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ZEPHYR project – Deliverable D7.6

Articles and peer-reviewed papers

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Executive summary

Zephyr project aims to introduce an innovative technology for the pre-cultivation of forest regeneration materials in a zero-impact and cost friendly production unit. This unit is not affected by outdoor climate and with LED lights providing an optimal spectrum for the photosynthesis. Light intensity, photo-period and further parameters can be automatically regulated by a control system that receives data from several sensors, while the energy is provided by solar panels.

The project's overall objective was the realization of a new technology that will produce high quality forest plants stocks ready to be transplanted in pots or in field at low costs.

In order to achieve the final goals of Zephyr, the study species were fully tested by means of morphological and biochemical analyses, as well as the physiological traits which have been considered for assessing new growth protocols to be used for indoor and outdoor cultivation. Conventional and LED lights have been compared to assess the plants response to different spectra and light intensity; and the selection of the best light spectrum was assessed by means of the abovementioned parameters. Likewise, the technological components that were developed have been fully tested and validated before being integrated in one functional unit.

As it is normal with an innovative concept and a project of this nature, a great deal of new knowledge has been generated. The results of the experiments have been presented to the international scientific community in order to share them but also to be validated and receive feedback. This exposure and dissemination activities have been done using the customary channels as written articles and thesis; oral presentations in conferences and visual posters and webpages, etc.

This report presents the scientific publications that have been done during the time of Zephyr project from all partners. They are organized by type of publication starting with an abstracts section and then including the full text versions in the final chapter. It is important to mention that, although the Zephyr project is coming to an end by the time of this report, much scientific material has been generated and more publications are to be expected even after the end of the project.

Table of Contents

1. Articles and peer-reviewed papers

1.1. Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success

Authors:

- Filippos Bantis
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  Horticulture and Product Physiology Group, Department of Plant Sciences, Wageningen University

- Kalliopi M Radoglou
  Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace

Type of publication: Peer-reviewed journal article

Published in: *Scientia Horticulturae* (in press)

Abstract:

The morphological and phytochemical characteristics of two Ocimum basilicum cultivars (Lettuce Leaf, and Red Rubin-mountain Athos hybrid) under artificial lighting were investigated. Four LED light treatments [AP673L (high red and high red:far-red), G2 (high red and low red:far-red), AP67 (moderate blue and red and low red:far-red), and NS1 (high blue and green, high red:far-red and 1% ultraviolet)] with different colours mixing ultraviolet, blue, green, red and far-red, and fluorescent tubes (FL, high blue, green and red:far-red) as Control were used in the growth chambers for 28 days under PPFD of 200 ± 20 μmol m-2 s-1 for all treatments at plant height. G2, Control and AP67 treatments for Lettuce Leaf, and G2 for Red Rubin hybrid had higher growth rate. Roots of Lettuce Leaf were significantly longer under AP673L compared to NS1, while Red Rubin hybrid showed no significant differences. Total biomass was significantly greater under NS1, AP67 and G2 compared to the Control, for both cultivars. For both Lettuce Leaf and Red Rubin hybrid, root:shoot ratio (R/S) was favored under NS1, whereas the Control had the lowest impact. Leaf area of both cultivars was greater under the Control. Root growth capacity evaluation was also assessed. Seedlings of Lettuce Leaf cultivated under the effect of the Control and AP673L, and seedlings of Red Rubin hybrid grown under AP673L (mainly) quickly developed new root system. This could offer them the advantage of fast exploitation of larger soil volume after transplanting. Total phenolic content of Lettuce Leaf was significantly higher under NS1 compared to the rest of the treatments, while in Red Rubin hybrid, NS1 had significantly higher total phenolic content compared to the Control and G2. Our study demonstrates that LEDs variably affected growth characteristics and increased total phenolic content compared to conventional fluorescent light for these two Ocimum basilicum cultivars.

Keywords: nursery production, LEDs, basil, seedling growth, phenolic compounds

1.2. Effects of cold stratification and GA3 on germination of *Arbutus unedo* seeds of three provenances

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  Elias Milios
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Type of publication: Peer-reviewed journal article

Published in: *African Journal of Traditional, Complementary and Alternative medicines (submitted)*

Abstract:

Background: *Arbutus unedo* is a valuable Mediterranean shrub as an ornamental plant as well as fruit tree. Fresh fruits of *A. unedo* are a good source of antioxidants, of vitamins C, E and carotenoids and also are characterized by the high content of mineral elements.

Materials and Methods: The effects of gibberellic acid (GA3) and cold stratification (CS) on seed germination performance were investigated in *A. unedo* seeds collected from three provenances in the Northern part of Greece. Seeds of each provenance were soaked in solutions of GA3 (500, 1000 or 2000 ppm) for 24 h and subsequently were subjected to CS at 3 – 5ºC for 0, 1, 2, and 3 months.

Results: Non-stratified seeds of the three *A. unedo* provenances which were not treated with GA3 solutions exhibited very low germination. However, seed germination was significantly improved after a one-month period of CS. Similarly, the non-stratified seeds of all three provenances became non-dormant after the treatment with 2000 ppm GA3 and they germinated at high percentages. However, in untreated seeds with GA3, after a one-month CS period the seeds of the Pieria provenance exhibited higher germination percentage than that of Rodopi provenance seeds. Furthermore, in non-stratified seeds, the Pieria provenance seeds treated with GA3 germinated at higher percentages and more rapidly than those of the other two provenances.

Conclusion: The results indicated that untreated seeds exhibited very low germination at 20/25ºC. However, in all three provenances seed germinability was significantly improved by a one-month period of CS or treatment of seeds with 2000 ppm GA3. Furthermore, there was a considerable variability among seed provenances in response to the treatments which were applied.

1.3. Stereo-vision image-based phenotyping for non-destructive analysis of tree seedlings growth

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Department of Biosciences and Territory, University of Molise, Italy

Type of publication: Peer-reviewed journal article

Published in: (in preparation)

Abstract:

Plant phenotyping is the identification of effects on the phenotype as a result of genotype differences and the environment. Plant biomass variability is considered as environmentally induced phenotypic variation in plants. Destructive direct method to measure plant biomass has become the major bottleneck for quantitative analysis of a large number of plants. In the present study a simple and economically affordable automated system was developed to monitor containerized tree seedlings biomass from seed germination to five weeks growth. Stereoscopic RGB images were taken and a new software for image analysis was developed. Results showed that the developed system is accurate enough to automatically monitoring seedlings growth. The best regression model to explain the relationship between direct biomass data and indirect measurements was based on parameters such as plant height for needle-leaved species and plant greenness for broad-leaved species. Finally, image analysis revealed information on the early seedlings developmental stage.

1.4. The effect of light-emitting diodes on the development of pomegranate seedlings

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Type of publication: Peer-reviewed journal article

Published in: (in preparation)

Abstract:

The objective of the present study was to investigate the impact of LED versus fluorescent (FL) lighting on morphological, physiological and phytochemical characteristics of pomegranate (*Punica granatum* L.) after six weeks pre-cultivation in growth chambers. Five light-emitting diodes (LED) treatments i.e. L20AP67 (moderate B, G and R and low R:FR), AP673L (high R and high R:FR), G2 (high R and low R:FR), AP67 (moderate B and R and low R:FR), and NS1 (high B and G, and high R:FR, and 1% UV) with different radiation spectra were used. Fluorescent (FL) tubes served as the control light treatment. Seedlings grown under L20AP67 exhibited the best morphological-agronomical characteristics showing rapid height increase, longer roots, greater fresh and dry weight, as well as greater leaf area compared to the rest of the treatments. Seedlings grown under the FL and L20AP67 showed higher photosynthetic efficiency, while greater root activity was revealed under NS1 and AP67. Higher chlorophyll and carotenoid content was found under the influence of the FL lighting. NS1 and AP67 seedlings produced a greater total and simple phenolic content respectively. Flavonoid formation was favored under G2 and AP67, while anthocyanins content was found greater under G2, AP67 and NS1. Root growth potential (RGP) estimation was also performed in order to evaluate the transplant response. After 31 days in the chamber where RGP was assessed, transplanted seedlings of AP67 treatment had the lengthiest and L20AP67 seedlings had the heaviest newly formed roots. Our study demonstrates that LEDs were more efficient in promoting a number of morphological and phytochemical characteristics than conventional fluorescent light in *Punica granatum*. In particular, L20AP67 LED is recommended for production of pomegranate seedlings under artificial lighting.

Keywords: nurseries, LED, photomorphogenesis, Punica granatum, secondary metabolism, phenolic compounds, pigments, transplanting

1.5. Cultivation of forest regeneration materials under artificial radiant sources - effects of light intensity on energy consumption and seedlings' development

Authors:

- Marco Hernandez Velasco
- Anders Mattsson
  Department of energy, forests and built environments, Dalarna University, Sweden

Type of publication: Peer-reviewed journal article from conference presentation

Published in: (in preparation

Abstract:

In times of major environmental challenges and increasing demand for forest products, planted forests have acknowledged advantages compared to other land uses. Despite not being able to substitute natural forests, planted ones have, if properly managed, a great potential to contribute in addressing this problems. Besides the ecological benefits such as carbon sequestration, planted forest can help coping with the wood products demand without further reduction of natural forest. Forest restoration, rehabilitation and reforestation are limited to the capacity of producing forest regeneration materials. Often, as the production is intensified at the forest nurseries, the practices start having an adverse impact on the environment and stop being truly sustainable. One of the main issues in nurseries is the energy consumption for grow lights in periods with short daylight times. By using high efficiency LED grow lamps and adjusting the light intensity, this study aimed to reduce the energy consumption from lighting per seedling without compromising their development. The pre-cultivation of *Picea abies* and *Pinus sylvestris* seedlings was done during 5 weeks under controlled conditions at 20°C and a relative humidity of 60%. The photoperiod was of 16 hours at an intensity ranging from 50 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Keywords: Containerized planting stock production, LED grow lights, *Picea abies, Pinus sylvestris*, energy consumption

1.6. Long-Night treatment for induction of cold hardiness in Scots pine and Norway spruce seedlings - effects of duration and photoperiod on energy consumption and storability

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Type of publication: Peer-reviewed journal article from conference presentation

Published in: (in preparation

Abstract:

Human-assisted forest regeneration in Nordic climates is considerably limited by the harsh outdoor conditions. There the local weather opens only a small time window during the summer for transplantation and establishment of the pre-cultivated seedlings in open land. Greenhouses and modern growth chambers aid to cope with this limitation by allowing year-round seedlings cultivation. Nonetheless, production levels are constrained to the cold storage capacity during the non-transplanting season. This storage is in turn dependent on the conifers' ability to adapt to freezing temperatures and withstand the overall stress associated with the cold hardening. Long night treatments can induce dormancy with growth cessation and terminal buds initiation, leading to a better cold resistance. When growing forest regeneration materials under artificial lights, the lengths of the long night treatment and the photoperiod will have a significant impact, not only on the biological response of the seedlings but also on the energy consumption and thus on the CO_2 emissions. The aim of this work was to explore different long night treatment regimes for induction of cold hardiness on *Picea abies* and *Pinus sylvestris* seedlings using artificial lights. This has been done with the purpose of studying the interplay between the energy consumption and the biological responses.

Keywords: Forest restoration, LED grow lights, forest nurseries, long-night treatment, cold hardiness

2. Oral presentations at conferences and workshops

2.1. Innovative approaches to increase biodiversity in forest plant production.

Authors:

- Kalliopi M Radoglou
  Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace

Type of publication: Oral presentation

Presented in: *International Final Conference of ZEPHYR. Zero –Impacts Technology to respond to Zero Hunger Challenge.* 21 October 2015. Milan, Italy, http://www.zephyr-project.eu/

Abstract:

A cost-effective regeneration effort in order to enhance forest biodiversity is priority for Greek Forestry. Nonetheless, overcoming transplanting stress in Mediterranean forest ecosystems is a great challenge, in adverse environments, which affects negatively the regeneration success. Nurseries target to produce the best seedlings that have the potential to overcome transplanting stress and successfully grow on a site. The introduction of new technology based on pre-cultivation protocols in mini-plugs and LED lamps in order to serve large-scale production of forest regeneration material for a wide range of species is an innovative approach for the nursery production. 18 species were studied and their germination and growth protocols were determined. All these species were studied under light-emitting diodes (LEDs) (L20AP67, AP673L,G2, AP67, NS1 -Valoya) or Fluorescent light (FL). Both morphological and physiological variables helped to determine the best growth conditions for each species in order to achieve the best seedling quality. Furthermore, these seedlings were transplanted to pots in the nursery and finally studied under field conditions in a selected site. The use of this new technology integrated to a build prototype in the frame of Zephyr project will allow a large scale production of seedlings that until now was unreachable through the use of the conventional techniques. The new technology, in conjunction with increased numbers of different forestry species will result to increased biodiversity levels that lead to more sustainable ecosystems.

Keywords: Mediterranean species, Nursery production, LEDs, seedlings

2.2. Innovation in seedling production of *Prunus avium* L. by using LED lights

Authors:

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- Kalliopi M Radoglou

Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace

Type of publication: Oral presentation

Presented in: *International Scientific Conference Reforestation Challenges. Belgrade 3-6 June 2015,* http://www.reforestationchallenges.org/Reforestation_Challenges.php

Abstract:

The objective of this research was the analysis of LED lighting effects in physiological and morphological characteristics of Wild cherry *Prunus avium* L. seedlings. Pre-germinated seeds were placed in mini-plug containers inside growth chambers under control conditions using five LEDs (G2, AP673L, L20AP67, AP67, NS1 -Valoya) with different irradiation spectra mixing ultraviolet, blue, green, red, far-red and infra-red, as well as fluorescent tubes (FL). In order to break the dormancy seeds were hydrated for 24-h and placed for 4 weeks in a phytotron chamber for warm stratification which was followed by 20 weeks cold stratification. Seedlings were cultivated in the growth chambers for six weeks. Seedlings grown under FL and L20AP67 LED formed significantly darker green colour compared to the rest of the treatments. No differences were observed regarding the leaf numbers. Shoot height was not affected by the different light treatments and significantly longer roots were formed under the illumination of NS1 LED. Dry biomass was improved when plants grew under NS1, AP67, G2 and AP673L LEDs. Furthermore, R/S ratio was found significantly higher under NS1 compared to L20AP67, while leaf area was not affected by the different light treatments. Root growth potential evaluation was additionally applied, as indicator of field performance after transplanting. The length and dry weight of new roots were considerably improved for seedlings cultivated under G2 compared to L20AP67 treatment. The study shows that the use of LEDs provide better growth and transplant success for the *Prunus avium* seedlings than using conventional fluorescent light.

Keywords: Mediterranean species, Nursery production, LEDs, seedlings

2.3. Light needs for seedling development in cork oak (*Quercus suber* L.)

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- Federico Canali
- Erica Chiummariello
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Type of publication: Oral presentation

Presented in: *International Scientific Conference Reforestation Challenges. Belgrade 3-6 June 2015, http://www.reforestationchallenges.org/Reforestation_Challenges.php*

Abstract:

Seed germination and seedling development of cork oak have specific light needs in terms of photoperiod, light quantity and quality. To reproduce optimal light conditions in a controlled environment, these parameters were analyzed in a mature cork forest in Central Italy (Viterbo) since November 2014 up to the spring. The distributional area of this species has a photoperiod ranging from 9h52'01'' (NW) to 15h15'17'' (SE); the total daytime is 4859 h in the northernmost point and 4762 h in the southernmost point. In Viterbo, during the period of analysis, photoperiod ranges from 11h 19'25''to 12h 9'26''.
Germination resulted to occur both in sunny and shaded areas, with light intensity ranging from 100 to 2000 PPFD and RED/FAR RED ratio ranging from 1(sun) to ~ 0.3 (shadow). Clouds effect was analysed in an open area showing a significant reduction of light intensity (up to 90 %) without great variations in light quality. In particular, RED/FAR RED ratio, very important for germination and first stages of seedling growth, remains invariable. Commercial plant lights provide spectra which are too different from that of the sun, especially for higher values of PAR and RED/FAR RED ratio. To evaluate the effect of different spectra on germination and seedling development, 7 light sources were tested for cork oak propagation with a photoperiod of 12L 12D. Data were compared to those collected into the forest. Germination and seedling development resulted to be speeded up under all artificial conditions. This may be caused by the lack of diurnal temperature variations so as to the high PAR and RED/FAR RED ratio values of the lamps.

Keywords: Light needs, germination, cork oak, photoperiod

2.4. Light quality in natural and artificial propagation of Oriental Plane (*Platanus orientalis* L.)

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- Kalliopi M Radoglou
Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace

Type of publication: Oral presentation

Presented in: *II Congresso Internazionale di Selvicoltura, Firenze, 25-29 November 2014*

Abstract:

A mature forest generally has several vertical layers: herb layer, shrub layer, undercanopy, canopy and emergents. Tree species occupy the last three, which are characterized by great differences in light availability, in terms of quality and quantity. Light intensity varies according to the seasons, the forest structure, clouds and wind. Light spectra depend on the photoperiod and on the tree cover, varying in relation to the height and density of the species. According to their light requirements, forest tree species can be divided in three main categories: heliophilous, sciaphilous and intermediate species. Moreover, forest structure and composition change constantly passing through different successional stages. Each stage creates the conditions for the next one. The first are dominated by pioneer heliophilous species, afterwards the shade-tolerant species, whose plantlets are very sensible to light and need some shade, take gradually place. These different behaviours are linked to physiological characteristics of the species. There are several studies on the light intensity required by forest species. Instead, almost no studies are available on light quality requirements. Such researches are essential for the definition of the best light conditions for in situ propagation. In fact, artificial lights for plant growth have been designed mainly for agricultural crops and are rich in blue and red wavelengths, in order to enhance the photosynthetic activity. Since all agricultural crops are heliophilous, these light sources may be considered as adaptable to heliophilous forest species. So, the indoor growth of a presumed heliophilous species, *Platanus orientalis*, under artificial lights was tested. Six different light sources were compared: 5 Valoya® LEDs vs OSRAM® fluorescent tubes. Seeds were grown under each light source, in quickpots containing a peat-based substrate, in a climatized growth chamber (22 ±1°C; 60-70% RH; 12L/12D; 100 µmol m-2s-1 PAR). A rapid yellowing and desiccation of cotyledons and primary leaves led to the hypothesis of a high light stress. To test this hypothesis, light conditions in an environment where natural regeneration occurs were analysed. The Natural Reserve of Pantalica (Sicily) was chosen as study area; in particular the watersides of Anapo river, characterized by a riparian vegetation rich in *Platanus orientalis*, *Salix pedicellata*, *Populus nigra* and *P. alba*. Two transects 20 x 6 m were selected. In each one, various points with and without natural regeneration were identified. Light spectra were collected for each point at different times during the day. No significant differences, in terms of light quality and quantity, were found in presence or absence of regeneration. The understory showed an homogeneous alternation of shade and full sunlight from 10 am to 2 pm, due to the movement of upper canopy. Therefore, *Platanus orientalis* is not completely heliophilous, as reported in literature, but grows in partial shade. This behaviour may be explained by the lack of a complete xantophyll cycle in the seedlings, responsible for the production of sunscreens like anthocyanins. Oriental Plane seedlings have only one protection against light excess: leaf hairs. Traditionally involved into the defense against biotic attack, water loss and overheating, they could be considered as a transient physical protection against photoinhibitory damage. The results of this research can contribute to understand the ecology of sciaphilous forest species and to choose the best light conditions for their indoor propagation.

Keywords: forest regeneration, light requirements, indoor propagation

2.5. New technology for pre-cultivation of forest seedlings under LED lamps – modification of light conditions to mitigate light shock stress after transplanting to open land

Authors:

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- Anders Mattsson
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- Titta Kotilainen
  Valoya OY, Finland

Type of publication: Oral presentation

Presented in: *2nd Restoring Forests Congress. 14-16 October 2014, Lafayette, USA.*

Abstract:

Forest restoration aims to cope with the increasing demand on forest products, as well as an aid in fighting climate change and compensating for accelerated deforestation. Funded by the European Commission under the Seventh Framework Program (FP7), the Zephyr project aims to introduce a zero-impact incubator for the pre-cultivation of forest regeneration materials. The consortium, involving 14 organizations of 10 different European countries, is developing innovative and cost-efficient technologies that will allow the production of standardized high quality forest seedlings ready to be transplanted. The technologies will be integrated into a functional and transportable unit not affected by the outdoor conditions and producing minimal emissions. To achieve this, the system will be powered mainly by solar energy and will recycle the water used. Specifically developed devices such as wireless sensors and LED lamps will be used to monitor and enhance the cultivation process, reduce the energy consumption and decrease the overall cost due to their high efficiency, long lifetime and low maintenance.

The LED grow lights used have a continuous spectrum that has been selected and specifically tailored to the plants' needs. Nevertheless, seedlings pre-cultivated under LED lights could face UV stress after transplanting to open land as these wavelengths are not included in the light spectrum used in the growth chamber. Moreover, light intensity levels during indoor cultivation are usually much lower compared to the outdoor conditions, which can cause a light shock to the plants. Juvenile plants are less efficient in the utilization of the absorbed light, and therefore, prone to photoinhibition by radiation fluxes that usually do not harm mature plants. Plant protective mechanisms against UV radiation and high PAR (400-700nm) light intensity are partly overlapping. Hence, exposure to UV or high light intensity before transplanting, or introducing a transient phase by using shading cloths during transplantation period could help to reduce this stress.

The aim was to reduce the transplanting stress of *Picea abies* and *Pinus sylvestris* seedlings grown under LED-lamps for the first 5 weeks of cultivation. We investigated how different methods; UV-A pre-treatment or high irradiance during the indoor cultivation or usage of shading cloths for the first week(s) after transplanting outdoors can be used to mitigate light shock stress. Different methods and exposure times showed varying ability in ensuring good seedling growth and survival.

Keywords: LED growth lights, forest nurseries, light shock, transplanting stress

2.6. Outdoor performance of forest seedlings pre-cultivated under artificial lights – effects of the light spectra used for pre-cultivation on the future establishment and development

Authors:

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  Department of energy, forests and built environments, Dalarna University, Sweden

Type of publication: Oral presentation

Presented in: *International Scientific Conference Reforestation Challenges. Belgrade 3-6 June 2015,* http://www.reforestationchallenges.org/Reforestation_Challenges.php

Abstract:

Forest nurseries are essential for producing good quality seedlings, thus being a key element in the reforestation process. With increasing climate change awareness, nursery managers are looking for new tools that can help reduce the effects of their operations on the environment. The ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), has the objective of finding new alternatives for nurseries by developing innovative zero-impact technologies for forest plant production. Due to their direct relationship to the energy consumption of the nurseries, one of the main elements addressed are the grow lights used for the pre-cultivation. New LED luminaires with a light spectrum tailored to the seedlings' needs are being studied and compared against the traditional fluorescent lamps. Seedlings of *Picea abies* and *Pinus sylvestris* were grown under five different light spectra (one fluorescent and 4 LED) during 5 weeks with a photoperiod of 16 hours at 100 µmol·m-2·s-1 and 60% humidity. In order to evaluate if these seedlings were able cope with real field stress conditions, a forest field trial was also designed. The terrain chosen was a typical planting site in mid-Sweden after clear-cutting. Two vegetation periods after the outplanting, the seedlings that were pre-cultivated under the LED lamps have performed at least as well as those that were grown under fluorescent lights. These results show that there is a good potential for lightning substitution in forestry nurseries.

Keywords: Forest regeneration materials, LED grow lights, nursery technologies, planted forest

2.7. Reforestation challenges in Scandinavia

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Type of publication: Oral presentation (Keynote)

Presented in: *International Scientific Conference Reforestation Challenges. Belgrade 3-6 June 2015, http://www.reforestationchallenges.org/Reforestation_Challenges.php*

Abstract:

In the keynote major reforestation challenges in Scandinavia will be high-lighted. In Scandinavia the following countries are included: Iceland, Norway, Sweden, Finland and Denmark. For Iceland, with only a forest cover of 2%, a major reforestation challenge is the deforestation and overgrazing in combination with land degradation and extensive soil erosion. The challenge includes the conflict with livestock farmers. For centuries, the commons were used for sheep and horse grazing. However more and more of the farmer grazing land have been fenced up, allowing regeneration of birch and plantations of other species. In Norway and Sweden, with a forest cover of 37 and 69% respectively, a major reforestation challenge has for decades been the risk of seedling damages from the pine weevil. If the seedlings are not protected it is common with survival rates less than 25% after planting. The pine weevil feed on the bark of young seedlings after they are planted at the regeneration site and if the seedling is girdled it will not survive. In Sweden, and soon in Norway, insecticides have been forbidden. In the keynote new methods and technology will be presented based on non-chemical protection. In Finland, with a forest cover of 75%, a major reforestation challenge is linked to the forest structure. The structure of Finnish forestry includes many private forest owners in combination with small regeneration sites. This implies a situation where logistics and methods for lifting and field storage provide a major challenge in order to preserve seedling quality until the planting date. Due to this situation new logistic systems and technology is being developed in Finland including new seedling cultivation programs (including cultivation under Light Emitting Diodes (LEDs)) to match the access of fresh planting stock to different planting dates. In Denmark, with a forest cover of 13%, a major reforestation challenge is the possibility of future plantations based on a wide range of relevant species. For this option new methods and technology have to be developed in reforestation activities that support this possibility. These methods and technology should make it possible not to be limited to certain species due to problems and limitations during field establishment. This due to the prospect of establishing stabile, healthy and productive stands of various forest species that also can be adapted to future climate change.

Keywords: Scandinavia, reforestation challenges, deforestation, degradation, erosion, pine weevil, pre-cultivation, transplanting, forest structure, lifting, field storage, alternative species, climate change

2.8. The effect of light-emitting diodes on the development of pomegranate seedlings

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Type of publication: Oral presentation

Presented in: *14th National Conference of Hellenic Botanical Society*. Patra, Greece, 8-11 October 2015

Abstract:

The impact of Light-Emitting Diodes (LED) versus fluorescent lighting (FL) on morphological, physiological and phytochemical characteristics of pomegranate (*Punica granatum* L.) after six weeks pre-cultivation in growth chambers was investigated. Five LED treatments with different irradiation spectra [L20AP67 (moderate B, G, R, low R: FR), AP673L (high R, R: FR), G2 (high R, low R: FR), AP67 (moderate B, R, low R:FR), NS1 (high B, G, R:FR, plus 1% UV)] were used. Seedlings grown under L20AP67 exhibited the best morphological-agronomical characteristics (height, root length, fresh and dry weight, leaf area). Seedlings of FL andL20AP67 treatments showed the highest photosynthetic efficiency; the FL had the highest chlorophyll and carotenoid content. The greatest total phenolic content was recorded inNS1, of simple phenolsinAP67, of flavonoids inG2 and AP67, and of anthocyanins inG2, AP67 and NS1. For evaluating the seedling transplanting capacity, root growth potential (RGP) estimation was performed following a 31-day incubation in a specialized chamber. Transplanted seedlings of AP67treatmenthad the lengthiest, of L20AP67 the heaviest newly formed roots. In concluding, L20 AP67 treatment promoted more efficiently than conventional FL lighting the pomegranate growth and transplanting potential, whereas a number of other LED treatments imposed stress in this species.

3. Poster presentations at conferences and workshops

3.1. A silvicultural practice to facilitate forest restoration – a new seedling cultivation technology for regeneration establishment

Authors:

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- Anders Mattsson
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Type of publication: Poster presentation

Presented in: *XXIV IUFRO World Congress - "Sustaining Forests, Sustaining People". 5-11 October 2014. Salt Lake City, USA.*

Abstract:

Forest restoration has become a primary task not only to cope with an increasing demand on forest products, but also to fight climate change and compensate an accelerated global deforestation. However, many of the current practices used in forestry nurseries to produce forest planting stock have adverse effects on the environment. The main objective of the ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), is to develop an innovative zero-impact technology for the pre-cultivation of forest regeneration materials that is not affected by the outdoor climate. Among the main components to be improved are the artificial lighting sources used for the cultivation. Traditional fluorescence lamps are to be replaced by LED grow lights with spectra tailored to the seedlings' needs. The present work investigates biological responses of *Picea abies* and *Pinus sylvestris* to six different light spectra. The pre-cultivation has been done following a standard growth protocols during 5 weeks with a photoperiod of 16 hours at 100 μmol·m-2·s-1. This has been done under controlled closed conditions with a room temperature of 20°C and a relative humidity of 60%. The analyses have shown clear differences among the treatments and their adapting capacity when being transplanted.

Keywords: Forest restoration, LED grow lights, nursery production, containerized planting stock

3.2. Analysis of *Corylus Avellana* l. growth under led lights for reforestation purposes

Authors:

- Tatiana Marras
- Bartolomeo Schirone

DAFNE Department, University of Tuscia, Viterbo, Italy

Type of publication: Poster presentation

Presented in: *109th Congress of Italian Botanical Society.* Florence, Italy. 2-5 September 2014

Abstract:

Reforestation is very important in contrasting landscape degradation and desertification processes due to climate changes. In the Mediterranean area, forest restoration is also important to reestablish and to preserve the rich biodiversity of the different forest ecosystems, which has been damaged for a long time due to not appropriated silvicultural activities. The production of great amounts of high quality forest plants stocks ready to be transplanted in pots or in field at low costs is a primary challenge in this field. Since 2012 the Zephyr EU-project (FP7-ENV.2012.6.3-1) is developing an automated high-density seedling production unit based on a combined action of optimal environmental conditions and LED lamps, with a noticeable reduction of emissions achieved through a low energy consumption, reduced by up to 70% in respect to the traditional nursery pre-cultivations.
After a first set of experiments on *P. granatum* L., which highlighted the possibility of using specific LED spectra to foster the development of roots and the production of secondary metabolites (two important elements favouring the adaption of plants in open-field), a second species was analyzed: *Corylus avellana* L.; in order to avoid the gradual loss of genetic variability of populations caused by the propagation by cuttings, propagation by seeds was chosen. After 2-months of cold stratification to break dormancy, 104 seeds were sown under each light source (5 Valoya® LED spectra vs OSRAM® fluorescent tubes as control, Fig.1) in quickpots containing a peat-based substrate, in a climate growth chamber at a temperature of 22 ±1°C, at 60-70% of RH, with 12/12h of photoperiod and with 100 µmol m-2 s-1 PAR. After 1 month morphological analysis were performed on seedlings, before transplanting them into a greenhouse. Results on C. avellana pointed out the best influence of LED spectra on stem growth, stem diameter (Fig.2), leaves, shoot and roots dry weights (Fig.3) in comparison with conventional fluorescent tubes. In details, higher far red percentages (AP67 spectra) best performed on shoot and root weights, while lower far red percentages (NS1 spectrum) did the same on leaves dry weight. Mean leaf area did not show statistical differences among light sources, probably because it is more linked to light quantity than quality. Once transferred into the greenhouse for outdoor adaption, the plants immediately stopped to grow and leaves started to yellow before falling down. They were subjected to an evident transplant shock reaction, differently from *P. granatum*. Reasons for transplant shock reactions of pre-cultivated forest seedlings to open land can be related to several factors, in particular the lack of protective mechanisms (as UV absorbing compounds) against higher UV-A and UV-B irradiance outdoors, that is absent/minimal during the indoor cultivation phase and the lack of protective mechanisms against high light intensity, which is much lower during indoor cultivation than outdoor. In fact, *P. granatum* seedlings were able to produce carotenoids and anthocyanins already under LED sources and these compounds led them to easily survive outdoor. Other species, as *C. avellana*, are not enough stimulated to produce secondary metabolites by LED spectra commonly used for plant growth. In this case, the exposure to UV radiation during pre-cultivation, for a short time, in order to avoid DNA damage so as the

exposure to higher light intensity before transplanting, or transient phase by using shading cloths during transplantation in open-field could be suitable solutions to reduce this stress. Trials to test these solutions are subject of ongoing research.

3.3. Analysis of seedlings growth under different LED lights

Authors:

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Type of publication: Poster presentation

Presented in: *109th Congress of Italian Botanical Society.* Florence, Italy. 2-5 September 2014

Abstract:

LED lights have a lower environmental impact than traditional lights due to a series of factors such as high energy-conversion efficiency, small volume, longer life, low thermal energy output. Concerning plant growth, the use of LED lights provide specific wavelength as well as the possibility to adjust light intensity/quality. The increasingly need to reduce energy consumption worldwide, raised the necessity to improve LED lights use. The present work aims to 1) examine the effect of different LED light spectra on seedlings growth of different species in order to define a species-specific cultivation protocol under optimal plant growth spectrum 2) compare direct measurements with non-destructive method by optical sensors. The plant species analyzed were Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), Holm oak (*Quercus ilex* L.) and Pomegranate (*Punica granatum* L.) Seedlings were left to grow in a growth chamber (16 h photoperiod, 120µmol m_{-2} s_{-1} PAR, temperature 21-22°C, humidity 80% germination - 55-70% growth) for 4 weeks under G2, AP67, AP67-3L, NS1 LED light (Valoya) and Fluorescent light (FL, control). Direct measurements of shoot height, root length, shoot and root biomass were carried. Non-destructive analysis was carried by measuring *Greenness* (percentage of shoot cover projected on tray ground) and *Plant height*. Greenness data were first obtained by a series of manually taken images (Nikon D70s digital camera) analysed with an open source software (Image*J*). Plant height was manually taken during the growth period to find a relationship with plant biomass. Furthermore, plant height was manually taken and compared with data obtained from images acquired by Optical sensors and analysed by *uEyeDualcam HeightMap* software (ACREO). Seedlings growth under AP673-L and G2 light, for Scots pine and Norway spruce, showed height values higher than values measured under control light and 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 total biomass and root length than seedlings growth under control light. In conclusion, seedling growth under G2 LED type shows 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. Therefore, in alternative to G2 type, AP67-3L LED type could represent the best option for a standard cultivation protocol. Results about non-destructive analysis show that greenness value for Scots pine and Norway spruce species in relation to different light type, showed highest values with AP67-3L. Preliminary results showed the same pattern also for Holm oak and Pomegranate species. Highly significant positive relationships between greenness, plant height and seedlings total biomass were found for Scots pine and Norway spruce for all LED

light types ($0.89 < R_2 < 0.99$). For all considered species tests were carried to relate plant height data obtained by *HeightMap* Software (ACREO) with plant height manually measured, and a good relationship was found. (Scots pine: $R_2 = 0.85$; Norway spruce lower relationship $R_2 = 0.59$; Holm oak $R_2 = 0.83$). Tests are in progress to relate Greenness data obtained by *uEyeDualcam* software with plant biomass. In conclusion tests about relationship between plant height and greenness, obtained by manual measurements and by Nikon digital camera-Image J software, highlighted that indirect analysis are good parameters for non-destructive quantification of plant biomass.

3.4. Analysis of the effects of gibberellic acid and cold stratification on the germination of two endemic species of Azorean Islands

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Type of publication: Poster presentation

Presented in: *Ecoplantmed Conference,* Beirut, 14-16 October 2015

Abstract:

Azorean islands show about 300 native species. Among them, 156 are considered to be rare and a significant number is disappearing for the expansion of agriculture, farming and the competition with invasive exotic species. In order to counteract their extinction and recover native habitats, some attempts of reforestation, based on artificial propagation of seedlings, because of a low dissemination of native species usually associated to the presence of seed dormancy, are underway.
Main purpose of this work is the analysis of the effects of gibberellic acid (GA3) and cold stratification on the germination of 2 endemic species of Azorean islands, *Hypericum foliosum* Aiton and *Laurus azorica* (Seub.) Franco, in terms of ability in promoting dormancy breaking or speeding up the germination process.
Four different pretreatments were tested: 30 or 60 days of cold stratification (4°C) without gibberellins; 18h of incubation in a GA3 solution with a concentration of 200 mg/L followed by 30 or 60 days of cold stratification. Pretreated seeds were then incubated at 20°C and 12L 12D in a phytoclimatic chamber. Two different controls were set up: direct incubation at 20°C and 12L 12D photoperiod (control 1); 18 h of immersion in a GA3 solution (200 mg/L) followed by incubation at 20°C and 12L 12D photoperiod (control 2).
H. foliosum showed a 100% of germination reached in only 3 days as a consequence of 30/60 days of cold stratification and 80% in the case of combined pretreatments (GA3 + 30/60 days of cold stratification). Control 1 and 2 showed a lower germination percentage (< 10%). *L. azorica* showed the best results (70% of germination) as a consequence of 60 days of cold stratification and GA3 pretreatment without stratification (control 2). Control 1 showed 30% of germination, as in the case of combined pretreatments. Moreover, the combination of the two pretreatments led to higher shoot height values of seedlings, measured 1 month after germination. These results are promising for the definition of a germination protocol which would allow, in a short time, to obtain a significant number of seedlings of *H. foliosum* and *L. azorica*, to be used in reforestation and restoration activities in Azorean islands.

Keywords: forest restoration, reforestation, germination, dormancy, artificial propagation

3.5. Climate control in the production of forest plants – using photovoltaics to power an innovative forestry incubator

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- Sreenivaasa R Pamidi
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Type of publication: Poster presentation

Presented in: *6th International Conference on Solar Air-Conditioning. 24-25 September 2015, Rome, Italy*

Abstract:

Forest ecosystems are currently challenged by fluctuations in the climate and an extreme exploitation of their resources. Forest restoration requires among other things, high amounts of healthy forest seedlings to replace the lost trees. However, the cultivation of these seedlings often involves intensive methods in forest nurseries which consume considerable amounts of energy for lighting, acclimatization and irrigation.

The ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), is developing innovative and cost-friendly technologies for the pre-cultivation of forest plants. Devices such as LED growth lights and a new generation of wireless sensors will be integrated into a functional and transportable system for large scale production of forest seedlings. The unit will have a very low impact on the environment, will be independent of the outdoor conditions and will be powered by solar energy. The whole concept represents a breakthrough in forest nursery production for reforestation purposes. It addresses issues such as energy use, water recycling, reduction of fertilizers and avoidance of pesticides. One of the main features of the Zephyr incubator is the fact that the seedlings will be pre-cultivated during the first stage in an isolated environment. A transportable and closed incubator possess several advantages: it provides a better climate control for the production of seedlings and reduces the need for pesticides and fertilizers. The closed-climate allows growing seedlings in places where it would not be possible otherwise (e.g. near deserts). The plants can be produced directly at the place where they are needed avoiding further transport to the reforestation/afforestation site. Additionally, it extends the production time throughout the whole year even during the winter. Moreover, it will allow a certified and standardized production of reforestation materials, with a noticeable increase in the success of the restoration actions.

In order to benefit from the advantages of a closed growing environment without having a negative impact on the environment, the incubator will be powered mainly by a solar photovoltaic (PV) system which will be mounted on the roof of the unit. In order to reduce the amount of air conditioning needed, the chamber has been isolated from the rest of the system. The growth protocols have been set to a certain temperature range that allows a more flexible operation of the air conditioning system without compromising the development of the seedlings. Finally, since the LED growth lamps do not produce as much additional heat compared to other lightning sources so there will be further savings.
Keywords: PV-driven Air Conditioning, Forestry incubator, indoor forest plants production

3.6. Cultivation of forest regeneration materials under artificial radiant sources - effects of light intensity on energy consumption and seedlings' development

Authors:

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Type of publication: Poster presentation

Presented in: *XXIV IUFRO World Congress - "Sustaining Forests, Sustaining People". 5-11 October 2014. Salt Lake City, USA.*

Abstract:

In times of major environmental challenges and increasing demand for forest products, planted forests have acknowledged advantages compared to other land uses. Despite not being able to substitute natural forests, planted ones have, if properly managed, a great potential to contribute in addressing this problems. Besides the ecological benefits such as carbon sequestration, planted forest can help coping with the wood products demand without further reduction of natural forest. Forest restoration, rehabilitation and reforestation are limited to the capacity of producing forest regeneration materials. Often, as the production is intensified at the forest nurseries, the practices start having an adverse impact on the environment and stop being truly sustainable. One of the main issues in nurseries is the energy consumption for grow lights in periods with short daylight times. By using high efficiency LED grow lamps and adjusting the light intensity, this study aimed to reduce the energy consumption from lighting per seedling without compromising their development. The pre-cultivation of *Picea abies* and *Pinus sylvestris* seedlings was done during 5 weeks under controlled conditions at 20°C and a relative humidity of 60%. The photoperiod was of 16 hours at an intensity ranging from 50 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Keywords: Containerized planting stock production, LED grow lights, *Picea abies, Pinus sylvestris*, energy consumption

3.7. Effects of different LED lights on the growth and phytochemical characteristic of *Myrtus communis* L. seedlings

Authors:

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- Kalliopi M Radoglou

Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace

Type of publication: Poster presentation

Presented in: *14th National Conference of Hellenic Botanical Society*. Patra, Greece, 8-11 October 2015

Abstract:

Five LED light qualities of continuous spectrum with a mixture of different wavelengths percentages L20AP67, AP673L, G2, AP67, NS1 and fluorescent lamps (FL) as control were used to investigate the effects on growth and phytochemical characteristics of myrtle seedlings cultivated in mini-plug containers inside growth chambers. After one month of indoor cultivation AP673L and NS1 LEDs significantly promoted the root development and induced an increase by 85% in the total dry weight accumulation of the myrtle seedlings compared to the rest of the light treatments. The contents of Chl a and carotenoid in myrtle leaves under different LEDs were shown no significant differences. Antioxidant status of myrtle by means of significantly higher phenolic and anthocyanin content was promoted by the L20AP67 LED among all the light treatments, while FL light showed the lowest. After the end of the indoor experiment root growth potential (RGP) test was held for another month, as a performance attribute for seedling quality evaluation. Initiation of new roots, significantly longer and heavier was found for the AP673L and NS1 LEDs, while the lowest was for the FL. In conclusion our results may provide an insight on the advantages of using LED lights as a regulation tool for seedlings quality in *M. communis* in controlled environments; hence those enhanced attributes of the seedlings could ultimately be useful for the demands of a potential high scale production.

3.8. Effects of LED lights on seedlings growth of *Pinus sylvestris* l. and *Picea abies* l.

Authors:

- Nicoletta Fulgaro
- Mattia Terzaghi
- Barbara Baesso
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Department of Biotechnology and Life Science, University of Insubria, Varese, Italy

- Gabriella Stefania Scippa

Department of Biosciences and Territory, University of Molise, Italy

Type of publication: Poster presentation

Presented in: *108th Congress of Italian Botanical Society.* Baselga di Pinè, Italy. 18-20 September 2013

Abstract:

LED lights have a lower environmental impact than traditional lights due to a series of factors such as high energy-conversion efficiency, small volume, longer life, low thermal energy output. Concerning plant growth, the use of LED lights provide specific wavelength as well as the possibility to adjust light intensity/quality. The increasingly need to reduce energy consumption worldwide, raised the necessity to improve LED lights use. The objective of the present study was to examine the effect of different LED light spectra on seedlings growth of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L.). The final goal is to define a species-specific cultivation protocol under optimal plant growth spectrum.

Materials and methods

Seeds of Scots pine and Norway spruce from Italian Pre-Alps Region were provided by National Forest Service (Peri, VR). The seeds were stored at 4°C and pretreated with a 24-hours hydratation before sowing in mini-plug container. Trays were placed in growth chambers (16 h photoperiod, 120 µmol m_{-2} s_{-1} PAR, temperature 21-26°C, and humidity 80% germination - 55-70% growth). Seedlings were left to grow for 6 weeks under G2, AP67, AP67-3L, NS1 LED light (Valoya) and Fluorescent light (FL). Different parameters were measured: plant height, shoot diameter, total root length, shoot and root total dry mass and greenness development. The plant greenness was estimated as percentage of shoot cover on tray ground by manually taken images which were analyzed by open source software (HTPheno plugin of ImageJ). In order to estimate total root length, roots were scanned and analyzed by WinRHIZO Pro V. 2007d.

Results and discussion

Our preliminary results show that for Norway spruce seedlings all measured parameters (plant height, root-shoot dry mass and root length) were promoted by AP67-3L spectra with exception of greenness where the highest value was found with AP67 spectra. Scots pine seedlings showed the highest values of all measured parameters (greenness, root-shoot dry mass and root length) under G2 spectrum with the exception of plant height where the highest value was found with AP67-3L. The two species responded differently to different light spectra. Moreover, we could assert that except for NS1 spectra, both species showed similar or higher values under all spectra compared to control light. Concluding, in the perspective of establishing a standard cultivation protocol, AP67-3L spectra could represent the optimal plant growth for both considered species.

3.9. Energy efficiency in intensified production of forest regeneration materials – design of a photovoltaic system for sustainably powering an innovative forestry incubator

Authors:

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Type of publication: Poster presentation

Presented in:
XXIV IUFRO World Congress - "Sustaining Forests, Sustaining People". 5-11 October 2014. Salt Lake City, USA.

VI Latin American Forestry Congress. 20-24 October 2014, Morelia, Mexico.

Abstract:

Planted forests can contribute addressing problems of global concern such as climate change mitigation, biodiversity lost and pressure on ecosystems due to high demand of forestry products. However, in order to be able to profit from these benefits sustainably, production rates of forest regeneration materials should be higher than the harvesting rates. Nevertheless, intensive production methods often bring along adverse consequences for the environment. In the frame of the ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), innovative and cost-friendly technologies for the pre-cultivation are being developed. They will be integrated into a functional and transportable system for a large scale production of seedlings, with zero-impact on the environment and not affected by outdoor conditions. To achieve this, high efficiency devices with low energy consumption will be used and the incubator will be powered by solar energy. This work aims to present the efforts made to reduce the energy loads and optimize the photovoltaic (PV) system. The power system will also be capable of connecting to the electricity grid, using a diesel generator as a back-up, and a battery bank with at least one day of autonomy (up to 7 kWh/day) in central European latitudes.

Keywords: Planted forest, photovoltaic system, climate change mitigation, incubator, forest regeneration materials

3.10. Enhanced growth characteristics of *Castanea sativa* mill. seedlings exposed to led lights with continuous spectrum during indoor cultivation

Authors:

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Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace

Type of publication: Poster presentation

Presented in: *the International Scientific Conference "Forestry: Bridge to the Future",* 6-9 May 2015, Sofia, Bulgaria

Abstract:

Previous studies demonstrated that combined effects of monochromic LEDs were an effective light source for plant growth of several species in artificial environments. However, there has not been discussion on the application of LED lights of continuous spectrum in plant growth chambers. This study examines the influence of five different LED light qualities (L20AP67, AP673L, G2, AP67 & NS1) that emitted a mixture of continuous spectrum based on various percentages of ultraviolet, blue, green, red, far-red and infra-red radiation or Fluorescent light (FL as control) on growth of sweet chestnut seedlings into mini-plug containers during one month indoor cultivation. Leaf characteristics of chestnut seedlings were better promoted under LEDs by means of faster leaf formation of greater area that showed significantly higher stomata and epidermal cell number compared to the FL light. Therefore among LEDs G2, NS1 and AP67 showed the greatest effect by inducing significantly higher stomatal density (SD), stomatal index (SI%) and cell density (CD) compared to the FL light and L20AP67 LED: Similar shoot development was found irrespective the light spectrum, however significantly longer roots were formed by the L20AP67 than the FL light that showed the shortest. Further root system architecture analysis revealed that NS1 and AP673L LEDs produced seedlings with significantly higher root fibrosity index compared to the FL. Dry weight accumulation especially of the shoots and roots of the seedlings treated with the AP673L was the highest by far. In contrast FL light obtained the lowest dry weight mass thus exhibited the lowest R7S ration compared to LEDs. These results presented, might provide new strategies for using LEDs of continuous spectrum for adequate cultivation protocols into growth chambers of *Castanea sativa* or other forest tree species.

Key Words: chestnut, growth chamber, LEDs, light quality, photomorphogenesis

3.11. *Frangula azorica* under different LED treatments

Authors:

- Raquel Ferreira
- Malgorzata Pietrzak

Azorina – Sociedade de Gestão Ambiental e Conservação da Natureza S.A.

Type of publication: Poster presentation

Presented in: Workshop: *Role of research and conservation projects in preservation of endangered species and biodiversity enhancement.* 15 September 2014, Furnas, Portugal

Abstract:

The effects of five light types on the growth of the aerial part and toots of micro propagated plants of *Frangula azorica* were studied during five weeks. The light types were: Osram L36W/77 Floura (Flourescent), 2 bar lamps Valoya AP67 (120cm), 2 bar lamps Valoya AP673 L (120cm), 2 bar lamps Valoya G2 (120cm), 2 bar lamps Valoya NS1 (120cm).

3.12. *Juniperus brevifolia* under different LED treatments

Authors:

- Raquel Ferreira
- Malgorzata Pietrzak

Azorina – Sociedade de Gestão Ambiental e Conservação da Natureza S.A.

Type of publication: Poster presentation

Presented in: Workshop: *Role of research and conservation projects in preservation of endangered species and biodiversity enhancement.* 15 September 2014, Furnas, Portugal

Abstract:

The effects of five light types on the growth of the aerial part and toots of micro propagated plants of *Juniperus brevifolia* were studied during five weeks. The light types were: Osram L36W/77 Floura (Flourescent), 2 bar lamps Valoya AP67 (120cm), 2 bar lamps Valoya AP673 L (120cm), 2 bar lamps Valoya G2 (120cm), 2 bar lamps Valoya NS1 (120cm).

3.13. Long night treatment for induction of cold hardiness using artificial lights - effects of photoperiod on seedlings' storability and energy consumption.

Authors:

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- Anders Mattsson
  Department of energy, forests and built environments, Dalarna University, Sweden

Type of publication: Poster presentation

Presented in: *XXIV IUFRO World Congress - "Sustaining Forests, Sustaining People". 5-11 October 2014. Salt Lake City, USA.*

Abstract:

Human-assisted forest regeneration in Nordic climates is considerably limited by the harsh outdoor conditions. There the local weather opens only a small time window during the summer for transplantation and establishment of the pre-cultivated seedlings in open land. Greenhouses and modern growth chambers aid to cope with this limitation by allowing year-round seedlings cultivation. Nonetheless, production levels are constrained to the cold storage capacity during the non-transplanting season. This storage is in turn dependent on the conifers' ability to adapt to freezing temperatures and withstand the overall stress associated with the cold hardening. Long night treatments can induce dormancy with growth cessation and terminal buds initiation, leading to a better cold resistance. When growing forest regeneration materials under artificial lights, the lengths of the long night treatment and the photoperiod will have a significant impact, not only on the biological response of the seedlings but also on the energy consumption and thus on the CO_2 emissions. The aim of this work was to explore different long night treatment regimes for induction of cold hardiness on *Picea abies* and *Pinus sylvestris* seedlings using artificial lights. This has been done with the purpose of studying the interplay between the energy consumption and the biological responses.

Keywords: Forest restoration, LED grow lights, forest nurseries, long-night treatment, cold hardiness

3.14. Reducing the impact of forest plant production - design of a stand-alone PV-hybrid system for powering an innovative forestry incubator

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Type of publication: Poster presentation

Presented in: *29th European Photovoltaic Solar Energy Conference. 22 - 26 September 201, Amsterdam, Netherlands*

Abstract:

Nowadays, the high demand of forestry products imposes a high pressure on the ecosystems and can derive in biodiversity lost and other ecological problems. Planted forests can contribute to more sustainable practices and help addressing other problems of global concern such as climate change, erosion and desertification. Large scale production of seedling is required to offset the high harvesting rates; however these intensive methods often have a negative impact on the environment. Funded by the European Commission under the Seventh Framework Programme (FP7), the ZEPHYR project consortium is developing innovative and cost-friendly technologies for the pre-cultivation of forestry species. These will be integrated into a functional and transportable system for pre-cultivation of seedlings, with zero-impact on the environment and not affected by outdoor conditions. To achieve this, the incubator will be powered mainly by solar energy. This work aims to present the efforts made to design and optimize the solar photovoltaic (PV) system which will be mounted on the roof of the unit. Especially developed devices such as LED growth lamps and wireless sensors will be used to reduce energy consumption and monitor the cultivation process. A load profile study was conducted and the growth protocols were adapted to perform most of the tasks during daytime to use the energy from the PV panels directly. A battery bank will be designed to provide at least one day of autonomy in central European latitudes. Moreover, the power system will also be capable of connecting to the electricity grid or use a diesel generator as a back-up.

Keywords: Stand-alone PV-system, climate change mitigation, forest plant production, forestry incubator

3.15. Zephyr Project: Phase I: Morphogenetic effects of led lights on seedlings growth

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Type of publication: Poster presentation

Presented in: *108th Congress of Italian Botanical Society.* Baselga di Pinè, Italy. 18-20 September 2013

Abstract:

Forests, a vital resource for many countries, play a key-role in regulating global climate. The main aim of Zephyr EU-project (FP7-ENV.2012.6.3-1) is the creation of a new technology to produce high quality forest plants stocks for transplantion in pots or in-field at low costs. 14 partners from 10 European countries are in the project consortium.
The production unit, not affected by outdoor climate, will be equipped with LED lamps providing the optimal spectrum for photosynthesis, a control system regulating light intensity, photoperiod and further environmental parameters, wireless sensors sending data on the parameters to be surveyed the control system, solar panels providing energy and an irrigation system based on water recycling.
The aim of this new integrated technology is a more sustainable and resource-efficient forest nursery production, contributing to the environmental and biodiversity protection through a strong reduction of fertilizers and the avoidance of pesticides. The first step of the Zephyr project is the growth analysis of seven target forest species under different wide continuous spectra provided by LED lamps in order to select the most suitable light source: the LED lamp effects must resemble the effects of the solar spectrum on plant growth. The overall plant response under different light treatments is difficult to assess due to the complicated interaction pattern of many variables (1). Therefore, different morphological, microscopic, eco-physiological and biochemical parameters must be compared.
A first species, *Punica granatum* L., was grown under 6 different light treatments: 5 different wide continuous spectra provided by Valoya® LED lights and 1 continuous spectrum provided by OSRAM® FLUORA T8 neon tubes (2). 104 seeds were sown per light treatment into plug trays containing a peat-based substrate (Jiffy® PREFORMA). Experiments are performed in a climate growth chamber at a temperature of 22 ±1°C, 60-70% of RH and a 12/12h photoperiod. PAR values at the ground level range between 100 and 300 µmol m-2 s-1. Morphological (shoot height, stem diameter, number of leaves, fresh and dry leaves weight, roots and stem, total and mean leaf area) and anatomical (stomata number, density and dimensions, leaf anatomy in transversal sections) parameters were measured after 50 days of growth. Preliminary results show that both fluorescent and LED lamps with different spectra affect differently plant growth at morphological level. The most variable parameters among spectra were those related to aboveground plant growth (shoot height and weight, stem diameter, number of leaves). Further biochemical and ecophysiological studies have to be planned. *P. granatum* showed also pigment variations (chlorophylls, carotenoids and anthocyanins), induced by light, determining different leaves colors under each spectrum. All these effects are probably due to the different percentage of red, far red and blue light provided by each lamp,

especially referring to red:blue and red:far red ratios, which affect predominantly the germination of seeds and the first phase of seedlings growth. In particular, it resulted that high percentage of far red promoted seedling growth, in terms of root and leaves biomass, while high percentage of blue light inhibited it. These data reflect on the strong quali-quantitative light requirement of *P. granatum* seedlings growth. Indeed, light quality regulates plant development in an intricate manner and it seems not possible to select a single optimal spectrum for seedling growth. In fact, each LED spectrum influences differently plant growth. For example, some spectra foster the aboveground elongation, some others the development of roots or the production of secondary metabolites, as the pigments of flowers or some antioxidant compounds. Therefore, the production unit must be equipped with many interchangeable LED lights, suitable for different purposes of the user.

4. Other Publications

4.1. Analysis of light needs for germination and first stages of growth of cork oak (*Q. Suber* l.) Seedlings in a forest of central Italy

Authors:

- Federico Canali
  DAFNE Department, University of Tuscia, Viterbo, Italy

Type of publication: Student Thesis

Presented in: *University of Tuscia,* Viterbo, Italy

Abstract:

Seed germination and seedling development of *Quercus suber* L. have specific light needs in terms of photoperiod, light quantity and quality. To reproduce optimal light conditions in a controlled environment, these parameters were analyzed in a mature cork forest in Central Italy (Tuscania, VT) since November 2014 up to July 2015.
Germination resulted to occur both in sunny and shaded areas. From the analysis of light spectra collected in sunny and shaded areas where natural regeneration of the species, a great variation in the quality and quantity of the sunlight spectrum reaching acorns onto the ground and then growing seedlings, was observed. This phenomenon depends on climatic conditions (sunny, cloudy or rainy days), shadow degree (shaded or sunny areas), canopy cover and on the season.
Leaves of mature trees which cover new seedlings are able to stop blue and red wavelengths of sunlight, reducing the photons useful for their photosynthesis. This causes, as observed in those areas characterized by deep shadow, more difficulties in seedling growth. Plantlets showed higher values of stem diameter and chlorophyll concentration than in terms of shoot height.
On the other hand, in sunny areas plantlets resulted to be higher with a low stem diameter value.
Thanks to the field surveys it was possible to define a protocol for indoor growth of *Quercus suber* L., using LED lamps, trying to reproduce into an artificial environment the best conditions in terms of temperature, humidity and photoperiod found in a natural forest.

Keywords: Light needs, germination, cork oak, seedling growth, LEDs

4.2. Germination processes of the endemic species of the Azorean Islands

Authors:

- Filippo Munno
  DAFNE Department, University of Tuscia, Viterbo, Italy

Type of publication: Student Thesis

Presented in: *University of Tuscia,* Viterbo, Italy

Abstract:

Azorean Islands show about 300 native species. Among them, 156 are considered to be rare and a significant number, including some endemisms, is disappearing for the expansion of agriculture, farmng and the competition with invasive exotic species. In order to contrast their extinction and recover native habitats of these species, some attempts of reforestation, based on artificial propagation of seedlings, because of a low dissemination of native species associated to the presence of seed dormancy and the strong competition from invasive species, are underway.

Main purpose of this work is the analysis of the effects of two different pretreatments, one based on the usage of gibberellic acid (GA3) and the other one on cold stratification, on the germination of 5 native species of Azorean Islands (*Hypericum foliosum, Prunus azorica, Laurus azorica, Frangula azorica, Morella faya*), in terms of ability in inducing dormancy breaking or speeding up the germination process.

The definition of a germination protocol for each species is a fundamental prerequisite for the following breeding of a huge number of seedlings to be used for reforestation purposes in Azorean Islands.

The results, obtained after 1 month of incubation of pretreated seeds at 20°C in a phytoclimatic chamber, showed that GA3 and cold stratification are not able to break the deep dormancy of *Morella faya e Prunus azorica. Frangula azorica* showed a weak response to the hormonal treatment, while *L. azorica* and *H. foliosum* showed high germination rates after both pretreatments. Moreover, for these last two species, the combination of the two pretreatments led to an increased seedling growth rate in the first stages after germination. These results are promising for the definition of a germination protocol which would allow, in a short time, to obtain a significant number of seedlings of *H. foliosum* and *L. azorica*, to be used in reforestation and restoration of Azorean landscapes

Keywords: Forest restoration, reforestation, germination, dormancy, artificial propagation

5. Full text versions

Elsevier Editorial System(tm) for Scientia Horticulturae

Manuscript Draft

Manuscript Number: HORTI14588R2

Title: Artificial LED lighting enhances growth characteristics and total phenolic content of Ocimum basilicum, but variably affects transplant success

Article Type: Research Paper

Section/Category: Greenhouse (cultivation, management, models)

Keywords: nursery production, LEDs, basil, seedling growth, phenolic compounds

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First Author: Filippos Bantis

Order of Authors: Filippos Bantis; Theoharis Ouzounis, Ph.D.; Kalliopi M Radoglou, Ph.D.

Abstract: The morphological and phytochemical characteristics of two Ocimum basilicum cultivars (Lettuce Leaf, and Red Rubin-mountain Athos hybrid) under artificial lighting were investigated. Four LED light treatments [AP673L (high red and high red:far-red), G2 (high red and low red:far-red), AP67 (moderate blue and red and low red:far-red), and NS1 (high blue and green, high red:far-red and 1% ultraviolet)] with different colours mixing ultraviolet, blue, green, red and far-red, and fluorescent tubes (FL, high blue, green and red:far-red) as Control were used in the growth chambers for 28 days under PPFD of 200 ± 20 μmol m-2 s-1 for all treatments at plant height. G2, Control and AP67 treatments for Lettuce Leaf, and G2 for Red Rubin hybrid had higher growth rate. Roots of Lettuce Leaf were significantly longer under AP673L compared to NS1, while Red Rubin hybrid showed no significant differences. Total biomass was significantly greater under NS1, AP67 and G2 compared to the Control, for both cultivars. For both Lettuce Leaf and Red Rubin hybrid, root:shoot ratio (R/S) was favored under NS1, whereas the Control had the lowest impact. Leaf area of both cultivars was greater under the Control. Root growth capacity evaluation was also assessed. Seedlings of Lettuce Leaf cultivated under the effect of the Control and AP673L, and seedlings of Red Rubin hybrid grown under AP673L (mainly) quickly developed new root system. This could offer them the advantage of fast exploitation of larger soil volume after transplanting. Total phenolic content of Lettuce Leaf was significantly higher under NS1 compared to the rest of the treatments, while in Red Rubin hybrid, NS1 had significantly higher total phenolic content compared to the Control and G2. Our study demonstrates that LEDs variably affected growth characteristics and increased total phenolic content compared to conventional fluorescent light for these two Ocimum basilicum cultivars.

Reply to Reviewer #2

Reviewer #2: The Authors of article entitled „Artificial LED lighting enhances…" made changes in the manuscript in accordance with comments of reviewers. The work is interesting and relates to current issues regarding the effect of the use of modern, energy-efficient LED light sources on some morphological and biochemical parameters of indoor-cultivated plants, which contributes to the aims and scope of the Journal. I find it suitable for publication after considering small additional remarks, which are as follows:

L.167. The centrifugation speed is given in RPM units, but better way to express it would be relative centrifugal force specified as g multiplication.
Reply: Now line 167. Centrifugation speed is now expressed in g.

L.232 - 234. The part of the sentence "As for the DWS, G2 and AP67 lights exhibited greater values than…" should be modified, for example: "As for the DWS, plants grown under G2 and AP67 lights exhibited greater values than…"
Reply: Now lines 232 - 234. Phrase was modified as suggested.

Fig.2. This figure shows the dry weight of leaves, shoots and roots of tested plants affected by used light treatments. Presentation of the results regarding the roots as bars below the horizontal axis is very illustrative (probably authors wanted to emphasize the opposite direction of the growth of shoots and roots), but the values described on the y-axis below zero are negative, which introduces the incorrect information about the negative results obtained in the measurement of dry matter of roots. I suggest to present this data in a different way.
Reply: Figure was modified as suggested.

Highlights:

- LEDs induce greater biomass production in two basil cultivars than FL light.
- LEDs increase total phenolic content in two basil cultivars compared to FL light.
- Root:shoot ratio of both cultivars was greater under LEDs.
- For both cultivars, root growth capacity was variably affected.

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Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success.

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Abstract

The morphological and phytochemical characteristics of two *Ocimum basilicum* cultivars (Lettuce Leaf, and Red Rubin-mountain Athos hybrid) under artificial lighting were investigated. Four LED light treatments [AP673L (high red and high red:far-red), G2 (high red and low red:far-red), AP67 (moderate blue and red and low red:far-red), and NS1 (high blue and green, high red:far-red and 1% ultraviolet)] with different colours mixing ultraviolet, blue, green, red and far-red, and fluorescent tubes (FL, high blue, green and red:far-red) as Control were used in the growth chambers for 28 days under PPFD of 200 ± 20 μmol m^{-2} s^{-1} for all treatments at plant height. G2, Control and AP67 treatments for Lettuce Leaf, and G2 for Red Rubin hybrid had higher growth rate. Roots of Lettuce Leaf were significantly longer under AP673L compared to NS1, while Red Rubin hybrid showed no significant differences. Total biomass was significantly greater under NS1, AP67 and G2 compared to the Control, for both cultivars. For both Lettuce Leaf and Red Rubin hybrid, root:shoot ratio (R/S) was favored under NS1, whereas the Control had the lowest impact. Leaf area of both cultivars was greater under the Control. Root growth capacity evaluation was also assessed. Seedlings of Lettuce Leaf cultivated under the effect of the Control and AP673L, and seedlings of Red Rubin hybrid grown under AP673L (mainly) quickly developed new root system. This could offer them the advantage of fast exploitation of larger soil volume after transplanting. Total phenolic content of Lettuce Leaf was significantly higher under NS1 compared to the rest of the treatments, while in Red Rubin hybrid, NS1 had significantly higher total phenolic content compared to the Control and G2. Our study demonstrates that LEDs variably affected growth characteristics and increased total phenolic content compared to conventional fluorescent light for these two *Ocimum basilicum* cultivars.

Keywords: nursery production, LEDs, basil, seedling growth, phenolic compounds

Abbreviations:

DWL, dry weight of leaves; DWS, dry weight of shoots; DWR, dry weight of roots; LED, light-emitting diode; NDWR, dry weight of new roots; NRL, new root length; RGC, root growth capacity; R/S, root:shoot ratio; TPC, total phenolic content

1. Introduction

Until today fluorescent, metal-halide and incandescent lamps are used as supplementary lighting for crop production. Even though these sources induce an increase of daily photosynthetic flux levels, they are not as energetically efficient as desired. Spectral quality can affect plant growth and development (Schuerger et al. 1997) and the aforementioned light sources do not offer the option of spectral manipulation. Therefore, a light source with high energy-conversion efficiency that offers the possibility of spectral setting would be of great value. Research has been conducted using light-emitting diodes (LEDs) as a primary source of light irradiation for plant growth chambers in space programs (Barta et al. 1992). LED light technology presents a number of advantages including long life, small volume, low heat emission, adjustable light intensity, high energy-conversion efficiency and wavelength specificity (Massa et al. 2008; Morrow 2008; Schuerger et al. 1997). Therefore, several plant species such as sweet basil and lemon balm (Fraszczak et al. 2014), pepper (Schuerger et al. 1997), pea (Wu et al. 2007), cucumber and tomato transplants (Brazaityte et al. 2010, 2009), lettuce (Kim et al. 2004; Li and Kubota 2009; Lin et al. 2013; Ouzounis et al. 2015) and rapeseed (Li et al. 2013), as well as ornamental plants such as roses, campanulas, chrysanthemums and *Phalaenopsis* (Ouzounis et al. 2014a, 2014b) have already been tested under LED light sources with promising results, although varying among the different species.

Basil (*Ocimum basilicum* L.) is an annual culinary and medicinal herb originating from India and Southeast Asia. Apart from being cultivated in Asia, Africa and South America it is also cultivated in the Mediterranean countries (Makri and Kintzios 2008). It is also cultivated as an ornamental plant and its leaves are used as a seasonal food in dry and fresh form (Lee et al. 2005). Basil is rich in phenolic compounds such as rosmarinic, caffeic, chicoric and caftaric acids (Kwee and Niemeyer 2011; Lee and Scagel 2009; Zgorka and Glowniak 2001) One of the most important cultivars is “Lettuce Leaf” (*Ocimum basilicum var. crispum*). Its large crinkled leaves have a sweet sense which makes it ideal for salad and cooking use. “Red Rubin” basil (*Ocimum basilicum var. purpurascens*) is a purple-leaf cultivar with strong flavor. Its leaves are edible and are also used in cooking. Although basil is an important culinary and medicinal herb only a few studies have been conducted using LEDs as a lighting source.

Little information is available regarding the relationship between the light absorbed by basil and the mechanisms underlying the physiology and secondary metabolism under the influence of different light spectra. Secondary metabolites are formed in order for the plant to overcome potential stressful conditions. In plant tissues such as stems and leaves, the secondary metabolite synthesis can change due to environmental, physiological, biochemical and genetic factors (Wink 2010; Zhao et al. 2005) with light being one of the most influential factors (Kopsell et al. 2004; Kopsell and Sams 2013). One of the main groups of secondary metabolites is the phenolic group. This group is among the most ubiquitous groups of secondary metabolites in the plant kingdom and represents an example of metabolic plasticity enabling plants to adapt to biotic and abiotic environmental changes (Wink 2010). Their concentration depends on season and varies at different stages of growth and development (Seigler 1998; Wink 2010). Phenolics are pigments exhibiting radical scavenging activity, as well as protective activity against fungi, bacteria, viruses and insects (Lattanzio et al. 2006; Seigler 1998). Physiological changes are triggered by exposure to varying wavelengths (Samuolienė et al. 2013). Red light is the primary light source affecting biomass production and elongation through the phytochrome photoreceptor (Sager and McFarlane 1997). Blue light also affects photomorphogenic responses (e.g. regulation of leaf flattening and compact appearance) through phototropins and cryptochromes acting in an independent and/or synergistic manner with the phytochromes (de Carbonnel et al. 2010; Kozuka et al. 2013).

The research hypothesis was that pre-cultivating basil seedlings under the effect of lights with varying irradiation wavelengths would differently affect morphological and phytochemical properties. In addition, different LED spectra would trigger various morphological and phytochemical reactions among different basil cultivars. The objective of this research was 1) to investigate the effect of LED lighting on morphological and phytochemical characteristics of two basil cultivars during pre-cultivation in mini-plug containers, 2) to determine the best light quality treatment for pre-cultivation of two basil cultivars, and 3) to evaluate the transplant success.

2. Materials and methods

2.1 Plant material, growth conditions and light treatments

Seeds of "Lettuce Leaf" basil (LL) and "Red Rubin" basil cultivars were supplied from a nursery (Geniki Fytotechniki of Athens, Athens, Greece) in 2012. Additionally, basil seeds were collected from mountain Athos, Greece in 2012. The "Red Rubin" and "mountain Athos" cultivars were hybridized (RR).The experiment was held in November and December 2013. In total, 100 basil seeds per cultivar and treatment were hydrated for 24 hours and two seeds were transferred into each cell of the mini-plug containers. After three days the germination rate of the "LL" cultivar was 85%, whereas the "RR" had a 55% germination rate. One week after the sowing, excessive seedlings were removed leaving one seedling per mini-plug cell (50 per cultivar and treatment). The mini-plug plastic container trays (QP D 104 VW QuickPot®, Herkuplast-Kubern, Germany) with identical dimensions (310 × 530 mm, density: 630 seedlings/ m^2; 27 cc) were filled with a stabilize peat (Preforma PP01, Jiffy® Products, Norway) containing a binding agent in order to facilitate the process of transplanting.

We examined the application of conventional fluorescent lighting (FL) or LED lighting for the first four weeks after sowing. In total, 50 seedlings per cultivar and treatment were used. After sowing, basil seedlings were transferred to environmentally controlled growth chambers for 28 days under five different light treatments as described in Table 1. The white fluorescent lamps (Osram, Fluora, Munich, Germany) were used as the Control treatment. Valoya (Valoya Oy, Helsinki, Finland) LED lights generate a wide continuous spectrum with a mixture of ultraviolet (UV, < 400 nm), blue (B, 400-500 nm), green (G, 500-600 nm), red (R, 600-700 nm) and far-red (FR, 700-800 nm). It is worth mentioning that G2 emits high proportion of red and far-red light which makes it unpleasant to the user's eyes when working inside the growth chambers. Environmental conditions in the chambers were set at a 20°C/15°C day/night temperature, air relative humidity (RH) of 80 ± 10% and 14 h photoperiod. Photosynthetic photon flux density (PPFD) was maintained at around 200 ± 20 $\mu mol\ m^{-2}\ s^{-1}$ for all treatments at plant height. Watering was applied every day with automated water sprinklers and the containers were rotated frequently in order to ensure equal growth conditions.

2.2 Morphological and developmental measurements

Height growth rate measurements were conducted during the four-week experimental period (28 days). Specifically, shoot height was measured on a weekly basis with the first measure taking place 7 days after sowing. After the four-week experimental period 10 randomly selected seedlings per cultivar and mini-plug container were sampled. Morphological characteristics such as leaf number, shoot height, root length, leaf area, leaf dry weight (DWL), shoot dry weight (DWS) and root dry weight (DWR) were measured. Additionally, root:shoot dry weight ratio (R/S) was estimated. The samples were dried in a drying oven for three days at 80 °C before weighing and the leaf area (cm^2) of every plant was measured on fresh leaves by leaf area meter LI-3000C (LI-COR biosciences, Lincoln, USA).

After four weeks six randomly selected seedlings for each treatment were transferred in containers placed on top of stainless steel boxes (35 cm × 26 × cm × 8 cm) having a 1:1 mixture of peat and sand and placed in a water tank in order to assess Root Growth Capacity (RGC) (Mattsson 1986). The water tank is used in order to maintain a stable temperature (20±1 °C) for the root system. Environmental conditions in the RGC room were set at 20°C temperature; air relative humidity (RH) of 60 ± 10% and 14 h photoperiod. Photosynthetic photon flux density (PPFD) was maintained at around 300 μmol m^{-2} s^{-1} for all treatments at plant height. Watering was applied every two days. Seedlings were harvested after 31 days. The characteristics measured were new root length (NRL) and dry weight of new roots (NDWR) after a three-day stay in a drying oven at 80 °C. The shoot height and root length, as well as the NRL were calculated with a Powerfix (Milomex, Pulloxhill, UK) digital caliper.

2.3 Quantification of total phenolic content (TPC)

Four weeks after initial germination, basil shoots and leaves were stored in polyethylene bags at -80 °C until processed. Specifically, five samples of shoots and leaves from four-five seedlings per cultivar and treatment were used (1 g fresh basil per treatment). TPC of the extracts was measured using the Folin-Ciocalteau colorimetric assay (Singleton and Rossi

1965) with gallic acid as calibration standard, by a UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA). Shoots and leaves weighing 1 g were ground using a mortar and pestle and then extracted into 10 mL of 80% aqueous methanol. This was followed by centrifugation at 16,000 g for 15 min and at 4 °C. Aliquot of 2.5 mL of Folin-Ciocalteau's reagent was added and the mixture was vortexed for 20-30 s. Aliquot of 2 mL of 7.5% sodium carbonate solution was added after 1 min and the mixture was then vortexed for 20-30 s. Samples were incubated in a thermoblock for 5 min at 50 °C. The absorbance of the colored reaction product was measured at 760 nm versus a blank consisting of 500 μL of methanol, 2.5 mL of Folin- Ciocalteau's reagent and 2 mL of 7.5% aqueous sodium carbonate. The TPC in the extracts was calculated from a standard calibration curve obtained with different concentrations of gallic acid (correlation coefficient: R^2= 0.998) and the results were expressed as mg of gallic acid equivalent per g (mg GAE/g) of fresh basil.

2.4 Statistical analysis

Statistical analysis was performed using SPSS (SPSS 15.0, SPSS Inc.). Growth rate data were analyzed using repeated measures. After the four-week experimental period data were analyzed by analysis of variance (ANOVA). Mean comparisons were conducted using a Bonferroni test at a = 0.05.

3. Results

3.1 Growth rate

The development and morphogenesis of the two cultivars were variably affected by the different light conditions. On the 7th day after sowing, LL seedlings grown under the Control and G2 lights had significantly higher growth rate compared to the rest of the treatments. Furthermore, AP67 showed significantly greater values than AP673L treatment. On the 14th

day, both NS1 and AP673L LEDs showed significantly lower growth rate than G2 and AP67 lights. On the 21^{st} day, the Control and G2 lights exhibited significantly higher values compared to AP673L. No significant differences were found on the 28^{th} day between the light treatments (Fig. 1A).

