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## Original article

# Gender, season and habitat: Patterns of variation in photosynthetic activity, growth and fecundity in *Thymelaea velutina*

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## ABSTRACT

Changes in the ecophysiological performance of a plant species due to different environmental conditions generally reflect adaptations to the habitat where the plant grows and are often related to its survival capacity in a particular place. We examined this with the dioecious shrub *Thymelaea velutina*, in two contrasting populations representing the extremes of the altitudinal gradient where the species lives (coastal dunes and mountain habitats over 1000 m). We measured net photosynthetic rates and stomatal conductance, estimated the level of plant stress by chlorophyll fluorescence, and assessed their correlations with growth rate, plant size, flower production and fruit set. We hypothesized that plants at high altitude were more photosynthetically stressed than at sea level and expected a gender  $\times$  habitat interaction in performance as females need more resources than males. Plants in the mountain experienced chronic photoinhibition during winter and a reduced photosynthetic performance both in winter and spring compared to plants in coastal dunes. However, there was no association between any of the fluorescence variables and either plant growth or fecundity, suggesting that other factors are involved determining performance. Mountain plants showed also an apparent lower capacity of heat dissipation to excessive radiation than dune plants. In the dunes, the greater leaf area and mass can lead to a higher photosynthetic carbon gain by whole individuals compared to plants in the mountain. No effect of gender was detected on the ecophysiological performance of this species, which we partly attribute to the small size of fruits of the female plants.

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## 1. Introduction

The ecophysiological versatility shown by most plant species allows them to grow in very different habitats where they can differently respond to seasonal and/or diurnal variations in the environmental conditions (Flexas et al., 2001; Nieva et al., 2003; Valladares et al., 2005). This versatility is observed in aspects such as gas exchange, midday stomatal closure and down-regulation of photochemical efficiency (to avoid water losses and damage in the photosynthetic apparatus; González-Rodríguez et al., 2002a,b), resource allocation (Sultan and Bazzaz, 1993; Haukos and Smith, 2006), and leaf longevity (Jonasson et al., 1997). Thus, changes in the photosynthetic rate due to changing environmental conditions reflect adaptations to the habitat where the plant grows and are in general related to its survival capacity in a particular place. For instance, the salt marsh plant *Spartina densiflora* is able to tolerate a wide elevational range across the tidal frame, adjusting its photochemical efficiency and net photosynthesis to the degree of salinity and to the duration of flooding periods (Nieva et al., 2003). In the Mediterranean shrub *Pistacia lentiscus*, the capacity to grow along a gradient of moisture and temperature is associated with a flexible gas exchange strategy; the main control of photosynthesis in this species is through stomatal limitations, which is in turn determined by water availability (Flexas et al., 2001). Still another example is found in *Swietenia macrophylla*, a tree species established on degraded lands in Central Amazonia, its photosynthetic capacity and growth is higher at open sites than in shady environments, which allows the species to tolerate high irradiances and the successful establishment of its seedlings in large gaps (Marenco et al., 2001). This has also been observed in a number of tropical and temperate shrubs and trees (Valladares et al., 2000, 2002, and references therein). Even when differences in ecological conditions are small, they can translate into large changes in the photosynthetic activity, at least during the period of higher stress, being able to limit the distributional area of a given species, as it occurs with Mediterranean Holm oak (*Quercus ilex*, Tretiach et al., 1997). By contrast, certain species can maintain rather constant photosynthetic rates despite limiting environmental conditions by means of remarkable morphological and physiological adjustments, as reported for *Metrosideros polymorpha*, the dominant tree species in Hawaiian forest ecosystem growing from sea level to 2500 m (Cordell et al., 1999). Thus, plants coping with contrasting environments can either adjust their photosynthetic performance to each particular environment or adjust a number of morphological and physiological traits to keep a homeostatic photosynthetic performance.

In the present study, we examined the physiological performance of a Balearic endemic, dioecious shrub, *Thymelaea velutina*, which lives from sea level to relatively high elevations (c. 1200 m). Population densities, plant size and fruit set of this species are higher at sea level (in dune systems) than in mountain sites (de la Bandera and Traveset, 2006a,b). Our main goal was to determine whether the species exhibits a different photosynthetic behavior in the two populations representing the two extremes of the altitudinal

gradient. Gender, habitat and season were explored as main sources of variation in photosynthetic features. Specifically, we aimed: (1) to determine if there are differences between the two sites in photochemical efficiency and overall level of stress; (2) to assess whether males and females have a different photosynthetic performance; and (3) to investigate whether photosynthetic features are related to growth, plant size, or fecundity as three surrogates of overall performance. Season and habitat are the primary sources of variation in ecophysiological features and a large number of studies have documented the responses of many species to these two factors (Larcher, 1995). Variations in ecophysiological traits between sexes of dioecious species have received far less attention despite evidences for potentially large differences in performance among sex forms (Retuerto et al., 2000; Zunzunegui et al., 2006). Gender-specific differences in ecophysiology may affect growth and reproduction, with a tendency for a greater physiological stress in the gender with greater reproductive burden (Obeso, 2002; Zunzunegui et al., 2006). Emphasis to *in situ* determinations of chlorophyll fluorescence parameters was given in this study, which was complemented with gas exchange measurements carried out also with adult plants in the field.

