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Isolation and Pathogenicity of *Phytophthora* Species from Poplar Plantations in Serbia

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Abstract: During a survey in three declining and three healthy poplar plantations in Serbia, six different *Phytophthora* species were obtained. *Phytophthora plurivora* was the most common, followed by *P. pini*, *P. polonica*, *P. lacustris*, *P. cactorum*, and *P. gonapodyides*. Pathogenicity of all isolated species to four-month and one-year-old cuttings of *Populus* hybrid clones I-214 and Pánnonia, respectively, was tested using both a soil infestation and stem inoculation test. Isolates of *P. polonica*, *P.* × *cambivora*, *P. cryptogea*, and *P.* × *serendipita* from other host plants were included as a comparison. In the soil infestation test, the most aggressive species to clone I-214 were *P. plurivora*, *P.* × *serendipita*, and *P. pini*. On clone Pánnonia, *P. gonapodyides* and *P. pini* were the most aggressive, both causing 100% mortality, followed by *P. cactorum*, *P.* × *cambivora*, and *P. polonica*. In the underbark inoculation test, the susceptibility of both poplar clones to the different *Phytophthora* species was largely similar, as in the soil infestation test, with the exception of *P. polonica*, which proved to be only weakly pathogenic to poplar bark. The most aggressive species to clone I-214 was *P. pini*, while on clone Pánnonia, the longest lesions and highest disease incidence were caused by *P. gonapodyides*. *Phytophthora cactorum* and *P. plurivora* were pathogenic to both clones, whereas *P.* × *cambivora* showed only weak pathogenicity. The implications of these findings and possible pathways of dispersion of the pathogens are discussed.

Keywords: soilborne pathogens; pathways; *Populus*; *Phytophthora plurivora*; *Phytophthora pini*; pathogenicity tests

1. Introduction

Phytophthora species are fungus-like organisms belonging to the kingdom Chromista (Stramenopiles) within the SAR (Stramenopiles, Alveolata, Rhizaria) super group [1]. These pathogens can infect numerous woody host plants in natural ecosystems, nurseries, and plantings [2–5]. The wide distribution of these damaging pathogens is mainly a consequence of the increasing international trade in living plants resulting in the introduction of non-native *Phytophthora* spp. into previously unaffected

regions on infected nursery stock [3–8]. Therefore, forests and plantations established via the planting of nursery stock are at high risk of *Phytophthora* diseases [4,5].

Poplar plantations are the most widespread, artificially established broadleaved stands in Serbia. In the alluvial plains along the large rivers in Serbia, natural diverse stands of *Populus alba* L. and *P. nigra* L. are also common and of high ecological and economic importance. Due to the high productivity rate of the different hybrid clones, the area of poplar plantations in Serbia has increased considerably [9], reaching 48,000 ha or 2.1% of the total forest area [10]. In the area of Public Enterprise (PE) "Vojvodinašume", poplar plantations along the Sava, Tisa, and Danube rivers are of particular importance. Wet soil conditions and seasonal floodings create favourable conditions for the spread, infection, and survival of *Phytophthora* species. In riparian poplar plantations along the Sava River and in central Serbia, decline and dieback symptoms indicative of *Phytophthora* root infections have been recorded during the previous decade. Apart from individual reports of *P. cactorum* (Lebert and Cohn) Schröter on white poplar in the Czech Republic and in Hungary [4,11], and of *P.* × *cambivora* (Petri) Buisman on poplar trees in Croatia [12], there are no studies of *Phytophthora* species distribution in poplar stands in Europe. In a preliminary investigation of the fungal and oomycete community associated with poplars in Serbia, *P. cactorum* and *P. plurivora* Jung and Burgess were recorded in the rhizosphere of poplar trees [13].

The present study aimed to (1) determine the occurrence and diversity of *Phytophthora* species in poplar plantations in Serbia; and (2) test the aggressiveness of the isolated *Phytophthora* spp. to two poplar clones widely used in Serbia.

2. Material and Methods

2.1. Studied Sites

Four sites were selected near Srem in the northern province of Vojvodina. The 5–33 year old plantations were located on alluvial forest sites with humogley soils along the Sava River and belonged to Public Enterprise (PE) "Vojvodinašume" (Table 1). In stands 1 and 2, poplars showed increased crown transparency and yellowing of leaves (Figure 1a,b), while stands 3 and 4 were healthy. Two additional sites were located in central Serbia, in the forests of PE "Srbijašume" (stand 5; 25 years old) and in a private property in the village Brzeće, near the Kopaonik mountain (stand 6; 2 years old), respectively (Table 1). The latter two stands showed severe dieback and mortality (Figure 1c,d). Due to their vicinity to local rivers, both stands were growing on wet alluvial soils. All six plantations were established via the planting of nursery stock. In four plantations, poplar clone *P.* × *euramericana* I-214, a hybrid between *P. nigra* and the North American *P. deltoides* Bartram ex. Marshall, was used, while the other two plantations contained *P. deltoides* (Table 1).

Table 1. Isolation of *Phytophthora* species from rhizosphere soil samples in poplar plantations in Serbia.

Stand, Location (River)	Tree No.	*Populus* Species/Clone	Age	Disease Symptoms	Sample	*Phytophthora* Species (No. Isolates)	GenBank Accession Numbers
Stand No. 1, Klenak 44°45′48″ N 19°48′18″ E 86 m asl (Sava River)	1	*Populus deltoides*	10	No symptoms	Rhizosphere soil	*P. plurivora* *P. cactorum*	
	2	*P. deltoides*	10	No symptoms	Wet rhizosphere soil	*P. lacustris*	
	3	*P. deltoides*	10	No symptoms	Wet rhizosphere soil	*P. gonapodyides*	
	4	*P. deltoides*	10	No symptoms	Rhizosphere soil	*P. plurivora* *P. cactorum*	
	5	*P. deltoides*	10	No symptoms	Wet rhizosphere soil	*P. gonapodyides*	
	6	*P. deltoides*	10	No symptoms	Rhizosphere soil	-	

