

The impact of *Phytophthora ilicis* on *Ilex* spp. in botanic garden collections: a case study at the Royal Botanic Garden Edinburgh

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Abstract

Ilex spp. (holly) are widely planted ornamental evergreen shrubs which perform both an aesthetic and an ecological function in gardens. In Edinburgh, *I. aquifolium* is the second most common tree after *Acer pseudoplatanus* (sycamore), providing food and shelter for a range of species. Unfortunately, holly is under attack from a disease caused by the pathogen *Phytophthora ilicis*. This understudied pathogen has been in the UK for at least 35 years, but the impact has not been clearly elucidated. We undertook a study at the Royal Botanic Garden Edinburgh to understand the impact of this disease on *Ilex* spp. within a botanical plant collection. Our survey showed 14% of the collection was confirmed to be infected with *P. ilicis*, although true prevalence is probably underrepresented due to seasonal factors and detection challenges. We discuss possible management strategies for this disease.

Introduction

Ilex aquifolium L. (common holly) is a native evergreen tree or shrub of deciduous woodlands and wood pasture, which is often a locally dominant undershrub (Wilmore & Walker, 2020). Significant planting over centuries obscures its native range and it is now widely distributed throughout Britain and Ireland.

According to a 2016 survey of trees with a diameter above 7 cm (at 130 cm above ground level) carried out by Forest Research in Edinburgh (Doick *et al.*, 2017), *I. aquifolium* is the second most common tree in the city after *Acer pseudoplatanus* (sycamore). It often does not become a tree of any great stature but rather is present as a large shrub. The evergreen *I. aquifolium* introduces the

benefit of year-round cover and provides shelter and food for a range of species. In addition, *I. aquifolium* ranks among the top ten ornamental species in Edinburgh for its ability to remove CO₂ from the air (Hutchings *et al.*, 2012).

The Royal Botanic Garden Edinburgh (RBGE) has a sizeable *Ilex* collection with 131 accessions comprising multiple cultivars and species, including *I. aquifolium* 'Monstrosa', *I. aquifolium* 'Silver Milkmaid', *I. × altaclerensis*, *I. pernyi*, *I. macrocarpa*, *I. fargesii* and *I. crenata*. Some of these individuals are more than a century old, including an *I. aquifolium* 'Pendula' that was planted in 1907 (Fig. 1).

Unfortunately, the *Ilex* collection is under pressure from a disease caused by a species of *Phytophthora* that is having

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Fig. 1 *I. aquifolium* 'Pendula', which was planted at RBGE in 1907. Photo: M. Elliot.

an impact at RBGE. Phytophthoras are fungus-like plant pathogens which have become an increasingly serious problem for a range of plant host species over the last 50 years (Jung *et al.*, 2024). There are currently 223 described species with more being discovered and described every year (Abad *et al.*, 2023a). As a result, *Phytophthora* species are recognised as major plant pathogens with serious implications for natural habitats and managed collections worldwide (Scott *et al.*, 2019; Abad *et al.*, 2023a; Jung *et al.*, 2024).

The specific disease-causing pathogen of *Ilex* is *Phytophthora ilicis*. This pathogen is responsible for leaf and twig blight of *Ilex* species and was first described in the USA in the 1950s (Buddenhagen & Young, 1957). It is currently not clear whether the species originates from the USA or it was introduced there. The first recorded findings in the UK

were in 1989 (Strouts *et al.*, 1989). This is not a well-studied pathogen and so its distribution across the UK is currently unknown. In recent years, *P. ilicis* seems to have widened its range, with findings in Corsica and Sardinia (Scanu *et al.*, 2014), Spain (Pintos *et al.*, 2012) and Slovenia (Bregant *et al.*, 2024). *P. ilicis* infection appears to be increasingly common, and so a better understanding of its distribution and impacts on collections, such as those at RBGE, is warranted.