Among different photosynthetic parameters, chlorophyll fluorescence has been shown to be a good tool to assess plant stress, photosynthetic performance and heat dissipation (Lichtenthaler and Rindele, 1988; Maxwell and Johnson, 2000).

## 2. Material and methods

### 2.1. Study species

*Thymelaea velutina* (Pouret. ex Cambess.) Endl. is a dioecious shrub, endemic to the two largest islands of the Balearics, Mallorca and Menorca (Western Mediterranean). In Menorca, with a lower altitude (the highest mountain is 356 m tall), most populations are found at the coast. The opposite occurs in Mallorca, where most coastal populations have disappeared due to habitat alteration (mainly urbanization; Alomar et al., 1997). Only a couple of populations remain in a dune system in the north of the island, and the species is currently more common in the mountains (along the Serra de Tramuntana mountain chain, which expands from northwestern to southwestern Mallorca). The mountain populations, however, usually have a low number of individuals (de la Bandera and Traveset, unpubl. data), and a previous study has shown that plant size and fruit set are significantly greater in the coastal dunes than in the mountain habitat (de la Bandera and Traveset, 2006a). Plants in the coastal dunes are taller than in the mountain ( $55.66 \pm 2.19$  cm,  $N = 71$  vs  $24.19 \pm 1.11$  cm,  $N = 91$ , respectively). The flowering period occurs during spring and early summer, plants producing inflorescences that bear three to five flowers each. Male flowers are yellow, bear eight stamens and present a rudimentary (aborted) ovary. Female flowers are greenish, have a unilocular ovary and are slightly smaller than male flowers. Flowers of both sexes produce small amounts of nectar. Flowering synchrony between males and females is almost absolute and the lifetime of a flower is three to 4 days.

The species is ambophilous, being pollinated by both insects and wind (de la Bandera and Traveset, 2006a). *T. velutina* is a heterocarpic species, the same female producing simultaneously dry (achenes) and fleshy (drupes) fruits in (de la Bandera and Traveset, 2006b). Although *T. velutina* is a perennial shrub, it can be considered a semi-deciduous species, as it loses a considerable part of foliar mass during the summer drought. A full description of the species can be found in Pedrol (1997), and more details on the reproductive ecology of the species can be found in de la Bandera and Traveset (2006a,b).

## 2.2. Study sites

The study was carried out in two Mallorcan localities representing the two types of habitat where the species lives. The coastal dunes locality was within S'Albufera Natural Park, near Ca'n Picafort (UTM: 31S EE10); here, *T. velutina* is rather abundant (with several hundred individuals) and lives on fixed dunes, coexisting with other species like *Cistus salvifolius* L., *Erica multiflora* L., *Halimium halimifolium* (L.) Willk., *Rosmarinus officinalis* L. and *Teucrium dunense* Sennen. Mean annual temperature and precipitation at this locality are 17 °C and 628 mm, respectively (Guijarro, 1986). Flowering of *T. velutina* takes place from February to early May, the flowering peak occurring in late March. Sex ratio is 1 female:1.5 males ( $N = 429$ ). The mountain locality is at Puig Major, the highest Mallorcan peak (1450 m), specifically in a site named Sa Coma de N'Arbona (UTM: 31S DE70, 1250 m) located within a military area protected of human disturbances. The predominant species here are *Ampelodesmos mauritanica* (Poiret) T. Durand and Schinz, *Hypericum balearicum* L. *Teucrium marum* L. and *Rosmarinus officinalis* L. The population of *T. velutina* here consists of less than 150 individuals and the sex ratio is 1 female:1.6 males ( $N = 120$ ). Mean annual temperature is 9 °C and mean annual precipitation is 1121 mm (Guijarro, 1986). The flowering period here is later and shorter than in the dune population, extending from May to June, with the peak in late May–early June.

## 2.3. Fluorescence and gas exchanged measurements

By means of a pulse-modulated fluorometer (FMS2, Hansatech Instruments Limited, Norfolk, UK), we determined the photochemical efficiency of photosystem II (PSII) and a set of fluorescence variables in fully expanded leaves of different individuals from each population. In the coastal dunes, measurements were taken on December 19th 2004 in a total of 10 males and 10 females and on April 2nd 2005 on the same individuals plus two extra males and females. In the mountain habitat, fluorescence measurements were obtained on December 18th 2004 (from eight males and eight females) and on May 21st 2005 (from a total of 11 males and 11 females, which included seven males and six females of those measured in December). All measurements were taken between 11:00 and 17:00 h. Spring measurements coincided with the peak of the flowering period in each population. Selected leaves from each plant were first darkened for 30 min with leaf clips bearing a shutter that could be opened when the fluorometer probe was attached and  $F_w/F_m$

