SEVENTH FRAMEWORK PROGRAMME

THEME [ENV.2012.6.3-1]

Innovative resource efficient technologies, processes and services

DUTH-WP3-D3.2-Intermediate report on growth tests

Project Acronym: **ZEPHYR**

Project Coordinator: **TUSCIA UNIVERSITY**

Proposal full title: **Zero-impact innovative technology in forest plant production**

Grant Agreement n°: **308313**

Document Title: **Intermediate Report for Partner no. 1 - UNITUS**

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Summary: **This document provides data referring to growth test conducted by UNITUS, DUTH, DU, and Uninsubria during the first 18 months of work.**

Status: Final

Distribution: **All Partners**

Document ID: **DUTH-WP3-D3.2-Intermediate report on growth tests**

Date: **31 March 2014**

Project start: 1st October 2012 **Duration: 36 Months**

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Executive Summary

The intermediate report of this project for WP3 presents the progresses done by the Partners in regard of the development of specific protocols to be used in new growth chamber equipped with: LED lamps, wireless sensors, and a robotic device. At the same time a description is presented about a number of experiments conducted for the selection of the best morphological, physiological, and biochemical parameters which could become indicative markers of plant growth rate and performance during the pre-cultivation time following seed germination. Furthermore, new tests have been conducted by all partners involved in WP3 in order to obtain a biological validation of the new growth protocols suggested for use in the growth chamber prototype.

In regard of the plant species to be tested in Zephyr project, this intermediate report shows that all 15 most important plant species indicated in the DOW for their importance for the European vegetation have been investigated. At the same extent, also all 8 additional plant species have been investigated with the addition of some more species which are still under investigation.

The results achieved by all the partners during the past 18 months are presented below separately in different chapters and such a scheme has been preferred to enable a more complete description of the work done within each single laboratory. Despite the fact that the work done by each partner up to date is presented separately, nevertheless the reader can realize that the objectives selected in each task of WP3 have been fully achieved or they are in a good advancement status in a way to be finished in time for the final report. In particular, on the basis of the present report is possible to conclude that:

- Task 3.1 which deals with “Definition of new growth protocols for cultural practices” (Task Leader DU) has been already completed as described in D3.1;

- Task 3.2 which deals with “Implementation of the new growth protocols” (Task leader DU) has been successfully performed and has produced optimal indications for responding to possible problems raising during plant growth. In particular, solution have been found to problems due to: a) need for seed pre-treatment; b) need for breaking seed dormancy; c) need for seedling acclimation and hardening after the pre-cultivation time passed in the growth chamber. It has been conducted an extensive comparative evaluation between several traditional growth protocols in order to select those which could be better adapted to the new overall condition characterizing the innovative growth chamber. The most important results emerging from this task indicate a series of possible protocols which enable to solving all possible problems raising from this new pre-cultivation method.

- Task 3.3 which deals with “Growth tests performed in normal growth chambers with LED lamps” (Task leader Unitus) has achieved an advanced status after that in each laboratory the setting with the new LED lamps has been realized. In fact the results presented by each partners in regard of pre-cultivation period with LED lamps indicate a good efficiency of this new micro-environmental growth condition which enable a good growth rate which could be even better than the one present in the control growing under normal fluorescent illumination. The problems related to the patchy LED light distribution on the trays observed by some partner will be resolved in the final growth chamber prototype for the reason that the trays will be rotated and all the seedlings will receive an equal duration

and intensity of light. Also the growth substrate for the seedlings has been subjected to an intense series of experiments which have demonstrated that several type of substrate could be used with the same degree of success. Furthermore, it has been shown that possible differences in water retention due to specific growth substrate could be eliminated by adjusting the time of watering. In this case the problems with the time and intensity of watering will be completely solved by the interaction between the loss of water from the trays and the signaling produced by the soil sensors. In this way, the data obtained indicate a very useful interaction between growth condition and sensor efficiency which will enable to enlarge the potential use of this autonomous growth chamber system.

- Task 3.4 which deals with " Growth test with the new sensors" (Task Leader Uninsubria) has started to investigate the efficiency of the new sensors. In this regard the optical sensor has achieved a more advanced status of development in respect to the soil sensor. In fact a number of experiments have been done with the optical system which have given the possibility to interact with the partners who have developed this sensor and to produce step by step several improvements in the software needed to use this sensor. The difficulties of detecting growth rate presented by broad-leaved species for the reciprocal shading effect have been eliminated by means of adjustments potential included in the software setting options. In regard to the new soil sensor the experiments have started only during the last few days but the preliminary results achieved seem to indicate the possibility to achieve a good performance also for this new sensor. The importance of the results obtained by this task is related to the fact that the experiments done so far with the optical sensor developed show that it is possible to make a good estimation of seedling growth rate during the pre-cultivation period with a good degree of correlation with the estimation obtainable by the old destructive and time-consuming approaches. Furthermore, the indications emerging from these preliminary results of the new sensors let foresee that their estimation will be easily integrated in the overall control system operation in the pre-cultivation prototype growth chamber. In this way the sensors will enable a real-time evaluation of seedlings performance with intervals of few hours and this evaluation will be possible also when the operator is distant from the location when the growth chamber prototype has been placed.

1. Input of UNITUS

1.1. Introduction

Review of existing growth protocols using conventional light and proposal of new protocols for study species included in Zephyr, in order to obtain the best indoor and outdoor growth performance

1.2. Materials and methods

Two trolleys made of 3 shelves (see picture below) have been used by UNITUS as indoor facilities, in order to carry out the experiments on the selected species. These were located into a room climatically controlled with a temperature of 24 °C and monitored by a datalogger which measures also the relative humidity of the air.

HerkuPlast QPD 104 VW trays were employed, with 4 trays per shelf (12 trays per trolley). Each trolley was equipped with three different lamps to test growth performance of the species according to the aims of the project. The full list of lamps is provided below:
OSRAM L36W/77 FLUORA (Fluorescent)
Valoya AP67 (bar, 2 lamps, length 120cm)
Valoya AP67 (tube, 4 lamps, length 120cm)
Valoya AP673L (bar, 2 lamps, length 120cm)
Valoya G2 (bar, 2 lamps, length 120cm)

Valoya NS1 (bar, 2 lamps, length 120cm)

The soil substrate chosen in all the tests was the VECO3 Preforma Plug-OB, composed by Jiffy soil and cocopeat-sphagnum peat.

1.2.1. Zephyr project soilless substrate

Physical and hydraulic properties of Jiffy Preforma substrate

Results Physical research

Hoek van Holland 29.07.2010

Data on analyzed product:

Company name :	Jiffy Products International BV
Type of product:	**VECO3 Preforma plug - OB**
Remarks:	PR-38655, 90%
Start date:	16.07.2010
Analysis nr. :	

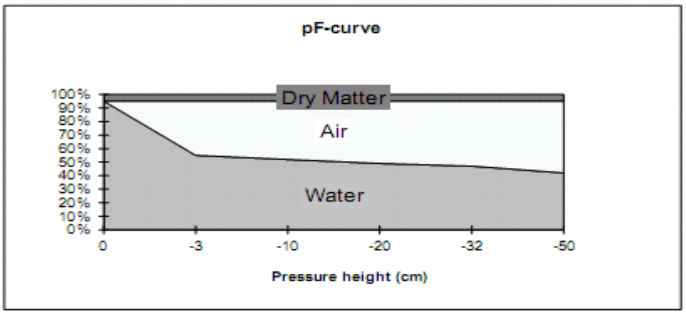
Analysis Results:		Composition
Moisture:	84 %	Cocopeat
Organic matter:	93 %	Peat
Bulkdensity:	82 kg/m³	
Shrinkage:	36 %	
Pore volume:	95 %	
EAW:*	10 %	

*Easy Available Water

Data at different pressure heights:

	Pressure height in centimeters				
	-3	**-10**	-20	-32	-50
Volume % water	55	**52**	49	47	42
Volume % air	40	**43**	46	48	53
Waterquantity in OM*		**6,8**			

*Waterquantity of organic matter in g/g OM



- Preforma is a peat based propagation material designed to work with both seed and cutting based crops.
- Preforma trays come pre-moistened. It's recommended that growers water the trays ahead of use so that moisture level in the micro-zone around the seed or cutting is optimal. It's suggested to water Preforma when the top of the media dries and lightens in color. Water quality is important to the crop. It's better to avoid watering with high chlorine levels or with water with extremely high bi-carbonate levels.
- Preforma is a sterile media. Rooting stimulators or hormones may be used, depending on grower preference. Preforma is pH balanced and it's recommended that the pH be kept in the range between 6 and 7.
- Preforma trays come shipped with a nutrient charge so it will not need any additional nutrients during propagation. (information provided by the manufacturer)

Our tests with Preforma substrate

Analysis of water retention properties of soil, under LED or FLUO lamps, without growing plants

In absence of growing plants, daily loss of water (corresponding to fresh weight loss) of soil is caused only by evaporation.
So, in order to value daily evaporation of soil plugs exposed to LED/fluorescent lamps in growth chamber, fresh weight of 12 saturated (~80% of moisture) Jiffy's soil plugs (27 cc) have been recorded at time 0 and after 3-5-6-7-10 days.

Growth chamber parameters:

Lighting	*LED lamps*	*Fluorescent lamps*
Air relative humidity (RH)	~70%	~70%
Temperature	22°C	22°C
Aeration	Independent aeration system (based on fans) for each shelf	Common aeration system (based on fans) for all the shelves

Test Results:

Table 1: Fresh weight variation

days of observation	*average fresh weight of plugs under fluorescent lamp (g)*	*average fresh weight of plugs under LED lamps (g)*
1	15,119	15
3	10,5	10,34
5	6,85	6,04
6	6,028	5,011
7	5,12	4,26
10	3,351	3,693

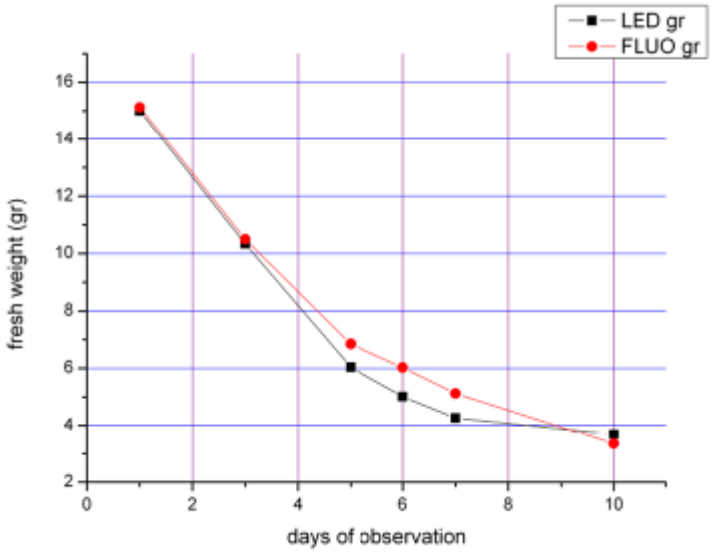
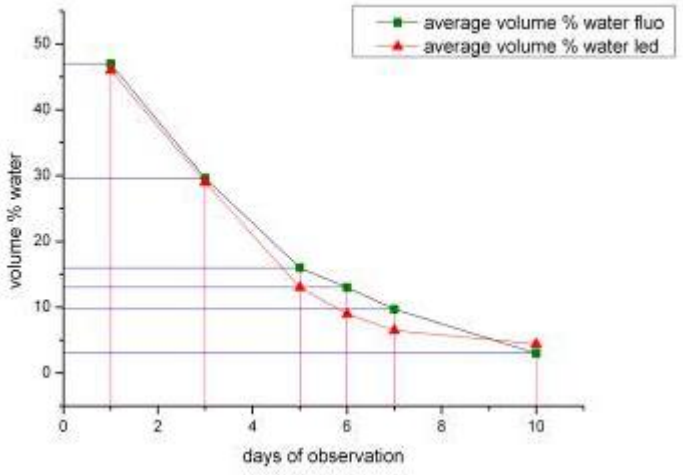


Table 2: Average volume % water variation

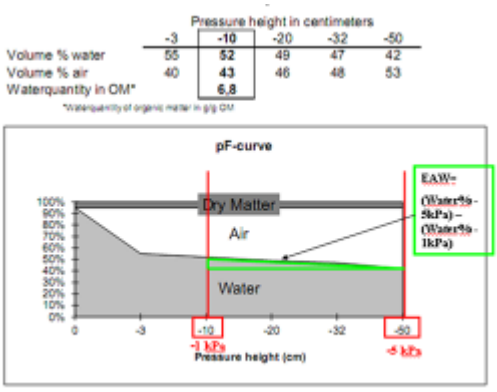
days of observation	*Average content of water (cc) – volume % - Fluorescent lamp*	*Average content of water - volume % - (cc) – LED lamp*
1	12,62 – 47%	12,5 – 46 %
3	8 – 29,6 %	7,84 – 29 %
5	4,35 – 16 %	3,54 – 13 %
6	3,53 – 13 %	2,5 – 9 %
7	2,62 – 9,7 %	1,76 – 6,5 %
10	0,851 – 3 %	1,19 – 4,4 %

*average air dry weight of a plug (27cc): 2,5 g



Interpretation of data

According to Jiffy's pF curve, Preforma substrate shows a very low EAW.



At 10 cm pressure height, the substrate contains 52% of water while at 50 cm pressure height it contains 42% of water. The EAW for this material is 10% (52% - 42%). This means there is 100 ml of water available per liter pot volume (2,7mL of water per 27cc pot).
For a good distribution of moisture through out the pot, it is important that the water permeability is high. If it is high, the water will quickly be transported to all the parts of the pot and create a homogenous moisture content. The water permeability is high if the pot is wet, but as the it gets dryer the difference between the different potting soil become more clear.

Some materials are easy to re-wet (sand, pumice and coco grinds) others not (peat, as Jiffy's soil). The main reason is that peat hardly takes up water after it dried in. Also within the group of peat there are big differences. Most of the time light peat's are less sensitive then the dark peats.

According to above mentioned data:

- Starting with a seeded pot with almost 50% of volume water, we have observed a fast decrease in available water content in 2 days (EAW is ½ of *total available water*, whose range varies from ~ 50% to ~30% volume water).
- The fast decrease in moisture content lasts a few days, until plug reaches ½ of its initial fresh weight (~ after 5 days of free evaporation). Remaining water is strongly retained, probably thanks to the great amount of starch, and daily fresh weight change becomes lower (the starch polymer is useful to stabilize soil mixture and it's an effective water management aid in all aspects of the horticultural and grower market. The polymer will absorb over two hundred times its weight in tap water. When applied at the recommended application rates, the polymer can reduce plant waterings by 50%).
- To keep soil moisture constant in EAW range, avoiding saturation and dry suboptimal condition, an irrigation protocol based on small amounts of water added frequently to plugs is required (~2,7 ml of water per pot when soil moisture is about 40%).
- The faster loss in water showed by plugs exposed to LED lamps than those exposed to Fluorescent lamps, is only due to the presence of an independent aeration system (based on fans) for each shelf, which increases the speed of drying process, especially in upper layers of soil.

1.2.2. Watering management

According to physical and hydraulic properties of Preforma soilless substrate, it is assessed to use in Zephyr project the main watering systems usually used for hydroponic cultures:

- Drip system
- Subirrigation systems (Ebb and flow)

The problem of the best irrigation method

Hardwood plants are characterized by broad leaves. This is a great problem when using growing trays with a high density of plugs (trays used in Zephyr project measuring 31 x 53 cm and containing 104 plugs). As observed during different experiments carried out by Unitus working group (DAFNE Department), if seeds are sown in all the plugs, in a few weeks plants will develop broad leaves which will overlap with leaves of the same plant but also with those of neighboring plants (Fig. 10). This causes difficulties in watering plants with drip systems, because water falls only on upper leaves, so it would be better to use an ebb and flow system.

Fig. 10 Effects of light treatments on the expansion of *Fagus sylvatica* leaves

When the seedlings are small, water is predominately lost by soil evaporation, but once they are well developed and leaves completely cover the soil, evaporation rate decreases and transpiration becomes the main process.
So in the first period of seedling growth, it's necessary to oppose evaporation keeping upper layers of soil steadily wet. Using an ebb and flow system associated with spray irrigation systems which use mini-sprinklers or fine mist spray devices could be a good solution.
Even if Zephyr prototype would be pesticide-free, a robotized spray device, as that shown in figure 11, would be useful to deliver pesticides in case of emergency.

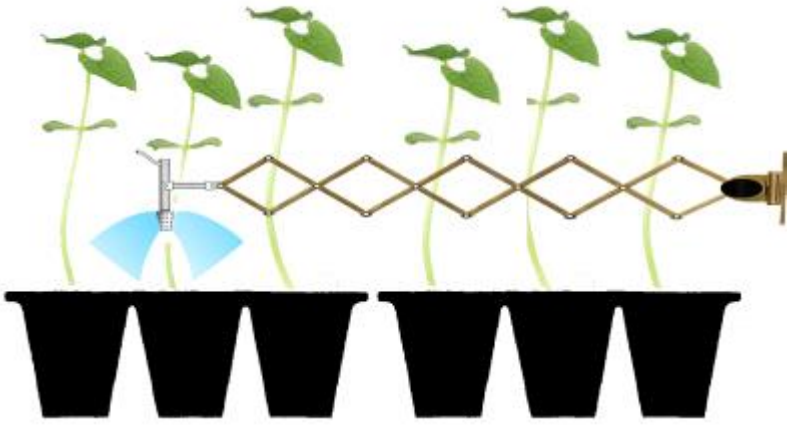
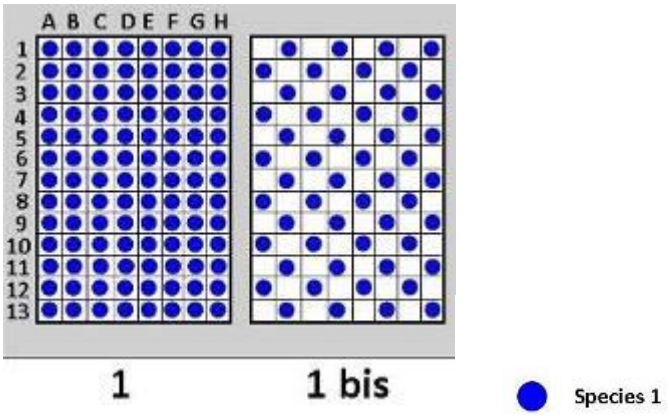


Fig. 11 Robotized spray device, able to irrigate above and below leaves

Upper leaves limit the amount of light available for lower leaves, causing reduction in their photosynthetic activity and growth.
This would be a problem for detecting above-ground seedlings parameters measured by optical camera as 'greeness', seedling height and biomass, fluorescence measurement.
In order to better understand the problem and try to solve it, in Zephyr project, the Unitus working group (DAFNE Department) tested each species in 2 trays, respectively containing 104 (Tray 1) and 52 seedlings (Tray 1 bis), as shown in the figure below.



1.2.3. Defining a watering protocol for Jiffy PREFORMA substrate .

A. Ebb and flow system: definition of the minimum flood time

Starting with a pot of Jiffy Preforma substrate stored for weeks at 4°C, characterized by an initial value of water volume content about 44,4%, we have measured the minimum time of flood needed by Jiffy soil to reach again the 52% (upper value of EAW range), using an ebb and flow system.
The following values are referred to a single pot of a QPD104VW tray (27 cc of soil/pot).
As you can see in the table below, the minimum time needed to reach 52% of water volume content is 60'' of flood; 90'' allow to reach 53%; 120'' allow to reach 54%. So, if we want to be sure to water enough the substrate, 120'' of flood would be good.
Under Valoya lamps a pot with a water volume content of 52%, reaches the lower value of EAW range (42%) in about 48 hours, but with an air conditioning system this time is smaller (24 hours).

Time of flood	Initial weight	Final weight	Water volume %
20''	11,55g (44,4%)	13,166g	51%
60''	11,55g	13,5g	52%
90''	11,55g	13,7g	53%
120''	11,55g	14,032g	54%
5 min	12,1g (46,7%)	14g	54%

Measurement of moisture content of soil (method chosen by Unitus group)

Main properties of Jiffy Preforma:
Bulk density: 0,082g/cc
Porosity: 95% of total volume
Dry matter: 5% of total volume

Type of tray: QPD 104 VW
Total volume: 27 cc
Pore volume: 95% of 27cc = 25,65 cc
Dry matter volume: 5% of 27 cc = 1,35 cc
Dry matter weight = Dry matter volume * bulk density= 1,35 cc * 0,082 g/cc= 0,11 g

Water volume % content (considering porosity) =

(WET WEIGHT – DRY MATTER WEIGHT) / PORE VOLUME

B. Water absorption rate of different substrates

In these first months of Zephyr project we have found some difficulties in defining a watering protocol for Jiffy Preforma soil, caused primarily by the fast hydration/dehydration of the soil. So we have decided to compare the hydraulic behaviour of Jiffy Preforma -when using an ebb and flood system- to 6 other substrates, commonly used in Italian nurseries (in particular, pozzolan and pumice are used also for the rooting of cuttings):

1. Jiffy Preforma soil
2. mixed substrate 1 (20:10:1; Brill substrate Type 3 Special*: perlite: fine river sand)

* http://www.brill-substrate.com/tray_substrates-121-133-131-2-147.html

3. 100% Perlite (http://www.perlite.it/)
4. 100% pozzolan
5. mixed substrate 2: ½ black peat ½ pozzolan
6. 100% fine river sand
7. 100% pumice

Using an ebb and flow system (1L of water/tray), the different substrates showed different water absorption rates (we have considered the time needed by the upper surface to become wet):

- only a few seconds (< 1 minute): JIFFY SOIL; SAND; PERLITE; POZZOLAN (fast and homogeneous absorption)
- 4 minutes: MIXED SUBSTRATE 1 (non homogeneous absorption)

No absorption: PUMICE and MIXED SUBSTRATE 2

1.2.4. Morphological and biochemical parameters

List of Parameters

Hereafter a list of parameters is presented to show the measures performed on each sample collected from the selected species in the project. These variables were chosen as the most representatives of the morphological and biochemical properties of each species:

Shoot length (SL)
Hypocotyl length (HL)
Primary root length (PRL)
Number of lateral roots (LR)
Total Root length (TRL)
Shoot fresh weight (SFW)
Shoot dry weight (SDW)
Root fresh weight (RFW)
Root dry weight (RDW)
Hypocotyl fresh weight (HFW)
Hypocotyl dry weight (HDW)
Number of leaves (NL)
Total Leaf Area (TLA)
Mean Leaf Area (TLA/NL)
Leaves fresh weight (LFW)
Leaves dry weight (LDW)
Growth curves (GC)
Stomata number (SN)
Relative Stomata density (SN/mm2)
Absolute Stomata density (SN/TLA)
Stomata dimensions (SD)
Leaf anatomy in transversal sections (LTS)
Photosynthetic capacity (PhotC)
Gas exchanges (GE)
Chlorophyll and carotenoid content (CCC)
Protein content (ProtC)
Rubisco (R)
Glutammine synthetase activity (GSA)
Lipid peroxidation level (LPL)
SPAD chlorophyll content (SCC)
Stomata checked by SEM (SSem)
CO_2 assimilation (CO_2)

Seedlings have been measured by selecting a specific number of samples on each tray. In details the used schema was the following:

Morphometry
#(104 measures on tray 1 and 2; 52 measures on tray 1bis and 2bis- non destructive tests)

- *Shoot length (SL)*
- *Hypocotyl length (HL)*
- *Number of leaves (NL)*
- *Mean Leaf Area (TLA/NL)*

#(26 measures on tray 1 and 2; 13 measures on tray 1bis and 2bis- non destructive tests)

- *Growth curves (GC)*

#(16 measures on tray 1 and 2; 8 measures on tray 1bis and 2bis, destructive tests)

- *Shoot length (SL)*
- *Hypocotyl length (HL)*
- *Primary root length (PRL)*
- *Number of lateral roots (LR)*
- *Total Root length (TRL)*
- *Shoot fresh weight (SFW)*
- *Shoot dry weight (SDW)*
- *Root fresh weight (RFW)*
- *Root dry weight (RDW)*
- *Htypocotyl fresh weight (HFW)*
- *Hypocotyl dry weight (HDW)*
- *Number of leaves (NL)*
- *Total Leaf Area (TLA)*
- *Mean Leaf Area (TLA/NL)*
- *Leaves fresh weight (LFW)*
- *Leaves dry weight (LDW)*

Microscopy
(5 measures on tray 1 and 2; 5 measures on tray 1bis and 2bis)

- *Stomata number (SN)*
- *Relative Stomata density (SN/mm2)*
- *Absolute Stomata density (SN/TLA)*
- *Stomata dimensions (SD)*
- *Leaf anatomy in transversal sections (LTS)*

Ecophisiology
(10 measures on tray 1 and 2; 5 measures on tray 1bis and 2bis)

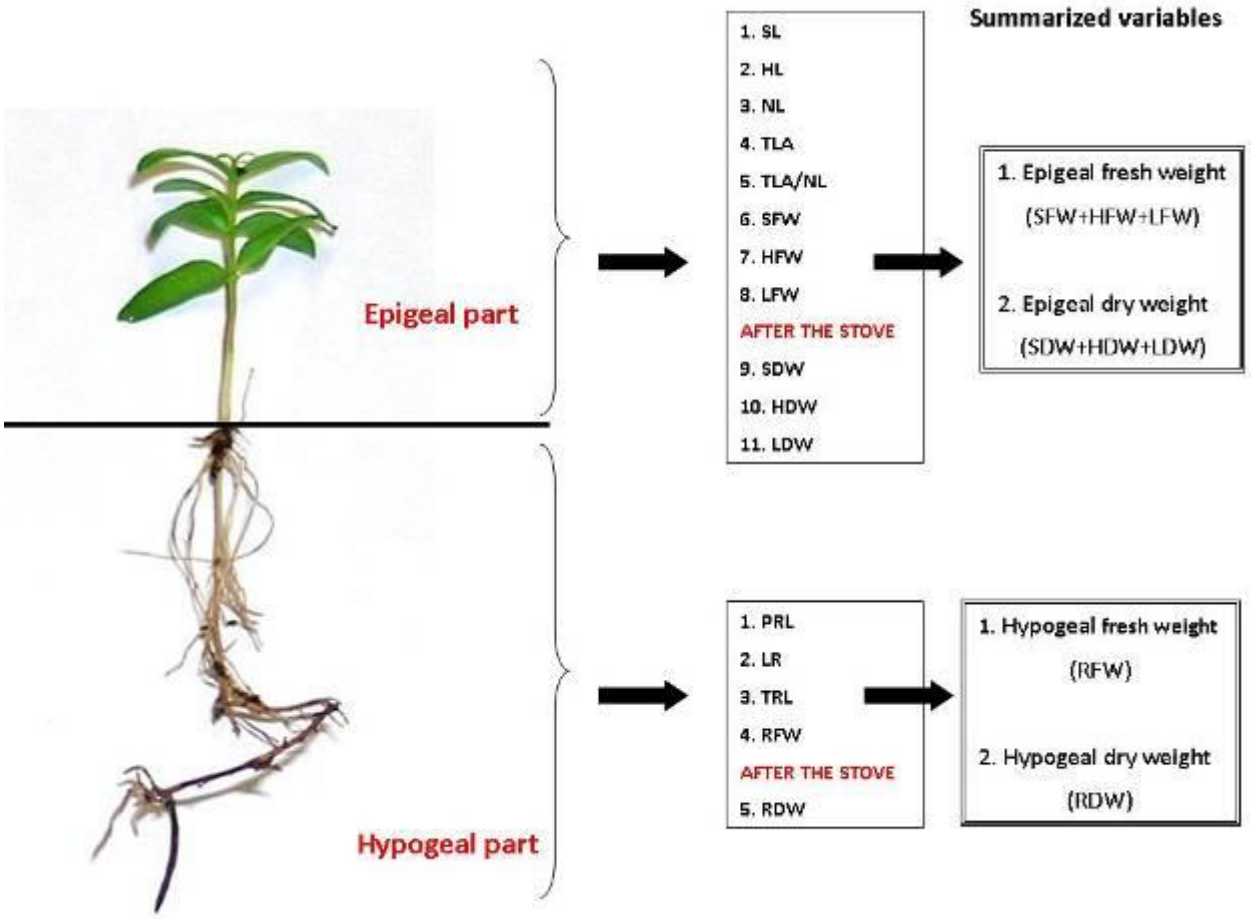
- *Photosynthetic capacity (PhotC)*
- *Gas exchange (GE)*

Biochemistry
(3 measures on tray 1 and 2; 3 measures on tray 1bis and 2bis)

- *Chlorophyll and carotenoid content (CCC)*
- *Protein content (ProtC)*
- *Rubisco (R)*
- *Glutammine synthetase activity (GSA)*
- *Lipid peroxidation level (LPL)*

Other

- *SPAD chlorophyll content (SCC)*
- *Stomata checked by SEM (SSem)*
- *CO_2 assimilation (CO_2)*



1.2.5. Biochemical methods

- *Biochemical analyses*

At the end of each experimental phase (30 days for beech, holm oak and cork oak and 52 days for common myrtle cultured in growth chambers and prototype; 90 days for holm oak grown in greenhouse and 120 days for beech planted in the field), some randomly selected seedlings per treatment were weighed and frozen in liquid nitrogen and submitted to the following assays.
Shoot tissue was used for the analysis of chlorophyll and carotenoids content, changes in extractable activities of key enzymes involved in N assimilation pathways (nitrate reductase and glutamine synthetase), and finally changes in lipid peroxidation level.

- *Enzyme extraction*

Frozen tissue (approximately 1 g FW) was ground to a fine powder in a pre-chilled mortar under liquid nitrogen. Cold extraction buffer, containing 50 mM HEPES-KOH (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 5 mM DTT, 1 mM PMSF and 1% (w/v) PVP, was added in a ratio of 1:7 (w/v). The brei was filtered through four layers of cheesecloth.
Samples of the homogenate (0.1 ml) were used to evaluate chlorophyll and β-carotene content, while the remaining amount was centrifuged at 1000 *g* for 5 min. at 2°C. The supernatant was recovered and then centrifuged at 30 000 *g* for 5 min at 2°C. The

supernatant was divided into 300 µl aliquots, which were then frozen in liquid nitrogen and stored at -80 °C until analysis

- *Determination of chlorophyll and carotenoids contents*

The ethanol was added to the homogenate (0.1 ml) to a final volume of 1.0 ml and centrifuged at 30 000 *g* for 5 min at 2°C. The absorbance of the samples was measured at 649 nm and 665 nm for chlorophyll measurement and at 470 nm for carotenoids measurement.
The values were calculated using the following expressions:

Chlorophyll a content (Chla) $(13.7 \times A_{665}) - (5.76 \times A_{649})$
Chlorophyll b content (Chlb) $(25.8 \times A_{649}) - (7.6 \times A_{665})$
Total Chlorophyll content $(6.1 \times A_{665}) + (20.04 \times A_{649})$
Carotenoids content $\frac{(1000 \times A_{470}) - (2.13 \times Chla) - (97.64 \times Chlb)}{209}$

Both chlorophyll and carotenoids content was expressed as mg g^{-1} fresh weight.

- *Determination of protein content*

Protein content was determined according to Bradford (1976) using BSA as standard.
The sample and distilled water were pipetted into a tube to a final volume of 1.0 ml. Coomassie Reagent (1.0 ml) was added to each tube and the mixture was mixed. The absorbance of all samples was measured at 595 nm. A BSA standard curve was used to determine the protein concentration of each sample. The protein content was expressed as mg g^{-1} fresh weight.

- *Nitrate reductase (NR) and Glutamine synthetase (GS) activity*

Nitrogen is likely the most important nutrient for higher plants, being involved in the production of organic compounds of high biological value such as amino acids, proteins and nucleic acids. It is well known that photosynthetic activity is closely relate to nitrogen metabolism. Indeed, the incorporation of ammonia for amino acid synthesis requires the availability of carbon skeletons from the photosynthetic process.
Nitrate reductase catalyzes the reduction of nitrate to nitrite, while of glutamine synthetase catalyzes the formation of glutamine acid from glutamate, ammonia and ATP.
Nitrate Reductase assay was determined as described by Faleiros and Cazetta (1996). In particular, leaves were cut into discs with a diameter of 1.0 cm (300 mg), were incubated in 10.0 ml of a medium containing 200 mM KH_2PO_4-NaOH (pH 7.4), 60 mM KNO_3, 1% (v/v) Isopropanol and 0.1% (v/v) Triton X-100 and subjected to vacuum infiltration. The incubation was carried out in the dark at 30°C for 60 minutes. The NO_2^- produced by action of the NR enzyme was determined in an 500 µl aliquot of the incubation medium, which was added to 500 µl of 1% sulfanilamide in 3 M HCl and 500 µl 0.02% NED (N-(1-naphtyl)-ethylenediamine dihydrochloride). After 20 minutes, the solution was diluted to 3 ml with deionized water, and the absorbance (540 nm) was measured using a spectrophotometer. In order to calculate the amount of NO_2^- contained in the sample, a standard curve was prepared in the same way as the sample, but using aliquots of 500 µl of $NaNO_2$ standard solutions (containing from 0 to 200 µl 0.1 mM NO_2^-). The NR activity was expressed as µmol h^{-1} g^{-1} fresh weight.
Glutamine synthetase (GS; EC 6.3.1.2) activity was determined as described in Astolfi *et al*. (2004). Briefly, 0.2 ml of the reaction buffer (containing 250 mM Tris, 50 mM $MgSO_4$, 2.5 mM Na_2EDTA, 125 mM NaGlutammic, 5 mM DTT and adjusted to pH 7.8 with HCl), 50 µl of 750 mM $HONH_3Cl$ (hydroxylamine hydrochloride) and 750 mM KOH in 1:1 ratio, 0.11 ml distilled

water and 40 µl of 200 mM ATP were added to 0.1 ml of the enzyme extract and placed at 30° C for 30 min. After 30 min. the samples were cooled on ice and 0.7 ml of the solution, containing 0.67 mM $FeCl_3$, 0.37 mM HCl, 20% (w/v) TCA, was added to stop the reaction.
The extract was added to the blank sample and all samples were centrifuged at 30 000 *g* for 5 min. at 2° C. The absorbance of the samples was measured at 540 nm.
The GS activity was measured using a standard curve obtained with increasing amounts of γ-Glutamyl hydroxamate. The GS activity was expressed as µmol min^{-1} mg^{-1} protein.

- *Determination of lipid peroxidation level*

The level of lipid peroxidation was expressed as malondialdehyde (MDA) content and was determined as TBA reactive metabolites as described in Astolfi and Zuchi (2012).
Fresh tissue (approximately 0.5 g FW) was ground to a fine powder in a mortar under liquid nitrogen and then homogenized in 5.0 ml extraction buffer, containing 0.25% TBA (thiobarbituric acid) made in 10% TCA (trichloroacetic acid).
The homogenate was placed in test tubes (which were sealed to prevent spillage of material) and placed in a preheated thermoblock at 95° C. After 30 minutes, the tubes were removed and quickly cooled on ice. The homogenate was filtered through two layers of cheesecloth and centrifuged at 10 000 g for 10 min at 2° C.
The absorbance of the supernatant was measured at 532 nm and the correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm.
The level of lipid peroxidation was expressed as nmol MDA g^{-1} fresh weight by using an extinction coefficient of 155 mM cm^{-1}.

1.2.6. Data treatment

Collected data can be managed in different ways in order to provide information about species' response to different light treatments. Generally, we start with simple descriptive statistics, computed for each measured variable. These include mean, minimum, maximum, quartiles, standard deviation and standard error, in addition to data distribution (skeweness or Kurtosis asymmetry). Graphic outputs may include histograms with error bars, box-plot, or frequency class histograms with density curve (to represent the distribution pattern).
Further statistic investigations would include comparisons between two treatments or among all treatments with the aim of investigating the more performing treatment and provide even information about the most important variables affecting differences in plant response. For these purposes, mean comparisons (t-Student test, Komolgorov-Smirnov test, Mann-Whitney U-test, etc), Analysis of variance by ANOVA one-way, Multivariate ANOVA (MANOVA), Principal Component Analysis (PCA), Categorical PCA, and Cluster Analysis (K-means or hierarchical) would be taken into account and used when or if appropriate.
Many software are able to perform simple descriptive statistics, while someone more specialized is devoted to multivariate statistic investigation. Here we can use the following freeware or shareware programs according to their availability:

1. Microsoft Excel with XLSTAT package installed. The commonly used software for descriptive statistic with the addition of a dedicated plug-in for multivariate analyses (You can downloaded from http://www.xlstat.com/en/ as a trial expiring after 30 days, then it requires a licence code).
2. R project for statistical computing, commonly named R. R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. R provides a wide variety of statistical (linear and nonlinear modelling, classical statistical tests, time-series analysis, classification, clustering, ...) and graphical techniques, and is highly extensible.

3. S-Plus. S-PLUS is a commercial implementation of the S programming language sold by TIBCO Software Inc. It features object-oriented programming capabilities and advanced analytical algorithms. It is the commercial version of R, differing by the first in some script syntax, but compatible with it.
4. SPSS Statistics. SPSS is among the most widely used commercial programs for statistical analysis in social science. It is used by market researchers, health researchers, survey companies, government, education researchers, marketing organizations and others. It is similar to Excel in terms of appearance, but differs substantially for the application performance and options. Indeed, it performs Descriptive statistics (Cross tabulation, Frequencies, Descriptives, Explore, Descriptive Ratio Statistics); Bivariate statistics (Means, t-test, ANOVA, Correlation (bivariate, partial, distances), Nonparametric tests); Prediction for numerical outcomes (Linear regression); Prediction for identifying groups (Factor analysis, cluster analysis (two-step, K-means, hierarchical), Discriminant.
The many features of SPSS are accessible via pull-down menus or can be programmed with a proprietary 4GL command syntax language. Command syntax programming has the benefits of reproducibility, simplifying repetitive tasks, and handling complex data manipulations and analyses. Additionally, some complex applications can only be programmed in syntax and are not accessible through the menu structure. The pull-down menu interface also generates command syntax; this can be displayed in the output, although the default settings have to be changed to make the syntax visible to the user.
5. SYSTAT Software. SYSTAT is another commercial software with the same performance as SPSS. It has a default organization for the menus and toolbars, based on similarity of features; The user interface of SYSTAT is organized into three spaces: Viewspace, Workspace and Commandspace. The graphic outputs are one the key points of SYSTAT, allowing to customize the results of all statistical computations.

1.2.7. Additional species

Taking advantage of the indoor and outdoor equipments, as well as the availability of seeds from extra-project sources, additional experiments have been carried out using two oak species (*Quercus suber*, evergreen, and *Quercus pubescens*, deciduous) and another Mediterranean maquis key species (*Myrtus communis*). Basically, such tests had the objective to implement the knowledge about light influence on a broader type of species belonging to Mediterranean ecosystems.

1.3. Results

Hereafter we present the results achieved during the first year project, divided by arguments and study species.

1.3.1. Proposal of new protocols under LEDs in the Zephyr project

Common Hazel (*Corylus avellana* L.)
1. Period of seed harvesting
- Second half of September (Cimini Mountains, Viterbo)

2. Seed storage
- Closed black plastic containers at +4 °C

3 Storage period
- Probably up to 1 year but still to be accurately defined due to the recalcitrant type of the seeds

4 Pretreatments

- Cold stratification in sterilized perlite medium at 4 °C for 4 months
- Gibberellic acid (GA_3) 15 mg/100 ml, under stirring condition for 18 h in darkness (test in progress)

5. Substrate

- Quickpot HerkuPlast QPD 104 VW with Pre-forma Jiffy Soil

6. Temperature

- 21 ± 1 °C

7. Light

- OSRAM L36W/77 FLUORA (Fluorescent)
- Valoya AP67 (bar, 2 lamps, length 120cm)
- Valoya AP67 (tube, 4 lamps, length 120cm)
- Valoya AP673L (bar, 2 lamps, length 120cm)
- Valoya G2 (bar, 2 lamps, length 120cm)
- Valoya NS1 (bar, 2 lamps, length 120cm)

8. Photoperiod

- 8 h/16 h (light/darkness)

9. Relative Humidity

- 70%

10. Watering

- Ebb and flow method: tap water, flood until substrate saturation (max 1 hour), three times a week.

11. Other recommendations

- Remove pericarp before the test.

Silver Fir (Abies alba Mill.)

1. Period of seed harvesting

- September up to mid-October in the Appennins

2. Seed storage

- Stored in glass jars at +4 °C

3. Storage period

- 2 years

4. Pretreatments

- Cold stratification in perlite for 1 month at 4°C

5. Substrate

- Quickpot HerkuPlast QPD 104 VW with Pre-forma Jiffy Soil

6. Temperature

-21 ± 1 °C

7. Light

- OSRAM L36W/77 FLUORA (Fluorescent)
- Valoya AP67 (bar, 2 lamps, length 120cm)
- Valoya AP67 (tube, 4 lamps, length 120cm)
- Valoya AP673L (bar, 2 lamps, length 120cm)
- Valoya G2 (bar, 2 lamps, length 120cm)
- Valoya NS1 (bar, 2 lamps, length 120cm)

8. Photoperiod

- 12 h/12 h (light/darkness)

9. Relative Humidity

- 70%

10. Watering

- Ebb and flow method: tap water, flood until substrate saturation (max 1 our), three times a week

11. Other recommendations

Pomegranate (Punica granatum L.)

1. Period of seed harvesting

- From mid-September to October

2. Seed storage

- Stored in glass jars at 4°C until cold stratification

3. Storage period

- Up to 1 month

4. Pretreatments

- Cold stratification in perlite for 40-60 days at 4°C

5. Substrate

- Quickpot HerkuPlast QPD 104 VW with Pre-forma Jiffy Soil

6. Temperature

- 22 °C

7. Light

- OSRAM L36W/77 FLUORA (Fluorescent)
- Valoya AP67 (bar, 2 lamps, length 120cm)
- Valoya AP67 (tube, 4 lamps, length 120cm)
- Valoya AP673L (bar, 2 lamps, length 120cm)
- Valoya G2 (bar, 2 lamps, length 120cm)
- Valoya NS1 (bar, 2 lamps, length 120cm)

8. Photoperiod

- 12 h/12 h (light/darkness)

9. Relative Humidity

- 70%

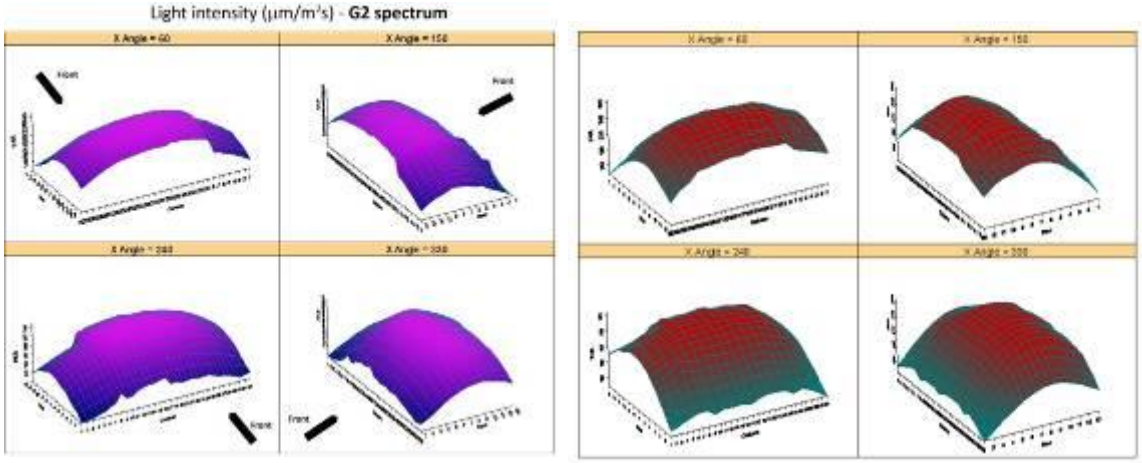
10. Watering

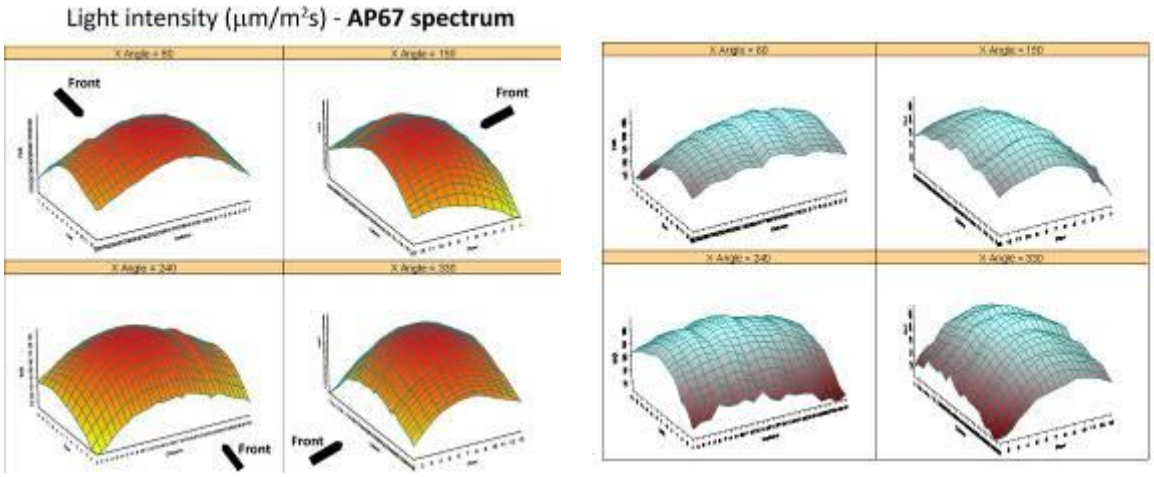
- Ebb and flow method: tap water, flood until substrate saturation (max 1 our), three times a week

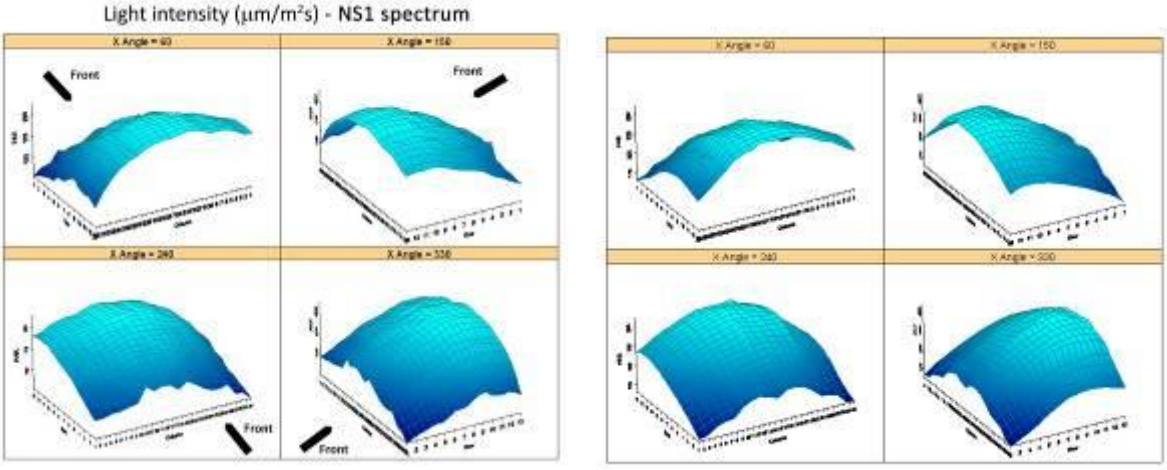
11. Other recommendations

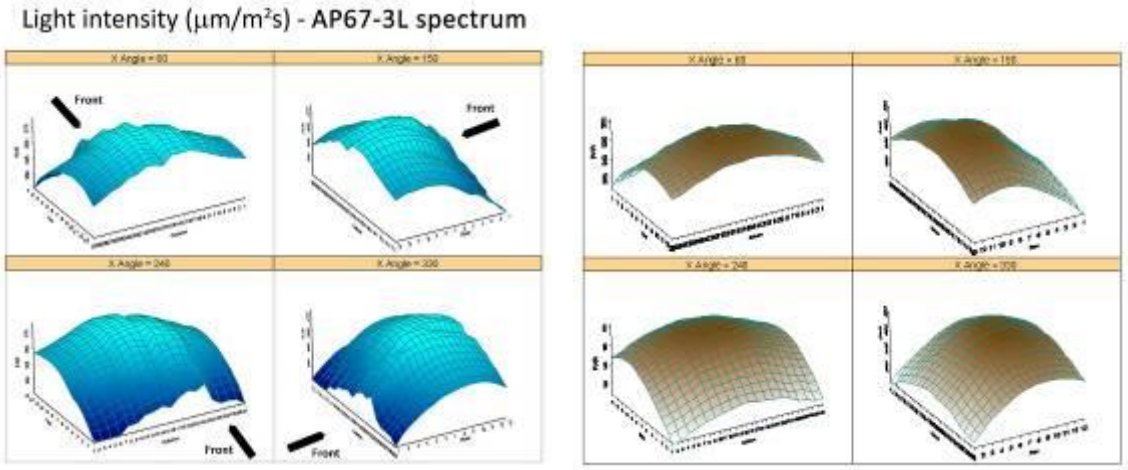
1.3.2. Performance of light intensity across space

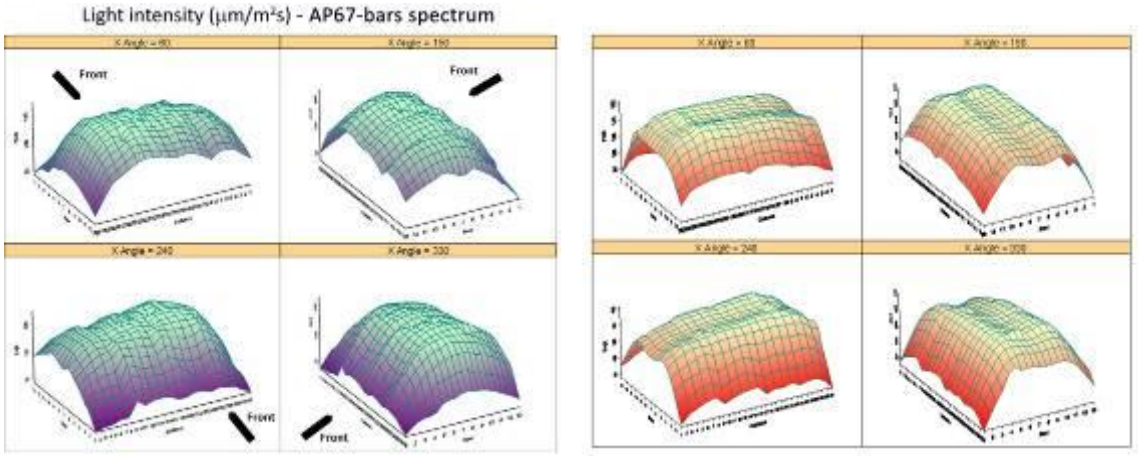
Results of LED light behaviours in space on the trolleys have been performed afterm measuring the light intensity per grid cell in the shelves, meaning grid cell with the relative position of each seedlings into the quickpots. Measurements at tray's level and 10 cm have been done. Summary results stated as light intensity tends to decrease in particular in those area in front of the operator, i.e. in the shelf's side not masked by white walls. Overall, a decrease is observed also in the other three side of the shelves, but with minor magnitude. This is partially due to light reflectance of white walls.
Although some slightly differences are pointed out among LED spectra, the overall feature is the one mentioned above.



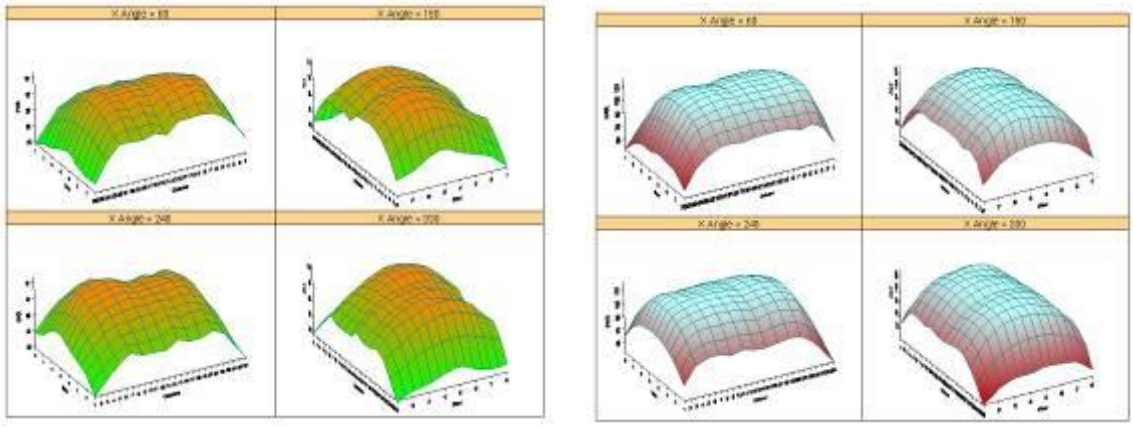








Cool white LED strips (additional experiment)



Lamp		Min	Position (col-row)	Max	Position (col-row)	Mean ± St. Dev.	Light intensity loss (%)
G2	**0**	117.4	1-1 (Front-left corner)	247.3	16-8	197.4 ± 32.3	52%
“ “	**10**	131.8	32-1 (Front-right corner)	274.7	16-9	224 ± 34	52%
AP67-Lamps	**0**	103.4	1-1 (Front-left corner)	198.9	17-8	166.8 ± 20.2	48%
“ ”	**10**	125	28-1	238	15-8	201.9 ± 24.3	47.5%
AP67-3L	**0**	172.3	3-1	289.6	15-9	249.1 ± 28	40%
“ “	**10**	173.2	3-1	325.6	17-8	272.5 ± 36.5	47%
NS1	**0**	127.6	32-1 (Front-right corner)	214.6	15-9	185.1 ± 20	40.5%
“ “	**10**	165.2	32-1 (Front-right corner)	261.6	15-10	224.8 ± 22.9	37%
AP67-Bars	**0**	70.9	1-1 (Front-left corner)	136.8	21-7	114.4 ± 16.6	48%
“ “	**10**	84.7	32-1 (Front-right corner)	168.5	23-7	140.2 ± 18.4	49%

Valoya LED lamps produce wide spectrum to the plants, from 400 nm to 800 nm. Many research papers studying LED lights have had light treatments with only blue and red combinations, occasionally with additional far-red, as narrow waveband peaks. Hence, the results of Valoya’s in-house experiments are not directly comparable to other LED studies, because the Valoya spectra are not constructed by combining narrow waveband peaks like many other LED’s available, instead Valoya LED lights have wide light spectra. As e.g. Kim et al. (2004) and Hogewoning et al. (2012) conclude, wide light spectrum can result to better plant growth responses.

Concerning the LED light features for plant growth, we can state as main conclusions:

- Light intensity distribution across space differs both longitudinally and latitudinally
- The patterns of such distributions are mainly affected by:

A. the kind of spectrum

B. the distance between lamps

C. the type of surfaces that enclosed a shelf (white panel, white wall, plexiglass, hollow surface, etc.)

D. the LED’s emission cones that partially overlap in some regions of the shelf

E. Other physical effects not considered in this study

- No significant differences in light distribution patterns were observed between tray’s level and 10 cm
- Maximum intensity loss occurs at the marginal areas of the shelf, in particular at the front side, while stable conditions were observed in the middle
- However, some spectra revealed to be more homogeneous across space than others (e.g. NS1, AP67, AP67-3L)

Possible noises that affect light intensity values on the shelves and corrective actions should be stated, e.g. dispersion by “edge effect”: it makes a bell-shaped distribution of the light intensity; white panels around the shelves: they reduce the light intensity loss by partially reflecting the light; complex pattern of overlapping light emission cones generate a non-homogeneous light surface, this is a critical aspect of LED fabrication. If lens are applied to the LED, this would be probably solve, or at least partially reduce the behaviours related to LEDs in plat growth application.

1.3.3. Vitality and germination tests

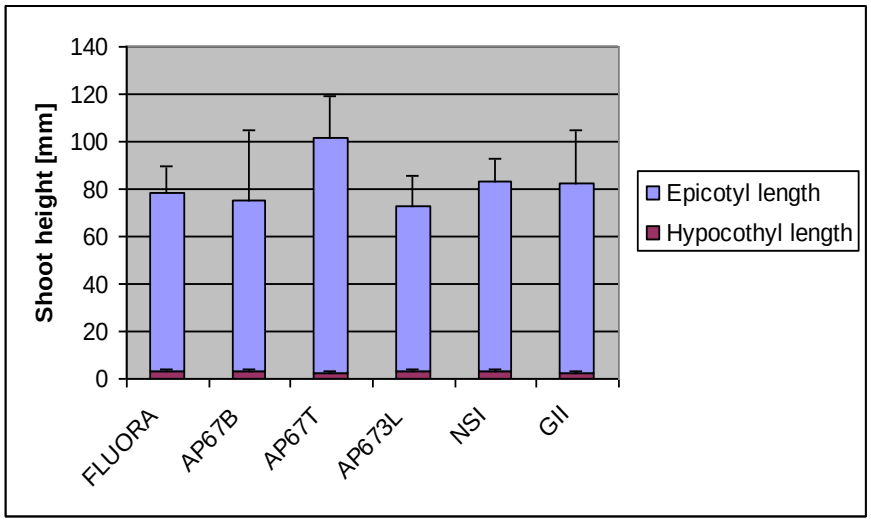
Among the 6 species cultivated in this first year of project, only 3 needed pre-tretaments in order to break dormancy through vernalization in moist agriperlite at 4°C : *P. granatum* (40-60 days), *C. avellana* (2-4 months) and *A. alba* (1 month). Seeds of the 3 species were treated just after their collection so that tetrazolium test, useful to check the residual vitality of seeds, was not needed, except for seeds of *A. alba* which were stored for months at 4°C before the usage. The tetrazolium test showed a vitality percentage equal to 91%.
For the other species (*Q. suber*, *Q. pubescens* and *M. communis*) fresh seeds were germinated in moist agriperlite at 22°C under G2 lamp, just after the collection without any pretreatment.

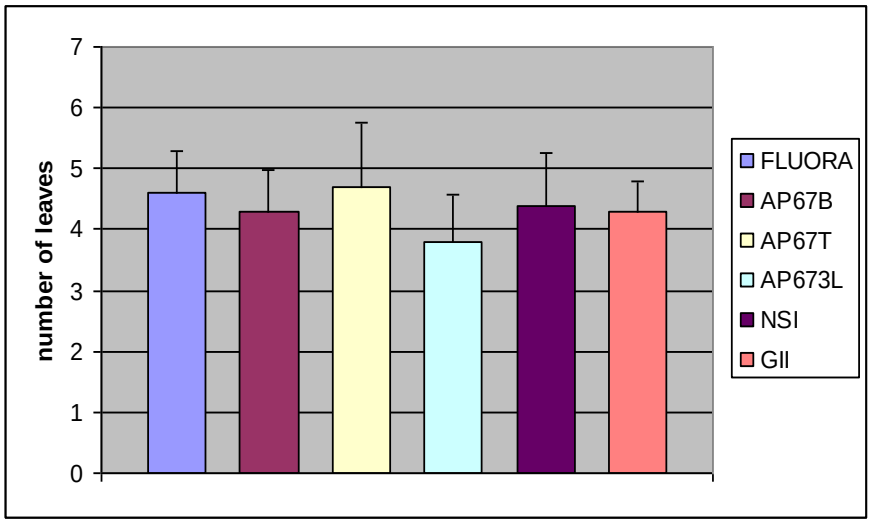
Four pictures showing the vitality tests of Abies alba seeds and Corylus avellana germination.

