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DIVERSITY OF FUNGI COLONIZING AND DAMAGING SELECTED PARTS OF RIBWORT (*Plantago lanceolata* L.)

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Abstract. Studies on diversity of fungi colonizing and damaging selected plant parts of ribwort (*Plantago lanceolata* L.) were carried out in 2009–2011 on plantations grouped in south-eastern Poland. Fungi were isolated from superficially disinfected roots, collar roots and leaves by means of a mineral medium. Fungi from *Fusarium* spp., *Boeremia exigua* var. *exigua* comb. nov. and *Rhizoctonia solani* were obtained from the roots and collar roots showing necrosis and tissue decay. *Phyllosticta plantaginis* was commonly obtained from the leaves with symptoms of small, regular, necrotic spots. This species had not been found in Poland earlier.

Key words: herbaceous plants, fungi, occurrence, *Phyllosticta plantaginis*

INTRODUCTION

Medicinal plants recently cultivated in south-eastern Poland include ribwort (*Plantago lanceolata* L.). Interest in the cultivation of this species results from the demand for the raw material of *Plantaginis lanceolatae folium,* which contains valuable iridoid glucosides, especially aucubin and catalpol [Senderski 2009]. The good quality of the raw material largely depends on the health condition of plants in the conditions of their cultivation. Plant pathogens, especially fungi overgrowing plant tissues, harm the secretory cells, which leads to the modification of the biologically active substances [Zechini et al. 1995]. It is known from rare information from literature that the fungi occurring on the plants of ribwort found in the United States include obligatory pathogens and specific pathogens of facultative character of parasitism [Farr et al. 1995]. The first group includes, for example: *Peronospora alta* and *Peronospora plantaginis*, which are the species causing downy mildew, and *Golovinomyces cichoracearum,* causing powdery mildew [Farr et al. 1995]. *Cercospora plantaginis, Septoria plantaginis* and *Phyllosticta plantaginis,* pathogens causing the spot of ribwort leaves, can be included within the second group [Farr et al. 1995].

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The present paper discusses the results of three-year-long studies on the fungi colonizing and harming the underground and above-ground parts of ribwort, with special regard to the species discovered in Poland for the first time.

MATERIAL AND METHODS

The studies were conducted in the years 2009–2011 on three one-year-old production plantations of ribwort in the communes of Fajsławice, Dziecinin and Suchodoły in the Lublin province. The forecrop on those plantations were usually other herbs, e.g. lemon balm, common thyme and motherwort. Each year the percentage of plants with disease symptoms was established twice during the vegetation period, directly on the plants. Plants with visible disease changes were taken for studies in the laboratory. The fungi presence was determined on the basis of etiological signs occurring on the infected plant parts and on the basis of the mycological analysis. The fungi were isolated from the superficially disinfected roots, collar roots and leaves. A 10% solution of sodium hypochlorite was used for disinfection, while a mineral medium was used for isolation [Zimowska and Machowicz-Stefaniak 2004]. After the fungi were brought to one-spore cultures, they were marked for the species on the medium used for the isolation or on standard media [Saccardo 1878, Rifai 1969, Ellis 1971, Ramirez 1982, Nelson et al. 1983, van der Aa and Vanev 2002, Boerema et al. 2004, Avescamp et al. 2010].

RESULTS

The studies pointed to the occurrence of differentiated disease symptoms on the plants of ribwort. Three types of disease symptoms were observed on the leaves. One of them were longitudal necrotic spots which were most often formed on the edge of the leaf blade and on the top. The leaf tissue around the symptoms described here got yellow and then died out (photo 1). Etiological signs were found in the place of such spots

