SHORT TERM MOBILITY 4-25 NOVEMBER 2015 – ANTONELLA FACCIO

The aim of the stage at the laboratory of Prof. Robert Roberson was to prepare ectomycorrhizal samples for transmission electron microscopy (TEM) with an improved protocol of fixation and embedding (high pressure freezing and freeze substitution). Obtaining a good ultrastructural preservation allows to better analyze the symbiotic interface typical of an ectomycorrhiza and to verify the impact of the fungal colonization on plant cell wall. To this aim, ectomycorrhizal samples have been processed as described in the next sections, where the different performed activities have been described in detail. The planned work has been done, and materials have been already cut and observation at TEM have been performed, showing the success of the applied protocols. Although some pictures have been obtained during this period (for some examples see the figures inside this report), the work on these samples is still in progress and immunolabeling will be performed with the aim to localize plant and fungal cell wall components during the ectomycorrhizal symbiosis. The preparation of samples from different plant/fungus combinations will allow to compare the impact of the same fungus on different plants.

<u>First week</u>: Fixation and embedding (Method 1 for Immunolabelling)

a) HIGH PRESSURE FREEZING PROTOCOL
The samples (i.e., 3 different ectomycorrhizal root samples) were transferred in the cavity of the aluminium carrier. Excess of water was drawn off with filter paper and the space was filled with 1-Hexadecene. The sandwich was completed with a flat speciment carrier and frozen in a HPM 100 high-pressure freezing machine (Bal-Tec Products). Frozen samples were stored in liquid nitrogen until further processing.

b) FREEZE SUBSTITUTION PROTOCOL
The frozen samples were processed as follow:

1. 0.2% Glutaraldehyde + 0.1% uranyl acetate in dry acetone
2. Incubate at -85 C for 48 to 72 h
3. Transfer to -20 C for 2 h
4. Transfer to 4 C for 2 h
5. Transfer to room temp for 1 h
6. Rinse in acetone three times
7. Infiltrate in London Resin White (LRWhite)

c) RESIN INFILTRATION PROTOCOL
10% increase of resin each step for one hour, for example
Day one:
10%resin:90% acetone 1 hour (hr) 20% resin: 80% acetone 1 h
etc. until 70% to 80% resin, at this time leave sample overnight.
Day two:
80% resin:20% acetone 1 h
90% resin:10% acetone 1 h
100% resin 1.5 h
100% resin 1.5 h
Embed and polymerize

<u>Second week</u>: Fixation and embedding (Method 2 for Ultrastructure)

HIGH PRESSURE FREEZING PROTOCOL (the same as Method 1)

FREEZE SUBSTITUTION PROTOCOL
The frozen samples were processed ad follow:

1. 1.0% glutaraldehyde + 1.0% tannic acid, in dry acetone
2. Incubate at -85 C for 48 to 72 h
3. Rinse in acetone at -85 C three times
4. Transfer to cold (-85 C) 1.0 % OsO4 in acetone and incubate for one h
5. Transfer to -20 C for 2 h
6. Transfer to 4 C for 2 h
7. Transfer to room temp for 1 h
8. Rinse in acetone three times
9. Infiltrate in Epon/Araldite resin

RESIN INFILTRATION PROTOCOL (the same as Method 1)

<u>Third week:</u> Sectioning and Transmission Electron Microscope observation

Samples were processed for ultramicrotomy: semi-thin sections (1 µm thick) were stained with 1% toluidine blu and observed under an optical microscope; ultra-thin sections (70nm thick) were counter-staining with uranyl acetate and lead citrate, and imaged using a JEOL 1200 transmission electron microscopy. Approximately 50 digital images were obtained.

Figure 1. TEM picture of a *Corylus avelana*/*T. melanosporum* ectomycorrhiza. Hartig net region.

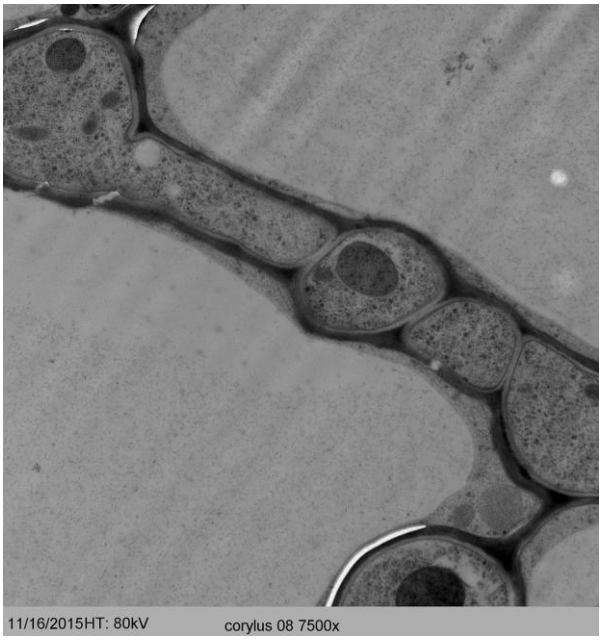

Figure 2. TEM picture of a *Pinus pinea*/*T. borchii* ectomycorrhiza. Hartig net region.

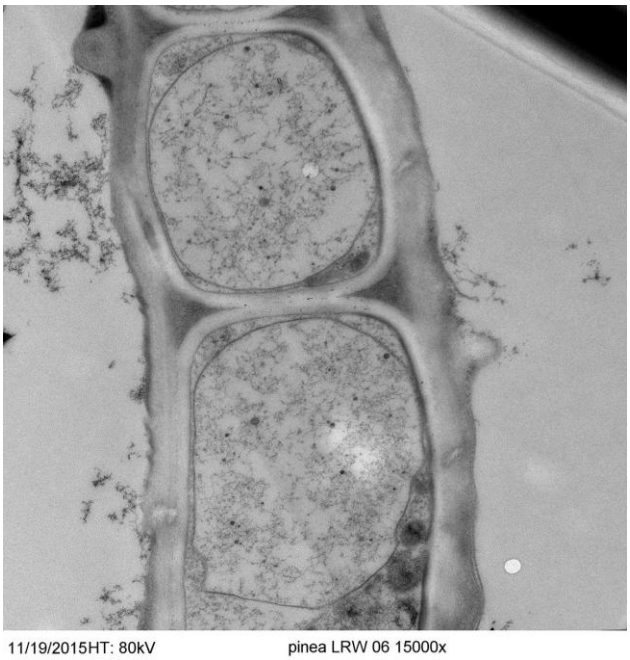

Figure 3. TEM picture of a *Quercus robur*/*T. melanosporum* ectomycorrhiza. Magnification of the plant/fungus interface.