J Plant Dis Prot (2025) 132:123
https://doi.org/10.1007/s41348-025-01118-w

SHORT COMMUNICATION

First report of *Paraconiothyrium brasiliense* and *Querciphoma minuta* causing stem cankers on urban and forest trees in Germany

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Received: 23 October 2024 / Accepted: 26 June 2025 / Published online: 16 July 2025

Abstract
In this study, we report the first identification of *Paraconiothyrium brasiliense* and *Querciphoma minuta* causing stem cankers on previously unreported tree host species. *P. brasiliense* and *Q. minuta* were isolated for the first time from *Carpinus betulus*, *Fagus sylvatica*, and *Aesculus hippocastanum*. Additionally, *P. brasiliense* was newly isolated from *Ulmus* spp. and *Platanus* × *hispanica*, while *Q. minuta* was also found for the first time on *Acer platanoides*. These trees originate from different cities in Germany, located in different geographical regions. Fungal isolates were initially grouped by morphotyping based on visually recognizable culture characteristics. The taxonomy of these fungi was verified by sequencing with multiple primers, including ITSu1/4, and LSU/LR6. The pathogenicity of each fungus was confirmed using Koch's postulates on the corresponding host trees. *P. brasiliense* and *Q. minuta* are opportunistic pathogens on several plant species, but this is the first report of their pathogenic activity on the corresponding urban trees, confirmed through stem inoculations with fungal agar plugs. This report indicates the emergence of fungal pathogens causing stem necrosis on tree hosts that have not previously been reported.

Keywords Molecular identification · Pathogenicity · Urban trees · Host range · Stem cankers

Introduction

Urban and forest trees provide vital environmental services and socioeconomic benefits, including carbon sequestration, adaptability, and natural cooling through microclimate regulation, especially in urban areas (Keeler et al. 2019). *C. betulus* is planted in urban landscapes for hedging and pollution mitigation, showing high tolerance to different soil conditions and contributing to airborne pollutant capture (European Commission. Joint Research Centre 2016; Imperato et al. 2019). *F. sylvatica* offers recreational value and soil fertility enhancement, while its drought-adaptive root system increases its resilience in changing urban climates (Holzwarth et al. 2011; Nikolova et al. 2020). *P.* × *hispanica* is favored for its fast growth, tolerance to compacted soils, and adequate shading capacity, making it a key component of the urban environment (Endreny 2018; Pauleit et al. 2002). *A. hippocastanum* improves urban aesthetics and biodiversity, especially through its long flowering period and appeal to pollinators (Karliński et al. 2014; Plichta et al. 2022). *Ulmus* species contribute to urban resilience with rapid regrowth and tolerance to pollution, drought, and salinity, which makes them ideal for reforestation and roadside planting (Jürisoo et al. 2021). *A. platanoides* is a common street tree valued for its tolerance to urban stressors, utility in slope stabilization, and requires low maintenance, and it has good wind resistance (European Commission. Joint Research Centre 2016; Mirabile et al. 2015). However, both natural and urban ecosystems are increasingly affected by climate change, resulting in reduced tree growth and higher mortality rates. The increasing frequency and severity of extreme events, such as heatwaves, flooding, and drought, further intensify tree dieback and mortality in urban and forested areas. These stresses also make trees more susceptible to opportunistic pathogens, which can spread more easily under such conditions and expand their host range (Esperon-Rodriguez et al. 2022).

P. brasiliense Verkley (2004) and *Q. minuta* (J.C. Carter) Crous & P.M. Kirk (2017) belong to the kingdom *Fungi* and phylum *Ascomycota*. *P. brasiliense* is classified within

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the class *Dothideomycetes*, order *Pleosporales*, and family *Didymosphaeriaceae*, while *Q. minuta* is within the same class and order but is assigned to the family *Leptosphaeriaceae*. These are two fungal species broadly distributed across different environments and host plants. However, only a few information can be found for both fungal species and their characteristics. *P. brasiliense* has been found as an endophyte in the leaves of *Ginkgo biloba* L., in the needles of various conifers, and reported to cause discolored wood of *P.* × *hispanica*, causing no disease symptoms to those host plants (Damm et al. 2008). This fungus is also reported as a pathogen causing necrotic wood of *P.* x *hispanica* in Switzerland (based on the NCBI database, no published record found) and several fruit trees (Damm et al. 2008).

