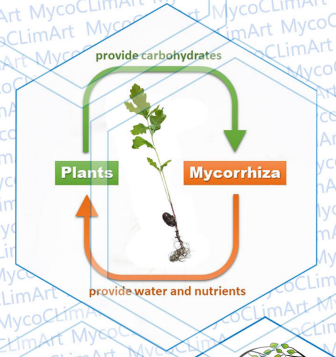
Development of Climate Smart Forestry (CSF) concept in the Republic of Serbia through mycorrhizal modulation of polyamine metabolism in pedunculate oak (*Quercus robur* L.) trees (MYCOCLIMArt)

THE USE OF MYCORRHIZA IN THE NURSERY PRODUCTION OF *Quercus robur* L. SEEDLINGS

A GUIDELINE FOR FOREST PRODUCERS

Republic of Serbia

Science Fund of the Republic of Serbia



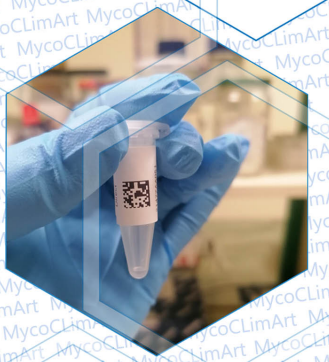
HAVE YOU HEARD ABOUT MYCORRHIZA?

Mycorrhiza is a symbiotic association between a fungus and a higher plant that brings numerous benefits to both partners.

Several forms of mycorrhiza are known, which are classified according to the way of contact between the fungus and the plant and the taxonomic groups of the fungal and plant partners.

In the temperate zone, the most common forms of mycorrhizae are:

- ARBUSCULAR MYCORRHIZA (AM) (endomycorrhiza - fungal hyphae penetrate cells of the root),
- ECTOMYCORRHIZA (ECM) (fungal hyphae envelop the surface of the root, but do not penetrate its cells) (white marks on the bottom left photo).



Although AM is the most widespread form of mycorrhiza, establishment, growth and survival of trees in most temperate forests depend on colonization with ectomycorrhizal fungi.

Mycelium of ECM fungi represents the main component of forest ecosystems, and links forest trees, ground vegetation, decomposers and different organic and inorganic resources in the forest floor, popularly called “wood wide web”.

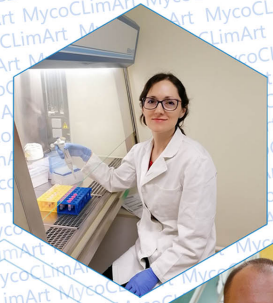
BENEFITS OF MYCORRHIZAE

The fungus obtains hydrocarbons from the plant and in return provides numerous direct and indirect benefits for its plant partner:

- ✔ Increase nutrient uptake (nitrogen and phosphorus),
- ✔ Enhance plant efficiency in absorbing water,
- ✔ Reduce fertilizer and irrigation requirements,
- ✔ Protect against damage from heavy metals and other pollutants,
- ✔ Minimize the effects of various biotic and abiotic stresses,
- ✔ Improve seedlings growth and survival,
- ✔ Contribute to maintaining soil quality and nutrient cycling,
- ✔ Improve soil structure by the extraradical hyphal network,
- ✔ Contribute toward carbon sequestration.

For more, please check following publication:

Milović, M., Kebert, M., Orlović, S. (2021). How mycorrhizas can help forests to cope with ongoing climate change? Šumarski list 145, 279-286.



ABOUT MYCOCLIMART PROJECT

Climatic changes are supposed to negatively affect majority of forest ecosystems in Republic of Serbia. Moreover, pedunculate oak (*Quercus robur* L.) has been projected to be the most endangered tree species from upcoming climate perturbations, causing great economic losses.