On the 7^{th} day after sowing, RR seedlings grown under NS1 and AP673L lights had significantly lower growth rate compared to the Control and AP67 light treatments. On the 14^{th} day, G2 exhibited higher growth rate than the Control, NS1 and AP673L light treatments. No significant differences were observed on the 21^{st} day with the Control showing the lowest average values among all the light treatments. However, on the 28^{th} day, significant differences were found only between NS1 that exhibited the lowest values, and AP67 light treatment (Fig. 1B).

3.2 Number of leaves, shoot height, root length, root:shoot ratio and leaf area

At day 28, leaf number of both cultivars was not affected by the different light treatments, with LL and RR forming around 4 and 5 leaves respectively (data not shown). Shoot height of LL was variably affected by the different light treatments. Seedlings grown under the Control, G2 and AP67 exhibited significantly higher shoots compared to AP673L and NS1 treatments. Regarding the root length, significant differences were found only between AP673L that exhibited the highest average value and NS1 treatment (Table 2). As for RR, significantly higher shoots were observed under G2 compared to AP673L, Control and NS1 lights, while AP67 promoted higher shoots than AP673L light. Pre-cultivation of basil seedlings showed no significant effect on the root length at any of the different light treatments (Table 2). For the LL seedlings the results revealed significant differences for R/S ratio between NS1 that showed the greatest values, and the rest of the light treatments. Furthermore, the Control showed significantly lower R/S ratio compared to AP67 and AP673L lights (Table 2). The R/S ratio of the RR seedlings was higher under NS1 light compared to the Control and G2. Additionally, AP673L and AP67 lights exhibited significantly greater values than the Control (Table 2). Regarding the leaf area, it is reduced by LED light independently from spectra and cultivar tested. LL had significantly greater leaf area under the Control compared to all other treatments (Table 2). As for the leaf area of RR seedlings, the Control fluorescent lamp promoted greater leaves' formation compared to AP673L and AP67 lights (Table 2).

3.3 Dry weights (DW)

For LL seedlings, there were significant differences regarding the DWL only between AP67 that showed the greatest values and the Control. G2 induced the highest values for the DWS compared to the Control, AP673L and NS1 light treatments. DWR of *Ocimum basilicum* seedlings benefited under the LEDs, especially under NS1 light that induced the heavier roots compared to the rest of the treatments. Furthermore, the Control induced lighter roots compared to AP673L, G2 and AP67 lights (Fig. 2A). Finally, total biomass was significantly lower under the Control compared to AP67, G2 and NS1.

The results showed significant differences between the light treatments for the DWL of RR. NS1 light induced heavier leaves than the Control and AP673L lights. As for the DWS, plants grown under G2 and AP67 lights exhibited greater values than the Control and AP673L lights, while the Control also induced lighter shoots than NS1. NS1 also promoted greater DWR compared to the Control, AP673L and G2 lights. In addition, the Control also induced lighter roots than AP67, G2 and AP673L lights (Fig. 2B). Total biomass was significantly greater under NS1 compared to the Control and AP673L treatments. In addition, the Control induced the formation of significantly less total biomass compared to G2 and AP67 light treatments.

3.4 Total phenolic content (TPC)

Different light qualities after the cultivation of LL into the growth chambers resulted in significantly higher TPC for seedlings grown under NS1 compared to all other treatments. Further, the Control showed lower TPC values compared to AP673L, AP67 and G2 lights. Finally, G2 light induced significantly less total phenolics than AP673L and AP67 lights. The results of RR showed significantly lower TPC for the Control compared to the rest of the treatments. Additionally, NS1 light exhibited significantly higher TPC than G2 light (Fig. 3).

3.5 Root Growth Capacity (RGC)

After 31 days of cultivation LL seedlings exhibited no significant differences in the NRL formation at any of the different light treatments. The measurements also revealed significantly lower NDWR for seedlings grown under NS1 compared to those grown under the Control and AP673L (Fig. 4A). For the RR seedlings, after 31 days in the RGC water tankNS1 induced significantly lower NRL than AP67, Control and AP673L. Additionally, roots of seedlings pre-cultivated under NS1 were found significantly lighter compared to G2, AP673L and AP67. Finally, Control seedlings formed significantly lighter new roots compared to G2 and AP673L (Fig. 4B).

4. Discussion

Light spectrum strongly affects plant growth and development (Whitelam and Halliday 2007). A great deal of different spectral distributions has been applied up to now in confined environments in order to characterize the effect of LEDs on numerous plants (Li and Kubota 2009; Johkan et al. 2010; Chen et al. 2014; Fraszczak et al. 2014). In general, the cultural conditions applied during this experiment for basil proved appropriate, as it was confirmed by the absence of morphological and developmental abnormalities during plant growth in the mini-plug containers. In both cultivars, AP673L and NS1 (the treatments with high R:FR ratio) induced the lowest growth rate resulting in the formation of the shortest shoots. The seedlings of the two cultivars grown under the same light treatments exhibited different responses, indicating that shoot height is species and/or cultivar dependent. Higher shoots were formed under G2 and AP67 (the treatments with low R:FR ratio) for both cultivars. It is no surprise that R and FR light have such effects as it has been reported that R light via R:FR ratio on phytochrome affects stem elongation, leaf area, germination and photomorphogenic responses of plants (Hogewoning et al. 2010; Sager and McFarlane 1997). Earlier work has shown that high R:FR ratio reduced dry weight, plant height and leaf area compared to natural light in chrysanthemum, tomato, and lettuce (Mortensen and Strømme 1987), as well as in begonia and campanula (Mortensen 1990). Later work has shown that sweet basil grown for 28 days under white LED induced shorter plants compared to FL lamps (Fraszczak et al. 2014). In lettuce plants, R LED lighting induced longer stems compared to the B, RB LEDs and the FL treatment (Chen et al. 2014). On the contrary, rapeseed plantlets showed no differences regarding the aforementioned parameter. However, in the same experiment rapeseed plantlets grown under RB LED (B:R=3:1 and B:R=1:1) exhibited greater root length values than plants cultivated under R LED and under FL light (Li et al. 2013).

Both for LL and RR, seedlings grown under the Control formed less total biomass compared to G2, AP67 and NS1 (treatments with relatively high R light portion) LEDs. Rapeseed plants grown under BR LED (B:R=3:1 and B:R=1:1) also exhibited greater total biomass, while FL promoted the lightest dry plants (Li et al. 2013). In roses and chrysanthemums increasing R light ratio resulted in greater biomass and taller plants (Ouzounis et al. 2014a). For both cultivars, G2 followed by the AP67 light recipes (treatments with high R portion and low R:FR ratio) promoted the greatest DWS. FL light after 45 days from sowing also induced the lowest DWS in red leaf lettuce, while the greatest values were found under the B LED and the BR (B:R=1:1) LED lights (Johkan et al. 2010). Furthermore, DWR for both basil cultivars was favored under NS1 light treatment (high R:FR ratio and 1% UV). The results are consistent with red leaf lettuce which also formed greater DWR under the R and the BR (B:R=1:1) LED treatments and lower under the FL light after 45 days from sowing (Johkan et al. 2010). On the contrary, FL light promoted the formation of more DWR in lettuce plants compared to the R, B and RB (R:B=1:1) LED treatments (Chen et al. 2014), as well as compared to the RB LEDs in hydroponically grown lettuce (Lin et al. 2013). In chrysanthemums total biomass was increased with higher B light ratio, whereas in roses more

R light induced the formation of greater total dry weight (Ouzounis et al. 2014a). All these results indicate that plant responses to LED lighting are species and/or cultivar dependent.

For both cultivars, leaf area of the seedlings was benefited under the Control. Studies with hydroponically grown lettuce also revealed greater leaf area for plants cultivated under FL compared to plants grown under RB LED (Lin et al. 2013), whereas cool white FL lamps induced the formation of smaller leaves compared to RGB treatment but larger compared to the RB LED (Kim et al. 2004). However, sweet basil after 28 days did not exhibit significant differences between the FL and white LED treatments (Fraszczak et al. 2014). In *Phalaenopsis* greater leaf formation was observed with increasing R light (Ouzounis et al. 2014b) which has been also reported for cucumber (Hogewoning et al. 2010). In roses greater leaf area was observed with increasing R light, while in chrysanthemums and campanulas greater values were observed under 20%B/80%R and white light respectively (Ouzounis et al. 2014a). As mentioned in the introduction, R and B lights acting in an independent and/or synergistic manner, affect photomorphogenesis. From the results it appears that leaf area is reduced under LEDs, whereas DWL is increased. This could be the result of higher carbohydrates and primary metabolites accumulation suggesting that LED light affects photosynthesis activity. Previous studies have shown that combinations of R and B light are favorable for plant growth and greater carbohydrates production in plants (Yorio et al. 2001; Ouzounis et al. 2014b).

Seedlings grown under low irradiation elongate quickly and form compact root system that does not absorb sufficient water and nutrients leading to plant growth decrease. Therefore, seedlings with low R/S ratio are not suitable for active growth (Johkan et al. 2010). Our results suggest that seedlings grown under the Control form inadequate roots compared to the plants grown under the rest of the treatments. In general, LEDs that emit more B light promoted greater R/S ratio indicating that B light affects this parameter. B light is reported to suppress stem elongation leading to more compact plants (Folta and Childers 2008). Results of R/S ratio in the literature are contradicting. Lettuce plants treated with FL showed greater S/R dry weight ratio (lower R/S) (Johkan et al. 2010) compared to the LED treatments, whereas in other studies with lettuce, RB (R:B=1:1) LED treatment promoted greater S/R dry weight ratio (lower R/S) compared to the FL light (Chen et al. 2014; Lin et al. 2013).

It has been reported that both morphological characteristics and RGC parameters are species dependent (Kostopoulou et al. 2010). A careful selection of quality seedlings is important for an increase of the survival rate and the growth rate after transplanting (Radoglou 2001). Further, selecting high quality seedlings can potentially mitigate the harmful effects of stressful environmental conditions resulting in successful establishment in the field, while using low quality seedlings can lead to unsuccessful transplanting even under favorable environmental conditions (Radoglou et al. 2009). The potential for new root growth is critical for a rapid absorbance of water and minerals after transplanting. RGC is a performance feature for quality assessment (Mattsson 1996, 1986) and can be defined as the ability of a seedling to increase the size of its root system through the formation of new roots and the elongation of already present roots (Mattsson 1986). Even though the NS1 light regime (relatively high B portion with the highest R:FR ratio and 1% UV) promoted the formation of heavier roots compared to the other light qualities for both cultivars, this was not the case after the RGC transplant, with NS1 seedlings having less new root biomass for both cultivars. The quick root system development under the effect of the Control and AP673L for LL basil, and mainly under AP673L for RR basil may offer the seedlings an advantage regarding the fast exploitation of larger soil amount after transplanting.

The highest TPC was obtained under NS1 light quality (relatively high B portion with the highest R:FR ratio and 1% UV) which was 3.9 times higher and 3.7 times higher than the Control's for the LL and the RR respectively. In plant tissues, phenolic compounds are reported to act protectively against UV radiation (Lattanzio et al. 2006), which might explain the increased total phenolic formation under NS1 light regime. Previous studies have shown that secondary metabolites are increased with additional B light, even though the B LED has been applied only as supplemental light (Ouzounis et al. 2014a), while secondary metabolites were reportedly enhanced in carrots and grapes under UV-B radiation (Gläβgen et al. 1998; Pezet et al. 2003). Our results indicate that LED applications with high B light portion create a physiological state that induces the accumulation of phenolic compounds. As far as concern the mechanism behind the increase of secondary metabolites under B light, it has been reported that the activity of phenylalanine ammonia-lyase (PAL, a key enzyme in the phenylpropanoid pathway) was stimulated (Heo et al. 2012). Moreover, PAL gene expression was activated by monochromatic B LED lighting in lettuce (Son et al. 2012). However, there are also contrasting examples in the literature, indicating that the amount of secondary metabolism under artificial lighting is light and species dependent. For example, no essential difference was found in pigment content in *Dieffenbachia amoena*, *Ficus elastica* and Boston lettuce (Heo et al. 2010; Martineau et al. 2012). Consequently, the production of phenolic compounds depends concurrently on the light environment, the physiological, and biochemical factors.

5. Conclusion

Two *Ocimum basilicum* cultivars (LL and RR) were grown under artificial LED and FL lighting. For both cultivars, total biomass was increased under LED lighting, while root:shoot ratio was favored under NS1 (high blue, high red:far-red and 1% UV) in comparison with fluorescent lighting. Leaf area was favored by the Control in both LL and RR. The treatments with high red and high red:far-red (AP673L) and moderate blue and red with low red:far-red (AP67) triggered greater new root length compared to NS1, but only in RR. Total phenolic content was also higher in treatments enriched with blue light. For both cultivars, root growth capacity was variably affected by the different light treatments. Our study demonstrates that artificial LED lighting enhanced (even though variably) the growth and increased total phenolic content of two *Ocimum basilicum* L. cultivars compared to fluorescent light, but the effects were cultivar dependent.

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Tables

Table 1. Spectral distribution and red:far-red (R:FR) ratio for the five light treatments

Light treatments	< 400 nm	400-500 nm	500-600 nm	600-700 nm	700-800 nm	R:FR
Control (FL)	0%	35%	24%	37%	4%	5.74
AP673L	0%	12%	19%	61%	8%	5.56
G2	0%	8%	2%	65%	25%	2.51
AP67	0%	14%	16%	53%	17%	2.77
NS1	1%	20%	39%	35%	5%	8.16

Table 2. Morphological and developmental parameters of *Ocimum basilicum* "LL" and *Ocimum basilicum* "RR" grown under the five different light treatments with the same abbreviations as in Table 1. Average values (n=10, ±SE) followed by different letters within a row differ significantly (a= 0.05).

Cultivars	Parameters	Light treatments				
		Control (FL)	**AP673L**	**G2**	**AP67**	**NS1**
Ocimum basilicum "LL"	**Shoot height (cm)**	3.22 ± 0.19a	2.34 ± 0.26b	3.52 ± 0.16a	3.42 ± 0.33a	2.43 ± 0.13b
	Root length (cm)	4.03 ± 0.12ab	5.69 ± 0.67a	4.50 ± 0.26ab	4.52 ± 0.36ab	3.73 ± 0.34b
	Root/Shoot ratio	0.09 ± 0.01c	0.29 ± 0.02b	0.26 ± 0.03bc	0.35 ± 0.09b	0.57 ± 0.04a
	Leaf area (cm^2)	10.02 ± 0.39a	6.56 ± 0.54b	6.56 ± 0.38b	6.91 ± 0.64b	5.70 ± 0.38b
Ocimum basilicum "RR"	**Shoot height (cm)**	4.09 ± 0.25bc	3.50 ± 0.28c	5.46 ± 0.22a	5.08 ± 0.28ab	3.93 ± 0.30bc
	Root length (cm)	4.59 ± 0.44a	6.23 ± 0.42a	4.42 ± 0.37a	4.94 ± 0.36a	6.53 ± 0.78a
	Root/Shoot ratio	0.16 ± 0.03c	0.49 ± 0.11ab	0.34 ± 0.03bc	0.46 ± 0.04ab	0.61 ± 0.04a
	Leaf area (cm^2)	8.25 ± 0.80a	5.59 ± 0.36b	6.70 ± 0.44ab	6.05 ± 0.29b	7.05 ± 0.39ab

Figures

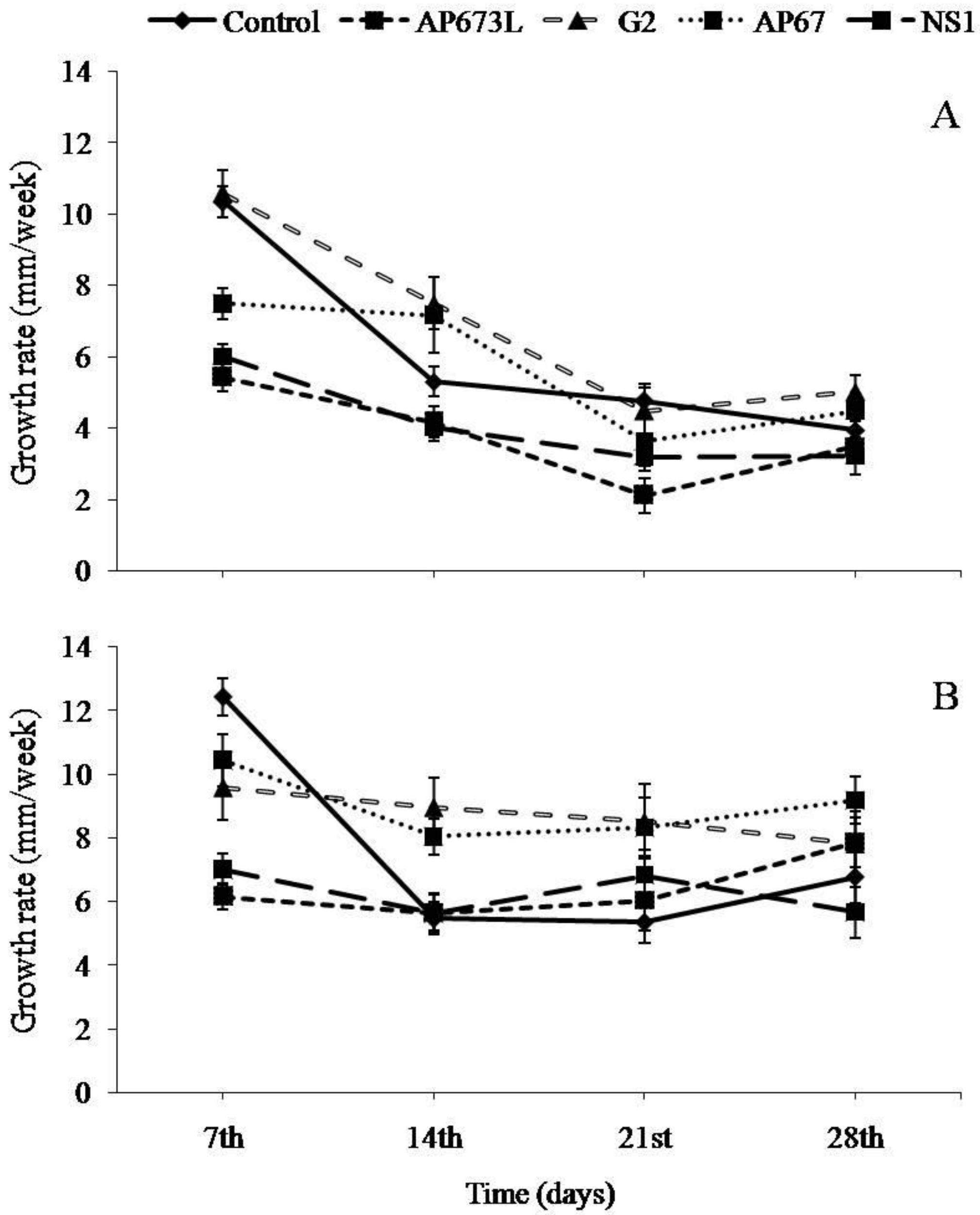

Figure 1. Growth rate from the 7th, 14th, 21st and 28th day of *Ocimum basilicum* "LL" (A) and "RR" (B) seedlings grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE.

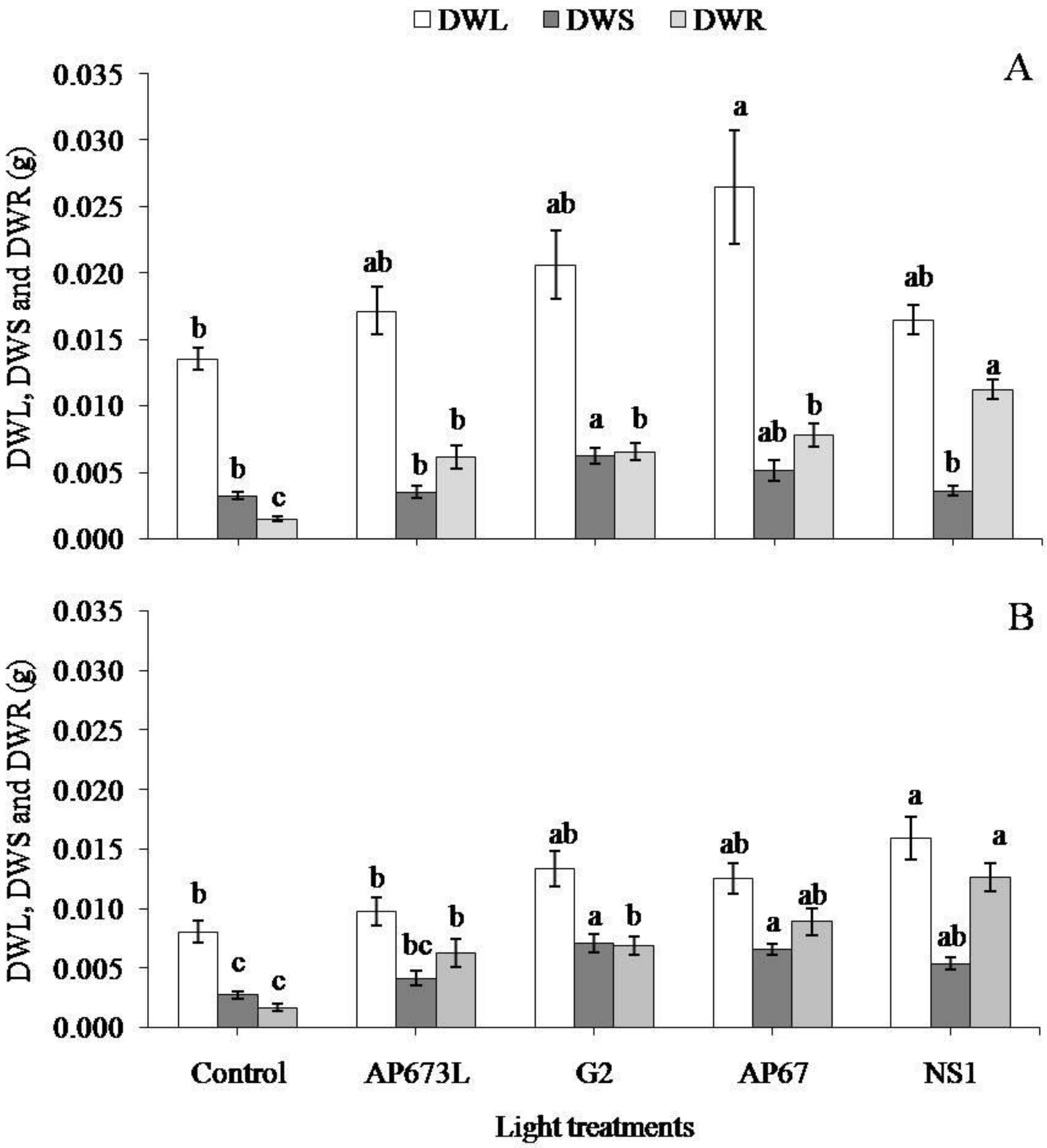

Figure 2. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of *Ocimum basilicum* “LL” (A) and “RR” (B) seedlings grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE. Bars followed by a different letter within a parameter differ significantly (a = 0.05).

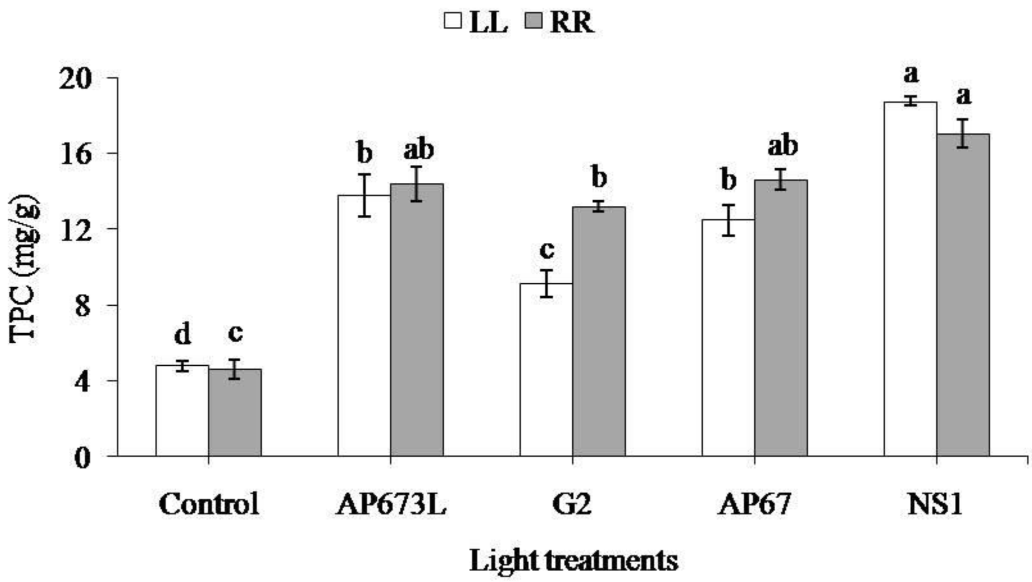

Figure 3. Total phenolic content (TPC) of *Ocimum basilicum* “LL” and “RR” seedlings grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values (n=5) ±SE. Error bars represent the SE. Bars followed by a different letter within a cultivar differ significantly (a = 0.05).

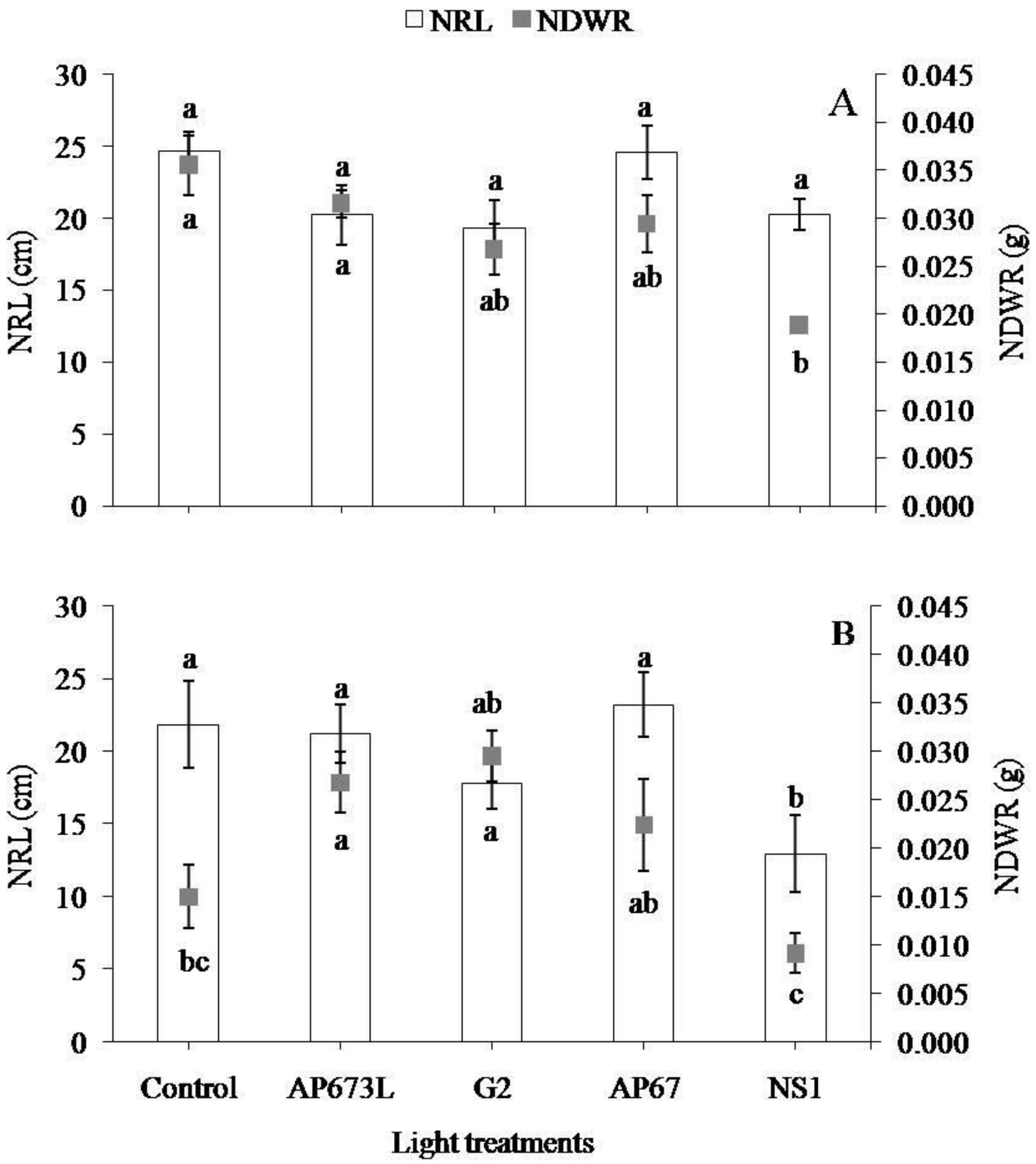

Figure 4. New root length (NRL) and new dry weight of roots (NDWR) of *Ocimum basilicum* "LL" (A) and "RR" (B) seedlings after 31 days in the RGC water bath after grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values (n=6) ±SE. Error bars represent the SE. Bars and lines followed by a different letter within treatments differ significantly (a = 0.05).

Effects of cold stratification and GA_3 on germination of *Arbutus unedo* seeds of three provenances

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Abstract

Background: *Arbutus unedo* is a valuable Mediterranean shrub as an ornamental plant as well as fruit tree. Fresh fruits of *A. unedo* are a good source of antioxidants, of vitamins C, E and carotenoids and also are characterized by the high content of mineral elements.

Materials and Methods: The effects of gibberellic acid (GA_3) and cold stratification (CS) on seed germination performance were investigated in *A. unedo* seeds collected from three provenances in the Northern part of Greece. Seeds of each provenance were soaked in solutions of GA_3 (500, 1000 or 2000 ppm) for 24 h and subsequently were subjected to CS at 3 – 5ºC for 0, 1, 2, and 3 months.

Results: Non-stratified seeds of the three *A. unedo* provenances which were not treated with GA_3 solutions exhibited very low germination. However, seed germination was significantly improved after a one-month period of CS. Similarly, the non-stratified seeds of all three provenances became non-dormant after the treatment with 2000 ppm GA_3 and they germinated at high percentages. However, in untreated seeds with GA_3, after a one-month CS period the seeds of the Pieria provenance exhibited higher germination percentage than that of Rodopi provenance seeds. Furthermore, in non-stratified seeds, the Pieria provenance seeds treated with GA_3 germinated at higher percentages and more rapidly than those of the other two provenances.

Conclusion: The results indicated that untreated seeds exhibited very low germination at 20/25ºC. However, in all three provenances seed germinability was significantly improved by a one-month period of CS or treatment of seeds with 2000 ppm GA_3. Furthermore, there was a considerable variability among seed provenances in response to the treatments which were applied.

Keywords: pre-germination treatment, seed dormancy, strawberry tree, Mediterranean species.

Introduction

The genus *Arbutus* L. (Ericaceae) includes about 12 to 20 species (depending on the author) distributed from the West coast of North America through Mexico and Central America, Western Europe, the Mediterranean region, Northern Africa and parts of the Middle East (Hileman et al., 2001; Torres et al., 2002). According to Hileman et al. (2001), three species of genus *Arbutus* are distributed in the Mediterranean region: *A. unedo* L., *A. andrachne* L. and their hybrid *A.* x *andrachnoides* Link. *Arbutus unedo* is an evergreen species, which usually exists as a shrub of a height up to 2 – 3 m or sometimes a small tree up to 10 - 12 m. In Greece, it is one of the most important components of maquis communities (Boratynski et al., 1992). *Arbutus unedo* is also a valuable species as an ornamental plant due to its attractive white flowers which appear simultaneously with the orange-red fruits in the fall and winter (Celikel et al., 2008) as well as fruit tree. Fresh fruits of *A. unedo* are a good source of antioxidants, of vitamins C, E and carotenoids (Alarcao-E-Silva et al., 2001; Pallauf et al., 2008) and also are characterized by the high content of mineral elements (Ca, K, MG, Na and P) (Ozcan and Haciseferogullari, 2007). Furthermore, the flowers of *A. unedo* are a significant source of nectar for bees (Soro and Paxton, 1999; Dalla Serra et al., 1999). Apart from the economic value of non-timber forest products, the ability of species to grow in dry areas (Hileman et al., 2001) and to vigorously resprout after fire (Espelta et al., 2012) makes it quite suitable for reforestation programs in Mediterranean regions.

Considering the above, in order to introduce the specific species in reforestation programmes, an easy propagation method has to be developed. For many species, propagation from seeds is the most common and the cheapest method used in nurseries (Macdonald, 2006). As a further benefit the genetic diversity is promoted by propagation from seeds. However, a major constraint to the sexual propagation of many species is the poor germination of their seeds. This is possibly due to low viability, although it is frequently due to seed dormancy (Mackay et al., 2002), which is a physiological state during which a viable seed fails to germinate even when the environment is favorable to germination (Macdonald, 2006). However, in the relevant literature there is conflicting information regarding the dormancy of *A. unedo* seeds. Several references report that *A. unedo* seeds show dormancy and that cold stratification is used to overcome it (Tilki, 2004; Smiris et al., 2006; Demirsoy et al., 2010; Ertekin and Kirdar, 2010). Furthermore, the treatment of seeds with GA_3 is effective to break dormancy and increase the germination in *A. unedo* and *A. andrachne* (Karam and Al-Salem, 2001; Tilki, 2004; Demirsoy et al., 2010). In contrast, Ricardo and Veloso (1987), Mesleard and Lepart (1991) and Bertsouklis and Papafotiou (2013) stated that non-stratified seeds of *A. unedo* germinate at high percentage when they are placed at alternating temperatures of 15 and 20ºC or at a constant temperature of 15ºC or less. Although seed dormancy has been studied by numerous researchers, no attention has been given on the variation in germination characteristics among populations of the species. Germination characteristics of seeds collected from different populations can vary in degree of dormancy, germination rates, environmental conditions (temperature, substrate moisture, pH, calcium, and salinity) required for germination as well as in the amount of cold stratification required to break dormancy (Baskin and Baskin, 1998). Applying the recommended germination protocol may incur the risk of poor germination, resulting in increased production cost when the variability in seed germination requirements among provenances is not taken into account.

The present study aims to evaluate the effect of gibberellic acid (GA_3) and cold stratification treatments (and their combinations) on germination of *A. unedo* seeds and also to reveal the existence of variability in germination of three *A. unedo* provenances by evaluating the germination characteristics (germination percentage and rate) of seeds subjected to cold stratification and gibberellic acid (GA_3) treatments (and their combinations).

Materials and Methods

Seed collection

Mature fruits of *A. unedo* were collected from shrubs growing in natural habitats from three provenances. In particular, fruits were collected from three prefectures located in the Northern part of Greece (Rodopi, Chalkidiki, Pieria) (Table 1). After collection, the fruits were pulped by hand and the separation of seeds from pulp was achieved using sieves and running water. In addition, floated seeds were removed during cleaning, and subsequently, the clean seeds were spread out on filter papers in laboratory conditions and left to dry for a week. After drying, the moisture content of seeds as well as the number of seeds per grammar were calculated for each provenance (Table 1) according to the rules of ISTA (1999) and then the seeds were stored in glass containers in the refrigerator (3 - 5ºC) until they were used in the experiments.

Seed treatment

Germination experiments were started the following February and conducted in the laboratory of Silviculture, Department of Forestry and Management of the Environment and Natural Resources, Democritus University of Thrace.

For each provenance, an experiment was carried out to determine the effects of gibberellic acid (GA_3), cold stratification (CS) and combination of GA_3 with CS on seed germination. Seeds of each provenance were soaked in solutions of GA_3 for 24 hours. The concentrations of GA_3 solutions were 500, 1000 and 2000 ppm. Subsequently, the treated seeds were placed between two moist layers of filter paper in plastic containers and given CS at 3 - 5ºC for 0, 1, 2, and 3 months. In addition, seeds from each provenance were soaked in distilled water for

24 hours (control) and then were subjected to CS for 0, 1, 2, or 3 months. For each provenance there were four plastic containers (3 of them corresponded to the three concentrations of GA_3 and one to control seeds). During stratification, filter papers moisture was checked periodically and water was added as needed.

Germination test

For each provenance, at the end of each CS period, a random sample of 120 seeds were taken out from each plastic container and randomly placed in 4 plastic Petri dishes (30 seeds per Petri dish). For each treatment, there were 4 replications of 30 seeds. Seeds were placed on two layers of filter paper moistened with distilled water in 9-cm plastic Petri dishes. The Petri dishes were randomly arranged on the shelves of the growth chamber and were watered with distilled water, as necessary. The temperature in the growth chamber was set at 20ºC for a 16-hour dark period and 25ºC for a 8-hour light period. Germinated seeds were counted once a week for a period of 6 weeks. A seed was considered as germinated when the radicle had emerged through the seed coat. Finally, for each treatment of each provenance the germination percentage (GP) and the mean germination time (MGT) were calculated as the average of the 4 replications. The MGT was calculated for each replication per treatment according to the following equation:

$$MGT = \Sigma(Dn)/\Sigma n$$

where *n* is the number of seeds which germinated on day *D* and *D* is the number of days counted from the beginning of the test (Hartmann et al., 1997).

Statistical analysis

For each provenance, a completely randomised experimental design was used. It is worth noting that, in all three provenances a CS period longer than one month was not used as, at the end of the two-month period of CS, germinated seeds appeared. In each provenance, the germination percentage data, which were firstly arc-sine square-root transformed (Snedecor and Cochran, 1980), as well as MGT data were analysed by one-way ANOVA. Furthermore, in each treatment (combinations of GA_3 and CS treatment) the germination percentage data as well as MGT data of the three provenances were analysed by one-way ANOVA. The means were compared using the Duncan test (Klockars and Sax, 1986). All statistical analyses were carried out using SPSS 21.0 (SPSS, Inc., USA).

Results

The number of seeds per gram was 510 at 5.07% moisture content for Rodopi provenance, 441 at 5.51% for Chalkidiki provenance and 405 at 5.05% for Pieria provenance (Table 1).

In all three provenances, the analyses of variance indicated that there were significant differences in germination percentages as well as in MGT (a=0.05) among the combinations of GA_3 concentrations and CS periods (Tables 2 and 3).

In all three provenances, non-stratified seeds of *A. unedo* which were not treated with GA_3 solutions exhibited very low germination (0.83 – 7.50%). In Rodopi and Chalkidiki provenances, regardless of GA_3 treatment, seeds stratified for 1 month exhibited higher ($p < 0.05$) GPs than that of non-stratified seeds. Whereas, in Pieria provenance, in seeds only treated with GA_3 solutions, there were no significant differences ($p > 0.05$) in GPs between non-stratified seeds and seeds stratified for 1 month. In all three provenances, GA_3 application significantly improved the germination of non-stratified seeds. Furthermore, in Rodopi and Chalkidiki provenances increasing the concentration of GA_3 resulted in a significant increase ($p < 0.05$) in GPs of non-stratified seeds. In Pieria provenance, non-stratified seeds which were treated with 2000 ppm exhibited the highest ($p < 0.05$) GP, whereas there were no significant differences ($p > 0.05$) in GPs between seeds treated with 500 and 1000 ppm. After a one-month period of CS, the germination percentage of seeds of Rodopi provenance which had been treated with 500 and 1000 ppm GA_3 was higher ($p < 0.05$) than that of the seeds which had not been treated with GA_3, whereas in Chalkidiki provenance the seeds treated with GA_3, regardless of concentration, exhibited higher ($p < 0.05$) GPs than those not treated with GA_3. In contrast, after a one-month CS period of Pieria provenance seeds, untreated seeds with GA_3 exhibited higher ($p < 0.05$) GP than those treated with 500 ppm GA_3, whereas there were no significant differences with seeds treated with 1000 or 2000 ppm.

In all three provenances, the seeds stratified for 1 month, regardless of GA_3 treatment and concentration, exhibited the lowest ($p < 0.05$) MGT. Only in Rodopi provenance the MGT of non-stratified seeds was affected by concentration of GA_3. Seeds treated with 1000 and 2000 ppm exhibited lower ($p < 0.05$) MGT than those treated with 500 ppm.

Statistical analysis also revealed significant differences in GPs and MGT among the three provenances (Tables 2 and 3). In non-stratified seeds which were treated with GA_3, regardless of concentration, Pieria provenance exhibited the highest ($p < 0.05$) GPs and the lowest ($p < 0.05$) MGT. After 1 month of CS, in seeds which were not treated with GA_3, Pieria provenance exhibited higher ($p < 0.05$) GP than the Rodopi provenance, whereas in seeds treated with 500 mg l^{-1} Pieria provenance exhibited lower ($p < 0.05$) GP than the other two provenances. In seeds treated with 1000 and 2000 ppm which were stratified for 1 month, Chalkidiki provenance exhibited the highest ($p < 0.05$) GPs.

As regards MGT, in seeds stratified for 1 month, regardless of GA_3 treatment and concentration, no significant differences ($p > 0.05$) were observed among the three provenances.

Discussion

Non-stratified seeds of the three *A. unedo* provenances, which were not treated with GA_3 solutions, exhibited very low germination (0.83 – 7.50%). The same results were also provided by Tilki (2004), Demirsoy et al. (2010), Ertekin and Kirdar (2010), who found very low germination percentages. In the present study, as in the studies of the above researchers, the seeds germinated in a temperature range between 18 and 25°C. Ricardo and Veloso (1987) and Bertsouklis and Papafotiou (2013) found that non-stratified seeds of *A. unedo* germinated at percentages about 40 and 30%, respectively when they were placed in constant temperature at 20ºC, while in both studies the seeds failed to germinate at 25ºC. However, in their experiment, non-stratified seeds germinated at very high percentage (> 80%) when they were placed at temperature equal or lower than 15ºC. Perhaps, *A. unedo* seeds are conditionally dormant and they germinate over a narrow range of low temperatures (10 – 15ºC).

For all three provenances, seed germination was significantly improved by cold stratification treatment. In untreated seeds with GA_3, after a one-month period of CS, the germination percentages of seeds of all three provenances were very high (> 80%). Although, Tilki (2004) reported that a six-week period of CS resulted in 50% germination and increasing CS duration to 9 or 12 weeks increased germination of *A. unedo* seeds (86 and 84%, respectively). Furthermore, Smiris et al. (2006) referred that increasing CS duration from 1 to 2 and 3 months increased germination of *A. unedo* seeds from 0 to 26 and 48%, respectively. In the present study, the response to longer period of CS (2 months) was similar for all three provenances, many seeds germinated during the moist stratification at 3 – 5ºC. After a CS treatment, seeds came out of dormancy and they germinated over a wide range of temperatures. According to Baskin and Baskin (1998) non-dormant seeds germinate over a wider range of conditions than do conditionally dormant seeds. Similarly, after the treatment with 2000 ppm GA_3 the seeds of all three provenances became non-dormant and they germinated at high percentages (≥ 80%) at 20/25ºC. Possibly, the treatment of seeds with a high concentration solution of GA_3 counteracted the inhibitory effect of high temperatures on germination. According to Ricardo and Veloso (1987), *A. unedo* seeds treated only with 500 ppm GA_3 germinated at percentages about 90% and 50% at 20ºC and 25ºC, respectively. Seeds of *Kalidium gracile*, which exhibit primary conditional dormancy, become non-dormant by a short period of CS or by GA_3 treatment (Cao et al., 2014). Furthermore, the CS, as well as the exogenous GA_3 application, has been reported to be effective in breaking dormancy in the seeds of *Arbutus* species (Karam and Al-Salem, 2001; Tilki, 2004; Smiris et al., 2006; Demirsoy et al., 2010; Ertekin and Kirdar, 2010). As far as the germination rate is concerned, the CS for 1 month of seeds, regardless of GA_3 treatment, resulted in the most rapid germination (the lowest values in MGT) for all three provenances. In practice, apart from high seed germination, uniform and rapid seed germination is also significant in order to avoid environmental hazards in the nursery. Thus, for propagation purposes a CS period of seeds is recommended as an effective treatment for maximum, rapid and uniform germination of *A. unedo* seeds over a wide range of temperatures.

The CS, as well as the GA_3 application, significantly improved the germination of the three seed provenances of *A. unedo*. However, there was a considerable variability among seed provenances in response to treatments which were applied. In untreated seeds with GA_3, after a one-month CS period the seeds of the Pieria provenance exhibited higher GP than those of Rodopi provenance seeds. Germination characteristics of seeds collected in various locations can vary in degree of dormancy, as reflected by GPs of fresh seeds (Baskin and Baskin 1998). As mentioned, in all three provenances a CS period longer than one month was not used as germinated seeds appeared at the end of the two-month period of CS. The germination percentage of Rodopi provenance seeds may be higher if a longer period of CS is used, for example one and half months. The effects of GA_3 application on seed germination varied among the three provenances. The results indicated that in non stratified seeds, the Pieria provenance seeds treated with GA_3, regardless of concentration, germinated at higher percentages and more rapidly (lower values in MGT) than those of the other two provenances.

The GA_3 application in seeds of Pieria provenance counteracted in greater degree the inhibitory effect of high temperatures on germination, than in seeds of the other two provenances. Whereas, in seeds stratified for 1 month, the Pieria provenance seeds treated with GA_3, regardless of concentration, exhibited lower GP than those of Chalkidiki provenance. Taking into account the results of the present and previous studies, variability in germination requirements due to provenance of *A. unedo* seeds is observed. Smiris et al. (2006) studying the germination of *A. unedo* seeds, which were collected in Western Greece, reported that the maximum percentage of germination (85.75%) was observed when the seeds were treated for 24 hours with 500 ppm GA_3 and then cold stratified for a period of 3 months. Furthermore, Bertsouklis and Papafotiou (2013), in *A. unedo* seeds collected in South Greece found that seeds stratified for 40 days germinated at about 80 and 15%, when they were placed in constant temperatures at 20 and 25ºC, respectively. This variability in germination of *A. unedo* seeds among provenances may reflect adaptations to differing environmental conditions. Similarly, Ricardo and Veloso (1987) observed variability in seed germination in high temperature among three provenances of *A. unedo* seeds from South Portugal.

Conclusions

Based on the results of the present research, it can be concluded that untreated seeds of the three *A. unedo* provenances exhibited very low germination at 20/25ºC. However, seed germination was significantly improved after a one-month period of CS. Similarly, the treatment of seeds only with 2000 ppm GA_3 was successfully overcome the dormancy in *A. unedo* seeds. There was a considerable variability among seed provenances in response to the treatments which were applied. In untreated seeds with GA_3, after a one-month stratification period the seeds of the Pieria provenance exhibited higher germination percentage than that of Rodopi provenance seeds. Furthermore, in non-stratified seeds, the Pieria provenance seeds treated with GA_3, regardless of concentration, germinated at higher percentages and more rapidly than those of the other two provenances.

In reforestation programs, emphasis is given in the use of local provenances as they have adapted to local environments. So, in an untested seed provenance of *A. unedo*, considering the variability in germination requirements among provenances of the species, in order to maximize seed germination the best duration of CS and GA_3 concentration have to be determined first on a small sample before treating all the seed lot.

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Table 1. Provenances of the collected *A. unedo* seeds.

Provenances	Altitude (m a.s.l)	Latitude	Longitude	Collection date	Number of seeds/g	Moisture content (%)
Rodopi	255	41º08´38´´N	25º15´35´´E	25/11/2013	510	5.07
Chalkidiki	15	40º35´13´´N	23º47´43´´E	11/12/2013	441	5.51
Pieria	500	40º11´26´´N	22º19´25´´E	7/12/2013	405	5.05

Table 2. Germination percentages of *A. unedo* seeds of the three provenances, after various combinations of GA_3 and cold stratification treatments

GA_3 (ppm)	Cold stratification (months)	Germination percentage (%, ± S.D.)		
		Rodopi	Chalkidiki	Pieria
0	0	0.83 f[1] **b**[2] ± 1.67	3.33 e **ab** ± 4.71	7.50 d **a** ± 4.19
	1	81.67 bc **b** ± 1.92	88.34 b **ab** ± 1.92	90.83 a **a** ± 5.69
500	0	49.17 e **b** ± 4.19	40.00 d **c** ± 4.71	79.17 c **a** ± 5.00
	1	91.67 a **a** ± 1.92	94.17 a **a** ± 5.00	81.67 bc **b** ± 6.38
1000	0	68.33 d **b** ± 6.39	67.50 c **b** ± 1.67	80.84 c **a** ± 6.31
	1	88.34 a **b** ± 3.33	95.84 a **a** ± 1.67	83.34 abc **b** ± 6.09
2000	0	80.00 c **b** ± 4.71	80.83 b **b** ± 5.69	90.00 ab **a** ± 6.09
	1	86.67 ab **b** ± 2.72	97.50 a **a** ± 1.66	85.83 abc **b** ± 3.19

[1] In a column, percentages are statistically different at $p < 0.05$, when they don't share a common letter (letters in normal font). [2] In a row, percentages are statistically different at $p < 0.05$, when they don't share a common letter (letters in bold font). The comparisons were made using Duncan test.

Table 3. Mean Germination Time of *A. unedo* seeds of the three provenances, after various combinations of GA_3 and cold stratification treatments

GA_3 (ppm)	Cold stratification (months)	Mean Germination Time (days, ± S.D.)		
		Rodopi	Chalkidiki	Pieria
0	0	*	*	14.00 b ± 4.95
	1	8.71 a[1] **a**[2] ± 0.51	8.84 a **a** ± 0.55	8.24 a **a** ± 0.54
500	0	22.81 c **b** ± 1.59	20.98 b **b** ± 1.94	15.57 b **a** ± 0.53
	1	9.47 a **a** ± 0.80	9.35 a **a** ± 0.55	9.55 a **a** ± 0.81
1000	0	20.29 b **b** ± 0.65	19.98 b **b** ± 2.19	16.03 b **a** ± 0.81
	1	8.13 a **a** ± 0.72	8.27 a **a** ± 0.63	8.20 a **a** ± 0.57
2000	0	21.16 b **b** ± 1.57	20.39 b **b** ± 1.86	16.12 b **a** ± 1.08
	1	9.43 a **a** ± 0.70	8.56 a **a** ± 0.80	8.65 a **a** ± 0.83

* MGT was not calculated because in one of the four replications, no seed germinated. [1] In a column, means are statistically different at $p < 0.05$, when they don't share a common letter (letters in normal font). [2] In a row, means are statistically different at $p < 0.05$, when they don't share a common letter (letters in bold font). The comparisons were made using Duncan test.

Title(s)

Stereo-vision image-based phenotyping for non-destructive analysis of tree seedlings growth

Authors

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Abstract

Plant phenotyping is the identification of effects on the phenotype as a result of genotype differences and the environment. Plant biomass variability is considered as environmentally-induced phenotypic variation in plants. Destructive direct method to measure plant biomass has become the major bottleneck for quantitative analysis of a large number of plants. In the present study a simple and economically affordable automated system was developed to monitor containerized tree seedlings biomass from seed germination to five weeks growth. Stereoscopic RGB images were taken and a new software for image analysis was developed. Results showed that the developed system is accurate enough to automatically monitoring seedlings growth. The best regression model to explain the relationship between direct biomass data and indirect measurements was based on parameters such as plant height for needle-leaved species and plant greenness for broad-leaved species. Finally, image analysis revealed information on the early seedlings developmental stage.

Introduction

Plant phenotype refers to an integrated function of morphological, ontogenetical, physiological and biochemical properties (Gratani et al. 2014). Plant phenotyping is the identification of effects on the phenotype (i.e., the plant appearance and behavior) as a result of genotype differences (i.e., differences in the genetic code) and the environment. In particular, variability in plant biomass partitioning may be considered as environmentally-induced phenotypic variation in plants (Coleman et al. 1994; Chiatante et al. 2015). Apart from the importance of root-growth analysis, a key descriptive parameter in plant physiology is the growth of plant shoots. Although there are numerous secondary traits describing the morphology of shoots in particular species and their developmental stages, the primary and universal trait is biomass formation. Plant biomass is defined as the total mass of all the above and below -ground plant parts at a given point in a plant's life [Reference]. This trait assessment involves the destruction of the measured plant thus only allowing end-point analyses. Similarly, leaf area and consequently the plant growth rate are usually determined by manual measurements of the dimensions of plant leaves [9-11]. Previously, the process of taking phenotypic measurements has been manual, costly, and time consuming, representing the bottleneck of large-scale measurement of plant traits. Today, rapid developments are taking place in the field of non-destructive, image-based method for phenotyping measurements that enable for a characterization of plant traits. These approaches use precise and sophisticated tools and methodologies to study plant growth and development. The most used measuring tool is RGB imaging, or integrative phenotyping, signifying two or more measuring tools. Similarly, leaf area and consequently the plant growth rate are usually determined by manual measurements of the dimensions of plant leaves [9-11]. Such measurements are highly time consuming and thus cannot be used for large scale experiments. For this reason, plant phenotyping facilities prefer to evaluate the growth rate using imaging methods which employ digital cameras with subsequent software image analysis. This enables a faster and more precise determination of both plant biomass growth and leaf expansion. In general, non-invasive techniques of shoot growth determination have proven very reliable, and high correlations between the digital area and the shoot fresh, or dry weights, respectively. Similarly, other common morphometric parameters such as stem high, number of tillers and inflorescence architecture can be assessed non-destructively and manually, but again the time requirements, limit the number of plants analysed Correct determination of digital plant growth area can be distorted by overlapping leaves, leaf twisting and curling, and circadian movement, especially when the RGB image is taken only from one view (e.g. from top view).

The objective of the present study was to develop a simple and economically affordable automated system to monitor containerized tree seedlings biomass from seed germination to five weeks growth. Seedlings growth and development of four different tree species -two broadleaved (*Fagus sylvatica* L., *Querculs ilex* L.) and two needle leaved (*Picea abies* L., *Pinus sylvestris* L.)- through measuring various morphological parameters. Further, a new software for automatic RGB image analysis of plant phenotype was developed and data obtained with manual measurements of the shoot height and leaf area were validated through correlation of pixel based plant height and greenness. Finally, data obtained from automated imaging analysis were compared with plant biomass as indicator of plant performance.

Materials and methods

Plant material and growth chamber characteristics

Seeds of four tree species (*Fagus sylvatica* L., *Quercus ilex* L., *Picea abies* L. and *Pinus sylvestris* L.) were provided by the National Forest Service (National Center for Study and Conservation of Forest Biodiversity- Peri, IT), sorted for uniform size and subject to pre-treatment. Seeds of *F. sylvatica* were first hydrated by soaking for 24 hours in tap water; then seeds surface were sterilised with 3,5% household bleach for 2 minutes, and rinsed with sterile water four times to remove all traces of bleach. Afterwards, seeds were treated with "Teldor" fungicide (3 ml in 1 l of sterile water per 10 minutes) and placed under hood for 3 hours in order to improve fungicide adhering to the seed coat. Finally seeds were subject to cold stratification in perlite at 4°C for 2 months. Seeds of *Q. ilex* were hydrated by soaking them for 24 hours in tap water. Seeds were then sowed after hydration period without further pre-treatment. *P. sylvestris* and *P. abies* seeds were directly sown without any pre-treatment. A total of 104 seeds were sown in 4 different mini-plug plastic container trays (QPD 104 VW - 104 cells; 33x33x45 mm; 40 mm/height; 27 cc) (QuickPot by HerkuPlast-Kubern, Germany), containing sterile stabilized peat growing medium Preforma VECO3 (Jiffy® Products). The temperature and humidity settings in the growth chamber are detailed in Table 1. The trays were placed on a steel table with a 50 mm-high edge in order to fill it up by water. The mini-plugs had drainage holes in their base, allowing watering from underneath. Watering operations were made every three days during germination and two day during growth period so as to maintain constant water content in each tray. Seed germination was 78 % for *Q. ilex*, 66% for *F. sylvatica*, 78 % for *P. sylvestris* and 96% for *P. abies*. Plants were grown under fluorescent light (FLUORA T8), yielding approximately 120 μmolm^{-2} s^{-1} (Light Meter sensor - HD2302.0 - Delta Ohm, IT) at tray height. Each plant species was grown independently in the same chamber until the harvest date. A single growth chamber was used to allow for a strict control of environmental factors (uniform conditions) and seedling development (coetaneous cohort). In order to avoid influence of near lights, each table was isolated by a black polyethylene panel.

Experimental design

For each species a number of four trays were grown for a total of 416 seedlings. To investigate the kinetics of plant growth, half tray was considered for destructive analysis and half tray for non-destructive image analysis. The first sampling point was 14, 15 and 21 days after germination (a.g.) depending on the plant species. Afterwards, sampling was carried at an interval of not less than 6 days and not more than 12 days depending on the plant species, for a total of four sampling point and four weeks of growth period.

Measurement of shoot height and plant biomass

At each sampling date, for all seedlings in the half tray for non destructive analysis (n=52), plant height was manually measured with a wooden measuring stick from the base of the seedling to the highest leaf. Furthermore, five seedlings per tray, for a total of twenty seedlings per species, were randomly collected at each sampling point. Leaves, shoots, and roots from each seedling were oven drying (52 h at 75 °C) and weighed in order to measure total plant biomass.

Image capture system and analysis – shoot height and greenness

Optical sensing system is based on image acquisition and data processing using in-house developed algorithms using hue-saturation-value analysis of the image data. Shoot height sensing is based on analyses of reflected light by using a stereoscopic imaging system (Figure 1). Total leaf area sensing or green biomass is based on analyses of reflected light by using the rate of green ground coverage by the foliage when observed from above. The optical system contains two identical colour cameras from Edmund Optics; 1/1.8” CMOS, 1280 x 1024 pixels, sensor area 6.79 x 5.43 mm, 5 mm fixed focal length lens, field-of-view of 65.5 degree. Rugged USB cable is used for both data transmission and supplying the current to the camera electronics. The same hardware is used for the extraction of plant greenness as for the stereoscopic analysis. The depth of focus of the image is a combination of the size of the sensor, the focal length of the lens, lens aperture and the distance between camera and object. This system can measure for various leave colours (e.g. green, red-brown) and different seedlings height (e.g. 4-5cm, 15-20cm). The control of the cameras is carried out using a vendor-supplied software library, uEye (from IDS GmbH). This library is linked to a graphical user interface in-house developed in Microsoft Visual C++, uEyeDualCam GUI. A separate set of processing tools (uEyeDualCam HeightMap) is also home-developed for the purpose of height-mapping of each stereoscopic pair. The same GUI can also extract the "green-only" information for each picture taken. Additionally, the GUI provides the percentage of green pixels for the currently processed image. The green-pixel selection is sensitive to the light source; the proper configuration is also controlled by the .ini file for the respective camera. A long enough sequence of these images can be used to provide a time-series of plant growth – either averaged over the entire scene, or for individual plants. The achieved resolution of the height map is about 1mm that is adequate to follow the plant development. We have used the hue-saturation-value analysis of the image data to extract the green colours related to plants in a digital photo. The repeatability of lighting conditions is an important to be taken in consideration.

Shoot stereoscopic images were taken at the same time of the destructive sampling. The trays were manually moved into the image capture cabinets where one stereoscopic image – top-view – of each experimental half tray was taken. After image capture, all images were analysed using uEyeDualcam and HeightMap (Acreo Swedish ICT). Plant greenness (%) were estimated by uEyeDualcam software and then HeightMAP software recalculate greenness using uEyeDualCam settings output and create a plant height map (cm) of the tray conferring a value to the pixel of the selected images.

Statistical analysis

Results and discussion

Shoot height and plant biomass

Shoot height throughout the experiment showed different pattern for needle- and broad-leaved species (Figure 2). In the case of both needle-leaved species, after the emergence of cotyledons, a significant increment of the plant height was not detected. This happened because internodes elongation did not occur during the consecutive emissions of new leaves at this early developmental stage. In particular, plant height for *P. abies* reached the maximum value of 3 cm at the first sampling point (day 14^{th} a.g.), without further increment during the duration of the experiment. Seedlings of *P. sylvestris* as *P. abies* reached almost the maximum height at the first sampling point (day 15^{th} a.g.) with a slight increment detectable at the last sampling point (day 42^{nd} after germination). In the case of both broad-leaved species, plant height showed a continuous increment throughout the experiment that reached the maximum value of 13 cm and 8 cm, for *F. sylvatica* and *Q. ilex* respectively, at the third sampling point (day 28^{th} and 40^{th} a.g.), without further increment until the end of the experiment. Results on plant height did not show significant difference between manual and software measurements for all four species and sampling points (Figure 2). Concerning the plant biomass development, all four species showed a linear increase throughout the experiment (Figure 3). Moreover, the two broad-leaved species were characterized by a total biomass 10-fold higher than needle-leaved species.

Shoot greenness

Seedlings greenness showed a significant variation throughout the experiment with different patterns for each of the considered species. In the case of *P. abies* maximum value was reached at the third sampling point (day 32 a.g.) remaining stable later until the end of the experiment. *F. sylvatica* seedlings showed a similar pattern of *P. abies* reaching its maximum greenness value at the third sampling point (day 28 a.g.). Both *P. sylvestris* and *Q. ilex* showed a continuous increment throughout the experiment reaching the maximum value at the last sampling point (day 42 and 49 a.g. respectively). In general, broad-leaved species showed values of greenness 10-20 time fold higher than needle-leaved species. Seedling leaves of *F. sylvatica* covered almost all trays at day 20 a.g. while *Q. ilex* covered the 80% of trays at day 49 a.g. On the opposite, P. abies and P. sylvestris covered less than 7% of the total trays area in 42 days.

Regression model

In order to test the non-destructive measurements as tool for monitoring forest seedling growth, patterns of both seedling tray greenness and height obtained by Software analysis were related to seedling biomass obtained by classical destructive analysis method. The relationship between tray greenness and seedling biomass showed good correlation for all species until the tray got almost fully covered. This was the case of *F. sylvatica* that, as previously stated, covered almost the whole tray in less than one month but its biomass still continue to increase after the full coverage.

As result, the relation between seedling height and biomass showed good results with the two broad-leaved species but no relation was found for the two needle-leaved species. Indeed, the constant height of P. abies L. and P. sylvestris L. didn't relate to the continuous increment of seedling biomass.

We report the best regression model developed to explain the relationship between lidar height metrics and field-measured height at the plot level. Linear regression indicated a multiple R2 of 0.87 (Figure 3a; slope test $p < 0.001$) The final model derived from height distribution data was based on the following equation

As well highlighted by previous studies (….), stereoscopic image analysis is a robust method to record three-dimensional information. Our study confirm this case, indeed in Figure "all species height” is showed the high correlation (R2 = 0.94) that we obtain between the actual seedling height and the estimated seedling height.

Conclusion

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Table 1 Percentage values (%) of a wavelength range composing the specific light spectrum for each LED light type.

Figure 1. (a) Schematics of optical sensing based on stereoscopic measurements. (b) Photos of the optical system for measuring the shoot height and ‘greenness’; zoom-in showing the dual cameras for stereoscopic imaging.

Figure 1

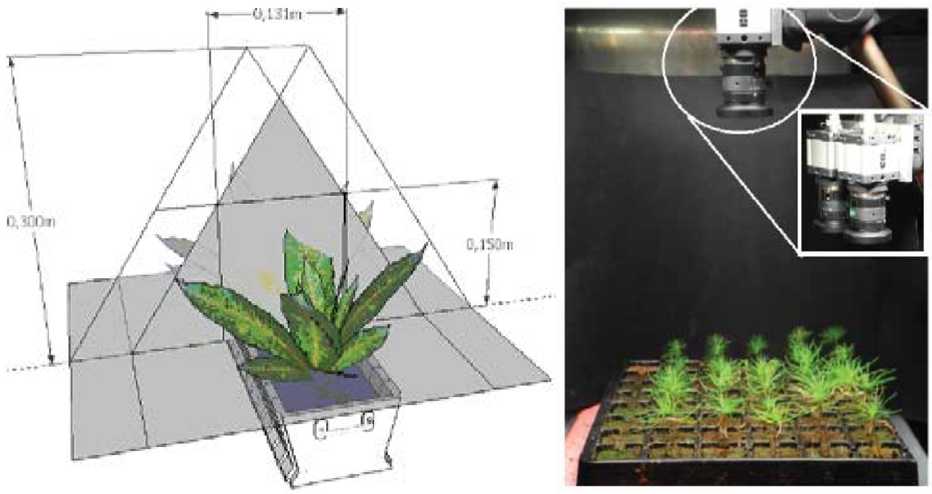


Figure 2

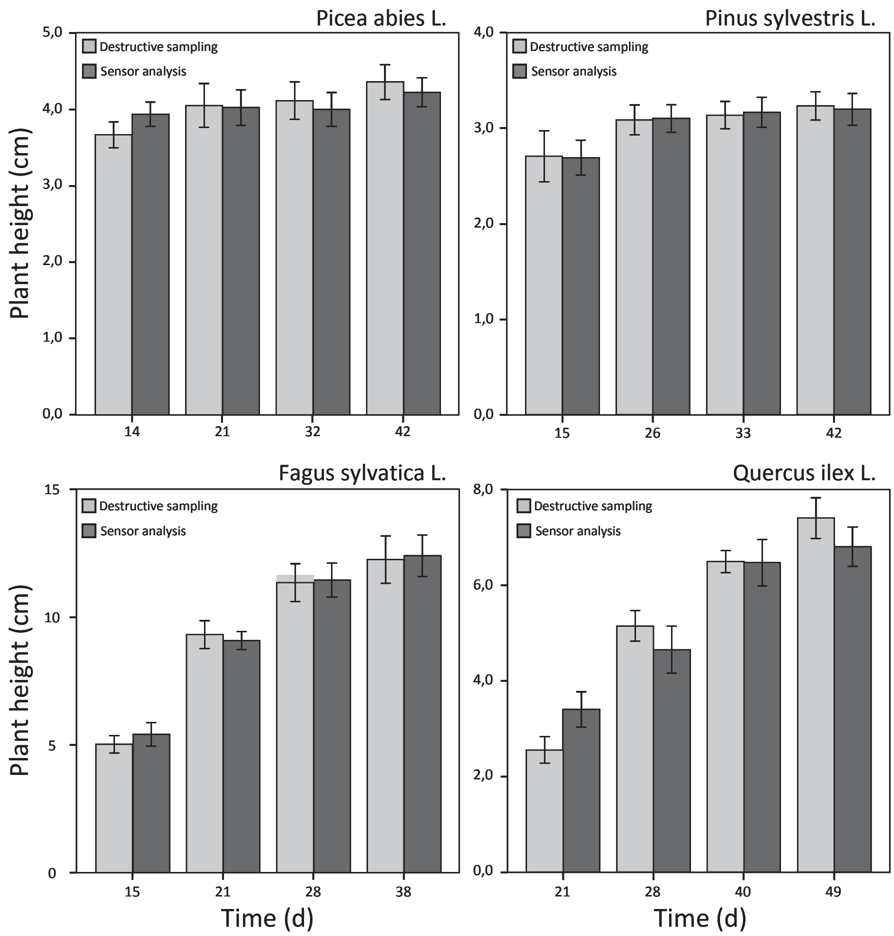


Figure 3

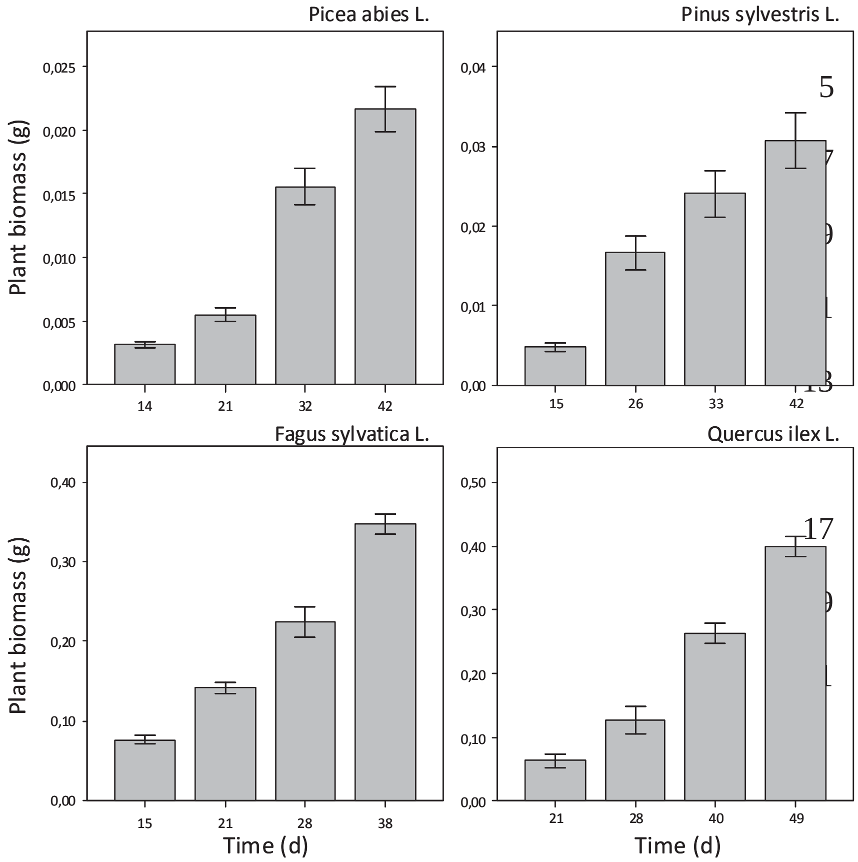


Figure 4

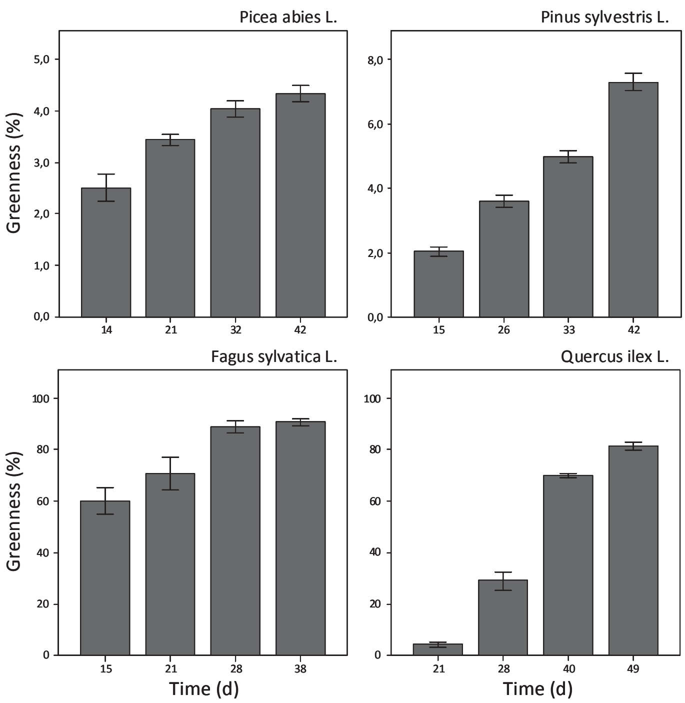
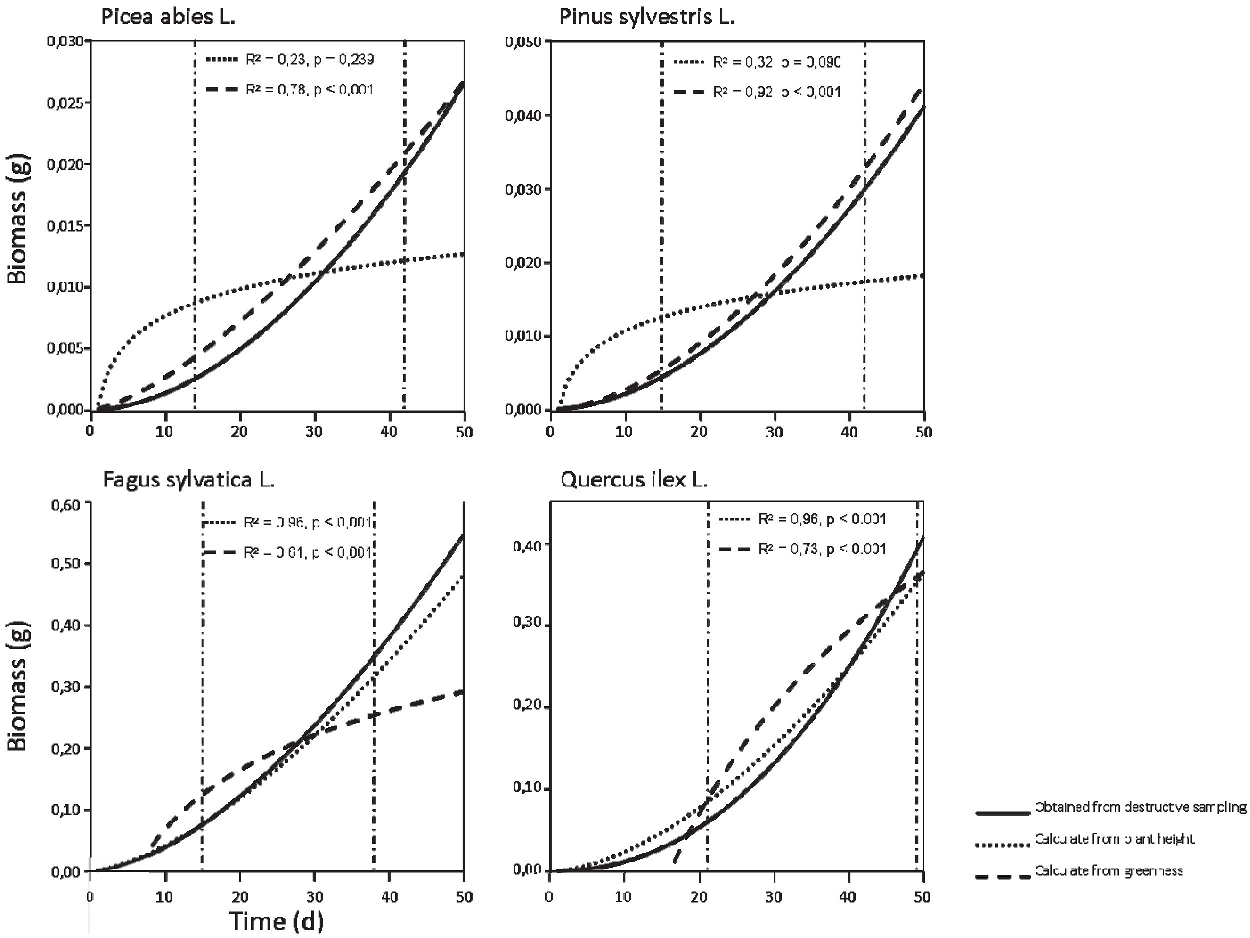
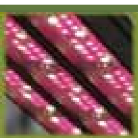


Figure 5



Picea abies L.
R² = 0,23, p = 0,239
R² = 0,78, p < 0,001
Pinus sylvestris L.
R² = 0,32, p = 0,090
R² = 0,92, p < 0,001
Fagus sylvatica L.
R² = 0,96, p < 0,001
R² = 0,61, p < 0,001
Quercus ilex L.
R² = 0,96, p < 0,001
R² = 0,73, p < 0,001
Biomass (g)
Time (d)
Obtained from destructive sampling
Calculate from plant height
Calculate from greenness



Effects of different LED lights on the growth and phytochemical characteristics of *Myrtus communis* L. seedlings

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Introduction-Hypothesis

Light is a vital environmental factor that affects plant growth, development and phytochemical accumulation by acting on plants not only as the sole energy source of photosynthesis, but also as an external signal. Light requirements of plants are subject to species, growth and developmental stages of plant, environmental conditions and manipulation target of yield and quality. Therefore, detailed studies on artificial lights are urgently needed for getting high yield and good quality in plants.

