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ORIGINAL PAPER

Revealing novel interactions between oak and *Tubakia* species: evidence of the efficacy of the sentinel arboreta strategy

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Abstract In the present study, the sentinel arboreta strategy was applied, and its efficacy was evaluated at the Atatürk Arboretum (Istanbul, Turkey), having as a study case the interaction *Tubakia* spp.—*Quercus* spp. Thirty-four oak species native of America and Eurasia were sampled within the Fagaceae collection of the arboretum. Isolation trials were conducted from leaf necroses, and High Throughput Sequencing for fungal taxa was carried out from asymptomatic leaf blades. Four *Tubakia* species were identified, *T. dryina*, *T. suttoniana*, *T. hallii,* and *T. macnabbii.* Three out of four are of recent description and the present study contributed to updating their host-range. Thirty-two oak-*Tubakia* interactions new to science were described. Hypotheses were formulated on the possible movement across geographic areas of these species and on the risk posed in case of introduction in the distribution range of susceptible host species. As a conclusive remark, the present study confirmed the efficacy of the sentinel arboreta strategy to highlight new host–pathogen interactions and the risk of host-shift events.

Keywords Sentinel plantation · Early warning · Pathogenicity tests · Host-shift · Invasive forest pathogens (IFP) · Metabarcoding

Introduction

Alien pathogens affecting trees in natural forests and plantations are the cause of the most devastating current and past epidemics worldwide. Trading of living plants and wood products represents the main pathways of introduction of plant pathogens into a new environment and provide the chances for invasion (Santini et al. 2013). The global phytosanitary and quarantine system provides standards aimed at managing the risk of introducing harmful organisms into a new environment (Eschen et al. 2015) mostly, if not all, based on the assumption that the identity and potential impact of the pathogen are known. However, as evidenced by Brasier (2008), most current and past epidemics are caused by pathogens 'unknown to

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science' at the time of their first introduction and described only after they become invasive. For many of them, the native range is still unknown or questionable; for others, it was individuated only after the invasion. Others were known in their native ranges but not recorded as harmful, as demonstrated for the Ash dieback fungus *Hymenoscyphus fraxineus* (Zhao et al. 2013; Zheng and Zhuang 2014). Based on current and past evidence, the current phytosanitary system lacks effectiveness in the timely identification of new invaders (Brasier 2008; Eschen et al. 2019). The sentinel tree concept aims to remediate the above flaws, providing early warning methods and procedures able to identify and rank possible risks to the native flora of a specific geographic area posed by exotic pathogens (and pests) before their introduction. Specifically, these methods provide most of the information needed to perform a Pest Risk Analysis (PRA) before the threat becomes effective (Eschen et al. 2019; Morales-Rodríguez et al. 2019a, b). Two main strategies apply to the sentinel tree concept: sentinel nurseries and sentinel plantations. A sentinel nursery ("in-patria" plantings sensu Eschen et al. 2019) is defined as a site where native traded plants are planted without phytosanitary treatments in their region of production (exporting country) and monitored to identify pests and pathogens which could be spread with their international trade (Kenis et al. 2018; Vettraino et al. 2017). A sentinel plantation ("ex-patria" plantings sensu Eschen et al., 2019) can be defined as a plantation of exotic plants grown in an environment and monitored to identify native pests & pathogens affecting the heathy status of these plants (Roques et al. 2015; Vettraino et al. 2015). A particular type of sentinel plantation is represented by the existing network of botanical gardens and arboreta: though not specifically designed as an early warning tool to detect potential plant pests or pathogens, arboreta and botanical gardens can offer another opportunity for sentinel research and contribute valuable information about novel pest–host associations (Mansfield et al. 2019; Morales-Rodríguez et al. 2019a, b). The importance of such plantings in the context of non-native species prevention has grown recently and the International Plant Sentinel Network (IPSN) has been launched (Barham et al. 2016) to coordinate surveys and activities carried out at botanic gardens on a global scale. In these sites, plant species from different areas of the world live in promiscuity, grouped per taxonomic or ecosystem relatedness, and interacting with the resident native and exotic microorganism communities, favoring host-shift events and, eventually the rise of new host–pathogen interactions.

These assumptions were tested at the Atatürk Arboretum, one of the largest and biodiverse arboreta, established in Istanbul, at the interface between Europe and Asia. A collection of hundreds of different tree species from different continents are represented, each of which is characterized by the geographic origin of the propagation material. The tree collection in the arboretum is organized according to large taxonomic groups. Therefore, related species from different continents grow together in the same plot, favoring interactions and interchanges. A collaborative research program was established within the COST Action FP1401 'Global Warning' between the University of Tuscia (Italy) and the Isparta University of Applied Science, Isparta (Turkey), to verify the above assumptions choosing the Fagaceae collection as the target taxonomic group to monitor and study host-shift events between co-generic species from different regions of the world. The present study aimed to demonstrate the efficiency of the sentinel arboreta in providing evidence of new host–pathogen interactions. For this purpose, the model *Tubakia* spp.—*Quercus* spp. was chosen. The genus *Tubakia* (Diaportales) included species that are commonly fungal endophytes and plant pathogens causing conspicuous leaf blade and vein necroses, spot, and blotch (Fig. 3) on a wide range of hosts most of which Fagaceae, and mostly oaks (Braun et al. 2018). The species with a pathogenic lifestyle commonly cause late-season symptoms. As described by Taylor and Clark (1996) for *T. dryina*, conidia can germinate on leaf surfaces and actively penetrate and colonize the leaf tissues causing necrotic spots that gradually coalesce in large necrotic areas. The pathogenic species produce phytotoxic metabolites (Venkatasubbaiah and Chilton 1992) and can impair host physiology as recently evidenced by Park et al. (2021). On the necrotic tissues *Tubakia* spp. differentiate a unique conidiomata called pycnothyrium that consists of a circular scutellum attached to the leaf surface by a central columella. Conidia, the main source of inoculum of the genus, are formed from conidiogenous cells beneath the scutellum (Braun et al. 2018). Broun et al. (2018) recognized 16 different species of *Tubakia* from different part of

the world, some of which with a restricted distribution range, other with a very broad one. More recently, Young Yun and Kim (2020) described a new species, *T. koreana*, pathogenic on leaves of *Q. acutissima*, *Q.* × *alienoserratoides*, *Q. mongolica*, and *Q. serrata* in Korea. Because of their affinity to oaks throughout their distribution range, from Asia to Europe and Americas, the species of *Tubakia* are an interesting model to study the host-shift events between hosts of different geographic origin. Tree's collections in arboreta represent the most suitable sites where to conduct such studies. The present study reports novel *Tubakia—Quercus* interactions and the proof of pathogenicity, from an extensive survey, carried out in the *Quercus* spp. collection of the Atatürk Arboretum.

Materials and methods

Sampling area

The Atatürk Arboretum located in Bahçeköy, Sarıyer, Istanbul Province, Turkey (41°10′35″N 28°59′06″E) covers an area of 296 ha at the southeast of Belgrade Forest which is classified under Meso-thermophilous oak, hornbeam, and beech forests (Colline mixed oak forests) (Coban and Willner 2019), and is mainly composed of *Quercus petraea*, *Q. frainetto*, *Q. robur*, *Fagus orientalis*, *Castanea sativa*, *Carpinus betulus*, *Alnus glutinosa*, *Salix alba* and *Ulmus minor* (Yaltırık 1966).

The arboretum was established in 1946 and is currently operated by the İstanbul Bahçeköy Forestry Enterprise. Samples were collected at the Fagaceae section, in the *Quercus* spp. plot. It consists of a collection of more than a hundred different oak species from America and Eurasia (Fig. 1). The collection was started from seedlings grown in the arboretum nursery and obtained from 50 different arboreta and botanical gardens around the world.

Collection of samples

The survey was carried out in September 2015. A total of 34 oak species were sampled within the following sections, Cerris (10 species); Lobatae (11 species); Quercus (11 species); Ilex (1 species), Virentes (1 species) (Fig. 2). From each species, symptomatic and asymptomatic leaves were sampled from different individuals where possible. Leaves from each tree and category (symptomatic and asymptomatic) were kept in a separate bag, tagged, and taken to the laboratory.

