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PHYTOPHTHORA CITRICOLA ON *RHODODENDRON* SPP. IN POLISH NURSERIES*

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Abstract: *Phytophthora citricola* dominated among 11 genera and fungal species isolated from *Rhododendron brachycarpum, R. catawbiense, R. impeditum* and *R. sepedonicum*. In greenhouse trial isolates from *Abies concolor, Chamaecyparis lawsoniana, R. catawbiense, R. impeditum* and *Thuya occidentalis* caused dieback of rhododendron. Inoculation of leaf blades with isolates of the pathogen from 4 cultivars resulted in the spread of necrosis about 0.63 mm/hr. *P. citricola* was pathogenic to all tested rhododendron cultivars.

Key words: *Phytophthora citricola*, rhododendron, occurrence, cultivar, pathogenicity

INTRODUCTION

Many diseases caused by *Phytophthora* species affect ornamental nursery crops but members of *Ericacae*, including *Rhododendron* spp., are the most frequent victims (Hoitink and Powell 1990). *Phytophthora* dieback of rhododendron may be caused by *P. cactorum* (Leb. and Coyn) Schroet., *P. citricola* Sawada, *P. cinnamomi* Rands, *P. cryptogea* Pethybr. et Laff., *P. gonapodyides* Peterson, *P. heveae* Thomp., *P. megasperma* Drechsler and *P. parasitica* Dast. (Benson and Jones 1980; Kuske and Benson 1983). Hoitink and Schmitthenner (1974) considered *P. citrophthora* (Smith and Smith) Leonian as minor dieback pathogen of rhododendron branches. Survey by Orlikowski et al. (1995) revealed the occurrence of *P. cinnamomi* and *P. citricola*, the first species being dominant on rhododendron with stem and root rot symptoms. Further studies (Orlikowski and Szkuta 2002b) indicated the occurrence of *P. citri-*

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cola and *P. ramorum* Werres, De Cock and Man in't Veld on rhododendron as causal agents of twig blight and foliar lesions.

The purpose of this study was to determine the incidence, distribution and pathogenicity characteristics of *P. citricola* causing rhododendron dieback.

MATERIALS AND METHODS

Disease symptoms, distribution and incidence. Five container-grown nurseries located in the middle and east parts of Poland were surveyed. In each nursery from 1 to 5 rhododendron species with 5 to 42 cultivars were grown. In the years 1999–2002 *Phytophthora* dieback or wilt symptoms were observed in 4 nurseries. Incidence of dieback varied greatly among nurseries with sporadic occurrence of diseased plants to 3–20%. Usually 2–3 inspections from July to September were done every year. Disease symptoms were observed on stems and leaf blades. Necrosis spread from stems to leaf blades was mainly observed on top parts of plants but sometimes on side parts, especially near passages. On stems first yellow-brown and later brown or dark-brown spots enlarging along and around individual twigs were observed at different points, being most common on branches. Invaded twigs died.

Isolation and identification of fungi. Partly diseased shoot samples taken from *Rhododendron brachycarpum* D. Don, *R. catawbiense* Michx., *R. impeditum* Balf. and *R. sepedonicum* L., were individually collected in plastic bags and transferred into laboratory, washed in running tap water, rinsed 3 times in distilled, sterile water and blotted dry with paper towels. Chosen shoot parts were sterilised over a burner flame, cut into 5 mm pieces and placed on Difco PDA in 90 mm Petri dishes (6 pieces/dish and 3 plates/plant). After 2–5-day-incubation at 24°C in the dark, parts of the grown colonies were transferred into PDA slants. Isolation and identification of fungi recovered from diseased plant parts was performed according to the method given by Orlikowski et al. (2001). Morphology of *P. citricola* was studied using procedure described by Orlikowski and Szkuta (2002b).

Pathogenicity of *P. citricola* toward rhododendron and colonisation of leaf blades by the pathogen. Isolates of *P. citricola* obtained from *Abies concolor* Lindl. et Gord., *Chamaecyparis lawsoniana* (Andr.) Parl., *R. catawbiense* Michx. (cv. Francesca), *R. impeditum* Balf. and *Thuya occidentalis* L. were used for peat infestation. Stock cultures were maintained at 24°C in the dark. Inocula were prepared on Quick oats using procedure of Orlikowski (1999). Population density of tested isolates in peat was estimated on the levels from 212 to 280 colony forming units (cfu)/g of air dry substratum using gallic acid selective medium (Flowers and Hendrix 1969). Rooted cuttings of rhododendron cv. Lachsgold were planted in pots filled with 1 dm^3 of infested peat. Control plants were grown in noninfested substratum. Plants were grown on greenhouse bench at temperature ranging from 17° to 25°C. Development of disease symptoms was observed weekly.

