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Micromycetes colonizing and damaging leaves of evergreen rhododendron (*Rhododendron* L.) in nursery

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Abstract

In May and October 2010–2012, mycological studies were conducted on 10 cultivars of rhododendron bushes growing in containers in the nursery of ornamental plants. Out of 3000 specimens of infested leaf fragments, 2566 fungal colonies belonging to 41 species were isolated. The following species colonizing the leaves and causing their necrosis were extracted in the largest number of colonies: *Alternaria alternata*, *Aspergillus niger*, *Epicoccum nigrum*, *Humicola grisea*, *Pestalotiopsis sydowiana*, *Phoma pomorum*, *Sordaria fimicola*, *Trichoderma koningii*, *Trichoderma polysporum*, *Truncatella truncata*, *Umbelopsis isabellina* and others. The research showed that the micromycetes colonies colonizing and damaging rhododendron leaves varied in species composition and number of colonies in different years and at different times. The study determined which rhododendron cultivars were characterized by good health and which had the greatest susceptibility to infection by micromycetes.

Keywords: rhododendron; cultivar; health status; leaves; fungi

Introduction

Evergreen rhododendron (*Rhododendron* L.) is a valuable plant in modern gardens. Their harmonious, fragrant flowers that vary in color, form and size are the pride of rhododendrons. Outside of the flowering period, however, their evergreen leaves determine the decorative aspects of these plants. Evergreen rhododendrons and other plants are susceptible to pathogenic organisms, such as the most common micromycetes. Currently, more than 200 mycobiota species have been identified in the phyllosphere of rhododendrons that can determine the health of the leaves.

These organisms deteriorate the condition of shrubs, contribute to defoliation and reduce the value of nursery stock in trade, often leading to the exclusion of cultivars from cultivation. To evaluate the usefulness of some cultivars of rhododendron for cultivation, research is carried out on the health status of nursery material [1–4].

The aim of the study was to identify micromycetes species inhabiting and damaging the leaves of selected cultivars of evergreen rhododendron (*Rhododendron* L.).

Material and methods

In the years 2010–2012, studies were carried out in May and October on the bushes of 10 rhododendron cultivars: *R. forrestii* 'Baden Baden', *R. calophytum* 'Dominik', *R. brachycarpum* 'Flautando', *R. wardii* 'Goldbukett', *R. yakushimanum* 'Golden Torch' and 'Sneezy', *R. catawbiense* 'Nova Zembla' and 'Roseum Elegans', *R. ponticum* 'Rasputin' and *R. campylocarpum* 'Simona', grown in containers in the nursery of ornamental plants. A total of 3000 affected leaf fragments were collected for mycological analysis. The leaf fragments were decontaminated in 70% ethanol. Micromycete isolation and culture were carried out according to the standard methods practiced in mycology [5].

The keys used in taxonomic identification were as follows: Domsch et al. [6], Ellis and Ellis [7], Guba [8], and Rifai [9]. The basis for the classification was the Kirk et al. [10] system. The researchers' findings were verified using the database of Index Fungorum 2014 [11].

Meteorological data contained in Fig. 1 and Fig. 2 came from weather station Davis Vantage Pro2, located in Kraków Fiołkowa.

Results

In 10 cultivars of 4–6-year *Rhododendron* L. evergreen rhododendron shrubs grown in the nursery of ornamental plants, light brown stains were mostly visible on the edges

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of the leaves and the top of the leaf blade as well as oval and irregular spots along the midrib of the leaf. The tissues developed necrotic spots and died. Leaves with necrosis fell off across a large area.

Micromycetes colonizing and damaging the leaves of rhododendron belonged to 41 species. 2566 micromycetes colonies were isolated from the infected tissues (Tab. 1). The most numerous fungal colonies belonged to the following species: *Alternaria alternata*, *Aspergillus niger*, *Epicoccum nigrum*, *Humicola grisea*, *Pestalotiopsis sydowiana*, *Phoma pomorum*, *Sordaria fimicola*, *Trichoderma koningii*, *T. polysporum*, *Truncatella truncate*, and *Umbelopsis isabellina*.