Symptoms consistent with *P. ilicis* infection have been observed in RBGE's collection for over a decade, but in the last few years its prevalence and severity appear to have increased. These symptoms include defoliation, blackening of stems and leaves, small discrete shoot cankers and bleeding trunk cankers (Scanu *et al.*, 2014) (Fig. 2). The increased observations of these



Fig. 2 Symptomatic holly. **A** Leaf infections. **B** A branch lesion. **C** Infected young branches. **D** Trunk lesions. Photos: A. Jones.

symptoms triggered the decision to carry out an assessment of the holly collection in the Garden, with a particular focus on *P. ilicis*.

Materials and methods

Collection, sampling and Phytophthora isolation

From January to March 2025, RBGE's *Ilex* accessions at RBGE's Edinburgh Garden were visually inspected for *P. ilicis* symptoms. In terms of experimental design, all plants that could be accessed were included in the survey. Accessions in areas of the Garden that were under construction at the time or housed active badger setts were not assessed. Symptomatic material was collected from trees and shrubs (generally two samples per accession, representing both leaf and twig or branch where possible). Samples were processed within 24 hours in the authorised pathology laboratory on site.

For isolation, six small pieces of leaf or stem tissue (c. 3 × 3 mm) were cut from the transition zone between dead and living plant tissue. These were surface sterilised using the following method: 60 sec. in 70% ethanol, followed by 60 sec. in sterile distilled water, and then air drying on paper towel (Scanu *et al.*, 2014). Material was then plated onto PARP(H)-V8 *Phytophthora* selective medium (Ferguson & Jeffers, 1999) and incubated at 20 °C in the dark.

Morphological characterisation

Colony morphology was assessed visually after c. 10 days. Initially, colonies that appeared to be *Phytophthora* were subbed onto CMA (cornmeal agar), 10% V8 agar and 10% CA (carrot juice agar) (Ferguson & Jeffers, 1999). The latter was made using the agar:water:juice ratio from Ferguson & Jeffers (1999) – modified to include 100 mL/L of juice obtained from squeezing freshly grated carrot

through a muslin bag. Latterly, CA alone was used for subs. Sporangia production was induced from pure cultures (c. 1 week old) using methods from Drenth & Sendall (2001), whereby 0.5 cm² plugs were cut from the growing edge and incubated in sterile distilled water at room temperature under continuous fluorescent light for 24 hours, then moved to ambient light/dark conditions (near a window) and checked daily for sporangia growth.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from pure cultures (potential *P. ilicis*, and a selection of secondary fungi) using a simple heat lysis method (adapted from standard TE heat lysis procedures and Katy Hayden, pers. comm., 2019). Approximately 1 cm² of actively growing mycelium was scraped from the agar surface with a sterile toothpick and transferred into a 1.5 mL microcentrifuge tube containing 100 µL of 0.1% TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). Tubes were vortexed briefly and incubated at 95 °C for 5 minutes. After heating, samples were cooled immediately on ice. The resulting suspension was used directly as a template for PCR.

Three genes were targeted for amplification/sequencing: ITS with primers ITS6 (Cooke *et al.*, 2000) and ITS4 (White *et al.*, 1990); COX1 using primers COIF-1 and COIR-1 (Robideau *et al.*, 2011); *Ypt1* (ras-related protein gene) using primers YPh1F and YPh2R (Schena *et al.*, 2006). Sanger sequencing was carried out at the University of Dundee. Sequence editing was performed using Geneious (v. 2025.0.3). Sequences were BLASTed (Basic Alignment Search Tool – NCBI, 2025) against sequences available on IDphy (online tool for molecular and morphological identification of

Phytophthora based on types) (Abad *et al.*, 2023b). Further verification of ID through phylogenetic analysis was performed. Sequences for each gene were aligned with a set of ex-type sequences from Clade 3 and an outgroup sequence from Clade 5 (Abad *et al.*, 2023a) (Table 1) (Geneious MAFFT plug-in), then concatenated to make a consensus tree (neighbour-joining tree using Tamura-Nei distance model and 100 replicates) (Fig. 3).

