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ORIGINAL RESEARCH PAPER

Micromycetes on ericaceous plant leaves

Maria Kowalik*, Joanna Bonio, Klaudia Duda-Franiak

Department of Plant Protection, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Cracow, Poland

* Corresponding author. Email: m.kowalik@ogr.ur.krakow.pl

Abstract

A two-year study was carried out on the ericaceous plant collection of the Botanic Garden of the Jagiellonian University in Cracow and the Rogów Arboretum of the Warsaw University of Life Sciences on the following plants: wild rosemary *Ledum palustre* L., leatherleaf *Chamaedaphne calyculata* (L.) Moench and American cranberry *Oxycoccus macrocarpus* (Ait.) Pursh. Diverse micromycetes species composition was specified on the leaves of tested ericaceous plants. The perpetrators of dying leaves were recognized, among which the dominant role was played by the necrotroph *Pestalotiopsis sydowiana*. Quantitative and qualitative comparative analyses of micromycetes in both locations were carried out, showing a comparable degree of plant colonization by these fungi in the botanic garden and arboretum. The study may be helpful in explaining the causes of dieback of protected and endangered plants in Polish flora.

Keywords

Ericaceae; leaves; fungi; botanic garden; arboretum

This issue of Acta Mycologica is dedicated to Professor Maria Lisiewska and Professor Anna Bujakiewicz on the occasion of their 80th and 75th birthday, respectively.

Introduction

The role of botanic gardens is to protect rare and endangered plant species, so one of the duties is the storage and reproduction of these species. The following plants are threatened by extinction: wild rosemary *Ledum palustre* L. (syn. *Rhododendron tomentosum* Harmaja), leatherleaf *Chamaedaphne calyculata* (L.) Moench and American cranberry *Oxycoccus macrocarpus* (Ait.) Pursh. (syn. *Vaccinium macrocarpon* Ait.).

Wild rosemary grows on peat bogs and swamp forests. This is a medicinal plant, often obtained from natural habitats, although in Poland is under partial protection. Leatherleaf is the only species in the genus *Chamaedaphne*. In Poland its natural habitat is the Kampinos National Park and Drawa National Park, where this species is under careful protection as a relic of glaciation. In the current Polish red book of plants, *Ch. calyculata* is classified in the group EN – endangered. The American cranberry grows in natural habitats and wetlands in the eastern part of North America but in Poland is recognized as domesticated plant.

These three plant species transferred to the collection of the Botanic Garden of the Jagiellonian University in Cracow and the Rogów Arboretum of the Warsaw University of Life Sciences, showed symptoms of poor health and revealed extensive necrosis on leaves. It resulted in leaf dieback and defoliation, and further dieback of sprouts and shrubs. Leatherleaf and wild rosemary were especially affected by a disease. Finally wild rosemary plants died in the botanic garden in 2013.

This poor condition of plants was the inspiration for starting mycological studies aiming to determine the species composition of micromycetes inhabiting the leaves of *L. palustre*, *Ch. calyculata* and *O. macrocarpus*, identification the perpetrators of leaf dieback and finally to conduct a comparative analysis of the mycobiota at these two locations.

Material and methods

The study was carried out in the collection of ericaceous plants of the Botanic Garden of the Jagiellonian University in Cracow (Cracow Botanic Garden) and the Rogów Arboretum of the Warsaw University of Life Sciences (Rogów Arboretum). Mycological analysis included the following plants: wild rosemary *L. palustre*, leatherleaf *Ch. calyculata* and American cranberry *O. macrocarpus*. The sources of the research material were leaves of leatherleaf and American cranberry with necrosis, collected in May, July and September 2012 and 2013 and wild rosemary in the same months in 2012. Micromycetes were isolated from a total of 120 wild rosemary leaf fragments and 240 leaf fragments of leatherleaf and American cranberry. On both tested sites twenty leaves from each plant species were collected at every time point. Leaf fragments were cut from the border of healthy and necrotic tissues from single spots and surface decontaminated in 70% ethanol for minute, then thoroughly rinsed three times for one minute in sterile water before being placed on a Petri dish with a 2% PDA medium. The Petri dishes were incubated for 7 days at 21–22°C. The microscope used for observation was a Delta Optical microscope, model Evolution 300. Cultures of representative species of fungi are held in the Department of Plant Protection of the University of Agriculture in Krakow.

