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THE EFFECT OF SEED HEALTH OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.) ON GERMINATION CAPACITY

ABSTRACT

The analysis of seed health and germination capacity of 7 varieties of *Lolium perenne*, originated from two breeding station and two seed company localized in different places of Poland, harvested in 2006, 2007 and 2008 were performed. Results of mentioned analysis indicated that seeds were contaminated by numerous species of fungi. The most numerous group of fungi were saprophytes or weak parasites such as: *Alternaria alternata*, *Epicoccum nigrum*, *Septonema chaetospira* and *Penicillium* spp. Pathogenic species were represented by species of *Drechslera*, *Fusarium*, *Phoma*, *Curvularia lunata* and *Bipolaris sorokiniana*. Seed germination capacity was influenced by contamination of fungi. It was found that seed more often contaminated by fungi germinated much poorer then seed with lower infection.

Key words: seed borne fungi, perennial ryegrass, germination capacity

INTRODUCTION

The primary species found worldwide and used for both lawns and as a forage crop is perennial ryegrass (*Lolium perenne* L.). Like many cool-season grasses, it is often infected by fungi as well as pathogens and saprophytes (Prończuk 2000, Wiewióra and Prończuk 2002). Some of these fungi associated with seeds, are of the potential ability to induce diseases of seedlings or plants (Agarwal and Sinclair 1997). Diseases play a major role in determining the success or failure of a turfgrass stand or seed crop. It is often the most important factor limiting the successful growth of a variety, therefore it is important in selection process of breeding (Vargas 1994).

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The most dangerous seedborne pathogens of perernnial ryegrass in turf maintenance are *Drechslera* spp., *Bipolaris* spp. and *Fusarium* spp. (Kućmierz and Gorajczyk 1991a, Kutrzeba 1994, Musial 1996, Wiewióra and Prończuk 2000, Weber and Urbański 2009). The Drechslera and *Bipolaris* fungi are widespread on both cool- and warm- season turfgrass species. They can attack seedlings and plants causing leaf spot (*Bipolaris sorokiniana*), net-blotch (*Drechslera dictyoides*) or brown blight (*D. siccans*) (Vargas 1994). *Fusarium* spp. are causal agents of post emergence damping off and *Fusarium* blight of grass during the summer months (Baldwin 1990). All these pathogens influence on field emergence and have negative effects on turf performance. It's commonly known that breeders have some problems with germination of sowing material of grasses and often they have no idea, what is the reason of this situation. There is no enough information in literature about the role of seedborne fungi in germination process.

The creation of the Common Catalogue of Varieties of Agricultural Plant Species allowed entry for Polish varieties into the EU market, as well as worldwide. It seems important that potential buyers can find not only short information for interesting varieties contained in this catalog, but also others such as seed health or germination of sowing materials. This contributed to the research topic in the work undertaken.

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

MATERIALS AND METHODS

Seeds of 7 varieties of *Lolium perenne* (fodder: Diament, Solen, Gagat, Naki and turf: Grilla, Nira, Pinia) originated from four locations in Poland harvested in 2006, 2007 and 2008 were tested (Fig. 1). All of them are on Common Catalogue of Varieties of Agricultural Plant Species (CCA) and all except of variety Pinia are on the OECD list.

The mycological assays were carried out on 100 seeds in three replications taken from sample of 2 g seed of each cultivar. Seeds were disinfected with 2% sodium hypochlorite for 1 minute and then washed with sterile water three times. Disinfected seeds were placed on malt-agar medium with streptomycin. Fungal colonies were grown at 18°C in alternating cycle of 12 h NUV radiation (360 um) and 12 h darkness. Developed colonies were transferred to malt-agar plates and incubated in 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). Data were presented as mean number of colonies of 100 seeds in 4 replication (50 seeds in every replication were tested).

