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Evaluation by optical and soil sensors of forest seedlings grown in controlled conditions under low-energy lighting (LED)

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ABSTRACT

Introduction

LED lights have a lower environmental impact than traditional lights due to a series of factors such as their wavelength specificity and narrow bandwidth, high energy-conversion efficiency, small volume, longer life, low thermal energy output. Concerning plant growth, the uses 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 study aims to 1) examine the effect of different LED light spectra on forest seedlings growth of different species, in order to define a species-specific cultivation protocols under optimal plant growth spectrum to enhance plant growth performance 2) compare direct measurements with indirect method by optical sensors system for automatic plant phenotyping 3) develop soil sensors system for automatic measurements of optimal soil water content. The plant species analysed, widely used in protective and productive planted forests, were: Scots pine Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), Beech (*Fagus sylvatica* L.), Holm oak (*Quercus ilex* L.), Pomegranate (*Punica granatum* L.), Strawberry-tree (*Arbutus unedo* L.), Firetree (*Morella faya* (Ait.) Wilbur), Sanguinho (*Frangula azorica* Tutin) and Azores laurel cherry *Prunus azorica* (Hort. ex Mouillef.).

Materials and methods

Seeds of almost all investigated species were pre-treated to removal dormancy before sowing in mini-plug container. Trays were placed in growth chambers (12-14-16 h photoperiod, 110 ±10 µmol m-2 s-1 PAR, temperature 20-30°C, humidity 55-80%). Seedlings were left to grow for 4-8 weeks under G2, AP67, AP67-3L, NS1, AP67tube LED light (Valoya) and Fluorescent light (FL). The LED lamps used in this study emitted a continuous spectrum thanks to a mixture of blue, green, red and far-red LEDs. Direct measurements of plant height, root length, shoot and root biomass were carried. In order to estimate total root length, roots were scanned and analyzed by WinRHIZO Pro V. 2007d. Non-destructive analysis was carried by measuring *Greenness* (percentage of shoot cover projected on tray ground) and *Plant height* by optical sensors. *Greenness* data were obtained by a series of images acquired by specific developed Optical sensors system (ACREO) and analysed by uEyeDualcam software. After that *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.

Plant height data, manually taken during the growth period, were used to find a relationship with plant biomass and with data from images analysed by uEyeDualcam HeightMap software (ACREO). Electronic soil sensors tested in the trays with and without seedlings helding watering for two weeks in order to evaluate the soil water content measurements efficiency of the soil. Soil water content measurement obtained by the software “Zephyr logger” (Acreo) was compared with the SWC calculated by gravimetric measurements.

Results and discussion

The best results recorded for all studied species were under AP67, AP67-3L and G2 LED light type for all morphological parameters analysed. The lowest values among all LED light type were obtained under NS1 LED type for almost all morphological parameters. Results showed a linear increment of seedling height in time for six species (*Pinus sylvestris*, *Picea abies*, *Quercus ilex*, *Fagus sylvatica* and *Punica granatum*) and for all different light types. *P. sylvestris, P. abies* and *A. unedo* showed interesting results in root length mainly under G2 LED type. *A. unedo* showed slightly higher biomass values for seedlings growth under G2 LED light type, in particular for root and leaves biomass. The lowest values among all LED light type were obtained under NS1 LED type for almost all studied morphological parameters. The low seeds germination *of Morella faya* species was detected under all different LED light types. In particular, *Morella faya* seeds did not show any germination under AP67-3L LED light type. Analysis of the total dry mass increment (g; shoot + root) showed the highest values for seedlings growth under AP67 light type (bar) among all LED light types and control light. The lowest values of total dry mass increment were measured for seedlings growth under AP67 tube. A high heterogeneity in seed germination and plant growth was observed. Concerning the optical analysis results, relation between greenness and seedling biomass showed good correlation for all species until the tray was fully covered. Instead, 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.) because internodes elongation did not occur during the consecutive emissions of new leaves, did not relate to the continuous increment of seedling biomass. This is probably due to the specie-specific characteristic. Thus, the best regression model to explain the relationship between direct biomass data and indirect measurements was based on parameters such as plant height, for broad-leaved species, and plant greenness for needle-leaved species. The main result of our study is that the relevance of relations between non-destructive parameters and forest seedlings growth is species specific.

Conclusion

- Plant growth performance with LED light is specie-specific.
- LED lights represent an efficient and valid alternative to the fluorescent light.
- The best performance for all studied species are observed with AP67, AP67-3L and G2 LED light type whereas NS1 LED type seems to not be suitable for this use.
- G2 gave some good results but due to its higher percentage of far-red/red, it can cause operators' eyes fatigue and could interfere with optical measurements such as greenness.
- Finally, AP67 and AP67-3L LED type could represent the best option for a standard cultivation protocol.

Data collected confirm that optical system (sensors and software) could represent a robust method to measure plant phenotype as alternative to the traditionally used destructive methods.

Protocol of seed germination developed during the present study and applied to *Morella faya* seeds, showed good results for the *ex-situ* plant species conservation objective.

Electronic soil sensors represent a good system to monitor the water content in the soil and when they are used in combination with LED light, and optical sensors the result is a complex system characterized by high level of cost-effectiveness coupled with a good possibility to save energy consumption and reduce pollution.

EXECUTIVE SUMMARY

1. INTRODUCTION

With impacts of climate change (Yeh, Chung 2009), a number of environmental concerns currently confront vegetation (Wang et al., 2005). These include soil salinization, drought and decrease in water quality, wind erosion, and losses of biodiversity.

Furthermore, issues such as more frequent and serious droughts, floods, and storms as well as pest and diseases are becoming more serious threats to agriculture (Yeh, Chung 2009). These threats along with shortage of food supply make people turn to indoor and urban farming (such as vertical farming) for help. With proper lighting, indoor agriculture eliminates weather-related crop failures due to droughts and floods to provide year-round crop production, which assist in supplying food in cities with surging populations and in areas of severe environmental conditions. (Yeh, Chung 2009).

Worldwide, an estimated 2 billion ha of forests are degraded (Minnemayer et al., 2011 in Stanturf et al. 2014). In addition to anthropogenic alterations of global ecosystems (Foley et al., 2005; Kareiva et al., 2007; Ellis et al., 2013), the anticipated effects of global climate change suggest the future need for restoration will be even greater (Steffen et al., 2007; Zalasiewicz et al., 2010; Stanturf et al. 2014).

Furthermore, Forests and other tree-based production systems such as agroforests have been estimated to contribute to the *livelihoods* of more than 1.6 billion people worldwide (World Bank, 2008). Indeed the role played by *forests* and trees in the lives of many people appears obvious through the many uses made of tree products, including foods, medicines, fodder, fibres and fuels, and for construction, fencing and furniture (FAO, 2010), but just how they contribute – and the varying levels of dependency of different communities on tree products and services and how these change over time – has often not been well defined (Byron and Arnold, 1997).

The vast diversity of forest products available includes not only those derived from trees, but a wide range of (often) “less visible” products from other plants, fungi, animals and insects. “Natural” forests, agroforests and other tree-based production systems not only provide such direct products, but contribute indirectly to support people’s livelihoods through the provision of a wide range of ecosystem services (FAO, 2010).

For these motive it is very important the environment protection, ecosystems preserving and biodiversity safeguarding. It can be also done through landscape Restoration intervention.

Thus, international efforts and strategies are needed toward restoring degraded ecosystems (CBD, 2010; WRI, 2012; Chazdon, 2008; Ciccarese et al., 2012; Stanturf et al. 2014; Astolfi et al. 2012).

Forest restoration (according to Italian Society for Forest Restoration) means all the active operations aimed to trigger the processes whereby optimal ecosystem functions and community composition in forests are returned to their native state.

Restoration strategies can be achieved by many techniques and tools (Stanturf et al. 2014). It is well known that vegetation restoration strategies are needed to recovery degraded areas as well as in post-fire restoration (Chazdon, 2008; Ciccarese et al., 2012). Among these strategies, abandoned farmland reforestation programs represent one of the most significant means in improving vegetation and controlling soil erosion (Wang et al., 2004).

Reforestation requires artificially regenerated forest planting stock material, since in adverse environments, planting seedlings of woody species increases the possibility of recovering ecosystem integrity in comparison to spontaneous successional colonisation or direct sowing (Cole et al., 2011; Wang et al., 2007).

Thus, there is the need to produce high-quality stock of seedlings, which can successfully survive and grow after outplanting (Wilson and Jacobs, 2006), contributes in stressing the importance of nursery culture treatments, namely growing media and fertilization practices, to improve the success of reforestation programs (Grossnickle, 2005; Navarro et al., 2006).

Plants require nutrients, water, CO^2, light and temperature at optimal level in order to grow and develop. It has been argued in several reports that changes in environmental conditions, mainly limited by light and water availability, directly affect plant growth and field performance (Niinemets, 2010; Yamori et al., 2010). The best studied case is most probably the influence of light which drives the processes of photosynthesis by supplying ATP and NADPH needed for CO^2 assimilation.

Many studies have clearly shown that modulation in light quality, quantity, and photoperiod can affect plant growth and development (Chen et al., 2004; Zuchi and Astolfi, 2012). On the other hand, it is well known that plants respond to irradiance changes through morphological, biochemical and physiological responses. Such responses lead to an adjustment of the growth rate according to the change in availability of energy in the environment (Ariz et al., 2010; Smith et al., 1999).

The illumination of plant growth chambers is typically based on conventional light sources such as fluorescent light (especially cool-white) often used in combination with additional high pressure sodium and/or incandescent lights, providing a broader light spectrum reproducing outdoors conditions (Bubenheim et al., 1988). However these light sources have some limitations due to their short lifetime, high electrical consumption and heat emission.

Recently the utilization of light emitting diodes (LEDs) for plant growth in controlled environment has emerged as an attractive low-cost alternative technology (Yeh and Chung, 2009). LEDs are particularly suitable for plant growth chambers, because of their light weight, small volume and long life (about 100,000 hours) (Tennessen et al., 1994; Yeh and Chung, 2009). Furthermore, LED lighting result in significant energy saving due to emission of very narrow bands of light intensity.

Despite these attractive features of the LEDs system, and the acquired knowledge about the effect of light quality on plant morphogenesis, chlorophyll synthesis and photosynthesis (Massa et al., 2008; Schuerger and Brown, 1994; Tennessen et al., 1994), studies on growth and development of forest trees under different LEDs systems are very limited (Nhut et al., 2002), whereas are more advanced on horticultural plant species (Li et al., 2012; Liu et al., 2011; Stutte et al., 2009).

The present PhD work is part of a wider European research project by acronym Zephyr (Zero impact innovative technology in forest plant production). The 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 these technologies into a functional system for large scale production of pre-cultivated forest regeneration materials adapted to transplanting and further growth at forest nurseries all over Europe.

The objectives of the present study are to examine the effect of different LED light spectra on forest seedlings growth of different species, choosing the best LED light type to enhance seedlings growth performance, consequently to develop species-specific growth protocols.

Furthermore, develop an automatic plant phenotyping control system composed of optical sensors to monitor the seedlings growth during the early life period. Automatized faster indirect method respect to direct destructive one (time consuming) which needs a lot of time. In the beginning non-destructive analysis by optical sensors and software analysis were carried out. Then direct destructive measurements with non-destructive method were compared.

Moreover develop wireless electronic soil sensors system for automatic measurements of optimal soil water content. in order to evaluate their efficiency in measuring the soil water content during the period when seedlings grow in the growth chamber.

Plant species tested in the experiments are part of different distribution areal (chorotype) and they have different ecology. They are widely used for wood production and environmental and social benefits: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), Beech (*Fagus sylvatica* L.), Holm oak (*Quercus ilex* L.), Pomegranate (*Punica granatum* L.), Strawberry-tree (*Arbutus unedo* L.), Firetree (*Morella faya* (Ait.) Wilbur), Sanguinho (*Frangula azorica* Tutin) and Azores laurel cherry *Prunus azorica* (Hort. ex Mouillef.) Rivas Mart., Lousa, Fer. Prieto, E. Dias, J.C. Costa, C. Aguiar.

The seedlings (miniplugs) were produced using a new forest nursery cultivation method based on pre-cultivation in growth chambers and in very small containers for a very short period of culturing (Mattsson et al., 2010).

In particular, considering that the early growth period (about 30-40 days) is strongly critical for the functional characteristics of seedlings and their field performance, we tested the application of LED lighting compared to the traditional fluorescent lighting in relation to growth (fresh and dry weight, shoot height, root length and leaf area).

1.1. FORESTS AND TREE-BASED SYSTEMS

1.1.1 PROBLEM STATEMENT: CAN FORESTS AND TREE-BASED SYSTEMS CONTRIBUTE TO FOOD SECURITY AND NUTRITION?

As population estimates for 2050 reach over 9 billion, issues of *food security* and *nutrition* have been dominating academic and policy debates, especially in relation to the global development agenda beyond 2015. A total of 805 million people are undernourished worldwide, even though the trend appears to be slowly reversing (FAO et al., 2014) and *malnutrition* – defined as either under-5 stunting, anaemia among women of reproductive age or adult obesity – affects nearly every country on the planet (IFPRI, 2014). Despite impressive productivity increases, there is growing evidence that conventional agricultural strategies fall short of eliminating global hunger, result in unbalanced diets that lack nutritional diversity, enhance exposure of the most vulnerable groups to volatile food prices, and fail to recognise the long-term ecological consequences of intensified agricultural systems (FAO, 2013; FAO et al., 2013). In parallel, there is considerable evidence that suggests that *forests and tree-based systems* can play an important role in complementing agricultural production in providing better and more nutritionally- balanced diets (Vinceti et al., 2013); woodfuel for cooking; greater control over food consumption choices, particularly during lean seasons and periods of vulnerability (especially for marginalised groups); and deliver a broad set of *ecosystem services* which enhance and support crop production (FAO 2011a; Foli et al., 2014). Already, while precise figures are difficult to come by, it has been estimated that approximately 1.2-1.5 billion people (just under 20 percent of the global population) are forest dependent (Chao, 2012, cited by FAO, 2014a; Agrawal et al., 2013). These estimates include about 60 million indigenous people who are almost wholly dependent on *forests* (World Bank, 2002). Despite these figures, much of these forests remain under government control (even if the trend suggests a slight increase in community control of forests. Ultimately, who controls forests has important implications for the role of forests in food security and nutrition. The loss and degradation of forests exacerbate the problem of food insecurity both directly and indirectly: directly, by affecting the availability of fruits and other forest and tree-based food products and indirectly by modifying ecological factors relevant for crop and livestock and thereby affecting the availability of food (van Noordwijk et al., 2014).

In 2012, at the UN Conference on Sustainable Development: Rio+20, the UN Secretary General proposed an ambitious goal to eliminate global hunger by 2025 – the so-called "Zero Hunger Challenge". Fulfilling this challenge requires not just providing universal and year-round access to food for the world's growing population, but doing so in a nutritionally-balanced way, while enhancing livelihood security for smallholders, reducing waste from consumption and production systems and also ensuring that these systems are sustainable. Evolving strategies to respond to this challenge primarily focus on achieving "sustainable intensification", by improving the productivity of agricultural systems, without causing ecological harm or compromising biodiversity and ecosystem services (FAO, 2011b; Garnett et al., 2013). However, a particular concern is those parts of the world that are characterised by deep-rooted hunger and malnutrition, where food security is a particular challenge, primarily in poorer nations and in the tropics.

1.1.2 Direct and indirect Contributions of Forests and Tree-based Systems to Food Security and Nutrition

Forests and tree based systems provide a steady supply of wild and cultivated fruit, vegetables, seeds, nuts, oils, roots, fungi, herbs and animal proteins, which complement more conventional staple diets derived from agricultural production systems (and, in some cases, provide dependable staple sources for food security and nutrition). Some 50 percent of the fruit consumed globally comes from trees (much of this collected by women and children) and recent studies show that access to forests and tree-based systems is associated with increased vitamin intake from fruit and vegetable consumption.

What this growing evidence suggests is that, while forests are not a solution for global hunger in themselves, in many circumstances they play a vital supplementary role, especially during periods of unpredictability (such as long dry spells).

In some regions, food from forests plays a central role in providing calorific staples (such as açai palm fruit in the Amazon; Brondizio, 2008). It is also increasingly recognised that food from forests provides micronutrients and contributes to dietary diversity, thereby supporting a shift away from calorific intake as the primary metric for food security, towards a broader understanding of nutritionally-balanced diets (FAO, 2013).

Forests provide not only food items, they are also critically important for providing fuel for cooking. In developing countries, 2.4 billion households still use conventional biofuels (firewood, charcoal, crop residues and cattle dung) for cooking and heating. This includes 90 percent of rural households in large parts of sub-Saharan Africa and 70–80 percent in China (Modi et al., 2005). The most important biofuel used as rural domestic fuel is firewood, and the numbers dependent on it and other traditional biofuels are expected to increase over time (IEA, 2004).

Firewood shortages can have negative nutritional effects, since efforts to economise on firewood can induce shifts to less nutritious foods which need less fuel to cook, or cause poor families to eat raw or partially cooked food which could be toxic, or to eat leftovers which could rot if left unrefrigerated, or even to miss meals altogether (Agarwal, 1986).

Apart from these direct roles, forests support the diversification of livelihoods through income earning opportunities that contribute to household food security. Their role in providing ecosystem services which underpin the agricultural production system – through soil formation, nutrient cycling and provision of green manure, water provisioning, pollination and microclimate regulation – further enhances synergies between the forest-tree landscape and the wider food production system (MA, 2005).

1.1.3 ENVIRONMENTAL TRANSFORMATION AND DEGRADATION

The effect of human activities on ecosystems has been profound, particularly during the past century. Many critical thresholds of the earth's biophysical systems have already been crossed as a result of human activities (Rockström et al., 2009; Steffen et al., 2015). Though the consequences are complex, there is considerable evidence that ongoing and future climate change will have drastic impacts, especially in the poorest regions of the world. People living directly off the production from the earth's ecosystems are particularly affected by these changes.

Forests are affected by increasing temperatures, variable precipitation, *fragmentation*, *deforestation*, loss of biological diversity and spread of *invasive species*. These factors affect not only the extent of forest but also the structure and species composition within forests (and therefore, forest products) thus impacting on the availability of food and nutrition.

Environmentally-induced changes affecting forest cover imply both direct and indirect consequences for food security and nutrition: direct consequences result from changes in the availability and quality of food and nutrition, while indirect consequences result from changes in income and livelihoods related to forest products.

1.1.4 PROVISIONS OF ECOSYSTEM SERVICES

Forests, agroforests and to a certain extent plantations, provide important ecosystem services including: soil, spring, stream and watershed protection; microclimate regulation; biodiversity conservation; and pollination, all of which ultimately affect food and nutritional security (Garrity, 2004; Zhang et al., 2007).

Forests, woodlands and trees elsewhere in landscapes play a vital role in controlling water flows, and preventing soil erosion and nutrient leaching, all of which are critical functions for food production systems (Bruinsma, 2003). At the same time, green manures in agroforestry systems maintain and enhance soil fertility, supporting crop yields when external fertiliser inputs are not available or are unaffordable (Garrity et al., 2010; Sanchez, 2002). Nitrogen-fixing trees have in particular received considerable attention for their ability to cycle atmospheric nitrogen in cropping systems (Sileshi et al., 2008; Sileshi et al., 2011; Sileshi et al., 2012). Microclimate regulation by trees in agroforestry systems, such as through the provision of a canopy that protects crops from direct exposure to the sun (reducing evapotranspiration), from extreme rainfall events and from high temperatures, can also promote more resilient and productive food-cropping systems (Pramova et al., 2012).

Forests, and frequently agroforests, are centres of plant and animal biodiversity, protecting species and the genetic variation that is found with them, which may be essential for future human food security (Dawson et al., 2013). As well as being sources of existing and “new” foods, many already cultivated tree species have their centres of genetic diversity within forests, and these resources may be crucial for future crop improvement.

Pollination is one of the most studied ecosystem services, with perhaps the most comprehensive reviews of animal pollination and how it underpins global food production being that of Klein et al. (2007). A diversity of trees in forests and in farmland can support populations of pollinator species such as insects and birds that are essential for the production of important human foods, including fruits in both forest and farmland, and a range of other important crops in farmland (Garibaldi et al., 2013; Hagen and Kraemer, 2010; for the specific case of coffee, see Ricketts et al., 2004; Priess et al., 2007). For communities living in or around forests, pollination is therefore a crucial ecosystem service (Adams, 2012).

Of course, forests and trees in agroforest provide important habitat for a range of other fauna that include the natural predators of crop pests (as well as sometimes being hosts for the crop pests themselves; Tscharntke et al., 2005).

1.1.5 INTERACTIONS BETWEEN LANDSCAPE COMPONENTS

Positive contributions of forests to agricultural productivity

Forests provide an array of direct and indirect contributions to agriculture at different scales (MA, 2005). At the broad scale, forests contribute to the recycling of nutrients, suppression of agricultural pests, detoxification of noxious chemicals, control of hydrological processes and genetic resources for future adaptation to climate change (Foley et al., 2005; MA, 2005; Plantegenest et al., 2007). In a study carried out in 56 countries in Africa, Asia and Central/ South America it was found that a ten percent increase in deforestation would result in a 4-28 percent increase in flood frequency (Bradshaw et al., 2007), with large impact on rural and agrarian populations (FAO and CIFOR, 2005; Jonkman, 2005). Forests also contribute to climate change mitigation, having the capacity to absorb a significant fraction of global carbon emissions which could have positive impacts on food production (FAO, 2012). At the local scale, forests and trees outside forests are essential for ecosystem services, as already talked about (discussed).

Impacts of other land use patches on forests

Forests can be impacted positively or negatively by other nearby or distant land uses in ways that affect their own role as food production systems, as habitat for biodiversity, or their structure and function more generally.

Forests located near farming and urban areas may be more exposed to air, water and other types of pollution. Forests are vulnerable to emissions of reactive pollutants such as SO2, NOx, HNO3 and NH3 as well as elevated levels of ozone and excessive mineral salts (Fowler et al., 1999; Likens et al., 1996). These potentially phytotoxic pollutants, largely studied in the northern hemisphere, are damaging to forest health although it is difficult to identify specific pollutant effects given the high level of interactivity between pollutants, and between pollutants and climate change (Bytnerowicz et al., 2007; Paoletti et al., 2010). Atmospheric pollutants can also severely damage forests through acid rain (Likens et al., 1996).

Proximity to human settlements and roads can increase the likelihood of invasive species being introduced to, and perhaps damaging, forest environments (Bradley and Mustard, 2006; Bartuszevige et al., 2006). In most cases the introduction of non-native species may have little impact since they often fail to survive in a new habitat. However, those that do become established and thrive can cause severe and widespread economic and ecological losses, such as a reduction in forest and agricultural productivity, species population declines and even extinctions. For example in French Polynesia and other Pacific islands, Miconia calvescens (an introduced tropical American tree), has shaded out native plant species in some areas and, due to its shallow rooting habit, increased erosion and frequency of landslides (Meyer and Malet, 1997; Environment Canada, 2004; Moore, 2005).

Scale and fragmentation issues

Many of the interactions described above are influenced by the scale and spatial configuration of different land use patches. The process of forest fragmentation, occurring when formerly forested lands are converted permanently to pastures, agricultural fields, or human-inhabited developed areas, can result in changes in ecosystem functions that alter the supply and distribution of ecosystem services vital for agriculture (Tscharntke et al., 2012). Reduced connectivity of forest patches affects the ability of pollinators, pest predators (Tscharntke et al., 2005b; Kremen, 2005), water and nutrients (Brauman et al., 2007; Power, 2010) to move across a landscape.

In some cases, managing landscape configuration to enhance forest fragment connectivity may be a more effective tool for optimising agricultural landscapes for multiple ecosystem services rather than simply limiting further forest loss (Mitchell et al., 2014). It is however important that sufficiently large forest patches and connectivity are maintained, as high levels of forest loss can result in abrupt landscape-scale loss of native forest specialist species in the long term (Pardini et al., 2010). In many parts of the world, traditional agricultural landscape management approaches have been developed to more closely link agricultural and forest (or woodland) management and ensure continuity in the provision of ecosystem services from forests.

1.2. A BIBLIOGRAPHY OF SPECIES CHOSEN

1.2.1. NEEDLE LEAVED SPECIES (SCOTS PINE AND NORWAY SPRUCE)

Scots pine (*Pinus sylvestris* L.)

Scots pine is an evergreen coniferous tree growing up to 35 m in height and 1 m trunk diameter when mature. The bark is thick, scaly dark, grey to brown on the lower trunk, and thin, flaky and orange on the upper trunk and branches (Rushforth, 1999). The lifespan is normally 150-300 years, with the oldest recorded specimens just over 700 years. The shoots are light brown with a spirally arranged scale-like pattern. On mature trees the needles are glaucous blue-green, often darker green to dark yellow-green in winter. The needles are 3-6 cm long and 1-2 mm broad, produced in fascicles of two with a persistent grey 5-10 mm basal sheath. Needle persistence varies from two to four years in warmer climates, and up to nine years in sub-artic regions (Farjon, 2005). The seed cones are red at pollination, then pale brown, globose and 4-8 mm diameter in their first year, expanding to full size in their second year with a length of up to 8 cm. The seeds are blackish, 3-5 mm long with a pale brown 12-20 mm wing.

This species has the largest geographical distribution of any pine species, is one of the most abundant trees in Europe and one of the most widespread conifer species on earth. It has a wide distribution that extends among almost all the width of Eurasia (Carlisle and Brown, 1968). In fact Scots pine is a species of pine (Mirov, 1967) native to Europe and Asia, ranging from Scotland, Ireland and Portugal in the west. In the east it range to eastern Siberia, south to the Caucasus Mountains, and as far north as well inside the Artic Circle in Scandinavia.