Micromycetes communities inhabiting the phyllosphere of selected evergreen rhododendron cultivars differed in species composition and number of colonies. From the material collected in 2010–2012, 1241 colonies were isolated in the spring study period, including 33 species (Tab. 2), while 1325 colonies of 30 species were isolated in the autumn (Tab. 3). In May the leaves of individual cultivars were colonized by 12 to 18 species, numbering from 76 to 181 colonies. The most micromycetes colonies were found on the leaves of 'Simona', with fewer found on 'Rasputin' and 'Nova Zembla'. The greatest number of species were found on 'Baden Baden' and 'Rasputin'. *Aspergillus niger* and *Pestalotiopsis sydowiana* inhabited the leaves of ten cultivars of rhododendron.

Between 51 and 205 colonies were isolated from the rhododendron leaves collected in October, and the number of colonies ranged from 8 to 19. 'Simona' leaves were colonized by the most micromycetes colonies and species. The fewest fungal colonies inhabited the 'Dominik' cultivar, while the fewest species were found on 'Golden Torch'. The leaves of all of the cultivars were inhabited at different frequencies by the following: *A. alternata*, *H. grisea*, *Ph. pomorum* and *U. isabellina*.

Discussion

The phyllosphere of evergreen rhododendrons was dominated by *P. sydowiana*, *U. isabellina* and *A. alternata*. *P. sydowiana* has been cited in many studies as a harbinger of disease symptoms that appears as the brown edges and tips of the leaves [2,4,12–14]. However, Łabanowski et al. [15] do not attribute pathogenic properties to these fungi. Kita and Mazurek [16] write about the occurrence of *P. fibricola*, *P. rhododendri* and *T. truncata* (syn. *P. truncata*) in the phyllosphere of azaleas with falling leaves and evergreen rhododendrons, whereas Kowalik [2,5] documents the strong participation of *P. sydowiana* and *T. truncata* on infected leaves of rhododendron and pontic azaleas in home gardens, arboretums, and natural habitats.

The mass colonization of the living and fallen leaves of evergreen rhododendron by *A. alternata* is mentioned by the above-named authors, while publications by Kowalik and Muras [17], Kowalik et al. [3,14] have documented the presence and the role of this necrotroph in the process of the death and severe premature fall of leaves. Kozłowska and Konieczny [18] have also written about the role of necrotrophs in these processes. The necrotrophs *E. nigrum*, *H. grisea* and *S. fimicola* played a significant role in the whole community, which has been confirmed by previous studies [2,3,5,13,14,19], whereas the saprotrophs *Ch. crispatum*, *Ch. globosum*, *H. fuscoatra* and *M. heterogamus* constituted a small percentage of the total fungal communities isolated from the infected leaves.

The high, but uneven colonization of rhododendron leaves by the hygrophilous fungus *U. isabellina* appears to be associated with a large amount of rainfall in the last year of the study, as this type of fungus prefers moist habitats, such as wet leaves [20].

The results do not confirm the previous studies concerning the participation of *A. niger* in colonizing and damaging rhododendron leaves [3,14,19].

The significant share of micromycetes of the *Trichoderma* and *Phoma* genera confirms that they stimulate progressive necrosis of leaves [5] and contribute to their death [14,19].

Frequent rainfall and continued high humidity were conducive to the intensified process of necrotizing rhododendron leaf tissue in October 2010–2012. The colonization and damage of leaves by micromycetes, especially by *P. sydowiana*, *S. fimicola* and *E. nigrum*, was conducive to the formation of fog and rain dew.

It can be assumed that the intensive sporulation and induction of necrosis by *A. alternata*, *A. niger*, *P. expansum*, *P. jensenii*, *T. koningii*, *T. polysporum*, *T. viride* and *T. truncata* in May 2010 was favored by a rapid increase in ambient temperature.

According to Kryczyński and Weber [21], natural environmental factors, such as water and temperature, affect both plants and pathogenic and saprotrophic micromycetes. During the vegetation period when the temperature rises, fungi grow faster, penetrate plant tissues faster and also reproduce faster.

