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Constraints of photosynthetic performance and water status of four evergreen species co-occurring under field conditions

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ABSTRACT. Leaf water status and photosynthetic characteristics were investigated in four evergreen species, i.e. *Laurus nobilis*, *Ligustrum japonicum*, *Nerium oleander* and *Pittosporum tobira*, grown under ambient conditions. The results reveal variations in photosynthetic traits in relation to the use of water, during the optimal period of growth, in the middle of the dry season, during the secondary growth period and in the middle of the cold and wet season. Photosynthesis was restricted by limitations of stomatal conductance, causing transpiration impairment in *L. nobilis*, *L. japonicum* and *P. tobira*; while, the opposite holds true for *N. oleander*. Stomatal conductance of *N. oleander* was higher than that of the three co-existing species, sustaining elevated rates of photosynthesis and transpiration, at the expense of water. As drought progressed, there was a reduction in photosynthesis and water use efficiency in *L. japonicum* and *P. tobira*. Leaf turgor of the four species was closely associated with leaf water potential and differences among species narrowed in the dry season. The results show that *L. nobilis* possess features that confer advantage for the maintenance of this species in the driest sites, *N. oleander* maximises gas exchanges in the dry season by exhibiting a capacity for water acquisition, while *L. japonicum* and *P. tobira* may be limited to the moist sites.

Keywords: Co-occurring evergreens; Photosynthesis; Transpiration; Water relations.

INTRODUCTION

Water scarcity is a major factor limiting plant development in Eastern Mediterranean ecosystems, which influences water relations, rates of gas exchange and water use efficiency (Lo Gullo and Salleo, 1988; Rhizopoulou and Mitrakos, 1990; Chaves et al., 2002; Serrano et al., 2005; Varone and Gratani, 2007; Arena et al., 2008; Maatallah et al., 2010). It has been argued that photosynthesis and transpiration are progressively reduced by water deficit, as a result of stomatal closure (Jones, 1998; Galmés et al., 2007); however large variations among species have been investigated (Faria et al., 1998; Niinemets et al., 2009).

Slowly growing, evergreen sclerophylls and relatively more rapidly growing, malacophylls evergreens have selected for central reservation and median strips (Barker, 1995; Nardini, 2001; Harrison et al., 2010; Meletiou-Christou et al., 2011); their response to fluctuations of climate in East Mediterranean ecosystems is based on empirical observation, while opportunities for water saving strategies based on measurements are obscure. Many wild species have inherently low growth rates because they are adapted to environments where limitation is imposed by water and other abiotic and biotic stresses. It seems likely that evolution favours strategies for survival, establishment, and reproductive success that are not necessarily associated with highest potential rates of growth and carbon gain (Murchie and Niyogi, 2011). Results of photosynthesis and leaf water potential are widely used in predicting plants' performance and physiological tolerance to the environmental conditions (Harrison et al., 2010; Zhang, 2010).

The main goal of this study was to investigate constraints in photosynthesis, transpiration and water use efficiency in four co-existing, woody evergreen species, with a closely-packed foliage density, i.e. *Laurus nobilis*, *Ligustrum japonicum*, *Nerium oleander* and *Pittosporum tobira*, throughout the seasons. Photosynthesis (P_n), transpiration (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were investigated in leaves, expanded under ambient conditions of the current year, at the same temperature and water regime. Leaf water potential (Ψ_w), turgor (Ψ_p) and water use efficiency (WUE) were estimated in same-aged leaves of the four species.

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MATERIALS AND METHODS

Plant species and sampling

The present study was carried out in five-year old shrubs of two native evergreens [*Laurus nobilis* L. (Lauraceae) and *Nerium oleander* L. (Apocynaceae)] and two naturalized evergreens [*Ligustrum japonicum* Thunb. (Oleaceae) and *Pittosporum tobira* (Thunb.) Aiton (Pittosporaceae)] in Mediterranean landscapes, which grow in the field (37°55′ N, 23°38′ E); the shrubs received natural precipitation from late September to early June and irrigated twice per week to fulfil water requirements without causing runoff, during the dry season (from late June to early September). Measurements were conducted with fully expanded, healthy leaves early in May (optimal period for growth), early in July (in the middle of the dry season), early in October (secondary growth period) and January (in the middle of the wet and cold season). The averaged air temperature and monthly rainfall was 21°C and 11 mm in May, 28°C and 2 mm in July, 17°C and in 34 mm in October and 10°C and 71 mm in January, respectively.

Gas exchange measurements

P_n in response to photosynthetic photon flux density (PPFD) was measured with a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA), on the 5th fully expanded sunlit leaf (from the upper canopy) attached to parent plant from each species at dawn; the leaves were illuminated by step-wise increase of PPFD from 50 μmol m^{-2} s^{-1} to 1600 μmol m^{-2} s^{-1} and in the case of *N. oleander* up to 2000 μmol m^{-2} s^{-1}, using a light source (LED, Li-Cor, Lincoln, NE, USA). Leaves, with a leaf size suitable for measurements with the gas-exchange cuvette, were sampled under field conditions (Long et al., 1996; Rodeghiero et al., 2007). Leaf temperature was adjusted to 25°C and CO_2 concentration at approximately 350 μmol mol^{-1} air. Also, E, C_i and g_s were concomitantly estimated (with the Li-6400 portable photosynthesis system) on the 5th fully expanded sunlit leaf (from the upper canopy) at dawn. Measurements were made on 45 individual leaves, i.e. five outer canopy leaves from each of nine different shrubs, during clear days.

