

## Water uptake dynamics, photosynthesis and water use efficiency in field-grown *Medicago arborea* and *Medicago citrina* under prolonged Mediterranean drought conditions

By E LEFI, H MEDRANO and J CIFRE\*

Departament de Biologia, Escola Universitària d'Enginyeria Tècnica Agrícola, Universitat de les Illes Balears, C/ Valldemossa km 7.5, 07122 Palma de Mallorca, Spain

(Accepted 22 March 2004; Received 12 June 2003)

### Summary

Drought is the main limiting factor of forage production in semi-arid areas. Annual variation of soil water availability (SWA), predawn leaf water potential ( $\Psi_{pd}$ ), stomatal conductance ( $g$ ), photosynthesis rate ( $A$ ) and the intrinsic water use efficiency ( $A/g$ ) were examined under irrigation (control) and rain fed conditions in Palma de Mallorca (Spain) for two Mediterranean shrubs: *Medicago arborea* (*M. arborea*), with a wide distribution and *Medicago citrina* (*M. citrina*), an endemic species to the Balearic islands. Experimental conditions were typically Mediterranean, with low annual precipitation.

In well-irrigated plants, SWA and  $\Psi_{pd}$  were similar for both species throughout the year but higher  $g$  was observed in autumn and in spring-summer. The photosynthesis rate was slightly higher in autumn and spring. Even though photosynthetic activity in *M. arborea* was higher than in *M. citrina* in spring,  $A/g$  was relatively higher for *M. citrina* for most of the year due to a lower transpiration rate.

Under rain fed conditions, SWA dropped after February from 70% to 10-20%. Variations in plant water status,  $g$  and  $A$  were mainly dependent on SWA. Moreover, differences for water extraction and water use efficiency between species were found. Under moderate drought (SWA > 60%),  $g$  was more severely reduced than  $A$  and  $\Psi_{pd}$  (October-March), as a consequence,  $A/g$  increased. Stomatal conductance variations for both species were more closely related to soil moisture rather than to leaf water status. Interestingly, *M. citrina* desiccated the soil more gradually and extracted water at deeper horizons (between 0.5 and 1 m). As a consequence, it maintained relatively higher  $\Psi_{pd}$ ,  $g$  and  $A$  than *M. arborea* throughout spring. For *M. arborea*, SWA declined more rapidly and leaf shedding in spring was much more substantial. When subjected to severe and prolonged summer drought (SWA < 50%), all parameters were severely reduced for both species and  $A$  had values near zero. Consequently,  $A/g$  was low and the senescence of the majority of leaves for *M. arborea* took place. On the basis of these results, *M. citrina* exhibits a higher water use efficiency under moderate drought.

**Key words:** Soil water availability, water potential, stomatal control, drought, *Medicago* shrubs

### Introduction

The Mediterranean climate is characterised by a dry summer with high temperatures and evapotranspiration (Hamdy & Lacirignola, 1999) and precipitation commonly concentrated in autumn-spring but largely unpredictable in amount and spatiotemporal distribution (Joffre *et al.*, 2001). Hence, the vegetation is affected by drastic seasonal variations of soil water availability (Rana & Katerji, 2000).

Drought stress strongly affects photosynthesis, growth and survival of plants growing in Mediterranean climates (Haase *et al.*, 2000; Chaves *et al.*, 2002). Under these conditions, the soil water deficit and air temperature are the main limiting factors that seriously reduce forage production and its seasonal availability (Papanastasis *et al.*, 1997;

Medrano *et al.*, 1998).

Several studies have demonstrated that Mediterranean legume shrubs may be promising forage crops (Correal *et al.*, 1988; Papanastasis *et al.*, 1997). The reasons for this enable: a deep root system with, a high soil water uptake; a capacity for integration in agro-forestry systems (González-Andrés & Ortiz, 1997; Papanastasis *et al.*, 1997); soil protection (Andreu *et al.*, 1998a,b); soil fertility improvement (Gimeno-García *et al.*, 2000; Wezel *et al.*, 2000) and adaptation to drought (Le Houérou, 1989).

Under dry environmental conditions, plants develop different mechanisms to resist and survive unpredictable rains. These mechanisms are commonly based on morphological and physiological responses that delay the water deficit (Joffre & Rambal, 1999). When the superficial

\*Corresponding Author E-mail: pep.cifre@uib.es

horizons do not assure a flow of water between the soil and the plant, the water lost by transpiration reduces the water potential and the extraction moves towards deeper layers (Joffre *et al.*, 2001). A deeper root system has been mentioned as a mechanism of adaptation to seasonal changes of precipitation in different Mediterranean species, and the promotion of root growth of several species improves water extraction (Blum & Sullivan, 1997; Volaire *et al.*, 1998; Chaves *et al.*, 2002). The work of Rambal (1984) and Chebbi *et al.* (1994) has emphasised the importance of this character as a drought-adaptation mechanism in *Quercus coccifera* and *Medicago* shrubs. Under drought conditions, changes in the root/shoot ratio favoring root growth in depth and/or density, contribute to improving carbon and nitrogen assimilation (Chaves *et al.*, 2002). In addition, stomatal control of water losses has been identified as an early event in plant response to water deficit under field conditions and a limitation to carbon uptake by the leaves (Chaves, 1991).

Seasonal variation in photosynthesis for Mediterranean shrubs and trees under drought stress has been previously reported (Haase *et al.*, 2000; Flexas *et al.*, 2001; Chaves *et al.*, 2002). Maximal net photosynthesis rate and stomatal conductance were observed in autumn and spring, while the lowest values were observed in winter and summer. In response to soil drying, stomatal conductance is reduced before any substantial change in water potential (Tardieu *et al.*, 1991; Socias *et al.*, 1997). However, because plant responses to stress are complex (Chaves *et al.*, 2003), the relationships among drought tolerance, stomatal sensitivity, root depth and soil water availability are not clear.

