

Original article

Fungal trunk pathogens and drought stress are serious threats to London plane (*Platanus x hispanica*) trees in northern ItalyGuglielmo Lione^a, Vladimiro Guarnaccia^a, Alina Veronica Martiniuc^a, Giorgio Costa^b, Pier Mario Travaglia^c, Paolo Gonthier^{a,*}^a Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, Grugliasco I-10095, Italy^b Azienda Servizi Territoriali (A.S.Ter.) Genova S.p.A., Via XX Settembre 15, Genova I-16121, Italy^c Unità verde pubblico - Patrimonio arboreo, Città di Novara, Via dei Tornielli 5, Novara I-28100, Italy

ARTICLE INFO

Handling Editor: Dr Cecil Konijnendijk van den Bosch

Keywords:

Botryosphaeriaceae
Climate change
Fungi
Latent pathogens
Neofusicoccum parvum
Syndrome
Virulence

ABSTRACT

Sudden diebacks and the mortality of London plane (*Platanus x hispanica*) trees have been investigated at two sites in northern Italy over several years. The main objective of this study was to test whether the observed syndrome was caused by fungal trunk pathogens whose virulence was triggered by drought. Seventy-six trees were inspected for several disease symptoms. The fungi associated with the symptoms were isolated and identified through macro-micromorphological and/or molecular methods, including multilocus phylogenetic analyses of relevant DNA loci. Overall, 1352 fungal isolates were obtained and assigned to 33 morphotypes and 24 species. The most prevalent fungal family associated with symptomatic tissues was *Botryosphaeriaceae* (51 % of isolates), followed by *Diaportheaceae* (11 %). Pathogenicity tests on healthy five-year-old London planes for the most common *Neofusicoccum parvum* species revealed that 60 % of isolates caused necrotic lesions of different size ($p < 0.05$), and this was interpreted as virulence diversity. Pathogenicity tests were conducted on five *Botryosphaeriaceae* species and *Seiridium aquaticum* (*Pestalotiopsidaceae*) under two irrigation regimes. All the tested isolates were re-isolated thereby fulfilling one of Koch's postulates, but *Diplodia seriata*, *Dothiorella iberica* and *S. aquaticum* isolates were not pathogenic under the test conditions, regardless of the irrigation regime. Instead, *Diplodia mutila* and *Lasiodiplodia theobromae* isolates, and virulent strains of *N. parvum* were pathogenic, irrespective of the irrigation regime, and their virulence was significantly increased on drought-stressed trees. The results indicate a clear role of fungal trunk pathogens and drought stress, the latter being further supported by climate analyses, in the onset of the syndrome.

1. Introduction

London plane (*Platanus x hispanica* Mill. ex Münchh.) is a hybrid of the American sycamore (*P. occidentalis* L.) and the oriental plane tree (*P. orientalis* L.), and it is widely used in urban forestry throughout the world. In addition to being a large and fast-growing tree, London plane has been reported to be able to tolerate urban microclimate conditions, soil compaction, air pollution, frost and drought (Franceschi et al., 2023; Hull, 2009), thus making it an ideal tree species for cities in temperate and Mediterranean climates. Not surprisingly, London plane is among the most frequently planted trees in Europe (Pauleit et al., 2002). The suitability of growing London plane in the near future has been pointed out by a recent study that has shown that this tree species has grown more than many others species, despite the current drought and climate

change conditions (Franceschi et al., 2023).

London plane is susceptible to several diseases, including foliar, shoot, root rot and wood decay diseases (Raupp and Gonthier, 2017). However, the health status of London plane is primarily threatened by a destructive fungal disease, namely the canker stain of plane trees, which is caused by the ascomycete *Ceratocystis platani* (J.M. Walter) Engelbr. & T.C. Harr. (Raupp and Gonthier, 2017). This fungus is native to eastern USA, but it was accidentally introduced into Europe during the last century (Raupp and Gonthier, 2017). *Ceratocystis platani* is a European Union quarantine pest (EU-QP) for which eradication and containment measures are compulsory, as stated in different legislations, including the Commission Implementing Regulation (EU) 2022/1629. Thanks to breeding efforts deployed in the past, a London plane cultivar (i.e. Vallis Clausa), which is resistant to *C. platani*, is now available (Vigouroux and

* Corresponding author.

E-mail address: paolo.gonthier@unito.it (P. Gonthier).<https://doi.org/10.1016/j.ufug.2025.128787>

Received 16 September 2024; Received in revised form 14 March 2025; Accepted 17 March 2025

Available online 19 March 2025

1618-8667/© 2025 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Olivier, 2004). Such a cultivar, patented as Platanor®, started to be grown in several cities in Europe in 2005.

A few years ago, in the framework of a monitoring campaign that targeted *C. platani* in the city of Geneva, Switzerland, Pelleteret et al. (2017) documented the dieback of a few London plane trees in association with bark cankers and four *Botryosphaeriaceae* species, namely *Diplodia mutila* (Fr.) Fr., *Diplodia serotia* De Not., *Dothiorella* sp., and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. However, only *N. parvum* was indicated, based on pathogenicity tests, as being an important fungal trunk pathogen on this tree species (Pelleteret et al., 2017). The *Botryosphaeriaceae* family includes important pathogens of trees and other woody plants that are known to infect by means of sexual or asexual spores through wounds and/or natural openings, including lenticels and stomata (Slippers and Wingfield, 2007). Starting from the late 1980's, an increasing body of literature has recognised these fungi as endophytes, i.e. they may be present in asymptomatic plant tissues as latent pathogens (Slippers and Wingfield, 2007). Although some species in the family can cause disease in unstressed plants (Mullen et al., 1991), various stresses, including drought, have been reported to enable these latent pathogens to shift from an endophytic to a parasitic phase, thereby causing disease (Slippers and Wingfield, 2007). The importance and impact of *Botryosphaeriaceae* on a range of agricultural crops and forest ecosystems under Mediterranean climates, often in association with environmental stressors, have recently been reported (Aiello et al., 2023; Galarneau et al., 2019; Linaldeddu et al., 2023; Manetti et al., 2024; Martino et al., 2024b). However, little is known about the impact, symptomatology or the course of the disease, i.e. the evolution of symptoms associated with *Botryosphaeriaceae*, on London plane trees, or whether such symptoms are prevalent in young or mature trees. In general terms, and regardless of the hosts, very little is also known about the variability in virulence of populations of these pathogens (Martino et al., 2024a). In addition, it is still unknown whether *N. parvum* or other *Botryosphaeriaceae* may behave as endophytes in London plane and whether disease expression can be enhanced by drought on the host.

Diebacks and the death of branches of London plane trees, associated with the appearance of bark cankers, which often result in the death of trees, have been observed in the cities of Genoa and Novara, in North West Italy, since 2017 and 2019, respectively. Such disease symptoms were also observed on individuals of the Platanor® cultivar in Genoa. However, it was excluded, on the basis of official inspections conducted by the Italian National Plant Protection Organisation (NPPO), that those symptomatic trees were affected by *C. platani*. The significance of such a syndrome, which involves high value trees, such as those belonging to the Platanor® cultivar in Genoa and monumental veteran trees in Novara, combined with its unknown aetiology, prompted the thorough investigation described in this paper.

The overall aims of this work have been to characterise the syndrome observed in North West Italy on London planes and to test the hypothesis that such a syndrome is caused by fungal pathogens whose virulence is triggered by drought stress. Specifically, our work was aimed at: 1) describing the symptomatology, the incidence and the course of the syndrome over a period of three years; 2) identifying and quantifying the fungi associated with the symptoms, and checking whether those fungi were also present in the asymptomatic trees; 3) assessing the variability of virulence of the most frequent fungus encountered on symptomatic trees; 4) inferring the role of climate change, with particular reference to drought, in the onset of the syndrome. To assess the effects of drought stress on plants, approaches based on quantitative indices such as the De Martonne Aridity Index (DMAI; Gavrillov et al., 2019) and the Environmental Disease Component Index (EDCI; Garbelotto, 2024) have been recommended. While DMAI is suitable to investigate climatic aridity at the site scale, EDCI was specifically designed to appraise the interaction between drought and fungal plant pathogens on the host plant. Hence, in this study, we used both DMAI and EDCI to fulfil the goal related to the point 4 above.

2. Material and Methods

2.1. Field observations and samplings

Surveys of London plane trees, including Platanor®, were carried out over three years both in Genoa, starting from autumn 2019, and in Novara, starting from autumn 2020. A total of 57 trees from five sites (Parco dell'Acquasola, Via Cecchi, Corso Galliera, Piazza Palermo, Corso Sardegna) and from one nursery (Vivaio Doria) in the city of Genoa were surveyed. A total of 19 trees from three sites (the Monumental Giardino dei Giusti - Parco dell'Allea, Largo Bellini, Viale Turati) in the city of Novara were surveyed. The surveys targeted dead, symptomatic and asymptomatic trees. The characteristics of the surveyed trees are presented in Table 1.

Each tree was carefully inspected to identify the presence of any disease symptom, including defoliation, dieback, epicormic shooting, trunk and branch cankers with or without liquid leakage, and death.

The samplings targeted the most relevant symptoms observed on each tree, with special emphasis on bark lesions, cankers and dead branches. After bark removal, wood chips (approximately 2.5×2.5×1.5 cm) were extracted, using a sterilised chisel, from the margin between healthy and necrotised cambium tissues, and were then placed individually into Falcon tubes before being transferred to the laboratory. Samplings were conducted on dead and asymptomatic trees using the same method, and randomly selected points were targeted on the main stems.

In order to investigate the course of the syndrome and determine whether its incidence and symptoms were worsening over time, 21 of the symptomatic and asymptomatic trees were re-inspected in Genoa and 12 in Novara. Re-inspection was conducted two years after the first survey (autumn 2021) in Genoa and annually in the autumn for an additional two years in Novara (2021 and 2022).

