From Forest Nursery Notes, Winter 2013

194. Effects of "short" photoperiods on seedling growth of *Pinus brutia*. Iakovoglou, V., Radoglou, K., Kostopoulou, P., and Dini-Papanatasi, O. Journal of Environmental Biology 33:149-154. 2012.

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Full paper available on: www.jeb.co.in

J. Environ. Biol.
33, 149-154 (2012)
ISSN: 0254-8704
CODEN: JEBIDP

Effects of "short" photoperiods on seedling growth of *Pinus brutia*

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Publication Data

Paper received:
13 August 2010

Revised received:
08 February 2011

Accepted:
16 February 2011

Abstract

This study investigated how nurseries could benefit by inducing "short" photoperiods as low as 4 hr to produce "better" seedlings characterized by more vigorous roots; a substantial feature to overcome transplanting stress. The carryover effect of the photoperiod was also investigated on seedlings that grew for 30 days more under the consistent 14 hr photoperiod. Seedlings of *Pinus brutia* were subjected to 4, 6, 8 and 14 hr photoperiod for 3 week. Fifteen seedlings were used to evaluate the leaf area, the root and shoot dry weight and their ratio. Six and sixteen seedlings were used to evaluate the shoot electrolyte leakage and the root growth potential, respectively. Based on the results, the 6 and 8 hr photoperiod indicated greater root allocation (4.8 and 4.9 mg, respectively) and chlorophyll content (3.7 and 4.4, respectively). They also indicated greater leaf area values (3.3 and 3.5 cm^2, respectively) along with the 14 hr (3.4 cm^2). The photoperiod effect continued even after seedlings were subjected at consistent photoperiod. Overall, "short" photoperiods could provide "better" *P. brutia* seedlings to accommodate immediate massive reforestation and afforestation needs.

Key words

Afforestation, *Pinus brutia*, Reforestation, Short photoperiod

Introduction

The success of reforestation and afforestation, especially during the first year of establishment, largely depends on both the quality of the planting material and the site conditions (Radoglou, 2001; Radoglou *et al.*, 2009). Drought conditions in semi-arid Mediterranean ecosystems impose another obstacle for seedling establishment (Pigott and Pigott, 1993; Mendoza *et al.*, 2009). Furthermore, the hot and dry summers make these ecosystems on a yearly base prone to both natural and human caused fires (Lloret *et al.*, 2002). Despite the fact that the dominant tree species are genetically well equipped to grow at these ecosystems, if fire events occur at frequent rates before the trees reach their reproductive age maturity, their ability to naturally regenerate the sites minimizes (Corona *et al.*, 1998; Thanos, 2000). Forest nurseries need to produce "better" seedlings with characteristics such as vigorous root systems that help overcome adverse growth conditions (Mendoza *et al.*, 2009; Thanos, 2000). In addition, the timing between fire events and regeneration should be as minimal as possible in order to reduce negative environmental impacts such as the extent of soil erosion, especially on steep non-vegetative slopes (Andren *et al.*, 2001; Pierce *et al.*, 2004). Under those circumstances it is of high priority to artificially regenerate those sites as soon as possible. Hence, it is vital that the forest nurseries not only provide "better" seedlings, but also, produce and transplant them within a short period right after the disturbance event, specifically fire.

One of the species frequently used for reforestation and afforestation purposes is *Pinus brutia*; a species that dominates highly disturbed semi-arid Mediterranean ecosystems of Eastern Greece (Thanos and Doussi, 2000; Panetsos, 1975). It's well adapted strategies and genetic characteristics have enabled it up till now to naturally regenerate and dominate open sites, particularly after fire events (Boydak, 2004; Thanos and Doussi, 2000). One of the strategies is the seratinity of the cones. The cones enclose seeds that are viable for extended periods that open and release the seeds only when subjected at high temperatures, similar to those during fire events (Boydak, 2004; Thanos and Doussi, 2000). Also, its ability to occupy open areas by massive recolonization enables the species to dominate the sites; a successful post-fire regeneration strategy especially under high frequency fire events (Thanos, 2000). In addition, the genetic characteristics of *Pinus brutia* to produce vigorous root systems with rather stunted epicotyls, allows it to overcome the adverse semi-arid environmental conditions, like water deficit situations and successfully regenerate the site. However, despite its ability to naturally regenerate the sites, high frequency of fire events in conjunction with the need to alleviate negative environmental impacts, like soil erosion, makes it of vital importance to artificially regenerate the sites.

