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FUNGI LIVING AT THE FALLEN LEAVES OF RHODODENDRON AND AZALEA (*Rhododendron* L.)

Maria Kowalik, Małgorzata Żołna, Barbara Kierpiec
University of Agriculture in Krakow

Abstract. Rhododendrons of evergreen leaves and rhododendrons of falling leaves (azaleas) are the most shrubs cultivated in modern gardens. Their values are not only decorative flowers but also leathery and glossy leaves. In a number of specimens during the vegetation season on leaves are visible blotch and necrosis, leading to their premature falling. Research was conducted in three successive vegetation seasons in gardens and parks in Malopolska. The object of research were the fallen leaves of rhododendrons and azaleas. For the mycological research 1500 infected leaves fragments was taken, and mycological isolation was performed according to standard methods used in phytopathology. From the fallen leaves of rhododendrons 1300 colonies of fungi, and from azaleas 1777 colonies respectively were isolated. Most frequently were isolated *Alternaria alternata, Pestalotia sydowiana*, *P. truncata* and *Epicoccum purpurascens* and fungi of the genera: *Aspergillus*, *Fusarium*, *Mortierella*, *Penicillium*, *Phoma* and *Trichoderma*.

Key words: evergreen rhododendron, azalea, phyllotaxis, mycobiota, blotch, necrosis

INTRODUCTION

Fungi that live in phyllosphere of both evergreen, and deciduous rhododendrons known as azaleas, (*Rhododendron* L.) cause a variety of discolorations, spotting and necrosis symptoms of leaves, which lower decorative value and lead to their decay and premature falling [Kita and Mazurek 2003, Kowalik and Muras 2007, Kowalik et al. 2010a]. Healthy, shiny, dark green leaves determine the attractiveness of rhododendrons and azaleas outside the period of flowering. Advanced defoliation of leaves indicates weaker condition of shrubs, which evoke the limited flower buds formation.

Previous reports show that necrosis symptoms on the rhododendrons and azaleas leaves are mostly brought about by fungi of the genera: *Alternaria*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Epicoccum*, *Cylindrocarpo*n, *Cylindrocladium*, *Fusarium*, *Humicola*, *Mortierella, Penicillium*, *Pestalotia*, *Phialophora*, *Phoma*,

Corresponding author – Adres do korespondencji: Maria Kowalik, Małgorzata Żołna, Barbara Kierpiec, Department of Plant Protection, University of Agriculture in Krakow, 29 Listopada 54, 31-425 Kraków, Poland, e-mail: mkowalik@ogr.ar.krakow.pl, e-mail: gosia_zolna@interia.pl, e-mail: barbara.kierpiec@wp.pl

Sordaria, *Trichoderma* which inhabit such peculiar ecological niche [Kita and Mazurek 2003, Kowalik and Muras 2007, Kowalik 2008, 2009, Kowalik et al. 2010a,b], thus those pathogenic fungi, that cause leaves necrosis, contribute to their earlier falling.

The aim of this research was to indentify the fungi present on the fallen leaves of *Rhododendron* L. and to compare species preferences in leaves colonizing of evergreen and deciduous rhododendrons.

MATERIAL AND METHODS

Research was performed in the years 2008–2010 in the twelve gardens and parks in Malopolska. 1500 fragments of necrotic fallen leaves of rhododendrons and azaleas were collected. Decontamination, isolation and cultivation of fungal colonies was performed according to standard methods used in mycology [Kowalik 2008]. Fungi were determined according to mycological keys: Guba [1961], Domsch et al. [1980], Ellis and Ellis [1985], Rifai [1987]. The study included the division of fungi on: dominants, influential and accessory based on Kowalik [1993].

RESULTS AND DISCUSSION

As a result of mycological analysis of fallen *Rhododendron* leaves with necrosis symptoms 3077 fungal colonies belonging to 81 species were isolated (tab. 1). In successive years of the research we have isolated from 371 to 641 colonies of fungi. On the leaves of rhododendrons and azaleas 54 species of fungi have been found.

Table 1. Fungi isolated from fallen leaves of rhododendron and azalea (*Rhododendron* L.) with the symptoms of necrosis

Tabela 1. Grzyby wyizolowane z opadłych liści różanecznika i azalii (*Rhododendron* L.) z objawami nekroz

Fungus Grzyb	Azalea Azalia			Rhododendron Różanecznik			Total Ogółem	Percentage of occurrence Udział, %
	2008	2009	2010	2008	2009	2010		
Acremonium kiliense				5			5	0.16
Alternaria alternata	76	92	79	170	148	160	725	23.56
Arthrinium phaeospermum		1		4	1		6	0.19
Aspergillus niger		4	4	6	2	10	26	0.84
Aspergillus versicolor		2			1		3	0.10
Chaetomium globosum		6			2		8	0.26
Cladosporium macrocarpum				10			10	0.32
Coleophoma empetri				2	2	3	7	0.23
Cylindrocarpon destructans	12		1	5			18	0.58
Epicoccum pururascens	72	33	38	50	14	35	242	7.86
Farrowia seminude		4					4	0.12
Fusarium culmorum	1		29				30	0.97
Fusarium flocciferum			7				7	0.23
Fusarium oxysporum			5				5	0.16