2.2. Fungal isolation and identification

Falcon tubes containing samples were stored at 4 °C before fungal isolations. Inner slivers (approximately 0.5X0.3X0.3 cm) were obtained from wood chips collected in the field, using a sterile scalpel, and under a biosafety cabinet (Gelaire ICN Biomedicals, Australia). The wood slivers were surface sterilised in a sodium hypochlorite aqueous solution (1 % w/v) for five seconds, and subsequently rinsed in sterile water for another five seconds and were then plated in 9 cm diameter Petri dishes filled with Potato Dextrose Agar (PDA; Merck KGaA, Darmstadt, Germany). Five slivers from each chip were plated in a single Petri dish, while maximising the distance between the slivers.

The Petri dishes were sealed with Parafilm®, incubated in the dark at 25 °C and checked for fungal growth daily for two months. The mycelia that grew from each sliver were transferred to 6 cm diameter Petri dishes filled with PDA to obtain pure fungal cultures. These pure fungal cultures were organised into morphotypes. A subsample of isolates per morphotype, consisting of at least one and up to eight randomly selected isolates classified as belonging to that morphotype, was chosen for macro- and micromorphological and/or molecular identification.

Table 1

Summary of the characteristics of London plane trees inspected and sampled in Genoa and Novara in 2019 and 2020, respectively. Out of brackets specifications for London planes other than Platanor®, in brackets for Platanor® trees.

City	Number of symptomatic trees	Number of asymptomatic trees	Number of dead trees	Minimum, mean and maximum diameter at breast height (DBH), cm
Genoa	29 (13)	21 (3)	7 (2)	6.1, 17.5, 81.5 (8.9, 11.6, 16.2)
Novara	7 (0)	11 (4)	1 (0)	70.1, 107.3, 162.4 (10.4, 11.2, 14.1)

Morphological identification was obtained, at the genus or at the species level, by using dedicated keys (Hanlin, 1990; Phillips et al., 2013; Von Arx, 1981).

Prior to DNA extraction, the isolates were sub-cultured in a 2 % (w/v) liquid malt extract (ME; AppliChem GmbH, Darmstadt, Germany) medium for approximately six days at room temperature for molecular identification purposes. Liquid cultures were harvested by filtration and lyophilised for 48 h. About 100 mg of lyophilised mycelium was pulverised in a 2-ml screw cap tube by shaking for 30 s at 4.5 m s^{-1} in a FastPrep FP120 Cell Disrupter (Qbiogene, Irvine, CA USA) with 2.00 and 6.00 mm-diameter glass beads. DNA extraction was performed on the thus obtained homogenised samples by using the E.Z.N.A.® Stool DNA Kit (Omega BIO-TEK), according to the manufacturer's instructions.

The selected isolates were identified by means of the molecular and phylogenetic analyses of four genomic loci: the nuclear ribosomal internal transcribed spacer (ITS), β -tubulin (*tub2*), the RNA polymerase II gene (*RPB2*) and translation elongation factor-1 α (*tef-1 α*). First, a preliminary sequence analysis of the ITS of all the selected isolates was conducted to identify the fungal genera. Second, isolates characterised as belonging to *Botryosphaeriaceae*, *Diaporthaceae* and *Cytosporaceae* were identified at the species level by means of single and multilocus phylogenetic analyses based on ITS, *tub2* and *tef-1 α* , as previously described (Fan et al., 2020; Marin-Felix et al., 2017, 2019). Namely, *Cytospora*, *Diaporthe*, *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* isolates were identified at the species level by multilocus phylogenetic analyses concatenating ITS, *tub2* and *tef-1 α* , whilst *Dothiorella* isolates were identified through ITS and *tub2* (Martino et al., 2024a). Likewise, isolates belonging to *Fomitiporia* and *Seiridium* were identified at the species level by means of multilocus phylogenetic analyses based on ITS and *RPB2* (Luo et al., 2019), whilst *Ambrosiella* and *Didymosphaeria* isolates were identified by means of ITS phylogenetic analysis (Martino et al., 2024a; Morales-Rodríguez et al., 2021). The employed primers are reported in Supplementary material 1. The PCR mixtures and cycling conditions for ITS, *tub2*, and *tef-1 α* were described by Martino et al. (2024a), whereas they were depicted by Sanna et al. (2023) for *RBP2*. The PCR amplification was checked by means of electrophoresis, on 1 % agarose (VWR Life Science AMRESCO biochemicals) gels stained with GelRed. PCR products were sequenced in both directions by BMR Genomics S.R.L. (Padua, Italy). The generated DNA sequences were analysed, and consensus sequences were computed using the Geneious v. 11.1.5 programme (Auckland, New Zealand). The sequences obtained in this study were analysed, through the BLASTn tool, against the NCBI GenBank nucleotide database to determine the closest relatives for a taxonomic identification of the studied isolates. Alignments of different gene regions, including sequences obtained in this study and sequences downloaded from GenBank, were initially performed with the MAFFT v. 7 online server (<https://mafft.cbrc.jp/alignment/server/index.html>) (Katoh and Standley, 2013) and then manually adjusted in MEGA v. 7 (Kumar et al., 2016). After an initial analysis on the ITS locus (data not shown), a subset of representative isolates was selected to distinguish the isolates at the species level. Phylogenetic analyses were performed in MEGA V.6 using Maximum Likelihood (ML). The phylogenetic trees for *Botryosphaeriaceae*, *Diaporthaceae* and *Cytosporaceae*, as well as for *Fomitiporia* and *Seiridium* were calculated first individually for each locus (data not shown) to verify the genealogical concordance phylogenetic species recognition, and subsequently in a concatenated tree.

2.3. Pathogenicity tests

Inoculation experiments were conducted on London planes to 1) characterise the variability in virulence of the most frequent fungal species encountered in the symptomatic trees, 2) fulfil Koch's postulates, i.e. to determine whether a fungus isolated from symptomatic plant tissues may be the cause of that symptom/disease, and 3) infer the role of drought in the onset of the syndrome.

Because of the higher incidence of fungi belonging to

Botryosphaeriaceae than to *Diaporthaceae* and *Cytosporaceae*, and considering that most of *Diaporthaceae* and *Cytosporaceae* isolates originated from plant tissues that were also infected by *Botryosphaeriaceae* (see the results), the inoculation experiments exclusively targeted five species of *Botryosphaeriaceae* as well as one species of *Seiridium* associated with clearly defined symptoms (see the results) (Table 2).

Inoculation experiments were performed on healthy, five-year-old *P. x hispanica* trees grown in pots (230 l). These trees had an average circumference of 8.4 cm at one meter from the base and an average height of 3.5 m. The trees were kept outdoors under a shade house in the University of Torino Campus of Grugliasco, Italy. Some of the trees were regularly irrigated, daily, with a volume of 2 litres of tap water, while the remaining trees were subjected to a reduced water supply (-60 %), which was provided as emergency irrigation every 7–10 days, depending on whether rainfall had occurred, to simulate drought conditions.

Fungal inocula were prepared by transferring mycelial plugs obtained from 13-day-old colonies into flasks filled with 2 % (w/v) liquid malt extract (ME; AppliChem GmbH, Darmstadt, Germany). The flasks were kept under constant shaking (100 rpm) at 25 °C for five days. The liquid medium was filtered using sterile Whatman filter paper with 0.45 μm pores. The resulting mycelium was collected, one day before the inoculations, in sterile vials stored at 4 °C before use.

The inoculation was performed 1 m above the tree collar. The bark at the inoculation point was disinfected with sterile gauze soaked in 90 % ethanol. Using a 5 mm diameter cork borer and a scalpel sterilised with a Bunsen burner, a piece of bark was removed down to the cambium layer. The resulting hole was filled with fungal inoculum with the aid of a sterilised spatula. The inoculation point was covered with sterile cotton soaked in sterile water to prevent dryness, and it was then sealed with Parafilm®.

Inoculation experiments aimed at characterising the variability in virulence of the most frequent fungal species encountered on symptomatic plants, i.e. *N. parvum*, were conducted by inoculating ten isolates of the fungus (Table 2) as described above, into each of the three trees subjected to emergency irrigation. Another three trees were inoculated with sterile ground malt extract agar to serve as controls. The trial started on July 6th, 2021, and ended on August 2nd, 2021, thereby lasting 27 days.

Inoculation experiments aimed at fulfilling Koch's postulates and inferring the role of drought in the onset of the syndrome were conducted by inoculating one isolate of each of the six selected fungal species (*Diplodia mutila*, *Diplodia seriata*, *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Neofusicoccum parvum*, *Seiridium aquaticum* Z.L. Luo, K.D. Hyde & H.Y. Su) (Table 2) into three trees per isolate that had been irrigated on a daily basis and into three trees per isolate that had been subjected to emergency irrigation. Another three trees of each of the two irrigation regimes were treated as controls, as described above. The trial started on August 3rd, 2021, and ended on October 4th, 2021, thereby lasting 65 days. The statistical plan included a total of 42 trees, split evenly between irrigated and drought-stressed trees; three replicates per each inoculated fungal isolate and water treatment, including controls; complete spatial randomisation of the treatments along a seven row and six column array of equally spaced tree pots.

Tree death was annotated at the end of the experiments, and the longitudinal length and the transversal width of necrotic lesions were measured around the inoculation point. In the few cases where lesions were not visible on the bark, debarking was performed before measuring any lesions that were observed below the bark. Re-isolation of the fungal isolate from the inoculated tree was attempted by extracting four wood slivers (0.5 \times 0.5 \times 0.5 cm), using a sterile scalpel, from the four outermost edges of each lesion and plating them in 9 cm diameter Petri dishes filled with PDA. The fungi growing out from wood slivers were identified on the basis of their macro- and micromorphological features. Re-isolation was deemed to have been fulfilled if the same fungal species inoculated into a tree was retrieved from at least one of the four wood

Table 2

Fungal isolates employed in the inoculation experiments conducted on London plane trees and their origin. GenBank accession numbers of all the isolates are reported in the [Supplementary material 4](#).

