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ORIGINAL ARTICLE

Incidence and pathogenicity of *Phytophthora* species in beech (*Fagus sylvatica* L.) stands in Slovakia

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Abstract

The common beech (*Fagus sylvatica* L.) is the main forest-forming species in Slovakia and its share accounts for over 31% of the total forest area in this country. Recently, there are more and more reports of the deterioration of the health of this species in Europe, incl. as a result of the action of pathogens of the genus *Phytophthora*. The aim of this study is to confirm the presence of pathogens of the genus *Phytophthora* in beech forests in Slovakia. Ten plots in central and western Slovakia were selected for the study. The presence of: *P. x cambivora*, *P. cactorum*, *P. plurivora*, *Globisporangium macrosporum*, and *G. heterothallicum* was confirmed in the samples taken. A pathogenicity test was performed to confirm Koch's postulates. After three months, the plants were gently taken out, and then: the pathogen was reisolated from the roots to confirm its presence in the tissues, the root systems were scanned and the image was analyzed with WinRhizo software, and finally the roots were dried to obtaining dry biomass. Additionally, during the course of the experiment, the degree of infection of the plants was assessed weekly in order to calculate the area under the disease-progress curve. The conducted research showed the greatest threat from *P. x cambivora*. In this variant, the symptoms of plant dieback were observed the fastest, as well as the pathogen, compared to the control variant, significantly damaged the root systems.

Keywords Dieback · *Phytophthora* · Root disease · European beech

Introduction

High shade tolerance and growth capacity as well as high climatic and geological amplitude of the European beech (*Fagus sylvatica* L.) make this species the most competitive in Western and Central Europe, especially in mountainous areas (Walentowski et al. 2004; Ammer et al. 2005; Kölling et al. 2005; Felbermeier and Mosandl 2006). *F. sylvatica* is the species with the largest share in the forests of the Slovakia, exceeding 31.6% (Schieber et al. 2013). Its vertical range extends from about 150 m to 1450 m above sea level however, its ecological and production optimum is narrower, from 450 to 900 m a.s.l. only. Due to the particularly favorable temperature and humidity, beech shows good vitality there. Until recently, beech was considered one of the most resistant to diseases of forest tree species (Leuschner 2020). There are reports of complex beech shoulder disease (BBD), and other factors that may cause the decline of this tree species, such as fungi, oomyceta or drought and heat (Butin 1996; Felbermeier and Mosandl 2006; Purahong et al. 2021; Frei et al. 2022; Langer and Bußkamp 2021; Meyer et al. 2022; Riolo et al. 2022). Despite this,, Dyderski et al. (2018), in his work on the impact of climate change on the occurrence of trees in Europe, included beech among others in the group of "winner" species, emphasizing the ability to adapt to new conditions.

However, the presence of *F. sylvatica* in humid habitats may also be the reason for observing problems related to the activity of pathogens of the *Phytophthora* genus. Infections from these dangerous pathogens and their participation in beech dieback have already been noted and confirmed in many studies (Brasier et al. 2005; Jung et al. 2005a, b; Orlikowski et al. 2006; Schmitz et al. 2007; Munda et al. 2007; Jung 2009; Jung and Burgessa 2009; Weilend et al. 2010; Milenković et al. 2012). These organisms are responsible for economic losses in many sectors

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of the economy around the world. *Phytophthora* can infect various tissues, including: fine roots, bark and cambium from woody roots and stems and shoots, and leaves of a very wide range of host species in nurseries, ornamental plantings, object and forest ecosystems (Erwin and Ribeiro 1996). Limiting the spread of these organisms is almost inevitable, especially due to the increase in international trade in live plants (Evans and Oszako 2006; Brasier 2008) and the introduction of *Phytophthora* with seedlings into parks, forests and natural ecosystems (Brasier and Jung 2006). Information on the occurrence of the *Phytophthora* species infecting the European beech in the Slovakia is very poor so far. So far, only Jung et al. (2016) published the results of the *Phytophthora* inventory in beech stands in Slovakia, but did not test their virulence. Nevertheless, symptoms typical of *Phytophthora* activity are often observed, such as: small, fine-grained, sparse and often yellowish leaves, crown dieback, root and root neck rot, and bleeding air cankers up to stem height > 20 m (Jung et al. 2005a, b).

