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ORIGINAL PAPER

Differences in morphology, gas exchange and root hydraulic conductance before planting in *Pinus canariensis* seedlings growing under different fertilization and light regimes

V. C. Luis · M. Llorca · E. Chirino · E. I. Hernández · A. Vilagrosa

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Abstract As the main forestry species in the Canary Islands (Spain), *Pinus canariensis* is frequently used in afforestation programs. Several nursery techniques are commonly employed to modify its morphology and physiology with the aim of improving post-planting survival and growth. In this work, we studied how fertilization and light regime treatments applied during the nursery period modify biomass allocation patterns and produce effects in gas exchange and root hydraulic conductance. Seedlings were grown for a 6-month period in the nursery under two light regimes (full sunlight and 40% PAR reduction), and three fertilization levels were applied in each light regime. Morphology, biomass allocation patterns, leaf gas exchange and hydraulic conductance of the whole root system were evaluated. Fertilization treatments produced significant changes in biomass allocation, gas exchange and root hydraulic conductance under both light regimes. In contrast, no differences were found between full sunlight and shade, except for a slight variation in the root:shoot ratio. Photosynthesis rate and WUE increased with fertilization in both light regimes, while *E* and gs maintained the same values. An opposite trend was observed for root hydraulic conductance, which showed lower values with high fertilization regimes. The results obtained indicate that fertilization is more important, determining high photosynthetic capacity than high hydraulic conductance rates before planting in *Pinus canariensis* containerized seedlings.

Keywords Fertilization · Gas exchange · Hydraulic conductance · Morphology · *Pinus canariensis* · Shade

Introduction

The endemic tree species *Pinus canariensis* is both the main forest species in the Canary Islands (Spain) and the one most used in reforestation programs. Its characteristic high fire resistance due to its resprouting capacity (Climent et al. 2004) makes this species very useful for recovering degraded and fire-sensitive areas. However, in forests destroyed after The Conquest of the Canary Islands (Parsons 1981) and later reforested, some studies have found regeneration problems, especially in degraded environments (Vaxevanidou et al. 2006; López 2009). In addition, IPCC projections (IPCC 2007) point to an increase in the vulnerability of Mediterranean-type ecosystems to degradation and an aggravation of unfavourable conditions for forest regeneration and restoration. Therefore, there is a need to improve forest restoration techniques, especially in these areas.

The application of innovative technologies during the nursery period can improve plant quality and seedling field performance (Cortina et al. 2006; Chirino et al. 2009). Recently, Luis et al. (2009) indicated that *Pinus canariensis* seedling quality influences reforestation success in dry and degraded areas in Tenerife (Canary Islands). Low-quality plants may exhibit morphological and physiological

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V. C. Luis (✉) · M. Llorca · E. Chirino · A. Vilagrosa
Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), Departamento de Restauración Forestal,
Facultad de Ciencias, University of Alicante,
Fase V, Ctra, San Vicente del Raspeig s/n,
San Vicente del Raspeig, CP 03690 Alicante, Spain
e-mail: vc.luis@ua.es

E. I. Hernández
Department of Ecology, University of Alicante,
Ap 99, 03080 Alicante, Spain

characteristics that impair their performance under stressful conditions such as low water availability during summer. In contrast, high seedling quality may enhance the survival of this species by improving morphological parameters and physiological behaviour (Puértolas et al. 2003; Villar-Salvador et al. 2008; Oliet et al. 2009b).

It is well known that fertilization during the nursery stage produces changes in biomass allocation and enhances survival and growth in the field (Landis 1985; Timmer 1991; Grossniclke 2005). Nevertheless, some authors found that high fertilization can also have negative consequences such as reducing plant drought tolerance (Tan and Hogan 1995; Hernández et al. 2009), thus affecting survival success in the field (Trubat et al. 2008). In this respect, previous studies with *Pinus canariensis* showed that seedlings fertilized during nursery culture produced more vigorous plants with higher survival rates and growth under field conditions (Luis et al. 2009). These results agree with those obtained by other authors with *Pinus halepensis* and *Quercus ilex* (Puértolas et al. 2003; Oliet et al. 2009a, b; Cuesta et al. 2010) and with *Retama sphaerocarpa* (Villar-Salvador et al. 2008) in Mediterranean ecosystems.