Species	Isolate ID [TUCC ¹ Accession Number]	City and site	Tree ID	Cultivar Platanor®	Tree health status	Aim of the inoculation experiment
<i>Neofusicoccum parvum</i>	21694L2C2 [00000800]	Novara, Parco dell'Allea	21694	No	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	21696L1A2 [00000864]	Novara, Parco dell'Allea	21696	No	Asymptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	D522 6	Genoa, Spianata Acquasola	D522	No	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	CS2 11 [00000801]	Genoa, Corso Sardegna	CS2	No	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	D717 C4	Genoa, Spianata Acquasola	D717	No	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	D510 A1 [00000917]	Genoa, Spianata Acquasola	D510	No	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	3285 22	Genoa, Vivaio Doria	3285	Yes	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	1AF802 33	Genoa, Spianata Acquasola	1AF802	Yes	Dead	Variability in virulence
<i>Neofusicoccum parvum</i>	D501 B1	Genoa, Spianata Acquasola	D501	No	Symptomatic	Variability in virulence
<i>Neofusicoccum parvum</i>	1AF783 5 [00000918]	Genoa, Spianata Acquasola	1AF783	Yes	Dead	Variability in virulence/Koch's postulates and role of drought
<i>Diplodia mutila</i>	21695L1A12345 [00000865]	Novara, Parco dell'Allea	21695	No	Symptomatic	Koch's postulates and role of drought
<i>Diplodia seriata</i>	3292 44 [00000867]	Genoa, Vivaio Doria	3292	Yes	Symptomatic	Koch's postulates and role of drought
<i>Dothiorella iberica</i>	1AF781 7 [00000920]	Genoa, Spianata Acquasola	1AF781	Yes	Symptomatic	Koch's postulates and role of drought
<i>Lasiodiplodia theobromae</i>	CS4 43 [00000802]	Genoa, Corso Sardegna	CS4	No	Dead	Koch's postulates and role of drought
<i>Seiridium aquaticum</i>	21695L1B5 [00000866]	Novara, Parco dell'Allea	21695	No	Symptomatic	Koch's postulates and role of drought

1 TUCC: Turin University Culture Collection.

slivers taken from the same tree. For each of the inoculated fungal species, the re-isolation percentage was calculated both as the ratio between the number of trees from which the fungal species was successfully re-isolated and the total number of inoculated trees, and as the ratio between the number of wood slivers from which the fungus was re-isolated and the total number of wood slivers tested.

2.4. Climate analyses to infer the role of drought in the onset of the syndrome

Climate analyses were conducted on data provided by the official meteorological network managed by the Regional Agency for the Protection of the Environment (Agenzia Regionale per la Protezione Ambientale - ARPA) of Liguria (for Genoa) and Piedmont (for Novara). The available data included the 16 year-long time series of the daily values of the mean, maximum and minimum temperatures (T_{mean} , T_{max} , T_{min} , °C) and cumulated rainfall precipitation (P , mm) from 2005 to 2020. Drought levels were assessed by calculating for each year the DMAI, whose values were scored in seven classes, that is, from 1 to 7, that corresponded to the lowest and highest aridity conditions, respectively (Gavrilov et al., 2019). The annual DMAIs were compared to the reference threshold values obtained from the summary meteorological data gathered from the historical time series starting from 1833 in Genoa (Acquaotta et al., 2018) and 1875 in Novara (Dal Bello and Perego, 2023). Binary recursive partitioning tree models (Hothorn et al., 2006) were fitted to T_{mean} , T_{max} , T_{min} , and P to test whether any clusters of years characterised by significant climatic differences could be detected.

2.5. Data interpretation and statistical analyses

The absolute and relative frequencies were calculated for each type

of symptom observed in the field. Absolute frequency was appraised as the number of trees that displayed a specific symptom, while relative frequency was considered the ratio (%) between the absolute frequency and the total number of surveyed symptomatic trees. The incidence of symptomatic trees expressed over the total number of living trees (%) was compared between the first survey and the following ones for the subsample of periodically re-inspected trees. Likewise, to appraise whether symptom evolution had occurred, the relative frequency (%) of each type of symptom was compared at different time periods, for the subsample of the periodically re-inspected trees.

The incidence of each fungus on symptomatic, asymptomatic and dead trees was expressed in terms of ratio (%) between the number of infected trees and the total number of trees sampled in each category. Sample size refers to the number of trees inspected in the surveyed sites (Table 1 and Supplementary material 2).

The size of the necrotic lesions, as determined for each fungus, was calculated for the inoculation experiments as the area of the elliptic surface (cm^2) resulting from the $A = 0.25\pi lw$ equation, with l and w representing, respectively, the longitudinal length (cm) and the transversal width (cm) of the lesions measured at the end of the experiments. The minima and maxima were measured for l and w , and calculated for A . The average values of the lesion size on trees inoculated with the isolates and the controls were compared by fitting an unbiased recursive partitioning tree model and using the c -statistic p -value as a metric to accept/reject the null hypothesis (i.e. H_0 : the graph split is significant) (Hothorn et al., 2006). A fungus was scored as pathogenic whenever it caused a necrotic lesion that was significantly larger in size than one that occurred in the control trees. Differences among the isolates, in terms of size of necrotic lesions, were regarded as differences in their virulence (i.e. levels of pathogenicity). The same unbiased recursive partitioning tree model was used to contrast the mean surfaces of the necrotic lesions and the mortality rates (in %, calculated as the ratio between the

number of dead trees and the number of inoculated trees) of the regularly irrigated and drought-stressed London planes. The above comparison was conducted for each of the six inoculated fungal species and the control. The significance threshold was set at 0.05 for all the analyses. Finally, the EDCI was calculated for each inoculated fungal species when significant differences between the lesion sizes of irrigated vs. drought-stressed trees were detected.

3. Results

3.1. Symptomatology, incidence and course of the syndrome

Overall, the most recurrent symptoms were cankers or necrotic lesions with liquid leakage on trunks (Fig. 1), observed in 44 % of symptomatic trees (16 out of 36 trees), followed by similar cankers without liquid leakage. Cumulatively, these symptoms on trunks were present on 69 % of the symptomatic trees. Up to 13 cankers per tree were observed. Other recurrent symptoms were dieback, bark discoloration, epicormic shooting and defoliation (Fig. 1). Symptoms such as sudden tree death, branch cankers and death of branches were also observed at lower frequencies, with the latter two being on mature trees (Fig. 1). All the above mentioned symptoms were also observed on Platanor® trees, with the exception of branch canker and the death of branches. More than one symptom was often present on the symptomatic trees. For instance, trunk cankers were often observed in combination with dieback. A detailed description of symptomatology of all the surveyed trees is provided in the Supplementary material 2.

The incidence of the symptomatic trees in Genoa rose from 57 % (12 out of 21 trees) in 2019 to 81 % (17 out of 21 trees) in 2021. Worsening of the symptoms mostly involved defoliation and trunk cankers or necrotic lesions without liquid leakage, whereas a reduction in the incidence of trunk cankers with liquid leakage was observed (Fig. 2a).

The incidence of the symptomatic trees in Novara rose from 42 % in 2020 (5 out of 12 trees), to 58 % in 2021 (7 out of 12 trees), up to 75 % in 2022 (9 out of 12 trees). Worsening of the symptoms mostly involved trunk cankers or necrotic lesions with liquid leakage, death of branches and, to a lesser extent, branch cankers (Fig. 2b).

3.2. Frequency of fungi and their association with symptoms

A total of 1352 fungal isolates, classified in 33 morphotypes, were found. The identification of a representative subsample of the isolates classified in those morphotypes led to the detection of 24 fungal species belonging to 21 genera (Table 3). Although some of these genera are known to accommodate ubiquitous fungi or fungi not known to be associated with symptoms on woody tissues, and were thus discarded from further analysis, some others are known to include plant pathogens

associated with woody tissues. These isolates were identified more accurately at the species level through phylogenetic analyses (Supplementary material 3). Reference isolates were deposited in the Turin University Culture Collection (TUCC) (Table 2). Relevant sequences were deposited in GenBank (Supplementary material 4). Detailed information on the fungi isolated from each tree is reported in Supplementary material 2.

Overall, *Botryosphaeriaceae* was the most frequently isolated family representing 51 % of isolates, followed by *Diaporthaceae* representing 11 % of isolates (Table 3). *Neofusicoccum parvum* was the most prevalent species in both Genoa and Novara, and it was also found on Platanor® trees. This species was isolated from 50.0 % of dead trees, 12.5 % of asymptomatic trees, and 47.2 % of symptomatic trees, mainly in association with trunk cankers or necrotic lesions, with or without liquid leakage and with dieback and death of branches (Table 3). Other *Botryosphaeriaceae* species, such as *D. mutila* and *D. seriata*, *L. theobromae* and *D. iberica*, were found associated with the same symptoms, but with a much lower incidence (Table 3). The same was found for *Diaporthaceae* and *Cytosporaceae* fungi, which were often observed on the same trees and occasionally on the same lesions infected by *N. parvum*. The isolation of *S. aquaticum* from necrotic lesions with liquid leakage on the trunks of two distinct trees and of *Fomitiporia mediterranea* M. Fisch. from a large trunk canker on a monumental tree are worthy of mentioning. A plate with representative symptoms and their associated fungi is provided in Fig. 3.

3.3. Pathogenicity tests

Necrotic wood lesions were generally visible on the bark at the end of inoculation experiments. It was found, on the basis of the outcomes of experiments that had been aimed at characterising the variability in virulence of the most frequent fungal species encountered in symptomatic trees, that four out of ten *N. parvum* isolates tested for virulence on London plane trees were not pathogenic, while the remaining six were pathogenic (Fig. 4). The three most virulent isolates caused 13-fold larger necrotic lesions than the least virulent isolate (13.4 vs. 1.0 cm²; $p < 0.05$) (Fig. 4). One of the three most virulent *N. parvum* isolates (ID 1AF783 5) was selected for the subsequent experiment aimed at inferring the role of drought in the onset of the syndrome.

