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Infestation of Micromycetes on the Flowers and Seeds of *Rhododendron* sp.

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Abstract: The study of micromycetes infestation on flower buds, flower petals and seeds from 10 taxa of *Rhododendron* sp. was conducted during 2010-2012. Mycological analysis was comprised of 1,500 specimens of buds and petals and 500 seeds of *Rhododendron* sp.. It was shown that the necrosis and dieback of buds were caused by complex micromycetes (43 species), with dominants *Pestalotiopsis sydowiana*, *Alternaria alternata*, *Truncatella truncata* and *Epicoccum nigrum*. Watery brown spots on the flower petals, resulting in the dieback of flowers, were caused by 38 species, including the most common *P. sydowiana*, *A. alternata* and *Trichoderma viride*. The seeds were contaminated by 18 species, and in addition to the above, the following species were associated: *Oidiodendron tennuissimum*, *Davidiella macrocarpa* and *Phoma leveillei*. The results of the mycological analysis confirmed the diversity spectrum of micromycetes species that inhabit the infested *Rhododendron* buds, flowers and seeds. The research revealed which taxa attracted the largest number of colonies and species of fungi.

Key words: *Rhododendron*, flower buds, flower petals, seeds, fungi, infestation, health status.

1. Introduction

Rhododendron sp. is one of the most attractive plants in contemporary gardens. The selective breeding of *Rhododendrons* takes into consideration not only the value of flowers, the decorativeness of leaves, frost resistance, tolerance to adverse climate and soil conditions, but also the low susceptibility to diseases caused by biotic factors, including filamentous fungal micromycetes.

The health evaluation of interspecific evergreen *Rhododendron* hybrids may give a basis to the exclusion of sensitive taxa from further breeding and indicate which taxa possess flower buds, flowers and seeds that are slightly susceptible to infection by micromycetes [1-5].

The aim of the study was to compare micromycetes biodiversity inhabiting the flowers and seeds of newly grown *Rhododendron* sp. hybrids and also diagnose the hybrids' susceptibility to colonization by pathogenic and saprophytic micromycetes species.

2. Materials and Methods

The research was carried out during 2010-2012 with the collection of ornamental plants from the Faculty of Biotechnology and Horticulture at the University of Agriculture in Krakow. The mycological assessment consisted of nine *Rhododendron* sp. evergreen *Rhododendron* interspecific hybrids, which were obtained from the selective breeding in hybridization of three East Asian species—*R. aureum*, *R. brachycarpum* and *R. purdomii*, with the "Catherine van Tol" cultivar from the *R. catawbiense* group, "Koichiro Wada" from *R. yakushimanum* and the *R. purdomii* species (Table 1). The study was carried out on 10 individual shrubs of each taxon, with the age of the shrubs being 11-13 years.

Dead flower buds were collected in October 2010-2012 and infested flowers were collected during the flowering period (from April to June). Seeds were collected once in November 2012.

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Table 1 Details of studied evergreen *Rhododendron* sp. taxa.

Hybrids		Color of flower
Mother plants	Male plants	
R. aureum	*R. brachycarpum*	Creamy-white
R. aureum	*R. catawbiense* "Catherine van Tol"	Pink
R. aureum	*R. yakushimanum* "Koichiro Wada"	Creamy-white
R. brachycarpum	*R. brachycarpum*	White and pink
R. brachycarpum	*R. purdomii*	White and pink
R. purdomii	*R. yakushimanum* "Koichiro Wada"	Pink
R. yakushimanum "Koichiro Wada"	*R. aureum*	Creamy-white
R. yakushimanum "Koichiro Wada"	*R. brachycarpum*	Pink
R. yakushimanum "Koichiro Wada"	*R. catawbiense* "Catherine van Tol"	Pink
R. purdomii	*R. purdomii*	Pink

Fifteen hundred pieces of buds, 1,500 pieces of flower petals and 500 seeds were allocated for mycological analysis. Fragments of buds and petals (taken from the border of living and dead tissue) and seeds were disinfected in 70% ethanol and then placed in Petri dishes with a solidified Difco® potato dextrose agar (PDA) medium. Micromycetes isolation and cultivation were conducted according to standard methods applied in mycology [5].