At an altitudinal scale, *P. sylvestris* occurs from sea level to 1000 m a.s.l. in the north of its range, while in the south of its range it is a high altitude mountain tree, growing at 1200-2600 m altitude. This wide distribution range encompasses the broad range of climatic conditions that Scots pine is able to tolerate, from the severe cold winters of northern Siberia to the Mediterranean climate of southern Spain; and from the wet, oceanic climate of the west coast of Scotland to the dry continental climate of central Europe and Asia (Steven and Carlisle, 1959). Scots pine is a very valuable species both from ecological and economic perspectives (Kuper, 1994). From an ecological point of view, it is the only native pine in northern Europe and a keystone species for many ecosystems such as the Caledonian forest, taiga or Mediterranean mountain forests and supports many species of lichens, mosses, fungus and insects (Archibold, 1995). From an economic point of view, this species is found in all member states of the EU, where it constitutes approximately 20% of the commercial forest area, and it is of considerable importance as a timber producing species, particularly in Nordic countries (Mason and Alia, 2000).

Norway spruce (*Picea abies* L.)

Norway spruce is a large, fast-growing evergreen coniferous tree growing to 35-55 m tall and with a trunk diameter of up to 1-1.5 m. The shoots are orange-brown and glabrous. The needles are 12-24 mm long, quadrangular in cross-section and dark green on all four sides with inconspicuous stomatal lines. The cones are 10-15 cm long and have bluntly to sharply triangular-pointed scale tips. They are green or reddish, maturing brown 5-7 months after pollination. The seeds are dark brown to black, 3-5 mm long, with a pale brown 15 mm wing (Farjon, 1990).

Norway spruce is the most important forest tree species throughout Central Europe, including Germany (Schelhaas *et al*., 2003). It was one of the comparably few tree species to survive the last glaciations in Europe, and spread from only four refugia: the Apennines, the Carparthians, the Dinaric Alps, and the area north of Moscow, Kostroma (Vendramin *et al.,* 2000; Ravazzi, 2002).

Norway spruce grows throughout Europe from Norway in the northwest and Poland eastward, and also in the mountains of central Europe. Southwest it grows to the western end of the Alps, and southeast in the Carpathians and Balkans to the extreme north of Greece. Its eastern limit in Russia is the Ural Mountains. In North America, Norway spruce is widely planted, specifically in the northeastern, Pacific coast, and Rocky Mountains states, as well as in southeastern Canada.

Favored by modern forestry as a fast-growing tree species with high growing stocks and valuable wood, spruce has been cultivated in large-scale plantations at lower elevations instead of the natural beech and oak trees, even far from its cooler montane and subalpine natural ranges (Walentowski *et al*., 2004).

The importance of Norway Spruce in European forests is reflected in the several fields that deal with its role as a host tree for arthropods. One field, pest management (Wermelinger, 2004), focuses on species living on Norway Spruce, particularly with regard to risk assessment (Schelhaas *et al*., 2003). Another field, modern forestry, aims not only at maximum timber production, but also at biodiversity (Brockerhoff *et al.,* 2008). A third field deals with climate change and its consequences on community composition, and this field touches on both pest management and conservation (Jönsson *et al*., 2009; Muller *et al*., 2009).

1.2.2 BROADLEAVED SPECIES

European beech (*Fagus sylvatica* L.)

European beech (*Fagus sylvatica* L.) is a deciduous tree reaching up to 30m tall with a stocky trunk and a round crown that lives in European mountain woodlands up to 2.000 m altitude. Leaves are alternate, simple, ovate or elliptical, 2 to 4 inches long, pinnately-veined (7 to 9 pairs), with a nearly entire to somewhat toothed or wavy margin. They have fine hairs present on margin with tomentum on veins. They are commonly shiny green or purple in color. Flowers are monoecious; male flowers borne on globose heads hanging from a slender stalk, female flowers borne on shorter spikes. Flowers appear just after leaves in the spring. Nuts are irregularly triangular, shiny brown and edible, found in pairs within a woody husk covered with spines, 1 inch long, maturing in the fall. Twig is slender, zigzag, light brown in colour, buds are long 1 inch, light brown, and slender, covered with overlapping scales that are tinged with tomentum, widely divergent from stems. Bark is smooth, thin, and dark gray-blue in colour.

European beech is native from the southern parts of Sweden and Norway to Spain, Italy, Greece and northeast Turkey and Ukraine. It is a common tree in the “old world” where it is often found in association with oaks, European fir and Norway spruce. Close relatives in the beech family (Fagaceae) include chestnuts (Castanea) and oaks (Quercus). European beech is a symbol of wisdom. It is widely distributed in Western, Central and Southern Europe, Northern Spain, Southeastern England, Denmark, Southern Sweden, Western Poland, in the mountains of Romania, the Balkan Peninsula, Italy and Corsica.

In Europe it represents about 10% of the total forest area; this corresponds to about 17 million hectares of beech forests.

Though not demanding of its soil type, the European Beech has several significant requirements: a humid atmosphere (precipitation well distributed throughout the year and frequent fogs) and well drained soil (it cannot handle excessive stagnant water). It prefers moderately fertile ground, calcified or lightly acidic. It tolerates rigorous winter cold, but is sensitive to spring frost.

Pomegranate (*Punica granatum* L.)

Pomegranate (*Punica granatum* L.) is a deciduous shrub or small tree that is a member of the family Punicaceae. It grows between 5 and 8 m tall usually with multiple stems. The deciduous leaves are shiny and about 3-7.6 cm long. This is a hermaphroditic species with beautiful orange-red trumpet shaped flowers with ruffled petals. The flowers are about 2-5 cm long, often double, and are produced over a long period (April, May, June). The fruits ripen 6 to 7 months after flowering. They are globose, 2-7.6 cm in diameter, and shiny reddish or yellowish green when mature. It has a persistent calyx opposite the stem end that looks like a little crown. The fruit is technically a berry. It is filled with crunchy seeds each of which is encased in a juicy, somewhat acidic pulp that is itself enclosed in a membranous skin. The seeds, juice and pulp are eaten, but the yellowish membrane is too astringent. Fruiting begins in the 7th or 8th year and fruits take 6-7 months to mature. The fruit collection is held from

September to February. The number of fruits may vary from 20-25 for young trees to 100-150 for 10 year old trees and even 200-250 fruits for older trees. Yield varies with tree size. The most popular cultivar is ‘Wonderful’ as it is large-fruited and fruits are well-colored.

It grows in Mediterranean woodlands and shrublands, semi-steppe shrublands and montane vegetation (Mt. Hermon), upwards from 200 m up to 1500m. Apart from the high oaks, in Mediterranean shrublands, trees and shrubs tend to grow in dense thickets or maquis. These species include pomegranate, pistachio or terebinth, carob, myrtle, fig-sycamore and other companion species, often drought-resistant features.

Pomegranate is native to Asia, from the Middle East (Iran and northern India) to the Himalayas and it is now cultivated for its fruit and showy flowers in much of the Mediterranean region and tropical America. The most important growing regions are Egypt, China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, India, Burma and Saudi Arabia.

Pomegranates do best in climates with long hot, dry summers and cool winters. They are very tolerant of sandy, clayey, acidic and even alkaline and salty soils but they prefers well drained, heavy, light and medium soils or calcareous soils, too. Pomegranate is susceptible to fire, frost (at -11 °C damage to trees is recoverable) and strongly alkaline soils but tolerates soil compaction, drought and seasonal waterlogging.

Strawberry tree (*Arbutus unedo* L.)

Strawberry tree (*Arbutus unedo* L.) is an evergreen tree belongs to the Ericaceae family. The bark, is much like the madrone tree, *Arbutus menzii*, becomes reddish brown and peels with age. It is in flower from October to December, and the seeds ripen from October to December. The flowers are hermaphrodite (have both male and female organs) and are pollinated by bees. Leaves are leathery and serrated, with red stems. Fruits are fleshly berries and suitable for endozoochoric dispersal by birds and mammals. Strawberry tree is tolerant of many soil types as long as there is good drainage and is fairly drought resistant. Generally is widespread through the Western Mediterranean as far as Greece and Lebanon (Mesleard and Lepart, 1991; Celikel et al., 2008).

1.2.3 AZOREAN SPECIES

The Azorean species reproduction and conservation is very important for several reasons. These species have an important role as key components of the Azorean Laurel-Forest. This broad-leaved evergreen forest is largely dominated by *Laurus azorica* (a

Macaronesian endemic) and *Morella faya* and is considered to be the natural climax vegetation up to an altitude of about 600m above the sea level. Furthermore, seeds germination for these species is extremely difficult representing a problem for genetic conservation and thus a challenge for an optimal new germination protocols development. Main threats faced by these species are: habitat degradation mainly due to invasive alien flora species, agriculture expansion and development of infrastructures. Main factors that inhibit the recovery of these species are the dramatic low density of the natural population and its extreme fragmentation resulting in a poor genetic diversity.

Firetree (*Morella faya* (Ait.) Wilbur), Sanguinho (*Frangula azorica* Tutin) and Azores laurel cherry *Prunus azorica* (Hort. ex Mouillef.) Rivas Mart., Lousa, Fer. Prieto, E. Dias, J.C. Costa, C. Aguiar.

Azores laurel cherry (*Prunus azorica*)

Azores laurel cherry (*Prunus azorica* (Hort. Ex Mouil.) Rivas Mart., Lousa Fer. Prieto, E.Dias, J.C. Costa, C. Aguiar) is a small perennial tree, growing up to 6 meters high. Young-twigs are reddish-brown colour. Leaves are simple, elliptical, up to 12 x 6 cm, acuminate and regularly crenate. Its inflorescence is a 20 (-30) flowered, elongate raceme, up to 20 cm long. With 5 petals, flowers are white, orbicular, up to 5 mm. The fruit is a black, ovoid drupe. The flowering period of this species ranges between May and July. This species is endemic to Azores Archipelago flora. It is estimated to exist about 50-250 mature individuals in all Azores (Martin et al., 2008). The species is absent from Corvo, Graciosa and Santa Maria Islands, due to its over-exploitation in the past. The population has been declining in terms of numbers, size as well as in terms of distribution area, since Azores occupation 500 years ago. The species can be found in Laurissilva forest remnants in shady ravines, water streams and craters between 500 – 600 meters. The middle altitude laurel forest is a type of forest that was largely replaced by other land uses. A low-growing tree which is found in deep narrow ravines and in stands of undisturbed laurel-juniper forest. Moreover, in Sa˜o Miguel Island, it is also one of the main food sources for the endangered bird *Pyrrhula murina*. The species in considered as one of the TOP 100 species endangered in Azores Archipelago (Martín et al, 2008). It is protected by the Habitats Directive, Annex II and by the Born Convention, Annex I. In terms of Regional Law is protected also under the Regional Diploma n.º 15/2012/A. The species is rated in the IUCN Red List as an “Endangered Species” (IUCN).

Firetree (*Morella faya* (Ait.) Wilbur)

Morella faya is also a dominant component of the Azorean Fayal forest mostly develops on recently developed lava soils rich in potassium. The local climate also has to be moderate with reduced exposure, moderate temperatures and good amounts of precipitation. They tend to have a simple structure but the endemic tree *Picconia azorica* (Oleaceae) is often a conspicuous component. At ground level the herbaceous layer typically includes the endemic *Carex hochstetteriana* (Cyperceae) and *Polypodium azoricum* (Polypodiaceae).
Sanguinho (*Frangula azorica* Tutin)

1.2.3.1 Azorean Laurel Forest

VEGETATION

The total knowledge of the Azorean vegetation is still incomplete. The classic understandings of altitudinal zonation have been changed by recent studies that proved the exposed to the winds and the soil’s humidity as the main factors for the communities' distribution. So, while the presence of the” placic” in altitude soils (horizon of deposition of oxides of iron and magnesium, that turn them impermeable) originates the existence of vast wet areas in the endorreic plateaus, the characteristic formation of seas of clouds, above 700 m of altitude, for the wet winds, allows the existence of subtropical forests (cloud forests). On the other hand, there’s information that, before the human presence, the best soils would have imposing forests, with more than 20 meters height (attested by the bars of indigenous wood found). With the agricultural occupation of these soils, are only possible find today forests with little more than 10 meters height.

The azorean forests belong to the macaronesian element, in its more Atlantic species and constitute relic elements of the Tertiary period in the European continent, of subtropical conditions, denominated by species” alive fossils” per-glaciers. While Lauraceae family possesses great importance in the formations of the other archipelagos, is, in Azores, substituted by more elements wet-temperate, as the Ilex, *Vaccinium*, *Taxus* and *Juniperus* species.

NATURAL FOREST VEGETATION

The Azores Natural Forest find theme self’s in a diffuse ecological position with a strict relation with the other types of Azorean vegetation.

Recent studies contradict the opinion of some scientist that visited the Azores islands in the last century, which defended the non-existence of forest formations in this archipelago. The historical descriptions show that different forest types covered most part of the island’s territory. Nowadays most of these formations, especially in low altitudes, were destroyed, but we still can distinguish several types of forests with different ecological tendencies; paleomediterranean, macaronesian, subtropical mountain and north-atlantic forests, that correspond to unique *sintaxas* with their own structure and dynamic process.

AZOREAN LAUREL FOREST

These broad-leaved evergreen forest, are largely dominated by *Laurus azorica* (a Macaronesian endemic) and *Myrica faya* and are considered to be the natural climax vegetation up to an altitude of about 600m. Similar laurel forests can be found in Madeira and the Canaries, and all are thought to be the relicts of vegetation that was once widespread in southern Europe before climatic cooling during the Pleistocene. These macaronesian forests are rich in endemics. On the Azores they include *Ilex perado* subsp. *azorica* (Aquifoliaceae) and *Notelaea azorica* (Oleaceae) among the trees, *Euphorbia stygiana* (Euphorbiaceae), *Picconia azorica* (Oleaceae), *Prunus lusitanics* ssp. *azorica* (Rosaceae), *Rubus hochstetterorum* (Rosaceae), *Vaccinium cylindraceum* (Ericaceae), *Viburnum tinus* var. *subcordatum* (Adoxaceae) among the shrubs and *Hypericum foliosum* (Hypericaceae), *Rubia peregrina* var. *azorica* (Rubiaceae), *Sanicula azorica* (Apiaceae), *Senecio malvaefolius* (Asteraceae), *Lactuca watsoniana* (Asteraceae) and *Selaginalla azorica* in the field layer.

In the central islands three sub formations are recognized – mesic forest, humid forest and hyper-humid forest. In mesic forest the environmental conditions are characterized by moderate winds, high levels of precipitation and low humidity, and usually have high levels of floristic diversity. Among the trees there is typically a high level of codominance. In addition to *Laurus azorica* include *Myrica faya* and the endemic *Frangula azorica* (Rhamnaceae) and *Picconia azorica* (Oleaceae).

1.2.4 WHY THE SPECIFIC SPECIES WERE CHOSEN

Scots pine (*Pinus sylvestris*)

Scots pine is an important tree in forestry. The wood is used for pulp and sown timber products. A seedling stand can be created by plantation, sowing or natural regeneration. Commercial plantation rotations vary between 50-120 years with longer rotations in the north eastern areas where growth is lower. In Scandinavian countries, Scots pine has also for many decades been used for making tar and as a source of rosin and turpentine. Scots pine has a quite wide range of pharmaceutical uses. It has been used especially for its antiseptic action and beneficial effect upon the respiratory system. The turpentine obtained from the resin is anti-rheumatic and antiseptic. Externally the resin is used in the form of liniment plasters and inhalers. The essential oil from the needles is used in the treatment of asthma, bronchitis and other respiratory infections. An essential oil obtained from the seed has diuretic and respiratory stimulant properties.

Norway spruce (*Picea abies*)

Norway spruce is one of the most widely planted spruces, both in and outside of its native range. It is also one of the most economically important coniferous species in Europe. It is used in forestry for timber and paper production, and as an ornamental tree in parks and gardens. It is also widely planted for use of Christmas trees. Norway spruce also has an interest for pharmaceutical reasons. The buds, leaves and resin are antibiotic and antiseptic. The resin from the trunk is used externally in plasters etc. for its healing and antiseptic properties. Because of the vitamin C, that are present in Norway spruce shoot tips, spruce tea from these tips has been used against tiredness or other symptoms of lack of vitamin C. Other substances that have been used for pharmaceutical reasons are turpentine and essential oil.

European Beech (*Fagus sylvatica* L.)

Fagus sylvatica L. (Beech) is one of the most important species in Europe. Although natural regeneration may be abundant, it is conditioned by long periods of non-fruiting (5-6 years) and the seed, hardly stored, presents a complex dormancy due to the immaturity of the embryo. It has been used for centuries for its wood that is hard, heavy, strong and very durable. It has a wide range of applications, including furniture, flooring and turnery. It makes a very good fuel, burning with a lot of heat, and yields a charcoal known as 'Carbo Ligni Pulveratus'. The wood has often been used as a source of creosote, tar, methyl alcohol, acetic acid, etc. A semi-drying oil (17 - 20%) is obtained from the seed and used as a fuel for lighting or as a lubricant, for polishing wood. The leaf buds harvested in the winter and dried on the twigs are used as toothpicks. The leaves are gathered in autumn and used as a stuffing material for mattresses. *Fagus sylvatica* has also medicinal uses. In fact, the bark contains antacid, antipyretic, antiseptic, antitussive, expectorant, odontalgic properties. A tar (or creosote), obtained by dry distillation of the branches, is stimulating and antiseptic. The plant is used also in Bach flower remedies.

Pomegranate (*Punica granatum* L.)

Punica granatum L. has a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. The plant as a whole and especially its flowers, have been extensively used in the ancient Ayurvedic health care system. Different researchers, such as Robert Longtin (2003), have referred to the pomegranate as “nature’s power fruit”. The fruit’s medical significance dates back to ancient times and is even noted in Egyptian mythology and art. Extracts of the juice, bark, leaves, immature fruit, and fruit rinds have all been noted to have some medical significance, most notably antioxidant activity, antibacterial properties, uses in diabetes, heart disease, and cancer. An important potential application for the anti-microbial properties of *Punica granatum*, is its use as a topical microbicide for HIV prevention. The bark of the branches and roots contain an alkaloid *pellatrierine* and tannic acid, highly useful in medicine to get rid of helminthes and in the treatment of tuberculosis, dysentery and diarrhea. Seeds are rich in oil, which have hormone producing effects and stimulate estrogen hormone. It is also used to prepare cosmetics and moisturizing body lotion. Rind powder is excellent source of beta-carotene, potassium, phosphorous and calcium. Fruit juice contains potassium, phosphorous and calcium as well as micronutrients like iron, manganese, zinc and copper. It stimulates appetite and is used in treatment of stomach disorders. It is also a good painkiller and it is well known as an excellent treatment for anemia. It has also a high content of riboflavin, the B2 vitamin that normalizes the nervous system and is used against radiation sickness. So the fruit juice is especially important in our present-day polluted environment, preventing the harmful effects of radioactive materials by producing biologically active substances. Moreover, antioxidant substances as tannins, anthocyanins and polyphenolics work together to benefit the arteries. It has also been found to increase levels of nitric oxide, important to prevent stroke and to help in curing erectile dysfunction. Other benefits include preventing premature aging, arthritis and Alzheimer disease. The juice, peel, and seed oil have been found to have anticancer properties (especially against skin, breast, lung and prostate cancer) inhibiting proliferation, cell cycle, and angiogenesis. Pomegranate is also useful in fields different from medicine, for example for the production of fodder (from leaves), fuel (from branches as firewood), timber (hard and durable wood, useful in making farm implements), dyestuff rich in tannins for leather dyeing (from root bark). This deep rooting tree is also important in soil erosion control and it is planted along rivers to stabilize banks, so also as in soil improvement, thanks to its leaf litter that decomposes slowly and is suitable for mulching.

Strawberry tree (*Arbutus unedo* L.)

Strawberry tree apart from its aesthetical value, its fruits are edible and leaves are used in pharmaceutical industry because of its strong antibiotic activity against Mycobacterium bacteria. Also its leaves, fruits and bark is full of tannins, wood is used in turning, making Greek flutes and makes a good charcoal (Hammami et al., 2005).

Azores laurel cherry (*Prunus azorica)*

Prunus azorica wood in the past was extremely appreciated for fine furniture making. There is evidence that the islands, when colonized had large trees, namely of Prunus azorica (Frutuoso, 1583; Guppy, 1917). However, their exploitation without allowing the populations to recovery has led to its present endangered status. Hence, it is extremely important to set growth protocols that allow improving the species status, but also to have excellent quality seedlings for afforestation promoting the diversification of Azores forest products. Main threats faced by the species are: habitat degradation mainly due to invasive alien flora species, agriculture expansion and development of infrastructures. Main factors that inhibit the recuperation of the species are populations with very low density, subpopulations are very isolated and a poor genetic diversity. Some proposals have been identified has useful to allow the species to recover from its endangered status (MARTÍN et al, 2008). Those actions are: reinforcement of the existing subpopulations and populations and the re-introduction of new populations. The fruits of this species are included in some of the Azorean bird endemic species and subspecies.

1.3 LED LIGHT - HOW DOES LIGHT EFFECT PLANT GROWTH?

Light is the most important environmental factor for plant growth and development (Jeong et al., 2012). Plants require light throughout their whole life-span from germination to flower and seed production (Singh et al. 2015).

Light characteristics (spectral quality, quantity and duration) have a profound influence on the metabolism and development of plants (Ouzounis et al. 2015).

Light quantity (*intensity*): Light quantity or intensity is the main parameter which affects photosynthesis, a photochemical reaction within the chloroplasts of plant cells in which light energy is used to convert atmospheric CO^2 into carbohydrate.

Light quality (*spectral distribution*): Light quality refers to the spectral distribution of the radiation, i.e. which portion of the emission is in the blue, green, red or other visible or invisible wavelength regions. For photosynthesis, plants respond strongest to red and blue light. Light spectral distribution also has an effect on plant shape, development and flowering (photomorphogenesis).

Light duration (*photoperiod*): Photoperiod mainly affects flowering. Flowering time in plants can be controlled by regulating the photoperiod (Singh et al. 2015).

Plants do not absorb all wavelengths of light (solar radiation), they are very selective in absorbing the proper wavelength according to their requirements. The most important part of the light spectrum is 400 to 700 nm which is known as photosynthetically active radiation (PAR), this spectral range corresponds to more or less the visible spectrum of the human eye. Chlorophylls (chlorophyll a and b) play an important role in the photosynthesis but they are not the only chromophores. Plants have other photosynthetic pigments, known as antenna pigments (such as the carotenoids β-carotene, zeaxanthin, lycopene and lutein etc.), which participate in light absorption and play a significant role in photosynthesis (Fig. 1).

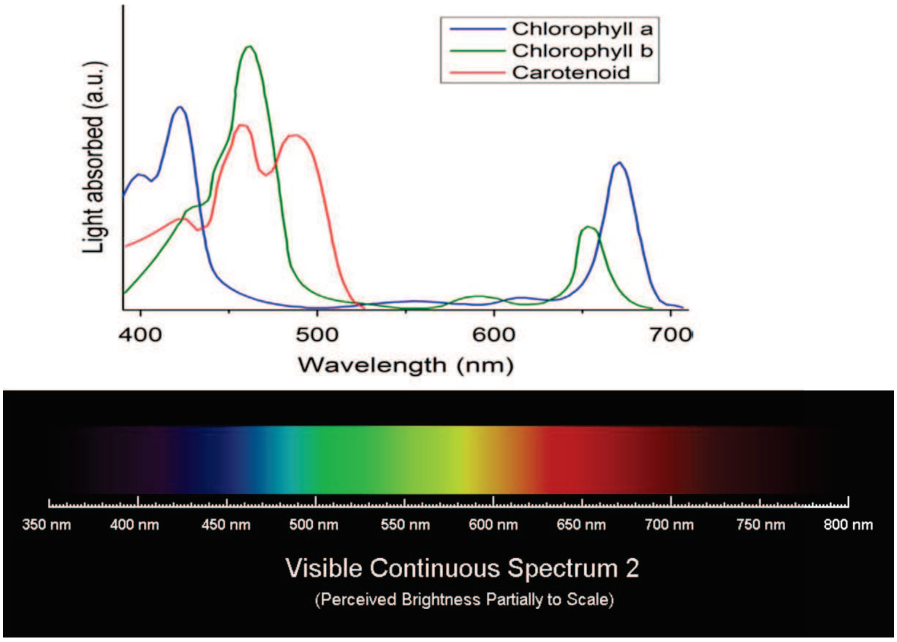


Fig. 1 Absorption spectrum of chlorophyll and antenna pigments

The solar radiation spectrum mainly consists of three parts: ultraviolet (UV), visible light, and infra-red.

200-280 nm (*ultraviolet C*): This part of the spectrum is harmful to the plant because of its high toxicity. UVC is blocked by the terrestrial ozone layer, so it does not reach the earth's surface.

280–315 nm (ultraviolet B): This part is not very harmful but causes plant colours to fade.

315–380 nm (ultraviolet A): This range does not have any positive or negative effect on plant growth.

380–400 nm (ultraviolet A/visible light): Beginning of visible light spectrum, process of light absorption by plant pigments (chlorophylls and carotenoids) begins.

400–520 nm (visible light): Contains violet, blue and green bands. Peak absorption by chlorophylls occurs in this range and it has a strong influence on vegetative growth and photosynthesis.

520–610 nm (visible light): This range contains green, yellow and orange bands. This range is less absorbed by the plant pigments and has less influence on vegetative growth and photosynthesis.

610–720 nm (visible light): Contains red bands and a large amount of absorption occurs at this range. This band strongly affects the vegetative growth, photosynthesis, flowering and budding.

720–1000 nm (far-red/infrared): Germination and flowering is influenced by this range but little absorption occurs at this band.

>1000 nm (infrared): All absorption in this region is converted to heat.

Researchers around the world are experimenting with different spectral compositions to optimize the plant growth. A controlled spectrum composition would be much more beneficial for the plants than white light because it would allow to better control the plants' performance such as flowering time, high photosynthetic efficiency, low heat stress etc. LED lighting offers a simple replacement of current light sources (HPS lamps) with better control on spectral composition.

