

## OCCURRENCE OF *PHYTOPHTHORA RAMORUM* AND OTHER *PHYTOPHTHORA* SPECIES IN NURSERIES, TRADE STANDS, FORESTS AND WATER

Leszek B. Orlikowski<sup>1\*</sup>, Tomasz Oszako<sup>2</sup>,  
Aleksandra Trzewik<sup>1</sup>, Teresa Orlikowska<sup>1</sup>

<sup>1</sup>Research Institute of Pomology and Floriculture  
Pomologiczna 18, 96-100 Skierniewice

<sup>2</sup>Forest Research Institute, Sękocin Stary 3, 05-090 Raszyn

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**Abstract:** Research over a three year period indicated that *P. ramorum* occurred rarely in Poland on *Rhododendron* spp., in spite of established monitoring in nurseries, trade stands, forest and water from early spring to late autumn each year. The pathogen was not found in forests on *Vaccinium vitis-idaea*, *Calluna vulgaris*, *Fagus sylvatica* and *Quercus rubra*, proving its limited spread. The species was detected, however, from 2 rivers. *P. citricola* was isolated from most of surveyed plants. Besides this *P. cactorum*, *P. cinnamomi*, *P. citrophthora* and *P. nicotianae* var. *nicotianae* were isolated from diseased plants. Additionally *Pestalotia sydowiana*, species of *Fusarium*, *Botrytis cinerea* and *Trichoderma* were often found in diseased plant tissues. Laboratory and glasshouse research showed slight differences in colonization of plants by *P. ramorum* and *P. citricola*. However, taking into account the range of host plants, and frequency of pathogen occurrence in infected plant material and water, it became clear that *P. citricola* poses a much greater danger than *P. ramorum* to the natural environment in Poland.

**Key words:** ericaceous plants, water, isolation, *Phytophthora ramorum*, colonization, characteristics

## INTRODUCTION

*Phytophthora ramorum*, identified as a new species by Weres *et al.* (2001), was found in Germany and Holland (1993–1994) on *Rhododendron* spp. and *Viburnum bodnatense* as well as in oak forests on the Californian coast. The same species was found in Poland several times between 1999 and 2002 (Orlikowski and Szkuta 2002) also on rhododendrons showing symptoms of top shoot dieback. Over the following 2 years,

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\*Corresponding address:  
lorlikow@insad.pl

*P. ramorum* was found on *Calluna vulgaris* and *Pieris fraseri* (Orlikowski and Szkuta 2004); it was also observed in dying shoots of *Vaccinium vitis-idaea*, however, the isolates were contaminated and had to be eliminated from the *Phytophthora* culture collection. *P. ramorum* was most frequently associated with *P. citricola* on rhododendrons and heathers, with *P. citrophthora* on *Pieris* and *P. cactorum* on *P. fraseri* (Orlikowski, not published). In Poland *P. ramorum* was isolated rarely from rhododendrons and only from a few varieties. Elsewhere, it was isolated more frequently from this host e.g., Moralejo and Werres (2002) isolated *P. ramorum* from 9 species of rhododendrons and a few hybrids of *R. catawbiense*. *Rhododendron* spp. are amongst the plants most vulnerable to *Phytophthora* infections. In the past 30 years 14 species of *Phytophthora* were found on rhododendrons, including *P. tropicalis* in 2006 (Hong *et al.* 2006). Research in Europe and the USA proved the occurrence of *P. ramorum* on more than 50 host-plant species, including *Aesculus hippocastanum*, *Quercus cerris*, *Q. falcata*, *Q. ilex*, *Q. rubra*, *Fagus sylvatica* and *Pseudotsuga menziesii* in Europe (Huberli *et al.* 2004). On the APHIS list ([www.aphis.usda.gov](http://www.aphis.usda.gov)) there are 46 genera and species listed as potential host-plants of *P. ramorum*, amongst which are *Acer pseudoplatanus*, *Fraxinus excelsior*, *Salix caprea* and *Syringa vulgaris*. In many countries, including Poland, there is a danger that transfer of a disease on a single forest or horticultural host-plant may introduce pathogens onto economically important trees or shrubs threatening a particular ecosystem. A sudden decline of 5 Californian oak species and a rapid spread of the disease in the states of Oregon and Washington provides an example of this phenomenon (Garbelotto *et al.* 2001; Parke *et al.* 2002). This process may be accelerated by the presence of *P. ramorum* in irrigation reservoirs in container nurseries of ornamental plants (Themann *et al.* 2002). The aim of this research, undertaken in 2005–2007 was to: 1) detect the occurrence of *P. ramorum* and other pathogens of the *Phytophthora* group and estimate the threat to *Calluna* plantings or other known host-species, 2) determine the virulence of *Phytophthora* species isolated from *Rhododendron*, *Photinia*, *Pieris*, heathers and nursery irrigation water on selected plant species.

