

ORIGINAL ARTICLE

The Potential Role of Acidic Soils in the Low Incidence of *Phytophthora* in Belgian Beech Forests

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ABSTRACT

Phytophthora monitoring was carried out across 20 beech stands in southern Belgium. Twenty dominant or co-dominant trees were observed at all 20 sites. In 2022, more than half of these trees displayed crown transparency and branch dieback. In each site, soil samples were collected from three trees representing different levels of decline to assess the presence of *Phytophthora* in the fine roots. Baiting tests were performed in 2019 (9 sites) and 2021 (20 sites). Among all baited samples, *P. cinnamomi* was the only *Phytophthora* species recovered. It was found in a single site (site 14, Mormont) located in a very humid area, in 2021, and its presence was confirmed in 2025. To our knowledge, this is the first report of this species in beech forests in Belgium. All baiting results at the other 19 sites were negative. Baiting tests conducted by artificially infecting the soils from eight of the 20 sites with various *Phytophthora* species associated with beech decline in Europe were negative, except for *P. cinnamomi*, showing a different behaviour of this species according to the characteristics of the soils studied. Replacing demineralised water with filtered river water or a seaweed solution for baiting tests, both of which increase the pH of the soil suspension, restored the infection potential of the *Phytophthora* species studied, as reflected by necrosis on baited leaves. However, when soils that had previously tested negative using demineralised water were reanalysed with filtered river water or a seaweed solution as baiting media, the baited leaves remained symptomless. These trials demonstrate that soils with a low pH limit the infective potential and the occurrence of *Phytophthora*. They also highlight the higher risk represented by *P. cinnamomi* for Belgian beech forests growing in acidic soils.

1 | Introduction

Beech (*Fagus sylvatica* L.) is a key hardwood species in European forests, widely distributed across diverse climatic regions (Caudullo et al. 2017). Under climate change, this species experiences abiotic stress, exhibits signs of decline and faces various phytosanitary challenges (Martinez del Castillo et al. 2022; Langer and Bußkamp 2023). In southern Belgium, visual observations conducted between 2019 and 2022 showed that beech stands displayed significant signs of dieback (Pirronitto et al. 2022). This situation raises concerns about the long-term

resilience of this tree species under changing environmental conditions.

Species of the oomycete genus *Phytophthora* represent a major threat to forest tree health, and the number of decline diseases caused by *Phytophthora* species has increased over the last few decades in Europe (Vettraino et al. 2008; Jung et al. 2018; Matsiakh et al. 2021). Monitoring conducted in woody plant nurseries in different European regions has shown the importance of this pathway for introducing *Phytophthora* species into forests, most of which are suspected to be alien invasive

species (Lyubenova et al. 2015; Jung et al. 2016; Antonelli et al. 2023). In trees, soil-borne *Phytophthora* infect the fine roots, inducing crown diebacks, wilting and yellowing of leaves. Infections progress into the cambium, leading to root and collar rot and bleeding cankers on the trunk, usually at the collar base (Ruffner et al. 2019). Depending on the species, these pathogens can survive for several years in the soil as resting structures like chlamydospores or oospores. Site conditions influence the occurrence of *Phytophthora* species in forest stands. The alternation of flooding and drier periods has shown to be a trigger for their development (Jung 2009). Climatic conditions also affect their diversity, with *Phytophthora* communities varying according to a latitude gradient in Europe (Caballol et al. 2024). Besides climatic and weather conditions, studies have highlighted the role of soil texture and pH on the development of these pathogens (Jung et al. 2000; Jung 2009; Corcobado et al. 2020).

Surveys conducted in 300 beech stands representing a range of site conditions in 17 European countries have shown that *Phytophthora* was present in most stands (Jung et al. 2018). Among the species associated with beech decline, *P. xcam bivora*, *P. plurivora* and *P. cactorum* are the most common, whereas at least 13 other species have also been reported less often (Jung et al. 2005; Jankowiak et al. 2023; Štraus et al. 2023).

Baiting *Phytophthora* species from rhizosphere samples using susceptible host plant material is the recommended method for detecting live *Phytophthora* in fine roots, and several methods are described in the literature, notably those of Erwin and Ribeiro (1996), Burgess et al. (2021), Pérez-Sierra et al. (2022) or

Antonelli et al. (2023). Although these methods have proved effective, various parameters influence the success of the baiting, and different studies have shown that molecular tests detected more species than traditional baiting followed by isolation (Sarker et al. 2021; Sarker, Burgess, et al. 2023; Sarker, McComb, et al. 2023).

This study had two objectives: first, to conduct surveillance in the Belgian Ardennes beech forests to evaluate the role of *Phytophthora* in the observed decline; and second, to assess the influence of soil pH on the infection potential of various *Phytophthora* species associated with beech decline.

2 | Materials and Methods

2.1 | Survey Units, Visual Observation and Collection of Soil Samples

Twenty pure beech stands were selected in the bioclimatic region of the Ardennes, where most of the Belgian beech forests are present (Figure 1, Table 1, sites no. 1–20). To evaluate the presence of *Phytophthora* in soil (objective ‘Surveillance’, Table 1), soil samples were taken at approximately 1 m from the trunk of three trees per stand as recommended by Jung (2009). The trees selected corresponded to three categories according to the condition of their crowns: slightly declining trees (less than 25% defoliation), moderately declining trees (between 25% and 50% defoliation) and declining trees (more than 50% defoliation). The selected trees had no bleeding bark cankers. The soil layer beneath the leaf litter was collected to a depth of approximately 20 cm at four locations around the tree (four

