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MK: concept of the study; KDF, MK: determination of the specimens; MK, KDF: writing the manuscript

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ORIGINAL RESEARCH PAPER

Micromycetes on climbing roses leaves (Rosa L.) in the Botanic Garden of the Jagiellonian University in Cracow

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Abstract

Micromycetes inhabiting the leaves of 20 cultivars of climbing roses (Rosa L.), grown in Botanic Garden of the Jagiellonian University in Cracow was investigated in the three successive years of research. Sixty-five taxa of of micromycetes was recorded with a few species dominating: Alternaria alternata, Epicoccum nigrum, Pestalotia rosae, Penicillium brevicompactum and Sordaria fimicola, accompanied by various other microfungi. A high abundance of rose black spot caused by Diplocarpon rosae was also observed. The affected leaves revealed advancing necrosis, substantially enhancing at the end of the growing season. Defoliation took place from June to October. Micromycetes inhabiting the leaves of climbing roses in Botanic Garden of the Jagiellonian University in Cracow considerably deteriorated the decorative aspect of the plants.

Keywords

microfungi; necrosis of leaves; rose black spot; defoliation

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

Botanic gardens form a special place within a city, established to conserve plants around the world and to support teaching, research and recreation. The aesthetic experience offered by the plant collections in botanic gardens contribute to raising public awareness about the threat posed by microorganisms that cause various diseases and thus reduce the decorative aspects of plants. Climbing roses make an elegant, beautiful covering for nearly any horizontal or vertical structure. The climbing roses found in the Botanic Garden of the Jagiellonian University in Cracow, represent a collection of old and rare cultivars, most of which, are no longer in cultivation. Collection found in green spaces, with their low biological stability and continuous and intensive pest pressure, are highly susceptible to infections caused by mycobiota, among which micromycetes play an important role [1-3].

Micromycetes inhabiting climbing roses cause deformities of above-ground parts of plants, dieback of shoots and flowers and dieback and premature leaf fall. Climbing roses deprived of lush foliage or with visible symptoms of disease on the leaves lose their ornamental features and become useless as covers for unsightly places in gardens [4,5].

Up to now there is no data about the occurrence of micromycetes on climbing roses in Botanic Garden of the Jagiellonian University in Cracow. This is why this study was undertaken.

Material and methods

The mycological research was carried out using the collection of climbing roses from the Botanic Garden of the Jagiellonian University in Cracow. The source of the research material were leaves of 55 climbing roses bushes, represented by 20 cultivars collected in June, August and October 2011–2013. Micromycetes were isolated from 1350 climbing rose leaves. Leaf fragments were cut from the border of healthy and necrotic tissues from single spots and surface sterilized in 70% ethanol for one 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. Micromycetes isolation and culture were carried out according to the standard methods practiced in mycology [6]. The microscope used for observation was a Delta Optical microscope, model Evolution 300.

The following keys was used in micromycetes taxonomic identification: Guba [7], Domsch et al. [8], Sutton [9], Sivanesan [10] and Ellis and Ellis [11]. The basis for the classification was the system of Kirk et al. [12] and the authors' epithets by fungal species names were verified according to Index Fungorum 2014 [13].

On the basis of micromycetes specification and considering the participation of individual species in the total fungal community, particular taxa were classified to the group of dominants (constituting of >5% of the entire community), influents (1–5%) and accessory fungi (<1%), according to Kowalik [14].

Similarity coefficient of the micromycetes communities on rose leaves was calculated including terms and years of research according to the formula given by Błaszkowski et al. [15]: 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

Community of micromycetes inhabiting leaves of climbing roses from the Botanic Garden of the Jagiellonian University, Cracow in 2011–2013 produced differentiated number of colonies and revealed diverse species composition. Among one thousand two hundred micromycetes colonies isolated from the infected fragments of climbing roses leaves 65 species have been identified. The number of fungal colonies in the years 2011, 2012 and 2013 amounted 320, 412 and 468 and 27, 45 and 37 taxa has been identified each year respectively. In June, August and October 36 to 181 colonies were isolated, which comprised 8 to 28 species (Tab. 1).

