P L A N T   B R E E D I N G   A N D   S E E D   S C I E N C E
Volume 53 2006

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OCCURRENCE OF ERGOT IN SEED LOTS OF GRASSES HARVESTED IN DIFFERENT REGIONS OF POLAND

ABSTRACT

The ergot (*Claviceps purpurea*) incidence in seed samples of *Poa pratensis*, *Lolium perenne*, *L. multiflorum*, *Festuca rubra* and *F. pratensis* cultivars originating from different regions of grass cultivation in Poland was detected in 1999 – 2002. Each year, about hundred twenty cultivars of different species were tested. It was found that number of seed replaced by sclerotia significantly varied and depended on the year of seed harvest, species and cultivar tested and region of grass grown for seed. The highest level of *Claviceps purpurea* sclerotia almost for all grass species was detected in 1999. The highest number of seed replaced by sclerotia was found in sowing material of *Poa pratensis* for all years of testing. Ergot in seed lots of *L. multiflorum* and *F. pratensis* were detected at trace levels. Differences in susceptibility to *Claviceps purpurea* among cultivars were observed. However, significant variation in occurrence of ergot sclerotia were noted for the same cultivar harvested in different region of Poland. It was found that size of sclerotia depended on the host - grass species and the year of seed harvest. Significant differences were noted particularly in the length and weight of sclerotia.

Key words: *Claviceps purpurea*, *Festuca rubra,* grass seeds, *Lolium perenne*, *Poa pratensis*, size of sclerotia

INTRODUCTION

Ergot, caused by *Claviceps purpurea* (Fr.) Tul. is a widespread floral disease of grasses grown for seed and can infect circa 200 species of wild and cultivated grasses (Alderman *et al.*, 2004). The fungus over-winters as sclerotia in the soil surface. Sclerotia germinate forming a stroma during spring to early summer (Bretag and Merriman, 1981). Ascospores are released from the ascoma in stroma during flowering time of grasses and are windblown or rain splashed to the flower. Infection occurs via the stigma and the fungus grows down the pollen tube into the ovary. Rainfall is an important factor in the process of infection. Higher rainfall in spring and during flowering stimulated development of this fungus (Mühle *et al.* 1975; Alderman, 1991; Alderman and Barker, 2003; Cagaš, 1992; Prończuk and Wiewióra, 1999). During early stage of infection the fungus produces conidia in “honeydew” that oozes from infected ovaries. Conidia as secondary inoculum are spread by direct contact with uninfected florets, insects and splashing rain. In the late stage of infection *C. purpurea* produces purplish–black sclerotia in infected florets. Yield losses from ergot can occur through seed

Communicated by Edward Arseniuk

replacement, reducing seed production in infected spikelet, lower seed weight, and loss of healthy seed during re-cleaning to remove ergot (Alderman *et al.* 1996, Schultz *et al.* 1993).

Recently, increase of grass infection by *C. purpurea* has been observed in Poland. Our previous investigations shown that some cultivars of ryegrass (*Lolium perenne*) were infected by the fungus in 35% (at Radzików) and smooth-meadow-grass (*Poa pratensis*) even in 60% (at Bartążek) in 1997 (Prończuk and Wiewióra, 1999).

The purpose of the research initiated in 1999 at Radzików was to examine the occurrence of ergot sclerotia samples in seed of grasses harvested in different regions of Poland, determine the losses of seed yield, and characterise the sclerotia of *C. purpurea* found in tested grass species.

MATERIALS AND METHODS

Data concerning the ergot contamination in grasses seed samples were collected in 1999-2002. Seeds of smooth-meadow-grass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*L. multiflorum* Lam.), red fescue (*Festuca rubra* L.) and meadow fescue (*F. pratensis* L.) cultivars originating from different regions of grass cultivation in Poland were tested. Four hundred sixty nine seed samples were examined. In each year, about hundred twenty cultivars of different species were tested (Table 1). Number of sclerotia in a sample containing 2500 grains for each cultivar, in 3 replication, according to the ISTA recommendation (ISTA, 1996) was estimated. Losses of yield expressed as a percentage of grain number replaced by ergot, and as a percentage of sclerotia mass in the most affected cultivars by ergot were determined. Sclerotia produced in inflorescence of different grass species were characterised and their biometrical characters such as: length, thickness and weight were measured. One hundred sclerotia originated from each grass species were analysed in consecutive years.

