Occurrence of *Phytophthora multivora* and *Phytophthora plurivora* in the Czech Republic

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Abstract

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Beginning in 2006, a survey of two related *Phytophthora* species, *P. multivora* and *P. plurivora,* was performed in the Czech Republic. Both pathogens were distributed throughout a broad range of environments including forest and riparian stands and probably became naturalised in the country. The two species differed in their frequency and elevational distribution. *P. multivora* was less frequent, but commonly occurred in the lowest regions such as Central Bohemia and South Moravia, i.e. areas which generally exhibit a high level of invasion. This species was isolated primarily from *Quercus robur* and found to be involved in oak decline. Moreover it poses a high risk to other forest trees. *P. plurivora* was distributed in a broad range of elevations over the entire area. A substrate specificity was detected in *P. plurivora* – the isolates from forest trees were more aggressive to such trees than the isolates from ericaceous ornamental plants.

Keywords: phytophthora disease; forest trees; oak decline; pathogenicity; Central Europe

Progress in molecular techniques based on DNA sequences and isozyme analysis has improved the taxonomic understanding of the genus *Phytophthora* (Scott *et al.* 2009). A number of newly described species have been listed since the publication of the encyclopedic book of Erwin and Ribeiro (1996) till the up-dating by Érsek & Ribeiro (2010). Also, the way how some traditional and well-known taxa are viewed has substantially changed. For instance, recent studies have revealed a high DNA variability in *Phytophthora citricola sensu lato* suggesting the distinction of several lineages (Kong *et al.* 2003; Bhat & Browne 2007; Gallegly & Hong 2008; Moralejo *et al.* 2008). Based on SSCP fingerprinting and isozyme analysis results, the original species were divided into distinct subgroups (Oudemans *et al.* 1994; Kong *et al.* 2003; Gallegly & Hong 2008). Further, DNA sequence analyses based on the ITS region of the rRNA operon, mitochondrial *cox*1 and β-tubulin gene regions demonstrated that the *P. citricola* complex comprised four lineages corresponding

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to *P. citricola sensu stricto*, *P. citricola* I, and two novel species – *P. multivora* and *P. plurivora* – that partially differed in their morphology, physiology, and geography, too (Jung & Burgess 2009).

P. multivora is widespread in natural ecosystems in Western Australia; it was recovered from dying or dead plants of 16 species and was responsible for the mortality of trees involved (Scott *et al.* 2009). In addition, this pathogen was sporadically recovered from European nurseries (Jung & Burgess 2009). *P. plurivora* is involved in several devastating declines of forests and semi-natural ecosystems and is associated with a widespread chronic decline and dieback of oak species across Europe (Hansen & Delatour 1999; Jung *et al.* 2000; Vettraino *et al.* 2002; Balci & Halmschlager 2003a,b; Jung & Burgess 2009).

This species is also the cause of a twig blight of understory beech trees (Nechwatal *et al.* 2011) and diseases of rhododendron and other ornamentals (Jung & Burgess 2009). Outside Europe, *P. plurivora* was predominantly recovered from highly managed plantations and nurseries in North America (Jung & Burgess 2009). Previous *P. citricola* s.l. isolations from European forest trees have usually been ascribed to *P. plurivora* (Jung & Burgess 2009), however some findings (Jung & Burgess 2009; Černý *et al.* 2011) indicated that *P. multivora* could have been introduced to European forest and riparian stands and potentially involved in tree decline.

The pathogenicity of *P. multivora* and *P. plurivora* isolates to host species was confirmed several times (e.g. Jung & Burgess 2009; Scott *et al.* 2009), but its variability in European isolates has not been thoroughly studied to date.

The aim of the present work is to provide a comparative study on the distribution and pathogenicity of both species in the Czech Republic and estimate of the potential risk ensuing from the naturalisation of *P. multivora* in European forest ecosystems.

MATERIAL AND METHODS

Sampling and isolation. Ornamental and forest woody plants were surveyed for the presence of *Phytophthora* disease symptoms such as leaf spot, shoot dieback, bark necrosis, and rot of feeding roots, usually indicated by foliage yellowing and thinning, in the Czech Republic. When the dieback of ornamental ericaceous shrubs was identified, damaged leaves, twigs, shoots, and whole branches or whole small plants were sampled. In the case of bark lesions on the stems and collars of trees, the outer bark from the apical part of active lesions was removed, the subcortical tissues were uncovered, and samples (100–200 cm^2) of the tissues (including cambium) were stripped from the wood using a chisel and placed into sterile polyethylene bags. In the case of root rot, soil samples containing damaged roots, 1–2 l each, were collected from a depth of 10–20 cm at three different locations within the root zone of the declining individual. In declining tree stands, a series of soil samples was collected from the root zones of 3–5 trees, at least 10 m apart. The samples were stored in a dark and cool place or boxed, transported to the laboratory, and processed immediately.

