

Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L.

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Received 25 February 2000; revised 25 October 2000; accepted 23 November 2000

Abstract – We examined temporal and spatial variations in net photosynthesis, stomatal conductance, intrinsic water-use efficiency, sub-stomatal CO₂ concentration, apparent carboxylation efficiency and chlorophyll fluorescence in the Mediterranean shrub *Pistacia lentiscus*. The study was done at the extremes of a precipitation and temperature gradient on the coast and in the mountains of Mallorca, Spain, with gas exchange measurements at different times of the year, and combined measurements of gas exchange and chlorophyll fluorescence in a controlled experiment. The objectives were to relate annual variation in photosynthetic functions to environmentally induced constraints and to quantify to which extent local differences in climate can affect photosynthesis in shrub populations. In the mountain population, net photosynthesis peaked in spring and autumn, when water was abundant and temperature was moderately high. It was reduced in winter paralleling reduced carboxylation efficiency. Photosynthesis was at the annual minimum in summer at both sites due to drought-induced stomata closure combined with impaired function of the Calvin cycle. The coastal population maintained high photosynthesis in mid winter but had a pronounced decline in spring, and the summer decline lasted longer than in the mountains. Integrated over the seasons, net photosynthesis was about 25 % lower in the coastal than in the mountain population, in spite of maintained high mid winter photosynthesis. Hence, the reduction at the coast was mainly due to early onset of drought in spring and a long period of summer drought, showing that local climatic differences can cause pronounced spatial differences in plant carbon balance. As a consequence, similar differences probably also occur as a function of year-to-year variability of precipitation patterns and temperatures. © 2001 Éditions scientifiques et médicales Elsevier SAS

carbon balance / climate stress / net photosynthesis / precipitation / stomatal conductance / temperature

1. INTRODUCTION

Pistacia lentiscus L. is a dioecious, evergreen Mediterranean small tree or shrub up to 8 m tall, common within the Mediterranean basin, and having typical attributes that characterise this common life form of Mediterranean plants [31, 46]. Previous research [23] performed along a moisture and temperature gradient on the island of Mallorca, Spain, has shown that leaf formation, loss, retention, longevity and leaf biomass production are site-dependent and vary across temperature and precipitation gradients. The leaf production and phenology seem, in turn, to be strongly

correlated with the leaf content of ¹³C, at least in males. These show a trend of decreasing leaf longevity and production coincident with increased δ¹³C as precipitation decreases and temperature rises, i.e. when drought increases. This pattern indicates that the leaf production and phenology are driven by constraints on the water-use efficiency affecting the photosynthesis. Hence, variations in the leaf traits appear to be induced by site-specific differential use of carbon across regional and local gradients of drought.

In this study, we examine temporal and spatial variations in a number of physiological variables related to carbon fixation and water use in *P. lentiscus* at the extremes of the same gradient [23]. The amount of precipitation and the precipitation pattern normally regulate summer net photosynthesis (A), while low temperature normally reduces winter photosynthesis in

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Mediterranean plants [16–18, 22]. We therefore hypothesised that the main control of gas exchange was drought-induced effects on stomatal function in summer, paired with non-stomatal constraints during winter. We also hypothesised that local differences in summer precipitation together with temperature differences of a few degrees in winter may cause disproportionately large differences in gas exchange and carbon balance between the populations. If this occurs, we can expect that predicted future changes in climate will have different effects on these populations, which we discuss in light of our results.

In summary, the main objectives of the study were:

- To identify the major climatic constraints to photosynthesis in the two contrasting populations of *P. lentiscus* that grow within a small distance from each other but, yet, are subjected to large differences in climate.
- To study the underlying photosynthetic mechanisms that are affected by these constraints.
- To test our previous hypothesis that the long-term integrated differences in leaf $\delta^{13}\text{C}$ were related to differences in stomatal control of the gas exchange between the local populations [23].

2. MATERIALS AND METHODS

2.1. Field measurements and statistical analyses

The study was conducted at a mountain site, Binifaldó, about 600 m above sea level and at a coastal, lowland site, Sa Vall, about 5 m above sea level situated 80 km from Binifaldó. The measurements were done on six occasions from June 1998 to late May 1999. The dates of measurements (*table I*) were chosen to represent the extremes of high temperature and strong drought in late summer, low temperature and non-limiting water supply in winter and the transition phases in autumn and spring when the precipitation increased and decreased, respectively.

Meteorological data of monthly mean precipitation, maximum and minimum temperatures were supplied by the 'Centre Meteorològic Territorial de ses Illes Balears' for Lluc and Sa Ràpita, the nearest meteorological station to the mountain and to the coastal site, respectively (*figure 1*).

The two sites and their vegetation were characterised by estimation of the percentage ground cover of rocks and the canopy cover of *P. lentiscus* and other shrubs within four randomly selected 400-m² plots at each site. For *P. lentiscus*, we also measured plant height and diameter, leaf area index (LAI), leaf nitrogen (N) concentration and leaf mass per area (LMA)

Table I. Dates of gas exchange measurements at the mountain (Binifaldó) and the coastal site (Sa Vall) and the set cuvette temperature.