According to findings by Boldt-Burisch and Douanla-Meli (2023), *Q. minuta* has been found to occur primarily in several oak species, where it causes dieback. It can also cause branch and stem cankers in *P.* × *hispanica*. Additionally, according to data from NCBI, it has been reported on *Acer pseudoplatanus*. These discoveries indicate that *Q. minuta* has a broader host range and distribution than previously recognized, with significant pathogenic potential in various tree species.

Given the prevalence of many unknown fungi and their potential impact on tree health, ecosystem stability, and urban forest management, it is important to better understand the characteristics of opportunistic fungi. The primary aim of this study is to investigate the presence and pathogenic potential of *P. brasiliense* and *Q. minuta* in symptomatic urban and forest trees. In this study, we describe our findings, including the isolation and identification methods used, and confirm pathogenicity through Koch's postulates.

Materials and methods

Sample collection

The inventories were carried out in urban areas in Germany, selected to represent the diverse geographical conditions, where different tree species are common. The monitoring covered several German cities: Frankfurt, Düsseldorf, Darmstadt, Freiburg, Dresden, Nuremberg, and Stuttgart. Several tree species showed signs of similar symptoms of necrotic canker tissue in different parts of these cities, including city parks and streets: *P.* × *hispanica* Münchh. (London plane; Fig. 1a), *F. sylvatica* L. (European beech; Fig. 1b), *C. betulus* L. (hornbeam; Fig. 1c), *A. platanoides* L. (Norway maple; Fig. 1d), *Ulmus* sp. (elm hybrid; Fig. 1e), *A. hippocastanum* L. (European horse chestnut; Fig. 1f), and *Q. robur* L. (pedunculate oak). Visually recognizable, partially bleeding cankers were observed on the stems and freshly pruned or unpruned branches of urban trees (Fig. 1), and samples of the moist tissue were taken for further analysis.

Wood chips from symptomatic bark and phloem tissue were cut using a sterilized hammer and chisel (Fig. 2a) and stored in sterile bags at − 20 °C for further analysis. Without surface sterilization, some of the wood chips were directly placed on MEA (Malt Extract Agar)-Thiabendazole media in the field to suppress excessive mold growth, particularly from fast-growing fungi such as *Mucor* spp. The canker tissue samples were collected with sterile cotton swabs, by swiping the necrotic area below the upper microbial canker surface until soaked or covered (Fig. 2b). The swabs were immersed into 2.0 ml Eppendorf tubes filled with 1 ml 1 × TAE (Tris-acetate-EDTA) buffer. Later the same day, half of the tubes containing the microbial buffer were poured onto two MEA Petri dishes to compare microbial activity before and after storage. The other half was stored in the refrigerator at 4 °C for further processing.

Laboratory analysis

The buffer containing the microorganisms was diluted with sterile water to obtain concentrations of 100% (undiluted), 50%, and 25%, and then poured onto MEA plates. Surface sterilization of the wood chips was performed using two different methods: (1) washing the samples for 1 min with 70% ethanol, followed by 5 min with sodium chloride, then 40 s in ethanol and 15 s in sterile water (2×), and (2) washing the sample with sterile water and applying flame sterilization for 2 s. After sterilization, all samples were cultured on 2% MEA. In cases where fungi and bacteria were inseparable, MEA plates supplemented with antibiotics (Streptomycin, Tetracycline, and Penicillin) were used to inhibit bacterial growth. Additionally, MEA-Thiabendazole medium was used when necessary to suppress mold growth. Pure cultures were obtained by sub-culturing visible mycelium, observed within 3 days to 1 week after plating the buffer and wood chips, onto MEA Petri dishes. Once in pure cultures, the fungi were grouped according to their morphological characteristics.