Table 1

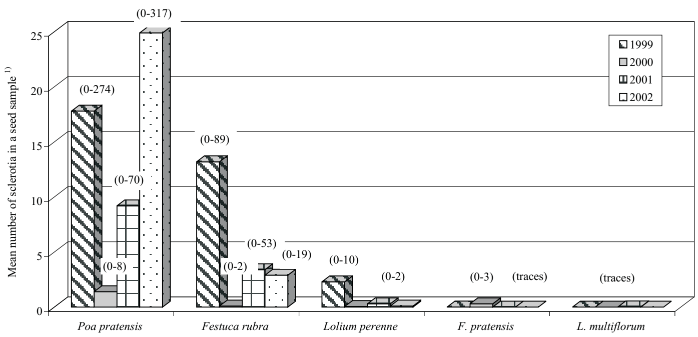
Number of seed crops and cultivars of grass species tested in 1999-2002.

Species of grass	Number of field crops				Number of cultivars			
	1999	2000	2001	2002	1999	2000	2001	2002
Poa pratensis	42	30	28	36	16	15	16	17
Festuca rubra	40	48	34	49	16	17	13	19
Festuca pratensis	10	7	7	3	6	4	7	3
Lolium perenne	29	27	27	32	16	12	11	12
Lolium multiflorum	5	3	8	4	4	2	7	3
Total	126	115	104	124	58	50	54	54

Data were summarised and subjected to the three factors ANOVA with the use of split-plot design according to Wójcik and Laudański (1989).

RESULTS

The studies have shown significant variation in disease prevalence in successive years. The highest level of ergot sclerotia was observed in 1999 in almost all tested species, except *Poa pratensis* seeds which were replaced by *C. purpurea* sclerotia in the highest number in 2002 also. The lowest detection of sclerotia in examined grass species was noted in 2000 (Fig. 1).



1) seed sample: 2500 seeds according to the ISTA recommendation
( ) range in seed crops

Fig. 1. Mean number of seed replaced by *Claviceps purpurea* sclerotia in different grass species during 1999-2002 in Poland

Table 2

Monthly mean temperatures and precipitation during spring 1999-2002 in comparison with long-term averages at Radzików (Central Poland)

Months	Mean daily temperatures [°C]					Precipitation [mm]				
	1999	2000	2001	2002	Long-term (1965-1995)	1999	2000	2001	2002	Long-term (1965-1995)
April	11.8	14.9	9.0	10.2	7.8	94.9	0.5	66.7	21.2	33.8
May	15.7	17.0	16.6	19.4	13.9	39.3	33.2	32.0	33.4	47.7
June	19.7	20.0	17.0	19.4	16.8	155.1	14.4	57.8	57.6	62.2

Weather conditions in 1999-2002 at the Central Poland are presented in the Table 2. In other regions of Poland the weather could be a little differ, but data in the Table 2 illustrated general tendency in weather condition in Poland. The spring in 1999 was very wet and moderately warm while 2000 was very dry and warm as compare to other years and the long-term averages. The spring in 2002 was warm like in 2001 with rainfall below or close the long–term average.

In all years the most infected was sowing material of *P. pratensis* (mean: 17.7 in 1999, 1,4 in 2000, 9.2 in 2001 and 24.9 in 2002 number of sclerotia in a seed sample). *F. rubra* and *L. perenne* were less infected (mean from 0.1 to 13.2 in *F. rubra* and from 0.06 to 2.3 number of sclerotia in seed sample of *L. perenne*). The lowest infection, only in one sclerotium in a sample, was detected in seed samples of *L. multiflorum* and *F. pratensis* (Fig. 1).

Prevalence of ergot sclerotia was differ in regions of Poland where grasses grown for seeds. Sowing material of *P. pratensis* originated from the North of Poland (Bartążek, Iława) shown the highest level of infection as compare to other regions. However, some differences in successive years were noted, as well. The highest number of seed replaced by ergot sclerotia was found in seed lots from Bartążek harvested in 1999 - mean 77,6 (range for cultivars from 17.7 to 274.0 number of sclerotia), from Turek 15.3 and Krotoszyn 13.6. In 2000 infection was very low, but relatively more infected was sowing material of *P. pratensis* originated from Nieznanice and Iława (respectively 3.8 and 3.3 number of sclerotia in seed samples). Whereas in 2001 ergot sclerotia were found in higher number in seed samples harvested in regions of Koło, Nieznanice, Iława and Bartązek. In 2002 the highest infection of *P. pratensis* was noted at Bartążek (mean 101.0) as compare to remaining regions. Number of sclerotia in a sample was reached to 317.0 in seed lots of cultivar Bila. In this year high level of ergot sclerotia was also detected in region of Koło (mean 70.9 sclerotia in a sample) (Table 3).

