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Fungi and fungi-like Oomycetes isolated from affected leaves of rhododendron

MARIA KOWALIK

Department of Plant Protection, University of Agriculture in Kraków
Al. 29. Listopada 54, PL-31-425 Kraków, mkowalik@ogr.ar.krakow.pl

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The aim of the work is to identify fungi and fungi-like Oomycetes occurring on affected leaves of rhododendron *Rhododendron* L. Mycological analyses were carried out on 200 leaves collected from green areas of Kraków from May till September 2005. Isolated fungi-like Oomycetes belonged to 67 taxa. The most frequently found fungi included: *Alternaria alternata, Aspergillus niger, Botrytis cinerea, Coelophoma empetri, Nigrospora sphaerica, Pestalotia sydowiana, Phialophora cyclaminis, Phomopsis archeri, Septoria azalea* and *Sordaria fimicola.* Among fungi-like organisms *Phytophthora cinnamomi* and *P. citricola* were isolated.

Key words: rhododendron, leaves, isolation, fungi-like Oomycetes

INTRODUCTION

Rhododendrons growing in urban green areas are susceptible to various pathogenes. Infected plants develop discolouration, brown spots and necroses, affecting their aesthetic value. The symptoms of infection, observable from spring to autumn, increase when the plants are in bloom resulting in dieback and leaf drop. The damage is caused by fungi-like Oomycetes of genera *Pythium* and *Phytophthora* and fungi of genera: *Botrytis, Cercospora, Colletotrichum, Cylindrocladium, Exobasidium, Microsphaera, Ovulinia, Pestalotia, Phomopsis, Phyllosticta, Pycnostysanus, Ramularia, Rhizoctonia* and *Septoria* (Farr et al. 1996; Werner, Kwaśna 1998; Werres 2000; Orlikowski 1999; Łabanowski et al. 2001; Kita, Mazurek 2003; Kowalik et al. 2006; Kowalik, Muras 2007).

The aim of this work was to identify the organisms occurring on affected rhododendron leaves in green areas of Kraków.

MATERIAL AND METHODS

Leaves of rhododendron with the symptoms of discoloration, brown spots or necroses were collected from the plants in the Botanical Garden of the Jagiellonian University, the Zoological Garden and town lawns in Kraków. 200 leaves from 50 plants were collected for mycological analysis. The numbers of leaves taken from the top part (T), central part (C) and lower part (L) of the plant were approximately equal. The leaf samples were disinfected in 70% ethyl alcohol and 5 parts of each leaf from the area between ill and healthy tissue were put on Petri dishes with 2% PDA medium. The colonies of fungi and fungi-like Oomycetes were identified using various media for identification: Guba (1961), Domsch et al. (1980), Sutton (1980), Ho (1981), Ellis and Ellis (1987).

RESULTS

From the collected material 721 colonies of fungi, 19 colonies of fungi-like Oomycetes and 150 colonies of bacteria (not being the subject of this paper) were isolated. The isolated fungi and Oomycetes belonged to 67 taxa within 37 genera.

Among the dominat fungi the following constitued more than 66 per cent of the total: *Alternaria alternata, Aspergillus niger, Botrytis cinerea, Cladosporium macrocarpum, Coelophoma empetri, Humicola grisea* v. *grisea, Nigrospora sphaerica, Pestalotia sydowiana, Phialophora cyclaminis, Ph. richardsiae, Phoma fimeti, Ph. macrostoma, Phomopsis archeri, Pycnostysanus azaleae, Septoria azalea* and *Sordaria fimicola.* Less frequent were: *Acremonium furcatum, Cladosporium sphaerospermum, Colletotrichum gloeosporioides, Cylindrocladium scoparium, Epicoccum purpurascens, Exobasidium vaccinii, Humicola fuscoatra* v. *fuscoatra, Phoma chrysanthemicola* and *Ph. putaminum.* Fungi belonging to 20 taxa occurred once or twice.

Fungi-like Oomycetes were represented by *Phytophthora cinnamomi* and *Ph. citricola* (Tab. 1).