Infectivity test

Pathogenicity of three *P. ilicis* isolates (confirmed with morphology and DNA) was tested by inoculating young *I. aquifolium* pot plants using combined methods from Scanu *et al.* (2014) and Pintos *et al.* (2012). Twelve plants were inoculated with a 5 mm plug of 7-day-old *P. ilicis* culture (three strains, three replicates each). A wound was cut in the upper stem and the plug was inserted with the culture side facing inwards. Damp paper towel was wrapped around the inoculated area, followed by sealing with parafilm. Control plants (3) were given the same treatment with plain (sterile) agar plugs. Plants were maintained at room temperature (c. 19 °C–22 °C) near a window with diffuse light and watered and misted regularly. Necrotic lesions were not measured; instead, plants were visually monitored and qualitative observations recorded throughout the two-week incubation period. Plants were then harvested and material from the inoculation/wound area of treatment plants was surface sterilised and plated out onto PARP(H)-V8 media. A representative colony from each plate was subbed onto CA and a subsample of plates (four cultures) was sequenced to confirm ID.

Results

Eighty-six of the 131 *Ilex* accessions were assessed for *P. ilicis* symptoms. Assessment and collection for all accessions was not possible due to lack of access caused by building work, storm damage and proximity to badger setts. Forty-seven of the trees or shrubs were considered to have *Phytophthora*-like disease symptoms and were subsequently sampled. These were primarily confined to *I. aquifolium*, with leaf and stem lesions frequent among the trees assessed. Bole cankers were less common. Many branches had brown (suberised) tissue around petiole lesions and girdling of lesions on stems, suggesting historic rather than active infection sites. An effort was made to collect the most active-looking symptomatic areas (a strategy which evolved and improved over time). Many of the *I. aquifolium* assessed were moderately or severely defoliated. Some non-*aquifolium* species presented leaf or stem spots and lesions. These were less commonly and less definitively *Phytophthora*-like.

Colony morphology of *Phytophthora* isolates grown on V8 and CA agreed with descriptions of *P. ilicis* in Abad *et al.* (2023b) – slow-growing and a light stellate pattern. Sporangia were produced readily in distilled water after 1–2 days (generally semipapillate and caducous) (Fig. 4). After one week, oogonia and antheridia were observed on the same plugs (amphigynous).

Identification of isolates and disease incidence

Colony morphology together with ITS BLAST matches of 100–99.9% confirmed the identification of *P. ilicis* isolates, which were recovered from 12 of the *Ilex* accessions sampled (14% disease incidence among trees assessed). Notably, the hybrid *I. × altaclerensis*

Table 1 GenBank accessions for *P. ilicis* included in this study. Study isolates not used in the phylogenetic analysis are marked with *. Additional reference sequences are from Abad et al. (2023).

