Biodiversity of fungi colonizing and damaging selected parts of motherwort (*Leonurus cardiaca* L.)

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S u m m a r y

Studies on biodiversity of fungi colonizing and damaging selected plant parts were conducted in 2004–2006. They were performed on productive plantations of motherwort (*Leonurus cardiaca* L.) grouped in south-eastern Poland. Fungi were isolated from superficially disinfected roots, stem bases and leaves by means of a mineral medium. Fungi from *Fusarium* spp., *Phoma* spp. and *Rhizoctonia solani* were obtained from the roots showing necrosis and tissue disintegration. *Phoma nepeticola* was commonly obtained from the leaves and stems with symptoms of small, regular, necrotic spots. This is reported for the first time in Poland.

Key words: motherwort, Leonurus cardiaca, fungi, biodiversity

INTRODUCTION

In recent years, in south-eastern Poland the spice and medicinal plants from the species of the family *Labiateae*, e.g. motherwort (*Leonurus cardiaca* L.) have been cultivated. Grouping of herbaceous plants in this region causes their frequent return to the same field which contributes to the accumulation of fungi decreasing the quality and quantity of the yield [1-5].

As it is known from literature, saprophytic species, facultative parasites and specific pathogens of this plant are found among the fungi occurring on the plants of motherwort in the United States [6]. The first group includes, among others, *Phoma exigua* var. *exigua*, while the second comprises *Cercospora leonuri, Ascochyta leonuri* and *Septoria lamiicola*, the species causing leaf spot of motherwort [6].

The present paper discusses the results of three-year-long studies on fungi colonizing and damaging the underground and aboveground parts of motherwort plants cultivated in south-eastern Poland.

MATERIAL AND METHODS

The studies were conducted in 2004–2006 on three productive plantations of motherwort in the second year of cultivation, at Fajsławice in Dziecinin commune (the Lublin district). Each year the percentage of plants with necrotic symptoms on the stems and leaves was determined twice during the vegetation period. Plants with visible disease changes were analyzed in the laboratory. The presence of fungi was assumed on the basis of etiological signs observed on the infected parts of plants and on the basis of the mycological analysis. Fungi were isolated from the roots, the stems up to the height of 25 cm from the base and the leaves disinfected on the surface. A 10% solution of sodium hypochlorite was used to disinfect the material, while a mineral medium was used to isolate the fungi [4]. Monospore cultures were identified as the species on the medium used for isolations or on standard medium [7-13].

RESULTS

Two types of disease symptoms in the form of necrotic spots were observed on the leaves of motherwort. Some of them were formed on the tops and edges of the leaf blade. Those were of irregular shape, extensive, occupying greater part of the leaf at full vegetation (fig. 1). Apart from such symptoms, smaller, regular spots of 3–5 mm diameter were observed. They were situated on the whole surface of the leaf blade (fig. 2). Intensification of these disease symptoms was observed at full vegetation, in second half of July. At that time, yellowing of such leaves and their earlier falling were observed (fig. 3). Similar small, necrotic, regular spots occurred on the stems. They were most frequently formed at the height of 5 to 20 cm from the base. Besides, the symptoms of necrosis and tissue softening were observed on the roots and lower parts of the stems up to the height of 5–7 cm. Conidia typical of *Altenaria alternata* were found on the leaves with the symptoms of extensive, irregular spots. On the other hand, on the leaves and stems with small regular spots perithecia (fig. 4) with asci- and ascopores typical for genus *Leptosphaeria* (fig. 5), and conidia typical of *Phoma* spp. were found out (fig. 6). Conidia of features typical fot *Fusarium* spp. were found on the roots and lower stem parts.

The above-described symptoms occurred in 10–20% of plants at the beginning of vegetation and on 25–40% of plants at full vegetation.

