Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Disponible on line en www.inia.es/srf

Investigación Agraria: Sistemas y Recursos Forestales 2008 17(1), 3-9
ISSN: 1131-7965

Elevated atmospheric CO_2 does not modify osmotic adjustment to light and drought in the Mediterranean oak *Quercus suber* L.

I. Aranda[1]*, J. Puértolas[2], M. Pardos and J. A. Pardos[1,2]

[1] *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA).*
Ctra. A Coruña, km 7,5. 28040 Madrid. Spain
[2] *Unidad de Anatomía, Fisiología y Genética Forestal. Escuela Técnica Superior de Ingenieros de Montes.*
Universidad Politécnica de Madrid. Ciudad Universitaria, s/n. 28040 Madrid. Spain

Abstract

The current ongoing increase in the atmospheric CO_2 concentration is an unquestionable fact. Thus, plants are bound to live in a more enriched CO_2 world in a not far-off future. In this new framework, regeneration of forest tree species may be modified as a consequence of the change in the current patterns of seedling response to other environmental resources, such as water or light. We studied the impact of an elevated CO_2 concentration on the interaction of drought and light upon the water relations of cork oak (*Quercus suber* L.) seedlings. In a complete factorial design of contrasting light (HL *vs* LL), water (WW *vs* S) and CO_2 levels (800 ppm *vs* 370 ppm), we analysed the influence of each factor and its interaction in the modification of different leaf water parameters in potted seedlings after a 6 months experimental period. These parameters were derived from the construction, with leaf materials, of the P-V curves: osmotic potential at full turgor ($\Psi\pi_{100}$), osmotic potential at zero turgor ($\Psi\pi_0$), modulus of elasticity at full turgor (ε_{max}), and the ratio dry/turgor weight (DW/TW).

Doubling of the CO_2 levels over the current concentration (380 ppm) did not change any of the studied leaf water parameters, while light and water availabilities had a significant influence. This result does not exclude changes in other basic physiological parameters that could modify the pattern of cork oak regeneration responding to a CO_2 enriched atmosphere in the future, and under climatic conditions different to the current ones.

Key words: cork oak, CO_2 enrichment, water availability, shade, PV curves.

Resumen

Niveles elevados de CO_2 no modifican el ajuste osmótico en respuesta a la luz y la sequía en *Quercus ruber*

El continuo aumento actual en la concentración de CO_2 en la atmósfera es un hecho incuestionable. Así, las plantas se verán abocadas a vivir en un mundo más enriquecido en CO_2 que el actual en un futuro no muy lejano. En este nuevo marco, la regeneración de las especies arbóreas puede modificarse como consecuencia del cambio en los patrones actuales de respuesta a otros recursos del medio como el agua o la luz. En relación con esto, se realizó un estudio para investigar el impacto de una concentración de CO_2 elevada sobre la interacción de la sequía y la luz en las relaciones hídricas de hojas de brinzales de alcornoque (*Quercus suber* L.). En un diseño factorial completo, y considerando niveles altos y bajos de: luz (HL-alto, respecto a LL-bajo), agua (WW-bien regadas, respecto a S-estresadas) y CO_2 (800 ppm *respecto a* 370 ppm), se analizó la influencia de cada factor individual, y su interacción, en la modificación de distintos parámetros hídricos en hoja tras un periodo experimental de 6 meses. Los diferentes parámetros se estimaron a partir de la construcción de curvas P-V en hojas: potencial osmótico a plena turgencia ($\Psi\pi_{100}$), potencial osmótico en el punto de marchitez ($\Psi\pi_0$), módulo de elasticidad a máxima tugencia (ε_{max}), y la relación entre el peso seco y el peso turgente (DW/TW).

Una duplicación de los niveles de CO_2 por encima de la concentración actual (380 ppm) no cambió ninguno de los parámetros hídricos estudiados, mientras que las disponibilidades de luz y de agua tuvieron una influencia significativa. Esto no excluye posibles modificaciones de otros procesos fisiológicos básicos que puedan alterar los actuales patrones de regeneración en el alcornoque en respuesta a una atmósfera más enriquecida en CO_2 en el futuro, y en marcos climáticos diferentes al actual.

Palabras clave: alcornoque, enriquecimiento en CO_2, disponibilidad de agua, sombra, curvas P-V.

