Plant and Soil **230:** 175–183, 2001.

Comparison of proline accumulation in two mediterranean shrubs subjected to natural and experimental water deficit

F. Ain-Lhout, M. Zunzunegui[1], M.C. Diaz Barradas, R. Tirado, A. Clavijo & F. Garcia Novo
Departamento de Biología Vegetal y Ecología. Universidad de Sevilla. Apartado 1095. 41080 Sevilla. Spain
[1]*Corresponding author*[*]

Received 9 November 1999. Accepted in revised form 6 November 2000

Key words: drought, *Halimium halimifolium*, leaf water potential, *Pistacia lentiscus*, plant responses, stomatal resistance

Abstract

The effect of water stress on proline accumulation was tested in two contrasted species of Mediterranean scrub: *Halimium halimifolium* (L.) Willk and *Pistacia lentiscus* L. Leaf water potential, stomatal resistance and proline content have been measured both in experimental and in natural water stress conditions. Both species accumulated proline in their leaves when leaf water potential dropped below a threshold value of −3.0 MPa, under natural as well as under experimental conditions. In the field, however, a time-lag between decrease of leaf water potential and proline accumulation could be observed. In *Halimium halimifolium*, proline accumulation appeared to be associated with severe stress conditions as most plants with high proline contents suffered irreversible wilting, especially in the greenhouse. *P. lentiscus* showed a different pattern, accumulating proline at two different times of the year, as a response to cold or to drought. The results of our study indicated that the role of proline in this species, rather than an osmotic agent, seems to be more related to a protective action in cases of severe stress conditions.

Introduction

Under Mediterranean type climate, the performance and distribution of shrub species depend on their ability to withstand drought, high temperatures and strong irradiance (Poole and Miller, 1975). Under these climatic conditions, two functional types of shrub species persist (Oliveira and Peñuelas, 2000; Verdú, 2000; Werner et al., 1998), having received different names: evergreen sclerophylls, chaparral, maquis, Herrera's type II (1984, 1992); *versus* drought semi-deciduous, malacophyllous, coastal sage scrub, phrygana, garriga and Herrera's type I.

Semi-deciduous species partially avoid water stress through major reductions of their foliage area, thus restricting their growth to the more favourable seasons. In contrast, evergreen sclerophylls go through the stress conditions with intact green leaves, by exhibiting a variety of morphological adaptations such as small, highly sclerophyllous leaves, thick cuticles, deep root systems and effective stomatal control of water loss (Margaris, 1981; Mooney, 1981; Werner et al., 1998).

The increase of proline levels during drought stress is unique compared to other free amino acids in the same tissue (Aspinall and Paleg, 1981; Handa et al., 1983; Sivaramakrishnan et al., 1988), although similar to other low molecular weight solutes such as sugars and organic acids (Ford, 1984; Newton et al., 1986; Sivaramakrishnan et al., 1988). These compounds may act as osmotic solutes (Hare et al., 1998).

Proline may protect protein structure and membranes from damage, and to reduce enzyme denaturation (Iyer and Caplan, 1998; Rajendrakumar et al., 1994; Saradhi et al., 1995; Smirnoff and Cumbes, 1989). Alternatively, proline accumulation has also been proposed to be nitrogen storage (Gil, 1995; Hare et al., 1998; Larcher, 1995).

Under the stressing summer conditions of Mediterranean climate (high temperature and irradiance,

[*] FAX No: +34-954626308. E-mail: zunzu@cica.es

together with water deprivation), plants tend to close stomata to avoid water lost by transpiration (Lange et al., 1971; Larcher, 1995; Poole and Miller, 1975), and therefore CO_2 uptake is prevented, under these conditions it has been proposed that proline can act as an electron acceptor, avoiding damage by photoinhibition (Hare and Cress, 1997; Hare et al., 1998).

However, a great controversy exists about the protective properties of proline accumulation. Hanson (1980) concluded that proline accumulation was not an adaptive trait, but only a symptom of stress.

The aims of this study were to compare the effects of experimental and natural water stress on proline accumulation in two Mediterranean shrub species belonging to different plant functional types (Díaz Barradas et al., 1999a): *Halimium halimifolium* (L.) Willk, a semi-deciduous shrub, and *Pistacia lentiscus* L., an evergreen sclerophyll.

Both Functional Types exhibit different responses to summer stress (Lansac et al., 1994; Oliveira and Peñuelas, 2000; Werner et al., 1998). Evergreen sclerophylls face summer stress by means of control mechanisms, while semi-deciduous let their water potential drop to very low values and reduce their transpiration surface by leaf shedding in stress conditions. *Pistacia lentiscus* and other species of *Halimium* (like *H. viscosum*) also accumulate proline under drought conditions (Lansac et al., 1994; Rhizopoulou et al., 1991).

With this background, it was hypothesised that sclerophyllous species have a better control over their physiological variables, showing a more conservative strategy (a gradual proline increment), while semi-deciduous species should respond following an opportunistic strategy (fast and great changes in proline concentration).

