



Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?

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Abstract

Tree shelters in Mediterranean environments have a two-sided effect. They not only protect seedlings from browsing but also ameliorate microclimatic conditions, improving post-planting survival and growth. However, the ecophysiological basis of these effects are poorly understood. A factorial experiment combining light transmissivity and shelter type (solid tube vs. mesh wall) was carried out to assess the impact of contrasting microclimatic characteristics on seedling performance and physiological stress levels of shelters in two Mediterranean shrubland species (*Quercus coccifera* and *Rhamnus lycioides*) planted in a semiarid site. Even though seedlings in solid tube shelters experienced higher temperature and were slightly more photoinhibited, they had higher predawn water potential and, in general, better survival and growth than in mesh wall shelters. However, these effects were species-specific, with *Rh. lycioides* more favoured by solid wall shelters than *Q. coccifera*. However, root growth cannot explain these interactions between species and shelter type on seedling survival. Since light transmission had a marginal effect compared with wall type, we proposed that the observed effects and interaction with species are not dependent on light intensity or temperature but on other microclimatic differences like air velocity or light quality and distribution. Further studies should assess the importance of these factors on post-planting growth and physiological stress levels, which can be critical for matching the correct tree shelters type for each species in plantations in semiarid environments.

Keywords Afforestation · Restoration · Water potential · Chlorophyll fluorescence · *Quercus coccifera* · *Rhamnus lycioides*

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Introduction

Animal browsing is an important threat to the successful establishment of planted seedlings (Burney and Jacobs 2018). The incidence of browsing is highly dependent on the ecological characteristics of the reforested area that affects the animal specific composition and their abundance. Landscapes such as cropland matrixes tend to support high amounts of rabbits, hares and other generalist species (Calvete et al. 2004) that can be very detrimental for young plantations and result in major economic losses and delays in the restoration process. Among the most common system to protect seedlings is the use of individual tree shelters (Devine and Harrington 2008). Tree shelters are usually plastic tubes enclosing seedlings shoots to preclude browsing. Broadly speaking, two types of tree shelters are commercially available: solid tubes and meshes. Solid tubes are made in a continuous plastic wall, while meshes are open nets that allows free air circulation throughout the seedling. Solid tubes can also be ventilated by several holes. These characteristics have a strong influence on the environmental conditions around the protected plant (Bergez and Dupraz 2009). Therefore, tree shelters not only play a mere physical barrier role, but also can affect plant establishment and growth in additional ways by the changes in temperature, light, vapor pressure deficit or others (Dupraz and Bergez 1999; Oliet and Jacobs 2007; Pemán et al. 2010; Puértolas et al. 2010; Mariotti et al. 2015). There are numerous studies from different geographical areas analyzing the response of planted seedlings to the use of solid tube shelters in relation to the micro-environmental conditions inside (Del Campo et al. 2006; Jacobs 2011; Close et al. 2009; Bellot et al. 2002; Bergez and Dupraz, 2000). As expected, plant response is species- and environment-specific (Oliet et al. 2003; Padilla et al. 2011 Devine and Harrington 2008; Defaa et al. 2015). In semiarid areas, the use of solid tubes has proven to be on average beneficial for survival and growth (Piñeiro et al. 2013). On the contrary, the number of studies analyzing the effect of meshes is much lower (but see Ward et al. 2000; Devine and Harrington 2008), despite this type of protector is broadly used in operational plantations (Taylor et al. 2006; Van Lerberghe 2014). Unlike solid tubes, meshes allows air circulation, which precludes greenhouse effect, while reducing radiation incidence on the leaves. Although these effects could be beneficial for plant establishment in harsh areas, we are only aware of two studies addressing plant response to both types of tree shelters under semiarid Mediterranean conditions (Close et al. 2009; Padilla et al. 2011). Results from these studies are opposite, probably due to different site conditions and species, which reinforces the necessity of improving the knowledge of physiological basis for that responses.

Constructive characteristics of tree shelters are variable. Apart from different heights to adapt to herbivory size (Van Lerberghe 2014), other characteristics such as ventilation, color or light transmissivity affect micro-environmental conditions inside. Ventilation reduces air overheating during midday (Bergez and Dupraz 2009). Light transmissivity affects the amount of incident radiation, with effects on the intensity of stress and on plant growth response that could be crucial for plant survival under harsh conditions (Oliet et al. 2003). For example, photoinhibition can occur in shade tolerant species when protected by highly transmissive solid wall shelters (Puértolas et al. 2010), and some studies reveal a species-specific response of resources allocation to shoot or root as a function of shade tolerance (Jiménez et al. 2005; Puértolas et al. 2010; Vázquez de Castro et al. 2014). This could explain differences in survival between species with contrasted functional traits planted in Mediterranean environments under a gradient of light transmissivity (Oliet et al. 2003, 2015). However, these studies have only compared gradients of light transmissivity

within solid wall tube shelters, but the characteristics of mesh shelters (higher ventilation, different quality of the transmitted light) might interact with light transmission to determine the effects on survival and performance.

The objective of our experiment is to compare the effect of both types of tube shelters (solid and mesh) on two species (*Quercus coccifera* L. and *Rhamnus lycioides* L.) under semiarid Mediterranean conditions. We assessed first year after planting survival, growth and physiological stress levels (water potential and photochemical efficiency) and under a gradient of light transmissivity (40, 60 and 80%) for both types of shelters. Testing a gradient of light transmissivity for both types of guards will help to characterize the tree shelter ecophysiological system by assessing the relative contribution of different environmental variables on seedling response during establishment. Despite the duration of the study is relatively short, it includes post planting summer, which under harsh Mediterranean conditions is the most critical period in terms of survival (Villar-Salvador et al. 2012). The selected species are sprouting shrubs widespread in the western Mediterranean Basin. In semi-arid environments, they are considered keystone species affecting community composition and ecosystem function (Maestre and Cortina 2003). *Q. coccifera* is widely used in afforestations in the semiarid areas of the Mediterranean basin (Maestre and Cortina 2004; Sakcali and Ozturk 2004), although frequently accounts for low planting success (Baquedano and Castillo 2006). So far, the use of *Rh. lycioides* has been constrained to small scale or experimental plantations (Trubat et al. 2011; Chirino Miranda et al. 2013). Semiarid areas of the Mediterranean Basin are among the most challenging zones for the establishment of woody vegetation. Numerous biotic and abiotic factors negatively affect survival of planted young trees in these zones. Summer drought combined with excess of radiation and high temperatures (Martínez-Ferri et al. 2000; Niinemets and Keenan 2014) can reduce post summer survival to very low levels (Villar-Salvador et al. 2012). Besides, predation by small mammals, birds or ungulates constitutes another major source of failure (Leverkus et al. 2013). All these factors dramatically reduce the efficiency of restoration efforts. In addition, the current scenario of climate change, with higher probabilities of extreme, harsh summers in these areas (Giorgi and Lionello 2008), suggests that the success of restoration programs in dry Mediterranean environments will require improvements in planting techniques (Cortina et al. 2011; Vallejo et al. 2012). Our study could contribute to improve establishment success of key woody species in these challenging areas by a better management of tree shelters.

