

## WATER AS POTENTIAL SOURCE OF *PHYTOPHTHORA CITRICOLA*

Leszek B. Orlikowski\*, Aleksandra Trzewik, Teresa Orlikowska

Research Institute of Pomology and Floriculture  
Pomologiczna 18, 96-100 Skierniewice, Poland

Accepted: May 30, 2007

**Abstract:** *Phytophthora citricola* constituted about 70% of all *Phytophthora* isolates recovered from rhododendron leaves used as baits for detection of that group of organisms in water. The species was found in 4 rivers, 2 hardy nursery water reservoirs and nursery drainage canal from May to October, 2006. Analysis of spots' number on rhododendron leaf baits as a measure of *P. citricola* density showed that place of holding baits had a significant influence on the species occurrence. Significantly more spots, especially in July survey, were observed on baits held in Skierniewka and Zwierzynka rivers swimming through agricultural and forest area than in Ner, the river of horticultural area. Significantly more spots/rhododendron leaves were observed when they were held in rivers downstream of nursery and in the middle of hardy nursery borders. In nursery water containers and drainage canal higher *Phytophthora* density was recorded in August than in other periods of surveying. Using water from reservoir for sprinkling of *Picea omorika* nursery trees caused the development of tip blight and from diseased twigs *P. citricola* was isolated.

**Key words:** river, nursery reservoir, canal, rhododendron bait, recovery, *Phytophthora*, occurrence

### INTRODUCTION

In the middle of summer 2005 in 2 hardy ornamental nursery stocks twig blight of *Picea omorika* (Panić) Purk., *Thuja occidentalis* L. and *Rhododendron* spp. was observed. Similar symptoms were also previously noticed on *Calluna vulgaris* (Orlikowski and Szkuta 2002). The occurrence of the disease was preceded by at least 2 days of drizzling rain and temperature 18–22°C. Mycological analysis of affected twig fragments showed the occurrence of *Phytophthora citricola* Sawada. The species had been previously noticed in nurseries on coniferous and some ericaceous plants (Orlikowski 2006a, b; Orlikowski and Szkuta 2003a, b). Several studies performed during past decades have indicated on water as the source of *Phytophthora* spp. Hunter and Kuni-

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\*Corresponding author:

Research Institute of Pomology and Floriculture,  
Pomologiczna 18, 96-100 Skierniewice, Poland, 96-100 Skierniewice, Poland,  
leszek.orlikowski@insad.pl

moto (1974), Keim et al. (1981) and Shea et al. (1983) concluded that periods of rainfall and sprinkler irrigation of plants, susceptible to *Phytophthora* spp., enhance conditions which encourage disease to become epidemic. Oudemans (1999) reported that *P. cinnamomi* Rands was widely distributed throughout streams, irrigation reservoirs and drainage canals in cranberry cultivation whereas the occurrence of *P. megasperma* Drechsler was more restricted. Themann et al. (2002) isolated from water reservoirs of German hardy ornamental nurseries at least 12 *Phytophthora* species with domination of *P. gonapodyides* (Petersen) Buisman, *P. drechsleri* Tucker, *P. cryptogea* Pethybr. et Laff. and *P. citricola* Sawada. *Phytophthora* spp. were detected during all seasons but most of them were found in November. Hansen and Delatour (1999) recovered from streams, puddles and forest shallow ditches mainly *P. gonapodyides* but *P. citricola* and *P. megasperma* were also present. The objectives of this study was to determine an occurrence of *Phytophthora citricola* in rivers, nursery water reservoirs and drainage canal in relation to sampling time and place of baiting.

## MATERIALS AND METHODS

### Sampling of water

Sampling for *Phytophthora* was carried out from May to October, 2006 in 4 rivers (Ner, Korabiewka, Skierniewka, Zwierzynka), 2 nursery water reservoirs and nursery drainage canal by baiting technique (Hansen and Delatour 1999). The first river swims through horticultural area including farms under covers and hardy ornamental nurseries whereas 3 others run through agricultural area and forests. Young rhododendron twigs at the stage of 4–6 leaves of cv. Nova Zembla from the plant tops were immersed in water about 1 m from the bank of rivers or nursery water reservoirs and held for 4–6 days (longer period in autumn). After that time leaves were removed from water, put in sterile plastic bags and transported to the laboratory. The same or next day they were rinsed under tap water and again in distilled water and blotted dry. On each of leaf blade number of necrotic spots were counted. Chosen leaf parts were sterilised over a burner flame, cut into about 3–5 mm diam. pieces and placed on PDA medium (8 pieces/90 mm diam. Petri dish). Plates were incubated in the dark at 24°C and examined daily for the presence of *Phytophthora* colonies. Small parts of cultures growing around leaf parts were transferred to PDA slants. After 7–10 days the obtained isolates were grouped by growth pattern and morphology into species types and representative cultures were chosen for further identification.