The following pathogens were not found in this study: *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Cylindrocladium scoparium*, *Exobasidium vaccinii*, *Phomopsis archeri*, *Pycnostysanus azalea* and *Septoria azalea*, whose appearance on the leaves of *Rhododendron* L. has been documented in many publications [3,5,15,22].

Comparing the species composition and the quantitative properties of mycobiota, including fungi and fungi-like organisms isolated from the leaves of *Rhododendron* spp., in backyard growing conditions, in the natural environment and in nursery production, it should be noted that the spectrum of micromycetes is much lower in the phyllosphere of the nursery material.

Conclusions

(***i***) The species composition and the number of colonies of micromycetes colonizing and damaging evergreen rhododendron leaves depend on year and time of the year.

(***ii***) A lower number of micromycetes colonies and species were found colonizing evergreen rhododendron leaves in container cultivation than under constant growing conditions, which shows less pathogen and saprotroph pressure in the ornamental plant nursery.

(***iii***) The necrosis symptoms caused by micromycetes decreased the suitability of nursery plants.

(***iv***) The rhododendron cultivars 'Sneezy', 'Golden Torch', 'Flautando', 'Baden Baden' and 'Goldbukett' were characterized by high health.

(***v***) The 'Simona' cultivar had the greatest susceptibility to infection by micromycetes, including *P. sydowiana*, which caused extensive leaf necrosis.

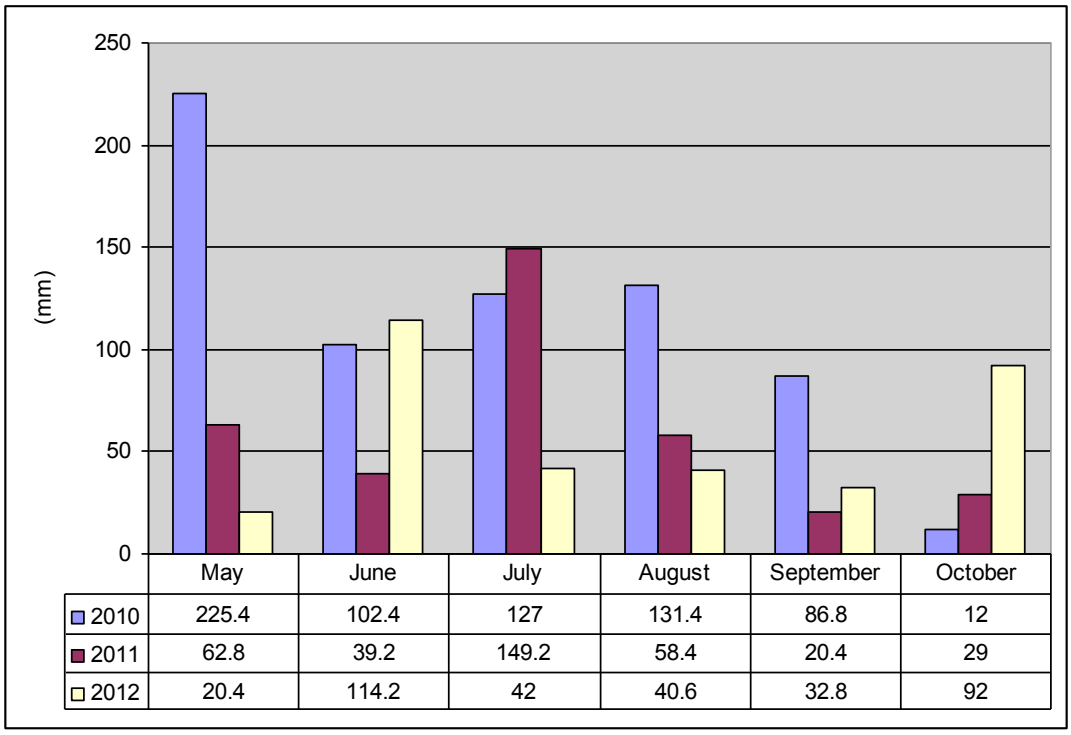

Fig. 1 Monthly rainfall in May–October 2010–2012 powered by a Davis Vantage Pro2 weather station in Kraków Fiołkowa.