The keys used for the taxonomic identification of the mycobiota were these aspects by the following authors: Guba [6], Domsch et al. [7], Sutton [8], Sivanesan [9] and Ellis, M. B., and Ellis, J. P [10]. The basis of classification was the system by Kirk et al. [11], and the authors' descriptions of fungal species names were verified according to Index Fungorum [12].

On the basis of micromycetes specification and considering the share of individual species within the total fungi community, they were classified into three groups, namely, dominants (constituting > 5% of the entire community), influents (1%-5%) and accessory fungi (< 1%), according to Kowalik [13].

3. Results and Discussion

Sixty-five filamentous fungal micromycetes were found on the infected buds, petals and seeds of 10 taxa of *Rhododendron.* The entire community consisted of 1,934 colonies (Tables 2-4).

The symptoms of infestations on flower buds were characterized by necrosis, which led to browning and bud dieback. Nine hundred and sixty micromycetes colonies comprising 43 species from 29 genera were isolated from infested flower buds. The most common species were *Pestalotiopsis sydowiana* (constituting 29.49% of the entire community), *Alternaria alternata* (19.58%), *Truncatella truncata* (10.10%) and *Epicoccum nigrum* (5.31%). These micromycetes were classified to the group of dominants. The following species were isolated in large numbers: *Broomella acuta*, *Isaria fumorosea*, *Paraphoma chrysanthemicola*, *Phialophora asteris*, *P. cyclaminis*, *Phoma eupyrena*, *Ph. pinodella*, *Trichoderma viride* and *Umbelopsis isabellina.* These species were classified to the group of influents. The other 31 species were classified into the accessory group.

The number of micromycetes colonies inhabiting the flower buds increased in the consecutive years of the study. The most fungi colonies (391 isolates) were isolated in 2012, representing 25 species. The fewest fungi colonies were isolated in 2010, at which time the flower buds were inhabited by 275 colonies and 28 species of fungi (Table 2).

Watery brown spots on the flower petals, resulting in the dieback of flowers, were caused by 38 species of micromycetes within 25 genera. Eight hundred and forty-five colonies of fungi were isolated from the infected petals, including the dominants *P. sydowiana*, *A. alternata* and *T. viride* (56.69% of the entire community).

Table 2 Isolation frequency of fungi isolated from the infested buds of evergreen *Rhododendron*.

Fungus	Number of fungi colonies in years			Percentage (%)
	2010	2011	2012	
Alternaria alternata (Fr.) Keissl.	31	49	108	19.58
Alternaria tenuissima (Kunze) Wiltshire	2	-	-	0.21
Aspergillus brasiliensis Varga, Frisvad & Samson	2	1	-	0.31
Aspergillus ustus (Bainier) Thom & Church	1	-	-	0.10
Aspergillus versicolor (Vuill.) Tirab.	4	3	-	0.73
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	1	-	6	0.73
Botrytis cinerea Pers.	1	-	6	0.73
Broomella acuta Shoemaker & E. Müll.	-	-	12	1.25
Calonectria morganii Crous, Alfenas & M.J. Wingf.	-	-	5	0.52
Cladosporium cladosporioides (Fresen.) G.A. de Vries	-	-	5	0.52
Cladosporium herbarum (Pers.) Link	2	-	-	0.21
Epicoccum nigrum Link	15	13	23	5.31
Fusarium poae (Peck) Wollenw.	4	2	-	0.63
Isaria fumosorosea Wize	-	-	34	3.54
Khuskia oryzae K.J. Huds	-	-	8	0.83
Mammaria echinobotryoides Ces.	4	3	-	0.73
Monodictys castaneae (Wallr.) S. Hughes	-	-	1	0.10
Mortierella alpina Peyronel	-	-	2	0.21
Mortierella bainieri Costantin	1	1	-	0.21
Mortierella hyalina (Harz) W. Gams	-	1	-	0.10
Mucor racemosus f. sphaerosporus (Hagem) Schipper	2	-	1	0.31
Myrothecium roridum Tode	-	-	2	0.21
Oidiodendron tenuissimum (Peck) S. Hughes	-	-	4	0.42
Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley	6	10	1	1.77
Penicillium expansum Link	3	2	-	0.52
Penicillium waksmanii K.M. Zaleski	2	3	-	0.52
Pestalotiopsis sydowiana (Bres.) B. Sutton	109	89	85	29.49
Phialophora asteris (Dowson) Burge & I. Isaac	13	21	2	3.75
Phialophora cinerescens (Wollenw.) J.F.H. Beyma	-	2	-	0.21
Phialophora cyclaminis J.F.H. Beyma	8	17	15	4.17
Phoma eupyrena Sacc.	3	3	8	1.46
Phoma leveillei Boerema & G.J. Bollen	-	-	8	0.83
Phoma pinodella (L.K. Jones) Morgan-Jones & K.B. Burch		3	19	2.29
Pleurostomophora richardsiae (Nannf.) L. Mostert, W. Gams & Crous	2	1	-	0.31
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	-	-	1	0.10
Talaromyces wortmannii C.R. Benj.	3	6	-	0.94
Trichoderma koningii Oudem.	1	-	-	0.10
Trichoderma pseudokoningii Rifai	1	1	-	0.21
Trichoderma viride Pers.	13	17	-	3.13
Truncatella truncata (Lév.) Steyaert	36	42	19	10.10
Ulocladium consortiale (Thüm.) E.G. Simmons	-	-	2	0.21
Umbelopsis isabellina (Oudem.) W. Gams	3	4	14	2.19
Umbelopsis nana (Linnem.) Arx	2	-	-	0.21
Total	275	294	391	100.00

