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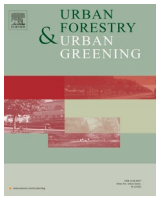

Original article

Phytophthora nicotianae and *Ph. mediterranea*: A biosecurity threat to *Platanus orientalis* and *P.* x *acerifolia* in urban green areas in Greece

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ABSTRACT

Field surveys conducted in two urban green spaces in Athens (Greece) revealed the presence of *Phytophthora*-related diseases on *Platanus orientalis* and *P.* x *acerifolia* trees. Declining trees showed a range of disease symptoms including root rot, stem bleeding cankers and extensive canopy dieback. Since there is little information about the etiology of these diseases, a study was conducted from 2016 to 2021 to define the main pathogens involved. A total of 71 *Phytophthora* isolates were obtained from bark tissues and rhizosphere samples collected from symptomatic plane trees. Based on morphological traits and DNA sequence data, *Phytophthora* isolates were identified as *Ph. mediterranea* (11 isolates) and *Ph. nicotianae* (60). Over a five-year period 41% of *P. orientalis* trees associated with *Phytophthora* infections died and were removed from the park. Pathogenicity tests confirmed that all *Phytophthora* species including *Ph. cinnamomi* used for comparison, are pathogenic on both *P. orientalis* and *P.* x *acerifolia* trees. *Phytophthora cinnamomi* was shown to be the most aggressive species on both *P. orientalis* and *P.* x *acerifolia*. *Phytophthora nicotianae* was the dominant species, whereas *Ph. mediterranea* was associated with a few trees. Our finding has contributed to expanding knowledge on the host and geographic range of *Ph. mediterranea*, an invasive pathogen with a high potential for diffusion in Mediterranean regions and highlights the importance of enhancing biosecurity measures to prevent and limit the spread of invasive pathogens in urban and natural ecosystems.

1. Introduction

Urban green spaces have represented an important component of the landscape for centuries, and are claimed to provide a number of environmental and social services. These include pollution absorption, atmospheric cooling, reduction of building energy-use, habitat provision and enhancement of human mental and physical health and well-being (Van Leeuwen et al., 2010; Roy et al., 2012; Dadvand et al., 2015; Sanusi and Livesley, 2020). All these benefits for cities, and the constant population migration from rural areas to cities, underline the importance of increasing urban green spaces such as parks, gardens and street trees in the future (Ramaiah and Avtar, 2019). However, there are several problems related to tree planting in urban areas, such as the selection of tree species, the adaptability of the species to the specific soil conditions, the functions of the tree and costs of establishment and management (Oldfield et al., 2013). Space assigned to urban trees is often limited and human activities have a negative impact on the growth and health status of the trees (Referowska-Chodak, 2019).

Stressful urban growth conditions, together with climate change, can enhance plant susceptibility to pests and pathogens often accidentally introduced through nursery material used for planting (Weed et al., 2013; Dale and Frank, 2017). In particular, during the last decades several studies have pointed out the introduction of *Phytophthora* spp. through infected nursery stock in urban green areas (Mascheretti et al., 2008; Burgess et al., 2017; Simamora et al., 2018; Pintos et al., 2023; Schiffer-Forsyth et al., 2023).

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The genus *Phytophthora* is a large group of fungus-like organisms, which includes important plant pathogens responsible for severe diseases on herbaceous and woody plants in agriculture, horticulture, natural ecosystems and urban forests (Moralejo et al., 2009; Lamour, 2013; Migliorini et al., 2019; Antonelli et al., 2022; Linaldeddu et al., 2023; Schiffer-Forsyth et al., 2023). To date, there are over 220 formally described *Phytophthora* species, many of which have been discovered in urban forests or nurseries during the last three decades (Abad et al., 2023; Bregant et al., 2023; Coomber et al., 2023).

In the event of *Phytophthora* species being introduced in urban areas, the risk of an outbreak is related to specific traits of the species, including polyphagia, breeding system, survival strategies and ability of developing in a very wide temperature and pH range (Marcot et al., 2023). Some species of *Phytophthora* such as *Ph. cinnamomi* and *Ph. nicotianae* are cosmopolitan and polyphagous, while others, e.g., *Ph. ilicis*, *Ph. lateralis*, and *Ph. quercina* are associated with few hosts (Jung et al., 1999; Vettraino et al., 2005; Brasier et al., 2010; Bregant et al., 2023).

In Greece, the majority of studies on *Phytophthora* related-diseases have focused on agriculture and forest environments, whereas very few have investigated the impact of *Phytophthora* on urban environments (Dale et al., 2017; Hulbert et al., 2017; Markakis et al., 2017; Thomidis, 2003). Since 2016, a severe decline affecting oriental plane trees (*Platanus orientalis*) and the hybrid London plane (*Platanus* x *acerifolia*) has been observed in public and private urban green areas in Athens (Greece). Subsequent field surveys revealed a range of disease symptoms indicative of *Phytophthora* infections including root rot, stem bleeding cankers and extensive branch dieback. Given the diversity of ecosystem services provided by urban trees and the potential losses caused by *Phytophthora* diseases, a study was conducted to establish the etiology of the decline affecting oriental plane and London plane trees in two urban green areas in Athens and to determine the virulence of the major species involved.

