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FIRST REPORT OF *PHYTOPHTHORA INFLATA* IN POLISH RHODODENDRON NURSERY

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Abstract: *Phytophthora inflata* Caros & Tucker was detected together with *P. citricola* Sawada from rhododendron cv. University of Helsinki with necrotic spots on leaves. Spots were enlarged on tip parts of the stems. The rhododendron plantswere taken from hardy ornamental stock. Inoculation of the leaf blades of 15 species and cultivars of rhododendron with *P. inflata* and *P. citricola* resulted in the development of necroses on all of them. Reaction of tested rhododendron species and cultivars for *P. inflata* and *P. citricola* was similar. The pathogen also colonized leaf blades of *Goultheria decumbens*, *Kalmia ostroseptice*, *Vaccinium corymbiferum* and *V. vitis-idaea*. The quickest spread of necroses was observed on *K. ostroseptice* and *V. corymbiferum.* In greenhouse trials *P. citricola* and *P. citrophthora* caused dieback of more rhododendron plants than *P. inflata.*

Key words: rhododendron, *Phytophthora*, isolation, ericaceous plants, pathogenicity

INTRODUCTION

Ericaceous plants are the most frequent victims of soil-borne pathogens (Hoitink and Powell 1990). Till now 17 species were described on rhododendron as causal agent of dieback, stem rot and tip blight (Benson and Jones 1980; Kuske and Benson 1983; Hoitink and Schmitthenner 1974). During the last 15 years in Polish hardy nursery stocks *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (Smith and Smith) Leonian and *P. ramorum* Werres, De Cock and Man in't Veld were recorded on diseased rhododendron (Orlikowski and Szkuta 2002a, b, 2003; Orlikowski *et al*. 1995). In the year 2009, in one nursery enlarged leaf spots on the main vain and on stems of the rhododendron "Helsinki University" were observed. The diseased parts of shoots died. *P. citricola* and unkown *Phytophthora* were isolated from affected leaf blades and stem parts. The purpose of this study was (1) to identify a species and (2) to estimate its pathogenicity toward ericaceous plants, including rhododendron, in laboratory and greenhouse trials.

MATERIALS AND METHODS

Isolation and identification of *Phytophthora* from diseased rhododendron

Partly diseased shoots of rhododendron cv. Helsinki Univ. were taken from 10 plants growing under a plastic tunnel, which was in central Poland. The plants were individually collected in plastic bags and transferred into the laboratory, washed under tap water, rinsed in distilled water, and blot dried. Chosen leaf blades and stem parts were sterilized over a burner flame, cut into 5 mm pieces and put on PDA medium in 90 mm diam Petri dishes (10 pieces/plate). Within 48 hrs of incubation in the dark at 24°C, small parts of colonies growing around inocula were transferred into PDA slants. After 10 days, obtained isolates where segregated. Chosen cultures were identified to genera and species on the base of their morphological feature, and confirmed using PCR techniques (Trzewik *et al.* 2006; Schubert *et al*. 1999). In addition, the ITS regions of detected pathogens were sequenced.

Phytophthora species

P. inflata Caros & Tucker and *P. citricola* were used as the main species in laboratory and greenhouse pathogenicity trials. Additionally, *P. cactorum* (Leberth et Cohn) J. Schrot. and *P. cinnamomi* from diseased stem parts of rhododendron, *P. cambivora* (Petri) Buisman from diseased stem bases of *Acer pensylvanicum*, and *P. citrophthora* from rotted tips of *Pieris japonica* were used. Stock cultures were maintained on PDA medium at 24°C in the dark, for 7 days. For infestation of peat substratum (Orlikowski 1999), the species were grown for 2 weeks on oats.

Plants

For laboratory trials, leaves were taken from 6 species and 13 cultivars of rhododendron. Additionally, leaves of *Goultheria decumbens*, *Kalmia ostroseptice, Vaccinium corymbiferum* and. *V. vitis-idaea* were used in the pathogenicity trials. In the greenhouse trails, rhododendron cultivars: Cunningham's White, Grandiflorum, Nova Zembla and University of Helsinki were used.