Water relations

Leaf samples of each species were measured at dawn. Ψ_w was measured using 6 mm diameter leaf discs, from the 5th fully expanded sunlit leaf (from the upper canopy), placed in C-52 psychrometric chambers (Wescor Inc. Logan, Utah, USA) attached to a microvoltmeter (HR-33T, Wescor Inc.); solute potential (Ψ_s) was determined from the same discs after freezing and thawing according to Rhizopoulou et al. (1991). Ψ_p was calculated by difference between Ψ and Ψ_s. The results are means of 45 measurements, i.e. from five outer canopy leaves from each of nine different shrubs.

Water use efficiency (WUE)

WUE was calculated as the ratio of P_n versus E at PPFD 1600 μmol m^{-2} s^{-1}.

Quantitative anatomical measurements

Images from cross sections of the 5th fully expanded sunlit leaves (from the upper canopy) were used for quantitative anatomical measurements according to Rhizopoulou and Psaras (2003). Measurements were made on 45 individual leaves, i.e. five outer canopy leaves from each of nine different shrubs.

Statistical analysis

All the presented data were measured using the same expanded leaves, collected under field conditions. Data were subjected to two-way analysis of variance (Anova) that was carried out by using the statistical software package OriginPro 8 (OriginLab) to ascertain significant differences between measurements and species. Duncun multiple range tests were used to compare means among species and months. Linear and non-linear regression analyses were fitted to data. All tests of significance were made at 5 % level.

RESULTS

P_n was increased over a range of increasing PPFD up to 1600 μmol m^{-2} s^{-1} in *L. nolibis* (Figure 1A), *L. japonicum* (Figure 1C) and *P. tobira* (Figure 1D), while in *N. oleander* an increase of P_n up to PPFD 2000 μmol m^{-2} s^{-1} was detected (Figure 1B). There were seasonal differences in the response of P_n to PPFD, among species (Figure 1, Table 1). In the middle of the dry season (July), trace amounts of P_n were measured in *L. nobilis* (Figure 1A), *L. japonicum* (Figure 1C) and *P. tobira* (Figure 1D), while in *N. oleander* P_n attained maximum values (Figure 1B). In *L. nobilis* maximum values of P_n were measured during the main (May) and the secondary (October) growth period (Figure 1A). P_n did not vary significantly at 1600 μmol m^{-2} s^{-1} among *L. nobilis* (Figure 1A), *L. japonicum* (Figure 1C) and *P. tobira* (Figure 1D) early in May and early in October (Table 1), while the opposite holds true for *N. oleander* (Figure 1B). In the middle of the cold season (January) the highest values of P_n were detected in *L. japonicum* (Figure 1C) and *P. tobira* (Figure 1D) and the lowest in *N. oleander* (Figure 1B). Values of g_s exceeded 0.2 mol m^{-2} s^{-1} in *N. oleander* early in July (Figure 2B) and in *P. tobira* early in January (Figure 2D), coinciding with elevating rates of P_n (Figure 2) and E (Figure 3); low values of g_s (0.1 mol m^{-2} s^{-1}) were recorded in *L. nobilis* (Figures 2A and 3A) and *L. japonicum* (Figures 2C and 3C). At the onset and in the middle of the dry season, E was consistently higher in *N. oleander* (Figure 3B), while intermediate values were detected in *P. tobira* (Figure 3D). The low rates of transpiration observed in *L. nobilis* (Figure 3A) and *L. japonicum* (Figure 3C) were restricted by g_s and this was more pronounced in the early days of May and July. In *P.*