## MATERIALS AND METHODS

### Sites and plant materials

Between 2005–2007 samples from plants showing symptoms of top dying and/or necrosis of leaves were taken (from January till November) from 4 container nurseries in central and south-eastern Poland, where heathers, *Viburnum*, *Syringa* and *Photinia fraseri* were cultivated. In addition from spring to autumn the health status of *C. vulgaris* and other plants was surveyed in trade stands of nursery plant material and in 8 private gardens. Similar observations in 17 forest stands were carried out and samples of *C. vulgaris*, *V. vitis-idaea*, red oak and beech were taken for further thorough analysis. Investigations on the occurrence of *Phytophthora* organisms (including *P. ramorum*) in 9 rivers, 2 canals and 10 irrigation water reservoirs were carried out.

### Mycological analyses of plants

Samples were placed in plastic bags and transported to the laboratory. On the same or the following day, plant material was washed under running tap water, followed by 3 rinses in distilled water and dried between sterile filtration tissues. Selected plant parts were flamed and 3–5 mm diam. tissue fragments placed on PDA.

Depending on the size of the trial, plant fragments were placed in 2–5 replicate 90 mm diam. Petri dishes and incubated in the dark at 24°C. Growth of microorganisms was monitored over 4 days and colonies emerging from plant tissues transferred to PDA slants in 30 ml test tubes. After preliminary screening, selected isolates were purified and identified to genus or species based on published keys. Identification of *Phytophthora* species was based on growth characteristics and morphology. Confirmation of classification to species was obtained by DNA analysis (Orlikowski *et al.* 2007; Trzeźwik *et al.* 2006).

#### **Isolation of *Phytophthora ramorum* from water**

The procedure described by Orlikowski *et al.* (2007) was used. Rhododendron leaf baits were held in the sample of water for 4–6 days, placed in sterile plastic bags and transported to laboratory. Washed and dried leaf blade parts were flamed, and 3–5 mm diam. fragments placed on PDA in 90 mm diam. Petri dishes. Isolates were characterized and identified as described above.

#### **Colonisation of plant parts tissues by isolates of *P. ramorum***

Isolates of *P. ramorum* from *Calluna vulgaris*, *Photinia fraseri*, *Pieris japonica*, *Rhododendron* sp., and 2 rivers (Ner, Rawka) were used for inoculation of leaves or/and stem parts of *Acer pseudoplatanus*, *Aesculus hippocastanum*, *Alnus glutinosa*, *Betula pendula*, *Fagus sylvatica*, *Fraxinus excelsior*, *Ligustrum vulgare*, *Rhododendron* sp., *Rosa multiflora*, *Salix caprea*, *Sambucus nigra*, *Syringa vulgaris* and *Taxus baccata*. In laboratory trials, the method described by Orlikowski and Szkuta (2002) was used. Leaves and stem parts were placed in plastic boxes on sterilized, moist, blotting paper, covered with plastic net and inoculated with 3 mm diam plugs taken from the edge of 7 day-old cultures grown on V8 agar at 24°C. Boxes were covered with plastic foil and incubated at 21–25°C in the dark. After 3 and 5 days (isolates from plants) or 5 and 8 days (cultures from water) incubation, diameter and length of necrosis was measured.