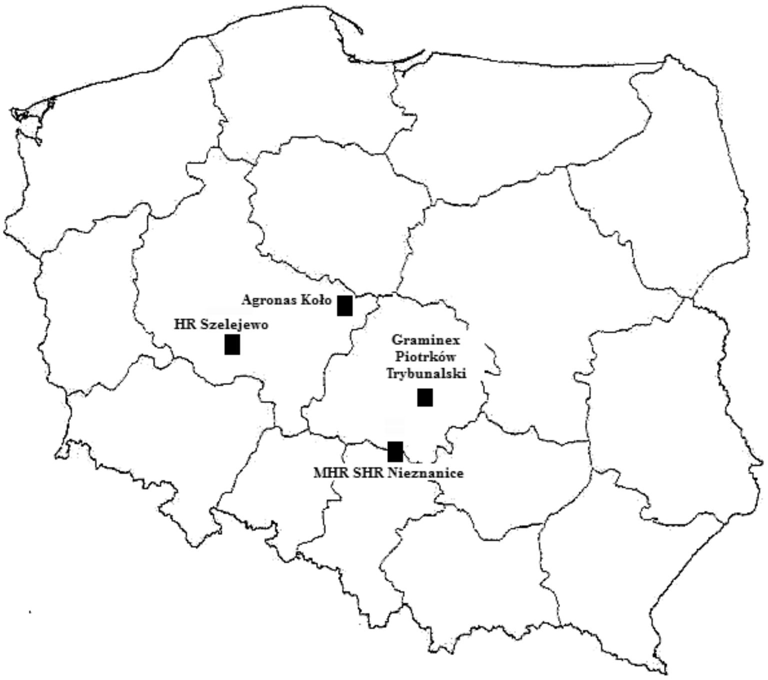

Fig. 1 Localization of breeding stations and seed companies from which come seeds for research

Germination capacity was estimated according to ISTA rules (ISTA, 2010). Seeds in germination test classified as abnormal seedlings and ungerminated seeds were examined for incidence of seedborne fungi.

All data were analyzed by means of analysis of variance followed by Duncan's multiple range test. Spermann correlation coefficient (r) was calculated with SAS® statistical package (SAS 2004a, SAS 2004b).

RESULTS

Seeds of *Lolium perenne* were contaminated by numerous fungi (mean 75.80 colonies/100 seeds): 38 fungal species belonging to 26 genera (from 10 to 25 depending on variety and seed origins) (Table 1). It was found that the most often fungi occurred on seeds from location 4 (breeding station in Nieznanice): mean 95.5 colonies/100 seeds (range from 87.9 to 103.1). The lowest number of fungal colonies was observed on seeds originated from location 1 (breeding station in Szelejewo): mean 51.9 colonies/100 seeds (from 46.7 to 55.8 depending on variety). The most numerous were saprophytes and weak parasites, such as: *Alternaria alternata*, *Epicoccum nigrum*, *Septonema chaetospira*, and *Penicillium* spp. *Alternaria alternata* was detected in 46.8% of seeds (from 20.0% for variety Diament to 73.3% for variety—Pinia). *Septonema chaetospira* occurred on seed of five examined varieties and contamination ranged from 0.3 to 17.0% (mean 5.56%). It should be noted that this species occurred only in varieties from locations 1 and 4. Seeds from the other two locations were free of this fungus. Infection by *Epicoccum nigrum* and *Penicillium* spp. was much lower: 3.74% (from 0.3 to 9.3%) and 2.40% (from 0.0 to 13.7%), respectively.