Colonisation of rhododendron (cv. Lachsgold) leaves by 4 tested isolates of *P. citricola* from different cultivars was observed in moisture chambers on sterile, moist blotting paper covered with plastic net. The same procedure was used with colonisation of leaves of 10 rhododendron cultivars by isolate obtained from cv. Francesca. Three mm diameter mycelial disks taken from the edge of *P. citricola* col-

onies were placed on bases of leaf blades. After 3 and 6-day-incubation at 24°C length of necrosis was measured.

Experimental design was completely randomised with 4 replications and 5 plants (greenhouse test) or 10 leaves in each replicate. Trials were repeated at least twice.

RESULTS

Identification of fungi. *R. catawbiense* was a dominant species in the surveyed nurseries whereas *R. brachycarpum* was found only in one place. *P. citricola* was the most often isolated species from all tested rhododendrons as it was found in about 88% of analysed plant parts (Tab. 1). On all tested species *Alternaria alternata, Botrytis cinerea, Penicillium* spp. and *Trichoderma* spp. were also found. *Fusarium* spp. represented by *F. avenaceum, F. oxysporum* and *F. solani* occurred rarely or only sporadically (Tab. 1).

Pathogenicity of *P. citricola*. In greenhouse trial the first discoloration of shoots was observed 2 weeks after planting rhododendron in peat infested with 5 *P. citricola* isolates (Tab. 2). Within the next 4 and 12 weeks significantly more dis-

Table 1. Fungi isolated from diseased stems of *Rhododendron* spp.; number of plants colonised by fungi (a) and number of isolates obtained (b)

Fungal species	*R. brachycarpum* (10 plants)		*R. catawbiense* (98 plants)		*R. impeditum* (32 plants)		*R. sepedonicum* (22 plants)	
	a	b	a	b	a	b	a	b
Alternaria alternata Nees	1	3	17	49	3	10	4	9
Botrytis cinerea Pers.	2	7	22	55	2	5	3	10
Chaetomium glubosum Kunze	–	–	7	11	1	3	1	3
Cladosporium herbarum (Pers.) Link.	–	–	–	–	2	4	–	–
Fusarium avenaceum (Fr.) Sacc.	1	2	3	8	–	–	2	5
Fusarium oxysporum Schlecht.	–	–	1	5	2	5	–	–
Fusarium solani (Mart.) Sny. et Hans.	2	3	2	7	–	–	4	7
Mucor spp.	–	–	11	18	7	19	9	16
Penicillium spp.	2	4	9	17	5	11	5	11
Phytophthora citricola Sawada	9	26	85	236	30	92	19	61
Trichoderma spp.	2	5	19	40	10	18	5	13
Brown, nonsporulating fungi	–	–	3	7	1	3	–	–

Table 2. Pathogenicity of *Phytophthora citricola* isolates toward rhododendron; number of diseased plants (n=5). Planting: 2001.07.04

Source of isolates	Weeks after planting			
	2	4	6	16
Abies concolor	0.5 a	0.5 a	0.5 a	1.0 a
Chamaecyparis lawsoniana	0.3 a	1.8 b	3.5 c	4.8 c
Rhododendron catawbiense	1.0 b	2.5 bc	3.3 c	4.5 c
Rhododendron impeditum	1.0 b	3.8 d	3.8 c	4.8 c
Thuja occidentalis	0.5 a	1.0 ab	2.3 b	3.3 b

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

eased plants were observed when they were grown in peat infested with isolates from *C. lawsoniana* and both rhododendron species than other host plants (Tab. 2). After 16-week-cultivation only 1/5 of rhododendron grown in peat infested with *P. citricola* obtained from *A. concolor* showed necrosis of stems and leaves (Tab. 2).