Shading is another nursery technique commonly used in Mediterranean nurseries to avoid the excessive radiation that can cause leaf damage in seedlings during the prolonged summer. Shade is applied during nursery culture mainly in the summer months, and/or in the field by means of treeshelters during the first years after outplanting (Valladares et al. 2000; Oliet and Jacobs 2007; Puértolas et al. 2009). Shade increases specific leaf area and shoot:root ratio (Ballare et al. 1990) and promotes lower drought resistance by increasing the vulnerability to cavitation (Cochard et al. 1999); however, it can also modify water transport capacity and gas exchange rates (Krasowski and Caputa 2005) and even enhance seedling survival in dry zones (Holmgren 2000).

Due to the large number of techniques used in nurseries and the wide variation in species response, it is becoming more necessary to evaluate seedling quality as a response to the techniques applied. Although several morphological features are currently being used to define seedling quality for each forest species in Spain, few physiological parameters have been selected as good predictors. In general, a plant showing higher carbon assimilation will have a greater survival probability, because its root system will grow faster and a water supply from roots to shoots will be assured (Burdett et al. 1983). In this sense, one of the most important parameters to take into account is the ability of roots to supply water to the aerial part of the plant, namely root hydraulic conductance (K_h). Root hydraulic limitations to water flow have been found to reduce gas exchange rates (Chirino et al. 2008; Hernández et al. 2009), affecting plant growth (Ryan et al. 2006) and increasing the risk of cavitation or cell damage (Vilagrosa et al. 2003, 2010). However, no studies on this have been carried out with *Pinus canariensis.*

The underlying hypothesis of the present work arose from results reported in Luis et al. (2009), which indicated that the success of reforestations with *Pinus canariensis* was much better with well-fertilized seedlings than with poorly fertilized ones. We thus hypothesized that fertilized seedlings would have a set of traits (morphological and physiological) that enhance survival and growth in the field. The objective of this study, then, was to identify these traits that show how fertilization treatments applied during the nursery period can modify seedling morphology and physiology, mainly in root hydraulic conductance, and find out whether there are also differences under two light regimes.

Materials and methods

Plant material and cultivation method

Pinus canariensis seeds of Vilaflor provenance (FS-27/01/38/004) were sown in a standard 300 cc forestry container in January 2005 in Santa Faz nursery (38°23′, 0°26′W, 80 m a.s.l.) in Alicante (Spain). The mean annual rainfall is 353 mm and the mean annual temperature is 18°C. Seedlings were grown for 6 months. The substrate used was a mixture of fertilized peat and coconut fibre (1:1 v/v). Seedlings were grown under two different environments and fertilized seedlings were assigned randomly to different light regimes. Thus, seedlings were subjected to six combinations of light and fertilization treatments (two levels of light x three levels of fertilization). A total of 96 seedlings were grown in each treatment. The irradiance regimes were full sunlight and a 40% reduction of photosynthetic active radiation (PAR) referred to as shade. PAR was measured with a Sunfleck Ceptometer (Decagon Devices Inc., Pullman, WA, USA). The watering regime was moderate according to seedling water demand (13 mm applied 2 days a week). Three levels of fertilization were applied: initial fertilization supplied in the growing medium (NO_3: 57 mg L^{-1}, NH_4: 69 mg L^{-1}, P: 60 mg L^{-1} and K: 344 mg L^{-1}), initial fertilization plus 2 g L^{-1} of Osmocote® (N:P:K 14:8:14) and initial fertilization plus 5 g L^{-1} of a slow-release fertilizer (Osmocote®).

Morphological traits

Seven seedlings per set were randomly selected for morphological parameter determination at the end of the culture period. The measured parameters included shoot height (cm) and root collar diameter (RCD, mm); the sturdiness index (SI, cm mm^{-1}) was calculated as the ratio between shoot height and RCD. Biomass fractions of needles, shoots and roots (g) were obtained after drying seedlings in a forced-air oven at 65°C up to constant weight. Biomass allocation was evaluated by calculating the root:shoot ratio (R/S) between root and shoot dry weight. Root length (RL, cm) and root surface area (RS, cm^2) were measured after washing out the substrate from the roots, scanning the root system on a professional scanner (EPSON Expression 1680 110 Pro scanner) with a transparency adapter and analyzing the image by means of specific analysis software (WinRhizo, Régent Instruments Inc., Quebec, Canada). The projected leaf area of seedlings (LA, m^2) was determined in the same way as the root system variables. Specific leaf weight (SLW) was calculated as the ratio between needle dry weight and needle projected surface area.