Among the species isolated from the leaves situated in the central part of the plants, the following were predominant: *A. alternata, B. cinerea, C. empetri, C. gloeosporioides, E. purpurascens, N. sphaerica, P. sydowiana, S. fimicola* and fungi belonging to genera: *Aspergillus, Chaetomium, Cladosporium, Humicola* and *Penicillium.*

From leaves situated in the lower parts of the plant, the following were isolated: *Cylindrocarpon destructans, C. scoparium, Ph. archeri, Rhizoctonia solani, S. fimicola, Trichothecium roseum* and fungi of genera: *Acremonium, Fusarium, Mortierella* and *Phialophora.* From the lower parts also *Ph. cinnamomi* and *Ph. citricola* were isolated.

From the leaves situated at the top part of the plant, numerous colonies of *A. alternata, C. empetri, S. azalea, S. fimicola* were isolated. Less frequent were: *E. vaccinii, Mucor hiemalis, P. sydowiana, Pycnostysanus azaleae* and fungi of genera *Trichoderma* and *Phoma.*

The frequency of species occurrence of fungi and fungi-like Oomycetes on leafblades was differentiated. The following species occurred at the highest frequency: *A. alternata, S. fimicola, P. sydowiana, S. azalea* and *C. empetri*. They were isolated (respectively) from 27, 11, 9, 8 and 7 leaves. Less frequently (on 5-6 leaves) occurred: *Aspergillus niger, B. cinerea, Cladosporium macrocarpum, C. gloeosporioides,*

Table 1
Fungi and fungi-like Oomycetes isolated from affected leaves of rhododendron

Species	Frequency of occurrence	Percentage of occurrence	The number of leaves the fungus species was isolated from	Localization of leaves*
Acremonium butyri (van Beyma) W. Gams	6	0.81	2	L
Acremonium furcatum F. et V. Moreau ex W. Gams	8	1.08	2	L
Acremonium fusidioides (Nicot) W. Gams	1	0.14	1	C
Acremonium kiliense Grütz	4	0.54	1	L
Alternaria alternata (Fr.) Keissler	131	17.70	27	TCL
Arthrinium pheosphermum (Corda) M. B. Ellis	2	0.27	1	C
Aspergillus niger van Tiegh.	21	2.84	6	C
Aspergillus wentii Wehmer	4	0.54	1	C
Botrytis cinerea Pers. ex Nocca et Balb.	21	2.84	6	C
Chaetomium globosum Kunze ex Steud.	3	0.41	1	C
Chaetomium indicum Corda	2	0.27	1	C
Chrysosporium asperatum Carm.	6	0.81	2	C
Chrysosporium merdarium (Link ex Grev.) Carm.	1	0.14	1	L
Cladosporium cladosporioides (Fres.) de Vries	6	0.81	2	C
Cladosporium macrocarpum Preuss	19	2.57	6	C
Cladosporium sphaerospermum Penz.	10	1.35	2	C
Coelophoma empetri (Rostr.) Petrak	25	3.38	7	TC
Colletotrichum gloeosporioides (Penz.) Sacc.	14	1.89	5	C
Coniothyrium minitans W. A. Campbell	6	0.81	2	C
Cylindrocarpon destructans (Zins.) Scholten	6	0.81	2	L
Cylindrocarpon tenue Bugn.	2	0.27	1	C
Cylindrocladium scoparium Morg.	8	1.08	2	L
Doratomyces stemonitis (Pers. ex Steud.) Morton	2	0.27	1	T
Epicoccum purpurascens Ehrenb. ex Schlecht.	12	1.62	4	C
Exobasidium vaccinii (Fuckel) Woron.	12	1.62	3	T
Fusarium culmorum (W. G. Sm.) Sacc.	5	0.68	1	L
Fusarium graminearum Schwabe	2	0.27	1	L
Fusarium stilboides Wollenw.	2	0.27	1	L
Gloeosporium rhododendri Briosi et Cav.	2	0.27	1	C
Humicola fuscoatra Traaen var. *fuscoatra*	8	1.08	4	C
Humicola grisea Traaen var. *grisea*	17	2.30	5	C
Mammaria echinobotryoides Ces.	7	0.95	2	C
Morenoina rhododendri J. P. Ellis	2	0.27	1	C
Mortierella alpina Peyronel	2	0.27	1	L
Mortierella horticola Linnem.	1	0.14	1	L
Mortierella isabellina Oudem.	1	0.14	1	L
Mortierella parvispora Linnem.	4	0.54	1	L
Mucor hiemalis Wehmer f. *hiemalis*	4	0.54	1	T
Myrothecium cinctum (Corda) Sacc.	1	0.14	1	C
Nectria inventa Pethybr.	2	0.27	1	C
Nigrospora sphaerica (Sacc.) Mason	21	2.84	5	C
Penicillium expansum Link ex Gray	7	0.95	2	C
Pestalotia sydowiana Bres.	40	5.41	9	TC
Phialophora cyclaminis van Beyma	23	3.11	5	L
Phialophora richardsiae (Nannf.) Conant	16	2.16	4	L
Phoma chrysanthemicola Hollòs	11	1.49	3	T
Phoma epicoccina Punithalingam, Tulloch et Leach	7	0.95	2	T
Phoma eupyrena Sacc.	6	0.81	2	T