The inoculation experiments of isolates of selected fungal species on London plane trees subjected to two different irrigation regimes revealed that the *D. seriata*, *D. iberica* and *S. aquaticum* isolates were not pathogenic under the test conditions, regardless of the irrigation regime (Table 4). Conversely, the *N. parvum* (virulent strain), *D. mutila* and *L. theobromae* isolates were always pathogenic, irrespective of the irrigation regime (Table 4). However, the size of the necrotic lesions and the mortality rates were significantly boosted ($p < 0.05$) by isolates of these

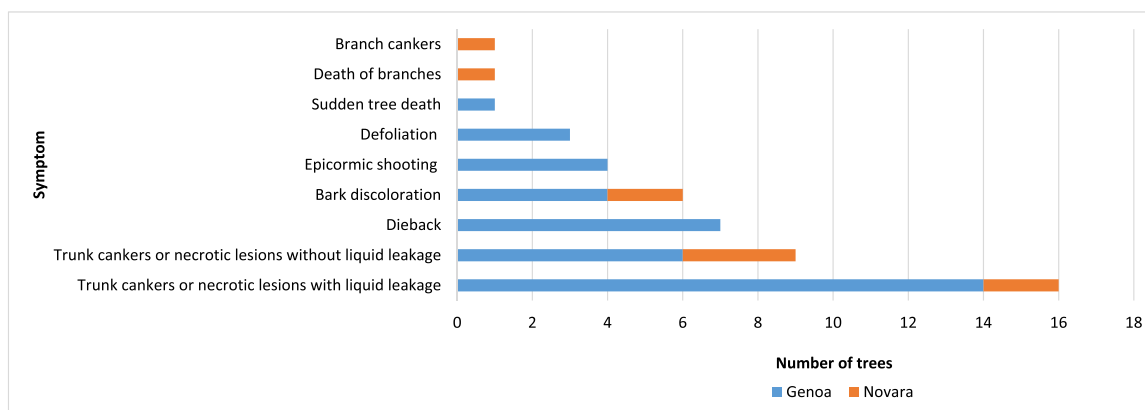


Fig. 1. Absolute frequency of living London planes, including Platanor® trees showing different disease symptoms at the first survey in Genoa (autumn 2019) and Novara (autumn 2020).

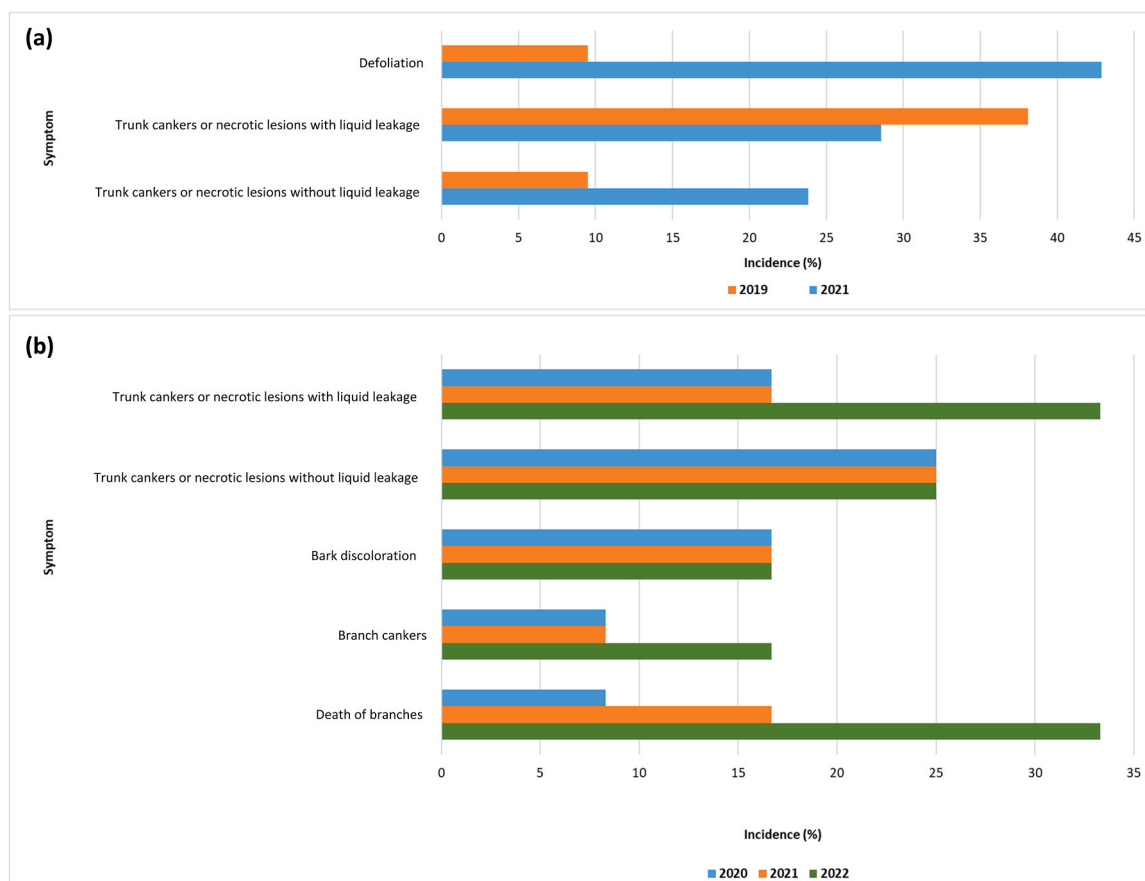


Fig. 2. Comparison of incidence of selected symptoms at two time periods (autumn 2019 and 2021) in a sample of 21 London plane trees in Genoa (a) and at three time periods (autumn 2020, 2021 and 2022) in a sample of 12 London plane trees in Novara.

species in the drought-stressed treatments. Indeed, the latter displayed average lesion areas that were up to 200-fold larger than those of the regularly irrigated trees, an EDCI close to 1 (i.e. ranging from 0.920 to 1.000) and mortality rates ranging from 67 % to 100 %, while no mortality was observed on the regularly irrigated trees (Table 4; Fig. 5).

At the end of both inoculation experiments, the re-isolation of the inoculated fungal isolates was accomplished from 100 % of the inoculated trees, and from 41.7 % to 91.7 % of the tested wood slivers depending on the isolate and the experiment (Table 4). With the exception of a few *Alternaria alternata* (Fr.) Keissl. isolates, no other fungi were isolated from the plated wood slivers.

3.4. Climate analyses to infer the role of drought in the onset of the syndrome

The DMAI obtained from the reference time series displayed threshold values of 49.2 in Genoa and 40.6 in Novara. Climate analyses showed that 88 % and 75 % of the years from 2005 to 2020 displayed lower DMAIs than the threshold values pertaining to Genoa and Novara, respectively, thus indicating higher drought conditions than in the reference time series. Similarly, although the DMAI values of the reference time series fell into class 2 for both cities, nine and seven years were observed with a DMAI falling into class 3–6 during the 2005–2020 period in Genoa and Novara, respectively. The maximum drought conditions (i.e. DMAI in class 6) were attained during 2006 and 2017 in both cities, but also during 2005 in Novara (Fig. 6).

The tree models revealed that both cities were characterised by one or two clusters of years significantly warmer and drier than others ($p < 0.05$) with average differences ranging from + 0.8 to + 2.2 °C for temperatures and from –1.52 to –1.58 mm for precipitations, in the

2005–2020 timeframe (Supplementary material 5).

4. Discussion

Among the fungi isolated from symptomatic London plane tissues, *N. parvum* was the most prevalent, followed by other *Botryosphaeriaceae* species and by species belonging to the *Diaporthaceae* family. The high prevalence of *N. parvum* over other fungi on London plane is new, as previous observations from Switzerland pointed to other *Botryosphaeriaceae* species such as *D. mutila* and *Dothiorella* sp. being the most frequent in symptomatic tissues (Pelletier et al., 2017). It should be noted that *N. parvum* had previously been reported as being predominantly associated with symptomatic samples from *Sequoiadendron giganteum* (Lindl.) J. Buchholz in Switzerland (Haenzi et al., 2021). In addition, the fungus had repeatedly been isolated from symptomatic *Acer pseudoplatanus* L. and *Quercus robur* L. trees in Italy (Moricca et al., 2012), and from London plane trees in China (Yu et al., 2018). We have documented, for the first time ever, the association of a range of fungi that are also known to have pathogenic behaviour, including *Ambrosiella xylebori* Brader ex Arx & Hennebert, *Cytospora acaciae* Oudem., *Diaporthe eres* Nitschke, *Diaporthe foeniculina* (Sacc.) Udayanga & Castl., *Didymosphaeria rubi-ulmifolii* Ariyaw., Camporesi & K.D. Hyde, *F. mediterranea*, *S. aquaticum*, but also *D. iberica* and *L. theobromae*, with the genus *Platanus* (U.S. National Fungal Databases, 2024). *Fomitiporia mediterranea* was isolated from a large stem canker that was similar to those caused on planes by the related *F. punctata* (P. Karst.) Murrill species, from which it is morphologically indistinguishable (Bernicchia and Gorgjón, 2020). The association of *S. aquaticum* with leaking cankers is worth noting. This observation, along with the remark that the *Seiridium* genus accommodates important tree pathogens, such as the

Table 3
Incidence of fungi isolated in this study on asymptomatic, symptomatic and dead London plane trees and other relevant information.