### DNA identification of *Phytophthora* species

Confirmation of classification to species was performed by DNA analysis. All the isolates were collected as pure cultures and DNA was extracted using the method of Aljanabi and Martinez (1997), modified by Wiejacha et al. (2002). We established a procedure in which PCR with non-specific primers – RADP and ISSR, were used to generate DNA profiles characteristic of 14 *Phytophthora* species, including *P. cinnamomi* and *P. citricola*. The primers RADP – C85, C92 (Lee et al. 1996) and ISSR – 808, 827, 889, 890 (University of British Columbia – UBC) were most useful (Trzewik et al. 2006). This first step was followed by confirmation of results using PCR with species-specific primers or the PCR-RFLP method, as described by Cooke and Duncan (1997).

Experimental design was completely randomized with 4 replications and 4 rhododendron leaves in each rep. Each time of water sampling baits were held in the same place of rivers, water reservoirs and nursery canal. Leaf parts from each replication were placed in 4 Petri dishes and number of *Phytophthora* colonies were counted on particular plates.

### **Relationship between places of baits holding in rivers, surveying time and number of *Phytophthora* spots on rhododendron leaves**

Two commercial hardy nurseries, which constituted the borders from one side of rivers were chosen for the study of relationship between *Phytophthora* spp. density in water, places of baits holding and surveying periods. Rhododendron leaf baits were placed in water in the first decades of May and October 10 m upstream the nursery borders, in the middle length and 10 m downstream of cultivated areas. In the first nursery coniferous and ericaceous plants were grown whereas in the second only ericaceous plants, mainly rhododendrons. Number of spots/rhododendron leaves were counted after 5 days of baits incubation in water. Additionally, *Phytophthora* species which settled the necrotic tissues were isolated and identified.

### **Surveying of twig blight spread on *Picea omorika***

Three-year-old plants were grown outdoors on container area covered with black mat. In the previous year disease symptoms were not observed on them. Plants were watered by sprinkling and during hot summer days water was supplied even 4 times a day. Nursery reservoir was the source of water. First browning of needles on some twigs was observed after 3-day-drizzling rain time and temperature about 20°C. On each plant number of diseased twigs was observed and number of spruce with such symptoms in each replication were counted during 3 observation dates. Experimental design was completely randomized with 4 replications and 20 spruces in each rep.

## **RESULTS AND DISCUSSION**

### **Recovery of *Phytophthora* spp. from rivers, reservoirs and canal water**

Rhododendron leaves were effective baits for isolation of *Phytophthora* species, mainly *P. citricola*. Benson (1980) and Kuske and Benson (1983) reported 8 *Phytophthora* species that caused foliar dieback root and stem rot diseases of hybrid rhododendron. Additionally *P. ramorum* was described by Werres et al (2001) and *P. kernoviae* by Brasier et al. (2005) as new rhododendron pathogens. *P. citricola* was recovered from water of 4 rivers between July and first decade of October 2005 from about 70% of leaf spots (Table 1). The most of necrotic spots on rhododendron leaf baits with the presence of *Phytophthora* spp., were observed in July and August. In July more spots were noticed on leaf baits placed in Skierniewka and Zwierzynka rivers than in Korabiewka and Ner (Table 1).

*Phytophthora* spp., mainly *P. citricola*, were present in both hardy nursery reservoirs surveyed from July to September (Fig. 1). Significantly more spots/leaf baits were observed in August (Fig. 1) but *Phytophthora* spp. was still present in water in December (Orlikowski et al. unpubl.).

Using of rhododendron leaves gave possibility to detect *P. citricola* from nursery drainage canal from June to October with the domination of the species in July

(Fig. 2). The pathogen was also sporadically found on diseased top leaves of rhododendron growing in surveyed hardy nursery. Probably such invaded, fallen leaves, infested substratum parts and/or zoospores were transported with surplus sprinkled or rainfall water to the drainage canal.