2. Materials and methods

2.1. Field survey and sampling

2.1.1. Sampling and isolation

Field surveys were conducted in a public park in Athens, where about 350 oriental plane trees (7–9 m tall) were planted in 2015, and in a private garden in Spata (suburb of Athens), where eight trees of London plane (4–5 m tall) were planted in 2014. In the park, field surveys were conducted in 2016, 2017 and 2021, while in the private garden in 2018 (Table 1). From both localities, samples were taken from the rhizosphere of symptomatic or recently dead trees (Table 1). Soil samples (500–700 g) containing also thin rootlets, were collected from 15 to 20 cm depth, and from deeper parts (50–60 cm) from dead trees during their removal. A portion of 100–120 gr of soil, randomly collected in the sampled areas, were flooded with 500 ml of distilled water in plastic containers; 10–12 healthy freshly cut young leaves of carob (*Ceratonia siliqua*) were placed on the water surface of each container as bait. After 4–7 days, leaves that were becoming blackish or showing dark spots were examined under a light microscope for the presence of sporangia. Leaves were blotted on filter paper, surface sterilized with 70% ethanol, cut into small pieces (5 mm^2) and placed on Petri plates containing PARPH-V8 selective medium for *Phytophthora* (Ferguson and Jeffers, 1999). Plates were incubated in the dark at 20 °C.

Necrotic inner bark samples, including a small part of sapwood, were taken from the edges of cankers on the main stem of living trees, using a surface-disinfected chisel (Table 1). These were placed directly on deionized water and transported to the lab on the same day. The water was replaced 3–4 times per day for a period of 2–3 days in order to remove excess polyphenols (Jung, 2009). The bark samples were blotted on filter paper, and treated as reported above for leaf samples. In addition, bark samples were also baited with carob leaves, the same way as with the soil samples. Petri dishes were examined daily under a dissection microscope and after 3–4 days single-hypha isolates with *Phytophthora* morphology were transferred onto V8 agar plates without amendments.

2.2. Identification of Phytophthora isolates

2.2.1. Morphology

The isolates obtained in pure culture were initially grouped in morphotypes based on the colony appearance after 7 days on V8 agar, potato dextrose agar (PDA) and carrot agar (CA) at 20 °C in the dark. For sporangia production, pieces of V8 agar (5 mm^2) with active *Phytophthora* mycelium were cut from 5-day-old cultures, flooded with 1.5% non-sterile soil extract and incubated at room temperature (23–25 °C) under daylight conditions for 1–2 days. Measurements of the main morphological structures (oospores, chlamydospores, hyphal swellings) were taken at 400× magnification and recorded using the software CellSens paired with an Olympus SC30 camera connected to a Zeiss Axiolab light microscope. Temperature-growth relationship was evaluated on CA and PDA plates incubated at 15, 25, 30, 32, 35 and 37 °C (±0.5 °C) in the dark for 96 h, for the isolate Z13, chosen as representative isolate of *Ph. mediterranea*-like' isolates. *Phytophthora cinnamomi* (Qi1) from Institute of Mediterranean Forest Ecosystems (IMFE) fungal collection was also included in the growth bioassay for comparison. The test was performed in triplicate. *Phytophthora* isolates obtained in this study were stored on PDA and CA in the culture collection of the Institute of IMFE, Athens, Greece.

2.2.2. DNA extraction, PCR amplification and sequencing

Three isolates (Z7, Z13 and Z14, obtained from *P. orientalis* bark tissues located at the park in Athens), were chosen as representative of the morphotypes obtained. They were incubated in V8 broth for a week, at 20 °C in the dark. Mycelia were harvested from the liquid culture by filtering through a miracloth and subsequently lyophilized overnight in a freeze dryer. DNA was extracted using a modified protocol described by Oñate-Sánchez and Vicente-Carbajosa (2008). The mycelium pellet was resuspended in TE buffer (Tris-HCl and EDTA, pH=8) supplemented with 10 μg/ml of RNAse A, instead of DEPC-treated H_2O and DNAse. PCR reactions for each isolate were performed using the primer sets ITS5/ITS4 (White et al., 1990) and OomCoxI-Levup/OomCoxI-Levlo (Robideau et al., 2011), targeting the rRNA internal transcribed spacer (ITS) locus containing the 5.8 S region and the cytochrome c oxidase subunit 1 (*cox*1), respectively. The final reaction volume was 50 μl, containing 1X KAPA Taq Buffer A (Code KK1014, Kapa Biosystems), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primer and 0.5 U of KAPA Taq DNA Polymerase. The PCR conditions for both primer sets consisted of an initial denaturation step at 95 °C for 3 min, and 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec and chain

Table 1
Details of the monitored sites and number of samples collected.

Locality	Tree species	Dates investigated	Number of trees sampled	Number of soil samples	Number of bark tissue samples
Athens, park	*Platanus orientalis*	October 2016	4	2	3
	P. orientalis	August 2017	14	12	4
	P. orientalis	October 2021	13	13	-
Spata, Private garden	*P.* x *acerifolia*	October 2018	4	3	2

elongation at 72 °C for 1 min. When all cycles were completed, the reaction was ended after a 7-min final elongation step. PCR products were purified, and subjected to sequencing of both strands. Sequences of the two strands were aligned using the MAFFT algorithm (Katoh, 2002), edited using the Benchling web-based software (https://www.benchling.com/) and the consensus sequences were compared to those of the GenBank database using the BLASTn analysis. The sequences obtained in this study (ITS and *cox*1) were deposited in the GenBank database under the Accession Numbers shown in Table 2.

2.2.3. Phylogenetic analysis

The phylogenetic analysis was performed in MEGA-X software (Kumar et al., 2018), for both single and concatenated loci sequences. Initially, the ITS, the *cox*1 and the concatenated sequences were aligned separately using the ClustalW multiple sequence alignment algorithm (Thompson et al., 1994) and the optimum substitution model for each case was examined. For the ITS locus, the Hasegawa-Kishino-Yano with Gamma Distribution (HKY+G) was used (Hasegawa et al., 1985), while the Hasegawa-Kishino-Yano with Invariant Sites (HKY+I) and the Hasegawa-Kishino-Yano model (HKY) for the respective concatenated sequences and *cox*1 locus, was applied. Phylogenetic trees were constructed using the Maximum-Likelihood statistical method with 1000 bootstrap replications. Table 2 reports the information regarding isolates used in this study.

