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FUNGI COLONIZING THE SOWING MATERIAL OF TURFGRASSES CONSIDERING SUSCEPTIBILITY OF CULTIVARS TO SELECTED PATHOGENS

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Abstract. One of the reasons lowering the esthetic appearance of a lawn are diseases caused by fungi. Studies were conducted on the sowing material of fifteen turfgrasses belonging to five species. Samples of seeds of the studied cultivars were from the harvest of 2007 and 2008. 573 fungi isolates belonging to 28 species and non-sporulating forms were obtained as a result of the mycological analysis during the two years of the experiment. In each year of studies, the greatest number of colonies belonging to *Alternaria alternata*. Within the group of pathogenic fungi: *Fusarium culmorum*, *F. avenaceum, F. equiseti, F. solani*, *F. crookwellense, F. graminearum F. sporotrichioides, B. sorokiniana, D. siccans* and *Rhizoctonia solani* were obtained. Studies on susceptibility of the seedlings of 8 cultivars of 4 grass species to infection by *D. avenae* No. 2, *D. siccans* No. 8 and *B. sorokiniana* No. 69 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 cultivars. Strain *D. siccans* No. 8 proved to be the most pathogenic towards cultivar Info of perennial ryegrass (92.20), and *B. sorokiniana* No. 69 towards wood bluegrass Pinokio cultivar (97.75). In the experimental combination with *D. avenae* the mean values of disease index ranged from 12.25 (Pinia) to 84.00 (Info).

Key words: healthiness of seeds, pathogenicity, *Bipolaris sorokiniana*, *Drechslera avenae, Drechslera siccans*, seedlings

INTRODUCTION

In recent years, a considerable interest in lawns set up for recreational, park or sport purposes has been observed [Wiewióra and Prończuk 2000].

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One of the reasons lowering the esthetic appearance of a lawn are diseases caused by fungi. In the case of sports areas such as golf courses, football and hockey pitches as well as tennis courts, diseases cause an uneven surface of the turf, even making it impossible to play games [Baldwin 1990 according to Prończuk 2000].

Pathogens damaging turfgrasses in the period of vegetation are very often carried with the sowing material. Grass seeds are the original source of inoculum of a number of fungi, including *Bipolaris sorokiniana* (Sacc.) Shoem., *Drechslera* spp. and the species from genus *Fusarium* Link, which cause necrosis of the roots and grass rhizomes [Kutrzeba 1994, Vargas 1994, Prończuk 2000, Wiewióra and Prończuk 2000, Czembor 2002]. Saprophytic species occurring in grass seeds include representatives of genera *Alternaria, Rhizopus*, *Mucor*, *Aspergillus* and *Penicillium*. The species of the latter ones are the so-called stored fungi, which in favourable conditions have a destructive effect on the stored sowing material [Christensen 1972 according to Łacicowa et al. 1991/1992].

Turfgrasses are seriously threatened by fungi from genus *Drechslera* Ito, including the species of *Drechslera siccans* (Drechsler) Shoem., *D. poae* (Baudys) Shoem., *D. dictyoides* (Drechsler) Shoem. and *D. avenae* (Eidam) Scharif [Paul 1995, Prończuk 2000, Czembor 2002]. There exists a certain kind of specialization to infect certain grass species [Lam 1985; Burhenne 1992 according to Paul 1995, Prończuk 2000]. The same pathogen may cause different symptoms of leaf brown spot on different grass species, and even on particular cultivars of the same species [Lam 1985; Burhenne 1992 according to Paul 1995].

Drechslera siccans (Drechsler) Shoem. (teleomorph *Pyrenophora lolii* Dovaston) is a dangerous pathogen towards *Lolium perenne* (perennial ryegrass) in lawn cultivations in England and Wales [Lam 1984 according to Paul 1995]. Despite the existence of specialization of pathogen-host, this fungus damages the leaves of grass from genera *Festuca* (fescue), *Dactylis* (cocksfoot), *Poa* (meadow grass), *Phleum* (timothy) in Europe, North America and Australia [Kwaśna 1995, Paul 1995, Prończuk 2000].

Drechslera dictyoides causes characteristic symptoms, mainly on red fescue [Vargas 1994].

Drechslera avenae (Eidam) Scharif (teleomorph *Pyrenophora avenae* Ito et Kurib.), the main pathogen towards oat seeds and leaves, can be the cause of leaf brown spot of some turfgrasses [Obst 1995, Paul 1995, Prończuk 2000, Mehta 2001, Carmona et al. 2004].

Bipolaris sorokiniana (Sacc.) Shoem. (teleomorph *Cochliobolus sativus* Ito et Kurib.) also causes leaf spot of turfgrasses both in lawn cultivation and in cultivation for sowing material [Vargas 1994, Krupinsky and Berdahl 2000, Wiewióra and Prończuk 2000, 2002, Pratt 2006]. This fungus is observed on all species of turfgrasses, showing smaller specialization towards the host plant than fungi from genus *Drechslera* [Vargas 1994].

In view of the fact that new species of lawn plants are introduced into commercial use, it was considered advisable to conduct studies on the healthiness of the sowing material of 15 species of turfgrasses that are most frequently used in lawn mixtures. Because of scarce information on the harmfulness of *B. sorokiniana* and *Drechslera* spp. towards the seedlings of turfgrasses, studies in growth chamber conditions were chosen.