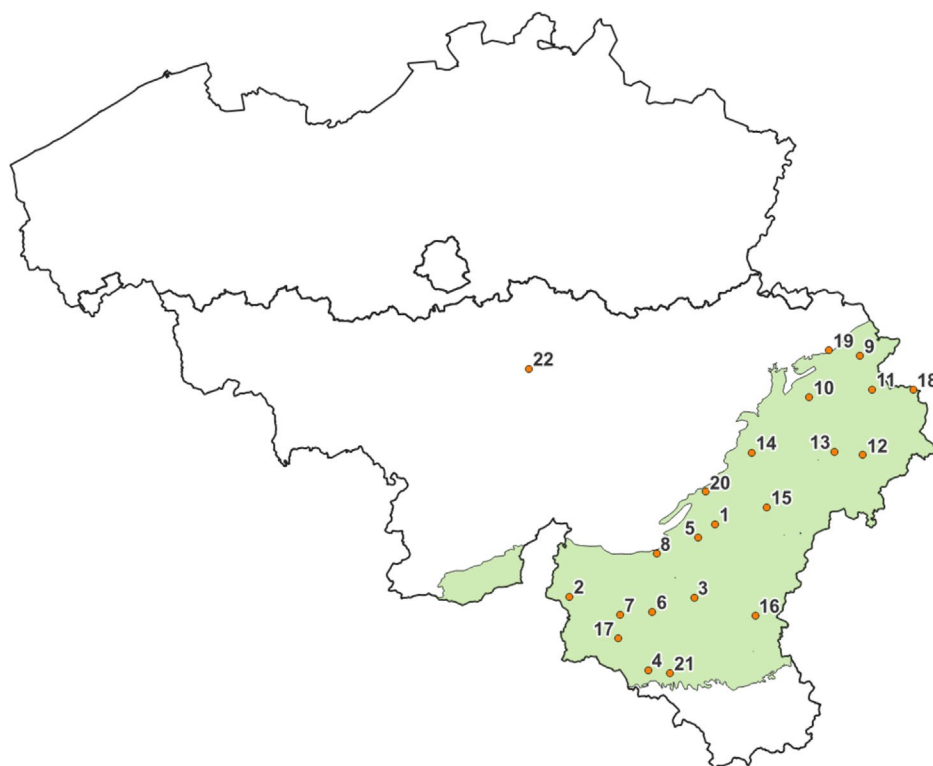


FIGURE 1 | Location of the different sites from which soil samples were collected. Sites 1 to 21 = beech stands in the Ardennes bioclimatic region (in green). Site 22 = meadow near the laboratory (in Gembloux) used as a control.

TABLE 1 | Characteristics of sites selected for the study.

No.	Site	Type	pH _{water}	Surveillance			Role of soil pH	
				2019	2021	2025	2023	2024
1	Vecquemont	Beech forest	3.8	(B1/2)	(B2)			
2	Louette	Beech forest	3.9	(B1/2)	(B2)			(B4)
3	Seviscours	Beech forest	4.1	(B1/2)	(B2)			
4	SteCecile	Beech forest	4.2	(B1/2)	(B2)			(B4)
5	Journal	Beech forest	4.2	(B1/2)	(B2)			
6	Jehonville	Beech forest	4.4	(B1/2)	(B2)			
7	Naome	Beech forest	4.5	(B1/2)	(B2)			
8	Mirwart	Beech forest	4.3	(B1/2)	(B2)		(B3)	(B4)
9	Eupen	Beech forest	3.9	(B1/2)	(B2)			(B4)
10	Spa	Beech forest	3.8		(B2)		(B3)	
11	Sourbrodt	Beech forest	4.2		(B2)			
12	Born	Beech forest	4.0		(B2)			
13	PetitThier	Beech forest	4.0		(B2)			
14	Mormont	Beech forest	4.0		(B2)	(B2)	(B3)	(B4)
15	Wibrin	Beech forest	4.0		(B2)			
16	Cobreville	Beech forest	4.4		(B2)			
17	Mogimont	Beech forest	4.3		(B2)			
18	Elsenborn	Beech forest	4.0		(B2)			
19	Jamhay	Beech forest	3.9		(B2)		(B3)	(B4)
20	Waharday	Beech forest	4.0		(B2)			(B4)
21	Epioux	Beech forest	4.4					(B4)
22	Gembloux	Meadow	6.6				(B3)	(B4)

Note: Different modalities of baiting were conducted depending on the year of analysis and research objective. B1 = soil placed in apple fruit; B2 = box with soil and demineralised water and different baiting plant tissues (Rhododendron leaves, young beech leaves, chestnut and oak leaves, and lupin germlings); B3 = box with soil and demineralised water or river water or filtered river water/demineralised water and Rhododendron leaves as baits and B4 = box with demineralised water, filtered river water/demineralised water, or seaweed solution and rhododendron leaves as baits.

monoliths of rhizosphere soil, approximately 20 × 20 × 20 cm). Soil samples from the same tree were pooled into a bulked sample (approximately 2–3 L). The soil samples were left in plastic bags in a greenhouse (18°C–25°C) and analysed within 48 h. To evaluate the presence of *Phytophthora* in soil using baiting, samples were taken in August/September 2019 (nine beech stands, no. 1–9, Table 1), in August/September 2021 (20 beech stands, no. 1–20, Table 1) and in May 2025 (beech stand no. 14, Table 1). To assess the infection potential of various *Phytophthora* species using different baiting modalities (objective ‘Role of soil pH’, Table 1), soil samples were taken from a limited number of sites in September 2023 and 2024, using only two trees per site and pooling the soil samples from the two trees into an average sample. Two other soil samples were analysed in 2023 and 2024: a soil sample coming from a beech stand not selected in 2019 (no. 21, Figure 1, Table 1), and a soil sample taken from a meadow found near the laboratory (no. 22, Figure 1, Table 1).