Light sources such as fluorescent, metal halide, high-pressure sodium, and incandescent lamps are generally used from fall to spring to enhance plant growth in greenhouse production and to ascertain a year round supply of good quality plants despite low natural solar radiation (Jeong et al 2012) (Ouzounis et al. 2015). Although these light sources, in particular high-pressure sodium (HPS), are the most commonly used lighting system, they are neither spectrally nor energetically optimal (Heuvelink et al. 2006, Marcelis et al. 2006). They contain only 5% blue light (400–500nm), which is low compared to the natural sun light percentage of around 18% blue (Heuvelink et al. 2006, Marcelis et al. 2006). These sources show several disadvantages such as low photosynthetic photon flux as well as limited lifetime of operation, low quantum yield, and less suitable wavelength spectra for plant growth In 1990, light-emitting diodes (LEDs) were investigated for the first time for plant growth and demonstrated to be an efficient alternative to traditional lamps used in lighting systems (Jeong et al 2012).

During the last decades, solid state lighting using light-emitting diode (LED) technology have arisen as a fundamentally different and energy efficient approach for the green house industry that has proficient advantages over gaseous discharge-type lamps currently used in most greenhouses (Schuebert and Kim 2005, Heuvelink et al 2006, van Ieperen and Trouwborst 2008) (Ouzounis et al. 2015 Physiol Plant)

The incandescent or fluorescent bulbs contain filaments that must be periodically replaced and consume a lot of electrical power while emitting radiant heat (infrared) in the direct environment (Singh et al 2015). LEDs do not have filaments and, thus, do not burn like incandescent or fluorescent bulbs. However, LEDs have the ability to precisely tune spectral quality and light intensity, producing high luminous flux with low radiant heat output and maintain their light output efficacy for years. The operational lifetime of fluorescent bulbs are in the order of 20,000 h while only 1000 h are expected for incandescent bulbs. Normally, LEDs are known to have lifetimes in the order of 30,000– 50,000 h and even beyond. Energy is an important factor which contributes about 20–30% of total production cost in greenhouse industry. Appropriate crop lighting is a necessity of the green house industry, particularly in regions where the seasonal photoperiod (natural day length) fluctuates and there is not sufficient light for optimal plant growth. Thus, a new technology which significantly reduces the electricity consumption and produces low radiant heat for crop lighting while maintaining or improving the crop value (growth and nutritional value) is of great interest to the greenhouse industry (Singh et al 2015). Climate strategies where ventilation is avoided and the growing temperature follows more the natural variation in day (DT) and night temperatures (NT) to reduce energy consumption are of current interest (Blanchard et al., 2011; Fitz-Rodríguez et al., 2010; Lund et al., 2006; Markvart et al., 2007). LEDs represent an energy efficient approach for greenhouse lighting that has technical advantages over traditional light sources (Singh et al 2015).

Therefore LEDs can provide several benefits to the green house industry:

Reduction in energy consumption up to 70% compared to traditional light sources.

Fast switching and steady state operation.

Simple electronic dimming function.

Reduction of cable gauge (and hence cost and weight).

High Relative Quantum Efficiency (RQE): Red light has the highest RQE, meaning it is the most efficient at photosynthesis.

Blue light is about 70–75% as efficient as red light.

Stable temperature inside the growth chamber and green-house.

Ability to control spectral composition of blue, green, red, and far-red wavelengths.

Reduction of heat stress on plants.

Reduction in watering and ventilation maintenance.

Life time, reliability, and compact size as the major technical advantages over traditional light sources. (Singh et al 2015).

Several reports have confirmed successful growth of plants under LED illumination. Different spectral combinations have been used to study the effect of light on plant growth and development and it has been confirmed that plants show a high degree of physiological and morphological plasticity to changes in spectral quality.

The most important wavelengths are in the blue and red region, where chlorophylls absorb light energy and use it for photosynthesis (McCree1972). Red (610-720 nm) light is required for the development of the photosynthetic apparatus and photosynthesis (Singh et al 2015). The use of red LEDs is universally accepted as red wavelengths (600–700 nm) are efficiently absorbed by plant pigments while the most efficient LED with the red wavelength peak at 660nm is close to an absorption peak of chlorophyll, which is 665nm (Sager and McFarlane 1997). Red light induces hypocotyl elongation and expansion in leaf area (McNellis and Deng, 1995; Johkan *et al.,* 2010).

Blue (400-500 nm) light is also important for the synthesis of chlorophyll, chloroplast development. Blue light acting through cryptochromes and phototropins affects fresh and dry matter accumulation, stomata opening, flowering and photomorphogenetic responses. Blue light suppresses hypocotyl elongation and induces biomass production (McNellis and Deng, 1995; Johkan *et al.,* 2010). The combination of red and blue LED lighting is favorable for the plant in many aspects (Yorio et al. 2001, Whitelam and Halliday 2007, Johkan et al. 2010) (Ouzounis et al. 2015). However, different wavelengths of red (660, 670, 680 and 690 nm) and blue (430, 440, 460 and 475 nm) light might have uneven effects on plants depending on plant species. Far-red LED light (700-725 nm) which is beyond the PAR has been shown to support the plant growth and photosynthesis (Singh et al 2015). Nevertheless, photosynthetically inefficient light qualities also convey important environmental information to a developing plant. For example, far-red light reverses the effect of phytochromes, leading to changes in gene expression, plant architecture, and reproductive responses (Yeh and Chung, 2009). Concerning plant growth, the use of LED lights provide specific wavelength in order to optimize photosynthetic processes and improve the plants growth as well as the possibility to adjust light intensity/quality.

1.4 What plant phenotyping is?

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. 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. 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. 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.

1.5 Zephyr project

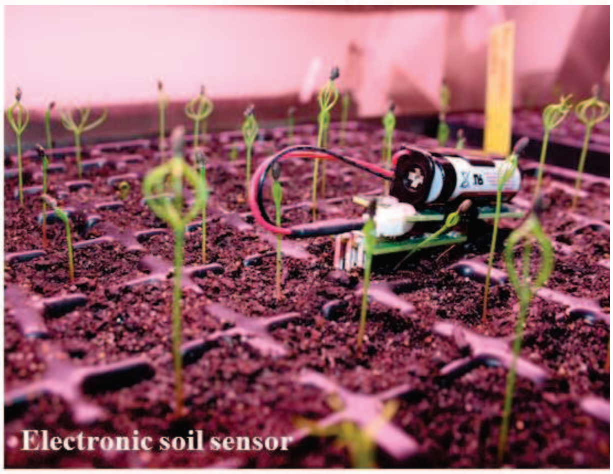
Zephyr (Zero impact innovative technology in forest plant production) is a European research project involving 14 partners from 10 countries. This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No 308313. The Zephyr project aims to introduce and integrate innovative technologies into a production unit functional system characterised by zero-impact and cost-friendly. The unit will provide for large scale production of certified and standardized pre-cultivated forest regeneration materials adapted to transplanting and further growth at forest nurseries all over Europe. In fact, the new Zephyr integrated technology will make a drastic change to state-of-the-art in forest nursery production for reforestation purposes. This production unit can be also used for the agronomic and flowering plant species.

Apart from being more resource-efficient, it will also contribute to the environmental protection through: biodiversity defending (developing specific growth protocols for different species), water recycling, strong fertilizers reduction and avoidance of pesticides.

The fulcrum of the project is a rotating automated growth chamber, equipped with LED lights, an automatic system (useful also by remote) for 1. the adjustment of light intensity and photoperiod, 2. control of wireless sensors for the regulation of soil water content (SWC) and conductivity (SC) related to total ions concentration, 3. control of environmental parameters such as air temperature (AT), relative humidity (RU) and CO_2 concentration (CDC). The growth chamber is equipped with photovoltaic panels (PVP) for the energy production and an irrigation system based on the water recycling. Finally an optical system composed by two stereoscopic cameras positioned on an automated robotic arm to monitor the seedling growth during the time.


ZEPHYR - rotating growth chamber


Optical sensor - Dualcam


Electronic soil sensor

2 MATERIAL AND METHODS

2.1 PLANT GROWTH WITH DIFFERENT LED LIGHT TYPE

2.1.1 GROWTH CHAMBER CHARACTERISTICS AND PLANT MATERIAL

Light characteristics

Different LED light types for the tested species were applied, and one fluorescence light as control light.

Valoya AP67 (bar, 2 lamps, length 120cm)

Valoya AP673L (bar, 2 lamps, length 120cm)

Valoya G2 (bar, 2 lamps, length 120cm)

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

Valoya L20AP67 (tube, 6 lamps, length 120cm)

OSRAM L36W/77 FLUORA (tube, 6 lamps, length 120cm)

OSRAM L 36W/77 Fluora: the control spectrum of the fluorescence lamps is widely used in horticultural plant production.
Valoya AP673L (B100): 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.
Valoya NS1 (B100): High intensity spectrum. of Valoya's spectra, 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.
Valoya G2 (B100): 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.
Valoya AP67 (B100): 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: Best known of the Valoya spectra. 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. **Valoya AP67 tube (L20):** 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.

Combined effects of the tested light treatments due to different percentages covering the specific light spectrum areas were shown in Figure 2.

Light intensity

In order to ensure a constant light intensity (PAR) equal to 110 ±10 µmol m^{-2} s^{-1} (Light Meter sensor - HD2302.0 - Delta Ohm, IT) per each table at the tray level, one or two LED light source per table at different distances from the trays were used.

In the experiment with optical sensors was used light intensity under fluorescent light (FLUORA T8), was yielding approximately 120 µmolm^{-2} s^{-1}

Mini-plug containers and Soil substrate

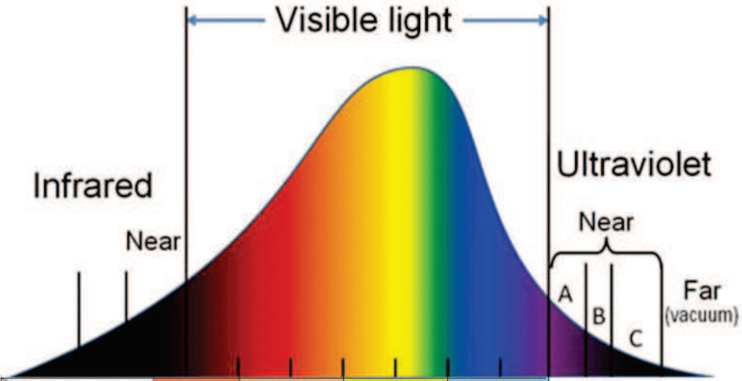
Mini-plug containers used for each of the experiment was ***QPD** (QuickPot) **104 VW** by HerkuPak*, Germany, with the following identical dimensions (tray dimension 310X530 mm ; cell size 33X33X45 mm; depth 40 mm; volume 27 cc). Two soil substrates was used: stabilized peat (Preforma VECO3, Jiffy® Products, Netherlands) pH of 5.0, and sphagnum and sand (VigorPlant) pH = 6,4.

Growth chamber

A single growth chamber (Figure 3) was used to allow for a strict control of environmental factors (uniform conditions) and seedling development (coetaneous cohort). So each plant species was grown independently in the same chamber until the harvest date. In order to avoid influence of near lights, each table was isolated by a white polyethylene panel.
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.
The photoperiod, temperature and humidity settings in the growth chamber are detailed in Table 1 for each species.

Species list, seeds and further details about growth protocols for each species please see sections 2.2, 2.3, 2.5

Species	Photoperiod	Temperature	Relative Umidity
Pinus sylvestris	16h/8h (light/darkness)	21-26°C	80% during germination 55-70% during growth
Picea abies	16h/8h (light/darkness)	21-26°C	80% during germination 55-70% during growth
Quercus ilex	14h/10h (light/darkness)	21-22°C	60-70%
Punica granatum	14h/10h (light/darkness)	21-22°C	60-70%
Fagus silvatica	14h/10h (light/darkness)	21-22°C	60-70%
Arbutus unedo	14h/10h (light/darkness)	21-22°C	60-70%
Morella faya	12h/12h (light/darkness)	30-20°C	60-70%
Frangula azorica	12h/12h (light/darkness)	30-20°C	60-70%



Light type \ Wave length (nm)	700 - 800	600 - 700	500 - 600	400 - 500
	Spectrum (%)			
AP67	18.1	53	15.1	13.8
AP673L	8.3	60.5	19.3	11.9
G2	25.5	64.4	2.4	7.7
NS1	5.2	35.7	38.9	20.2
FLUORA T8	4.4	36.7	24.1	34.8

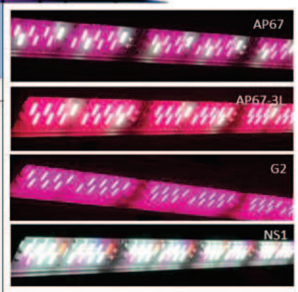


Fig. **2** Light treatments used in the experiments such as Fluorescent (FL), AP673L, G2, AP67, NS1 and the different percentages of wave length covering specific color bands in the light spectrum.

Fig.**3** seedling growth chamber equipped with different LED light type, with table isolated by a white polyethylene panel and environmental controlled.

2.1.1 EXPERIMENTAL DESIGN

A total of 208 seeds for each needle-leaved species (*P. sylvestris* and *P. abies*), two for each mini-pot, were sown in 2 different trays, for a total of 416 seedlings. Two trays under 5 light type (4 LED light plus 1control light) for a total of 2080 seedlings.

A total of 104 seeds for each broad-leaved species (*Q. ilex, F. sylvatica, P. granatum and A. unedo*) were sown in 4 different trays for a total of 416 seedlings. Two trays under 5 light type (4 LED light plus 1control light) for a total of 2080 seedlings.

To investigate the kinetics of plant growth, each tray was considered for destructive analysis. The first sampling point was 14, 13, 29, 42, 21 and 22 days after germination (e.g.) depending on the plant species (*P. sylvestris, P. abies, Q. ilex, P. granatum, F. sylvatica* and *A. unedo* respectively). Afterwards, sampling was carried at an interval of 7 days (only in one case after 14 days), for needle leaved species and at an interval of not less than 6 days and not more than 12 days depending on the plant species, for the leaved species, for a total of four or five sampling point and four or five weeks of growth period.

2.1.2 PLANT MEASUREMENT (SHOOT HEIGHT, PLANT BIOMASS AND ROOT LENGTH)

At each sampling date, a statistically significant number of seedlings were considered for destructive direct analysis. 26 seedlings per tray (for the needle leaved tested), for a total of 104 and 130 seedlings per species (respectively for *P. sylvestris*, *P. abies*); and five seedlings per tray (for all the broad leaved species tested), for a total of twenty seedlings per species, were randomly collected at each sampling point. Before collecting plant height was manually measured with a wooden measuring stick from the base of the seedling to the highest leaf. Leaves, shoots, and roots from each seedling were oven drying (52 h at 75 °C) and weighed in order to measure total plant biomass. In order to estimate root length, roots were scanned and analysed by WinRHIZO Pro V. 2007d software (Fig 4)

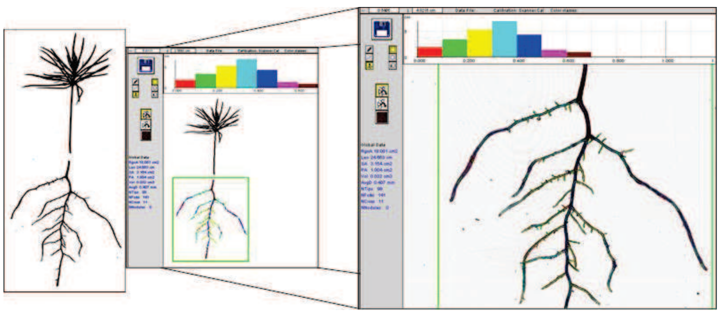


Figure 4

2.2 GROWTH PROTOCOLS OF NEEDLE LEAVED SPECIES (*PINUS SYLVESTRIS, PICEA ABIES*)

2.2.1 SEEDS PRE-TREATMENT, REMOVAL OF DORMANCY

Pinus sylvestris L. and *Picea abies* L. seeds were provided by the National Forest Service (National Center for Study and Conservation of Forest Biodiversity- Peri, IT),

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

2.2.2 SEEDS GERMINATION AND GROWTH CONDITIONS GERMINATION CONDITIONS

A total of 208 seeds for each species (*P. sylvestris* and *P. abies*), two for each mini-pot, were sown in 2 different mini-plug plastic container trays. Germination conditions for Scots pine and Norway spruce seed are schematically reported in the following sections

- Germination conditions:

Substrate: stabilized peat (Preforma VECO3, Jiffy® Products, Netherlands)

Light: 4 different LED lights (spectra) (Valoya) and Fluorescent light (or tube spectrum) (Osram) as a control

LED: - AP67 (bar, 2 lamps)

- AP67-3L (bar 1 lamp)
- G2 (bar, 2 lamps)
- NS1 bar (bar 1 lamp)

Control: OSRAM L36W/77 FLUORA (Fluorescent)

PAR: 120 μmol m^{-2} s^{-1} (All spectra were setted to give 120 μmol m^{-2} s^{-1} over the substrate surface)

Photoperiod: 16/8 hours (light/darkness)

Temperature: 21-26°C

Relative humidity: For both species: 80% during germination - 55-70% during growth

Watering: For both species the flood method was used with tap water until substrate saturation (from half an hour, to 1 hour) per two times a week so as to maintain constant water content in each tray.

2.3 GROWTH PROTOCOLS OF BROADLEAVED SPECIES (*QUERCUS ILEX FAGUS SYLVATICA, PUNICA GRANATUM, ARBUTUS UNEDO*)

Seeds pre-treatment protocols for *Fagus sylvatica* (Beech), *Quercus ilex* (Holm oak), *Punica granatum* (Pomegranate) and *Arbutus unedo* (Strawberry tree) are reported below. Protocols resulted from integration of 'Zephyr Deliverable 3.1 – New growth protocols' and seed provider indications.

2.3.1 SEEDS PRE-TREATMENT, REMOVAL OF DORMANCY

***Fagus sylvatica* L.**

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.

Quercus ilex L.

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.

Punica granatum L.

Seeds of *P. granatum* were hydrated by soaking them for 24 hours in tap water. Seeds were then placed in Petri dishes on bibulous paper at environment temperature and light for 10 days. After that were directly sowed.

***Arbutus unedo* L.**

Seeds of *A. unedo* 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 two 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 at 4°C for 2 months.

2.3.1 Seeds germination and growth conditions Germination conditions

A total of 104 seeds for each broad-leaved species (*Q. ilex*, *F. sylvatica*, *P. granatum* and *A. unedo*) were sown in 4 different mini-plug plastic container trays. Germination conditions for all broad-leaved species seeds are schematically reported in the following sections

- Germination conditions:

Substrate: sphagnum and sand (VigorPlant) pH = 6,4

Light: 4 different LED lights (spectra) (Valoya) and Fluorescent light (or tube spectrum) (Osram) as a control

LED:
- AP67 (bar, 2 lamps)
- AP67-3L (bar 1 lamp)
- G2 (bar, 2 lamps)
- NS1 bar (bar 1 lamp)

Control: OSRAM L36W/77 FLUORA (Fluorescent)

PAR: 120 µmol m^{-2} s^{-1} (All spectra were setted to give 120 µmol m^{-2} s^{-1} over the substrate surface)

Photoperiod: 14/10 hours (light/darkness)

Temperature: 21-22°C

Relative humidity: For all four species: 60-70% during growth

Watering: For both species the flood method was used with tap water until substrate saturation (from 15 to 30 minutes) per two times a week so as to maintain constant water content in each tray.

2.4 Seeds pre-treatment, of others Broadleaved species

Seeds pre-treatment protocols for the other broadleaved species *Prunus avium* (Wild Cherry), *Taxus baccata* (European Yew), *Abies alba* (Silver FIr), *Platanus orientalis* (Oriental Plane), *Corylus avellana* (Hazel), are reported below. Protocols resulted from integration of ‘Zephyr Deliverable 3.1 – New growth protocols’ and seed provider indications. For some species two alternative protocols are given.

These pre-treatments were not so efficient in order to remove dormancy of seeds.

Abies alba

Hydration (soaking per 24-hours)

Sowing after hydration (without further pre-treatment)

Acer pseudoplatanus

Hydration (soaking per 24-hours)

Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 10 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat.

Moist Stratification without medium (on bibulous paper) for 7 months at 4°C (Zephyr Protocol D 3.1)

Taxus baccata

Hydration (soaking per 24-hours)

Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 5 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat.

Moist stratification in perlite at 22°C for 3 months, then at 4°C for 4 months

Alternatively

Hydration (soaking per 24-hours)

Seeds surface sterilized for 2 min in 3.5% household bleach, and rinsed once with sterile water

Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 5 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat

Store at cold temperature (3°- 4°C) for 3 months

Corylus avellana

Hydration (soaking per 24-hours)

Seeds surface sterilized for 2 min in 3,5% household bleach, and rinsed once with sterile water

Treatment with “Teldor” fungicide: (3 ml in 1 l of water per 10 minutes). Afterwards dry seeds under hood for 3 hours in order to improve fungicide adhering to the seed coat

Store at cold temperature (3°- 4°C) for 2 months

Platanus orientalis

Hydration (soaking per 24-hours)

Seeds surface sterilized for 2 min in 3,5% household bleach, and rinsed once with sterile water

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

Cold stratification in perlite at 4°C for 2 months (Zephyr)

2.5 Growth protocols of three Azorean plant species (*Frangula* azorica, *Morella faya* and *Prunus azorica*)

Three Azorean plant species *Frangula azorica* (Tutin), *Morella faya* (Ait.) Wilbur, and *Prunus azorica* (Hort. ex Mouillef.) Rivas Mart., Lousa, Fer. Prieto, E. Dias, J.C. Costa, C. Aguiar, were tested. Seeds germination and growth protocols of the Azorean endemic species are almost unknown. In the present work, one of existing Azorean species protocol was modified. Others protocols were developed according to protocols developed for the same *genus* and similar ecological characteristic species. *Frangula azorica* and *Morella faya* protocol based on the protocol indications of *Frangula alnus Myrica rubra* respectively and ongoing experiments. *Prunus azorica* protocol based on the existent protocol indications and ongoing experiments.

2.5.1 Seeds pre-treatment, removal of dormancy

***Frangula azorica* (Tutin) (Sanguinho)**

Frangula azorica seeds were provided by Azorina Sociedade Gestão Ambiental e Conservação da Natureza, of the year 2014. *Frangula azorica* seeds were mixed in a moist draining substrate with 50/50 mixture of compost and sharp sand with enough volume of material to keep the seeds separated. After that the seeds mixture were placed in a clear plastic bag (freezer bags) closed but with a little gap left to provide air exchange. Later, seeds were kept in refrigerator at 4°C for two months.

Following seeds sowed in mini-plug plastic trays with a growth substrate made of sphagnum and sand and transferred in the environmentally controlled growth chamber and under different LED light type.

***Morella (Myrica) faya* (Ait.) Wilbur (Firetree)**

Morella faya seeds were provided by Azorina Sociedade Gestão Ambiental e Conservação da Natureza, of the year 2014. Seeds are orthodox and for breaking the dormancy were pre-treated in warm (moist sphagnum) stratification for 8 weeks. Seeds of *M. faya* were mixed with moist sphagnum (water content of the sphagnum was about 400% of dry mass). Sphagnum was boiled per half an hour to sterilize it, and left become cold before to mix seeds. Then seeds were sealed inside polyethylene bags (0.04 mm in thickness) and incubated at a day/night temperature of 30/20°C with a 12-h photoperiod supplied by fluorescent light (100-120 μmol). After the two months of stratification seeds were treated with 5.2 mM GA3 solution at room temperature before germination. Seeds washed before in a strainer to remove the stratification substrate. A solution with 5.2 mM GA3, with 0.54 gr of GA3 (potassium salt) and 300 ml of distilled water (ddH_2O), was prepared. Washed seeds kept in the GA3 solution per 20 h at room temperature. Following seeds directly sowed in mini-plug plastic trays with a growth substrate made of sphagnum and sand and transferred in the environmentally controlled growth chamber at the same photoperiod and temperature conditions of the warm stratification waiting the germination.

***Prunus azorica* (Hort. ex Mouillef**

seeds were provided by Azorina Sociedade Gestão Ambiental e Conservação da Natureza, of the year 2014. After endocarp removal, *Prunus azorica* seeds were transferred into an incubator at temperature of 10°C with a photoperiod of 12 hours provided by a fluorescent lamps (19 - 22 μmol $m^{-2} \cdot s^{-1}$) for 3 month. After that no one radicle appeared so no one seed germinated but one seed was transferred into each cell of the mini-plug trays and transferred into the growth chamber in environmental controlled conditions and under different LED light to try to stimulate the germination.

2.5.2 SEEDS GERMINATION AND GROWTH CONDITIONS

A total of 208 seeds for each Azorean species two for each mini-pot were sown in 2 different trays for a total of 416 seedlings. Two trays under 6 light type (5 LED light plus 1control light).

Germination and rate conditions for Azorean species seeds are schematically reported in the following sections.

Prunus azorica seeds dormancy was not removed, despite the pre-treatment applied because after 3 months none seedling appeared and the trays were full covered by algae and mildew.