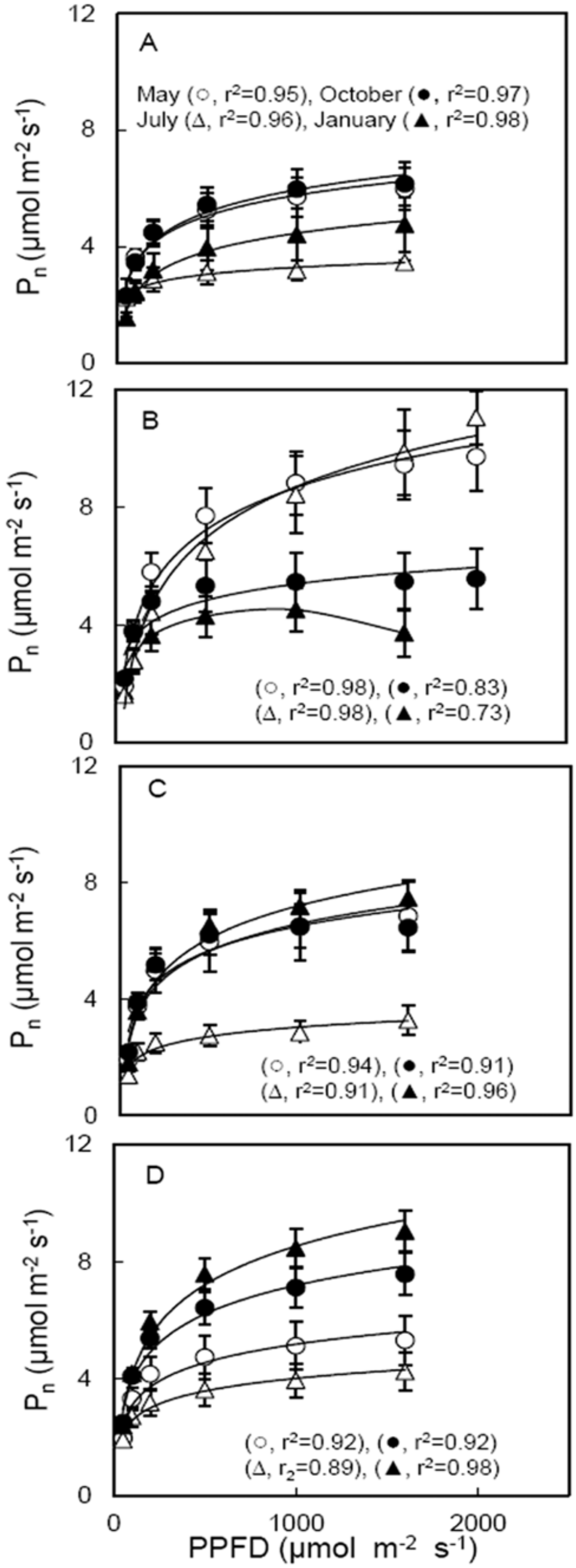

Figure 1. Response of P_n to increasing PPFD in expanded leaves of *L. nobilis* (A), *N. oleander* (B), *L. japonicum* (C) and *P. tobira* (D). Means ± standard error (S.E.) (n=45) are reported.

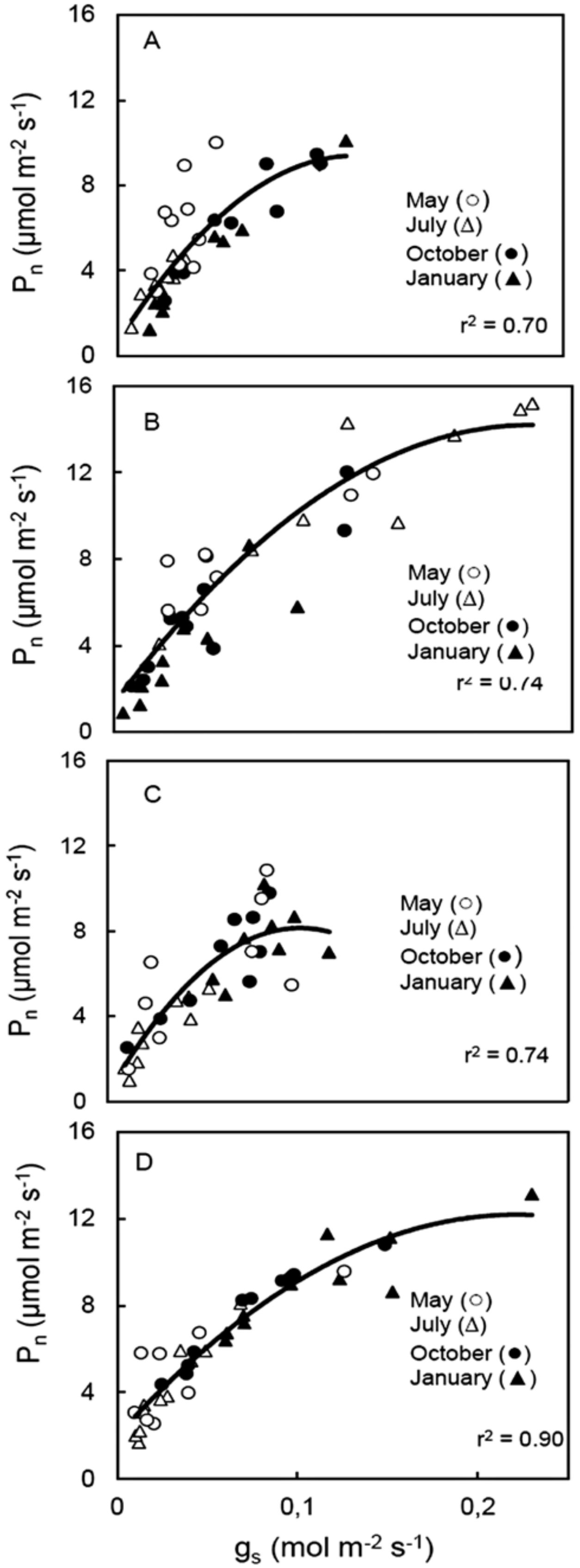

Figure 2. P_n versus g_s in expanded leaves of: *L. nobilis* (A), *N. oleander* (B), *L. japonicum* (C) and *P. tobira* (D), in May (○), in October (●), in July (△) and January (▲).