#### **Development of leaf blight on rhododendron leaves inoculated with *P. ramorum* (greenhouse test)**

Cultivar Nova Zembla was used in the trial. Three mm diameter plugs, taken from the edge of 7 day-old cultures isolated from *C. vulgaris*, *P. fraseri*, *P. japonica* and *Rhododendron* sp. were transferred to the base of leaves at shoot tips. In addition, for comparison of the dynamics of spread of necrosis, *P. citricola* was used for rhododendron inoculation (Table 9). Plants growing on the greenhouse bench were covered with a plastic cloche to increase relative humidity to approx. 92%. After 7 and 14 days of incubation, length of necrosis was measured.

The experimental design was completely randomized with 4 replications. In laboratory trials 10 leaves and stem parts were used in each replicate, whereas in the greenhouse experiment 3 plants were inoculated on at least 4 leaf blades each. Trials were repeated twice.

## RESULTS

### Fungi and Algae – like *Oomycetes* isolated from plant material

Seven hundred and twenty six symptomatic plants were analyzed in 2005–2007 to investigate the presence of *Phytophthora*. Heathers (32 varieties) were the dominant species tested, but lilacs (*Syringa*) and beech (*Fagus*) were also included. They were taken from 52 sites (Table 1).

Table 1. Plant species and sites surveyed in 2005–2007  
First survey: 2005.01.11  
Last survey: 2007.10.17

Specification	<i>Calluna vulgaris</i>	<i>Kalmia angustifolia</i>	<i>Gaultheria procumbens</i>	<i>Pieris japonica</i>	<i>Rhododendron</i> spp.	<i>Vaccinium vitis-idaea</i>	<i>Syringa vulgaris</i>	<i>Fagus sylvatica</i>
Number of diseased plants	164	21	16	99	303	39	74	12
Number of analysed cultivars	11	1	1	5	14	1	4	–
Number of surveyed sites	18	6	4	17	43	11	2	3

Thirteen genera and species of fungi and 6 species of *Phytophthora*: *P. cactorum*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. nicotianae* var. *nicotianae* and *P. ramorum* were isolated from infected plants (Table 2). *P. citricola* was the most common species isolated being found in all plant species except *Syringa*. The other *Phytophthora* spp. were isolated from *Rhododendron* – 4 species; from *Pieris* – 2 species and 1 species of each from the remaining investigated plants (Table 2). *P. ramorum* was isolated only from necrotic leaf blades and petioles of *Rhododendron* obtained from distal plant parts. *P. citricola* was isolated from about 50% of affected plants and from 4/5 tested rhododendrons. *P. cinnamomi* was isolated more frequently from diseased heathers than rhododendrons. *P. ramorum*, *P. cactorum* and *P. citrophthora* were found sporadically on rhododendrons (Table 3). Analyses of relationships between the occurrence of *Phytophthora* spp. and the monitored research sites suggested that *P. citricola* occurred in every group of research objects except nursery No 4, *P. cinnamomi* was recorded in 2 nurseries and 4 private gardens. *P. citrophthora* was only found on plants growing in trade stands of nursery material and in private gardens (Table 4).

### The occurrence of *P. ramorum* in water

*Phytophthora* species were found in all investigated rivers, water reservoirs and ditches, with *P. citricola* clearly dominating. *P. ramorum* was found in 2 rivers: Rawka (2006 and 2007) and Ner (2007) (Table 5). The first river flows mainly through agricultural lands, meadows and woods, the second one flows near by horticultural areas with ornamental nurseries, greenhouses and plastic tunnels.

Table 2. *Phytophthora* spp. and fungi isolated from diseased tissues of tested plants in 2005–2007

