
Suitability of Drought-Preconditioning Techniques in Mediterranean Climate

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Abstract

Arid and semiarid ecosystems in the Mediterranean are under high risk of desertification. Revegetation with native well-adapted evergreen shrubs is desirable, but techniques for successful establishment of these species are not fully developed. Transplant shock is a key hurdle to plantation success. The application of a drought-preconditioning treatment during the last months of nursery culture is a potential technique for reducing transplant shock. This technique has been widely applied in boreal habitats and humid temperate areas. Three representative Mediterranean species (*Pistacia lentiscus*, *Quercus coccifera*, and *Juniperus oxycedrus* seedlings) were exposed to classic drought-preconditioning treatment consisting of reductions in the watering regime. The effects of preconditioning on seedling quality were assessed by cell water relationships (pressure-volume curves), minimal transpiration, leaf capacitance, chlorophyll fluorescence, and gas exchange. Moreover, seedlings were exposed to transplant shock (intense drought period) during which water potential (predawn

and midday) and maximal photochemical efficiency were evaluated to establish seedling performance. Results showed that preconditioning did not affect cell water relationships and minimal transpiration in any of the three species. Preconditioned seedlings of *P. lentiscus* maintained higher water content during desiccating conditions as a consequence of an increase in leaf water content at full turgor. These changes allowed plants to maintain higher net CO₂ assimilation rates and an elevated photosystem II status, facilitating an increase in drought survival. Preconditioning improved the performance of *Q. coccifera* and *J. oxycedrus* seedlings, but these two species were much less responsive than *P. lentiscus* seedlings. Finally, results suggest that sensitivity to drought preconditioning may be related to drought tolerance or avoidance strategy of each species. Drought-related strategies should be considered to optimize management scale preconditioning.

Key words: land restoration, drought preconditioning, drought tolerance, drought avoidance, evergreen species.

Introduction

Arid and semiarid ecosystems in the Mediterranean have been subjected to a long history of intense human uses (e.g., fuel extraction) and wildfires, resulting in land degradation (Dregne 1991). As a consequence, vegetation cover has been reduced and erosion processes intensified (United Nations Environment Programme 1992). These processes have often led to reductions in the moisture available for vegetation, thus initiating succession from semiarid types of vegetation to arid types of vegetation (Dick-Peddie 1991). Under these circumstances, the introduction of evergreen shrubs with a high potential cover may be of particular value for soil protection because of their capacity to develop a dense canopy, to accumulate litter, to quickly recover from disturbances such as fire, to protect soil against erosion, and to confer an increased resilience to the whole community (Naveh & Lieberman

1993). In the western Mediterranean, sprouting shrubs and trees, such as *Quercus*, *Pistacia*, *Rhamnus*, and *Juniperus* species, are major components of what is considered the potential vegetation (Le Houérou 1981).

Recently, the use of sprouting species for ecosystem restoration has increased (Vilagrosa et al. 1997; Vallejo et al. 2000). However, the techniques for a successful establishment of these species are not fully developed, and failures in the form of unacceptable mortality rates and meager growth are common.

On the other hand, soil water availability represents a major environmental constraint under Mediterranean conditions (Di Castri 1973). Soil drought leads to water deficits in the leaf tissue, affecting many physiological processes with ultimate consequences for the plant yield and survival (Hsiao 1973). Much evidence suggests that a key hurdle in plantation success is transplant shock, that is, the initial short-term stress experienced by seedlings as they are transferred from favorable nursery conditions to the adverse field environment (Burdett 1990). Nursery techniques to avoid transplant shock include manipulations of the watering regime and radiation environment that may precondition the seedling to unfavorable field conditions (Duryea & McClain 1984). Landis et al. (1998) pointed out that seedlings can be naturally preconditioned by exposing

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them to ambient conditions, but it is possible to achieve a greater level of stress resistance in a shorter time by imposing cultivation treatments in the nursery.

As a general procedure, drought preconditioning should be carried out during the last months of nursery culture before outplanting (from the end of summer to autumn) (Brissette et al. 1991; Johnson & Cline 1991). According to Landis et al. (1998), preconditioning has four main objectives: to manipulate seedling morphology and to induce dormancy, to acclimate seedlings to the natural environment, to develop stress resistance, and to improve seedling survival and growth after outplanting.

Several studies have analyzed the responses of forest woody species to drought preconditioning. Most of these studies are from boreal habitats and humid temperate areas (Parker & Pallardy 1988; van den Driessche 1991; Edwards & Dixon 1995; Stewart & Bernier 1995; Stewart et al. 1995; Tschaplinski et al. 1995; Khan et al. 1996; Landhäusser et al. 1996; McKay & White 1997). In contrast, few studies have focused on Mediterranean species (Nunes et al. 1989; Ksontini et al. 1998; Fernández et al. 1999, 2000; Villar et al. 1999), despite water availability being the predominant selective force for plant survival in these environments (Mooney et al. 1975). On the other hand, poor results obtained so far suggest that drought preconditioning may not be of interest for the production of seedlings of Mediterranean drought-resistant species.