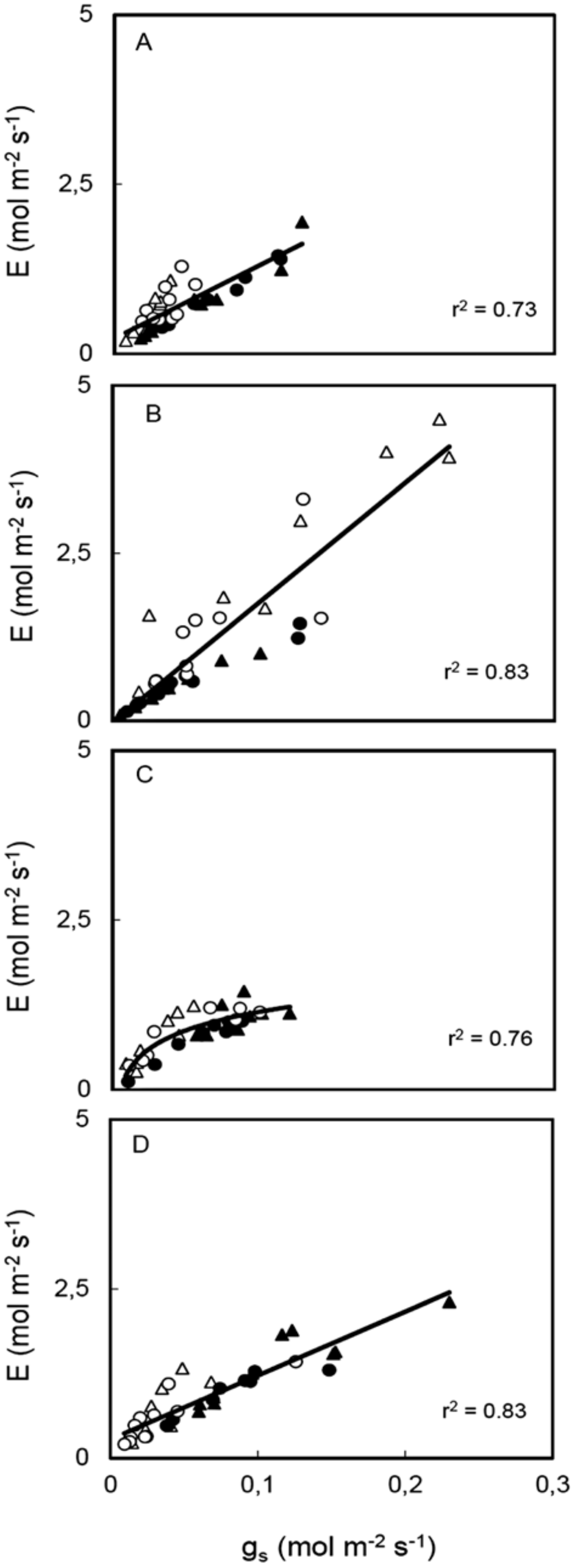

Figure 3. E versus g_s in *L. nobilis* (A, r^2=0.73)*, N. oleander* (B, r^2=0.83), *L. japonicum* (C, r^2=0.76) and *P. tobira* (D, r^2=0.83). Individual symbols as in Figure 2.

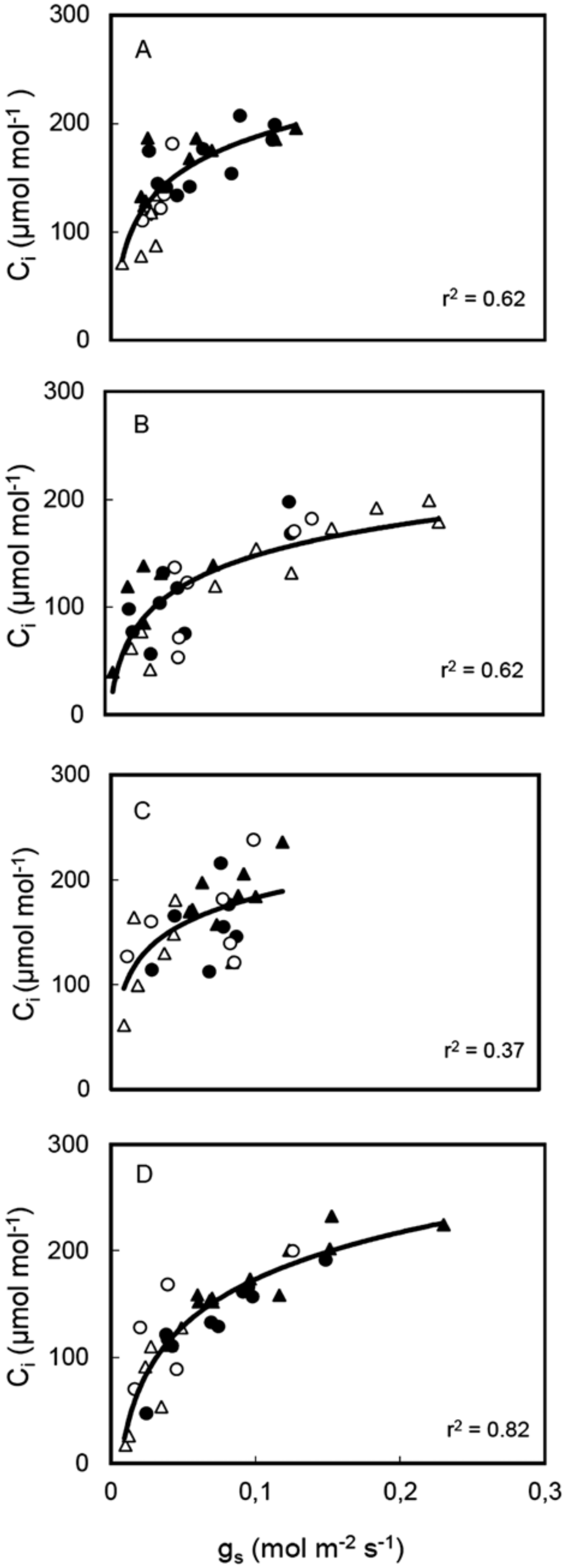

Figure 4. C_i versus g_s in *L. nobilis* (A, r^2=0.62)*, N. oleander* (B, r^2=0.62), *L. japonicum* (C, r^2=0.37) and *P. tobira* (D, r^2=0.82). Individual symbols as in Figure 2.

