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RELATIONSHIP BETWEEN SEEDBORNE FUNGI OF RED FESCUE (*FESTUCA RUBRA*) AND SEED GERMINATION CAPACITY

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Abstract

The analysis of seed health and germination capacity of seven cultivars of *Festuca rubra* harvested in 2006, 2007 and 2008 was performed. Seeds deriving from five different localizations in Poland were contaminated by different species of fungi. The most numerous group were saprotrophs or weak parasites such as: *Alternaria alternata, Epicoccum nigrum, Aspergillus* spp. and *Penicillium* spp. Pathogenic fungi were represented by species of *Drechslera, Fusarium, Phoma, Rhizoctonia solani* and *Bipolaris sorokiniana*. Seed germination was influenced by infection of fungi. Seeds more often infected by fungi germinated much poorer than seeds with lower infection degree.

Key words: abnormal seedlings, pathogenic fungi, seed infection, ungerminated seeds

Introduction

Red fescue (*Festuca rubra*) is a perennial and long-living grass species, which can be used both for fodder and lawn. It has low water and soil requirements, and therefore is used as a basic component of mixtures for poor and periodically dry soil. It has also been widely used for turf and is suitable on slopes, roadsides and highways (Rognli et al. 2010).

In Poland there are about 20 species of pathogens, which might contribute to losses in grass cultivation (Kućmierz 1977, Kutrzeba 1994 a, 1994 b, Prończuk 2000). Some of them are associated with the seeds and may play a potential role in causing diseases of seedlings or plants (Agarwal and Sinclair 1997). Contamination of seed lots may be caused by external contamination of the seed coat, but is mainly

Phytopathologia 60: 47–56

ISSN 2081-1756

due to infection of the seeds by these fungi. Therefore, a certain percentage of contaminated seeds may act as an important source of crop infection (Labruyére 1977).

The most dangerous seedborne pathogens of red fescue in turf maintenance are *Drechslera* spp., *Bipolaris* spp. and *Fusarium* spp. (Musiał 1996, Wiewióra and Prończuk 2000, Weber and Urbański 2009). *Drechslera siccans* for example causes brown blight disease on ryegrass during cool weather of spring and fall (Vargas 1994). Species of *Bipolaris* often cause leaf spots as well as stem, crown, and root rots. Disorders caused by *Bipolaris* spp. are often referred to as "melting-out" or "Helminthosporium leaf and crown diseases" and can cause significant damage to turf during prolonged periods of warm wet weather (Smiley et al. 1992). *Fusarium* spp. are causal agents of post emergence damping off and Fusarium blight of grass during summer months (Baldwin 1990). *Rhizoctonia solani* causes brown patch or Rhizoctonia blight disease (Burpee and Martin 1996). All these pathogens may affect field emergence and have negative effects on turf performance. There is not enough information in literature about the role of seedborne fungi in germination process.

The purpose of this work was to investigate seedborne fungi infecting red fescue and their importance for germination capacity.

Materials and methods

Seeds of seven cultivars of *F. rubra* ('Nil', 'Nimba', 'Leo', 'Areta', 'Dorosa', 'Gross', 'Raisa') harvested in 2006, 2007 and 2008 were tested. All of them are on Common Catalogue of Cultivars of Agricultural Plant Species (CCA) and on the OECD list. Seed samples originated from plantations localized in five different locations in Poland:

1 – 19°31'E, 50°92'N,
2 – 19°01'E, 52°07'N,
3 – 16°44'E, 51°12'N,
4 – 20°37'E, 51°36'N,
5 – 17°43'E, 51°18'N.

The mycological assays were carried out on 300 seeds in three replicates of 100, taken from sample of 2 g seed of each cultivar. Seeds were disinfected with 2% sodium hypochlorite for 1 min and then washed with sterile water three times. Disinfected seeds were placed on malt-agar (Malt Extract Agar) medium with 0.003% streptomycin sulphate. Seeds were incubated at 18°C in alternating cycle of 12 h NUV radiation (360 nm) and 12 h darkness. Developed colonies were transferred to malt-agar plates and incubated in the above-mentioned conditions to stimulate sporulation. Fungi were identified after 15–20 days of incubation according to the descriptions of Chidambaram et al. (1974), Malone and Muskett (1997) and Kwaśna et al. (1991).

