Phytophthora and *Pythium* Species Detected in Rivers of the Polish-Ukrainian Border Areas

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Abstract

There are many records in the literature demonstrating that species belonging to the genus *Phytophthora* and *Pythium* cause damage to fine root and trunk collars of many forest tree species. Natural water courses can be an important but often neglected source of these phytopathogens. The detection of pathogenic *Oomycetes* organisms in the Ukrainian and Polish rivers was performed with the methods of baiting and filtering of water. The former was applied in Ukraine, and the latter in Poland. Branches with young leaves of beech, oak and black alder were used as baits and floated on the river surfaces for 3 days. Three *Phytophthora* species (*Ph. gonapodyides*, *Ph. lacustris* and *Ph. cactorum)* were detected in Ukrainian rivers presumably moving naturally towards the EU border. Species of the *Pythium* genus, and among them the most common ones like *P. lycopersicum*, *P. sylvaticum*, *P. citrinum* and *P. terrestris,* were detected in Polish rivers.

Keywords: water courses, *Oomycetes*, phytopathogens, seedlings, watering, nurseries

Introduction

Invasive plant pathogens, especially those of the genus *Phytophthora* are recognized as an emerging threat to natural ecosystems, as well as to horticultural production systems (Hansen 2015, Jankowiak et al. 2015, Rahman et al. 2015). Species of the genus *Phytophthora* can be divided into three groups: airborne, soil-borne and waterborne (Erwin and Ribeiro 1996, Martin et al. 2012, Park et al. 2013). The first group has the advantage of being able to transfer zoospores via air and water splashes, and can survive in soil. The biological life cycle of the second group is strongly linked with specific soil properties, most of these organisms are fine root pathogens that infect plant tissues via zoospores, especially when soil is saturated with water, e.g. Huai et al. (2013) identified several *Phytophthora* species baited and isolated from forest soil in north-western Yunnan province, China. The third group of *Phytophthora* resides in water ecosystems, which are fundamental to their biological lifecycle (sporangia with zoospores). Copes et al. (2015) recovered many organisms of the *Phytophthora* genus from irrigation reservoirs in Mississippi and Alabama, including three new species. In spite of these findings, watercourses as natural spread pathways of phytopathogens are still very often neglected in contemporary forest protection prevention. The first record dates back as early as 1921, when *Phytophthora cryptogea* was found in the water used for watering plants in a nursery greenhouse (Bewley and Buddin 1921). Later, e.g. a survey by Hüberli et al. 2013 in Western Australia waterways and survey of soil and water in South African natural ecosystems by Oh et al. 2013 revealed a wide *Phytophthora* distribution and diversity. In addition, the species responsible for alder decline was frequently found in riparian ecosystems of western Oregon, USA (Sims et al. 2015). Especially, plants like the aforementioned alders (nota bene considered to be a forest as an ornamental species), growing along rivers are in danger because of a new species, *Ph. alni,* spreading within the water courses. This species specializes in damaging only alders and probably, being very virulent, has already killed millions of trees across Europe (Streito et al. 2002). This opinion was accepted at the end of 20th century by other scientists, when mass dying of alder plantations (known as alder dieback) has occurred in Britain, France and Germany, especially near the river banks in riparian ecosystems (Gibbs 1999, Jung 2000). In Poland, the possibility of spread of zoosporic organisms via water courses has been demonstrated several times in the horticulture sector, especially in ornamental plant cultivation (Nowak et al. 2015, Orlikowski 2006, Orlikowski et al. 2007, 2008, Ptaszek and Orlikowski 2015). In the case of oaks the greatest danger exists, when zoospores multiple quickly in forest soil and together attack the fine roots, i.e. in such a case even 80-90% of fine roots could be lost by adult trees. Periodical stand flooding or even a precipitation in the period of the optimal temperature favours the development of sporangia releasing zoospores into the water accumulated among soil particles. In addition, structures such as oospores and chlamydospores can survive a long time without any rain and can readily infect plants in the next favourable period (Schlenzing et al. 2014).

In Ukraine, disease symptoms indicative of *Phytophthora* infections, have been recorded during the last decade in forest stands and nurseries. They included appearance of lesions and cankers, as well as the atrophy of leaves in the tree crowns. Taking into account the economic and ecological threats posed by *Phytophthora* species, a study in Ukrainian ecosystems started in 2010, aiming to determine the diversity of these pathogens (Kramarets et al. 2011). The first report of *Phytophthora* species in water sources in Ukraine and the occurrence of *Phytophthora* species in the rivers Stryj and Yasenytsya and in the lake situated in „Skoliwski Beskidy" National Park was documented in 2012 (Matsiakh et al. 2012). The detection of the pathogen was carried out in October and November in 2011 using baiting technique for the isolation of microorganisms from water with rhododendron leaves, the cultivar Nova Zembla. Our studies showed the occurrence of *Phytophthora gonapodyides*, *P. lacustris* and *Pythium litorale* in analysed water sources.

The aim of this work was to study the diversity of pathogenic *Oomycetes* with special regard to species of *Phytophthora* genus, in the border areas between Poland and Ukraine.

Materials and Methods

Sampling sites

The water to be studied for the occurrence of Oomycetes was taken from six rivers crossing the Polish-Ukrainian border (or being their tributaries). The geographic coordinates of eight observation plots are given below (Table 1) and their localizations are marked in the map (Figure 1). The rivers were adjacent to the forests, where *Phytophthora* symptoms were observed. Riparian vegetation was dominated mainly with such tree species like alder, ash and willow, residential or agricultural development was not pronounced upstream and downstream of the sampling sites. In general, in the analysed rivers the thread of stream is from east to west direction

***Isolation of Phytophthora* spp.**

The detection of Oomycetes in the Ukrainian and Polish rivers was performed with two methods: baiting and filtering of water with the help of "multilayer" microfilters. The baiting method was performed in Ukraine and water filtration in Poland (Figures 2, 3).

