ARTIFICIAL PROPAGATION OF ORIENTAL PLANE THROUGH LEDS

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

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

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1. Introduction

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

2. Materials and methods

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

2.1 Growth protocols of DUTH

2.1.1 Growth conditions

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

2.1.2 Growth analysis

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

2.2 Growth protocols of UNITUS

2.2.1 Growth conditions

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

2.2.2 Growth analysis

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

2.3 Light spectra collection

2.3.1 Study area

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

2.3.2 Transect surveys

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

2.4 Statistical analysis

2.4.1. Growth analysis

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

2.4.2 Light spectra analysis

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

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

3. Results and discussion

3.1 DUTH growth analysis

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

3.2 UNITUS growth analysis

After 13 days, seedlings grown under NS1 and AP673L show the lowest values for shoot height while AP67B and L20-AP67 show the highest values. After 16 days FLUORA still shows significant lower height values while the other spectra reach the same average value, recovering the initial slowing down. Between day 16 and day 50, AP673L and L20-AP67 show a faster growth rate, reaching significant higher values of shoot height, in particular if compared to FLUORA, which exhibits an arrest in plant growth (Fig. 3). Since day 13 to day 40, FLUORA shows a faster emission of leaves and therefore a significant higher number, while at day 50 there are no more significant differences among all the spectra (Fig. 4). After 50 days a sudden chlorosis started to cause the loss of leaves of some seedlings. No changes in leaf colour were detected.

3.3 Light spectra analysis

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

4. Conclusion

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

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

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

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

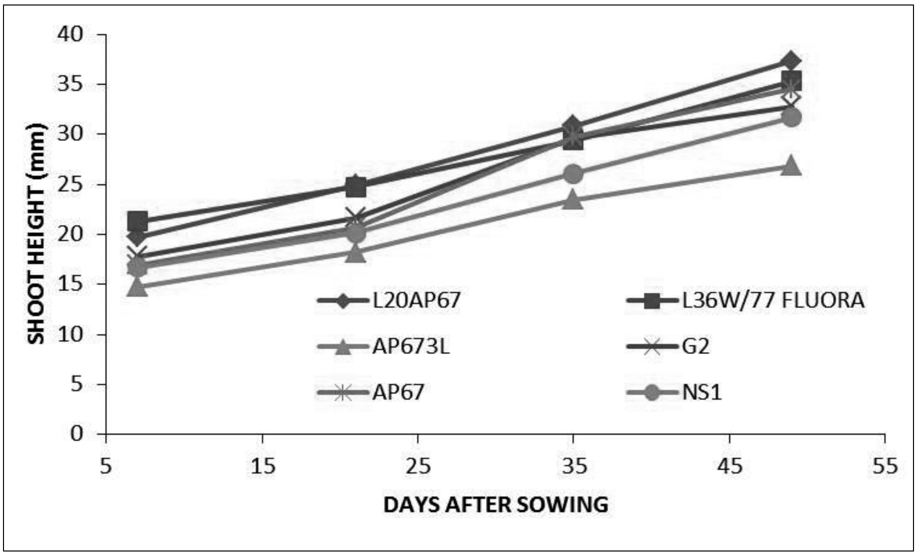

Figure 1. Shoot height of oriental plane seedlings during 7 weeks of cultivation in phytotron chamber (DUTH).
Figura 1. Altezza del fusto di semenzali di Platano orientale nel corso di 7 settimane di coltivazione in fitotrone (DUTH).
Figure 1. Hauteur de la tige des bourgeons de *P. orientalis* pendant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