To increase the reforestation and afforestation success, researchers have focused on producing “better” seedlings to withstand transplanting stress by manipulating their growing conditions under controlled chamber environments. The photoperiod duration is one of the growing conditions that can easily be altered. However, only a few studies have addressed its effect on a range of photoperiod durations for forestry species. Verma and Tandon (1984) studied the photoperiod effect for durations of 8, 10, 12, 16, 18 and 20 hr on *Pinus kesiya* Royle ex. Gord. seedlings. They found that maximum seedling growth occurred at 16 hr. Researchers have also focused on the effect of “short” photoperiod durations, with “short” being defined as minimum as 8 hr (Webb, 1976; Rostad *et al.*, 2006) and 9 hr of photoperiod (Jacobs *et al.*, 2008). Researchers have also found that by exposing seedlings to “short” photoperiods there was an initiation on bud dormancy that coincided with cold hardiness and further growth cessation (Borthwick, 1957; Cambell and Sugano, 1975). This is one of the common nursery practices that has been used to reduce seedling damage during storage in order to facilitate spring plantings (Jacobs *et al.*, 2008; Rostad *et al.*, 2006). Along with the arrest of seedling growth, “short” photoperiods have been used for conifer seedlings to induce a shift to the carbohydrate allocation to further root growth (Burdett, 1990; Wu *et al.*, 2004). That implies that by manipulating the photoperiod duration it could be possible to induce a carbohydrate shifting to greater root allocation. Further, seedling with “greater” root systems could be more resilient to transplanting stress.

The main objective of this study was to investigate the effect of “short” photoperiod on seedling growth of *P. brutia*. Further, it was investigated if there was a carryover effect of the photoperiods after the seedlings were grown under a consistent photoperiod.

Materials and Methods

Different photoperiods: Seeds of *P. brutia* were provided by the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens, Greece), with collection year of 2004 and seed sampling from trees located in Thasos island (40 46’40’’N 24 40’00’’E). The seed lot retained 75% germination, 10% seed moisture content and average weight of 46.15 g per 1,000 seeds (ISTA, 2008). The experiment started in August, 2007. After seeds were soaked in water (15-20^{o}C) for 24 hr, they were sown in 9 cm^3 cavities of mini-plug plastic trays (310 x 530 mm, 1820 plants m^{-2}) (QuickPot®, Herkuplast-Kubern GmbH, Ering, Germany). Each cavity retained one seed and was filled with stabilized growing medium (Preforma PP01, EC=0.1 ms cm^{-1} and pH=4, Jiffy Products International AS, Stange, Norway). After seeds were sown, the trays were placed in environmentally controlled growth chambers (400 lt KB8000FL, Termaks AS, Bergen, Norway) and let germinate for 4 weeks under 14 hr of light (250 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)) at 20^{o}C altered with 10 hr at 15^{o}C of dark and relative humidity (RH) of 80±10%. Watering was applied every other day with no fertilizer application.

Continuously, the seedlings were transferred and let grown for 3 weeks under the photoperiods of 4, 6, 8 and 14 hr at 20^{o}C altered respectively with 20, 18, 16 and 10 hr of dark at 15^{o}C, under RH of 80±10%. Sampling was taken place every week. Each sampling time, 15 seedlings were used for each photoperiod to evaluate the root dry weight (RDW), the shoot dry weight (SDW) and their ratio (RDW/SDW) by oven drying the samples at 70^{o}C for 48 hr (Brønnum, 2005). For those seedlings, the leaf area (LA) was also estimated (cm^2) by using an area meter (AM100, ADC Bioscientific Ltd., Herts, UK).

The shoot electrolyte leakage (SEL) was also evaluated (McKay, 1992) for a total of six seedlings for each studied photoperiod. After following standard seedling cleaning procedure, the shoots were placed in 16 ml tubes and were incubated at room temperature (25^{o}C) for 24 hr. The electrical conductivity of the solution where the shoots were immersed was measured before and after the incubation with an electrical conductivity meter (EC 215, Hanna Instruments® Inc., Leighton, UK). The total electrolytes of the samples were also measured after autoclaving at 121^{o}C.

Finally, 16 seedlings were used for each photoperiod to evaluate the root growth potential (RGP). The mini-plug plastic trays were placed into stainless boxes filled with equal volumes of peat (Klassmann Base Substrate 250l, Klassmann-Delmann GmbH, Geeste, Germany) and sand. According to the standardized RGP technique, the trays were immersed in stainless water bath. The water and air temperatures were maintained at 21 ± 2^{o}C, with air RH at 40 ± 10%, while the photoperiod was set at 14 hr of light (PPFD at plant level of 300 μmol m^{-2} s^{-1}). Seedlings were watered every other day with no fertilizer application. At the end of the third week, the root parts that developed outside the mini-plug trays were washed and cut. The RGP was assessed based on the dry weights of those root parts that were grown outside the mini-plug containers. The samples were oven dried at 70^{o}C for 48 hr.

