iForest
Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor1848-009
vol. 9, pp. 946-953

Evergreen species response to Mediterranean climate stress factors

Loretta Gratani, Rosangela Catoni, Laura Varone

Recent climatic projections predict a decline in rainfall mainly during the summer period and an increase in air temperature for the Mediterranean basin, resulting in extended periods of soil water deficit. Mediterranean evergreen species attain drought resistance through different traits or combination of traits. The main objective of this research is to analyze the response of the evergreen species co-occurring in the Mediterranean maquis to variations in water availability and air temperature during the year. The results show that leaf structural traits significantly affect physiological traits as confirmed by the Partial Least Squares Regression analysis (PLS). In particular, the considered species have a similar leaf respiration (R_L) trend during the year with the lowest rates in winter (mean 0.95 ± 0.44 µmol m^{-2} s^{-1}) and the highest in drought (mean 3.05 ± 0.96 µmol m^{-2} s^{-1}). Nevertheless, a different R_L effect on gross photosynthesis (P_G) during drought was observed. *C. incanus*, *E. multiflora*, *R. officinalis* and *S. aspera* have the highest R_L/P_G ratio (mean 0.54 ± 0.08), while *Q. ilex*, *P. latifolia*, *P. lentiscus*, *A. unedo* and *E. arborea* have the lowest (mean 0.22 ± 0.07). R_L/P_G ratio variations depend on the sensitivity of both the two parameters to drought. Considering the increase of the length and intensity of drought in the Mediterranean basin, and that the photosynthesis of Mediterranean evergreen species is frequently limited by sub-optimal conditions (*i.e.*, water deficit, high light intensity and high air temperature), it is important to improve knowledge on R_L, since it has a critical function in modulating carbon balance of Mediterranean species.

Keywords: Global Climate Change, Mediterranean Evergreen Species, Net Photosynthesis, Gross Photosynthesis, Leaf Respiration

Introduction

The projected global climate warming could affect a wide range of species and ecosystems (Hamann & Wang 2006). Considering that the implications of global climate change are characterized by strong latitudinal variations (IPCC 2014), regional studies are recommended to provide an essential tool for scientists and decision-makers (Giannakopoulos et al. 2009). The Mediterranean basin is especially vulnerable to climate change (Goubanova & Li 2007). Due to its unique geographic location, this region is a transitional zone between the very hot and dry African climate in the South and the mild and humid European climate in the North (Goubanova & Li 2007). Recent climatic projections for the Mediterranean basin predict an air temperature increase and a decline in rainfall mainly during summer (IPCC 2014). Moreover, the Mediterranean basin undergoes more prolonged dry periods than in the past. This produces a negative soil water balance as the amount of water that evaporates overcomes the amount of water intercepted by soil through rainfall and summer humidity (Brunetti et al. 2002, Moretti et al. 2015). The Mediterranean basin is characterized by a high plant diversity of growth forms, habits and phenology (Galmés et al. 2007). In particular, physiological adaptations involve regulation of the water status by stomatal control (Gratani & Varone 2004). Morphological and anatomical adaptations involve leaf protective structures (*i.e.*, hairs, thick cuticle, sclerenchymatic cells), steep leaf inclination, low leaf surface area (Castro-Díez et al. 1998, Karabourniotis 1998, Gratani & Bombelli 2000) and a high stomatal density of small size (Rotondi et al. 2003, Gratani & Varone 2004). Such traits improve drought resistance by decreasing photochemical damage of the photosynthetic apparatus and limiting leaf transpiration (Castro-Díez et al. 1998). Among the Mediterranean species, the sclerophyllous have a high construction cost of their leaf protective structures (*i.e.*, high leaf consistency) and a long leaf longevity (Gratani & Crescente 1997), while the semi-deciduous species have a lower leaf consistency and a shorter leaf longevity. Moreover, they have leaf morphological and anatomical differences between summer and winter leaves (Catoni et al. 2012). Leaf longevity may be a source of variation in the relationship among the different leaf traits (Reich et al. 1991). Nevertheless, if the dry season lasts too long, water deficit may negatively affect Mediterranean evergreen species carbon assimilation, as a result of the lowest photosynthetic rates and leaf surface areas produced (Pereira et al. 2007). Thus, plant survival in Mediterranean environments depends largely upon their capacity to photosynthesize and keep water loses lower than the supply. It is important to take into account the key role of leaf respiration when plants experience intense drought periods, since it has a critical function in modulating carbon balance (Sun et al. 2014). The effects of leaf respiration on

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Department of Environmental Biology, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome (Italy)

@ Loretta Gratani (loretta.gratani@uniroma1.it)

Received: Sep 01, 2015 - Accepted: Apr 06, 2016

Citation: Gratani L, Catoni R, Varone L (2016). Evergreen species response to Mediterranean climate stress factors. iForest 9: 946-953. - doi: 10.3832/ifor1848-009 [online 2016-07-07]

Communicated by: Elena Paoletti

gross photosynthesis, particularly under drought conditions (Flexas et al. 2005) may allow more reliable climate change scenarios of ecosystem functioning.

