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Canker Stain: A Lethal Disease Destroying Iconic Plane Trees

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Iconic Plane Trees

"The fame of the plane-tree fills all antiquity, east and west, and is still echoed in the descriptions of ancient and modern travellers" (Hehn 1888). This iconic tree, oriental plane (*Platanus orientalis* L.), with its enormous size, was held sacred in ancient Egypt, Greece, and Persia and it was planted in gardens, avenues, and around tombs (Wells 2010).

The name *Platanus* appears in the writings of Cicero (1th century BC) and was used by Linnaeus to describe the genus. It derives from the Greek name "platanos" used by Theophrastus (4th-3rd century BC), while in Homer's (9th century BC) epic poem "The Iliad" it appears as "platanistos." Etymologically, the name platanos in Greek stems from the term "platys" meaning broad or wide, referring to the wide palmate leaves of this tree (Hehn 1888; Wells 2010).

The Oriental plane forms part of various stories and legends. The Greek historian Herodotus (5th century BC) describes an oriental plane of impressive size that was greatly admired by the Persian emperor Xerxes, who adorned its branches with golden ornaments (Hogg 1834). According to legend, it was under an evergreen plane tree at Gortyna in Crete that Zeus (Jupiter) was united with Europa, a princess from Phoenicia and after which the continent of Europe was named (Hehn 1888).

Many legendary plane trees of great size have been recorded to occur in Europe and Asia. In Kashmir, where *P. orientalis* is known as "chinar," there are four very large plane trees on an island on Dal Lake in the Srinagar district. In the district of Budgam, a chinar tree of enormous size and with a circumference of 31.82 m at ground level is considered to be the largest tree in Asia (Kozgar and Khan 2011). According to legend, the plane tree growing close to the harbor (Fig. 1A) on the island of Kos provided shade to Hippocrates (5th century BC), who is considered the "father of rational medicine," to teach the art of medicine.

Platanus is the sole genus in the family Platanaceae (order Hamamelidales) and includes 10 species and varieties, all of which occur in the Northern hemisphere (Nixon and Poole 2003). The genus *Platanus* has a rich fossil record extending back to the early Cretaceous (about 115 million years ago), with many extinct species (Grimm and Denk 2008). The modern species of *Platanus* are divided into two subgenera: the subgenus *Castaneophyllum*, which includes a single species, *Platanus kerrii* Gagnep., an evergreen tree with elliptical to lanceolate leaves, present in Indochina and considered an ancestral species; and the subgenus *Platanus*, which accommodates all remaining species of the genus (Nixon and Poole 2003).

In Europe, *P. orientalis* is the only species occurring naturally in the southeastern part of the continent and also extends to southwestern Asia. The remaining eight taxa in the subgenus *Platanus* all occur in North America. *P. orientalis* is well distributed in the southern part of the Balkan Peninsula up to latitude 42°, and some remnants of the species can be found in southern Italy (including Calabria and Sicily) (Grueva and Zhelev 2011; Tutin et al. 1964). This tree species extends eastwards from Greece to Asia Minor, Iran, and central Asia up to Kashmir; however, its native range is not very clear because it has been widely planted since ancient times (Santamour and McArdle 1986). There is an evergreen variety of *P. orientalis* with palmate lobed leaves on Crete Island of Greece, *P. orientalis* var. *cretica* Dode, which is known from ancient times (Nikolakaki and Hajaje 2001).

The most common species in North America is *P. occidentalis* L. (American sycamore), described as *P. occidentalis* var. *occidentalis* in a new taxonomic account by Nixon and Poole (2003). This species is distributed in the eastern United States and southern Ontario in

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Canada and is considered as one of the largest indigenous tree species in the deciduous forests of eastern North America. George Washington in his journal in 1770 describes a sycamore tree of extraordinary size near the junction of the Kanawha and Ohio Rivers in West Virginia (then Virginia) (Spurr 1951).

Another variety of *P. occidentalis*, *P. occidentalis* L. var. *palmeri* (Kuntze) Nixon & Poole, is found in Texas and Mexico. *P. racemosa* Nutt. var. *racemosa* (California sycamore) is distributed throughout California and *P. racemosa* Nutt. var. *wrightii* (S. Wats.) Benson (Arizona sycamore) is present in Arizona and New Mexico; both species extend south into Mexico. Three more species (one with two varieties) are also present in Mexico: *P. gentryi* Nixon & Poole, *P. mexicana* Morie. var. *mexicana*, *P. mexicana* Morie. var. *interior* Nixon & Poole, and *P. rzedowskii* Nixon & Poole (Nixon and Poole 2003).

The most popular and best-known plane tree worldwide is the hybrid between *P. orientalis* and *P. occidentalis*, known as *Platanus* × *hispanica* Mill. ex Münchh., *P.* × *acerifolia* (Ait.) Willd., *P.* × *hybrida* Brot., and *P.* × *intermedia* Hort. ex Chow. and commonly known collectively as "London plane." The fact that this hybrid shows a remarkable adaptability to different climatic conditions, and has proven to be very tolerant to urban conditions, has resulted in its popularity worldwide as an important landscape tree. These trees have been widely planted in urban and ornamental environments in Europe, North America, and Asia for the past three centuries and they remain a dominant feature of streets, parks, and city squares in many cities globally (Santamour and McArdle 1986).

In Europe, both Oriental plane and London plane trees are seriously threatened by the invasive fungal pathogen *Ceratocystis platani* (Walter) Engelbr. & T.C. Harr., the causal agent of canker stain disease (CSD) of plane trees. The fungus is considered to be indigenous to North America (Engelbrecht et al. 2004; Walter et al. 1952) and was accidently introduced into Europe during World War II (Cristinzio et al. 1973; Panconesi 1999) where it continues to spread clonally (Ocasio Morales et al. 2007; Santini and Capretti 2000).

The impact of CSD in Europe can be compared with notorious tree diseases such as Dutch elm disease, chestnut blight, and more recently Ash dieback, which have all caused devastating losses to natural woody ecosystems and ornamental trees (Anagnostakis 2012; Brasier and Kirk 2001; Kowalski 2006; Ocasio Morales et al. 2007; Walter et al. 1952). In Italy and France, *C. platani* has caused widespread mortality to London plane trees and the pathogen has also been recorded in Switzerland and Spain (EPPO 2014; Panconesi 1999; Vigouroux 2013). However, the most dramatic impact of the disease has been in Greece (Fig. 2) in natural stands of Oriental plane (Ocasio-Morales et al. 2007; Tsopelas and Soulioti 2011, 2014).

The objective of this feature article is to review current knowledge regarding CSD and to highlight the dramatic and devastating nature of the disease. An important aim is also to highlight the risk of *C. platani* spreading northward in Europe and eastward to Asia in the natural and cultivated range of oriental and London plane.

Occurrence of *Ceratocystis platani* Worldwide

CSD was originally described in 1935 in the northeastern United States in Delaware County, PA (Jackson and Sleeth 1935) on *P.* × *hispanica*, although initially incorrectly reported as *P. orientalis* (Fowler 1939). According to Walter et al. (1952), symptoms of this disease had been noticed on London plane trees in the city of Gloucester, NJ, since 1929, possibly infected around 1926. In subsequent years, the disease became

Fig. 1. Iconic *Platanus orientalis* trees threatened by canker stain disease. **A,** The legendary Hippocrates plane tree on the Island of Cos in Greece. **B-C,** A monumental large plane tree dying in Peloponnese, Greece. A church (C) established in the hollow trunk of the tree known as "Virgin Mary of Plataniotissa" for many centuries and accommodating about 20 people.

Fig. 2. Extensive tree mortality in a natural stand of *Platanus orientalis* in Greece caused by *Ceratocystis platani*.

widespread in many areas of the northeastern states as well as other parts of the United States. By 1940, the disease had been reported in Washington D.C., New York, Delaware, Maryland, Pennsylvania, Ohio, Virginia, West Virginia, North Carolina, Tennessee, and Kentucky (Crandal 1935; Crone 1962; Walter 1946; Walter et al. 1952).

In the 1960s and 1970s, *C. platani* was reported in North Carolina, Georgia, Mississippi, Alabama, Arkansas, and Louisiana in plantations established for short rotation coppicing, and in natural stands of *P. occidentalis* (McCracken and Burkhardt 1977; Ross 1971). In the western United States, the pathogen has been known since 1961 in the city of Modesto, California, in urban tree plantings (Perry and McCain 1988).

In Europe, CSD was reported for the first time in 1972 in the city of Forte dei Marmi in Tuscany, Italy (Panconesi 1972), and in 1974, it was detected in Marseilles in southern France (Ferrari and Pichenot 1974). However, disease symptoms and extensive plane tree mortality were observed in both countries well before the time that the first records were published. It has been speculated that the pathogen was introduced into both countries in Europe during World War II, presumably with wood packaging material used by the U.S. military forces (Cristinzio et al. 1973; Ferrari and Pichenot 1976; Panconesi 1999).

