– Master's training course of Salvatore Tirrito –

"*Leaf-to-air* and *root-to-soil* interphaces in *Quercus pubescens* Willd.
as related to acidic irrigation and heavy metal contamination in the soil"

– Research Project of Framework "From Cell-to-Tree" –

This abstract is aimed at resuming my personal stage experience based on a contribute in the research project developed by Dr. Elena Paoletti at Plant Protection Institute (IPP) of *National Research Council* (CNR), in Firenze and titled "*Leaf surface micromorphologies of Quercus pubescens Willd. as related to acidic irrigation and heavy metal contamination in the soil*", recently modified to include also fine roots and mycorrhizas.

Organization: This project is a part of a big framework carried out at the Swiss Federal Research Institute WSL in Birmensdorf near Zürich. The framework leader is Dr. Madeleine Goerg-Günthardt of the research group Bioindications. Research groups from WSL and other institutes in Switzerland and Europe carry out research projects within the framework analysis of the effects of soil toxicity on the level of cells, organs, organisms and ecosystems in view of *Phytoremediation* of polluted soils by woody plants.

Currently, there are 16 partner projects working to 30 different projects within the "From Cell to Tree" framework.

Problem: Waste deposition in dumps and deponies, immissions from industrial production, and agricultural practices including the use of sludge have led to many soils being polluted by heavy metals. There are not only inherited problems from improperly disposed waste, which urgently need remediation, but also insidious and regionally expanded heavy metal inputs over a long time. In agricultural land alone the area which exceeds the critical levels for heavy metals and organic pollutants is estimated to be 37 – 65 %. Solutions have to be found all over Europe, in order to stabilish, to decontaminate and to reuse moderately contaminated soils (energy farming, recreation areas). Therefore we urgently need data on the fluxes, allocation and metabolism of the pollutants in relation to the planting, soil type and rain acidity. However, until now there was a lack of experiments using an ecosystem approach, i.e. which takes into account the fact that the plants' reactions to pollutants are greatly modified by the competition between different plants for light, water and nutrients and by the effects of associated organisms (pests, pathogens, mycorrhiza, bacteria).

Aim: This large project aims to investigate the biofluxes in model ecosystem chambers and to trace and better understand the reactions of the plants and associated organisms to the chronic influence of important soil pollutants (Zn, Cu, Cd, Pb) and rain acidity. This project is intended to improve the accuracy of the estimation of benefit and risk when polluted soils, dumps and deponies are regreened/reforested using so-called 'phytoremediation'.

Experiments: The model ecosystem chambers are 16 Open-Top-Chambers (OTCs - area 6 m^2, height 2 m, depth 1.5 m built as lysimeters) with mobile roofs which exclude rain automatically (see picture below), and 20 Field Plots (FPs).

In the following page there is a briefly description of experimental plants.

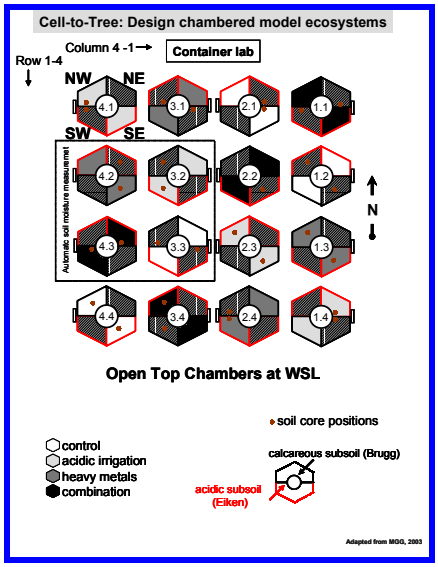
Treatments into the OTCs are 4 with 4 replication:

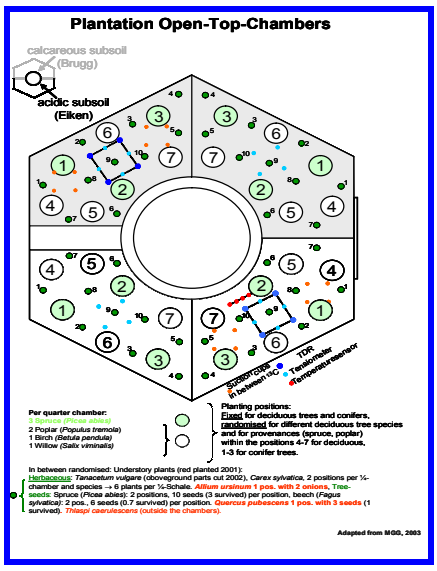
1. (**C**) control,
2. (**AR**) acidic irrigation,
3. (**HM**) heavy metals in the upper part of soil – 15 cm
4. (**HMAR**) combination of AR & HM.

Each chamber is split into 2 soil compartments, filled with an acidic (pH 4.5) or a calcareous (pH 7.5) subsoil.

← Inside an OTC after broadleaves cut

Layout of the OTCs →

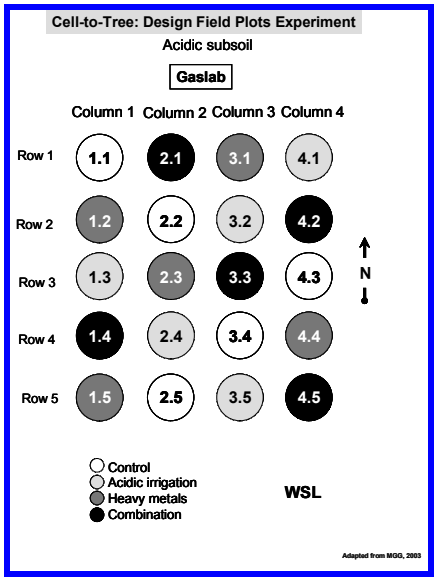




Each model ecosystem is formed with the same collective of trees, including Norway spruce (*Picea abies*), birch (*Betula pendula*), willow (*Salix viminalis*), and poplar (*Populus tremula*), seedlings from 3 different tree species (among which there is "our" *Quercus pubescens*) and many herbaceous understory plant species differing in their growth strategies.