Materials and methods

Study species

The main morpho-physiological features of the studied species, *Halimium halimifolium* (L.) Willk and *Pistacia lentiscus* L. (Valdés et al., 1987) are summarised in Table 1.

Greenhouse experiment

Sixty young *H. halimifolium* plants (25–30 cm high at the beginning of the experiment) and 9 *P. lentiscus* plants (20–35 cm high at the beginning of the experiment) were transplanted from the field to the greenhouse in 5 litre plastic pots with their original sandy soil, to which 10% of perlite was added to improve field capacity. The plants were left in the same pots, during 5 months before the experiment started, for acclimatisation purposes. The greenhouse was located in the Campus of Sevilla University, made with glass, and covered with an awning to avoid excessive increase of temperature (under direct sun, air temperature within the enclosure can reach 45 °C in many summer days).

All plants were collected on the dune fields of Doñana National Park (SW Spain, in the Atlantic coast), where soil texture is dominated 80% w/w by aeolian quartz sands (0.2–0.02 mm) with a field capacity of 12–14% (García Novo, 1997). Due to the low regeneration rate of *P. lentiscus*, there was a reduced number of young plants in the study area; under these conditions, we could only transplant a limited number of individuals to the greenhouse.

The treatment was carried out during 9 weeks, from early May to July. After the 9th week, no further measurements could be carried out due to the generalised wilting of the plants. Plants were separated in 3 sets subjected to different irrigation intensities:

1. A set: daily watering (control).
2. B set: twice a week watering during the first 4 weeks and once a week until the end of the experiment.
3. C set: once a week watering during the first 4 weeks and once every 2 weeks until the end of the experiment.

Plants were irrigated with tap water and they did not receive any nutrition during the 8 months (acclimatisation plus experiment).

Measurements

The following environmental variables were recorded weekly in the greenhouse: Air and soil temperature (with an electronic sensor connected to a LI-1000 data logger), air relative humidity (with a porometer) and midday PFD (Photosynthetic Flux Density) with a quantum sensor (model Li-190 SB LI-COR, located above the plants) connected to a LI-1000 data logger. Soil temperature was measured at a depth of 5 cm and 5 cm from the inner edge of the pot.

Midday leaf water potential was measured in fully expanded leaves by means of a Scholander pressure chamber (Scholander et al., 1965). Five plants of *H. halimifolium* and 3 plants of *P. lentiscus* were meas-

Table 1. Structural, phenological and physiological features of *Halimium halimifolium* and *Pistacia lentiscus*. References in brackets

Characteristics	*Halimium halimifolium*	*Pistacia lentiscus*
Plant form	Chamaephyte or phanerophyte (1)	Phanerophyte (1)
Specfic leaf mass	Low (2)	High (2)
Leaves	Relatively thin (2, 3)	Relatively thick (2, 3)
Trichome density	Both surfaces (4)	Absent (4)
Leaf longevity	$<$ 1 year (1)	$>$ 2 year (1)
Root depth	Shallow (5)	Deep (5)
Leaf water potential	Relatively low (6, 7, 8)	Relatively low (6, 7, 8)
Stomatal control	High (2, 8)	High (2, 8)
Osmotic potential	Not known	Relatively low (10)
Regeneration after fire	Seeder (9)	Sprouter (9)
Canopy reduction	Medium (2)	Slight (2)

1: Orshan (1989) 2: Díaz Barradas et al. (1999a) 3: Merino J. in Specht. (1988) 4: Valdés et al. (1987) 5: Martínez & Rodríguez (1988) 6: Merino J. et al. (1976) 7: Merino O. et al. (1995) 8: Zunzunegui et al. (2000) 9: García Novo (1977) 10: Rhizopoulou et al. (1991).

ured weekly for each set, with two replicates per plant.

Midday resistance to diffusion was measured with a MK3 Delta-Device portable diffusion porometer (type: transit-time instrument). Five plants of *H. halimifolium* and 3 plants of *P. lentiscus* were measured weekly for each set, with two replicates per plant.

Proline concentration was estimated using the acid-ninhydrin method (Bates et al., 1973). Fully expanded leaves were excised and immersed in liquid nitrogen while being transferred from the greenhouse to the laboratory. Tissues were stored at -25 °C in a freezer until they were analysed. Two 0.5 g leaf samples were taken from each plant and were ground with liquid nitrogen. Sulphosalicylic acid (10 ml, 3%) was added and the extract was then vacuum filtered through Whatman no. 2 filter paper. Then, 2 ml of the filtered extract were taken for the analysis, to which 2 ml of acid-ninhydrin and acetic acid were added. The mixture was oven incubated at 100 °C for 1 h and the reaction was finished in an ice bath. The reaction mixture was extracted with 4 ml toluene and absorbance was read at 517 nm, using toluene as a blank. Proline concentration was calculated using L-proline (Panreac) for the standard curve.