C. platani has continued to spread throughout the Italian peninsula and Sicily (Panconesi 1999), while in France the disease has been reported in five regions in the southern part of the country: Provence Alpes Côtes d'Azur, Rhône Alpes, Languedoc-Roussillon, Midi Pyrénées, and Aquitaine. It was also reported in Corsica in 1992, but is considered to have been eradicated (Chapin and Arcangioli 2007).

CSD has been known in Switzerland since 1986 (Gessler and Mauri 1987), although it has a rather limited distribution in this country (EPPO 2014). There have also been unconfirmed reports of the disease in Spain since the mid-1970s (Cadahia 1983; Fernandez de Aña Magan and Gil 1977; Ferrari and Pichenot 1976; Ruperez and Muñoz 1980). However, the only confirmed report was in 2010 in Girona, Catalonia, which borders with known infected areas in southern France (EPPO 2014; Riba 2011).

In Greece, CSD was initially detected in 2003 in Peloponnese (southern part of the country) where it has affected natural stands of *P. orientalis* as well as shade trees in urban areas (Ocasio Morales et al. 2007; Tsopelas and Angelopoulos 2004). The pathogen has gradually spread into a major part of Peloponnese and in 2010 it was found in the Epirus region in northwestern Greece, close to the border of Albania (Tsopelas and Soulioti 2014). In 2014, *C. platani* was recorded in Albania, where it appears to have been established for many years on *P. orientalis* in natural stands and on shade trees (Tsopelas et al. 2015a). Recently the pathogen was detected in the European part of Turkey (H. T. Dogmus, *personal communication*) There are also unconfirmed reports of *C. platani* in Asia: Armenia (Simonian and Mamikonyan 1982) and Iran (Salari et al. 2006).

The Pathogen

C. platani resides in the Ceratocystidaceae (Sordariomycetes) as defined by de Beer et al. (2014). Together with the Ophiostomatales, these fungi are broadly referred to as the ophiostomatoid fungi (Seifert et al. 2013; Wingfield et al. 1993). This terminology arises from the fact that species in the genera *Ceratocystis sensu lato* and *Ophiostoma sensu lato* were the subject of considerable taxonomic confusion and controversial argument for many years (de Beer et al. 2013; Wingfield et al. 1993). The confusion primarily centered on the fact that species of *Ophiostoma* and *Ceratocystis* share many morphological features that have arisen from convergent evolution associated with life cycles where insects act as their vectors. For example, most species in the two aggregate genera have globose ascomatal bases and long necks with masses of sticky ascospores at their apices. The asci in these fungi deliquesce early and are seldom seen; many species in both genera also have variously ornamented ascospores (de Beer and Wingfield 2013; de Beer et al. 2014). It is, therefore, not surprising that they were confused for almost a century.

DNA sequence data have made it possible to clearly define and separate *Ceratocystis* and *Ophiostoma*. Thus, subsequent to the publication of a book emerging from an important symposium on *Ceratocystis* and *Ophiostoma* in 1990 (Wingfield et al. 1993), the genera have been accepted as different. Hausner et al. (1993) and Spatafora and Blackwell (1994) were the first to provide DNA sequence data to show that *Ceratocystis* and *Ophiostoma* are not only different genera, but that they reside in different orders in the Sordariomycetes: *Ceratocystis* in the Microascales and *Ophiostoma* in the Ophiostomatales (de Beer et al. 2013).

Until recently, *Ceratocystis sensu lato* has included groups of fungi that are morphologically, ecologically and phylogenetically very distinct. Wingfield et al. (2013) provided clear DNA-based evidence to show that these fungi were unrelated and that grouping them in a single genus was not only incorrect, but that it was also taxonomically confusing. de Beer et al. (2014) provided additional and robust data based on multiple gene regions to define these genera. In this treatment, *Ceratocystis* is defined based on the type species *C. fimbriata sensu stricto*, a sweet potato and root crop pathogen. The genus includes 33 species, most of which have been described primarily based on DNA sequence data and where *C. platani* is clearly defined as a distinct species (Figs. 3 and 4).

Although CSD was described in earlier reports (Jackson and Sleeth 1935; Walter 1946), the causal agent of the disease was only described in 1952 by Walter et al. (1952) as *Endoconidiophora fimbriata* f. *platani*. From this description, it was evident that the fungus was morphologically indistinguishable from *C. fimbriata*, but that it differed based on host susceptibility. However, Walter et al. (1952) was seemingly unaware of the fact that Bakshi (1951) had reinstated the name *Ceratocystis* with *Endoconidiophora* as a synonym. Following Bakshi's (1951) study, several additional species were transferred to *Ceratocystis* (Hunt 1956; Moreau 1952), but *E. fimbriata* f. *platani* was not among those. In his monograph of *Ceratocystis*, Hunt (1956) mentioned '*E. fimbriata* f. *platani*' in the discussion of *C. fimbriata*, but he also did not transfer the name to *Ceratocystis*. To the best of our knowledge, the name was never formally transferred to *Ceratocystis*, and after 1 January 1953, the Botanical Code required that a new combination should be accompanied by the original name (basionym) as well as the full reference and page number of the original description (McNeill et al. 2012). The first reference that we could obtain where '*C. fimbriata* f. *platani*' was used (May and Palmer 1959) did not meet these requirements; neither did any subsequent publication using this form of the name and variations thereof (e.g., '*C. fimbriata* f. sp. *platani*'). Thus, despite the fact that '*C. fimbriata* f. *platani*' became widely used for the next four decades (e.g., Alami et al. 1998; Crone and Bachelder 1961; Mutto Accordi 1986; Panconesi 1976), the name was never validated.

Baker et al. (2003) provided the first DNA sequence-based evidence to show that the causal agent of CSD was in fact distinct from the sweet potato pathogen, *C. fimbriata*. Based on these data, as well as mating experiments and host susceptibility, Baker Engelbrecht and Harrington (2005) elevated the name from *forma specialis* to species level as *C. platani*. The name that is currently applied and its formal synonyms are as follows:

Ceratocystis platani (Walter) Engelbr. & T.C. Harr., Mycologia 97: 65. 2005.
≡ *Endoconidiophora fimbriata* f. *platani* Walter, Phytopathology 42:236. 1952.
= *Ceratocystis fimbriata* f. *platani* (Walter) C. May & J.G. Palmer, Plant Disease Reporter 43:565. 1959. *nom. inval.* [Art. 41.5].

The sexual state of *C. platani* (Fig. 5A to C) is characterized by ascomata that are dark brown in color, superficial or partly immersed in the substrate, with a globose base (120 to 330 µm), ornamented with hyphal filaments (sometimes consisting of the conidiophores themselves), and with long necks (400 to 1,000 µm) that are dark and wide at the base, and lighter and narrower at the tips. The apices of the necks are adorned with erect hyaline hyphae (48 to 102 µm) surrounding the ostioles from which the ascospores are expelled at maturity in a cream colored mass (Fig. 5B and C). Asci are evanescent and typically not seen. Ascospores are hyaline, one-celled, hat-shaped (Fig. 5A), 4 to 6.5 mm long, 3 to 4.5 mm wide, and 3 to 4.5 mm tall (Baker-Engelbrecht and Harrington 2005).

In culture (potato dextrose agar [PDA], 25°C), the mycelium of *C. platani* is at first hyaline becoming brownish-green with time and producing a pronounced banana odor (Jackson and Sleeth 1935). Mycelial growth in vitro is relatively fast (radial growth 15 to 20 mm per week). Three different forms of conidia (Fig. 5D to H) are produced by the asexual state of *C. platani*. Endoconidiophores arise laterally from vegetative hyphae, scattered or in clusters, are 1 to 4 septate, 55 to 165 µm long including the basal cells, and 3.5 to 7.5 µm wide at the base. Two forms of phialides can be present. The more common of these are lageniform, 24 to 90 µm long, 3.5 to 9 µm wide in the middle, 2.5 to 7.5 µm wide at the tip, and hyaline to pale brown. The cylindrical endoconidia (Fig. 5D and E) produced by these structures are unicellular, smooth, primarily cylindrical with flattened ends, straight, biguttulate, 11 to 22 × 3 to 5 µm, borne in chains of variable length, and hyaline to light brown. Wide-mouthed phialides are 35 to 50 µm long, 5.5 to 6.5 µm wide at the tip, 4.5 to 5 µm wide at the base, usually around the base of ascomata. The doliform endoconidia (Fig. 4E and F) produced from wide-mouthed phialides often persist in