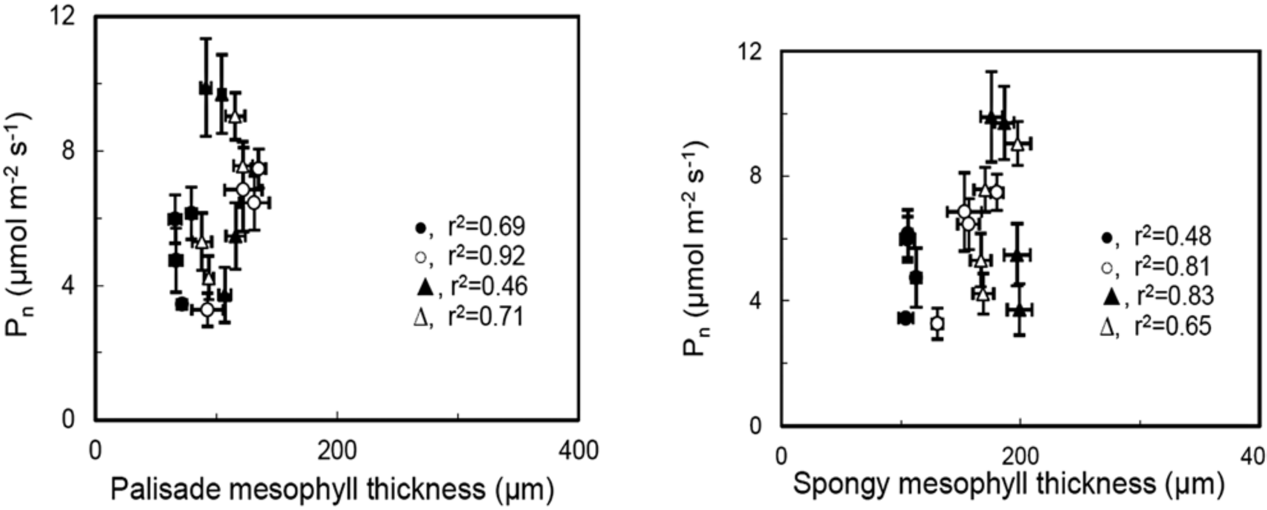

Figure 5. P_n versus thickness of palisade and spongy mesophyll in *L. nobilis* (●), *L. japonicum* (○), *N. oleander* (▲) and *P. tobira* (△). Means ± S.E. (n=45) are reported.

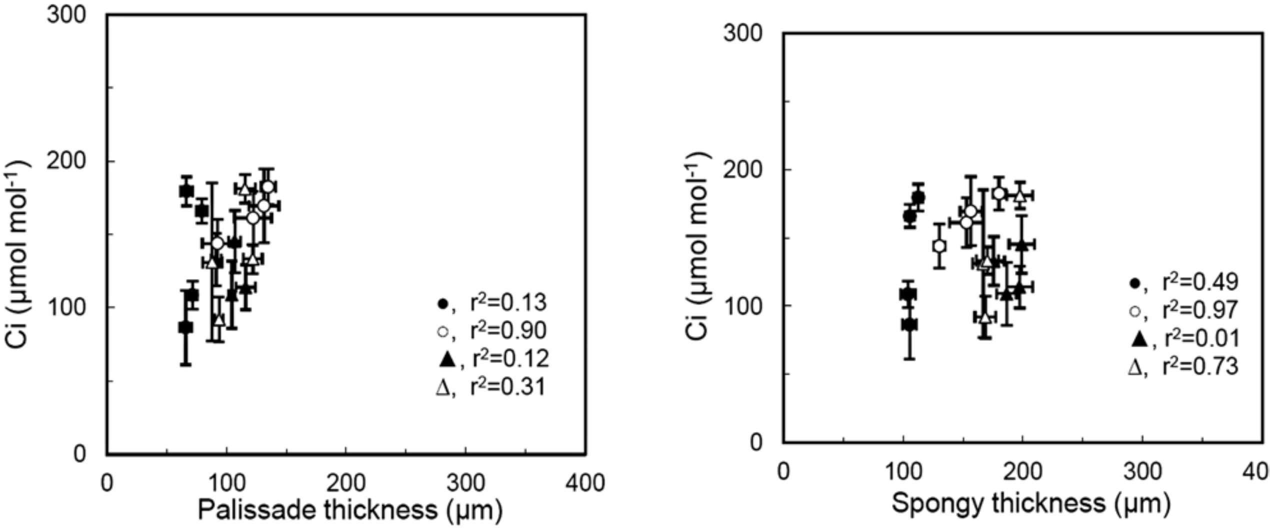

Figure 6. C_i versus thickness of palisade and spongy mesophyll in *L. nobilis* (●), *L. japonicum* (○), *N. oleander* (▲) and *P. tobira* (△). Means ± S.E. (n=45) are reported.

tobira (Figure 3D) a restriction in g_s was observed early in July, whereas the opposite holds true for *N. oleander* (Figure 3B). The best fit of C_i on g_s was curvilinear rather than linear in *L. nobilis* (Figure 4A), *N. oleander* (Figure 4B) *L. japonicum* (Figure 4C) and *P. tobira* (Figure 4D), indicating that stomata were not the primary factor limiting C_i.