The dominant (with abundance above 5% of all recovered colonies) micromycetes isolated from leaves of climbing roses comprised the following species (in the descending order): Alternaria alternata, Epicoccum nigrum, Diplocarpon rosae, Penicillium brevicompactum and Pestalotia rosae. The group of influents (abundance 1–5% of all recovered colonies), included: Arthrinium sphaerospermum, Aspergillus flavus, A. niger, Chaetomium funicola, Cladosporium cladosporioides, C. herbarum, Humicola grisea, Ilyonectria radicicola, Mortierella parvispora, Penicillim expansum, P. glabrum, P. herquei, Pestalotiopsis sydowiana, Phialophora cyclaminis, Phoma leveillei, Ph. medicaginis, Pleurostomophora richardsiae and Sordaria fimicola.

The accessory group, amounting less than 1% of the isolated colonies, included species from the following genera: *Acremonium*, *Coelophoma*, *Gliomastix*, *Isaria*, *Mucor*, *Nectria*, *Oidiodendron*, *Paraphoma*, *Rhizopus*, *Stemphylium*, *Thanathephorus*, *Trichoderma*, *Trichothecium*, *Umbelopsis* and others.

Aspergillus chevalieri, Boeremia exigua, Chaetomim cochlioides, Ch. piluliferum, Humicola fuscoatra, Nectria inventa, Mucor hiemalis, Penicillium citrinum, Rhizopus stolonifer, Stemphylium vesicarium, Trichothecium roseum were occasionally found in the phyllosphere of roses as single or double colonies.

Alternaria alternata and C. cladosporioides constantly inhabited leaves with necrosis symptoms, although with a different frequency. The pathogen D. rosae appeared continuously on the leaves (except year 2011), while P. rosae was found from August to October, with a greater number of colonies recorded in October. The

Tab. 1 Micromycetes isolated from the infected leaves of ci	limbing roses	(Rosa L.).									
	Fungi freq	uency on le	aves in:								
	2011			2012			2013				
Fungus	June	August	October	June	August	October	June	August	October	Total	%
Acremonium rutilum W. Gams	ı	ı	1	1	7	1	1	ı	I	ß	0.42
Alternaria alternata (Fr.) Keissl.	16	20	30	24	62	64	62	17	40	335	27.92
Alternaria botrytis (Preuss) Woudenberg & Crous	1	I	1	I	I	2	I	T	I	2	0.17
Arthrinium sphaerospermum Fuckel	I	I	1	7	6	ı	I	I	I	16	1.33
Aspergillus chevalieri Thom & Church	1	I	I	1	I	I	T	I	I	1	0.08
Aspergillus flavus Link	I	I	1	I	I	3	1	S	S	14	1.17
Aspergillus niger Thiegh.	1	I	I	T	I	I	1	×	7	16	1.33
Aspergillus sydowii (Bainier & Sartory) Thom & Church	1	4	1	1	I	I	1	1	I	9	0.50
Aspergillus versicolor (Vuill.) Tirab.	1	I	2	T	I	I	2	2	I	6	0.50
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	I	1	1	1	I	1	I	1	I	1	0.08
Botrytis cinerea Pers.	1	I	I	I	I	I	5	4	1	10	0.83
Chaetomium cochliodes Palliser	I	I	1	I	I	1	I	I	I	1	0.08
Chaetomium funicola Cooke	I	4	3	2	I	2	I	1	2	13	1.08
Chaetomium globosum Kunze	I	I	2	I	1	ı	I	I	2	5	0.42
Chaetomium piluliferum J. Daniels	1	I	1	I	I	ı	I	I	2	2	0.17
Cladosporium cladosporioides (Fresen.) G.A de Vries	1	1	2	5	14	6	4	5	5	43	3.58
Cladosporium herbarum (Pers.) Link	2	I	5	1	I	4	6	ы	3	29	2.42
Cladosporium sphaerospermum Penz.	I	I	1	I	З	ı	I	2	I	5	0.42
<i>Coleophoma empetri</i> (Rostr.) Died.	1	ı	1	1	3	ı	I	I	I	4	0.33
Diplocarpon rosae F.A. Wolf	I	10	21	3	1	2	1	21	3	62	5.17
Epicoccum nigrum Link	4	10	49	1	T	1	4	1	6	78	6.50