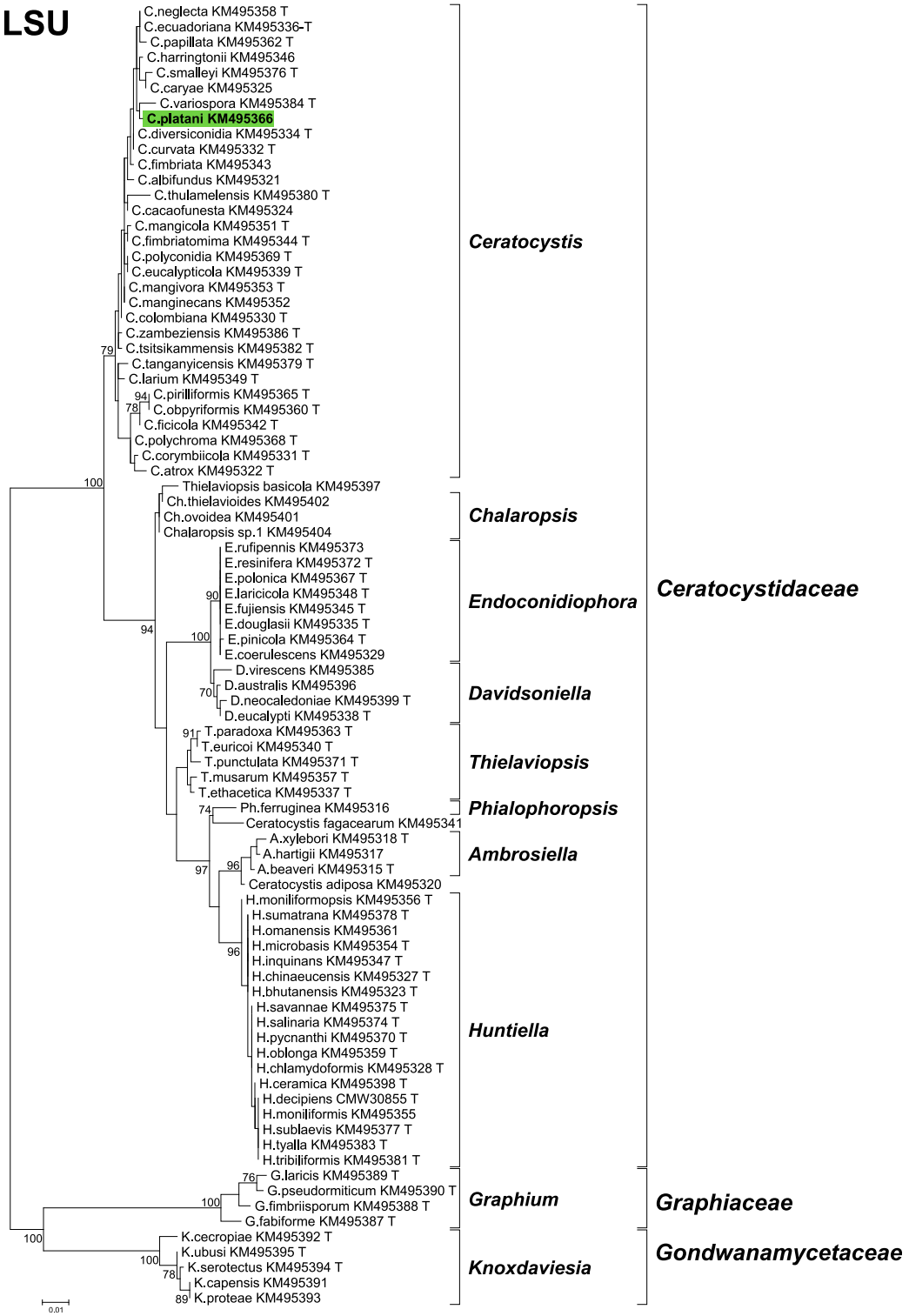

Fig. 3. Phylogenetic tree based on ribosomal large subunit (LSU) sequences showing the position of *Ceratocystis platani* within *Ceratocystis* in relation to other genera in the Ceratocystidaceae. The maximum likelihood tree was generated in MEGA 7 (Kumar et al. 2016) using the general time reversible (GTR) model. Only bootstrap values (1,000 replicates) above 75% are shown at the nodes.

chains (Fig. 5F), are 6 to 10 µm × 3.5 to 5 µm, and hyaline to light brown. Aleurioconidiophores are less often produced and sometimes arise laterally from the mycelium, with 0 to 14 septa, 10 to 285 µm × 4 to 7 µm. The aleurioconidia (Fig. 5G and H) are brown, globose to pyriform, 10 to 20 µm × 6 to 12 µm, occurring singly or in short chains (Baker Engelbrecht and Harrington 2005).

The asexual state of *C. platani* has never been provided with a binomial, probably because the name *Endoconidiophora*, the initial sexual genus in which it was originally treated, was descriptive of asexual structures. However, the anamorph of *C. fimbriata*, and of which *C. platani* was treated as a *forma specialis*, was accommodated for many years in *Chalara* (de Hoog and Scheffer 1984; Nag Raj and Kendrick 1975; Upadhyay 1981). Paulin and Harrington (2000) showed that the type species of *Chalara* is not related to *Ceratocystis*, and thus Paulin-Mahady et al. (2002) redefined *Thielaviopsis* to accommodate asexual states of *Ceratocystis*. de Beer et al. (2014)

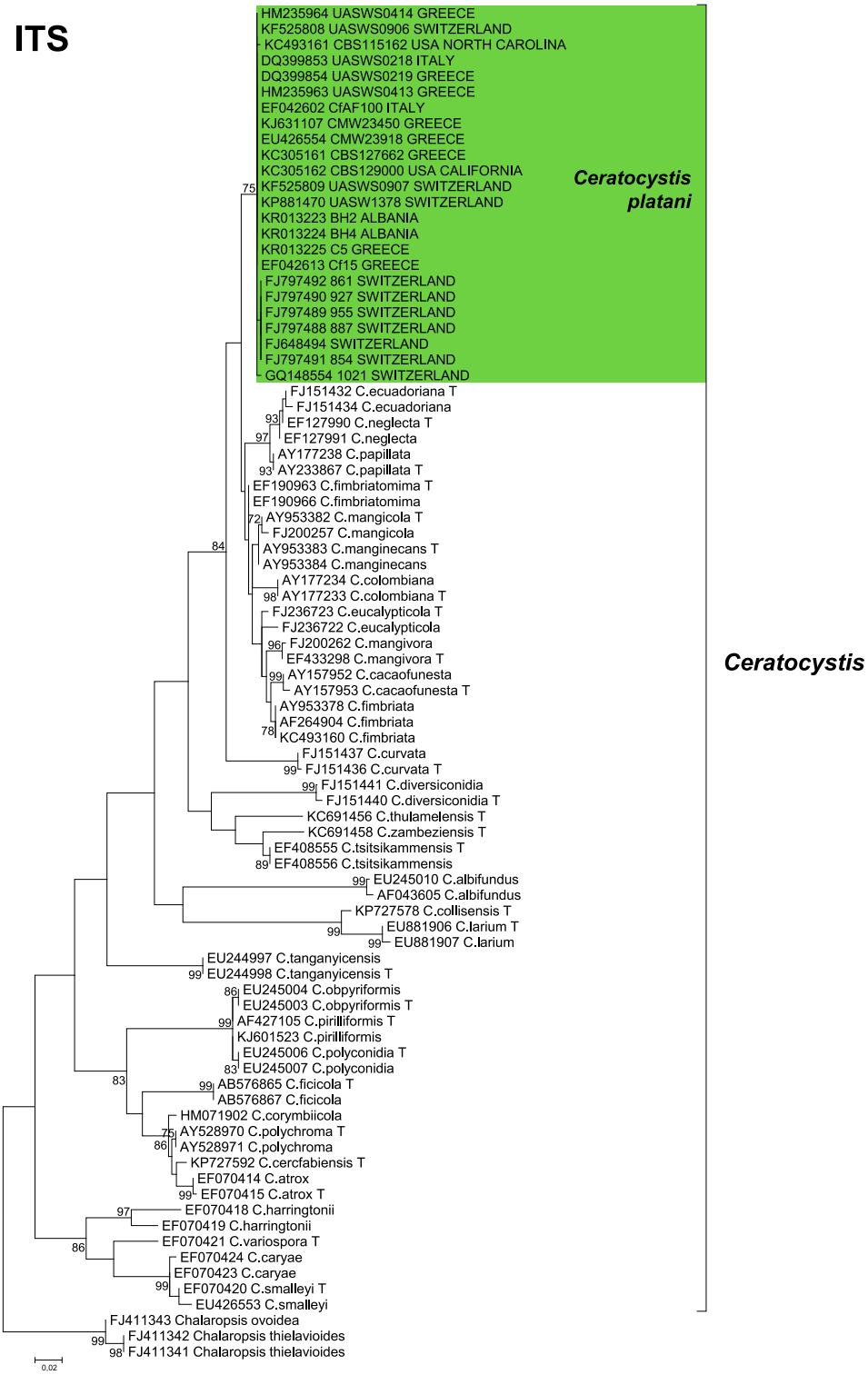

Fig. 4. Phylogenetic tree based on sequences of the internal transcribed spacer (ITS) regions of the ribosomal DNA operon, showing the *Ceratocystis platani* sequences from various countries in relation to other species of *Ceratocystis*. The maximum likelihood tree was generated in MEGA 7 (Kumar et al. 2016) using the general time reversible (GTR) model. Only bootstrap values (1,000 replicates) above 75% are shown at the nodes.