MATERIAL AND METHODS

Studies were conducted on the sowing material of fifteen turfgrasses belonging to five species (tab. 1). The sowing material came from the Plant Cultivation Ltd. – Nieznanice Section. Samples of seeds of the studied cultivars were from the harvest of 2007 and 2008. 100 seeds randomly chosen from 50 g samples were analyzed for each cultivar.

The plate method was applied to isolate the fungi colonizing the seeds, with the subsoil being the mineral medium [Mielniczuk et al. 2010], prepared on a brew from seeds (100 g of perennial ryegrass was used per $1000 \cdot ml^{-1}$ of the medium). Fungi from *Fusarium* genus were marked according to the recommendations by Nelson et al. [1983], Burgess et al. [1988] and Leslie and Summerell [2006]. Species from genera *Drechslera, Alternaria alternata* (Fr.) Keissler and *Bipolaris sorokiniana* were marked by means of the monograph by Ellis [1971]. Other fungi species were marked to the species according to the monograph by Ramirez [1982], Domsch et al. [1980], Hanlin [1992], Skirgiełło et al. [1979], Thom and Raper [1945].

Studies on susceptibility of the seedlings of 8 cultivars of turfgrasses: red fescue (*Festuca rubra* L.) Nimba and Nil, perennial ryegrass (*Lolium perenne* L.) Info, Inka and Pinia, wood bluegrass (*Poa nemoralis* L.) Pinokio and Cień, and sheep fescue (*Festuca ovina* L.) Sima to infection by *Drechslera avenae* No. 2 obtained from oat leaves, *Drechslera siccans* No. 8 obtained from seeds of perennial ryegrass and *Bipolaris sorokiniana* No. 69 isolated from the grass roots were conducted in a growth chamber, at the temperature of 22–23°C and with relative humidity of the air of 85%. In the studies were used those strains whose pathogenicity had been earlier tested in the laboratory by the Mishra and Behr's method [1976]. The inoculum of the fungi were 14-day-old cultures of *D. avenae*, *D. siccans* as well as 21-days' culture of *B. sorokiniana*, growing on PDA medium in Petri dishes at the temperature of 22°C.

In the experiment were used plastic pots with the diameter of 10 cm filled with the universal subsoil with sand in the proportion 2:1, with pH 6.5, previously sterilized in an autoclave for two hours at the temperature 121°C, under the pressure of 0.12 MPa [Mańka 1989].

Seed material of the analyzed grass cultivars whose sprouts reached the length of 10 mm and were normally formed were used for the studies. The selected material was placed on plasters of the medium with the analyzed strain of each fungi species, and next it was covered with the medium according to Mańka [1989]. The control were the pots where slightly germinated seeds were placed on medium plasters without the fungus. The experiment was set up on 18 December 2008 and each combination of the experiment had four replications, with 25 plants in each. The plants grew for 25 days, after which the numbers of healthy, infected plants and those that died out before emergence were established. The infection degree of plants with disease symptoms was determined according to a 4^o scale:

– 1^o seedlings with necrosis on 1–3 roots,
– 2^o seedlings with necrosis on more than 3 roots,
– 3^o seedlings with completely necrotized roots and spots on leaf sheaths,
– 4^o completely necrotized seedlings.

Disease indexes were calculated using the formula of McKinney [Łacicowa 1969].

The obtained results were statistically analyzed using T-Tukey's confidence semi-intervals [Żuk 1989].

Ten seedlings with disease symptoms from each combination of the growth chamber experiment were taken for mycological analysis. Fifty 3-milimeter-long fragments of roots and leaf sheaths of the diseased seedlings of each cultivar were analyzed. The same monographs were used to mark the fungi isolated from the diseased plants in the growth chamber experiment as in the case of the mycological analysis of the sowing material.

RESULTS

573 fungi isolates belonging to 28 species and non-sporulating forms were obtained as a result of the mycological analysis of seeds of 15 cultivars of turfgrasses during the two years of the experiment, including 165 colonies from sporulating seeds and 408 from non-sporulating ones (tab. 1, 2).