➢This study examines the influence of five different LED light qualities (L20AP67, AP673L, G2, AP67 & NS1) that emit a mixture of continuous spectrum based on various percentages of ultraviolet, blue, green, red, far-red and infra-red radiation or Fluorescent light (FL as a control) on growth of common myrtle into mini-plug containers during one month indoor cultivation. (Table 1)

➢*Aim: Plant specification cultivate-protocols under the optimal spectrum for growth.*

➢*Innovation: determining quality specific effects of LEDs with continuous spectrum in regulating growth and physiology of forest tree species.*

Light treatments / Areas of light spectrum	400-500 nm	500-600 nm	600-700 nm	700-800 nm	R:FR ratio
FL	34.0%	24.1%	36.7%	4.4%	5.74
L20AP67	10.9%	26.2%	40.9%	14.4%	2.91
AP673L	11.9%	18.2%	60.9%	8.3%	5.56
G2	7.7%	2.4%	64.4%	25.5%	2.51
AP67	13.0%	15.1%	[illegible]	18.1%	2.27
NS1	31.3%	31.3%	35.7%	6.2%	8.18

Table 1 . Percentages covering different areas of the light spectrum

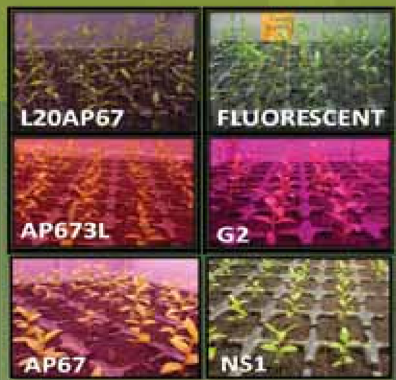

Photo 1 . *Myrtus communis* seedlings under different light treatments into the growth chambers

Materials & Methods

➢Tested species: *Myrtus communis* L.

•Area of collection: Skioni, Chalkidiki (39° 57' 0", 23° 32' 0")

•*Breaking myrtle dormancy*:

-Seed coating was carefully cracked and removed. The seeds were allowed to dry and were stored at 4^0 C

- 24 hrs hydration

-placed in phytotron chamber till germination

➢Experimental design

•Seed material: Pre-germinated

•Mini-plug trays & dimensions: QPD 104 VW (HerkuPak, Germany): 310X530 mm; cell size 33X33X43 mm; depth 12 mm; volume 27 cc; 630 plants/m^2

•Growing medium: enriched peat soil substrate (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany)

•Growth chambers conditions

17 hrs photoperiod, 140 μmol m^{-2} s^{-1} PAR, 80%, air relative humidity (RH) and a 22°C/18°C day/night temperature (Photo 1)

• Watering: twice a day by automatic sprinklers

•Continuous spectrum of LEDs & FL light qualities:

Selected percentages covering different areas of the light spectrum shown in Table 1.

✓*At the end of the growth chamber experiment*:

•Randomly selection of 10 seedlings per light

• Morphological parameters: shoot height (SH) & root length (RL) were defined as the distance from the top of the root plug to the upper and lower end of a seedling, respectively.

•Dry weight of leaves, shoots & roots were assessed after oven drying at 70 °C for 48 h.

•Phytochemical measurements:

•*Extraction of Chlorophyll a (Chl a), Chlorophyll b (Chl b) & Carotenoid content*:

-Leafs were submersed into liquid nitrogen for 5 min and then placed in 3 ml N,N'-dimethylformamide at 4 °C for 24 h.

-Absorbance of each related parameter was detected by a UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA), at 663, 647 and 480 nm, respectively.

-Following the obtained concentrations of Chl a, Chl b and carotenoids were calculated using the equations described by Porra *et al*., 1989 shown below:

Chl a: $C_a = 12 * A_{663.2} - 3.11 * A_{646.8}$

Chl b: $C_b = 20.78 * A_{646.8} - 4.88 * A_{663.2}$

Carotenoids: $C_{x+c} = (1000 * A_{480} - 1.12 * C_a - 34.07 * C_b) / 245$

•*Phenol content determination*

-PC of the extracts was measured using the Folin-Ciocalteau colorimetric assay with gallic acid as calibration standard (R^2= 0.998).

-Leaves were extracted into 10 mL of 80% aqueous methanol and centrifugation at 15,000 rpm for 15 min followed. 2.5 mL of Folin-Ciocalteau's reagent was added in each sample and after 1 min, 2 mL of 7.5% sodium carbonate solution was added.

-The absorbance of the colored reaction product was measured at 760 nm versus a blank.

•*Extraction of Anthocyanin content (AC)*

-Determined spectro-photometrically as A_{530}-0.24A_{563} (Murray & Hackett, 1991) versus a blank containing 6M HCl : H2O : MeOH (7 : 23 : 70).

- AC in the extracts was calculated from a standard calibration curve obtained with different concentrations of cyanidin glycoside (correlation coefficient: R^2= 0.992) and the results were expressed as μg of cyaniding glycoside per g of fresh myrtle leaves.

✓*Further investigation of the physiological status of the myrtle seedlings*

•*31 days RGP test*: determine the potential capacity of seedlings to initiate new roots by measuring the new root length (NRL) & new root dry weight (NRDW):

10 seedlings per species/light treatment were selected, following the standardized RGP technique for containerized seedlings described by Mattsson (1986) (Photo 2).

Photo 2. *M. communis* seedlings into the RGP bath

Results & Discussion

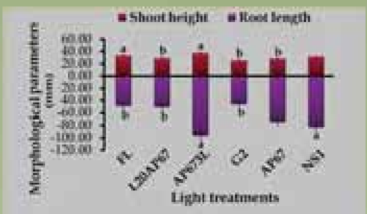

Fig.1.Differences between means in *shoot height* and *root length* under different lights for the *Myrtus* seedlings at the end of one month. Vertical bars represent the standard deviation.

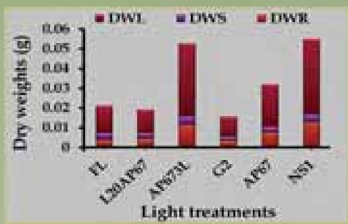

Fig.2.Differences between means in *dry weight leaves, shoots & roots* under different lights for the *Myrtus* seedlings at the end of one month.

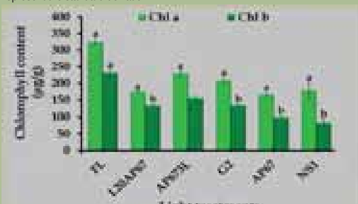

Fig.3.Differences between means in *Chl a* and *Chl b content* under different lights for the *Myrtus* seedlings at the end of one month. Vertical bars represent the standard deviation.

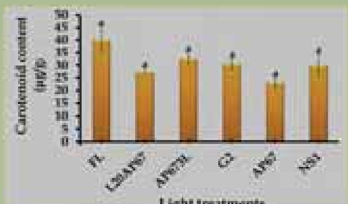

Fig.4.Differences between means in *Carotenoid content* under different lights for the *Myrtus* seedlings at the end of one month. Vertical bars represent the standard deviation.

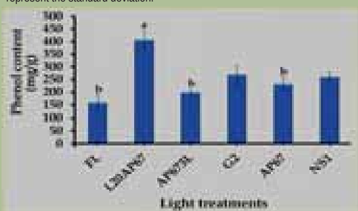

Fig.5.Differences between means in *Phenol content* under different lights for the *Myrtus* seedlings at the end of one month. Vertical bars represent the standard deviation.

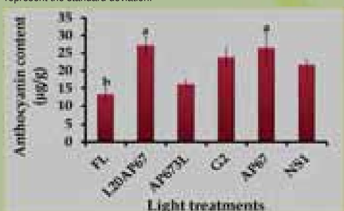

Fig.6.Differences between means in *Anthocyanin content* under different lights for the *Myrtus* seedlings at the end of one month. Vertical bars represent the standard deviation.

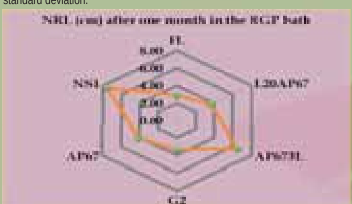

Fig.7.Differences between means in *new root length* of *Myrtus* seedlings *pre-cultivated* under different lights after one month in the RGP bath.

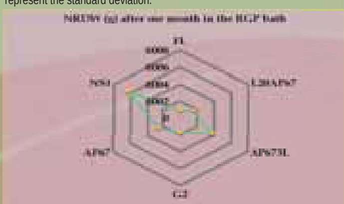

Fig.8.Differences between means in *new root dry weight* of *Myrtus* seedlings *pre-cultivated* under different lights after one month in the RGP bath.

➢Results in the present study, showed significant differences on the morphological, physiological & phytochemical state of *M. communis* seedlings after one month cultivation under different light treatments into environmentally controlled growth chambers affirming the key role factor of light in early developmental stages.

➢Both AP673L & FL lights positively affected the SH of myrtle compared to G2, AP67, L20AP67 LEDs & G2 (Fig.1).

➢ Also combined effects of AP673L & NS1 LEDs that are modest on FR and had high R:FR ratios relatively to the rest of the lights, significantly promoted the RL compared to G2, FL and L20AP67 (Fig.1) and induced an increase by 85% in the total dry weight accumulation of the myrtle seedlings compared to the rest of the light treatments (Fig.2). High ratios of red to far-red illumination can stimulate phytochrome responses in plants including stem elongation, flowering, and changes in stomatal conductance or plant anatomy. On the other hand, findings showed also an increase in shoot elongation and shoot dry mass of plants grown in reduced R:FR ratio, such as in plants that might be found in shade; this response has been attributed to a reduced phytochrome photostationary state but the amount of blue remained constant. According to other experiments when blue light levels varied the resultant R:FR effect on shoot growth was different. Our results also agree with the previous statement that blue light or the interaction of blue and other wavelengths are critical in determining photomorphogenetic response.

➢Chlorophyll content is one of the most important factors to estimate net photosynthesis and dry matter production. Suppression of photosynthesis is due to reduction of Chl levels, particularly Chla, which is directly involved in determination of photosynthetic activity and accumulation of carbohydrates, while chl b is responsible for gathering light energy and transfer it to chl a. Therefore in the absence of chl b the light harvesting complex proteins of the plants are decreased. Carotenoid is the auxiliary pigment of antenna Chls in chloroplasts and can help Chl to receive light energy. Our results showed no significant differences in the contents of Chl a (Fig.3) and carotenoid (Fig.4) in myrtle leaves under different lights, except from the Chl b that was significantly higher for the FL light compared to the AP67 and NS1 LEDs (Fig.3). Despite the fact that FL light induced significantly higher chl b content, the dry weight accumulation of the seedlings was not the highest at all (Fig.2)

➢Plants typically respond to environmental stressors by inducing antioxidant production as a defense mechanism. High light treatments also resulted in increased contents of phenolic compounds and antioxidant activity with no adverse effect on growth or yield. Indeed, LED lights showed high PC & AC, while FL showed the lowest. Specifically L20AP67 showed significantly higher PC compared to FL, AP673L & AP67 (Fig.5). Significantly higher AC was found for the L20AP67 & AP67 LEDs compared to the FL (Fig.6)

➢Thus according to the RGP test, significantly longer and heavier new roots were found for the AP673L and NS1 LEDs, while the lowest were for the FL (Fig.7, Fig.8).

Conclusion

***M. communis* seedlings after one month cultivation under five different LED and fluorescent lights, into environmentally controlled growth chambers, exhibited better performance under AP673L & NS1 based on morphological parameters. L20AP67 showed significantly higher PC & AC compared to FL, AP673L & AP67. RGP test yielded significantly longer and heavier new roots for the NS1 & AP673L compared to the other light treatments.**

The effect of light-emitting diodes on the development of pomegranate seedlings

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Abstract

The objective of the present study was to investigate the impact of LED versus fluorescent (FL) lighting on morphological, physiological and phytochemical characteristics of pomegranate (*Punica granatum* L.) after six weeks pre-cultivation in growth chambers. Five light-emitting diodes (LED) treatments i.e. L20AP67 (moderate B, G and R and low R:FR), AP673L (high R and high R:FR), G2 (high R and low R:FR), AP67 (moderate B and R and low R:FR), and NS1 (high B and G, and high R:FR, and 1% UV) with different radiation spectra were used. Fluorescent (FL) tubes served as the control light treatment. Seedlings grown under L20AP67 exhibited the best morphological-agronomical characteristics showing rapid height increase, longer roots, greater fresh and dry weight, as well as greater leaf area compared to the rest of the treatments. Seedlings grown under the FL and L20AP67 showed higher photosynthetic efficiency, while greater root activity was revealed under NS1 and AP67. Higher chlorophyll and carotenoid content was found under the influence of the FL lighting. NS1 and AP67 seedlings produced a greater total and simple phenolic content respectively. Flavonoid formation was favored under G2 and AP67, while anthocyanins content was found greater under G2, AP67 and NS1. Root growth potential (RGP) estimation was also performed in order to evaluate the transplant response. After 31 days in the chamber where RGP was assessed, transplanted seedlings of AP67 treatment had the lengthiest and L20AP67 seedlings had the heaviest newly formed roots. Our study demonstrates that LEDs were more efficient in promoting a number of morphological and phytochemical characteristics than conventional fluorescent light in *Punica granatum*. In particular, L20AP67 LED is recommended for production of pomegranate seedlings under artificial lighting.

Keywords: nurseries, LED, photomorphogenesis, *Punica granatum*, secondary metabolism, phenolic compounds, pigments, transplanting

Abbreviations:

FWL, FWS and FWR, fresh weight of leaves, shoots and roots; DWL, DWS and DWR, dry weight of leaves, shoots and roots; NDWR, dry weight of new roots; NRL, new root length; RGC, root growth capacity; R/S, root:shoot ratio; TPC, total phenolic content; SPC, simple phenolic content

1. Introduction

Light is one of the most important factors affecting plant growth and development (Kendrick and Kronenberg 1994; Mc Donald 2003). Various lamp types such as fluorescent (FL), incandescent and metal halide are used in crop production in order to increase photoperiod and subsequently photosynthesis. These lamp types are not as energetically efficient as desired. Further, they do not offer the option of spectral manipulation which is very important for plant growth and development (Schuerger et al. 1997). In the last years, light-emitting diodes (LEDs) have been facilitating photobiology studies and have proven to be an efficient light source for commercial crop production (Nhut et al. 2003). Compared to a conventional light source, a LED has small weight and volume and a long lifespan (about 50.000 h). Its spectral efficiency is continuously increased (Bourget 2008; Morrow 2008). Heat produced by a LED can be dissipated through an external source, thus allowing the LED to be placed close to a plant without increasing its temperature or causing plant burns (Bourget 2008; Massa et al. 2008; Morrow 2008). Numerous plant species have already been tested using LED lights, in particular tree species such as wild cherry, holm oak and beech (Astolfi et al. 2012), fruits and vegetables such as lettuce (Chen et al. 2014; Johkan et al. 2010; Lin et al. 2013; Samuoliene et al. 2013), cucumber (Hogewoning et al. 2010), strawberry (Choi et al. 2015), rapeseed (Li et al. 2013), as well as ornamental plants such as *Phalaenopsis*, roses, chrysanthemums and campanulas (Ouzounis et al. 2014a, 2014b).

Light absorption triggers photosynthesis. Chlorophyll a is one of the main molecules absorbing light energy. Apart from primary metabolites such as chlorophylls, plants produce a variety of chemical compounds called secondary metabolites. Carotenoids, which belong to the terpenoid group, are accessory pigments. that have orange, yellow or red colour. Carotenoids protect a plant by energy dissipation and radical detoxification when it is exposed to excessive light intensity, thus limiting membrane damages. Proteins, membranes, and other molecules may be damaged by excessive light intensity if carotenoids are absent. Phenolic compounds are non photosynthetic pigments (Edreva 2005) that prevent damages caused by light. Furthermore, they facilitate photosynthesis in various environmental conditions (Solovchenko and Merzlyak 2008). These compounds are characterized by metabolic plasticity helping the plants to adapt in biotic and abiotic environmental changes (Taiz and Zeiger 2010). Their accumulation in vacuoles and their biosynthesis in chloroplasts are triggered by exposure to light (Kefeli et al. 2003). In plants, phenolic compounds serve as blue and red pigments, as antioxidants and as protective compounds from UV radiation (Lattanzio et al. 2006). The flavonoid group is a group of phenolic compounds affecting the nutritional value of many plant species (Ebisawa et al. 2008; Hichri et al. 2011). Their biological role is linked to the plant adaptation in biotic and abiotic environmental changes and to stressful conditions. Flavonoids act as defensive and signaling compounds, as well as protective from UV radiation and oxidative damage neutralizing reactive oxygen species (ROS). Flavonoids and tannins exhibit antimicrobial and antioxidant action and have metal ion chelating properties (Seigler 1998; Karamanoli et al. 2011). Anthocyanins, which belong to the flavonoid group, contribute to flower and fruit colouring and to insect attraction. Moreover, anthocyanins exhibit antimicrobial action and protect cells from damages caused by blue and UV radiation (Seigler 1998).

Pomegranate (*Punica granatum* L.) is a shrub or small tree growing from 5 to 10 m. It is cultivated all around the world but the main centre of its cultivation are Mediterranean countries followed by Asian and former USSR countries (Texeira da Silva et al. 2013). Pomegranate fruits are eaten fresh and are used for the production of juice that is consumed worldwide. Pomegranate is one of the main anthocyanin sources (Fischer et al. 2011). Its fruit is characterized by high phenolic compound content and antioxidant activity (Ozgen et al. 2008; Madrigal-Carballo et al. 2009; Qu et al. 2010; Zaouay et al. 2012). Parts of the tree have been used for dyeing fabrics and as a tannin source for repairing leather. Due to its high antioxidant activity pomegranates are a good source of reducing agents required for the formation of nanoparticles which is a new trend in the nanoparticle technology and is promoted as green technology (Texeira da Silva et al. 2013).

It is generally accepted that light effects on the plant morphology, physiology and secondary metabolism is species dependent. Little information is available regarding the relationship between varying light spectra absorbed by pomegranate and the aforementioned parameters. Therefore, the objective of this study was:

1) the investigation of the effects of five LED light sources having different light spectra and FL (Control) on the morphological, physiological and phytochemical properties of pomegranate seedlings immediately after the germination,

2) the determination of the best quality among the tested light treatments for the pre-cultivation of pomegranate seedlings,

3) the examination of the response of the seedlings after transplantation by evaluating their root growth capacity (RGC) after their cultivation under controlled environmental conditions.

2. Materials and methods

2.1 Plant material, growth conditions and light treatments

The study was carried out at the Forest Research Institute in the provenance of Thessaloniki, Greece, in 2014. Ripe *Punica granatum* var. Wonderful fruits were collected from Riza, Chalkidiki provenance (40°30’214’’N, 23°26’612’’E), Greece, in October 2013. The seeds were depulped, dried and stored at 4°C in polyethylene bags. For the experiment only pre-germinated material was used in order to ensure the maximum uniformity of germination. Pomegranate seeds were hydrated for 24h and placed at 4°C for 2 months in Petri dishes containing moist sand. Afterwards, the seeds were transferred in a phytotron chamber (20°C, 70% RH, 8h photoperiod) (Piotto, 2003). The germination rate was 46% and 78% after 3 and 5 weeks respectively. The mini-plug plastic container trays (QP D 104 VW, Herkuplast Kubern GmbH, Germany) with identical dimensions (310×530 mm, density: 630 seedlings/ m^2; 27 cc) were filled with enriched peat (EP, pH 6.0). This substrate is suitable for young seedlings and transplanting material (TS1, Klassmann-Deilmann GmbH, Geeste, Germany).

We tested the application of fluorescent lighting or LED lighting for the first six weeks after germination. LED lights produced by Valoya (Valoya Oy, Helsinki, Finland) generate a wide continuous spectrum having a mixture of ultraviolet (UV, < 400 nm), blue (B, 400-500 nm), green (G, 500-600 nm), red (R, 600-700 nm) and far-red (FR, 700-800 nm). In total, 200 seedlings per treatment were used. Pre-germinated pomegranate seeds were placed in the mini-plug containers and transferred to environmentally controlled growth chambers for 42 days under Valoya LED [L20AP67 (moderate B, G and R and low R:FR), AP673L (high R and high R:FR), G2 (high R and low R:FR), AP67 (moderate B and R and low R:FR), and NS1 (high B and G, and high R:FR, and 1% UV)] lights with different light spectra or white fluorescent lamps (Osram Fluora, Munich, Germany) that were used as the Control treatment (table 1). It is worth mentioning that G2 light's high emission of R and FR makes it unpleasant for people working inside the growth chambers. In the growth chambers environmental conditions were stable and set at 14h photoperiod, 20°C and air relative humidity of 80 ± 10%. Photosynthetic photon flux density (PPFD) was maintained at around 200±20 μmol m^{-2} s^{-1} at plant height. Automated water sprinklers were used every day for watering. The containers were rotated once per day in order to ensure equal growth conditions.

2.2 Morphological and developmental measurements

Height growth rate measurements were applied during the six-week experimental period (42 days). Specifically, shoot height was evaluated every two weeks. The first measurement was carried out 14 days after sowing. After the six-week experimental period, 10 randomly selected seedlings from each treatment were sampled. Morphological characteristics were measured such as leaf number, shoot height, root length, leaf area, as well as fresh and dry weight of shoots (FWS and DWS), leaves (FWL and DWL) and roots (FWR and DWR). Furthermore, root:shoot dry weight ratio (R/S) was estimated. Dry weight was measured after samples were dried in a drying oven (80 °C) for three days, and the leaf area was measured by leaf area meter LI-3000C (LI-COR biosciences, Lincoln, USA).

2.3 Root growth capacity (RGC) evaluation

After six weeks, 10 randomly selected seedlings for each treatment were moved in another growth chamber (20°C temperature; 60 ± 10% air relative humidity; 14 h photoperiod; 300 μmol m^{-2} s^{-1} PPFD for all treatments at plant height; watering was applied every two days), transferred on top of steel boxes (35 cm × 26 × cm × 8 cm) with a mixture of peat and sand (1:1) and placed in a water tank in order to assess their root growth capacity (RGC). The water tank was used in order to maintain a stable temperature for the root system (20±1 °C). The seedlings were divided into two groups. The first group was harvested after 15 days and the second group was harvested after 31 days. The characteristics measured for the RGC assessment were new root length (NRL) and dry weight of new roots (NDWR). The growth rate, shoot height and root length, as well as the NRL were calculated with a Powerfix (Milomex, Pulloxhill, UK) digital caliper.

2.4 Physiological measurements

2.4.1 Root activity determination

For the determination of the root activity, roots were excised from 5 randomly selected seedlings of each treatment. The roots were treated in 2.5 ml of 1% 2,3,5-triphenyltetrazolium chloride (TTC) and 2.5 ml of potassium phosphate buffer (PBS) and then mixed thoroughly and incubated for 2.5 h (T) at 37 ◦C. The reaction was terminated with 1 ml of H_2SO_4, and the root was extracted with 3 ml ethyl acetate twice. The samples were subsequently placed in a mortar and ground down. The optical density was measured at 490 nm versus a blank containing ethyl acetate. For calibrations, different concentrations of TTC were used. Two standard curves were used (R^2= 0,979 and R^2= 0,996) because the reaction was not linear for all concentrations. The root activity was determined using the following equation: Root activity (mg/(g h))= ρV/WT (Zhang et al., 2009), where ρ is the optical density (mg/ml), V is the total volume of extract (ml), W is the fresh weight (g) of the sample, and T is the reaction time (h).

2.4.2 PSII photosynthetic efficiency

Six weeks after initial germination, chlorophyll fluorescence measurement was applied in 10 randomly selected seedlings in order to assess their PSII photosynthetic efficiency under the light environment that they have been cultivated. The measurement was conducted using the chlorophyll fluorescence meter FluorPen FP100 (Photon Systems Instruments, Drasov, Czech Republic).

2.4.3 Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to Burits (2001). Briefly, 30 μl of extracts were added to 5 ml of 0.004% methanol solution of the stable 2,2'-diphenylpicrylhydrazyl (DPPH) radical. After a 30 min incubation period at dark and room temperature the decrease of the absorbance was measured at 517 nm versus a blank containing 6M HCl:H_2O:MeOH (7:23:70). Different concentrations of ascorbic acid were used for the calibration curve (R^2= 0.980) and the results were expressed as mg of ascorbic acid equivalents antioxidant capacity per g of fresh weight.

2.5 Quantification of phytochemical content

2.5.1 Total phenolic content (TPC)

Six weeks after initial germination 10 randomly selected pomegranate seedlings were processed for the determination of the total and simple phenolic content (TPC and SPC), the flavonoid content and the anthocyanin content. TPC of the extracts was measured by a UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) using the Folin-Ciocalteau colorimetric assay (Singleton and Rossi 1965). For the extraction, seedlings were submersed into liquid nitrogen for 5 min to perforate the waxy cuticle and rupture cell membranes and then placed in plastic containers with 3 ml 6M HCl:H_2O:MeOH (7:23:70). The containers remained in the dark at 4 °C for 24 h. This was followed by addition of 2.5 mL of Folin- Ciocalteau's reagent and vortex of the mixture. 2 mL of 7.5% sodium carbonate solution was added after 1 min, the mixture was vortexed again and samples were incubated for 5 min at 50 °C. The absorbance of the colored reaction product was measured at 760 nm versus a blank consisting of 500 μL of methanol, 2.5 ml of Folin- Ciocalteau's reagent and 2 mL of 7.5% aqueous sodium carbonate. The TPC in the extracts was calculated from a standard calibration curve obtained with different concentrations of tannic acid (R^2= 0.998) and the results were expressed as mg of tannic acid equivalent per g (mg/g) of fresh pomegranate.

Simple phenolic content of the extracts was determined using insoluble polyvinylpolypyrrolidone (PVPP) which is a tannin binding reagent. Aliquot of 60 mg PVPP was added in plastic vials containing 0.6 ml distilled water and 0.6 ml of the pomegranate extract and the mixture was vortexed. The vials remained at 4 °C for 15 min, then centrifuged (3000 g for 10 min) and the supernatant was collected. The SPC was then measured at 760 nm as described above.

2.5.2 Flavonoid content

The flavonoid content was determined according to the Arvouet-Grand et al. method (1994) using a 96-well microplate reader. Aliquot of 100 μl of each seedling extract was mixed with a methanolic (2%) solution of aluminium trichloride ($AlCl_3$) (100 μl). The absorbance of each mixture was measured at 510 nm versus a blank reagent of plant extract (100 μl) without $AlCl_3$, and methanol (100 μl). For calibrations different concentrations of quercetin solution (R^2= 0.991) were used and results were expressed as μg of quercetin equivalents per g (μg/g) of fresh weight.

2.5.3 Anthocyanin content

Anthocyanin content of the extracts was determined spectrophotometrically as A_{530}-0.24A_{563} (Murray and Hackett 1991) versus a blank containing 6M HCl:H_2O:MeOH (7:23:70). The anthocyanin content in the extracts was calculated from a standard calibration curve obtained with different concentrations of cyanidin glycoside (R^2=0.992) and the results were expressed as μg of cyanidin glycoside per g (μg/g) of fresh pomegranate.

2.5.4 Chlorophyll a, chlorophyll b and carotenoid content

After six weeks stay in the growth chambers, 10 pomegranate seedlings were randomly selected and processed for the determination of the total chlorophyll a (chl a), chlorophyll b (chl b) and carotenoid content. For the extraction, the seedlings were treated in liquid nitrogen as described above and then placed in 3 ml N,N'- dimethylformamide to extract at 4 °C for 24 h. Absorptions were measured at 663, 647 and 480 nm. The concentrations of chl a, chl b and carotenoids were calculated using the equations described by Porra et al. (1989).

Chl a: $C_a = 12 * A_{663.2} – 3.11 * A_{646.8}$

Chl b: $C_b = 20.78 * A_{646.8} – 4.88 * A_{663.2}$

Carotenoids: $C_{x+c} = (1000 * A_{480} – 1.12 * C_a – 34.07 * C_b) / 245$

2.6 Statistical analysis

For statistical analysis SPSS (SPSS 15.0, SPSS Inc.) was used. Growth rate data were analyzed using repeated measures. After the six-week experimental period, data were analyzed by analysis of variance (ANOVA). Mean comparisons were conducted using a Bonferroni test at a < 0.05.

3. Results

3.1 Growth rate

Both on the 14th and on the 28th day after sowing, L20AP67 promoted significantly higher growth rate compared to the rest of the treatments. In addition, on the 28th day AP67 had significantly lower growth rate than AP673L. On the 42nd day G2 seedlings showed significantly lower growth rate compared to AP673L, Control and L20AP67 treatments (fig. 1).

3.2 Number of leaves, shoot height, root length, root:shoot ratio and leaf area

Leaf colour differences were observed between the light treatments, after visual examination. Specifically, seedlings grown under the Control and L20AP67 only formed green leaves, whereas seedlings under the rest of the treatments formed green and reddish leaves. Regarding the number of leaves, no significant differences were observed with seedlings forming between 11 and 15 leaves irrespective of the different light treatment (data not shown). After six weeks in the growth chambers, shoot height was significantly greater under L20AP67 compared to the rest of the treatments (table 2). Root length was also significantly greater under the influence of L20AP67 compared to the Control, AP67 and NS1 (table 2). No significant differences were observed regarding the R/S among the light treatments (table 2). However, greater values were found under G2 treatment. As far as leaf area is concerned, seedlings pre-cultivated under L20AP67 exhibited significantly greater values compared to all other treatments (table 2).

3.3 Fresh and dry weights

Seedling pre-cultivation under the influence of L20AP67 resulted in the formation of significantly greater total fresh weight compared to the rest of the treatments. Specifically, FWL and FWS were significantly favored under L20AP67 compared to the other treatments, while FWR was also greater under L20AP67 compared to AP67, G2, AP673L and NS1 (fig. 2A).

Total dry weight was also significantly greater under L20AP67 compared to the rest of the treatments. Additionally, seedlings grown under the Control formed less total biomass compared to AP673L, NS1 and G2. Specifically, DWL was significantly favored under L20AP67 compared to the Control, AP67 and G2, while the Control also induced the formation of less DWL compared to NS1, AP673L and G2. L20AP67 promoted the formation of significantly greater DWS compared to all other treatments, while seedlings grown under the Control also formed significantly less DWS compared to AP673L, G2 and NS1. The Control seedlings exhibited significantly less DWR than L20AP67, AP673L, NS1 and G2, whereas AP67 also had significantly lower values compared to L20AP67 (fig. 2B).

3.4 Root activity

Regarding the root activity evaluation, seedlings grown under AP67 and NS1 exhibited significantly greater values compared to the Control (fig. 3).

3.5 PSII Photosynthetic efficiency

The chlorophyll fluorescence measurement revealed significantly higher photosynthetic efficiency under the Control and L20AP67 compared to the rest of the LEDs. Moreover, NS1 also exhibited significantly lower values compared to AP673L (fig.).

3.6 Antioxidant activity

The results of the antioxidant activity did not reveal any significant differences among the different light treatments (data not shown).

3.7 Total and simple phenolic content (TPC and SPC)

Different light qualities after the cultivation of pomegranate seedlings into the growth chambers resulted in significantly higher TPC for seedlings grown under NS1 compared to the Control, L20AP67 and AP673L. In addition, G2 induced the formation of greater TPC compared to the Control (fig. 4). SPC was significantly favored under AP67 compared to L20AP67, Control and AP673L (fig. 4).

3.8 Flavonoid and anthocyanin content

Results of the flavonoid determinations revealed significant differences between G2 and AP67 that had the greatest values, and the rest of the treatments (fig. 5). Quite similarly, anthocyanin content was significantly greater under G2, AP67 and NS1compared to all other treatments (fig. 5).

3.9 Chlorophyll a and b, and carotenoid content

Seedlings grown under the influence of the Control formed significantly greater chlorophyll a values compared to the rest of the treatments, while L20AP67 showed significantly greater values compared to NS1 (fig. 6). Further, Control seedlings also formed significantly more chlorophyll b and carotenoids compared to all other treatments (fig. 6).

3.10 Root growth capacity (RGC)

After 15 days in the growth chamber were RGC was determined, neither NRL nor NDWR were significantly affected by the different light treatments. However, after 31 days, seedlings pre-cultivated under AP67 exhibited significantly greater NRL compared to all other treatments, while G2 also had significantly lower NRL compared to NS1 (fig. 7A). Further, after 31 days, NDWR was significantly greater under L20AP67 compared to NS1, G2 and Control (fig. 7B).

4. Discussion

Plant growth and development is affected by light (Whitelam and Halliday 2007). During pre-cultivation in the mini-plugs no abnormalities were observed indicating that the cultural conditions applied were appropriate. In general, L20AP67 (moderate B, G and R and low R:FR) promoted greater growth rate compared to the rest of the treatments. Seedlings grown under L20AP67 rapidly increased in height, whereas seedlings grown under AP673L, G2, AP67 and NS1 (relatively high R portion) had more compact appearance. However, the greater height increase of L20AP67 seedlings was not followed by greater leaf formation, with leaf number having no differences among the light treatments. Similarly, no differences were observed in lettuce regarding the leaf number between FL and LED 45 days from sowing (Johkan et al. 2010). After 42 days, shoot height was also favored under L20AP67 confirming the results of the growth rate measurements. In wild cherry, holm oak and beech shoot height was greater under AP67 compared to FL (Astolfi et al. 2012), while no differences were observed between FL and LED treatments in rapeseed (Li et al. 2013). L20AP67 promoted the longest root formation among the different light treatments. However, in rapeseed plantlets root length was found greater under FL and R LED compared to the B containing light treatments (Li et al. 2013).

Total fresh weight of pomegranate seedlings was found greater under L20AP67 (moderate B, G and R and low R:FR). In greenhouse roses greater total fresh weight was found under 20%B/80%R and 100%R treatments compared to W LED (Ouzounis et al. 2014b). FWS of beech seedlings was greater under AP67 compared to FL, while FWS of wild cherry and holm oak were not affected by the different light treatments (Astolfi et al. 2012). In lettuce, FWR was found greater under RB LED compared to RBW LED (Lin et al. 2013). Similarly to the fresh weight, total dry biomass production was also favored under L20AP67 compared to the rest of the treatments. In rapeseed, greater biomass was found under B:R=3:1 and B:R=1:1 LEDs compared to FL (Li et al. 2013). In roses, BR and R LED treatments led to greater biomass production compared to W LED, while in campanulas no differences were observed (Ouzounis et al. 2014b). DWL, DWS and DWR were found lower under the Control compared to the LEDs. In beech seedlings, DWS was lower under FL compared to AP67, while in wild cherry and holm oak no differences were found (Astolfi et al. 2012). It is revealed that seedlings grown under the Control and L20AP67 had the highest loses in total weight after drying. These weight loses might be the result of higher water and volatile concentration in seedlings grown under the Control and L20AP67. Seedlings grown under AP673L, G2, AP67 and NS1 maintained their weight differences after drying and proved to be more compact plants. It is reported that R light affects biomass production and elongation through the phytochrome photoreceptors (Sager and McFarlane 1997). B light acting through cryptochromes and phototropines affects photomorphogenic responses (de Carbonnel et al. 2010; Kozuka et al. 2013).

Leaf area of pomegranate plants was benefited under L20AP67 light treatment (moderate B, G and R and low R:FR). In other experiments, leaf area of beech seedlings was favored under AP67 compared to FL, while in holm oak FL induced greater leaf area compared to AP67, and wild cherry was not affected by the different light treatments (Astolfi et al. 2012). In cucumber greater leaf area was formed with increased R light (Hogewoning et al. 2010). As mentioned before, R and B lights affect photomorphogenic responses.

A well developed root system is important for the plants to absorb sufficient amounts of water and nutrients. In our case, no significant differences were found among the light treatments. In lettuce, plants grown under FL showed greater S/R (lower R/S) compared to LED treatments (Johkan et al. 2010), while also in lettuce, RB LED treatment promoted the formation of greater S/R (lower R/S) compared to RBW LED and FL (Lin et al. 2013). Moreover, high root activity is crucial for water and mineral absorbance. NS1 and AP67 treatments (relatively high B and R portions) promoted the highest root activity. Similarly, in rapeseed B:R=3:1 induced greater root activity compared to FL light (Li et al. 2013). It is likely that root activity is intensified by the presence of B and R radiation. However, more research is needed in order to determine the factors affecting the parameter.

It has been reported that the maximum quantum efficiency of PSII, Fv/Fm for 44 species is 0.83 on average (Bjorkman and Demmig 1987). In our case, Fv'/Fm' ranged from 0.18 to 0.60. This was not a surprise as chlorophyll fluorescence is environmental and species dependent (Bjorkman and Demmig 1987). In *Phalaenopsis* greater Fv'/Fm' values were observed under 32%B/W treatment compared to 0%B/R and 40%B/R treatments (Ouzounis et al. 2014a). The results of *Phalaenopsis* suggest that B radiation is needed for better PSII photosynthetic mechanism function (Hogewoning et al. 2010). However, NS1 (high

B, G and R:FR, and 1% UV) induced the lowest Fv'/Fm' values. Low Fv'/Fm' values indicate possible photoinhibition when plants are exposed at stressful conditions (Ouzounidou and Constantinidou 1999, Maxwell and Johnson 2000, Baker and Rosenqvist 2004).

The highest TPC was found in seedlings grown under NS1 (high B, G and R:FR, and 1% UV) which was 1.5 fold higher than the Control's. Phenolic compounds have been reported to act protectively against UV radiation (Lattanzio et al. 2006), which is likely to explain the higher phenolic concentration under the UV containing NS1 treatment. Both in growth chamber and in greenhouse strawberry, higher TPC was found under R LED compared to B and RB LEDs (Choi et al. 2015), while in baby leaf lettuce cultivated in growth chamber lower TPC was found under B-R-FR LED compared to B-R-FR + UV LED (Samuoliene et al. 2013). It has previously been reported that B supplementing light leads to increased secondary metabolites production (Ouzounis et al. 2014a). Regarding the mechanisms leading to higher formation of phenolic compounds, it has been reported that under B radiation phenylalanine ammonia-lyase (PAL) activity was triggered, which is an important enzyme in the phenylpropanoid pathway (Heo et al. 2012).

Regarding the flavonoids, G2 and AP67 (relatively high R and low R:FR) exhibited the highest concentrations. In roses, chrysanthemums and campanulas, higher flavonoid synthesis was observed under increased B light (Ouzounis et al. 2014b), while in growth chamber grown strawberries, no differences were observed between the R, B and RB treatments (Choi et al. 2015). The results of the anthocyanin quantification revealed that seedlings cultivated under G2, AP67 and NS1 (relatively high R and B portions) formed more anthocyanins compared to the Control, L20AP67 and AP673L (relatively high R, and less B portion). In strawberry grown in growth chamber, B and RB LEDs induced the formation of more anthocyanins compared to R LED (Choi et al. 2015), while in red leaf lettuce greater anthocyanins content was also found under B containing LED treatments (Johkan et al. 2010). It has been reported that B light is very effective in regulating anthocyanins synthesis in tomato, and cryptochromes are related acting as B and UV-A photoreceptor (Ninu et al. 1999; Giliberto et al. 2005). The results above indicate the importance of B light in the anthocyanins synthesis.

Plant pigments have been studied extensively due to their major role in light absorbance and plant stress physiology. In our case, seedlings cultivated under the Control had the highest chl a and chl b content. L20AP67 (moderate B, G and R and low R:FR) followed having significant greater values only compared to NS1 light regime (high B, G and R:FR, and 1% UV). In beech and holm oak, FL seedlings produced more chlorophyll than AP67 light, while wild cherry was not significantly affected (measurement with SPAD chlorophyll meter) (Astolfi et al. 2012). Carotenoid formation was favored under the Control. In beech, wild cherry and holm oak seedlings, no differences were observed between FL and AP67 light treatments (Astolfi et al. 2012). In cucumber, chlorophyll and carotenoid content increased with increasing B light, until B was 50% (Hogewoning et al. 2010), while in Green oak leaf lettuce chlorophyll and carotenoid content was greater under FL+R LED and FL+B LED (Chen et al. 2014).

Toxic reactive oxygen species (ROS) are formed when plants use oxygen as electron receiver. When ROS accumulation speed is greater than their scavenging from the antioxidant mechanisms, the result is oxidative damage. Similarly to the results in pomegranate regarding the antioxidant activity, no significant differences were found in lettuce between B LED, R LED and FL 45 days after sowing (Johkan et al. 2010). Phenolic compounds and carotenoids represent two major groups of non enzymic antioxidant compounds. In pomegranate, even though there were differences between the light treatments regarding the phenolic compounds and carotenoids, no differences were exhibited in the antioxidant activity. It is possible that the high carotenoid content of the Control (mainly) and L20AP67 (secondarily) seedlings and the high phenolic compound concentration under the rest of the LEDs led to similar antioxidant activity values in all light treatments.

During pre-cultivation, seedlings grown under the Control and L20AP67 only formed green leaves, while the rest of the treatments (relatively high R portion) induced the formation of green and reddish leaves. In *Phalaenopsis* reddish leaves were observed with increasing B radiation (Ouzounis et al. 2014a). This colouring difference can be the result of increased flavonoid and especially anthocyanin concentration in seedlings cultivated under the influence of AP673L, G2, AP67 and NS1. Higher chlorophyll concentration might also explain the absence of reddish leaves for seedlings grown under the Control and L20AP67.

In Mediterranean regions where drought stress is one of the main limiting factors, producing seedlings with vigorous root system is very important for the transplant success (Radoglou et al. 2003; Raftoyannis et al. 2006). RGC is an index of the seedling performance after transplanting, by which the plant's establishment success can be predicted. RGC could be defined as the ability of a seedling to expand its root system through new root formation and/or through present roots elongation (Mattsson 1986, Mattsson 1996). Both morphological and RGC parameters have been reported to be species dependent (Kostopoulou et al. 2010). After 15 days from transplant, both the length and the dry weight of new roots were not affected by pre-cultivation under the different light treatments, indicating that more time is needed for the root system to respond to the varying light qualities. However, 31 days after transplanting, seedlings grown under AP67 (moderate B and R, and low R:FR) formed the longest roots but L20AP67 (moderate B, G and R, and low R:FR) promoted greater new root biomass production. RGC is mainly determined by the new root dry weight because it refers both to the mass of the primary roots and to the mass of the secondary roots. The secondary roots are crucial for the plant's ability to successfully exploit the surrounding soil. The results suggest that L20AP67 may provide the seedlings with an advantage in exploiting greater soil volume compared to the rest of the treatments, in a short period of time after transplanting.

5. Conclusion

Punica granatum var. Wonderful seedlings were cultivated under six different LED lights and fluorescent lamps. In general, L20AP67 (moderate blue, green and red, and low red:far-red) treatment promoted better morphological characteristics with high growth rate, shoot height, root length, fresh and dry weight, and leaf area values. Moreover, L20AP67 along with the Control induced the highest PSII photosynthetic efficiency. The most chlorophylls and carotenoids were formed under the Control light treatment. As for the root activity, seedlings grown under NS1 and AP67 showed the highest values. Total phenolic, flavonoid and anthocyanin contents were higher under G2, AP67 and NS1 (relatively high red portion) indicating possible stress. After 31 days from transplanting, L20AP67 had the heaviest new roots, thus better root system development indicating that they may better exploit larger soil volume faster after transplanting. Overall, L20AP67 LED promoted better seedling growth and development compared to the Control and the rest of the LEDs.

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Table 1. Spectral distribution and red:far-red (R:FR) ratio for the six light treatments

Light treatment	< 400 nm	400-500 nm	500-600 nm	600-700 nm	700-800 nm	R:FR
Control	0%	34.8%	24.1%	36.7%	4.4%	5.74
L20AP67	0%	10.5%	26.2%	48.9%	14.4%	2.91
AP673L	0%	11.9%	19.3%	60.5%	8.3%	5.56
AP67	0%	13.8%	15.1%	53%	18.1%	2.77
G2	0%	7.7%	2.4%	64.4%	25.5%	2.51
NS1	1%	20.2%	38.9%	35.7%	5.2%	8.16

Table 2. Morphological and developmental parameters of *Punica granatum* grown under the six different light treatments with the same abbreviations as in Table 1. Average values (n=10, ±SE) followed by different letters within a row differ significantly (a= 0.05).

Parameters	Light treatments					
	Control (FL)	**L20AP67**	**AP673L**	**G2**	**AP67**	**NS1**
Shoot height (cm)	46.75 ± 2.95b	77.28 ± 1.75a	42.95 ± 3.74b	45.11 ± 2.97b	41.03 ± 2.22b	45.33 ± 2.40b
Root length (cm)	44.96 ± 5.67b	93.31 ± 7.61a	65.01 ± 5.11ab	64.42 ± 10.58ab	51.92 ± 5.36b	61.38 ± 4.76b
Root/Shoot ratio	0.16 ± 0.01a	0.17 ± 0.01a	0.17 ± 0.02a	0.20 ± 0.02a	0.16 ± 0.01a	0.18 ± 0.01a
Leaf area (cm^2)	5.71 ± 0.65b	13.55 ± 1.44a	6.80 ± 0.92b	5.54 ± 0.81b	5.77 ± 0.70b	7.99 ± 0.77b

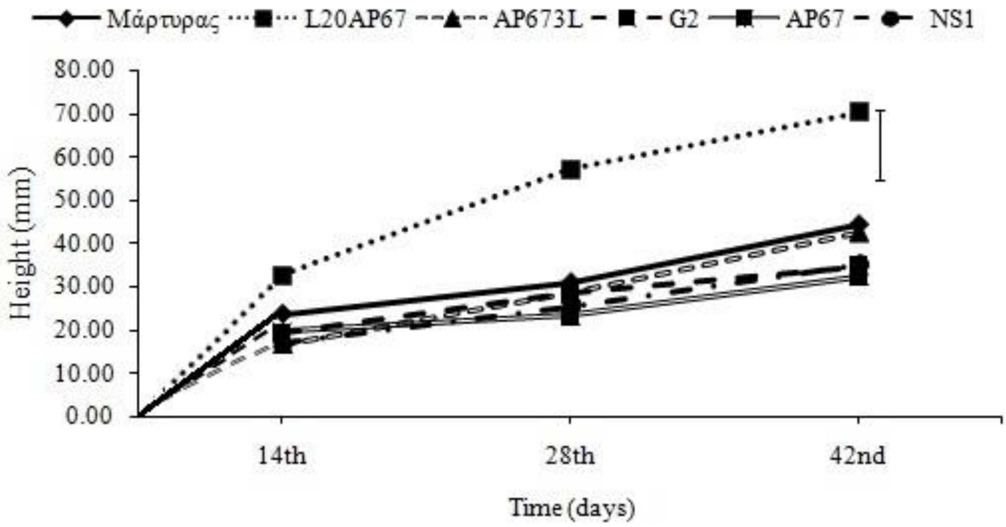

Figure 1. Growth rate from the 14th, 28th and 42th day of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Vertical bar represents the LSD.

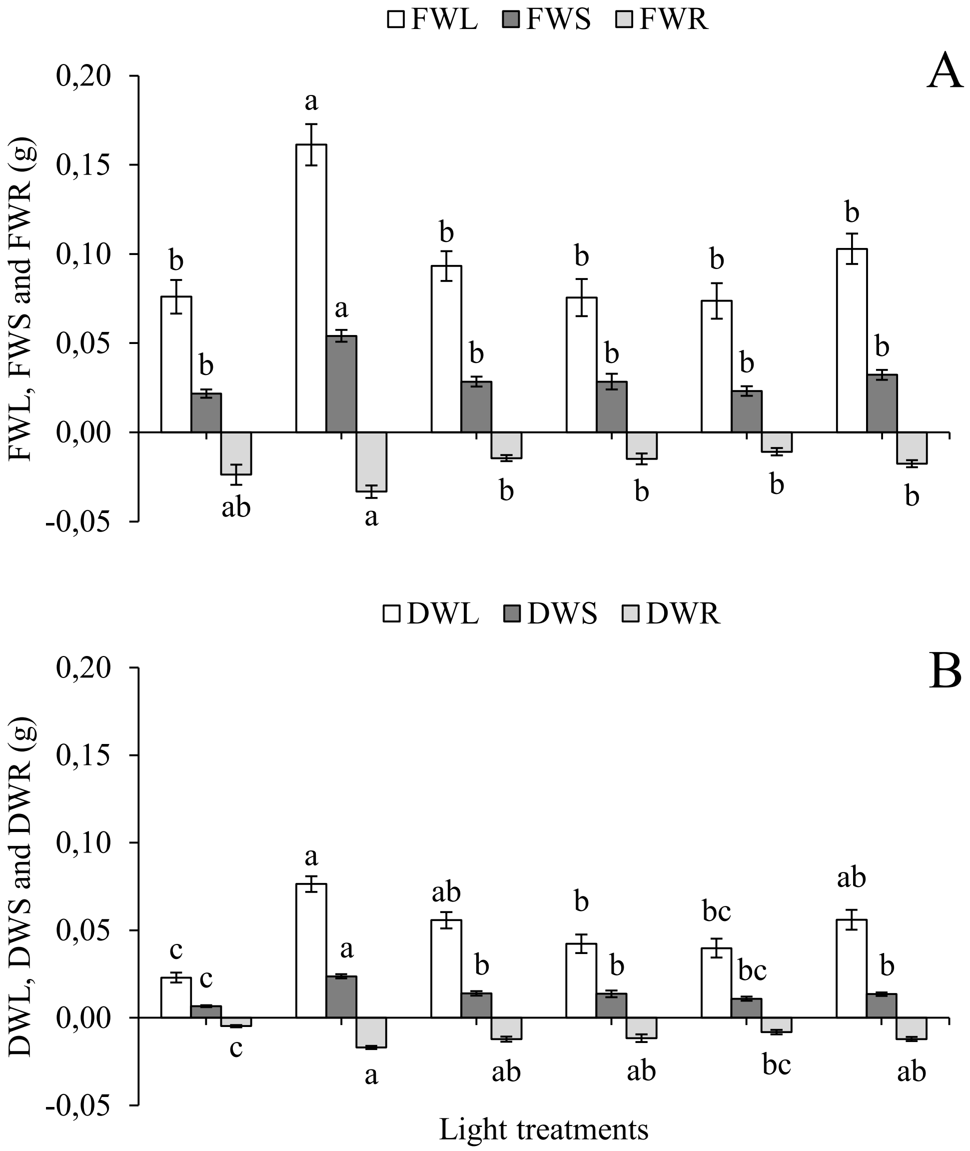

Figure 2. Fresh weight of leaves (FWL), shoots (FWS) and roots (FWR) (A), and dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (B) of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE. Bars followed by a different letter within a parameter differ significantly (a = 0.05).

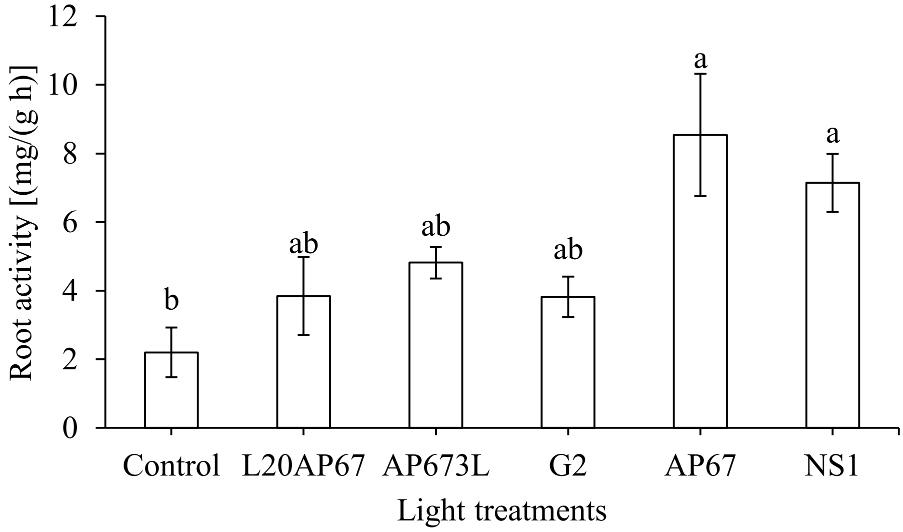

Figure 3. Root activity of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=5) ±SE. Error bars represent the SE. Bars followed by a different letter differ significantly (a = 0.05).

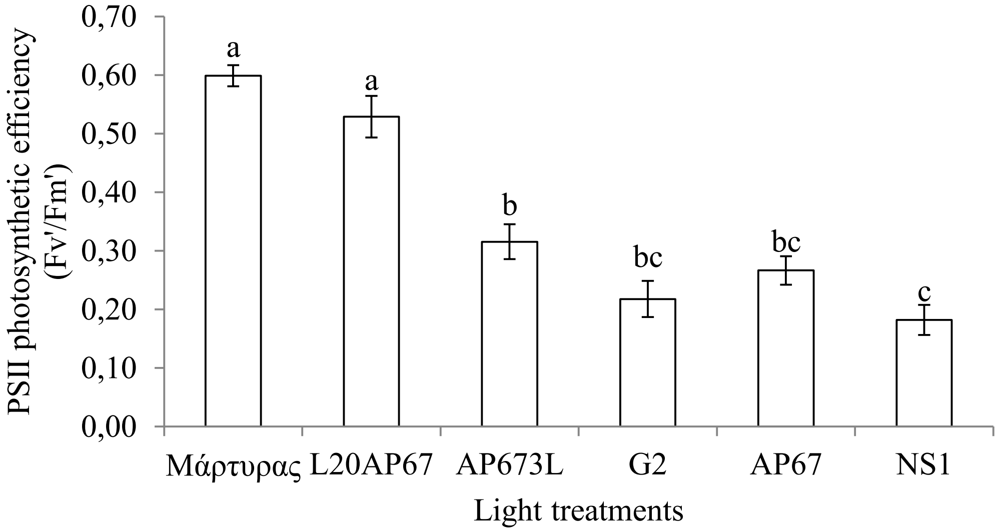

Figure 4. PSII photosynthetic efficiency of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=5) ±SE. Error bars represent the SE. Bars followed by a different letter differ significantly (a = 0.05).

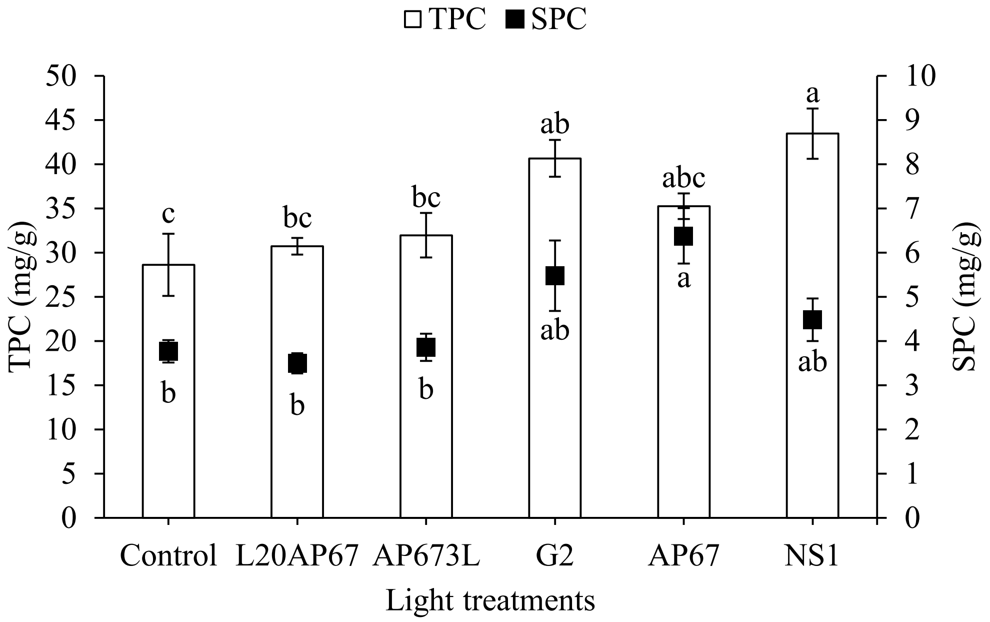

Figure 5. Total and simple phenolic content (TPC and SPC) of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE. Bars followed by a different letter differ significantly (a = 0.05).

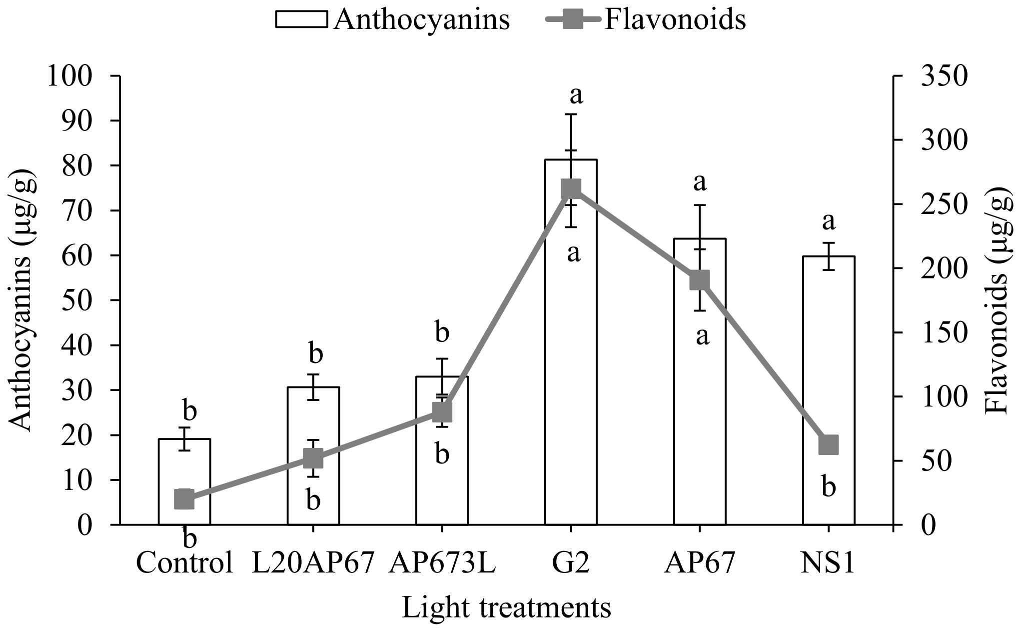

Figure 6. Flavonoid and anthocyanin content of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE. Bars and lines followed by a different letter differ significantly (a = 0.05).

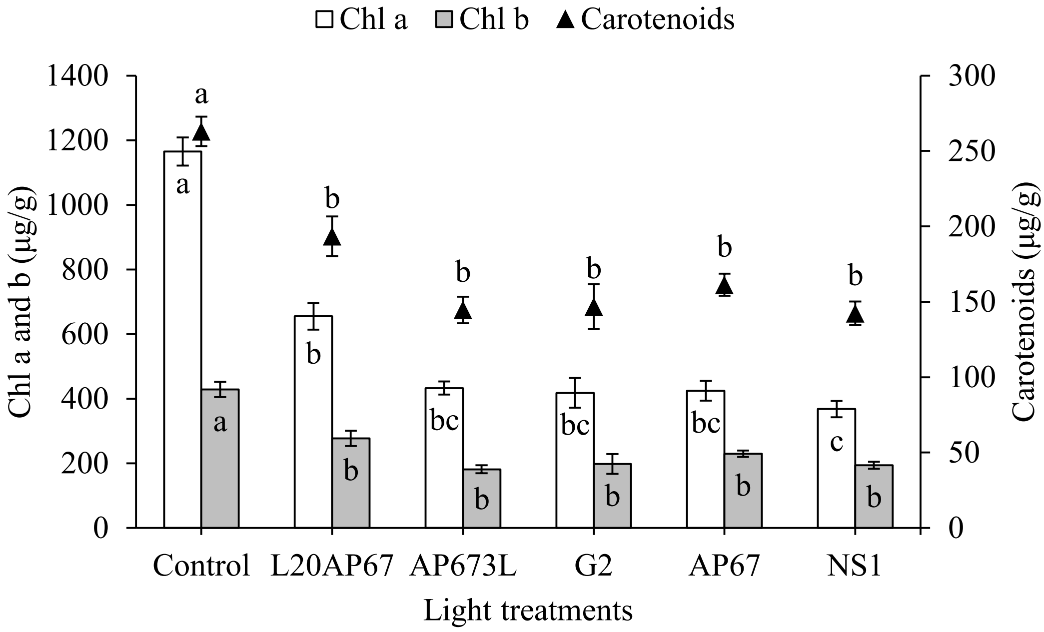

Figure 7. Chlorophyll a and b (chl a and chl b), and carotenoid content of *Punica granatum* seedlings grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE. Bars and lines followed by a different letter differ significantly (a = 0.05).

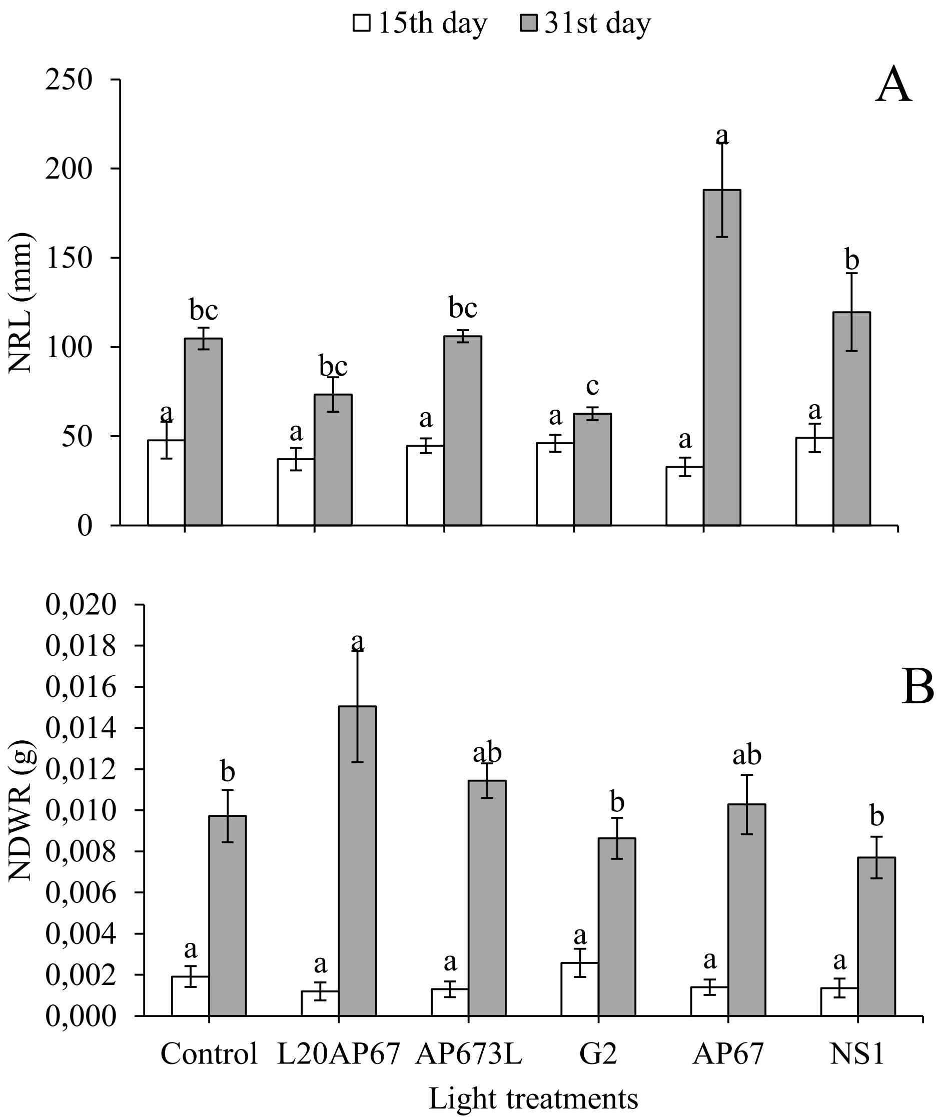

Figure 8. New root length (NRL) (A) and dry weight of new roots (NDWR) (B) of *Punica granatum* seedlings after 15 and 31 days in the RGC water bath after grown under the six different light treatments with the same abbreviations as in Table 1. Data are mean values (n=10) ±SE. Error bars represent the SE. Bars followed by a different letter differ significantly (a = 0.05).

Frangula azorica under different LED treatments

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The effects of five light types on the growth of the aerial part and roots of micropropagated plants of *Frangula azorica* were studied during five weeks. The light types were: Osram L36W/77 FLUORA (Fluorescent), 2 bar lamps Valoya AP67 (120 cm), 2 bar lamps Valoya AP673 L (120cm), 2 bar lamps Valoya G2 (120cm), 2 bar lamps Valoya NS1 (120cm).

Materials and methods

The micropropagated plants were put in Quickpot HercuPlast QPD 104 VW trays with preforma Jiffy substrate. They were placed in a dark room on shelves of two inox trolleys, each shelf equipped with a certain light type, photoperiod set for 10 hours of light. In the first 17 days, the trays with the plants were inside a plastic bag to maintain a high humidity environment. The room temperature was about 22ºC.

Ten days after the transplant of the micropropagated plants of *F. azorica* the measurements started and were taken once a week, during five weeks. For that, seven plants growing under each light type were taken off the trays at random and their roots were washed over a mesh screen to take out the soil particles and immediately dried in paper towel. After that the height of the 35 plants were obtained (aerial part), the plants were separated in the aerial and root parts.

Each fraction was than weighted, in fresh and after drying for two days at 55ºC, in a weighing scale that measured in mg.

Data were analysed using one way ANOVA of the StatPlus:mac , Copyright 2009 AnalystSoft Inc.

The means were compared with the test of Tukey-Kramer at $P \leq 0.05$

Fig. 2. The micropropagated plants, cordiality of the Azores Biotechnology Centre

Fig. 3. Plants under various light treatments

Fig. 4. Weekly sampling and analysing of root and shoot growth

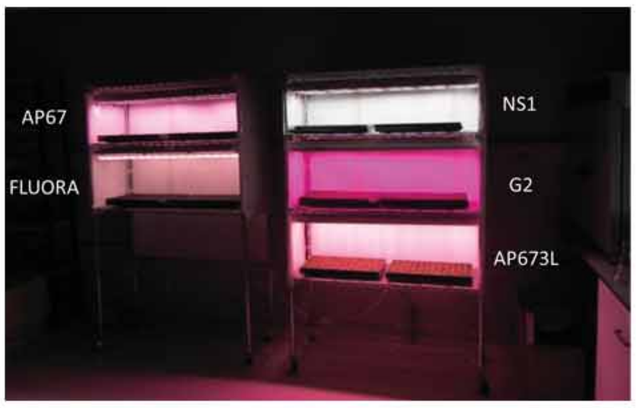

Fig. 5. Distribution of LED lights

Fig. 6. Preparation of samples

Fig. 7. The 5-week grown plants under Fluora light

Fig. 8. The 5-week grown plants under AP67 light

Results

The differences in the dry weight of stems and leaves of *Frangula azorica* under different light types were statistically different only in the last harvest although AP67 and NS1 had higher weights than the other plants in the 4th harvest but not yet significantly different. In the 5th harvest the weights of the plants under the lights AP 67 were significantly higher than the weights of the plants under the other light types, except NS1 (Table 1).

The differences in the dry weight of the roots of *Frangula azorica* under different light types were statistically different only in the last harvest although AP 67 and NS1 had higher weights than the others in the 4th harvest but not yet significantly different. In the 5th harvest the weights of the roots under the lights AP 67 were significantly higher than the weights of the roots under the other light types, except NS1 (Table 1).

The dry matter content (DM) of the plants under Fluora light, since the first harvest, was significantly lower than the DM of the plants under AP67 (Table 3). Although the plants in the Fluora treatment were as tall as the plants under AP67 and NS1 (Fig. 1) , their dry weights were lower and it was possible to see that the plants were less lignified (Fig. 7) that the plants under the other light sources, specially under AP67 (Fig. 8), AP 673 L and NS1. In average, over all harvests, NS1 had more leaves (14) than all the other treatments (11). For each light type, after the second harvest, the number of leaves was almost constant, since as new leaves were formed some died.

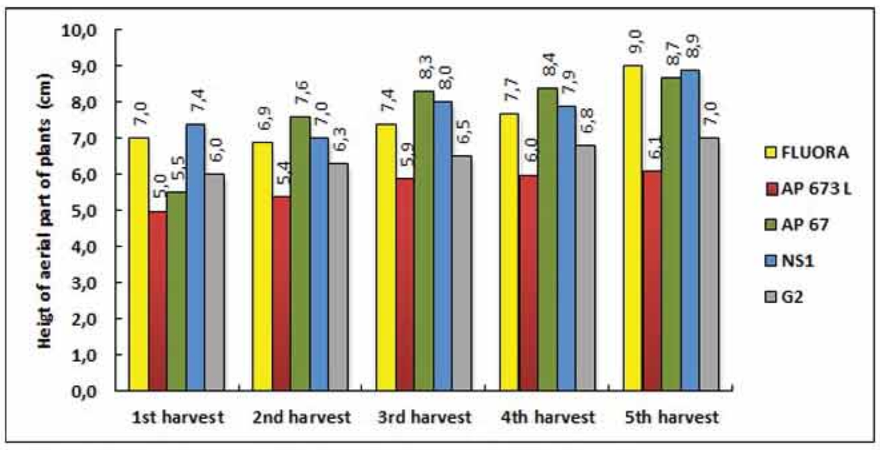

Fig. 1. Height of aerial part of plants (cm) in the five harvests

Table 1. Dry weights (g) of stems and leaves of *Frangula azorica* in the five harvests.

Dry weights of stems and leaves (g)						
LIGHT TYPE	1stharvest	2nd harvest	3rdharvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0236 ^{a}	0.0257 ^{a}	0.0374 ^{a}	0.0423 ^{a}	0.0645 ^{b}	0.0387
AP 673 L	0.0135 ^{a}	0.0252 ^{a}	0.0338 ^{a}	0.0309 ^{a}	0.0436 ^{b}	0.0294
AP 67	0.0225 ^{a}	0.0477 ^{a}	0.0369 ^{a}	0.0595 ^{a}	0.1194 ^{a}	0.0572
NS1	0.0215 ^{a}	0.0273 ^{a}	0.0335 ^{a}	0.0564 ^{a}	0.0699 ^{ab}	0.0417
G2	0.0182 ^{a}	0.0197 ^{a}	0.0194 ^{a}	0.0251 ^{a}	0.0299 ^{b}	0.0225
AVERAGE	0.0199	0.0291	0.0322	0.0428	0.0655	

Note 1: The average dry weight of stems and leaves at start was 0.0084 ± 0.0041 g
Note 2: In each harvest column the means with the same letters were similar at P=0.05

Table 2. Dry weights (g) of roots of *Frangula azorica* in the five harvests.

Dry weights of roots (g)						
LIGHT TYPE	1stharvest	2nd harvest	3rdharvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0045^{a}	0.0047 ^{a}	0.0085 ^{a}	0.0106 ^{a}	0.0159 ^{c}	0.0088
AP 673 L	0.0034^{a}	0.0062 ^{a}	0.0110 ^{a}	0.0115 ^{a}	0.0220 ^{c}	0.0108
AP 67	0.0050^{a}	0.0090 ^{a}	0.0117 ^{a}	0.0161 ^{a}	0.0490 ^{a}	0.0182
NS1	0.0038^{a}	0.0062 ^{a}	0.0057 ^{a}	0.0183 ^{a}	0.0267 ^{ab}	0.0121
G2	0.0045^{a}	0.0036 ^{a}	0.0055 ^{a}	0.0089 ^{a}	0.0140 ^{bc}	0.0073
AVERAGE	0.0042	0.0059	0.0085	0.0131	0.0255	

Note1: The average dry weight of roots at star was 0,0025 ± 0.0015 g
Note 2: In each harvest column the means with the same letters were similar at P=0.05

Table 3. Stems and leaves dry matter content (%) in each harvest.

Stems and leaves dry matter (%)					
LIGHT TYPE	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	21.29 ^{c}	26.76 ^{b}	29.72 ^{b}	32.80 ^{b}	27.64
AP 673 L	27.90 ^{ab}	32.56 ^{a}	34.50 ^{ab}	36.54 ^{ab}	32.88
AP 67	25.11 ^{b}	34.79 ^{a}	35.10 ^{a}	40.49 ^{a}	33.87
NS1	28.81 ^{ab}	32.18 ^{a}	32.36 ^{ab}	36.45 ^{ab}	32.45
G2	28.82 ^{a}	33.43 ^{a}	31.03 ^{b}	31.84 ^{b}	31.28
AVERAGE	26.39	31.94	32.54	35.62	

Note: In each harvest column the means with the same letters were similar at P=0.05

Juniperus brevifolia under different LED treatments

Raquel Ferreira and Malgorzata Pietrzak
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The effects of five light types on the growth of the aerial part and roots of micropropagated plants of *Juniperus brevifolia* were studied during five weeks. The light types were: Osram L36W/77 FLUORA (Fluorescent), 2 bar lamps Valoya AP67 (120 cm), 2 bar lamps Valoya AP673 L (120cm), 2 bar lamps Valoya G2 (120cm), 2 bar lamps Valoya NS1 (120cm).

Materials and methods

The micropropagated plants were put in Quickpot HercuPlast QPD 104 VW trays with preforma Jiffy substrate. They were placed in a dark room on shelves of two inox trolleys, each shelf equipeed with a certain light type, photoperiod set for 10 hours of light. In the first 17 days the trays with the plants were inside a plastic bag to maintain a high humidity environment. The room temperature was about 22ºC.

Ten days after the transplant of the micropropagated plants of *J. brevifolia* the measurements started and were taken once a week, during five weeks. For that, seven plants growing under each light type were taken off the trays at random and their roots were washed over a mesh screen to take out the soil particles and immediately dried in paper towel. After that the height of the 35 plants were obtained (aerial part), the plants were separated in the aerial and root parts. Each fraction was than weighted, in fresh and after drying for two days at 55ºC, in a weighing scale that measured in mg.