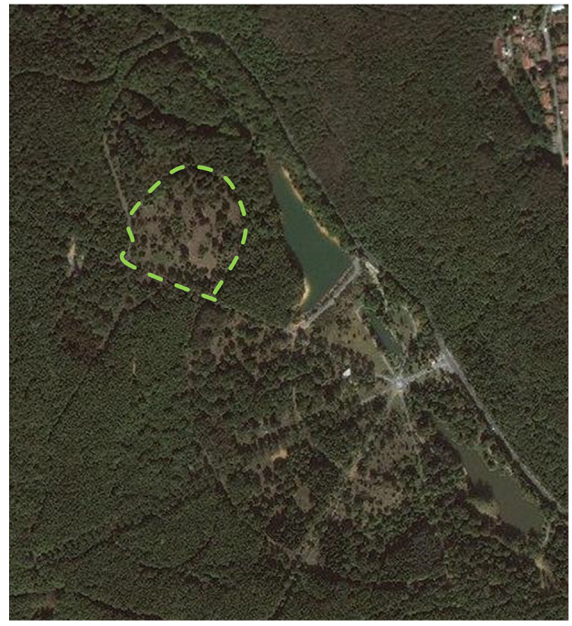

Fig. 1 Satellite image of the Atatürk Arboretum located in Bahçeköy, Sarıyer, Istanbul Province, Turkey (41°10′35″N 28°59′06″E). The oak collection from which the samples were collected is delimited by the dotted line (source Google Earth©)

Symptom assessment and isolation

Symptom assessment was carried out on leaves following the keys as reported by Roques et al. (2017).

Fungal isolation from leaf necrosis

Isolations were taken from the margin of the necrosis with the healthy tissues in areas free of fungal reproductive structures. Small leaf fragments (2 × 2 mm) were excised, sterilized in 75% EtOH (1 min); 2% sodium hypochlorite (3 min); 75% EtOH (30 s); washed 3 times in sterile, distilled H_2O (sdH_2O), dried on filter paper and plated on potato dextrose agar with streptomycin (0.02 g/L) (PDAs) in 9-cm diameter Petri dishes. Cultures were incubated at 24 °C at 12/12 h photoperiod for 1 week. Colonies grown from leaf fragments were isolated in single

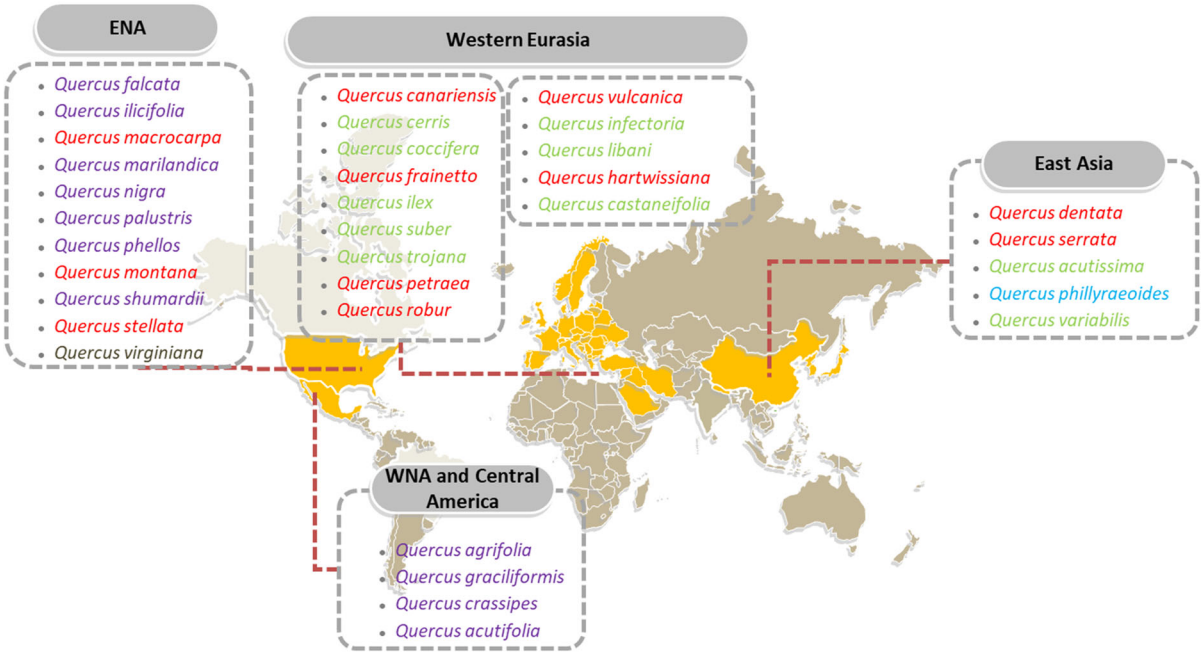

Fig. 2 Geographic distribution of the 34 oak species sampled at the Atatürk Arboretum. Colors refer to the section: Lobatae (purple); Quercus (red); Cerris (green); Ilex (blue), and Virentes (brown)

culture on PDAs and then grouped per colony morphotype.

Molecular identification and phylogenetic analysis

DNA was extracted from fresh mycelium grown on PDB (potato dextrose broth) with the NucleoSpin Plant II mini kit (Macherey Nagel, Germany) following the manufacturers' instructions. DNA concentration was assessed by gel electrophoresis, and DNA was diluted 1:10 to perform PCR. The nuclear rDNA operon spanning the end of the 18S nrRNA gene, the first internal transcribed spacer region, the 5.8S nrRNA gene, the second internal transcribed spacer region and the end of the 28S nrRNA gene (ITS), the partial β-tubulin (TUB), and partial translation elongation factor 1-alpha (TEF) genes were amplified using primer pairs ITS5/ITS4 (White et al. 1990), T1/Bt-2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997) and EF1–728F/EF-2 (Carbone and Kohn 1999; O'Donnell et al. 1998). Amplification conditions for ITS, TEF and TUB followed by Braun et al. (2018). Amplicons were purified with NucleoSpin Gel and PCR Cleanup (Macherey Nagel). Sequencing reactions were performed by Eurofins Scientific (Luxemburg) and forward and reverse sequences assembled and edited using BioEdit (Ibis Bioscience, CA, USA) and compared to the NCBI database (https://blast.ncbi.nlm.nih.gov/). For phylogenetic analysis, the sequences generated in this study (ITS, TUB and TEF) were supplemented with additional sequences of *Tubakia* spp. obtained from GenBank (Table S1) based on blast searches and literature (Braun et al. 2018).

Sequences were aligned using ClustalW, included in MEGA v. 7 (Kumar et al. 2016), under default settings, all the alignments were inspected and adjusted manually if required (Alignments available at TreeBASE: ID25838). A Bayesian phylogenetic analysis was done using MrBayes v. 3.2.7a (Ronquist et al. 2012). As reported by Morales-Rodríguez et al. (2019a, b), evolutionary history was inferred using the maximum-likelihood method based on the general time-reversible model (Nei and Kumar 2000) according to the result obtained using jModelTest v. 2.1.7 (Darriba et al. 2012). Maximum likelihood analyses were conducted with MEGA v. 7.

HTS analysis

High-throughput sequencing analysis was carried out on asymptomatic leaf blades disinfected and surface washed as described above. Total DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany), following the manufacturer's instructions. The ITS1 region was amplified with a dual indexing primer using the tagged primer pair ITS1F (5′-xxxxCTYGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5-xxxxGCHRCGTTCTTCATCGDTGC-3′), where xxx represents the barcoding key. The PCR reaction mixture comprised 12.5 µl of Maxima Hot Start PCR Master Mix (2X) (Thermo Fisher Scientific, USA) and 1 µM of each primer in a total volume of 25 µl containing 24 µl of the reaction mixture and 1 µl template. The thermal cycle was an initial denaturation at 94 °C for 10 min followed by 30 cycles of 95 °C for 40 s, 60 °C for 40 s and 72 °C for 1 min, and a final elongation step of 72 °C for 10 min. Eight PCRs were carried out and pooled per sample. Amplicons were purified using the MagJET NGS Cleanup (Thermo Scientific, USA), quantified with the Qubit Quantitation kit (Invitrogen, USA), and pooled at equal concentrations for sequencing. Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq sequencer by Eurofins Genomics GmbH (Germany). Two mock communities ('even' and 'staggered') were used as an internal control to evaluate HTS results. Both were constituted by a DNA mix from the same fungal taxa (7 Ascomycota and 1 Mortierellales). In the'even' mock community DNA concentration was equal among isolates, while in the 'staggered' the DNA concentration differed by 5 folds among members. The composition and characteristics of the Mock community are reported in Table S2.