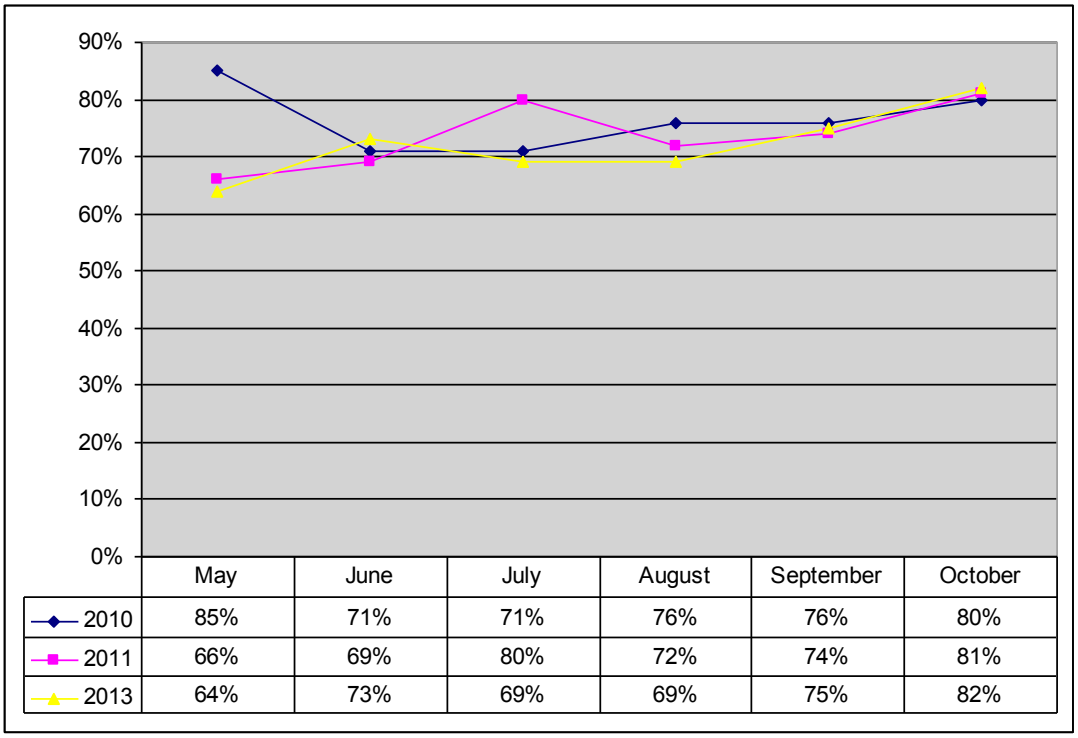

Fig. 2 Monthly average humidity in May–October 2010–2012 powered by a Davis Vantage Pro2 weather station in Kraków Fiołkowa.

Tab. 1 Micromycetes isolated from leaves of evergreen rhododendron (*Rhododendron* L.) shrubs in 2010–2012.