2.2 | Baiting Tests From Soil Samples

All baiting trials were conducted in a greenhouse (18°C–25°C) for 6–7 days. Soil samples were homogenised by gently crumbling them. Large roots and stones were removed. A subset of fine roots (diameter ≤ 2–3 mm) from the survey conducted in 2021 (60 samples) was retained for molecular detection of *Phytophthora*. For the part of the study dealing with surveillance (in 2019, 2021 and 2025, Table 1), baiting tests were conducted using two methods. In 2019, apple fruits (cultivar Granny Smith) were inoculated with soil samples according to the method of Lilja et al. (2014). Baiting tests were also conducted in plastic boxes with baiting plant tissues on 200 g of soil in the presence of 600 mL of demineralised water according to the method of Jung et al. (2000). In 2021 and 2025, tests were conducted in plastic boxes only, in the same conditions as in 2019. Plant tissues used for the baiting tests in plastic box included mature *Rhododendron* (Catawbiense Cunningham white) leaves, young beech leaves (obtained from seedlings prepared in

TABLE 2 | List of *Phytophthora* species used in this study and parameters of the standard curves for qPCR.

Species	CRAW code	Original host	Location	Collect. year	qPCR standard curves			
					Slope	Constant	R ²	LOD (fg/PCR)
<i>P. xcambivora</i>	P4773	Beech	Goé	2014	−3.50	41.24	0.998	10
<i>P. pseudosyringae</i>	P4549	Beech	Brussels	2013	−3.70	42.20	0.998	10
<i>P. plurivora</i>	P5753	Beech	Brussels	2014	−3.37	42.04	0.998	100
<i>P. cactorum</i>	P4798	Pear fruit	Aubel	2015	−3.47	42.03	0.999	100
<i>P. gonapodyides</i>	P4689	Beech	Mochamps	2013	−3.55	41.51	0.997	10
<i>P. cinnamomi</i> 1	P5700	Soil beech forest	Mormont	2021	−3.69	43.26	0.998	100
<i>P. cinnamomi</i> 2	P3793	Rhododendron	Antwerpen	2008	nd	nd	nd	nd
<i>P. cinnamomi</i> A1	P3231	JKI collection ^a , isolate BBA69094			nd	nd	nd	nd
<i>P. cinnamomi</i> A2	P3232	JKI collection ^a , isolate BBA62660			nd	nd	nd	nd

Abbreviations: LOD, limit of detection; nd, not done.

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the greenhouse), chestnut (*Castanea sativa*) leaves collected from trees near the laboratory, as well as germinating lupin (*Lupinus angustifolius*). For the part of the study dealing with the role of soil pH (in 2023 and 2024, Table 1), tests were conducted in plastic boxes with four types of baiting solutions (demineralised water, river water, filtered river water/demineralised water mixture, or a seaweed solution) and mycelium plugs of different *Phytophthora* species (Table 2) taken from the edge of a 5-day-old culture on V8 medium (five plugs per box). River water was taken from a watercourse near the laboratory the day before the test and stored at 2°C–4°C. Filtered river water was obtained by successive filtrations using a vacuum pump filtration system with 60, 11 and 1.2µm nylon filters (Merck Millipore Ltd.). The filtered water was used to avoid any contamination with zoospores present in the water at the time of collection in the watercourse. It was mixed with demineralised water (330/270 mL v/v in 2023, and 360 mL filtered river water/240 mL demineralised water v/v in 2024) due to the difficulty in getting a sufficient volume of water for the experiments (rapid saturation of the 1.2µm filter). The seaweed solution consisted in diluting seaweed granules (DCM, Belgium) to 0.2 g/L in demineralised water. DCM seaweed is a commercial product used by farmers and gardeners to raise soil pH. Only rhododendron leaves were used as baiting material as the aim was not to determine the community of *Phytophthora* present in the sample, but to evaluate the infection potential of selected *Phytophthora* species in the presence of soil from the Ardennes beech forest. Preliminary tests showed that rhododendron leaves could be infected with the *Phytophthora* species selected in this study (data not shown). The proportion of necrotic area on the rhododendron leaves was assessed visually (Figure 2).

2.3 | Baiting Tests in Stream

They were conducted by placing rhododendron leaves in mosquito netting fitted with floats and leaving them in the stream for 5–6 days. These tests were conducted in October 2021 in two streams crossing beech stand 14, and in June, August, and October 2022 in a watercourse downstream from site 14 (Figure 1).

2.4 | Measurement of Soil Suspension pH for Baiting Tests

Six grams of crumbled, dried soil (after pooling soil samples from the same site) were suspended in 30 mL of the liquid used for baiting, depending on the trial (demineralised water, river water, filtered river water mixed with demineralised water, and the seaweed solution). The tubes were shaken vigorously and left to stand for 20 min. The operation was repeated twice. The suspension was then measured using a calibrated pH meter at 20°C–22°C.

2.5 | Isolation of *Phytophthora*

For all trials, isolation was performed exclusively on bait leaves exhibiting necrotic lesions, as recommended by Pérez-Sierra et al. (2022). These tests were carried out to identify the *Phytophthora* species involved. Leaves were cleaned under running water, then dried with paper towels. Pieces of tissue at the junction between healthy and necrotic areas were plated onto semi-selective *Phytophthora* medium PARP-V8 (2019, 2021) or PARP-carrot agar (2023, 2024, 2025). Plates were incubated at 20°C–22°C for a maximum of 7 days. Subcultures were done on V8 or carrot agar.