Gas exchange traits

Gas exchange measurements were carried out after 6 months of cultivation in five plants per set under controlled conditions in a climatic chamber Fitotron (Sanyo, Gallenkamp PLC, Leicester). Five seedlings per treatment were selected and submitted to constant climatic conditions. The conditions were constant temperature (25°C) and water vapour pressure deficit (VPD = 1.6 kPa). Seedlings were irrigated to field capacity the night before the measurements. Measurements were taken at mid-morning with a portable infrared gas analyzer IRGA model Li-6400 with the 6400-05 Conifer Chamber (LI-COR, Lincoln, NB, USA) after the plants had been exposed for 3 h to a CO_2 concentration of 380 ppm and a photon flux density of 1,500 $\mu mol\ m^{-2}\ s^{-1}$. The parameters measured were net photosynthesis (A, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$), transpiration ($E$, $mmol\ H_2O\ m^{-2}\ s^{-1}$) and stomatal conductance (gs, $mmol\ H_2O\ m^{-2}\ s^{-1}$). Water use efficiency (WUE, $\mu mol\ CO_2\ mmol\ H_2O\ ^{-1}$) was estimated as the ratio between A and E.

Root hydraulic conductance traits

Root system hydraulic conductance was measured in seven plants per set using the high pressure flow meter method (HPFM, Dynamax, USA) in a transient mode within a range of 0–500 kPa pressures at a constant rate of 3–5 kPa s^{-1}, and the flow rate (F) was recorded every 3 s as described in Tyree et al. (1995). Flow (F) was plotted versus pressure (P), and root hydraulic conductance was estimated as the slope of a linear regression between both variables (ΔF, ΔP). We estimated specific root hydraulic conductance on a projected leaf area basis (K_L) and specific root hydraulic conductance scaled by total root surface area (K_R) (Nardini and Salleo 2000). Hydraulic conductance was determined at mid-morning in seedlings irrigated to field capacity the night before the measurements.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the effects of light regime on plant morphology, gas exchange and root hydraulic conductance variables; then, the data were separated according to light regime, and one-way ANOVA was applied to each set of data to test fertilization effects. Differences among fertilization levels were assessed with a Tuckey *b* posthoc test at $P < 0.05$. All statistical tests were performed with SPSS (v. 15.0, SPSS Inc., Chicago, IL, USA).

Results

The results showed three groups of parameters based on their range of variation with the applied treatments. The first group consisted of parameters that changed with fertilization under both light regimes. These parameters were highly sensitive to nutrient availability: height, RCD, biomass fractions, leaf area, root length, root surface area, photosynthesis and WUE. The second group comprises parameters that changed in response to fertilization under one light regime but not the other. In this group, SLW varied only under shade conditions, and root:shoot ratio and root hydraulic conductance (K_L and K_R) changed under full sunlight but not under shade conditions. The third group held parameters that showed no change with the applied treatments: transpiration and stomatal conductance.

Fertilization produced significant alterations in the vast majority of analysed parameters (Table 1). On the contrary, the shading treatment did not modify any of the morphological parameters except for the root:shoot ratio. Fertilization doses linearly increased the size (height and RCD) of seedlings (Table 2). Under full sunlight, seedling height increased from 2.5 ± 0.7 cm at the lowest fertilization level to 24.9 ± 1.5 cm at the highest treatment. The high fertilization treatment modified seedling biomass allocation and produced the largest seedlings with the highest biomass values and the lowest root:shoot ratios. R/S ratio decreased linearly with fertilization at full sunlight, while no differences were observed under shade. In the shade treatment, the morphological parameters showed no differences between the medium and the high level of fertilization, except for height. SLW did not change significantly with fertilization under full sunlight, but it was significantly lower in the less fertilized treatment under shade.