Morphological identification

In order to classify and differentiate fungal isolates, morphotyping was performed with cultures growing on the same type of media to ensure uniform growth conditions. The fungi were classified on the basis of phenotypic characteristics such as growth rate, mycelial texture, pigmentation, the presence of pycnidia and fruiting bodies as well as the visible secondary metabolites. Their production, including liquid droplets recognizable as clear beads on the surface of the medium, differs between fungi in terms of amount, distribution, and production period, while pigment extraction

Fig. 1 Canker and stem necroses (**a**: *P.* × *hispanica,* **b**: *F. sylvatica,* **c**: *C. betulus,* **d**: *A. platanoides,* **e**: *Ulmus* sp., **f**: *A. hippocastanum)*

serves as a visible marker, with some cultures producing a strong halo and others showing a faint hue diffusing into the agar. Isolates with similar traits are grouped and designated as distinct morphotypes. Their visual appearances were recorded using high-resolution photography to be added to our photograph database. For molecular identification, at least one representative isolate of each morphotype was selected for further analysis using molecular markers from multiple loci.

Molecular identification

DNA extraction was performed based on the protocol by Keriö et al. (2020) with some modifications. Fungal mycelium growing on culture was collected using a scalpel and transferred into 1.5 ml tubes. The heat block was set to 65 °C, and 500 µl of PVP extraction buffer was added to the samples. The tubes were incubated at 65 °C for 15 min with continuous mixing at 800 rpm. After incubation, each sample was vortexed for 20 s and then subjected to two rounds of 2-s pulses using an ultrasonic homogenizer. The samples were then incubated at 65 °C for 15 min without mixing. This is followed by a further 20-s vortex. SDS was added to 2 ml tubes, which were then warmed on the heat block at 65 °C. The samples were centrifuged at 5000 rpm for 10 min, and 250 µl of the supernatant was transferred into fresh 1.5 mL tubes. SDS wash buffer (250 µl) was added, followed by

Fig. 2 Sampling methods **a**: sampling with hammer and chisel; **b**: sampling with cotton swab

vortexing for 20 s, then centrifugation at 13,300 rpm for 15 min. The supernatant (300 µl) was transferred into new tubes, and 255 µl of isopropanol was added. The samples were mixed by inversion 20 times and centrifuged again at 13,300 rpm for 15 min. The supernatant was discarded, and the pellet was washed with 200 µl of cold 70% ethanol. The samples were centrifuged at 13,300 rpm for 10 min, after which the ethanol was removed. The pellets were dried in a heat block at 37 °C (with the lids open) for 15 min and subsequently resuspended in 50 µl of nuclease-free water.

PCR was completed using 7.5 µl of DNA, 2.5 µl of the corresponding primer pairs—ITSu1/ITSu4 and LSU/LR6, and 12.5 µl of FastGene Optima PCR HotStart ReadyMix Master Mix. The primer sequences and their respective annealing temperatures were as follows: ITSu1 (5′-GGA AGK ARA AGT CGT AAC AAG G-3′) and ITSu4 (5′-RGT TTC TTT TCC TCC GCT TA-3′) at 57 °C; LSU (5′-GAC CCG AAA GAT GGT GAA-3′) and LR6 (5′-CGC CAG TTC TGC TTA CC-3′) at 55 °C (Blumenstein and Korkmaz 2025). PCR amplification was tested using gel electrophoresis. Amplified samples were purified using magnetic beads from the HighPrep PCR clean-up protocol. Purified PCR products were sent for sequencing to Microsynth Seqlab in Göttingen, Germany. All sequences were checked against the NCBI database.