Fungus	Number of colonies				%
	2010	2011	2012	Total	
Alternaria alternata (Fr.) Keissl.	137	20	54	211	8.22
Alternaria tenuissima (Kunze) Wiltshire	2	-	1	3	0.12
Aspergillus niger Thiegh.	57	33	11	101	3.94
Chaetomium crispatum (Fuckel) Fuckel	-	1	12	13	0.51
Chaetomium globosum Kunze	4	21	1	26	1.01
Cladosporium alliicola H.D. Shin & U. Braun	-	-	1	1	0.04
Drechslera poae (Baudyš) Shoemaker	-	18	-	18	0.70
Epicoccum nigrum Link	46	3	3	52	2.03
Fusarium poae (Peck) Wollenw.	2	-	-	2	0.08
Humicola fuscoatra Traaen	-	5	-	5	0.19
Humicola grisea Traaen	37	34	-	71	2.77
Isaria farinosa (Holmsk.) Fr.	-	5	3	8	0.31
Monographella nivalis (Schaffnit) E. Müll.	5	-	-	5	0.19
Mortierella alpina Peyronel	18	31	1	50	1.95
Mortierella hyalina (Harz) W. Gams	-	11	9	20	0.78
Mucor heterogamus Vuill.	2	-	-	2	0.08
Mucor hiemalis Wehmer	-	18	9	27	1.05
Oidiodendron tenuissimum (Peck) S. Hughes	-	3	-	3	0.12
Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley	-	-	1	1	0.04
Penicillium expansum Link	24	17	-	41	1.60
Penicillium herquei Bainier & Sartory	4	-	10	14	0.55
Penicillium jensenii K.M. Zaleski	23	9	1	33	1.29
Penicillium miczynskii K.M. Zaleski	4	-	-	4	0.16
Penicillium waksmanii K.M. Zaleski	-	10	1	11	0.43
Pestalotiopsis sydowiana (Bres.) B. Sutton	348	377	173	898	35.00
Phialophora cyclaminis J.F.H. Beyma	3	-	-	3	0.12
Phoma eupyrena Sacc.	9	15	5	29	1.13
Phoma glomerata (Corda) Wollenw. & Hochapfel	-	14	2	16	0.62
Phoma leveillei Boerema & G.J. Bollen	28	4	-	32	1.25
Phoma medicaginis Malbr. & Roum.	-	-	11	11	0.43
Phoma pomorum Thüm.	-	-	74	74	2.88
Pleurostomophora richardsiae (Nannf.) L. Mostert, W. Gams & Crous	-	2	27	29	1.13
Sarocladium kiliense (Grütz) Summerb.	14	4	-	18	0.70
Sordaria fimicola (Roberge ex Desm.) Ces. & de Not.	39	3	41	83	3.23
Trichoderma asperellum Samuels, Lieckf. & Nirenberg	-	12	-	12	0.47
Trichoderma koningii Oudem.	64	32	7	103	4.01
Trichoderma polysporum (Link) Rifai	24	-	34	58	2.26
Trichoderma viride Pers.	33	-	-	33	1.29
Truncatella truncata (Lév.) Steyaert	38	13	1	52	2.03
Umbelopsis isabellina (Oudem.) W. Gams	95	163	115	373	14.54
Umbelopsis vinacea (Dixon-Stew.) Arx	-	18	2	20	0.78
Total	1060	896	610	2566	100.00

Tab. 2 Micromycetes isolated from leaves of evergreen rhododendron (*Rhododendron* L.) shrubs in May 2010–2012.