Tab. 1 Continued											
	Fungi freq	uency on lea	ives in:								
	2011			2012			2013				
Fungus	June	August	October	June	August	October	June	August	October	Total	%
Gliomastix luzulae (Fuckel) E.W. Mason ex S. Hughes	ı	ı	I	I	ı	ı	5	ю	1	9	0.50
Humicola fuscoatra Traaen	I	1	I	I	I	1	ı	I	ı	1	0.08
<i>Humicola grisea</i> Traaen	I	1	I	1	I	3	4	I	7	16	1.33
<i>Ilyonectria radicicola</i> (Gerlach & L. Nilson) P. Chaverri & C. Salgado	1	œ	10	I	I	10	ı	ı	ı	28	2.33
Isaria fumosorosea Wize	I	T	I	I	T	7	I	1	1	æ	0.67
Khuskia oryzae H.J. Huds.	I	I	1	4	I	ı	1	I	1	5	0.42
<i>Mortierella alpina</i> Peyronel	I	T	I	I	2	I	1	5	I	5	0.42
Mortierella hyalina (Harz) W. Gams	I	I	I	6	I	ı	I	I	I	6	0.75
Mortierella parvispora Linnem.	3	5	5	2		8		п	4	28	2.33
Mucor hiemalis Wehmer	I	I	I	I	ı	-1	ı	I	I	1	0.08
Myrothecium verrucaria (Alb. & Schwein.) Ditmar	3	I	I	I	I	ı	ı	I	I	3	0.25
<i>Nectria inventa</i> Pethybr.	I	I	1	I	I	ı	ı	I	ı	1	0.08
Oidiodendron echinulatum G.L. Barron	ı	ī	I	I	ī	ı	ı	I	4	7	0.58
Paraconiothyrium minitans (W.A. Campb.) Verkley	ı	6	I	I	2	ı	ı	I	ı	8	0.67
Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley	I	7	ı	ı	ī	7	1	ı	1	4	0.33
Penicilium dodgei Pitt	I	T	I	I	T	3	ı	I	ı	3	0.25
Penicillium brevicompactum Dierckx	I	I	I	I	I	I	13	29	19	61	5.08
Penicillium citrinum Thom	ı	ı	I	I		ı		ı	-1	п	0.08
Penicillium expansum Link	I	10	I	I	6	I	ı	I	I	19	1.58
Penicillium glabrum (Wehmer) Westling	I	I	I	I	ı	2	6	16	17	44	3.67

Continued	
1	
Tab.	

	Fungi freq	uency on lea	ives in:								
	2011			2012			2013				
Fungus	June	August	October	June	August	October	June	August	October	Total	%
Penicillium herquei Bainier & Sartory	I	ı	I	I	ı	ı	6	15	8	29	2.42
Penicillium javanicum J.F.H. Beyma	I	ī	I	I	T	1	I	I	I	1	0.08
Penicillium jensenii K.M. Zalessky	I	I	I	1	Э	T	I	I	I	4	0.33
Penicillium miczynski K.M. Zalessky	I	I	I	I	T	2	1	I	I	з	0.25
Penicillium restrictum J.C. Gilman & E.V. Abbott	I	T	I	I	T	T	I	1	7	8	0.67
Periconia macrospinosa Lefebvre & Johnson	1	T	4	I	T	T	I	T	I	4	0.33
Pestalotia rosae Westend.	I	8	12	I	7	20	2	2	10	61	5.08
Pestalotiopsis sydowiana (Bres.) B. Sutton	I	I	I	I	I	T	13	I	1	14	1.17
Phialophora cyclaminis J.F.H. Beyma	I	10	I	4	T	T	I	I	I	14	1.17
Phoma complanata (Tode) Desm.	I	I	I	I	I	з	I	I	1	ŝ	0.25
Phoma eupyrena Sacc.	I	2	4	I	I	1	I	ı	I	7	0.58
Phoma leveillei Boerema & G.J. Bollen	3	ъ	5	2	4	19	I	I	I	38	3.17
Phoma medicaginis Malbr & Roum.	I	I	I	I	8	I	3	1	I	12	1.00
Phoma putaminum Hollós	I	I	I	2	I	T	I	I	I	2	0.17
Pleurostomophora richardsiae (Nannf.) L. Mosrert, W. Gams & Crous	4	11	I	ß	I	I	I	I	ı	20	1.67
Rhizopus stolonifer (Ehrenb.) Vuill.	I	I	I	1		I	I	I	1	1	0.08
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not	I	ı	6	11	12	4	6	6	1	48	4.00
Stemphylium vesicarium (Wallr.) E.G. Simmons	I	п	I	I	ı	ı	I	I	ı	1	0.08
Thanatephorus cucumeris (A.B. Frank) Donk	I	I	I	I	ı	ı	2	I	ı	2	0.17
Trichoderma viride Pers.	1	ı	I	I	ı	5	1	ı	ı	9	0.50
Trichothecium roseum (Pers.) Link	I	I	I	I	1	I	1	I	I	2	0.17