Field measurement

Two sites were selected in Doñana National Park: A dry area where only *H. halimifolium* was present, and a more humid one, where both species grew (Díaz Barradas et al., 1999a, b). Ten *H. halimifolium* plants in the drier site and 10 plants of *H. halimifolium* and 9 plants of *P. lentiscus* in the moister site were selected and marked for field measurements.

Leaf water potential, proline content and stomatal resistance were measured using the same equipment and methods as described above. Field measurements were carried out at midday in January, July, September and November 1998 and February, May, September and October 1999. These sampling months were chosen according to previous ecophysiological studies on these species (Correia and Catarino, 1994; Correia and Díaz Barradas, 2000; Rhizopoulou et al., 1991; Zunzunegui et al., 2000): January and February, when the lowest temperatures take place, together with high levels of irradiance; May and July correspond to the beginning of the summer conditions; in September, the summer accumulated stress showed its most extreme consequences; October and November represent the recovery from summer stress after the first autumn rains.

A meteorological station located in the Doñana National Park recorded environmental variables.

Data analysis

The results were subjected to a repeated measures ANOVA, on the effects of treatment and shrub species on water potential, proline concentration and stomatal resistance data. Tukey test was used for comparison

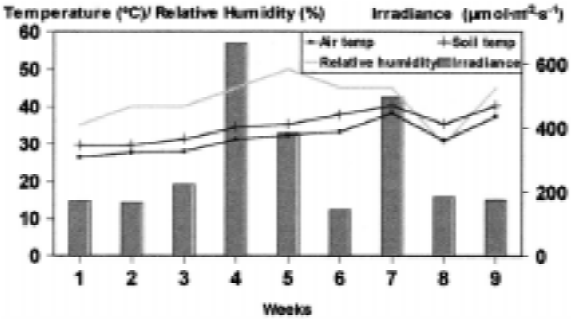

Figure 1. Temporal changes of temperature, relative humidity and irradiance during the experiment measured at midday, at the same time that physiological variables.

between pairs of treatments. All variables were compared by means of Pearson correlation. The analyses were carried out using the statistical SPSS computer program. Regressions were calculated between water potential, proline content, mortality of study plants and stomatal resistance.

Results

Greenhouse experiment

Air and soil temperatures increased gradually during the experiment (27 °C to 37 °C) with small fluctuations, following the usual pattern from May to July (Figure 1). Relative humidity and irradiance did not show any clear trend, rather they widely fluctuated throughout the whole experiment.

Set A plants did not show any evidence of stress during the experiment in either species. In *P. lentiscus*, however, levels of proline in leaves of 15–20 μmol g^{-1} d.w. were measured during all the experiment, but specially between the 4th and 7th weeks.

Leaf water potential values in *H. halimifolium* sets B and C plants did not show significant differences. Differences only appeared in the 9th week. In both cases, this was not a gradual process (Figure 2), but rather a sudden one, as water potential dropped to low values (-2.9 ± 1.1 and -5.0 ± 1.7 MPa in sets B and C, respectively). In *P. lentiscus* plants, leaf water potential values, contrarily to the case of *H. halimifolium*, decreased gradually since the first week, including set A plants. This decrease was more pronounced in sets B and C, where the lowest values recorded were -3.45 ± 0.55 and -3.25 ± 0.45 MPa, respectively.

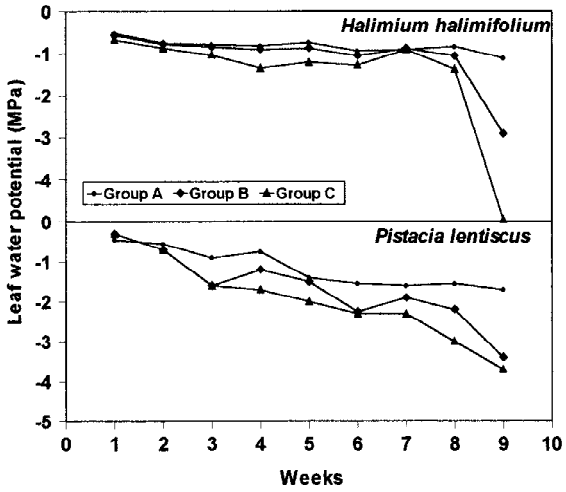

Figure 2. Changes in leaf water potential in control plants (A) and stressed plants (B and C) during the experiment in *Halimium halimifolium* and *Pistacia lentiscus.*