Table l

Seed health of perennial ryegrass originated from different localities of Poland

Species	Locality							Mean
	1			2	3	4		
	Variety							
	Solen	Dia-ment	Gagat	Naki	Grilla	Nira	Pinia	
Acrenloniella atra (Corda) Sacc.					1.0	0.3		0.18
Acremonium spp.							0.3	0.04
Alternaria alternata (Fr) Keissler	27.3	20	28.3	68.7	48.7	61.3	73.3	46.8
Ascochyta sp.		1		0.3	1			0.33
Aspergillus spp.			0.7		2.3			43.00
Aureobasidium pullulans (de Bary) Arn.	1.3	0.3	5.0	0.3	0.7	0.3	0.3	1.17
Bipolaris sorokiniana (Sacc.) Shoem.				0.3		0.3	0.3	0.13
Botrytis cinerea Pers. ex Fr.	-	-	0.3	-	-	0.3	-	0.08
Chaetomium sp.	-	0.3	-	-	-	-	-	0.04
Cladosporium herbarum (Pers.) Link ex Fr.	3.0	1.3	2.3	1.0	-	1.0	2.0	1.51
Curvularia lunata Wakker) Bedijn	-	0.3	-	-	0.3	0.3	0.3	0.17
Drechslera dictyoides (Drechs.) Shoem.	-	-	-	-	1.0	2.3	0.3	0.51
*Drechslera siccans (*Dreechs.) Shoem.	3.0	1.7	3	1.7	2.3	7.7	2.0	3.06
Drechslera triseptata (Drechs.) Subram et Jain.	-	-	-	-	-	-	0.3	0.04
Drechslera spp. — TOTAL	3.0	1.7	3.0	1.7	3.3	10.0	2.6	3.61
Epicocum nigrum Link.	1.3	0.3	3.0	2.3	9.3	4.0	6.0	3.74
Fusarium avenaceum (F r.) Sacc.	-	-	-	0.7	4.7	-	1.0	0.91
Fusarium culmorum (W.G. Smith) Sacc.	-	-	0.3	-	0.7	-	0.7	0.24

Table l

Continued

Species	Locality							Mean
	1			2	3	4		
	Variety							
	Solen	Diament	Gagat	Naki	Grilla	Nira	Pinia	
Fusarium equiseti (Corda) Sacc.	-	-	0.3	0.3	1.0	0.7	2.0	0.61
Fusarium graminearum Schwabe	-	0.3	0.3	0.7	0.7	0.7	0.3	0.43
Fusarium oxysporum Schl. cmend. Snyd et Hans	-	0.3	-	-	0.3	-	-	0.24
Fusarium poae (Peck) Wollenw.	-	-	-	1.3	0.3	-	-	0.28
Flusarium semitectum Berk. et Rav.	-	-	0.3	-	-	-	0.3	0.08
Fusarium solani (Mart) Sacc. (G,BJ)	0.3	0.3	0.3	0.3	1.7	0.7	2.0	0.80
Fusarium sporotrichioides Sherb.	-	-	-	0.3	1.3	0.3	-	0.27
Fusarium tricinctum (Corda) Sacc.	-	-	-	2.0	1.0	1.0	0.3	0.61
Fusarium spp. — TOTAL	0.3	0.9	1.5	5.6	11.7	3.4	7.9	4.47
Gliocladium sp.	2.0	0.3	1.3	0.7	-	-	0.3	0.66
Melanospora sp.	-	0.3	-	-	-	-	-	0.04
Mucor spp.	-	-	-	-	0.7	-	-	0.10
Papularia arundinis (Corda) Fr.	-	-		1.7	0.7	-	0.3	0.38
Papulaspora sp.	-	-	0.7	-	-	-	-	0.10
Penicillium spp.	-	-	1.7	13.7	0.7	0.7	-	2.40
Phoma sp.	-	-	-		0.3	0.7	-	0.14
Rhizopus spp.	-	-	-	1.7	-	1.0	-	0.38
Septonema chaetospira (Grove) Hughes	16.30	17.0	4.0	-	-	0.3	1.3	5.56
Sordaria fimicola (Rob.) Ces. ex de Not.	0,30	-	0,6	-	-	-	4.3	0.70
Stemphylium botryosum Wallr.	1.00	0.3	1.0	0.7	1.0	0.7	2.3	1.00
Stemphylium consortiale (Thürn.) Gr. et Skol.	-	1.7	-	1.0	1.3	3.3	1.3	1.23
Trichoderma viridae Pers. ex F r.	-	-	-	-	0.7	-	-	0.10
Non sporulating colonies	-	0.7	-	-	0.3	-	0.3	0.18
Total fungi	55.8	46.7	53.1	99.7	84.7	87.9	103.1	75.8

Pathogenic fungi were represented by *Drechslera* spp., *Fusarium* spp., *Curvularia lunata* and *Bipolaris sorokiniana*. According to mycological assays, *Drechslera* spp. occurred on seeds of perennial ryegrass at an incidence of 3.61 %, however, the strongest infection was observed on seeds from location 4 - an average of 6.3%. Three species of this genus were identified: *D. dictyoides*, *D. triseptata* and *D. siccans*. The latter was observed most often (mean infection 3.06%). Different incidence of seed infection by *Drechslera* fungi were found among varieties from 1.7% on Naki and Diament to 10.0% on Nira. *D. triseptata* was found only on seeds of variety Pinia from location no. 4 (Table 1).