Tab. 1 Continued

	Fungi freq	uency on lea	ves in:								
	2011			2012			2013				
Fungus	June	August	October	June	August	October	June	August	October	Total	%
Ulocladium consortiale (Thüm) E.G. Simmons	ı	ı	1	ı	ı	ı	ı	I	2	2	0.17
<i>Umbelopsis isabellina</i> (Oudem.) W. Gams	I	1	I	I	1	3	1	I	1	5	0.42
Umbelopsis vinacea (Dixon-Stew.) Arx	I	I	I	I	I	I	ı	1	ı	1	0.08
Total colonies	36	119	165	87	144	181	155	149	164	1200	100.0
Total species	8	19	17	20	18	28	25	23	24	65	ı
Total colonies in year	320			412			468			1200	ı
Total species in year	27			45			37			65	I

necrotroph *E. nigrum* colonized massively the leaves of climbing roses in October 2011. Species of *Cladosporium* and *Phoma*, including *C. cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *Ph. companata*, *Ph. eupyrena*, *Ph. leveillei*, *Ph. medicaginis* and *Ph. putaminum*, inhabited roses leaves in large numbers.

The similarity between two of the compared communities (within three terms for each year of the study and between the same terms) ranged from 0.13 to 0.60 (Tab. 2). In the growing season 2011 the similarity indices ranged from 0.29 to 0.38, in 2012 from 0.21 to 0.31, and in 2013 from 0.44 to 0.6. The lowest similarity coefficient, calculated for the micromycetes communities on the leaves of climbing roses was found in June 2011 and August 2012, and the highest was for the community found in June and August 2013. Eleven compared communities were characterized by very low similarity coefficients (in the range 0.1 to 0.2), 15 communities were characterized by low coefficient (0.21-0.3), seven communities as middle coefficient (0.31-0.4), two as high coefficient (0.41–0.5), and only a single community as the highest (0.51 - 0.60).

Discussion

The phyllosphere of climbing roses was dominated by fungus A. alternata for three consecutive years. This polyphagous species has been mentioned in many studies as the cause of extensive necrosis on leaves [6,14,16,17]. Scheffer [18] speculates that this may be the most widespread species in the world, both in temperate and tropical climates. It is considered to be a very persistent species because its dried spores are able to survive for a years [8]. Dominance of A. alternata in community structure of micromycetes on the leaves of climbing roses proved to be particularly harmful and as a consequence caused numerous necrosis on leaves. This fungus can also colonize the dead tissue of rose leaves that was previously necrotized by pathogens. Therefore in this study we collected fragments from the edges of the living and dead leaf tissue of roses. Such approach excludes the isolation of endophytes because they inhabit the asymptomatic tissue of their hosts. The high frequency of A. alternata on the leaves of roses, confirmed by the isolation of 335 colonies, favored a significant level of infectivity and pathogenicity of this necrotroph. The proximity of many host plants also created favorable conditions for its spread, causing increased colonization and infection, as documented in earlier studies [2,14,17]. The production of host specific toxins had an impact on dieback and premature leaf fall, showing its toxic properties in relation to host plants, which is emphasized by Wakuliński [19].