In this context, the main goal of our research was to analyze the response of the evergreen species co-occurring in the Mediterranean maquis to seasonal variations in water availability and air temperature during the year. In particular, the effects of leaf respiration on gross photosynthesis were considered. The Mediterranean maquis is largely distributed in areas around the Mediterranean Basin and its structure and composition is strongly influenced by air temperature and water availability (Gratani et al. 2013).

Material and methods

Study area and plant materials

The study was carried out in the period January – December 2014 in the Mediterranean maquis inside the Presidential Estate of Castelporziano (SSW of Rome, Italy; 4° 45′ N, 12° 26′ E – http://palazzo.quirinale.it/residenze/c_porziano_en.html).

The Presidential Estate of Castelporziano is included in the "Natura 2000" network and is classified as a ZPS (Special Protection Area, "Tenuta Presidenziale", IT603 0084) and a SIC (Sites of Community Importance) with two areas: "Querceti igrofili" (IT6030028) and "Fascia costiera" (IT6030027). The maquis under study was characterized by the presence of the following species: *Arbutus unedo* L., *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Quercus ilex* L. (typical sclerophyllous species), *Cistus incanus* L. (drought semi-deciduous species), *Erica arborea* L., *Erica multiflora* L., *Rosmarinus officinalis* L. (narrow-leaves species), and *Smilax aspera* L. (liana – Gratani & Crescente 1997).

The climate of the area is Mediterranean, with the mean minimum air temperature (T_{min}) of the coldest months (February) of 3.7 ± 1.8 °C (mean ± SD), the mean maximum air temperature (T_{max}) of the hottest months (August) 30.3 ± 1.4°C, and the mean yearly air temperature (T_m) 15.6 ± 5.9 °C. Total annual rainfall is 738 mm, mainly occurring in autumn and winter. Drought period is from the end of May to the beginning of September (60 mm in the period). During the study period, T_{min} of the coldest month (March) was 5.0 ± 2.1°C, T_{max} of the hottest month (August) 28.0 ± 2.4°C and the total rainfall during the drought period (from the end of July to the end of August) was 15 mm (Fig. 1). Weather and climate data (1985-2014) were obtained from the Meteorological Station located inside the Estate.

Structural, morphological, anatomical and physiological leaf traits were measured on twelve representative shrubs per each of the considered species, randomly distributed in three representative areas (100 m^2 each). Mature leaves were detached from the southern, upper portion of each shrub at the end of May 2014 for measurements.

Structural shrubs traits

Measurements of shrub structure included total height (H_S, defined as the maximum vertical distance from the ground to the highest point of the shrub), height of the shrub crown (Ch, defined as the vertical distance from the lowest leaf insertion to the highest point of the shrub), depth of the shrub crown (CD, excluding the central non-foliated branch portion, according to Schulze et al. 1977), major axis (A) and minor axis (a, orthogonal to A) of the shrub crown. Shrub volume (V_S) was derived from the measured traits, by assigning simple geometric solids to shrub form, such as cone, semisphere and ellipsoid which appeared to give the best fit of the natural shape of the crown, according to Sternberg & Shoshany (2001); the volume of the shrub crown (V_{crown}) was calculated excluding the central non-foliated branch portion of shrub. For *S. aspera* was measured the length (l_s).