Branches with young leaves of beech, oak and black alder were used as baits and floated on the river surfaces for 3 days (Figure 2). After appearance of the first necrotic spots on the leaves, pieces were aseptically cut out

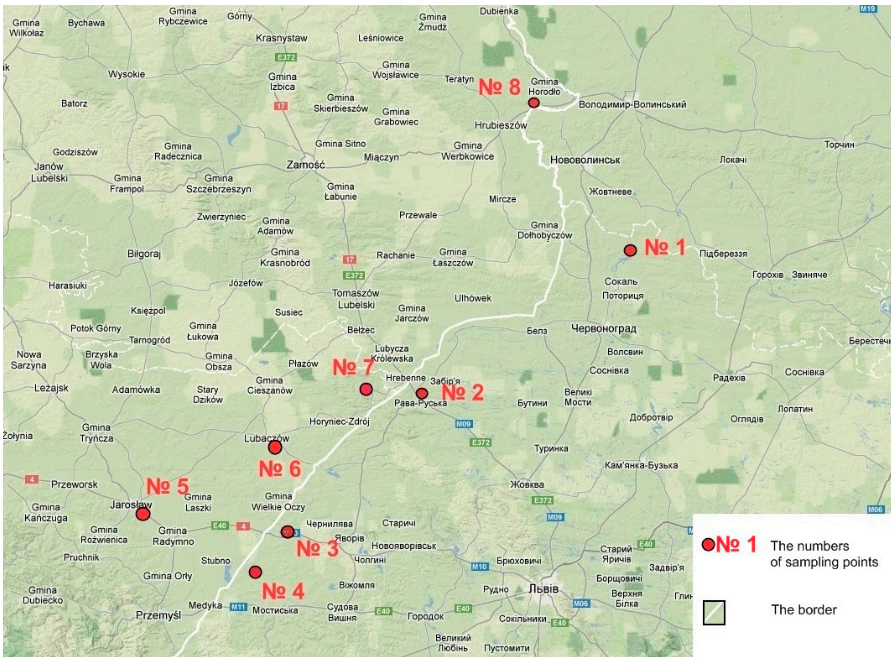

Figure 1. Water sampling points are situated on the both sites of the Polish-Ukrainian border (white line)