Dates of measurements		Cuvette temperature (°C)
Mountain	Coast	
15 June	3 June	25
19 August	31 July	30
14 October	6 October	25
5 February	15 January	15
1 April	3 April	20
25 May	19 May	25

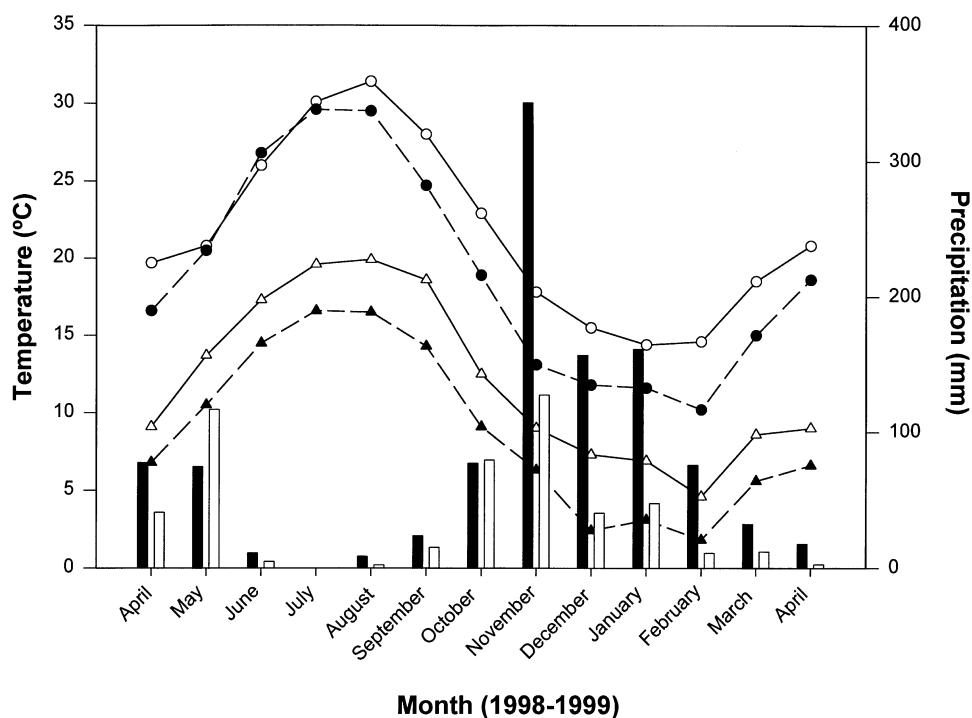
on the same eight specimens for which we measured gas exchange (see below). LAI was estimated with a LAI-2000 plant canopy analyzer (Li-Cor Inc., Nebraska, USA) [45]. Leaf N concentration was analysed colorimetrically after Kjeldahl digestion [23] and LMA was determined with an AM100 leaf area meter (ADC, Herts, UK) followed by drying and weighing of leaves.

Gas exchange, including net photosynthesis (*A*) and stomatal conductance (*g*), was measured by using a Li-6400 system (Li-Cor Inc., Nebraska, USA), a portable, open-circuit infrared gas analyser. One leaf was measured on eight different female shrubs at each site. The choice of female individuals only was due to our desire to avoid possible effects of sex on photosynthesis [6, 23]. Measurements were done on leaves formed the previous year to reduce age effects on photosynthesis. The measurements were always taken on the south facing side of well-lit adult shrubs during mid morning on sunny days. The cuvette temperatures were fixed at levels that approximated the maximum mean monthly temperature at each measuring occasion (*table I*), and light intensity was set at 1 500 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by using a LED source coupled to the measuring system. From previously constructed light response curves for well-irrigated healthy plants, we know that this light intensity is more than enough to reach light saturated photosynthesis.

Sub-stomatal CO_2 concentration (C_i) was calculated using conventional formulas [27, 35]. Intrinsic water-use efficiency was calculated as A/g and apparent carboxylation efficiency as A/C_i .

Two-way analyses of variance (ANOVA) with site and time of measurement as main factors were carried out to reveal differences in responses between the two sites, seasonal differences and their interactions, and the Tukey's HSD test was used to identify differences over time for each site. Before the analyses, we applied suitable data transformations to obtain homogeneity of variances in cases of heterogeneous vari-

Figure 1. Monthly mean maximum and minimum temperatures ($^{\circ}\text{C}$) and monthly precipitation (mm) from April 1998 to April 1999 at the coastal meteorological station at Sa Ràpita (open symbols) and the mountain station at Lluc (filled symbols). Circles are maximum temperatures, triangles are minimum temperatures and bars are precipitation.



ances in the non-transformed data. All analyses were performed with SAS, using the GLM procedure [40].

2.2. Measurements under controlled conditions

Chlorophyll fluorescence of 1-year-old leaves was measured, together with net photosynthesis and stomatal conductance, in six different 3-year-old potted plants kept at the University of the Balearic Islands (UIB), in order to examine the detailed photosynthetic responses to mild water stress. UIB is located in the inland at about a similar distance from the mountain and the coastal site, and has an intermediate climate between these two sites.

