Establishment and field growth performance of *Quercus rubra* seedlings inoculated with selected ectomycorrhizal fungi

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Figure 1: Serra da Cabreira experimental site, Northern Portugal.

Introduction

Successful tree establishment is a critical step on reforestation, being a difficult, unpredictable and spatially variable process. Previous studies have suggested that fungal inoculation enhances seedling growth and survival on the first years after out planting. This is mostly because nursery grown seedlings inoculated with ectomycorrhizal (ECM) fungi often mitigate stress factors, increasing seedlings capacity to capture nutrients. However, their performance may not be straightforward, thus an increased effort is needed to understand ECM fungi roles and behaviour.

The present study aims at

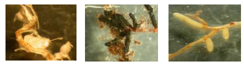
1. Assessing growth and establishment of pre-inoculated *Quercus rubra* seedlings on a reforestation site.
2. Monitoring ECM fungal persistence dynamics between selected ECM fungi and native fungal community.

Methodology

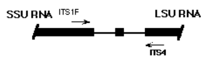
- Nursery grown *Quercus rubra* seedling were inoculated with a mixture of selected ECM fungi: *Cenococcum geophilum* (CG1), *Hebeloma crustuliniforme* (HO1), *H. mesophaeum* (HME), *H. velutipes* (HV1), *Paxillus involutus* (PAX), *Scleroderma citrinum* (SC);
- Persistence of inoculated ECM fungi was assessed two years after planting in Serra da Cabreira region, Northern Portugal (Fig. 1), using ITS-DGGE;
- Oak saplings growth (height and root collar diameter) of inoculated and non-inoculated control plots were compared using Student's t-test (P<0.05).

Experimental site design:
1 – ECM inoculated plot
2 – Non-inoculated control plot

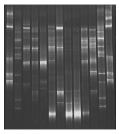
Soil cores (n=18) from inoculated and non-inoculated control plots were sampled



Total ECM root tips were counted and bulk DNA extracted (n=9)



ITS region 18S rDNA amplified using ITS1f-GC / ITS2



DGGE fingerprint

ECM fungal spp. identification by comparing DGGE bands positions against reference positions of known ECM fungal species

Results and Discussion

A) Seedlings performance

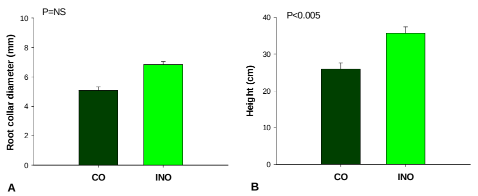


Figure 2: *Quercus rubra* inoculated (INO) and non-inoculated (CO) growth performance: Root collar diameter (A) and shoot height (B).

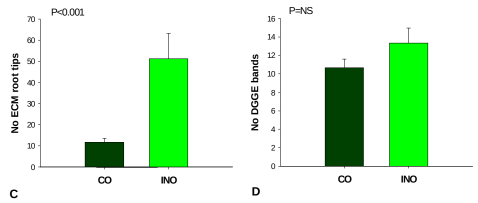


Figure 3: Comparison between *Q. rubra* inoculated (INO) and non-inoculated (CO) sapling: ECM root tips (C) or number DGGE bands (D).

✓Successful *Q. rubra* establishment was observed on both plots. Inoculated oak saplings showed significant higher growth than non-inoculated oak samples (Fig. 2);

✓Non-inoculated oak saplings showed some ECM root colonisation, but significantly higher ECM root tips were found on inoculated oak samples (Fig.3);

✓No relationship was found between the number of ECM root tips and DGGE bands, suggesting that any detected pattern is not a sampling artefact and represents true differences in ECM fungal community composition.

B) ECM fungal community ordination analysis

✓ Ordination analysis (Fig. 4) showed that ECM fungal community of inoculated and non-inoculated samples are significantly different;

✓Joint biplot suggests that a relationship exists between axis 1 of DCA and environmental data (saplings height and number of ECM root tips) on the inoculated plot;

✓Results suggest that ECM fungi enhanced oak seedling establishment and better performance after out planting.

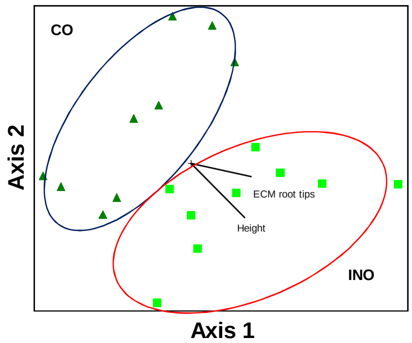


Figure 4: Detrended correspondence analysis of *Q. rubra* inoculated (INO) and non-inoculated (CO) samples. Vectors from joint biplot represent strength and direction of correlated seedling performance.

C) Persistence of ECM fungi

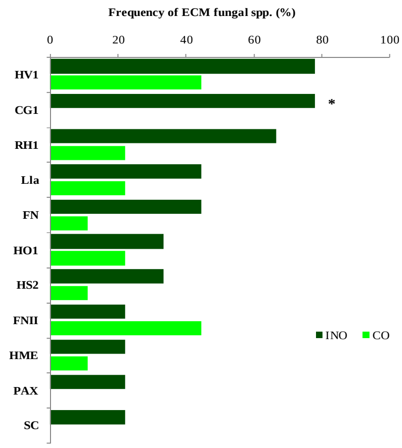


Figure 5: Frequency of some identified ECM fungal species on *Q. rubra* inoculated (INO) and non-inoculated (CO) root samples. Legend: *H. sinapizans* (HS2), *Laccaria laccata* (Lla3), *Rhizopogon roseolus* (RH1), Unknown ECM spp. (FN and FNII). * Represent statistically significant indicator species P<0.05.

✓ Fungal inoculum persisted on inoculated (INO) oak root samples after two years (Fig. 5);

✓CG1 was significantly associated with inoculated oak plot. HV1, HO1 and HME were also detected on non-inoculated oak samples;

✓Other ECM fungi species were also identified on both plots. i.e. RH1 was found mostly on inoculated plot;

✓Results suggest that ECM fungal community and plant establishment performance could be related.

Conclusions:

❑ Inoculation with selected ECM fungi at nursery stage can improve field growth performance of *Q. rubra* seedlings. Inoculated ECM fungi persisted on oak roots two years after field transplanting. Further monitoring is needed to increase knowledge on communities persistence and succession dynamics.

❑ ECM fungi are a successful biotechnological tool aiding reforestation projects.

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