In each year of studies, the greatest number of colonies belonging to *Alternaria alternata* (Fr.) Keissler, whose isolates constituted 24.20% in 2007, while 18.53% of all isolations in 2008, were isolated from the analyzed sowing material of turfgrasses. Within the group of fungi pathogenic towards grasses in both vegetation seasons, species from genus *Fusarium* were obtained. Isolates of those fungi constituted 16.57% in 2007, while in 2008 8.12% of all isolations (tab. 1, 2). The dominating species in 2007 was *Fusarium culmorum* (W.G.Sm.) Sacc., whose isolates constituted 9.55% of all isolations (tab. 1), while in 2008 *F. avenaceum* (Fr.) Sacc. – 4.25% of all fungi – was isolated in the greatest numbers (tab. 2). *Fusarium equiseti* Corda Sacc. and *F. solani* (Mart.) Appel et Wollenw., whose isolates constituted, respectively, 0.64% and 0.64% of all isolations in 2007, while in 2008 r. 1.93% and 0.39%, were obtained in both years of studies (tab. 1, 2). Besides, in 2007, *F. crookwellense* Burgess, Nelson, Toussoun and *F. graminearum* Schwabe were isolated, whose colonies constituted, respectively, 0.96% and 4.78% (tab. 1), whereas species *F. sporotrichioides* Sherb., whose isolates constitutes 0.39% of all obtained fungi, was also obtained in 2008 (tab. 2). In both years of studies, pathogenic species of *Drechslera siccans* were obtained, whose isolates constituted 3.18% in 2007, and 0.39% in 2008 of all isolations (tab. 1, 2). On the other hand, in 2008, *B. sorokiniana* and *Rhizoctonia solani* Kühn were also obtained, whose isolates constituted, respectively, 2.32% and 5.40% of all isolations of all fungi (tab. 2). Genus *Penicillium* was represented by the following: *P. decumbens* Thom, *P. notatum* Westling, *P. puberulum* Bainier, *P. verrucosum* Dierckx var. *cyclopium* (Westling.) Samson et al., *P. verrucosum* Dierckx var. *verrucosum* Samson, Stolk et Hadlok, whose isolates constituted, respectively in the years 2007 and 2008: 3.50% and 1.54%, 0 and 3.86%, 10.51% and 6.56%, 3.18% and 0.77%, 4.78% and 0 (tab. 1, 2). The other fungi species colonizing the sowing material in the analyzed years were represented by the following: *Acremoniella atra* Corda, *Aspergillus flavus* Link, *Aspergillus niger* van Tiegh, *Aureobasidium pullulans* (de Bary) Arnaud., *Chaetomium globosum* Kunze, *Cladosporium cladosporioides* (Fres.) de Vries, *Culvularia protuberata* Nelson &

Table 1. Fungi isolated from grains of 15 turfgrass cultivars on mineral medium prepared on a brew from seeds in 2007
Tabela 1. Grzyby wyizolowane z ziarniaków 15 odmian traw gazonowych na pożywce mineralnej z dodatkiem wyciągu z ziarniaków traw w 2007 r.

Fungi species Gatunki grzybów	Number of isolates obtained from germinating seeds (non germinating) Liczba izolatów uzyskanych z ziarniaków kiełkujących (niekiełkujących)																
	species and cultivars of turfgrasses – gatunki i odmiany traw gazonowych															Total Razem	Total of isolates Ogółem (%)
	Festuca rubra			*Festuca ovina*			*Poa nemoralis*			*Poa pratensis*			*Lolium perenne*				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Acremoniella atra Corda	-	-	-	-	0(3)	-	-	-	-	-	0(1)	-	-	-	9(20)	9 (24)	33 (10.51)
Alternaria alternata (Fr.) Keissler	-	2(2)	1(5)	-	-	1(0)	1(2)	2(19)	-	0(37)	0(2)	-	-	-	2(0)	9 (67)	76 (24.20)
Aspergillus flavus Link	-	2(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	2 (1)	3 (0.96)
Aspergillus niger van Tiegh	-	-	-	-	-	-	-	-	-	0(1)	-	-	-	-	-	0 (1)	1 (0.32)
Chaetomium globusom Kunze	9(1)	-	-	3(4)	-	0(1)	-	-	-	0(1)	-	-	-	-	-	12 (7)	19 (6.05)
Drechslera siccans (Drech.) Shoem.	-	-	-	-	-	-	-	-	-	-	-	-	8(0)	-	0(2)	8 (2)	10 (3.18)
Fusarium crookwellense Burgess, Nelson, Toussoun	-	-	-	-	-	-	-	-	-	0(3)	-	-	-	-	-	0 (3)	3 (0.96)
Fusarium culmorum (W.G.Sm.) Sacc.	0(10)	-	-	-	0(16)	-	-	-	-	-	-	0(4)	-	-	-	0 (30)	30 (9.55)
Fusarium equiseti Corda Sacc.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0(2)	0 (2)	2 (0.64)
Fusarium graminearum Schwabe	1(10)	-	-	-	-	-	-	-	-	-	-	-	-	2(2)	-	3 (12)	15 (4.78)
Fusarium solani (Mart.) Appell et Wollenw.	-	-	-	-	-	-	0(1)	-	-	-	-	-	-	0(1)	-	0 (2)	2 (0.64)
Penicillium decumbens Thom	7(0)	-	-	-	-	-	-	0(2)	0(2)	-	-	-	-	-	-	7 (4)	11 (3.50)
Penicillium puberulum Bainier	-	-	-	-	0(4)	-	-	-	0(6)	0(2)	0(10)	-	0(3)	-	0(8)	0 (33)	33 (10.51)
Penicillium verrucosum Dierckx var. *cyclopium* (Westling.) Samson et al.	-	1(0)	1(0)	-	0(1)	-	-	-	3(0)	-	-	-	-	-	4(0)	9 (1)	10 (3.18)
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	3(4)	4(4)	-	-	-	-	-	-	-	-	-	-	-	-	-	7 (8)	15 (4.78)
Papularia sphaeosperma (Pers.) Höhn	5(0)	-	0 (8)	-	0 (1)	-	-	-	-	-	-	-	-	-	-	5 (9)	14 (4.46)
Stemphylium botryosum Wallv.	0 (2)	2(3)	-	-	-	-	-	-	-	0 (9)	-	-	-	1(0)	-	3 (14)	17 (5.41)
Mycelia sterylia	2(14)	2(2)	-	-	-	-	-	-	-	-	-	-	-	-	-	4 (16)	20 (6.37)
Total	27(41)	13(12)	2(13)	3(4)	0(25)	1(1)	1(3)	2(21)	3(8)	0(53)	0(13)	0(4)	8(3)	3(3)	15(32)	78 (236)	314 (100)