Table 3 Isolation frequency of fungi isolated from the infested petals of evergreen *Rhododendron*.

Fungus	Number of fungi colonies in years			Percentage (%)
	2010	2011	2012	
Absidia spinosa Lendn.	-	-	3	0.36
Acremonium rutilum W. Gams	1	2	5	0.95
Alternaria alternata (Fr.) Keissl.	76	98	28	23.91
Alternaria tenuissima (Kunze) Wiltshire	-	-	3	0.36
Arthrinium phaeospermum (Corda) M.B. Ellis	-	1	-	0.12
Aspergillus brasiliensis Varga, Frisvad & Samson	-	-	15	1.78
Aspergillus ustus (Bainier) Thom & Church	-	-	1	0.12
Aspergillus versicolor (Vuill.) Tirab.	-	-	2	0.24
Botrytis cinerea Pers.	2	6	3	1.30
Chaetomium funicola Cooke	1	2	10	1.54
Chaetomium globosum Kunze	3	4	1	0.95
Cladosporium cladosporioides (Fresen.) G.A. de Vries	-	1	-	0.12
Coleophoma rhododendri Syd.	-	-	1	0.12
Epicoccum nigrum Link	16	23	-	4.62
Fusarium culmorum (Wm.G. Sm.) Sacc.	-	7	-	0.83
Fusarium oxysporum Schltdl.	-	-	1	0.12
Gibberella avenacea R.J. Cook	4	9	-	1.54
Humicola fuscoatra Traaen	2	3	-	0.59
Mortierella alpina Peyronel	-	3	-	0.36
Mortierella hyalina (Harz) W. Gams	6	3	-	1.07
Mucor hiemalis Wehmer	-	-	4	0.47
Penicillium citrinum Thom	-	-	10	1.18
Pestalotiopsis sydowiana (Bres.) B. Sutton	78	91	65	27.69
Phialophora cyclaminis J.F.H. Beyma	-	-	38	4.50
Phoma eupyrena Sacc.	7	1	16	2.84
Phoma laundoniae Boerema & Gruyter	-	-	8	0.95
Phoma leveillei Boerema & G.J. Bollen	-	1	9	1.18
Phoma pinodella (L.K. Jones) Morgan-Jones & K.B. Burch	-	2	2	0.47
Phoma putaminum Speg.	-	-	2	0.24
Sclerotinia sclerotiorum (Lib.) de Bary	3	12	-	1.78
Scopulariopsis fusca Zach	-	-	1	0.12
Tetracoccosporium paxianum Szabó	-	1	-	0.12
Trichoderma hamatum (Bonord.) Bainier	-	-	1	0.12
Trichoderma harzianum Rifai	-	4	13	2.01
Trichoderma koningii Oudem.	10	12	9	3.67
Trichoderma viride Pers.	2	5	36	5.09
Truncatella truncata (Lév.) Steyaert	12	15	3	3.55
Umbelopsis isabellina (Oudem.) W. Gams	7	18	1	3.08
Total	230	324	291	100.00

The influents group included *Aspergillus brasilensis*, *Botrytis cinerea*, *Chaetomium funicola*, *E. nigrum*, *Gibberella avenacea*, *Mortierella hyalina*, *Penicillium citrinum*, *Ph. cyclaminis*, *Ph. eupyrena*, *Ph. leveillei*, *Sclerotinia sclerotiorum*, *Trichoderma harzianum*, *T. koningii*, *T. truncata* and *U. isabellina*. Colonies of these micromycetes constituted more than 32% of the total isolated fungi.