Genus/species	Family	Number of morphotypes	Number of isolates per morphotype	Incidence on asymptomatic hosts (%)	Incidence on symptomatic hosts (%)	Incidence on dead hosts (%)	Association with symptoms*	Association with London planes other than Platanor® (L) and with Platanor® (P) trees	Presence in Genoa (G) and Novara (N)
<i>Acremonium</i> sp.	<i>Incertae sedis</i>	1	13	3.1	0	0	-	L	N
<i>Alternaria alternata</i> (Fr.) Keissl.	<i>Pleosporaceae</i>	2	42; 73	9.4	5.6	12.5	SD, TCWL	L, P	G
<i>Ambrosiella xylebori</i> Brader ex Arx & Hennebert	<i>Ceratocystidaceae</i>	1	39	0	5.6	12.5	TC, TCWL	L, P	G
<i>Apiospora espori</i> ensis (Pintos & P. Alvarado) Pintos & P. Alvarado	<i>Apiosporaceae</i>	1	10	0	2.8	0	DI, TCWL	L, P	G
<i>Apiospora marii</i> (Larrondo & Calvo) Pintos & P. Alvarado	<i>Apiosporaceae</i>	1	13	0	2.8	0	TC	P	G
<i>Aureobasidium</i> sp.	<i>Sacrotheciaceae</i>	1	77	0	5.6	12.5	BD, TC, TCWL	L	N
<i>Clonostachys rosea</i> (Link) Schroers, Samuels, Seifert & W. Gams	<i>Bionectriaceae</i>	1	53	0	2.8	12.5	DI, TC	L	G
<i>Cytospora acaciae</i> Oudem.	<i>Cytosporaceae</i>	3	10; 23; 29	6.2	8.4	0	DI, ES, SD, TCWL	L, P	G
<i>Diaporthe eres</i> Nitschke	<i>Diaporthaceae</i>	2	25; 52	0	5.6	12.5	DE, TCWL	L, P	G
<i>Diaporthe foeniculina</i> (Sacc.) Udayanga & Castl.	<i>Diaporthaceae</i>	2	11; 56	0	5.6	12.5	DI, TCWL	L, P	G
<i>Diplodia mutila</i> (Fr.) Fr.	<i>Botryosphaeriaceae</i>	1	102	3.1	8.4	12.5	BD, DI, TC, TCWL	L, P	G, N
<i>Diplodia seriata</i> De Not.	<i>Botryosphaeriaceae</i>	1	24	0	5.6	0	DI, TC	L, P	G
<i>Dothiorella iberica</i> A.J.L. Phillips, J. Luque & A. Alves	<i>Botryosphaeriaceae</i>	1	25	3.1	2.8	0	TC	P	G
<i>Didymosphaeria rubi-ulfifolii</i> Ariyaw., Camporesi & K.D. Hyde	<i>Didymosphaeriaceae</i>	1	13	3.1	0	0	-	L	N
<i>Epicoccum</i> sp.	<i>Didymellaceae</i>	1	15	0	2.8	0	DE, TCWL	L, P	G
<i>Fomitiporia mediterranea</i> M. Fisch.	<i>Hymenochaetaceae</i>	1	9	0	2.8	0	TC	L	N
<i>Fusarium</i> sp.	<i>Nectriaceae</i>	2	9; 16	3.1	2.8	0	DI, TCWL	L	G
<i>Lasiodiplodia theobromae</i> (Pat.) Griffon & Maubl.	<i>Botryosphaeriaceae</i>	1	70	0	2.8	12.5	TC	L	G
<i>Mortierella</i> sp.	<i>Mortierellaceae</i>	1	12	0	2.8	0	DB	L	N
<i>Neofusicoccum parvum</i> (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips	<i>Botryosphaeriaceae</i>	3	57; 89; 324	12.5	47.2	50.0	BD, DI, ES, SD, TC, TCWL	L, P	G, N
<i>Neosetophoma italica</i> W.J. Li, Camporesi & K.D. Hyde	<i>Phaeosphaeriaceae</i>	1	14	3.1	0	0	-	P	G
<i>Nigrospora oryzae</i> (Berk. & Broome) Petch	<i>Apiosporaceae</i>	1	12	0	2.8	0	DE	L	G
<i>Pseudocamarosporium</i> sp.	<i>Didymosphaeriaceae</i>	1	11	0	2.8	0	General decline	L	N
<i>Seiridium aquaticum</i> Z.L. Luo, K. D. Hyde & H.Y. Su	<i>Pestalotiopsidaceae</i>	2	11; 13	0	5.6	0	BD, TC, TCWL	L	N

*BC, branch cankers; BD, bark discoloration; DB, death of branches; DE, defoliation; DI, dieback; ES, epicormic shooting; SD, sudden tree death; TC, trunk cankers or necrotic lesions without liquid leakage; TCWL, trunk cankers or necrotic lesions with liquid leakage.



Fig. 3. Plate of representative symptoms observed on London plane and their associated fungi. Dieback (a and b), trunk cankers and necrotic lesions with and without liquid leakage (c and d, respectively), and the combination of both (e) associated with *Neofusicoccum parvum*. Necrotic lesions or cankers associated with *Diplodia mutila* (f), *Cytospora acaciae* (g) and *Seiridium aquaticum* (h). Large stem canker associated with *Fomitiporia mediterranea* (i).

cypress canker agent (Graniti, 1998), prompted us to further investigate the role of *S. aquaticum* in the onset of the syndrome.

Apart from being prevalent in symptomatic host tissues, *N. parvum* and other *Botryosphaeriaceae* species, including *D. mutila* and *D. iberica*, but also *C. acaciae*, were also isolated from asymptomatic London planes, thus demonstrating the occurrence of endophytic stages in these host/microbe model systems. We were not able to isolate other *Botryosphaeriaceae* or *Diaporthaceae* species in their endophytic state at

the sampling intensity used in this study.

Inoculation experiments of drought-stressed London planes with multiple *N. parvum* isolates revealed that 40 % of the isolates were non-pathogenic, whereas the remaining ones displayed a considerable virulence diversity, consistently with results obtained with isolates collected from and inoculated in grapevines (Baskarathevan et al., 2012). Although this aspect was beyond the aim of this study, no correlation was observed between the virulence of isolates and their origin (i.e. site, cultivar, type of symptom or health condition of the hosts).

Overall, the inoculation experiment on London planes with five *Botryosphaeriaceae* species and *S. aquaticum* pointed to the *N. parvum* isolate as being the most virulent, followed by isolates of *D. mutila* and *L. theobromae*, whereas *D. seriata*, *D. iberica* and *S. aquaticum* isolates were non-pathogenic under the test conditions. Although *S. aquaticum* had never been reported before in Italy, Italian isolates of *D. seriata* had previously been found to be pathogenic on apple and olive trees (Manetti et al., 2024; Martino et al., 2024a), while those of *D. iberica* were non-pathogenic on hazelnut (Linaldeddu et al., 2016). Italian isolates of *N. parvum*, *D. mutila* and *L. theobromae* were reported as pathogenic on several plant species, including grapevine, mango, litchi and pomegranate plants (Aiello et al., 2022; Bregant et al., 2024; Burruano et al., 2008). Compared to previous data (Pelletier et al., 2017; Yu et al., 2018), we have proved, for the first time, the pathogenicity of *D. mutila* isolates on London plane, and we have confirmed the pathogenicity and the non-pathogenicity of *N. parvum* and *D. seriata* isolates, respectively, on the same host species. The relatively high virulence of *N. parvum* is in agreement with previous results on plane trees (Pelletier et al., 2017). However, caution should be taken in concluding that *N. parvum* is more virulent than other *Botryosphaeriaceae* species because, as clarified in this study, the virulence of *N. parvum* is largely dependent on the isolate, and it cannot be excluded that the same is true for other *Botryosphaeriaceae* species. Further experiments, using additional isolates for each species, are needed to comparatively test virulence on London plane.

A clear limitation of our study hinges on the limited number of isolates and fungal species tested for their pathogenicity on London plane. Other fungi, belonging to the *Diaporthaceae* and *Cytosporaceae* species detected in this study, could play a role in the onset of the syndrome, and this aspect deserves further investigations. From a broader perspective, an additional limitation refers to the fact that the entire study focused only on fungi, and it cannot be excluded that other microbes (e.g. bacteria) may be involved in the syndrome.

A solid outcome of our inoculation experiments, for all the tested pathogenic fungi, is that their virulence on drought-stressed trees was overwhelmingly higher than on trees irrigated on a daily basis. This experimental evidence supports the notion that drought enhances symptom expression, mortality, and possibly infections by these fungi on plane trees, consistently with other studies that focused on *Botryosphaeriaceae* species on other hosts (Galarnau et al., 2019; Garbelotto, 2024; Manetti et al., 2024). It should be noted that the EDCI clearly indicated that the pathogenicity of *N. parvum*, *D. mutila* and *L. theobromae* was almost entirely driven by drought as an environmental stressor (Garbelotto, 2024). The results of the climate analyses corroborated the observations resulting from the inoculation trials conducted by comparing irrigated and drought-stressed trees. Indeed, such analyses showed not only that drought conditions have substantially increased over the last two centuries in both cities, but also that repeated episodes of severe aridity occurred during the years when the onset of symptoms were observed on London planes. When aridity scores from class 3–6, according to the De Martonne index, as we frequently observed from 2005 to 2020, irrigations are strongly recommended (Charalampopoulos et al., 2023), although they were not conducted in the surveyed sites. Drought may have compromised the health status of London planes to different extents, depending on the age of the tree. Indeed, most of the considered London planes, including the Platanor® trees in Genoa, were planted in the 2016–2017 period, during

