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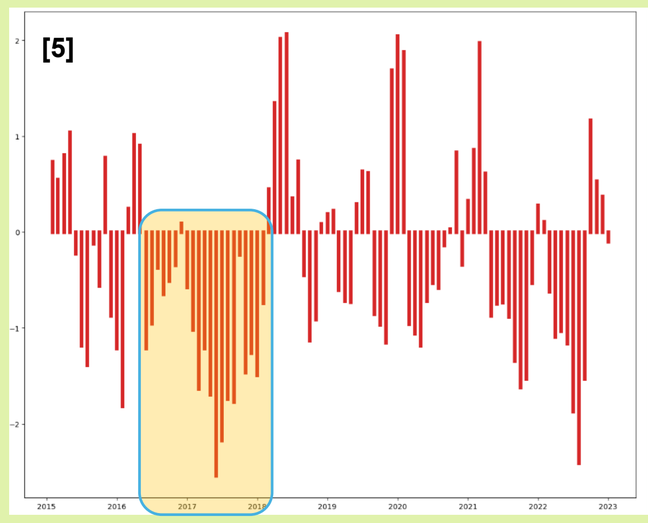
Monitoring the biotic stressors in a Mediterranean Holm oak forest: outcomes of the project SpecFor

[1] Location of the study areas in the Parco regionale della Maremma (Tuscany, Italy): LD and HD indicate the two sampled stands with a different grade of holm oak decline (LD: low decline; HD: high decline)
[2-3] Charcoal cankers caused by *Biscogniuaxia mediterranea*
[4] Representative symptoms observed in the HD area.

INTRODUCTION

Holm oak (*Quercus ilex* L.) forests are currently a key element in the ecological and socio-economic sustainability of forest ecosystems in the Mediterranean area, but over the last few decades, extensive dieback and mortality episodes of the trees have been documented. The study area of the project SpecFor was located in the Maremma Regional Park in Tuscany (Italy): our surveys reported dieback and decline in different stands in the park (Fig 1), suggesting a correlation with extreme climatic events occurred in the region and the decline status of the site (Fig 5). Holm oak decline is reported as a complex disease caused by multiple interaction of biotic and environmental contributing factors.



[5] Time series of the SPI (Standardized Precipitation Index). From 2016 to 2018, the region experienced a persistent and continuous drought. The plot is based on analysis of ERA5 Land climatological dataset (ECMWF – Copernicus Climate Service). Data related to 2016-2017, when the onset of decline was reported, have been highlighted.

In addition to drought, heat waves, shift of rainfall pattern, human pressure, microbiome alteration, several primary, weak and opportunistic pathogens on predisposed hosts have been reported to be involved. The project Specfor aim to elucidate the biotic factors contributing to the decline syndrome in Tuscany.

Materials and Methods

Sampling was conducted in May 2024 in two stands, differing by the number of symptomatic trees per area, namely respectively “lowly diseased area” (LD) and “highly diseased area” (HD). In each stand, a total of 10 plant has been surveyed (5 declining, 5 non-declinig): the rate of decline has been evaluated by visual assessment by using phenotypic descriptors according to ICP-Forests (2016).

Few branches with leaves, representative of the healthy state of each tree, were sampled by the 4 cardinal point of the crown. No evidence of cankers or necrosis were observed externally on the sampled branches. Inner bark (50 small pieces per tree) was taken from superficially sterilized twigs and placed on Potato Dextrose Agar (PDA) plates. After 10 days incubation, the obtained fungal colonies were examined for their morphological characterization and grouped into a different morphotype. Molecular characterization of each morphotype was carried out by DNA extraction and PCR amplification of at least three barcoding regions with specific primers (ITS1/ITS4, EF1-728/EF1-986, BT2A/BT2B, rpb2-5f2/ rpb2-7cr)

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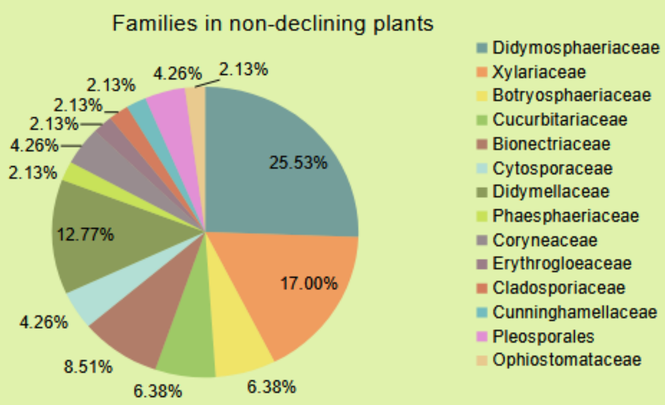

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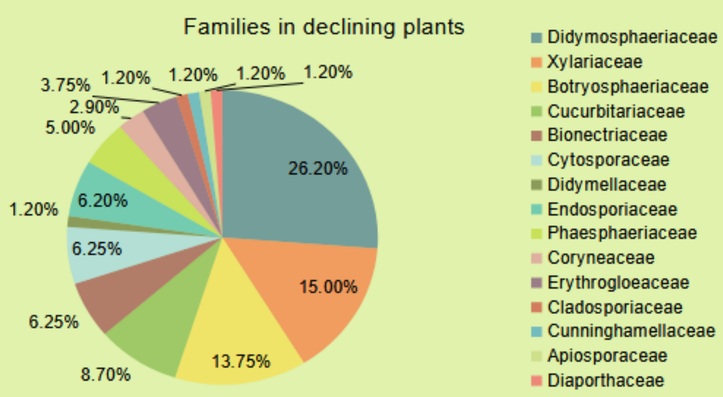

FINDINGS

A total of 24 fungal taxa were identified based on DNA sequence data and morphological features, belonging to 15 families (Fig. 6 - 7). The fungal genera most frequently isolated were *Biscogniauxia*, *Paraconiothyrium* and *Neocucurbitaria* (Fig 8 - 10): all of them are fungi that can persist endophytically on healthy host tissues, and act as pathogens only in stressed plants. *Biscogniauxia mediterranea*, for instance, is a weak pathogen, frequently observed on *Quercus* and in other plants of the Mediterranean maquis. During the in situ sampling, fungal fruiting structures of *B. mediterranea* were observed on dead oaks , causing evident charcoal cankers on the bark. Together with *Diplodia* sp., it is reported to be associated with “holm oak decline” also in other parts of Italy. Other fungi belonging to *Botryosphaeriaceae*, a family of cosmopolitan fungi frequently associated with cankers and dieback, have been reported in this study and in particular in declining plants (for instance *Dothiorella iberica*, a fungal strain that can cause branch cankers on trees).

[8]

[9]

[10]

[6 -7] Pie chart representing the relative abundance (percentage) of the identified families which the fungal colonies isolated from declining plants (Fig. 6) and from non-declining plants (Fig. 7) belong to.
[8 - 9 - 10] Colonies of Paraconiothyrium nelloi (Fig. 8), Biscogniauxia mediterranea (Fig. 9) Neocucurbitaria juglandicola (Fig. 10).

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