The most micromycetes in the number of 324 colonies and 24 species were isolated from infected flowers in 2011. The lowest micromycetes was isolated from infected flowers in 2010, inhabiting 16

Table 4 Isolation frequency of fungi isolated from the seeds of evergreen *Rhododendron*.

Fungus	Number of fungi colonies in 2012	Percentage (%)
Alternaria alternata (Fr.) Keissl.	29	22.48
Broomella acuta Shoemaker & E. Müll.	1	0.78
Davidiella macrocarpa Crous, K. Schub. & U. Braun	10	7.75
Epicoccum nigrum Link	13	10.08
Haematonectria haematococca (Berk. & Broome) Samuels & Rossman	6	4.65
Isaria fumosorosea Wize	1	0.78
Khuskia oryzae K.J. Huds	2	1.55
Mortierella alpina Peyronel	3	2.33
Oidiodendron tenuissimum (Peck) S. Hughes	11	8.53
Pestalotiopsis sydowiana (Bres.) B. Sutton	20	15.50
Phialophora asteris (Dowson) Burge & I. Isaac	1	0.78
Phialophora cyclaminis J.F.H. Beyma	2	1.55
Phoma eupyrena Sacc.	1	0.78
Phoma leveillei Boerema & G.J. Bollen	8	6.20
Sordaria fimicola Roberge ex Desm.) Ces. & De Not.	1	0.78
Stachybotrys elegans (Pidopl.) W. Gams	4	3.10
Truncatella truncata (Lév.) Steyaert	10	7.75
Umbelopsis isabellina (Oudem.) W. Gams	6	4.65
Total	129	100.00

species and representing 230 colonies (Table 3).

The seeds were contaminated by 18 micromycetes species. Species found in large numbers were *A. alternata*, *P. sydowiana*, *E. nigrum*, *Oidiodendron tenuissimum*, *Davidiella macrocarpa* and *Ph. leveillei.* These dominants constituted more than 72% of the fungi colonies inhabiting seeds (Table 4).

The authors found differences among the taxa when comparing the number of micromycetes colonies and species on the infested organs of 10 *Rhododendron* taxa. From 60 to 130 colonies and from 7 to 21 species were isolated from dead flower buds. From 30 to 127 colonies and from 8 to 18 species were obtained from infected flowers. From 7 to 21 colonies and from 1 to 7 fungi species were obtained from the seeds (Tables 5-7).

The greatest number of fungi colonies was isolated from the buds flowers of hybrid obtained from *R. brachycarpum* × *R. brachycarpum*. The dominants among the 18 species were *A. alternata*, *P. sydowiana*, *Ph. asteris* and *Ph. cyclaminis*. The lowest number of fungi colonies in each year of the analysis was reported on *R. purdomii* and the lowest number of species (seven) was reported on *R. yakushimanum* "Koichiro Wada" × *R. aureum*. The most numerous fungi species (21) were isolated from the hybrid *R. yakushimanum* "Koichiro Wada" × *R. catawbiense* "Catherine van Tol" (Table 5).

The most numerous fungi species were isolated from the petals of *R. aureum* × *R. catawbiense* "Catherine van Tol". Among the 12 species found, the following dominated: *A. alternata*, *P. sydowiana*, *S. sclerotiorum*, *E. nigrum* and *P. citrinum*. Only 30 micromycetes colonies belonging to 11 species were isolated from the flowers of the hybrid obtained from *R. brachycarpum* × *R. brachycarpum* hybrid, which was not correlated with the high number of fungi colonies isolated from the flower buds. The most abundant in terms of micromycetes species diversity were the flowers of hybrid *R. yakushimanum* "Koichiro Wada" × *R. brachycarpum*, of which 18 fungi species representing only 60 colonies were isolated.

