Fungi threatening the cultivation of sage (*Salvia officinalis* L.) in south-eastern Poland

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S u m m a r y

In 2004–2006 there were studies conducted on fungi colonizing and harming different parts of plants on plantations of sage (*Salvia officinalis* L.) grouped in south-eastern Poland. Fungi were isolated from roots, the stem base and leaves. The surface was disinfected by means of a mineral medium. Fungi from genera *Fusarium, Rhizoctonia solani, Phoma exigua* var. *exigua* were isolated from roots and the lower parts of stems with the symptoms of necrosis and tissue disintegration. The species *Phomopsis sclarea*, which had not been observed earlier in Poland, was obtained from the stems with the symptoms of necrosis, peeling off and bark breaking. *Altenaria alternata* was commonly isolated from the leaves with necrotic symptoms.

Key words: sage, Salvia officinalis, fungi

INTRODUCTION

Sage (*Salvia officinalis* L.) from the *Labiatae* family is a herb frequently cultivated in south-eastern Poland. A good phytosanitary state of plants during their cultivation is a condition for a high quality yield and raw material of *Folia salviae* and *Herba salviae* [1, 2]. The most important infectious diseases of sage in European countries are antracnosis caused by *Colletotrichum dematium*, ascochitosis caused by *Ascochyta sclarea* and root rot caused by *Rhizoctonia solani* [3, 4]. Economically important pathogens in Italy and Spain include *Phomopsis sclarea, Phodosphaera inequalis, Erysiphae polygoni* and *Sclerotinia sclerotiorum* [3]. In 1995 massive dying out of sage seedlings infected by *Fusarium oxysporum* was observed in California, USA [3].

Results of three-year-long studies presented here on fungi occurring and injuring the underground and aboveground parts of sage are a part of a continued cycle of studies on mycoses of herbaceous and medicinal plants from the family *Labiateae* cultivated in south-eastern Poland [5-7].

MATERIAL AND METHODS

The studies conducted in 2004–2006 concerned three plantations of sage in the second year of cultivation, located in the communes of Diecinin and Suchodoły in the Lublin district. The forecrops on the examined plantations were other herbs, i.e. lemon balm, thyme and motherwort. The percentage of plants with necrotic symptoms on the stems and the leaves was determined each year at the beginning of vegetation and at full vegetation, directly in the fields. Plant samples with disease symptoms were taken for laboratory studies. The presence of fungi was established on the basis of etiological symptoms occurring on the infected parts of plants and on the basis of mycological analysis conducted by the method of artificial cultures. Fungi were isolated from roots, the stem base and laves, which were disinfected on the surface by means of a mineral medium [8]. After segregation and after making single-spore cultures, the obtained fungi colonies were identified to the species on the maltose medium or standard media [9-12].

RESULTS

Irregular necrotic spots between the nerves as well as on the edges and tops of the leaf blade were observed on sage leaves (fig. 1). At full vegetation such spots often merged, covering a greater part of the leaf. At the same time drying out and premature defoliation of infected leaves took place, especially in the lower parts of plants. Characteristic disease symptoms were observed on the stems at the height of 10 to 18 cm from the base. Those were brown oblong spots that frequently merged together, covering the stem around . Peeling off and breaking of the bark occurred in such places (fig. 2). Besides, twisting and dying out of the leaves found above the disease symptoms described above were observed (fig. 3). Necrotic symptoms also occurred on the plants roots. Such were frequently accompanied by the signs of disintegration and softening of tissues, which also moved onto lower parts of stem up to the height of 5 cm from the base. Spores with the features typical for *Altenaria alternata* species were found on the infected leaves, whereas spores with the features typical of genus *Phomopsis* occurred in the cracks of the stem bark. Conidia with the features typical of genera *Fusarium* and *Phoma* were found on the roots and the stems with the symptoms of tissue necrosis and disintegration. The percentage of plants with the disease symptoms described above ranged from 10 to 20% at the beginning of vegetation, and from 15 to 40% at full vegetation in the studied period.