2.6 | Detection of *Phytophthora* DNA Using Molecular Testing

Root samples (recovered from the soil samples collected from the beech stands in 2021) and cleaned *Rhododendron* leaves without necrosis (coming from the baiting tests conducted in 2023 and 2024) were ground in liquid nitrogen. DNA extractions were performed either with the DNeasy PowerSoil Pro Kit (Qiagen) for beech roots or with the NucleoSpin Plant II kit (Macherey-Nagel) for rhododendron leaves. DNA quantity and purity were assessed using a spectrophotometer

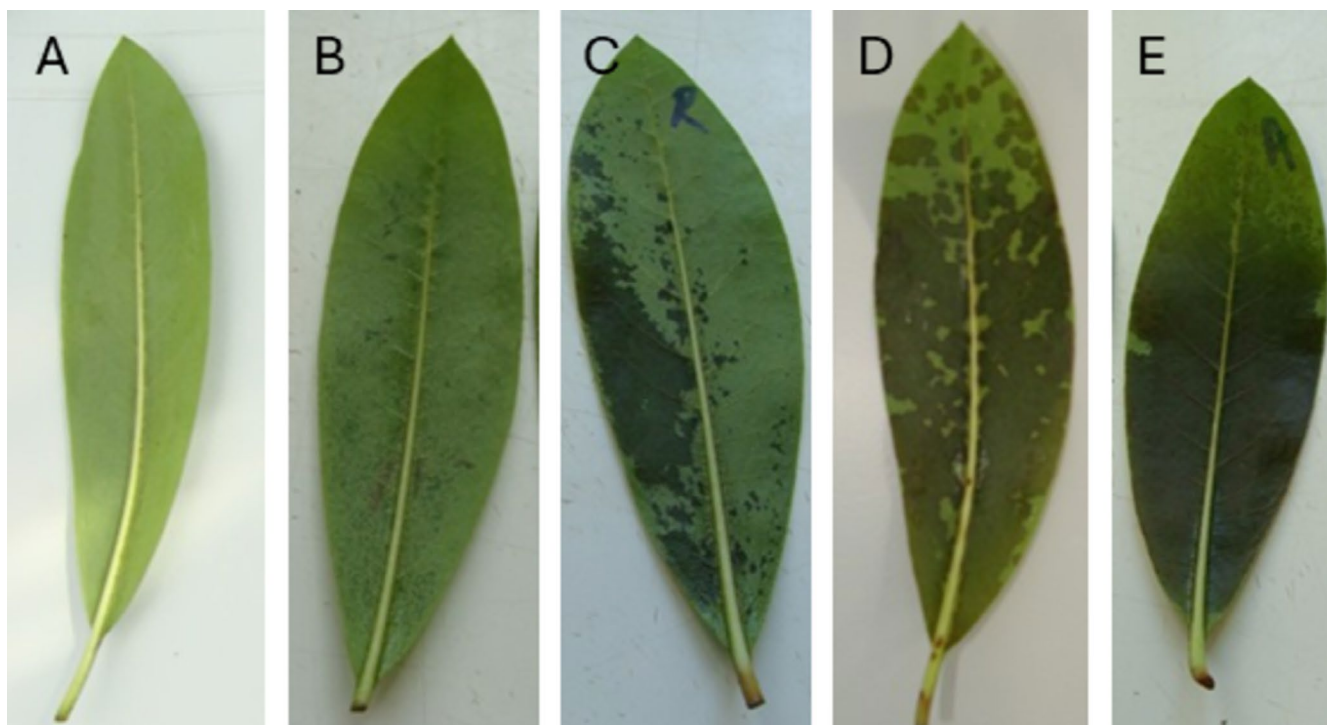


FIGURE 2 | *Rhododendron* leaves recovered from a baiting test, showing different percentages of necrotic areas (A: 0%, B: Approximately 20%, C: Approximately 40%, D: Approximately 80%, E: Approximately 95%).

(Biospectrometer, Eppendorf). For root samples (objective ‘Surveillance’, Table 1), three DNA extractions were performed, and two PCR methods were used. A qPCR test for the detection of all *Phytophthora* species (Bilodeau et al. 2014) was carried out on the three replicates using the Probe 2x kit from Nippon Genetics and a BioRad CFX thermocycler. Amplifications were carried out in 20 μ L with 0.5 μ M primers and 0.25 μ M Taqman probe. Cycling conditions included a first denaturation at 95°C for 3 min, 40 cycles of 95°C for 15 s and 62°C for 1 min. A nested PCR approach targeting the ITS region with primers of Scibetta et al. (2012) for the first PCR and primers of Scibetta et al. (2012) and Cooke et al. (2000) for the second PCR was also used on one of the three DNA replicates with the protocol described by Burgess et al. (2021). The PCR reactions were conducted with the GoTaq G2 Flexi DNA Polymerase kit (Promega) and a Biorad T100 thermocycler. Amplification products were visualised on a 1.5% agarose gel stained with GelRed. For DNA from *Rhododendron* leaf samples (objective ‘Role of soil pH’, Table 1), only the qPCR method was performed. For each *Phytophthora* species used as inoculum, the limit of detection (LOD) and the corresponding C_q value ($C_{q_{LOD}}$) were determined by performing qPCR on successive dilutions of DNA (from 1 ng to 10 fg/PCR) extracted from pure culture, taking three replicates per DNA concentration level into account. The LOD corresponds to the concentration for which all replicates provided a C_q value (Table 2).

2.7 | Mating Type Determination

Phytophthora cinnamomi isolates from mating types A1 and A2 (Table 2) were paired with the isolate identified from site 14 on

V8 agar medium. After 3 weeks of incubation at 20°C–22°C in the dark, observations were made using an inverted microscope to check the presence of oospores at the junction zone between the two mycelia.