Fusarium spp. infected almost 5% seeds of *Lolium perenne*. These fungi occurred more abundantly on seeds of variety Grilla and Pinia (11.7 and 7.9%, respectively). Ten species were isolated in this genus: *F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. poae, F. semitectum, F. solani, F. sporotrichioides* and *F. tricinctum*. Among them *F. avenaceum* and *F. solani* were the most frequent ones. Other pathogens as well as *Curvularia lunata* and *Bipolaris sorokiniana* were isolated in a smaller number from perennial ryegrass seeds.

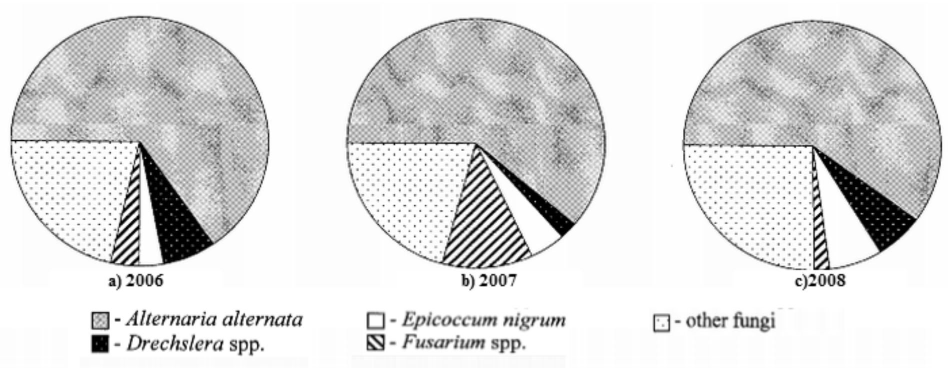

Fig. 2. Share of selected species of fungi contaminating seeds of perennial ryegrass depending on the year of harvest

Table 2

Results of two-way ANOVA for identified sources of variation

Sources of variation	DF	Mean Square Errors
Localities	3	181.65**
Years	2	143.39
Localities × Years	6	260.06**

' * - significance of variation at probability of 95 and 99% respectively

It was also found that composition of fungal communities was different in respect of year of harvest. The smallest amounts of fungi were found seed harvested in 2006: mean 55.1 colonies per 100 seeds. Seeds harvested in 2008 and 2007 were contaminated by fungi in significantly higher range: from 84.3 to 88.2 respectively. The most often isolated fungus *Alternaria alternata* was observed in the largest number on seeds harvested in 2007 (mean 53.8 colonies/ 100 seeds) in comparison to seeds harvested in

2008 and 2006 (mean respectively 50.6 and 36.0 colonies/100 seeds). Interesting relations were observed for seed infection by *Drechslera* and *Fusarium* fungi. It was found that on seed with the stronger infection caused by *Fusarium* spp., *Drechslera* fungi were observed less frequently. Also, the greater infection by *Drechslera* was associated with fewer occurrences of *Fusarium* fungi. Seeds harvested in 2008 was mostly infected by *Drechslera* spp. (mean 5.1%), but infection by *Fusarium* spp. of these seeds was the smallest (mean 1.7%) in comparison to seeds harvested in other years (Fig. 2c). The similar relation was observed for seeds harvested in 2007, which were infected by *Fusarium* to the largest extent number (mean 9.9% infected seeds), but *Drechslera* fungi were found on these seeds in the smallest amount (mean 2.1%) (Fig. 2b). Only seeds from 2006 year were infected by fungi from both genera in medium level (3.6% for *Drechslera* spp. and 2.0% for *Fusarium* spp.) (Fig. 2a).

