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Original Research Article

Tree pathogens and their insect-mediated transport: Implications for oak tree die-off in a natural park area

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ABSTRACT

Successful infection by a tree pathogen depends both on species-specific traits, related to its infection biology, and on external factors, i.e. the biotic and abiotic components of the ecosystem. Among species-specific traits, virulence, biomass (propagule) production and dispersal ability all contribute to pathogen establishment and spread. Among the external factors, two crucial roles are played by an environment conducive to disease and by vector occurrence. We investigated the endophytic fungi and xylophagous beetles associated with declining oaks in a natural stand of central Italy, particularly focusing on the fungi isolated from insects' body. This paper extends and reinterprets the outcome of a previous study, which looked into the whole mycota (pathogenic/neutral/beneficial) found on these oaks. A rich assemblage of fungi was found on declining trees on which a number of wood-boring beetles (Buprestidae and Cerambycidae) also occurred. In the laboratory two-thirds of the insects proved to convey propagules of prominent fungal pathogens (*Botryosphaeriaceae*, *Pestalotiopsisaceae*, *Plectosphaerellaceae*, *Pleosporaceae*); this confirms pathogen pervasiveness in oak stands is enhanced by their association with xylophagous insects. The outcome of this research will benefit forest manager and other stakeholder efforts to manage stands and species invasions, also in the light of climate change.

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1. Introduction

Successful infection by a tree pathogen depends on a number of factors, among which dispersal ability plays a central role. The most important factor in pathogen spread is long-distance dispersal of spores across the landscape. Propagules capable of moving long distances may increase pathogens' average dispersal distance and promote the establishment of new infection loci, where they may give rise to satellite populations (Lockwood et al., 2007). Given this, wind-borne pathogens have a higher capacity for spreading over vast areas compared to other types of pathogens (e.g. soil-borne ones) that are constrained to their original environment. However, only a small percentage of wind-dispersed propagules move a very long way, whereas most fall in close proximity to the source of sporulation (Ragazzi et al., 2007). It is indeed a fact in plant disease epidemiology that the rate of spread of a pathogen at a local scale strongly depends on the distance between plants (Begon et al., 1990; Ragazzi

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et al., 2007). Nonetheless, the limits of unassisted spore dispersal can to some extent be overcome by animal vectors; particularly, those that fly greatly enhance the possibilities of a microorganism reaching distant, still uninfected, hosts.

In natural ecosystems, insects and microbes have co-evolved to benefit from each other. Plant pathogens, in particular, have astonishingly co-adapted with a range of insects for optimal dispersion (Mommaerts and Smagghe, 2011): there is a copious literature that highlights the key role of insects as vectors of, specifically, forest tree diseases. An intimate relationship exists between some phytophagous insects and forest pathogens: its importance in the development of tree diseases has been ascertained in a plethora of investigations (Tiberi et al., 2002; Kirisits, 2004; Klepzig and Six, 2004). While in obligate relationships the importance of the vector in pathogen movement is evident, knowledge is still scarce and fragmentary regarding facultative relationships: for example, it is still uncertain how these insect-fungus associations function in complex systems, such as in forest ecosystems. It is also not yet clear if, and to what extent, insect-fungus relationships are frequent, and if these are affected by current climate change.

Climate warming is disrupting forest ecosystems, with increases in drought, fire, storms, and biotic invasions (IPCC, 2001). These disturbances strongly impact tree health (causing, for example, reduced growth, altered phenology, impaired physiology and poor resistance to biotic constraints), as well as growth, survival and reproduction of many organisms inhabiting these trees. In fact, as climate changes, the pathogenic fungi and phytophagous insects inhabiting forest trees also change in their rates of occurrence, population density, and aggressiveness (Sturrock et al., 2011).

A type of forest in which the above alterations turned out to be particularly evident are Mediterranean oak forest ecosystems. Indeed, over the last few decades, coinciding with the climate trend towards warming, we have witnessed unprecedented disease and pest outbreaks in Mediterranean oak stands (Moricca et al., 2016; Tiberi et al., 2016). This phenomenon, known worldwide as Oak Decline, can be triggered by the adverse environmental conditions like those induced by climate change, and subsequently exacerbated by inciting and/or contributing factors (Manion, 1991; Panzavolta et al., 2017), among which a prominent role is played by disease and pest attacks (Moricca et al., 2016; Tiberi et al., 2016). The objective of this current research was to extend a previous study carried out in the same area (Maremma Regional Park - Italy), which, regarding the fungi, was limited only to members of the *Botryosphaeriaceae* family (Panzavolta et al., 2017). In the present study, the whole endophytic assemblage was taken into consideration in order to have a more complete understanding of the possible interactions among biotic stressors, specifically latent pathogens and xylophagous insects, in the onset of decline/die-back of oak species.