<i>Calluna vulgaris</i> (nurseries, garden centers, private gardens)	<i>Kalmia angustifolia</i> (nurseries, garden centers)	<i>Pteris japonica</i> (nurseries, garden centers)	<i>Rhododendron</i> spp. (nurseries, garden centers)	<i>Vaccinium vitis-idaea</i> (nurseries)	<i>Syringa vulgaris</i> (nurseries)	<i>Fagus sylvatica</i> (8 forests)
<i>Alternaria alternata</i>	<i>Alternaria alternata</i>	<i>Alternaria alternata</i>	<i>Acremonium</i> sp. <i>Alternaria alternata</i>	<i>Alternaria alternata</i>	<i>Alternaria alternata</i> <i>Botrytis cinerea</i>	<i>Fusarium culmorum</i> <i>F. equiseti</i> <i>F. solani</i>
<i>Botrytis cinerea</i>	<i>Botrytis cinerea</i>	<i>Botrytis cinerea</i>	<i>Botrytis cinerea</i>	<i>Botrytis cinerea</i>	<i>Cladosporium</i> spp.	<i>Phytophthora citricola</i>
<i>Cladosporium</i> spp.	<i>Fusarium avenaceum</i>	<i>Cladosporium</i> spp.	<i>Cladosporium</i> spp.	<i>Mucor</i> spp.	<i>Fusarium oxysporum</i> <i>F. solani</i>	<i>Trichoderma</i> spp.
<i>Fusarium avenaceum</i> <i>Fusarium poae</i>	<i>Mucor</i> spp.	<i>Fusarium avenaceum</i> <i>Fusarium culmorum</i>	<i>Fusarium avenaceum</i> <i>F. culmorum</i> <i>F. equiseti</i> <i>F. poae</i> , <i>F. solani</i>	<i>Phytophthora citricola</i>	<i>Phytophthora citricola</i> , <i>P. citrophthora</i>	
<i>Mucor</i> spp.	<i>Penicillium</i> spp.	<i>Mucor</i> spp.	<i>Gliocladium roseum</i>	<i>Trichoderma</i> spp.		
<i>Pestalotia sydowiana</i>	<i>Phytophthora citricola</i>	<i>Penicillium</i> spp.	<i>Mucor</i> spp.			
<i>Phytophthora cinnamomi</i> <i>P. citricola</i> <i>P. nicotianae</i> var. <i>nicotianae</i>		<i>Pestalotia sydowiana</i>	<i>Penicillium</i> spp.			
<i>Trichoderma</i> spp.		<i>Phytophthora citricola</i> <i>P. citrophthora</i>	<i>Pestalotia sydowiana</i>			
			<i>Phytophthora cactorum</i> <i>P. cinnamomi</i> <i>P. citricola</i> <i>P. ramorum</i>			
<i>Rhizoctonia solani</i>			<i>Trichoderma</i> spp.			
<i>Trichoderma</i> spp.						

Table 3. Frequency of *Phytophthora* spp. isolation from diseased ericaceous plant tissues in 2005–2007

<i>Phytophthora</i> species	<i>Calluna vulgaris</i> (108 plants)	<i>Pieris japonica</i> (26 plants)	<i>Rhododendron</i> spp. (85 plants)	<i>Vaccinium vitis-idaea</i> (11 plants)
<i>P. cactorum</i>	–	–	8	–
<i>P. cinnamomi</i>	29	–	9	–
<i>P. citricola</i>	24	4	66	3
<i>P. citrophthora</i>	–	18	3	–
<i>P. nicotianae</i> var. <i>nicotianae</i>	6	–	–	–
<i>P. ramorum</i>	–	–	8	–

Table 4. Relationship between surveyed sites and occurrence of *Phytophthora* spp. in diseased plant tissues

Surveyed objects	<i>P. cactorum</i>	<i>P. cinnamomi</i>	<i>P. citricola</i>	<i>P. nicotianae</i>	<i>P. citrophthora</i>	<i>P. ramorum</i> *
Nursery No 1 (C)	–	+	+	–	–	+
Nursery No 2 (W)	–	–	–	+	–	–
Nursery No 3 (D)	–	+	+	–	+	–
Nursery No 4 (K)	–	–	–	–	–	–
Garden centers (22)	–	–	+(10)**	–	+(2)**	–
Forests (17)	–	–	+(2)	–	–	–
Private gardens (8)	+(2)**	+(4)**	+(3)**	–	+(2)**	–

\* the species isolated from diseased upper leaves of rhododendron only in 2005

\*\*number of objects with diseased plants invaded by *Phytophthora* spp.