Fusarium poae	28	2		2	1		33	1.07
Fusarium stilboides				6		3	9	0.29
Gilmaniella humicola		6	3				9	0.29
Humicola fuscoatra v. *fuscoatra*	16	2		1			19	0.62
Humicola grisea v. *grisea*	4	10	2				16	0.52
Mortierella alpina	16	5			4		25	0.81
Mortierella bainieri			4				4	0.13
Mortierella hyalina				5			5	0.16
Mortierella isabellina	12	41			9		62	2.01
Mortierella parvispora	8				8	6	22	0.71
Mortierella ramaniana v. *ramaniana*				4			4	0.13
Mortierella vinacea					10		10	0.32
Mucor hiemalis f. *hiemalis*	8			2			10	0.32
Oidiodendron tenuissimum			6				6	0.19
Paeciliomyces farinosus				6			6	0.19
Papulaspora immersa				4			4	0.13
Papulaspora irregularis			7				7	0.23
Penicillium expansum	16	23	125				164	5.33
Penicillium herquei		8	14				22	0.71
Penicillium implicatum		6					6	0.19
Penicillium jensenii				4			4	0.13
Penicillium lividum		71					71	2.31
Penicillium steckii					3		3	0.10
Penicillium vaksmanii		8	5				13	0.42
Penicillium verrucosum v. *cyclopium*		18			3		21	0.68
Pestalotia sydowiana	55	88	79	47	59	20	348	11.31
Pestalotia truncata	72	79	93	54	69	98	465	15.1
Phialophora cinerescens		1	1		5		7	0.23
Phialophora cyclaminis	8	7	16		2		33	1.07
Phialophora richardsiae	4		6	2	17		29	0.94
Phoma chrysanthemicola			3	6	1		10	0.32
Phoma epicoccina				9			9	0.29
Phoma eupyrena				24	2	2	28	0.91
Phoma exigua v. *exigua*		6	5	1			12	0.39
Phoma herbarum	12	2	1				15	0.49
Phoma leveillei					29	23	52	1.69
Phoma macrostoma		11	53		8		72	2.34
Phomopsis archeri				4	2		6	0.19
Pycnostysanus azaleae	27						27	0.88
Rhizomucor pusillus			3				3	0.10
Rhizopus nigricans	16						16	0.52
Septaria azalea	28						28	0.91
Sordaria fimicola	55	7		42	5	7	116	3.77
Talaromycetes wortmannii		3	5				8	0.26
Trichoderma polysporum		2			3	1	6	0.19
Trichoderma harzianum					5		5	0.16
Trichoderma koningii	11	20	36		10		77	2.50
Trichoderma longibrachiatum				4			4	0.13
Trichoderma piluiferum			3		4		7	0.23
Trichoderma pseudokoningi				4	4	3	11	0.36
Trichoderma viride	1	4	6	1			12	0.38
*Other species – Pozostałe gatunki	2	4	2	5	7		20	0.65
Total – Ogółem	560	576	641	489	440	371	3077	100%

*Other species: *Aspergillus sclerotiorum*, *Aspergillus terreus*, *Botrytis cinerea*, *Chaetomium crispatum*, *Cladosporium cladosporioides*, *Coniothyrium minitans*, *Drechslera poae*, *Fusarium heterosporum*, *Fusarium nivale*, *Mortierella ramaniana* v. *angulispora*, *Nigrospora sphaerica*, *Oidiodendron griseum*, *Paeciliomyces carenus*, *Penicillium cremeo-griseum*, *Rhizopus oryzae*, *Ulocladium consortiale*.

Among the isolated fungal colonies, five species dominated: *Alernaria alternata* (constituting 23.56% of the total fungal communities), *Pestalotia truncata* (15.1%), *P. sydowiana* (11.31%), *Epicoccum purpurascens* (7.86%) and *Penicillium expansum* (5.33%). These fungi were a total of 63.16% of all communities. Into the group influential which are from 1 to 5% of the total community included: *Fusarium poae*, *Mortierella isabellina*, *Penicillium lividum*, *Phialophora cyclaminis*, *Phoma leveillei*, *Ph. macrostoma*, *Sordaria fimicola* and *Trichoderma koningii.* Influential accounted for 16.76% of the total community. The remaining 68 species of fungi were classified as accessory, their colonies constituted 20.08% of the total community.