Materials and methods

Study site and plant material

The study site is located in an old cropland of central Spain in Toledo province (39°39'8"N, 3°28'5"W, elevation 660 m a.s.l.). The slope of the planting area is North aspect with a moderate 14% steep. Soils are mostly Inceptisols (Gómez-Miguel and Badía-Villas 2016). In accordance with a sample from study site, soils are deep (0–110 cm) of mostly loamy texture (22.4–35.2% sand, 44.0–48.7% silt and 19.7–29.1% clay). Horizons are light-colored highly basic (pH from 7.9 to 8.3) and calcic, with organic matter ranging from 2.14 (upper) to 0.86% (deepest horizon). Maximum electric conductivity of deepest horizon is low (170 $\mu\text{S cm}^{-1}$). Permeability is high for the first two horizons (0–30 cm) and moderate below this depth, according to Gandullo (1985). The climate is Mediterranean semiarid,

with mean annual precipitation of 418 mm and mean annual temperature of 14.2 °C. Summers are very hot and dry, with drought periods lasting 4 months, mean maximum temperature of 33 °C in July and absolute maximum temperature reaching 43 °C. Winters are cold with frequent frosts. Temperature can drop to -11 °C and the mean minimum temperature is 0.9 °C in January (Ninyerola et al. 2005). During the planting year (2014) annual rainfall was much lower than average (252 mm), with a prolonged dry period from May to September of accumulated rainfall as low as 32 mm (data from National Agency of Meteorology, Agriculture and Environment Department, Spanish Government).

Seedlings of *Q. coccifera* and *Rh. lycioides* were raised from seeds of provenance region ES29 Montes de Toledo (Alía Miranda et al. 2009) and cultivated in 200 cm³ cells (plant density 370 m⁻² Plasnor, Spain). After one year in the nursery and prior to planting, seedling height, root collar diameter, total biomass and root biomass were 22.1 ± 1.5 cm, 5.5 ± 0.4 mm, 8.1 ± 0.4 g and 5.0 ± 0.4 g, respectively, for *Q. coccifera*, and 22.9 ± 1.5 cm, 3.8 ± 0.3 mm, 4.7 ± 0.6 g and 1.4 ± 0.2 g, respectively, for *Rh. lycioides* (n = 10). These values fall within the recommended ranges for both species according to Pemán García et al. (2013).

Field experiment

The site was cross subsoiled prior to planting at a 60 cm depth with two rippers 1 m apart to reduce soil compaction. Subsoiling was conducted following contour lines separated by two meters and perpendicular directions. Seedlings were planted on January 11, 2014 every 1 m along contour lines (spacing was 2 × 1 m), after manually opening holes (0.3 m × 0.3 m × 0.3 m) in the junction of the subsoiling furrows. No weed control were conducted during the experiment, as the seed bank of weeds was weak in this area, specially under the arid conditions of the planting year. Both species were alternated within each planting row, and shelter treatments were randomly assigned to each seedling. The experimental design was a 2 × 2 × 3 factorial design, with the following factors and levels: (1) species (*Q. coccifera* vs. *Rh. lycioides*), (2) tree shelter type (solid wall vs. mesh) and (3) light transmissivity of the solid plastic or mesh (values around 40, 60 or 80%). The solid wall tube shelters were made from plastic material supplied by Repsol Química (Spain). Additives were added to the copolymer base to reach the light transmissivities tested in this experiment, maintaining the red/far red ratio around 1 (neutral shade) (Vázquez de Castro et al. 2014). Hand-made tubes using the plastic sheets were circular, single-walled tubes, 50 cm tall × 10 cm wide, with four ventilation holes facing each other of 2.5 cm width and situated at 18 and 36 cm in height. Minimum value of 40% light transmissivity was considered as a target when designing shelters that promote biomass allocation to roots and improve water balance of Mediterranean seedlings (Vázquez de Castro et al. 2014). Solid plastic tubes were stabilized by fixing a plastic stake with clamps and burying the shelter in the soil. Plastic meshes were chosen among available polyethylene products in the market. Mesh of 80% was a 60 cm tall × 15 cm wide cylindrical blue net with holes 8 × 8 mm (Protec Blaunet model, Projar SA, Spain). 60% light transmissivity mesh was also a 60 cm tall × 15 cm wide cylindrical black net with holes 2.4 × 2.4 mm (P40 model, Projar S.A., Spain). And 40% light transmissivity mesh was a was a 60 cm tall × 12 cm wide cylindrical black net with holes 4 × 3 mm (V8 model, Improfort Limited, Spain). Meshes were stabilized by using two plastic stakes. Actual light transmissivity of solid tubes and meshes under field conditions was determined in several daily cycles of photosynthetically active radiation (PAR) measurements with two or three sensor replicates (QSO-SUN, Onset,

USA) per shelter type connected to a U12 data-logger (Onset, USA). Values were registered every 10–15 min. Light transmissivity was averaged along the mean daily cycle. Mean transmissivity percentages were 77 (named solid tube 80%), 58 (named solid tube 60%) and 36 (named solid tube 40%) % for solid tubes and 83 (plastic mesh 80%), 56 (plastic mesh 60%) and 46 (plastic mesh 40%) % for meshes. A total of 300 seedlings per species were planted, of which 50 seedlings were randomly assigned to each combination of type of shelter \times transmissivity. As experimental plot was small and homogeneous, arrangement of treatments was fully randomized, and no blocking or grouping as a mean to control experimental error was necessary.

Monitoring plant response and microclimatic conditions for shelter types

Seedlings survival was measured four times along the study, from June 2014 to February 2015. Some apparently dead seedlings resprouted after measurements and were accounted as live in the following assessment. Height and basal stem diameter were measured on every plant at the end of October 2014. Seedlings biomass and root development were evaluated from five randomly chosen seedlings per treatment and species (60 plants in total) that were destructively harvested on February 28, 2015. Using small hand tools, root systems were carefully excavated from soil up to a depth of 70 cm and taking care to retain roots > 1 mm diameter. Shoots were separated from the roots at the root collar and all parts were frozen until processing. Roots protruding out of the plug were excised and washed free from soil with tap water. Leaves and protruding roots were scanned and leaf area and root length measured with an image analyzer (ImageJ V1.48[®], National Institutes of Health, USA). After these measurements, dry mass of each component (leaves, stem, plug roots and protruding roots) was determined by oven drying them at 65 °C for 48 h and weighing.

