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PHYTOPHTHORA CITRICOLA ON EUROPEAN BEECH AND SILVER FIR IN POLISH FOREST NURSERIES

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Abstract: *Phytophthora citricola* was isolated from diseased seedlings of European beech and Silver fir taken from the most of surveyed nurseries. *Fusarium* species, *Pythium ultimum* and *Rhizoctonia solani* were also found in diseased plant tissues. Isolates of *P. citricola* from both plants and additionally from heather and rhododendron colonised leaf blades, needles and stem parts of beech and fir. In greenhouse trials on inoculated 1-year-old seedlings necrosis spread about 2 mm/24 hr on beech stems whereas on fir about 1.5 mm/24 hr.

Key words: forest nursery, survey, isolation, *Phytophthora*, colonisation, beech, fir

INTRODUCTION

European beech (*Fagus sylvatica* L.) and Silver fir (*Abies alba* Mill.) are important plants in Polish forests. Several species of *Phytophthora* damage trees in several areas of the world in ornamental and forest nurseries (Hensen et al. 1980; Orlikowski et al. 1995). Novotielnova (1974) in her monograph of *Phytophthora* mentioned beech as the host of *P. cactorum* (Leb. and Cohn) Schr., *P. cambivora* (Petri) Buisman and *P. cinnamomi* Rands. Hudler et al. (2002) isolated *Phytophthora* spp. from trunk canker of European beech. Isolates obtained were equally closely related to *P. inflanta* Caroselli and Tucker and *P. citricola* Sawada. Erwin and Ribeiro (1996) classified *P. cambivora* Drechsler as causal agent of root rot of European beech. In the group of coniferous plants, species of fir were mentioned as hosts of *P. cactorum, P. cambivora, P. cinnamomi, P. citricola, P. cryptogea* Pethybr. *et* Laff., *P. drechsleri* Tucker, *P. gonapodyides* (Peterson) Buisman and *P. megasperma* (Adams and Bielenin 1988; Chastagner et al. 1995; Shew and Benson 1981). *P. citricola* was isolated from

Fraser fir seedlings by Shew and Benson (1981). *Phytophthora* spp. caused root rot, foliage discoloration, their necrosis and wilting of new growth. Similar symptoms were observed on 1-3-year-old seedlings of European beech and Silver fir in some Polish forest nurseries. Such plants were found in different places of each nursery. After removing of affected plants from soil, rot was observed on all roots or was limited to a few roots. On some plants basal stem rot was also observed.

In this study we attempted to isolate *Phytophthora* sp. from diseased tissues and to determine its possible involvement in plant death.

MATERIALS AND METHODS

Nursery survey. Twenty-one forest nurseries in different parts of Poland were surveyed for the presence of *Phytophthora* spp. European beech was grown in 6 nurseries whereas Silver fir in 7 places (Tabs. 1, 2). In most of nurseries both coniferous and deciduous plants were grown. Three-four-year rotation system was used in each nursery. Plants were fertilised and watered as required. Seedlings with discoloration of leaves or needles and with root and stem base rot were collected between April 2002 and October 2003. A total 59 diseased European beech seedlings and 107 Silver fir seedlings 1–3-year-old were collected. Plants were placed into plastic bags and transferred to the laboratory.

Isolation of fungi. Pieces of diseased roots and stems were washed in tap water and rinsed 3 times in distilled water. After drying between blotting paper they were disinfected over a burner flame and 5 mm diam. pieces were placed on potato-dextrose agar (PDA) in 90 mm Petri dishes (6 pieces/dish and 3 plates for each plant). Plates were incubated at 24°C in the dark and checked every day for the presence of colonies. Small fragments of colonies, growing around of tissue pieces, were transferred to PDA slants. Additionally, green apples were used as alternative medium (Orlikowski et al. 2003). After segregation of isolates obtained, representative cultures were cleaned and identified to genera and species (Orlikowski and Szkuta 2003).