Figure 1.
Necrotic spots of leaves from which *Alternaria alternata* was isolated (photo: E. Zalewska)

Figure 2.
Necrosis and cracks in the bark of stems from which *Phomopsis sclarea* was isolated (photo: E. Zalewska)

Figure 3.
Decay of the stems top inhabiting by *Phomopsis sclarea* (photo: B. Zimowska)

Totally, 2743 fungi isolates were obtained from the analyzed parts of sage, and the most cultures were obtained from the stem base, slightly less from the leaves and the least from the plant roots (tab. 1). Fungi from genus *Fusarium* belonged to the species that were most frequently isolated from all the examined parts. They constituted 52.09, 37.14 and 0.42 of all isolates obtained from the roots, the stem base and the leaves, respectively (fig. 4). *Phoma exigua* var. *exigua* was isolated from all the examined plant parts, and the cultures of this fungus constituted 5.82, 3.91 and 0.34% of all fungi obtained from the roots, stem base and leaves, respectively (fig. 4). *Botrytis cinerea* was isolated from the stem base and leaves of sage. The isolates of this species constituted 4.32 and 4.75% of all fungi obtained from those parts, respectively (fig. 4). *R. solani* was isolated from the roots and the stem base, and the cultures of this species constituted 1.26 and 1.23% of all fungi obtained from those parts, respectively (fig. 1). The species most frequently isolated from the leaves was *A. alternata*. Cultures of this fungus constituted as much as 85.11% of isolations obtained from this part. Isolates of the fungus was obtained less frequently from the roots and the stem base (tab. 1, fig. 4). Species *Phomopsis sclarea* was isolated only from the stem base, and isolates of this species constituted 8.86% of all isolates obtained from that part (fig. 1).

Table 1.

Fungi isolated from sage (*Salvia officinalis*) in 2004–2006

species of fungus	number of isolates			total
	roots	stem bases	leaves	number (%)
Alternaria alternata (Fr.) Keissler	89	317	806	1212 (44.72)
Botrytis cinerea Pers.		42	45	87 (3.21)
Chaetomium globosum Kunze			1	1 (0.04)
Cladosporium cladosporioides (Fres. De Vries)	3	14	17	34 (1.25)
Cylindrocarpon didymum (Hartig) Wollenw.	48			48 (1.77)
Cylindrocarpon heteronema (Berk. et Br.)	8	3		11 (0.4)
Cylindrocarpon obtusisporum (Cooke et Harkness) Wollenw.	28	2		30 (1.11)
Epicoccum purpurascens Ehrenberg	6	9	35	50 (1.85)
Fusarium avenaceum (Fr.) Sacc.	78	52	1	131 (4.83)
Fusarium culmorum (W.G.Smith) Sacc.	112	125		237 (8.75)
Fusarium equiseti (Corda) Sacc.	126	103	3	232 (8.56)
Fusarium oxysporum Schlecht.	87	78		165 (6.09)
Fusarium solani (Mart.) Appel et Wollenw emend Snyd et Marasas	9	3		12 (0.44)
Gliocladium catenulatum Gilman et Abbott	21	13	2	36 (1.33)
Gliocladium roseum Bainier	13	11		24 (0.89)
Penicillium decumbens Thom	8	5		13 (0.48)
Penicillium chrysogenum Thom	6	7		13 (0.48)
Penicillium verrucosum, Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	4			4 (0.15)
Phoma capitulum Pawer, Mathur et Thirumalachar	8			8 (0.29)
Phoma exigua Desm. var. *exigua*	46	38	4	88 (3.25)
Phoma glomerata (Cda.) Wollenw. et Hochapf	9	8	2	19 (0.71)
Phoma labilis Sacc.	8	9		17 (0.63)
Phoma subglomerata Boerema, de Gruyter & Noordel.	8	11	15	34 (1.25)
Phoma pareupyrena de Gruyter, Noordel & Boerema	10	5		15 (0.55)
Phomopsis sclarea Sarwar.		89		89 (3.04)
Rhizoctonia solani Kühn	10	12		22 (0.81)
Talaromyces flavus (Köcker) Stolk et Samson	4	3		7 (0.26)
Trichoderma aureoviride Rifai	15	8	6	29 (1.07)
Trichoderma harzianum Rifai	8	4		12 (0.41)
Trichoderma koningii Oud.	15	24	10	49 (1.81)
Trichoderma polysporum Link et Pers. Rifai	4	10		14 (0.52)
total	791	1005	947	2743

