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**Response to seasonal drought in three cultivars of *Ceratonia siliqua* L.:
leaf growth and water relations**

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Running head: Leaf growth and water relations in carob tree

Summary

The responses of leaf growth and leaf water relations to seasonal drought were monitored, during two successive years, in three cultivars of *Ceratonia siliqua* L. growing in the field, in the South of Portugal. In leaves from the current year growth bulk leaf water relations parameters were characterised using pressure-volume analysis, and morphometric measurements of xylem in petioles were undertaken. The three cultivars under study (Galhosa, Espargal and Mulata) differed as to the seasonal pattern of leaf initiation: in Galhosa a sharp peak of leaf initiation occurred in June, soon before the period in which the highest leaf expansion rates were determined; on the other two cultivars (Espargal and Mulata), not only the onset of leaf growth occurred earlier, but they continued producing new leaves throughout the summer period. The diurnal pattern of water relations determined in recently-expanded leaves indicated that Galhosa was the only cultivar in which, during midsummer, leaf water potential was not decreased below the turgor loss point and leaf relative water content remained above 90%. Although no conclusive evidence was found for the occurrence of osmotic adjustment in recently-expanded leaves, during the dry season following leaf formation a seasonal decrease in osmotic potential sufficient to maintain turgor was detected in the leaves of Galhosa but not for the other cultivars. As to the anatomy of the xylem at the petioles, the widest xylem conduits were recorded in Galhosa. This might contribute to explain why in this cultivar midday leaf water potential never decreased below -2.0 MPa even at the end of summer drought season.

Keywords

carob tree, osmotic adjustment, water stress, xylem anatomy.

Introduction

Carob tree (*Ceratonia siliqua* L.) grows successfully in areas with a Mediterranean type of climate, characterised by a long dry season during which negligible rainfall is associated with high evaporative demand. Despite those stressful conditions, the foliage of field-grown *C. siliqua* is frequently found to remain green and apparently turgid as soil dries during summer, which suggests that this evergreen broad-leaved tree is well adapted to drought conditions.

A variety of mechanisms may be responsible for increased drought resistance, and previous studies evidenced that carob may withstand drought by avoiding water stress. At the whole plant level, drought avoidance in *C. siliqua* seems to rely on its ability to develop a deep root system, together with its xylem being highly efficient for water transport. When compared with other evergreen tree species, *C. siliqua* was found to possess relatively wide xylem conduits both at the twig (Lo Gullo and Salleo 1988) and the main leaflet vein (Kikuta et al. 1997), resulting in relatively high specific hydraulic conductivity of the xylem. In addition carob seems to be also very resistant to cavitation, tolerating leaf water potential (Ψ) values below the turgor loss point with only minor losses in hydraulic conductivity, and promptly restoring xylem continuity following rewatering of moderately droughted plants (Salleo and Lo Gullo 1989). As to the root system, Rhizopoulou and Davies (1991) have found that root growth of *C. siliqua* seedlings is not much affected by soil drying, and deep penetration of some roots is able to supply a substantial amount of water to the shoot, even when the plants are rooted in dry soil.

In regions with a Mediterranean-type of climate, plants have to cope both with seasonal drought and short-term water stress conditions associated with large diurnal

changes in air temperature and evaporative demand occurring during summer days. As Ψ declines with increasing evaporative demand, the maintenance of cell volume is critical for normal cell function and plant survival. Carob trees have been found to maintain high stomatal conductance throughout the dry season, but in spite of losing considerable amounts of water during summer days, the relative water content (θ) of mature leaves remained above 94% (Lo Gullo and Salleo 1988). Osmotic adjustment and low tissue elasticity are frequently cited as mechanisms enabling droughted plants to sustain cell volume and to avoid deleterious relative water content (Tyree and Jarvis 1982). Previous reports (Lo Gullo et al. 1986, Lo Gullo and Salleo 1988, Kikuta et al. 1997) indicate that mature carob leaves possess rather rigid cell walls (maximum bulk elastic modulus over 25 MPa) and the osmotic potential (Ψ_{π}) at full turgor is below -1.5 MPa. This combination of characteristics may allow large decreases in Ψ to occur, facilitating continued water uptake, while θ is maintained at a high level (Tyree and Jarvis 1982). In contrast, similarly to what occurs in other species, expanding leaves of *C. siliqua* appear to be very vulnerable to water deficits, exhibiting higher Ψ_{π} , less rigid cell walls, thinner cuticle (Lo Gullo et al. 1986), and hence being prone to suffer detrimental water deficits. However, according to Lo Gullo et al. (1986), restricting leaf growth to winter and spring, might allow young *C. siliqua* leaves to escape the effects of drought.

In the South of Portugal, *C. siliqua* has long been grown as a non-irrigated tree crop, most of the trees growing in Algarve being grafts from a single cultivar (Mulata). Although other cultivars were identified as having a high potential agronomic value, due to their high seed quality and yield, the physiological characterisation of those cultivars is scarce. To our knowledge no study has been conducted to assess whether those

cultivars differ as to their tolerance to seasonal drought. However, evidence for genotypic variation within other woody species has been found as to their drought tolerance, namely in which concerns tissue elasticity and the ability to undergo osmotic adjustment (Parker and Pallardy 1985, Abrams 1988).

The purpose of the present work was to compare leaf response to seasonal water deficits in three cultivars of *C. siliqua*: Mulata, Espargal and Galhosa. With that objective, seasonal progression of leaf growth, leaf water potential and osmotic potential were determined in adult trees growing in the field. Mature leaves from the three cultivars were also compared as to their bulk water relations parameters, characterised from pressure-volume curves, and their xylem anatomy at the petiole level.

Materials and methods

Site description and plant material

This study was undertaken, during 1998 and 1999, in a non-irrigated orchard with ten-year-old *Ceratonia siliqua* trees (about 3 m height), located at sea level, near Tavira (37° 07' N; 7° 39' W). The climate (summarised in Figure 1) is of the Mediterranean type, tempered by a maritime influence due to the proximity of the ocean. The accumulated rainfall from September 1997 to May 1998 reached 616 mm, surpassing the average annual rainfall in the region (around 500 mm). This rainy season was also characterised by moderate temperature, the monthly average minimum temperature (T_{\min}) only dropping clearly below 10°C in January. The following winter was drier (only 210 mm rainfall) and cooler: T_{\min} remained below 10°C from December to February, and during that period decreased below 5°C for 24 days. During both

summer periods no precipitation was recorded from June until the end of September. Although seasonal maximum temperatures (T_{\max}) were similar, the spring rise in daily T_{\max} and evaporation occurred earlier in 1999, and evaporation values during summer drought were higher in 1999 than those in 1998 (Figure 1).

