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PHYTOPHTHORA CAMBIVORA ON *ALNUS GLUTINOSA*: ISOLATION AND COLONISATION OF PLANTS

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Abstract: *Phytophthora cambivora* was isolated from the bark lesions of two 10- and 15-year-old of analysed alder trees. Additionally, *Botrytis cinerea*, 3 *Fusarium* species, *Mucor* spp., *P. alni* and *Trichoderma* spp. were recovered from diseased tissues. Isolates of *P. cambivora* from six plant species, used for inoculation of alder seedlings and plant parts, caused the development of necrosis. Isolate from *Chamaecyparis lawsoniana* was the weakest pathogen whereas those from *Abies alba*, *Acer pennsylvanicum* and *Alnus glutinosa* were the strongest.

Key words: *Phytophthora*, alder, isolation, bark lesions, seedling, plant parts, pathogenicity

INTRODUCTION

Phytophthora cambivora (Petri) Buisman known already in 1917 as *Blepharospora cambivora* is the causal agent of seedling blight, root rot, collar and root rot, trunk cancer, ink disease and wilt of 30 plant species including *Acer pennsylvanicum* L., *Castanea* spp., *Fagus sylvatica* L., *Juglans* sp., *Malus* spp., *Prunus* spp. and *Rhododendron* sp. (Erwin and Ribeiro 1996). In Poland the pathogen was detected from diseased trunk of *Acer pennsylvanicum* L., bases of rotted stems of *Abies alba* Mill., *Chamaecyparis lawsoniana* (And.) Parl. and *Cotoneaster horizontalis*. Dcne., grown in container ornamental nurseries (Orlikowski et al. 2002; Orlikowski and Szkuta, nonpubl.). In Germany the species was detected from diseased trunks of common alder (*Alnus glutinosa* L. Gaertn.) and from alder rhizosphere (Hartmann 1995; Von Schumacher 2003). The pathogen was found in nursery where 3 species of alder were grown, including *A. glutinosa*. *P. cambivora* was isolated from diseased oak and beech stands (Jung et al. 2000) and the species was the most aggressive to root systems of young plants (Jung and Blaschke 1996, 2004). In biotests, several *Phytophthora* species re-

leased toxic substances into their culture medium that induced wilting and intercostal chlorosis and leaf necrosis of oak (Jung et al. 2000).

This paper presents preliminary results of isolation of *Phytophthora cambivora* from *Alnus glutinosa* in south-east part of Poland and pathogenicity of its isolates from different host plants toward alder.

MATERIALS AND METHODS

Survey of alder diseased trees. Survey was conducted between late of September and October 2004. On some trees 10 to 30-year old crown rot symptoms enlarging on trunks, usually on one side were observed. On 5 trees black, oval or irregular spots with tarry exudations up to 80 cm from soil level were seen. Six trees had small, yellow and sparse leaves described earlier by Brasier et al. (1995), Gibbs (1995) and Orlikowski et al. (2003).

Isolation and identification of fungi and *Algae* like *Oomycetes* from diseased trees. Samples were collected from diseased, darkbrown lesions at the bases of trunks. From one tree samples from 2–4 lesions were taken at the outer edge of spots, collected in plastic bags and transferred to laboratory. The same or next day the bark was removed from samples and they were washed under running tap water 4 hrs and after that dried in sterile blotting paper. Individual samples were disinfected over a burner flame and fragments of ca 5 mm were put on Difco potato-dextrose agar (PDA) in 90 mm Petri dishes (six–nine inocula per dish). Within 5-day-incubation of plates at 22–24°C colonies grown around the inocula were transferred into PDA slants. Additionally apple fruits were used as the bait for isolation of *Phytophthora* spp., using procedure described by Szkuta (2004). After segregation, chosen isolates were identified using available monographs. *Phytophthora* spp. were identified to species on the base of morphological characteristics and with molecular methods (Szkuta 2004; Wiejacha et al. 2004).

Colonisation of alder seedlings and plant parts by isolates of *Phytophthora cambivora*. Pathogenicity of six isolates from *A. alba, A. pennsylvanicum, A. glutinosa, Castanea sativa, Chamaecyparis lawsoniana* and *Cotoneaster horizontalis* (Tables 2–4) was evaluated. In laboratory trials 2-week-old seedlings of common alder were inoculated on the border of stem and root with 3 mm diameter disks of tested isolates, taken from 7-day-old colonies grown on V8 juice agar at 24°C. Seedlings were incubated on moist, sterilized blotting paper covered with plastic net in polystyrene boxes, covered with foil. After 3 and 5 day-incubation at 24°C length of necrosis was measured. In the second trial, one-year-old stem parts and leaf blades were inoculated with 6 isolates. The same procedure as in the previous trial was used. Diameter and length of necrosis was measured after 5-day-incubation. In greenhouse trial peat was infested with 6 isolates of *P. cambivora* (Orlikowski 1995) and population densities was established on levels 250–310 colony forming units/g, using gallic acid selective medium (Flowers and Hendrix 1969). After 20-day-incubation at 20–27°C 1 dmł pots were filled with infested substratum and 10 seeds were sown to each of them. Control seeds were sown to noninfested peat. After 18 and 26-day-incubation of pots on greenhouse bench at 18–23°C number of diseased seedlings were counted.