The above-ground tissue samples were carefully cleaned under running tap water and the margins of the active lesions and acutely damaged plant parts were identified. The tissues were cut into segments (approximately 5 × 5 × 5 mm), which were repeatedly washed in sterile water and 95% ethanol, blotted on sterile filter paper or pulp, and plated onto selective PARPNH V8-juice agar medium. The medium consisted of 100 ml V8 juice (Campbell Grocery Products Ltd., Ashford, UK), 15 g agar, 3 g $CaCO_3$, 200 mg ampicillin, 10 mg rifampicin, 25 mg pentachloronitrobenzene (PCNB), 50 mg nystatin, 50 mg hymexazol, and 1 l of deionised water (Jung *et al.* 1996). The soil samples were processed via a baiting method. The fine roots with attached substrate were separated from the samples, placed in plastic trays, and flooded with deionised water to 1–2 cm above the samples. Young healthy rhododendron leaves were rinsed under tap water and used as baits floating over the flooded samples. Baiting was performed at approx. 20°C for one week; if needed, the water was changed to prevent bacterial upsurge. The necrotised leaves were cleaned, cut, and their segments were incubated on PARPNH V8-juice agar medium as described above.

The PARPNH plates with segments of necrotised host tissues and baits were incubated at 20°C in the dark and repeatedly examined under a stereomicroscope for the presence of *Phytophthora*-like coenocytic hyphae. The hyphal tips from the margin of characteristic colonies were transferred onto V8-juice agar (100 ml V8 juice, 15 g agar, 3 g $CaCO_3$, 1 l deionised water) and carrot agar (50 g sliced carrot and 15 g agar per litre of deionised

water) plates. Isolates were purified and stored on oatmeal agar (50 g oatmeal and 15 g agar per litre of deionised water) in tubes under mineral oil at approximately 12°C.

Identification by DNA sequences, morphology, and growth characteristics. The isolates were identified by sequencing the nuclear ribosomal DNA of the ITS region after amplification with the primer pair ITS1/ITS4 (White *et al.* 1990). The DNA was extracted from fresh cultures using DNA extraction kits (DNeasy Plant Mini kit; Qiagen, Hilden, Germany and Ultra Clean Microbial DNA Isolation Kit; Mo-Bio, Carlsbad, USA) and amplified by PCR using a Mastercycler® ep thermocycler (Eppendorf AG, Hamburg, Germany). The PCR amplifications were performed in a 25 µl reaction volume containing 50 ng DNA, 20 pmol each primer, 0.2mM dNTPs, and 1 U DynaZyme™ polymerase with the appropriate buffer (Finnzymes, Espoo, Finland). The PCR was run under the following conditions: 94°C for 3 min, 50°C for 30 s, and 72°C for 1 min (1 cycle); 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s (33 cycles); and 94°C for 30 s, 50°C for 30 s, and 72°C for 5 min (1 cycle). The PCR products were sequenced using the services of Macrogen Inc. (Seoul, Korea) and were analysed using the BLAST algorithms in the GenBank database.

Morphology and growth characteristics were investigated in 5 strains of *P. multivora* and 20 strains of *P. plurivora* (Table 1). The sexual structures (oogonia, oospores, antheridia) were observed on V8A after 10–14 days of cultivation in the dark at 20°C. Sporangial production was induced by the cultivation of approx. 1 cm^2 segments cut from the margin of 1-week-old colonies on CA plates in filtered pond water in the dark, at approx. 15°C for 2–5 days. The dimensions and features of 20 randomly selected mature structures were measured as described in Jung *et al.* (1999) under Olympus BX51 microscope with a camera using QuickPHOTO CAMERA 2.3 software (Promicra, Prague, Czech Republic). The oospore wall index was calculated as the ratio between the volume of the oospore wall and the volume of the entire oospore (Dick 1990). Data thus obtained were compared with the original descriptions (Jung & Burgess 2009; Scott *et al.* 2009). The growth characteristics and colony morphology were determined for 7-day-old cultures growing on V8A, CA, MEA (15 g malt extract, 5 g peptone, and 15 g agar per litre of deionised water) and PDA (4 g potato extract, 20 g dextrose, and 15 g agar/l deionised water) (both Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) at 20°C in the dark. The radial growth rate was recorded in two replications along two lines intersecting the centre of the inoculum at right angles after 5–7 days of incubation on V8A plates in the dark. The cardinal temperatures were determined using V8A plates between 0°C and 35°C.