2. Materials and methods

2.1. Field sampling

The investigation was carried out in a mixed oak woodland composed of *Quercus pubescens* Willd., *Quercus cerris* L., *Quercus suber* L., and sporadic individuals of *Quercus ilex* L. in the Maremma Regional Park (42°37'03" N, 11°06'47" E: Alberese, Tuscany, central Italy). This is a mature stand, with large, old trees, dominated by *Q. pubescens*, with a few individuals of the other three oak species and a poor understory degraded by animal grazing. The stand exhibited evident signs of decline, with many of the trees showing advanced crown transparency with leaf yellowing and shedding, partial dieback of branches and twigs, epicormic shoot formation, tarry exudates on the bark, cankers on the main stems and on the branches, and signs of xylophagous insect attacks.

To identify the endophytic mycota, 280 current-year twigs were taken during the 2015 spring-summer period (from April to October) from 10 trees (six *Q. pubescens*, two *Q. cerris*, and two *Q. suber* trees). All these trees showed the decline/dieback symptoms described above. Tree sampling was performed according to the specific composition of the population and the relative occurrence of individual taxa. Specifically, four twig samples (up to 4 cm in diameter) were collected once a month from each tree, at the height of 2.50 m. To minimize sampling subjectivity, twigs were randomly taken in each of the four cardinal points, without considering their health conditions, but only excluding necrotic ones. The samples were taken to the laboratory, within 12 h of collection, and further processed, to isolate the associated endophytic fungi within 72 h.

In the selected oak woodland the occurrence of xylophagous beetles (particularly Cerambycidae and Buprestidae) was also monitored twice a week during the same study period. To gather insects to be utilized for fungal isolation, only methods for collecting live insects were taken into consideration. Adult xylophagous beetles with flower-visiting behavior were collected by sweeping directly host plants or flowers. Twilight or nocturnal flying beetles were captured with light (UV lighting equipment, mercury vapor bulbs), attracting them onto a vertical white sheet. Finally, trees with signs of xylophagous attacks were inspected carefully to detect attacked twigs, branches or stems. These samples were debarked or kept in rearing cages at outdoor conditions to collect adults. Sampled specimens were stored in sterile plastic tubes and promptly taken to the laboratory.

2.2. Fungal isolation

For fungal isolation from plant twigs, the same methodology used in Panzavolta et al. (2017) was applied. The sample material was sterilized by immersion in 10% H₂O₂ for 3 min, then it was washed twice with sterilized distilled water, and dried on sterile tissue paper. A 2-cm-long tissue piece was removed from each twig, excluding the bark. From each piece, 15 wood samples (roughly 5 mm long and 1 mm thick) were removed and placed, in groups of five, in three Petri dishes (90 mm in

diameter) containing Potato Dextrose Agar (PDA) (Difco, Detroit, MI, USA) medium with 0.06 g/l streptomycin. In total 600 explants were placed into the nutrient medium each month. The plates were incubated at 20 °C in darkness for a week. Fungal colonies growing from the wood samples were isolated and subcultured on Oak Leaf Agar (OLA) (30 g oak leaves boiled in 350 ml water for 30 min, 8 g agar, pH 6.6, and impoverished PDA, consisting of 6 g/l potato dextrose broth and 20 g/l agar). Colony morphology was assessed under a dissecting microscope (ZEISS, West Germany) and distinguishing micro-morphological features were determined under a compound microscope at $\times 100$, $\times 400$ and $\times 1000$ magnifications (ZEISS, West Germany) using the keys provided by Booth (1971), Gams (1971), Carmichael et al. (1980), Sutton (1980), and Von Arx (1987). The isolation frequency of each fungus species per each month was calculated using the following formula:

$$Fi = \left(\frac{Ni}{Nt} \right) \times 100 \quad (1)$$

Where F_i is the fungus species frequency, N_i is the number of times the species was isolated and N_t is the total number of wood samples placed in PDA.