Table 1. The locations of water sampling sites in Ukraine and Poland

<table>
<tr><td></td><td>UKRAINE</td><td>POLAND</td></tr>
<tr><td></td><td>The WESTERN BUG RIVER</td><td>The WESTERN BUG RIVER</td></tr>
<tr><td>Nearest city, town</td><td>town Sokal</td><td>town Strzyżów</td></tr>
<tr><td>Geographic coordinates</td><td>35U 309191,34 m East
5602298,36 m North</td><td>35U 289035,55 m East
5636415,54 м m North</td></tr>
<tr><td>Additional information on the sampling areas</td><td colspan="2">The Western Bug r. (ukr. Західний Буг, Zachidnyj Buh, belar. Заходні Буг, Zachodni Buh) (its name derives from ‘Old God’ as a worshiped river) flows through territories of Ukraine, Belarus and Poland (where it is called simply Bug), and is the left tributary of the Narew r. (Vistula). The length of the Western Buh is 772 km (the Ukrainian part is 392 km, and the Polish one is 587 km), the river basin is 73,500 km^2 (in Ukraine: 11,205 km^2, in Poland: 39.420 km^2). The inclination makes 0,3 m/km. The Western Buh is the plain river that is characterized by significant meandering, swampy floodplains and many basin lakes, including the Shatski Lakes. In the territory of Ukraine, the Western Bug is flowing through the territory of the Small Polesie (within Nadbuzhanskiy Basin), between Sokal and the strand of the Nadbuzkiy Upland (a part of the Volyn Upland) and along the western edge of the Polesie lowland. In the territory of Poland, the Western Bug is flowing into the artificial lake Zegrzyńskie (previously in the Narew r.).
The interesting fact is that the Vistula r. in Poland could be a tributary of the Bug r. because the Bug r. is longer than the Vistula r. However, it prevails the Vistula flows and sinks into the Baltic Sea. Opposite to the other rivers, the Western Bug r. does not begin on small streams but on a spring and relatively affluent flow from the well in village Verkhobuzh. In the past, once for diversion of the river a water mill was working, this means that it was enough water to turn the heavy millstones of mills.</td></tr>
<tr><td></td><td>The RATA RIVER</td><td>The RATA RIVER</td></tr>
<tr><td>Nearest city, town</td><td>village Borove</td><td>city Prusie</td></tr>
<tr><td>Geographic coordinates</td><td>34U 682831,45 m East
5568080,17 m North</td><td>34U 678262,92 m East
5569132,47 m North</td></tr>
<tr><td>Additional information on the sampling areas</td><td colspan="2">The Rata r. (ukr. Рата, Rata) is flowing in Poland and in Ukraine (within the Zhovkva and Sokal districts, Lviv region). It is the left tributary of the Western Bug r. (the Vistula basin). The length of the Rata is 76 km, basin area is 1790 km^2. Its floodplain sometimes is swampy, covered with meadow vegetation. Its riverbed is meandering, mostly 15-20 m wide (up to 50 m in its lower part). Also, there are a lot of islands. The depth is 2.3-2.5 m, the inclination makes 1.2 m/km. The Rata r. begins on the territory of Poland, near the Polish-Ukrainian border, on the east part of Verkhrata village. It is entering into the Western Buh r. on the south of Chervonohrad city.
The Rata flows mainly from the west to east, just before entering to the Western Buh, then it returns to the north. The sources of the river are located among the hills of Roztoczczia. After crossing the Polish-Ukrainian border, the Rata r. enters the Nadbuzhansk valley (the West Little Polesie). The main tributaries are the following: the Telytsia, Moschanka, Bila, Zheldets (on the right bank) and the Bolotnya (on the left one) r. Many small towns are situated along the river: Rava-Ruska, Velyki Mosty and Hirnyk village. The environmental state of the river is not satisfactory since it is under the influence of many negative factors from local population and industry. In the headwater of the river Potelytsky hydrological reservation is located.</td></tr>
<tr><td></td><td>The SHKLO r.</td><td>The SAN r.</td></tr>
<tr><td>Nearest city, town</td><td>town Krakovets</td><td>town Jarosław</td></tr>
<tr><td>Geographic coordinates</td><td>34U 657327,56 m East
5535650,57 m North</td><td>34U 621959,17 m East
5542214,59 m North</td></tr>
<tr><td>Additional information on the sampling areas</td><td>The Shklo r. is a Ukrainian (Javoriv district) and Polish river (a right tributary of the San r., the Vistula basin). The length of the river is 76 km, basin area is 863 km^2. The valley of the river is expanding gradually from 0.5-0.8 km to 1.5 km. Its floodplain is well expressed in the middle of river, and its lower places are swampy. The Shklo r. begins on the northern outskirts of the Novoyavorivsk town, near the south-western slopes of the Roztoczczya. It flows from the east to the west through the Nadsianski lowlands. The river crosses the Ukrainian-Polish border near (on the north) Krakovets village and enters into the San r. on the north of Radymno town.
Its major tributaries are the following: the Tereshko, Retychyn (on the right bank) and the Vyzhomlia r. (on the left one). There are many ponds. On the banks of the river Shklo village and Yavoriv town are located.</td><td>The San r. (Ukr. Сян, Germ. Saan) begins on the territory of Ukraine (Turka district, Lviv region), and is flowing on the territory of Poland. It is the most right Carpathian tributary of the Vistula r. (the Baltic Sea basin). The length of the river is 444 km, and basin area is 16,861 km^2 (14,390 km^2 in Poland and 2,471 km^2 in Ukraine). The average water flow rate is 124 m^3/s. The gradient of the river is 8 m/km in the upper river and up to 0.5 m/km on the reaches near Carpathian foothills and 0.25 m/km on the Nadsianska lowlands. The river is navigable in its lower reaches (90 km). In the mountainous course of the San r. two reservoirs, the Solynske and Mychkovske ones, are built.
It begins on the Eastern Beskidy on the verge of the Bieszczady and the Verkhovynsky Vododil, near the Užocký pass. The sources of the river are located at an elevation of 950 m a.s.l. The river is flowing on the north-west between the Beskidy and the Stryisko-Sianska Verkhovyna (where it created several longitudinal valleys and bends) in the Sianitsky basin, and hills change its thread of stream. After that it flows on the north and east, below Przemyśl town it flows north-westward crossing the Nadsianskie lowlands. It runs into the Vistula r. below Sandomierz town. There are many towns, which are located in the San valley, the most important among them are Lesko, Zagórz, Sanok, Dynów and Przemyśl.</td></tr>
</table>

	The WYSHNIA RIVER	The LUBACHÓWKA RIVER
Nearest city, town	village Mazury	town Lubaczów
Geographic coordinates	34U 649716,73 m East 5526485,62 m North	34U 651984,41 m East 5557940,45 m North
Additional information on the sampling areas	**The Wyshnia r.** is a Ukrainian (districts of Sambir, Gorodok and Mostyska, Lviv region) and Polish river (a right tributary of the San r., the Vistula basin). The total length of the river is about 100 km (according to other sources is 80 km), of which15 km are in Poland. The total basin area makes up 1220 km². The river valley in its upper part is 800 m wide and in the lower part reaches 1-2 km. The characteristic of the lower part of the river is water-logging in the floodplain. It rises on the south-east of Rudki town. The river is created by confluence of several streams; one of them begins on just 3 km away from the Dniester r., which is entering into the Black Sea, while the water of the Wyshnia r. flows towards the Baltic Sea. The Wyshnia r. flows on the Siansko-Dniester watershed plain, mostly from the southeast to northwest. Then it is flowing to the north and after 6 km back to the northwest towards Sudowa Wyshnia town. The river is crossing the Ukrainian-Polish border between two villages, Staryava and Zahorby. Then it falls the San r. in front of Radymno town (Poland).	**The Lubachówka r.** (in its upper reaches known as the Zavadivka r.) is a Ukrainian (within Yavoriv Region) and Polish river. The total length of the river is 88,2 km, of which 67,3 km are in Poland. It is the right tributary of the Sian (the Vistula basin). Its basin area comprises 1,129 km². This is a plain river, sometimes swampy and forms floodplains. There are many ponds, for example, near villages Kalytiaky and Grushiv. The river rises on the west of Koty village, in the southwestern part of the Roztoczczia (Ukraine). The river flows on the northwest, crossing the Polish-Ukrainian border on the north of Hrushiv village. In Lubaczów city, it turns on the south and then gradually returns to the west. Then it runs into the San r. on the north of Jaroslav town.

Figure 2. The baiting (set of branches with leaves) floating in the Ukrainian river. Photo I. Matsiakh

Figure 3. Equipment for water filtering used in Poland. Photo I. Matsiakh

and plated onto selective agar media (V8PARPNH) (Jung 2009, Jung et al. 1996). The water taken from Polish rivers was filtered with 5 μm diameter pores filters and each time 3 liters of water was pumped through them each time (Figure 3). After filtration of water, filters were plated onto selective agar media to get pure cultures.