---

* Corresponding author: aranda@inia.es

Received: 20-11-07; Accepted: 08-02-08.

Introduction

In Mediterranean forests, water stress and light may be considered as the main climatic limiting factors that control regeneration success of forest tree species. Both factors may act in a non-coordinate manner rendering a segregation in species regeneration niches (Sack and Grubb, 2002; Sack *et al*., 2003). However, a new environmental factor must currently be taken into account, the progressive increase in the levels of the atmospheric carbon dioxide concentration (IPCC, 2007). Such increase, in conjunction with other greenhouse gases, is considered to be responsible for climatic change. One of the effects predicted in the future is the likely increase in the occurrence and intensity of drought in the Mediterranean basin (Pastor and Post, 1988; Peñuelas *et al*., 1998). Whether these changes may induce displacements in the actual recruitment of seedlings in the understory of forest ecosystems and the patterns of segregation in the regeneration niches is an important issue that needs to be unravelled (Bazzaz and Miao, 1993; Hättenschwiler and Körner, 2000). Indeed, the issue is much more complex when considering the interaction between shade and drought on performance of seedlings (Valladares and Pearcy, 2002; Aranda *et al*., 2005; Pardos *et al*., 2005; Sack *et al*., 2003). How the interaction of both factors, co-occurring in nature, will be modified in the future enriched CO_2 world is uncertain, as are the changes promoted in forest composition.

Under this change in the future climatic scenario, tolerance to drought must be analysed taking into account its interaction with light availability (Abrams and Knapp, 1986; Augé *et al*., 1990; Tschaplinski *et al*., 1998), along with the increase in the atmospheric CO_2 concentration. Whereas a lot of information is available on the change in water relations of forest tree species responding to drought under current environmental conditions (Aranda *et al*., 1996; Bahari *et al*., 1985; Davis and Mooney, 1986; Tschaplinski *et al*., 1998), and for variable levels of CO_2 (Tognetti *et al.,* 2002; Tschaplinski *et al.,* 1995), the interaction of both factors with light availability has been assessed to a lesser extent (Hättenschwiler and Körner, 2000).

The increase of atmospheric CO_2 concentration during the next few decades will have a direct impact on the physiology of tree species in environments with complex interactions with other resources. Different changes in basic physiological processes such as photosynthetic capacity or stomatal conductance to water vapour have been described to respond to increasing CO_2 (Wullschleger *et al*., 2002). Furthermore, an enhancement in the ability of plants to face water stress by increasing, for example, water use efficiency has been frequently observed (Tyree and Alexander, 1993; Eamus and Jarvis, 1989; Osborne *et al*., 2000). Thus, it is also expected that an increase in CO_2 levels might enhance the ability to develop mechanisms of dehydration tolerance such as osmotic adjustment (Morgan, 1984; Picon *et al*., 1997). In this sense, the lower osmotic capacity observed in shade conditions under actual atmospheric CO_2 concentration (Vance and Zaerr, 1991; Tschaplinski *et al*., 1998; Aranda *et al*., 2001), may be compensated in part by the increase in the CO_2 concentration in the air. This would render a higher accumulation of osmotic solutes such as soluble carbohydrates by increasing carbon uptake, thereby lowering the osmotic potential at full and zero turgor. In the short-term, the enhancement in carbon fixing would provide a mechanism of increasing the tolerance of seedlings that experience the effects of drought under shade environments (Aranda *et al*., 2005; Tschaplinski *et al*., 1998; Tschaplinski and Gebre, 2003). However, no clear trends have been found in this respect. Indeed, even a decrease in osmotic potential at full turgor has been observed when growing under high CO_2 environments (Tschaplinski *et al*., 1993). This result was probably explained by a stimulation of growth, which brought about a depletion in the concentration of osmotically-active organic solutes (Tschaplinski *et al*., 1995). In other cases, no changes were observed in the osmotic adjustment ability responding to a CO_2 enrichment of the atmosphere (Polley *et al*., 1999).