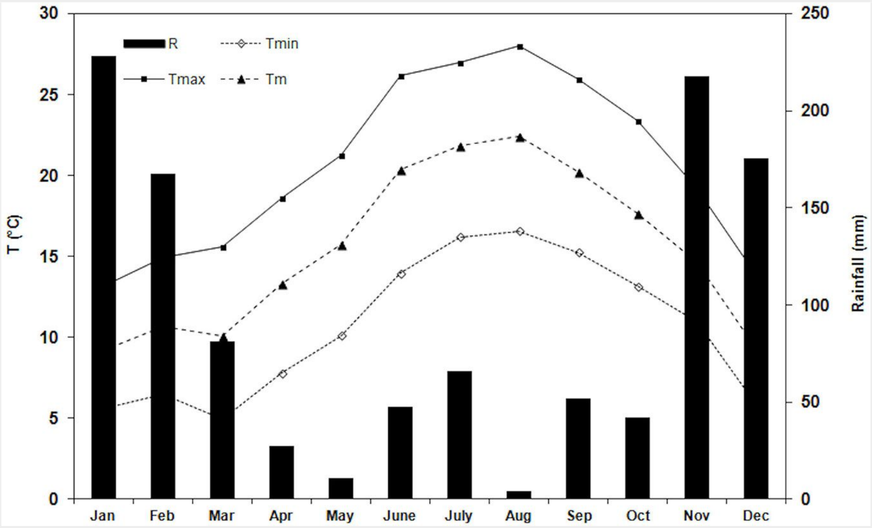

Fig. 1 - Monthly trend of air temperatures and total monthly rainfall during the study period (January - December 2014); (R): total monthly rainfall (T_{min}): minimum air temperature; (T_{max}): maximum air temperature; (T_m): mean air temperature.

Anatomical leaf traits

The following parameters were considered for leaf anatomical measurements (n = 3 leaves per shrub per species): total leaf lamina thickness (LT); palisade and spongy thickness; thickness of the upper and lower cuticle and epidermis (CET, µm). Measurements were restricted to vein-free areas. The fraction of mesophyll volume occupied by the intercellular air spaces (f_{ias}, %) was calculated according to Syvertsen et al. (1995) as follows (eqn. 1):

$$f_{ias} = 1 - A_m / lW$$

where A_m is the cross-sectional area of the mesophyll cell, W the width of the measured section and l is the mesophyll thickness.

The following parameters were measured for stomata: guard cell length and width (L_A and W_B, respectively), according to Stojnić et al. (2015), and the stomatal pore surface of stomata guard cell (SPS_{LAWB}, µm²), according to Balasooriya et al. (2009) as (eqn. 2):

$$SPS_{LAWB} = (L_A \cdot W_B \cdot \pi)/4$$

The potential conductance index (PCI) was calculated according to Holland & Richardson (2009), as follows (eqn. 3):

$$PCI = L_A^2 \cdot SD \cdot 10^{-4}$$

where SD is the stomatal density (stomata mm^{-2}), measured from nail varnish impressions of the inferior lamina, according to Sack et al. (2003), each of them 0.5 × 1.0 cm, obtained by a Zeiss Axiocam MRc 5® digital camera (Carl Zeiss, Jena, Germany), equipped with the software Axiovision AC® (release 4.5).

Morphological leaf traits

The following parameters were measured for leaf morphological measurements (n = 5 leaves per shrub per species): projected fresh leaf surface area excluding petioles (LA, cm^2), obtained by the Image Analysis System® (Delta-T Devices, Burwell, UK) and leaf dry mass (DM, mg), drying leaves at 80 °C to constant mass.

Leaf mass per unit leaf area (LMA, mg cm^{-2}) was calculated by the ratio of DM and LA and leaf tissue density (LTD, mg cm^{-3}) by the ratio of LMA and total lamina thickness.

Gas exchange

Gas exchange measurements were carried out using an infrared gas analyzer (LCPro+®, ADC Bioscientific Ltd., Hoddesdon, UK) equipped with a conifer leaf chamber (PLC, Parkinson Leaf Chamber) for *E. arborea, E. multiflora* and *R. officinalis*, and with a broad leaf chamber (PLC) for *Q. ilex, A.unedo, C. incanus, P. latifolia, P. lentiscus* and *S. aspera*. Measurements were taken on fully expanded sun leaves (n = 6 per each sampling occasion for *A. unedo, C. incanus, P. latifolia, P. lentiscus, Q. ilex*, and *S. aspera*) and on sun apical shoots (n= 6 per each sampling occasion for *E. arborea, E. multiflora* and *R. officinalis*). Net photosynthetic rate (P_N, μmol CO$_2$ m^{-2} s^{-1}), stomatal conductance (g_s, mol H$_2$O m^{-2} s^{-1}), leaf transpiration (E, mmol H$_2$O m^{-2} s^{-1}), sub-stomatal CO$_2$ concentration (C_i, μmol CO$_2$ mol^{-1} air), leaf temperature (T_l, °C) and photosynthetic photon flux density ($PPFD$, μmol photons m^{-2} s^{-1}) were measured from 9.00 to 11.00 a.m., under natural conditions, on cloud-free days ($PPFD >$ 1000 μmol m^{-2} s^{-1}, saturating level) to ensure that the near maximum daily photosynthetic rates were measured (Reich et al. 1999). The apparent carboxylation efficiency of Rubisco (P_N/C_i, mol CO$_2$ m^{-2} s^{-1}) was determined according to Arena et al. (2013).

