Forest Pathology

For. Path. 45 (2015) 367–372

doi: 10.1111/efp.12176

Platypus cylindrus, a vector of *Ceratocystis platani* in *Platanus orientalis* stands in Greece

By N. Soulioti[1,2,3], P. Tsopelas[1] and S. Woodward[2]

[1]ELGO 'DEMETER' – Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28, Ilissia, Athens, Greece; [2]Department of Plant and Soil Science, School of Biological Sciences, University of Aberdeen, Cruickshank Building, Aberdeen AB24 3UU, UK; [3]E-mail: soulioti@fria.gr (for correspondence)

Summary

The ambrosia beetle *Platypus cylindrus* was very common in stands of *Platanus orientalis* trees in Greece, infesting trees already infected by *Ceratocystis platani*. The fungus *C. platani* was isolated from 59% of adult beetles of *P. cylindrus* collected on the wing in a heavily infected stand of *P. orientalis*. Transmission of *C. platani* was demonstrated in 80% of *P. orientalis* seedlings challenged with *P. cylindrus* beetles, which were previously fed in cultures of the fungus. In most of the cases, the beetles had bored tunnels in stems, where abundant perithecia of *C. platani* were observed. *Platypus cylindrus* adults regularly visited artificially wounded *P. orientalis* trees in a natural stand. Although this ambrosia beetle normally infests stressed or dead trees, it appears to play a role as a vector of *C. platani*, especially by visiting wounded trees.

1 Introduction

Canker stain, caused by the Ascomycete *Ceratocystis platani* (JM Walter) Engelbrecht & TC Harrington is the most destructive disease of *Platanus* trees known. Although probably native to the south west of North America, the pathogen was found in ornamental plantings of *Platanus* spp. in Italy and France following the Second World War (Panconesi 1972; Ferrari and Pichenot 1976) and has since spread throughout the Italian peninsula and many areas of France; it has also been reported in Switzerland and Spain (Ruperez and Munoz 1980; Matasci and Gessler 1997). In late 2003, the disease was first observed in natural stands and ornamental plantings of *Platanus orientalis* L. (oriental plane) in the Peloponnese, Greece, causing widespread mortality (Tsopelas and Angelopoulos 2004). Since then, *C. platani* has spread in the native range of *P. orientalis* in southern and north-western Greece causing massive epidemics and extensive ecosystem degradation in natural stands of *P. orientalis*, which is very susceptible to this pathogen (Ocasio-Morales et al. 2007; Tsopelas and Soulioti 2011).

Platanus orientalis is the most important riparian tree species in Greece and other countries of the Balkan Peninsula and eastern Mediterranean, with a natural distribution extending eastwards to Anatolia, north Iran to central Asia and west Himalayas (Strid and Tan 2002). As the pathogen is now established in the western range of the natural distribution of *P. orientalis*, millions of individual trees of this species are at risk; serious ecosystem degradation is already underway in Greece, and the pathogen is likely to continue spreading eastwards. In the absence of prompt and intensive management methods to control this major ecological hazard, the pathogen has the potential to eliminate the host species.

The disease is transmitted principally by human activities in both urban and rural environments. Tree wounding with contaminated tools and terracing machinery is the main pathway for infection (Ocasio-Morales et al. 2007). Subsequent spread of the fungus to neighbouring trees is known to occur through root grafting (Mutto Accordi 1986). Infections by *C. platani* can be also initiated through wounds resulting from weather damage, rolling pebbles or other material damaging the roots of trees in riparian ecosystems; in addition, infected dead logs and branch pieces that are carried by the water downstream create new infection centres (Ocasio-Morales et al. 2007).

Ambrosia beetles are species of subfamilies Scolytinae and Platypodinae (Fam. Curculionidae) that transport and favour the growth of certain ambrosia fungi on the walls of the galleries they create, providing a significant food source to both larvae and adults (Inácio et al. 2011). Etymologically, the term 'ambrosia' is derived from Greek mythology, meaning the food of the Olympian gods and thought to preserve their immortality. The genus *Platypus* Herbst. includes many species of ambrosia beetles in different regions of the globe. *Platypus cylindrus* Fab. is a polyphagous species and the most common ambrosia beetle of the Platypodinae in southern Europe attacking mostly stressed or recently killed hardwood trees (Cassier et al. 1996; Tilbury 2010; Inácio et al. 2011).