A first set of measurements was taken in December 1995, during a cold period, on well-watered and sun-exposed plants. A second set of measurements was taken on different individuals in June 1999 on well-irrigated plants, representing optimum conditions of temperature and water availability. A third set of measurements was taken on the same plants in early September (when air temperature and maximum radiation are quite similar to the conditions in June) after a period of mild water stress, achieved by withholding watering. The gas exchange was measured regularly at midday during the period when water was withheld and chlorophyll fluorescence was measured when the net photosynthesis reached a level comparable to that

previously found in December 1995. This level was reached after about 1 week without watering. For these data, we compared: (i) physiological responses in plants under optimum conditions with the responses in plants under mild drought and chilling stress; and (ii) responses under mild drought with the responses to chilling at a comparable rate of net photosynthesis.

The chlorophyll fluorescence was measured at pre-dawn to obtain the full-night relaxed value of the intrinsic quantum yield efficiency of photosystem II, F_v/F_m , by using a portable fluorometer PAM-2000 (Walz, Effeltrich, Germany). This is the ratio of variable (F_v) and maximum (F_m) fluorescence [15, 26]. At midday, we determined the electron transport rate (ETR) from data of quantum yield, using conventional methods [26]. Details of the measurements have been given previously [12].

2.3. Physiological background and data interpretation

We interpret the responses of a given stress on the basis of expected effects on photochemistry, CO_2 capture by stomata, and the functioning of the Calvin cycle.

Light energy is captured by antenna pigments and transferred to the reaction centres, from which the energy passes through the electron transport chain,

Table II. Percent ground cover of rocks and shrub canopy cover and characteristics of two populations of *P. lentiscus* at a coastal and a mountain site. The cover data are means \pm SE within four random 400 m² plots per site. All other measurements are means \pm SE of eight specimens for which gas exchange data are presented.

Parameter	Coastal site	Mountain site	Significance
Ground cover of rocks (%)	32.5 \pm 4.3	53.8 \pm 8.5	ns
Canopy cover of <i>P. lentiscus</i> (%)	27.5 \pm 4.8	16.3 \pm 3.1	ns
Canopy cover of other shrubs (%)	23.8 \pm 2.4	51.3 \pm 2.4	ns
Plant height (cm)	94.5 \pm 6.0	167.3 \pm 14.3	$P < 0.001$
Plant diameter (cm)	317.5 \pm 33.8	270.9 \pm 26.8	ns
LAI (m ² leaves·m ⁻² ground)	1.7 \pm 0.2	1.8 \pm 0.1	ns
LMA (g·m ⁻²)	238.4 \pm 5.9	213.5 \pm 7.3	$P < 0.05$
Leaf N (mg·g ⁻¹ dry weight)	13.2 \pm 1.0	11.2 \pm 1.0	ns

generating ATP and finally reducing NADP⁺ to NADPH, which may be used for CO₂ assimilation in the Calvin cycle [14]. This is a step occurring in the thylakoid membranes of chloroplasts, and it can be affected basically by two constraints: photoinhibition and thermal energy dissipation in the antenna. Photoinhibition results from a degradation of the chlorophyll-protein complexes as a result of excess light. It is reflected by lowered values of pre-dawn F_v/F_m and occasionally by reduced ETR [29, 33]. The thermal energy dissipation in the antenna is a photo-protective mechanism, which lowers the excitation pressure on PSII reaction centre [7]. Hence, if the main limitation is at the photochemical level, we expect low F_v/F_m values indicating photoinhibition and, occasionally, low ETR values but not necessarily any major effect on gas exchange parameters.

CO₂ enters the leaves through the stomata, which can be more or less open depending on environmental conditions including water availability [9]. The degree of stomata opening is measured by stomatal conductance (g). If the main limitation is stomata closure, we expect low g and, consequently, decreased C_i. The intrinsic water-use efficiency (A/g) is expected to increase during the initial phase of drought due to higher reduction of g than of A. The apparent carboxylation efficiency (A/C_i) is likely to remain unaffected or be only slightly reduced. F_v/F_m and ETR are likely to remain high or to be slightly reduced due to electron consumption by other processes than carbon assimilation. These could be increased photorespiration and/or direct O₂ reduction at the level of photosystem I, a process known as the Mehler reaction [32, 34]. These reactions are possible due to the high O₂ concentration inside the chloroplast despite stomata closure and are reflected by an increase in the ETR/A ratio [12, 13].

The ATP and NADPH produced and the CO₂ captured by the stomata are used for carbon fixation in

the stroma through the Calvin cycle. In contrast to stomatal limitation, impairment of the Calvin cycle components and enzymes like Rubisco will lead to increased C_i and, consequently, low A/C_i. Because this can occur regardless of water availability, water may be abundant and, hence, A/g can be low, indicating higher limitation of the photosynthetic capacity per se than a reduction due to stomata closure, resulting in high A/g [28, 30].

3. RESULTS

3.1. Site and populations characteristics and meteorological conditions

Both sites were rocky (*table II*) and had an open canopy cover of shrubs. In the mountains, the shrubs of *P. lentiscus* were higher than at the coastal site and had slightly lower LMA but similar area extension, LAI and leaf N concentration. In spite of the differences in shrub height, LAI did not differ between the sites because the shrubs at the mountain site had less and very few leaves on the basal part of the branches.

The monthly averaged minimum temperatures were 2–5 °C higher at the coastal site than in the mountains (*figure 1*). The monthly maxima, however, were similar from May to the beginning of August, after which there was a more pronounced decline in temperatures in the mountains.