Physiological measurements took place in two consecutive sunny days of June (14 and 15) and July (15 and 16) 2014 in five seedlings per shelter type, transmissivity and species (60 seedlings in total, 30 per day). A small window was opened in the solid or mesh wall of the shelters to facilitate sampling for water potential and chlorophyll fluorescence measurements; the window was otherwise closed. Shoot xylem water potential was measured at predawn (Ψ_{pd}). A 3–7 cm healthy twig of the upper third of the plant was excised between 05.00 and 07.00 AM, wrapped in aluminum foil, kept in sealed polyethylene bags and stored refrigerated in an ice box. Water potential was measured within 3 h using a pressure chamber (Model 1000[®], PMS Instruments Company, USA). To check potential confounding effect of time since twig excising and measurement on water potential, both variables were plotted and no significant correlation was found (data not shown). Chlorophyll fluorescence was evaluated on the same plants. The ratio of variable to maximum fluorescence (F_v/F_m) as a surrogate of maximum photochemical efficiency of photosystem II was measured through the opened window with a fluorometer (FMS, Hansathech Instruments, UK). A fully expanded leaf of the upper third of the seedling was chosen. Prior to F_v/F_m measurements, that were done at predawn (07.00 AM) and midday (13.30 PM) leaves were dark acclimated for 30 min (Kalaji et al. 2014).

To assess the effect of light transmissivity of tube shelters on internal microclimate conditions, air temperature and relative humidity (RH) data logger sensors (U23-001 Onset, USA) were installed in the shelters from 14 to 25 June 2014. All this period was cloudless. Sensors were randomly installed inside two shelters of each shelter type \times light transmissivity combination (12 sensors in total), attached to a stake at a height between both pairs

of ventilation holes for solid tubes and the same height for meshes. Temperature and RH were recorded every 15 min. Vapor pressure deficit (VPD) was calculated from temperature and RH data following Rosenberg et al. (1983) method.

Data processing and statistical analysis

Post summer (October 2014) and 13 months (February 2015) after planting survival data were analyzed using a generalized lineal model based upon a binomial errors distribution with a logit link function. Full model included shelter type, light transmissivity, species, and all interactions among factors as predictors. Post hoc comparisons among treatments for last measurement (February 2015) were done using Bonferroni correction for paired comparisons.

In case of plant development (height, diameter, biomass and root length), a general lineal model with three main fixed factors as per survival were applied by running a three ways analysis of variance (ANOVA). Physiological data were analyzed similarly, although a fourth fixed factor (summer month, June and July) was included in the model. Non-normal data (basal stem diameter in October 2014 and all data from February 2015 plant excavation) were previously converted to logarithmic forms to fulfill normality and variance homogeneity requirements. When ANOVA showed significance, differences among means were identified using a Tukey post hoc test. Differences were considered statistically significant if $P < 0.05$. Results are given as mean \pm SE throughout the paper.

Data from temperature and relative humidity inside tube shelters were averaged per sensor and time to represent a mean daily cycle.

All the statistical analyses were performed using software R version 3.1.1 (R Core Team 2014 Vienna, Austria). Figures were produced using Sigmaplot, Version 12.0 (Sigma Plot 2012, Inc., San Jose, CA, USA).

Results

Physiological response to shelters (maximum photochemical efficiency and water potential) and microclimate in summer

Maximum photochemical efficiency (F_v/F_m) at both pre-dawn and midday was significantly affected by all factors of the study during June and July. Besides, significant interactions were found between month of measurement and shelter type (mesh or solid tube) and those two factors and species for predawn F_v/F_m (Table 1). Seedlings growing in solid tubes showed lower values of both predawn and midday F_v/F_m than those in meshes, although differences were lower for predawn (0.02) than for midday (0.06) (Fig. 1). A drop in F_v/F_m was found with increasing light transmissivity, with plants growing in lightest tubes (80%) having lowest values of both pre-dawn and midday maximum photochemical efficiency. In addition fluorescence plants response to light transmissivity was higher for midday F_v/F_m , as seedlings in 60% light transmissivity shelters had also minimum values as per lightest shelters (Fig. 1). On average, F_v/F_m dropped from June to July (data not shown) and, by species, predawn and midday F_v/F_m for *Rh. lycioides* was higher (0.83 ± 0.00 and 0.72 ± 0.01) than that of *Q. coccifera* (0.77 ± 0.00) and 0.69 ± 0.01 , respectively).

Predawn water potential during summer months was only affected by shelter type (Table 1). Seedlings in meshes were significantly more water stressed, with

Table 1 Results from ANOVA (Snedecor F and associated probability *P*) test for the effects of month of measurement (June and July), species (*Q. coccifera* and *Rh. lycioides*), shelter type (solid tube and plastic mesh) and transmissivity (80–60–40%) during 2014 summer on predawn water potential (Ψ_{pd}) and leaf fluorescence at predawn ($F_v/F_{m_{pd}}$) and midday ($F_v/F_{m_{md}}$)

	df	Ψ_{pd}		$F_v/F_{m_{pd}}$		$F_v/F_{m_{md}}$	
		F	<i>P</i> > <i>F</i>	F	<i>P</i> > <i>F</i>	F	<i>P</i> > <i>F</i>
Month (M)	1	0.02	0.89	6.10	0.01	5.40	0.02
Species (S)	1	1.80	0.18	60.94	< 0.001	5.08	0.02
Shelter type (ST)	1	22.49	< 0.001	6.11	0.01	19.80	< 0.001
Transmissivity (T)	2	0.15	0.85	6.30	0.002	3.64	0.02
Month × species	1	0.11	0.73	0.01	0.93	2.30	0.13
Month × shelter type	1	0.001	0.97	10.83	0.001	0.58	0.44
Species × shelter type	1	0.47	0.49	0.01	0.92	0.40	0.52
Month × transmissivity	2	0.17	0.83	0.12	0.88	0.14	0.86
Species × transmissivity	2	1.17	0.31	2.64	0.07	0.72	0.48
Shelter type × transmissivity	2	0.01	0.99	0.37	0.68	0.98	0.37
M × S × ST	1	0.01	0.93	4.75	0.03	3.49	0.06
M × S × T	2	0.250	0.77	0.08	0.91	0.53	0.58
M × ST × T	2	0.12	0.88	0.41	0.66	0.11	0.89
S × ST × T	2	1.05	0.35	0.71	0.49	0.24	0.78
M × S × ST × T	2	0.16	0.85	1.24	0.29	0.65	0.52

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

values of -3.11 ± 0.12 MPa, while plants growing in solid tubes were more hydrated (-2.32 ± 0.11 MPa).

Averaged temperature in solid wall shelters during the daylight period in late June was higher than that in meshes, with maximum differences (pooling the three light transmissivity levels) reaching 7.3 °C at 13:15 h solar time (Fig. 2). Temperature differences among light transmissions were minor, with the 80% shelter tending to have higher temperatures than the rest (1.1 °C, data not shown). Differences in RH among type of shelters and light transmissivities were minimum (Fig. 2). As a consequence, VPD inside shelters follow the same pattern as temperature, with solid tubes having maximum VPD differences (after averaging by light transmissivity) of 1.86 kPa at 13:15 h solar time (Fig. 2).