Phytophthora species	Collection number	Host/substrate/isolation source	Location	GenBank accession				Type
				ITS	COX1	Ypt1		
<i>Phytophthora ilicis</i>	022(EDNA25-0071723)	<i>Ilex aquifolium</i>	Scotland	PX583118	PX666712	PX620746	---	
<i>Phytophthora ilicis</i>	024(EDNA25-0071724)	<i>Ilex x altaclerensis</i> Maderensis Group	Scotland	PX583117	PX666711	PX620747	---	
<i>Phytophthora ilicis</i>	027(EDNA25-0071725)	<i>Ilex aquifolium</i>	Scotland	PX583116	PX666708	PX620750	---	
<i>Phytophthora ilicis</i>	028(EDNA25-0071726)	<i>Ilex aquifolium</i>	Scotland	PX583115	PX666709	PX620749	---	
<i>Phytophthora ilicis</i>	046(EDNA25-0071727)	<i>Ilex aquifolium</i>	Scotland	PX583114	PX666710	PX620748	---	
<i>Phytophthora ilicis</i>	047(EDNA25-0071728)	<i>Ilex x altaclerensis</i>	Scotland	PX583113	PX666707	PX620745	---	
<i>Phytophthora ilicis</i>	H-2(EDNA25-0071729)	<i>Ilex aquifolium</i>	Scotland	PX583128	PX666706	PX620751	---	
<i>Phytophthora ilicis</i>	H-7(EDNA25-0071730)	<i>Ilex aquifolium</i>	Scotland	PX583127	PX666705	PX620744	---	
<i>Phytophthora ilicis</i> *	021(EDNA25-0071722)	<i>Ilex x altaclerensis</i> 'Hendersonii'	Scotland	PX583119	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	017(EDNA25-0071718)	<i>Ilex x altaclerensis</i> 'Belgica'	Scotland	PX583120	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	011(EDNA25-0071715)	<i>Ilex x altaclerensis</i> 'Wilsonii'	Scotland	PX583121	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	004(EDNA25-0069719)	<i>Ilex aquifolium</i> 'Pendula'	Scotland	PX583122	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	003(EDNA25-0069717)	<i>Ilex aquifolium</i> 'Laurifolia'	Scotland	PX583123	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	001(EDNA25-0069715)	<i>Ilex aquifolium</i> 'Laurifolia'	Scotland	PX583124	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	H-12(EDNA25-0071732)	<i>Ilex aquifolium</i>	Scotland	PX583125	n.a.	n.a.	---	
<i>Phytophthora ilicis</i> *	H-11(EDNA25-0071731)	<i>Ilex aquifolium</i>	Scotland	PX583126	n.a.	n.a.	---	
<i>Phytophthora pluvialis</i>	ATCC MYA-4930	Rain collected under forest canopy	USA	n.a.	LC595935.1	OP104654.1	ex-type	
<i>Phytophthora castaneae</i>	CPHST BL 47G	<i>Castanea crenata</i>	Japan	MG865470.1	MH136866.1	MH975006.1	ex-type	
<i>Phytophthora ilicis</i>	CPHST BL 103	<i>Ilex aquifolium</i>	USA	MG865510.1	MH136904.1	MH443246.1	selected co-identical strain to ex-type	
<i>Phytophthora nemorosa</i>	CPHST BL 27	<i>Notholithocarpus densiflorus</i>	USA	MG865548.1	MH136941.1	MH988451.1	ex-type	
<i>Phytophthora pseudosyringae</i>	CPHST BL 51G	Soil, under <i>Quercus robur</i>	Germany	MG865574.1	MH136966.1	OP104658.1	ex-type	
<i>Phytophthora psychrophila</i>	CPHST BL 52	Rhizosphere of decaying <i>Quercus robur</i>	Germany	MG865576.1	MH136968.1	OP104660.1	ex-type	



Fig. 3 Concatenated neighbour-joining tree (ITS, COX1, *Ypt1*) – Clade 3 species with Clade 5 *P. castaneae* as outgroup (ex-type sequences from Abad *et al.*, 2023a), showing branch support percentage values. Produced in Geneious with MAFFT plug-in.

had a much higher rate (5 out of the 11 trees assessed: ~45%) of disease incidence than *I. aquifolium*. Given the similarity of ITS sequences to other *Phytophthora* species (for example, *P. nemorosa* and *P. pseudosyringae*), further identity support was provided by a concatenated three-gene tree (ITS, COX1 and *Ypt1*), showing a well-supported clade with the *P. ilicis* reference sequence (Fig. 3). A fungus identified as *Neonectria punicea* was isolated from roughly 50% of the sample

material (confirmed by sequencing on two occasions). Less commonly isolated fungal species identified in sequence included *Fusarium* spp. and *Phoma* spp.

