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Plant Pathogenic Fungi Associated with *Coraebus florentinus* (Coleoptera: Buprestidae) Attacks in Declining Oak Forests

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Abstract: The black-banded oak borer, *Coraebus florentinus*, is an emerging pest of oak trees in the western Mediterranean region. Larvae of the insect are xylophagous and progressively excavate an annular gallery that interrupts sap flow, resulting in the death of the attacked branches. Until now, limited information has been available regarding the ecological interactions between *C. florentinus* and the main plant pathogenic fungi involved in the etiology of oak decline. Knowledge of these interactions is important in understanding their impact in natural ecosystems and developing appropriate management strategies. Therefore, in this study, we characterized the fungal communities occurring in the exoskeleton of adults and larvae of *C. florentinus* and associated with the necrotic wood tissues surrounding the branch galleries of declining oak trees. A total of 29 fungal species were identified based on DNA sequence data and morphological features, of which 14 were from symptomatic woody tissues, six from insect exoskeleton, and nine from both insects and symptomatic wood tissues. The most frequent fungal species, *Cryphonectria naterciae* (15.9% of isolates), *Dothiorella iberica* (11.3%), and *Diplodia corticola* (9.9%), were isolated from both insect and gallery systems. All three species are well-known oak pathogens and are reported here, for the first time, to be associated with *C. florentinus*. At the same time, 89.6% of the fungal taxa were isolated from one or two sites, highlighting the site-dependence of fungal community assemblages.

Keywords: Buprestidae; wood-boring insect; emerging diseases; *Botryosphaeriaceae*; *Diplodia corticola*

1. Introduction

Over the past 30 years, Mediterranean oak forest ecosystems have been greatly impacted by severe decline phenomena and extensive mortality events, revealing the high vulnerability of these formations [1–3]. Severe decline and mortality events have the potential to drastically alter oak ecosystems, with important implications for the plant community dynamics, soil carbon–water balance, and structure and composition of soil food webs [4–7]. Oak decline is commonly considered a multifactorial disease in which many interacting abiotic and biotic factors such as drought, frost, insect pests and pathogens variable in intensity and frequency even at site level are involved [8–13]. Among the biotic factors involved in the onset of oak decline events, several invasive insects, fungi, and oomycetes play a primary role. In particular, the bark- and wood-boring beetles *Coraebus florentinus* Herbst, *Coraebus undatus* Fabricius, *Cerambyx cerdo* Linnaeus and *Platypus cylindrus* Fabricius are frequently implicated in oak declines in southern Europe [14–16]. At the same time, in recent years,

many independent surveys have demonstrated the involvement of some canker-causing agents, such as *Biscogniauxia mediterranea* (De Not.) Kuntze and *Diplodia corticola* A.J.L. Phillips, A. Alves & J. Luque, and root rot agents, such as *Phytophthora cinnamomi* Rands and *Phytophthora quercina* T. Jung, in the oak decline processes [17–20]. However, the ecological interactions between beetles and pathogenic fungi, as well as the role played by insects as a vector of the main fungal pathogens, are generally poorly investigated.

In recent decades, there has been a constant expansion of oak decline events in Sardinia, Italy, mainly in cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L.) woodlands. *Diplodia corticola* and *P. cinnamomi* have been identified as the major pathogens involved [21,22]. Furthermore, the black-banded oak borer (*Coraebus florentinus*) is considered an emerging pest of oaks in Sardinia [23]. This xylophagous beetle attacks chestnut (*Castanea sativa* Miller) and both evergreen and deciduous oaks, with a particular affinity towards holm oak and cork oak trees [24]. The larvae of *C. florentinus* burrow into the branches forming elliptic galleries, resulting in the destruction of water- and sap-conducting tissues and consequent dieback of 6–8-year-old branches [25]. Along and surrounding the insect galleries, it is often possible to observe the presence of large necrotic lesions of woody tissues caused by fungal pathogens that may contribute to accelerating the branch death.