The taxonomic identification of micromycetes was conducted after: Domsch et al. [1], Ellis and Ellis [2], Guba [3], and Sutton [4]. The basis for the classification was the Kirk et al. [5] system, and author citations for each taxon are abbreviated according to the Index Fungorum database accessed November 2014 [6].

The similarity coefficient was calculated for the micromycetes communities isolated from the leaves of the tested plants, including habitats and years of research according to the formula given by Błaszkowski et al. [7]: $S = w / a + b - w$, where: S – similarity of compared communities (range of coefficient variation 0–1), a, b – number of species in communities A, B, w – number of shared species in both communities.

Results

Micromycetes communities inhabiting the leaves of wild rosemary, leatherleaf and American cranberry growing in the Cracow Botanic Garden and the Rogów Arboretum differed in species composition and number of isolated colonies.

In total 583 micromycetes colonies, comprising 31 species, were isolated from the leaves of ericaceous plants. One hundred and sixty micromycetes colonies isolated from 120 infected wild rosemary leaves fragments resulted in identification of 15 species (Tab. 1). The dominant fungi were: *Pestalotiopsis sydowiana* (35% of all of the fungi communities), *Alternaria alternata* and *Epicoccum nigrum*. The leaves of wild rosemary from the botanic garden and arboretum were inhabited by fungi in a comparable rates. Seventy-eight isolates belonging to 9 species and 82 isolates representing 11 species were isolated from the botanic garden and arboretum, respectively. Five species – *A. alternata*, *E. nigrum*, *Penicillium expansum*, *P. sydowiana* and *Phialophora cyclaminis* – were common for both tested sites.

From 187 colonies of micromycetes, isolated from the dying leaves of leatherleaf, 18 species has been determined (Tab. 2). *Pestalotiopsis sydowiana* played a major role (as well as on wild rosemary) and constituted almost 30% of the entire fungi community. *Pestalotia rhododendri*, *A. alternata*, *E. nigrum*, *Umbelopsis isabellina*, *Sordaria fimicola*, *Ph. cyclaminis* and *Phoma medicaginis* were also numerous. Their participation in the community of identified fungi was above 57%. The similar number of

Tab. 1 Micromycetes on leaves of wild rosemary *Ledum palustre* L.

Fungus	Fungi frequency on leaves in:						Total	Percentage (%)
	Cracow Botanic Garden			Rogów Arboretum				
	2012			2012				
	May	July	September	May	July	September		
Pestalotiopsis sydowiana (Bres.) B. Sutton	6	15	13	10	2	10	56	35.00
Alternaria alternata (Fr.) Keissl.	4	1	5	-	-	6	16	10.00
Epicoccum nigrum Link	2	4	2	4	2	2	16	10.00
Penicillium expansum Link	-	-	2	-	-	9	11	6.88
Phialophora cyclaminis J.F.H. Beyma	-	-	6	4	-	-	10	6.25
Phoma medicaginis Malbr. & Roum.	-	-	-	6	3	-	9	5.63
Leptosphaeria maculans (Tul.) Ces. & de Not.	-	-	8	-	-	-	8	5.00
Sordaria fimicola (Roberge ex Desm.) Ces. & de Not.	-	-	-	4	2	-	6	3.75
Fusarium flocciferum Corda	-	-	6	-	-	-	6	3.75
Fusarium oxysporum Schltdl.	-	-	-	-	-	6	6	3.75
Mortierella alpina Peyronel	-	-	-	-	5	1	6	3.75
Acremoniella atra (Corda) Sacc.	-	-	-	4	-	-	4	2.50
Fusarium culmorum (Wm.G. Sm.) Sacc.	-	-	2	-	-	-	2	1.25
Umbelopsis isabellina (Oudem.) W. Gams	-	-	-	-	2	-	2	1.25
Mortierella parvispora Linnem.	-	-	2	-	-	-	2	1.25
Total in month	12	20	46	32	16	34	-	-
Total	78			82			160	100.00

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Tab. 2 Micromycetes on leaves of leatherleaf *Chamaedaphne calyculata* (L.) Moench

