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Dieback of *Pieris japonica* caused by *Phytophthora citrophthora*

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Phytophthora citrophthora (Sm. et SM.) Leonian was mainly isolated from rotted twigs of pieris. The species used for artificial inoculation of leaf petiols and shoots of pieris, azalea and rhododendron spread very fast, especially on leaves coming from the top of the plants. In greenhouse trial, plants grown in peat, artificially infested with the species, showed discoloration and wilt symptoms already 2–4 weeks after planting.

Key words: twig, isolation, inoculation, *Phytophthora*, pathogenicity.

INTRODUCTION

Gerlach et al. (1974) are of the opinion that *Phytophthora* spp. are among the most serious soilborne pathogens of pieris [*Pieris japonica* (Thunb.) D. Don]. In the years 2000–2001 severe losses occurred in ornamental plants nurseries where plants were grown in containers under covering and in open field. Blighting of young leaves on individual twigs (Fig. 1) or plants (Fig. 2) were observed usually at the end of summer. Subterranean infection usually extended up the main stem and branches.The disease spread from single pierises sometimes onto 5–20 neighbouring plants during the vegetation period. Sprinklers irrigation, wind splushing, transport of *Phytophthora* by insects, snails and rodents resulted in fast spread of the pathogen. On fallen, rotted leaves zoosporangia production was often observed. Streams of water droplets falling onto the zoosporangia were able to disperse them from affected leaves. During the first routine isolation on apple fruits, *Phytophthora* sp. was the fungus most often isolated from diseased twigs.

Gerlach et al. (1974) reported that the dieback of pieris could be attributed to a number of causes including *Botryosphaeria dothidea*, *Phyto-*

phthora citricola and *P. citrophthora* whereas Robertson (1970) mentioned also *P. cinnamomi.*

In this paper we report *Phytophthora citrophthora* (Sm. et SM.) Leonian as a new pathogen of *Pieris japonica* in Polish nurseries.

MATERIALS AND METHODS

Isolation of fungi from diseased plants. Affected plants collected in August 2000–2001 in 2 ornamental plants nurseries were put individually to plastic bags and transported to the laboratory. After removing of roots, pieris twigs were washed in tap water, blotted dry with paper towels and surface sterilised over a burner fire. About 5 mm in diameter fragments of tissues, taken from the border of healthy and invaded stems were put on the surface of potato-dextrose-agar (PDA) in 90 mm Petri dishes. Tissue parts from each analysed plant were plated on 3 dishes (6 pieces/plate) and incubated in the dark at 25°C. Grown colonies were transferred into PDA slants. After separation and cleaning of fungal cultures, they were identified to species using available monographs and keys.

In vitro and *in vivo* estimation of *Phytophthora citrophthora* pathogenicity towards pieris, azalea and rhododendron. The isolate PJ/1/00, obtained from rotted twig of pieris was used in all trials. Stock culture was maintained on PDA at 25°C. Five mm in diameter mycelial disks, taken from the edge of 7-day-old cultures were used for inoculation of shoots and leaf petioles (Orlikowski 1996). Inoculated organs were incubated in moist chambers at 25°C. After 2 and 4-day-incubation the length of necrosis was measured. In 2 greenhouse trials development of disease symptoms was observed on plants grown in artificially infested peat. The fungus grown 2 weeks on rolled Quick oats was blended with minimum of distilled water and the thick slurry was mixed with peat. The population density of the species was estimated using gallic acid selective medium (Orlikowski 1999) on the level 220 colonies forming units (cfu)/g of air dry peat. After 2-week-storage at 22°C one dm^3 pots were filled with infested peat and pierises at the stage of 2–3 shoots were planted. Control plants were planted in noninfested peat. Plants were grown on greenhouse bench at the temperature range from 17° to 28°C. During 10-week-growth development of disease symptoms was observed. Plants with first discoulration of leaves or wilt symptoms were examined for the presence of *P. citrophthora* in an invaded tissue.