Very little is known about the responses of *Medicago* shrubs to variations in soil water availability and their drought-adaptation mechanisms under Mediterranean conditions. Chebbi *et al.* (1994) reported that *M. citrina* was more resistant to drought than *M. arborea*. The objective of this work was to examine the plant water status and the gas exchange parameters of *M. arborea* and *M. citrina* under irrigation and rain fed conditions in a field experiment. The relationships between these parameters and some related drought-adaptation mechanisms throughout the year are also discussed.

## Material and Methods

### *Plant material*

The experimental field was located at the University of the Balearic Islands in Spain (39°34'N; 2°40'E and 28 m of altitude). Seeds of *M. arborea* from Mallorca island (Spain) and *M. citrina* from Cabrera island (near Mallorca) were germinated under controlled conditions and then transplanted

into small pots. Seedlings were grown in a glasshouse (12 h per day of light), irrigated with Hoagland's solution for 1 month and then transplanted to the experimental field in October 1998. The soil type was characterised by 24% v/v of field capacity, 12.8% of permanent wilting point and a pH of 7.6. The clay contents at 30, 60, and 90 cm of depth were 22.4%, 37% and 39% respectively. The plantation was made on two adjacent plots, separated by 35 m. One remained under irrigation, the other one for rain fed treatment. Inside each plot the plants of *M. arborea* and *M. citrina* were distributed on two adjacent subplots, planted in rows with a mark 1.5 m/1.5 m between rows and plants. During the first year of growth, all plots remained under a drip irrigation system (two drippers per plant).

### *Treatments and measurements*

All measurements were made on two year-old plants (from October 1999 to September 2000). During the second year, one plot was maintained under a permanent irrigation regime in order to maintain the soil moisture near the field capacity, while the other plot was kept under rain fed conditions. The applied treatments were: *M. arborea* and *M. citrina* irrigated and rain fed.

Soil moisture was measured in four locations per treatment, from tubes placed inside the row at 0.5 m from the neighbouring plant (1 m from the next one), every 2 wks during the year, at two horizons (among 0 and 0.5 m and among 0.5 and 1 m of depth) with TDR equipment (Time Domain-Reflectometry, TRIME®-SYSTEM). Measurements were made 2 days after irrigation. Soil water availability (SWA) was calculated after calibration of TDR values with respect to measurements of soil moisture, taking into account the soil water content at the permanent wilting point (13%). Meteorological data was collected by the National Meteorological Institute of Palma de Mallorca.

Leaf water potential ( $\Psi_{pd}$ ) was measured monthly at predawn on the most recent fully expanded leaves (4<sup>th</sup> or 5<sup>th</sup> leaf on the stem at the top of the plant canopy) using a Schölander pressure chamber (Soil Moisture Equipment Corp., CA, USA), following Fais (1983). The gas exchange parameters were measured in intact leaflets using an IRGA Li-6200 portable photosynthesis system (Li-cor Inc., Lincoln, NE, USA) with a 1/4 litre chamber in the closed circuit mode. These measurements were made at mid-morning on sunny days, when the photosynthetic photon flux density ranged between 1700 and 2100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . These measurements began in October 1999 and were repeated every 45 days in autumn, winter and spring, and monthly in the summer. Water use efficiency was estimated as intrinsic water use efficiency A/g (A, net

photosynthesis rate  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ;  $g$ , stomatal conductance  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ).

#### Statistical analysis

The data obtained during the comparative measurements of irrigated and rain fed plants was submitted to the analysis of variance (ANOVA) with a factorial fixed model taking into account the effects of species and treatment (rain fed or irrigated). The models were analysed using the SPSS statistical package (Anon., 1990). Regression analysis and adjustment between variables (Fig 7, 8 and 9) were solved using Sigma Plot 8.0 statistical package (Anon, 1993).

### Results

#### Weather conditions

The period between October 1999 and September 2000 was characterised by a low annual rainfall (229.7 mm, 49% below the mean annual rainfall) and 74% of it fell in autumn and winter (Fig. 1). Monthly average minimum temperatures of the cold months were recorded in January (7°C) and February (9°C), and maximum ones were recorded in July (29°C) and August (30.8°C). The driest seasons were spring and summer with low rainfall and high evapotranspiration (values around 8.8 and 8.5 mm in June and July respectively, Fig. 1).

#### Soil water availability

In well-irrigated plants, SWA at 0.5 and 1 m depth was maintained around 90% between October and June, then, higher transpiration rates in summer decreased it to values around 75% (Fig. 2a & 2b). Throughout the experiment there were no significant differences between *M. arborea* and *M. citrina* for SWA and the soil moisture was always maintained over 70%. Under rain fed conditions SWA decreased significantly for *M. arborea* and *M. citrina* ( $P < 0.001$ ). It remained at 45% in spring and dropped to 20% in summer (Fig. 2a & 2b). However, the pattern of soil drying throughout the dry period was different between species. For *M. arborea*, SWA decreased between February and March from 76% to 53% for the first and the second horizon; then it fell to 39% and 48% in April- June, decreased to 28% in July and maintained around 20% in summer. For *M. citrina*, SWA decreased significantly ( $P < 0.001$ ) and gradually between February and July (Fig. 2b): it decreased from 77% to 28% for the first horizon and from 78% to 20% for the second one; then SWA was maintained around 20%. In contrast to *M. arborea*, *M. citrina* roots extracted more water from the deeper horizon than the superficial one from March to September, with maximum differences between them around 18% and 24% in May and June respectively.

#### Leaf water potential

Under irrigated conditions, plant water status, as measured by predawn leaf water potential ( $\Psi_{pd}$ ), showed no significant differences between species throughout the year. Means varied between  $-0.23$  MPa and  $-0.44$  MPa for both species (Fig. 3). Summer reductions in SWA under high temperature and evapotranspiration (Fig. 1 and 2) did not reduce  $\Psi_{pd}$  at predawn.