Tab. 1 cont.

Phoma fimeti Brun.	18	2.43	6	T
Phoma herbarum Westd.	3	0.41	1	T
Phoma leveillei Boerema et Bollen	1	0.14	1	T
Phoma macrostoma Mont.	17	2.30	6	T
Phoma medicaginis Malbr. et Roum.	5	0.68	1	T
Phoma pomorum Thüm.	5	0.68	1	T
Phoma putaminum Speg.	9	1.22	2	T
Phomopsis archeri Sutton	23	3.11	6	L
Phytophthora cinnamomi Rands	12	1.62	3	L
Phytophthora citricola Sawada	7	0.95	2	L
Pseudeurotium zonatum van Beyma	2	0.27	1	C
Pycnostysanus azaleae (Peck) E. Mason	16	2.16	5	T
Rhizoctonia solani Kühn	2	0.27	1	L
Septoria azalea Voglino	33	4.46	8	T
Sordaria fimicola (Rob.) Ces. et de Not.	53	7.16	11	TCL
Trichoderma harzianum Rifai	2	0.27	1	T
Trichoderma pseudokoningii Rifai	2	0.27	1	T
Trichoderma viride Pers. ex Gray	4	0.54	1	T
Trichothecium roseum (Pers.) Link ex Gray	3	0.41	1	L
Total	740	100.00	-	-

Abbreviations: * – localization of leaves: T – top part of the plant; C – central part of the plant; L – lower part of the plant.

Humicola grisea v. *grisea, N. sphaerica, Ph. cyclaminis, Ph. fimeti, Ph. macrostoma, Ph. archeri* and *P. azaleae*. On 3-4 leaves occurred *E. purpurascens, E. vaccinii, H. fuscoatra* v. *fuscoatra, Ph. richardsiae, Ph. chrysanthemicola* and *Ph. cinnamomi*. The remaining were isolated form 1-2 leaves (Tab. 1).

Rhododendron symptoms caused by fungi and fungi-like Oomycetes were varied. From chlorosis affected leaves with discoloration changes alongside the main veins, *C. destructans, C. tenue, C. scoparium*, and also three species of *Fusarium* were isolated. Fungus *R. solani*, causing the rhizoctoniosis in rhododendron, was isolated from the leaves with irregular brown-grey spots, surrounded by a large yellow area. *B. cinerea*, the cause of *Botrytis* petal blight, was isolated from rapidly dying leaves, with the symptoms of necrosis, such as large brown spots covered with dense grey mould. Fungus *E. vaccinii*, was isolated from deformed leaves with small reddish and brownish galls, typical of azalea leaf and flower gall. The cause of large oval necrotic spots with dark linings and visible picnidia was identified as *Ph. archeri*.

Other pathogens occurring in phyllosphere, such as *C. gloeosporioides* (causing rhododendron anthracnose)*, Gloeosporium rhododendri, Morenoina rhododendri, C. empetri* and *S. azalea* (the reason for leaf scorch) caused round, oval or irregular necrotic spots of different shapes. A sign of *C. gloeosporioides* infection was grey spots, with visible rings and picnidia. In case of *S. azalea,* small oval, grey-brown spots with darker lining were seen. Necrotic spots caused by *Ph. cyclaminis* and *P. richardsiae* were small irregular with uneven edges. *P. sydowiana* was isolated from large light brown necrotic spots appearing on the edges and the tops of the leaves.