Consistent photoperiod: After the seedlings were grown for 3 weeks under the different photoperiods, continually they were transferred at a consistent 14 hr photoperiod growth chamber and let grown for additional 4 weeks (20°C altered by 10 hr of dark at 15°C and RH of 80±10%). At the end of those 4 weeks, for each studied photoperiod 15 seedlings were used to evaluate again the RDW, SDW, RDW/SDW ratio, LA, SEL and RGP. In addition, the seedlings were evaluated for chlorophyll content by using a chlorophyll content meter (CCM-200, Opti-Sciences Inc., Hudson NH). Also, the chlorophyll fluorescence (CF) levels were estimated in order to evaluate the photosynthetic capacity of the seedlings. Chlorophyll fluorescence measurements were done at a sample of 5 dark adapted (30 min) and 8 light adapted seedlings before transplanting to destination containers (11th week after sowing). The variable to maximal fluorescence ratio (F_v/F_m) and the effective quantum yield of photosystem II ($\ddot{O}_{PSII}=(F2_m-F_t)/F2_m$) ($F2_m$ is the fluorescence maximum in the light and F_t the steady-state fluorescence immediately prior to the flash) (Maxwell and Johnson, 2000), were measured for a cluster of needles by using a pulse amplitude modulated photosynthesis yield analyzer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany).

Statistical analysis was conducted by using analysis of variance (ANOVA) with the SPSS® statistical software v.15.0 (SPSS, 2006). The error was assumed to be identically and independently normally distributed. Differences among means and the Duncan's multiple range tests were done with significant differences at p<0.05. Pearson correlation matrixes were computed for determining the significant coefficients among the studied variables at probability level of p<0.05 and p<0.01.

Results and Discussion

Different photoperiods: Based on the results, the different photoperiods significantly impacted the root and the shoot dry weights that varied according to the sampling time (days), with greater means for the photoperiods of 6 and 8 hr (Fig. 1). For the RDW, differences were found at the first sampling week with greater values for the 8hrs photoperiod, while at the third week greater values were found for 6 and 8 hr photoperiod. The SDW indicated differences with greater values for the photoperiods of 4, 6 and 8 hr at the first week, while the second and the third week had greater values for the 8 and 6 hr of photoperiods, respectively. Differences were also indicated by the SEL at the third week and the RGP at the first week, with greater values for the 8 hr photoperiod.

Specifically, the third week indicated greater root biomass production (RDW) for the 6 and 8 hr than the other photoperiods, suggesting a carbohydrate shift to greater root allocation. That was also evident by the overall increase of the RDW/SDW ratios at the third week. Luoranen *et al.* (2007) also found for *Picea abies* an increase in the root biomass for seedlings that were exposed under both "short" day period (defined as 10 hr daylight when compared to 20 hr daylight) and water stress conditions. In this study, the seedlings grew under much shorter photoperiods compared to Luoranen's *et al.* (2007) work. This suggests that by subjecting seedlings to shorter photoperiod levels, a carbohydrate shift to greater root allocation could be encouraged. Hence, within a few weeks nurseries could produce seedlings with more vigorous roots; a characteristic that can enhance water absorption, utilization efficiency and increased ability of the seedlings to tolerate drought conditions (Villar-Salvador *et al.*, 2008). Those characteristics are even more important for shade intolerant species, such as *Pinus brutia*, not only for helping these seedlings to overcome transplanting stressed conditions, but also for enhancing their ability to compete with other vegetative species. The shifting to greater root allocation was also evident by the simultaneous reduction of the mean SDW values (Fig. 1). That was also indicated by the reduced LA, with a greater LA reduction for the 8 hr photoperiod (0.8 cm^2 LA reduction from the second to the third sampling week). Further, multi-correlation analyses revealed a positive correlation of the SDW with the LA for all studied photoperiods (0.98, 0.96 and 0.88 at p<0.05 for the 4, 6 and 8 hr of photoperiod, respectively), except the 14 hr based on the third week of sampling. That suggests that the growth shifting in producing more roots has been done in the expense of leaf growth (possibly needle loses).

