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SHORT COMMUNICATION

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Molecular analysis of *Phytophthora* species found in Poland

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Abstract

Pathogens of *Phytophthora* genus are common not only in forest nurseries and stands, but also in water courses. Species of *Phytophthora* spread with plants for plantings (and soil attached to them) and with water courses as well, attacking the plants growing in riparian ecosystems. Several specialized organisms damaging only one tree species were identified like *P. alni* on alders or *P. quercina* on oaks. Some *Phytophthora* species can develop on several hosts like *P. plurivora* and *P. cactorum* on oaks, beeches, alders, ashes and horse chestnuts. Other oomycetes like *P. gallica* species was found for the first time in Poland in water used for plant watering in forest nursery. Species *P. lacustris* and *P. gonapodyides* were found in superficial water. *Phytophthora* species *P. polonica* was identified in the declining alder stands for the first time in the world, and *P.* taxon *hungarica* and *P. megasperma* were found in the rhizosphere of seriously damaged ash stands for the first time in Poland. The most often isolated species were *P. plurivora* (clade 2) with frequency 37% and *P. lacustris* with frequency 33% (clade 6). The best represented clade 6 revealed the occurrence of 6 species: *P. gonapodyides, P. lacustris, P. megasperma, P.* sp. *raspberry*, *P.* taxon *hungarica* and *P.* taxon oak soil.

Key words

fine root pathogens, sequencing DNA, alien, invasive, emerging

Introduction

An increase in trade of plants and globalization pose a risk of plant disease epidemics, resulting from introductions of exotic plant pathogens. An associated risk that accelerates pathogen evolution may occur as a consequence of genetic exchange between introduced or introduced and resident pathogens (Brasier et al. 1999). There is a likelihood of such evolutionary events occurring in Poland, as well. On the other hand, new diagnostic methods based on molecular tools are currently sufficiently sensitive to allow detection of new phytopathogens. Recently, in forestry, emerging diseases are caused by invasive, alien oomycetes, which are soil-borne fine root pathogens, sometimes specializing in damage of certain forest tree species as their host. As established in

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Central Europe, *Phytophthora quercina* is often recovered from declining oaks proved to be more pathogenic to European oaks *Q. robur* than any other *Phytophthora* species (Jung et al. 1999). The common species *Phytophthora plurivora* occurs all over Italy, while *P. quercina* is the species significantly associated with declining of oak trees (Vettraino et al. 2002). In Italy, eleven soil-borne species of *Phytophthora* were detected in oak forests with 35% as the frequency of isolations, being also correlated with soil pH and longitude of the sites. *P. cactorum* was recovered from sites in central and southern Italy, whereas *P. quercina* was isolated in the northern and central part of the country. In Denmark, several species of *Phytophthora* were found in the rhizosphere of declining ash trees (Orlikowski et al. 2011); earlier, they were also found in nurseries (Jung et al. 2016).

Since pathogens from genus *Phytophthora* are responsible for serious diseases world-wide and can occur on a wide range of hosts, in the present study, we concentrated on an assessment of the occurrence of these pathogens in the Polish forest nurseries and stands.

Material and methods

Soil, together with the root system, was sampled in plastic bags weighing 0.5 kg each and isolation tests were performed using rhododendron, oak or beech leaves as baits. Water was collected with 1.5 l plastic bottles, which were sterilized with 70% ethanol and washed with distilled water. The sampled water was filtered in the lab, using the Millipore vacuum pump with nylon filters of 5 µm pore-size. Filters with biological sediment as well as the fragments of discoloured bait tissues were placed on selective PARPNH medium (potato dextrose agar amended with 10 µg ml^{-1} pimaricin, 200 µg ml^{-1} ampicillin, 10 µg ml^{-1} rifampicin, 25 µg ml^{-1} pentachloronitrobenzene (PCNB), 50 µg ml^{-1} nystatin, and 50 µg ml^{-1} hymexazol).