Experimental design was completely randomised with 4 replications with 10 leaves, shoots or plants in each one. Trials were repeated twice.

Fig. 1. Fall down of pieris leaves infested with *Phytophthora citrophthora*

Fig. 2. Dieback of pieris infested with *P. citrophthora*

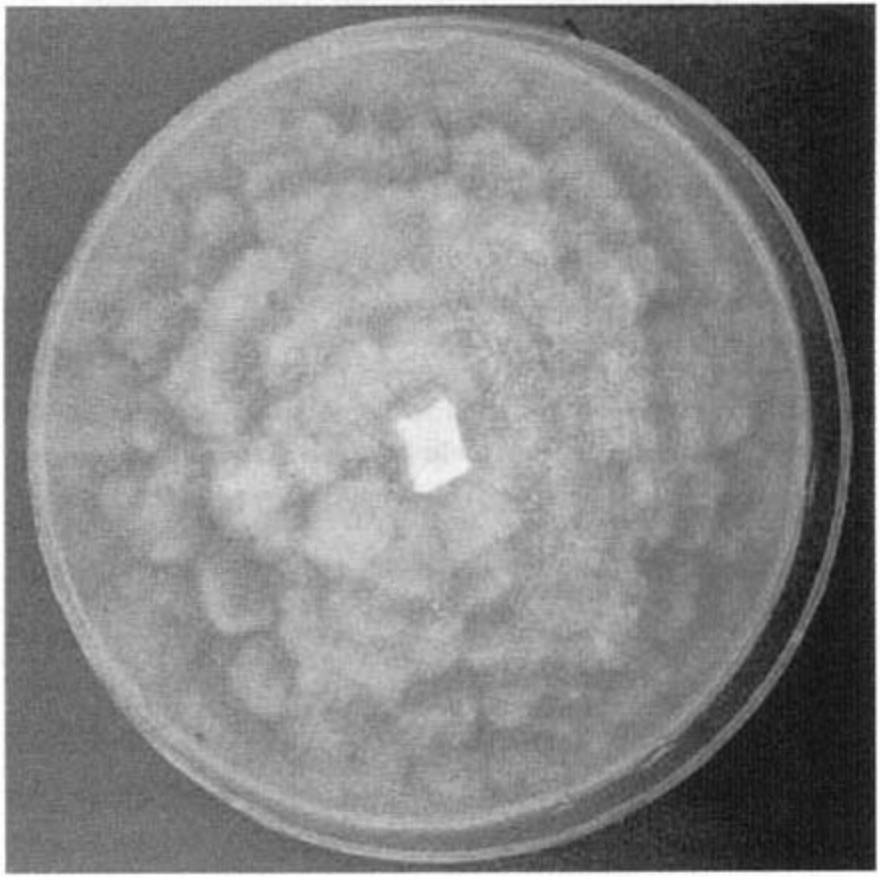

Fig. 3. Seven-day-old *P. citrophthora* on potato-dextrose agar

RESULTS AND DISCUSSION

Fungi isolated from diseased pieris. Only 5 species were isolated from diseased twigs of plants examined (Table 1). Such small number of fungal species was probably connected with sterilisation of plant parts over a burner fire. In both years *Phytophthora citrophthora* dominated in diseased tissues. The species was isolated from most plants tested and analysed tissue parts (Table 1). *Botrytis cinerea*, also the pieris pathogen (Łabanowski et al. 2001), occurred on plants analysed in both years but especially in 2001 (Table 1). Unidentified *Pestalotia* species was also isolated in both years. Fungi from the genus *Pestalotias* are known to be leaf pathogens but they can colonise weak or already diseased plant parts (Coyier and Roane 1988). Our trials (Orlikowski and Szkuta unpubl.) with inoculation of pieris and rhododendron leaves with *Pestalotia* sp. have not indicated on that fungus as a potential pathogen. *Chaetomium globosum* and *Trichoderma* sp. were isolated from diseased plants rarely or sporadically (Table 1).