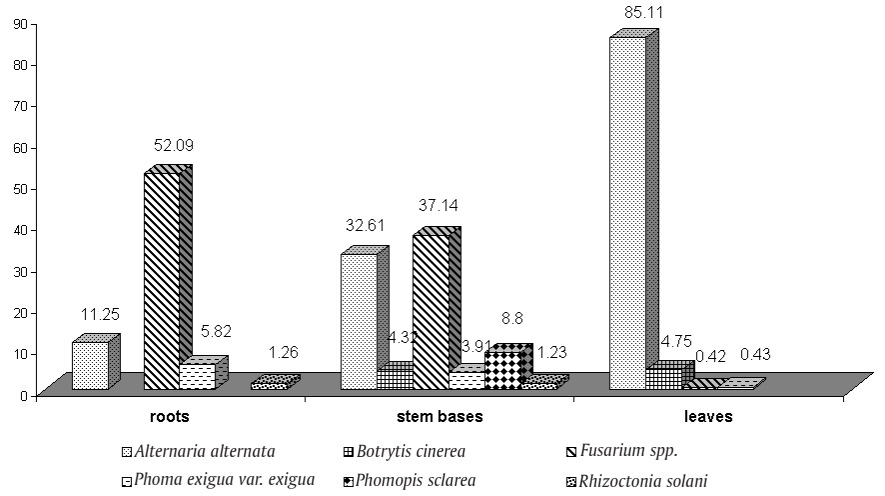

Figure 4.
Participation some species of fungi isolated from examined parts of sage (Salvia officinalis L.) in 2004-2006

DISCUSSION

The presented studies confirmed the occurrence of disease symptoms caused by fungi on the plants of sage cultivated on plantations in South-Eastern Poland. Symptoms of the necrosis of roots and lower parts of stems, connected with tissue disintegration and softening, were most probably the consequence of pathogenic effect of soil-borne fungi. This conclusion is justified by the fact of frequent isolation from those plant parts of fungi from genus *Fusarium* and species of *P. exigua* var. *exigua,* and *R. solani,* familiar polyphagous species of many cultivated plants, including spices and herbaceous [2, 5, 8]. The species of *F. avenaceum, F. culmorum* and *F. equiseti* isolated in the present studies, were recognized – on the basis on pathogenicity tests – as the cause of dying out of the shoots and the seedlings of lemon balm and thyme [13, 14]. Results of mycological analysis showed that the roots and the stem base were colonized by *F. oxysporum*. It is just this species that was recognized in California and Oregon, USA, as the main cause of sage dying out as a result of root rot and wilting of plants [3]. Considering the progressing warming of the climate in Poland, the thermophilous character of the pathogen as well as reports from literature on the forma speciales of the fungus specialized to infect definite species of herbaceous plants [15, 16], it can be supposed that the obtained isolates of *F. oxysporum* could have contributed to the above described symptoms on the examined sage plants. However, pathogenicity tests according to Koch's postulates are necessary in order to confirm this thesis.

R. solani, obtained from the roots and the stem base, can confirm the harmfulness of this species towards the enumerated parts of sage. This is indicated by the results of earlier studies on fungi infecting plants of thyme, lemon balm, St. John's wort and peppermint [2, 5, 6, 8] as well as by reports from literature on the pathogenic effect of *R. solani* on the plants of mint and trefoil cultivated in India [17, 18]. In Poland, considerable harmfulness of the fungus towards the roots and the stems of ginseng [19] and towards the seedlings of basil in the regions of the latter's cultivation was shown [20].

On the basis of the studies as well as reports from literature, *P. exigua* var. *exigua* can be included within the complex of fungi responsible for above-mentioned symptoms on the roots and lower parts of stems. It was experimentally proved that this species may cause the necrosis of the shoots and seedlings of thyme and lemon balm [21]. In connection with occurrence of a complex of fungi discussed above in the cultivation environment of the studied sage plants, the polyphagous character of parasitism of those fungi species should be taken into consideration while deciding upon the proper crop rotation. The root and pulse crops [22] recommended as proper forecrop for sage are very often the hosts of the fungi species described above [24]. Considering the above, introduction of phytosanitary plants, i.e. maize or a grass mixture, should be taken into account, with a simultaneous one-year interval in the cultivation of sage. Such a system of cultivation brought positive results, as far as the healthiness of plants is concerned, on mint plantations in India [17].