In recent years, the condition of beech stands has been constantly deteriorating in Slovakia (Kunca 2020). One of the factors responsible for this state of affairs may be the presence of pathogens of the genus *Phytophthora*, but so far no studies have been carried out to confirm or exclude the involvement of these dangerous pathogens in the weakening of beech stands in Slovakia. For this reason, the presented research focuses on: (1) to inventorying the presence and diversity of *Phytophthora* in beech stands in Slovakia, (2) to test the aggressiveness of the isolated species.

Methods

Study sites and disease symptoms

After consulting with forest managers, it was decided to designate ten mature European beech stands located in the central and western part of Slovakia, where the progressive tree weakening has been observed in the last decade (Table 1). All the selected stands are managed stands with the dominant share of European beech. Although the stands were characterized by weakened conditions and contained symptomatic trees (e.g. yellowing of leaves, crown transparency, aerial cankers), declining symptoms were not uniformly observed in all stands; seemingly healthy individual trees with no visible crown symptoms occurred throughout.

Sampling and isolation

Samples were collected in April 2022. On each plot, 4 soil samples were collected along with the roots, and where possible, water and tissue samples (if aerial cankers were observed) were taken. A total of 40 soil and root samples, 2 water samples and 3 tissue samples were collected from all locations. The sampling and isolation methods were performed according to that described by Jung (1998, 2009) and Jung et al. (1996) methodology. Tissue fragments from trees with visible aerial cankers were taken from necrotic parts, superficially sterilized for one minute in 1% sodium hypochlorite solution and placed directly on a V8-PARPNH selective medium (16 g/l agar, 2 g/l $CaCO_3$, 100 ml/l vegetable juice with the addition of antibiotics 10 µg ml^{-1}

Table 1 Study locations and samples taken in beech stands in Slovakia

Location	No	Coordinates	Disease symptoms	Number of samples	Number of positive samples	
					N	%
Banská Štiavnica	1	48° 27′ 41.8″ N 18° 56′ 37.6″ E	Aerial cankers, crown transparency	4S/1T5	1	20
Zvolen	2	48° 30′ 56.6″ N 19° 06′ 21.2″ E	Yellowing of leaves and crown transparency	4S	3	75
	3	48° 31′ 15.7″ N 19° 06′ 58.5″ E	No symptoms	4S/1W/1T6	2	33
Liptovská Osada	4	48° 58′ 33.0″ N 19° 12′ 29.5″ E	Crown transparency	4S	1	25
Brezno	5	48° 54′ 15.2″ N 19° 42′ 04.7″ E	Crown transparency	4S	0	0
Partizánske	6	48° 32′ 30.9″ N 18° 31′ 58.9″ E	Crown transparency	4S/1W5	2	40
Bánovce nad Bebravou	7	48° 42′ 56.1″ N 18° 02′ 37.4″ E	No symptoms	4S	0	0
	8	48° 50′ 39.1″ N 18° 23′ 53.5″ E	Yellowing of leaves and crown transparency	4S/1T5	1	20
Trenčianske Teplice	9	48° 51′ 11.6″ N 18° 11′ 43.2″ E	Crown transparency	4S	2	50
Nováky	10	48° 43′ 22.0″ N 18° 30′ 34.7″ E	Crown transparency	4S	1	25

S, soil and root samples; W, water samples; T, tissue samples

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). Soil and root system samples were collected using soil monoliths with dimensions of about 20 × 20 × 20 cm. In the laboratory, from each sample 200 g of soil was flooded with 500 ml of distilled water. Young beech leaves were used as baits. Five to seven leaves were placed on the water surface in each soil and water container. After 3–7 days, brown spots appeared on the surfaces of some leaves. These were then cut into smaller pieces (approximately 5 × 5 mm) and placed on V8-PARPNH selective media (Jung et al. 1996, 2000; Jung 2009). The Petri dishes were incubated for a minimum of 48 h in the dark at 20 °C. After this time, individual mycelial hyphae were transferred to the V8 media without the addition of antibiotics, where they remained for further growth. The water samples were taken from watercourses flowing across the surface and collected in 1L plastic bottles that were previously sterilized in 70% ethanol and washed with distilled water. The taken water was processed in the laboratory, also using baiting techniques as described above.