Table 3

Distribution of ergot in *Poa pratensis*. *Festuca* spp. and *Lolium* spp. grown for seed in different regions and years in Poland

Region	*Poa pratensis* [1])				*Festuca* spp. [1])				*Lolium* spp. [1])			
	1999	2000	2001	2002	1999	2000	2001	2002	1999	2000	2001	2002
Bartążek	77.6	2.1	9.3	101.0	0.1	0	6.9	0	3.7	0	0.3	0.1
Iława	-	3.3	13.9	0	0	0.5	0	3.2	0.2	0.3	0.1	0.1
Krotoszyn	13.6	0	1.0	1.6	2.8	0	0.6	0	0	0	0	0
Koło	12.9	-	21.9	70.9	2.7	-	9.1	1.6	0	0	0	0.1
Turek	15.3	-	2.8	18.4	16.2	-	0.1	5.8	10.3	-	1.0	0.3
Kalisz	8.7	0.2	-	11.5	4.0	0.3	-	8.0	1.9	0	-	0.1
Nieznanice	11.2	3.8	14.2	23.3	44.3	0	2.6	4.2	0	0.2	0.9	0
Antoniny	-	-	-	-	0	-	0	0	-	-	-	-
Radzików	2.3	0.1	-	0	19.2	0.1	-	0	4.1	-	-	-
Skrzeszowice	0.3	0	1.4	0	0	0	0	3.3	0	0	0	0
Bydgoszcz	-	-	-	22.9	-	-	-	-	-	-	-	-

[1)] a seed sample – 2500 seeds according to the ISTA recommendation; - not tested

Considerably lower seed infection by *C. purpurea* occurred in sowing materials of red fescue and perennial ryegrass. However, in these species the variation in disease prevalence in years and regions of grass cultivation were observed. Like red fescue the sowing material of perennial ryegrass was more infected in 1999 than harvested in the other years. In this year ergot sclerotia were detected at higher number in a samples of red fescue seeds originated from Nieznanice, Radzików and Turek (mean 44.3, 19.2 and 16.2 sclerotia in a seed sample, respectively) (Table 3) and in samples of perennial ryegrass seeds harvested in Turek, Radzików and Bartążek (mean 10.3, 4.1 and 3.7 sclerotia, respectively) (Table 3).

Statistical analysis revealed significant differences in ergot incidence over the years, regions of grass cultivation and tested cultivars. Particularly high significant differences were obtained in response of *L. perenne* and *F. rubra* to *C. purpurea* in years and *L. perenne* in regions (at high *F-value)* (Table 4). Differences in response of *P. pratensis* to ergot in years, regions and cultivars were significant too, but at lower *F-value.* The analysis also pointed out the interaction within years and regions for all tested species, but within cultivars, years and regions only for *F. rubra*.

Table 4

***F-value* from ANOVA for ergot incidence in seeds of main grass species in relation to the year of testing. region of grass cultivation and grass cultivar**

Species	F-value for year of testing (A)	F-value for region(B)	F-value for cultivar(C)	F-value for interactions	
				(A × B)	A × B × C)
Poa pratensis	17.87****	14.17****	13.53****	5.56****	0.00
Festuca rubra	88.97****	15.34****	18.76****	22.63****	4.8****
Lolium perenne	91.97****	64.45****	29.57****	10.76****	0.00

****Significant at P < 0.0001

Table 5

Number of seed replaced by ergot sclerotia in selected cultivars during 1999-2002 in Poland.