The annual precipitation was about 2-fold higher in the mountains than at the coast. The greatest difference was between autumn and spring, while there was a pronounced period of drought during summer at both sites. In the growing season of 1998–1999, the autumn rains started in early October in the mountains and in late October at the coast. The rain ceased at the coast

already in December and only 29 mm fell from February to April, while the precipitation in the mountains was several-folds higher (figure 1).

3.2. Annual variation of photosynthesis and stomatal conductance at the contrasting sites

Net photosynthesis (A) and stomatal conductance (g) differed significantly ($P < 0.001$) across the annual cycle but with differences in patterns between the two sites (figure 2A, B), shown by highly significant site \times date interactions (table III). Similarly, the intrinsic water-use efficiency (A/g) differed significantly ($P = 0.005$) over the seasons but not between the sites (table III), except during spring. In spring, A/g was significantly higher ($P = 0.037$) at the coastal site than at the mountain site (figure 2C). This effect was even larger in new, developing leaves ($P = 0.006$, data not shown).

At the coastal site, both A and g decreased from about $7 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $60 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, in June 1998 to a significant minimum of $1.9 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $22 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in October (figure 2A, B). These reductions also coincided with a significant decrease in the intrinsic water-use efficiency (A/g) to an annual minimum in October (figure 2C). After the autumn rains, A and g increased significantly to an annual peak of about $12 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $160 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in January, while A/g increased less and non-significantly. After the peak in January, A and g decreased significantly, and A/g increased in April and May 1999 when very little rain fell (figure 1). Averaged over the six dates of measurements, the means of A and g were $6.7 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $73 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively.

At the mountain site, the June values of both net photosynthesis and stomatal conductance reached $11 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $110 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ respectively (figure 2A, B). A declined to a minimum of slightly above $1.6 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and g to $16 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in August and had increased again already in October to about $8.4 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $95 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These levels were maintained at the subsequent measurements in early February, after which they showed an increasing trend to an annual peak of $12.6 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $148 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by the end of May. The intrinsic water-use efficiency decreased non-significantly from June to October, after which it stayed approximately constant. Hence, compared with the coastal site, the summer depression of the gas exchange was appreciably shorter, and mid winter gas exchange was appreciably lower but recovered in spring to levels close to those in January at the coast. Hence, there was no pronounced mid winter peak and no depression in spring as at the

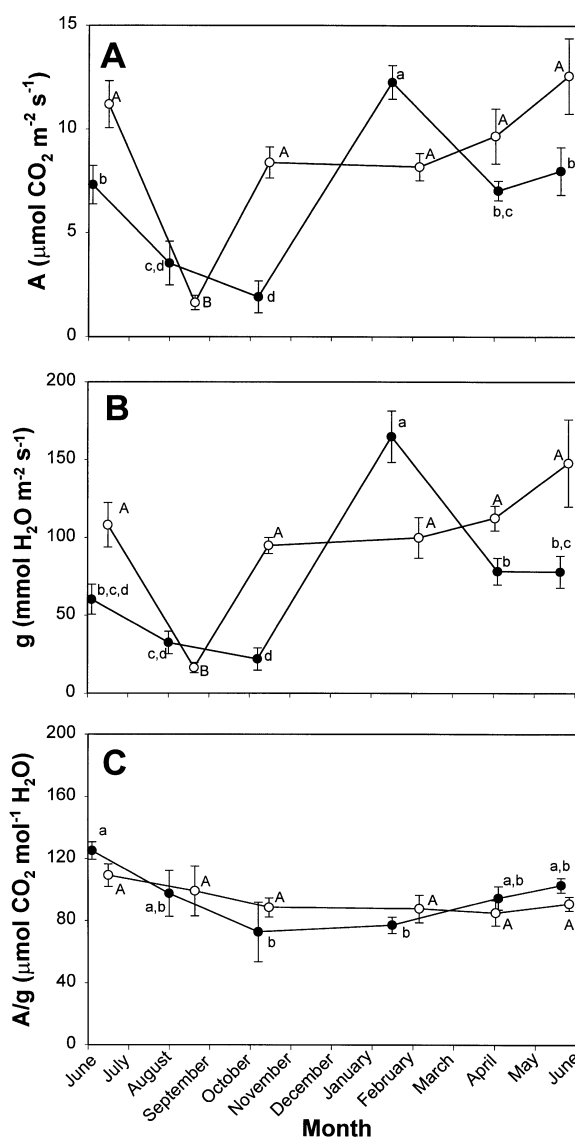


Figure 2. Annual variations of A) net CO_2 assimilation (A, $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); B) stomatal conductance (g, $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); C) intrinsic water-use efficiency (A/g, $\mu\text{mol CO}_2\cdot\text{mol}^{-1}\cdot\text{H}_2\text{O}$) in *Pistacia lentiscus* at a coastal site, Sa Vall (filled symbols) and a mountain site at Binifaldó (open symbols). Data are means of eight replicates \pm SE. Different letters show significantly different levels among dates within each site, with capital letters used for the mountain site and lower case letters for the coastal site.

coastal site. Averaged over the six dates of measurements, the means of A and g were $8.6 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $97 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, i.e. appreciably higher than the values at the coastal station.