Data were analysed using one way ANOVA of the StatPlus:mac , Copyright 2009 AnalystSoft Inc. The means were compared with the test of Tukey-Kramer at $P \leq 0.05$

Fig. 3. The micropropagated plants, cordiality of the Azores Biotechnology Centre

Fig. 4. Distribution of LED lights

Fig. 5. Harvested and identified samples

Fig. 6. Weighing of aerial part

Fig. 7. The 5-week grown plants under AP673L light

Fig. 8. The 5-week grown plants under NS1 light

Results

The differences in the dry weight of stems and leaves and roots of *Juniperus brevifolia* under different light types were not statistically different but AP 67 and AP 673 L had generally lower weights than the others in the last three harvests (Table 1). For each light source the aerial part of the plants and the roots always had the same pattern of growth (Fig 1 and 2).

In the 5th harvest the plants of *J. brevifolia* had 5.1 cm in the NS1 light, 4.6 cm in the G2 and 4.2 cm in the other lights. The micropropagated plants had 2 or 3 branches and the plants continue to ramificate. Since the 2nd harvest the plants under Fluora light have had significantly lower dry matter content (Table 3) than the plants under the other lights.

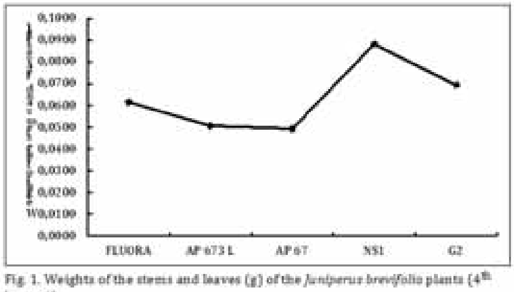

Fig. 1. Weights of the stems and leaves (g) of the *Juniperus brevifolia* plants (4th harvest)

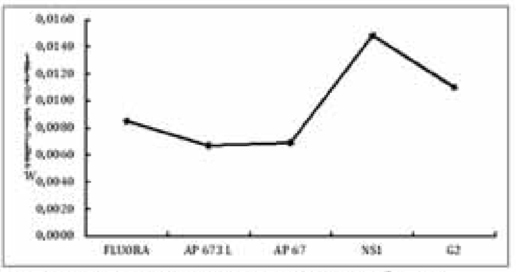

Fig. 2. Weights of the roots (g) of *Juniperus brevifolia* plants (4th harvest)

Table 1. Dry weights of stems and leaves of *Juniperus brevifolia* in the five harvests.

Stem and leaves dry weights (g)						
LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0526 ^{a}	0.1020 ^{a}	0.0796 ^{a}	0.0614 ^{a}	0.0588 ^{a}	0.0709
AP 673 L	0.0401 ^{a}	0.0551 ^{a}	0.0459 ^{a}	0.0505 ^{a}	0.0602 ^{a}	0.0504
AP 67	0.0529 ^{a}	0.0435 ^{a}	0.0564 ^{a}	0.0492 ^{a}	0.0625 ^{a}	0.0529
NS1	0.0825 ^{a}	0.0689 ^{a}	0.0708 ^{a}	0.0881 ^{a}	0.0915 ^{a}	0.0804
G2	0.0701 ^{a}	0.0571 ^{a}	0.0467 ^{a}	0.0694 ^{a}	0.1027 ^{a}	0.0692
AVERAGE	0.0596	0.0653	0.0599	0.0637	0.0751	

Note 1: The average dry weight of the aerial part of plants at start was 0.0467 ± 0.0193 g
Note 2: In each harvest column the means with the same letters were similar at P=0.05

Table 2. Dry weights of the roots of *Juniperus brevifolia* in the five harvests.

Roots dry weights (g)						
LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0073 ^{a}	0.0157 ^{a}	0.0089 ^{a}	0.0085 ^{a}	0.0103 ^{a}	0.0101
AP 673 L	0.0054 ^{a}	0.0088 ^{a}	0.0042 ^{a}	0.0067 ^{a}	0.0085 ^{a}	0.0067
AP 67	0.0077 ^{a}	0.0058 ^{a}	0.0075 ^{a}	0.0069 ^{a}	0.0082 ^{a}	0.0072
NS1	0.0097 ^{a}	0.0083 ^{a}	0.0098 ^{a}	0.0148 ^{a}	0.0136 ^{a}	0.0112
G2	0.0090 ^{a}	0.0071 ^{a}	0.0059 ^{a}	0.0110 ^{a}	0.0175 ^{a}	0.0101
AVERAGE	0.0078	0.0091	0.0073	0.0096	0.0116	

Note 1: The average dry weight of the roots at start was 0.0076 ± 0.0038 g
Note 2: In each harvest column the means with the same letters were similar at P=0.05

Table 3. Stems and leaves dry matter content in the five harvests (%)

Stems and leaves dry matter content (%)						
LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	32.22 ^{a}	29.02 ^{b}	29.04 ^{b}	29.24 ^{b}	28.57 ^{b}	28.97
AP 673 L	34.68 ^{a}	29.66 ^{b}	32.34 ^{ab}	30.88 ^{ab}	29.13 ^{ab}	30.50
AP 67	30.26 ^{ab}	27.17 ^{b}	33.51 ^{ab}	30.51 ^{ab}	29.56 ^{ab}	30.19
NS1	31.65 ^{ab}	36.92 ^{a}	35.35 ^{a}	32.94 ^{a}	32.71 ^{a}	34.48
G2	24.79 ^{b}	29.33 ^{b}	34.41 ^{a}	31.27 ^{ab}	30.45 ^{ab}	31.37
AVERAGE	30.72	30.42	32.93	30.97	30.08	

Note: In each harvest column the means with the same letters were similar at P=0.05

REDUCING THE IMPACT OF FOREST PLANT PRODUCTION
- design of a stand-alone PV-hybrid system for powering an innovative forestry incubator

ZEPHYR

Zero-impact Innovative Technology in Forest Plant Production

Sreenivaasa R Pamidi [1], Marco Hernández Velasco [2]*

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[2] Solar Energy Research Center; Dalarna University, 791 88 Falun, Sweden
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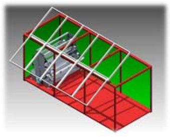
Background

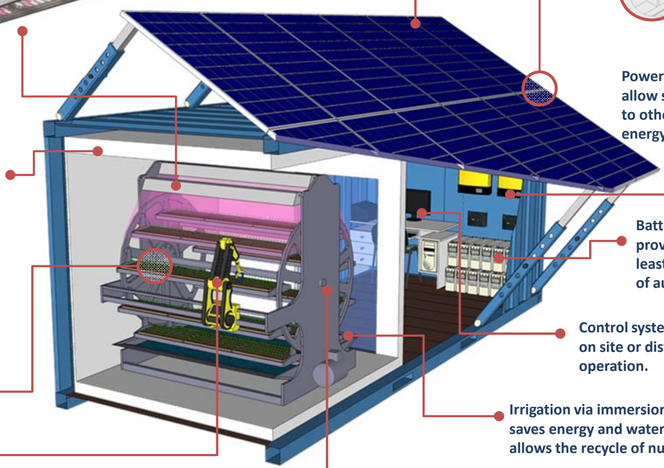
Nowadays, the high demand of forestry products imposes a great pressure on the ecosystems and can derive in biodiversity loss and other ecological problems. Planted forests can contribute to more sustainable practices and help addressing other issues of global concern such as climate change, erosion and desertification. Large scale production of seedling is required to offset the high harvesting rates; however these intensive methods often have a negative impact on the environment.

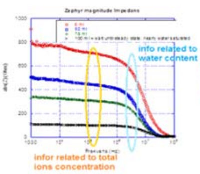
Objectives

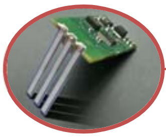
Funded by the European Commission under the Seventh Framework Programme (FP7), the ZEPHYR project consortium has the goal of developing innovative and cost-friendly technologies for the pre-cultivation of forestry species. These will be integrated into a functional and transportable system for pre-cultivation of seedlings, with zero-impact on the environment and not affected by outdoor conditions.

Growth differences in *Fagus sylvatica* using LED lights (left) and fluorescent lights (right)









2,5 kWp photovoltaic system and low energy consumption devices contribute to an operation with a zero-impact on the environment

Foldable structure mounted on a standard TEU container for practical transport.

Passively cooled LED-lamps provide a continuous light spectrum optimized for the seedlings' growth.

Power electronics allow switching to other auxiliary energy sources.

Extra insulation in chamber gives better temperature and humidity control. Closed environment eliminates the need for pesticides.

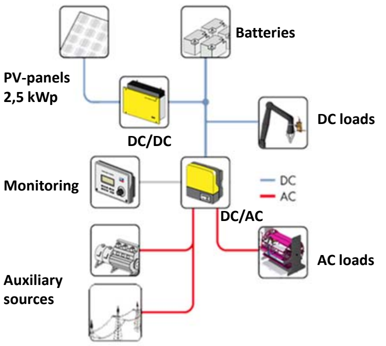
Battery bank provides at least one day of autonomy.

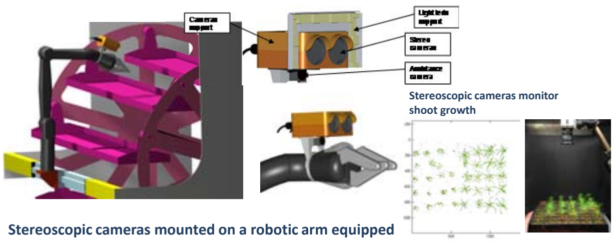
New family of miniaturized wireless sensors placed in the pots to measure the soil conditions

Control system allows on site or distance operation.

Irrigation via immersion bath saves energy and water and allows the recycle of nutrients.

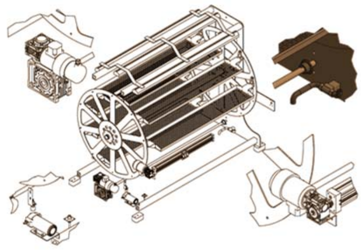
PV-system design



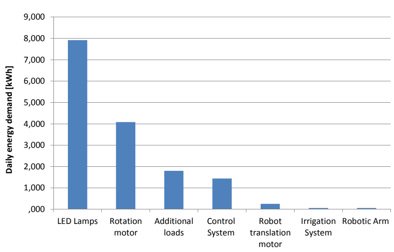


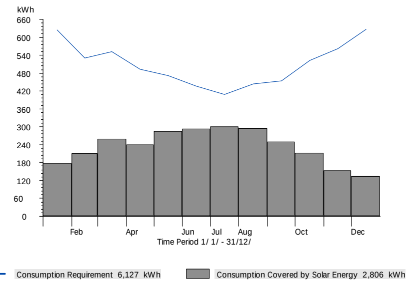
Stereoscopic cameras mounted on a robotic arm equipped with a gripper for operations inside the controlled environment

The rotating system saves space and ensures homogeneous temperature and humidity. Intermittent light has proven to give excellent growth results in forest seedlings.



Energy analysis for PV-system design





*Excluding HVAC which is dependant of the location and species cultivated

Comparison to traditional system

	Swedish forest nursery	ZEPHYR incubator
Technology	Modern Greenhouse	Rotating shelves with LED lightning
Production capacity	10.000.000 seedling per year	80.000 seedlings per year
Energy consumption	2.500.000 kWh	13.500 kWh
Energy per seedling	0,25 kWh/seedling	0,17 kWh/ seedling
Energy source	Oil (250 m^3)	Electricity (assuming tariff 0,90 SEK/kWh)
Cost of energy	0,25 SEK/seedling	0,15 SEK/seedling

Assuming peak power consumption of all devices and HVAC running 24 hours.

Project Outcomes

- Innovative and light growth chamber with a production capacity of up to 12000 plantlets per cycle in a fully automatized and controlled environment.
- New family of wireless sensors studied for controlling the growth status
- High energy efficiency, due to space saving through the rotating system and the use of LED lights; up to 70% respect to traditional nurseries.
- Full automatized cycle production inside a standard TEU container powered with PV panels
- Flexible: more units can be placed in series for a bulk production

European Commission

www.zephyr-project.eu
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Total Cost: 4,284,275 € - EC Contribution: 3,438,252 €
Duration: 36 months - Start Date: 01/10/2012
Consortium: 14 partners from 10 countries
Project Coordinator: Tuscia University – DAFNE Department (Italy)

Environmental technologies

Università Tuscia · Torsanlorenzo · azorina · Swedish ICT Acreo · Fraunhofer IFAM · Dalarna University · robosoft · Valoya · advanticsys · exergy · CETMA · Cemet Art

REDUCING THE IMPACT OF FOREST PLANT PRODUCTION - DESIGN OF A STAND-ALONE PV-HYBRID SYSTEM FOR POWERING AN INNOVATIVE FORESTRY INCUBATOR

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ABSTRACT: Nowadays, the high demand of forestry products imposes a high pressure on the ecosystems and can derive in biodiversity loss and other ecological problems. Planted forests can contribute to more sustainable practices and help addressing other problems of global concern such as climate change, erosion and desertification. Large scale production of seedling is required to offset the high harvesting rates; however these intensive methods often have a negative impact on the environment. Funded by the European Commission under the Seventh Framework Programme (FP7), the ZEPHYR project consortium is developing innovative and cost-friendly technologies for the pre-cultivation of forestry species. These will be integrated into a functional and transportable system for pre-cultivation of seedlings, with zero-impact on the environment and not affected by outdoor conditions. To achieve this, the incubator will be powered mainly by solar energy. This work aims to present the efforts made to design and optimize the solar photovoltaic (PV) system which will be mounted on the roof of the unit. Especially developed devices such as LED growth lamps and wireless sensors will be used to reduce energy consumption and monitor the cultivation process. A load profile study was conducted and the growth protocols were adapted to perform most of the tasks during daytime to use the energy from the PV panels directly. A battery bank will be designed to provide at least one day of autonomy in central European latitudes. Moreover, the power system will also be capable of connecting to the electricity grid or use a diesel generator as a back-up.
Keywords: Stand-alone PV Systems, Off-grid system, forestry incubator

1 INTRODUCTION

Currently, forest ecosystems are facing many sustainability problems due to drastic climate changes and extreme exploitation of their resources. Unfortunately, forests have a limited capacity of adaptation and it means that forest ecosystems and therefore forest biodiversity are seriously in danger. Thus, the problem of forest regeneration materials is becoming even more important.

At the moment, there exist several alternatives such as greenhouses or plant growth chambers that allow producing forest materials more rapidly. However these systems consume considerable high amounts of energy for lighting, acclimatization and irrigation having a negative impact on the environment.

1.1 Aims

Funded by the European Commission under the Seventh Framework Programme (FP7), the ZEPHYR project.aims to introduce an innovative technology built on pre-cultivation of forest regeneration materials in a zero-impact and cost-friendly production unit. The project will integrate several technologies into a functional and transportable system for large scale production of pre-cultivated forest regeneration materials adapted to transplanting and further growth at forest nurseries.

The expected project outcomes are:

- Innovative and light growth chamber with a production capacity of up to 12000 plantlets per cycle in a fully automatized and controlled environment.
- New family of wireless sensors studied for controlling the growth status
- High energy efficiency, due to space saving through the rotating system and the use of LED lights; up to 70% with respect to traditional nurseries.
- Full automatized cycle production inside a standard TEU container powered with PV panels
- Flexible: more units can be placed in series for a bulk production

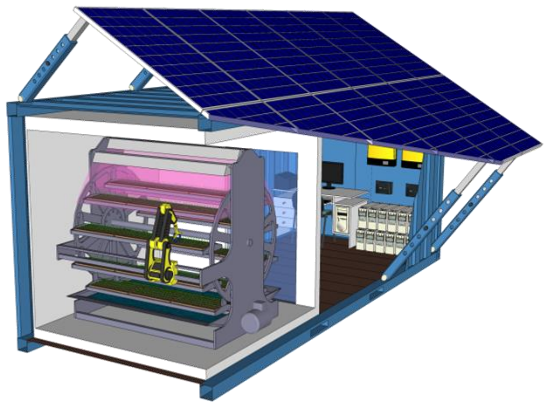

Figure 1: Concept of the incubator

The new integrated technology will make a drastic change to state-of-the-art in forest nursery production for reforestation purposes: apart from being more resource-efficient, it will also contribute to the environmental protection through: biodiversity defending, water recycling, strong reduction of fertilizers and avoidance of pesticides.

A transportable and closed incubator independent from the outdoor climate provides a better control on the seedlings production. The plants can be produced directly at the place where they are needed avoiding further transportation to the reforestation/afforestation zone. The closed-climate allows seedlings pre-cultivation in places where it would not be possible otherwise (e.g. near deserts). Additionally, it extends the production time throughout the whole year even during the winter. Moreover, it will allow a certified and standardized production of reforestation materials, with a noticeable increasing of the efficiency of the reforestation operations.

Specially developed LED growth lamps and wireless sensors will be used to reduce energy consumption and monitor the cultivation process. The main part of the energy will be provided by solar PV-panels, depending from the geographic and climatic area the power system should be able to provide at least one day of autonomy (in central Europe). The energy savings will result in a reduction of greenhouse gas emissions; moreover, since the LED lamps do not produce additional warming, there will be further energy saving through the reduction of air conditioning costs.

2 DESIGN OF THE PV-SYSTEM

The aim of the technical design for the power supply system in the Zephyr project is mainly to enable a standalone off-grid system with Solar PV as the primary energy source along with a Battery unit as energy storage. Integration of additional sources (Diesel generator set or local Grid) when available can be used to support the demand levels and ensure continuous energy supply to the unit.

The design considerations for a typical solar photovoltaic system, both off-grid and on-grid, depends on solar resource or weather data, system mechanical and electrical characteristics, load characteristics and economic parameters. These four parameters are essential for the design of an optimal PV system. The Zephyr design has considered limitations with availability of space, both on the roof and inside the transport unit, enabling continuous energy flow along with safe mode of transportation. These challenges regarding required flexibility of the design for Zephyr project has required several issues to be confronted and changes to the design of the power supply system to be made.

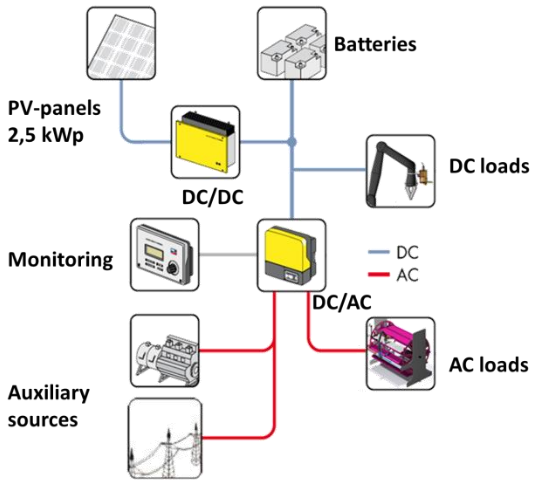

Figure 2: Basic Power system design

Options were considered to design the system in a way which can incorporate additional energy sources, i.e. diesel generator set and Grid supply, whenever available. In order to incorporate these options, new products/devices which can integrate and provide smarter power management to switch to additional sources when required without interrupting the energy flow to the subsystems, are required. Products such as a bidirectional inverter there which incorporate easy installation are difficult to find (especially in support of DC systems) and also results in increased complexity of the system layout or design.

Conventional Inverter manufacturers provide products to support standalone and off-grid applications along with the possibility to incorporate diesel generator and grid connectivity. Identification of the product and reducing the design complexity can be observed through this option, the products can regulate the energy flow from the battery bank, Solar PV and additional sources when connected. The design of the system using these products to provide output at 24V DC is not feasible; options are to be considered only in using AC loads.

The solar PV power supply system to power the Zephyr unit is a stand-alone solar PV-Hybrid system with battery backup and capability to use additional sources like local grid or diesel generator (DG set) whenever available, mainly depending on the location. The main power source is from the PV-battery system and the system is designed to be compatible with local grid (mainly in Europe) or DG set at different locations across Europe based on the requirement.

The PV-Hybrid system has greater reliability with regards to electricity production than PV systems with power generation from different sources. The system consists of solar panels, battery bank, MPPT charge controller and inverter, and assumes there is no shading and negligible snow and soiling losses associated for calculation and design purpose.

Typically, sizing an off-grid system is usually done by sizing the PV system alone or in conjunction with supplementary power source(s), to match the load requirements. The main conditions to be satisfied while designing an off-grid PV system are:

- Sufficient inverter capacity to meet peak power demands (especially for AC systems)
- PV Array sized to meet energy needs after a minimum number of days of autonomy under seasonal weather patterns.
- PV array or PV-Hybrid system sized to meet maximum allowable days of backup generator use

System sizing and integration is the process of matching the specifications of various individual components to the operating boundary conditions listed for the location. It is the complex and important part of a PV design as not all components available are compatible with each other and certainly not in all environments. Understanding the location data is the starting point as these are natural variables and uncontrollable.

The battery and the inverter part of the design are mainly sized to match the load requirement and number of days of autonomy. Engineering the design of the system from the back (i.e. loads) to the energy source(s) to match the daily requirement is optimal for an off-grid unit, taking into consideration the limited space on the roof of the container, relying on the PV source alone is not feasible. Once the basic requirement is considered in sizing the inverter and battery bank to match the load requirement, the PV panels have been compared with different components/manufactures in order to understand the performance of the entire system. Later, depending on the space availability, the number of panels is calculated, and the remaining additional energy is supplied through additional sources (local grid or DG set) depending on the location and accessibility factors. Additionally, simulations were performed using the components from the sizing data in PV*SOL Expert 6.0.

2.1 Load Characteristics

For off-grid systems, load characteristics indicate the storage needs, PV system size and backup power capacity, if needed. The design will typically include enough storage capacity to satisfy energy requirements during extended periods of poor weather conditions, otherwise known as days of autonomy; some also refer to as loss of load probability (LOLP) instead of days of autonomy. In this design, one day of autonomy is considered for the estimation of number of batteries in Zephyr unit.

Below is the chart of the loads for the Zephyr subsystems, based on the load profiles of individual components, an estimation of daily energy requirement is estimated.

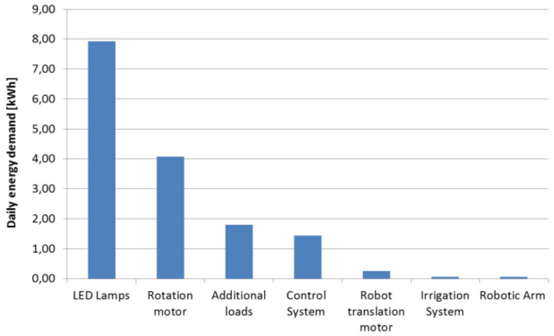

Figure 3: Load analysis of the different components

The loads are classified into AC and DC as the requirement of Robotic arm is specified in DC. Taking in to account these considerations the total daily energy requirement is calculated below:

- The total load from Zephyr unit = 34755Wh/day (AC) +60Wh/day (DC)
- The efficiency of the inverter chosen is assumed to be 93%; therefore, total required power to be delivered from the power source:
  (PVBattery/DG set/Local Grid) = 34755/0.93 + 60 = **37.431 kWh/day** is to be supplied for the Zephyr unit.
- With the data available on the daily loads of the system, design and minimum requirements for/comparing 2 zones in Europe in Belgium (Brussels) and Italy (Viterbo) is studied.

3 RESULTS

In this section, the simulation results and analysis is made for both the locations (year round data is considered). Considerations made out of the PV output analysis on the number of panels and battery are used accordingly for simulation in PV*Sol, keeping in mind the roof space available.

PV*Sol calculates the electrical layout of the system and accordingly calculates the size of a charge controller, it also checks for any faulty connections, voltage drops, minimizes technical losses and derives maximum output, but the details with regards to losses are less well represented. The design consideration and assumptions in PV*Sol are listed below:

- The system considered 10 panels for 250Wp and 20 batteries along with the use of backup diesel generator for charging the batteries and supplying additional loads as the simulation is performed over a year.
- The same inverter data is uploaded and corresponding simulations are performed.

3.1 Results Brussels

Simulation data for climatic conditions at Brussels using 250Wp panel is analyzed. The optimal angle for design of a PV system in Brussels is 35°. Figure 4presents the results of the simulation showing that the energy from the solar PV system is 1,739 kWh. Besides, this simulation shows the consumption covered by the DG set is 11,917 kWh and is distributed among the load and the battery bank as storage.

The battery bank used has 85% charge efficiency and the efficiency of the inverter used is 92%.

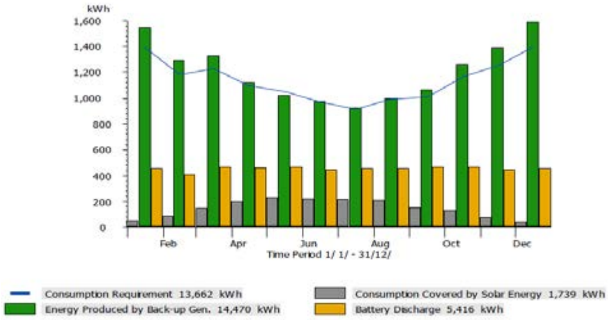

Figure 4: Simulation results for the location Brussels, Be

3.2 Results Viterbo

The climatic conditions in PV*Sol Expert 6.0 are changed to Viterbo from Brussels, without changing the system configuration. The climatic data for Viterbo is quite different from that of Brussels, as it is located south (to Brussels) the solar radiation is comparatively high and the average sun hours per day are also high which results in more consumption covered by the system

Though the consumption covered by the system is increased compared to the system at Brussels with the increase in solar radiation, the consumption requirement is still not completely satisfied by the system. This requirement can be satisfied by the same methods employed for Brussels i.e. by diesel generator set.

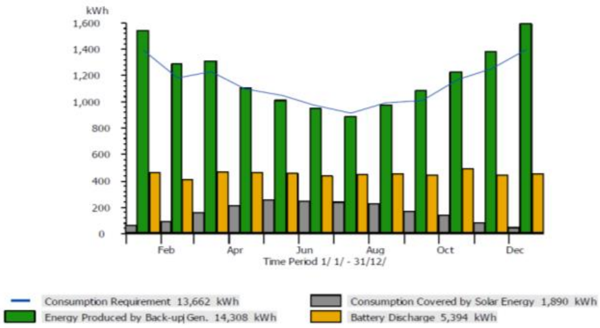

Figure 5: Simulation results for the location Viterbo, It

4 CONCLUSIONS

The typical system discussed in this paper is a solar PV-battery system for Brussels; this is a basic design of the power supply system for Zephyr project. The proposed system is designed and analysed using PV*Sol Expert 6.0 with energy backup supply and simulations are performed for different locations as well. The solutions proposed for various locations for the Zephyr unit include Solar PV-Hybrid system to provide continuous power supply to the Zephyr subsystems.

In focus to define the technical specifications of the power supply system for Zephyr project. The study also aimed to compare the design of the system for different locations in Brussels and Viterbo as the main project site where demonstration and installation activities scheduled to take place.

The system was designed to supply continuous power to the subsystems involved in the plant production unit. The design of the system is focused on flexibility to connect and operate using different power source options. Based on the technical specifications and use of foldable or sliding mounting structure have been studied and presented.

The design of the system using different panels is compared in order to compare the performance and the flexibility in installation & operation during the later stages of the project. As the Zephyr unit is a prototype, part of a research project, the prototype will be built to perform tests under realistic conditions/field before giving the final specification for the possible future production, thus this study allows the design of power supply system for the challenging situation as a framework of the unit design.

Based on the results of various simulations discussed in the project, the basic Solar PV-Battery system is highly infeasible and additional sources of power supply for continuous generation is necessary.

6 REFERENCES

[1] ZEPHYR | Zero-impact innovative technology in forest plant production [Internet]. Available from: <http://www.zephyr-project.eu/>

A SILVICULTURAL PRACTICE TO FACILITATE FOREST RESTORATION
– a new seedling cultivation technology for regeneration establishment

ZEPHYR

Zero-impact Innovative Technology in Forest Plant Production

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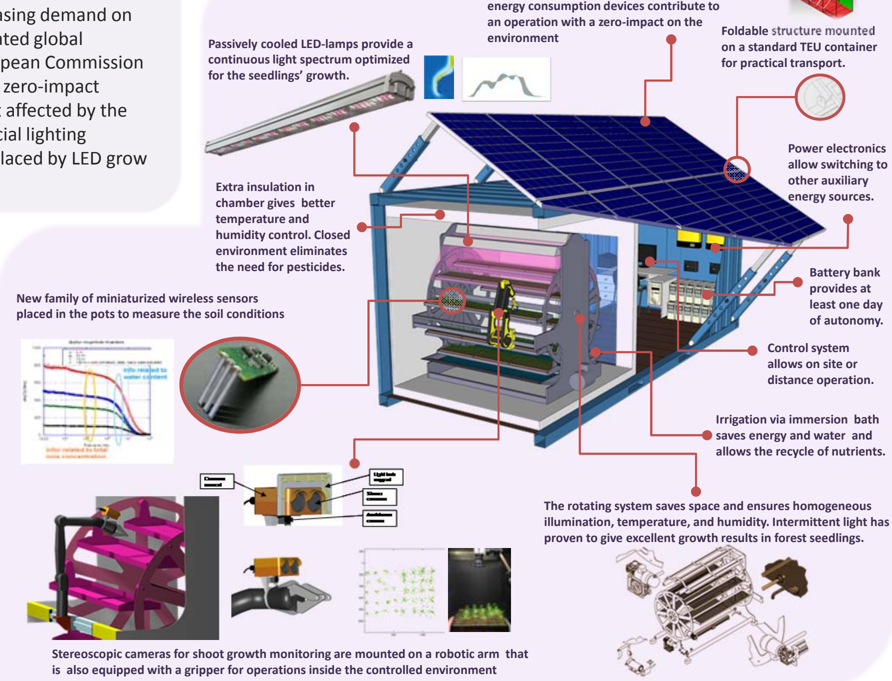
Background

Forest restoration has become a primary task not only to cope with an increasing demand on forest products, but also to fight climate change and compensate an accelerated global deforestation. The main objective of the ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), is to develop an innovative zero-impact technology for the pre-cultivation of forest regeneration materials that is not affected by the outdoor climate. Among the main components to be improved are the artificial lighting sources used for the cultivation. Traditional fluorescence lamps are to be replaced by LED grow lights with spectra tailored to the seedlings' needs.

ZEPHYR Concept*



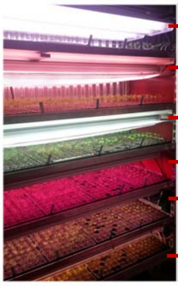
* For more details on the ZEPHYR incubator please visit Poster 1767

Material and Methods

The present work investigates biological responses of *Picea abies* and *Pinus sylvestris* to fivedifferent light spectra. The pre-cultivation has been done following a standard growth protocols during 5 weeks with a photoperiod of 16 hours at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This has been done under controlled closed conditions with a room temperature of 20°C and a relative humidity of 60%.

Parameters for finding the correct "Light regime"

Different light spectra tested



Flourescence: the control spectrum of the fluorescence lamps is widely used in horticultural plant production.

LED 1: High red spectra, with far-red and moderate blue. Good growth results with lettuce and herbs. Peach-tone appearance to human eyes. Reason for selection in test: Good reference spectra to AP67, as this has less green and less far-red.

LED 2: High intensity spectrum that matches more closely the spectrum of the sun. White appearance to human eyes. Reason for selection in test: Sun-shock capability test, i.e. can this light prepare plants for outdoor cultivation.

LED 3: High on far-red and red, low on blue. Pink appearance to human eyes. Reason for selection in test: Good results with several tree species, good root development. Good results from previous project with tree seedlings. Different from other selected spectra, in terms of low blue and high far-red.

LED 4: General growth spectrum, with documented good impact on vegetable and flower biomass production. Induces flowering. Good root development. Light pink appearance to human eyes.
Reason for selection in test: Good results with growing lemon tree seedlings – this is an indication on good performance with southern European trees (sun plants – high light intensity) as well as other tree seedlings. Good results from previous project with tree seedlings.

LED 4 (tube): The tube version has the same spectrum and can also have significant commercial interest since the tube fits into any standard fittings for conventional fluorescent tubes.

Forest field trial

Pinus sylvestris

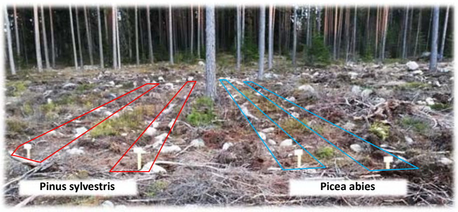
3:5	5:1	2:2	1:4	2:5	1:3	2:1	2:4	1:2	3:1	3:2								
2:3	4:3	6:1	6:4	1:1	1:5	5:2	4:1	3:4	4:4	6:2	5:3	4:5	5:4	3:3	4:2	6:3	6:5	5:5

Picea abies

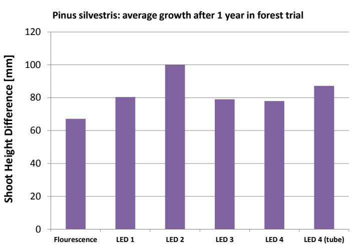
3:5	5:1	2:2	1:4	2:5	1:3	2:1	2:4	1:2	3:1	3:2	2:3	6:1	4:3	6:4	1:1	1:5
5:2	4:1	3:4	4:4	6:2	5:3	4:5	5:4	3:3	4:2	6:3	5:5	6:5				

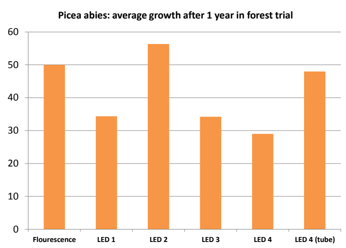
light treatment : replication (with 3 seedlings)

In order to evaluate how the "Zephyr seedlings" pre-cultivated under the artificial lights are able cope with real field stress conditions, a forest field trial was designed. The terrain presents an average planting site in mid-Sweden after clear-cut.

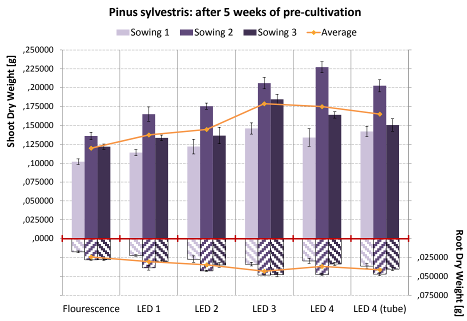


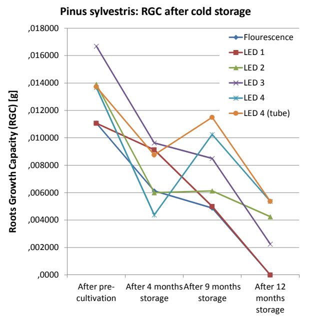
Location of field trial
Latitude: 60.562845,
Longitude 15.477584

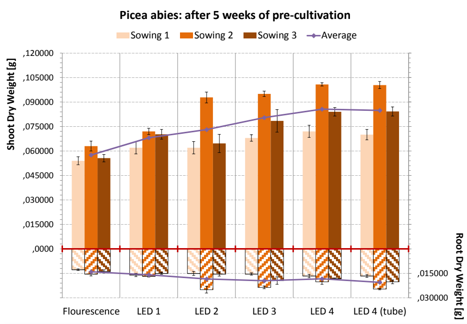


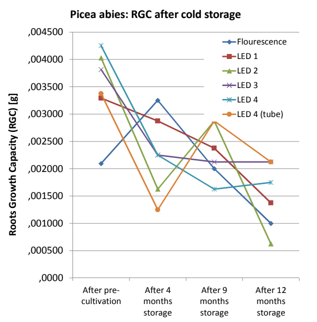


Pre-cultivation results









Conclusions

- The results after pre-cultivation under different light spectra showed differences in the seedlings growth and storability. However, the RGC was negatively affected for all light spectra when comparing results for pre-cultivated Scots pine and Norway spruce seedlings cold stored for 4, 9 and 12 months. After analyzing seedling development for one vegetation period on a forest field trial, both *Pinus sylvestris* and *Picea abies* seedlings had a better development when they had been pre-cultivated under the spectra LED 2 and LED 4 (especially the tube version) compared to the other light sources.

European Commission

www.zephyr-project.eu
This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No 308313

Total Cost: 4,284,275 € - EC Contribution: 3,438,252 €
Duration: 36 months - Start Date: 01/10/2012
Consortium: 14 partners from 10 countries
Project Coordinator: Tuscia University – DAFNE Department (Italy)

Environmental technologies

DALARNA UNIVERSITY

LONG NIGHT TREATMENT FOR INDUCTION OF COLD HARDINESS USING ARTIFICIAL LIGHTS
– effects of photoperiod on seedlings' storability and energy consumption.

Marco Hernández Velasco, Anders Mattsson*

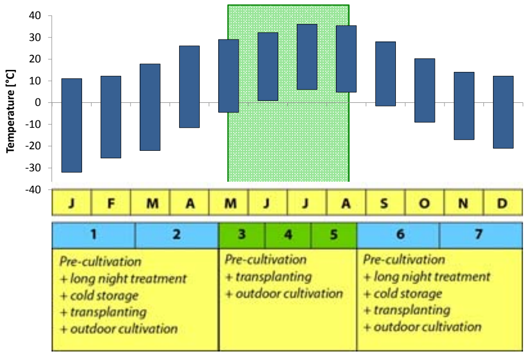
Energy, Forests and Built Environments; Dalarna University, 791 88 Falun, Sweden
** E-mail: mhv@du.se*

Background

Human-assisted forest regeneration in Nordic climates is considerably limited by the harsh outdoor conditions. There the local weather opens only a small time window during the summer for transplanting and establishing of the pre-cultivated seedlings in open land. Greenhouses and modern growth chambers aid to cope with this limitation by allowing year-round seedlings cultivation. Nonetheless, production levels are constrained to the cold storage capacity during the non-transplanting season. This storage is in turn dependent on the conifers' ability to adapt to freezing temperatures and withstand the overall stress associated with the cold hardening.

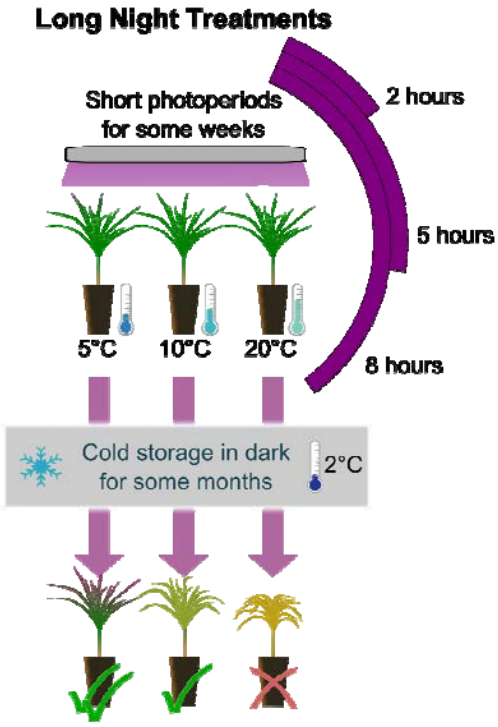
Long night treatments can induce dormancy with growth cessation and terminal buds initiation, leading to a better cold resistance. When growing forest regeneration materials under artificial lights, the lengths of the long night treatment and the photoperiod will have a significant impact, not only on the biological response of the seedlings but also on the energy consumption.

Transplanting window based on the temperatures in Sweden which determine the year-round cultivation programs

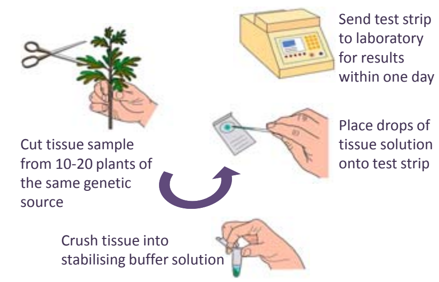


Material and Methods

The aim of this work was to explore different long night treatment regimes for induction of cold hardiness on *Picea abies* and *Pinus sylvestris* seedlings using artificial lights. The pre-cultivation has been done following a standard growth protocols during 5 weeks with a photoperiod of 16 hours at 100 μmol·m^{-2}·s^{-1}. This has been done under controlled closed conditions with a room temperature of 20°C and a relative humidity of 60%. After the pre-cultivation, various daily photoperiod regimes where applied during different durations ranging from 3 to 7 weeks. The cold tolerance and storability of the seedlings was measured using a molecular marker method developed by *Stattin et al. (2012)* and carried out by the Dutch company *NSure*.



nsure

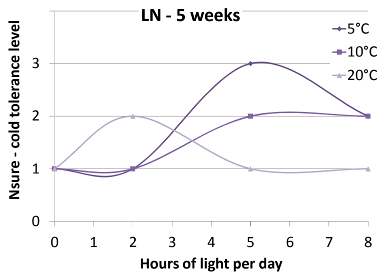


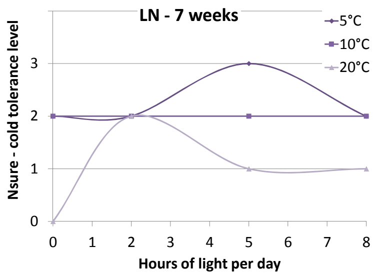
NSure defines four stages of cold tolerance based on the activity profile:

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.

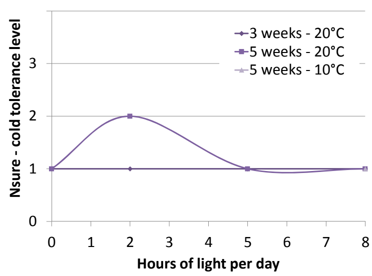
Preliminary Results

Pinus sylvestris

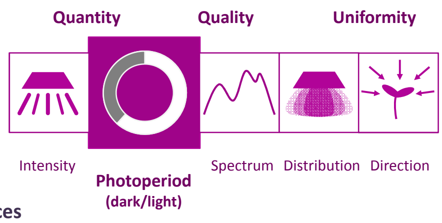




Picea abies



Parameters for finding the correct "Light regime"



Discussions

- If the development of the seedlings is not affected by an even shorter photoperiod (2 or 5 hours compared to 8 hours), then energy savings are also possible by reducing the amount of time the lamps are used.
- Cold tolerance appears to be highly dependent not only on the photoperiod but also on the temperature during LN treatment, specially for *Pinus sylvestris*.
- 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.

References

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.

CULTIVATION OF FOREST REGENERATION MATERIALS UNDER ARTIFICIAL RADIANT SOURCES – effects of light intensity on energy consumption and seedlings' development.

DALARNA UNIVERSITY

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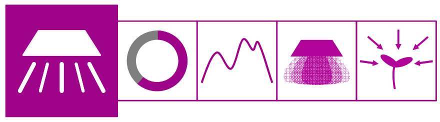
Background

In times of major environmental challenges and increasing demand for forest products, planted forests have acknowledged advantages compared to other land uses. Despite not being able to substitute natural forests, planted ones have, if properly managed, a great potential to contribute in addressing this problems. Besides the ecological benefits such as carbon sequestration, planted forest can help coping with the wood products demand without further reduction of natural forest. Forest restoration, rehabilitation and reforestation are limited to the capacity of producing forest regeneration materials. Often, as the production is intensified at the forest nurseries, the practices start having an adverse impact on the environment and stop being truly sustainable. One of the main issues in nurseries is the energy consumption for grow lights in periods with short daylight times.

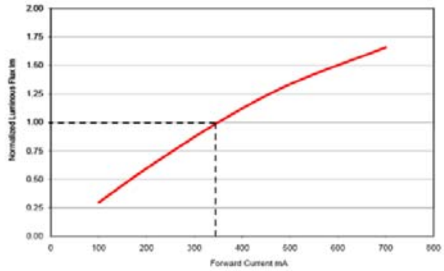
Parameters for finding the correct "Light regime"

Quantity | Quality | Uniformity



Intensity | Photoperiod | Spectrum | Distribution | Direction

In LEDs, luminous flux is almost linearly proportional to the current over the usable range

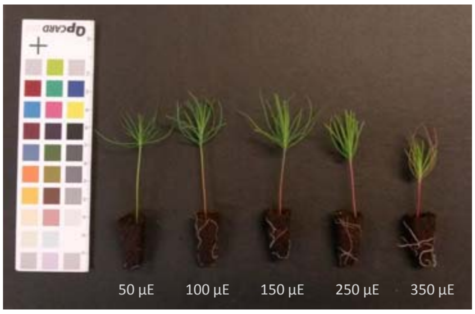


Material and Methods

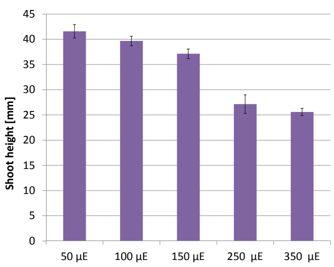
By using high efficiency LED grow lamps and adjusting the light intensity, this study aimed to reduce the energy consumption from lighting per seedling without compromising their development. The pre-cultivation of *Picea abies* and *Pinus sylvestris* seedlings was done during 5 weeks under controlled conditions at 20°C and a relative humidity of 60%. The photoperiod was of 16 hours at an intensity ranging from 50 to 350 µmol·m^{-2}·s^{-1}.

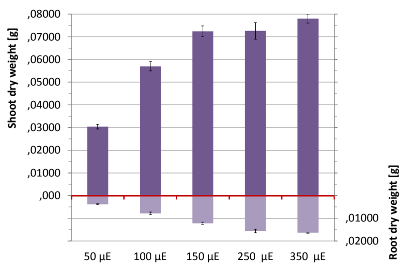
Preliminary Results

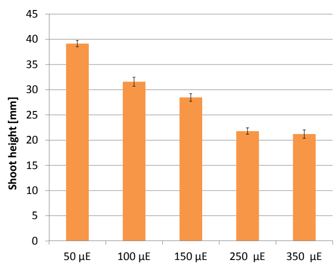
Pinus sylvestris

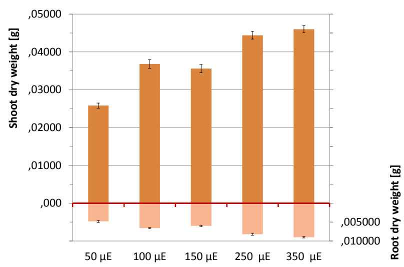


Picea abies





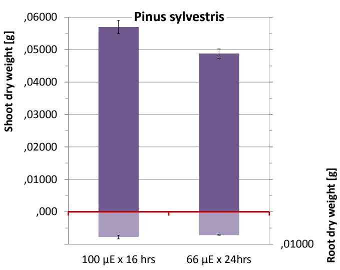


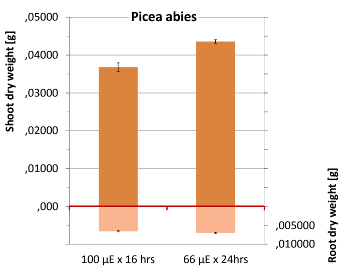


Daily Light Integral (DLI)

The *Daily Light Integral (DLI)* measures the total amount of photons received in a certain area during one day. Different light intensities can yield the same DLI if the photoperiods are accordingly adjusted.

The following charts compared two groups treated with different light intensity - photoperiod combinations but an equal DLI of 5,76 mol m^{-2} day^{-1}.





Discussions

- Due to the direct relationship between light intensity and energy consumption, it is important to optimize the light level at which the seedlings are pre-cultivated when using artificial radiant sources. For both species studied, the increasing light intensity had a negative effect on the shoot height but a positive on the dry weight matter.
- With equal daily amount of photons or *Daily Light Integrals* (DLI), the two species responded differently depending on the photoperiod. The sampled seedlings of *Picea abies* that were exposed to 24 hrs. of light had a greater dry matter weight compared to those exposed for only 16 hrs. ; meanwhile *Pinus sylvestris* showed opposite results. This could be explained the contrasting light adaption of the two species.
- Choosing the correct intensity-photoperiod combination is not only important for the seedlings' growth but also for the energy management and overall use of the lamps. Extended photoperiods with lower intensities allow for example to use less lamps and to buy electricity during low price times of the day. Shorter photoperiods with high intensities allow using energy that is available only at certain times (e.g. Solar-PV) without needing to store it.

ENERGY EFFICIENCY IN INTENSIFIED PRODUCTION OF FOREST REGENERATION MATERIALS
- design of a photovoltaic system for sustainably powering an innovative forestry incubator

Zero-impact Innovative Technology in Forest Plant Production

Marco Hernández Velasco [1], Sreenivaasa R Pamidi [2], Anders Mattsson [1]*

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[2] Exergy Ltd.; The Technocentre, Coventry University Technology Park, Coventry CV1 2TT, United Kingdom
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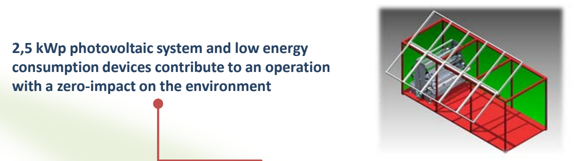
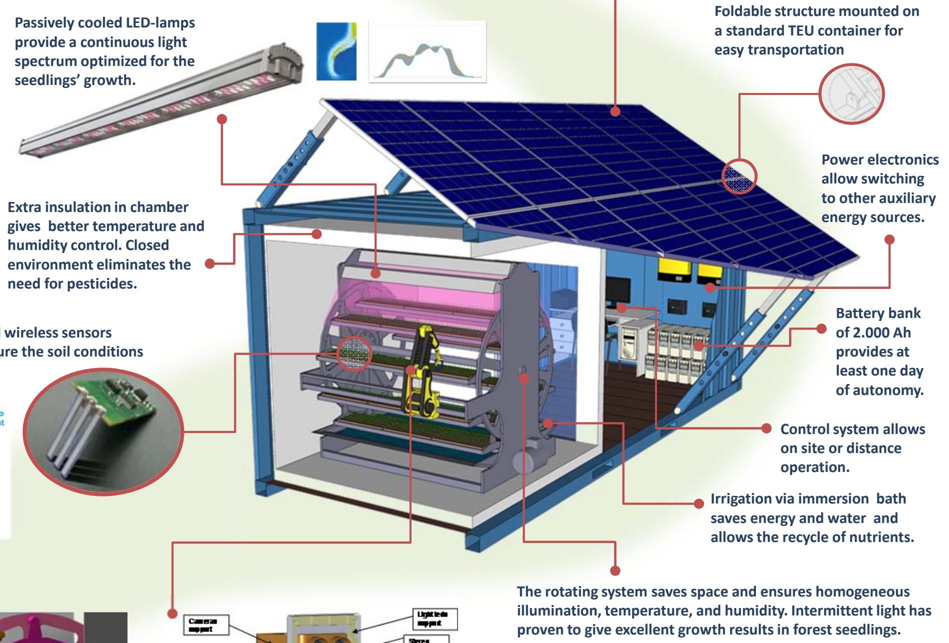
Background

Planted forests can contribute addressing problems of global concern such as climate change mitigation, biodiversity lost and pressure on ecosystems due to high demand of forestry products. However, in order to be able to profit from these benefits sustainably, production rates of forest regeneration materials should be higher than the harvesting rates. Nevertheless, intensive production methods often bring along adverse consequences for the environment.

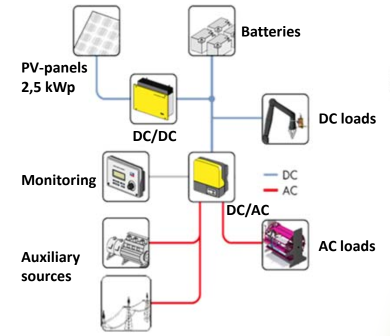
Objectives

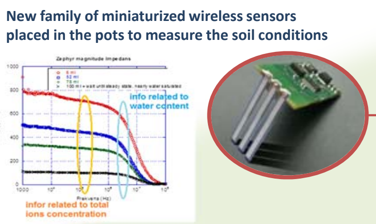
In the frame of the ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), innovative and cost-friendly technologies for the pre-cultivation are being developed. They will be integrated into a functional and transportable system for a large scale production of seedlings, with zero-impact on the environment and not affected by outdoor conditions. To achieve this, high efficiency devices with low energy consumption will be used and the incubator will be powered by Photovoltaic (PV) solar energy.

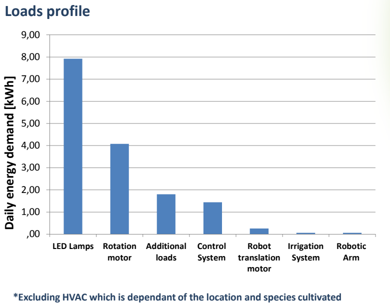


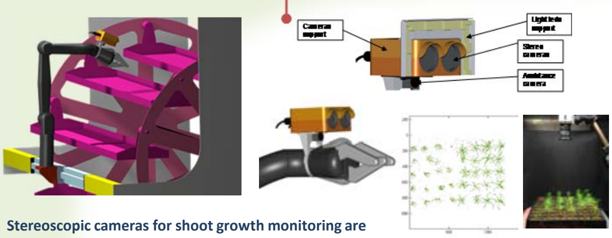


PV-system design

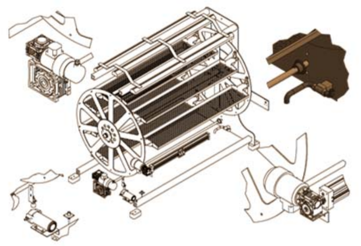




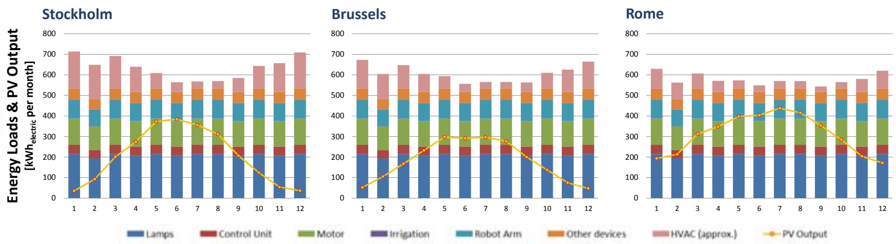




Stereoscopic cameras for shoot growth monitoring are mounted on a robotic arm that is also equipped with a gripper for operations inside the controlled environment



Energy analysis for PV-system in different European locations



Comparison to traditional system

	Swedish forest nursery	ZEPHYR incubator
		
Technology	Modern Greenhouse	Rotating shelves with LED lighting
Production capacity	10.000.000 seedling per year	80.000 seedlings per year
Energy consumption	2.500.000 kWh	13.500 kWh
Energy per seedling	0,25 kWh/seedling	0,17 kWh/ seedling
Energy source	Oil (250 m^3)	Electricity (assuming tariff 0,90 SEK/kWh)
Cost of energy	0,25 SEK/seedling	0,15 SEK/seedling

Assuming peak power consumption of all devices and HVAC running 24 hours.

Project Outcomes

- Innovative and light growth chamber with a production capacity of up to 12 000 plantlets per cycle in a fully automatized and controlled environment.
- New family of wireless sensors studied for controlling the growth status
- High energy efficiency, due to space saving through the rotating system and the use of LED lights; up to 70% respect to traditional nurseries.
- Full automatized cycle production inside a standard TEU container powered with PV panels
- Flexible: more units can be placed in series for a bulk production

European Commission

www.zephyr-project.eu

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No 308313

Total Cost: 4,284,275 € - EC Contribution: 3,438,252 €
Duration: 36 months - Start Date: 01/10/2012
Consortium: 14 partners from 10 countries
Project Coordinator: Tuscia University – DAFNE Department (Italy)

Environmental technologies

REFORESTATION CHALLENGES

International conference, Belgrade, Serbia – June 3-6, 2015

Anders Mattsson
Dalarna University
Department of energy, forests and built environments
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Reforestation challenges in Scandinavia
Keynote address

In my keynote major reforestation challenges in Scandinavia will be high-lighted. In Scandinavia I include the following countries: Iceland, Norway, Sweden, Finland and Denmark.

Figure 1

Iceland

Iceland once had a forest cover up to 60% mostly with birch forest. Today with only a forest cover of 2% due to extensive cuttings for fire wood, land degradation and soil erosion Iceland face a major reforestation challenge. Some of the most common species included in this challenge are birch, Siberian larch, Sitka spruce and Lodgepole pine.

Figure 2

The challenge also includes the conflict with livestock farmers. For centuries the commons were used for horse and sheep grazing. However more and more of the grazing land have been fenced up allowing regeneration of birch and other species.

Figure 3a

Regarding land degradation and eroded soils, forest regeneration often starts by planting of Siberian larch to establish a humus layer by litter from the needles falling of each year followed by for example planting of Sitka spruce. Some forest nurseries have also been established in recent years adding to the picture of a raising hope for increased future efforts regarding forest regeneration in Iceland.

Norway

In Norway with a forest cover of 37% the most common tree species are Norway spruce, Scots pine and silver or downy birch.

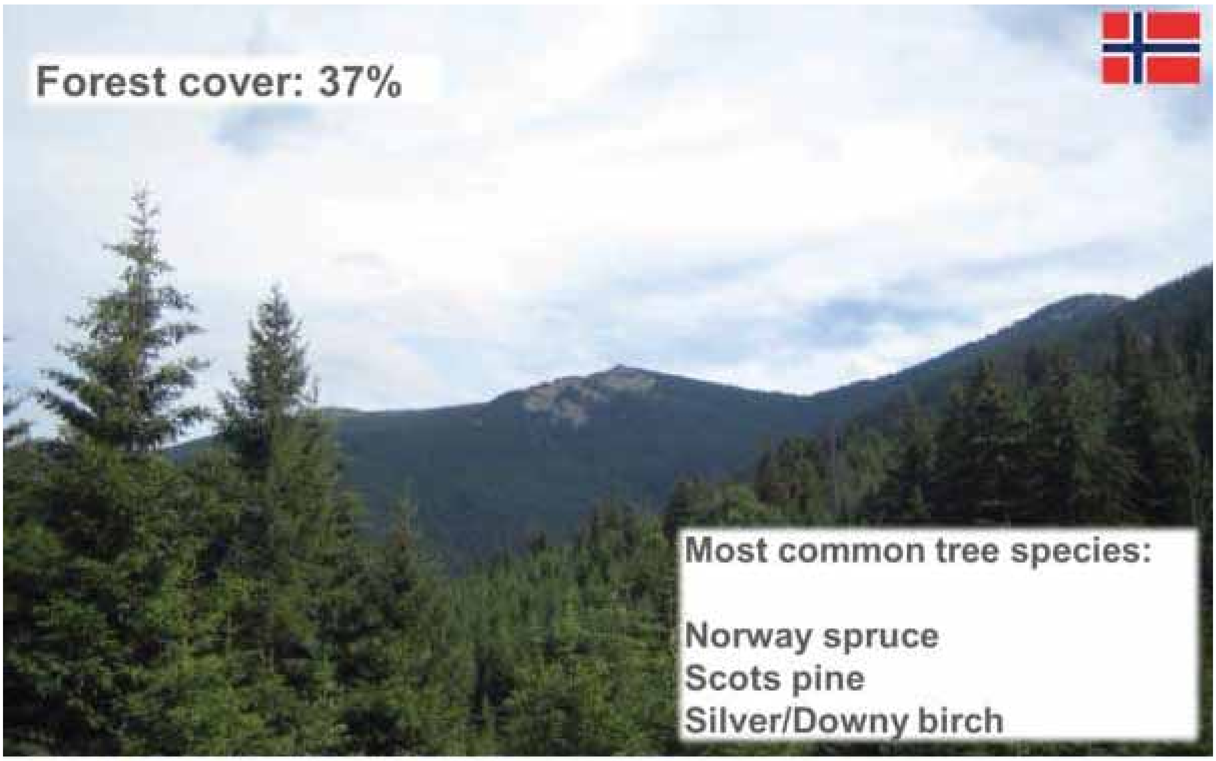

Figure 3b

The forest is often very productive along the coast but often grows in a steep terrain. Therefore logging operations are often done by cable cranes and transportation of logs done by helicopter

Figure 4

A major reforestation challenge in Norway has for decades been the risk of seedling damages from the pine weevil. If the seedlings are not protected after planting it is common with survival rates less than 25% after planting. The pine weevil feed on the bark of young seedlings after they are planted at the regeneration site and if the seedling is girdled it will not survive. In Sweden, and soon in Norway, insecticides have been forbidden so future protection of seedlings against the Pine weevil has to be based on silvicultural or non-chemical methods.

Figure 5

Since it also is one of the major reforestation challenges in Sweden, impacts and possible methods to reduce Pine weevil damage will be discussed under the heading of Sweden.

Sweden

Sweden is a country where almost 70% of the land area is covered by forest. As in Norway the most common tree species is Norway spruce, Scots pine and silver or downy birch.

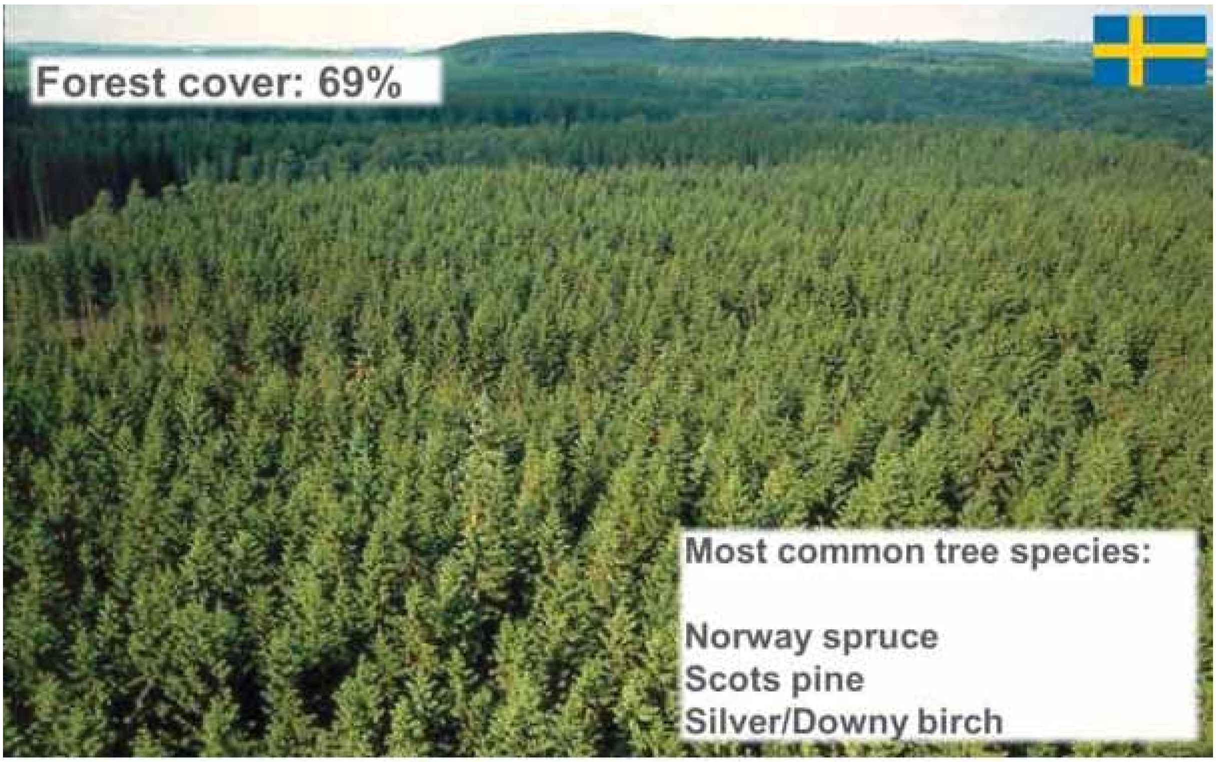

Figure 6

In many parts of Sweden, especially in the south, we have forest stands with a good volume growth. This implies intensive forest harvesting operations for delivery to our pulp and saw mills. To manage the reforestation challenge after extensive harvesting operations Swedish forest nurseries produce about 400 million seedlings each year for planting operations. The production is almost only based on containerized seedlings making us one of the largest producers in the world of this plant type.

Before discussing the mutual reforestation challenge in Norway and Sweden regarding how to reduce seedling damage from the Pine weevil, the additional reforestation challenge for Sweden in producing millions of seedlings each year for planting operations will be discussed.

Today seedlings are produced in large conventional plastic greenhouses limiting the production time to the short vegetation period in Sweden. Also environmental and cost issues are involved in a discussion of how future production technology should be outlined.

As almost all greenhouses are heated by oil the consumption for a greenhouse is very high. This based on that production normally have to start in March for requested volumes when outside temperature can be -20°C and the temperature inside the greenhouse has to be +20°C for a proper seed germination.

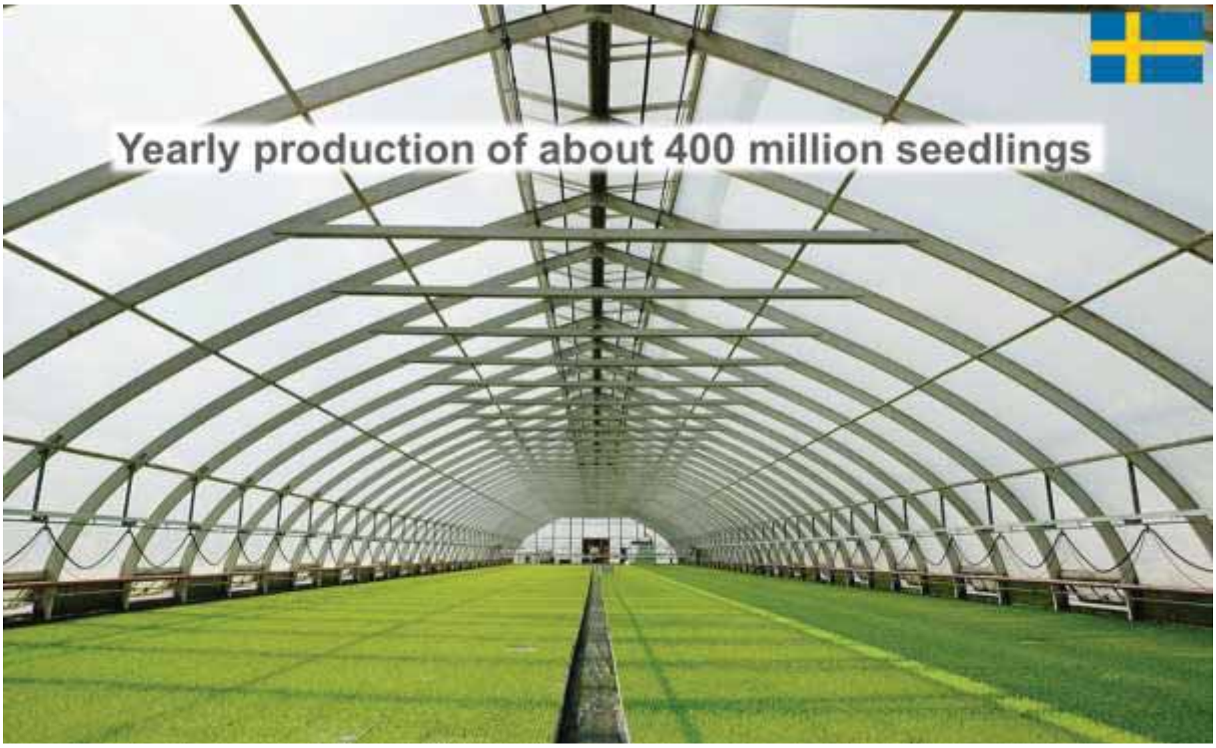

Figure 7

Therefore Dalarna University in Sweden is involved in an EU-project (acronym Zephyr) regarding new innovative technology for production of forest seedlings without using conventional greenhouses. This project can really be seen a major reforestation challenge. The technology is based on a short pre-cultivation period in a growth chamber at high seedling density. For our major species Scots pine and Norway spruce 3500 seedlings/m², followed by automatic transplanting to any optional container system and after transplanting final growth outdoors without using conventional greenhouses.

In addition the concept includes, among other things, LED lights providing maintenance free, energy efficient and low heat emittance as an alternative to traditional light sources. Also the life span of a LED light is almost 10 times compared to conventional lighting. The new technology also includes a photovoltaic system and wireless sensors for control of the climate and soil conditions. With this system it would be possible for Sweden to produce forest seedlings on a year-around basis without using conventional greenhouses.

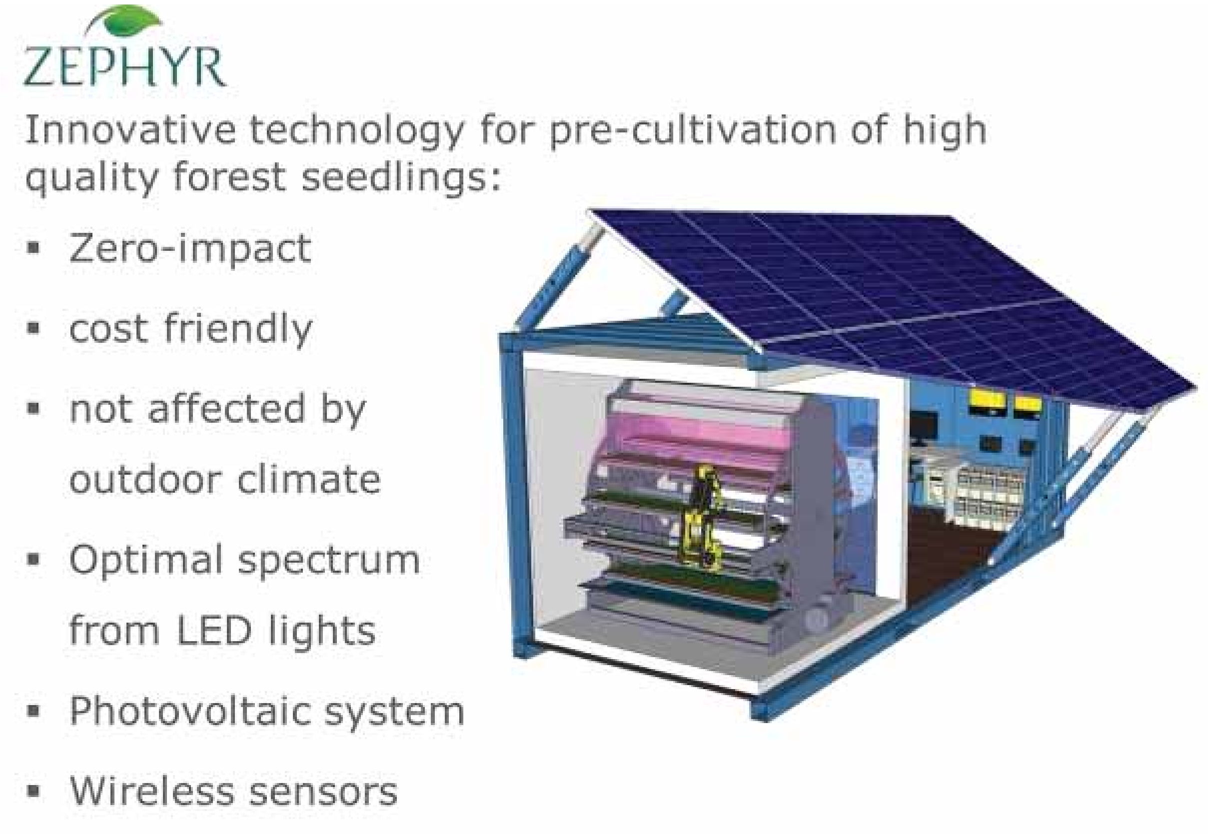

Figure 8

In co-operation between Dalarna University and one of the major forest companies in Sweden the technology with pre-cultivation followed by transplanting to outdoor cultivation has been introduced commercially at one of its forest nurseries. As can be seen in the Figure the total yearly production of the nursery of 14 million seedlings can be pre-cultivated on an area of about 100 m² without using conventional greenhouses.

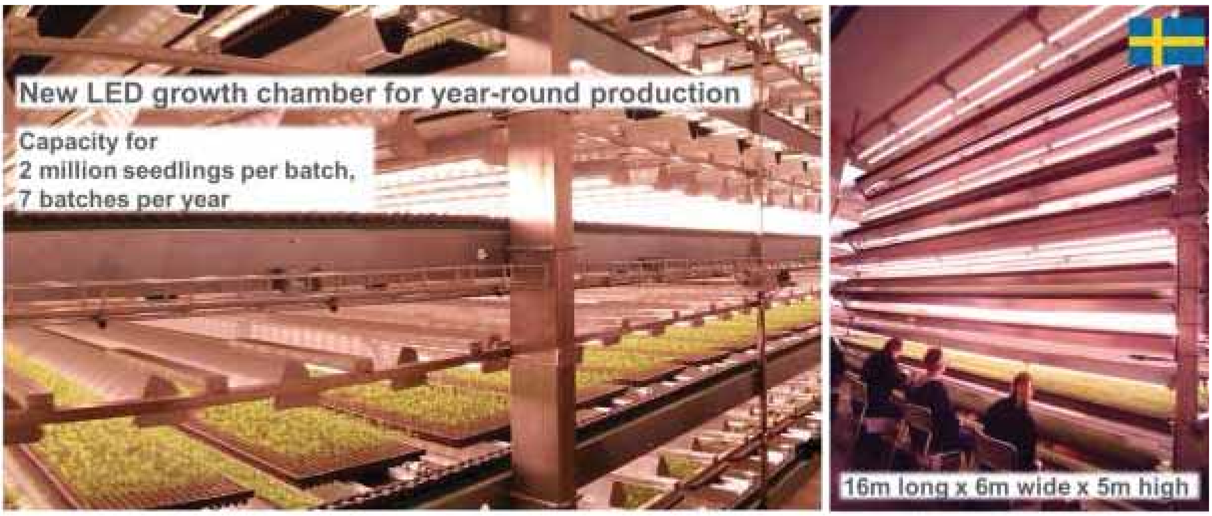

Figure 9

Now back to the other reforestation challenge that we share with Norway that is how to reduce the effect of seedling damage caused by the Pine weevil. The pine weevil is attracted to fresh clear-cuts by the smell of monoterpenes and ethanol released after the cutting. After feeding on fresh slash the weevil mate and the female put her eggs in the roots of the stumps. The new generation hatches the second autumn and start feeding on the fresh bark of the newly planted seedlings.