Molecular identification of isolates

One-week-old isolates were sorted into morphotypes based on colony growth. After this stage, 7 morphotype groups were determined for further analysed and one isolate was selected from each group. The internal transcribed spacer (ITS) region of the nuclear rDNA of 5 isolates was amplified with universal ITS4 (White et al. 1990) and ITS 6 (Cooke et al. 2000) primers in direct PCR (diPCR) using Phire™Plant Direct PCR Kits (Thermo Fisher Scientific Inc., Waltham, MA, USA). Mycelium from 7 d.o. colonies growing on V8A was scrapped with a sterile tip and placed in 0.2 ml Eppendorf tubes, with 30 µl of Dilution Buffer (ThermoFisher Scientific Inc., Waltham, MA, USA). The 20 ul Phire PCR reaction mixture consisted 0.5µl of Dilution Buffer with young hypha (DNA template), 1 µl 0.5 µM of primers ITS4/ITS6, 10 µl 1 × Phire Plant PCR Buffer, and 0.4 µl Phire Hot Start II DNA Polymerase. The PCR conditions were as follows: 98 °C for 5 min; 40 cycles of 98 °C for 5 s, 55 °C for 5 s, and 72 °C for 50 s; and 72 °C for 7 min. The presence and size of PCR products were confirmed by analyzing 1 µl of product by electrophoresis in 1% TAE-agarose gel, stained with GelRed™NulceidAcid Dye (Biotium, Inc., Fremont, CA, USA). Prior sequencing, PCR product was purified with the AntyInhibitor kit (A&A Biotechnology, Gdynia, Poland), following the manufacturer'sprotocol, and sequenced with ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Obtained sequences were checked and trimmed in FinchTV software (Geospiza) and compared to other sequences deposited in GenBank using BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). Isolates were assigned to a *Phytophthora* species when sequence identities were above a 99.7% cut-off in respect to those of extype isolates or key isolates.

Table 2 Oomycetes species on European beech in Slovakia, origin of isolates, and number of isolates

Location	No	Origin and number of isolates			Oomycete species (number of isolates)	GenBank Accession Numbers of the representative isolates
		Soil and roots	Water	Tissue		
Banská Štiavnica	1	3		1	*P. x cambivora* (1)	OP175954
					P. plurivora (3)	–
Zvolen	2	61			*P. cambivora* (9)	–
					P. plurivora (17)	OP175952
					P. cactorum (11)	OP175953
					G. macrosporum (14)	–
					G. heterothallicum (10)	–
	3	3	1		*P. plurivora* (4)	–
Liptovská Osada	4	3			*G. macrosporum* (3)	–
Brezno	5				–	–
Partizánske	6	15			*P. cactorum* (4),	OP175950
					P. plurivora (7),	–
					G. heterothallicum (4)	–
Bánovce nad Bebravou	7				–	–
	8	1			*P. plurivora* (1)	OP175951
Trenčianske Teplice	9	2			*P. plurivora* (2)	–
Nováky	10	6			*P. plurivora* (6)	–

All ITS sequences obtained in this study were submitted to GenBank (Table 2).

Pathogenicity tests with *Phytophthora* species

The pathogenicity tests were performed using the standardized soil infestation protocol according to Jung et al. (1996). One-year-old *F. sylvatica* seedlings were grown from seeds in a mixture of peat, sand and perlite (v:v:v = 1:1:1) in single one-liter containers. Additionally, glass tubes were left in the containers near each plant in order to prepare holes into which the inoculum would then be introduced. The inoculation substrate was prepared from a mixture of fine vermiculite, millet seeds and V8 liquid juice medium (200 ml of filtered multi-vegetable juice, 800 ml of water) and then autoclaved at 120 °C for 20 min. The substrate prepared in this way was inoculated with 5 day old cultures of *Phytophthora* species isolated from the rhizosphere soil of beech trees, that were previously molecularly identified to species level. The substrate was previously selected for molecular identification were used for inoculation. The substrate thus inoculated was incubated for 4 weeks. The soil was inoculated by filling the cavities of the previously prepared glass 20–25 cm^3 test tubes of the inoculated substrate. Twelve plants were infested per treatment. In the control variant, twelve plants were inoculated with the sterile substrate (without adding any pathogens). After the plants had been inoculated, the entire containers were flooded with water to stimulate *Phytophthora* sporulation and left there for 72 h. This operation was repeated regularly every 3 weeks. Observations of the ground symptoms (leaf discoloration) were carried out once a week.