- Germination conditions:

Frangula azorica and *Morella faya* seeds were sowed in the mini-plug trays and left to germinate and grow for a period of 43 days and 66 days from the sowing day respectively. An environmental settings follow reported

Substrate: sphagnum and sand (VigorPlant) pH = 6,4

Light: 5 different LED lights (spectra) (Valoya) and Fluorescent light (or tube spectrum) (Osram) as a control

LED: - AP67 (bar, 2 lamps)
- AP67 V1 model L20 (tube, 6 lamps)
- AP67-3L (bar 1 lamp)
- G2 (bar, 2 lamps)
- NS1 bar (bar 1 lamp)

Control: OSRAM L36W/77 FLUORA (Fluorescent)

PAR: 90-100 µmol m^{-2} s^{-1} (over the substrate surface)

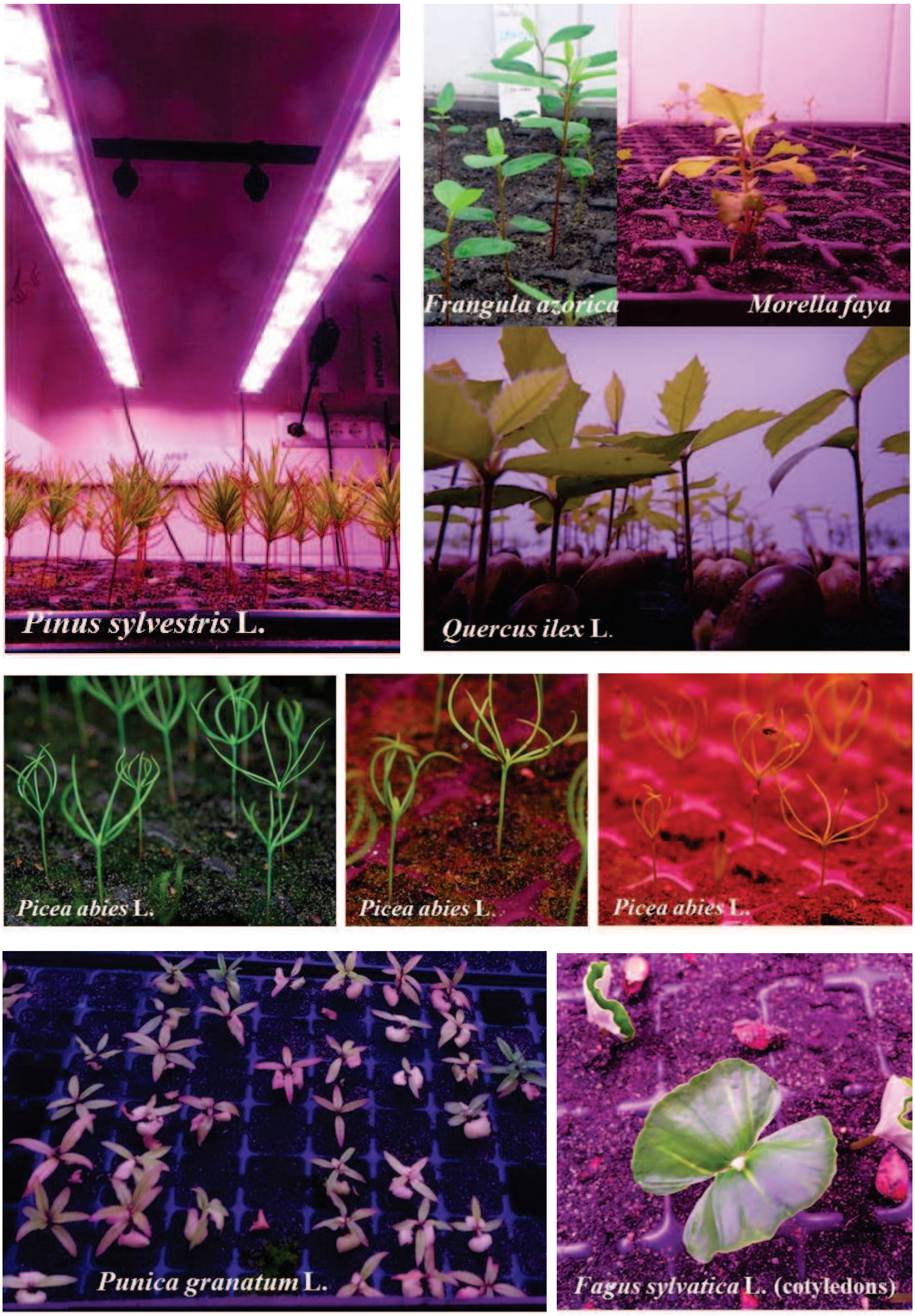
Photoperiod: 12/12 hours (light/darkness)

Temperature: 30/20°C day/night

Relative humidity: For both species: 70 +/- 10% during growth

Watering: For both species the flood method was used with tap water until substrate saturation (from 10 to 15 minutes) per two times a week.

Seedling of different species growing under different LED light types



Optical Sensors

2.5.3 Plant growth with optical sensors

2.5.3.1 Experimental design

In order to evaluate the efficiency of the optical sensors for the indirect plant phenotyping, six species, four broadleaved (*Quercus ilex* L. and *Fagus sylvatica* L. *Punica granatum* L. and *Arbutus unedo* L.) and two needle-leaved (*Picea abies* L. and *Pinus sylvestris* L.) were tested. We tested pomegranate seedlings (*Punica granatum* L.) for the reddish colour of their leaves, which would provide us with an efficiency test able to indicate if the software developed could be used also when growing non green-leaved plant species. Plants were grown under fluorescent light (FLUORA T8), yielding approximately 120 $\mu molm^{-2}\ s^{-1}$ at tray height. Seed germination was 78 % for *Q. ilex*, 66% for *F. sylvatica*, 78 % for *P. sylvestris* and 96% for *P. abies*.

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 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.

A certain number of plants, from which information can be obtained, are contained in the measured field of view by optical cameras. In order, to find out the minimum number of seedlings representative for plant growing of the entire tray, different numbers of seedlings (1, 2, 4, 9, 16, 36 and 104-full tray) were analysed for greenness for each plant species. This information is important for optimal positioning of the camera system in relation to the plants to be measured as well as optical components to be used (Figure 5).

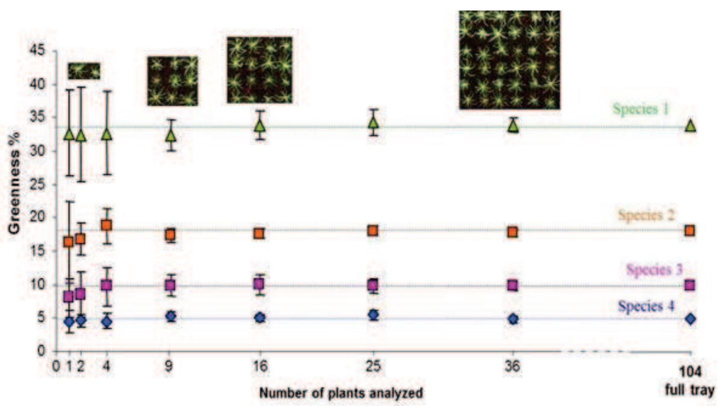


Fig. **5** Influence of the number of plants in the filed view on the greenness measured values.

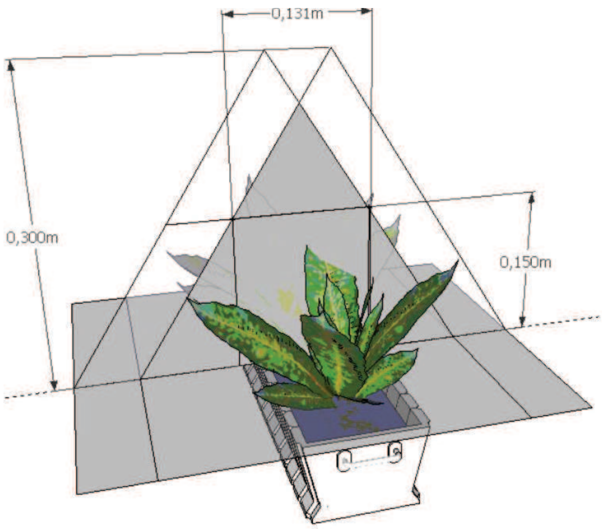
2.5.3.1 Optical Sensors - Image capture system and analysis

Optical sensing system is based on image acquisition and data processing using in-house developed algorithms using hue-saturation-brightness analysis of the image data. Shoot height sensing is based on analyses of reflected light by using a stereoscopic imaging system (Figure 6). 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. *Greenness* can be defined as percentage of shoot cover projected on tray ground and detected as amount of green pixels referred to leaves present on the tray compared to the darker pixels referring to the soil/tray.

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- brightness 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.

2.5.3.1 Non-destructive analysis by optical sensors: plant height and greenness

The trays were manually moved into the image capture cabinets where one stereoscopic image – top-view – of each experimental half tray was taken (Figure 6). Shoot stereoscopic images were taken at the same time of the destructive sampling, (immediately before). After image capture, all images were analysed using uEyeDualcam and HeightMap (Acreo Swedish ICT). Plant greenness (%) were estimated by uEyeDualcam software (Figure 7) and then HeightMAP software (Figure 8) 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 mages. Therefore, the optical sensors acquired images of a plant tray, after that software measured two parameters: 1) greenness intensity detected as amount of green pixels referred to leaves present on the tray compared to the darker pixels referring to the soil/tray; 2) plant height detected as distance between soil and stem apex.



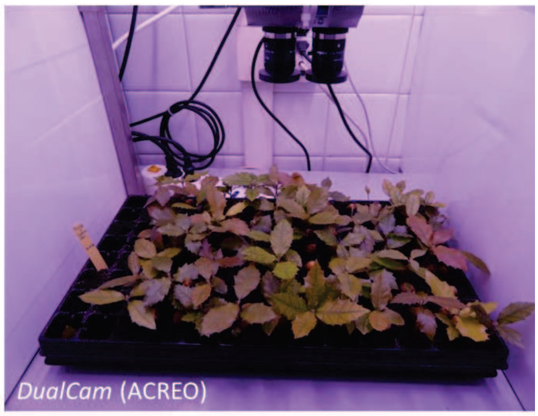


Fig. **6** (left) Schematics of optical sensing based on stereoscopic measurements. (right) Photos of the optical system for measuring the shoot height and 'greenness'; above showing the dual cameras for stereoscopic imaging.

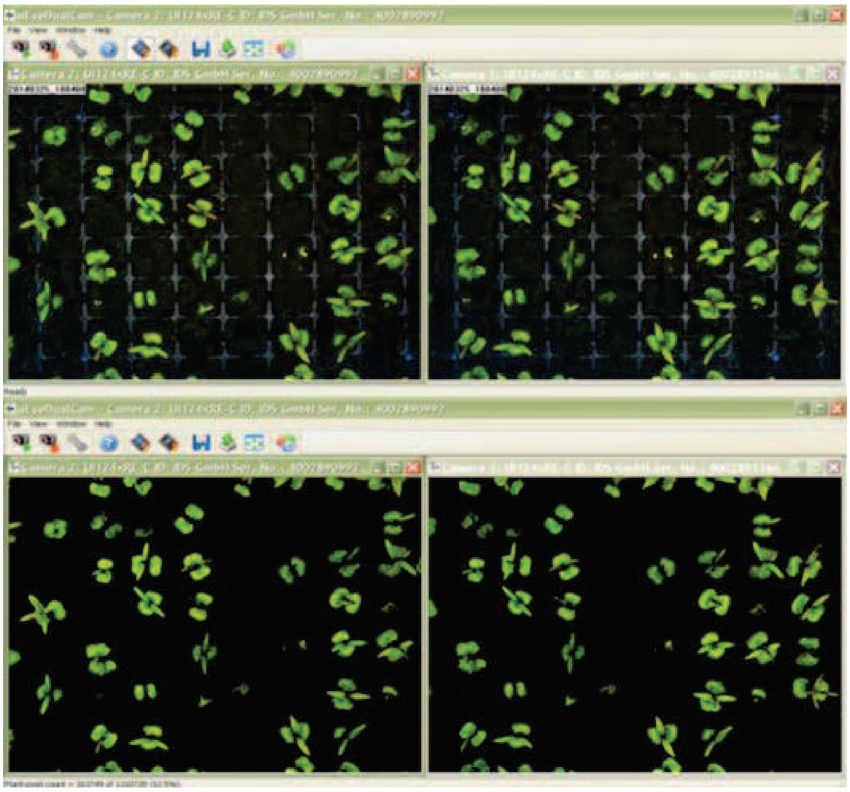


Fig. **7** Images of greenness analysis for Pomegranate (*uEyeDualCam* software)

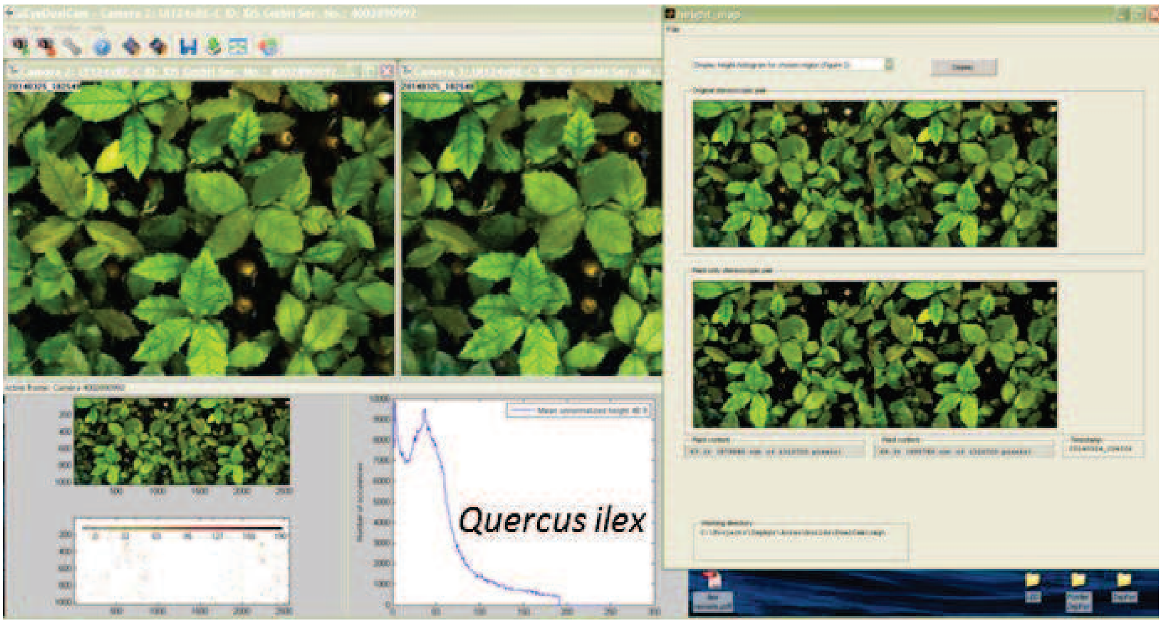


Fig. **8** Above, stereoscopic photo of Holm oak plant tray. Below, Height map and Height histogram obtain by *Height MAP* software analisys

2.5.3.1 Destructive analysis: plant biomass and shoot height

Five seedlings per tray, for a total of twenty seedlings per treatment-species combination, 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. Furthermore, 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.

2.6 WIFI Soil Sensors

2.6.1 Experimental design

In order to evaluate the soil water content measurements efficiency of the soil stick sensors, we have tested them in the trays with and without seedlings when watering was withheld for two weeks. The soil water content measurement obtained by the software "Zephyr logger" (Acreo) was compared with the SWC calculated by gravimetric measurements (θm, g g–1) based on weighing the amount of water lost (water mass - Mw) by oven drying the soil at 75°C until constant weigh.

2.6.2 Soil sensors characteristics

The soil stick sensors mainly consist in a 'two module' device: one module with the metal electrodes on ceramic substrate, (Silver electrode structures on Al2O3 substrate coated with dielectric glass-ceramic; Measurement Unit - MU), and one module for sensor electronics & communication & power (Communication Module - CM). The CM provides energy to the MU, a wide range of battery types (small factor size, high capacity, low voltage level) have been used in order to provide the sensor stick maximum autonomy.

2.6.2.1 Electromagnetic interaction

During the water content measurements a quick signal change on the performance curve was detected. This might be due to the electromagnetic interaction of the LED light on the soil stick sensors. In order to test the possible influences of the LED light electromagnetic field, soil water content measurement (SWC theta $\theta = m^3/m^3$) was measured in a pot with grass-type plant and a fixed stick sensor. Three different tests were carried: 1. LED lamp (NS1) was positioned at different distances from the pot; 2. LED lamp was positioned at 1 meters distance from the pot and the power pack at different distances (10, 20, 30, 40, 50 and 60 cm); 3. both LED lamp and power pack were positioned together at different distances (10, 20, 30, 40, 50 and 60 cm). In brief, the measurement of SWC was made every 10 cm from the pot per 15 minutes each until the maximum distance of 60 cm. During the 15 minutes measurement were made with the light on for 7 minutes and light off for 7 minutes.

3 RESULTS

3.1 SEED GERMINATION RATE OF NEEDLE LEAVED (*PINUS SYLVESTRIS*, *PICEA ABIES*) AND BROADLEAVED SPECIES (*FAGUS SYLVATICA*, *QUERCUS ILEX*, *PUNICA GRANATUM AND ARBUTUS UNEDO*)

3.1.1 SEED GERMINATION RATE OF NEEDLE LEAVED SPECIES (*PINUS SYLVESTRIS* L. AND *PICEA ABIES* L.)

During the growth chamber experiments for both needle leaved species the number of germinated seed after 21 days was compared to the total number of seeds (Figure 9)

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

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

Total seed germination was 59 % for *P. sylvestris* and 75% for *P. abies*.

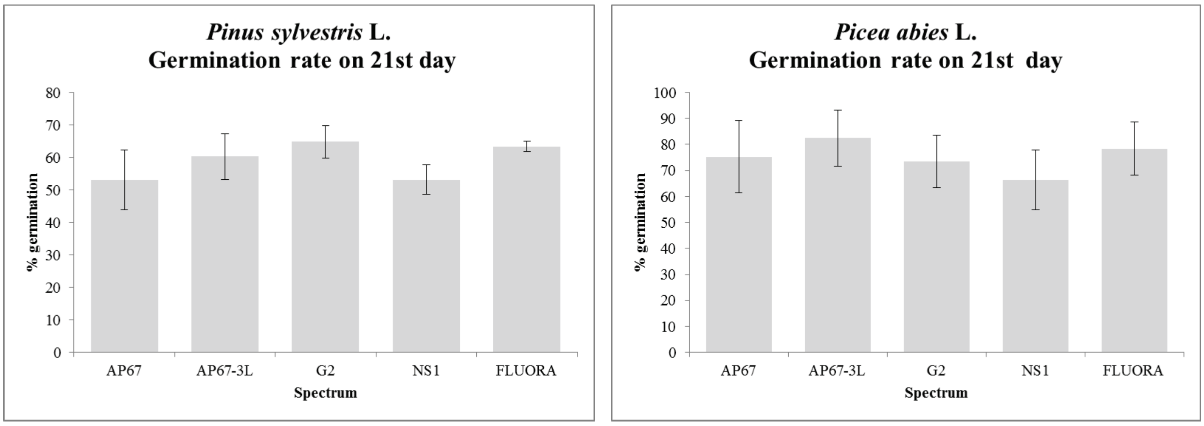


Figure 9 Seeds germination rate for *Pinus sylvestris* L. and *Picea abies* L. on the 21st day from sowing, under different LED light type

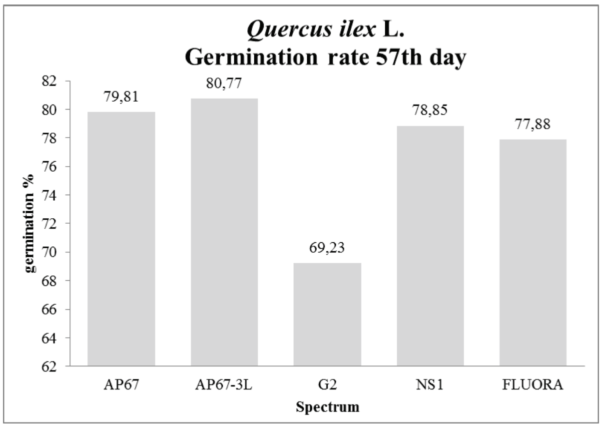
3.1.1 SEED GERMINATION RATE OF BROADLEAVED SPECIES (*QUERCUS ILEX* L., *PUNICA GRANATUM* L., *FAGUS SYLVATICA* L. AND *ARBUTUS UNEDO* L.)

During the growth chamber experiments for broadleaved species *Quercus ilex* and *Punica granatum* the number of germinated seed after 57 and 50 days respectively, was compared to the total number of seeds. Due to the heterogeneous growth the germination data was considered at the end of the experiment. (Figure 10)

In the case of *Quercus ilex* seeds the highest percentage of germination was observed under AP67-3L LED light type. Similar germination rate value was measured for AP67 and NS1. Moreover, germination rates for these LED light types were similar to germination rate measured under control light. Instead the lower germination percentage was obtained under G2 LED type.

In the case of *Punica granatum* seeds the highest percentage of germination was observed under AP67-3L LED light between LED types, but lower than control light. Similar germination values were measured for both AP67 and G2 LED light types. Instead the lower germination percentage was obtained under NS1 LED type.

Total seed germination was 77 % for *Quercus ilex* and 49% for *Punica granatum*.



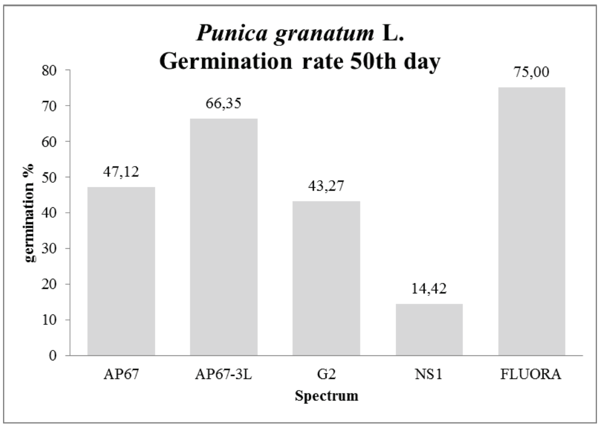


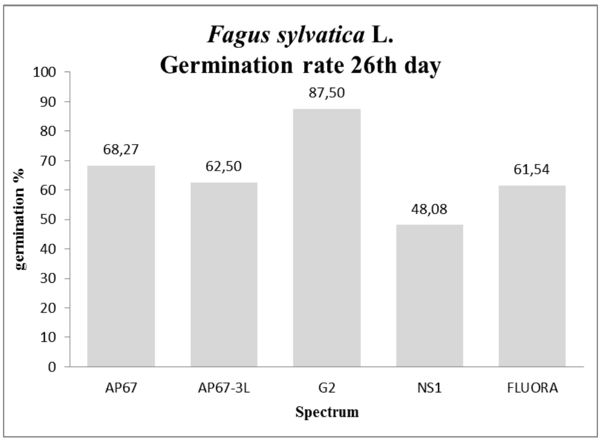
Figure 10 Seeds germination rate for *Quercus ilex* L. and *Punica granatum* L. on the 57^{st} and 50^{th} day from sowing, under different LED light type.

During the growth chamber experiments for broadleaved species *Fagus sylvatica* and *Arbutus unedo* the number of germinated seed after 26 and 11 days respectively, was compared to the total number of seeds (Figure 11)

In the case of *Fagus sylvatica* seeds the highest percentage of germination was observed under G2 LED light type (87%). Similar germination rate value was measured for AP67 and AP67-3L. Both LED light types were comparable to germination measured under control light. The lower germination percentage was obtained under NS1 LED type.

In the case of *Arbutus unedo* seeds the germination rate was very high already before the 21st day. The highest percentage of germination (98%) was observed under G2 LED type between LED light. Similar germination values were measured between all the others LED type. All germination value were highest than control light.

Total seed germination was 66 % for *Fagus sylvatica* and 92% for *Arbutus unedo*.



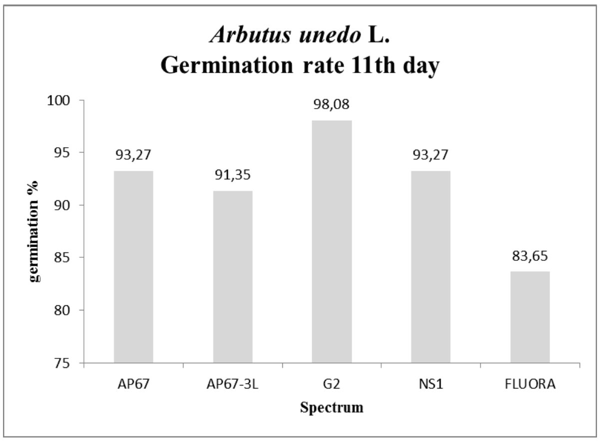


Figure 11 Seeds germination rate for *Fagus sylvatica* L. and *Arbutus unedo* L. on the 26th and 11th day from sowing, under different LED light type.

3.2 Seedlings growth performance under different LED illumination type (*Pinus sylvestris*, *Picea abies* *Quercus ilex*, *Punica granatum*, *Fagus sylvatica* and *Arbutus unedo*)Seedling height development

Following are reported data of seedling height development (at leaves level) during the experimental time for needle leaves (Figure 12) and broadleaved (Figure 13 and 14) species.

Data showed, for all species and all different light types, a linear increment of seedling height in time. For all six species final seedling height, at the end of the growth period, showed values higher than control light (FLUORA).

In the case of *Pinus sylvestris* and *Picea abies* seedling height showed first a fast growth and then a minimum variation, during the entire life time of 42 days. In the case of *P. sylvestris* seedling height showed similar values between all LED light types and under control light. In the case of *P. abies* seedling height showed the highest values under AP76 and AP67-3L LED light respect to control light.