Therefore, given the growing recrudescence of oak decline events in Sardinian woodlands and the limited information about the ecological interactions among *C. florentinus* and plant pathogenic fungi, a study was carried out to isolate and identify the fungal species occurring in insect exoskeletons and associated with necrotic wood tissues surrounding gallery systems on the branches of declining oak trees.

2. Materials and Methods

2.1. Study Sites, Field Surveys, and Sampling Procedure

Six oak woodlands located in the central and northern part of Sardinia, Italy, where oak decline phenomena and attacks of *C. florentinus* were previously reported [23], were selected to collect living and just-emerged insects and symptomatic oak branches. In all woodlands, cork oak and/or holm oak were the dominant species, whereas pubescent oak (*Quercus pubescens* Willd.) was sporadically present in only two study areas, as seen in Table 1.

From autumn 2016 to spring 2017, oak trees that had been attacked by *C. florentinus* at each site were identified by the presence of bored branches (4–7 cm in diameter) showing dead leaves still attached scattered among healthy foliage (Figure 1). In order to evaluate the infestation level, in each study site, 3 transects of 10 trees each were traced, at random, inside the woodland. All oak plants along the transect have been identified at the species level (holm oak, cork oak, and pubescent oak) and the occurrence of damaged branches recorded. The infestation level was evaluated as the percentage of damaged trees among the total number of oak trees monitored.

A total of 133 bored branches were randomly collected from April to June 2017, prior to the emergence of adults (Table 1). Sampled branches were cut approximately 10 cm below the pupation chamber. The branches were debarked and cut to find larvae. Thirty-seven branches were stored in the laboratory in cages until the emergence of adults. The cages consisted of plastic cylinders 55 cm high and 30–40 cm in diameter, closed with two breathable metallic nets with small meshes to prevent adult exit. Cages were checked regularly to collect the adults of *C. florentinus*. As soon as the adults of *C. florentinus* escaped from branches, they were sexed by observing their abdomen and used for fungal isolation.

2.2. Fungal Isolation and Identification

Fungal isolation was achieved from insect exoskeletons of 37 adults (26 males and 11 females), from preimaginal insect stages (17 larvae), and from 133 necrotic woody tissues surrounding the insect gallery systems on oak branches (Figure 1).

To avoid contamination, insects (adults and larvae) were sampled individually with sterilized tweezers, placed singly in an Eppendorf tube, and killed by freezing at −20 °C. For fungal isolation, insects were surface-disinfected for 2–3 sec with ethanol (90%), rinsed in sterile water, dried out on sterilized filter papers and then placed by pressing the whole bodies onto the surface of a potato dextrose agar (PDA, Oxoid Ltd., Basingstoke, UK) medium in 90 mm Petri dishes, using one insect per Petri dish.

Longitudinal and transverse sections were made to observe any internal symptoms (necrotic lesions) along the insect gallery system of branch samples. Isolations were achieved from approximately 5 mm^2 chips of xylem tissues cut, aseptically, from the margin of necrotic lesions along galleries. All plant samples were cultured on PDA in 90 mm Petri dishes.

After incubation at 25 °C for 1 week, all emerging mycelia from insects and plant material were subcultured to obtain pure cultures. Purified fungal isolates were subcultured onto half-strength PDA in 60 mm Petri dishes and kept on the laboratory bench at about 20–25 °C, where they received indirect sunlight to enhance sporulation. Fungal isolates were initially grouped and identified to genus/species level by comparison of macro- and micro-morphological features, such as colony appearance and size and shape of spores with species descriptions available in literature.

Representative isolates of each taxon were stored on PDA slants, under oil, in the culture collection of the Sez. di Patologia vegetale ed Entomologia, Dipartimento di Agraria at the University of Sassari.

Table 1. Study sites information and number of symptomatic branch samples collected from cork oak (c), holm oak (h), and pubescent oak (p) trees.