Leaf dark respiration rate (R_L, μmol CO$_2$ m^{-2} s^{-1}) measurements were carried out contemporary to photosynthesis measurements, by darkening the leaf chamber with a black paper for 30 min prior to each measurement to avoid transient post-illumination bursts of CO$_2$ releasing. Measurements were carried out in four sampling days with the same weather conditions for each month.

Gross photosynthetic rates (P_G, μmol CO$_2$ m^{-2} s^{-1}) were calculated as the sum of the average values for P_N, photorespiration and R_L, based on the assumption that R_L (excluding photorespiration) were similar in the light and in the dark (Van Iersel 2003). Photorespiration rates (P_r, μmol CO$_2$ m^{-2} s^{-1}) were evaluated considering that under natural conditions C3 plants lose about 20% of the photosynthetically acquired CO$_2$ in the form of photorespiratory CO$_2$ (Larcher 2003). Total yearly P_N and R_L (P_{Ny} and R_{Ly}, respectively) were calculated on the basis of the daily photosynthetic and respiratory activity, according to Van Iersel (2003), extending data over the whole year. The ratio R_L/P_G was calculated according to Chastain et al. (2014). Total yearly P_G (P_{Gy}) and the fraction of total yearly R_{Ly} on P_{Gy} (R_{Ly}/P_{Gy}) were also calculated.

Leaf water status

Leaf water potential at pre-dawn (Ψ_{pd}) was measured on leaves of *A. unedo, C. incanus, P. latifolia, P. lentiscus, Q. ilex*, and *S. aspera* and on shoots of *E. arborea, E. multiflora, R. officinalis* (five leaves and five apical shoots per species, respectively, per each sampling occasion). Ψ measurements were carried out using a portable pressure chamber (SKPM 1400®, Skye Instruments, Llandrindod Wells, Powys, UK). Relative water content at pre-dawn (RWC_{pd}) was calculated at the same time and on the same leaves used for Ψ measurements as (eqn. 4):

$$RWC = \frac{FM - DM}{TM - DM} \cdot 100$$

where FM is the leaf fresh mass, DM the leaf mass after drying at 90 °C until constant mass was reached, and TM the leaf mass after rehydration until saturation for 48 h at 5 °C in the darkness (Bacelar et al. 2007). Ψ_{pd} and RWC_{pd} measurements were carried out in April and May (during the favorable period) and in August (during drought), simultaneously with gas exchange measurements.

Tab. 1 - Mean values and standard deviation of shrub height (H_s, m), shrub volume (V_s, m^3) and volume of shrub crown (V_{crown}, m^3) in the considered species (n =12).

Species	H_s (m)	V_s (m^3)	V_{crown} (m^3)
A.unedo	1.01 ± 0.22	1.58 ± 1.41	0.59 ± 0.43
C.incanus	0.76 ± 0.11	0.32 ± 0.24	0.20 ± 0.12
E. arborea	1.72 ± 0.24	3.55 ± 1.27	1.74 ± 0.75
E. multiflora	0.85 ± 0.23	1.24 ± 0.75	0.38 ± 0.23
P.latifolia	1.32 ± 0.31	1.77 ± 0.84	0.48 ± 0.25
P.lentiscus	0.90 ± 0.13	0.60 ± 0.46	0.22 ± 0.10
Q.ilex	1.45 ± 0.32	7.16 ± 5.20	2.72 ± 1.24
R.officinalis	0.87 ± 0.18	1.48 ± 1.35	0.85 ± 0.55
S.aspera	1.50 ± 0.40	-	-

Statistical analysis

All statistical tests were performed using the software package Statistica® v. 10.0 (Statsoft Inc., Tulsa, OK, USA).

The differences in physiological leaf traits were determined by the analysis of variance (ANOVA) and the *post-hoc* Tukey's test for multiple comparisons ($\alpha \leq 0.05$). Data were tested for normality and homogeneity of variances before carrying out the statistical analysis, with $\alpha \leq 0.05$.