Cultivars – odmiany: 1 – Adio, 2 – Nil, 3 – Nimba, 4 – Noni, 5 – Sima, 6 – Witra, 7 – Cień, 8 – Niuans, 9 – Pinokio, 10 – Alicja, 11 – Ani, 12 – Nandu, 13 – Info, 14 – Inka, 15 – Pinia

Values before brackets determine number of isolates obtained from germinating seeds, in brackets from non germinating seeds – Wartości podane przed nawiasem określają liczbę izolatów uzyskanych z ziarniaków kiełkujących, zaś w nawiasach z ziarniaków niekiełkujących

Table 2. Fungi isolated from grains of 15 turfgrass cultivars on mineral medium prepared on a brew from seeds in 2008
Tabela 2. Grzyby wyizolowane z ziarniaków 15 odmian traw gazonowych na pożywce mineralnej z dodatkiem wyciągu z ziarniaków traw w 2008 r.

Fungi species Gatunki grzybów	Number of isolates obtained from germinating seeds (non germinating) Liczba izolatów uzyskanych z ziarniaków kiełkujących (niekiełkujących)																
	species and cultivars of turfgrasses – gatunki i odmiany traw gazonowych															Total Razem	Total of isolates Ogółem (%)
	Festuca rubra			*Festuca ovina*			*Poa nemoralis*			*Poa pratensis*			*Lolium perenne*				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Acremoniella atra Corda	-	-	-	-		-	-	-	-	-	-	-	-	-	2(0)	2(0)	2 (0.77)
Alternaria alternata (Fr.) Keissler	-	1 (0)	1(0)	-	5(5)	2(0)	0(1)	0(7)	-	0(4)	0(1)	0(5)	-	-	16(0)	25(23)	48 (18.53)
Aspergillus flavus Link	-	-	-	-	0(1)	-	-	-	-	-	-	-	-	-	-	0(1)	1 (0.39)
Aspergillus niger van Tiegh	-	-	-	-	-	-	-	-	-	-	0(1)	0(2)	0 (2)	-	1(0)	1(5)	6 (2.32)
Aureobasidium pullulans (de Bary) Arnaud.	-	-	-	-	-	-	-	-	-	-	-	-	5 (5)	-	-	5(5)	10 (3.86)
Bipolaris sorokiniana (Sacc.) Shoem.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0(6)	0(6)	6 (2.32)
Chaetomium globusom Kunze	-	-	1(0)	-	-	-	-	-	-	-	-	-	-	-	-	1(0)	1 (0.39)
Cladosporium cladosporioides (Fres.) de Vries	0(8)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0(8)	8 (3.09)
Culvularia protuberata Nelson & Hodges	-	-	-	-	-	-	-	-	-	-	0(1)	-	-	-	-	0(1)	1 (0.39)
Drechslera siccans (Drech.) Shoem	-	-	-	-	-	-	-	-	-	-	-	-	1 (0)	-	-	1(0)	1 (0.39)
Epicoccum nigrum Link ex Link	-	-	-	-	-	-	-	-	0(1)	-	-	-	-	-	1(2)	1(3)	4 (1.54)
Fusarium avenaceum (Fr.) Sacc.	-	-	-	-	-	-	-	0(1)	-	0(1)	0(9)	-	-	-	-	0(11)	11 (4.25)
Fusarium culmorum (W.G.Sm.) Sacc.	-	-	-	-	-	-	-	-	-	-	-	0(3)	-	-	-	0(3)	3 (1.16)
Fusarium equiseti Corda Sacc.	-	-	-	1(1)	-	-	1(0)	-	-	-	-	-	-	0(1)	1(0)	3(2)	5 (1.93)
Fusarium solani (Mart.) Appell et Wollenw.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(0)	1(0)	1 (0.39)
Fusarium sporotrichioides Sherb.	-	-	-	-	-	-	-	0(1)	-	-	-	-	-	-	-	0(1)	1 (0.39)
Mucor hiemalis Wehmer	0(1)	4 (1)	-	-	-	0(6)	-	0(1)	0(1)	-	-	0(1)	-	0(1)	0(1)	4(13)	17 (6,56)
Penicillium decumbens Thom	2(0)	-	1(0)	-	-	-	-	-	-	0(1)	-	-	-	-	-	3(1)	4 (1.54)
Penicillium notatum Westling	2(2)	2 (0)	-	-	-	-	-	-	-	0(4)	-	-	-	-	-	4(6)	10 (3.86)
Penicillium puberulum Bainier	-	-	-	0(7)	-	-	-	-	-	0(9)	0(1)	-	-	-	-	0(17)	17 (6.56)
Penicillium verrucosum Dierckx var. *cyclopium* (Westling.) Samson et al.	-	-	-	-	-	-	-	-	-	-	-	-	-	0(1)	0(1)	0(2)	2 (0.77)
Papularia sphaeosperma (Pers.) Höhn	-	-	-	-	0(2)	-	-	-	-	-	-	-	-	-	-	0(2)	2 (0.77)
Rhizoctonia solani Kühn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5 (9)	5(9)	14 (5.40)
*Sordaria fimicola (*Roberge ex. Desman.) ces. & de Not.	-	-	-	-	3(7)	-	-	-	-	-	-	-	-	-	1 (0)	4(7)	11 (4.25)
Stemphylium botryosum Wallv.	1(0)	-	1(0)	-	-	2(0)	-	-	-	0(5)	-	-	-	0(4)	9(0)	13(9)	22 (8,49)
Mycelia sterylia	2(1)	3(0)	2(2)	0(5)	0(9)	1(8)	-	0(1)	-	1(2)	-	0(3)	2 (0)	0(4)	3(2)	14(37)	51 (19.69)
Total	7(12)	10(1)	6(2)	1(13)	8(24)	5(14)	1(1)	0(11)	0(2)	1(26)	0(13)	0(14)	8(7)	0(11)	40(21)	87(172)	259 (100)