The objective of this study is focused on several watering manipulations to produce drought-preconditioned seedlings native to Mediterranean climates that are more able to overcome the first stages of outplanting. We analyzed the effect of drought preconditioning on three representative Mediterranean shrub species—mastic tree (*Pistacia lentiscus*), kermes oak (*Quercus coccifera*), and prickly juniper (*Juniperus oxycedrus*)—over a wide range of variables related to drought-tolerance and drought-avoidance adaptations. We discuss whether the drought-preconditioning techniques extensively applied to humid or boreal species are suitable to Mediterranean drought-resistant species and how species-dependent drought strategies can determine the results.

Materials and Methods

Plant Material and Growing Conditions

Seeds from all three species were supplied by the forest services (Seed Bank, Regional Government of Valencia, Spain) and collected in the same area where the experiment was carried out. Seedlings were grown in 250-cm³ containers filled with a mixture of light peat, coconut fiber, and perlite (50:45:5). Seedlings were cultivated in the open air for 16 months and fertilized twice a week with a generic 8:8:8 (N:P:K) fertilizer solution enriched with micronutrients (Nutrileaf, Agrodán S.A., Spain), applied in the irrigation water (3 cm³/L). Following Landis et al. (1989), we applied a commercial 4:25:35 (N:P:K) fertilizer (CONIFER Finisher, Peters

Professional, The Scotts Co., Marysville, OH, U.S.A.) fixed to 50 ppm of nitrogen dose during the preconditioning phase. This type of fertilization is recommended during the last phases of culture to reduce shoot growth and facilitate seedling hardening (Peñuelas & Ocaña 1996; Landis et al. 1998).

Drought-Preconditioning Procedure

Drought preconditioning was carried out from October 1997 to December 1997, following common procedures reported in the literature. The drought-preconditioning treatment was established to provide the seedlings with a mild level of stress. We let the seedlings dry out to a pre-dawn water potential (PWP) that was 50% of the loss of turgency point, thus avoiding catastrophic xylem cavitation (Tyree & Sperry 1988) and deleterious effects associated with complete loss of hydraulic conductivity (Vilagrosa et al. 1998).

During the preconditioning period, water stress was applied through five drought cycles (10–15 days long each), which consisted of withholding water until seedling weight (plant + container) was reduced to 35–40% of their initial weight. This value corresponded to an average PWP of -1.5 ± 0.2 MPa for *P. lentiscus*, -0.9 ± 0.2 MPa for *Q. coccifera*, and -0.9 ± 0.1 MPa for *J. oxycedrus*. In preliminary experiments, we observed that substrate rehydration was difficult when seedling weight (plant + container) decreased below 40% of initial weight, presumably due to hydrophobic characteristics of the culture medium.

Control seedlings were irrigated every 2 days as a normal procedure, and the PWP was never lower than -0.31 ± 0.02 , -0.24 ± 0.02 , and -0.16 ± 0.10 MPa for *P. lentiscus*, *Q. coccifera*, and *J. oxycedrus*, respectively. At the end of the preconditioning period, we evaluated ecophysiological traits and performance of seedlings.

Pressure–Volume Curves

To identify seedling adjustments resulting from changes in cell water relations, we analyzed pressure–volume relationships in three randomly chosen seedlings per treatment and species. Because Mediterranean species have petioles too short, one leafy twig with five to six leaves for each seedling was selected to carry out pressure–volume determinations. Samples were rehydrated in distilled water during approximately 12 hr at 4°C and dark conditions. Recommendations given by Kubiske and Abrams (1991) were also taken to prevent leaf oversaturation.

Pressure–volume curves were carried out following Hammel's technique according to Tyree and Hammel (1972) and Kyriakopoulos and Richter (1981), following Turner (1988) recommendations. From each curve, the parameters estimated were relative water content at turgor loss point (RWC_{tlp}), water potential at turgor loss point (ψ_{tlp}), osmotic potential at full turgor (Π_o), bulk modulus of elasticity (E_{max}), and symplasmic water deficit at turgor loss point (SWD_{tlp}) (Tyree & Karamanos 1980).

Leaf Characteristics and Minimal Transpiration

Five seedlings per treatment were randomly chosen to measure leaf characteristics and minimal transpiration. From these, five mature and representative leaves (one per seedling) of *P. lentiscus* and five small twigs (one per seedling) in *Q. coccifera* and *J. oxycedrus* were fully hydrated in distilled water for 24 hr at 4°C (Slavik 1974). Specific leaf weight (SLW), water capacitance at full turgor (WC_{ft}) expressed as a function of water content at full turgor on a leaf area basis (Larcher 1995), and minimal transpiration (MT) were measured. MT was taken as water loss of both leaf sides under controlled microclimatic conditions (22°C and vapor pressure deficit as the difference between leaf and surrounding air = 0.82 kPa) after stomata closure (Kramer 1983).

