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Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought

A. Vilagrosa[1,*], J. Bellot[2], V. R. Vallejo[3] and E. Gil-Pelegrín[4]

[1] *CEAM-Department de Ecologia, Universitat d'Alacant, POB 99, E-03080 Alacant, Spain*

[2] *Department de Ecologia, Universitat d'Alacant, POB 99, E-03080 Alacant, Spain*

[3] *CEAM, C/Ch. Darwin 14, Parque Tecnológico, E-46980 Paterna, Spain*

[4] *Unidad de Recursos Forestales, SIA-DGA, POB 727, E-50080 Zaragoza, Spain*

Abstract

Seedling shrubs in the Mediterranean semi-arid climate are subjected to intense droughts during summer. Thus, seedlings often surpass their limits of tolerance to water stress, resulting in the loss of hydraulic conductivity due to xylem cavitation. The response in terms of stomatal conductance, vulnerability to cavitation, leaf dieback, and survival were analysed in two co-occurring seedlings of mastic tree (*Pistacia lentiscus* L.) and kermes oak (*Quercus coccifera* L.) during an intense drought period. Both species reacted to drought with steep decreases in stomatal conductance before the critical water potential brought about the onset of cavitation events. *Q. coccifera* showed wider safety margins for avoiding runaway embolism than *P. lentiscus* and these differences could be related to the particular drought strategy displayed by each species: water saver or water spender. The limits for survival, resprout capacity and leaf dieback were also analysed in terms of loss of conductivity. By contrast with previous studies, the species showing higher seedling survival in the presence of drought also showed higher susceptibility to cavitation and operated with a lower safety margin for cavitation. Both species showed a leaf specific conductivity (*LSC*) threshold below which leaf biomass had to be regulated to avoid runaway embolism. However, each species displayed a different type of response: *P. lentiscus* conserved total leaf area up to 100% loss of *LSC*, whereas *Q. coccifera* continuously adjusted leaf biomass throughout the drought period in order to maintain the *LSC* very close to the maximum values recorded without loss of conductivity. Both species maintained the capacity for survival until the loss of conductivity was very nearly 100%.

Key words: Drought stress, leaf dieback, Mediterranean shrubs, stomatal conductance, survival, xylem cavitation.

Introduction

Soil water availability represents a major environmental constraint under Mediterranean conditions. Drought leads to water deficit in the leaf tissue, which affects many physiological processes and can have ultimate consequences for plant growth and survival. Among these processes, the loss of hydraulic conductivity in the xylem has been recognized as playing an important role in drought resistance (Tyree and Sperry, 1989). This phenomenon is due to xylem cavitation, i.e. breakage of the water column under negative xylem pressure (Zimmermann, 1983). Once a conduit cavitates and becomes air-filled (embolized), it is not available for water transport (Tyree and Sperry, 1989). Thus, cavitation reduces hydraulic conductivity in the xylem, and plants cannot supply their leaves with water properly.

Plants differ widely in their susceptibility to cavitation, and it has been suggested that a high cavitation resistance results in a higher tolerance to water deficit (Pockman and Sperry, 2000). Therefore, the survival of the species in climates with water limitations would be related to the

* To whom correspondence should be addressed. Fax: +34 965 903 464. E-mail: a.vilagrosa@ua.es

resistance of their xylem to cavitate (Davis *et al.*, 1998; Hacke *et al.*, 2000). In this sense, it has been observed that vulnerability to embolism determines the patterns of survival in different species and that these patterns could affect species distribution (Pockman and Sperry, 2000). Moreover, after applying a survival model in Mediterranean species, Martínez-Vilalta (2001) reported that vulnerability to cavitation was the crucial variable for explaining differences in drought tolerance and survival.

In a Mediterranean context, global warming models predict generalized temperature increases as well as increases in the frequency of intense drought episodes and, in effect, both phenomena have already been observed (Piñol *et al.*, 1998). Prolonged climatic changes could produce cavitation-induced species declines and, consequently, alterations in species composition at the community level (Tognetti *et al.*, 1998). Therefore, differences in vulnerability to cavitation among species could have important implications for the survival of adult individuals, and these differences could be even more critical for the recruitment of young individuals that have less access to deep water reserves in the soil (Williams *et al.*, 1997; Davis *et al.*, 1998). In this sense, after analysing a wide range of Mediterranean species, Vallejo *et al.* (2000) found that seedling mortality during summer in the semi-arid Mediterranean (eastern Spain) increased with the length of the rainless period, and rainless periods longer than 120 d produced mortality rates above 80%. In fact, previous studies with *Pistacia lentiscus* and *Quercus coccifera* at the seedling stage showed steep decreases in water potential (i.e. less than –5 MPa) during a period of several months without rainfall in drought years, resulting in high mortality rates (Fonseca, 1999).

In addition, there is increasing evidence that xylem embolism limits gas exchange (Jones and Sutherland, 1991; Nardini and Salleo, 2000; Sperry *et al.*, 2002), and can act as a control mechanism which, in connection with stomatal activity, regulates the amount of water extracted by the plant (Salleo *et al.*, 2000; Cochard *et al.*, 2002). It has generally been considered that species tend to operate near the point at which water potential causes catastrophic xylem dysfunction and that they must regulate transpiration to avoid the positive feedback that would increase the loss of hydraulic conductivity and runaway embolism (Sperry *et al.*, 2002).

The principal aim of the present study was to investigate how seedlings of the co-occurring Mediterranean sclerophyllous shrubs, *P. lentiscus* L. (Anarcadiaceae) and *Q. coccifera* L. (Fagaceae), regulate water losses during an intense drought period in relation to the mechanisms for avoiding cavitation. A second objective was to attempt to ascertain the limits to survival in terms of loss of conductivity. Thus, changes in water potential, stomatal conductance, hydraulic conductivity parameters, leaf biomass, and survival capacity were monitored simultaneously.