In addition, we used growth patterns on PDA to identify species. The morphology and growth rates of colonies on artificial media was observed. For morphological examination, cultures were grown on carrot agar medium at 20 °C in the dark for three days, after which 1×1 cm pieces were cut from the growing edge and flooded with non-sterile soil extract for 6 hours (Erwin and Ribeiro 1996, Jung et al. 1996). The soil extract was changed after additional 6 and 12 hours with distilled water, and after approx. 24 hours, typical structures of *Phytophthora* and/or *Pythium* spp. (sporangia, hyphal swellings, chlamydospores) were observed under the microscope at ×400 magnification (Nikon®).

DNA amplification

No DNA isolation was performed, instead it was amplified directly from mycelia. Samples were subjected to Phire Plant Direct PCR kits (FINNZYMES®) and sequenced in order to identify the species. The Phire Plant Direct PCR Kit was used to perform the PCR directly from pure culture mycelia prior to DNA sequencing. The PCR reaction consisted of 10 µl Phire Plant PCR Buffer, 1 µM of each primer ITS6/ITS4 (Cooke et al. 2000, White et al. 1990) and 0.4 µM Phire Hot Start II DNA Polymerase and 0,5µl of DNA extract (filled with water up to 20 µl). The program cycle was as follows: an initial denaturation at 98 ºC for 5 min; subsequent 40 cycles of denaturation at 98 ºC for 5 sec, annealing at 55 ºC for 5 sec and extension at 72 ºC for 20 s; followed by final extension step at 72 ºC for 1 min.

DNA sequencing and analyses

The PCR products were purified using "Clean-up" kit (A&A Biotechnology, Poland), according to manufacturer's procedure and they were checked using a spectrophotometer (NanoDrop®ND-1000) in order to estimate their quality and quantity. The reaction mixture of 20 µl contained 65 ng of purified PCR product (the amount recommended for fragments length 600-1,000 bp), 4 µl DTCS Quick Start Master Mix (including 4 fluorochromes, buffer and Taq polymerase), and 3.2 pmol primer ITS6 or ITS4. The mixture was amplified in a PTC-200™ Programmable Thermal Controller (MJ Research, Inc.) programmed for 30 cycles of 20 s at 96 ºC, 20 s at 55 ºC and 4 min at 60 ºC. PCR reactions were stopped by adding 5 µl mixture to each sample: 2 µl of 100 mM Na_2-EDTA (SIGMA cat. No. S 7899), 2 µl 3M sodium acetate (SIGMA cat. No E 7889) and 1 µl glycogen (20 mg/ml). Finally, DNA was precipitated in 60 µl of 95% ethanol, centrifuged at 14000 rpm at 4 ºC for 15 min and rinsed 2 times with 200 µl of 75% ethanol. Samples were dried for about 15 min and re-suspended in 40 µl SLS (Beckman Coulter®). Samples were transferred on sequencing plate and overlaid with one drop of mineral oil.

The DNA sequencing was done in automated sequencer CEQ™8000, version 9.0.25 (Beckman Coulter®, Fullerton, USA) according to manufacturer's procedure. Sequences were analysed using CEQ™8000 Genetic Analysis System software (Beckman Coulter®, Fullerton, USA). The final sequences were compared and consensus sequences were made with BioEdit v 7.1.3 software. All obtained sequences, corresponding to the given isolates, were verified in the gene bank NCBI (www.ncbi.nlm.nih.gov/genbank/).

Results

In total, 51 isolates (including *Phytophthora* and *Pythium* species) from Ukrainian and Polish rivers were obtained (Table 2). Generally, 5 *Phytophthora* and 44 *Pythium* isolates were obtained (5% and 75% of total isolates, re-

Table 2. Identification of species obtained from water samples (Ukraine and Poland)

UKRAINE			POLAND		
Species	No NCBI	Similarity	Species	No NCBI	Similarity
The RATA RIVER			**The RATA RIVER**		
Phytophthora gonapodyides	EU194380.1	100%	*Pythium lycopersicum*	HQ832773.1	96%
Phytophthora lacustris	JQ755226.1	100%	*Pythium lycopersicum*	HQ832773.1	95%
Phytophthora lacustris	JQ755226.1	99%	*Pythium lycopersicum*	HQ832773.1	96%
Pythium oopapillum	JQ898469.1	99%	*Pythium citrinum*	HQ643380.1	100%
Pythium oopapillum	FJ655177.1	99%	*Pythium citrinum*	HQ643380.1	100%
Pythium oopapillum	FJ655176.2	100%			
Pythium angustatum	HQ643437.1	99%			
Pythium oopapillum	FJ655177.1	99%			
Pythium oopapillum	FJ655177.1	99%			
Pythium longicarpum	HM747029.1	99%			
Pythium oopapillum	FJ655177.1	99%			
Pythium spp.	DQ230903.1	99%			
Pythium spp.	DQ403786.1	99%			
Pythium spp.	HQ261735.1	98%			
The WESTERN BUG RIVER			**The WESTERN BUG RIVER**		
Pythium marsipium	HQ643690.1	98%	*Pythium sylvaticum*	HQ643851.1	100%
Pythium lutarium	JQ898467.1	100%/	*Pythium sylvaticum*	HM051065.1	100%
Pythium spp.	DQ230903.1	99%	*Pythium terrestris*	HQ643858.1	100%/
Pythium spp.	DQ230903.1	100%	*Pythium terrestris*	HQ643858.1	100%/
Pythium spp.	DQ230903.1	100%			
The WISHNIA RIVER			**The LUBACHÓWKA RIVER**		
Phytophthora cactorum	EU194422.1	100%	*Fusarium equiseti*	JQ690085.1	100%