Previously Duda and Bonio [16] and Kowalik et al. [17,20,21] have documented the high frequency of *A. al-ternata* and other potentially pathogenic micromycetes in the Botanic Garden of the Jagiellonian University in Cracow. The most frequently isolated species from the leaves of Lamiaceae family herbs, *Azalea pontica* and saucer

Year		2011			2012			2013		
	Month	VI	VIII	Х	VI	VIII	Х	VI	VIII	Х
	VI	-	0.29	0.32	0.27	0.13	0.20	0.14	0.19	0.19
-	VIII	0.29	-	0.38	0.30	0.23	0.34	0.19	0.20	0.23
201	Х	0.32	0.38	-	0.32	0.25	0.36	0.24	0.29	0.28
	VI	0.27	0.30	0.32	-	0.31	0.26	0.18	0.16	0.19
7	VIII	0.13	0.23	0.25	0.31	-	0.21	0.26	0.24	0.17
201	Х	0.20	0.34	0.36	0.26	0.21	-	0.33	0.28	0.30
	VI	0.14	0.19	0.24	0.18	0.26	0.33	-	0.60	0.44
ŝ	VIII	0.19	0.20	0.29	0.16	0.24	0.28	0.60	-	0.47
201	Х	0.19	0.23	0.28	0.19	0.17	0.30	0.44	0.47	-

Tab. 2 Similarity coefficient between micromycetes communities isolated from the leaves of climbing roses (Rosa L.).

magnolia were *A. alternata*, *E. nigrum*, *S. fimicola*, *P. expansum* and *Pestalotiopsis sydowiana*. The colonization of leaves of roses by fungal species found on the other plant species grown in the vicinity of rosaceous plants indicates on the strong liaison of pathogenic fungi and saprobes in phyllospheres of different plants. According to Jędryczka [22], the *Alternaria* and *Cladosporium* genera are particularly frequent in the air, and the *Aspergillus* and *Penicillium* genera are often listed as aerosols [23]. Bonio and Duda [23], evaluating the number of colony forming units in the Botanic Garden of the Jagiellonian University in Cracow and the Rogów Arboretum of the Warsaw University of Life Sciences, reported that *A. alternata* was the dominant fungus in the air near these two locations. Ogórek et al. [24] indicated that *A. alternata* is a ubiquitous fungus with a very high concentration of spores in the air of mountain areas.

Cladosporium cladosporioides, *C. herbarum* and *C. sphaerospermum* inhabit the leaves of climbing roses quite frequently. Jędryczka [22] states that their presence in the atmospheric air in the form of bioaerosols favors their infectious potential, causing necrosis on colonized plant tissue [25,26]. The weather and local climate can cause an increase density of *Alternaria*, *Cladosporium* and *Epicoccum* spores in the air [23]. These studies may suggest that fungi mentioned above colonize the leaves of plants under conditions of slight moisture. A somewhat higher colonization of leaves of roses in October than in the summer months by fungi from the genus *Mortierella* is probably related to their preferences in terms of higher humidity.

More than 60 colonies of *P. rosae* were isolated in the present study. This micromycete is monophagous and was found on roses in the vicinity of Ghent and described for the first time in 1859 [7]. In Polish literature this fungus has not been described up to now.

According to mycological analyses, the most frequent disease occurring on climbing roses leaves was rose black spot caused by *D. rosae*, which resulted in dieback and drooping of leaves in June for three consecutive years of the study (particularly evident in 2011).

Symptoms of grey mould caused by *B. cinerea* were visible on the leaves until June 2013, which confirms the opinion that the pathogen is not dangerous for strong, well-fed shrubs [2].

The number of isolated micromycetes colonies was almost five times higher in October 2011 than in June of 2011, twice as high in October 2012 than in June 2012 and similar in both terms of 2013. This reflects the increasing infestation towards the end of the growing season that appears as spots and leaf necrosis, resulting in defoliation [5]. The increase of the number of colonies and species of micromycetes obtained in the third year of the study may indicate on the accumulation of micromycetes propagules on tested climbing roses.

Conclusions

- (i) Communities of micromycetes inhabiting the leaves of climbing roses in the Botanic Garden of the Jagiellonian University in Cracow were characterized by different species composition in the three successive years of research.
- (*ii*) The dieback of climbing rose leaves was caused by numerous micromycetes. The dominant role was played by the pathogen *D. rosae* and necrotrophs *A. alternata*, *E. nigrum*, *P. brevicompactum* and *S. fimicola*.

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