2.3. Pathogenicity test

2.3.1. Phytophthora isolates

Three *Phytophthora* species were used for this trial: *Ph. cinnamomi* (isolates: Ph28 and CB225), *Ph. nicotianae* (isolates: PGr1 and PGr2) and *Ph. mediterranea* (isolate: CB84). The isolates were obtained from the culture collection of Professor Anna Maria Vettraino (isolates Ph28, PGr1, PGr2 - Laboratory of Plant Protection-DIBAF, University of Tuscia, Viterbo, Italy) and Professor Benedetto Linaldeddu (isolates CB225 and CB84 - Laboratory of Plant Pathology - Department of Land, Environment, Agriculture and Forestry-TESAF, University of Padova, Italy). Isolates were inoculated on plane tree stems to renew their virulence and reisolated on PARPH-V8 for use in later experiments (Erwin and Ribeiro, 1996).

2.3.2. Plant material

In June 2022, 48 seedlings (two years old) of *P. orientalis*, and 48 seedlings (two years old) of *P.* x *acerifolia*, were purchased from a commercial nursery located in central Italy. Seedlings were maintained in the DIBAF greenhouse and regularly watered before starting the pathogenicity tests. After 1 month, they were transplanted in 2 L plastic pots containing a mixture of soil/perlite (3:1, vol/vol). All plants were grown under greenhouse conditions at a temperature range of 24 °C ± 2 °C and regularly watered.

2.3.3. Pathogenicity test

Phytophthora inoculum was prepared according to Vettraino et al. (2001). Sterilized millet seeds moistened with V8 broth (200 ml V8 juice, 3 g $CaCO_3$, 800 ml distilled H_2O) were placed into 500 ml Erlenmeyer flasks and autoclaved twice at 121 °C for 20 min over two consecutive days, and then inoculated. Inoculum consisted of 10 agar discs (5 mm^2) of the specific *Phytophthora* isolates, obtained from the edge of actively growing colonies. Flasks were incubated for 2–3 weeks at 25 °C (*Ph. cinnamomi* and *Ph. nicotianae*) and 30 °C (*Ph. mediterranea*). The final inoculum for each *Phytophthora* species consisted of an equal amount of millet seeds inoculated with two isolates (20 ml/*Phytophthora* species). The inoculated soil was prepared by adding infected millet seeds to the potting mix at the rate of 2% (vol/vol) when plants were transplanted into new pots. The day after the inoculation, pots were flooded for 24 h to promote *Phytophthora* sporulation and zoospores release. The flooding was repeated every month for 48 h. A total of 12 seedlings/treatment were used. Control seedlings (12) were inoculated with sterile millet seeds. Pots were arranged in a randomized complete block design on benches in the glasshouse with twelve replicates per treatment and control (not inoculated seedlings). The experiment lasted five months and the presence of *Phytophthora* on inoculated plants was confirmed by plating 8 small root portions per seedling from each pot on PARPH-V8. At the end of the experiment, seedlings were harvested. The aerial parts were separated from the roots. Whole root systems were washed under tap water, blotted onto paper towels to dry and sorted into: mother roots (diam. >5 mm), small woody roots (diam. 1–5 mm), fine roots (diam. 0–1 mm). Aerial parts and roots were oven-dried at 65 °C until weights were constant.

In order to evaluate the effect of treatments, for each individual seedling, the length of the stems and the dry weight of the aerial (stem and leaves) and roots portions, were measured. Whole root systems were visually rated (root damage) for root rot on a scale 0–3 (0=healthy root system, no root dieback; 1=10–49% fine root losses, reduced root dieback; 2=50–75% fine root losses, moderate root dieback; 3=>75% fine root losses, advanced root dieback) (Fig. 1). In addition, the number of dead seedlings was recorded.

2.4. Data analysis

Statistical analysis of data was performed using the Graph Pad Prism software (version 8.0.1 for Windows, GraphPad Software, California, USA). Data were first checked for normality by the Shapiro-Wilk test and then subjected to analysis of variance (ANOVA) and compared using Tukey's Test at a significance level of 5%, assuming $p<0.05$ as a significant value.

3. Results

3.1. Field survey

In the public park in Athens, a gradual decline and mortality of *P. orientalis* trees was observed. In 2016, a single tree had died and some showed symptoms of infection; the following year, a rapid spread was detected with another 18 dead trees and 38 with symptoms of infection.

Table 2
GenBank accession number of the *Phytophthora* isolates obtained in this study and included in the Maximum Likelihood (ml) analyses.

Species	Code	GenBank Accession Number	
		ITS	*cox*1
Phytophthora nicotianae	*Z7*	*OR731362*	*OR744861*
Ph. mediterranea	*Z13*	*OR731369*	*OR744862*
Ph. mediterranea	*Z14*	*OR731363*	*OR744863*

Fig. 1. Rating scale of disease symptoms on plane roots inoculated with *Phytophthora* spp. Pictures of root seedlings representing the following disease ratings: 0=healthy root system, no root dieback; 1=10–49% fine root losses, reduced root dieback; 2=50–75% fine root losses, moderate root dieback; 3>75% fine root losses, advanced root dieback).

In a five-year period (2016–2021) a total of 144 out of about 350 (41%) *P. orientalis* trees died and were removed, while many others showed symptoms of decline. During the removal of dead trees, roots showed extensive root rot. The main roots were dark brown with soft dead bark and the fine feeder roots were limited in numbers, also discolored and brittle. On the main stem of infected living trees, bleeding cankers were extending from the collar up to a height of 1.8 m. The bark was dark in color and watery in appearance; wood discoloration was also under the bark, but very limited in width, extending only a few mm (Fig. 2). In some of the living affected trees, symptoms of branch dieback and yellow sparse foliage were observed. However, in several trees there were no symptoms on the crown, although extensive cankers were present on the main stem. Similar *Phytophthora*-related symptoms were also observed in almost all (8) trees of *P.* x *acerifolia* in the private garden in the Spata locality (Greece). These trees were smaller in size in comparison to those in the park and all of them showed symptoms of sparse foliage and branch dieback. Extensive cankers were also observed, but none of the trees were completely dead.