The levels of sub-stomatal leaf CO_2 concentration (C_i) did not differ significantly between the sites but

Table III. Analysis of variance on net photosynthesis (A), stomata conductance (g), intrinsic water-use-efficiency (A/g), substomatal CO₂ concentration (C_i) and apparent carboxylation efficiency (A/C_i) at a mountain (Binifaldó) and a coastal (Sa Vall) site over six sampling occasions (time) during a year.

	A		g		A/g		C _i		A/C _i	
	F	P	F	P	F	P	F	P	F	P
Site	10.43	0.002	11.98	0.001	0.09	0.764	0.00	0.954	4.85	0.031
Time	27.01	< 0.001	20.59	< 0.001	3.61	0.005	3.92	0.003	11.60	< 0.001
Site × time	10.27	< 0.001	9.61	< 0.001	0.88	0.498	0.70	0.623	3.74	< 0.004

they varied significantly across the season (*table III*) with an annual minimum in early summer (*figure 3A*). At the coastal site, C_i increased significantly until October and declined again until May. At the mountain site, the annual variation was much less pronounced but with a weak tendency to lower concentrations in late spring and summer compared to autumn and winter.

The apparent carboxylation efficiency, i.e. the ratio A/C_i, followed the seasonal variation in photosynthesis closely with significant differences between sites and dates and also a significant site × date interaction (*table III*). The highest averaged annual mean of 0.047 mol·mol⁻¹ was recorded at the mountain site compared with 0.038 mol·mol⁻¹ at the coastal site (*figure 3B*). Also the largest annual range was measured at the mountains, where the A/C_i declined from 0.068 mol·mol⁻¹ in early summer of 1998 to 0.010 mol·mol⁻¹ in August, after which it increased again in October and stayed at an even level until late spring when it rose further to 0.065 mol·mol⁻¹. At the coastal site, the range was between 0.012 mol·mol⁻¹ in October and 0.057 mol·mol⁻¹ in January.

3.3. Responses to chilling and mild water stress

In the controlled experiment, the values of net photosynthesis and A/C_i ratios in watered plants were close to values measured in the field at times of the year with low stress (cf. *table IV* and *figures 2* and *3*). However, g was higher as a consequence of the watering, resulting in lower A/g values. The intrinsic quantum yield efficiency of photosystem II (F_v/F_m) was 0.8, i.e. a level that is expected for healthy plants without any physiological constraint on photosynthesis [3]. The electron transport rate (ETR) was 128 mmol·m⁻²·s⁻¹, a rate that is expected from other measurements in field-grown Mediterranean evergreen shrubs [36, 45] and in other broad-leaved shrubs [11, 21, 45]. Also, the values of ETR/A were consistent with those found in well-watered C₃ plants [11, 26].

Plants subjected to low temperatures in winter showed 1/3 lower net photosynthesis of 8.0 μmol

CO₂·m⁻²·s⁻¹ compared to the June value of 12.5 μmol CO₂·m⁻²·s⁻¹, but similar stomatal conductance and C_i. This led to lower intrinsic water-use efficiency and A/C_i than in June (*table IV*). The mean F_v/F_m value

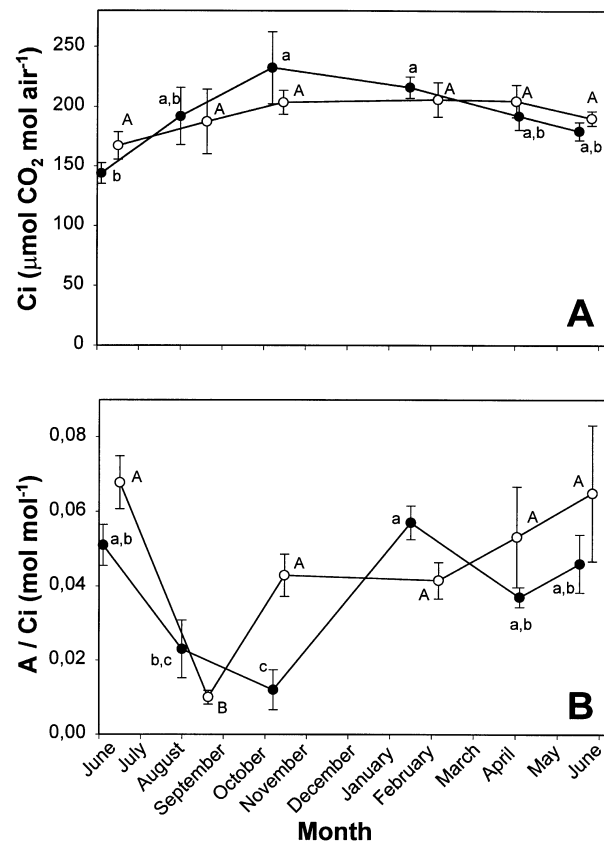


Figure 3. Annual variations of A) sub-stomatal CO₂ concentration (C_i, μmol CO₂·mol⁻¹ air); B) apparent carboxylation efficiency (A/C_i, mol CO₂ fixed·mol⁻¹ CO₂ present at the sub-stomatal space) in *Pistacia lentiscus* at a coastal site, Sa Vall (filled symbols) and a mountain site, Binifaldó (open symbols). Data are means of eight replicates ± SE. Different letters show significantly different levels among dates within each site, with capital letters used for the mountain site and lower case letters for the coastal site.