Table 1 One-way analysis of variance ANOVAs to test light regime and fertilization effects under full sunlight and shade in morphological, gas exchange and hydraulic conductance parameters

Factors	Light regime		Fertilization (full sunlight)		Fertilization (shade)	
Variable	*F*	*p*	*F*	*p*	*F*	*p*
Height	1.117	0.297	353.169	**<0.001**	271.396	**<0.001**
Diameter (RCD)	0.201	0.656	11.872	**0.001**	8.195	**0.004**
Sturdiness index	2.933	0.095	75.790	**<0.001**	44.815	**<0.001**
Needle weight	0.315	0.578	24.401	**<0.001**	10.789	**0.001**
Shoot weight	0.367	0.548	17.383	**<0.001**	5.125	**0.019**
Root weight	0.000	0.991	9.299	**0.002**	6.877	**0.007**
Leaf projected area	1.137	0.293	23.001	**<0.001**	15.483	**<0.001**
Specific leaf weight (SLW)	0.960	0.333	2.103	0.153	8.371	**0.003**
Root length	0.044	0.836	9.868	**0.001**	10.344	**0.001**
Root surface area	0.168	0.684	15.021	**<0.001**	9.678	**0.002**
Root:shoot	5.830	**0.021**	25.010	**<0.001**	3.050	0.075
Photosynthesis	1.073	0.316	23.029	**0.002**	22.565	**0.002**
Transpiration	1.327	0.266	0.872	0.465	5.152	0.050
Water use efficiency (WUE)	1.002	0.332	10.314	**0.011**	54.009	**<0.001**
Stomatal conductance	0.008	0.932	0.186	0.835	0.871	0.466
K_L	0.025	0.876	10.161	**0.001**	3.239	0.066
K_R	1.372	0.249	6.772	**0.007**	0.311	0.737

Significant *P* values ($P < 0.05$) are in bold

K_L specific root hydraulic conductance on a projected leaf area basis, K_R specific root hydraulic conductance scaled by total root surface area

Table 2 Morphological parameters for different light regimes (full sunlight and shade) at low, medium and high fertilization levels

Variables	Full sunlight			Shade		
	Low	Medium	High	Low	Medium	High
Height (cm)	2.5 ± 0.7a	12.1 ± 1.9b	24.9 ± 1.4c	4.4 ± 1.1A	18.1 ± 1.4B	22.5 ± 1.6C
Diameter, RCD (mm)	1.3 ± 0.2a	1.9 ± 0.3b	2.2 ± 0.4b	1.4 ± 0.3A	1.7 ± 0.3AB	2.0 ± 0.1B
Sturdiness index	1.8 ± 0.5a	6.3 ± 1.2b	11.4 ± 1.8c	3.2 ± 1.0A	10.6 ± 2.5B	10.8 ± 0.8B
Needle weight (g)	0.329 ± 0.107a	0.770 ± 0.202a	1.570 ± 0.410b	0.393 ± 0.087A	1.250 ± 0.430B	1.420 ± 0.550B
Shoot weight (g)	0.025 ± 0.010a	0.100 ± 0.051a	0.306 ± 0.161b	0.047 ± 0.018A	0.227 ± 0.146B	0.254 ± 0.152B
Root weight (g)	0.263 ± 0.076a	0.541 ± 0.204b	0.766 ± 0.302b	0.264 ± 0.060A	0.556 ± 0.181B	0.651 ± 0.261B
Leaf area ($\times 10^{-2}$ m^2)	28.9 ± 3.4a	82.2 ± 9.4b	149.9 ± 25.3c	43.4 ± 3.7A	117.1 ± 6.2B	164.3 ± 19.6B
SLW (g cm$^{-2)}$	0.010 ± 0.004a	0.014 ± 0.009a	0.028 ± 0.024a	0.009 ± 0.002A	0.016 ± 0.004B	0.013 ± 0.002B
Root length (cm)	0.45 ± 0.05a	1.51 ± 0.17b	2.35 ± 0.02b	0.55 ± 0.06A	1.71 ± 0.41B	1.64 ± 0.04B
Root surface area (cm^2)	86.8 ± 9.7a	231.0 ± 27.0b	388.2 ± 33.5b	90.6 ± 11.0A	283.5 ± 58.1B	259.5 ± 17.0B
Root:shoot	0.75 ± 0.07a	0.60 ± 0.10b	0. 40 ± 0.05c	0.60 ± 0.06A	0.38 ± 0.05A	0.42 ± 0.25A