Table 5. Detection of *Phytophthora ramorum* in the Ner (a) and Rawka (b) rivers

Years of survey	Months of surveying									
	04		06		08		10		11	
	a	b	a	b	a	b	a	b	a	b
2005	–	–	–	–	–	–	–	–	–	–
2006	–	–	–	–	–	–	+	–	–	–
2007	+	–	–	+	–	–	–	–	–	–

### Colonisation of plant tissues by *P. ramorum* isolates

Isolates of *P. ramorum* from *Calluna*, *Pieris*, *Photinia* and *Rhododendron* colonized tissue of 9 plant species, including 6 known host-plants of *P. ramorum*. The isolates obtained from *Rhododendron* appeared to develop more rapidly than isolates obtained from other host-plants (Table 6).

Table 6. Colonisation of leaves (a) and stem segments (b) by isolates of *Phytophthora ramorum*; diameter/length of necrosis in mm after 6-days incubation. Inoculation: August 2006

Plant species	Source of isolates							
	<i>Calluna vulgaris</i>		<i>Pieris japonica</i>		<i>Photinia faseri</i>		<i>Rhododendron</i> sp.	
	a	b	a	b	a	b	a	b
<i>Acer pseudoplatanus</i>	6.8 c	12.5 d	5.8 d	10.5 d	4.0 b	8.8 cd	9.0 b	15.5 d
<i>Alnus glutinosa</i>	5.0 b	10.0 bc	3.5 bc	8.0 bc	4.3 b	7.5 c	12.3 c	16.8 d
<i>Fagus sylvatica</i>	6.5 bc	8.8 b	8.8 e	8.0 bc	8.0 d	7.3 c	10.3 b	11.5 c
<i>Fraxinus excelsior</i>	7.0 c	5.5 a	5.8 d	4.5 a	3.8 b	5.0 ab	5.5 a	6.8 a
<i>Ligustrum vulgare</i>	11.5 d	14.0 e	8.8 e	9.5 c	6.3 c	8.0 c	14.3 d	11.8 c
<i>Rhododendron</i> sp.	16.0 d	21.5 f	14.5 g	17.0 d	12.5 e	15.5 e	18.8 e	23.5 e
<i>Sambucus nigra</i>	6.3 bc	4.0 a	5.5 d	5.0 a	3.8 b	4.3 a	7.5 ab	9.0 ab
<i>Syringa vulgaris</i>	13.0 de	8.3 b	11.3 f	7.0 b	8.8 d	7.5 c	10.8 b	8.7 ab
<i>Taxus baccata</i>	3.5 a	4.8 a	2.0 b	3.5 a	4.3 bc	3.5 a	5.8 a	7.5 a

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

Table 7. Colonisation of plant leaves by 2 isolates of *Phytophthora ramorum* from the Rawka (A) and Ner (B) rivers. Trial: September 2007

Plant species	Days after inoculation			
	5		8	
	A	B	A	B
<i>Acer pseudoplatanus</i>	4.0 b	5.2 bc	11.3 b	11.0 b
<i>Aesculus hippocastanum</i>	9.0 d	8.5 de	9.0 b	23.0 c
<i>Alnus glutinosa</i>	8.6 d	6.2 b-d	21.0 c	13.3 b
<i>Betula verrucosa</i>	6.6 c	5.0 b	23.3 c	15.8 b
<i>Fagus sylvatica</i>	6.0 c	6.2 b-d	9.3 b	15.8 b
<i>Fraxinus excelsior</i>	11.5 e	4.5 b	31.3 c	23.5 c
<i>Rhododendron</i> sp.	8.5 d	23.0 g	21.3 c	46.8 e
<i>Rosa multiflora</i>	7.6 cd	7.7 cd	21.0 c	25.0 c
<i>Salix caprea</i>	3.7 b	10.5 e	4.0 a	11.5 b
<i>Sambucus nigra</i>	0 a	0 a	49.8 f	32.8 d
<i>Syringa vulgaris</i>	16.5 f	17.4 f	41.8 e	45.0 e
<i>Taxus baccata</i>	6.3 c	0 a	13.3 b	0 a