Pure cultures of *Phytophthora* sp. isolates obtained from the water and soil samples were grown in the liquid V8 media (100 ml clarified V8 juice in 900 ml distilled water, amended with 2 g of $CaCO_3$ for 3–5 days in the dark at 22–25°C. The mycelium was subsequently rinsed in sterile distilled water, dried and disrupted in liquid nitrogen prior to the DNA extraction. Total DNA was extracted from mycelium by using GenElute^{TM} Plant Genomic DNA Miniprep Kit (Sigma-Aldrich® GmbH, Germany), following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification of the ITS region of the template DNA was performed using primers ITS6 and ITS4 (White et al. 1990; Cooke et al. 2000) in a 50 µl reaction containing 50–100 ng genomic DNA, 250 nM of each primer, 200 µM of each dNTP, 1 mM $MgCl_2$, 1U *Taq* polymerase, 1xQ solution and 1xPCR buffer (Qiagen Ltd., Valencia, CA, USA). The reaction was performed in a PTC-200Ô Programmable Thermal Controller (MJ Research, Inc.) for 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 50 s, with initial denaturation of 3 min at 94°C before cycling and a final extension of 10 min at 72°C after cycling. The PCR product was purified using the Clean-up kit (A&A Biotechnology), following the manufacturer's protocol. Sequencing was conducted on a CEQ™8000 9.0.25 automated sequencer, (Beckman Coulter®, Fullerton, USA). Forward and reverse sequences were linked in BioEdit software and the resulting sequences were aligned with NCBI Nucleotide collection.

All the collected sequences were compared in ITS1 region by using the ClustalW algorithm provided in the BioEdit software; further phylogenetic analysis was performed using MEGA5. The Maximum Likelihood method based on the Tamura-Nei model was used. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Joining and BIONJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 117 nucleotide sequences (116 *Phytophthora* sequences and *Pythium sterilum* JX271797 sequence as an outgroup).

Results

As given in the table below, the *Phytophthora* isolates were identified to species on the basis of sequence alignment with NCBI database (Tab. 1). Among the list of identified *Phytophthora* isolates, there is a new species in Poland – *Phytophthora gallica,* which is considered to be moderately aggressive to *Alnus glutinosa* and *Fagus sylvatica*, weakly aggressive to *Quercus robur* and *Salix alba* and non-pathogenic to *Fraxinus excelsior* (Jung and Nechwatal 2008). The origin of *P. gallica* and its ecological role in wet ecosystems remain unclear.

Table 1. List of *Phytophthora* isolates used in the study

NCBI №	Isolate	Country	Location	Host	Sample	IBL №
1	2	3	4	5	6	7
JX276034	*P. alni*	Poland	Sękocin	*Alnus glutinosa*	forest	IBL/2011/1/1
JX276035	*P. alni*	Poland	Sękocin	*Alnus glutinosa*	forest	IBL/2011/2/1
EF152518	*P. alni*	Poland	Żyrardów	*Alnus glutinosa*	forest	825a
EF152517	*P. alni*	Poland	Żyrardów	*Alnus glutinosa*	forest	825b
EF152516	*P. alni*	Poland	Żyrardów	*Alnus glutinosa*	forest	825c
JX276022	*P. cactorum*	Poland	Konstantynowo	*Fraxinus excelsior*	forest	IBL/2011/212
JX276028	*P. cactorum*	Poland	Konstantynowo	*Quercus robur*	forest	IBL/2011/220
JX276029	*P. cactorum*	Poland	Konstantynowo	*Fraxinus excelsior*	forest	IBL/2011/221
JX276030	*P. cactorum*	Poland	Konstantynowo	*Quercus robur*	forest	IBL/2011/223
JX276031	*P. cactorum*	Poland	Konstantynowo	*Fraxinus excelsior*	forest	IBL/2011/225
JX276019	*P. cactorum*	Poland	Krotoszyn	*Quercus robur*	forest	IBL/2011/210
EU240056	*P. cactorum*	Poland	Moszczanka	*Fagus sylvatica*	forest	798
EU240060	*P. cactorum*	Poland	Radziejowice	*Aesculus hippocastanum*	park	813
EU240045	*P. cactorum*	Poland	Wilanowice	*Fraxinus excelsior*	nursery	748
EU240182	*P. cactorum*	Poland	Wilanowice	*Quercus robur*	forest	753
EU240061	*P. cactorum*	Poland	Wilanowice	*Fagus sylvatica*	forest	764A
JX271803	*P. gallica*	Poland	Kiejsze	*riparian area*	water	IBL/2011/28/2
JX276033	*P. gonapodyides*	Poland	Chojnów	*Alnus glutinosa*	forest	IBL/2011/232
JX276038	*P. gonapodyides*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/10/4/1
JX276041	*P. gonapodyides*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/8/1/10
JX276036	*P. gonapodyides*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/8/1/6
EU240125	*P. lacustris*	Poland	Bug river	*Quercus robur*	water	WD40A
EU240126	*P. lacustris*	Poland	Bug river	*Quercus robur*	water	WD40C
EU240137	*P. lacustris*	Poland	Bug river	*Quercus robur*	water	WD40E
EU240042	*P. lacustris*	Poland	Bug river	*Quercus robur*	water	WD41a
EU240175	*P. lacustris*	Poland	Bug river	*Quercus robur*	water	WD43A
EU240152	*P. lacustris*	Poland	Chojnów	*Quercus robur*	nursery	GD15A
EU240088	*P. lacustris*	Poland	Dąbie	*Alnus glutinosa*	forest	GD7B
EU240089	*P. lacustris*	Poland	Dąbie	*Alnus glutinosa*	forest	GD7C
EU240091	*P. lacustris*	Poland	Dąbie	*Alnus glutinosa*	forest	GD7G
EU240166	*P. lacustris*	Poland	Kanał Królewski	*Alnus glutinosa*	water	WD47A
EU240179	*P. lacustris*	Poland	Kiejsze	*riparian area*	water	920
EU240180	*P. lacustris*	Poland	Kiejsze	*riparian area*	water	921
EU240181	*P. lacustris*	Poland	Kiejsze	*riparian area*	water	922