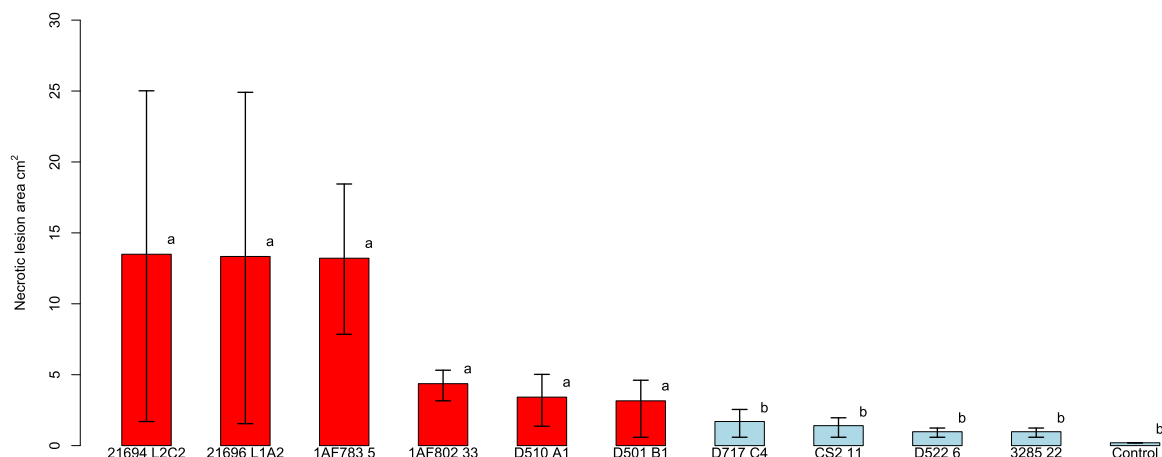


Fig. 4. Size of the necrotic lesions on London plane trees caused by 10 isolates of *Neofusicoccum parvum* 27 days post-inoculations. Different letters indicate significant differences ($p < 0.05$), while different colors split pathogenic and non-pathogenic isolates, marked in red and azure, respectively. Error bars refer to the 95 % confidence intervals.

Table 4

Average size of lesions [minimum, maximum] and mortality rates caused on London plane trees by one isolate each of six fungal species 65 days post-inoculations. For each fungus inoculated, average values of the lesion area and the overall mortality rate are compared between trees subjected to two irrigation regimes. Different letters indicate significantly different values ($p < 0.05$). The 95 % confidence intervals are reported in brackets, if applicable. When applicable, the Environmental Disease Component index (EDCI) associated with each fungal species is reported. For each fungus inoculated, the re-isolation percentage expressed both as the ratio between the number of trees from which the fungal species was successfully re-isolated and the total number of inoculated trees, and as the ratio between the number of wood slivers from which the fungus was re-isolated and the total number of wood slivers tested is also reported.

Species (EDCI)	Irrigation regime	Necrotic lesion width (cm)	Necrotic lesion length (cm)	Necrotic lesion area (cm ²)	Mortality rate (%)	Re-isolation from trees (%)	Re-isolation from wood slivers (%)
<i>Neofusicoccum parvum</i> (1.000)	Irrigation	1.1 [0.8, 1.2]	1.3 [0.9, 1.8]	1.2 ^b [0.6, 1.7] (0.6; 1.5)	0 ^b (0; 63.2)	100	58.3
	Drought	9.3 [9.0, 10.0]	32.5 [27.5, 40.0]	240.0 ^a [194.3, 314.0] (194.3; 280.0)	100 ^a (36.8; 100)	100	91.7
<i>Diplodia mutila</i> (0.989)	Irrigation	1.1 [0.8, 1.5]	2.0 [1.5, 2.3]	1.7 ^b [1.4, 1.8] (1.4; 1.8)	0 ^b (0; 63.2)	100	66.7
	Drought	6.2 [1.9, 8.5]	22.6 [8.2, 31.5]	134.2 ^a [12.2, 210.2] (12.2; 200.2)	66.6 ^a (13.5; 98.3)	100	83.3
<i>Diplodia seriata</i> (-)	Irrigation	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	100	50.0
	Drought	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	100	58.3
<i>Dothiorella iberica</i> (-)	Irrigation	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	100	41.7
	Drought	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	100	58.3
<i>Lasiodiplodia theobromae</i> (0.920)	Irrigation	1.1 [0.9, 1.5]	7.9 [4.6, 13.6]	7.8 ^b [3.6, 16.0] (3.6; 12.0)	0 ^b (0; 63.2)	100	75.0
	Drought	4.6 [0.9, 8.0]	20.2 [6.0, 29.5]	95.8 ^a [4.2, 185.3] (4.2; 156.2)	66.6 ^a (13.5; 98.3)	100	83.3
<i>Seiridium aquaticum</i> (-)	Irrigation	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	100	41.7
	Drought	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	100	50.0
Control	Irrigation	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	-	-
	Drought	0.5 [0.5, 0.5]	0.5 [0.5, 0.5]	0.2 ^a [0.2, 0.2] (-)	0 ^a (0; 63.2)	-	-

a peak of aridity, and this possibly led to acute post-transplanting stress. It should also be mentioned that most of the symptomatic trees in Genoa grew in soils that were inadequate in retaining water. Conversely, the older London planes of Novara might have suffered from chronic stress associated with an overall increase in the drought levels. In this case, aridity peaks that occurred during late maturity or senescence, when the trees were notably weaker and physiologically less reactive, might have amplified the effects of the drought-related stress. However, drought is

unlikely to have been the primary cause of decline *sensu* Manion and Lachance (Manion and Lachance, 1991) on either young or old London planes, but it may instead have been a factor that predisposed or incited the onset of the syndrome. In this framework, latent fungal pathogens could have been the contributing factor, as suggested by the results of our inoculation experiments. It should be noted that, technically, in this study, we were unable to satisfy all Koch's postulates because, for instance, some of the fungi associated with symptoms were also detected

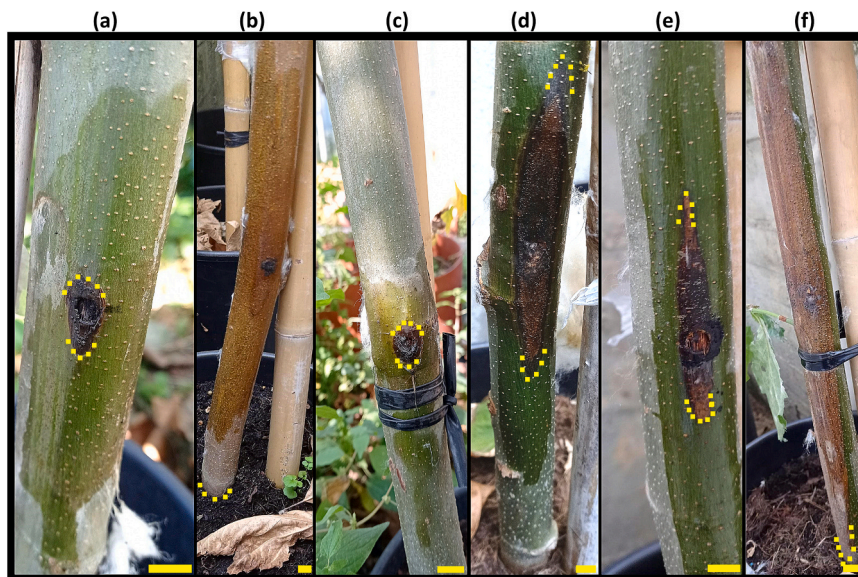


Fig. 5. Necrotic lesions caused by selected fungal isolates on London planes subjected to different irrigation regimes. Panels refer to trees inoculated with *Neofusicoccum parvum* (isolate ID 1AF783 5) (a) and (b), *Diplodia mutila* (isolate ID 21695L1A12345) (c) and (d), and *Lasiodiplodia theobromae* (isolate ID CS4 43) (e) and (f). Irrigated trees are shown in panels (a), (c) and (e), while drought-stressed trees are displayed in panels (b), (d) and (f). The bar represents 1 cm in all pictures. The boundaries of the upper and lower areas of the necrotic lesions are marked with dotted lines.

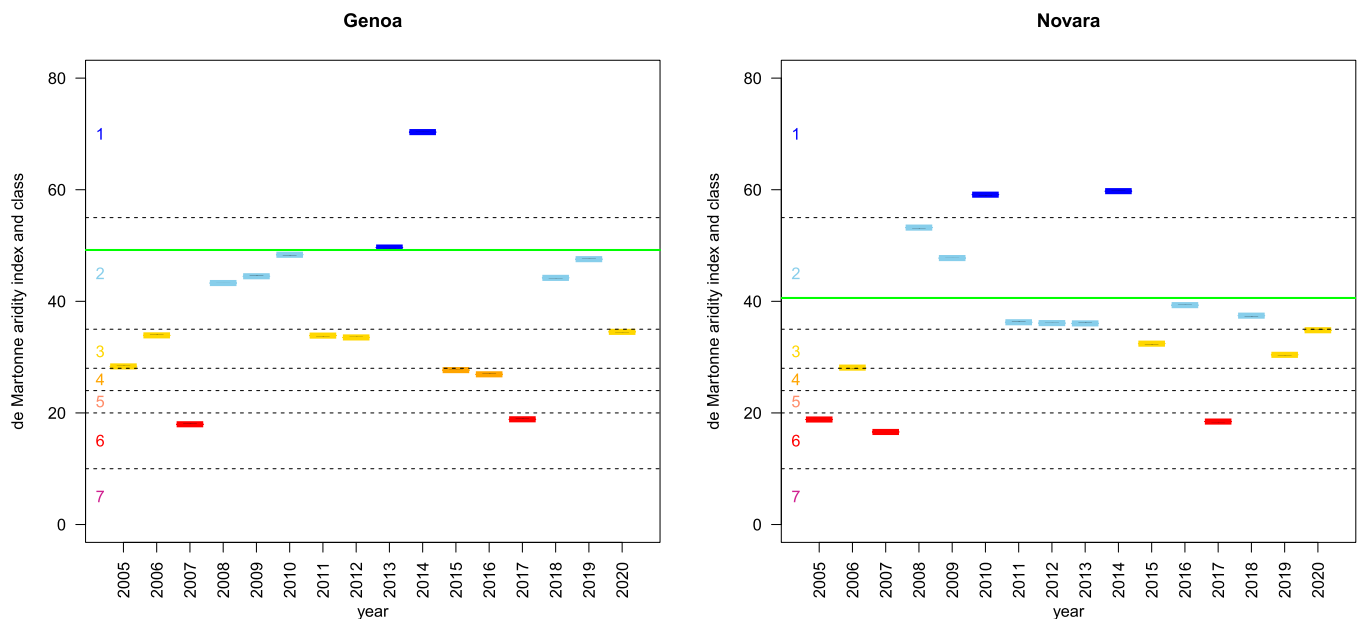


Fig. 6. De Martonne aridity index (DMAI) from 2005 to 2020 in the cities of Genoa and Novara. Low DMAI values indicate high aridity levels, while increasing values of the corresponding class (from 1 to 7) increasing drought conditions. DMAIs included in different classes are represented with different colors. The bounds of each DMAI class are marked with horizontal dashed lines, while the reference threshold of DMAI resulting from the historical time series since the 19th century to present is shown as a green line.

