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Studies on the occurrence and colonisation of plants by *Phytophthora ramorum* in Poland

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Occurrence of *Phytophthora ramorum* on *Rhododendron*, *Vaccinium*, *Viburnum* and *Quercus* species in ornamental nurseries and forest stands in 2001-2002 and necrosis spread on plant parts and seedlings were studied. Only *P. citricola* was isolated from *Rhododendron* spp. and *V. vitis-idaea*. Shoot necrosis and dieback symptoms were not observed on *Viburnum* species in surveyed nurseries. From diseased *Quercus* trunks among others *Armillaria* spp. and *Fusarium* spp. were isolated. Inoculation of leaves and stem parts of *Rhododendron* cultivars and other ericaceous plants with *P. ramorum* resulted in the development of leaf and stem rot. The species caused stem necrosis of *Fagus sylvatica*, *Q. rubra* and *Pseudotsuga menziesii* but symptoms developed slowly.

Key words: isolation, nursery, forest, colonisation, necrosis, plants

INTRODUCTION

Nine years ago unkown *Phytophthora* species was isolated from diseased *Rhododendron* twigs and leaves and *Vaccinium bodnantense* stems in Germany and the Netherlands (Werres et al. 2001). The discovery of a new pathogen on those hosts, growing widely in natural conditions and in ornamental arrangements, has serious implications. It may be present at the ecosystem scale and its appearance on ornamentals suggests the possibility of wider dissemination (Garbelotto et al. 2001). In 2000 the pathogen was isolated sporadically from diseased, container-grown rhododendrons in Poland (Orlikowski and Szkuta 2002). Additionally it was reported on rhododendron in UK, France and Spain (Werres 2002a). In 1994 unknown *Phytophthora* species was found on diseased rhododendron and oaks in California, USA. Within the next 8 years 17 host plants for that pathogen have been described. In 2001 Werres et al. identified a species as *Phytophthora ramorum* Werres,

de Cock and Man in't Veld sp. nov. The pathogen is the most dangerous threat of oak species (*Lithocarpus densiflorus*, *Quercus agrifolia*, *Q. kelloggii* and *Q. parvula* var. *shrevei*) in California and during the last 2 years in Oregon, USA (Rizzo et al. 2002). The disease known as sudden oak death (SOD) is currently patchy in its regional distribution but can affect 40 to 80% of trees in any given stand (Garbelotto et al. 2001). Till now the pathogen has not been observed on oaks in Europe. It is still not known whether the pathogen was transmitted from California to Europe, or vice versa, or originated from third, unknown location. Rizzo et al. (2002) do not dismiss the hypothesis that *P. ramorum* has been in California for many years, but the changes in the environment had led to an increase in its agressiveness or prevalance. Another potential hypothesis is a change in host specificity or host preferences by local *Phytophthora* spp. Hybrids of this genera are thought to occur in nature and may show a marked change in host range (Brasier et al. 1999). Every year new host plants for *P. ramorum* are described. Werres (2002b) observed the pathogen on 6 species of *Rhododendron* including *R. catawbiense, R. ferrugineum x hirsutum, R. forrestri, R. repens, R. wardii, R. Yakushimanum* and 4 species of *Viburnum (V. fragans, V. plicatum, R. tinus, Viburnum* sp.). Maloney et al. (2002) isolated *P. ramorum* from diseased *Sequoia sempervirens* whereas Davidson et al. (2002) from *Pseudotsuga menziesii.* The formation of sympodial, semipappilate and caducous sporangia by that species combined with the production of terminal and intercalary chlamydospores is unreported within genus *Phytophthora* (Rizzo et al. 2002). Under moist conditions and temperature between 101-241C zoosporangia and at times chlamydospores can be formed on rhododendron leaves and infected twig parts. Sporangia may be rain, wind or irrigation splashed and spread aerially or by water to infect new plants. Movement of plant material including infected nursery stock, firewood, timber and transport of soil or substrata containing propagules on shoes, tires of trucks, bikes or feet of animals may spread the pathogen often on a large area (Davidson et al. 2002). Thus, foliar host, especially ericaceous plants in the country, may allow for the rapid establishment of the pathogen in nurseries, Garden Center, gardens and other plant arrangements.

The objectives of this study were to determine (1) the occurrence of *P. ramorum* in ericaceous and container-grown *Viburnum* nurseries and other plants, (2) possibility of ericaceous plants, European beech, Douglas-fir and oaks colonization by the pathogen.