Net CO₂ Assimilation and Transpiration Rates at Full Hydration

Five plants per treatment and species were fully hydrated overnight. On the following day, the net CO₂ assimilation rates ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and transpiration rates ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were measured with an infrared gas analyzer (IRGA, ADC-LCA 2, Hoddesdon, U.K.) as previously described in Long et al. (1996), using equations developed by von Caemmerer and Farquhar (1981). Measurements were taken in the morning (08:00–09:00 solar time) and midday (12:00–13:00 solar time). Plant water potential was also measured in the same seedlings by using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, U.S.A.) (Scholander et al. 1965). During photosynthesis and transpiration measurements, weather conditions were hot and sunny (photosynthetic photon flux density $713 \pm 36 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the morning and $1,008 \pm 63 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at midday; air temperatures $17.5 \pm 0.4^\circ\text{C}$ and $21.8 \pm 0.2^\circ\text{C}$ in the morning and at midday, respectively).

Maximal Photosystem II Photochemical Efficiency in Relation to Cell Dehydration

Chlorophyll *a* fluorescence of photosystem (PS)II was measured using a pulse amplitude modulated fluorometer (Pam-2000, Walz, Effeltrich, Germany). Maximal PSII photochemical efficiency in the dark-adapted state (F_v/F_m) was calculated according to Genty et al. (1989).

To test the effects of the RWC of leaves on the maximal photochemical efficiency (F_v/F_m), five leaves per treatment and species were rehydrated (24 hr, 4°C) and allowed to dry in the dark under room conditions (21°C and 60% relative humidity). Every 30 minutes the leaves were weighed and F_o (minimum) and F_m (maximum dark-adapted fluorescence) were measured by applying a pulse of saturating white light (1.2 seconds, $>15,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Then, regression analysis was used to compare the relationship between F_v/F_m and RWC. These measurements could not be performed in *J. oxycedrus* because of the small leaf size and the impossibility of analyzing the same amount of leaf area in each measurement.

Transplant Shock

To evaluate overall seedling performance, seedlings of all three species and treatments were subjected to a drought period lasting until seedling death. About 50 seedlings per species and treatment were left unwatered under sunny conditions. Every 2 days, five seedlings per species and treatment were selected at random for ecophysiological measurements. Variables selected to reflect seedling status were PWP, midday xylem water potential (MWP) (Scholander et al. 1965), and maximal PSII photochemical efficiency (F_v/F_m) at predawn (Genty et al. 1989).

Experimental Design and Data Analysis

Seedlings of each species (1,500 plants per treatment) were randomly assigned to eight different blocks. Randomly, four blocks were assigned to regular watering fre-

Table 1. Cell water relationships (pressure–volume curves) and parameters of control (C) and drought-preconditioned (DP) seedlings at the end of the drought-preconditioning period.

	Ψ_{tp} (–MPa)	Π_o (–MPa)	E_{max} (MPa)	SWD_{tp} (%)	RWC_{tp} (%)
<i>P. lentiscus</i>					
C	3.8 ± 0.1 a	3.2 ± 0.03 a	31.7 ± 2.1 a	16.4 ± 2.1 a	85.6 ± 1.3 a
DP	4.1 ± 0.1 a	3.3 ± 0.2 a	25.9 ± 6.7 a	21.5 ± 1.4 a	83.3 ± 1.7 a
<i>Q. coccifera</i>					
C	3.6 ± 0.1 a	2.8 ± 0.2 a	20.9 ± 5.6 a	20.4 ± 5.0 a	88.5 ± 0.9 a
DP	4.0 ± 0.3 a	3.1 ± 0.2 a	30.1 ± 10.5 a	23.3 ± 5.9 a	85.4 ± 2.0 a
<i>J. oxycedrus</i>					
C	2.7 ± 0.6 b	1.9 ± 0.3 b	10.8 ± 1.5 b	32.5 ± 1.9 b	65.2 ± 4.8 b
DP	2.8 ± 0.3 b	1.9 ± 0.2 b	9.4 ± 1.0 b	32.1 ± 3.5 b	75.1 ± 3.3 b

Values are means \pm SE of $n = 3$. Within each variable and species, same letter indicates no statistical differences ($p > 0.05$) between control and preconditioned seedlings. A significant species effect was found in all parameters, in which *J. oxycedrus* was significantly different from *Q. coccifera* and *P. lentiscus* ($p < 0.01$, Tukey-B test).

Ψ_{tp} , water potential at turgor loss point; Π_o , osmotic potential at full turgor; E_{max} , bulk modulus of elasticity; SWD_{tp} , symplast water deficit at turgor loss point; RWC_{tp} , relative water content at turgor loss point.

quency (control) and the others to low watering (drought-preconditioning treatment).