Colonisation of plant parts and stem base by *Phytophthora citricola* isolates. Isolates of *P. citricola* from *Abies alba* Mill. , *Fagus sylvatica* L. and additionally from *Calluna vulgaris* (L.) Salisb. and *Rhododendron* sp. (Orlikowski and Szkuta 2003; Orlikowski et al. 2004) were used. Stock cultures were maintained on PDA at 24°C in the dark. In laboratory trials 3 mm diam. plugs, taken from the edge of 7-day-old colonies were placed on leaf blades, needles and stem parts of beech and fir. Plant parts were placed on the moist blotting paper covered with plastic net in the polystyrene boxes and covered with foil. Length of necrosis was measured after 4 and 8-day-incubation at 20–23°C. In greenhouse trials 3-mm-diam. plugs of different isolates were used for inoculation of stem bases of 1-year-old seedlings of beech and fir. Plugs were placed under the cut part of bark and covered with tape. After 12 and 28 days of incubation of inoculated plants on greenhouse bench at 19–27°C under plastic tunnel and RH about 92%–95% the length of necrosis was measured.

Experimental design was completely randomised with 4 replications and 5 plant parts or seedlings (greenhouse trials) in each replication. Trials were repeated 2–3 times.

Table 1. Fungi and fungi-like organisms isolated from diseased roots and stem bases of European beach seedlings; number of settled seedlings (a) and isolates obtained (b). Isolation: 2003.04.03–2003.10.26

Species	Nurseries											
	I.	12 plants	II.	17 plants	III.	11 plants	IV.	5 plants	V.	8 plants	VI.	6 plants
	a	b	a	b	a	b	a	b	a	b	a	b
Alternaria alternata Nees	8	27	–	–	1	3	4	9	4	10	5	11
Botrytis cinerea Pers.	–	–	3	7	–	–	1	1	–	–	–	–
Fusarium avenaceum (Fr.) Sacc.	–	–	11	18	–	–	–	–	3	7	–	–
Fusarium culmorum (W.G.Sm.) Sacc.	1	3	–	–	–	–	1	3	–	–	–	–
Fusarium oxysporum Schlecht.	3	8	–	–	–	–	–	–	–	–	3	7
Fusarium solani (Mart.) Sny. et Hans.	–	–	–	–	5	9	–	–	–	–	–	–
Mucor spp.	7	13	2	5	3	7	2	5	–	–	3	7
Mucor circinelloides van Tiegh.	–	–	1	4	–	–	–	–	–	–	–	–
Penicillium spp.	3	7	–	–	6	15	1	3	–	–	–	–
Phomopsis sp.	–	–	–	–	–	–	–	–	–	–	1	1
Phytophthora citricola Sawada	3	12	12	25	4	21	–	–	5	23	–	–
Pythium ultimum Trow	–	–	–	–	–	–	3	7	–	–	2	5
Rhizoctonia solani Kühn	–	–	–	–	–	–	–	–	–	–	2	5
Trichoderma spp.	3	7	3	5	7	19	5	11	–	–	–	–

Table 2. Fungi and fungi-like organisms isolated from diseased roots and stem bases of Silver fir; number of settled seedlings (a) and isolates obtained (b). Isolation: 2002.04.03–2003.10.26

Species	Nurseries															
	I. 18 seedlings 2-year-old		II. 8 seedlings 3-year-old		III. 18 seedlings 2-year-old		IV. 25 seedlings 2-year-old		V. 8 seedlings 2-year-old		VI. 20 seedlings 3-year-old		VII. 15 seedlings 1-year-old		VIII. 8 seedlings 1-year-old	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Alternaria alternata Nees	–	–	–	–	–	–	–	–	2	5	–	–	–	–	–	–
Chaetomium globosum Kunze	–	–	–	–	–	–	–	–	–	–	2	4	–	–	1	2
Fusarium avenaceum (Fr.) Sacc.	–	–	1	2	3	7	2	5	2	4	5	15	–	–	–	–
Fusarium culmorum (W.G.Sm) Sacc.	–	–	–	–	–	–	–	–	–	–	4	8	7	29	1	1
Fusarium equiseti (Cda) Sacc.	–	–	1	1	–	–	–	–	1	3	–	–	–	–	1	4
Fusarium oxysporum Schlecht.	9	12	2	4	4	28	–	–	–	–	–	–	2	5	–	
Mucor spp.	4	7	1	2	–	–	1	3	–	–	–	–	7	13	1	2
Penicillium spp.	–	–	–	–	3	3	2	4	–	–	–	–	6	13	–	–
Phomopsis sp.	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–
Phytophthora citricola Sawada	–	–	–	–	3	7	–	–	4	11	15	31	9	17	2	12
Pythium ultimum Trow	7	28	–	–	–	–	–	–	3	6	–	–	–	–	–	–
Rhizoctonia solani Kühn	–	–	–	–	–	–	15	42	–	–	–	–	3	5	–	–
Trichoderma spp.	15	47	–	–	9	27	–	–	5	12	2	6	3	7	3	7