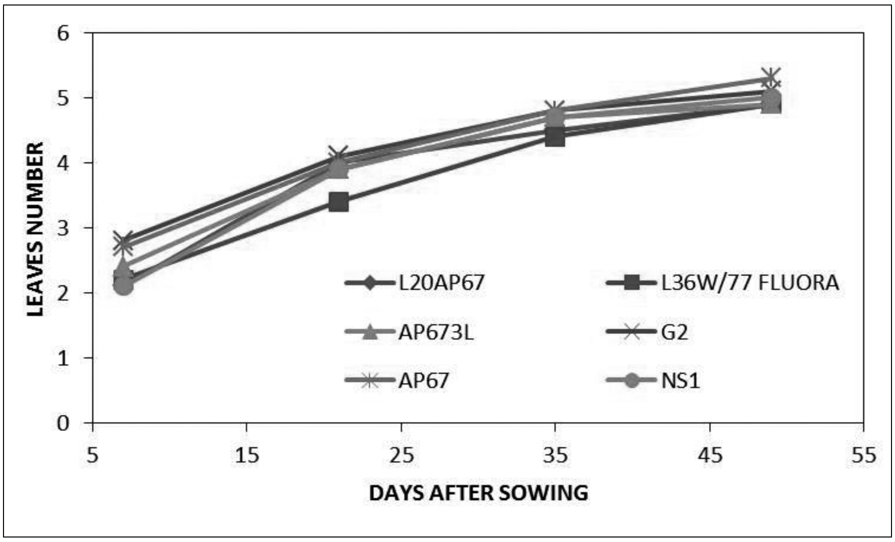

Figure 2. Leaves emission of oriental plane seedlings during 7 weeks of cultivation in phytotron chamber (DUTH).
Figura 2. Emissione delle foglie di semenzali di Platano orientale nel corso di 7 settimane di coltivazione in fitotrone (DUTH).
Figure 2. Emission des feuilles des bourgeons de *P. orientalis*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

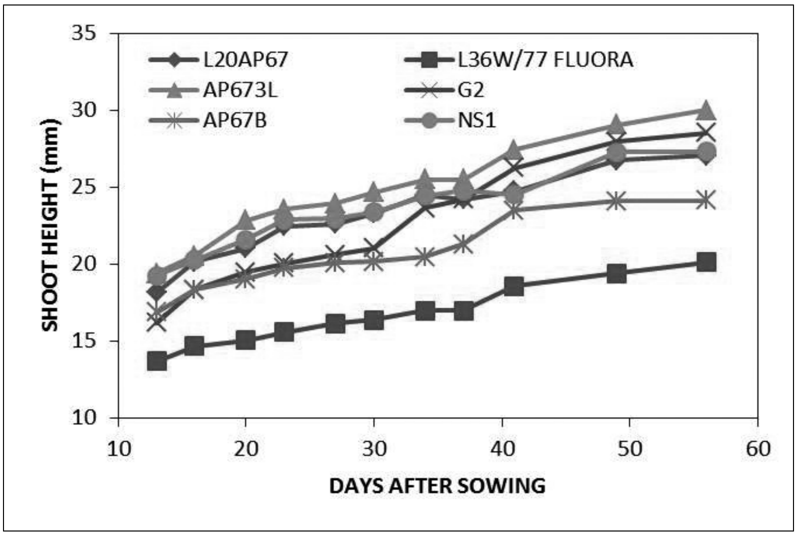

Figure 3. Shoot height of oriental plane seedlings during 8 weeks of cultivation in a climatized growth chamber (UNITUS).
Figura 3. Altezza del fusto di semenzali di Platano orientale nel corso di 8 settimane di coltivazione in camera di crescita climatizzata (UNITUS).
Figure 3. Hauteur de la tige des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