Survival and growth

Post summer survival (October 2014) was significantly affected by type of shelter and light transmissivity, although shelter type interacted with species (Table 2). 1 year after planting (February 2015), the interaction between shelter type and transmissivity became also significant (Table 2). Survival was almost double in solid wall shelters ($84 \pm 3\%$) than in meshes ($45 \pm 4\%$) for *Rh. lycioides* (Fig. 3). In contrast, for *Q. coccifera* only the mesh of 80% light transmissivity had significantly lower survival (Fig. 3). In February 2015, this combination of shelter type and transmissivity had the lower survival in both species (Fig. 3). Overall survival of *Rh. lycioides* in February 2015 was slightly superior to *Q. coccifera* (68 ± 3 versus $60 \pm 4\%$, respectively), but survival in the most favorable shelter (solid wall tube 60%) was higher for *Rh. lycioides* ($89 \pm 4.6\%$) than for *Q. coccifera* ($75 \pm 5.8\%$).

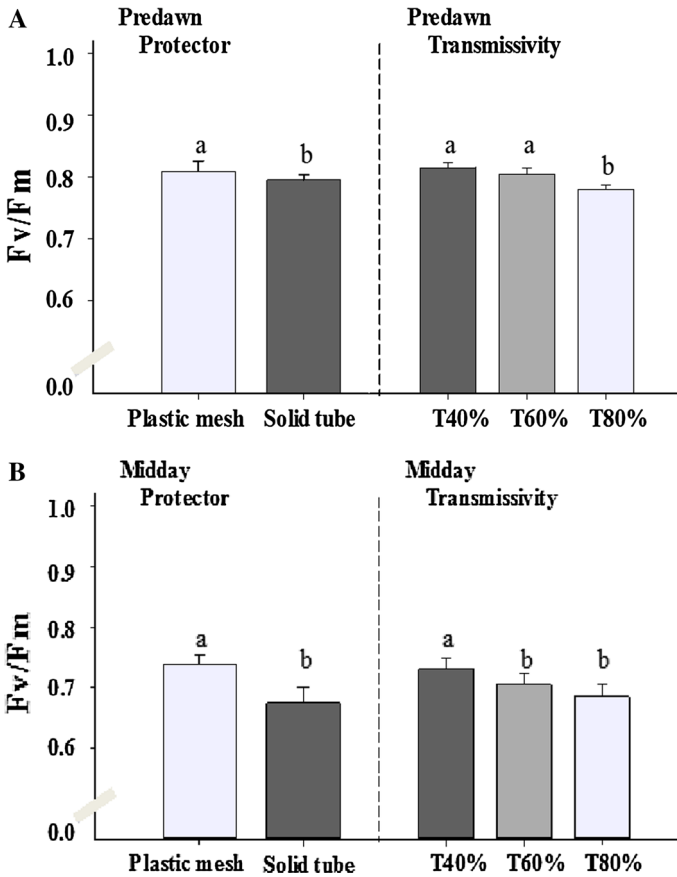


Fig. 1 Summer predawn (a) and midday (b) photochemical efficiency (F_v/F_m) as affected by shelter type (plastic mesh or solid tube) and light transmissivity (T40–T60–T80%). Data are averaged by species and month of measurement (June and July 2014). Different letters between Shelter type and among transmissivity levels denote differences among levels of factors after Tukey's post hoc test. Error bars represent \pm SE

Post summer height was significantly affected by all factors and by almost every second order interactions among them ($P < 0.001$), with the exception of species \times shelter type ($P = 0.07$). Seedlings growing under 40 and 60% light transmissivity grew similarly in height, while a detrimental effect of height growth appears when plants grew under maximum light transmissivity level. Besides, this pattern is much more intense for seedlings growing in meshes and for *Rh. lycioides* (Fig. 4a). Thus, differences ranged between almost same height across transmissivities for *Q. coccifera* and plants growing in solid tubes, to a significant depletion in height under 80% transmissivity for *Rh. lycioides* and those growing in meshes (Fig. 4a). Post-summer basal stem diameter (BSD) was significantly affected by shelter type ($P < 0.001$) and by a light transmissivity \times shelter type interaction ($P = 0.03$). BSD of plants within solid tubes were on average 18% larger than that in meshes with no differences between light transmissivity within shelter types except for the 80% mesh, which were significantly smaller for *Rh. lycioides* and almost significant for *Q. coccifera* (Fig. 4b). No significant third order

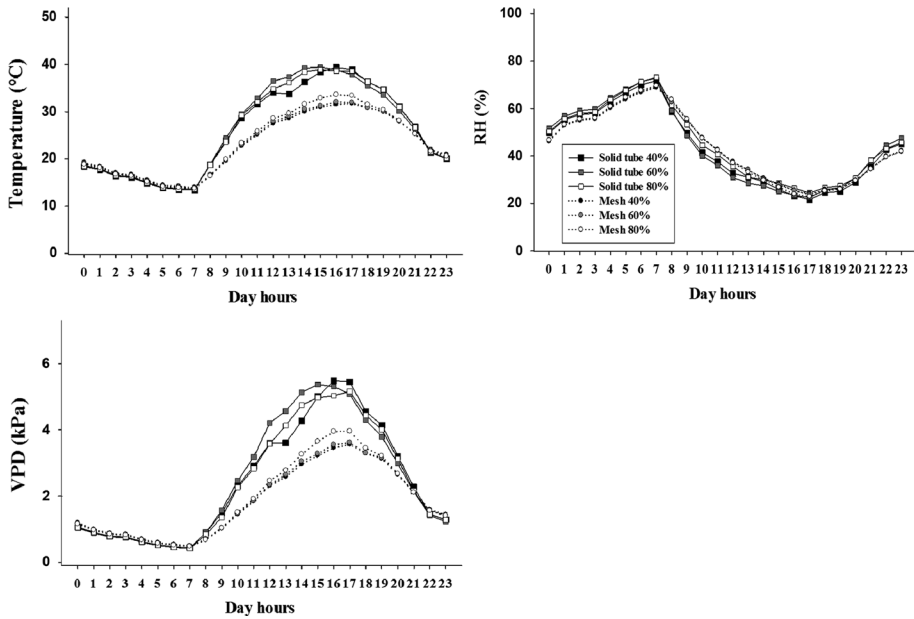


Fig. 2 Temperature, relative humidity (RH) and vapor pressure deficit (VPD) during a mean daily cycle (GMT +2) of June 2014 within different combinations of shelters types and light transmissivity. Data from two sensors per shelter type and transmissivity, 11 days of measurements and 15 min frequency are averaged to one point per hour

Table 2 Contrasts of the generalized linear model effects for survival in October 2104 and February 2015 of *Q. coccifera* and *Rh. lycioides* species planted with solid tubes or meshes (shelter type) under three levels of light transmissivity (40–60–80%)

	df	October 2014		February 2015	
		Wald χ^2	$P > \chi^2$	Wald χ^2	$P > \chi^2$
Species (S)	1	6.07	0.014	3.04	0.081
Shelter type (ST)	1	31.67	0.000	38.86	0.000
Transmissivity (T)	2	7.25	0.027	12.18	0.002
Species \times shelter type	1	11.00	0.001	14.12	0.000
Species \times transmissivity	2	1.17	0.556	0.90	0.637
Shelter type \times transmissivity	2	3.76	0.153	7.37	0.025
S \times ST \times T	2	0.60	0.742	0.21	0.898

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

interaction appeared among three factors for height or diameter. Cross values for this three factors combination are presented in Supplementary material.