Under rain fed conditions, water stress was absent during autumn and winter in spite of some SWA reductions in February (Fig. 2a & 2b). During March, the soil water deficit effects were clear, thus  $\Psi_{pd}$  dropped to  $-0.96$  MPa for *M. arborea* and  $-0.68$  MPa for *M. citrina*. In addition, differences between species for  $\Psi_{pd}$  were observed ( $P < 0.001$ ). The most significant reduction was observed in April and May, leading to  $-3.35$  MPa for *M. arborea* and  $-2.54$  MPa for *M. citrina*. These decreases coincided with maintenance of SWA between 40% and 30% for *M. arborea* (Fig. 2a). In summer,  $\Psi_{pd}$  was below  $-4$  MPa for *M. arborea* and  $-3.66$  MPa for *M. citrina*. In general, throughout the dry period,  $\Psi_{pd}$  decreased continuously for both species, but *M. citrina* always maintained higher values than *M. arborea* ( $P < 0.001$ ). In addition, throughout the entire experiment, plant water status changed with SWA for *M. citrina* ( $r = 0.89$  at 0.5 m and  $r = 0.85$  at 1 m of depth,  $P < 0.001$ ) and *M. arborea* ( $r = 0.78$  at 0.5 m and  $r = 0.88$  at 1 m of depth,  $P < 0.001$ ). Since July, considerable leaf senescence was induced in *M. arborea*, but *M. citrina* maintained a major fraction of leaf biomass. Late summer rainfall enabled the recovery of plant water status in *M. citrina*.

#### Net photosynthesis rate and stomatal conductance

In well-irrigated shrubs, stomatal conductance decreased from around  $350 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in autumn to  $150 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in winter in coincidence with low temperatures (Fig. 4). From winter to summer, *M. arborea* increased  $g$  to  $550 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  but *M. citrina* remained around  $250 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  during the same period, with an increase at the beginning of autumn to  $471 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . For most of the time, *M. arborea* maintained significantly higher  $g$  values than *M. citrina* in irrigated plants (Fig. 4).

When subjected to soil water deficit, reductions of  $g$  were observed, beginning in February for both species ( $P < 0.001$ ) and reaching 44% and 33% for *M. arborea* and *M. citrina* respectively, in March. In April,  $g$  strongly decreased for both species, in coincidence with reductions in  $\Psi_{pd}$ , progressive reductions in SWA for *M. citrina* and maintenance of SWA for *M. arborea*. In summer,  $g$  was maintained around  $70 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , thus at low SWA and  $\Psi_{pd}$  there were no significant differences between species (Fig. 4). Compared to the control,

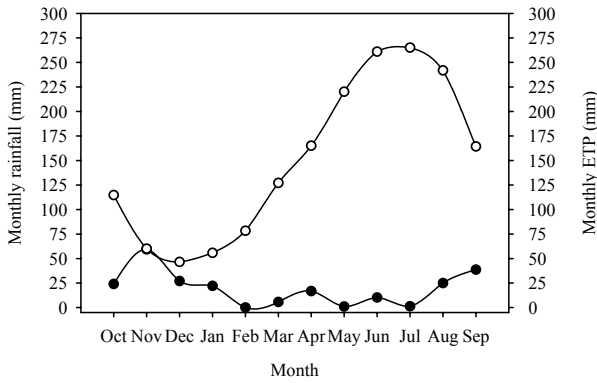


Fig. 1. Seasonal variation in monthly rainfall (●) and monthly evapotranspiration (○).

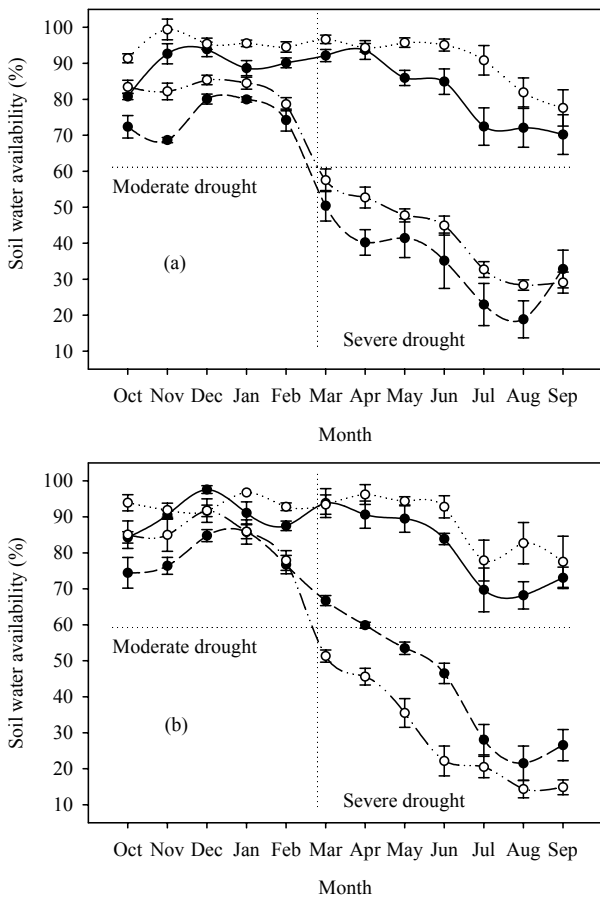


Fig. 2. Seasonal variation in soil water availability (SWA) for *Medicago arborea* (a) and *Medicago citrina* (b) under irrigated regime at 0.5 (—) and 1 m (····) of depth, and under rain fed conditions at 0.5 (---) and 1 m (----) of depth. Each value is the average of four replicates and vertical bars represent standard errors.

the average reductions of *g* in spring and in summer were respectively 69% and 83% for *M. arborea* and 48% and 71% for *M. citrina*.