Table 1
Fungi isolated from diseased twigs of *Pieris japonica* in years 2000 (A) and 2001 (B)

Species	A (32 plants)		B (25 plants)	
	number of settled plants	number of isolates	number of settled plants	number of isolates
Botrytis cinerea Pers.	3	7	7	9
Chaetomium globosum Kunze	2	3	–	–
Pestalotia sp.	9	21	4	12
Phytophthora citrophthora (Sm. et Sm.) Leonian	28	111	22	94
Trichoderma sp.	4	6	3	4

Morphology of *Phytophthora citrophthora*. On V8 agar and PDA the hyphae were smooth and only sometimes coarse, 4–7 μm in diameter. The culture patterns were rosette or stellate, both on V8 and PDA (Fig. 3). The growth of the species colony was observed at the temperature range from 5° to 35°C with optimum at 23°C (radial growth rate about 1/5 mm/hr). In sterilised, 1% soil leachate, zoosporangia (Fig. 4) born singly on irregularly branched sporangiophores or in loose sympodia were observed after 48 hr incubation at 23°C. They were papillate, persistent and not caducous, spherical, ovoid or ellipsoid, 20–40 × 16–36 μm (average 31.2 × 24.1 μm). The decrease of temperature resulted in releasing of zoospores from zoosporangia (Fig. 5). Chlamydospores and oospores were not observed.

Pathogenicity of *Phytophthora citrophthora*. In the laboratory trials, inoculation of leaf petioles of pieris with the fungus

resulted in the fast development of necrosis (Table 2). The spread of petiols rot was significantly faster on leaves taken from the top of shoots than from the base. The spread of necrosis on inoculated shoots was like on the leaves from the base of pieris (Table 2). Similar pattern of the development of necrosis on tested leaves was observed on azalea and rhododendron. On the shoots of rhododendron the necrosis development was significantly slower than on the leaf petioles (Table 2).

Table 2

Development of necrosis on pieris, azalea and rhododendron leaves and shoots inoculated with *Phytophthora citrophthora*; length of necrosis 4 days after inoculation

Plant part	*Pieris*	*Azalea*	*Rhododendron*
Top leaves	28.2 b	46.4 b	50.5 c
Leaves from the base of plant	21.6 a	26.0 a	42.4 b
Shoots	28.8 b	22.6 a	29.5 a

Explanations: means in columns, followed by the same letter, do not differ with 5 level of significance (Duncan's multiple range test).

In the greenhouse trials pieris was planted into the infested peat at 24-day-intervals. In the first experiment discolouration of leaves into green-yellow colour was already observed after 2-week-growth and within the next 14 days 1/4 of plants tested in each replicate showed leaf discoloration or wilt symptoms (Table 3). About the half of plants tested wilted or died after 2-month-growth (Table 3). In the second trial the development of disease was much faster than in the first experiment (Table 3). 2/5 of plants wilted or died already after 4-week-growth whereas after 10 weeks most of them showed *Phytophthora* rot, both on twigs and some leaves. Faster development of disease symptoms on pieris in the second trials was probably connected with higher substratum temperature, optimal for the pathogen growth and sporulation. Plants cultivated in noninfested peat did not show any disease symptoms.

Table 3

Development of *Phytophthora* rot on pieris grown in the infested peat; number of plants (n = 10) with disease symptoms

Weeks after planting	Planting time	
	2001.07.04	2001.07.28
2	0.75 a	0.75 a
4	2.5 b	4.0 b
8	5.5 c	7.0 c
10	7.5 d	8.5 cd

Explanations: see Table 1

The present paper is the first report of *P. citrophthora* from pieris in Poland. The pathogen has been already reported on rotted shoots of *Radermachera*