Treatment effects were evaluated by using one-way analysis of variance (ANOVA). Data transformations were performed when necessary to ensure the validity of the assumptions of normality, linearity, and homoscedasticity. When this was not possible, a nonparametric Kruskal-Wallis test was performed. Regression analysis was used to compare the relationship between Fv/Fm and RWC; differences between treatments were tested with analysis of covariance of Fv/Fm with RWC. Repeated ANOVA measurements (ANOVAR) were used to analyze statistical differences in minimal transpiration and relative water content of detached leaves during water loss under controlled microclimatic conditions. When interactions between treatment and species were significant or when we sought to gain accuracy, statistical analyses were decomposed by species. All statistical tests were performed by using SPSS (v. 9.0, SPSS Inc., Chicago, IL, U.S.A.).

Results

Cell Water Relationships (Pressure–Volume Curves)

Drought preconditioning did not affect cell water relationships (Table 1). *Pistacia lentiscus* and *Q. coccifera* lost turgor between -3.5 and -4.1 MPa. Osmotic potentials at full turgor (Π_0) ranged between -2.8 to -3.2 MPa and did not show differences between treatments. E_{\max} values corresponded to relatively nonelastic cell walls, with high gradients of water potential associated with small losses in cell volume. Therefore, in both species and treatments, SWD_{tip} remained relatively low and RWC_{tip} did not decrease below 83%. For *J. oxycedrus*, turgor loss occurred at less negative water potentials (-2.7 to -2.8 MPa) and RWC_{tip} decreased to values as low as 65–75%.

Leaf Characteristics and Minimal Transpiration

Excised leaves from drought-preconditioned *P. lentiscus* seedlings showed a trend toward a better maintenance of water losses than control seedlings. This was reflected in a lower rate of decrease in RWC under controlled conditions (Fig. 1), although the differences were not statistically significant ($F = 1.81$, $G-G = 0.219$). Lower water losses were not the result of lower minimal transpiration rates (Table 2; $F = 1.1539$, $G-G = 0.324$) but rather reflected a higher capacity to store water at full turgor (Table 2; $F = 9.01$, $p = 0.017$). Preconditioning produced higher SLW in *P. lentiscus* than control seedlings (Table 2; $F = 4.81$, $p = 0.028$). Drought-preconditioned *J. oxycedrus* seedlings also showed lower minimal transpiration than control seedlings, but the differences were not statistically significant (Fig. 1 and Table 2; $F = 0.625$, $G-G = 0.544$ and $F = 1.169$, $G-G = 0.22$, respectively). In leaves excised from *Q. coccifera*, no differences in water loss were ob-

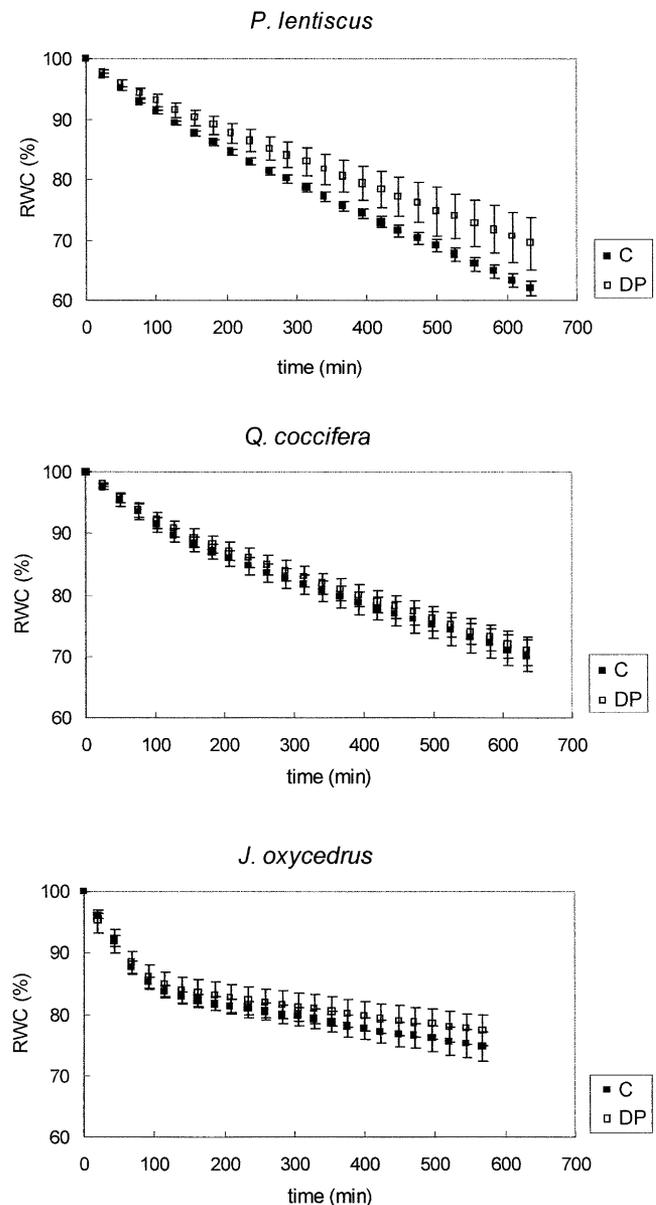


Figure 1. Changes in relative water content (RWC) of leaves detached from *P. lentiscus*, *Q. coccifera*, and *J. oxycedrus* seedlings subjected to desiccating conditions of drought-preconditioning (\square) or control (\blacksquare) treatments. Values are means \pm SE of $n = 5$.

served between treatments (Fig. 1 and Table 2; $F = 0.152$, $G-G = 0.837$ and $F = 1.524$, $G-G = 0.233$, respectively).