Study Sites	Locality	Elevation (m a.s.l.)	Coordinates (°N, °E)		Number of Branches Sampled
1	Gavoi	710	40° 07′ 43.6″	9° 11′ 14.1″	0 (c), 13 (h), 2 (p)
2	Buddusò	780	40° 34′ 01.4″	9° 19′ 06.9″	19 (c), 11 (h), 0 (p)
3	Pattada	630	40° 33′ 48.6″	9° 08′ 58.2″	6 (c), 0 (h), 0 (p)
4	Monte Lerno	600	40° 35′ 17.2″	9° 10′ 10.4″	12 (c), 8 (h), 0 (p)
5	Bottidda	310	40° 20′ 56.8″	9° 04′ 11.6″	2 (c), 0 (h), 0 (p)
6	Abbasanta	370	40° 08′ 28.9″	8° 45′ 40.1″	60 (c), 0 (h), 0 (p)

2.3. DNA Extraction, PCR Amplification, and Sequencing

Molecular analysis was used to confirm the identification of all isolates at the species level. InstaGene Matrix (BioRad Laboratories, Hercules, CA, USA) was used to extract genomic DNA from 5-day-old cultures grown on PDA and incubated at 25 °C in the dark. The primers ITS1 and ITS4 [26] were used to amplify and sequence the internal transcribed spacer (ITS) regions, including the complete 5.8s gene. Polymerase chain reaction (PCR) mixtures and amplification conditions were as described by Linaldeddu et al. [27]. The PCR products were purified using the EUROGOLD gel extraction kit (EuroClone S.p.A., Pero, Italy) following the manufacturer's instructions. The ITS regions were sequenced by the BMR Genomics DNA sequencing service (www.bmr-genomics.it), in both directions, with the primers used for amplification. The nucleotide sequences were read and edited with FinchTV 1.4.0 (Geospiza, Inc. http://www.geospiza.com/finchtv) and then compared with reference sequences (chiefly derived from type material) retrieved from GenBank using the BLAST search function [28]. Isolates were assigned to a species when their sequences were at least 99.8% identical to the sequence of type material or representative isolates. ITS sequences from representative isolates obtained in this study were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank).

2.4. Data Analysis

The species diversity of the insect-associated fungal communities was measured using the taxonomic richness (S), Shannon diversity index (H), and Simpson dominance index (D) [29]. In addition, the similarity of fungal communities between insect exoskeletons and gallery systems was evaluated

using the Jaccard similarity coefficient (Jc) [30]. The species diversity analyses were performed using the software Past® version 3.24 [31].

Similarities in fungal taxonomic richness between communities were summarized in Venn diagrams using GeneVenn software (http://genevenn.sourceforge.net/).