Fungus	Fungi frequency on leaves in:												Total	Percentage (%)
	Cracow Botanic Garden						Rogów Arboretum							
	2012			2013			2012			2013				
	May	Jul	Sep	May	Jul	Sep	May	Jul	Sep	May	Jul	Sep		
Pestalotiopsis sydowiana (Bres.) B. Sutton	2	-	3	-	7	7	5	10	14	-	5	3	56	29.95
Pestalotia rhododendri (D. Sacc.) Guba	-	-	-	5	-	-	-	-	-	7	4	5	21	11.23
Alternaria alternata (Fr.) Keissl.	-	-	-	4	-	5	-	-	-	1	4	6	20	10.70
Epicoccum nigrum Link	6	1	1	-	-	2	2	-	2	1	-	2	17	9.09
Umbelopsis isabellina (Oudem.) W. Gams	-	2	5	-	-	-	10	-	-	-	-	-	17	9.09
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	-	5	-	2	-	3	-	-	-	-	1	2	13	6.95
Phialophora cyclaminis J.F.H. Beyma	6	3		-	-	-	-	-	1	-	-	-	10	5.35
Phoma medicaginis Malbr. & Roum.	4	1	-	-	-	-	4	-	1	-	-	-	10	5.35
Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley	4	-	1	-	-	-	-	-	-	-	-	-	5	2.67
Trichoderma pseudokoningii Rifai	-	4	-	-	-	-	-	-	-	-	-	-	4	2.14
Chaetomium globosum Kunze	-	-	-	3	-	-	-	-	-	-	-	-	3	1.60
Penicillium expansum Link	-	-	-	-	-	-	-	-	-	-	1	2	3	1.60
Aspergillus versicolor (Vuill.) Tirab.	-	1	-	-	-	-	-	-	1	-	-	-	2	1.07
Humicola fuscoatra Traaen	-	-	-	-	2	-	-	-	-	-	-	-	2	1.07
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	-	-	-	-	1	-	-	-	-	-	-	-	1	0.53
Botrytis cinerea Pers.	-	-	-	-	1	-	-	-	-	-	-	-	1	0.53
Sclerotinia sclerotiorum (Lib.) de Bary	-	-	-	-	1	-	-	-	-	-	-	-	1	0.53
Trichoderma harzianum Rifai	-	-	-	1	-	-	-	-	-	-	-	-	1	0.53
Total in month	22	17	10	15	12	17	21	10	19	9	15	20	-	-
Total	49			44			50			44			187	100.00

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colonies was isolated from leaves of leatherleaf and 17 and 10 species were identified from the botanic garden and from the arboretum respectively. Nine fungi species were common for leatherleaf leaves in both sites. Two hundred thirty six micromycetes colonies were isolated from infected and brown American cranberry leaf tissues, including 16 species (Tab. 3). The dominant fungus was *P. sydowiana*, which accounted for more than 63% of all of the colonies. Other species found in large number of colonies were *P. rhododendri* and *A. alternata*, while fungi from *Giberella*, *Epicoccum*, *Phialophora* and *Sordaria* types appeared in the lower abundance. *Pestalotiopsis sydowiana*, *A. alternata*, *P. rhododendri*, *Giberella stilboides* and *E. nigrum* were common species for both locations. A greater diversity of fungi species was found in the phyllosphere of American cranberry in the Cracow Botanic Garden than in the Rogów Arboretum.

Only four species – *A. alternata*, *E. nigrum*, *P. sydowiana* and *P. rhododendri* – inhabited the leaves of the ericaceous plants during both years of the study in the botanic garden and the arboretum.

Aspergillus versicolor, *Boeremia exigua*, *Botrytis cinerea*, *Davidiella macrocarpa*, *Humicola fuscoatra*, *Sclerotinia sclerotiorum* and *Trichoderma harzianum* were occasionally found on the ericaceous plants.

The similarity between the studied micromycetes communities (within locations and year of study) ranged from 0.1 to 0.88 (Tab. 4). The lowest rate of similarity was calculated for the micromycetes communities inhabiting the leaves of leatherleaf in the botanic garden and American cranberry in the arboretum in 2012. Micromycetes inhabiting leatherleaf and American cranberry in both locations and years as well as wild rosemary and leatherleaf in the botanic garden were also characterized by low similarity coefficients.

The highest similarity coefficient (0.88) was calculated for the micromycetes communities on leatherleaf leaves from the botanic garden and arboretum in 2012. A slightly lower similarity coefficient (0.55) was found for American cranberry in both locations in 2013.