Although both species are characteristic of the macchia on the Mediterranean arid range (Le Houérou, 1981), they show some interesting differences between them. Seedlings of *P. lentiscus* registered higher survival rates than those of *Q. coccifera* after plantation in reforestation programmes (Vilagrosa *et al.*, 1997; Fonseca, 1999; Vilagrosa, 2002). Moreover, *P. lentiscus* is semi-ring-porous (Villar-Salvador, 2000), and it has been observed to follow a strategy of drought-avoidance by water-spending (Levitt, 1980; Vilagrosa, 2002). *Q. coccifera*, on the contrary, is diffuse-porous (Villar-Salvador, 2000), and shows a strategy of drought-avoidance by water-saving (Vilagrosa *et al.*, 1997). Taking into account these differences, it was investigated whether stomatal conductance was limited by xylem cavitation in these species, what the safety margins were for avoiding the onset of cavitation events and what mechanisms were displayed to avoid runaway embolism after stomata closure.

Materials and methods

Plant material

The local Forest Service supplied seeds of both species (from the Valencia, Spain, Regional Government seed bank) from the same area where the experiment was carried out (Mediterranean semi-arid climate, precipitation: 250–300 mm year^{-1} and average temperature: 17–19 °C). Two hundred 2-year-old seedlings for each species were grown in 8.0 l containers filled with forest soil under full sunlight conditions, and they were watered and fertilized as needed.

The drought period took place during summer in full sunlight at the SIA (Servicio de Investigaciones Agroalimentarias-DGA, Spain) experimental fields. Daily temperatures and relative humidity ranged between 20 and 37 °C and 40 and 80%, respectively. Photon flux density at midday was from 1700–2200 µmol m^{-2} s^{-1}. Before the drought period began, the seedlings of both species were watered to field capacity and then allowed to dehydrate freely during the intense drought period.

Cell-water relationships

Pressure–volume (*P–V* curves) analysis was conducted in order to establish the critical point at which seedlings lost turgor. Since Mediterranean species have very short petioles, one leafy shoot with 5–6 leaves was selected to carry out *P–V* determinations. Five *P–V* curves were analysed in each species according to the methods of Tyree and Hammel (1972). From each curve, relative water content at turgor loss point (RWC_{tlp}), water potential at the turgor loss point (Ψ_{tlp}), osmotic potential at full turgor (Π_o), and bulk modulus of elasticity (E_{max}) were estimated.

Stomatal conductance and water potential

Every three days throughout the drought period, five plants of each species were chosen randomly to measure stomatal conductance (g_s) and predawn water potential (Ψ_{pd}). A model LI-1600 Steady State Porometer (Li-Cor Inc., Lincoln, NE, USA) was used to measure stomatal conductance. For determination of maximal stomatal conductance ($g_{s\text{-max}}$), measurements were taken at 3 h intervals throughout the day, from 06.00 to 18.00 h solar time. Measurements were made on the abaxial side of the leaves, and the sensor head of the porometer was held at the natural position and angle of the leaf during measurement. $g_{s\text{-max}}$ was related to Ψ_{pd} according to Acherar and Rambal (1992), who established that Ψ_{pd} determines the daily

maximum values of g_s ($g_{s\text{-}max}$). Water potential in leafy shoots was assessed by means of a pressure chamber. To avoid tissue dehydration during measurements, the walls of the pressure chamber were covered with wet filter paper. Another set of plants (*n*=19 for *P. lentiscus* and *n*=15 for *Q. coccifera*) was used to establish the relationship between Ψ_{pd} and midday water potential (Ψ_{md}).

Leaf dieback, survival and resprout capacity

Leaf dieback was recorded in another set of plants that were subjected to the same intense drought period (*n*=22 and *n*=20 for *P. lentiscus* and *Q. coccifera*, respectively). Leaf dieback was computed as a percentage of reduction in leaf area as a function of water potential (Ψ_{xil}). During the drought period, after seedlings attained a certain Ψ_{xil}, they were rewatered to analyse survival and resprout capacity.

Vulnerability to embolism and hydraulic parameters

A total of *n*=20 and *n*=24 seedlings of *P. lentiscus* and *Q. coccifera*, respectively, were used to carry out the vulnerability curves. Vulnerability to embolism was measured in current-year twigs by constructing vulnerability curves through the dehydration method (Tyree and Sperry, 1989). For measuring xylem conductivity during the drought period, ten current-year twigs of one seedling were collected in which water potential (Ψ_{xil}) had previously been measured. To avoid additional embolism the twigs were cut underwater in segments of 30–50 mm in length, and both ends were shaved with a razor blade. Since both species showed low relative growth rates, it was not possible to choose longer segments. However, preliminary determinations of maximum vessel length showed that the length of the longest vessel was very similar to the segment lengths chosen in this study. The segments were placed in a tubing manifold similar to the one described by Cochard *et al*. (1996). A more accurate description of the device can be found in Vilagrosa (2002). The manifold with the twigs was immersed in distilled water to prevent desiccation and to maintain a near constant temperature. The segments were perfused with a degassed HCl solution (0.5 ml l^{-1}, pH≈2) at low pressure (5.48 kPa) to measure initial hydraulic conductivity (K_{hi}, kg m^{-1} s^{-1} MPa^{-1}). The acid was used to minimize microbial growth in the tubing system and to avoid artefacts due to salt solutions used in previous studies (Alder *et al*., 1997; van Ieperen *et al*., 2000). Recent studies (Zwieniecki *et al*., 2001) found that acidic solutions could increase the flow throughout the stems after 40 min of flushing. In a previous study, several flushing times were tested (up to 20 min), finding no significant variations in the flow. However, to minimize possible artefacts, measurements were performed at short time intervals, about 15 min for the whole measurement in each segment.

