

OCCURRENCE OF *PHYTOPHTHORA CINNAMOMI* ON ERICACEOUS PLANTS IN CONTAINER-GROWN ORNAMENTAL NURSERIES IN POLAND

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Abstract: *Phytophthora cinnamomi* dominated among isolates obtained from diseased 9 species of ericaceous plants. Inoculation of leaves or shoot parts by that species resulted in the fast development of necrosis. In greenhouse trials the pathogen caused root and shoot rot within 10–12-week-growth. The source of isolate had significant influence on the development of *Phytophthora* rot.

Key words: *Phytophthora*, isolation, pathogenicity, incubation time

INTRODUCTION

Phytophthora cinnamomi Rands, first discovered from the bark canker of *Cinnamomum burmanni* Bl. in Sumatra and identified by Rands in 1922, is the most devastating and cosmopolitan species (Ho and Zentmyer 1977) that attacks at least 1000 of host plants throughout the world (Zentmyer 1983). In some nurseries root rot or dieback caused by *P. cinnamomi* may reach epidemic proportion (Van Steekelenburg 1974). Vegh (1985) found *P. cinnamomi* on 16 genera of ornamental plants including *Azalea*, *Calluna*, *Daboecia*, *Erica*, *Rhododendron* and *Vaccinium* as its hosts. Symptoms of infection of ericaceous plants by *P. cinnamomi* include decay of roots and a reddish – brown necrosis of the cambial region at the root collar extending several centimetres up the shoots and down the larger roots. Foliar symptoms include chlorosis, stunting, eventually reddish-brown discoloration and in some cases wilting of individual current year's growth. In opinion of Davison et al. (1994) the common feature for plant infection by *P. cinnamomi* is zoospore attraction by root exudates, cyst germination in the root cap cell zone and development of mycelium in the cortical cells, phloem and xylem of infected roots, although the pathogen is

not able to hydrolyse lignified cell walls. Reduced stomatal conductance and transpiration by that species have been also demonstrated on some infected plants. Additionally, water deficiency symptoms which can result in plant death have been observed on some trees (Robin et al. 2001). The pattern of invasion and the histopathological changes in primary root tissues after infection by *P. cinnamomi* was described by Cahill et al. (1989). Zoospores germinated on and penetrated plant roots and lesion formed within 8–16 hr at 20°–24°C. Root growth ceased within 24 hr. Progressive symptoms developed including water soaking of tissues, lesion extension through the root to hypocotyl. This was accompanied by wilting and chlorosis of leaves, dieback of shoots and plant death. Sporulation of the pathogen occurred between 24 and 72 hr after plant inoculation.

First report on the occurrence of *P. cinnamomi* in Poland was published by Orlikowski et al. (1995). The species was found as the causal agent of wilt and death of *Abies alba*, *Chamaecyparis lawsoniana*, *Pinus nigra*, *P. mugho* var. *pumilio*, *Rhododendron* spp. and *Calluna vulgaris*. The objectives of this study were (1) to search healthiness of ericaceous plants in 6 ornamental nurseries and isolation of fungi occurring in diseased shoots, (2) identification of obtained fungi to genera and species, (3) to investigate pathogenicity of *Phytophthora cinnamomi* toward chosen plants.

MATERIAL AND METHODS

Isolation and identification of fungi. Diseased *Azalia japonica* L., *Andromeda polifolia* L., *Daboecia cantabrica* (Huds.) K. Koch, *Hebe inbricata* cv. Green Globe, *Calluna vulgaris* L., *Empetrum nigrum* L., *Kalmia angustifolia* L., *Ledum palustre* L. and *Vaccinium vitis-idaea* L. grown in containers were analysed. Most of chosen plants showed wilting of individual shoots or/and brown discoloration of stems and leaves. Their roots, especially small lateral and feeder, were partly or completely rotted. Plants with described symptoms occurred in nurseries singly in different places or in groups with at least 3 to 50 (*Vaccinium*) in some places. Such plants were collected in individual plastic bags and transferred to laboratory. Samples were collected 2 times at the end of July and beginning of September. Chosen shoots were sterilised over a burner flame, cut into 5 mm pieces and placed on Difco PDA in 90 mm Petri dishes (6 shoot parts/dish and 3 plates/plant). After 3–5-day-incubation at 24°C in the dark parts of grown colonies were transferred into PDA slants. Isolation and identification of fungi, recovered from symptomatic plant parts, followed the method given by Orlikowski et al. (2001).