Table 5 Isolation frequency of fungi isolated from the infested flower buds of particular evergreen *Rhododendron* taxa.

Fungi taxon	Number of fungi colonies										
	R. aureum × *R. brachycarpum*	*R. aureum* × *R. catawbiense*	*R. aureum* × *R. yakushimanum*	*R. brachycarpum* × *R. brachycarpum*	*R. brachycarpum* × *R. purdomii*	*R. purdomii* × *R. yakushimanum*	*R. yakushimanum* × *R. aureum*	*R. yakushimanum* × *R. brachycarpum*	*R. yakushimanum* × *R. catawbiense*	*R. purdomii*	Total
A. alternata	35	17	12	27	31	13	1	34	11	7	188
A tennuissima	2	-	-	-	-	-	-	-	-	-	2
A. brasiliensis	-	-	-	-	-	3	-	-	-	-	3
A. ustus	-	-	-	-	-	-	-	1	-	-	1
A. versicolor	-	-	-	-	-	1	-	-	6	-	7
B. exigua	-	-	-	6	-	-	-	-	1	-	7
B. cinerea	3	1	-	-	2	-	-	-	-	1	7
B. acuta	-	1	11	-	-	-	-	-	-	-	12
C. morganii	-	-	-	5	-	-	-	-	-	-	5
C. cladosporioides	-	-	-	-	-	5	-	-	-	-	5
C. herbarum	-	-	-	-	-	2	-	-	-	-	2
E. nigrum	10	4	7	2	3	4	-	-	20	1	51
F. poae	-	4	-	2	-	-	-	-	-	-	6
I. fumosorosea	-	-	2	-	2	-	-	9	14	7	34
K. oryzae	-	-	-	1	-	-	-	-	6	1	8
M. echinobotryoides	3	4	-	-	-	-	-	-	-	-	7
M. castaneae	-	-	-	-	1	-	-	-	-	-	1
M. alpina	1	-	-	-	-	-	1	-	-		2
M. bainieri		1	-	-	-	-	-	-	1	-	2
M. hyalina	-	-	-	-	-	-	-	-	1	-	1
M. racemosus	-	-	-	1	-	-	-	2	-	-	3
M. roridum	-	-	-	-	-	-	-	2	-	-	2
O. tenuissimum	-	-	-	-	-	-	-	1	-	3	4
P. chrysanthemicola	9	-	-	-	-	8	-	-	-	-	17
P. expansum	-	-	-	-	-	-	-	-	5	-	5
P. waksmanii	1	-	-	-	-	-	-	-	4	-	5
P. sydowiana	37	25	41	12	18	36	58	22	4	30	283
Ph. asteris	-	-	1	13	16	-	-	-	5	1	36
Ph. cinerescens	-	-	-	1	-	-	-	-	1	-	2

(Table 5 continued)

Fungi taxon	Number of fungi colonies										
	R. aureum × *R. brachycarpum*	*R. aureum* × *R. catawbiense*	*R. aureum* × *R. yakushimanum*	*R. brachycarpum* × *R. brachycarpum*	*R. brachycarpum* × *R. purdomii*	*R. purdomii* × *R. yakushimanum*	*R. yakushimanum* × *R. aureum*	*R. yakushimanum*× *R. brachycarpum*	*R. yakushimanum* × *R. catawbiense*	*R. purdomii*	Total
Ph. cyclaminis	-	1	2	21	2	-	3	4	7	-	40
Ph. eupyrena	-	-	3	9	-	-	-	-	2	-	14
Ph. leveillei	-	5	-	-	1	1	-	-	1	-	8
Ph. pinodella	-	2	2	11	-	-	-	-	-	7	22
P. richardsiae	1	-	-	1	-	-	-	-	1	-	3
S. fimicola	-	-	-	1	-	-	-	-	-	-	1
T. wortmannii	-	-	-	9	-	-	-	-	-	-	9
T. koningii	-	-	-	-	-	-	-	-	1	-	1
T. pseudokoningii	-	-	-	-	-	2	-	-	-	-	2
T. viride	-	-	-	-	-	8	10	-	12	-	30
T. truncata	15	7	22	7	10	4	11	20	-	1	97
U. consortiale	-	-	-	-	-	-	-	-	1	1	2
U. isabellina	-	-	-	-	-	-	15	5	1	-	21
U. nana	-	-	-	1	-	-	-	1	-	-	2
Total colonies /species	117/ 11	72/ 12	103/ 10	130/ 18	86/ 10	87/ 12	99/ 7	101/ 11	105/ 21	60/ 11	960/ 43

Table 6 Isolation frequency of fungi isolated from the infested petals of particular evergreen *Rhododendron* taxa.