produced phylogenetic data that distinguished between *Ceratocystis* and *Thielaviopsis* (Fig. 3). Based on the one fungus one name principles adopted in the Melbourne Code (Hawksworth et al. 2011; McNeill et al. 2012), de Beer et al. (2014) redefined *Thielaviopsis* to also include species with sexual states, and emended the description of *Ceratocystis* to encompass asexual states as well (de Beer et al. 2014). Although the asexual state of *C. platani* can be described as thielaviopsis-like, current practice prohibits the treatment of the asexual state in a genus other than *Ceratocystis*.

Host Susceptibility

C. platani was first reported on *P.* × *hispanica*, which is a highly susceptible host. Walter et al. (1952) indicated that all infected trees of this host died from the disease in the Philadelphia area and other parts of North America and recovery was never observed. Similar reports have also been published in several other cases in Europe where the pathogen has killed trees of all sizes and ages; some of them of enormous size and many centuries old (Cristinzio et al. 1973; Ferrari and Pichenot 1976; Panconesi 1999; Vigouroux 1979a). In an inoculation trial in Italy, Pilotti et al. (2009) reported that 93% of the seedlings of *P.* × *hispanica* died within approximately 6 months after inoculation, while most of the remaining plants died in the following years. However, 0.4% of the seedlings in this trial survived, showing that some resistance exists in the hybrid species.

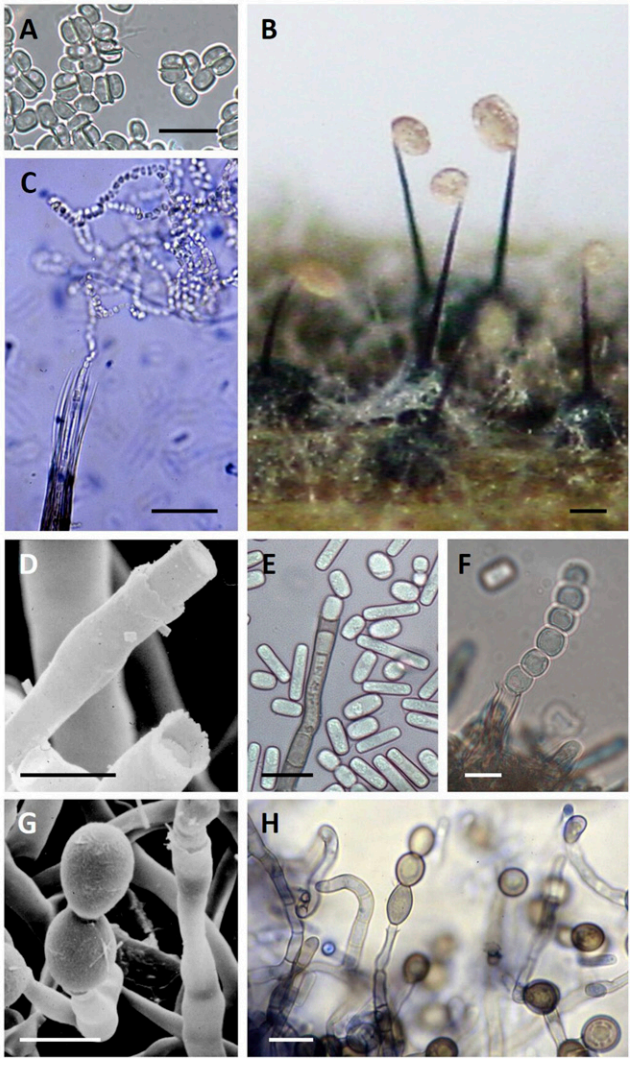

Fig. 5. Fruiting structures of *Ceratocystis platani*. **A,** Hat-shaped ascospores. **B,** Ascomata on wood with sticky droplets of ascospores at their apices. **C,** Threads of ascospores emerging from an ostiole surrounded by ostiolar hyphae at the apex of an ascomatal neck. **D,** Cylindrical endoconidium protruding from a phialidic endoconidiophore. **E,** Endoconidiophore surrounded by cylindrical and doliform conidia. **F,** Chain of doliform endoconidia protruding from an endoconidiophore. **G-H,** Pigmented aleurioconidia. Scale bars A, D-H = 10 µm; B = 100 µm; C = 50 µm. Pictures C, D, G, H were kindly provided by Dr. A. Panconesi.

P. orientalis, the Eurasian parent of the hybrid, is also highly susceptible to *C. platani*. Extensive tree mortality has been reported in natural stands of this species in Sicily, Italy (Granata and Pennisi 1989), as well as in Greece and Albania (Ocasio-Morales et al. 2007; Tsopelas et al. 2015a). Walter (1946) demonstrated the susceptibility *P. orientalis* in a limited scale inoculation trial. In this trial, he failed to detect any differences in susceptibility between *P. orientalis* and *P.* × *hispanica*. The extreme susceptibility of *P. orientalis* has also been shown in several inoculation tests in Greece (Tsopelas and Angelopoulos 2004; Tsopelas et al. 2015a; N. Soulioti and P. Tsopelas, *unpublished*).

P. occidentalis, the North American parent of *P.* × *hispanica*, is considered moderately susceptible to infection by *C. platani*. Walter (1946) had indicated that this species is less susceptible than *P.* × *hispanica*, although the disease had destroyed many street trees in cities of North America. The disease has been reported in the southeastern United States in natural stands and plantations of *P. occidentalis*, but incidence was relatively low (McCracken and Burkhardt 1977). These authors, in an inoculation trial, reported differences in susceptibility among individual trees of *P. occidentalis*, although all inoculated trees died by the end of the experiment. In the following years, F. I. McCracken (*unpublished*) selected several individuals of *P. occidentalis* showing resistance to *C. platani*. Some of these trees were crossed with susceptible *P. orientalis* plants from Greece, as part of a breeding for resistance program in France. About 2% of these hybrids showed resistance to the pathogen, surviving two successive inoculation trials. A *P.* × *hispanica* clone highly resistant to *C. platani* (Platanor 'Vallis clausa') was ultimately selected and released to the market (Vigouroux and Olivier 2004).

Among the remaining *Platanus* species, only *P. racemosa* var. *racemosa*, the California sycamore, is a known host of *C. platani*; extensive street tree mortality has been reported in the city of Modesto in California (Perry and McCain 1988). However, the relative susceptibility of *P. racemosa* var. *racemosa* in comparison with other hosts has not been investigated. Information on susceptibility of the other *Platanus* species occurring in the southwestern United States, Mexico, and Guatemala, as well as *P. kerri* occurring in Laos and Vietnam, remains to be determined.

Disease Diagnosis

Symptoms. CSD is fatal; *C. platani* has the ability to kill trees of all ages and sizes. In plantations and natural stands, groups of dead trees can be observed with adjacent trees in different stages of infection and crown symptoms. In small trees, the entire tree may fail to develop leaves in the spring or the leaves wither and die very soon after emergence (Fig. 6D). In contrast, large trees can take a few years to die (Panconesi 1981, 1999).

The development of symptoms in the crowns of diseased trees depends on the manner in which infections are initiated. In large trees, when the infection is initiated from pruning wounds or other wound types on one side of the tree, the first symptoms of the disease develop on that side of the tree; one or more branches show chlorotic foliage and they die progressively (Fig. 6C) (Panconesi 1999; Tsopelas and Soulioti 2013). Very often, these types of symptoms in large trees also appear when infections have been initiated from rootgrafts. Here, they usually develop on the side of the tree where the roots have become infected from those of neighboring trees. The disease develops progressively over the entire crown (Fig. 6A and B) with thinning and yellowing of the foliage as well as symptoms of microphyllia (undersized leaves) (Ocasio-Morales et al. 2007; Panconesi 1981). Usually, these symptoms are more pronounced on the side of the tree where the infection first appeared (Fig. 6C); however, in some cases, trees show these symptoms evenly over the entire crown (Fig. 6A) (P. Tsopelas, *unpublished*).