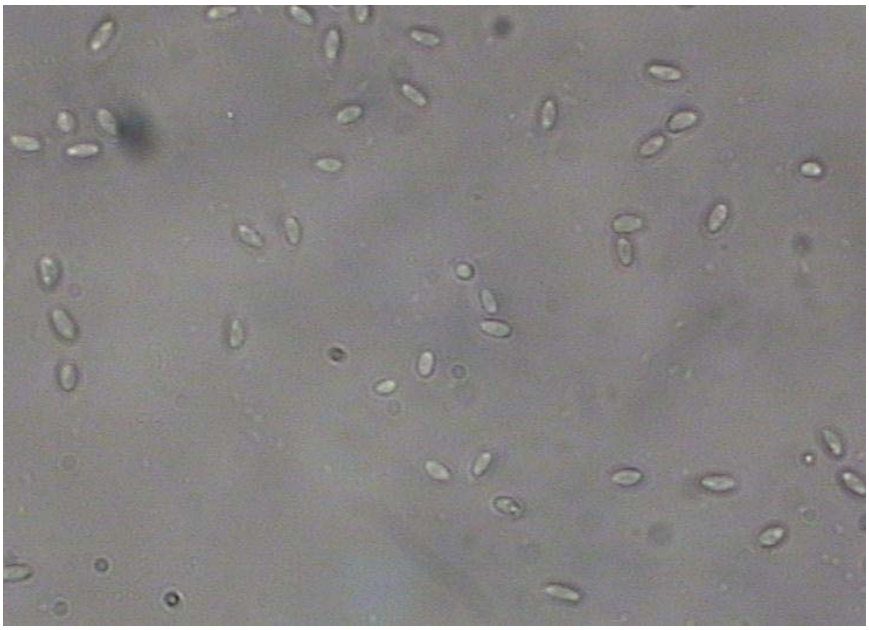
Phot. 1. Oblong, necrotic spots on which etiological sings in the form of conidiophores and conidia *Cercospora plantaginis* were found. Photo E. Zalewska

Phot. 2. Conidia of *Cercospora plantaginis* × 500. Photo E. Zalewska

Phot. 3. Conidiophores of *Cercospora plantaginis* × 500. Photo E. Zalewska

Phot. 4. Necrotic, regular spots from which *Phyllosticta plantaginis* was isolated. Photo B. Zimowska

Phot. 5. Pycnidia *Phyllosticta plantaginis*. Photo E. Zalewska



Phot. 6. Conidia *Phyllosticta plantaginis* × 450. Photo E. Zalewska

Phot. 7. Necrotic, irregular spots from which *Alternaria alternata* was isolated. Photo E. Zalewska

Phot. 8. White powdery mildew and necrosis of ribwort leaves caused by *Golovinomyces cichoracearum*. Photo. B. Zimowska

Phot. 9. Necrosis of leaves rossete of ribwort caused by *Golovinomyces cichoracearum*. Photo. B Zimowska

in the form of conidia (photo 2) and conidiophores (photo 3) typical of the species *Cercospora plantaginis.* The second type of disease symptoms observed on the leaves were small, regular, necrotic spots with the diameter of 3–5 mm (photo 4), where etiological sings in the form of pycnidia (photo 5), including conidia typical of genus *Phyllosticta* (photo 6), were observed. Besides, symptoms in the form of irregular necrotic spots (photo 7), including etiological signs in the form of conidiophores and spores characteristic of species *Alternaria alternata,* were observed on the plant leaves. All types of disease symptoms were observed both during the first analysis of plants, i.e. at the beginning of vegetation, and in full vegetation, with an increased number of spots on the leaves during the second observation. In addition, such spots merged together, which led to the drying out of the leaves. Besides the aforementioned disease symptoms, the symptoms caused by *Golovinomyces cichoracearum* were observed on the studied plants. The leaves were covered with a white powdery mildew (photo 8), consisting of the pathogen's hyphae and conidia. The disease symptoms also included a slowly progressing necrosis, which resulted in the dying out of the leaves and even the whole leaf rosette (photo 9). Necrosis and tissue decay were observed on the collar root (photo 10). The roots of such plants showed similar disease symptoms. The percentage of plants with the above-mentioned disease symptoms ranged in the years 2009–2011 from 10 to 20 % at the beginning of the vegetation to 20 to 40% at full vegetation. The most development of *G. cichoracearum* was observed in the year 2011 during low rainfall and high temperature (tab. 1).

Phot. 10. Collar root of ribwort; a – without diseases symptoms; b – with symptoms of necrosis and decay of tissues from witch *Fusarium* spp., *Rhizoctonia solani* and *Boeremia exiqua* var. *exiqua* comb. nov. were isolated. Photo. E. Zalewska

Table 1. Comparison of average value of monthly temperature of air and rainfalls with average many years in vegetation periods in 2009–2011

Month	Means of the years 1963–1992		Difference of mean air temperature in comparison with means of the years			Percentage of the average annual rainfalls		
	air temperature °C	rainfalls mm	2009	2010	2011	2009	2010	2011
May	13.3	60.9	-0.2*	0.7	0	1.8	108.9	-6.1*
June	16.4	78.3	-0.2*	1.1	1.7	58.5	-23.2*	0.9
July	17.8	77.9	1.8	1.8	0.75	-9.6*	3.0	92.8
August	17.3	69.3	-0.3*	-0.3*	1.05	-14.3*	33.0	-38.0*
September	13.1	36.0	-0.6*	-0.6*	1.75	-23.5*	104.4	-30.7*