Table IV. Net photosynthesis (A , $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (g , $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), intrinsic water-use-efficiency (A/g , $\mu\text{mol CO}_2\cdot\text{mol}^{-1}\text{ H}_2\text{O}$), substomatal CO_2 concentration (C_i , $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$ air), apparent carboxylation efficiency (A/C_i , mol CO_2 fixed· $\text{mol}^{-1}\text{ CO}_2$ present at the sub-stomatal space), intrinsic photochemical efficiency of PSII (F_v/F_m), electron transport rate (ETR) and ETR/A measured in well-watered *Pistacia lentiscus* in June 1999, under mild drought in September 1999 and under low temperatures in December 1995. Data are means \pm SE.

	Well-watered	Mild water stress	Low temperatures
A	12.5 \pm 2.3	7.7 \pm 0.4	8.0 \pm 0.2
g	243.8 \pm 28.8	86.6 \pm 6.0	224.2 \pm 9.3
A/g	50.4 \pm 4.5	91.9 \pm 4.0	36.0 \pm 0.9
C_i	255.5 \pm 10.0	181.8 \pm 5.5	244.4 \pm 8.89
A/C_i	0.050 \pm 0.010	0.043 \pm 0.002	0.033 \pm 0.002
F_v/F_m	0.798 \pm 0.004	0.776 \pm 0.004	0.660 \pm 0.007
ETR	128.1 \pm 8.4	115.3 \pm 5.5	138.6 \pm 7.3
ETR/A	10.2 \pm 2.5	14.9 \pm 1.7	17.4 \pm 1.4

was 0.66, indicating constrained photochemical function. Similar low winter values of F_v/F_m , ranging from 0.61 to 0.67, and much higher spring values of above 0.80 have been measured at other occasions and sites on Mallorca (data not shown). However, the ETR values were not affected and, as a consequence, ETR/A increased.

When net photosynthesis was reduced by mild water stress to 7.7 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, i.e. close to the winter values (table IV), stomatal conductance declined to 86.6 $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. A/g increased to 91.9 $\mu\text{mol CO}_2\cdot\text{mol}^{-1}\text{ H}_2\text{O}$ compared to 50 and 36 $\mu\text{mol CO}_2\cdot\text{mol}^{-1}\text{ H}_2\text{O}$ in June and winter, respectively. However, A/C_i was maintained at a relatively high level of 0.043 $\text{mol}\cdot\text{mol}^{-1}$ compared with 0.050 $\text{mol}\cdot\text{mol}^{-1}$ in June because A and C_i were reduced by about the same proportion, but it was above the ratio of 0.033 $\text{mol}\cdot\text{mol}^{-1}$ measured in winter. The F_v/F_m ratio of 0.78 was little affected in comparison with the level of irrigated plants, but it was appreciably above the winter value of 0.66. ETR of 115 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was slightly reduced in comparison to irrigated plants and was appreciably lower than 139 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ measured in winter. As a consequence, ETR/A increased in comparison to irrigation, but it was lower than in winter.

4. DISCUSSION

4.1. Site characteristics, annual and site-related variations in climate and gas exchange: stomatal regulation

The data show a seasonal pattern of strong depression of gas exchange and stomatal conductance during

the summer followed by a recovery in autumn. Hence, this pattern suggests stomata-controlled gas exchange triggered by variations in soil water availability, which is commonly found in shrubs of the Mediterranean region [4, 17, 18], including *Pistacia lentiscus* [5].

However, the annual variation in gas exchange is site-related, with a shorter summer depression at the mountain site than at the coast. Indeed, at the mountain site both net photosynthesis and stomatal conductance had recovered by the October measurements, while the gas exchange reached the seasonal minimum at that time at the coast. It also appears that this variation coincides with between-site variation in precipitation, as the onset of the autumn rain was delayed at the coast compared to the mountains. After the autumn recovery, the mountain population showed a longer period of high net photosynthesis and stomatal conductance with A and g values increasing until June. In contrast, the coastal population presented a pronounced peak in January, when net photosynthesis and stomatal conductance were about 1.5 times higher than in the mountain site. However, the values decreased to a level below that observed in the mountain population by April, coincident with a trend of increasing water-use efficiency. Again, this pattern is consistent with local variation in precipitation. The rain had fallen abundantly in the mountains throughout the winter, while the coast had experienced a drought period from late January on with almost no rain to replenish the soil reserves (figure 1). The differences in gas exchange rate between the mountain and the coastal population in spring and early summer is probably related to soil water availability. The drought started much earlier at the coastal site, when the available soil water was still abundant in the mountain site. Due to the late onset of the autumn recovery and the lower A

and g in spring, the net photosynthesis and stomatal conductance integrated over the entire year were 22 and 25 % lower, respectively, at the coastal site than in the mountain site. This suggests, first, much higher constraints to the photosynthetic function at the coast than in the mountains. Second, there was no noticeable effect of differences in soils or any adjustments in the vegetation that cancelled out the response to the climatic differences at leaf level between the two sites. Hence, the climatic conditions that translated into pronounced differences in soil water availability, apparently was the main driving force on the major pattern of gas exchange through climatic control of stomatal function, rather than adjustments of shrub density or transpiring leaf area for example [38].