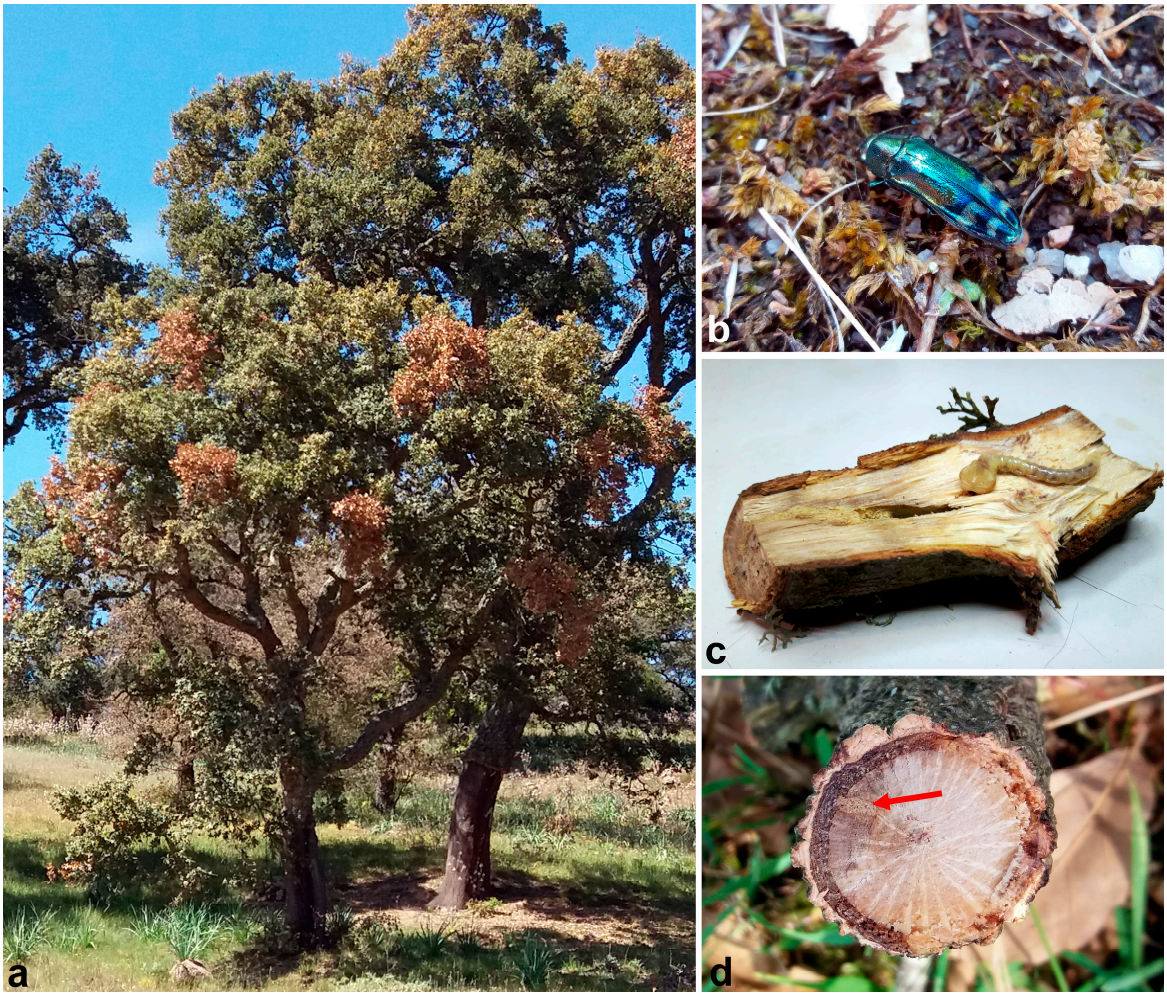

Figure 1. Cork oak tree showing multiple branch dieback symptoms (**a**), adult and larvae of *Coraebus florentinus* (**b**,**c**), and necrotic wood tissues surrounding the beetle gallery (red arrow) on a cork oak branch (**d**).

3. Results

3.1. Field Survey

Insect infestation was very variable among the investigated sites (Table 2). The most infested woodland was site 6. This woodland is chiefly composed of cork oak trees (87%) and, to a lesser extent, by pubescent oak trees (13%). At this site, 86.7% of oaks were attacked by the buprestid pest *C. florentinus*, with an average of 5.5 bored branches per tree.

A severe infestation level was also recorded in the mixed oak woodland of site 4 (10% holm oaks, 87% cork oaks, 3% pubescent oaks) and 1 (63% holm oaks, 27% cork oaks, 10% pubescent oaks), with an average number of bored branches of 2.6 and 4.8, respectively.

At the other sites, the infestation level ranged from 3.3% to 43.3%, with an average number of bored branches per tree that ranged from 1.5 to 2 (Table 2).

Table 2. *Coraebus florentinus* infestation level (%) recorded at the study sites.