← Layout of the plantation of used species inside each OTC (see list)

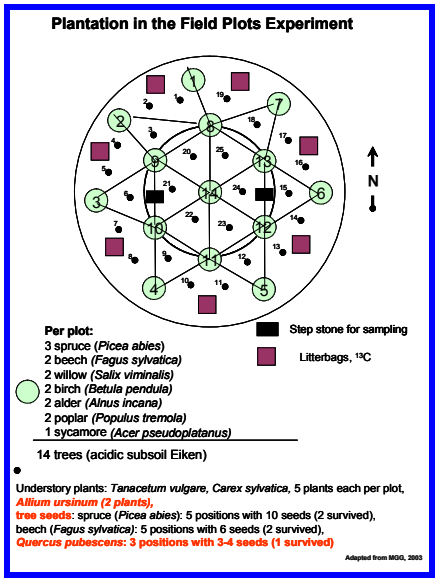
Some FPs →



To study the reactions in the field to spontaneous and controlled pests, particularly sucking and feeding insects, the model ecosystems are repeated in 20 FPs nearby. Here the plantations are completed by beech, alder (measuring symbiontic N_2 fixation), and maple. The results from the experimental plots are validated by comparable studies at Swiss field sites with polluted soils.

← Layout of the FPs

Layout of the plantation of used species inside each OTC (see list) →



Personal Stage experience: My first interesting Stage experience was the transfer to WSL in Birmensdorf with the aim of collecting 31 soil cores immediately at the stem base: this number corresponds to one seedling per each of the two sub-soils per each OTC. A 445 cm3 cylindrical steel sampler with cutting borders was utilised to collect the same soil volume. In fact it was not possible to excavate the whole plant, as it was used by other project partners. Each soil sample with roots was sealed in a plastic bag and kept at 4°C temperature. At the Florence laboratories, each sample was carefully washed in order to divide soil and roots, and to clean root without damaging mycorrhizas and fine roots. Some samples were put in a 1% solution of sodium esametaphosphate (a deflocculant agent) to favour the dispersion of clay and organic particles. By means of a cryo-microtome, some 30 µm thick sections were cut from roots that appeared morphologically different in order to assess only Q. pubescens roots. Comparisons were performed by an optical microscope to evaluate the secondary structure. For each sample the roots were separated into: "other trees" put all together; *Q. pubescens* coarse roots (Ø > 2 mm) and *Q. pubescens* fine roots (Ø < 2 mm). Part of the latter, was casually cut off in fragments of 3-4 cm length which were transversely put in a Petri dish (Ø = 12 cm) having a grid at the bottom with 1 cm^2 mesh. By means of a stereomicroscope roots in the dish were scored and the following parameters recorded: mycorrhized tips, not mycorrhized tips, dead tips, living axes and dead axes. Horizontal and vertical lines were scored and the above-mentioned parameters were recorded at each intersection point using a mechanical counter. To perform comparable measurements, work was carried out at constant magnification (18 X) and observations were made inside the microscope graticule. Then other parameters were then calculated and referred to the total fine root dry mass, obtained in an oven at 80°C until a constant weight was reached. The findings were subjected to analysis of variance througth the STATISTICA™ software.

Another exiting exerience was the "discovery" of the electronic microscopy using two innovative intruments of Ce.M.E. (Center of Electronic Microscopy): the ESEM QUANTA Fei – ENVIRONMENTAL-SEM – (with EDAX probe and hot chamber) and the SEM XL20 Fei – CRYO-SEM – (with EDAX probe and CRYO-GATAN ALTO 2100 technology).

Returning to the pure research project, the leaves were sampled on October 2002 (by Dr.Paoletti), using the following methodology. It was decided to sample one seedling per subsoil in OTCs and one seedling per plot in FPs. From the top of the 2nd growth flush, three mature leaves per seedling were collected. The samples were placed in paper bags, transported to the lab and air-dried to prevent alteration of epicuticuticular waxes. My job started from this point. A strip inside the middle-right part of each leaf was removed by a sharp scalpel. The lower sides of these leaf-portions were placed upon special supports (aluminium stubs) and gold-sputtered using EDWARDS S 150 sputter coater. Obviously, great care was taken to protect the leaves against mechanical damages: all materials were gently handled at all stages since the harvest, during preparation, until observation. This operation was carried out using a PHILIPS 515 Scanning Electron Microscope (SEM) at 20 kV. Following a transect along each leaf' portion, 50 stomata were observed at random, giving a total of 150 stomata per seedling. Changes were quantified by assigning each stoma to one of the four arbitrary stomatal damage classes (*Class 0 = healty stomata; Class 1 = slightly damaged stomata; Class 2 = damaged stomata;Cclass 3 = severely damaged stomata which had losed its physiological role*), to evaluate the Stomatal Damage Index (SDI). Another important parameter such as density of stomata was also measured: working at constant magnification (745 X), they were counted inside the main monitor of SEM in 10 random interveinal areas of each leaf. Like for roots, the findings were subjected to analysis of variance.

At the and, in order to search traces of Zn, Cu, Cd and Pb in the leaf surface, it was applied x-ray microanalisys by means of EDAX pv 9900 electron probe connected to the SEM: it was found only Cu in leaves of treated seedlings.