For all biomass and growth traits measured after plant excavation in February 2015 (13 months after planting), no significant effect was found for light transmissivity (Table 3). However, leaf area and shoot biomass was significantly bigger in solid wall tubes than in meshes (64 and 33% respectively) (Tables 3, 4). Protruding roots length was also greater in solid tubes and affected by the interaction of this factor with species: it was 78% longer in *Q. coccifera* and not significantly different from meshes in *Rh. lycioides* (Tables 3, 4). Shoot:root

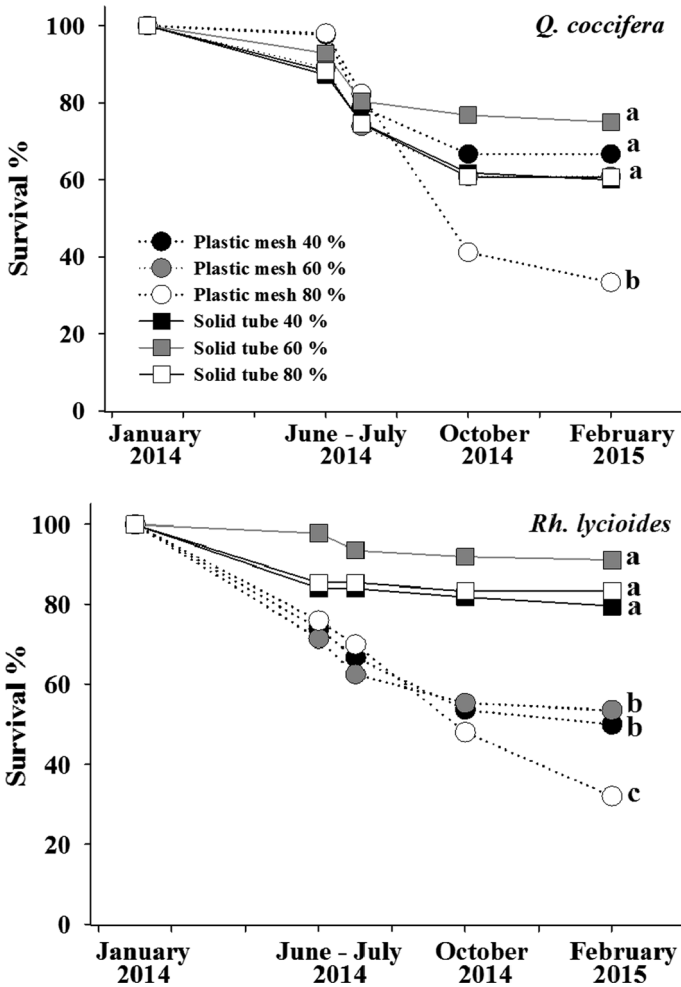


Fig. 3 Survival of *Q. coccifera* (top) and *Rh. lycioides* (bottom) along the study period as affected by type of shelter (plastic mesh and solid tube) and transmissivity (40, 60 and 80%). Letters at the end of each treatment denote significant differences during last measurement (February 2015)

ratio (g g^{-1}), specific leaf area and specific root length of seedlings were unaffected by shelter type or light transmissivity. Most of the evaluated traits from excavation were species-specific: *Q. coccifera* leaf area was 65% higher, while protruding roots length, shoot:root ratio and specific root length were 36, 60 and 40% lower than those of *Rh. lycioides*. Nevertheless, data from plant excavation 13 months after planting show high variability levels (Table 4), precluding the declaration of additional significant responses to tested factors.

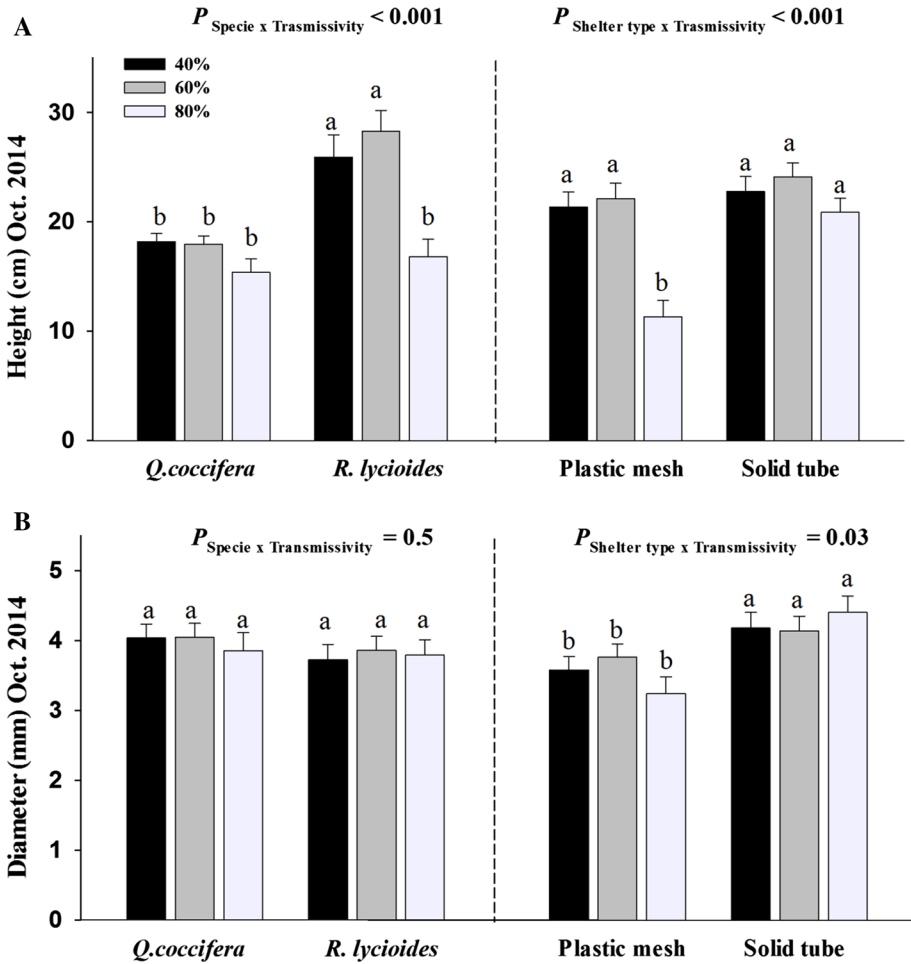


Fig. 4 Post summer height (a) and basal diameter (b) of tested species (*Rh. lycioides* and *Q. coccifera*) in October 2014 as affected by shelter type (plastic mesh or solid tube) and light transmissivity (40–60–80%). Different letters denote differences among levels of treatments after Tukey’s post hoc test. Error bars represent \pm SE. *P* values for second order interaction are shown on top of each subfigure