Fungal isolation from each insect body (only adults) was performed as in Panzavolta et al. (2017). Each beetle was surface-washed by vortexing for 1 min in 300 μ l of sterile distilled water with 1% of Tween-80 detergent. The resulting solutions were used to inoculate the PDA medium by pouring a volume of 50 μ l per plate with a micropipette and uniformly spreading the inoculum on the agar surface with a bacteriology loop. All the Petri dishes were incubated at 20 °C in darkness for five days. Emerging fungal colonies were subcultured in a pure OLA medium. After one week of growth, colonies were observed under the microscope as described above and identified by means of their macro- and micro-morphological characters, and by the DNA-based method described below. All adult beetles were identified in the laboratory using specific keys (Curletti et al., 2003; Pesarini, 2004).

2.3. DNA-based identification of fungal taxa

Molecular identification was necessary for discriminating among fungal taxa whose micro-morphological characteristics alone proved inconclusive for species determination. However, this was carried out only for those taxa we considered of interest because of their belonging to genera that include important fungal antagonists or tree pathogens known from the literature and from our experience for their aggressiveness. Hyphal-tip-derived, fresh cultures were incubated in the dark for one week on an MEA (Difco) medium: genomic DNA was then extracted (Moricca et al., 2000), after which, the rDNA region encoding the 5.8S gene and its flanking ITS1 and ITS2 spacers was PCR-amplified using the ITS1 (5'-TCCGTAGGT-GAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) universal primers (White et al., 1990); PCR cycling conditions and subsequent amplicon sequencing were as in Moricca et al. (2012). Next, sequences were processed in the GenBank database (Benson et al., 2013), with a BLAST search for the highest homologies (in terms of percentage of nucleotide similarity) in order to identify the taxa at the species level, with a minimum threshold of 98% sequence similarity (Sánchez Márquez et al., 2008).

2.4. Statistical analysis

Isolation frequencies of each fungal species, per each month, were arcsine transformed and analyzed with ANOVA. The differences were examined for significance using the Duncan's New Multiple Range Test (SPSS software).

3. Results and discussion

3.1. Fungi isolated from trees

A diverse assemblage of mycota was found in twigs of declining oaks (Table 1). These were sorted, based on the types of ecological interaction they perform with the host plant, into three main guilds: tree pathogens; indifferent saprophytes; and fungal antagonists. Isolation frequencies revealed 68.96% of the fungi fell into the first group, 11.9% were fungal antagonists, while 19.13% proved to be indifferent saprophytes. We further divided the tree pathogens into two subgroups: one included the *Botryosphaeriaceae* family, due to their prominent role as tree pathogens (this subgroup alone totaled 29.27% of the isolations), and another one comprising the rest of the pathogenic fungi (39.69%). Five species were identified in the *Botryosphaeriaceae*, with *Diplodia corticola* A.J.L. Phillips, A. Alves and J. Luque and *Botryosphaeria dothidea* (Moug.) Ces. and De Not being the most represented in this subgroup (31.59% and 26.69% respectively) (Fig. 1). The rest of the pathogenic fungi included eight taxa, two of which (*Camarosporium* and *Cephalosporium*) were identified only at the genus level. Among these, more than half were represented by *Verticillium dahliae* Kleb. (31.28%) and *Fusarium solani* (Mart.) Sacc. (26.27%) (Fig. 2). As regards fungi known for their antagonistic behavior against plant pathogenic fungi, three fungal taxa, namely *Clonostachys rosea* (Link.: Fr.) Schroers, Samuels, Seifert & Gams (formerly *Gliocladium roseum* Bainier), *Trichoderma viride* Pers. and *Gonatorrhodiella* sp., were recorded, with a high prevalence within the group of *T. viride* (67.77%) (Fig. 3). Finally, six taxa of common contaminant fungi were also isolated, three of which, *Alternaria alternata* (Fr.) Keissl. (36.84%), *Aspergillus* sp. (20.69%), and *Cladosporium* sp. (23.04%), predominated (Fig. 4).

Table 1

Frequency of isolation (% of 600 monthly samplings during the 2015 growing season) of endophytic fungi obtained from oak twig samples in the Maremma Regional Park at Alberese (Tuscany – Italy).