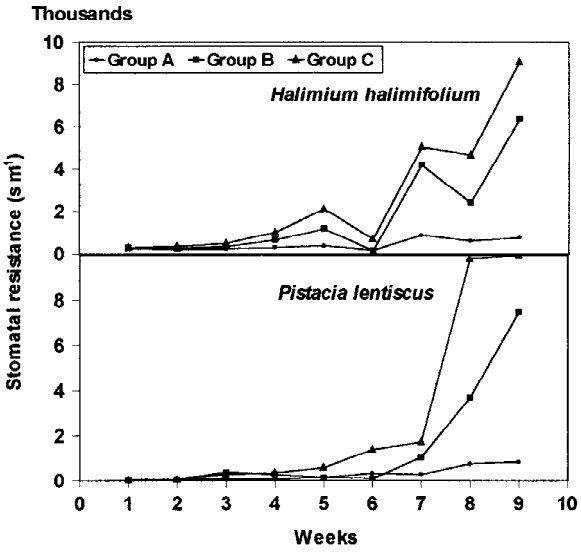

Figure 3. Stomatal resistance in control plants (A) and stressed plants (B and C) during the experiment in *Halimium halimifolium* and *Pistacia lentiscus.*

Stomatal resistance behaved differently. It started to increase in the 4th week, in a more marked way in *H. halimifolium* plants, reaching its maximum in the 9th week (6360 ± 3440 and 9070 ± 1860 s m^{-1} for sets B and C). A decrease of the resistance was recorded the 6th and 8th weeks, in parallel with a reduction of the irradiance and a decrease of air and soil temperatures (Figure 3) in certain cloudy days. Resistance values in *P. lentiscus* increased since the 5th week in set C and since the 6th week in set B, reaching a final

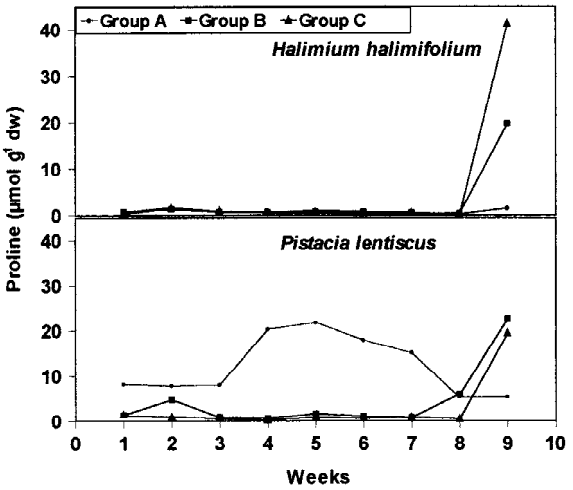

Figure 4. Leaf proline accumulation in control plants (A) and stressed plants (B and C) during the experiment in *Halimium halimifolium* and *Pistacia lentiscus.*

maximum of 7440±2520 and 10000±3240 s cm^{-1} in sets B and C, respectively.

Proline accumulation in *H. halimifolium* leaves shot up to high values the last week (19.7±14.3 and 41.19±24.8 μmol/g dw in sets B and C). In *P. lentiscus* plants, proline concentration values increased sharply towards the 8th week in set B and towards the 9th week in set C (22.6±5.5 and 29.4±1.5 μmol/g dw, respectively). Set A showed a different proline accumulation pattern throughout the whole experiment (Figure 4).

By the end of the experiment, set C plants suffered a high mortality: 75% in *H. halimifolium* and 100% in *P. lentiscus*. Mortality in set B plants was smaller: 20% in *H. Halimifolium* and 66% in *P. lentiscus*. And no mortality occurred in set A plants, in either species. Considering both species, a significant correlation was found between plant mortality and proline accumulation (r=0.869, p<0.025).

Proline accumulation was dependent on leaf water potential in *H. halimifolium* but not in *P. lentiscus*. In the former, a significant cubic regression with r^2=0.995 was found. No correlation was found between proline accumulation and stomatal resistance for both species.

The results of the repeated measures ANOVA test for *H. halimifolium* showed that differences among treatments for proline accumulation, leaf water potential and resistance were significant (Table 2). Tukey test showed differences among the three treatments for leaf water potential and resistance. But for proline accumulation differences only appeared when comparing A and B with C. In *P. lentiscus*, the results of the repeated measures ANOVA test were significant for the three variables (Table 3). The results of the Tukey test showed that treatment A was different from C only in terms of leaf water potential.

Table 2. Results of the repeated measures ANOVA of *Halimium halimifolium* experiment

	Variable	Df	MS	*F*	*P*
Water potential	Time	8	506.7	133.9	0.001
	Time * treatment	16	113.8	3.7	0.001
	Error	96	3.7		
Resistance	Time	8	4959.3	20.5	0.001
	Time * treatment	16	1118.4	4.6	0.001
	Error	96	242.4		
Proline	Time	8	558.4	17.6	0.001
	Time * treatment	16	267.1	8.401	0.001
	Error	96	31.8		

Table 3. Results of the repeated measures ANOVA of *Pistacia lentiscus* experiment

	Variable	Df	MS	*F*	*P*
Water potential	Time	8	541.7	131.7	0.001
	Time * treatment	16	34.1	8.3	0.023
	Error	48	4.11		
Resistance	Time	8	4990.8	51.8	0.001
	Time * treatment	16	758.0	7.9	0.001
	Error	48	96.3		
Proline	Time	8	420.5	14.4	0.001
	Time * treatment	16	311.8	10.7	0.001
	Error	48	29.2		

The results of the repeated measures ANOVA comparing the responses of both species showed significant differences in all the measured variables (Table 4).

