

Relationship between source of water, occurrence, and pathogenicity of *Phytophthora plurivora*

LESZEK B. ORLIKOWSKI, ALEKSANDRA TRZEWIK
MAGDALENA PTASZEK and TERESA ORLIKOWSKA

Research Institute of Horticulture

Konstytucji 3 Maja 1/3, PL-96-100 Skierniewice, leszek.orlikowski@inhort.pl

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Phytophthora plurivora was the most often detected species from water using rhododendron baits. The species was isolated from water of two rivers, Jasieniec and Korabiewka, a water pond and a drainage canal from March to November, 2008 (in Korabiewka river also in December). The highest population density of *P. plurivora* was observed in March and April in water pond and canal, and in May in both analysed rivers. In laboratory trials all tested isolates colonized rhododendron and poplar leaves. Isolates from drainage canal were the most pathogenic for rhododendron. Isolates detected in March from water pond and two rivers caused the quickest spread of necrosis on leaf blades. On poplar leaves the fastest development of necrotic spots was observed when isolates obtained in June and November were used for inoculation, while the isolate from September sample was less pathogenic.

Key words: soilborne pathogens, water sources, bait, detection, pathogenicity

INTRODUCTION

Phytophthora species are soilborne pathogens able to affect several hundred plant species worldwide. Currently about 160 species belong to this genus (Jung 2011 unpubl., pers. communic. on COST Meeting, Sękocin 2011). They occur on all continents except of Antarctica, causing several losses in forests and ornamental nurseries. Hong and Moorman (2005), detected 17 *Phytophthora* and 26 *Pythium* species, 27 fungi determined to the genus level, 8 bacteria species, 10 viruses and 13 species of parasitic nematodes from water used for plant sprinkling. Among them *P. plurivora* Jung & Burgess is one of the most dangerous pathogen (Orlikowski et al. 2004, 2006; Orlikowski, Ptaszek 2010; Orlikowski et al. 2011a). The pathogen was described for the first time in 1927 by Sawada on Sekkan orange fruit as *P. citricola*

Sawada. Many isolates described as *P. citricola* in the world collections differ from each other, so Jung and Burgess (2009), on the basis of molecular, morphological and physiological methods described new species named as *P. plurivora* in the *P. citricola* complex. Nowadays most of *P. citricola* isolates were renamed as *P. plurivora* and this species is one of the most often isolated from the *P. citricola* complex.

P. citricola is known in Poland as the main reason for ericaceous and coniferous ornamental plants dying as well as the cause of stem base rot of forest trees (Orlikowski et al. 1995; Orlikowski, Szkuta 2003a, b; Orlikowski et al. 2004, 2006; Orlikowski, Ptaszek 2010; Orlikowski et al. 2011b). Among different sources of plant pathogens, including nursery seedlings, infested soil and transmission on machine parts, water in the opinion of Hong and Moorman (2005) is a primary if not the sole, source of inoculum for *Phytophthora* diseases of numerous nursery, fruit and vegetable crops. The aim of current studies was to estimate the occurrence of *P. plurivora* in different water sources in relation to sampling time, and pathogenicity of the species toward *Rhododendron* sp. and *Populus alba* L.

MATERIAL AND METHODS

Water sources. Studies were conducted in two rivers in Łódź and Warsaw districts (Jasieniec [RJ] and Korabiewka [RK]), in a water pond [S1] localized in ornamental nursery, from which the water is taken for plant sprinkling, and in a drainage canal [C1] to which surplus water from plant watering is running off. Jasieniec is swimming through small forests, fields, but also among horticulture farms growing ericaceous, deciduous and coniferous plants. Korabiewka is forest small river contaminated by plant pathogens during spring time and the local floods.

Isolation of *Phytophthora* species from water. Sampling of *Phytophthora* spp. was carried out during whole year, each month in 2008. For pathogen's isolation baiting technique described by Themann and Werres (1998) and Orlikowski (2006) was used. Twigs of *Rhododendron* cv. Nova Zembla were immersed in water for 4-7 days (depending on year season). Thereafter leaves were taken out from water, put in plastic bags and transported to the laboratory. Leaves were washed in tap and in distilled water and dabbed dry. On each leaf blade the number of necrotic spots was counted as a measure of *Phytophthora* infection density. In sterile laminar bench chosen leaves were sterilized over a burner flame and small fragments of *Rhododendron* sp. tissues were cut from individual necrotic spots and put onto PDA medium in Petri dishes (90 mm in diam.). Plates were incubated in the dark at 20°C. Within the next 2 days colonies growing around plant tissues were transferred onto PDA slants. After a few days isolates were grouped on the basis of growth pattern and representative cultures were taken for further identification.