measurements made in the absence of any light. Preliminary tests revealed that NPQ (non-photochemical quenching) values did not significantly change for longer darkening periods. We recorded the fluorescence parameters  $F_o$  and  $F_m$  at darkness, and then switched on the internal halogen lamp of the fluorometer without removing the leaf from the leaf clip. The evolution of fluorescence under light was monitored until the fluorescence signal became stable, which typically took 3–5 min. After this period of exposure to an actinic light of  $269 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  a far red short pulse followed by an over-saturating pulse ( $>6000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were provided to obtain the fluorescence parameters  $F$  (baseline fluorescence under actinic light),  $F_m'$  and  $F_o'$  (maximum and minimum fluorescence when over-saturating pulse was applied under actinic light). The same protocol was followed with a saturating actinic light of  $1810 \mu\text{mol m}^{-2} \text{s}^{-1}$ . We then calculated the following chlorophyll fluorescence variables: (1) photochemical efficiency ( $F_v/F_m = (F_m - F_o)/F_m$ ), a measure of the maximum efficiency of PSII; (2) apparent electron transport rate at saturating light ( $\text{ETR} = \text{quantum yield} * 1810 \mu\text{mol m}^{-2} \text{s}^{-1} * 0.5 * 0.84$ ; quantum yield being the photochemical efficiency under actinic light and 0.84 being the expected mean leaf absorbance); (3) photochemical quenching ( $qP = (F_m' - F)/(F_m' - F_o')$ ), which gives an indication of the proportion of PSII reaction centers that are open; and (4) non-photochemical quenching ( $\text{NPQ} = (F_m - F_m')/F_m'$ ), a measure of the efficiency of apparent heat dissipation relative to the dark-adapted state (Maxwell and Johnson, 2000). NPQ determinations in the field can be problematic because  $F_m$  may be sensitive to the previous history of the plant. We took the measurements when plants were not exposed to punctual or transient stress, although we cannot preclude this source of error completely. Thus, when we refer to NPQ we imply apparent heat dissipation since estimates of real absolute heat dissipation would require measurements under controlled conditions in the laboratory (Maxwell and Johnson, 2000).

Net photosynthesis ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $g$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) were measured in April 24th 2001 (from eight males and eight females) and January 29th (from nine males and seven females) 2002 in Ca'n Picafort and in April 6th 2002 at the mountain site (from eight males and eight females), by using a Li-6400 system (Li-Cor Inc., NE, USA), a portable open-circuit infrared gas analyser. Light intensity was held constant at  $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  to ensure light-saturated photosynthesis and the  $\text{CO}_2$  partial pressure was that of the atmosphere (360–370 ppm). Measurements of  $A$  and  $g$  were recorded between 11:30 and 13:00 h both days and in both populations.

## 2.4. Growth and plant size

In spring, we recorded annual stem growth -by measuring the extension from the previous year of selected branches- and flower numbers in five branches from 66 (33 males and 33 females) and 38 (19 males and 19 females) individuals in Ca'n Picafort and Puig Major, respectively.

We further randomly chose and cut a short branch from each individual, keeping them in separate paper bags and carrying them to the laboratory where, after separating leaves

and stems, we measured: stem length and weight, area and perimeter of leaves, and fresh weight of leaves. Dry weight of leaves and stems was obtained after placing them in a stove at 60 °C for a minimum of 48 h. We then calculated the following ratios: leaf area to leaf dry mass (specific leaf area, SLA), leaf area to total dry mass (leaf area ratio, LAR) and leaf dry mass to total dry mass respectively (leaf weight ratio, LWR).

Parameters describing plant size (height, crown diameter, and stem diameter) and fecundity (flower production and fruit set) from the measured individuals of *T. velutina* were obtained from a previous study directed to investigate the reproductive system of the species in both environments (de la Bandera and Traveset, 2006a).

2.5. Statistical analysis

Differences between populations and sexes in the fluorescence variables  $F_v/F_m$ , ETR, qP and NPQ were tested by means of repeated-measurements ANOVA. Only individuals measured in both seasons were included in these analyses as the repeated subject. The Pearson correlation was used to test for an association between the fluorescence variables and plant growth rate, size, flower production and fruit set. Net photosynthesis (A), stomatal conductance (g)

and intrinsic water use efficiency (A/g) were compared between populations and sexes by means of a two-way ANOVA. Seasonal differences in A, g and A/g in the dune population were tested with t-tests. All statistical analyses were performed with STATISTICA (v. 6.0).

3. Results

3.1. Spatial and seasonal variations of fluorescence variables

Photochemical efficiency ( $F_v/F_m$ ) was significantly higher in the dune than in the mountain population ( $F_{1,29} = 39.66$ ,  $**P < 0.01$ ; Table 1). Differences between sexes were not significant ( $F_{1,29} = 2.51$ ,  $P >> 0.05$ ) and there was no significant interaction between population and plant sex ( $F_{1,29} = 0.42$ ,  $P >> 0.05$ ). By contrast, differences existed in  $F_v/F_m$  between seasons, though only in the mountain site ( $F_{1,29} = 16.46$ ,  $**P < 0.01$ ); this is why the interaction season × population was significant ( $F_{1,29} = 25.53$ ,  $**P < 0.01$ ; Fig. 1A).