Pathogenicity test to fulfill Koch's postulates

An *in planta* infection test was conducted to assess the pathogenicity potential of the isolated fungi. Five healthy tree species, 3 years old and grown in containers under greenhouse conditions, were inoculated with the fungal cultures derived from stem cankers of the corresponding tree species as described above. Three trees of the respective host species were used for each fungal isolate. Additionally, both the *Q. minuta* and the *P. brasiliense* isolates were inoculated onto all the five tree species to evaluate potential cross-host pathogenicity, regardless of whether they were initially isolated from those tree species or not. The fungal cultures used for inoculation were 14 days old and actively growing on MEA. Each tree was inoculated three times at different heights along the stem, with adequate spacing between inoculation sites to avoid overlap of necrotic tissue. Small incisions were made on the stem using a sterilized cork borer (Fig. 3a). Agar plugs (6 mm) (Fig. 3b) containing the fungal mycelium were inserted into the wounds, and the site was sealed with parafilm (Fig. 3c) to maintain moisture and prevent contamination. Sterile gloves were worn throughout the procedure, and cork borers and scalpels were disinfected between inoculations by dipping in 100% ethanol and flaming. One additional tree per species was treated identically for controls but inoculated with sterile MEA plugs instead

Fig. 3 Infection method **a**: using a sterile cork borer to stance out the bark in a standardized shape. **b**: An agar plug with the corresponding fungal species facing toward the phloem is inserted into the whole. **c**: Parafilm is wrapped around the stem to protect the infection area from cross-contamination and the agar plug from drying out

of fungal cultures. All trees were kept in the greenhouse and watered daily. After about 30 days, all trees, including the controls, were harvested. The bark at the inoculation sites was carefully removed to examine phloem tissue for necrotic lesions. To fulfill Koch's postulates, fungi were re-isolated from the margin of the necrotic areas. The tissue pieces were first surface-sterilized as described above before plating on MEA to ensure isolation of the internal pathogen. Morphological comparison between the re-isolates with the original cultures confirmed the identity. Identification of the re-isolated fungi was conducted after 14 days of plating. Disease symptoms in the inoculated trees were recorded and compared to those in the control group, which showed no signs of infection.

Results

Field observations

We characterized the necrotic areas from freshly pruned or unpruned branches as a reddish oozing, which was observed on both lower and higher branches. The tissues of the stem cankers had a slimy texture (Fig. 1a–f) that could be above or below DBH (Diameter at Breast Height—130 cm above ground). Following this study, we observed that the oozing from the corresponding trees can be visible from spring to autumn. Field observations revealed that the spatial distribution of canker development did not show a consistent or predictable pattern. Sometimes, cankers were observed on individual trees spatially isolated from conspecifics or closely related species.

Conversely, other cases involved multiple trees affected within a localized area, such as a valley, where the disease spread irregularly, impacting either a single tree or several nearby individuals. Cankers were typically confined to one side of the tree; however, in some instances, they were dispersed across multiple locations on the same tree, manifesting at various points along the stem and branches, indicating a nonuniform progression of the infection.

Laboratory findings

Sequence comparisons with the NCBI database confirmed the identification of the fungal isolates. Two primer pairs were used to confirm the taxonomy of the fungal isolates. In addition to molecular methods, the two fungi *P. brasiliense* and *Q. minuta* were morphologically matched to previously isolated species in our laboratory, further supporting the identification (Table 1). The fungal isolates were obtained from six host tree species (Table 1). *P. brasiliense* was identified on *C. betulus, F. sylvatica*, *P.* × *hispanica*, *A. hippocastanum*, and *Ulmus* species, and *Q. minuta* was found on *P.* × *hispanica*, *A. platanoides*, *F. sylvatica*, *A. hippocastanum*. *Q. robur* and *C. betulus*.

Morphologically, the isolates showed distinctive features based on mycelial growth and colony appearance, documented through photographs taken before DNA extraction (Fig. 4). All cultures were grown on MEA media. The re-isolated cultures were also plated on MEA to maintain consistency; as different media types can influence morphological traits.