Maximal PSII Photochemical Efficiency in Relation to Cell Dehydration

Initial values of Fv/Fm for *Q. coccifera* and *P. lentiscus* seedlings ranged between 0.6 and 0.7. Fv/Fm decreased exponentially as leaves dried out, particularly as RWC fell below 80% (Fig. 2). The lowest Fv/Fm values reached by *P. lentiscus* and *Q. coccifera* were close to 0.2. We found no treat-

Table 2. Foliar water capacitance at full turgor (WC_{ft}), specific leaf weight (SLW), and minimal transpiration (MT) in all three species and treatments.

	WC_{ft} (mg/cm^2)	SLW (mg/cm^2)	MT ($g\cdot m^{-2}\cdot hr^{-1}$)
<i>P. lentiscus</i>			
C	19.5 ± 0.7 a	15.9 ± 0.4 a	3.12 ± 0.08
DP	24.9 ± 1.6 b	18.7 ± 1.2 b	3.22 ± 0.12
<i>Q. coccifera</i>			
C	23.8 ± 1.2	26.7 ± 2.1	2.67 ± 0.09
DP	23.9 ± 1.0	27.5 ± 1.1	2.75 ± 0.07
<i>J. oxycedrus</i>			
C	33.9 ± 2.0	25.6 ± 2.2	1.81 ± 0.10
DP	31.6 ± 1.9	27.8 ± 1.2	1.39 ± 0.06

Values are means ± SE of $n = 5$. Different letters indicate statistical differences between control and drought preconditioned for a given species and variable at $p < 0.05$ level. C, control; DP, drought preconditioned.

ment effect on Fv/Fm for a given RWC in *Q. coccifera* leaves ($F = 0.01, p = 0.937$). In contrast, drought-preconditioned *P. lentiscus* seedlings showed higher Fv/Fm than control seedlings at any RWC ($F = 16.82, p < 0.01$).

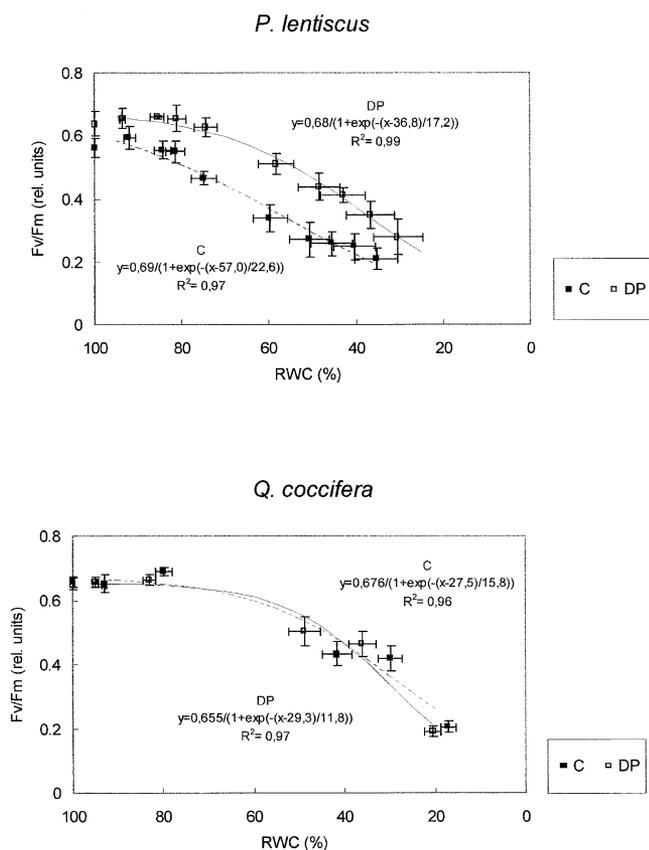


Figure 2. Relationship between relative water content (RWC) and maximal PSII photochemical efficiency (Fv/Fm) in leaves from control (■) and drought-preconditioned (□) seedlings of *P. lentiscus* and *Q. coccifera*. Values are means ± SE of $n = 5$.

Table 3. Net CO_2 assimilation rate (A) and transpiration rates (TR) at full hydration in all three species and treatments.