Table 1. *Cont.*

Stand, Location (River)	Tree No.	*Populus* Species/Clone	Age	Disease Symptoms	Sample	*Phytophthora* Species (No. Isolates)	GenBank Accession Numbers
Stand No. 2, Kupinovo, Kupinski Kut 44°40′01″ N 19°59′34″ E 76 m asl (Sava River)	7	*Populus* × *euramericana* clone I-214	31	No symptoms	Wet rhizosphere soil	*P. gonapodyides* *P. cactorum*	
	8	I-214	31	No symptoms	Rhizosphere soil	*P. plurivora*	
	9	I-214	31	No symptoms	Rhizosphere soil	-	
	10	I-214	31	No symptoms	Rhizosphere soil	*P. pini* (2) *P. plurivora* *P. lacustris*	KF234654 KF234736
	11	I-214)	31	No symptoms	Rhizosphere soil	*P. lacustris* *P. plurivora*	
	12	I-214	31	No symptoms	Rhizosphere soil	*P. pini* *P. plurivora* (2)	KF234655 KF234737
	13	I-214	31	High crown transparency	Rhizosphere soil	*P. plurivora* *P. pini*	
Stand No. 3, Kupinovo, Kupinski Kut 44°39′52″ N 19°59′43″ E 80 m asl (Sava River)	14	I-214	33	No symptoms	Rhizosphere soil	*P. plurivora* *P. pini* (3)	KF234740 KF234656
	15	I-214	33	No symptoms	Wet rhizosphere soil	*P. lacustris*	
	16	I-214	33	No symptoms	Rhizosphere soil	*P. plurivora* *P. cactorum*	JX276094
	17	I-214	33	No symptoms	Rhizosphere soil	*P. pini* *P. pini*	KF234660 KF234657
	18	I-214	33	No symptoms	Rhizosphere soil	*P. plurivora* (2) *P. pini*	KF234658
Stand No. 4, Kupinovo, Jasenska Belilo44°43′02″ N 20°06′20″ E 91 m asl (Sava River)	19	*P. deltoides*	5	No symptoms	Rhizosphere soil	-	
	20	*P. deltoides*	5	Yellowing of leaves	Rhizosphere soil	*P. plurivora* *P. polonica*	KF234729 KF234759
	21	*P. deltoides*	5	Yellowing of leaves	Rhizosphere soil	-	
	22	*P. deltoides*	5	Yellowing of leaves	Rhizosphere soil	*P. polonica* (2)	
	23	*P. deltoides*	5	High crown transparency	Rhizosphere soil	*P. plurivora* *P. polonica*	KF234727
	24	*P. deltoides*	5	Crown transparency	Rhizosphere soil	*P. plurivora* *P. polonica*	KF234760
	25	*P. deltoides*	5	Yellowing of leaves	Rhizosphere soil	*P. plurivora*	KF234728
Stand No. 5, Veliki Jastrebac-Blace43°21′19″ N 21°15′36″ E 492 m asl (stream Popovačka reka)	26	I-214	25	Dieback	Wet rhizosphere soil	*P. gonapodyides* *P. plurivora*	
	27	I-214	25	Crown transparency and dieback	Wet rhizosphere soil	*P. gonapodyides*	
Stand No. 6 Brus, Brzeće 43°18′07″ N 20°53′08″ E 1011 m asl (stream Bela reka)	28	I-214	2	Dieback, root necroses	Rhizosphere soil	*P. gonapodyides*	
No. of positive samples No. of obtained isolates No. of sequenced isolates						24 46 15	

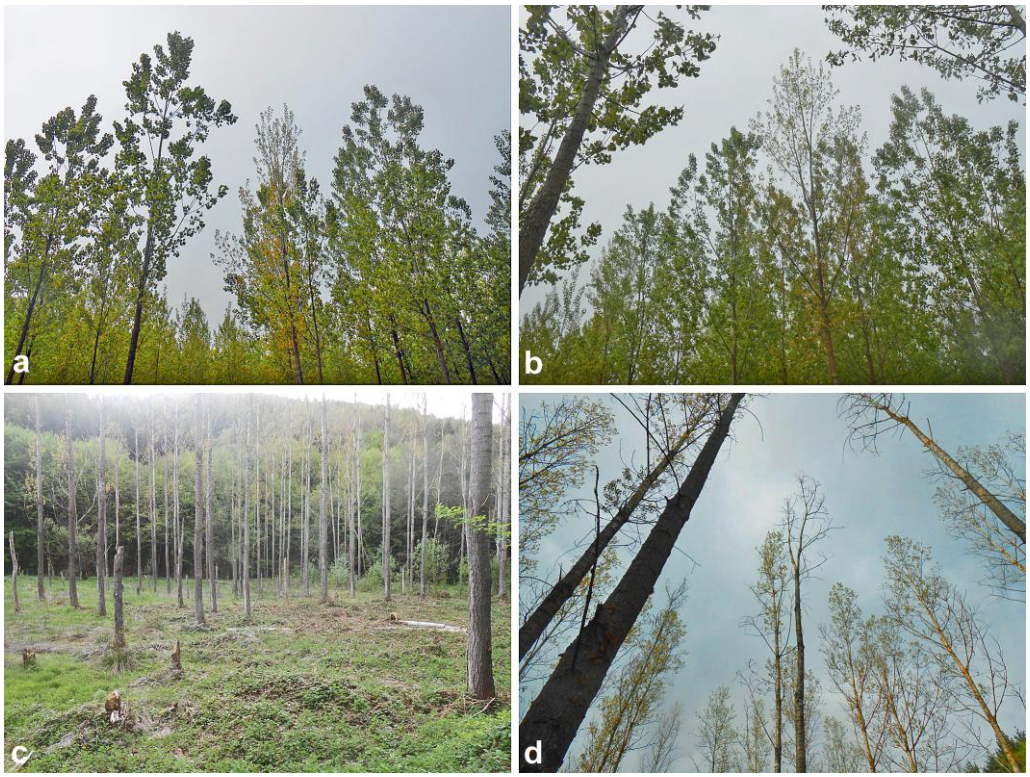

Figure 1. Disease symptoms in poplar plantations in Serbia: (**a**,**b**) increased crown transparency and yellowing of leaves on five-year old trees of *Populus deltoides* in stand No. 4; (**c**,**d**) severe mortality and dieback of 25-years old trees of *Populus* × *euramericana* clone I-214 in stand No. 5.

2.2. Sampling, Isolation, and Morphological Identification of Phytophthora Spp.

Sampling and isolation methodology were performed according to [14,15]. In the four plantations in the Srem forest area, 25 poplar trees were randomly sampled in May 2011 and May/June 2012, while in the two stands in central Serbia, three trees were sampled in the spring and summer 2017. Three to four soil monoliths were taken from the rhizosphere of each tree, mixed, and ca. 3–4 L of soil per tree was taken to the laboratory. Both symptomatic and asymptomatic trees were sampled. Each soil sample was thoroughly mixed and a subsample of ca. 200 mL used for the isolation test using young leaves of *Quercus robur* L. and *Fagus sylvatica* L., as baits. The baiting test was performed at 20 °C and natural light. After the appearance of the first necrotic spots, baiting leaves were examined for the presence of *Phytophthora* sporangia under the light microscope. Small pieces from the necroses were then plated onto selective PARPNH agar [14–16]. First hyphae from plated leaves were subcultured onto V8-agar (V8A) and carrot juice agar (CA) (800 mL distilled water, 200 mL carrot or vegetable juice (Biotta®, Tägerwilen, Switzerland), 18 g agar (Torlak, Belgrade, Serbia) and 3 g $CaCO_3$; [15]), and stored at 20 °C for further examinations.

For classical species identification, the morphology of obtained isolates was examined at × 400 magnifications using a light microscope (CETI®MAGNUM-T/Trinocular Microscope, Oxon, UK). Structures were measured using a camera (Si3000®, Medline Scientific, Oxon, UK) and the XliCap® (Xl Imaging Ltd., Swansea, UK) imaging software. Sporangia were produced by flooding agar discs of young CA colonies for 24–48 h in non-sterile soil extract according to [14]. Gametangia were studied from four-week-old CA cultures, incubated at 20–22 °C in the dark. Self-sterile isolates were paired with known tester strains of *P. cryptogea* Pethybridge and Lafferty (A1 mating type: BBA 65909; A2 mating type: BBA 63651) to clarify whether they were sterile or heterothallic, and to which mating type heterothallic isolates belong. Colony growth patterns were examined after the growth of isolates for one week at 20 °C in the dark on four different agar media, including CA, V8A, malt-extract-agar

(MEA; 48 g/L malt-extract-agar; Merck KGaA, Darmstadt, Germany), and potato-dextrose-agar (PDA; 39 g/L potato-dextrose-agar; Merck KGaA, Darmstadt, Germany). Morphological features and colony growth patterns were compared with descriptions in the literature [2,17–22].