Flow rate through the twig segment was measured gravimetrically with an analytical balance (Metlher AE 40) connected to a computer which calculated the flow rate of each twig segment. Hydraulic conductivity was calculated as the mass flow rate of the solution through the twig segment divided by the pressure gradient along the segment (K_h, kg m^{-1} s^{-1} MPa^{-1}).

After the initial hydraulic conductivity (K_{hi}) was measured, the twig segments were flushed with pressurized solution (100 kPa for 10 min) to remove any air emboli. It was verified that longer flushing times did not produce significant variations in the flow rates. Then, the same procedure as in K_{hi} was followed to measure the maximum flow of water (K_{hmax}). The percentage loss of conductivity (*PLC*) was computed as: $(1-K_{hi})\times100/K_{hmax}$. *PLC* was used to plot the reduction in cavitation-induced leaf specific conductivity (*LSC*) versus water potential (Ψ_{xil}) as a new type of vulnerability curve. This type of vulnerability curve illustrated how large the reduction was in the capacity to supply water to leaves during the drought period. Modelled reductions (sigmoid regression) computed in

Table 1. *Comparison of cell-water relationships (pressure–volume curves) between both species*

No statistical differences were found for any parameter. Number of samples was five for each species. Mean ± (SEs). See Materials and methods for abbreviations.

	Ψ_{tlp} (–MPa)	π_o (–MPa)	E_{max} (–MPa)	RWC_{tlp} (%)
P. lentiscus	3.3 (0.2)	2.6 (0.1)	29.2 (1.8)	86.0 (1.0)
Q. coccifera	3.5 (0.1)	2.7 (0.1)	25.4 (3.4)	87.0 (1.8)

drought-induced leaf dieback were used to correct the modelled decrements in *LSC* due to cavitation and then to calculate the real capacity of the xylem to supply water to leaves (*LSC*$_{corr}$). The same current-year twig segments were measured in length, diameter without bark, and leaf biomass supplied, to compute the main hydraulic architecture parameters: hydraulic conductivity (K_h), specific conductivity (K_s), and *LSC*.

Statistical analysis

All statistical analysis were performed by using SPSS version 10.0 package (SPSS Inc., Chicago, Illinois, USA). Data from *P–V* curves and hydraulic parameters were subjected to analysis of variance (one-way ANOVA) to detect differences between the species. Regression analysis was used to fit stomatal conductance, vulnerability to cavitation curves, *LSC*, and leaf dieback with water potential. Differences between species were tested with analysis of covariance since this technique combines regression analysis with ANOVA (Underwood, 1997). The lineal adjustment of the data was verified previously. The natural logarithm of water potential was the concomitant variable, and it was compared for the equality of slopes through the interaction of the concomitant variable and the factor. Data transformations were made when necessary to ensure the validity of the assumptions of normality, linearity and homoscedasticity.

Results

Analysis of pressure–volume curves showed similar values for both species (*P* >0.05). Both species lost turgor between –3.3 to –3.5 MPa, and Π_o at full turgor was –2.6 MPa (Table 1). E_{max} values corresponded to relatively non-elastic cell walls, with high gradients of water potential associated with small losses in cell volume. RWC_{tlp} remained relatively high for both species, with values ranging between 86 and 87% at the turgor loss point.

Figure 1 shows the relationship between $g_{s\text{-}max}$ measured during the day and Ψ_{pd}. Generally, the $g_{s\text{-}max}$ was recorded during the early hours of the day. *P. lentiscus* showed higher rates of stomatal conductance with high water availability (491±99 mmol m^{-2} s^{-1}) than *Q. coccifera* (173±15 mmol m^{-2} s^{-1}). These values decreased suddenly when Ψ_{pd} diminished only slightly (i.e. $g_{s\text{-}max}$ around 100 mmol m^{-2} s^{-1} at –1 MPa). After 24 d of intense drought, *P. lentiscus* showed lower $g_{s\text{-}max}$ values (26±12 mmol m^{-2} s^{-1}), which corresponded to a Ψ_{pd} of –4 MPa. Even with high water availability, *Q. coccifera*
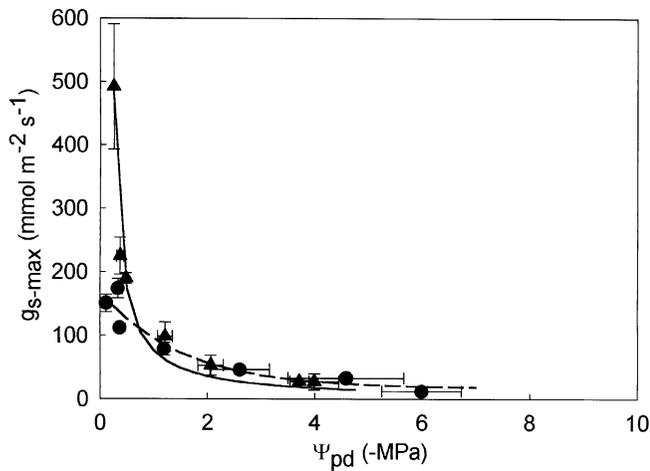
Fig. 1. Relationship between maximal stomatal conductance ($g_{s\text{-max}}$) registered throughout the day and predawn water potential (Ψ_{pd}) in *P. lentiscus* (triangles) and *Q. coccifera* (circles). Data were fitted to the best model: $g_{s\text{-max}}=1000/(14.8-1.64\ \Psi_{pd})$ (r^2=0.98, F=249.1, P <0.01) in *P. lentiscus* (solid line) and $g_{s\text{-max}}=179.8\ e^{-(0.58\ \Psi_{pd})}$ (r^2=0.91, F=20.3, P <0.01) in *Q. coccifera* (dashed line). Each point represents the average of five plants, and bars are SEs.