1	2	3	4	5	6	7
EU240197	*P. lacustris*	Poland	Kiejsze	*riparian area*	water	923
EU240159	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD37A
EU240099	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD37B
EU240160	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD37C
EU240123	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD38A
EU240124	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD38B
EU240100	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD39A
EU240161	*P. lacustris*	Poland	Narew river	*riparian area*	water	WD39B
JX271790	*P. lacustris*	Poland	Orlanka river	*riparian area*	water	IBL/2011/10/1
JX271791	*P. lacustris*	Poland	Orlanka river	*riparian area*	water	IBL/2011/10/2
EU240153	*P. lacustris*	Poland	Pomiechówek	*Acer pseudoplatanus*	forest	GD18G
EU240138	*P. lacustris*	Poland	Rządza river	*Acer pseudoplatanus*	water	WD44B
EU240164	*P. lacustris*	Poland	Rządza river	*Acer pseudoplatanus*	water	WD44C
EU240177	*P. lacustris*	Poland	Rządza river	*Acer pseudoplatanus*	water	WD45A
EU240101	*P. lacustris*	Poland	Rządza river	*Acer pseudoplatanus*	water	WD45B
EU240165	*P. lacustris*	Poland	Rządza river	*Acer pseudoplatanus*	water	WD45C
EU240167	*P. lacustris*	Poland	Sękocin	*Acer pseudoplatanus*	water	B02
EU240094	*P. lacustris*	Poland	Sękocin	*Acer pseudoplatanus*	water	B04
EU240037	*P. lacustris*	Poland	Sękocin	*Acer pseudoplatanus*	water	B14
EU240102	*P. lacustris*	Poland	Sokołówka	*Quercus robur*	nursery	GD36D
EU240067	*P. lacustris*	Poland	Sokołówka	*Quercus robur*	nursery	GD36F
EU240066	*P. lacustris*	Poland	Sokołówka	*Quercus robur*	nursery	GD40A
EU240184	*P. lacustris*	Poland	Sokołówka	*Quercus robur*	nursery	GD40C
EU240065	*P. lacustris*	Poland	Sokołówka	*Quercus robur*	nursery	GD40D
JX271796	*P. lacustris*	Poland	Zuzela	*Quercus robur*	water	IBL/2011/13/2
JX274423	*P. megasperma*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/9/3
EU240052	*P. plurivora*	Poland	Biechów	*Fraxinus excelsior*	forest	785
EU240053	*P. plurivora*	Poland	Biechów	*Fraxinus excelsior*	forest	786
EF152519	*P. plurivora*	Poland	Biernatów	*Quercus robur*	forest	790a
EU240075	*P. plurivora*	Poland	Biernatów	*Quercus robur*	forest	791A
JX276018	*P. plurivora*	Poland	Broniszew	*Pyrus*	orchard	IBL/2011/206
EU240085	*P. plurivora*	Poland	Buków	*Pyrus*	nursery	754A
EU240054	*P. plurivora*	Poland	Dębowiec	*Pyrus*	forest	788
EU240077	*P. plurivora*	Poland	Dębowiec	*Pyrus*	forest	787A
EU240076	*P. plurivora*	Poland	Dębowiec	*Pyrus*	forest	787B
JX276023	*P. plurivora*	Poland	Konstantynowo	*Fraxinus excelsior*	forest	IBL/2011/213
JX276024	*P. plurivora*	Poland	Konstantynowo	*Fraxinus excelsior*	forest	IBL/2011/214
JX276025	*P. plurivora*	Poland	Konstantynowo	*Quercus robur*	forest	IBL/2011/216
JX276027	*P. plurivora*	Poland	Konstantynowo	*Quercus robur*	forest	IBL/2011/219
EU240188	*P. plurivora*	Poland	Korczew	*Alnus glutinosa*	forest	6’’a
EU240189	*P. plurivora*	Poland	Korczew	*Alnus glutinosa*	forest	6’’b