2.3. Molecular identifIcation of Phytophthora Spp.

The ITS1-5.8S-ITS2 rDNA region of 15 selected isolates representative of all morphotypes was sequenced after direct PCR using Phire™ Plant Direct PCR Kits (Thermo Fisher Scientific Inc., Waltham, MA, USA). Mycelium from three to five days old V8A colonies was scraped without agar using a sterile needle and placed in 2 mL Eppendorf tubes, with 30 µL of previously added Dilution Buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA). PCR reactions were performed with a 20 µL volume, containing 10 µL 1 × Phire Plant PCR Buffer-a, 1 µL 0.5 µM of primers ITS4/ITS6 [23,24], 0.4 µL Phire Hot Start II DNA Polymerase, 0.5 µL of Dilution Buffer with diluted young hypha, and water (mQ) up to 20 µL, according to the manufacturer's recommendation. Reactions were performed in a PTC-200™ machine (MJ Research Inc., Waltham, MA, USA) with three-step PCR protocol, according to the manufacturer's recommendations. The PCR program was 5 min at 98 °C followed by 40 cycles of 5 s at 98 °C, 5 s at 55 °C, and 50 s at 72 °C. The presence and size of PCR products was confirmed by analyzing 1 µL of product by electrophoresis in 1% TAE-agarose gel, stained with GelRed™ Nulceid Acid Dye (Biotium, Inc., Fremont, CA, USA), with FastRuler MR DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA) as the molecular mass standard of DNA. For sequencing, 20 µL PCR product was purified with the CleanUp Kit (A&A Biotechnology, Gdynia, Poland), following the manufacturer's protocol, and sequenced with ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Obtained sequences were aligned using the ClustalW algorithm of the BioEdit program subjected to an NCBI BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence analysis was done with MEGA6 software [25]. Isolates were assigned to a *Phytophthora* species when sequence identities were above a 99% cut-off in respect to those of extype isolates or key isolates. All ITS sequences obtained in this study were submitted to GenBank (Table 1).

2.4. Soil Infestation Test

One-year old cuttings of *P.* × *euramericana* clones I-214 and Pánnonia, commonly used in Serbian forestry, were rooted and grown for four months in the laboratory at 22–25 °C and natural daylight in 15 l plastic containers containing an autoclaved mixture of peat (Florabella, AGRO-FertiCrop d.o.o., Subotica, Serbia) and perlite (4–6 mm; Agro Perlit Extra, Termika, Zrenjanin, Serbia) with a 3:1 volume ratio. Isolates of all *Phytophthora* species recovered from the rhizosphere of poplar trees in this study, and, for comparisons, each isolate of *P. polonica* Belbahri et al. from *Q. robur*, *P. cryptogea* from *Q. petraea* (Matt.) Liebl., *P.* × *cambivora* from *F. sylvatica*, and *P.* × *serendipita* Man in't Veld et al. from *Pyrus pyraster* (L.) Burgsd. were included (Table 2). Twelve plants per clone and per treatment and control were used (Table 2). Inocula of 12 isolates from nine different *Phytophthora* species were prepared using fine vermiculite, millet seeds, and V8 juice [14]. The substrate in the controls received a sterile mixture of fine vermiculite, millet seeds, and V8 juice [14]. After the inoculation, plants were immediately flooded for 72 h, and flooding was repeated at three-week intervals. After each flooding period, the water was removed and sterilized with bleach. During the second and third flooding, leaves of *Prunus laurocerasus* L. and *Q. robur* were floated on the water surface in order to check the ability of the *Phytophthora* strains to produce sporangia and cause infections.

Table 2. Pathogenicity of nine *Phytophthora* species to *Populus* × *euramericana* clones I-214 and Pánnonia in the soil infestation test after 10 weeks.

Poplar Clone	*Phytophthora* Species (Isolates)	No. of Inoculated Plants	No. of Dead Plants	No. of Plants with Bark Necroses	No. of Plants with 100% Root Rot	Dry Weight of Small Roots (g)	Re-Isolation Frequency (%)	
							Necroses	Fine Roots
I-214	Control	12	0	0	0	3.591	0	0
	P. cactorum (JX276094)	12	8	2[2]	9	2.073	100	100
	P. cryptogea (KF234765)	12	7	1 [a]; 2 [b]	9	2.199	100	100
	P. gonapodyides (2011/Pop.04)	12	9	1 [a]; 1 [b]	10	1.506	83.3	100
	P. lacustris (2011/Pop.Blato.03)	12	9	2 [a]; 1 [b]	10	1.59	66.67	100
	P. pini (KF234655)	12	10	2 [a]	9	1.155	100	100
	P. pini (KF234658)	12	10	3 [a]	10	1.143	100	100
	P. plurivora (KF234737)	12	7	4 [a];1 [b]	10	1.092	100	100
	P. plurivora (KF234740)	12	11	3 [a]	11	1.26	100	83.3
	P. polonica (JX276065)	12	9	2 [a]; 1 [b]	9	1.542	100	100
	P. polonica (KF234760)	12	6	4 [a]; 1 [b]	8	1.773	100	100
	P. × *cambivora* (JX276088)	12	5	4 [a]	5	2.112	100	100
	P. × *serendipita* (KM272262)	12	11	2 [a]	10	2.199	100	100
Pánnonia	Control	12	0	0	0	4.014	0	0
	P. cactorum (JX276094)	12	10	1 [a]; 1 [b]	4	1.245	100	100
	P. cryptogea (KF234765)	12	9	1 [b]	10	1.239	100	100
	P. gonapodyides (2011/Pop.04)	12	12	0	12	1.02	100	100
	P. lacustris (2011/Pop.Blato.03)	12	8	2 [a]; 1 [b]	9	1.209	50	100
	P. pini (KF234655)	12	9	2 [b]	11	1.509	100	100
	P. pini (KF234658)	12	12	0	12	1.077	100	100
	P. plurivora (KF234737)	12	7	3 [a]; 1 [b]	8	1.158	100	100
	P. plurivora (KF234740)	12	7	3 [a]; 2 [b]	9	1.357	100	100
	P. polonica (JX276065)	12	10	2 [a]	10	1.626	100	100
	P. polonica (KF234760)	12	10	1 [b]	10	1.434	100	100
	P. × *cambivora* (JX276088)	12	10	1 [b]	9	1.359	100	91.67
	P. × *serendipita* (KM272262)	12	9	3 [a]	11	1.131	100	100

[a] girdling necroses; [b] longitudinal necroses.

The experiment was performed in the laboratory at 22–25 °C and natural daylight. After ten weeks and three flooding periods, all plants were carefully excavated and the roots washed. Photos were taken and re-isolations were made by plating small pieces from the edges of necrotic lesions and segments of fine roots from each infested and control plant onto PARPNH. Then, all roots were harvested from the cuttings, dried at 65 °C for 72 h, and their dry weight measured using a fine scale (Exacta 300 EB, Tehtnica, Železniki, Slovenia).