3.1.1. *Phytophthora* isolations

Phytophthora spp. were recovered from 76% of *P. orientalis* samples (21 rhizosphere and 5 bark positive samples). Interestingly, over 7 tissue samples collected, 3 (42.85%) resulted *Phytophthora* positive when directly plated on PARPH-V8, yielding up to 5 positive samples when they were baited with carob leaves (71,42%). All *P.* x *acerifolia* samples were *Phytophthora* positive. Most of the isolates morphologically resembled previously known *Phytophthora* species. A total of 60 *Ph. nicotianae*-resembling and 11 *Ph. mediterranea*-resembling cultures were obtained (Table 3). *Ph. mediterranea*-resembling cultures were isolated only from *P. orientalis* trees in Athens.

3.1.2. Morphological identification of isolates

Phytophthora nicotianae-resembling cultures formed petaloid colonies on PDA. Spherical oospores (23–30 µm in diameter) with characteristic thick walls and amphigynous antheridia were observed in a few single cultures (3–4 weeks old) (Fig. 3a). Sporangia were broadly ovoid in shape with well-developed papilla, 40–55 ×25–35 µm and length to width ratio 1.2–1.4 (Fig. 3b). Chlamydospores were terminal, spherical in shape 30–40 µm in diameter. *Phytophthora mediterranea*-resembling isolates formed a coralloid mycelium with abundant hyphal swellings (irregular and catenulate) very similar to the mycelium of *Ph. cinnamomi* (Fig. 3c). Sporangia were in different shapes; non-papillate, persistent and ovoid 30 ×21µm (17–47 ×12–29 µm), sometimes elongated (Fig. 3d). Sporangial proliferation was mostly external and very rarely internal.

Phytophthora mediterranea Z13 differed from *Ph. cinnamomi* by higher maximum and optimum temperature values for growth (Fig. 4).

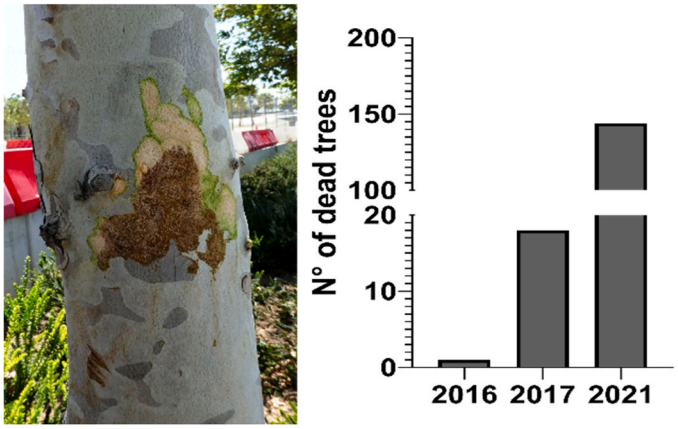

Fig. 2. Inner bark necrosis detected on *P. orientalis* in a public park in Athens (Greece) (left) and number of dead *P. orientalis* trees detected during surveys 2016–2021 (right).

Table 3
Number of isolates of *Phytophthora* species obtained from stem and rhizosphere of *Platanus orientalis* tree and *P.* x *acerifolia* samples in the investigated sites.

Species	*Platanus orientalis*		*P.* x *acerifolia*	
	Stem	Rhizosphere	Stem	Rhizosphere
Phytophthora nicotianae	11	38	3	8
Ph. mediterranea	-	11	-	-

3.1.3. Molecular identification of isolates

The ITS and *cox*1 sequence analysis confirmed the morphological identification of all *Phytophthora* species. The phylogenetic trees obtained from the ITS (Fig. 5a), *cox*1 (Fig. 5b) and concatenated sequences of the two genetic loci produced similar topologies (Fig. 5c). The phylogenetic analysis revealed that isolate Z7 belongs to the species *Ph. nicotianae*, while Z13 and Z14 are grouped into the species *Ph. mediterranea* (Fig. 5). A 26-nucleotide difference was observed between the *cox*1 locus sequences of Z13 and Z14 isolates (this study) and *Ph. cinnamomi* sequences (CBS41196) retrieved from the GenBank database. No nucleotide differences were detected between *cox*1 sequences of the ex-type culture of *Ph. mediterranea* (CB84) and the strains Z13 and Z14, indicating that the colonies belong to the recently described species *Ph. mediterranea*.

3.2. Greenhouse trial

3.2.1. Ascending portion growth

At the end of the experimental period, the control plants appeared the healthiest, whilst *Ph. nicotianae* and *Ph. mediterranea*-inoculated seedlings were either visually less vigorous compared with controls or were dead (*Ph. cinnamomi-P. orientalis* = 42% of dead trees; *Ph. cinnamomi-P.* x *acerifolia* = 25% of dead trees). No significant differences were found between treatments in terms of height, both for *P. orientalis* and *P.* x *acerifolia* seedlings (Fig. 6a) (ANOVA, $p>0.05$). The biomass of the ascending portion (stem and leaves) of *P. orientalis* was significantly reduced by *Ph. mediterranea* when compared to control plants and other *Phytophthora* treatments (Fig. 6b) (ANOVA, $p<0.05$), confirmed by the absence of leaves in 50% of the seedlings.