Germination capacity was estimated according to ISTA rules (International rules... 2010). In germination test abnormal seedlings and ungerminated seeds (healthy ungerminated and dead seeds) were examined for incidence of seedborne fungi. Results are expressed as an average of the three years of harvest.

All data were analysed by means of analysis of variance followed by Duncan's multiple range test. Spermann correlation coefficient (*r*) was calculated with SAS® statistical package (SAS 9.1... 2004, SAS/STAT 9.1... 2004).

Results

Grasses seeds were infested by numerous fungi: 30 fungal species belonging to 26 genera were identified (Table 1). The highest number of colonies was isolated from seeds of cultivar 'Gross' from location number 4 (an average of 79.8 colonies per 100 seeds), and the lowest for seeds of cultivar 'Dorosa' from location 3 (an average of 14.2 colonies per 100 seeds). Species found most often were saprotrophs and weak parasites, such as: *Alternaria alternata, Epicoccum nigrum, Aspergillus* spp. and *Penicillium* spp. *Alternaria alternata* occurred on 42% of seeds, ranging from 21.1% (seeds from 3rd location) to 63.9% (seeds from 4th location; Fig. 1).

Pathogenic fungi were represented by *Drechslera* spp., *Fusarium* spp., *Rhizoctonia solani* and *Bipolaris sorokiniana*. *Drechslera* spp. occurred on seeds of red fescue with mean incidence of 1.5%, however, the strongest infection occurred on seeds from location number 3 – an average of 4.9% (Fig. 1). Three species of this genus were identified: *D. dictyoides, D. triseptata* and *D. siccans*. Different incidence of seed infection by *Drechslera* fungi was found among cultivars: from 0.0 on 'Raisa' (location 5) to 1.0 colony per 100 seeds on 'Leo' (location 2). *Drechslera triseptata* was found only on seeds of cultivar 'Leo' from location 2 (Table 1).

Fusarium spp. infected on average 0.15% seeds of *F. rubra*. These fungi occurred only on seeds of cultivars 'Nil' and 'Areta' (both 0.3 colony per 100 seeds). Two species were isolated: *F. poae* and *F. tricinctum*. Also *B. sorokiniana* was isolated in a small number from red fescue seeds and only from seed sample of cultivar 'Dorosa' (Table 1).

Germination capacity ranged from 44.0% for 'Nil' cultivar harvested in 2006 to 97.3% for 'Areta' from 2007 year of harvest. There were no differences in germination capacity between the examined years of harvest (Fig. 2). The germination capacity values were statistically different between cultivars and ranged from 70.7% for 'Nil' (location 1) to 92.9% for 'Areta' (location 2). The highest seed germination capacity values were obtained also for cultivars 'Gross' and 'Nimba' (90.7 and 88.9%, respectively). 'Nil', 'Dorosa' and 'Raisa' germinated below 80% (Table 2). Besides the cultivars, major factors affecting germination variation were locations (Table 3).

Total number of 16 different species of fungi (saprotrophic and pathogenic, esp. *A. alternata* – mean 6.36%, *Fusarium* spp. – mean 3.34%, *Epicoccum nigrum* – mean 1.54% and *Penicillium* spp. – mean 0.93%) were found on seeds classified in germination test as abnormal seedlings and ungerminated seeds: healthy ungerminated and dead seeds (Table 4). The most often fungi were isolated from seed

Table 1

Fungi infecting red fescue seeds originating from different locations of Poland (mean number of colonies per 100 seeds)