2.5. Underbark Inoculation Test

One-year-old cuttings of *P.* × *euramericana* clones I-214 and Pánnonia were grown for one year in an autoclaved mixture of peat (Florabella, AGRO-FertiCrop d.o.o., Subotica, Serbia) and perlite (4–6 mm; Agro Perlit Extra, Termika, Serbia) with a 2:1 volume ratio. After sterilising the bark at a 10–15 cm distance from the collar with 70% ethanol, the cuttings were inoculated under the bark using a sterile 7-mm metal cork borer. Same-sized plugs cut from the edges of three to five -day old CA colonies, were used as inocula. In total, ten isolates of seven different *Phytophthora* species were used (Table 3), including isolates of all *Phytophthora* species recovered from the rhizosphere of poplar trees in this study, and, as comparisons, one isolate each of *P. polonica* from *Q. robur* and *P.* × *cambivora* from *F. sylvatica*. Control plants were inoculated with sterile CA plugs. The agar plugs were covered with the removed piece of bark and sterile moistened cotton, and sealed with Parafilm (Merck KGaA, Darmstadt, Germany). The plants were incubated in the laboratory at 22–25 °C and natural daylight, and checked weekly for the appearance of symptoms. After 11 weeks, the experiment was finished and necroses lengths were recorded after removal of the outer bark. Reisolations were made from all the inoculated and control plants by plating small pieces from the upper and lower margins of necrotic lesions or, in the absence of necroses, from the margins of the inoculation places onto PARPNH. Measuring of necrosis length was performed using a precise ruler, while necrosis width was measured using a flexible measurement tape.

Table 3. Results of the underbark inoculation test with *Populus* × *euramericana* clones I-214 and Pánnonia and seven *Phytophthora* spp. after 11 weeks.

Poplar Clone	*Phytophthora* Species (Isolates)	Number of Inoculated Plants	Stem Diameter (x^{-} ± SE (mm))	Plant Height (x^{-} ± SE (mm))	Number of Plants with Lesions (Bleeding)	Number of Plants with Secondary Shoots at Necroses Margins	Number of Plants with Dieback	Reisolation Frequency (%)
I-214	Control	12	10.6 ± 0.29	103.6 ± 3.16	0	0	0	0
	P. cactorum (JX276094)	12	9.3 ± 0.26	95.7 ± 3.47	12 (9)	2	0	100
	P. gonapodyides (2011/Pop.04)	12	9.7 ± 0.21	93.7 ± 2.74	12 (2)	0	0	83.3
	P. lacustris (2011/Pop.Blato.03)	12	9.1 ± 0.36	90.1 ± 3.72	12 (3)	0	0	83.3
	P. pini (KF234655)	12	11.2 ± 0.49	103.8 ± 3.58	12 (8)	5	5	100
	P. pini (KF234658)	12	10.6 ± 0.31	100.2 ± 2.16	12 (11)	10	1	100
	P. plurivora (KF234737)	12	9.3 ± 0.34	89 ± 3.02	12 (6)	0	1	100
	P. plurivora (KF234740)	12	10.1 ± 0.3	100.5 ± 3.49	12 (9)	4	1	100
	P. polonica (JX276065)	12	9.3 ± 0.22	94.1 ± 2.57	7 (0)	0	0	91.67
	P. polonica (KF234760)	12	10.1 ± 0.37	95.7 ± 2.15	9 (0)	0	0	100
	P. × *cambivora* (JX276088)	12	9.8 ± 0.51	100.6 ± 3.87	10 (0)	1	0	58.3

Table 3. *Cont.*

Poplar Clone	*Phytophthora* Species (Isolates)	Number of Inoculated Plants	Stem Diameter (x^{-} ± SE (mm))	Plant Height (x^{-} ± SE (mm))	Number of Plants with Lesions (Bleeding)	Number of Plants with Secondary Shoots at Necroses Margins	Number of Plants with Dieback	Reisolation Frequency (%)
Pánnonia	Control	12	9.4 ± 0.56	110.7 ± 5.74	0	0	0	0
	P. cactorum (JX276094)	12	10.9 ± 0.64	111.2 ± 6.11	12 (2)	3	2	100
	P. gonapodyides (2011/Pop.04)	12	9.9 ± 0.4	118 ± 5.48	12 (2)	0	7	91.67
	P. lacustris (2011/Pop.Blato.03)	12	7.9 ± 0.28	74.8 ± 2.27	12 (3)	5	0	66.67
	P. pini (KF234655)	12	9.9 ± 0.42	103.7 ± 4.49	12 (6)	0	1	100
	P. pini (KF234658)	12	9.8 ± 0.55	113 ± 7.39	12 (4)	2	1	100
	P. plurivora (KF234737)	12	8.4 ± 0.29	98.6 ± 6.06	12 (7)	0	0	100
	P. plurivora (KF234740)	12	9.9 ± 0.56	114.1 ± 6.01	12 (5)	0	1	100
	P. polonica (JX276065)	12	9.2 ± 0.25	103.6 ± 3.57	11 (4)	2	0	100
	P. polonica (KF234760)	12	10.3 ± 0.54	104.4 ± 6.45	10 (0)	0	0	91.67
	P. × *cambivora* (JX276088)	12	10.5 ± 0.53	104.2 ± 7.28	8 (0)	2	0	83.3

2.6. Statistical Analyses

Based on the length and width, the surface of each necrosis was calculated using the mathematical formula for elliptic surfaces. For each treatment, means (x^{-}) and standard errors (±SE) of necrosis length, width, and surface were calculated. Analysis of variance was performed using a Generalized Linear Model (GLM), with a significance level of $\alpha = 0.05$. Testing of significance of differences in mean necrosis length, width, and surface between different treatments was performed using Tukey's HSD post hoc test ($\alpha = 0.05$). Statistical procedures were performed with the RStudio software version 1.1.383 (Integrated Development for R. RStudio, Inc., Boston, MA, USA).

3. Results

3.1. Phytophthora Species in Poplar Plantations

In total, 45 isolates of six different *Phytophthora* species were isolated from 24 of 28 soil samples (86%) (Table 1). The most common was *P. plurivora* (15 samples = 53.6%), followed by *Phytophthora pini* Leonian (7 samples = 25%), *Phytophthora gonapodyides* (H.E. Petersen) Buisman (6 samples = 21.4%), and *P. polonica*, *Phytophthora lacustris* Nechwatal et al. and *P. cactorum* (each 4 samples = 14.3%) (Table 1). In the mainly healthy stands 1 and 3, all samples were taken underneath non-symptomatic trees. Additionally, in stand 2, five of the six samples originated from non-symptomatic trees. In contrast, six of the seven samples in stand 4 were taken from trees with high crown transparency and yellowing of leaves. In the rhizosphere of each four declining trees, *P. plurivora* and *P. polonica* were found (Table 1). The three samples taken in stands 5 and 6 originated from trees showing severe dieback. *Phytophthora gonapodyides* was isolated from all three samples, while *P. plurivora* was present in one sample (Table 1). Co-occurrence of two different *Phytophthora* species was found in 11 of the 25 samples, while in one sample, three *Phytophthora* spp. co-occurred (Table 1).

