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BOOK OF ABSTRACTS

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P-152 - DETECTION AND QUANTIFICATION BY REAL-TIME PCR OF ECTOMYCORRHIZAL FUNGI IN INOCULUM FORMULATIONS FOR URBAN TREES APPLICATION

Cindy Serafim[1]; Miguel Ramos[1]; Nadine Sousa[1]; Paula Lima Castro[1]

1 - Escola Superior de Biotecnolgia

Background
Urban trees are key elements in mitigating the common environmental problems in urban areas, through provisioning crucial ecosystem services such as air quality improvement, decrease of water runoff and microclimate mitigation. Because of their well-known host tree benefits, Ectomycorrhizal Fungi (EcM) may play an important role in urban tree management, improving tree vigor, and thus the extent and resilience of ecosystem services delivered by urban trees under stress. Therefore it is important to develop dedicated EcM-inocula to improve urban tree health and its associated ecosystem service delivery. To support the study of the effect of inoculum application in urban context it is necessary to establish new biotechnology tools and test their efficacy in laboratory trials. The major aims of this work were (i) to isolate EcM species known to associate with the selected tree species chosen (*Tilia tomentosa*) and (ii) to develop and test (in situ & ex situ) a specific RT-qPCR assays for detection and quantification of ectomycorrhizal RNA. The latter will allow fast, quantitative monitoring of the selected target species over time, space or different environmental conditions and can be directly applied to RNA samples from *in-vitro* and field experiments.

Method
Two key-criteria were used in the fungi species selection: EcM species that are present on healthy urban trees, but absent on the unhealthy ones; and (ii) EcM species from forest/old park trees that are not found on the unhealthy street trees. Fungi were isolated from Northern Portugal and identified by molecular techniques (DNA extraction, PCR, sequencing). For each fungal species selected, a real-time reverse-transcription quantitative PCR (RT-qPCR) assay was developed based on the ITS sequences available in NCBI and UNITED data base enabling specific monitoring and quantification of viable or active EcM of interest during the experiments that will be conducted.

Results & Conclusions
Ten new fungal isolates were successfully isolated, comprising the genera *Russula*, *Pisolithus* and *Paxillus*. The standard curves obtained for mycelial quantification of the chosen EcM species satisfy the requirements for real-time PCR, showing high reaction efficiency. Under the conditions established in the present work, mycelium concentration was detectable to levels adequate for biomass in situ quantification.

References & Acknowledgments
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Detection and quantification by real-time PCR of ectomycorrhizal fungi in inoculum formulations for urban trees application

Keywords: real-time PCR