on asymptomatic trees. In addition, we were only able to reproduce some of the symptoms observed in nature in the pathogenicity tests, possibly because of the young age of the trees used for the inoculation experiments and the limited duration of the experiments themselves. However, as reported by Bonello (Bonello, 2024) in his conceptual paper on the subject, we came to a reasonable and acceptable conclusion on the basis of the preponderance of evidence, even without fulfilling all the Koch's postulates.

In summary, by integrating field observations, laboratory diagnostics, and experiments conducted under controlled conditions, we have documented a clear role of the most prevalent *N. parvum* fungus

and of a cohort of other *Botryophariaceae* species in the onset of the deadly syndrome observed on London plane trees in northern Italy, including on those of the Platanor® cultivar. Furthermore, by combining climate analyses and inoculation experiments on hosts under different irrigation regimes, we have shown that drought, i.e. a major climate change condition, significantly increased the virulence of such fungi on London planes, thereby suggesting a predisposing role of climate change and providing a clue to help explain why this syndrome has only recently been observed.

5. Conclusions

In this paper we have described a deadly syndrome on London planes that was clearly triggered by drought and driven by a cohort of *Botryosphaeriaceae* species, although a possible role of other fungal species that were detected in this study but not tested for their pathogenicity and of other non-target microbes cannot be ruled out. Although robust and conclusive evidence about the higher virulence of *N. parvum*, compared to other fungi, is still lacking, it is expected that this fungus will play a major role, as clearly supported by its remarkable field prevalence. Given the role of drought in the onset of the syndrome, we believe that the mortality of London plane, caused by *Botryosphaeriaceae*, could rise in the future because of climate change.

The obtained results of this study may help to define practical guidelines for the prevention and management of this syndrome. For instance, the implementation of any solution aimed at minimising water stress in plants may help prevent the onset of the syndrome. Therefore, nature-based solutions in the framework of sponge city policies (Richter et al., 2024) may be desirable. Although the infection biology of *N. parvum* on London plane still needs to be elucidated, precautionary wound dressings and the disinfection of pruning tools may help reduce infections, considering the documented ability of this fungus to infect other hosts through wounds (Otoya-Martinez et al., 2023). The detection of *N. parvum* and other *Botryosphaeriaceae* in one nursery and the documented ability of *N. parvum* and *D. mutila* to colonise endophytically asymptomatic planes support the notion that the disease may also spread through the movement of plants for planting. Diagnostics tools and sampling protocols pertaining to detection may be needed to prevent the movement of infected London planes. However, the development and optimisation of such tools and protocols require information that is currently lacking, on the histopathology of *Botryosphaeriaceae* on London plane.

Funding

This work was funded and logistically supported by the Azienda Servizi Territoriali (ASTER) Genova and by the City of Novara. The management and characterisation of fungal isolates was also partially supported by the European Commission—NextGenerationEU, Project SUS-MIRRI.IT “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy” [IR0000005].

CRedit authorship contribution statement

Gonthier Paolo: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Travaglia Pier Mario:** Resources, Project administration, Investigation. **Costa Giorgio:** Resources, Project administration, Investigation. **Martiniuc Alina Veronica:** Methodology, Investigation. **Guarnaccia Vladimiro:** Writing – review & editing, Methodology, Investigation. **Lione Guglielmo:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing interest

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

Acknowledgments

The authors wish to thank the funding bodies and all the technicians who logistically supported the surveys and samplings in Genoa and Novara. The authors also thank Marianna Giraud and Daniele Giunta for assistance in the management of fungal isolates and sequences, and

Ilaria Martino for supporting the sequence editing process.

Data statement

All data relevant to this work are included in the [supplementary material](#). Raw data are available upon request to the Authors.

Appendix A

Supplementary material 1. Primer combinations used in this study.

Supplementary material 2. Characteristics of London plane trees inspected and sampled in Genoa and Novara in the autumn 2019 and 2020, respectively, including symptoms and the associated fungi. Trees that were re-inspected periodically (see text) are marked with an asterisk in the column tree ID. Types of symptoms are as follows: BC, branch cankers; BD, bark discoloration; DB, death of branches; DE, defoliation; DI, dieback; ES, epicormic shooting; SD, sudden tree death; TC, trunk cankers or necrotic lesions without liquid leakage; TCWL, trunk cankers or necrotic lesions with liquid leakage

Supplementary material 3. Phylogenetic trees developed by Maximum Likelihood method.

Supplementary material 4. GenBank accession numbers of relevant isolates for the following loci: β -tubulin (*tub2*), Nuclear ribosomal internal transcribed spacer (ITS), RNA polymerase II gene (*RPB2*), Translation elongation factor-1 α (*tef1 α*).

Supplementary material 5. Climate analysis of mean, maximum and minimum temperatures (T_{mean} , T_{max} , T_{min} , °C) and rainfall precipitation (P, mm) from 2005 to 2020 in Genova and Novara. For each city, the model algorithm identified clusters of years displaying significantly different averages of the climatic variables (marked with different letters in the column “Cluster average”). The symbol “-” indicates a range of consecutive years included within the cluster.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ufug.2025.128787](https://doi.org/10.1016/j.ufug.2025.128787).

References

- Acquaotta, F., Faccini, F., Fratianni, S., Paliaga, G., Sacchini, A., 2018. Rainfall intensity in the Genoa Metropolitan Area: secular variations and consequences. *Weather* 73, 356–362. <https://doi.org/10.1002/wea.3208>.
- Aiello, D., Bregant, C., Carlucci, A., Guarnaccia, V., Gusella, G., Linaldeddu, B.T., Mugnai, L., Raimondo, M.L., Polizzi, G., 2023. Current status of Botryosphaeriaceae species in Italy: Impacts on agricultural crops and forest ecosystems. *Phytopathol. Mediterr.* 62, 381–412. <https://doi.org/10.36253/phyto-14711>.
- Aiello, D., Guarnaccia, V., Costanzo, M.B., Leonardi, G.R., Epifani, F., Perrone, G., Polizzi, G., 2022. Woody canker and shoot blight caused by Botryosphaeriaceae and Diaporthaceae on mango and litchi in Italy. *Horticolturae* 8, 330. <https://doi.org/10.3390/horticolturae8040330>.
- Baskarathevan, J., Jaspers, M.V., Jones, E.E., Cruickshank, R.H., Ridgway, H.J., 2012. Genetic and pathogenic diversity of *Neofusicoccum parvum* in New Zealand vineyards. *Fungal Biol.* 116, 276–288. <https://doi.org/10.1016/j.funbio.2011.11.010>.
- Bernicchia, A., Gorgjón S.P., 2020. Polypores of the Mediterranean Region, Second ed. Romar, Rome, Italy.
- Bonello, P., 2024. Complex forest tree diseases – diagnostics beyond Koch’s postulates. *Curr. For. Rep.* 10, 273–280. <https://doi.org/10.1007/s40725-024-00220-w>.
- Bregant, C., Rossetto, G., Montecchio, L., Tundo, S., Raiola, A., Linaldeddu, B.T., 2024. First report of *Neofusicoccum parvum* and *Phytophthora palmivora* causing fruit rot of pomegranate in Italy. *Ital. J. Mycol.* 53, 65–74. <https://doi.org/10.6092/issn.2531-7342/19082>.
- Burruano, S., Mondello, V., Conigliaro, G., Alfonso, A., Spagnolo, A., Mugnai, L., 2008. Grapevine decline in Italy caused by *Lasiodiplodia theobromae*. *Phytopathol. Mediterr.* 47, 132–136. https://doi.org/10.14601/Phytopathol_Mediterr-2616.
- Charalampopoulos, I., Droulia, F., Evans, J., 2023. The bioclimatic change of the agricultural and natural areas of the Adriatic coastal countries. *Sustainability* 15, 4867. <https://doi.org/10.3390/su15064867>.
- Dal Bello, L., Perego, L., 2023. La serie climatica ultracentenaria di Novara (1875 – 2022). (<http://www.centrometeolombardo.com/content.asp?ContentId=7788>).
- Fan, X.L., Bezerra, J.D.P., Tian, C.M., Crous, P.W., 2020. *Cytospora* (Diaporthales) in China. *Pers. Mol. Phylogeny Evol.* 45, 1–45. <https://doi.org/10.3767/persoonia.2020.45.01>.