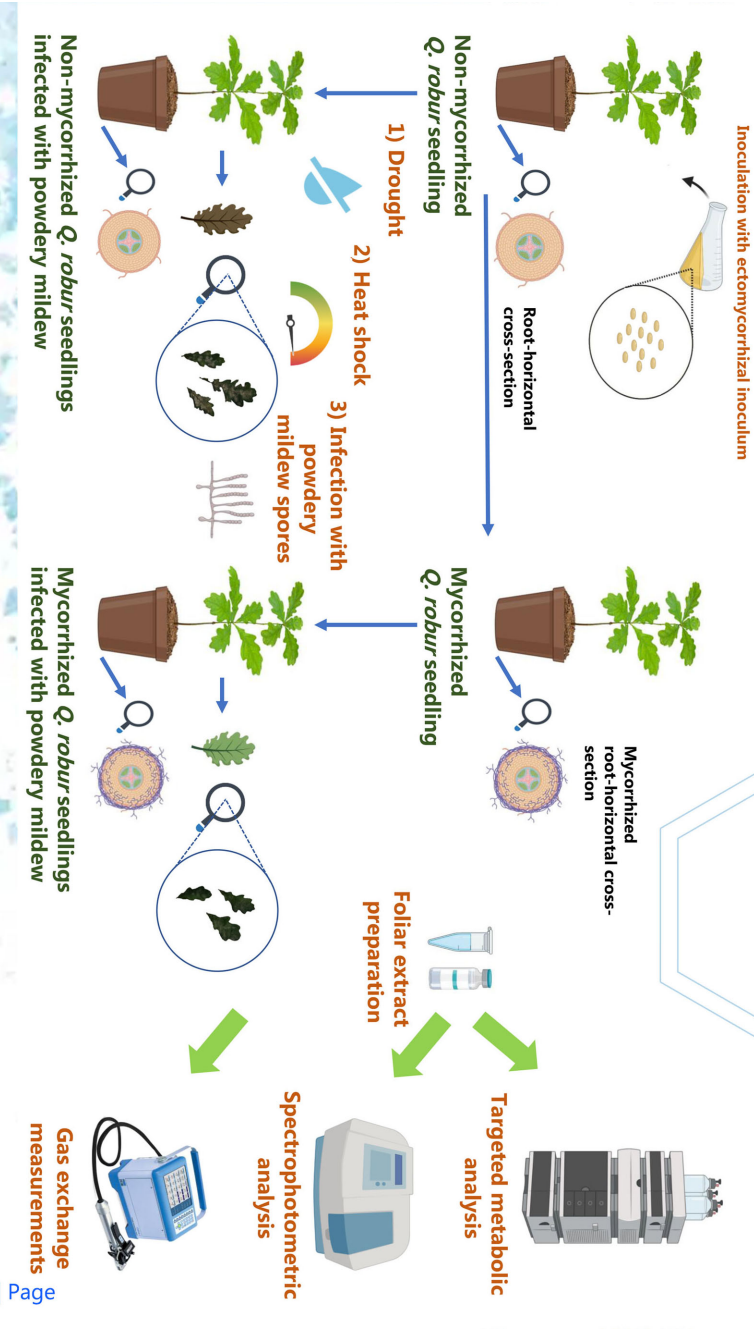
Mycorrhizal fungi can improve trees tolerance to unfavorable abiotic stress factors, such as heat and drought, as well as boost trees immunity and increase its resistance to pathogens.

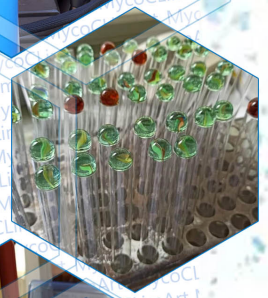
Therefore, the MYCOCLIMArt project is focused on the following specific objectives:

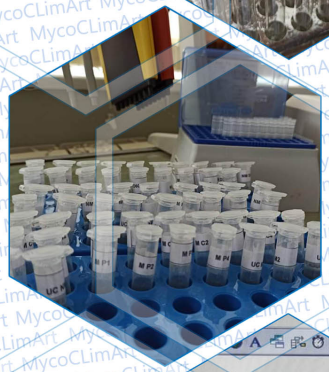
- **Objective 1. To evaluate the effects of mycorrhyza on pedunculate oaks' (*Quercus robur* L.) biological response to abiotic stress factors.**
- **Objective 2. To explore bioprotective properties of mycorrhyza in suppression of powdery mildew disease casused bz *Erysiphe alphitoides* (Griffon & Maublanc 1912).**

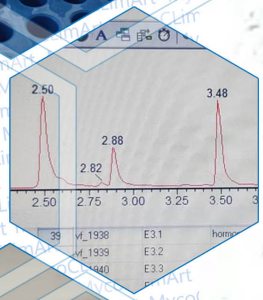
The higher level objective of the project is to form a multidisciplinary research team and to strengthen research capacities of project team members.

