POPmodels

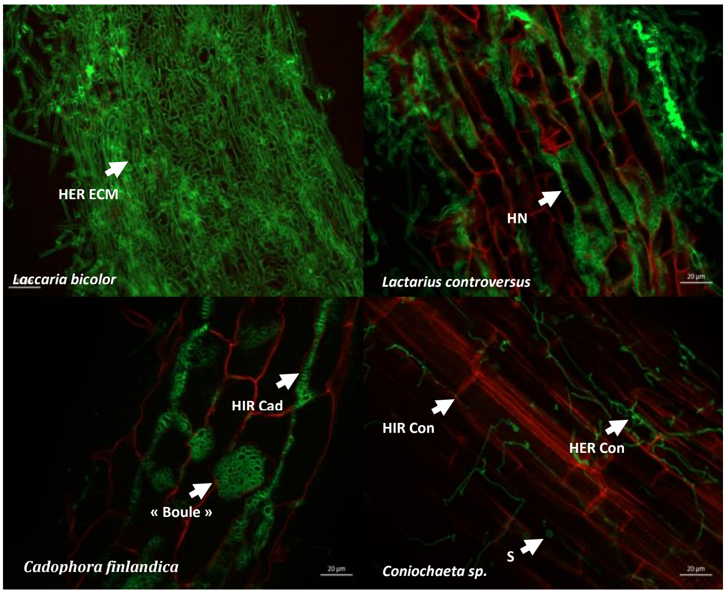

Figure 1: *Populus tremula x alba* roots inoculated with an ectomycorrhizal fungus or an endophyte. Root cells were stained with propidium iodide (red) and fungal cells with WGA-Alexa fluor 488 (green). HER= Extra-radicular Hyphae; HIR: Intra-radicular Hyphae; HN: Hartig net

Understanding Poplar-Microbe Interfaces: From model systems to model synthetic communities

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Thematic action concerned: WP1

Context — Thousands of species of fungi occur in the soil, but only a small fraction is able to form mutualistic symbioses promoting tree growth. Ectomycorrhizal fungi (ECM), but also endophytes with positive, negative or neutral effects on their hosts, are becoming increasingly recognized as important members of the tree microbiome. Despite their importance for tree growth and resistance to stresses, we still know very little about the mechanisms by which these fungi associate and communicate among each other and with their host trees, or the mechanisms by which they contribute to ecosystem processes such as nutrient cycling, among others.

Objectives — The current knowledge on the molecular signalling in ECM interactions was obtained with a few *in vitro* model systems, including our model *Laccaria bicolor*-Poplar. While significant progress was made in the elucidation of signals that are essential for ECM development, very few is known at the molecular level for endophytic interactions and the possible inhibitory or synergistic effects that these fungi may have on ECM fungi and towards the host tree. To assess if common or different mechanism of interaction are found for different types of mutualistic plant-fungal interactions, one challenge will be to extend our molecular studies from simple *in vitro* systems to more complex and finally natural systems. In this project, we aim at determining whether plants differentially recognize beneficial microorganisms and, further, how do plants engage at the same time with a wide range of microorganisms?

Approaches — In this study, we analysed the colonisation of naïve *Populus tremula x alba* roots by two ectomycorrhizal fungi and two endophytes either by single inoculation or by different combinations of dual and multiple inoculations. In sterile pots, 3-weeks-old poplar were grown in 200 ml of gamma irradiated sterile soil plus 5% volume of fungal inoculum for 30 days. For each treatment, five pools composed of the arial parts or roots from five plants were harvested for molecular analysis and 6 roots were kept for microscopy analyses. In addition, soil samples from each pot were snap frozen in order to analyse the fungal transcriptome in the soil compartment. Fresh biomass was determined and stained root samples were observed by confocal microscopy (the root cell walls stained with propidium iodide (red) and the fungal cell walls with Alexa fluor (green)). Aerial part and root samples were processed to analyse their metabolome and transcriptome.