The ambrosia beetle *P. cylindrus* has been observed to infest oriental plane trees in Greece infected by *C. platani*. Species in the genus *Ceratocystis* produce long-necked perithecia, with passive discharge of adhesive ascospores from evanescent asci, morphological characteristics that have been suggested to result from convergent evolution selected for arthropod dispersal (Rollins et al. 2001; de Beer et al. 2014). Wood of *Platanus* spp. colonized by *C. platani* has a fruity odour attractive to insects, a feature common with other species of *Ceratocystis*, such as *Ceratocystis fagacearum* (Bretz) Hunt, the causal agent of oak wilt disease in North America (Juzwik et al. 2008). *C. platani* has been isolated from frass of *P. cylindrus,* and it has been suggested that it can be dispersed by wind or stream water to tree wounds causing new infections (Ocasio-Morales et al. 2007).

Having a fungal pathogen and an insect occurring in large numbers at infection sites, we examined the possibility whether this beetle acts as a vector of the pathogen causing canker stain disease of *Platanus*. The aim of this work was to investigate whether *P. cylindrus* adults trapped on the wing carried inoculum of *C. platani* and if these beetles were able to

Received: 11.11.2014; accepted: 22.1.2015; editor: O. Holdenrieder

transmit the disease to trees of *P. orientalis*. An artificial wounding experiment was also conducted to determine whether *P. cylindrus* is attracted to fresh wounds on healthy trees.

2 Materials and methods

2.1 Occurrence of *Platypus cylindrus*

The presence of *P. cylindrus* was investigated from 2007 to 2013, in natural stands and ornamental plantings of oriental plane infected with *C. platani* in several sites in the Peloponnese and Epirus regions of Greece. Infected trees were examined for the presence of frass from boring insects on the main stem and the branches. Adult beetles of *P. cylindrus* were collected from galleries, by excavating the infested wood and bark. Species identity was confirmed using stereomicroscope (Zeiss, Stemi 2000-C) observations, and in many cases, the sex of the beetles was also determined.

2.2 Collection of beetles

Beetles were trapped in a stand of oriental plane heavily infected by *C. platani*, alongside the Rhodia stream, a riparian area in Messenia Prefecture, SW Peloponnese (WGS84′; latitude: N 37°12′/longitude: E 21°43′, altitude: 390 m). Many of the recently dead and dying trees were infested by *P. cylindrus*; this was recognized by the presence of abundant frass on the trunks of the trees arising from gallery entrances.

Two intercept flight bark beetle traps (Thyssen, Trofolio-M.gmbH) were placed under the canopy of selected trees at approximately 1.6 m above the ground. Each trap was baited with two cultures of an isolate of *C. platani* previously obtained from an infected tree at the same site. Cultures were maintained in 90 mm diameter Petri dishes on 2% malt extract agar (MEA; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and incubated at 25°C for 14 days before use, when abundant production of perithecia had occurred. Immediately prior to placing in the traps, the lids of the Petri dishes were removed and the dishes wrapped in clear food film (Sanitas-Sanitas S.A.). A Vapona® insecticide sheet was placed in the base of the collection container of each trap to kill trapped insects. Traps were inspected daily, and any insects caught stored in sterilized 1.5-ml Eppendorf tubes at 4–6°C until transferred to the laboratory. Collections were made during August 2008. Diurnal temperature fluctuations during collection were from 23 to 36°C.

Living adult beetles were also collected from two infested tree trunks in the same area, using disinfected tools (axe, chisel and forceps) to recover galleries; a 30-ml entomological pooter was used to catch the insects. Insects were placed individually in clean sterilized 20-ml tubes adding also a small amount ($10 \times 10 \times 50$ mm^3) of healthy freshly cut material consisting of wood with attached bark, from the host plant. Tubes were stored at 4–6°C in a cool box, to facilitate survival during transfer to the laboratory.