Discussion

Unexpectedly, seedlings in solid wall tubes showed a higher survival and growth for almost every combination of light transmissivity and species. Microclimatic conditions inside mesh shelters (T, VPD) during the hot Mediterranean summer were expected to decrease heat and water stress compared to solid wall shelters. In fact, chlorophyll fluorescence data suggests a higher degree of photoinhibition in solid tubes with differences deepening from the beginning (June) to midsummer (July). Maximum photochemical efficiency is more sensitive to thermal than to hydric stress, as shown in a wide variety of woody species that presented significant reduction in F_v/F_m under higher temperatures (Matias et al. 2017; Methy et al. 1997). However, values were always close to 0.8, which is considered the

Table 3 Results from ANOVA (Snedecor F and associated probability *P*) test for the effects of Species (*Q. coccoifera* and *Rh. lyciooides*), shelter type (solid tube and plastic mesh) and Transmissivity (80–60–40%) on morphological traits of seedlings excavated in February 2015

<i>df</i>	Leaf area		Protruding roots length		Shoot weight		Shoot/root		Specific leaf area		Specific root length		
	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	
Species (S)	1	12.59	0.001	6.68	0.01	2.48	0.12	83.30	0.001	0.007	0.97	16.43	0.001
Shelter type (ST)	1	23.86	< 0.001	3.88	0.05	8.14	0.006	0.81	0.37	1.13	0.29	1.02	0.31
Transmissivity (T)	2	0.57	0.56	0.13	0.87	1.34	0.26	1.83	0.16	0.13	0.87	0.62	0.54
Species × shelter type	2	0.03	0.86	4.15	0.04	0.03	0.86	0.57	0.45	0.16	0.68	1.29	0.26
Species × transmissivity	1	0.31	0.73	0.30	0.74	0.06	0.93	0.09	0.91	1.05	0.35	0.46	0.63
Shelter type × transmissivity	2	1.66	0.19	0.95	0.39	0.45	0.63	2.7	0.07	1.54	0.22	0.27	0.78
S × ST × T	2	2.07	0.13	2.35	0.08	1.43	0.24	1.20	0.30	2.66	0.08	1.5	0.21

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

Table 4 Morphological traits by species (*Q. coccifera* and *Rh. lycioides*) as affected by shelter type (plastic mesh or solid tube) of seedlings excavated in February 2015

	<i>Q. coccifera</i>		<i>Rh. lycioides</i>	
	Plastic mesh	Solid tube	Plastic mesh	Solid tube
Leaf Area (cm ²)	60.60 ± 10.9	127.03 ± 20.43	36.15 ± 10.93	77.25 ± 19.0
Protrud. roots length (mm) ¹	82.93 ± 14.60 ^b	147.50 ± 20.77 ^a	171.47 ± 49.33 ^a	189.30 ± 56.83 ^a
Shoot weight (g)	3.44 ± 0.47	5.11 ± 0.80	2.98 ± 0.93	4.39 ± 1.20
Shoot/Root ratio (g g ⁻¹)	0.58 ± 0.0	0.59 ± 0.0	1.40 ± 0.23	1.55 ± 0.27
Specific leaf area (cm ² g ⁻¹)	74.07 ± 15.37	58.48 ± 5.23	72.84 ± 15.27	63.09 ± 6.5
Specific root length (cm g ⁻¹)	159.59 ± 25.97	180.80 ± 55.63	323.94 ± 63.80	246.09 ± 46.83

¹As species × shelter type was significant for this variable (see Table 3), different letters following mean values ± SE denote differences among combination treatments after Tukey's post hoc test

optimum value (Bjorkman and Demmig 1987) and the differences between both shelter types were small. Deactivation of reaction centers of photosystem II is part of the acclimation process to avoid photodamage when photosynthesis is impaired (Demmig-Adams and Adams 1992). The small changes observed here might only reflect slight differences in this deactivation but with no impact on plant capacity to survive and grow.

In contrast, differences in predawn water potential were in agreement with seedling performance. Average predawn water potential registered in summer for both species in plastic mesh (−3.1 MPa) indicates a moderate level of water stress. *Q. coccifera* shows drastic reduction of assimilation rate with ψ_{pd} values below −2 MPa (Baquedano and Castillo 2006) which implies reduction of root growth. Even though these values are higher than the critical water potential values (−6 MPa) inducing fatal embolism (Vilagrosa et al. 2003), they could impair plant functioning and internal carbon budget, putting plants in higher risk of reaching those critical values.

Several hypotheses can be formulated to explain the lower ψ_{pd} in meshes. Air velocity inside solid wall shelters is negligible, even for ventilated tubes (Bergez and Dupraz 2000). Restricted air movement creates a thicker and less conductive boundary layer that reduces foliar water loss and improves hydric status. This effect has been demonstrated in previous works with solid wall shelters under controlled conditions (Kjelgren and Rupp 1997; Bergez and Dupraz 1997), where water losses of seedlings in tube shelters was much lower than in unprotected ones. Even though mesh shelters might restrict air movement, they allow some air circulation through leaves. This would decrease leaf-air boundary layer conductance (Lambers et al. 2008), accelerating soil water depletion in the root-zone. Within this hypothesis the increase in transpiration demand due to air movement in meshes would be more intense than the higher VPD inside solid wall shelters provoked by temperature.