ETR values did not differ between populations or sexes ( $F_{1,29} = 1.64$ , and  $F_{1,29} = 0.01$ , respectively  $P >> 0.05$ ; Fig. 1B). However, ETR was significantly greater in spring than in winter ( $F_{1,29} = 19.72$ ,  $**P < 0.01$ ; Fig. 1B). The interaction

**Table 1 – Values of chlorophyll fluorescence variables, plant size and fecundity of *Thymelaea velutina* in the two populations. Numbers in parentheses indicate sample sizes. Abbreviations:  $F_v/F_m$  = maximum photochemical efficiency of photosystem II; ETR = electron transport rate under 1810  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; qP = photochemical quenching; NPQ = non-photochemical quenching; H = height (cm); Crown D = crown diameter (cm); Stem D = stem diameter (mm); Flowers = flower production per plant, and fruit set. Letters w and s account for winter and spring.  $***P < 0.001$ ;  $**P < 0.01$ ;  $*P < 0.05$**

Variable	Sex	Coastal dunes	Mountain habitat	F-ratio
$F_v/F_m$ w	♂	0.809 ± 0.010 (10)	0.664 ± 0.026 (7)	$F_{1,15} = 34.19$ $***P$
	♀	0.832 ± 0.006 (10)	0.710 ± 0.023 (6)	$F_{1,14} = 39.69$ $***P$
$F_v/F_m$ s	♂	0.809 ± 0.012 (10)	0.793 ± 0.028 (7)	$F_{1,15} = 0.37$ ns
	♀	0.808 ± 0.012 (10)	0.798 ± 0.018 (6)	$F_{1,14} = 0.20$ ns
ETR w	♂	111.2 ± 6.0 (10)	39.0 ± 7.8 (7)	$F_{1,14} = 48.26$ $***P$
	♀	101.7 ± 14.1 (10)	48.0 ± 11.3 (6)	$F_{1,14} = 7.10$ $**P$
ETR s	♂	118.8 ± 15.9 (10)	127.18 ± 37.4 (7)	$F_{1,14} = 2.06$ ns
	♀	96.457 ± 7.7 (10)	153.6 ± 12.9 (6)	$F_{1,14} = 16.76$ $***P$
qP w	♂	0.3 ± 0.0 (10)	0.1 ± 0.0 (7)	$F_{1,15} = 48.55$ $***P$
	♀	0.26 ± 0.0 (10)	0.1 ± 0.0 (6)	$F_{1,14} = 41.19$ $***P$
qP s	♂	0.3 ± 0.0 (10)	0.3 ± 0.1 (7)	$F_{1,15} = 0.091$ ns
	♀	0.3 ± 0.0 (10)	0.4 ± 0.0 (6)	$F_{1,14} = 23.34$ $***P$
NPQ w	♂	3.6 ± 0.2 (10)	2.1 ± 0.1 (7)	$F_{1,14} = 43.26$ $***P$
	♀	3.7 ± 0.2 (10)	2.1 ± 0.2 (6)	$F_{1,14} = 41.19$ $***P$
NPQ s	♂	3.6 ± 0.2 (10)	3.2 ± 0.5 (7)	$F_{1,14} = 1.55$ ns
	♀	3.7 ± 0.2 (10)	3.5 ± 0.2 (6)	$F_{1,14} = 0.29$ ns
H	♂	55.5 ± 4.7 (10)	26.6 ± 3.3 (7)	$F_{1,20} = 35.76$ $***P$
	♀	53.5 ± 4.0 (10)	21.0 ± 2.2 (6)	$F_{1,20} = 79.29$ $***P$
Crown D	♂	98.5 ± 11.256 (10)	60.9 ± 7.9 (7)	$F_{1,20} = 5.14$ $*P$
	♀	84.9 ± 2.207 (10)	62.7 ± 10.1 (6)	$F_{1,20} = 2.58$ ns
Stem D	♂	15.8 ± 1.7 (10)	34.0 ± 18.7 (7)	$F_{1,20} = 0.06$ ns
	♀	15.0 ± 1.9 (10)	10.5 ± 1.3 (6)	$F_{1,20} = 5.42$ $*P$
Flowers	♂	36,125 ± 5768 (8)	53,000 ± 33,500 (6)	$F_{1,18} = 0.04$ ns
	♀	94,125 ± 18,898 (8)	48,333 ± 11,230 (6)	$F_{1,18} = 5.29$ $*P$
Fruit set	♂			
	♀	0.75 ± 0.05 (16)	0.57 ± 0.07 (15)	$F_{1,29} = 4.27$ $*P$

population  $\times$  season was significant ( $F_{1,29} = 18.77$ ,  $**P < 0.01$ ); differences in ETR values between spring and winter were much higher at the mountain site (Fig. 1B). Females in the mountain site showed a higher ETR than those in the dune, but only in spring (Table 1).