Cankers may appear on the surface of the trunk and the branches as darkened, pale-brown flattened areas of the bark that often become cracked with no wound callus formation at the margins (Panconesi 1981; Walter et al. 1952). Cankers are more evident in *P. occidentalis*

and *P.* × *hispanica* trees with smooth bark (Fig. 7A), while cankers can usually not be distinguished on the trunks of most *P. orientalis* trees (Fig. 7C). This is because the outer bark of the trunks is thick and roughened (Tsopelas et al. 2006). In *P. orientalis* trees infected for long periods of time (several months or even years), frass from wood boring insects can be seen in the lower parts of the trunks (Soulioti et al. 2015).

The most characteristic symptom of the disease is the extensive wood staining that is present under the cankered areas of the bark (Fig. 7B and C). This is very often also the case in parts of the trunk or branches that are free from typical external cankers (Panconesi 1999; Tsopelas et al. 2006). Wood staining is also present in the roots of infected trees (Fig. 8B). Wood discoloration becomes evident after removal of the bark; the sapwood is stained bluish black to purple or reddish to dark brown, very often taking the form of elliptical to flame-shaped patterns (Fig. 7C). In cross sections (Fig. 7D), the staining along the rays in the sapwood typically forms a wedge-shape pattern pointing toward the center of the trunk (Panconesi 1999; Walter et al. 1952). However, these symptoms can be seen only in infected living or recently dead trees, while wood staining cannot be distinguished in trees that have been dead for long periods of time (Ocasio-Morales et al. 2007).

Fungal detection-isolation. Isolation of *C. platani* is possible from the wood of living infected or recently dead trees. Direct fungal isolation is very difficult from wood tissues that have been dead for long periods of time. This is due to colonization by many saprophytic and

Fig. 6. External symptoms of trees suffering from canker stain disease. **A-B,** Dead trees on the left and infected trees with symptoms of crown thinning, chlorosis, and microphyllia on the right. **C,** A dead branch and more intense symptoms on the right side of a tree crown from which infection started. **D,** A young tree that died within the growing season and still retaining leaves.

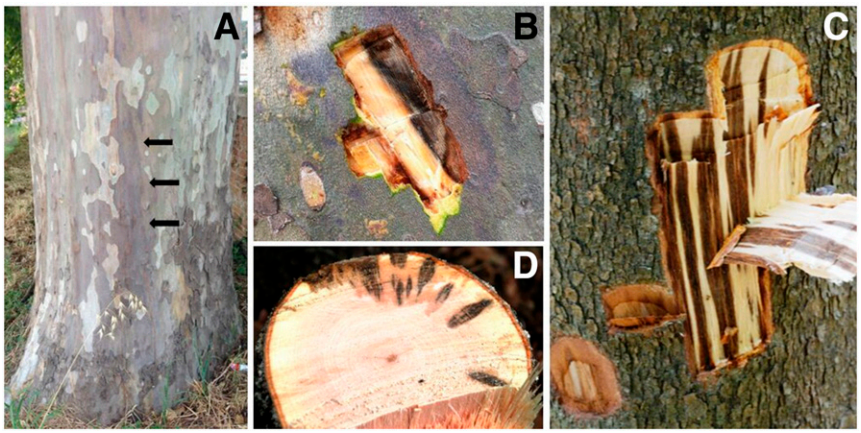

Fig. 7. Disease symptoms on the surfaces of tree trunks. **A-B,** *Platanus* × *hispanica* showing discoloration (arrows) under the smooth and thin bark. **C,** Thick and rough bark of *Platanus orientalis* that must be removed to show wood discoloration. **D,** Characteristic wood discoloration in a cross-section of a tree trunk.

wood decay fungi that outgrow the colonies of *C. platani* (Panconesi 1981). Isolation of the pathogen is possible on potato dextrose agar (PDA), malt agar (MA), malt yeast extract agar (MYEA), and V8 agar (EPPO 2014).

C. platani can be detected by placing chips or cores of stained wood in a moist chamber at 20 to 25°C. Ascomata of the fungus are formed after 7 to 10 days on the surface of the wood, while the endoconidia and aleurioconidia (chlamydospores) can be observed after 2 to 4 days incubation (Vigouroux 1979b). The fungus can be baited from infected wood or insect frass in slices of carrot placed in moist chambers (Moller and De Vay 1968; Ocasio-Morales et al. 2007). However, the most efficient method of *C. platani* detection and isolation from infected plant material, contaminated soil and water, or insect and insect frass is by using the trapping technique developed by Grosclaude et al. (1988). In this method, freshly cut *Platanus* twigs are used as baits; they are stripped of their bark and placed in conical flaks with water along with infected material. Fresh air is continuously pumped into the flask through a Pasteur pipette and after 7 to 10 days incubation at 22 to 25°C, the fungus forms mycelia and spore structures on the twigs and can be positively identified or even isolated (EPPO 2014; Soulioti et al. 2015).

In recent years, techniques other than direct isolation have been established in order to detect *C. platani*. Boddi et al. (2004) optimized a serological assay to confirm the presence of the CP protein from *C. platani* ascospores and mycelium, but it is not used for diagnostic purposes. More recently, two molecular approaches based on qPCR to detect *C. platani* from infected *Platanus* wood and from airborne samples have been reported (Luchi et al. 2013; Pilotti et al. 2012). These techniques provide useful tools to study both the infection process as well as the epidemiology of the disease. Because they are able to detect even very small quantities of *C. platani* DNA, they could be used in surveillance and monitoring operations in order to prevent its spread into new environments.

Infection Process

C. platani infects trees through wounds or other injuries made in the branches, the trunk, or the roots by biotic or abiotic agents (Vigouroux and Stojadinovic 1990). All spore types of the fungus can produce infections when they come into contact with freshly wounded tissue. After spore germination, the developing mycelium colonizes the exposed tissues; it then advances into the xylem tissues of the underlying sapwood, where it develops both longitudinally and tangentially. On the surface of the wounded infected tissues, spores of the pathogen are formed within 2 to 8 days following infection in the form of an ash-colored, powdery layer (Clérivet and El Modafar 1994; Clérivet et al. 2003; Panconesi 1999).

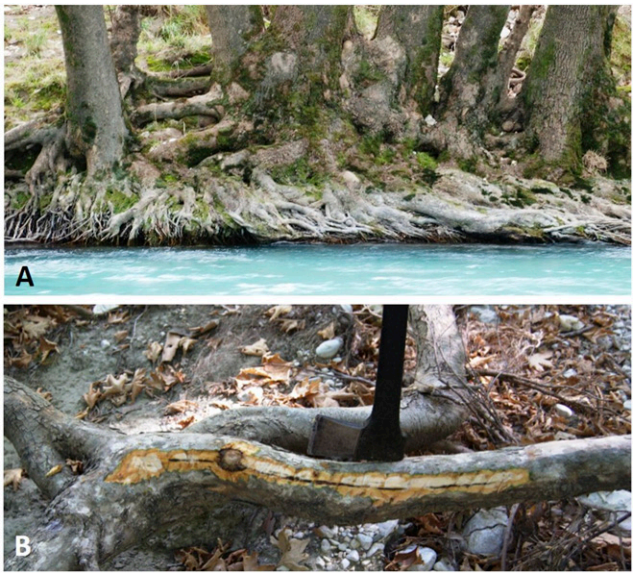

Fig. 8. A, Root grafting of *Platanus orientalis* trees on a river bank. **B,** Root graft and associated disease symptoms on roots.

Once in the host, *C. platani* moves through the xylem as a wilt pathogen, but it also kills the cambium and inner bark tissues forming long cankers. The mycelium spreads along the medullary ray elements causing discoloration of the sapwood tissues, showing a radial fashion in cross section of the stem (Fig. 7D). The host responds with vessel wall thickening and the formation of pectin-containing gels and tyloses resulting in occlusion of the xylem elements (Clérivet and El Modafar 1994; Clérivet et al. 2003). Phytotoxic compounds have also been implicated in necrosis and discoloration of wood and bark tissues (Bürki et al. 2003).

According to Panconesi (1999), in *P.* × *hispanica*, the fungus can grow longitudinally up to 2 to 2.5 m long within a year and can kill a tree of 30 to 40 cm in diameter within 2 to 3 years; however, in *P. orientalis*, trees have been observed with wood discoloration extending up to 5 m within a single year (P. Tsopelas, *unpublished*). The fungal mycelium also invades the root system of the infected trees and spreads through root anastomosis (functional root graft) (Fig. 8A and B) to the roots of neighboring trees (Mutto-Accordi 1986).