An evaluation of infection severity was performed once a week starting from seven days after the inoculation process. Severity was assessed on the following scale: 0—asymptomatic plants, 1—leaf discoloration, 2—wilting, dieback, 3—dead plants (Jönsson et al. 2003). During each evaluation, the number of plants in each class was counted, and then the mean value for each variant was calculated. With this in mind, it was possible to calculate the area under the disease-progress curve (AUDPC) (Campbell and Madden 1990).

Re-isolation and examination

Three months after inoculation with *Phytophthora* species, symptoms of beech dieback (leaf yellowing, wilt and dieback) were observed on 50% of the seedlings, therefore all the plants (symptomatic and asymptomatic) were removed from the substrate and then the roots washed under running water. After washing, the necroses observed on the roots were re-isolated in order to confirm the presence of pathogens in the roots. Two to five small pieces of fine roots from each plant were placed on selection medium (V8-PARPNH) after being dried with filter paper (Jung et al. 1996). The fine root pieces from the control groups were also laid out on selective media agar.

In the next step, all the roots were scanned with the EPSON Perfection V700 Photo Scanner software and analyzed using the WinRhizo® software (Regent Instruments, Canada). Then the scanned roots were dried at 65 °C in a constant weight dryer (Termaks Series 2000, Norway), and the dry biomass was measured as fine roots (whose diameter did not exceed 2 mm) and mother roots (whose diameter was greater than 2 mm) using the Sartorius analytical scale A200S (GMBH, Germany). As a result of these activities, a number of parameters were obtained, such as: fine roots tips (FRT), total roots length (TRL), fine roots length (FRL), mother roots length (MRL), the ratio of the length of fine roots to the length of mother roots (FRL / MRL)), the ratio of the number of fine roots to the length of the mother roots (FRT / MRL), the ratio of the length of the fine roots to the dry weight of the mother roots (FRL / DWMR), fine roots surface area (FRSA), dry weight of fine roots (DWFR) and dry weight of mother roots (DWMR) (Bouma et al. 2000). These parameters were used to assess the damage caused by pathogens of the *Phytophthora* genus, with which beech seedlings were infected.

Statistical analysis

All parameters obtained at the scanning stage were analyzed in terms of meeting the assumptions for parametric tests (compliance with the normal distribution and homogeneity of variance). After verifying the assumptions, a one-way analysis of variance was performed at $p = 0.05$. Differences between mean root parameters were investigated using Duncan's multi-range post hoc test ($\alpha = 0.05$). All calculations were performed with the STATISTICA 13.1 package (Dell Inc., Tulsa, OK, USA). Additionally, the Rstudio program was used to calculate the area under the disease-progress curve (AUDPC), using the agricolae package.

Results

In total, 96 isolates of various oomycetes species were obtained from all the collected samples (Table 2). Most of the isolates came from the beech stand marked as plot number two (Zvolen). On the remaining plots, the number of obtained isolates ranged from one to fifteen, and no pathogens were isolated on two plots. Most of the obtained isolates (96%) were isolated from locations where symptomatic trees were grown, while 4 isolates (4%) were obtained from asymptomatic trees. After detailed molecular analysis, five different species of oomycetes were

identified, including *Phytophthora cactorum* (Lebert & Cohn) J. Schröt, *P. plurivora* (T. Jung &. T. I Burgess), *P. x cambivora* Petri (Buisman), *Globisporangium macrosporum* (Vaartaja & Plaäts-Nit.) Uzuhashi, Tojo & Kakish., and *G. heterothallicum* (W.A. Campb. & F.F. Hendrix) Uzuhashi, Tojo & Kakish.. Detailed information on the number of isolates and their origin is provided in Table 2.