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Laboratory trials

The purpose of these studies was to estimate pathogenicty of *P. inflata* in comparison to *P. citricola*. They are often isolated together from diseased tissues. Collected leaves were put into a tray covered with a sterile, moist blotting paper and plastic net. Five mm diam mycelium disks, taken from the edge of 7 old cultures, were put in the middle part of leaf blades. Ashes were covered with foil and incubated at 22–24°C in the dark. The diameter of necrotic spots was measured within 8 days of incubation.

Greenhouse trials

The purpose of theses studies was to estimate the development of shoot rot of rhododendron inrelation to 4 tested *Phytophthora* species and 4 cultivars. Rhododendron plants containing 4–6 leaves were planted in 1 l pots. The pots contained substratum infested with *P. cinnamomi, P. citricola, P. citrophthora* and *P. inflata*. The control rhododendrons were planted into noninfested peat. Plants were grown on greenhouse benches for 9 weeks. At the end of the 9 weeks, the number of plants with rotted leaves and stem parts were counted.

The experimental design was completely randomized with 4 replications, and 5 leaves or plants in each replication. Trials were repeated twice.

RESULTS

Mycological analyses of diseased rhododendron

P. inflata was isolated from 9/10 analysed plant parts whereas *P. citricola* from 4/5 analysed plant parts. Both species were often isolated from the same tissues of diseased leaves or stem parts. *Botrytis cinerea, Penicillium* spp. were rarely found in the diseased plants.

Colonisation of leaves by the *Phytophthora* species

In the first trial, inoculation of rhododendron leaves by *P. inflata* and *P. citricola* resulted in the development of necrotic spots on all species and cultivars (Table 1). The reaction was similar for both pathogens. The quickest spread of necrotic spots was noticed on Hachmann's Charmant (about 9 mm/24 hrs) and next, on cultivars Nova Zembla, Golden Buckett, Bonde Nerge (which was about twice slower than on the first cultivar). On cv. Helsinki University, from which *P. inflata* and *P. citricola* were detected, necrotic spots spread about 4.5 mm/24 hrs (Table 1). The smallest necrotic spots were observed on cv. April Gloche (Table 1). In the second trial, the leaves of 15 cultivars and species were inoculated with 4 *Phytophthora* species (Table 2). Necrotic spots developed very slowly on leaves inoculated with *P. cactorum*. The quickest spread of disease was only on cv. Nova Zembla whereas significantly slower than on blades inoculated with 3 other *Phytophthoras* (Table 2). Leaf blades of the Nova Zembla cultivar were used as the bait for detection of *Phytophthora* spp. from water and substratum because of its similar reaction with different species (Orlikowski 2006). The tested cultivars and species reacted similarly to *P. inflata, P. citricola* and *P. citrophthora* (Table 2).

In the 3rd trial (Table 3) the reaction of different ericaceous plants, including rhododendron, to *P. inflata* and *P. citricola* was estimated. The quickest development of necrotic spots was noticed on the leaves of *R. carolinianum* (P.J.M. Elite), *K. ostroseptice, V. corymbiferum* and *G. decumbens* inoculated with *P. inflata* (Table 3).

Table 1. Colonization of rhododendron leaves by *Phythtophora* spp. in relation to pathogen species and cultivars, in laboratory trial. Diameter of necrotic spots [mm] 6 days after inoculation are noted

Rhodendron species\cultivars	*Phythophtora inflata*	*Phythophtora citricola*
April Gloche	9.1 a	15.0 bc
Baden Baden	27.6 h–j	25.2 g–i
Bonde Nerge	33.6 l–o	31.7 k–m
Fantastica	27.8 h–j	25.7 g–i
Golden Buckett	35.4 m–p	24.8 g–i
Grandiflorum	28.0 i–k	31.8 k–m
Hachmann's Charmant	54.6 r	61.6 s
Helsinki University	26.3 g–j	30.0 j–l
Nova Zembla	37.2 op	43.4 q
Poh. Doughter	14.3 b	19.3 de
Rhododendron brachycarpum	23.9 f–h	20.2 d–f
Rhododendron insigne	18.5 cd	23.2 fg
Tamarindos	39.0 p	36.0 n–p
Walküre	22.6 e–g	32.7 l–n