Studies were conducted in three cultivars: Espargal, Galhosa and Mulata. Mulata is a regional cultivar with a wide distribution through Algarve, whereas Espargal and Galhosa are local cultivars. The origin of both local cultivars is the “barrocal”, an area of limestone hills with red soils and typical Mediterranean climate, located in the center of Algarve. For each cultivar, three trees were selected for study. During the two years in which this study was undertaken, no significant cultivar effect was detected as to pod yield, each selected tree yielding around 20 kg of pods per year, irrespective of the cultivar.

Leaf growth

In order to develop a non-destructive way to assess leaf area, four branches per cultivar were harvested during the summer of 1997, the sampled shoots bearing leaves with variable developmental stage. The area of each leaflet (A) was determined with a leaf area meter (Delta-T Devices, Cambridge, U.K.), along with their length (L) and breadth (B). A linear relationship between leaflet areas and their linear dimensions was then established for each cultivar: $A=0.00+0.74*B*L$ ($r^2=0.99$), $A=0.19+0.70*B*L$ ($r^2=0.96$) and $A=0.21+0.70*B*L$ ($r^2=0.98$), respectively for cv. Espargal, Galhosa and Mulata. Approximately 650 leaflets were measured for each cultivar.

In January 1998, two shoots per tree were selected in the outer part of the canopy, at a height of about 2 m above the ground level. An additional set of shoots was selected in 1999. At 7-20 day intervals, leaf growth was monitored on the marked shoots

by determining: the number of new leaves, their respective number of leaflets, as well as the length and breadth of the leaflets located on one side of the rachis (*C. siliqua* has compound-pinnate leaves). The leaflet area was estimated using the previously determined linear relationship between the area and the linear dimensions of the leaflets. The mean relative expansion rate was then calculated as $RER=(\ln A- \ln A_0)/\Delta t$, where A_0 and A are the leaflet areas determined in two successive sampling days, and Δt is the time interval elapsed between sampling days. Estimates of RER were only performed using measurements undertaken at one week time intervals. In order to detect seasonal changes in expansion rate, and to allow comparison between cultivars exhibiting a large number of leaf growth stages, RER was then plotted against leaflet area normalized with respect to final leaflet size (Randall and Sinclair 1989).

Xylem morphometric measurements

In October 1998, nine leaves expanded during the previous spring were selected per cultivar (three leaves per tree), in order to carry out anatomical measurements at the petiole xylem. Transverse petiole thin sections were prepared across its middle length, with a hand-held scalpel, bleached with a diluted chlorine solution and stained with methylene blue. One section per leaf was selected for morphometric measurements performed using enlargements of photomicrographs. Total xylem cross-sectional area, including the xylem parenchyma (A_x), the number of xylem conduits and the diameters of their lumina were determined. The theoretical maximum hydraulic conductance per unit length (K_h) was estimated from $K_h=(\pi \sum r^4)/8\eta$, where r is the xylem conduits radius, and η is the viscosity of water taken at 20 °C (Jones 1992). The surface area of all the leaflets supplied by the sectioned petiole (A_L) was also measured, and the investment into xylem development relative to the leaf surface area supplied was estimated by

calculating the Huber value as A_x/A_L . Leaf specific conductivity (LSC) was calculated as $LSC=K_h/A_L$.

Pressure-volume analysis

Leaf tissue water relations were studied using the pressure-volume (P-V) technique (Tyree and Hammel 1972), in mid-July and at the end of September 1998. Recently expanded leaves were sampled in July, whereas in September sampling involved leaves that had reached full expansion during the previous month. Details on procedure used to collect leaves avoiding the introduction of vapour gaps into the xylem, as well as resaturation methods used, were similar to those described by Correia et al. (1989). P-V curves were generated by the sap expression procedure (Pallardy et al. 1990) using two pressure chambers (PMS, Corvallis, OR, USA and Manofrígido, Lisboa, Portugal). After being sealed in the pressure chamber, the leaves were subjected to a repeated sequence of operations which involved: (a) determination of balance pressure (BP); (b) sap removal by applying, during 10 minutes, an overpressure of approximately 0.3 MPa; (c) slow release of pressure (usually 0.1 MPa) until sap exudation ceased; (d) determination of the new balance pressure. Measurements were taken until at least four points were obtained on the linear part of the $1/BP$ vs volume expressed curve. Precautions taken in order to minimise evaporation from the leaf enclosed in the pressure chamber were described by Correia et al. (1989). A moisture release curve (Ψ plotted against leaf fresh mass) for each leaf was examined for the presence of a plateau near full hydration that would indicate the presence of excess apoplastic water (Dreyer et al. 1990, Kubiske and Abrams 1991). Despite rehydrating the leaves prior to P-V analysis, none of the samples exhibited plateau.

The osmotic potential at full turgor ($\Psi_{\pi 100}$), the apoplastic water content at full turgor (θ_a), the water potential at the turgor loss point (Ψ_{tlp}) and the symplastic water content (F) were determined as described in Correia et al. (1989). The relative water content at the turgor loss point (θ_{tlp}) was estimated using a modified exponential equation (Schulte and Hinckley 1985): $\Psi_p = \exp[-a(\theta - \theta_{tlp})] - 1$, where Ψ_p is pressure potential, and a is a parameter. The plots of Ψ_p against F displayed linearity at high values of Ψ_p and curvilinearity at low Ψ_p , the slope decreasing as turgor approached zero. Therefore, maximum bulk modulus of elasticity (E_{max}) was evaluated as the slope of the linear fits of observations of Ψ_p and F, for turgor higher than 0.5 MPa.

Leaf water relations under field conditions

Diurnal courses of leaf water status under field conditions were determined one week following leaf sampling for P-V analysis. Leaf Ψ was determined, using a pressure chamber (PMS, Corvallis, OR, USA), four times the day defined as: predawn (immediately before sunrise), morning (4 h after sunrise), midday, and afternoon (3 h before sunset). At each time of day, four leaves per cultivar were sampled. In the morning and at midday the leaves were sampled from an east-facing branch, whereas at predawn and afternoon the sampled leaves were located in a west-facing branch. The leaves sampled were located immediately below (predawn and morning) or above (midday and afternoon) the insertion point of the leaves used for P-V analysis. Leaf relative water contents were estimated by determining the values of θ corresponding to the measured values of Ψ , using the potential isotherms derived from P-V analysis. Diurnal osmotic adjustment was assessed by determining the diurnal variation in the total solute content on a leaf dry mass basis. Leaf discs were collected, at predawn and in the afternoon, from the same leaf where Ψ was measured in the afternoon. The osmotic

potential of frozen-thawed leaf discs was determined by thermocouple psychrometry, using C-52 chambers connected with a HR-33T dew-point microvoltmeter (Wescor Inc., Logan, UT, USA). The amount of solutes in leaf lamina (N_s) was calculated as $N_s = -\Psi_\pi(W/RT)$, where R is the universal gas constant, T is temperature and W is the mass of water in the fresh tissue, as determined in another set of leaf discs punched from the same leaflets.