Cultivars – odmiany: 1 – Adio, 2 – Nil, 3 – Nimba, 4 – Noni, 5 – Sima, 6 – Witra, 7 – Cień, 8 – Niuans, 9 – Pinokio, 10 – Alicja, 11 – Ani, 12 – Nandu, 13 – Info, 14 – Inka, 15 – Pinia
Values before brackets determine number of isolates obtained from germinating seeds, in brackets from non germinating seeds – Wartości podane przed nawiasem określają liczbę izolatów uzyskanych z ziarniaków kiełkujących, zaś w nawiasach z ziarniaków niekiełkujących

Table 3. Pathogenicity of the strains *Drechslera avenae* No. 2, *D. siccans* No. 8 and *Bipolaris sorokiniana* No. 69 to seedlings of selected turfgrass cultivars in growth chamber conditions

Table 3. Patogeniczność szczepów *Drechslera avenae* nr 2, *D. siccans* nr 8 i *Bipolaris sorokiniana* nr 69 dla siewek wybranych odmian traw gazonowych w warunkach doświadczenia fitotronowego

Turfgrass species and cultivars Gatunki i odmiany traw	Experimental combination – Kombinacja doswiadczenia											
	Drechslera avenae			*Drechslera siccans*			*Bipolaris sorokiniana*			Control – Kontrola		
	z	p	o	z	p	o	z	p	o	z	p	o
Lolium perenne Info	0	49	51	0	100	0	40	19	41	82	10	8
Lolium perenne Inka	64	34	2	14	72	14	11	41	48	96	0	4
Lolium perenne Pinia	78	16	6	11	73	16	9	73	18	99	0	1
Festuca rubra Nimba	40	18	42	29	43	28	5	95	0	86	0	14
Festuca rubra Nil	5	60	35	3	57	40	11	45	44	90	0	10
Poa nemoralis Pinokio	57	12	31	4	41	55	2	1	97	90	0	10
Poa nemoralis Cień	63	9	28	17	28	55	7	15	78	81	0	19
Festuca ovina Sima	47	26	27	8	34	58	6	11	83	78	0	22

z – number of healthy plants, p – number of diseased plants, o – number of died plants
z – liczba roślin zdrowych, p – liczba roślin porażonych, o – liczba roślin obumarłych

Hodges, *Mucor hiemalis* Wehmer, *Papularia sphaerosperma* (Pers.) Hőhn, *Stemphylium botryosum* Wallv., *Sordaria fimicola (*Roberge ex. Desman.) ces. & de Not. as well as non-sporulating forms (tab. 1, 2).

Within the two years of studies, the cultivar Pinia of perennial ryegrass was distinguished by the greatest infection of seeds. Its seeds colonized such pathogenic species as *B. sorokiniana*, *D. siccans*, *F. equiseti* and *A. alternata*. On the other hand, in 2007, the cultivar Adio of red fescue, whose seeds colonized such pathogenic species as *F. culmorum* and *F. graminearum* (tab. 1, 2), was characterized by frequent infections.

Results of the studies conducted in a growth chamber indicated that infected plants occurred in all experimental combinations with infection of seeds and the subsoil by *D. avenae, D. siccans* and *B. sorokiniana* (tab. 3).

The diseased plants in the experimental combination with *D. avenae* No. 2 were characterized by necrosis of the roots and the hypocotyl, while growth inhibition and the reduction of the root system occurred with strong infection of more sensitive cultivars. Sometimes, the narrowing of the hypocotyl part and the yellowing and necrosis of the leaf tops were observed.