The morphology of all isolates of *P. cactorum*, *P. pini*, *P. plurivora*, and *P. polonica* conformed with the original descriptions [2,18–21]. In accordance with [22], *P. lacustris* and *P. gonapodyides* did not form gametangia in pure cultures or in the mating tests. This is the first report of *P. lacustris*, *P. gonapodyides*, *P. pini*, and *P. polonica* on poplars in Serbia.

3.2. Soil Infestation Test

Five weeks after the inoculation (pi), the first symptoms like slight yellowing and atrophy of leaves were noticed in clone I-214 in the substrate infested by *P. plurivora*. Eight weeks pi, the first dieback of plants occurred in both clones in the substrate infested by *P. plurivora*, *P. pini*, and *P. gonapodyides*. Ten weeks pi, dieback also started in clone I-214 infested by *P. cactorum* and in clone Pánnonia in the substrate infested by the *P. polonica* strain originally isolated from oak. These diebacks were followed by premature shedding of leaves and total plant collapse. In addition, in the treatment Pánnonia/*P. plurivora* (KF234737), collar necroses were observed on two plants (Figure 2o). Ten weeks pi and after three flooding periods, numerous plants started to decline in most of the treatments (Table 2), and the experiment was assessed.

Figure 2. Representative cuttings of poplar clone I-214 after ten weeks in soil infested with nine *Phytophthora* spp.: (**a**,**b**) *P. cactorum*; (**c**,**d**) *P. cryptogea*; (**e**) *P. gonapodyides*; (**f**,**g**) *P. lacustris*; (**h**,**i**) *P. pini* KF234655; (**j**,**k**) *P. pini* KF234658; (**l**,**m**) *P. plurivora* KF234737; (**n**,**o**) *P. plurivora* KF234740; (**p**,**q**) *P. polonica* JX276065; (**r**,**s**) *P. polonica* KF234760; (**t**,**u**) *P.* × *serendipita*; (**v**,**w**) *P.* × *cambivora*; (**x**,**z**) control.

The most aggressive species to poplar clone I-214 were *P. plurivora* (KF234740) and *P.* × *serendipita*, each causing the decline of 11 poplar plants (91.67%), followed by both strains of *P. pini* (10 declining

plants = 83.3%) (Table 2). In several treatments, necrotic bark lesions were recorded (Figure 2). Most *Phytophthora* species caused root rot and the loss of fine roots (Figure 2). The reduction of total root dry weight compared to the control was most severe for the two isolates of *P. plurivora* (30.4% and 35.1%, respectively) and the two isolates of *P. pini* (31.8% and 32.2%, respectively). Both pathogens caused 100% root rot in 75–91.2% of plants (Table 2). In the case of clone Pánnonia, the most aggressive species were *P. gonapodyides* and one isolate of *P. pini* (KF234658), which both caused 100% mortality, followed by *P. cactorum*, *P.* × *cambivora* and *P. polonica* each killing 10 plants (83.3%) (Table 2). Root rot and loss of fine roots were recorded in all treatments (Figure 2). The highest reduction of total root dry weight was caused by *P. gonapodyides* (25.4% compared to the control) and one isolate of *P. pini* (26.8%) (Table 2). These two species also killed all plants (Table 2). Additionally, all other *Phytophthora* species/isolates caused severe reductions of total root dry weight (28.2–40.5% compared to the control) and 100% root rot in 33.3–91.7% of plants (Table 2). In addition, several *Phytophthora* species caused girdling and longitudinal bark lesions (Figure 3).

Figure 3. Representative cuttings of poplar clone Pánnonia after ten weeks in soil infested with nine *Phytophthora* spp.: (**a**,**b**) *P. cactorum*; (**c**,**d**) *P. cryptogea*; (**e**,**f**) *P. gonapodyides*; (**g**,**h**) *P. lacustris*; (**i**,**j**) *P. pini* KF234655; (**k**,**l**) *P. pini* KF234658; (**m**,**o**) *P. plurivora* KF234737; (**p**,**r**) *P. plurivora* KF234740; (**s**) *P. polonica* JX276065; (**t**) *P. polonica* KF234760; (**u**,**v**) *P.* × *cambivora*; (**w**) *P.* × *serendipita*; (**x**,**y**) control.

3.3. Underbark Inoculation Test

Recorded symptoms and numbers of plants with dieback are shown in Table 3 and Figures 4 and 5. Four weeks after inoculation (pi), the first discrete bark necroses were observed, soon followed by the oozing of exudates. In clone I-214, these symptoms appeared first in the treatments with both *P. pini* isolates and one isolate of *P. plurivora* (KF234737). In clone Pánnonia, the first bleeding lesions were caused by *P. cactorum*. Six weeks pi, intensive bleeding lesions on both clones were recorded in the treatments with *P. pini*, *P. plurivora*, and *P. cactorum*. In addition, in clone I-214, small necroses were caused by *P. lacustris*, while in clone Pánnonia, bleeding cankers started to appear on individual plants infected by *P. lacustris*, *P. gonapodyides*, and the *P. polonica* isolate from oak (JX276065). Eight weeks pi, symptoms had progressed and numerous secondary shoots started to develop at the margins of necrotic zones (Figure 5b,c). In addition, the first plants with dieback were observed in the I-214/*P. pini* (KF234655) and the Pánnonia/*P. gonapodyides* treatments. Eleven weeks pi, more plants of both clones showed dieback and other symptoms, and the experiment was finished. Reisolations were successful in all treatments except of the controls, although with slightly lower reisolation rates in some cases (Table 3).

Figure 4. Representative symptoms caused by seven *Phytophthora* spp. on cuttings of poplar clone I-214 in the underbark inoculation test: (**a**,**d**) bleeding bark lesions after eight weeks: (**a**,**b**) *P. pini*; (**c**) *P. cactorum*, with beginning formation of secondary shoot; (**d**) *P. plurivora*, with beginning formation of secondary shoot; (**e**) cuttings inoculated with *P. pini* (KF234655) after 11 weeks; (**f**,**r**) necrotic lesions of the inner bark after 11 weeks: (**f**,**g**) *P. cactorum*; (**h**) *P.* × *cambivora*; (**i**) *P. gonapodyides*; (**j**) *P. lacustris*; (**k**) *P. polonica* JX276065; (**l**) *P. polonica* KF234760; (**m**) *P. pini* KF234658; (**n**) *P. pini* KF234655; (**o**,**p**) *P. plurivora* KF234740; (**q**) *P. plurivora* KF234737; (**r**) control.