Table 1. Occurrence of *Phytophthora* spp., mainly *P. citricola*, in river water in relation to surveying time

Rivers	Mean number of necrotic spots/rhododendron leaf in surveying time			
	2005.07.12	2005.07.29	2005.08.11	2005.10.20
Korabiewka	27 a	–	83 c	11 a
Ner	32 a	37 a	23 b	4 a
Skierniewka	130 c	123 c	38 b	11 a
Zwierzynka	71 b	62 b	12 a	7 a

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

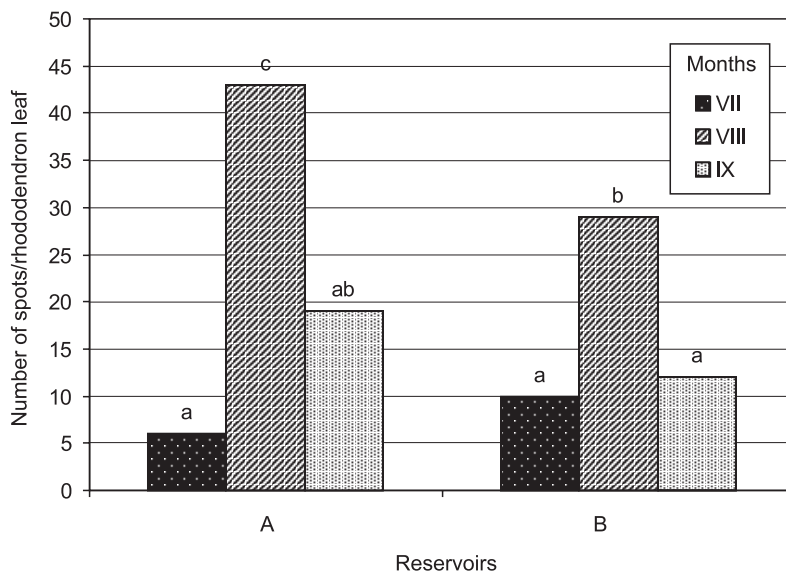


Fig. 1. Influence of nursery reservoir location, time of water surveying and number of *Phytophthora* spots/rhododendron leaf baits

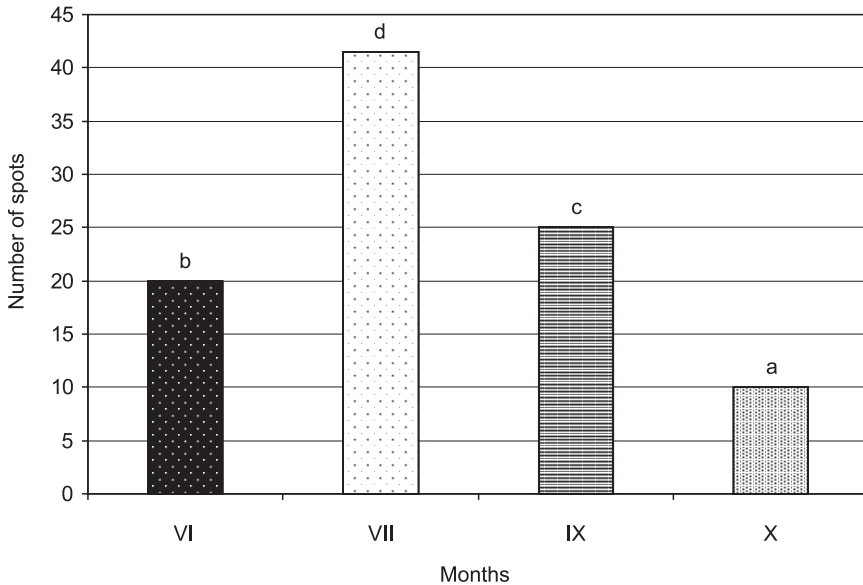


Fig. 2. Relationship between period of nursery canal water surveying and number of *Phytophthora* spots/rhododendron leaf baits

### Relationship between holding of baits in rivers, surveying time and number of *Phytophthora* spots on rhododendron baits

At least twice more necrotic spots occurred on twigs held in the middle section of rivers swimming near both nurseries (Table 2). More *Phytophthora* spots were observed on leaf baits held downstream the nursery cultivated areas. It indicates that surplus water from nurseries may be an additional source of *Phytophthora* propagules in rivers.

Table 2. Relationship between holding of baits in river water, surveying time and number of *Phytophthora* spots on rhododendron leaf

Place of baits holding	Rivers and months of water surveying			
	Kurówka		Ner	
	May	October	May	October
10m upstream of nursery border	10 a	8.3 a	2 a	9.3 a
In the middle of nursery border	21.3 b	24.5 b	25.8 c	36.8 c
10m downstream of cultivated areas	29 bc	20.8 b	15 b	24 b

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

### Spread of *Phytophthora* tip blight of *Picea omorika*

First, sporadic necrosis on the top of twigs was noticed on July 8th, 2005. After 3 days disease symptoms occurred on about 1/6 of observed plants and 1/5 affected tips/spruce (Table 3). Within the next 9 days tip blight was noticed on about 1/3 of analysed spruces and 6 tips/plant (Table 3). From diseased twig parts *P. citricola* was isolated. Additionally, *Pestalotia sydowiana* Bres. occurred on diseased twig parts. Investigations on the pathogen source in nursery indicate sprinkled water, taken from the water reservoir, because shoot rot caused by that species was not observed on other nursery plants.