Inoculation experiments. In total, 2 isolates of *P. multivora* and 13 isolates of *P. plurivora* (Table 1) were used in these tests. The isolates were first revived on V8A plates and then transferred to CA plates and cultivated for 5 days. The pathogenicity was restored on leaves of young rhododendron plants as follows: the adaxial side of young rhododendron leaves was surface-sterilised by wood pulp rinsed in 95% ethanol and the cuticle was mechanically damaged with a sterile scalpel near the midrib for approximately half of the length of the leaf blade. Agar segments (1 cm^2) from the actively growing margins of colonies were placed with the mycelium downward on the damaged leaves and sealed with parafilm. The plants with inoculated leaves were cultivated in a greenhouse at approximately 25°C for two weeks. The strains were re-isolated from segments of the necrotised leaf tissue placed on PARPNH agar, as described above. The isolates were then purified, transferred onto CA plates, cultivated for one week in the dark at 20°C, and prepared for artificial inoculations.

The pathogenicity test was conducted in June and July of 2009 using 2-year-old saplings, 40–60 cm in height, of six forest tree species (*Acer pseudoplatanus*, *Alnus glutinosa*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus robur*, and *Tilia cordata*) grown in containers. The stems of the saplings were wiped with sterile pulp soaked with 95% ethanol. Wounds (5 mm in diameter) were made using a sterilised cork borer in the stems at approx. 5 cm above the collar and the covering tissues, including cambium, were removed. Plugs (5 mm in diameter) from the margins of actively growing colonies on CA plates were inserted into the wound site, with the mycelium inward, and covered with parafilm. The control plants were inoculated in the same way using sterile agar plugs. A total of 15 plants were used for each treatment and the experiment was completely randomised. The saplings were cultivated in a greenhouse at a temperature of 20–25°C, at 40–60% relative humidity, and watered with tap water as needed. The length of the lesions developed on the stems was measured 6 weeks after

inoculation. The lesion length caused by particular isolates and both species on particular hosts was compared. Moreover, *P. plurivora* isolates were classified into two groups according to their origin (forest trees and *Ericaceae*) and the differences in damages were investigated.

The data were digitalised and processed using Statistica 7.1 (StatSoft Inc., Tulsa, USA). As the assumptions of normality were not satisfied after transformation in some data groups, the data were processed using nonparametric statistics.

RESULTS

Distribution and hosts

Since 2006, the presence of *P. citricola* s.l. has been confirmed in 46 locations on the territory of the Czech Republic (Figure 1). There have been detected over 160 isolates from 20 host taxa belonging to 13 genera from 9 families – *Abies*, *Acer*, *Alnus*, *Fagus*, *Fraxinus*, *Pieris*, *Pinus*, *Quercus*, *Rhododendron*, *Salix*, *Tilia*, *Ulmus*, and *Vaccinium* (Table 1). The ITS analysis revealed that two species were present in the collection – *Phytophthora multivora* P.M. Scott & T. Jung and *Phytophthora plurivora* T. Jung & T.I.Burgess. Seventy-eight isolates were deposited in our culture collection that comprised 12 isolates (13.0%) of *P. multivora* and 66 isolates (87.0%) of *P. plurivora*, both isolated from the same stands in certain cases.

P. multivora was recorded in two areas, i.e. Central Bohemia and South Moravia, representing the lowest, warmest, and, moreover, dry regions in the Czech Republic (Figure 1). The pathogen was found in a very narrow range of elevations

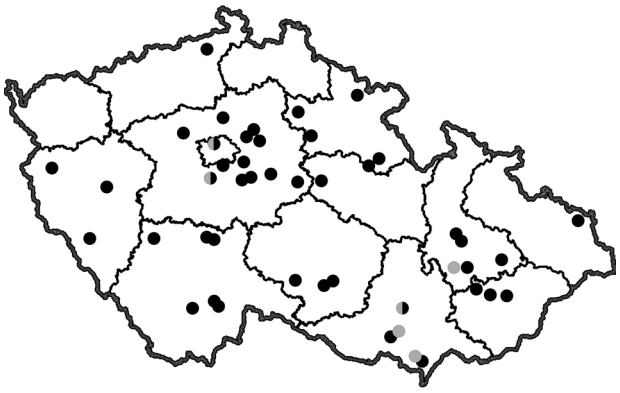

Figure 1. Distribution of *P. multivora* (grey dots) and *P. plurivora* (black dots) in the Czech Republic