Values represent mean ± SE ($n = 7$)

Different letters indicate single effects within each light level determined by the Tukey *b* test $P < 0.05$ (lower-case letters for full sunlight and upper-case letters for shade treatment)

Photosynthesis (*A*) and instantaneous water use efficiency (WUE) were significantly increased by fertilization under both light regimes, but no differences were found for transpiration rate (*E*) or stomatal conductance (gs) (Table 1; Fig. 1). Seedlings from the medium and high fertilization treatments showed around three times higher photosynthesis values than the low fertilized seedlings. Furthermore, fertilization enhanced WUE up to 80% in the high fertilized plants. Low fertilized plants had significantly lower values than medium and high treatments under both light regimes (Fig. 1). The opposite trend was found for transpiration rate and stomatal conductance, which tended to decrease with fertilization although no significant differences were found.

Water transport capacity of the whole root system (K_L and K_R) was modified by fertilization under full sunlight, but no differences were found between fertilization levels within the shade treatment (Table 1; Fig. 2). Low fertilized plants grown in full sunlight showed the highest values of K_L, while the other treatments (medium and high) did not

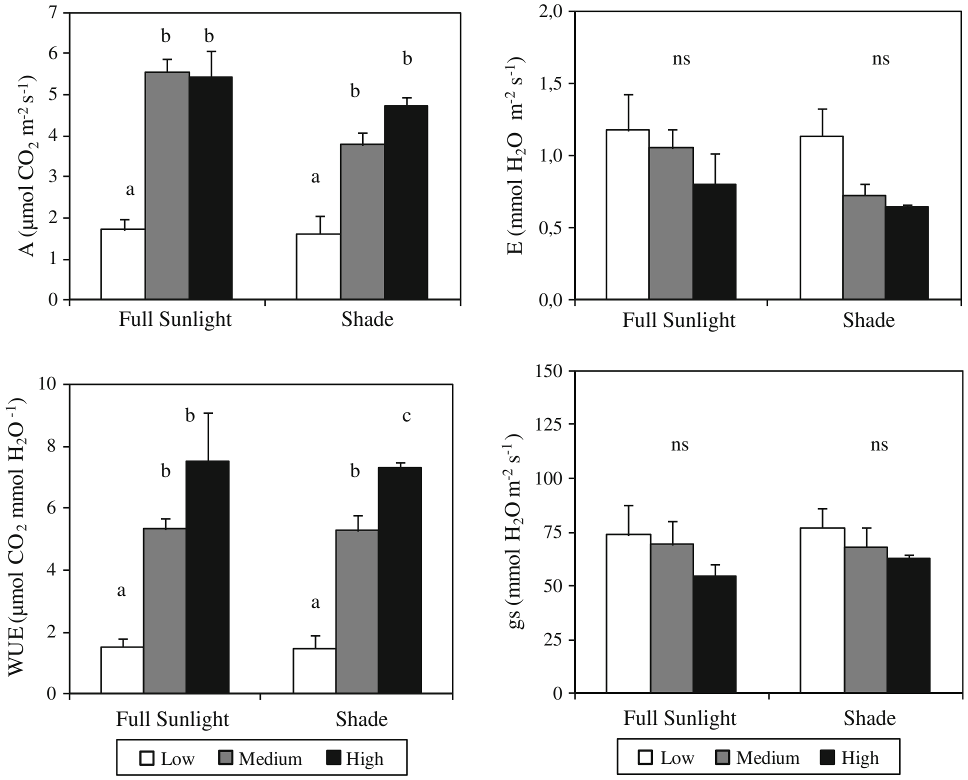

Fig. 1 Net photosynthesis (*A*), transpiration (*E*), water use efficiency (WUE) and stomatal conductance (gs) for different light regimes (full sunlight and shade) at low, medium and high fertilization level. *Different letters* indicate single effects within each light level determined by the Tukey *b* test $P < 0.05$

present any differences between each other. No differences in K_L were found between fertilization levels in the shade treatment. K_R showed the same response pattern as K_L (Fig. 2).