Phytophthora cactorum	EU194422.1	100%	*Fusarium solani*	FR878062.1	100%
Pythium angustatum	HQ643437.1	100%	*Trichoderma asperellum*	JQ617303.1	100%/
Pythium aquatile	HQ643446.1	100%	*Trichoderma hamatum*	JQ040347.1	100%
Pythium aquatile	EU240074.1	99%			
Pythium monospermum	JN630508.1	100%			
Pythium sukuiense	HQ643836.1	99%/			
The SHKLO RIVER			**The SAN RIVER**		
Pythium aquatile	HQ643446.1	100%	*Saprolegnia ferax*	JN400035.1	100%/
Pythium aquatile	HQ643446.1	100%	*Saprolegnia diclina*	HQ643980.1	100%
Pythium monospermum	JN630508.1	100%	*Hypocrea lixii*	JF501658.1	99%/
Pythium sukuiense	HQ643836.1	100%/	*Trichoderma aureoviride*	HQ596945.1	99%
Pythium sukuiense	HQ643836.1	100%/			
Pythium oopapillum	HQ643717.1	100%			
Pythium oopapillum	FJ655177.1	99%			
Pythium spp.	DQ232768.1	100%			
Pythium spp.	HQ261735.1	96%			
Pythium spp.	JN863967.1	96%			
Pythium spp.	HQ261735.1	98%			

spectively). *Ph. gonapodyides*, *Ph. lacustris* and *Ph. cactorum* were the most often found species in Ukrainian water samples. Some of morphological features of these isolates are presented below (Figure 4). *Pythiums* were the most abundant in the investigated samples. Not the same species were detected on both the Ukrainian and Polish sides of the same rivers. In the Ukrainian part of the river Rata *P. oopapillium, P. angustatum* and *P. longicarpum* were identified as well as *Ph. lacustris* and *Ph. gonapodyides,* while in Polish part of the river only *P. lycopersicum* and *P. citrinum.*

The baits used in the detection were beech, oak and black alder but there were no observations about which bait detected which species and if there were any differences in species detection between bait species.

Discussion

Our monitoring of rivers focused on species of the genus *Phytophthora* (from Greek: φυτόν (phyton) "plant" and φθορά (phthora), "destruct, destruction," that is "plant destroyer"), because they are primary parasites of fine roots, and cause root and collar rots, bark necrosis, stem and aerial cankers, stunting of shoots and twigs of many species of young and mature trees and shrubs (Jung et al. 1999, Jung and Blaschke 2004). *Pythium* and *Phytophthora* species are considered as "fungus-like organisms" or "pseudo-fungi" and are placed in the kingdom Chromista or kingdom Stramenopila, distinct from the kingdom Fungi (Martin et al. 2012). In Ukraine, prior to

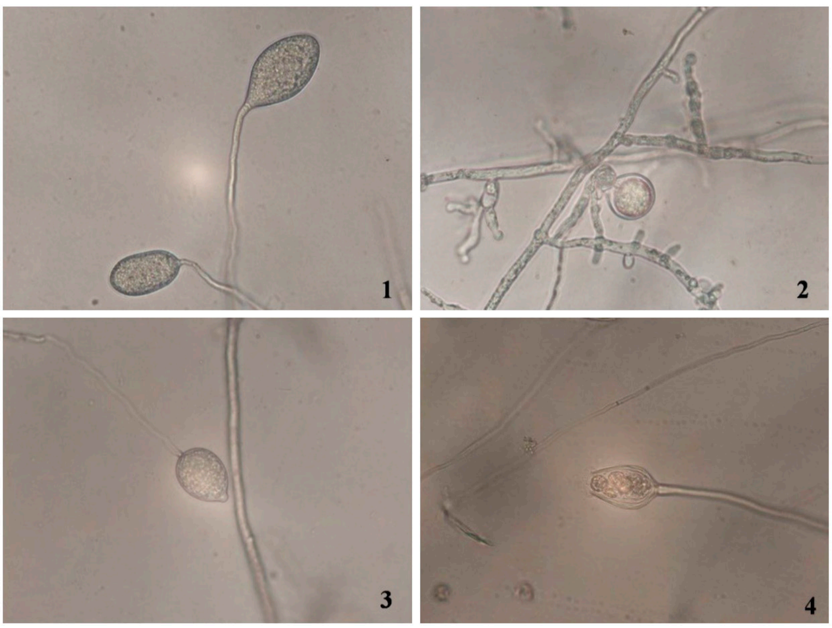

Figure 4. Morphological structures of *Phytophthora* species: 1 – nonpapillate, ellipsoid or obpyriform, non-caducous sporangia of *Ph. gonapodyides*; 2 – oogonia and oospore with paragynous antheridia of *Ph. cactorum*; 3 – papillate, terminnaly born, ovoid to spherical, caducouos with a short pedicel sporangia of *Ph. cactorum*; 4 - nonpapillate sporangia of *Ph. lacustris*. Photo I. Matsiakh

this study the above pathogens were found in many tree species, such as *Fagus*, *Abies*, *Betula* and *Alnus* (Kramarets et al. 2011). The observed symptoms in the Ukrainian riparian forests (dominated by alders) could be acute, when trees die within one year, or very often chronically, when they are declining within several seasons. The external symptoms included increased crown transparency, dieback of crowns and branches, dying of shoots and wilting of leaves, which are abnormally small, changing colour from green to yellow-pale. The disease can be spread through the contact of zoospores (travelling with the current of rivers) with the bark tissues, usually started at the water level (Oszako and Zakrzewska 2005). Our study was conducted to check water courses as potential *Phytophthora* and *Pythium* pathways between countries and the entrance point to the EU. The topic seems to be both timely and important in light of the increasing importance of emerging pathogens belonging to these genera.