A Partial Least Squares Regression analysis (PLS) was carried out to explore the pattern of co-variation between morphological and physiological leaf traits and to establish the order of the variable importance (i.e. VIP). PLS was carried out using the structural leaf traits (LMA, LTD, LT, CET, PCI and f_{ias}) as predictor variables and physiological traits (P_N, R_L, R_L/P_G, Ψ and RWC) as the response variables.

Results

Structural shrub traits

Structural shrub traits of the considered species are shown in Tab. 1. In particular, among the species analyzed, *E. arborea* showed the highest H_S (1.72 ± 0.24 m) and *C. incanus* the lowest (0.76 ± 0.11 m). *Q. ilex* was the species with the highest V_S and V_{crown} values (7.16 ± 5.20 m^3 and 2.72 ± 1.24 m^3, respectively), while *C. incanus* the lowest ones (0.32 ± 0.24 m^3 and 0.20 ± 0.12 m^3, respectively).

Anatomical and morphological leaf traits

E. arborea showed the lowest LT (200 ± 19 μm), followed by *C. incanus* (217 ± 9 μm), *P. lentiscus* (254 ± 18 μm), *A. unedo* (300 ± 20 μm), *Q. ilex, P. latifolia* and *S. aspera* (316 ± 5 μm, mean value) and by *R. officinalis* and *E. multiflora* (335 ± 7 μm).

CET was the highest in *P. latifolia* (43 ± 3 μm), followed by *E. multiflora* (41 ± 2 μm), *A. unedo* (35 ± 4 μm), *E. arborea* and *R. officinalis* (26 ± 3 μm, mean value), *S. aspera* (25 ± 2 μm), *Q. ilex* (22 ± 1 μm), *P. lentiscus* (16 ± 2 μm) and *C. incanus* (14 ± 2 μm).

A. unedo and *C. incanus* showed the highest f_{ias} (37 ± 1 %, mean value), followed by *P.lentiscus, Q. ilex* and *P. latifolia* (31 ± 3 %, mean value), *R. officinalis, E. multiflora* and *E. arborea* (26 ± 1 %, mean value) and *S. aspera* (15 ± 3%).

SPS_{LAWB} and PCI varied significantly among the considered species (Tab. 2), *P. latifolia* having the highest SPS_{LAWB} (574 ± 96 μm^2) and *E. arborea* the lowest one (155

Tab. 2 - Mean values and standard deviation of stomatal pore surface of stomata guard cell (SPS_{LAWB}, μm^2) and potential conductance index (PCI) in the considered species (n = 36). Mean values with the same letters are not significantly different after Tukey's test (P ≥ 0.05).

Species	SPS_{LAWB} (μm^2)	PCI
A.unedo	428 ± 50 ^{a}	18.2 ± 2.8 ^{a}
C.incanus	207 ± 47 ^{c}	7.8 ± 2.0 ^{d}
E. arborea	155 ± 21 ^{c}	6.0 ± 0.8 ^{d}
E. multiflora	238 ± 20 ^{c}	6.5 ± 0.7 ^{d}
P.latifolia	574 ± 96 ^{b}	23.5 ± 4.0 ^{b}
P.lentiscus	242 ± 25 ^{c}	15.4 ± 1.6 ^{a}
Q.ilex	389 ± 56 ^{a}	31.2 ± 3.0 ^{c}
R.officinalis	219 ± 47 ^{c}	5.5 ± 1.4 ^{d}
S.aspera	400 ± 45 ^{a}	9.2 ± 1.4 ^{d}

Tab. 3 - Mean values and standard deviations of leaf mass per area (*LMA*, mg cm^{-2}) and leaf tissue density (*LTD*, mg cm^{-3}) in the considered species (n = 60). Mean values with the same letters are not significantly different after Tukey's test ($P \geq 0.05$).

Species	*LMA* (mg cm^{-2})	*LTD* (mg cm^{-3})
A. unedo	15.8 ± 0.6 ^{c}	419 ± 20 ^{b}
C. incanus	15.1 ± 0.7 ^{bc}	580 ± 16 ^{e}
E. arborea	12.0 ± 1.1 ^{a}	510 ± 12 ^{c}
E. multiflora	26.3 ± 1.7 ^{f}	760 ± 23 ^{f}
P. latifolia	20.1 ± 1.2 ^{de}	515 ± 18 ^{c}
P. lentiscus	18.9 ± 1.0 ^{de}	548 ± 25 ^{d}
Q. ilex	20.4 ± 1.1 ^{e}	622 ± 16 ^{f}
R. officinalis	20.8 ± 0.6 ^{de}	580 ± 15 ^{e}
S. aspera	13.9 ± 0.8 ^{b}	355 ± 32 ^{a}

± 21 μm^2). *Q. ilex* had the highest *PCI* (31.2 ± 3.0) while *R. officinalis*, *E. multiflora* and *E. arborea* the lowest (6.0 ± 0.5, mean value).