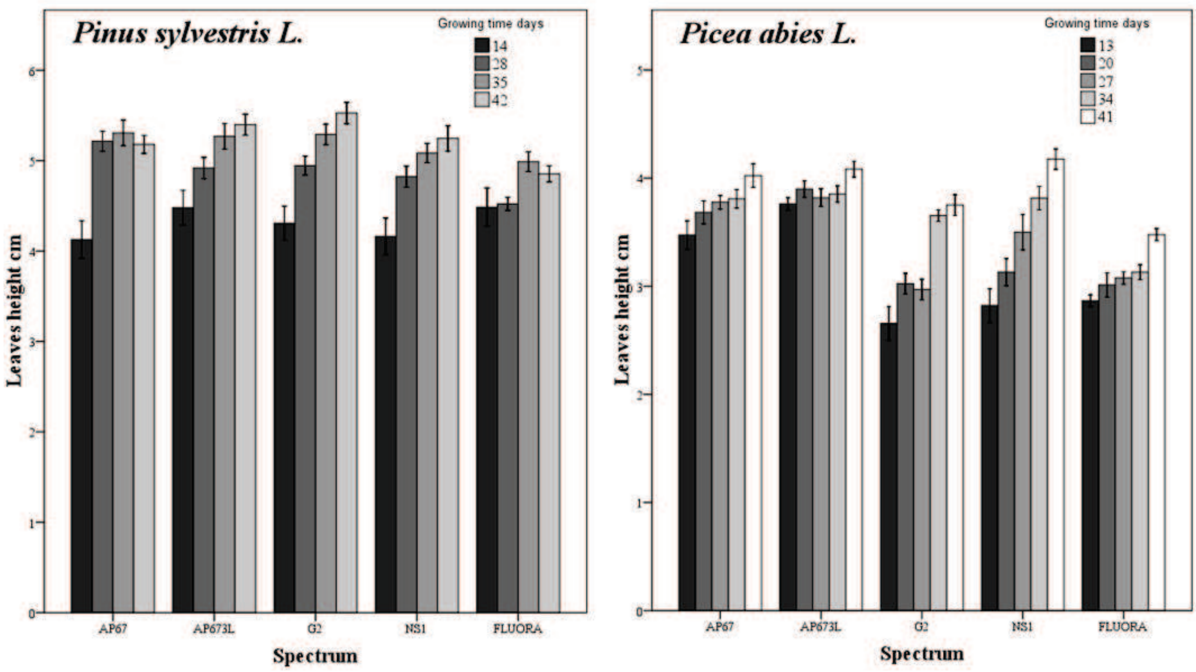


Figure 12 Leaves height measured every 7 days during the 4 weeks of growth for *Pinus sylvestris* L. and *Picea abies* L. seedlings

In the case of *Q. ilex*, *F. sylvatica* and *P. granatum* the highest values were obtained for seedlings growth under AP76, AP67-3L and G2 LED light types. In the case of *A. unedo* height under all LED light types showed values similar to control light with the only exception of height under G2 LED light type that showed the highest values. Moreover *Q. ilex* and *P. granatum* showed a slow growth in fact they reached a height of 9 cm and 4 cm respectively, in a period of 57 day. Instead *F. sylvatica* has a faster growth in a shorter period of 39 days. In this same period *A. unedo* reached a very short height of 2,5cm.

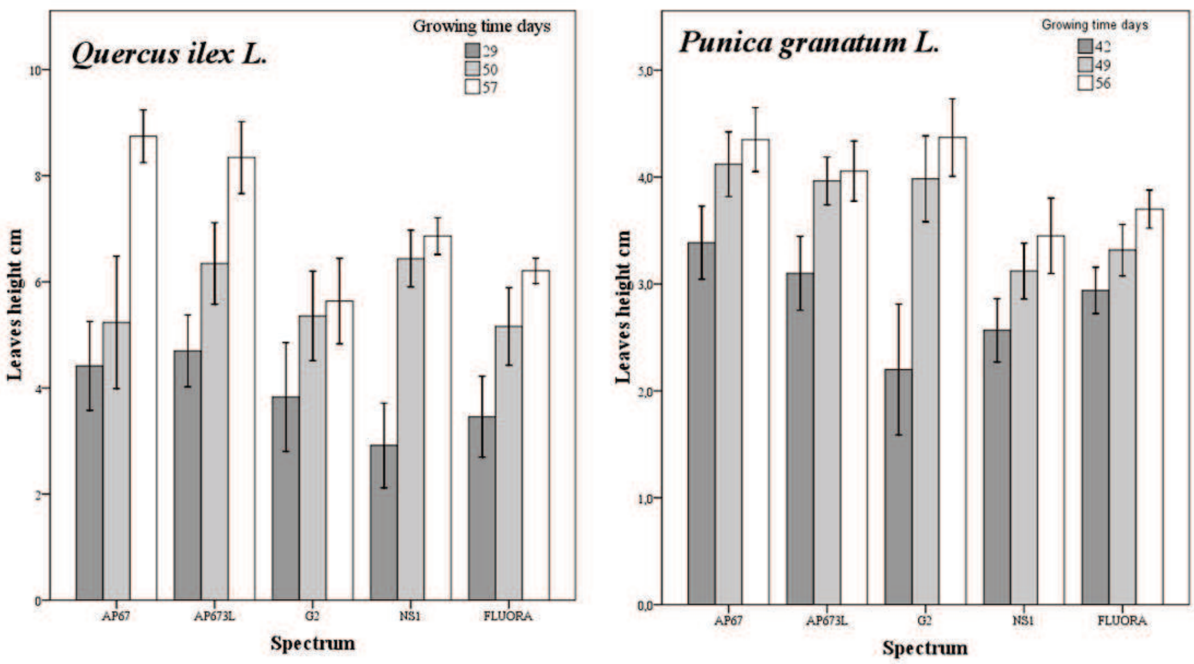


Figure 13 Leaves height measured during 57 days of growth for *Quercus ilex* L. and *Punica granatum* L. seedlings

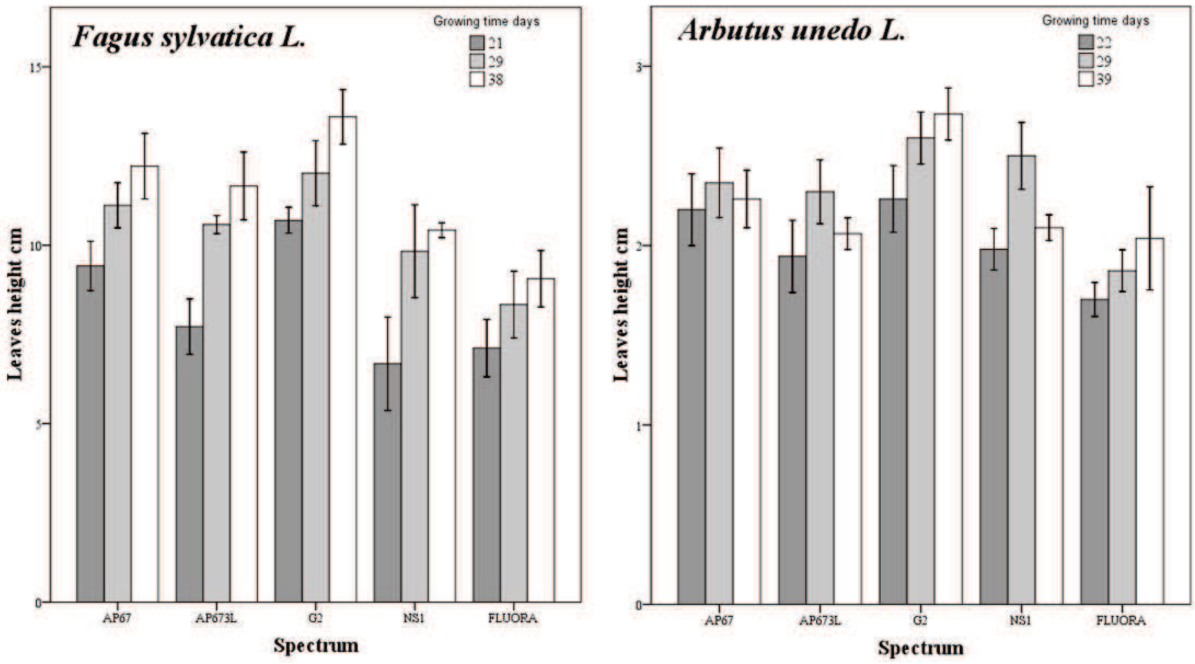


Figure 14 Leaves height measured during the growth period of 39 days for *Fagus sylvatica* L. and *Arbutus unedo* L. seedlings

3.2.2 SEEDLING ROOT LENGTH

Following are reported data of seedling root length development during the experimental time for needle leaves (Figure 15) and broadleaved (Figure 16 and 17) species.

For both *Pinus sylvestris* and *Picea abies* species, root length under control light showed the highest value from the beginning until the end of the growth period. Concerning LED lights, seedling growth under G2 light type showed the highest values of root length followed by both AP67 and AP67-3L. The lowest values were measured under NS1 LED type.

In the case of *Q. ilex* and *P. granatum* root length under all LED light types showed values similar to control light

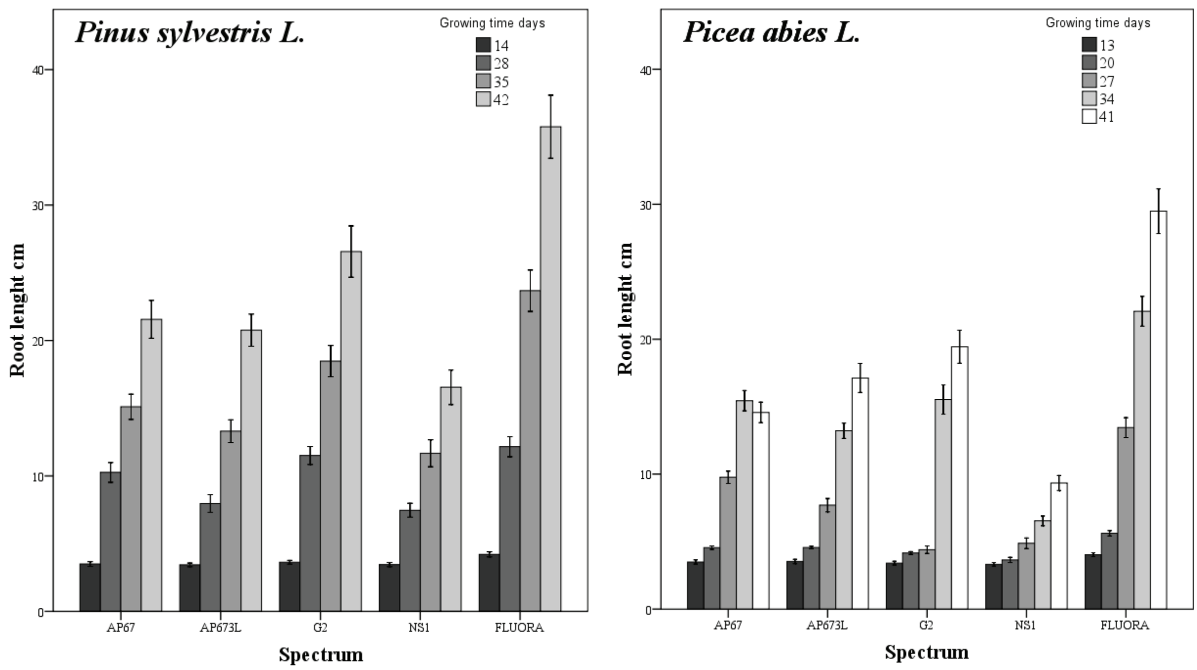


Figure 15 Root length measured every 7 days during the 4 weeks of growth for *Pinus sylvestris* L. and *Picea abies* L. seedlings.

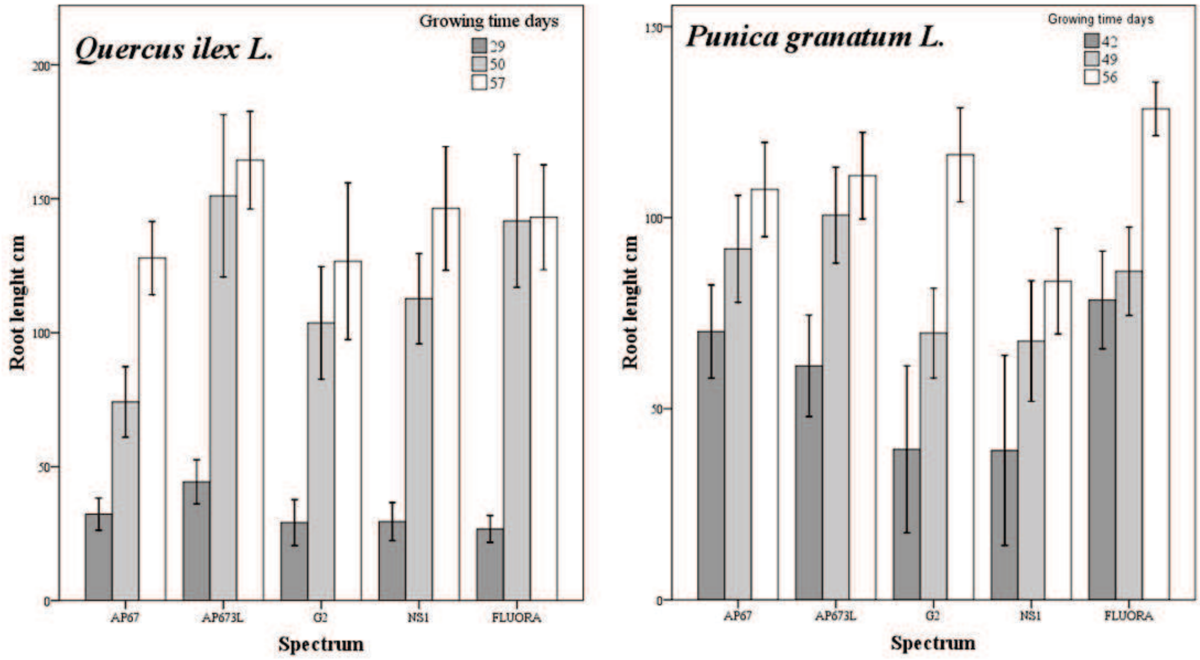


Figure 16 Root length measured during 57 days of growth for *Quercus ilex* L. and *Punica granatum* L. seedlings

In the case of *F. sylvatica* at the end of the growth period root length values are higher under AP67and NS1 LED light. Others LED types showed similar values to control light.

In the case of *A. unedo* root length showed similar values under all LED light types and under control light. The only exception of G2 LED light type showed slightly higher values of root length at the end of growth period.

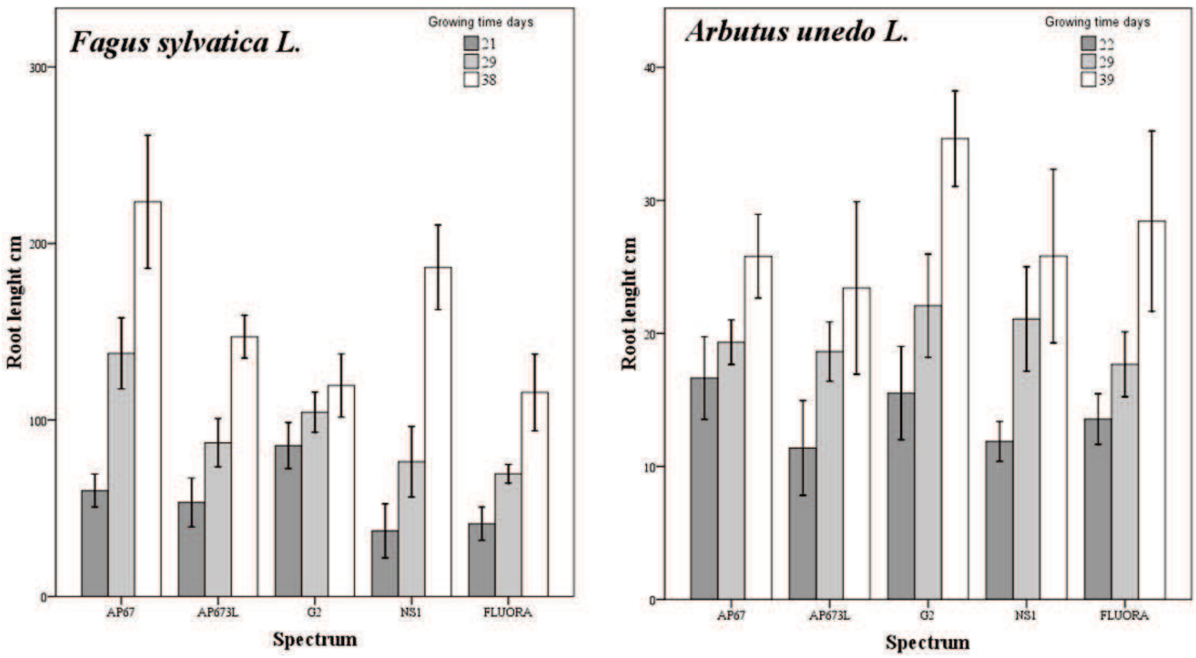


Figure 17 Root length measured during the growth period of 39 days for *Fagus sylvatica* L. and *Arbutus unedo* L. seedlings

3.2.3 SEEDLING BIOMASS DEVELOPMENT

Results on seedling biomass development during the experiment showed species-specific differences for total biomass and biomass of each seedling component.

Pinus sylvestris showed the highest values of biomass for seedlings growth under control light (FLUORA). Seedlings growth under both AP67-3L and G2 LED light types showed the highest values between all the other LED light types. Shoot biomass was 4 fold higher than root biomass (Figure 18 a, b).

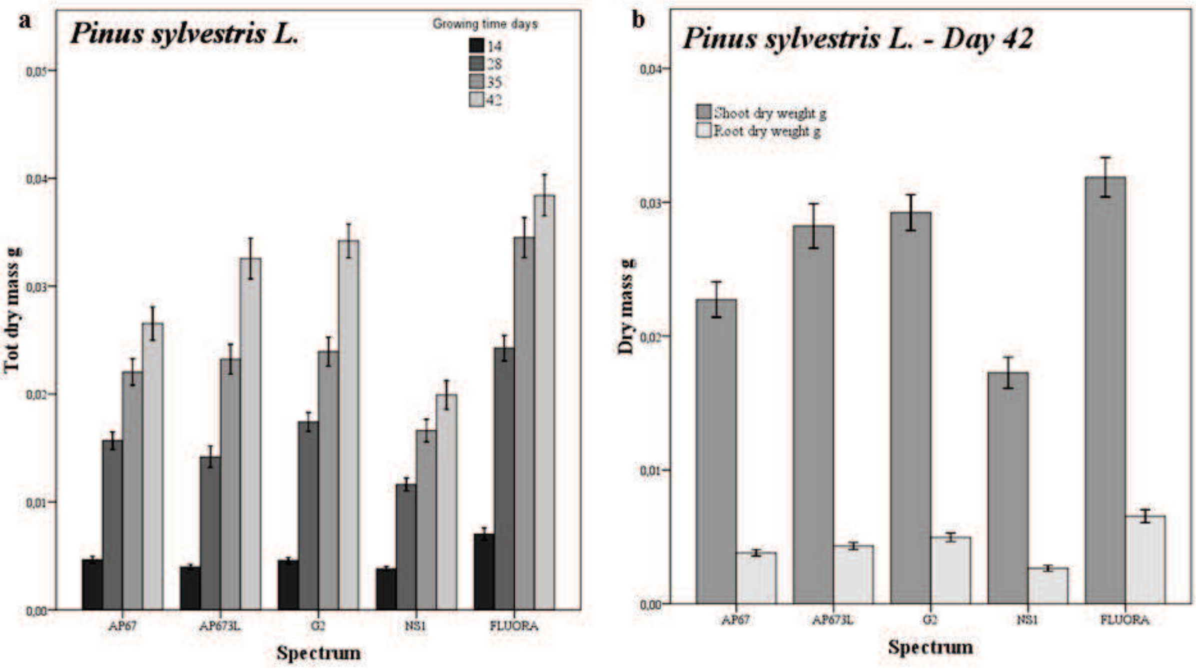


Figure 18 (**a**) total dry mass, (**b**) dry mass of shoot and root, measured every 7 days during the 4 weeks of growth for *Pinus sylvestris* L. seedlings

Picea abies showed the highest values of biomass for seedlings growth under control light (FLUORA). Seedlings growth under AP67 LED light type showed the highest values between all the other LED light types. Shoot biomass was 4 fold higher than root biomass. Total biomass showed that, with the only exception of NS1, all LED light type had similar values (Figure 19 a, b).

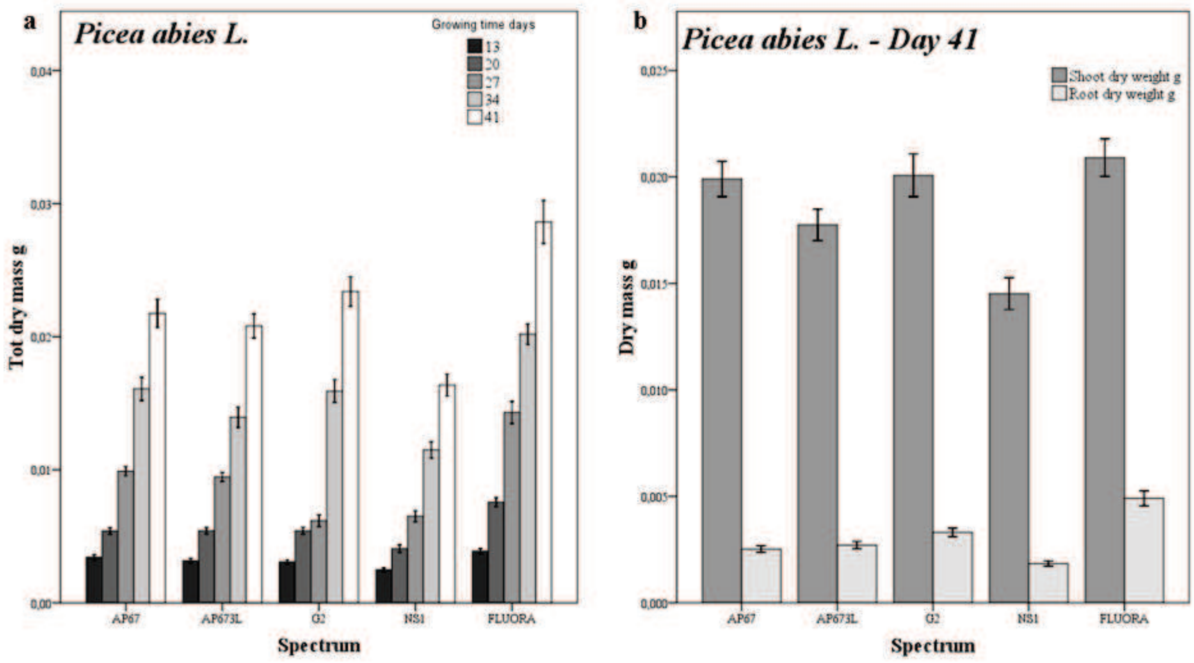


Figure 19 (**a**) total dry mass, (**b**) dry mass of shoot and root, measured every 7 days during the 4 weeks of growth for *Picea abies* L. seedlings

Quercus ilex showed the highest value for leaves biomass while the shoot dry mass was the lowest. Root dry mass showed intermediate values (Figure 20 b). Seedling growth under different LED light type showed differences in biomass component. In particular seedling growth under AP67-3L showed the highest values of leaves and root biomass compared to the other LED light type. Moreover, root biomass was almost similar to leaves biomass showing an higher development with this spectrum. Total dry mass for *Q. ilex* seedlings (Figure 20a) showed the highest values for plants growth under AP67-3L LED light type. Seedlings growth under all the other LED light type showed values similar or higher than control light (FLUORA) with the only exception of AP67-3L with values similar to AP67.

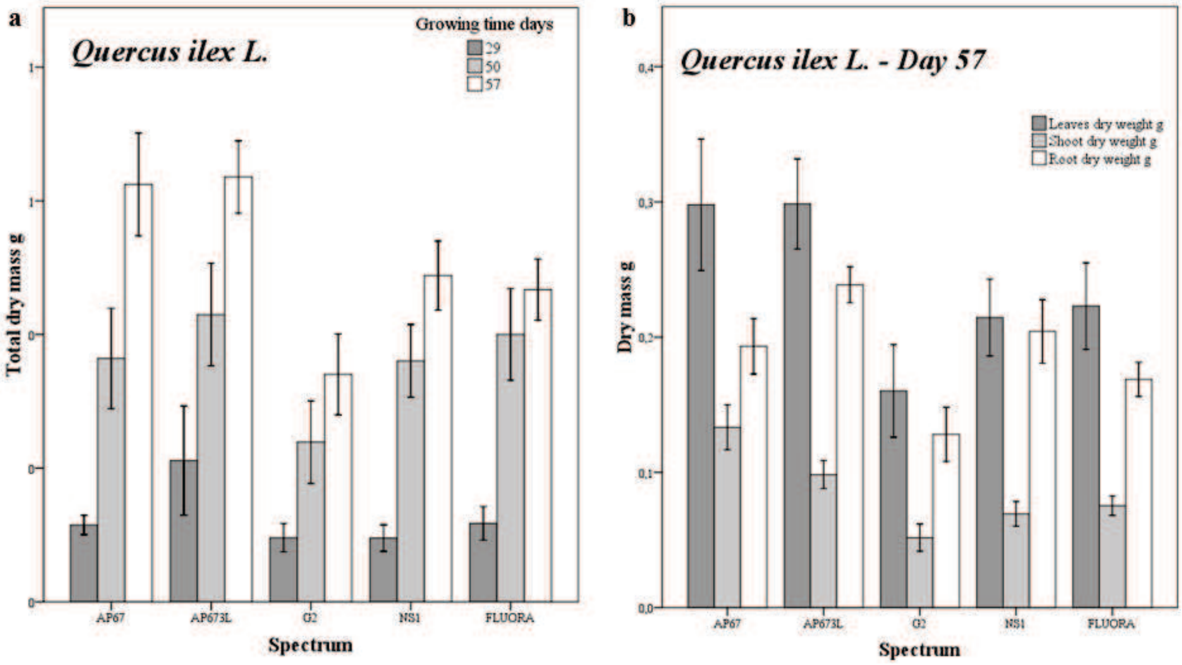


Figure 20 (**a**) total dry mass, (**b**) dry mass of leaves, shoot and root, measured during 57 days of growth for *Quercus ilex* L. seedlings.