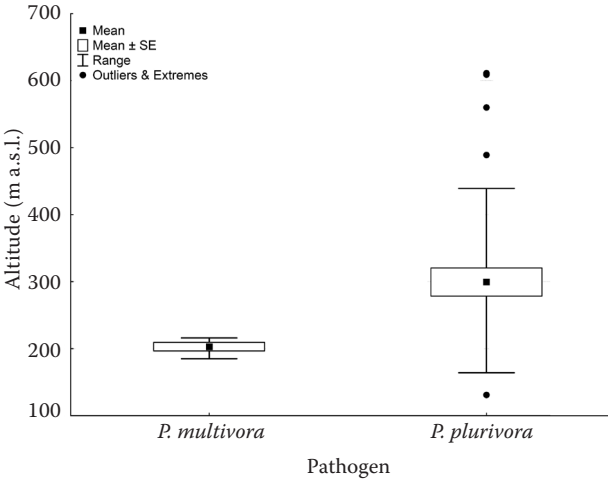

Figure 2. Vertical distribution of *P. multivora* and *P. plurivora* in the Czech Republic

162–216 m a.s.l. (212 m on average). The different distribution of *P. multivora* and *P. plurivora* with regard to the elevation was clear (Figure 2) and the Mann-Whitney U test was conclusive (P = 0.03). *P. multivora* was found in park, natural forest and riparian stands and in garden centre. Its most frequent host was oak; 9 out of the 11 stored isolates were obtained from this host. One natural lowland oak forest highly affected by *P. multivora* was also identified in South Moravia. Moreover, *P. multivora* was also isolated from rotten feeding roots and the rhizosphere of *Alnus* and *Salix* and from *Rhododendron* leaf lesions.

P. plurivora was found in 43 stands (Figure 1) of different types, such as ornamental and forest nurseries, garden centres, private gardens, parks, seminatural or natural forest stands and riparian stands. More than 1/3 of the isolates (39%) originated from natural forest and riparian stands, whereas 28% of the isolates were acquired from parks and 33% from nurseries (including forest nurseries) and gardens. The presence of the pathogen was recorded in a broad range of elevation (131–611 m a.s.l.; Figure 2), with an average of 317 m. In total, *P. plurivora* was isolated from 20 hosts, predominantly (22%) from *Rhododendron*, *Acer* (20%), *Quercus* (15%), *Fraxinus*, and *Tilia* (both 11%).

Analysis of pathogenicity

The pathogenicity test showed that both *P. multivora* and *P. plurivora* are able to cause damage in forest tree species. However, *P. plurivora* was usu-

Table 1. Host range of *P. multivora* and *P. plurivora* in the Czech Republic. Isolates marked by asterisk were used in the infection experiment