Field measurement

Figure 5 shows the hydrological differences between the cycles 1997–98 and 1998–99, an extremely dry one, in which precipitation was 50% lower than the average. In September 1999, ground-water table depth was 2.75 m (1 m deeper that in September 1998) in

Table 4. Results of the repeated measures ANOVA comparing *Pistacia lentiscus* and *Halimium halimifolium*, both in the field and in the greenhouse experiment

	Variable	Df	MS	*F*	*P*
Field	Time	6	19.6	37.9	0.001
Water	Time * species	6	1.6	3.1	0.01
potential	Error	78	0.51		
Proline	Time	6	224.8	3.7	0.003
	Time * species	6	322.1	5.3	0.001
	Error	78	60.9		
Greenhouse	Time	8	908.3	233.3	0.001
Water	Time * species	8	148.9	38.4	0.001
potential	Error	144	3.9		
Resistance	Time	8	8718.6	45.1	0.001
	Time * species	8	1239.4	6.4	0.001
	Error	144	193.5		
Proline	Time	8	869.5	28.1	0.001
	Time * species	8	74.9	2.4	0.017
	Error	144	30.4		

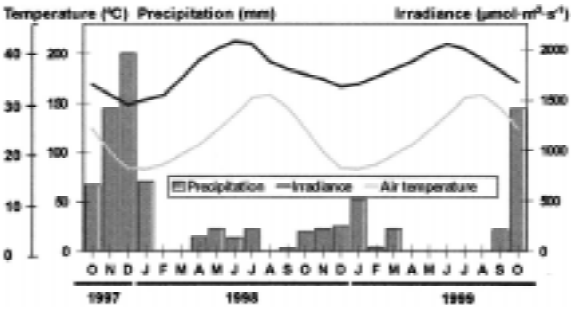

Figure 5. Temporal changes of midday temperature, midday irradiance and monthly precipitation during the study period at Doñana National Park.

the wet site and more than 6 m in the dry site. In the summer of 1998, small rainfall events were recorded during July and August, very atypical in summer in the south of Spain.

H. halimifolium individuals of the wet site only showed symptoms of stress in September 1999, when low values of leaf water potential were recorded together with proline accumulation. In September 1998 the low depth of ground water table (less than 2 m) allowed to the roots reach the ground water.

In the dry site, *H. halimifolium* plants showed low leaf water potential values and accumulation of proline

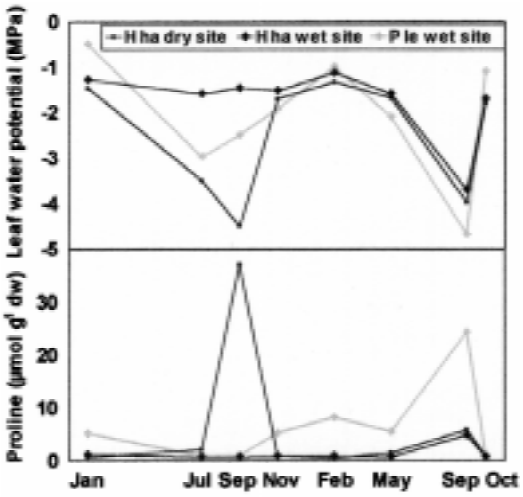

Figure 6. Temporal changes under field conditions in leaf water potential and proline accumulation in the three populations under study at Doñana National Park: 1) dry site population of *Halimium halimifolium* (Hha dry site), 2) wet site population of *H. halimifolium* (Hha wet site) and 3) wet site population of *Pistacia lentiscus* (P. le wet site).

in July and September 1998 and in September 1999. In autumn, winter and spring, no evidence of stress was observed.

Proline accumulation pattern in *P. lentiscus* was different than in *H. halimifolium*. In this species, proline mainly accumulated in cold months – November, January and February. Despite the low leaf water potential values measured in July and September 1998, no accumulation of proline was found. However, proline accumulation was observed in May and September 1999, a dryer year than 1998, as it has been described above.

In natural conditions, mortality rate was much lower than in the greenhouse experiment: After the summer of 1998, 40% of the *H. halimifolium* population from the dry site died and no plant mortality was found in both species on the moist site. However, after the summer of 1999, 40–50% of *H. halimifolium* plants died on both sites and no mortality occurred in *P. lentiscus*.

There was no significant correlation between proline and mortality for both species. In *H. halimifolium*, the significant regression between proline and leaf water potential (r^2=0.586, p<0.0001) was exponential, with an increase of proline concentration for leaf water potential under −4 MPa. In *P. lentiscus*, on the contrary, the correlation between proline and leaf water potential was not found to be significant.

The results of the repeated measures ANOVA comparing the responses of both species under field conditions were significant for proline and leaf water potential (Table 4).