The pathogenicity tests were completed after three months of incubation, when symptoms of *Phytophthora* infection were observed on some of theseedlings. The presence of inoculated pathogens in the roots of seedlings was confirmed according to Koch's postulate. During the re-isolation, the presence of *Phytophthora* pathogens in the root fragments was confirmed. Reisolation rate for *P. x cambivora* was 72%, for *P. plurivora* 88% and for *P. cactorum* 67%. Of all treated plants, roots necrosis was observed in three plants plants (two seedlings in the variant infected with *P. x cambivora* and one for *P. plurivora*). The *Phytophthora* cultures were not recovered from the roots of the control seedlings.

The severity of infection and the calculated AUDPC values is shown in Fig. 1. The first symptoms of infection were observed after three weeks (leaf discoloration) in the variant where plants were inoculated with *P. x cambivora*. It was also the only variant for which a dying plant (classified into class 3) was observed on day 84 from the inoculation of the plants, which was equivalent to the end of the experiment. The AUDPC value for this variant was the highest and amounted to 32.45. For the remaining species, the first signs of infection were observed on 35 day after infection (d.a.i.), and on the day of completion of the experiment, the AUDPC values for *P. cactorum* and *P. plurivora* were 17.96 and 25.62, respectively.

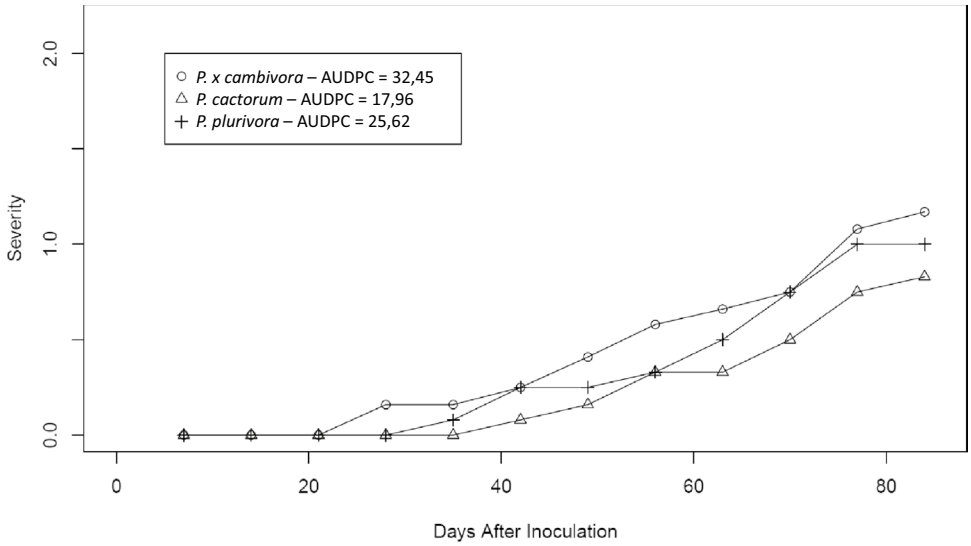

Fig. 1 Disease severity of three isolates of *Phytophthora* 84 days after inoculation. Severity was evaluated using the following scale: 0—asymptomatic plants; 1—leaf discoloration; 2—wilting, dieback; and 3—dead plant

Table 3 Results of the soil infestation test of *Phytophthora* spp. on *Fagus sylvatica* after 3 months: mean values with standard deviation (SD) of the measured root parameters

	Control	*P. x cambivora*	*P. plurivora*	*P. cactorum*	*p*-value
FRT	576 ± 283 a	187 ± 128 c	367 ± 169 bc	459 ± 100 ab	0.000
TRL	154 ± 71 a	77 ± 50 b	151 ± 76 a	100 ± 23 ab	0.004
FRL	141 ± 66 a	67 ± 44 b	139 ± 69 a	90 ± 21 ab	0.003
MRL	10 ± 4	6 ± 3	9 ± 5	10 ± 4	0.067
FRL/MRL	12 ± 2 ab	10 ± 3 b	15 ± 5 a	9 ± 3 b	0.005
FRT/MRL	53 ± 18 a	29 ± 13 b	43 ± 18 ab	47 ± 14 ab	0.007
FRL/DWMR	857 ± 321	967 ± 687	819 ± 550	699 ± 444	0.655
FRSA	23 ± 12 a	11 ± 7 b	22 ± 12 a	17 ± 4 ab	0.011
DWFR	0.11 ± 0.07	0.05 ± 0.04	0.1 ± 0.07	0.08 ± 0.05	0.145
DWMR	0.17 ± 0.08	0.1 ± 0.08	0.2 ± 0.09	0.16 ± 0.08	0.057