Leaf petioles and blades, with brown discolouration and necroses forming a cline shape, were the reflection of *Ph. cinnamomi* and *Ph. citricola* presence. These pathogens were also isolated from fast expanding brown oval spots along the leaf margin.

DISCUSSION

Different discolorations, brown spots and necroses, visible on rhododendron leaves in green areas of Kraków, were caused by many organisms classified as Fungi and Oomycetes. According to Farr et al. (1996), Werner and Kwaśna (1998), Werres et al. (2000), Łabanowski et al. (2001) rhododendron leaves can be infected by fungi and fungi-like organisms of different genera including: *Botrytis*, *Cercospora, Colletotrichum, Cylindrocladium, Exobasidium, Gloeosporium, Macrophoma, Microsphaera, Monochaetia, Pestalotia, Pestalotiopsis, Phomopsis, Phyllosticta, Phytophthora, Septoria, Rhizoctonia* and others. While Kita and Mazurek (2003) isolated fungi of genera: *Alternaria, Botrytis, Cladosporium, Epicoccum, Fusarium, Humicola, Mucor, Penicillium, Pestalotia, Phoma, Rhizopus, Sclerotinia* and *Trichoderma* from the phyllosphere of rhododendron and azalea growing in the botanical garden and arboretum. This research work confirms that most of fungi and fungi-like Oomycetes isolated from the affected leaves of rhododendron belong to the mentioned above taxa.

The determination of organisms inhabiting affected rhododendron leaves enabled to show the relation between pathogens and pathologic symptoms. The isolation of fungi such as: *C. destructans, C. tenue, C. scoparium* in the leaves with the discolouration alongside the main vein indicates that the root system of the plants was firsty attacked, causing root rot, as mentioned by Werner (2005) and Łabanowski et al. (2001). Also in the work by Kowalik and Muras (2007) fungi *C. destructans, C. scoparium* and numerous species of *Fusarium* were numerously isolated from dropped rhododendron leaves. Fungus *R. solani* was the reason for rhododendron rhizoctoniosis, *B. cinerea* caused grey mould, *C. gloeosporioides* – anthracnose, and *S. azalea* – rhododendron leaf scorch (Łabanowski et al. 2001), while fungus *E. vaccinii* spending winter in flower and leaf buds of rhododendrons, in spring caused the disease called azalea leaf and flower gall (Orlikowski 1999).

The occurrence in phyllosphere of pathogens such as *C. gloeosporioides, G. rhododendri, M. rhododendri, C. empetri* and *S. azalea* (numerously isolated in this reasearch work) and leaf symptoms caused by them were documented in the papers by Werner and Kwaśna (1998), Łabanowski et al. (2001), Kita and Mazurek (2003).

In this work *P. sydowiana* was frequently isolated. Łabanowski et al. (2001) do not attribute necroses to this species. The role of *P. sydowiana* in causing necroses of heathers in nurseries and in permanent sites was described by Kowalik and Sagan (2005) as well as Kowalik and Wandzel (2005), while Kita and Mazurek (2003), in phyllosphere of azaleas and rhododendrons found quite numerously occurring: *Pestalotia fibricola, P. rhododendrii* and *P. truncata.* In the works by Kowalik and Muras (2007) fungus *P. sydowiana* together with *A. alternata*, occurred causing spots and necroses of rhododendron leaves and intensifying the process of leaf drop were isolated at large frequency.

The description of phytophtorosis symptoms on leaves and petioles of rhododendrons and their effects in rhododendron plantings are confirmed in the works by Łabanowski et al. (2001), Werres (2000), Werner (2005), Kowalik et al. (2006). The occurrence of *Ph. citricola* in rhododendron nurseries and susceptibility of many rhododendron taxa to this pathogen is described by Orlikowski and Szkuta (2003),

while Orlikowski and Szkuta (2002) highlight the role *Ph. cinnamomi* in causing phytophtorosis on container-grown ericaceous plants in ornamental nurseries.