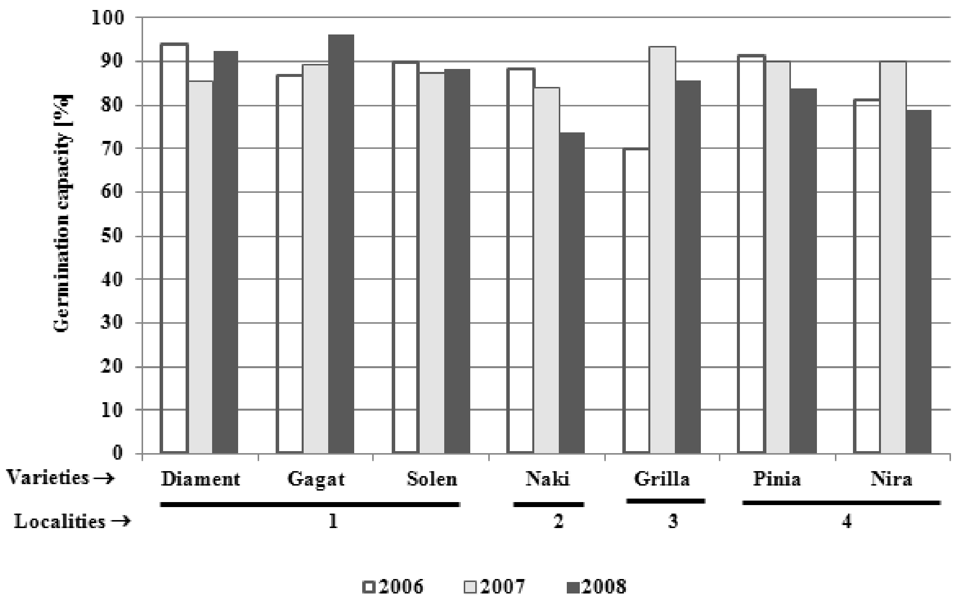

Fig. 3. Germination capacity of perennial ryegrass depending on cultivars, localities and a year of harvest

Germination of all tested perennial ryegrass varieties were not statistically different and ranged from 70.7% for Grilla variety in 2006 to 97,3% for Gagat variety in 2008 (Table2 and Fig. 3). The seed samples of two varieties from all examined germinated below 80%: Grilla harvested in 2006 (70.7%) and Naki from harvest 2008 (74.7%). It was found that germination capacity was correlated with fungi occurrence on perennial ryegrass seeds, especially *Alternaria alternata* and *Fusarium* spp. (Table 3). Total number of 16 different species of fungi (saprophytic and pathogenic, esp. *Alternaria alternata*, *Epicoccum nigrum*, *Fusarium* spp. and *Septonema chaetospira*) were found on abnormal seedlings and ungerminated seeds used to germination test (Table 4). Also, two species from genus *Drechslera*: *D. diclyoides* and *D. siccans*, were noted but in small amounts. The statistical analysis revealed highly significant negative correlation (r = - 0.88***) between germination capacity and number of fungi that colonized tested seeds (Fig. 4).

Table 3

Correlation coefficient between germination capacity and fungi occurring on perennial ryegrass seeds

Fungi	Correlation coefficient
Alternaria alterrnata	-0.660**
Drechslera spp.	-0.403*
Fusarium spp.	-0.617**
Total fungi	-0,716***

***Significant at α = 0.01; **Significant at α = 0.05, *Significant at α = 0. I

Table 4

Occurrence of fungi on seeds of perennial ryegrass classified during germination as ungerminated seeds and abnormal seedlings