3.2.2. Root damage

All *Phytophthora* species were re-isolated from the roots of inoculated trees, fulfilling Koch's postulates, but not from control trees. Pathogen identity was confirmed by morphological and molecular analysis. At the end of the trial, root systems of control plants were generally healthy and well developed, with a root damage class of 0.25 ± 0.13 and 0.33± 0.14 for *P. orientalis* and *P.* x *acerifolia*, respectively (Fig. 7a). *Phytophthora cinnamomi* treatments had a much more negative effect compared to other treatments, although when the damage was visually assessed there were not significant differences between pathogens, with the exception of the combination *P.* x *acerifolia-Ph. mediterranea*. ANOVA results confirmed that *Ph. cinnamomi* treatments had a significant effect on root dry weight with values higher than *Ph. nicotianae* and *Ph. mediterranea* treatments. It is worth noticing that the tree species did not differ in their susceptibility to *Phytophthora* spp. (root dry weight - Fig. 7b).

All pathogens caused significant small woody and fine root losses compared to controls (ANOVA, $p<0.05$), although *Ph. cinnamomi* significantly reduced mother roots compared to *Ph. nicotianae* and *Ph. mediterranea*, both on *P. orientalis* and *P.* x *acerifolia* seedlings, and caused small woody roots losses on *P.* x *acerifolia* plants (Fig. 8) (ANOVA, $p<0.05$).

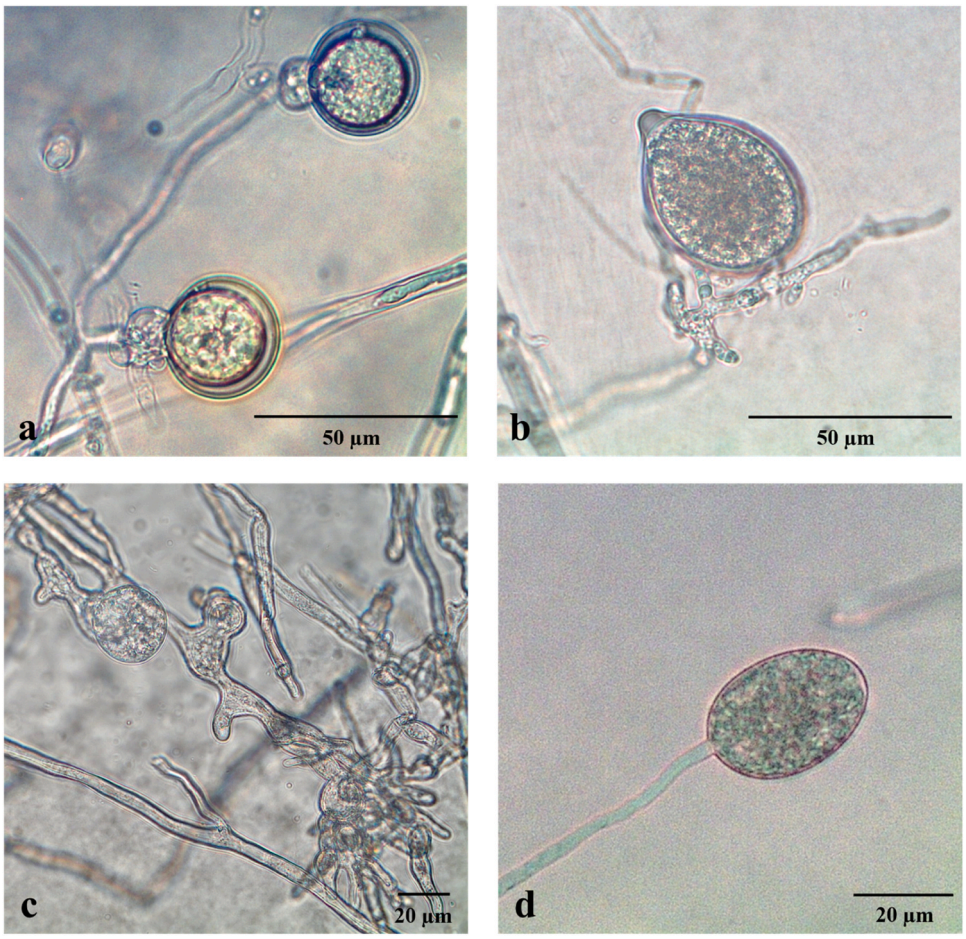

Fig. 3. Oogonia (a) and sporangium (b) of *Phytophthora nicotianae* (Z7) and hyphal swellings (c) and sporangium (d) of *Ph. mediterranea* (Z13).

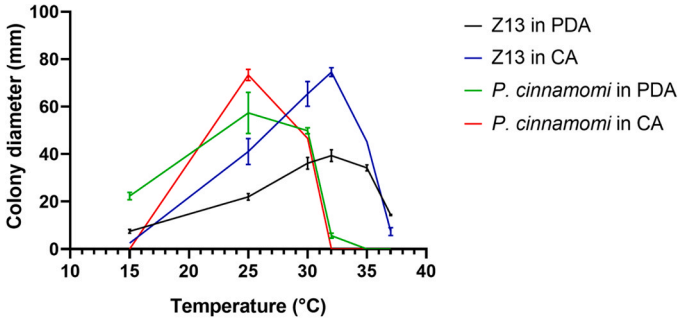

Fig. 4. Mean colony diameter (± SD) of *Phytophthora mediterranea* (Z13) and *Ph. cinnamomi* (Qi1) after 96 h on PDA and CA at different temperatures.

4. Discussion

To our knowledge, this is the first detailed study reporting the extended damage of *Phytophthora* spp. associated with plane decline in public parks in Greece and evaluating the susceptibility of both *Platanus orientalis* and *P.* x *acerifolia* to *Phytophthora cinnamomi*, *Ph. nicotianae* and *Ph. mediterranea*.