Figure 1. Necrotic irregular spots of the leaves from which *Alternaria alternata* was isolated. Photo: B. Zimowska

Figure 2. Regular necrotic spots from which *Phoma nepeticola* was isolated. Photo: E. Zalewska

Figure 3. Yellowing of the diseased leaves. Photo: B. Zimowska

Figure 4. Perithecia of *Leptosphaeria rubicunda*. Photo: E. Zalewska

Figure 5. Asci and ascospores of *Leptosphaeria rubicunda* (640 x). Photo: B. Zimowska

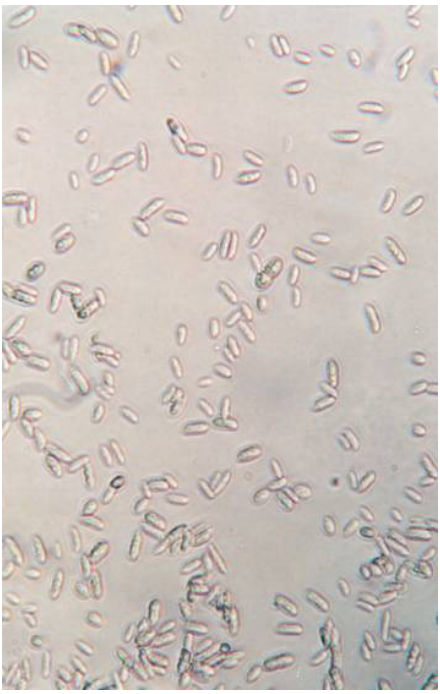

Figure 6. Conidia of *Phoma nepeticola* (640 x). Photo: B. Zimowska

In total, 2933 isolates of fungi represented by 30 species were obtained from the analyzed parts of motherwort plants (tab.1). The cultures of *A. alternata, Phoma nepeticola* and *Botrytis cinerea* were most frequently obtained from the leaves, and their proportion was, 72.24%, 8.57% and 5.20%, respectively of all fungi obtained from this organ (fig. 7). The above-mentioned fungi species were also isolated from the motherwort stems. They coustituted 25.44, 11.74 and 4.21% of the cultures obtained from this part of plants, respectively (fig. 7). However, species from *Fusarium* genus were isolated from the stems most frequently, since those fungi constituted as much as 36.60% of the cultures obtained from this organ (fig. 7). *Fusarium* spp. was also isolated from the roots of motherwort most frequently, and the cultures of these fungi constituted almost half of all isolates obtained from this part of plants (fig. 7). The cultures of *Phoma* spp., including *P. exigua* var.*exigua, Rhizoctonia solani, A. alternata, Cylindrocarpon* spp., *Trichoderma* spp. and *Gliocladium* spp. were obtained from the roots with much less frequency (tab.1, fig. 7).

Table 1.

Fungi isolated from motherwort plants (*Leonurus cardiaca* L.) in 2004–2006

fungus species	number of isolates			
	roots	stems	leaves	total (%)
Alternaria alternata (Fr.) Keissler	143	260	781	1184 (40.37)
Botrytis cinerea Pers.		43	54	97 (3.31)
Chaetonium globosum Kunze			1	1 (0.03)
Cladosporium cladosporioides (Fres. De Vries)	8	14	22	44 (1.50)
Cylindrocarpon heteronema (Berk. et Br.)	10			10 (0.35)
Cylindrocarpon didymum (Harting) Wollenw.	14			14 (0.48)
Cylindrocarpon obtusisporum (Cooke et Harkness) Wollenw.	26	1		27 (0.92)
Epicoccum purpurascens Ehrenberg		4	42	46 (1.57)
Fusarium avenaceum (Fr.) Sacc.	94	81	2	177 (6.03)
Fusarium culmorum (W.G.Sm.) Sacc.	76	137	2	215 (7.33)
Fusarium equiseti (Corda) Sacc.	127	103	4	234 (7.98)
Fusarium oxysporum Schlecht.	92	51	2	145 (4.94)
Fusarium solani (Mart.) Appel et Wollenw. Snud et Marasas	10	2		12 (0.41)
Gliocladium catenulatum Gilman et Abbott	5			5 (0.17)
Gliocladium roseum Bainier	25	14		39 (1.31)
Penicillium decumbens	1		4	5 (0.17)
Phoma exigua Desm. var. *exigua*	26	46	3	75 (2.56)
Phoma glomerata (Corda) Wollenw&Hochapfel	13	9		22 (0.75)
Phoma labilis Sacc.	15	26		41 (1.39)
Phoma nemophilae Neerg.	3			3 (0.10)
Phoma nepeticola (Melnik) Dorenb.& de Gruyter		120	89	209 (7.13)
Phoma septicidalis Boerema	31	8	11	50 (1.70)
Phoma subglomerata Boerema	3	13		16 (0.55)
Rhizoctonia solani Küchn	41	11		52 (1.77)
Talaromyces flavus	4	1		5 (0.17)
Trichotecium roseum	2	1	5	8 (0.27)
Trichoderma aureoviride Rifai	24	15	2	41 (1.39)
Trichoderma harzianum Rifai	22	22	2	46 (1.57)
Trichoderma koningii Oud.	55	39	12	106 (3.61)
Trichoderma polysporum (Link ex Pers. Rifai)	3	1		4 (0.14)
total	873	1022	1038	2933