1	2	3	4	5	6	7
EU240192	*P. plurivora*	Poland	Korczew	*Alnus glutinosa*	forest	6’’’c
EU240193	*P. plurivora*	Poland	Korczew	*Alnus glutinosa*	forest	6’’’d
EU240194	*P. plurivora*	Poland	Korczew	*Alnus glutinosa*	forest	6’’’e
EU240195	*P. plurivora*	Poland	Korczew	*Alnus glutinosa*	forest	6’’’f
EU240055	*P. plurivora*	Poland	Lipowa	*Alnus glutinosa*	forest	794
EU240057	*P. plurivora*	Poland	Moszczanka	*Fagus sylvatica*	forest	801
EU240050	*P. plurivora*	Poland	Opawice	*Fagus sylvatica*	forest	776
EU240051	*P. plurivora*	Poland	Opawice	*Quercus robur*	nursery	778
EU240080	*P. plurivora*	Poland	Opawice	*Quercus robur*	nursery	777A
EU240079	*P. plurivora*	Poland	Opawice	*Fagus sylvatica*	nursery	779A
EU240078	*P. plurivora*	Poland	Opawice	*Fraxinus excelsior*	nursery	780A
EU240183	*P. plurivora*	Poland	Pokrzywna	*Fraxinus excelsior*	forest	772
EU240187	*P. plurivora*	Poland	Pokrzywna	*Fraxinus excelsior*	forest	769A
EU240058	*P. plurivora*	Poland	Szklary	*Fraxinus excelsior*	forest	804
EU240059	*P. plurivora*	Poland	Szklary	*Fraxinus excelsior*	forest	806
EU240044	*P. plurivora*	Poland	Wilanowice	*Fagus sylvatica*	nursery	747
EU240046	*P. plurivora*	Poland	Wilanowice	*Fagus sylvatica*	forest	755
JX274421	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/3/4
JX274427	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/5/3
JX274422	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/5/5
JX274420	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/6/2
JX274425	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/7A
JX274426	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/7c
JX274424	*P. plurivora*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/8/1
JX276032	*P. plurivora*	Poland	Zabuże	*Alnus glutinosa*	forest	IBL/2011/231
JX276057	*P. plurivora*	Poland	Zuzela	*Alnus glutinosa*	water	IBL/2011/11
JX276051	*P. plurivora*	Poland	Zuzela	*Alnus glutinosa*	water	IBL/2011/1/2
JX276052	*P. plurivora*	Poland	Zuzela	*Alnus glutinosa*	water	IBL/2011/2/2
EU240063	*P. polonica*	Poland	Dąbie	*Alnus glutinosa*	forest	GD7A
EU240198	*P. polonica*	Poland	Dąbie	*Alnus glutinosa*	forest	GD7D
EU240093	*P. polonica*	Poland	Dąbie	*Alnus glutinosa*	forest	GD7I
JX276017	*P. polonica*	Poland	Kwidzyń	*Alnus glutinosa*	forest	IBL/2011/204
JX276020	*P. quercina*	Poland	Piaski	*Quercus robur*	forest	IBL/2011/211/1
JX276021	*P. quercina*	Poland	Piaski	*Quercus robur*	forest	IBL/2011/211/2
EU240068	*P. sp. raspberry*	Poland	Dobieszyn	*Quercus petraea*	nursery	GD23B
JX274428	*P. taxon hungarica*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/9/7
JX274429	*P. taxon hungarica*	Poland	Wolica	*Fraxinus excelsior*	forest	IBL/2012/9/8
JX276040	*P. taxon oaksoil*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/6/1/7
JX276042	*P. taxon oaksoil*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/7/1/4
JX276043	*P. taxon oaksoil*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/7/1/5
JX276037	*P. taxon oaksoil*	Poland	Oborniki	*Quercus robur*	forest	IBL/2011/9/1/2

As many as 12 species of *Phytophthora* belonging to 7 different clades were found based on the sequence analysis (Tab. 2). The most abundant clades present in Poland are clade 6 (43.1%) and 2 (37.1%). *Phytophthora* species in clade 6 have non-papillate sporangia and are mostly infectious to roots or present in the rhizosphere. *Phytophthora plurivora,* the only representative of clade 2, is considered to be the cause of several devastating declines and diebacks of major forest tree species.

