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SHORT COMMUNICATION

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Susceptibility of silver birch and black alder to several *Phytophthora* species isolated from soils in declining broadleaf forests in western Ukraine

Iryna Matsiakh[1,2] | **Noelia López-García**[1] | **Magdalena Kacprzyk**[3] | **Michelle Cleary**[1]

[1]Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Alnarp, Sweden

[2]Institute of Forestry and Park Gardening, Ukrainian National Forestry University, Lviv, Ukraine

[3]Department of Forest Ecosystems Protection, University of Agriculture in Krakow, Krakow, Poland

Correspondence
Iryna Matsiakh, Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre, Sundsvägen 3, 23053, Alnarp, Sweden.
Email: iryna.matsiakh@slu.se

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Abstract

In declining broadleaf forests in western Ukraine, several *Phytophthora* species including *P. plurivora*, *P. bilorbang*, *P. polonica*, *P. gonapodyides* and *P. cactorum* were recovered using soil baiting assays and identified using morphological and molecular methods. Pathogenicity tests of selected isolates were performed on black alder (*Alnus glutinosa* (L.) Gaerth.) and silver birch (*Betula pendula* Roth.) to assess susceptibility of these two tree species to the newly detected *Phytophthora* species. *Phytophthora plurivora*, *P. bilorbang* and *P. polonica* showed higher pathogenicity in both alder and birch compared to the other tested *Phytophthora* species.

KEYWORDS
black alder, pathogenicity, *Phytophthora*, silver birch, Ukraine

1 | INTRODUCTION

Phytophthora species are important pathogens that have been implicated in causing tree decline globally (Jung et al., 2018). In western Ukraine, symptoms of dieback including crown transparency and chlorosis, bleeding cankers on trunks and tree mortality have been observed in several forest types comprised of different broadleaved tree species during the last decade (Matsiakh et al., 2021). Rhizosphere soil samples collected from these declining broadleaved forests revealed the presence of seven *Phytophthora* species (*P. bilorbang*, *P. cactorum*, *P. gallica*, *P. gonapodyides*, *P. lacustris*, *P. plurivora* and *P. polonica*), some of which had never before been reported in Ukraine (Matsiakh et al., 2021). Among these *P. bilorbang*, *P. gonapodyides*, *P. plurivora* and *P. polonica* were isolated from declining silver birch (*Betula pendula* Roth.) and black alder (*Alnus glutinosa* [L.] Gaertn.) forest stands (Matsiakh et al., 2021) and *P. cactorum* was recovered from a declining chestnut (*Castanea sativa* Mill.) stand (Matsiakh et al., 2021).

In this area of western Ukraine, both silver birch and common alder are the main forest species of native woodlands and wetlands, particularly in the Polisia region of Ukraine. Silver birch naturally regenerates and is commonly planted together with Scots pine (*Pinus* *sylvestris* L.) forming pure or mixed stands. Common alder is an important riparian tree species and has an important role in maintaining stability for riparian habitats. The recent detection of *Phytophthora* species from declining birch and alder forests is a concern for both the future productivity and disease management strategies. A better understanding of which *Phytophthora* species are most aggressive on tree species would help guide future species choice for these sites. In particular, information about tree species susceptibility to *P. bilorbang* which is among the opportunistic aggressive pathogens in this area of Europe is rather limited (Aghighia et al., 2012; Deidda et al., 2022).

The aim of the study was to determine the pathogenicity of five *Phytophthora* species (*P. bilorbang*, *P. cactorum*, *P. gonapodyides*, *P. plurivora* and *P. polonica*,) commonly isolated from rhizosphere soils of declining broadleaf forests in western Ukraine on black alder and silver birch and tree susceptibility to tested *Phytophthoras*.

2 | MATERIALS AND METHODS

2.1 | *Phytophthora* isolates and plant material

Five *Phytophthora* species, namely *P. bilorbang* (UKR01), *P. cactorum* (UKR05), *P. gonapodyides* (UKR04), *P. plurivora* (UKR03) and *P. polonica* (UKR02), and previously isolated from declining broadleaved forests in western Ukraine (Table 1) were used for inoculations of black alder and silver birch (Matsiakh et al., 2021). Two isolates *P. plurivora* (UKR06) and *P. bilorbang* (UKR07) of a diverse origin were included in the experiment (Table 1). To obtain mycelial agar discs for inoculations, colonies of each of the *Phytophthora* isolates were subcultured onto 90–mm Petri dishes containing sterilized potato dextrose agar (PDA) and maintained for 3–4 weeks at 20°C in the dark. Plants used for the inoculation test were one-year-old black alder and silver birch produced from seeds (Ramlösa Plantskola AB). Plants were maintained in a controlled climate chamber with a 16 h photoperiod of 23/18°C (day/night) for 5 weeks prior to inoculations.