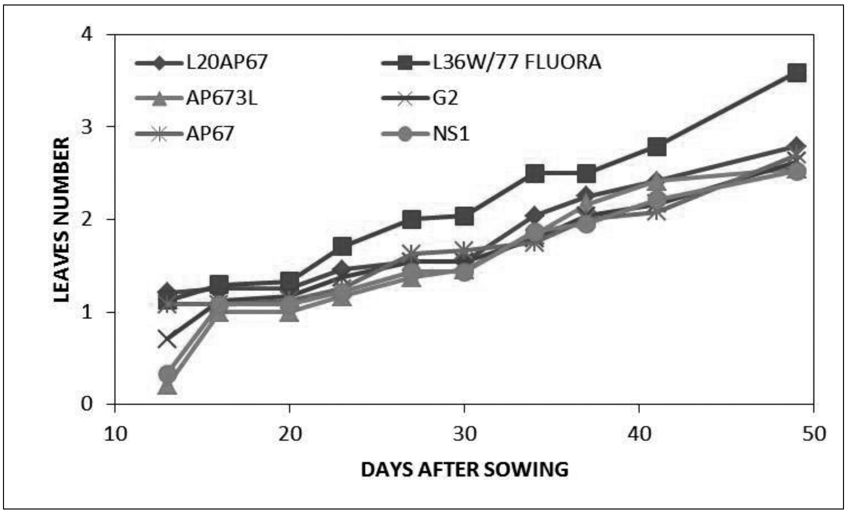

Figure 4. Leaves emission of oriental plane seedlings during 8 weeks of cultivation in a climatized growth chamber (UNITUS).
Figura 4. Emissione delle foglie di semenzali di Platano orientale nel corso di 8 settimane di coltivazione in camera di crescita climatizzata (UNITUS)
Figure 4. Emission des feuilles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

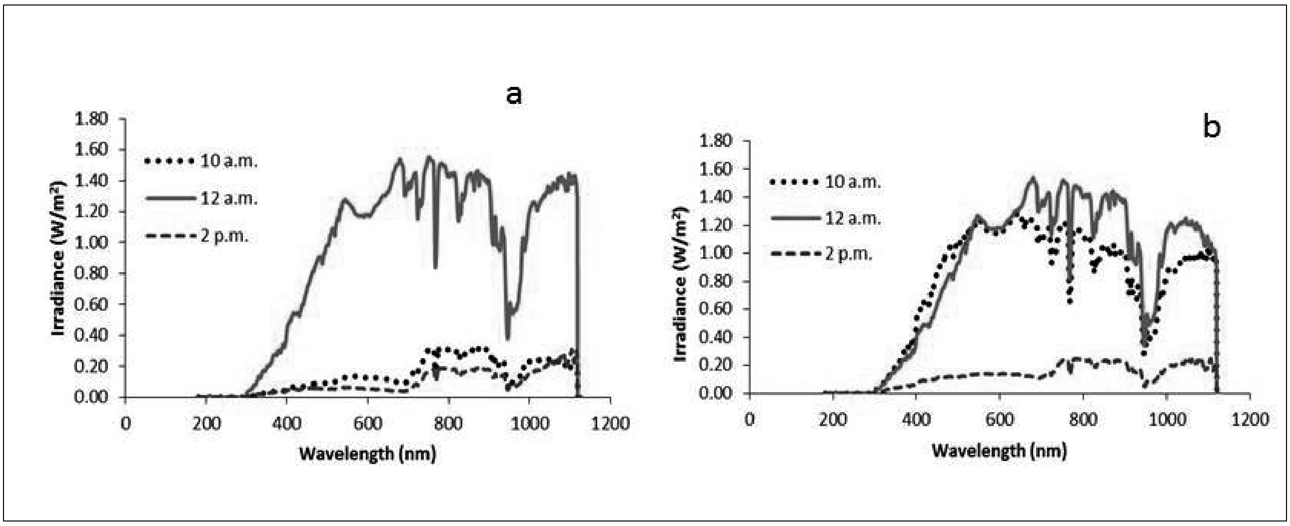

Figure 5. Alternation of different light conditions at soil level in a *P. orientalis* forest, in correspondence of points with (a) and without (b) regeneration, from 10 a.m. to 2 p.m.
Figura 5. Alternanza di differenti condizioni di luce a livello del suolo in una foresta di *P. orientalis*, in corrispondenza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

RIASSUNTO

Propagazione artificiale del Platano orientale mediante LED

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

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