Table 2. *Hydraulic xylem characteristics of current-year twigs in* P. lentiscus *and* Q. coccifera

Different letters (a, b) indicate significant differences at P <0.01 level. Number of samples were n=169 and n=203 for *P. lentiscus* and *Q. coccifera*, respectively. Mean ±SEs. See Materials and methods for abbreviations.

	K_h (10^{-7}) (kg m s^{-1} MPa^{-1})	K_s (kg s^{-1} m^{-1} MPa^{-1})	LSC (10^{-4}) (kg m^{-1} s^{-1} MPa^{-1})
Q. coccifera	5.0 (0.6)[a]	0.29 (0.02)[a]	1.46 (0.11)[a]
P. lentiscus	29.8 (5.2)[b]	0.73 (0.09)[b]	3.01 (0.40)[b]

maintained conservative stomatal conductance rates which decreased more gradually with lower Ψ_{pd}. The shape of the curves was significantly different between species ($F_{1,15}$=7.84; P=0.01).

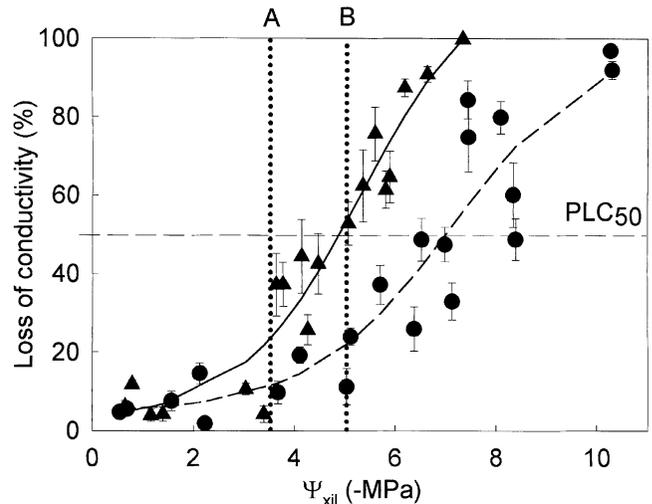According to $g_{s\text{-max}}$ results, *P. lentiscus* was more efficient in conducting water to leaves than *Q. coccifera* (P <0.01; Table 2). *P. lentiscus* exhibited much higher K_h than *Q. coccifera*, i.e. 29.8×10^{-7} and 5.0×10^{-7} kg m s^{-1} MPa^{-1}, respectively. The differences between both species decreased when K_h was expressed as a function of sapwood area (specific conductivity, K_s) or leaf area supplied (leaf specific conductivity, LSC). Nonetheless, the differences were still significant; *P. lentiscus* showed two times higher values than *Q. coccifera* for both parameters (P <0.01).

In order to evaluate the reduction in the water supply to leaves as the plants got drier, the vulnerability to cavitation curves and the reduction in LSC due to cavitation as a function of Ψ_{xil} were analysed (Figs 2, 3). Both species were very resistant to cavitation events, but *P. lentiscus* was more susceptible to water stress-induced cavitation than *Q. coccifera* ($F_{1,36}$=8.34, P <0.01) (Fig. 2). Cavitation events started at the critical water potential (Ψ_{crit}) of –3.5 MPa for *P. lentiscus*, whereas loss of conductivity was close to 100% at –7.5 MPa. For *Q. coccifera*, Ψ_{crit} was –5 MPa and all hydraulic conductivity was lost when Ψ_{xil} was brought down below –10 MPa. As a consequence, *P. lentiscus* reduced LSC sooner than *Q. coccifera* and both curves crossed at –5.5 MPa (Fig. 3). At that point, both species showed the same LSC, about 1.1×10^{-4} kg m^{-1} s^{-1} MPa^{-1}, but while *P. lentiscus* had lost 50% of LSC, *Q. coccifera* had lost only 20%. From this point,

Fig. 2. Vulnerability curves to cavitation for *P. lentiscus* (triangles) and *Q. coccifera* (circles). Pairs of data were adjusted to sigmoid regression (Loss of conductivity= $100/(1+\exp(c(\Psi_{xil}-d)))$ according to Pammenter and Van der Willigen (1998), where 'c' and 'd' coefficients are the slope of the regression and the 50% loss of hydraulic conductivity (PLC_{50}), respectively. Regression coefficients were c= –0.96 and d=4.81 (r^2=0.93, F=242.8, P <0.01) for *P. lentiscus* and c= –0.64 and d=6.96 (r^2=0.86, F=111.6, P <0.01) for *Q. coccifera*. Dotted lines A, for *P. lentiscus*, and B, for *Q. coccifera*, represent the critical water potential (Ψ_{crit}) from which the onset of cavitation events began (slope≠0) in both species. Number of samples was n=19 and n=21 for *P. lentiscus* and *Q. coccifera*, respectively.

Q. coccifera continued losing LSC slightly until the Ψ_{xil} decreased to –10 MPa. By contrast, *P. lentiscus* lost LSC completely in a short interval of Ψ_{xil}, between –5.5 and –7.5 MPa.