During 1999, field measurements of leaf water relations from east-facing branches were conducted from May until the end of summer, on selected sunny days. At each sampling time, six leaves expanded in the previous year were harvested per cultivar. Ψ was determined both at predawn and midday. Leaf disc samples for Ψ_π were also collected at midday, and pressure potential calculated as $\Psi_p = \Psi - \Psi_\pi$. No correction factor was applied in order to avoid turgor underestimation due to dilution of cell sap with apoplastic water following freezing and subsequent thawing of leaf tissues. Therefore, we can not exclude the possibility of values shown for Ψ_π being overestimated, whereas pressure potential may be underestimated (Wenkert 1980).

Data statistical analysis

To test the effect of cultivar on leaf production, final leaf size and xylem anatomy, data were subjected to one-way analysis of variance (ANOVA). The effects of cultivar and time of sampling on leaf water relations parameters were tested by two-way ANOVA. Statistical analysis was carried out using SigmaStat 2.0 (SPSS Science, Chicago, IL).

Results

Leaf growth

As shown in Table 1, the three cultivars under study did not differ as to the total number of leaves produced during the whole growth season of 1998, each selected branch producing about 11 new leaves, irrespective of the cultivar. No new leaf growth was recorded either during the autumn of 1998 or the spring of 1999.

During the growth season of 1998, significant differences between cultivars were found as to full-expanded leaflet area, which was significantly higher in Galhosa than in the other two cultivars. The same cultivar ranking was observed as to the whole leaf full-expanded area, despite Espargal producing leaves with a higher number of leaflets (Table 1). The seasonal pattern of leaf emergence (defined as the time of leaflet unfolding) also varied with cultivar: in May the lowest number of new leaves was found in Galhosa, but this cultivar exhibited the highest rate of leaf production during June; from July onwards Galhosa did not produce any more leaves, in contrast to the other two cultivars that continued producing new leaves (although in reduced number) throughout the whole summer period (Figure 2).

When the relative expansion rate was plotted as a function of leaflet area normalised with respect to final size at full expansion (Figure 3), data points from different cultivars fitted to the same line, i.e., the mean RER of leaflets of comparable size was similar regardless of cultivar. However, as shown in Figure 3, the relationship between RER and leaflet size changed throughout the season: irrespective of the cultivar, for each particular normalized leaflet size, their relative expansion rates were higher at the beginning of summer than both during spring and at the end of drought season.

Anatomical characterisation of xylem petiole and Hagen-Poiseuille predictions

Results from morphometric measurements at the xylem petiole are shown on Table 2, together with Hagen-Poiseuille predictions. Significant differences were found between cultivars as to xylem transectional area, this parameter being about 50% higher in Galhosa than in the other cultivars. However, the investment into xylem development in relation to leaf area supplied, as indicated by the Huber value, was similar in the three cultivars. The mean number of xylem conduits per xylem cross-sectional area varied widely between cultivars, the xylem in petioles of Espargal leaves exhibiting the highest conduit density, whereas the lowest value was found in Galhosa (Table 2). As shown in Figure 4, cultivars also differed as to the distribution of the diameters of the xylem conduits: in the petioles of both Espargal and Mulata, the diameter of almost 70% of the conduits was lower than 25 μm , whereas this percentage decreased to 50% in Galhosa; in contrast, wider xylem conduits (between 25 and 40 μm) were more abundant in Galhosa (about 45%) than in the other cultivars (less than 30% of the total number of conduits); Galhosa also presented a higher percentage (about 5%) of the widest xylem conduits (40 to 55 μm in diameter) than Espargal and Mulata in which conduits of that diameter class represented only 2% of total xylem conduits. In accordance to the observed differences in conduit number and diameters, the mean theoretical maximum hydraulic conductivity (K_h) of the petiole was about 2-fold higher in Galhosa than in Mulata, whereas K_h at the petioles of Espargal trees was about 60% of values found in Galhosa (Table 2). Despite the magnitude of cultivar differences being attenuated when variation in leaf area was taken into account, Galhosa was also the cultivar exhibiting the highest ability for water supply to the sustained leaf area: as shown in Table 2, in Galhosa mean leaf specific conductivity was 50% higher than in Mulata and about 30%

higher than in Espargal, although those differences were not significant at the 0.05 probability level.

Pressure-volume analysis of current-year leaves

Summary of bulk leaf water relations parameters derived from pressure-volume analysis is shown in Table 3. No significant cultivar effect was detected as to Ψ_{π} determined at full turgor but the value of this water relations parameter decreased as the season progressed, its mean value being approximately 0.1 MPa lower in September than in July. A similar pattern of variation was observed as to Ψ at the turgor loss point (Table 3). The apoplastic water content at full saturation not only increased from July to September, but it was also significantly higher in Galhosa than in the other two cultivars. Consequently, in both sampling periods, Galhosa exhibited the highest relative water content at the turgor loss point (Table 3). The observed differences in θ_{tlp} were not a result of Galhosa having less elastic cell walls: as shown in Table 3, all three cultivars exhibited high values of maximum bulk modulus of elasticity (≥ 30 MPa) and reached the wilting point at a relative symplastic water content of about 93%.

Diurnal variation in the water relations of current-year leaves

Figure 5 shows the diurnal time course of leaf water potential and relative water content determined in midsummer and one week after the first autumnal rainfall that occurred at the end of September. In midsummer, all three cultivars underwent a rapid decrease in Ψ , from -0.4 MPa at predawn to about -1.8 MPa soon before midday. The later value was in every cultivar very close to Ψ at the turgor loss point determined in the previous week in leaves sampled at predawn (Table 3). However, as can be seen from Figure 5, the evolution of Ψ during the afternoon was dependent on the cultivar. In Galhosa Ψ recovered during the afternoon to values above Ψ_{tlp} . In the case of Espargal

and Mulata, however, Ψ remained below the turgor loss point during the afternoon, the minimum diurnal Ψ being about 0.2 MPa lower than in Galhosa. As shown in Figure 5, whereas in the later cultivar leaf relative water content was maintained well above 90% throughout the day, in the case of both Espargal and Mulata θ reached a minimum diurnal value of about 86%. One week following the first rainfall event late in the season (59 mm in two days), leaf water potential promptly increased to about -0.25 MPa at predawn, and remained well above Ψ_{tlp} throughout the day (Figure 5).

The osmotic potential and the concentration of osmotically active solutes in leaf lamina, determined both at predawn and late in the afternoon in the same leaves, are shown in Table 4. Although Ψ_{π} dropped significantly from predawn to afternoon, no evidence was found for the occurrence of diurnal osmotic adjustment, since irrespective of the cultivar, the amount of osmotically active solutes per unit dry mass did not significantly change throughout the day.