Identification of *Phytophthora* species. DNA was extracted from mycelium using the method described by Aljanabi and Martinez (1997), modified by Wiejacha et al. (2002). At a first step PCR with non-specific primers: RAPD and ISSR were used (Trzewik et al. 2006). Results were confirmed using PCR with species specific primers for *P. citricola* complex CITER1/CITER2 (Schubert et al. 1999).

Pathogenicity of *P. plurivora*. Trials were conducted in laboratory conditions using method described by Orlikowski and Szkuta (2001). Isolates of *P. plurivora* obtained from different water sources in subsequent months were used. Rhododendron and poplar leaf blades were washed under tap and distilled water, dabbed dry and put to plastic trays onto moist sterile blotting paper covered with plastic net. Three mm diameter discs, overgrown by *P. plurivora*, taken from margins of 7-days old PDA cultures, were put in wounded central part of leaf blade. Clean PDA medium was used as the control. Trays were covered with foil and incubated in 22-24°C. Within 7 days the diameter of necrosis was measured. Experimental design was completely randomized with 4 replications and 5 leaf blades in each replication. Duncan multiple range test was used for mean separation.

RESULTS AND DISCUSSION

Detection of *Phytophthora plurivora* from water. During one-year studies 384 leaf baits were analysed on the occurrence of *P. plurivora* in 4 sources of water (Tab. 1). The species was detected from drainage canal, water reservoir and 2 rivers from March to November, 2008 (in Korabiewka river also in December), except water pond in June and Jasieniec river in November. The highest number of this species isolates was noticed in March, April and May. Decrease of *Phytophthora* population from summer to autumn Themann et al. (2002) indicated on the presence of chemical compounds for *Oomycetes* in water, and mixture of *Phytophthora* contaminated pond water with fresh water from well. Orlikowski et al. (2007) provided that *Phytophthora* species were never isolated from a well.

The analysis of *Phytophthora* isolates number from water showed, that baiting time and place of baits holding were not correlated with the frequency of these pathogens group detection. The number of *P. plurivora* isolates obtained from a canal, water pond and Korabiewka river was similar and varied from 76 to 83 (Tab.

Table 1
Occurrence of *Phytophthora plurivora* in water from different sources
in relation to detection time

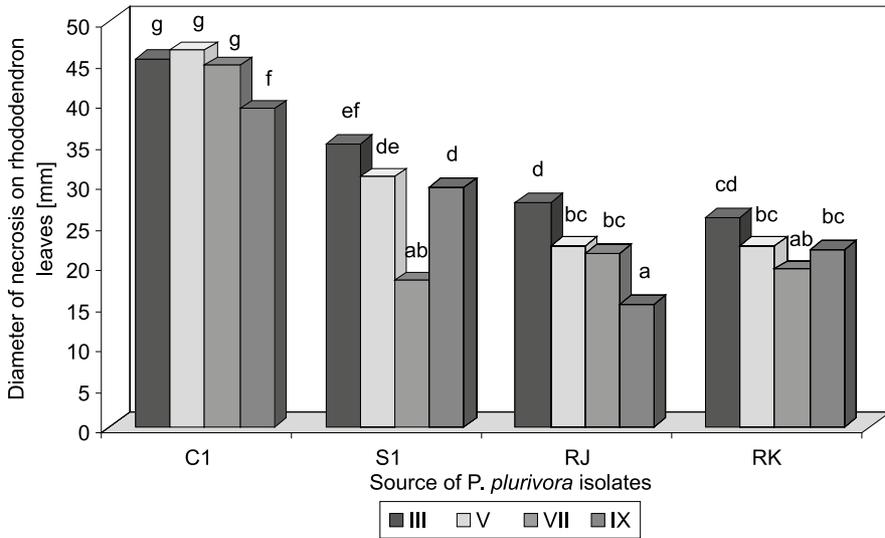
Sampling time (months)	Number of <i>Phytophthora</i> isolates (a), including <i>P. plurivora</i> (b)							
	Drainage canal		Water pond		Jasieniec river		Korabiewka river	
	a	b	a	b	a	b	a	b
I	-	-	-	-	-	-	4	4
II	-	-	3	3	-	-	-	-
III	35	28	33	20	13	9	6	6
IV	31	27	27	25	11	9	2	1
V	6	6	13	13	16	14	13	13
VI	3	2	4	0	5	5	12	12
VII	6	6	3	3	3	2	7	7
VIII	2	1	5	3	11	11	8	8
IX	5	3	5	4	11	5	9	9
X	7	4	3	3	2	2	12	12
XI	9	6	8	2	-	0	3	3
XII	-	-	-	-	-	0	11	8