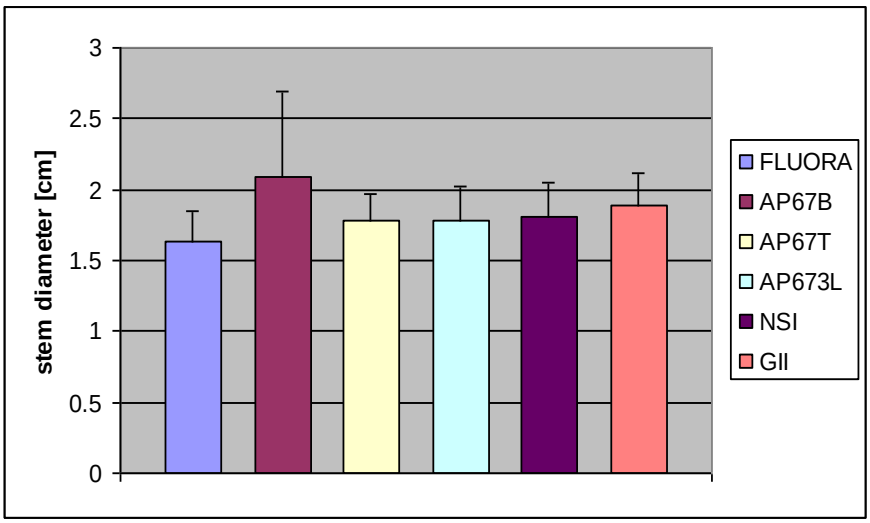
1.3.4. Morphological measures

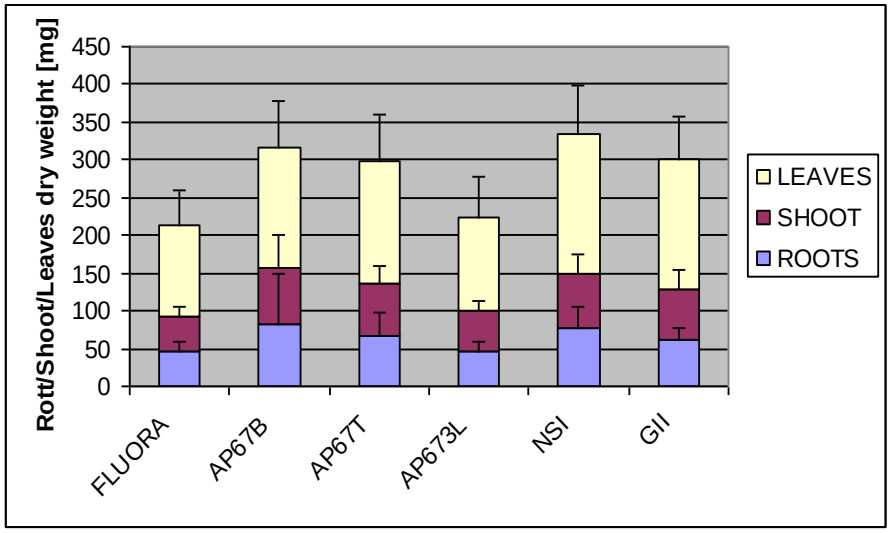
Results concerning the morphological analyses are hereafter showed separating the species and the variables. Histograms showing mean values with standard deviation related to each light source were made. Short comments are also provided to describe the achieved results.

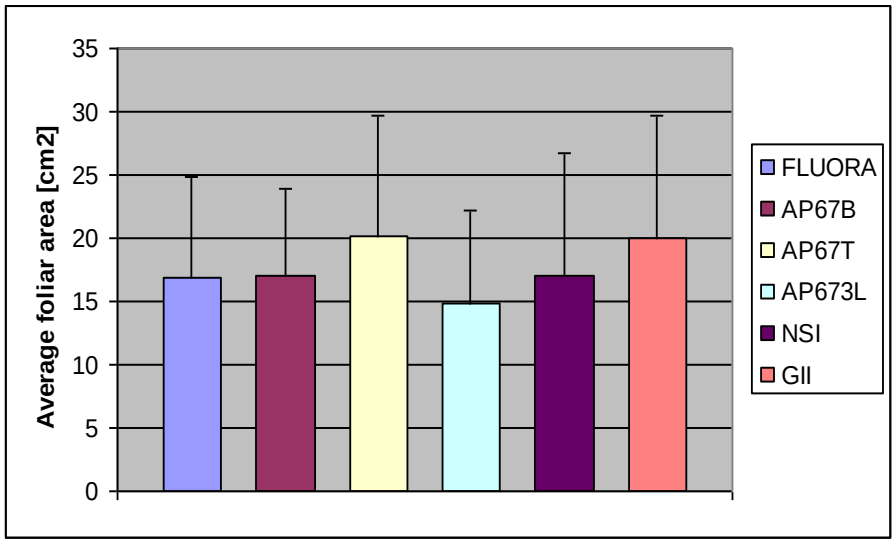
Corylus avellana

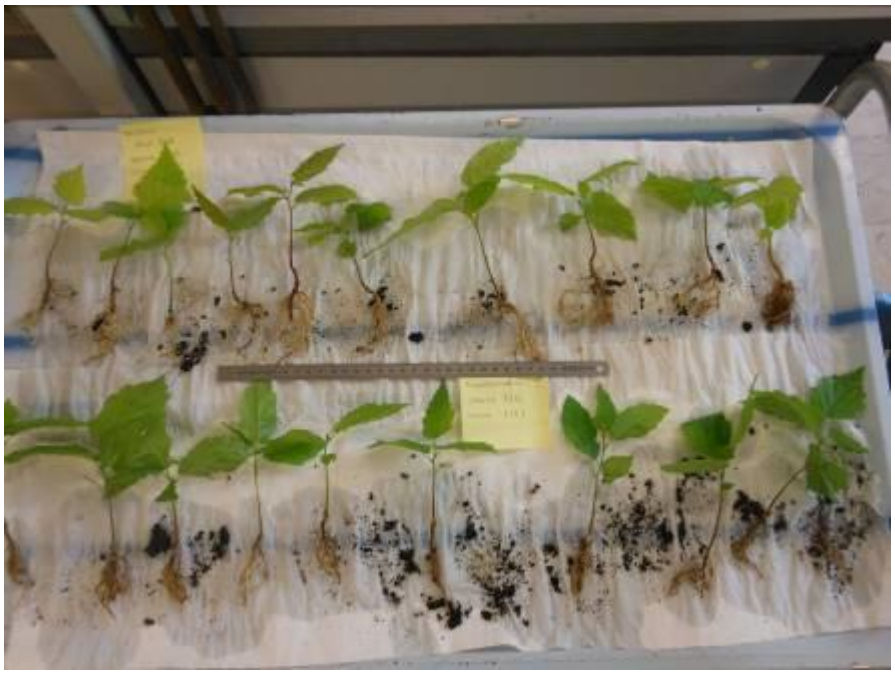







Rott/Shoot/Leaves dry weight [mg]
450
400
350
300
250
200
150
100
50
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII
LEAVES
SHOOT
ROOTS

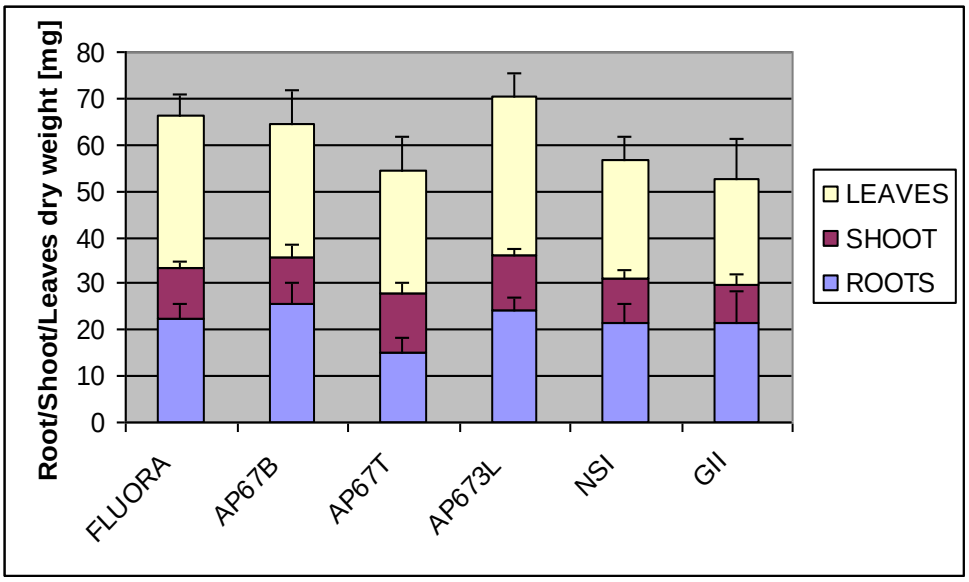

Average foliar area [cm2]
35
30
25
20
15
10
5
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII

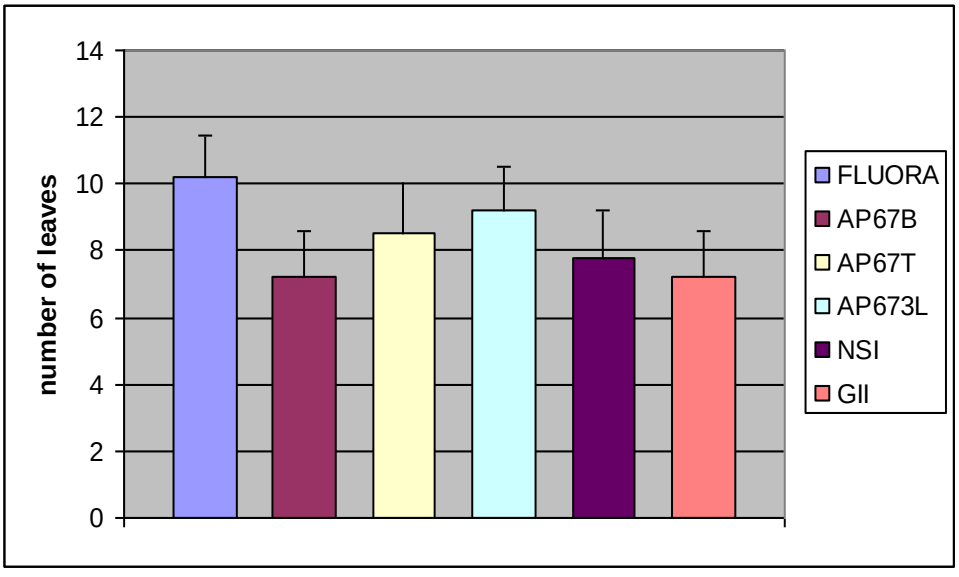


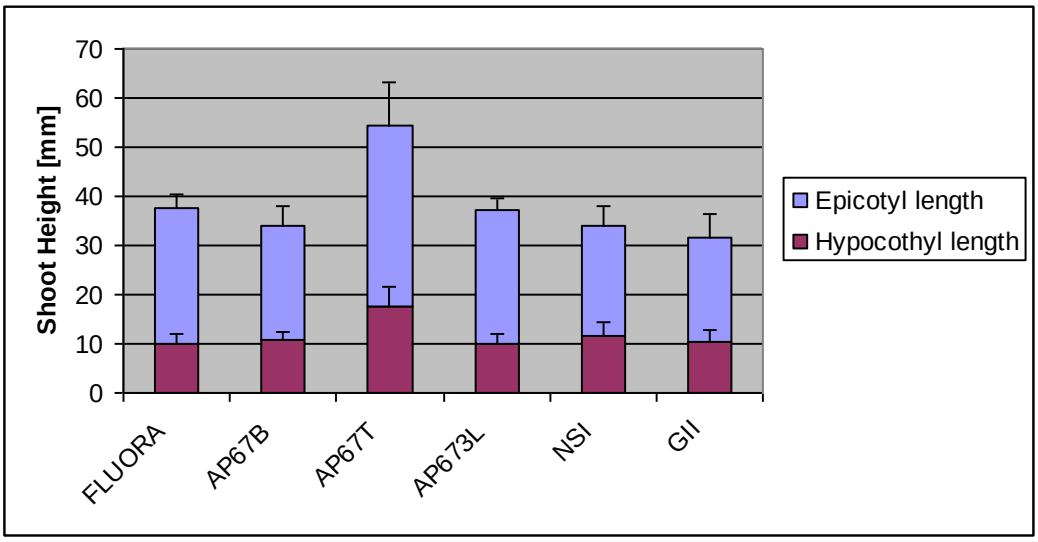
Common hazel seedlings used for morphometric measurements.

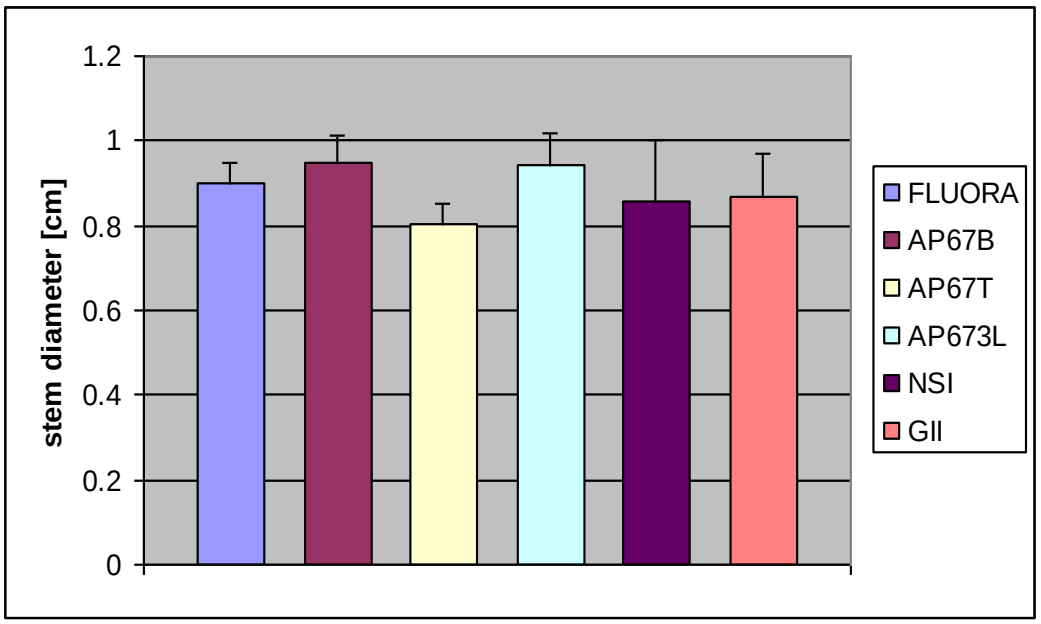
Results on *Corylus avellana* pointed out the best influence of AP67T, NS1 e G2 on stem growth in comparison with Fluora. In details, the minimum value of the hypocothyl was observed for G2, while the maximum was for Fluora. Epicotyl length was highest for AP67 tubes and lowest for AP673L. Concerning the stem diameter, common hazel responded positively under all LED lights, especially under AP67 bars, while the lowest value was achieved under Fluora light. On the other hand, no significant differences were retrieved from biomass. Moreover, LED lights showed higher values of leaves, shoot and roots dry weights than Fluora conventional light; in details, AP67 bars best performed on shoot and root weights, while NS1 did the same on leaves dry weight. Mean leaf area did not show statistical differences among light sources, although AP67 Tubes and G2 resulted more performing than Fluora, with AP673L having the lowest value.

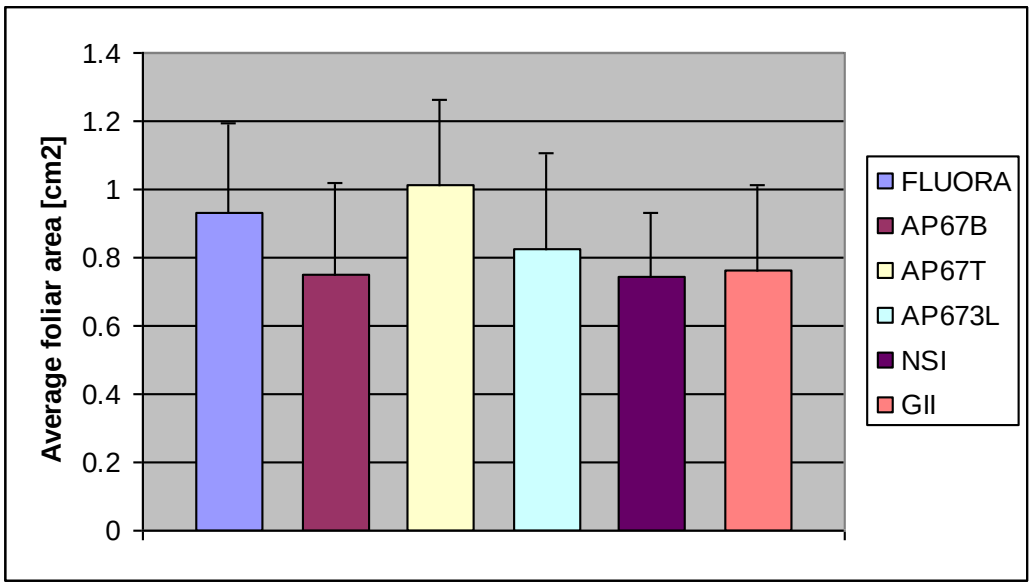
Punica granatum





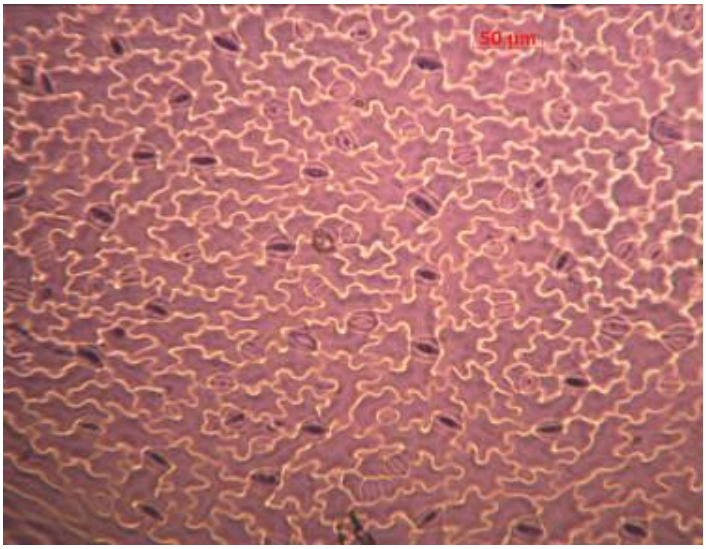

Shoot Height [mm]
70
60
50
40
30
20
10
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII
Epicotyl length
Hypocothyl length


stem diameter [cm]
1.2
1
0.8
0.6
0.4
0.2
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII


Average foliar area [cm2]
1.4
1.2
1
0.8
0.6
0.4
0.2
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII

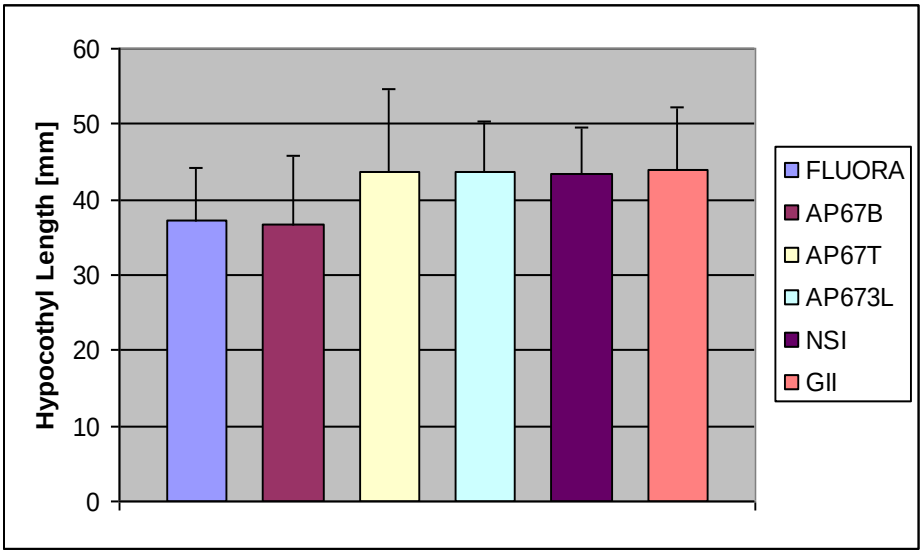
Punica granatum seedlings sampled from different LED light sources as they appeared in the miniplug container (above) and employed in morphological measures (below).

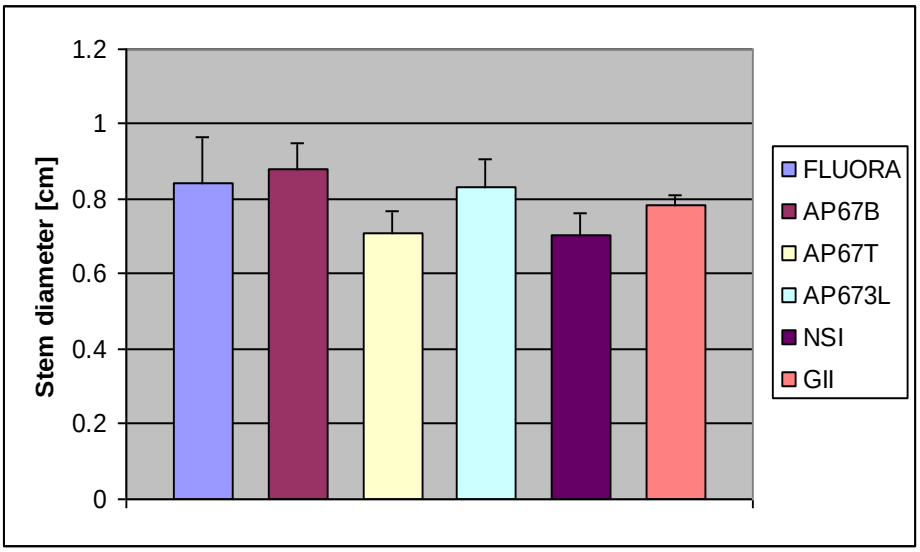
Results on pomegranate showed that AP67 tubes affected stem height better than other LED spectra and Fluora as well. Lowest values were obtained under AP673L and G2 for hipocothyl and epicotyl respectively. Stem diameter resulted highest for AP67 bars and AP673L with respect to Fluora, while AP67 tubes had the lowest performance.
Concerning the number of leaves, Fluora performed best, the minimum was achieved for AP67 bars and G2. The biomass parameters pointed out as AP67 spectra mostly affected the stem, roots and leaves dry weight; in details, AP67 tubes was the most performing about stem weight (minimum resulted from G2), AP67 bars about roots (minimum was under AP67 tubes), and AP673L about leaves weight (minimum was obtained for G2).
Moreover, the mean leaf area resulted highest for AP67 tubes, especially compared to Fluora, while the minimum was obtained from NS1. The stomata density revealed a best performance of all LED spectra if compared with Fluora, in particular AP673L which had the highest number of stomata per mm^2.

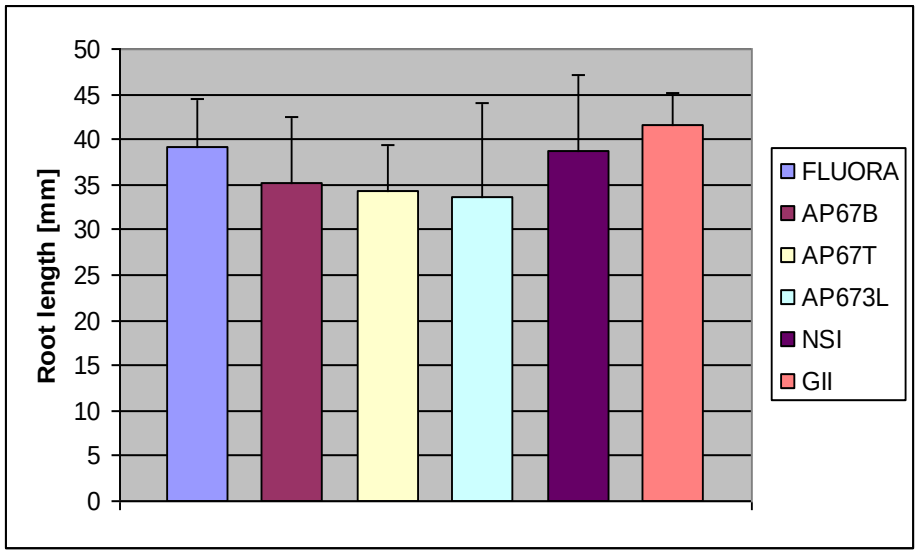


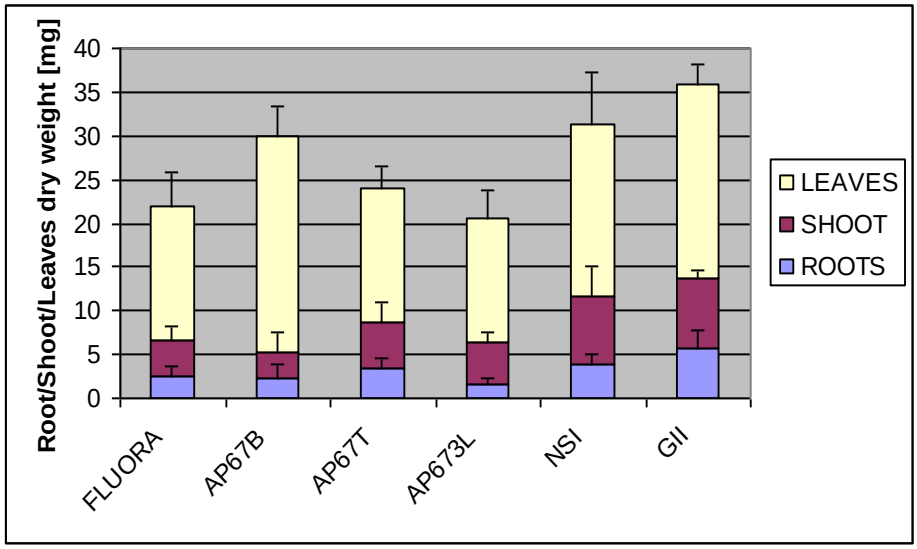
Picture of leaf stomata in a pomegranate sample growing under AP67 bars.

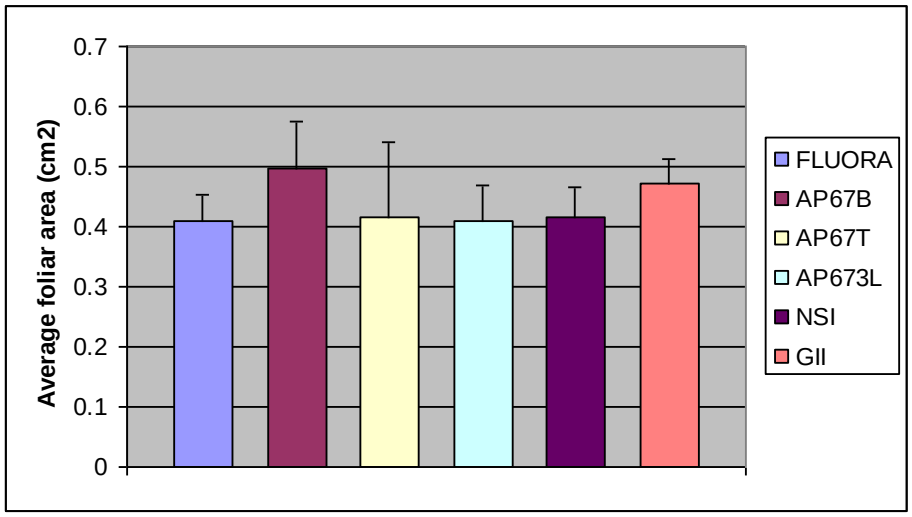
Abies alba





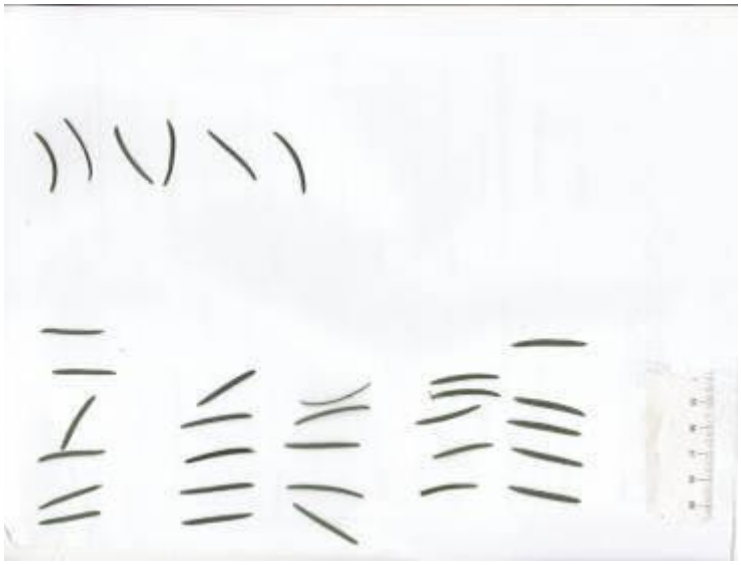






Silver fir seedlings did not statistically differ under experimented light conditions if hypocothyl, and root length are considered. However, G2 gave the maximum value for both parameters (AP67 bars the minimum for hypocothyl and AP673L for root length); if related to Fluora, LED

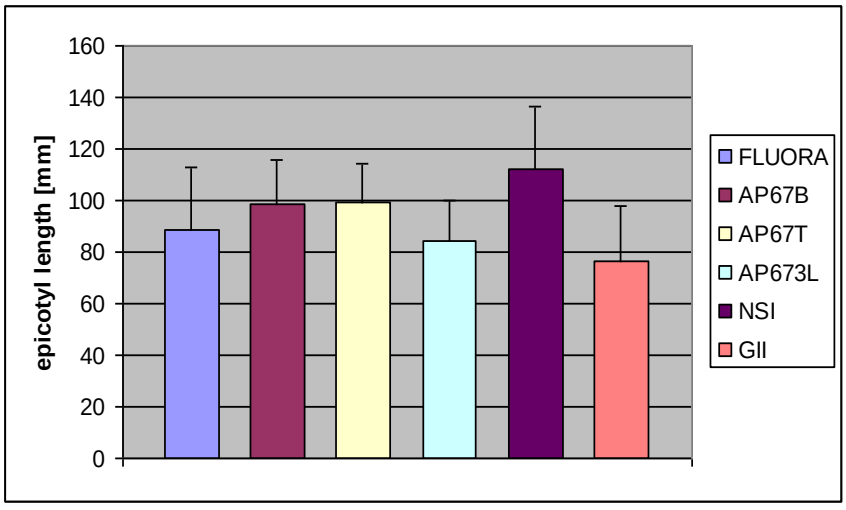
lights well performed in all cases about hypocothyl, AP67 bars excepted, while only NS1, G2 gave best results in terms of root length.
Concerning stem diameter, not statistically differences were retrieved as well, but a slightly variation could be observed if AP67 bars and AP673L are compared with Fluora. It is just to note that NS1 was the worst performing spectrum for stem diameter growth in silver fir.
Biomass variables confirm the good performance of G2 for this species, because it affected stem and roots dry weight more than the other spectra; additionally, AP67 bars showed the lowest values for stem dry weight, and AP673L did the same for roots. About leaves dry weight, the maximum value was obtained under AP673 bars, while the minimum was under AP673L.
Mean leaf area also confirmed the good performances of LED lights if compared with Fluora, which had the minimum value, while AP67 bars had the highest one.

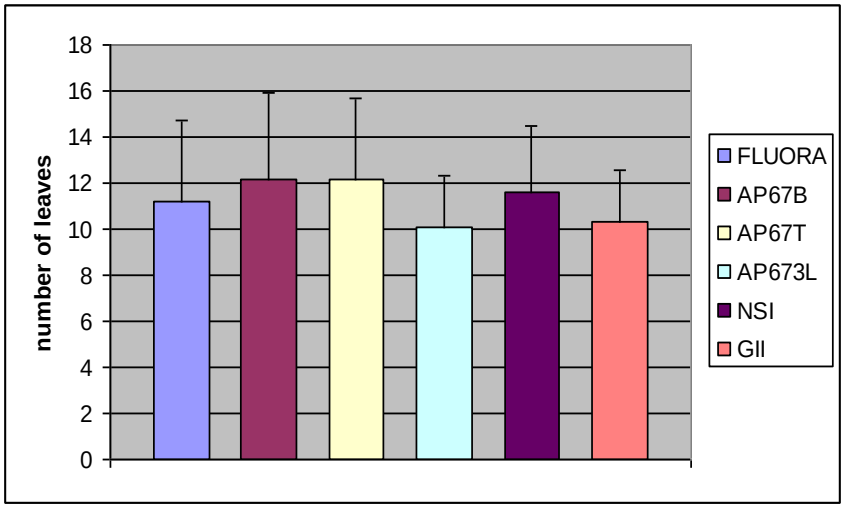


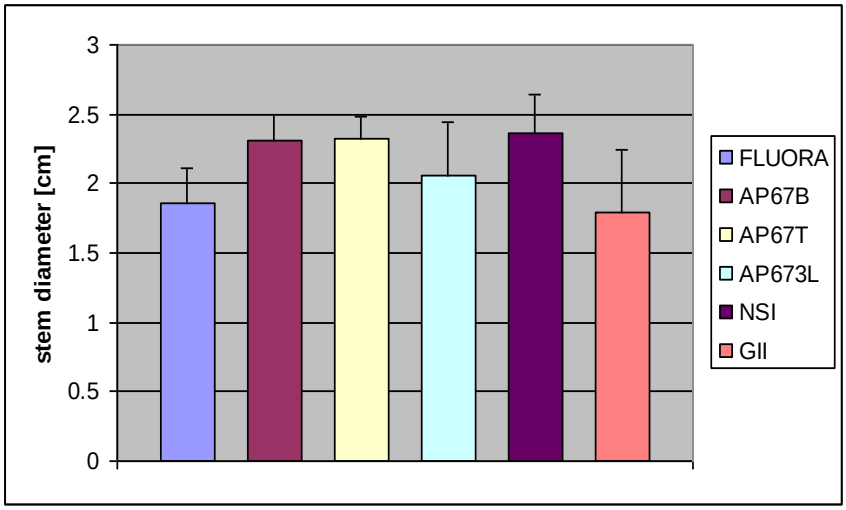
Leaves of Abies alba scanned using Digimizer Image Analyzer to measure mean leaf area.

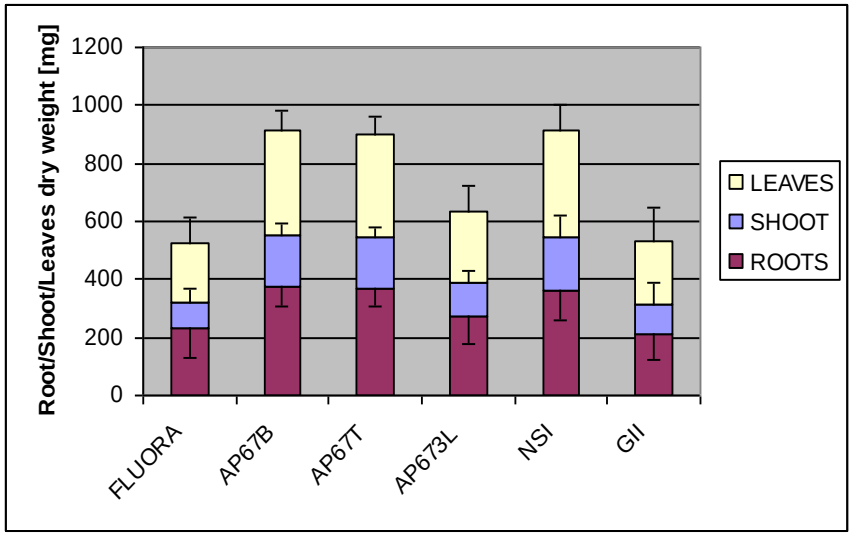
1.3.5. Supplementary species – morphological results

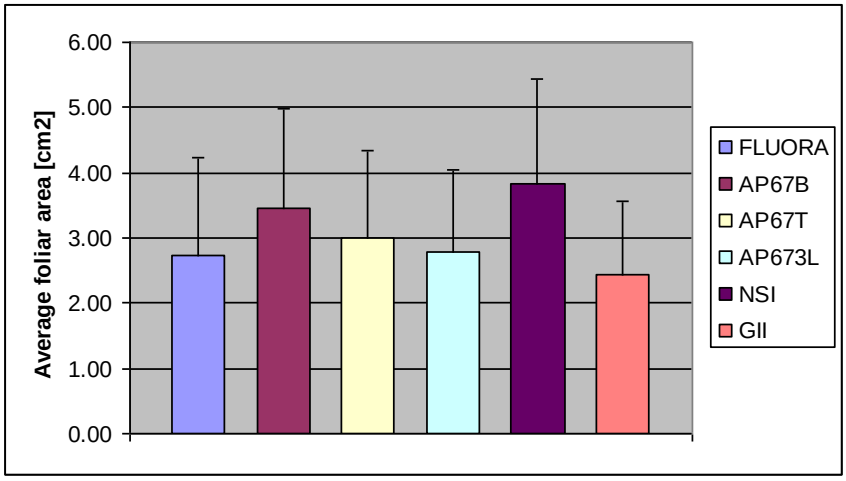
Quercus suber




number of leaves
18
16
14
12
10
8
6
4
2
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII

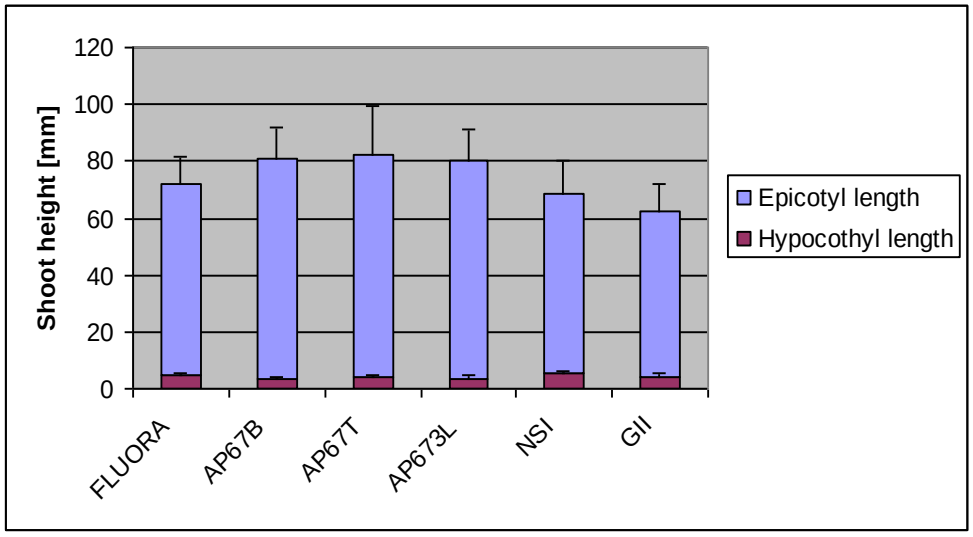

stem diameter [cm]
3
2.5
2
1.5
1
0.5
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII

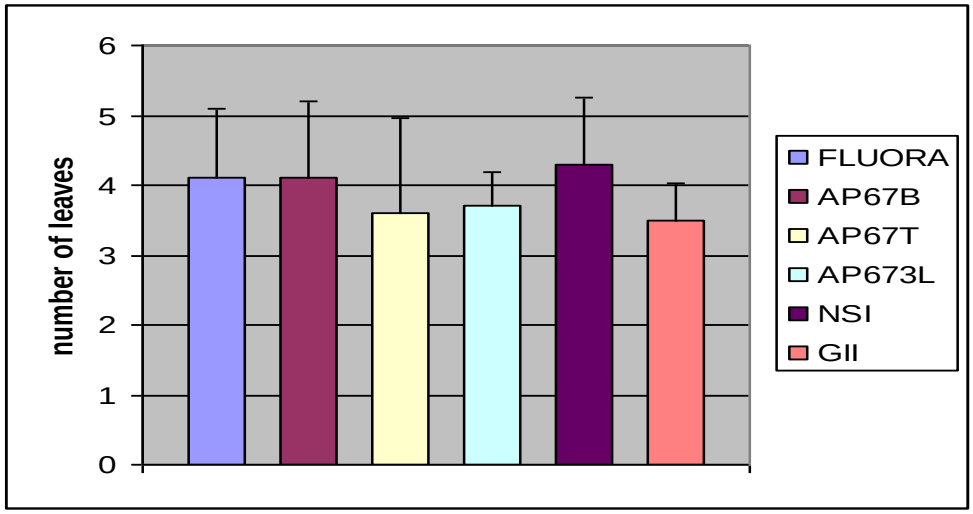

Root/Shoot/Leaves dry weight [mg]
1200
1000
800
600
400
200
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII
LEAVES
SHOOT
ROOTS

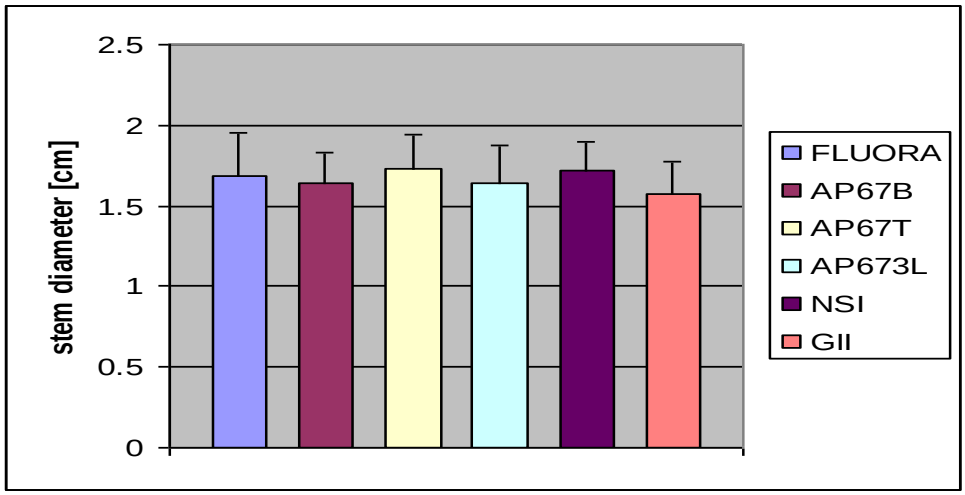


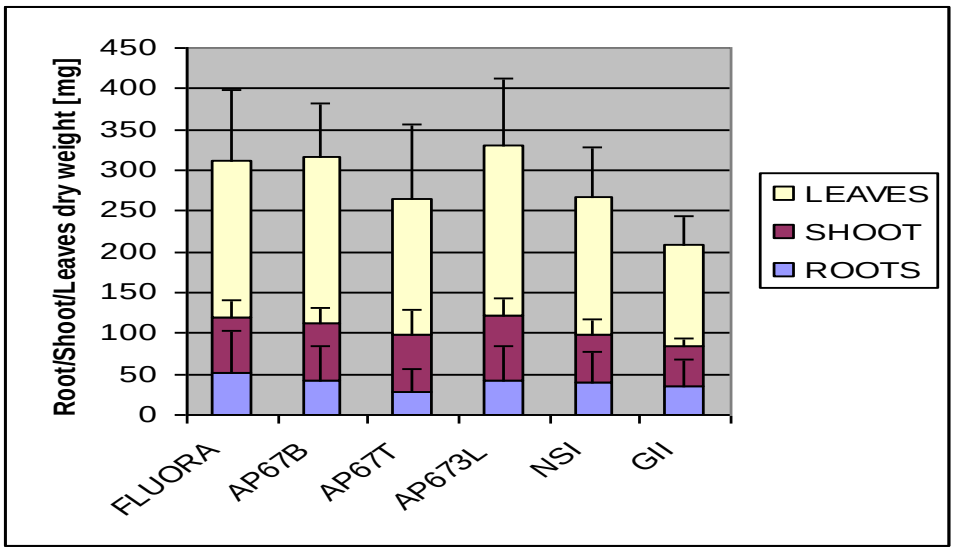
Epicotyl length measured on cork oak samples showed good performances of almost the LED spectra, G2 and AP673L excepted; in particular NS1 is statistically more performing than Fluora and the other LEDs, while G2 showed the lowest results.
The number of leaves resulted not statistically significant; however, all LEDs lights had better results than Fluora with the exception of AP673L and G2 (maximum value achieved from AP67 tubes). Also the stem diameter resulted larger under LEDs spectra than Fluora, G2 excepted; the highest values was obtained from NS1.
About the biomass measurements, the stem and the leaves dry weights were higher in all LEDs than Fluora, with the maximum value for NS1. Only the roots weight showed a certain variation, with G2 as the worst performing spectra and AP67 bars as the best.
Good results were achieved also about leaf area, with LEDs spectra generally better performing than Fluora (maximum value for NS1), G2 excepted.

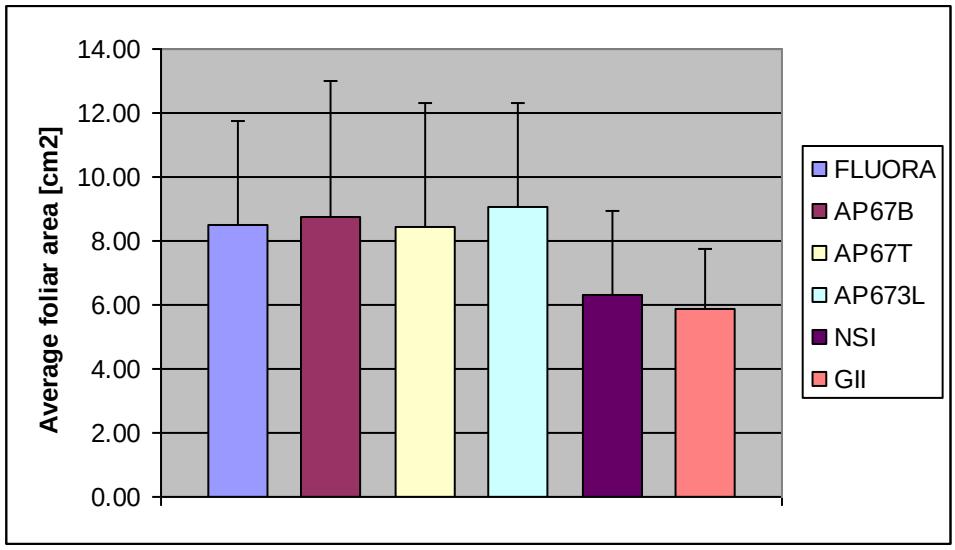
Quercus pubescens




number of leaves
0
1
2
3
4
5
6
FLUORA
AP67B
AP67T
AP673L
NSI
GII


stem diameter [cm]
0
0.5
1
1.5
2
2.5
FLUORA
AP67B
AP67T
AP673L
NSI
GII


Root/Shoot/Leaves dry weight [mg]
0
50
100
150
200
250
300
350
400
450
FLUORA
AP67B
AP67T
AP673L
NSI
GII
LEAVES
SHOOT
ROOTS

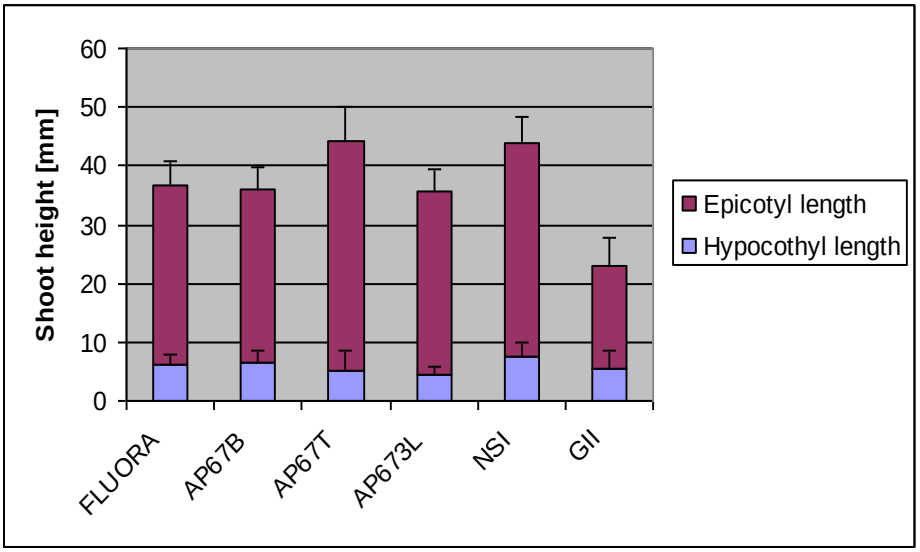


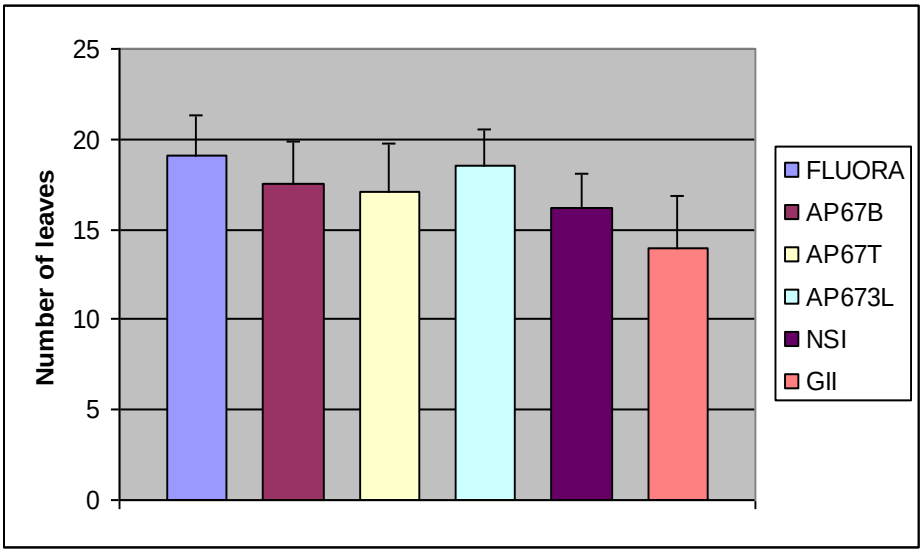
Seedlings of Quercus pubescens grown under Fluora light spectra and used for morphometric measures.

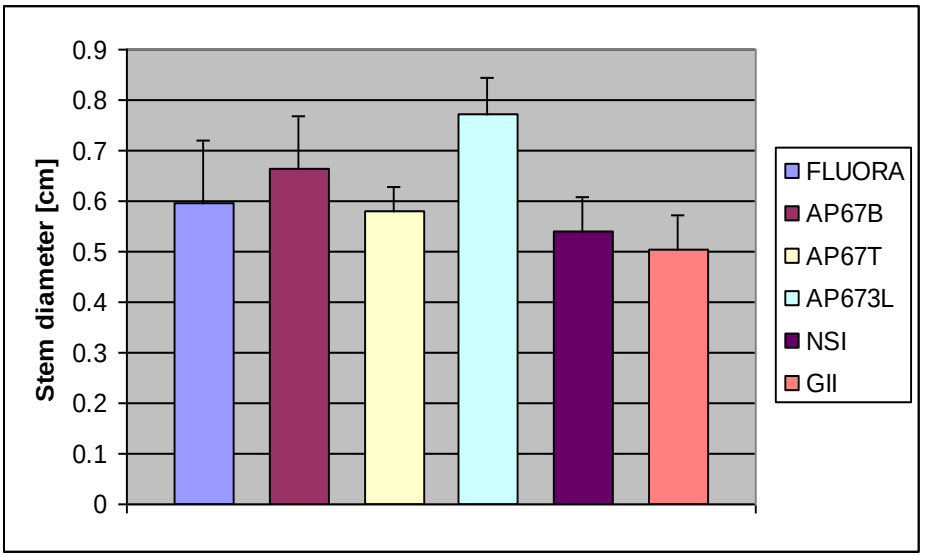
Leaves of Quercus pubescens collected and scanned using Digimizer Image Analyzer to calculate mean leaf area.

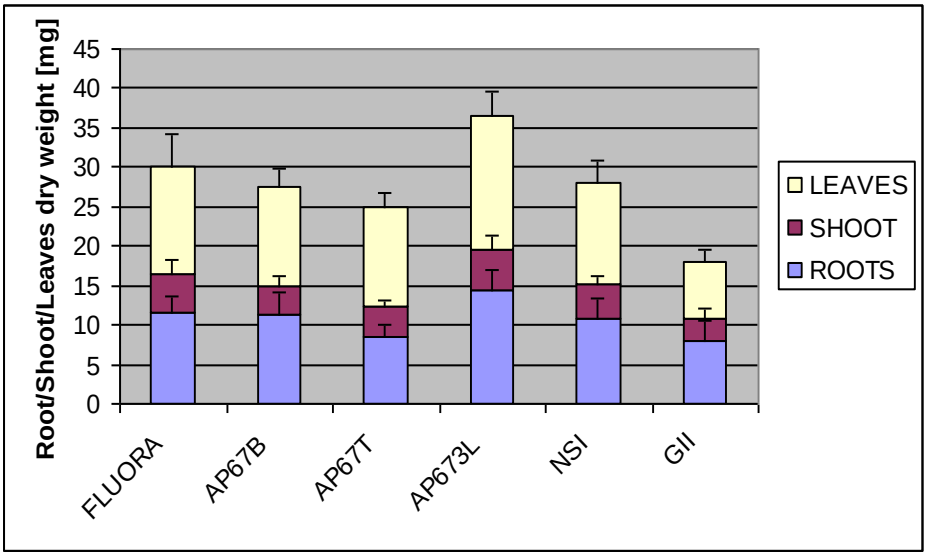
Morphometric investigation on *Quercus pubescens* pointed out some variables statistically significant and some others not; in details, shoot length was highest for NS1 (hypocothyl) and AP67 tubes (epicotyl) respectlively, while the minimum values were obtained for AP67 bars and G2. Fluora light was less performing than AP67 spectra (tubes, bars and 3L).
On the other hand, stem diameter and leaves number showed not significant differences, although AP67 tubes and NS1 had highest values than Fluora (overall minimum observed for G2) about stem diameter, and the same NS1 spectrum best performed on leaves number (followed by Fluora and having G2 as less effective spectrum).
Furthermore, biomass variables were not significant on *Quercus pubescens* response to light treatments. In fact, all dry weights showed similar values among spectra, with large standard deviations. Stem dry weight resulted higher for AP673L and lower for G2; roots weight was higher for NS1 and lower for AP67 tubes; leaves weight was higher for AP673L and lower for G2.

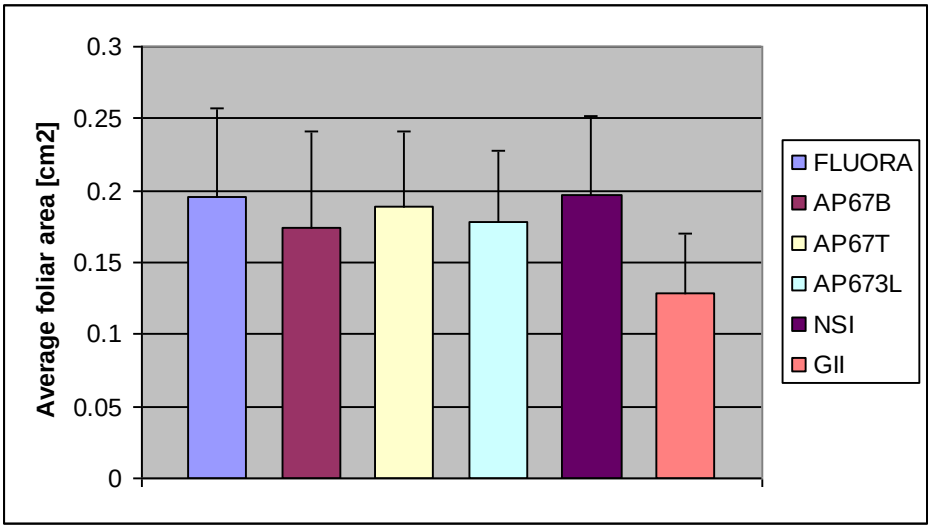
Myrtus communis

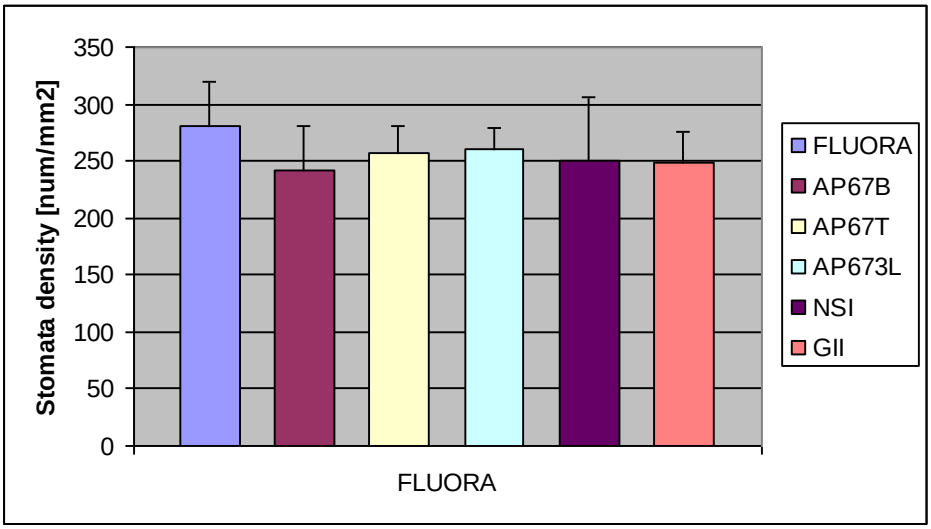


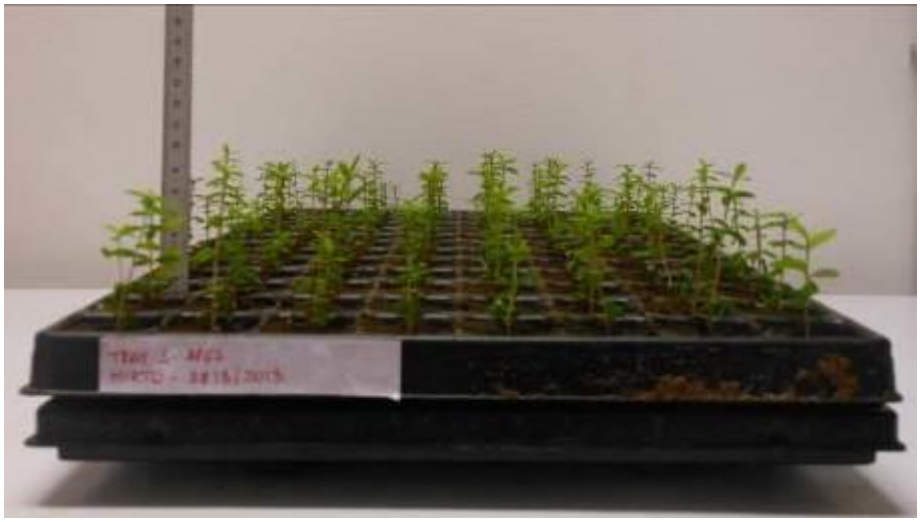



Stem diameter [cm]
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII

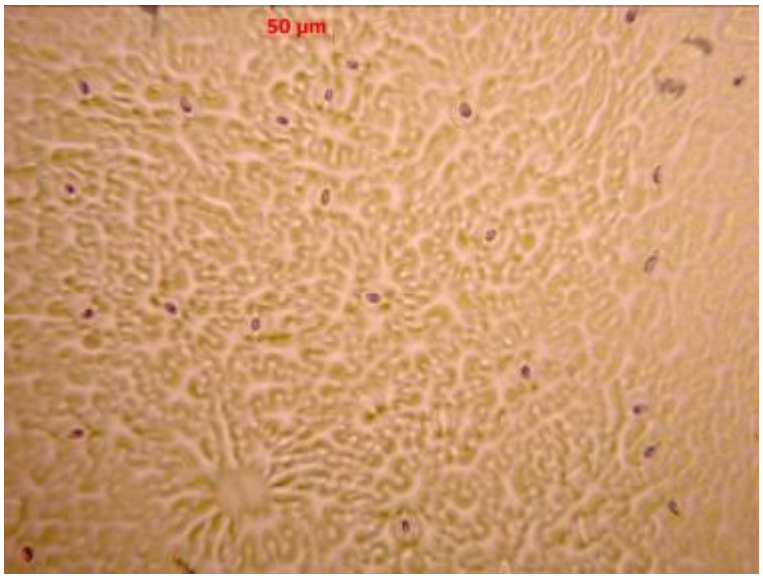

Root/Shoot/Leaves dry weight [mg]
45
40
35
30
25
20
15
10
5
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII
LEAVES
SHOOT
ROOTS


Average foliar area [cm2]
0.3
0.25
0.2
0.15
0.1
0.05
0
FLUORA
AP67B
AP67T
AP673L
NSI
GII





Quickpot with Myrtus communis seedlings ready to be measured.