Discussion

It has been widely proved that proline accumulates in leaves of plants undergoing from water deprivation (Iyer and Caplan, 1998; Lansac et al., 1994; Rhizopoulou et al., 1991), although the role of this compound is still obscure.

Most of proline concentration values found in leaves of *H. halimifolium* under field conditions were lower than 5 μmol g^{-1} dw, but in September of 1998 plants from the dry site exhibited values higher than 30 μmol g^{-1} dw. The absence of intermediate figures and the exponential regression with leaf water potential suggests the existence of a fast accumulation mechanism. These maximum values of proline accumulation are similar to the results for *H. viscosum* (Lansac et al., 1994) with an average of 35.4 μmol g^{-1} dw in September.

Blum and Ebercon (1976), Sivaramakrishnan et al. (1988) and Lansac et al. (1994) have described this kind of response. They suggested a threshold leaf water potential value below which proline accumulation occurred.

Low values of leaf water potential recorded in July were not accompanied by proline accumulation. But after a more prolonged dry period, proline accumulation was indeed observed, what suggests that both variables are not in phase, but rather, delayed. This suggests that proline accumulation should not act as a relevant active osmolyte for this species.

The highest proline concentrations in *P. lentiscus* in the field were also recorded in September, together with the lowest leaf water potential. Working with *P. lentiscus*, Rhizopoulou et al. (1991) found the lowest leaf water potential figures in June, but did not find proline to accumulate until September (22 μmol g^{-1} dw).

Roots of the wet site plants are used to have phreatic water available throughout the year. But in September 1999, the higher descent of the groundwater table may be responsible for the lower leaf water potential, a higher proline accumulation and mortality in the *H. halimifolium* plants than September 1998.

P. lentiscus accumulated proline twice a year, as a response to cold and drought respectively (Rhizopoulou et al., 1991). It is well documented that the association of light with stresses induces the accumulation of proline. Proline accumulation in cold stress is promoted in light and suppressed in the dark (Chu et al., 1978; Hare and Cress, 1997). The high irradiance level (1600 μmol m^{-2} s^{-1}) measured in the study area during cold winter months affected more to *P. lentiscus* plants than to *H. halimifolium* ones. In the Mediterranean basin, sclerophyll taxa as *P. lentiscus* evolved in the Tertiary under a tropical climate (Herrera, 1984; Verdú, 2000). Therefore, they are worse adapted to cold temperatures, as also happen with *Myrtus communis* (Diamamtoglou and Rhizopoulou, 1992).

In the greenhouse, the water stress induced in the *H. halimifolium*, experiment was so fast that no delay could be observed between water potential and proline accumulation. When leaf water potential dropped down below -2.9 MPa significant changes in proline concentration occurred. In this case, no intermediate proline concentration values were recorded.

Leaf water potential decreased throughout the experiment according to a more gradual pattern in *P. lentiscus*, in contrast with an exponential decrease in *H. halimifolium*.

Proline concentration in *P. lentiscus* set A showed a different pattern and it is presumable that some kind of relation should exist with other processes, as, for instance, the size or age of plants. Proline contents increased from the bigger to the smaller plants (Lansac et al., 1994) and from the elder to the younger ones (Amberg-Ochsenbauer and Obendorfer, 1998). As *P. lentiscus* group A individuals (controls), were the smallest and perhaps the youngest they could present a higher proline accumulation than bigger ones subjected to the water deprivation experiment (groups B and C).

Stomatal resistance increase was evident 3–4 weeks before leaf water potential and proline concentration were affected. The first and most sensitive response to water deficit seems to be the sensibility of stomata to water deficit (Sala and Tenhunen, 1994; Tenhunen et al., 1990) as it happened in both species. Of the daily fluctuating environmental factors, incoming solar radiation is the best correlated to transpiration, followed by temperature and relative humidity (Gil, 1995). This would explain the decrease in stomatal resistance occurred in 6th and 8th week, during a descent in irradiance and temperatures, in spite of the maintained water stress. Campos et al. (1999) studying the answer to drought in 4 genotypes of *Vigna* with dif-

ferent drought resistance found that the only genotype able to accumulate significant amounts of proline also showed a more gradual stomatal closure.

The results discussed here support the hypothesis that proline accumulation is a part of a physiological response of the plant to an intense stress (Hare et al., 1998). The high mortality rate, both in the greenhouse experiment and in field, suggests that proline concentration only occurred when plants were under severe stress, close to permanent withering, an irreversible situation for a high percentage of the plants under study. Singh et al. (1973), Blum and Ebercon (1976) and Sivaramakrishnan (1998) suggested that the role of proline might be in the intermediate recovery of plants after the stress.