Discussion

Biomass allocation between root and shoot changed as a consequence of the fertilization treatments. High fertilization levels produced the largest plants, as previously reported (Oliet et al. 2009b; Villar-Salvador et al. 2008; Luis et al. 2009). R/S ratio decreased gradually according to the fertilization regime only under full sunlight as a consequence of the bigger shoots and the relatively lower development of roots. Root system development (both in terms of biomass and extension) is considered a key step for the establishment of woody seedlings in the field (Lloret et al. 1999; Tsakaldimi et al. 2005). According to some authors, a higher allocation to aboveground biomass and a lower root:shoot ratio can decrease the capacity of plants to overcome drought conditions (Leiva and Fernández-Ales 1998; Aranda et al. 2001). However, previous results with *P. canariensis* (Luis et al. 2009) found the opposite trend under semi-arid field conditions, where high fertilization treatments with lower root:shoot ratios had lower mortality after planting. Supporting these results, several studies have reported the positive influence of nursery fertilization in enhancing survival and growth after outplanting in Mediterranean ecosystems (Puértolas et al. 2003; Villar-Salvador et al. 2004; Oliet et al. 2009a, b). In contrast, under intense water limiting conditions, other works found a worse response in fertilized seedlings, based on their earlier mortality during the first year in the field (Cortina et al. 1997; Trubat et al. 2008).

Shade did not produce any changes in morphology except in biomass allocation patterns (R/S ratio), as has been observed in another Mediterranean species in a similar study (Villar-Salvador et al. 2004). The fact that the shading treatment did not produce any morphological changes indicates that the PAR reduction was not severe enough for this species. However, the same levels of shading applied in a parallel experiment (Hernández et al. 2009) to other Mediterranean species such as *Pistacia lentiscus* and *Quercus suber* produced changes in morphology, indicating that the response would be species-specific. Other works reporting significant morpho-functional acclimation processes in response to shade were the consequence of intense levels of shade, with average values around 10–20% of external radiation (Valladares et al. 2005; Quero et al. 2006, 2008). In addition, *P. canariensis* has been considered a species with some degree of shade tolerance at the seedling stage due to its capacity to germinate and grow under the pine forest canopy (Peters 2001; Luis 2000;

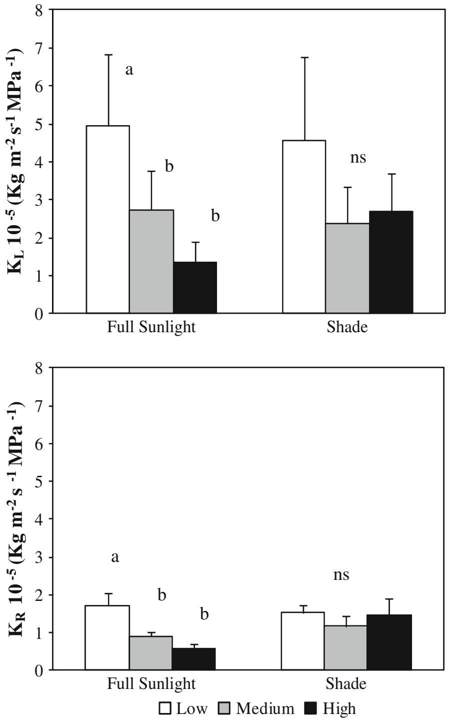

Fig. 2 Leaf-specific hydraulic conductance (K_L) and root-specific hydraulic conductance (K_R) for full sunlight and shade treatments at low, medium and high fertilization levels. *Different letters* indicate single effects within each light level determined by the Tukey *b* test $P < 0.05$

Arevalo and Palacios 2005) and even under 7% of the incident PAR in greenhouse conditions (Luis 2000). In spite of this tolerance to shade, specific leaf weight showed a different pattern of response between the full sun light and the shade treatment, indicating some effect of combined shade and nutrient availability.