The maximum *A* for *M. arborea* and *M. citrina* under irrigation was recorded in autumn and spring, and minimum values were observed in winter and summer (Fig. 5). The differences between species and seasons were significant ( $P < 0.001$ ) in some

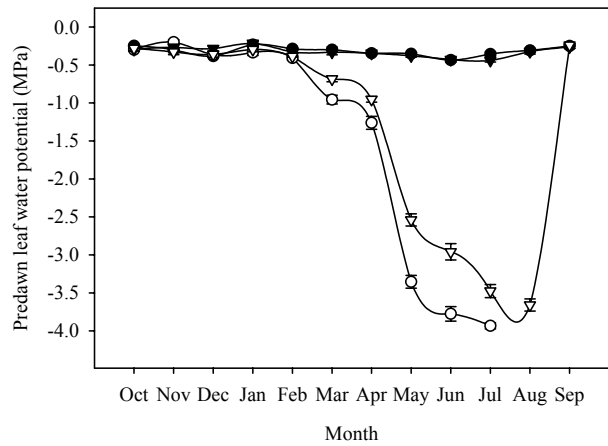


Fig. 3. Seasonal variation in predawn leaf water potential for irrigated (●) and rainfed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical bars represent standard errors.

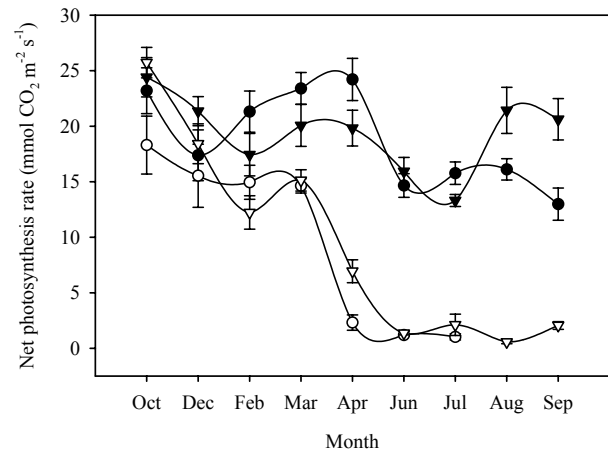


Fig. 4. Seasonal variation in stomatal conductance (*g*) for irrigated (●) and rain fed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical bars represent standard errors.

months. Under low temperatures, *A* reached values around 18  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , then increased during spring to reach 25.5  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for *M. arborea* and 21  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for *M. citrina* (Fig. 5). In summer, *A* remained at 15  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for both *M. arborea* and *M. citrina*. Afterwards, higher *A* values were recorded for *M. citrina* than for *M. arborea* at the end of summer (August and September).

Under soil water deficit and in March, reductions in *A* were 45% for *M. arborea* and 30% for *M. citrina*. Again, in April *A* was severely reduced for both species, but higher values were maintained for *M. citrina* (7  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) than *M. arborea* (2.3  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Similarly as for *g*, *A* did not show significant differences between species at the lowest SWA and  $\Psi_{pd}$  values. During spring, high *g* reductions coincided with the step SWA depletion.

In contrast  $A$  was more reduced than  $g$  in summer.

#### Intrinsic water use efficiency

The intrinsic water use efficiency ( $A/g$ ) at different sampling times exhibited significant differences between species ( $P < 0.001$ ), seasons ( $P < 0.001$ ) and treatments ( $P < 0.05$ ). In well-irrigated shrubs, maximum values were observed in winter and spring (between 100 and 125  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ), but the lowest were in autumn and summer (Fig. 6). For most of the sampling times, *M. citrina* was more efficient in water use than *M. arborea* because it maintained lowest stomatal conductance. Moderate soil water deficit, between February and April, induced increases in  $A/g$ . During this period, *M. citrina* maintained higher  $A/g$  than *M. arborea*, but with lower SWA in summer,  $A/g$  fell to around 15  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$  for both species. Under these conditions, stressed plants maintained lower  $A/g$  than well-watered ones (Fig. 6). For all seasons,  $A/g$  was dependent on SWA ( $r = 0.75$ ,  $P < 0.001$ ) and  $\Psi_{pd}$  ( $r = 0.61$ ,  $P < 0.001$ ) for *M. citrina* and for *M. arborea* ( $r = 0.50$  and  $r = 0.53$  respectively,  $P < 0.01$ ).

### Discussion

The experiment was characterised by large variations of climatic conditions in rainfall, temperatures and evapotranspiration. Prolonged drought (almost 7 months at SWA below 60%) accompanied by high irradiance, temperature and evapotranspiration led to a broad reduction in all measured parameters in spring and summer.

#### General pattern of stomatal conductance and photosynthesis without water stress

In well-irrigated shrubs, SWA (at 0.5 and 1 m depth) and  $\Psi_{pd}$  were almost the same for both species

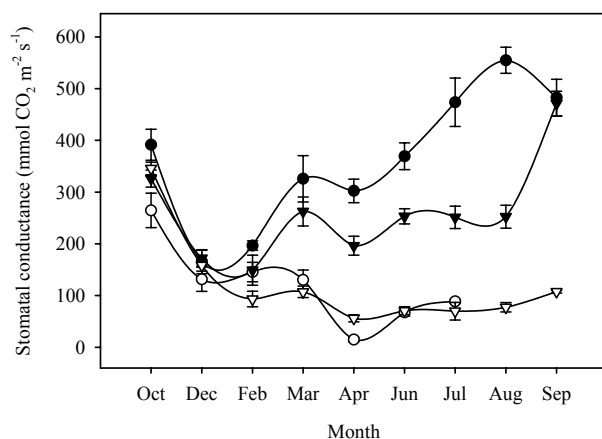


Fig. 5. Seasonal variation in the net photosynthesis rate ( $A$ ) for irrigated (●) and rain fed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical bars represent standard errors.

