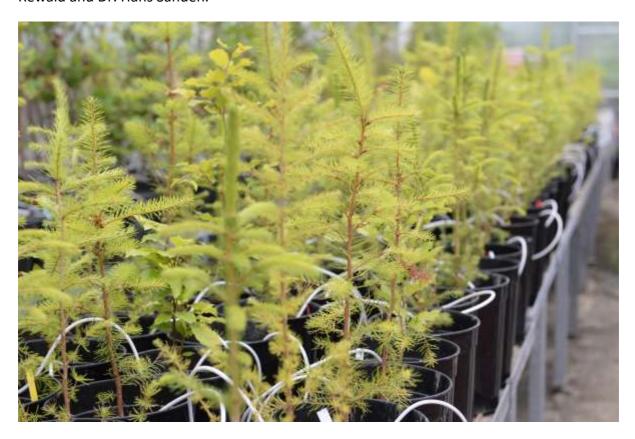
**Application of Polyter in forestation**: performance test of Polyter GR on four tree species under simulated drought and its effect on root growth and mycorrhizae.

Green Legacy GmbH thanks the Institute of Forest Ecology (IFE) of the University of Natural Resources and Life Sciences of Vienna (BOKU), the Austrian Research Promotion Agency (FFG) and LIECO GmbH & Co KG for their vital support.

The work presented was part of a master thesis under the supervision of Ass Prof. Boris Rewald and Dr. Hans Sandén.



# **Background**

Green Legacy GmbH believes agricultural hydrogels/super absorbent polymers have enormous potential to address some of the key problems Austria's forestry and agriculture face. Market research and feedback from key prospective customers indicate great interest in this water saving products.

Polyter is unique in that it is the only commercially available agricultural hydrogel that is mostly organic. This makes it more biocompatible and environmentally friendly than conventional agricultural hydrogels; and its application to help newly planted tree seedlings in the forest to better survive droughts very appealing. Polyter is also the only super water absorber authorised by the Austrian Bundesamt für Ernährungssicherheit (BAES) without restrictions and can be applied to food crops.

### **Problem**

It is a key problem of forestry that newly planted tree seedlings do not survive the first or second summer because the root system being still small, makes the plants sensitive to drought events.

## **Proposed solution**

Polyter GR applied to the planting pit absorbs large amounts of water and any nutrients dissolved in it, that the plant can then draw on during a drought. Polyter also contains preloaded nutrients that are available for the plant to take as it requires right from day one. This continuous availability of nutrients on demand can improve plant growth, decreasing nutrient leaching at the same time.

#### **Tests**

As a BOKU research unit specialized on forest ecology with a strong focus on below-ground systems, IFE embarked on an objective evaluation of Polyter GR and its potential to improve seedling growth and root system development during drought.

The purpose of the tests was to gather scientific evidence on the performance of Polyter GR in practice, especially its effect on trees' roots system and mycorrhiza development; to provide an objective evaluation of Polyter and its potential to improve seedling growth and root system development during drought.

The study had the following specific objectives:

- to critically evaluate whether addition of Polyter GR to the planting pit affects plant growth during drought periods;
- to test the effect of Polyter GR on water absorption and fertilization;
- to examine the effect of Polyter GR on root system and mycorrhiza development and spatial distribution; and
- to compare the added value (innovation potential) of Polyter GR on plant growth visa-vis a conventional hydrogel available on the market (Stockosorb).

### Work

Practical work was carried out by two bachelor students and one master student as well as additional personnel for lab analysis, with additional help from Green Legacy GmbH personnel. Supervision of the work, control and evaluation of data was performed by Prof. Dr. Boris Rewald and PD Dr. Hans Sandén.

# The glasshouse test

Eco-physiological assessment: effect of Polyter on plant growth during drought, and its effect on root and mycorrhiza distribution

To test the effect of Polyter on plant performance, a pot experiment in a greenhouse was established.

Four silvicultural important species were selected: Beech (*Fagus sylvatica*), Douglas fir (*Pseudotsuga menziesii*), Larch (*Larix decidua*) and Norway spruce (*Picea abies*). Planting was done on Wednesday 19th April in BOKU's green house in Tulln, Austria.

Two-year old container seedlings, courtesy of LIECO, were planted in seven-liter pots, including the pre-existing peat soil plug to mimic natural transplanting conditions. The pots

were filled with a mixture of 75% B-horizon soil, 25% sand and, in two thirds of the pots, 2.5g of either one of the two polymers (Polyter and Stockosorb) was added to the planting pit.



This resulted in three different combinations of soil and polymers:

- 1) B-horizon and sand (Control group C).
- 2) B-horizon and sand + 2.5 g Stockosorb© (Stockosorb group S).
- 3) B-horizon and sand + 2.5 g Polyter (Polyter group P).

The polymers were added right under the rootstock/peat plug, to simulate the simplest method of application in the field.



Assuming a weight of 1.2 kg per liter substrate and a pot filling of 6 liter, the weight of earth per pot was approximately 7.2 kg. An addition of 2.5 g polymer per pot equals ca. 0.035%. Each of the 3 combinations was replicated 24 times per species. To prevent stand- influenced results, the species and treatments were arranged in a randomized block design. The pots were connected to an automatic irrigation system, which supplied sufficient water for growth during the plant's establishment phase. 0.5 litres were provided every second day in the morning.