The results of our study indicated that the proline role in this species, rather than an osmotic agent with an osmotic potential depressing activity, seems to be more related to a protective action in cases of severe stress conditions. Sánchez et al. (1998) found that free proline increased as much as 40 times in response to water stress. However the contribution of this amino acid to the osmotic potential to was small (approximately 1%). On the other hand, sugars increased as much as until 7 times and their contribution to osmotic potential was 17.3%. The stimulation of sugar levels induced by drought was proportional to osmotic potential. They concluded that the role of proline would be minimising the damage caused by dehydration. Similar results were concluded by Wang et al. (1999).

The results of this study support the initial hypothesis. *H. halimifolium*, the semi-deciduous species, accumulated proline by pulses, in response to a severe water deprivation, according to an opportunistic pattern. Therefore, in *H. halimifolium*, proline accumulation was induced when dehydration was so severe that the process was hardly reversible, with a mortality of 50% under natural conditions. In contrast, *P. lentiscus* presented a gradual pattern of proline accumulation in response to drought and to cold. In this species, the process was reversible and no mortality was found under natural conditions.

Acknowledgements

Rafael Sarmiento was of great help in the methodology of proline analysis. Yon Jauregui has helped in the preparation of the manuscript. And two anonymous referees have helped with their comments to improve the manuscript.

References

Amberger-Ochsenbauer S and Obendorfer J 1998 Levels of free proline in Ornamental Plants: I. Influence of plant age, leaf age and leaf region in *Saintpaulia* and *Chrysanthemum.* J. Plant Physiol. 132, 758–761.

Aspinall D and Paleg L D 1981 Proline accumulation. Physiological aspects. *In* Physiology and Biochemistry of Drought Resistance in Plants. Eds. LD Paleg and D Aspinall. pp. 206–240. Academic Press, Sydney.

Bates L S, Waldren R P and Teare I D 1973 Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207.

Blum A and Ebercon A 1976 Genotypic response in sorghum to drought stress. III. Free proline accumulation and drought resistance. Crop Sci. 16, 428–431.

Campos P S, Ramalho J C, Lauriano J A, Silav M J and Mateos M D 1999 Effects of Drought on photosynthetic performance and water relations of 4 V*igna* genotypes. Photosynthetica 36, 79–87.

Correia O A and Catarino F M 1994 Seasonal changes in soil-to-leaf resistance in *Cistus sp*. And in *Pistacia lentiscus.* Acta Oecol. 15, 289–300.

Correia O and Díaz Barradas M C 2000 Ecophysiological differences between male and female plants of *Pistacia lentiscus* L. Plant Ecol. 149: 131–142.

Chu T M, Jusaitis M, Aspinall D and Paleg L G 1978 Accumulation of free proline at low temperatures. Physiol. Plant. 43, 254–260.

Diamantoglou S and Rhizopoulou S 1992 Free proline accumulation in sapwood, bark and leaves of three evergreen Sclerophylls and comparison with evergreen conifer. J. Plant Physiol. 140, 361–365.

Díaz Barradas M C, Zunzunegui M, Ain-Lhout F Z, Tirado R and García Novo F 1999a Plant functional types and ecosystem function in Mediterranean scrub. J. Veg. Sci. 10, 709–716.

Díaz Barradas M C, Zunzunegui M and García Novo F 1999b Autoecological traits of *Halimium halimifolium* under Mediterranean type climate. Folia Geobot. 34, 189–208.

Ford C W 1984 Accumulation of low molecular weight solutes in water stressed tropical legumes. Phytochemistry 23, 1007–1015.

García Novo F 1997 The ecosystem of Doñana National Park. *In* The Ecology and Conservation of European Dunes. Eds. F García Novo, RM Crawford and MC Díaz Barradas. pp 97–116. Universidad de Sevilla. Sevilla.

Gil Martínez F 1995 Elementos de Fisiología Vegetal. Ediciones Mundi-Prensa. Madrid.

Handa S, Bresan R A, Handa A K, Carpita N C and Hasegawa P M 1983 Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. Plant Physiol. 73, 834–843.

Hare P D and Cress W A 1997 Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul. 21, 79–102.

Hare P D, Cress W A and Van Staden J 1998 Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21, 535–553.

Hanson A D 1980 Interpreting the metabolic responses of plants to water stress. Hort. Sci. 15, 623–629.

Herrera C 1984 Tipos morfológicos y funcionales en plantas del matorral mediterráneo del sur de España. Studia Oecol. V, 7–34.

Herrera C 1992 Historical effects and sorting processes as explanations for contemporary ecological patterns: character syndromes in Mediterranean woody plants. Am. Nat. 140, 421–446.

Iyer S and Caplan A 1998 Products of proline catabolism can induce osmotically regulated genes in ricel.

Lange O L, Lösch R, Schulze E D and Kappen L 1971 Responses of stomata to changes in humidity. Planta 100, 76–86.

Lansac A R, Zaballos J P and Martín A 1994 Seasonal water potential changes and proline accumulation in Mediterranean shrubland species. Vegetatio 113, 141–154.

Larcher W 1995 Physiological Plant Ecology. pp. 506. Springer-Verlag. Berlin.