It is generally accepted that plants operate near the point at which Ψ_{xil} causes catastrophic xylem dysfunction (Pockman and Sperry, 2000; Sperry *et al.*, 2002). The minimum water potential (midday water potential, Ψ_{md}) as a function of predawn water potential (Ψ_{pd}) was analysed in order to establish the responses to high water availability and intense drought stress in both species (Fig. 4). *P. lentiscus* showed a linear regression between Ψ_{pd} and Ψ_{md}, displaying a constant increment ($\Psi_{md} - \Psi_{pd}$ about 1.6 MPa) independent of the Ψ_{pd} values. This species operated near the critical water potential (Ψ_{crit}), up to
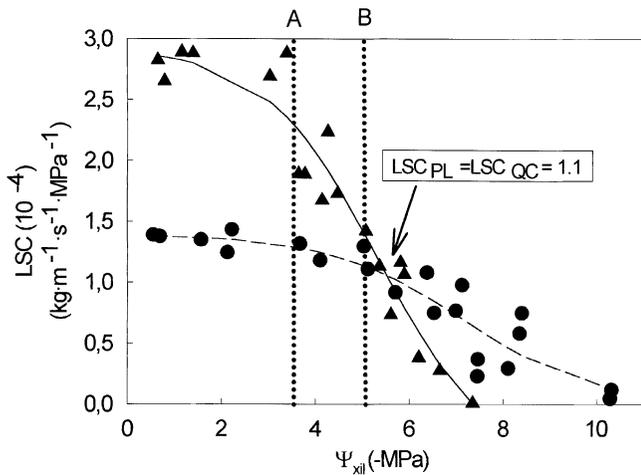
Fig. 3. Loss of leaf specific conductivity (*LSC*) induced by cavitation as a function of xylem water potential (Ψ_{xil}) in *P. lentiscus* (triangles) and *Q. coccifera* (circles). Data were fitted by a sigmoid curve for *P. lentiscus* ($LSC = -0.52+3.45/(1+\exp((-\Psi_{xil}-5.32)/-1.18))$; $r^2=0.93$, $F=70.6$, $P <0.001$) and *Q. coccifera* ($LSC = -0.003+1.39/(1+\exp((-\Psi_{xil}-7.15)/-1.39))$, $r^2=0.86$, $F=34.4$, $P <0.001$). Dotted lines A, for P. lentiscus, and B, for Q. coccifera, represent the critical water potential (Ψ_{crit}). Number of samples was $n=19$ and $n=21$ for *P. lentiscus* and *Q. coccifera*, respectively. See text for more details.

-2 MPa, at which point the Ψ_{md} decreased under the Ψ_{crit} and cavitation occurred. *Q. coccifera* showed an exponential regression indicating differential increases in Ψ_{md} throughout the drought period. This species showed an increment ($\Psi_{md}-\Psi_{pd}$) of about 2 MPa with high water availability, which was progressively reduced (1 MPa at $\Psi_{pd} \leqslant -4$ MPa). Moreover, during the turgor phase, *Q. coccifera* operated very far from the point at which Ψ_{xil} causes catastrophic xylem dysfunction.

Leaf dieback and resprout capacity were also recorded during the drought period (Fig. 5; Table 3). Leaf dieback started when Ψ_{xil} was between -5 and -6 MPa and *LSC* was the same for both species (around 1.1×10^{-4} kg m^{-1} s^{-1} MPa^{-1}). Afterwards, *P. lentiscus* lost all leaves in a short interval of Ψ_{xil} (i.e. between -5 and -8 MPa). By contrast, leaf dieback was more progressive in *Q. coccifera* and this species lost all the leaves when Ψ_{xil} reached values around -10 MPa. To clarify drought stress effects on loss of conductivity and resprout capacity, four phases were identified (Table 3). Phase 1 was characterized by a moderate loss of conductivity without visible damage on the plants. This phase continued until both species fell to -5 MPa and the loss of conductivity was 55 and 20% for *P. lentiscus* and *Q. coccifera,* respectively. During Phase 2, some leaf dieback occurred, but after rewatering, 100% of seedlings remained alive and no resprouts were observed. In this phase, Ψ_{xil} remained between -5 and -7 MPa for both species. Loss of leaf area in *P. lentiscus* reached 50% when Ψ_{xil} fell to -7 MPa and loss of conductivity was nearly 95%. For the same Ψ_{xil}, loss of leaf area was only
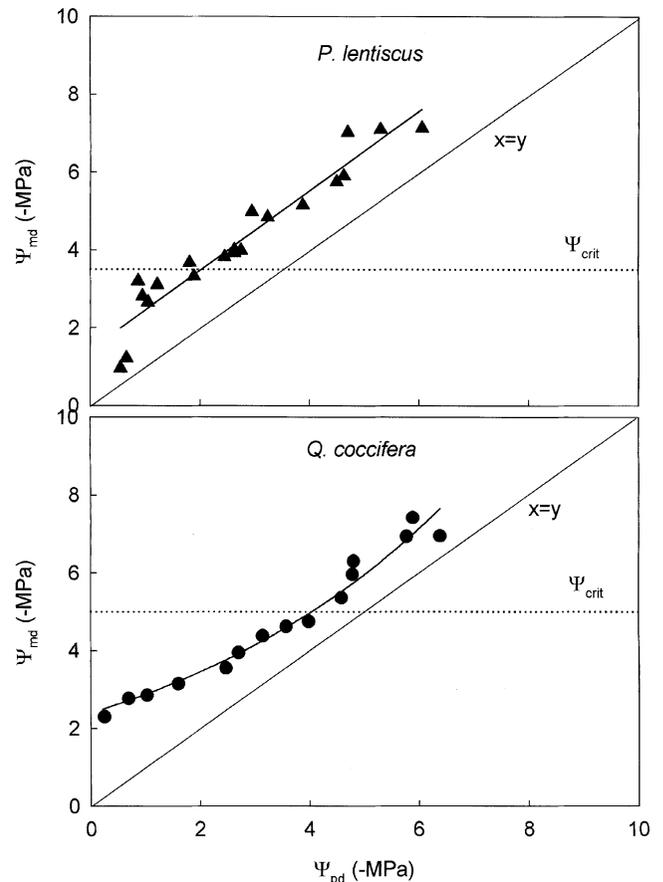
Fig. 4. Relationship between the minimum water potential, defined as midday water potential (Ψ_{md}), and the predawn water potential (Ψ_{pd}) reached by both species. The graphs show regression lines $x=y$ where Ψ_{md} would show the same values as Ψ_{pd}, and the cavitation threshold (Ψ_{crit}). In order to illustrate tendencies, values were adjusted to linear model in *P. lentiscus* ($r^2=0.92$) and exponential model in *Q. coccifera* ($r^2=0.97$). Number of samples was $n=19$ and $n=15$ for *P. lentiscus* and *Q. coccifera*, respectively.