Discussion

There is little information in the literature about the diseases, their potential causes and the fungi inhabiting the leaves of *L. palustre*, *Ch. calyculata* and *O. macrocarpus* [8–11].

The phyllosphere of ericaceous plants in the Cracow Botanic Garden and the Rogów Arboretum was dominated by *P. sydowiana* (syn. *Pestalotia sydowiana*, *Pestalotia guepini* var. *rhododendri*). This fungus is also mentioned in other papers as common on the leaves of *Rhododendron*, *Gaultheria*, *Epigaea*, *Oxycoccus*, *Kalmia*, *Calluna*, and *Erica* [2–4,12–19].

The previous research on the causes of leatherleaf and wild rosemary leaf death indicates on rust fungi: *Chrysomyxa ledi*, *Ch. cassandrae*, *Ch. vaccinii* and *Ch. reticulata* [10]. However in our studies we did not isolated these fungi, and symptoms of rust on plants grown in the botanic garden and arboretum were not observed.

Bristow and Windom [8], Caruso and Ramsdell [9] and Polashock [20] write about cranberry as susceptible to pathogen infections of leaves, sprouts, fruits and roots. American cranberry in its natural habitats is infected by *Monilinia oxycocci*, *Lophodermium oxycocci*, *Exobasidium oxycocci*, *Phomopsis vaccinii*, *Fusicoccum putrfaciens*, *Glomerella cingulata*, *Coleophoma empetri*, *Botryosphaeria vaccinii* and *Phyllosticta elongata* [11,21,22]. Caruso et al. [23] ascribe a special role to the genera *Phomopsis*, *Fusicoccum*, *Colletotrichum*, *Gloeosporium*, *Aureobasidium* and *Pestalotia* in the yellowing and falling leaves, and Caruso and Ramsdell [9] provide information about the fungi *Protoventuria myrtilli*, *Pyrenobotrys compacta*, *Exobasidium rostrupii* and *E. perenne* on low-lying leaves of cranberries. In the present study, there was no incidence of these pathogens on *O. macrocarpus* in the botanic garden and arboretum.

A comparative analysis of fungal communities indicate that the species *A. alternata*, *B. cinerea*, *E. nigrum*, *P. sydowiana*, *P. rhododendri*, *Ph. cyclaminis*, *Ph. medicaginis* and *S. fimicola* (with a high frequency) and numerous species belonging to

Tab. 3 Micromycetes on leaves of American cranberry *Oxycoccus macrocarpus* (Ait.) Pursh.

Fungus	Fungi frequency on leaves in:												Total	Percentage (%)
	Cracow Botanic Garden						Rogów Arboretum							
	2012			2013			2012			2013				
	May	Jul	Sep	May	Jul	Sep	May	Jul	Sep	May	Jul	Sep		
Pestalotiopsis sydowiana (Bres.) B. Sutton	13	5	15	-	25	18	14	16	16	-	13	15	150	63.56
Alternaria alternata (Fr.) Keissl.	-	3	2	1	1	3	1	2	-	1	1	2	17	7.20
Pestalotia rhododendri (D. Sacc.) Guba	-	-	-	4	2	5	-	-	-	3	-	-	14	5.93
Epicoccum nigrum Link	1	-	-	-	2	3	-	-	-	-	1	2	9	3.81
Gibberella stilboides W.L. Gordon ex C. Booth	-	-	-	1	-	3	-	-	-	-	3	-	7	2.97
Phialophora cyclaminis J.F.H. Beyma	1	3	3	-	-	-	-	-	-	-	-	-	7	2.97
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	-	-	-	-	-	-	-	-	-	5	-	-	5	2.12
Umbelopsis isabellina (Oudem.) W. Gams	-	-	-	-	2	3	-	-	-	-	-	-	5	2.12
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	-	-	-	-	-	-	-	-	-	1	3	-	4	1.69
Phoma leveillei Boerema & G.J. Bollen	4	-	-	-	-	-	-	-	-	-	-	-	4	1.69
Cladosporium cladosporioides (Fresen.) G.A. de Vries	-	-	1	-	-	2	-	-	-	-	-	-	3	1.27
Phoma medicaginis Malbr. & Roum.		3	-	-	-	-	-	-	-	-	-	-	3	1.27
Trichoderma hamatum (Bonord.) Bainier	3	-	-	-	-	-	-	-	-	-	-	-	3	1.27
Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley	-	2	-	-	-	-	-	-	-	-	-	-	2	0.85
Phialophora cinerescens (Wollenw.) J.F.H. Beyma	-	-	-	-	1	-	-	-	-	1	-	-	2	0.85
Davidiella macrocarpa Crous, K. Schub. & U. Braun	-	-	-	-	-	-	-	-	-	1	-	-	1	0.42
Total in month	22	16	21	6	33	37	15	18	16	12	21	19	-	-
Total	59			76			49			52			236	100.00