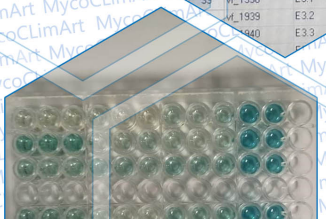
EXPERIMENTAL DESIGN











PARAMETERS MONITORED IN PLANTS WITHIN THE PROJECT

Osmolyte amounts:
- Glycine-betaine (GB)
- Proline (PRO)
- Dimethylsulphoniopropionic acid (DMSP)

Poliamines:
- Putrescine
- Spermidine
- Spermine

Parameters of oxidative stress:
- ABTS assay
- DPPH assay
- FRAP assay
- Total phenolic content (TPC)
- Total flavonoid content (TFC)
- Condensed tannins (CT)
- Total non-protein thiols
- Lipid peroxidation (LP)

Plant hormones:
- Abscisic acid (ABA)
- Indole-3-acetic acid (IAA)
- Salicylic acid (SA)
- Jasmonic acid (JA)
- 12-oxo-phytodienoic acid (OPDA)

Physiological traits:
- Leaf relative water content
- Gas exchange parameters
- Chlorophyll fluorescence

THE ROLE OF MYCORRHIZAE IN IMPROVING SEEDLINGS PRODUCTIVITY UNDER STRESS CONDITIONS


Artist: Jakov Jakovljević

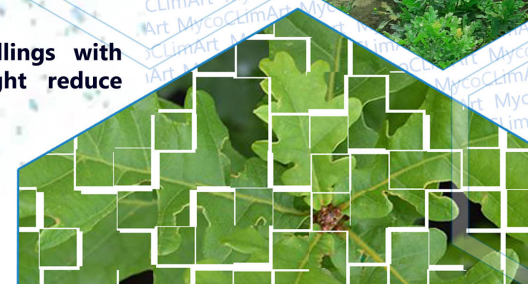
Establishment, survival, and growth of seedlings depend significantly on colonisation with mycorrhizal fungi.

Seedlings colonised with mycorrhizal fungi are in advantage compared to uncolonized ones because they can form more successful contact with water and nutrients and comm-unicate with other organisms in forest soil.

This is especially important in stress conditions, such as drought or lack of nutrients when mycorrhizal fungi can provide seedlings with additional water or nutrients that plant could not reach by itself.

Some species of mycorrhizal fungi can prevent heavy metal from entering plant shoots through various mechanisms of metal detoxification.

Likewise, inoculation of seedlings with some mycorrhizal fungi might reduce infections of pathogens.



PHASE 1.
PRODUCTION OF THE SEEDLINGS FROM THE ACORNS


1

Place the seed (acorn) in small pots (cca. 0,5 litres) previously filled with the vermiculite to germinate the seedlings.

The acorn should be sown in the autumn of the previous year, immediately after collection, or during February/early March of the current year.


2

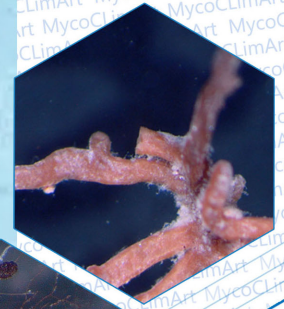
NOTE:
To achieve a higher percentage of germination it is desirable to soak the acorn in the water for 24 hours before sowing (see Photo 2).


3

Plant one to two acorns in a pot filled almost to the top with the vermiculite. The acorn should be sown 1-2 cm below the vermiculite surface. Vermiculite should be kept wet during seed germination and growth of the seedlings by watering it regularly once to twice per week, depending on the climatic conditions.

To be sufficiently developed the seedlings should be grown cca. 2 months in vermiculite (Photo 1) before the inoculation and transplantation to a new substrate (Photo 3).