Comparing the leaves colonization it was found that *A. alternata*, *P. truncata*, *P. sydowiana* and *E. purpurascens* inhabited fallen leaves of both host plants every year. This was particularly the case for aging plants or weakened by adverse environmental conditions, and as reported Płażek [2011] pathogens infecting weakened plants are characterized by low virulence. In studies of Kowalik et al. [2006], Kowalik and Muras [2007], and Kowalik [2008] showed that the dominant role in the process of leaves necrotization and decay, so thus earlier defoliation of rhododendrons, is brought about by *A. alternata*, the fungus producing toxins. Kozłowska and Konieczny [2003] emphasize the necrotrophic role of this pathogen which produces tenotoxin. In the present research this fungus has been recorded on numerous of evergreen rhododendrons, which may indicate the greater resistance to infection with this pathogen by azaleas.

Phyllosphere of rhododendrons was also dominated by *A. alternata* and *E. purpurascens* in the Botanical Garden in Wroclaw and Arboretum in Wojsławice [Kita and Mazurek 2003].

Among the pathogens occurring only on the leaves of evergreen rhodendrons were a large number of *Phoma leveillei* colonies. There has also occasionally occurred: *Aspergillus sclerotiorum*, *Botrytis cinerea*, *Chaetomium crispatum*, *Coleophoma empetri*, *Coniothyrium minitans*, *Drechslera poae*, *Fusarium nivale*, *F. stilboides*, *Mortierella hyalina*, *M. ramaniana*, *M. vinacea*, *Nigrospora sphaerica*, *Paecilomyces farinosus*, *Papulaspora immersa*, *Penicillium jensenii*, *P. steckii*, *Phoma epicocciana*, *Ph. eupyrena*, *Ph. exigua*, *Phomopsis archeri*, *Trichoderma harzianum* and *T. pseudokoningii.*

In the group of pathogens that occurring with considerable frequency and attacking only the leaves of azaleas were: *Fusarium culmorum*, *Humicola grisea* v. *grisea*, *M. isabellina*, *P. expansum*, *P. herquei*, *P. lividum*, *P. waksmanii*, *Phoma herbarum*, *Pycnostysanus azaleae* and *Septoria azalea.* With low frequency and singly were noted: *Cladosporium cladosporioides*, *Fusarium flocciferum*, *F. heterosporum*, *F. oxysporum*, *Gilmaniella humicola*, *Mortierella bainieri*, *Oidiodendron griseum*, *O. tenuissimum*, *Paecilomyces carneus*, *Penicillium implicatum*, *Rhizomucor nigricans*, *R. oryzae* and *Talaromyces wortmannii.*

A significant contribution of fungi of the genus *Penicillium*, including *P. expansum* and *P. lividum*, inhabiting the fallen leaves of azaleas show preferences in colonizing green leaves, and action of toxins leading to their falling. In the publication of Kowalik and others [2010b] fungi of the genus *Penicillium* were classified to the saprotrophes, which increasing necrosis of the live evergreen leaves of rhododendrons. In the group of fungi isolated from fallen leaves of rhododendron and azaleas numerous colonies of fungi producting mycotoxins were identified: *A. alternata* – tenotoxin, *F. culmorum* –

deoxynivalenol and trichothecene, *F. poae* – trichothecene, *P. verrucosum* – ochratoxin, *P. expansum* – patulin and aflatoxin, *A. niger* – aflatoxin. About the role of toxins produced by necrotrophic pathogens occurring in plants, writes Płażek [2011], stressing the induction of extensive necrosis in infected plant material. Łukanowski and Sadowski [2005] underline the possibility of *F. poae* and *F. culmorum* (numerously living on falling azaleas leaves) to produce mycotoxins from the trichothecene group. It can be suspected that fungi which produce toxins were the real cause of premature leaves falling.

This research shows that evergreen and deciduous rhododendrons are the kind of shrubs susceptible to infection by pathogenic fungi and saprotrophes. It was evidenced by the extensive colonization of host tissues, by the biodiversity of inhabiting fungi (as reflected in the isolation of more than 80 species), by plant cell damage produced by toxins, and by dead, necrotic tissue which appear as large spots on the fallen plant leaves.

CONCLUSIONS

1. The most commonly occurring fungi on evergreen leaves of rhododendron and fallen leaves of rhododendrons (azaleas) (*Rhododendron* L.) were *Alternaria alternata, Epicoccum purpurascens*, *Pestalotia truncata*, *P. sydowiana* and *Penicillium expansum.*

2. Necrotization process and falling of evergreen rhododendrons leaves were intensified by fungi *Phoma leveillei* and *Sordaria fimicola*, and by numerous species of: *Fusarium*, *Mortierella*, *Penicillium*, *Phoma* and *Trichoderma*.