Host	Culture No.	Disease	Locality, region	Altitude (m a.s.l.)	Stand
P. multivora					
Alnus glutinosa	P355.09	root and collar rot	Tovačov, Olomouc	191	riparian stand
Rhododendron catawbiense 'Grandiflorum'	P030.06	leaf spot	Brno, South Moravian	216	nursery
Quercus robur	P159.07*	root rot, rhizosphere soil	Praha, Praha	208	park
	P242.08*	root rot, rhizosphere soil	Pouzdřany, South Moravian	185	natural forest
	P374.10	root rot, rhizosphere soil	Tovačov, Olomouc	191	riparian stand
	P554.11	root rot, rhizosphere soil	Lednice, South Moravian	162	park
Salix fragilis	P366.09	root rot, rhizosphere soil	Pikovice, Central Bohemian	215	riparian stand
P. plurivora					
Abies koreana	P560.12	root rot	Lhotky, Central Bohemian	359	nursery
Acer campestre	P305.09	root rot, rhizosphere soil	Troubky, Olomouc	209	natural forest
	P593.12	root rot, rhizosphere soil	Kopidlno, Hradec Králové	233	park
Acer platanoides	P241.08*	root rot, rhizosphere soil	Milovice, Central Bohemian	210	natural forest
	P593.12	root rot	Kopidlno, Hradec Králové	233	park
Acer pseudoplatanus	P232.08*	root rot, rhizosphere soil	Praha, Praha	208	park
	P294.09	root and collar rot	Růženín, Central Bohemian	286	riparian stand
	P334.09	root and collar rot	Křivošín, South Bohemian	609	pond bank
	P401.10	root and collar rot	Stružinec, South Bohemian	611	pond bank
	P474.11	root and collar rot	České Budějovice, South Bohemian	389	park
	P573.12	root rot, rhizosphere soil	Kostelec nad Orlicí, Hradec Králové	278	park
	P557.12	root rot, rhizosphere soil	Telč, Vysočina	521	park
Alnus glutinosa	P139.07*	root and collar rot	Březinka, Pardubice	395	pond bank
Fagus sylvatica	P201.08	root and collar rot	Praha, Praha	208	Park
	P558.12	root rot, rhizosphere soil	Lhotky, Central Bohemian	359	nursery
	P598.12	root rot	Čermná nad Orlicí, Hradec Králové	305	nursery
Fraxinus excelsior	P165.07*	root rot, rhizosphere soil	Praha, Praha	208	park
	P306.09	root rot, rhizosphere soil	Troubky, Olomouc	209	natural forest
	P405.10	root rot, rhizosphere soil	Dětmarovice, Moravian-Silesian	208	riparian stand
	P410.10	root rot, rhizosphere soil	Tisová, Plzeň	489	riparian stand
	P480.11	root rot, rhizosphere soil	Olomouc, Olomouc	216	park
Pieris floribunda	P126.07*	leaf spot	Průhonice, Central Bohemian	310	nursery
Pieris sp.	P455.11	root rot, rhizosphere soil	Plzeň, Plzeň	332	garden centre
Pinus strobus	P559.12	root and collar rot	Lhotky, Central Bohemian	359	nursery
Quercus robur	P070.07*	root rot, rhizosphere soil	Třeboň, South Bohemian	439	natural forest
	P164.07*	root rot, rhizosphere soil	Neratovice, Central Bohemian	185	natural forest
	P256.09	root rot, rhizosphere soil	Ivaň, South Moravian	175	natural forest
	P258.09	root rot, rhizosphere soil	Břeclav, South Moravian	164	natural forest
	P303.09	root rot, rhizosphere soil	Troubky, Olomouc	209	natural forest
	P309.09	root rot, rhizosphere soil	Chropyně, Zlín	201	natural forest
Quercus rubra	P167.07*	root rot, rhizosphere soil	Praha, Praha	208	park
	P493.11	root rot, rhizosphere soil	Blatná, Plzeň	442	park
	P566.12	root rot, rhizosphere soil	Děčín, Ústí nad Labem	131	park

Table 1 to be continued

Host	Culture No.	Disease	Locality, region	Altitude (m a.s.l.)	Stand
Rhododendron catawbiense 'Grandiflorum'	P029.06*	shoot blight and leaf spot	Tuřany, South Moravian	222	garden centre
Rhododendron 'Nicoline'	P301.09	root and collar rot	Markvartice, Vysočina	560	nursery
Rhododendron 'Roseum Elegans'	P033.06	leaf spot	Tuřany, South Moravian	222	garden centre
Rhododendron sp.	P162.07	leaf spot	Jevany, Central Bohemian	396	nursery
	P009.06	branch necrosis	Třebíč, Vysočina	435	nursery
	P034.06*	root and collar rot	Hvězdonice, Central Bohemian	288	private garden
	P036.06*	root and collar rot	Trutnov, Hradec Králové	418	private garden
	P102.07	root rot, rhizosphere soil	Kladno, Central Bohemian	371	garden centre
	P456.11	stem necrose	Martinice, Zlín	252	nursery
	P467.11	dieback	Olomouc, Olomouc	216	garden centre
	P494.11	dieback	Třeboň, South Bohemian	435	park
	P507.11	dieback	Čáslav, Central Bohemian	263	garden centre
Salix fragilis	P363.09	root rot, rhizosphere soil	Pikovice, Central Bohemian	215	riparian stand
Tilia cordata	P215.08*	root rot, rhizosphere soil	Ostrá, Central Bohemian	176	natural forest
	P304.09	root rot, rhizosphere soil	Troubky, Olomouc	209	natural forest
	P330.09	root rot, rhizosphere soil	Lysá n. Labem, Central Bohemian	179	seminatural forest
	P414.10	root rot, rhizosphere soil	Týn n. Bečvou, Olomouc	230	riparian stand
	P493.11	root rot, rhizosphere soil	Klatovy, Plzeň	416	park
	P546.11	root rot, rhizosphere soil	Kvasice, Zlín	190	park
Vaccinium sp.	P127.07*	shoot blight	Průhonice, Central Bohemian	310	nursery
Ulmus laevis	no stored	root rot, rhizosphere soil	Troubky, Olomouc	209	natural forest