We found significant differences in photochemical quenching (qP) between populations ( $F_{1,29} = 5.13$ ,  $P = 0.03$ ; Fig. 1C). Values of qP were higher in the dune in winter but the opposite occurred in spring (Fig. 1C). There were no differences between sexes ( $F_{1,29} = 0.02$ ,  $P \gg 0.5$ ). However, there was a marginal interaction population  $\times$  sex ( $F_{1,29} = 4.27$ ,  $P = 0.048$ ): males showed consistently greater qP in the dune than in the mountain in the two seasons; by contrast, females had higher qP in the mountain site than in the dune, especially in spring (Table 1).

Overall values of qP were higher in spring than in winter ( $F_{1,29} = 33.65$ ,  $**P < 0.01$ ), but this was found only at the mountain site (interaction:  $F_{1,29} = 25.38$ ,  $**P < 0.01$ ; Fig. 1C). Likewise, dune plants showed a greater non-photochemical quenching (NPQ) than mountain plants ( $F_{1,29} = 25.34$ ,  $**P < 0.01$ ; Fig. 1D). Consistently in the two sites, females and males had similar NPQ values. NPQ was greater in spring than in winter ( $F_{1,29} = 8.20$ ,  $**P < 0.01$ ), but again this was observed only in the mountain population (interaction population  $\times$  season:  $F_{1,29} = 23.84$ ,  $**P < 0.01$ ; Fig. 1D).

### 3.2. Leaf traits

Leaf area, perimeter, and length were greater in the coastal dunes than in the mountain habitat ( $F_{1,190} = 92.202$ ,  $F_{1,190} = 36.270$ ,  $F_{1,190} = 105.275$ ,  $***P < 0.001$ ; Table 2). Moreover, dune plants showed higher SLA, LAR and LWR than mountain plants ( $F_{1,25} = 6.356$ ,  $F_{1,25} = 12.1140$ , and  $F_{1,25} = 5.730$ , respectively,  $P < 0.02$ ; Table 2). We found significant differences between sexes in leaf area ( $F_{1,190} = 4.346$ ,  $P = 0.038$ ); leaves are greater in females than in males, although only at the mountain site (interaction population  $\times$  sex:  $F_{1,190} = 4.997$ ,  $P = 0.0265$ ). No differences in leaf or stem water content were found either between populations or sexes (all  $P > 0.05$ ).

### 3.3. Gas exchange variations between populations

Both net photosynthesis (A) and conductance (g) were significantly higher in the dune than in the mountain ( $F_{1,30} = 6.06$ ,  $P = 0.02$ , and  $F_{1,30} = 5.23$ ,  $P = 0.03$ , respectively; Fig. 2B,C). A separate analysis (only data from the dune available) showed that both parameters were greater in spring than in winter, although differences were significant only for g ( $t = 2.47$ ,  $df = 10$   $*P < 0.05$ ; Fig. 2A,B). Differences in intrinsic water use efficiency were non-significant either between populations, sexes or seasons ( $F_{1,28} = 0.02$ ,  $P = 0.88$ ;  $F_{1,28} = 4$ ,