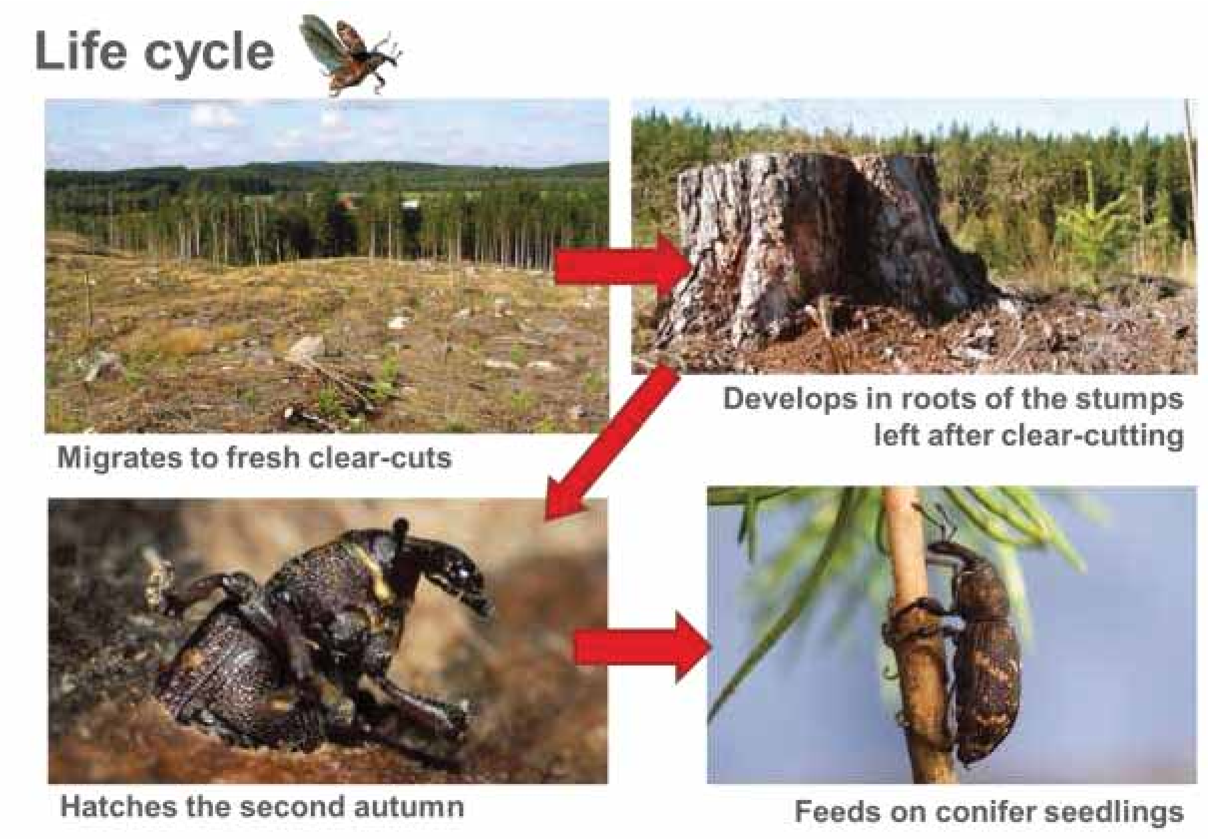

Figure 10

Actually there is a potential risk of Pine weevil attacks in many countries in Europe. The magnitude of the problem is connected to the fact if chemical treatment is allowed or not in a specific country.

This Figure show a risk ranking made in a unique European project. As can be seen there were no data available from most of the Balkan countries at the time so of course there could be a potential risk for attacks also in this part of Europe.

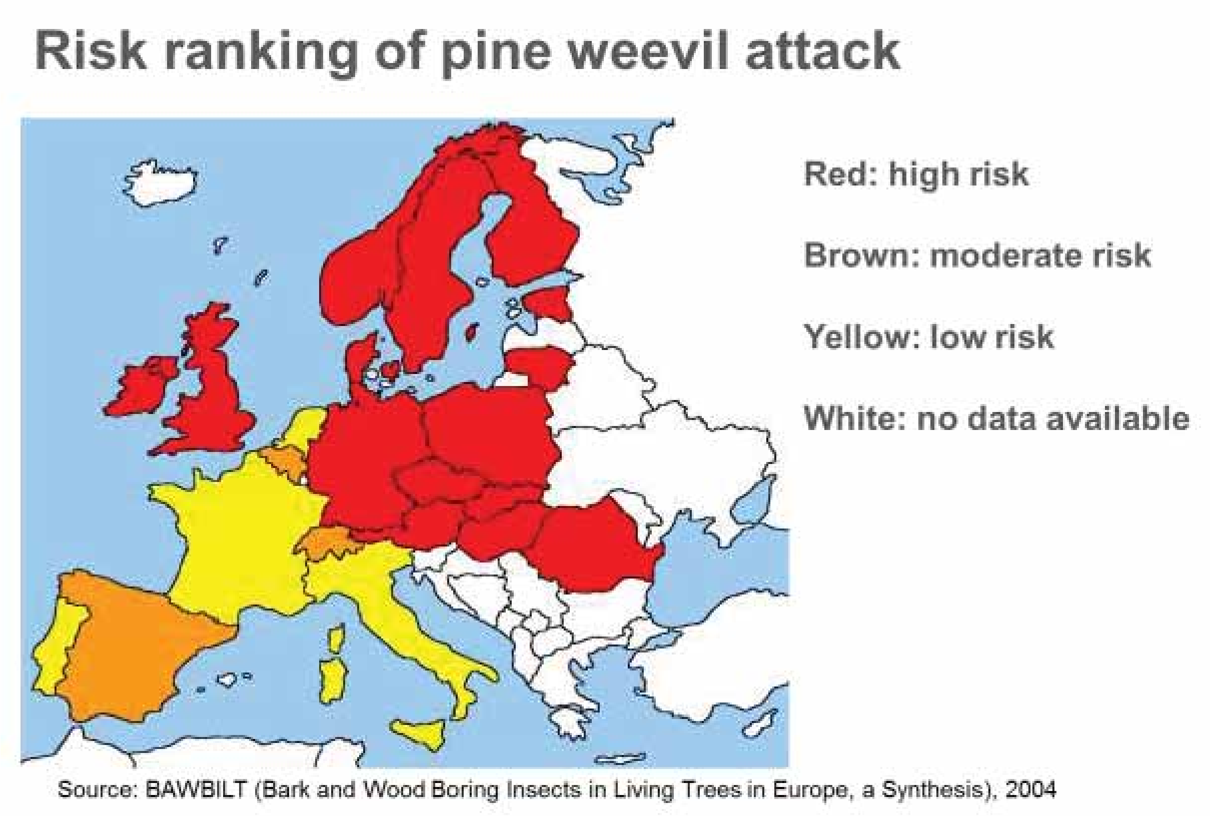

Figure 11

In Sweden the Pine weevil damage is estimated to a cost of about 30 million USD per year. In recent years a lot of efforts and money have been allocated to the problem and methods to reduce Pine weevil damage without using chemicals have been developed or are developing.

These includes as shown in this Figure both silvicultural methods and direct protection of single seedlings.

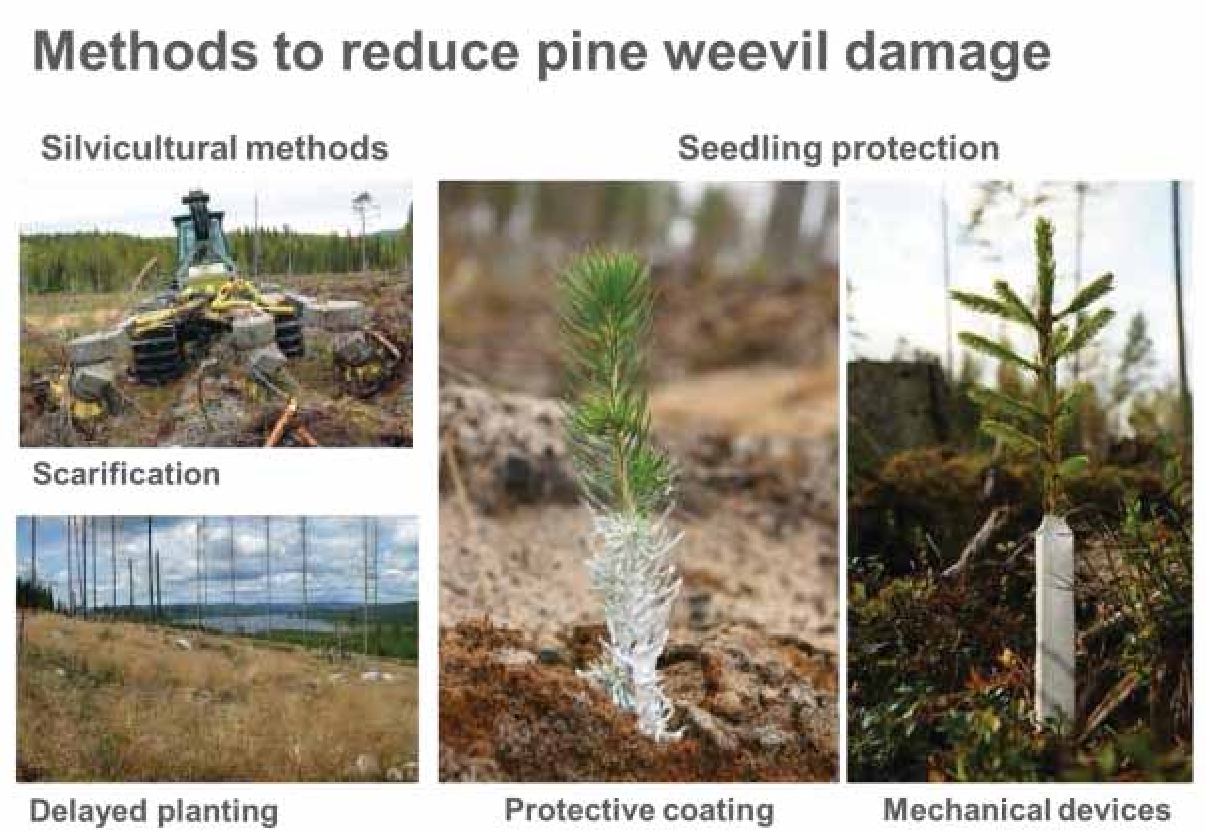

Figure 12

In the first category scarification and delayed planting have been used. Scarification has shown to be quite effective since if the seedling is planted in the middle of an area with pure mineral soil the Pine weevil do not find such an area attractive to enter for approaching the seedling. This compared to a seedling planted in an intact humus layer with more vegetation and humid conditions.

Delayed planting can reduce the attacks since the amount of weevils will be reduced for each year due to lack of suitable feeding materials. The problem is the fast introduction of weeds making the establishment of young seedlings difficult due to increasing competition of light, water and nutrients,

A very promising method has been the introduction of direct protection of each seedling by coating or mechanical devices. These methods have now been introduced in large-scale at Swedish forest nurseries. In spite of higher costs for application in the nursery results have shown that in the end the costs for reforestation are calculated to be lower than just using silvicultural methods.

Finland

Finland has a lot of forest with a total cover of 75%. As in Norway and Sweden you can find the same common species. That is Norway spruce, Scots pine and silver and downy birch. In contrast to Norway and Sweden the amount of birch planting and birch stands are quite significant in Finland. Besides forestry Finland is also known as the land of the thousand lakes which also is illustrated by the following Figure.

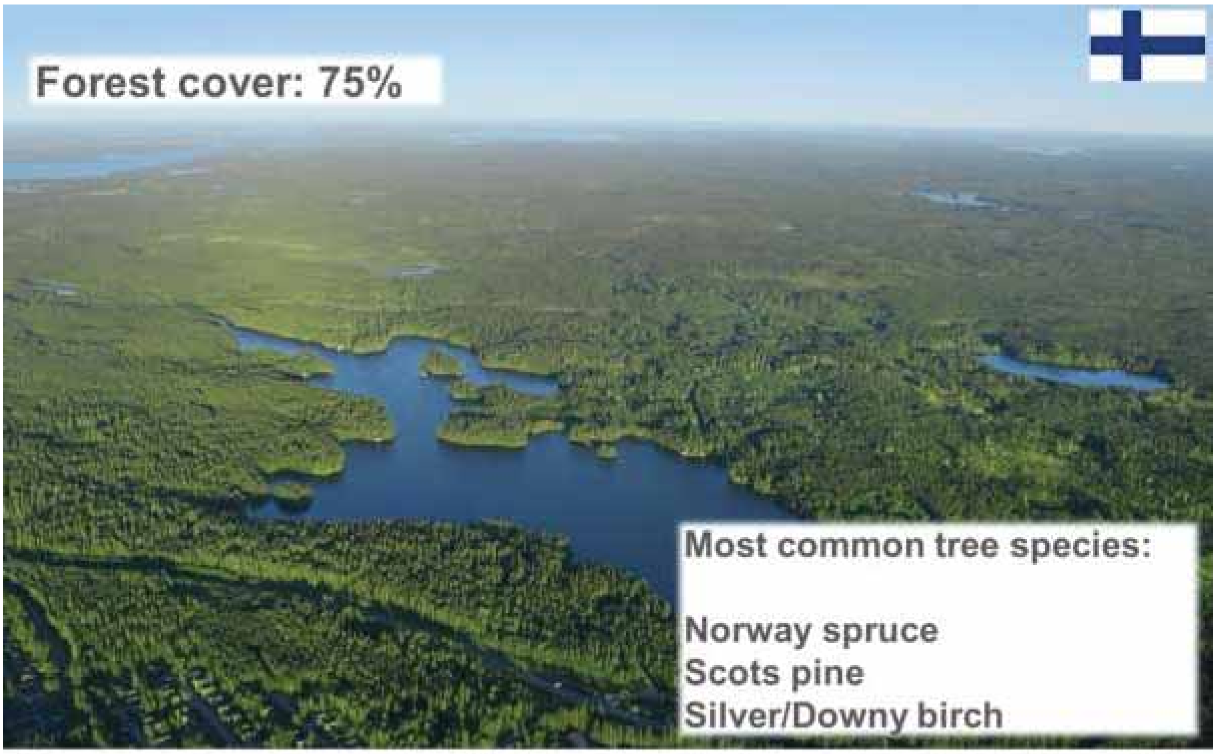

Figure 13

Finland has a long tradition in producing and plant birch seedlings. A typical forest stand is a mix of birch and spruce with birch as a nurse stand and Norway spruce establishing as an understory. In Finland the birch is therefore known as the mother of the spruce since the conditions under a birch stand is very good for spruce development considering light and other conditions.

Figure 14

A major reforestation challenge in Finland is linked to the forest structure. The structure of Finnish forestry includes many private forest owners in combination with small regeneration sites. In the southern part of Finland private forest owners are as high as 80%. This implies a situation where logistics and methods for lifting in combination with field storage at the site provide a major reforestation challenge in order to preserve seedling quality until the planting date.

Small quantities of seedlings have to be delivered to each small regeneration site also in a relatively short planting season due to climate conditions. This implies problems to be able to take care of the seedlings in a way that they do not rapidly loose in quality. Often forest owners therefore have to water the plants at the field storage with portable water systems.

Figure 15

Due to this situation a major reforestation challenge for Finnish forestry is to develop new logistic systems and technology, including new seedling cultivation programs, to match the access of fresh planting stock to a lot of small regeneration sites in combination with different planting dates.

Denmark

Denmark has today a forest cover of 13% but the climate and soil conditions allow more forest cover and also more variations in species compared to other Scandinavian countries. As an example of species, Norway spruce, beech, oak, Sitka spruce and different firs as Nordmann and Noble fir can be mentioned.

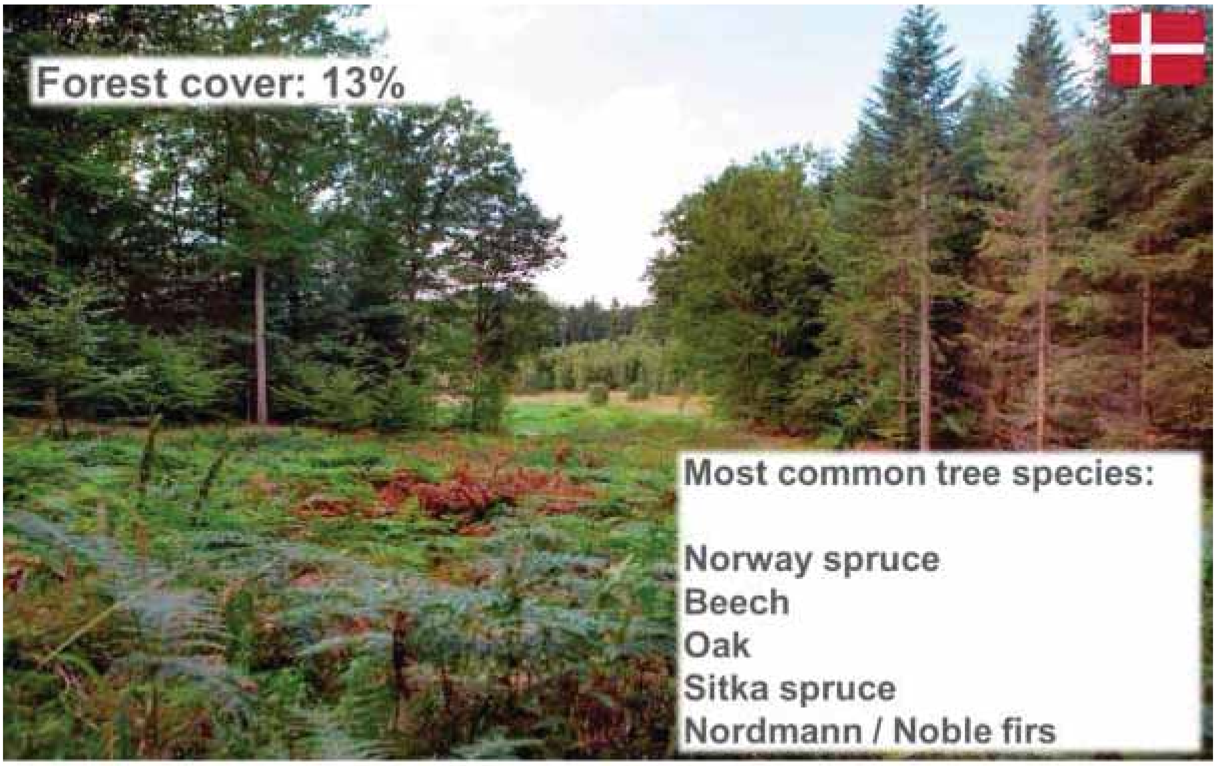

Figure 16

Although the conditions allow more variation spruce monocultures have been established in many places mainly due to economic reasons. A major future reforestation challenge for Denmark is therefore the possibility to establish plantations based on a wide range of relevant species. For this option new methods and technology have to be developed in reforestation activities that support this possibility. These methods and technology should make it possible not to be limited to certain species due to problems and limitations during field establishment.

Figure 17

In a forest with a wide range of species the risk of specific species being attacked by for example a fungi disease will be limited. Also a wide range of species will in general make the forest more stabile, healthy and productive and in that way also be more adapted to future climate change.

With this I conclude my presentation of forestry in Scandinavia and the reforestation challenges laying ahead.

Keywords:
Scandinavia, reforestation challenges, deforestation, degradation, erosion, pine weevil, pre-cultivation, transplanting, forest structure, lifting, field storage, alternative species, climate change

Climate control in the production of forest plants
– using PV to power an innovative forestry incubator

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1 INTRODUCTION

Forest ecosystems are currently challenged by changes in climate, natural disasters and an extreme exploitation of their resources. Forest restoration requires among other things, high amounts of healthy seedlings to replace the lost or extracted trees. However, the cultivation of these seedlings is often done through intensive methods in forest nurseries. These techniques consume considerable amounts of energy for lighting, acclimatization and irrigation applying also significant amounts of fertilizers and pesticides.

The ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (*FP7, Grant Agreement n° 308313*), is developing innovative and cost-friendly technologies for the pre-cultivation of forest plants. Devices such as LED growth lights and a new generation of wireless sensors will be integrated into a functional and transportable system for large scale production of seedlings. The unit will have a very low impact on the environment, will be independent of the outdoor conditions and will be powered by solar energy. The whole concept represents a breakthrough in forest seedlings production for reforestation purposes. It addresses key issues such as energy usage, water recycling, reduction of fertilizers and avoidance of pesticides.

1.1 Project Objectives

One of the main features of the Zephyr incubator is the fact that the seedlings will be pre-cultivated during the first stage in an isolated environment. A transportable and closed incubator possesses several advantages compared to traditional greenhouses: it provides a better climate control and reduces the need for pesticides and fertilizers; it allows growing seedlings in places where it would not be possible otherwise (e.g. near deserts); and the plants can be produced directly at the place where they are needed avoiding damage from the transport and storage at the reforestation/afforestation site. A closed environment extends the cultivation time throughout the whole year, even during the winter or dry seasons. Furthermore, it

allows a standardized and certified production of high quality reforestation materials increasing the chances of success of the restoration actions.
The main expected project outcomes are:

- Growth chamber with a production capacity of up to 12.000 plantlets per cycle in a fully automatized and controlled environment.
- New family of wireless sensors designed for controlling the growth status of the seedlings.
- High energy efficiency, due to space saving through the rotating system and the use of LED lights; up to 70% with respect to traditional nurseries.
- Full automatized production cycles inside a standard TEU container powered with PV panels
- Flexible production: more units can be placed in series for a bulk production

Figure 1: Original concept of the incubator and first working prototype

Specially developed LED growth lamps and wireless sensors are used to reduce energy consumption and monitor the cultivation process. The main part of the energy is provided by solar PV-panels, which are designed to provide at least one day of autonomy depending from the geographic and climatic area (in central Europe). The energy savings will result in a reduction of greenhouse gas emissions; moreover, since the LED lamps do not produce additional warming, there will be further energy savings through a reduced need of air conditioning.

2 PV-SYSTEM

The goal when designing the power supply system of the Zephyr incubator was to enable a standalone off-grid system with Solar PV as the primary energy source along with a battery bank as energy storage. Integration of additional sources (diesel generator set or local grid when available) should be used to support the demand levels and ensure continuous energy supply to the unit.

The design considerations for a typical solar photovoltaic system, both off-grid and on-grid, depends on solar resource of the place, system mechanical and electrical characteristics, load characteristics and economic factors. These four parameters are essential for the design of an optimal PV system. The Zephyr design has considered limitations with availability of space and weight, both on the roof and inside the transport unit, enabling continuous energy flow along with safe mode of transportation. These challenges regarding required flexibility of the design for Zephyr project has required several issues to be confronted and changes to the design of the power supply system to be made.

Figure 2: Basic Power system design and final installation

Different options were considered to design the system in a way which could incorporate additional energy sources, i.e. diesel generator set and grid supply, whenever available. In order to incorporate these options, new devices which can integrate and provide smarter power management to switch to additional sources when needed without interrupting the energy flow to the subsystems, were required. Products such as a bidirectional inverter which incorporate easy installation proved to be difficult to find (especially in support of DC systems) and also resulted in increased complexity and cost of the system design.

Table 1: Load analysis of the different components

Main Loads	Unit power (W)	Quantity	Total Power (W)	Average daily hours of usage (h)	Wh/day
DC Loads					
Robotic Arm	40	1	40	1,5	60
			40		**60**
AC Loads					
LED Lamps	165	3	495	16	7920
Control System	60	1	60	24	1440
Motor (rotation)	170	1	170	24	4080
Motor (x-axis)	170	1	170	1,5	255
Irrigation System	60	1	60	1	60
HVAC system	700	1	700	24	16800
Fans (additional loads)	150	1	150	12	1800
			1905		**32355**

The battery and the inverter sizing were mainly done by matching the load requirements and number of days of autonomy. Engineering the design of the system from the back (i.e. loads) to the energy source(s) to match the daily requirement is optimal for an off-grid unit. Nevertheless, due to the limited space on the roof of the container, relying on the PV source alone was not feasible for many European locations.

Once the basic requirement was considered for pre-sizing the inverter and battery bank to match the load requirements, PV panels of different technologies have been compared with different components/manufactures in order to optimize the performance of the entire system. Later, depending on the space availability, the number of panels was calculated, and the remaining additional energy should be supplied through additional sources depending on the location and accessibility factors.

Additionally, simulations for several sites were performed using the components from the sizing data in PV*SOL Expert 6.0. It is important to notice that the load for the HVAC was overestimated assuming it would be operating constantly at maximum power. This was done to make sure the incubator was able to provide ideal growing climate regardless of the outdoor conditions.

The final installed system has a MPPT charge controller and an off-grid bidirectional inverter regulating 20 PV modules, each of 135 Wp, connected in 5 parallel strings of 4 modules. The battery bank is formed by 20 batteries of 12V and 230Ah. The total installed power is 2,7 kWp.

3 HVAC SYSTEM

The design and installation of the HVAC system was commissioned to the company *NUOVA CRIOTECNICA AMCOTA S.N.C.* with headquarters in Rome, Italy. Since 1968 in the company has specialized in equipment for climate control of laboratories and growth chambers.

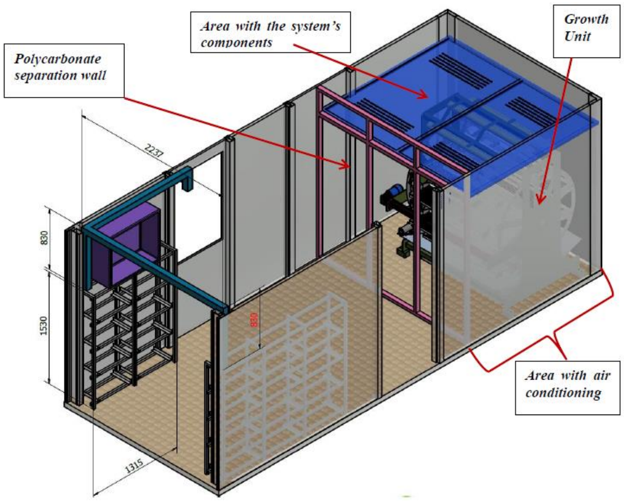

Figure 3: Overview of the container and the area with climate control

The system was designed for air conditioning only the part of the container with the growth unit in it, meaning the part separated by the polycarbonate wall, as shown in Figure 3. During the ordinary use, no human presence is foreseen in the other section of the container so there is no real need to control the climate there. This solution allows lower energy consumption by reducing the cooling/heating volume.

The system is composed by a condensing unit, an evaporating unit and a control panel. The condensing unit is composed by the refrigerant compressor, the cooling fan for the condenser and 2 solenoid valves for reversing the refrigerant cycle. It has a power of 700 watts at 220V / 50Hz single phase. The evaporating unit is composed by 2 electric fans with a power consumption of 100 Watts.

4 FURTHER ACTIONS

Together with the design and construction of the prototype, many biological experiments were conducted in order to test the feasibility of the concept and find the best growing conditions for different tree species (see Table 2). However, these tests were carried out in laboratories using traditional growth chambers and need to be validated in the ZEPHYR incubator.

In these laboratories, the climate conditions were strictly controlled without considering the energy consumption of the growth chambers. Instead of defining a fixed temperature and using the HVAC continuously to maintain it; a greater temperature range for each plant species could be defined to increase the flexibility of the system. Allowing a certain temperature fluctuation would mean that the HVAC system would only work when the temperature or humidity goes out of the range in turn reducing the load from HVAC.

Table 2: Overview of growth conditions for some of the species tested

Species	**Ideal cultivation temperatures**		**Photoperiod**	**Relative Humidity**
	Day	**Night**	hours of light per day	
Azores laurel cherry (*Prunus azorica*)	15 ± 1 °C	15 ± 1 °C	12	70%
Norway spruce (*Picea abies*)	20°C	20°C	16	60%
Scots pine (*Pinus sylvestris*)	20°C	15°C	16	60%
White oak (*Quercus pubescens* Wild.)	21 ± 1 °C	21 ± 1 °C	14	80 ± 10%
Hungarian oak (*Quercus frainetto* Ten.)	21 ± 1 °C	21 ± 1 °C	14	80 ± 10%
European Beech (*Fagus sylvatica* L.)	22°C	22°C	12	70%
Pomegranate (*Punica granatum* L.)	22°C	22°C	12	70%
European Yew (*Taxus baccata* L.)	22°C	20°C	14	70%

Since all growth protocols foresee relatively constant values of humidity and temperature for the whole cycle, the HVAC system has been designed with a simplified control panel, independent from the central control system of the new growth chamber. The convenience of integrating it into the central control will be analyzed before the exploitation phase.

It is expected that the actual operation of the prototype will give a more clear insight of the true energy requirements for the operation and provide clues for further improvements and optimizations.

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ZEPHYR

Zero-impact Innovative Technology
in Forest Plant Production

Climate control in the production of forest plants - using PV to power an innovative forestry incubator

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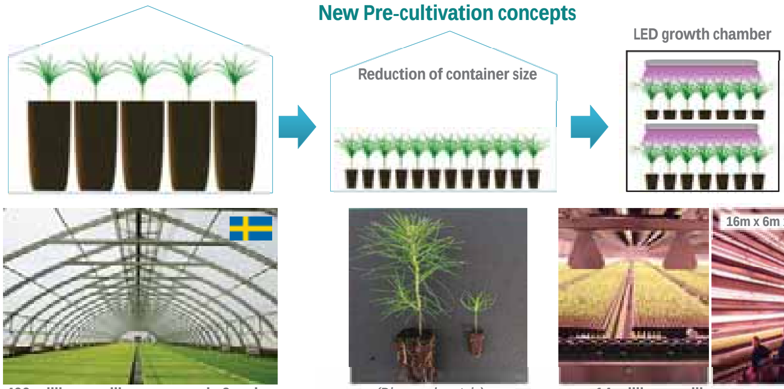
Background

Forest ecosystems are currently challenged by changes in climate, natural disasters and an extreme exploitation of their resources. Forest restoration requires among other things, high amounts of healthy seedlings to replace the lost or extracted trees. However, the cultivation of these seedlings is often done through intensive methods in forest nurseries. These techniques consume considerable amounts of energy for lighting, acclimatization and irrigation applying also significant amounts of fertilizers and pesticides.

Objectives

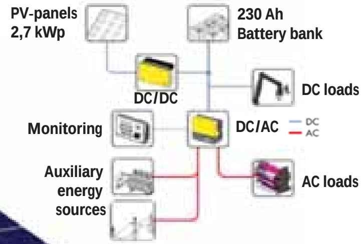
In the frame of the ZEPHYR project, funded by the European Commission under the Seventh Framework Programme (FP7), innovative and cost-friendly technologies for the pre-cultivation are being developed. They will be integrated into a functional and transportable system for a large scale production of seedlings, with zero-impact on the environment and not affected by outdoor conditions. To achieve this, high efficiency devices with low energy consumption will be used and the incubator will be powered by Photovoltaic (PV) solar energy.

New Pre-cultivation concepts



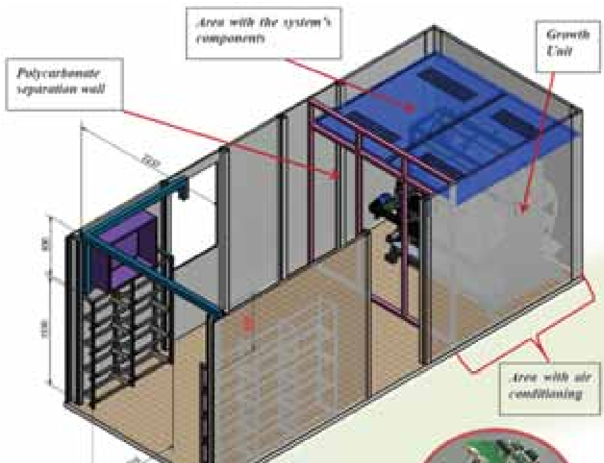
400 million seedlings per year in Sweden — *(Pinus sylvestris)* — 14 million seedlings per year

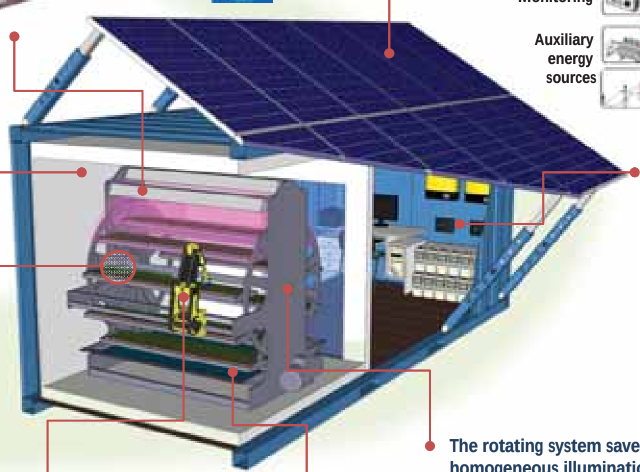
PV system and low energy consumption devices contribute to an operation with a zero-impact on the environment



Passively cooled LED-lamps provide a continuous light spectrum optimized for the seedlings' growth.

Closed environment eliminates the need for pesticides and gives better temperature and humidity control. Biological tests have been conducted to determine the best growing conditions for several species.





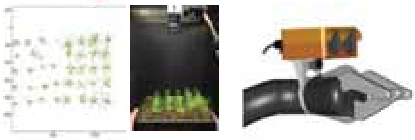
Battery bank provides at least one day of autonomy and allows using other auxiliary energy sources.

The rotating system saves space and ensures homogeneous illumination, temperature, and humidity. Intermittent light has proven to give excellent growth results in forest seedlings.

Irrigation via immersion saves water and allows to recycle the nutrients.

New family of miniaturized wireless sensors placed in the pots to measure the soil conditions

Stereoscopic cameras for shoot growth monitoring are mounted on a robotic arm which is also equipped with a gripper for operations inside the controlled environment



Project Outcomes

- Innovative and light growth chamber with a production capacity of up to 12 000 plantlets per cycle in a fully automatized and controlled environment.
- New family of wireless sensors studied for controlling the growth status
- High energy efficiency, due to space saving through the rotating system and the use of LED lights; up to 70% respect to traditional nurseries.
- Full automatized cycle production inside a standard TEU container powered with PV panels
- Flexible: more units can be placed in series for a bulk production

www.zephyr-project.eu

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No 308313

Total Cost: 4,284,275 € - EC Contribution: 3,438,252 €
Duration: 36 months - Start Date: 01/10/2012
Consortium: 14 partners from 10 countries
Project Coordinator: Tuscia University – DAFNE Department (Italy)

Environmental technologies

Analysis of the effects of gibberellic acid and cold stratification on the germination of two endemic species of Azorean Islands

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Introduction

Azorean islands (Fig.1) show about 300 native species. Among them, 156 are considered to be rare and a significant number is disappearing for the expansion of agriculture, farming and the competition with invasive exotic species (Pérez, 1999). In order to counteract their extinction and recover native habitats, some attempts of reforestation, based on artificial propagation of seedlings, because of a low dissemination of native species usually associated to the presence of seed dormancy, are underway.

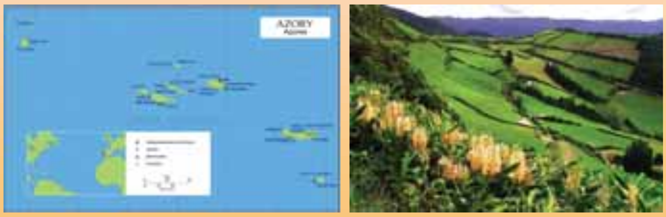

Fig.1 Azorean islands (Portugal)

Fig.2 a) *Hypericum foliosum*; **b)** *Laurus azorica*

Main purpose of this work is the analysis of the effects of gibberellic acid (GA3) and cold stratification on the germination of 2 endemic species of Azorean islands, *Hypericum foliosum* **Aiton (Moura, 1998) and** *Laurus azorica* **(Seub.) Franco (Fig.2), in terms of ability in promoting dormancy breaking or speeding up the germination process. Gibberellic acid is in fact known as a possible alternative treatment to that based on cold stratification with the advantage of a reduced time needed to induce germination (Bacchetta** *et al***., 2006).**

Materials and Methods

Four different pretreatments were tested: 30 or 60 days of cold stratification (4°C) without gibberellins; 18h of incubation in a GA3 solution with a concentration of 200 mg/L followed by 30 or 60 days of cold stratification. Pretreated seeds were then incubated at 20°C and 12L 12D in a phytoclimatic chamber. Two different controls were set up: direct incubation at 20°C and 12L 12D photoperiod (control 1); 18 h of immersion in a GA3 solution (200 mg/L) followed by incubation at 20°C and 12L 12D photoperiod (control 2).

Results and Discussion

H. foliosum **showed a 100% of germination reached in only 3 days as a consequence of 30/60 days of cold stratification and 80% in the case of combined pretreatments (GA3 + 30/60 days of cold stratification).**

Control 1 and 2 showed a lower germination percentage (< 10%) (Fig.3a). *L. azorica* **showed the best results (70% of germination) as a consequence of 60 days of cold stratification and GA3 pretreatment without stratification (control 2). Control 1 showed 30% of germination, as in the case of combined pretreatments (Fig.3b). Moreover, the combination of the two pretreatments led to higher shoot height values of seedlings, measured 1 month after germination.**

a

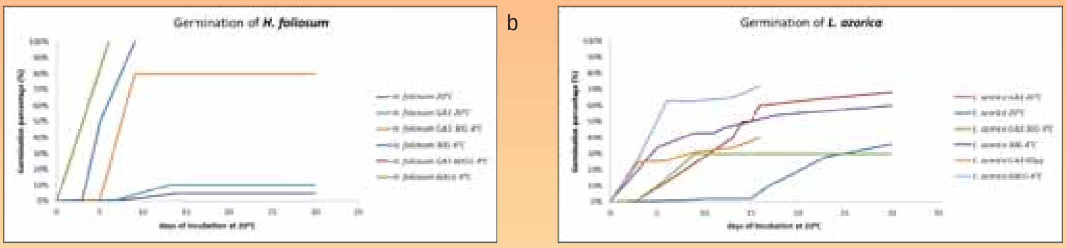

Fig.3 Percentage of germination of the two species after different pretreatments: a) *H. foliosum*; **b)** *L. azorica*

Conclusions

These results are promising for the definition of a germination protocol which would allow, in a short time, to obtain a significant number of seedlings of *H. foliosum* **and** *L. azorica***, to be used in reforestation and restoration activities in Azorean islands.**

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ZEPHYR

DAFNE
Dipartimento di scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia

B6 = ZEPHYR PROJECT: PHASE I, MORPHOGENETIC EFFECTS OF LED LIGHTS ON SEEDLINGS GROWTH

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Introduction

The main aim of Zephyr EU-project (FP7-ENV.2012.6.3-1) is the creation of a more sustainable and resource-efficient technology for forest nursery production. Plant growth will be not affected by outdoor climate thanks to LED lamps providing the optimal spectrum for photosynthesis and the production unit will be totally automated thanks to a control system based on wireless sensors regulating light intensity, photoperiod and further environmental parameters. It will be equipped with solar panels providing energy and with an irrigation system based on water recycling.

A strong reduction of fertilizers and the avoidance of pesticides will contribute to the environmental and biodiversity protection. The first step of the project is the selection of the most suitable LED light source by the growth analysis of target forest species under different wide continuous spectra.

Figure 1: Growth of seedlings under LED lights

	400-500 nm	500-600 nm	600-700 nm	700-800 nm
LED 1	11,9 %	19,3 %	60,5 %	8,3 %
LED 2	13,8 %	15,1 %	53 %	18,1 %
LED 3	7,7 %	2,4 %	64,4 %	25,5 %
LED 4	20,2 %	38,9 %	35,7 %	5,2 %
LED 5	10,5 %	26,2 %	48,9 %	14,4 %
Osram FLUORA T8	34,8 %	24,1 %	36,7 %	4,4 %

Figure 2: Spectral composition of light sources used in Zephyr, provided by Valoya® and Osram®

Materials and Methods

A first target species, *Punica granatum* L., was grown under 6 different light treatments: 5 different wide continuous spectra provided by Valoya® LED lights (Fig.1) and 1 continuous spectrum provided by OSRAM® FLUORA T8 neon tubes as control (Fig. 2). 104 seeds were sown per light treatment into plug trays containing a peat-based substrate (Jiffy® PREFORMA). Experiments were performed in a climate growth chamber at a temperature of 22 ±1°C, 60-70% of RH, a 12/12h photoperiod and PAR values at the ground level ranging between 100 and 300 µmol m^{-2} s^{-1}.

After 50 days of growth the morphological (epicotyl and hypocotyl length, stem diameter, number of leaves, fresh and dry weight of shoot, root and leaves, total and mean leaf area) and anatomical (leaf stomata density and area) parameters were measured. Microscopic analysis of leaf anatomy in transversal sections are in progress. Further biochemical and ecophysiological studies have to be planned.

Results and Discussion

The data obtained for each parameter under the 6 different light spectra were analysed via ANOVA to identify those characterized by a statistical relation with light. Among all the 14 measured parameters, only "*stem diameter*" resulted not to be affected by light. Subsequently, the dependence from light was analysed by "lm" R function and the light source able to ensure the highest values for one or more than one parameter was selected.

Among the five LED light sources, LED 5 showed the highest values for all the light-dependent parameters except for "*leaf stomata density*" (Fig. 3a) that was more promoted by LED 4. However, when compared with Osram FLUORA T8, LED 5 showed higher values for shoot biomass but lower values for root and leaves biomass (Fig. 3b, c, d).

P. granatum showed also different colors of leaves under each spectrum due to different percentage of chlorophylls, carotenoids and anthocyanins. These data reflect on the strong quali-quantitative light requirement of *P. granatum* seedlings growth but also point out that light regulates plant growth in an intricate manner. In fact, some spectra foster more the aboveground elongation than the development of roots and viceversa or the production of secondary metabolites.

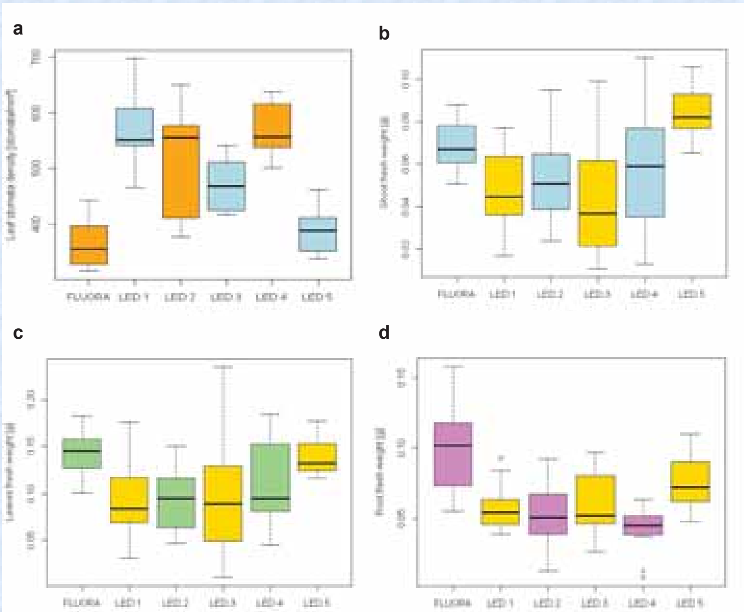

Figure 3: Box-plots of most significant morphological and anatomical parameters measured after 50 days of growth of *Punica granatum* L. under different light sources: a) Leaf stomata density; b) Shoot fresh weight; c) Leaves fresh weight; d) Root fresh weight.

Conclusions

According to these preliminary results, it seems not possible to select a single optimal spectrum ensuring the highest values for all the parameters of interest. Therefore it is convenient to equip the production unit with many interchangeable LED lights, suitable for different purposes of the user.

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2.1 = ANALYSIS OF *CORYLUS AVELLANA* L. GROWTH UNDER LED LIGHTS FOR REFORESTATION PURPOSES

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[2] SIRF, Società Italiana di Restauro Forestale

Introduction

Reforestation is very important in contrasting landscape degradation and desertification processes due to climate changes. In the Mediterranean area, forest restoration is also important to reestablish and to preserve the rich biodiversity of the different forest ecosystems, which has been damaged for a long time due to not appropriated silvicultural activities. The production of great amounts of high quality forest plants stocks ready to be transplanted in pots or in field at low costs is a primary challenge in this field (Astolfi *et al.*, 2012). Since 2012 the Zephyr EU-project (FP7-ENV.2012.6.3-1) is developing an automated high-density seedling production unit based on a combined action of optimal environmental conditions and LED lamps, with a noticeable reduction of emissions achieved through a low energy consumption, reduced by up to 70% in respect to the traditional nursery pre-cultivations.

After a first set of experiments on *P. granatum L.* (Marras *et al.*, 2013), which highlighted the possibility of using specific LED spectra to foster the development of roots and the production of secondary metabolites (two important elements favouring the adaption of plants in open-field), a second species was analysed: *Corylus avellana* L. In order to avoid the gradual loss of genetic variability of populations caused by the propagation by cuttings, propagation by seeds was chosen.

Materials & methods

After 2-months of cold stratification to break dormancy, 104 seeds were sown under each light source (5 Valoya® LED spectra vs OSRAM® fluorescent tubes as control, Fig.1b) in quickpots containing a peat-based substrate, in a climate growth chamber at a temperature of 22 ±1°C, at 60-70% of RH, with 12/12h of photoperiod and with 100 mμmol $m^{-2} s^{-1}$ PAR (Fig.1a).

a

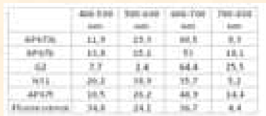

b


Fig.1 a) climate growth chamber;
b) spectra compositions

Fig. 2 Morphological analysis

After 1 month of growth, morphological analysis were performed on seedlings, before transplanting them into a greenhouse (Fig.2)

Results & Discussion

Results on *C. avellana* pointed out the best influence of LED spectra on stem growth (Fig.3a), stem diameter (Fig.3b), shoot, roots and leaves dry weights in comparison with conventional fluorescent tubes. In details, higher far red percentages (AP67 spectra) best performed on shoot and root weights (Fig.3c), while lower far red percentages (NS1 spectrum) did the same on leaves dry weight (Fig.3d). Mean leaf area did not show statistical differences among light sources, probably because it is more linked to light quantity than quality.

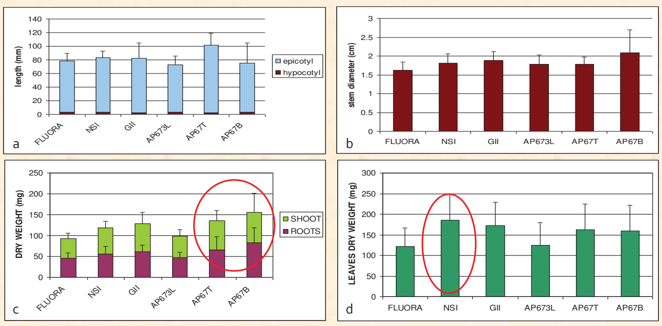


Fig.3 – Light quality effects on: a) stem growth; b) stem diameter; c) shoot and roots dry weight; d) leaves dry weight

Once transferred into the greenhouse for outdoor adaption, the plants immediately stopped to grow and leaves started to yellow before falling down (Fig.4). They were subjected to an evident transplant shock reaction, differently from *P. granatum*. Reasons for transplant shock reactions of pre-cultivated forest seedlings to open land can be related to several factors.

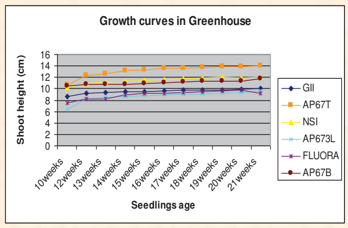


Fig.4 Growth curves of *C. avellana* in greenhouse

In particular the lack of protective mechanisms (as UV absorbing compounds) against higher UV-A and UV-B irradiance outdoors, that is absent/minimal during the indoor cultivation phase (Chalker-Scott, 2002; Solovchenko and Chivkunova, 2011) and the lack of protective mechanisms against high light intensity, which is much lower during indoor cultivation than outdoor. In fact, *P. granatum* seedlings were able to produce carotenoids and anthocyanins already under LED sources and these compounds led them to easily survive outdoor.

Conclusions

Other species, as *C. avellana*, are not enough stimulated to produce secondary metabolites by LED spectra commonly used for plant growth. In this case, the exposure to UV radiation during pre-cultivation, for a short time, in order to avoid DNA damage so as the exposure to higher light intensity before transplanting, or transient phase by using shading cloths during transplantation in open-field , could be suitable solutions to reduce this stress. Trials to test these solutions are subject of ongoing research.

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L. Chalker-Scott, Do anthocyanins function as osmoregulators in leaf tissues? In: K.S. Gould and D.W. Lee (eds.), Why Leaves Turn Red: Anthocyanins in Vegetative Organs, Advances in Botanical Research (2002), Academic Press, London
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UNIVERSITÀ Tuscia

ZEPHYR

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No 308313

The 4 Rs: Reduce, Reuse, Recycle … Reforest!

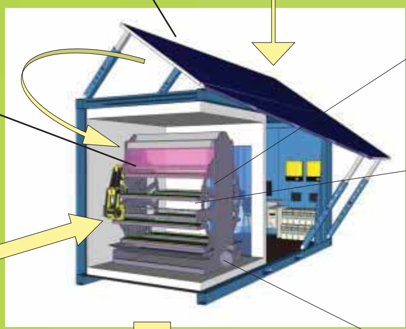
Instrument: FP7, Collaborative Project
Total Cost: 4,284,275 € - EC Contribution: 3,438,252 €
Duration: 36 months - Start Date: 01/10/2012
Consortium: 14 partners from 10 countries
Project Coordinator: Tuscia University – DAFNE Department (Italy)
Project Web Site: www.zephyr-project.eu

The main aim of Zephyr EU-project (FP7-ENV.2012.6.3-1) is the creation of a more sustainable and resource-efficient technology for forest nursery production. Plant growth will be not affected by outdoor climate and strong reduction of fertilizers and the avoidance of pesticides will contribute to the environmental and biodiversity protection.

REUSE: Renewable energy source (PV panels)

REDUCE human intervention: Wireless sensors connected to a central control system regulating environmental parameters

REDUCE energy consumption: LEDs



REDUCE space: rotating system and multiplug trays

Forest seeds (not cuttings to ensure genetic diversity)

BIODIVERSITY ensures best adaptation to environmental changes

RECYCLE: irrigation system based on water recycling

RESTORE FOREST & ENVIRONMENT

Direct planting (*in situ*)

Benefits and Services

- CO2 absorption
- Soil erosion control
- Tourism and recreation

Delivery and planting (*ex situ*)

NATURAL FORESTS/PLANTATIONS

URBAN FORESTS

- **GAME ANIMALS**
- **FUR-BEARERS**
- **NUTS**
- **SEEDS**
- **BERRIES**
- **MUSHROOMS**
- **OILS**
- **FOLIAGE**
- **MEDICINAL PLANTS**
- **PEAT**
- **FUELWOOD**
- **FORAGE**

1) **Food** (for humans and animals)

2) **Energy**

3) **Health**

ARTIFICIAL PROPAGATION OF ORIENTAL PLANE THROUGH LEDS

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Light intensity required by forest species has been widely studied, leading to their classification in heliophilous, sciaphilous and intermediate species. Conversely, few studies are available on light quality requirements. Artificial lights for plant growth have been designed mainly for agricultural crops that are all heliophilous, with high percentages of blue and red wavelengths in order to increase the photosynthetic activity. These light sources may be considered adaptable to heliophilous forest species; in order to test this hypothesis, a presumed heliophilous species, *Platanus orientalis*, was cultivated under different LED and fluorescent light sources, commercially available, in a controlled growth chamber. Some seedlings showed a progressive yellowing or reddening of leaves, leading to the hypothesis of a light stress. Therefore, the real light conditions in which natural regeneration occurs were analysed. The Natural Reserve of Pantalica (Sicily), was chosen as study area. Light spectra were collected along Anapo river, in July, from 10 a.m. to 2 p.m., in correspondence of different points with and without natural regeneration. Seedlings resulted to grow in slight shadow, frequently interrupted by short sunflecks. The spectra associated to shadow and sunflecks resulted to be different, both in terms of quality and quantity. Therefore, it seems that *Platanus orientalis* is not properly heliophilous, as reported in literature; this fact may explain why, as some sciaphilous species, it lacks of a complete xantophyll cycle, showing only leaf hairs as a protection against light excess. The results of this research show that the analysis of light requirements for each species is essential to define the best light conditions, in terms of quality and quantity, for artificial propagation.

Keywords: forest regeneration, light requirements, indoor propagation.
Parole chiave: rinnovazione forestale, esigenze luminose, propagazione artificiale.

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1. Introduction

P. orientalis L. belongs to the family of Platanaceae. It is a deep rooting tree with green alternate leaves that are usually lobed with a smooth margin. In autumn the leaves of many trees assume a ochre yellow pigmentation. The flowering period occurs in March – April. The species is monoecious. The fruit is a spherical infructescence, which is about 2 cm in diameter. The fruits often hang late as the spring on the trees. The twigs are greenish to brown with small lenticels. The buds are green, thick and protruding. The bark is grey-brown, small-scaly and flaking. It grows in sunny locations without tolerating continuous shadow. It is suitable for sandy, loamy and clay soils and tolerates pH from neutral to strongly alkaline. It prefers moist soil but can tolerate drought. The plant can tolerate strong winds but not maritime exposure. It can tolerate atmospheric pollution, too. *P. orientalis* grows naturally in the Balkan peninsula up to the 42° parallel. Eastward, it grows naturally in Turkey, Cyprus, in the countries of Western Asia as far as the western Himalayas (Panetsos, 1984). It is an element of lowland riparian forests (Zangheri, 1976; Pignatti, 1982; Tutin *et al*., 1993), from 0 to 600 m a.s.l. (Pignatti, 1982). *Platanus orientalis* is a deciduous species in all the area of its natural distribution, exhibiting the seasonal alternation of growth and dormancy, which is a characteristic of the trees of the temperate zone. One exception is represented by the evergreen Oriental Plane of Crete island (Nikolakaki and Hajaje, 2001). In Italy, where the species has the westernmost limit of its distribution, it occurs in Sicily, Calabria, Campania and has been recently excluded from Tuscany. *Platanus orientalis* has conservation significance as a characteristic species for the habitat 92C0 – *Platanus orientalis* and *Liquidambar orientalis* woods (*Platanion orientalis*) (All. I dir. 92/43 CEE) = G1.38 [*Platanus orientalis*] woods (EUNIS), and is also included in the Italian Red Data List as a vulnerable species (Caruso *et al.*, 2008). A big enemy which this species has to face in Italy is *Ceratocystis platani*, the agent of the canker stain of Plane. *Platanus* spp. are the only hosts of this pathogen. Penetration only occurs through wounds and the fungus colonizes the bark and also the wood. The main intervent which has been used in Italy against the expansion of the infection is to cut and burn the infected tree leaving the coppice on the soil, removing also residual sawdust that is highly infective. The low number of residual fertile trees and the creation of gaps make easier the expansion of other species, in particular of invasive ones.

2. Materials and methods

Artificial propagation of *Platanus orientalis* L. under LED and fluorescent light sources was tested in parallel in Viterbo (Italy) in the laboratories of University of Tuscia (UNITUS) and in Thessaloniki (Greece) at Democritus University of Thrace (DUTH) within the European "Zephyr" project
(http://www.zephyr-project.eu/node/1).

2.1. Growth protocols of DUTH

2.1.1. Growth conditions

P. orientalis L. seeds were collected from Thermi, Thessaloniki, Greece, in March 2013. After having been smashed, seeds were stored in sealed glass containers at a low temperature (+5° to +7°C). Then seeds were deprived of their hairs, hydrated for 24 hours and placed in petri dishes with wet sand. They were then subjected to 50 days of cold stratification at 3-5°C. Afterwards, petri dishes were transferred in a phytotron chamber with 16L:8D photoperiod and a temperature of 20°C during the day and 15 °C during the night, under cool-white fluorescent lamps, in order to induce germination. Germination percentage after cold stratification was 70%. Pre-germinated seeds were transferred into a stabilized medium (Jiffy® peat-based substrate) in Herkuplast® trays (QPD 104 VW type: tray dimension 310x530; cell size 38.5 mm; plant centre 43/43 mm; depth 50 mm; volume 50 cc; 510 plant/m^2) and grown for 7 weeks under artificial lights in a climatized growth chamber (one tray per each light treatment) with 14L:10D photoperiod, 80 ± 10% of relative humidity and a temperature of 20°C during the day and 15 °C during the night. Six different light sources were tested: 5 different Valoya® LED lights (L20-AP67 tubes, AP67 bars, AP673L bars, NS1 bars, G2 bars) and 1 OSRAM® Fluorescent light (L36W/77 FLUORA tubes). Light PAR of tubes (L20-AP67 and L36W/77 FLUORA) was set around 50±20 μmol m^{-2} s^{-1} while that of LED bars was set around 200±20 μmol m^{-2} s^{-1}. Trays were watered twice a day with automatic sprinklers.

2.1.2. Growth analysis

Shoot height and leaves number were measured every 2 weeks on a sample of 10 seedlings, randomly chosen.

2.2. Growth protocols of UNITUS

2.2.1. Growth conditions

Fresh seeds collected from Thermi, Thessaloniki, Greece were sent to Italy in March 2013. Seeds were stored at 4°C in a juta bag for 4 months. In July 2014, seeds were deprived of their hairs, hydrated for 24 hours and subjected to cold stratification using perlite as substrate for 60 days at 3-5°C. Afterwards, they were transferred into a growth chamber at 22°C under fluorescent tubes (100 μmol m^{-2} s^{-1}) to induce germination. Germinated seeds were transferred in a sand-based substrate (river sand: peat: pozzolana: coal, 50:15:25:10) in Herkuplast® trays (QPD 144/6R type: tray dimension 310 × 530 mm; cell size 31 mm; depth 60 mm; volume 31 cc; 870 plants/m2) and grown for 8 weeks under artificial lights in a climatized growth chamber (one tray per each light treatment) with a 12L:12D photoperiod, 60 ± 5% of relative humidity and constant temperature equal to 22 ± 1°C. Six different light sources were tested: 5 Valoya® LED lamps: L20-AP67 tubes, AP67 bars, AP673L bars, NS1 bars, G2 bars) and 1 OSRAM® Fluorescent light (L36W/77 FLUORA tubes). Light PAR was set at 50 ±10 μmol m^{-2} s^{-1} for all the lamps.

2.2.2. Growth analysis

Shoot height and leaves number were measured twice a week for 63 days on a sample of 24 seedlings randomly chosen.

2.3. Light spectra collection

2.3.1. Study area

A Sicilian plane forest was chosen as study area to analyse which light conditions allow the natural regeneration of oriental plane,. This forest is located in the Natural Reserve of Anapo Valley, that is also an UNESCO site. The Oriented Natural Reserve of Pantalica, Anapo river valley and Cava Grande torrent (founded in 1997 to preserve the association of *Platanelia orientalis*) occupies an area of 3,712 hectares through the territories of Sortino, Ferla, Cassaro, Buscemi and Palazzolo Acreide (in the province of Siracusa). The predominant vegetation is the typical Mediterranean maquis, characterized by the presence of oriental plane trees, black and white poplars, willows and a rich and fragrant underwood; the less steep slopes are colonized by large oaks and holm oaks. In the past centuries, *Platanus orientalis* was distributed along the river borders but since the last decades the species has been hugely threatened by the stain canker which has progressively decimated the population.

2.3.2 Transect surveys

Two transects 20 x 6 m, characterized by the presence of more than one adult plane tree, were selected along the watersides. In each one, 3 points with and 3 points without natural regeneration were identified. Light spectra, ranging from 180 nm to 1100 nm, were collected for each point at different times (10 consecutive measurements in 60 s per each point) during the day (10 a.m.; 12 a.m.; 2 p.m.) with a Stellarnet spectraradiometer, at soil level.

2.4 Statistical analysis

2.4.1. Growth analysis

Both DUTH and UNITUS, compared shoot height and leaves number per each day of measurement through ANOVA and Tuckey range test, in order to identify significant differences among the light sources ($P > 0.001$).

2.4.2. Light spectra analysis

Light spectra were subdivided into 8 regions, identified by specific ranges of wavelengths corresponding to a specific colour: UV (ultraviolet) < 400 nm; violet, from

400 to 430 nm; blue, from 430 to 480 nm; green, from 480 to 560 nm; yellow from 560 to 590 nm; orange from 590 to 630 nm; red from 630 to 750 nm; IR (infrared) > 750 nm. The irradiance (W/m^2) of each region so as the irradiance of the whole spectrum, were compared among the spectra taken for all the points at the same hour and for each point at different hours during a day, through ANOVA and Tuckey range test, in order to identify significant differences.

3. Results and discussion

3.1 DUTH growth analysis

The comparison of shoot height after the 1st week of growth shows that G2 spectrum is able to fasten shoot growth if compared to all the other spectra which are able to fill the gap in 20 days, except for fluorescent tubes which need 35 days. After day 35 there are no more significant differences among the different treatments (Fig. 1). No significant differences in leaves number were detected among all the spectra during 7 weeks (Fig. 2) but seedlings developed a reddish or purple colour during the last two weeks of growth under LED bars, not under LED tubes. This effect may therefore be linked to the different values of intensity of the two types of lights.

3.2 UNITUS growth analysis

After 13 days, seedlings grown under NS1 and AP673L show the lowest values for shoot height while AP67B and AP67T show the highest values. After 16 days FLUORA still shows significant lower height values while the other spectra reach the same average value, recovering the initial slowing down. Between day 16 and day 50, AP673L and AP67T show a faster growth rate, reaching significant higher values of shoot height, in particular if compared to FLUORA, which exhibits an arrest in plant growth (Fig. 3). Since day 13 to day 40, FLUORA shows a faster emission of leaves and therefore a significant higher number, while at day 50 there are no more significant differences among all the spectra (Fig. 4). After 50 days a sudden chlorosis started to cause the loss of leaves of some seedlings. No changes in leaf colour were detected.

3.3 Light spectra analysis

Significant differences, in terms of light quality and quantity, were found comparing the spectra collected at the same hour, in correspondence of different points, with and without regeneration of Oriental Plane, so as those collected in correspondence of the same point but at different hours during the day. In fact, no points characterized by continuous shadow or by continuous full sun from 10 am to 2 pm, were detected. Every selected point, independently from the presence or absence of regeneration, is characterized by the alternation of two main conditions: a slight shadow, due to the forest canopy and frequent brief sunflecks (brief bursts of light on a ms scale) due to the movement of leaves in the upper layers induced by the wind, so that intensity varies from 120 W/m^2 in the shadow to 1800 W/m^2 during sunflecks (Fig. 5). The wavelength regions which show the most significant differences between the two light conditions are those of red and infrared light, followed by the green and orange regions. In fact, slight shadow shows a 10% of red light and a 72% of infrared light, while sunflecks are characterized by a 20% of red light and 50% of infrared light (Tab. 1). Therefore, red : far red ratio results to be lower in slight shadow (0.1) than in full sun (1).

4. Conclusion

Natural regeneration of oriental plane takes place along rivers, on sandy substrates and in slight shadow, frequently interrupted by brief sunflecks all over the day. Therefore, *Platanus orientalis* is not properly heliophilous, as reported in literature.

As some sciaphilous species, its seedlings lack of a complete xantophyll cycle and transient leaf hairs are a sufficient protection against full sun (Bisba *et al*., 1997), which directly arrives at seedlings level only for a few milliseconds during sunflecks. Commercially available lamps for plant growth have high percentages of red and blue light, even higher than the natural percentages characteristic of full sunlight. Therefore, even if their intensity is not too high (100-300 PAR), the continuous exposition for 12/14 hours a day to these high values may stress *P. orientalis* seedlings lacking in high light protection systems, causing a forced reddening of leaves. On the other hand, because of the fact that the species grows in slight and not deep shadow, also too low intensities may stress plants, determining a progressive chlorosis which may be increased by the calcareous substrate. Nevertheless, artificial lamps result to be able to fasten oriental plane seedlings growth if compared to natural conditions, in which plants may take several months to reach the same growth stage obtainable after 2 months of growth in a controlled growth chamber. Moreover, because of the fact that artificial spectra are only slight different one from each other in terms of light quality, similar results in terms of shoot height and leaves number were detected during the growth period under all the tested light sources. The main difference between natural and artificial light, that probably mostly influences seedlings growth, is related to the red:far red ratio. In fact artificial lamps show high values of this parameter, which equal if not surpass the natural maximum value which is reached only in full sunlight. It should be noted that *P. orientalis* seedlings generally grow in slight shadow which is characterized by low values of this ratio and are exposed to the maximum value only during brief sunflecks. The results of this research show that the analysis of light requirements of a particular species is essential to define the best light conditions, in terms of quality and quantity, for artificial propagation.

Table 1. Composition of light spectra corresponding to shadow and full sunlight conditions, expressed as irradiance percentage of different colour regions.
Tabella 1. Composizione degli spettri luminosi corrispondenti a condizioni di ombra e sole pieno, espressa come percentuale di irraggiamento delle differenti bande cromatiche.
Table 1. Composition des spectres correspondant à l'ombre et au soleil, exprés comme pourcentages de irradiation des différent régions de coleur.

	shadow	*sunfleck*
ultraviolet	3 %	3 %
violet	2 %	2 %
blue	4 %	5 %
green	6 %	12 %
yellow	2 %	5 %
orange	2 %	7 %
red	9 %	19 %

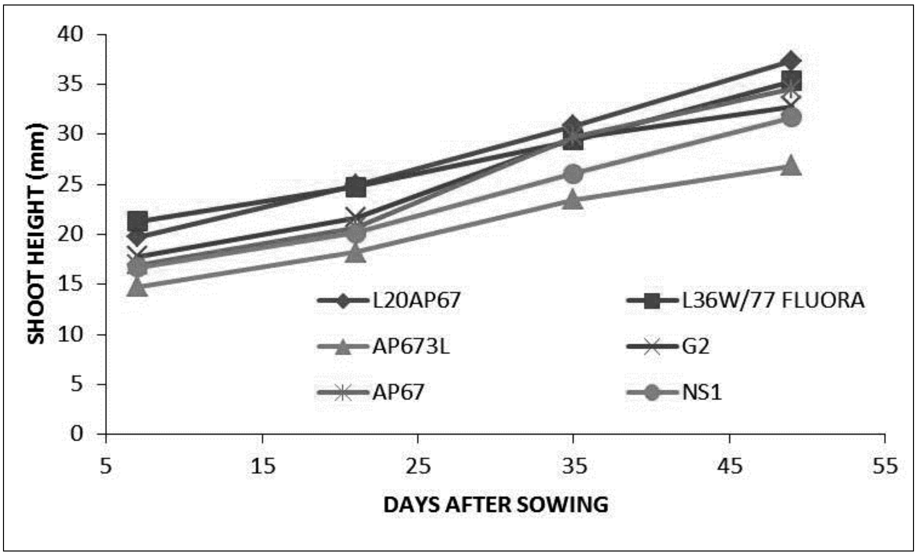

Figure 1. Shoot height of oriental plane seedlings during 7 weeks of cultivation in phytotron chamber (DUTH).
Figura 1. Altezza del fusto di semenzali di Platano orientale nel corso di 7 settimane di coltivazione in fitotrone (DUTH).
Figure 1. Hauteur de la tige des bourgeons de *P. orientalis* pendant 7 semaines de culture dans un fitotron (DUTH).

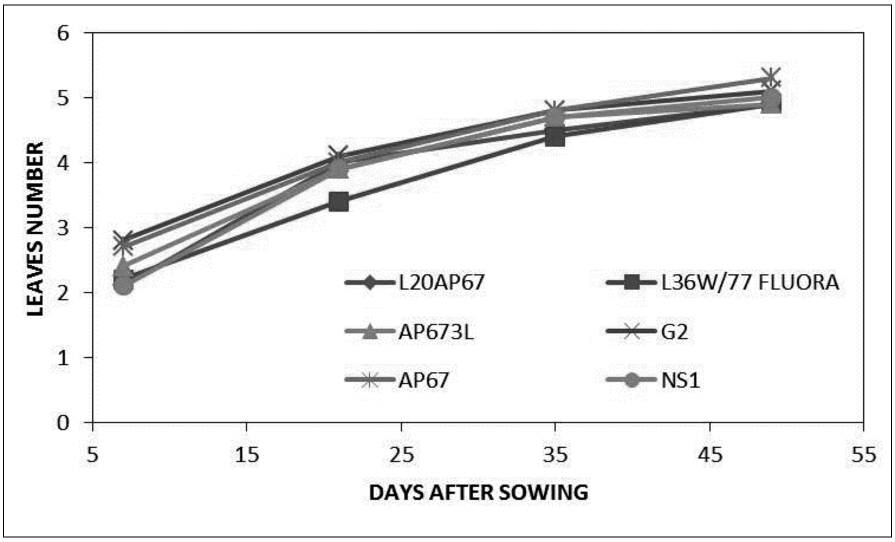

Figure 2. Leaves emission of oriental plane seedlings during 7 weeks of cultivation in phytotron chamber (DUTH).
Figura 2. Emissione delle foglie di semenzali di Platano orientale nel corso di 7 settimane di coltivazione in fitotrone (DUTH).
Figure 2. Emission des feuilles des bourgeons de *P. orientalis* pendant 7 semaines de culture dans un fitotron (DUTH).

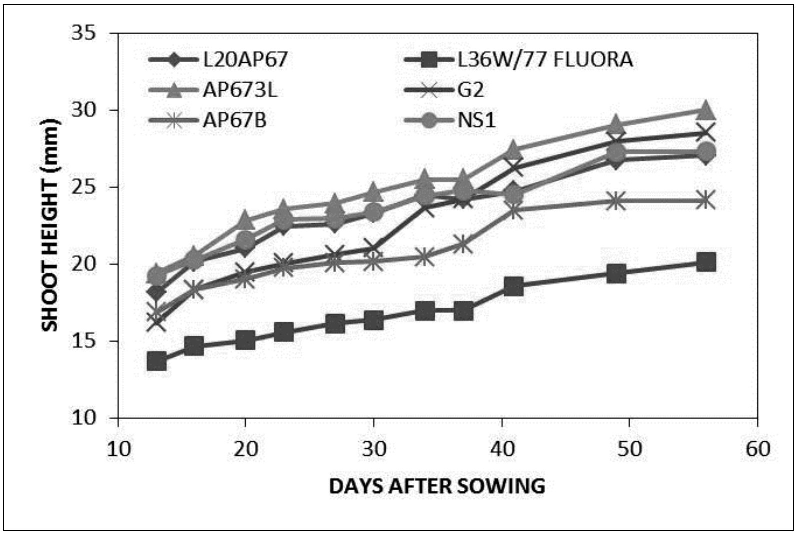

Figure 3. Shoot height of oriental plane seedlings during 8 weeks of cultivation in a climatized growth chamber (UNITUS).
Figura 3. Altezza del fusto di semenzali di Platano orientale nel corso di 8 settimane di coltivazione in camera di crescita climatizzata (UNITUS).
Figure 3. Hauteur de la tige des bourgeons de *P. orientalis* pendant 8 semaines de culture dans une chambre de culture (UNITUS).

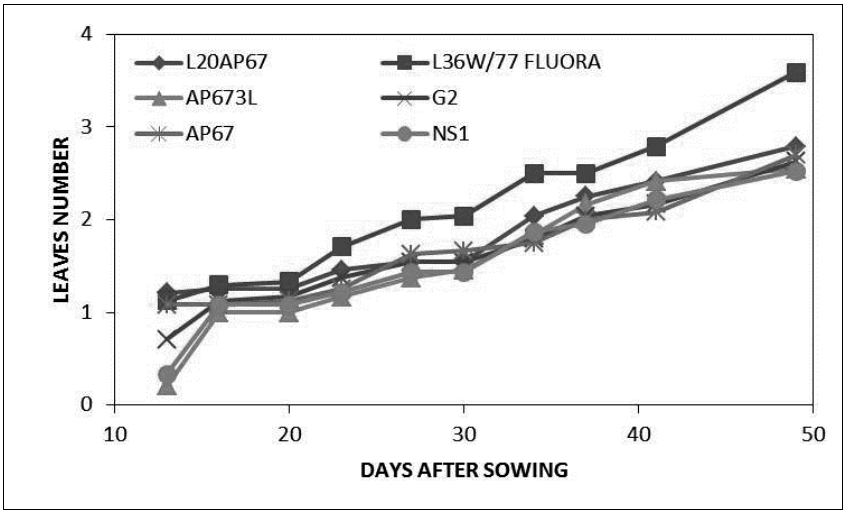

Figure 4. Leaves emission of oriental plane seedlings during 8 weeks of cultivation in a climatized growth chamber (UNITUS).
Figura 4. Emissione delle foglie di semenzali di Platano orientale nel corso di 8 settimane di coltivazione in camera di crescita climatizzata (UNITUS)
Figure 4. Emission des feuilles des bourgeons de *P. orientalis* pendant 8 semaines de culture dans une chambre de culture (UNITUS).

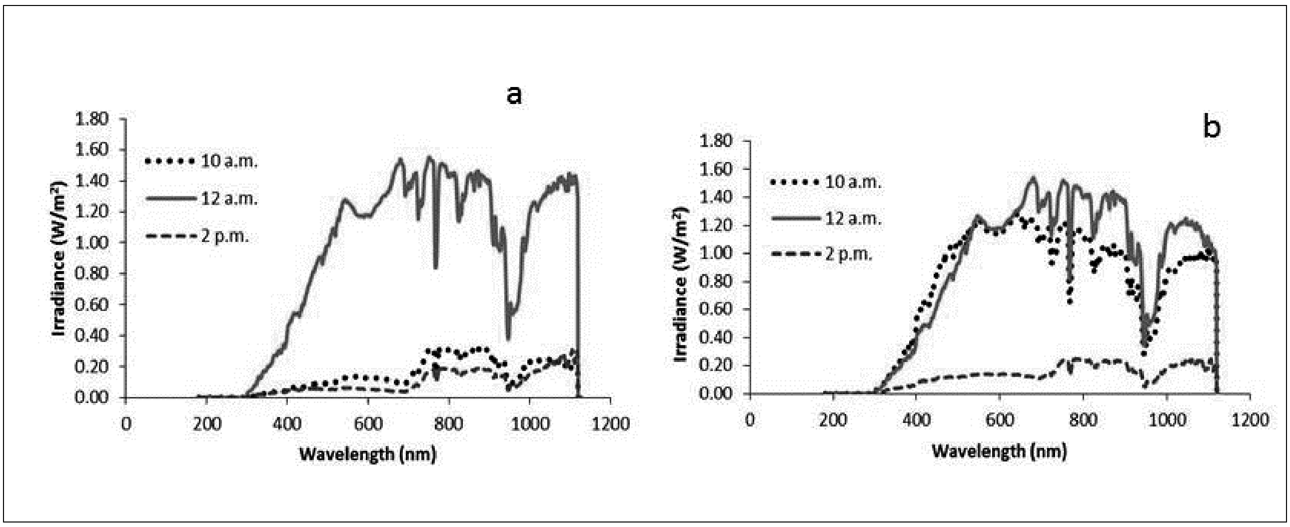

Figure 5. Alternation of different light conditions at soil level in a *P. orientalis* forest, in correspondence of points with (a) and without (b) regeneration, from 10 a.m. to 2 p.m.
Figura 5. Alternanza di differenti condizioni di luce a livello del suolo in una foresta di *P. orientalis*, in corrispondenza di punti con (a) e senza (b) rinnovazione, dalle 10 a.m. alle 2 p.m.
Figure 5. Alternance de différent conditions de lumière a terre dans un forêt de *P. orientalis,* en correspondence avec points avec (a) et sans (b) regeneration.