- *Q. minuta* forms medium brown colonies (Fig. 4a) characterized by the development of dense pycnidial structures (Fig. 4c), which first appear around the central agar plug and gradually expand outward. Pycnidia typically become visible around the ninth day, containing conidial masses within. Their growth often follows a circular pattern (Fig. 4a), emerging in intervals from the center toward the colony edge. These patterns are also evident on the underside of the Petri dish (Fig. 4b),

Table 1 Specifications for the fungal species and their identification based on two primers (with the corresponding Accession Numbers at NCBI) or based on morphology

FUNGAL SPECIES	Host species	City of origin	ITSu1/u4	LSU/LR6	Morphology
P. brasiliense	*Platanus* x *hispanica*	Frankfurt	PQ468734	PQ468773	
P. brasiliense	*Fagus sylvatica*	Düsseldorf			+
P. brasiliense	*Aesculus hippocastanum*	Frankfurt			+
P. brasiliense	*Carpinus betulus*	Düsseldorf			+
P. brasiliense	*Ulmus* sp.	Darmstadt	PQ468729	PQ468767	
P. brasiliense	*Platanus* x *hispanica*	Freiburg	PQ468727	PQ468765	
Q. minuta	*Platanus* x *hispanica*	Frankfurt	PQ468733	PQ468771	
Q. minuta	*Platanus* x *hispanica*	Freiburg	PQ468737	PQ468777	
Q. minuta	*Platanus* x *hispanica*	Dresden	PQ468730	PQ468768	
Q. minuta	*Acer platanoides*	Düsseldorf			+
Q. minuta	*Fagus sylvatica*	Düsseldorf			+
Q. minuta	*Aesculus hippocastanum*	Frankfurt	PQ468732	PQ468770	
Q. minuta	*Carpinus betulus*	Düsseldorf		PQ468772	
Q. minuta	*Acer platanoides*	Nuremberg			+
Q. minuta	*Fagus sylvatica*	Stuttgart			+
Q. minuta	*Carpinus betulus*	Nuremberg			+
Q. minuta	*Quercus robur*	Nuremberg			+