Alternatively, increased air velocity also induces stomatal closure and hence decreased carbon gain, which along with lower air temperature during spring in mesh shelters could explain the observed reduction in growth in this type of shelters. Besides the effects related to differences in air movement between mesh and tube shelters, growth reduction could be linked to light quality and distribution. Even though the experimental factorial design allowed the comparison of both types of shelters with similar total radiation levels, light inside tubes was exclusively diffuse while in meshes there was a mixture of direct and indirect radiation. Moreover, leaves in meshes were exposed not only to those patches of different light quality and intensity, but also to relatively rapid changes as the sun moved

during the day or the leaves were shaken by wind. These rapid changes similar to sunflecks within or beneath canopies can decrease photosynthetic efficiency and water use efficiency, as the capacity of photoacclimation to contrasting light conditions cannot cope with such rapid changes (Townsend et al. 2017). Potentially, these two negative effects on carbon gain described above could impair root growth, which is essential for summer survival in dry environments (Padilla et al. 2007), and has been linked to survival rates in *Q. coccifera* across a range of different tree shelters (Bellot et al. 2002). However, unlike the latter study, no relationship between root growth and survival was observed here. For *Rh. lycioides*, even though survival was much higher in tubes, root length was similar to mesh shelters, while *Q. coccifera* roots grew less in mesh shelters but only survival was impaired for the 80% transmissivity.

This species-specific effect of the type of shelter on seedling performance confirms that the effect of tube shelters on seedling performance depend on the ecophysiological features of the species (Puértolas et al. 2010; Vázquez de Castro et al. 2014). These previous studies pointed towards shade tolerance as the main trait explaining plant responses to tree shelters. Shade tolerant species were more benefited from protection, as root growth was less negatively affected by the reduction in light compared to intolerant species. Moreover, light reduction during summer could explain increased survival in *Q. ilex* (Puértolas et al. 2010). Also, *Rh. lycioides* benefits from shade in semiarid plantations (Soliveres et al. 2008). However, our results suggest that other characteristics might also contribute to explain differential effects across species. Lack of knowledge on the physiological characteristics of *Rh. lycioides* makes difficult to understand the basis of these differences. It seems that the higher overall survival rates of this species compared to *Q. coccifera*, which are coincident with previous studies (Trubat et al. 2008, 2011), could be linked to faster root growth. Adults from *Rh. lycioides* show higher assimilation rates than *Q. coccifera* during spring, which could explain larger growth and root development (Bellot et al. 2004). The reason for the clear differences in survival between mesh and tube shelters within *Rh. lycioides*, which are less evident than in *Q. coccifera*, are not easy to explain with the current information available. Further studies should investigate which functional attributes other than shade tolerance determine seedling response to tube shelters.

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References

- Alfía Miranda R, García Del Barrio JM, Iglesias Sauce S, Mancha Núñez JA, de Miguel y Del Ángel J, Nicolás Peragón JL, Pérez Martín F, Sánchez de Ron D (2009) Regiones de procedencia de especies forestales españolas. Organismo Autónomo Parques Nacionales, Madrid
- Baquedano FJ, Castillo FJ (2006) Comparative ecophysiological effects of drought on seedlings of the Mediterranean water-saver *Pinus halepensis* and water-spenders *Quercus coccifera* and *Quercus ilex*. *Trees* 20:689–700
- Bellot J, Ortiz de Urbina JM, Bonet A, Sánchez JR (2002) The effects of treeshelters on the growth of *Quercus coccifera* L. seedlings in a semiarid environment. *Forestry* 75(1):89–106
- Bellot J, Maestre FT, Chirino E, Hernández N, de Urbina JO (2004) Afforestation with *Pinus halepensis* reduces native shrub performance in a Mediterranean semiarid area. *Acta Oecol* 25:7–15
- Bergez JE, Dupraz ZC (1997) Transpiration rate of *Prunus avium* L. Seedlings inside an unventilated treeshelter. *For Ecol Manag* 97:255–264

- Bergez JE, Dupraz ZC (2000) Effect of ventilation on growth of *Prunus avium* seedlings grown in treeshelters. *Agric For Meteorol* 104:199–214
- Bergez JE, Dupraz ZC (2009) Radiation and thermal microclimate in tree shelter. *Agric For Meteorol* 149:179–186
- Bjorkman O, Demmig B (1987) Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* 170:489–504
- Burney OT, Jacobs DF (2018) Species selection—a fundamental silvicultural tool to promote forest regeneration under high animal browsing pressure. *For Ecol Manag* 408:67–74
- Calvete C, Estrada R, Angulo E, Cabezas-Ruiz S (2004) Habitat factors related to wild rabbit conservation in an agricultural landscape. *Landscape Ecol* 19:531–542
- Chirino Miranda E, Puertolas Simon E, Garcia Vinas JL, Gaston Gonzalez A, Prada Saez MA (2013) *Rhamnus alaternus* L. y *Rhamnus lycioides* L. In: Navarro Cerrillo RM, Nicolás Peragón JL, Prada Sáez MA, Serada Hierro R, Pemán García J (eds) Producción y Manejo de semillas y plantas forestales. Naturaleza y Parques Nacionales. Serie Forestal. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid
- Close DC, Ruthrof KX, Turner S, Rokich DP, Dixon KW (2009) Ecophysiology of species with distinct leaf morphologies: effects of plastic and shadecloth tree guards. *Restor Ecol* 17(1):33–41
- Cortina J, Amat B, Castillo V, Fuentes D, Maestre FT, Padilla FM, Rojo L (2011) The restoration of vegetation cover in the semi-arid Iberian southeast. *J Arid Environ* 75:1377–1384
- Defaa C, Elantry S, El Alam SL, Achour A, El Mousadik A, Msanda F (2015) Effects of tree shelters on the survival and growth of *Argania spinosa* seedlings in Mediterranean arid environment. *Int J Ecol*. <https://doi.org/10.1155/2015/124075>
- Del Campo A, Navarro RM, Aguililla A, Gonzalez E (2006) Effect of tree shelter design on water condensation and run-off and its potential benefit for reforestation establishment in semiarid climates. *For Ecol Manag* 235:107–115
- Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43:599–626
- Devine W, Harrington CA (2008) Influence of four tree shelter types on microclimate and seedling performance of Oregon white oak and western redcedar. Research Paper PNW-RP-576, USDA Forest Service, Pacific NorthWest Research Station
- Dupraz C, Bergez JE (1999) Carbon dioxide limitation of the photosynthesis of *Prunus avium* L. seedlings inside an unventilated treeshelter. *For Ecol Manag* 119:89–97
- Gandullo JM (1985) *Ecología Vegetal*. Fundación Conde del Valle de Salazar, Madrid
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Global Planet Change* 63:90–104
- Gómez-Miguel VD, Badía-Villas D (2016) Soil distribution and classification. In: Gallardo JF (ed) *The soils of Spain*, World soils book series. Springer, Berlin, pp 11–48
- Jacobs DF (2011) Reforestation of a salvage-logged high-elevation clearcut: Engelmann spruce seedling response to tree shelters after 11 growing seasons. *West J Appl For* 26(2):53–56
- Jiménez MN, Navarro FB, Ripoll MA, Bocio I, De Simón E (2005) Effect of shelter tubes on establishment and growth of *Juniperus thurifera* L. (*Cupressaceae*) seedlings in Mediterranean semi-arid environment. *Ann For Sci* 62:717–725
- Kalaji HM, Schansker G, Ladle RJ et al (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynth Res* 122:121–158
- Kjelgren R, Rupp LA (1997) Establishment in treeshelters I: shelters reduce growth, water use, and hardiness, but not drought avoidance. *HortScience* 32:1281–1283
- Lambers H, Chapin FS, Pons TJ (2008) *Plant physiological ecology*, 2nd edn. Springer, New York, p 604
- Leverkus A, Castro J, Puerta-Piñero C, Rey Benayas JM (2013) Suitability of the management of habitat complexity, acorn burial depth, and a chemical repellent for post-fire reforestation of oaks. *Ecol Eng* 53:15–22
- Maestre F, Cortina J (2003) Small-scale spatial variation in soil CO₂ efflux in a Mediterranean semiarid steppe. *Appl Soil Ecol* 23:199–209
- Maestre FT, Cortina J (2004) Are *Pinus halepensis* plantations useful as a restoration tool in semiarid Mediterranean areas? *For Ecol Manag* 198:303–317
- Mariotti B, Maltoni A, Jacobs DF, Tani A (2015) Tree shelters affect shoot and root system growth and structure in *Quercus robur* during regeneration establishment. *Eur J Forest Res* 134(4):641–652
- Martínez-Ferri E, Balaguer L, Valladares F, Chico JM, Manrique E (2000) Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. *Tree Physiol* 20:131–138
- Matías L, Castro J, Villar-Salvador P, Quero JL, Jump AS (2017) Differential impact of hotter drought on seedling performance of five ecologically distinct pine species. *Plant Ecol* 218:201–212
- Methy M, Gillon D, Houssard C (1997) Temperature-induced changes of photosystem II activity in *Quercus ilex* and *Pinus halepensis*. *Can J For Res* 27:31–38

