ACTA AGROBOTANICA
Vol. 66 (1), 2013: 61–64
DOI: 10.5586/aa.2013.007

FUNGI COLONIZING DEAD LEAVES OF HERBS

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Received: 16.10.2012

Abstract

The material was collected from the Botanical Garden and the Collegium Medicum Medicinal Plant Garden of the Jagiellonian University in Krakow. The investigated species were: lemon balm (*Mellisa officinalis* L.), common lavender (*Lavendula angustifolia* Mill.), horsemint (*Mentha longifolia* L.), sage (*Salvia officinalis* L.), sweet basil (*Ocimum basilicum* L.), and wild marjoram (*Origanum vulgare* L.). The aim of the investigation was to identify fungi causing the death of leaf tissues of herbs from the mint family Lamiaceae. In mycological investigations, 180 fragments of each plant leaves (1,080 dead leaf fragments in total) were placed in a 2% PDA medium. Over 970 colonies of fungi belonging to 48 species were isolated from the dead leaf tissues of the six herb species. *Alternaria alternata* (toxin-producing), *Epicoccum nigrum* and *Sordaria fimicola* were the most frequently isolated. The largest numbers of colonies and species of fungi were isolated from horsemint, while the lowest numbers were from wild marjoram leaves. It was shown that the death of leaves of selected herb species from the Lamiaceae family was caused by various fungi. The results of the mycological analysis confirmed the diversity of species colonizing the leaves of the herbs.

Key words: Lamiaceae, herbs, leaves, fungi, necroses

INTRODUCTION

Herbs, like all plants, are susceptible to infectious disease pathogens. Disease symptoms manifested as necrotic tissue are common in herbal plants, despite the anti-fungal properties of active substances derived from this group of plants (Garibaldi et al. 1997; Machowicz-Stefaniak et al. 2003). Infection can lead to the reduction in the decorative qualities of plants, significant crop loss, and the deterioration of medicinal raw materials. The pathogens most commonly found on herbs are fungi and fungi-like Chromista organisms (Machowicz-Stefaniak et al. 2003; Zalewska and Machowicz-Stefaniak, 2004; Łabanowski et al. 2005; Adamska, 2006; Kołodziej, 2010; Zimowska, 2010).

The aim of this study was to investigate the microorganisms that colonize dead leaves of herbs.

MATERIALS AND METHODS

The research material was collected from the Botanical Garden of the Jagiellonian University and the Medicinal Plants Garden of the Jagiellonian University in Krakow, from May to September 2011 and 2012. The study was conducted on: lemon balm (*Mellisa officinalis* L.), common lavender (*Lavendula angustifolia* Mill.) horsemint (*Mentha longifolia* L.), sage (*Salvia officinalis* L.), sweet basil (*Ocimum basilicum* L.), and wild marjoram (*Origanum vulgare* L.). In mycological investigations, 180 fragments of herb leaves, taken from the edges of living and dead tissue, were placed in a 2% PDA medium. The taxonomic identification of isolated species of fungi was determined using the classifications by Booth (1971), Ellis and Ellis (1985) as well as Domsch et al. (1980). The system by Kirk et al. (2008) for determinate verification in naming fungi according to the Index Fungorum (2012) was used as the basis of classification.

RESULTS AND DISCUSSION

Brownish or brown necrotic spots were visible on the blades and petioles of the tested herb species, sometimes with a narrow lighter border. With time, the leaf tissue dried out and disintegrated.

The mycological analysis of dead tissue covering large areas of leaves showed that these symptoms were caused by fungi from the kingdom Fungi, belonging to 48 species. Over 970 colonies of fungi were isolated from the infected tissue. Most of colonies and

species of fungi were isolated from horsemint (205 and 27) and fewest of all from wild marjoram (117 and 10). A comparable number of species of fungi (21–22) were isolated from lemon balm, lavender, sage and basil, with varying numbers of colonies (135–189).