Table 4. Mean values of the disease index for seedlings of analysed turfgrass cultivars obtained in growth chamber conditions with artificial infection of subsoil with *Drechslera avenae, D. siccans* and *Bipolaris sorokiniana*

Tabela 4. Średnie wartości wskaźników chorobowych dla siewek analizowanych odmian traw gazonowych w doświadczeniu fitotronowym ze sztucznym zakażaniem podłoża przez *Drechslera avenae, D. siccans* i *Bipolaris sorokiniana*

Turfgrass species and cultivars Gatunki i odmiany traw	Experimental combination – Kombinacja doświadczenia			
	Drechslera avenae No. 2	*Drechslera siccans* No. 8	*Bipolaris sorokiniana* No. 69	Control Kontrola
Lolium perenne Info	84.00*d	92.20*c	51.25*a	15.70
Lolium perenne Inka	15.00*a	71.5*b	73.75*b	4.00
Lolium perenne Pinia	12.25*a	64.25*ab	71.50*b	1.00
Festuca rubra Nimba	49.25*bc	51.75*a	80.20*bc	14.00
Festuca rubra Nil	67.75*c	75.50*bc	72.50*b	22.50
Poa nemoralis Pinokio	33.75*b	70.25*b	97.75*c	22.50
Poa nemoralis Cień	29.75*b	66.50*ab	86.25*bc	19.00
Festuca ovina Sima	39.25*b	78.75*bc	89.75*c	22.00

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

*średnie różnią się istotnie w porównaniu do kontroli przy $p \leq 0,05$

a, b, c – mean values in columns followed by the different small letters differ significantly at $p \leq 0.05$ – średnie wartości w kolumnach oznaczone różnymi małymi literami różnią się istotnie przy $p \leq 0,05$

Table 5. Fungi isolated from roots and leaf sheaths of selected turfgrass cultivars obtained from experiment with artificial infection of subsoil
Tabela 5. Grzyby wyizolowane z korzeni i pochew liściowych siewek wybranych odmian traw gazonowych uzyskanych z doświadczenia ze sztucznym zakażaniem podłoża

Fungi species Gatunki grzybów	*Drechslera avenae*				*Drechslera siccans*				*Bipolaris sorokiniana*				Control – Kontrola				Total of isolates Ogólna liczba izolatów
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	
Alternaria alternata (Fr.) Keissler	2	1	2	1	1		1						5	6	2	1	22
Aspergillus flavus Link						1		2					1	4	2		10
Aspergillus niger van Tieghem													1				1
Bipolaris sorokiniana (Sacc.) Shoem.					1				93	96	76	41	8	6	8	3	332
Drechslera avenae (Eidam) Scharif	74	62	53	55													244
Drechslera siccans (Drechsler)					89	73	63	46									271
Epicoccum nigrum Link ex Link	3																3
Fusarium culmorum (W.G.Sm.) Sacc.	4								2	1	1	1	2	1	1	1	14
Fusarium equiseti (Corda) Sacc.	6	1											4	3			14
Penicillium decumbens Thom		8											1	1			10
Penicillium verrucosum Dierckx var. *cyclopium* (Westling) Samson, Stolk et Hadlok	1	6	3	2		3	1		2	2	1	1	4	2	1	2	31
Rhizoctonia solani Kühn									1	2							3
Trichoderma vride Rifai													9	3			12
Trichothecium roseum Link															5		5
Mycelia sterylia – Formy niezarodnikujące	1	3	2	1	2	3	3	1					1	2	1	1	21
Total	91	81	60	59	93	80	68	49	98	101	78	43	36	28	20	8	993

Turfgrass species – gatunki traw gazonowych : I – perennial ryegrass, II – red fescue, III – wood bluegrass, IV – sheep fescue

In the case of seedlings from the experimental combination with *D. siccans* No. 8, the root system reduction and necrosis of the hypocotyl part were observed. Plants with the symptoms of brown spots with a chlorotic ring on the surface of the leaf blades also occurred. The yellowing and dying out of the leaves were observed in some cultivars. The infected seedlings were also sometimes inhibited in their growth and deformed.

In the experimental combination with *B. sorokiniana* No. 69, plants with the necrosis and reduction of the root system and necrosis of leaf sheaths and leaf spots were observed. The statistical analysis of the disease indexes, differentiated susceptibility of the cultivars to the analyzed species of *D. avenae, D. siccans* and *B. sorokiniana* was observed.

The statistical analysis of disease indexes for plants that grew in the experimental combination with artificial infection of the subsoil with *D. avenae, D. siccans* and *B. sorokiniana* as compared to the control indicated significant differences in all studied grass cultivars (tab. 4). In the conditions of controlled temperature and humidity, strain *D. avenae* No. 2 showed the highest pathogenicity towards cultivar Info of perennial ryegrass and cultivar Nil of red fescue, for which the disease indexes were, respectively, 84.00 and 67.75. Cultivars Pinia and Inka of perennial ryegrass, whose disease indexes were, respectively, 12.25 and 15.00, were the least susceptible to *D. avenae* (tab. 4).

Strain *D. siccans* No. 8 proved to be the most pathogenic towards cultivar Info of perennial ryegrass, Sima of sheep fescue and Nil of red fescue, whose values of disease indexes were, respectively, 92.20, 78.75 and 75.50. Cultivar Nimba of red fescue, whose disease index was 51.75, was the least susceptible to the seedling infection by *D. siccans.*

Strain *B. sorokiniana* No. 69 proved the most pathogenic towards wood bluegrass Pinokio cultivar (97.75). Cultivar Info of perennial ryegrass was the least susceptible to infection by *B. sorokiniana* No. 69 (51.25) (tab. 4).