2.3 Isolation of *Ceratocystis platani* from beetles

The presence of the pathogen on *P. cylindrus* adults was examined using a modification of the *C. platani* trapping technique described by Grosclaude et al. (1988). Each insect was rinsed with sterile distilled water to remove wood particles and other debris. Conical flasks (500 ml), each containing 300 ml deionized water and a Pasteur pipette, were plugged with cotton wool and autoclaved at 121°C, 105 kPa for 30 min. Three freshly cut healthy twigs of *P. orientalis* (*circa* 10 cm long and 7–10 mm diam) were added to each flask. These twigs were previously stripped of bark over approximately 2/3 of their total length and surface disinfected with 70% ethanol for 10 min, then rinsed with sterile distilled water. A dead adult beetle was placed in each flask and fresh air was continuously pumped into the flask through the Pasteur pipette, using a regular aquarium pump (Aqua-Air AP4, Interpet); this air flow also kept dead beetle circulating into the water. In total, 22 flasks were prepared and incubated at approximately 25°C for 2 weeks. After incubation, twigs were examined under a stereomicroscope for the presence of perithecia of *C. platani*. The fungus was isolated by transferring ascospore masses from the tip of the perithecial neck with a sterilized needle to Petri dishes with MEA amended with 200 mg/l streptomycin sulphate (MEA-S).

2.4 Vector trial

Living adult beetles collected from galleries were also sexed; subsequently, females and males were treated separately. Beetles were transferred into 14-day-old cultures of *C. platani* in Petri dishes with perithecia for 30 min, prior to being used to artificially infest healthy trees. No ‘clean’ beetles were used as reference as these could not be grown in dedicated laboratory facility. Each beetle was placed in a ‘cage’ comprising a small piece of silicon tube (15 mm long, 5 mm diam.), one end of which was capped with a mesh screen (30×150 Micronic filter cloth ‘T-316’ stainless steel). The open end of the tube was firmly attached over a wound using Parafilm®, prepared using a 5-mm cork borer to remove the bark, on the stem of a healthy 2-year-old *P. orientalis* seedling. Mean stem diameter was 8.6 mm (5–14 mm) at the point where the cage was placed (Crone 1962; Fraedrich et al. 2008).

The seedlings used in this experiment were raised in a nursery in Attica prefecture, from a local seed source of *P. orientalis* and planted into 7.5 l pots. At the time of inoculation, they were one and a half year old, 70–100 cm tall, with a stem diameter at the root collar of 5–12 mm.

Ten female and 10 male *P. cylindrus* adults were used on a total of 20 plants. Cages were left in place for 3 weeks. During the interaction period, temperatures ranged from 19 to 37°C, and rainfall occurred twice. All seedlings were observed for symptoms during this period. After the interaction period, the plants were harvested and transferred to the laboratory before removal of cages. The condition of each beetle was recorded and the bark around the wound removed to observe symptoms and signs of infection, and tunnel development. Lesion sizes were measured, the presence of perithecia around the wound checked under a stereomicroscope and isolations made from the lesion boundaries onto MEA-S. The development of healing callus around the wound, if present, was also recorded.

2.5 Wounding experiment

Late summer of 2009, in the same heavily infested stand where beetles were collected (Rhodia stream), 10 healthy *P. orientalis* were selected upstream from the infected trees and artificial wounds made on the stems at approximately 1.3 m above ground. One *P. orientalis* recently infected by *C. platani* was also selected at the borders of the nearby locus of infection, as a control; this tree was wounded and treated in the same way as the other ten. A disinfected chisel was used to remove the bark in blocks of approximately 15 cm^2 exposing the sapwood (Barnes et al. 2003). All wounds were made on the opposite side of the trees relative to the closest infected trees, to reduce the chance of accidental airborne infection by frass or other means. The wounds were covered and secured with a plastic transparent mesh × 750 μm (Plastok® Plastok Ltd, Birkenhead, UK) sprayed with an odourless non-setting adhesive (Oecospray® Oecos, Kimpton, UK). Wounded trees were visited after 1, 3 and 5 weeks and inspected for the presence of *P. cylindrus* adults adhering to the plastic mesh.

3 Results

3.1 Occurrence of *Platypus cylindrus* in infected stands

The presence of *P. cylindrus* on plane trees was evident from the copious frass in the form of sawdust on the bark of trees, mainly at the base of trunks. The entrance holes of galleries were also evident as round holes, 1.5–1.7 mm in diameter. Inside the galleries, perithecia as well as aleurioconidia and endoconidia of *C. platani* were observed covering the walls. Live adult beetles were found inside the galleries on bark and wood 1–5 cm from the entrance holes.