Species of grass	Cultivar	Number of seed crops examined in				Number of sclerotia - mean in sample[1]) (range in seed crops)			
		1999	2000	2001	2002	1999	2000	2001	2002
Poa pratensis	Skrzeszowicka	8	2	3	6	11.8 (0-35.7)	0 0	10.7 (1.0-28.3)	8.5 (0-30.7)
	Opal	6	9	-	6	11.8 (2.3-47.7)	0.3 (0-1.7)	- -	15.5 (8.3-23.7)
	Alicja	8	1	4	7	22.0 (0-85.0)	7.7 (7.7)	7.5 (0.7-27.3)	14.1 (0-25.0)
	Bila	2	1	2	3	22.7 (2.7-22.7)	0 (0)	9.8 (9.3-10.3)	139.3 (0-317.0)
Festuca rubra	Nakielska	5	2	4	4	13.7 (2.7-50.3)	0 (0)	0.2 (0-0.3)	3.8 (0-15.3)
	Olivia	5	7	3	9	2.5 (0-7.3)	0.2 (0-0.7)	0 (0)	8.9 (0-18.7)
	Areta	6	4	11	8	6.8 (0-16.0)	0 (0)	0.6 (0-5.7)	0.3 (0-1.3)
	Nimba	3	5	2	2	37.6 (0-81.7)	0 (0)	0.3 (0-0.5)	5.8 (5.7-6.0)
Lolium perenne	Stadion	7	4	8	11	1.6 (0-10.3)	0.8 (0-2.3)	0.5 (0-2.3)	0.1 (0-1.0)
	Nadmorski	2	3	5	8	0 (0)	0 (0)	0.1 (0-0.3)	0.1 (0-0.3)
	Niga	2	3	1	1	0.7 (0-1.3)	0.1 (0-0.3)	0.3 (0.3)	0.2 (0.2)
	Solen	2	3	3	-	0 (0)	0.1 (0-0.3)	0 (0)	- -

[1)] a seed sample – 2500 seeds according to the ISTA recommendation
- not tested

Tested cultivars of *P. pratensis, F. rubra* and *L. perenne* showed differences in response to *C. purpurea*, when they grow for seed in one location. However, a great variation in level of infection was observed for the same cultivar in different districts of Poland - for example Alicja cultivar of *P. pratensis* in the year 1999 was infected at a level of 22.0 sclerotia in a seed sample, but among eight seed plantations of this cultivar, the infection ranged from 0 to 85.0 sclerotia in a sample. The same tendency was observed for other cultivars of *P. pratensis*, *F. rubra* and *L. perenne* (Table 5).

Ergot sclerotia were most frequently detected in seed samples of *P. pratensis* cultivars. Some strains and cultivars of this species were very susceptible to *C. purpurea* e.g. strain BA-2245 contained 274.0 sclerotia in a sample and Bila cultivar reached even 317.0. Among tested cultivars of *F. rubra* the most infected were cultivars Noni and Nimba (mean 88.7 and 81.7 sclerotia in sample in 1999). Relatively small number of ergot sclerotia were found in seed sample of *L. perenne* but cultivar Stadion was the most susceptible to this pathogen as compare to other examined cultivars (Table 6)

Table 6

Number of sclerotia in a seed sample of selected cultivars. seed yield losses expressed as percentage of seeds number replaced by sclerotia and sclerotia mass in seed of selected cultivars in 1999-2002

Species of grass	Cultivar	Number of seed crops examined in:				Number of sclerotia (mean in sample1) (range in seed crops)			
		1999	2000	2001	2002	1999	2000	2001	2002
Poa pratensis	Skrzeszowicka	8	2	3	6	11.8 (0-35.7)	0 0	10.7 (1.0-28.3)	8.5 (0-30.7)
	Opal	6	9	-	6	11.8 (2.3-47.7)	0.3 (0-1.7)	- -	15.5 (8.3-23.7)
	Alicja	8	1	4	7	22.0 (0-85.0)	7.7 (7.7)	7.5 (0.7-27.3)	14.1 (0-25.0)
	Bila	2	1	2	3	22.7 (2.7-22.7)	0 (0)	9.8 (9.3-10.3)	139.3 (0-317.0)
Festuca rubra	Nakielska	5	2	4	4	13.7 (2.7-50.3)	0 (0)	0.2 (0-0.3)	3.8 (0-15.3)
	Olivia	5	7	3	9	2.5 (0-7.3)	0.2 (0-0.7)	0 (0)	8.9 (0-18.7)
	Areta	6	4	11	8	6.8 (0-16.0)	0 (0)	0.6 (0-5.7)	0.3 (0-1.3)
	Nimba	3	5	2	2	37.6 (0-81.7)	0 (0)	0.3 (0-0.5)	5.8 (5.7-6.0)
Lolium perenne	Stadion	7	4	8	11	1.6 (0-10.3)	0.8 (0-2.3)	0.5 (0-2.3)	0.1 (0-1.0)
	Nadmorski	2	3	5	8	0 (0)	0 (0)	0.1 (0-0.3)	0.1 (0-0.3)
	Niga	2	3	1	1	0.7 (0-1.3)	0.1 (0-0.3)	0.3 (0.3)	0.2 (0.2)
	Solen	2	3	3	-	0 (0)	0.1 (0-0.3)	0 (0)	- -