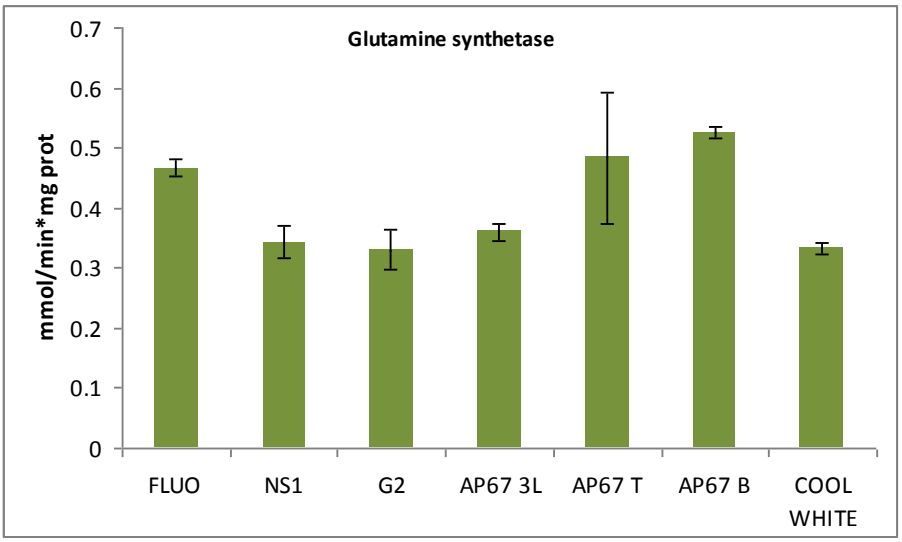
Picture of stomata in a Myrtus communis leaf captured at the microscope.

Myrtus communis represents the third supplementary species investigated from a morphological and anatomical point of view. The stem length (epycotyl) was significantly

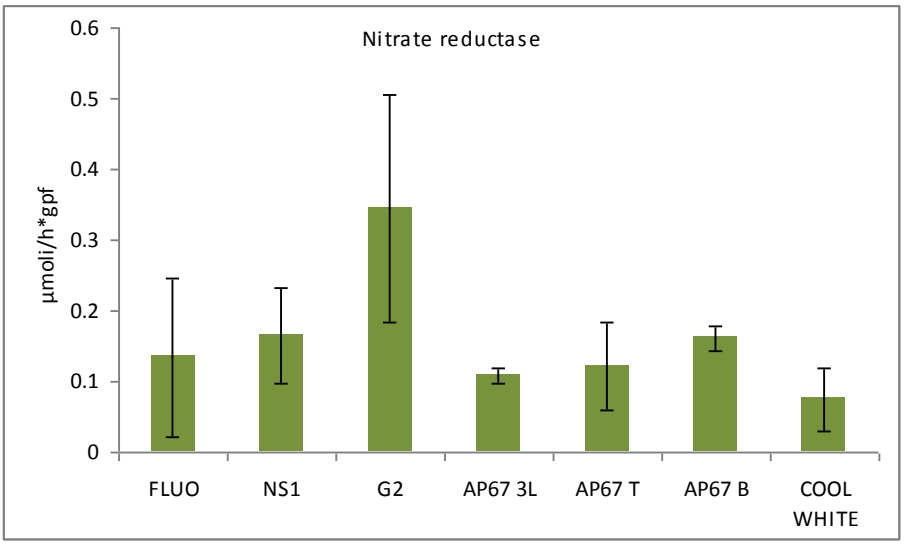
different among spectra, in particular AP67 tubes had the highest value, while G2 had the lowest; conversely, the hypocothyl measures did not differ significantly, even if AP67 tubes and NS1 got higher results than Fluora (minimum value for AP673L).
On the other hand, Fluora spectrum best performed about number of leaves, with G2 having the lowest result. Finally, the stem diameter showed best results for AP67 bars and AP673L if compared with Fluora, while G2 performed as the worst.
Biomass variables showed as AP673L was the most effective light (stem, roots and leaves dry weights) while G2 performed as the worst once again. Concerning mean leaf area, NS1 showed the highest result, but not statistically differed from Fluora, which was however more performing than the other LEDs.
The anatomic investigation by means of stomata density revealed as LEDs spectra had lower values than Fluora, with AP67 bars as the worst performing, although the differences retrieved from this test were not statistically significant.

1.3.6. iochemical results

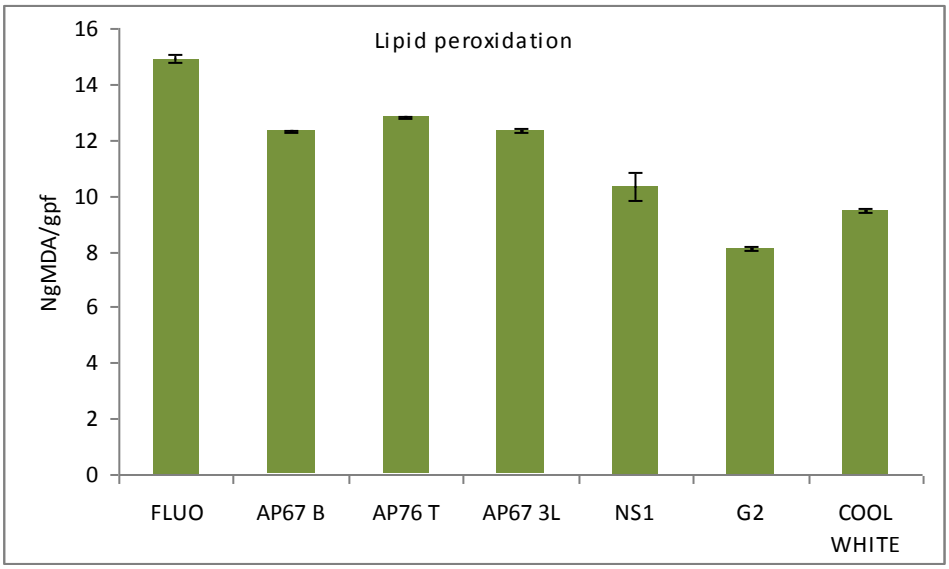
Corylus avellana



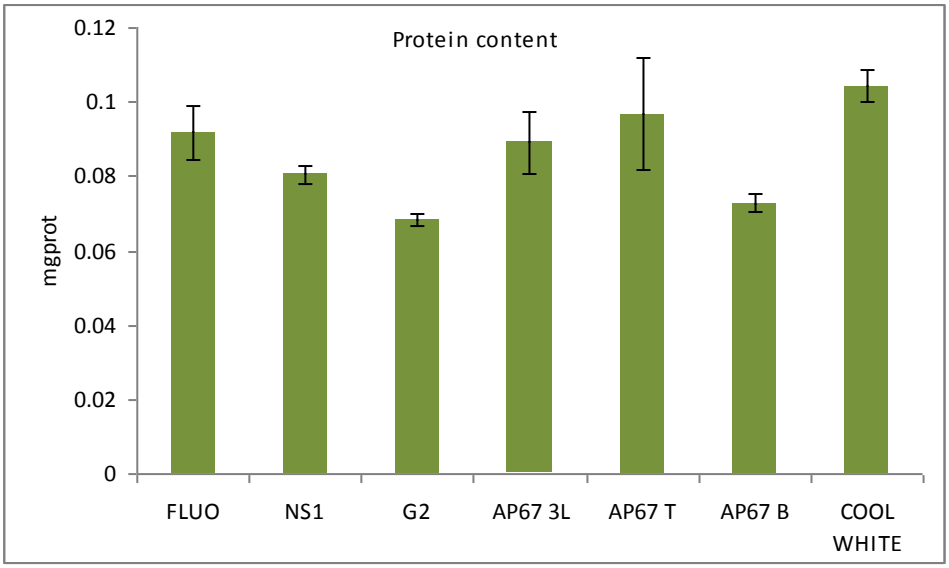
FLUO	0.467	0.014
NS1	0.343	0.027
G2	0.331	0.032
AP67 3L	0.360	0.015
AP67 T	0.484	0.110
AP67 B	0.525	0.009
COOL WHITE	0.332	0.009



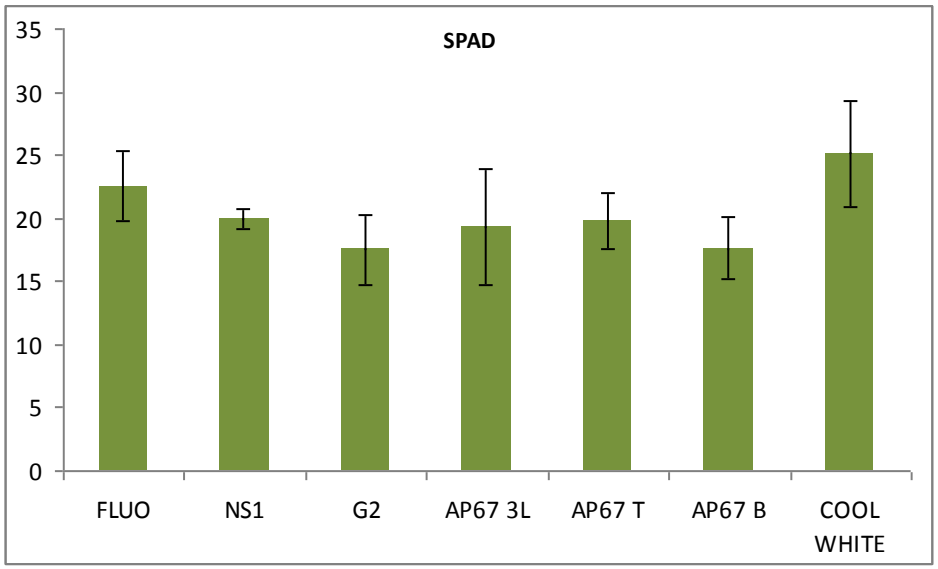
FLUO	0.135	0.112
NS1	0.164	0.068
G2	0.345	0.161
AP67 3L	0.108	0.010
AP67 T	0.122	0.062
AP67 B	0.162	0.018
COOL WHITE	0.074	0.046



FLUO	14.946	0.152
AP67 B	12.312	0.049
AP76 T	12.817	0.019
AP67 3L	12.344	0.067
NS1	10.355	0.504
G2	8.108	0.067
COOL WHITE	9.495	0.081



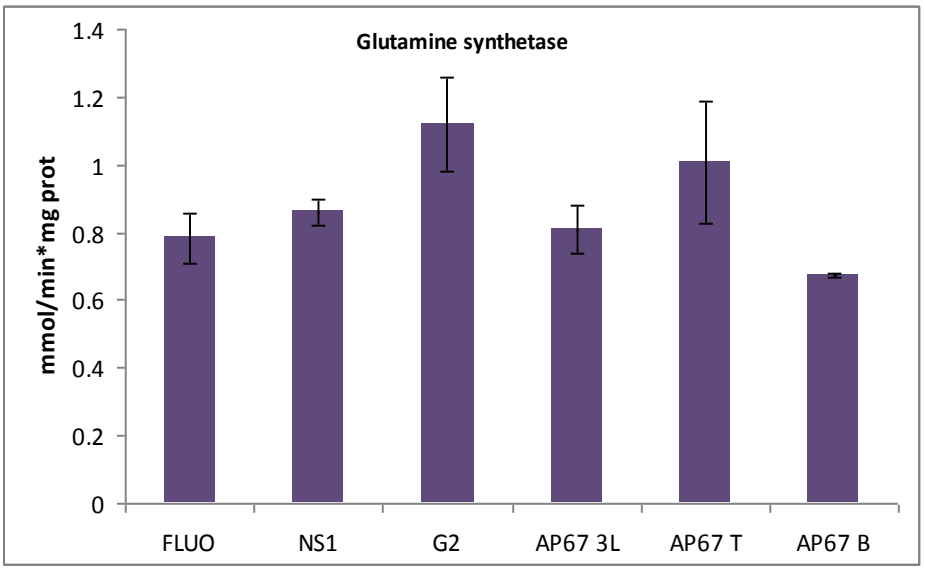
FLUO	0.092	0.007
NS1	0.081	0.002
G2	0.068	0.002
AP67 3L	0.089	0.008
AP67 T	0.097	0.015
AP67 B	0.073	0.002
COOL WHITE	0.104	0.004



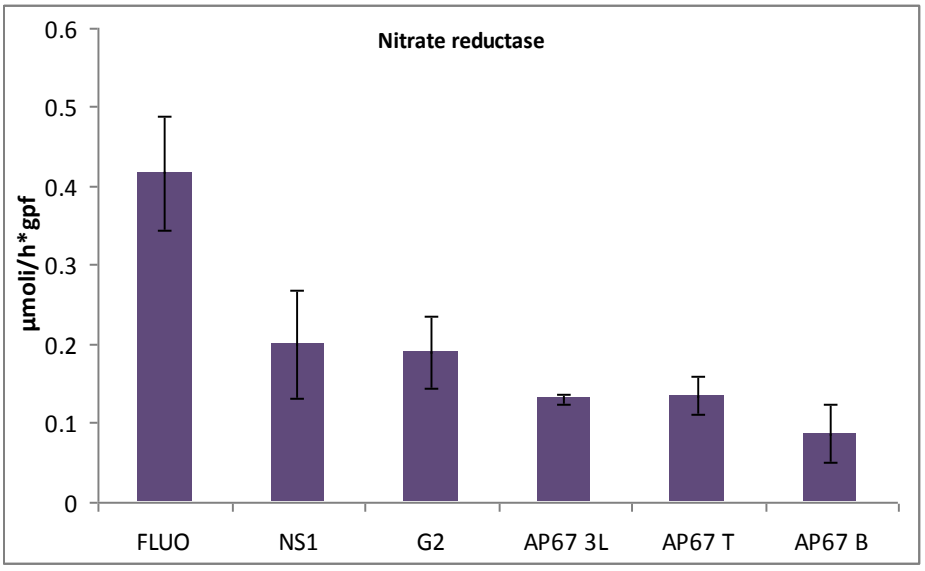
FLUO	22.56	2.834
NS1	19.90	0.771
G2	17.56	2.766
AP67 3L	19.26	4.608
AP67 T	19.78	2.196
AP67 B	17.64	2.470
COOL WHITE	25.12	4.223

Common hazel biochemical results stated that light spectra affected differently the concentration and activities of the study chemical compounds. In details, the glutamine synthetase activity was highest for AP67 bars, followed by AP67 tubes and Fluora with a slightly lower value; the lowest value was obtained for G" and Cool White respectively.
The nitrate reductase showed G2 as the best performing spectrum, although its value is linked to a high standard deviation. However, the mean value reported is significantly higher than the other spectra. Among them, Cool White got the lowest value, mosltly in line with the AP67 spectra and Fluora. Lipid peroxidation analysis showed as Fluora is the most performing spectra and G2 the worst one, while AP67 LEDs showed almost the same results; in all cases, standard deviations are extremely reduced among the spectra. Protein content resulted highest under Cool White treatment, while Fluora, AP67 tubes and AP673L were a bit less performing. G2 obtained the lowest value as for lipid peroxidation mentioned above.

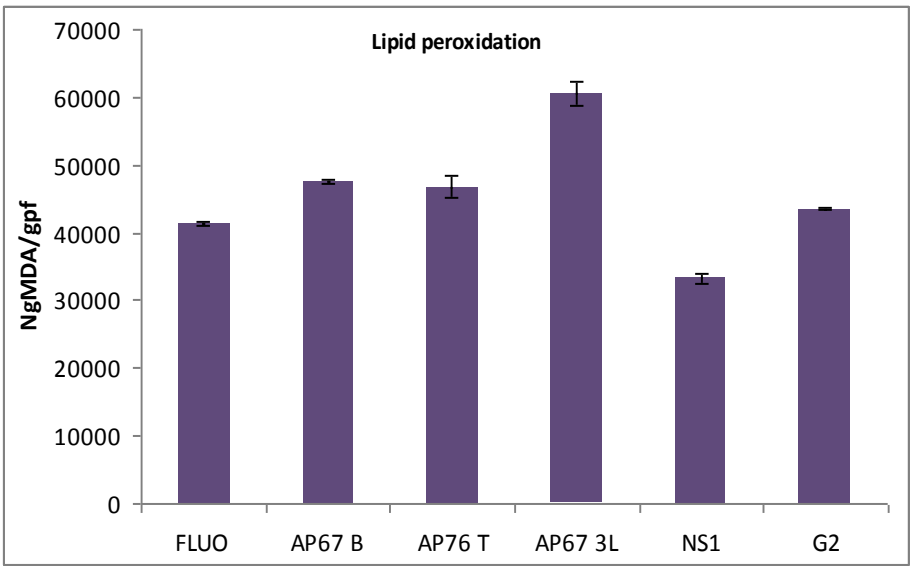
Punica granatum



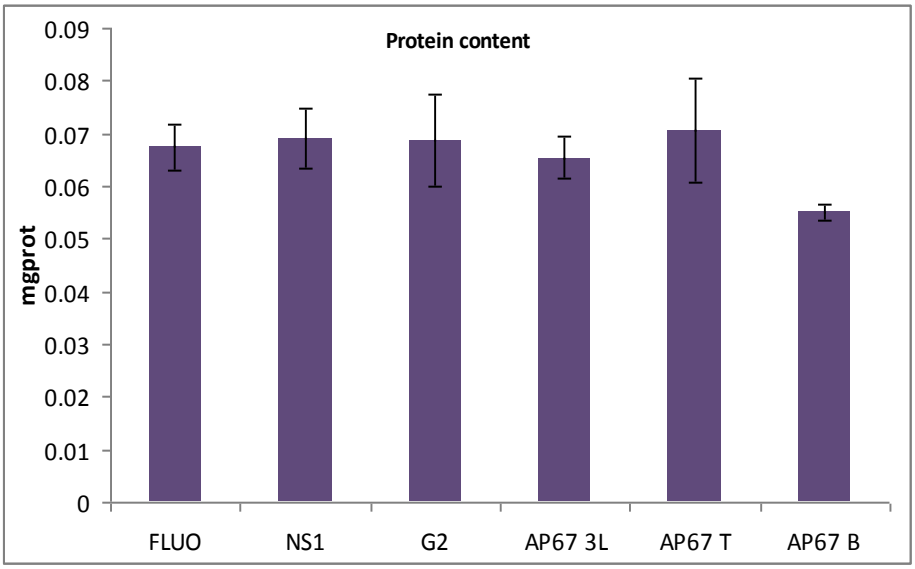
FLUO	0.784	0.074
NS1	0.860	0.040
G2	1.120	0.139
AP67 3L	0.811	0.071
AP67 T	1.008	0.179
AP67 B	0.675	0.006



FLUO	0.417	0.071
NS1	0.200	0.069
G2	0.190	0.046
AP67 3L	0.131	0.007
AP67 T	0.135	0.024
AP67 B	0.087	0.036



FLUO	41461.63	296.32
AP67 B	47595.85	299.64
AP76 T	46746.58	1590.23
AP67 3L	60498.95	1761.06
NS1	33317.39	698.18
G2	43488.97	130.41



FLUO	0.068	0.004
NS1	0.069	0.006
G2	0.069	0.009
AP67 3L	0.065	0.004
AP67 T	0.071	0.010
AP67 B	0.055	0.002

Pomegranate biochemical tests were performed on four variables, thus excluding SPAD with respect to common hazel. For this species, Cool White light spectrum was excluded from the analysis. Data about the glutamine synthetase pointed out as G2 is the best spectrum affecting the glutamine synthetase activity, followed by AP67 tubes; the other spectra had almost the same values, with AP67 bars as the worst performing spectrum.
The activity measured on the nitrate reductase enzyme stated as Fluora significantly got highest results, while the LEDs spectra had less than the half value of Fluora; in particular NS1 was the best spectrum among the LEDs treatments and AP67 bars the worst one.
The lipid peroxidation resulted highest for AP673L, followed by the other two AP67 lights (bars and tubes respectively), G2, Fluora, and finally NS1. As for the common hazel, also in the test on pomegranate the replicas performed on lipid peroxidation had narrow standard deviations for all spectra.
Finally the protein content did not evidence a specific spectrum as best performing, because the values obtained were almost equal and the standard deviations large enough to consider such results as statistically not significant. Indeed, the LED lights, AP67 bars excepted,

ranged between 0.065 (AP673L) and 0.071 (AP67 tubes), a narrow range in which Fluora is included.

Overall, it is possible to make a comparison among the obtained results concerning the biochemical properties of the investigated species with the following table that summarize the abovementioned tests. Hereafter mean values and standard deviations, in brackets, are reported.

		FLUO	NS1	G2	AP67 3L	AP67 T	AP67 B	COOL WHITE
Corylus avellana	**SPAD**	22.560	19.900	17.560	19.260	19.780	17.640	25.120
		(2.834)	(0.771)	(2.766)	(4.608)	(2.196)	(2.470)	(4.223)
	Protein	0.092	0.081	0.068	0.089	0.097	0.073	0.104
		(0.007)	(0.002)	(0.002)	(0.008)	(0.015)	(0.002)	(0.004)
	Lipid per.	14.946	12.312	12.817	12.344	10.355	8.108	9.495
		(0.152)	(0.049)	(0.019)	(0.067)	(0.504)	(0.067)	(0.081)
	NR	0.135	0.164	0.345	0.108	0.122	0.162	0.074
		(0.112)	(0.068)	(0.161)	(0.010)	(0.062)	(0.018)	(0.046)
	GS	0.467	0.343	0.331	0.360	0.484	0.525	0.332
		(0.014)	(0.027)	(0.032)	(0.015)	(0.110)	(0.009)	(0.009)
Punica granatum	**Protein**	0.068	0.069	0.069	0.065	0.071	0.055	--
		(0.004)	(0.006)	(0.009)	(0.004)	(0.010)	(0.002)	--
	Lipid per.	41461.63	47595.85	46746.58	60498.95	33317.39	43488.97	--
		(296.32)	(299.64)	(1590.23)	(1761.06)	(698.18)	(130.41)	--
	NR	0.417	0.200	0.190	0.131	0.135	0.087	--
		(0.071)	(0.069)	(0.046)	(0.007)	(0.024)	(0.036)	--
	GS	0.784	0.860	1.120	0.811	1.008	0.675	--
		(0.074)	(0.040)	(0.139)	(0.071)	(0.179)	(0.006)	--

1.4. **Conclusions**

- After a comparison of the huge amount of data obtained through morphological and microscopic analysis of the 6 species, cultivated under fluorescent and LED lights, it is possible to state that each species shows a different performance of growth under the different spectra. Therefore, it is not easy to define a best spectrum for all the species and, moreover, for all the parameters which we have analysed.

- All the species show better growth performances under LED lights than under Fluorescent lights.
- The two shrub species, *P. granatum* and *C. avellana*, show the best growth performance under AP67 spectra. In particular, the highest values of shoot height and average foliar area are reached under AP67TUBE while the highest diameter is reached under AP67BARS. High dry weights for roots, stem and leaves are associated alternatively to AP67TUBE and AP67BARS. For these species, some biochemical results are also available and in this case, they show very different results. For example, lipid peroxidation, which identifies a stress state of the plant, is really high under fluorescent lamps for *C. avellana* and under AP673L for *P. granatum*.
- The conifer species, *A. alba*, shows different results, growing better under G2 lights, in terms of shoot height and root and stem biomass. AP67B is another time associated to the highest value of stem diameter.
- The 3 species not included in Zephyr's list show different results, too. *Q. suber* and *Q. pubescens* seem to growth better, in terms of shoot height, stem diameter and stem and root biomass, under NS1 lights. While *M. communis,* like the two shrub species described earlier, shows the highest value of shoot height under AP67TUBE, but for the other parameters the best spectra are AP673L (root, stem and leaves biomass) and FLUORA (number of leaves, average foliar area and stomata density).

2. Input of DUTH

2.1. Removal of dormancy

***Arbutus unedo* L.**

Arbutus unedo L. seeds were collected from Skioni, Chalkidiki in 2013. Fruits are difficult to be stored because of the fleshly stain, which is susceptible of subjecting to fungal infection. Thus propagation with seeds involves a process for the pulp removal, prior to maceration in water for several hours of the ripe fruits, rubbed between fingers. Following the seeds are pressed through a sieve. After drying the seeds were stored in a refrigerator at 2-5 ^{0} C. In order to remove the dormancy seeds were hydrated for 24 hours in room conditions and cold stratified for 50 days in the refrigerator at 3-5º C. The germination results were quite enough around to 60-70% (Photo 1.).

Photo 1. *Arbutus unedo fruits and pregerminated seeds after 50 days of cold stratification pretreatment*

***Myrtus communis* L.**

Myrtle *Myrtus communis* L. seeds were provided from Skioni, Chalkidiki of the year 2013. Seed coating was carefully cracked and removed, they were allowed to dry and stored at 4^{0} C Generally the Greek variety of myrtle shows no dormancy so seeds were only hydrated with distilled water for 24 hours at room temperature and were placed in a germination chamber set at 20^{0}C for 16 hours with light and at 15^{0}C for 8 hours without light, while light was provided by cool-white fluorescent lamps on both side walls of the chamber. Good germination results were succeeded around 75-80 %. Further we tried another pretreatment, a 30 days cold stratification at 2-5 ^{0} C that showed higher germination percentage around 80-90% (Photo 2.).

Photo 2. *Myrtus communis fruits and pregerminated seeds after 30 days of cold stratification pretreatment*

***Abies borisii-regis* Mattf.**

The seeds of *Abies borisii-regis* were provided by the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens). They were collected from Peukoto, Pella in Central Greece (22^0 05′ 00″ N, 410 07 00 E). For breaking the dormancy the seeds of *Abies* were pretreated for a 4-month cold stratification period at 3-5^0C with 48% germination success (Photo 3).

Photo 3. *Abies borisii-regis individual at Pieria Mountains in Greece and pregerminated seeds after 4 months of cold stratification pretreatment*

***Platanus orientalis* L.**

Platanus orientalis L. seeds were collected from Thermi, Thessaloniki, Greece on March of the 2013. After having been air-dried until the moisture content has been reduced to 7-10%,

seeds were stored in sealed containers at a low temperature (+5° to +7°C). Seeds that hydrated for 24 hours showed a 58% germination success while been cold stratified for 50 days (6-8 weeks) showed better results, around 70% (Photo 4).

Photo 4. *Platanus orientalis pregerminated seeds after 50 days of cold stratification pretreatment*

***Picea abies* Karst.**

Picea abies seeds were collected from Karadere, Drama in Northeastern Greece on December the 17th of the year 2012. After removing the inherent fin of the seeds were hydrated for 24 hours in room temperature and germination test was done with very good results (after 7 days the germination percentage was high) (Photo 5).

Photo 5. *Picea abies pregerminated seeds after 24 hrs hydration and placed into phytotron chamber for 8 days.*

***Pinus sylvestris* L.)**

Pinus sylvestris L. seeds were provided by the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens). The seeds were collected from Laylia, Serres in central Greece (23^0 34′ 00″ N, 41^0 17′ 00″ E) in 2012. The seed germination ability, determined according to the policies of ISTA (2008). *Pinus* seeds were hydrated for 24 h with 75% germination success (Photo 6).

Photo 6. Pinus sylvestris pregerminated seeds after 24 hrs hydration and placed into phytotron chamber for 8 days.

2.1.1. Germination

After pretreatment seeds of the species *Myrtus communis, Platanus orientalis, Picea abies and Pinus sylvestris*, were placed on filter paper over sand saturated with distilled water, in 9 cm covered Petri dishes. All dishes were placed in a germination chamber set at 20^0C for 16 hours with light and at 15^0C for 8 hours without light, while light was provided by cool-white fluorescent lamps on both side walls of the chamber (Photo 7.) Germination test was performed on seeds of that species and the germination is achieved in a short time.

During pre-cultivation of all species the same type of plastic container was used, specifically the ***QPD*** *(QuickPot)* ***104 VW*** *by HerkuPak*, Germany, with the following identical dimensions *(tray dimension 310X530 mm ; cell size 33X33X43 mm; depth 12 mm; volume 27 cc; 630 plants/m^2)* (Photo 7.).

As growing substrate stabilized peat was used for all studied species. Stabilized peat (SP) is produced by Jiffy International (Preforma PP01, Jiffy Products International AS, Stange, Norway) being the world leading producer of growing substrate to forest and horticultural nurseries. The substrate had a pH of 5.0. With a technology based on pre-cultivation and transplanting SP is very favorable in regard of reducing the risk of losing part of the substrate around the roots or compression of the root system during the automatic transplanting operation.

For all the experiments only pre-germinated material was used in order to ensure the maximum uniformity of germination. All seeds were allowed to germinate under the conditions recommended by the International Seed Testing Association (ISTA) and the procedures followed for each species were mentioned previously.

Photo 7. Phytotron chamber used for seed and the mini-plug size that is used in the experiments (QPD 104 VW)

A pre-germinated seed was transferred into each cell of the mini-plug plastic trays. Continuously, mini-plug trays were transferred to the environmentally controlled growth chambers (chamber 1, chamber 2) for a cultivation period of 5 weeks. Environmental conditions in both chambers were set at a 14-h photoperiod of a photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis.

Both chambers reach 2 m height and consist of three shelves. Each shelve has 1.20 m length, 0.60 m height, and 0.55 m depth. Also, the distance from the lights until the bottom of the mini-plugs is 0.52 m. At the chamber 1, L20AP67 light was applied with a T8 fixation. In the middle shelve four Fluorescent tubes (Osram Fluora Philips-TLD (36W/54 daylight)-(PILUX&DANPEX, MAL 1.30 DTHSR, 1 X 30W, 230 V, 50Hz) were placed as the reference lighting type, with 30 cm space between them. At the bottom shelve a new tested light was applied the AP673L that is a high red spectra, with far-red and moderate blue. Good growth results with lettuce and herbs. It has peach-tone appearance to human eyes. Also is a good reference spectra to AP67, as this has less green and less far-red.

At Chamber 2 the top shelve had the G2 light, the second the AP67 light and third shelve the NS1 light that is also a new one tested light spectrum. It is a high intensity spectrum; of Valoya's spectra, matches more closely the spectrum of the sun. Also it has white appearance to human eyes and can prepare plants for outdoor cultivation. Selected percentages covering different areas of the light spectrum were shown in Table 1.

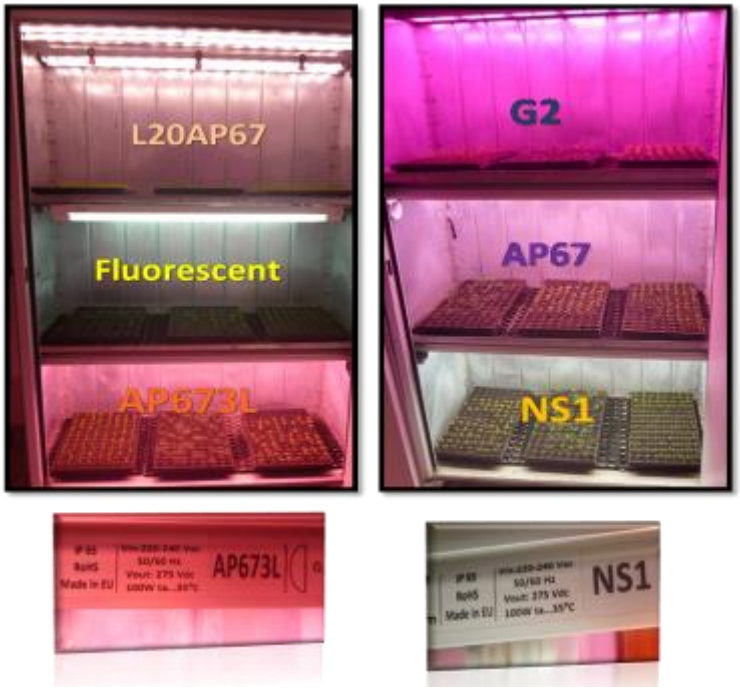


Photo 8. Visual indication of the growth chambers; chamber used for seed and the mini-plug size that is used in the experiments (QPD 104 VW)

Light treatments/	400-500 nm	500-600 nm	600-700 nm	700-800 nm	R:FR ratio
FL	34.8%	24.1%	36.7%	4.4%	5.74
L20 AP67	10.5%	26.2%	48.9%	14.4%	2.91
AP673L	11.9%	19.3%	60.5%	8.3%	5.56
G2	7.7%	2.4%	64.4%	25.5%	2.51
AP67	13.8%	15.1%	53%	18.1%	2.77
NS1	20.2%	38.9%	35.7%	5.2%	8.16

Table 1. Light treatments used in the experiments such as Fluorescent (FL), L20 AP67, AP673L, G2, AP67, NS1 and the different percentages covering different area of the light spectrum.

2.1.2. Growth kinetics

In the previous experiments seedlings were kept for a five week period into the growth chambers. However it was expanded for another couple of weeks in order to monitor changes in their growth. During the 7 weeks of cultivation in the growth chambers the seedlings were evaluated once a week based on the morphological parameter of seedling height, in order to estimate the growth rate (mm/week) that was computed through a formula entered in the statistical program based on maximum of ten randomly selected seedlings per species and light treatment.

```
 COMPUTE gr_1 = Height.1 / 7.
COMPUTE gr_2 = (Height.2 - Height.1) / 7.
COMPUTE gr_3 = (Height.3 - Height.2) / 7.
COMPUTE gr_4 = (Height.4 - Height.3) / 7.
COMPUTE gr_5 = (Height.5 - Height.4) / 7.
COMPUTE gr_6 = (Height.6 - Height.5) / 7.
COMPUTE gr_7 = (Height.7 - Height.6) / 7.
EXECUTE.
```

The leaf/needle number and the visual evaluation of leaf/needle colour (1=pale, 2=light green, 3=dark green). For the estimation of leaf/needle colour the following R:G:B ratios were used (Photo 3). The yellow- pale: i) R:255, G:255, B:204, ii) R:238, G:221, B:130, iii) R:238, G:232, B:170 and iv) R:214, G:219, B:112 (Photo 9a). The light green combinations: i) R:153, G:204, B:0, ii) R:102, G:204, B:0 and iii) R:102, G:153, B:0 (Photo 9b) Finally the dark green combinations, i) R:0, G:100, B:0, ii) R:85, G:107, B:47 and iii) R:110, G:139, B:61 (Photo 9c). Furthermore, by taking photographs from above and from the side of the trays for all tested species.

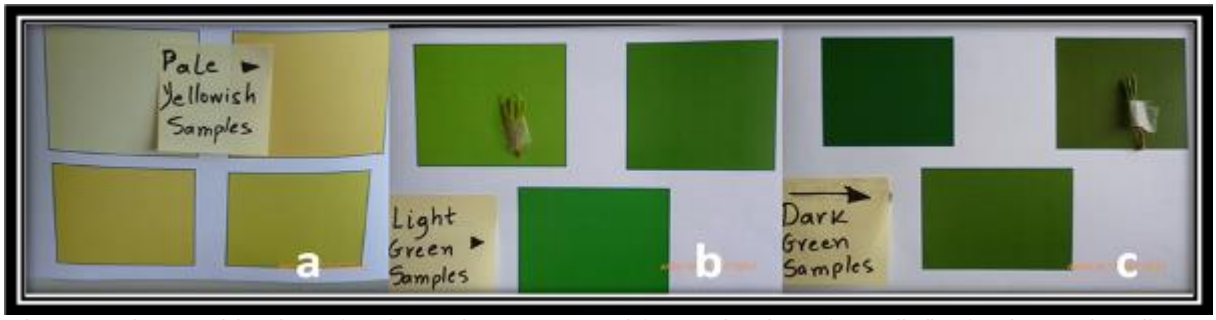


Photo 9. The combination of Colours that were used for evaluating of needle/leaf colour; a.) Yellow-pale, b.) Light green and c.) Dark green.

Also it should be mentioned that during the cultivation of the broad-leaved species *Arbutus unedo* and *Platanus orientalis,* different leaf coloration was observed especially during the last two weeks under the effect of LED treatments that was characterized by optical assumption as reddish with color rating (4) and purple with color rating (5) (Photo 10).

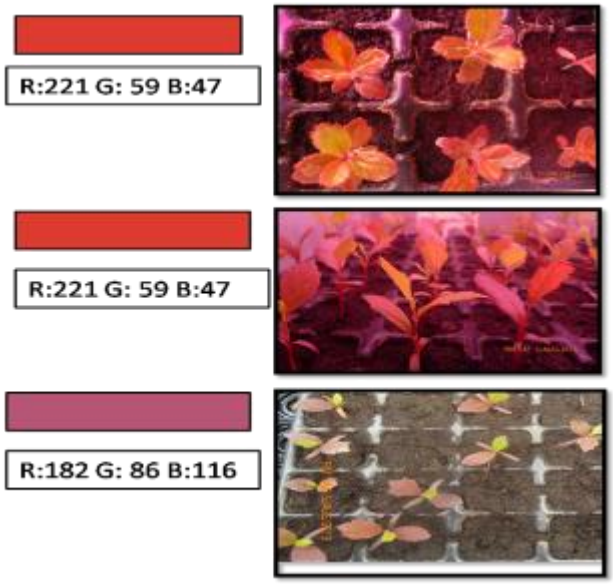


Photo 10. The combination of Colours that were used for evaluating of leaf colour; a.) Reddish for Arbutus ubedo & Platanus orientalis seedlings b) Purple for Platanus orientalis seedlings

Also, seedlings were measured for the Shoot height (SH) (mm) and the Root length (RL) (mm) using a digital caliper. In addition to the morphological variables, the seedlings were also evaluated based on dry weight (g), of the leaves (DWL), shoots (DWS) and roots (DWR) that were assessed after oven-drying at 70 °C for 48 h. The root-to-shoot ratio (R/S) was calculated on a dry weight basis.

Furthermore the root growth potential (RGP) test was carried out to determine the potential capacity of seedlings to initiate new roots. This test was implemented immediately after the end of the cultivation period in the growth chambers using the plant material from the same populations. At random, 10 seedlings per species per light treatment were selected and transplanted into mini-plug containers of same size, following the standardized RGP technique for containerized seedlings described by Mattsson (1986). The containers were placed on top of stainless steel boxes (35 × 26 × 8 cm) filled with equal volumes of peat (Klassmann Base Substrate 250I, Klassmann-Delmann GmbH, Geeste, Germany) and sand.

The boxes were immersed in a stainless water bath. The seedlings remained in the RGP bath for 31 days at an air temperature of 21±2 °C, RH of 60±10%, and 16-h photoperiod, with a PPFD at plant level of 300 μmol m^{-2} s^{-1} (Photo 11). Finally seedlings per species per light treatment were carefully removed from the RGP boxes. All new roots protruding from the root plug (new roots that were formed during the RGP test) were cut and cleared from the peat remains. The root growth potential (RGP) of each seedling was assessed by measuring the new root length (NRL) and new root dry weight (NRDW) of these roots.

Photo 11. The Root Growth Potential Bath

2.1.3. Selection of plant species to be further investigated

The species that would be further investigated under the illumination of LED lights and the conventional light are the *Prunus avium* L. (wild cherry), a variety of the species *Ocimum basilicum* L. specifically the var. “Lettuce leaf ”, *Cornus sanguinea* L. L. and the Quercus sp L.

2.2. Initial evaluation of growth performance of LED illumination on plants growth

2.2.1. Performance of LED spectra on height growth rate compared to control light

***Arbutus unedo* L.**

Results showed that the two way interaction time*light F (30, 324) = 6.67, p=.000 suggested a significant time effect for the height growth rate of *Arbutus unedo* seedlings between the different light treatments. Therefore at the first week into the growth chambers seedlings of *Arbutus* shown significantly higher growth rate (p< .000) under the L20AP67 (2.37 mm) and the FL lights (2.19 mm) compared to the rest of lights such as G2 (1.74 mm), AP673L (1.64 mm), AP67 (1.62 mm) and NS1 (1.56 mm). At the second week situation was different and the growth rate of the seedlings was significantly lower under the FL light (0.16 mm) compared to those grown under the NS1 (0.51 mm) (p<. 005), AP673L (p<. 0.28) and AP67 (0.46 mm) (p<. 026). At the third week seedlings under the FL light (0.39 mm) continued showing lower growth rate that differed significantly from those grown under the G2 (0.82 mm) (p<. 004), NS1 (0.77 mm) (p<. 017) and the AP67 (0.74) (p<. 034). At the fourth week seedlings grown under the G2 (0.88 mm) and the FL lights (0.84 mm) showed significantly higher growth rate than those under the NS1 (0.51 mm) (p<. 025), AP67 (0.49 mm) (p<. 015) and the L20AP67 (0.46 mm) (p<. 007). Following at the fifth week as it could be shown at the Figure 1, the L20AP67 light obtained higher growth rate (0.78 mm) only compared to G2 and NS1 lights (0.38 mm) (p<. 022). At the sixth week no significant differences found for the growth rate between the lights however the highest was for the FL light with 0.50 mm and the lowest for the AP673L only with 0.23 mm. Finally at the end of the cultivation period height growth rate of Arbutus seedlings was found significantly higher under the L20AP67 (0.65 mm) and the FL (0.63 mm) (like at the beginning of the experimental period) compared to NS1 (0.24 mm) (p<. 016), AP673L (0.26 mm) (p<. 015) and the G2 (0.40) (p<. 001).

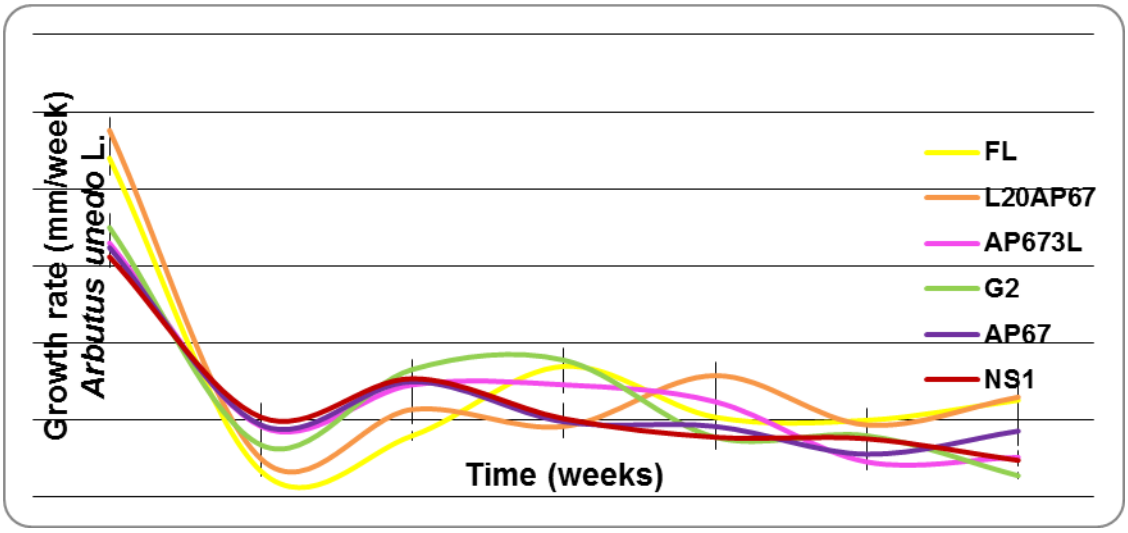


Figure 1. The growth rate of Arbutus unedo seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 7week experimental period.

***Myrtus communis* L.**

The main findings for the analysis are that the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (20) = 74.43, p= .000. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=. 68). The results showed

that there was significant effect of different light treatments F (20.61, 222.59) =147.87, p=.000, on the growth rate of *Myrtus* seedlings over time (Figure 2). Time effect was more intense at the begging of the experimental period, at the middle (4th week) and at the end (7th week). More specifically at the first week into the growth chambers seedlings grown under the FL light (2.11 mm) and the L20AP67 (2.07 mm) showed significantly higher growth rate than the rest of LED treatments (p<. 000). At the fourth week the situation was different while seedlings grown under all LEDs showed higher growth rate than under the FL; especially under the AP67 light with 1.15 mm that differed significantly with the FL (0.59 mm). Finally a significant decrease (almost the half) was observed for the growth rate of all the LED treatments except from the L20AP67 (1.33 mm) and the FL light (1.21 mm).

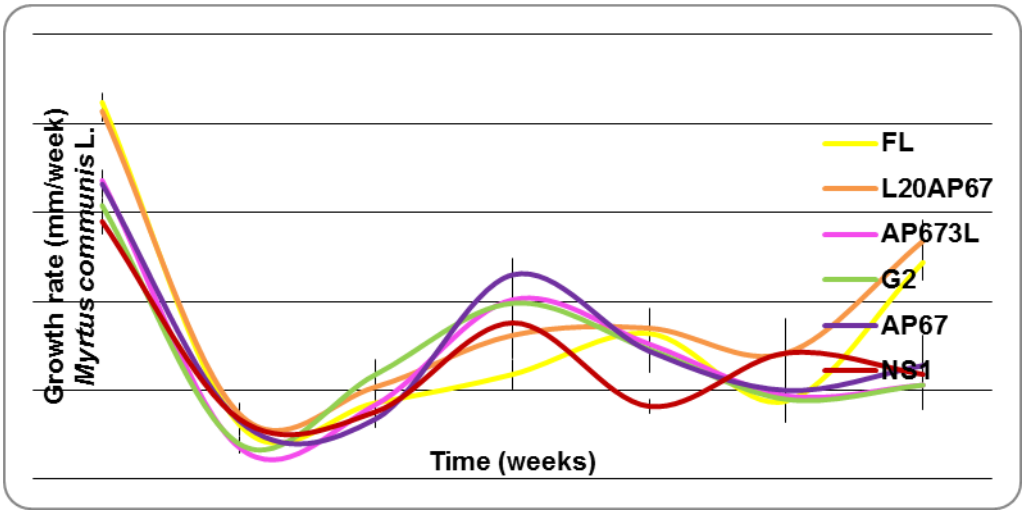


Figure 2. The growth rate of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 7 week experimental period.

***Abies borisii-regis* Mattf.**

The main findings for the analysis are that the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (9) = 89.72, p= .000. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=. 57). The results showed that there was significant effect of different light treatments F (11.40, 123.54) =1099.92, p=.000, on the growth rate of *Abies* seedlings over time (Figure 3) more specifically at the begging of the experimental period and at the third week. At the first week seedlings grown under the L20AP67 (4.58 mm) and the FL (4.27 mm) lights showed significantly higher growth rate than the AP67 (3.42 mm) (p<. 001), NS1 (3.51 mm) and the AP673L (3.65 mm) (p<. 0.13). Also at the third week significantly higher growth rate was found for the seedlings grown under the NS1 light with 0.57 mm compared to the L20AP67 with 0.14 mm (p<. 018).

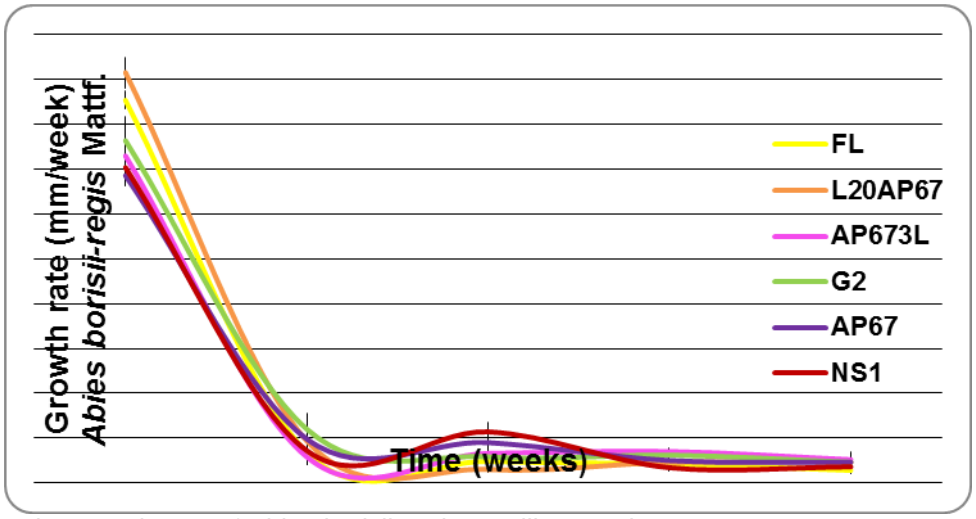


Figure 3. The growth rate of Abies borisii-regis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 5 week experimental period.

***Myrtus communis* L. (second time)**

According to the results Mauchly's test indicated the assumption of sphericity had not been violated, χ^2 (5) = 8.68, p= .123. The results showed that there was significant effect of different light treatments F (15, 162) =343.151, p=.000, on the growth rate of *Myrtus* seedlings over time specifically at the first, fifth and the seventh week of measurements. At the begging myrtus seedlings showed significantly higher growth rate under the effect of FL light (1.32 mm) compared to the rest of the light treatments except from the L20AP67 (1.22 mm). However, significant differences also found among the LED treatments where NS1 light (0.88 mm) showed lower growth rate than the G2 (1.12 mm) (p<. 000) and the AP67 (1.07 mm) (p<. 006). Further at the fifth week height growth rate was significantly higher under the G2 illumination (0.88 mm) compared to the FL (0.52 mm), AP673L (0.55 mm) and the NS1 (0.40 mm) (p<. 000) (Figure 4). Also the same reaction was observed under the AP67 light (0.77 mm) compared to the FL and the NS1 lights (p<. 000). Additionally L20AP67 light (0.76 mm) showed also significantly higher growth rate than the NS1 light (p<. 000). At the seventh week of the experimental period L20AP67 illumination (0.93 mm) showed significantly higher growth rate compared to all the light treatments (p<. 000). Further FL light (0.58 mm) differed significantly for the height growth rate only from the NS1 LED light (0.32 mm) (p<. 008).

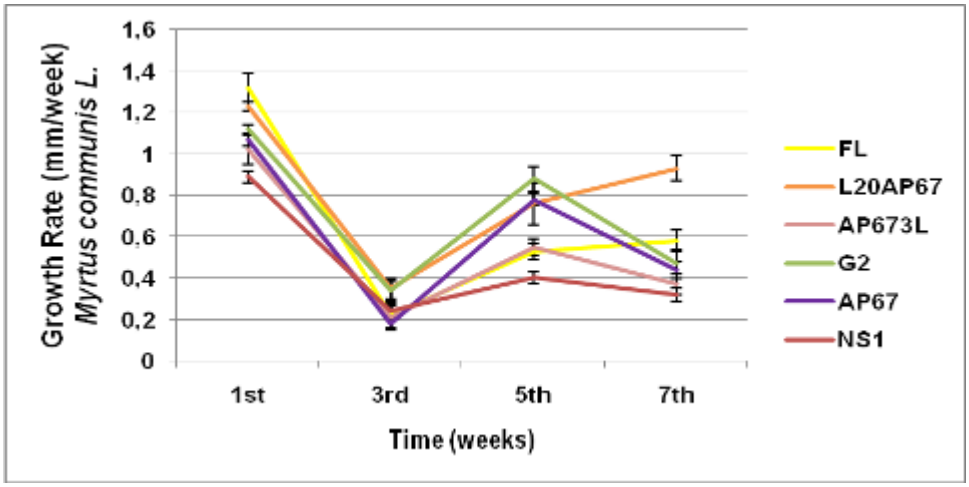


Figure 4. The growth rate of Myrtus communis (second time) seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 5 week experimental period.

***Platanus orientalis* L.**

The main findings for the analysis are that the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (5) = 13.68, p= .018. Therefore degrees of freedom were corrected using Huynh- Feldt estimates of sphericity (ε=1). The results showed that there was significant effect of different light treatments F (15, 162) =344.57, p=.000, on the growth rate of *Platanus* seedlings over time (Figure 5) more specifically at the first and the fifth week of measurements. At the begging of the experimental period seedlings of *Platanus* showed significantly higher growth rate under the effect of FL light (1.51 mm) only compared to the AP673L LED light (1.05 mm) (p<. 002). The situation was altered at the fifth week where seedlings grown under the illumination of the AP67 LED (0.64 mm) showed significantly higher growth rate almost double compared to the FL light (0.33 mm) (p<. 019).

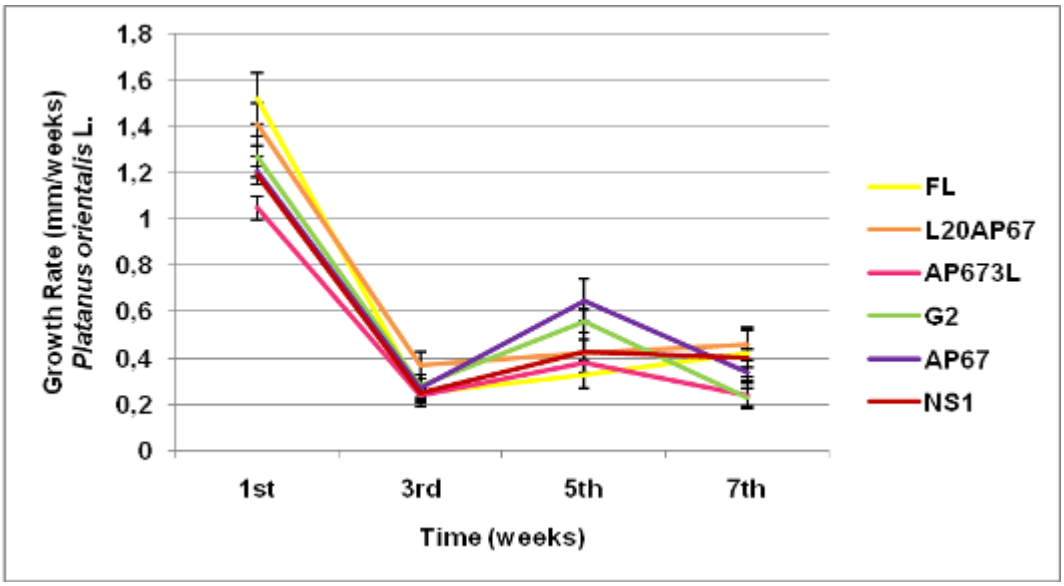


Figure 5. The growth rate of Platanus orientalis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 7 week experimental period.

***Picea abies* Karst.**

The main findings for the analysis are that the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (5) = 33.86, p= .000. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=. 73). The results showed that there was significant effect of different light treatments F (11.06, 119.50) =864.34, p=.000, on the growth rate of *Picea* seedlings only at the begging of the experimental period (Figure 6.) Specifically height growth rate of the *Picea* seedlings was significantly higher under the illumination of the L20AP67 (2.29 mm) compared to the rest of LED treatments (p<. 000) except from the FL light (2.06 mm). LED treatments such as G2, AP67 and NS1 showed approximately the same growth rate (1.56 mm) at the first week, while AP673L showed in average 1.74 mm.

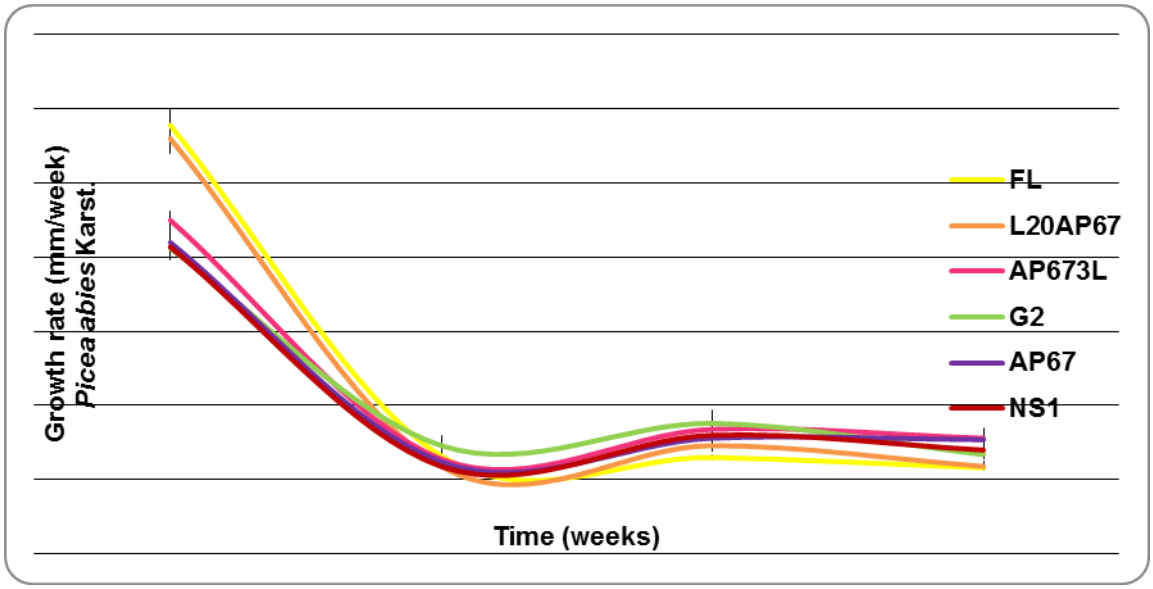


Figure 6. The growth rate of Picea abies seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 7 week experimental period.

***Pinus sylvestris* L.**

Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (5) = 48.27, p= .000. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=. 59). The results showed that there was significant effect of time F (7.1, 79.89) =1182.54, p=.016, on the growth rate of *Pinus* seedlings only at the begging of the experimental period (Figure 7.) that explained from the significant difference between the FL light (2.39 mm) and the NS1 (1.94 mm) (p<. 014). No significant differences found between the light treatments for the rest of the time measured. Height growth rate was approximately the same for all the light treatments 0.64 mm.