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

Very clear differences in the rates of colonization of 12 plant species were found for *P. ramorum* isolates originating from the rivers Rawka and Ner (Table 7). Necrosis on leaves was visible on all tested plants 8 days after inoculation but isolates from the river Ner failed to colonize needles of yew after incubation of 5–8 days (Table 7). After 8 days, the largest diameter of necrotic lesions were found on leaves of *S. vulgaris* and *S. nigra*. The isolate from the river Ner also caused rapid development of necrosis on leaves of *Rhododendron* sp., *A. hippocastanum* and *F. excelsior*. The slowest colonization occurred on leaves of *A. pseudoplatanus* and *S. caprea* (Table 7).

### Development of shoot blight of *Rhododendron* in glasshouse inoculations

Necrosis was observed on petioles and leaf blades of *Rhododendron* 7 days after inoculation with *P. ramorum* (Table 8). Development of necrosis was significantly faster on leaves inoculated with the isolate originating from rhododendron compared with cultures from other host-plants. After 14 days, however, differences in size of necroses were no longer significant. Those caused by the *Rhododendron* isolate still were developing slightly faster than the others (Table 8). In the glasshouse inoculations of rhododendron leaves with isolates of 2 species of *Phytophthora* and *P. ramorum* originating from heather or rhododendron shoots, development of necrosis was faster with *P. citricola* than *P. ramorum* (Table 9). Fourteen days after inoculation there were no significant differences in the necrosis length on leaves inoculated with both species of *Phytophthora* obtained from 2 different host plants (Table 9).

Table 8. Spread of necrosis (mm) on rhododendron leaves in relation to source of *P. ramorum* isolate and incubation time; glasshouse trial

Source of isolates	Days of incubation	
	7	14
<i>Calluna vulgaris</i>	23.5 ab	35.8 a
<i>Pieris japonica</i>	22.3 a	36.0 a
<i>Photinia fraseri</i>	18.9 a	34.5 a
<i>Rhododendron</i> sp.	28.3 c	39.5 ab

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

Table 9. Spread of necrosis (mm) on rhododendron leaf petioles and blades inoculated with *Phytophthora citricola* (A) and *P. ramorum* (B); glasshouse trial

Source of isolates	Length of necrosis after days of incubation			
	7		14	
	A	B	A	B
<i>Calluna vulgaris</i>	25.0 a	21.3 a	38.9 a	35.5 a
<i>Rhododendron</i> sp.	29.5 b	26.0 a	42.9 a	38.9 a

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

## DISCUSSION

*P. ramorum* was observed only sporadically during health status monitoring and mycological analyses of ericaceous ornamental plants in 4 nurseries. This species was not found in horticultural shops, smaller trade stands and private gardens, or in forests with natural *C. vulgaris* and *V. vitis-idaea* shrub layers, or in beech stands. It is possible that such sporadic findings of *P. ramorum* on diseased plant tissues in nurseries result from a few cases of disease spread and measures undertaken to minimize the disease, such as cutting and burning affected tops of shoots directly after first of disease symptoms are noted. The research of Neubauer *et al.* (2006) suggests that such worries are unnecessary, because active spread of *P. ramorum* is slow. Our own observations indicated possible passive ways of spread: for example, infected *Rhododendron* leaves shed much earlier than leaves infected by *P. citricola*. Strong wind may carry infected leaves some distance far from the infection source. Heavy rains, irrigation water delivered through sprinklers or drip systems may also play important role in the spread of this pathogen. Even in containerized plants on shallow slopes, pathogens may spread in run-off water and wash into ditches, streams or rivers, as well as into water reservoirs in the lowest part of a nursery. The presence of *P. ramorum* in closed water nurseries was described previously (Themann *et al.* 2002).

