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Short note

Medicinal wood decay fungi on large old oaks in the Madonie Mountains (Sicily)

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Abstract
Periodic observations made on two 800-plus-year-old *Quercus pubescens* trees in the Madonie Mountains (Sicily) revealed the presence of two saprotroph and occasionally weak parasite fungi. The basiodiomycetes *Laetiporus sulphureus* and *Pseudoinonotus dryadeus* were identified by combining morphological and observation of microscopic characters with molecular analysis. The two mushrooms fall in the so-named category of "medicinal mushrooms" showing many properties such as antioxidant, antimicrobial, anticancer, cytotoxic, hypoglycemic and anti-inflammatory. Actions are proposed to safeguard old trees and, simultaneously, preserve ex situ the wood-decay fungi of potential medicinal interest.

Keywords
Old trees, *Quercus pubescens*, wood-decay fungi, medicinal mushrooms, Sicily

Introduction

In Italy there is a growing interest in large old trees, which are a resource globally threatened by intensive land use. Large old trees are distinguished by their high biological and ecological value (age, size, morphology, species rarity, habitat for certain animal species), historical, cultural and, religious importance, their close relationship with architectural type emergencies, and their ability to characterize the landscape in both aesthetic and identity terms. These old trees are recognized an important cultural and biodiversity values deserving of protection in the frame of the National Law

No. 10/2013 (Italian Official Gazette, 2013) and the Ministerial Decree of October 23, 2014, which also includes Italy's first official list of large old trees (Camarda and Brundu 2021).

In large old trees aging and decay and the consequent presence of rotting branches, cavities, deep cracks, etc., have traditionally been regarded as malformations. However, these degraded parts of the tree promote the attraction of insects and their larvae, fungi, molds, lichens, birds and, small rodents (Alexander et al., 2006). Regarding fungi, old trees are known to harbor Basidiomycetes and Ascomycetes with unknown and/or untapped medicinal potential (Gilhen-Baker et al., 2022) along with a wide range of rare fungal species (Berg et al., 1994). Older and weaker trees are more susceptible to decay fungi, which reduce wood strength and adversely affect deposition and conductive tissues in the sapwood (Ding et al., 2020). Among the different species of aged trees, old oaks are subject to different attacks by white and brown rot-causing fungi, and this is particularly evident in old-growth forests (Marchetti et al., 2010; Frati et al., 2022).

In this contribution we analyzed the presence of *Pseudoinonotus dryadeus* (Pers.) T. Wagner & M. Fisch. [Hymenochaetaceae] and *Laetiporus sulphureus* (Bull.) Murrill [Laetiporaceae] on year-old monumental *Quercus pubescens* Willd. s.l. in Piano Sempria (Castelbuono, Madonie, northern Sicily) evaluating their role in the natural process of aging of the oaks and at the same time their potential as medicinal fungi.

Materials and Methods

Field observations and fungal identification

Field observations were carried out in Piano Sempria, a forest of Madonie mountains located in Castelbuono, Sicily (37°54’11’’N 14°04’06’’E, Fig. 1), province of Palermo. During observation the presence of lignicolous fungi was detected on the trunks of two living monumental downy oak (*Q. pubescens*), considered to be of exceptional historical value (Fig. 2a, b).

The basidiomata were collected then transferred to the laboratory and identified by morphological and molecular analysis as reported by Mirabile et al. (2023). Dried basidiomata were cut into slices and stored in the Herbarium SAF of Department of Agricultural, Food and Forest Sciences (SAAF) of the University of Palermo. For fungal nomenclature Index Fungorum database was used.

DNA was extracted from 7-days-old pure colonies, obtained from basidiomata, using the Extract-N-Amp™ kit (Sigma-Aldrich, St. Louis, USA) as reported by Gargano et al. (2024). The primers ITS1F and ITS4 were used for the amplification of the internal transcribed spacer region of rDNA (ITS) by polymerase chain reaction (PCR). Phylogenetic analyses were conducted using our obtained sequences, representative sequences of genus *Laetiporus* Murril from a previous published paper (Song et al., 2018), representative sequences of *Pseudoinonotus* T. Wagner & M. Fisch., and related genus *Inonotus* P. Karst. retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Fig. 1. – Piano Sempria forest located in the Madonie mountains of Castelbuono, Sicily.

Fig. 2. – a) century-old *Q. pubescens* with a cavity in the trunk used as a votive chapel; b) Old large tree of *Q. pubescens* located in Piano Sempria with lignicolous fungi growing at the base of the trunk.