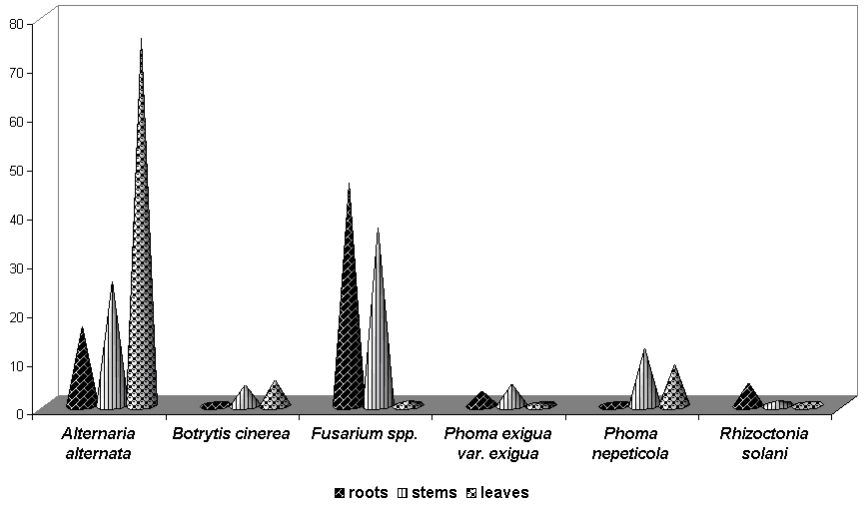

Figure 7. Means of some fungi isolated from examined organs of motherwort in 2004-2006

DISCUSSION

The studies pointed to big diversity of fungi occurring in the studied, cultivated plants of motherwort. They included the species from *Fusarium, Phoma* and *R. solani* genera, which are known as facultative parasites of plants [5, 12, 14, 15]. The occurrence of necrosis starting with the roots and the softening of lower parts of stems may suggest that the soil-borne fungi caused the disease [4, 16]. Results of the mycological analysis showed that those fungi were colonized by a complex of fungi, the most important of which were the species from genera *Fusarium, Phoma* and *R. solani.* Much attention was devoted to the role of fungi of *Fusarium* spp. in the soil and the problem of their colonization of the underground parts of plants, including the spices and medicinal ones [3, 5, 17-19]. The saprophytic forms of fungi living in the soil are pathogenic of different degrees and may cause an infection in favorable conditions [10].

R. solani also belongs to facultative species isolated in the present studies. The fungus is known to induce the symptoms of rot, and then dying-out of the roots, stems and even leaves of different species of plants [4, 20-22].

Documented in the present differentiation of species from *Phoma* spp. occurring in the cultivated environment of motherwort studies deserves attention. The commonly occurring saprophytes developing on different substrates include the species isolated in the present studies, namely: *P. glomerata, P. subglomerata* and *P. septicidalis*, with the latter also causing the necrosis of plants from the family of *Gramineae* [23-25]. The fact that *P. labilis* isolates were obtained from the roots and the stems of motherwort points to the presence of this species in the cultivated

environment of the examined plants. Cultures of the fungus were earlier obtained from the soil and the aboveground parts of herbaceous and lignified plants in Italy, Turkey and Israel. This species is considered as a frequently occurring necrotroph, especially in the regions of warm climate [13]. Probably, this fungus found suitable conditions for growth and development during vegetation periods observed in recent years in Poland, characterized by fairly high temperatures, which is testified to be the obtained isolates.

The fact that the species *P. nepeticola* (*Ascochyta nepeticola, A. nepetae*) was obtained from the leaves and stems with symptoms of small, regular necrotic spots deserves special attention. The fungus is described in the literature as a commonly occurring pathogen of catmint (*Nepeta cataria*) and other species from the *Nepeta* genus [12]. A monograph on fungi of *Ascochyta* genus prepared by Mel'nik in 1977 includes information that species discussed may infect other plants from the *Labiateae* family, for example motherwort and peppermint (*Mentha piperita)* [12, 13, 26]. On the synonyms list of *P. nepeticola* species provided by Mel'nik one is found under the name of *Ascochyta leonuri*, which could point to pathogenic relation of this species with *Leonurus cardiaca* [13, 16]. Common isolation of *P. nepeticola* from the leaves and stems of motherwort showing the characteristic disease symptoms in the form of regular, necrotic spots, and the information from the literature presented above suggest that such symptoms could have been the consequence of the pathogenic effect of the obtained isolates of the fungus. However, in order to confirm this hypothesis it is necessary to carry infection tests according to the postulates by Koch.