3. Necrotization process and falling of azaleas leaves were intensified by fungi *P. expansum*, *P. lividum*, *Trichoderma koningii*, *Fusarium culmorum*, *S. fimicola*, and by numerous species of the genera: *Humicola*, *Mortierella*, *Phoma*, *Pycnostysanus* and *Septoria.*

REFERENCES

Domsch K.H., Gams W., Anderson T.H., 1980. Compendium of Soil Fungi. Acad. Press. London, New York, Toronto, Sydney, San Francisco.

Ellis M.B., Ellis J.P., 1985. Microfungi on Land Plants. An Identification Handbook. Croom Helm. London, Sydney.

Guba E.F., 1961. Monograph of *Monochaetia* and *Pestalotia*. Harvard Univ. Press, Cambridge.

Kita W., Mazurek J., 2003. Skład gatunkowy fyllosfery różanecznika w Ogrodzie Botanicznym we Wrocławiu i w Arboretum w Wojsławicach. Erica Polonica 14, 25–36.

Kowalik M., 1993. Grzyby gleby inicjalnej industroziemnej rekultywowanego w kierunku rolnym i leśnym zwałowiska Kopalni Siarki „Machów". Zesz. Nauk. AR w Krakowie, Rozpr. hab. 180, 1–78.

Kowalik M., 2008. Fungi and fungi-like *Oomycetes* isolated from affected leaves of rhododendron. Acta Mycologica 43(1), 21–27.

Kowalik M., 2009. Bioróżnorodność grzybów występujących w fyllosferze różanecznika zimozielonego *Rhododendron* L. Zesz. Probl. Post. Nauk Roln. 539, 341–348.

Kowalik M., Muras P., 2007. Grzyby zasiedlające opadłe liście różanecznika. Rocz. AR Poznań 333, Ogrodnictwo 41, 69–73.
Kowalik M., Muras P., Kierpiec B., Żołna M., 2010a. Zdrowotność liści różaneczników zawsze zielonych *Rhododendron* L. Zesz. Probl. Post. Nauk Roln. 551,117–123.
Kowalik M., Muras P., Żołna M., Kierpiec B., 2010b. Grzyby wyosobnione z nekrotycznych plam na liściach różaneczników zawsze zielonych *Rhododendron* L. Zesz. Probl. Post. Nauk Roln. 554, 49–55.
Kowalik M., Oliwa A., Paliga A., 2006. Choroby różaneczników (*Rhododendron* L.) na terenach zieleni Krakowa. Erica Polonica 17, 33–41.
Kozłowska M., Konieczny G., 2003. Biologia odporności roślin na patogeny i szkodniki. Wyd. AR w Poznaniu.
Łukanowski A., Sadowski Cz., 2005. Wykorzystanie metody PCR do badania jakości ziarna pszenicy ozimej uprawianej w systemach ekologicznym, integrowanym, konwencjonalnym oraz w monokulturze w aspekcie fitopatologicznym. Acta Agrobotanica 58 (2), 55–70.
Płażek A., 2011. Patofizjologia roślin. Wyd. UR w Krakowie, Kraków.
Rifai M.A., 1987. A revision of the genus *Trichoderma*. Mycol. Papers 116, 1–56.

GRZYBY BYTUJĄCE NA OPADŁYCH LIŚCIACH RÓŻANECZNIKA I AZALII (*Rhododendron* L.)

Streszczenie. Różaneczniki o liściach zimozielonych i o liściach opadających na zimę (azalie) to krzewy najczęściej uprawiane we współczesnych ogrodach. Ich walorami są nie tylko dekoracyjne kwiaty, ale również skórzaste, błyszczące liście. Na licznych krzewach w sezonie wegetacyjnym obserwowano plamistości i nekrozy liści prowadzące do ich przedwczesnego opadania. Badania prowadzono w trzech kolejnych sezonach wegetacyjnych w ogrodach i parkach na terenie Małopolski. Obiektem badań były opadłe liście różaneczników i azalii. Do badań mikologicznych pobrano po 1500 fragmentów porażonych liści. Izolację mikologiczną przeprowadzono zgodnie ze standardowymi metodami stosowanymi w fitopatologii. Z opadłych liści różaneczników wyodrębniono 1300 kolonii grzybów należących do 54 gatunków, a z azalii 1777 kolonii, również należących do 54 gatunków. Do najliczniej izolowanych należały: *Alternaria alternata*, *Pestalotia sydowiana*, *P. truncata* i *Epicoccum purpurascens* oraz grzyby z rodzajów: *Aspergillus*, *Fusarium*, *Mortierella*, *Penicillium*, *Phoma* i *Trichoderma.*

Słowa kluczowe: różaneczniki zimozielone, azalie, ulistnienie, mikobiota, plamistości, nekrozy

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