Gas exchange variables were partially modified by fertilization. Photosynthesis rate increased as a result of fertilization but no variations in either transpiration or stomatal conductance were found, leading to a significant increase in WUE in fertilized seedlings. Higher photosynthetic rates in fertilized seedlings during the post-transplanting conditions in the field could be related to the higher growth of these seedlings in comparison with low fertilized seedlings (Leiva and Fernández-Ales 1998; Oliet et al. 2009a). In fact, Luis (2006) observed that well-fertilized seedlings had higher relative growth rates than low fertilized seedlings, with high fertilized plants reaching a shoot height average of 20.36 ± 1.39 cm in contrast with the 8.5 ± 0.23 cm shoot height reached by low fertilized seedlings, after 8 months of cultivation.

Previous studies with other Mediterranean species have shown that fertilization can modify the hydraulic capacity of water transport at root level (Trubat et al. 2006; Hernández et al. 2009). In addition, several works have linked the maintenance of transpiration rates to variations in xylem hydraulic properties (Hubbard et al. 1999; Chirino et al. 2008). In the present work, root hydraulic conductance was modified by fertilization, with low fertilized seedlings reaching the highest values of K_L and K_R at full sunlight and with no trend observed in the shade. A high root hydraulic conductance may facilitate efficient water and nutrient transport to leaves, avoiding negative water potentials and the risk of xylem cavitation during periods of drought stress (Sperry 2000). Therefore, the reduced K_L and K_R in fertilized seedlings would seem a priori to be a disadvantage for maintaining an adequate water supply to leaves. However, the fact that no effect was observed when transpiration and stomatal conductance were analysed under well-watered conditions indicates that under high water availability this species is able to supply water to leaves efficiently. Low fertilized seedlings showed more developed roots than shoots—as expressed in the root:shoot ratio—and thus a higher K_L and K_R was reached. The observed differences in hydraulic conductance rates could be explained by the differences found in resource allocation. When the aerial part of the plant grows due to fertilization, but the root system does not, hydraulic conductance can decrease (Bucci et al. 2006). Because the highly fertilized plants had more aboveground biomass to supply with water, their hydraulic conductivity was reduced. These results agree with others found for *Pistacia lentiscus* and *Quercus suber* seedlings where high fertilization significantly increased the leaf area and dry mass invested in leaves and shoots in both species without changing the root biomass, and lower rates of K_L were reached (Hernández et al. 2009). In another study with *P. lentiscus*, N deficiencies had little effect on belowground morphology or biomass allocation, and no significant effect on K_L was found (Trubat et al. 2006). However, after field planting, the root:shoot ratio differences between high and low fertilization treatments become reduced (Luis et al. 2009). Taking into account the feedback model for *Pinus canariensis* establishment in semiarid environments (Luis et al. 2009) when seedlings are transferred to the field, both the carbon allocation pattern could change with the different environmental conditions and the hydraulic properties could vary as the root system starts to grow to access soil water. In fact, other authors have pointed out that root system confinement could restrict the water flow (Pemán et al. 2006), and recently, Cuesta et al. (2010) suggested that larger, highly fertilized seedlings could have greater root hydraulic conductivity after planting due to their enhanced new root growth, also other studies reported increased aboveground woody biomass and hydraulic conductance with fertilization in *Pinus taeda* plantations (Samuelson et al. 2008). According to the results found in Luis et al. (2009) for outplanted *Pinus canariensis* seedlings, highly fertilized seedlings will have faster growing roots than low fertilized seedlings during the wet season after planting, but the root:shoot ratio of both will end up being similar. Therefore, the relevance of this species's hydraulic conductance at planting is reduced because it can change dramatically before the onset of the summer drought period due to different growing patterns in the roots. In contrast, other physiological parameters, such as the photosynthesis rate at planting, are essential because they can determine carbon gain and root and shoot growth after outplanting.

Conclusions

Fertilization significantly enhanced growth, biomass allocation, photosynthesis and water use efficiency in *Pinus canariensis* seedlings. Shade affected only one parameter (root:shoot ratio), indicating that the applied PAR reduction was not enough to modify morphology and physiology. Unpredictably, fertilization caused a significant reduction in root hydraulic conductance at the nursery. However, this reduced water flow at root level did not limit gas exchange at leaf level. The obtained results suggest that high photosynthesis at outplanting is of greater importance than high hydraulic conductance for ensuring *Pinus canariensis* seedling survival under Mediterranean conditions.

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