Table 2. *Phytophthora* species found in Poland

Species	Clade	N	%
P. alni	7a	5	4.31
P. cactorum	1a	11	9.48
P. gallica	10	1	0.86
P. gonapodyides	6	4	3.45
P. lacustris	6	38	32.76
P. megasperma	6	1	0.86
P. plurivora	2	43	37.07
P. polonica	9	4	3.45
P. quercina	4	2	1.72
P. sp. raspberry	6	1	0.86
P. taxon hungarica	6	2	1.72
P. taxon oaksoil	6	4	3.45

As shown, many of the discovered *Phytophthora* species were found on different hosts, including important forest tree species (Tab. 3). Also, there was a diversity in the age of host species – *Phytophthora* species were found in mature forest stands and on tree seedlings in forest nurseries. The identification of species like *P. gallica, P. lacustris, P. gonapodyides* and *P. alni* in riparian ecosystems was possible due to the use of water filtration techniques, plating and DNA (ITS) analysis.

Since 2000, an increasing decline and dieback of alders has been observed in Poland. Ten different species of obtained *Phytophthora* isolates, including those shown in Table 3, originated from diseased trunks and from rhizosphere (Trzewik et al. 2015). Phylogeny of Polish *Phytophthora* species is shown on the dendrogram created based on Maximum Likelihood method (Fig. 1). The new for knowledge oomycete species *P. polonica* was found in declining alder stands along the river Ner (Belbahri et al. 2006).

Table 3. Host range of *Phytophthora* species fund in Poland

Species	Hosts
P. alni	*Alnus glutinosa*
P. cactorum	*Aesculus hippocastanum*
	Fagus sylvatica
	Fraxinus excelsior
	Quercus robur
P. gonapodyides	*Alnus glutinosa*
	Quercus robur
P. lacustris	*Acer pseudoplatanus*
	Alnus glutinosa
	Quercus robur
P. megasperma	*Fraxinus excelsior*
P. plurivora	*Alnus glutinosa*
	Fagus sylvatica
	Fraxinus excelsior
	Pyrus sp.
	Quercus robur
P. polonica	*Alnus glutinosa*
P. quercina	*Quercus robur*
P. sp. raspberry	*Quercus petraea*
P. taxon hungarica	*Fraxinus excelsior*
P. taxon oaksoil	*Quercus robur*

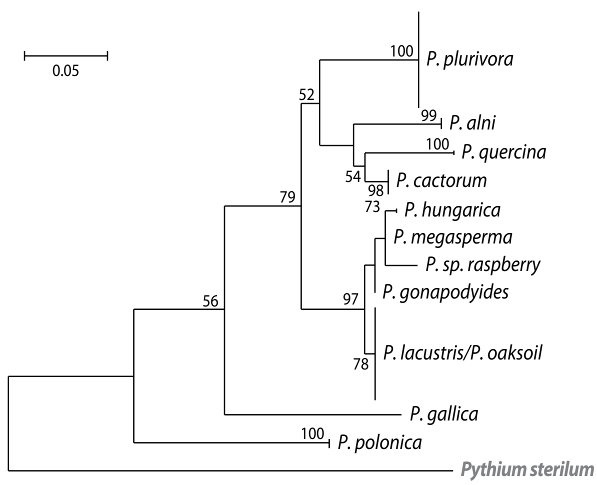

Figure 1. Phylogeny of *Phytophthora* isolates (ML method, bootstrap = 1000)