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Tab. 4 Similarity coefficient of the micromycetes communities isolated from the leaves of ericaceous plants (BG – Cracow Botanic Garden; A – Rogów Arboretum).

Species			*Oxycoccus macrocarpus*				*Chamaedaphne calyculata*				*Ledum palustre*	
	Year		2012		2013		2012		2013		2012	
		Stand	BG	A	BG	A	BG	A	BG	A	BG	A
Oxycoccus macrocarpus	2012	BG	-	0.22	0.31	0.20	0.39	0.36	0.18	0.25	0.39	0.33
		A	0.22	-	0.25	0.22	0.10	0.14	0.18	0.33	0.22	0.18
	2013	BG	0.31	0.25	-	0.55	0.21	0.27	0.27	0.40	0.21	0.27
		A	0.20	0.22	0.55	-	0.20	0.15	0.43	0.50	0.29	0.25
Chamaedaphne calyculata	2012	BG	0.39	0.10	0.21	0.20	-	0.88	0.18	0.25	0.20	0.43
		A	0.36	0.14	0.27	0.15	0.88	-	0.13	0.20	0.25	0.42
	2013	BG	0.18	0.18	0.27	0.43	0.18	0.13	-	0.42	0.18	0.22
		A	0.25	0.33	0.40	0.50	0.25	0.20	0.42	-	0.36	0.42
Ledum palustre	2012	BG	0.39	0.22	0.21	0.29	0.20	0.25	0.18	0.36	-	0.33
		A	0.33	0.18	0.27	0.25	0.43	0.42	0.22	0.42	0.33	-

the genera *Chaetomium*, *Cladosporium*, *Davidiella*, *Fusarium*, *Mortierella*, *Umbelopsis* and *Trichoderma* were common fungi inhabiting the leaves of azalea, evergreen rhododendron, wild rosemary, leatherleaf and American cranberry in previous vegetation periods in the Cracow Botanic Garden [12,13,15,16]. The research carried out in the Rogów Arboretum 2011 [14] shows the similar results.

The previous results showed that a dozen micromycetes species were common for the leaves and flowers of Saucer magnolia in the botanic garden in 2013 [24] and for the leaves of ericaceous plants. *Alternaria alternata*, *E. nigrum*, *Mortierella parvispora*, *P. expansum*, *Ph. medicaginis* inhabited the infected organs with a high frequency.

Comparison of mycological analysis of air carried out in the botanic garden and arboretum in 2012 [25] with the fungal communities inhabiting the ericaceous plants revealed very low similarity in terms of colony-forming units for the air and tested plants. The air of the botanic garden and the leaves of wild rosemary, leatherleaf and American cranberry contained *A. alternata*, *E. nigrum*, *P. expansum* and *C. cladosporioides*, while the air of the arboretum and the ericaceous plants contained three species: *A. alternata*, *E. nigrum* and *P. expansum*. Additionally *F. oxysporum* was found on the leaves of wild rosemary.

The low and very low similarity coefficients calculated for the micromycetes communities inhabiting wild rosemary, leatherleaf and American cranberry in the two years of research and locations is an evidence of the diverse species composition in the phyllosphere of ericaceous plants.

Conclusions

(***i***) Micromycetes communities inhabiting the leaves of wild rosemary, leatherleaf and American cranberry , growing in the Cracow Botanic Garden and the Rogów Arboretum, differed in their species composition and the number of colonies.

(***ii***) The dieback of wild rosemary, leatherleaf and American cranberry was caused by a variety of micromycetes, among which necrotroph *P. sydowiana* played a dominant role.

(***iii***) Most of the micromycetes communities inhabiting the leaves of ericaceous plants in the Cracow Botanic Garden and the Rogów Arboretum were characterized by a low and very low coefficient of similarity.

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