Values of disease indexes in control plants ranged from 1.00 in the case of cultivar Pinia of perennial ryegrass to 22.50 for cultivars Pinokio of wood bluegrass and Nil of red fescue (tab. 4).

The mycological analysis of seedlings with disease symptoms allowed to consider the studied strain of *D. avenae, D. siccans* and *B. sorokiniana* to be the cause of injuries (tab. 5).

Apart from the species used for artificial infection of the seeds and subsoil, scare isolates of the following were obtained as a result of the mycological analysis: *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Epicoccum nigrum* Link ex Link, *Fusarium culmorum*, *F. equiseti*, *Penicillium decumbens*, *P. verrucosum* var. *cyclopium*, *Rhizoctonia solani*, *Trichoderma viride* Rifai, *Trichothecium roseum* Link and mycelia sterylia (tab. 5).

DISCUSSION

Alternaria alternata deserves special attention in the community of fungi obtained from seeds of the analyzed cultivars of 15 species of turfgrasses. That fungus was the cause of lowered ability to sporulate, especially of cultivar Alicja of common meadow

grass and cultivar Niuans of wood bluegrass in 2007. On the other hand, in the case of the other cultivars of the analyzed species of turfgrasses, this species was obtained both from sporulating and non-sporulating seeds. *A. alternata* established a close contact with seeds of the analyzed turfgrasses, which was indicated by the isolation of his fungus from the sowing material which was disinfected on the surface. Kućmierz and Gorajczyk [1991] as well as Wiewióra and Prończuk [2000] report on frequent colonization by *A. alternata* seeds of *Poa pratensis*, *Lolium perenne*, *Poa* spp. and *Festuca* spp., which came from the Polish cultivation, and the grass species from abroad. Pathogenicity of this fungus is connected with the formation of secondary metabolites, including tenauzonic acid and tentoxin, playing a role in pathogenesis of plant diseases [Logrieco et al. 2003, Thomma 2003].

The sowing material of the analyzed grasses was colonized by pathogenic species from genus *Fusarium.* Colonization of grass seeds by those fungi can be the cause of local dying of plants as a result of fusarium blight on the lawns. These pathogens can damage grasses in different periods of their growth and in different seasons [Baldwin and Margot 1990 according to Prończuk 2000].

A mineral medium prepared on grass seeds brew proved useful in the isolation of fungi from genus *Fusarium*. The use of that medium made it possible to isolate the colonies of seven species, namely *F. avenaceum*, *F. culmorum*, *F. crookwellense, F. graminearum*, *F. equiseti*, *F. solani* and *F. sporotrichioides*. *Fusarium avenaceum*, *F. graminearum* and *F. culmorum* are known for their harmfulness towards plants from family *Poaceae* [Kiecana and Kocyłak 1999, Kiecana and Mielniczuk 2001, Gołębniak 2001, Pląskowska et al. 2006]. In the present studies, *F. avenaceum* especially decreased the sporulating ability of common meadow grass cultivar Ani in 2008. In an infection experiment in glasshouse conditions, on the other hand, it caused strong reduction of emergences of *Lolium perenne* [Gołębniak 2001]. That fungus also proved to be a dangerous pathogen of grasses on football pitches [Pląskowska et al. 2006]. The species that especially lowered the sporulation ability of seeds of red fescue Adio cultivar in 2007 included *F. culmorum* and *F. graminearum*. The harmfulness of those fungi towards the plants from family *Poaceae* is reflected, for example, in causing the post-emergence blight of seedlings [Clarke and Eagling 1994, Kiecana and Kocyłak 1999, Kiecana and Mielniczuk 2001, Gołębniak 2001]. *Fusarium graminearum* is considered to be a dangerous pathogen to such grasses as *Agrostis, Bromus, Digitaria, Lolium, Panicum, Poa, Setaria* [Pereyra and Dill-Macky 2008].

The analyzed sowing material of grasses was colonized by *Bipolaris sorokiniana.* Infections of grain by this pathogen occurs via two ways – direct infection of the outer layers of the cell walls of the pericarp and through entering the stigma into the pericarp cells. Secretion of host cell wall hydrolitic enzymes at the apex of the penetrating hyphae may facilitate the spread of the fungus. In addition, toxins secreted by the fungus might explain the rapid death of host cells in contact with or distant to fungall cells [Han et al. 2010].

Special attention should be directed to *Rhizoctonia solani*, which colonized seeds of perennial ryegrass of Pinia cultivar. Studies by Wiewióra and Prończuk [2002] pointed to seeds of perennial ryegrass as a significant source of inoculum of *R. solani.*

Species from genus *Penicillium* were obtained in the present studies both from sporulating and non-sporulating seeds. According to Logrieco et al. [2003], secondary metabolites with the character of phytotoxins produced by species from genus *Penicillium* could have had an effect on the decrease of the sporulating ability of the seeds.

Different methods of infection are used to establish susceptibility of plant genotypes to pathogens, and their choice depends on the quantity of the tested plant material and the conditions in which the studies are conducted [Frank and Christ 1988, Mańka 1989, Almgren et al. 1999, Cegiełko 2008, Kiecana and Mielniczuk 2010].