In the present study, the influence of CO_2 levels on osmotic adjustment and other leaf water parameters of cork oak seedlings in response to water stress under different light environments was analysed. A factorial design was established to assess the interaction of the three environmental factors: light, water and atmospheric CO_2. These three factors sum up the main environmental constraints in the future regeneration of Mediterranean forest tree species. Two hypotheses were tested: (i) an increase in CO_2 may ameliorate the drought response of cork oak seedlings by enhancing osmotic adjustment, and (ii) the effect will be dependent on the light environment. Both questions may be important in the context of the climatic change and its impact on Mediterranean forest regeneration. The niches of regeneration may be altered in the future, not only by a change in the main climatic variables, but by a differential effect of the increasing CO_2 on the physiological response of forest tree species at the seedling state (Hättenschwiler and Körner, 2000, 2003).

Material and Methods

Acorns of cork oak (*Quercus suber* L.) from a population at the center of the Iberian Peninsula (39°58'N, 5°6'W) were collected in the 2003 fall and kept in moist plastic bags at 4°C. Two hundred acorns were seeded in a seedbed inside a climatic chamber at 25°C. Two weeks after seeding, and when seed radicules had emerged 2 cm, 120 acorns were selected and transplanted to 3 L pots containing a mixture of peat and sand (3:1, v:v). A six-months controlled release fertilizer (N:P:K, 20:10:20 +micronutrients) was added to the growing medium (3 g l^{-1}). Seedlings were randomly assigned to each of two climatic chambers containing either ambient air (370 µmol mol^{-1}) or ambient air enriched with CO_2 to a target level of 800 µmol mol^{-1}. CO_2 concentration into the climatic chambers was checked regularly at two weeks intervals. Temperature (25-20°C day/night) and vapour pressure deficit (1.5-1 KPa day/night) were maintained constant during the experiment.

Seedlings were divided in two groups inside each chamber. One group was maintained under low light (LL: 3 mol m^{-2} d^{-1} ~60 µmol m^{-2} s^{-1}) during the experiment, and the other was grown under high light (HL: 27 mol m^{-2} d^{-1} 600 µmol m^{-2} s^{-1}). Low light levels were achieved by growing the seedlings under a neutral shade-cloth. Light was provided by lamps of high vapour pressure and fluorescent tubes as light sources, without observing differences on both climatic chambers in daily irradiance, temperature and vapour pressure deficit during the experiment. To avoid position effects, seedlings were rotated once a week inside the growing chamber.

Within each light treatment, watering was withdrawn on half of the seedlings (S), maintaining the rest well-watered (WW) during all the experiment. Because weekly water consumption in HL seedlings was slightly higher than in LL, additional water was added to HL seedlings to reach the same weekly target average soil moisture content as in LL. This watering regime was applied to well-watered and to water stressed seedlings. The protocol was followed until the minimum soil water content in the pots of stressed seedlings (LLS and HLS) reached a mean gravimetric soil moisture content of 34%. Afterwards, this target level of water stress was maintained until the end of the experiment. Average soil water content on well-watered seedlings was around 84% during the experiment. Water content in the pots was controlled by gravimetric estimation weighing the pots twice per week.

Five seedlings per treatment combination were harvested at three dates, selecting one leaf per seedling from the upper third of the plant. For construction of P-V curves, one fully expanded leaf was sealed with parafilm and the base of the petiole was placed in distilled water in a beaker after re-cutting under water. Leaves were allowed to re-hydrate for an hour at room temperature. Special care was taken to prevent over-saturation of apoplasmic and intercellular spaces in leaves because of immersion. Over-saturation during the first steps of dehydration causes the shift in leaf saturation deficit due to water losses without changes in measured water potential (Dreyer *et al*., 1990; Abrams and Menges, 1992). The repeat pressurization technique (Robichaux, 1984) was used to construct PV curves from a series of parallel fresh weight and pressure chamber Ψ measurements. After each Ψ measurement, leave samples were removed from the chamber, weighed immediately, and allowed to air dry between consecutive Ψ determinations. When approximately 7-8 data points on the apparent linear portion of the PV curves were obtained, samples were oven dried at 70°C for 48h to obtain leaf dry weight (DW). Sample relative water content (RWC) was calculated as (fresh weight – dry weight) / (weight at full turgor – dry weight). Weight at turgor was derived from the relationship between fresh weight and water potential. Data points above loss of turgor were identified and weight at full turgor determined by linear regression ($r^2 > 0.98$) (27).