The 8 hr photoperiod also showed greater SEL mean value compared to the other photoperiods. According to Binder and Fielder (1995), conifer seedlings with greater SEL values indicated post-planting and heat needle damage. Greater leakage values have also been associated with needle browning and loss, or even bud death (Sutinen *et al.*, 1992; Raisanen *et al.*, 2009). To our

Table - 1: The level of significance on seedling variables and florescence after the seedlings were grown for additional four weeks under the same photoperiod of 14 hr.

Photoperiods (hr)	Seedling variables						Florecence		
	RDW (mg)	SDW (mg)	RDW/SDW	LA (cm^2)	SEL (%)	RGP (mg)	CHL	Fv/Fm	Yield
4	3.14^b	14.64^c	0.22^a	2.88^b	3.95^b	6.81^a	3.54^{ab}	0.70^b	0.71^b
6	4.80^a	20.21^a	0.25^a	3.31^a	4.97^{ab}	7.15^a	3.73^a	0.69^b	0.71^b
8	4.92^a	21.16^a	0.23^a	3.46^a	3.69^b	8.05^a	4.41^a	0.71^b	0.71^{ab}
14	3.53^b	17.51^b	0.20^a	3.43^a	5.79^a	8.24^a	2.44^b	0.73^a	0.72^a
p-value	<0.001	<0.001	0.09	<0.001	0.01	0.44	0.04	0.01	0.05

Root dry weight (RDW), shoot dry weight (SDW), and their ratio (RDW/SDW), leaf area (LA), shoot electrolyte leakage (SEL), root growth potential (RGP) and chlorophyll content (CHL); Means with the same letter are not significantly different at p <0.05

