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Phytophthora root and stem rot – new disease of *Ilex aquifolium* "Myrtifolia" in Poland

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Phytophthora cinnamomi was often isolated from rotted roots and stems of English holly "Myrtifolia" together with *Alternaria alternata*, *Cylindrocarpon destructans*, *Fusarium avenaceum* and other fungal species. Inoculation of leaf blades and stem parts of 4 species and 12 holly cultivars with *P. cinnamomi* showed the spread of rot symptoms on the most of them. On *Ilex crenata* tissues necrosis did not develop or spread slowly. Isolation of *P. cinnamomi* only from one holly cultivar in surveyed nursery indicate on transmission of the pathogen with imported young plants.

Key words: *Phytophthora cinnamomi,* occurrence, isolation, colonisation, cultivars

INTRODUCTION

Several fungi affect hollies (*Ilex* spp.) in nurseries, including *Thielaviopsis basicola* (Berk) Ferr., *Rhizoctonia solani* Kuhn and *Colletotrichum gloeosporioides* (Penzig.) Sacc. (Chase 1991; Frisina and Benson 1987; Lambe and Wills 1976). A leaf and twig disease incited by *Phytophthora ilicis* was described by Buddenhagen and Young (1957). The pathogen has been reported only on *Ilex aquifolium* L. The authors observed purple black spots on leaves and berries, defoliation and twig cankers. Affected twigs dieback and canker developed on larger stems. This paper presents studies concerning detection, identification and pathogenicity of *Phytophthora cinnamomi* Rands toward holly.

MATERIALS AND METHODS

Diseased plants. Diseased English hollies were found in one container grown nursery in eastern part of Poland. First disease symptoms were observed in Aug. 2002 on shoots which changed colour on yellow-green and within the next 2 weeks

on yellow-brown or dark brown. After the next 4 weeks leaves were dark brown whereas stems, especially on one side, black from the base to the top. The analysis of root system showed that 2-4 roots on all length or partly were brown or darkbrown. From such affected roots discoloration spread on stems. On longitudinal section of invaded stems black discoloration of one side, spread from the base to the top, was observed. Among 1500 plants, 3-year-old, about 30% died within one growth-season.

Isolation and identification of fungi from diseased plants. Affected plants were collected twice, on Aug. and Sept. 2002 (together 22 hollies). After removing the substratum from roots plants were put individually to plastic bags and transported to the laboratory. Diseased roots and shoot parts were washed under running tap water, blotted dry with paper towel and surface sterilised over a burner flame. About 5 mm diam. parts of tissues taken on the border of healthy and affected roots and stems were put on the surface of potato-dextrose agar (PDA) in 90 mm Petri dishes (6 pieces/dish and 3 plates/root or stem parts). Plates were incubated at 24°C in the dark and after 2-6 days small parts of grown colonies were transferred into PDA slants. After separation and cleaning of fungal cultures they were identified to species using available monographs and keys. *Phytophthora* sp. was identified to species by comparison of colony growth patterns on Difco PDA, CMA and V8 juice at the temperature from 2° to 35° C. Additionally, morphological features according to Stamps et al. (1990) and Erwin and Ribeiro (1998) confirmed by isozyme electrophoresis of MDH and MDHP (Oudemans and Coffey 1991, Man in't Veld et al. 1998) was used.

Colonisation of leaf blades and stem parts of *Ilex* spp. by *P. cinnamomi*. Four holly species: *Ilex altaclerensis* (Loud.) Dallim., *I. aquifolium* L., *I. crenata* Thumb., *I. meserveae* S.Y.Hu and 12 cultivars (Tab. 2) were used in *in vitro* trials. Leaves and stem parts were taken from the top of plants, washed 3 min under tap water and rinsed twice in distilled water and blotting dried. Plant parts were placed on moist blotting paper covered with plastic net in the polystyrene boxes. Isolate of *P. cinnamomi* isolated from diseased stem base was used for leaves and stem inoculation. Three mm diam mycelial disks, taken from the edge of colonies were applied on leaf petioles and base of stem parts. Boxes were covered with foil and incubated at 20°-23°C. Length of necrosis was measured after 7 and 11-day-incubation.

Experimental design was completely randomised with four replications and five plant parts in each rep. The trial was repeated twice at 3-week-interval.

RESULTS AND DISCUSSION

Isolation and identification of fungi. Most of fungi obtained were isolated from diseased roots and stem parts (Tab. 1). From some stem fragments even 3-5 different fungi were isolated. *Phytophthora cinnamomi* dominated among 9 species and genera. The species was isolated from all tested plants but especially from stem pieces (Tab. 1). *Alternaria alternata*, *Cylindrocarpon destructans* and *Fusarium avenaceum* were isolated also very often. The presence of *Gliocladium roseum* and *Trichoderma* spp. on affected stems indicated that plants were not often treated with chemical compounds.