For the derivation of PV parameters, paired observations of Ψ and RWC were plotted using ($1/\Psi$) transformations to identify data points to be included in simple regression analysis of the linear portion of PV curves. For the regression analysis, $1/\Psi$ and RWC were used as the dependent and independent variables, respectively. The x- and y- intercepts yielded estimates of the water content in the apoplast at full turgor (RWC_a) and inverse of osmotic potential at full turgor ($\Psi\pi_{100}$). The x- and y- coordinates of the first data point of the linear portion of the PV curve corresponded to relative water content at the turgor-loss point (RWC_0), and inverse of osmotic potential at the turgor-loss point ($\Psi\pi_0$). From the relationship between $1/\Psi$ and RWC, the relationship between turgor pressure (P) and RWC was calculated. Values for the bulk tissue elastic modulus (ε) were calculated from this latter relationship. The bulk tissue elastic modulus is defined as the change in tissue turgor pressure for a given fractional change in symplastic content [$\varepsilon = dP/dRWC\ (RWC\text{-}RWC_a)$]. Osmotic and elastic adjustments, defined as the decrease in osmotic potential at full turgor or in ε, respectively, in response to water deficits were calculated. Estimates of $\Psi\pi_{100}$ and ε were used to characterize osmotic and elastic adjustments.

Water stress was assessed through the measurement of predawn water potential on leaves from the same seedlings sampled for P-V curves construction. The first harvest was made 45 days after reaching the target soil moisture content on stressed seedlings. The following harvests were carried out in two 45-days intervals following the first one.

Data were analysed by ANOVA, with CO_2, watering and light as main fixed effects. Comparison of mean treatment differences was done by LSD test. Linear regressions of osmotic potential at full turgor ($\Psi\pi_{100}$) and at the turgor-loss point ($\Psi\pi_0$) with predawn water potential (Ψ_{pd}), as surrogate of water stress, were made considering only the effect of light and watering.

Results

No differences on water parameters between the three harvests were observed, although in the first harvest, Ψ_{pd} was slightly lower for water stressed seedlings compared to the second and third harvests (Fig. 1). Water availability, in terms of Ψ_{pd}, was linked to watering regime without differences in Ψ_{pd} related to the light environment or the CO_2 level (Table 1). The water stress experienced by seedlings, in terms of water availability, was the same across the light environments. Harvest date was not significant in the ANOVA analysis, so data were pooled for subsequent analysis.

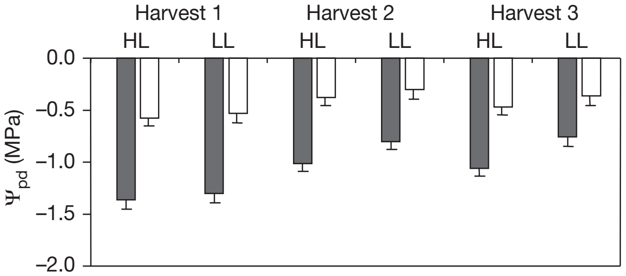

Figure 1. Predawn water potential (Ψ_{pd}-MPa) in each harvest. HL: seedlings under high light, 27 mol m^{-2} d^{-1}. LL: seedlings under low light, 3 mol m^{-2} d^{-1}. Black bars refer to water-stressed seedlings (S) and white bars to well-watered (WW). Data are averages values (± SE, n = 5). CO_2 treatments were pooled because no differences were found between CO_2 concentrations.

CO_2 was not significant in promoting any change on the different water parameters studied. Thus, only light environment and watering were significant (Table 1). $\Psi\pi_0$ and $\Psi\pi_{100}$ decreased in response to water stress in HL, as well as in LL seedlings (Table 2). However, the decrease was steeper in HL seedlings, since the slopes and intercepts from the relationships between Ψ_{pd} and $\Psi\pi_0$ or Ψ_{pd} and $\Psi\pi_{100}$ were higher for HL (Fig. 2). The significance of the interaction L × W in the ANOVA analysis ($P < 0.01$) reinforces this result (Table 1).