	Cultivar										
	'Baden Baden'	'Dominik'	'Flautando'	'Goldbuckett'	'Golden Torch'	'Nova Zembla'	'Rasputin'	'Roseum Elegans'	'Simona'	'Sneezy'	Total
Fungus	**Number of colonies**										
Alternaria alternata (Fr.) Keissl.	16	-	30	8	15	9	4	25	9	9	125
Alternaria tenuissima (Kunze) Wiltshire	-	-	-	-	-	-	1	-	-	-	1
Aspergillus niger Thiegh.	15	12	1	17	6	3	2	12	15	1	84
Chaetomium crispatum (Fuckel) Fuckel	-	-	-	2	1	-	1	-	2	5	11
Chaetomium globosum Kunze	-	-	1	-	-	-	-	-	-	-	1
Cladosporium alliicola H.D. Shin & U. Braun	-	-	-	-	-	-	-	-	-	1	1
Epicoccum nigrum Link	2	-	3	7	-	-	-	-	19	-	31
Humicola fuscoatra Traaen	1	-	3	-	-	-	1	-	-	-	5
Humicola grisea Traaen	-	-	-	-	-	-	-	-	1	1	2
Monographella nivalis (Schaffnit) E. Müll.	-	-	-	-	-	-	1	1	-	3	5
Mortierella alpina Peyronel	5	6	-	2	-	12	-	5	-	-	30
Mortierella hyalina (Harz) W. Gams	2	-	-	4	1	-	-	2	-	-	9
Mucor hiemalis Wehmer	1	-	-	4	-	2	5	-	-	-	12
Oidiodendron tenuissimum (Peck) S. Hughes	-	-	-	-	-	3	-	-	-	-	3
Penicillium expansum Link	2	-	-	5	4	-	-	-	5	1	17
Penicillium herquei Bainier & Sartory	-	-	-	-	-	-	-	4	-	-	4
Penicillium jensenii K.M. Zaleski	-	2	-	-	2	-	4	8	-	8	24
Penicillium miczynskii K.M. Zaleski	4	-	-	-	-	-	-	-	-	-	4
Penicillium waksmanii K.M. Zaleski	1	-	1	-	-	-	6	-	-	3	11
Pestalotiopsis sydowiana (Bres.) B. Sutton	33	42	32	53	31	53	32	41	45	21	383
Phoma eupyrena Sacc.	-	4	1	-	-	4	-	5	-	-	14
Phoma glomerata (Corda) Wollenw. & Hochapfel	-	1	-	-	7	4	-	-	2	-	14
Phoma leveillei Boerema & G.J. Bollen	1	1	5	1	8	-	3	-	7	2	28
Phoma medicaginis Malbr. & Roum.	-	-	-	-	-	10	-	1	-	-	11
Pleurostomophora richardsiae (Nannf.) L. Mostert, W. Gams & Crous	-	-	3	-	-	5	5	-	16	-	29
Sarocladium kiliense (Grütz) Summerb.	4	-	6	-	-	-	-	5	3	-	18
Sordaria fimicola (Roberge ex Desm.) Ces. & de Not.	5	-	-	-	7	5	14	-	-	-	31
Trichoderma koningii Oudem.	5	6	-	10	1	4	26	5	9	7	73
Trichoderma polysporum (Link) Rifai	3	19	1	-	-	7	11	8	9	-	58
Trichoderma viride Pers.	-	5	-	5	-	-	12	-	11	-	33
Truncatella truncata (Lév.) Steyaert	3	9	6	4	1	6	6	5	-	-	40
Umbelopsis isabellina (Oudem.) W. Gams	12	5	2	10	-	23	14	8	23	14	111
Umbelopsis vinacea (Dixon-Stew.) Arx	-	3	6	-	-	4	-	-	5	-	18
Total colonies	115	115	101	132	84	154	148	135	181	76	1241
Total species	18	13	15	14	12	16	18	15	16	13	33

Tab. 3 Micromycetes isolated from leaves of evergreen rhododendron (*Rhododendron* L.) shrubs in October 2010–2012.