Pathogenicity of isolates

By seven days after inoculation, darkened lesions had developed beyond the parafilm inoculation sites on all nine plants infected with *P. ilicis*, and by the tenth day the same plants exhibited

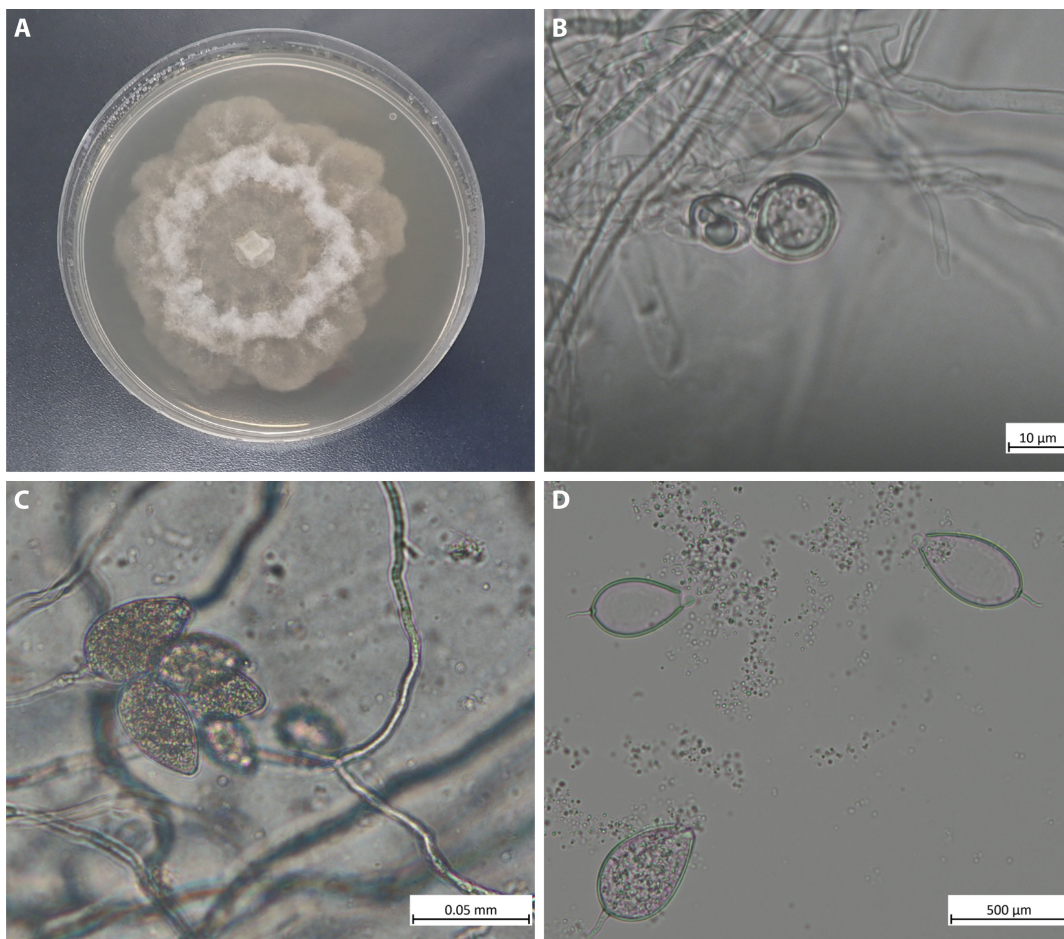


Fig. 4 Morphology of *P. ilicis*. **A** Colony growth on CA (at c. 2 weeks). **B** Oogonium/antheridium ($\times 100$). **C** Sporangia ($\times 100$). **D** Sporangia and zoospores ($\times 40$). Photos: A. Jones.

pronounced leaf drop. In contrast, none of the three control plants displayed lesion darkening or notable leaf drop. Although lesion progression was assessed qualitatively, symptom occurrence differed markedly between treatments, with 100% of inoculated plants vs 0% of controls exhibiting visible lesions and leaf drop when evaluated as presence/absence data. *P. ilicis* was re-isolated from all nine inoculated plants with the identity of four representative cultures confirmed by DNA sequencing, thereby fulfilling Koch's postulates for the three *P. ilicis* strains tested.

Discussion

Although a large proportion of the *I. aquifolium* accessions at the Edinburgh Garden appeared to be symptomatic for *P. ilicis*, only 12 accessions (14%) were confirmed as positive (Fig. 5).