Growth of *C. platani* in the host tissues is greatly influenced by the season of infection. Pilotti et al. (2016) reported that inoculations in spring and summer periods resulted in more rapid fungal growth and death of young *P.* × *hispanica* trees, in comparison with inoculations in late autumn. Very slow growth of *C. platani* during winter end early spring were observed in large *P. orientalis* trees inoculated in late autumn in Greece; however, the fungus grew very rapidly in the following late spring and summer (P. Tsopelas, *unpublished*).

In 1999, Pazzagli et al. (1999) purified cerato-platanin (CP), a noncatalytic protein of 12.4 kDa from culture filtrates of *C. platani*. CP is a stable component of the fungal cell wall (Boddi et al. 2004; Pazzagli et al. 1999). The protein is secreted when the fungus grows both in axenic culture and on plane leaves; in the latter situation, the CP gene is expressed earlier (Bernardi et al. 2011; Scala et al. 2004). CP elicits defense-related reactions from both host and nonhost plants; in plane leaves, it causes cell plasmolysis, programmed cell death, production of hydrogen peroxide, nitric oxide, and phenolic compounds, localized resistance, and overexpression of defense-related genes (Bennici et al. 2005; Fontana et al. 2008; Lombardi et al. 2010; Pazzagli et al. 1999; Scala et al. 2004). CP appears to behave as a pathogen-associated molecular pattern (PAMP), able to trigger the basal defense system. CP expression is positively correlated to fungal growth and the formation and differentiation process of aleurioconidia. Because it is localized in the fungal cell wall, it can be assumed that CP plays a role in growth and developmental processes of *C. platani* (Baccelli et al. 2012). Cerato-platanin was the first member of the CP family to be characterized. Members of the CP protein family are now known to be secreted by a number of phytopathogenic fungi, animal pathogens, and nonpathogenic fungal species (de Oliveira et al. 2011). The role of these proteins remains unknown but they are reported as virulence factors and effectors, and they play a major role in animal-fungus and fungus-fungus interactions (Gomes et al. 2015).

Pathways of Pathogen Spread

All spore types of *C. platani* are formed on the surface of wounded tissues, such as pruning wounds, cut stumps, and also under the bark of the cankered areas (Baker Engelbrecht and Harrington 2005; Panconesi 1999). Aleurioconidia of the fungus are also produced in abundance inside the xylem vessels of infected trees 10 to 20 days following infection. *C. platani* survives for several months or years in the form of aleurioconidia in the wood of diseased trees (Engelbrecht et al. 2004; Grosclaude et al. 1996). The pathogen could also survive for more than 105 days in soil during the winter, but temperatures of 35 to 40°C negatively affected the survival of the fungus in soil (Mutto Accordi 1989).

All evidence suggests that spores of this fungus, as well as in other species of the *C. fimbriata* complex, are not airborne (Kile 1993; Panconesi 1999). Although spores can be transmitted by rain splash, hail, insects, etc., the pathogen appears to spread primarily in infected

wood fragments (sawdust, wood chips, etc.) arising from human activity (Grosclaude et al. 1996; Vigouroux 1979a).

Contaminated pruning and cutting tools are the main pathways of disease transmission, in the case of both long and short distance spread. Spores of *C. platani* can remain viable on metal surfaces for a few weeks and still give rise to infections (Crone 1962; Walter 1946). However, the pathogen is mostly transferred from diseased to healthy trees by infected sawdust on chainsaws or other pruning and cutting tools (Panconesi 1999; Vigouroux 1979a). Pruning tools were responsible for disease spread in urban environments in the northeastern United States in 1930s and 1940s (Walter et al. 1952) and also in Italy and France after the introduction of the pathogen in the 1940s (Panconesi 1999). In many areas of Greece, *C. platani* has been spread by teams undertaking line-clearing in infected sites and then moving to disease-free areas (Tsopelas and Soulioti 2011).

Terracing machinery also play a major role in disease spread. When used in contaminated sites, this type of machinery can carry wood debris or even contaminated soil into disease-free areas and cause new infections on the wounded roots of plane trees (Ocasio Morales et al. 2007; Vigouroux 1979a). In this way, *C. platani* can be spread for long distances. It has thus been speculated that the pathogen was transferred from Peloponnese in southern Greece into Epirus in northwestern Greece (about 350 km) with terracing machinery (Tsopelas and Soulioti 2014). Also, in Spain, the first confirmed outbreak of *C. platani* was caused by a machine during the installation of a gas pipe line in Catalonia, which is adjacent to infected areas in France (Maire 2011).

In riparian woodlands, plane trees grow in close proximity to each other and *C. platani* spreads readily via root grafts (Fig. 8A). It also moves via water; logs and branches from infected dead trees that are carried downstream cause root wounding and new infections, especially during flood events (Ocasio Morales et al. 2007). Spores of the fungus, especially aleurioconidia, can survive in river water and may cause infections on roots damaged by rolling pebbles and other material carried by the water (Grosclaude et al. 1991; Vigouroux and Stojadinovic 1990).

C. platani can be transported long distances on wood used for packaging, as has been widely accepted as the mode of introduction of the fungus into Europe from the United States (Cristinzio et al. 1973; Panconesi 1999). Movement of firewood from infected to disease-free areas can contribute to pathogen spread. Sawdust produced during firewood preparation or even sanitary operations can be carried by the wind to freshly wounded healthy plane trees (pruning or any other type of wounds) where new infections are then initiated (Panconesi 1999). Luchi et al. (2013), using a real-time PCR assay, demonstrated the presence of *C. platani* at distances up to 200 m from infected trees during sanitary felling operations. Sawdust and other forms of contaminated wood debris can also be transported long distances when they enter water courses (Panconesi 1999).

C. platani can be moved to disease-free areas with infected nursery stock and this is a possible pathway for international transport of the fungus (Ocasio Morales et al. 2007). Infected young plants show symptoms within a few weeks after infection and die rapidly, but during winter months the fungus grows slowly in plant tissues (Pilotti et al. 2016). Disease symptoms are also not evident during the winter because plane trees are deciduous and consequently, infected nursery stock can escape the attention of inspections. *C. platani* can survive for long periods of time in the soil (Mutto-Accordi 1989) and in this way it can be transferred with nonhost plants for planting to other areas. However, it is very unlikely for fungal propagules in soil to come into contact with wounded roots of plane trees and to initiate new infections.

Wood infected by *C. platani* has a fruity odor that is also very common in the case of many species in the Ceratocystidaceae (de Beer et al. 2014) and other ophiostomatoid fungi (Wingfield et al. 1993), which is considered as an adaptation for fungal dispersal by insects. Bark and ambrosia beetles are thus attracted to diseased trees and can carry spores of these fungi to healthy trees initiating infections (Ploetz et al. 2013). Crone (1962) showed that *C. platani* can be transmitted by sap-feeding beetles of the Nitidulidae. This was also demonstrated recently for the ambrosia beetle *Platypus cylindrus* Fab. (Soulioti et al. 2015). Spores of the fungus, especially aleurioconidia, can also survive passage through the guts of insects. *C. platani* has been isolated from the frass of *P. cylindrus* (Ocasio-Morales et al. 2007), while Crone (1962) demonstrated that frass of the nitidulid *Carpophilus lugubris* Murray could infect wounded plane trees. Aerial dispersal or river-water dispersal of frass thus represent effective means for new infections to arise (Ocasio-Morales et al. 2007), as has also been suggested for other species in the Ceratocystidaceae (Harrington 2013; Iton 1966; Kile 1993).

The lace bug *Corythucha ciliata* Say (Tingidae) is native to North America and has spread into many areas of southern and central Europe. It feeds on the undersides of the leaves of *Platanus* spp. causing a white stippling that can develop into intense bronzed foliage and premature leaf fall (CABI 2014). Panconesi (1999) speculated that this insect does not play a role in *C. platani* transmission since it does not come in contact with infected woody tissues in its life cycle. This is also the view of the senior author arising from observations in areas in Greece close to *C. platani* infection centers that were heavily infested by *C. ciliata*.

Disease Impact

CSD is a devastating disease, causing high levels of mortality of plane trees in both the United States and Europe. Huge losses of trees have been recorded in urban areas, including trees of considerable aesthetic value in city parks and streets where *C. platani* occurs. The economic cost is substantial for tree removal and replacement in cities affected by the disease. Extensive damage has also been reported in natural stands as well as plantations of plane trees. Mass tree death in the riparian vegetation has a tremendous negative impact on the environment in certain areas of Greece, where the disease has assumed epidemic proportions (Ocasio-Morales et al. 2007; Panconesi 1999; Tsopelas and Soulioti 2013; Walter 1946).