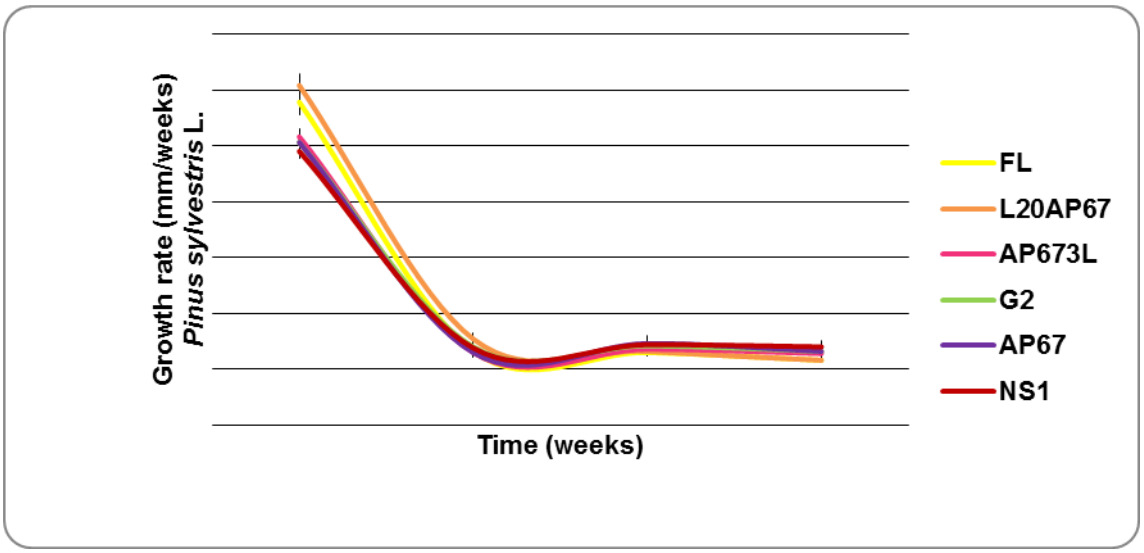


Figure 7. The growth rate of Pinus sylvestris seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size during the 7 week experimental period.

2.2.2. Performance of LED spectra on leaf/needle colour and formation compared to control light

***Arbutus unedo* L.**

The main findings for the analysis are that the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (20) = 188.27, p= .000. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=. 51). The results showed that there was significant effect of different light treatments F (15.44, 166.83) =84.11, p=.000 on the leaf colour of *Arbutus* seedlings over time. Specifically the AP673L LED light showed significantly different colour rating during the first five weeks. Seedlings appeared with light green leaves (p< .001) compared to the dark green leaves formed under the FL, L20AP67, G2, AP67, NS1 lights. However during the last two weeks seedlings of *Arbutus unedo* grown under the effect of AP673L, G2, AP67 and NS1 appeared with reddish leaf colour compared to the dark green colour formed under the FL and L20AP67 lights (Figure 8). Further the results shown significant differences for the formation of leaves almost every week, F (13.80, 149.08) =474.20, p=.014. At the begging seedlings formed two leaves under all the light treatments; however at the second week LED treatments such as AP673L, G2, AP67 and NS1 induced four leaves (p<. 000) compared to the FL light that still induced two. Further at the third week G2 LED induced six leaves (p<. 030) formed and significant differences found with the FL, L20AP67 and NS1 lights where seedlings formed four leaves. At the fourth week FL and L20AP67 lights induced four to five leaves but still differed significantly from the LEDs AP673L (p<. 024), G2 (p<. 000) and AP67 (p<. 012). Following at the fifth week seedlings under the FL light formed five leaves and significant differences found with those grown under the AP673L (p<. 021), G2, AP67 (p<. 006) and the NS1 (p<. 034) that formed seven leaves. At the end of the experimental period seedlings of *Arbutus* grown under the FL light formed six leaves and significant differences found with those grown under the AP673L (p<. 028), G2, AP67, NS1 (p<. 004) that formed eight to nine leaves (Figure 8).

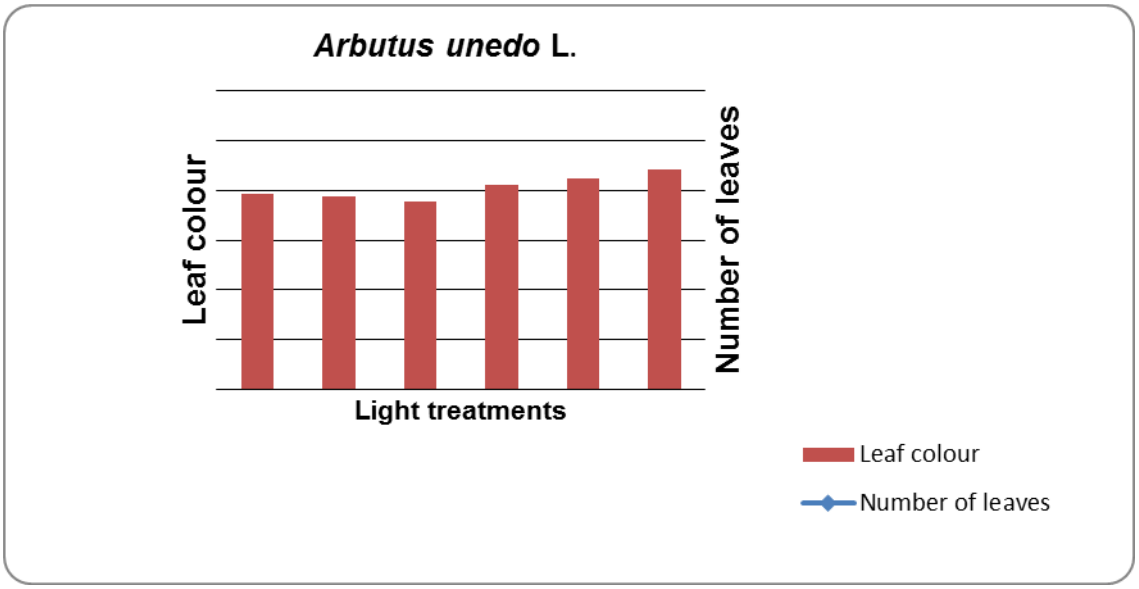


Figure 8. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Arbutus unedo seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 7th week of the experimental period.

***Myrtus communis* L.**

The Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (20) = 72.75, p= .000. Therefore degrees of freedom were corrected using Huynh-Feldt estimates of sphericity (ε=. 83). The results showed that there was not significant effect of different light treatments over time and eventually the leaf colour of myrtus seedlings was characterized dark green. However there was significant effect of different light treatments over time for the leaf formation, F (17.49, 188.90) =635.21, p=.000. At the begging myrtus seedlings had formed two leaves under all the light treatments. At the second week FL and L20AP67 lights induced two to three leaves formed and differed significantly with AP673L, G2, (p<. 001), AP67 (p<. 006), NS1 (p<. 000) that formed eight to four to five leaves. Further at the third week myrtus seedlings grown under the FL and L20AP67 lights formed four leaves and significant differences found with those grown under the G2 (p<. 000) and the AP67 (p<. 001) that formed six leaves. At the fourth week FL light induced five leaves formed and significant difference found with the AP67 LED that induced seven leaves. At the fifth week FL light induced six leaves and significant differences found with the LEDs AP673L (p<. 009) (seven leaves formed) and G2, AP67 (p<.000) that induced eight leaves formed. L20AP67 LED light also induced six leaves and differed significantly from the G2, AP67 (p<.000). Finally at the last week seedling grown under the FL light (induced seven leaves) differed significantly with all the LEDs (induced nine to ten leaves) for the leaf formation (Figure 9).

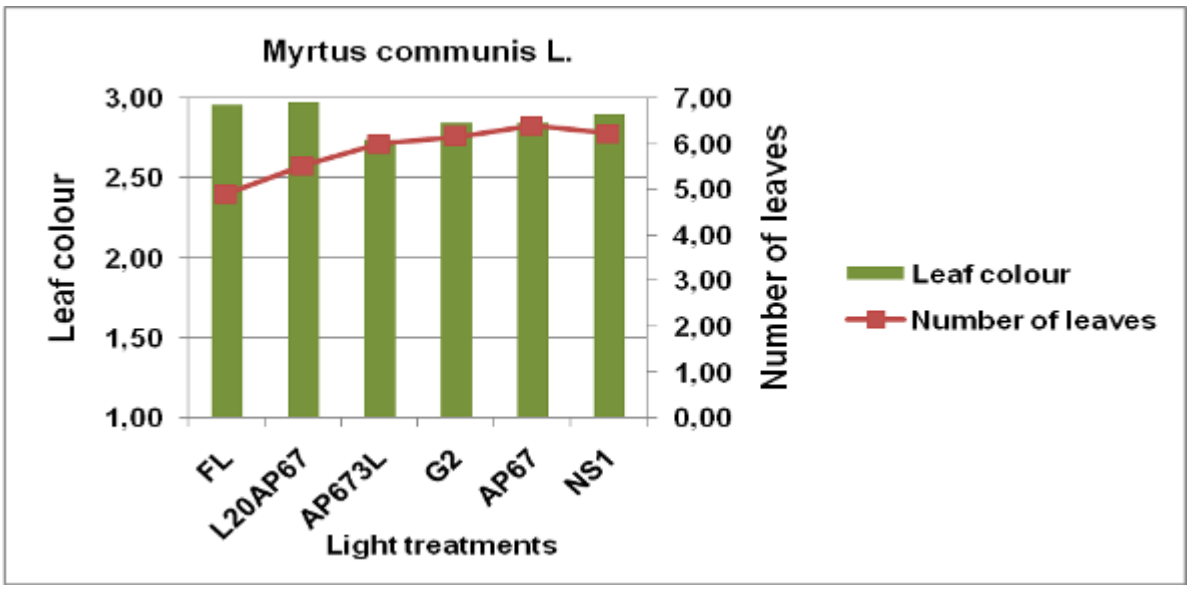


Figure 9. The leaf colour rating (1=pale, 2=light green,3=dark green) and number of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 7th week of the experimental period.

***Abies borisii-regis* Mattf.**

The results showed that there was significant effect of different light treatments over time F (9.57, 103.40) =2.91, p=.003. Significant differences for the needle colour of *Abies* seedlings were found at the fourth and the fifth week where, AP673L LED showed significantly more light green needlles compared to the rest of the light treatments that induced dark green needles (Figure 10). That was not the case for the needle formation of *Abies* seedlings where no significant differences found between the light treatments over time. Less number of needles were formed under the G2 and FL illumination with six needles in an average scale and more under the NS1 LED that induced seven needles formed (Figure 10).

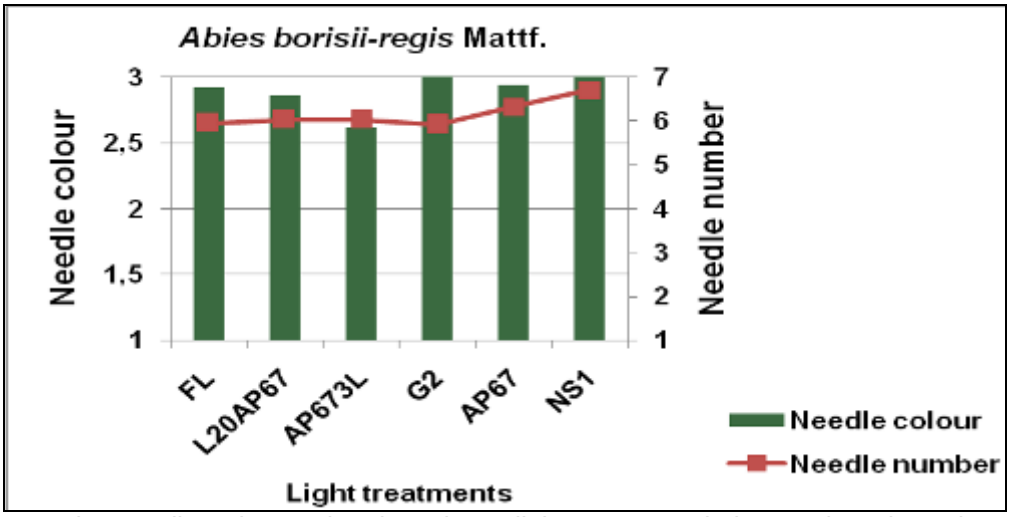


Figure 10. The needle colour rating (1=pale, 2=light green, 3=dark green) and number of Abies borisii-regis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 5th week of the experimental period.

***Myrtus communis* L. (second time)**

According to the results no significant differences found between the light treatments over time for the myrtus seedlings for the leaf colour. The effect of the different light treatments was the same and induced dark green leaves (Figure 11). There was significant differences for the leaf formation over time F (15, 162) = 798.92, p=. 000. At the begging *myrtus* seedlings formed significantly more leaves almost double under the illumination of the G2 light (four leaves formed) compared to all the light treatments. At the second week of the experiment FL light induced four leaves and significant differences found with all the LEDs that induced six to seven leaves. At the third week FL and NS1 light induced six leaves and significant differences found with the rest of the light treatments that induced eight leaves. At the end seedlings grown under the FL light formed seven leaves and significant differences found with the G2 (p<. 010) and the AP67 (p<. 002) that formed nine leaves. Further AP67 light differed significantly with the NS1 LED (p<. 010) (Figure 11).

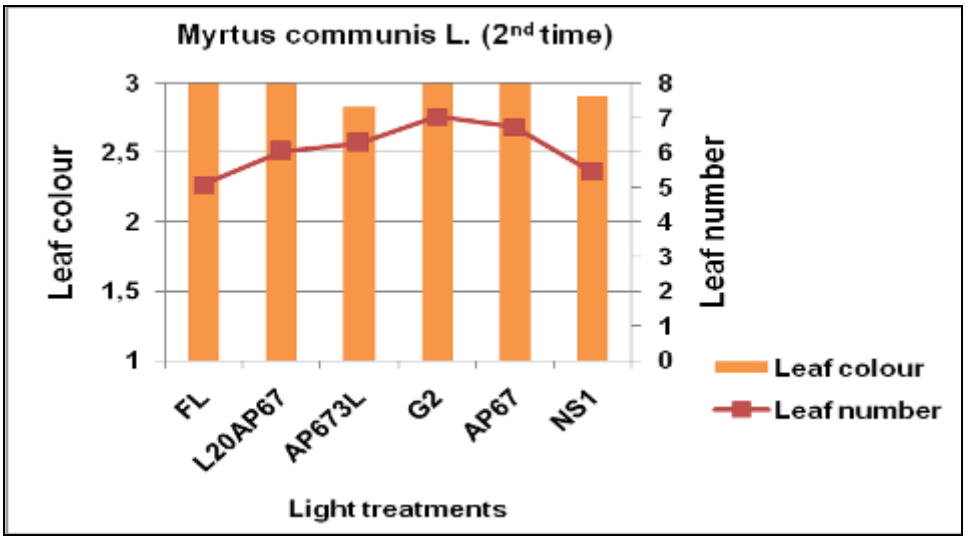


Figure 11. The leaf colour rating (1=pale, 2=light green,3=dark green) and number of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 7th week of the experimental period.

***Platanus orientalis* L.**

The main findings for the analysis are that the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (5) = 114.63, p= .000. Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=. 44). The results showed that there was significant effect of different light treatments F (6.72, 72.65) =37.20, p=.000 on the leaf colour of *Platanus* seedlings only at the end of the experimental period. Specifically FL and L20AP67 lights induced dark green colour on the *Platanus* seedlings leaves and significant differences found both with AP673L (p<. 000) and NS1 (p<. 034) that induced reddish colour on the leaves (Figure 12). Also significant differences found for the leaf formation over time F (15, 162) = 654.772, p= .000. Specifically at the first week of the experimental period FL and NS1 (p<. 035), L20AP67 (p<. 007) lights induced two leaves formed and significant differences found with AP67 and G2 LEDs that induced three to four leaves. At the second week FL light induced three leaves and significant differences found with the LEDs such as the L20AP67 and AP67 (p<. 008), G2 (p<. 001) that induced four to five leaves (Figure 12).

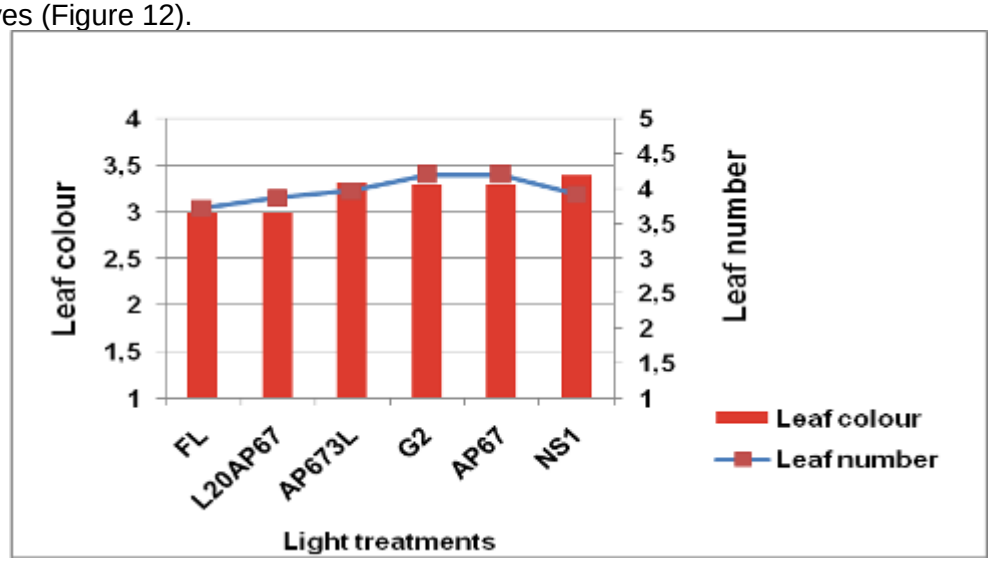


Figure12. The leaf colour rating (1=pale, 2=light green,3=dark green 4=reddish) and number of Platanus orientalis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 7th week of the experimental period.

***Picea abies* Karst.**

According to the results no significant differences found between the light treatments over time for the needle colour of *picea* seedlings that were characterized dark green (Figure 13). However significant differences found for the needle formation over time F (13.11, 141.63) = 715.44, p= .000. At the begging of the experiment all seedlings formed seven to eight needles regardless the light treatment. At the third week seedlings grown under the FL light induced fifteen needles and significant differences found with all the LEDs (p<. 000) that induced twenty four to thirty three needles (AP67 and NS1 lights). Also significant differences found between the AP67 light that formed thirty three needles compared to L20AP67 (p< .039) that induced twenty four. Finally at the seventh week of the experiment FL light induced twenty three needles and significant differences found with all the LEDs (p<. 000), AP67 (forty four), AP673L and NS1 (thirty nine), G2 (thirty eight) and L20AP67 (thirty six) (Figure 13).

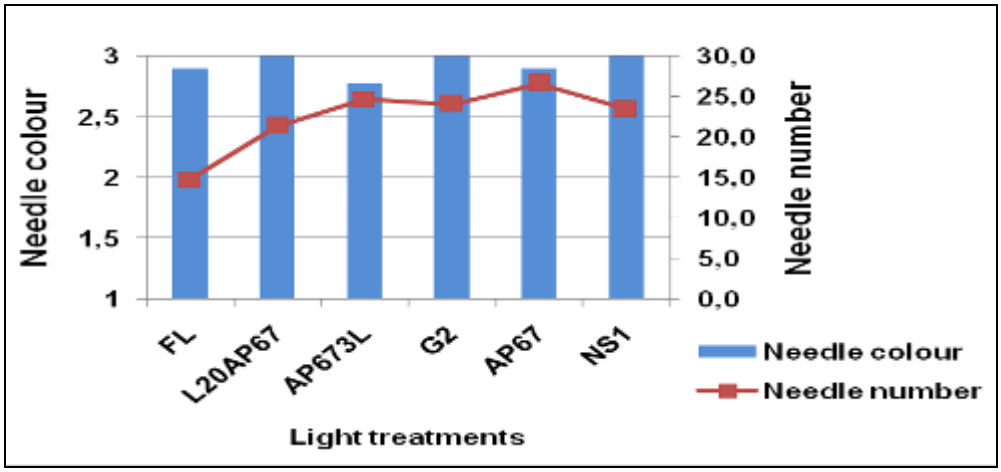


Figure 13. The needle colour rating (1=pale, 2=light green,3=dark green) and number of Picea abies seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 7th week of the experimental period.

***Pinus sylvestris* L.**

No significant differences found for the needle colour of *Pinus sylvestris* seedlings between the light treatments over time. It was characterized dark green for all the lights (Figure 14). Further the Mauchly's test indicated the assumption of sphericity had been violated, χ^2 (5) = 27.62, p= .000. Therefore degrees of freedom were corrected using Huynh-Feldt estimates of sphericity (ε=. 84). The results showed that there was significant effect of different light treatments over time for the needle formation of *pinus* seedlings F (10.17, 114.45) =878.83, p=.000. At the first week of measurements seedlings under G2 light induced eight needles formed and significant differences found with the FL and NS1(five needles) (p<. 001) and the AP67 (six needles) (p<. 015). Following at the third week seedlings grown under the G2 and AP67 LEDs induced significantly more needles (nineteen) and significant differences found with those grown under the FL (twelve needles) (p<. 000), AP673L (thirteen) (p<. 000) and NS1 (fourteen) (p<. 000). At the third week G2 and AP67 LEDs that induced thirty needles formed continued to differed significantly with the FL (eighteen needles) (p<. 000), AP673L (twenty two) (p<. 000) and NS1 (twenty five) (p<. 022). Finally G2 and AP67 LEDs induced thirty five needles and significant differences found with the FL (twenty six) (p<. 000), AP673L (twenty eight) (p<. 001) and the NS1 (thirty needles) (p<. 013) (Figure 14).

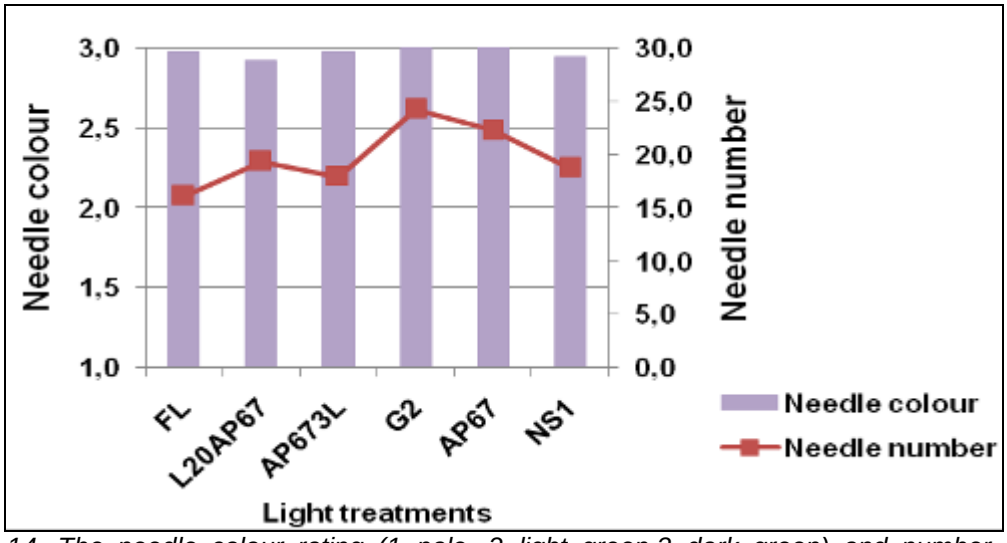


Figure 14. The needle colour rating (1=pale, 2=light green,3=dark green) and number of Pinus sylvestris seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the 7th week of the experimental period.

2.2.3. Performance of LED spectra on shoot and root development compared to control light

***Arbutus unedo* L.**

According to the results significant differences found both for the shoot height and the root length (p<. 000) of *Arbutus* seedlings at the end of the cultivation period into the growth chambers. Specifically for the shoot height, seedlings grown under the L20AP67 light (32.11 mm) showed significantly taller seedlings compared to those grown under the AP67 (25.67 mm) (p<. 009) and the NS1 (26.06 mm) (p<. 018). FL light (30.88 mm) also induced taller seedlings than all LEDs (except from L20AP67) but no significant differences found. For the root length FL light (56.28 mm) induced the shortest roots compared to LEDs and significant differences found such as with the G2 (87.45 mm) (p<. 000), NS1 (84.44 mm) (p<. 000), AP67 (81.62 mm) (p<. 001) and the AP673L (76.22 mm) (p<. 022). L20AP67 light showed an average value of 69.11 mm for the root length, however no significant difference found with the rest of light treatments (Figure 15).

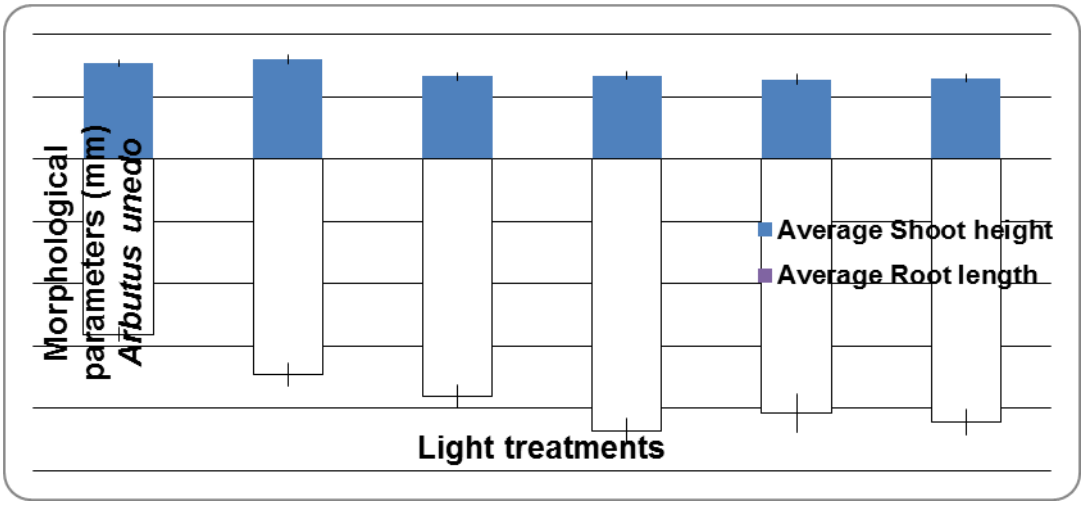


Figure 15. Morphological parameters (mm) of Arbutus unedo seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Myrtus communis* L.**

Myrtus communis seedlings showed significant differences for both of morphological parameters tested. FL light (44.12 mm) induced significantly higher seedlings compared to NS1 (32.06 mm) (p<. 001), G2 (32.91 mm) (p<. 002), AP673L (33.70 mm) (p<. 004) and AP67 (35.56 mm) (p<. 036). Also L20AP67 light (44.66 mm) showed the tallest seedlings among the light treatments and significant differences found with NS1 (p<. 000), G2 (p<. 001), AP673L (p<. 002) and AP67 (p<. 020). Root length was found significantly higher under the illumination of AP67 light with 77.02 mm and significant differences found with the FL light (51.18 mm) (p<. 000), L20AP67 (54.13 mm) (p<. 001) and the NS1 LED (59.42 mm) (p<. 019) (Figure 16). As for the G2 and AP673L LEDs showed average values of 63.91 mm and 64.79 mm, respectively.

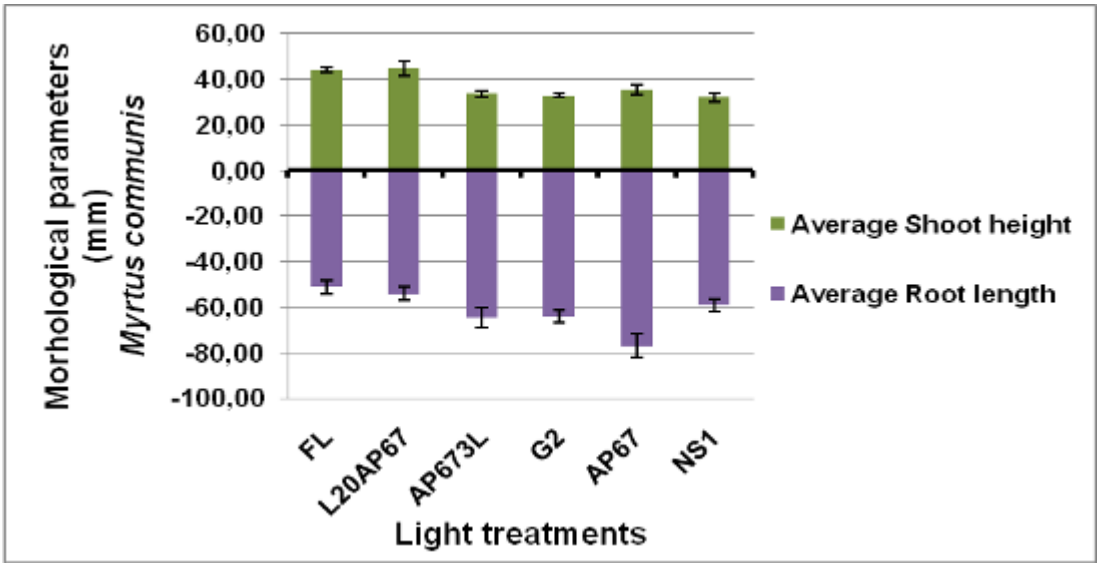


Figure 16. Morphological parameters (mm) of Myrtus communis seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Abies borisii-regis* Mattf.**

Significant differences found for both of the morphological parameters tested of *Abies* seedlings grown in different light environments (p<. 000). L20AP67 LED induced the tallest seedlings with 41.48 mm shoot height compared to the rest of the LEDs and significant differences found with the AP67 (27.18 mm), NS1 (28.9 mm), G2 (29.83 mm) and AP673L (31.28 mm) (p<. 000). Further FL light also induced taller seedlings with 37 mm shoot height than those grown under the AP67 (p<.000), NS1 (p<. 001) and G2 (p<. 005) (Figure 17). Root length of *Abies* seedlings was found significantly longer under the NS1 LED with 77.57 mm only compared to the FL and L20AP67 lights with 48.34 mm and 52.09 mm (p<. 000), respectively. As for the rest of LEDs such as AP67, G2 and AP673L induced average values for the root length 67.40 mm, 66.85, 61.79 mm, respectively. (Figure 17).

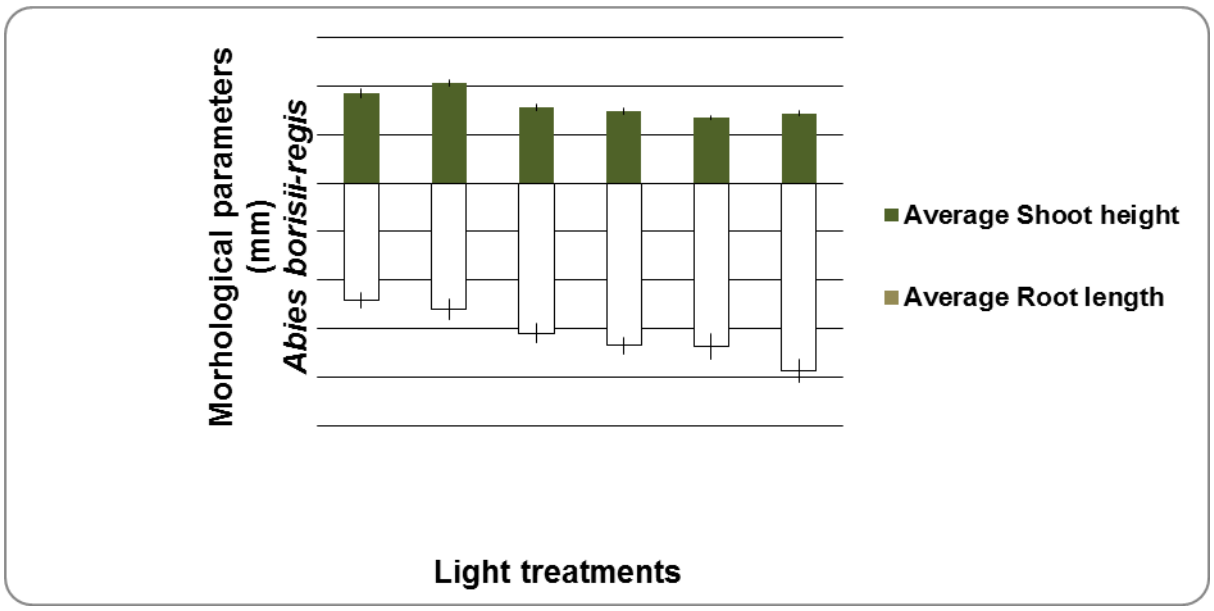


Figure 17. Morphological parameters (mm) of Abies borisii-regis seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Myrtus communis* L. (second time)**

For both morphological parameters significant differences were found between the different light treatments for *Myrtus* seedlings (p<. 000). L20AP67 LED light induced significantly taller seedlings with an average of 45.23 mm compared to the rest of LEDs such as NS1, AP673L, AP67 with 27.47 mm, 30.15 mm, 33.48 mm (p<. 000) respectively, and G2 with 37.91 mm (p<. 013). Also FL light showed significantly taller seedlings with 40.71 mm compared to NS1, AP673L (p<. 000) and AP67 (p<. 021) (Figure 18). Root length obtained under the FL light was significantly lower than the AP67 with 61.89 mm, AP673L with 61.51 mm (p<. 000) and the G2 with 61 mm (p<. 003). Further significant differences for the root length were found among LEDs such as the AP67 (p<. 020) and the G2 (p<. 0.35) with the NS1 LED light that shown lower value. Seedlings grown under the L20AP67 LED showed an average root length of 48 mm (Figure 18).

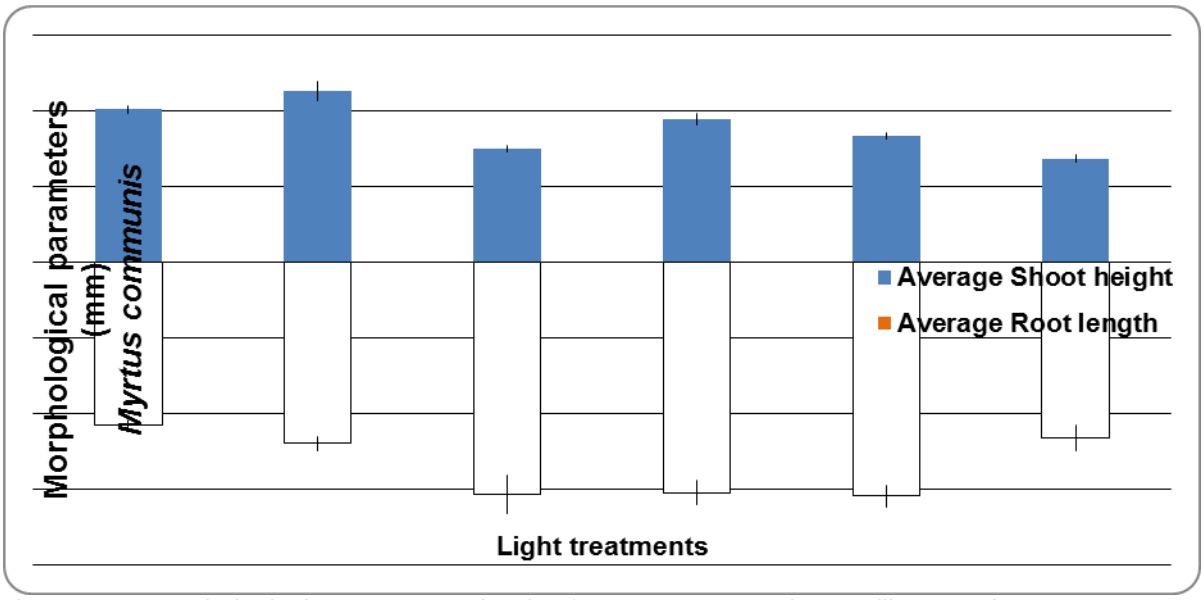


Figure 18. Morphological parameters (mm) of Myrtus communis seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Platanus orientalis* L.**

For the shoot height of *Platanus orientalis* seedlings grown under the effect of L20AP67 light (39.22 mm) differed significantly with those grown under the AP673L LED (26.95 mm) (p<. 019). AP67, FL, NS1 and G2 lights showed average shoot height of 36.49 mm, 34.33 mm, 31.33 mm and 28.92 mm, respectively (Figure 19). FL light obtained the lowest average root length of 37.29 mm and significant differences found with all LEDs (p<. 000) except from the L20AP67 that shown an average value of 50.96 mm. On the other hand AP67 LED illumination obtained the highest average root length value of 68.46 mm and significant differences found also with the L20AP67 (p<. 007). The rest of LEDs such as NS1, AP673L and G2 showed average root length values of 61.59 mm, 58.72 and 57.34 mm, respectively (Figure 19).

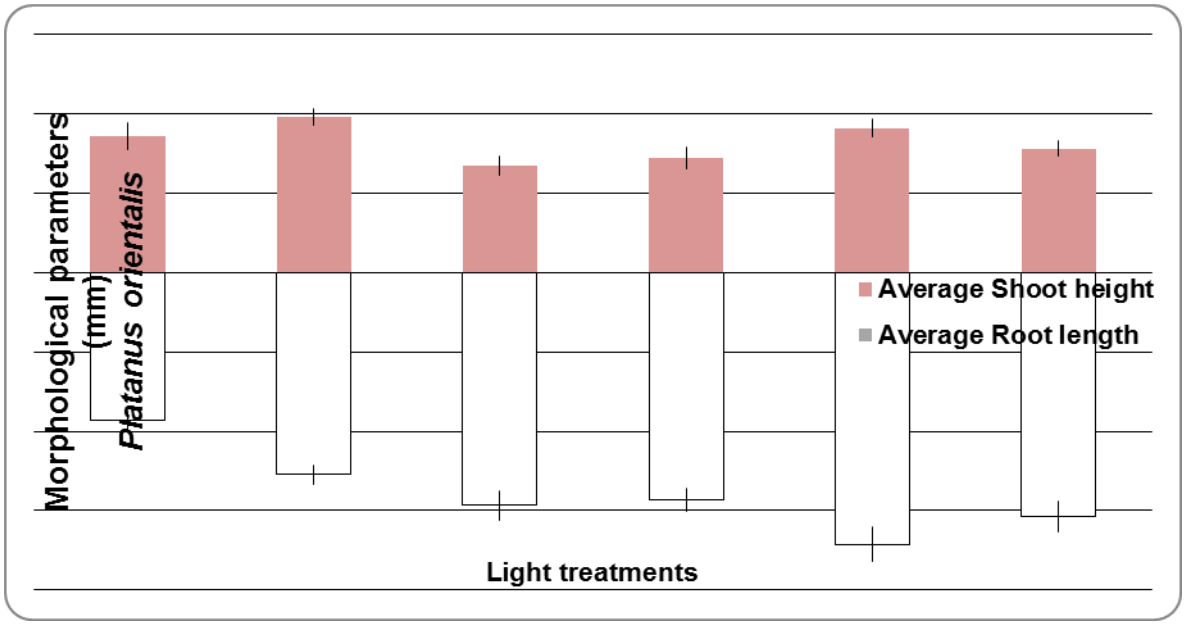


Figure 19. Morphological parameters (mm) of Platanus orientalis seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Picea abies* Karst.**

For both morphologocal parameters tested of *Picea* abies seedlings significant differences were found (p<. 000). Specifically L20AP67 LED obtained significanly taller seedlings with an average value of 33.90 mm compared to AP67 with 23.98 mm (p<. 000), NS1 with 25.85 mm (p<. 003), AP673L with 26.05 mm (p<. 002) and G2 with 26.74 mm (p<. 005) LEDs. Also FL light obtained significantly taller seedlings with 33.37 mm than AP67 (p<. 000), NS1 (p<. 001), AP673L (p<. 004) and G2 with 26.74 mm (p<. 013) LEDs. However FL light induced the shortest roots of 40.51 mm and significant differences found with all LED treatments (p<. 000) except from L20AP67 with 47.19 mm. significant differences also found between the L20AP67 and the NS1 with 73.75 mm (p<. 000), AP67 with 68.34 (p<.005), G2 with 67.31 mm (p<. 010)and AP673L with 66.63 (p<. 013) (Figure 20).

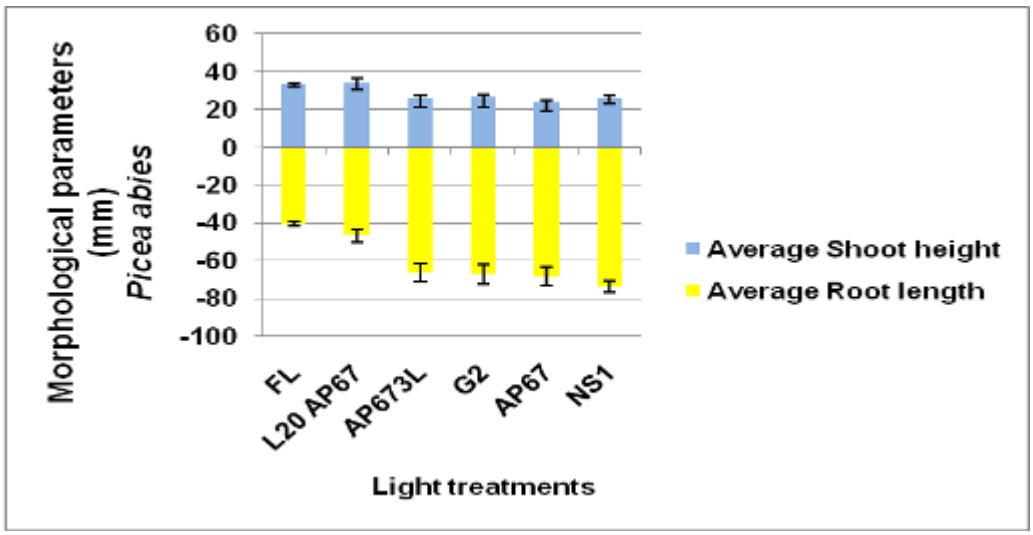


Figure 20. Morphological parameters (mm) of Picea abies seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Pinus sylvestris* L.**

No significant differences found for the shoot height, however numerically greater average value was found for the G2 LED with 42.16 mm, following by FL, L20AP67, AP67, AP673L and NS1 with 41.82 mm, 38.87 mm, 33.61 mm, 33.54 mm and 33.18 mm, respectively. On the other hand for the root length significant differences between the light treatments were found (p<. 000) Specifically FL light showed significantly lower average value of 35.5 mm compared to all LEDs (p<. 000) except from the L20AP67 with 52.14 mm; the longest roots found under the NS1 LED with 104.17 mm, following the G2, AP67 and AP673L with 89.31 mm, 82.21 mm and 81.28 mm, respectively (Figure 21).

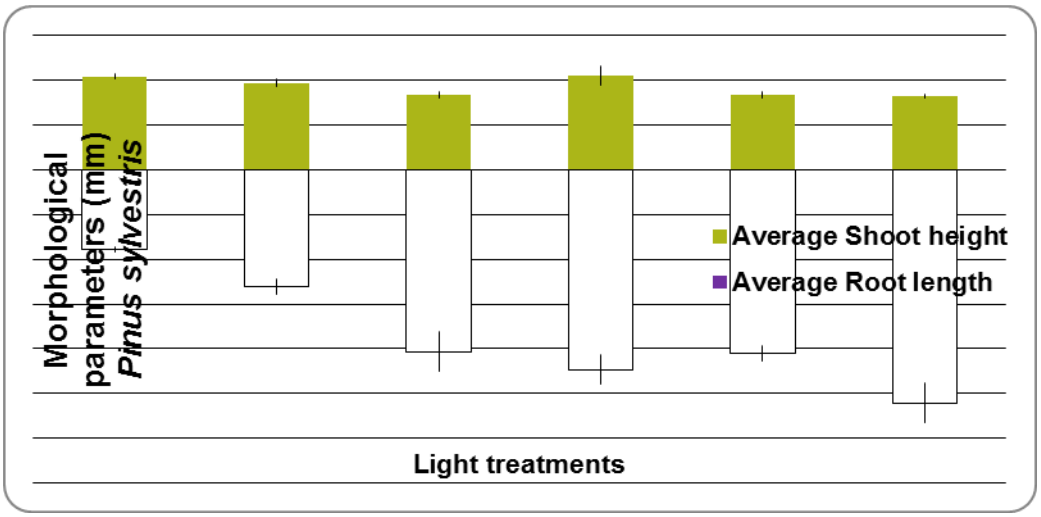


Figure 21. Morphological parameters (mm) of Pinus sylvestris seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

2.2.4. Performance of LED spectra on dry weight of leaves, shoots and roots compared to control light

***Arbutus unedo* L.**

Examining the response of *Arbutus unedo* seedlings under the different light treatments was found significant lower average value for the DWL under the FL light with 0.0092 g (p< .000) and the L20AP67 with 0.0167 g (p<. 000) compared to LEDs AP67, AP673L, G2, NS1 with average values of 0.0372 g, 0.0335 g, 0.0334g and 0.0289 g, respectively (Figure 22). For the DWS also significant lower average values found under the FL with 0.0026 g and the L20AP67 with 0.0029 g (p<. 000) compared to LEDs G2, AP67, AP673L and NS1 with 0.0051 g, 0.0049 g, 0.0048 g, 0.0047 g, respectively. The same reaction also found for the DWR where both FL light with 0.0025 g (p< .000) and the L20AP67 with 0.0060 g (p<. 000) induced lower dry weight mass compared to AP67, G2, NS1, AP673L with 0.016 g, 0.015 g, 0.014 g, 0.013 g, respectively.

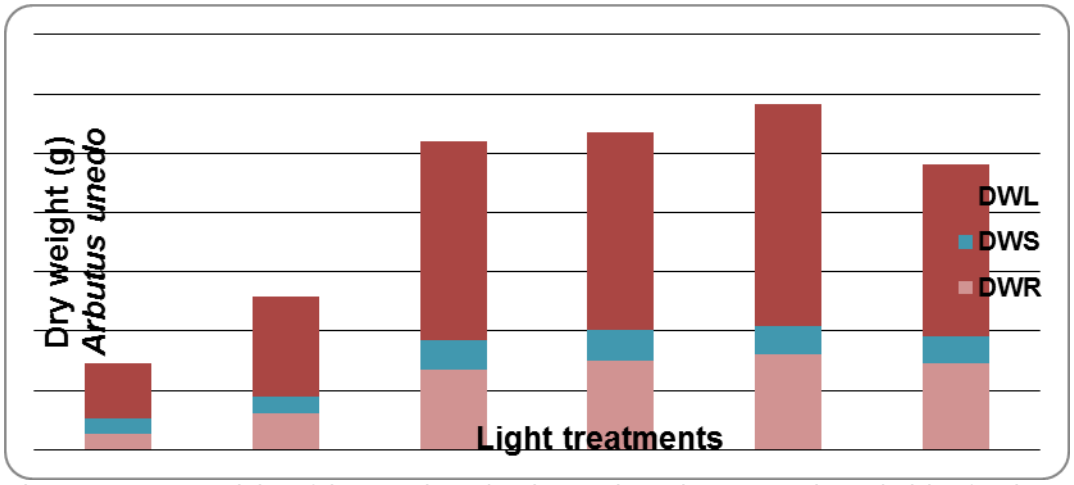


Figure 22. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Arbutus unedo seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Myrtus communis* L.**

FL light induced significantly lower average value for the DWL such as 0.009 g than the AP67 with 0.028 g (p<. 000), AP673L with 0.021 g (p<. 001) g, G2 with 0.020 g (p<. 003) and NS1 with 0.019 g (p<. 019). Also AP67 light differed significanty with L20AP67 with 0.014 g (p<. 000) and the NS1 (p<. 026). For the DWS significant differences found only between the FL and the AP67 LED (p<. 003) light that shown an average of 0.002 g and 0.004 g, respectively. For the DWR both FL and L20AP67 lights showed significantly lower average values of 0.002 g and 0.003 g, respectively contrast to LEDs AP67 with 0.011 g, G2 and AP673L with 0.008 g and the NS1 with 0.007 g (p<. 000) (Figure 23). Myrtus communis seedlings favored more for the dry weight mass of the seedlings under the AP67 LED illumination.

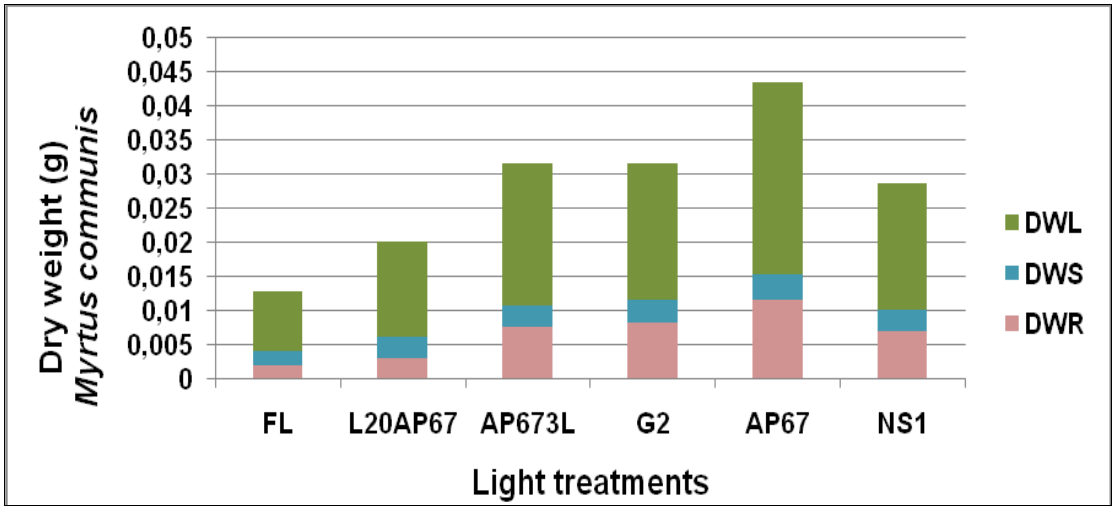


Figure 23. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Myrtus communis seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Abies borisii-regis* Mattf.**

For the DWL of *Abies* seedlings FL light and L20AP67 LED induced significantly lower average values of 0.015 g and 0.018 g compared to G2 with 0.046 g, NS1 with 0.042 g,

AP673L with 0.040 g and AP67 with 0.039 g (p<. 000) (Figure 24). Significanlty higher average values for the DWS were induced under the G2 and AP673L LEDs with 0.009 g only compared to the FL light with 0.006 g (p<. 002). Also for the DWR significalty lower average values of 0.005 g and 0.007 g were found for the FL and L20AP67 LED compared to the rest of LED lights G2 with 0.021 g, AP673L with 0.018 g, AP67 and NS1 0.017 g (Figure 24).

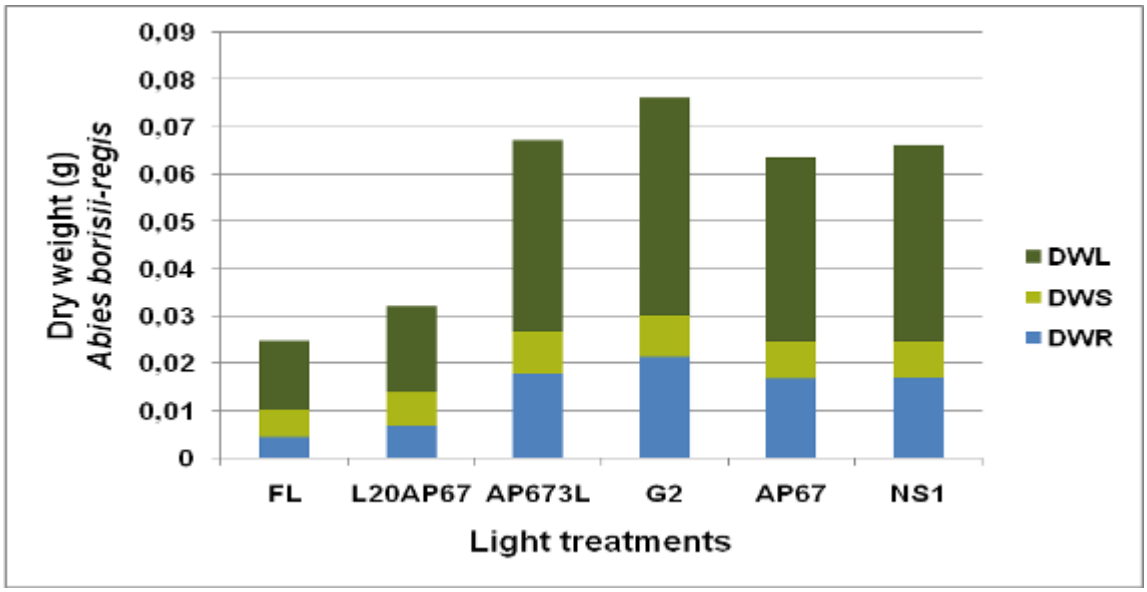


Figure 24. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Abies borisii-regis seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Myrtus communis* L. (second time)**

FL light obtained significantly lower average of 0.006 g value for the DWL compared to all LED treatments except from the NS1 that shown an average value of 0.009 g. Under G2 LED illumination obtained the highest average value for the DWL with 0.020 g and significant differences found with NS1, AP673L with 0.009 g, 0.015 g (p<. 000); also with the AP67 that shown 0.016 g (p<. 013). Further it should be mentioned that AP673L and AP67 LEDs also differed significantly with the NS1 (p<. 000). G2 LED obtained significantly higher average value of 0.004 g for the DWS compared to the FL NS1 and AP673L with 0.002 g (p<. 000) and with L20AP67 0.003 g (p<. 008). Additionally AP67 LED also differed significantly with FL (p<. 005) and NS1 (p<. 014) for the same parameter. Finally for the DWR significantly lower average values were obtained under the FL and L20AP67 lights with 0.001 g and 0.002 g compared to the rest of LEDs (p<. 000), such as G2, AP673L with 0.006 g, AP67 with 0.005 g and the NS1 with 0.004 g. Also LEDs of G2 and AP673L had significantly higher average value than the NS1 (p<. 000) (Figure 25).

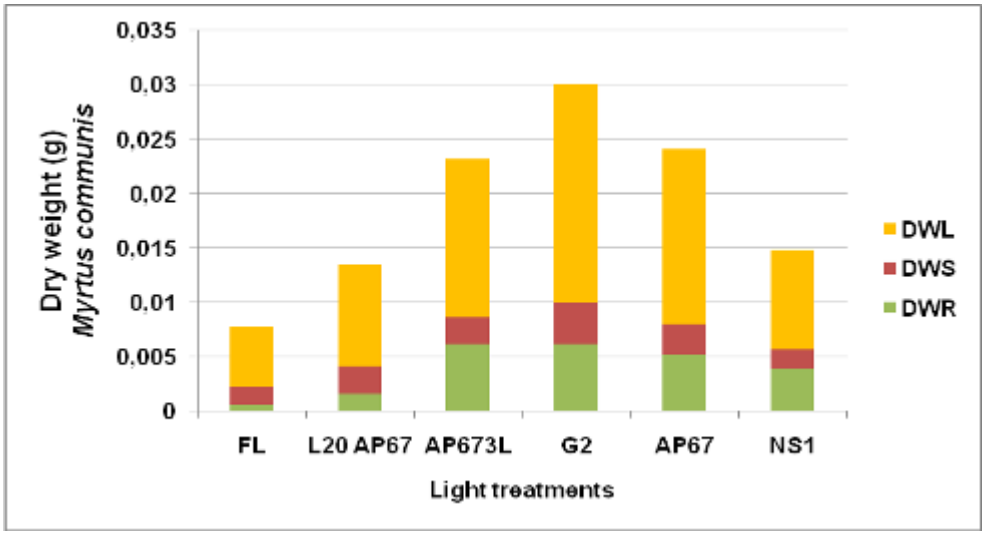


Figure 25. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Myrtus communis seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Platanus orientalis* L.**

FL light induced the lowest average value of 0.008 g for the DWL and significant differences found with LEDs of AP67, G2 and AP673L with 0.025 g, 0.024 g, and 0.022 g (p<. 000), and with the NS1 LED with 0.017 g (p<. 003). Further L20AP67 showed an average value of 0.015 g and also differed from those recorded under the AP67 (p<.000), G2 (p<. 004) and AP673L (p<. 026). FL light also obtained the lowest average value of 0.002 g for the DWS and significant differences found with the AP67 with 0.005 g (p<. 000) and the NS1 with 0.004 g (p<. 029). Also L20P67 LED obtained significantly lower average value of 0.003 g only compared to the AP67 LED (p<. 025). Finally for the DWR lowest average value was found under the FL light with 0.001 g and significant differences found with all LEDs (p<. 000) except from L20AP67 that had 0.005 g. G2 and AP67 LED illuminations with 0.010 (p<. 005) g and 0.011 g (p<. 002) showed significant differences with the L20AP67 (Figure 26)

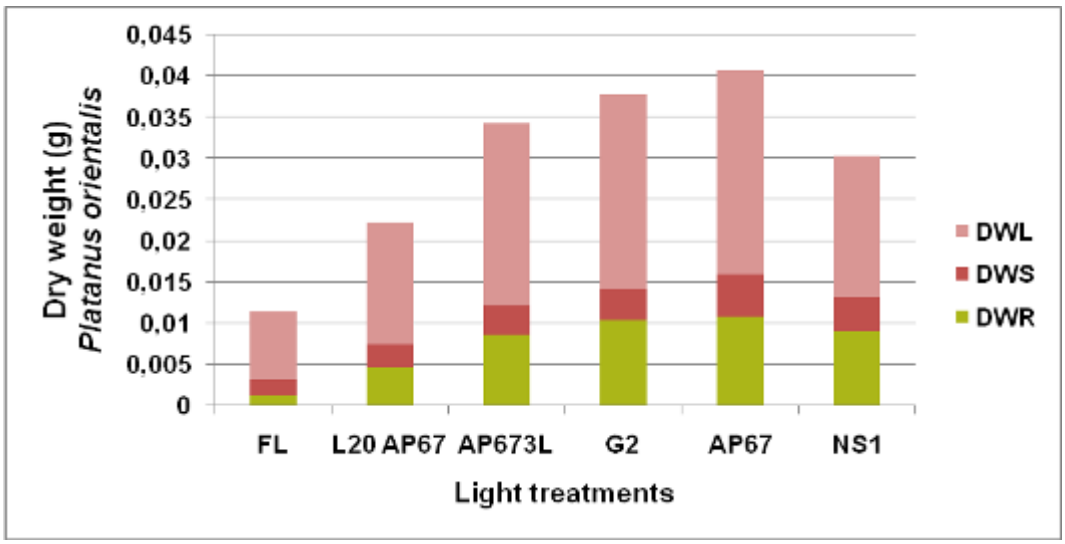


Figure 26. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Platanus orientalis seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Picea abies* Karst.**

The lowest average value for the DWL of *Picea* seedlings was found for the FL light with 0.006 g and significant differences found with all LEDs (p<. 000). Moreover L20AP67 with an average value of 0.012 g also showed significantly lower compared to the rest of LEDs (p<. 000) AP673L, AP67, NS1 and G2 with 0.025 g, 0.023 g and 0.021 g, respectively (Figure 27). Further FL light obtained lower average value of 0.0018 g for the DWS and signifcant differences found with the AP673L with 0.0039 g (p<. 000), G2 with 0.0034 g (p<. 000) and AP67 with 0.0030 g (p<. 008). Also significant differences found between the L20AP67 with 0.0023 g with the G2 (p<.009) and AP673L (p<. 023) LEDs. Significantly lower average value (p<.000) was found for the DWR under the FL light only with 0.0015 g compared to LEDs of AP673L with 0.0096 g, NS1 with 0.0091 g, G2 with 0.0083 g and the AP67 with 0.0076 g. Moreover L20AP67 with 0.0034 g differed significantly with the rest of LED treatments (p<. 000) (Figure 27).