Punica granatum showed similar biomass values for seedlings growth under AP67, AP67-3L and G2 LED light type. The lowest values recorded with NS1 In particular, leaves biomass resulted the highest of the three considered component (Figure 21 b). Root and shoot biomass were similar each other and did not show differences between different LED light type and with control light (FLUORA). Seedlings growth under FLUORA light type showed the highest biomass of all the LED light type (Figure 21a).

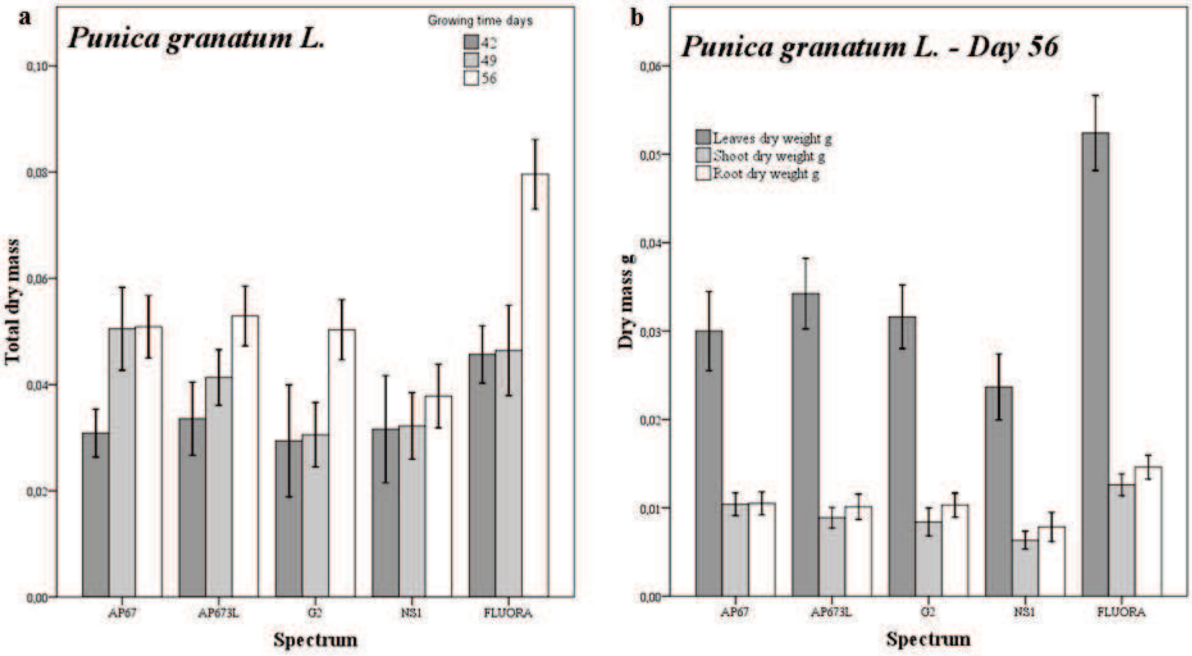


Figure 21 (**a**) total dry mass, (**b**) dry mass of leaves, shoot and root, measured during 57 days of growth for *Punica granatum* L. seedlings

Fagus sylvatica showed the highest value for the leaves biomass while shoot and root showed similar values each other almost 4 fold lower (Figure 22 b). Total dry mass for *F. sylvatica* seedlings (Figure 22 a) showed the highest values for plants growth under AP67 LED light type at the end of growth period. Seedlings growth under all the other LED light type showed values similar or higher than control light (FLUORA).

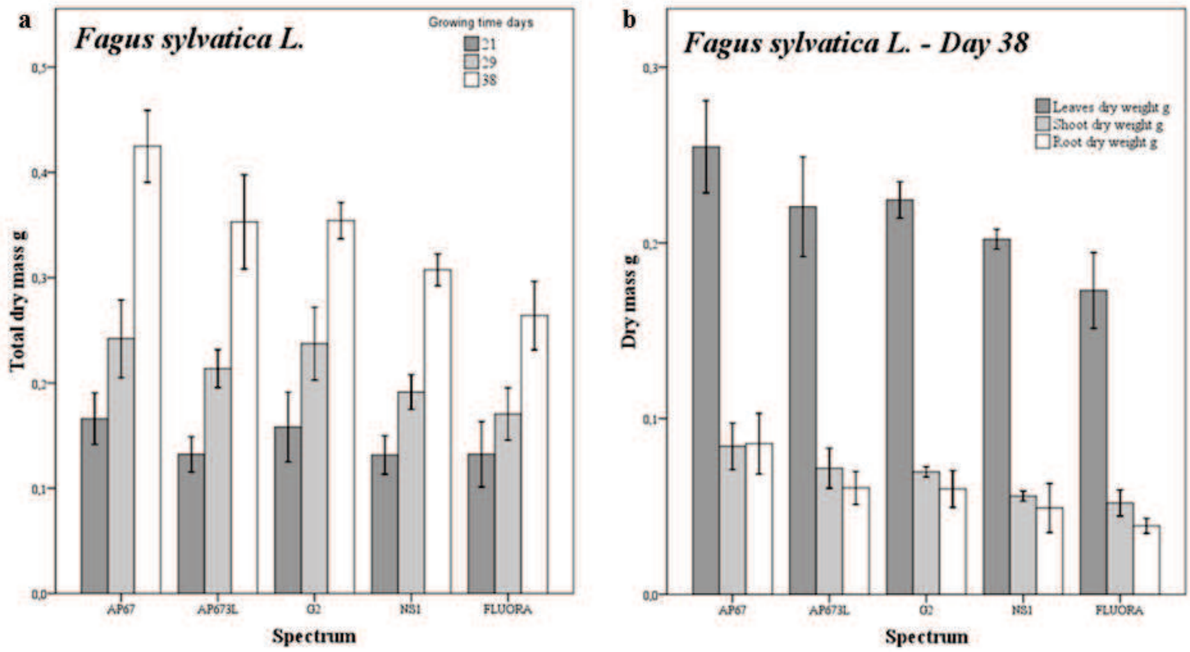


Figure 22 (**a**) total dry mass, (**b**) dry mass of leaves, shoot and root, measured during the growth period of 39 days for *Fagus sylvatica* L. seedlings.

Arbutus unedo showed slightly higher biomass values for seedlings growth under G2 LED light type. In particular, leaves biomass resulted the highest of the three considered component (Figure 23 b). Root and shoot biomass were similar each other with the only exception of G2 LED light type where root biomass was slightly higher than shoot biomass. Seedlings growth under all the other LED light type showed similar values of seedlings growth under control lights (FLUORA; Figure 23 a)

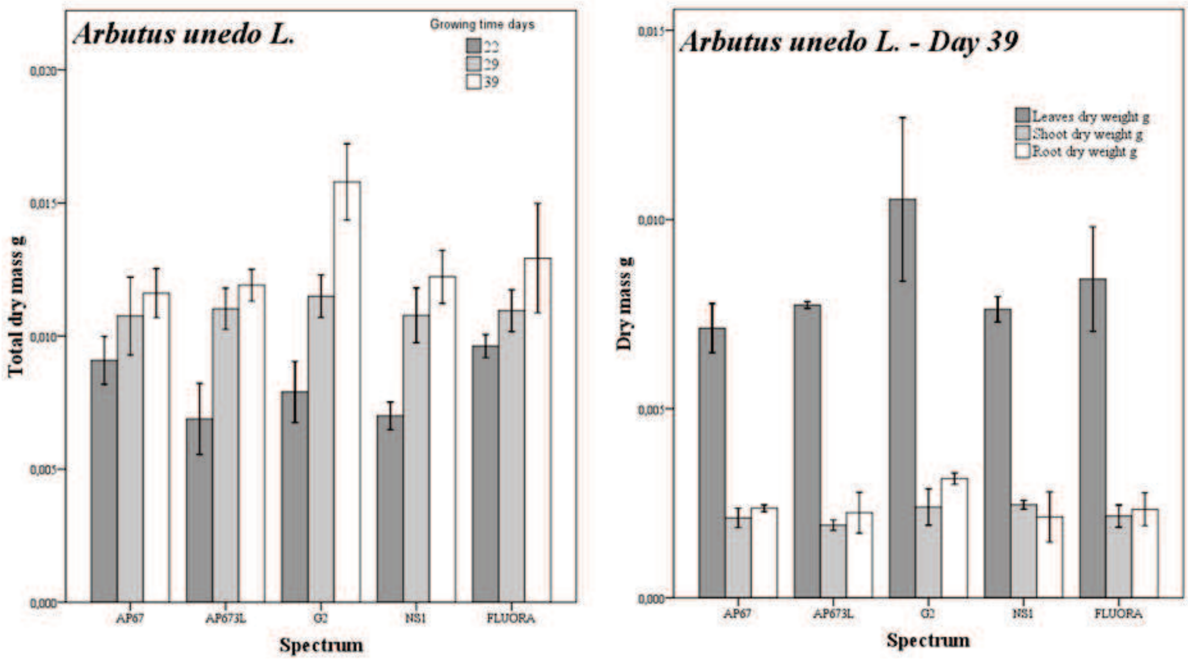


Figure 23 (**a**) total dry mass, (**b**) dry mass of leaves, shoot and root, measured during the growth period of 39 days for *Arbutus unedo* L. seedlings.

3.3 Seed germination rate and growth kinetics of Azorean species (*Frangula azorica*, *Morella faya* and *Prunus azorica*)

3.3.1 Seed germination rate

Unfortunately none of the pre-tread seeds of *Prunus azorica* germinated after the treatment under different LED light type. This might be due to the incubation period and would need more time to repeat the protocol. The other two species (*Morella faya* and *Frangula azorica*) showed a highly heterogeneous seed germination in time. In fact, *M. faya* showed a total of 81 seeds germinated in 66 days of treatment (Figure 24a) corresponding to a 6% circa of germination rate (Figure 24b). Finally, seed germination showed the highest value of germination (%; Figure 24c) with AP67 LED light type. Seeds under AP67-3L did not germinate at all underlining the strong influence of light type on the whole life cycle of seedlings.

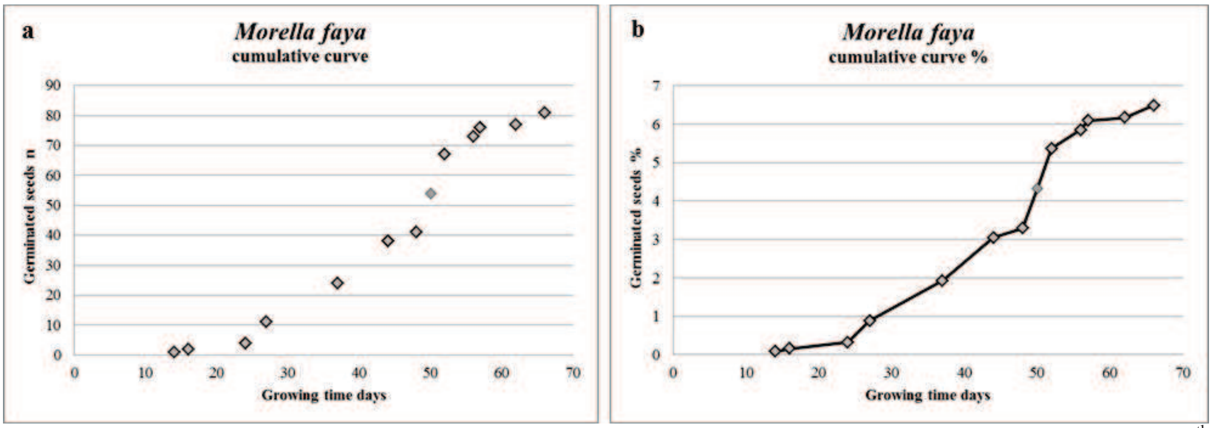


Figure 24 a) Cumulative curve of germinated seeds and **b**) seeds germination rate for *Morella faya* on the 66th day from sowing

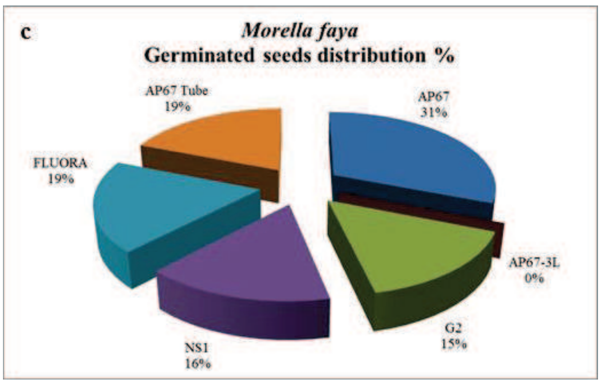


Figure 24 c) Germinated seeds distribution (%) for *Morella faya* on the 66th day from sowing, under different LED light type.

In the case of *F. azorica*, number of germinated seed was even lower than *M. faya* with a number of 54 seed germinated in 43 days of treatment (Figure 25a) corresponding to less than 4% of germination rate (Figure 25 b). Finally, seed germination showed the highest value of germination (%; Figure 3c) with control light (FLUORA), and between LED light, with AP67 LED type. Seeds under other LED types showed very low germination.

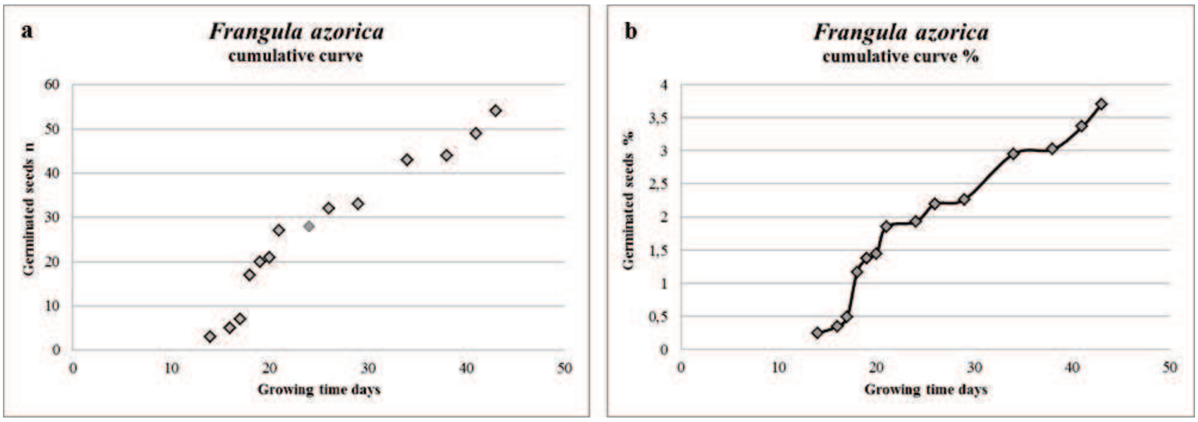


Figure 25 a) Cumulative curve of germinated seeds and **b**) seeds germination rate for *Frangula azorica* on the 43th day from sowing.

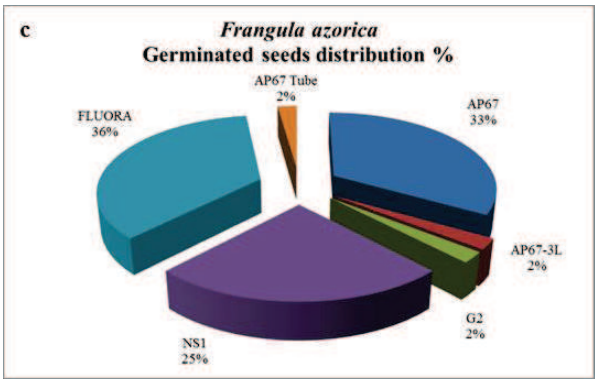


Figure 25 c) Germinated seeds distribution (%) for *Frangula azorica* on the 43th day from sowing, under different LED light type

3.3.2 MORPHOLOGICAL PARAMETERS

Direct measurements of total biomass, plant height, leaves area and root length, were carried by destructive sampling. Due to the heterogeneous seedlings growth, and to the reduced sample number, sampling was carried at the end of the growth period (98 and 43 days respectively for *Morella faya* and *Frangula azorica*); and all morphological parameters were considered as increment of each parameter and were calculated as ratio between each parameter and growing time (day).

In the case of *Morella faya* all considered morphological parameters (Figure 26 a, b, c and d) showed the highest value for seedlings grown under AP67 LED type. G2 and NS1 LED light types showed values similar to the control lights (FLUORA), only for leaves height values were slightly higher than control lights. The lowest value was found for AP67 tubes LED type, except in the case of the leaves height they are similar to the control light values.

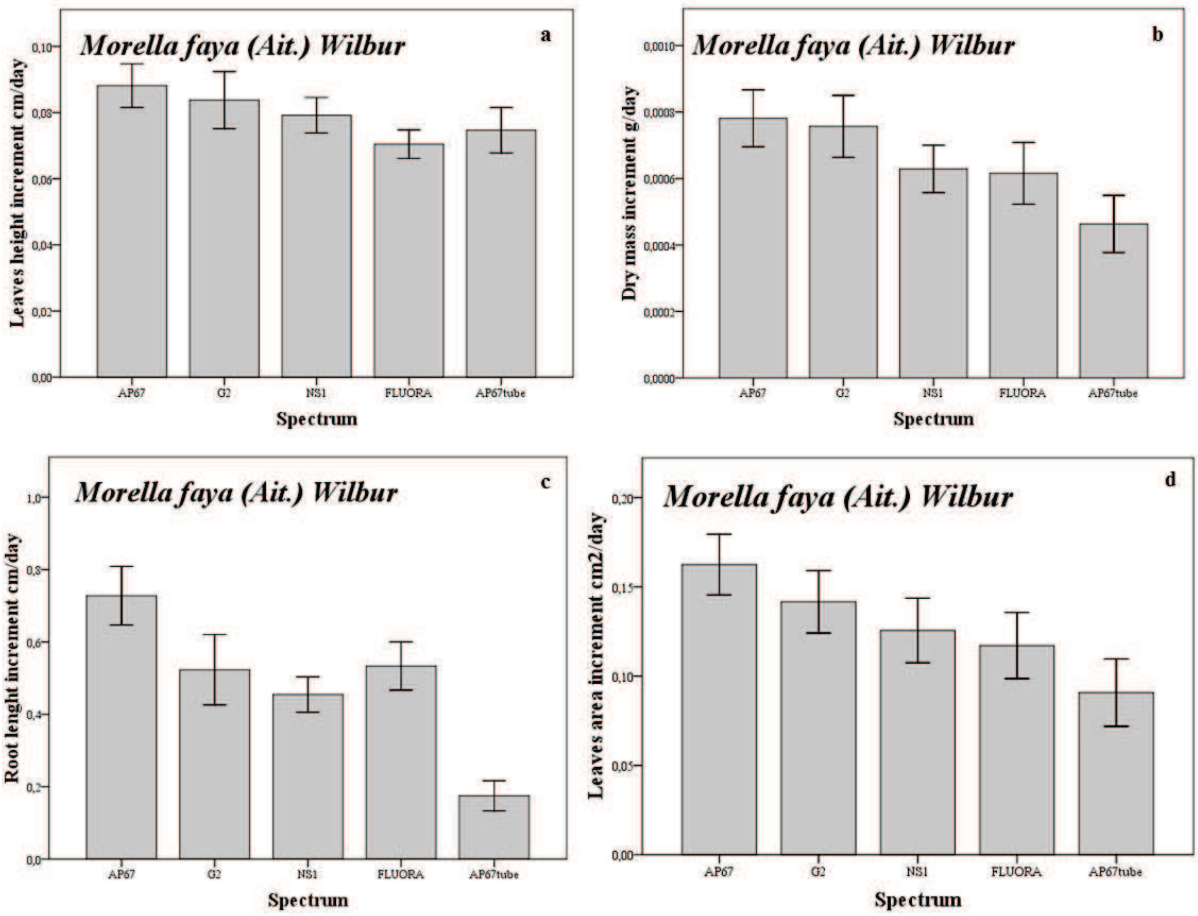


Figure 26 (**a**) Leaves height increment, (**b**) dry mass increment, (**c**) root length increment and (**d**) leaves area increment measured on the 98th days from the sowing for *Morella faya* seedlings.

In the case of *Frangula azorica* all considered morphological parameter (Figure 27 a, b, c, and d) showed that seedlings growth under AP67 LED light type had similar or higher values (plant height) than seedlings growth under control light (FLUORA). Seedlings growth under NS1 LED light type showed the lowest values for all considered parameters with the only exception of plant height. The other LED don’t compare due to the very low value. Standard errors bars were very high for both Total biomass and Root length parameters highlighting, as in the case of seed germination rate, the high variability of these seedlings of Azorean species.

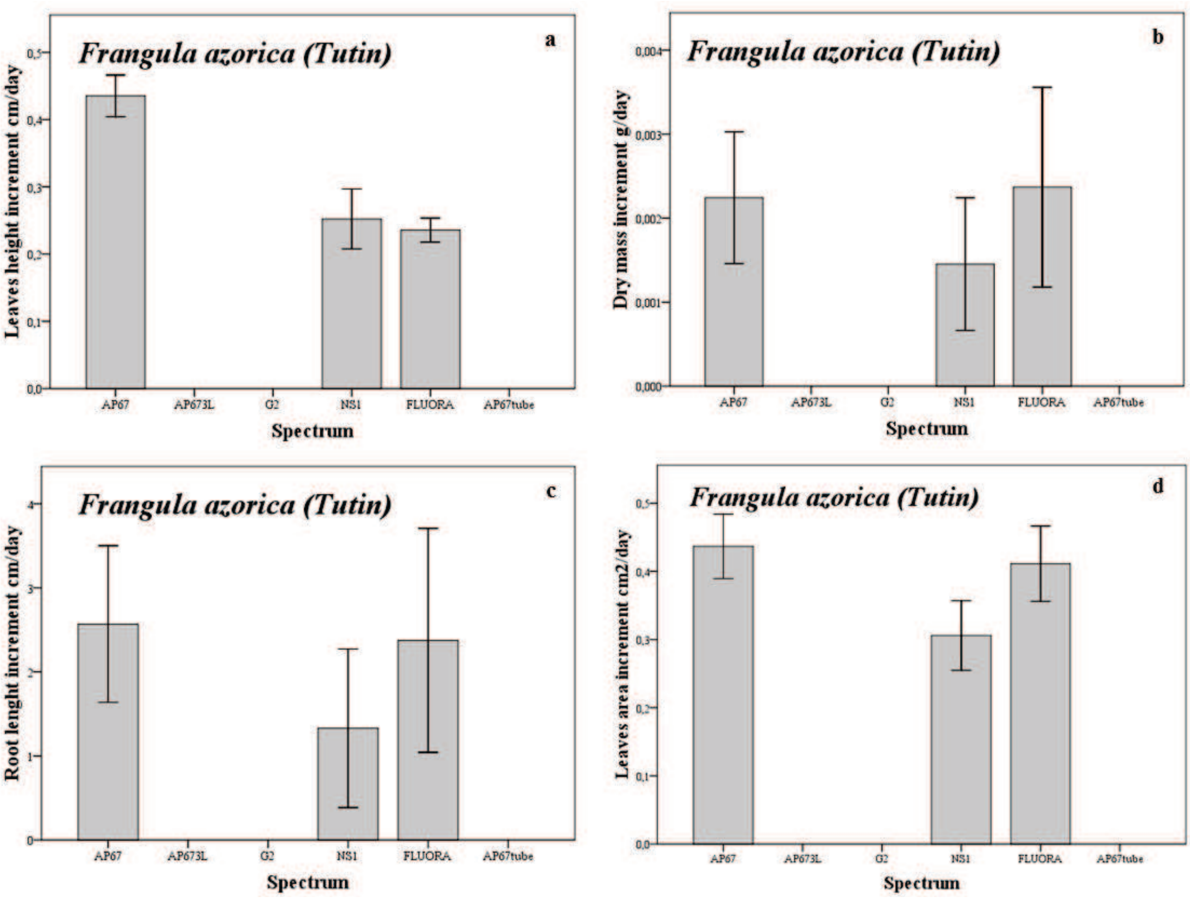


Figure 27 (**a**) Leaves height increment, (**b**) dry mass increment, (**c**) root length increment and (**d**) leaves area increment measured on the 43th days from the sowing for *Frangula azorica* seedlings.

3.4 Seedling phenotyping measurements with optical sensors in growth chamber

3.4.1 Shoot height and plant biomass

Shoot height throughout the experiment showed different pattern for needle- and broad-leaved species (Figure 28). In the case of both needle-leaved species, after the emergence of cotyledons, a significant increment of the plant height was not detected.