*P. ramorum* was present in 2 river systems analysed in the present work, although the presence or absence of nurseries or plastic tunnels near these water courses had no influence on isolation frequency. Detection of *P. ramorum* in river water during spring and autumn was probably connected with temperatures below or around 20°C, which is optimal for development of this pathogen (Orlikowski and Szkuta 2002). It is likely that zoospores of *P. ramorum* could have been washed into the river flowing through agricultural areas, meadows and woods from infected plants taken from orchards, and from composted plant debris around private houses and gardens. Where a river flows near or through a horticultural area, rare recovery of *P. ramorum* from the water may be related to the use of metalaxyl or other chemicals, residues of which may flow into the water course.

Inoculation tests on 12 plant species, including a majority recognized in the literature as hosts for *P. ramorum*, showed little difference in virulence of the isolates obtained from the 2 rivers. Eight days after inoculation of leaves or needles with a culture from Ner river, necrotic symptoms were observed on all plants except yew. Necrosis developed on *Rhododendron* leaves twice as fast as on leaves inoculated with the culture from Rawka river. In contrast, *P. ramorum* isolated from the river Rawka more rapidly colonized leaves of alder, birch, ash and common elder. The occurrence of these plants in the vicinity of rivers, shedding leaves into the water, may increase the amount of *P. ramorum* inoculum.

Analysis of the relationship between the origin of the isolates and rate of colonization of leaves or fragments of stems of 9 plant species revealed slight differences in the diameter of observed spots or length of necrosis. Generally, isolates from rhododendrons and heather colonized tissues faster than cultures from pieris or photinia. These phenomena were also observed in glasshouse tests with rhododendron on leaves 7 days after inoculation; however, after 14 days differences were not significant. The differences in colonization of plant tissues by *P. ramorum* isolates may suggest 2 or 3 initial sources of the pathogen such as rhododendron, heather and

peris. The latter host may have spread the pathogen into nearby plantings of photinia (Orlikowski and Szkuta 2004).

Currently, *P. ramorum* is on the EPP0 pathogens 'alert list'. Results from the work reported here, and from previous research, suggest, however, that this alert is not necessary. The current work supports previous findings (Orlikowski and Szkuta 2002; 2004; Orlikowski and Wiejacha 2005) indicating that *P. ramorum* is limited in occurrence; this species was found only on rhododendrons, despite frequent monitoring from early spring till late autumn each year for 3 years. The lack of this harmful factor in forests on cranberry, heathers, beech and red oaks supports these conclusions. *P. citricola*, however, the species occurring together with *P. ramorum* on diseased *Rhododendron*, was the most frequent *Phytophthora* found in the research, occurring in forest nurseries (Orlikowski *et al.* 2004a, 2004b) and in forest stands on beech (Orlikowski *et al.* 2006). This species is also frequently found in rivers, canals and water reservoirs (Orlikowski 2007). The present laboratory and glasshouse research indicated slight differences in colonization of plants by these two pathogenic species. Taking into account the range of host-plants, however, and frequency of occurrence of the *Phytophthora* species in infected plant material and water, it became clear that *P. citricola* presents a much higher danger than *P. ramorum* to the natural environment in Poland.

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## REFERENCES

- APHIS list of regular hosts and plants associated with *Phytophthora ramorum*. 2007. [www.aphis.usda.gov](http://www.aphis.usda.gov)
- Garbelotto M., Svirha P., Rizzo D.M. 2001. Sudden oak death syndrome kills three oak species. *Calif. Agric.* 55: 9–19.
- Hong C.X., Richardson P.A., Kong P. 2006. *Phytophthora tropicalis* isolated from diseased leaves of *Pieris japonica* and *Rhododendron catawbiense* and found in irrigation water and soil in Virginia. *Plant Dis.* 90, p. 525.
- Huberli D., Reuther K.D., Smith A., Swain S., Tse J.G., Garbelotto M. 2004. First report of foliar infection of *Rosa gymnocarpa* by *Phytophthora ramorum*. *Plant Dis.* 88, p. 430.
- Neubauer C., Gliessmann J., Beltz H. 2006. Zu den Ausbreitungswegen von *Phytophthora ramorum* an *Rhododendron* und *Viburnum* auf Container-Stellflächen. *Gesunde Pflanzen* 58: 185–191.
- Orlikowski L.B., Duda B., Szkuta G. 2004a. *Phytophthora citricola* on European beech and silver fir in Polish forest nurseries. *J. Plant Protection Res.* 44: 57–64.
- Orlikowski L.B., Oszako T., Duda B., Szkuta G. 2004b. Występowanie *Phytophthora citricola* na jesionie wyniosłym w szkółkach leśnych. *Leśne Pr. Bad.* 4: 129–136.
- Orlikowski L.B., Oszako T., Szkuta G. 2006. First record of *Phytophthora* spp. associated with the decline of European beech stand in south-west Poland. *Phytopathol. Pol.* 42: 37–46.
- Orlikowski L.B., Szkuta G. 2002. First record of *Phytophthora ramorum* in Poland. *Phytopathol. Pol.* 25: 69–79.
- Orlikowski L.B., Szkuta G. 2004. First notice of *Phytophthora ramorum* on *Calluna vulgaris*, *Photinia fraserii* and *Pieris japonica* in Polish container-ornamental nurseries. *Phytopathol. Pol.* 43: 87–92.
- Orlikowski L.B., Trzewik A., Orlikowska T. 2007. Water as potential source of *Phytophthora citricola*. *J. Plant Protection Res.* 47: 125–132.

