supported by Poland's Ministry of Science and Higher Education as part of the statutory activities of University of Life Sciences in Lublin

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GRZYBY PORAZAJĄCE TRAWY OZDOBNE I PATOGENICZNOŚĆ *Fusarium culmorum* (W.G.Sm.) Sacc. I *Fusarium equiseti* (Corda) Sacc. DLA WYBRANYCH GATUNKÓW

Streszczenie. Jedną z przyczyn obniżających estetyczny wygląd trawnika są choroby powodowane przez grzyby. Badania przeprowadzono w latach 2009–2010 na materiale siewnym 11 gatunków traw ozdobnych. W ciągu dwóch lat badań uzyskano 681 izolatów grzybów należących do 22 gatunków oraz form niezarodnikujących. Spośród grzybów patogenicznych dla traw, szczególnie w 2010 r., uzyskiwano gatunki z rodzaju *Fusarium*. Izolaty tych grzybów w tym roku badań stanowiły 29,94% wszystkich wyizolowanych kolonii. Często wyosabniano również kolonie *Alternaria alternata*, którego izolaty w 2009 i 2010 r. stanowiły odpowiednio 25,36 i 12,57% ogółu wyosobnień. Badania nad zdrowotnością traw ozdobnych przeprowadzone w warunkach polowych wykazały występowanie roślin z nekrozą korzeni i dolnych międzywęźli. Odsetek takich roślin wahał się od 12% dla *Coix lacryma-jobi* do 69% w przypadku *Festuca ovina*. Natomiast średnie wartości wskaźników chorobowych wynosiły od 2,6% w przypadku *Coix lacryma-jobi* do 39,6% dla *Lagurus ovatus* i różniły się istotnie. Z porażonych roślin uzyskiwano głównie gatunki z rodzaju *Fusarium*. Większość izolatów stanowił *F. oxysporum*, izolowano również gatunki: *F. culmorum*, *F. crookwellense*, *F. graminearum*, *F. solani*, *F. avenaceum*, *F. sporotrichioides* oraz *Bipolaris sorokiniana* i *Exserohilum pedicellatum*. Badania podatności siewek siedmiu gatunków traw ozdobnych na porażenie przez *Fusarium culmorum* nr 34 i *F. equiseti* nr 62 przeprowadzono w fitotronie. Analiza statystyczna wskaźników chorobowych dla roślin wyrosłych w kombinacji doświadczenia ze sztucznym zakażaniem podłoża przez wyżej wymienione szczepy w porównaniu z kontrolą wykazała istotne różnice u wszystkich badanych gatunków traw w przypadku *F. culmorum* i u 5 gatunków w kombinacji doświadczenia z *F. equiseti*. Szczep *Fusarium culmorum* nr. 34 okazał się najbardziej patogeniczny w stosunku do *Festuca glauca* (98,25%), natomiast *F. equiseti* nr 62 dla *Phalaris canariensis* (89,5%).

Słowa kluczowe: zdrowotność ziarniaków, patogeniczność, *Fusarium culmorum*, *Fusarium equiseti*, siewki, nekroza korzeni i dolnych międzywęźli

Accepted for print: 2.06.2014