Leaves of *L. nobilis* exhibited the thinnest palisade and spongy tissues among the examined species (Figures 5 and 6), while negligible changes in palisade mesophyll influence P_n and C_i (Figure 5). In *L. japonicum*, P_n and C_i were linearly correlated with the thickness of palisade (r^2=0.92 and r^2=0.90, respectively) and spongy (r^2=0.81 and r^2=0.97, respectively) mesophylls. In *P. tobira,* P_n was linearly correlated with thickness of palisade (r^2=0.71); also, P_n and C_i were linearly correlated with spongy mesophyll (r^2=0.65 and r^2=0.73, respectively). In *N. oleander*, P_n and C_i were weakly and negatively coordinated to the thickness of palisade mesophyll (r^2=0.46 and r^2=0.12, respectively), while the opposite holds true for P_n and the thickness of the spongy mesophyll (r^2=0.83), with irregularly-shaped cells and numerous intracellular spaces (Figure 5).

In May values of Ψ_w exceeded -1.52 MPa in *L. nobilis*, while Ψ_w of *N. oleander* was approximately -0.98 MPa (Figure 7); more substantial differences were detected for the leaf osmotic potential (data not shown). In May, the highest value of Ψ_p was calculated for *L. nobilis* (Figure 7). In July, Ψ_w of leaves of the four evergreen species decreased sharply as soil was dried and minima of Ψ_p were estimated at approximately 0.11 MPa for *L. nobilis* (i.e. the lowest value), 0.17 MPa for *N. oleander*, 0.21 MPa for *L. japonicum* and 0.22 MPa for *P. tobira* (Figure 7). The values of Ψ_w vary significantly among the species (Table

1) and especially those recorded at the onset and in the middle of the dry season. There were differences in the response of g_s to the leaf water potential among species. As water became less available, at values of Ψ_w as low as -2.2 MPa in *L. nobilis* and *L. japonicum*, and -2.0 MPa in *P. tobira* trace amounts of g_s and E were estimated. In contrast, in *N. oleander* values of Ψ_w as low as -1.3 MPa did not influence g_s and E, indicating that stomatal aperture of leaves of *N. oleander* was sustained under declining leaf water potentials.

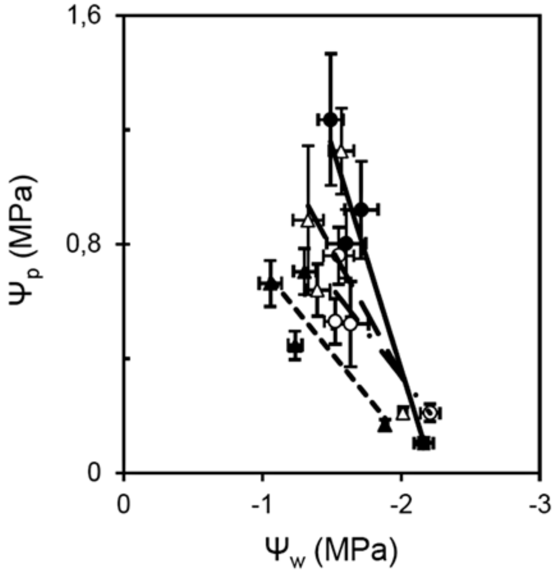

Figure 7. Ψ_w versus Ψ_p, in *L. nobilis* (●, continuous line, r^2=0.93), *L. japonicum* (○, dashed-dotted line, r^2=0.79), *N. oleander* (▲, dotted line, r^2=0.76) and *P. tobira* (△, dashed line, r^2=0.59). Means ± S.E. (n=45) are reported.

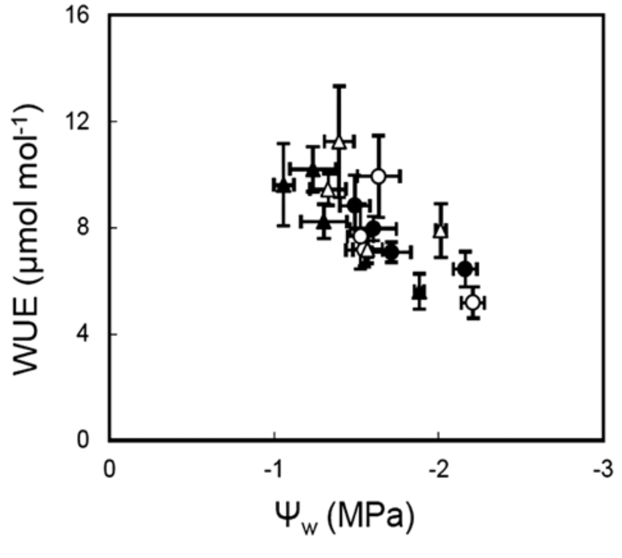

Figure 8. WUE versus Ψ_w in *L. nobilis* (●, r^2=0.99), *L. japonicum* (○, r^2=0.94), *N. oleander* (▲, r^2=0.90) and *P. tobira* (△, r^2=0.56). Means ± S.E. (n=45) are reported.