- Franceschi, E., Moser-Reischl, A., Honold, M., Asrafur Rahman, M., Pretzsch, H., Pauleit, S., Rötzer, T., 2023. Urban environment, drought events and climate change strongly affect the growth of common urban tree species in a temperate city. *Urban For. Urban Green.* 88, 128083. <https://doi.org/10.1016/j.ufug.2023.128083>.
- Galarneau, E.R.A., Lawrence, D.P., Travadon, R., Baumgartner, K., 2019. Drought exacerbates *Botryosphaeria* dieback symptoms in grapevines and confounds host-based molecular markers of infection by *Neofusicoccum parvum*. *Plant Dis.* 103, 1738–1745. <https://doi.org/10.1094/PDIS-09-18-1549-RE>.
- Garbelotto, M., 2024. Drought heightens severity of diseases caused by *Botryosphaeria dothidea* and *Cryptostroma corticale* and needs to be factored in to properly assess pathogenicity or fulfill Koch's postulates. *J. Plant Pathol.* <https://doi.org/10.1007/s42161-024-01702-3>.
- Gavrilov, M.B., An, W., Xu, C., Radaković, M.G., Hao, Q., Yang, F., Guo, Z., Perić, Z., Gavrilov, G., Marković, S.B., 2019. Independent aridity and drought pieces of evidence based on meteorological data and tree ring data in southeast Banat. *Vojv., Serb. Atmosphere* 10, 586. <https://doi.org/10.1016/j.ufug.2023.128083>.
- Graniti, A., 1998. Cypress canker: a pandemic in progress. *Annu. Rev. Phytopathol.* 36, 91–114. <https://doi.org/10.1146/annurev.phyto.36.1.91>.
- Haenzi, M., Cochard, B., Chablais, R., Crovadore, J., Lefort, F., 2021. *Neofusicoccum parvum*, a new agent of sequoia canker and dieback identified in Geneva, Switzerland. *Forests* 12, 434. <https://doi.org/10.3390/f12040434>.
- Hanlin, R.T., 1990. *Illustrated Genera of Ascomycetes*. The American Phytopathological Society, St. Paul, Minnesota. ISBN: 9780890541074.
- Hothorn, T., Hornik, K., Zeileis, A., 2006. Unbiased recursive partitioning: a conditional inference framework. *J. Comput. Graph. Stat.* 15, 651–674. <https://doi.org/10.1198/106186006X133933>.
- Hull, R., 2009. A Short Guide to the London Plane. (https://treetree.co.uk/treetree_downloads/The_London_Plane.pdf).
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. <https://doi.org/10.1093/molbev/mst010>.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. <https://doi.org/10.1093/molbev/msw054>.
- Linaldeddu, B.T., Deidda, A., Scanu, B., Franceschini, A., Alves, A., Abdollahzadeh, J., Phillips, A.J.L., 2016. Phylogeny, morphology and pathogenicity of *Botryosphaeria* spp., *Diatrypaceae* and *Gnomoniaceae* associated with branch diseases of hazelnut in Sardinia (Italy). *Eur. J. Plant Pathol.* 146, 259–279. <https://doi.org/10.1007/s10658-016-0912-z>.
- Linaldeddu, B.T., Rossetto, G., Maddau, L., Vatrano, T., Bregant, C., 2023. Diversity and pathogenicity of *Botryosphaeria* spp. and *Phytophthora* species associated with emerging olive diseases in Italy. *Agriculture* 13, 1575. <https://doi.org/10.3390/agriculture13081575>.
- Luo, Z.L., Hyde, K.D., Liu, J.K., Maharachchikumbura, S.S.N., Jeewon, R., Bao, D.F., Bhat, D.J., Lin, C.G., Li, W.L., Yang, J., Liu, N.G., Lu, Y.Z., Jayawardena, R.S., Li, J.F., Su, H.Y., 2019. Freshwater Sordariomycetes. *Fungal Divers* 99, 451–660. <https://doi.org/10.1007/s13225-019-00438-1>.
- Manetti, G., Brunetti, A., Sciarroni, L., Lumia, V., Bechini, S., Marangi, P., Reverberi, M., Scorticini, M., Pilotti, M., 2024. *Diplodia seriata* isolated from declining olive trees in Salento (Apulia, Italy): pathogenicity trials give a glimpse that it is more virulent to drought-stressed olive trees and in a warmth-conditioned environment. *Plants* 13, 2245. <https://doi.org/10.3390/plants13162245>.
- Manion, P.D., Lachance, D., 1991. Forest decline concepts: an overview. In: Manion, P. D., Lachance, D. (Eds.), *Forest Decline Concepts*. APS Press, St. Paul, Minnesota, pp. 181–190.
- Marin-Felix, Y., et al., 2017. Genera of phytopathogenic fungi: GOPHY 1. *Stud. Mycol.* 86, 99–216. <https://doi.org/10.1016/j.simyco.2017.04.002>.
- Marin-Felix, Y., et al., 2019. Genera of phytopathogenic fungi: GOPHY 3. *Stud. Mycol.* 94, 1–124. <https://doi.org/10.1016/j.simyco.2019.05.001>.
- Martino, I., Agustí-Brisach, C., Nari, L., Gullino, M.L., Guarnaccia, V., 2024a. Characterization and pathogenicity of fungal species associated with dieback of apple trees in Northern Italy. *Plant Dis.* 108, 311–331. <https://doi.org/10.1094/PDIS-04-23-0645-RE>.
- Martino, I., Spadaro, D., Guarnaccia, V., 2024b. Fungal trunk pathogens of fruit and nut tree crops: identification, characterization, detection and perspectives for a critical global issue. *Plant Dis.* <https://doi.org/10.1094/PDIS-10-24-2069-FE> in press.
- Morales-Rodríguez, C., Sferazza, I., Aleandri, M.P., Dalla Valle, M., Speranza, S., Contarini, M., Vannini, A., 2021. The fungal community associated with the ambrosia beetle *Xylosandrus compactus* invading the mediterranean maquis in central Italy reveals high biodiversity and suggests environmental acquisitions. *Fungal Biol.* 125, 12–24. <https://doi.org/10.1016/j.funbio.2020.09.008>.
- Moricca, S., Uccello, A., Ginetti, B., Ragazzi, A., 2012. First report of *Neofusicoccum parvum* associated with bark canker and dieback of *Acer pseudoplatanus* and *Quercus robur* in Italy. *Plant Dis.* 96, 1699. <https://doi.org/10.1094/PDIS-06-12-0543-PDN>.
- Mullen, J.M., Gilliam, C., Hagan, A., Morgan-Jones, G., 1991. Canker of dogwood caused by *Lasiodiplodia theobromae*, a disease influenced by drought stress or cultivar selection. *Plant Dis.* 75, 886–889. <https://doi.org/10.1094/PD-75-0886>.
- U.S. National Fungal Databases, 2024. (<https://fungi.ars.usda.gov/>).
- Otoya-Martinez, N., Leite, L.G., Harakava, R., Touray, M., Hazir, S., Chacon-Orozco, J., Bueno, C.J., 2023. Disease caused by *Neofusicoccum parvum* in pruning wounds of grapevine shoots and its control by *Trichoderma* spp. and *Xenorhabdus szentirmai*. *Fungal Biol.* 127, 865–871. <https://doi.org/10.1016/j.funbio.2022.12.002>.
- Pauleit, S., Jones, N., Garcia-Martin, G., Garcia-Valdecantos, J.L., Rivière, L.M., Vidal-Beaudet, L., Bodson, M., Randrup, T.B., 2002. Tree establishment practice in towns and cities - results from a European survey. *Urban For. Urban Green.* 1, 83–96. <https://doi.org/10.1078/1618-8667-00009>.
- Pelleteret, P., Crovadore, J., Cochard, B., Pasche, S., Bovigny, P.-Y., Chablais, R., Lefort, F., 2017. Urban London plane tree dieback linked to fungi in the *Botryosphaeria* spp. *Urban For. Urban Green.* 22, 74–83. <https://doi.org/10.1016/j.ufug.2017.01.014>.
- Phillips, A.J.L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M.J., Groenewald, J. Z., Crous, P.W., 2013. The *Botryosphaeria* spp.: genera and species known from culture. *Stud. Mycol.* 76, 51–167. <https://doi.org/10.3114/sim0021>.
- Raup, M., Gonthier, P., 2017. Biotic factors – Pests and Diseases. In: Ferrini, F., van den Bosch, C.C.K., Fini, A. (Eds.), *Routledge Handbook of Urban Forestry*. Routledge, Taylor and Francis Group, Oxon, UK, pp. 251–272. <https://doi.org/10.4324/9781315627106>.
- Richter, M., Heinemann, K., Meiser, N., Dickhaut, W., 2024. Trees in sponge cities—a systematic review of trees as a component of blue-green infrastructure, vegetation engineering principles, and stormwater management. *Water* 16, 655. <https://doi.org/10.3390/w16050655>.
- Sanna, M., Martino, I., Guarnaccia, V., Mezzalama, M., 2023. Diversity and pathogenicity of *Fusarium* species associated with stalk and crown rot in maize in Northern Italy. *Plants* 12, 3857. <https://doi.org/10.20944/preprints202310.0504.v1>.
- Slippers, B., Wingfield, M.J., 2007. *Botryosphaeria* spp. as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol. Rev.* 21, 90–106. <https://doi.org/10.1016/j.fbr.2007.06.002>.
- Vigouroux, A., Olivier, R., 2004. First hybrid plane trees to show resistance against canker stain (*Ceratocystis fimbriata* f. sp. *platani*). *For. Pathol.* 34, 307–319. <https://doi.org/10.1111/j.1439-0329.2004.00372.x>.
- Von Arx, J.A., 1981. The genera of fungi sporulating in pure culture. Cramer, Vaduz, Germany. ISBN: 9783768206938.
- Yu, L., Liu, J.N., Chen, Z.B., Xu, S.G., Ren, Z., Wang, D.K., Geng, K.Y., Zhang, Q., Pei, W. H., Kong, C.S., 2018. First report of stem die-back on *Platanus × acerifolia* caused by *Neofusicoccum parvum* in China. *Plant Dis.* 102, 1446. <https://doi.org/10.1094/PDIS-06-15-0642-PDN>.