2.8 | Statistical Analyses of the Data

All the tests were conducted with R (R Core Team 2024) using the ggplot2 packages for the graphics. To compare the pH among baiting solutions (demineralised water, a mixture of filtered river water/demineralised water, and the seaweed solution) on soil from beech stands, analyses were performed separately for each year (2023 and 2024) using the non-parametric Kruskal-Wallis test. When significant differences were detected ($p < 0.05$), pairwise comparisons were conducted using Dunn’s post hoc test with Bonferroni correction. To evaluate the relationship between the percentage of leaf area with necrosis and the pH of baiting solution, a separate Generalised Additive Model (GAM) was fitted for each *Phytophthora* isolate and year combination. The degree of smoothing of the model was determined using the GCV (Generalised Cross-Validation) criterion, that is, the default method of the mgcv package (Wood 2017). To evaluate the effect of the baiting solution on the proportion of necrotic leaf area, a mixed model was used, with the percentage of necrosis area as response, the type of baiting solution as fixed effect and the species and substrate as random effects (R package lme4; Bates et al. 2015). All pairwise comparisons between the three types of liquid were then performed, with p -values adjusted for multiple comparisons using the default ‘one-step’ method in the R package multcomp (Hothorn et al. 2008). Only data from 2024 was used for this analysis because not all isolates were tested in 2023.

TABLE 3 | Percentage of rhododendron leaf area with necrosis in different *Phytophthora* baiting solutions (D = demineralised water, R = River water, F = mixture of river water/demineralised water (55/45v/v in 2023 and 60/40v/v in 2024) and S = seaweed solution).

Year	Soil	Pcin1			Pcam			Pplu			Pgon			Pcin2			Pcac			Ppse			No inoculum		
		D	R	F	D	R	F	D	R	F	D	R	F	D	R	F	D	R	F	D	R	F	D	R	F
2023	None	15	15	—	70	100	—	95	85	—	40	99	—	—	—	—	—	—	—	—	—	—	0	0	—
	Meadow	15	15	—	95	95	—	95	100	—	50	100	—	—	—	—	—	—	—	—	—	—	0	0	—
		S8	10	30	50	0	100	5	0	80	0	0	40	0	—	—	—	—	—	—	—	—	0	0	0
	S10	0	5	0	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	—	—	0	0	0	
	S14	70	100	60	0	100	100	0	100	0	0	99	0	—	—	—	—	—	—	—	—	0	0	0	
	S19	50	100	30	0	100	5	0	100	0	0	99	0	—	—	—	—	—	—	—	—	0	5	0	
2024		D	F	S	D	F	S	D	F	S	D	F	S	D	F	S	D	F	S	D	F	S	D	F	S
		D	F	S	D	F	S	D	F	S	D	F	S	D	F	S	D	F	S	D	F	S	D	F	S
	None	20	20	20	40	75	95	70	95	95	30	70	50	15	20	15	30	70	80	40	70	30	0	0	0
	Meadow	40	70	40	100	90	100	95	90	95	60	70	70	50	5	10	20	40	20	40	40	0	0	0	0
		S2	0	90	40	0	40	70	0	90	90	0	10	60	0	30	10	0	0	70	0	—	50	0	0
	S4	70	80	70	0	90	75	0	85	85	0	30	75	20	40	40	0	0	30	0	30	15	0	0	0
	S8	70	80	50	0	95	95	0	95	85	0	20	20	30	30	70	0	0	15	0	—	30	0	0	0
	S9	0	80	40	0	20	60	0	80	75	0	0	5	5	40	0	0	5	15	—	—	0	0	0	0
	S14	0	85	15	10	90	65	0	90	90	0	30	35	5	5	0	15	15	0	50	30	0	0	0	0
	S19	0	50	10	5	5	15	0	80	70	0	10	30	20	40	60	0	0	0	—	30	0	0	0	0
	S20	10	75	80	0	65	80	0	80	95	0	40	60	0	20	40	0	0	20	0	60	30	0	0	0
	S21	40	70	70	0	85	70	0	95	95	0	30	40	15	50	50	0	10	0	—	60	0	0	0	0

Abbreviations: —, not done; Pcam, *P. cactorum*; Pcin1, *P. cinnamomi* isolate 1; Pcin2, *P. cinnamomi* isolate 2; Pgon, *P. gonapodyides*; Pplu, *P. plurivora*; Ppse, *P. pseudosyringae*; S2–S21, soil from beech stands (list of stand numbers in Table 1).

3 | Results

3.1 | Surveillance of *Phytophthora* Species in Belgian Beech Forests

Approximately half of the surveyed trees displayed clear symptoms of dieback, with crown transparency, sparse ramifications and chlorotic foliage indicating root problems. None of the trees displayed bleeding cankers on the trunks.

In 2019, none of the baiting assays yielded positive detection. In 2021, *P. cinnamomi* was isolated from all plant material used as baits and for three trees from the same site (site 14, Figure 1). Real-time PCRs and nested PCRs conducted on fine roots recovered from the 60 soil samples collected in 2021 confirmed the baiting results, with only three trees (from site 14) for which C_q values were below the $C_{q_{LOD}}$ (qPCR) or for which a signal was visible on agarose gel (nested PCR). Site 14 is in a wetland area crossed by two small streams. Baiting tests conducted in streams crossing the stand in October 2021, and in a water-course located downstream of the stand in 2022, allowed the baiting of several *Phytophthora* species including *P. plurivora*, *P. xcambivora*, *P. gonapodyides*, *P. lacustris*, *P. syringae*, *P. pseudo-syringae* and *P. chlamydospora*. However, *P. cinnamomi* was not detected. A survey conducted at site 14 in May 2025 confirmed the presence of *P. cinnamomi* in the rhizosphere of one of the three trees sampled in 2021. Mating type analysis showed that the *P. cinnamomi* isolate belonged to mating type A2.

Soil pH values in demineralised water (i.e., used for baiting) ranged from 3.8 to 4.5 for all 20 sites with a value of 4.0 for site 14 (Table 1). These data, indicate markedly acidic conditions.