Morphological leaf traits varied significantly among the considered species (Tab. 3). In particular, *LMA* value ranged from 26.3 ± 1.7 mg cm^{-2} (*E. multiflora*) to 12.0 ± 1.1 mg cm^{-2} (*E. arborea*) and *LTD* from 760 ± 23 mg cm^{-3} (*E. multiflora*) to 355 ± 32 mg cm^{-3} (*S. aspera*).

Gas exchange

The considered species had the same R_L trend during the year. The lowest rates were measured in winter (January-February: 0.95 ± 0.44 μmol m^{-2} s^{-1}, mean value) and the highest in August (3.05 ± 0.96 μmol m^{-2} s^{-1}, mean value). In particular, *R. officinalis* had the highest R_L in August (4.5 ± 1.6 μmol m^{-2} s^{-1}) and *P. latifolia* the lowest (1.58 ± 0.05 μmol m^{-2} s^{-1}).

The species analyzed shared the same P_G trend during the year (Fig. 2), though some differences were observed. A relatively low P_G value (12.4 ± 4.3 μmol m^{-2} s^{-1}, mean value) was monitored in winter (January-February), *R. officinalis* having the lowest rate (7.9 ± 1.2 μmol m^{-2} s^{-1}) and *C. incanus* the highest (19.0 ± 3.7 μmol m^{-2} s^{-1}). The highest P_G (22.0 ± 7.1 μmol m^{-2} s^{-1}, mean value) was measured in April-May, *C. incanus* having the highest P_G (39.5 ± 1.3 μmol m^{-2} s^{-1}), fol-

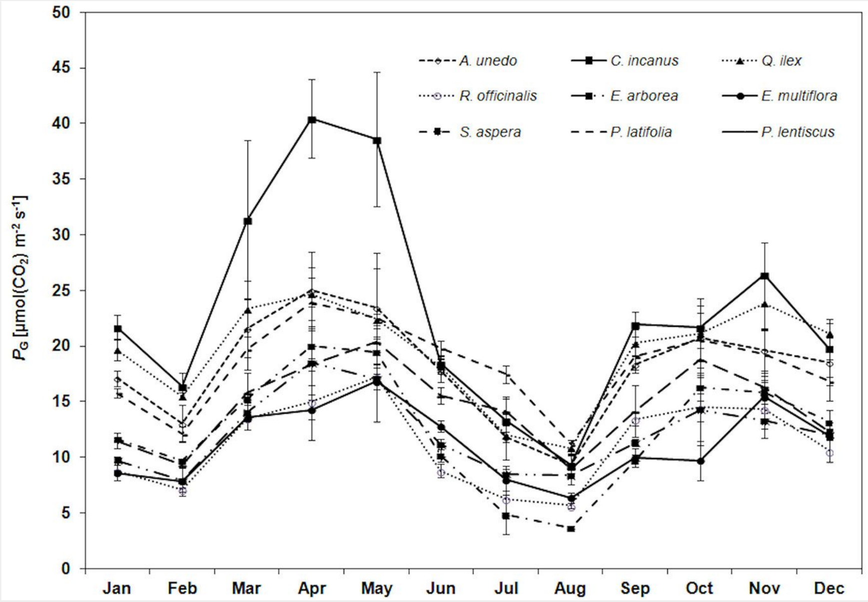

Fig. 2 - Trend of leaf gross photosynthesis (P_G, μmol CO$_2$ m^{-2} s^{-1}) during the study period for the considered species. Each point is the mean value of four sampling days per months (n = 24). Mean values (points) and standard deviation (error bars) are shown.