The *Phomopsis sclareae* species was considered as the main cause of specific symptoms on sage stem in the form of necrotic spots, peeling off and bark breaking [4, 11]. Such a conclusion is justified by the fact of isolating the fungus cultures from the infected places and the presence of etiological signs on them in the form of the fungus conidia. Among many species of *Phomopsis,* more than 60 are recognized as important pathogens of different host plants, including spices and herbaceous [11]. They include, for example, *P. diachenii*, causing dying out of caraway umbels [25], *P. subordinaria*, causing necrosis and dying out of the inflorescences of ribwort plantain [26], *P. levandulae*, lavender pathogen commonly occurring in Europe [27] and *P. sclarea*, isolated in present studies [4, 11]. It is known from literature that *Phomopsis* spp. may infect all aboveground parts of plants but the most typical symptoms include those on herbaceous and lignified stems [11]. Besides, as typical pathogens with facultative character of parasitism, they infect plants through natural openings, young undamaged tissues and first of all through fresh injuries [28]. The occurrence of *P. sclarea* in present studies, especially on the stems of the examined plants at full vegetation stage, confirms the above information since in early spring plants' rejuvenation took place on plantation, consisting in cutting of the plants to the height of 8–10 cm [22]. The injuries made in such a way probably became the gate for penetration of the fungus spores and consequently a development of the disease.

The *A. alternata* species was recognized as a cause of leaf necrosis and defoliation. Common isolation of the fungus cultures from the infected organs, the presence of the pathogen conidia on them and reports from literature on the occurrence of the discussed species on different herbaceous plants prove the thesis set above [17, 29]. Besides, the harmful effect of *A. alternata* on the yield quantity and quality results from the fact that the fungus produces toxic substances, which accumulate in plant tissues already during their growth [17, 30].

Isolation of *B. cinerea* from the leaves and the stems may point to the contribution of this species to sage diseases. It is the most dangerous pathogen of many herb species, e.g. basil cultivated in Italy as well as lemon balm, marjoram and sage cultivated in Israel [31, 32]. Since the fungus spores penetrate into the plants through its injuries, and the presence of a water drop is necessary for infection to occur, cutting of the plants should be performed in dry weather [32].

The occurrence of fungi from *Gliocladium* and *Trichiderma* genera, known for their antagonistic effect towards different pathogens of cultivated plants, including herbaceous ones, in the cultivation environment of the studied plants of sage should be viewed as positive [29, 33, 34].

The present studies made getting to know the fungal species colonizing and injuring the underground and aboveground parts of sage cultivated in south-eastern Poland possible. Isolation of *P. sclarea* from sage stems for the first time in Poland can be viewed as dangerous. Besides, on the basis of the present studies and reports from literature, *A. alternata* was recognized as an economically important species.

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GRZYBY ZAGRAŻAJĄCE UPRAWIE SZAŁWII LEKARSKIEJ (*SALVIA OFFICINALIS* L.) W POŁUDNIOWO-WSCHODNIEJ POLSCE

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

W latach 2004–2006 na plantacjach produkcyjnych szałwii lekarskiej (*Salvia officinalis* L.) zgrupowanych w południowo-wschodniej Polsce przeprowadzono badania nad grzybami zasiedlającymi i uszkadzającymi różne części roślin. Grzyby izolowano z powierzchniowo odkażonych korzeni, podstawy łodygi oraz liści przy zastosowaniu pożywki mineralnej. Z korzeni i dolnych części łodyg z objawami nekrozy i dezintegracji tkanek izolowano grzyby z rodzaju *Fusarium, Rhizoctonia solani, Phoma exigua* var. *exigua*. Z łodyg wykazujących symptomy nekrozy oraz łuszczenia i pękania kory otrzymano nie notowany wcześniej w Polsce gatunek *Phomopsis sclarea*. Z liści z objawami nekrozy powszechnie izolowano *Alternaria alternata*.

Słowa kluczowe: szałwia, Salvia officinalis, grzyby