	A ($\mu mol CO_2\cdot m^{-2}\cdot sec^{-1}$)		TR ($mmol\cdot m^{-2}\cdot sec^{-1}$)	
	Morning	Midday	Morning	Midday
<i>P. lentiscus</i>				
C	3.7 ± 0.7	0.5 ± 0.1 a	0.16 ± 0.05 a	0.65 ± 0.15
DP	3.7 ± 1.3	2.4 ± 0.4 b	0.66 ± 0.12 b	0.54 ± 0.06
<i>Q. coccifera</i>				
C	5.4 ± 1.1	5.1 ± 0.8	0.29 ± 0.10	1.51 ± 0.42
DP	5.3 ± 2.4	4.7 ± 0.7	0.24 ± 0.08	1.30 ± 0.07
<i>J. oxycedrus</i>				
C	3.8 ± 0.4	5.7 ± 0.7	0.03 ± 0.03	1.76 ± 0.15
DP	4.2 ± 1.1	4.1 ± 0.9	0.06 ± 0.05	1.47 ± 0.08

Values are means ± SE of $n = 5$. Different letters indicate statistical differences between control and drought preconditioned for a given species and variable at $p < 0.01$ level. C, control; DP, drought preconditioned.

Net CO_2 Assimilation and Transpiration Rates at Full Hydration

Early morning net CO_2 assimilation rate for both the control and the drought-preconditioned seedlings was 3.7, 5.4, and 4.0 $\mu mol CO_2\cdot m^{-2}\cdot s^{-1}$ for *P. lentiscus*, *Q. coccifera*, and *J. oxycedrus*, respectively (Table 3). At midday, control *P. lentiscus* substantially reduced the net CO_2 assimilation rate compared with drought-preconditioned seedlings ($F = 33.32, p < 0.01$). No treatment effect was found in *Q. coccifera* and *J. oxycedrus* seedlings ($F = 0.08, p = 0.778$ and $F = 1.99, p = 0.195$, respectively).

Transpiration rates did not show statistical differences between treatments, either in the morning or at midday (Table 3; $F = 3.26, p = 0.085$ and $F = 0.96, p = 0.337$, respectively). But when analyzed separately, preconditioned *P. lentiscus* showed higher transpiration rates than control seedlings in early morning (Table 3; $F = 12.12, p = 0.01$).

PWP ranged between -0.11 and -0.25 MPa for all three species with no significant differences between treatments (data not shown). At midday, a reduction in MWP was observed (MWP of -0.77 to -0.95 MPa) for all three species but it was insensitive to preconditioning.

Transplant shock

During the first 7 days of drought, the PWP of control and drought-preconditioned *P. lentiscus* seedlings was similar (Fig. 3). PWP decreased thereafter, and on the tenth day control seedlings showed lower PWP than drought-preconditioned seedlings (Fig. 3; $F = 11.28, p = 0.012$). We observed the same pattern in Fv/Fm (Fig. 4; $F = 3.84, p = 0.05$). *Quercus coccifera* showed a similar trend, but differences were significant only on day 12 for Fv/Fm (Figs. 3 and 4; $F = 7.51, p = 0.034$). For *J. oxycedrus*, PWP in preconditioned seedlings showed higher decrease than in control seedlings on day 10 (Fig. 3; $F = 10.98, p = 0.011$).

After day 10 in *P. lentiscus* and day 12 in *Q. coccifera*, water potential decreased suddenly and all seedlings died in a short period of time. Visual observations indicated that

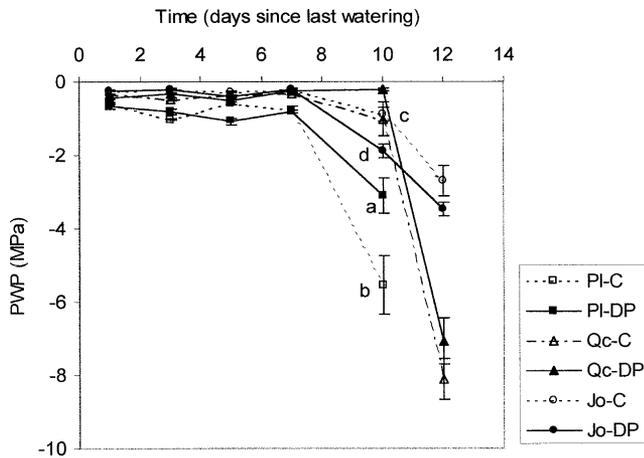


Figure 3. Changes in predawn water potential (PWP) during the severe drought cycle in *P. lentiscus* (PI), *Q. coccifera* (Qc), and *J. oxycedrus* (Jo), irrigated regularly (control, C) and drought preconditioned (DP). Different letters indicate a significant treatment effect (see text for details). Values are means \pm SE of $n = 5$.

P. lentiscus control seedlings died sooner than drought-preconditioned seedlings. No differences were observed in *Q. coccifera*. Control and drought-preconditioned *J. oxycedrus* seedlings did not die after 12 days of desiccation.