Phytophthora cinnamomi has already been reported as a causal agent of plane diseases. In previous investigations in the USA, a not well described *Phytophthora* species, probably *Ph. cambivora* or *Ph. cinnamomi*, was reported in *P. orientalis* seedlings affected by wedge-shaped cankers (Crandall, 1936). During the 20th century, *Ph. cinnamomi* was isolated from plane trees showing root rot (Frezzi, 1950, 1977) or resinous canker (Erwin and Ribeiro, 1996). Stem cankers and black-stained necrosis on roots by *Ph. cinnamomi* were observed on *P.* x *acerifolia* trees in an Italian nursery (Pilotti et al., 2014). Apparently, a callus formation stopped the disease development along the stem. These symptoms have never been reported by other studies. A few years later, in 2016, *Ph. cinnamomi* was isolated from soil samples and roots of symptomatic *P.* x *acerifolia* trees in the city of Curitiba, in Brazil (Santos and Santos, 2019). Symptoms consisting of bark alteration and necrosis of the inner bark were also recorded from *P. orientalis* plants threatened by *Ph. cinnamomi* in Australia, both in urban areas and nurseries (Greenhalgh and Challen, 1979). Nevertheless, other *Phytophthora* species can affect plane tree plants. *Phytophthora tentaculata* and *Ph. cactorum* cause significant cankers on stems of California sycamore (*P. racemosa*) (Sims and Garbelotto, 2018), while *Ph. bilorbang* has been isolated from *P. orientalis* plants in natural areas in Sicily (Riolo et al., 2020). Our investigations showed that both *P. orientalis* and *P.* x *acerifolia* are susceptible to the *Phytophthora* spp. tested, with *Ph. cinnamomi* isolates producing higher mortality rates and root losses. In addition, *Ph. nicotianae* and *Ph. mediterranea* differ in pathogenicity with respect to the controls, leading us to consider them as potential additional elements in the decline of *Platanus* spp. Those species have already been reported as causal agents of several broadleaves' diseases (Belisario et al., 2003; Lucero et al., 2006; Barber et al., 2013; Bregant et al., 2021; Trouillas et al., 2022).

Phytophthora nicotianae was the predominant species isolated (85% of the total *Phytophthora* isolates) from both *P. orientalis* and *P.* x *acerifolia* sites in Greece. It was recovered both from the soil samples and stem tissues and it caused significant root damage in artificial inoculation tests. This pathogen is the most common *Phytophthora* species in

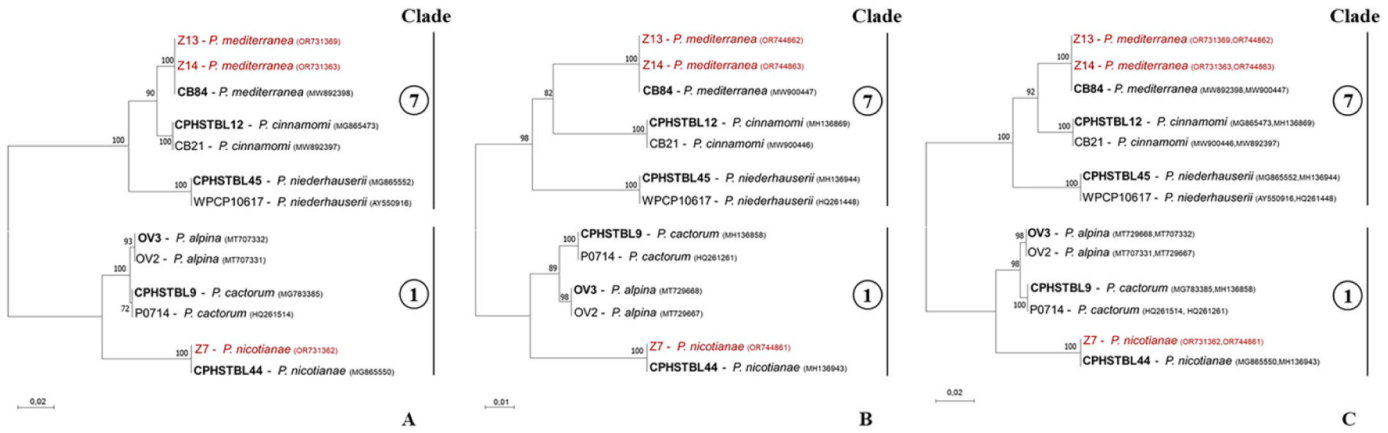

Fig. 5. Maximum likelihood tree obtained from ITS (A), *cox*1 (B) and concatenated ITS+*cox*1 (C) sequences of different *Phytophthora* species belonging to the clades 1 and 7. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support values in percentage (1000 replicates) are given at the nodes. Ex-type cultures are in bold, and isolates obtained in this study in red. GenBank accession numbers are in brackets.