ally more aggressive than *P. multivora* (Figure 3), as is evident from stem lesion lengths on tested host species of *Acer*, *Alnus*, *Fraxinus*, *Quercus*, and *Tilia* (Mann-Whitney U test; $P \leq 0.05$). The stem lesions caused by *P. plurivora* on *Fagus sylvatica* were also longer than those caused by *P. multivora*, but the difference was not significant ($P = 0.72$; data not shown).

Conclusively, the tested tree species differed in their susceptibility to the two pathogens (Kruskal-Wallis test; $P \leq 0.05$; Figure 3). As to *P. multivora*, *Fagus sylvatica* appeared to be the most sensitive host, whereas *Alnus glutinosa* and *Tilia cordata* were moderately susceptible, and *Acer pseudoplatanus*, *Quercus robur*, and *Fraxinus excelsior* seemed to be the least sensitive hosts. Susceptibility to *P. plurivora* was more or less similar: *Fagus sylvatica*, *Alnus glutinosa*, and *Tilia cordata* appeared to be the most sensitive, *Acer pseudoplatanus* and *Quercus robur* moderately sensitive, and *Fraxinus excelsior* the least sensitive hosts.

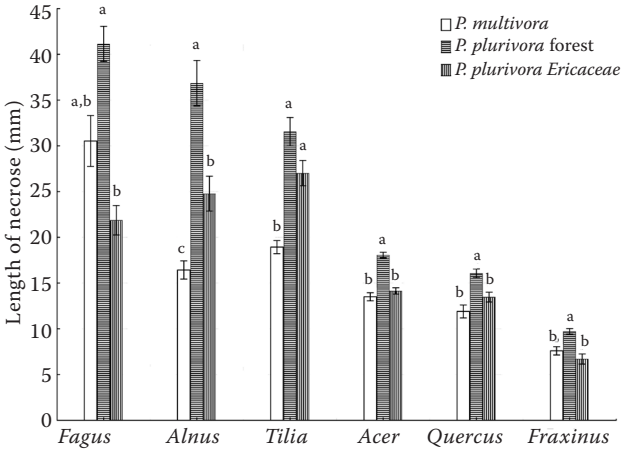

Figure 3. The lesion length caused by *P. multivora* and *P. plurivora* (isolates from forest trees and *Ericaceae*) in stems of forest tree seedlings after six-week infection experiment

The columns indexed by the same letter in individual hosts are not statistically different; there were inoculated and measured 15 seedlings in each isolate/host species combination; SE – standard error

Substrate specificity

Two isolates of *P. multivora* (P159.07 and P242.08, both isolated from *Quercus robur*) differed in the extent of damage caused in all of the tested tree species. The P159.07 isolate was consistently more aggressive than the P242.08 one, however the differences were only statistically supported in *Fagus* and *Fraxinus* (Mann-Whitney U test; $P < 0.05$). The mean length of lesions for P159.07 and P242.08 in *Fagus* was 37.47 and 23.60 mm, respectively, and in *Fraxinus* 9.20 and 6.00 mm, respectively.

The experiment confirmed the significant variability in the lesion length caused by the *P. plurivora* isolates in all six tested host taxa (Kruskall-Wallis test; $P < 0.001$). All of the tested tree species (i.e. *Acer pseudoplatanus*, *Alnus glutinosa*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus robur*, and *Tilia cordata*) were more damaged by the isolates obtained from the same host species (isolate P139.07 and P165.07) or other forest tree species (isolates P164.07, P167.07, and P241.08) than by the isolates from *Ericaceae*. When the isolates were classified into two groups according to the origin of isolation, i.e. forest trees and *Ericaceae*, it turned out that the isolates from the forest trees were more aggressive in each of the six of host taxa tested. The difference was statistically supported (Mann-Whitney U test; $P < 0.01$) in five host species (except for *Tilia*; Figure 3). The length of necrotic lesions caused by the isolates from *Ericaceae* varied between 6.71 mm (in *Fraxinus*) and 27.01 mm (in *Tilia*) after six weeks, whereas the lesion length caused by the isolates from the forest trees varied between 9.72 mm (*Fraxinus*) and 41.13 mm (*Fagus*) after six weeks (Figure 3).