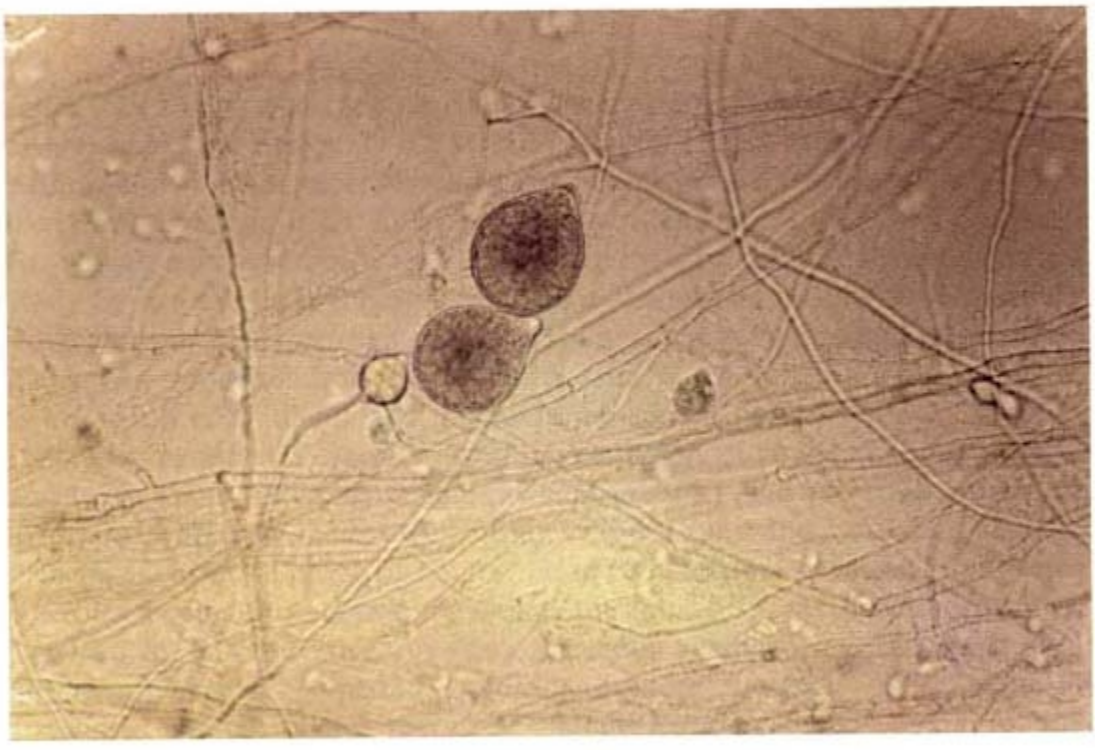

Fig. 4. Young and mature zoosporangia of *P. citrophthora*

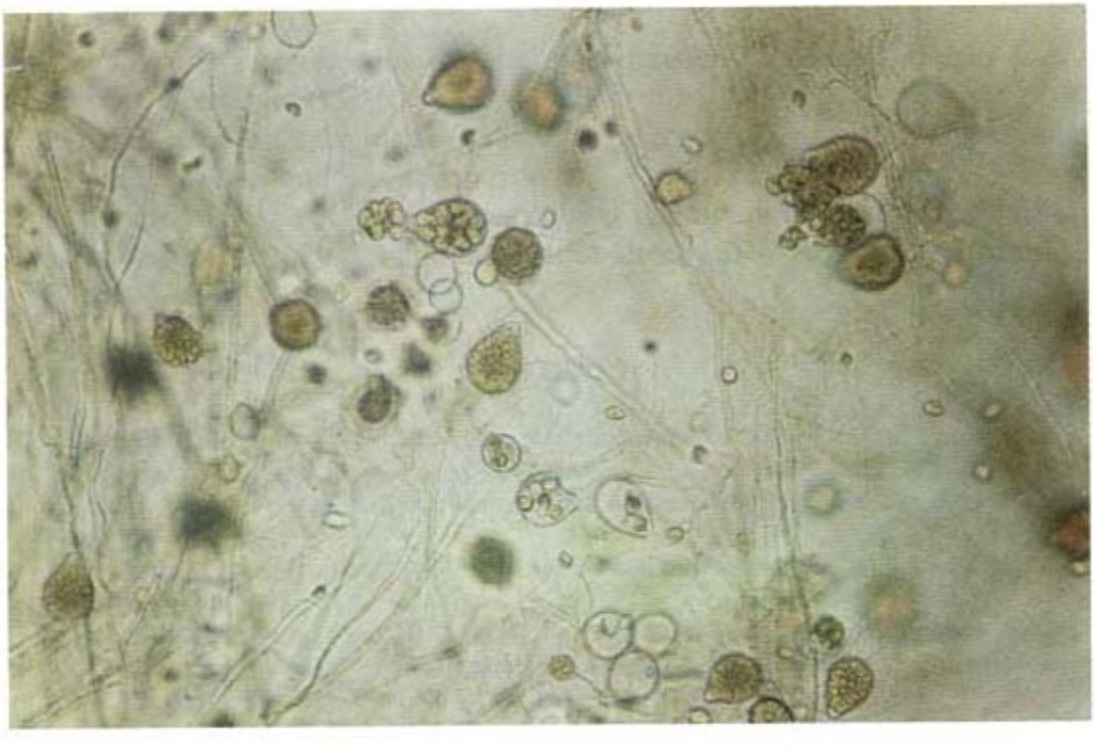

Fig. 5. Zoosporangia of *P. citrophthora* releasing their zoospores

sinica (O r l i k o w s k i et al. 2001). The species is known as a pathogen of rather wide host range. N o v o t i e l n o v a (1974) announced it as the pathogen of 48 plant genera from 28 families whereas E r w i n and R i b e i r o (1996) described it on 83 species. S m i t h and S m i t h (1906) described the fungus, isolated from rotted lemons, as *Pythiacystis citrophthora*. Further study by L e o n i a n (1925) showed that the species should be clasified as *Phytophthora*. The species is mainly known as the minor pathogen of citrus, pieris and rhododendron (H o i t i n k and S c h m i t t h e n n e r 1974; G e r l a c h et al. 1974). On the container grown pieris G e r l a c h et al. (1974) distinguished 3 types of symptoms: a blight of young succulent foliage and twigs, spots on leaves of intermediate maturity and root and crown rot associated with twig dieback. According to B e n s o n and J o n e s (1980) the dieback may be one phase of *Phytophthora* complex that develops in nurseries when the temperature is near 30°C and the rainfall or overhead irrigation is frequent. In 2 Polish ericaceus plant nurseries overhead irrigation was used during all vegetation period. Additionally, the