MATERIALS AND METHODS

Isolation and identification of fungi from diseased plants. Eight container - grown ericaceous plants (mainly *Rhododendron catawbiense*, *R. impeditum*, *R. sepedonicum*, *Vaccinium vitis-idaea*) and *Viburnum*-grown nurseries were surveyed at least twice during the spring and August 2001-2002. Additionally, 6 stands of *Quercus robur* and *Q. rubra* with inhibition of growth and necrotic spots and exudates on tranks were surveyed. All or parts of diseased plants were collected individually in plastic bags and transferred into laboratory. After cleaning, washing and disinfection tissue parts were transferred on potato-dextrose agar (PDA) and into green apples using procedure described by Orlikowski and Szkuta (2002). Isol-

ates obtained were identified to genera and species using the available monographs. *Phytophthora* sp. was identified by comparison of colony growth patterns. Additionally, morphological identification was confirmed by isozyme electrophoresis (Orlikowski et al. 2002).

Colonisation of plant parts by *Phytophthora ramorum*. Leaves and stem parts of *Azalea japonica* (azalia), *Andromeda polifolia* (andromeda), *Calluna vulgaris* (heather), *Ledum palustre* (Dutch myrthe), *Leucothoe walteri* (leucothae), *Rhododendron* cultivars, *Vaccinium vitis-idaea* (cowberry), *Fagus sylvatica* (European beech), *Pseudotsuga menziesii* (Douglas-fir), *Q. rubra* (red oak), were used in an *in vitro* and greenhouse trials. *Quercus robur* (Common oak) and *Q. petraea* (Sessile oak) were not used in these trials because in previous study they had not reacted to *P. ramorum* infection. Isolates of *P. ramorum* were used for inoculation of plant parts (Orlikowski and Szkuta 2002). In greenhouse trials 6-month-old seedlings of European beech and red oak were inoculated near the base (Orlikowski et al. 2002) by isolate RH 122. The spread of necrosis was measured on infected plant parts and stem bases. Experimental design was completely randomized with 4 replications and 5 plant parts or seedlings in each rep. Trials were repeated at least twice.

RESULTS AND DISCUSSION

Fungi isolated from analysed plant parts. *Phytophthora citricola* was isolated from the most of diseased twigs of rhododendron species and cowberry (Tab. 1). The species was easily isolated on PDA medium as well as on green apples. The pathogen was isolated mainly from diseased plant parts taken for mycological analysis on August (Figs 1,2). In the spring the species was isolated rarely. *P. ramorum* was not found even in the material coming from the nursery where the species was found on rhododendron first time in Poland (Orlikowski and Szkuta 2002). *Botrytis cinerea* was isolated especially from died twigs of rhododendron and cowberry. Other species including *Fusarium avenaceum, F. culmorum, F. equiseti, F. oxysporum, F. solani, Mucor, Pestalotia, Penicillium* and *Trichoderma* were isolated rarely or

Table 1

Fungi isolated from diseased twigs of *Rhododendron* spp. and *Vaccinium vitis-idaea* on PDA medium; number of colonised plants
Isolation: spring and Aug. 2001-2002

Fungal species	Plant species and number of diseased samples							
	Rhododendron hybrids		*R. impeditum*		*R. sepedonicum*		*Vaccinium vitis-idaea*	
	2001 (74)*	2002 (56)	2001 (18)	2001 (18)	2001 (14)	2002 (18)	2002 (26)	2002 (35)
Botrytis cinerea Pers.	19	27	5	3	6	5	3	10
Fusarium spp.	3	8	3	4	2	3	-	5

only sporadically (Tab. 1). *Viburnum* species, grown in 5 nurseries, did not show any discolouration of stems or wilt symptoms in 2 vegetation seasons. In surveyed nurseries, however, *V. bodnantense* was not grown. *Phytophthora* spp. were not found on diseased parts of oak tranks. *Armillaria* spp. and *Fusarium* spp. were isolated, however, from diseased tissue parts.