Fungal Taxa	Month of Collection						
	Apr.	May	June	July	Aug.	Sept.	Oct.
Tree pathogens							
Botryosphaeriaceae							
<i>Botryosphaeria dothidea</i> (Moug.) Ces. and De Not	1,75	3,75	6,90	9,00	8,78	10,25	12,50
<i>Diplodia corticola</i> A.J.L. Phillips, A. Alves and J. Luque	3,37	4,90	4,75	4,50	15,25	13,89	16,00
<i>Diplodia seriata</i> De Not.	3,50	3,78	3,25	4,25	6,75	7,65	11,97
<i>Dothiorella sarmentorum</i> (Fr.) A.J.L. Phillips, A. Alves and J. Luque	1,37	3,12	1,89	3,25	3,12	3,50	8,97
<i>Neofusicoccum parvum</i> (Pennycook and Samuels) Crous, Slippers and A.J.L. Phillips	1,87	1,50	1,25	2,00	3,00	3,50	3,25
Other fungal pathogens							
<i>Biscogniauxia mediterranea</i> (De Not.) Kuntze	0,75	0,50	1,90	2,87	3,55	3,12	3,75
<i>Camarosporium</i> sp.	0,87	0,50	0,67	1,20	1,35	0,57	0,87
<i>Cephalosporium</i> sp.	20,37	15,12	11,12	5,12	6,78	5,75	6,37
<i>Fusarium solani</i> (Mart.) Sacc.	1,87	3,50	6,00	8,37	6,25	2,12	3,62
<i>Pestalotiopsis versicolor</i> (Speg.) Steyaert	4,75	4,00	2,70	3,80	2,45	2,10	3,00
<i>Rizhoctonia solani</i> J.G. Kühn	3,50	4,75	5,25	5,62	5,05	4,37	4,12
<i>Ulocladium consortiale</i> (Thüm.) E.G. Simmons	24,00	22,25	16,87	14,25	2,75	0,50	3,50
<i>Verticillium dahliae</i> Kleb.	0,75	0,50	1,90	2,87	3,55	3,12	3,75
Fungal antagonists							
<i>Clonostachys rosea</i> (Link.: Fr.) Schroers, Samuels, Seifert & Gams	4,65	4,85	4,75	3,50	3,00	0,62	0,50
<i>Gonatorrhodiella</i> sp.	1,12	1,30	1,00	0,70	0,00	0,00	0,00
<i>Trichoderma viride</i> Pers.	6,50	7,75	8,24	8,85	7,90	6,50	8,90
Indifferent saprophytes							
<i>Alternaria alternata</i> (Fr.) Keissl.	6,37	6,87	7,00	6,92	7,13	6,74	6,73
<i>Aspergillus</i> sp.	3,00	3,25	6,87	3,58	3,30	3,25	3,57
<i>Candida</i> sp.	0,75	1,50	0,00	0,50	0,00	0,00	0,00
<i>Cladosporium</i> sp.	4,12	5,60	4,70	3,40	3,00	3,00	6,05
<i>Pollularia</i> sp.	0,87	1,20	0,60	0,80	0,00	0,00	0,00
<i>Rhizopus</i> sp.	2,37	3,30	3,70	4,10	1,20	2,50	1,80



Fig. 1. Occurrence of botryosphaeriaceous fungi in oak twig samples from the Maremma Regional Park at Alberese (Tuscany–Italy) based on their isolation frequencies throughout the 2015 growing season.

Data on monthly isolation frequencies revealed a different occurrence, from April to October, among the different groups of fungi (Fig. 5). While isolation frequencies of indifferent saprophytes and fungal antagonists were almost constant over time, those of pathogenic fungi turned out strongly variable throughout the growing season. In fact, the *Botryosphaeriaceae*

Other fungal pathogens



Fig. 2. Occurrence of latent pathogens other than *Botryosphaeriaceae* in oak twig samples from the Maremma Regional Park at Alberese (Tuscany – Italy) based on their isolation frequencies throughout the 2015 growing season.

increased dramatically in July, peaking in October (52.69%). On the contrary, the other pathogens had their highest isolation frequencies in April (56.11%), continuously dropped over the subsequent months, until bottoming out in September (19.53%), and then rose markedly in the last month (October).