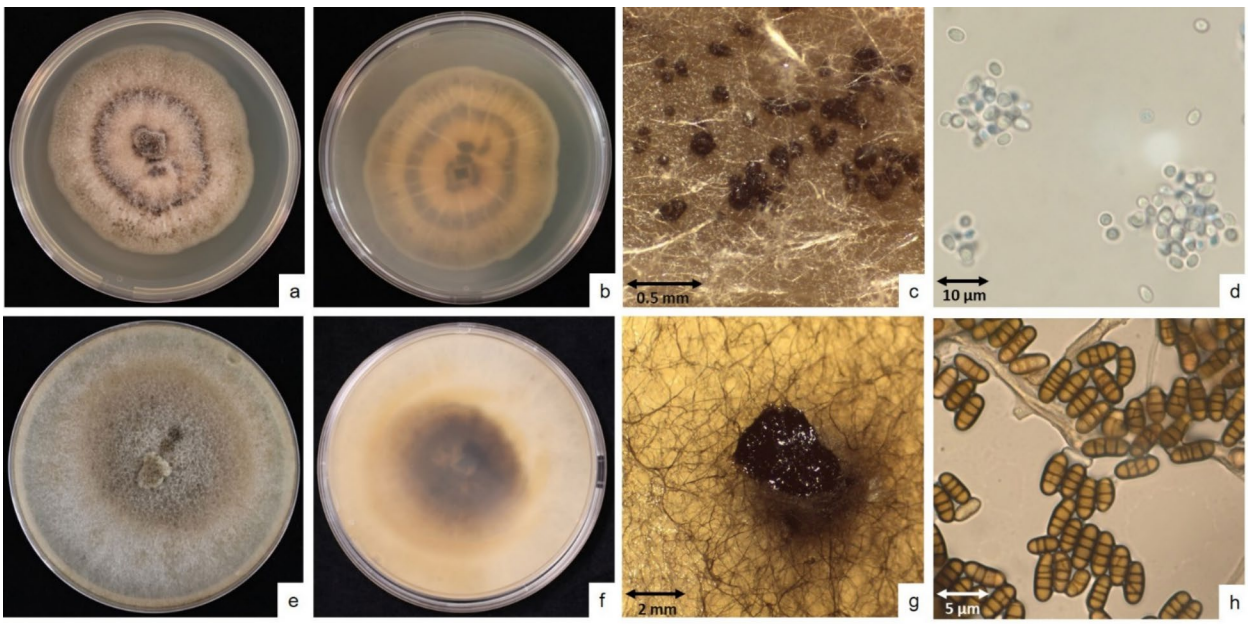

Fig. 4 Morphological features of *Q. minuta* and *P. brasiliense*. **a–d** *Q. minuta*: **a** colony on MEA, upper side; **b** colony on MEA, underside; **c** pycnidia on host tissue; **d** conidia under light microscopy. **e–h.** *P. brasiliense*: **e** colony on MEA, upper side; **f** colony on MEA, underside; **g** pycnidia on host tissue; **h** conidia under light microscopy

where the central agar plug is surrounded by darker brown pigmentation, which in turn is encircled by light brown mycelium. This circular pattern continues outward toward the colony edges. The colony surface displays a layered structure: light brown mycelium extends from the center to the margins, overlaid by fluffy, whitish to grayish mycelium that gives a pyramidal appearance radiating outward. Toward the edges, the mycelium transitions into a creamy color. Based on our observations, growth may slow or appear to halt around the 18th day, full colony development generally occurs between 21 to 24 days under room temperature conditions, without the need for specific lighting. The conidia shape ranges from circular to slightly elliptical with a diameter of 3 µm and a white pigmentation (Fig. 4d).

- *P. brasiliense* initially forms a dense, whitish mycelial mat across the surface of the plate. Gray aerial mycelium spreads outward from the center, gradually blending into a surrounding brownish hue (Fig. 4e). As the colony matures, the central region becomes dark brown to blackish, partially masked by overlying white mycelium. A distinctive feature of this fungus on this medium is the development of black mycelium along the colony edge. The remaining area of the plate is covered with creamy white mycelium. On the underside of the Petri dish, pigmentation ranges from light brown to dark brown, particularly concentrated at the center (Fig. 4f). Full colony growth is typically achieved within 12 to 14 days at room temperature, without specific light conditions. Pycnidial structures (Fig. 4g) begin to form after full colony growth, generally becoming visible around 20 days later, containing brownish, septated conidial spores of 3–5 µm length and 1.5 µm width (Fig. 4h).

Pathogenicity test

The results of the pathogenicity test showed that all five tree species exhibited varying degrees of susceptibility to the fungal isolates. Necrotic areas were observed on the phloem tissues of the inoculated stems, although the severity of symptoms differed among tree species (Fig. 5). The re-isolation of fungi from necrotic areas confirmed the presence of the initially inoculated fungal species, fulfilling Koch's postulates and thereby verifying their pathogenic potential. *P. brasiliense* showed an overall success rate of 90%, with re-isolation rates of 83% in horse chestnut, 100% in plane, carpinus, and beech trees, but only 33% in elm. *Q. minuta* had an overall success rate of 86%, with 100% re-isolation from maple, plane, carpinus, beech, and elm trees, but only 16% in horse chestnut. These results indicate that most inoculations were successful, although susceptibility varied among host tree species. This variation highlights both the broad pathogenic potential and the potential host specificity of the isolates tested.

In contrast, the control groups, which were treated with only agar plugs, showed no signs of necrosis or disease symptoms, further confirming the pathogenicity of the fungal isolates. The cross-species inoculations revealed that several fungal cultures could infect multiple tree species, indicating a potential risk for a broader host range and future disease outbreaks in diverse tree species. While these fungi were originally isolated from different urban trees, extended inoculation experiments helped to test other tree species to explore their pathogenic potential. For instance, *Q. minuta* inoculation on elm trees showed pathogenic activity. This finding further emphasizes the adaptability of these fungi and the need for vigilance regarding their potential impact on different tree species.