during the year. However, there were significant differences between seasons and species for stomatal conductance, photosynthesis and water use efficiency. In winter stomatal conductance was severely reduced. However, in spring and summer,  $g$  increased in coincidence with higher irradiance, temperature and evapotranspiration. This increase was more pronounced in *M. arborea* than in *M. citrina*. The difference in  $g$  between species is not explained by SWA nor  $\Psi_{pd}$ . It should be noticed, however that plant leaf area remained around 5.5  $\text{m}^2 \text{ plant}^{-1}$  for *M. citrina* but only 2.5  $\text{m}^2 \text{ plant}^{-1}$  in *M. arborea* (Lefi *et al.*, 2004). Thus even if the reduction in SWA did not induce stress, the high summer daily evaporative demand could lead to a higher leaf water

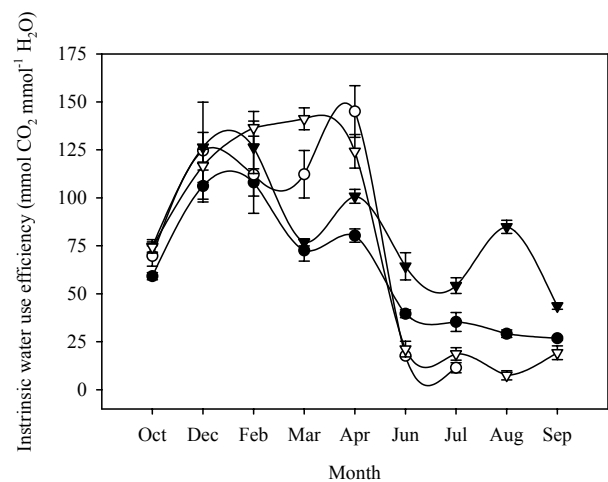


Fig. 6. Seasonal variation in intrinsic water use efficiency for irrigated (●) and rain fed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical bars represent standard errors.

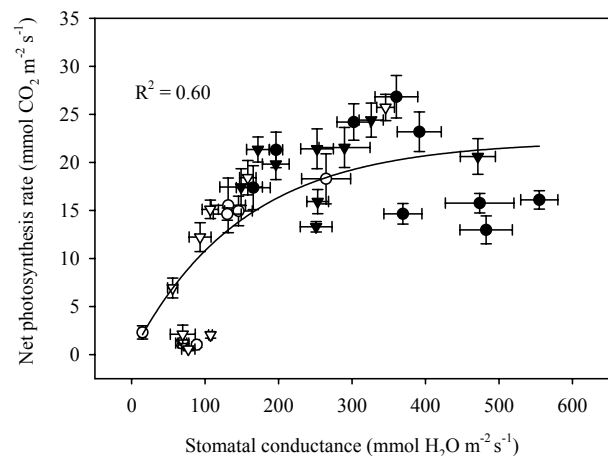


Fig. 7. Relations between stomatal conductance ( $g$ ) and the net photosynthesis rate ( $A$ ) for irrigated (●) and rain fed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical and horizontal bars represent standard errors.

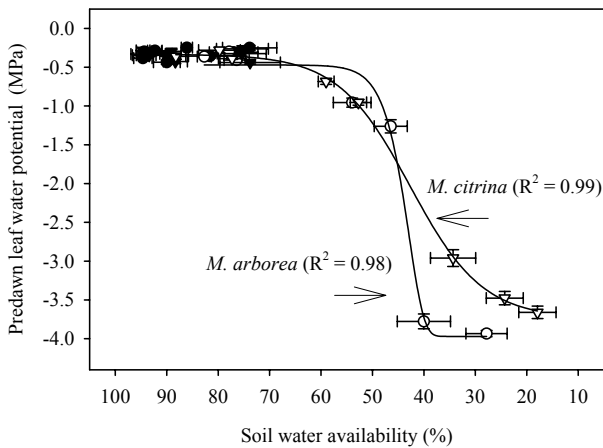


Fig. 8. Relations between soil water availability and predawn leaf water potential for irrigated (●) and rain fed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical and horizontal bars represent standard errors.

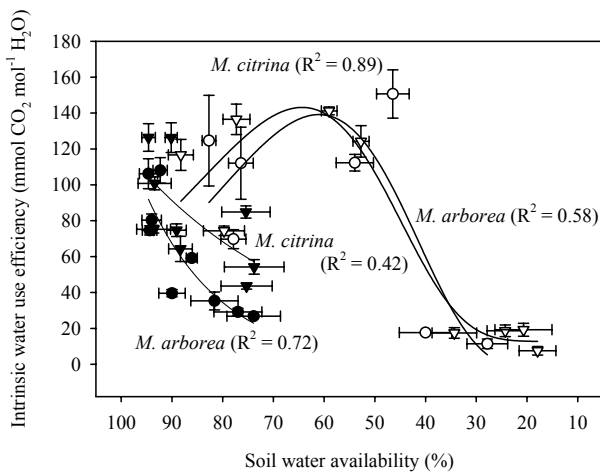


Fig. 9. Relations between soil water availability and intrinsic water use efficiency for irrigated (●) and rain fed (○) *Medicago arborea* plants and irrigated (▼) and rain fed (▽) *Medicago citrina* plants. Each value is the average of six replicates and vertical and horizontal bars represent standard errors.

deficit in *M. citrina* than in *M. arborea* around midmorning (sampling time). In fact, the  $g$  increase for September sampling is coincident with an important reduction of plant leaf area to  $3.5 \text{ m}^2 \text{ plant}^{-1}$  in *M. citrina* (leaf senescence), at the time when there was an increase to similar plant leaf area values for *M. arborea* (Lefi *et al.*, 2004).