In total (Table 1), *P. cylindrus* was detected in 37 infection sites in the Peloponnese (southern Greece) and 22 sites in Epirus (north-western Greece). Only in 3 infection sites (Table 1), the insect was not detected from a total of 63 sites examined in Greece, those were small infection foci with recently infected trees and treated with glyphosate herbicide to eradicate the disease (Grosclaude et al. 1992). Besides *P. orientalis* trees infested by *P. cylindrus*, in two cases in Peloponnese trees of *Platanus* x *acerifolia* Wild. (London plane) were also found infested by this insect. This ambrosia beetle was not found on non-infected plane trees and was rare on trees recently infected by *C. platani*; it was present in large numbers on trees infected for longer periods of time (1–3 years), some of which were already killed by the disease. On recently infected trees, entrance holes were on the infected (stained) part of the wood but not on healthy wood of the same tree.

3.2 Presence of *Ceratocystis platani* on *Platypus cylindrus*

Using the trapping technique, *C. platani* was positively detected from 13 of 22 (59.1%) adults of *P. cylindrus* caught on the wing; 11 males of 14 (78.5%) and 2 females of 8 (25%) were carrying the fungus. Under the stereomicroscope, abundant perithecia were observed on the plane twigs used as baits; the fungus was consistently isolated from ascospore masses.

3.3 Vector trials

Three weeks after the plane seedlings were challenged with pathogen-infested *P. cylindrus* beetles, 16 of the 20 plants used were infected by *C. platani*, with both sexes of insects showing equal success in transferring the fungus to the host trees

Table 1. Occurrence of *Platypus cylindrus* on *Platanus* spp. in different areas of Greece.

		No of localities		
Region	Prefecture	Present	Not Detected	*Platanus* sp.
Peloponnese	Achaea	10		*P. orientalis*
	Arcadia	9	1	*P. orientalis*
	Ilia	6		*P. orientalis*
	Corinthia	2		*P. orientalis*
	Laconia	1		*P. orientalis*
	Messenia	7	1	*P. orientalis*
		2		*P. x acerifolia*
Epirus	Arta	2		*P. orientalis*
	Ioannina	11	1	*P. orientalis*
	Preveza	2		*P. orientalis*
	Thesprotia	7		*P. orientalis*
Total		59	3	

Table 2. Infection by *Ceratocystis platani* of *Platanus orientalis* plants challenged with *Platypus cylindrus* adults fed on cultures of the fungus.

	No of beetles	No of challenged plants	No of infected plants
	10 ♂	10	8
	10 ♀	10	8
Total	20	20	16

(Table 2). Necrotic lesions of 2–62 mm in length were formed on the stems around the point at which the cages were attached. Four seedlings remained lesion free and collenchyma callus closed the wounds. In contrast, no callusing occurred at the point of wounding in seedlings showing lesion development.

At the time of harvesting, one male beetle remained alive; in the remaining traps, five males were dead in the cages or on the host stems, and four had bored through the stem and escaped. Of the female beetles used, seven were dead in the cages or on the stems, whereas three had escaped by boring directly through the stem. Frass was observed inside the cages in most cases, indicating boring activity. No frass was found in the cages of the four uninfected seedlings. In most of the wounds and in the beetle-bored tunnels, abundant perithecia were observed (Fig. 1). *Ceratocystis platani* was consistently re-isolated from stem tissues at the lesion boundaries of the infected stems.

3.4 Wounding experiment

After the 5-week period, the 10 healthy *P. orientalis* were, in most of the cases, regularly visited by *P. cylindrus* adults. More specifically, on 7 of 10 healthy *P. orientalis, P. cylindrus* adults were found stuck in the glue on the plastic mesh (70%). In total 37 *P. cylindrus* adults visited the seven healthy *P. orientalis* (mean 5.3, range 2 to 12). The control (infected) *P. orientalis* was also visited by five *P. cylindrus* adults in total, over the same 5-week period.

4 Discussion

The ambrosia beetle *P. cylindrus* was very common in southern as well as in north-western Greece, found in almost all *P. orientalis* sites infected by *C. platani*, and was also found on a few *P. x acerifolia* trees. *Platypus cylindrus* was also associated with *P. x acerifolia* trees infected by *C. platani* in the Pisa area of Italy (Åke Lindelöw, personal communication). As this beetle is very common in many parts of Europe (Cassier et al. 1996; Tilbury 2010; Inácio et al. 2011), it may be important in other countries where the disease occurs. Infestations by this beetle in Greece were more intense on trees 2–3 years after infection by *C. platani*; this is probably why *P. cylindrus* has escaped wider attention on infected trees in Italy and France, as the number of trees infected by this pathogen in these countries is relatively small and they are usually removed soon after infection.