*values with minus are low than the means of the years

Table 2. Fungi isolated from plants of ribwort (*Plantago lanceolata* L.) in 2009–2011

Fungus species	Number of isolates			Total number and (%)
	roots	collar roots	leaves	
Alternaria alternata (Fr.) Keissler	7	32	93	132 (13.08)
Boeremia exigua var. *exigua* (Desm.) Avescamp, Gruyter&Verkley, comb. nov.	24	18	19	61 (6.06)
Botrytis cienerea Pers.	4	3	18	25 (2.48)
Cladosporium cladosporioides (Fresen.) de Vries		4	12	16 (1.59)
Chaetomium globosum Kunze		2	13	15 (1.49)
Epicoccum nigrum Link			17	17 (1.68)
Fusarium avenaceum (Fr.) Sacc.	39	30	9	78 (7.73)
Fusarium culmorum (W.G.Smith) Sacc.	33	43	7	83 (8.23)
Fusarium equiseti (Corda) Sacc.	58	54	17	129 (12.78)
Fusarium oxysporum Schlecht.	21	12		33 (3.23)
Gliocladium fimbriatum Gilman & Abbott	3		15	18 (1.78)
Gliocladium roseum Bainier	6	13	12	31 (3.07)
Mucor hiemalis Wehmer	7		2	9 (0.89)
Phoma glomerata Corda Wollenw. et Hochapf.	3	2	3	8 (0.79)
Phoma capitulum Pawar. Mathur et Thirum.	2	1	2	5 (0.49)
Phoma heteroderae Sen Y. Chen. D. W. Dicks. & Kimbr	4	5	14	23 (2.28)
Phyllosticta plantaginis Sacc.			161	161 (15.96)
Rhizoctonia solani Kühn	27	11		38 (3.77)
Sphaceloma plantaginis Jenkins & Bitanc.			5	5 (0.49)
Trichothecium roseum (Pers.) Link		5	7	12 (1.19)
Trichoderma harzianum Rifai	10	9	16	35 (3.47)
Trichoderma koningii Oud.	4	14	19	37 (3.67)
Trichoderma viride Pers.	1	10	27	38 (3.77)
Total	253	268	488	1009 (100%)

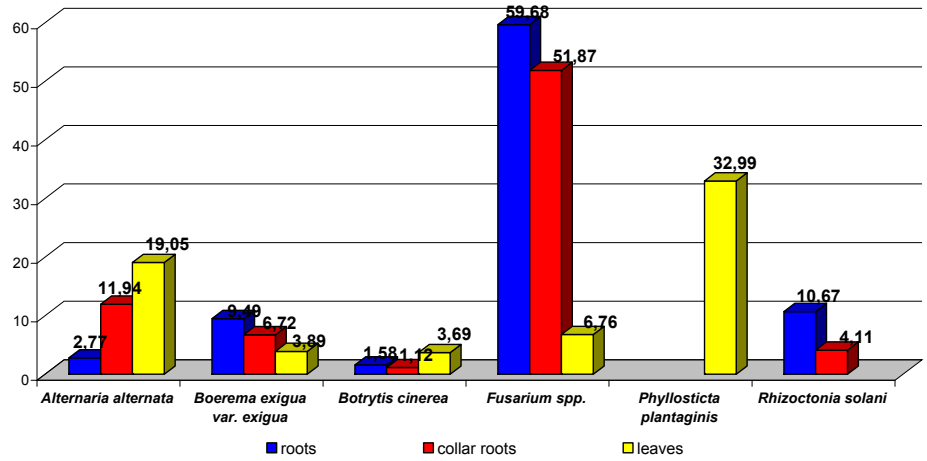


Fig. 1. Mean participation of some fungi isolated from examined organs of ribwort in 2009-2011