In the 1930s and 1940s, CSD caused extensive tree mortality in many of the cities of the northeastern United States where *P.* × *hispanica* was widely planted as an ornamental. In Gloucester, NJ, where the disease was first detected in the 1920s, over 90% of the trees were infected and killed by the pathogen by 1949. Likewise in Philadelphia, PA, about 10,000 plane trees had died due the disease by 1940 (Walter 1946; Walter et al. 1952). Plane tree mortality was also recorded during the same period in many of the cities in Pennsylvania, Maryland, and New York, as well as Washington, D.C. and other localities of the east coast of the United States (Crone 1962). These epidemics in urban areas of the United States were significantly reduced in the following years by applying effective sanitation practices (Engelbrecht et al. 2004; Walter et al. 1952).

Other than *P.* × *hispanica* in urban plantings, the native plane tree *P. occidentalis*, which is considered moderately susceptible to CSD, has also been affected in the southeastern United States. Infection by *C. platani* has caused significant losses in plantations in Mississippi, Alabama, and Arkansas as well as in natural stands of Louisiana, where rates of infection have reached up to 30% in some cases (McCracken and Burkhardt 1977). *C. platani* has also been very destructive on the California sycamore, *P. racemosa* var. *racemosa* in street plantings of Modesto, CA, where the pathogen has also affected *P.* × *hispanica* and *P. occidentalis* trees (Perry and McCain 1988).

The impact of *C. platani* has been most severe in Europe, where it is an invasive alien pathogen. Since its introduction in the 1940s, *C. platani* has caused extensive mortality of *P.* × *hispanica* trees planted in urban and rural environments (Panconesi 1999). However, the disease has been most devastating in natural stands of the native species *P. orientalis* (Ocasio Morales et al. 2007; Tsopelas and Soulioti 2013).

One of the first disease foci in Italy was reported in the Caserta area close to the port of Naples, where the American Army was stationed during and after World War II. Cristinzio et al. (1973) reported that most of the 200-year-old plane trees lining the Vialone Carlo III, the broad avenue connecting the Royal Palace of Caserta with Naples, were destroyed by the disease. In the city of Forte dei Marmi in Italy, where *C. platani* was recorded for the first time in Europe in 1972, the pathogen destroyed 90% of the plane trees between 1972 and 1991 (Panconesi 1999).

Extensive plane tree mortality caused by *C. platani* has been reported in many areas of Italy; there are several reports from the 1970s to the present showing that the disease is causing considerable losses throughout the country from north to south including Sicily. CSD has been reported in urban plantings in many of the major cities of Italy including Rome, Pisa, Livorno, Florence, Padua, Verona, Parma, Bologna, and Turin (Panconesi 1999). In a recent study in Florence, it was concluded that 39% of plane trees had been felled between 1998 and 2013 mainly due to the disease (Feducci et al. 2013). In Sicily, *C. platani* has affected natural stands of *P. orientalis*; in one of the rivers close to Syracuse, 51% of the plane trees were found to be infected by the disease (Granata and Pennisi 1989).

CSD was very destructive in the areas of southern France where it has spread rapidly. One of the regions that has experienced extensive losses is Provence Alpes Côte d'Azur in southeastern France. In the city of Marseilles where *C. platani* was originally reported in the country, Ferrari and Pichenot (1976) reported that 1,822 plane trees had been destroyed over a 12-year period (1960 to 1972). In more recent years, it has been estimated that about 1,500 to 1,700 infected plane trees were felled annually in the department of Vaucluse due to infection by *C. platani* (Chapin and Arcangioli 2007). The same authors reported that by 2007, more than 30,000 plane trees had been removed over a 25-year period in the region of Provence Alpes Côte d'Azur.

Of particular concern is the spread of CSD along the Canal du Midì that runs through the Midi Pyrénées and the Languedoc-Roussillon regions of France. This is a 240 km long canal constructed in the 17th century that joins the Atlantic Ocean to the Mediterranean Sea through the Garone River. It has been declared a UNESCO World Heritage site and is lined with about 42,000 plane trees. In 2006, *C. platani* was detected in a few foci close to Toulouse (Bonnet and Collet 2007), but the pathogen spread to several locations in the following years. According to a more recent report (VNF 2014), a total of about 13,000 trees were affected by the disease in the Canal du Midì and 9,850 of them had been felled.

In Greece, CSD has had a devastating impact on natural populations of *P. orientalis*, which is considered highly susceptible to infection by *C. platani* (Vigouroux and Olivier 2004). As mentioned earlier in this review, the pathogen has also been reported in Sicily on the same host, but in Greece *P. orientalis* is more common, being the key component tree species in riparian vegetation throughout the country (Panetsos and Alizioti 1996). Since the first detection of *C. platani* in 2003 in southern Greece, the pathogen has been spread throughout the Peloponnese region causing extensive damage. The disease has also been severe in natural stands of *P. orientalis* in the Epirus region of northwestern Greece, where *P. orientalis* is more common than it is in Peloponnese (Tsopelas and Soulioti 2013, 2014).

C. platani has invaded all the major rivers in the Peloponnese and Epirus regions as well as smaller rivers and streams. In some of these rivers, the numbers of *P. orientalis* trees have been drastically reduced; the disease has spread for many kilometers, with several infection foci including 20 to 50 dying trees. In some localities, disease foci have merged with hundreds of dead and dying trees found at a single site. There are no precise inventory data for the disease in Greece, but the number of dead trees in both regions can be estimated to be in the range of tens of thousands (Ocasio Morales et al. 2007; Tsopelas and Soulioti 2011, 2013).

Besides its impact on natural stands in Greece, *C. platani* has caused considerable losses in residential areas and recreational sites. Many amenity plane trees in village and town squares and along roads have died due to the disease. Some of these trees have been of great aesthetic value; the deaths have included trees of enormous size and in some cases more than 5 centuries old, which had been declared as "Monuments of Nature" (Fig. 1B and C) (Tsopelas and Soulioti 2013; P. Tsopelas, *unpublished*).

CSD has also become a very serious problem in Albania. *C. platani* infections are widespread in the southern part of the country neighboring the infected areas of the Epirus region in northwestern Greece. However, there is no detailed information for plane tree mortality in other parts of the country. What is known is that the disease in southern Albania is causing extensive tree mortality on *P. orientalis* trees in natural stands and in residential areas (Tsopelas et al. 2015a).

Disease Management

Quarantine measures are applied in member states of the European Union to prevent the introduction or spread of *C. platani* and there are specific regulations for the movement of plants for planting and wood of *Platanus* sp. (Council Directive 2000/29/EC). The pathogen is also included in the A2 list of quarantine pests and diseases of the European and Mediterranean Plant Protection Organization (EPPO/CABI 1997). Quarantine zones are established in the infected areas, where eradication measures are applied and intensive monitoring for the presence of the pathogen take place (Bouhot-Delduc 2007).

A key issue in disease management is the early detection of the pathogen. In small disease foci, when the disease is detected at the initial stages, the pathogen can be eradicated and the risk of further spread into disease free areas can be minimized (Harrington 2013; Tsopelas et al. 2015b; Vigouroux 2013). Control measures are very difficult to apply when the disease has spread over large areas. In certain sites in natural ecosystems of *P. orientalis* in Greece, the disease has already assumed epidemic proportions and eradication is clearly impossible (Ocasio-Morales et al. 2007; Tsopelas et al. 2015b). However, even in such cases, it is important to apply phytosanitary measures that will result in disease containment and thus avoid further spread of the pathogen into new areas.

Sanitation measures for CSD have been applied since the 1940s in the United States. These included the removal of all infected trees in order to eliminate sources of inoculum and avoid pathogen spread to healthy trees (Harrington 2013; Walter 1946; Walter et al. 1952). Good practice would be to fell and destroy all affected trees as rapidly as possible after they become infected (Vigouroux 2013). When infections are initiated at the branches and are limited to these parts, trees can be saved by careful pruning (Panconesi 1999; Walter 1946). However, in most cases the pathogen has spread in to the trunk and possibly to the roots by the time that symptoms are evident on the crown of the trees and such practices are not very effective (P. Tsopelas, *unpublished*).

During felling operations, an enormous amount of sawdust is produced, which is then carried in air currents or wind, facilitating dispersal of *C. platani* (Luchi et al. 2013; Panconesi 1999). It is, therefore, important to collect and destroy the sawdust and small wood particles along with the wood of infected trees. This can be achieved using incineration or by burying infected plant material in sanitary landfills. The use of plastic sheets for the collection of sawdust and other debris has been recommended (Panconesi 1999).