Species	Location							Mean
	1		2		3	4	5	
	'Nil'	'Nimba'	'Leo'	'Areta'	'Dorosa'	'Gross'	'Raisa'	
Acremoniella atra	–	–	–	–	–	0.3	–	0.04
Acremonium spp.	1.3	–	–	–	–	–	1.0	0.33
Alternaria alternata	28.0	18.3	21.3	11.3	3.0	51.0	6.3	19.88
Ascochyta sp.	–	–	–	0.3	–	4.0	–	0.61
Aspergillus spp.	0.7	0.3	2.3	10.7	5.0	0.3	3.0	3.18
Aureobasidium pullulans	1.3	3.7	0.7	0.3	2.0	1.3	0.7	1.43
Bipolaris sorokiniana	–	–	–	–	0.3	–	–	0.04
Botrytis cinerea	–	–	–	–	–	0.3	–	0.04
Chaetomium spp.	–	10.7	2.0	1.0	–	0.3	–	2.00
Cladosporium herbarum	1.0	1.0	0.3	0.3	0.3	0.3	0.3	0.50
Drechslera dictyoides	–	–	–	–	0.7	0.3	–	0.14
Drechslera siccans	0.7	0.3	0.3	0.3	–	–	–	0.23
Drechslera triseptata	–	–	0.7	–	–	–	–	0.10
Epicoccum nigrum	0.7	4.3	1.7	5.0	–	8.7	–	2.91
Fusarium poae	–	–	–	0.3	–	–	–	0.04
Fusarium tricinctum	0.3	–	–	–	–	–	–	0.04
Mucor spp.	–	–	–	0.3	–	–	–	0.04
Nigrospora sp.	–	–	–	0.3	–	–	1.0	0.18
Papularia arundinis	0.7	–	6.7	3.7	–	2.0	1.3	2.06
Papulaspora sp.	–	–	0.3	–	–	–	–	0.04
Penicillium spp.	0.3	0.3	10.0	3.0	1.3	5.0	5.3	3.60
Phoma sp.	1.0	0.7	–	0.3	0.3	0.7	–	0.43
Rhizoctonia solani	–	2.0	–	2.3	–	0.3	–	0.66
Rhizopus spp.	–	–	–	0.7	–	0.3	–	0.14
Septonema chaetospira	–	–	–	0.7	–	–	–	0.10
Septoria sp.	–	–	0.3	–	–	–	–	0.04
Stemphylium botryosum	0.3	0.3	0.7	0.3	–	–	–	0.23
Trichoderma viride	–	–	0.3	–	–	–	–	0.04
Trichothecium roseum	0.7	–	0.7	–	–	–	0.3	0.24
Ulocladium consortiale	1.3	–	–	–	–	2.0	0.3	0.51
Non sporulating colonies	0.3	1.3	2.3	1.0	1.3	2.7	1.0	1.41
Total fungi	38.6	43.2	50.6	42.1	14.2	79.8	20.5	41.23

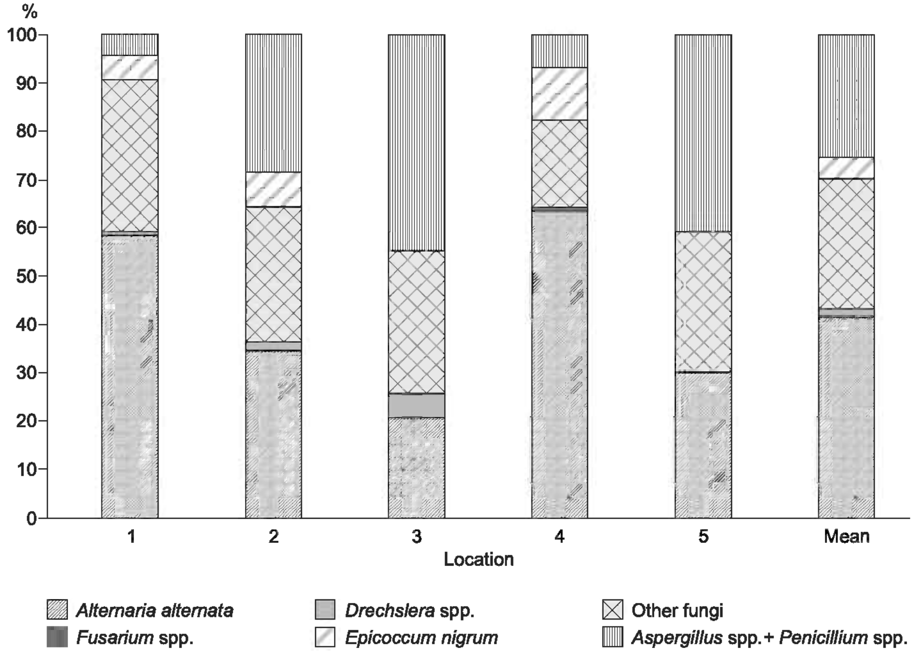

Fig. 1. Share of selected species of fungi infecting seeds of red fescue depending on the location