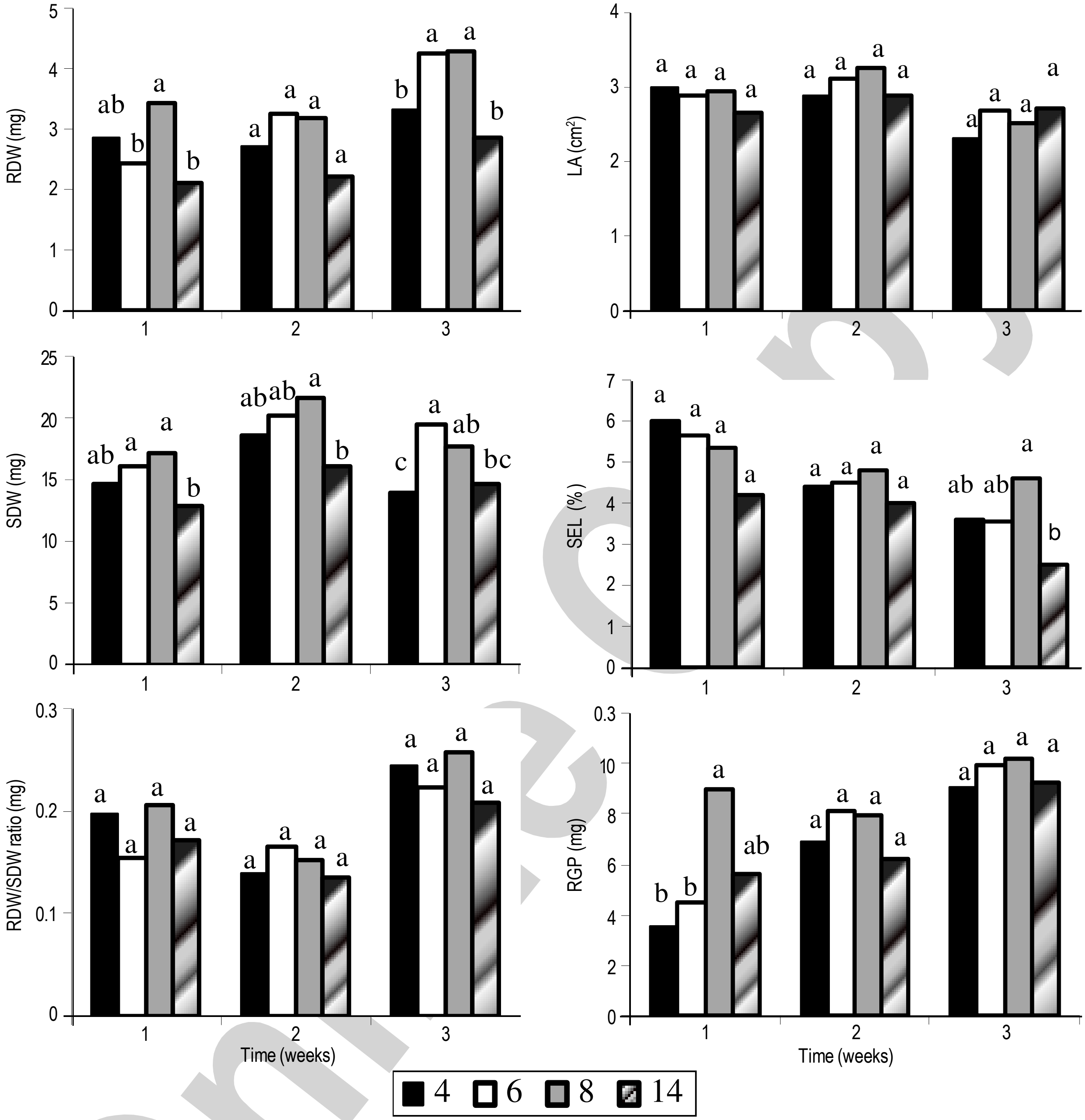

Fig. 1: The effect of the 4, 6, 8 and 14 hr photoperiod on three week period on seedling variables of root dry weight (RDW), shoot dry weight (SDW) and their ratio (RDW/SDW), leaf area (LA), shoot electrolyte leakage (SEL) and root growth potential (RGP); Means with the same letter are not significantly different at p<0.05

understanding, the higher SEL values for the 8 hr indicated the abrupt reduction of the LA at the specific sampling time. Hence, shifting to greater root allocation could be accompanied by a physiological response such as reduction in leaf area induced by leaf loss. Based on the results, the RGP indicated a photoperiod effect with greater value for the 8 hr only for the first sampling week that did not carry over for the rest of the sampling weeks. That might suggest that although the RGP is a strong tool to study the potential of increased growth of the roots (McCreary and Duryea, 1987; Simpson and Ritchie, 1997), it might not be the best indicator when evaluating the photoperiod effects on seedlings.

Consistent photoperiod: When seedlings were grown for additional four weeks under the consistent photoperiod of 14 hr, the RGP did not indicate any differences among photoperiods. Unlike the RGP, the rest of the variables showed differences, suggesting a carryover effect of the photoperiod even after the four weeks of consistent photoperiod (14 hr) growth conditions (Table 1).

Again, for the two photoperiods of 6 and 8 hrs the RDW and SDW indicated more vigorous seedlings. These photoperiods along with the 14 hrs had greater LA values, while the chlorophyll content was greater only for the 6 and 8hrs photoperiod (Table 1). According to Awada *et al*. (2003), greater chlorophyll content was a characteristic for two year old *Pinus brutia* seedlings that were grown under shade conditions (25% full sunlight) when compared to seedlings that were grown under higher ambient photoperiod levels (100% full sunlight). That could suggest that those shade intolerant seedlings that were subjected to "short" photoperiods (6 and 8 hr), in addition to greater dry weights, have also altered their chlorophyll content. These seedlings with greater chlorophyll level might be able to tolerate shade conditions better during the first year of establishment. That is also supported by the lower SEL values for the 6 and 8 hrs photoperiod, indicating that those seedlings should be able to overcome transplanting stress better (Binder and Fielder, 1995). At this point, further field research should help understand the impact of chlorophyll content under competitive and non-competitive field conditions and the potential threshold for the SEL that can guarantee "better" seedlings for future plantings.

However, it also needs to be mentioned that the chlorophyll fluorescence (Fv/Fm) and the effective quantum yield value were greater for 14 hr, with those two variables being negative correlated (-0.9 at $p<0.01$). This negative correlation suggests that this photoperiod duration might exceed the light needs of the *P. brutia* seedlings since there is an increase at the reflected light quantity as expressed by the chlorophyll fluorescence values. Nevertheless, it has also been suggested that the Fv/Fm ratio is a more sensitive indicator when studying seedling responses under cold stress as oppose to photoperiod (Fernandez *et al*., 2003).

Based on the research findings, it seems that the short photoperiod could be used in order to produce better *P. brutia* seedling and also increased leaf area (LA) and chlorophyll content to accommodate forestry practices, like reforestation efforts after fire events. These characteristics are very important for shade intolerant species like *P. brutia*, to help overcome the transplanting stressed conditions and enhance their ability to compete and outgrow other competitors.

Acknowledgments

This study was part of the PRE-FOREST project funded by European Commission under the CRAFT FP6 (Contact No 032820). The authors like to thank the project's partners for their collaboration and the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens) for providing seeds.

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