Seasonal variation in the water relations of previous-year leaves

The evolution during the dry season of 1999 of predawn Ψ from leaves expanded in the previous year is shown in Figure 6. Reflecting the rainfall deficit during the previous autumn and winter (Figure 1), the leaves of all three cultivars exhibited in May values of predawn Ψ very close to -0.40 MPa, *i.e.* similar to those recorded at midsummer in 1998 (Figure 5). The way predawn water potential varied as season progressed was dependent on the cultivar (Table 5 and Figure 6). In Galhosa predawn Ψ decreased from May to the beginning of July, reaching a mean value of -0.56 MPa, but no further decrease was detected thereafter. In contrast, predawn Ψ values determined in Mulata declined throughout the entire summer season, reaching a mean value of about -0.78 MPa near the end of summer. As shown in Figure 6, from midsummer onwards the

values of Ψ determined at predawn in Espargal were intermediate to those exhibited by the other two cultivars.

Irrespective of the cultivar, minimum values of midday Ψ were recorded at the end of July, but similarly to what has been observed at predawn, the seasonal decline in midday Ψ tended to be less accentuated in Galhosa than in the other cultivars (Figure 6). Values of midday Ψ recorded in September were about 0.2 MPa higher than those determined in July, although no precipitation occurred during that period. The above mentioned recovery in midday Ψ was associated with the decline in the evaporative demand of the air recorded in September, when maximum diurnal temperature was close to 25°C, compared to 32°C in the previous days of measurements (Figure 1). Although the effect of cultivar on the evolution of midday Ψ was not statistically significant, a significant effect of cultivar was detected at the level of both Ψ_{π} and Ψ_p (Table 5). In Espargal and Mulata, the seasonal decrease in Ψ was not counterbalanced by a proportional decline in Ψ_{π} , and consequently turgor decreased from May to July. In contrast, in the case of Galhosa Ψ decreased by a lesser extent than Ψ_{π} and, hence turgor tended to increase rather than decreasing as the dry season progressed (Figure 6).

Discussion

In the present work, during the summer of 1999, field-grown non-irrigated carob trees were found to maintain predawn Ψ above -0.8 MPa (Figure 6), despite the severe rainfall deficit that occurred during the previous autumn and winter (Figure 1). These results are in accordance with previous work with field-grown mature *C. siliqua* trees, in which this evergreen tree was found to maintain, at the peak of summer drought, predawn values of Ψ ranging from -0.4 MPa (Lo Gullo and Salleo 1988) to -1.1 MPa

(Correia and Martins-Loução 1995). The ability of *C. siliqua* to maintain high predawn Ψ was interpreted by Correia and Martins-Loução (1995) as suggesting that this species achieves drought avoidance through deep rooting. As shown in Figure 6, the ability to maintain high predawn Ψ was particularly evident in the case of Galhosa, in which Ψ determined before dawn was never lower than -0.55 MPa. These results suggest that it is worth investigating the possibility of the three cultivars under study differing as to the capacity of their roots to penetrate deep soil layers.

As shown in Figure 6, Galhosa was also the cultivar in which the highest values of midday Ψ were recorded during midsummer. The maintenance of high Ψ during periods of high evaporative demand of the air may be achieved by reducing transpiration through stomatal closure. Another condition that may contribute to the maintenance of high Ψ in transpiring leaves is an efficient xylem transport system. The structural analysis of the water conducting system at the petiole level showed that, within the three cultivars under study, Galhosa was the one with the highest percentage of wide xylem conduits (Figure 4), and the highest values of hydraulic conductance at the petioles (Table 2). Whether those differences also occur in other parts of the xylem system, they may contribute to explain why Galhosa was able to maintain, at the end of drought period, midday Ψ values higher than those determined on the other cultivars (Figure 6), although the possibility of Galhosa exhibiting a more conservative stomatal behaviour should not be discarded.

In the present work, recently-expanded leaves of *C. siliqua* were found to exhibit a bulk volumetric elastic modulus of about 30 MPa or even higher (Table 3). These values are similar in magnitude to those previously reported for mature carob leaves (Lo Gullo et al. 1986, Lo Gullo and Salleo 1988, Kikuta et al. 1997), and are among the

highest values reported for evergreen woody species (Dreyer et al. 1990, Kikuta et al. 1997, Salleo et al. 1997). As a result of their rigid cell walls, the Ψ of mature leaves of field-grown *C. siliqua* has been reported not to decrease below the turgor loss point, and their θ to remain above 94%, even at the end of summer (Lo Gullo and Salleo 1988). In the present work Galhosa was the only cultivar under study which recently-expanded leaves behaved in such a way during a hot midsummer day (Figure 5). In contrast, in the other two cultivars Ψ decreased below the turgor loss point at midday, while their relative water deficit increased to about 15%. The differences between cultivars as to the value of minimum diurnal θ were not associated with any difference in the elasticity of cell walls or Ψ_{π} (Table 3). The three cultivars differed however as to the diurnal trend in Ψ , Galhosa being the only cultivar in which Ψ tended to increase at midday compared to midmorning values (Figure 5). It remains to be seen whether the partial recovery of Ψ observed in Galhosa is the result of midday stomatal closure being more accentuated in this cultivar.

Evidence in the literature for the ability of mature leaves of *C. siliqua* to undergo drought-induced osmotic adjustment is contradictory: whereas Nunes et al. (1989) found a significant decrease in the Ψ_{π} at full turgor when potted carob trees were subjected to drought, other authors found no evidence of field-grown trees exhibiting osmotic adjustment in response to seasonal drought (Lo Gullo and Salleo 1988). In the present work $\Psi_{\pi 100}$ decreased by 0.1 MPa from midsummer to the end of season (Table 3). However, as mentioned in Material and Methods section, the leaves sampled at the end of summer stopped expanding longer ago than leaves sampled earlier in the season. Since significant changes in leaf tissue water relations may occur with ontogeny (Lo Gullo et al. 1986), the observed decrease in $\Psi_{\pi 100}$ may be attributable to differences in

leaf age. No evidence was also found for full-expanded carob leaves from the current year growth experiencing osmotic adjustment on a diurnal basis, as assessed by the diurnal variation in the concentration of osmotically active solutes (Table 4). Therefore, no compelling evidence was found for a significant drought-induced osmotic adjustment in recently-expanded leaves of carob tree. However, during the following dry season, a seasonal decrease in the Ψ_{π} , resulting in turgor maintenance as the dry season progressed was detected in the leaves of Galhosa (Figure 6). No such a seasonal decline in Ψ_{π} was detected on the other cultivars, and hence their leaves tended to loose turgor at the peak of the dry season.