3.2. Fungi isolated from insects

Several insect species were sampled during the study period. However, only xylophagous insects known to feed on living trees or on dead organs (twigs and branches) of still living trees were taken into consideration. Among these, eight species belonged to the Buprestidae family and eight species to Cerambycidae (Table 2). Of the 16 insect species tested (145 individuals in total), 10 (62.5% of the total) bore fungi (7 species) on their bodies. With the exception of the fungus *C. rosea*, all the other fungi found are known to be plant pathogenic. Three of these (*D. corticola*, *Diplodia seriata* De Not., *B. dothidea*) belonged to the *Botryosphaeriaceae* family, while the others belonged to the *Pleosporaceae* [*Ulocladium consortiale* (Thüm.) E.G. Simmons], the *Pestalotiopsidaceae* [*Pestalotiopsis versicolor* (Speg.) Steyaert] and the *Plectosphaerellaceae* (*Verticillium dahliae* Kleb.) families. In total, 20 individuals presented fungi propagules, of which 12 featured members of the *Botryosphaeriaceae* family. The known fungal antagonist *C. rosea*, instead, was isolated from three individuals (two species of longhorn beetles), and in all cases it was consistently associated with *D. seriata*; furthermore, in one case *V. dahliae* was also found. Finally, the two most harmful beetle species, namely *Coraebus fasciatus* (Villers) [syn. *Coraebus florentinus* (Herbst)] and *Cerambyx welensii* (Küster), were both associated with the pathogenic botryosphaeriaceous fungi, *D. seriata* and *D. corticola* respectively.

4. Discussion

The fungi inhabiting woody plants establish with their hosts a range of ecological interactions, ranging from beneficial (in the case of endophytic symbionts antagonistic to plant enemies), to neutral (nonpathogenic, perpetual occupants of inner tree tissues) to pathogenic (i.e. parasitic colonizers of host tissues) (Wilson, 2000). Furthermore, the relationship between the latent pathogens and their hosts may vary along a continuum of interactions in which they switch from symptomless, temporally latent infections to aggressive colonization of host tissues, ending with pervasive horizontal spread (through sporulation over the tree surfaces) (Saikkonen et al., 1998). In this study, the largest component of the endophytic mycota found in twigs of declining oaks was represented by pathogens. Among these, the *Botryosphaeriaceae* species predominated. This is a diverse and large family including more than 2000 taxa (Index fungorum) whose members share some attributes, like aggressiveness towards water-stressed hosts and tolerance to hot and dry climates (Slippers and Wingfield, 2007). *D. corticola* and *B. dothidea* were the most frequently isolated members of this family. *D. corticola* is an emerging pathogen that

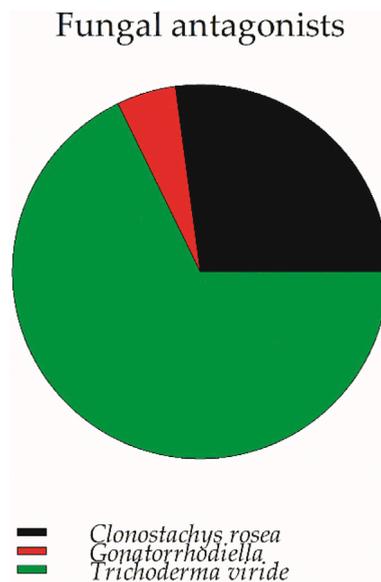


Fig. 3. Occurrence of fungal antagonists in oak twig samples from the Maremma Regional Park at Alberese (Tuscany – Italy) based on their isolation frequencies throughout the 2015 growing season.