Given the frequency of symptoms (more than half of the holly assessed was sampled), the number of infected trees in the garden is probably much higher. *P. ilicis* is most active during cooler, wetter months. Although sampling was done during winter/early spring, it was unseasonably dry, and some of the material sampled was likely to have

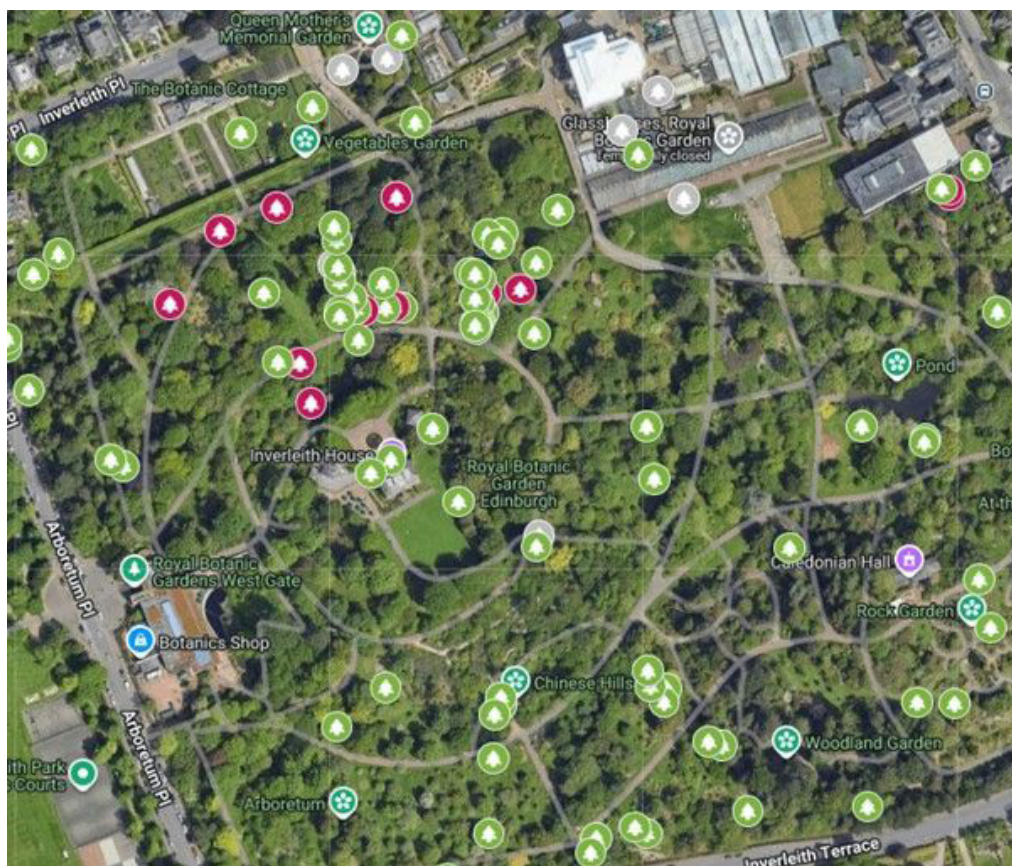


Fig. 5 Accessions of *Ilex* spp. at the Edinburgh Garden (those coloured magenta are confirmed positives for *P. ilicis*). Produced using Google Maps and RBGE's plant records database.

inactive or historic infections. Many of the shoot dieback and lesioned areas had scarred over or suberised. It appears that *P. ilicis* – like other homothallic species – can survive warmer, dryer periods by forming resting spores in infected foliage and dead twigs, and resuming growth in wetter, cooler conditions (Buddenhagen & Young, 1957). Additional surveys in wetter periods could give a more comprehensive picture of infection frequency.