When compared to mature leaves, expanding leaves of *C. siliqua* were reported to have much lower bulk modulus of elasticity and higher $\Psi_{\pi 100}$ (Lo Gullo et al. 1986). Therefore, these young leaves are expected to be potentially very vulnerable to water stress, since they will be subjected to large reductions in symplasmic water content. However, data in the literature indicate that most of carob leaves are formed during spring and hence reach maturity before drought becomes severe (Lo Gullo et al. 1986, Nunes et al. 1992). This was interpreted by Lo Gullo et al. (1986) as indicating that young leaves of carob are drought-escaping organs, developing the drought avoidance strategy as they mature and their cell walls become less elastic. No such a drought-escaping strategy was evidenced in the present work in Espargal and Mulata, which continued to form new leaves until the end of summer (Figure 2). In contrast, after mid July no new leaves were initiated in the case of Galhosa, the only cultivar which young leaves may be considered to be drought-escaping organs as suggested by Lo Gullo et al. (1986). Besides stopping leaf initiation early in the season, Galhosa also exhibited a slower rate of leaf formation at the beginning of the growth season (Figure 2). It is

noteworthy that T_{\min} remained below 15°C during that period (Figure 1). Although water availability is generally considered as the main factor that limits tree growth in Mediterranean type of climates, previous reports indicate that carob tree is more cold sensitive than most others Mediterranean evergreens (Mitrakos 1980, Larcher 1981). It deserves further investigation whether different sensitivity to low temperature underlies the delay in the onset of leaf growth in Galhosa compared to the others cultivars.

Despite the shortening of leaf growth season in Galhosa, it was this cultivar that produced the highest values of new leaf area during the growing season of 1998 (Table 1). The high final areas reached by full-expanded leaves of Galhosa may be partly attributable to the highest proportion of new leaves produced in June by this cultivar when compared to Espargal and Mulata (Figure 2), because the period of most intensive leaf formation in Galhosa slightly preceded the period during which the highest leaf expansion rates were recorded (Figure 3).

The slowing down of leaf expansion during summer drought peak is not unexpected, since soil drying may decrease the plasticity of the cell walls from young leaves, including in *C. siliqua* (Rhizopoulou and Davies 1991). In addition, the ability to maintain turgor has been reported to be lower in expanding than in mature leaves of carob trees (Lo Gullo et al. 1986). Since, Ψ of expanded leaves (Figure 5) was found to decrease to values near or below the turgor loss point (Table 3), it is likely that expanding leaves will also loose turgor then. Therefore, being driven by turgor leaf expansion would be expected to stop during daytime. However, as shown in Figure 3, once the leaflets have unfolded, they kept expanding even at the end of summer, although at slightly lower rates than those determined early in the season. It is noteworthy that darkened carob leaves have been found to expand at higher leaf

expansion rates than those recorded during daylight (Rhizopoulou and Davies 1991). Such a diurnal pattern of leaf growth, attributable to the maintenance of loosened cell walls upon darkening, has been found in other tree species (Taylor and Davies 1985). This may be a crucial factor allowing the leaves to expand despite turgor loss point being reached during daytime, provided that the nocturnal recovery of Ψ is substantial, such as the case of *C. siliqua*.

Conclusions

The results obtained in the present work confirmed previous findings as to *Ceratonia siliqua* having a high ability to avoid severe leaf water deficits during summer. However, this study evidenced the existence of genotypic variability on the extent of development of drought resistance mechanisms that have been previously attributed to leaves of this evergreen tree species. Galhosa was the only cultivar under study which young leaves exhibited a tendency for a drought-escaping strategy, achieved by accomplishing leaf maturation before drought becomes too severe. It was also in Galhosa that the development of a drought-avoidance strategy was more evident soon after the leaves reached full expansion. By that time, this cultivar exhibited not only the highest predawn Ψ and diurnal minimum θ , but also the highest percentage of wide xylem conduits at the petiole. In addition, only in leaves of Galhosa did we detect a seasonal decrease in the Ψ_{π} , resulting in turgor maintenance during the dry season following leaf formation. These characteristics may contribute to increased drought tolerance in Galhosa. However, the seasonal pattern of leaf initiation evidenced a delay in the onset of leaf growth in Galhosa compare to the other cultivars. This might suggest that low temperature is a limiting factor particularly important in the case of Galhosa.

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Table 1

Characterisation of leaf growth in three cultivars of *Ceratonia siliqua* during the growth season of 1998. Values shown for the number of leaves produced per branch, the number of leaflets per leaf, and the full-expanded area of both leaves and leaflets, are means \pm standard error of measurements made on 6 branches. Results from one-way ANOVA are also shown as the F values for the effect of cultivar, and their probability level (P).

	Espargal	Galhosa	Mulata	F	P
leaves per branch	10.6 \pm 1.2	10.3 \pm 1.4	11.8 \pm 1.9	0.26	0.774
leaflets per leaf	8.6 \pm 0.2	7.2 \pm 0.1	7.7 \pm 0.1	22.6	<0.001
leaflet area (cm ²)	8.0 \pm 0.3	12.4 \pm 0.5	7.9 \pm 0.5	31.6	<0.001
leaf area (cm ² /leaf)	69.9 \pm 3.4	88.4 \pm 3.8	60.4 \pm 3.7	15.3	<0.001

Table 2

Xylem cross-sectional area (A_x), Huber value (HV), number of conduits per xylem cross-sectional area (N/A_x), theoretical maximum hydraulic conductance per unit path length (K_h) and leaf specific conductivity (LSC) determined at the petioles of three *C. siliqua* cultivars. Values shown are means \pm standard error of measurements made on 9 leaves. Results from one-way ANOVA are also shown as the F values for the effect of cultivar, and their probability level (P).

	Espargal	Galhosa	Mulata	F	P
A_x (mm ²)	1.04 \pm 0.06	1.50 \pm 0.12	1.02 \pm 0.07	10.6	<0.001
HV (10 ⁻⁴)	0.90 \pm 0.04	1.07 \pm 0.07	0.98 \pm 0.10	1.4	0.279
N/A_x (mm ⁻²)	291 \pm 22	174 \pm 10	222 \pm 13	12.6	<0.001
K_h (10 ⁻⁹ m ⁴ MPa ⁻¹ s ⁻¹)	3.16 \pm 0.44	5.25 \pm 0.96	2.42 \pm 0.31	6.5	0.007
LSC (10 ⁻⁷ m ² MPa ⁻¹ s ⁻¹)	2.77 \pm 0.37	3.57 \pm 0.33	2.34 \pm 0.35	3.0	0.072

¹⁾

Table 3

Summary of bulk leaf water relations parameters derived from pressure-volume analysis in leaves of three cultivars of *Ceratonia siliqua* sampled in July and September 1998. θ_a is the apoplastic water content at full turgor, $\Psi_{\pi 100}$ is the osmotic potential at full turgor, $\Psi_{t_{lp}}$, $\theta_{t_{lp}}$ and $F_{t_{lp}}$ are, respectively, the water potential, the relative water content and the symplastic water content determined at the turgor loss point, and E_{max} is the maximum bulk modulus of elasticity. Values shown are means \pm standard errors of measurements made on 5-6 leaves. Results of two-way analysis of variance (ANOVA) are also shown as the F-values and the probability level (P) for the effect of cultivar, time of sampling and their interaction.