1), while from Jasieniec river, only 57 cultures were detected. It was probably connected with the localization of this river further than other already mentioned river and plant species growing along it. Ghimire et al. (2009) claimed, that diversity and population density of *Phytophthora* spp. occurring in water depends on the range and number of cultivated plants and geographic localisation of nurseries. Our current studies affirm that thesis. Jasieniec is flowing through horticulture area, but nurseries are placed about 200-1000 m from this river, so zoospores from affected plants are not transported to the water. The occurrence of *P. plurivora* in analysed water sources is probably connected with the presence of this species on deciduous, coniferous and ericaceous plants as well as on trees growing along river banks. The pathogen was probably introduced to water with parts of soil and substrata, fragments of diseased roots or infected leaves which could be a source of energy for *Phytophthora*. In river water zoospores released from zoosporangia are transported for a long distance (Miligroom, Peever 2003) and they could infect others, new host plants such as alder, poplar or willow growing along banks. In nurseries, propagules from diseased plants are washed off and transported with excess water and rain to water ponds and canals, usually situated in their lowest points. During hot summer, when plants are being watered few times per day, effluents in ponds are mixed with well water or river water before sprinkling and use for plant irrigation. Such situation cause the threat for the spread of *Phytophthora* zoospores on new host plants in nursery. Previous observation of Orlikowski (2006) indicated the spread of *P. citricola* propagules, mainly zoospores, together with effluent water used for sprinkling of *Thuja occidentalis* L. cv. *fastigiata* Jaeger, *Buxus sempervirens* L. and *Rhododendron* sp. Occurrence of tip blight of thuja and rhododendron and shoot and root rot of boxwood was observed to be preceded by drizzly weather and air humidity about 90% during at least 2 days. On thuja within 8 days disease symptoms spread from 7% (initial observation) to 40%. On *Rhododendron* sp. during 2 months necrotic spots were observed on 13% of crop whereas on boxwood first yellowing was noticed in the middle of July and after 15 weeks the disease occurred on 15% of plants.

P. citricola was detected from forest rivers and streams in north-east part of France (Hansen, Delatour 1999), from water-recycling irrigation system at a perennial container nursery in south-western Virginia (Bush et al. 2003) and also from reservoirs in German commercial hardy ornamental nurseries with water recirculation system (Themann et al. 2002). The species was commonly occurring plant pathogen accommodated to various environmental conditions.