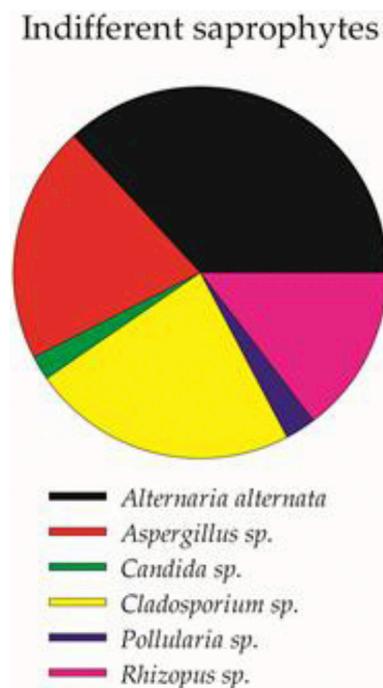


Fig. 4. Occurrence of indifferent saprophytes in oak twig samples from the Maremma Regional Park at Alberese (Tuscany – Italy) based on their isolation frequencies throughout the 2015 growing season.

has, in recent years, been more and more frequently associated with cases of oak tree mortality in various Mediterranean countries, thereby taking a prominent role in the decline/dieback of oak stands in the whole Mediterranean area (Alves et al., 2004). *B. dothidea* is a cosmopolitan pathogen that has been isolated from a number of woody hosts worldwide: this fungus ability to induce disease following the onset of stress by adverse environmental conditions, and to switch easily from one host to another, makes it particularly aggressive (Slippers and Wingfield, 2007). The group labelled “other fungal pathogens” included several species of weakly pathogenic endophytes, some of which are symptomless, at least for a considerable part of their life. However, others, such as *R. solani*, *V. dahliae* and *F. solani*, are not secondary plant pathogens. These fungi have been

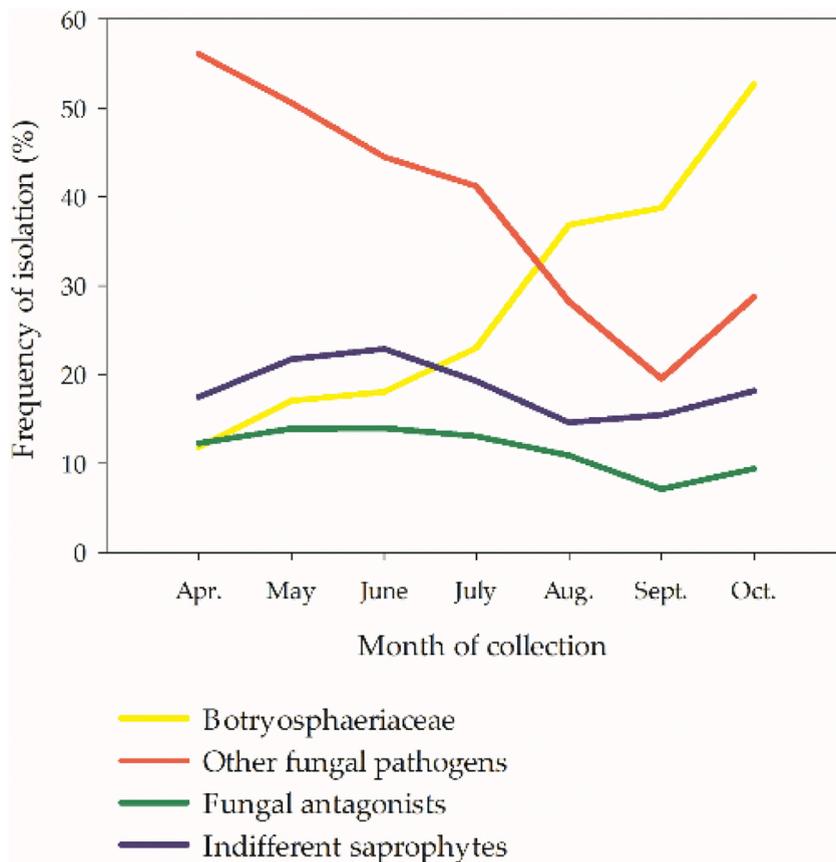


Fig. 5. Occurrence of the various groups of oak-associated fungal endophytes (sorted according to their interaction with the host) in oak twig samples from the Maremma Regional Park at Alberese (Tuscany – Italy) based on their isolation frequencies throughout the 2015 growing season.

identified in other studies at several locations, from oaks with various disease symptoms, and several times they have been considered involved in the development of oak decline syndrome (Kowalski and Kehr, 1992; Ragazzi et al., 1995).