Study Sites	Locality	N° of oak Tree Monitored	N° of Tree Damaged	Infestation Level (%)	Bored Branches per Tree (Mean ± SD)
1	Gavoi	30	20	66.7	4.8 ± 3.9
2	Buddusò	30	13	43.3	1.7 ± 1.1
3	Pattada	30	4	13.3	1.5 ± 0.6
4	Monte Lerno	30	23	76.7	2.6 ± 2.2
5	Bottidda	30	1	3.3	2
6	Abbasanta	30	26	86.7	5.5 ± 3.9

3.2. Fungal Isolation

Isolations carried out from insect exoskeletons and symptomatic branch samples yielded a total of 151 fungal colonies belonging to 12 orders, including *Ascomycetes*, *Basidiomycetes*, and *Zygomycetes*. About a quarter of the isolates (24.5%) were included within the *Botryosphaeriales* order, followed by *Pleosporales* (23.8%) and *Diaporthales* (21.2%); the remaining isolates were distributed across nine orders.

A total of 26 genera belonging to 19 families were identified. *Botryosphaeriaceae*, *Cryphonectriaceae*, and *Pleosporaceae* were the most abundant taxonomic groups, with 37, 24, and 21 isolates, respectively.

A total of 29 fungal species were identified based on DNA sequence data and morphological features, of which 14 were from symptomatic woody tissues, 6 from insect exoskeletons, and 9 from both insects and symptomatic wood tissues taken from bored branches (Tables 3 and 4). The most frequent fungal isolates, with a value of 15.9%, were identified as *Cryphonectria naterciae* Bragança, E. Diogo & A.J.L. Phillips. The second most frequent species corresponded to *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, representing 11.3% of isolates, followed by *Diplodia corticola*, with a value of 9.9% (Table 4). The other *taxa* were represented by a limited number of isolates.

Nine fungal species, namely *Alternaria doliconidium* J.F. Li, Camporesi & K.D. Hyde, *Alternaria hordeicola* E.G. Simmons & Kosiak, *Botrytis cinerea* Pers., *C. naterciae*, *D. corticola*, *Do. iberica*, *Gnomoniopsis paraclavulata* Sogonov, *Penicillium* sp., and *Trichoderma harzianum* Rifai, were isolated from both insect exoskeletons and symptomatic woody tissues of oak branches (Table 4). Interestingly, the species *Do. iberica* and *C. naterciae* were isolated from adult males, whereas *D. corticola* was isolated from the females. *Alternaria doliconidium*, *A. hordeicola*, and *Penicillium* sp. were the only species isolated from both adults and larvae.

Table 3. Percentage of internal transcribed spacer (ITS) sequence identity between representative isolates of each species obtained in this study and their closest matching references in GenBank.