WUE decreased as Ψ_w declined at -2.0 MPa (Figure 8), in the four evergreen species and it appeared to be linearly correlated with Ψ_w (Figure 8, considering all data points: $y = 3.84x + 14.27$, r^2=0.68). The dry season significantly affected P_n (Figure 2) and E (Figure 3), which showed a progressively decrease in *L. nobilis*, *L. japonicum* and *P. tobira*. In *N. oleander* high rates of P_n, sustained in May and July (9.8 μmol m^{-2} s^{-1} and 9.7 μmol m^{-2} s^{-1}, respectively), were coupled with elevated values of E (2.2 mol m^{-2} s^{-1} and 1.3 mol m^{-2} s^{-1}, respectively). In July, there was a noticeable narrowing of the differences in WUE among the species and more precisely between *L. nobilis*, *L. japonicum* and *P. tobira*, when Ψ_w fell below -2.0 MPa, while less negative values were recorded for *N. oleander*. It is indicative that the reduction in Ψ_w coincides with the suppression of E in *L. nobilis*, *L. japonicum* and *P. tobira*, but with an increase in E of *N. oleander*. As turgor of leaves approached zero in July (Figure 7), a coordination of WUE and Ψ_w, at values lower than -2.0 MPa was detected (Figure 8).

DISCUSSION

Stomatal conductance (g_s) is an important element in quantitative evaluation of P_n and E (Jones, 1998). P_n and C_i were affected by g_s, thus elevated values were detected in the wet season and low in the dry season for *L. nobilis*, *L. japonicum* and *P. tobira*. In the case of *N. oleander* enhanced values were obtained in the dry season and low values in the cold season. Values of E of *L. nobilis*, *L. japonicum* and *P. tobira* seem to be affected by water shortage, while the opposite holds true for *N. oleander*; this has important implications for the control of water loss from shrubs under drought conditions. It is noteworthy that leaves of *N. oleander* exhibited high g_s in July, which permitted much higher transpiration rate in comparison with the similar-sized leaves of *L. nobilis* and the larger leaves of *L. japonicum*. This indicates that stomata of *N. oleander* arranged in crypts with trichomes may be partially open (Lakušić et al., 2007; Roth-Nebelsick et al., 2009). Although, the adaptive significance of stomatal encryption is still under debate, it has been shown that crypts facilitate CO_2 diffusion in the photosynthetic mesophyll of thick leaves (Hassiotou et al., 2009), while the presence of stomata on one surface increases the distance of CO_2 diffusion to the photosynthetic mesophyll cells. P_n of leaves *N. oleander* is supported by elevated g_s, in considering the relatively thick mesophyll of this species (approximately 290 μm) that enhances the overall gas-exchange parameters, in comparison with leaves of *L. nobilis* that possess a thinner mesophyll (approximately 178 μm) prone to dehydration under high evaporative demand or limited water supply (Oppenheimer and Leshem, 1966; Christodoulakis and Mitrakos, 1987; Zwieniecki et al., 2002).

High values of P_n and E of *N. oleander* in July may also indicate the maintenance of elevated hydraulic conductivity that has been shown to modulate stomatal behaviour

Table 1. Differences between measurements and species were statistically significant at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Source of variation: all species		MS	*F*	*P*
Measurements: May	P_n	32.98	3.41	*
	Ψ_w	0.46	7.05	***
	C_i	21.45	0.89	n.s.
Measurements: July	P_n	98.86	13.94	***
	Ψ_w	0.22	7.78	***
	C_i	13.96	2.45	n.s.
Measurements: October	P_n	7.56	1.15	n.s.
	Ψ_w	0.40	2.35	*
	C_i	9.77	1.20	*
Measurements: January	P_n	59.18	10.97	***
	Ψ_w	0.29	2.24	n.s.
	C_i	2.78	1.01	n.s.
Source of variation: all months		MS	*F*	*P*
Laurus nobilis	P_n	15.66	2.96	**
	Ψ_w	0.86	7.29	***
	C_i	10.46	1.95	n.s.
Ligustrum japonicum	P_n	31.06	5.01	**
	Ψ_w	1.04	12.51	***
	C_i	34.22	3.25	n.s.
Nerium oleander	P_n	91.03	7.29	***
	Ψ_w	1.28	11.57	***
	C_i	41.88	4.14	**
Pittosporum tobira	P_n	47.11	8.69	***
	Ψ_w	0.94	12.14	***
	C_i	32.51	2.15	*

(Badger et al., 1982; Jones, 1998; Medrano et al., 2002; Sack et al., 2003). It seems likely that in *N. oleander* coordination of P_n and E with g_s supports the concept that stomatal structure and function has been honed through evolution to optimise the ratio of CO_2 uptake to water lost through photosynthesis (Driscoll et al., 2006; Delaney, 2008). According to Roth-Nebelsick et al. (2009), it is unlikely that the primary function of crypts and crypt trichomes is to reduce E. In contrast, P_n and E of *L. nobilis* were restricted by g_s and the minima occurred in the dry season, are probably driven by traits that reduce transpiration and water use processes (Beerling and Franks, 2010). It is noteworthy that maximal P_n and E of *L. nobilis* coincided with the main and the secondary growth period of Mediterranean evergreens (Rhizopoulou et al., 1991).