3.2 | Influence of Baiting Solution pH on the Infection Potential of *Phytophthora* Species

As shown in Table 3, in soil from the beech stands flooded with demineralised water, the species *P. cactorum*, *P. gonapodyides*, *P. pseudosyringae* and *P. plurivora* produced no visible necrosis on bait leaves. The results of qPCR conducted on these leaves were negative or above the $C_{q_{LOD}}$. For *P. xcambivora*, there was no necrosis on leaves in 2023 and only a few rare spots on the leaves for two sites in 2024 (surface area with necrosis at 10% for site 14 and 5% for site 19) (Table 3). By performing qPCR on leaves without symptoms, five additional samples with a *P. xcambivora* DNA concentration above the LOD were found (sites 14 and 19 in 2023, and sites 4, 8 and 21 in 2024). However, the C_q values were close to the end of the PCR run (> 35). For *P. cinnamomi* isolate 1 in 2023, necroses were observed on rhododendron leaves for three of the four beech soils tested (sites 8, 14 and 19, Table 3). No necrosis was detected at site 10. In 2024, there was no visible necrosis in four of the eight beech soil samples tested (sites 2, 9, 14 and 19, Table 3). These results were confirmed by qPCR. For *P. cinnamomi* isolate 2, there was no visible necrosis in leaves used as baits for two beech soil samples (sites 2 and 20, Table 3), and the samples were negative using qPCR. The seven *Phytophthora* isolates tested caused necroses in rhododendron leaves used as baits during tests in the soil collected from a meadow (with a higher pH) and in tests conducted without a substrate for the 2 years of the

study when demineralised water was used as baiting solution (Table 3).

By replacing demineralised water by river water or by a mixture of filtered river water and demineralised water, the pH of the soil suspension increased (Figure 3), and the infection potential was restored for those *Phytophthora* species that did not induce necrosis when soils collected in beech stands were suspended in demineralised water (Table 3). The proportion of necrotic area on leaves was increased for *P. cinnamomi* using these alternative baiting solutions (Table 3). Site 10 in 2023 remained suppressive to *Phytophthora* infection across all treatments (Table 3). However, unlike other beech forest soils, replacing demineralised water with river water or a mixture of river water and demineralised water led to a decrease in the pH of the soil suspension for this site (Figure 3).

Infection potential also improved markedly when using a seaweed-based alkaline solution (Table 3). For *P. cactorum*, the seaweed-based solution was even more efficient than a mixture of filtered river water and demineralised water, with positive results for seven sites compared to only two with the mixture of filtered river water and demineralised water (Table 3).

Baiting tests were also performed on soil samples collected in 2023 and 2024 without adding *Phytophthora* inoculum, using demineralised water, river water, a mixture of filtered river water and demineralised water, or a seaweed solution as the baiting medium. Only one sample tested positive for *Phytophthora* in 2023, when river water was used as the baiting solution (necrosis at 5% for site 19, Table 3).

For the samples artificially infected by a *Phytophthora* species, isolations conducted on symptomatic leaves confirmed that the *Phytophthora* used as inoculum caused the necroses in all cases.

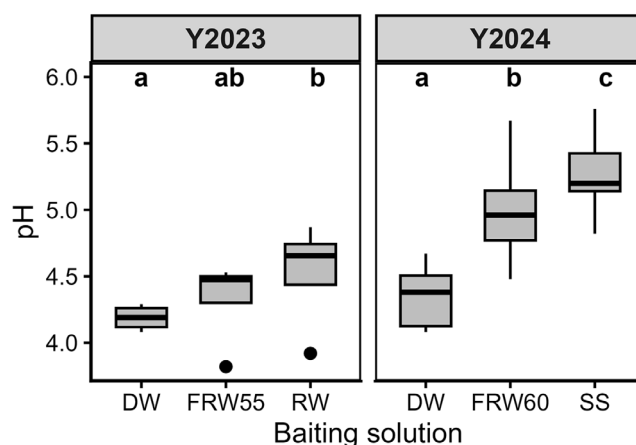


FIGURE 3 | Boxplot showing the distribution of pH in soil samples from beech forests suspended in different solutions (DW=demineralised water, FRW55=55/45v/v mixture of river water and demineralised water, FRW60=60/40v/v mixture of river water and demineralised water, RW=river water, SS=seaweed solution). Each box represents the interquartile range of the pH, with the center line indicating the median. Data were collected in 2023 and 2024. Outlier values correspond to site 10 (2023). Letters above each box indicate results of pairwise comparisons. Groups sharing the same letter are not significantly different ($p > 0.05$).