Means in columns, followed by the same letter do not differ at 5% of significance (Duncan's multiple range test)

Table 2. Relationship between *Phytophthora* species *Rhododendron* species/cultivars and colonisation of leaves 6 days after inoculation

Rhodendron species\ cultivars	Diameter of necrotic spots on leaves			
	P. cactorum	*P. citricola*	*P. citrophthora*	*P. inflata*
April Gloche	2.9 a	8.3 c–e	8.9 d–f	9.9 e–g
Baden Baden	3.0 a	15.8 k–n	14.1 j–l	16.5 l–p
Bonde Nerge	8.0 c–e	23.2 x	20.3 s–w	22.9 wx
Fantastica	6.1 bc	16.2 l–p	15.7 k–n	14.1 j–l
Golden Buckett	5.9 bc	21.5 u–x	21.0 s–x	14.8 j–m
Grandiflorum	2.6 a	19.3 q–u	20.4 s–w	18.5 o–t
Hachmann's Charmant	6.5 b–d	18.7 p–t	15.8 k–n	18.3 n–s
Helsinki University	3.2 a	15.8 k–n	13.4 h–k	12.6 h–j
Nova Zembla	16.0 k–o	30.8 z	25.5 y	27.3 y
Poh. Doughter	2.7 a	13.9 i–l	12.6 h–j	10.8 f–h
Thododendron brachycarpum	5.2 ab	17.3 m–r	15.3 k–m	12.3 g–j
Thododendron insigne	4.5 ab	17.1 m–q	11.3 f–i	14.0 j–l
Eilberwolke	2.8 a	21.2 t–x	19.7 q–v	18.6 p–t
Tamarindos	2.6 a	22.1 v–x	21.1 t–x	19.9 r–v
Walküre	4.3 ab	16.5 l–p	12.7 h–j	13.3 h–k

Means in columns, followed by the same letter do not differ at 5% of significance (Duncan's multiple range test)

Table 3. Colonisation of ericaceous plant leaves by the *Phytophthora* species; diameter of necrosis [mm] 4 (a) and 8 (b) days after inoculation

Plant species	Diameter of necrosis after inoculation; in days			
	P. citricola		*P. inflata*	
	a	b	a	b
G. decumbens	6.5 ef	14.6 c	10.8 g	15.6c
K. ostroseptice	10.3 g	21.4 d	11.0 g	18.0 d
R. carolinianum	2.6 a–c	19.8 d	2.5 a–c	23.0 d
R. repens	1.1 a	11.3 bc	1.2 a	10.5 b
R. simsii	7.5 ef	11.3 bc	16.8 h	–
R. yakushimanum	3.6 bc	7.8 ab	3.7 bc	7.2 ab
V. corymbiferum	5.6 de	12.1 c	8.2 f	17.2 d
V. vitis- idaea	4.2 cd	21.5 d	3.3 bc	6.0 a

Means in columns, followed by the same letter do not differ at 5% of significance (Duncan's multiple range test)

Table 4. Relationship between *Phytophthora* species, rhododendron cultivars and development of leaf and stem rot; number of diseased plants (n = 5) after 9 weeks of growth

Cultivars	*P. cinnamomi*	*P. citricola*	*P. citrophthora*	*P. inflata*
Cunningham's White	3.8 b	4.1 b	4.5 b	3.8 a
Grandiflorum	3.0 ab	2.5 a	1.8 a	3.3 a
Helsinki University	3.3 ab	4.1 b	4.8 b	1.8 a
Nova Zembla	1.8 a	1.9 a	3.0 ab	2.8 a

Means in columns, followed by the same letter do not differ at 5% of significance (Duncan's multiple range test)

Reaction of rhododendron cultivars to 4 *Phytophthora* species in greenhouse trials

Analysis of the relationship between the tested cultivars, *P. inflata* and development of disease on plants, indicated the lack of significant differences in the number of affected rhododendrons (Table 4). On cv. Helsinki Univ. *Phytophthora* rot occurred on about 2/5 of tested plants whereas the diseases occurred on about 4/5 of Cunningham's White.