The relative water content at zero turgor (RWC^0) decreased only in response to drought. The modulus of elasticity at maximum turgor (ε_{max}) changed also in response to drought in a similar manner, though

Table 1. Three-way ANOVA of the different water parameters on leaves from cork oak seedlings growing under high and low light regimes interacting with two levels of water availability and two CO_2 atmospheric concentrations

Treatment	Ψ_{pd}	$\Psi\pi_0$	$\Psi\pi_{100}$	RWC_0	ε_{max}	DW/TW
Light (L)	n.s.	***	***	n.s.	n.s.	***
Watering (W)	***	***	***	**	**	***
[CO_2]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L × W	n.s.	***	***	n.s.	n.s.	**
L × [CO_2]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
W × [CO_2]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L × W × [CO_2]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Ψ_{pd}: predawn water potential. $\Psi\pi_0$: osmotic potetial at zero turgor. $\Psi\pi_{100}$: osmotic potetial at full turgor. CHR_0: relative water content at zero turgor. ε_{max}: modulus de elasticity at maximum turgor, leaf dry to turgid weight ratio (DW/TW). Data from different harvests were pooled as no significant differences were related to harvest. n.s.: not significant. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Table 2. Mean values (± SE) of different water parameters derived from P-V curves

	Light	Watering	$\Psi\pi_0$	$\Psi\pi_{100}$	RWC^0	ε_{max}	DW/TW
$[CO_2]$- 370 ppm	HL	WW	–2.29 ± 0.04 b	–1.74 ± 0.03 b	82.75 ± 0.66 ab	6.58 ± 0.38 a	0.44 ± 0.006 b
		S	–3.20 ± 0.05 d	–2.39 ± 0.03 d	81.19 ± 0.76 ab	9.71 ± 0.66 c	0.47 ± 0.004 c
	LL	WW	–2.06 ± 0.05 a	–1.64 ± 0.04 a	83.00 ± 0.79 b	6.87 ± 0.75 ab	0.42 ± 0.005 a
		S	–2.68 ± 0.05 c	–1.99 ± 0.04 c	80.76 ± 0.70 a	8.49 ± 0.72 bc	0.42 ± 0.005 a
$[CO_2]$-800 ppm	HL	WW	–2.39 ± 0.06 b	–1.79 ± 0.05 b	83.17 ± 0.73 b	8.05 ± 0.65 a	0.44 ± 0.007 b
		S	–3.32 ± 0.08 d	–2.47 ± 0.08 d	80.43 ± 0.63 a	9.19 ± 0.89 a	0.48 ± 0.005 c
	LL	WW	–2.12 ± 0.08 a	–1.62 ± 0.04 a	81.64 ± 0.86 ab	7.10 ± 0.42 a	0.42 ± 0.003 a
		S	–2.59 ± 0.05 c	–1.95 ± 0.05 c	81.06 ± 0.84 ab	7.74 ± 1.01 a	0.41 ± 0.005 a

Differences between harvests were not significant, so data were pooled ($n = 15$, mean ± s.e.). Different letters indicate significant differences between treatments within each CO_2 atmospheric concentration ($P < 0.05$). Symbols refer to water parameters as in the previous table.

increasing in response to water stress (Table 2). The ratio of leaf dry weight to turgid weight (DW/TW) was affected by watering and light environment. DW/TW increased with light and tended to decline in well watered seedlings. Moreover, as the interaction between both factors was significant ($L \times W$, $P < 0.01$), DW/TW decrease with water stress was steeper in seedlings growing under high light.