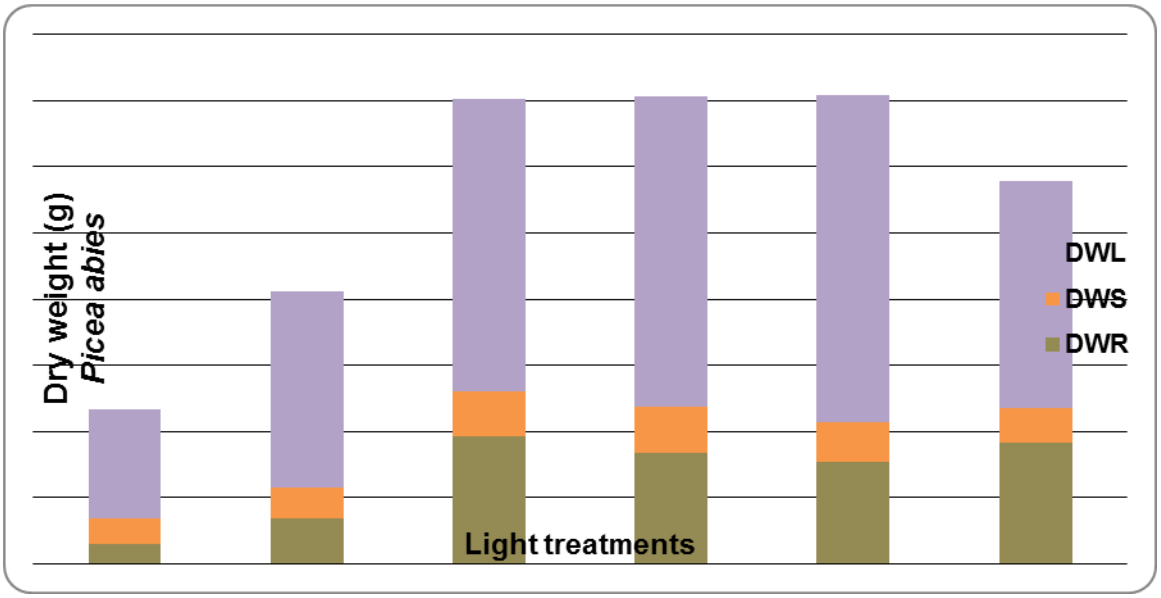


Figure 27. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Picea abies seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Pinus sylvestris* L.**

FL and L20AP67 lights obtained the lowest average values of 0.0092 g and 0.015 g. for the DWL of *Pinus* seedlings compared to all LEDs (p<. 000). Further G2 LED with an average value of 0.047 g was significantly greater than the AP673L (p<. 000) and NS1 (p<. 005) with 0.030 g and 0.033 g, respectively. As for the AP67 LED showed an average value of 0.041 g. Further FL light also obtained the lowest average value of 0.0032 g for the DWS compared to all LEDs (p<. 000) except from the L20AP67. L20AP67 with an average value of 0.0045 g was significantly lower than the G2 with 0.0073 (p<. 000) g and the AP673L 0.0066 g (p<. 018). DWR found significantly lower under the FL and L20AP67 lights with 0.0007 g and 0.0033 g, respectively compared to the rest of LEDs (p<. 000). Further G2 LED showed the highest average value of 0.016 g and significant differences also found with two more LEDs those of AP673L with 0.011 g (p<. 000) and NS1with 0.012 g (p<. 026) (Figure 28).

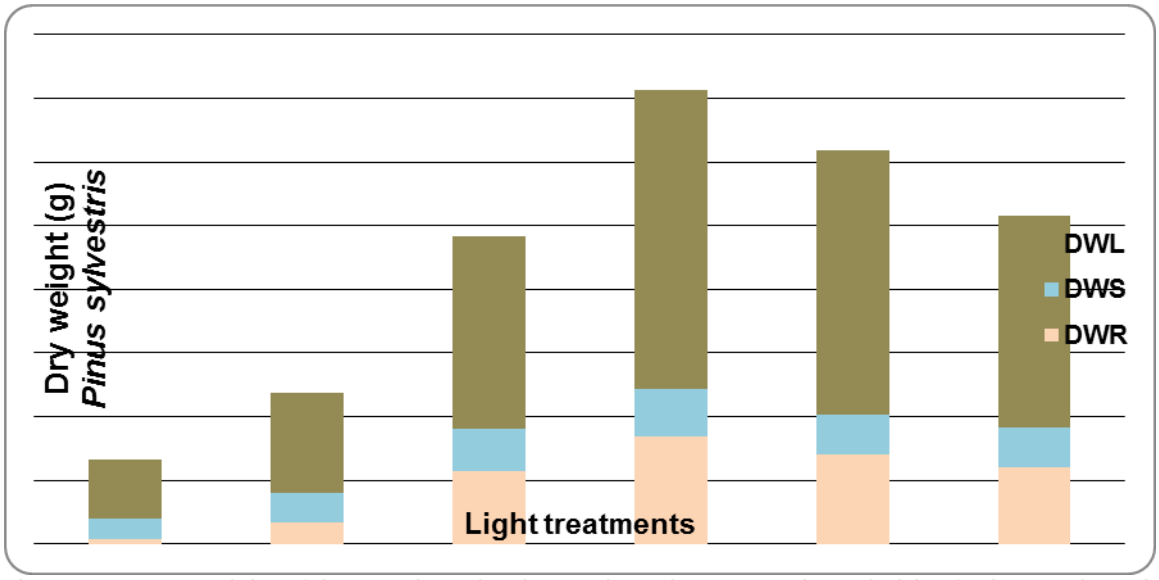


Figure 28. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Pinus sylvestris seedlings under FL, L20AP67,AP673L, G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

2.2.5. Performance of LED spectra on the root-to-shoot ratio compared to control light

***Arbutus unedo* L.**

Seedlings of *Arbutus unedo* grown under LEDs had significantly higher R/S ratio contrast to those under FL light with 0.22 (p<.000). However no significant differences found among the LED treatments; numerically greater allocation to the roots found under the NS1 LED with 0.43, following by the G2 with a ratio of 0.40, AP67 with 0.39, AP673L with 0.35 (Figure 29). L20AP67 light induced a R/S ratio of 0.32; however no significant differences found with the rest of lights.

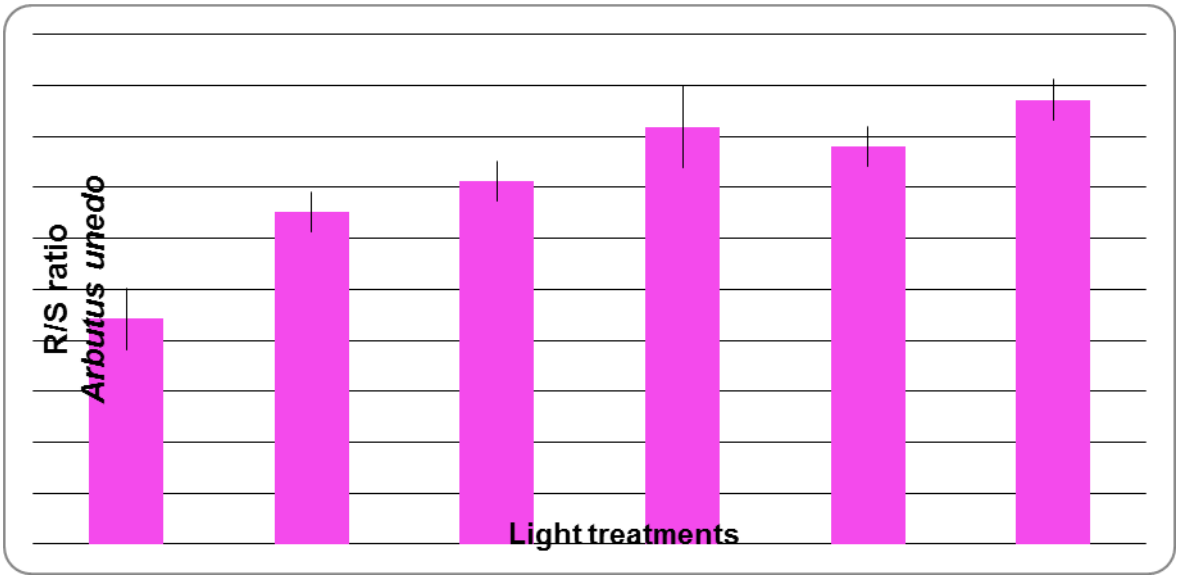


Figure 29. Root-shoot ratio of Arbutus unedo seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Myrtus communis* L.**

Myrtus communis seedlings showed significantly higher allocation to the roots under AP67 light with 0.38 (p<. 000), G2 with 0.35 (p<. 001), NS1 with 0.34 (p<. 002) and the AP673L with 0.32 (p<. 006) compared to FL light with 0.16 ratio at the end of the experimental period (Figure 30). Also L20AP67 LED induced significantly lower allocation to the roots of 0.17 compared to LEDs AP67 light (p<. 000), G2 (p<. 002), NS1 (p<. 004) and the AP673L (p<. 015).

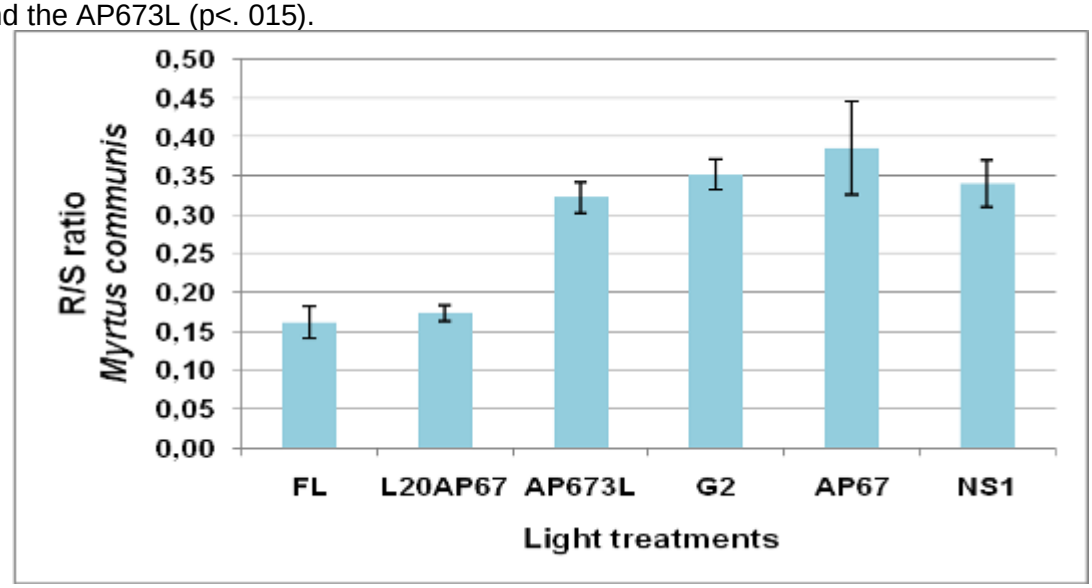


Figure 30. Root-shoot ratio of Myrtus communis seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Abies borisii-regis* Mattf.**

Abies borisii-regis seedlings showed significantly lower allocation to the roots under the FL illumination with 0.22 compared to G2 with 0.39 (p<. 004), AP673L with 0.38, AP67 and NS1 with 0.36 (p<. 0.22). L20AP67 induced R/S ratio of 0.27 but no significant differences found with any of the light treatments (Figure 31).

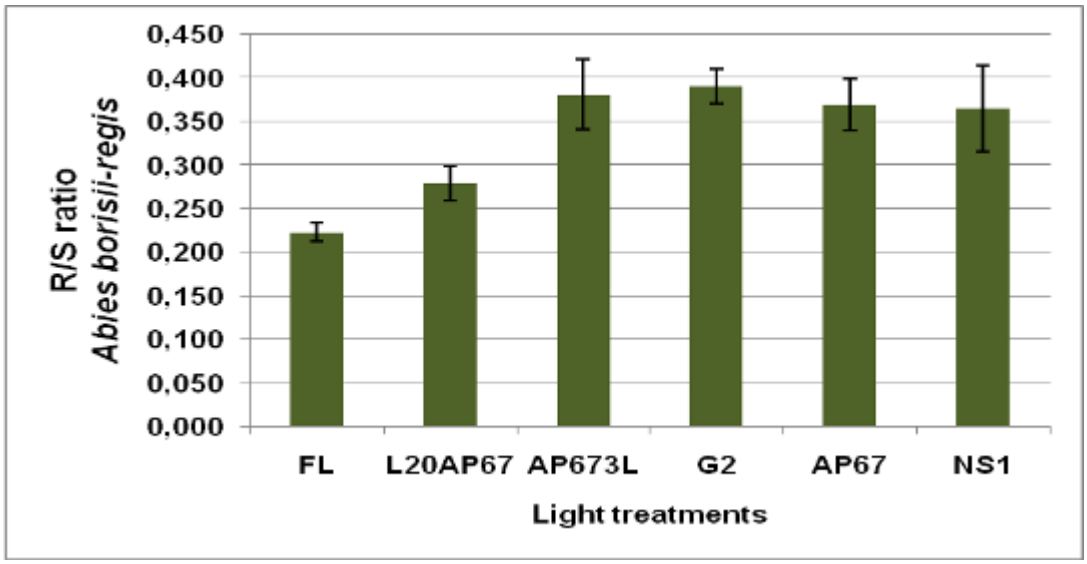


Figure 31. Root-shoot ratio of Abies borisii-regis seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Myrtus communis* L. (second time)**

Under the FL light was obtained the lowest R/S ratio of 0.08 for *Myrtus communis* seedlings and significant differences found with all the LEDs (p<. 000) except from the L20AP67 with a ratio of 0.14. AP673L and NS1 LEDs obtained the highest allocation with 0.37 ratio to the roots and significant differences found also with G2 LED that shown a ratio of 0.27 (Figure 32).

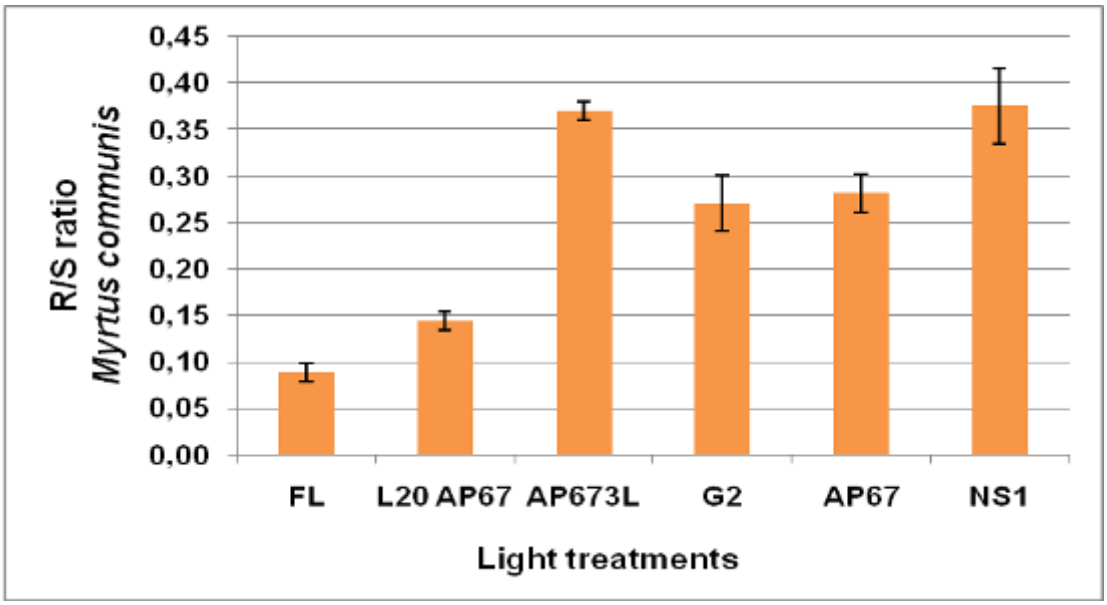


Figure 32. Root-shoot ratio of Myrtus communis seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Platanus orientalis* L.**

The lowest R/S ratio of 0.12 for *Platanus* seedlings was obtained under the FL light and significantly differences found with NS1, G2, AP67 with 0.42, 0.39 and 0.36 ratios (p<. 000) and with the AP673L with 0.33 (p<. 002). Further the L20AP67 showed a R/S ratio of 0.27; however no significant differences found with the other lights (Figure 33).

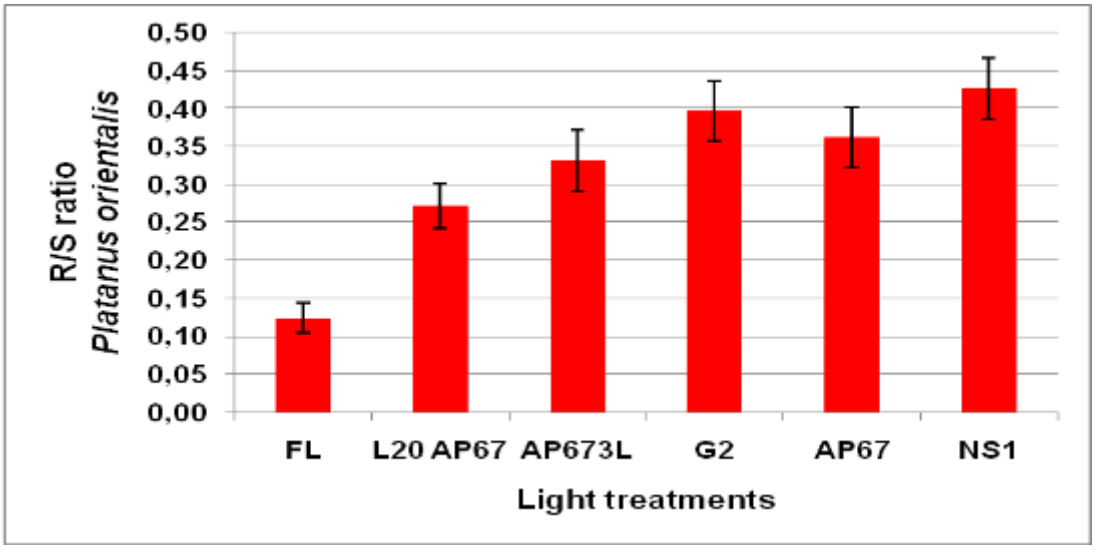


Figure 33. Root-shoot ratio of Platanus orientalis seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Picea abies* Karst.**

Picea abies seedlings obtained the lowest allocation to roots under the effect of FL light only with 0.18 and significant differences found with the NS1 with a ratio of 0.36 (p<. 000), G2 with 0.34 (p<. 002) and AP673L with 0.33 (p<. 005). Also L20AP67 LED obtained significantly lower R/S ratio of 0.24 (p<. 037) compared to the NS1 LED (Figure 34).

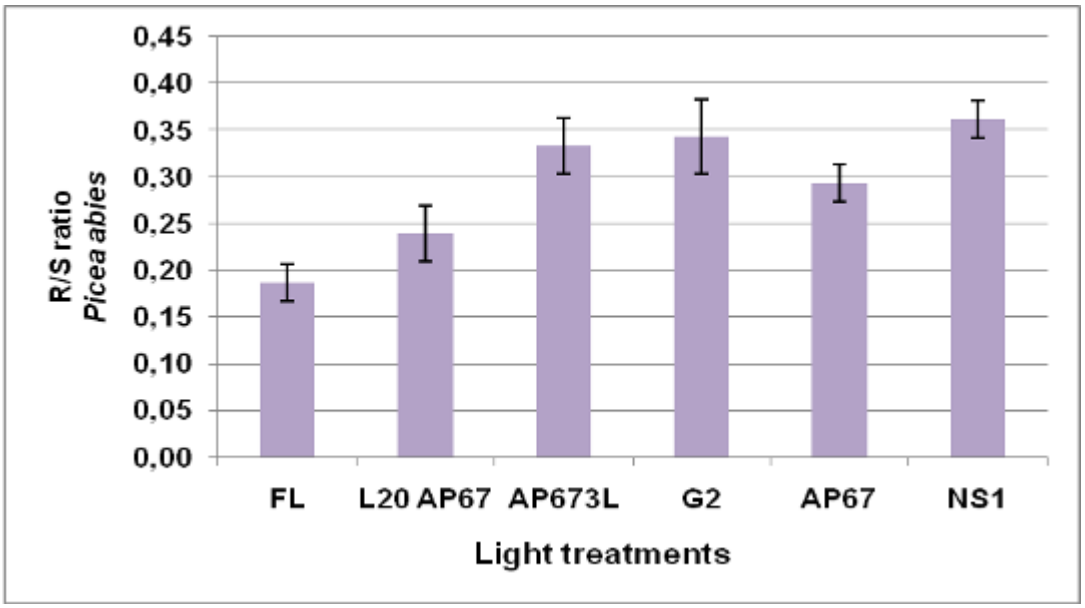


Figure 34. Root-shoot ratio of Picea abies seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

***Pinus sylvestris* L.**

The lowest allocation to roots was found under the FL and L20AP67 lights with 0.06 and 0.17, respectively and significant differences found with the rest of LED treatments (p<. 000). The highest R/S ratio was found under the AP673L with 0.31 following by G2 with 0.309, AP67 with 0.304 and the NS1 with 0.30 (Figure 35).

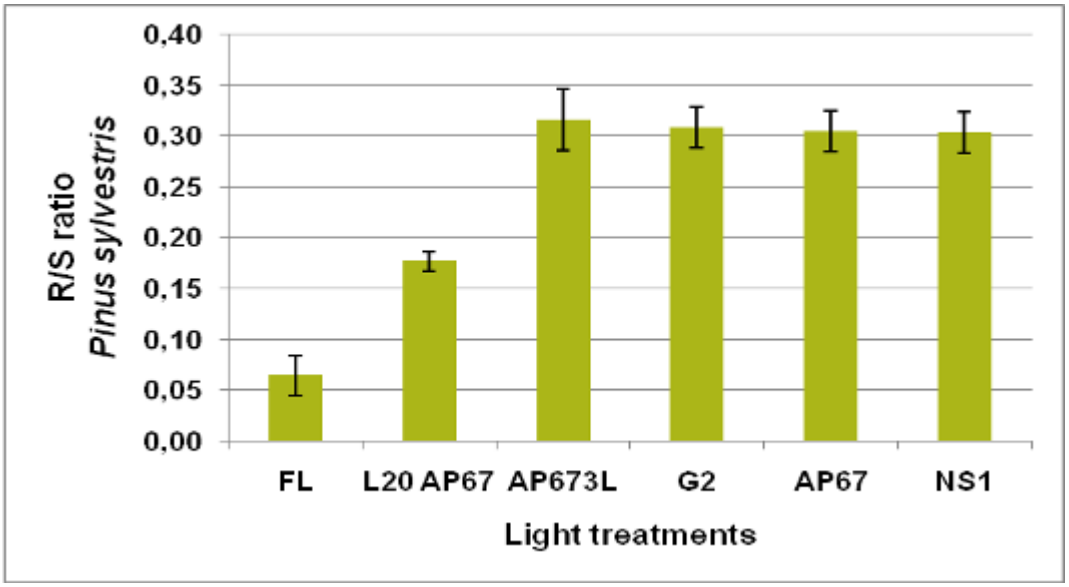


Figure 35. Root-shoot ratio of Pinus sylvestris seedlings under FL, L20AP67, AP673L G2, AP67& NS1 light treatments in stabilized peat (SP) soil substrate and in QPD 104 VW mini-plug size at the end of the experimental period.

2.2.6. Performance of LED spectra on the Root Growth Potential compared to control light

***Arbutus unedo* L.**

- **New Root length (NRL)**

After 15 days into the RGP bath *Arbutus unedo* seedlings formed longer roots under the L20AP67 with 37.22 mm, following by the G2 with 33.29 mm, AP67 with 32.81 mm, AP673L with 30.01 mm, NS1 with 23.59 mm and the shortest under the FL light with 15.17 mm. However no significant differences found between the different spectra. At the 31st day significantly longer roots were formed for the seedlings grown under the G2 LED almost doubled with 61.53 mm compared to the rest of light treatments that showed in order L20AP67 with 38.39 mm (p<. 035), NS1 with 34.11 mm (p<. 005), AP673L with 32.56 mm (p<. 003), AP67 with 27.86 mm (p<. 000) and the FL with 22.6 mm (p<.000). Between the measurements significant difference found only for the G2 LED (p<. 000) (Figure 36).

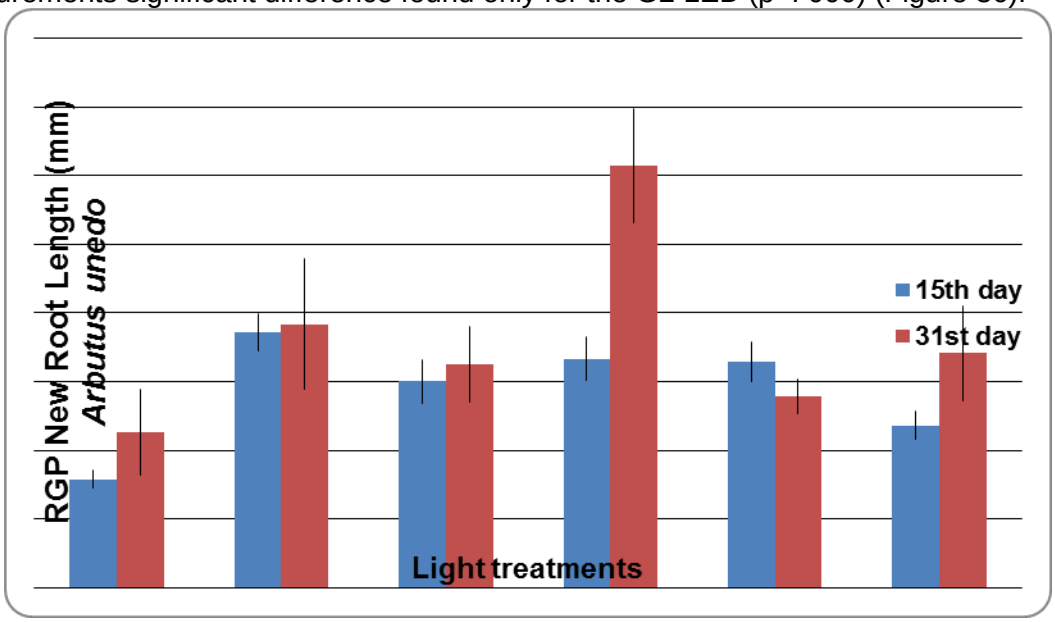


Figure 36. New Root Length of Arbutus unedo seedlings after 15 and 31 days in the RGP bath

- **New Root Dry Weight (NRDW)**

At the 15th day no significant differences found for the NRDW, however numerically heavier roots were found for the G2 LED with 0.0041 g, following by the NS1 0.0038 g, AP67 with 0.0032 g, AP673L with 0.0023 g, L20AP67 with 0.0022 g and the lighter for the FL light with 0.0011 g. At the 31st day significantly lighter roots were formed for the seedlings grown under the FL light with
0.0011 g compared to G2 (p<. 000) and L20AP67 (p<.005) LEDs with 0.0083 g and 0.0060 g, respectively. Also G2 LED obtained significantly heavier roots compared to AP673L (p<. 000) and the NS1 (p<. 004) with 0.0024 g and 0.0034 g, respectively (Figure 37). Between the two measurements for the NRDW significant differences in amount were found for the G2 (p<.002) and the L20AP67 (p<. 005) LEDs.

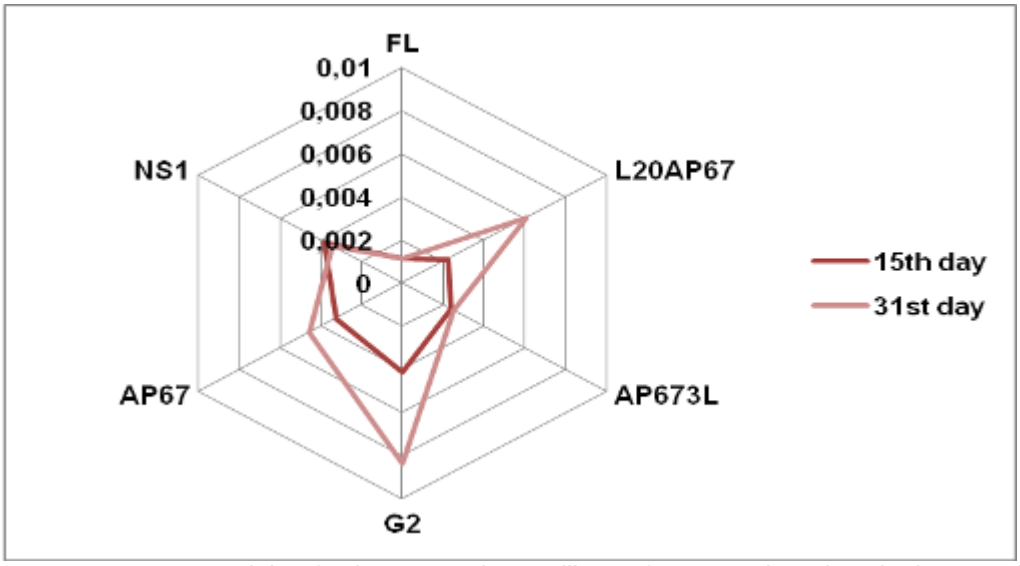


Figure 37. New Root Dry Weight of Arbutus unedo seedlings after 15 and 31 days in the RGP bath

***Myrtus communis* L.**

- **New Root length (NRL)**

At the 15th day the NRL was significantly lower under the FL light with 18.18 mm compared to AP673L (p<. 005) with 37.66 mm and the G2 (p<. 009) with 36.72 mm. As for the rest of lights such as NS1, AP67 and L20AP67 the average values in order were 31.51 mm, 31.27 mm and 24.17 mm (Figure 38). At the 31st day in the RGP bath significantly shorter root was formed for the FL light with 25.31 mm compared to the AP673L (p<. 003), L20AP67 (p<. 003) and G2(p<. 000) LEDs with 53.43 mm, 51.02 mm and 45.64 mm, respectively. Further significantly longer roots were formed for the AP673L (p<.003) and the L20AP67 (p<. 016) compared to NS1 LED with 33.37 mm; between the two measurements significant differences were found for the New root length formed for the AP673L (p<. 003) and the L20AP67 (p<. 000).

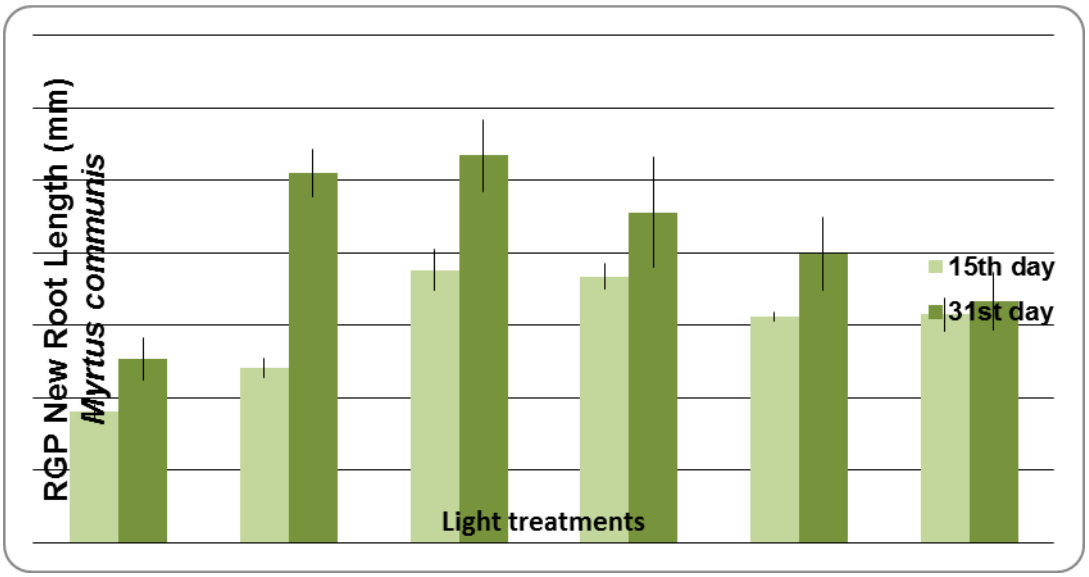


Figure 38. New Root Length of Myrtus communis seedlings after 15 and 31 days in the RGP bath

- **New Root Dry Weight (NRDW)**

No significant differences found between the light treatments for the NRDW; however numerically heavier roots were formed for the G2 LED with 0.0046 g, following by the AP67 with 0.0042 g, AP673L with 0.0030 g, NS1 with 0.0024 g, L20AP67 with 0.0021 g and the lighter for the FL light with 0.0012 g (Figure 39). Further at the 31st day significantly heavier roots were formed for the G2 LED with 0.014 g compared to all the lights (p<. 000) such as AP673L, AP67, L20AP67, NS1 and FL that showed average values in order 0.0047 g, 0.0038 g, 0.0036 g, 0.0018 g and 0.0012 g (Figure 39). Between the two measurements significant differences for the NRDW found only for the G2 LED.

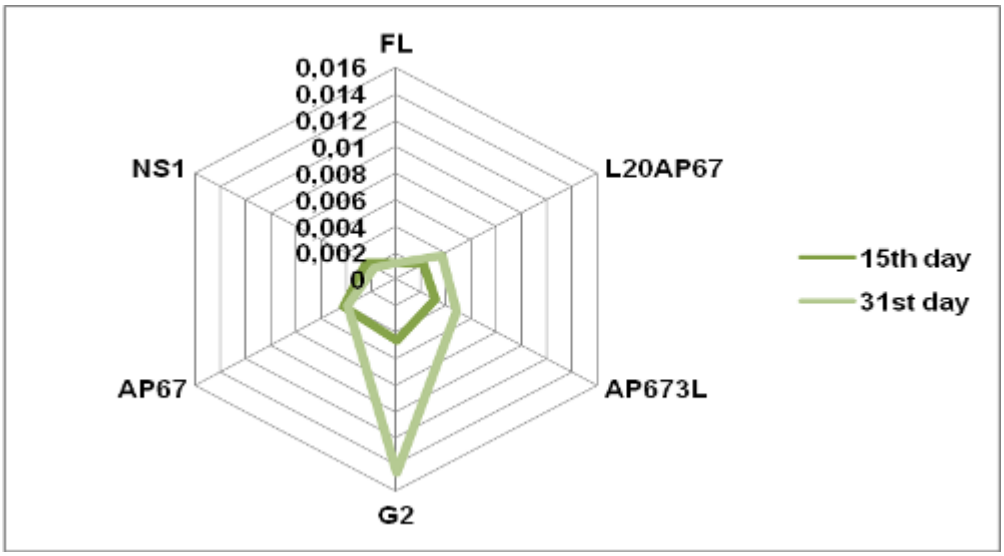


Figure 39. New Root Dry Weight of Myrtus communis seedlings after 15 and 31 days in the RGP bath

***Abies borisii-regis* Mattf.**

- **New Root length (NRL)**

Significantly longer roots for *Abies* seedlings were formed for the NS1 LED with 60.78 mm (p<. 000) only compared to those formed for the AP67 with 37.68 mm. As for the rest of lights such as L20AP67, AP673L, G2, and FL showed average values in order 50.66 mm, 49.09 mm, 47.96 mm and 46.9 mm (Figure 40).

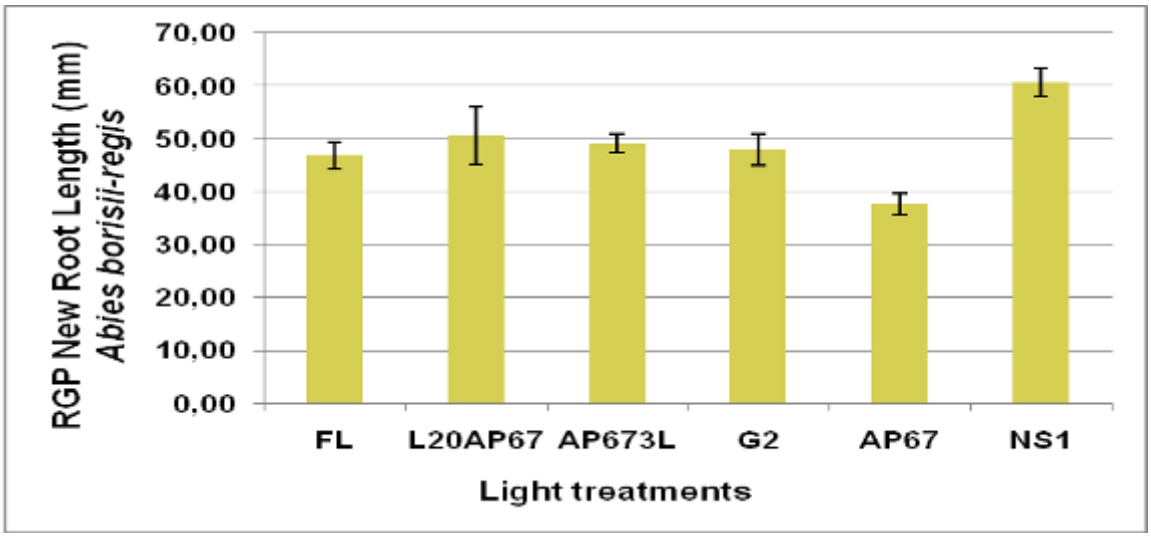


Figure 40. New Root Length of Abies borisii-regis seedlings after 31 days in the RGP water bath

- **New Root Dry Weight (NRDW)**

No significant differences found for the NRDW of abies seedlings after 31 days in the RGP bath; however numerically greater values were found for the G2 LED with 0.018 g, following by the AP673L with 0.013 g, NS1 with 0.010 g, AP67 WITH 0.009 g, L20AP67 with 0.007 g and the lowest for FL light with 0.006 g (Figure 41).

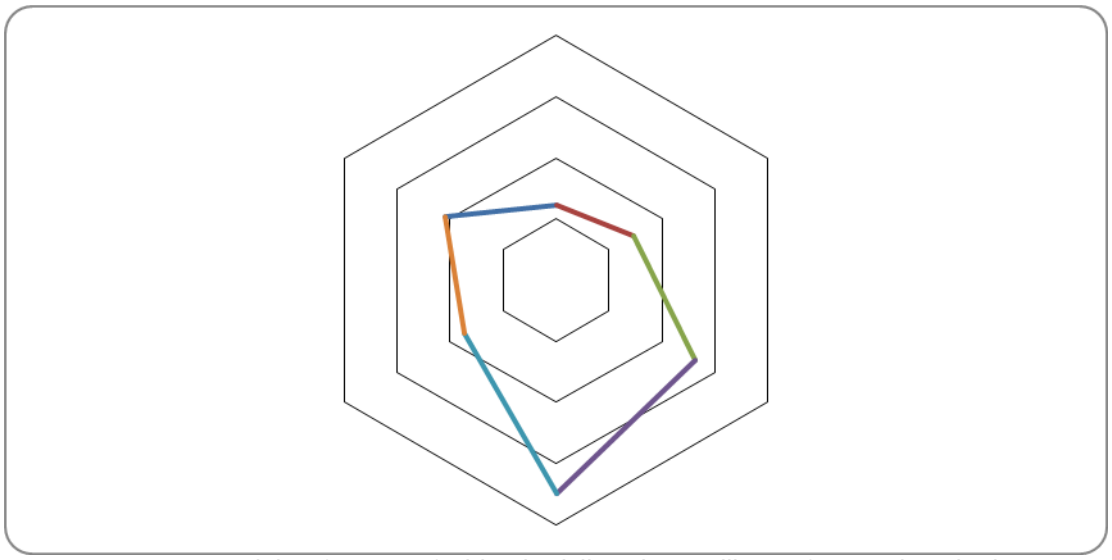


Figure 41. New Dry Weight of Roots of Abies borisii-regis seedlings after 31 days in the RGP water bath

***Myrtus communis* L. (second time)**

- **New Root length (NRL)**

All the light treatments showed significant increase (p<.000) almost doubled in amount for the NRL from the 15th day to 31st day in the RGP bath. However in every measurement the light treatments did not showed significant effect on the NRL; numerically longer roots were found at the 15th day for the G2 light with 53.8 mm, following by the AP67 with 51.22 mm, AP673L with 50.34 mm, NS1 with 46.99 mm, L20AP67 with 45.39 mm and the shorter for the FL light with 38.69 mm (Figure 42). According to the 31st day in the RGP bath the NRL for the G2, L20AP67, AP67, NS1, AP673L and the FL light were in average 95.20 mm, 86.94 mm, 86.54 mm, 86.04 mm, 80.97 mm, 69.46 mm.

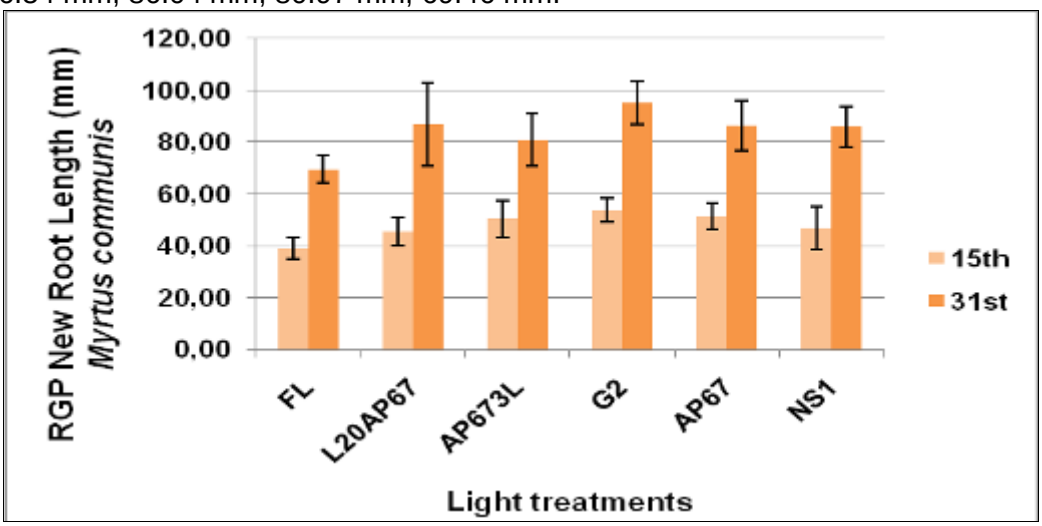


Figure 42. New Root Length of Myrtus communis seedlings after 15 and 31 days in the RGP water bath

- **New Root Dry Weight (NRDW)**

Significant differences for the NRDW were found only at the 31st day for the G2 light with 0.010 g compared to AP673L (p<. 014) that had 0.0034 g (Figure 43).

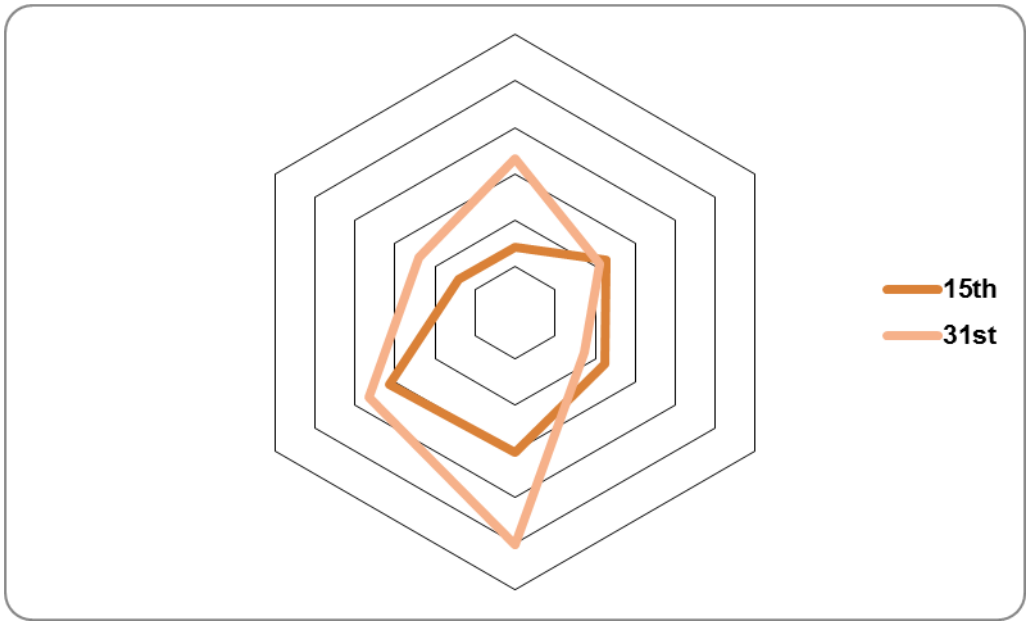


Figure 43. New Dry Weight of Roots of Myrtus communis seedlings after 15 and 31 days in the RGP water bath

***Platanus orientalis* L.**

- **New Root length (NRL)**

At the 15th day no significant differences found between the light treatments for the NRL. However significant differences found at the 31st day into the RGP bath where seedlings for the G2 LED showed longer roots of 124 mm compared to those formed for the NS1 with 56.78 mm (p<. 005) and the FL light with 68.08 mm (p<. 038). Between the measurements significant increase in the amount of NRL was found for the G2 (p<. 027) AP67 (p<. 025) and L20AP67 (p<. 022) lights (Figure 44).

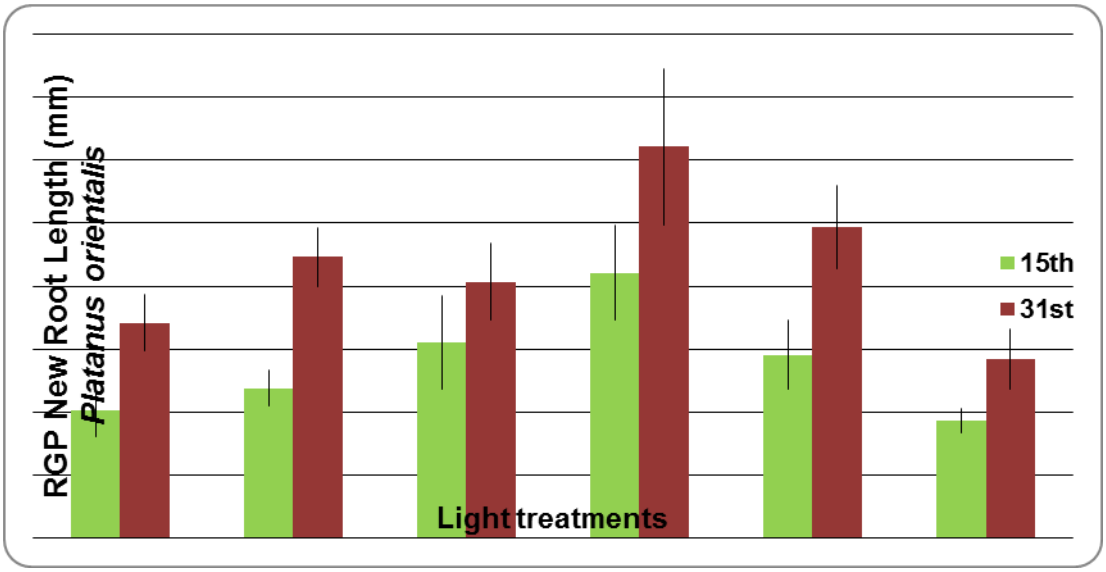


Figure 44. New Root Length of Platanus orientalis seedlings after 15 and 31 days in the RGP water bath

- **New Root Dry Weight (NRDW)**

Significant differences for the NRDW were found for the *platanus* seedlings of G2 that had heavier roots 0.024 g compared to those of NS1 with 0.005 g. between the two measurements significant increase for the NRDW was found for the G2 LED (p<. 030) from 0.013 g to 0.024 g and for the FL light from 0.005 g to 0.017 g (Figure 45).

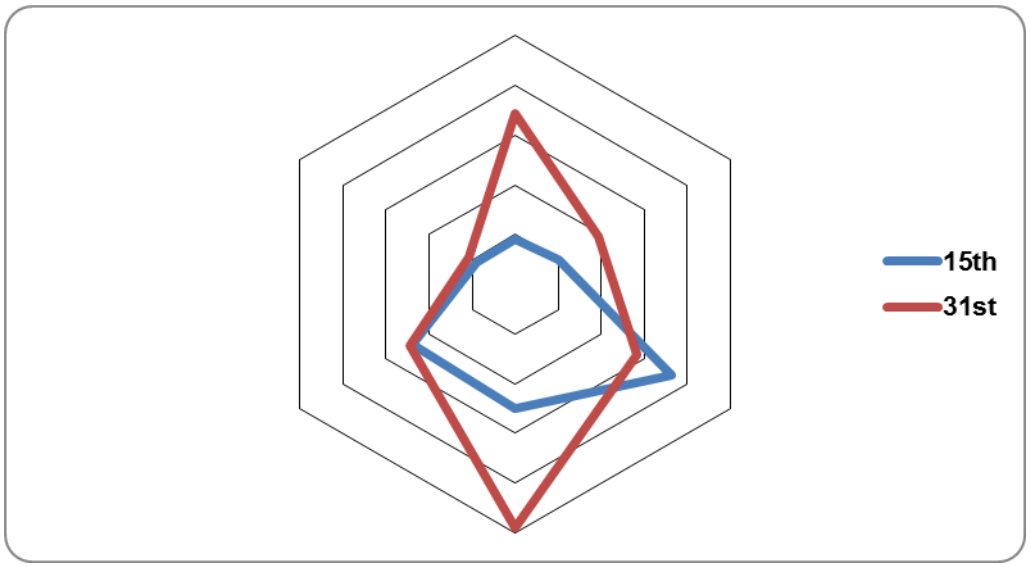


Figure 45. New Dry Weight of Roots of Platanus orientalis seedlings after 15 and 31 days in the RGP water bath

***Picea abies* Karst.**

- **New Root length (NRL)**

At the 15th day no significant differences found for the NRL between the lights. However seedlings of FL and L20AP67 significantly increased the amount from the first to the second measurement. FL light increased in average NRL value from 60.28 mm to 80.44 mm (p<. 016) and L20AP67 from 50.34 mm to 74.58 mm (p<. 004) (Figure 46).

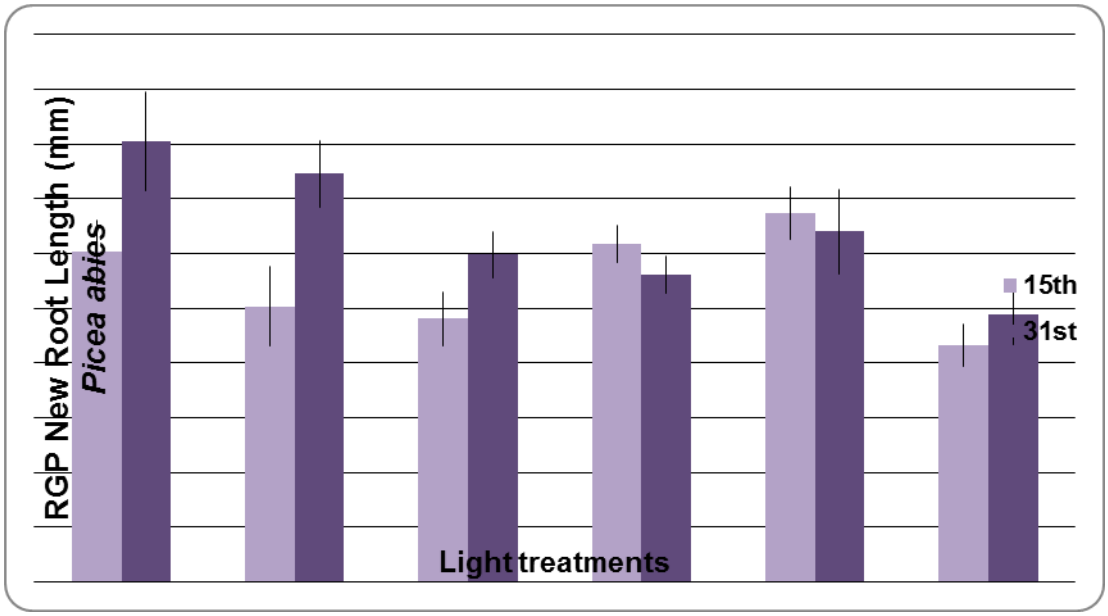


Figure 46. New Root Length of Picea abies seedlings after 15 and 31 days in the RGP water bath

- **New Root Dry Weight (NRDW)**

Significant differences found at the 31st day in the RGP bath for the AP673L with 0.015 g compared to FL, L20AP67 with 0.010 g (p<. 004) and the NS1 with 0.011 g (p<. 004) (Figure 47). Between the two measurements significant decrease was found for the AP67 from 0.027 g to 0.013 g (p<. 000) and the AP673L from 0.025 g to 0.015 (p<. 008).G2 LED showed the highest average value with 0.017 g and the lowest for the FL light with 0.0006 g.

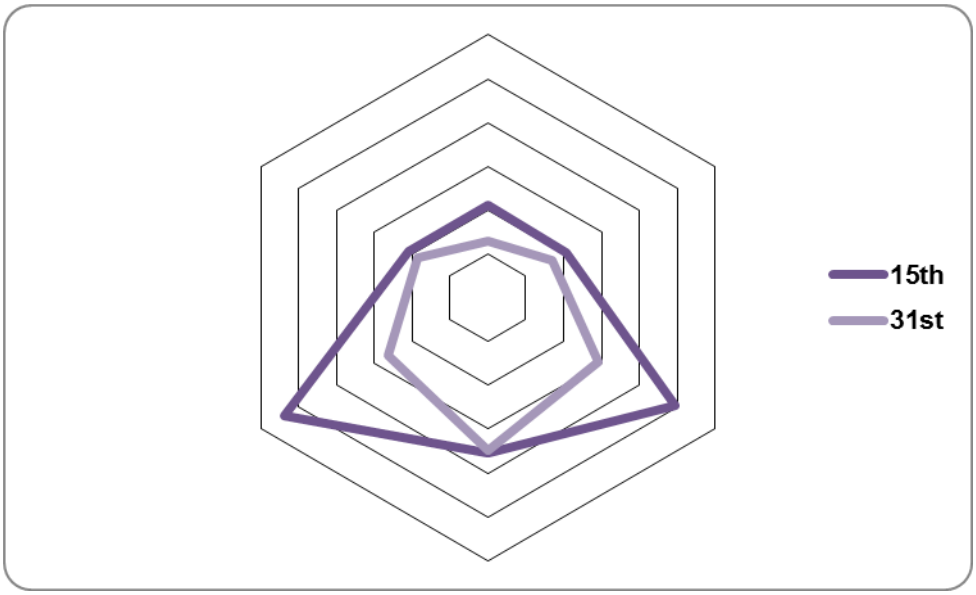


Figure 47. New Dry Weight of Roots of Picea abies seedlings after 15 and 31 days in the RGP water bath

***Pinus sylvestris* L.**

- **New Root length (NRL)**

No significant differences found between the light treatments neither at the 15th or the 31st day in the RGP bath of *Pinus sylvestris* seedlings. However significant increase observed between the two measurements for the FL light from 59 mm to 132.18 mm (p<. 000), the NS1 from 54.25 mm to 108.47 mm and for the L20AP67 from 49 mm to 126.61 (50-60% increase) Figure 48.

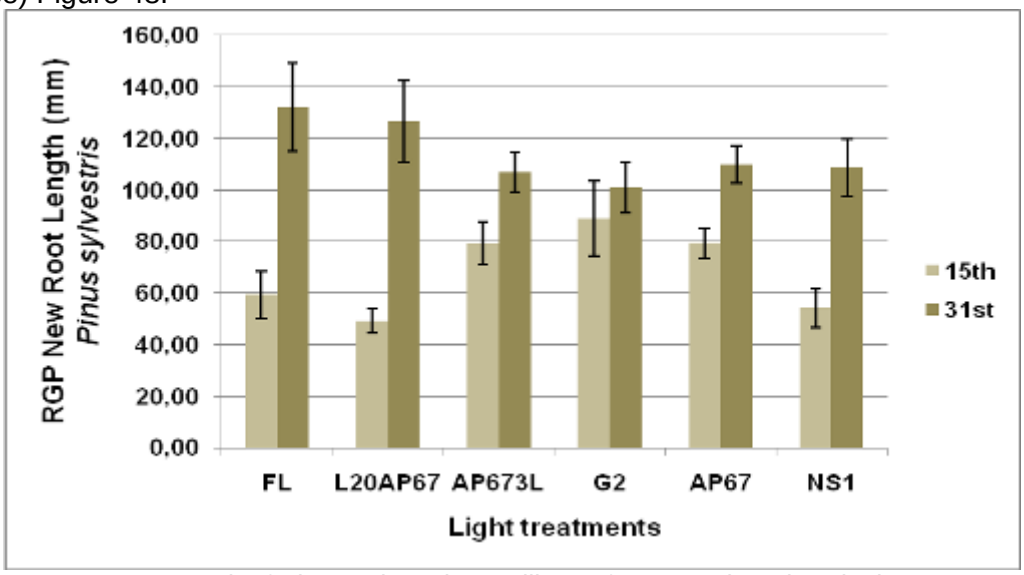


Figure 48. New Root Length of Pinus sylvestris seedlings after 15 and 31 days in the RGP water bath

- **New Root Dry Weight (NRDW)**

No significant differences found at the 15th day for the NRDW between the light treatments. However significant increase in NRDW observed for AP67 light from 0.013 g to 0.020 g (p<. 037) and for NS1 from 0.006 g to 0.015 g (p<. 013). Significant differences found for the NRDW of *Pinus* seedlings at the 31st day in the RGP bath for the G2 LED with 0.020 g compared to the FL and NS1 with 0.006 g (p<. 002) (Figure 49). The highest average value found for the AP67 with 0.020 g and the lowest for the L20AP67 with 0.010 g.