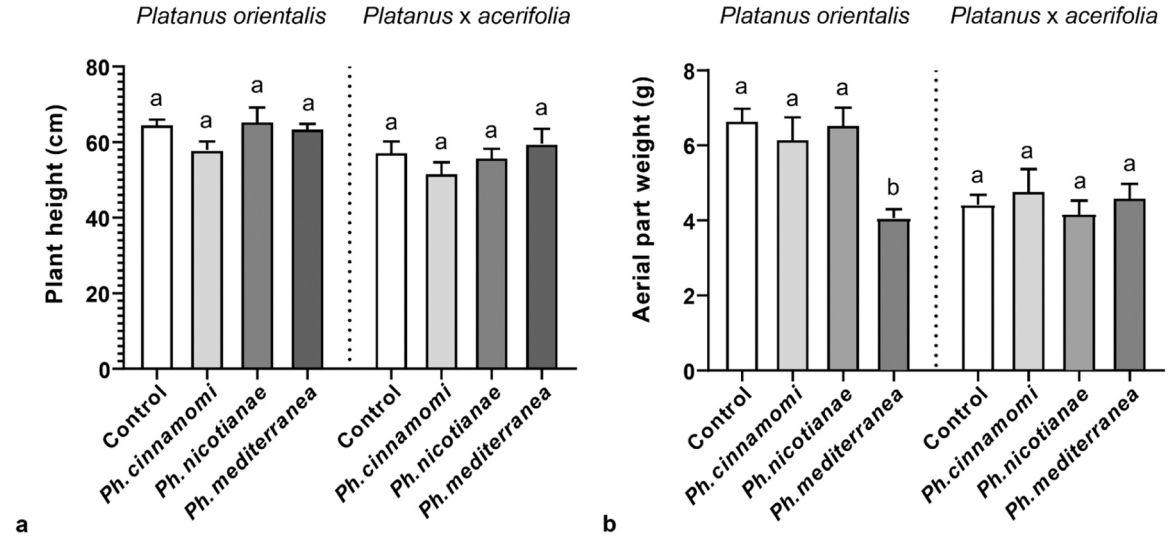

Fig. 6. Plant height (a) and mean weight of aerial part (b) of *Platanus orientalis* and *P.* x *acerifolia* plants after 5 months growth in uninfected soil (control) and in soil infested by *Phytophthora cinnamomi*, *Ph. nicotianae* and *Ph. mediterranea*, respectively. Data are mean ± standard error; different alphabets on top of error bars of mean represent significant differences (ANOVA, $p<0.05$).

Greece, associated with ornamental, horticulture and forest plants (Elena, 1999; Tsopelas et al., 2011; Jung et al., 2016). The pathogen has been reported to be associated with more than 30 plant taxa (Elena, 1999). According to Jung et al. (2016), *Ph. nicotianae* is also one of the most common *Phytophthora* species found in European nurseries. Thus, we hypothesize that the pathogen was introduced through nursery plants, not necessarily plane trees, as many ornamental plants were settled in the studied areas (such as *Arbutus, Cistus, Euphorbia, Lavandula, Pinus, Rosmarinus, Salvia* plants).

The presence of *Ph. mediterranea* in a limited number of *P. orientalis* trees suggests that this pathogen was probably introduced with infected nursery stock. *Phytophthora mediterranea* was formally described as a distinct species in 2021, from nursery plants of *Myrtus communis,* a species of the Mediterranean maquis in Sardinia (Italy) (Bregant et al., 2021), and later as a pathogen of fruit and nut crops in California (Trouillas et al., 2022). Almost all findings of *Ph. mediterranea* come from geographical areas characterized by a Mediterranean climate, highlighting the high potential for diffusion of this species in the middle latitude zone given its ability to grow optimally at temperatures between 15 and 35°C. This represents the first report of *Ph. mediterranea* in Greece and as a plane tree pathogen. Concerning the morphology of *Ph. mediterranea,* the isolates obtained in this study showed slightly smaller sporangia than the ones described by Bregant et al. (2021). All the other morphological characters were according to Bregant et al. (2021). Phylogenetically speaking, *Ph. mediterranea* shares a close relationship with *Ph. cinnamomi,* yet it exhibits a higher level of thermophily. This critical trait gives an advantage to this pathogen for its easier establishment in Mediterranean cities as in the case of Athens. *Platanus orientalis* and *P.* x *acerifolia* are commonly planted trees in parks, along streets and in historical gardens in southern Europe and middle Asia cities (Pourkhabbaz et al., 2010; Ciaffi et al., 2018; Marzano, 2020). Plane trees were planted in the studied park and private garden in Athens when they were already adult trees, probably obtained from asymptomatic infected nursery stock. In the Athens park, *Phytophthora* infections were detected in various sections of the area, with distances between them exceeding 300 meters. Introduction of *Phytophthora* through infected nursery plants was also evident in the private garden, where the *P.* x *acerifolia* trees began to die four years after being planted.

This observation confirms data derived from the literature, infact, it is well known that plant trade plays a major role in the spread of *Phytophthora* pathogens into many countries (Jung et al., 2016; Antonelli et al., 2022). For example, genetic analysis demonstrated that *Ph.*

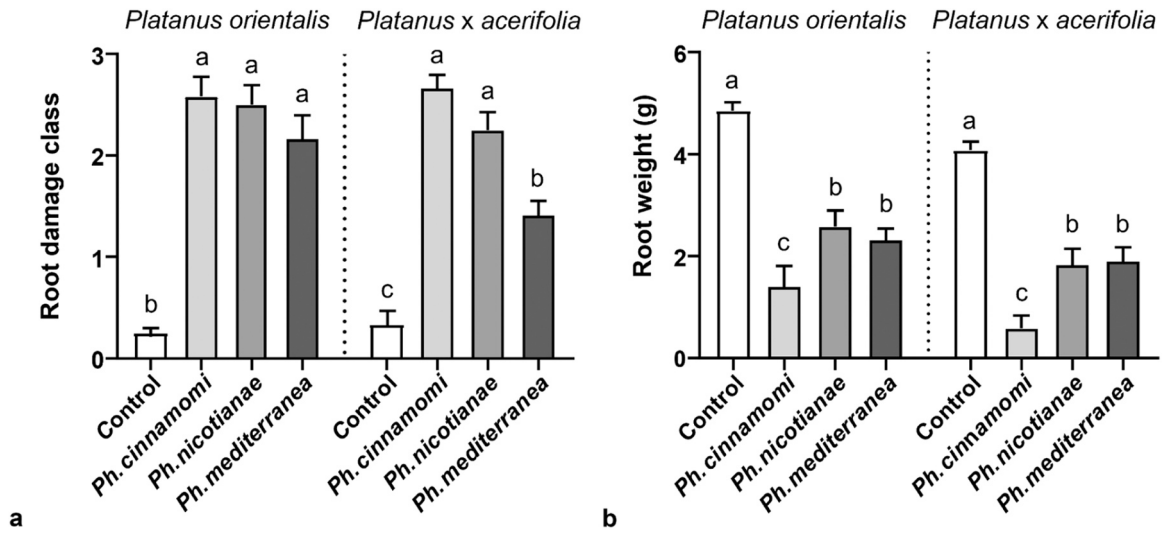

Fig. 7. Mean root damage (a) and mean root weight (b) of *Platanus orientalis* and *P.* x *acerifolia* plants after 5 months growth in uninfected soil (control) and in soil infested by *Phytophthora cinnamomi*, *Ph. nicotianae* and *Ph. mediterranea*, respectively. Data are mean ± standard error; different alphabets on top of error bars of mean represent significant differences (ANOVA, $p<0.05$).