Totally, 1009 isolates of fungi represented by 23 species were obtained from the analyzed parts of ribwort during the three years of studies (tab. 2). The biggest number of isolates was obtained from ribwort leaves and their proportion was almost 50% of all fungi obtained during the studies (tab. 2). Among all the isolated species, *Alternaria alternata* was the commonly occurring species. The fungus was isolated both from the roots and the collar roots but the biggest number of isolates was obtained from the leaves (tab. 2). Their proportion was, respectively, 2.77%, 11.94% and 19.05% of all fungi obtained from those parts (fig. 1). Fungi from genus *Fusarium* were mainly isolated from the roots and the collar roots, and their proportion was, respectively, 59.68% and 51.87% of all fungi obtained from those plant parts (tab. 2, fig. 1). Species *Boeremia exigua* var. *exigua* comb. nov. was isolated from all analyzed parts, and the biggest number of fungus isolates were obtained from the roots since their proportion was 9.49% of all fungi obtained from that part (tab. 2, fig. 1). Isolates of *Botrytis cinerea* were obtained from all plant parts, with the biggest number of cultures obtained from the leaves whose proportion was 3.89% (fig. 1). *Rhizoctonia solani* cultures were obtained from the roots and the collar roots, and their proportion was, respectively, 10.67% and 4.11% (tab. 2, fig. 1). Species *Phyllosticta plantaginis* was obtained only from the leaves of ribwort. Isolates of the fungus constituted 32.99% of all fungi obtained from that part (tab. 2, fig. 1). Species from genus *Phoma*, for example *Phoma capitulum, P. glomerata* and *P. heteroderae* (tab. 2) were also isolated. Single isolates of *Sphaceloma plantaginis* were obtained from ribwort leaves. Cultures of *Trichoderma* spp. and *Gliocladium* spp. were obtained from all parts of plants (tab. 2).

DISCUSSION

The present studies confirmed the occurrence of disease symptoms caused by fungi on the plants of ribwort cultivated on the plantations in south-eastern Poland. The symptoms of necrosis and the rot of the roots and the collar roots were most probably the consequence of the pathogenic effect of soil-borne fungi. Results of the mycological analysis indicated that those parts were colonized by complexes of fungi. The greatest importance should be ascribed to the species from genera *Fusarium, Boeremia exigua* var. *exigua* comb. nov. and *Rhizoctonia solani.* A lot of attention has been devoted to the role of fungi from genus *Fusarium* in the soil and to the problem of colonization of the underground parts of plants, including spices and medicinal plants, by them [Filoda et al. 1998, Machowicz-Stefaniak et al. 2002, Machowicz-Stefaniak and Zalewska 2004, Zalewska and Machowicz-Stefaniak 2004, Zimowska 2007]. Saprophytic forms living in the soil include the forms of various degrees of pathogenicity able to infect in favourable conditions [Nelson et al. 1983].

The fact of obtaining *R. solani* from the roots and the collar root can indicate the harmfulness of this species towards ribwort. The fungus was isolated earlier from the plants of summer savory, garden sage, common thyme, peppermint and motherwort showing the signs similar to those observed on ribwort plants [Machowicz-Stefaniak et al. 2004, Zimowska 2007, Zimowska 2008ab, Zimowska 2010].

Basing on the conducted studies and information from literature, *B. exigua* var. *exigua* comb. nov. can be included within the complex of fungi responsible for the symptoms of necrosis and the rot of the roots and the collar root [Avescamp et al. 2010]. The harmfulness of the species under discussion was shown towards common thyme and lemon balm. It consisted in inhibiting the germination of schizocarps and the emergence of plants through the necrosis of the sprouts and the hypocotyls of the seedlings [Machowicz-Stefaniak et al. 2008]. Besides, the fungus colonized the underground parts of herbs from the families of Lamiaceae*,* Apiaceae*,* Asteraceae and Hypericaceae while the disease symptoms were similar to those found on ribwort plants [Zimowska and Machowicz-Stefaniak 2004, Machowicz-Stefaniak and Zalewska 2007, Zimowska 2008 a, b].

What deserved special attention is obtaining the species that was not noted in Poland before, namely *Phyllosticta plantaginis,* from ribwort leaves with the symptoms of small necrotic regular spots. The fungus is described in literature as a pathogen causing the spots of ribwort and broadleaf (*Plantago major* L.) [Saccardo 1878, Farr et al. 1995]. Isolation of numerous fungus cultures from the leaves showing specific disease symptoms, the presence of etiological signs on them and the fulfillment of Koch's postulates for selected isolates of the fungus in pathogenicity tests in *in vitro* conditions [Zimowska unpublished] allow to consider *P. plantaginis* as a pathogen of ribwort. The studies conducted on the effect of temperature on the growth and sporulation of isolates of the fungus indicated its eurythermic character and the ability of the mycelium hyphae to survive even in sub-zero temperatures [Zimowska unpublished]. The fungus probably overwinters on the residues of the infected ribwort leaves while in spring it begins to form pycnidia with propagation spores in them which cause the primary infection of the leaves. Hence, it is recommended to make a deep plough in autumn so that the infected residues should be introduced deeper in order to minimize the source of primary infec-

tion. Besides, agrotechnical methods, including crop rotation, should take an importance place in the case of all pathogens of herbaceous plants in the program of plant protection, especially in the aspect of using the principles of Good Agricultural Practice (GAP) in herb production, which were established and introduced in legislature of the European Union in 2004 [Buchwald 2012] .