Key results —

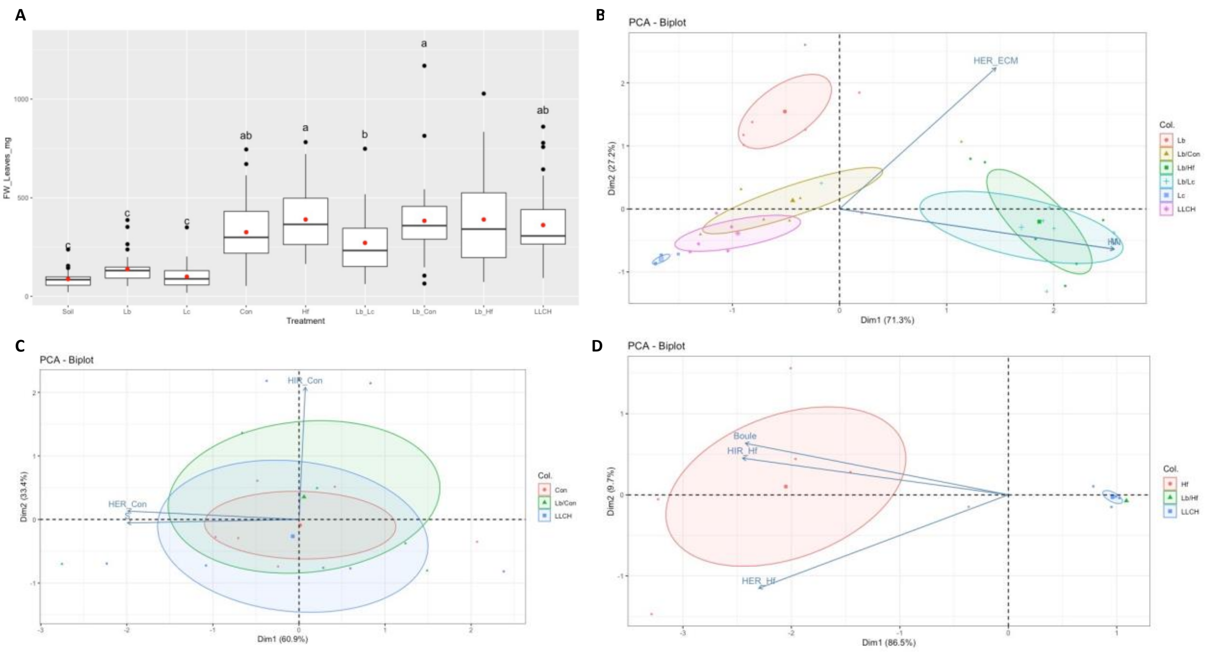

Figure 2 : Aerial fresh biomass and root colonization of Populus tremula x alba. Aerial fresh biomass was determined at the harvest (A). Typical fungal structures were counted using the McGonigle method (1990) and their frequency calculated. We segregated Mantle (M), Hartig Net (HN) and extra-radicular hyphae (HER) for ECM fungi (B), intra-radicular and extra-radicular hyphae (HIR and HER respectively) for the endophytes plus typical structures like spores (S) and globular structures ("Boules") (D) belonging to Coniochaeta sp. (C) and H. finlandica (D) respectively. Single inoculation : L. bicolor (Lb), L. controversus (Lc), H. finlandica (Hf), Coniochaeta sp. (Con), dual inoculations : L. bicolor/ L. controversus (Lb/Lc), L.bicolor/Coniochaeta sp. (Lb/Con) et L. bicolor/H. finlandica (Lb/Hf), all strains (LLCH).

- Endophytes and co-inoculations (dual and multiple) between ECM fungi and endophytes showed a plant growth promoting effect. (Fig.2A)
- In our experimental set-up, ECM fungi formed ectomycorrhiza with *P. tremula x alba* root only when ECM fungi where in dual inoculation with either another ECM or an endophyte. (Fig.2B)
- Both endophytes, *Hyaloscypha finlandica* and *Coniochaeta* sp, colonized poplar roots but with very distinct structures. *Coniochaeta* displayed highly melanized, thin extraradicular hyphae and spore-like structures, while *Hyalopscypha* formed intercellular punctuated by globular structures (Fig. 1).
- In dual inoculation with the ECM *L. bicolor* and one endophyte, two different trends were observed. Co-inoculation with *L. bicolor* and *H. finlandica* showed only ECM structures, while *L. bicolor* - *Coniochaeta* co-inoculation led to less frequent ECM structures (Fig.2, B-D)
- Using the most complex inoculum, all four fungal strains interact with poplar roots, with low frequency of ECM structures and intracellular structures, with the exception of the *Coniochaeta* extracellular hyphae which interacts with poplar roots at the same rate whatever fungal combination. (Fig.2, B-D)
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Main conclusions including key points of discussion —

- ECM fungi formed ectomycorrhiza with *P. tremula x alba* only in combination with either an ECM or an endophyte. Thus, their ability to colonize the roots could be promoted by synergistic or competition mechanisms between fungi or by their growth rate which allowed them to get access to the root system before the other or a priming or defense effect by the first fungus colonizing the root system allowing or preventing, respectively, the colonization by another fungus

- Despite a plant-growth promoting effect by the two endophytes, they displayed very distinct colonization patterns. While *H. finlandica* alone formed intra-radicular structures, *Coniochaeta* hyphae stayed mainly in the surrounding of the root as an epiphyte. This suggests that two fungi belonging to the same trophic guild can (1) form different kind of interactions at the cellular level and it remains to be demonstrated if they allow bi-directional exchange of nutrients or not and (2) promote plant growth with different mechanisms (direct transfer of nutrients at the fungus/plant interface vs. indirect transfer through fungal degradation of nutrients present in the rhizosphere or soil, thus contributing to their bioavailability to the plant, or production of metabolites or hormones). depending on the environmental conditions.
  Using the most complex inoculum, we mainly observed fungal structures from *Coniochaeta*, suggesting that poplar roots would preferentially associate with this fungus which confers growth benefit without generating carbon costs (but more experiments on carbon costs are necessary).

Perspectives

In addition to confocal microscopy, transcriptome and metabolome analyses will be conducted to elucidate the differences in perception of the fungi by the host tree and in comparing interaction-induced gene sets of ectomycorrhizal fungi and endophytes.

Valorization Presentation of first results in lab seminars and to collaborators of the PMI project

Leveraging effect of the project—The POPmodel project is closely linked to our international long-term project Plant-Microbe Interfaces (PMI; https://pmiweb.ornl.gov/; since 2010). The intermediate results presented here were already discussed in the frame of PMI and were considered for future directions of the project.