30%, and loss of conductivity was 50% in *Q. coccifera.* Both species lost all leaves at the end of Phase 3. But, at rewatering, both species resprouted from the base of the shoots and all seedlings remained alive. This phase was very short for *P. lentiscus* (Ψ_{xil} between -7 and almost -8 MPa), whereas for *Q. coccifera* it ranged from -7 to -10 MPa. Loss of conductivity was between 95–100% in *P. lentiscus* and between 50–90% in *Q. coccifera*. Finally, during Phase 4, all seedlings lost their resprouting capacity and died when Ψ_{xil} was close to -8 MPa or lower in *P. lentiscus* and -10 MPa or lower in *Q. coccifera*.
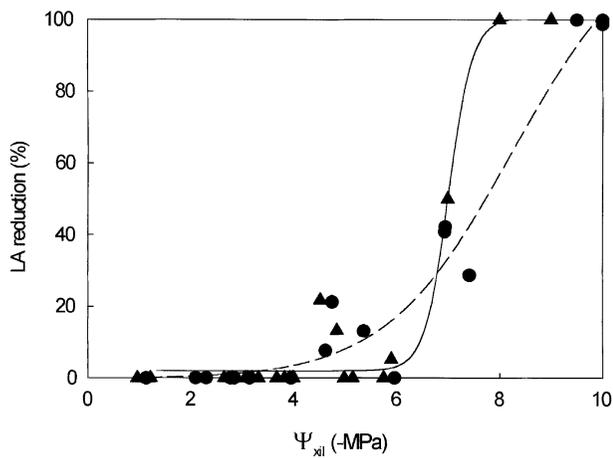
In order to analyse the effect of leaf dieback on the capacity of the xylem to supply water to leaves, the *LSC* was corrected for the reduction in leaf area (LSC_{corr}) that plants suffered with the decrease in Ψ_{xil} (Fig. 6). *P. lentiscus* did not lose leaves until Ψ_{xil} reached -5 MPa; as a consequence, *LSC* and LSC_{corr} decreased in a similar way. When Ψ_{xil} fell to -5 MPa, leaf abscission took place and LSC_{corr} showed slightly higher values than

LSC. The moderately high values of LSC_{corr} were the consequence of the loss in the *LSC* that had previously been very high (55–95%) and, therefore, leaf abscission had little effect on *LSC* recovery. On the other hand, the gradual leaf abscission in *Q. coccifera* permitted the maintenance of LSC_{corr} values similar to the maximum *LSC* values registered at the beginning of the drought period without loss of conductivity. As a result, the capacity of this species to supply water to leaves remained nearly constant throughout the whole drought period.

Discussion

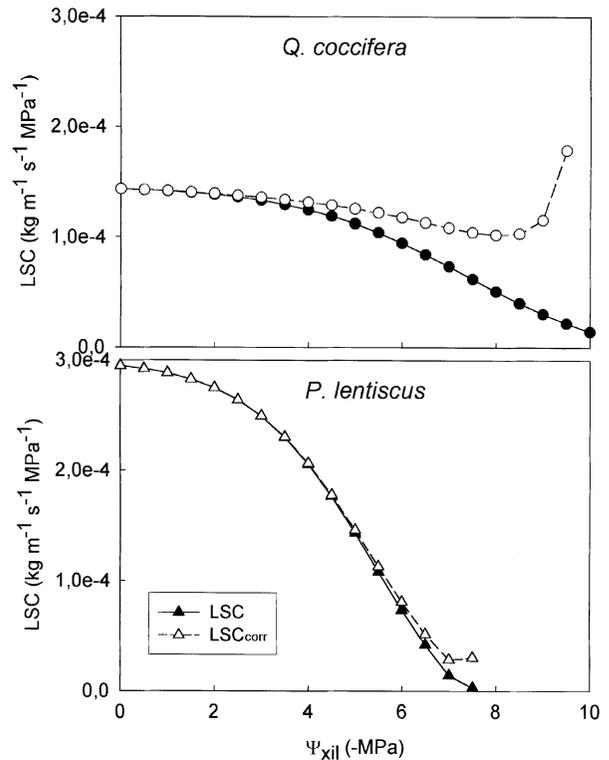### Drought strategies, stomatal conductance and vulnerability to cavitation

Several studies have reported that plants must regulate transpiration through stomatal conductance in order to

avoid xylem cavitation and runaway embolism (Sperry and Pockman, 1993; Cochard *et al.*, 1996; Salleo *et al.*, 2000). Jones and Sutherland (1991) proposed that plants could be classified in two different categories according to their stomatal behaviour: (1) plants that regulate stomatal closure to avoid any loss of hydraulic conductivity; and (2) plants that maximize stomatal conductance even if their stomatal behaviour involves some loss of hydraulic conductance. Cavitation avoidance has been interpreted

Fig. 5. Leaf dieback expressed as a percentage in reduction of leaf area as a function of Ψ_{xil} in *P. lentiscus* (triangles) and *Q. coccifera* (circles). Data were fitted by a sigmoid curve for *P. lentiscus* (solid line) ($LSC = -0.52 + 3.45/(1+\exp((-\Psi_{xil}-5.32)/-1.18))$); $r^2 = 0.93$, $F = 70.6$, $P < 0.001$) and *Q. coccifera* (dashed line) ($LSC = -0.003 + 1.39/(1+\exp((-\Psi_{xil}-7.15)/-1.39))$, $r^2 = 0.86$, $F = 34.4$, $P < 0.001$). Modelled loss of leaf area was used to recalculate LSC_{corr} in Fig. 6. Number of samples was $n = 22$ and $n = 20$ for *P. lentiscus* and *Q. coccifera*, respectively.