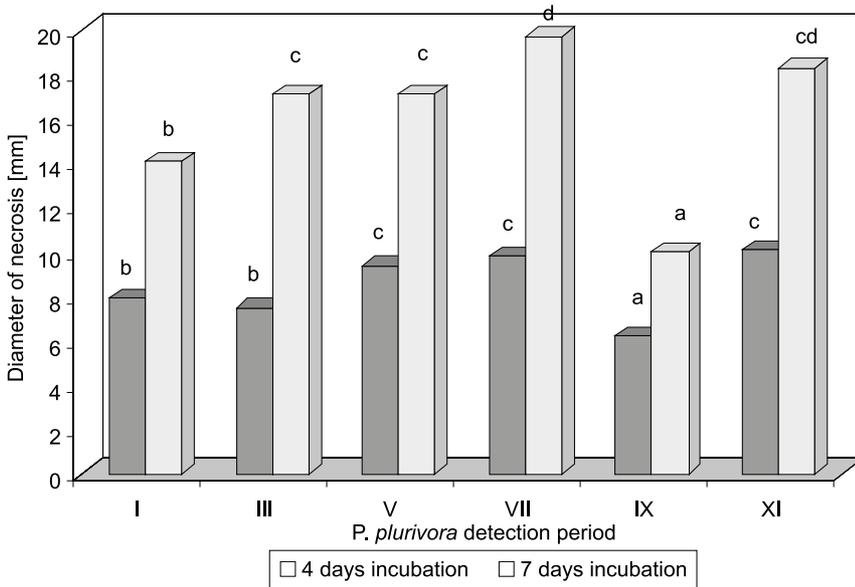
Rhododendron leaves were found to be effective baits for *P. plurivora* detection from all water sources, what is in agreement with study of Orlikowski et al. (2011b), who indicated that leaves of this plant are the most efficient bait for *Phytophthora* species detection. These authors showed that more than 70% of *Phytophthora citricola* isolates from different water sources were obtained using rhododendron leaf bait.

Colonisation of plant parts by *P. plurivora* isolates from river. In the laboratory trials all tested isolates colonized rhododendron and poplar leaf blades (Figs 1, 2). In the trials with rhododendron the quickest spread of necrosis was observed when cultures from drainage canal were used. In the case of other water sources more pathogenic were isolates detected in March than in other months. The slowest rate of necrosis development was observed on rhododendron leaves inoculated with isolates from Jasieniec river detected in September (Fig. 1). In the studies with poplar



Note: means followed by the same letter (a-g) do not differ with 5% of significance acc. to Duncan's multiple range test

Fig. 1. Relationship between source of *P. plurivora* isolates, baiting time and colonization of rhododendron leaves.



Note: means followed by the same letter (a-d) do not differ with 5% of significance acc. to Duncan's multiple range test

Fig. 2. Colonization of poplar leaves by *P. plurivora* isolates obtained from different months in 2008 from Korabiewka river.

the quickest spread of necrotic spots was observed for cultures from July and November and the slowest for September isolate (Fig. 2).

Our results confirm earlier data on the occurrence and pathogenicity of *P. plurivora*. Orlikowski et al. (2010) showed significant variation in the necrosis development on inoculated alder leaves by *P. plurivora* from water obtained in different year periods. Cultures of *P. plurivora* isolated from the Ner river, flowing mainly through Łódź district, in March, May and August colonized alder tissues quicker than an isolate from October. Isolates from water pond and canal obtained in March were more pathogenic for alder leaves than those from other months. Such relationships are probably connected with contamination of water with some chemicals which influenced on isolates pathogenicity (Themann et al. 2002). Orlikowski et al. (2010) showed also that the isolates of this species colonized rhododendron, poplar and willow tissues with the fastest spread of necrosis on rhododendron leaf blades as the most sensitive plant species for this pathogen. Moreover, among three *Phytophthora* species isolated from water in 2007, *P. plurivora* was the most pathogenic for birch leaves and stem fragments as well as for alder leaves (Orlikowski et al. 2008). Pathogenicity of that species toward different plants in relation to detection period will be evaluated in the nearest future.

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Współzależność pomiędzy źródłem wody a występowaniem i patogennością *Phytophthora plurivora*

Streszczenie

Phytophthora plurivora izolowano od marca do listopada 2008 roku z dwóch rzek Jasieniec (RJ) i Korabiewka (RK), zbiornika wodnego (S1) zlokalizowanego na terenie szkółki oraz kanału (C1) odprowadzającego nadmiar wody z kontenerowni. Największą liczbę izolatów uzyskano w marcu i kwietniu z kanału i zbiornika, a w maju z obu rzek. W warunkach laboratoryjnych wszystkie testowane kultury kolonizowały blaszki liściowe różanecznika i topoli. Izolaty z kanału okazały się najbardziej patogenicznymi dla różanecznika. Kultury z marca, uzyskane z trzech źródeł wody (S1, RJ, RK), powodowały szybszy rozwój nekrozy niż pochodzące z prób z innych miesięcy. Najszybsze tempo zasiedlania tkanek liści topoli obserwowano po inokulacji kulturami uzyskanymi z prób z lipca i listopada, a najwolniejsze w przypadku izolatu z września.