2.2 | Pathogenicity tests

Ten replicates of alder and birch plants were inoculated with an isolate of either *P. bilorbang* ($n=2$), *P. cactorum*, *P. gonapodyides*, *P. plurivora* ($n=2$) or *P. polonica* according to a previously described protocol (Cleary et al., 2016). Control plants were wound inoculated with a sterile PDA disc. Plants were kept in the greenhouse for approximately 16 weeks and watered twice per week. Symptom development (e.g. foliar yellowing, wilting, necrotic lesions, defoliation, bleeding cankers) was checked periodically throughout the study period. After 16 weeks, seedlings were destructively sampled by carefully scraping off the outer bark around the inoculation site to expose the entire lesion. Total lesion length above and below the point of inoculation of both the outer– and inner bark was measured, as was the width of necrosis around the circumference of the stem lesion. At the end of the experiment, attempts to re-isolate *Phytophthora* spp. were made from a subset of plants by plating small pieces of necrotic bark dissected from the margins of the longitudinal lesion on selective V8-PARPH media. The identity of the *Phytophthora* spp. was confirmed by examining the colony morphology and growth compared to known isolates.

2.3 | Statistical analyses

For each host tree and *Phytophthora* species, means and standard deviation (±SD) values of inner and outer length of bark necrosis, and circumference width were calculated. Stem lesion sizes were analysed using Kruskal–Wallis one-way analysis of variance by ranks and Dunn's post-hoc test to show any significant difference within and among treatment (inoculation) and host species. For each treatment, differences among inner– and outer bark necrosis length were analysed using the Mann–Whitney U test. Statistical significance was assigned at $\alpha=0.05$. All of the statistical procedures were performed with TIBCO Statistica™ 13.3 software.

3 | RESULTS AND DISCUSSION

Inoculated seedlings first began to show symptoms 10 days after inoculation with gradual yellowing and wilting of the leaves. The first symptoms appeared on five alder plants inoculated with *P. bilorbang* showed some wilting of leaves by the third week of post–inoculation. Necrotic lesions were observed on the stems of all inoculated seedlings, appearing as dark depressions that progressively became more

TABLE 1 *Phytophthora* spp. isolated from western Ukraine used in the inoculation experiment with alder and birch plants.

Species	Isolate name	Host species[a]	Locality in Ukraine	GenBank Accession No.
Phytophthora bilorbang	UKR01	*Alnus glutinosa*	Klesiv Forestry, Rivne region	MT420377
Phytophthora polonica	UKR02	*Alnus glutinosa*	Klesiv Forestry, Rivne region	MT420383
Phytophthora plurivora II	UKR03	*Betula pendula*	Klesiv Forestry, Rivne region	MT420379
Phytophthora gonapodyides	UKR04	*Betula pendula*	Strashiv, Rivne region	MT420398
Phytophthora cactorum	UKR05	*Castanea sativa*	Lavkivske Forestry, Transcarpathia region	MT420389
Phytophthora plurivora	UKR06	*Alnus glutinosa*	Kukly Forestry, Volyn region	MT420404
Phytophthora bilorbang II	UKR07	*Alnus glutinosa*	Kukly Forestry, Volyn region	MT420401

[a]Declined host forests where soil samples were collected.

sunken over time (Figure S1). On two of ten control plants of alder and on three of ten birch plants, lesions were also observed around the site of injury though they appeared less pronounced than on *Phytophthora*-inoculated seedlings. All *Phytophthora* species used for inoculation were re–isolated from stem lesions of black alder and silver birch. They were identified and confirmed based on the morphology of isolates. Sixteen isolates obtained from control alder plants and 10 isolates from control birch plants were not identified as *Phytophthora* spp.

After 5 months of inoculation, crown mortality due to girdling occurred in nine black alder and six silver birch plants caused by *P. plurivora* and *P. bilorgbang*. No mortality occurred in the control plants. Despite necrotic lesions that encircle the alder or birch stems, the root systems were still alive. Significant differences were found in the length of lesions among the alder and birch seedlings inoculated with different *Phytophthora* species/isolates (Table 2). *Phytophthora plurivora*, *P. bilorbang* and *P. polonica* showed the largest necrostic lesions compared to *P. gonapodyides* and *P. cactorum*, regardless of host species. For these three *Phythophthora* species, the length of outer- and inner bark necrosis differed significantly from the control (Table 2).

The largest lesions were noted in alder plants inoculated by *P. plurivora* (9.8 mm ±2.86 mm) and *P. plurivora* II (6.6 mm ±2.68 mm) (Table 2, Figure S1), whereas with birch plants the widest lesions was observed with *P. gonapodyides* (6.1 mm ±1.49 mm) and *P. bilorbang* (6.7 mm ±2.37 mm) (Table 2, Figure S1). The mean width of lesions caused by all four mentioned *Phythophthora* species/isolates (*P. plurivora*, *P. plurivora* II, *P. gonapodyides* and *P. bilorbang*) differed significantly from the control. Among the studied *Phytophthora* species/isolates on alder and birch, *P. bilorbang* II produced the smallest lesions in all measurements (Table 2, Figure S1).

The mean length of inner bark necrosis was significantly ($p < .05$) higher than the outer bark necrosis but only for *P. plurivora* in alder and for *P. plurivora*, *P. bilorbang* and *P. polonica* for birch (Table 2).