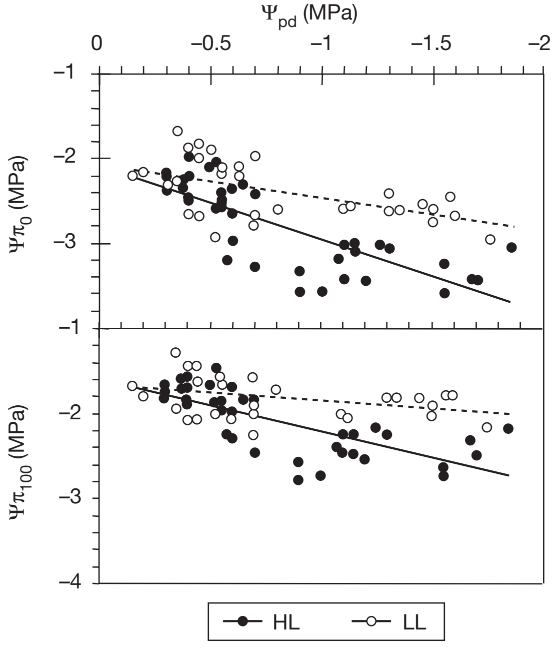

Figure 2. Relationship between osmotic potentials at full ($\Psi\pi_{100}$) and zero turgor ($\Psi\pi_0$), and predawn water potential (Ψ_{pd}-MPa). HL: seedlings under high light. LL: seedlings under low right. Data from both CO_2 treatments were pooled because of absence of differences related to atmospheric CO_2 concentrations. The depicted linear regressions are: HL-$\Psi\pi_0 = -2.07 + 0.88\Psi_{pd}$, $r^2 = 0.60$ $P < 0.001$; LL-$\Psi\pi_0 = -2.08 + 0.38\Psi_{pd}$, $r^2 = 0.30$ $P < 0.001$, and HL-$\Psi\pi_{100} = -1.60 + 0.61\Psi_{pd}$, $r^2 = 0.53$ $P < 0.001$; LL-$\Psi\pi_{100} = -1.67 + 0.18\Psi_{pd}$, $r^2 = 0.14$ $P = 0.02$.

Discussion

The enhancement of the osmotic adjustment in response to drought under elevated CO_2 may be determined by a low demand of solutes by other plant sinks (Wullschleger *et al.*, 2002). However, in the present study, there was no effect of doubling CO_2 levels on the water relations of cork oak, including osmotic potential at full ($\Psi\pi_{100}$) and zero turgor ($\Psi\pi_0$). This was in accordance with previous results where no clear effect of elevated CO_2 was noted in promoting a change in water relations (Tschaplinski *et al.*, 1993; 1995; Polley *et al.*, 1996; 1999; Picon-Cochard and Guehl, 1999; Tognetti *et al.*, 2000). Nevertheless, it must be considered that this was a short-term study about the effects of CO_2 enhancement on osmotic adjustment to light and drought, although there is evidence that the slow growth in Mediterranean environments may cancel in the practice the responses to high CO_2.

Light and drought were key factors controlling osmotic adjustment in cork oak seedlings, as previously reported (Salleo and Lo Gullo, 1990; Aranda *et al.*, 2005; Pardos *et al.*, 2005). Enhancement in dehydration tolerance in response to a change in light environment is important in order to withstand the more stressful environment achieved by sun leaves (Augé *et al.*, 1990; Aranda *et al.*, 2001; Niinemets, 2001). Under these conditions, leaves must withstand lower water potentials as a consequence of higher heating and higher water losses by transpiration (Ellsworth and Reich, 1992; Groom and Lamont, 1997; Niinemets, 2001).

Low light coupled with a decrease in water availability may limit the capacity of osmotic adjustment by decreasing net carbon assimilation rates (Aranda *et al*., 2004; Pardos *et al.,* 2005; Aranda *et al*., 2005). Thus, in extreme climatic years and under intense drought brought about by phenomena such as *El Niño* (Valladares and Pearcy, 2002), seedlings may be more drought stressed under shady conditions because competence from overstory vegetation may prevail over facilitation (Aranda *et al*., 2005). Under this scenario, carbon balance may decline, endangering long-term survival of seedlings, not only from a starvation of reserves, but also by a decreased drought tolerance as a consequence of a lesser capacity of the involved mechanisms in it, such as turgor maintenance by osmotic adjustment.

The slope and intercept were significantly different for HL and LL seedlings in the relationships of $\Psi\pi_0$ and $\Psi\pi_{100}$ with Ψ_{pd}. In previous works with this species, only the difference on the intercept of these relationships when comparing seedlings growing under low and high light environments was significant (Aranda *et al*., 2005; Pardos *et al*., 2005). This was likely a consequence of the shorter span in the water stress experienced by seedlings in terms of Ψ_{pd} in the cited works. Interaction between different environmental resources in modifying the physiological response has long been recognized (Bazzaz and Miao, 1993). Thus, it may be hypothesized the additive character of soil dryness and high sunlight exposure as environmental factors which would bring about the triggering of drought tolerance mechanisms such as the decrease of $\Psi\pi_0$ (Groom and Lamont, 1997). On the other hand, seedlings growing under drought and low light face a greater challenge because both act as stress factors. A physiological trade-off may emerge from the lower capacity of cork oak seedlings to develop mechanisms of tolerance to water stress under shade (Aranda *et al*., 2001, 2005; Pardos *et al*., 2005). This fact, jointly with other shortcomings linked to growing under low light, such as a limited capacity for carbon uptake or low water use efficiency (Aranda *et al.,* 2007), might compromise the long-standing of seedlings in the understory of close forests under recurrent dry periods.