	Cultivar										
	'Baden Baden'	'Dominik'	'Flautando'	'Goldbuckett'	'Golden Torch'	'Nova Zembla'	'Rasputin'	'Roseum Elegans'	'Simona'	'Sneezy'	Total
Fungus	**Number of colonies**										
Alternaria alternata (Fr.) Keissl.	11	6	10	6	-	13	12	4	21	3	86
Alternaria tenuissima (Kunze) Wiltshire	-	-	-	-	-	-	-	-	2	-	2
Aspergillus niger Thiegh.	4	-	8	-	1	1	-	3	-	-	17
Chaetomium crispatum (Fuckel) Fuckel	-	1	-	-	-	-	-	-	1	-	2
Chaetomium globosum Kunze	4	-	1	1	-	3	10	2	4	-	25
Drechslera poae (Baudyš) Shoemaker	2	1	2	-	-	6	4	-	2	1	18
Epicoccum nigrum Link	1	-	-	3	-	8	2	2	5	-	21
Fusarium poae (Peck) Wollenw.	-	-	-	-	-	2	-	-	-	-	2
Humicola grisea Traaen	12	6	2	5	5	1	16	18	4	-	69
Isaria farinosa (Holmsk.) Fr.	1	-	2	-	-	-	-	3	1	1	8
Mortierella alpina Peyronel	-	1	4	1	7	2	-	4	-	1	20
Mortierella hyalina (Harz) W. Gams	-	4	1	1	2	-	1	-	2	-	11
Mucor heterogamus Vuill.	-	-	-	-	-	-	-	2	-	-	2
Mucor hiemalis Wehmer	-	2	-	-	-	8	-	5	-	-	15
Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley	-	-	-	-	-	-	1	-	-	-	1
Penicillium expansum Link	1	-	5	-	-	12	-	-	6	-	24
Penicillium herquei Bainier & Sartory	-	-	-	-	-	-	-	-	6	4	10
Penicillium jensenii K.M. Zaleski	1	-	-	2	-	-	-	1	2	3	9
Pestalotiopsis sydowiana (Bres.) B. Sutton	65	18	28	58	56	38	69	54	87	42	515
Phialophora cyclaminis J.F.H. Beyma	-	-	-	-	-	3	-	-	-	-	3
Phoma eupyrena Sacc.	6	-	-	-	-	4	5	-	-	-	15
Phoma glomerata (Corda) Wollenw. & Hochapfel	-	-	-	2	-	-	-	-	-	-	2
Phoma leveillei Boerema & G.J. Bollen	-	-	-	-	-	-	-	-	4	-	4
Phoma pomorum Thüm	6	-	2	7	17	20	3	10	7	2	74
Sordaria fimicola (Roberge ex Desm.) Ces. & de Not.	3	-	-	4	6	2	6	17	14	-	52
Trichoderma asperellum Samuels, Lieckf. & Nirenberg	2	1	-	3	-	2	-	1	-	3	12
Trichoderma koningii Oudem.	1	-	2	1	-	10	7	7	2	-	30
Truncatella truncata (Lév.) Steyaert	-	-	-	8	-	-	-	-	4	-	12
Umbelopsis isabellina (Oudem.) W. Gams	22	11	53	11	26	31	31	39	31	7	262
Umbelopsis vinacea (Dixon-Stew.) Arx	-	-	-	2	-	-	-	-	-	-	2
Total colonies	142	51	120	115	120	166	167	172	205	67	1325
Total species	16	10	13	16	8	18	13	16	19	10	30

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Authors' contributions
The following declarations about authors' contributions to the research have been made: concept of the study: MK, BKB; determination of the specimens: BKB, MK, KDF; writing the manuscript: MK, KDF.

Competing interests
No competing interests have been declared.

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Micromycetes kolonizujące i uszkadzające liście różaneczników zimozielonych *Rhododendron* L. w szkółce

Streszczenie
Badania mykologiczne przeprowadzono w latach 2010–2012 wykorzystując krzewy różanecznika zimozielonego (*Rhododendron* L). Rośliny pochodziły z uprawy pojemnikowej, ze szkółki roślin ozdobnych. Celem badań była weryfikacja gatunków micromycetes bytujących na liściach dziesięciu odmian różanecznika. Stwierdzono, że zbiorowiska grzybów kolonizujących i powodujących nekrozę blaszki liściowej oraz opadanie liści poszczególnych odmian, różniły się składem gatunkowym i liczebnością kolonii w latach i terminach badań. Z porażonych liści różanecznika najliczniej izolowano: *Alternaria alternata*, *Aspergillus niger*, *Epicoccum nigrum*, *Humicola grisea*, *Pestalotiopsis sydowiana*, *Phoma pomorum*, *Sordaria fimicola*, *Trichoderma koningii*, *Trichoderma polysporum*, *Truncatella truncata*, *Umbelopsis isabellina*. Stan zdrowotny krzewów różanecznika był dobry, jednak symptomy nekrozy, będącej skutkiem uszkodzenia blaszki liściowej przez kolonizujące micromycetes obniżały przydatność materiału szkółkarskiego do uprawy. Wysoka zdrowotność cechowała krzewy odmian: 'Sneezy', 'Golden Torch', 'Flautando', 'Baden Baden' i 'Goldbukett'. Odmiana 'Simona' była szczególnie podatna na porażenie przez micromycetes, w tym przez *Pestalotiopsis sydowiana*, sprawcę rozległych nekroz liści.