Fungal *taxa* (Strain Number)	Accession Number	The Closest Matching NCBI GenBank Entry			
		Taxon	Accession Number	(%) Identity	References
Alternaria doliconidium (cp54)	MK796128	*Alternaria doliconidium**	MG828864	100%	[32]
Alternaria hordeicola (cp72.1)	MK796129	*Alternaria hordeicola**	NR_136019	100%	[33]
Arthrinium sp. (cp62.2)	MK796130	*Arthrinium kogelbergense**	KF144892	96.1%	[34]
Aureobasidium pullulans (cp73.2)	MK796131	*Aureobasidium pullulans**	FJ150906	100%	[35]
Biscogniauxia rosacearum (cp73.1)	MK796132	*Biscogniauxia rosacearum**	KT253493	100%	[36]
Botryosphaeria dothidea (cp2)	MK796133	*Botryosphaeria dothidea**	AY236949	100%	[37]
Botrytis cinerea (cp67)	MK796134	*Botrytis cinerea*	MH860108	100%	[38]
Cladosporium sp.§					
Cryphonectria naterciae (cp71.1)	MK796135	*Cryphonectria naterciae**	NR_159875	100%	[39]
Cryptovalsa ampelina (cp12)	MK796136	*Cryptovalsa ampelina*	JN975335	100%	[40]
Cytospora sp. (cp68.2)	MK796137	*Cytospora fraxinigena**	MF190134	99.04%	[41]
Diatrype sp. (cp11)	MK796138	*Diatrype stigma*	KU721866	99.42%	[42]
Diplodia corticola (cp60)	MK796139	*Diplodia corticola**	AY259100	100%	[43]
Dothiorella iberica (cp89.1)	MK796140	*Dothiorella iberica**	AY573202	100%	[44]
Fusarium sp. (cp79.6)	MK796141	*Fusarium solani*	MH864425	99.58%	[38]
Gnomoniopsis paraclavulata (cp71.5)	MK796142	*Gnomoniopsis paraclavulata**	EU254839	100%	[45]
Kalmusia sp. (cp46)	MK796143	*Kalmusia variispora**	NR_145165	99.6%	[46]
Neocucurbitaria cava (cp75.3)	MK796144	*Neocucurbitaria cava*	AY853248	100%	[47]
Neonectria coccinea (cp70)	MK796145	*Neonectria coccinea**	KC660521	99.81%	[48]
Nigrospora osmanthi (cp22)	MK796146	*Nigrospora osmanthi**	KX986010	99.79%	[49]

Table 3. *Cont.*

Fungal *taxa* (Strain Number)	Accession Number	The Closest Matching NCBI GenBank Entry			
		Taxon	Accession Number	(%) Identity	References
Penicillium sp.[§]					
Phaeoacremonium angustius (cp77.3)	MK796147	*Phaeoacremonium angustius**	KU060813	100%	[50]
Pseudocamarosporium piceae (cp48)	MK796148	*Pseudocamarosporium piceae**	KJ747046	100%	[51]
Querciphoma carteri (cp53)	MK796149	*Querciphoma carteri*	KF251209	100%	[52]
Stemphylium amaranthi (cp73.3)	MK796150	*Stemphylium amaranthi**	KU850505	100%	[53]
Stemphylium vesicarium (cp78.1)	MK796151	*Stemphylium vesicarium*	KU850565	100%	[53]
Stereum armeniacum (cp35)	MK796152	*Stereum armeniacum**	MH862626	100%	[38]
Trichoderma harzianum (cp25)	MK796153	*Trichoderma harzianum*	MH865862	100%	[38]
Unidentified (*Mortierellales*) (cp91.1)	MK796154	*Mortierella hyalina*	HQ630355	82%	[54]

[§] Not sequenced. * From ex-type culture.

Among the most frequently recovered species, *Do. iberica* was isolated from the galleries of all three oak species examined, while *C. naterciae* and *D. corticola* were only isolated from cork oak trees. *Dothiorella iberica* and *C. naterciae* were the most widespread species, and positive samples were derived from four of the six examined sites. Interestingly, 89.6% of the *taxa* were isolated from one or two sites.

No significant matches were generated in the BLAST analysis for the sequence of the zygomycetous strain and, hence, it was not identified to a species and genus level (Table 3).

Table 4. Number of fungal isolates obtained from insects and wood tissues of bored oak branches.