C_i increased with g_s up to 0.1 mol m^{-2} s^{-1} in *L. nobilis* and *L. japonicum*, and up to 0.2 mol m^{-2} s^{-1} in *N. oleander* and *P. tobira*. Niinemets et al. (2005) argued that differences in g_s and C_i that limit P_n may reflect properties of the mesophyll. It has been shown that C_i in mature leaves is correlated with the surface area of mesophyll cells and exposed mesophyll cell walls facing to intercellular spaces of the photosynthetic leaf lamella, as an increase in the above mentioned parameters would lead to a rise in P_n (Hanba et al., 1999; Praras and Rhizopoulou, 1995; Miyazawa and Terashima, 2001; Niinemets et al., 2005). In *L. nobilis,* a weak coordination of C_i with spongy mesophyll thickness (r^2=0.49) was detected. C_i was linearly correlated with the thickness of the palisade (r^2=0.90) and the spongy (r^2=0.97) mesophyll of *L. japonicum*, thus accelerating P_n. In *P. tobira*, a coordination of C_i with the thickness of spongy mesophyll (r^2=0.73) was detected. In the case of *N. oleander*, C_i did not coordinate with mesophyll thickness. In earlier work, it has been shown that leaf mesophyll of *N. oleander* is differentiated into a thick palisade (with two to three layers of palisade cells) and numerous layers of spongy parenchyma (Openeheimer and Lesmen, 1966; Lakušić et al., 2007; Roth-Nebelsick et al., 2009). Also, leaf anatomy of *L. nobilis*, *L. japonicum* and *P. tobira* has been studied (Christodoulakis, 1993; Martin et al., 1994;

Lakušić et al., 2007; Christodoulakis et al., 2009); however, functional analyses of leaf structural traits of the above mentioned evergreens have not been made.

The highest values of WUE of the examined species coincide with elevated values of Ψ_w during spring, when water can be withdrawn from the soil. In contrast, the lowest values of WUE were detected in July, in the middle of the dry season; in such situation when sufficient amount of water can be lost by transpiration, high WUE is disadvantageous. *L. japonicum* and *P. tobira* exhibited higher WUE than that of *L. nobilis* and *N. oleander* at elevated values of Ψ_w, under ambient conditions. Also, WUE of *L. japonicum* and *P. tobira* at Ψ_w as low as -1.5 MPa indicate that P_n was sustained under adequate water status. It has been argued that plants showing high WUE at high Ψ_w might not compete successfully on drier sites (Lima et al., 2003); while, low WUE indicates that CO_2 was adversely influenced by leaf water status.

WUE of *L. nobilis* may be enhanced by partial closure of stomata, so that C_i is just sufficient for saturation of P_n, while the rate of water loss can be concomitantly lowered; this is in agreement with earlier results (Rhizopoulou and Mitrakos, 1990), further suggesting that this species might compete successfully on drier sites (Salleo et al., 2009). Stomatal closure at low Ψ_w, with a consequent decrease in P_n could put plants at a selective advantage on drier sites. WUE of *N. oleander* was lower than that of *L. nobilis* in July. During the dry period maximal g_s was obtained in *N. oleander* concomitantly with declining WUE; this may be related to the control of stomatal response rather in the sense of maximising carbon assimilation in the prevailing circumstances, than in the sense of conserving water. Leaves of *N. oleander* were capable of elevated P_n by increasing g_s, which leads to higher rates of transpirational water loss.

In this work limitations of photosynthetic rates and transpiration imposed by water shortage and g_s have been detected in *L. nobilis*, *L. japonicum* and *P. tobira* grown under ambient conditions. In contrast, *N. oleander* maximise gas exchanges during the dry season. It has been argued that *N. oleander* possesses a high potential for photosynthetic acclimation to elevated temperature (Badger et al., 1982) and when supplied with water it remains photosynthetically active during the dry season. The photosynthetic capacity of *N. oleander* was found to be unrelated to stomatal and mesophyll traits that also did not influence C_i. Hence, photosynthetic rates of *N. oleander* during the hot and dry season may be attributed to thermal stability of its photosynthetic machinery (Badger et al., 1982; Raison et al., 1982). Further work is required to illustrate traits contributing to the functional adaptation of the long-lived leaves of the four evergreens to the fluctuations of the Mediterranean climate.

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在田野生長環境下共存之四種長綠植物：其光合作用表現和水利用狀態的限制

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共同生長在田野環境下之四種長綠植物，即 *Laurus nobilis*, *Ligustrum japonicum*, *Nerium oleander* 及 *Pittosporum tobira*，我們探討其水利用狀態和光合作用之諸多性狀。植物之生長期劃分為最佳生長期，乾旱季之中期，次要之生長期，以及冷、溼季之中期共四期。在這四期我們發現伴隨著水分之利用，光合作用之諸多性狀均有上下起伏之現象。對 *L. nobilis*, *L. japonicum* 及 *P. tobira* 三種長綠植物而言，光合作用受制於氣孔導度 (stomatal conductance) 之限制，構成蒸散過程之不順；相對之下，*N. oleander* 的情形剛好相反。*N. oleander* 之氣孔導度比共存之三種其他長綠植物都高，因此長時間維持高速率之光合作用和蒸散作用：付出之代價乃多消耗水。當旱季進行時，光合作用和水利用效率在 *L. japonicum* 及 *P. tobira* 均有所下降。所觀察之四種長綠植物其葉張壓 (leaf turgor) 和葉之水潛能 (water potential) 密切相關。而四種植物之葉張壓之差異在乾旱季有縮小的現象。我們的結果顯示：*L. nobilis* 具有著干特性使得此種植物在最乾旱之地點得以維持族群，*N. oleander* 在乾旱季節之所以能極大化其氣體交換能力乃歸功於其獲取水資源之能耐，而 *L. japonicum* 及 *P. tobira* 這兩種可能僅限於長在潮溼地點。

關鍵詞：共存之長綠植物；光合作用；蒸散作用；水之利用機制。