The occurrence of symptoms on the examined ribwort leaves in the form of necrotic, longitudal spots as well as the presence on etiological signs on them in the form of conidia and conidophores of *Cercospora plantaginis* suggest that those symptoms were the result of the infection of the leaf tissue by the aforementioned fungus species. *C. plantaginis* was considered as a species pathogenic towards the plants of *Plantago lagopus* in Turkey [Sert et al. 2004]. Although characteristic symptoms and the presence of etiological sings were observed on the leaves of ribwort, no fungus cultures were isolated during the mycological analysis. The fungus probably belongs to slowly growing species and during the isolation it was dominated by other fast growing species.

Species *A. alternata* should be viewed as the cause of the type of disease symptoms on ribwort leaves in the form of irregular necrotic spots. Common isolation of the fungus cultures from the infected organs, the presence of etiological sings on them as well as information from literature on the occurrence and harmfulness of this fungus towards different species of herbaceous plants confirm the hypothesis [Kalra et al. 2004, Zimowska 2007, Zimowska 2008ab, Zimowska 2010].

Besides facultative pathogens, another threat for the plantations of ribwort can be an obligatory pathogen, *G. cichoracearum,* which was also found in the studies. This species is a frequently encountered polyphagous species on the plantations of herbaceous plants [Machowicz-Stefaniak 2001]. As a result of their parasitism, it causes dying out of ribwort leaves thus affecting a decrease of the yield.

The present studies pointed out for the next time to the species diversity of fungi from genus *Phoma* colonizing herbaceous plants, including ribwort [Zimowska 2010, 2011]. Species *P. heteroderae* obtained mainly from ribwort leaves belongs to frequently observed saprotrophs colonizing different organic and inorganic substrates. It was isolated in Europe, North America and Asia [Boerema et al. 2004]. Besides, the cultures of species *P. glomerata* and *P. capitulum*, which were frequently isolated from herbs, were obtained from the analyzed plants of ribwort [Zimowska 2007, Zimowska 2008 a, b]. The fact that single isolates of *Sphaceloma plantaginis* were obtained from the leaves testifies to the presence of this species in the phyllosphere of *Plantago lanceolata* L. The species under discussion was observed on ribwort leaves with the symptoms of spots in the Unites States [Farr et al. 1995]. Obtaining only a few isolates of this fungus can testify to its poor competitive properties.

It should be considered a positive phenomenon that species from genera *Trichoderma* and *Gliocladium*, known for their antagonistic effect towards different pathogens of cultivated and herbaceous plants, were obtained from the examined parts of ribwort [Sandoval et al. 2006, Singh et al. 2007].

CONCLUSIONS

The satisfactory and repeatable quality of raw materials, including ribwort, obtained from herbaceous plants depends on a number of abiotic and biotic factors. One of them is the phytosanitary condition of plants during their cultivation. The present studies pointed to the diversity of fungi, including pathogenic species, which – as a result of injuring the plants – exerts a negative effect on the quality and quantity of *Plantaginis lanceolatae folium.* In the light of GAP principles, all injured plants or their parts must be eliminated from the further process of production.

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RÓŻNORODNOŚC GRZYBÓW ZASIEDLAJĄCYCH I USZKADZAJĄCYCH WYBRANE ORGANY BABKI LANCETOWATEJ (*Plantago lanceolata* L.)

Streszczenie. W latach 2009–2011 na plantacjach produkcyjnych zgrupowanych w południowo-wschodniej Polsce przeprowadzono badania nad grzybami zasiedlającymi i uszkadzającymi wybrane organy babki lancetowatej (*Plantago lanceolata* L.). Grzyby izolowano z powierzchniowo odkażonych korzeni, szyjki korzeniowej oraz liści, używając pożywki mineralnej. Z korzeni oraz szyjki korzeniowej wykazujących objawy w postaci nekrozy i rozpadu tkanek uzyskano *Fusarium* spp., *Boeremia exigua* var. *exigua* comb. nov. oraz *Rhizoctonia solani.* Z liści z objawami drobnych, regularnych, nekrotycznych plam powszechnie izolowano nie notowany wcześniej w Polsce gatunek *Phyllosticta plantaginis*.

Słowa kluczowe: rośliny zielarskie, grzyby, występowanie, *Phyllosticta plantaginis*

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