Pathogenicity of *Phytophthora cinnamomi* toward ericaceous plants. In an *in vitro* trials isolate ER 18 of *P. cinnamomi*, obtained from diseased heather runner, was used for inoculation of leaf petioles of andromeda, azalea, heather, daboecia, cowberry, kalmia and cranberry. Stock culture was maintained on Difco PDA at 24°C. Five mm diam discs, taken from the edge of 7-day-old cultures were transferred on the base of leaf petioles placed in moist chambers. Development of necrosis on leaves was estimated after 4 and 6-day-incubation at 24°C.

In greenhouse trials the mentioned, freshly rooted plants species were planted into 1 dm pots containing peat artificially infested with isolate ER 18. Procedure de-

scribed by Orlikowski (1999) was used for substratum infestation. Initial population density of the pathogen was established on the level 315 colony forming units/g of air dry peat.

In the next trial isolates of *P. cinnamomi* obtained from azalea, heather, Lawson cypress, cowberry and rhododendron were used for peat infestation. To such substratum rooted heathers were planted. In both trials plants were placed on the bench covered with a black mat and grown over 8–12 weeks at 17°–25°C. Healthy stand of plants was assessed at 7-day-intervals.

Experimental design was completely randomised with 4 replications and 10 leaves and 5 plants in each rep. Trials were repeated at least twice.

RESULTS

Identification of fungi. *Phytophthora cinnamomi* dominated among 15 fungal species isolated from diseased tissues of 9 tested ericaceous plants species (Tab. 1). The pathogen was found on the most of tested plants. From others fungi, known as plant pathogens, *Botrytis cinerea* occurred on all tested plants species whereas *Fusarium avenaceum* was not found only on hebe and ledum. From the base of kalmia and cowberry shoots *Pythium* sp. was isolated (Tab. 1).

Pathogenicity of *P. cinnamomi* toward ericaceous plants. *In vitro* trials with inoculation of leaves or top parts of shoots showed, that *P. cinnamomi* caused their browning and necrosis. First disease symptoms were already observed 24 hr after inoculation of leaf petioles. Within 4 days length of necrosis varied from 8.9 (hebe) to 16.7 (cowberry) mm (Tab. 2). Six days after inoculation further spread of necrosis was noticed on all tested plants but especially on ledum and cranberry (Tab. 2).

In greenhouse trial first diseases symptoms on plants, grown in peat infested with *P. cinnamomi* from heather, were observed already 3 weeks after planting. One week later 1/5 of daboecia and 2/5 of kalmia plants showed diseases symptoms (Tab. 3). After 8-week-growth at least 1/3 but also 4/5 plants wilt and/or died. Most of plants died after 10-week-cultivation (Tab. 3).

Source of isolate of *P. cinnamomi* had significant influence on disease severity of heather during 12-week-growth (Tab. 4). When plants were grown in peat infested with isolate from rhododendron, after 6 weeks more than 2/5 of heather plants had reddish-brown runners whereas such symptoms were observed only sporadically when substratum was infested with isolates from azalea and Lawson cypress (Tab. 4). Two weeks later such difference was not evident with rhododendron isolate but disease symptoms developed only on some plants when grown in the presence of *P. cinnamomi* from azalea and cypress. After 3 months most of plant died except heathers cultivated in peat infested with isolate from Lawson cypress (Tab. 4).