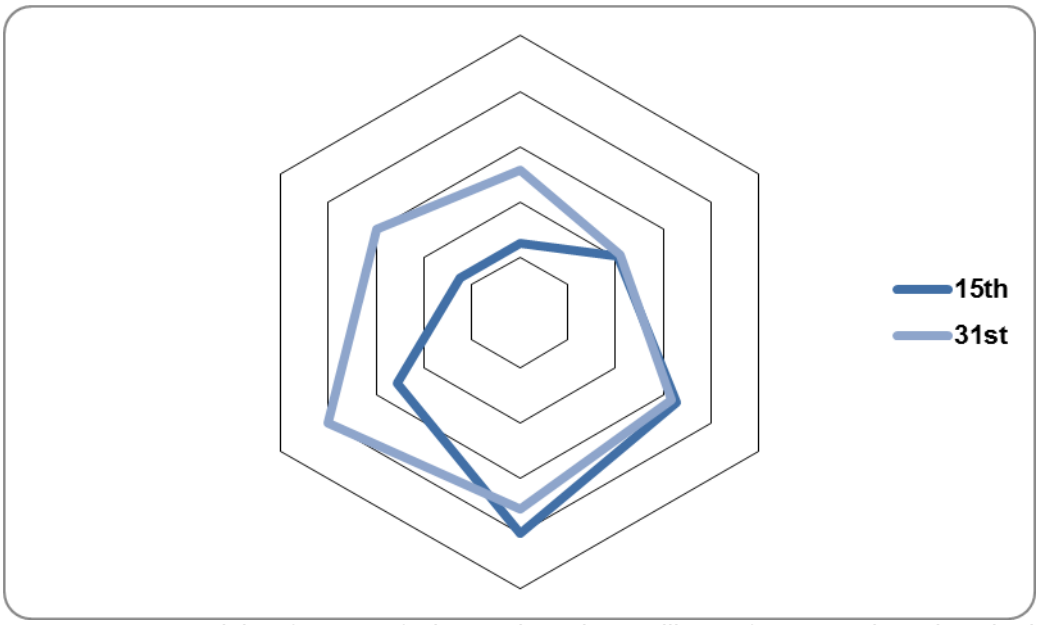


Figure 49. New Dry Weight of Roots of Pinus sylvestris seedlings after 15 and 31 days in the RGP water bath

2.3. Conclusions

- Higher growth rate tendency under the L20AP67 LED and the FL light especially at the first week of the experimental period. This phenomenon is altered at the middle (3rd week) of the experimental period both for G2&AP67 LEDs that shown higher height increment
- Generally more leaves/needles formed under the illumination of G2&AP67 LEDs
- Dark green colour in leaves/needles reached under the FL in a later phase than under LEDs. Also LEDs G2, AP67, AP673L and NS1 induced reddish coloration for *Arbutus unedo* and *Platanus orientalis* seedlings.
- Generally taller seedlings obtained cultivated under the L20AP67 LED & FL lights Some exceptions: G2 light (*Myrtus communis - P.sylvestris*)
- Conifer species favored more for the root development under the NS1 LED, while the broad-leaved under the AP67 LED. In contrast FL light induced less effect
- Generally lower dry mass of leaves, shoots & roots for all the tested species was found under the FL & L20 AP67 light treatments.
- G2&AP67 LEDs induce higher dry weight mass of leaves, shoots, roots for all species with an exception of *Picea* that seemed to be better adopted under the new AP673L LED.

- Higher allocation to roots obtained under LEDs, especially for the NS1, AP673L. in contrast lowest under FL
- New root length obtained after 31 days in RGP bath for the seedlings pre-cultivated under the G2 and L20AP67 LEDs
- New dry root weight obtained after 31 days in RGP bath for the seedlings pre-cultivated under the G2 and AP67 LEDs

3. Input from Dalarna University

3.1. Background to a Scandinavian outline of growth protocols

To adapt a new cultivation technology for forest seedlings based on pre-cultivation and transplanting, as being developed within the Zephyr project, to the Scandinavian climate certain adjustments have to be considered. Since the climate only allows a narrow transplanting window to open land direct pre-cultivation followed by transplanting can only be done between the beginnings of May to the end of August in order to avoid for example frost damages. Therefore, to use the new cultivation technology on a year around basis a period of cold storage has to be introduced after pre-cultivation for batches that cannot be directly transplanted within the transplanting window.
To be able to cold store pre-cultivated seedlings a period of long-night (LN) treatment has to be introduced to induce cold tolerance in the seedlings (Figure 1). This treatment is based on a cultural regime including a short photoperiod combined with a long-night simulating the seedlings to prepare for the winter climate. Besides to identify growth protocols for this LN treatment the pre-requisites for a long term cold storage, with a storage time adapted to the transplanting window, has to be analyst.
Therefore Dalarna University in Sweden has to adapt to this situation and outline the growth protocols for identified species considering what has been stated above.

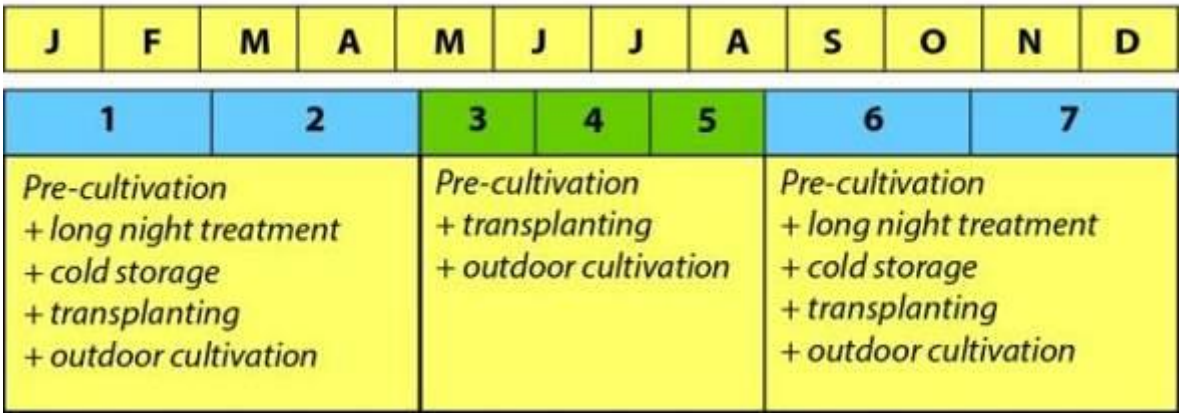


Figure 1: Cultivation programs adapted to Scandinavian species and climate conditions.

Regarding identified species Dalarna University has performed growth tests on Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* Karst.) seedlings. These species are dominating species for reforestation in Scandinavia. In Sweden they refer to over 95 % of the total plant production of about 400 million seedlings a year where Scots pine accounts for 40 % and Norway spruce for 60 %. The high quality seed material for cultivation of Scots pine and Norway spruce was collected from a seed orchard of a mid-Swedish provenance.
In this **intermediate report on growth tests** (D3.2) the outline of tests for identifying the growth protocols in the **final report on growth tests and biological validation** (D3.3) follows the background presented regarding adapting the protocols for the pre-cultivation and transplanting concept to the special conditions in Scandinavia including Sweden.
The growth tests at Dalarna University, performed as a foundation for the growth protocols that will be presented in Deliverable D3.3 in August 2015, have been divided under the following headings:

- Pre-cultivation
- Direct transplanting to open land
- Long-Night (LN) treatment before cold storage
- Cold storage before transplanting to open land
- Forest field trial

- Future measurements

3.2. Pre-cultivation

Pre-cultivation of Scots pine and Norway spruce was performed under artificial light for 5 weeks. According to earlier studies in identifying optimal conditions for germination and growth of Scots pine and Norway spruce seedlings the temperature was held at 20°C and the relative humidity was 80 % during germination and lowered to 60% during the growth phase. As light sources a standard fluorescent tube for indoor cultivation was used as a control. In addition 5 different LED lamps with different spectra were used to evaluate the most optimal spectra for cultivation of the species mentioned (Figure 2).

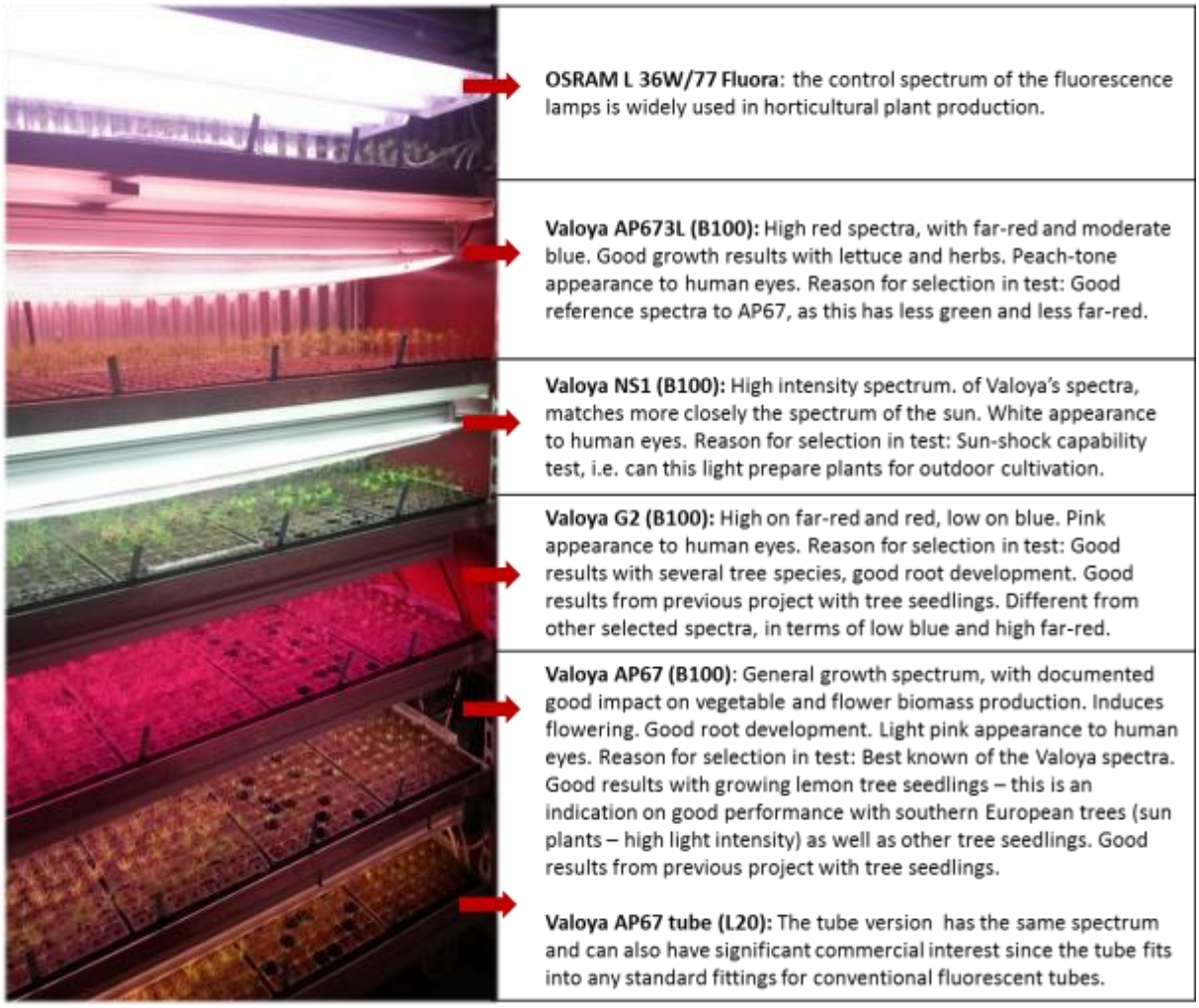


Figure 2: Trolley with different light sources and description of the corresponding spectra

Scots pine

The following figures show the growth result for 3 different sowings after 5 weeks of pre-cultivation of Scots pine under the different light sources. The repetition of sowings was introduced to have a more solid validation of the results obtained compared to just use one single sowing.

As stated the seeds were collected from an orchard with an excellent germination rate without any pre-treatment of almost 100 % for each treatment.

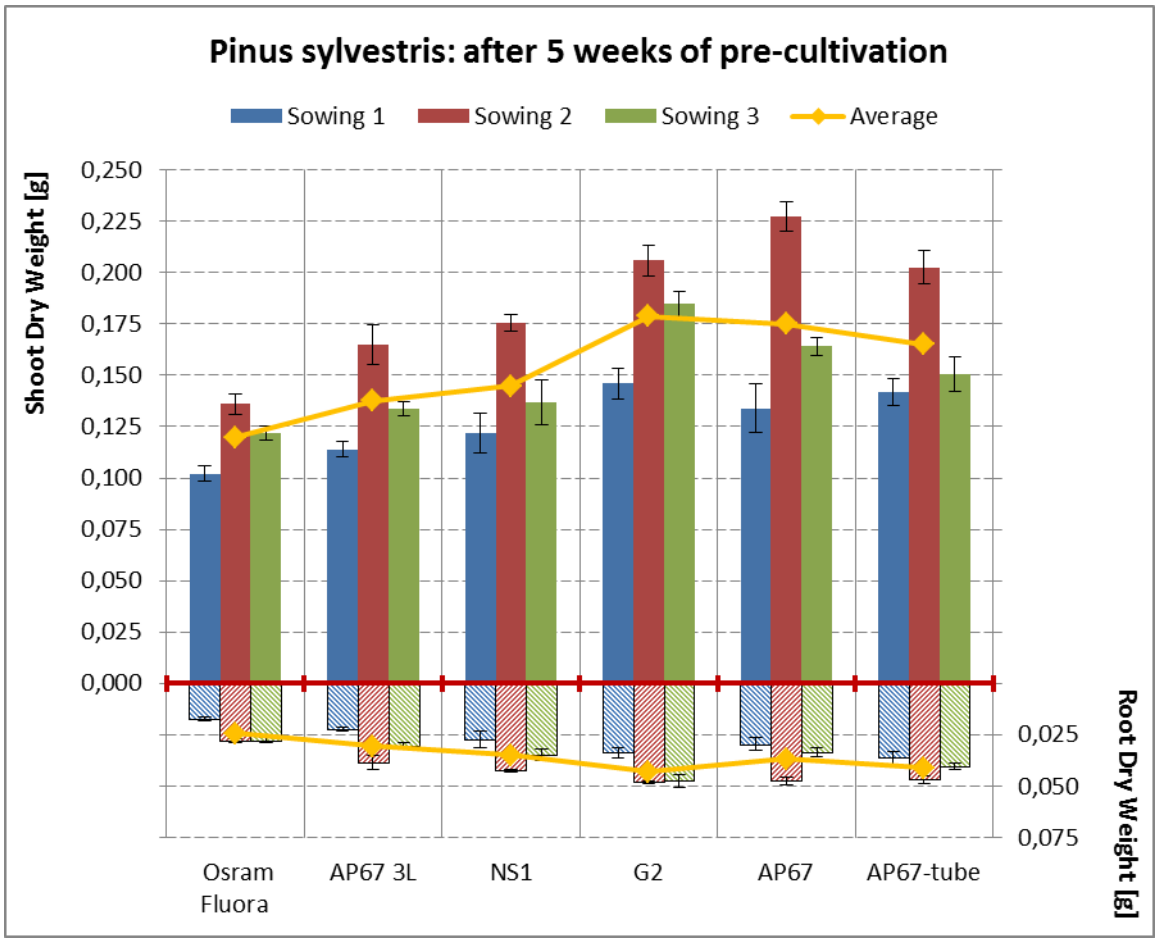


Figure 3: Shoot dry weight and roots dry weight comparison of 3 sowings of Pinus sylvestris seedlings after pre-cultivation under different light spectra

As can be seen in Figure 3 the shoot dry weight varied somewhat between the different sowings indicating minor differences in the growing environment for the different sowings. The results between the different light sources were however consistent with the highest values for G2 and AP67 (both bar and tube) indicating a positive growth effect when the seedlings were pre-cultivated under these spectra. The measurements of the root dry weight followed the pattern as for shoot dry weight with higher values for the spectra

mentioned.

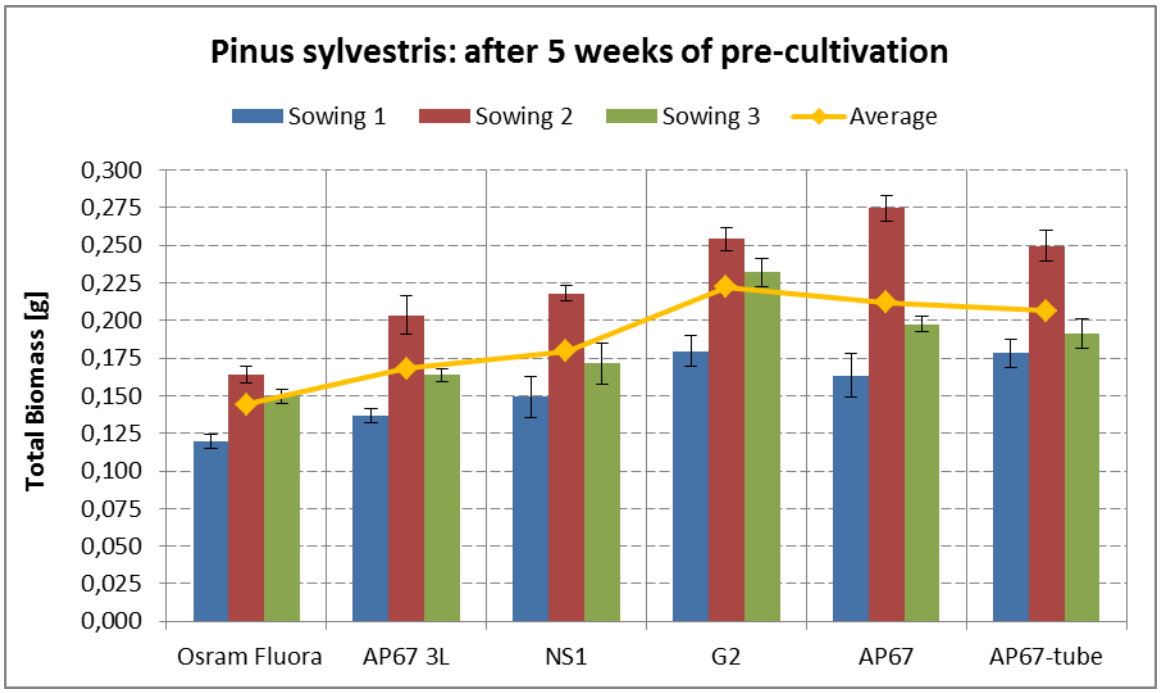


Figure 4: Total biomass (dry weight) comparison of 3 sowings of Pinus sylvestris seedlings after pre-cultivation under different light spectra

In Figure 4 the total biomass for all treatments is shown. The results confirm in all essentials the findings presented in Figure 3. That is a positive biomass development when the seedlings were grown under the LED spectra G2 and AP67 (bar and tube).

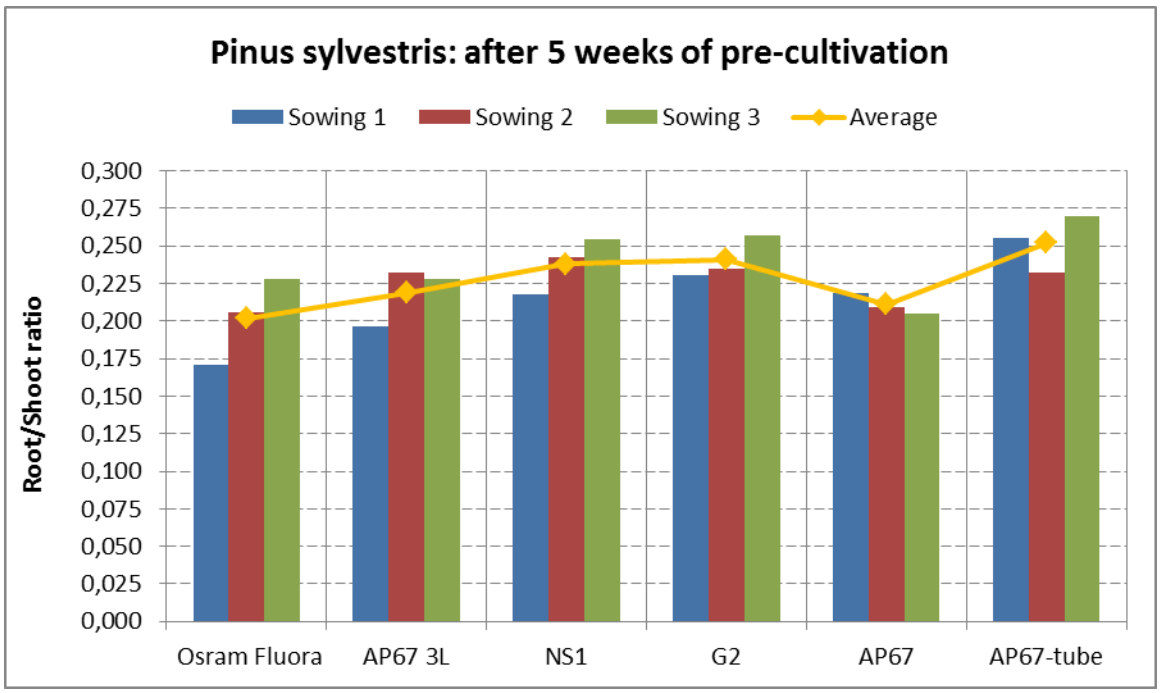


Figure 5: Roots/shoot comparison of 3 sowings of Pinus sylvestris seedlings after pre-cultivation under different light spectra

In Figure 5 the root/shoot ratio is shown for the different treatments. The figure shows that the differences between treatments had levelled out indicating a good balance between the root and the shoot although some of the treatments had higher values regarding the total biomass.

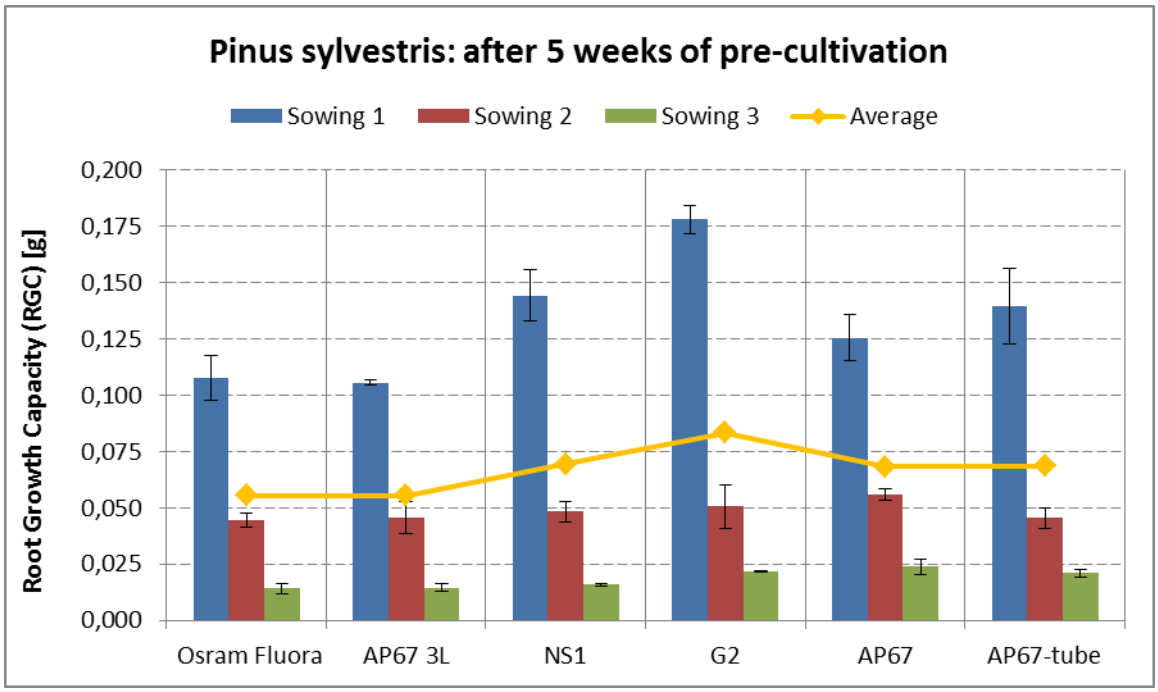


Figure 6: Root Growth Capacity comparison of 3 sowings of Pinus sylvestris seedlings after pre-cultivation under different light spectra

The Root Growth Capacity (Mattsson, 1986) for the different treatments is shown in Figure 6. The high values for sowing 1 is difficult to explain since the root dry weight presented in Figure 3 was lower for all treatment compared to sowing 2 and 3. Some error in the measurement procedure must therefore be assumed giving the high values for sowing 1. Anyhow there was a systematic error for all the different spectra resulting in that the average values for all 3 sowings shows the same picture as for all the other measurements presented in Figure 3 and Figure 4. That is, a positive effect of pre-cultivation under the LED spectra G2 and AP67.

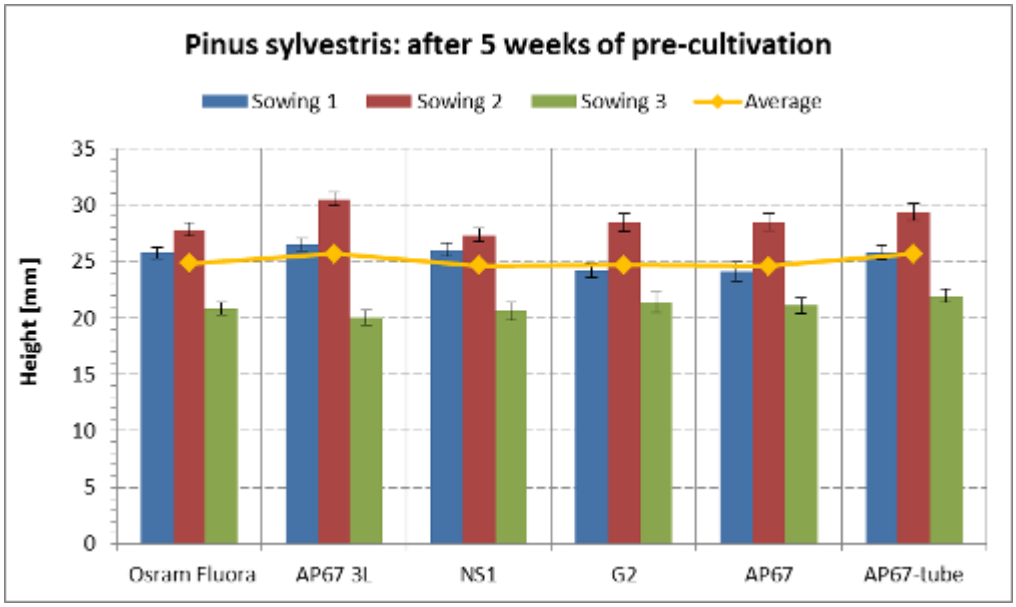


Figure 7: Height comparison of 3 sowings of Pinus sylvestris seedlings after pre-cultivation under different light spectra

The height development for the different sowings and light treatments is shown in Figure 7. As can be seen the differences between sowings and treatments are very small with an average close to 25 mm. This indicates that although there are differences in biomass between treatments the shoot elongation in Scots pine the first year is not so affected of changes in light spectra.

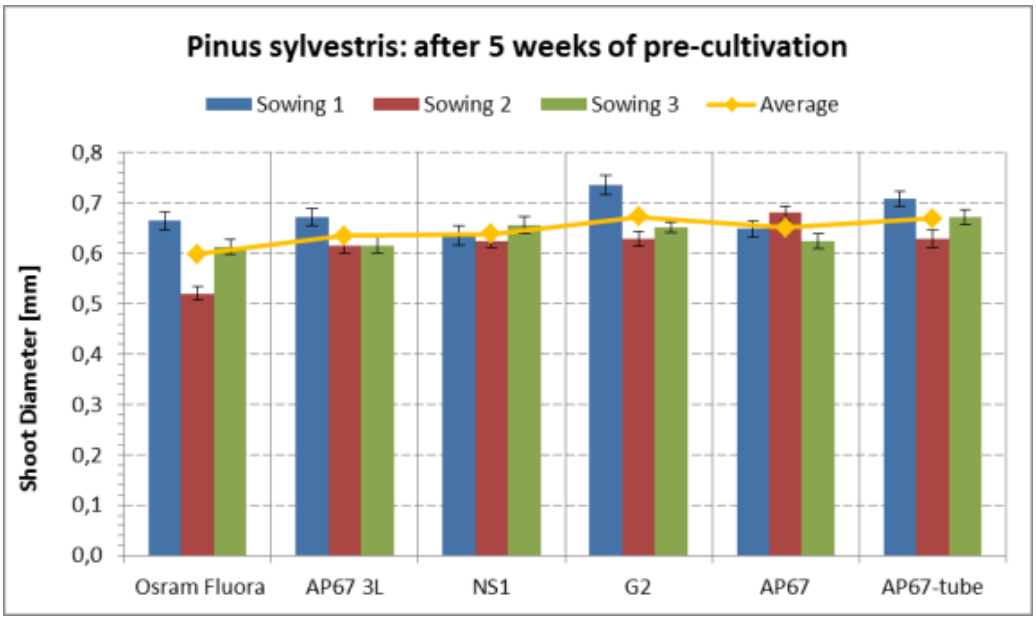


Figure 8: Shoot diameter comparison of 3 sowings of Pinus sylvestris seedlings after pre-cultivation under different light spectra

The results for shoot diameter in Figure 8 are very similar to the height measurements presented in Figure 7. In general the stem is very thin after 5 weeks of pre-cultivation, in this case about 0.6 mm for each treatment, making it difficult to identify any major differences within a resolution of 0.1 mm.

Norway spruce

The following figures show the growth results for 3 different sowings after 5 weeks of pre-cultivation of Norway spruce under different light sources. The repetition of sowings was introduced to have a more solid validation of the results obtained compared to just one single sowing.

The seeds were collected from a seed orchard with an excellent germination rate without any pre-treatment of almost 100 % for each treatment.

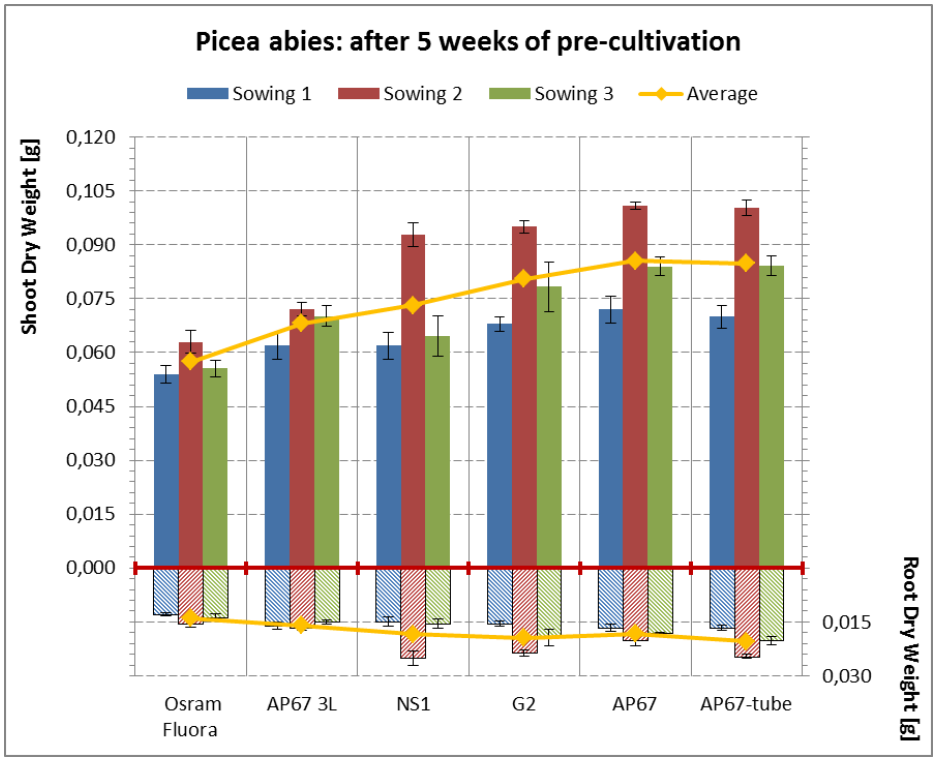


Figure 9: Shoot dry weight and roots dry weight comparison of 3 sowings of Picea abies seedlings after pre-cultivation under different light spectra

As for Scots pine the values for shoot and root dry weight varied somewhat between the different sowings probably due to some variations in the growing environment. However, as for Scots pine the important thing in analysing the result was the fact that the difference between the different light sources was consistent between the different sowings.

This means that like the results from dry weight measurements after pre-cultivation for 5 weeks of Scots pine, dry weight development in Norway spruce benefit from cultivation under G2 and AP67 (bar and tube). These LED spectra therefore seems to have a positive growth effect on both species.

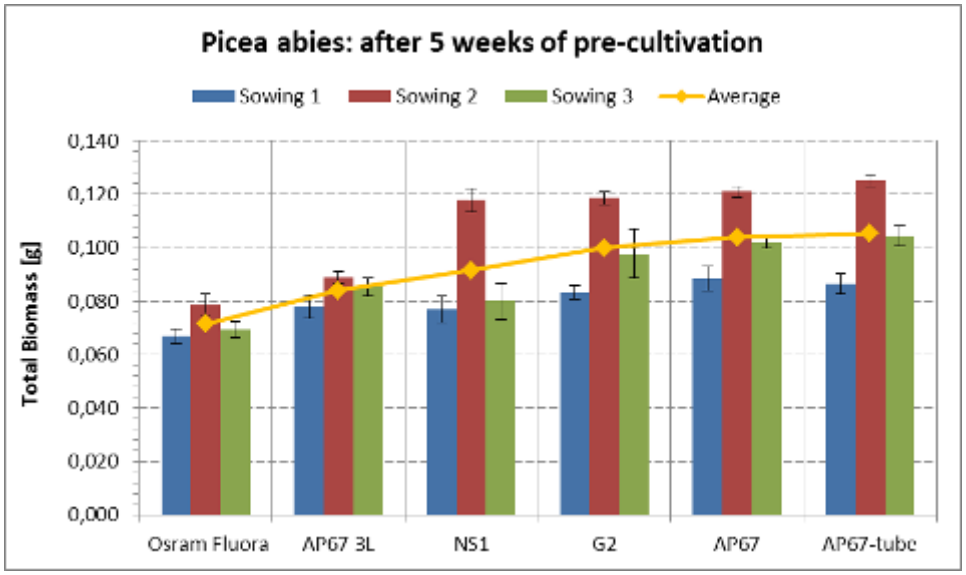


Figure 10: Total biomass (dry weight) comparison of 3 sowings of Picea abies seedlings after pre-cultivation under different light spectra

The measurements of the total biomass confirm the results presented in Figure 10 that is a positive effect of the LED spectra G2 and AP 67 when Norway spruce is pre-cultivated under these spectra for 5 weeks.

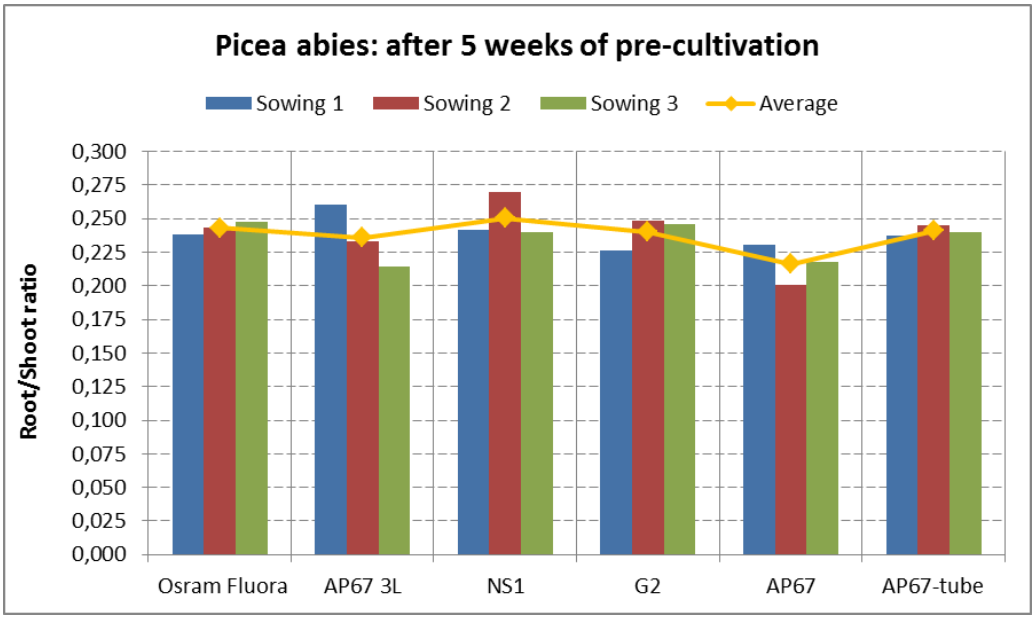


Figure 11: Roots/shoot comparison of 3 sowings of Picea abies seedlings after pre-cultivation under different light spectra

As for Scots pine the differences in root/shoot ratio levelled out between treatments indicating a good balance between the shoot and root development independent of light source during pre-cultivation.

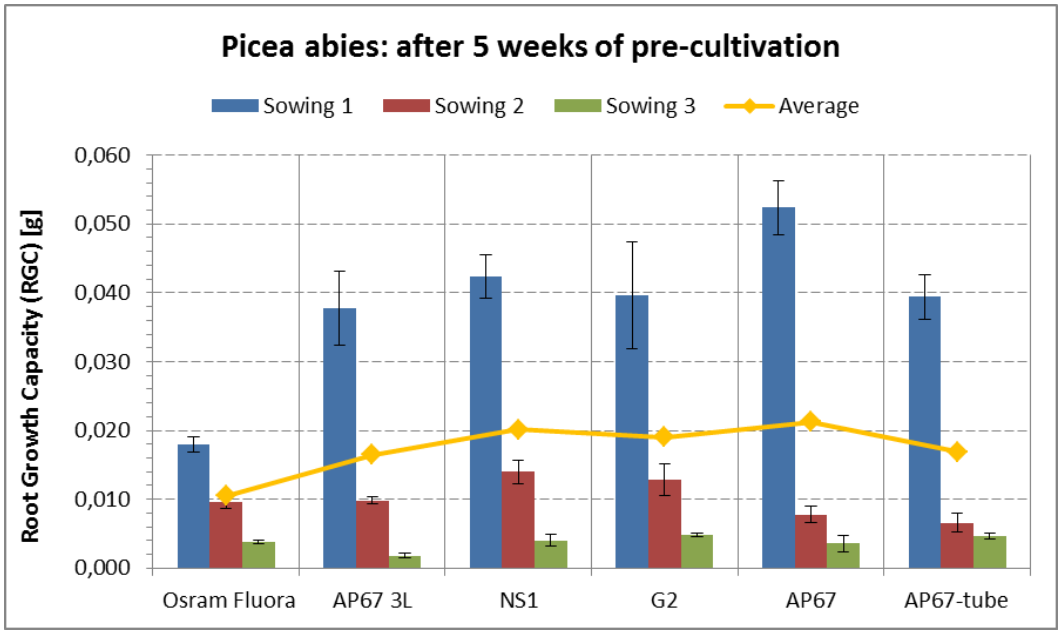


Figure 12: Roots Growth Capacity comparison of 3 sowings of Picea abies seedlings after pre-cultivation under different light spectra

Root Growth Capacity for the respective treatments is shown in Figure 12. Also these results confirm an error in the measurements regarding sowing 1 of the dry weight of new roots produced under the RGC growth period. As for Scots pine measured at the same time, root dry weight for Norway spruce presented in Figure 9 was lower or equal than the other sowings indicating that the presented values for RGC regarding sowing 1 are not correct. However since there was a systematic error for all spectra the average values confirm the results from Figure 9 and Figure 10. That is, a positive effect when the seedlings were pre-cultivated under LED spectra in particular AP67.

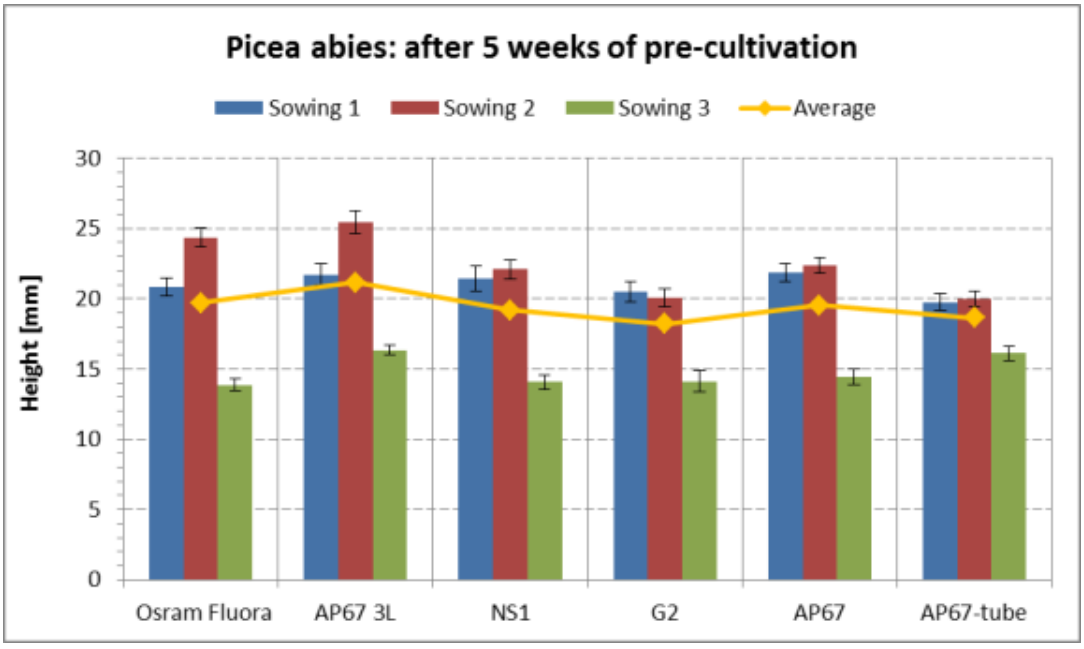


Figure 13: Height comparison of 3 sowings of Picea abies seedlings after pre-cultivation under different light spectra

As for Scots pine differences in height after 5 weeks of pre-cultivation was also small for Norway spruce when comparing sowings and light sources. This can be explained in the same way that is that the first 5 weeks of height development is not so dependent of light spectra compared to biomass development as shown in figure 9 and 10.

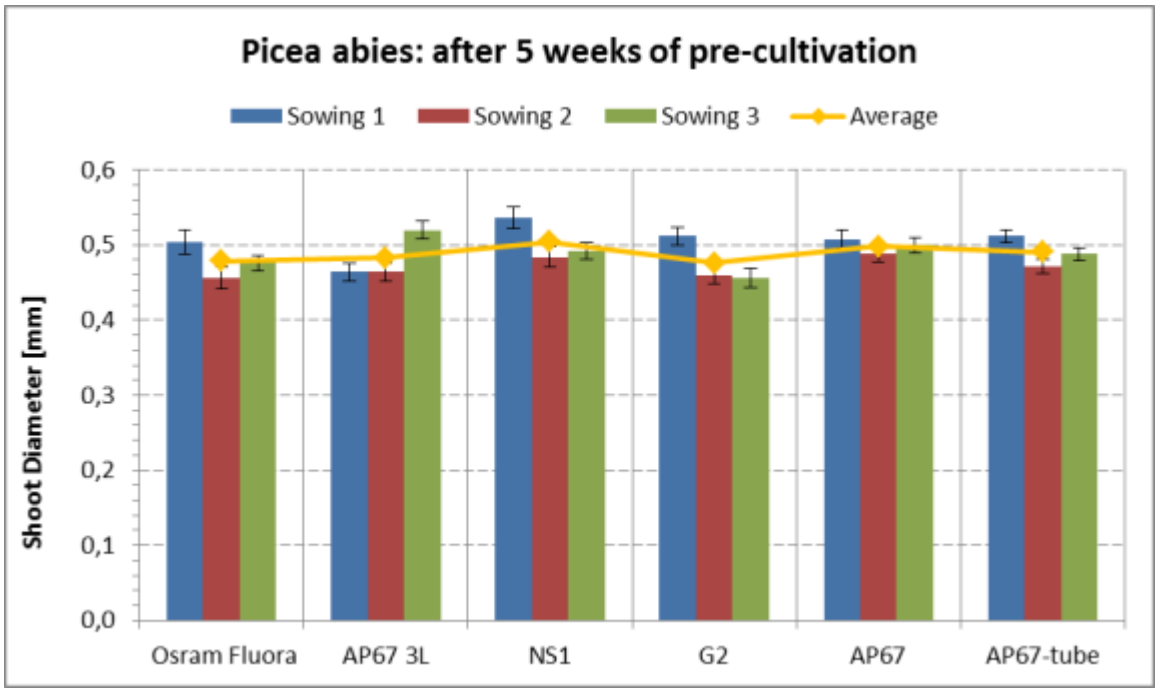


Figure 14: Shoot diameter comparison of 3 sowings of Picea abies seedlings after pre-cultivation under different light spectra

Figure 14 shows that differences in diameter are minor between sowings and light spectra. All treatments have a diameter of about 0.5mm. The reason for this results can be referred to as the same as discussed for Scots pine.

3.3. Direct transplanting to open land

After pre-cultivation and direct transplanting to open land Scots pine and Norway spruce seedlings, pre-cultivated under different light sources, were grown for one vegetation period and shoot height and diameter were measured in October after the end of the vegetation period.

Scots pine

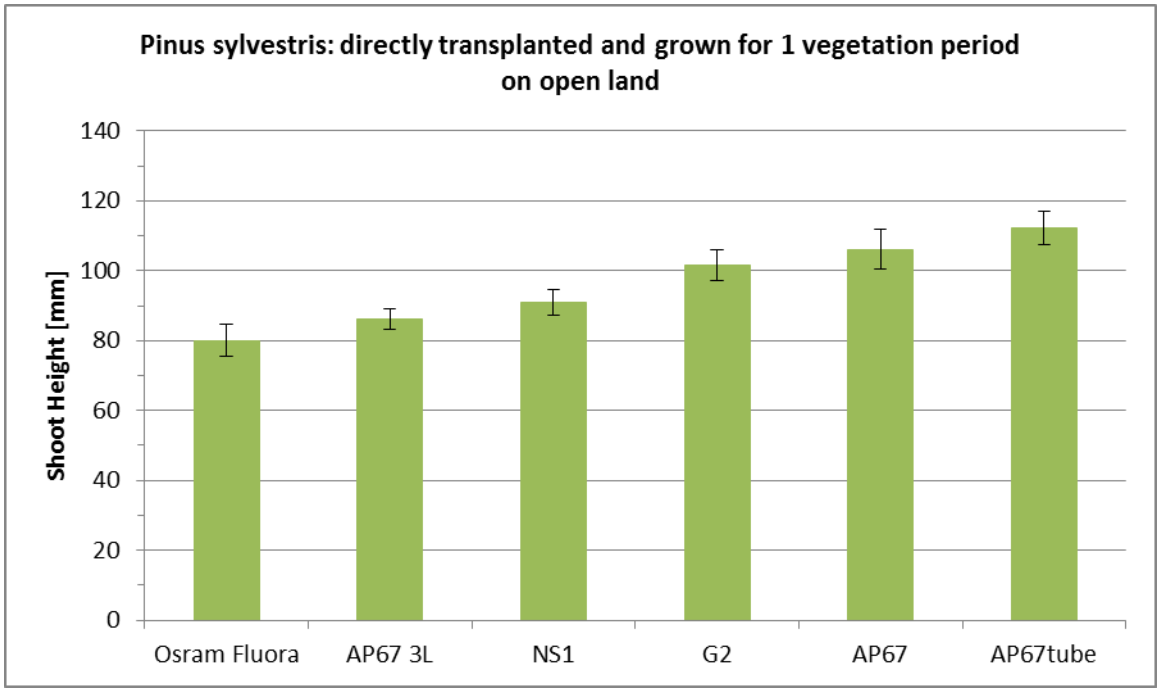


Figure 15: Shoot height comparison of Pinus sylvestris seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land.

The results reflect the measurement of biomass after pre-cultivation with a more positive height development for the LED light spectra G2 and AP67 and within that spectrum especially for the tube version.

One explanation for the, in general, positive results when pre-cultivating under AP67 tube can be that when mounted close to the seedlings the more diffuse light from the inside coated tubes will imply that the full favourable spectrum from the different LED diodes mounted in the tube will hit the seedlings. When mounted in a bar with a clear glass protecting the different LED diodes, spectrum from different individual diodes will hit the seedling to a greater extent compared to the when the same diodes are mounted in a tube.

The seedling shoot height was significantly higher for the LED spectra G2 and AP67 (bar and tube version) compared to the other spectra implying that the higher biomass values after pre-cultivation have had a positive effect the rate of photosynthesis. Also the higher root biomass after pre-cultivation can have implied a better uptake of water and nutrients due to more active root tips.

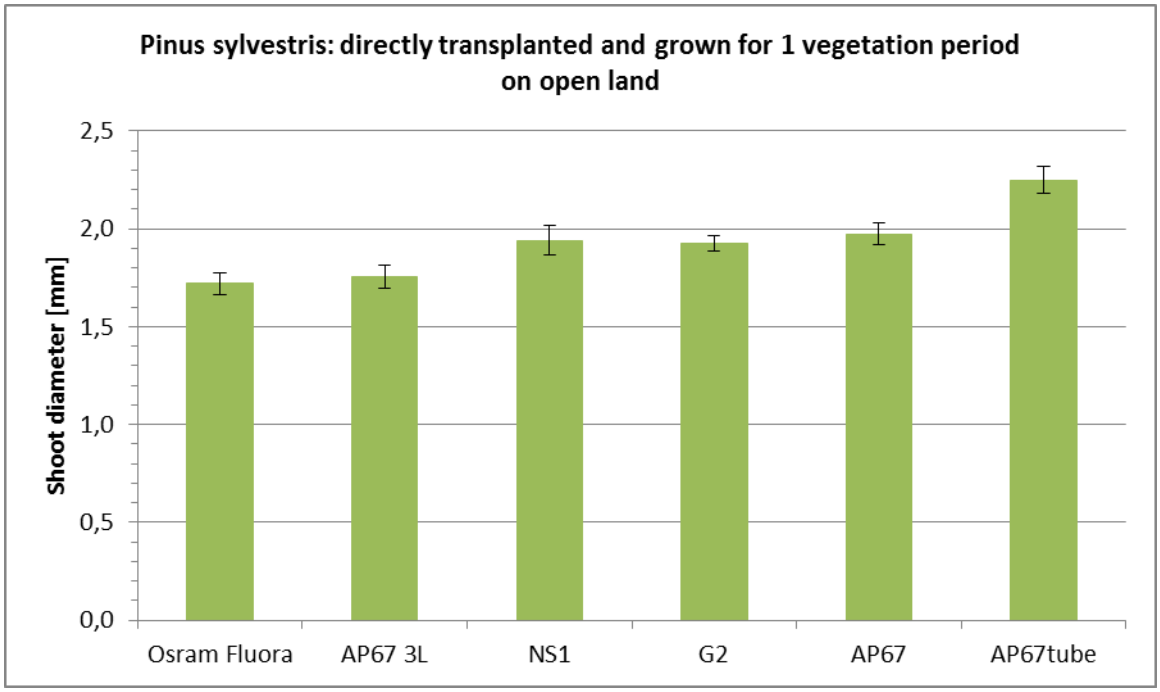


Figure 16: Shoot diameter comparison of Pinus sylvestris seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land

The measurement of shoot diameter after one vegetation period confirmed the results from the height measurements. That is a positive effect on diameter growth for seedlings pre-cultivated under the LED spectra G2 and AP67 and especially for the tube version. Reasons for these results can in all essentials be explained from what has already been discussed regarding the height development.

Norway spruce

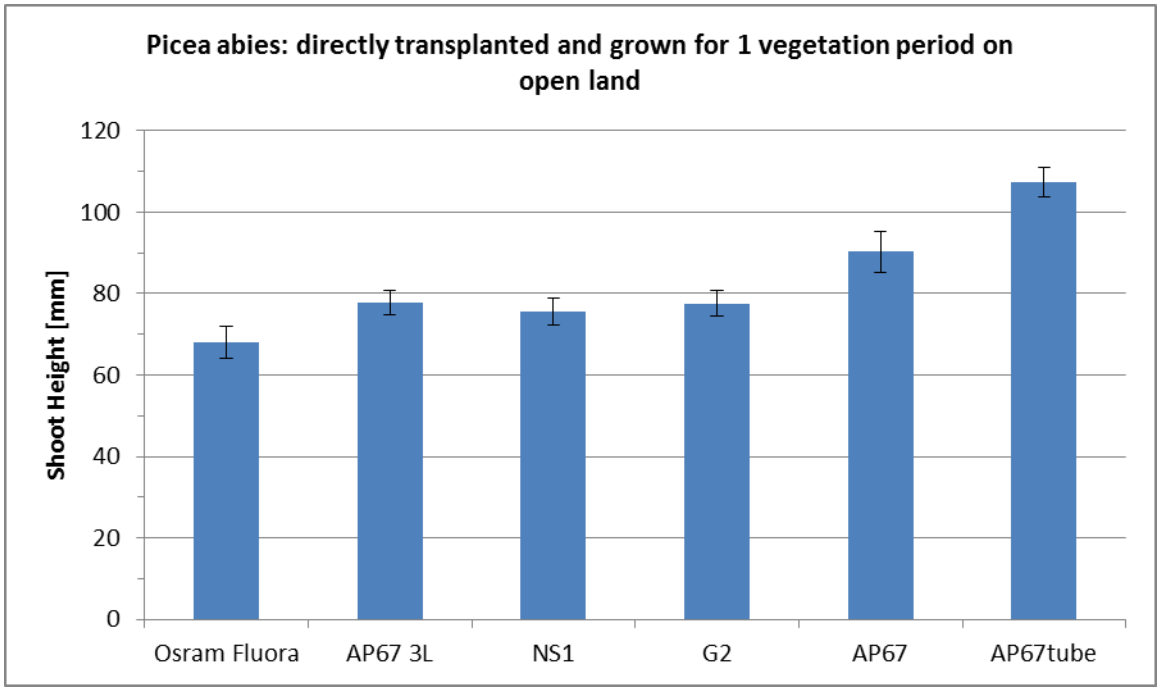


Figure 17: Shoot height comparison of Picea abies seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land.

The results from the follow up of shoot development in Norway spruce pre-cultivated and grown for one vegetation period on open land after transplanting confirmed in all essentials the results from the measurements of Scots pine. Also here there was a positive effect of pre-cultivating under the LED spectra G2 and AP 67 especially for the tube version. Also here the results probably are an effect of a larger shoot and root biomass affecting seedling development in the way already described for Scots pine.

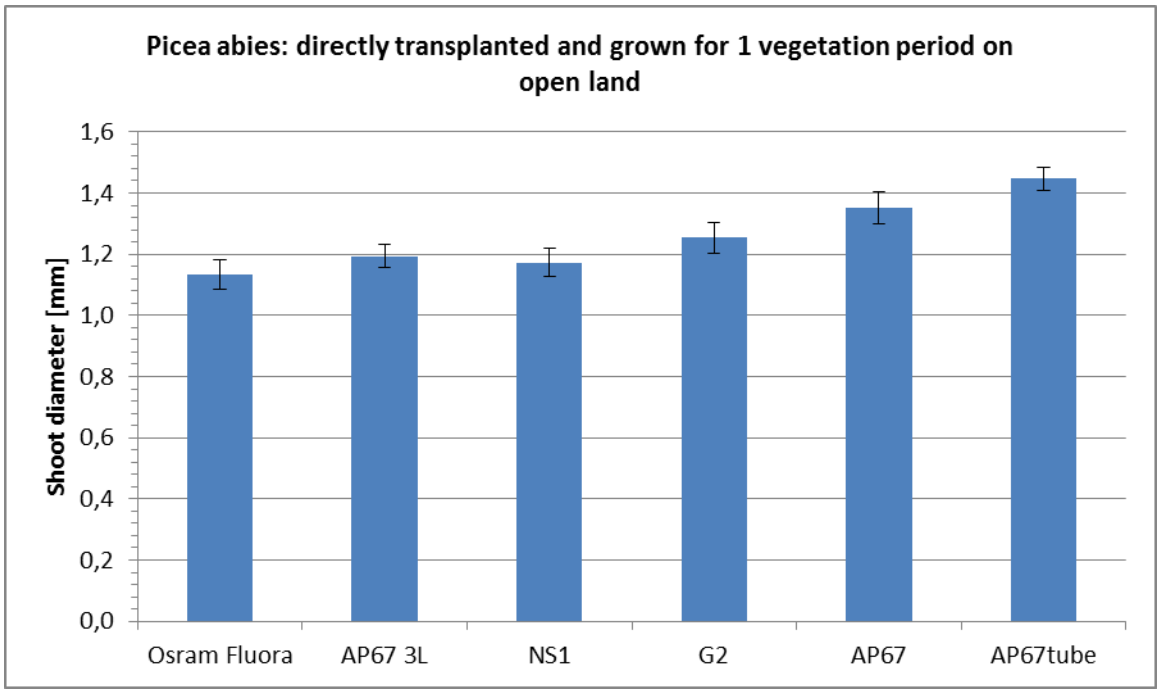


Figure 18: Shoot diameter comparison of Picea abies seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land

The measurement of shoot diameter gave the same results as for height development. That is showing a positive effect when seedlings have been pre-cultivated under the LED spectra G2 and AP67. The reason for these results can most probably be referred to what already have been discussed in regard to the results for Scots pine after one vegetation period on open land after transplanting.

3.3.1. Light shock trial

Regarding direct transplanting from pre-cultivation under artificial light to full exposure to sunlight on open land after transplanting can imply a light shock that could result in for example needle damages affecting seedling development negatively. Therefore before presenting final growth protocols for Scots pine and Norway spruce in D3.3, cultural regimes for reducing this risk must be identified. The reasons for a potential light shock when moving the pre-cultivated seedlings to open land in connection to transplanting can be explained in a simplified manner as follows.

In general the photosynthetic apparatus is extremely good at adapting to different light sources and intensities. A tree seedling in the forest understorey for example may experience sudden sunflecks during which the photon flux may increase up to 100 times in matter of seconds (Pearcy, 2000). Likewise during its lifetime growth to the top of the canopy, the light received will vary depending on the season and disturbances in neighbouring trees. The temporal scale in which these light changes occur requires flexibility and diverse short- and long-term mechanisms to cope with eventual excess of energy (Huner et al., 2006).

While normally in charge of harvesting light; the photoreceptors are also responsible for short-term photoprotection mechanisms with diverse enzymes to avoid damaging the cells during sudden light increments (Horton & Ruban, 2005). Excess energy should be re-emitted (known as energy quenching), either in the form of fluorescence or in the form of heat (called *non-photochemical quenching, NPQ*) (Reece & Campbell, 2011). While light-induced damage (*photoinhibition*) hinders growth by reducing the photosynthetic efficiency; it is also a powerful short-term solution against the formation of toxic reactive oxygen formed when more photons are captured than the electrons that can be effectively transported (W. Adams III et al., 2006).

During longer exposures to high irradiance levels (e.g. transplanting to open land), long-term solutions are needed. These involve reducing the amount of energy harvested by the photoreceptors by changing their size and amount. The size of the receptors or antenna is directly related to the light they are grown under. Plants that are grown under low light intensity try to catch as much photons as possible by developing bigger harvesting systems than those that are grown under higher intensities (Anderson et al., 1995). Modulation of antenna size in response environmental changes is part of a complex and time consuming response called *photoacclimation* (Huner et al., 2003). The commonly used terms “shade tolerant” and “shade intolerant” refer in part to the plants ability to adapt and stand low or high light levels respectively.