Fig. 6. Modelled decline in *LSC* due to cavitation as a function of Ψ_{xil}. Black symbols correspond to *LSC* decline due to cavitation assuming leaf area constant, and the white symbols are *LSC*-corrected (LSC_{corr}) for the leaf area remaining on the plants after leaf dieback occurred for each Ψ_{xil}. Curves were plotted from modelled regression lines on Figs 3 and 4.

Table 3. *Observed plant damages attributed to leaf dieback, survival and resprout capacity after rewatering for each species throughout the intense drought period*

The range of Ψ_{xil} and loss of conductivity for the different phases observed are shown.

Phase	Plant damage	*Q. coccifera*		*P. lentiscus*	
		Ψ_{xil} (–MPa)	Loss of conductivity (%)	Ψ_{xil} (–MPa)	Loss of conductivity (%)
1	No visible damage	–0.1 to –5	0–20	–0.1 to –5	0–55
2	Damaged leaves Loss of some leaves 100% seedlings alive No resprouts	–5 to –7	20–50	–5 to –7	55–95
3	Progressive loss of all leaves Resprouts from the base of shoots 100% seedlings alive	–7 to –10	50–90	–7 to –8	95–100
4	All seedlings dead	<–10	>90	<–8	100

as the consequence of an efficient stomatal control over transpiration (Cochard *et al*., 1996), but it has also been considered an ideal condition for actively transpiring plants growing in natural conditions (Nardini and Salleo, 2000; Salleo *et al*., 2000). However, this topic is still being subjected to extensive study (e.g. Hubbard *et al*., 2001; Cochard *et al*., 2002; Sperry *et al*., 2002). The results of this work show that both species maintained an efficient stomatal control with steep decreases in stomatal conductance, well before reaching Ψ_{crit} that would cause the onset of cavitation events. This behaviour could be considered as cavitation avoidance since stomatal closure prevents xylem cavitation (Sperry *et al*., 1993). The stomatal response observed in both species would be linked to a conservative use of water resources, mainly in *Q. coccifera*. In fact, both species displayed drought-avoidance strategies, by water-saving in *Q. coccifera* and by water-spending in *P. lentiscus* (Levitt, 1980; Vilagrosa *et al*., 1997; Vilagrosa, 2002). These strategies are frequently reported in species from habitats with strong water limitations (Salleo and Lo Gullo, 1993). This conservative use of resources is further supported by the results of the *P–V* curves, which showed low cell wall elasticity with high gradients of water potential associated with small losses of cell volume. These results would be in accordance with other authors (Tenhunen *et al*., 1987; Pereira and Chaves, 1993) who suggested that a prompt response of plants to drought is crucial in order to prevent severe tissue dehydration and plant damage under Mediterranean severe drought conditions.

In addition to the role of stomatal conductance, it has been reported that plants operate near the point at which water potential causes catastrophic xylem dysfunction (Pockman and Sperry, 2000; Sperry *et al*., 2002). Moreover, Hacke *et al*. (2000) proposed that plants from arid climates would maintain wider safety margins than plants from moist habitats, and that this will improve the survival during drought periods. Although these observations coincide with the behaviour observed in *Q. coccifera*, the same does not apply for *P. lentiscus*, which maintained narrower intervals between Ψ_{md} and Ψ_{crit}. *P. lentiscus* takes advantage of episodes with high water availability, and would maintain narrower limits than *Q. coccifera* with its conservative strategy based on water saving. Furthermore, several studies (Vilagrosa *et al*., 1997; Fonseca, 1999; Vilagrosa, 2002) have shown that seedlings of *Q. coccifera* showed higher mortality rates than those of *P. lentiscus* during the first summer on experimental plots established in semi-arid climate. Therefore, wider safety margins to xylem dysfunction as observed in this study do not always imply higher survival under field conditions. Moreover, the results of this study suggest that it is possible to maintain wide or narrow margins independently of the habitat occupied, since both species are characteristic of the same Mediterranean dry and semi-arid

environments. Martínez-Vilalta *et al*. (2002) obtained similar results with several Mediterranean shrub and tree species that are closely related to the species studied in this work. However, these authors linked wide or narrow margins to water-conducting efficiency and phylogenetic characteristics. The results here do not fully agree with these observations since *P. lentiscus*, with higher water-conducting efficiency, was the species showing narrow safety margins and high survival under field conditions. The observations of the present study reinforce the idea that the different drought strategies exhibited by the species would establish the safety margins that each species can maintain, since stomatal closure entails a high cost for species to pay for maintaining xylem integrity (Jones, 1998).

LSC *maintenance, leaf dieback and survival capacity.*

According to Tyree and Ewers (1991) vulnerability to cavitation can be considered the most important physiological parameter explaining drought tolerance in plants. However, this hypothesis has never been tested (Tyree and Zimmermann, 2002). Several studies have related hydraulic failure to leaf shedding (Tyree *et al*., 1993) or branch dieback (Davis *et al*., 2002), and Williams *et al*. (1997) related embolism with seedling mortality in *Adenostoma fasciculatum*. Moreover, Sperry and Pockman (1993) have reported the existence of a critical value of leaf specific conductance in *Betula occidentalis* at which a leaf would be in danger of dieback. As far as is known, the limits for leaf dieback, whole plant mortality, and survival capacity have not yet been related to the loss of hydraulic conductivity. The present study shows that leaf dieback started after the plants reached the turgor loss point and the stomata remained closed, when Ψ_{xil} decreased below –5 MPa. This fact would represent a mechanism to avoid runaway embolism through the reduction in overall passive water losses (i.e. cuticular transpiration). On the other hand, whereas leaf dieback began at different rates of loss of conductivity (55% and 20% for *P. lentiscus* and *Q. coccifera*, respectively), the coincidence in *LSC* values around 1.1×10^{-4} kg m^{-1} s^{-1} MPa^{-1} might support the existence of an *LSC* threshold at which plants cannot properly supply their leaves with water and thus must regulate the leaf biomass. In addition to these results, both species showed similar values of residual transpiration when stomata remained closed (Vilagrosa *et al*., 2003).