RESULTS AND DISCUSSION

Fungi and fungi – like organisms isolated from European beech and Silver fir. Fourteen genera and species were obtained from diseased roots and stem parts of beech seedlings (Tab. 1). *P. citricola* was found on plants in 4 nurseries using both PDA and green apples. The species colonised most of diseased plants taken from the 2nd nursery. *Pythium ultimum* was detected from plants from 2 nurseries whereas *Rhizoctonia solani* only from one place. Both species are known as causal agents of seedlings damping-off. Four *Fusarium* species were isolated from plants only occasionally. *Trichoderma* species, known as antagonist of *P. citricola*, were isolated from analysed plants taken from 4 nurseries (Tab. 1).

Thirteen genera and species were isolated from diseased Silver fir (Tab. 2). *P. citricola* was isolated from diseased plants taken from 4 nurseries. The species colonised most of analyzed plants from 6th and 7th nurseries (Tab. 2). *P. ultimum* and *R. solani* were recovered from plants in 4 nurseries. *Fusarium* species, but especially *F. avenaceum* and *F. oxysporum* were found on plants in 5 and 4 places, respectively. *Trichoderma* species were found on plants from 5 nurseries (Tab. 2).

Colonisation of plant parts and stem base by isolates of *Phytophthora citricola*. Inoculation of leaves of European beech by isolates of *P. citricola* from 4 different plants resulted in the spread of necrosis about 2.5 mm/day (Tab. 3). On inoculated stem parts necrosis spread faster (about 3–4 mm/day) than on leaf blades. Isolate from *A. alba* caused the fastest spread of the disease symptoms, both, on leaves and stem parts of beech (Tab. 3). In greenhouse trial first small spots on stem bases were observed 2 days after inoculation and within 28 days necrosis spread on at least 25.8 mm of stem length (mean spread about 1 mm/day). Isolate from *F. sylvatica*, obtained from M nursery, caused significantly longer necrosis than from other plants (Tab. 4). After 8-day-incubation of needles and stems of *A. alba* the slowest necrosis spread was observed when isolate from heather was used for inoculation (about 2.6 mm/day). Beech isolate, obtained from R nursery, was more aggressive than others, especially to stem parts (Tab. 5). On stems of Silver fir, inoculated in greenhouse test, necrosis spread about 0.7 mm/day (Tab. 6). On plants inoculated with European beech isolate from M nursery, necrosis developed faster than on seedlings inoculated with other cultures (Tab. 6). In Werres (1995) study

Tabela 3. Colonisation of leaf blades and stem parts of European beech by different isolates of *Phytophthora citricola*; diam./length of necrosis in mm 4 (a) and 8 (b) days after inoculation
Inoculation: 2003.09.08

Source of isolates	Leaf blades		Stem parts	
	a	b	a	b
Abies alba	16.5 c	23.4 c	16.1 c	34.7 c
Calluna vulgaris	15.2 b	22.0 bc	15.4 b	31.5 b
Fagus sylvatica M	14.0 a	19.5 a	13.5 a	28.3 a
Fagus sylvatica R	15.0 b	21.2 b	16.3 c	34.9 c
Rhododendron sp.	14.1 a	20.0 a	15.0 b	32.1 bc

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

Table 4. Development of necrosis on stem base of 1-year-old European beech seedlings inoculated with different isolates of *Phytophthora citricola* (greenhouse trial)
Inoculation: 2003.07.18