The occurrence of *Phytophthora cactorum* in tested water samples proves such a pathway, as the pathogen is typical in forest and ornamental nurseries (Leonberger et al. 2013). It is likely, that in Ukraine, the Wishnia river flows through forests, where some trees could already be affected by this pathogen. The story demonstrates how this pathogen can spread naturally, this is often neglected by the risk assessors dealing with forest protection. Moreover, other phytopathogens can use the same pathway to find new host plants (Themann et al. 2002). When pathogens enter a nursery (e.g. with irrigation water) sooner or later they tend to spread via soil residues on tools, machinery parts, especially wheels of tractors or cars, and even men's shoes (McNeill 2011).

The aim of this study was to assess the diversity of pathogenic Oomycetes in rivers crossing the Ukrainian/Polish border as potential inoculum travelling with this pathway. The detection and identification of phytopathogens were performed in classical way (morphological features), as well as with PCR and DNA sequencing. Among the detected organisms, there are pathogens of plants including forest tree species but also those that attack amphibians or fish.

No *Phytophthora* species were detected in Polish water samples. The monitoring success of oomycetes in the environment might depend on the method used (but it was not the aim of this work). We did not compare the methods in the same sites (instead baiting was used in one part of the rivers and filtering was used in the other), although baiting seems to sample a larger volume of water and also better reflect the ecology of the species. Probably, from above reasons, the classical baiting method was more successful for *Phytophthora* monitoring. However, there are also limitations associated with the baiting method, for example, depending on the leaves included as baits, species able to infect those particular plants and some slow growing species will be difficult to detect. Keeping plant leaves floating for a few days in water also better reflects the biology of *Phytophthoras*. In the case of water filtering on the spot (3 liters each sample), there is little chance of detecting an organism if it is not abundant in the environment at the time of sampling. The genus *Pythium*, with just over 140 described species, has been classified traditionally with other filamentous, coenocytic, sporangia-producing fungi as "Phycomycetes" (Chenari Bouket et al. 2015). It is also worth noting that with the filtering method, many different species of the genus of *Pythium* were found in water samples, for which, leaves as baits are not necessary attractive enough to infect them. It means that they are very common in the aquatic environment and are transported with the river current for long distances. Among them the most widely represented were the following ones: *P. oopapillum, P. aquatile* and *P. sukuiense*. Also, *P*. *angustatum.* and *P. monospermum* (twice)*,*and *P. lutarium* (once) were detected and identified in the investigated water samples. While *Pythium* species are not considered as forest pathogens, at least some of them cause damping off in nursery settings (Weiland et al. 2013). Many species are usually present in nurseries, but damping off usually occurs only when conditions are too wet. However, they are most likely spread by irrigation water.

In the water samples, a part from Oomycetes, also fungi like *Fusarium equiseti* and few species of the genus of *Trichoderma* (*T. asperellum, T. aureoviride, T. hamatum*) were found. Especially, fungi of *Fusarium* genus could have a negative impact on forest tree species. Recently, among other organisms responsible for the oak decline phenomenon are fungi belonging to *Fusarium* species (Wit et al. 2015). The sampling of symptomatic shoots of *Quercus robur* L. performed in 2013 by Wit and others, respecting different parts of tree crowns (top, central and bottom) showed that the *Fusarium* spp. group of fungi dominated with an average frequency of 32%. Considering the large scale dieback of oak shoots the continuation of research on the etiology of this phenomenon seems fully justified. The scarce literature reporting the harmfulness of these fungi to oaks, especially as being the pathogen of buds, suggests need for further studies on this subject. Although, *Fusarium* species detected in this study (*F. equiseti, F. solani*) were not the same found in infected oak tissues, like *F. avenaceum* (Wit et al. 2015), but our study shows that *Fusarium* species may travel with water courses. However, these Fusariums found in rivers are most likely soil saprotrophs and potentially agricultural pathogens.

The *Saprolegnia* genus found in the investigated rivers consist of fresh water organisms often called a "cotton mould", because of the characteristic white or grey fibrous patches it forms. *Saprolegnias* are saprotrophs as well as opportunistic pathogens. Current taxonomy puts

Saprolegnia as a genus of the heterokonts in the order Saprolegniales. In the river San, *Saprolegnia ferax* was found. This species causes severe diseases of fish in Switzerland (Steciow et al. 2014). Moreover, in Poland *Saprolegnia ferax* and *Pythium aquatile* were observed on the eggs of European whitefish *Coregonus lavaretus* in a lake in Poland (Czeczuga et al. 2004). This study describes and confirms the spreading of pathogenic organisms with water that could be as dangerous for plants as for fish.