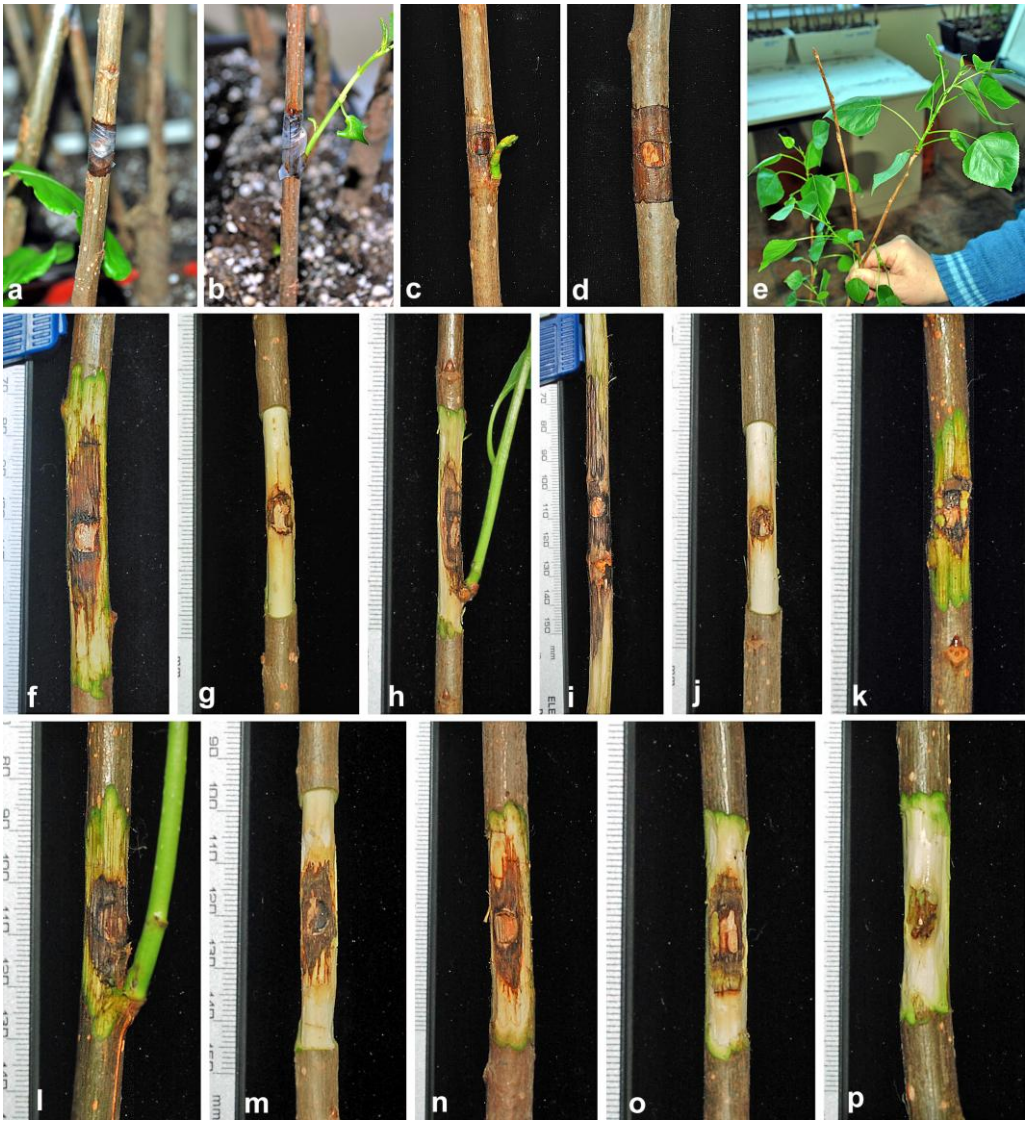

Figure 5. Representative symptoms caused by seven *Phytophthora* spp. on cuttings of poplar clone Pánnonia in the underbark inoculation test: (**a**,**b**) bleeding bark lesions after eight weeks: (**a**) *P. cactorum*; (**b**) *P. lacustris*, with formation of secondary shoot. (**c**,**d**) necrotic lesions of outer bark after 11 weeks: (**c**) *P. plurivora*, with beginning formation of secondary shoot; (**d**) *P. gonapodyides.* (**e**) declining and healthy plants inoculated with *P. pini* after eight weeks. (**f**–**p**) necrotic lesions of the inner bark after 11 weeks: (**f**) *P. cactorum*; (**g**) *P.* × *cambivora*; (**h**) *P. lacustris*; (**i**) *P. gonapodyides*; (**j**) *P. polonica* JX276065; (**k**) *P. polonica* KF234760; (**l**) *P. pini* KF234658; (**m**) *P. pini* KF234655; (**n**) *P. plurivora* KF234737; (**o**) *P. plurivora* KF234740; (**p**) control.

The statistical analyses showed that in clone I214, most *Phytophthora* species significantly influenced most of the tested parameters. Exceptions were found for *P. gonapodyides* and *P. polonica* and lesion lengths; *P. gonapodyides*, *P. lacustris*, *P. polonica* and *P.* × *cambivora* and lesion widths; and *P. gonapodyides*, *P. lacustris*, and *P. polonica* and the surface areas of the lesions (Table 4). In the case of clone Pánnonia, only in plants infected with *P. polonica* (JX276065) and *P.* × *cambivora* did the lesion lengths differ significantly from the control. In contrast, plants infected with *P. gonapodyides*, *P. pini* (KF234655), and *P. polonica* (KF234760) showed significantly higher lesion widths compared to the control plants (Table 4). Lesions caused by these three *Phytophthora* spp. and *P. cactorum* also showed

significantly higher surface areas compared to the control (Table 4). Results of Tukey's test are shown in Table 5.

Table 4. Estimates, *t* values, *p* values, and Residual deviances from GLM for tested parameters in the underbark inoculation trial with poplars. In clone I-214, degrees of freedom (df) for Residual deviance was 114; in clone Pánnonia df for Residual, deviance was 109. Significant effects are marked in bold type.

Poplar Clone Host	Treatment	Length			Width			Surface		
		Estimate	*t* Value	(*p*)	Estimate	*t* Value	(*p*)	Estimate	*t* Value	(*p*)
I214	Control	2.32	19.44	**0.000**	1.95	33.29	**0.000**	4.03	21.78	**0.000**
	P. cactorum	1.66	10.04	**0.000**	0.63	7.70	**0.000**	2.35	9.17	**0.000**
	P. gonapodyides	0.11	0.67	**0.506**	-0.07	-0.84	0.405	0.05	0.18	0.855
	P. lacustris	0.45	2.71	**0.008**	0.01	0.14	0.890	0.49	1.92	0.057
	P. pini (KF234655)	1.72	9.96	**0.000**	0.67	7.91	**0.000**	2.47	9.21	**0.000**
	P. pini (KF234658)	1.46	8.65	**0.000**	0.56	6.69	**0.000**	2.05	7.82	**0.000**
	P. plurivora (KF234737)	0.89	5.24	**0.000**	0.32	3.89	**0.000**	1.25	4.78	**0.000**
	P. plurivora (KF234740)	1.45	8.59	**0.000**	0.57	6.98	**0.000**	2.0	7.62	**0.000**
	P. polonica (JX276065)	-0.03	-0.18	0.861	0.01	0.07	0.947	-0.02	-0.09	0.933
	P. polonica (KF234760)	-0.02	-0.12	0.909	-0.08	-0.99	0.323	-0.1	-0.38	0.703
	P. × *cambivora*	0.50	2.96	**<0.010**	-0.05	-0.56	0.579	0.66	2.53	**0.013**
	Residual deviance		12.4			4.05			27.33	
Pánnonia	Control	2.29	14.83	**0.000**	1.99	22.85	**0.000**	4.05	15.02	**0.000**
	P. cactorum	1.07	4.76	**0.000**	0.19	1.48	0.141	1.32	3.38	**<0.010**
	P. gonapodyides	1.11	3.89	**0.000**	0.56	3.45	**<0.010**	2.16	4.33	**0.000**
	P. lacustris	0.57	2.62	**<0.010**	0.02	0.16	0.870	0.64	1.68	0.096
	P. pini (KF234655)	1.03	4.61	**0.000**	0.48	3.81	**0.000**	1.72	4.41	**0.000**
	P. pini (KF234658)	0.53	2.36	**0.020**	0.14	1.11	0.270	0.68	1.73	0.086
	P. plurivora (KF234737)	0.55	2.51	**0.014**	0.16	1.26	0.212	0.72	1.88	0.063
	P. plurivora (KF234740)	0.64	2.85	**<0.010**	0.16	-0.43	0.210	0.79	-0.02	**0.045**
	P. polonica (JX276065)	0.05	0.22	0.826	-0.05	2.66	0.670	-0.01	2.95	0.982
	P. polonica (KF234760)	0.50	2.26	**0.026**	0.33	1.18	**<0.010**	1.12	1.61	**<0.010**
	P. × *cambivora*	0.26	1.20	0.232	0.15	1.26	0.241	0.61	2.02	0.111
	Residual deviance		20.89			7.36			53.52	