Many of the fungi isolated from declining oaks are known to prefer weakened and physiologically impaired trees. This is a fundamental characteristic of *Botryosphaeriaceae* (Ragazzi et al., 1997; Slippers and Wingfield, 2007). These opportunistic pathogens increase their incidence on hosts stressed by high temperatures, heat waves and extended drought, in other words, in environmental conditions that occur at Mediterranean latitudes in summer. In fact, while most of the fungi slow down or completely stop their activity during this period (known as “oversummering”), botryosphaeriaceous fungi, on the other hand, because of their thermotolerance, are not inhibited and indeed become more pervasive. These characteristics give *Botryosphaeriaceae* a preeminent role among those biotic factors responsible for the extensive die-off of trees in Mediterranean oak forest ecosystems.

As for the wood-boring insects captured in the study area, only a few of the species found are considered true pests, whereas the others are considered of secondary importance. Specifically, *C. fasciatus* and *C. welensii* were the most damaging species occurring in the oak forest that was investigated. In the same way as the above-mentioned fungal endophytes, these insects attack trees stressed by adverse environmental factors. However, under some circumstances, they also attack trees in apparently good health (Sallé et al., 2014; Torres-Vila et al., 2016a). The other insects, on the contrary, play a minor role, feeding only on dead trees or on dead twigs and branches of still living trees (Curlletti, 1994; Contarini, 2014). Finally, *T. holosericeus* is considered a pest of dry wood (Palanti et al., 2010).

Significantly, pathogenic fungi and wood-boring beetles are often found together on impaired oak trees, cooperating to weaken them and trigger a state of decline. In the past, the contribution of each biotic stressor was usually considered separately, with little research focused on the synergy between fungus-insect interactions. In our study, instead, we noted that roughly 66% of the beetle species tested (10 out of 16) were associated with some of the most aggressive pathogens in the oak endophytic fungi. Some species in the Buprestidae and Cerambycidae families are among the most damaging xylophagous insects in forest ecosystems. Two of the beetles in our stand, namely *C. fasciatus* and *C. welensii*, have been extensively reported as being involved in oak decline (Jurc et al., 2009; Sallé et al., 2014; Torres-Vila et al., 2016a). Both these wood boring beetles were found here to transport the fungal pathogens *D. corticola* and *D. seriata*. These interactions might be harmful to

Table 2

Xylophagous beetles caught in the oak woodland in the Maremma Regional Park at Alberese (Tuscany – Italy) and sampled in the laboratory for their association with fungal species.

Insect species	N. of insects		Fungal species
	tested	associated with fungi	
Buprestidae			
<i>Acmaeoderella adspersula</i> (Illiger, 1803)	3	0	
<i>Acmaeoderella flavofasciata</i> (Piller and Mitterpacher, 1783)	8	0	
<i>Anthaxia millefolii polychloros</i> Abeille de Perrin, 1894	15	3	<i>U. consortiale</i>
<i>Anthaxia scutellaris</i> Gene, 1839	15	0	
<i>Anthaxia thalassophila</i> Abeille de Perrin, 1900	11	0	
<i>Anthaxia umbellatarum</i> (Fabricius, 1787)	6	2	<i>B. dothidea</i>
<i>Coraeus fasciatus</i> (Villers, 1789)	12	4	<i>D. seriata</i>
<i>Latipalpis plana</i> (Olivier, 1790)	3	1	<i>P. versicolor</i>
Cerambycidae			
<i>Cerambyx welensii</i> (Küster, 1845)	8	3	<i>D. corticola</i>
<i>Chlorophorus sartor</i> (Müller, 1766)	4	1	<i>D. seriata</i> , <i>C. rosea</i>
<i>Deilus fugax</i> (Olivier, 1790)	1	0	
<i>Phymatodes testaceus</i> (Linnaeus, 1758)	13	0	
<i>Purpuricenus kaehleri</i> (Linnaeus, 1758)*	23	1	<i>D. seriata</i> , <i>C. rosea</i>
		1	<i>D. seriata</i> , <i>C. rosea</i> , <i>V. dahliae</i>
<i>Stenopterus rufus</i> (Linnaeus, 1767)	9	0	
<i>Stictoleptura cordigera</i> (Fuessly, 1775)	2	1	<i>V. dahliae</i>
<i>Trichoferus holosericeus</i> (Rossi, 1790)	12	3	<i>V. dahliae</i>
Total	145	20	

host tree populations, as insects can favor pathogen dispersal, transporting its propagules in a more targeted way on trees already debilitated by adverse weather conditions. The fungus-insect associations may thus result in an increased pervasiveness of harmful fungal pathogens, which would have serious consequences on forest health.