In the case of the tree seedlings studied in the Zephyr project, the light intensity at which they are being pre-cultivated (100μmol/m^2·s) is considerably low compared to the levels that they can encounter outside the growth chamber (>1000μmol/m^2·s even in cloudy days). It is thus necessary to find solutions that help them reduce the immediate *light shock* after transplanting and give them time to *photoacclimate* to the new lighting conditions without hindering their growth.

3.3.2. Trial design

To study the possibility of reducing the light shock after transplanting the following pilot trial was performed. More trials within this important area will be performed during the continuation of the Zephyr project with the aim of finding suitable cultivation regimes to be implemented in the growth protocols in D3.3.

For the pilot trial, all seedlings were pre-cultivated under Valoya's AP67-tube spectrum at 100μmol/m^2·s at 20°C during 5 weeks. After the pre-cultivation, the seedlings were transplanted to open land dividing them in different treatments. The control group was placed directly under the sunlight whereas the other groups were first treated either with an evaporation protection solution, placed under a shading cloth or both. The shading cloth reduced to half the incoming irradiance. As evaporation protection, the seedlings were sprayed with a liquid that protect them from high levels of evaporation after outplanting (product name: *NA Avdunstningsskydd*; active substance Styren-butadien polymer 480g/l).

The seedlings under the shading cloth remained there either 3 or 5 weeks before being placed also under direct sunlight for the rest of the vegetation period. Due to a limited amount of seedlings, the 3 weeks treatments were omitted for Norway spruce. A more detailed explanation of the experimental design can be found in Table 1 and Figure 19.

At the end of the vegetation period (October) an inventory of the seedling was done. For this, 15 seedlings per treatment divided in 5 replications were selected to measure:

- shoot height
- shoot diameter
- needles dry weight
- stem dry weight
- roots dry weight
- survival %

Table 1: Experimental design for trial to reduce light shock after transplanting

	Pine	Spruce
1. Control	X	X
2. Evaporation protection	X	X
3. Shading cloth (50% of light intensity) 3 weeks (15/7-7/8)	X	
4. Shading cloth (50% of light intensity) 5 weeks (15/7-22/8)	X	X
5. Evaporation protection + Shading cloth 3 weeks (15/7-7/8)	X	
6. Evaporation protection + Shading cloth 5 weeks (15/7-22/8)	X	X

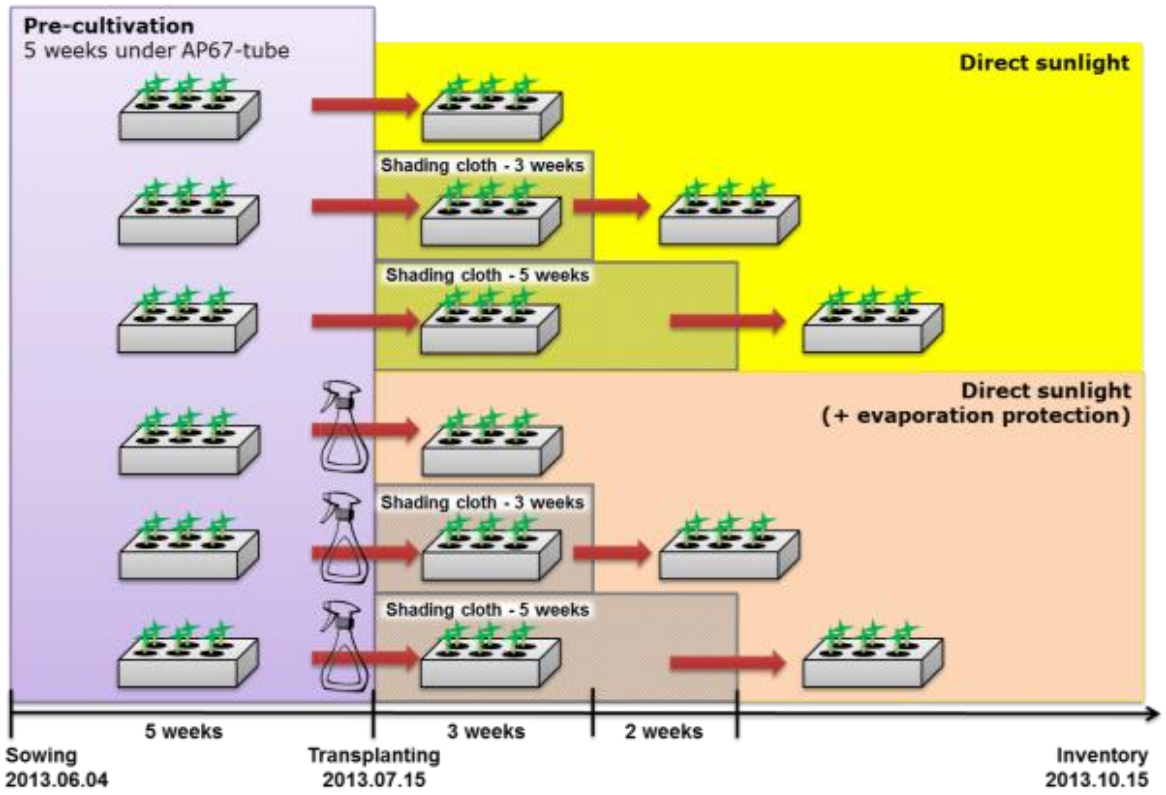


Figure 19: Diagram of light shock trial

A visual inspection of the seedlings was done after 3 weeks of transplanting. It was possible to observe clear differences between the seedlings that have been under the shading cloth compared to those that haven't (Figure 20). In order to further study the acclimation time, some of the trays were taken out of the shading cloth to open land while others were left there for two more weeks before taking them out to direct sunlight for the rest of the vegetation period.

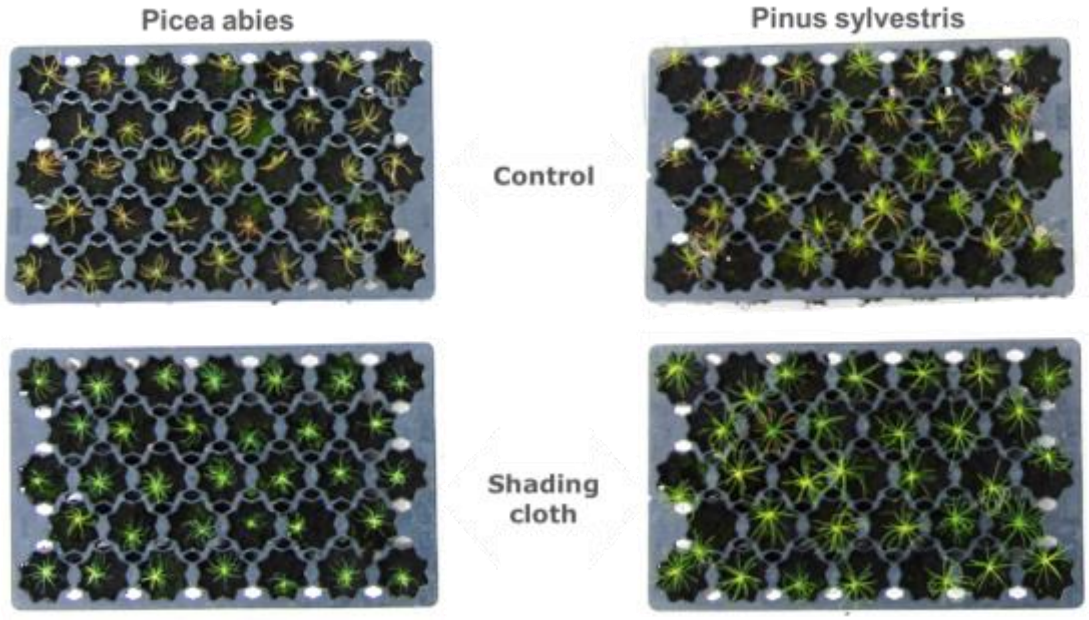


Figure 20: Preliminary results after 3 weeks of transplanting

Scots pine

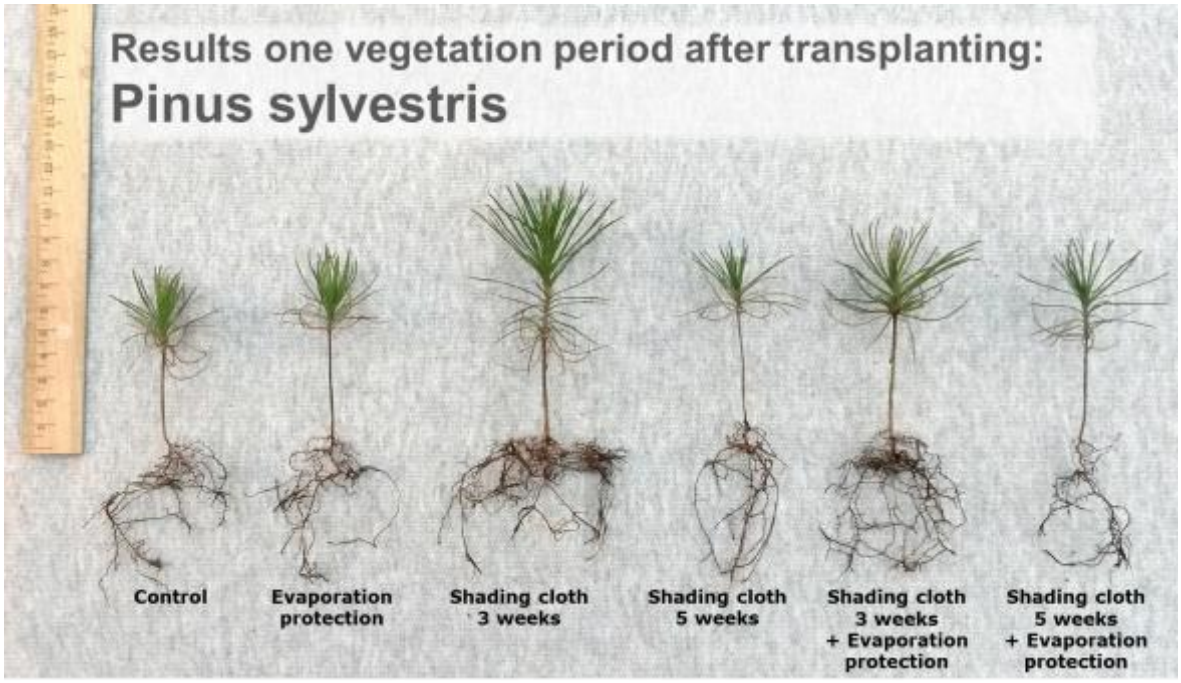


Figure 21: Pinus sylvestris seedlings comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

At the end of the vegetation period an inventory was made to evaluate the effects of the different treatments. Even with the naked eye it was possible to find notable differences among the various groups (Figure 21). This confirms the importance of considering the transplanting shock and include the best solutions in the growth protocols in order to obtain better quality seedlings.

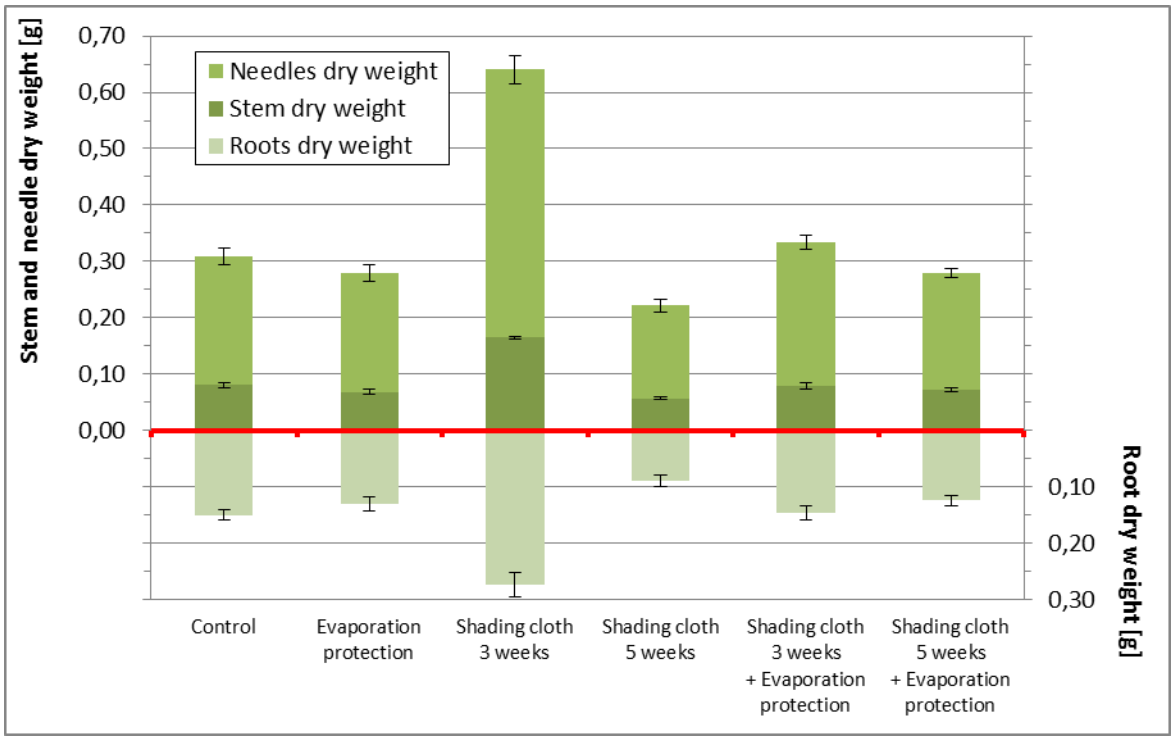


Figure 22: Pinus sylvestris stems, needles and roots dry weight comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

As it can be seen in Figure 22, the seedlings treated with the shading cloth during three weeks without evaporation protection exhibited the best results. It seems that the shading cloth was effective in protecting them from the immediate light shock. This way the seedlings might have been able to continue their growth with low photoinhibition and better photosynthetic rates.

Also, the good results give a hint of the time this species needs to *photoacclimate*. Being a rather shade intolerant tree, the *Pinus sylvestris* seedlings that were kept under the cloth for too long (5 weeks) did not presented the same positive results. In fact, the seedlings treated under the shading cloth for 5 weeks without evaporation protection had a lower biomass (Figure 22) and shorter height (Figure 23) than any other treatment. Their shoot diameter was smaller than the control group (Figure 24) and their survival rate only as good as the control but lower than the 3 weeks group (Figure 25).

The evaporation protection liquid did not bring the expected benefits. Instead the results suggest it has a negative effect on seedling growth.

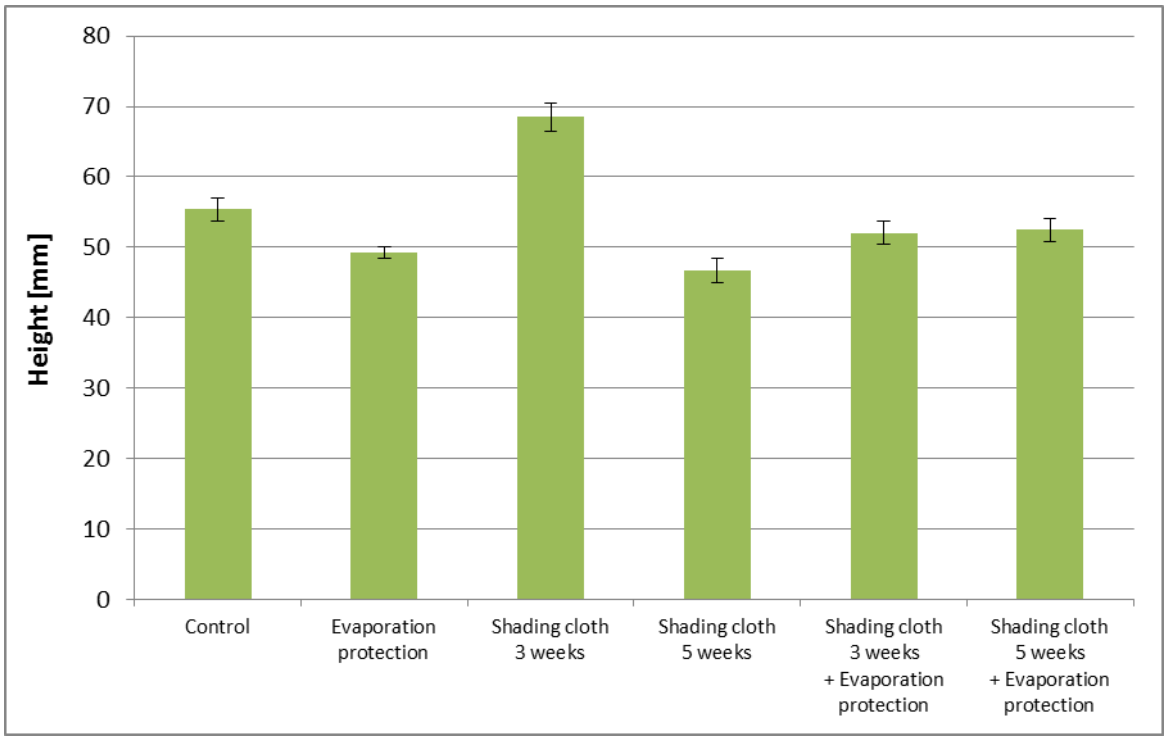


Figure 23: Pinus sylvestris shoot height comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

The height and diameter differences (Figure 23 and Figure 24) for Scots pine show a similar trend and a clear advantage for the seedlings shaded only for three weeks without evaporation protection.

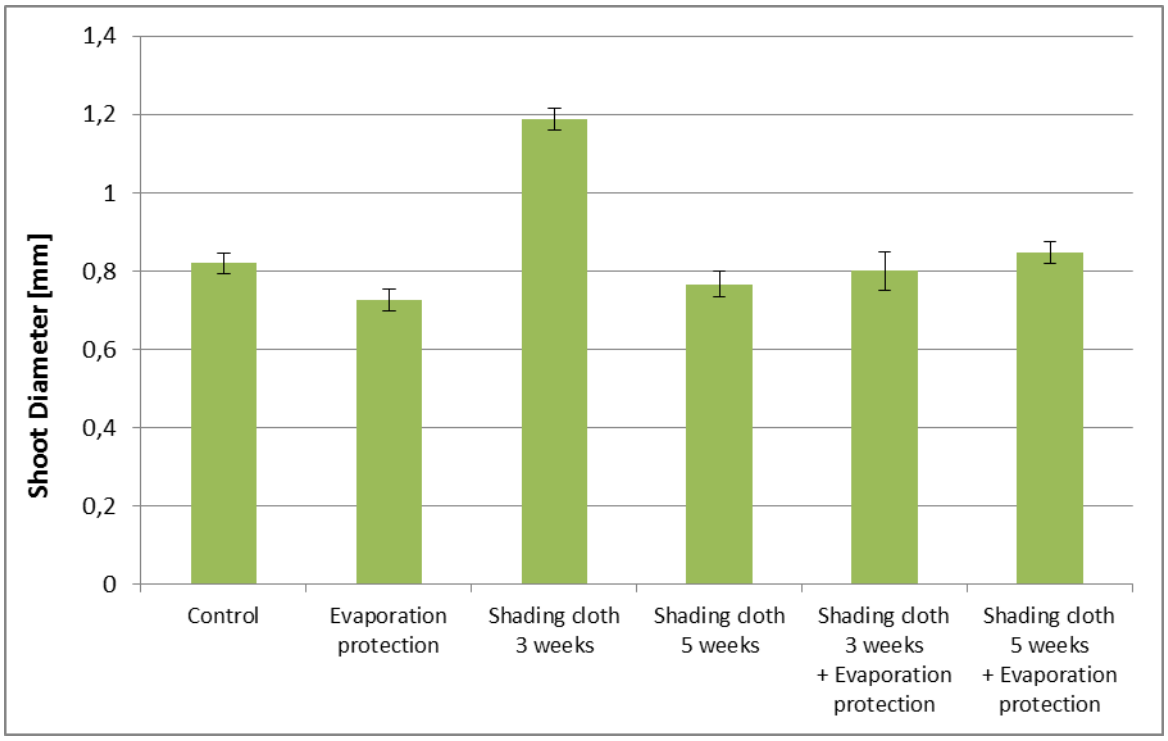


Figure 24: Pinus sylvestris shoot diameter comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

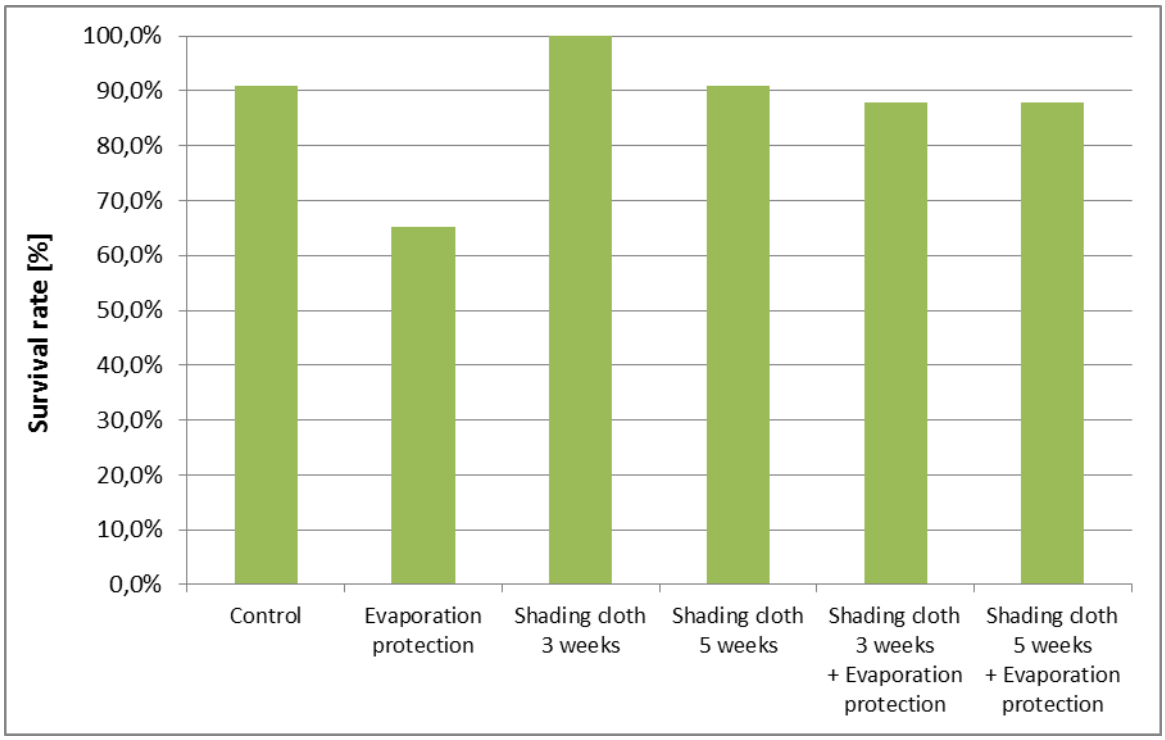


Figure 25: Pinus sylvestris survival rate comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

The high survival rates of the seedlings under the cloth for 3 weeks confirm the previous results concerning the need of a shading cloth at the beginning but only for a limited period of time.

The low survival rates of the treatments with evaporation protection liquid compared to their counterparts gives reasons to discourage its use in this type of applications. This product is mainly used in golf courses where it is important that the grass stays green and fresh but is not important that it grows. It appears that in the attempt of blocking water evaporating from the needles, the substance also blocks other functions and the incoming light reducing the photosynthesis.

Norway spruce

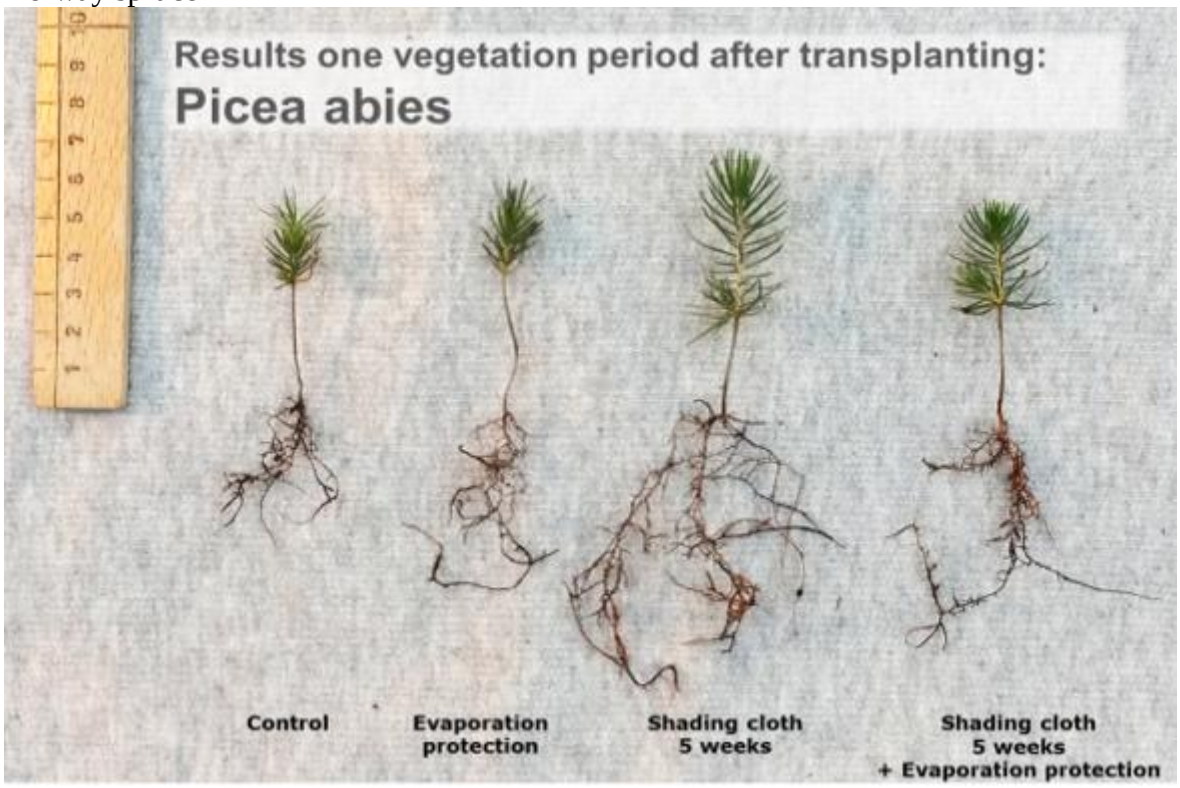


Figure 26: Pinus sylvestris seedlings comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

Analogous to Scots pine, the results for Norway spruce indicate that there is a need to take action when transplanting directly to open land in order to prevent a light shock.

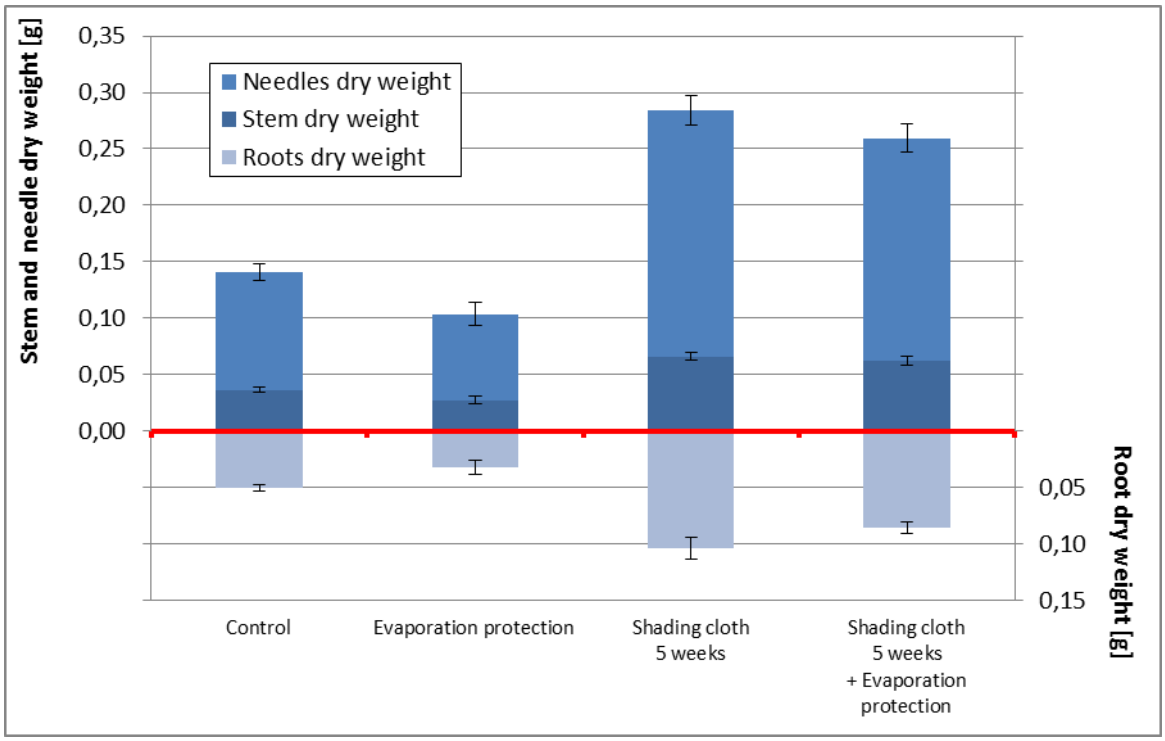


Figure 27: Picea abies stems, needles and roots dry weight comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

In contrast to Scots pine that had its lower growth when being under the shading cloth for 5 weeks; Norway spruce seedlings had their best results in this same treatment. Since it was a pilot study it was not possible to study the effect of only three weeks of shading cloth for Norway and have a better picture of the time each species needs to *photoacclimate*. Nevertheless, the results obtained clearly contrast the nature of the two species studied.

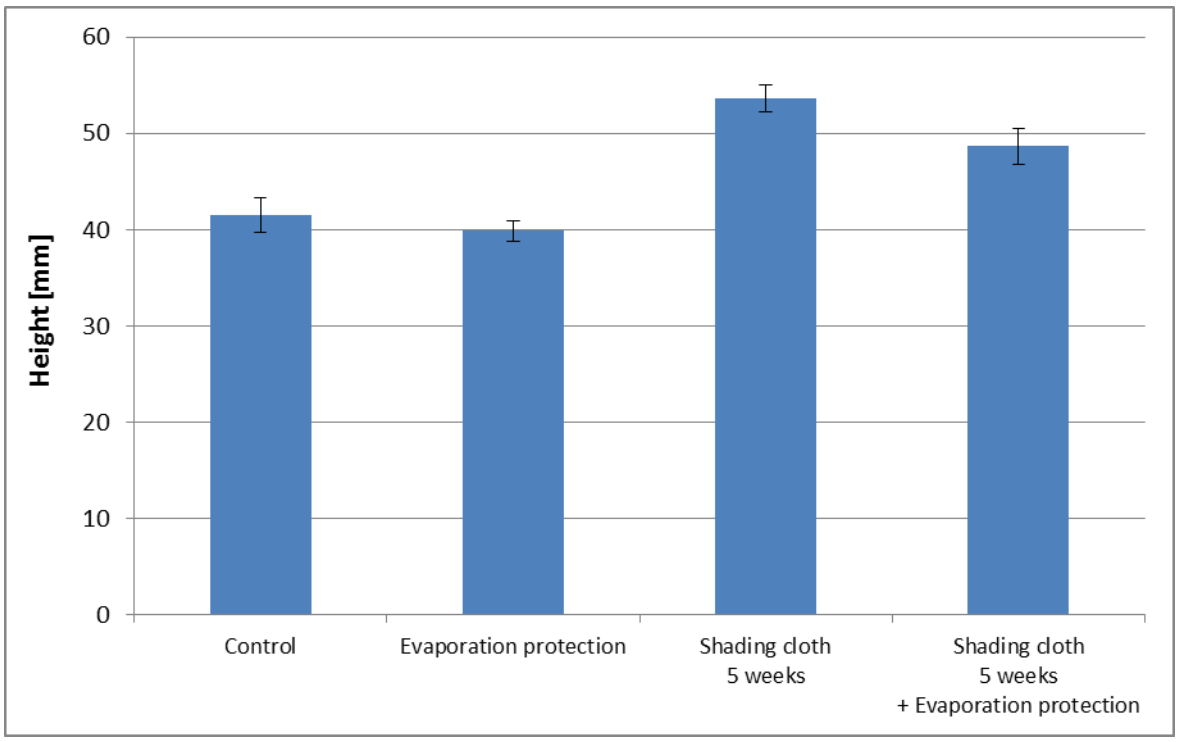


Figure 28: Picea abies shoot height comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

Being a more shade tolerant specie, Norway spruce seedlings benefited from a longer shading period and had a higher biomass dry weight (Figure 27), a taller average height (Figure 28), a bigger diameter (Figure 29) and a better survival rate (Figure 30) than the control seedlings.

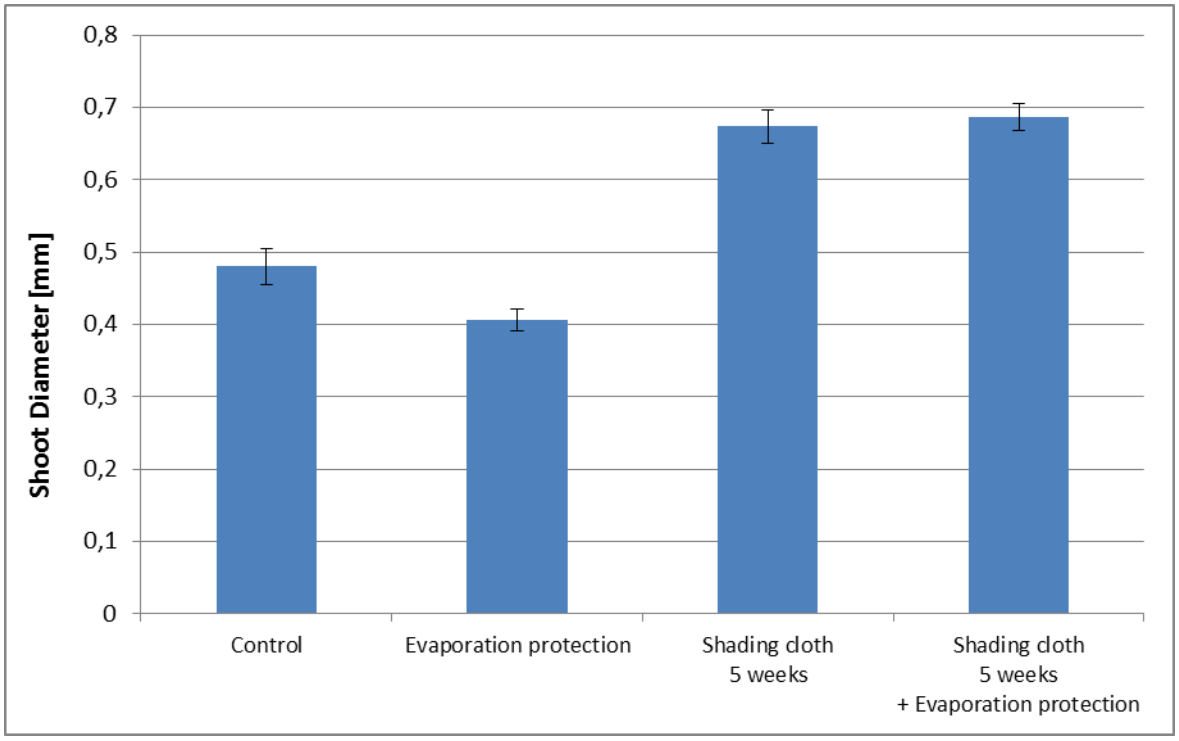


Figure 29: Picea abies shoot diameter comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

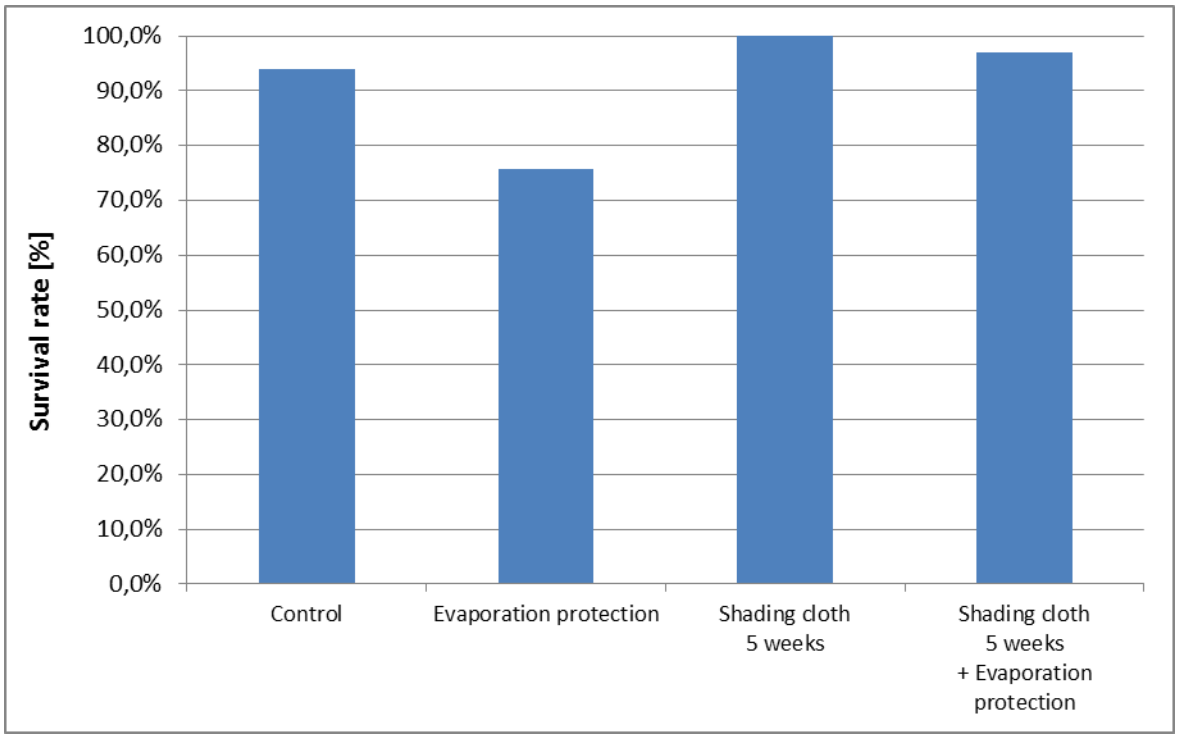


Figure 30: Picea abies survival rate comparison after transplanting and growing for 1 vegetation period under different light shock prevention treatments

The survival rate of Norway spruce was benefited by the shading cloth and as with pine, it was reduced by the use of the evaporation protection liquid when compared to their counterparts.

3.4. Long-night (LN) treatment before cold storage

As described in the background cultural practices for preparing pre-cultivated Scots pine and Norway spruce seedlings for long-term cold storage (2°C) is of utmost importance for the introduction of the pre-cultivation and transplanting cultivation concept in northern Europe. Since most of the batches in a year around cultivation layout have to be cold stored methods have to be tested and validated that ensure that seedling quality will be maintained during and after cold storage. This implies methods to be used both to identify cold tolerance before cold storage and also to identify seedling vitality during and after cold storage.

Methods that have been used include for example freeze tests (Van den Driessche, 1976) and measurement of electrolyte leakage (McKay, 1992) for identifying cold tolerance. Regarding vitality the RGC (Burdett, 1987; Feret & Kreh, 1985; Sutton, 1987; Mattsson, 1991) test and the OSU (Hermann et al., 1979; McCreary & Duryea, 1985) test have been used. All of these tests have the major disadvantage that you have to wait for a long time for the results making it difficult to take quick decisions what actions that have to be done.

Therefore Dalarna University in cooperation with Dutch researcher have developed a method for rapid definition of cold tolerance in Scots pine and Norway spruce seedlings. Together we have identified the genes in the respective species that are active during the hardening phase and by validation with parallel conventional freeze tests followed by vitality measurements, a reliable test have been developed (Stattin et al., 2012). In the test the level of expression of those genetic indicators are identified as molecular markers and four stages of cold tolerance from cold sensitive to cold tolerant have been outlined according to the table below.

Table 2: Definition of the four stages of cold tolerance based on the activity profile of the genetic indicators

0	Cold sensitive	The indicator profiles match the profiles of lots that are actively growing and no sign of cold tolerance development could be recognized.
1	Developing cold tolerance	Early signs of frost tolerance development can be recognized.
2	Developing cold tolerance	Frost tolerance level approaches full cold tolerance.
3	Cold tolerant	The indicator profiles match the profiles of lots that have ceased growth and that are fully tolerant, ready for lifting and storage.

In the following figures the procedures for the test is explained including the advantages to earlier tests.

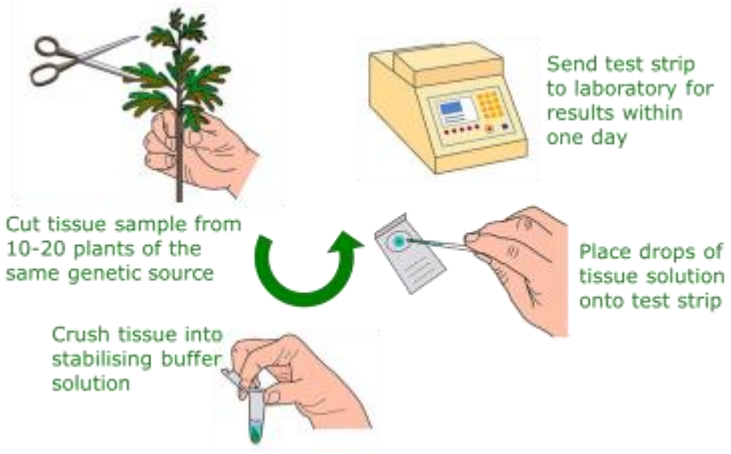


Figure 31: Procedure for making the gene test

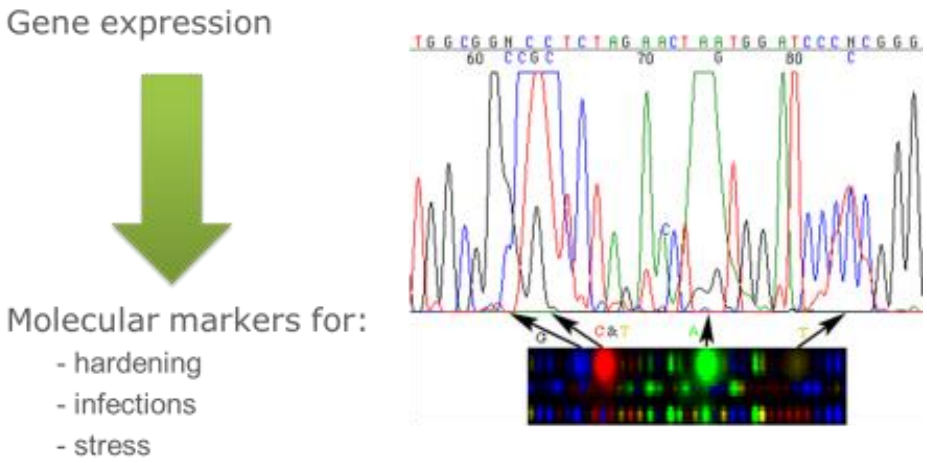


Figure 32: Molecular attributes of gene test

Advantages with the gene test over earlier procedures:

- Sampling is done in the nursery
  (no changes in plant status during transport etc.)
- None-destructive method
  (a few needles are used from each plant)
- Rapid method
  (results within 24h after the sample has arrived at the laboratory)

3.4.1. LN protocol tests for Norway spruce

In Swedish forest nurseries LN treatment of one year old conventional cultivate containerized Norway seedlings have been practised during many years to prepare the seedlings for cold storage. In these treatment seedlings are LN treated during 5 weeks with a daily photoperiod of 8 hours and a night period of 16 hours at an ambient temperature of 20°C.

To test if this treatment also could be applicable for young pre-cultivated Norway seedlings the same procedure was applied. As the LED light source AP67 in the tube version had proven to be favourable this light source was also chosen during the 5 weeks of pre-cultivation of Norway spruce seedlings. After pre-cultivation LN treatment was conducted during 5 weeks under the same conditions as described for conventionally grown Norway spruce seedlings. The treatment showed to be very effective and after the gene test the result showed that the seedlings were cold tolerant and ready for cold storage. Therefore the LN treatment described will be part of the final growth protocols for pre-cultivated Norway spruce

3.4.2. LN protocol tests for Scots pine

Pre-cultivated Scots pine did not react as Norway spruce on the described LN treatment and was in stage 0 after the gene test. That meant that no sign of cold tolerance development could be recognized. In general Scots pine has shown to be more difficult to induce cold hardiness compared with Norway spruce. This can be due to the history of how these species found its way into Sweden after the latest glacial period. Scots pine found its way mainly from central Europe while Norway spruce was coming to Sweden from the very north parts of Europe and therefore genetically different in there adaptation to a cold climate.

To test if cold tolerance also could be induced in Scots pine seedlings a broad experimental design was conducted including both variations in the duration, photoperiod and temperature during LN treatment. All treatments were pre-cultivated under AP67-tube spectrum that has proven to be favorable during pre-cultivation of Scots pine. Light intensity was as for Norway spruce set at 100 µmol/m^2·s.

Table 3: Experimental design for new LN-protocol of Pinus sylvestris

		Photoperiod for LN							
Species	**LN -treatment (duration weeks)**	2 hrs light 22-hrs dark	5 hrs light 19-hrs dark			8 hrs light 16-hrs dark			
Scots pine	Short: 5 weeks	20°C *	5°C	10°C	20°C	5°C *	10°C	20°C	
	Long: 7 weeks	20°C *	5°C	10°C	20°C	5°C *	10°C	20°C	

**currently ongoing treatments*

Table 3 shows the experimental design including 2 different durations, 3 photoperiods and 3 different temperatures during the LN-treatment.

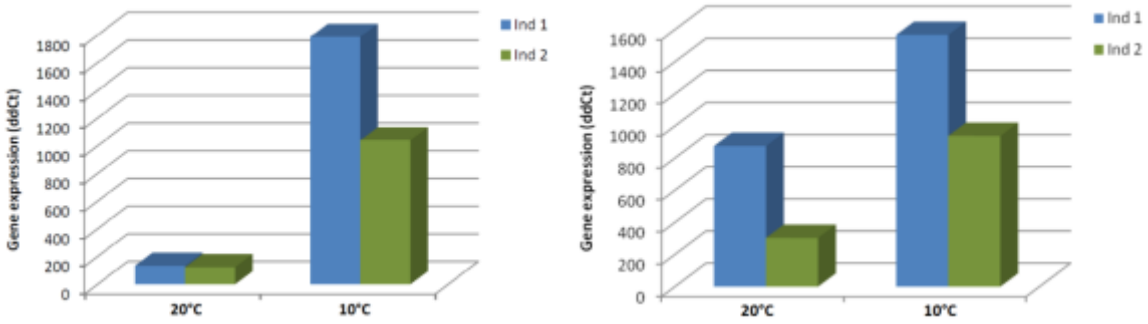


Figure 33: Gene expressions for cold tolerance: Pinus sylvestris LN treated at 20°C and 10°C for 5 weeks (left) and 7 weeks (right)

Figure 33 shows the result of the gene test after 5 respective 7 weeks LN treatment at a photoperiod of 8 hours. As can be seen from the figure there were some influence of the duration for seedlings LN treated at 20°C but still both results was within stage 1 that is developing cold tolerance in an early stage. The major differences in the figure refer to the effect of temperature during LN treatment. There was a higher level of gene expression regarding cold tolerance for seedlings treated at 10°C compared to 20°C and the seedlings was in stage 2 that is approaching full cold tolerance.

One interpretation of the results could be that it seems that Scots pine seedlings needs a cooler climate during the hardening phase which perhaps can be due to the genetic constitution of a species that in its evolution has been adapted to a warmer climate compared to northern Europe.

To our knowledge no studies have been published within this field showing that young Scots pine seedlings (10-12 weeks) can be positively affected regarding cold tolerance by introduction of a low temperature during LN-treatment.

Therefore we see this study as a breakthrough in the possibility of outlining growth protocols that will make it possible to cold store pre-cultivated Scots pine seedlings within the new cultivation technology developed within the Zephyr project.

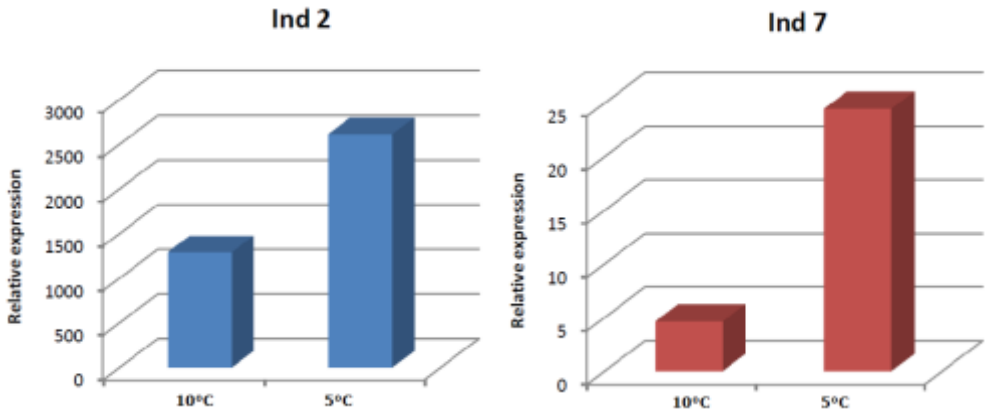


Figure 34: Gene expressions for cold tolerance: Pinus sylvestris LN treated at 10°C and 5°C for 5 weeks

In Figure 34 results are shown where we lowered the temperature down to 5°C to see if we could have an additional effect regarding the development of cold hardiness. Still the photoperiod was set to 8 hours. As can be seen we gained an additional level in the expression of genes active in the hardening phase. This also meant that seedlings LN-treated at 5°C had reached stage 3 and was fully cold tolerant. The positive reaction

regarding cold tolerance to a temperature lower than 10°C can most certainly be due to what has been discussed in regard to the results shown in figure 19.

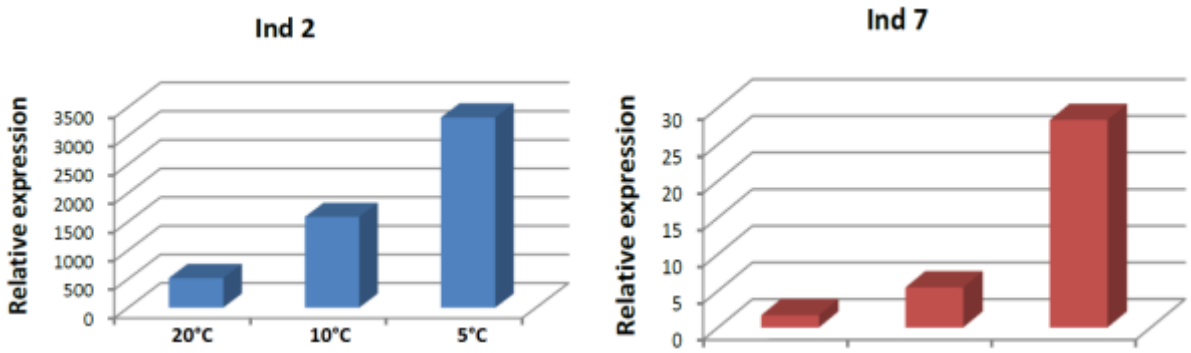


Figure 35: Gene expressions for cold tolerance: Pinus sylvestris LN treated at 20°C, 10°C and 5°C for 7 weeks

Figure 35 is showing the results after LN-treatment during 7 weeks at all three temperatures with a photoperiod of 8 hours. For both of the two gene expressions seedlings treated at 20°C was in stage 1, seedlings treated at 10°C in stage 2 and finally seedlings treated at 5°C was in stage 3. The reasons for these results have in all essentials been discussed in connection to the previous figures.

Table 4: Results of LN-treatment: cold tolerance according to the gene test.

	Photoperiod for LN-treatment				
LN -treatment (duration weeks)	5 hrs light 19-hrs dark			8 hrs light 16-hrs dark	
5 weeks	5°C	10°C	20°C	10°C	20°C
Cold Tolerance stage	3	2	1	2	1
7 weeks	5°C	10°C	20°C	10°C	20°C
Cold Tolerance stage	3	2	1	2	1

Table 4 summarizes all the tests performed so far regarding variations in duration, photoperiod and temperature during LN-treatment. As can be seen cold tolerance in Scots pine seedlings seems to be highly dependent on the temperature during LN treatment of pre-cultivated Scots pine seedlings and not so much on the photoperiod and duration of the treatment.

3.5. Cold storage before transplanting to open land

The cold storage environment was defined at +2°C with a tolerance of ±1°C with a relative humidity of 90% ±5%. Cold storage will be carried out from July 2013 to June 2015. Batches from different treatments will be currently stored at various durations from three months up to 12 months. For each batch validation of seedling vitality will be conducted using gene test, RGC and shoot dry weight development. Dry weight of the shoot will be recorded since the respiration in the storage will affect the dry weight due to the consumption of for example carbohydrates.

In this intermediate report two figures has been chosen to illustrate interesting differences in survival between Scots pine and Norway seedlings pre-cultivated and LN-treated under different light sources and then cold stored for 5 or 8 months. LN-treatment was conducted at a duration of 5 weeks, a photoperiod of 8 hours and a temperature of 20°C for the respective species.

3.5.1. Survival rates depending of storage time

Scots pine

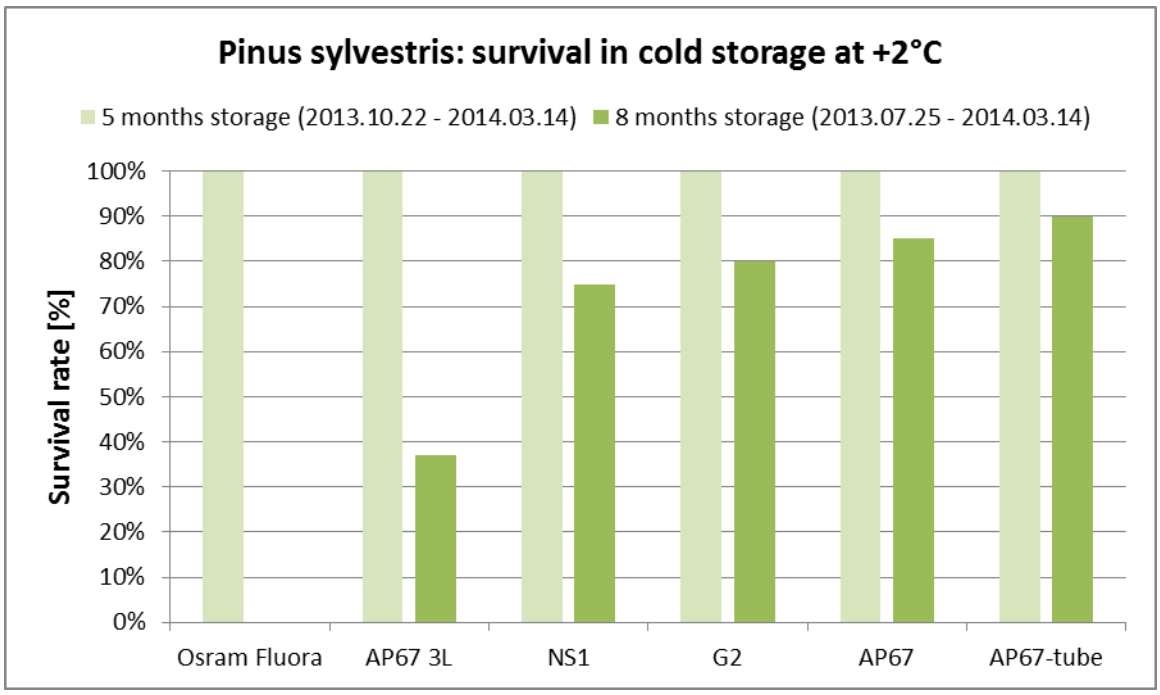


Figure 36: Pinus sylvestris survival rate comparison in cold storage

Figure 36 shows survival rate after 5 respective 8 months of storage for Scots pine seedlings pre-cultivated and LN treated under 6 different light sources. As can be seen all seedlings survived 5 months of storage but after 8 months there were a reduction in survival. The reduction was less severe when cultivated under the LED spectra G2 and AP67 (both bar and tube). For the control all seedlings had died after 8 months while only about 35% had survived for the LED spectra AP67 3L and about 75% for the LED spectra NS1.

This indicates that different spectra had influenced the hardening process in various ways and because of that also the cold tolerance for the seedlings. This are to us new findings that has not been reported earlier and therefor has to further analysed and validated in future tests within the Zephyr project. If different spectra can affect the cold tolerance in young

Scots pine seedlings interesting new possibilities opens within the field of inducing cold hardiness in coniferous seedlings.

Norway spruce

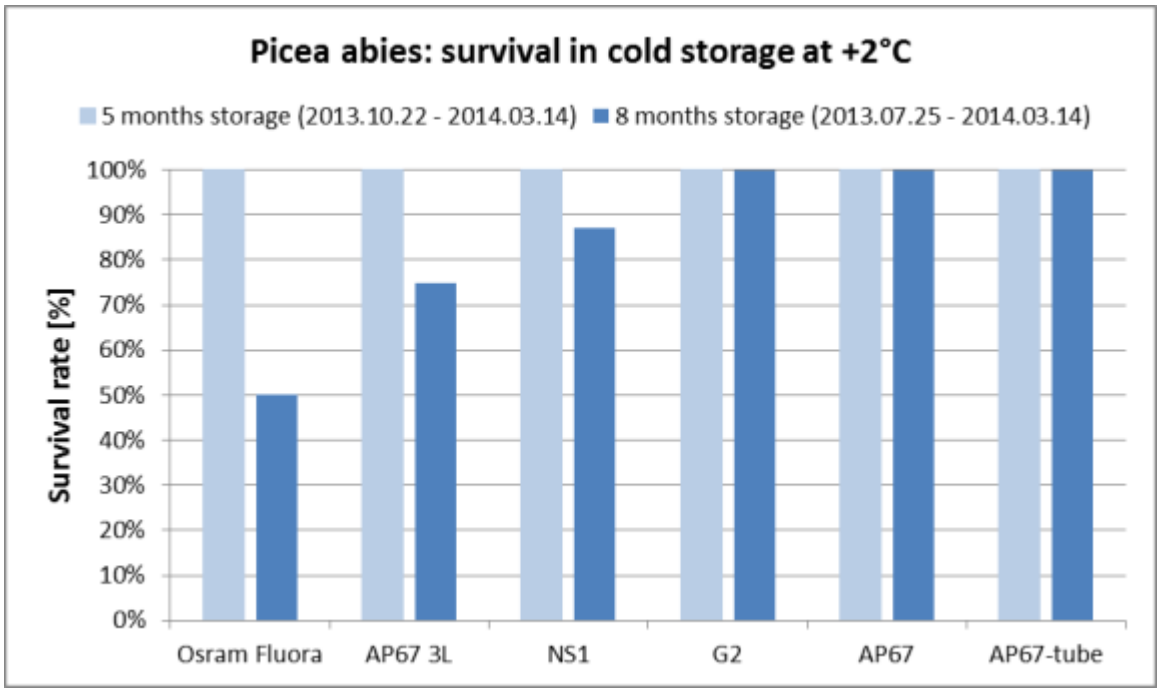


Figure 37: Picea abies survival rate comparison in cold storage

Figure 37 shows the same results as for Scots pine with a little bit less drastic results in the rate of survival. This can be due to the earlier discussion of Norway spruce being more adapted to a cold climate.