Source of isolates	Length of necrosis (mm) after days of inoculation	
	12	28
Abies alba	11.3 a	26.5 a
Calluna vulgaris	10.9 a	25.8 a
Fagus sylvatica M	14.2 bc	30.2 d
Fagus sylvatica R	15.1 c	28.9 c
Rhododendron sp.	13.6 b	27.3 b

Note – see table 3

Table 5. Colonisation of needles and stem parts of Silver fir by different isolates of *Phytophthora citricola*; length of necrosis in mm 4 (a) and 8 (b) days after inoculation

Source of isolates	Needles		Stem parts	
	a	b	a	b
Abies alba	13.8 a	19.3 b	17.3 b	21.8 b
Calluna vulgaris	13.5 a	17.8 a	15.4 a	20.9 a
Fagus sylvatica M	14.7 ab	18.8 ab	16.8 b	22.2 b
Fagus sylvatica R	14.1 a	20.1 bc	16.2 ab	23.4 bc
Rhododendron sp.	14.0 a	19.5 b	16.1 ab	21.3 a

Note – see table 3

Table 6. Development of necrosis on stem base of 1-year-old Silver fir seedlings inoculated with different isolates of *Phytophthora citricola* (greenhouse trial)

Source of isolates	Length of necrosis (mm) after days of inoculation	
	12	28
Abies alba B	10.4 b	20.3 ab
Abies alba J	8.9 a	19.0 a
Calluna vulgaris	8.7 a	21.0 b
Fagus sylvatica M	11.3 bc	23.5 c
Rhododendron sp.	8.0 a	18.9 a

Note – see table 3

average of 90% of beech seedlings inoculated with *P. citricola* were damaged or dead. The author, however, used seedlings with fully developed cotyledons but undeveloped first foliage leaves whereas in our trials one-year-old plants were used. Additionally, the beech seed source influenced the development of seedling blight but had less effect than isolates tested (Werres 1995). In Shew and Benson (1981) trial with one-year-old Fraser fir, inoculation of seedlings already with 1×10^4 zoospores of *P. citricola*/cm^3, 70% infection was obtained and 40% of plants died after 10-day-incubation. Increasing of zoospore number resulted in the increase of seedling death. Microscopic and cultural examination of roots of some diseased plants by Jung and Blaschke (1996) showed that *Phytophthora* spp. are the main cause of progressive destruction of rootlets. Additionally *Phytophthora* spp. infect bark, roots

and collar and are able to induce tyloses in large xylem vessels thus reducing their conductivity for water and nutrients. Both, root decay and plugging of vessels are probably the main cause of seedling mortality of beech and fir in Polish nurseries. Production of phytotoxins by *Phytophthora* species can also play a role in symptom expression (Wood et al. 1972). In the opinion of Jung and Blaschke (1996) excess of nitrogen which reduced mycorrhiza, the frequent occurrence of mild-humid period during winter and springtime has favoured *Phytophthora* spp. development, increasing population and progressive destruction of roots. *P. citricola* is considered a warm-soil pathogen (Benson et al. 1976) and its occurrence in most of surveyed European beech and Silver fir nurseries in 2003 was probably connected with hot spring and summer. Mycological analyses of diseased plants showed the occurrence of other known plant pathogen like *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium* species. It is possible that they had a strong influence on the development of seedling blight, both on beech and fir. This is the first report of *P. citricola* in Polish forest nurseries.

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POLISH SUMMARY

PHYTOPHTHORA CITRICOLA W SZKÓŁKACH LEŚNYCH BUKA ZWYCZAJNEGO I JODŁY POSPOLITEJ

Gatunek *Phytophthora citricola* izolowano z większości szkółek leśnych buka i jodły z objawami zgnilizny korzeni i podstawy pędu. Obok tego patogena z porażonych tkanek wyosobniano inne grzyby patogeniczne z rodzaju *Fusarium* oraz *Pyhtium ultimum* i *Rhizoctonia solani*. Izolaty *P. citricola* z buka i jodły oraz z wrzosa i różanecznika kolonizowały liście i igły oraz części łodyg buka i jodły. W doświadczeniu szklarniowym, na zakażonych u nasady 1-rocznych siewkach nekroza rozwijała się około 2 mm/dobę na buku i 1,4 mm na jodle.