Previous studies showed the ability of wood-boring beetles to transport fungal propagules. Tiberi et al. (2002) proved that *C. fasciatus* is able to transport the propagules of the botryosphaeriaceous fungus *Diplodia mutila* (Fr.) Mont. While Martín et al. (2005) determined the relationship between *Cerambyx* spp. damage and *B. mediterranea* infection. In our study *B. mediterranea* was isolated from oak samples but it was never isolated from *C. welensii* adults. However, our finding is not in disagreement with Martín et al. (2005), inasmuch this insect-fungus association seemed to be linked mainly to the large exit holes bore by the adults rather than to fungus vectoring.

Though the insect-fungus relationship may be an additional factor of damage in woodlands, they can also sometimes play important, even essential, roles: for example, insects can serve as carriers of beneficial (antagonistic) fungi which act as “body guards” to their host plants. In fact, one important finding in this study was the isolation of the fungal antagonist *C. rosea* from the body of two cerambycids, *C. sartor* and *P. kaehleri*, both of whom also carried *D. seriata*. Therefore, interestingly, these individuals vectored both a pathogenic fungus and a fungal antagonist. *C. rosea* is in fact an effective biological control agent (BCA) of various plant pathogens, and many studies have focused on its use in entomovectoring: this technology makes use of pollinating insects to effectively transport BCAs over crops in order to protect cultivated plants from pathogen attacks (Mommaerts and Smagghe, 2011). Insect vectors ability to transmit viruses, fungi and bacteria of various forest tree diseases are well-known, but few investigations have explored if, and to what extent, they also contribute to short- or long-distance dispersal of beneficial agents (e.g. BCA). Further investigation is still needed to clarify the interactions between carrier insects and pathogenic or beneficial fungi, as well as the effects of such interactions on plant health.

5. Conclusions

Climate change and other forest disturbance processes (e.g. fungal and insect outbreaks) are inducing serious perturbations to forest ecosystems worldwide. Tree dieback and die-off as a consequence of rising temperatures and recurrent droughts have been largely documented in the Mediterranean area (Allen et al., 2010). Oak stands, a widespread and key component of the Mediterranean flora, have been profoundly affected by climate warming in vast portions of their range, with several species exhibiting physiological impairment due to general stress and, especially, higher predisposition to insect and fungal attacks (Ragazzi et al., 1999, 2001). The impact of these biotic stressors on oak forest ecosystems has historically been assessed separately or, at most, cumulatively (Moricca et al., 2016; Tiberi et al., 2016). Instead, research should explore their synergy, as such information could be critical to effective stand management. In fact, the cooperative interaction between species belonging to these two categories of causative agents that emerged in this study, suggests that some fungus-insect associations impact the onset and exacerbation of oak decline in Mediterranean forests.

The chronology of reports provides evidence that many endophytic pathogens, particularly some members of the *Botryosphaeriaceae*, have become in recent years more widespread in the Mediterranean area (Ragazzi et al., 2003; Moricca and Ragazzi, 2008; Moricca et al., 2018). Undoubtedly, climate change has favored some thermo-tolerant organisms, such as endophytic fungi and some wood-boring beetles. Other climate-mediated factors, however, must also be involved in such a

complex phenomenon; for example, the rate of dispersal is likely to be critical in the spread of forest pathogens. This study confirmed that wood-boring beetles are involved in the dispersal of latent pathogens in oak forests. Although some wood-boring beetles have a limited capacity of dispersal (Torres-Vila et al., 2016b), certain cerambycid species are capable of flying at great distances from the point of emergence (Haack et al., 2017), increasing the dispersal distance of fungal pathogens, thus potentially enhancing their pervasiveness in oak forests.

This study provides a new perspective on the close-knit cooperation between certain fungal endophytes and wood-boring beetles: the demonstration that the life strategies of these causative agents are strongly intermingled widens our understanding of their ecological role in native plant communities like Mediterranean oak forests. Forest management often takes into consideration pest problems only when damages are significant and clearly perceived; instead, a proactive forest management approach which deals with pest management issues is urgently needed. Information from this study should be used to inform policies and programs aimed at protecting that valuable resource which is the Mediterranean oak forest.

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