Starting in June, half of the pots per species and treatment were exposed to a series of four drought periods followed by recovering time. The first event took place from 23.6 to 8.7.2017, the second from 18 to 24.7.2017, the third from 29.7 to 5.8.2017, and the last from 16 to 23.8.2017. The duration of droughts was made dependent on the stress-level of the plants.

Loss of turgor in the beech leaves and the decrease in CO2 uptake measured with a closed chamber technic and a EGM CO2-IRGA analyzer were used as indicator of severe water stress.

Overview experimental set-up: 4 species x 3 polymer treatments x 2 water levels x 12 replicates = 288 pots + 24 control pots without trees = 312 pots.

#### Harvest

In mid-September, the plants were harvested. The shoots were cut right above the soil surface, sampled into paper bags, and dried to constant weight (at 70°C). Subsequently, branches and leaves were separated manually. The newly developed root systems were harvested, using a method developed exclusively for this experiment. First, the core of the pot, with a diameter of eight centimetres, was extracted utilizing a sharpened PVC-tube and placed on the washing table.

The core was split in two sections: the "plug", corresponding to the original rootstock and some surrounding soil, and the "polymer zone", corresponding to the area where the polymers were located. The "outside" part was the remaining part of the pot. The roots were collected separately for each section of the pot, by rinsing the soil through a sieve with a mesh size of 2 mm.



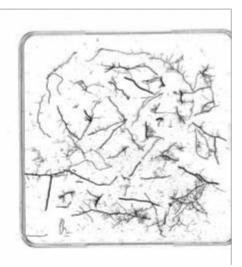
The roots were stored in water-filled plastic bags in the fridge until processing. Mycorrhiza ingrowth bags were collected and stored at 4°C until further evaluation in a local fridge. Every evening the samples were brought to the lab in Vienna.



In the laboratory, the roots from the polymer zone were analysed for ectomycorrhiza root tip abundance (degree of mycorrhysation).

Subsequently, the roots from the polyter and the outer zones were scanned to asses the morphology, architecture and root surface area/length of newly established fine roots during the growing season.





The biomass above (wood and leaves separately) and below ground (polymer zone and outer zone) was measured (dry weight; accuracy: +-0.01g for shoots, 0.001g for roots). The leaves were milled and analysed for nutrients concentrations and the soil was analysed for nutrient content using acid digestion followed by ICP, and C and N analysis followed grinding and homogenisation. The data was statistical analysed using SPSS and R.

# Results

The results show that the experiment setup worked, as there was a significant negative effect on tree growth by the drought treatments.

### Above-ground biomass

The addition of polymers to the planting pit did not have a measurable effect on the development of above-ground biomass as compared to the control group, due to the large variation in tree size at the start of the experiment. Potential minor differences/trends in growth could not be confirmed by strict statistical analysis.

# Root development

Below ground, the applied detailed sampling method of separating the root plug from new grown roots served as a good criteria to measure plant growth. Similar to above-ground biomass, the simulated drought spells had a negative effect on root system establishment measurable in root biomass, length and root surface area.

The effect of drought was strongest in beech and Douglas fir. The effect of both polymers was visible in that significantly more roots were allocated to the polymer zone as compared to the outer zone. Because the outer zone did not have less roots, as compared with control, it must be concluded that the application of hydrogels/polymers does not negatively affect

the root system development. Instead of resulting in unsustainable accumulation of roots in the polymer zone as feared, it triggered the establishment of additional fine roots in and around the hydrogels. The nutrient content tended to be increased by both polymers, but only Polyter GR released nutrients to the surrounding solution when measured with resin bags. The advantage of Polyter GR compared to Stockosorb© can be confirmed, as the enhanced root growth mentioned above was more significant in trees treated with Polyter GR. The biomass increase in the polymer zone was mainly caused by thicker roots as evidenced by the lower specific root length (SRL; cm root/g root). It has been suggested that roots with a lower SRL feature a greater desiccation tolerance compared to thinner/less dense fine roots, additionally underlining the potential benefits of the induced morphological changes for tree seedling resilience to drought events.

Importantly, no negative effects on mycorrhiza colonization by either polymer was found, but rather a positive effect, significant in spruce seedlings. The mycorrhiza ingrowth bags showed no difference in the production of external mycelia. This indicated that the trees' nutrient uptake capacity via mycorrhizal symbionts is not hampered by using hydrogels.



Spruce root tip with Polyter shows mycorrhizae development

# **Ongoing tests**

Having ruled out any negative effects on plants and ectomycorrhizal establishment, and seen that Polyter GR has potential to increase survival in dry periods, a mortality test on tree seedlings was established to supplement the knowledge acquired.

A large mortality test supervised by the same IFE/BOKU staff is currently under way, results are expected in September 2019. The tests will determine the effect of adding Polyter GR to the planting pit on the length of time a tree can survive in a severe draught, and its longer-term effect on growth over 5 to 7 years. The tests are done both in pots in glasshouse (Tulln, Lower Austria), to be able to control watering, and on the field (Litschau, Waldviertel), to observe performance in real-life conditions.

The glasshouse study will run over a two-year period, and will involve trees planted in year 1 and year 2 of the test, to examine the effect of Polyter in the first and in the second year after application. Results are expected in October 2019.

The field tests currently underway will run over a 5 to 7-year period, to examine Polyter's longer term effect on tree development. Trunk diameter and height will be monitored and recorded to be able to calculate change in biomass. The first results are expected in Spring 2019.