Fungal *taxa*	Ecology *	Adults		Larvae	Wood Tissues	Number of Sites
		Male	Female			
Alternaria doliconidium	unk	1		2	3	1
Alternaria hordeicola	unk	8		3	2	1
Arthrinium sp.	unk				1	1
Aureobasidium pullulans	ocr				8	1
Biscogniauxia rosacearum	pp				3	1
Botryosphaeria dothidea	pp				5	2
Botrytis cinerea	pp			1	2	2
Cladosporium sp.	unk				1	1
Cryphonectria naterciae	pp	5			19	4
Cryptovalsa ampelina	pp				1	1
Cytospora sp.	unk				1	1
Diatrype sp.	unk				5	2
Diplodia corticola	pp		5		10	1
Dothiorella iberica	pp	1			16	4
Fusarium sp.	unk			6		1
Gnomoniopsis paraclavulata	unk			1	6	2
Kalmusia sp.	unk				2	1
Neocucurbitaria cava	pp	7	4			1
Neonectria coccinea	pp			5		2
Nigrospora osmanthi	unk				2	1
Penicillium sp.	unk	1		1	1	1
Phaeoacremonium angustius	pp	2				1
Pseudocamarosporium piceae	ocr				1	1
Querciphoma carteri	unk				1	1
Stemphylium amaranthi	pp				1	1
Stemphylium vesicarium	pp	1				1
Stereum armeniacum	ocr				1	1
Trichoderma harzianum	ocr		1		3	3
Unidentified *Mortierellales*	unk		1			1

* Ecology: pp = plant pathogen; ocr = other ecological roles; unk = no data about ecology available.

3.3. Fungal Community Diversity

The Shannon diversity index value was highest for the fungal community associated with symptomatic wood tissues surrounding feeding galleries on oak branches (Table 5). For both communities (insect and wood tissues), low values of the Simpson dominance index were measured, demonstrating that both communities had a high microbial diversity and richness. The Simpson dominance index was slightly higher in insects than the gallery systems community.

Table 5. Diversity indices of fungal communities.

Index	Insect	Wood Tissues
Taxonomic richness	15	23
Shannon diversity index	2.352	2.636
Simpson dominance index	0.119	0.101
Jaccard similarity coefficient *	0.31	

* Between fungal community of insect and wood tissues.

The Jaccard similarity coefficient showed a low similarity value between insect and plant fungal communities, indicating that only a few fungal species could be efficiently vectored by *C. florentinus* (Table 5). The highest overlap (Jc = 0.259) was observed for the fungal communities from adults and wood tissues, indicating that many of the isolated fungi are not essential for larvae development (Figure 2).

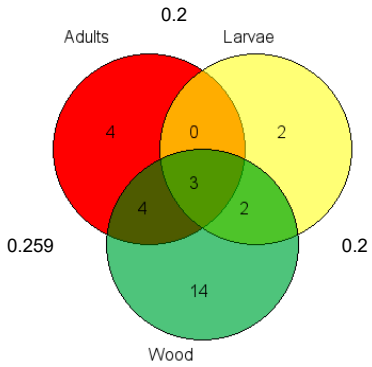

Figure 2. Venn diagram illustrating the unique and shared fungal taxa among insect adults (red), larvae (yellow), and wood tissues (green). Outside numbers are the Jaccard similarity coefficient.

4. Discussion

The findings obtained in this study allowed us to characterize, for the first time, the fungal communities associated with *C. florentinus* attacks in declining oak woodlands in Sardinia, contributing to expanding the knowledge on the bioecology of this emerging insect pest in the Mediterranean region. *Coraebus florentinus* is an insect pest able to attack apparently healthy plants and cause extensive wilting and branch dieback, which contribute to a general and progressive decline of oak trees [24,55]. After mating in summer, *C. florentinus* females lay eggs in the bark of twigs and after about 2 weeks, emerging larvae feed in the inner bark, burrowing downward sinuous galleries in the sapwood [25]. The results of this study show that the feed gallery system excavated by the insect represents a substrate on which several pathogenic fungi can develop, causing necrotic lesions.

In this study, a total of 29 fungal species were isolated from six different sites located across northern and central Sardinia. In particular, three of the main oak canker-causing agents, namely *Diplodia corticola*, *Dothiorella iberica*, and *Cryphonectria naterciae*, were isolated from bored branches.