Colonisation of plant parts by *Phytophthora ramorum*. Development of necrotic, black-brown spots was observed on all tested rhododendron cultivars but the disease spread significantly slower on "Charmant" and "Lumina Jakushirm" both 3 and 6 days after leaf blade inoculation (Tab. 2). On other 6 species of ericaceous plants development of necrosis was observed, both, on leaf blades and stem parts (Tab. 3). The spread of necrosis was significantly faster on azalea, heather and cowberry than on Dutch myrthe and leucothae (Tab. 3). Browning of European beech and Douglas-fir branches (about 3 mm diam.) from the base to top developed rather slowly. After 14-day-incubation necrosis spread about 1.3 mm/24 hr (Tab. 4). Ashy-brown discolouration of Douglas-fir needles, inoculated with *P. ramorum*, was also observed. Inoculation of red oak stem parts with 3 *P. ramorum* isolates (Tab. 5) indicates that isolate RH 2, obtained from diseased leaf blade of rhododendron, did not cause any disease symptoms. When isolate RH 122 was used for inoculation, necrosis spread faster than on stem parts treated with RH6 (Tab. 5). Inoculation of red oak seedlings with *P. ramorum* resulted in the development of plant base canker (Tab. 5). Necrosis spread about 1.2 mm/24 hr.

Mycological analysis of diseased rhododendrons, cowberry and other potential host plants for *P. ramorum* showed that the species was not present either in diseased twigs or necrotic parts of tree trunks. It indicates that the pathogen found in 2000 (Orlikowski and Szkuta 2002) occurred only in one nursery and on limited

Table 2

Spread of necrosis on the leaves of *Rhododendron* cultivars inoculated with *Phytophthora ramorum*, isolate RH 122; length of necrosis in mm
Inoculation: 2002.03.18

Cultivars	Days after inoculation	
	3	6
Cunningam's White	16.2 cd	28.9 b
Haaga	15.0 c	28.6 b
Helliki	12.2 b	25.4 b
H. Charmant	8.5 a	16.0 a
Lumina Jakushim	8.8 a	18.6 a
Mikkeli	15.4 c	27.4 b
Nova Zembla	16.1 cd	26.1 b
Pohjola's Daughter	17.7 cd	27.5 b
Purple Splendour	16.0 cd	26.8 b
Tiger Stedli	18.8 d	30.3 b

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

Fig.1. Dieback of rhododendron infested with *Phytophthora ramorum.* Phot. Orlikowski and Skrzypczak.

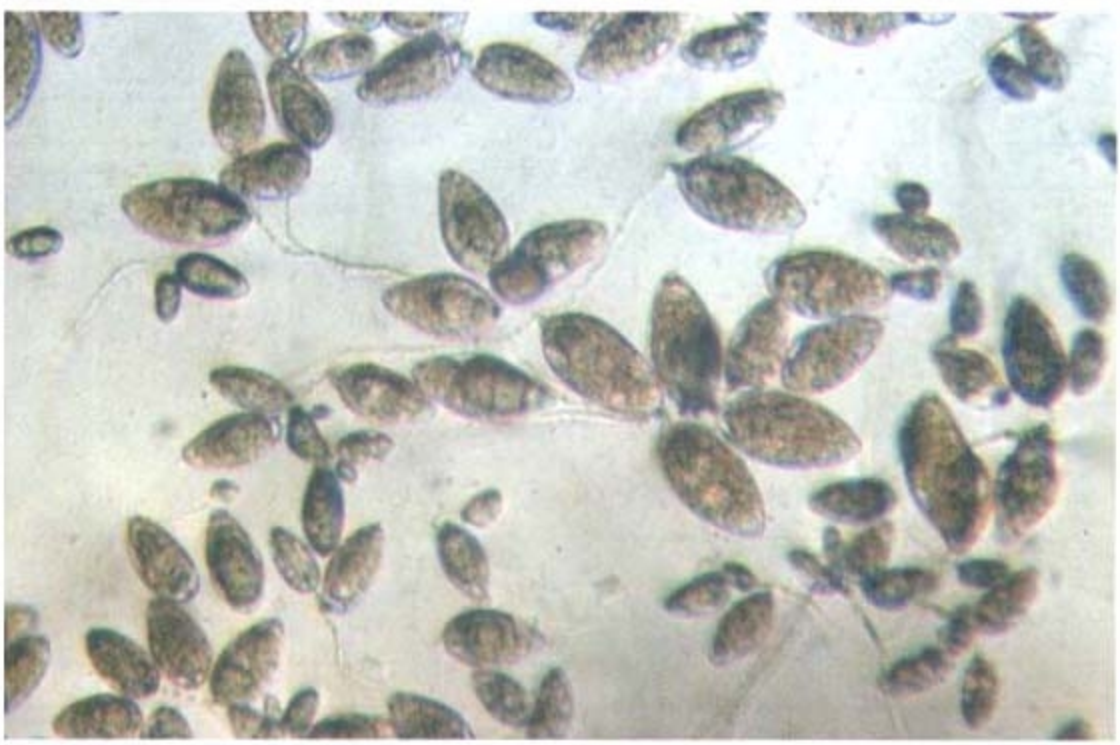

Fig.2. Zoosporangia of *Phytophthora ramorum.* Phot. Orlikowski and Skrzypczak.