DISCUSSION

The data obtained indicated on *P. cinnamomi* as the causal agent of *Phytophthora* root and shoot rot of 9 ericaceous plant species. The pathogen dominated among isolated fungi and was found on the most of analysed plants. Other *Phytophthora* species were not isolated. In Vegh (1985) studies *P. cinnamomi* was the prime pathogen of ericaceous plants but *P. cactorum* and *P. citricola* occurred as well on azalea and

Table 1. Fungi isolated from diseased ericaceous plants in the years 2000–2001; number of affected plants (a) and number of isolates obtained (b)

Species of fungi	<i>Andromeda polifolia</i> (42 plants)		<i>Azalia japonica</i> (68 plants)		<i>Calluna vulgaris</i> (65 plants)		<i>Daboecia cantabrica</i> (37 plants)		<i>Hebe inbricata</i> <i>Green Globe</i> (28 plants)		<i>Empetrum nigrum</i> (19 plants)		<i>Kalmia augustifolia</i> (31 plants)		<i>Ledum palustre</i> (25 plants)		<i>Vaccinium vitis-idaea</i> (25 plants)		
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
<i>Alternaria alternata</i> Nees	–	–	–	–	2	3	2	2	–	–	–	–	–	–	–	–	–	7	15
<i>Botrytis cinerea</i> Pers.	11	18	4	7	7	11	4	9	2	5	3	7	2	5	2	3	6	9	
<i>Chaetomium funiculum</i> Cooke	–	–	1	1	2	3	–	–	1	2	–	–	–	–	–	–	–	–	
<i>Chaetomium globosum</i> Kunze	3	5	–	–	–	–	–	–	–	–	1	3	–	–	–	–	4	7	
<i>Fusarium avenaceum</i> (Fr.) Sacc.	5	9	3	7	3	4	6	9	–	–	1	4	2	5	–	–	4	9	
<i>Fusarium equiseti</i> (Cda) Sacc.	1	1	–	–	–	–	2	3	–	–	1	2	2	3	–	–	–	–	
<i>Fusarium solani</i> (Mart.) Sny et Hans.	–	–	–	–	1	1	1	2	–	–	–	–	5	8	2	5	2	3	
<i>Gliocladium roseum</i> (Link.) Thom	–	–	–	–	1	3	–	–	4	7	–	–	–	–	4	7	2	3	
<i>Mucor circinelloides</i> van Tieghem	–	–	3	7	–	–	1	3	–	–	1	3	–	–	2	3	–	–	
<i>Mucor hiemalis</i> Wehmer	4	7	–	–	3	4	–	–	6	11	–	–	–	–	3	8	4	9	
<i>Penicillium</i> spp.	–	–	7	11	7	16	2	4	–	–	3	3	8	1	1	3	–	–	
<i>Pestalotia</i> sp.	–	–	3	5	4	7	–	–	–	–	–	–	3	3	–	–	–	–	
<i>Phytophthora cinnamomi</i> Rands	34	79	53	148	51	136	34	84	23	48	19	46	29	41	24	53	22	67	
<i>Pythium</i> sp.	–	–	–	–	–	–	–	–	–	–	2	3	8	14	–	–	–	–	
<i>Trichoderma</i> spp.	–	–	9	14	3	7	3	6	7	18	5	14	7	9	9	17	5	12	
Brown, nonsporulating fungi	–	–	2	5	–	–	1	2	–	–	4	9	1	3	4	9	2	5	

Table 2. Development of necrosis on leaves or shoots of ericaceous plants inoculated with *Phytophthora cinnamomi* isolate ER 18 from heather; length of necrosis in mm

Plant species	Days after inoculation	
	4	6
<i>Andromeda polifolia</i>	12.9 d	18.4 bc
<i>Azalea indica</i>	10.0 bc	19.2 bc
<i>Calluna vulgaris</i>	8.8 ab	13.7 a
<i>Daboecia cantabrica</i>	12.8 d	19.9 c
<i>Empetrum nigrum</i>	7.9 a	15.4 ab
<i>Hebe inbricata</i>	8.9 ab	15.7 ab
<i>Kalmia anqustifolia</i>	11.7 cd	18.9 bc
<i>Ledum palustre</i>	14.8 e	23.9 d
<i>Vaccinium vitis-idaea</i>	16.7 f	25.0 d

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

Table 3. Pathogenicity of *Phytophthora cinnamomi* isolated from *Calluna vulgaris* (isolate ER18) toward ericaceous plants; number of diseased plants (n=5). Planting: 2001.06.08