Such summer increases of  $g$  in *M. arborea* did not correspond with increases in  $A$  but with important decreases, obviously not induced by soil water deficit. At the end of summer,  $A$  was higher in irrigated *M. citrina* plants as compared with *M. arborea*. This could be related to some defoliation of *M. citrina*, which would improve the nitrogen status of young (measured) leaves (Lefi *et al.*, 2004). The crucial role of reproductive development at a

time of generalised leaf senescence could explain such reductions in  $A$  (Pic *et al.*, 2002). In addition, changes in *M. citrina* leaf characteristics, such as thickness, breakable and yellowish blade (data not shown) could also explain the reductions in  $A$  (under irrigation) in summer. A recent study of endemic species in the Balearic Islands showed a reduced photosynthetic capacity of endemics, with respect to widespread species, associated with lower specific leaf area and photosynthetic nitrogen-use efficiency (Gulías *et al.*, 2003).

Differences between species for  $g$  and  $A$  coincided with the reported differences for morphological characters (Robledo *et al.*, 1993; Chebbi *et al.*, 1994, 1995). During the experiment, *M. citrina* maintained lower and more stable  $g$  than *M. arborea*. Moreover, large seasonal variations in temperature (between  $11^\circ\text{C}$  and  $27^\circ\text{C}$ ) caused slight variations in the photosynthesis rates, for both species. This can be an important characteristic to favor plant growth under mild winter conditions, when soil water availability is usually not limiting.

After the autumn-winter months, a progressive decline in intrinsic water use efficiency ( $A/g$ ) was observed in well-irrigated plants in relation to  $g$  increases. *M. citrina* always maintained higher instantaneous water use efficiency (Fig. 6 and 9) than *M. arborea*. This is in accordance with the data obtained from previous experiments in pots, in which *M. arborea* performed less efficiently in water use than *M. citrina* and *Medicago strasseri* (*M. strasseri*) during plant establishment (Lefi *et al.*, 2003).

Under adequate water supply, and for both species, stomatal conductance was more dependent than  $A$  on irradiance, temperature and evapotranspiration. Consequently, in winter and spring these two species achieved a high intrinsic water use efficiency. Under these conditions, but also during the rest of the year, *M. citrina* had a greater water use efficiency.

#### Responses to soil water deficit

Very little has been published concerning the responses of *M. arborea* and *M. citrina* to drought stress. A general pattern of SWA extraction showed two different phases during the experiment (Fig. 2a & 2b): moderate drought (SWA > 60%) between October and March and severe drought (SWA < 50%) between April and September. For the first phase, *M. citrina* exhibited a great capacity to use water at deeper horizons (> 0.5 m). Several authors have shown that *M. citrina* has a more developed root system than *M. arborea* and *M. strasseri* (Robledo *et al.*, 1993; Chebbi *et al.*, 1994), which allows the roots to explore into deeper horizons for greater soil water extraction. In a second phase, progressive reductions in SWA for *M. citrina* enabled the plant to maintain smaller reductions in leaf water potential during spring and summer and higher

photosynthesis in spring, compared to *M. arborea*.

The capacity of *M. citrina* roots to extract soil water from deep horizons confirms its ability to survive on a small rocky island (Cabrera, Balearic Islands, Spain) under a low annual rainfall (between 193 and 555 mm) and Columbretes Islands (Spain) with 265 mm, its second area of distribution. The data of Sponchiado *et al.* (1989) then indicated that in drying soils, the rapid vertical penetration of roots and the greater maximum rooting depth increased the access to deep soil water. Moreover, Blum & Sullivan (1997) showed that stress conditions increased total root length in wheat. Thus, under prolonged Mediterranean summer drought, the deep root system and the greater water uptake at depth, are traits strongly associated with the superior survival of perennial forage grasses (Volaire *et al.*, 1998). Rambal (1984) also emphasised the importance of this character as a drought-adaptation mechanism of *Quercus coccifera*. Such a response is a typical drought-avoidance mechanism (Levitt, 1972; Ludlow, 1989) under Mediterranean climates.

Between October and March, with a slight soil water deficit, high reductions in stomatal conductance (71%) and relatively moderate reductions in photosynthesis (52%) occurred before any changes in leaf water potential took place (Fig. 7 and Fig. 8). These reductions were similar for both species and treatments (Fig. 4) most likely in response to the common limiting factor, the low winter temperatures.

As soil water depletion progressed,  $g$  dropped to lower values than when under irrigation. Several authors have indicated that when the soil is drying, some chemical signals, such as abscisic acid (ABA) generated by dehydrating roots, are transmitted by the xylem to the plant shoot in order to control the stomatal conductance and leaf growth (Saab & Sharp, 1989; Gowing *et al.*, 1990; McDonald & Davies, 1996; Munns *et al.*, 2000), without changes in plant water status. Our results agreed with various studies on clover (Socias *et al.*, 1997), maize (Tardieu *et al.*, 1991) and wheat (Blum & Sullivan, 1997), that stomatal responses are more closely related to soil moisture than to leaf water status.

Under moderate drought, stomatal conductance was more affected than photosynthesis; consequently, water use efficiency increased for both species in spring (Fig. 6). The first and important drop of  $g$  corresponded with small reductions in  $A$  (Fig. 7) and increasing water use efficiency (WUE), which is similar to the pattern established for other Mediterranean species such as *Trifolium subterraneum* (Vadell *et al.*, 1995), *Pistacia lentiscus* L. (Granati, 1995; Gulías *et al.*, 2002), *Cistus albidus*, *Cistus montpelienis* and *Olea europaea* (Gulías *et al.*, 2002).