Since *C. platani* spreads to neighboring trees through rootgrafts, Panconesi (1999) recommended the removal of the roots after tree felling. However, where the fungus has spread into the root system, complete removal of infected roots is not possible and quite often the pathogen has already spread into the roots of surrounding trees by the time that felling operations take place (Tsopelas et al. 2015b). The use of herbicides on the living parts of the infected trees and on the surrounding healthy trees has proven to be an effective method to avoid spread of the pathogen to neighboring trees (Ferrieu and Miniggio 2007; Harrington 2013). The fungus does not spread to roots killed by the herbicide through rootgrafts. In this manner, it is possible to establish a "buffer zone" around the infected trees (Grosclaude et al. 1992; Tsopelas et al. 2015b). The fungus survives in the roots of the infected trees for up to 5 years (Maire and Vigouroux 2004), but it slowly deteriorates, due to competition by saprophytic fungi that colonize the infected roots (Grosclaude et al. 1992).

Stem injection (drill and fill) with the herbicide glyphosate has been used effectively in France and Greece. It is applied on healthy trees neighboring diseased trees up to a distance of 35 to 50 m. Using this technique, undiluted glyphosate (36%) or dense concentrations (25 to 50% of the product) are applied directly into the bark and sapwood tissues, by drilling holes in the periphery of the lower part of the stem or even directly on buttress roots (Ferrieu and Miniggio 2007; Grosclaude et al. 1989, 1992; Tsopelas et al. 2015b). Another method

used in southern France is girdling the trunks with a chainsaw, making a horizontal groove through the bark and sapwood followed by the application of undiluted glyphosate (Ferrieu and Miniggio 2007).

In southern France, the best management results have been achieved when herbicide was used in late spring and summer, while applications of glyphosate in early spring (March) were not very effective (Grosclaude et al. 1992). In Greece, glyphosate was very effective in killing healthy *P. orientalis* trees with applications at different periods of the year from mid-April to the end of November (Tsopelas et al. 2015b).

Glyphosate can also be used on the stumps of healthy plane trees neighboring those that are infected and thus preventing infections via root contact. The application of herbicides on the uninfected parts of diseased trees can also reduce the inoculum potential of the pathogen, since the uninfected parts of the tree do not become colonized by the fungus. If the herbicide is used on trees at an early stage where infections have been initiated on the branches, complete eradication of the fungus can be achieved (Tsopelas et al. 2015b).

An undesirable effect of herbicide applications is the damage caused on nontarget trees in the vicinity of those that have been treated. This is because the herbicide moves through root grafts to neighboring trees, affecting their growth or even killing them, especially when the doses are high (Tsopelas et al. 2015b). Therefore, herbicides should be used with care, especially where they are applied in the vicinity of valuable trees.

Transmission of *C. platani* through root anastomoses can be avoided by severing root connections between neighboring plane trees by digging deep trenches between trees. This method is commonly used in the United States for the prevention of spread of the oak wilt pathogen, *C. fagacearum*, between oak trees. Trenches are placed between healthy oak trees at least 30 m away from symptomatic trees (Juzwik et al. 2011). Herbicides can also be used along with the formation of trenches for the control of CSD.

C. platani is mostly dispersed by human activities and precaution measures based on tool and machinery disinfestation have been recommended to minimize the risk of new infections (Harrington 2013; Walter 1946). All hand tools and machinery used at infected sites must be cleaned carefully and disinfested before leaving the site. Disinfested tools should always be used in pruning and tree removal operations of *Platanus* spp. trees in disease-free areas (Bonnet and Collet 2007; Vigouroux 2013).

Different products have been recommended as disinfectants. In France, 70% denatured alcohol has been recommended for the treatment of small tools. For large machinery, disinfectants containing the quaternary ammonium compounds, ortho-phenyl-phenol and 8-hydroxyquinoline sulfate, have been recommended (EPPO/CABI 1997; Ferrieu and Miniggio 2007). A readily available disinfectant is house bleach used at concentrations 10% or higher; however, it is corrosive to metal surfaces and it should be used with care since it is toxic to the skin (Spotts and Peters 1980).

Several fungicides have been tested in vitro and were effective in inhibiting mycelial growth as well as spore germination (Causin et al. 1995; Minervini et al. 2001; Tawil et al. 1982). However, the use of these fungicides for the control of CSD is very limited, since they cannot be applied externally for the protection of healthy trees (Panconesi 1999). Some of these products could be used as disinfectants or they can be incorporated into wound dressings.

Therapeutic treatments have been applied on plane trees through intravascular injection of fungicides, in a way similar to those used to control the oak wilt pathogen, *C. fagacearum* and the Dutch elm disease pathogens, *Ophiostoma ulmi* and *O. novo-ulmi* (Haugen and Stennes 1999; Juzwik et al. 2011). Some of the fungicides (propiconazole, carbendazim, Imazalil) reduced the extension of *C. platani* in the vascular system in comparison with untreated plants. However, there were no cases where the fungicides were able to eradicate the pathogen in the wood or bark tissues (Causin et al. 1995; Minervini et al. 2001; Panconesi 1999; P. Tsopelas, *unpublished*).

As mentioned previously, some work has been done in France and Italy to exploit host-resistance (Pilotti et al. 2009; Vigouroux and Olivier 2004). The resistant hybrid clone developed in France, as well as those that could emerge from the genetic improvement program in Italy, could be used in diseased areas, especially in replanting infected sites by *C. platani*. However, they cannot replace oriental plane trees in natural stands and the magnificent centuries-old plane trees declared as Protected Monuments of Nature in many areas of Europe and Asia (Tsopelas and Soulioti 2013).

Conclusions

C. platani is indigenous to North America. In the 1930s and 1940s, the disease assumed epidemic proportions in the northeastern United States affecting mainly *P.* × *hispanica*, a susceptible tree that had been widely planted as an ornamental in many cities. In contrast, CSD occurred only at low levels in natural forests of the native host *P. occidentalis*, although it was quite frequent in plantations of this tree species in the southeastern states (McCracken and Burkhardt 1977; Walter et al. 1952).

P. × *hispanica* was also the main host tree affected in Italy and France and the disease also occurred in natural stands of *P. orientalis* in Sicily. *C. platani* is believed to have been introduced into Italy and France in the 1940s, but was only detected in the early 1970s. This late diagnosis of the disease had the unfortunate result of allowing the pathogen to spread widely both in Italy and France, killing tens of thousands of plane trees in urban areas as well as in natural forests. In stark contrast, the early detection of *C. platani* in Switzerland in 1986 and the implementation of eradication measures made it possible to contain the disease and ensured only limited spread (Chapin and Arcangioli 2007; Panconesi 1999).

C. platani has resulted in very severe damage in many areas of Italy and France, but the pathogen resulted in an even more catastrophic situation when it reached the native *P. orientalis* in Greece and Albania. The result has been that many iconic trees of impressive size have died; this includes trees that had been declared "monuments of nature" (Tsopelas and Soulioti 2013; P. Tsopelas, *unpublished*). In Greece, this was also the first time that the pathogen had invaded extensive natural ecosystems of this very susceptible host. Accepting the fact that the disease is only in the first stages of expansion in most localities, the impact of the pathogen is likely to be much greater in the near future. In all likelihood, it will result in the complete elimination of *P. orientalis* in many areas of the region (Ocasio Morales et al. 2007; Tsopelas et al. 2015a).

There are unconfirmed reports for the presence of *C. platani* in Armenia and Iran and it was recently detected in the European part of Turkey (H. T. Dogmus, *personal communication*); it is also possible that the pathogen is already present in other countries of western Asia where it has not yet been reported. The spread of the pathogen in the Balkan Peninsula represents a very critical threat. All available evidence suggests that *C. platani* will spread to other neighboring Balkan countries and through Turkey to other countries in southwestern Asia where *P. orientalis* occurs naturally. Likewise, the pathogen could also easily spread northward in central Europe on *P.* × *hispanica*, placing one of the most commonly planted urban tree species in the world at risk.

Canker stain disease can be managed where it is detected in the early stages of infection and where the number of affected trees is limited. The use of herbicides to kill infected as well as neighboring trees and limit spread has proven to be effective in disease eradication in France, Italy, and Greece (Ferrieu and Miniggio 2007; Tsopelas et al. 2015b). In addition, the disinfection of pruning and cutting tools as well as terracing machinery can greatly limit pathogen spread. Eradication methods are more efficient in residential areas and parks where the number of host trees is limited. In natural stands and plantings along rivers and canals, disease eradication or even containment is difficult, if not impossible. *C. platani* is spreading rapidly along the Canal du Midi in southern France irrespective of the intensive control measures that are being applied. The situation is even more difficult in the natural stands of *P. orientalis* in Greece and Albania; in some of the rivers and streams in Greece, the disease has reached unmanageable levels with thousands of trees dead and dying. Clearly this is a situation that is out of control.

A major ecological disaster is in progress in natural stands of *P. orientalis* in southeastern Europe and it is one that will likely extend to western Asia. This is due to the introduction of a non-native and very aggressive pathogen that remains virtually unknown to most forest pathologists, let alone plant pathologists globally. It

clearly deserves much more attention both in terms of scientific investigation and also from a sociological standpoint.

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