The most frequently isolated fungi were: *Alternaria alternata* (33.02%), *Epicoccum nigrum* (12.55%), and *Sordaria fimicola* (representing 5.86% of the total isolated colonies). Fungi belonging to the genera *Chaetomium*, *Cladosporium*, *Humicola*, *Mortierella*, *Penicillium* and *Umbellopsis* were isolated from leaves of most of the herb species (Table 1).

The toxin-producing species *A. alternata* was found on all tested herbs, causing the symptoms of alternaria blight on leaves. For basil leaves, this fungus amounted to over 50% of isolates. An equally high proportion of the pathogen was found in the fungal communities isolated from the leaves of wild marjoram, lavender and sage, in which isolates from communities of *A. alternata* made up over 42, 38 and 30% of isolated fungi, respectively. An even higher proportion of *A. alternata* (over 76%) was reported by Machowicz-Stefaniak and Zalewska (2007) on dying dill plants. The necrotrophs *E. nigrum* and *S. fimicola* also occurred frequently on all studied herb species, though their percentage in all communities was lower and more variable. *E. nigrum* ranged from 7% (in fungal colonies isolated from horsemint leaves) to 24% (from lemon balm leaves). The occurrence of *S. fimicola* was highest among fungi colonies isolated from leaves of wild marjoram (over 18%) and lowest among fungi found on lavender, horsemint and basil (1–2%). Fungi of the genera *Fusarium* and *Giberella* also occurred on dead herb leaves, including *F. chlamydosporum* var. *chlamydosporum* on horsemint. The species *Mortierella* and *Umbellopsis* (syn. *Mortierella*) were found on the leaves of all herbs, with the exception of wild marjoram, accounting for over 7% of all isolated colonies. These fungi colonize the youngest, most sensitive leaf blades under conditions of high humidity. Fungi of the species *Penicillium* and *Aspergillus* did not colonize dead marjoram leaves, either, but were occasionally found inhabiting other herbs. Fungi of the genera *Phoma* and *Boeremia* were found on common lavender, horsemint and sage. The study also detected a small share of fungi from the genus *Cladosporium*, which has been confirmed by Machowicz-Stefaniak et al. (2003), Machowicz-Stefaniak and Zalewska (2007) as well as Zimowska (2010). Single *Thanatephorus cucumeris* colonies were isolated from horsemint, although in the study by Zimowska (2010) the pathogen appeared with a frequency of up to 15% on peppermint and sage. The fungus *Trichoderma viride*, noted in many studies on herbal crop health, was not found on dead lavender and lemon balm leaves. *Septoria melissae*, which affects *Melissa*, was occasionally found on the oldest leaves (Table 1).

The incidence of *A. alternata* was particularly dangerous on basil leaves, because it led to premature drying and loss of leaves and to a lower quality of raw material, which has been confirmed by Machowicz-Stefaniak et al. (2003) as well as Mazur and Szczeponek (2006). The herbal literature also highlights the extensive damage caused by *B. cinerea* and *E. nigrum* to basil (Garibaldi et al. 1997; Mazur and Szczeponek, 2006). Machowicz-Stefaniak et al. (2002), Zalewska and Machowicz-Stefaniak (2004), Mazur and Szczeponek (2006), Frużyńska-Jóźwiak and Andrzejak (2007) reported the risk of disease caused by *Fusarium* spp. for herbs. In the present study, only a few colonies of *Fusarium* spp. were isolated from dead leaves of mint and lavender, and its presence was not confirmed on the leaves of basil, marjoram or sage, while Frużyńska-Jóźwiak and Andrzejak (2007) reported that lemon balm plants were often colonized by *F. avenaceum*, *F. oxysporum*, *B. cinerea* and *S. melissae*, peppermint plants by *F. avenaceum* and *F. oxysporum*, and sage by *F. culmorum*, *F. oxysporum* and *T. cucumeris*.