RIASSUNTO

Propagazione artificiale del Platano orientale mediante LED

Molteplici studi sull'intensità luminosa richiesta dalle specie forestali, hanno condotto alla loro suddivisione in specie eliofile, sciafile e intermedie. Rari sono invece gli studi sulla qualità della luce. Le luci artificiali per la crescita vegetale sono state create per specie agricole eliofile, con un'alta percentuale di rosso e blu al fine di promuoverne l'attività fotosintetica. Pertanto tali lampade potrebbero ritenersi adattabili alle specie eliofile forestali.
Una presunta specie eliofila, il Platano orientale, è stata coltivata sotto 5 fonti luminose LED ed 1 fluorescente, disponibili in commercio, in una stanza climatizzata. Alcuni semenzali hanno mostrato un progressivo ingiallimento o arrossamento fogliare, caratteristici dello stress luminoso. Si è quindi deciso di analizzare le naturali condizioni luminose in cui avviene la rinnovazione.
La Riserva Naturale di Pantalica (Sicilia) è stata scelta come area di studio. Spettri luminosi sono stati raccolti lungo il fiume Anapo nel mese di Luglio, tra le ore 10 e 14, in punti con e senza rinnovazione. Le plantule sono risultate crescere in condizioni di ombra, interrotta frequentemente da brevi *sunflecks*. Gli spettri corrispondenti a condizioni di luce e ombra sono apparsi molto differenti, sia in termini qualitativi sia quantitativi. Pertanto, il Platano orientale non risulta essere realmente eliofilo, come descritto in letteratura. Come molte specie sciafile, le plantule mancano di un ciclo completo delle xantofille mentre una transiente pubescenza fogliare risulta essere l'unica protezione contro l'eccesso di luce.
I risultati di questa ricerca mostrano la necessità di analizzare i requisiti luminosi delle singole specie forestali per definire le migliori condizioni luminose, in termini di intensità e qualità, per la propagazione artificiale.

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Light needs for seed germination and early development of seedlings in cork oak (*Quercus suber* L.)

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Abstract. Seed germination and seedling development of cork oak have specific light needs in terms of photoperiod, light quantity and quality. To reproduce optimal light conditions in a controlled environment, these parameters were analyzed in a mature cork forest in Central Italy (Viterbo) from November 2014 to the spring 2015. The species range has a photoperiod between from 9h52'01'' (NW) and 15h15'17'' (SE); the total daytime is 4859 h in the northernmost point and 4762 h in the southernmost. In Viterbo, during the period of analysis, photoperiod ranges from 11h 19'25''to 12h 9'26''. Germination resulted to occur both in sunny and shaded areas, with light intensity ranging from 100 to 2000 PPFD and RED/FAR RED ratio ranging from 1(sun) to ~ 0.3 (shadow). Clouds effect was analysed in an open area showing a significant reduction of light intensity (up to 90 %) without great variations in light quality. In particular, RED/FAR RED ratio, very important for germination and first stages of seedling growth, remains invariable. Commercial plant lights provide spectra which are too different from that of the sun, especially for higher values of PAR and RED/FAR RED ratio. To evaluate the effect of different spectra on germination and seedling development, 7 light sources were tested for cork oak propagation with a photoperiod of 12L 12D. Data were compared to those collected into the forest. Germination and seedling development resulted to be speeded up under all artificial conditions. This may be caused by the lack of diurnal temperature variations and by the high PAR and RED/FAR RED ratio values of the lamps.

Keywords: Light needs, germination, cork oak, photoperiod

1. Introduction

Forest restoration programs include the reintroduction of trees in degraded sites by means of two methodologies: 1) fostering the natural regeneration from mother plants; 2) supporting the regeneration, i.e. planting new seedlings produced in nurseries. Different natural factors affect plant growth, both in natural environment and in nurseries: light, nutrients, temperature, wind, soil moisture and composition, animal predation and species competition, the latter only related to natural environment. An innovative way to avoid or reduce some of these limiting factors is the application of the *plant factory* concept also into forest plant production. Plant factories are closed growing systems producing high-quality vegetables during the whole year, due to an artificial control of the cultivation environment (i.e. light intensity, photoperiod, temperature, carbon dioxide concentration, relative air humidity, culture substrata). Thanks to the total independence from climate and multiple cultivation shelves, these systems lead to a faster and more plentiful production than traditional outdoor cultivations, also for exotic species. Another important advantage of such systems is that pesticides are not needed. These systems are energy saving technologies, able to reduce vehicles emissions thanks to the local production, as well as the consumption of soil and water, the latter also possible to be recycled. In Japan, about 200 plant factories are currently producing lettuce, herbs, tomatoes, strawberries and other agricultural species (Kim, 2010; Lee, 2010). Presently, no plant factories producing forest species are available. The first European project focusing on this topic is Zephyr (www.zephyr.project.eu). This project, started in 2012, aims to the introduction of an innovative technology inspired by the plant factory concept for the pre-cultivation of forest regeneration materials in a zero-impact and cost friendly mobile production unit. During a 3-years work, about 20 different forest species have been cultivated under artificial lights in order to define the optimal growth protocols for a mass production of seedlings into the final prototype. For each species the best values of temperature, relative air humidity and photoperiod have been defined according to data from literature and field surveys. While the optimal values of the abovementioned parameters for a target species are easily achievable, the definition of the best quali-quantitative light conditions for its growth is a challenging hint. Indeed, forest species are divided into two main classes basing on their light needs: heliophilous species (or shade intolerant) and sciaphilous species (or shade tolerant). Generally, this classification is valid for adult trees, but sometimes the behavior of the seedlings is divergent from the mother plants (Loach, 1970). To understand the real light needs of seedlings, field surveys are needed in forests where natural regeneration occurs. This study focuses on cork oak (*Quercus suber* L.), a Mediterranean evergreen oak growing up to 20 meters; an emblematic species of many landscapes of the Mediterranean Biome, sustaining rich biodiversity, ecological processes, ecosystem preservation and representing a valuable source of income from its bark, the cork. The ongoing repercussions of human impacts and climate change are still shaping the cork oak distribution, thus increasing the threatens for the species permanence in many areas of the Mediterranean. In view of this, reforestation programs are becoming a primary need. Different studies are available on the effects of various degrees of shade on cork oak seedlings growth (Benayas, 1998; Cardillo *et al*., 2006) but no attempts of cultivating this species under artificial lights have been made up to now. This study is the first focusing on the effectiveness to produce a great amount of cork oak seedlings, in a controlled environment, ready to be used in reforestation programs.

2. Materials and methods

2.1. Selection of light sources

Different spectra provided by commercially available lamps generally used in greenhouses to promote photosynthesis were compared. Each commercial lamp was characterized by a specific wide continuous spectrum ranging from 180 to 1100 nm, almost lacking in UV and infrared and with different percentages of blue, green, yellow, orange and red wavelengths. Among these, FLUORA (Fluorescent lamp, Osram) and NS1 (LED lamp, Valoya) resulted to be the most resembling sunlight. Moreover, NS1 was preferred because of the lower energy consumption, the longer life, and the lower heat production of LED in comparison to fluorescent lights.

2.2. Study site

A natural cork oak forest located in the Natural Reserve of Tuscania (Viterbo, Central Italy, 42° 25’ 08’’N, 11°52’06’’E, 189 m. a.s.l.), was chosen as study site (Fig.1). This forest, recognized as SCI (Site of Community Importance), occupies 40 ha of the total 1,901 ha of the Reserve, which is divided into two halves by the Marta River. Acorns production is abundant and natural regeneration as well, even if new seedlings have to face the high stress of Mediterranean summer drought, which is one of the main causes of seedlings’ death during the first year of life (Pausas *et al*., 2009). Canopy cover is too dense, so that seedlings do not grow in open areas but in different degrees of shade.

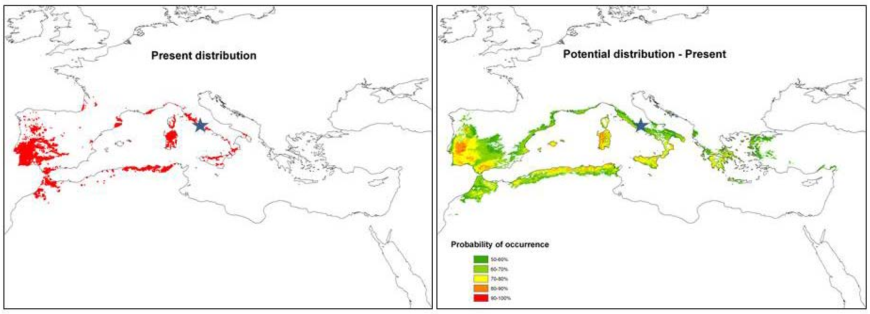

Fig.1 Present and potential distribution of cork oak

2.2.1. Collection of climatic parameters

Daily climatic parameters were downloaded from a meteorological website (www.ilmeteo.it) for the period from November 2014 (corresponding to acorns fall and beginning of germination) to May 2015 (corresponding to the first apical bud closing of new seedlings): Tmin and Tmax; humidity percentage; precipitation; photoperiod and irradiance.

Theoretical daily irradiance was calculated using the *Solar radiation* ARCGIS toolbox, under standard conditions of clear sky and considering topographical features as secondary variables. Photoperiod was calculated as day length between sunrise and sunset, using the exact times for sunrise and sunset, daily provided by the above-mentioned website.

2.2.1. Light analysis

Six areas with abundant cork oak regeneration were selected inside the forest, 3 of them well-lighted at noon (HL) and 3 shaded at noon (LL, 50-60% of full PAR). At the beginning of November 2014, 6 metal cages (50*50cm) were placed, one per area, to avoid animal predation of acorns. Light spectra (from 180 nm to 1100 nm) were hourly collected, from 9 am to 2 pm, in a sunny day to avoid possible interferences by clouds, per each cage. Two different spectra were collected as controls in an open area during a sunny and a cloudy day (control 1 and control 2, respectively). Light spectra collected at noon were subdivided into 8 regions, identified by specific ranges of wavelengths corresponding to a specific color: UV (ultraviolet) < 400 nm; blue-green (400-520 nm); yellow-orange (520-610 nm); red (610-720 nm); Far red (720-850 nm); IR (infrared) > 850 nm. A quantitative comparison of irradiance (W/m^2) of single regions was carried on among the 6 cages and controls.

2.2.2. Seedlings analysis

Dates of germination, shoot apex emergence and first bud closing, as well as the percentages of germination and of emergence, during a period ranging from January (early emergence) and May (late emergence), were detected for each cage. Moreover, after the first leaves sprouting, shoot height and number of leaves were monthly measured until the first apical bud closing. At apical bud closing stage, thinness coefficient was evaluated, as ratio between shoot height and shoot diameter.

2.3. Indoor growth

2.3.1. Growth protocols

Acorns were collected in the cork oak forest of Tuscania at the beginning of November 2014 and stored at 4°C in a dark aerated box until February 2015. At the beginning of February, they were immersed for 24 hours in tap water in order to rehydrate them and to select the ones eligible for sowing. Then, 52 acorns with pericarp and 52 deprived of pericarp, were disinfected with a solution of 20% sodium hypocloride and sown into a multi-plug tray (Herkuplast, QPD104VW, 104 pots) using a peat-based substrate. The tray was incubated into a phytoclimatic chamber under NS1 light. Light intensity, air temperature and relative air humidity were kept constant, respectively at 150±50 PAR, 22±2°C and 50±10%. A 12L 12D photoperiod was applied.

2.3.2. Seedlings analysis

Seedlings growth was weekly monitored measuring shoot height and number of leaves. The percentage of germination and the date of germination, shoot apex emergence and first bud closing were detected. At apical bud closing stage, thinness coefficient was calculated.

3. Results

3.1. Study site

3.1.1. Collection of climatic parameters

Table 1 shows minimum, maximum and mean value of daily Tmin, Tmax, precipitation, humidity and irradiance for the study site, between November 2014 and May 2015.

Tab.1. Climatic parameters referred to the study site of Tuscania (Nov 2014-May 2015)

Parameter	**Minimum value**	**Maximum value**	**Mean value**
Daily Tmin	-2°C	+16°C	6.1°C
Daily Tmax	0°C	25°C	13.9°C
Daily precipitation (Total precipitation Nov 2014-May 2015: 941 mm)	0 mm	72 mm	5.19 mm
Daily humidity	37%	97%	75%
Daily irradiance	726.39 kWh/m^2	5,562.48 kWh/m^2	2,381.83 kWh/m^2

Fig.2a shows the annual photoperiod for different latitudes into the range of cork oak. Fig.2b highlights the annual curve referred to Tuscania. Fig.2c shows the photoperiod referred to Tuscania, in the period of analysis (Oct 2014-May 2015).

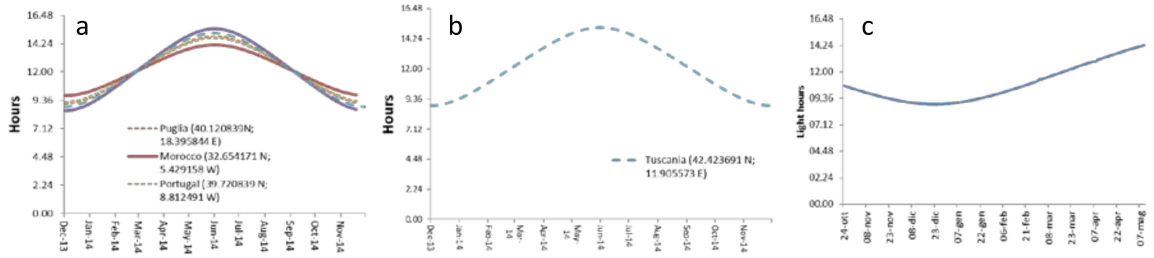

Fig.2: a) annual photoperiod for different latitudes into the range of cork oak; b) annual photoperiod for Tuscania; c) Photoperiod for Tuscania (Oct 2014- May 2015)

3.1.2. Light analysis

Spectra collected for each cage from 9 am to 2 pm in a sunny day, showed as no cage has been constantly exposed to the same level of light during the day, rather to different degrees of partial shade, whose depth depends on the canopy density (Fig.3). Spectra collected at noon were quantitatively and qualitatively compared to control 1 and 2 (Fig.4) as shown in table 2.

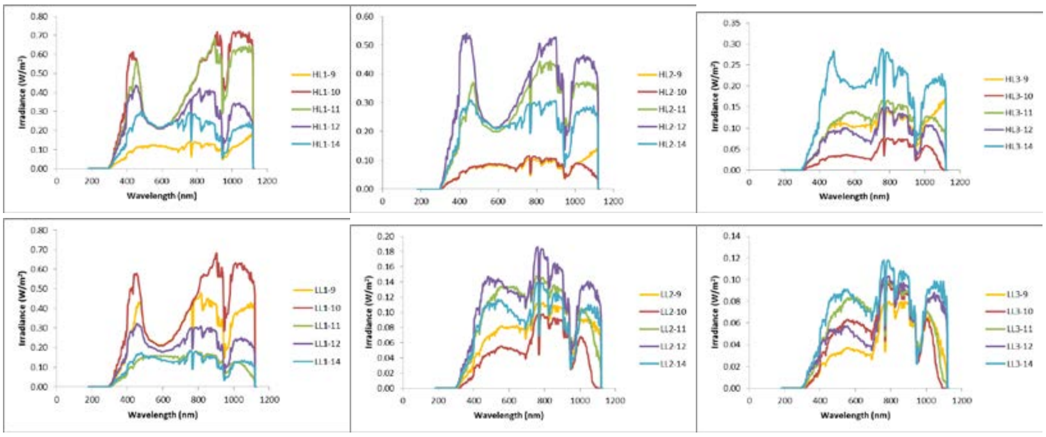

Fig.3. Spectra collected from 9 am to 2 pm in correspondence of high light cages (HL1-HL2-HL3) and low light cages (LL1-LL2-LL3)

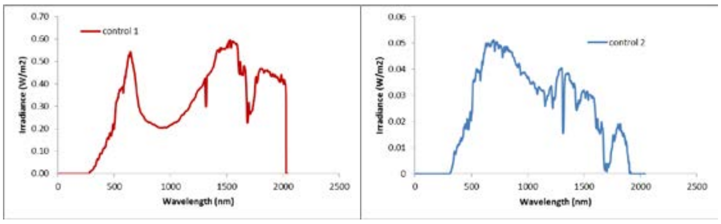

Fig.4. Control spectra collected at noon in a sunny day (control 1) and in a cloudy day (control 2)

Table 2. Comparison of the irradiance of the different colour regions of the spectra collected at noon in correspondence of each cage, expressed as W/m^2 and percentage of total irradiance

	HL1	**HL2**	**HL3**	**LL1**	**LL2**	**LL3**	**control 1**	**control 2**
UV	27.16 (2.5 %)	37.14 (3.49%)	19.41 (2.38%)	5.67 (1.09%)	7.75 (1.24%)	3.79 (0.78%)	54.55 (5.28%)	0.77 (0.46%)
Blue-Green	95.63 (8.82%)	117.27 (11.03%)	73.39 (9.01%)	22.48 (4.32%)	33.98 (5.42%)	13.21 (2.74%)	133.64 (12.93%)	3.18 (1.91%)
Yellow-Orange	43.97 (4.06%)	44.12 (4.15%)	37.51 (4.60%)	18.45 (3.55%)	26.67 (4.25%)	10.47 (2.17%)	44.12 (4.27%)	2.11 (1.27%)
Red	58.24 (5.37%)	62.92 (5.92%)	48.00 (5.89%)	18.00 (3.46%)	29.04 (4.63%)	10.20 (2.11%)	63.55 (6.15%)	3.04 (1.83%)
Far Red	98.31 (9.07%)	116.38 (10.94%)	75.79 (9.30%)	34.48 (6.63%)	43.22 (6.89%)	23.77 (4.93%)	129.61 (12.54%)	4.37 (2.63%)
Infrared	273.71 (25.26%)	350.98 (33%)	204.43 (25.08%)	86.59 (16.65%)	113.96 (18.17%)	65.49 (13.57%)	472.45 (45.71%)	11.28 (6.79%)
Tot (180-1000 nm)	1083.73	1063.64	814.98	520.13	627.26	482.71	1033.64	166.11

3.1.3. Seedlings analysis

No significant differences among cages were found in terms of phenological stages. Germination of acorns started in November 2014. Shoot apex emergence of seedlings was detected in the first half of January 2015, after winter solstice and first winter frosts. First apical bud closing started in the first half of May 2015, as shown in Fig.5. Well-light cages (HL) and low-light cages (LL) were compared in terms of germination, early and late emergence percentages. For all the parameters HL cages showed higher results, mainly for germination and late emergence (Fig.6). Seedlings growing into HL and LL cages, showed no significant differences in terms of shoot height but a higher number of leaves was found in HL seedlings (Fig.7).

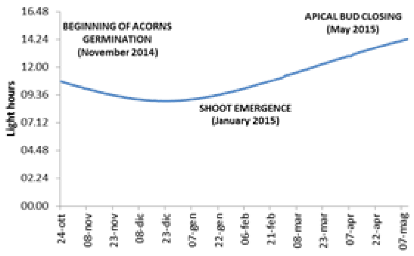

Fig.5 Phenological stages detected into the forest

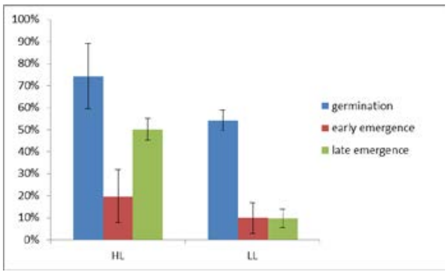

Fig.6 Germination and shoot emergence in HL and LL

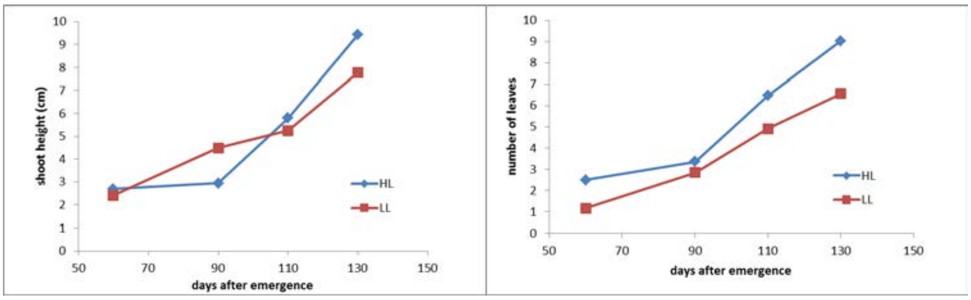

Fig.7 Shoot height and number of leaves of seedlings in HL and LL cages

3.2. Comparison between seedlings growth indoor and in the forest

Germination under NS1 light started only 24 hours after sowing and lasted for 1 week. Acorns without pericarp showed a higher germination rate if compared to those with pericarp (Fig.8). Emergence started after ~10 days since the germination, while first apical bud closing occurred after 70 days. A second growth phase started after 100 days and lasted only 20 days. A second brief stop in active growth occurred therefore between 120 and 125 days since the germination, followed by a third growth phase. Conversely, the emergence in the field started after 90 days from the germination and the active growth continues for 180 days after the germination, at the beginning of May 2015 (Fig.9). In terms of growth performance, acorns with pericarp became seedlings characterized by higher values of shoot height and number of leaves (Fig.10).

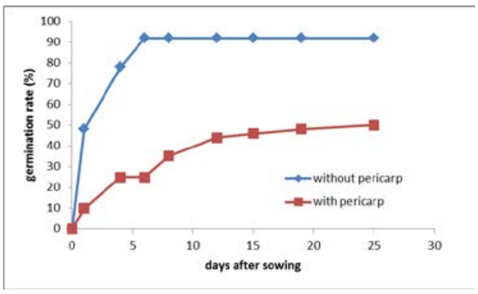

Fig.8 Germination of acorns with and without pericarp

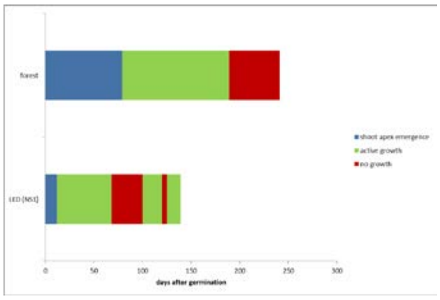

Fig.9 Phenology comparison between forest and growth chamber

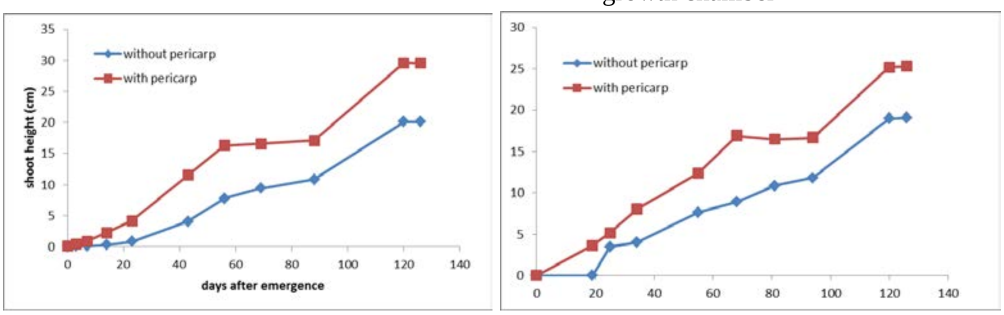

Fig. 10 Shoot height and number of leaves of seedlings deriving from acorns with and without pericarp

A comparison between artificially and naturally grown seedlings in correspondence of their first apical bud closing (respectively 60 and 120 days after the emergence), showed a significant difference in terms of morphological parameters. In particular, seedlings deriving from acorns deprived of pericarp, showed similar values of shoot height, number of leaves, diameter and thinness coefficient to those grown in nature. On the other side, seedlings from acorns with pericarp showed almost double values for each parameter (Table 3).

Table 3. Comparison of shoot height, shoot diameter, number of leaves and thinness coefficient (height/diameter ratio) between seedlings grown indoor and in the forest at first apical bud closing

	shoot height (cm)	**diameter (cm)**	**number of leaves**	**thinness coefficient**
without pericarp NS1	9.1	1.46	8.8	6.23
with pericarp NS1	18.1	2.25	16.9	8.04
forest	9.66	1.54	9.14	6.27

The comparison of seedlings growth in terms of days after the emergence showed a large difference both in shoot height and in number of leaves between seedlings naturally and artificially growing (Fig.11). When first apical bud closing occurred in forest (120 days after the emergence, red circles in Fig.11) seedlings under LEDs were at the beginning of their second growth phase and both shoot height and number of leaves showed double values when compared to those growing into the forest.
Moreover, at the first apical bud closing under LEDs (60 days after the emergence, blue circles in Fig.11), seedlings growing into the forest showed a very slow growth rate. An increase was noticeable only after 90 days from the emergence (April 2015).

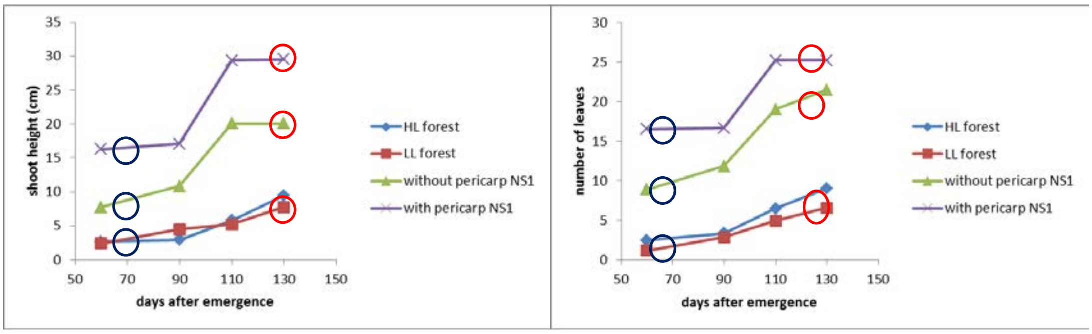

Fig.11 Comparison in terms of shoot height and number of leaves between seedlings grown in the forest (in HL and LL cages) and under NS1 light from acorns with and without pericarp

4. Discussion

Cork oak seedlings, growing under full sun (HL) and 50-60% of full PAR (LL) into a natural forest, showed higher germination and emergence percentages under HL conditions. Since these first stages are not directly linked to the photosynthetic activity, the higher results could rely on a thermal effect due to the IR region of sunlight spectrum. In the following stage of active growth, seedlings showed no significant differences among HL and LL areas, in terms of shoot height but a higher number of leaves was found in HL seedlings. According to some previous studies carried on in nurseries to analyze the shade tolerance of cork oak, it was assessed as seedlings are able to grow without any negative effects up to 2-5% of full PAR (Aranda *et al.*, 2005; Pardos *et al.*, 2005). Under this limit, plants start to suffer showing clear symptoms of etiolation and a drastic reduction of net assimilation rate which can also reach zero value (Cardillo and Bernal, 2006). Seedlings growth rhythms in HL and in LL conditions resulted to be phenologically synchronized. The acorns germination occurred at the beginning of November, under mild temperatures and high humidity, followed by a period of growth break due to winter frosts. After the first days of January 2015, in which acorns were still exposed to negative Tmin, milder temperatures and the increasing photoperiod favoured seedlings shoot apex emergence. Seedlings growth rate was very slow up to the spring equinox, when a sudden increase was observed. The average monthly percentage of sunny days between January and March was only of ~25%, while that of cloudy days ~45% and that of rainy days ~30%, thus a possible role of clouds on limiting seedlings growth has to be considered. Clouds are in fact able to reduce sunlight intensity up to 90% (in rainy days with high cloudiness), affecting the whole spectrum, similarly to deep shadow induced by canopy cover, reducing the amount of wavelengths driving photosynthesis and thermal radiation. First apical bud closing occurred into the forest at the beginning of May, when climate became hotter and drier. Under artificial lights, the time between germination and shoot apex emergence was reduced up to 84%, and up to 50% between emergence and first apical bud closing. Moreover, thanks to a higher growth rate, artificially grown seedlings showed double values for shoot height, shoot diameter and number of leaves, compared to those grown into the forest at apical bud closing.

5. Conclusions

Artificial lights resulted to be an innovative solution to obtain a high amount of seedlings in a very short time and to increase the standard growth rate of cork oak seedlings into a forest or into a traditional nursery, due to the absence of climatic stresses linked to eventual variations in temperature or humidity which generally stop seedlings growth. The possible growth of seedlings both in high and low light conditions assessed in the forest allows to use low PAR values in artificial growth chamber, with a relevant energy-saving effect.

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Università degli Studi di Viterbo
Facoltà di Agraria

Corso di Laurea in Scienze e Tecnologie per la Conservazione delle Foreste e della Natura

PROCESSI GERMINATIVI IN SPECIE ENDEMICHE DELLE ISOLE AZZORRE

Relatore
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Anno Accademico 2013 - 2014

A mio padre…

INDICE

RIASSUNTO

Su un totale di 300 specie native delle Isole Azzorre, 156 risultano oggi rare e un numero consistente, comprensivo di importanti endemismi, sta scomparendo negli ultimi anni, in particolare a causa dell'espansione del suolo utilizzato a scopi agricoli e pastorali, in particolare lungo le zone costiere, nonché alla competizione con specie esotiche, nelle zone di montagna. Per contrastare la loro estinzione e allo stesso tempo tentare di recuperare habitat nativi di tali specie da alcuni anni sono in corso nell'arcipelago tentativi di rimboschimento con specie native, basato su rinnovazione artificiale dei semenzali, a causa della limitata capacità disseminativa delle specie native, associata alla presenza di dormienza del seme e alla difficoltà di competizione delle piantine eventualmente germinate con le specie invasive.

Scopo principale di questo lavoro di tesi è stata l'analisi degli effetti di due tipologie di pretrattamenti, nello specifico di un trattamento ormonale a base di acido gibberellico (GA3) e di un periodo di stratificazione a freddo, sulla germinazione di 5 specie native delle isole Azzorre (*Hypericum foliosum, Prunus azorica, Laurus azorica, Frangula azorica, Morella faya*), in termini di efficacia nella rottura di eventuali dormienze o di accelerazione dei processi germinativi.
La messa a punto di un protocollo di germinazione per ognuna di tali specie, risulta difatti essere un presupposto essenziale per il successivo allevamento di un considerevole numero di piantine, necessarie nei processi di rimboschimento nelle Isole Azzorre.

I risultati ottenuti dopo 1 mese di incubazione a 20°C in cella fitoclimatica dei semi pretrattati, hanno mostrato un'inadeguatezza del GA3 e della vernalizzazione nella rottura della profonda dormienza delle specie *Morella faya* e *Prunus azorica*. *Frangula azorica* ha mostrato una debole risposta al pretrattamento ormonale, mentre *L. azorica* e *H. foliosum* hanno mostrato elevati tassi di germinazione in seguito sia a pretrattamento con GA3 che stratificazione a freddo. Inoltre, per queste ultime due specie, la combinazione dei due pretrattamenti ha condotto a un incremento nel tasso di accrescimento dei germogli nelle prime fasi di sviluppo post germinativo. Tali risultati risultano promettenti per la messa a punto di un protocollo idoneo di germinazione che consenta, in un breve periodo di tempo, l'ottenimento di un significativo numero di semenzali di *H. foliosum* e *L. azorica*, utili a opere di rimboschimento e resaturo forestale nelle Isole Azzorre.

Parole chiave: restauro forestale, rimboschimento, germinazione, dormienza, rinnovazione artificiale

ABSTRACT

Azorean Islands show about 300 native species. Among them, 156 are considered to be rare and a significant number, including some endemisms, is disappearing for the expansion of agriculture, farmng and the competition with invasive exotic species. In order to contrast their extinction and recover native habitats of these species, some attempts of reforestation, based on artificial propagation of seedlings, because of a low dissemination of native species associated to the presence of seed dormancy and the strong competition from invasive species, are underway.

Main purpose of this work is the analysis of the effects of two different pretreatments, one based on the usage of gibberellic acid (GA3) and the other one on cold stratification, on the germination of 5 native species of Azorean Islands (*Hypericum foliosum, Prunus azorica, Laurus azorica, Frangula azorica, Morella faya*), in terms of ability in inducing dormancy breaking or speeding up the germination process.

The definition of a germination protocol for each species is a fundamental prerequisite for the following breeding of a huge number of seedlings to be used for reforestation purposes in Azorean Islands.

The results, obtained after 1 month of incubation of pretreated seeds at 20°C in a phytoclimatic chamber, showed that GA3 and cold stratification are not able to break the deep dormancy of *Morella faya* e *Prunus azorica*. *Frangula azorica* showed a weak response to the hormonal treatment, while *L. azorica* and *H. foliosum* showed high germination rates after both pretreatments. Moreover, for these last two species, the combination of the two pretreatments led to an increased seedling growth rate in the first stages after germination. These results are promising for the definition of a germination protocol which would allow, in a short time, to obtain a significant number of seedlings of *H. foliosum* and *L. azorica*, to be used in reforestation and restoration of Azorean landscapes.

Key words: forest restoration, reforestation, germination, dormancy, artificial propagation

1. Introduzione

1.1. Flora delle Isole Azzorre

1.1.1. Geografia delle Isole Azzorre

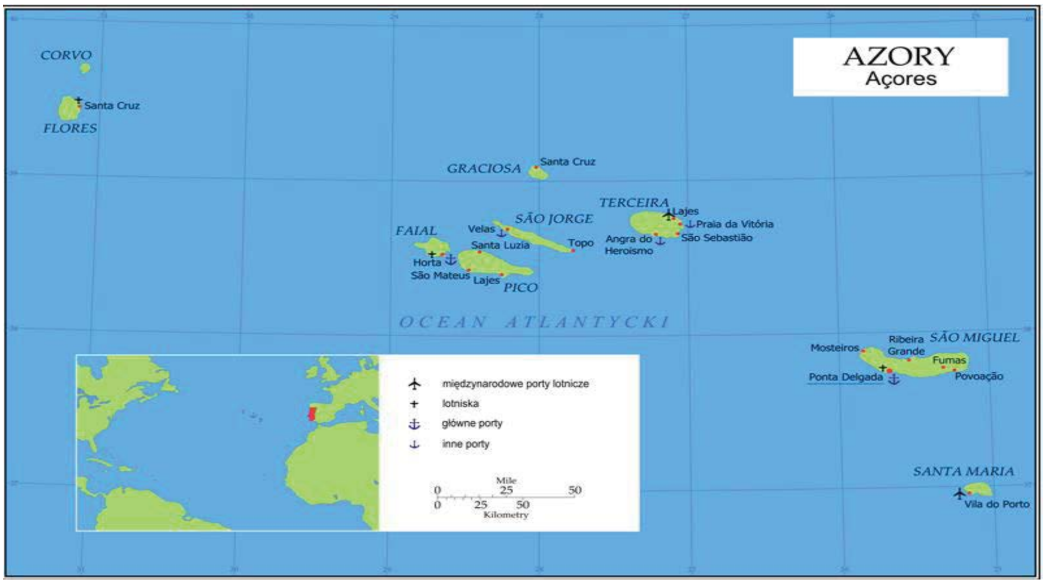

Fig. 1 Atlantico Settentrionale con le 9 isole Azzorre

Le Azzorre sono un arcipelago di 9 isole di origine vulcanica più alcuni piccoli isolotti, localizzati nell'Oceano Atlantico del Nord (Fig.1). L'area totale, irregolarmente distribuita tra le 9 isole, ammonta a 2.325 km^2.: le tre isole maggiori sono S. Miguel, Pico e Terceira, che includono il 69% dell'area totale. Anche l'altitudine è variabile da isola a isola, andando da 0 a 2351 metri, raggiunti in corrispondenza della Montagna dell'Isola di Pico, che rappresenta un cono vulcanico isolato di dimensioni straordinarie. L'arcipelago è situato a 1.815 km di distanza dal continente europeo (Portogallo) e a 2.625 km da quello nordamericano (Canada) e su di esso vivono circa 250.000 persone Questo territorio fa parte dello stato portoghese costituendone la Regione Autonoma delle Azzorre. Tutte le nove isole si trovano fra i 37° e 40° di latitudine nord e i 25° e 31° di longitudine ovest; l'isola di Flores rappresenta l'estremo confine occidentale del continente europeo. Corvo, l'isola più settentrionale dell'arcipelago, si trova ad una latitudine di +39° 41', all'incirca la stessa di Cosenza mentre Santa Maria, la più meridionale è posta alla latitudine di +36° 58', quanto la città di Siracusa.

Le isole dell'arcipelago sono divise in tre gruppi geografici: il Gruppo Orientale (composto da Santa Maria e São Miguel), il Gruppo Centrale (che comprende le isole di Terceira, Graciosa, São Jorge, Pico e Faial) ed il Gruppo Occidentale (costituito dalle isole di Corvo e Flores) (Fig.2).

Le Azzorre, insieme agli arcipelaghi di Madeira, delle Canarie e di Capo Verde, costituiscono la regione biogeografica della Macaronesia, designazione greca che significa "isole fortunate". Le Azzorre occupano l'area nord-occidentale di tale Regione, che è anche la più distante dalla terraferma nonché la più recente dal punto di vista geologico.

Fig. 2 I tre gruppi geografici delle isole Azzorre

Nonostante la loro origine vulcanica, le isole Azzorre mostrano una orografia diversificata in quanto materiali vulcanici continuano a rimodellare la superficie. Le forme dominanti del paesaggio sono rappresentate dalle ampie caldere (dallo spagnolo *caldera*, caldaia), ovvero ampie conche o depressioni, spesso occupate da un lago e di forma circolare o ellittica, che si formano normalmente dopo lo sprofondamento di un edificio vulcanico a seguito di un'imponente eruzione.

La maggior parte del suolo è rappresentato da andosuoli ma i litosuoli occupano comunque una superficie significativa. Suoli evoluti, i cosiddetti vertisuoli, sono stati identificati in alcuni antichi depositi. In ogni modo, la classificazione completa dei suoli delle Azzorre e la relativa cartografia risultano incomplete.

La vegetazione naturale è attualmente ristretta nelle zone a suolo meno fertile a causa dell'ampio utilizzo di suolo a scopo agricolo e pastorale.

Il clima delle Azzorre è fortemente oceanico, con limitati cambiamenti termici, abbondanti precipitazioni e alti valori di umidità nel corso di tutto l'anno. La temperatura media annuale è di circa 17.5°C. Febbraio rappresenta il mese più freddo, con valori medi attorno a 13.8°C, nelle aree più vicine alla costa. Le gelate sono rare al di sotto dei 600 metri di altitudine. Le precipitazioni mostrano profonde differenze tra l'area orientale e occidentale dell'arcipelago, andando dai 710 mm/anno ai 1592 mm/anno. Il valore di umidità dell'aria mostra valori annui vicini al 75-80% a livello della costa. Livelli di saturazione vengono raggiunti durante molti giorni dell'anno. Il fattore climatico di maggiore importanza per le comunità naturali è il vento. I venti dominanti raggiungono alte intensità; provengono dal quadrante S-W con alti valori di umidità. Il vento determina la struttura e la distribuzione delle comunità forestali.

Il paesaggio delle Isole Azzorre ha subito un rimaneggiamento ad opera dell'uomo soltanto a partire da 5 secoli fa, quando furono scoperte dalle caravelle portoghesi. In accordo con descrizioni storiche, le isole erano essenzialmente coperte da dense foreste. I primi cambiamenti apportati dall'uomo ancor prima dell'occupazione delle terre, sono stati determinati dall'introduzione degli allevamenti (Fig.3). Inizialmente, la colonizzazione si limitò all'area costiera. Le estese foreste furono essenzialmente esplorate per recuperare legname e frutti. I periodi di ricchezza economica delle Azzorre, legati alla produzione di urzela, grano, poi arance e infine di latte, mostrano la crescente trasformazione del paesaggio naturale in aree agricole, dalla costa verso l'entroterra (Fig.4).

Fig. 3 Bovini al pascolo

Fig. 4 Paesaggio tipico delle Isole Azzorre con campi coltivati e specie invasive

1.1.2. Flora endemica delle isole Azzorre

A partire dalle prime esplorazioni delle Azzorre, all'inizio del XIX secolo, sono state riconosciute 1007 specie vascolari, di cui 300 indigene e 70 endemiche delle Isole Azzorre e della Macaronesia. Circa il 50% delle specie naturali è oggi a rischio di estinzione. Inoltre vi sono 275 specie diverse di muschi e 175 di epatiche.

La vegetazione delle Azzorre è per gran parte determinata dal clima Atlantico, caratterizzato da inverni miti ed estati relativamente fresche con pesanti precipitazioni distribuite lungo tutto l'anno, che determinano una costante disponibilità di acqua per la vegetazione. In ogni caso, le specie presenti sulle isole non sono solo rappresentate da quelle caratteristiche dell'ambiente atlantico ma anche delle zone mediterranee, tropicali e da un ampio spettro di specie cosmopolite. Molte specie sono esotiche e sono state introdotte dai primi colonizzatori come fonti di cibo. Crescendo bene in un ambiente mite e umido furono in grado di espandersi velocemente in assenza di nemici naturali. In particolare, lo zenzero (*Hedychium gardenerarum*) che ha origine sull'Himalaya, compete con molte specie endemiche nel loro habitat naturale ed è divenuto una minaccia significativa alla sopravvivenza di molte di loro. Molte altre specie sono state introdotte durante i secoli tra queste ricordiamo le *Acacia melanoxylon* e *Cryptomeria japonica,Rhododendron indicum* e le magnifiche ed eleganti *Camelia Japonica,* gli *Agapantus praecox* e la *Brunsvigia rosea* ed inoltre una grande quantità di variopinte ortensie *(Hydrangea macrophylla)* (Fig.5-6).

Fig. 5 Specie invasive sull'isola di Sao Miguel

Fig. 6 Ortensie invasive sull'isola di Sao Miguel

La maggiore biodiversità si ritrova nelle foreste endemiche della Laurisilva mesica. La sua diversità strutturale, con più di 7 strati, numerose epifite, piante rampicanti e felci, la rende simile ad una foresta subtropicale. Associati a questa elevata diversità strutturale vi sono numerosi habitat ricchi di specie vegetali e animali rare o in via di estinzione. Le aree residue di tali formazioni sono estremamente piccole e solitamente ricadono in proprietà private.

Molte specie indigene delle Azzorre sono comuni all'arcipelago di Madeira, che è considerato un naturale semenzaio delle Azzorre. Molte delle specie endemiche sono dei "fossili viventi", filogeneticamente primitive, legate alle famiglie che dominavano la flora europea nel Terziario, parzialmente estinte durante le glaciazioni.

I delicati fiori delle specie endemiche presentano particolari adattamenti legati all'insularità, come la perdita dei colori forti, la diminuzione della taglia o l'attrazione degli impollinatori e degli insetti, come nel caso del fiore di *Tolpis azorica*. In questo caso, anche il frutto ha perso la caratteristica peculiare di questo tipo di piante, che è la capacità di dispersione a lunga distanza, in modo che il vento non possa portarli fuori dall'isola.

Le felci sono un gruppo interessante della flora dell'arcipelago e ne esistono circa 80 differenti specie di cui però solo 53 sono considerabili come indigene. Diverse specie, veri e propri relitti del Terziario, sono molto rare nel continente europeo.

Delle 300 specie native, 156 possono essere considerate rare e un numero consistente di queste sta scomparendo negli ultimi anni. Pertanto, il numero delle specie a rischio d'estinzione è molto elevato, superiore a 60, ed include importanti endemismi.
Le specie si distribuiscono dalla linea di costa fino alle montagne all'interno delle isole. Testimonianze del passato narrano della presenza, prima dell'arrivo dell'uomo, di immense foreste, alte più di 20 metri (le cui prove sono rappresentate dai ritrovamenti di barre lignee usate dagli indigeni). Lo sfruttamento agricolo di questi suoli ha causato la drastica riduzione della superficie coperta da foreste, che non superano ad oggi i 10 metri di altezza.
La vegetazione costiera è rappresentata da tre principali tipologie di comunità: la vegetazione delle dune, dominata d *Spartina versicolor*; la vegetazione che si instaura su colate laviche, cui appartengono piccole specie alofite come la *Spergularia azorica* e arbusti come l'*Erica azorica* e quella tipica delle scogliere, le cui specie dominanti dipendono dalla geomorfologia della scogliera anche se sono presenti, di norma, la *Festuca petraeae* e l*'Erica azorica*.
Le foreste delle Azzorre appartengono alla tipologia macaronesica e costituiscono elementi relitti del periodo terziario del continente europeo, di condizioni subtropicali, tanto da essere considerate "fossili viventi" preglaciali. Mentre la famiglia delle Lauraceae possiede una grande importanza nella formazione degli altri arcipelaghi, nelle Azzorre essa è sostituita da elementi più legati ad un clima umido-temperato, come *Ilex, Vaccinium, Taxus* e *Juniperus*. Studi recenti identificano quattro tipi principali di foreste. Alle quote più basse persistono formazioni mesiche di *Myrica Faya-Picconia azorica*, strutturalmente povere. La Laurisilva umida è di sicuro la tipologia forestale più ricca. Si sviluppa ad altitudini maggiori e gli esemplari possono raggiungere i 10 metri di altezza. Possiede una complessità strutturale elevata: fino a 7 diversi strati e forte biodiversità, che include anche endemismi rari. Le specie dominanti sono *Laurus azorica, Vaccinium cyiidraceum, Frangula azorica* e *Erica azorica*. Queste formazioni sono oggigiorno estremamente rare, con una presenza a macchie sull'isola di San Miguel, Terceira e Pico. La "foresta delle nubi" è una Laurisilva iperumida, dominata da *Laurus azorica, Ilex perado ssp. Azorica* e *Juniperus brevifolia*, con un'altezza media superiore ai 5 metri e, strutturalmente complessa e densa, a tratti impenetrabile. Molto difficile è la

definizione degli strati in quanto molte specie sono epifite o si sono sviluppate in tronchi morti sul suolo, permanentemente inumiditi dall'acqua raccolta dalle chiome. Pertanto, i muschi, che ricoprono tutte le superfici disponibili, rappresentano un anello chiave nel ciclo dei nutrienti a livello del suolo e nel controllo del ciclo dell'acqua. Persistono ancora aree intatte di questa tipologia forestale. La foresta di cedro è una formazione montana, tollerante lo stress idrico legato alla permanente saturazione del suolo. Nella sua struttura tipica, è costituita da una foresta *di Juniperus brevifolia* associato ad un tappeto di muschio (*Spagnum spp*.). Le formazioni sub-alpine possono essere ritrovate solo sulla montagna di Pico sull'isola omonima, al di sopra di 1900 metri. Sono caratterizzate da specie arbustive, dominate da *Calluna vulgaris*, *Daboecia azorica* e *Erica azorica*. Molte specie sono a forte rischio di estinzione.

In buone condizioni, il processo successionale può essere sorprendentemente rapido, portando in 400 anni alla formazione di foreste mature.

1.1.3. Specie endemiche oggetto di studio

- **HYPERICUM FOLIOSUM**

Ordine: Malpighiales

Famiglia: Hypericaceae

Genus: Hypericum

Species: *Hypericum foliosum* Aiton

Fig. 7 *Hypericum foliosum* Aiton

La specie *Hypericum foliosum* è anche comunemente detta “Furalha” o “Malfurada” (Fig.7). Si tratta di una fanerofita endemica delle Isole Azzorre, distribuita su tutte le isole dell’arcipelago. Si tratta di una pianta legnosa che cresce preferenzialmente al di sopra dei 400 metri e solo occasionalmente al di sotto dei 100 metri di altitudine, sia in aree riparate che in spazi aperti (Moura, 1998). Ha un portamento arbustivo, con un’altezza compresa tra 0-5 e 1 metro, con rami eretti o diffusi. Il tronco mostra una corteccia fissurata. Le foglie sono sessili o sub sessili, spesso amplessi cauli, con forma variabile da ovale a triangolare-lanceolata, a margine piano, base arrotondata, con la lamina inferiore più pallida della superiore. Le infiorescenze si compongono di un numero di fiori variabile tra 1 e 9, corimbi formi o ombrelliformi, senza fiori accessori; il pedicello ha una lunghezza tra 7 e 12 mm; le brattee sono ridotte, da lineari-lanceolate a lineari-subulate; i petali appaiono di un colore giallo oro. I semi, di colore marrone-giallo, sono lunghi 1.2-1.5 mm dotati di appendici alari.

Usi: Come tutte le specie del genere Hypericum, anche *H. foliosum* mostra proprietà antidepressive, antibatteriche e antiossidanti, legate a sostanze estraibili dalla parte aerea. Una delle sostanze più note è l’ipericina, che ha dimostrato di possedere promettenti proprietà antitumorali e ha la capacità di inibire la crescita di gliomi (tumori cerebrali), del cancro al polmone e del cancro della pelle in vitro (in laboratorio). Le sue proprietà fotodinamiche potrebbero portare all'utilizzo di ipericina in combinazione con il laser nel trattamento del cancro.

- **PRUNUS LUSITANICA AZORICA**

Ordine: Rosales

Famiglia: Rosaceae

Genere: Prunus

Specie: *Prunus lusitanica azorica*

Fig. 8 ***Prunus lusitanica azorica*** **(fiore)**

Fig. 9 ***Prunus lusitanica azorica*** **(drupa)**

Questa sottospecie di alloro portoghese si presenta come un robusto albero sempreverde con foglie più spesse e ampie delle altre sottospecie, ma con i medesimi affascinanti gruppi di fiori bianchi profumati (Fig. 8). Le foglie giovani mostrano una pigmentazione rossastra ma diventano verde scuro una volt mature, mentre lo stelo continua a rimanere rosso. Nonostante il nome comune utilizzato per tale specie, alloro portoghese, implichi in maniera scorretta che essa appartenga alla famiglia delle Lauraceae, tale sottospecie è una tipologia di ciliegio. A differenza degli altri ciliegi però, essa è una sempreverde, simile agli allori. Inoltre, I veri allori portoghesi, non fiorisce finchè nuove foglie non siano state emesse in primavera, mentre la maggior parte dei ciliegi fiorisce prima di emettere nuove foglie. I fiori che sbocciano a Giugno, hanno organi sia maschili sia femminili e sono impollinati dagli insetti. Dal fiore originano le ciliegie verdi che eventualmente virano ad un colore nero brillante in autunno (Fig. 9). Tale sub specie è presente sulle isole di Sâo Miguel, Terceira, Sâo Jorge e Pico. Cresce in gole profonde e strette e foreste indisturbate di alloro e ginepro.

E' stata inserita come subspecie a rischio (EN B1 + 2ce) nella Lista rossa della IUCN (2004) nonchè nell'allegato II della Direttiva europea Habitat e nell'appendice 1 della Convenzione sulla conservazione della vita selvatica e dell'ambiente naturale in Europa.

I maggiori rischi per tale sub-specie sono rappresentati dall'abbattimento degli alberi per lasciare spazio ad agricoltura e piantagioni nonché la pressione generata dalla competizione con specie esotiche e dalla predazione animale.

Usi: Le foglie di *P. lusitanica azorica* contengono cianuro che può essere rilasciato nell'ambiente in seguito a incendio o rottura della foglia. Il frutto è commestibile se totalmente maturo, invece quando è ancora un po' duro, risulta tossico.

- **LAURUS AZORICA**

Ordine: Laurales

Famiglia: Lauraceae

Genere: Laurus

Specie: *Laurus azorica*

Fig. 10 *Laurus azorica* (fiore)

Fig. 11 ***Laurus azorica*** **(foglie)**

Laurus azorica (comunemente detto *alloro delle Azzorre*) è una specie appartenente alla famiglia delle Lauraceae. Si tratta di un vigoroso albero di forma conica che può variare significativamente in dimensioni e altezza, raggiungendo talvolta i 10-18 metri di altezza. Come risultato di recenti variazioni tassonomiche, *Laurus azorica* è stato ristretto all'arcipelago delle Azzorre, ma è stata descritta la presenza di popolazioni passate appartenenti a tale specie nella parte occidentale delle isole Canarie, inclusa Gran Canaria così come è stata identificata una nuova specie nell'arcipelago di Madeira, chiamata *Laurus novocanariensis*.

Laurus azorica è una specie dioica. Con fiori maschili e femminili su piante separate (Fig.10). Ogni fiore è odoroso, color bianco crema, circa 1 cm di diametro e sono portati in coppia accanto ad una foglia. Le foglie sono ampie, lucenti (Fig.11). Di un colore verde scuro, fortemente ovoidali, lunghe 7-14 cm e larghe 4-8cm, con margine continuo. Il frutto è una drupa nera di circa 1-2 cm di lunghezza.

<u>Usi:</u> le bacche e l'olio da esse estratto sono utilizzati come preparazione cutanea antibatterica; l'olio viene applicato esternamente come anti reumatico e cicatrizzante per ferite e è assunto oralmente per il trattamento dell'apoplessia; l'olio è anche utilizzato per trattare le bolle, come emostatico e per problemi dell'apparato respiratorio.

- **FRANGULA AZORICA (SANGUINHO)**

Ordine: Rosales

Famiglia: Rhamnaceae

Genere: Frangula

Specie: *Frangula azorica*

Fig. 12 ***Frangula azorica*** **(bacca)**

Si tratta di un piccolo albero con foglie semplici alternate, comunemente chiamato "sanguinho". Manca delle spine caratteristiche della famiglia delle Rhamnaceae. I fiori hanno 5 petali e il frutto è rappresentato da bacche blu scuro (Fig.12). La specie appare vigorosa e persiste su terreni degradati. Si ritrova su tutte le isole dell'arcipelgo delle Azzorre, fatta eccezione per le isole Graciosa e Corvo. Nel passato è stato rinvenuto anche a Madeira, dove oggi si ritiene estinto. La specie si sviluppa nelle foreste di alloro e ginepro ad altitudini fino a 1000 metri. E' classificata come specie a basso rischio nella Lista Rossa della IUCN ed inserita nell'allegato II della Direttiva europea Habitat e nell'appendice 1 della Convenzione sulla conservazione della vita selvatica e dell'ambiente naturale in Europa.
La rarefazione della specie è legata alla perdita degli habitat, in quanto gli alberi vengono eliminati per lasciare spazio all'agricoltura e per il recupero di legname. Anche l'espansione di specie invasive sta mettendo in seria difficoltà la specie.

Usi: Le bacche mostrano un effetto lassativo, probabilmente causato da sostanze chimiche in grado di danneggiare anche le cellule del colon e promuovere la formazione di tumori. Pertanto, è sconsigliato l'utilizzo di tali frutti per periodi protratti.

- **MORELLA FAYA**

Ordine: Fagales

Famiglia: Myricaceae

Genere: Myrica

Specie: *Myrica faya* o *Morella faya*

Fig. 13 ***Morella faya*** **(drupa)**

Morella faya, comunemente chiamata albero del fuoco, è una specie nativa delle Azzorre, di Madeira e delle Canarie. E' stata introdotta in svariati luoghi quali le Hawaii, Nuova Zelanda e Australia.

E' un arbusto sempreverde o un piccolo albero alto 3-8 metri, raggiungendo raramente i 15 metri. Fusto e rami sono coperti da una peluria rossastra. Le foglie, coriacee e oblanceolate, sono solitamente di un verde scuro, lunghe 4-11cm e larghe 1-3cm, con margine continuo e apice appuntito. Risultano aromatiche e alternate lungo il fusto. Cresce facilmente su vari tipi di suolo. E' una specie dioica, con fiori maschili e femminili prodotti in larga parte su piante separate anche se talvolta sono presenti seppur in basso numero, fiori dell'altro sesso (Bingeìgeli, 1997). I fiori maschili hanno 4 stami e sono normalmente raggruppati vicino a un ramo. I fiori femminili, che solitamente si mostrano in gruppi similari, crescono leggermente oltre l'estremità del ramo

Il frutto è rappresentato da una drupa di 5-6 mm di diametro, di colore rossastro-violaceo che vira al viola scuro e nero (Fig.13). In Macaronesia è abbondantemente presente ad altitudini comprese tra 600 e 900 metri. La popolazione portoghese è probabilmente nativa o naturalizzata in seguito alla importazione da Madeira o dalle azzorre. Sulle isole Hawai è una specie invasiva (Vitousek *et al.* 1987), e compete con specie arboree native come *Metrosideros polymorpha*, con profondo impatto sul ciclo dell'azoto (Vitousek & Walker, 1989).

Usi: Le drupe sono commestibili e utilizzate tradizionalmente per la produzione di un vino molto particolare, mentre il legname è utilizzato come legna da ardere.

1.2. La germinazione dei semi

1.2.1. Il seme

Il **seme** è il corpo riproduttivo delle piante spermatofite, derivato dall'ovulo fecondato. In genere si sviluppa sulla pianta madre e se ne distacca dopo la maturazione. Schematicamente, in un seme si distinguono tre parti: l'embrione, il tessuto nutritivo o parenchima, e i tegumenti, che circondano e proteggono il tutto (Fig.14). L'**embrione** riproduce la struttura della futura pianta adulta; in esso sono riconoscibili un abbozzo del futuro fusto, uno della radice e una o più foglie embrionali o cotiledoni.

Il **tessuto nutritivo** è rappresentato dall'endosperma o albume, carico di sostanze di riserva. Contiene glucidi, lipidi e proteine, in proporzioni variabili a seconda delle specie. A maturità, nei semi delle dicotiledoni, tali sostanze sono accumulate nelle foglie cotiledonari, che si ingrossano fino a sostituire l'endosperma. Nel complesso, le sostanze di riserva sono di varia natura: nei cereali, come frumento e mais, prevalgono i glucidi (circa il 75% della sostanza fresca); in legumi, quali il fagiolo, sono prevalenti le proteine (sino al 40%), mentre i lipidi prevalgono nei semi di arachide e di colza (circa il 40%). Esso deriva dall'endosperma triploide nelle angiosperme o dall'accrescimento del gametofito femminile dell'ovulo nelle gimnosperme.

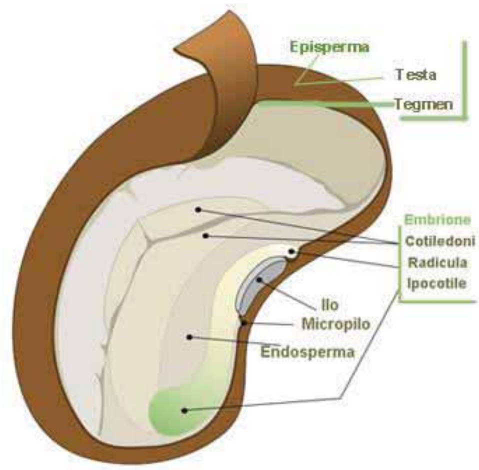

Fig. 14 La struttura del seme

I **tegumenti (episperma)**, che prendono il nome di "testa" (sostantivo maschile), proteggono le parti vitali del seme dall'azione immediata degli agenti esterni e garantiscono ad esso una fase di dormienza prima della germinazione, affinché questa avvenga nelle condizioni ambientali più favorevoli.

1.2.2. Il processo germinativo e le sue fasi

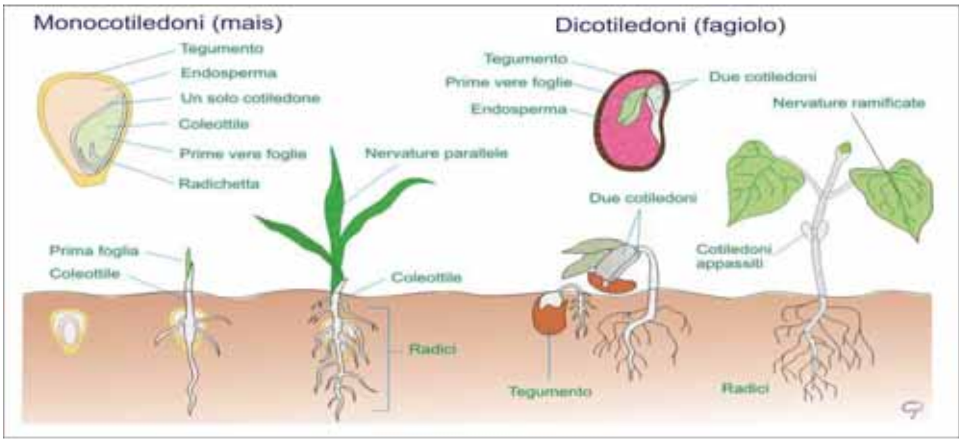

Fig. 15 Processo germinativo delle monocotiledoni e dicotiledoni

La germinazione è un processo evolutivo che ha inizio quando il seme, grazie a condizioni di temperatura e di umidità adeguate, inizia ad assorbire acqua (imbibizione) che lo fa aumentare di volume provocando la rottura del tegumento (tessuto) (Fig.15).

Le condizioni necessarie alla germinazione sono:

- presenza di ossigeno (il quale permette di demolire il glucosio e partecipa al processo di fotosintesi assieme alla luce);
- acqua (che permette di riprendere i processi metabolici ed, in tal modo, è possibile la crescita dell'embrione)
- la temperatura, la quale deve rientrare in un range compreso tra i 20° e i 24° C per le piante dei climi temperati.

Le fasi a cui un seme va incontro durante la germinazione sono:

- assorbimento di acqua (imbibizione)
- sintesi di proteine ed enzimi e riattivazione degli enzimi preesistenti
- utilizzazione delle riserve accumulate
- attivazione delle vie biosintetiche
- rottura dell'involucro seminale

Al termine di queste fasi la prima struttura a fuoriuscire dal seme è la radichetta con ruolo di ancoraggio ed assorbimento (Fig.16).

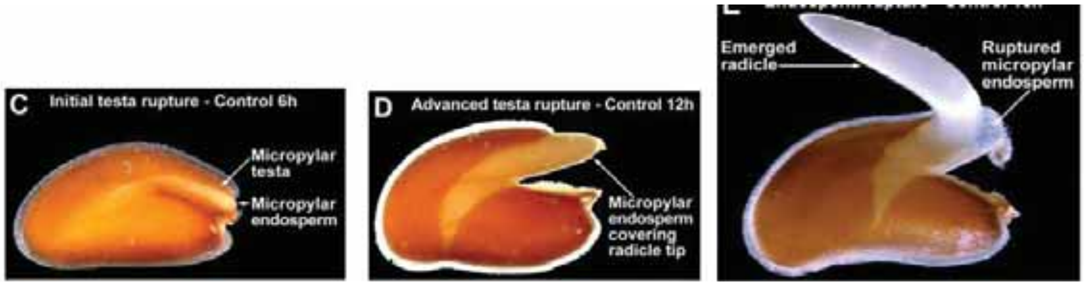

Fig. 16 Fase dello sviluppo della radichetta

Tra piante monocotiledoni e dicotiledoni intercorre qualche differenza ma il processo di germinazione è pressoché identico. Le monocotiledoni tendono a far crescere quasi simultaneamente sia la radice che la guaina protettiva da cui poi si svilupperanno le foglie, dando precedenza di pochissimo alla radice; mentre nelle dicotiledoni l'accrescimento interessa prima la radice e in un momento successivo viene spinto all'esterno del seme il germoglio, il cui piccolo stelo viene leggermente incurvato durante l'attraversamento del suolo al fine di proteggere le giovani foglie.

Le monocotiledoni hanno una germinazione tipicamente "ipogea" il seme germina sotto terra, l'ipocotile si allunga poco e il cotiledone resta sotto terra e svolge l'importante ruolo di trasferire le riserve dal seme all'embrione.

Le dicotiledoni hanno generalmente germinazione "epigea". Questo tipo di germinazione prevede l'allungamento dell'asse ipocotile fuori dal terreno con esposizione dei cotiledoni. A questo punto i cotiledoni portati in superficie possono funzionare da foglie (svolgendo fotosintesi) per un breve periodo, fino cioè alla emissione delle prime foglie vere. A questo punto i cotiledoni si riducono in dimensioni e cadono.

1.2.3. Luce e germinazione

L'azione della luce sul processo di germinazione è chiamato fotoblastismo. Alcuni semi necessitano di luce per la germinazione, quindi, sono chiamati fotoblastici altri, tuttavia, non hanno tale necessità e sono chiamati afotoblastici, altri mostrano un comportamento neutro.

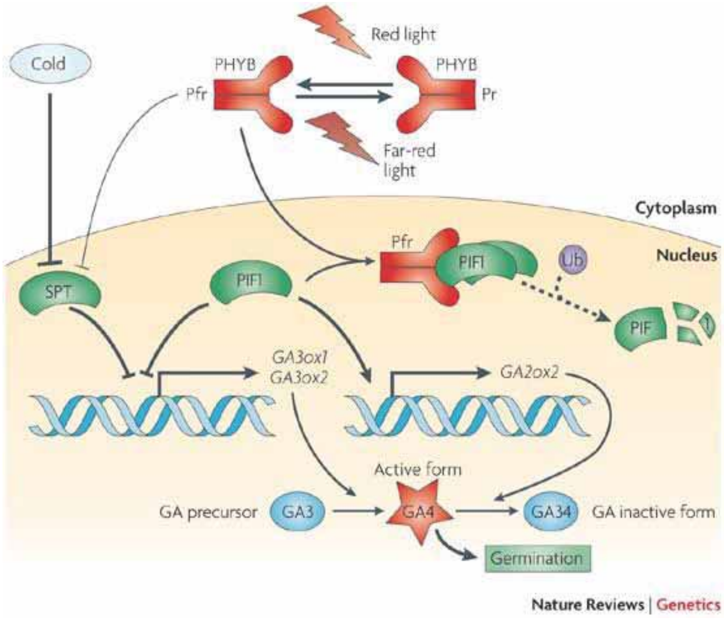

Fig. 17 Le due forme del Fitocromo

La reattività di piante a stimoli luminosi è associata al fitocromo, una proteina presente nelle cellule vegetali. Esistono due forme del fitocromo (Fig.17): il **Fitocromo Red (Pr)** e il **Fitocromo Far-red (Pfr)**. Le due forme si differenziano per il posizionamento dei picchi nel loro spettro di assorbimento della luce; il **Pr** ha il picco nella regione del rosso visibile (lunghezza d'onda circa 660nm) mentre il **Pfr** ha il suo picco nella regione dell'infrarosso (circa 730nm). La forma sintetizzata dalle piante è la **Pr.** Essa si converte nella forma **Pfr** quando è esposta alla luce rossa, la quale a sua volta si converte nella forma **Pr** in seguito all'esposizione a luce infrarossa; le due forme sono in equilibrio dinamico tra loro. Nei semi fotoblastici la germinazione è avviata dall' assorbimento della luce rossa, che converte il Pr in Pfr.

La dipendenza della germinazione dalla luce è adattamento caratteristico di molti semi di piccole dimensioni.
D'altra parte, i semi di molte specie sono afotoblastici. In tali casi, la luce inibisce la germinazione dei semi che necessitano dunque di una condizione di buio o di una semina in profondità nel terreno.
Per quanto riguarda i semi neutri, questi germinano sia in presenza che in assenza di luce.

1.3. La dormienza

La dormienza è lo stato fisiologico in cui si trova un seme o un embrione che, pur in condizioni favorevoli alla germinazione, sono incapaci di germinare.

Si individuano due grandi gruppi di dormienza:

- di tipo *endogeno*, che coinvolge l'embrione. Può essere morfologica se dovuta a incompleto sviluppo dell'embrione, o fisiologica se associata a meccanismi fisiologici di inibizione della germinazione;
- di tipo *esogeno*, che coinvolge solo alcune strutture (endocarpo legnoso, tegumenti seminali, endosperma, etc.) che impediscono la germinazione del seme. Gli inibitori tegumentali sono principalmente costituiti da sostanze aromatiche o composti fenolici. Spesso i tegumenti stessi possono costituire un fattore di inibizione della germinazione ostacolando l'imbibizione e gli scambi gassosi o impedendo la fuoriuscita della radichetta.

Possono esserci, inoltre, combinazioni di dormienze diverse e per ognuna di esse è necessario un particolare trattamento.

Da ricordare che, dopo la semina, se i semi non più dormienti sono esposti a condizioni ambientali sfavorevoli, possono attivarsi meccanismi fisiologici di blocco della germinazione. Questo fenomeno viene chiamato "dormienza secondaria". Spesso i semi soggetti a questo tipo di dormienza prediligono per germinare cicli giornalieri di temperature alternate, come avviene a fine inverno-inizio primavera.

1.3.1. Principali trattamenti di rottura della dormienza

Con il termine pretrattamento si indica l'insieme di processi, cure, manipolazioni o altri condizionamenti che precedono la semina, effettuati con l'obiettivo di rendere massima l'entità, la velocità e l'uniformità della germinazione. I Metodi ufficiali di analisi per le sementi (Ministero Agricoltura e Foreste, op. cit.) indicano il/i pretrattamento/i a cui sottoporre i semi di numerose specie erbacee, arbustive, arboree e officinali presenti in Italia. Nello stesso modo autorevoli Istituzioni come l'International Seed Testing Association (ISTA), l'International Plant Genetic Resources Institute (IPGRI), il Natural Resources Conservation Service dell'United States Department of Agriculture, The Native Plant Network, The Reforestation, Nursery and Genetic Resources Team, Kew Gardens, e tante altre istituzioni studiano e aggiornano permanentemente protocolli per ottimizzare i risultati delle procedure atte a propagare le piante oppure per determinare la qualità dei semi, compresa la germinazione ed i pretrattamenti per rimuovere eventuali dormienze. Tuttavia, per molte specie non ci sono notizie in merito (Bacchetta *et al.*, 2006).

Si descrivono di seguito i pretrattamenti più comunemente usati per rimuovere la dormienza dei semi prima della semina o dei test di germinazione.

- **Stratificazione a freddo o vernalizzazione**

Con il termine *prechilling* (sinonimo di vernalizzazione o stratificazione fredda) si intende l'esposizione dei semi dormienti a temperature variabili da +2° a +5°C in condizioni umide ed arieggiate (nudi o mischiati ad un substrato soffice) per periodi caratteristici per ogni specie o lotto. Il *prechilling* simula l'azione che l'inverno esercita su alcuni semi.

Una soluzione alternativa alla stratificazione fredda, che può durare anche diverse settimane, è talvolta l'applicazione di ormoni quali gibberelline (GA3) (Bacchetta *et al.*, 2006).

- **Pretrattamento con gibberelline**

L'acido gibberellico gioca un ruolo fondamentale nel favorire la germinazione di tutte quelle specie di semi che, per un motivo o per un altro, hanno lunghi tempi di germinazione. Si è dimostrato che trattamenti con GA3 possono indurre la germinazione nei semi che necessitano di vernalizzazione, di affumicatura, estivazione, esposizione alla luce rossa e possono accelerare e promuovere la germinazione in specie con tempi molto lunghi. Si tratta di un importante ormone vegetale appartenente al gruppo delle gibberelline la cui formula chimica è $C_{19}H_{22}O_6$ e il peso molecolare è 346.37 g/mol.

Sono state identificate almeno 136 gibberelline (GA) naturali aventi una struttura chimica simile, ma solo poche hanno dimostrato avere un'attività biologica intrinseca. Molte di queste infatti sono dei precursori metabolici delle GA bioattive o i loro prodotti di degradazione. Ogni gibberellina possiede uno scheletro tetraciclico che può essere ent-gibberellanico, contenente 20 atomi di carbonio, oppure 20-nor-ent-gibberellanico, contenente 19 atomi di carbonio (Fig.18). Il prefisso ent sta per ricordare che lo scheletro è derivato da un enantiometro del Kaurene, un idrocarburo tetraciclico presente in natura. Le gibberelline formate da 20 atomi di carbonio sono chiamate C20-GA mentre le gibberelline con soli 19 atomi di carbonio, poiché hanno perso il C in posizione 20, si chiamano C19-GA.

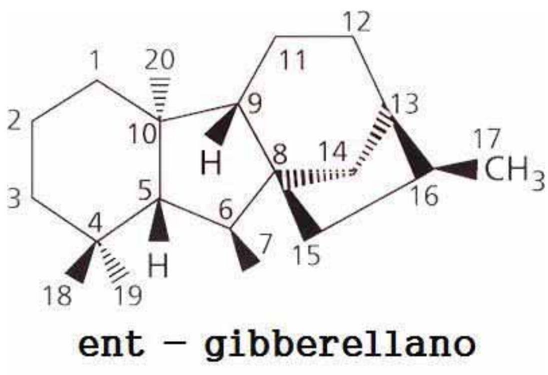

Fig. 18 Scheletro tetraciclico delle gibberelline

E' interessante notare che le GA maggiormente bioattive (GA1, GA3, GA4, GA7) sono state fra le prime ad essere scoperte e sono tutte delle C19-GA (Fig.19). Di queste poi la GA3, maggiormente attiva che, comunemente si reperisce in commercio per usi agronomici, orticultura e altri scopi scientifici. L'attività di questi composti è profondamente influenzata dalla posizione e stereochimica dei gruppi funzionali (R) che possiedono.

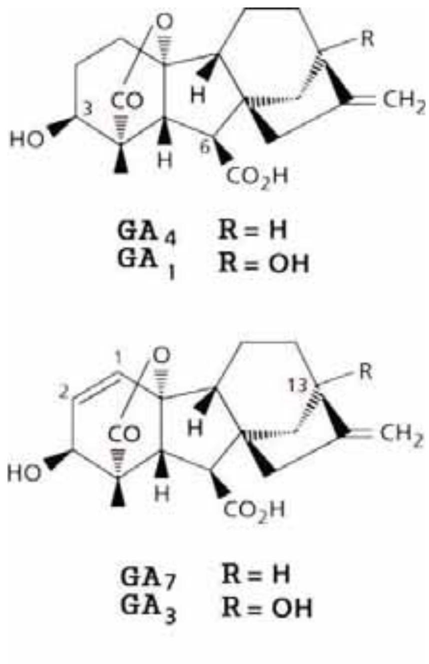

Fig. 19 Acido gibberellico bioattive

Il meccanismo mediante cui l'acido gibberellico è in grado di interrompere la dormienza è molto complesso e coinvolge numerosi secondi messaggeri e recettori ancora sconosciuti. E' molto probabile che alcuni di essi si trovino all'esterno della parete cellulare.

Le GA agiscono principalmente sull'embrione e sul'aleurone, una sostanza di riserva formata principalmente da proteine che compone l'endosperma. Nell'embrione il GA induce la divisione e l'allungamento cellulare. Si è dimostrato che le gibberelline non hanno effetto sui parametri osmotici ma piuttosto provocano un aumento sia dell'estensibilità meccanica delle pareti cellulari, sia del rilassamento da tensione di pareti cellulari vive (Fig.20); la GA quindi diminuisce la forza minima indispensabile a causare l'estensione della parete: ne abbassa la soglia di cedevolezza. La cellula così allungata viene indotta a dividersi mediante induzione dell'espressione dei geni di numerose proteine CDK, chinasi ciclina-dipendenti, che regolano la transizione dalla fase G1 ad S e da G2 a M favorendo in questo modo la divisione cellulare.