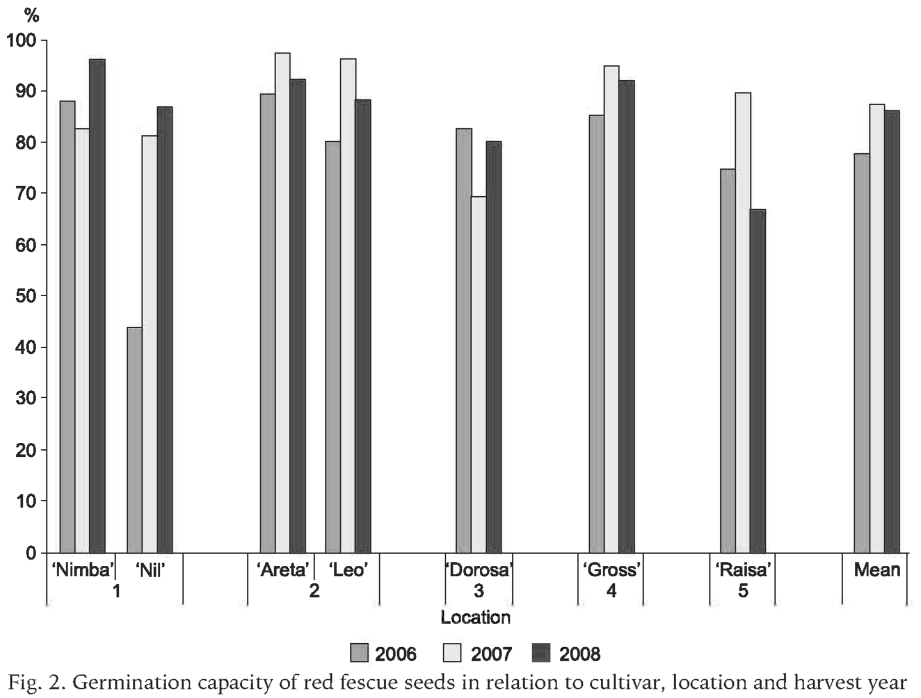

Fig. 2. Germination capacity of red fescue seeds in relation to cultivar, location and harvest year

Table 2

Mean germination capacity of *Festuca rubra* cultivars (%)

Cultivar	Germination
'Areta'	92.9 A
'Dorosa'	77.3 BCD
'Gross'	90.7 A
'Leo'	87.8 ABC
'Nil'	70.7 D
'Nimba'	88.9 A
'Raisa'	76.9 DC
Mean	84.1
LSD	10.71**

The same letters following different means indicate insignificant differences.

Table 3

Results of two-way ANOVA for identified sources of variation

Sources of variation	df	*Festuca rubra*
Locations	4	518.37**
Years	2	225.75
Locations × years	8	272.31**

**Significance of variation with probability of 95%.

Table 4

Occurrence of fungi on ungerminated seeds and abnormal seedlings (mean number of colonies per 100 seeds)