FRT, fine roots tips; TRL, total roots length; FRL, fine roots length; MRL, mother roots length; FRL/MRL, fine roots length/mother roots length; FRT/MRL, fine roots tips/mother roots length; FRL/DWMR, fine roots length/dry weight of mother roots; FRSA, fine roots surface area; DWFR, dry weight of fine roots; DWMR, dry weight of mother roots. Different letters in the rows denote statistical significance at $p < .05$ (Duncan's multiple range test)

The results of the comparison of the morphological features of the roots are presented in Table 3. Statistically significant differences were confirmed for the parameters fine roots tips (FRT), total roots length (TRL), fine roots length (FRL), fine roots length per mother roots length (FRL / MRL), fine roots tips / mother root length (FRT / MRL) and fine root surface area (FRSA). In all the discussed cases, *P. x cambivora* differed significantly from the control variant. The values of the analyzed features were significantly lower than the values for the control variant, which may indicate a high aggressiveness of this pathogen towards roots of *F. sylvatica*. Other pathogens (*P. plurivora* and *P. cactorum*) also had an impact on lower values of the described parameters, however, than not always, these differences were significantly smaller than the control variant. For example, *P. cactorum*, despite the lower values of individual parameters, did not differ significantly from the control variant. For *P. plurivora*, significant differences compared to the control variant were noted only for fine roots tips (FRT).

Discussion

The study presented in this article provides a first look at the problem of the occurrence of species of the genus *Phytophthora* in beech stands in Slovakia. Pathogens of the genus *Phytophthora* were isolated on most of the areas where symptoms related to the deterioration of the health condition of beech trees were observed (yellowing of the crowns, superficial cankers, etc.). Only in the area near Brezno, where symptoms of crown thinning were observed, it was not possible to isolate pathogens of the genus *Phytophthora*. However, this does not necessarily mean that the rhizosphere soil in this stand is free from the presence of oomycetes. Cooke et al. (2007) report that the success of traditional isolation may be influenced by many factors (the timing of sampling). For example, O'Brien et al. (2009) and Jung et al. (2002) suggest that for some *Phytophthora* species, detection efficiency may vary with the season. In this study, samples were collected only in one spring period (April 2022). This may have contributed to the fact that it was not possible to isolate pathogens of the genus *Phytophthora* on this plot. We do not therefore rule out that the rhizosphere communities are actually more diverse than what we could detect with traditional soil enticing techniques.

Another important aspect is the use of different leaves as baits. In their research, Matsiakh et al. (2021) confirmed variable success in isolating *Phytophthora* species from different bait hosts. *Rhododendron* spp. and *Quercus* proved to be more efficient than other hosts, accounting for more than half of the obtained isolates. Aghighi et al. (2015) confirming that soil enticing the youngest fully developed oak leaves (*Q. ilex* and *Q. suber*) allowed the isolation of up to seven *Phytophthora* species from dying *Rubus anglocandicans*. Differences in isolation success have been similarly reported in other studies (Jung et al. 2002; Vettraino et al. 2005). Due to the preservation of specificity for pathogens occurring in the beech rhizosphere, in our research, we focused on testing only young beech leaves, which could have had a certain impact on the number of isolates obtained.