Sage leaves were mostly colonized by *A. alternata*, *E. nigrum*, *Humicola grisea* var. *grisea*, *Ilionectria radicicola*, *Phoma medicaginis* and *S. fimicola*. A comparison of the results to those of Zimowska (2010) found that the occurrence of the first two species were similar to that on sage plants in plantations and in the collections of the Botanical Garden and the Garden of Medicinal Plants of the Jagiellonian University. The study by Zimowska (2010) also confirmed the significant role of *A. alternata*, *B. cinerea*, *E. nigrum* and *Fusarium* and *Trichoderma* fungi in the death of mint leaves.

According to Chełkowski (2012), tissue decay occurs due to the effects of enzymes, or toxins, as well as the effects of necrogenic substances. Mycotoxins produced by *Aspergillus* and *Penicillium* inhibit the metabolism of plants and are also produced by pathogens of the genera *Alternaria*, *Phoma* and *Fusarium*, which are characterised by strong phytotoxicity, causing symptoms of necrosis and leaf drop. It can be assumed that *A. alternata* mycotoxins, or alternariol and tenuazonic acid, played a significant role in causing leaf necrosis in the investigated herb species. Chełkowski (2012) determined that toxin-producing fungi are generally accompanied by various fungi that are not toxin-producing. This is evidenced by the relatively large number of different species of fungi inhabiting the herb phyllosphere and damaging their leaves.

The present study showed that on the test material, which consisted of selected species of herbs from the Lamiaceae family, leaf death was caused by a complex of fungi and mycological analysis confirmed the diversity of fungi colonizing them.

Table 1
Fungi isolated from dead leaves of herbs

Fungus	*Lavendula angustifoliaa*	*Melissa officinalis*	*Mentha longifolia*	*Ocimum basilicum*	*Origanum vulgare*	*Salvia officinalis*	Total	Percentage [%]
Alternaria alternata (Fr.) Keissl.	60	25	38	96	50	52	321	33.02
Arthrinium phaeospermum (Corda) M.B. Ellis			1				1	0.10
Aspergillus brasiliensis Varga, Frisvard & Samson			2				2	0.21
Aspergillus versicolor (Vuill.) Tirab			1				1	0.10
Aureobasidium pullulans (de Bary) G. Arnaud				1			1	0.10
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley var. *exigua*			12				12	1.23
Botrytis cinerea Pers.			11	5	1		17	1.75
Chaetomium globosum Kunze		1	11	2	1		15	1.54
Chetomium nozdrrenkoae Sergeeva						5	5	0.51
Cladosporium cladosporioides (Fresen.) G.A. de Vries	11	3	2			1	17	1.75
Cladosporium herbarum (Pers.) Link					11	1	12	1.23
Cladosporium sphaerospermum Penz		2	9	1			12	1.23
Davidiella macrocarpa Crous, K. Schub. & U. Braun		2			6		8	0.82
Epicoccum nigrum Link	19	33	15	17	12	26	122	12.55
Fusarium chlamydosporum var. *chlamydosporum* Wollenw. & Reinking		1	5				6	0.62
Fusarium oxysporum E.F. Sm. & Swingle	1						1	0.10
Giberella baccata (Wallr.) Sacc.						1	1	0.10
Giberella stilboides W.L. Gordon ex C. Booth	6						6	0.62
Humicola fuscoatra var. *fuscoatra* Traaen				7	5	1	13	1.34
Humicola grisea var. *grisea* Traaen	1	7		5		14	27	2.78
Ilyonectria radicicola (Gerlach & L. Nilsson) Chaverri & C. G. Salgado	8	5	2			11	26	2.67
Isaria farinosa (Homsk.) Fr.				2			2	0.21
Khuskia oryzae H.J. Huds.				1		1	2	0.21
Mammaria echinobotryoides Ces.		1		1			2	0.21
Mortierella alpina Peyronel	1	1	2				4	0.41
Mortierella horticola Linnem.	6						6	0.62
Mortierella parvispora Linnem.			1	1		1	3	0.31
Mucor hiemalis var. *hiemalis* Wehmer	3	5	10	7			25	2.57
Penicillium expansum Link	2		11	4		1	18	1.85
Penicillium glabrum (Wehmer) Westling					2	1	3	0.31
Penicillium hirsutum Dierckx var. *hirsutum*		4	3	2			9	0.93
Penicillium verrucosum var. *verrucosum* Dierckx	3	5	2	4		2	16	1.65
Phoma eupyrena Sacc.						5	5	0.51
Phoma herbarum Westend	1	4				7	12	1.23
Phoma medicaginis Malbr. & Roum.	13					12	25	2.57
Preusia aemulans (Rehm) Arx	1						1	0.10
Rhizopus stolonifer (Ehrenb.) Vuill.	3			3			6	0.62
Septoria mellissae Desm.		5					5	0.51
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not	2	16	4	4	22	9	57	5.86
Stemphylium botryosum Sacc.	9	4	2				15	1.54
Talaromyces wortmannii C.R. Benj.						5	5	0.51
Thanatephorus cucumeris (A.B. Frank) Donk			2				2	0.21
Trichoderma viride Pers.			10	12	7	6	35	3.60
Truncatella truncata (Lév.) Steyaert	1	3	26				30	3.09
Umbellopsis ramaniana (Möller) W. Gams	1	3	7	2			13	1.34
Umbellopsis vinacea (Dixon-Stew.) Arx			4				4	0.41
Umbelopsis isabellina (Oudem) W. Gams	4	5	10	11		5	35	3.60
Umbelopsis nana (Linnem.) Arx	1		2	1		2	6	0.62
Total	157	135	205	189	117	169	972	100.0