Our study confirmed that several *Phytophthora* species are pathogenic on both black alder and silver birch, and adds to the knowledge on their potential host range. In other studies, *P. plurivora* is associated with alder and birch mortality and was reported to cause collar rot, bark cankers, extensive fine root losses and/or dieback of crowns on young and mature broadleaf trees and shrubs (Jung et al., 2018). Infection of alder and birch trees by *P. plurivora* may predispose them to other stressing factors including wood-inhabiting fungi or insects that can further weaken the trees.

Though *P. polonica* caused significant lesions on both alder and birch stems (Table 2), the extent of inner vs. outer necrosis was much greater on alder than on birch (Figure S1).

An interesting finding in this study was the pathogenicity of *P. bilorbang* in silver birch, though differences were observed between the two isolates that were used. Until now, *P. bilorbang* has been mainly associated with natural vegetation and tree crops in Australia and Mediterranian region (Aghighia et al., 2012). Our results suggest that *P. bilorbang* may have a much wider geographic range and a broader host range that includes both black alder and silver birch.

TABLE 2 Mean (±SD) values and results of Kruskall–Wallis test* for measured bark necrosis following inoculation by *Phytophthora* species/isolates and control on one-year-old alder and birch plants.

	Alder				Birch			
		Necrosis characteristics (mm)				Necrosis characteristics (mm)		
Species	Number of inoculated plants	Outer bark length	Inner bark length	Width	Number of inoculated plants	Outer bark length	Inner bark length	Width
Phytophthora bilorbang	10	$11.2^{abc} \pm 2.91$	$35.3^{ab} \pm 15.17$	$5.8^{acd} \pm 1.79$	10	$18.9^{cd} \pm 2.18$	$34.0^{ab} \pm 12.84$	$6.7^{a} \pm 2.37$
Phytophthora polonica	10	$13.4^{ab} \pm 5.02$	$36.4^{ab} \pm 13.15$	$6.3^{ac} \pm 1.19$	10	$20.4^{d} \pm 3.53$	$26.3^{ab} \pm 5.53$	$5.3^{a} \pm 1.23$
Phytophthora plurivora II	10	$8.1^{bcd} \pm 3.82$	$27.4^{ab} \pm 9.26$	$6.6^{ac} \pm 2.68$	10	$14.4^{abcd} \pm 2.67$	$26.1^{ab} \pm 10.29$	$5.1^{ac} \pm 0.97$
Phytophthora gonapodyides	10	$8.9^{abcd} \pm 4.67$	$26.7^{abc} \pm 13.41$	$4.5^{bc} \pm 2.06$	10	$14.7^{acd} \pm 4.01$	$17.7^{abc} \pm 5.98$	$6.1^{a} \pm 1.49$
Phytophthora cactorum	10	$7.6^{bcd} \pm 3.50$	$15.6^{bc} \pm 7.83$	$5.1^{abcd} \pm 1.98$	10	$15.9^{acd} \pm 5.52$	$18.7^{bc} \pm 5.32$	$5.4^{ac} \pm 1.78$
Phytophthora plurivora	10	$36.5^{a} \pm 32.76$	$62.0^{a} \pm 28.16$	$9.8^{a} \pm 2.86$	10	$20.5^{acd} \pm 10.18$	$35.4^{a} \pm 19.37$	$4.8^{abc} \pm 2.23$
Phytophthora bilorbang II	10	$3.9^{cd} \pm 3.66$	$20.6^{bc} \pm 8.63$	$2.3^{bd} \pm 2.13$	10	$8.8^{ab} \pm 6.42$	$12.6^{bc} \pm 9.05$	$2.6^{bc} \pm 1.49$
Control	10	$1.2^{d} \pm 2.58$	$6.7^{c} \pm 6.67$	$0.7^{b} \pm 1.59$	10	$2.3^{b} \pm 3.87$	$3.6^{c} \pm 4.15$	$1.0^{b} \pm 1.68$

*Different small letters in the upper index of the mean values mean significant differences ($\alpha = 0.05$).

Phytophthora gonapodyides was more aggressive on black alder than silver birch (Table 2, Figure S1). Cleary et al. (2016) reported that *P. gonapodyides* can also be pathogenic on *Fagus sylvatica* causing bleeding canker symptoms in southern Sweden.

Lesions caused by *P. cactorum* on birch stems were more than twice as long as that on black alder (Table 2). *Phytophthora cactorum* is one of the most common species found in declining broadleaf forests across Europe and has been previously associated with causing root rot of *Alnus* spp. (Jung & Blaschke, 2004). Its pathogenicity on silver birch has also been confirmed by Lilja et al. (1998).

In conclusion, five *Phytophthora* species isolated from soil of declining forests in western Ukraine showed pathogenicity with variable degrees of damage towards black alder and silver birch seedlings in a cut-stem inoculation experiment. Both tree species demonstrated their susceptibility to tested *Phytophthora* species. The presence of these *Phytophthora* species in soil of declining broadleaf forests may pose a threat to local plant communities in western Ukraine.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Michelle Cleary https://orcid.org/0000-0002-0318-5974

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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