substrate temperature in containers is often higher than 25°C. Under such epidemic conditions, the incidence of *Phytophthora* disease on delicate, fast growing twigs of pieris reached a level even 40%.

CONCLUSIONS

1. *Phytophthora citrophthora* was the most frequently isolated species from diseased pieris twigs.
2. In the *in vitro* trials the fungus caused the necrosis of leaf petiols and shoots of pieris, azalea and rhododendron.
3. In the greenhouse trials the fungus caused the discoloration of pieris leaves as well as rotting of twigs and leaf blades already 2–4 weeks after planting.

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Phytophthora citrophthora jako przyczyna zamierania pierisa

S t r e s z c z e n i e

Niekiedy masowe zamieranie pierisa w pojemnikowej uprawie w szkółkach było powodem rozpoczęcia badań nad określeniem przyczyny choroby. W grupie grzybów wyizolowanych z porażonych pędów pierisa dominował gatunek *Phytophthora citrophthora*. Zakażenie ogonków liściowych i pędów pierisa, azalii oraz różanecznika izolatem tego gatunku powodowało bardzo szybkie gnicie tych organów. Nekroza rozwijała się najszybciej na liściach pobranych z wierzchołków pędów. W doświadczeniu szklarniowym, na pierisie rosnącym w substracie torfowym zakażonym przez *P. citrophthora*, zmiana zabarwienia liści oraz więdnięcie i zamieranie pędów obserwowano już po 2–4 tygodniach uprawy.