Discussion

A morphological evaluation of preconditioning in the studied species was carried out in a previous work (Fonseca 1999). Aboveground biomass slightly increased in preconditioned *P. lentiscus*, whereas it decreased in *Q. coccifera* (from 10.6 to 8.0 g dry weight), and *J. oxycedrus* maintained the same values in all parameters evaluated. None of the three species showed modifications in the

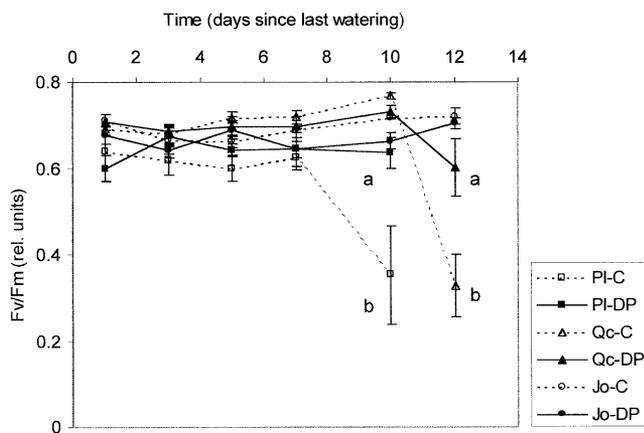


Figure 4. Maximal PSII photochemical efficiency (Fv/Fm) during the severe drought cycle in *P. lentiscus* (PI), *Q. coccifera* (Qc), and *J. oxycedrus* (Jo) irrigated regularly (control, C) and drought preconditioned (DP). Different letters indicate a significant treatment effect (see text for details). Values are means \pm SE of $n = 5$.

root-to-shoot ratio, with values around 0.6, 1.5, and 1.1 for *P. lentiscus*, *Q. coccifera*, and *J. oxycedrus*, respectively.

In terms of ecophysiological response, the benefits of preconditioning were particularly evident for *P. lentiscus*. These were not related to changes in the cell water relations, because neither *P. lentiscus* nor the other two species showed significant modifications in cell wall elasticity and osmotic adjustment. Increases in E_{max} and Π_o are frequently observed in water-stressed plants (Nunes et al. 1989; Collet & Guehl 1997). They represent an increase in the driving force for water transport, and thus they may allow higher water uptake under drought (Blake et al. 1991; Michael-Gebre et al. 1998). However, several studies from mesic to semiarid environments have failed to observe such responses (Stewart et al. 1995; Villar et al. 1999), suggesting that acclimation to drought through changes in cell water relations may be species dependent. In this sense, several authors (Davis & Mooney 1986; Saruwatari & Davis 1989) found that changes in osmotic potential and bulk modulus of elasticity were due to ontogenetic effects rather than to osmotic adjustments for turgor maintenance.

It has been reported that species with high water capacitance are buffered from short-term changes in water availability (Nilsen & Orcutt 1996). Drought preconditioning resulted in an increase in leaf capacitance in *P. lentiscus*. This larger quantity of stored water in the leaves may be particularly relevant during short-term droughts and may allow a short-time increase in transpiration rates. This is supported by the higher transpiration rates measured in preconditioned *P. lentiscus* seedlings early in the morning. Preconditioned seedlings of this species maintained 10% more water in the leaves after 10 hr of intense desiccation. This corresponds to an approximately 8-hr surplus of minimal transpiration under these intense drying conditions, and thus it may represent an advantage for these seedlings. The increase in apoplasmic water storage after drought has been described for *Picea mariana* (black spruce) (Blake et al. 1991) and *Laurus nobilis* (sweet bay or Grecian laurel) (Lo Gullo & Salleo 1988; Salleo et al. 1997). The latter authors suggested that apoplasmic water could help the plant to avoid short-term water stress such as that experienced at midday. In addition, when tension disappears (e.g., during the night), apoplasmic water could contribute to symplast rehydration (Dixon et al. 1984). On the other hand, preconditioned *P. lentiscus* seedlings showed somewhat higher SLW than control seedlings. This increment of mechanical tissues in the leaf may represent an additional force for leaf rehydration (Siau 1984).

The acclimation of preconditioned seedlings to drought in terms of photosynthetic rates and stomatal conductance has been widely described in the literature and has been considered an important regulatory mechanism to enhance better performance of seedlings (Van den Driessche 1991; Stewart et al. 1995). The Fv/Fm dynamics observed in the present study (i.e., a sharp decline when the water potential decreased below -4 MPa and RWC was approximately 70%) are similar to those described by other

authors (Méthy et al. 1997). Preconditioned leaves of *P. lentiscus* showed higher maximal PSII photochemical efficiency for a given relative water content than control seedlings. Chlorophyll *a* fluorescence is considered a reliable indicator of the photosynthetic electron transport in intact leaves (Krause & Weis 1991), and decreases in Fv/Fm have been related to photoinhibitory damages (Demmig-Adams & Adams 1992). A higher sensitivity to photoinhibitory damages may result from a lower efficiency of the repairing mechanisms in the reaction centers of damaged PSII (Tystjärvi et al. 1992) and may promote nonphotochemical dissipation of energy (Björkman & Demmig-Adams 1995). Thus, preconditioning presumably facilitated more efficient mechanisms for PSII repair and for nonphotosynthetic electron transport, particularly in *P. lentiscus* seedlings. These mechanisms were reflected in the maintenance of higher midday photosynthetic rates with similar transpiration rates, because photoinhibitory processes commonly hamper carbon fixation (Epron & Dreyer 1993).