	θ_a (%)		$\Psi_{\pi 100}$ (MPa)		$\Psi_{t_{lp}}$ (MPa)		$\theta_{t_{lp}}$ (%)		$F_{t_{lp}}$ (%)		E_{max} (MPa)	
July												
Espargal	24.5	± 1.5	-1.67	± 0.05	-1.80	± 0.05	94.5	± 0.2	92.7	± 0.3	30.3	± 2.6
Galhosa	27.2	± 2.1	-1.70	± 0.03	-1.82	± 0.03	95.4	± 0.1	93.6	± 0.2	36.2	± 1.5
Mulata	24.3	± 2.6	-1.65	± 0.04	-1.77	± 0.04	94.7	± 0.3	93.0	± 0.4	31.5	± 1.6
September												
Espargal	27.2	± 3.4	-1.75	± 0.05	-1.89	± 0.05	94.8	± 0.2	92.7	± 0.5	34.5	± 3.9
Galhosa	42.4	± 4.0	-1.82	± 0.02	-1.97	± 0.02	95.8	± 0.1	92.5	± 0.6	36.0	± 3.4
Mulata	27.0	± 3.1	-1.73	± 0.03	-1.86	± 0.04	95.2	± 0.4	93.4	± 0.8	40.5	± 5.1
ANOVA	F	(P)	F	(P)	F	(P)	F	(P)	F	(P)	F	(P)
Cultivar	6.1	(0.008)	2.3	(0.126)	2.2	(0.136)	7.4	(0.004)	0.4	(0.664)	1.0	(0.391)
Time	7.6	(0.012)	11.0	(0.003)	11.6	(0.003)	4.0	(0.059)	0.3	(0.608)	1.2	(0.295)
Interaction	2.9	(0.075)	0.1	(0.889)	0.4	(0.679)	0.1	(0.949)	1.1	(0.340)	0.6	(0.539)

Table 4

The osmotic potential of frozen-thawed leaf discs (Ψ_{π}) and the amount of osmotically active solutes per unit dry mass (N_s/DM) determined, at predawn and in the afternoon, in leaves of three cultivars of *Ceratonia siliqua* sampled in 23 July and 2 October 1998. Values shown are means \pm standard errors of measurements made on 4 leaves. Results of two-way analysis of variance (ANOVA) are shown as the F-values and the probability level (P) for the effects of cultivar, time of day and their interaction.

	Ψ_{π} (MPa)		N_s/DM (osmol kg ⁻¹)	
	July	October	July	October
Predawn				
Espargal	-1.46 \pm 0.06	-1.51 \pm 0.08	0.88 \pm 0.06	0.85 \pm 0.05
Galhosa	-1.55 \pm 0.01	-1.58 \pm 0.06	1.04 \pm 0.03	0.99 \pm 0.03
Mulata	-1.51 \pm 0.06	-1.52 \pm 0.06	1.00 \pm 0.06	0.97 \pm 0.01
Afternoon				
Espargal	-1.82 \pm 0.08	-1.72 \pm 0.13	0.88 \pm 0.07	0.89 \pm 0.06
Galhosa	-1.80 \pm 0.03	-1.69 \pm 0.06	1.05 \pm 0.05	0.97 \pm 0.04
Mulata	-1.89 \pm 0.13	-1.81 \pm 0.03	1.02 \pm 0.06	1.05 \pm 0.04
ANOVA	F (P)	F (P)	F (P)	F (P)
Cultivar	0.35 (0.711)	0.23 (0.797)	4.75 (0.022)	6.39 (0.008)
Time	31.51 (<0.001)	10.46 (0.005)	0.03 (0.858)	0.98 (0.335)
Interaction	0.55 (0.587)	0.69 (0.514)	0.02 (0.981)	0.97 (0.397)

Table 5

Two-way analysis of variance for leaf water potential (Ψ), osmotic potential (Ψ_{π}) and pressure potential (Ψ_p) determined, during the dry season of 1999 (from 12 May to 15 September), in leaves expanded during the previous year. The F-values for the effect of cultivar, time of season and their interaction are shown for the different parameters, together with their probability level (P).

	cultivar		time		interaction	
	F	P	F	P	F	P
Ψ predawn	5.12	0.009	27.66	<0.001	5.04	<0.001
Ψ midday	1.07	0.351	6.41	<0.001	1.57	0.173
Ψ_{π} midday	12.19	<0.001	3.87	0.013	1.13	0.359
Ψ_p midday	7.87	<0.001	4.21	0.009	2.56	0.028

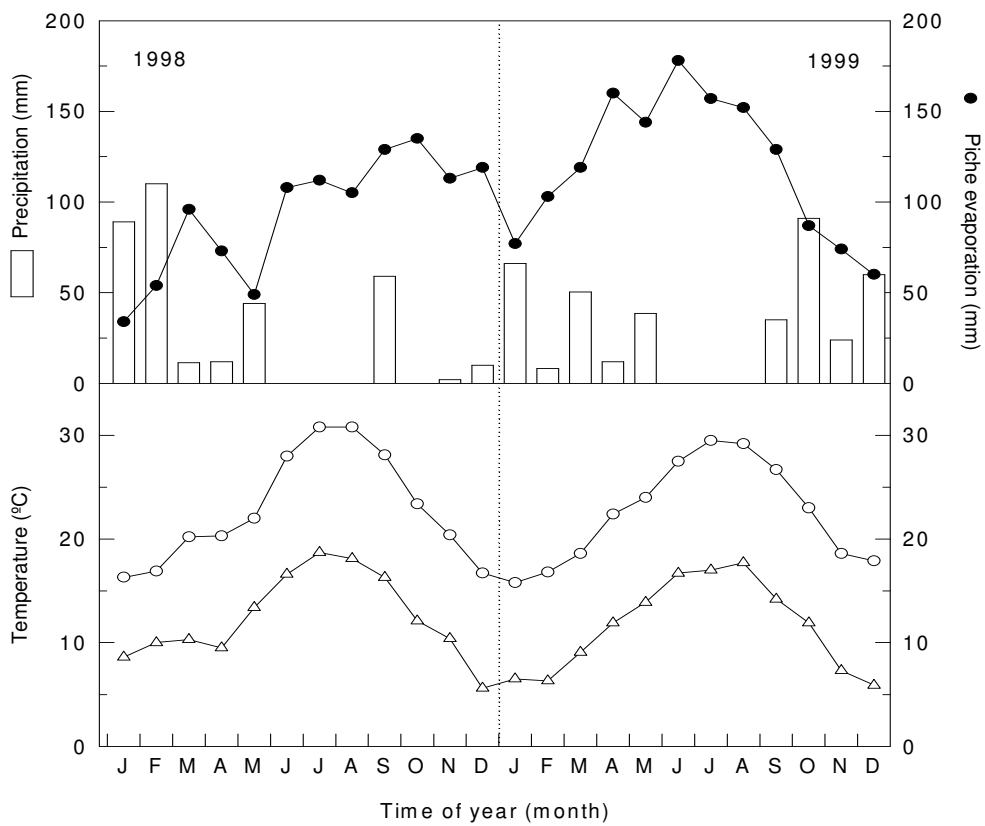


Figure 1

Monthly accumulated precipitation (bars) and piche evaporation (closed circles), monthly average minimum (open triangles) and maximum (open circles) temperatures obtained from a weather station located nearby (5 km) the study site.