Discussion

This study presents the first confirmed records of *P. brasiliense* and *Q. minuta* as tree pathogens of different urban trees. *P. brasiliense* is recorded for the first time on Elm hybrids, London plane, European common horse chestnut, European beech, and European hornbeam. *Q. minuta* is

Fig. 5 Necrotic symptoms on tree stems 30 days after fungal inoculation. **a**: *Carpinus betulus* showing localized necrosis around the infection site. **b**: *Acer platanoides* with extensive necrotic tissue under the bark. **c, d**: *Aesculus hippocastanum* with stem cankers, **c**: surface lesion; **d**: sunken canker with a darkened core

found for the first time on European common horse chestnut, European beech, European hornbeam, and Norway maple, according to our literature research and the CABI (Centre for Agriculture and Biosciences International) and EPPO (European and Mediterranean Plant Protection Organization) databases.

During our nationwide study in Germany focusing on stem necroses in urban areas, we identified *P. brasiliense* and *Q. minuta* in different cities, emphasizing their widespread occurrence in association with other fungal pathogens. This finding is of particular concern, as it indicates that these pathogens are expanding their host range while co-occurring with other pathogens, potentially complicating disease dynamics in affected trees.

The identification process confirms the presence of these fungi on these host trees for the first time in Central Europe. It supports previous studies highlighting their ability to infect a broader variety of species, expanding our understanding of their ecological range. Previously, *P. brasiliense* and *Q. minuta* had been primarily documented on specific hosts like oaks and conifers or fruit trees (Damm et al. 2008). Discovering these fungi on deciduous urban trees suggests a broader host range than described so far, indicating their adaptability and potential to affect a wider variety of tree species.

While this report focuses on identifying *P. brasiliense* and *Q. minuta* on these new hosts, the inoculation experiments confirmed Koch's postulates, demonstrating that these fungi are pathogenic on multiple tree species. The high success rate of these inoculations, particularly across a wide range of hosts, is alarming and suggests that these pathogens could cause a significant risk to urban trees in the future.

Beyond the sampling areas in this project, similar bleeding or slimy canker tissues have additionally been observed and reported on the same tree species in Hamburg, Berlin, and Göttingen. This suggests that *P. brasiliense* and *Q. minuta* on urban trees might be widespread across Germany and countries with similar climate conditions and tree species composition. Such widespread distribution highlights the importance of monitoring and managing this pathogen to protect urban tree health and maintain the ecological and recreational benefits that urban trees provide.

Conclusion

Understanding the dynamics of these pathogens is crucial for enhancing our knowledge of tree-pathogen interactions and informing urban tree management strategies aimed at mitigating potential disease outbreaks and broader ecological consequences. This finding underscores the importance of ongoing surveillance of fungal biodiversity and its implications for tree health. Urban trees are vital components of city environments, offering ecological, aesthetic, and recreational benefits. Introducing new pathogens, such as *P. brasiliense* and *Q. minuta*, raises significant concerns regarding the health and sustainability of these urban forests. Therefore, detailed monitoring and proactive management practices are essential to address the risks posed by these pathogens and to preserve the myriad benefits provided by urban trees.

Acknowledgements We are especially grateful to Sabine Diener for her great support in the laboratory. We also like to express our gratitude to the Eva-Mayr-Stihl Foundation for financing our project and supporting the field of tree pathology. We also like to thank the Faculty of Environment and Natural Resources of the University of Freiburg.

Funding Open Access funding enabled and organized by Projekt DEAL. The Eva-Mayr-Stihl Foundation and the Faculty of Environment and Natural Resources of the University of Freiburg supported this study.

Declarations

Competing interests The authors declare that there are no conflicts of interest.

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