Species	Location							Mean
	1		2		3	4	5	
	'Nil'	'Nimba'	'Leo'	'Areta'	'Dorosa'	'Gross'	'Raisa'	
Alternaria alternata	8.9	5.8	2.2	2.7	7.5	8.5	8.9	6.36
Bipolaris sorokiniana	0.4	–	–	–	–	–	–	0.06
Cladosporium herbarum	0.8	0.8	–	–	0.8	0.4	–	0.40
Colletotrichum sp.	–	–	–	–	0.4	–	–	0.06
Curvularia sp.	0.9	–	–	–	4.0	–	–	0.70
Drechslera dictyoides	–	–	–	–	0.9	0.4	–	0.18
Drechslera siccans	0.4	–	0.4	–	0.4	–	–	0.17
Drechslera triseptata	–	–	0.4	–	–	–	–	0.06
Epicoccum nigrum	3.6	0.9	1.3	0.9	1.4	–	2.7	1.54
Fusarium spp.	5.8	3.1	4.4	3.1	3.5	3.5	–	3.34
Mucor spp.	–	–	–	–	0.4	–	0.4	0.11
Penicillium spp.	0.4	0.4	0.9	–	1.7	1.3	1.8	0.93
Phoma sp.	1.3	0.8	–	0.4	0.9	–	0.4	0.54
Rhizopus spp.	2.2	0.9	0.4	0.4	0.9	0.4	0.4	0.80
Trichothecium roseum	0.9	0.4	–	–	–	–	–	0.18
Ulocladium consortiale	–	–	0.4	–	–	–	–	0.06
Total fungi	25.6	13.1	10.4	7.5	22.8	14.5	14.6	15.49

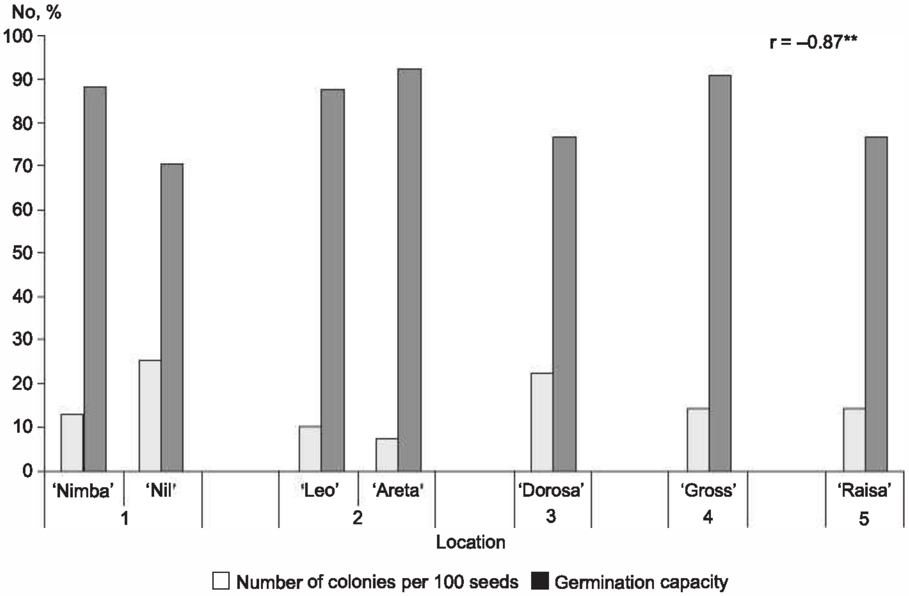

Fig. 3. Relationship between germination capacity and fungal abundance on abnormal seedlings and ungerminated seeds in germination test (mean for three years of the investigation)

samples of two cultivars: 'Nil' (location 1) – 25.6 colonies and 'Dorosa' (location 3) – 22.8 colonies per 100 abnormal seedlings and ungerminated seeds. Also, three species from genus *Drechslera*: *D. dictyoides, D. siccans* and *D. triseptata* were noted, but in small amounts. The statistical analysis revealed highly significant negative correlation (r = –0.87**) between germination capacity and number of fungi that colonized tested seeds (Fig. 3).

Discussion

The studies revealed that seeds of red fescue originating from different growing areas of Poland might be contaminated by saprotrophs as well as by pathogens. The most important saprotrophs and parasites infecting seeds of *F. rubra* – *A. alternata, E. nigrum, Fusarium* spp., *Drechslera* spp. and *Penicillium* spp. – were previously found on grass seeds in Poland by Kućmierz et al. (1992), Kutrzeba (1994 b), Wiewióra and Prończuk (2000) or Pańka et al. (2010).