Conclusions

1. Pathogens of *Phytophthora* genus are common not only in nurseries and forest stands, but also in parks and orchards.
2. Species of *Phytophthora* spread with plants for plantings (and soil attached to them) and with water along water courses as well, attacking the plant associations or shelterbelts of the riparian ecosystems, especially alders.
3. Several specialized organisms damaging only one tree species were identified like *P. alni* on alders or *P. quercina* on oaks.
4. Some *Phytophthora* species can develop on several hosts like *P. plurivora* and *P. cactorum* on oaks, beeches, alders, ashes and horse chestnuts.
5. Other oomycetes like *P. gallica* species was found for the first time in Poland in water used for plant watering in the Kiejsze nursery (Koło Forest District).
6. In water ecosystems, species like *P. lacustris* and *P. gonapodyides* were found. The pathogenicity of these species is not fully recognized yet.
7. For the first time in the world, the new *Phytophthora* species *P. polonica* was identified in the declining alder stands (Koło FD); and for the first time in Poland, two other species *P.* taxon *hungarica* and *P. megasperma* were found in the rhizosphere of seriously damaged ash stands (showing ash dieback).
8. The most often isolated species were *P. plurivora* (clade 2) with frequency 37% and *P. lacustris* with frequency 33% (clade 6).
9. The best represented clade 6 revealed the occurrence of 6 species: *P. gonapodyides*, *P. lacustris*, *P. megasperma*, *P.* sp. *raspberry, P.* taxon *hungarica* and *P.* taxon oak soil.

References

Belbahri L., Moralejo E., Calmin G., Oszako T., García J.A., Descals E., Lefort F. 2006. *Phytophthora polonica*, a new species isolated from declining *Alnus glutinosa* stands in Poland. *FEMS Microbiology Letters*, 261 (2), 165–174.

Brasier C.M., Cooke D.E.L., Duncan J.M. 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences*, 96 (10), 5878–5883.

Cooke D.E.L., Duncan J.M., Williams N.A., Weerdt M., Bonants P.J.M. 2000. Identification of *Phytophthora* species on the basis of restriction enzyme fragment analysis of the internal transcribed spacer regions of ribosomal RNA. *EPPO Bulletin*, 30 (3/4), 519–523.

Jung T., Cooke D.E.L., Blaschke H., Duncan J.M., Osswald W. 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. *Mycological Research*, 103 (7), 785–798.

Jung T., Nechwatal J. 2008. *Phytophthora gallica* sp. nov., a new species from rhizosphere soil of declining oak and reed stands in France and Germany. *Mycological research*, 112 (10), 1195–1205.

Jung T., Orlikowski L., Henricot B., Abad-Campos P., Aday A.G., Aguín Casal O., Bakonyi J., Cacciola S.O., Cech T., Chavarriaga D., Corcobado T., Cravador A., Decourcelle T., Denton G., Diamandis S., Doğmuş-Lehtijärvi H.T., Franceschini A., Ginetti B., Green S., Glavendekić M., Hantula J., Hartmann G., Herrero M., Ivic D., Horta Jung M., Lilja A., Keca N., Kramarets V., Lyubenova A., Machado H., Magnano di San Lio G., Mansilla Vázquez P. J., Marçais B., Matsiakh I., Milenkovic I., Moricca S., Nagy Z.Á., Nechwatal J., Olsson C., Oszako T., Pane A., Paplomatas E.J., Pintos Varela C., Prospero S., Rial Martínez C., Rigling D., Robin C., Rytkönen A., Sánchez M.E., Sanz Ros A.V., Scanu B., Schlenzig A., Schumacher J., Slavov S., Solla A., Sousa E., Stenlid J., Talgø V., Tomic Z., Tsopelas P., Vannini A., Vettraino A.M., Wenneker M., Woodward S., Peréz-Sierra A. 2016. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. *Forest Pathology*, 46 (2), 134–163.

Orlikowski L.B., Ptaszek M., Rodziewicz A., Nechwatal J., Thinggaard K., Jung T. 2011. Phytophthora root and collar rot of mature *Fraxinus excelsior* in forest stands in Poland and Denmark. *Forest Pathology*, 41 (6), 510–519.

Vettraino A.M., Barzanti G.P., Bianco M.C., Ragazzi A., Capretti P., Paoletti E., Luisi N., Anselmi N., Vannini A. 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology,* 32 (1), 19–28.

Trzewik A., Orlikowski L.B., Oszako T., Nowakowska J.A., Orlikowska T. 2015. The characterization of *Phytophthora* isolates obtained from diseased *Alnus glutinosa* in Poland. *Baltic Forestry*, 21 (1), 44–50.

White T.J., Bruns T., Lee S.J.W.T., Taylor J.L. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18 (1), 315–322.