Fungi taxon	Number of fungi colonies										
	R. aureum × *R. brachycarpum*	*R. aureum* × *R. catawbiense*	*R. aureum* × *R. yakushimanum*	*R. brachycarpum* × *R. brachycarpum*	*R. brachycarpum* × *R. purdomii*	*R. purdomii* × *R. yakushimanum*	*R. yakushimanum* × *R. aureum*	*R. yakushimanum* × *R. brachycarpum*	*R. yakushimanum* × *R. catawbiense*	*R. purdomii*	Total
A. spinosa	-	-	-	1	-	2	-	-	-	-	3
A. rutilum	-	2	1	-	-	-	-	5	-	-	8
A. alternata	36	43	29	8	11	10	14	8	28	15	202
A tennuissima	-	-	-	-		3	-	-	-	-	3
A. pheospermum	-	-	-	-	1	-	-	-	-	-	1
A. brasiliensis	-	-	-	-	1	9	-	-	-	5	15
A. ustus	-	-	-	-	-	-	-	-	-	1	1
A. versicolor	-	1	-	-	1	-	-	-	-	-	2

(Table 6 continued)

Fungi taxon	Number of fungi colonies										
	R. aureum × *R. brachycarpum*	*R. aureum* × *R. catawbiense*	*R. aureum* × *R. yakushimanum*	*R. brachycarpum* × *R. brachycarpum*	*R. brachycarpum* × *R. purdomii*	*R. purdomii* × *R. yakushimanum*	*R. yakushimanum* × *R. aureum*	*R. yakushimanum* × *R. brachycarpum*	*R. yakushimanum* × *R. catawbiense*	*R. purdomii*	Total
B. cinerea	-	-	-	-	1	3	-	7	-	-	11
Ch. funicola	8	2	-	3	-	-	-	-	-	-	13
Ch. globosum		1	-	-	-	3	1	2	1	-	8
C. cladosporioides	-	-	-	-	-	-	-	-	1	-	1
C. rhododendri	-	-	-	-	-	-	-	1	-	-	1
E. nigrum	5	10	4	1	-	-	7	2	5	5	39
F. culmorum	-	-	-	-	-	-	7	-	-	-	7
F. oxysporum	-	-	-	-	-	-	-	-	1	-	1
G. avenacea	-	-	2	-	3		6	2	-	-	13
H. fuscoatra	-	-	1	-	1	1	1	1	-	-	5
M. alpina	-	-	-	-	-	-	-	3	-	-	3
M. hyalina	-	-	-	-	6	-	-	3	-	-	9
M. hiemalis	-	-	-	-	-	-	-	-	4	-	4
P. citrinum	-	10	-	-	-	-	-	-	-	-	10
P. sydowiana	29	32	32	7	15	15	57	6	28	13	234
Ph. cyclaminis	-	-	5	-	3	9	8	4	2	7	38
Ph. eupyrena	-	-	9	2	1	2	3	3	-	4	24
Ph. laundoniae	-	-	-	-	-	-	-	-	-	8	8
Ph. leveillei	-	-	-	2	1	1	4	2	-	-	10
Ph. pinodella	-	-	-	-	2	1	-	-	-	1	4
Ph. putaminum	-	-	-	-	-	-	2	-	-	-	2
S. sclerotiorum	-	12	-	-	-	-	-	3	-	-	15
S. fusca	1	-	-	-	-	-	-	-	-	-	1
T. paxianum	-	-	1	-	-	-	-	-	-	-	1
T. hamatum	-	-	-	1	-	-	-	-	-	-	1
T. harzianum	-	-	10	-	-	-	-	-	6	1	17
T. koningii	8	8	-	3	6	-	-	3	1	2	31
T. viride	26	-	-	-	4	2	-	-	11	-	43
T. truncata	-	4	12	1	2	-	3	1	5	2	30
U. isabellina	3	2	-	1	-	-	-	4	14	2	26
Total colonies/ species	116/ 8	127/ 12	106/ 11	30/ 11	59/ 16	61/ 13	113/ 12	60/ 18	107/ 13	66/ 13	845/ 38

Table 7 Isolation frequency of fungi isolated from the seeds of particular evergreen *Rhododendron* taxa.