From a previous study showing increased leaf $\delta^{13}\text{C}$ with increasing drought along the same gradient, we expected to find differences in A/g between the sites [23]. Contrary to our expectations, we did not find such differences on an annual basis, even though A and g viewed separately differed (*table III*). However, de-correlation between $\delta^{13}\text{C}$ and A/g is usually found in studies covering long time-scales like ours and different reasons have been suggested for this [37, 39]. We have strong reasons to believe that, in our study, it was because the level of $\delta^{13}\text{C}$ was determined most of all by the proportion of ^{13}C incorporated into the leaves during the period of leaf formation and accumulation of structural tissue in spring. Indeed, at this time A/g in the 1-year-old leaves was higher at the coastal site than in the mountain (*figure 2C*). Moreover, unpublished data show that the difference in A/g in the developing leaves was even greater (about 60 %) at the same time as the $\delta^{13}\text{C}$ level was about 1.5–2 % higher. This difference in $\delta^{13}\text{C}$ persisted throughout the first year of the leaves' life-time.

4.2. Non-stomatal regulated reductions of photosynthetic function

Although the precipitation pattern and the soil water availability apparently trigger the summer decline and autumn rise of gas exchange, water availability and stomatal regulation cannot explain the detailed variations in annual and site-related gas exchange. For instance, the high rate of photosynthesis and water exchange at the coastal site during winter cannot be explained by higher availability of water at the coast than in the mountains. Instead, it is likely that the level of gas exchange was controlled mainly by temperature [2], particularly in the interval just above freezing. The monthly maximum temperature in January and February, the two coldest months, were about 14.5 °C at the coast, and the minimum temperature was at or above 5 °C (*figure 1*). In the mountains, the maxima for

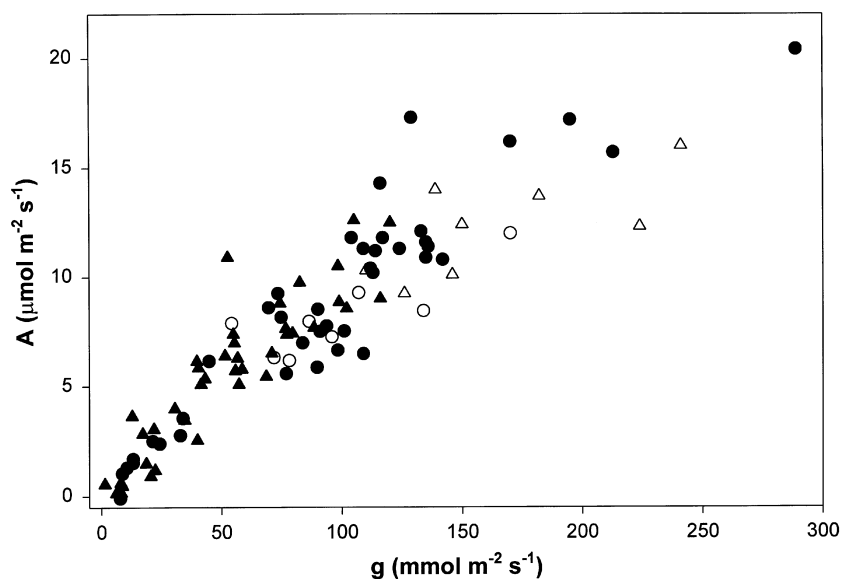
January and February were only around 11.5 and 10 °C and the minima were 3.1 and 1.8 °C (*figure 1*). This implies that the mountain site has several days with minimum temperatures below freezing. Indeed, snowfall is common at this site in winter, but extremely rare on the coast. Hence, the lower temperature at the mountain site probably explains the much lower rate of gas exchange in mid winter than found at the coast.

Low winter temperature is well known to limit photosynthesis in Mediterranean evergreen plants [22, 42]. Furthermore, a high winter depression has been measured previously in *P. lentiscus* by Gratani [16], who reported lower winter photosynthetic rates than in July and October. Tenhunen et al. [42] and Harley et al. [17, 18] have predicted very low values of carboxylation efficiency for evergreen sclerophylls as a consequence of low temperatures during winter.

We found an appreciably lower mid winter carboxylation efficiency (A/C_i) and net photosynthesis in the mountain population than in the coastal population (*figure 3B*), coinciding with 3–4 °C lower temperature and a monthly minimum temperature just above freezing. At the same time, net photosynthesis was much less sensitive to variations in g (*figure 4*), indicating non-stomatal limitation of A . As the temperature increased, A/g at the mountain site remained low through April to May, while the carboxylation efficiency increased. Hence, it appears that the photosynthetic capacity rather than the stomatal function restricted photosynthesis in mid winter, which is a general response to low temperatures in chilling-sensitive plants [19]. This limitation decreased with increasing temperature in spring.