- Niinimets U, Keenan T (2014) Photosynthetic responses to stress in Mediterranean evergreens: mechanisms and models. *Environ Exp Bot* 103:24–41
- Ninyerola M, Pons X, Roure JM (2005) Atlas Climático Digital de la Península Ibérica, Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Bellaterra
- Oliet JA, Jacobs DF (2007) Microclimatic conditions and plant morpho-physiological development within a tree shelter environment during establishment of *Quercus ilex* seedlings. *Agric For Meteorol* 144:58–72
- Oliet JA, Navarro R, Contreras O (2003) Evaluación de la aplicación de mejoradores y tubos en repoblaciones forestales. Consejería de Medio Ambiente de la Junta de Andalucía, Andalucía
- Oliet JA, Vázquez de Castro A, Puértolas J (2015) Establishing *Quercus ilex* under Mediterranean dry conditions: sowing recalcitrant acorns versus planting seedlings at different depths and tube shelter light transmissions. *New Forest* 46:869–883
- Padilla FM, Miranda J, Pugnaire FI (2007) Early root growth plasticity in seedlings of three Mediterranean woody species. *Plant Soil* 296:103–113
- Padilla FM, Miranda JD, Ortega R, Hervás M, Sánchez J, Pugnaire FI (2011) Does shelter enhance early seedling survival in dry environments? A test with eight Mediterranean species. *Appl Veg Sci* 14:31–39
- Pemán García J, Navarro Cerrillo RM, Nicolás Peragón JL, Prada Sáez MA, Serrada Hierro R (eds) (2013) Producción y Manejo de semillas y plantas forestales. Naturaleza y Parques Nacionales. Serie Forestal. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid
- Pemán J, Peguero-Pina JJ, Valladares F, Gil-Pelegrín E (2010) Evaluation of unventilated treeshelters in the context of Mediterranean climate: insights from a study on *Quercus faginea* seedlings assessed with a 3D architectural plant model. *Ecol Eng* 36:517–526
- Piñeiro J, Maestre FT, Bartolomé L, Valdecantos A (2013) Ecotechnology as a tool for restoring degraded drylands: a meta-analysis of field experiments. *Ecol Eng* 61(2013):133–144
- Puértolas J, Oliet JA, Jacobs DF, Benito LF, Peñuelas JL (2010) Is light the key factor for success of tube shelters in forest restoration plantings under Mediterranean climates? *For Ecol Manag* 260:610–617
- Rosenberg ND, Blad BL, Berma SB (1983) *Microclimate: the biological environment*, 2nd edn. Wiley, New York, p 495
- Sakcali MS, Ozturk M (2004) Eco-physiological behaviour of some mediterranean plants as suitable candidates for reclamation of degraded areas. *J Arid Environ* 57:1–13
- Soliveres S, Monerris J, Cortina J (2008) El uso de parches artificiales mejora el rendimiento de una repoblación de *Rhamnus lycioides* en medio semiárido. *Cuad Soc Esp Cienc For* 28:125–130
- Taylor TS, Loewenstein EF, Chappelka AH (2006) Effect of animal browse protection and fertilizer application on the establishment of planted Nuttall oak seedlings. *New Forest* 32:133–143
- Townsend AJ, Retkute R, Chinnathambi K, Randall JW, Foulkes J, Carmo-Silva E, Murchie EH (2017) Suboptimal photosynthetic acclimation in wheat. *Plant Physiol*. <https://doi.org/10.1104/pp.17.01213>
- Trubat R, Cortina J, Vilagrosa A (2008) Short-term nitrogen deprivation increases field performance in nursery seedlings of Mediterranean woody species. *J Arid Environ* 72:879–890
- Trubat R, Cortina J, Vilagrosa A (2011) Nutrient deprivation improves field performance of woody seedlings in a degraded semi-arid shrubland. *Ecol Eng* 37:1164–1173
- Vallejo VR, Smanis A, Chirino E, Fuentes D, Valdecantos A, Vilagrosa A (2012) Perspectives in dryland restoration: approaches for climate change adaptation. *New Forest* 43:561–579
- Van Lerberghe P (2014) Proteger los árboles contra los daños de la fauna cinegética. Los protectores de malla Proyecto Pirinoble. Unión Europea. Institut pour le Développement Forestier, Paris
- Vázquez de Castro A, Oliet JA, Puértolas J, Jacobs DF (2014) Light transmissivity of tube shelters affects root growth and biomass allocation of *Quercus ilex* L. and *Pinus halepensis* Mill. *Ann For Sci* 71:91–99
- Vilagrosa A, Bellot J, Vallejo VR, Gil-Pelegrín E (2003) Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. *J Exp Bot* 54:2015–2024
- Villar-Salvador P, Puértolas J, Cuesta B, Peñuelas JL, Uscola M, Heredia-Guerrero N, Rey Benayas JM (2012) Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. *New Forest* 43(5–6):755–770
- Ward JS, Gent Martin PN, Stephens GR (2000) Effects of planting stock quality and browse protection-type on height growth of northern red oak and eastern white pine. *For Ecol Manag* 127:205–216