Nevertheless, the conducted research confirmed the presence of three species belonging to the genus *Phytophthora*: *P. plurivora*, *P. cactorum* and *P. x cambivora* in the rhizosphere soil and tissues of symptomatic beech trees. The isolated pathogens have been described many times in the literature as the perpetrators of beech stands dieback (Jung et al. 2005a, b; Jung 2009). Their activity is related to damage to the roots (in particular fine roots), which limits the tree's ability to uptake water and minerals. The most aggressive of the identified species turned out to be *P. x cambivora* (belonging to clade 7 among other highly aggressive plant pathogens such as *P. cinnamomi*), which significantly reduced the parameters of the roots of the inoculated trees. This pathogen turned out to be pathogenic for beech roots after inoculation on seedlings, thus meeting Koch's postulate. *P. x cambivora* is commonly known as a highly aggressive pathogen towards beech, contributing to a decline in health both in North America (USA) and in several European countries (Day 1939; Jung and Blaschke 1996; Jung et al. 2005a, b; Orlikowski et al. 2006; Jung 2009; Nelson et al. 2010; Nechwatal et al. 2011; Milenković et al. 2012; Telfer et al. 2015; Jung et al. 2017a, b; Oszako et al. 2019; Corcobado et al. 2022). In Slovakia, this species was confirmed by Jung et al. (2016).

Another isolated species is *Phytophthora plurivora* (clade 2). This species was the most frequently isolated organism during the research this pathogen has been successfully reisolated from the roots of infected beech seedlings. Analyzes statistically confirmed lower values for a number of root parameters, however, significant differences were observed only for fine root tips, however, this species did not cause as much damage as *P. x cambivora*. *P. plurivora* is an organism with a very wide range of host plants (Jung et al. 2016). So far, in Slovakia, this species has been confirmed in the rhizosphere soil of oaks, maples and alders (Jung et al. 2016; Tkaczyk et al. 2020, 2021, 2023). It probably comes from the regions of South and East Asia (Jung et al. 2017a, b; Jung et al. 2020). It is also one of the most common *Phytophthora* species in European forest nurseries, from where it is transferred along with the plant material to mature stands (Jung et al. 2016).

The last of the isolated species is *P. cactorum* (clade 1a), which, like *P. plurivora*, is largely associated with the occurrence in forest nurseries, from where it can spread to mature stands (Orlikowski et al. 2006; Jung et al. 2016). *P. cactorum* was the least aggressive to *F. sylvatica* of all

pathogens isolated. Although the roots were more damaged by infection than in the control variant, which was manifested by lower parameter values, no significant differences were observed for this variant. Perhaps, if the pathogenicity test lasted longer, the differences would be statistically significant, although on the other hand *P. x cambivora*, which is the main pathogen of beech trees, caused statistically significant root damage. Nevertheless, *P. cactorum* was also previously recorded in the rhizosphere soil of the common beech (Weiland et al. 2010; Milenković et al. 2012). According to the research of Milenković et al. (2012), this species is not particularly aggressive towards beech, which was also confirmed in our research. In Slovakia, this species was confirmed by Jung et al. (2016) also in beech stands and by Tkaczyk et al. (2020) in oak stands.

The other two isolated species belonging to the genus *Globisporangium* (*G. macrosporum* and *G. heterothallicum*), due to their biology, were not included in the pathogenicity tests. Organisms belonging to the genus *Globisporangium* (previously known to belong to *Pythium* sensu lato) are known as saprotrophs or weak pathogens (Uzuhashi et al. 2010). So far, no connection of any of these organisms with the decline of beech stands has been confirmed.

The isolated species of *Phytophthora* show a significant relationship with the phenomenon of beech stands dieback in many European countries (Jung et al. 2016), which may indicate that they are also involved in this phenomenon in Slovakia. Pathogens of the genus *Phytophthora* are mainly responsible for damage to fine roots, thus limiting the plant's ability to uptake water. Water shortages observed in recent years may weaken beech trees and cause infections by pathogens belonging to the genus *Phytophthora*. These organisms are able to survive in the form of chlamydospores (Erwin and Ribeiro 1996) during unfavorable weather conditions (high temperatures and lack of moisture in the soil). At the same time, due to lack of water, beeches are subjected to long-term stress, which weakens them. On the other hand, when after a long dry period there is rainfall (even in small amounts), pathogens of the genus *Phytophthora*, thanks to the ability to actively move in water (even capillary), can easily spread and infect already weakened plants (Erwin and Ribeiro 1996).

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Data availability The data underlying this article will be shared on reasonable request to the corresponding author.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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