The presence of *P. cylindrus* in large numbers on infected sites in Greece clearly shows that this polyphagous ambrosia beetle takes advantage of the abundance of suitable breeding material to increase its population. Similarly, in southern England *P. cylindrus* increased rapidly following a hurricane in 1987 that provided large numbers of damaged oak trees, because this insect normally attacks trees that are stressed or recently dead (Tilbury 2010; Inácio et al. 2011). In the present work, *P. cylindrus* was noticed only on infected trees and not on healthy ones; it is worth mentioning though that, in recently infected trees, galleries of *P. cylindrus* were found only on infected wood of the trunk. It is highly likely that the insect was attracted by the fruity volatiles produced by *C. platani* in colonized wood (Harrington 2009) and was boring its galleries only on this part of the trunk. Inside the galleries *C. platani* produces an abundance of perithecia as well as aleurioconidia and endoconidia that provide food for larvae and adults of *P. cylindrus*.

In our study, it was clearly demonstrated that *P. cylindrus* adults carry the fungus *C. platani* when flying close to infected trees. The fungus appeared to be carried more commonly by male (78.5%) than female (25%) adult beetles on the wing; however, sampling of greater numbers of beetles is required to confirm these differences. It was not examined in our work if the fungus was carried in the mycangia or the exoskeleton of the beetles. Earlier works have shown the presence of many different ophiostomatoid fungi (*Raffaelea* spp., *Graphium* spp. and *Ophiostoma* spp.) in the mycangia of *P. cylindrus* (Cassier et al. 1996; Bellahirech et al. 2014). Spores of *C. platani* though, especially ascospores that are produced in sticky masses in the perithecial necks, can easily stick on the exoskeleton of the insects (Harrington 2009).

Ambrosia fungi are highly species specific in relation to insect vectors and are known to be associated with certain members of the Scolytinae and all Platypodinae (Batra 1963). A given species of ambrosia beetle, however, does not necessarily feed on a single fungal species exclusively throughout its lifecycle (Batra 1966). *Platypus cylindrus* is typical ambrosia beetle in terms of feeding habits (Baker 1963); in fact, the mutualistic relationship between *P. cylindrus* and ambrosia fungi is well characterized (Cassier et al. 1996; Inácio et al. 2012).

In our case, an invasive fungal pathogen, *C. platani*, joined a pre-existing beetle, *P. cylindrus*, and a putative mutualism between the insect and the pathogen may starting to happen. Ploetz et al. (2013) presented many examples of this type of pathogen–vector shifts with bark beetle vectors, the most well-known being the Dutch elm disease pathogens (*Ophiostoma* spp.).

Fig. 1. Tunnel bored into stem of *Platypus orientalis* by *Platypus cylindrus* adult. Perithecia of *Ceratocystis platani* are growing inside the tunnel after 14 days and the infected wood around the tunnel is heavily stained.

Ambrosia beetles are not common vectors of fungal pathogens, as they usually attack recently dead or weakened trees (Harrington 2009; Ploetz et al. 2013). However, recent reports of serious pathogens being transported and inoculated by ambrosia beetles are increasing worldwide, for example *Raffaelea quercivora* Kubono & Shin with *Xyleborus quercivorous* in Japan and *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva with *Xyleborus glabratus* in the USA (Kubono and Ito 2002; Harrington et al. 2008). Results in this study showed that *P. cylindrus* is able to transmit the fungus *C. platani* into healthy *P. orientalis* trees and start new infections. Similarly, Crone (1962) in the USA proved that beetles of the family Nitidulidae were able to transmit *C. platani*, but there are no other reports concerning insect vectors of this particular pathogen in North America or Europe where the disease has been present (Panconesi 1999).

Platypus cylindrus usually does not colonize healthy trees; a fact that makes it less likely that *C. platani* is transmitted into healthy *P. orientalis* trees by this beetle. The results did, however, show that regular visits of healthy wounded *P. orientalis* trees were made by *P. cylindrus* adults. Taking into account the large increase of *P. cylindrus* population in infected sites, it is more likely for this ambrosia beetle to transmit *C. platani* into healthy trees wounded by humans or by natural causes. In Portugal, *P. cylindrus* was reported to attack apparently healthy cork oak (*Quercus suber* L.) trees, mostly the recently decorked trees (Inácio et al. 2011). Ambrosia beetles that transmit *Ceratocystis fagacearum*, the causal agent of the oak wilt disease in the USA, are known to be attracted from certain volatiles arising from the sap in fresh wounds of live *Quercus* trees (Kuntz and Drake 1957).