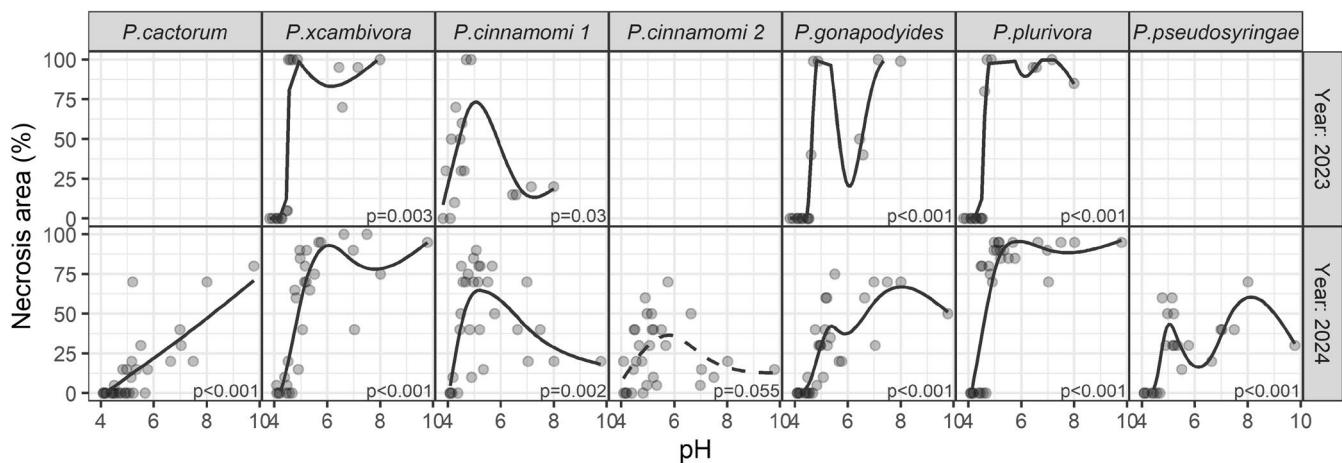


FIGURE 4 | Relationship between necrotic leaf area (%) and the pH of baiting solution for different *Phytophthora* species infecting beech trees. Data were obtained from tests using baiting solutions in beech soil, meadow soil and substrate-free solution artificially inoculated with various *Phytophthora* species. For four of the seven *Phytophthora* isolates, the tests were repeated over 2 years (2023 and 2024). The grey lines represent the fit of a Generalised Additive Model (GAM). The corresponding p -values are shown at the bottom right corner of each panel. Dashed lines indicate that the p -value is not significant ($p > 0.05$).

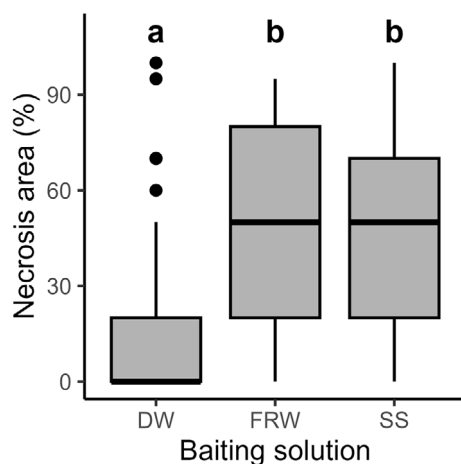


FIGURE 5 | Boxplot representing the distribution of necrotic area on rhododendron leaves in baiting solutions (DW = demineralised water, FRW = filtered river water/demineralised water, SS = seaweed solution). Data were obtained from tests in beech soil, meadow soil and substrate-free solution artificially inoculated with various *Phytophthora* species in 2024. Each box represents the interquartile range of the pH, with the center line indicating the median. Modalities with the same letters are not significantly different ($p > 0.05$).

The species identified in the 2023 sample of site 19, without adding a *Phytophthora* species to the soil, was *P. lacustris*.

The relationship between baiting solution acidity and infection potential, as reflected by the percentage of necrotic leaf surface, was evaluated across the different *Phytophthora* species selected for this study (Figure 4). Most of the *Phytophthora* species showed a trend towards increasing capacity to infect the bait plant with increasing pH, with a minimum pH for necrosis development around 4.3–4.5. *P. cinnamomi* showed a different profile compared to the other species, with a maximum around 5.5 for the two isolates. The p -values were significant for all *Phytophthora* × year tested combinations except for *P. cinnamomi* isolate 2 in 2024 (Figure 4).

The global effect of the baiting solution on necrosis area was highly significant ($F = 55.6$, $p < 0.0001$). The necrosis area was significantly lower with demineralised water (on average 13%) than with the mixture of filtered river water/demineralised water or the seaweed solution (Figure 5).

4 | Discussion

This study sampled 20 beech stands for *Phytophthora* species in the fine roots, selecting three trees with varying levels of crown die-back per site. Only one site was infected with *P. cinnamomi*. This species affects a broad range of host plants worldwide (Hardham and Blackman 2018) and is often detected in ornamental plants in European nurseries (Antonelli et al. 2023; Jung et al. 2016). Until recently, this pathogen was present in the natural environment in the Mediterranean zone due to its sensitivity to deep frost (Erwin and Ribeiro 1996). However, reports from more northerly countries, notably Poland, Austria and Germany (Khaliq et al. 2020; Nechwatal and Jung 2021), suggest that climate change may be extending its range across Europe. To our knowledge, it has not previously been reported in beech forests in Belgium although it has been detected on ornamental plants in nurseries. The origin of *P. cinnamomi* in a forest area is unclear. The stand is far from any nursery, private garden, or park where this exotic pathogen might have been introduced via ornamental plants. Severe flooding in southern Belgium in summer of 2021 may have caused root infection if the pathogen was present in the river water. However, baiting tests performed in the streams within the stand in October 2021 (3 months after the flooding) and in a downstream stream in 2022 did not detect the pathogen. On the contrary, various other *Phytophthora* species, including species implicated in beech decline in Europe, were detected. None of the three trees infected by *P. cinnamomi* had bleeding cankers on the trunks, and only one of the three sampled trees showed important crown degradation. The lack of correlation between symptoms and the presence of the pathogen in the roots could result from an early detection. Indeed, *P. cinnamomi* often causes no immediate damage (Sena et al. 2018) and can grow saprophytically, waiting for favourable conditions to

sporulate and develop (Hardham and Blackman 2018). Continued monitoring of the infected stand will be essential to track pathogen dynamics over time. The isolate identified in the beech stand belongs to the A2 mating type, the only mating type currently reported in Europe (Nechwatal and Jung 2021).