Species	Locality							Mean
		1		2	3	4		
	Variety							
	Diament	Solen	Gagat	Naki	Grilla	Pinia	Nira	
Acrenloniella atra (Corda) Sacc.	-	-	-	0.9	-	-	-	0.13
Altemaria alternate (Fr) Keissler	4.3	4.0	4.8	15.8	13.0	9.4	7.6	8.41
Aspergillus spp.	-	-	-	0.9	-	-	-	0.13
Botryris cinerea Pers. ex Fr.	0.4	0.9	0.4	1.3	1.7	-	0.3	0.71
Cladosporium herbarum (Pers.) Link ex Fr.	0.9	0.4	0.4	0.3	0.4	-	0.4	0.40
Drechslera dictyoides (Drechs.) Shoem.	-	-	0.4	-	0.4	-	0.7	0.21
*Drechslera siccans (*Drechs.) Shoem.	0.3	0.3	-	-	-	-	0.9	0.21
Epicocum nigrum Link.	1.2	1.7	1.7	0.9	2.1	0.4	2.2	1.46
Fusarium spp.	1.3	1.4	1.3	2.6	3.2	0.3	1.9	1.71
Mucor spp.	0.4	1.7	0.4	-	-	-	-	0.36
Penicillium spp.	-	-	-	1.8	-	-	-	0.26
Phoma sp.	-	0.4	-	-	-	0.3	0.8	0.21
Rhizopus spp.	-	0.3		0.4	0.4		0.4	0.21
Septonema chaetospira (Grove) Hughes	2.7	1.3	3.1	-	-	-	-	1.01
Stemphylium botryosum Wallr.	-	0.3	-	-	-	-	-	0.04
Stemphylium consortiale (Thürn.) Gr. et Skol.	-	-	-	0.8	1.4	-	1.4	0.51
Total	11.5	14.5	10.7	24.8	22.6	10.4	16.6	15.97

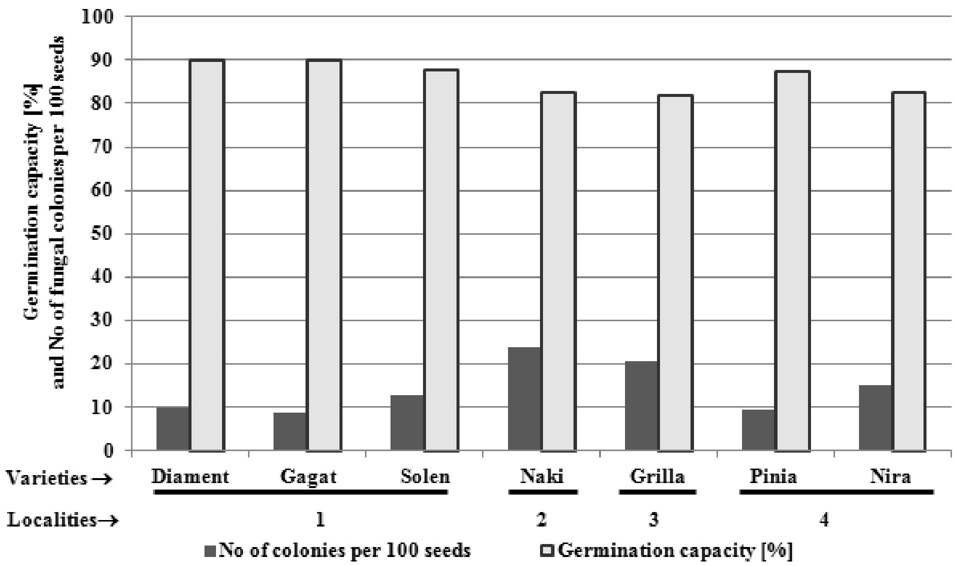

Fig. 4. Relationship between germination capacity and fungal colonies occurred on seeds using during germination test (average for three-year investigation)

DISCUSSION

General conclusion from above study is that perennial ryegrass seed from different growing areas of Poland might be contaminated by saprophytes as well as by pathogens. Most of fungi reported in this paper were previously found on grass seeds in Poland by Kućmierz *et al.* (1992), Kutrzeba (1994) or Wiewióra and Prończuk (2000).