- Orlikowski L.B., Wiejacha K. 2005. *Phytophthora ramorum*, nowy inwazyjny czynnik chorobotwórczy dla roślin na świecie i w Polsce. Post. Nauk Rol. 6: 3–14.
- Parke J.L., Linderman R.G., Hansen E.M. 2002. Susceptibility of *Vaccinium* to *Phytophthora ramorum*, cause of sudden oak death. Phytopathology 92, p. 63.
- Themann K., Werres S., Luttmann R., Diener H.A. 2002. Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. Eur. J. Plant Pathol. 108: 337–343.
- Trzewik A., Wiejacha K., Orlikowski L.B., Szkuta G., Orlikowska T. 2006. The identification of five *Phytophthora* species on the basis of DNA markers obtained via the PCR technique with non-specific primers. Phytopathol. Pol. 41: 27–37.
- Werres S., Marwitz R., Man int't Veld W.A., de Cock A.W.A.M., Bonants P.J.M., de Werdt M., Themann K., Iljeva E., Baayen R.P. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycol. Res. 105: 1155–1165.

## POLISH SUMMARY

### WYSTĘPOWANIE *PHYTOPHTHORA RAMORUM* I INNYCH GATUNKÓW *PHYTOPHTHORA* W SZKÓŁKACH, PUNKTACH HANDLOWYCH, LASACH I W WODZIE

Trzyletnie badania wykazały sporadyczne występowanie *Phytophthora ramorum* tylko na różanecznikach w szkółce oraz w 2 rzekach. Gatunku nie stwierdzono na wrzosach, borówce brusznicy, fotinii, buku oraz dębie czerwonym mimo prowadzenia obserwacji w szkółkach pojemnikowych, punktach sprzedaży roślin ozdobnych, ogrodach działkowych oraz w lasach. Najczęściej stwierdzanym był gatunek *P. citricola* na wszystkich badanych roślinach wrzosowatych, a także na buku. Ponadto stwierdzano *P. cactorum*, *P. cinnamomi*, *P. citrophthora* i *P. nicotianae* var. *nicotianae*. Obok nich często izolowano *Pestalotia sydowiana*, gatunki rodzaju *Fusarium*, *Botrytis cinerea* i rzadziej *Trichoderma* spp. Izolaty *P. ramorum* z różanecznika, fotinii, pierisa i wrzosu kolonizowały 9 badanych gatunków roślin znanych jako żywicieli tego patogena przy czym nekroza rozwijała się najszybciej na różaneczniku. Z kolei izolaty uzyskane z 2 rzek kolonizowały 12 badanych gatunków roślin przy czym nekroza rozwijała się najwolniej na platanie, wierzbie iwie i cisie. Z przeprowadzonych badań wynika, że *P. citricola* jest najczęściej występującym gatunkiem rodzaju *Phytophthora* w Polsce i stanowi znacznie większe zagrożenie dla roślin aniżeli *P. ramorum*.