The tested cultivars reacted differently on 3 other *Phytophtoras*. The smallest number of cv. Grandiflorum plants died in substratum infested with *P. citrophthora*, whereas Nova Zembla died in peat infested with *P. cinnamomi* and *P. citricola.* Cv. Grandiflorum reacted in a similar way on 4 *Phytophthora* specie.s About 4/5 of the plants of the cv. Grandiflorum died within a 9-week-growth on greenhouse bench (Table 4).

DISCUSSION

The results obtained from these studies indicated *P. inflata* as the sixth pathogen, including *P. cactorum, P. cinnamomi, P. citricola, P. citrophthora* and *P. ramorum*, detected from rhododendron nurseries in Poland. The species was isolated from diseased rhododendron shoots together with *P. citricola*. There were no differences in disease symptoms occurring on shoots invaded by particular species. Laboratory trials on rhododendron leaves indicated the lack of differences in pathogenicity of both species toward rhododendron cultivars. A similar reaction concerning both pathogens was observed on other ericaceous plants. Laboratory trials and especially greenhouse trials, showed that cv. Helsinki University is not the most susceptible cultivar to *P. inflata*. It is possible, that the pathogen was brought in with imported plant materials and spread on young cuttings during rhododendron multiplication. The first description of *P. inflata* on elm with canker symptoms, was done by Caroselli and Tucker (1949) in the USA. In Europe, the species was reported on rhododendron in the United Kingdom by Hall *et al*. (1992), in Italy by Testa *et al*. (2006) and in Finland by Lilja and Rytkonen (2007). Results obtained, indicated *P. inflata* as the pathogen of other ericaceous plants. The species colonized leaf blades of *Goultheria decumbens, Kalmia ostroseptice* and 2 *Vaccinium* spp. The quickest spread was on *K. ostroseptice*, *V. corymbiferum* and *G. decumbens*. The necroses spread very slowly on *V. vitis-idaea*. The occurrence of *P. inflata* on *G. shalon* and *V. vitis-idaea* in Scotland was reported by Schlenzig (2005). Both Schlenzig's description of the species and our results, indicated ericaceous plants as a potential source of that pathogen in European ornamental nurseries. Studies of Jung *et al*. (2005) showed the occurrence of tested species on beech (*Fagus sylvatica*) in the USA forests. It is possible the presence of that pathogen in forest stands may be connected with its spread from ericaceous plants on beech and probably other trees. In Lilja and Rytkonen's (2007) trial, the species caused necrotic lesions on *Alnus glutinosa, A. incana, Betula pendula* and *Picea abies*.

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POLISH SUMMARY

PIERWSZE DONIESIENIE O WYSTĘPOWANIU *PHYTOPHTHORA INFLATA* W SZKÓŁKACH RÓŻANECZNIKÓW W POLSCE

Gatunek *Phytophthora inflata* wykryto razem z *P. citricola* na różaneczniku odmiany University of Helsinki z objawami plamistości liści, rozszerzającej się na wierzchołkowe części łodyg. Inokulacja liści 15 gatunków i odmian różaneczników izolatami *P. inflata* i *P. citricola*, spowodowała rozwój nekrotycznych plam na ich powierzchni. Reakcja testowanych gatunków i odmian różanecznika na oba gatunki była podobna. Gatunek *P. inflata* kolonizował również blaszki liściowe *Goultheria decumbens, Kalmia ostroseptice, Vaccinium corymbiferum* i *V. vitis-idaea*, przy czym nekrotyczne plamy rozwijały się najszybciej na *V. corymbiferum*. W doświadczeniu szklarniowym *P. citricola* i *P. citrophthora* powodowały zamieranie większej liczby roślin aniżeli *P. inflata*.