CONCLUSIONS

1. Leaf death in lemon balm, lavender, horsemint, sage, common basil and wild marjoram was caused by numerous fungi found on the leaves, representing a variety of species.
2. *Alternaria alternata*, *Epicoccum nigrum* and *Sordaria fimicola* were considered to be particularly dangerous, as evidenced by widespread colonization on herb leaves.
3. The presence of toxin-producing *A. alternata* species on the herb phyllosphere often indicated the intensity of leaf damage.
4. Assessing the health of herbs growing in the same soil and climatic conditions, it was found that fungi prefer the environment of horsemint and basil plants.

Acknowledgements

Research supported by the Ministry of Science and Higher Education of Poland as the part of statutory activities of Department of Plant Protection, University of Agriculture in Krakow.

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Grzyby zasiedlające obumarłe liście ziół

Streszczenie

Materiał badawczy pobrano w Ogrodzie Botanicznym UJ i w Ogrodzie Roślin Leczniczych Collegium Medicum UJ. Badania prowadzono na: bazylii pospolitej (*Ocimum basilicum* L.), lawendzie wąskolistnej (*Lavendula angustifolia* Mill.), lebiodce pospolitej (*Origanum vulgare* L.), melisie lekarskiej (*Mellisa officinalis* L.), mięcie długolistnej (*Mentha longifolia* L.) i szałwii lekarskiej (*Salvia officinalis* L.). Celem pracy było poznanie grzybów zasiedlających obumarłe liście ziół z rodziny jasnotowatych Lamiaceae. W badaniach mykologicznych wyłożono na 2% pożywkę PDA po 180 fragmentów liści ziół, w sumie 1080 obumarłych fragmentów liści. Z martwych tkanek liści sześciu gatunków ziół wyodrębniono ponad 970 kolonii grzybów, należących do 48 gatunków. Najczęściej izolowanymi grzybami z porażonych liści były: *Alternaria alternata* (toksynotwórczy), *Epicoccum nigrum* i *Sordaria fimicola*. Najwięcej kolonii i gatunków grzybów wyizolowano z mięty długolistnej, a najmniej z lebiodki pospolitej. Wykazano, że na materiale badawczym, jakim były wybrane gatunki ziół z rodziny Lamiaceae, obumieranie liści powodował najczęściej kompleks grzybów, a wyniki analizy mykologicznej potwierdziły różnorodność grzybów je zasiedlających.