3.6. Forest field trial

In order to test the performance in field conditions for seedlings pre-cultivated under artificial light sources, a forest field trial was designed. The place chosen is in a clear cut located just a couple of kilometres away from the Dalarna University research station (Figure 38). The terrain presents an average planting site in mid-Sweden and will allow to evaluate how the "Zephyr seedlings" pre-cultivated under the artificial lights are able to cope with the field stress conditions.

Figure 38: Location of field trial: Latitude: 60.562845, Longitude 15.477584.
Satellite image source: Bing Maps

The trial was divided into two plots, one for Scots pine and one for Norway spruce seedlings which were planted in two scarified rows each as shown in Figure 39.

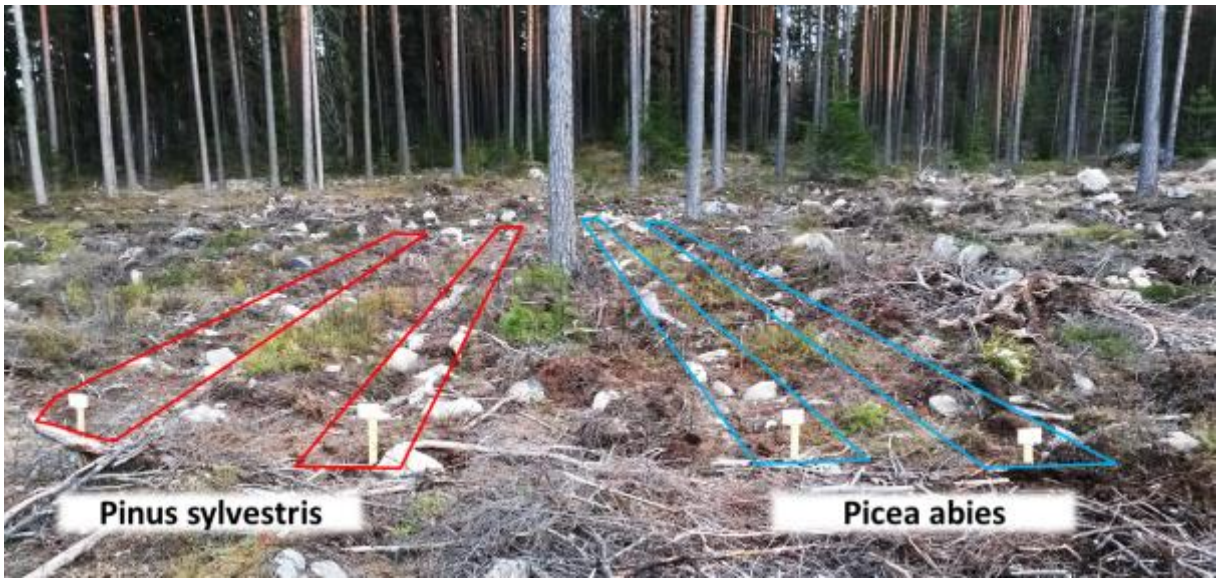


Figure 39: Plot distribution in the forest field trial

Pinus sylvestris	3:5	5:1	2:2	1:4	2:5	1:3	2:1	2:4	1:2	3:1	3:2							
	2:3	4:3	6:1	6:4	1:1	1:5	5:2	4:1	3:4	4:4	6:2	5:3	4:5	5:4	3:3	4:2	6:3	6:5	5:5
Picea abies	3:5	5:1	2:2	1:4	2:5	1:3	2:1	2:4	1:2	3:1	3:2	2:3	6:1	4:3	6:4	1:1	1:5		
	5:2	4:1	3:4	4:4	6:2	5:3	4:5	5:4	3:3	4:2	6:3	5:5	6:5						

Treatment : Replication (3 seedlings)

Figure 40: Experiment layout for the forest trial

Due to the homogeneity of the terrain and proximity of the two rows assigned per species, the seedlings were randomly distributed among both rows as shown in Figure 40. For each treatment, 5 replications of 3 seedlings each were taken and planted according to the layout. These seedlings will be followed for at least one vegetation period.

The seedlings used for this trial were all pre-cultivated under the 6 different spectra during 5 weeks, then transplanted to open land and grown there for one vegetation period before being outplanted in the field.

The seedlings used were taken from the batches studied under heading 3. The discrepancies between the measurements are due to the fact that the field trial seedlings were measured after outplanting. Obviously the various planting depths bias the results comparable to the controlled conditions in the laboratory.

Scots pine

In order to have a starting reference point, the shoot height and diameter of all the seedlings was measured directly in the field after they had been outplanted.

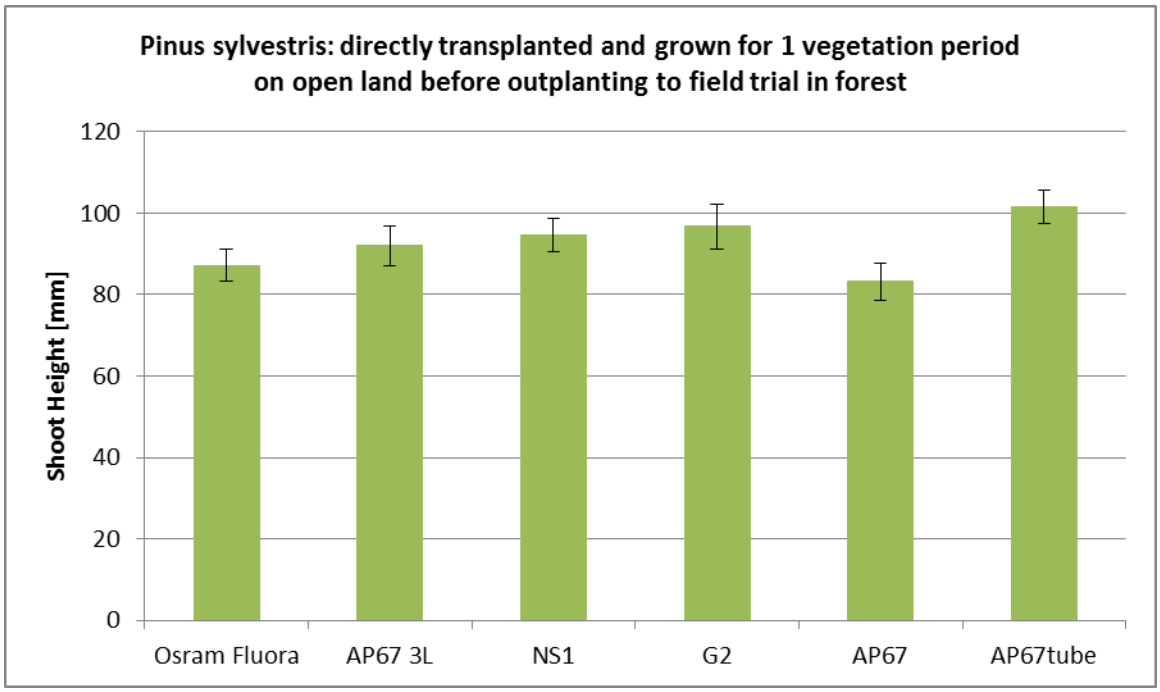


Figure 41: Shoot height comparison of Pinus Sylvestris seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land. Measurements made after outplanting to field trial in forest

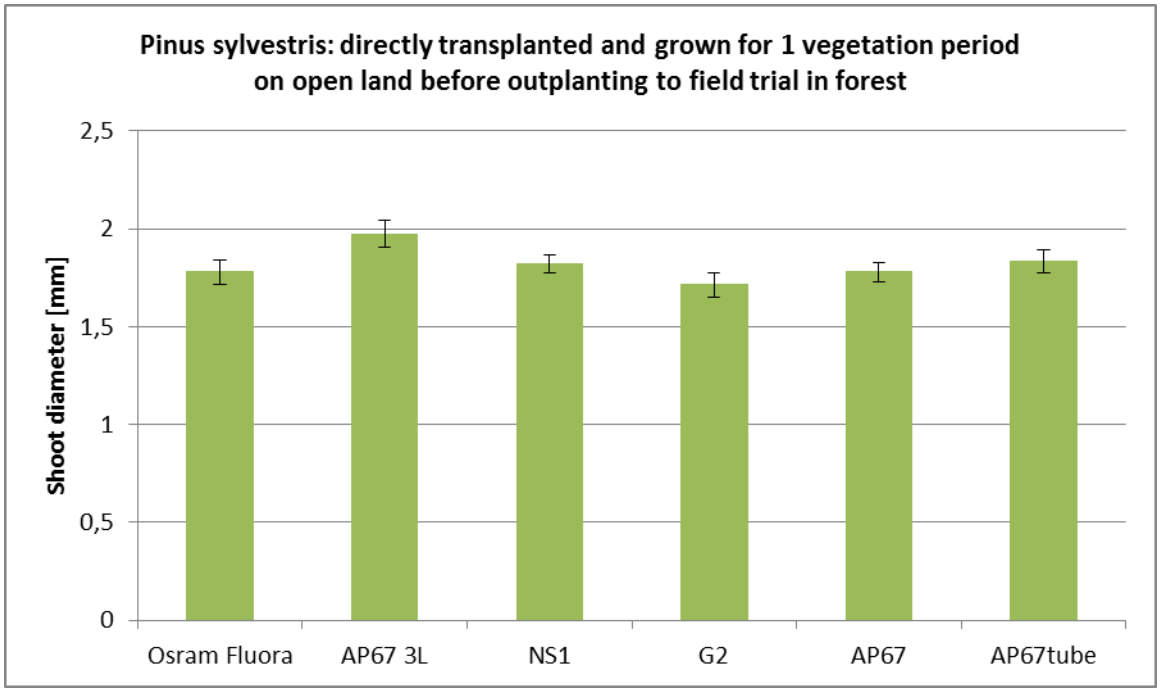


Figure 42: Shoot diameter comparison of Pinus Sylvestris seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land. Measurements made after outplanting to field trial in forest

Norway spruce

Similarly to Scots pine, the height and diameter of the planted Norway spruce seedlings were measured as a reference starting point for the field trial.

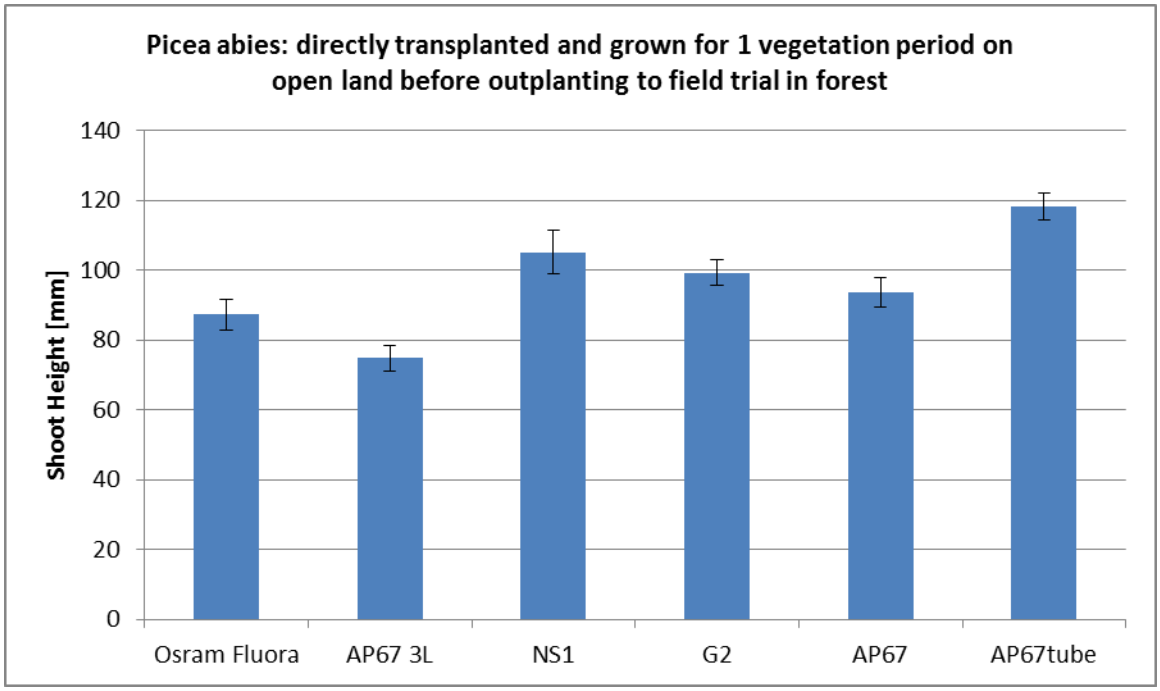


Figure 43: Shoot height comparison of Picea abies seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land. Measurements made after outplanting to field trial in forest

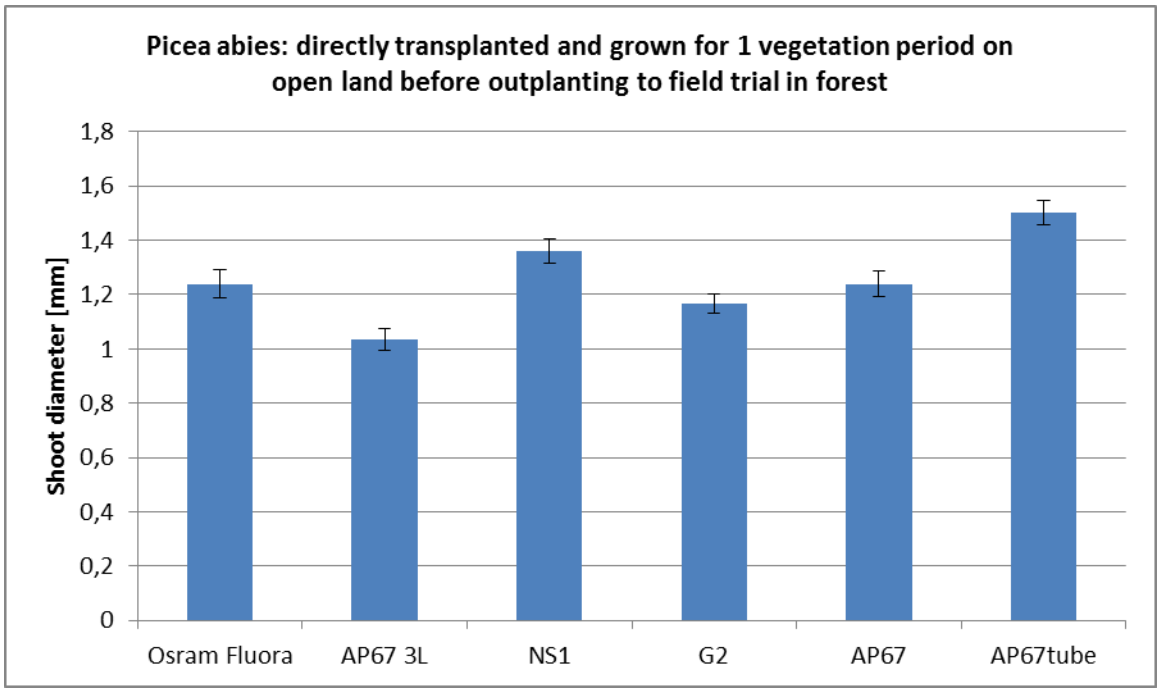


Figure 44: Shoot diameter comparison of Picea abies seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land. Measurements made after outplanting to field trial in forest

3.7. Future measurements

3.7.1. Gas Exchange and Chlorophyll Fluorescence Equipment

In order to obtain a better understanding of the seedlings development and performance in the field, state of the art equipment has been acquired in Dalarna University. It consists of PP-Systems CIRAS-3 open gas exchange system with a tailor-made PLC3 conifer cuvette and a CPY4 canopy for studying gas exchange of complete mini seedlings in the nursery (Figure 45) or in the field (Figure 47). For studying the status of the seedlings Photosystem II, a Hansatech FMS2 pulse modulated chlorophyll flourometer was obtained.

Figure 45: A tailor-made PLC3 conifer cuvette allows the integration of the CIRAS-3 open gas exchange system with the FMS2 pulse modulated chlorophyll flourometer

The recently acquired equipment allows a simultaneous measurement of gas exchange and chlorophyll fluorescence in conifer seedlings thanks to the unique PLC3 cuvette.This combination makes it possible to understand in a more powerful and elegant manner how the plant responds to its environment and gives thus a broader picture of the reactions involved. A detailed methodology for these parallel gas exchange and chlorophyll fluorescence measurements is explained by Maxwell & Johnson (2000) and will be implemented for the coming trials.

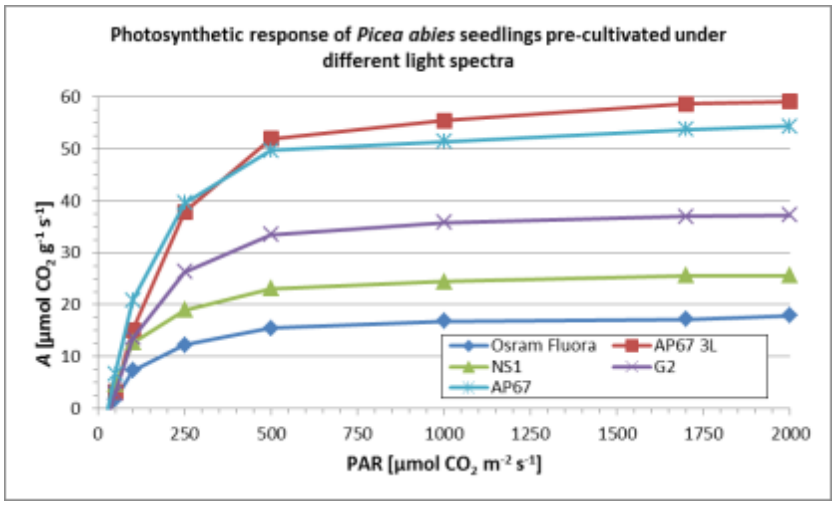


Figure 46: Example of gas exchange measurements: photosynthetic response of Picea abies seedlings pre-cultivated under different light spectra

Figure 47: Use of CIRAS-3 open gas exchange system with CPY-4 canopy assimilation chamber during forest field trial

3.8. Conclusions

- LN-treatment and cold storage have to be included in the growth protocols to adapt the Zephyr cultivation technology to the Scandinavian climate.

- During pre-cultivation the LED spectra G2 and AP67 (especially the tube version) showed promising results regarding seedling development for Scots pine and Norway spruce seedlings.

- The results after pre-cultivating regarding light spectra were also confirmed when analysing seedling development after one vegetation period on open land following transplanting. That is, both Scots pine and Norway spruce seedlings had a better development when pre-cultivated under the LED spectra G2 and AP67 (especially the tube version) compared to the other light sources.

- It is important to consider the light shock when moving Scots pine and Norway spruce seedlings from artificial growth light during pre-cultivation to full sun light exposure on open land after transplanting. In the future growth protocols cultivation regimes must be identified that prevent or reduce the risk of needle damages due to intense sun light.

- It is important to develop solid protocols for LN-treatment prior to cold storage to prevent or reduce the risk for seedling damages during long-term cold storage. These protocols are especially important for Scots pine seedlings.

- During cold storage it is important to measure seedling vitality in order to detect vitality reductions in an early stage.

- For a final validation of suitable cultivation regimes in regard to the new Zephyr technology it is important to establish forest field trials.

- For the new cultivation technology that is being developed in the Zephyr project this intermediate report on growth tests provides an important foundation to the final growth protocols for Scots pine and Norway spruce the will be presented in deliverable D3.3.

4. Input from UNISUBRIA

4.1. Data analysis regarding newly selected growth protocols and their relative efficiency

The species analysed in the first part of project are *Pinus sylvestris* (Scots pine) and *Picea abies* (Norway spruce). For both above mentioned species new growth protocols under LEDs light were used. Growth protocols used in the present work relied on the Deliverable 3.1 – New growth protocols slightly modified according to seeds providers indications and ongoing experiments.

4.1.1. Removal of dormancy

Seeds of Scots pine and Norway spruce are recalcitrant and does not benefit from specific pretreatment to remove the dormancy. Thus, seed were only soaked in water per 24-hours to enhance hydration.

4.1.2. Germination

Germination conditions and rate for Scots pine and Norway spruce seed are schematically reported in the following sections

- Germination conditions:

- **Pretreatments**: soaking per 24 hours
- **Substrate**: stabilized peat (Preforma VECO3, Jiffy® Products, Netherlands)
- **Light**: 4 different LED lights (spectra) (Valoya) and Fluorescent light (or tube spectrum) (Osram) as a control

  LED: - AP67 (bar, 2 lamps)

  - AP67-3L (bar 1 lamp)

  - G2 (bar, 2 lamps)

  - NS1 bar (bar 1 lamp)

  Control: OSRAM L36W/77 FLUORA (Fluorescent)

  PAR: 120 µmol m^{-2} s^{-1} (All spectra were setted to give 120 µmol m^{-2} s^{-1} over the substrate surface)
- **Photoperiod**: 16/8 hours (light/darkness)
- **Temperature**: 21-26°C
- **Relative humidity**: For both species: 80% during germination - 55-70% during growth
- **Watering**: For both species the flood method was used with tap water until substrate saturation (from half an hour, to 1 hour) per two times a week.

- Germination rate:

During the growth chamber experiments for both species the number of germinated seed after 21 days was compared to the total number of seeds (Figure 1)

- In the case of Scots pine seeds the highest percentage of germination was observed under G2 LED type. Similar germination rate value was measured for AP67-3L. Both LED light types were comparable to germination measured under control light.

- In the case of Norway spruce seeds the highest percentage of germination was observed under AP67-3L LED type. Similar germination values were measured for both AP67 and G2 LED light types. Moreover, germination rates for these LED light types were similar to germination rate measure under control light.

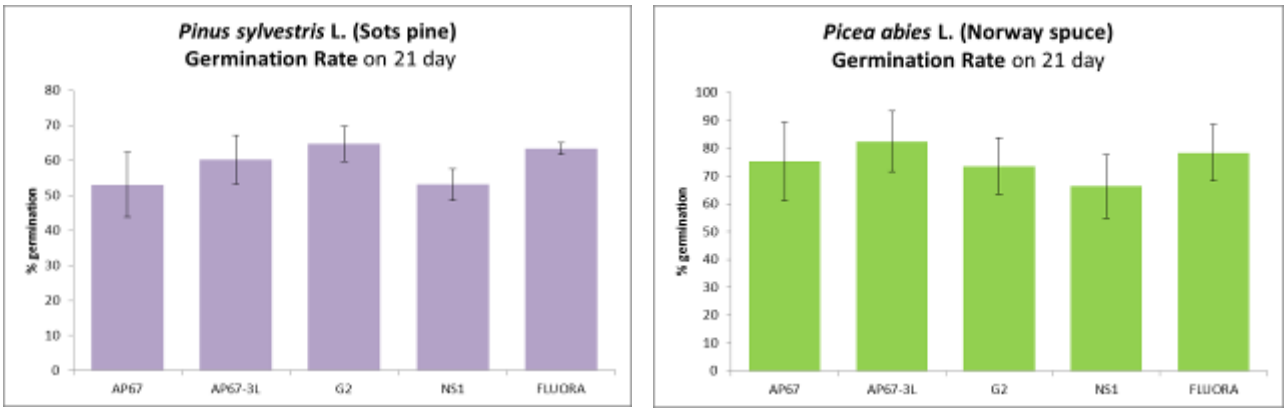


Figure 1. Seeds germination rate for *Pinus sylvestris* L. and *Picea abies* L. on the 21st day from sowing.

4.1.3. Growth kinetics

Root length, shoot and roots biomass of Scots pine and Norway spruce seedlings were measured every 7 days for a total of 4 weeks growing period. First sampling date was at the second week after seeds germination.

- Root length (Figure 2):

  For both species seedlings growth under control light (red line) showed the highest value from the beginning until the end of the growth period. Concerning LED lights, plant growth under G2 light type (orange line) showed the highest values of root length followed by both AP67 (blue line) and AP67-3L (green line). The lowest values was measured for plant growth under NS1 LED light (purple line).

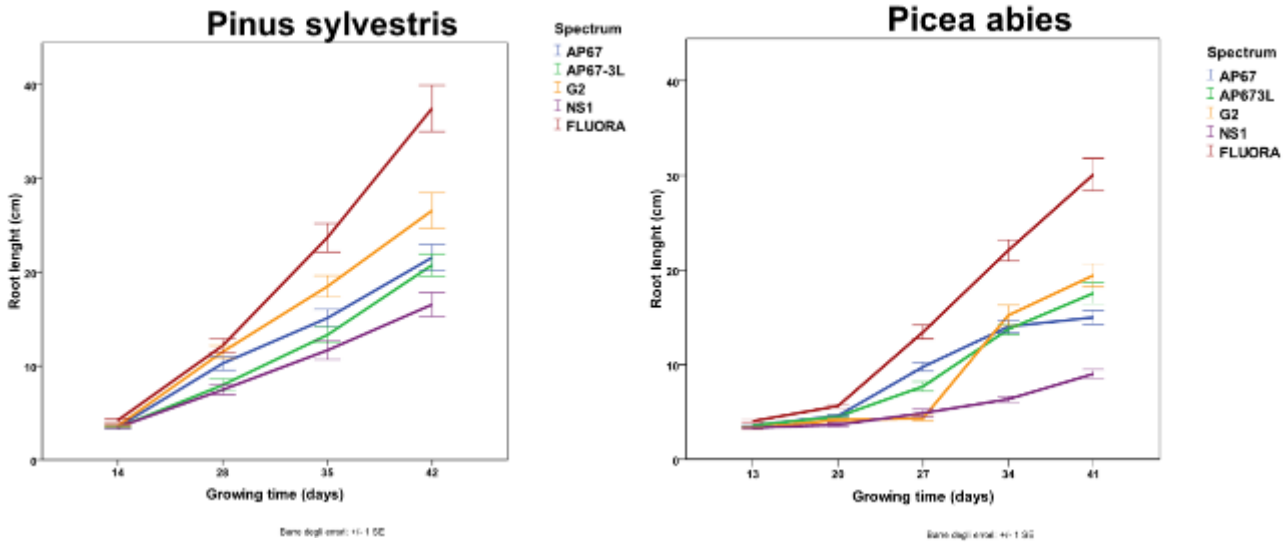


Figure 2. Root length measured every 7 days during the 4 weeks of growth for *Pinus sylvestris* L. and *Picea abies* L. seedlings.

- Shoot and Root biomass (Figure 3):

  Shoot and Root biomass for both considered species showed the same pattern of root length. Also in this case plant growth under control light (red line) showed the highest values. For seedlings growth under LED lights, G2 type (orange line) showed the highest values followed by AP67-3L (green line). Seedlings growth under NS1 LED light type (purple line) showed the lowest biomass values.

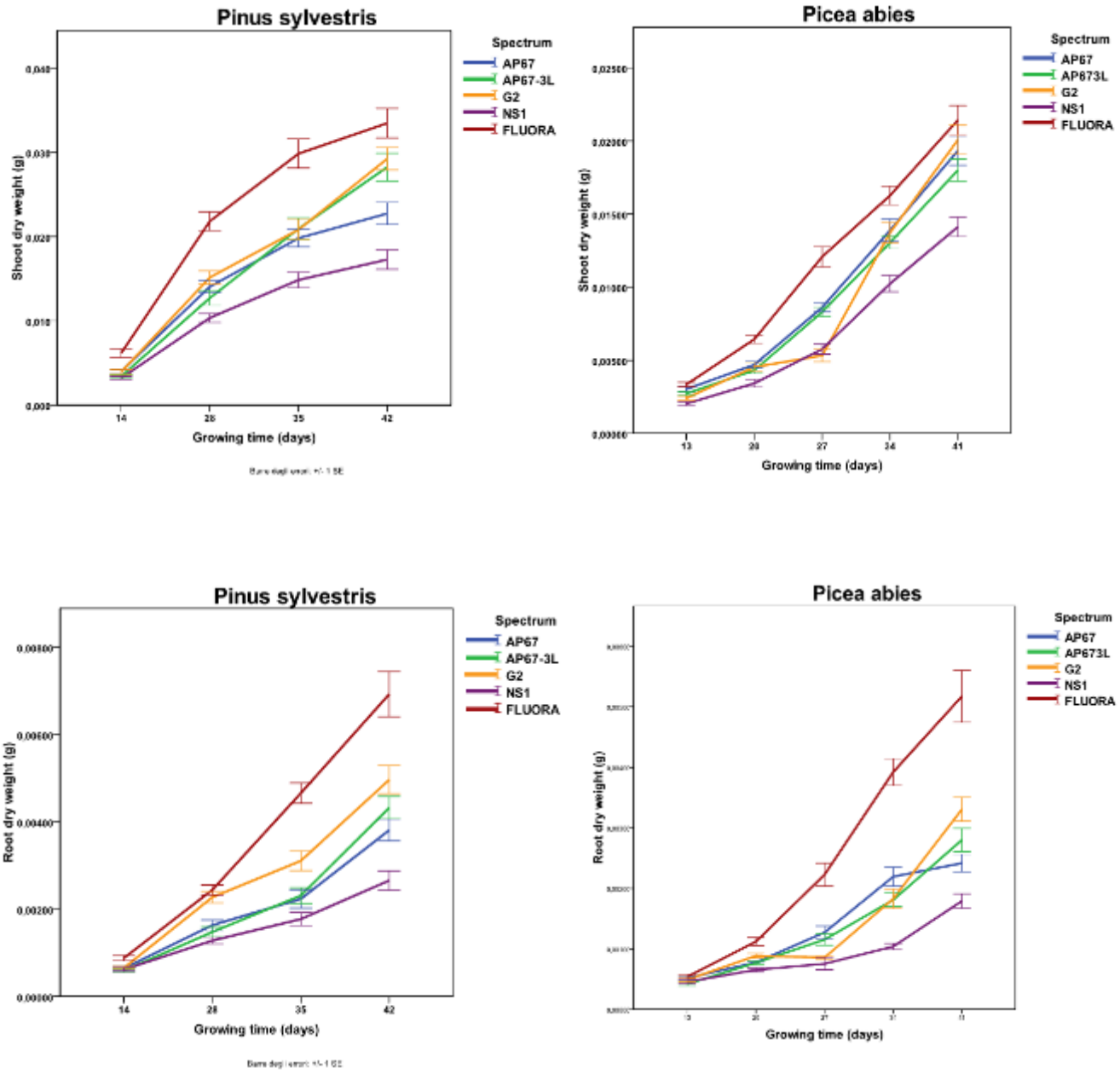


Figure 3. Shoot and Root biomass measured every 7 days during the 4 weeks of growth for *Pinus sylvestris* L. and *Picea abies* L. seedlings.

4.1.4. Selection of plant species to be further investigated

Plant species that will be further investigated are:

Prunus avium (Wild Cherry), *Taxus baccata* (European Yew), *Abies alba* (Silver FIr), *Fagus sylvatica* (Beech), *Platanus orientalis* (Oriental Plane), *Corylus avellana* (Hazel), *Punica granatum* (Pomegranate), *Arbutus unedo* (Strawberry tree), *Quercus ilex* (Holm oak)

Seeds pretreatment protocols only for the tested species are reported below. Protocols resulted from integration of 'Zephyr Deliverable 3.1 – New growth protocols' and seed provider indications. For some species two alternative protocols are given.

Quercus ilex

- Hydration (soaking per 24-hours)
- Sowing after Hydration period (without further pretreatment)

Abies alba

- Hydration (soaking per 24-hours)
- Sowing after hydration (without further pretreatment)

Punica granatum

- Hydration (soaking per 24-hours)
- Place seeds in Petri dishes on bibulous paper at environment temperature and light for 10 days
- Sowing

Acer pseudoplatanus

- Hydration (soaking per 24-hours)
- Treatment with "Teldor" fungicide: (3 ml in 1 l of water per 10 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat.
- Moist Stratification without medium (on bibulous paper) for 7 months at 4°C (Zephyr Protocol D 3.1)

Taxus baccata

- Hydration (soaking per 24-hours)
- Treatment with "Teldor" fungicide: (3 ml in 1 l of water per 5 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat.
- Moist stratification in perlite at 22°C for 3 months, then at 4°C for 4 months

Alternatively

- Hydration (soaking per 24-hours)
- Seeds surface sterilized for 2 min in 3.5% household bleach, and rinsed once with sterile water

- Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 5 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat
- Store at cold temperature (3°- 4°C) for 3 months

Fagus sylvatica

- Hydration (soaking per 24-hours)
- Seeds surface sterilized for 2 min in 3,5% household bleach, and rinsed once with sterile water
- Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 10 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat
- Cold stratification in perlite at 4°C for 2 months (Zephyr)

Corylus avellana

- Hydration (soaking per 24-hours)
- Seeds surface sterilized for 2 min in 3,5% household bleach, and rinsed once with sterile water
- Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 10 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat
- Store at cold temperature (3°- 4°C) for 2 months

Platanus orientalis

- Hydration (soaking per 24-hours)
- Seeds surface sterilized for 2 min in 3,5% household bleach, and rinsed once with sterile water
- Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 5 minutes). Afterwards dry seeds under hood for 4 hours in order to improve fungicide adhering to the seed coat
- Cold stratification in perlite at 4°C for 2 months (Zephyr)

Arbutus unedo

- Hydration (soaking per 24-hours)
- Seeds surface sterilized for 2 min in 3,5% household bleach, and rinsed once with sterile water
- Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 5 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat
- Sore at cold temperature (3°- 4°C) for 2 months

4.2. **Initial evaluation of growth performance of LED illumination on plants growth**

Seedlings growth for both Scots pine and Norway spruce was tested under different LED illumination. Performance of shoot and root development was analysed through shoot height, root length, shoot biomass and root biomass measurement. Data refer to values measured at the end of the growth period (4 weeks).

Shoot height (green bar) and root length (violet bar) are reported in the above panels; shoot (blue bar) and root (fuchsia bar) dry weight are reported in the panels below. The two species Scots pine and Norway spruce on the right and left columns respectively. (Figure 4)

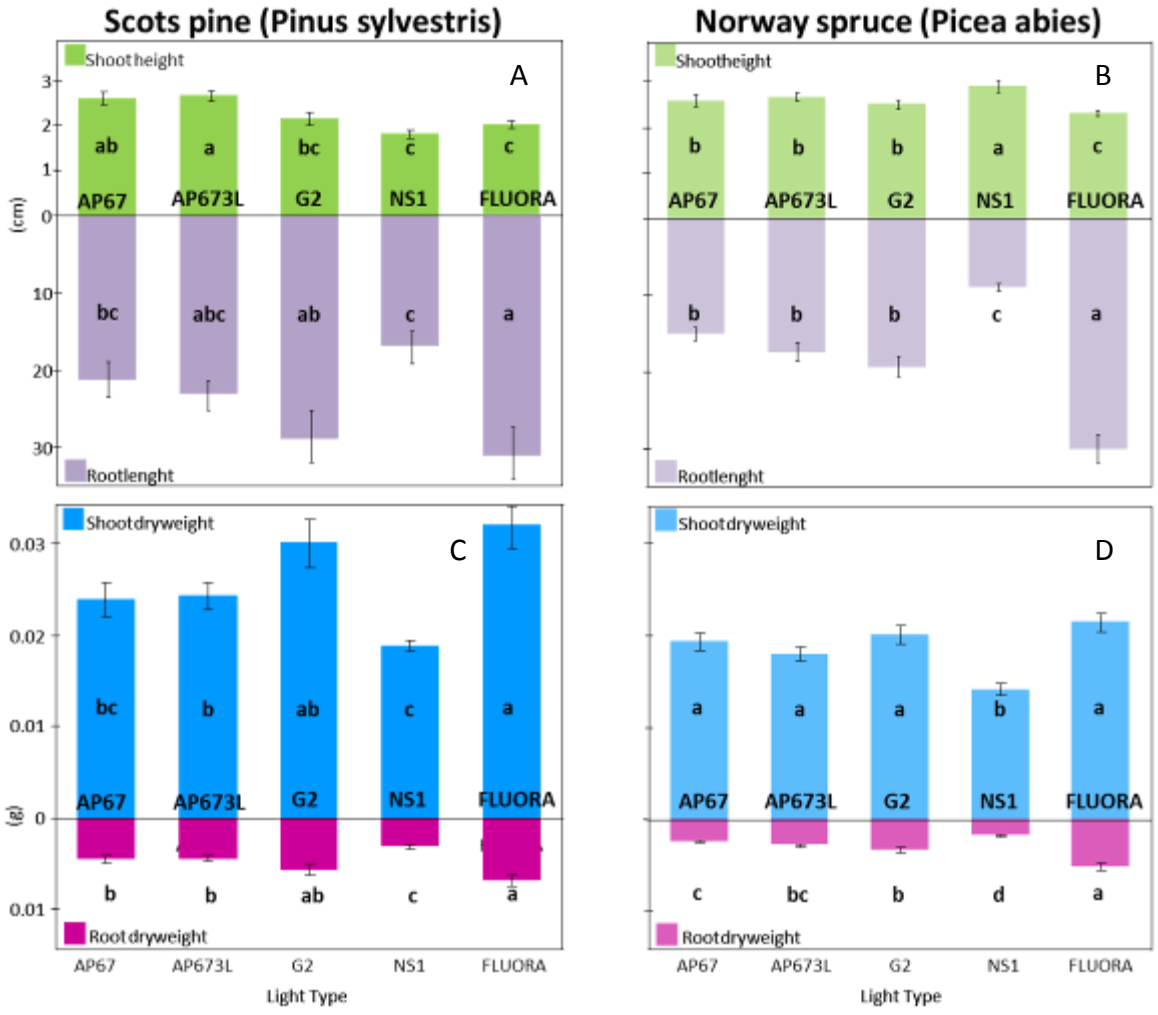


Figure 4. Shoot height and root length (A, B), shoot and root dry weight (C,D), under different light types for the seedlings of *Pinus sylvestris* L. and *Picea abies* L. at the end of the 4 [th] week of growth.

4.2.1. Performance of LED spectra compared to control light

Schematic report of parameters for Scots pine and Norway spruce seedling growth performance under different LED light compared to control light

Scots pine:

AP67: seedlings height values measured under AP67 lights were higher than values measured under control light. Instead root length, shoot and root dry weight values were lower than values measured under control light

AP673-L: seedlings height values measured under AP67-3L lights were higher than values measured under control light. Root length values were similar to control light. Shoot and root dry weight values were lower than values measured under control light.

G2: seedlings height values measured under G2 lights were higher than values measured under control light. Root length, shoot and root dry weight values were similar to values measured under control light.

NS1: seedlings height values measured under NS1 light were similar to values measured under control light. Root length, shoot and root dry weight values were lower than values measured under control light.

Norway spruce:

AP67, AP673-L, G2: seedlings height values measured under AP67, AP67-3L and G2 lights were higher than values measured under control light. Root length and root dry weight values were lower than values measured under control light. Shoot dry weight values were similar to values measured under control light.

NS1: seedlings height values measured under NS1 light was significantly higher than values measured under control light. Root length, shoot and root dry weight values were significantly lower than values measured under control light.

In conclusion we can say that seedlings growth under AP67, AP673-L, G2 light, for both species, showed seedlings height values higher than values measured under control light. Moreover, seedlings growth under AP673-L and G2 light showed root length and shoot and root dry weight values similar to the values measured under control light. Seedlings growth under NS1 light, for both species, showed significantly lower biomass and root length values than values measured under control light with the only exception of seedlings height values that were higher than values under control light.

4.2.2. Performance of LED spectra on shoot and root development

- Morphological parameters (plant height and root length):

- Scots pine seedlings growth under AP67-3L and AP67 lights showed a plant height significantly higher than seedlings growth under NS1 light and control light.

  Scots pine seedlings growth under both G2 and AP67-3L lights showed root length values similar to the seedlings growth under control light.

- Norway spruce seedlings growth under all LED light types showed higher values of shoot height than seedlings growth under control light. In particular seedlings growth under NS1 light showed the highest values among all.

Norway spruce seedlings growth under all light type showed root length values significantly lower than seedlings growth under control light. In particular, seedlings growth under NS1 light type showed the lowest values among all.

- Biomass (shoot and root dry weight):

  - Scots pine seedlings growth under G2 light type showed both shoot and root dry weight similar to the values measured for seedlings growth under control light. Both G2 and control light had the highest biomass values, while seedlings growth under NS1 had the lowest. Seedling growth under AP67 and AP67-3L showed intermediate values.
  - Norway spruce seedlings growth under all light type showed the same shoot dry mass except seedlings growth under NS1 light that showed lowest values. Seedlings growth under all light type showed lower values of root dry mass than control light. Seedlings growth under NS1 light had the lowest root mass.

In conclusion we can say that, for a standard cultivation protocol, G2 light type could represent the optimal spectrum for seedlings growth for both considered species. G2 light has the higher percentage of far-red/red (600-800 nm) wavelength (λ). Hence, it could interfere with chlorophyll fluorescence measurements. Therefore, in alternative to G2 light type AP67-3L LED type could represent the best option.

4.3. Trial tests with new growth sensors

Indirect and non-destructive analysis of *Pinus sylvestris* and *Picea abies* seedlings growth were carried by measuring *Greenness* and *Plant height*. Greenness was estimated as percentage of shoot cover projected on tray ground.
In the first part of the work (Section 3.1), greenness data were obtained by a series of manually taken images (Nikon D70s digital camera) analysed with an open source software (Image*J*). Moreover, plant height was manually taken during the growth period to find a relationship with plant biomass. During the second part of the work (Section 3.2) plant height data were manually taken and compared with data obtained from images acquired by Optical sensors and analysed by *uEyeDualcam HeightMap* software (by ACREO).

4.3.1. Optical sensor

Photocamera - *IMAGEJ* Software

- **Greenness analysis**

Bar graphs show the greenness value for both species in relation to different light type.
In the case of Scots pine (Figure 5; left panel, violet bars) the highest greenness value was found for seedlings growth under G2 light type. Seedling growth under AP67-3L light showed the second highest value. Seedling growth under NS1 light type showed the lowest greenness value.
Norway spruce (Figure 5; right panel, green bars) showed the highest greenness value for seedling growth under AP67 light type. Also in this case seedlings growth under AP67-3L light showed the second highest value. For both species seedlings growth under control lights showed the lowest value.

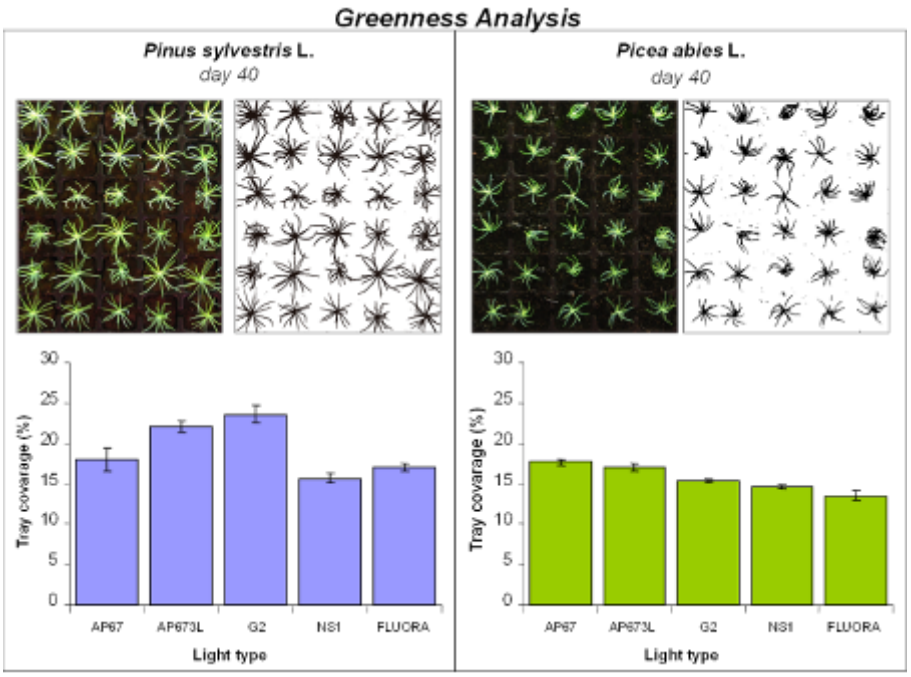


Figure 5. Greenness analysis for *Pinus sylvestris* L. and *Picea abies* L. in relation to different light type.

- **Time course Greenness and Height analysis**

Figure 6 show the pattern of Scots pine and Norway spruce greenness and height during the growth period. Measurements were taken every 7 days during 4 weeks. For both species greenness increased from the beginning until the end of the growth period. On the contrary for both species plant height increased almost three times during the first week remaining almost stable for the rest of the growing period. Therefore, increase of greenness is mainly due to continuous leaves development. Finally Scots pine seedlings showed values of Greenness and higher than Norway spruce

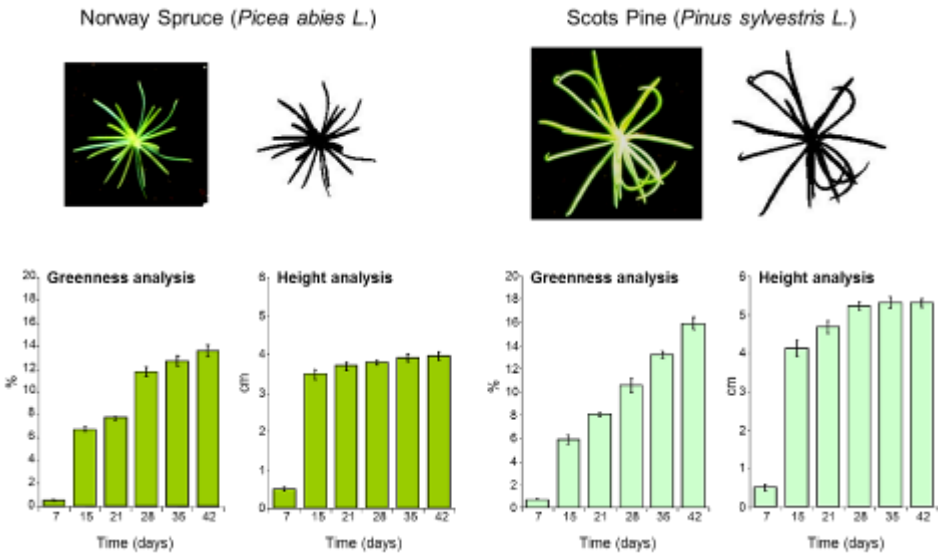


Figure 6. Time course Greenness and Height analysis for *Pinus sylvestris* L. and *Picea abies* L.. Measurements were taken every 7 days during 4 weeks

- Relationship between greenness, height and total biomass

Highly significant positive relationships between greenness and seedlings total biomass were found for both species and all LED types (Figure 7). These relationships highlighted that indirect measurements of seedling height and greenness are both good parameters for non-destructive quantification of plant biomass.

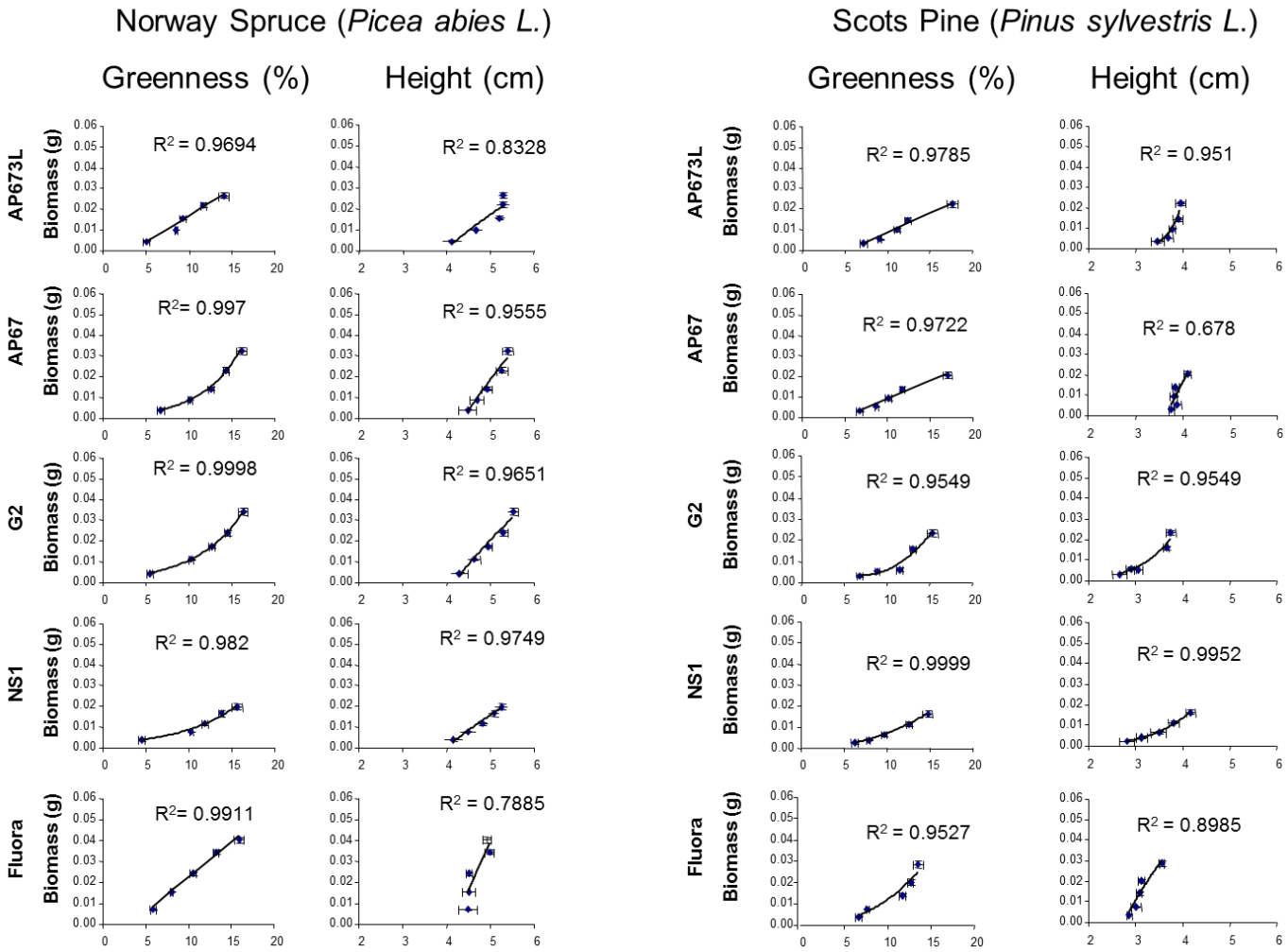
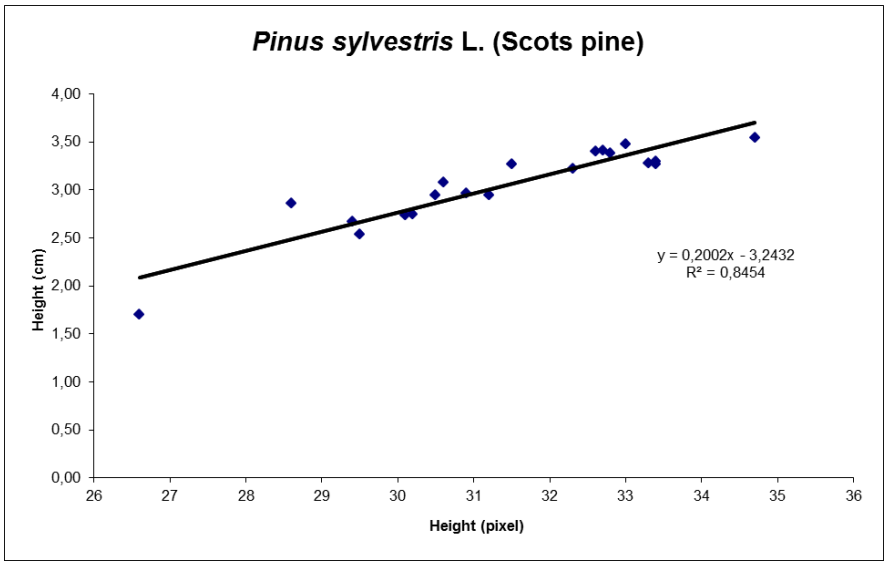


Figure 7. Greenness and plant height, relationship with total biomass of *Pinus sylvestris* L. and *Picea abies* L., for each light type.

4.3.2. uEyeDualcam Height Map Software (by Acreo)

For both Scots pine and Norway spruce preliminary tests were carried to relate plant height data obtained by HeightMap Software with plant height manually measured. In the case of

Scots pine a high relationships was found (R^2 = 0.85). Up to now for Norway spruce seedlings a lower relationship (R^2 = 0.59) was found (Figure 8)



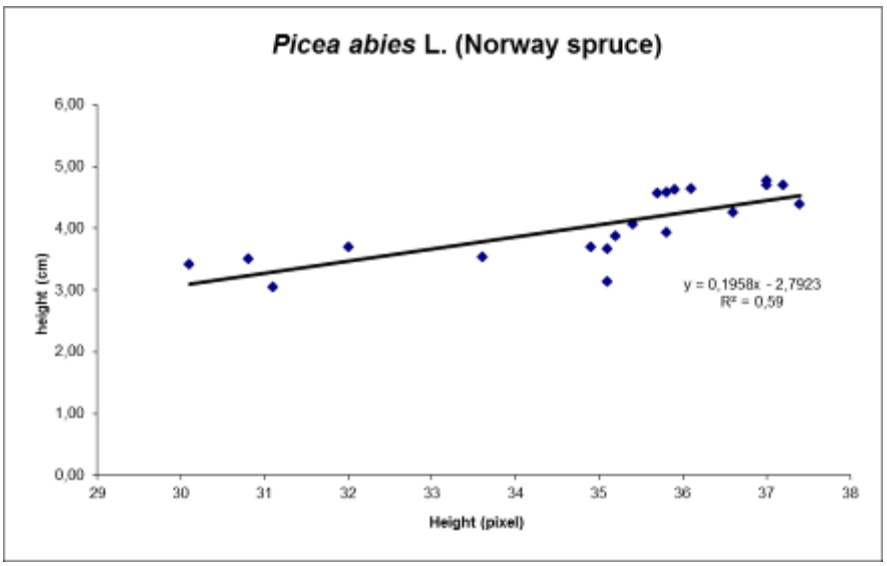


Figure 8. Relationship between HeightMap software plant height and manual measured plant height for *Pinus sylvestris* L. and *Picea abies* L..

4.4. Conclusions

- In general, seedling growth under G2 LED type show the highest performance, representing the optimal spectrum. Similar values were found for seedling growth under AP67-3L.
- G2 light has the higher percentage of far-red/red (600-800 nm) wavelength (λ). Hence, it could interfere with optical measurements such as greenness and fluorescence. Therefore, in alternative to G2 type, AP67-3L LED type could represent the best option.
- Relationship between height and greenness, obtained by manual measurements and by Nikon digital camera and Image J software respectively, highlighted that indirect analysis are good parameters for non-destructive quantification of plant biomass.
- Preliminary tests resulted in a good relationship between plant height data obtained by HeightMap Software (Acreo) with plant height manually measured. Improvements of softwares are probably still needed. Further analysis will be carried with last version of both uDualCam and Height Map softwares.

References

Anderson, J.M. et al. 1995. The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis Research*. **46**(1-2),pp.129–139.

Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Burdett, A.N. 1987. Understanding root growth capacity: theoretical considerations in assessing planting stock quality by means of root growth tests. *Canadian Journal of Forest Research*. **17**(8),pp.768–775.

Van den Driessche, R. 1976. Prediction of cold hardiness in Douglas fir seedlings by index of injury and conductivity methods. *Canadian Journal of Forest Research*. **6**(4),pp.511–515.

Feret, P.P. and Kreh, R.E. 1985. Seedling Root Growth Potential as an Indicator of Loblolly Pine Field Performance. *Forest Science*. **31**(4),pp.1005–1011.

Hermann, R.K. et al. 1979. *Testing the vigor of coniferous planting stock* [online]. Corvallis, Or. : Oregon State University, School of Forestry. Available from: http://ir.library.oregonstate.edu/xmlui/handle/1957/8123 [Accessed March 22, 2014].

Horton, P. and Ruban, A. 2005. Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. *Journal of Experimental Botany*. 56(411),pp.365–373.

Huner, N.P.A. et al. 2006. Photoprotection of Photosystem II: Reaction Center Quenching Versus Antenna Quenching *In*: B. DEMMIG-ADAMS et al., eds. *Photoprotection, Photoinhibition, Gene Regulation, and Environment* [online]. Advances in Photosynthesis and Respiration. Springer Netherlands, pp. 155–173. Available from: http://link.springer.com/chapter/10.1007/1-4020-3579-9_11 [Accessed March 21, 2014].

Huner, N.P.A. et al. 2013. Photostasis in Plants, Green Algae and Cyanobacteria: The Role of Light Harvesting Antenna Complexes *In*: B. R. GREEN and W. W. PARSON, eds. *Light-Harvesting Antennas in Photosynthesis* [online]. Advances in Photosynthesis and Respiration. Springer Netherlands, pp. 401–421. Available from: http://link.springer.com/chapter/10.1007/978-94-017-2087-8_14 [Accessed March 22, 2014].

Mattsson, A., 1986. Predicting field performance using seedling quality assessment. New Forests 13: 223-248.

Mattsson, A. 1991. Root growth capacity and field performance of Pinus sylvestris and Picea abies seedlings. *Scandinavian Journal of Forest Research*. **6**(1-4),pp.105–112.

Mattsson, A. 1986. Seasonal variation in root growth capacity during cultivation of container grown Pinus sylvestris seedlings. *Scandinavian Journal of Forest Research*. **1**(1-4),pp.473–482.

Maxwell, K. and Johnson, G.N. 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*. **51**(345),pp.659–668.

McCreary, D.D. and Duryea, M.L. 1985. OSU vigor test: principles, procedures, and predictive ability. Available from: http://agris.fao.org/agris-search/search.do?f=1989%2FUS%2FUS89493.xml%3BUS8851006 [Accessed March 22, 2014].

McKay, H.M. 1992. Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant vitality following cold storage. *Canadian Journal of Forest Research*. **22**(9),pp.1371–1377.

Pearcy, R.W. 2000. Acclimation to sun and shade *In*: A. S. RAGHAVENDRA, ed. *Photosynthesis: A Comprehensive Treatise*. Cambridge University Press.

Reece, J.B. and Campbell, N.A. 2011. *Campbell Biology*. Boston: Benjamin Cummings : imprint of Pearson.

Stattin, E. et al. 2012. Development of a molecular test to determine the vitality status of Norway spruce (Picea abies) seedlings during frozen storage. *New Forests*. **43**(5-6),pp.665–678.

Sutton, R.F. 1987. Root growth capacity and field performance of jack pine and black spruce in boreal stand establishment in Ontario. *Canadian Journal of Forest Research*. **17**(8),pp.794–804.

W. Adams III, W. et al. 2006. Energy Dissipation and Photoinhibition: A Continuum of Photoprotection *In*: B. DEMMIG-ADAMS et al., eds. *Photoprotection, Photoinhibition, Gene Regulation, and Environment* [online]. Advances in Photosynthesis and Respiration. Springer Netherlands, pp. 49–64. Available from: http://link.springer.com/chapter/10.1007/1-4020-3579-9_5 [Accessed March 22, 2014].