Data sets were analyzed following the pipeline described by Morales-Rodríguez et al. (2021). To reduce the phenomenon of cross-contamination and false assignments, only the reads containing the combination of 5'barcode and forward primer as well as the expected 3'barcode and reverse primer were paired and used in the analyses; moreover, for the identification of barcode and primer sequences no mismatches were allowed. Raw read pairs were quality filtered (limit = 0.05) and trimmed using CLC Genomic Workbench Version 8.5.1 (QIAGEN bioinformatics, Aarhus, Denmark) filtering out all sequences containing "N"s and sequences with a minimum length of 100 nucleotides or a maximum length of 400 nucleotides. After this, the paired-end reads were assembled. If there were mismatches between the overlapping fragments of the forward and reverse reads, these were corrected according to the base call with the higher sequencer-assigned quality score.

After quality filtering, paired-end assembly, and demultiplexing, the sequences were processed, and similarity clustering was performed based on the UPARSE pipeline of USEARCH v8 (Edgar 2010) using a 97% clustering threshold (Lindahl et al. 2013). Sequences failing alignment or identified as chimeric were removed before downstream analysis. Consensus OTUs were identified using the BLAST tool in the Genbank database with the algorithm parameters: word size = 11, match/mismatch scores = 2,-3, gap cost existence = 5, and gap cost extension = 2. The xml file from the BLAST and the blasted fasta files were imported into MEGAN (Huson et al. 2007) to compute and explore the taxonomical content of the data set, employing the NCBI taxonomy to summarize and order the results. The lowest common ancestor parameters were: Min score = 170; Max. expected = 0.01; Top percent = 2.0, Min support percent = 0.3; Min support = 1 and LCA percent = 40) and with the following minimum requirements of similarity to accept the proposed taxonomy: Species 99%, Genus 97%, Family 95%, Order 90%, Class 85%, and Phylum 80%.

The reads generated in this work are available in the NCBI Sequence Read Archive (SRA) http://www.ncbi.nlm.nih.gov/bioproject/646359 under the project name PRJNA646359: Fungal community of oak collection at Ataturk arboretum.

Pathogenicity tests

Healthy (asymptomatic) mature leaves for pathogenicity tests were excised from 10 oak species at the 'Tor Vergata' Arboretum (Botanical Garden of the second University of Rome—Italy) selected among the available species in the list of the Atatürk Arboretum from which *Tubakia* spp. was found associated with leaf necrosis. The oak species included *Q. trojana*, *Q. acutissima*, *Q. castaneifolia* (section Cerris); *Q. marilandica*, *Q. graciliformis*, *Q. palustris* (section Lobatae); *Q. robur*, *Q. macrocarpa*, *Q. dentata*, and *Q. montana* (section Quercus). Pathogenicity tests were carried out following the protocol of Munkvold and Neely (1990) slightly modified. Oak leaves were excised at the base of the

petiole, rinsed in sterile distilled water, and let dry on filter paper in a flow chamber. Inoculation was carried out in sterilized plastic trays lined with moistened filter paper to reach the dewpoint into the boxes; leaves (5) were placed on a plastic net at about 4 cm from the bottom of the tray with the adaxial surface up. Four *Tubakia* isolates were used for pathogenicity tests: *T. dryina*, isolate 4c from *Q. robur*; *T. halli*, isolate 14c from *Q. robur*; *T. macnabbii*, isolate 15a from *Q. dentata*; *T. suttoniana*, isolate 39ct from *Q. palustris*. Inoculum of *Tubakia* spp. was prepared by growing colonies on Carrot Agar (CA) for 15 days at room temperature and constant light. Conidia were collected by pipetting 1 mL of sdH_2O on the center of the colony and gently scraping the colony with the tip of the pipette to suspend conidia. The suspension was collected and adjusted to 10^6 conidia per mL. A small paintbrush was used to distribute the suspension (2 mL) evenly over the leaf surface. Leaves inoculated with distilled water were used as the negative control. After inoculation, the trays were sealed in transparent plastic bags and incubated at room temperature (23 °C) on the laboratory bench for 30 days. Three repetitions were performed for each combination *Tubakia—Quercus* and the test was repeated twice.

Statistics

The 2 × 2 Contingency table analysis was carried out using the Fisher Exact test with the Haldane correction in case of zero values according to Weber et al. (2020)

Results

Including both biological (isolation) and molecular (HTS) detection, *Tubakia* spp. were identified from 28 out of 34 oak species investigated (Table 1). Indeed, *Tubakia* spp. was not isolated/detected from *Q. suber*, *Q. variabilis* (section Cerris), *Q. nigra*, *Q. phellos* (section Lobatae), *Q. stellata* (section Quercus), and *Q. virginiana* (section Virentes). Twenty-nine *Tubakia* isolates were obtained from symptomatic leaf necrosis of 20 out of 34 *Quercus* spp. (Table 1). *Tubakia* spp. were isolated from leaf spots ranging from small to large round necrosis with definite margin, dark brown or reddish, or irregular angular necrosis affecting leaf blade sectors between veins (Fig. 3).

Seventeen isolates were identified by molecular barcoding as *Tubakia suttoniana* U.Braun & Crous; nine isolates as *Tubakia dryina* (Sacc.) B. Sutton; two isolates as *Tubakia hallii* T.C. Harr. & McNew; one isolate as *Tubakia macnabbii* T.C. Harr.& McNew (Table 1). A multilocus (ITS, TUB, and TEF) phylogenetic tree including all the *Tubakia* species is shown in Fig. 4. *Tubakia suttoniana* is considered a species with a limited distribution in Europe and New Zealand (Farr and Rossman 2021); *T. dryina* is a widespread species detected either in Europe, Asia, Americas, Oceania; *T. hallii* and *T. macnabbii* have a distribution limited to North America (Farr and Rossman 2021). Culture morphology of the four species on PDA is showed in Fig. 5.

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (− 2921.17) is shown (Fig. 3) Bayesian and Maximum likelihood tree resulted in the same topology. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. A discrete Gamma distribution was used to model evolutionary rate differences among sites (4 categories (+ G, parameter = 0.8108)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 28.73% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 63 nucleotide sequences. There were 569 positions in the final dataset.

The isolations of *T. dryina* associated with leaf necrosis of *Q. trojana* and *Q. vulcanica* are new records. The identification of *T. hallii* from leaf necrosis on the Euroasian *Q. robur* and *Q. frainetto* are new records as well. All the 14 combinations of *T. suttoniana—Quercus* spp. from leaf necrosis were new to science (Table 1). Finally, the recovery of *T. macnabbii* from leaf necrosis of the far east species *Quercus dentata*, is also a new record. The bar chart in Fig. 6A shows the percentage of detection by isolation from leaf necrosis of *T. suttoniana, T. dryina and T. hallii* from members of each oak section Quercus, Lobatae, and Cerris (section Ilex was not included in the analysis since represented by only one oak species). *Tubakia suttoniana* was the most represented

Table 1 *Tubakia* spp. isolated from leaf necroses and/or detected from asymptomatic leaf blades of 34 *Quercus* spp. sampled at the Atatürk Arboretum in Istanbul (Turkey)