Acknowledgments

This work was carried out thanks to the financial support of the Educational and Science Ministry of Spain by the project AGL 2001-2720.

References

ABRAMS M.D., KNAPP A.K., 1986. Seasonal water relations of 3 gallery forest hardwood species in northeast Kansas. Forest Science 32, 687-696.

ABRAMS M.D., MENGES E.S., 1992. Leaf ageing and plateau effects on seasonal pressure-volume relationships in three sclerophyllous *Quercus* species in south-eastern USA. Functional Ecology 6, 353-360.

ARANDA I., CASTRO L., PARDOS M., GIL L., PARDOS J.A., 2005. Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quecus suber* L.) seedlings. Forest Ecology and Management 210, 117-119.

ARANDA I., GIL L., PARDOS J.A., 1996. Seasonal water relations of three broadleaved species [*Fagus sylvatica* L., *Quercus petraea* (Mattuschka) Liebl. and *Quercus pyrenaica* Willd.] in a mixed stand in the centre of the Iberian Peninsula. Forest Ecology and Management 84, 219-229.

ARANDA I., GIL L., PARDOS J.A., 2001. Effects of thinning in a *Pinus sylvestris* L. stand on foliar water relations of *Fagus sylvatica* L. seedlings planted within the pinewood. Trees 15, 358-364.

ARANDA I., GIL L., PARDOS J.A., 2004. Improvement of growth conditions and gas exchange of *Fagus sylvatica* L. seedlings planted below a recently thinned *Pinus sylvestris* L. stand. Trees 18, 211-220.

ARANDA, I., PARDOS M., PUÉRTOLAS J., JIMÉNEZ M.D., PARDOS J.A., 2007. Water use efficiency in cork oak (*Quercus suber* L.) is modified by the interaction of water and light availabilities. Tree Physiology 27, 671-677.

AUGÉ R.M., STODOLA A.J.W., PENNELL B.D., 1990. Osmotic and turgor adjustment in *Rosa* foliage drought-stressed under varying irradiance. Journal of American Society Horticultural Science 115, 661-667.

BAHARI Z.A., PALLARDY S.G., PARKER W.C., 1985. Photosynthesis, water relations, and drought adaptation in six woody species of oak-hickory forests in Central Missouri. Forest Science 31, 557-569.

BAZZAZ F.A., MIAO S.L., 1993. Successional status, seed size, and responses of tree seedlings to CO_2, light, and nutrients. Ecology 74, 104-112.

DAVIS S.D., MOONEY H.A., 1986. Tissue water relations of four co-occurring chaparral shrubs. Oecologia 70, 527-535.

DREYER E., BOUSQUET F., DUCREY M., 1990. Use of pressure volume curves in water relation analysis on woody shoots: influence of rehydration and comparison of four european oak species. Annales des Sciences Forestieres 47, 285-297.

EAMUS D., JARVIS P.G., 1989. The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. Advanced Ecological Research 19, 1-55.

ELLSWORTH D.S., REICH P.B., 1992. Water relations and gas exchange of *Acer saccharum* seedlings in contrasting natural light and water regimes. Tree Physiology 10, 1-20.

GROOM P.K., LAMONT B.B., 1997. Xerophytic implications of increased sclerophylly: interactions with water and light in *Hakea psilorrhyncha* seedlings. New Phytologist 136, 231-237.

HÄTTENSCHWILER S., KÖRNER C., 2000. Tree seedling responses to in situ CO_2-enrichment differ among species and depend on understorey light availability. Global Change Biology 6, 213-226.

HÄTTENSCHWILER S., KÖRNER C., 2003. Does elevated CO_2 facilitate naturalization of the non-indigenous *Prunus laurocerasus* in Swiss temperate forests? Functional Ecology 17, 778-785.

IPCC, 2007: Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Solomon S., Qin D., Manning M., Chen Z., Marquis M., Avery K.B., Tignor M., Miller H.L., eds). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 996 pp.

MORGAN J.M., 1984. Osmoregulation and water stress in higher plants. Annual Review Plant Physiology 35, 299-319.

NIINEMETS Ü., 2001. Climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs at the global scale. Ecology 82, 453-469.