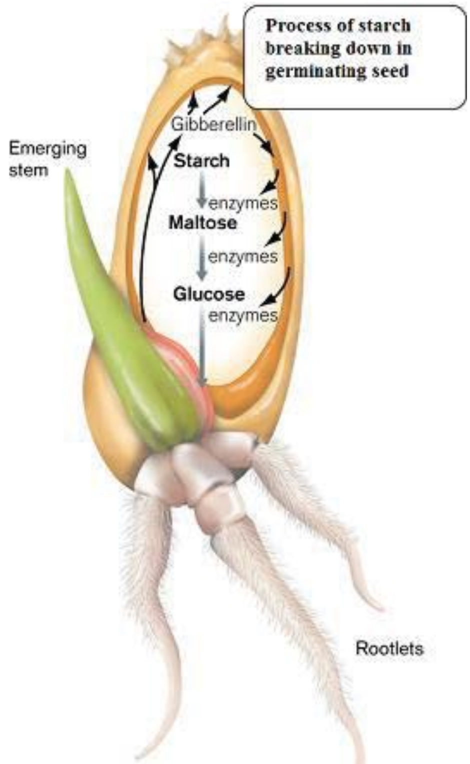

Fig. 20 Funzionamento del GA 3 nel seme

Nell'aleurone invece la GA induce l'espressione dei geni dell'α-amilasi aumentando a quantità di mRNA traducibile di α-amilasi e quindi la sua sintesi. La α-amilasi è un enzima idrolitico in grado di digerire l'endosperma, in questo modo le riserve del seme vengono mobilitate e rese disponibili per l'embrione in accrescimento mentre l'endosperma, che forma una barriera meccanica all'emergenza della radichetta, viene progressivamente meno.

- **Stratificazione calda o estivazione**

Con il termine *preheating* (sinonimo di stratificazione calda, estivazione o *warming*) si intende l'esposizione dei semi ad una temperatura non superiore a 30-35°C (generalmente 15-20°C) in condizioni umide, ma con una libera circolazione d'aria per un periodo variabile a seconda della specie. La stratificazione calda condotta artificialmente sostituisce quello che avviene durante l'estate e quasi sempre precede quella fredda. Quando si applicano più cicli di stratificazione calda e stratificazione fredda, l'ultimo pretrattamento applicato è quello freddo (Bacchetta *et al*., 2006).

- **Scarificazione**

I semi di alcune specie appartenenti a famiglie con tegumenti seminali o endocarpi legnosi molto duri e impermeabili devono essere sottoposti ad abrasione attraverso trattamenti di natura meccanica, chimica e fisica per consentire loro l'assorbimento dell'acqua. La scarificazione può essere eseguita prima dell'inizio del test o, quando si sospetta che il trattamento possa danneggiare i semi già idratati, alla fine della prova e solo per quei semi che non si sono imbibiti.

La scarificazione meccanica prevede la foratura, il taglio o l'abrasione con carta vetrata dei tegumenti esterni al fine di permettere l'imbibizione dei semi. La porzione del seme più adatta per la scarificazione meccanica è quella situata immediatamente al di sopra dell'apice dei cotiledoni.

La scarificazione di tipo chimico prevede l'immersione dei semi in acido solforico al 96 % per un tempo variabile, al fine di assottigliare i tegumenti. Dopo il trattamento i semi devono essere risciacquati in acqua corrente prima di avviare il test di germinazione o procedere alla semina.

La scarificazione fisica dei semi consiste essenzialmente in un trattamento in acqua bollente e in un successivo ammollo di 12-24 ore al fine di ammorbidire i tegumenti e favorire l'imbibizione. L'acqua deve essere allontanata dalla fonte di calore prima di versare i semi e la massa, generalmente costituita da dieci parti d'acqua per ogni parte di seme, deve essere mescolata di tanto in tanto fino al suo raffreddamento (Bacchetta *et al*., 2006).

- **Rimozione dei tegumenti**

Per alcune specie che presentano dei tegumenti particolarmente duri, la sola scarificazione non determina un indebolimento tale da permettere la fuoriuscita della radichetta. In questi casi è bene utilizzare una pinza per rimuovere questi tegumenti avendo cura di non danneggiare l'embrione (Bacchetta *et al*., 2006).

- **Rimozione delle sostanze inibitrici della germinazione**

La presenza di sostanze chimiche all'interno dei semi o nei tegumenti può ritardare o inibire la germinazione. La presenza di tali sostanze può essere rivelata dalla formazione di aloni colorati attorno ai semi sul substrato di germinazione che si sta utilizzando. Le sostanze inibitrici della germinazione possono essere rimosse con un prelavaggio in acqua o in alcool (90°) ad una temperatura di 25°C e facendo riasciugare i semi prima di effettuare il test.

Le sostanze fenoliche sono spesso responsabili dell'inibizione della germinazione perchè agiscono diminuendo l'apporto di ossigeno a livello embrionale, soprattutto quando le temperature sono superiori a 10°C. La loro eliminazione si può effettuare mediante lavaggi successivi in acqua o alcool, ma in particolar modo impiegando temperature di germinazione sufficientemente basse al fine di aumentare la dissoluzione dell'ossigeno nell'acqua di imbibizione.

L'ammollo dei semi in un potente ossidante (es.: acqua ossigenata, candeggina) permette l'ossidazione di molte sostanze inibitrici rendendole inefficaci oltre all'eliminazione di alcuni patogeni (Bacchetta *et al*., 2006).

1.4. Difficoltà di germinazione delle specie endemiche oggetto di studio

Le specie endemiche delle Isole Azzorre oggetto di studio, risultano accomunate da difficoltà nella germinazione del seme, legata alla presenza di dormienza (*Morella faya, Frangula azorica, Prunus azorica*), di una rapida perdita della capacità germinativa nel corso della conservazione (*Hypericum foliosum*) o di una germinazione molto lenta *(Laurus azorica*) con conseguente necessità di idonei pretrattamenti che consentano di rompere la dormienza o di accelerare i processi germinativi. Per tale ragione, la pratica della propagazione per talea o della micropropagazione è più diffusa della propagazione da seme. Ciò determina in letteratura un profondo deficit di informazioni relative a protocolli di germinazione o pretrattamenti idonei a rompere la dormienza del seme

Di seguito, un’analisi delle informazioni disponibili a tal riguardo, per singola specie.

Specie	**Informazioni disponibili in letteratura**
Hypericum foliosum	• Consigliato l’uso della micropropagazione (Moura, 1998) • 67% di germinazione a 15°C con fotoperiodo 8L 16B
Laurus azorica	• Germinazione senza pretrattamenti dopo 40-90 giorni alla luce e 12°C • Germinazione senza pretrattamenti dopo 6 settimane a 20°C • Necessità di stratificazione a freddo
Morella faya	• Non sono presenti informazioni relative alla germinazione indoor, ma soltanto in serra (55-63% di ombra)
Prunus azorica	• Necessità di rimozione dell’endocarpo • Incubare a 10/5°C o 15/10°C con fotorperiodo 12L12B (Moreira et al., 2012)
Frangula azorica	• Non sono presenti informazioni relative alla germinazione indoor, ma soltanto in serra

1.5. Scopo del lavoro

L'obiettivo principale di questo lavoro di tesi è l'analisi degli effetti di due tipologie di pretrattamenti, nello specifico di un trattamento ormonale a base di acido gibberellico (GA3) e di un periodo di stratificazione a freddo, sulla germinazione di 5 specie native delle isole Azzorre (*Hypericum foliosum, Prunus azorica, Laurus azorica, Frangula azorica, Morella faya*), in termini di efficacia nella rottura di eventuali dormienze o di accelerazione dei processi germinativi.

La messa a punto di un protocollo di germinazione per ognuna di tali specie, risulta difatti essere un presupposto essenziale per il successivo allevamento di un considerevole numero di piantine, necessarie nei processi di rimboschimento nelle Isole Azzorre.

2. Materiali e metodi

2.1. Raccolta e conservazione dei semi

I semi delle specie oggetto di studio sono stati inviati all'Università degli Studi della Tuscia dal Centro de Monitorizaçao e investigaçao das Furnas (Isola di Sao Miguel, Azzorre) nel settembre 2014, subito dopo la raccolta degli stessi.

I semi sono stati conservati in barattoli di vetro a 4°C in cella frigo fino all'utilizzo (Dicembre 2014).

2.2. Test del tetrazolio (TTC test)

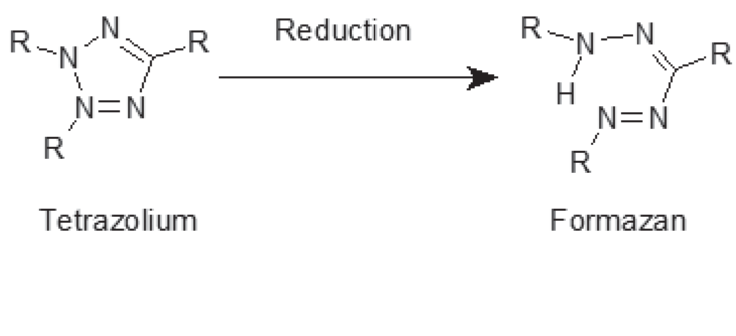

Fig. 21 Test ai Sali di tetrazolio

La vitalità dei semi è stata valutata attraverso il test ai Sali di tetrazolio. Tale test (detto TTC) si basa sulla riduzione del sale di tetrazolio 2,3,5-trifenil-tetrazolio cloruro (TTC), un colorante biologico che rivela l'attività respiratoria (Fig.21): in presenza di deidrogenasi, il TTC, da incolore, si trasforma in una sostanza rossa insolubile, il formazano.

Nello specifico, è stato possibile effettuare tale preliminare test solo su alcune delle specie in utilizzo, ovvero quelle per cui è stata possibile l'asportazione del pericarpo con consequenziale possibilità di penetrazione dei Sali di tetrazolio a livello embrionale (*Prunus azorica, Laurus azorica*).

E' stata preparata una soluzione all'1% di Sale di tetrazolio in acqua distillata, facendo attenzione a non esporre la soluzione a luce diretta, utilizzando una bottiglia sterile rivestita totalmente di alluminio.

20 semi per specie sono stati inizialmente privati del pericarpo, quindi messi a bagno per 48 ore a 4°C per consentirne la reidratazione. Dopo 48 ore sono stati immersi nella soluzione di Sali di tetrazolio e incubati per 48 ore al buio, così da consentire la penetrazione del colorante nei tessuti embrionali.

Dopo 48 ore di incubazione, i semi sono stati estratti dalla soluzione, asciugati su carta da filtro, quindi sezionati con un bisturi a metà, al fine di esporre l'embrione, così da poterne analizzare l'eventuale colorazione.

Sono stati ritenuti vitali semi il cui embrione abbia acquisito una colorazione rossastra.

2.3. Pretrattamenti

Circa 600 semi per ognuna delle specie in esame (*Prunus azorica, Laurus azorica, Frangula azorica, Morella faya*) e 1 g di semi nel caso *dell'Hypericum foliosum,* data la difficoltà di conteggio legata alle minime dimensioni del seme, sono stati messi 24 ore a bagno a 4°C in acqua distillata (Fig.22).

Nello specifico, *Prunus azorica* e *Laurus azorica* sono stati precedentemente privati del pericarpo e poi messi a bagno.

Fig. 22 Imbibizione dei semi in acqua distillata

Dopo 24 ore, sono stati eliminati i semi galleggianti in quanto vacui, mentre i restanti sono stati sterilizzati con ipoclorito di sodio al 20% per 20 minuti. Sono stati quindi risciacquati con acqua corrente (con un ultimo risciacquo effettuato con acqua distillata sterile) e quindi sottoposti ai diversi pretrattamenti atti a favorire la germinazione.

2.3.1. Pretrattamento con acido gibberellico (GA3)

E' stata preparata una soluzione di acido gibberellico (GA3, 346.37 g/mol), con concentrazione pari a 200 mg/L (Fig.23). Pertanto, sono stati solubilizzati 100 mg di GA3 in polvere in una minima quantità di etanolo 100% sotto cappa chimica e portati a volume con acqua distillata sterile. Circa 300 semi per specie (circa 300 mg per quanto riguarda l'*Hypericum foliosum*) sono stati immersi nella soluzione di GA3 200mg/L ed incubati per 18 ore al buio in agitazione (Fig.24).

Fig. 23 Acido gibberellico

Fig. 24 Incubazione semi trattati con GA3 su agitatore

Al termine dell'incubazione sono state allestite per ciascuna specie 6 piastre, contenenti 50 semi l'una (circa 40 mg nel caso dei semi di *H. foliosum*), posti su carta bibula inumidita con acqua sterile. Le piastre sono state sigillate con Parafilm e sottoposte a 2 differenti condizioni di incubazione (3 piastre per ciascuna condizione):

1) 30 giorni a 4°C in cella frigorifera (stratificazione a freddo)
2) A 20°C sotto lampada fluorescente con fotoperiodo 12L 12B

Come controllo, sono state allestite 6 piastre per specie, contenenti 50 semi (circa 40 mg nel caso dei semi di *H. foliosum*) non trattati con GA3 ma immediatamente trasferiti in piastra dopo le 24 ore di reidratazione (vedi paragrafo 2.3.); 3 piastre sono state incubate a 20°C sotto lampada fluorescente con fotoperiodo 12L 12B; le restanti 3 sono state poste a 4°C per un periodo di 30 giorni.

2.4. Prove di germinazione

Fig. 25 Camera fitologica

Al termine del periodo di stratificazione a freddo (30 giorni), le piastre sono state trasferite a 20°C, in camera fitologica illuminate da lampade a fluorescenza con fotoperiodo 12L 12B, al fine di indurre la germinazione dei semi (Fig.25).

2.5. Analisi dell'effetto del GA3 sull'accrescimento del germoglio

H. foliosum: i semi germinati in piastra in seguito a stratificazione a freddo con e senza pretrattamento con GA3, sono stati trasferiti in vasetti contenenti terriccio a base di torba (torba: perlite: sabbia; 20:10:1) e posti sotto lampade LED (spettro G2, Valoya ®) e a fluorescenza, con intensità ~ 50 PAR, fotoperiodo 12L 12B, temperatura costante di 22±2 °C e umidità relativa dell'aria pari a 50 ±10%.

Tale trattamento non è stato esteso ai semi germinati in piastra in assenza di vernalizzazione, a causa del numero limitato degli stessi che non avrebbe consentito di ottenere risultati statisticamente significativi.

La crescita dei germogli è stata monitorata prelevando dopo 15 giorni un campione di 9 individui da sottoporsi a misure morfometriche (lunghezza epicotile, lunghezza ipocotile, lunghezza radice, numero di foglie).

Attraverso il confronto di tali parametri è stato possibile identificare un eventuale effetto di incremento dell'accrescimento dei germogli, indotto dal pretrattamento del seme con GA3.

Allo stesso tempo è stato possibile valutare l'effetto di due differenti fonti luminose artificiali sull'accrescimento delle piantine.

Laurus azorica: i semi germinati in piastra in seguito ai diversi pretrattamenti, sono stati trasferiti in un vassoio QUICKPOT QPD104VW Herkuplast® contenente terriccio a base di torba (torba: perlite: sabbia; 20:10:1) e posti sotto lampade LED (spettro coolwhite, Paullmann®), con intensità ~ 50 PAR, fotoperiodo 12L 12B, temperatura costante di 22±2 °C e umidità relativa dell'aria pari a 50 ±10%.

La crescita dei germogli è stata monitorata misurando l'accrescimento dell'epicotile nel corso di 15 giorni.

In tal modo, è stato possibile identificare un eventuale effetto di incremento dell'accrescimento delle piantine, indotto dal pretrattamento del seme con GA3.

3. Risultati

3.1. Test del tetrazolio (TTC test)

Al termine delle 48 ore di incubazione nella soluzione di Sali di tetrazolio, i semi di *Prunus azorica* e *Laurus azorica* hanno mostrato le seguenti percentuali di vitalità (Fig. 26):

Specie	Percentuale di vitalità del seme
Prunus azorica	100 %
Laurus azorica	80%

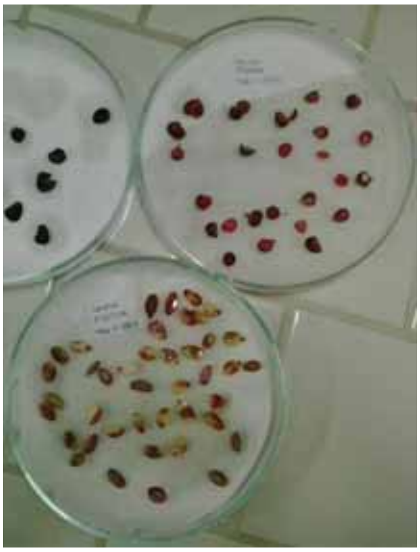

Fig. 26 TTC test su *P. azorica* e *L. azorica*

3.2. Prove di germinazione

La specie *Laurus azorica* ha mostrato un'evidente influenza positiva da parte dell'acido gibberellico (GA3) sulla velocità di germinazione del seme.

Nelle piastre di controllo incubate a 20°C, difatti, la germinazione si è mostrata molto lenta, iniziando dopo 7 giorni di incubazione dei semi. Dopo 10 giorni di incubazione si è raggiunto una percentuale pari al 2% di germinazione, che si è mantenuta tale fino al 15° giorno. E' quindi seguita una seconda fase esponenziale di germinazione, che ha condotto al raggiungimento di una percentuale finale, al 30° giorno di analisi, pari circa al 35%.

Nel caso di trattamento con GA3 seguito da incubazione a 20°C, la germinazione è iniziata dopo soli 3 giorni di incubazione, raggiungendo una percentuale pari al 25% dopo 10 giorni e 50% dopo 14 giorni. A tale fase esponenziale ha fatto seguito una fase di rallentamento nella germinazione, con raggiungimento di una percentuale finale pari circa al 70% dopo 30 giorni.

I semi sottoposti a 30 giorni di stratificazione a freddo, hanno raggiunto una percentuale di germinazione pari al 35% dopo soli 3 giorni di incubazione a 20°C e al 50% dopo 15 giorni. Tra il 15° e il 30° giorno si è assistito ad un rallentamento nella germinazione dei semi, con il raggiungimento di una percentuale finale di germinazione pari circa al 60% dopo 30 giorni.

I semi sottoposti a 30 giorni di stratificazione a freddo in seguito a trattamento con GA3, hanno mostrato i primi segni di germinazione dopo 3 giorni circa dal trasferimento a 20°C, raggiungendo una percentuale di germinazione pari al 30% dopo 7 giorni, che si è mantenuta invariata nelle settimane successive (Fig. 27).

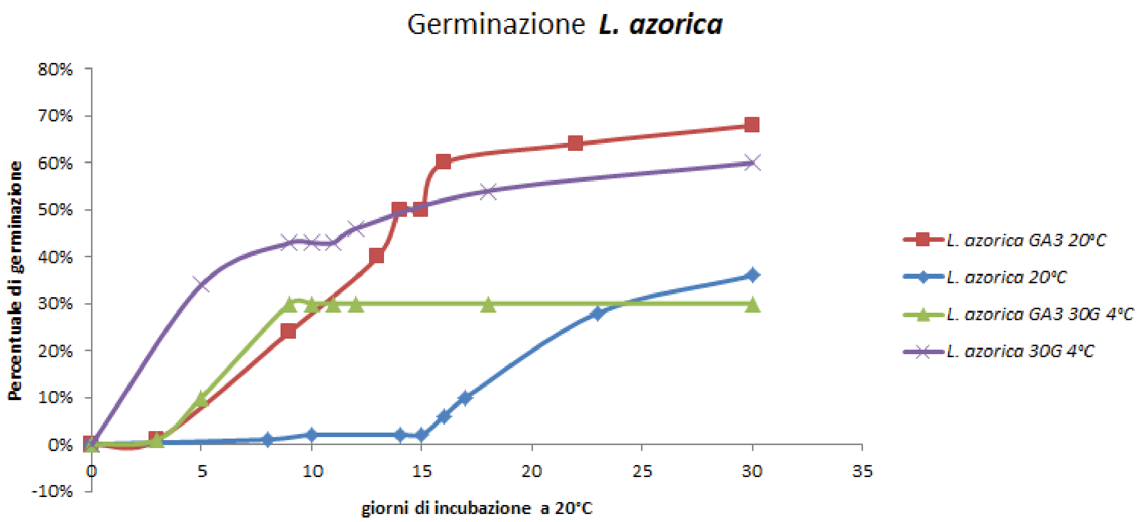

Fig. 27 Curve di germinazione dei semi di *L. azorica*

La specie *Hypericum foliosum* ha mostrato un'evidente efficacia della stratificazione a freddo come pretrattamento in grado di aumentare drasticamente la percentuale nonché la velocità di germinazione dei semi.

Nelle piastre di controllo incubate a 20°C, la germinazione è iniziata dopo una settimana di incubazione, raggiungendo un 5% dopo 15 giorni, valore rimasto invariato nelle successive settimane.

Comportamento simile è stato riscontrato nelle piastre contenenti semi trattati con GA3 e quindi incubati a 20°C in assenza di vernalizzazione. Anche in questo caso la germinazione è iniziata dopo una settimana di incubazione, raggiungendo un 10% dopo circa 15 giorni, valore rimasto costante nelle successive settimane.

I semi sottoposti a 30 giorni di stratificazione a freddo, invece, hanno mostrato segni immediati di germinazione (dopo 2-3 giorni di incubazione a 20°C) raggiungendo il 100% di germinazione in 7 giorni.

I semi sottoposti a 30 giorni di stratificazione a freddo dopo trattamento con GA3, hanno mostrato i primi segni di germinazione dopo 5 giorni circa dal trasferimento a 20°C, raggiungendo una percentuale di germinazione pari all'80% dopo 1 sola settimana, rimasta poi invariata nelle successive settimane (Fig.28). Nonostante la percentuale di germinazione sia risultata lievemente inferiore al semplice trattamento di stratificazione a freddo, i germogli hanno mostrato tassi di accrescimento decisamente superiori.

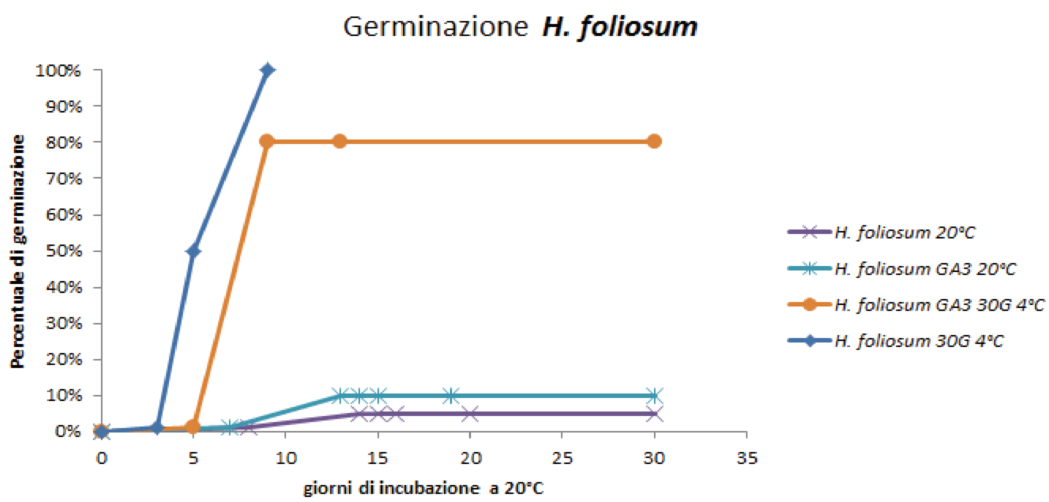

Fig. 28 Curve di germinazione dei semi di *H. foliosum*

Per quantificare tale effetto, è stata misurata la lunghezza totale (porzione epigea più ipogea del germoglio) di 10 germogli germinati nelle 4 condizioni sopra descritte, mediante l'uso del software Digimizer.

Nel caso delle piastre sottoposte a vernalizzazione, i germogli pretrattati con GA3 hanno mostrato una lunghezza media pari a 0.55 cm, circa il doppio della lunghezza media dei germogli nati da semi sottoposti a stratificazione a freddo in assenza di GA3 (0.26 cm) (Fig. 29-30-31-32).

Nel caso delle piastre non vernalizzate, non è stata riscontrata una significativa differenza tra semi trattati e non trattati con GA3, con un valore medio comune pari a 0.3 cm, a sua volta non significativamente diverso dal valore medio mostrato dai semi sottoposti a stratificazione a freddo.

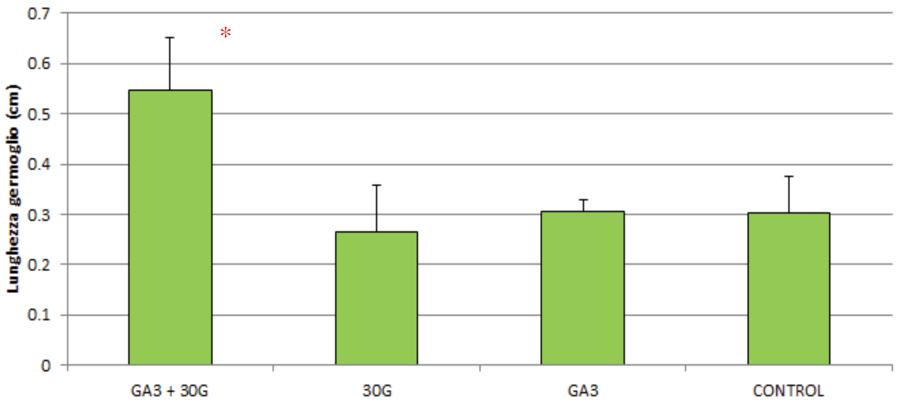

Fig. 29 Lunghezza media dei germogli di *H. foliosum* prima del trasferimento in terriccio

Fig. 30 Particolare dei germogli di *H. foliosum* germinati dopo 30 giorni di vernalizzazione

Fig. 31 Particolare dei germogli di *H. foliosum* germinati dopo vernalizzazione preceduta da trattamento con GA3

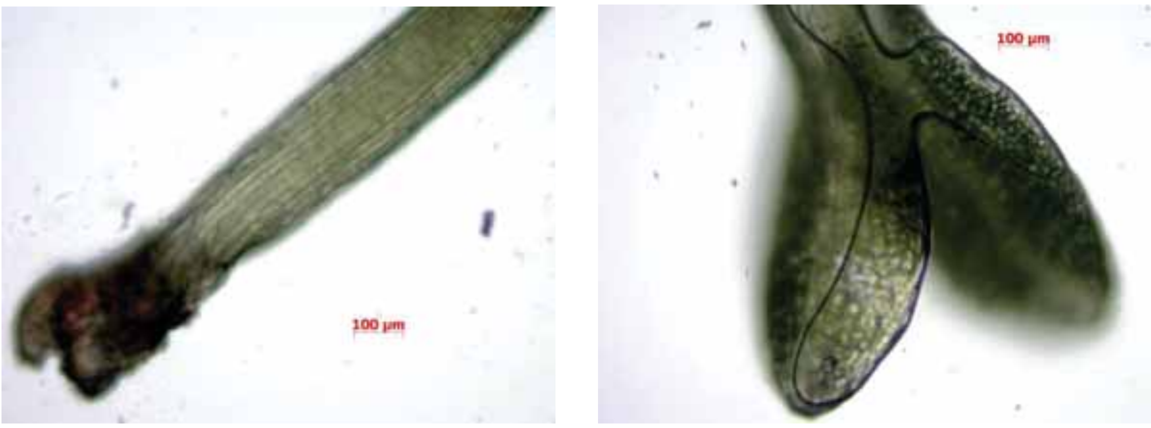

Fig. 32 Germogli di *H. foliosum* al microscopio ottico (10X)

Le specie *Frangula azorica,* ha mostrato i primi segni di germinazione soltanto dopo un mese di incubazione a 20°C, nel caso di semi pretrattati con GA3 (1 seme su 50).

Le specie *Morella faya* e *Prunus azorica* non hanno mostrato segni di germinazione né in seguito a stratificazione a freddo né all'applicazione delle gibberelline né all'associazione dei due trattamenti.

3.3. Analisi dell'effetto del GA3 sull'accrescimento delle plantule

Il monitoraggio dell'accrescimento dei germogli di *L. azorica*, trasferiti in terriccio in seguito all'avvenuta germinazione in piastra dei semi sottoposti ai 3 pretrattamenti precedentemente descritti (vedi paragrafo 2.3) ha mostrato un effetto sinergico indotto dall'associazione tra gibberelline e stratificazione a freddo sulla crescita della porzione epigea del germoglio. Infatti, i germogli derivanti da semi pretrattati con GA3 e quindi sottoposti a 30 giorni di vernalizzazione, hanno mostrato dimensioni dell'epicotile circa doppie rispetto ai valori mostrati dai germogli nati da semi trattati soltanto con GA3 o soltanto con 30 giorni di stratificazione a freddo. Tutte e tre le tipologie di pretrattamento hanno condotto a valori significativamente superiori a quelli mostrati da germogli nati da semi germinati senza alcun pretrattamento. In questo caso, è stato possibile misurare l'epicotile soltanto a partire dal 15° giorno dalla semina in terriccio, al contrario dei germogli nati da semi pretrattati, che hanno raggiunto dimensioni misurabili già dopo 10 giorni dalla semina (Fig. 33-34-35).

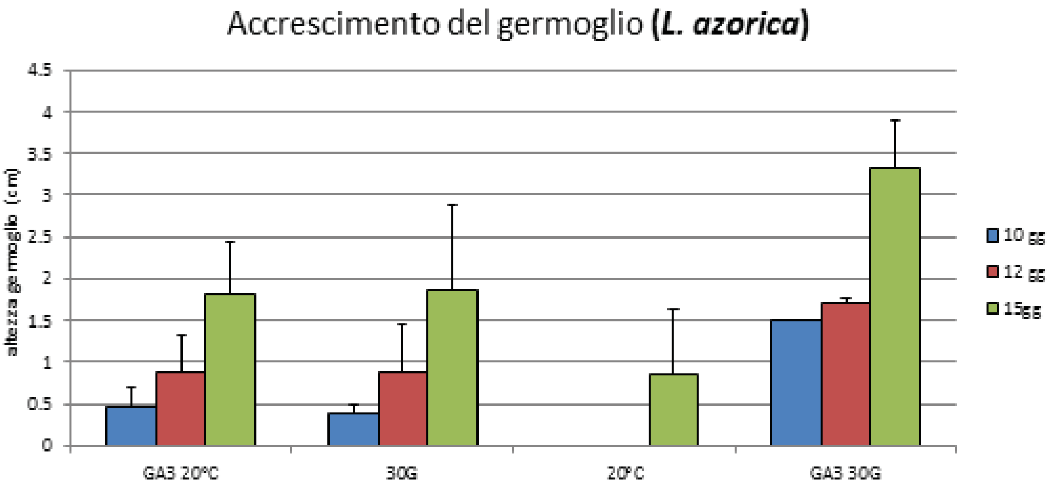

Fig. 33 Valutazione dell'accrescimento del germoglio di *L. azorica* dopo 10-12-15 gg dalla semina

Fig. 34 Germogli di *L. azorica* germinati senza alcun pretrattamento (15 gg dalla semina)

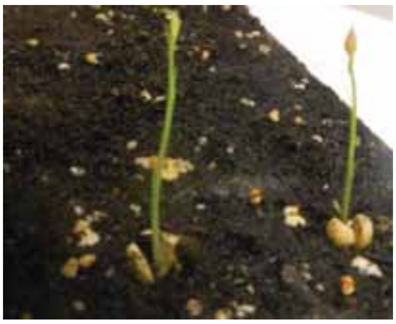

Fig. 35 Germogli di *L. azorica* germinati dopo pretrattamento con GA3 seguito da 30 giorni di vernalizzazione (15 gg dalla semina)

Per quanto concerne la specie *H. foliosum*, le misure morfometriche (lunghezza epicotile, lunghezza ipocotile, lunghezza radice, numero di foglie) effettuate sui germogli nati da semi sottoposti a 30 giorni di stratificazione a freddo con e senza precedente trattamento con GA3, e quindi trasferiti in terriccio sotto lampade LED e a fluorescenza, hanno mostrato, sotto entrambe le fonti luminose, un marcato effetto indotto dalle gibberelline sull'accrescimento dell'epicotile e dell'ipocotile. Non risultano invece influenzate dall'effetto degli ormoni né la misura della radice né il numero delle foglie.

Ponendo a confronto le due fonti luminose, è possibile osservare come lo spettro LED G2, mostri un effetto positivo sull'accrescimento dello stelo del germoglio piuttosto che della radice, indipendentemente dal pretrattamento in analisi; al contrario, la luce a fluorescenza sembra indurre un accrescimento maggiore nella radice piuttosto che nello stelo.

Confrontando le due fonti luminose a parità di pretrattamento (30 giorni di vernalizzazione o trattamento con GA3 seguito da 30 giorni di vernalizzazione), risulta evidente come, nel caso della sola stratificazione a freddo, la lampada a fluorescenza sia in grado di stimolare maggiormente lo sviluppo dell'epicotile e della radice rispetto alla lampada G2. I germogli cresciuti sotto quest'ultima, difatti, al 15° giorno dalla semina, non hanno mostrato ancora l'emergenza dell'epicotile e la dimensione della radice primaria è apparsa circa la metà rispetto al risultato ottenuto sotto lampada a fluorescenza. I germogli pretrattati con la combinazione di ormoni (GA3) e stratificazione a freddo, non hanno mostrato significative differenze dopo 15 giorni dalla semina, per quanto riguarda la lunghezza della radice e dell'epicotile. Una significativa differenza è stata invece identificata relativamente alla misura dell'ipocotile, che ha mostrato sotto lampada G2 dimensioni pari a circa il doppio di quelle ottenute sotto lampada a fluorescenza (Fig. 36).

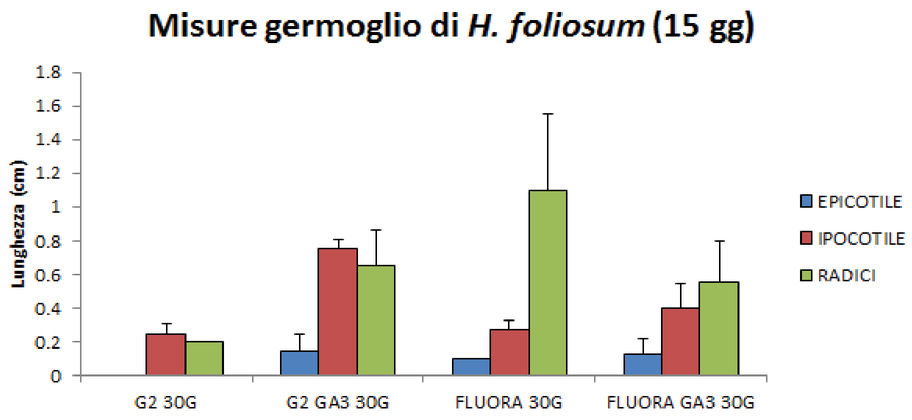

Fig. 36 Misure dei germogli di *H. foliosum* (lunghezza epicotile in azzurro, ipocotile in rosso, radice in verde) dopo 15 giorni dal trasferimento sotto lampade LED (G2) e a fluorescenza dei semi germinati a seguito di due pretrattamenti: incubazione con GA3 seguita da 30gg di vernalizzazione; vernalizzazione 30gg.

Per quanto riguarda il numero delle foglie, non sono state evidenziate significative differenze né tra diverse fonti luminose né tra diversi pretrattamenti (Fig. 37).

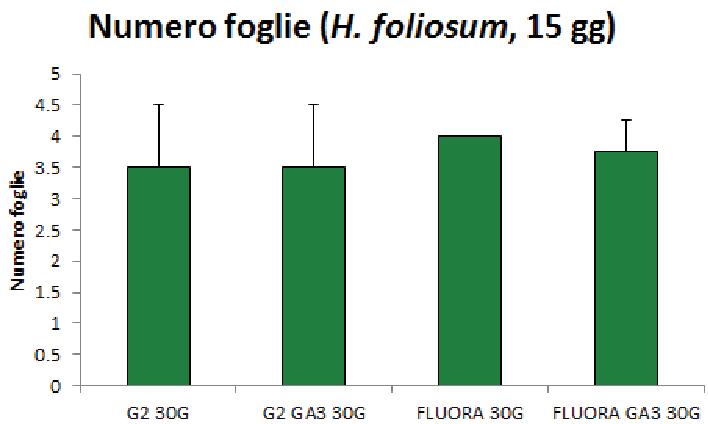

Fig. 37 Numero di foglie dei germogli di *H. foliosum* dopo 15 giorni dal trasferimento sotto lampade LED (G2) e a fluorescenza dei semi germinati a seguito di due pretrattamenti: incubazione con GA3 seguita da 30gg di vernalizzazione; vernalizzazione 30gg.

4. Discussione

Su un totale di 300 specie native delle Isole Azzorre, 156 possono essere considerate rare e un numero consistente di queste sta scomparendo negli ultimi anni. Pertanto, il numero delle specie a rischio d'estinzione è molto elevato, superiore a 60, ed include importanti endemismi.

Le cause principali di tale rarefazione sono legate all'espansione del suolo utilizzato a scopi agricoli e pastorali, in particolare lungo le zone costiere, nonché alla competizione con specie esotiche, nelle zone di montagna. Tali specie, crescendo bene in un ambiente mite e umido, sono state in grado di espandersi velocemente in assenza di nemici naturali.

Per contrastare la loro estinzione e allo stesso tempo tentare di recuperare habitat nativi di tali specie, senza danneggiare l'economia locale che, come già detto, si sviluppa soprattutto nelle zone costiere, da alcuni anni sono in corso nell'arcipelago tentativi di rimboschimento con specie native nelle aree di montagna presenti nell'entroterra, un tempo occupate da fiorenti foreste.

I nuovi impianti boschivi vengono comunemente chiamati rimboschimenti o imboschimenti, a seconda che si tratti di ripristino del soprassuolo boschivo o di un effettivo nuovo impianto su terreni prima dedicati ad altra coltura.

Quando il rimboschimento di una superficie, precedentemente sottoposta al taglio, avviene per mezzo della disseminazione da piante preesistenti o da quelle circostanti, si parla di rinnovazione naturale.

Questa è la forma migliore per perpetuare il bosco; tuttavia non sempre ciò è possibile, sia perché mancano le piante disseminatrici, sia per cause intrinseche alla stazione (terreno eroso, presenza eccessiva di animali che si cibano del seme), o anche da errata pratica nel trattamento del bosco. Quando non si hanno le condizioni idonee alla germinazione del seme e allo sviluppo della piantina, si procede con la messa a dimora di piantine o semi e si parla allora di rinnovazione artificiale o impianto artificiale di un bosco.

L'opera di rimboschimento, per l'impianto artificiale di nuovi boschi, appare spesso necessaria soprattutto quando la copertura vegetale è particolarmente degradata, i suoli erosi e le condizioni complessive della stazione non consentono di avere, in tempi relativamente brevi, la ricostituzione spontanea del manto forestale.

Nel caso specifico delle Isole Azzorre, la limitata capacità disseminativa delle specie native, associata alla presenza di dormienza del seme e alla difficoltà di competizione delle piantine eventualmente germinate con le specie invasive, dotate di un accrescimento decisamente più rapido, è risultato necessario optare per un rimboschimento basato su rinnovazione artificiale.
Per la precisione, si parla di rinnovazione artificiale protetta, mediante l'utilizzo di shelter in plastica (Fig. 38), che assicurano risultati decisamente superiori in termini sia di sopravvivenza che di accrescimento delle piante in rinnovazione, rispetto alla messa a dimora senza alcuna protezione, fenomeno tanto più evidente in quelle aree in cui maggiore sia la competizione con le specie invasive o la presenza di erbivori.


Fig. 38 Rinnovazione artificiale con shelter

La rinnovazione artificiale presuppone la produzione di piantine allevate in vivaio o camera fitologica. Le tre forme di propagazione utilizzabili sono le seguenti:

- da seme (rinnovazione **gamica**) quando gli esemplari giovani nascono da semi disseminati da piante adulte.
- per talea (rinnovazione **agamica**) se invece si tratta di riproduzione asessuale. Propagare una pianta per **talea** significa riprodurla per mezzo di una porzione di ramo con una o più foglie, o addirittura di una sola foglia o anche di un pezzo di fusto o di radice. Esistono talee legnose, semilegnose, fogliari ed

erbacee. Il sistema per talea è però il più usato perché permette di ottenere piante perfettamente identiche alla pianta madre, cosa impossibile partendo dal seme.

- micropropagazione, una tecnica di propagazione di una pianta che permette di ottenere un clone della pianta stessa, ovvero un insieme di individui dotati dello stesso patrimonio genetico, tramite l'utilizzo dei metodi moderni di coltura *in vitro* di cellule e tessuti vegetali.

La prima cosa da considerare è che la propagazione per talea non è possibile con tutte le piante perché vi sono specie le cui talee non emettono radici o lo fanno in tempi troppo lunghi. Inoltre, sia il metodo di propagazione per talea che la micropropagazione non comportano alcuna unione fra corredi cromosomici diversi e il nuovo individuo (clone) avrà lo stesso patrimonio genetico della pianta che l'ha generato, non assicurando quindi la tutela della biodiversità della specie, principale finalità della Convenzione sulla diversità biologica firmata a Rio de Janeiro il 5 giugno 1992, tutela assicurata al contrario dalla propagazione da seme. La riproduzione sessuale di una pianta, infatti, prevede una fecondazione che porta alla formazione di un seme con caratteristiche ereditate da entrambi i genitori. L'individuo che si origina sarà simile a essi, ma non uguale, poiché l'unione dei cromosomi maschili e femminili determina nuove caratteristiche.

Per tali motivi, è preferibile, in ambienti a forte rischio di "estinzione", come le foreste delle Azzorre, scegliere la via della propagazione per seme. Nel caso delle specie oggetto di studio (*Hypericum foliosum, Prunus azorica, Laurus azorica, Frangula azorica* e *Morella faya*), tale metodo presenta delle limitazioni legate alla dormienza del seme, alla rapida perdita di capacità germinativa nonchè alle scarse informazioni reperibili in letteratura che descrivano idonei protocolli di germinazione.

Pertanto si è deciso di testare l'efficacia dell'applicazione di gibberelline, impiegate da sole o unitamente ad un periodo di stratificazione a freddo (30 giorni) per rompere la dormienza del seme o accorciare il tempo di germinazione.

I risultati ottenuti dopo 1 mese di incubazione dei semi a 20°C, hanno mostrato l'efficacia di un pretrattamento con acido gibberellico nel ridurre i tempi di germinazione e aumentare la percentuale di semi germinati nel caso di specie non caratterizzate da dormienza profonda, come *Laurus azorica* e *Hypericum foliosum.* Nel caso della specie *Frangula azorica,* il pretrattamento con GA3 ha mostrato i primi segni di induzione della germinazione soltanto dopo 1 mese di incubazione a 20°C. Tale risultato è comunque da considerarsi promettente, dal momento che in letteratura non sono disponibili protocolli di germinazione per tale specie. Le specie *Morella faya* e *Prunus azorica*, invece, necessitano probabilmente di pretrattamenti più aggressivi o di una combinazione di pretrattamenti, a causa di dormienze più profonde, sulle quali non vi è quasi alcuna informazione reperibile in letteratura. Presso il Jardim Botanico do Faial (Parco Natural do Faial, Horta, Azzorre, Portogallo), nell'ambito del progetto europeo Zephyr (Zero Impact Innovative Technology in Forest Plant Production; www.zephyr-project.eu), coordinato Dipartimento DAFNE, Università degli studi della Tuscia, sono in corso test sperimentali per incrementare le conoscenze relative alla dormienza di tali specie e selezionare dei pretrattamenti atti a interromperla, consentendone la germinazione. Tale step risulta necessario per il raggiungimento del target principale di tale progetto, che è quello della produzione ad alta densità di piantine di specie forestali in un ambiente controllato e totalmente automatizzato in un solo mese di crescita indoor sotto lampade artificiali, al fine di poter disporre di materiale sufficiente per opere di rimboschimento con specie endogene. Nello specifico, i ricercatori stanno testando l'effetto di diversi periodi (30-60-90-120 giorni) di stratificazione a caldo e a freddo, nonché l'effetto dato dalla combinazione di tali pretrattamenti. Al momento non sono ancora disponibili risultati.

Nel caso della specie *L. azorica*, l'incubazione con acido gibberellico ha mostrato, come già noto dalla letteratura, la possibilità di sostituire un processo di stratificazione a freddo, con risparmio sui tempi di incubazione. In entrambi i casi, è stata raggiunta una percentuale di germinazione pari a circa il 60-70%. C'è però da aggiungere che la vernalizzazione ha indotto un'accelerazione nel processo germinativo, tanto da raggiungere il picco di germinazione, cui è seguita una fase di plateau, in soli 8 giorni, valore raggiunto dai semi trattati con GA3 soltanto dopo 14 giorni di incubazione.

Il monitoraggio dell'accrescimento dei germogli di *L. azorica*, in seguito a trasferimento in terriccio dei semi germinati in piastra, ha mostrato un effetto sinergico indotto dall'associazione tra gibberelline e stratificazione a freddo sulla crescita della porzione epigea del germoglio.

Per quanto concerne la specie *H. foliosum*, il pretrattamento risultato più efficace nell'incremento della percentuale di germinazione e nella velocità di quest'ultima, è risultato essere la stratificazione a freddo per 30 giorni. Il processo germinativo è iniziato dopo soli 3-5 giorni (3 nel caso di stratificazione a freddo in assenza di GA3 e 5 nel caso di applicazione di GA3). Nel caso della stratificazione non preceduta da applicazione di GA3, è stato raggiunto il 100% di germinazione nel corso di soli 7 giorni a partire dal trasferimento delle piastre vernalizzate a 20°C in camera fitologica. Nel caso di applicazione di GA3 prima dell'inizio della stratificazione a freddo, si è raggiunta una percentuale di germinazione pari all'80% in soli 7 giorni che si è mantenuta costante nelle successive settimane. La minore percentuale di germinazione, in questo caso, potrebbe non essere dovuta ad una minore efficacia del pretrattamento, quanto alla possibilità che parte dei semi non fossero vitali. Non è stato difatti possibile effettuare un test di vitalità a causa delle dimensioni minime di questi ultimi. Risulta dunque evidente quanto, al contrario della specie *L. azorica*, le gibberelline non mostrino un effetto significativo sul processo germinativo. L'applicazione di GA3 in assenza di vernalizzazione non ha mostrato effetti significativi sulla germinazione del seme, mostrando un comportamento similare a quello osservato nelle piastre di controllo (incubazione a 20°C in assenza di pretrattamenti), con un inizio nel processo germinativo ritardato rispetto alle piastre vernalizzate (15 giorni di incubazione). In entrambi i casi la percentuale di germinazione è risultata inferiore al 10%.

Misurazioni effettuate sui germogli, prima del trasferimento in terriccio, hanno mostrato un significativo effetto sinergico del pretrattamento con ormoni e della vernalizzazione sull'accrescimento del germoglio nelle sue prime fasi di sviluppo post germinazione.

Le misure morfometriche (lunghezza epicotile, lunghezza ipocotile, lunghezza radice, numero di foglie) effettuate sui germogli nati da semi sottoposti a 30 giorni di stratificazione a freddo con e senza precedente trattamento con GA3, e quindi trasferiti in terriccio sotto lampade LED e a fluorescenza, hanno mostrato, sotto entrambe le fonti luminose, un marcato effetto indotto dalle gibberelline sull'accrescimento dell'epicotile e dell'ipocotile. Al contrario, né la dimensione delle radici né il numero delle foglie hanno mostrato significative differenze tra i vari pretrattamenti. L'effetto sinergico dei due pretrattamenti, che era risultato evidente nelle prime fasi post germinative, non si è conservato in questa seconda fase di crescita in terra, a differenza di quanto mostrato dalla specie *L. azorica*.

Il confronto tra le due tipologie di fonti luminose, ha mostrato un maggiore effetto di accrescimento dello stelo indotto dai LED, al contrario della lampada a fluorescenza che ha mostrato un'influenza più marcata sull'accrescimento radicale. Tale differenza è da ricondurre al differente spettro luminoso emesso dalle due lampade (Fig.39-40). In particolare, lo spettro della lampada a fluorescenza appare più ricco in blu rispetto allo spettro G2, che mostra invece superiori percentuali di rosso e infrarosso. Notevole è inoltre la differenza nel rapporto red: far red (5.72 per la fluorescenza e 1.93 per lo spettro G2), che come noto risulta strettamente legato all'attività dei fitocromi, proteine che partecipano alla regolazione delle prime fasi di sviluppo delle piantine.

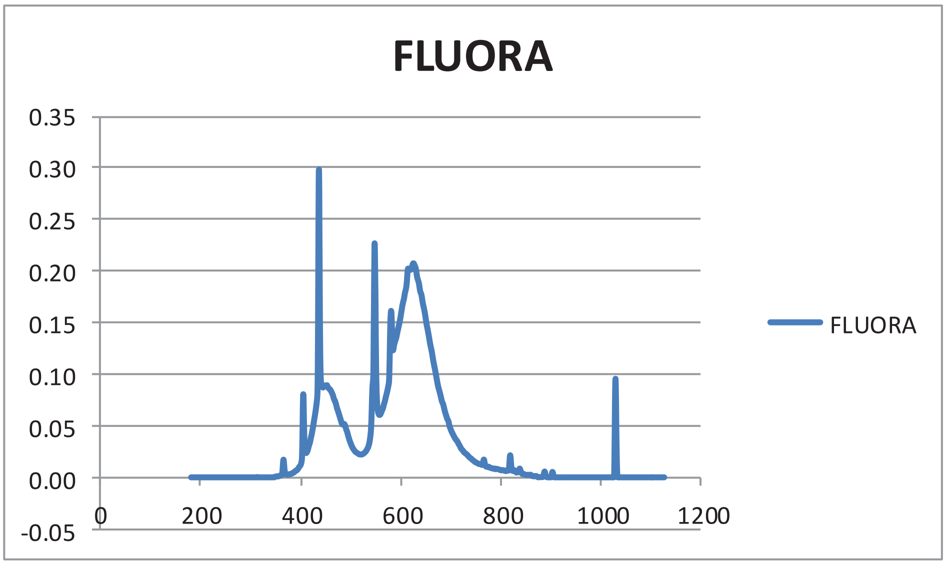

Fig. 39 Spettro luminoso della lampada a fluorescenza OSRAM FLUORA

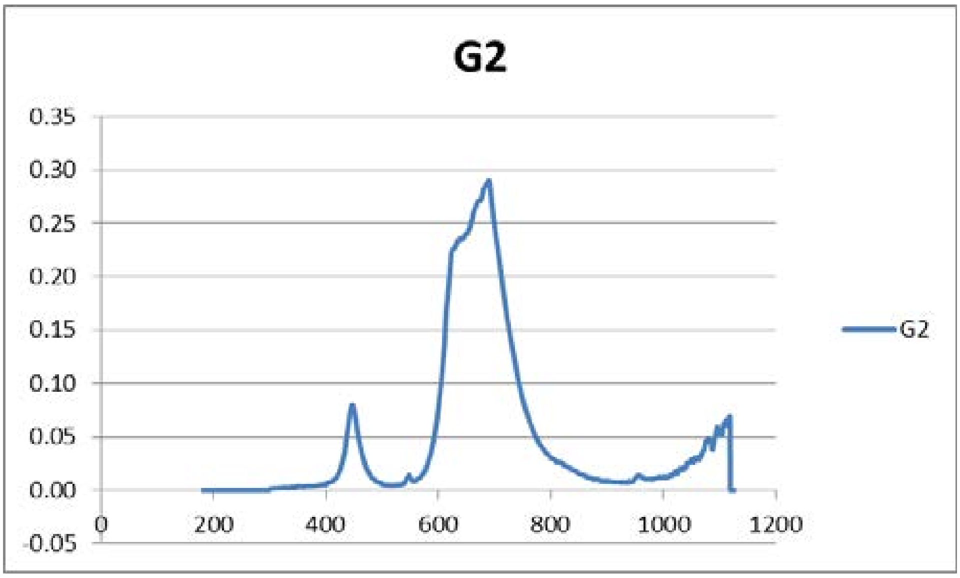

Fig. 40 Spettro luminoso della lampada LED G2

5. Conclusioni

Le specie native delle isole Azzorre sono oggigiorno caratterizzate da un rischio di estinzione molto elevato, a causa di una serie di fattori sia di origine antropica che non, quali l'espansione del suolo utilizzato a scopi agricoli e pastorali, in particolare lungo le zone costiere, nonché la competizione con specie esotiche, molto più rapide nella crescita rispetto alle specie endemiche, nelle zone di montagna.

Per contrastare la loro estinzione e allo stesso tempo tentare di recuperare habitat nativi di tali specie, risultano necessarie rapide opere di rimboschimento con specie native nelle aree di montagna, un tempo occupate da fiorenti foreste.

La limitata capacità disseminativa delle specie native delle Azzorre, associata alla presenza di dormienza del seme e alla difficoltà di competizione delle piantine con le specie invasive, determina la necessità di rimboschimenti basati su rinnovazione artificiale, ovvero su propagazione delle specie in ambiente controllato (vivaio o camera fitologica) e allevamento di semenzali da trasferire poi in campo aperto. La propagazione per seme assicura la tutela della biodiversità, pertanto in tale studio si è cercato di trovare idonei protocolli di rottura della dormienza e di germinazione di quattro specie endemiche (*Hypericum foliosum, Prunus azorica, Laurus azorica, Frangula azorica* e *Morella faya*), caratterizzate da dormienza del seme, germinazione molto lenta o rapida perdita di capacità germinativa.

Pertanto si è deciso di testare l'efficacia dell'applicazione di acido gibberellico (GA3) e di un periodo di stratificazione a freddo (30 giorni) nonché l'associazione dei due pretrattamenti, per rompere la dormienza del seme o accorciare il tempo di germinazione. I risultati ottenuti dopo 1 mese di incubazione dei semi a 20°C, hanno mostrato che tali pretrattamenti non risultano né singolarmente né in combinazione idonei a rompere la dormienza di specie quali *Morella faya* e *Prunus azorica*, che necessitano probabilmente di pretrattamenti più aggressivi.

Nel caso della specie *L. azorica*, sia il GA3 sia la stratificazione a freddo hanno mostrato buoni risultati in termini di velocità e tasso di germinazione. L'associazione dei due trattamenti ha inoltre mostrato ottimi effetti sull'accrescimento del germoglio nelle prime fasi dopo la germinazione.

Nel caso della specie *H. foliosum*, la stratificazione a freddo ha condotto a un 100% di germinazione nell'arco di una settimana. In questo caso, l'associazione dei due pretrattamenti ha condotto a un incremento nel tasso di accrescimento dei germogli nelle prime fasi di sviluppo post germinativo.

La combinazione dunque del trattamento con gibberelline e di un periodo di stratificazione a freddo si mostra molto promettente come metodo per l'ottenimento, in un breve periodo di tempo, di un significativo numero di semenzali di *H. foliosum* e *L. azorica*, utili a opere di rimboschimento nelle Isole Azzorre, non solo in virtù dell'alta percentuale di germinazione raggiunta ma anche grazie al potenziamento nell'accrescimento del germoglio nelle prime fasi di sviluppo post germinativo indotto dagli ormoni.

Relativamente alla specie *Frangula azorica*, i primi segni di germinazione indotta dall'acido gibberellico, sono apparsi al termine dei 30 giorni stabiliti di norma per lo svolgimento di una prova di germinazione. Pertanto non è stato possibile nel corso di questo studio analizzare la dinamica di germinazione di tale specie. Tali analisi sono al momento in corso. In ogni caso, l'efficacia del trattamento ormonale come metodo di rottura della dormienza di tale specie, risulta di grande interesse, data l'assenza di protocolli di germinazione disponibili in letteratura.

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RINGRAZIAMENTI

La presente tesi di laurea, eseguita presso il Centro Vivaistico Forestale viterbese dell'Università della Tuscia, ha per oggetto la germinazione e lo studio delle specie legnose protette ed in via di estinzione, Europee e delle isole Azzorre.

Durante il lavoro di laboratorio, da me svolto, ho eseguito, in collaborazione con il Prof. Schirone, con i tecnici e biologi nonché con il Corpo Forestale dello Stato, Test relativi alla germinazione e rimboschimento delle specie:

Ci siamo recati presso la tenuta vivaistica dell'Università della Tuscia per eseguire i test di verifica attestanti l'attecchimento delle specie in esame.

Durante il lavoro di laboratorio, ho svolto altri test tra cui germinazione, test di vitalità del seme, analisi della curva di accrescimento, misure morfologiche e piastre per germinazione e vernalizzazione.

Il presente studio, che ho trovato molto interessante mi ha permesso di approfondire le mie conoscenze in materia forestale. In particolare ho verificato che le specie arboree europee differiscono notevolmente da quelle delle isole delle Azzorre.

Queste ultime, pur facendo parte dell'Europa, si trovano infatti nell'Atlantico ed appartengono al Portogallo, hanno un ecosistema particolare che favorisce una flora del tutto diversa da quella del continente europeo.

Università degli Studi della Tuscia
Viterbo

Dipartimento di Scienze e tecnologie per
l'Agricoltura, le Foreste, la Natura e l'Energia
(DAFNE)

Corso di Laurea in
Scienze e Tecnologie per la Gestione Forestale e
Ambientale

ANALISI DELLE CONDIZIONI DI LUCE NECESSARIE ALLA GERMINAZIONE E ALLO SVILUPPO DEI SEMENZALI DI SUGHERA (Quercus suber L.) IN UN BOSCO DEL CENTRO ITALIA

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Laureando
Federico Canali

a.a. 2014-2015

Primo o ultimo non conta…
L’importante è aver dato
il meglio di sé in ogni
singolo giro.
(Marco Simoncelli)

Sommario

1. Introduzione

1.1. Importanza delle foreste e loro rischi

Le foreste hanno un ruolo insostituibile a livello biologico ed economico: contribuiscono alla tutela della biodiversità, svolgono un ruolo fondamentale nella conservazione delle acque e del suolo (difesa da frane e erosioni), forniscono innumerevoli prodotti non solo legnosi e cibo a centinaia di milioni di persone, sono tra i principali serbatoi di assorbimento del carbonio.
Le minacce principali alle foreste sono la loro conversione in terreni agricoli e destinati all'allevamento zootecnico, il taglio e il commercio illegale di prodotti forestali, gli incendi, l'avanzamento dell'urbanizzazione, in poche parole una insostenibile gestione di questo patrimonio unico. Proprio per le molteplici funzioni che svolgono, la perdita e la degradazione delle foreste sono fenomeni preoccupanti.
(http://www.wwf.it/ambiente/foreste/valore_foreste/)

1.2. La foresta mediterranea

La foresta mediterranea cresce a sud della foresta temperata nella zona costiera del Mediterraneo, in California, in alcune zone del Cile, in Africa meridionale e in Australia occidentale, dove viene anche detta "bushland" (= prateria o boscaglia).
In queste zone il clima è caratterizzato da estati calde e asciutte e inverni miti e piovosi. Il suolo è per lo più povero di sostanze nutritive e le attività dell'uomo, come l'eccessivo sfruttamento del legname, la necessità di creare nuovi pascoli e l'introduzione di specie esotiche sono responsabili del degrado del suolo (peggioramento qualitativo del suolo).

La fase di sviluppo di queste piante, quasi tutte sempreverdi, è in primavera, quando le temperature sono abbastanza alte e c'è ancora sufficiente umidità residua. Piante tipiche della foresta mediterranea sono il leccio, il carrubo, la quercia da sughero, gli ulivi o conifere come il pino marittimo, il cipresso o il cedro. Benché la foresta mediterranea rappresenti una parte modesta delle foreste globali, essa ospita una svariata quantità di specie animali e vegetali che si sono adattate alle estati lunghe, calde e asciutte. Qui troviamo circa il 10% di tutte le specie vegetali conosciute.

La maggior parte delle piante si è abituata agli incendi occasionali o addirittura possono sopravvivere solo grazie al fuoco. Alcune piante infatti hanno bisogno del calore delle fiamme per poter aprire le capsule dei semi. Nelle foreste mediterranee troviamo una gran quantità di piante endemiche, che crescono cioè esclusivamente in quelle zone e in nessuna altra parte del mondo.

1.2.1. Processi di riforestazione in ambiente mediterraneo

Il primo rapporto FAO sull'argomento "Lo Stato delle Foreste del Mediterraneo" (2013), ha previsto che le foreste del Mediterraneo saranno tra le più colpite dagli effetti del cambiamento climatico, in quanto già sottoposte a forte pressione a causa dell'incremento demografico della regione. Nell'area del Mediterraneo nel corso del ventesimo secolo le temperature sono aumentate di un grado mentre in certe aree le piogge sono diminuite del 20%. Per la fine di questo secolo si prevede che le temperature saliranno ancora di altri due gradi, fattore che con tutta probabilità metterà alcune specie boschive a rischio d'estinzione con grave perdita di biodiversità. Frequenti annate siccitose contribuiscono infatti all'indebolimento degli ecosistemi forestali. Come risultato le foreste riducono le loro capacità produttive, sono più soggette a fenomeni di degrado secondario quali gli attacchi parassitari. Inoltre i contesti economico-sociali possono acuire il degrado con la diffusione di uno scorretto uso della risorsa (tagli boschivi, pascolamento) e con la diffusione degli incendi boschivi.

In tale rapporto viene descritto anche il grande ruolo di assorbimento di carbonio svolto dalle foreste del Mediterraneo. Nel 2010 si calcola che abbiano immagazzinato circa 5 miliardi di tonnellate di carbonio, che rappresenta 1.6% dell'assorbimento globale di carbonio dalle foreste. Esse forniscono inoltre preziosi servizi ambientali come il controllo delle risorse idriche e del clima, la fornitura di prodotti legnosi e non e la conservazione della biodiversità.

Il rapporto sottolinea che il valore delle foreste mediterranee ed il loro ruolo vitale nella mitigazione e nell'adattamento al cambiamento climatico debba essere riconosciuto a livello locale, regionale e nazionale. Fa appello ai governi e agli operatori forestali affinché promuovano l'uso di prodotti forestali legnosi e non legnosi, ad esempio il sughero, per l'immagazzinamento di carbonio nel lungo periodo, e per potenziare un possibile investimento dei piccoli proprietari che lavorano con i prodotti legnosi e non, e con industrie basate sulle foreste (pinoli, erba alfa, funghi, miele etc).

Il rapporto sollecita gli operatori forestali ad usare nelle loro pratiche di silvicoltura quelle varietà di risorse genetiche boschive e quelle specie che meglio si adattano al cambio delle condizioni climatiche.

A livello locale, essi dovrebbero anche migliorare la pianificazione nella gestione degli ecosistemi forestali con una densità ottimale di alberi, affrontando al tempo stesso il problema della scarsità delle risorse idriche , laddove le attività su ampia sala dovrebbero includere la prevenzione degli incendi boschivi.

Per preservare i sistemi forestali in ambiente mediterraneo dai rischi derivanti dai cambiamenti climatici, bisogna da un lato intervenire sul bosco aumentandone la capacità di resilienza, migliorandone l'efficienza ecosistemica e favorendo la salvaguardia della biodiversità. Dall’altro effettuando dei programmi di rimboschimento su aree precedentemente destinate a scopo agricolo, per espandere e ripristinare l’originale superficie forestale mediterranea.

Nell'UE sono in atto intensi programmi di rimboschimento e imboschimento che sono essenziali per ridurre le emissioni di gas serra, in continuo aumento, contribuendo a rallentare i cambiamenti climatici e alla tutela della biodiversità.

http://www.resilformed.eu/it/progetto.html

http://www.fao.org/news/story/it/item/172636/icode/

Il termine **rimboschimento** indica il processo di reintroduzione di specie arboree e arbustive in terreni che, in un passato più o meno recente, hanno ospitato complessi boscati distrutti o degradati per cause naturali o per l'intervento dell'uomo.

Il termine **imboschimento** indica invece l'impianto di specie arboree e arbustive su terreni da lungo tempo privi di vegetazione legnosa (ex coltivi, terreni marginali abbandonati, terreni dissestati).

La scelta delle specie è sempre più orientata verso quelle autoctone, con preferenza per le provenienze locali, mentre nel passato sono state utilizzate prevalentemente specie esotiche, che talvolta non sono riuscite ad acclimatarsi o sono divenute infestanti, sconvolgendo gli ambienti locali e dando origine al cosiddetto inquinamento verde. Un caso particolare di imboschimento molto in voga all'inizio del 20° sec., che ha provocato la scomparsa o l'alterazione di numerosi habitat costieri di grande rilevanza naturalistica, è per esempio quello eseguito per il consolidamento delle dune sabbiose mediante l'impianto di pini o di eucalipti.

1.3. *Quercus suber L.*

La quercia da sughero è una specie termofila che predilige gli ambienti caldi e moderatamente siccitosi, rifugge gli ambienti di siccità estrema o soggetti a frequenti gelate invernali (gli esemplari giovani muoiono a T°< -5°C). Vegeta prevalentemente su suoli derivati da rocce a matrice acida (graniti e granitoidi, trachiti, scisti granitici, filladi) diventando sporadica nei suoli basaltici e in quelli calcarei (disponendo però di una buona adattabilità). Originaria dell'Europa sud-occidentale e dell'Africa nord-occidentale è da tempi remoti naturalizzata e spontanea in tutto il bacino occidentale del mar Mediterraneo, in Italia vegeta nella sottozona calda e media del Lauretum (classificazione fitoclimatica del Pavari) spingendosi fino ai 900 metri d'altitudine in alcune zone della Sicilia e della Sardegna sud-occidentale.
I boschi di sughera riescono a svilupparsi ed affermarsi solo in determinate aree con specifiche caratteristiche ambientali. Boschi puri di *Quercus suber* non sono generalmente presenti, ma spesso la specie si trova associata ad altre come acero e leccio.

Specie sempreverde con portamento arboreo, mostra un' altezza che può raggiungere i 20 metri e una chioma lassa ed espansa. La vita media è di 250-300 anni, diminuisce negli esemplari sfruttati per la produzione di sughero.
Le foglie sono semplici, ovali acute, di 3-7 cm di lunghezza, coriacee, a margine spesso revoluto, con denti mucronati; la pagina inferiore è grigia tomentosa, il picciolo peloso ha una lunghezza

Figura 1: Individuo maturo di *Quercus suber* L.

media di 1 cm e mostrano una inserzione alterna. Le infiorescenze sono unisessuali e la fioritura avviene a maggio. Le ghiande sono ovali di 1,5-3 cm di lunghezza, la cupola va a coprire 1/3 della ghianda, con squame poco appressate fra loro. Affonda le sue radici a profondità insospettabili e ciò le permette di adattarsi alla siccità, mentre resiste agli incendi grazie alla protezione del sughero. Quest'ultimo elemento ne determina una grande importanza a livello economico.
In seguito alla lavorazione del ritidoma si ottiene un prodotto finito destinato ad essere esportato in tutto il mondo.

Le foreste di sughera sono ad oggi considerate a rischio per via dei cambiamenti climatici che portano le piante ad esporsi a temperature e livelli di precipitazioni a loro non favorevoli. Altro grande danno è causato dai continui interventi dell'uomo, che indebolendo le difese naturali delle piante, favoriscono l'attacco da parte di patogeni come *Cytsodendron dryophilum, Lembosia quercina, Armillaria mellea*, l'attacco da parte di insetti *Thaumetopea processionea* e la *Lymantria dispar* ed infine l'assenza di insetti pronubi alla specie.
Questo porta le piante ad una crescita non corretta, sia nei tempi che nel quantitativo di ritidoma prodotto, determinando di conseguenza anche un danno economico all'imprenditore.

1.3.1. Areale della specie

Attualmente l'areale della quercia da sughero ha subito una riduzione a causa dei cambiamenti climatici e dell'esbosco che l'ha coinvolta fortemente. Principalmente si trova nella zona mediterranea nelle aree costiere degli stati come l'Italia (presente dalla Toscana fino ala Sicilia, Sardegna e con piccole popolazioni anche in Puglia), sulle coste della Tunisia, Algeria e Francia. Per quanto riguarda la Spagna e il Portogallo, paesi che hanno la maggior presenza della specie, sappiamo che si affaccia sull'Oceano Atlantico e si addentra molto anche nell'entroterra, trovando li le condizioni ambientali ideali al suo sviluppo.

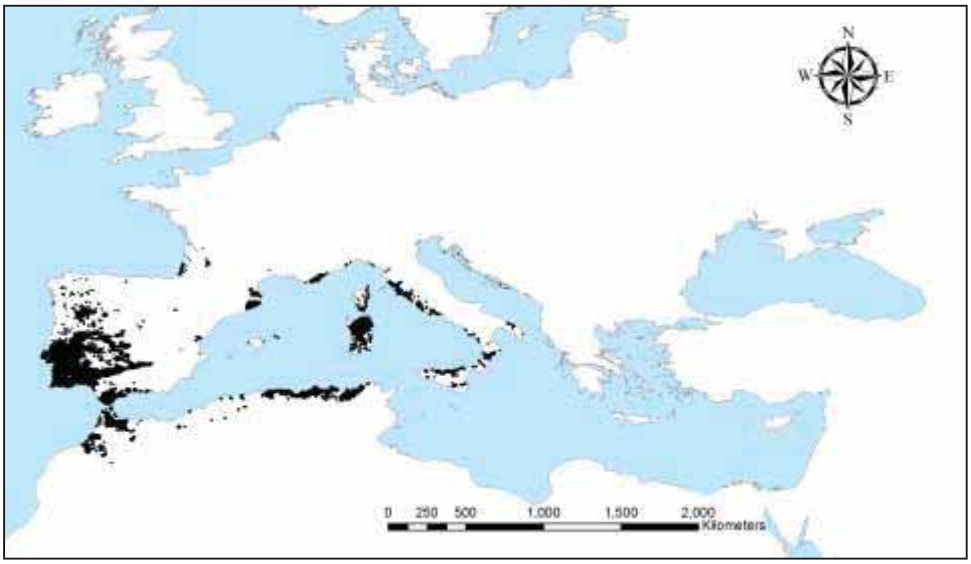


Figura 2: Areale di *Quercus suber* L.

1.4. Parametri che influenzano la rinnovazione naturale della specie

La crescita delle piante è regolata in maniera ben precisa: esse sono intimamente legate all'ambiente dove si trovano e sono condizionate da una serie di fattori ecologici e storici che giustificano o meno la loro presenza in un determinato luogo. Ogni specie vegetale ha nei confronti di ciascun fattore ecologico un ambito di tolleranza entro il quale può svolgere le proprie funzioni vitali. L'ampiezza di tale ambito varia da specie a specie: quelle ad ecologia ampia prendono il nome di euriecie, mentre quelle più esigenti, ad ecologia ristretta, sono dette stenoecie e sono quelle che danno il contributo più utile in termini di bioindicazione. *Q.suber* L. può essere considerata in tale ambito quale una specie frugale.

ACQUA	± 1200mm acqua/anno
NUTRIENTI	Non mostra esigenze particolari
TEMPERATURA	20°C (±2°C)
SUOLO	Siliceo - Argilloso
LUCE	Soleggiato

Tabella 1: Riassunto dei fattori utili per lo sviluppo di *Quercus suber* L.

I fattori ecologici possono agire sulle dimensioni del singolo individuo, sulla sua forma, possono influenzare le manifestazioni biologiche cicliche e la stessa durata della vita. Inoltre essi possono controllare la consistenza delle popolazioni, agendo sul tasso di riproduzione, sulla competitività, sulla capacità di germinazione e sulla velocità di crescita. Di contro gli organismi vegetali possono influire sull'ambiente modificando l'entità e la qualità di alcuni fattori, come ad esempio limitando la quantità di radiazione solare nelle vegetazioni stratificate o incrementando la quantità di sostanza organica con accumulo di necromassa o ancora acidificando il suolo come accade per alcuni boschi di conifere.

Come già accennato in precedenza i fattori ecologici possono essere idealmente suddivisi in abiotici e biotici come schematizzato nella tabella sottostante:

Fattori abiotici		Fattori biotici
Fisici	**Chimici**	
Luce	Comp. Chim. Acqua	Competizione interspecifica
Temperatura	Comp. Chim. Suolo	Competizione intraspecifica
Pioggia, Umidità	Comp. Chim. Aria	Simbiosi
Granulometria suolo		Microbiologia suolo
Vento		Disturbo antropico
(Altitudine)		
(Esposizione)		
(Inclinazione)		

Tabella 2: Fattori ecologici abiotici e biotici che condizionano la rinnovazione e la crescita delle specie vegetali

1.4.1. Acqua

L'acqua è la sostanza più diffusa sulla terra: è stato calcolato che solo negli oceani sono presenti circa 1400 Km^3 di H_2O. Essa è indispensabile per la vita in genere ed in particolare per quella degli organismi vegetali, permettendo i principali processi fisiologici come assorbimento e trasporto delle sostanze nutritive, fotosintesi, traspirazione, ecc. Gli adattamenti delle piante rispetto al fattore acqua sono innumerevoli e sorprendenti: basti

pensare alla succulenza, alla sclerofillia, al metabolismo CAM delle crassulacee; WARMING nel 1895 introdusse una classificazione empirica delle piante in base al loro adattamento ad ambienti con diverse disponibilità di acqua; seppur limitativa e schematica tale terminologia è tuttora in uso.
Il ruolo dell'acqua come fattore ecologico esce dagli schematismi in quanto può essere considerata indipendentemente fattore chimico, se si considera la composizione dei sali in soluzione, fattore fisico, quando è intesa come mezzo di trasporto, fattore climatico se si valutano le entità medie di pioggia, neve, umidità atmosferica.
Un deficit protratto di acqua può condurre la pianta a morte per disidratazione (appassimento), mentre un suo eccesso può condurre fino alla morte per asfissia radicale.

1.4.1.1. Pioggia – La piovosità ha influenza a livello regionale ed è quindi di grande importanza nella distribuzione di specie ed associazioni vegetali. In base all'andamento ed alla quantità di piogge annuali si caratterizzano i regimi delle precipitazioni. In Italia si distinguono quattro principali regimi pluviometrici: *regime continentale* tendenzialmente arido con precipitazioni concentrate ad inizio estate (Valtellina, valli a Pinus sylvestris), *regime prealpino* con precipitazioni massime durante gli equinozi (pre-alpi e pianura padana), *regime appenninico* senza un evidente periodo di aridità con piogge ridotte nel periodo estivo e *regime mediterraneo* con periodo di aridità estiva e massimo di precipitazioni invernale.

1.4.1.2. Umidità atmosferica e nebbia – La presenza di nebbia ed alta umidità atmosferica ha sulle piante l'effetto di limitazione della traspirazione. Alti tassi di umidità incrementano la crescita e la formazione delle foglie mentre hanno effetto opposto su fioritura e fruttificazione.

1.4.1.3. Neve – Il principale effetto della copertura nevosa sulle piante è quello di isolante dalla luce e dalle basse temperature esterne. Per le specie delle alte montagne una buona copertura nevale è provvidenziale per superare i mesi più freddi, in quanto essa previene il gelo del suolo e permette agli organismi vegetali di sopravvivere

1.4.2. Nutrienti

I nutrienti sono distinguibili in micro e macro elementi presenti nel suolo e svolgono un importante ruolo nello sviluppo della pianta. I nutrienti principali sono rappresentati da Azoto, Fosforo, Potassio, Calcio e Magnesio, mentre i microelementi sono rappresentati da Ferro, Manganese, Rame e Boro.
Tutti questi elementi devono essere presenti in concentrazioni definite e differenti per singola specie nel suolo. Al di là di tale range essi possono comportare danni da deficit o eccesso. Il livello ottimale è detto OPTIMUM.