Section	Species	Common name	Distribution range	Isolated from symptoms	HTS of healthy tissues	New record
Cerris	*Quercus acutissima* Carruth	Sawtooth oak	EA	*T. suttoniana 8c*		Yes
Cerris	*Quercus castaneifolia* C.A. Mey	Chestnut-leaved oak	WEA		*T. dryina*	No (Zahedi et al. 2011)
				T. suttoniana 6-2a; 6c	*T. suttoniana cx**	Yes
Cerris	*Quercus cerris* L	Turkey oak	WEA		*T. suttoniana cx*	No (Farr and Rossman 2021)
Cerris	*Quercus coccifera* L	Kermes oak	WEA	*T. dryina 31°*	*T. dryina*	Yes
					T. suttoniana cx	nd**
Cerris	*Quercus ilex* L	Holm oak	WEA	*T. dryina 22 k*		no (Fisher et al. 1994)
Cerris	*Quercus libani* Oliv	Lebanon oak	WEA		*T. dryina*	Yes
					T. hallii	Yes
					T. suttoniana cx	nd
Cerris	*Quercus suber* L	Cork oak	WEA			
Cerris	*Quercus trojana* Webb	Macedonian oak	WEA	*T. dryina* 10-2d	*T. dryina*	Yes
				T. suttoniana 10b	*T. suttoniana cx*	Yes
Cerris	*Quercus variabilis* Bl	Chinese cork oak	EA			
Lobateae	*Quercus agrifolia* Nee	Coast live oak	WNA	*T. suttoniana 18a*		Yes
Lobateae	*Quercus acutifolia* Nee		CA		*T.dryina*	Yes
				T. suttoniana 49a, 49b	*T. suttoniana cx*	Yes
Lobateae	*Quercus crassipes* Humb. & Bonpl		CA		*T. suttoniana cx*	nd
Lobateae	*Quercus falcata* Michx	Southern red oak	ENA		*T. dryina*	No (Farr and Rossman 2021)
					T. suttoniana cx	nd
Lobateae	*Quercus graciliformis* C.H. Mull	Chisos oak	WNA		*T. suttoniana cx*	nd
Lobateae	*Quercus ilicifolia* Wangenh	Bear oak	ENA	*T. suttoniana 9b*	*T. suttoniana cx*	Yes
Lobateae	*Quercus marilandica* Muench	Blackjack oak	ENA		*T. hallii*	Yes
				T. suttoniana 21-2d		Yes
Lobateae	*Quercus nigra* L	Water oak	ENA			
Lobateae	*Quercus palustris* Muench	Pin oak	ENA	*T. suttoniana 39ct*		Yes
Lobateae	*Quercus phellos* L	Willow oak	ENA			
Lobateae	*Quercus shumardii* Buck	Shumard oak	ENA	*T. suttoniana 19a, 19b*		Yes

Table 1 continued

Section	Species	Common name	Distribution range	Isolated from symptoms	HTS of healthy tissues	New record
Quercus	*Quercus canariensis* Wild	Mirbeck's oak	WEA		*T. dryina*	Yes
					T. hallii	Yes
				T. suttoniana 35b	*T. suttoniana cx*	Yes
Quercus	*Quercus dentata* Thunb	Daimyo oak	EA		*T. dryina*	No (Kobayashi 2007)
					T. hallii	Yes
					T. suttoniana cx	nd
				T. macnabbii 15a		Yes
Quercus	*Quercus frainetto* Ten	Hungarian oak	WEA		*T. dryina*	Yes
				T. hallii 29c	*T. hallii*	Yes
				T. suttoniana 29-3b;	*T. suttoniana cx*	Yes
Quercus	*Quercus vulcanica* Boiss. & Heldr	Kasnak oak	WEA	*T. dryina 26-2a*	*T. dryina*	Yes
					T. hallii	Yes
Quercus	*Quercus serrata* Thunb	Konara oak	EA		*T. suttoniana cx*	nd
Quercus	*Quercus hartwissiana* Stev	Strandzha oak	WEA		*T. dryina*	No (Huseyinov and Selçuk 2001)
					T. suttoniana cx	nd
Quercus	*Quercus macrocarpa* Michx	Bur oak	ENA		*T. hallii*	No (Farr and Rossman 2021)
				T. suttoniana 20c	*T. suttoniana cx*	Yes
Quercus	*Quercus petraea* (Mattuschka) Liebl	Sessile oak	WEA	*T. dryina 51b; 4c; 11b*	*T. dryina*	No (Farr and Rossman 2021)
					T. hallii	Yes
					T. suttoniana cx	nd
Quercus	*Quercus montana* Willd	Chestnut oak	ENA	*T. suttoniana 36b*	*T. suttoniana cx*	Yes
Quercus	*Quercus robur* L	Pedunculate oak	WEA	*T. dryina 50-1a; 59-2d*	*T. dryina*	No (Farr and Rossman 2021)
				T. hallii 14ct	*T. hallii*	Yes
					T. suttoniana cx	nd
Quercus	*Quercus stellata* Wangh	Post oak	ENA			
Quercus	*Quercus infectoria* Oliv	Aleppo oak	WEA		*T. dryina*	Yes
					T. hallii	Yes
					T. suttoniana cx	nd
Virentes	*Quercus virginiana* Mill	Southern live oak	ENA			

Table 1 continued

Section	Species	Common name	Distribution range	Isolated from symptoms	HTS of healthy tissues	New record
Ilex	*Quercus phillyraeoides* Gray	Ubame oak	EA		*T. dryina*	No (Kobayashi 2007)
				T. suttoniana 42a	*T. suttoniana cx*	Yes

The status of the record (new or not new) is described in the last column along with the reference for those already present in the literature. Taxonomy and distribution range of oak species obtained from Denk et al. (2017)

EA, Eastern Asia; WEA, Western Eurasia; WNA, Western North America; ENA, Eastern North America; CA, Central America

**Tubakia suttoniana* complex

**Not determinable; HTS analysis of ITS libraries cannot resolve species within the *T. suttoniana* complex

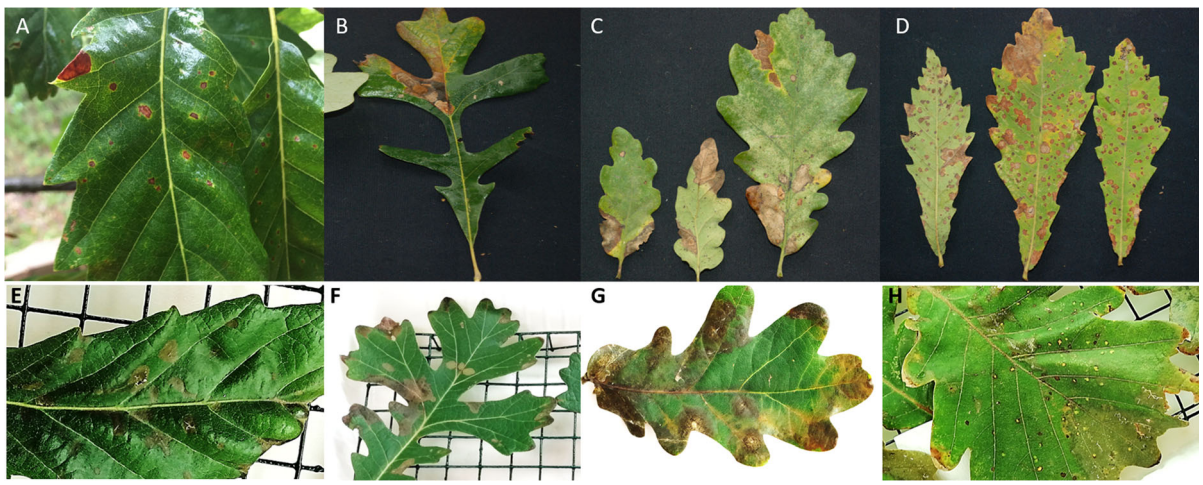

Fig. 3 Examples of leaf symptoms observed at the Atatürk Arboretum (**A**–**D**) and as results of artificial inoculation with *Tubakia* spp. (**E**–**H**). *Q. castaneifolia* (**A**); *Q. castaneifolia* x *T. suttoniana* (**E**); *Q. macrocarpa* (**B**); *Q. macrocarpa* x *T. hallii* (**F**); *Q. robur* (**C**); *Q. robur* x *T. dryina* (**G**); *Q. dentata* (**D**); *Q. dentata* x *T. macnabbii* (**H**)

species (from 13 oak species) and the only one detected in the 3 sections. Moreover *T. suttoniana* was the only species recovered from hosts of the section Lobatae. *Tubakia dryina* was isolated from necroses of oak species from sections Cerris and Quercus (6 oak species), while *T. hallii* was detected from section Quercus only (2 oak species).

High-throughput sequencing from asymptomatic leaves identified 218 OTU's. All members of the 'even' and staggered' mock communities were confirmed although *Fusarium oxysporum*, *F. aveacenarum*, *Verticillium dahliae,* and *V. tricorpus* were resolved only at the genus level by HTS analysis. The number of reads did not correlate with the DNA concentration (Table S2). No false positives were generated from the mock communities.