OSBORNE C.P., MITCHELL P.L., SHEEHY J.E., WOODWARD F.I., 2000. Modelling the recent historical impacts of atmospheric CO_2 and climate change on Mediterranean vegetation. Global Change Biology 6, 445-458.

PARDOS M., JIMÉNEZ M.D., ARANDA I., PUÉRTOLAS J., PARDOS J.A., 2005. Water relations of cork oak (*Quercus suber* L.) seedlings in response to shading and moderate drought. Annals Forest Science 62, 377-384.

PASTOR J., POST W.M., 1988. Response of northern forests to CO_2-induced climate change. Nature 334, 55-58.

PEÑUELAS J., FILELLA I., LLUSIA J., SISCART D., PIÑOL J., 1998. Comparative field study of spring and summer leaf gas exchange and photobiology of the Mediterranean trees *Quercus ilex* and *Phillyrea latifolia*. Journal Experimental Botany 49, 229-238.

PICON-COCHARD C., FERHI A., GUEHL J.M., 1997. Concentration and $\delta^{13}C$ of leaf carbohydrates in relation to gas exchange in *Quercus robur* under elevated CO_2 and drought. Journal Experimental Botany 19, 1547-1556.

PICON-COCHARD C., GUEHL J.M., 1999. Leaf gas exchange and carbohydrate concentrations in *Pinus pinaster* plants subjected to elevated CO_2 and a soil drying cycle. Annals of Forest Science 56, 71-76.

POLLEY H.W., TISCHLER C.R., JOHNSON H.B., PENNINGTON R.E., 1999. Growth, water relations, and survival of drought-exposed seedlings from six maternal families of honey mesquite (*Prosopis glandulosa*): responses to CO_2 enrichment. Tree Physiology 19, 359-366.

ROBICHAUX R.H., 1984. Variation in the tissue water relations of two sympatric Hawaiian *Dubautia* species and their natural hybrid. Oecologia 65, 75-81.

SACK L., GRUBB P.J., 2002. The combined impacts of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. Oecologia 131, 175-185.

SACK L., GRUBB P.J., MARAÑON T., 2003. The functional morphology of seedlings tolerant of deep shade plus drought in three Mediterranean-climate forests of southern Spain. Plant Ecology 168, 139-163.

SALLEO S., LO GULLO M.A., 1990. Sclerophylly and plant water relations in three mediterranean *Quercus* species. Annals Botany 65, 259-270.

TOGNETTI R., RASCHI A., JONES D.M.B., 2000. Seasonal patterns of tissue water relations in three Mediterranean shrubs co-occurring at a natural CO_2 spring. Plant Cell and Environment 23,1341-1351.

TOGNETTI R., RASCHI A., JONES M.B., 2002. Seasonal changes in tissue elasticity and water transport efficiency in three co-occurring Mediterranean shrubs under natural long-term CO_2 enrichment. Functional Plant Biology 29, 1097-1106.

TSCHAPLINSKI T.J., GEBRE G.M., SHIRSHAC T.L., 1998. Osmotic potential of several hardwood species as affected by manipulation of throughfall precipitation in an upland oak forest during a dry year. Tree Physiology 18, 291-298.

TSCHAPLINSKI T.J., NORBY R.J., WULLSCHLEGER S.D., 1993. Responses of loblolly pine seedlings to elevated CO_2 and fluctuating water supply. Tree Physiology 13, 283-296.

TSCHAPLINSKI T.J., STEWART D.B., NORBY R.J., 1995. Interactions between drought and elevated CO_2 on osmotic adjustment and solute concentrations of tree seedlings. New Phytologist 131, 169-177.

TYREE M.T., ALEXANDER J.D., 1993. Plant-water relations and the effects of elevated CO_2: a review and suggestions for future research. Vegetatio. 104/105, 47-62.

VALLADARES F., PEARCY R., 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. Plant Cell Environment 25, 749-759.

VANCE N.C., ZAERR J.B., 1991. Influence of drought stress and low irradiance on plant water relations and structural constituents in needles of *Pinus ponderosa* seedlings. Tree Physiology 8, 175-184.

WULLSCHLEGER S.D., TSCHAPLINSKI T.J., NORBY R.J., 2002. Plant water relations at elevated CO_2-implications for water-limited environments. Plant Cell and Environment 25, 319-331.