The most important saprophytes and weak parasites contaminating seeds of *Lolium perenne* were: *Alternaria alternata*, *Epicoccum nigrum* and *Septonema chaetospira*. The similar results were obtained by Tulloch and Leach (1972), Kućmierz and Gorajczyk (199la), and Varga and Fischl (2005). According to Tulloch and Leach (1972) and Labruyere (1980) the saprophytic mycoflora can be used as a rough indicator of the quality of seed. A marked increase in common saprophytic fungi were 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 made difficulty in isolation of pathogens. *Septonema chaetospira* (Grove) Hughes is a dematiaceous hyphomycete reported from wood, arthropod droppings, soil, and roots of plants collected from their natural habitats in Canada, Europe, Japan and Russia (Ellis, 1976; Domsch and Gams, 1972; Wilson *et al.*, 2004) as well as from the roots of herbaceous species grown as bait plants in field soils collected in Japan (Narisawa *et al.* 1998, 2002). Nothing has been found in literature concerning the prevalence of this fungus on seeds, but it is commonly known that many species occurred on the seed as well as in soil, e.g. *Bipolaris sorokiniana*, *Fusarium* spp. or *Rhizoctonia* (Singleton *et al.* 1992). Seed infection of *Lolium perenne* by *S. chaetospira* might relate also to the degree of plant lodging and fungus survives as mycelium or spores in soil or plant root (Narisawa *et al.* 2007). Plant lodging could make the contact of plants with fungus easier. The ability of *S. chaetospira* to grow in the roots of annual bait plants and in the roots of perennial species suggests little host specificity (Jumpponen and Trappe 1998).

The mycological assay showed also that seed of *L. perenne* were often contaminated by *Fusarium* species. These fungi were represented by ten species, especially *F. avenaceum, F. solani, F. equiseti* and *F. tricinctum*. It was observed that seed from the location no. 3 were the most often infected by *Fusarium* fungi (mean 11.7% infected seeds) with more abundantly occurring *Fusarium avenaceum*, which infected almost 5% of all examined seeds. The similar results were obtained by Vargas (1994) and Wiewióra and Prończuk (2002).

Three *Drechslera* species were isolated from seeds: *D. siccans*, *D. diclyoides* and *D. triseptata* where the first one was most frequently observed. These fungi were isolated from *L. perenne* seeds by many researches (Kućmierz and Gorajczyk 1991a, Vargas 1994, Musial 1996). Vargas (1994) reported, that *D. siccans* causes brown blight disease on perennial ryegrass. That disease is one of the factors limiting the successful adaptation of some of the new perennial ryegrass varieties, selected for their finer texture and better mow ability, with little regard for potential disease problems. The share of pathogens: *Fusarium* spp. and *Drechslera* spp. colonizing the seeds was much smaller in comparison to occurrence of saprophytes, especially *Alternaria alternata* on these seeds. According to Labruyere (1980) the presence of slower-growing pathogenic fungi might be masked by faster-growing saprophytes when routine health testing was carried out.

Major factors affecting variation of germination capacity of perennial ryegrass were localities. Therefore, not the climatic conditions during seed development and harvest but specific local conditions (soil, pre-crop, management, harvest etc.) were major forces driving variation between tested varieties. Polish quality standards define the minimum germination for perennial ryegrass at 80% (Official Gazette of the Republic of Poland No. 29, 2007). Germination capacity for the two tested samples of seeds did not meet the requirements for perennial ryegrass sowing material, and it can be assumed that the reason of the low germination could be their infection by fungi.

This study shows that abnormal seedlings and ungerminated seeds were often infected by fungi as well as saprophytic and pathogens: *Alternaria alternata*, *Epicoccum nigrum*, *Fusarium* spp. and *Septonema chaetospira*. It was also found that they affected on the level of germination. Lewis (1988) reported that *Fusarium culmorum* was the main fungal pathogen isolated from ungerminated seeds and dead seedlings of perennial ryegrass. The similar results were obtained by Zang *et al.* (2006) in the investigation about the effects of mycoflora on the germination and seedling vigour. They found that some seedborne fungi of perennial ryegrass, especially *Fusarium* spp. and *Bipolaris sorokiniana* significantly reduced germination through the production of secondary metabolites or by directly killing the seed tissues. The same effect was observed for *Alternaria alternata*, *Cladosporium herbarum* and *Drechslera phlei*, but it was less than caused by *Fusarium* spp. and *B. sorokiniana*. Kućmierz and Gorajczyk (l99lb) found also that *A. alternata* greatly inhibited germination but decreased their germination energy only to a small degree. *C. herbarum*, often considered to be saprophyte, also showed a pathogenicity to germinating caryopses and permanent ryegrass seedlings.

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