Margaris N S 1981 Adaptative strategies in plants dominating Mediterranean- type ecosystems. *In* Mediterranean Type Shrublands. Ecosystems of the World 11. Eds. F Di Castri, DW Goodall and RL Specht. pp 309–315. Elsevier Scientific Publishing, Amsterdam.

Martínez García F and Rodríguez J M 1988 Distribución vertical de las raíces del matorral de Doñana. "Vertical distribution of the roots in Doñana scrub". Lagascalia 15, 549–557.

Merino J, García Novo F and Sánchez Díaz M 1976 Annual fluctuation of water potential in the xerophytic shrub of Doñana Biological Reserve (Spain). Oecol. Plant. 11, 1–11.

Merino O, Villar R, Martín A, García D and Merino J 1995 Vegetation response to climatic change in a dune ecosystem in Southern Spain. *In* Global Change and Mediterranean Type Ecosystems. Ecological Studies 117. Eds. J M Moreno and WC Oechel. pp 225–238. Springer-Verlag, Berlin.

Mooney H A 1981 Primary production in Mediterranean-climatic regions. *In* Mediterranean Type Shrublands. Ecosystems of the World 11. Eds. F Di Castri, DW Goodall and RL Specht. pp 249–256. Elsevier Scientific Publishing, Amsterdam.

Newton R J, Bhaskaran S, Puryeas J D and Smith R H 1986 Physiological changes in cultured sorghum cells in response to induced water stress. II. Soluble carbohydrates and organic acid. Plant Physiol. 81, 626–629.

Specht R L (ed.) 1988 Mediterranean-type ecosystems a data source book. Kluwer Academic Press. Dordrecht, The Netherlands. 248 p.

Oliveira G and Peñuelas J 2000 Comparative photochemical and phenomorphological responses to winter stress of an evergreen (*Quercus ilex* L.) and a semi-deciduous (*Cistus albidus* L.) Mediterranean woody species. Acta Oecol. 21, 97–107.

Orshan G (ed.) 1989 Plant pheno-morphological studies in Mediterranean type ecosystems. Kluwer Academic Press, Dordrecht, The Netherlands.

Poole D K and Miller P C 1975 Water relations of selected species of chaparral and coastal sage communities. Ecology 56, 1118–1128.

Rajendrakumar C S V, Reddy B V D and Reddy A R 1994 Proline-protein interactions: Protection of structural and functional integrity of M4 lactate dehydrogenase. Biochem. Biophys. Res. Comm. 201, 957–963.

Rhizopoulou S, Meletiou-Christou M S and Diamantoglou S 1991 Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls. J. Exp. Bot. 42, 627–635.

Sala A and Tenhunen J D 1994 Site-specific water relations and stomatal response of *Quercus ilex* L. in a Mediterranean watershed. Tree Physiol. 14, 601–654.

Sánchez F J, Manzanares M, Deandress E F, Tenorio J L and Ayerbe L 1998 Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. Field Crops Res. 59, 225–235.

Saradhi P P, Arora S and Prasad K V S K 1995 Proline accumulates in plants exposed to U V radiation and protects them against induced peroxidation. Biochem. Biophys. Res. Comm. 290, 1–5.

Scholander P F, Hammel H T, Bradstreet E D and Hemmingsen E A 1965 Sap pressure in vascular plants. Science 148, 339–346.

Singh T N, Paleg L G and Aspinall D 1973 Stress metabolism. III. Variations in response to water deficit in the barley plant. Aust. J. Biol. Sci. 26, 65–76.

Sivaramakrishnan S, Patell V Z, Flower D J and Peacock J M 1988 Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress. Physiol. Plant. 74, 418–426.

Smirnoff N and Cumbes Q J 1989 Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28, 1057–1060.

Tenhunen J D, Sala Serra A, Harley P C, Dougherty R L and Reynolds J F 1990. Factors influencing carbon fixation and water use by Mediterranean sclerophyll shrub during summer drought. Oecologia 82, 381–393.

Valdés B, Talavera S and Fernández Galiano E 1987 Flora Vascular de Andalucía Occidental. "Vascular Flora of West Andalucía". Ketres Editora. Barcelona.

Verdú M 2000 Ecological and evolutionary differences between Mediterranean seeders and resprouters. J. Veg. Sci. 11, 265–268.

Wang H L, Lee P D, Liu L F and Su J C 1999 Effect of sorbitol induced osmotic stress on the changes of carbohydrate and free amino acid pools in sweet potato cell suspension cultures. Bot. Bulletin Acad. Sinica 40, 219–225.

Werner C, Correia O, Ryel R J and Beyschlag W 1998 Modelling whole plant primary production of macchia species: Assessing the effects of photoinhibition and foliage orientation. Revista Biol. 16, 247–257.

Zunzunegui M, Díaz Barradas M C and García Novo F 2000 Different phenotypic response of *Halimium halimifolium* in relation to groundwater availability. Plant Ecol 148, 165–174.

Section editor: H. Lambers