Results and Discussion

According to morphological analysis and analytical keys, the observed species were identified as *L. sulphureus* (accession number PP809774) (Fig. 3a) and *P. dryadeus* (accession number PP809775) (Fig. 3b).

Laetiporus sulphureus (Fig. 3a) is a lignicolous species that is pathogenic as long as the plant remains alive, then acts as a saprotroph by disintegrating the now-dead plant. It generates a cubic brown wood rot, which extends into the central part of the trunk and takes several years for the plant

on which it grows to die. *Laetiporus sulphureus* is characterized by a yellow/bright orange shelf-like structure basidioma that presented short tubules (2–3 mm) and pores producing a white spore print. The flesh, yellowish in colour, is succulent and presents a strong fungal odour. It appeared sessile, but the shelves of the cap is instead united in a stem-like structure that keeps the fungus anchored to the trunk on which it grows. Microscopically present hyaline, smooth and ellipsoidal to ovoid basidiospores measuring 5–7 × 3–4 µm produced by basidia with dimensions 15–17 × 4.5–7 µm size (Fig. 3c).

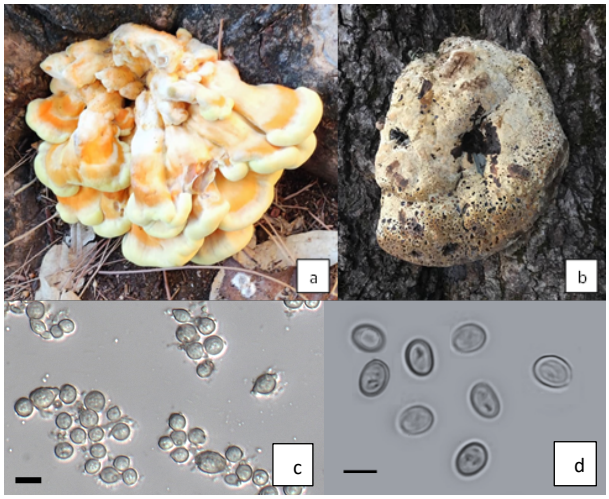

Fig. 3. – Basidiomata of *L. sulphureus* (a) and *P. dryadeus* (b) observed on the trunks of two monumental downy oak trees growing in Piano Sempria; c) *L. sulphureus* basidiospores (Bar 10 µm); d) *P. dryadeus* basidiospores (Bar 5 µm).

Pseudoinonotus dryadeus (Fig. 3b) is a fearsome pathogen, agent of white rot of tree species, especially oaks or, more rarely, spruces and white fir, and usually grows at the base of their trunks or on outcropping roots. It is an infrequent species, and its findings record single basidiomes or, at most, a few specimens developing on the same plant. *Pseudoinonotus dryadeus* is characterized by a huge, sessile basidioma (20 cm in width) with irregular shape. The surface, cream to yellowish in colour is tomentose with scattered brown patches and big tubes from which it produced a brown liquid. The flesh is leathery in texture and fibrous. The hymenophore with tubules and pores produced hyaline, smooth and subglobose basidiospores of 5–8 × 5–6.5 µm size (Fig. 3d).

Due to the presence of several similar species, phylogenetic trees were useful to confirm morphological analysis, showing that our isolates clustered with *L. sulphureus* and *P. dryadeus* with high bootstrap percentages (Fig. 1Sa,b).

Both investigated fungal species by the action of their ligninolytic enzymes may contribute, along with other concomitant biotic and abiotic factors, to the decay of the two centuries-old oaks. Therefore, interventions are needed to safeguard the trees with protection of wounds generated accidentally or due to human action. Concurrently, *L. sulphureus* and *P. dryadeus* are recognized as "medicinal mushrooms". Both *P. dryadeus* and *L. sulphureus*, in fact, are known for anti-cancer, antioxidant, immune-modulating, cardiovascular, anti-hypercholesterolemic, anti-inflammatory,

antimicrobial, and anti-hyperglycemic properties (Petrović et al., 2014; Cateni et al., 2015; Arsenijević et al., 2024).

In our opinion, is therefore necessary to safeguard monumental oaks with targeted dendrosurgery practices (safeguarding the compartmentalization areas) to improve the health status of these trees and at the same time safeguard the biodiversity of fungi growing on large old trees through mycelium isolation and their *ex situ* cultivation to secure for current and future generations a vital share of bioactive compounds in the treatment and prevention of various diseases and to provide alternatives to antibiotic resistance.

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