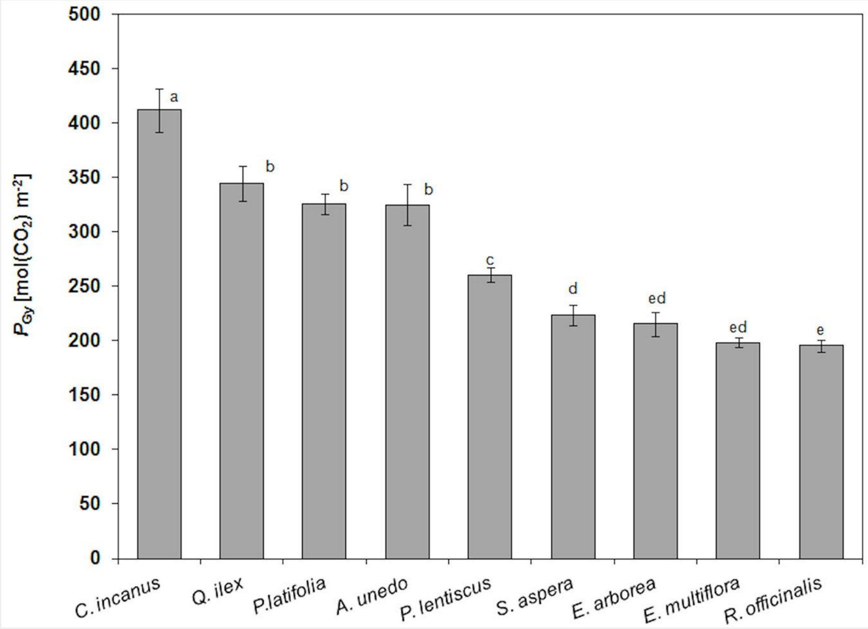

Fig. 3 - Mean values and standard deviation (error bars) of yearly gross photosynthesis (P_{Gy}, mol CO$_2$ m^{-2}) in the considered species (n= 12). Mean values with the same letters are not significantly different after Tukey's test ($P \geq 0.05$).

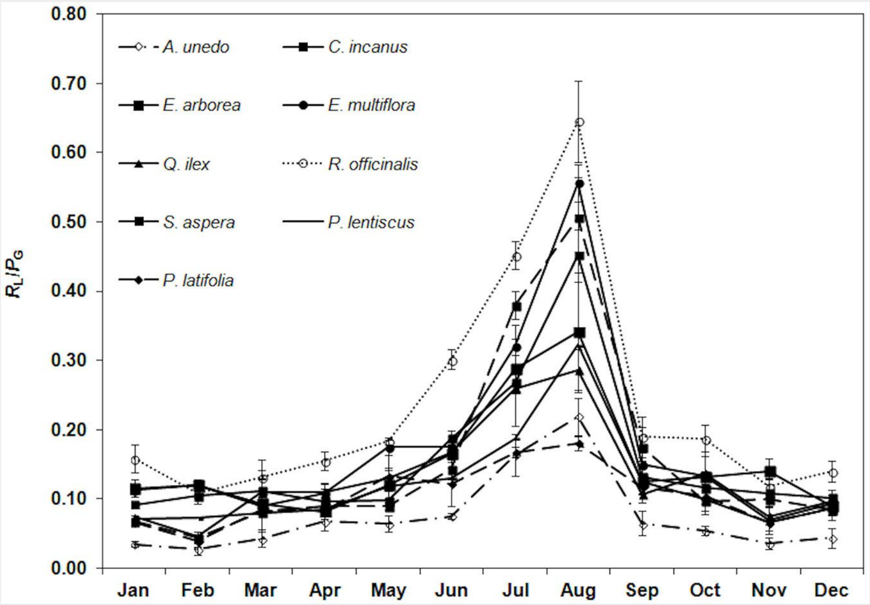

Fig. 4 - Trend of the ratio of leaf respiration to gross photosynthesis (R_L/P_G) during the study period. Each point is the mean value of four sampling days per months (n = 24). Error bars represent the standard deviation.

lowed by *A. unedo, Q. ilex, P. latifolia* and *P. lentiscus* (22.6 ± 7.7 µmol m^{-2} s^{-1}, mean value), *S. aspera, R. officinalis, E. multiflora* and *E. arborea* (17.3 ± 2.0 µmol m^{-2} s^{-1}, mean value). A 61% P_G decrease (mean value) than the spring maximum was monitored in August (*i.e.*, during drought), with *C. incanus* and *S. aspera* having the highest P_G decrease (79%, mean value) and *P. latifolia* the lowest (46%). After the first rainfall, at the beginning of September, P_G increased, on average, by 92%, *S. aspera* having the highest increase (> 100%).