Table 1
Fungi isolated from diseased *Ilex aquifolium* "Myrtifolia"

Fungal species	Roots (11 plants)		Shoots (14 plants)	
	a	b	a	b
Alternaria alternata Nees.	2	5	9	17
Botrytis cinerea Pers.	1	3	3	7
Cylindrocarpon destructans (Zins.) Scholten	7	23	5	7
Fusarium avenaceum (Fr.) Sacc.	5	9	7	16
Gliocladium roseum (Link.) Thom.	3	7	3	5
Mucor spp.	2	4	5	7
Penicillium spp.	-	-	4	9
Phytophthora cinnamomi Rands	6	17	10	34
Trichoderma spp.	3	5	4	12

Explanation: number of colonised plants (a) and number of isolates obtained (b)

Colonisation of leaves and stem parts of *Ilex* spp. by *P. cinnamomi*. Inoculation of leaf blades resulted in different spread of necrosis (Tab. 2). Disease symptoms were not observed or necrosis spread very slowly on *Ilex crenata* "Convexa" and "Golden Gem". The fastest spread of leaf rot were noticed on *I. altaclerensis* "Golden King" and 4 cultivars of *I. aquifolium* ("Myrtifolia", "Pyramidalis Aurea Marginata","Rubricaulis" and "Silver Milkboy"). Lack of stem rot was noticed on both cultivars of *I. meserveae* (Tab. 2). Necrosis spread very slowly on *I. aquifolium* "Bacciflava" and "Pyramidalis Aurea Marginata", *I. crenata* "Golden Gem". The fastest development of stem rot occurred on *I. altaclerensis* "Golden King" and *I. aquifolium* "Rubricaulis Aurea" (Tab. 2).

Table 2
The spread of necrosis on leaves and stem parts of *Ilex* species and cultivars inoculated with *Phytophthora cinnamomi*

Species and cultivars	Days after inoculation			
	Leaf blades		Stems	
	7	11	7	11
Ilex altaclerensis „Belgica Aurea"	8.0 cd	14.3 e	6.4 b-d	23.0 g
I. altaclerensis „Golden King"	16.3 ef	52.9 j	16.8 f	45.2 j
I. aquifolium „Bacciflava"	4.8 bc	14.5 e	3.5 b	6.8 cd
I. aquifolium „Golden van Tol"	5.3 bc	25.0 g	3.5 b	22.6 g
I. aquifolium „Myrtifolia"	27.5 g	40.3 i	5.5 bc	12.5 e
I. aquifolium „Pyramidalis Aurea Margarita"	5.0 bc	37.7 i	4.0 bc	8.8 d
I. aquifolium „Rubricaulis Aurea"	10.1 d	40.3 i	6.8 cd	38.3 i
I. aquifolium „Silver Milkboy"	18.6 f	37.0 i	7.0 cd	33.0 h
I. crenata „Convexa"	0 a	0 a	6.3 b-d	8,2cd
I. crenata „Golden Gem"	3.3 ab	3.5 ab	0 a	3.5 b
I. meserveae „Blue Angel"	4.5 bc	6.3 b-d	0 a	0 a
I. meserveae „Blue Princess"	2.8 ab	3.8 b	0 a	0 a

Explanations: means followed by the same letter do not differ at 5% of significance (Duncan's multiple range test); diam/length of necrosis in mm (mean values from 2 trials)

Mycological analysis of diseased holly showed that *P. cinnamomi* colonised, both, invaded roots and stems. *Botrytis cinerea, Cylindrocarpon destructans* and *Fusarium avenaceum* were probably the second invaders of tissues already settled by *P. cinnamomi.* The data obtained with inoculation trials of leaves and stem parts indicated *P. cinnamomi* as the main holly pathogen and this is the first information about *Ilex* spp. as potential host of that species. Our trials with *P. cinnamomi* did not confirm results of Buddenhagen and Young (1957) indicated *I. aquifolium* as only one host of *P. ilicis.* Leaf spot and stem rot development were also observed on other holly species but especially on *I. altaclerensis.* Cultivars of *I. crenata* were resistant, however, to the pathogen or tissue rot developed slowly. Inoculation trials showed large differences between reactions of holly cultivars on *P. cinnamomi.* The pathogen spread especially fast on cultivars with gold and silver leaves. Studies of Ticknor et al. (1980) indicated that among 196 holly species, cultivars and inter-specific hybrids all cultivars of *I. aquifolium* were susceptible to *P. ilicis.* Lack of any *Phytophthora* root and stem rot symptoms on *I. altaclerensis, I. crenata* and most of *I. aquifolium* cultivars in surveyed nursery indicated that *P. cinnamomi* was transmissed on imported, young *I. aquifolium* "Myrtifolia". It is also possible that the pathogen spread in the nursery from diseased Lawson cypress or other coniferous plants onto English holly.

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Fytoftoroza korzeni i łodyg – nowa choroba *Ilex aquifolium* w Polsce

Streszczenie

Chorobę stwierdzono na ostrokrzewach odm. Myrtifolia w jednej ze szkółek pojemnikowych w drugiej połowie lata. Pędy szarzały i żółkły, a następnie zmieniały zabarwienie na jasnobrązowe i brunatne. Po jednej stronie porażonych łodyg występowała wyraźna, ciemna smuga od podstawy do wierzchołka. Z porażonych korzeni i pędów izolowano głównie *Phytophthora cinnamomi*. Wśród innych organizmów występowały *Alternaria alternata, Cylindrocarpon destructans i Fusarium avenaceum*. Do badań nad kolonizacją liści i łodyg przez *P. cinnamomi* wybrano 4 gatunki ostrokrzewu i 12 odmian najczęściej uprawianych w szkółkach. Zgnilizna rozwijała się na większości zakażonych odmian, przy czym najszybciej *na I. altaclerensis* Golden King, *I. aquifolium* Rubricaulis Aurea i Silver Milkboy. Objawów nekrozy nie stwierdzono na łodygach *I. meserveae*.