Plant species	Weeks after planting		
	4	8	10
<i>Andromeda polifolia</i>	0.5 ab	3.0 bc	4.5 ab
<i>Azalea japonica</i>	0 a	2.25 a	3.75 a
<i>Calluna vulgaris</i>	0 a	3.5 c	5.0 b
<i>Daboecia cantabrica</i>	1.0 b	4.0 c	5.0 b
<i>Empetrum nigrum</i>	0.25 a	1.75 a	4.25 a
<i>Kalmia anqustifolia</i>	1.75 c	2.75 b	4.5 ab
<i>Vaccinium vitis - idaea</i>	0 a	2.5 ab	5.0 b

Note: see table 2

Table 4. Pathogenicity of *Phytophthora cinnamomi* isolates from different plants toward *Calluna vulgaris*; number of diseased plants (n=5). Planting: 2001.06.25

Source of isolate	Weeks after planting			
	4	6	8	12
<i>Azalea japonica</i>	0.5	0.75 a	3.5 ab	4.8 b
<i>Calluna vulgaris</i>	1.25 b	2.0 b	3.0 a	4.5 b
<i>Chamaecyparis lawsoniana</i>	0.5 a	1.0 a	2.5 a	3.3 a
<i>Empetrum nigrum</i>	1.75 bc	2.0 b	3.75 b	5.0 bc
<i>Rhododendron catawbiense</i>	2.25 c	2.25 b	3.0 a	4.5 b

Note: see table 2

heather. The discovery of *P. cinnamomi* on all ericaceous plant species cultivated in Polish nurseries has serious implications. The presence of pathogen at nurseries ecosystem and its appearance on various ornamentals (Orlikowski et al. 1995) suggests the possibility of its wider dissemination. Patterns and mechanisms of the pathogen dispersal was not included in this research. It is possible that *P. cinnamomi* was expanded throughout country by cuttings or mother plants. In container –

grown nurseries recycling water, used for plant sprinkling, may be a source of zoospores (Orlikowski, unpubl.). Also rainwater may spread the pathogen throughout nursery. Results obtained indicated that source of the pathogen is not so important in the development of *Phytophthora* rot. Even isolate of *P. cinnamomi* from diseased Lawson cypress was pathogenic to heather whereas the strain from that plant caused *Phytophthora* rot of all tested ericaceous. Studies of Vegh and Bourgeois (1976) showed different reaction of coniferous plants, *Erica* and *Calluna* cultivars on *P. cinnamomi*. Most of heather cultivars were susceptible to the pathogen.

Losses caused by *P. cinnamomi* in ericaceous plant nurseries was not easy to survey. Growers usually eliminate plants with a first disease symptoms and only empty places indicate on the lack of them. In our opinion, the pathogen effected, however, from 2 to even 70% of plants.

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POLISH SUMMARY

WYSTĘPOWANIE *PHYTOPHTHORA CINNAMOMI* W POJEMNIKOWEJ UPRAWIE OZDOBNYCH ROŚLIN WRZOSOWATYCH W POLSCE

Celem badań było określenie przyczyny zamierania andromedy, azalii, bagna, bażyny, borówki ozdobnej, dabeccji, hebe, kalmii i wrzosów oraz określenie chorobotwórczości *Phytophthora* w stosunku do wybranych gatunków roślin wrzosowatych. Gatunek *P. cinnamomi* był najczęściej izolowany z porażonych tkanek analizowanych gatunków roślin. Z innych gatunków, znanych jako patogeny roślin, izolowano m.in. *Botrytis cinerea*, *Fusarium avenaceum* i *Pythium* sp. Gatunek *P. cinnamomi*, użyty do inokulacji ogonków liściowych lub fragmentów pędów wierzchołkowych, powodował bardzo szybki rozwój nekrozy na tych organach. W doświadczeniach szklarniowych, izolat *P. cinnamomi* z wrzosów, powodował zamieranie większości badanych gatunków roślin w ciągu 12-tygodniowej uprawy. Gdy do zakażenia podłoża użyto izolatów z różnych gatunków roślin, stwierdzono zróżnicowaną reakcję wrzosów na patogena.