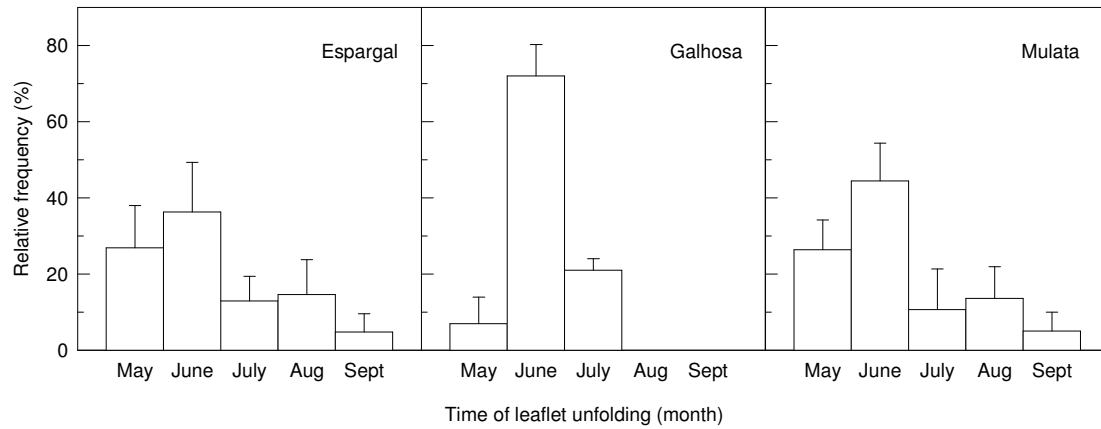


Figure 2

Seasonal variation in the relative frequency of leaf emergence, taken as the time of leaflet unfolding, determined during the growth season of 1998 in three cultivars of *C. siliqua*. Values shown are means \pm standard error of measurements made on 6 branches.

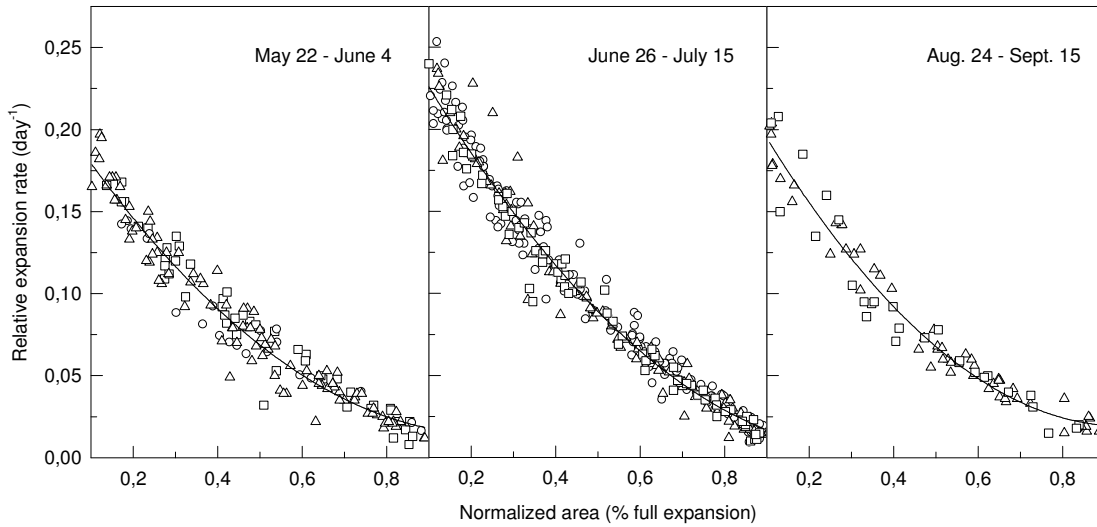


Figure 3

Plots of mean relative expansion rate against leaflet area normalized with respect to final area. Values shown were determined in 1998 during spring (left panel), beginning of summer (central panel) and end of summer (right panel). Each point represents measurements done, at one week interval, on individual leaflets of three cultivars of *C. siliqua*: Espargal (triangles), Galhosa (circles) and Mulata (squares). The lines were fitted by second order regression analysis: $y=0.216-0.389x+0.187x^2$, $r^2=0.950$ (spring), $y=0.270-0.463x+0.202x^2$, $r^2=0.974$ (beginning of summer) and $y=0.239-0.465x+0.246x^2$, $r^2=0.964$ (end of summer).

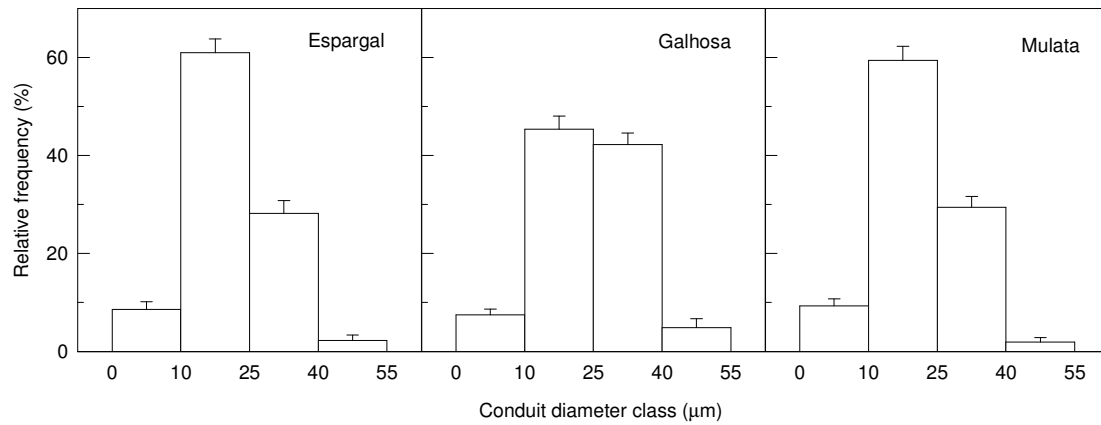


Figure 4

Relative frequency of xylem conduits of different diameter class on petiole sections from three cultivars of *C. siliqua*. Values shown are means \pm standard error of measurements made on 9 leaves.