Table 3
Development of necrosis on some ericaceaus plants inoculated with *Phytophthora ramorum*, isolate RH 122; length of necrosis in mm on leaves (a) and stems (b)
Inoculation: 2002.07.02

Plant species	Days after inoculation			
	3		6	
	a	b	a	b
Azalia japonica	9.3 b	5.3 b	16.5 b	13.8 c
Andromeda polifolia	3.0 a	3.0 a	3.5 a	8.8 b
Calluna vulgaris	-	13.8 c	-	16.4 d
Ledum palustre	3.0 a	5.0 b	5.3 a	5.3 a
Leucothoe walteri	3.0 a	0 a	4.5 a	6.3 a
Vaccinium vitis-idaea	10.6 b	5.8 b	18.8 b	16.4 d

Explanations: see Table 2

Table 4
Spread of necrosis (in mm) on stem parts of beech and Douglas-fir inoculated with *Phytophthora ramorum*, isolate RH 122; length of necrosis in mm
Inoculation: 2002.11.25

Plant	Days after inoculation	
	7	14
Fagus sylvatica	8.0 a	17.8 a
Pseudotsuga menziesii	10.0 a	19.8 a

Explanations: see Table 2

Table 5
Spread of necrosis on red oak stem parts of 6-month-old seedlings inoculated with *Phytophthora ramorum*; length of necrosis in mm
Inoculation: 2002.08.09

Isolates	Laboratory trials after day - incubation		Greenhouse trial after day - inocubation	
	4	6	14	28
RH 2	0 a	0 a	-	-
RH 6	4,0 b	8,0 b	17,0 a	33,8 a
RH 122	5.3 b	11.0 c	19.2 a	36.4 a

Explanations: see Table 2

number of rhododendrons. Burning of diseased plants in 2000, eliminated the pathogen from the nursery. Our data indicate, however, *P. citricola* as the main threat of rhododendron and cowberry in Polish nurseries.

Development of necrotic spots on leaves and stem parts, inoculated with *P. ramorum* indicate that the species introduced to nursery may colonise twigs of ericaceous plants, especially azalea, cowberry, heather and rhododendron but also kalmia and pieris (Orlikowski and Szkuta 2002). According to Swiecki (2002) at temperature 20°C and below and in the presence of water *P. ramorum* zoosporangia release a number of zoospores that can swim through water to reach susceptible infection sites. Increase of temperature caused direct germination of zoosporangia and decrease of propagule numbers in plant environment (Swiecki 2002). *In vitro* and *in vivo* colonisation of European beech and red oak tissues by *P. ramorum* indicated those species as potential hosts. The pathogen, however, did not colonise Common and Sessile oaks neither seedlings nor older plant parts (Orlikowski unpubl.). Swiecki's (2002) observation showed that oak decline occurs most commonly in stressed plants, and might even be limited to stressed trees. Also local differences in soil type, hydrology, and competition that affect levels of stress could help explain the distribution of the disease.

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Studia nad występowaniem i kolonizacją roślin przez *Phytophthora ramorum* w Polsce

Streszczenie

W latach 2001-2002 prowadzono badania nad występowaniem *P. ramorum* na różanecznikach, borówce, kalinach i dębach w szkółkach roślin ozdobnych oraz w drzewostanach leśnych. Dodatkowo oceniano możliwości zasiedlania różnych gatunków roślin przez tego patogena. Z lustrowanych gatunków roślin z objawami nekrozy pędów wierzchołkowych, osłabienia wzrostu, przejaśnienia liści i nekrotycznych plam na pniach nie izolowano *P. ramorum*. Z porażonych różaneczników i borówki ozdobnej izolowano natomiast *P. citricola*. Z pni dębów, wśród szeregu gatunków grzybów, izolowano *Armillaria* spp. oraz gatunki z rodzaju *Fusarium*. W nasadzeniach kaliny nie obserwowano roślin z objawami zamierania pędów. Inokulacja ogonków liściowych i części łodyg różanecznika i innych gatunków roślin wrzosowatych przez *P. ramorum* powodowała rozwój nekrozy na tych organach. Omawiany patogen powodował również nekrozę łodyg buka, dębu czerwonego i daglezji ale zbrunatnienie tkanek rozwijało się powoli.