Experimental design was completely randomised with 4 replications and 5 seedlings or plant parts in each rep. In greenhouse trial 50 alder seeds were sown in each replication. Experiments were repeated twice.

RESULTS AND DISCUSSION

Fungi and *Algae* like *Oomycetes* isolated from diseased trees. Eleven genera and species were recovered from twelve diseased alder trunks (Table 1). *Botrytis cinerea*, *Mucor* spp. *Penicillium* spp. and *Trichoderma* spp. dominated in diseased trunk tissues. Also *Fusarium* species, recovered earlier from diseased trees in Poland and France by Orlikowski et al. (2003) and Streito et al. (1999), were found in bark lesions. Using Difco PDA *Phytophthora alni* was isolated from 1/4 of analysed trees whereas *P. cambivora* from 1/6 of trunks with necrotic spots on their bases (Table 1). The last mentioned species was recovered from 10- and 15-year-old of alder trees. Additionally, using apple bait, *P. alni* was detected from 1/3 of trees whereas *P. cambivora* from 1/6 of analysed trunk tissues. It indicates that both, PDA and apple fruits were available media for isolation of *Phytophthora* spp. from diseased alder trees. Results of mycological analyse of diseased alder trees indicate on the first recovering of *P. cambivora* from *A. glutinosa* in Poland. In previous 3 year study no *Phytophthora* or only *P. alni* was recovered from tree parts taken from at least hundred riparian and forest stands in Poland (Orlikowski et al. 2003; Oszako and Orlikowski 2004). The pathogen was not also isolated from diseased bark lesions by Brasier et al. (1995) and Jung and Blaschke (2004). Von Schumacher (2003), however, isolated the species from diseased cambium of alder trunk among 3 other *Phytophthora* spp. Studies of Brasier et al. (1995) indicated on similarity of *P. alni* and *P. cambivora*. Especially zoosporangia are morphologically similar. In contrast *P. alni* has optimum temperature for growth of ca 22.5–25°C whereas *P. cambivora* 27.5–30°C (Brasier et al. 1995). In authors study *P. alni* exhibited an upper limit of growth ca 29–30°C whereas that for *P. cambivora* was 33–34°C. High requirements of *P. cambivora* to temperature probably limited its occurrence on bark lesions of alder. The pathogen has been recovered, however, from diseased bases of some ornamental plants grown in black containers in which temperature is usually higher than in soil (Orlikowski et al. 2002, Orlikowski and Szkuta, nonpubl.).

Table 1. Fungi and *Algae* like *Oomycetes* isolated from 12 diseased, 10–30-year-old *Alnus glutinosa* trees; number of settled trees (A) and isolates obtained (B). Isolation: autumn, 2004

Genera/species	A	B
Alternaria alternata Nees	7	13
Botrytis cinerea Pers.	9	22
Chaetomium globosum Kunze	3	6
Fusarium culmorum (W.G.Sm.) Sacc.	4	11
Fusarium equiseti (Cda) Sacc.	2	6
Fusarium solani (Mart.) Sny. et Hans.	3	7
Mucor spp.	10	25
Penicillium spp.	6	9
Phytophthora alni Brasier and Kirk	3	10
Phytophthora cambivora (Petri) Buisman	2	7
Trichoderma spp.	6	15

Colonisation of alder seedlings and plant parts by isolates of *P. cambivora*. Inoculation of 2-week-old alder seedlings by 6 isolates of *P. cambivora* resulted in the spread of lesions already 24 hr after inoculation and after 5 days the length of necrosis varied from18.5 to 24.4 mm (Table 2). There were no significance differences in the length of

Table 2. Colonisation of 2-weeks-old *Alnus glutinosa* seedlings (n=5) by isolates of *Phytophthora cambivora* from different host plants; laboratory trial