In earlier work carried out in the same area of the Peloponnese *C. platani* was isolated from frass samples collected from two of three diseased *P. orientalis* trees (Ocasio-Morales et al. 2007); in this same work, the authors stated the risk posed by this frass material produced by *P. cylindrus* during aerial dispersal may result in the disease being carried to fresh wounds caused on the trees by other agents (Silveira et al. 2006; Ocasio-Morales et al. 2007).

Although close symbiotic relationships between ambrosia beetles and fungi have been known since 1844 (Hartig 1844), in most cases, fungi associated with ambrosia beetles are not well characterized or remain unknown. More knowledge on the subject will arise as further serious tree pathogens emerge, as in this case with canker stain disease of *Platanus* caused by *C. platani*. In this work, Leach's rules (Leach 1940) were completed for the ambrosia beetle *P. cylindrus* as vector of *C. platani*. It was demonstrated that (i) a close association between the beetle and infected plants, (ii) regular visits of the insect to wounded healthy plants, (iii) the presence of the pathogen on the insect following visitation to a diseased plant and (iv) the development of the disease with pathogen-infested insects. It is probable that other Coleoptera are also involved in transmission of *C. platani* in *P. orientalis* in Greece. Work is currently underway to test this hypothesis and identify other insect species found in association with infected trees.

Acknowledgements

We would like to express our gratitude to Dr Panos V. Petrakis, Research Entomologist at IMFE – Athens, for advice during entomological field and laboratory work. The authors are also grateful to anonymous reviewers for their constructive comments. NS was funded by the Greek scholarship foundation 'IKY'.

References

Baker, J. M., 1963: Ambrosia beetles and their fungi, with particular reference to *Platypus cylindrus* Fab. In: “Symbiotic Associations”, 13th Symp. Soc. General Microbiol., London: UK pp. 232–264.

Barnes, I.; Roux, J.; Wingfield, B. D.; Dudzinski, M. J.; Old, K. M.; Wingfield, M. J., 2003: *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. Mycologia **95**, 865–871.

Batra, L. R., 1963: Ecology of ambrosia fungi and their dissemination by beetles. Trans. Kans. Acad. Sci. **66**, 213–236.

Batra, L. R., 1966: Ambrosia fungi: extent of specificity to ambrosia beetles. Science **153**, 193–195.

de Beer, Z. W.; Duong, T. A.; Barnes, I.; Wingfield, B. D.; Wingfield, M. J., 2014: Redefining Ceratocystis and allied genera. Stud. Mycol. **79**, 187–219.

Bellahirech, A.; Inacio, M. L.; Bonifácio, L.; Sousa, E.; Ben Jamâa, M. L., 2014: Comparison of fungi associated with *Platypus cylindrus* F. (Coleoptera: Platypodidae) in Tunisian and Portuguese cork oak stands. IOBC/WPRS Bull **101**, 149–156.

Cassier, P.; Lévieux, J.; Morelet, M.; Rougon, D., 1996: The mycangia of *Platypus cylindrus* Fab. and *P. oxyurus* Dufour (Coleoptera: Platypodidae). Structure and Associated Fungi. J. Insect Physiol. **42**, 171–179.

Crone, L. R., 1962: Symptoms, spread, and control of canker stain of plane trees. PhD thesis. Rutgers-the State University, USA.

Ferrari, J. P.; Pichenot, M., 1976: The canker stain disease of plane-tree in Marseilles and in the south of France. Eur. J. Forest Pathol. **6**, 18–25.

Fraedrich, S. W.; Harrington, T. C.; Rabaglia, R. J.; Ulyshen, M. D.; Mayfield, A. E. I. I. I.; Hanula, J. L.; Eickwort, J. M.; Miller, J. D. R., 2008: A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Dis. **92**, 215–224.

Grosclaude, C.; Olivier, R.; Pizzuto, J. C.; Romiti, C.; Madec, S., 1988: Detection of *C. fimbriata* f. sp. *platani* by trapping. Application to the study of the persistence of the parasite in infected wood. Eur. J. Forest Pathol. **18**, 385–390.

Grosclaude, C.; Olivier, R.; Pizzuto, J. C.; Romiti, C., 1992: La devitalisation du platane. Essais realises avec le glyphosate. Phytoma **440**, 37–38.