Some of the detected species are likely endemic, so should not pose a significant threat to natural ecosystems, however, they may cause damage to plants in agricultural or nursery settings if such water is used for irrigation. The quarantine species were not detected in this study, but it is not known which of the species can be introduced or have a potential to cause the significant damage in natural or semi-natural ecosystems in suitable conditions. The above records stress that the monitoring of rivers could be crucial to keep nurseries and forest stands free of plant pathogens. Since the 19th century more than 20 species of *Phytophthora*, and representatives of the genus of *Pythium*, *Fusarium*, *Rhizoctonia* have been isolated from irrigation water (Hong 2005, Themann 2002). Hong and others believe that water used for watering of plants is the main source of *Phytophthora* in nurseries causing damage to orchards and vegetables (Hong 2005). Our studies confirmed that such a pathway exists for *Phytophthora* species, which were found in the rivers. Next step is to know more about their ecology or where else they have been found, what diseases they cause and what is their general significance. For example, *Ph. lacustris* is an interesting species, because it has been found also elsewhere in Europe, the USA, China and Africa, also in remote areas (Huai et al. 2013, Nagel et al. 2015). It belongs to *Phytophthora* clade 6, which includes aquatic species that are found in natural waterways also beyond agricultural areas, and do not seem to be harmful to residents until suitable conditions occur (usually floods), being probably opportunistic pathogens. Also, *Ph. gonapodyides* is a clade 6 species. High diversities of *Phytophthora* species have also been found in natural waterways all over the world (Sims et al. 2015, Nagel et al. 2015, Hüberli et al. 2013). In Poland, taking water from surface sources for plant watering is a popular practice, in forestry alone around 40% of nurseries use rivers or lakes as natural water reservoirs. Even if a nursery possesses an underground well, the water is usually pumped and stored (for warming up) in open ponds and could be re-infected e.g. with pathogenic *Phytophthora* species. Symptomless potted plants can also carry soil-borne plant pathogens into the environment via planting (Migliorini et al. 2015). The future consequences could be reflected in the context of health, sustainability and biodiversity of European forests.

Conclusions

1. Several *Oomycetes* (also pathogenic species) were shown to occur in Ukrainian rivers likely spreading across the EU border. Some species (e.g. *P. cactorum*) can potentially cause the negative consequences in natural forest ecosystems too.
2. Three *Phytophthora* species: *Ph. gonapodyides*, *Ph. lacustris* and *Ph. cactorum* were prevalent in Ukrainian rivers as well as many species of the *Pythium* genus.
3. In Polish rivers, 4 species of *Pythium*: *P. lycopersicum*, *P. sylvaticum*, *P. citrinum* and *P. terrestris* are very common and their pathogenicity to fine roots of forest tree species require further research.
4. The above findings suggest the pre-treatment of water taken from the rivers (e.g. with slow sand filters), prior to using for plant watering in forest or ornamental nurseries.

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References

Bewley, W.F. and Buddin, W. 1921. On the fungus flora of greenhouse water supplies in relation to plant disease. *Annals of Applied Biol*ogy 8: 10-19.

Erwin, D.C and Ribeiro, O.K. 1996. *Phytophthora* Diseases Worldwide. The APS, St. Paul., 561 pp.

Chenari Bouket, A., Arzanlou, M., Tojo, M., and Babai-Ahari, A. 2015. A web-based identification programme for *Pythium* species. *Archives of Phytopathology and Plant Protection* 48 (6): 475-484.

Cooke, D. E. L., Drenth, A., Ducan, J. M., Wagels, G. and Brasier, C. M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30: 17-32.

Copes, W., Yang, X., and Hong, C. 2015. *Phytophthora* species recovered from irrigation reservoirs in Mississippi and Alabama nurseries and pathogenicity of three new species. *Plant Disease* (*in press*); http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-11-14-1197-RE.

Czeczuga, B., Kiziewicz, B., and Godlewska, A. 2004. Zoosporic fungi growing on eggs of *Coregonus lavaretus holsatus* Thienemann, 1916 from Lake Wdzydze in Kaszuby. *Polish Journal of Environmental Studies* 13 (4): 355-359.

Gibbs, J.N. 1999. The impact of *Phytophthora* disease on riparian population of common alder (*Alnus glutinosa*) in southern Britain. *European Journal of Plant Pathology* 29: 39-50.

Hansen, E. M. 2015. *Phytophthora* species emerging as pathogens of forest trees. *Current Forestry Reports* 1 (1): 16-24.

Hong, C.X. and Moorman, G.W. 2005. Plant pathogens in irrigated water: challenges and opportunities. *Critical Reviews in Plant Science* 24: 189-208.

Huai, W. X., Tian, G., Hansen, E. M., Zhao, W. X., Goheen, E. M., Grünwald, N. J., and Cheng, C. 2013. Identification of *Phytophthora* species baited and isolated from forest soil and streams in northwestern Yunnan province, China. *Forest Pathology* 43 (2): 87-103.

Hüberli, D., Hardy, G. S. J., White, D., Williams, N. and Burgess, T. I. 2013. Fishing for *Phytophthora* from Western Australia's waterways: a distribution and diversity survey. *Australasian Plant Pathology* 42 (3): 251-260.

Jankowiak, R., Stępniewska, H. and Bilański, P. 2015. Notes on some *Phytopythium* and *Pythium* species occurring in oak forests in southern Poland. *Acta Mycologica* 50 (1): 1052.

Jung, T. 2009. Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. *Forest Pathology* 39: 73-94.

Jung, T. and Blaschke, M. 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies. *Plant Pathol*ogy 53: 197-208.

Jung T., Blaschke, H. and Neumann, P. 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* 26: 253-272.

Jung, T., Blaschke, H. and Oßwald, W. 2000.Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology* 49: 706–718.

Jung, T., Cooke, D.E., Blaschke, H., Duncan, J.M. and Osswald, W. 1999. *Phytophthora quercina* sp. non causing root rot of European oaks. *Mycological Research* 103: 785-798.

Kramarets, V., Matsiakh, I. and Oszako, T. 2011. Pathogens of the *Phytophthora* genus as a potential threats to forest plants in Ukraine. *RVV NLTU* (9): 137-142 (in Ukrainian).

Leonberger, A. J., Speers, C., Ruhl, G., Creswell, T. and Beckerman, J. L. 2013. A survey of *Phytophthora* spp. in midwest nurseries, greenhouses, and landscapes. *Plant Disease* 97 (5): 635-640.