P. ilicis was isolated only from *I. aquifolium* hybrids, cultivars or crosses in this study. No clear pattern was discerned regarding habit, foliage type or location in the garden. For example, infections were not found to coincide with areas of heavy footfall or

poor drainage. A significant number of *I. × altaclerensis* (a hybrid of *I. aquifolium* and *I. perado*) were confirmed as positive (45% of those assessed), compared with the generally low *P. ilicis* disease incidence across *Ilex* accessions (14%). This may have implications for understanding susceptibility of *Ilex* cultivars (for example, *I. perado* is a Macronesian species), but this requires more investigation.

According to Oregon State University data, all cultivars of *I. aquifolium* appear to be susceptible to *P. ilicis*, but there are differences in infection severity (Ticknor, 1986). The Royal Horticultural Society (RHS) (2026) suggests that other holly species are

affected in the UK: *I. aquifolium*, *I. crenata*, *I. × altaclerensis*, *I. dipyrena*, *I. kingiana*, *I. colchica*, *I. pernyi* var. *veitchii* and some clones of *I. opaca*. Considering the lack of studies on *P. ilicis*, it could be that more host species and cultivars or crosses are susceptible.

Several species of holly are thought to be resistant to *P. ilicis* infection and defoliation, including: *I. cassine*, *I. ciliospinosa*, *I. cornuta*, most *I. crenata* tested, *I. glabra*, *I. intricata*, *I. latifolia*, *I. perado*, *I. sugeroki* and *I. vomitoria* (Ticknor, 1986). Likewise, some interspecific hybrids of *I. aquifolium* with *I. cornuta*, *I. integra*, *I. latifolia* and *I. pernyi* have shown tolerance to *P. ilicis* with relatively low amounts of infection and defoliation. This research is quite dated; more susceptibility work needs to be carried out on hybrids produced since the 1990s.

The infectivity test performed in this study showed that *I. aquifolium* was readily infected with *P. ilicis*. Symptoms progressed quickly, which may indicate rapid transmission and infection when translated to field conditions.

Another important finding of this study was the frequency of *Neonectria* (probably *N. punicea*) isolation during this study. This warrants further investigation, as it may be a co-infecter with *P. ilicis* and could be contributing to the general poor health and appearance of the holly collection at RBGE. *Neonectria* spp. can infect via wounds and eventually kill trees (Talgø *et al.*, 2012). In the context of botanic garden collections, non-lethal diseases are still a concern, as the health and aesthetics of the collections may be compromised. A significant number of RBGE holly accessions are in poor condition. Although *Phytophthora* thrives in damp, often cool conditions, abiotic stressors associated with climate change could increase disease susceptibility (Jung *et al.*, 2024).

Apart from the obvious aesthetic impacts on botanic garden collections, *P. ilicis* could also be impacting biodiversity in the wider city of Edinburgh. Holly provides an important biodiversity function as an evergreen tree, not least in its provision of berries for animals to eat.

This work highlights the need for plant health monitoring to be in place in gardens so that disease incidence and impact can be understood and managed. Holly which is showing the symptoms of *P. ilicis* should be recorded and considered for removal to prevent spread onto other *Ilex* spp. within botanical collections. Staff should have training in biosecurity, and robust processes – such as boot and tool cleaning – should be put in place to limit inadvertent spread by staff and volunteers. In addition, when selecting species and cultivars to grow, consideration of their susceptibility to *P. ilicis* is important for their long-term resilience.

Conclusions

Although the confirmed infection rate of *P. ilicis* was relatively low in this survey, true prevalence is probably underrepresented due to seasonal factors and detection challenges. In addition, many symptomatic trees have been removed from the collection over the last 10 years and so were not counted here. This highlights the need for further research into the pathogen's role in tree health decline, aesthetic degradation and interactions with other microorganisms – all observed in the collection at the Edinburgh Garden. Despite presumably being non-lethal, *P. ilicis* may have a greater impact than is currently recognised. In the UK, it is increasingly noted by horticultural and plant health bodies such as the RHS. A deeper understanding of the pathogen's ecology, pathogenicity and management seems critical for

protecting botanic gardens and urban spaces where visual and structural integrity are fundamental. This is particularly relevant in Edinburgh, given the abundance of holly at the Edinburgh Garden and in the city's many landscapes.

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