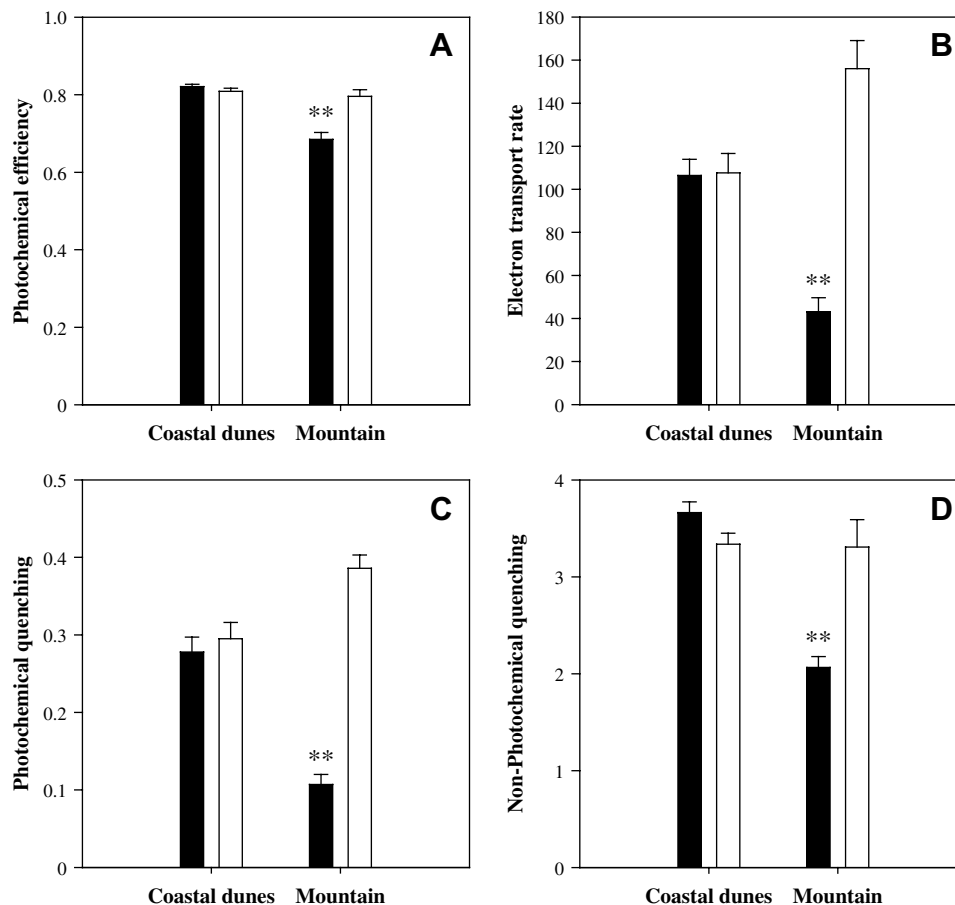


Fig. 1 – Mean values of  $F_v/F_m$  (A), ETR (B), qP (C) and NPQ (D) measured to  $6000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *Thymelaea velutina* in winter (black bars) and spring (white bars) in the two study populations. One standard error bars are shown.  $**P < 0.01$ .

**Table 2 – Mean and standard error of leaf area, perimeter, length, specific leaf area (SLA), leaf area ratio (LAR) and leaf weight ratio (LWR) of *Thymelaea velutina* in the two populations. Numbers in parentheses indicate sample sizes. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$**

	Coastal dunes	Mountain habitat	F-ratio
Leaf area (cm <sup>2</sup> )	0.3 ± 0.0 (20)	0.2 ± 0.1 (10)	$F_{1,190} = 92.202^{***}$
Leaf perimeter (cm)	2.8 ± 0.1 (20)	2.3 ± 0.1 (10)	$F_{1,190} = 36.270^{***}$
Leaf length (cm)	1.0 ± 0.0 (20)	0.8 ± 0.0 (10)	$F_{1,190} = 105.275^{***}$
SLA (cm <sup>2</sup> g <sup>-1</sup> )	100.9 ± 3.2 (20)	88.2 ± 3.2 (10)	$F_{1,25} = 6.356^*$
LAR (cm <sup>2</sup> g <sup>-1</sup> )	52.7 ± 2.2 (20)	39.5 ± 3.0 (10)	$F_{1,25} = 12.114^*$
LWR (g g <sup>-1</sup> )	0.5 ± 0.0 (20)	0.5 ± 0.0 (10)	$F_{1,25} = 5.730^{**}$

$P = 0.06$ ); and  $t = 1.78$ ,  $df = 10$   $P > 0.1$  (interaction population × sex:  $F_{1,28} = 0.59$ ,  $P > 0.45$ ). Mean values ranged from  $56.17 \pm 3.70$  to  $65.53 \pm 5.08$   $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ .

### 3.4. Growth rate-fluorescence measures correlation

We found no correlation between  $F_v/F_m$ , ETR, qP and NPQ and growth rate neither in the dune nor in the mountain site ( $r = 0.38$ ,  $r = 0.15$ ,  $r = 0.23$ ,  $r = 24$ , and  $r = 0.19$ ,  $r = 0.09$ ,  $r = 0.10$ ,  $r = 0.07$ , respectively; all  $P \gg 0.05$ ). NPQ was found to be positively correlated with plant crown diameter ( $r = 0.85$ , \* $P < 0.05$ ), at least in winter, but only at the mountain site. In spring, ETR and qP were lower in taller plants ( $r = -0.71$  and  $r = -0.67$ , respectively, \* $P < 0.05$ ). There was no significant effect of gender on growth at either site. Males in the coastal dunes produced greater number of flowers per branch ( $F_{1,64} = 6.225$ ,  $P = 0.015$ ) and a higher number of flowers per stem length ( $F_{1,64} = 5.177$ ,  $P = 0.026$ ) than at the mountain habitat. Females produced a greater amount of flowers per branch and per stem length than males, but differences were only observed at the mountain site and were marginally significant ( $F_{1,36} = 3.565$ ,  $P = 0.067$ ;  $F_{1,36} = 4.519$ ,  $P = 0.040$ ).

