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 olive pests: isolation, characterization and selection for biological control. 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: phyllophagous (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

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

<table>
<thead>
<tr>
<th>Generation</th>
<th>Simpson</th>
<th>Shannon-Wiener</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phyllophagous</td>
<td>0.13</td>
<td>2.47</td>
</tr>
<tr>
<td>Antophagous</td>
<td>0.14</td>
<td>2.50</td>
</tr>
<tr>
<td>Carpophagous</td>
<td>0.06</td>
<td>2.93</td>
</tr>
<tr>
<td>Total</td>
<td>0.11</td>
<td>2.63</td>
</tr>
</tbody>
</table>

**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 diversity of species was very different between the three generations (Table 1). Higher diversity was found in the carpophagous generation;
- The diversity of fungal species was very similar between the phyllophagous and antophagous generations.
- The diversity of fungal species differed between the three generations (Table 1).
- The diversity of fungal species accounted for the majority of isolates. In all three generations of P. oleae (Fig.8), EF isolates are most abundant in the phyllophagous 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.

**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 phyllophagous 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 olives of the species showed.

- 25 fungal species were associated to the phyllophagous 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 entomopathogenic 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.

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

**Fig. 10** – Percentage of isolates obtained in the three generations of P. oleae, belonging to each of the considered ecological roles.