In the present study, only the OTUs within the genus *Tubakia* were considered. Within the *Tubakia* genus, *T. halli* and *T. dryina* were distinguished in two separate OTUs. Differently, ITS sequences could not distinguish within the *T. suttoniana* species complex (cx) and related species including *T. suttoniana* nom. nov., *T. melnikiana*, *T. seoraksanensis*, *T. japonica,* and *T. macnabbii* (Braun et al. 2018). Specifically, *T. dryina* was detected from 15 oak species of which 5 represented new records for literature, 3 confirmed the new record of isolation from symptomatic tissues, and 7 were already reported in the literature. *T. hallii* was identified from 10 oak species, of which 7 represented

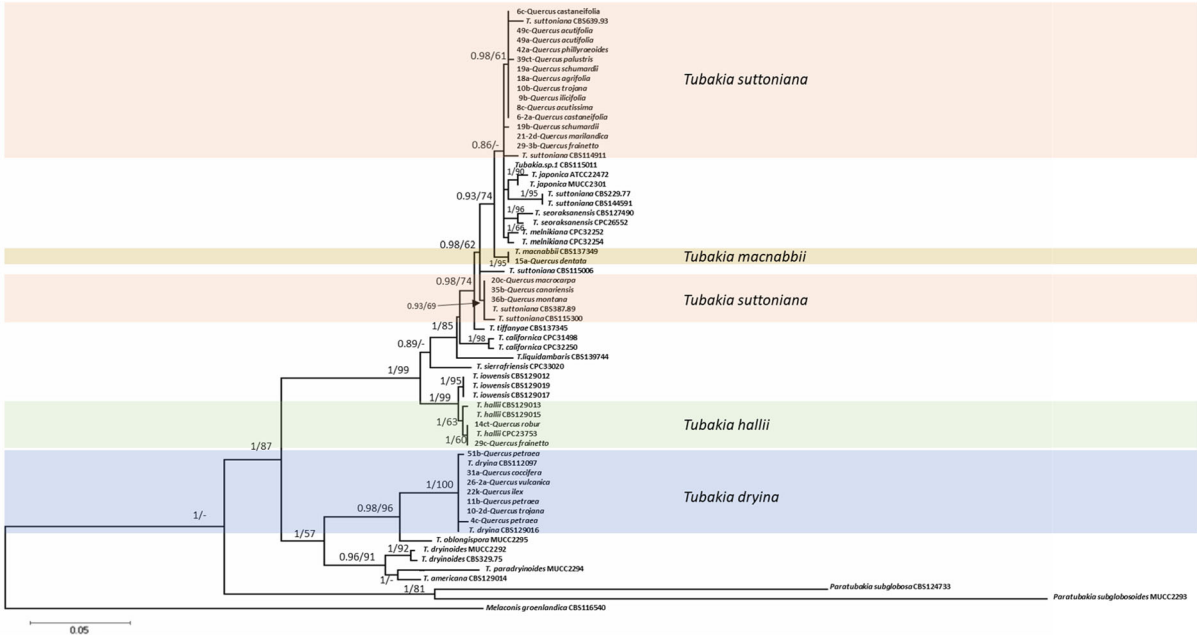

Fig. 4 Bayesian tree for *Tubakia* species produced from concatenated sequences of the ITS, beta-tubulin, and elongation factor using GTR + G model. Maximum likelihood was conducted on the same dataset with MEGA v. 7 and resulted in the same topology. Numbers above the branches reflect support obtained from the analysis of the same dataset (Bayesian posterior probabilities/Bootstrap values estimated by MEGA v. 7). *Paratubakia* spp. and *Melanconis groenlandica* were used as outgroups. The scale bar corresponds to substitutions per nucleotide site

new records for literature, 2 confirmed the new records of isolation from symptomatic tissues, and 1 was already reported in the literature. Finally, *T suttoniana* cx was identified from 22 oak species. Since the species complex includes several species of *Tubakia* (Braun et al. 2018) not resolved with ITS, it is impossible at the moment to determine which detection represents a new record. It must be underlined that new associations determined exclusively with DNA barcoding, cannot be necessarily classified as endophytic ones. The bar chart in Fig. 6B shows the percentage of detection by HTS analysis of *T. suttoniana cx*, *T. hallii* and *T. dryina* from asymptomatic leaf blades of each oak section Quercus, Lobatae, and Cerris. The 3 taxa were detected, at different percentages, from all the oak sections. The section Quercus displayed a higher percentage of detection numbers (*T. dryina* absolute frequency 9 over 12 members of the Sect. (9/12), 75%; *T. suttoniana* 11/12, 91.7%; *T. hallii* 8/12, 66.7%) for all the three *Tubakia* spp. compared to each other section.

The number of *Tubakia—Quercus* interactions new to science is shown in Fig. 7. Only the interactions identified with multi-locus phylogeny (isolates in pure culture) or distinguishable as an OTU (HTS reads) were considered (thus excluding the *T. suttoniana* cx OTUs).

In Fig. 8 the frequency of isolation/detection of *Tubakia* spp. is grouped per continental distribution of the oak species (America and Eurasia). HTS analysis evidenced a higher diversity and abundance of *Tubakia* spp. on the Euroasian than American oaks. Results of isolation from necroses also confirmed a higher diversity on Euroasian oaks and higher abundance for *T. dryina* and *T. hallii*. *T. suttoniana* abundance was higher on American oaks. However, differences in abundance were significant at the Fisher Exact test only for *T. dryina* in both biological (A) and molecular (B) detection.

Table 2 summarizes the results of the pathogenicity tests. Fourteen *Tubakia—Quercus* associations were tested. Eleven out of fourteen demonstrated the pathogenicity on detached leaves. In eleven combinations the pathogen was re-isolated from necrotic tissues thus satisfying the Koch postulates. An exception is the combination *T. suttoniana* x *Q.*

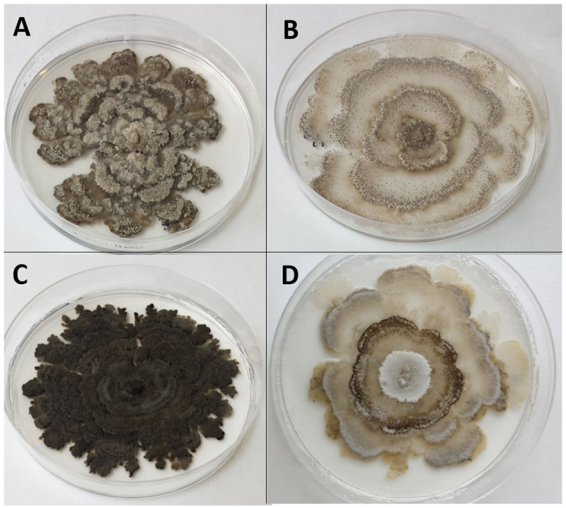

Fig. 5 Colony morphology on PDAs of the four *Tubakia* spp. Isolated from necrotic lesion on oak leaves: *Tubakia suttoniana*, isolate 39ct from *Quercus palustris* (A); *Tubakia hallii*, isolate 14c from *Quercus robur* (B); *Tubakia dryina*, isolate 4c from *Quercus robur* (C); *Tubakia macnabbii*, isolate 15a from *Quercus dentata* (D)

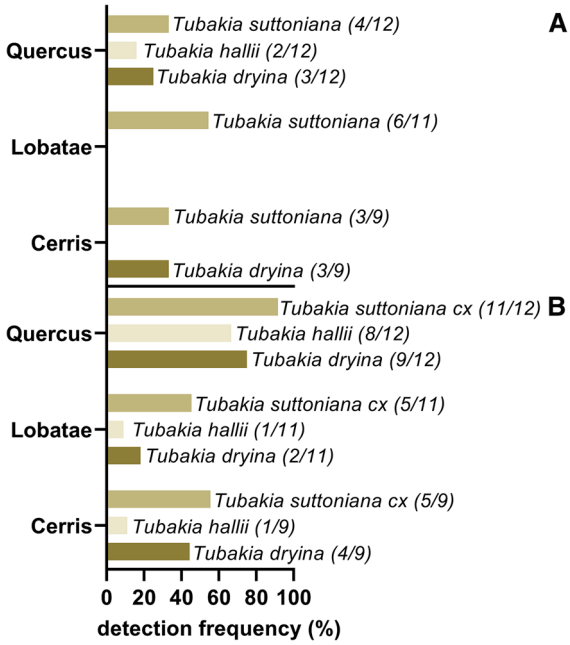

Fig. 6 Percentage of detection of *Tubakia* spp. from symptomatic (**A**), determined by isolation from necrosis) and asymptomatic (**B**), determined by HTS analysis) leaf blades of oak species grouped per section. In brackets, the number of members of the oak section with positive detection over the total number of members of the section

graciliformis where it was not possible to re-isolate the pathogen from the necrotic tissues. The combination *T. hallii* x *Q. robur* did not induce any symptom on detached leaves, as well as the combination *T. suttoniana* x *Q. trojana*. Eight out of eleven pathogenic interactions were new to science and confirmed with pathogenicity tests. Examples of symptom development on inoculated leaf blades are shown in Fig. 2.