The isolates of *P. plurivora* from forest trees were also more pathogenic to the tested tree species than the isolates of *P. multivora*. The difference in pathogenicity of *P. multivora* and *P. plurivora* from

Table 2. Morphological characteristics of Czech isolates of *P. multivora* and *P. plurivora* compared to original descriptions

	P. multivora		*P. plurivora*	
	Czech isolates	Scott *et al.* (2009)	Czech isolates	Jung & Burgess (2009)
No of isolates investigated	5	6	20	7
Sporangia				
l × b mean	43.0 ± 7.4 × 28.4 ± 5.3	51.0 ± 10.4 × 30.0 ± 5.1	40.1 ± 10.0 × 26.9 ± 4.3	47.4 ± 7.7 × 33.5 ± 5.9
range of isolate means	35.3–52.1 × 21.5–34.5	44.2–62.1 × 26.2–34.2	27.6–65.8 × 19.2–36.8	39.6–52.3 × 28.9–38.8
total range	22.2–71.1 × 14.6–47.8	36–58 × 13–33	17.1–105.8 × 11.1–45.5	27.5–80.5 × 16.7–69.6
l/b ratio	1.54 ± 0.15	1.7 ± 0.22	1.49 ± 0.24	1.43 ± 0.19
Oogonia				
mean diam	26.5 ± 2.8	26.5 ± 1.9	26.7 ± 1.4	28.5 ± 3.3
diam range	22.4–34.8	19–37	18.7–36.8	15.0–37.5
range of isolate means	23.4–29.9	25.5–27.8	24.4–29.2	27.5–29.9
Oospores				
aplerotic oospores	51% (30–80%)	45% (36–52%)	52% (25–89%)	44% (22–62%)
mean diam	24.3 ± 2.4	23.6 ± 1.8	24.9 ± 1.3	25.9 ± 3.1
diam range	20.6–32.3	17.3–33.1	14.7–33.6	14–35.8
wall diam	2.0 ± 0.5	2.6 ± 0.5	1.31 ± 0.15	1.45 ± 0.35
oospore wall index	0.41 ± 0.06	0.52 ± 0.07	0.28 ± 0.03	0.30 ± 0.06
Antheridia				
l × b mean	11.4 ± 0.7 × 8.7 ± 0.6	12.9 ± 1.9 × 8.7 ± 1.3	10.1 ± 0.9 × 7.3 ± 0.7	11.1 ± 4.4 × 8.4 ± 3.1
l × b range	7.2–15.8 × 5.9–12.1	8–20 × 5–14	4.5–16.2 × 3.9–14.8	7–21 × 5.3–16
Maximum temperature (°C)	32	32	32	32
Optimum temperature (°C)	25	25	25	25
Growth rate on V8A at optimum (mm/day)	7.3 ± 1.8	6.5 ± 0.02	8.3 ± 0.3	8.1 ± 0.2
Growth rate on V8A at 20°C (mm/day)	7.1 ± 1.5	4.8 ± 0.6	6.3 ± 0.9	6.3 ± 0.1

Ericaceae was rather minor (Figure 3) and significant ($P < 0.05$) only in *Alnus* and *Tilia*. Moreover, *P. multivora* was slightly more damaging to *Fagus* and *Fraxinus*, than to *P. plurivora* originated from *Ericaceae*.

Morphological analysis

The morphological and growth characteristics of the Czech isolates of *P. multivora* and *P. plurivora* usually corresponded to the original descriptions (Jung & Burgess 2009; Scott *et al.* 2009), as shown in Table 2. The differences between the two sorts of data were mostly negligible, regarding *P. plurivora* in particular. As for *P. multivora*, however, some differences between the Czech and Australian isolates were notable. The first difference was found for the thickness of the oospore wall and oospore wall index. In general, the values of these two characteristics were lower for the Czech isolates. The thickness of the oospore wall averaged 2.0 ± 0.5 µm for the Czech isolates, whereas it was 2.6 ± 0.5 µm for the Australian isolates. Similarly, the oospore wall index averaged 0.41 ± 0.06 for the Czech and 0.52 ± 0.07 for the Australian isolates. Another difference was a higher radial growth rate for the Czech than for the Australian isolates, which was particularly apparent at 20°C. The growth rate of the Czech isolates was 7.1 ± 1.5 mm/day as compared to 4.8 ± 0.6 mm/day for the Australian isolates. However, the two Czech *P. multivora* isolates, P30.06 (nursery finding from *Rhododendron*) and P366.09 (from *Salix* in riparian stand), grew similarly to the Australian isolates, whereas the growth rate of the other three isolates (from *Quercus* and *Alnus*) averaged 7.6 mm/day (Table 2).