Treatment effects on water storage and loss allowed preconditioned seedlings of *P. lentiscus* to maintain higher water potential and PSII status during an intense drought period. Neither *Q. coccifera* nor *J. oxycedrus* showed modifications in chlorophyll fluorescence, leaf capacitance, cell water relationships, transpiration rates, or transplant shock with drought-preconditioned treatment. Preconditioned *J. oxycedrus* seedlings showed very low values of minimal transpiration after stomata closure. In contrast with *Q. coccifera* and *P. lentiscus*, *J. oxycedrus* survived after 12 days of drought. Possibly, stomata closure at relatively high water potentials and low permeability to water losses represents an efficient water-conserving strategy in this species.

The resistance of *J. oxycedrus* seedlings to the imposed drought suggests that this species may be a good alternative for the restoration of Mediterranean semiarid areas. Other features of this species like relative plasticity in root growth and morphology (Fonseca 1999) make it suitable for this purpose. In accordance with this, *J. oxycedrus* usually shows relatively good survival rates when planted in the field under semiarid conditions, that is, higher survival than *Q. coccifera* but lower than *P. lentiscus* (Fonseca 1999).

The fact that plant species in Mediterranean areas are well adapted to long periods of water scarcity has been documented extensively (Tenhunen et al. 1985). Two main mechanisms for drought resistance in nonephemeral plants have been described: avoidance and tolerance (Levitt 1980). Several works (Lo Gullo & Salleo 1988; Nardini et al. 1999) have shown the presence of species with different strategies in the same environment. These authors indicated that species with different drought strategies could cohabit from the point of view of physiological adaptations within plant communities. In relation to drought strategy, *Q. coccifera* seedlings close stomata at very negative water potentials, and the stem xylem is relatively resistant to cavitation, suggesting that this species adopts a

drought-tolerant strategy (Ksontini et al. 1998; Vilagrosa et al. 1998). In contrast, the early stomatal closure as determined by pressure–volume curves and a relative stability of Fv/Fm during transplant shock in *J. oxycedrus* seedlings are typical traits of a drought-avoider water-saver species. This behavior has been described for other Mediterranean species such as *Pinus halepensis* (Martínez-Ferri et al. 2000; Calamassi et al. 2001). On the other hand, *P. lentiscus* is considered a drought-avoider species with water-spender strategy (Levitt 1980), although some eco-physiological traits such as strong resistance to xylem embolism (Vilagrosa et al. 1998) are characteristics of tolerant species.

Moderate responses to drought preconditioning have been described for *Q. coccifera* (Ksontini et al. 1998) and *Pinus halepensis* (Villar et al. 1999). In contrast, *Ceratonia siliqua*—a drought-avoider water-spender species according to Lo Gullo and Salleo (1988)—and *Pinus pinaster* respond to suboptimal irrigation with changes in cell wall elasticity and osmotic adjustment (Nunes et al. 1989; Fernández et al. 1999). Thus, it seems that preconditioning, as commonly applied, may not be suitable for highly drought-tolerant and water-saver Mediterranean species. However, *P. lentiscus* and *C. siliqua*, which show drought-avoider water-spender strategy, modify some physiological characteristics, showing similarities in acclimation and indicating that preconditioning treatments may be of practical benefit in such species. This greater capacity of response that these species have to preconditioning may be due to a strategy based on avoiding drought by spending water, thereby forcing these species to be more sensitive to water availability in the environment (Levitt 1980). On the contrary, drought-tolerant species (i.e., *Q. coccifera*) and water-saver species (i.e., *J. oxycedrus* and *P. halepensis*) establish their strategy to resist intense water deficits by means of tolerance or water conserving strategies, respectively, predisposing them to a lower sensitivity to the availability of water. Considering this evidence, we hypothesize that drought-avoider water-spender species are more responsive to preconditioning than drought-tolerant or water-saver species. If this is the case, preconditioning could be incorporated into routine nursery practices to improve establishment success of species with drought-avoider and water-spender strategy.

In conclusion, preconditioning can be used to enhance the functional traits that promote the resistance of seedlings of Mediterranean species to drought. But the efficacy of this technique seems to be species dependent, and it is probably related to species strategy to withstand drought. In this case, the intensity, duration, and time for optimal irrigation should be defined depending on species strategy.

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