D. corticola has emerged as an aggressive and potentially invasive fungal pathogen in southern Europe, North Africa, and North America [18,56–58]. The frequency of its attacks on oak trees

has significantly increased over the past decades, especially in mature cork oak forests after cork extraction [59,60]. *Diplodia corticola* is a necrotrophic pathogen whose infections induce extensive inner bark and xylem necrosis associated with blackish exudation from the outer bark [61]. Despite the various studies conducted on this emerging pathogen, the forces driving spore dissemination remain poorly understood. Recently, Inácio et al. [62] have demonstrated that *P. cylindrus* is a vector of *D. corticola*, whereas Kostovcik et al. [63] found that *D. corticola* is one of the fungi most frequently isolated from the mycangia of *Xyleborus affinis* Eichhoff and *Xylosandrus crassiusculus* Motschulsky, two invasive ambrosia beetles in Florida. In this study, *D. corticola* was the second most abundant species obtained from the exoskeleton of *C. florentinus* females, and the third most abundant from the woody tissues of bored oak branches. Interestingly, *D. corticola* was only isolated at site 6, which was characterized by the highest infestation level of *C. florentinus*.

The species of *Dothiorella* are considered weak pathogens or endophytes of different woody plants worldwide [64]; however, some species, such as *Do. iberica*, can cause branch cankers on oak trees [65,66]. In this study, *Do. iberica* was the second most abundant species obtained from necrotic tissues along the insect gallery systems.

Isolates of *C. naterciae* dominated the beetle-associated fungal communities in our investigation. *C. naterciae* was originally described from cork oak and sweet chestnut in Portugal [39], and was recently reported to be a cork oak pathogen in Algeria by Shami et al. [58]. To date, little information is available about the geographic distribution, oak host range, and bioecology of this species. All three species, *D. corticola*, *Do. iberica*, and *C. naterciae* are here reported for the first time, to be associated with *C. florentinus*.

Attacks of plant pathogenic fungi and xylophagous beetles are often found together on declining oak trees. A recent investigation on the causes underlying the severe decline of coast live oak (*Quercus agrifolia* Née) trees in Californian woodlands demonstrated the co-occurrence of *D. corticola*, *Do. iberica*, and the goldspotted oak borer (*Agrilus auroguttatus* Schaeffer), an emerging Buprestid species, at several infested sites [65]. Furthermore, it has been demonstrated that exit holes produced by cerambycid pests could represent a mode of entry for opportunistic pathogens such as *B. mediterranea* [15], a fungal species frequently recovered from declining oak trees [67]. Interestingly, in this study, the species *Biscogniauxia rosacearum* M.L. Raimondo & Carlucci, a recently described pathogen known to occur only on rosaceous hosts [36], was isolated from insect gallery systems.

The insect-associated fungal communities obtained in this study seem to be influenced by site factors, given that the majority of the species were sporadic and were isolated from a single site. Lynch et al. [68] obtained similar results when studying the occurrence and incidence of fungal species in declining coast live oak trees infested by *A. auroguttatus* in California.

5. Conclusions

In conclusion, the findings obtained in this study highlight that *C. florentinus* plays a role in the diffusion of different plant-pathogenic fungi and, in particular, of *D. corticola*; one of the main fungal pathogens involved in the etiology of oak decline in southwestern Europe and North America. Therefore, in addition to ambrosia beetles and cerambycid pests, Buprestid species such as *C. florentinus* could also contribute to fungal dispersal and influence fungal community assembly in declining oak ecosystems.

Whether the fungal species isolated in this study can promote the attacks of *C. florentinus* remains to be clarified, but the combined effect of *C. florentinus* and *D. corticola* on Mediterranean oak ecosystems could have a strong ecological impact, as recently observed in Spain by Torres-Vila et al. [55]. The co-occurrence of multiple adverse factors in the oak decline onset, such as *C. florentinus* and *D. corticola*, together with inappropriate agroforestry activity [69] and climatic change [70], suggest that a multidisciplinary approach is essential for developing adequate management strategies to preserve these important and vulnerable ecosystems in the future.

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