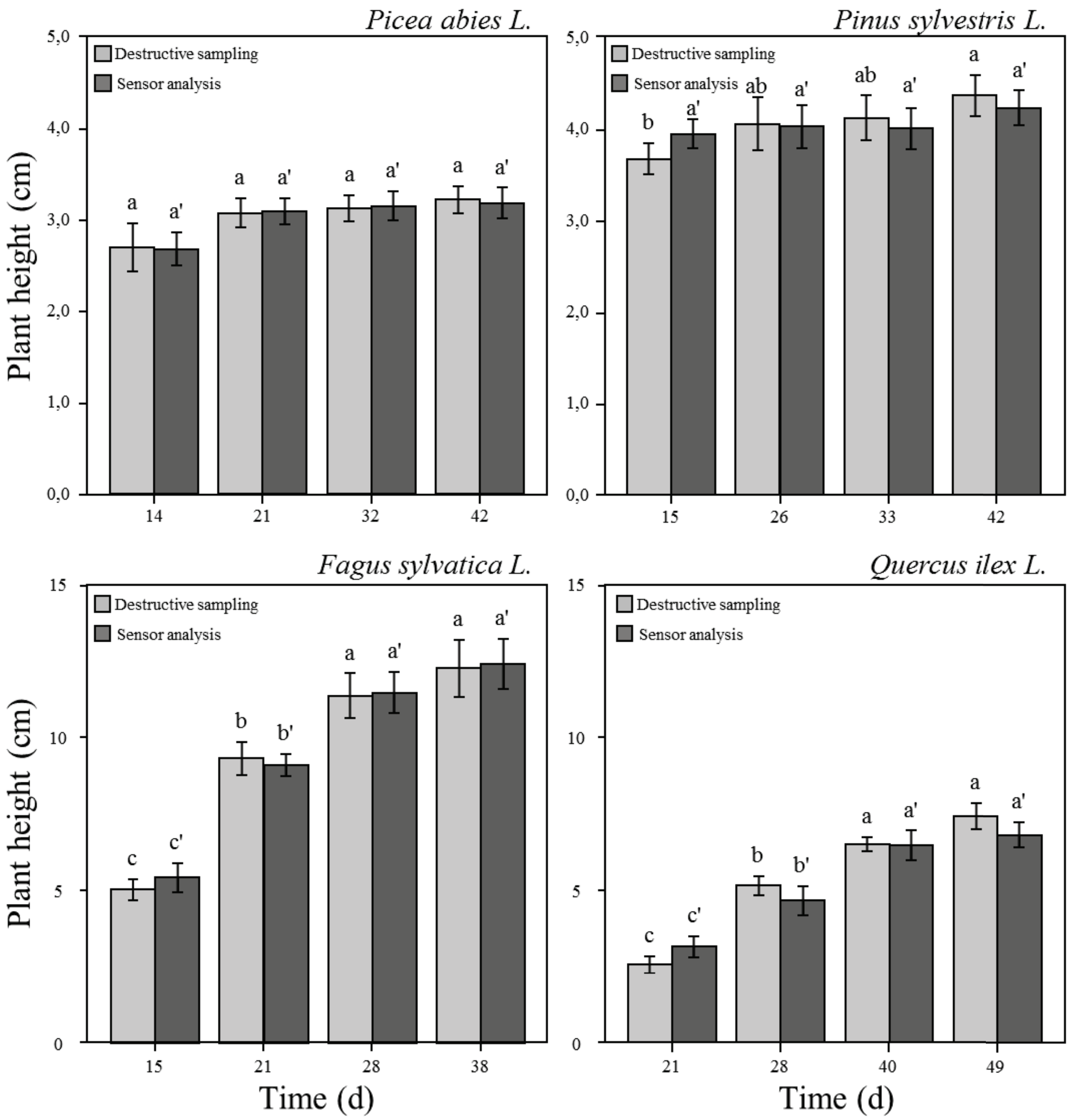


Figure 28 Direct (destructive sampling) and indirect (sensor analysis) measurement of seedlings height during the growth period for *P. abies*, *P. sylvestris*, *F. sylvatica* and *Q. ilex* plant species.

In particular, plant height for *P. abies* reached the maximum value of 3 cm at the first sampling point (day 14^{th} e.g.), without further increment during the duration of the experiment. Seedlings of *P. sylvestris* as *P. abies* reached the maximum height at the first sampling point (day 15^{th} e.g.) with a slight increment detectable at the last sampling point (day 42^{nd} after germination) (Figure 28).

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} e.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 28).

Concerning the plant biomass development, all four species showed a linear increase throughout the experiment (Figure 29). Moreover, the two broad-leaved species were characterized by a total biomass 10-fold higher than needle-leaved species.

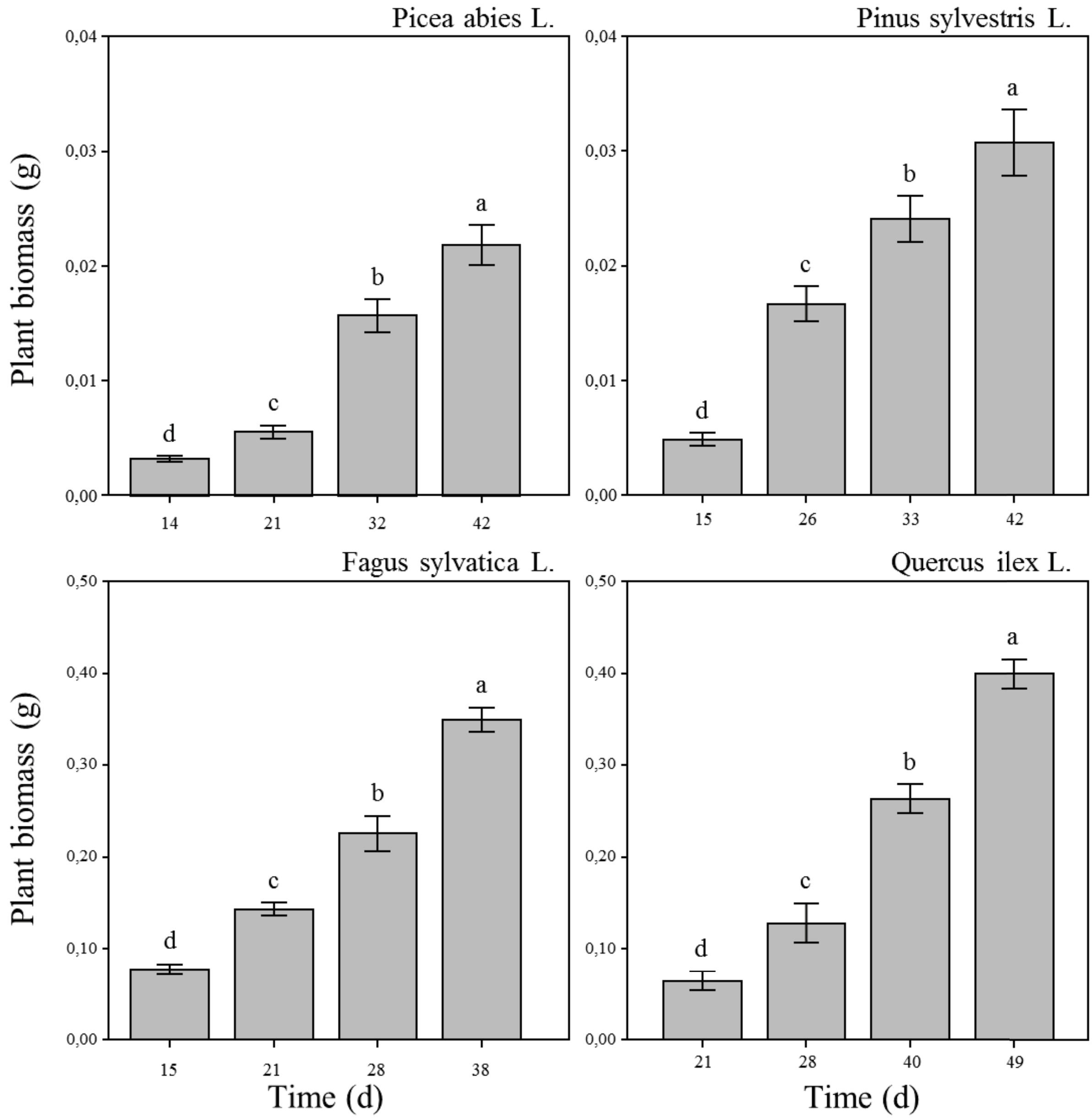


Figure 29 Seedling biomass destructive measurements during the growth period for *P. abies*, *P. sylvestris*, *F. sylvatica* and *Q. ilex* plant species.

3.4.1 SHOOT GREENNESS

Seedlings greenness (Figure 30) 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^{nd} e.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^{th} e.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^{nd} and 49^{th} e.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^{th} e.g. while *Q. ilex* covered the 80% of trays at day 49^{th} e.g.. On the opposite, *P. abies* and *P. sylvestris* covered less than 7% of the total trays area in 42 days.

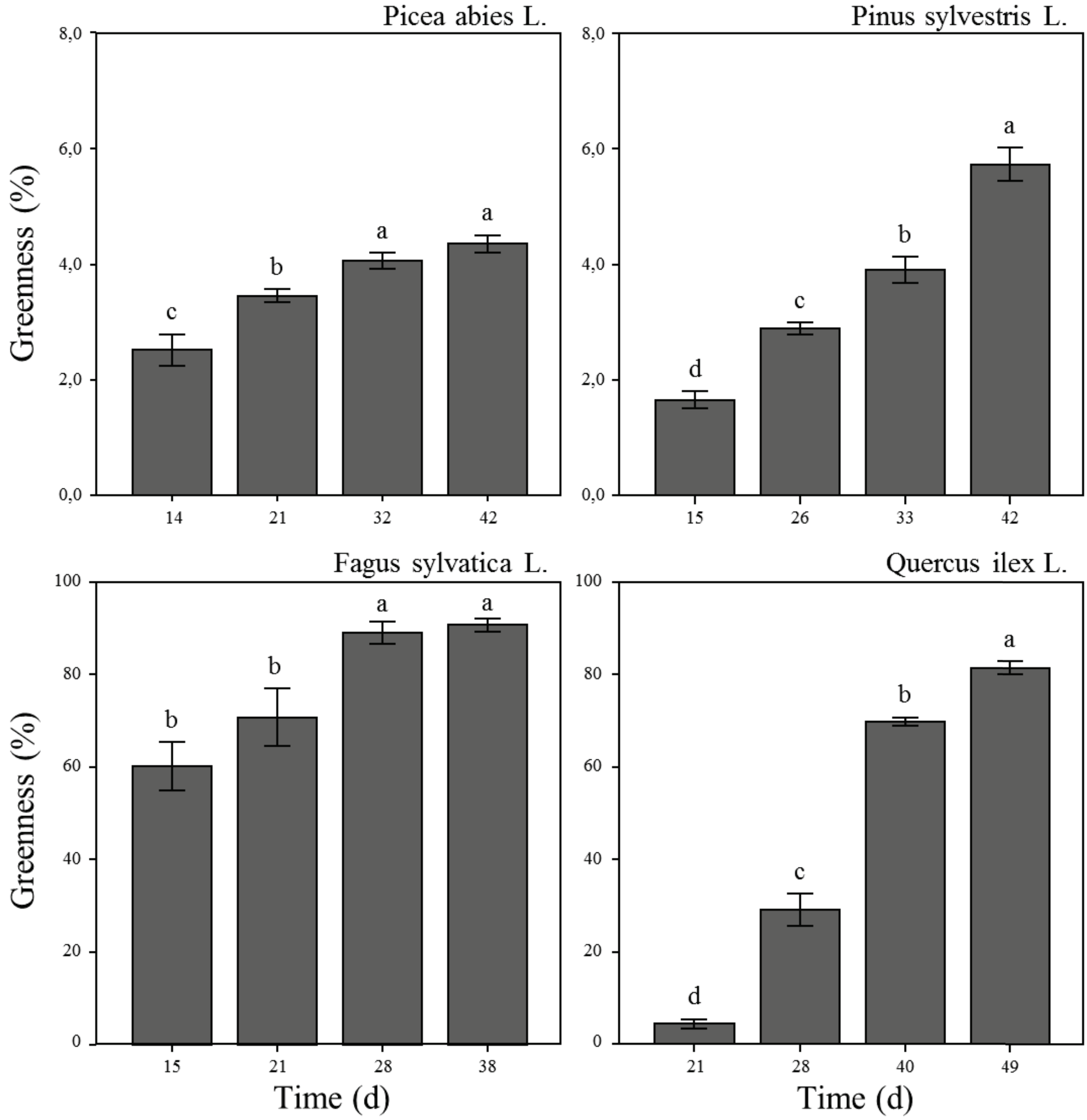


Figure 30 Greenness measurements during the growth period for *P. abies*, *P. sylvestris*, *F. sylvatica* and *Q. ilex* plant species.

3.4.2 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 (Figure 31a) 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.

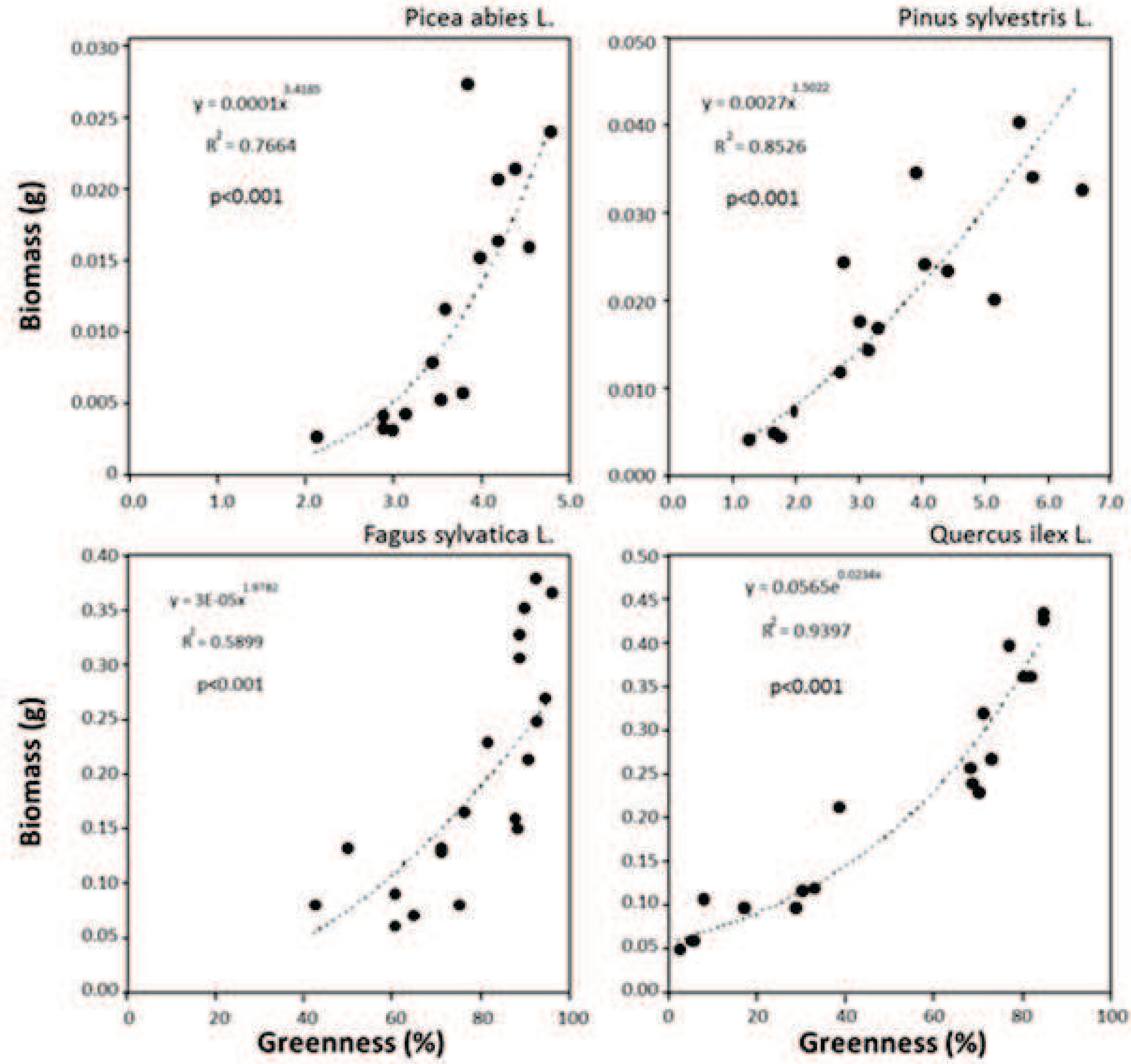


Figure 31 (**a**) Regression model relative to the relationship between seedling greenness and biomass for *P. abies* and *P. sylvestris*, *F. sylvatica* and *Q. ilex* species.

As result, the relation between seedling height and biomass (Figure 32 b) showed good results with the two broad-leaved species but no relation were 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. On the opposite, relationship between seedlings greenness and biomass showed a good result for needle-leaved species (Figure 31a).

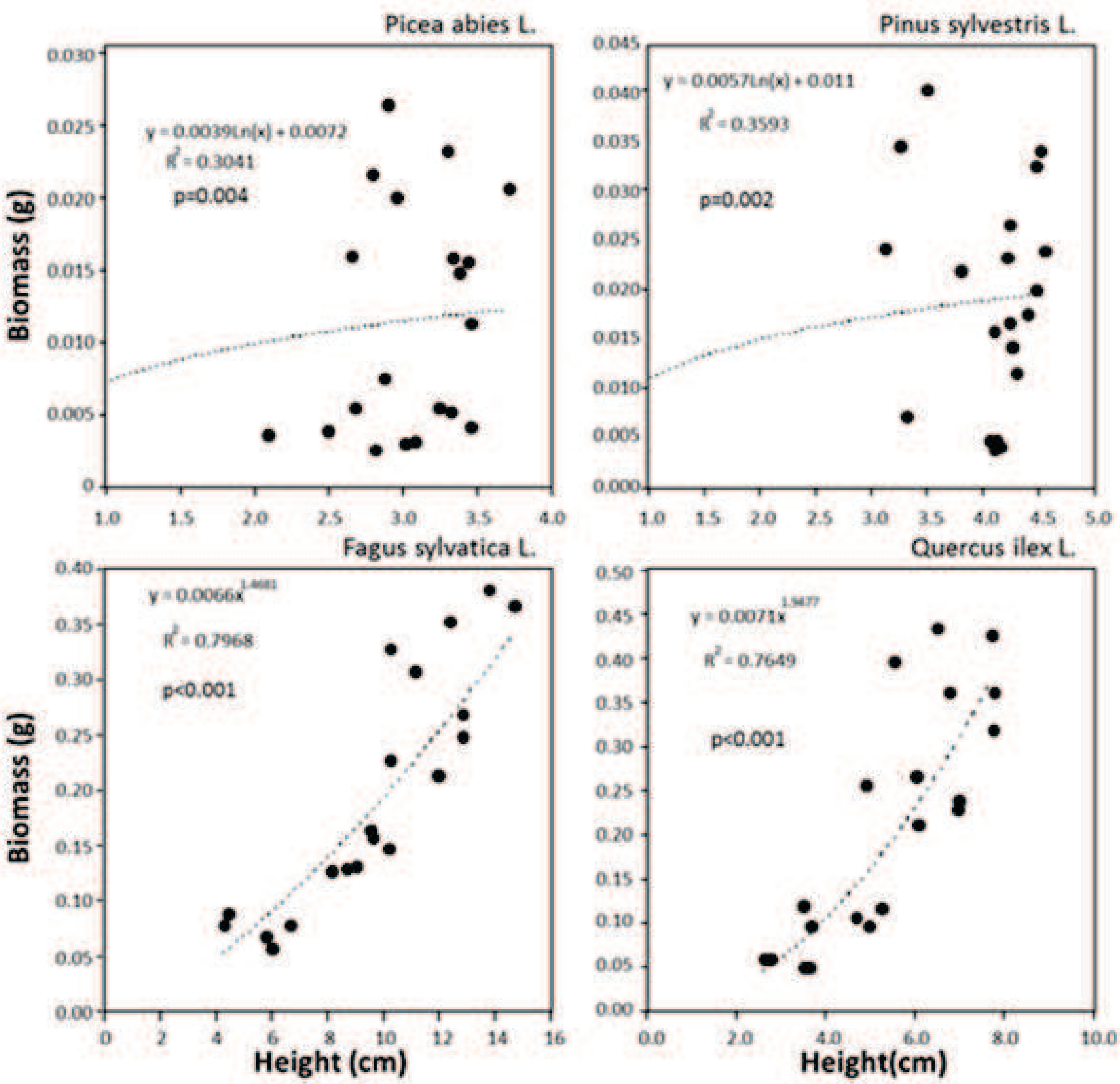
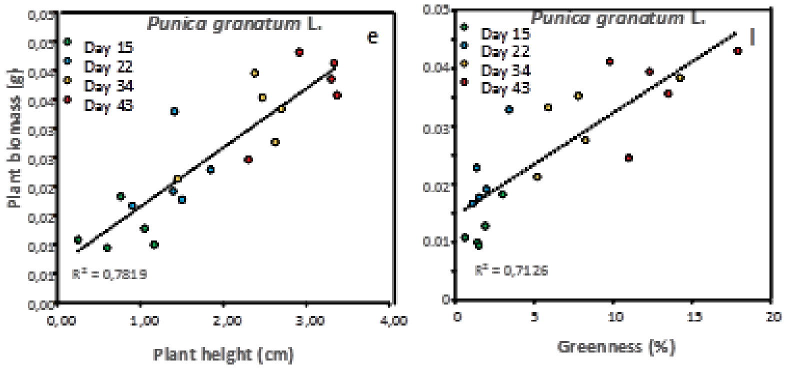


Figure 32 (**b**) Regression model relative to the relationship between seedling height and biomass for *P. abies*, *P. sylvestris*, *F. sylvatica* and *Q. ilex* plant species.



In the case of *Punica granatum* a good relation was found between plant biomass and plant height, and plant biomass and greenness.

Thus, the best regression model to explain the relationship between direct biomass data and indirect measurements was based on parameters such as plant height for broad-leaved species and plant greenness for needle-leaved species (Figure 33). Finally, image analysis revealed information on the early seedlings developmental stage.

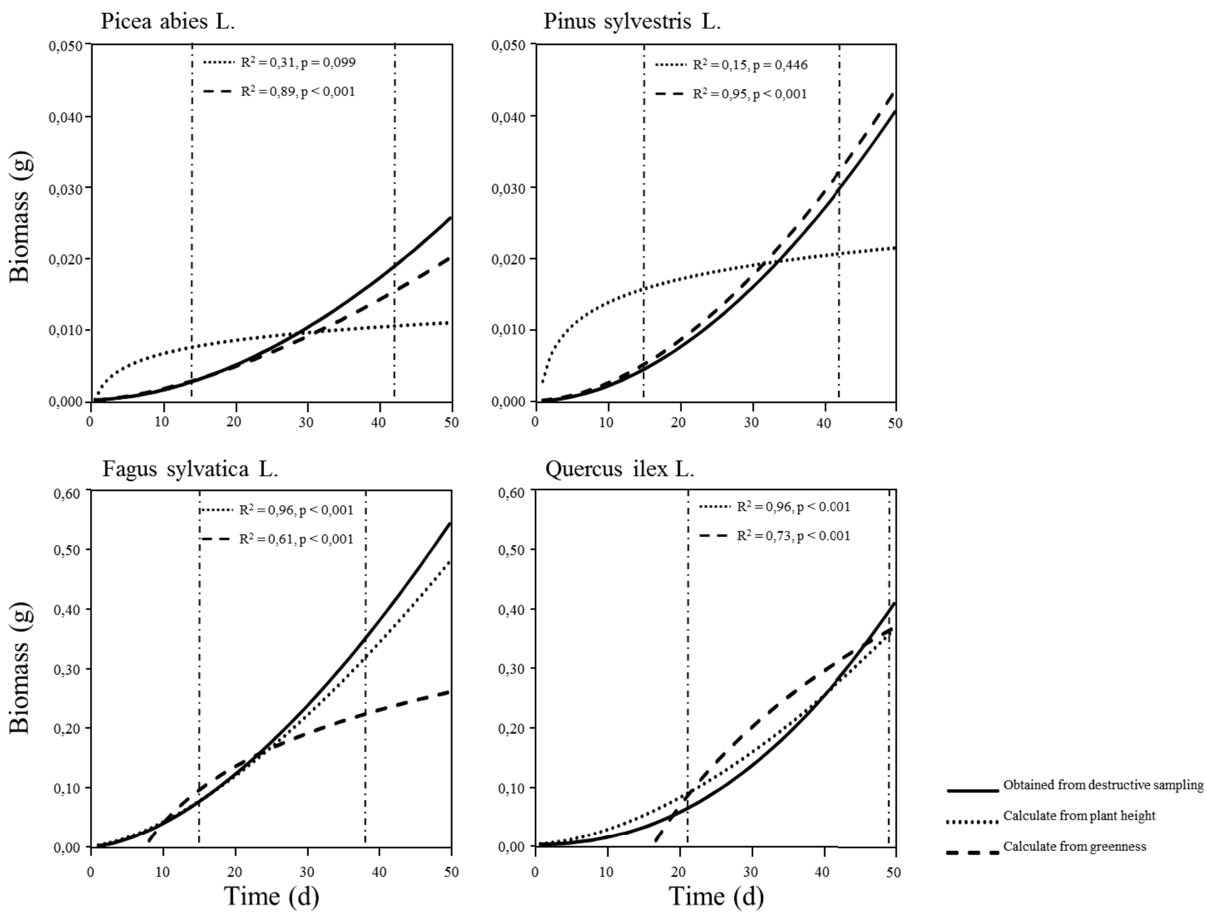


Figure 33 Regression model relative to the relationship between seedling greenness, height and biomass for *P. abies*, *P. sylvestris*, *F. sylvatica* and *Q. ilex* plant species.

3.5 SOIL WATER CONTENT MEASUREMENT

The value of soil water content (SWC)at beginning of the treatments indicates the amount of soil water content being present in the trays under a normal growth condition after trays had been watered with a constant watering regime for a number of days. The two paths (Figure 34 A and B) presented a comparable decrease in soil water content during the first four days despite the initial lower value was observed always in the trays with plants in respect to the trays without plants. After four days of treatment a considerable difference emerged between the two paths with soil water content which continued to decrease at the same rate in the trays without plants whereas showed a very rapid decrease (drop) in the majority of trays with plants.