Fungi taxon	Number of fungi colonies										
	R. aureum × *R. brachycarpum*	*R. aureum* × *R. catawbiense*	*R. aureum* × *R. yakushimanum*	*R. brachycarpum* × *R. brachycarpum*	*R. brachycarpum* × *R. purdomii*	*R. purdomii* × *R. yakushimanum*	*R. yakushimanum* × *R. aureum*	*R. yakushimanum* × *R. brachycarpum*	*R. yakushimanum* × *R. catawbiense*	*R. purdomii*	Total
A. alternata	1	8	1	2	2	10	-	-	4	1	29
B. acuta	-	1	-	-	-	-	-	-	-	-	1
D. macrocarpa	-	-	-	-	-	-	10	-	-	-	10
E. nigrum	5	-	2	-	1	1	-	-	3	1	13
H. haematococca	-	-	-	-	3	3	-	-	-	-	6
I. fumosorosea	1	-	-	-	-	-	-	-	-	-	1
K. oryzae	-	-	-	-	1	-	-	-	-	1	2
M. alpina	-	-	-	-	-	3	-	-	-	-	3
O. tenuissimum	-	-	-	-	-	-	-	10	-	1	11
P. sydowiana	-	-	5	3	6	1	-	-	5	-	20
Ph. asteris	-	-	-	-	-	-	-	-	-	1	1
Ph. cyclaminis	-	-	-	1	-	-	-	-	-	1	2
Ph. eupyrena	-	1	-	-	-	-	-	-	-	-	1
Ph. leveillei	-	-	-	-	-	-	-	5	1	2	8
S. fimicola	-	-	-	-	1	-	-	-	-	-	1
S. elegans	-	-	-	1	-	3	-	-	-	-	4
T. truncata	-	-	10	-	-	-	-	-	-	-	10
U. isabellina	-	3	-	-	-	-	-	3	-	-	6
Total colonies/ species	7/3	13/4	18/4	7/4	14/6	21/6	10/1	18/3	13/4	8/7	129/18

A. alternata and *P. sydowiana* inhabited the buds and flowers of all of the investigated taxa in every year.

The most numerous fungi colonies (21) were isolated from the seeds of the *R. purdomii* × *R. yakushimanum* "Koichiro Wada" hybrid. Among the six species, *A. alternata* dominated. Few colonies inhabited the seeds of the *R. aureum* × *R. brachycarpum* and *R. brachycarpum* × *R. brachycarpum* hybrids. The seeds of *R. purdomii* were a reservoir for the largest number of micromycetes, because seven species were isolated from the seeds of this taxon in contrast to *R. yakushimanum* "Koichiro Wada" × *R. aureum* seeds, which were inhabited only by *D. macrocarpa* (Table 7).

Shrubs of newly bred taxa of evergreen *Rhododendron*, growing in the collection of ornamental plants of the Faculty of Biotechnology and Horticulture at the University of Agriculture in Krakow, were found to be under pressure from saprotrophic and pathogenic fungi, which cause the dieback of buds and flowers and reduce the decorative value of the plants. The following authors have written about the health of evergreens: Farr et al. [14], Werner et al. [15], Garibaldi et al. [16], Żołna et al. [17], Czekalski and Frużyńska-Jóźwiak [1] and Kowalik [5, 13], emphasizing the diversity of micromycetes species inhabiting infested organs.

The following species dominated on the infested organs of 10 evergreen rhododendron taxa: *A. alternata*, *P. sydowiana* (syn. *Pestalotia sydowiana*), *T. truncata* (syn. *Pestalotia truncata*) and *E. nigrum*, which has been confirmed in other studies concerning the health of leaves, buds and flowers [1, 5, 13-15, 17-20]. Farr et al. [14] have written about the prevalence of *P. sydowiana* on the leaves and stems of *Rhododendrons*, e.g., in Chile, China and Canada.