Discussion

Among the four species of *Tubakia* identified at the Atatürk Arboretum, two, *T. hallii* and *T. macnabbii* were firstly described by Harrington and McNew (2018); one, *T. suttoniana*, was described by Braun et al. (2018). The type species, *T. dryina* has been then epitypified (Braun et al. 2018). In the present study, *T. dryina* was significantly more abundant in Euroasian oaks both as asymptomatic detection and isolation from leaf necrosis. This finding is in line with the statement of Braun et al. (2018) that speculated that *T. dryina* is a primarily European species introduced in other regions of the world with the main host *Q. robur*. The host-range reported in the present study refers to the potential of infection of *T. dryina* and canÿt be considered an indication of the effective geographic distribution of the species. However, it provides a picture of the possible risk of introduction of this

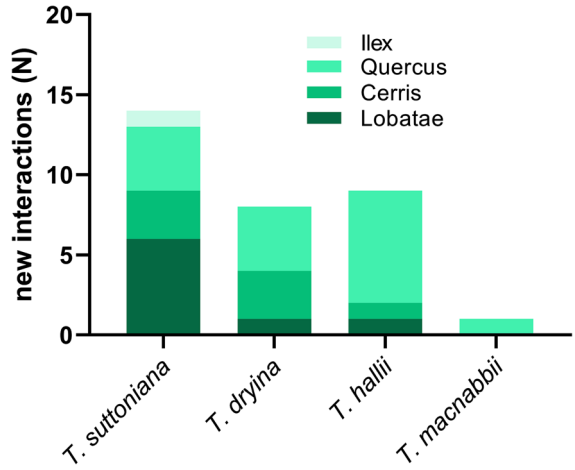

Fig. 7 Number of interactions new to science per *Tubakia* sp. and per *Quercus* section determined with the isolation/detection trials from leaves at the Atatürk Arboretum

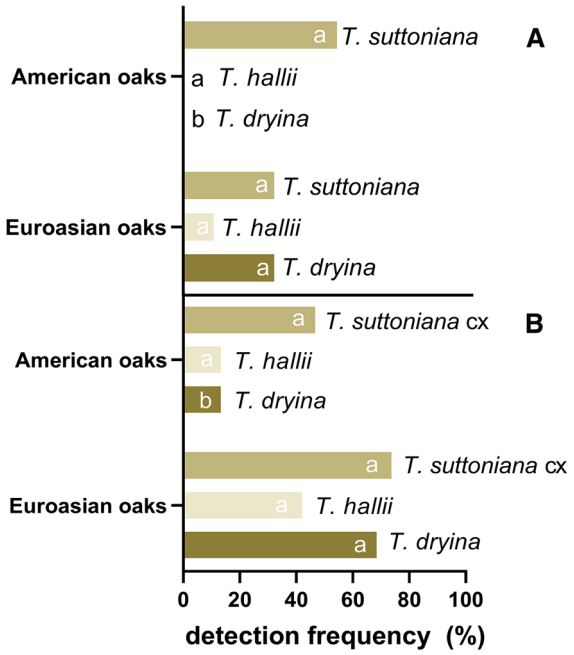

Fig. 8 Percentage of detection of *Tubakia* spp. from symptomatic (**A**) (determined by isolation from necroses), and asymptomatic (**B**) (determined by HTS analysis) leaf blades of oak species grouped per continental distribution. Different letters indicate for each *Tubakia* sp. a significant difference at the Fisher Exact test with Haldane correction in the percent of detection between the two groups of oaks (American and Euroasian)

species in other regions of the world to specific hosts, at least under the conditions present Atatürk Arboretum.

Due to the recent revision of the genus *Tubakia*, the knowledge of the effective geographic distribution and host-range of the newly described species is limited. *T. hallii* for example is a monophyletic group with a host range restricted to leaf spots and leaf veins necroses of oak species of the section Quercus in the East of US (Braun et al. 2018). In our study, *T. halli* was detected in three out of five oak sections but with a preference for the section Quercus. Furthermore, *T. hallii* was isolated from necroses on two Euroasian oaks of the section Quercus, confirming the observations of Harrington and McNew (2018) about the oak section, but leaving open the question on the origin of the species.

The isolation of *T. macnabbii* from necroses of *Quercus dentata*, a species native of East Asia, and the proof of pathogenicity on this host, are also aspects of great interest. Indeed, *T. macnabbii* is considered a species with a restricted range in the eastern USA, where it appears to be indigenous, and widespread on oaks belonging to section Lobatae (Braun et al. 2018). The isolation and the proof of pathogenicity to *Q. dentata* (section Quercus), supports a host-shift event and a possible new pathogenic association between fungal and plant species of different geographic origin.

Table 2 Results of pathogenicity tests carried out with 14 combinations of 10 *Quercus* spp. and the 4 *Tubakia* spp. All the host species—*Tubakia* spp. combinations tested were previously detected with the isolation trials from leaves of the trees at the Atatürk Arboretum

Isolate/host	*Tubakia* spp.	Host	Section	Symptoms	Re-isolation	Pathogenicity new to science
4c/Q. robur	*T. dryina*	*Q. trojana*	Cerris	Yes	Yes	Yes
		Q. robur	Quercus	Yes	Yes	No
14c/Q. robur	*T. halli*	*Q. robur*	*Quercus*	No	Nd	Nd
		Q. macrocarpa	*Quercus*	Yes	Yes	No
15a/Q. dentata	*T. macnabbi*	*Q. dentata*	Quercus	Yes	Yes	Yes
		Q. marilandica	Lobatae	Yes	Yes	No
39ct/Q. palustris	*T. suttoniana*	*Q. acutissima*	Cerris	Yes	Yes	Yes
		Q. macrocarpa	Quercus	Yes	Yes	Yes
		Q. montana	Quercus	Yes	Yes	Yes
		Q. robur	Quercus	Yes	Yes	Yes
		Q. palustris	Lobatae	Yes	Yes	Yes
		Q. graciliformis	Lobatae	Yes	No	Nd
		Q. castaneifolia	Cerris	Yes	Yes	Yes
		Q. trojana	Cerris	No	Nd	Nd

Unfortunately, we could not assess the presence of *T. macnabbii* from asymptomatic leaf blades since HTS analysis was not able to discriminate this species from *T. suttoniana*. Thus, its presence in the oak collection investigated might be underestimated. An open question is where the inoculum of *T. macnabbii* comes from. It can be argued that saplings or seeds from North American oak species were contaminated/infected at the time of shipping and transplant in the arboretum. It is well known that *Tubakia* can colonize buds, twigs and even seeds in absence of evident signs or symptoms (Gennaro et al. 2001; Harrington and McNew 2018; Yang et al. 2020); moreover, the colonization of twigs and buds represent the pathway through which these pathogens are introduced with living deciduous plant hosts that are commonly shipped without leaves.

The Atatürk Arboretum represents the hottest spot of *T. suttoniana* in Eurasia. According to Braun et al. (2018), the first record of *T. suttoniana* was from leaves of *Q. rubra* (section Lobatae) in Italy. Before the present study, and in addition to the Italian record on *Q. rubra*, the presence of *T. suttoniana* was limited to few records in The Netherlands and New Zealand (Farr and Rossman 2021) on oaks of the sections Cerris and Quercus. In the present study, *T. suttoniana* was by far the most frequent species detected/isolated from symptomatic oak leaves of oaks belonging to four sections, and on many of which the pathogenicity was confirmed with laboratory tests.