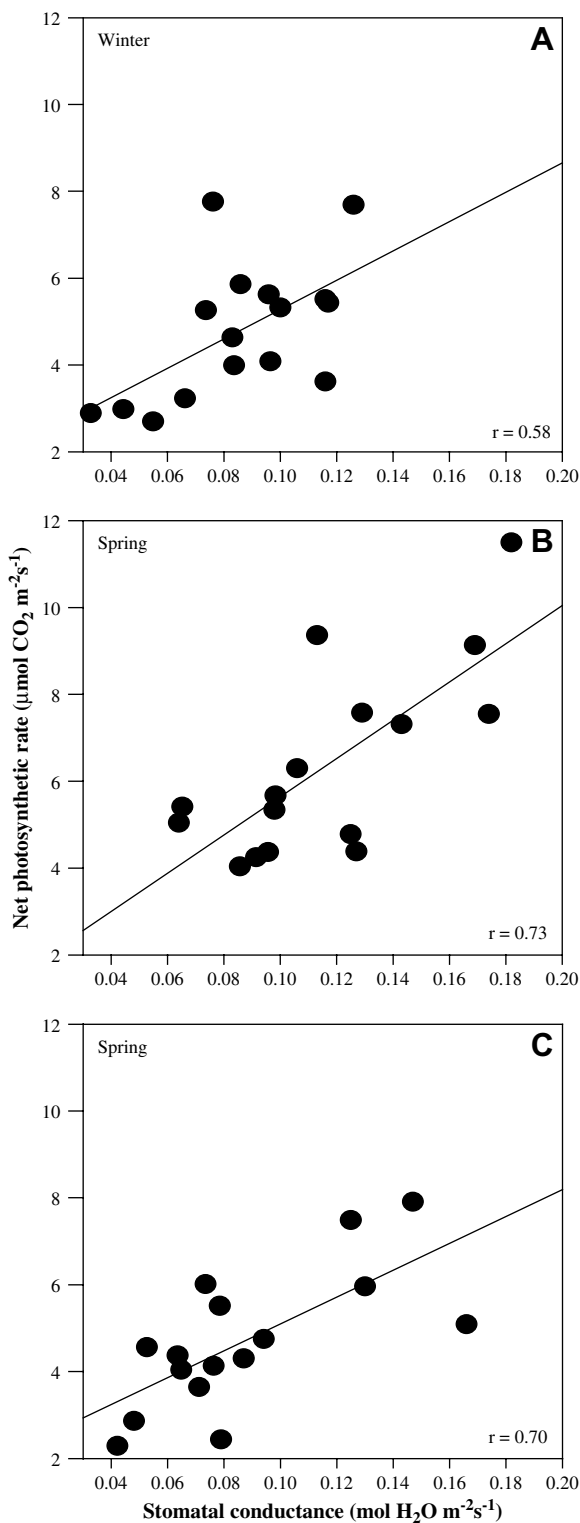
## 4. Discussion

Unlike other semi-deciduous Mediterranean species (Gulías et al., in press), rates of net photosynthesis and stomatal conductance of *T. velutina* recorded were rather low (average of  $6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Photosynthetic behavior did not appear to be homeostatic, but depended upon the specific conditions of the habitat in which the species lives and upon the season of the year. Even though the photosynthetic features explored exhibited a range of variation with significant differences for some of the factors explored, they were not strongly related to any of the surrogates of performance quantified here. Low values of net photosynthesis have also been found in other endemic Balearic species, like *Rhamnus ludovici-salvatoris* (Gulías et al., 2003). It has been argued that evolving under conditions of low competition, as it occurs in islands (Cox and Moore, 1993), leads to endemic species to present low photosynthetic capacity that would not be favorable under high competitive pressures (Gulías et al., 2003). However, opposite results have been obtained in recent studies (Galmés, 2006), indicating a more idiosyncratic pattern regarding

photosynthetic features of endemic plants than previously thought.

The photochemical efficiency of photosystem II estimated by the chlorophyll fluorescence  $F_v/F_m$  ratio can be used as a sensitive indicator of plant photosynthetic performance. Since optimal values for this variable are around 0.83 for most plant species (Björkman and Demmig, 1987; Johnson et al., 1993), lower values indicate photoinhibition as a result of plants stress (Maxwell and Johnson, 2000). The photochemical efficiency of *Thymelaea velutina* showed to be around the optimal value in the coastal dunes, both in winter and spring, indicating that environmental differences between seasons in this population were not sufficiently important to induce a strong photosynthetic stress. By contrast, at the mountain site, winter temperatures negatively and significantly affected the photosynthetic activity of *T. velutina*, which exhibited suboptimal  $F_v/F_m$  values. Photosynthesis sensitivity to chilling has actually been found in other Mediterranean species (Harley et al., 1987a; Tretiach, 1993; Gratani, 1995).

Differences in photosynthetic activity between populations due to local climatic variability have also been reported for other Mediterranean species, such as *Pistacia lentiscus* (Flexas et al., 2001). These ecotypic differentiations in photosynthetic features seem to have a genetic basis, at least in certain cases (e.g. *Quercus coccifera*, Balaguer et al., 2001), since it is maintained in seedlings grown under controlled conditions. It has been shown that small-scale spatial differences in climate and soil can produce differences in chlorophyll fluorescence features among individuals, with important consequences for carbon assimilation and storage and for the vegetation dynamics in Mediterranean semi-arid shrublands (Bellot et al., 2004). Environmental conditions during winter and autumn in Mediterranean semi-arid areas (with cold temperatures and relatively high light intensities) are known to induce photoinhibition in some evergreen woody species (Oliveira and Peñuelas, 2000; Valladares et al., 2005). However, more studies are needed to better understand this pattern and its generality across growth forms and truly Mediterranean vs Tertiary lineages that coexist in many Mediterranean habitats. In *T. velutina*,  $F_v/F_m$  values at the mountain site recovered during spring, when temperatures increase, revealing that the chronic photoinhibition and potential damage induced to the photosynthetic apparatus (by the combination of high light and low temperatures during winter) are not permanent, as it has also been reported for other species (González-Rodríguez et al., 2005). Despite the