Necrotrophs were diagnosed on infected evergreen *Rhododendron* organs from the following genera: *Alternaria*, *Pestalotiopsis*, *Epicoccum* and *Sordaria*; pathogens were from the genera: *Botrytis*, *Fusarium* and *Gibberella*; saprophytes were from the genera: *Aspergillus*, *Boeremia*, *Chaetomium*, *Cladosporium*, *Coleophoma*, *Humicola*, *Mortierella*, *Paraphoma*, *Penicillium*, *Phoma*, *Trichoderma* and *Umbelopsis*, proving that the leaves were a source of infection for forming buds in autumn [5, 13, 14, 16, 20-22].

These results confirm the pathogenicity and aggressiveness, which many of these fungi have for flowers buds and petals as well as the ability to contaminate seeds. According to Płażek [23], necrotrophic pathogens kill the plant using toxins and then feed on dead tissue, which is overgrown by the network of mycelium. In this study, necrosis on the covering tissue of flowers buds was visible in the three years of research in the growing season and preceded the dying of flowers buds. The necrosis of buds formed as a symptom of hypersensitivity—hypersensitive response (HR), which is one of the main defense mechanisms that are activated by the plant during pathogen attack [4, 23].

In the discussion on the health of rhododendrons, the importance of phyllosphere is emphasized, where competition between pathogens and saprotrophs results in the dieback of affected organs. In the present study, there was no bud infection by *Pycnostysanus azaleae*, what is mentioned by Czekalski and Frużyńska-Jóźwiak [1] and Garibaldi et al. [16]. The following pathogens were not found on newly grown *Rhododendron* hybrids: *Exobasidium vaccinii*, *Microsphaera penicillata*, *Colletotrichum gloeosporioides*, *Chrysomyxa* spp., *Pucciniastrum vaccinii*, *Puccinia rhododendri*, *Septoria azaleae*, *Cercospora handelii*, *Phyllosticta cunninghamii*, *Ovulinia azalea* and *Briosia azaleae*; these pathogens are mentioned in numerous scientific description [8-10, 14, 15, 19, 24].

Analyzing the number of micromycetes on the infested organs of different *Rhododendrons* taxa should take into account their sporulation, because a weak plant can keep its vital functions but it is not able to prevent the formation of the generative stage of parasite or conidia. Therefore, it appears that in the assessment of the susceptibility to colonization by micromycetes of *Rhododendron* taxa, mainly the size of colony should be taken into account [5, 13].

Because of the documented existence of numerous micromycetes colonies and species on the flower buds and petals of the taxa *R. aureum* × *R. brachycarpum* and *R. aureum* × *R. yakushimanum* “Koichiro Wada”, resulting in dieback, these hybrids can be considered to have little use in selective breeding research. Hybrids of *R. brachycarpum* × *R. brachycarpum* and *R. yakushimanum* “Koichiro Wada” × *R. catawbiense* “Catherine van Tol” also proved to have little use, because their flower buds were colonized by the largest number of colonies and species of micromycetes communities. The seeds of *R. purdomii* × *R. yakushimanum* “Koichiro Wada”, *R. aureum* × *R. yakushimanum* “Koichiro Wada” and *R. yakushimanum* “Koichiro Wada” × *R. brachycarpum* were contaminated by numerous micromycetes colonies, which is associated with poor germination. The most useful *Rhododendron* hybrid for breeding was found to be seeds derived from *R. aureum* × *R. brachycarpum*, *R. brachycarpum* × *R. brachycarpum* and *R. purdomii.*

4. Conclusions

The low susceptibility of newly grown taxa *R. brachycarpum* × *R. purdomii*, *R. purdomii* × *R. yakushimanum* and *R. purdomii* species to the infection by micromycetes confirms their usefulness in further breeding work.

The propagating work should also be concentrated on the hybrids *R. aureum* × *R. catawbiense* “Catherine van Tol”, *R. brachycarpum* × *R. brachycarpum* and *R. brachycarpum* × *R. purdomii*, which showed variable quantitative parameters of micromycetes, concerning infected organs.

The present study confirmed the diversity of the micromycetes species that inhabit the infested *Rhododendron* buds, flowers and seeds of newly grown evergreen *Rhododendron* hybrids and compared their infection by micromycetes with the suitability for further breeding work.

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