

FUNGAL DIVERSITY AMONG DIFFERENT ANNUAL GENERATIONS OF *PRAYS OLEAE*



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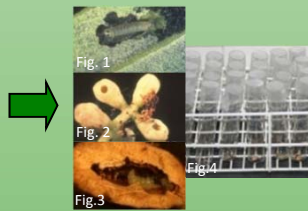
INTRODUCTION and OBJECTIVES

In sustainable agricultural production systems, entomopathogenic fungi (EF) present a promising alternative to chemical insecticides, for the control of insect pests. In Portugal, where olive production is one of the main agricultural activities, orchards are strongly affected by *Prays oleae* Bern. This lepidopteran has three generations per year (phylophagous, antophagous and carpophagous), synchronized with the olive tree life cycle. As a result, larvae of *P. oleae* damage the olive tree in different organs (leaves, flowers and fruits).

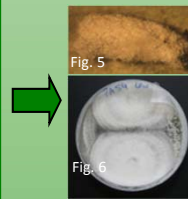
This work intends to evaluate the diversity of fungi associated to each one of the different generations of *P. oleae*, with special relevance to entomopathogenic fungi. Ecological roles (entomopathogenic, antagonistic and phytopathogenic) of the identified species are presented.

MATERIALS and METHODS

Six olive orchards were considered for collecting larvae and pupae of *P. oleae*, from the three generations: phylophagous (Fig.1), antophagous (Fig.2) and carpophagous (Fig.3). Collected larvae and pupae were placed in sterile tubes (Fig.4).



Whenever fungal growth was visible in the surface of larvae and pupae of *P. oleae* (Fig. 5), the isolation of fungus was performed to PDA culture medium (Fig.6).



DNA extraction was performed from fungal pure cultures, PCR amplifications and sequencing were done using the ITS region of the rDNA, using the universal primers *ITS1* and *ITS4* (White *et al.* 1990). The identification was achieved by comparison with the sequences present in the NCBI database. Sequence analysis was done using DNASTAR, v. 7.0.0. software. Principal component analysis (PCA) was performed using SPSS, v.17.0.

RESULTS

Fungal species recovered

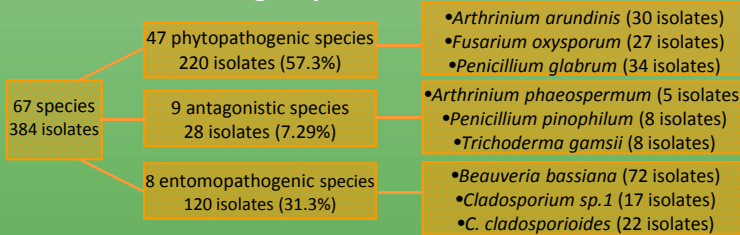


Fig. 7 – Number of species, total abundance and relative percentage of fungal taxa belonging to each of the three ecological roles. The remaining 3 species present an unknown ecological role. For each ecological role, only the 3 most important species are presented.

- From all moth generations, a total of 384 fungal isolates were identified, belonging to 67 species;
- The majority of species are described as being phytopathogenic;
- EF represent more than 31% of the total isolates, and the species *B. bassiana* was the most abundant (72 isolates).

Association of fungal species with *P. oleae* generation

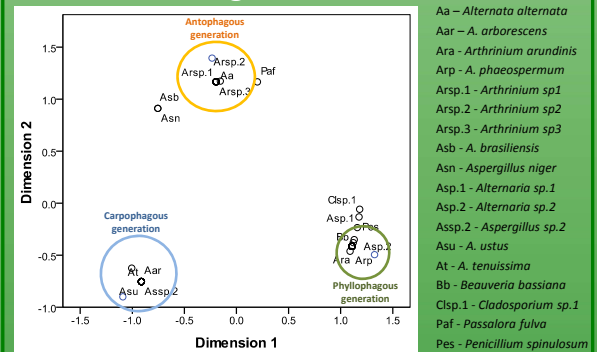


Fig. 9 – Principal component analysis (PCA) of the identified fungal species, according to the moth generation from which they were isolated. Some species are not represented, as coordinates match the ones of the species showed.

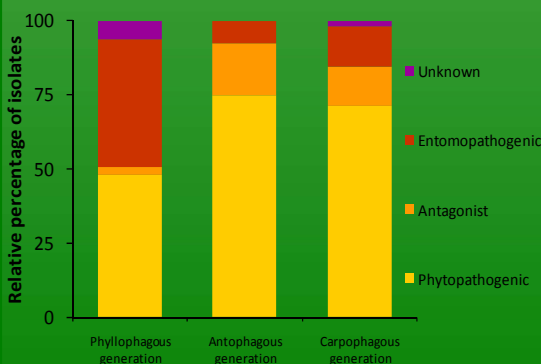
- 25 fungal species were associated to the phylophagous generation, including *B. bassiana* and *C. cladosporioides*;
- 23 species were found to be associated to the carpophagous generation, which includes *Penicillium pinophilum* and *Aspergillus ustus*;
- The antophagous generation includes the remaining 19 species, as *Fusarium oxysporum* and *Trichoderma gamsii*.
- From the 8 EF species found, only *B. bassiana* was isolated from the three generations. In contrast, 5 species are associated to only one of the *P. oleae* generations.

Fungal diversity in each *P. oleae* generation

- The diversity of fungal species differed between the three generations (Table 1). Higher diversity was found in the carpophagous generation;
- Diversity of fungal species was very similar between the phylophagous and antophagous generations.

Table 1 – Diversity indexes of fungal species, obtained from each generation of *P. oleae*.

Generation	Simpson	Shannon-Wiener
Phylophagous	0.13	2.47
Antophagous	0.14	2.50
Carpophagous	0.06	2.93
Total	0.11	2.63



- Phytopathogenic species account for the majority of isolates. In all three generations of *P. oleae* (Fig.8);
- EF isolates are most abundant in the phylophagous generation and reduced on the others (Fig.8). This may be due to the higher presence of their conidia on the leaves, but also to the influence of climatic conditions, known to influence EF activity, fitness and persistence.

Fig. 8 – Percentage of isolates obtained in the three generations of *P. oleae*, belonging to each of the considered ecological roles.

Conclusions

- The diversity of fungal species associated to *P. oleae* differed between moth generations;
- There is a clear association between fungal species and moth generation, being this fact probably related to the life cycle of the insect, as well as to climatic factors;
- The presence of both entomopathogenic and antagonistic fungi provide a variety of potential biocontrol agents, that could be used against pests and diseases occurring in olive orchards;
- *Beauveria bassiana* can be a promising fungus to be used as biocontrol agent against *P. oleae*, especially on larvae and pupae of the phylophagous generation.