1.4.3. Suolo

Le piante assumono i nutrienti dal substrato, che ha anche il compito di trattenere acqua e aria. In base alla tessitura del terreno, i nutrienti saranno più o meno disponibili, pertanto ogni specie vegetale predilige una certa tipologia di substrato.

Col termine fattori edafici si indicano tutti quei fattori ecologici riferibili al substrato sul quale si sviluppa un organismo vegetale, si avranno quindi fattori fisici (granulometria, acqua, aria, temperatura del suolo), chimici (pH, ioni, sali, nutrienti, ecc.) e biotici (microrganismi, micorrize, ecc.).

Esistono diverse tipologie di substrato:

- **suolo sabbioso**: la quantità di sabbia è superiore al 65%.
- **suolo argilloso**: la quantità di argilla è superiore al 30%.
- **suolo calcareo**: la quantità di calcare è superiore al 20%.
- **suolo umifero**: la quantità di humus è superiore al 15%.

Ognuno di questi tipi di suolo presenta delle caratteristiche che dipendono soprattutto dalla loro permeabilità, ovvero la capacità di una sostanza di farsi attraversare dall’acqua.

Dal diverso grado di permeabilità dipendono le principali caratteristiche dei 4 tipi di suolo.

- Un suolo sabbioso, molto permeabile, è generalmente povero di sostanze nutritive, è poco fertile vi crescono solo le piante dotate di radici lunghe , capaci di assorbire l'acqua in profondità.
- Un suolo argilloso, poco permeabile, trattiene l'acqua e per questo motivo è abbastanza fertile; è molto compatto e vi crescono piante con radici brevi e sottili.
- Un suolo calcareo, discretamente permeabile, è generalmente fertile, perchè trattiene il calcio, elemento indispensabile alla vita delle piante. E' particolarmente adatto alla coltivazione delle leguminose, dell' ulivo e della vite.
- Un suolo umifero, è generalmente umido, perchè l'humus assorbe e trattiene l'acqua, è molto fertile e adatto a qualsiasi tipo di coltivazione.

I terreni si possono distinguere anche in dolci ed acidi in funzione del pH.

1.4.4. Temperatura

La temperatura condiziona la distribuzione delle specie vegetali in senso geografico, altitudinale ed anche a livello di microhabitat. Essa influenza le principali funzioni fisiologiche di una pianta, prime fra tutte fotosintesi, respirazione e traspirazione. Le piante sono organismi eterotermi per cui hanno temperature organiche simili a quelle dell’ambiente circostante, a parte alcuni casi particolari (es. *Arum*).

I limiti di temperatura entro i quali si svolgono le attività biologiche delle piante si attestano all’incirca tra 0 e +70° C: al di sotto di 0° C la fotosintesi è pressochè nulla e la pianta entra in vita latente ed al di sopra di 70° C iniziano fenomeni di coagulazione del protoplasma.

Gli areali di distribuzione di alcune specie spesso mostrano interessanti correlazioni con le isoterme del mese più caldo o più freddo (Pavari, 1916): ad esempio il limite settentrionale del ***Fagus sylvatica*** coincide con l’isoterma di –2° C di gennaio e quello del ***Picea abies*** con l’isoterma di 10° C di luglio.

Esistono vari sistemi per stabilire categorie vegetali in relazione alla temperatura, quello di maggior uso in geobotanica definisce specie ***termofile*** quelle con optimum a temperature relativamente elevate, specie ***mesoterme*** quelle che si attestano su valori intermedi e specie ***microterme*** o ***criofile*** quelle di ambienti con valori bassi.

1.4.5. Luce

La luce rappresenta l'unica sorgente di energia disponibile per gli organismi vegetali: essa deriva quasi totalmente dal sole e giunge sulla terra sotto forma di radiazione solare. La quantità di energia che ci arriva dal sole può essere considerata costante e prende il nome di "costante solare": 1,983 cal/cm^2/min. Il bilancio netto della radiazione solare prevede che circa il 30 % del totale viene riflesso, il 50 % è assorbito dal suolo come calore, il 20 % è assorbito dall'atmosfera e solo lo 0,02 % è utilizzato per i processi biologici degli organismi autotrofi.
Con il termine luce si intende nello specifico la porzione dello spettro elettromagnetico percettibile dall'occhio umano e approssimativamente compresa tra 400 e 700 nanometri di lunghezza d'onda. Tale definizione è antropocentrica, tanto che è anche detta Luce Bianca. Essa è data dalla sovrapposizione di tutte le componenti spettrali della luce visibile.
L'azione della luce sulla vita vegetale si esplica principalmente in due modi: sulla crescita delle piante, in quanto la luce influenza la fotosintesi, e sui fenomeni periodici della specie attraverso il fotoperiodismo.
Prima ancora dello sviluppo, la luce appare come elemento influenzante la germinazione dei semi. In particolare, esistono semi che necessitano di poca luce e alcuni che preferiscono un'esposizione prolungata per iniziare i propri processi germinativi.

In base all'adattamento alle diverse intensità luminose si distinguono piante **sciafile**, che prediligono ambienti ombrosi quali sottoboschi (Primula vulgaris, Scilla bifolia, Anemone nemorosa, ecc) e rupi stillicidiose (Adiantum capillus-veneris) e piante **eliofile**, che hanno il loro optimum in pieno sole come gran parte delle specie di prateria.

Alcune specie, soprattutto tra quelle arboree, possono presentare all'interno di uno stesso individuo adattamenti morfologici diversi per le foglie esposte direttamente al sole e quelle ombreggiate dal resto della chioma (es. Quercus ilex). Tale dimorfismo fogliare è espresso nelle foglie da sole in quanto si presentano più coriacee, sclerificate, con disposizione dei cloroplasti perpendicolari alla superficie esposta e stomi infossati, mentre le foglie da ombra sono più flaccide, più espanse, spesso di colore più chiaro e con cloroplasti paralleli.

L'illuminazione è fondamentale per le piante in quanto gestisce e regola il processo chimico della ***fotosintesi***, tramite il quale convertono l'anidride carbonica (CO_2) e l'acqua (H_2O) in materiale vegetale, mediante l'impiego dell'energia luminosa, per poterlo poi conservare sotto forma di sostanza organica (zuccheri, grassi). Il processo inverso della fotosintesi è detto respirazione. Nel corso di tale processo chimico l'ossigeno brucia il glucosio prodotto dalla fotosintesi per liberare energia, che può essere spesa per tutte le attività cellulari indispensabili per i processi di crescita, sviluppo e produzione di biomassa degli organismi vegetali.

$$6\,CO_2 + 6\,H_2O + 2810\ kj \longrightarrow C_6H_{12}O_6 + 6\,O_2 \quad \text{FOTOSINTESI}$$

$$C_6H_{12}O_6 + 6\,O_2 + 2810\ kj \longrightarrow 6\,CO_2 + 6\,H_2O \quad \text{RESPIRAZIONE}$$

Oltre che come fonte di energia la luce svolge, per le colture, una importante funzione di informazione per i fenomeni fotomorfogenetici che si verificano nei diversi stadi della crescita.

I più importanti, maggiormente conosciuti e studiati sono:

1. *fotoperiodo* (tempo di esposizione alla luce)
2. *intensità luminosa* (quantità di energia luminosa ricevuta)
3. *qualità della luce.* (effetto della luce sull'accrescimento della pianta)

Come anticipato, il concetto classico di "luce" è prettamente antropocentrico. In realtà, le piante sono in grado di percepire un range di lunghezze d'onda maggiore di quello umano, compreso tra 200nm e 800nm (Lercari 1990). Questa differenza è dovuta alla presenza, all' interno delle piante, di particolari molecole dette fotorecettori in grado di catturare la radiazione luminosa a lunghezze d'onda diverse rispetto al campo del visibile.
In generale, i fotorecettori delle piante vengono ulteriormente suddivisi in:

1. fotorecettori **fotosintetici**, localizzati nei tilacoidi dei cloroplasti e responsabili dell'assorbimento della luce come energia.
2. **fotosensori,** specifici pigmenti responsabili delle risposte fotomorfogeniche.

I fotorecettori ad oggi conosciuti sono:

1. *Fitocromi*, capaci di assorbire le lunghezze d'onda corrispondenti alle regioni della luce rossa e rosso lontano.
   La luce si suddivide nel rosso lontano, con una lunghezza d'onda di circa 730 nm, al confine con i limiti della percezione umana, e nella luce rossa, la cui lunghezza d'onda è di circa 660 nm.
   Ci sono due forme di fitocromi tra loro interconvertibili grazie alla luce. La forma che assorbe prevalentemente la luce rossa è chiamata Pr. Quando assorbe un fotone di luce rossa, una molecola di Pr viene convertita in Pfr; al contrario, quando un Pfr assorbe la luce nel rosso lontano viene riconvertita nella forma Pr.
   I fitocromi partecipano alla regolazione delle prime fasi della crescita di una pianticella, a partire dalla germinazione del seme nel terreno.
2. *Fotorecettori Blu/UV-A*, che assorbono radiazioni inferiori a 500nm. La luce blu determina effetti significativi sulla morfogenesi delle piante (chiusura stomatica, sviluppo floreale,...). La capacità delle piante di percepire la luce blu e i raggi ultravioletti è stata probabilmente sviluppata molto tempo fa quando la concentrazione di questi raggi era molto più alta di quella attuale e le piante dovevano possedere sistemi di protezione contro queste radiazioni. Esistono diversi tipi di recettori per la luce blu che agiscono in parallelo, ma in modo indipendente l'uno dall'altro; tuttavia solo alcuni di essi sono stati individuati e caratterizzati. I **Criptocromi** sono pigmenti fotorecettori gialli che assorbono la luce blu e ultravioletta. Si trovano nel nucleo della cellula ma la loro funzione e i loro meccanismi sono tuttora sconosciuti, probabilmente essi hanno la funzione di substrati per i fitocromi che si comportano come delle proteine chinasi. Le **Fototropine** sono due pigmenti che partecipano alla ricollocazione dei cloroplasti in relazione alle variazioni dell'intensità di luce. Se esposti ad una luce con bassa intensità, i cloroplasti si dispongono con la superficie perpendicolare rispetto alla direzione della luce. Se invece sono esposti ad una luce più intensa i cloroplasti si dispongono parallelamente alla luce. L'apertura degli stomi viene determinata oltre che dalle fototropine anche da un pigmento plastico definito **Zeaxantina**, sensibile alla luce blu. Essa si forma nelle cellule di guardia in risposta all'esposizione alla luce. I fotorecettori hanno inoltre un ruolo importante per la regolazione della fioritura, assieme ad un altro fattore rappresentato dalla temperatura.
3. *Fotorecettori UV-B*, in grado di assorbire nell' ultravioletto con un picco oscillante intorno ai 290nm e insensibili a lunghezze d'onda superiori a 350nm.

1.5. Metodi di propagazione della Quercia da sughero

La quercia da sughero può essere propagata per seme o per via agamica.
A livello selvicolturale è possibile un impianto per semina diretta, ma dal momento che esso si accompagna generalmente a numerose fallanze tra il secondo e il terzo anno di età, per cause climatiche (prolungata siccità estiva, inverni rigidi e ventosi) o biologiche (predazione), si predilige la scelta della piantagione, utilizzando semenzali allevati in vivaio a radice nuda o più raramente in contenitore.
La propagazione per via agamica è generalmente sconsigliata in quanto la forma di governo più vantaggiosa per la specie risulta essere quella a fustaia, che assicura un maggior ricavo di sughero di qualità, mentre generalmente i cedui sono in grado di fornire esclusivamente legna da ardere. Tale effetto è legato allo stress imposto alla pianta dai tagli successivi, che determina un allungamento dei tempi necessari alla produzione di sughero.

1.5.1. Propagazione in vivaio

La crescente spinta europea e italiana ai rimboschimenti (vedi par. 1.2.1.) ha determinato una crescente domanda di materiale vivaistico forestale di alta qualità.
Per garantire la qualità dei semenzali prodotti, i responsabili dei vivai devono ottemperare a due principi fondamentali:

- Scegliere accuratamente l'origine del materiale, molto importante sul piano della qualità, vitalità e futura performance in pieno campo. Per garantire maggiormente quest'ultimo punto si utilizza materiale di provenienza locale in quanto già climaticamente adatto e predisposto alla resistenza nei confronti di determinate patologie o insetti presenti nell'area.
- Evitare l'introduzione e movimentazione di materiale non di origine locale che può portare ad un inquinamento degli ecotipi endemici e all'insediamento di patogeni assenti in precedenza. Per questo motivo sono state emanate direttive a livello nazionale per il controllo del materiale in ingresso, con periodi di stazionamento e controlli fitosanitari.

Ad oggi in vivaio la sughera viene tradizionalmente allevata a terra, direttamente da seme o per via agamica. Non viene utilizzato se non di rado l'allevamento in contenitore per evitare lo stress derivante dal taglio del fittone (necessario per via dell'elevata crescita) alle piccole piante. Non subiscono alcun tipo di cura culturale, ma vengono semplicemente irrigati.
Lo stazionamento minimo in vivaio è di 3 anni, al termine dei quali i semenzali possono essere utilizzati per molti scopi (ornamentale, rimboschimenti..).
Quando si coltivano semenzali a scopo di rimboschimento, a volte si opta per l'utilizzo di teli ombreggianti che verranno poi rimossi prima dell'impianto per poter dare alle piante la possibilità di adattamento a luce diretta.
In alcuni casi si attua l'innesto su leccio (*Quercus ilex L.*) ma tale pratica è sconsigliabile in quanto non in grado di fornire buoni risultati come le piante ottenute da seme.

1.5.2. Propagazione in ambiente controllato

In concomitanza con la ripresa della produzione vivaistica tradizionale, nell'ultimo decennio si è assistito allo sviluppo di innovative camere di crescita basate sull'utilizzo di fonti luminose artificiali, per la produzione indoor di specie forestali.

Il dipartimento DAFNE dell'Università degli Studi della Tuscia è uno dei centri di ricerca più attivi in tale settore.

Nel corso dell'ultimo decennio è stato infatti coordinatore di una serie di progetti basati sulla propagazione di specie forestali sotto lampade artificiali.

Il primo progetto finanziato dalla comunità europea è stato il progetto Filas, seguito dai progetti europei Regen Forest e Zephyr .

Produrre piante forestali in ambiente controllato presenta molti vantaggi ed alcuni svantaggi, seppur di poco conto.

I vantaggi possono riassumersi in 8 punti:

- Possibilità di coltivare tutto l'anno
- Possibilità di coltivare specie non adatte al clima locale
- Riduzione dei consumi di carbonio per il trasporto dei prodotti (produzione locale)
- Utilizzazione più efficiente delle risorse per la crescita delle piante (es. grazie all'utilizzo di fonti luminose a basso consumo come i LED, sistemi di riciclaggio dell'acqua ed eventualmente pannelli fotovoltaici per l'alimentazione elettrica)
- Facile gestione dei fattori ambientali (condizionamento dell'aria, che garantisce il controllo di temperatura e umidità)
- Illuminazione artificiale (LED o fluorescenza)
- No pesticidi
- Riduzione degli spazi necessari alla coltivazione, con incremento della densità di produzione grazie ad un sistema di coltivazione 3-D, basata sull'utilizzo di vassoi multiplug su scaffali a più ripiani

Mentre per quanto riguarda gli svantaggi, i principali sono rappresentati da:

- Costo attrezzatura
- Limitato spazio di crescita (altezza)
- rimozione del fittone (nelle specie che lo presentano)

Ogni specie ha esigenze di crescita differenti, in termini di substrato, temperatura, umidità dell'aria e del suolo. I parametri vanno dunque settati in funzione delle specie da coltivare. Nelle celle di ultima generazione, i semenzali sono esposti ad un'illuminazione LED con intensità variabile, in base alle necessità della specie, dai 10 ai 300 PAR. Tale intensità, a confronto con i circa 2000 PAR caratteristici della luce solare diretta in un giorno sereno, risulta essere molto bassa. Eppure il corretto bilanciamento delle lunghezze d'onda delle lampade utilizzate, che mostrano alte percentuali di luce blu e rossa con funzione stimolante la fotosintesi a discapito delle altre lunghezze della regione del visibile, consente un rapido e ottimale sviluppo dei semenzali anche a tali valori. Si ottiene dunque un doppio vantaggio, un forte risparmio energetico in virtù del basso consumo delle lampade e un più rapido accrescimento rispetto alle condizioni naturali.

La crescita dei semenzali avviene in contenitori ad alta densità (anche oltre le 100 celle per vassoio) così da poter ottenere un notevole numero di semenzali per ciclo di crescita.
Il volume di suolo assai limitato fa sì che il tempo di coltivazione sotto lampade, prima che le piante inizino a mostrare segni di stress, vari tra i 30 e i 60 giorni a partire dalla semina, in base alla specie. Specie quali la quercia da sughero, raggiungono tale stato limite in un tempo decisamente anticipato rispetto a specie dal seme piccolo quali ad esempio il *Myrtus communis* o *Punica granatum*.

Raggiunto tale stadio le piante vengono trasferite in vassoi più grandi in serra o, a seconda della frugalità della specie, a luce diretta. In tal modo si garantisce uno sviluppo radicale adeguato per il successivo trapianto in campo aperto.
Numerose sono le specie forestali la cui crescita è stata già favorevolmente testata in ambiente controllato. Tra queste annoveriamo faggio, leccio, acero, sughera, platano.

1.5.2.1. Definizione dei protocolli di crescita per singola specie

Per poter definire i protocolli di crescita di una qualsiasi specie vegetale si necessita di accurati studi di campo, svolti in più aree laddove la specie mostri una autonoma capacità rigenerativa, così da poter acquisire un quantitativo di dati sufficiente da definire per ogni parametro ambientale da riprodursi in cella, un protocollo adeguato agli standard naturali.

I fattori principalmente analizzati sono: Temperatura, tipologia di suolo, Umidità dell'aria e del suolo, grado di precipitazioni annuali ed eventuali periodi di siccità nonché le condizioni luminose.

Figura 3: Prototipo del progetto Zephyr per la propagazione sotto luci LED di specie forestali

Un'importante considerazione da farsi in relazione alle specie forestali, è che le condizioni ottimali di crescita possono variare in funzione della fase di sviluppo della pianta e che dunque condizioni che appaiono ottimali per una pianta adulta possono non corrispondere a quelle necessarie nelle prime fasi di germinazione e accrescimento delle plantule.

Ad esempio, una ghianda di sughera contiene una grande quantità di riserve energetiche che garantiscono al germoglio di potersi accrescere anche in assenza di attivi processi fotosintetici, finchè siano presenti i cotiledoni. Tali processi saranno alla base della sopravvivenza del semenzale dopo la caduta dei cotiledoni. Ne deriva che, a partire da tale step, la luce giocherà un ruolo fondamentale nello sviluppo della pianta, ruolo pressocchè assente nelle prime fasi.

Dal momento che la produzione di semenzali a scopo di rimboschimento va effettuata possibilmente a partire da seme, è dunque importante analizzare quali condizioni siano necessarie alla germinazione e alle prime fasi di sviluppo delle singole specie forestali e a partire da queste strutturare dei protocolli di crescita indoor.

2. Obiettivo della tesi

Definire ottimali condizioni di substrato, luce (qualità, quantità e fotoperiodo), temperatura e umidità per la coltivazione della quercia da sughero in ambiente controllato sotto luci artificiali, sulla base di studi di campo.

3. Materiali e metodi

3.1. Studi di campo

3.1.1. Sughereta riserva

Nel comune di Tuscania (VT) è presente una riserva naturale. Costituita nel 1997, si estende per 1901 ha compresi tutti nel comune di Tuscania, con altitudine che varia dai 40 m s.l.m. fino a 224 m s.l.m.. All'interno della riserva si trovano due zone SIC (zone speciali di conservazione) che sono il "Corso del fiume Marta" e la "Sughereta di Tuscania". Sono presenti numerose specie animali (cinghiale, moscardino, istrice, volpi) e vegetali (corniolo, sughera, biancospino, cerro, leccio, rosa selvatica..).
Il nostro interesse si è focalizzato sull'area della sughereta, che si estende per circa 40 ha nella zona nord-est della riserva. Si tratta di una piantagione oggi naturalizzata di *Quercus suber L.*, attualmente allo stato maturo (adulto), caratterizzata da un ricco sottobosco, con abbondanza di ginestra odorosa (*Spartium junceum* L.), ligustro comune (*Ligustrum vulgare* L.), orchidee selvatiche, asparago selvatico. Anche la fauna risulta rilevante. La specie predominante è rappresentata dal cinghiale (*Sus scrofa* Linneaus), molto dannoso per il sottobosco e per la rinnovazione della sughera, in quanto in forte aumento numerico per l'assenza di predatori e di adeguate campagne di abbattimento.
Attualmente la sughereta non è gestita, pertanto presenta una forte densità dovuta all'assenza di diradamenti negli ultimi 10-15 anni. Tale condizione ha determinato un'alta competizione, con riduzione della rinnovazione, affermazione del novellame e indebolimento delle piante adulte con conseguente facilitazione dell'attecchimento di patogeni, quali Hypoxylon mediterraneum, agente del cancro carbonioso.

Laddove in seguito a schianti si siano aperte spontaneamente delle buche, il sottobosco ha avuto modo di espandersi e il novellame di affermarsi grazie alla disponibilità di luce. Si nota con facilità che il problema è scaturito dalla chiusura delle chiome andando ad analizzare quelle aree dove si sono aperte buche dovute a schianti di alberi adulti, in quanto il sottobosco li è presente e anche molto affermato per via della ricezione della luce.

(www.areeprotette.vt.it)

3.1.2. Analisi dati meteorologici

Nel periodo compreso tra Novembre 2014 e Settembre 2015, corrispondente ad una stagione vegetativa della specie, sono stati raccolti i dati meteorologici utili per lo studio delle condizioni ottimali per la rinnovazione e la crescita della quercia da sughero, avvalendosi dei siti internet www.meteo.it e www.ilmeteo.it. Nello specifico, la stazione meteorologica di riferimento selezionata, è stata quella di Tuscania, sita a 166 metri s.l.m. con coordinate geografiche 42°25'N e 11°52'E.

I parametri considerati sono stati i seguenti:

- Condizioni meteorologiche giornaliere
- Temperature (minima, massima e media giornaliere)
- Umidità (media giornaliera)
- Precipitazioni (giornaliere)
- Fotoperiodo giornaliero

Per condizioni meteorologiche si intende il numero di ore di sole e di nuvolosità giornaliere. A tal scopo le ore di luce del giorno sono state suddivise in due periodi: uno compreso tra le ore 6 e ore 12 e uno dalle ore 12 alle ore 18. Ad ogni periodo è stato assegnato un valore categorico, sulla base delle condizioni meteorologiche:

- 0, cielo sereno;
- 1, cielo nuvoloso;
- 2, cielo nuvoloso con pioggia.

Ad ogni giorno è stato dunque assegnato un valore complessivo tra 0 (giornata di sole continuo dalle 6 alle 18) a 4 (giornata di pioggia continua dalle 6 alle 18). A partire da tali valori giornalieri, è stata calcolata una somma mensile, indicativa della percentuale di copertura mensile nel corso delle ore di luce.

Il grado di umidità fornito dalla stazione meteorologica ovviamente si riferisce ad un ambiente aperto. Esso sarà dunque paragonabile all'umidità a livello delle chiome degli alberi, ma inferiore al valore rilevabile a livello di sottobosco.
Grazie all'ausilio del sito www.ilmeteo.it, sono stati raccolti giornalmente i valori di umidità relativa nelle fasce orarie 6-12 e 12-18. A partire da tali due valori è stata ricavata la media del valore di umidità relativo alle ore di luce del giorno.
Tali valori medi giornalieri sono stati alla base del calcolo delle medie mensili.

In riferimento alle precipitazioni sono stati raccolti dati giornalieri al fine di calcolare i millimetri di pioggia totali caduti nell'arco di ogni singolo mese nonché la media delle precipitazioni giornaliere per singolo mese, così da identificare eventuali periodi di stress idrico per la specie. I dati sono stati raccolti tramite il sito www.idrografico.roma.it.annali.it.
Il fotoperiodo è stato calcolato tramite il sito www.eurometeo.com , che fornisce dati basati su coordinate satellitari del punto scelto, garantendo così una precisione maggiore del dato. Sono stati registrati gli orari precisi di alba e tramonto e quindi calcolato il fotoperiodo come differenza tra l'orario del tramonto e dell'alba.

Per le temperature si sono registrate giornalmente i valore minimo, massimo e medio. A partire dai dati giornalieri sono state calcolate le medie termiche mensili.

3.1.3. Deposizione gabbiette

All'inizio di Dicembre 2014, sono state definite 6 aree di saggio, in seguito al periodo di massima caduta delle ghiande.

Ogni area di saggio, di dimensioni 50*50 cm, è stata protetta dalla predazione dei cinghiali e roditori, con una gabbia metallica di 50*50*10 cm con maglie della rete metallica di 1 cm di lato. Le gabbie sono state fissate al suolo mediante utilizzo di 4 paletti metallici lunghi 70 cm, posizionati ai 4 vertici del quadrato.

Figura 4: Gabbiette di protezione delle aree di saggio

Il posizionamento delle gabbiette è avvenuto rispettando la naturale distribuzione delle ghiande cadute al suolo e scegliendo punti che non potessero ricevere alcun disturbo antropico, quindi lontani (o ai margini) di sentieri e strade.

Le aree sono state definite come segue:

- G1-G2-G3 con esposizione al sole alle ore 12:00 in un giorno sereno.
- G4-G5-G6 con esposizione all'ombra alle ore 12 in un giorno sereno.

A ciascuna gabbietta è stato associato un punto GPS, mediante utilizzo di un GPS Oregon® 550t.

3.1.4. Analisi luce

Al momento della messa a dimora delle gabbiette è stato effettuato, in corrispondenza di ciascuna di esse un rilievo quali-quantitativo dello spettro solare (180-1100 nm), mediante utilizzo di uno spettroradiometro Stellarnet®. Nello specifico, lo spettro è stato acquisito in corrispondenza di 5 punti per gabbietta. Sono state difatti costruite due diagonali orientate verso i 4 punti cardinali. La fibra ottica dello spettroradiometro è stata quindi posizionata in corrispondenza del baricentro e a metà della distanza tra i 4 vertici e il baricentro.

Figura 5: Identificazione dei punti di acquisizione degli spettri luminosi per gabbietta

Gli spettri luminosi sono stati rilevati alle ore 9-10-12 e14 , così da poter ricostruire l’andamento dello stato di illuminazione delle 6 are nel corso delle ore centrali di sole della giornata.

Ogni misura spettrale effettuata in ognuno dei 5 punti è stata ripetuta 3 volte nell’arco di 1minuto.

Il totale degli spettri collezionati nei 5 punti ad ognuno degli orari sopra definiti, è servito per calcolare una media di illuminazione della gabbietta.

Come controllo, sono stati rilevati uno spettro solare alle ore 12 in un punto senza copertura vegetativa (controllo positivo) e in un punto in ombra profonda (controllo negativo).

3.1.5. Analisi dei semenzali

3.1.5.1. Percentuale di germinazione ed emergenza

Ad inizio primavera (21/3/2015) è stato effettuato un sopralluogo accurato per ogni singola gabbietta, andando a rimuovere la copertura metallica, per valutare la percentuale di germinazione al termine dei mesi invernali.
E’ stato contato il numero totale di ghiande presenti nell’area di saggio e successivamente sono state suddivise in 3 distinte categorie: non germinate, germinate e plantule.
Sono state considerate germinate le ghiande con radichetta di lunghezza minima pari a 0.5cm; plantule quelle con apice caulinare di lunghezza pari minimo a 1.5cm.
Il numero di plantule è servito a valutare la percentuale di emergenza.

Per poter rimuovere la ghianda con tutta la radice senza andarla a danneggiare o rompere e poterla poi riporre nella sua sede naturale per consentirle di continuare la crescita è stato necessario l'utilizzo di piccole palette.
Le ghiande danneggiate (per cause fisiche o biologiche) sono state considerate non germinate.

3.1.5.2. Misure dei semenzali

L'accrescimento dei semenzali all'interno delle gabbiette è stato monitorato mediante analisi dei seguenti parametri, con cadenza mensile, da Marzo a Giugno 2015, ovvero al raggiungimento della prima chiusura annuale della gemma apicale:

- Altezza (espressa in cm)
- Diametro (espresso in mm, mediante utilizzo di calibro digitale)
- Numero di foglie
- Quantità di clorofilla (espressa in unità SPAD, mediante SPAD METER Konica Minolta®)

Tramite i dati ottenuti si sono potute costruire le curve di crescita dei semenzali presenti in ogni area di saggio, per poter valutare l'eventuale incidenza del grado di esposizione luminosa sull'accrescimento dei semenzali.

3.2. Analisi statistiche

Al fine di valutare il legame tra condizioni luminose e accrescimento dei semenzali, le 6 gabbiette sono state suddivise in due gruppi, definiti "AREE DI LUCE" e "AREE DI OMBRA". I valori medi relativi ai parametri descritti nel par. 3.1.5.2. sono stati quindi confrontati tra di loro mediante applicazione del test t di Student, dopo aver applicato lo Shapiro-wilks test al fine di valutare la normalità delle distribuzioni e la derivante possibilità di applicare il t-test.

3.3. Definizione del protocollo di crescita indoor

A partire dai dati metereologici raccolti (vedi par. sopra) sono stati ricavati i valori medi di temperatura, umidità, fotoperiodo e precipitazioni, relativi alla stazione di Tuscania, per il periodo compreso tra Marzo e Giugno 2015 (dalla germinazione ed emergenza alla chiusura della gemma apicale). Tali valori saranno alla base della definizione di un idoneo protocollo di coltivazione in ambiente controllato di querce da sughero (periodo previsto per l'inizio dei test: Novembre 2015).

4. Risultati

4.1. Analisi dei dati meteorologici

4.1.1. Temperatura

Dall'analisi delle temperature nel periodo compreso tra Novembre 2014 e Luglio 2015 in relazione alla stazione di Tuscania, Febbraio è risultato essere il mese più freddo, con temperatura minima media mensile pari a 3.39°C mentre il mese più caldo è risultato essere Luglio con temperatura massima media mensile pari a 31°C.

La temperatura minima assoluta, registrata nei mesi di dicembre, gennaio e Febbraio , è stata di -2°C.

La temperatura massima assoluta, registrata nel mese di Luglio è stata pari a 36°C.

		Media		
		T° min	T°max	T media
2014	novembre	9,76	17,3	13,8
	dicembre	5,58	11,61	8,74
2015	gennaio	3,51	10,9	7,38
	febbraio	3,39	10,64	7,46
	marzo	5,54	13,38	10,19
	aprile	7,53	17,6	13,83
	maggio	11,96	23,03	19,12
	giugno	16,7	27,7	23,93
	luglio	20,87	33,67	29,22
	agosto	19,45	31,06	26,58

Tabella 3: Tmax, Tmin, T media mensili del periodo d'analisi (Novembre 2014-Agosto 2015)

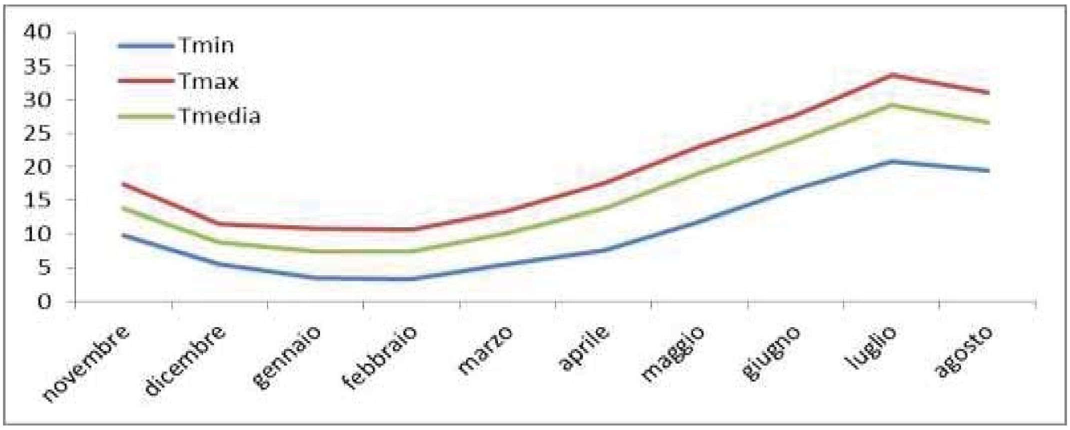


Grafico 1: Tmax, Tmin, T media mensili del periodo d'analisi (Novembre 2014-Agosto 2015)

4.1.2. Precipitazioni

Analizzando il grafico relativo alle precipitazioni cumulate mensili, l'intervallo compreso tra Novembre e Marzo, è risultato essere il periodo più piovoso, con picchi di 300 mm di pioggia cumulata mensile per Novembre e 160 per Marzo. Nei mesi successivi le precipitazioni hanno subito una rarefazione con un picco minimo a maggio, pari a 18 millimetri cumulati in un mese. Il mese di Luglio, che in termini termici è risultato essere il più caldo, è però stato caratterizzato da un valore cumulato di pioggia pari a circa 50 millimetri.

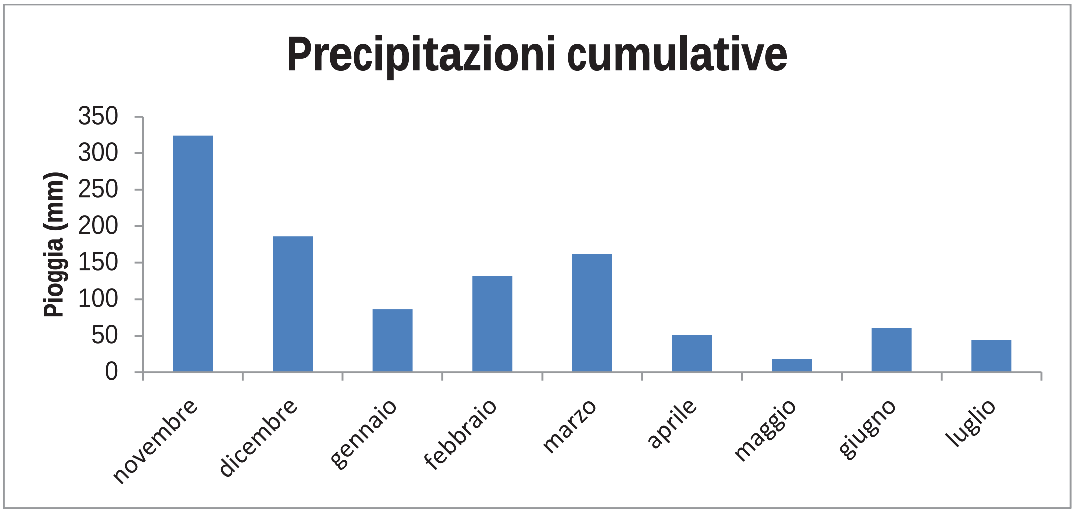


Grafico 2: Precipitazioni cumulative del periodo d'analisi (Novembre 2014-Luglio 2015)

4.1.3. Condizioni meteorologiche giornaliere

In termini di copertura, il valore mensile più alto si è rilevato per il mese di Novembre, così come in termini di precipitazioni. Per il periodo compreso tra Dicembre e Giugno è stato rilevato un grado di copertura mensile costante, di conseguenza non collimante con l'andamento delle precipitazioni. Il mese di Luglio ha invece mostrato il minimo di copertura, associata a temperature elevate e piogge scarse.

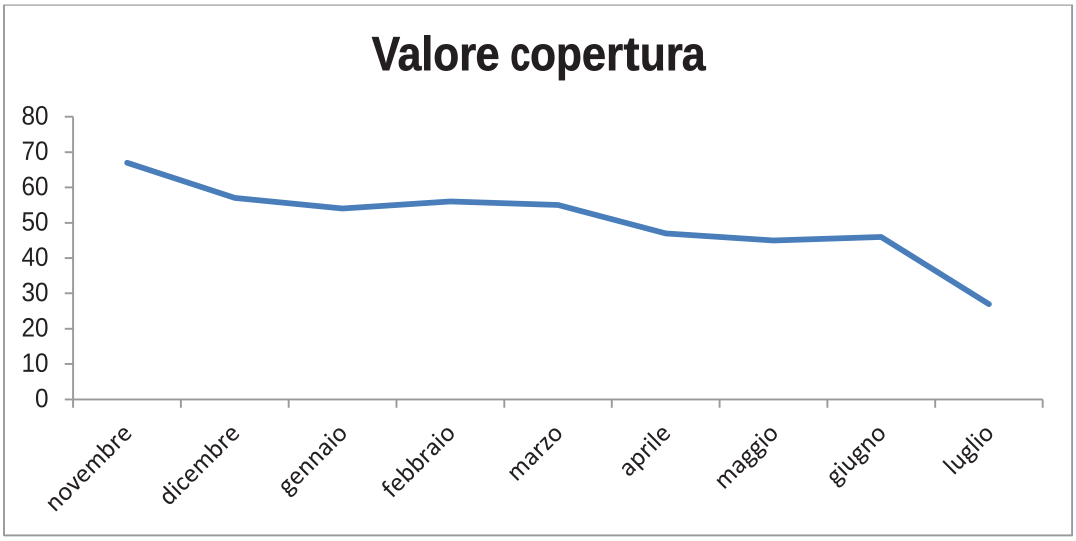


Grafico 3: Valore percentuale di copertura media mensile del periodo d'analisi (Novembre 2014-Luglio 2015)

4.1.4. Umidità

I valori di umidità media mensile sono risultati più elevati nel periodo invernale – primaverile, ovvero dal mese di Novembre al mese di Aprile, all'interno di un range 60-80%, per poi subire una rapida discesa nel periodo compreso tra Maggio e Luglio, arrivando a valori minimi pari a 47% (Luglio).

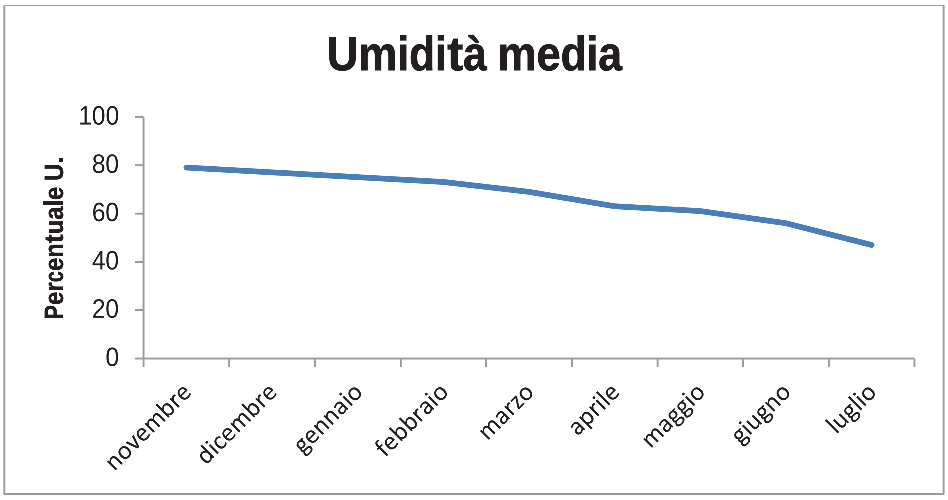


Grafico 4: Percentuale di umidità media mensile nel periodo d'analisi (Novembre 2014-Luglio 2015)

4.1.5. Fotoperiodo

Nel periodo compreso tra Novembre 2014 e Luglio 2015, il fotoperiodo ha mostrato un primo decremento da 10h 24' (1 Novembre 2014) a 9h 04' (21-25 Dicembre 2014), seguito da un allungamento del giorno iniziato il 26 Dicembre 2015 e proseguito fino al 25 Giugno 2015 (15h 18'). A partire da tale, l'accorciamento progressivo dei giorni ha portato a una diminuzione del fotoperiodo fino a 14h 29' al termine del periodo in analisi (31 luglio 2015).

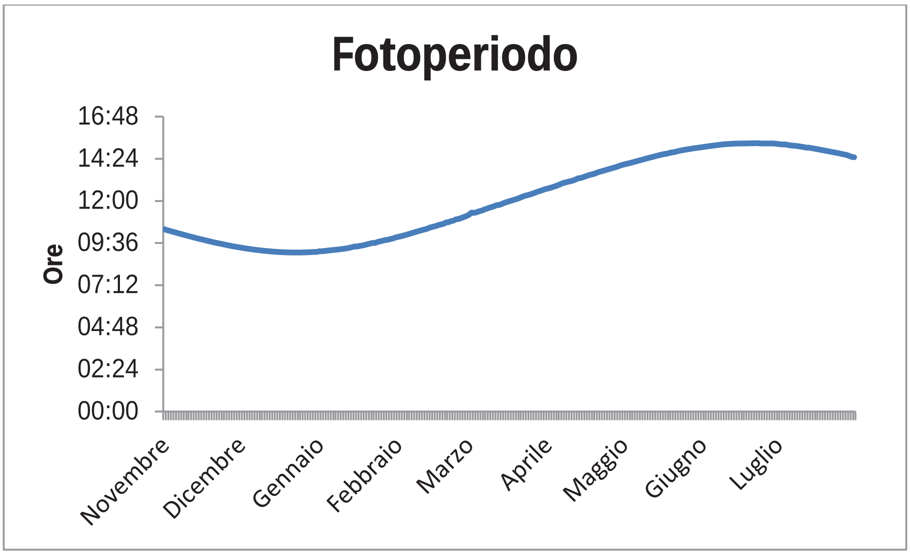


Grafico 5: Analisi del fotoperiodo relativo al periodo d'analisi (Novembre 2014-Luglio 2015)

4.2. Analisi della luce

I grafici degli spettri luminosi relativi alle singole gabbiette hanno mostrato profonde differenze quali-quantitative tra aree di saggio esposte alla luce e aree in ombra. In condizioni di ombra, l'intensità luminosa può difatti essere pari a 1/10 dell'intensità presente in aree assolate.

In termini qualitativi, le lunghezze d'onda maggiormente abbattute in condizioni di ombra, sono risultate essere quelle del blu, del rosso e dell'infrarosso, come effetto dell'assorbimento delle suddette da parte della copertura delle piante madri.

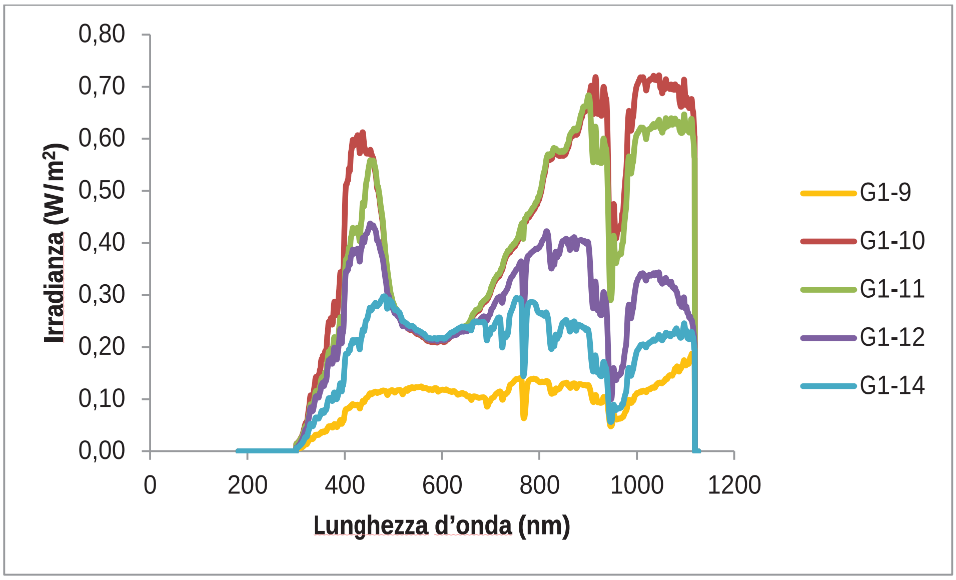


Grafico 6: Spettri luminosi raccolti in corrispondenza della gabbietta G1 alle ore 9-10-11-12-14

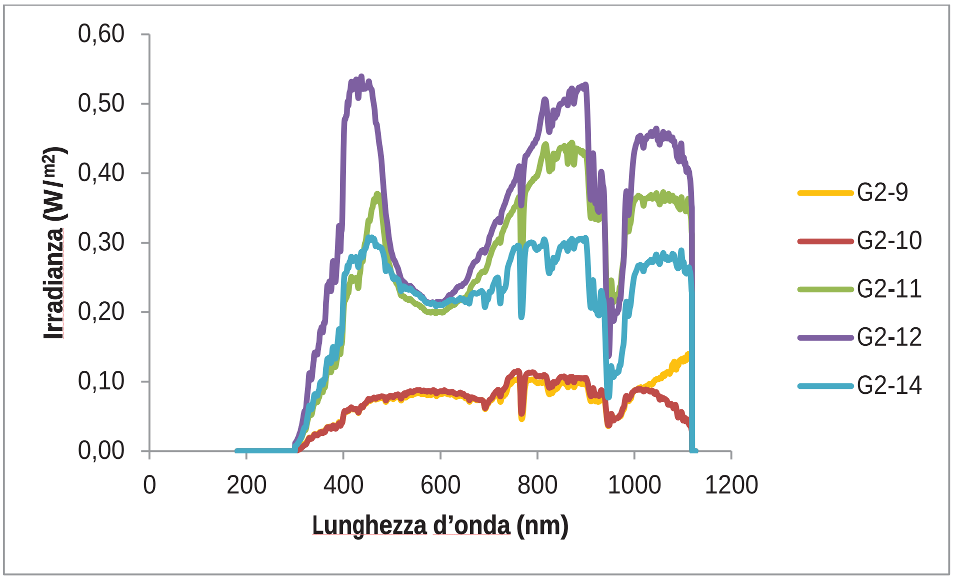
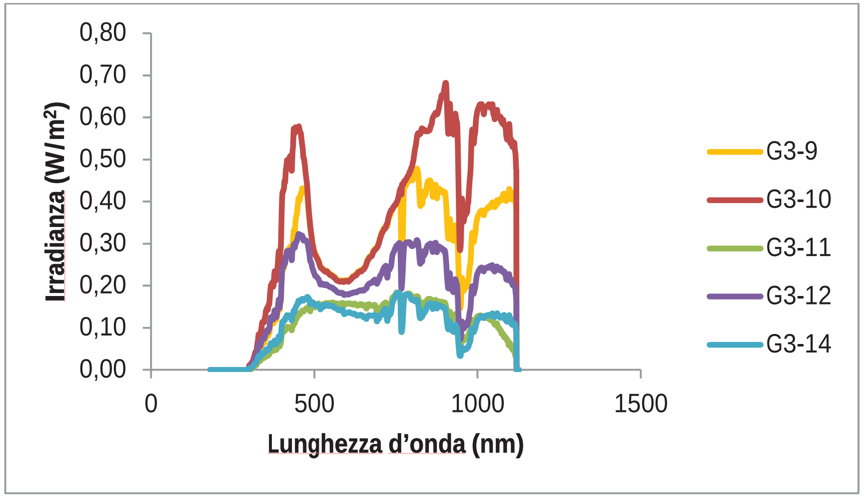


Grafico 7: Spettri luminosi raccolti in corrispondenza della gabbietta G2 alle ore 9-10-11-12-14



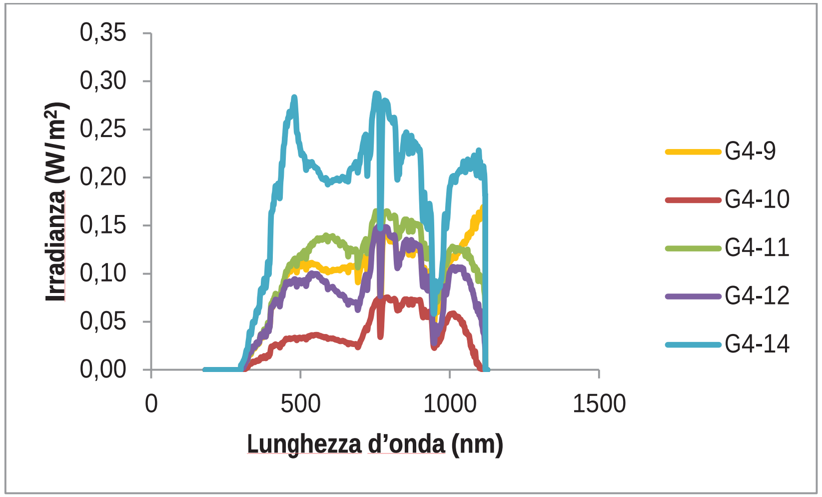
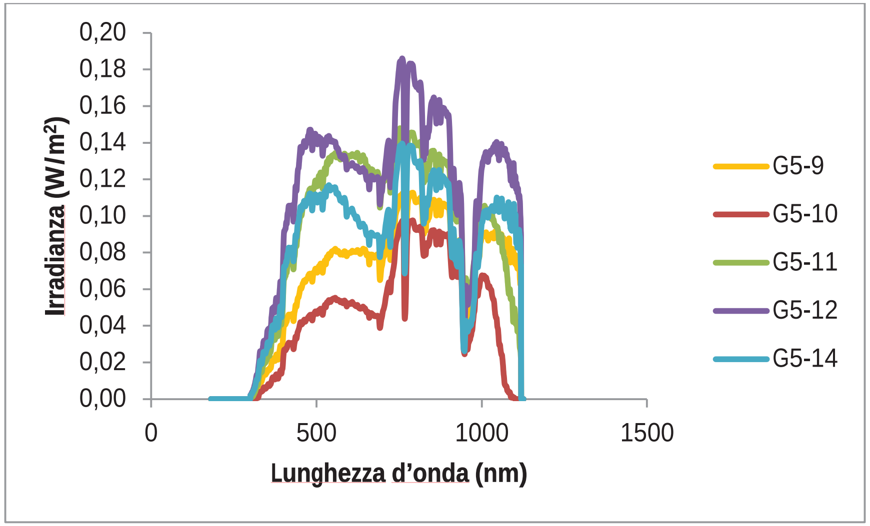


Grafico 8: Spettri luminosi raccolti in corrispondenza della gabbietta G3 9-10-11-12-14

Grafico 9: Spettri luminosi raccolti in corrispondenza della gabbietta G4 alle ore alle ore 9-10-11-12-14



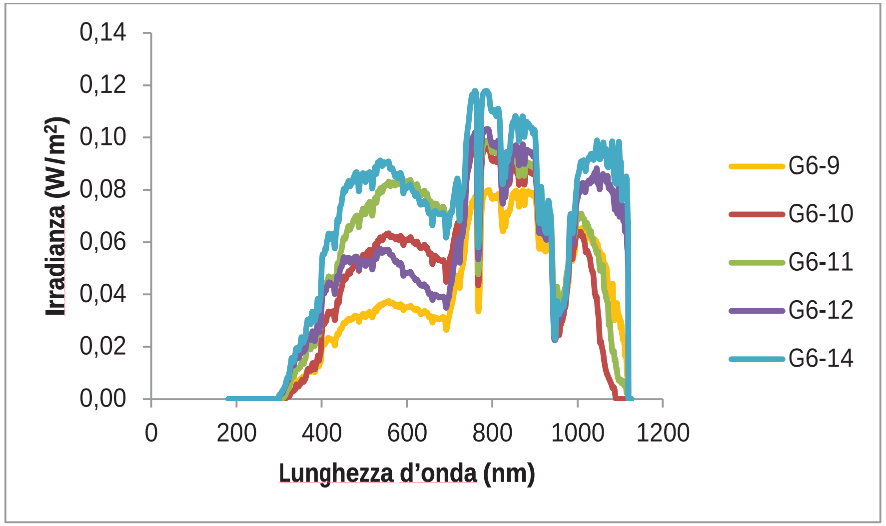


Grafico 10: Spettri luminosi raccolti in corrispondenza della gabbietta G5 9-10-11-12-14

Grafico 11: Spettri luminosi raccolti in corrispondenza della gabbietta G6 alle ore alle ore 9-10-11-12-14

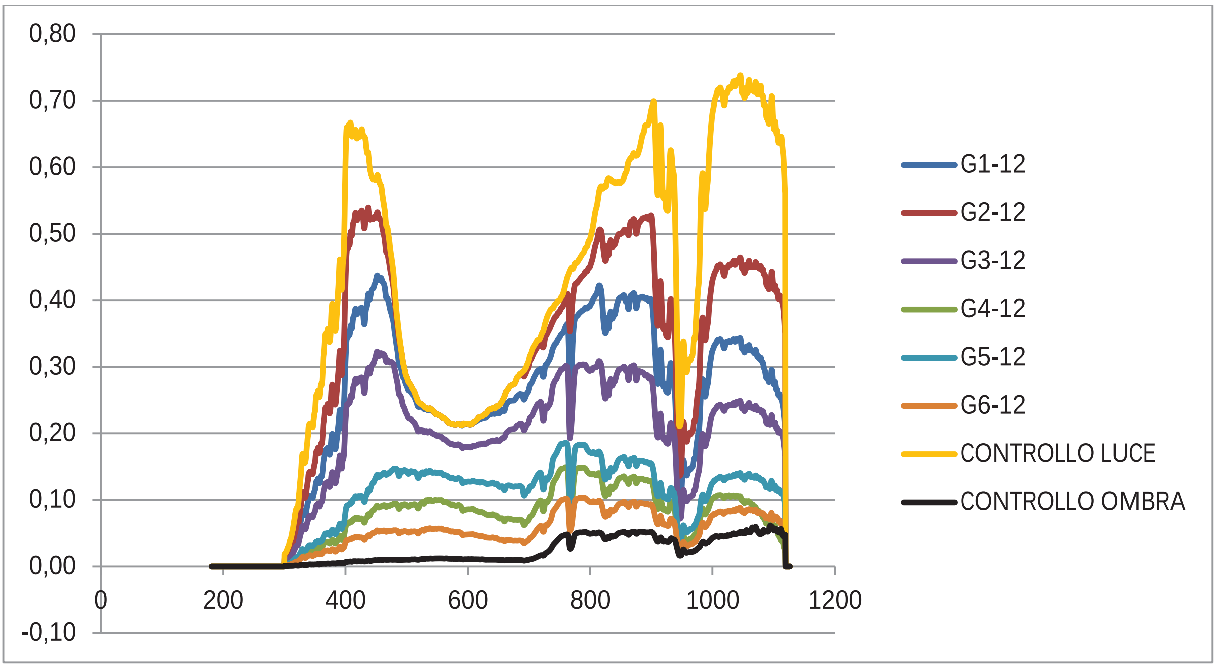


Grafico 12: Riepilogo spettri luminosi raccolti alle ore 12

4.3. Analisi dei semenzali

4.3.1. Analisi della germinazione e dell'emergenza

La differenza tra la percentuale di germinazione in aree di saggio esposte alla luce e aree in ombra è risultata statisticamente significativa, con un valore medio pari circa al 70% alla luce e 50% in ombra.
Per l'emergenza si è riscontrato un comportamento analogo alla fase di germinazione, con un valore medio di circa 20% alla luce e di circa 10% in ombra.

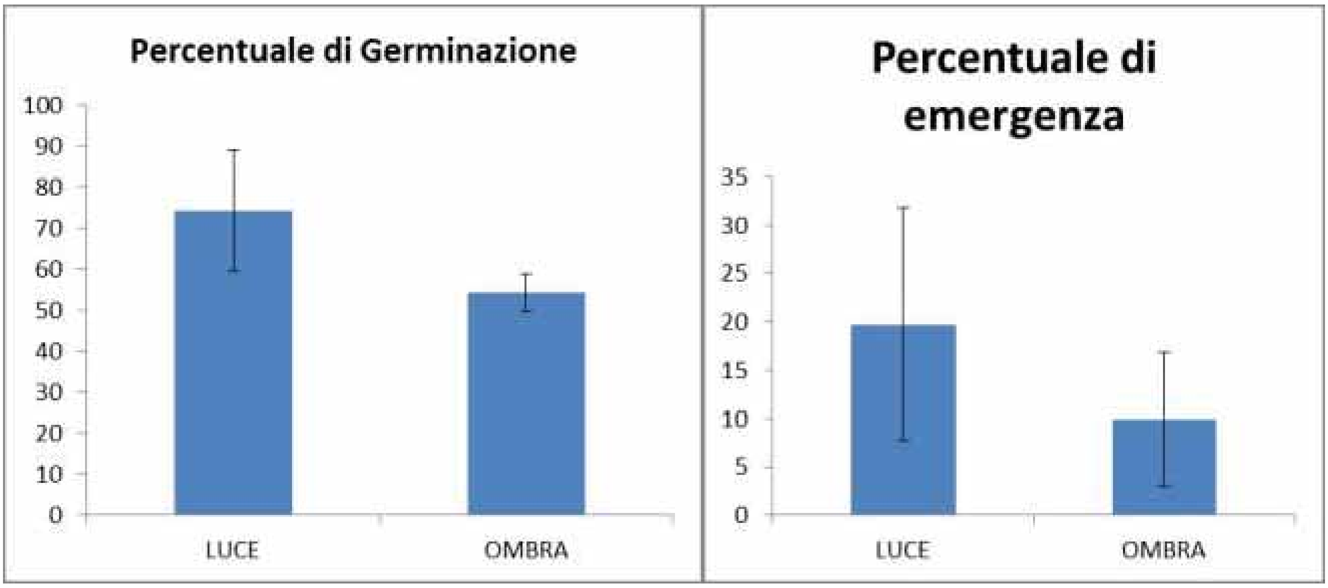


Grafico 13: Percentuale di germinazione ed emergenza delle ghiande di *Q.suber* L. in condizioni di luce e ombra in bosco

4.3.2.Misure dei semenzali

4.3.2.1. Altezza

I risultati hanno evidenziato che le plantule con esposizione alla luce abbiano un accrescimento più veloce e più elevato raggiungendo il valore massimo di altezza (circa 12 cm), in corrispondenza della fase di chiusura della gemma apicale, nel mese di Luglio. Al contrario, le plantule in ombra hanno mostrato un accrescimento minore, raggiungendo un'altezza massima di circa 6 cm, con una chiusura anticipata della gemma apicale verificatasi a maggio.

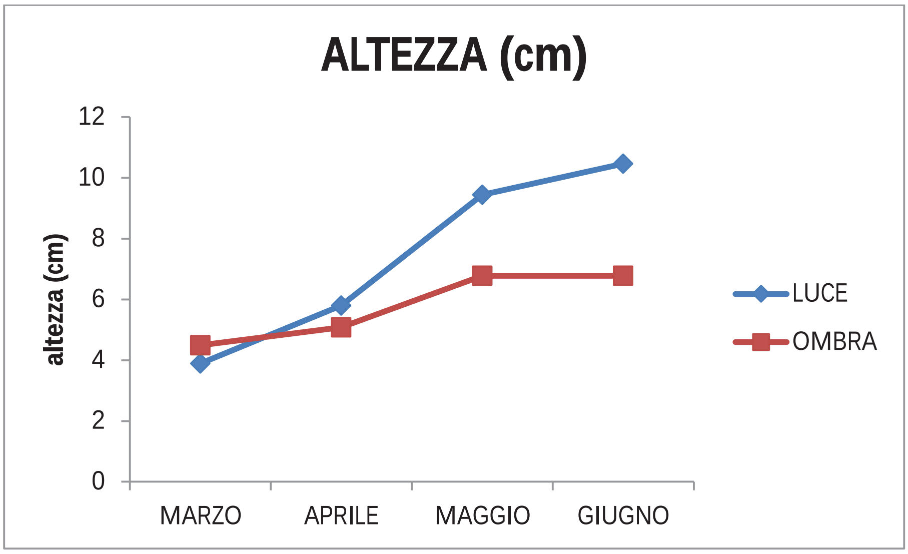


Grafico 14: Curve di crescita (parametro in analisi: altezza) dei semenzali di *Q. suber* L. nel periodo Marzo 2015-Giugno 2015 in condizioni di luce e ombra in bosco

4.3.2.2. Diametro

In corrispondenza della chiusura della gemma apicale sia le plantule esposte alla luce sia quelle in ombra, mostrano differenze non significative in termini di diametro al colletto, con valori medi attorno a 1.5 mm.

Tale valore è però raggiunto con velocità differenti nel corso dei mesi precedenti. Le plantule in ombra mostrano difatti un inspessimento più rapido a discapito dell'accrescimento in altezza.

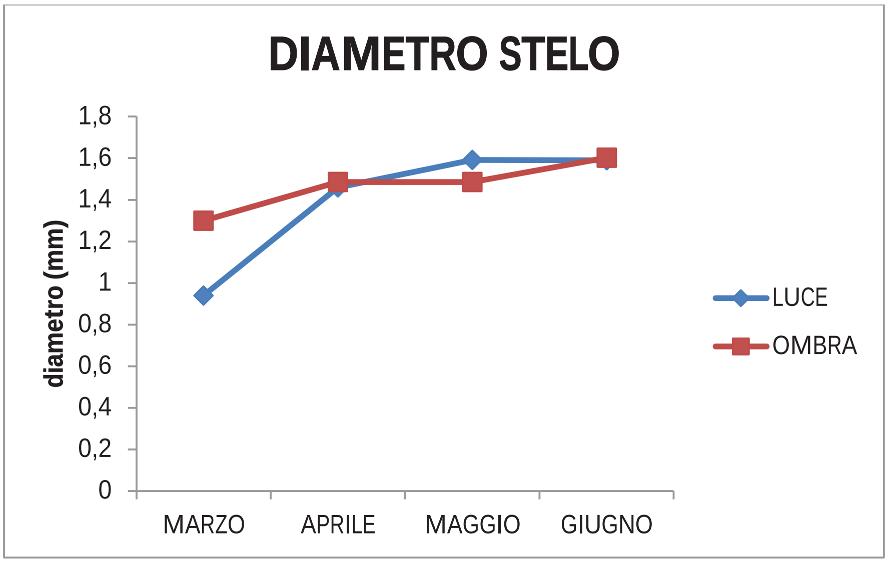


Grafico 15: Curve di crescita (parametro in analisi: diametro a livello del colletto) dei semenzali di *Q. suber* L. nel periodo Marzo 2015-Giugno 2015 in condizioni di luce e ombra in bosco

4.3.2.3. Numero di foglie

Tra aree di luce e di ombra è stata registrata una significativa differenza in termini di numero di foglie. Partendo nel mese di Marzo (periodo dell'emergenza) da un valore comune di 2-4 foglie, le plantule cresciute alla luce hanno raggiunto un valore medio di 10 foglie a Luglio (chiusura gemma apicale) mentre quelle cresciute in ombra hanno raggiunto un valore medio di 6 foglie a Maggio (chiusura gemma apicale).

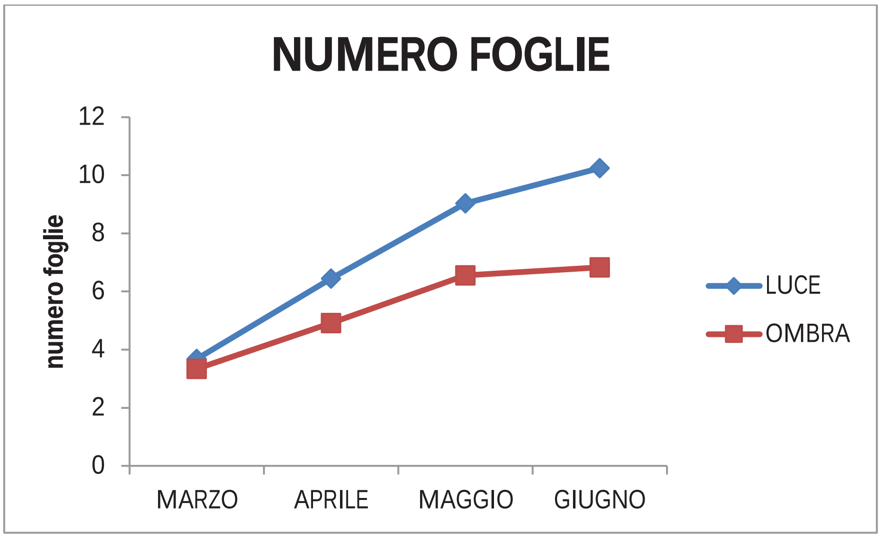


Grafico 16: Curve di crescita (parametro in analisi: numero di foglie) dei semenzali di *Q. suber* L. nel periodo Marzo 2015-Giugno 2015 in condizioni di luce e ombra in bosco

4.3.2.4. Quantità di clorofilla (espressa in unità SPAD)

Al termine della stagione vegetativa sia le plantule cresciute alla luce sia quelle in ombra hanno mostrato valori simili di SPAD, pari a circa 35 unità SPAD. Tale valore limite è stato però raggiunto con modalità diverse nel corso dei mesi precedenti. Le piante in ombra hanno mostrato fin da Marzo valori superiori di concentrazione di clorofilla, fino al mese di Maggio, quando si è assistito a un'inversione del fenomeno, con rapido incremento della clorofilla nelle plantule alla luce.

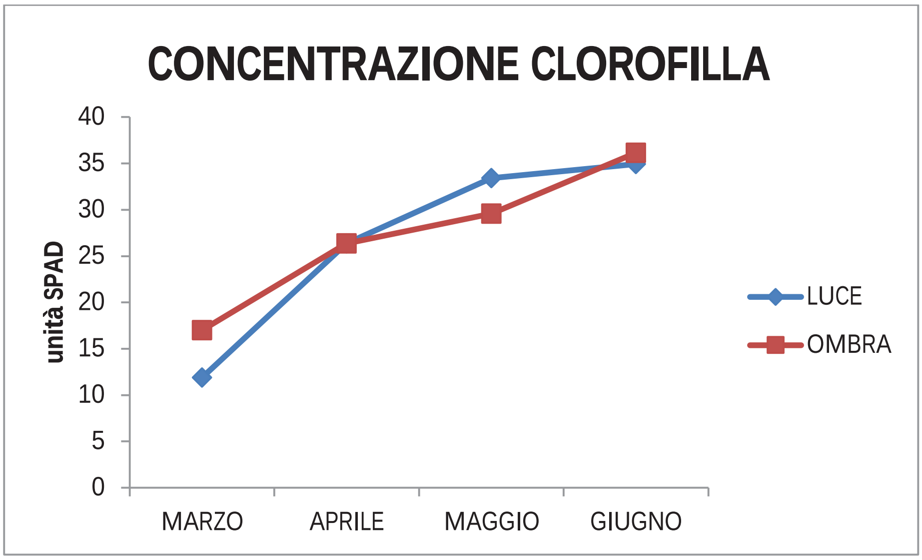


Grafico 17: Curve di crescita (parametro in analisi: quantitativo di clorofilla espressa in unità (SPAD) dei semenzali di *Q. suber* L. nel periodo Marzo 2015-Giugno 2015 in condizioni di luce e ombra in bosco.

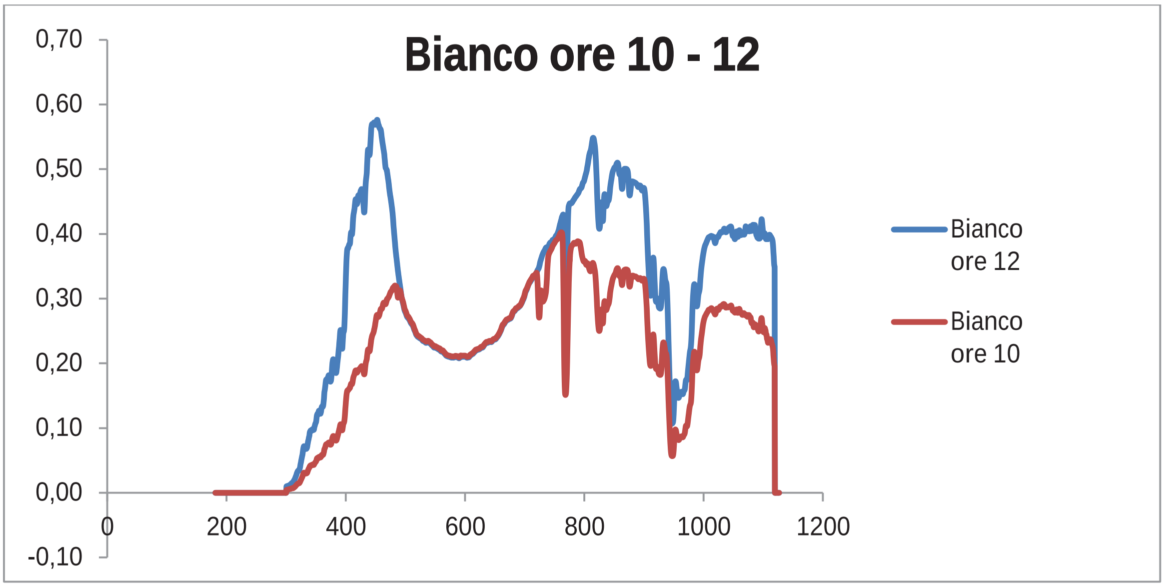


Grafico 18: Analisi spettro luminoso in pieno campo alle ore 10-12

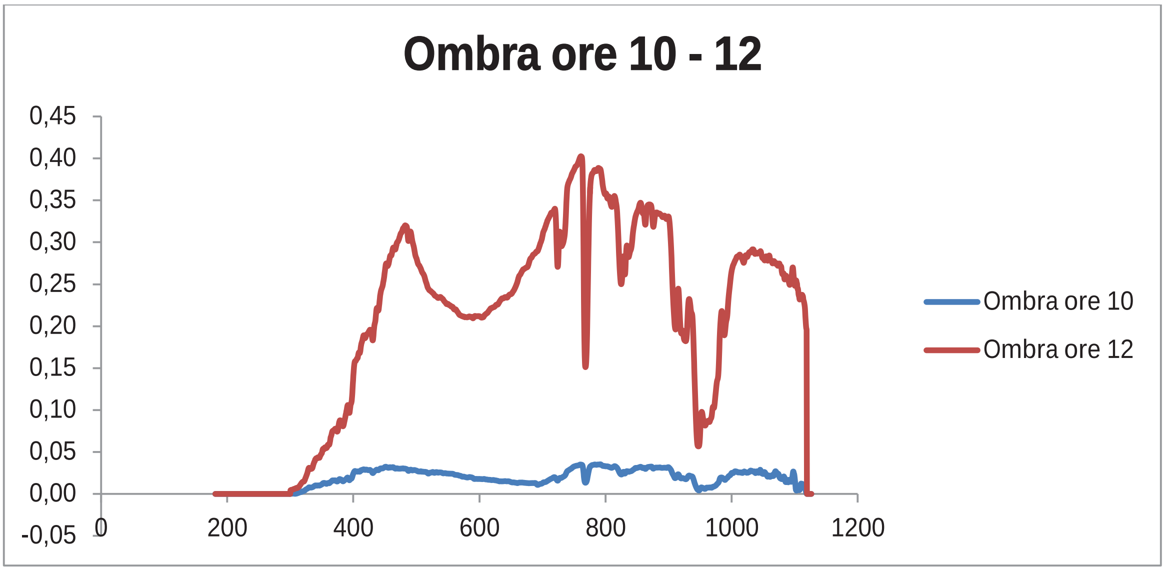


Grafico 19: Analisi spettro luminoso in ombra alle ore 10-12

4.4. Definizione del protocollo

A partire dai dati meteorologici rilevati nel corso della stagione vegetativa 2014-2015, sono stati scelti i seguenti parametri, per effettuare un test di coltivazione in ambiente controllato di *Q. suber* L.

T: 20°C (±2°C)

U. stanza: 60% (±5%)

Fotoperiodo: 12L 12 B

I valori di temperatura e umidità sono stati definiti sulla base dell'osservazione che i semenzali in bosco (esposti alla luce) abbiano mostrato il massimo accrescimento nel periodo Maggio-Giugno, prima della chiusura delle gemme. La media termica di tale periodo risulta essere pari a 21°C e la media dell'umidità pari al 60%.

Il valore del fotoperiodo è stato definito come media dei valori medi del fotoperiodo mensile da Novembre a Luglio.

5. **Discussione**

Al termine del periodo di studio effettuato sui fattori ambientali (Marzo-Luglio) si è potuto constatare come il principale, per la rinnovazione e lo sviluppo dei semenzali, risulti essere la luce, rendendo secondari gli altri, anche se indispensabili. Essa si è visto che può variare di molto a seconda delle condizioni climatiche (cielo sereno, nuvoloso, pioggia), di esposizione delle piante (piena luce o ombra), della loro copertura da parte di vegetazione affermata e dalla stagione in cui ci si trova. I grafici ottenuti dall'analisi degli spettri, fatti a diverse ore (8-9-10-12-14) in bosco, mostrano come siano nettamente inferiori rispetto a ricezioni in pieno campo, quindi senza copertura circostante. Lo spettro che riesce ad arrivare a terra, viene fortemente filtrato dalla copertura e privato maggiormente del Blu, Rosso e Infrarosso. I primi due si perdono per intercettazione da parte delle chiome, mentre per quanto riguarda l'infrarosso sappiamo che viene assorbito da qualsiasi materiale, riesce ad arrivare in forti dosi solo in aree aperte e con condizioni dell'aria perfette (ovvero assenza di foschia, nebbia ecc). Andando a confrontare i grafici degli spettri luminosi con quelli riguardanti lo sviluppo delle piante si nota come le gabbiette 4-5-6, ovvero quelle in ombra, riscontrino maggiori difficoltà nel loro sviluppo, concentrando tutto sul diametro dello stelo (per affermarsi il necessario alla sopravvivenza) e sul valore SPAD utile all'assorbimento di quella poca luce che arriva. Mentre per le 3 esposte alla luce si riscontrano accrescimenti più lenti per alcuni fattori (diametro e clorofilla) e elevate per altri (numero foglie e altezza), questo è dovuto da un abbondare di quantità luminosa che non le obbliga alla competizione per ottenerla. Le altre caratteristiche non risultano determinati allo stesso livello ma pur sempre importanti e necessariamente presenti. Tramite questi dati si sono potuti svolgere test di laboratorio per provare a riprodurre le migliori condizioni ambientali e climatiche utili all'accrescimento della specie. Tutto ciò avviene in camere di crescita dove i parametri sono controllati e basati sulla realtà in modo tale da consentire il massimo dello sviluppo ai semenzali.

6. **Conclusioni**

Grazie allo studio di campo effettuato direttamente in sughereta al fine di identificare le migliori condizioni di germinazione e sviluppo dei semenzali di *Quercus suber* L. in ambiente naturale, è stato possibile identificare i range di temperatura, umidità, fotoperiodo da riprodurre in camera di crescita per propagare artificialmente tale specie.

L'analisi delle condizioni luminose ha, in particolare, consentito di stabilire la possibilità di un utilizzo di basse intensità luminose per l'accrescimento dei semenzali nei primi mesi dopo la germinazione delle ghiande, garantendo così minori consumi energetici e di conseguenza minori costi di gestione.

Il protocollo messo a punto in tal modo, sarà testato nel corso del prossimo autunno, su ghiande appena raccolte dunque dotate di alta facoltà germinativa.

L'accrescimento sotto lampade artificiali sarà monitorato in concomitanza con lo sviluppo naturale dei semenzali in bosco, al fine di valutare l'efficacia del protocollo di propagazione indoor.