Source of isolate	Isolate number	Length of necrosis (mm) after days of inoculations	
		3	5
Abies alba	PO138	15.0 c	23.3 a
Acer pennsylvanicum	PO028	15.8 c	24.3 a
Alnus glutinosa	PO360	14.3 bc	24.0 a
Castanea sativa	PO065	9.8 ab	18.5 a
Chamaecyparis lawsoniana	PO003	9.0 a	18.5 a
Cotoneaster horizontalis	PO155	13.0 bc	23.8 a

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

lesions. Such differences were observed, however, on inoculated alder parts (Table 3). Spots did not developed on leaves inoculated with isolate from *Castanea sativa* or necrosis spread very slowly on leaf blades treated with isolate from *Chamaecyparis lawsoniana*. The quickest spread of leaf spot was observed when isolates from *Acer pennsylvanicum* and *Alnus glutinosa* were used for leaves inoculation. All tested isolates caused stem rot but the quickest necrosis spread was observed when tissues were inoculated with isolates from *Abies alba* and *A. pennsylvanicum* (Table 3). In greenhouse trial only isolate of *P. cambivora* from *Ch. lawsoniana* did not caused necrosis of alder seedlings (Table 4). The quickest spread of disease was observed on alder seedlings grown in peat infested with isolates from *A. alba, A. pennsylvanicum* and *C. sativa* (Table 4). Results from 3 trials showed that *P. cambivora* from Lawson cypress was the weakest isolate whereas those from fir maple and alder were the most pathogenic. In forest or ornamental nurseries, growing of different plant species, may be connected with transmission of *Phytophthora* spp., including *P. cambivora*, especially in soil. Brasier et al. (1995) showed that on 5-year old alders, *P. cambivora* caused the development of lesions (1.05 cm/week) marked by a depression in the bark and by production of black exudate. The pathogen may cause destruction of rootlets, necrosis of bark and collar rot. It is able to induce tyloses in large xylem vessels reducing its conductivity for water and nutrients (Jung and Blaschke 1996).

Table 3. Colonisation of *Alnus glutinosa* leaf blades and stem parts (n=5) by isolates of *Phytophthora cambivora* 5 days after inoculation; laboratory trial

Source of isolates	Isolate number	Diam/length of necrosis (mm)	
		Leaves	Stem parts
Abies alba	PO138	19.8 c	22.0 c
Acer pennsylvanicum	PO028	36.5 e	18.0 bc
Alnus glutinosa	PO360	29.3 d	11.5 a
Castanea sativa	PO065	0 a	15.3 ab
Chamaecyparis lawsoniana	PO003	4.5 a	10.6 a
Cotoneaster horizontalis	PO155	12.5 b	21.4 c

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

Table 4. Colonisation of *Alnus glutinosa* seedlings (n=50) in peat artificially infested by isolates of *Phytophthora cambivora* from different host plants; greenhouse trial

Source of isolates	Isolate number	Days after sowing of seeds	
		18	26
Control, non infested peat	–	3.5 a	6.0 a
Abies alba	PO138	24.8 d	35.3 d
Acer pennsylvanicum	PO028	17.3 c	32.8 d
Alnus glutinosa	PO360	19.0 bc	27.8 c
ctlpar*Castanea sativa*	PO065	22.5 cd	34.0 d
Chamaecyparis lawsoniana	PO003	2.8 a	4.3 a
Cotoneaster horizontalis	PO155	11.0 b	19.0 b

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

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POLISH SUMMARY

PHYTOPHTHORA CAMBIVORA NA *ALNUS GLUTINOSA*: IZOLACJA I KOLONIZACJA ROŚLIN

Phytophthora cambivora wyizolowano z dwóch 10- i 15-letnich drzew z nekrotycznymi plamami na korze u podstawy pni w południowo-wschodniej części Polski. Dodatkowo z porażonych tkanek uzyskano *Botrytis cinerea*, trzy gatunki rodzaju *Fusarium*, *Mucor* spp., *Phytophthora alni*, *Penicillium* spp. i *Trichoderma* spp. W minionych 3 latach przeprowadzono analizę mikologiczną porażonych tkanek pni olszy, pochodzących z co najmniej 100 stanowisk nadrzecznych i leśnych i jest to pierwsze stwierdzenie *P. cambivora* na *Alnus glutinosa* w Polsce. Izolaty tego gatunku, pochodzące z sześciu roślin żywicielskich, powodowały zgniliznę siewek liści i fragmentów jednorocznych pędów. Izolat z *Chamaecyparis lawsoniana* najwolniej kolonizował tkanki olszy natomiast wyosobnienia tego gatunku z *Abies alba, Acer pennsylvanicum* i *A. glutinosa* powodowały najszybszy rozwój nekrozy na zainokulowanych tkankach.