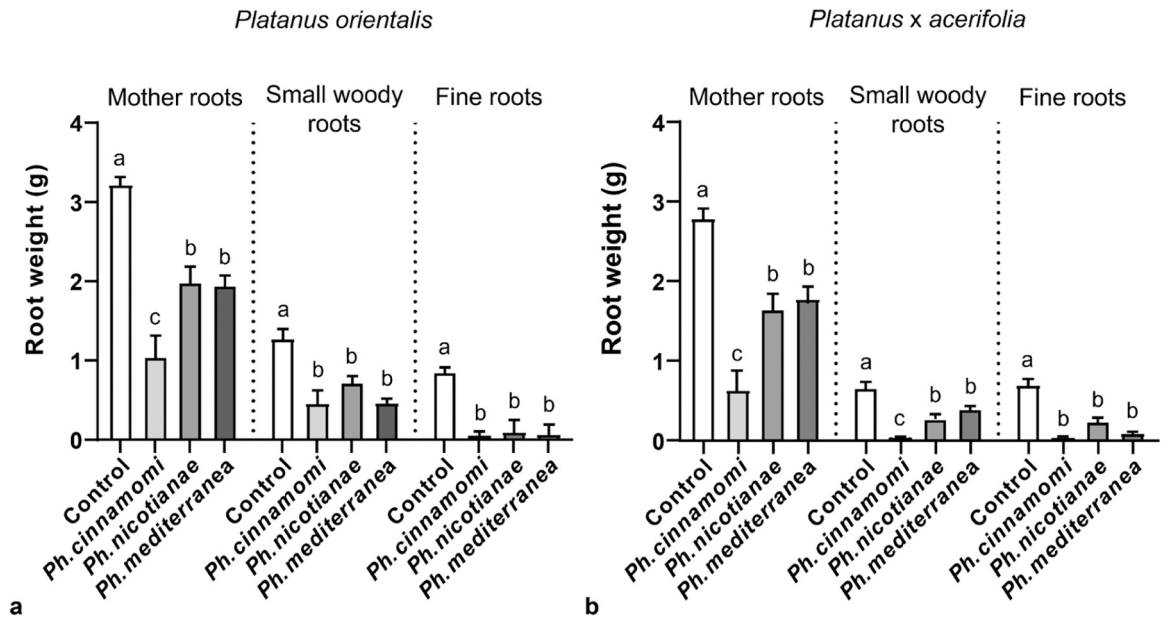

Fig. 8. Mean weight of mother, small woody, and fine roots of *Platanus orientalis* (a) and *P.* x *acerifolia* (b) plants, after 5 months growth in uninfected soil (control) and in soil infested by *Phytophthora cinnamomi*, *Ph. nicotianae* and *Ph. mediterranea*, respectively. Data are mean ± standard error; different alphabets on top of error bars of mean represent significant differences (ANOVA, $p<0.05$).

ramorum, which causes widespread mortality on oaks and tanoaks and ornamental plants, spread throughout nurseries and forests in North America and Europe via the plant trade, probably associated with asymptomatic plants (Mascheretti et al., 2008, 2009; Goss et al., 2011; Grünwald et al., 2012). Similarly, it is likely that *Ph.* x *alni* originated from a nursery, has spread throughout natural populations and plantations of *Alnus* species in Europe, causing massive mortality rates (Brasier et al., 2004; Jung and Blaschke, 2004). In California, *Ph. tentaculata* was firstly detected in 2012 in a nursery, associated to a semi woody shrub (*Diplacus aurantiacus*) and then, in 2014, on outplanted native plants (Rooney-Latham et al., 2019).

The outbreak of *Phytophthora* species in parks and gardens in Greece highlights the high risk posed by nursery plants in urban reforestation.

5. Conclusions

In conclusion, in order to maintain the tree population in an urban area, trees' vulnerability to pests and diseases should also be included within the main selection criteria for plant choice within a reforestation plan. *Phytophthora* species are prominent in lists of emerging threats to urban ecosystems, whose impact can be favored by climate change. Therefore, to assess the risk posed by these pathogens is a good strategy to establish biosecurity measures to prevent the introduction of diseases all along the urban reforestation process. Moreover, surveys focused on *Phytophthora* detection should be carried out regularly in urban areas, using both traditional and new detection tools. It has been proven, for instance, that the knowledge of the richness of the *Phytophthora* community was significantly improved when both baiting and metabarcoding approaches, were adopted, confirming the need for integrated

detection strategies (Vettraino et al., 2012; Khaliq et al., 2018; Khdiar et al., 2020).

CRediT authorship contribution statement

Chiara Antonelli: Writing – review & editing, Methodology, Investigation. **Christos Tsoukas:** Writing – review & editing, Methodology, Investigation. **Epaminondas Paplomatas:** Methodology, Investigation. **Elena Kuzminsky:** Writing – review & editing. **Anna Maria Vettraino:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Data curation, Conceptualization. **Nikoleta Soulioti:** Writing – review & editing, Methodology, Investigation. **Benedetto Teodoro Linaldeddu:** Writing – review & editing, Writing – original draft, Data curation. **Panaghiotis Tsopelas:** Writing – review & editing, Methodology, Investigation. **Margherita Biscontri:** Methodology, Investigation.

Declaration of Competing Interest

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

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