The present study represents one of the few examples of the application of the sentinel arboreta strategy to highlight new host-fungal interactions and to demonstrate pathogenicity. Although the sentinel arboreta concept has been developed in the first decade of this century (Britton et al. 2010), and the establishment of the IPSN, as part of a EUPHRESCO project (EUropean PHytosanitary RESearch COordination) Phytosanitary ERA-Net, is dated 2013 (Barham et al. 2015), still, many of the scientific papers on the sentinel arboreta strategy focus on the development of the concept (Barham et al. 2016; Eschen et al. 2019; Paap et al. 2017), its ecological pillars (Kirichenko and Kenis 2016; Kirichenko et al. 2013), and the methodology of collection and processing of the samples (Morales-Rodríguez et al. 2019a, b; Roques et al. 2017). Definitions, concepts, and methods are part of the recently issued PM 3/91(1) 'Sentinel woody plants: concepts and application' (Anonymous 2020). Besides the availability of concerted sampling procedures (Roques et al. 2017), and detailed protocols of sample processing (both biological and molecular) (Morales-Rodríguez et al. 2019a, b), the complexity of the approach reflects the complexity of its implementation. Difficulties rely on the high amount of data to manage and elaborate (specifically where metabarcoding and HTS analysis are employed); the time needed to complete the whole process, from sampling to pathogenicity assessment; the strong dependence on seasonality for sampling; and last but not the least the sensible cost in term of person-months and material to carry out the whole activity. In absence of a structured network of sentinel arboreta with shared duties and agreements for sampling and processing, the researchers need to travel often far from their laboratory also abroad with evident problems of logistic (need of a local laboratory/team for samples/specimens processing, authorization to transport specimens or cultures in between different countries, even in consideration of the entering in force of the Nagoya Protocol in 2014). Besides this, some experimental papers have been published mostly dealing with herbivore damages on nonnative trees in botanical gardens/arboreta in different parts of the world (Mansfield et al. 2019). These authors reported up to four publications dealing with the specific use of sentinel arboreta for risk assessment. One on insects (Redlich et al. 2019); one on nematodes (Aalders et al. 2006); one on fungal pathogens (Tomoshevich et al. 2013), and one specifically on known pathogen/vector association, *Xylella fastidiosa*/*Homalodisca vitripennis* (Groenteman et al. 2015). The only study on sentinel arboreta as a tool to identify novel pathogen/plant interactions was published by Tomoshevich et al. (2013). The authors, in their valuable work, found 102 associations between foliar symptoms and 67 fungal species on 50 tree species. In the present study involving only 34 oak species, 141 fungal species were isolated including the *Tubakia* spp. (Morales-Rodriguez, unpublished). These numbers provide the dimension of the problem. More than one species can be isolated from a single necrotic lesion, and all must be checked for their functionality (pathogen, saprotroph, other symbionts, antagonists, etc.) and origin (native, exotic of cryptic). With few exceptions often related to biotrophic pathogens and the presence of visible signs on the host (Vettraino et al. 2015), there is the need of

satisfying the Koch postulates to demonstrate pathogenicity.

Beyond these difficulties, the application of the sentinel arboreta strategy seems a very promising approach to highlight fungal-pathogen interactions new to science. In their study, Tomoshevich et al. (2013) found that 28% of the total associations between foliar symptoms and fungal species were new to science. Groenteman et al. (2015) in a survey carried out in a botanical garden in Southern California found 26 interactions new to science between New Zealand native plants and the pathogenic bacteria *Xylella fastidiosa* still not present in New Zealand. In the present study, which was restricted to the interaction between the genera *Tubakia* and *Quercus*, 32 associations were new to science (Table 1) many of which were determined in absence of evident symptoms. Dealing with microbes, a record new to science does not necessarily mean a recently established association. Having the genus *Tubakia* as a model, different studies, including the present one, demonstrated the transitory endophytic behavior of species of *Tubakia* in host tissues, in leaves as well as in annual shoots and buds, in absence of any external symptom (Braun et al. 2018; Gennaro et al. 2001). Thus, the time of the establishment of a new association is extremely difficult to be determined and cannot be dated by the appearance of symptoms. However, the determination of a new fungal-host association has an intrinsic ecological and evolutive value specifically when a native species interacts with an exotic one (the host in this case). The extent of colonization of an exotic plant by the microbial community within the environment of introduction is an important descriptor of the potential of adaptation to the new environment (Agrawal et al. 2005). In fact, based on the Enemy Release Hypothesis (ERH) (or the Natural Enemies Hypothesis) (Keane and Crawley 2002) exotic plants introduced in a new environment have a better fitness compared to native ones since they escape from their natural enemies (predators and pathogens) and are less impacted by local parasites community than the native ones. Looking at the *Tubakia* model of the present study, the analysis of healthy oak tissues reveals an overall higher detection of *Tubakia* spp. from Eurasian oak collections compared with the American ones, basically accepting one the postulate of ERH. Moreover, the overall biodiversity and abundance of *Tubakia* spp. isolated from necroses was found higher in Euroasian than American oaks, also supporting the ERH.

However, co-generic host-shift events of parasites from native to exotic plants also occur although less frequently (Agrawal et al. 2005; Keane and Crawley 2002; Kirichenko et al. 2013). In this study, the only species associated with foliar necroses of American oaks (mostly in the Lobatae section) was *T. suttoniana*, a species with a distribution range restricted to Europe and New Zealand. One of the pillars of the" ex-patria" sentinel concept postulates that when an exotic plant species is established in into new environment with (co-generic) native flora, it may fall prey of plant pathogens with which it has no recent co-evolutionary history, and with which it lacks specific defense mechanisms (Eschen et al. 2019). One relevant example was highlighted by Vettraino et al. (2015) in a sentinel planting in China where European oaks were exposed to the powdery mildew *Erysiphe quercicola*, which is prevalent in Asia and Australasia. This is an intriguing aspect; indeed, *T. suttoniana* is phylogenetically related to *T. macnabbii* (Braun et al. 2018) also mentioned as widespread on oaks of the Lobatae section and considered indigenous to North America.

In this study, the record of *T. macnabbii* on a *Q. dentata*, suggests a host-shift of a pathogen from an exotic to a native tree. Similarly, a putative American *Tubakia* species such as *T. hallii* has been detected with HTS at higher frequency in Euroasian oaks, suggesting host-shifts from the exotic American oaks to the Euroasian ones. It is evident that the ecological value of a new interaction in terms of both, time of establishment, and origin of the host-shift (native to native, native to exotic, exotic to native) is complicated and to be determined. Indeed, most of these shifts may go unnoticed, in absence of evident symptoms.

In the present study, the ability of *T. suttoniana* to cause leaf necroses on *Quercus* species of different natural geographical distributions was evident from the pathogenicity tests that confirmed for *T. suttoniana* six new pathogenic interactions out of 8 tested. However, it is also evident that not all the associations between host and pathogen observed in the field may reflect a pathogenic interaction.

In general, the sentinel arboreta strategy must be considered a powerful tool to identify host-parasite associations new to science. When applied to

herbivores it often allows determination of associations between damage and causal agent directly with field observations, thus arriving soon to possible recommendations useful for biosecurity (Redlich et al. 2019). Differently, the application of the strategy to plant pathogens is much more complicated and time-consuming. This study on sentinel arboreta is one of the few that shifted from concept to application, demonstrating the power of the strategy but also highlighting its limits specifically when applied to pathogens. As a conclusive remark, the existing network of sentinel arboreta at a global scale must be kept and reinforced, the protocols of their monitoring adapted to overcome the limits. This will facilitate the inclusion of the sentinel arboreta within the complex of strategies for the evaluation of the risk and the prevention of the introduction of new invasive pests and pathogens.

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Author contributions CMR and AV planned and designed the research; CMR, AV, FO, conducted fieldwork; CMR, AV, FO, and HTDL conducted the first processing of samples; CMR, MPA, GB performed most of the experiments; AV and CMR analyzed and elaborated the data; AV and CMR wrote the manuscript; FO and HTDL contributed to the critical review of the manuscript.

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Availability of data and materials The reads generated in this work are available in the NCBI Sequence Read Archive (SRA) http://www.ncbi.nlm.nih.gov/bioproject/646359 under the project name PRJNA646359: Fungal community of oak collection at Ataturk arboretum.

Declarations

Conflicts of interest Not applicable.