The two isolates of *P. pini* were most aggressive to clone I-214, causing bleeding lesions in 66.7 and 91.3% of plants and dieback in 8.3% and 41.7% of plants, respectively (Table 3). Also, *P. cactorum* (bleeding lesions in 75% of plants) and the two isolates of *P. plurivora* (bleeding lesions in 50% and 75% of plants, respectively) showed considerable aggressiveness to clone I-214 (Table 3). These three *Phytophthora* species also caused the largest bark lesions with mean necrosis areas that were statistically significantly different from all other treatments and the control, ranging between 415.5 ± 60.2 and 668.1 ± 128.1 mm^2 (Table 5). *Phytophthora gonapodyides* and both isolates of *P. polonica* were non-pathogenic to the bark of clone I-214, while *P.* × *cambivora* was mildly pathogenic (Tables 3 and 5).

Table 5. Mean values, standard errors, and results of Tukey's post hoc tests for the bark necroses caused by seven *Phytophthora* spp. in the underbark inoculation test on poplar clones I-214 and Pánnonia after 11 weeks. Different letters behind values indicate significant differences ($\alpha = 0.05$).

Phytophthora Species (Isolates)	Poplar Clone I-214 (Mean ± SE+Tukey's Test)			Poplar Clone Pánnonia (Mean ± SE+Tukey's Test)		
	Necrosis Length (mm)	Necrosis Width (mm)	Necrosis Area (mm^2)	Necrosis Length (mm)	Necrosis Width (mm)	Necrosis Area (mm^2)
Control	10.2 ± 0.18f	7 ± 0.18F	56.5 ± 1.87f	9.9 ± 0.38d	7.3 ± 0.19c	57.5 ± 3.30d
P. cactorum	53.7 ± 5.61abcd	13.2 ± 1.13abcd	592.9 ± 104.52abcd	28.8 ± 5.14abc	8.9 ± 0.42abc	214.9 ± 47.02abcd
P. gonapodyides	11.4 ± 0.61f	6.6 ± 0.14f	59.2 ± 3.88f	30 ± 17.43 [a]abc	12.8 ± 3.61 [a]abc	496.4 ± 404.56 [a]abcd
P. lacustris	16 ± 1.69ef	7.1 ± 0.29f	92.4 ± 13.19ef	17.6 ± 1.54abcd	7.5 ± 0.46c	109.3 ± 16.97abcd
P. pini (KF234655)	57.2 ± 6.39abcd	13.8 ± 1.12abcd	668.8 ± 128.10abcd	27.8 ± 6.24abc	11.9 ± 1.50abc	321.5 ± 118.21abcd
P. pini (KF234658)	44 ± 3.69abcd	12.3 ± 0.52abcde	437.1 ± 59.78abcd	16.8 ± 0.96abcd	8.4 ± 0.54cbc	113.2 ± 11.83abcd
P. plurivora (KF234737)	24.7 ± 2.93ef	9.7 ± 0.46cde	197.2 ± 33.26cdef	17.2 ± 0.70abd	8.6 ± 0.40cbc	117.7 ± 10.72abcd
P. plurivora (KF234740)	43.5 ± 4.76abcd	11.6 ± 0.72abcde	415.5 ± 60.22abcde	18.8 ± 1.27abcd	8.6 ± 0.38cbc	126.8 ± 6.29abcd
P. polonica (JX276065)	9.9 ± 0.25f	7.1 ± 0.22f	55.3 ± 2.57f	10.4 ± 0.41d	7 ± 0.15c	57 ± 2.67d
P. polonica (KF234760)	10 ± 0.24f	6.5 ± 0.20f	51.2 ± 2.22f	16.3 ± 4.35abcd	10.2 ± 1.31cbc	177 ± 93.63abcd
P. × *cambivora*	16.8 ± 4.99ef	6.7 ± 0.59f	109.4 ± 51.31ef	12.9 ± 2.14ad	8.5 ± 1.20cbc	106.2 ± 39.73abcd

[a] Due to the mortality of seven plants only five plants had clearly visible necroses margins which were measured.

In contrast, on clone Pánnonia, *P. gonapodyides* was by far the most aggressive species, causing the dieback of 58.3% of plants and the largest bark lesions with a mean necrosis area of 496.37 ± 404.56 mm^2, followed by *P. cactorum* with dieback in 16.7% of plants and *P. pini* and *P. plurivora* each causing dieback in 8.3% of plants (Table 3). All *Phytophthora* species and isolates, except of the *P. polonica* isolate from oak (JX276065), caused bleeding bark lesions with sizes significantly different from the control (Figure 5; Table 5).

4. Discussion

Prior to this study, very little was known about the occurrence and role of *Phytophthora* species in poplar stands. *Phytophthora cactorum* had been isolated from bleeding cankers of *Populus alba* in the Czech Republic [11] and from the rhizosphere of young *P. alba* trees in Hungary [4], while *P.* × *cambivora* was recovered from poplar trees in Croatia [12]. A preliminary study demonstrated the presence of *P. cactorum* and *P. plurivora* in the rhizosphere of poplar trees in Serbia [13].

In the present work, a community of six *Phytophthora* species, *P. cactorum*, *P. gonapodyides*, *P. lacustris*, *P. pini*, *P. plurivora* and *P. polonica*, was found in the rhizosphere of 85% of sampled trees in six riparian poplar plantations in Serbia. While *P. gonapodyides* and *P. lacustris* might be native to Europe, the other four *Phytophthora* species are considered as introduced invasive pathogens in Europe [3–5,20]. The known host-*Phytophthora* associations of *P. cactorum* and *P. plurivora* and *Populus* spp. [4,11–13] were confirmed by this study. In both the soil infestation and the underbark inoculation test, *P. cactorum* and *P. plurivora* demonstrated high aggressiveness to the roots and bark of poplar clones I-214 and Pánnonia. In addition, this study also extended the knowledge on the distribution and host ranges of several *Phytophthora* species with *P. gonapodyides*, *P. lacustris*, *P. pini*, and *P. polonica* being the first records from poplar trees, and the latter two *Phytophthora* species also being the first records from Serbia and the Balkan region in general.