When subjected to severe drought (SWA < 50%,

Fig. 7), lower SWA coincided with high irradiance, temperature and evapotranspiration; consequently, the plant water status was severely affected in both species. The  $g$  reductions were then steeper and photosynthesis rates declined to practically zero. Decreases in  $g$  from  $150 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  to around  $70 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  (Fig. 7) corresponded to the important reduction in SWA and  $\Psi_{pd}$  following the model of Flexas & Medrano (2002). These  $A$  reductions corresponding to the  $g$  ones are similar to those reported for other species, such as *Vitis vinifera* (Flexas *et al.*, 2002) *Pistacia lentiscus* (Flexas *et al.*, 2001) and *Anthyllis cytisoides* L. (Haase *et al.*, 2000).

As these data support, moderate water stress response is quite similar for both species and relatively similar to evergreen shrubs, such as *Pistacia lentiscus* (Flexas *et al.*, 2001), summer deciduous shrubs as *Anthyllis cytisoides* (Haase *et al.*, 2000) and evergreen trees as *Quercus suber* (Chaves *et al.*, 2002) under Mediterranean climates.

However, under severe drought conditions (SWA < 50%), the  $A$  drop is steeper (Fig. 7). Consequently, the water use efficiency decreased (Fig. 9). Under these conditions, the advantage of deeper horizon water use was not reflected in an improvement of stomatal conductance and photosynthesis. However, in general terms,  $A/g$  was highly dependant on SWA, especially under drought. Haase *et al.* (2000) have shown that instantaneous water use efficiency practically equals zero at predawn water potentials below  $-3.5 \text{ MPa}$  in the deciduous shrub, *Anthyllis cytisoides* in the semi-arid southern Spain.

A large reduction in  $A/g$  was accompanied in summer with a greater leaf senescence in *M. arborea* than *M. citrina*. The summer-deciduous or semi-deciduous leaf habit, under Mediterranean climates, improved the survival of these species for prolonged and severe drought by eliminating transpiring leaf surface.

## Conclusions

The soil water deficit affected the plant water status and gas exchange parameters for both species. Stomatal closure followed the variations in soil water availability. Moreover, differences between species have been found for plant access to soil water at deeper horizons and water use efficiency. *M. citrina* exhibited a better capacity to extract water at deeper horizons and to maintain a relatively higher water use efficiency than *M. arborea*, thus representing a promising forage shrub species for Mediterranean conditions.

## References

- Andreu V, Rubio J L, Cerni R. 1998a. Effects of Mediterranean shrub cover on water erosion (Valencia, Spain). *Journal of Soil Water Conservation* **53**:112-120.