Table 3. The spread of *Phytophthora* tip blight of *P. omorika* in relation to surveying time

Observation time	Number of diseased plants (n = 20)	Number of diseased tips/plant
2005.07.11	3.4 a	1.5 a
2005.07.14	5.4 b	4.8 b
2005.07.20	7.0 c	6.0 bc

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

This study has established that water in rivers, nursery reservoirs and drainage canal was contaminated with *Phytophthora* spp. with *P. citricola* as a predominant species. From Ner and Korabiewka rivers the species was even isolated from 90% of spots. In Themann et al (2002) studies *P. citricola* constituted 1/14 of *Phytophthora* isolates obtained from recirculated water and the species was mainly isolated in July. The species was found among at least 11 *Phytophthora* spp. with domination of *P. gonapodyides*. The authors indicated a dramatic increase in *Phytophthora* isolates in water reservoirs from autumn to spring probably being connected with lack of water dilution and no application of chemical compounds specific to *Oomycetes* during that period. In our study (Orlikowski et al. unpubl.) only sporadic *Phytophthora* detection was noticed during late autumn and winter time. The frequency of occurrence of *Phytophthora* spp. is correlated with seasonal changes of water temperature (Thomson and Allen 1974). Authors indicated that *P. parasitica* was a predominant species isolated when water temperature was over 20°C whereas *P. citrophthora* was not detected at water temperature over 23°C. Additionally, they found, using sieving technique on 8  $\mu$ m Millipore filter, that only zoospores were present in irrigation water. In trials of Gerlach et al. (1976) zoospores of *P. citrophthora* were also dispersed to potted *Pieris japonica* by splashing water. In our study dispersed zoospores of *P. citricola* could establish on the twigs of some plants, including *P. omorika*, by initiating colonization of needles and stem tips in favourable air temperature and humidity.

In this study we used rhododendron leaf traps for *Phytophthora* with possibility of isolation at least 4 species. Some other baits including citrus leaf pieces, lemon fruits, lupine seeds and seedlings were used (Kliejunas and Ko 1976; Thomson and Allen 1974). In opinion of Thomson and Allen (1974) leaf baits are more efficient in recovery of zoospores in water because chlamydospores, zoosporangia and oospores sank and did not infect leaf tissues.

These studies constitute the first step for recognizing the significance of *Phytophthora* species in different water sources in Poland. It is already known that contaminated water used for plant irrigation could easily provide *Phytophthora* inoculum necessary for infection and dispersal of propagules in a new locations.

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## POLISH SUMMARY

### WODA JAKO POTENCJALNE ŹRÓDŁO *PHYTOPHTHORA CITRICOLA*

Gatunek *Phytophthora citricola* stanowił około 70% izolatów *Phytophthora* spp. uzyskanych z liści różanecznika stosowanych jako pułapki do wykrywania przedstawicieli tego rodzaju w wodzie. Gatunek stwierdzono w 4 rzekach, 2 zbiornikach wodnych w szkółkach oraz kanale odprowadzającym nadmiar wody ze szkółki od maja do października. Analiza liczby plam na liściach pułapkowych jako miara liczebności *P. citricola* w wodzie wykazała, że miejsce zastawienia pułapek miało istotne znaczenie na częstotliwość izolowania badanego patogena. Istotnie więcej plam stwierdzono na liściach pułapkowych umiejscawianych w rzekach Skiernewce i Zwierzynce, przepływających przez lasy i tereny rolnicze aniżeli w Nerze – rzeki terenów ogrodniczych. Więcej plam na liściach pułapkowych stwierdzono, gdy pułapki zastawiano w rzekach poniżej szkółek umiejscowionych w ich pobliżu. W zbiornikach wodnych w szkółkach i kanale melioracyjnym wyższą liczebność *Phytophthora* spp. stwierdzano w sierpniu aniżeli w innych miesiącach. Stosowanie wody ze zbiornika w szkółce do podlewania świerka serbskiego, spowodowało pojawienie się objawów zarazy wierzchołków pędów.