1) a seed sample – 2500 seeds according to the ISTA recommendation; - not tested

Seed losses expressed as percentage of seed number replaced by sclerotia and percentage of sclerotia by weight in selected cultivars were demonstrated in Table 6. The highest seed losses were found in *P. pratensis* cultivar Bila harvested in 2002 (12.7% seed replaced by ergot and 28.5% ergot in seed weight) and strain BA

2245 harvested in 1999 (10.9% seed replaced by ergot and 15% ergot in seed weight). However, seed losses of these genotypes varied in years and ranged from 0 to 28.5% in seed weight (Table 6). Considerably lower seed losses were noted for *F. rubra* cultivars, which reached 4,6% seed weight for cultivar Noni and 4.2% for cultivar Nimba harvested in 1999. Relatively the lowest seed losses caused by *C. purpurea* were obtained for *L. perenne* cultivars (Table 6).

Table 7

Characters of *Claviceps purpurea* sclerotia from different grass species (hosts) and years of seed harvest.

Species of grass (host)	Mean lenght[1)] [mm]				Mean thickness[1)] [mm]				Mean weight[1)] [g]			
	1999	2000	2001	2002	1999	2000	2001	2002	1999	2000	2001	2002
Poa pratensis	2.9	2.6	2.7	2.4	1.0	1.0	1.0	1.0	0.00120	0.00110	0.00129	0.00131
	(2-5)	(1-5)	(1-5)	(1-4)	(1.0)	(1.0)	(1.0)	(1.0)	(0.001-0.003)	(0.001-0.003)	(0.001-0.002)	(0.001-0.003)
Festuca rubra	5.8	5.4	5.9	5.2	1.0	1.1	1.1	1.2	0.00179	0.00296	0.00305	0.00284
	(4-10)	(2-9)	(2-10)	(2-11)	(1-2)	(1-2)	(1-2)	(1-2)	(0.001-0.005)	(0.001-0.008)	(0.001-0.008)	(0.001-0.01)
Lolium perenne	4.5	5.2	4.0	3.5	1.2	1.2	1.2	1.1	0.00357	0.00417	0.00223	0.00175
	(2-9)	(3-9)	(2-6)	(2-6)	(1-2)	(1-2)	(1-2)	(1-2)	(0.001-0.011)	(0.001-0.011)	(0.001-0.004)	(0.001-0.004)

[1)] Mean of 100 sclerotia; ( ) - range – minimum and maximum

Table 8

F-value from ANOVA for size of *Claviceps purpurea* sclerotia in relation to the host –grass species and the year of testing.

Characters	F-value for host- grass species (A)	F-value for years of testing (B)	F-value for interactions (A × B)
Length	492.69****	0.92	10.69****
Weight	221.26****	13.98****	17.16****
Thickness	43.45****	1.36	1.00

****Significant at P < 0.0001

Biometrical characteristic of *C. purpurea* sclerotia revealed that their traits such as length, thickness and weight differed depending on the host grass species and the year of seed harvest (Table 7). Result of *F*- test in combined ANOVA showed that differences were particularly evident for the length and weight of sclerotia in dependence on host species and year of testing (Table 8). The longest sclerotia were found in seed sample of *F. rubra* (from 5.2 to 5.9 mm) whereas the smallest were separated from spikes of *P. pratensis* (from 2.0 to 2.9 mm). Statistical analysis indicated also on a significant interaction between years of testing and species where sclerotia were born. The longest sclerotia were isolated in 1999 and 2001 from seed samples of *P. pratensis* and *F. rubra*. Sclerotia from *L. perenne* ears were the longest in 2000 (mean 5,2) as compare to other years (Table 7). The weight of sclerotia ranged from 0.0011 to 0.00131g of *P. pratensis*, from 0.00179 to 0.00305 g of *F. rubra* and from 0.00175 to 0.00417g of *L. perenne* depending on year of seed harvest. The most weighty sclerotia were found in seed samples of *L. perenne* harvested in 1999 and 2000, of *F. rubra* harvested in 2000 and 2001 and of *P. pratensis* in 2001 and 2002. Also significant differences, but at lower *F-value* were found for thickness of sclerotia originated from particular species. Their thickness ranged from 1.0 mm for *P. pratensis* to 1.2 mm for *L. perenne*. However, in this case the interaction between grass species and year of seed harvest was not significant.