DISCUSSION

We found that *P. plurivora* is widely distributed in the Czech Republic, which is in agreement with the overall European data (Jung & Burgess 2009). In contrast, *P. multivora* is reported to be infrequent in Europe and more or less limited to nurseries (Jung & Burgess 2009). However, the pathogen was isolated from 3 natural stands in the present study and was also isolated from an oak forest in Hungary by I. Szabó (Jung & Burgess 2009). Thus, the population of *P. multivora* is most likely established in natural stands in Central Europe.

Although the introduction of *P. multivora* to Europe is apparently more recent than that of *P. plurivora* (Jung & Burgess 2009) and limited information about its distribution is available, it can be speculated that the areas and niches of these pathogens partially differ. The distribution of *P. multivora* in the Czech Republic seems to be more or less restricted to the two warm and dry areas in the Czech Republic, i.e. Central Bohemia and South Moravia (Figure 1; Tolasz *et al.* 2007). This finding is in accordance with the report of Jung & Burgess (2009) in that *P. multivora* is likely more adapted to a dry climate. It is inferred that the thick oospore wall of *P. multivora* is an adaptation to the extremely dry soil conditions in its native area in Western Australia (Scott *et al.* 2009). However, the presence of *P. multivora* in wet riparian stands in Central Europe indicates that humidity of the environment cannot play an important role in its distribution.

All the Czech *P. multivora* isolates were acquired in lower regions and in anthropogenic stands (nurseries, parks) or in river corridors (fluvisols in riparian stands and alluvial plains) – i.e. in areas and stands that generally exhibit a high level of plant invasion (Chytrý *et al.* 2009). Moreover, all natural findings of the pathogen – in riparian stands (the distance from the water line was up to 4 m) and in one alluvial oak forest indicate that the pathogen is able to spread via river- and floodwater in the natural environment. The character of the present *P. multivora* distribution in the Czech Republic seems to be primarily determined by higher level of human activities and higher chance of introduction in lower areas and by the presence of potential pathways (watercourses) through following spread. The character of the pathogen distribution in the area also indicates that *P. multivora* is in an early stage of invasion into the environment.

The observed close relation of *P. multivora* to oak in comparison to *P. plurivora* may be a consequence of higher frequency of the host in lower vegetation stages in the area.

The pathogenicity test on 6 native forest tree species revealed that *P. multivora* is usually less aggressive to the tested hosts than *P. plurivora*. In comparison with other inoculation experiments (e.g. Werres 1995; Vettraino *et al.* 2001; Fleischmann *et al.* 2002, 2004; Holub *et al.* 2010), however, the aggressiveness of *P. multivora* to forest trees might be at a level similar to that of *P. cactorum*

or *P. cambivora*. Therefore, *P. multivora* may also pose a potential risk to European forests, at least in Central and Mediterranean Europe, because the examined oak forest in South Moravia was highly affected by this species. The probable high impact of *P. multivora* and *P. plurivora* on oak lowland forests in South Moravia can be related to changes in temperature and rainfall (Jung 2009) and also to the canalisation of the Moravian lowland rivers in the 1970s and 1980s. These changes led to a significant decrease of the underground water table, to the ensuing water stress in the affected lowland forests, and to an increase of plants' susceptibility to fungal diseases (Čermák & Prax 2001).

Very likely, *P. multivora* will rank among other alien or cryptogenic *Phytophthora* pathogens, such as *P. plurivora*, *P. cactorum*, and *P. cambivora*, damaging the forest trees and causing forest decline in Central Europe (e.g. Jung *et al.* 2000, 2005; Jung 2009).

The presented broad range of affected hosts and stands of *P. plurivora* are in accordance with extensive reports on its distribution (Jung 2009; Jung & Burgess 2009). The common distribution of *P. plurivora* in natural and seminatural riparian and forest stands raises much concern. Furthermore, its partial substrate specificity was verified in that the isolates from forests trees were more aggressive to such trees than the isolates from ericaceous plants from nurseries and gardens. Thus, it is hypothesised that two subpopulations (in anthropogenic stands and natural stands) may exist on and are specialised for the accessible hosts in these different environments.

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