In summer, as stomatal conductance decreased, a similar decline of the A/C_i ratio also took place, followed by a new rise after the onset of the autumn rain (*figure 3B*). This suggests a combined effect of stomatal and non-stomatal controlled photosynthetic depression [17, 18, 41–43]. Unfortunately, we have no data on g and C_i from late June and July when the drought increased. However, it is likely that the first response to initial summer drought is a closure of stomata, and that prolonged exposure to low water availability and high temperatures and irradiance leads to a progressive loss in photosynthetic capacity through reduced Rubisco activity and/or enzyme degradation. Indeed, in the controlled experiment we performed, the imposed mild water stress led to a large reduction of g , increased A/g and a reduction of C_i . Hence, it appears that the first response to water deficit was stomata closure [4, 8, 43]. However, carboxylation efficiency remained almost unaffected. This supports the idea that the decreased A/C_i as summer progressed reflects a photosynthetic adjustment that

Figure 4. Relationship between net CO₂ assimilation (A , $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and stomatal conductance (g , $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in *Pistacia lentiscus* from June 1998 to May 1999. Triangles correspond to the coastal site, and circles to the mountain site. Open symbols are measurements from mid winter (January) and filled symbols are measurements from spring to autumn. The slope of the linear regression for the spring to autumn measurements was 0.081 and for the mid winter measurements 0.048.



takes place not as an immediate response to water shortage, but as a consequence of prolonged stomata closure.

4.3. Photochemical reduction of photosynthesis

F_v/F_m and the electron transport rate (ETR) remained unaffected by the imposed mild water stress (table IV), showing that reductions in photochemical capacity (photoinhibition) were not responsible for depression of net photosynthesis. This is consistent with the high resistance to photoinhibition exhibited by other Mediterranean sclerophylls under summer drought, contrary to the higher susceptibility of malacophyll shrubs [45]. As a consequence of the reduced A and unaffected ETR, the ratio ETR/ A increased. This could be due to increased photorespiration [12, 13, 44], which is a common response to water stress [28], and may be a mechanism to avoid photoinhibitory damage when photosynthesis is reduced by carbon availability [25, 34]. Indeed, Correia and Díaz-Barradas [6] showed that F_v/F_m in *P. lentiscus* remained high in July at the early stages of summer drought but that the ratio decreased to less than 0.7 in August, probably as a consequence of reduced carboxylation efficiency and/or photosynthetic capacity.

Low temperatures reduced F_v/F_m , suggesting photoinhibition (table IV). However, photoinhibition may not be the main cause for photosynthesis limitation, but rather a consequence of it, because ETR was higher than expected if photoinhibition were important. We measured F_v/F_m and ETR on a cloudy day, while the day before the measurement was sunny. It is

likely therefore that the high irradiance the previous day had led to photoinhibitory damage and reduction of carboxylation capacity, but ETR might have been able of recovering from dawn to midday. If so, the proximate limitation to photosynthesis was a reduced activity of the Calvin cycle enzymes [13, 19] rather than photoinhibition. As observed under mild water stress, ETR/ A increased. In contrast to mild water stress, however, such an increase probably reflected increased electron transport through the Mehler reaction [1, 13, 24] and not photorespiration. This is because reductions in the activity of Calvin cycle enzymes under chilling temperatures would also reduce the photorespiratory activity of the leaves.

5. CONCLUSIONS

Our results agree with well-known patterns of annual variation of gas exchange in Mediterranean evergreen shrubs [16–18]. The general pattern of photosynthesis was characterised by high rates in autumn and spring and lower rates in winter and, particularly, during the summer drought. Thus, the main control of photosynthesis apparently is through stomatal limitation, triggered by variation in water availability and second the function of the Calvin cycle affected mainly by temperature conditions, possibly overlaid by photoinhibition, particularly in winter. Initially, stomatal control and, later, impaired Calvin cycle activity are the major causes of photosynthetic depression during summer. In winter, impaired Calvin cycle activity seems to be the main

mechanism involved in photosynthetic reduction. Photoinhibition does not seem to be important at any time of the year in this species.

In addition, we show that local variability in the climate can cause dramatic differences in photosynthesis between populations within short distances. These differences are likely to be translated into large differences in the annual carbon balance between local populations of the same species. The coastal population showed lower net photosynthesis during most of the year than the mountain population due to more constrained water availability. This agrees with earlier demonstrated differences in $\delta^{13}\text{C}$ and leaf formation rate between populations along moisture gradients [23]. However, as the temperature was higher at the coast than in the mountains, winter net photosynthesis in the coast temporarily exceeded the net photosynthesis in the mountain population until it was reduced by low water availability. The pronounced differences we measured may not be equally pronounced from year to year because the temperature and rainfall patterns in the Mediterranean zone are subject to strong inter-annual variations and stochasticity [10].

However, in a perspective of a likely temperature increase and decrease in precipitation due to anthropogenic changes in atmospheric gas composition [20], we can foresee pronounced differences in carbon balance between local populations of Mediterranean evergreen species. This is because both variables would tend to prolong the periods of drought in summer and reduce the period of high water availability in autumn and winter. This would, logically, affect populations that already grow under high drought stress more than populations growing under less drought stress conditions, paralleling the differences we found between the coastal and the mountain sites.

Acknowledgments. Don G. Bonet kindly gave permission to perform part of the fieldwork at the Sa Vall estate, and Dr A. Michelsen helped with data processing. S.J. is grateful for logistic support from 'Institut Mediterrani d'Estudis Avançats' (CSIC-UIB).

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