PHASE 2.
PREPARATION OF ECTOMYCORRHIZAL INOCULUM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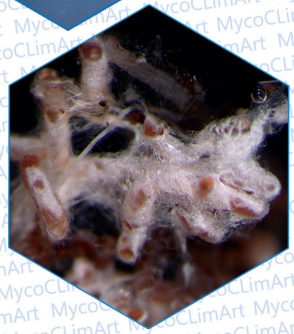
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The nursery producers are able to use either a commercial inoculum, which contain propagules of different species of ectomycorrhizal fungi prepared in advance for a certain group of tree species, or to obtain a specific inoculum based on autochthonous mycorrhizal fungi recorded on target tree species from specific site, which is going to be afforested with mycorrhizal seedlings.

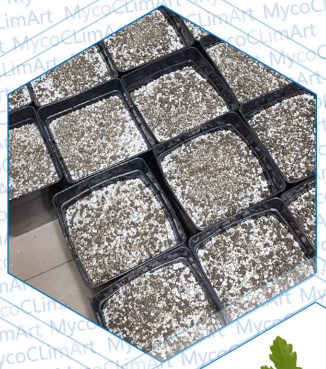
Photos 4-7 show some of the ectomycorrhizal fungi that can be found on pedunculate oak (see Milović et al. 2022).

SCAN ME

Source:

Milović, M., Kovačević, B., Pekeč, S., Pilipović, A., Kesić, L., Gavranović Markić, A, Orlović, S. (2022). Diversity of ectomycorrhizal fungi in young pedunculate oak stand from Morović, Serbia. South-east European Forestry 13(1): 19-25.

PHASE 3.
INOCULATION OF THE SEEDLING'S ROOT WITH ECTOMYCORRHIZAL INOCULUM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9

Prepare the medium (soil) where the seedlings will be transplanted. We used mixture of sterilized forest soil, vermiculite and perlite in the ratio 3:2:1 (Photo 8).

The seedlings are ready for inoculation and transplanting when they have well-developed roots and at least 4 fully developed leaves (Photo 9).


10

Producers of commercial inoculants commonly provide the instructions about its dosage and preparation. Certain producers recommend to soak the roots of the seedlings in a prepared gel to cover the whole surface of the roots thoroughly, giving the mycorrhiza propagules opportunity to make close contact with fine roots (Photo 10).


11

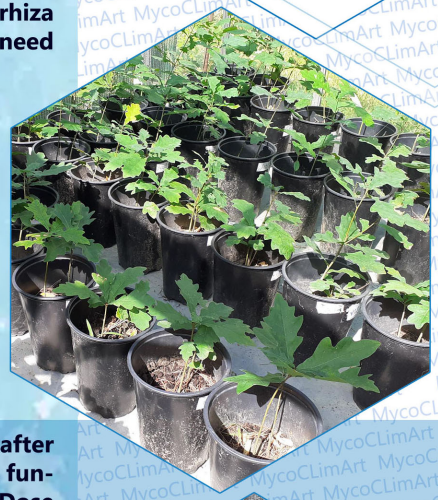
Place the inoculated seedling in a pot, in which the soil is filled to 1/3, and then lightly add the rest of the soil until the pot is completely full and the root of the plant is completely covered with soil (Photo 11).

PHASE 4.
CULTIVATION OF THE MYCORRHIZED SEEDLINGS

MYCOCLIMArt

Inoculating seedlings with mycorrhiza does not mean that they no longer need our help!

Do not forget that the main purpose of the seedling's mycorrhization is to improve their overall growth and health status, as well as to make them less vulnerable to abiotic and biotic stress factors. Inoculated seedlings should be cultivated regularly, applying all the measures commonly applied in the nursery production of the seedlings of *Quercus robur*.



Important! For the first 3 weeks after application, avoid the use of systemic fungicides as they may harm mycorrhiza. Dose chemical fertilizers carefully.

Want to know how mycorrhiza modulate physiological and biochemical responses of *Quercus robur* L. seedlings infected by powdery mildew disease?

Check the following reference:

Kebert, M., Kostić, S., Zlatković, M., Stojnić, S., Čapelja, E., Zorić, M., Kiprovski, B., Budakov, D., Orlović, S. (2022). Ectomycorrhizal fungi modulate biochemical response against powdery mildew disease in *Quercus robur* L. Forests 13(9): 1491.

Within the project, a new piece of equipment, a high-performance liquid chromatograph (HPLC), coupled with PhotoDiodaArray (SPD-M20A-PDA) and fluorometric (RF-20AXS) detectors, have been purchased.

MYCOCLIMArt research team

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