Except for the site infected by *P. cinnamomi* (site 14), no *Phytophthora* species associated with beech decline could be isolated from the other surveyed sites. Sarker, Burgess, et al. (2023) reported that the number of *Phytophthora* species detected using molecular methods was consistently higher than that obtained through baiting. For this reason, molecular tests were conducted on a part of the fine root samples of the 60 trees monitored. To limit sampling bias when comparing the two techniques (baiting followed by isolation and direct molecular testing), the rhizosphere samples (from soil samples taken at four points around the tree) were thoroughly homogenised before part of the fine roots were collected for molecular analysis. Two molecular tests, one targeting mitochondrial DNA (Bilodeau et al. 2014) and the other targeting ITS (Scibetta et al. 2012; Cooke et al. 2000), were conducted, as different results can be obtained depending on the genome region targeted (Burgess et al. 2021). The qPCR tests were also performed on three DNA replicates to take account of stochastic PCR amplification in case of low concentration of target DNA in the DNA sample. Apart from site 14, the findings were negative, regardless of the molecular test used or the replicate, showing concordance between baiting and molecular tests. These results also rule out the hypothesis of dormant structures, which would require soil samples to be pretreated before baiting as recommended by Pérez-Sierra et al. (2022). Surveillance results from this study, particularly the very low occurrence of infection by *Phytophthora* and the absence of marked symptoms in infected trees, suggest that *Phytophthora* species are not associated with the observed decline of Ardennes beech forests in Belgium. This conclusion contrasts with other studies conducted in Europe (Jung 2009; Jung et al. 2018; Corcobado et al. 2020; Tkaczyk et al. 2023) or North America (Nelson et al. 2010).

As the soils pH in the 20 beech stands monitored was low, it was hypothesized that the soils of Ardennes beech stands are suppressive to *Phytophthora* infection. Attempts were therefore made to bait *Phytophthora* from soil samples collected in various beech stands of our survey, which were inoculated with mycelium plugs of *Phytophthora*. The *Phytophthora* species selected were those associated with the decline of beech trees in Europe (*P. xcambivora*, *P. cactorum*, *P. plurivora*, *P. pseudosyringae* and *P. gonapodyides*) (Jung 2009; Jung et al. 2018; Corcobado et al. 2020) as well as two isolates of *P. cinnamomi*, one isolated from a leaf bait assay from beech soil (this study), and the other from a rhododendron in a nursery. The baiting test was conducted using demineralised water as is usually recommended (Jung 2009; Burgess et al. 2021; Pérez-Sierra et al. 2022). The two *P. cinnamomi* isolates induced necrosis on rhododendron leaves used as bait leaves. *P. xcambivora* induced very small spots on leaves compared to *P. cinnamomi* and only at two sites (out of eight) in 2024, whereas none of the other tested *Phytophthora* species infected the rhododendrons. As bait leaves might be infected without necrosis being visible (Pérez-Sierra et al. 2022; Sarker, McComb, et al. 2023), the molecular qPCR test targeting

Phytophthora species was conducted on asymptomatic bait leaves, with negative results (no C_q value) or doubtful results (C_q values close to the end of the PCR run) for most of the samples. On the contrary, rhododendron leaves in the experimental controls (baiting in water without beech soil) showed necrosis for all *Phytophthora* species. Moreover, in a control soil with a pH close to neutral, the bait leaves also showed necrosis, and the results were confirmed by isolation.

Collectively, these trials confirm that soil pH is a key factor affecting the infection potential of *Phytophthora*, consistent with previous observations linking soil acidity to sporangia formation and zoospore viability (Blaker and MacDonald 1983; Kong et al. 2009). Different behaviours emerged for the different *Phytophthora* species evaluated. *P. cinnamomi* exhibited a maximum infection potential, as indicated by leaf necrosis, around $\text{pH}_{\text{water}} = 5.5$ with a steep decrease at higher pH. This aligns with Blaker and MacDonald (1983) who reported that sporangium production in *P. cinnamomi* is maximal at pH ~5 and nearly zero at pH 6.5. It also supports Serano's findings of inhibited sporangia and chlamydospore production in cultures exposed to Ca^{2+} fertilisers (Socorro Serrano et al. 2012). The other species failed to infect the bait leaves at $\text{pH}_{\text{water}} < 4.3\text{--}4.5$, and their infection potential remained stable at intermediate pH, with successful infection occurring at pH_{water} above 6.5. This result suggests that beech stands in the bioclimatic region of the Ardennes are more at risk of infection with *P. cinnamomi* than with other *Phytophthora* species due to the acidic nature of the soil. However, a survey conducted in 2007 led to the isolation of *P. xcambivora* from trunk bleeding cankers in a few beech stands in Southern Belgium including some rare sites in Ardennes (Schmitz et al. 2007). Nevertheless, none of the Ardennes sites showed infections of multiple beeches by this soilborne *Phytophthora*. Moreover, although long-established, *P. xcambivora* was not observed causing bleeding cankers in the Ardennes during a four-year study more than 10 years later (Pirronitto et al. 2022). These occasional detections may reflect a lower sensitivity to acidic pH compared with the other species tested (*P. cactorum*, *P. plurivora*, *P. gonapodyides* and *P. pseudosyringae*). Although very rare, *P. xcambivora* was detected in bait leaves from two beech soil samples (site 14 and site 19) flooded with demineralised water in 2024 (Table 3), supporting this hypothesis.

5 | Conclusion

During 4 years of *Phytophthora* surveillance in declining beech stands in southern Belgium, no bleeding cankers were observed on trunk; however, soil analyses revealed the presence of *P. cinnamomi* in the rhizosphere of a beech stand in 2021. Its confirmed presence in the same stand 4 years later suggests that this species is expanding northward in Europe in response to global warming. This observation aligns with predictive models assessing the future risk of *P. cinnamomi* in Europe under different climate change scenarios (Cidre-González et al. 2025). The study also showed that tolerance to pH stress varies among *Phytophthora* species, with *P. cinnamomi* more tolerant of acidic soils than other species commonly associated with beech decline in Europe, but less tolerant of neutral or alkaline soils. These results highlight the need to

consider soil pH in future surveillance and monitoring strategies for the forest pathogen *P. cinnamomi*. More broadly, they suggest that soil pH should be integrated into surveillance approaches, as this factor may also be critical for detecting other *Phytophthora* species of interest.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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