Colonisation of leaf blades by *P. citricola* isolates from 4 rhododendron species. There were no significant differences in the spread of necrosis after 3 and 6-day-incubation on rhododendron leaves inoculated with 4 isolates (Tab. 3). On 10 tested cultivars of rhododendron necrosis spread significantly slower on H. Charmant and Lumina Jakushim than on other 8 varietes, both 3 and 6 days after inoculation (Tab. 4).

Table 3. Development of necrosis on leaves of rhododendron cv. Lachsgold inoculated with isolates of *Phytophthora citricola*; length of necrosis in mm. Inoculation: 2001.09.04

Source of isolates	Days after inoculation	
	3	5
Rhododendron "Album Novum"	21.6 a	36.9 a
Rhododendron "Francesca"	22.0 a	40.5 a
Rhododendron "Lugano"	22.4 a	40.4 a
Rhododendron "Percy Wiseman"	25.0 a	48.3 ab

Note: see table 2

Table 4. Development of necrosis on leaves of rhododendron cultivars inoculated with *Phytophthora citricola* (isolate from cv. Francesca); length of necrosis in mm. Inoculation: 2002.03.21

Cultivars	Days after inoculation	
	3	6
Cunningam's White	21.3 b	38.5 b
Haaga	20.4 b	40.1 b
Hellikki	18.9 b	37.9 b
H. Charmant	11.5 a	20.5 a
Lumina Jakushirm	12.6 a	22.3 a
Mikkeli	23.4 b	40.6 b
Nova Zembla	24.0 bc	43.5 b
Pohjola's Daughter	22.3 b	41.0 b
Purple Splendour	24.8 bc	44.0 b
Tiger Stedli	25.0 bc	43.8 b

Note: see table 2

DISCUSSION

Epidemics of *Phytophthora* dieback of rhododendron depend on the presence of already infected plants in nursery as a possible source of the pathogen. Relative humidity and moisture as well as temperature 20–30°C affect both sporulation of *P. citricola* on diseased stems or leaves and dissemination of the pathogen in nursery. In all inspected nurseries, growing of plants in containers was connected with higher substratum temperature than in soil during spring-autumn time. Addi-

tionally, overhead irrigation increased air humidity and shoot moisture. In such conditions *P. citricola* has almost optimal conditions for sporulation and zoospore splash dispersal from twigs and substratum surface. Production of oospores by *P. citricola* caused that in this form the pathogen may be more persistent than chlamydospores (Hoitink and Schmitthenner 1969). This may be one of the reasons why *P. cinnamomi*, observed earlier on rhododendron (Orlikowski et al. 1995; Orlikowski and Szkuta 2002a), was not discovered from analysed diseased twigs. Isolation of *A. alternata*, *B. cinerea* and *Fusarium* spp. from rhododendron indicated that those species settled on already week shoot parts, invaded by *P. citricola*.

Our study showed that isolates of *P. citricola* from other host plants, including Lawson cypress and arborvitae were also pathogenic toward rhododendron. It may suggest on dissemination of the pathogen zoospores in nursery from one host to another by overhead irrigation, rain, wind-blown rain (Kuske and Benson 1983) or/and by water collected in nursery containers. Gerlach et al. (1976) demonstrated that during rainstorms, propagules of *P. citrophthora* were splashed up from colonized leaves of pieris on the soil surface up to 60 cm from the infected container base.

The data obtained from greenhouse trial indicate the lack of host specialisation among *P. citricola* strains. This confirms results of Hoitink and Schmitthenner (1969) indicating on the lack of host specialisation among *P. cinnamomi*, *P. citricola* and *P. palmivora*. Our observation showed, however, that the isolate of *P. citricola* from *A. concolor* was significantly weaker pathogen than isolates from other host plants. Relationship between the source of isolates and their pathogenicity toward different host plants will be the aim of further study. The data obtained indicate the lack of differences between pathogenicity of isolates from 4 rhododendron cultivars toward cv. Lachsgold, very susceptible to *P. citricola*. We found, however, differences between the necrosis spread caused by *P. citricola* on 10 tested rhododendron cultivars. It may, in part, explain why dieback symptoms were observed only on some cultivars whereas on others the disease was not noticed or occurred only sporadically. The data obtained showed that *P. citricola* appears to be the primary pathogen of rhododendron species grown in Poland and must be considered in *Phytophthora* dieback control programs.

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