References

Aalders L, Bell N, Barratt B, et al. (2006) Using expatriate New Zealand flora as predictors of nematode threats to New Zealand natural ecosystems. In: XXVIII European Society of Nematologists International Symposium, p 155

Agrawal AA, Kotanen PM, Mitchell CE et al (2005) Enemy release? An experiment with congeneric plant pairs and diverse above-and belowground enemies. Ecology 86:2979–2989

Anonymous (2020) PM 3/91(1) Sentinel woody plants: concepts and application. EPPO Bulletin 50:429–436

Barham E, Sharrock S, Lane C et al (2016) The international plant sentinel network: a tool for regional and national plant protection organizations. EPPO Bulletin 46:156–162

Barham E, Sharrock S, Lane C et al (2015) An international plant sentinel network. Sibbaldia J Bot Garden Hortic 13:83–98

Brasier C (2008) The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol 57:792–808

Braun U, Nakashima C, Crous P et al (2018) Phylogeny and taxonomy of the genus Tubakia s. lat. Fungal Systemat Evolut 1:41–99

Britton K, White P, Kramer A et al (2010) A new approach to stopping the spread of invasive insects and pathogens: early detection and rapid response via a global network of sentinel plantings. N Z J for Sci 40:109–114

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Coban S, Willner W (2019) Numerical classification of the forest vegetation in the Western Euxine Region of Turkey.

Darriba D, Taboada GL, Doallo R et al (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772–772

Denk T, Grimm GW, Manos PS, et al. (2017) An updated infrageneric classification of the oaks: review of previous taxonomic schemes and synthesis of evolutionary patterns. Oaks physiological ecology. Exploring the functional diversity of genus Quercus L. Springer, Berlin, pp 13–38

Edgar R (2010) Usearch. Lawrence Berkeley National Lab.(LBNL), Berkeley, CA (United States),

Eschen R, Britton K, Brockerhoff E et al (2015) International variation in phytosanitary legislation and regulations governing importation of plants for planting. Environ Sci Policy 51:228–237

Eschen R, O'Hanlon R, Santini A et al (2019) Safeguarding global plant health: the rise of sentinels. J Pest Sci 92:29–36

Farr D, Rossman A (2021) Fungal Databases, US National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases

Fisher P, Petrini O, Petrini L et al (1994) Fungal endophytes from the leaves and twigs of Quercus ilex L. from England, Majorca and Switzerland. New Phytol 127:133–137

Gennaro M, Gonthier P, Nicolotti G et al (2001) First report of Tubakia dryina in buds and shoots of Quercus cerris and Quercus robur. Plant Dis 85:1289–1289

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Groenteman R, Forgie SA, Hoddle MS et al (2015) Assessing invasion threats: novel insect-pathogen-natural enemy associations with native New Zealand plants in southern California. Biol Invasions 17:1299–1305

Harrington T, McNew D (2018) A re-evaluation of Tubakia, including three new species on Quercus and six new combinations. Antonie Van Leeuwenhoek 111:1003–1022

Huseyinov E, Selçuk F (2001) Contribution to study of mycoflora of Turkey-I. Coelomycetes of orders Melanconiales and Sphaeropsidales on forest trees and shrubs in the Black Sea coast (Rize and Trabzon provinces). Mikol Fitopatol 35:28–33

Huson DH, Auch AF, Qi J et al (2007) MEGAN analysis of metagenomic data. Genome Res 17:377–386

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Kenis M, Li H, Fan J-t et al (2018) Sentinel nurseries to assess the phytosanitary risks from insect pests on importations of live plants. Sci Rep 8:1–8

Kirichenko N, Kenis M (2016) Using a botanical garden to assess factors influencing the colonization of exotic woody plants by phyllophagous insects. Oecologia 182:243–252

Kirichenko N, Péré C, Baranchikov Y et al (2013) Do alien plants escape from natural enemies of congeneric residents? Yes but not from all. Biol Invasions 15:2105–2113

Kobayashi T (2007) Index of fungi inhabiting woody plants in Japan. Zenkoku-Noson-Kyoiku Kyokai Publishing, Tokyo

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lindahl BD, Nilsson RH, Tedersoo L et al (2013) Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. New Phytol 199:288–299

Mansfield S, McNeill MR, Aalders LT et al (2019) The value of sentinel plants for risk assessment and surveillance to support biosecurity. NeoBiota 48:1

Morales-Rodríguez C, Sferrazza I, Aleandri MP et al (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

Morales-Rodríguez C, Anslan S, Auger-Rozenberg M et al (2019a) Forewarned is forearmed: Techniques and diagnostic approach for early detection of potentially invasive pests and pathogens in sentinel plantings. NeoBiota

Morales-Rodríguez C, Dalla-Valle M, Aleandri M et al (2019b) Pestalotiopsis biciliata, a new leaf pathogen of Eucalyptus spp. recorded in Italy. For Pathol 49:e12492

Munkvold G, Neely D (1990) Pathogenicity of Tubakia dryina. Plant Dis 74:518–522

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press

O'Donnell K, Kistler HC, Cigelnik E et al (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci 95:2044–2049

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungusfusariumare nonorthologous. Mol Phylogenet Evol 7:103–116

Paap T, Burgess TI, Wingfield MJ (2017) Urban trees: bridgeheads for forest pest invasions and sentinels for early detection. Biol Invasions 19:3515–3526

Park M, Yun HY, Kim YH et al (2021) Non-linear reduction of photosynthetic ability in oak trees infected with Tubakia koreana causing Quercus leaf blight. Forest Pathol 51:e12665

Redlich S, Clemens J, Bader MK-F et al (2019) Identifying new associations between invasive aphids and Pinaceae trees using plant sentinels in botanic gardens. Biol Invasions 21:217–228

Ronquist F, Teslenko M, Van Der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542

Roques A, Fan J-t, Courtial B et al (2015) Planting sentinel european trees in eastern asia as a novel method to identify potential insect pest invaders. PLoS ONE 10:e0120864

Roques A, Cleary M, Matsiakh I et al (2017) Field guide for the identification of damage on woody sentinel plants. CABI, Wallingford

Santini A, Ghelardini L, De Pace C et al (2013) Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytol 197:238–250

Taylor J, Clark S (1996) Infection and fungal development of *Tubakia dryina* on sweet gum (*Liquidambar styraciflua*). Mycologia 88:613–618

Tomoshevich M, Kirichenko N, Holmes K et al (2013) Foliar fungal pathogens of European woody plants in Siberia: an early warning of potential threats? Forest Pathol 43:345–359

Venkatasubbaiah P, Chilton WS (1992) Phytotoxins produced by *Tubakia dryina*. Mycopathologia 120:33–37

Vettraino A, Roques A, Yart A et al (2015) Sentinel trees as a tool to forecast invasions of alien plant pathogens. PLoS ONE 10:e0120571

Vettraino AM, Li H-M, Eschen R et al (2017) The sentinel tree nursery as an early warning system for pathway risk assessment: Fungal pathogens associated with Chinese woody plants commonly shipped to Europe. PLoS ONE 12:e0188800

Weber F, Knapp G, Ickstadt K et al (2020) Zero-cell corrections in random-effects meta-analyses. Res Synthesis Methods 11:913–919

White TJ, Bruns T, Lee S et al (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols Guide Methods Appl 18:315–322

Yaltırık F (1966) Floristic analysis of Belgrad Forest and main stand composition. General Directorate of Forestry Publication

Yang Q, Jiang N, Liang L et al (2020) First report of Tubakia americana causing oak seed rot on Quercus variabilis in China. Plant Dis 104:2724

Young Yun H, Kim YH (2020) *Tubakia koreana* sp. nov. causing *Quercus* leaf blight. Mycotaxon 135:223–229

Zahedi M, Elahinia S, Khodaparast S et al (2011) Introduction of some new mitosporic fungi causing leaf spot on broad leaf trees in Guilan province (N Iran). Rostaniha 12:63–71

Zhao Y-J, Hosoya T, Baral H-O et al (2013) Hymenoscyphus pseudoalbidus, the correct name for Lambertella albida reported from Japan. Mycotaxon 122:25–41

Zheng H-D, Zhuang W-Y (2014) Hymenoscyphus albidoides sp. nov. and H. pseudoalbidus from China. Mycol Prog 13:625–638