In this study, four phases were identified in relation to loss of conductivity, leaf dieback, resprouting capacity, and seedling mortality (Table 3). During the first two phases, loss of conductivity and leaf dieback did not represent a problem for the species studied, and they continued living after rewatering, even though the loss of conductivity was as high as 95% in *P. lentiscus*. During Phase 3, both species were able to resprout after

rewatering, possibly indicating that the loss of leaf biomass had been excessive and that they needed to recover leaf photosynthetic biomass to be able to survive. However, loss of conductivity in *P. lentiscus* ranged between 95 and almost 100%, whilst *Q. coccifera* only lost between 50–90%. Finally, during Phase 4, loss of conductivity was very close to 100% in both species, and they were unable to resprout and died. Therefore, these results show that cavitation can be considered a good predictor of the limits to drought tolerance of both species since they died when loss of conductivity was almost complete. However, several authors (Hacke *et al.*, 2000; Martínez-Vilalta *et al.*, 2002; Tyree and Zimmermann, 2002) have indicated that loss of conductivity could be considered a good predictor for species survival. Contrary to expected results, the higher resistance to xylem cavitation in *Q. coccifera* was not accompanied by higher survival under field conditions. These results might indicate that the different behaviour evident between both species could be related to differences in drought tolerance beyond xylem resistance to cavitation. Moreover, *P. lentiscus* with a water-spender strategy maintained its entire leaf biomass until the almost 100% loss of conductivity while *Q. coccifera* with a water-saver strategy adjusted its leaf biomass to maximize its capacity to supply water to leaves during the whole drought period (Fig. 6). Therefore, *P. lentiscus* would have to recover hydraulic conductivity to be able to supply water properly to its leaves when conditions of water availability returned again, whereas *Q. coccifera* would be able to maintain maximum rates of gas exchange with the remaining leaf biomass. But the latter species with lower loss of conductivity (*c.* 50–90%) would have to produce new leaves to maintain a positive carbon balance after rewatering, e.g. autumn rainfalls (Table 3).

The results of this study cannot explain the underlying mechanisms of such observations. However, both strategies may be related to different morpho-functional traits in both species. *P. lentiscus* develops a semi-ring-porous softwood whilst *Q. coccifera* develops a diffuse-porous hardwood (Villar-Salvador, 2000). According to Fahn (1982), it is cheaper to build a xylem structure based on ring-porous softwood than on diffuse-porous hardwood, and the formation of new vessels can be decoupled from phloem formation quicker in ring-porous (several days) than in diffuse-pore species (several weeks). Consequently, the construction of new vessels in *P. lentiscus* would suppose lower energy expenditures than in *Q. coccifera*. In this sense, a good correlation has been observed between starch metabolism and cambium activity in *Q. coccifera*, indicating a high construction cost for new vessels (Arianoutsou-Faraggitaki *et al.*, 1984). These observations agree with Hacke *et al.* (2001) who found that higher resistance to cavitation would be related to higher wood density for resisting the mechanical strength generated at low water potentials and, conse-

quently, to a higher construction cost of the xylem. In addition, it has been observed that *P. lentiscus* is more plastic than *Q. coccifera* in response to drought conditions. This species was able to modify above and below-ground biomass, and increase leaf water storage and photochemical efficiency under conditions of drought (Vilagrosa *et al.*, 1997, 2003; Rubio *et al.*, 2001). On the other hand, the type of root system developed by both species could also contribute to the observed results. *P. lentiscus* developed a dense root system along the soil profile while *Q. coccifera* developed a deep but sparse root system (Vilagrosa *et al.*, 1997; Vilagrosa, 2002). In semi-arid environments, a dense root system can take advantage of small rainfall episodes improving water adsorption and permitting a better rehydration after summer drought (Canadell and Zedler, 1995). In this sense, Fonseca (1999) observed under field conditions that *P. lentiscus* was able to show higher water potentials than *Q. coccifera* after autumn rainfalls. In addition to these observations, recent studies have shown that xylem refilling associated with high water potentials could be one of the mechanisms for restoring hydraulic conductivity after xylem cavitation (Salleo *et al.*, 1996; Tyree *et al.*, 1999; Cochard *et al.*, 2001; Vogt, 2001). Therefore, the higher water potentials, as observed in *P. lentiscus* could facilitate the mechanisms of xylem refilling or the construction of new xylem in this species. This fact would allow the species to maintain its entire leaf biomass until the almost 100% loss of conductivity.

In conclusion, the results of this study indicated that, before the onset of cavitation events, both species maintained an efficient stomatal control linked to a conservative use of water resources. However, wide safety margins do not always imply higher survival rates under field conditions. The results obtained in this study introduce the idea that, independent of the habitat occupied, the drought strategies exhibited by the different species could establish the safety margins that each species can maintain.

According to previous studies, loss of conductivity could be considered a good predictor of survival limits in both species since they both died when the loss of conductivity was very close to 100%. But higher resistance to xylem cavitation was not accompanied by higher survival under field conditions, and it is postulated that this fact could be related to the mopho-functional traits developed for the species. This study also reports the existence of a leaf specific conductivity threshold below which plants cannot supply water to their leaves properly and must regulate leaf biomass to avoid runaway embolism. It would be desirable in order to determine the exact role of xylem cavitation in the resistance of plants to drought by combining field observations on survival, xylem-refilling processes and root system features, with those on vulnerability to cavitation.

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