The occurrence of perithecia and ascopores of *Leptosphaeria rubicunda* on motherwort stems [6,27] led to the supposition that his species may be a teleomorph of the obtained *Phoma* cultures. However, after a thorough procedure based on the present principles of classifying the taxons of *Phoma* spp., the obtained isolates were marked as *P. nepticola,* whose teleomorph belongs to *Didymella* genera [11,12]. It is known from the literature that *L. rubicunda* commonly occurs on the stems of common teasel (*Dipsacus sylvestris*), purple loosestrife (*Lythrum salicaria*), elder (*Sambucus nigra*) and motherwort [6, 28]. No information on the pathogenicity of the discussed species was found in the available literature. It is only known that ascospores of *L. rubicunda* are spread by winter or with raindrops, and the fungus overwinters on plant residues [28]. However, other species of *Leptosphaeria* genus are described as dangerous pathogens of cultivated plants. Dying out of the raspberry, blackberry and rose stems is caused by *L. coniothyrium* [29]. *L. maculans* belongs to most important plant pathogens of the *Cruciferae* family [30]. In Africa, Australia and India, *L. leucadendri* causes leaf spot of plants from the *Proteaceae* family [31].

The fact that the cultures of *P.exigua* var. *exigua* were obtained from the roots and the lower parts of stems of the studied motherwort plants may indicate the participation of this species in causing disease symptoms on those organs. Such a conclusion is justified by numerous reports from the literature and experimentally

proved harmfulness of the isolates of the fungus towards the sprouts and seedlings of garden thyme and lemon balm [32].

On the basis of the mycological analysis and the etiological signs occurring on plants, the cause of the necrotic irregular spots formed on the leaves was determined as the species *A. alternata*. Reports from literature on the occurrence of the fungus on different species of herbaceous plants [22, 23] as well as earlier information given by the author on the harmfulness of *A. alternata* constitute the basis to consider this fungus as an economically important species in herb production [5, 22, 34].

Botrytis cinerea should be included to species that may have a negative effect on the quality of the raw material of *Herba Leonuri cardiaceae*. Such a conclusion was justified by the fact of isolating the fungus cultures from the studied motherwort plants and information from literature on the pathogenic effect of the fungus on different species of spice and medicinal plants [5, 35, 36].

Present studies pointed to biodiversity of the species of fungi occurring in the cultivated environment of the examined plants of motherwort. They contributed to discovering species that had not been noted in Poland so far, namely *P. nepeticola,* which considerably lowers size and quality of *Herba Leonuri cardiaceae* raw material. Besides, the obtained results draw attention to the danger resulting from the common colonization of leaves and stems of motherwort by toxin-forming species of *A. alternata*.

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BIORÓŻNORODNOŚĆ GRZYBÓW ZASIEDLAJĄCYCH I USZKADZAJĄCYCH WYBRANE ORGANY SERDECZNIKA POSPOLITEGO (*LEONURUS CARDIACA* L.)

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S t r e s z c z e n i e

W latach 2004–2006 na plantacjach produkcyjnych serdecznika pospolitego (*Leonurus cardiaca* L.) zgrupowanych w południowo-wschodniej Polsce przeprowadzono badania nad grzybami zasiedlającymi i uszkadzającymi wybrane organy roślin. Grzyby izolowano metodą sztucznych kultur z powierzchniowo odkażonych korzeni, łodyg oraz liści przy zastosowaniu pożywki mineralnej. Z korzeni oraz dolnych części łodyg z objawami nekrozy i dezintegracji tkanek izolowano grzyby z rodzaju *Fusarium, Phoma* oraz *Rhizoctonia solani*. Z łodyg oraz liści z symptomami drobnych regularnych plam uzyskano nie notowany wcześniej w Polsce gatunek *Phoma nepeticola*.

Słowa kluczowe: serdecznik pospolity, Leonurus cardiaca, grzyby, bioróżnorodność