**Fig. 2 – Relationship between net photosynthesis (A) and stomatal conductance (g) measured to  $1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in *Thymelaea velutina* in winter in coastal dunes (A) and during spring in coastal dunes (B) and mountain (C).**

lower winter  $F_v/F_m$  values in this population, the high values of ETR in spring (Fig. 1B) indicate that the radiation energy received exceeds the amount that can be used in photosynthesis and, thus, plants must dissipate it non-photosynthetically.

In addition to changes in photochemical efficiency, changes were also observed in the NPQ; these changes can result from the effect of both internal and external factors (Maxwell and Johnson, 2000). Compared to the dune population, mountain plants showed a lower apparent capacity of heat dissipation of excessive radiation, which further suggest a certain degree of damage to the PSII exerted by the combination of irradiance and low temperatures. Some studies have found that summer semi-deciduous shrubs have a photosynthetic apparatus that is very resistant to both high radiation and extreme temperatures (Karavatas and Manetas, 1999), which is considered of adaptive value since these plants must assimilate the largest possible amount of carbon during winter (Oliveira and Peñuelas, 2000; Gulías et al., 2004). Vulnerability to low temperatures over the winter in *T. velutina* seems to exert an important limitation in overall photosynthetic performance and growth since this species exhibits a pronounced shedding of the foliage during the summer. Low temperatures are an important factor limiting photosynthesis and plant growth in Mediterranean ecosystems, but this is often overlooked or underestimated (Mitrakos, 1980; Harley et al., 1987a,b; Gratani, 1995; Gulías et al., 2004). Summer drought is another important limiting factor of the photosynthetic activity in Mediterranean species (Gulías et al., 2004), though this particular effect is not examined in this study.

The fact that the hot season does not coincide with the wet season causes that plants in Mediterranean habitats can only grow during a short period of the year, when temperature is not limiting and water is available (Mitrakos, 1980; Gallardo, 2001). Like most Mediterranean species, *T. velutina* begins flowering in spring, using the reserves accumulated during the winter. The importance of  $\text{CO}_2$  assimilation during winter for annual  $\text{CO}_2$  assimilation has been revealed in a number of previous studies (e.g. Miyazawa and Kikuzawa, 2005). It would thus be expected that plants in the mountain, where the capacity of assimilation is low in winter, had a lower size and a reduced fecundity. However, we found no association between any of the fluorescence variables and any of the plant growth or fecundity estimates, suggesting that growth and reproduction are not directly coupled with immediate photosynthetic performance and that other factors are involved. One possibility is that plants invest most of the resources acquired by photosynthesis into structures that allow them to resist the harsh season (e.g. pubescence to reduce the absorption of irradiance and the loss of water) and in recovering the important amount of leaves shed during the summer. This may particularly apply to plants in the mountain habitat, which exhibited distinctive morphological features like dwarf, compact habit and small, densely pubescent leaves often observed in plants growing at high elevations (Cordell et al., 1999 and references therein). Many studies have found no direct relationships between photosynthetic capacity and relative plant growth (Poorter and Garnier, 1999), since

differences in photosynthetic capacity are not the main cause of differences in relative growth rate (Lambers et al., 1998). However, a direct relationship has been frequently documented between photosynthetic capacity (CO<sub>2</sub> assimilation rate by leaf mass unit) and leaf characteristics such as SLA, nitrogen content and the average life span (Reich et al., 1997). A positive correlation between SLA and relative growth rate (RGR) has also been reported by Roderick and Cornelissen (1997) and Wright and Westoby (2000). In *T. velutina*, the lower photochemical efficiency and photosynthetic activity at the mountain site also corresponded to lower SLA values, which suggests that leaves in this population are thicker or denser, which is known to reduce CO<sub>2</sub> diffusion towards the mesophyll tissue (Lloyd et al., 1992; Terashima and Hikosaka, 1995). This, together with the poor photosynthetic performance over winter, might be the reason for the smaller plant size in the mountain habitat. In the coastal dunes, the greater leaf area and mass fraction of the branch represented by the foliage can lead to a higher photosynthetic carbon gain by whole individuals compared to plants in the mountain.

Finally, it is noteworthy the lack of significant ecophysiological differences between male and female individuals of *T. velutina*. We expected a greater physiological stress and a poorer performance of the sex form with a greater reproductive effort as has been found for instance in *Ilex aquifolium* (Retuerto et al., 2000). However, male and female plants responded similarly to environmental conditions in both populations and there was no significant effect of gender on fluorescence, gas exchange and growth at either habitat. A possible reason for this lack of a gender effect on vegetative performance in *T. velutina* might be the relatively small size of the fruits, which are fleshy only in part, and thus might represent a minor burden in comparison with species from other studies.

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