DISCUSSION

Our results confirmed that ergot may be serious disease of grasses in Poland. Infection by this fungus reached to 13% of seed replaced by sclerotia and 28.5% by weight in some cultivar and strain of *P. pratensis*. Our previous investigations indicated that losses of seed by ergot could reached even 60% in the North of Poland (Prończuk and Wiewióra 1999). Schultz *et al.* (1993) reported that reduction of seed yield by ergot could be even 80-90%, although losses were usually much less. Alderman *et al.* (1998) stated, that additional seed losses might occur during harvest, if honeydew was present because the sticky exudates tended to aggregate seeds and debris and stick to machinery and equipment.

Ergot was most frequently observed in sowing material of *P. pratensis* for all years of study. This result has confirmed our previous observations (Prończuk and Wiewióra, 1999). Similar results were obtained by other researches. According to Alderman at al. (1996) ergot was important disease of Kentucky bluegrass grown for seed in the North-Eastern Oregon in the USA. Cagaš (1995) claimed that since 1979 *C. purpurea* hampered the seed yield and quality of Kentucky bluegrass in Czech Republic.

In our study sclerotia were not recorded or recorded only in trace number in *F. pratensis* and *L. multiflorum* samples. Both species have been susceptible to *C. purpurea* (Raynal *et al.* 1989, Cagaš, 1998) and it is not clear why ergot sclerotia were absent in seed samples of these species during four years of our studies.

It was found considerable yearly variation in ergot incidence and severity. Alderman (1991), Alderman and Barker (2003) and Cagaš (1992) reported that this variation could be expected depending on yearly variation in environmental conditions. According these authors the development of ergot is enhanced by high air condition during spring and in the duration of cultivars and species flowering period. Such weather conditions appeared sometimes in Poland and favoured development of the disease what happened in 1999 as compare to other years of our study. Cagaš and Horn (1994) and Cagaš and Machac (2002) observed that the level of ergot infection depended not only on the weather conditions during the growing period, but also on cultivars susceptibility to ergot and pathogenicity of *C. purpurea* isolates present in the region. On the other hand Shultz *et al.* (1993) reported that resistance to ergot among Kentucky bluegrass cultivars was incomplete and always available in desirable turf or seed production types were more susceptible to ergot. According to the opinion of these authors presently there were no cultivars resistant to ergot. However, Cagaš at al. (1999) reported that Czech cultivar Slezanka was resistant to ergot.

It was found considerable variation in level of infection for the same cultivar in different districts of Poland. The same results were obtained by Alderman *et al.* (1998). These authors claimed that distributions of disease was independent on cultivar because in many cases the same cultivars were free of ergot in the northern range of investigated county while in the southern were infected. Cultivars vary in number of flowers per panicle and in timing and duration of flowering. Environmental conditions, especially timing of rainfall, may have main effect on level of cultivar infection.

The study has shown that size of *C. purpurea* sclerotia differed depending on host - grass species and year of seed harvest. Differences were observed especially in the length and weight of sclerotia. Tanda reported (cit. Mühle *et al.*, 1975) that size of sclerotia depended on glumes structure of grass host, number of sclerotia in floret and wet conditions during sclerotia development. According to Alderman (2003) species of Claviceps differ not only by sclerotia morphology, but by a colour of stroma produced by sclerotium and also morphology of conidia. The size of conidia of *C. purpurea* var. *purpurea* was found to be relatively sTable across host and geographical regions. However, conidia of this fungus from hosts in the Aveneae and Meliceae (generally associated with wet habitats) were more variable in size and generally larger, than those from other tribes in the Pooidae (Alderman *et al.*, 2004).

CONCLUSIONS

The study confirmed that ergot may be a serious disease of grasses grown for seed in Poland, if the local and seasonal weather conditions will favour the development of *Claviceps purpurea*.

Poa pratensis appears to be the most susceptible species to ergot.

Differences in susceptibility to *Claviceps purpurea* among cultivars were found. However, high variation in disease prevalence in different regions and years of grass cultivation were noticed.

Selection of resistant and susceptible cultivars to *Claviceps purpurea* require a long term of study.

Biometrical analysis of *Claviceps purpurea* sclerotia showed their variability in relation to the host-grass species and the year of seed harvest. Significant differences were noted, particularly in the length and weight of sclerotia.

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