Phytophthora pini, like *P. plurivora*, belongs to the '*P. citricola* complex' within *Phytophthora* phylogenetic Clade 2 [21,25]. It was previously recorded on at least seven different plant species in North America and Europe, mainly ornamentals, but also *Pinus resinosa* Ait. and mature *F. sylvatica* trees [4,5,21,26–29]. However, its host range is most likely considerably wider since many plant

diseases assigned in the past to *P. citricola s.l.* Sawada were most likely caused by *P. plurivora* and *P. pini* [20,21]. *Phytophthora pini* also occurs in water courses and irrigation reservoirs in North America [21]. The findings of this species in riparian poplar stands along the Sava River in Serbia are in accordance with its preference of wet sites. Like *P. plurivora*, in both the soil infestation and the underbark inoculation test, *P. pini* was highly aggressive to both tested poplar cultivars, and in particular to clone I-214. This is of high concern since I-214 is one of the most common clones used in poplar plantations in Serbia. Due to their high aggressiveness to poplars and other tree species, their wide host ranges, and homothallic production of oospores, acting as enduring survival structures [3,5,20,21], the presence of *P. pini* and *P. plurivora* most likely poses a serious risk to planted and natural stands of poplars and other tree species in Serbia.

Phytophthora polonica from Clade 9 [30] is also homothallic and was originally described from the rhizosphere of declining *Alnus glutinosa* (L.) Gaertn. trees in Poland [19]. Recently, this species was isolated from soils of declining *Juglans nigra* L. and *Q. robur* stands in Hungary and Poland [31,32], respectively. Furthermore, in Serbia, *P. polonica* was previously isolated in 2011 from declining *Q. robur* trees [33]. In previous pathogenicity tests, *P. polonica* proved only weakly pathogenic to *Alnus* shoots and was non-pathogenic to shoots of *Fraxinus angustifolia* Vahl and three *Quercus* spp. [19]. In the present study, *P. polonica* was isolated from the rhizosphere of symptomatic poplar trees and caused significant root rot, extensive loss of fine roots, and dieback on poplar clones I-214 and Pánnonia in the soil infestation test. In contrast, this pathogen was almost non-pathogenic to poplar bark in the underbark inoculation test. These results suggest the involvement of *P. polonica* in the complex of poplar dieback as a serious fine root pathogen which is not progressing into the suberised roots. In previous studies, a similar aetiology was demonstrated for *P. quercina* Jung and European oak decline [14,16,34–36].

Interestingly, in both pathogenicity tests, *P. gonapodyides* from Clade 6 [30] was the most aggressive species to clone Pánnonia, causing extensive root rot, fine root loss, bark lesions, and dieback. *Phytphthora gonapodyides* is considered as an opportunistic pathogen with a mainly aquatic lifestyle [37,38]. However, *P. gonapodyides* is also involved in the declines of oak and beech stands on mesic sites in Germany and the decline of *Quercus ilex* L. in xeric conditions in Spain [3,14–16,39]. Due to its ubiquitous presence in watercourses across Europe and its high aggressiveness to clones Pánnonia and I-214, this pathogen poses a significant threat to riparian poplar stands. *Phytophthora lacustris* is another Clade 6 species [30] commonly occurring in waterways and in riparian stands [22]. In the soil infestation test, *P. lacustris* caused extensive fine root damage, girdling and longitudinal bark lesions developing from the infected root system into the stem, and dieback on both poplar clones. In contrast, in the underbark inoculation trial, this species was only slightly pathogenic to both clones, indicating flooding as an indispensable requisite for successful infections in this aquatic *Phytophthora* species. As with *P. gonapodyides*, the ubiquitous presence of *P. lacustris* in European waterways might pose a risk to the health of riparian poplar stands in Serbia and elsewhere. *Phytophthora cactorum*, *P. lacustris* (referred to as *P.* taxon salixsoil Brasier et al.), and *P. plurivora* also proved to be pathogenic to the roots and bark of *Fraxinus excelsior*, another common species in riparian forest stands [40].

It is possible that the six isolated *Phytophthora* species were already present at the sites before the six poplar plantations sampled in this study were established. River water appears as the most likely natural pathway of introduction since all six plantations are located in the flood plains of the Sava River, experiencing in most years strong floodings [41], or smaller rivers in central Serbia, respectively. On a global scale, river water is ubiquitously infested with a wide array of *Phytophthora* species [37,42–49]. The importance of water as a source of *Phytophthora* inoculum was convincingly demonstrated by several studies [50–56]. Severe and long-during floodings such as those affecting wide regions of Serbia in the years 2014 and 2016 are, hence, of particular concern as they enable the spread of a diverse range of harmful *Phytophthora* species from large catchments to forests and plantations situated in the floodplains downstream. However, against the background of almost ubiquitous infestations of nursery stands in Europe with a total of more than 50 *Phytophthora* species [4,7,57], including

P. cactorum, *P. gonapodyides*, *P. lacustris*, *P. pini*, *P. plurivora*, and *P. polonica*, a possible introduction of these *Phytophthora* species to the Serbian poplar plantations with infested nursery stock cannot be ruled out. Unfortunately, it was not possible to identify and sample the nurseries from where the plants, used for the establishment of the *Phytophthora*-infested poplar plantations in Serbia, originated.

In conclusion, the presence of the six *Phytophthora* species in the sampled poplar stands poses a serious threat to poplars, in particular to plants in young plantations, and potentially also to other riparian tree species. Continued monitoring of the presence and diversity of *Phytophthora* species in riparian poplar stands and other hygrophilic stands and in water courses in Serbia is urgently required in order to assess the magnitude of the problem and develop appropriate management concepts for the disease. Since the riparian poplar plantations are seasonally flooded with *Phytophthora*-infested river water, the repeated introduction and spread of *Phytophthora* spp. into and within the poplar plantations cannot be prevented. Direct control of *Phytophthora* spp. with fungizides like metalaxyl is impossible since their application is not permitted in riparian stands. Furthermore, the use of potassium phosphite, which is globally the most efficient control measure for forest *Phytophthora* diseases [5,58], is no longer possible due to its recent registration as a fungizide in Europe. Therefore, the most promising management measure will be the use of less susceptible poplar clones or other less susceptible riparian tree species, which requires extensive host range testing with the six *Phytophthora* species and other poplar clones and riparian tree species.

5. Conclusions

(1) A community of six different *Phytophthora* species, *P. cactorum*, *P. gonapodyides*, *P. lacustris*, *P. pini*, *P, plurivora*, and *P. polonica*, was detected in each of the three symptomatic and healthy, riparian poplar plantations in Serbia.

(2) In both a soil infestation test and an underbark inoculation test, all six *Phytophthora* species proved their pathogenicity to four-month and one-year-old cuttings of poplar clones I-214 and Pánnonia, respectively.

(3) The results suggest the involvement of soilborne *Phytophthora* species as fine root and bark pathogens in the decline of poplar plantations. The presence of these *Phytophthora* species in riparian poplar plantations might also pose a serious risk to other riparian forest communities, in particular the natural stands of *Quercus robur* and *Fraxinus angustifolia*.

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