Harrington, T. C., 2009: The genus Ceratocystis. Where does the oak wilt fungus fit? In: Proceedings of the 2nd National Oak Wilt Symposium. Ed. By Appel, D. N.; Billings, R. F. Austin, TX: International Society of Arboriculture – Texas Chapter, pp. 27–41.

Harrington, T. C.; Fraedrich, S. W.; Aghayeva, D. N., 2008: *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauraceae. Mycotaxon **104**, 399–404.

Hartig, T., 1844: Ambrosia des *Bostrychus dispar*. Allg. Forstund Jagdzeitung **13**, 73–74.

Inácio, M. L.; Henriques, J.; Guerra-Guimarães, L.; Gil Azinheira, H.; Lima, A.; Sousa, E., 2011: *Platypus cylindrus* Fab. (Coleoptera: Platypodidae) transports Biscogniauxia mediterranea, agent of cork oak charcoal canker. Bol. San. Veg. Plagas **37**, 181–186.

Inácio, M. L.; Henriques, J.; Lima, A.; Sousa, E., 2012: Ophiostomatoid fungi associated with cork oak mortality in Portugal. IOBC/WPRS Bull **76**, 89–92.

Juzwik, J.; Harrington, T. C.; McDonald, W. L.; Appel, D. A., 2008: The origin of *Ceratocystis fagacearum*, the oak wilt fungus. Annu. Rev. Phytopathol. **46**, 13–26.

Kubono, T.; Ito, S., 2002: *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle (*Platypus quercivorus*). Mycoscience **43**, 255–260.

Kuntz, J. E.; Drake, C. R., 1957: Tree wounds and long distance spread of oak wilt. Phytopathology **47**, 22.

Leach, J. G., 1940: Insect Transmission of Plant Diseases. New York and London: McGraw-Hill.

Matasci, M.; Gessler, C., 1997: Ein pilz bedroht die existenz der platane. Acta Vet. Hung. **45**, 69–75.

Mutto Accordi, S., 1986: Diffusione di *Ceratocystis fimbriata* attraverso le anastomosi radicali. Inf. Fitopatol. **36**, 53–58.

Ocasio-Morales, R.; Tsopelas, P.; Harrington, T. C., 2007: Origin of *Ceratocystis platani* on native *Platanus orientalis* in Greece and its impact on natural forests. Plant Dis. **91**, 901–904.

Panconesi, A., 1972: I nostri platani sono in pericolo. Inf. Fitopatol. **22**, 10–13.

Panconesi, A., 1999: Canker stain of plane trees: a serious danger to urban plantings in Europe. J. Plant Pathol. **81**, 3–15.

Ploetz, R. C.; Hulcr, J.; Wingfield, M. J.; De Beer, W. Z., 2013: Destructive tree diseases associated with ambrosia beetles: black Swan events in Tree Pathology? Plant Dis. **97**, 856–872.

Rollins, F.; Jones, K. G.; Krokene, P.; Solheim, H.; Blackwell, M., 2001: Phylogeny of asexual fungi associated with bark and ambrosia beetles. Mycologia **93**, 991–996.

Ruperez, A.; Munoz, C., 1980: Nuevas causas de desaparicion del platano. Bol. Serv. Def. Plag. Insp. Fitopatol. **6**, 106–107.

Silveira, S. F.; Harrington, T. C.; Mussi-Dias, V.; Engelbrecht, C. J. B.; Alfenas, A. C.; Silva, C. R., 2006: *Annona squamosa*, a new host of *Ceratocystis fimbriata*. Fitopatol. Bras. **31**, 394–397.

Strid, A.; Tan, K. (eds), 2002: Flora Hellenica, Vol. **2**. Ruggell: A.R.G. Gantner Verlag K. G, pp. 358.

Tilbury, C., 2010: Oak pinhole borer *Platypus cylindrus* (Coleoptera: Curculionidae). Tree Health division - Tree pest. Advisory Note. Forest Research.

Tsopelas, P.; Angelopoulos, A., 2004: First report of canker stain disease of plane trees, caused by *Ceratocystis fimbriata* f. sp. *platani* in Greece. Plant. Pathol. **53**, 531.

Tsopelas, P.; Soulioti, N., 2011: New records on the spread of canker stain disease in natural ecosystems of oriental plane in Peloponnese and Epirus, Greece. In: Proc. 15th Nat. Forestry Congress, Karditsa. October, 16-19, 2011. pp. 350–359. (in Greek)