- Andreu V, Rubio J L, Gimeno-García E, Llinares J V. 1998b.** Testing three Mediterranean shrub species in run off reduction and sediment transport. *Soil Tillage Research* **45**:441-454.
- Anon. 1990.** *SPSS® Base system user's guide*. Chicago, USA: SPSS Inc.. 949 pp.
- Anon. 1993.** *Sigma Plot scientific graphic software. Transforms and curve fitting*. Germany: Jandel Scientific.
- Blum A, Sullivan C Y. 1997.** The effect of size on wheat responses to agents of drought stress. I. Root drying. *Australian Journal of Plant Physiology* **24**:35-41.
- Chaves M M, Maroco J P, Pereira J S. 2003.** Understanding plant responses to drought—from genes to the whole plant. *Functional Plant Biology* **30**:239-264.
- Chaves M M, Pereira J S, Maroco J P, Rodrigues M L, Ricardo C P P, Osório M L, Carvalho I, Faria T, Pinheiro C. 2002.** How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany* **89**:907-916.
- Chaves M M. 1991.** Effect of water deficits on carbon assimilation. *Journal of Experimental Botany* **42**:1-16.
- Chebbi H, Pascual-Villalobos M J, Cenis J L, Correal E. 1995.** Caractérisation morphologique et moléculaire des espèces ligneuses du genre *Medicago*. *Fourrages* **142**:191-206.
- Chebbi H, Ríos S, Pascual-Villalobos M J, Correal E. 1994.** El grupo *Medicago arborea* en la cuenca Mediterránea: II. Comportamiento frente a la sequía. *Pastos* **24**:177-188.
- Correal E, Sánchez-Gomez P, Alcaraz F. 1988.** Woody species (trees and shrubs) of multiple value for the arid and semi-arid zones of northern Mediterranean EEC countries. In *Agriculture, Programme de recherche agrimed, commission des Communautés Européennes*, EUR 11770 FR-EN-DE pp. 1-39.
- Fais S M A. 1983.** Use of pressure bomb in the determination of soil water potential. *Plant and Soil* **73**:257-264.
- Flexas J, Medrano H. 2002.** Energy dissipation in C<sub>3</sub> plants under drought. *Functional Plant Biology* **29**:1209-1215.
- Flexas J, Bota J, Escalona J M, Sampol B, Medrano H. 2002.** Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology* **29**:461-471.
- Flexas J, Gulías J, Jonasson S, Medrano H, Mus M. 2001.** Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. *Acta Oecologica* **22**:33-43.
- Gimeno-García E, Andreu V, Rubio J L. 2000.** Changes in organic matter, nitrogen, phosphorus and cations in soil as a result of fire and water erosion in a Mediterranean landscape. *European Journal of Soil Science* **51**:201-210.
- González-Andrés F, Ortiz J-M. 1997.** Phenology of species belonging to the genus *Cystisus* and allies (Genistaceae: Leguminosae). *Israeli Journal of Plant Science* **45**:59-69.
- Gowing D J G, Davies W J, Jones H G. 1990.** A positive root-sourced signal as an indicator of soil drying in apple, *Malus × domestica* Borkh. *Journal of Experimental Botany* **41**:1535-1540.
- Granati L. 1995.** Structural and ecophysiological plasticity of some evergreen species of the Mediterranean maquis in response to climate. *Photosynthetica* **31**:335-343.
- Gulías J, Flexas J, Abadía A, Medrano H. 2002.** Photosynthetic responses to water stress in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. *Tree Physiology* **22**:687-697.
- Gulías J, Flexas J, Mus M, Cifre J, Lefi E, Medrano H. 2003.** Relationships between leaf photosynthesis, nitrogen content and specific leaf area in 78 Mediterranean species. Effects of evolutionary history, life form and leaf habit. *Annals of Botany* **92**:215-222.
- Haase P, Pugnaire F I, Clark S C, Incoll L D. 2000.** Photosynthetic rate and canopy development in the drought-deciduous shrub *Anthyllis cytisoides* L. *Journal of Arid Environments* **46**:79-91.
- Hamdy A, Lacirignola C. 1999.** Mediterranean water resources: major challenges towards the 21<sup>st</sup> Century. Bari, Italy: CIHEAM-IAM. 570 pp.
- Joffre R, Rambal S, Winkel T. 2001.** Respuestas de las plantas Mediterráneas a la limitación de agua: desde la hoja hasta el dosel. In *Ecosistemas Mediterráneos*, pp. 37-65. Eds R Z Rodríguez and F I Pugnaire de Iraola. España: CSIC, Castillo and Edisart, S L Publishers.
- Joffre R, Rambal S. 1999.** Functional attributes in Mediterranean-type ecosystems. In *Handbook of functional plant ecology*. pp. 347-380. Eds P I Pugnaire and F Valladars. New-York: Marcel Dekker.
- Lefi E, Gulías J, Ribas-Carbo M, Hipólito M. 2003.** Soil water deficit effects on photosynthesis, water use efficiency and growth of three Mediterranean shrubs: *Medicago arborea*, *Medicago citrina* and *Medicago strasseri*. *11<sup>th</sup> meeting of the FAO-CIHEAM subnetwork on mediterranean pastures and fodder crops*: 30, Djerba, Tunisia.
- Lefi E, Gulías J, Cifre J, Ben Younes M, Medrano H. 2004.** Drought effects on the dynamics of leaf production and senescence in field-grown *Medicago arborea* and *Medicago citrina*. *Annals of Applied Biology* **144**:169-176.
- Le Houérou H N. 1989.** Agrosilvicultura y silvopastoralismo para combatir la degradación del suelo en la cuenca mediterránea. In *Degradación de zonas áridas del entorno mediterráneo*. MOPU, Madrid (Spain), pp. 105-116. Monografía, Dirección General del Medio Ambiente.
- Levitt J. 1972.** Stress interactions—back to future. *Horticulture Science* **25**:1363-1365.
- Ludlow M M. 1989.** Strategies of response to water stress. In *Structural and Functional Responses to Environmental Stresses*. pp. 269-281. Eds H Richter and T M Hinckley. The Hague, the Netherlands: SPB Academic Publishing.
- McDonald A J S, Davies W J. 1996.** Keeping in touch: responses of the whole plant to deficit in water and nitrogen supply. *Advances in Botanical Research* **22**: 229-300. New York: Academic Press.
- Medrano H, Chaves M M, Porqueddu C, Caredda S. 1998.** Improving forage crops for semi-arid areas. *Outlook on Agriculture* **27**:89-94.
- Munns R, Passioura J B, Guo J, Chazen O, Cramer G R. 2000.** Water relations and leaf expansion: importance of time scale. *Journal of Experimental Botany* **51**:1495-1504.
- Papanastasis V P, Platis P D, Dini-Papanastasis O. 1997.** Productivity of deciduous woody and fodder species in relation to air temperature and precipitation in a Mediterranean environment. *Agroforestry Systems* **37**:187-198.
- Pic E, Teyssendier de la Serve B, Tardieu F, Turc O. 2002.** Leaf senescence induced by mild water deficit follows the same sequence of macroscopic, biochemical, and molecular events as monocarpic senescence in pea. *Plant Physiology* **128**:236-246.
- Rambal S. 1984.** Water balance and pattern of root water uptake by a *Quercus coccifera* L. evergreen shrub. *Oecologia* **62**:18-25.
- Rana G, Katerji N. 2000.** Measurement and estimation of actual evapotranspiration in the field under Mediterranean climate: a review. *European Journal of Agronomy* **13**:125-153.
- Robledo A, Ríos S, Correal E. 1993.** El grupo *Medicago arborea* en la cuenca Mediterránea: I. Origen, Distribución y Morfología. *Pastos* **23**:55-67.
- Saab I N, Sharp R E. 1989.** Non hydraulic signals from maize roots in drying soil: Inhibition of leaf elongation but not stomatal conductance. *Planta* **179**:466-474.
- Socias X, Correia M J, Chaves M M, Medrano H. 1997.** The role of abscisic acid and water relations in drought responses of subteneum clover. *Journal of Experimental Botany* **48**:1281-1288.

- Sponchiado B N, White J W, Castillo J A, Jones P G. 1989.** Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. *Experimental Agriculture* **25**:249-257.
- Tardieu F, Katerji N, Bethenod J, Zhang J, Davies W J. 1991.** Maize stomatal conductance in the field: its relationship with soil and plant water potentials, mechanical constraints and ABA concentration in the xylem sap. *Plant, Cell and Environment* **14**:121-126.
- Vadell J, Cabot C, Medrano H. 1995.** Diurnal time course of leaf gas exchange rates and related characters in drought-acclimated and irrigated *Trifolium subterraneum*. *Australian Journal of Plant Physiology* **22**:461-469.
- Voltaire F, Thomas H, Lelievre F. 1998.** Survival and recovery of perennial forage grasses under prolonged Mediterranean drought. I. Growth, death, water relations and solute content in herbage and stubble. *New Phytologist* **140**:439-449.
- Wezel A, Rajot J L, Herbrig C. 2000.** Influence of shrubs on soil characteristics and their function in Sahelian agroecosystems in semi-arid Niger. *Journal of Arid Environments* **44**:383-398.