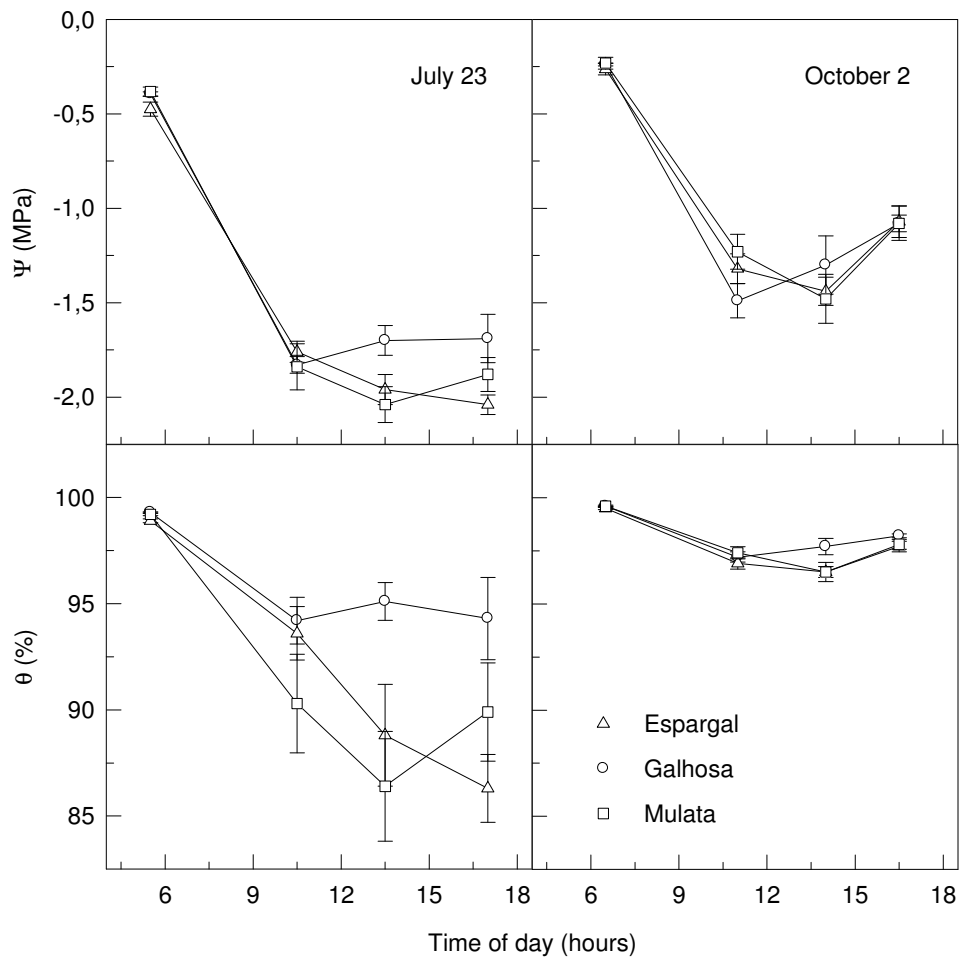


Figure 5

Diurnal time-course of leaf water potential (Ψ) and relative water content (θ) determined during midsummer (left panels) and one week following the first rainfall event (right panels) in three cultivars of *Ceratonia siliqua*: Espargal (triangles), Galhosa (circles) and Mulata (squares). Values shown are means \pm standard error of measurements made on 4 leaves.

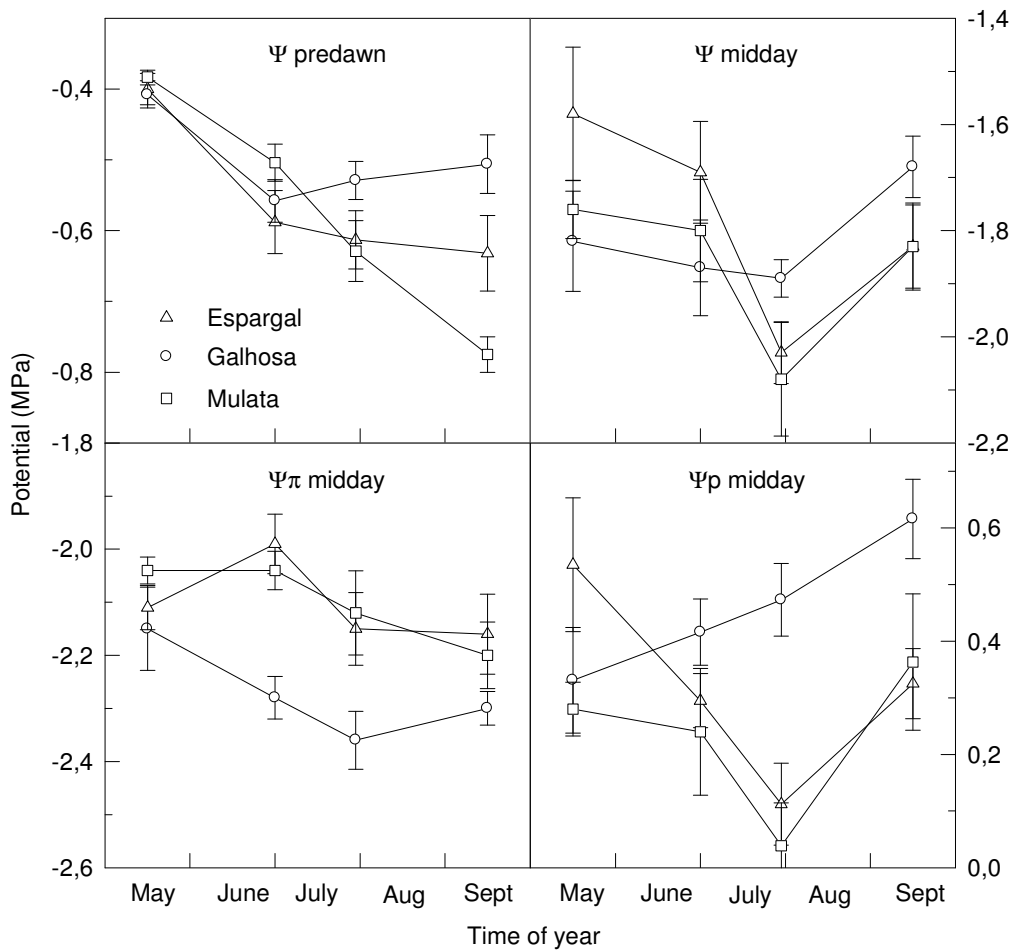


Figure 6

Evolution of predawn leaf water potential (Ψ) and midday leaf water potential (Ψ), osmotic potential (Ψ_{π}) and pressure potential (Ψ_p) determined during the dry season of 1999 in field-grown trees of three cultivars of *Ceratonia siliqua*: Espargal (triangles), Galhosa (circles) and Mulata (squares). Values shown are means \pm standard error of measurements made 6 leaves formed in the previous year.