The most often occurring saprotrophs were *A. alternata* and *E. nigrum*. According to Tulloch and Leach (1972) and Labruyére (1980) the saprotrophic fungi can be used as a rough indicator of the seed quality. A marked increase in common saprotrophic fungi was noticed when seed was harvested under adverse weather conditions. These microorganisms, due to their competitive and hiperparasitic properties can facilitate the control of pathogens or cause difficulty in isolation of pathogens.

The mycological assay showed also that seedborne pathogens of red fescue, especially *Drechslera* spp., *B. sorokiniana* and *Fusarium* spp. occurred rarely. According to Labruyére (1980) the presence of slower-growing pathogenic fungi could have been masked by faster-growing saprotrophs, when routine health testing was carried out. Madsen and Hodges (1980) found that *B. sorokiniana* reduced the amount and rate of seedling emergence and increased seedling mortality. However, Musiał (1996) claimed that both field and storage fungi caused decrease of seed value.

Major factors affecting variation of red fescue germination capacity were locations. It means that local conditions: soil, pre-crop, management or harvest conditions play an important role in seeds development and sowing value. Polish quality standards define the minimum germination capacity for red fescue at 75% (Rozporządzenie... 2007). In this work germination capacity for the one tested sample of seeds did not meet the requirements for sowing material, and it can be assumed that the reason of the low germination capacity could be their infection by fungi.

This study shows that abnormal seedlings and ungerminated seeds were often infected by fungi – both saprotrophs and pathogens: *A. alternata, E. nigrum, Fusarium* spp. and *Penicillium* spp. Their occurrence affected the level of germination. Similar results were obtained by Zang et al. (2006) for perennial ryegrass. They found that some seedborne fungi, especially *Fusarium* spp. and *B. sorokiniana* significantly reduced germination producing secondary metabolites or directly killing seed tissues. The same effect was observed for *A. alternata, Cladosporium herbarum* and *Drechslera phlei*, but it was less distinct than that caused by *Fusarium* spp. and *B. sorokiniana*. Kućmierz and Gorajczyk (1991) found also that *A. alternata* greatly inhibited germination capacity, but decreased their germination energy only to a small degree.

Streszczenie

GRZYBY ZASIEDLAJĄCE NASIONA KOSTRZEWY CZERWONEJ (*FESTUCA RUBRA*) A ZDOLNOŚĆ KIEŁKOWANIA NASION

Materiał do badań stanowiły nasiona siedmiu odmian gazonowych *Festuca rubra*: 'Nil', 'Nimba', 'Leo', 'Areta', 'Dorosa', 'Gross' i 'Raisa' ze zbioru w latach 2006, 2007 i 2008. Nasiona otrzymano od hodowców lub z firm nasiennych, których plantacje były zlokalizowane w pięciu różnych miejscach w Polsce. Przeprowadzono analizę fitopatologiczną, która wykazała zasiedlenie badanego ziarna przez 10–18 gatunków grzybów, należących do 26 rodzajów. Najwięcej grzybów wyizolowano z nasion odmiany 'Gross' z plantacji położonej w lokalizacji nr 4, najmniej zaś oznaczono z nasion odmiany 'Dorosa' (lokalizacja nr 3). Najliczniejszą grupę stanowiły grzyby znane jako saprotrofy lub pasożyty okolicznościowe, takie jak: *Alternaria alternata, Epicoccum nigrum* i *Penicillium* spp. Spośród grzybów patogenicznych wystąpiły gatunki należące do rodzajów *Drechslera* i *Fusarium*, jednak stanowiły one niewielką część wszystkich wyizolowanych grzybów. Odmiany istotnie

różniły się zdolnością kiełkowania: od 70,7% w przypadku odmiany 'Nil' (lokalizacja plantacji nr 1) do 92,9% w przypadku odmiany 'Areta' (lokalizacja nr 2). Na ziarniakach zakwalifikowanych w trakcie oceny zdolności kiełkowania jako nienormalnie kiełkujące, zdrowe niekiełkujące bądź martwe najczęściej występowały *A. alternata, E. nigrum* oraz grzyby rodzajów *Fusarium* i *Penicillium*, które mają istotny wpływ na zdolność kiełkowania nasion zbóż i mogą stanowić dodatkowe źródło infekcji roślin w polu.

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Accepted for publication: 17.05.2011