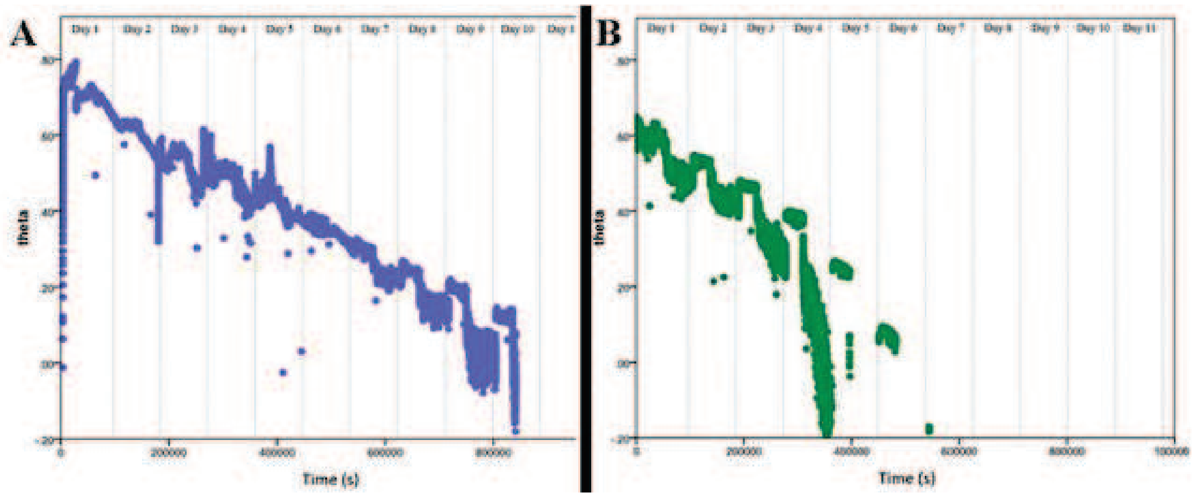


Figure 34 A SWC measurements in the tray with only soil. **B** SWC measurements in the tray with plants

3.5.1 ELECTROMAGNETIC INTERACTION

In these three experiments considering that the SWC values during the 7 minutes with light off are constant we obtained followed results.

In the case with LED lamp positioned at different distances from the pot the electromagnetic filed interaction decreased of very low values with distance between LED and soil sensor.

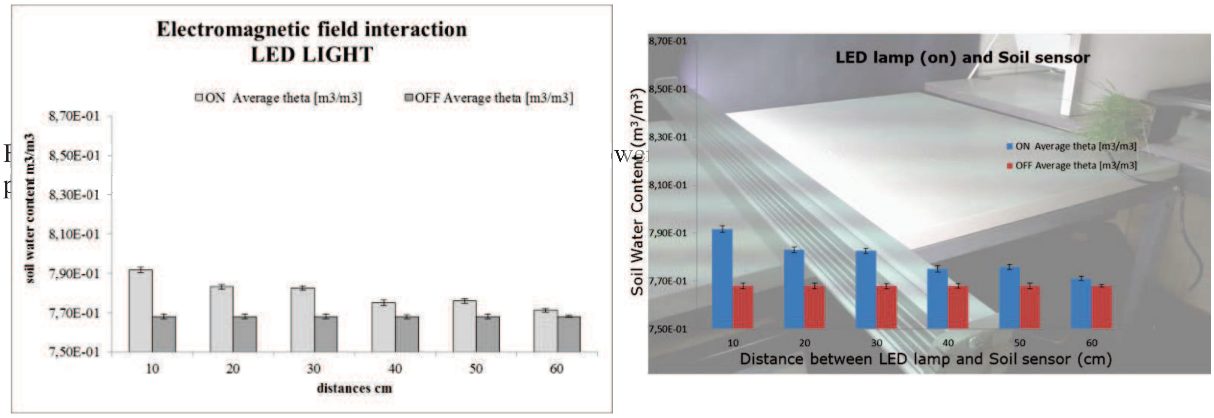


Figure 20 Electromagnetic field interactions with LED lamp positioned at different distances from the pot.

At the same way in the case with LED lamp positioned at 1 meters distance from the pot and the power pack at different distances from the pot, the electromagnetic filed interaction decreased of very low values with distance between power pack and soil sensor.

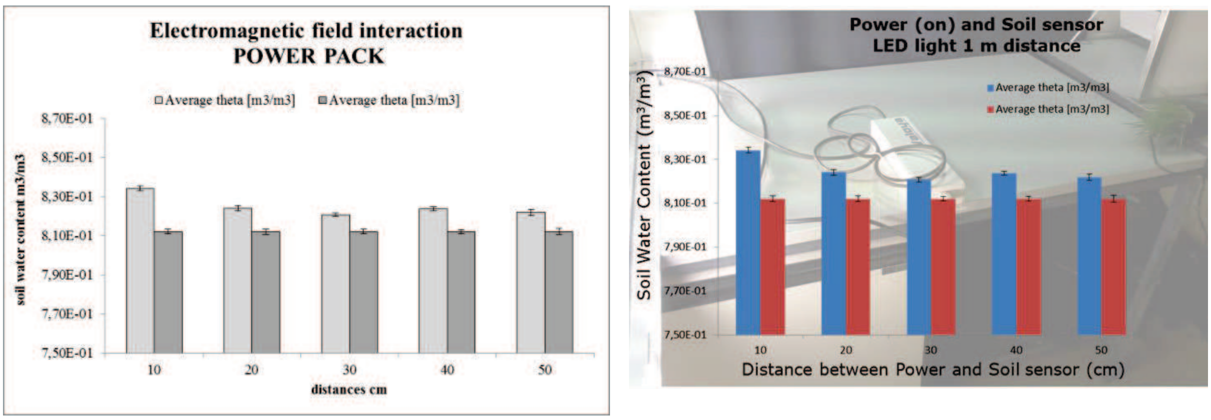
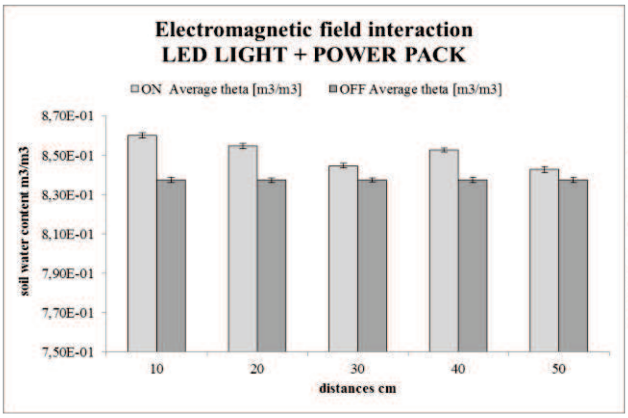
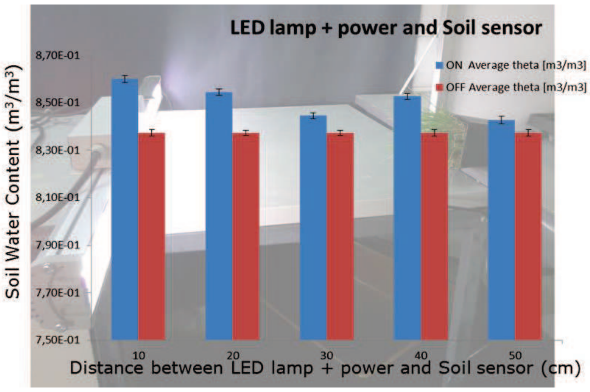


Figure 22 Electromagnetic field interactions with both LED lamp and power pack positioned together at different distances from the pot.

Again in the last case with both LED lamp and power pack positioned together at different distances the electromagnetic filed interaction decreased of very low values with distance from the soil sensor.

The maximum value recorded is 0,2E-01 m3/m3, not significant for all three cases.





Results concerning electromagnetic field interaction measurements showed that interaction of the LED light on the soil water content measurements is dependent of the distances between both LED lamp and power pack, together or separated, and soil stick sensors. In fact, electromagnetic field interaction was inversely related to the distance between LED light and soil sensor.

3.6 DISCUSSION

The work for this thesis has been characterized by two main aims: a) a comparative analysis of efficiency of LED light upon seedling growth; b) the implementation of an optical system able to measure the growth of seedling during the first weeks following germination honest.

In regard to the first aim the experiments attempted to investigate the effect of different LED lights spectra upon morphological parameters of seedlings of different plant species used traditionally for both agricultural- and forestry-purpose. The comparison was done not only among different LED lights spectra but it was extened also to fluorescent light in an attempt to understand if LED lights could represent a valid alternative to the use of growth rooms of nurseries premises. The rationale was to attempt to demonstrate the potential advantage to use more cost-effective and energy-saving light which offer environmental and social benefits when compared with the present type of lights. In regard of plant species to be tested we selected a number of species which present different distribution-areal (chorotype) with different ecological specificities. In this case, there was the need to understand if this type of approach could be used everywhere. LEDs light spectra lamps used in these experiments were manufactured by Valoya company (Finland) and they were selected because they are characterized by a mixture of red, far-red, blue and green wavelength so ton generate a continuous spectrum unlike from the one obtained by single LED ligth which produce a rather narrow light band.

The second aim of this work was develop an automatic plant phenotyping control system composed of optical sensors to monitor the seedlings growth in the growth chamber during the early weeks following germination period. In this case, the rationale was to understand if an automatized, faster, indirect method to measure growth could provide a consistent alternative to the traditional destructive and time consuming methods used at present in nurseries around the world. In the beginning non-destructive analysis by optical sensors and software analysis were carried out. Then direct destructive measurements with non-destructive method were compared.

Plant height

Results showed a linear increment of seedling height in time for six species studied (*Pinus sylvestris*, *Picea abies*, *Quercus ilex*, *Fagus sylvatica* and *Punica granatum*) and for all different light types. Furthermore the seedling height observed at the end of the growth period was higher using any LED light than using control light in all these species. Conversely, no LED lights showed improvements in *P. sylvestris* seedling height when compared to the control light. *P. abies*, *Q. ilex*, *F. sylvatica* and *P. granatum* showed the highest values under AP76 and AP67-3L LED light respect to control light, and *A. unendo* under G2. Furthermore, *F. sylvatica* and *P. granatum* showed high values also under G2 LED light. Also in the case of the Azorean species (*Morella faya* and *Frangula azorica*), the highest leaves height increment was observed under AP67 LED type, followed, only for *M. faya*, by G2 LED type. The increased shoot height observed with AP67 LED type and then with AP67-3L and G2 LED type may be related to the presence of a higher percentage of red wavelengths in the light spectrum than the others LED types and control light. In fact, red light helps plants to grow healthier and taller (Nair *et al.,* 2015) and induces hypocotyl elongation as well as expansion in leaf area (McNellis and Deng, 1995; Johkan et al., 2010).

Growth speed showed different values in different species. In the case of *P. sylvestris* and *P. Abies*, the seedling height soon showed a fast growth in a very short period (14 days) followed by minimum variations during the residual life time of 42 days. After the emergence of cotyledons, there weren't significant changes in internode elongation among consecutive emissions of new leaves. So, only a non-significant increment in plant height was recorded, probably due to specie-specific characteristics. *Q. ilex* and *P. granatum* showed a slow growth in the long period (57 days). Conversely, during the last observation time, *F. sylvatica* had a faster growth than in the previous 39 days. During the same period, *A. unedo* reached a very short height. As for coniferous species, these behaviors could be plausibly linked to specie-specific characteristics needed to be investigated in details in future studies.

Anyway, it was observed that **green** and **far-red** wavelengths cause **elongation** growth and flowering, whereas **blue** light produces a compact growth habit (Zhang et al. 2011, Hoenecke et al. 1992, Franklin and Whitelam 2005).

The **red:far-red ratio** influences **elongation** and flowering of plants (Franklin and Whitelam 2005).

<u>Root length</u>

P. sylvestris, P. abies and *A. unedo* showed interesting results in root length mainly under G2 LED type, then under both AP67 and AP67-3L.

In the case of *F. sylvatica* highest values were recorded with AP67 and NS1 at the end of the growth period. NS1 LED type shows great performance only for these species. In the case of *M.faya* highest values were recorded with AP67

NS1 LED spectrum, in analogy with the light emitted by fluorescent lamps, is more similar to the solar spectrum, but enables a level of seedling growth rate similar to the one induced by the others LED types. The fact that at the same level of light intensity (PAR), the amount of green wavelength is higher than the other wavelengths suggests that the green wavelength doesn't stimulate the plant photosynthetic process unlike red and blue wavelengths which are actively absorbed by chlorophyll pigments enabling photosynthetic reaction.

Plant biomass

Conifers species show the highest values of biomass under G2 LED light, followed by AP67-3L for *P. sylvestris* and AP67 for *P. abies*. Highest biomass values were recorded under control light with shoot biomass which presented a value 4 fold higher than root biomass

In the case of *Q. Ilex* highest values of total biomass were found when seedlings were grown under AP67-3L and AP67 LED types In particular, leave- and root-biomass show the highest values with AP67-3L compared to the other LED types Moreover, in this species, roots biomass shows values almost similar to the ones showed by leaves biomass when grown under all LED light types.

This plant species, among all the others studied, presented the lowest values with G2 LED type whose spectrum is characterized by a lower (7.7 %) percentage of blue wavelengths. Since blue light suppresses hypocotyl elongation and induces biomass production (McNellis and Deng, 1995; Johkan*et al.,* 2010), it is not unreasonable to speculate that probably this plant species requires a higher amount of this wavelength in the spectrum.

For *P. granatum* total plant biomass values obtained are similar with AP67, AP67-3L and G2 LED light type, but always lower than with fluorescent control light.

In the case of *F. sylvatica* total plant biomass values with LED light were higher than control light despite a slightly higher value was obtained with AP67 LED type.

A. unedo shows slightly higher total plant biomass values with r G2 LED light type, in particular when root and leaves biomass are considered. Total plant biomass values with all the other LED light types did not differ from control.

Leaves biomass values found in r *P. granatum*, *F. sylvatica* and *A. unedo* are the higher than shoot- and root-values. In particular with *F. Sylvatica* les biomass we observe a value 4 fold higher than root and shoot biomass values and this result is probably dependent upon e specific differences in biomass distributions between the different plant-body compatments. At this stage the effect of light-wavelength upon biomass compartimentation has not been investigated but it could explain the differences observed in our experiment. In regard to this is know that blue light, induces a more homogeneous biomass distribution (Zhang et al. 2011, Hoenecke et al. 1992, Franklin and Whitelam 2005). All growth parameters of *A. unedo* species are lowest among all others studied species.

Germination Percentage

Regarding the germination, the highest percentage among all the LED light tested with *P. abies*, *Q. ilex* and *P. Granatum* is observed with AP67-3L LED type. Instead with *P. sylvestris*, *F. sylvatica* and *A. unedo* the highest germination percentage is observed with G2 LED type. These two LED type are the one characterized by the highest percentage of red wavelength in their spectrum, and therefore we suggest that despite the presence of the red wavelength is necessary to induce germination in all the plant species tested it cannot be excluded that species-specific differences exists in relation to the effect of other wavelength components of the spectra upon the germination metabolic events. These differences could explain the germination percentage difference observed in our experiments. Unfortunately, the results obtained with the two Azorean plant species tested (*M. faya* and *F. azorica*) do not provide clear-cut indications about the LED ligth type performance with this physiological parameter. In fact, standard errors bars were t considerably high for both total biomass and root length parameters, In particular, seeds of *M. faya* r did not germinate at all with AP67-3L LED type, whereas the higher germination percentage value was observed with AP67 with both species with a value lower with *F. Azorica in respect to M. faya*.

In summary the data referring to the first aim of this thesis seem to indicate that growth of different plant species with LED light represents an efficient and valid alternative to the use of fluorescent light as traditionally used today in growth chambers present in nursery premises. However, it is important to highlight that the data obtain demonstrate that plant growth performance with LED light is specie-specific. Therefore, it is necessary to try to produce in future a suitable LED with variable wavelengths spectrum which could be adapted to the needs of each plant species in order to obtain the best growth performance, the data obtained suggest thatAP67 LED light is the best candidate, followed by AP67-3L and G2. These LED lights ensure a good production of seedlings in terms of plant height, biomass and root length of seedlings in a short time, to be used for transplanting.

The red:far-red ratio influences elongation and flowering of plants (Franklin and Whitelam 2005).

Red light helps plants to grow healthier and taller, whereas blue light helps leaves to grow, multiply and expand. (Nair *et al.,* 2015)
With blue light, it is possible to produce a compact growth habit (Zhang et al. 2011, Hoenecke et al. 1992, Franklin and Whitelam 2005).

In regard to the second aim of this work, the overall data obtained with broadleaved species indicate that the non-destructive approach based upon the optical sensors could replace efficiently the destructive one by maintaining an optimal correlation value during all the period of permanence of the seedlings in the growth chamber. Moreover, efficiency of the optical sensors seemed to be independent from the parameter measured: plant height or greenness.

Different is the consideration regarding the efficiency of the optical sensors in respect to needle-leaved species. Indeed, in this case the optical sensors shows a loss of efficiency when the plant height parameter is measured with correlation loss probably due to the specific peculiarities of physiological elongation rate in both *P. abies* L. and *P. sylvestris* L.. In fact, after a period of constant growth the plant height seemed to drop to zero value till the end of the cotyledons deployment and this behaviour contrasted with data collected by destructive approach where the biomass continued its growth rate undisturbed for all the period tested. When the optical sensors efficiency measured the greenness level then its efficiency was not lost despite the values were lower than those showed with broad-leaves species.

Therefore, in the case of both needle-leaved species (*P. sylvestris*and*P. abies*) after the emergence of cotyledons, shoot height value remains constant because internodes elongation did not occur during the consecutive emissions of new leaves at this early developmental stage. This is probably due to the specie-specific characteristic.

The preliminary data referring to a wireless electronic soil sensor able to measure soil water content indicate that this sensor evaluates very well the water content in the soil during the period when seedlings were grown in the growth chamber. The level of efficiency of this sensor is demonstrated by the fact that it is able to evindenciate also a difference in water content decrease when the seedlings are present in the container. In fact, the more rapid decrease of water content in respect to containers without seedlings is attribitable to the loss of water through transpiration activity.

In conclusion

This thesis shows that plant response to LED light is related to the plant species at least during the first weeks following germination and therefore for this period of plant life-cycle LED light can cost-efficiently replace the fluorescent light in the growth chambers. It is necessary to choose the right LED light spectrum for each species, according to their environmental needs. The best performance are observed with AP67, AP67-3L and G2 LED light type whereas NS1 LED type seems to not be suitable for this use. In alternative to the use of a single type of LED light between the one found to be effective here, a possible alternative could be a future development of LED light characterized by a variable spectrum which should present all the possible adaptation to the plant species to be used. Furthermore, it emerges the indication that electronic soil sensors represent a good system to monitor the water content in the soil and when they are used in combination with LED light, and optical sensors the result is a complex system chacterized by high level of cost-effectiveness coupled with a good possibility to save energy consumption and reduce pollution.

CONCLUSIONS

Results obtained regarding growth performances of different forest species under different LED light type indicate that LED lights represent an efficient and valid alternative to the fluorescent light in growth chambers. The use of artificial lights allows the production of a high amount of seedlings, in a real short time, ready to be used for reforestation programs.

It is important to highlight that the plant response to light quality is specie-specific. In fact, all LED types tested gave good results of seedlings' growth, but specific spectrum, according to their ecological behaviour and therefore to their light needs, provide best performance of growth. The best results recorded for all studied species were for seedlings growth under AP67, AP67-3L and G2 LED light type. A good seedlings' growth resulted under G2 LED light type but, due to its higher percentage of far-red/red, can cause operator's eye fatigue and interference with optical measurements such as greenness. The lowest values among all LED light types were obtained under NS1 LED type for almost all studied morphological parameters. This is probably due to higher percentage of green wavelength that does not stimulate the plant photosynthetic process.

Protocols of seed germination developed during the present study and applied to *Morella faya* seeds, showed good results for the *ex-situ* plant species conservation objective.

Furthermore, results showed that the optical sensors and related software represent a valid alternative to the destructive methods used traditionally to measure the growth performance of seedlings. Moreover the possibility of using two parameters (plant height and greenness) by means of the same optical sensor provides a versatility level which enables this approach to monitor the growth performance in the growth chamber in any plant species independently from their specific phenotype traits.

The soil stick sensors represent a good system to monitor the SWC and could provide an additional indicator of the seedlings growth performance in a growth chamber.

References

Orlanda Cristina Barros Moreira, Jose´ Martins, Lui´S Silva, and Monica Moura - Seed Germination and Seedling Growth of the Endangered Azorean Cherry *Prunus azorica* - HORTSCIENCE 47(9):1222–1227. 2012.

Shun-Ying Chen, Shing-Rong Kuo And Ching-Te Chien - Roles of gibberellins and abscisic acid in dormancy and germination of red bayberry (*Myrica rubra*) seeds. – Tree Physiology 28, 1431–1439 2008.

Devesh Singh, Chandrajit Basun, Merve Meinhardt-Wollweber, Bernhard Roth - LEDs for energy efficient greenhouse lighting. – Renewable and Sustainable Energy Reviews 49 (2015) 139–147.

Theoharis Ouzounis, Xavier Fretté, Carl-Otto Ottosen and Eva Rosenqvist - Spectral effects of LEDs on chlorophyll fluorescence and pigmentation in Phalaenopsis 'Vivien' and 'Purple Star' - Physiologia Plantarum 154: 314–327. 2015.

Astolfi S., Marianello C., Grego S., Bellarosa R.- Preliminary investigation of LED lighting as growth light for seedlings from different tree species in growth chambers - Not Bot Horti Agrobo, 2012, 40(2): 31-38

Sung Woo Jeong, Semin Park, Jong Sung Jin, On Nuri Seo, Gon-Sup Kim, Yun-Hi Kim, Hanhong Bae, Gyemin Lee, Soo Taek Kim, Won Sup Lee, and Sung Chul Shin - Influences of four different light-emitting diode lights on flowering and polyphenol variations in the leaves of Chrysanthemum (*Chrysanthemum morifolium*) – Journal of Agricultural and Food Chemistry. 2012, 60, 9793−9800

Naichia Yeh, Jen-Ping Chung - High-brightness LEDs-Energy efficient lighting sources and their potential in indoor plant cultivation - Renewable and sustainable energy reviews 13 (2009) 2175-2180

Forests, Trees and Landscapes for Food Security and Nutrition - A Global Assessment Report. - IUFRO World Series Vol. 33, *Editors: Bhaskar Vira, Christoph Wildburger, Stephanie Mansourian* Publisher: International Union of Forest Research Organizations (IUFRO)

Eduardo Dias Cândida Mendes Cecília Melo Rui B Elias - Azores Central Islands vegetation and flora field guide- *Quercetea 7: 123-173, 2005 ALFA, Lisboa. Portugal*

Reyes M. Perez-Sanchez, Joel Flores, Enrique Jurado, and Claudia Gonzalez-Salvatierra - Growth and ecophysiology of succulent seedlings under the protection of nurse plants in the Southern Chihuahuan Desert- Ecosphere 6(3):36 - 2015.

Field Estimation of Soil Water Content A Practical Guide to Methods, Instrumentation and Sensor Technology TRAINING COURSE SERIES No. 30 International Atomic Energy Agency, Vienna, 2008

Marco Bittelli - Measuring Soil Water Content: A Review June 2011 21(3)

Brumfield R. Dealing with rising energy costs. GPN 2007;17:24–31.

Mitchell CA, Both A, Bourget CM, Kuboto C, Lopez RG, Morrow RC, Runkle S. LEDs: the future of greenhouse lighting. ChronHortic 2012; 55:6–12.

Montagnoli A., A. Di Iorio, M. Terzaghi, D. Trupiano, G. S. Scippa, D. Chiatante. Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. Eur J Forest Res (2014) 133:957–968 DOI 10.1007/s10342-014-0814-6

Montagnoli A., M. Terzaghi, A. Di Iorio, G. S. Scippa, D. Chiatante. Fine-root morphological and growth traits in a Turkey-oak stand in relation to seasonal changes in soil moisture in the Southern Apennines, Italy Ecol Res (2012) 27: 1015–1025 DOI 10.1007/s11284-012-0981-1

Morrow RC. LED lighting in horticulture. HortScience 2008; 43:1947–50.

Singh D., Basu C., Meinhardt-Wollweber M., Roth B. LEDs for energy efficient greenhouse lighting. Renewable and Sustainable Energy Reviews 49 (2015)139–147

Ouzounis T., Fretté X., Ottosen C-O, Rosenqvist E. Spectral effects of LEDs on chlorophyll fluorescence and pigmentation in Phalaenopsis 'Vivien' and 'Purple Star'. Physiologia Plantarum 154: 314–327. 2015

Schuebert EF, Kim JK (2005) Solid-state light sources getting smart. Science 308: 1274–1278

Heuvelink E, Bakker MJ, Hogendonk L, Janse J, Kaarsemaker R, Maaswinkel R (2006) Horticultural lighting in the Netherlands: new developments. Acta Hortic 711: 25–34

van Ieperen W, Trouwborst G (2008) The application of LEDs as assimilation light source in greenhouse horticulture: a simulation study. Acta Hortic 801: 1407–1414

Tennessen DJ, Singsaas EL, Sharkey TD. Light-emitting diodes as a light source for photosynthesis research Photosynth Res 1994; 39:85–92.

Langton A, Plackett C, Kitchener H. Energy saving in poinsettia production. Horticultural Development Council Fact Sheet - 7, 2006, p.1–12.

Opdam JG, Schoonderbeek GG, Heller EB, Gelder A. Closed greenhouse: a starting point for sustainable entrepreneurship in horticulture. Acta Hortic 2005; 691:517–24.

M. Ashraful Islama, Goutam Kuwara, Jihong Liu Clarkeb, Dag-Ragnar Blystadb, Hans Ragnar Gislerøda,Jorunn E. Olsena, Sissel Torrea - Artificial light from light emitting diodes

(LEDs) with a high portion of blue light results in shorter poinsettias compared to high pressure sodium (HPS) lamps – Scientia Horticulturae 147 (2012) 136-143

Ming-Chang Wu et al.; 2007 A novel approach of LED light radiation improves the antioxidant activity of pea seedlings

Kuan-Hung Lin et al., 2013;

http://www.treeseedonline.com/store/p146/Alder_Buckthorn_%28frangula_alnus_syn_rhamnus_frangula%29.html

http://www.terrestrial-biozones.net/Holarctic%20Ecosystems/Azorean%20Ecosystems.html

http://www.researchgate.net/profile/Rui_Elias/publication/228973592_Azores_Central_Islands_vegetation_and_flora_field_guide/links/09e41510a6e2dccc15000000.pdf

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