Considering the differentiated virulence of strains within the fungi populations from genera *Drechslera* and *Bipolaris sorokiniana* [Buchannon and Mc Donald 1965; Makela 1977 according to Gacek 1979, Arabi et al. 1992, Almgren et al. 1999], strains with tested pathogenicity were used in growth chamber studies, using the Mishra and Behr's method [1976].

In studies on the harmfulness of *Drechslera avenae*, *D. siccans* and *Bipolaris sorokiniana* towards the seedlings of turfgrasses in the conditions of a growth chamber experiment, the inoculum of cultures of the analyzed strains of *D. avenae* No. 2, *D. siccans* No. 8 and *B. sorokiniana* No. 69 was used on potato – dextrose medium according to studies by Mańka [1989]. This is a fast and easy way of obtaining large quantities of the infection material. The method proved effective in the studied conditions.

High values of disease indexes, especially in the experimental combination with artificial infection of grass seeds by *D. siccans* and *B. sorokiniana* in the conditions of a growth chamber make it possible to suppose that the thermal conditions favoured the development of pre- and post-emergence blight because the range of temperature for the development of disease symptoms on plants after *D. siccans* infection is from 3°C to 27°C [Paul 1995], whereas for *B. sorokiniana* ranged from 23°C to 30°C [Prates and Fernandes 2001]. The occurrence of brown spots with a chlorotic ring and leaf necrosis was especially observed on the leaves of Inka and Pinia cultivars of perennial ryegrass.

Results obtained in the studies confirmed high harmfulness of *D. siccans* towards turfgrasses, including perennial ryegrass [Lam 1984 according to Paul 1995, Prończuk 2000]. In the conditions of a strict growth chamber experiment, with an increased temperature, that fungus also considerably infected the cultivars of sheep fescue and red fescue.

In the same conditions of temperature and soil, the harmfulness of *Drechslera avenae* towards the seedlings of turfgrasses proved less marked that in the studies by Cegiełko [2006] and Cegiełko et al. [2011] towards oat roots and leaves. Pathogenicity of *D. avenae* is determined by the production of toxic metabolites, including pyrenophorin [Sugawara and Strobel 1986]. In the studies by Kastanias and Chrysayi-Tokousbalides [1999], that compound at the concentration of $7 \cdot 10^{-5}$M caused inhibition of the root growth and it inhibited chlorophyll synthesis in the case of different plant species from genus *Avena*.

Studies on the harmfulness of *Bipolaris sorokiniana* towards the analyzed cultivars of turfgrasses proved a considerable importance of this species like in the case of barley and oat [Almgren et al. 1999, Prończuk 2000, Kiecana and Cegiełko 2007]. The patho-

genicity of *B. sorokiniana* is associated with production of sorokinianin and prehelminthosporol, which are phytotoxic [Nakajima et al. 1998].

CONCLUSIONS

The use of seeds of turfgrasses colonized by pathogenic species from genus *Fusarium* as the sowing material creates a potential danger for the esthetic appearance of the lawn.

Colonization of the sowing material of lawn grasses by *Alternaria alternata*, which is considered a necrotroph with tendencies to conditional parasitism in high humidity, points to the necessity of drying up excessively moist seeds before storing and the need to store them in conditions of little humidity of the air.

Due to considerable harmfulness of *Drechslera siccans* and *Bipolaris sorokiniana* towards the analyzed grasses, these pathogens should be considered in the cultivation of new cultivars of turfgrasses.

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GRZYBY ZASIEDLAJĄCE MATERIAŁ SIEWNY TRAW GAZONOWYCH Z UWZGLĘDNIENIEM PODATNOŚCI ODMIAN NA WYBRANE PATOGENY

Streszczenie. Jedną z przyczyn obniżających estetyczny wygląd trawnika są choroby powodowane przez grzyby. Badania przeprowadzono na materiale siewnym 15 odmian traw gazonowych należących do 5 gatunków. Próby ziarniaków pochodziły ze zbiorów w latach 2007 i 2008. W wyniku analizy mikologicznej w ciągu dwóch lat badań uzyskano 573 izolaty grzybów należących do 28 gatunków i form niezarodnikujących. Z analizowanego materiału siewnego w każdym roku badań uzyskiwano najwięcej *Alternaria alternata*. Spośród grzybów patogenicznych wyizolowano: *Fusarium culmorum*, *F. avenaceum, F. equiseti, F. solani*, *F. crookwellense, F. graminearum, F. sporotrichioides* oraz *Bipolaris sorokiniana*, *Drechslera siccans* i *Rhizoctonia solani.* W warunkach fitotronowych przeprowadzono badania podatności siewek 8 odmian, 4 gatunków traw na porażenie przez *D. avenae* nr 2, *D. siccans* nr 8 oraz *B. sorokiniana* nr 69. 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 odmian. Szczep *D. siccans* nr 8 okazał się najbardziej patogeniczny dla odmiany Info życicy trwałej (92,20), zaś *B. sorokiniana* nr 69 dla odmiany Pinokio wiechliny gajowej (97,75). W kombinacji doświadczenia z *D. avenae* średnie wartości wskaźników chorobowych wynosiły od 12,25 (Pinia) do 84,00 (Info).

Słowa kluczowe: zdrowotność ziarniaków, patogeniczność, *Bipolaris sorokiniana*, *Drechslera avenae, Drechslera siccans*, siewki

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