Martin, F. N., Abad, Z. G., Balci, Y. and Ivors, K. 2012. Identification and detection of *Phytophthora*: reviewing our progress, identifying our needs. *Plant Disease* 96 (8): 1080-1103.

Matsiakh, I., Kramarets, V., Orlikowski, L. B. and Trzewik, A. 2012. Pierwsze doniesienie o występowaniu *Phytophthora spp.* i *Pythium litorale* w rzekach Beskidu Ukraińskich Karpat [First report on *Phytophthora* spp. and *Pythium litorale* occurrence in Beskid's rivers (of the Ukrainian Carpathians)]. *Progress in Plant Protection* 52 (4): 1200-1203. (in Polish with English abstract).

McNeill, M., Phillips, C., Young, S., Shah, F., Aalders, L., Bell, N., Gerard, E. and Littlejohn, R. 2011. Transportation of nonindigenous species via soil on international aircraft passengers' footwear. *Biological Invasions* (13): 2799–2815.

Migliorini, D., Ghelardini, L., Tondini, E., Luchi, N. and Santini, A. 2015. The potential of symptomless potted plants for carrying invasive soilborne plant pathogens. *Diversity and Distributions* (in press); http://dx.doi.org/10.1111/ddi.12347.

Nagel, J. H., Slippers, B., Wingfield, M. J. and Gryzenhout, M. 2015. Multiple *Phytophthora* species associated with a single riparian ecosystem in South Africa. *Mycologia* (*in press*); http://www.mycologia.org/content/early/2015/06/26/13-380. abstract.

Nowak, K. J., Trzewik, A., Tułacz, D., Orlikowska, T. and Orlikowski, L. B. 2015. Characterization of Polish *Phytophthora lacustris* isolates obtained from water environments. *Polish Journal of Environmental Studies* 24 (2): 619-630.

Oh, E., Gryzenhout, M., Wingfield, B. D., Wingfield, M. J. and Burgess, T. I. 2013. Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African natural ecosystems. *IMA Fungus* 4 (1): 123.

Orlikowski, L.B. 2006. Relationship between source of water used for plant sprinkling and occurrence of *Phytophthora* shoot rot and tip blight in container-ornamental nurseries. *Journal of Plant Protection Research* 46 (2): 163-168.

Orlikowski, L.B., Ptaszek, M. and Trzewik, A. 2008 Water as the source of *Phytophthora* species in rivers and their pathogenicity to some plants. *Ecological Chemistry and Engineering* A, Vol. 15 No 9: 945-949.

Orlikowski, L.B., Trzewik, A. and Orlikowska, T. 2007. Water as potential source of *Phytophthora citricola. Journal of Plant Protection Research* 47 (2): 125-132.

Oszako, T. and Zakrzewska, W. 2005. Fytoftoroza – groźna choroba wielu gatunków drzew leśnych. **[***Phytophthora* rot: a dangerous disease of many species of forest trees**].** *Ochrona Roślin* 3: 31-32 (in Polish).

Park, B., Martin, F., Geiser, D. M., Kim, H. S., Mansfield, M. A. and Nikolaeva, E. 2013. *Phytophthora* database 2.0: update and future direction. *Phytopathology* 103 (12): 1204-1208.

Ptaszek, M. and Orlikowski, L.B. 2015. Occurrence of *Phytophthora* species in watercourses and reservoirs in Poland and the threat to cultivated plants by this genera. *Progress in Plant Protection* 55: 64-70.

Rahman, M. Z., Uematsu, S., Suga, H. and Kageyama, K. 2015. Diversity of *Phytophthora* species newly reported from Japanese horticultural production. *Mycoscience* 56: 443-459.

Schlenzig, A., Campbell, R. and Chard, J. 2014. *Phytophthora* species infecting hardy ornamentals in nurseries and the managed environment in Scotland. *Journal of Phytopathology* 163: 686-689.

Sims, L.L., Sutton, W., Reeser, P.W. and Hansen, E.M. 2015.The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. *Mycologia* (*in press*); http://www.mycologia.org/content/early/2015/06/26/14-255. abstract.

Steciow, M.M., Lara, E., Paul, C., Pillonel, A. and Belbahri L. 2014. Multiple barcode assessment within the Saprolegnia-Achlya clade (Saprolegniales, Oomycota, Straminipila) brings order in a neglected group of pathogens. *IMA Fungus* 5 (2): 439-448.

Streito, J.C., Legrand, Ph., Tabary, F. and Jarnouen de Villartay, G. 2002. *Phytophthora* disease of alder (*Alnus glutinosa*) in France: investigation between 1995 and 1999. *Forest Pathology* 32: 179-191.

Themann, K., Werres, S., Luttmann, R. and Diener, H.A. 2002. Observation of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. *European Journal of Plant Pathology* 108: 337-343.

Weiland, J.E., Beck, B.R. and Davis, A. 2013. Pathogenicity and virulence of Pythium species obtained from forest nursery soils on Douglas-fir seedlings. *Plant Disease* (97): 744-748.

Wit, M., Sierota, Z., Oszako, T., Mirzwa-Mróz, E. and Wakuliński, W. 2015. *Fusarium* spp. na nadziemnych organach zamierających dębów – nowe zagrożenie [*Fusarium* spp. on the above-ground organs of dying oaks: a new threat]. *Sylwan* 159 (5): 403-410 (in Polish).

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. The PCR Protocols: A guide to methods and applications. W: PCR Protocols: A guide to methods and applications. Innis M.A.D.H. Gelfand, J.J. Sninsky, and T. J. White Eds. Academic Press, Inc., New York, p. 315-322.

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