While analysing the plant material, it was found out that, in addition to the above mentioned causes of discolorations, brown spots and necroses, the secondarily invading fungi on pathological leaves were identified. They were the following: *A. alternata*, *S. fimicola, N. sphaerica, A. niger* and the species of genera: *Acremonium, Cladosporium, Humicola, Mortierella, Mucor, Penicillium, Phoma* and *Trichoderma.* According to Werner and Kwaśna (1998) these fungi can intensify pathogenic processes. Inhabiting small necrotic areas in a short time they rapidly intensify the process of necrosis. A large participation of fungi from genera: *Alternaria, Cladosporium, Epicoccum* and *Phoma* in the phyllosphere of rhododendrons is mentioned by Kita and Mazurek (2003). Their work indicates that these saprotrophs dominate in the structure of the populations of isolated fungi. The obtained results confirm considerable participation of *A. alternata, E. purpurascens, C. macrocarpum, C. sphaerospermum* and many species of *Phoma* in the populations of fungi isolated from necrotic tissues of rhododendrons.

It can be concluded that a large biodiversity of fungi and fungi-like Oomycetes isolated from the affected rhododendron leaves in the green areas of Kraków caused different pathologic symptoms and decreased decorative value of the plants.

CONCLUSIONS

1. The most numerous fungi occurring in the rhododendron plantings in the green areas of Kraków were: *Alternaria alternata, Aspergillus niger, Botrytis cinerea, Coelophoma empetri, Nigrospora sphaerica, Pestalotia sydowiana, Phialophora cyclaminis, Phomopsis archeri, Septoria azalea* and *Sordaria fimicola*
2. Among the fungi-like Oomycetes *Phytophthora cinnamomi* and *Ph. citricola* were isolated from the affected rhododendron leaves.
3. The causes of discolorations, brown spots and necroses of the rhododendron leaves were the pathogens belonging to genera: *Botrytis, Colletotrichum, Cylindrocarpon, Cylindrocladium, Exobasidium, Fusarium, Pestalotia, Phomopsis, Phytophthora, Rhizoctonia* and *Septoria.*
4. Commonly occurring saprotrophs of genera: *Acremonium, Alternaria, Cladosporium, Coelophoma, Epicoccum, Humicola, Nigrospora, Phoma, Sordaria* and others, intensified the process of leaf necrosis.
5. On the rhododendron plants the symptoms of phytophthorosis, grey mould, rhizoctoniosis, anthracnose, leaf scorch, azalea leaf and flower gall were found. The effect was leaf drop and, in consequence decreased decorative value of the plants.

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Grzyby i organizmy grzybopodobne Oomycetes wyizolowane z porażonych liści różanecznika

Streszczenie

Celem pracy była identyfikacja grzybów i organizmów grzybopodobnych Oomycetes występujących na porażonych liściach różanecznika *Rhododendron* L. Materiał do badań stanowiło 200 liści (z objawami przebarwień, plamistości i nekroz) zebranych z 50 krzewów na terenie Ogrodu Botanicznego UJ, Ogrodu Zoologicznego oraz na skwerkach miejskich Krakowa. Wyodrębniono grzyby i organizmy grzybopodobne Oomycetes (w liczbie 740 kolonii), należące do 67 taksonów. Na porażonych liściach najliczniej występowały grzyby: *Alternaria alternata, Aspergillus niger, Botrytis cinerea, Coelophoma empetri, Nigrospora sphaerica, Pestalotia sydowiana, Phialophora cyclaminis, Phomopsis archeri, Septoria azalea* i *Sordaria fimicola.* Spośród organizmów grzybopodobnych, z porażonych liści różanecznika wyizolowano *Phytophthora cinnamomi* i *Ph. citricola*. Sprawcami przebarwień, plamistości i nekroz na liściach różanecznika były patogeny z rodzajów: *Botrytis, Colletotrichum, Cylindrocarpon, Cylindrocladium, Exobasidium, Fusarium, Pestalotia, Phomopsis, Phytophthora, Rhizoctonia* i *Septoria.* Licznie występujące saprotrofy z rodzajów: *Acremonium, Alternaria, Cladosporium, Coelophoma, Epicoccum, Humicola, Nigrospora, Phoma, Sordaria* i inne intensyfikowały proces nekrotyzacji liści. Na różanecznikach rozpoznano objawy fytoftorozy, szarej pleśni, rizoktoniozy, antraknozy, septoriozy, powłocznika azaliowego, w wyniku których dochodziło do zamierania i opadania liści, a tym samym obniżenia walorów dekoracyjnych roślin.