P_{Gy} for the considered species are shown in Fig. 3. In particular, *C. incanus* showed the highest P_{Gy} (412 ± 20 mol m^{-2}), followed by *Q. ilex, A. unedo, P. latifolia* and *P. lentiscus* (314 ± 37 mol m^{-2}, mean value), *S. aspera, E. arborea, E. multiflora* and *R. officinalis* (208 ± 13 mol m^{-2}, mean value). The ratio R_{Ly}/P_{Gy} was the highest in *R. officinalis* (28%), followed by *E. multiflora* and *E.arborea* (16%), *C. incanus* (13%), *S .aspera* (12%), *Q. ilex, P. latifolia* and *P. lentiscus* (11%, mean value) and *A. unedo* (5%).

Among all the species, the lowest R_L/P_G ratio (0.07 ± 0.03, mean value) was monitored in winter and the highest in August (0.40 ± 0.15, mean value). *R. officinalis* had the highest ratio (0.65 ± 0.06) and *A. unedo* the lowest (0.22 ± 0.03 - Fig. 4).

Trend of apparent carboxylation efficiency is reported in Fig. 5. During the study period, the highest C_E was monitored, in all the considered species, in April-May (0.080 ± 0.025 mol m^{-2} s^{-1}, mean value) and the lowest in August (0.016 ± 0.007 mol m^{-2} s^{-1}, mean value). The mean yearly C_E value was the highest in *C. incanus* (0.063 ± 0.034 mol m^{-2} s^{-1}) and the lowest in *E. multiflora* (0.026 ± 0.014 mol m^{-2} s^{-1}).

Trends of stomatal conductance and transpiration rates are displayed in Fig. 6 (A, B). In particular, g_s showed the same P_N trend with the highest rates in April-May (0.140 ± 0.06 mmol m^{-2} s^{-1}, mean value) and the lowest in August (0.028 ± 0.011 mmol m^{-2} s^{-1}, mean value). The lowest E value were monitored in winter (January-February) in all the considered species (0.639 ± 0.143 mol m^{-2} s^{-1}, mean value).

Leaf water status

The highest Ψ_{pd} were measured in April-May (-0.11 ± 0.03 MPa, mean value), *A. unedo* and *E. multiflora* having the highest Ψ_{pd} (-0.07 ± 0.02 MPa, mean value) and *R. officinalis* the lowest (-0.15 ± 0.01 MPa). Ψ_{pd} decreased in August (-0.89 ± 0.31, mean value) *P. lentiscus* having the highest value (-0.40 ± 0.09 MPa) and *R. officinalis* the lowest (-1.50 ± 0.05 MPa).

RWC_{pd} followed the same trend showed by Ψ_{pd} during the study period, with the highest values in April-May (93.2 ± 1.1 % mean values) and a significant decrease in August. *Q. ilex, P. latifolia, P. lentiscus* and *A. unedo* had the highest values (92.0 ± 1.1 %) and *R. officinalis* the lowest (78 ± 4 %).

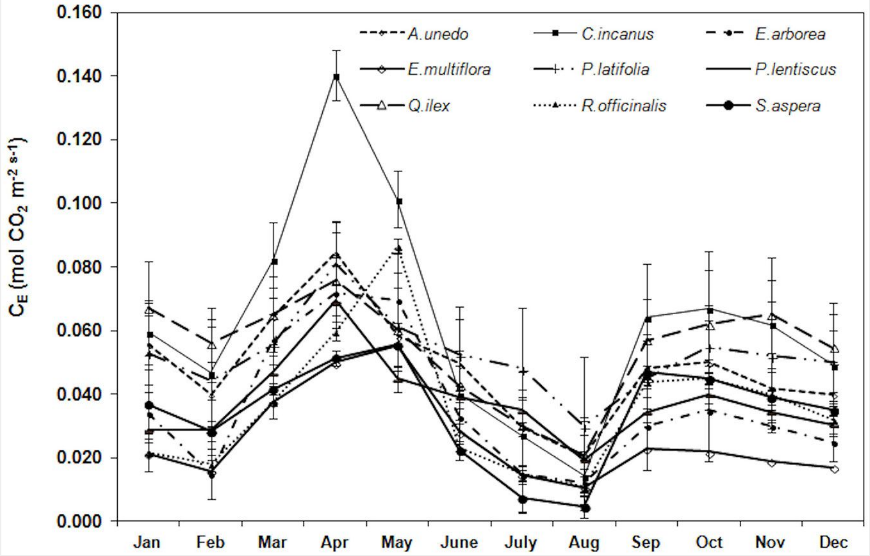

Fig. 5 - Trend of the apparent carboxylation efficiency (C_E, mol CO$_2$ mol^{-1} CO$_2$) during the study period. Each point is the mean value of four sampling days per months (n = 24). Error bars represent the standard deviation.

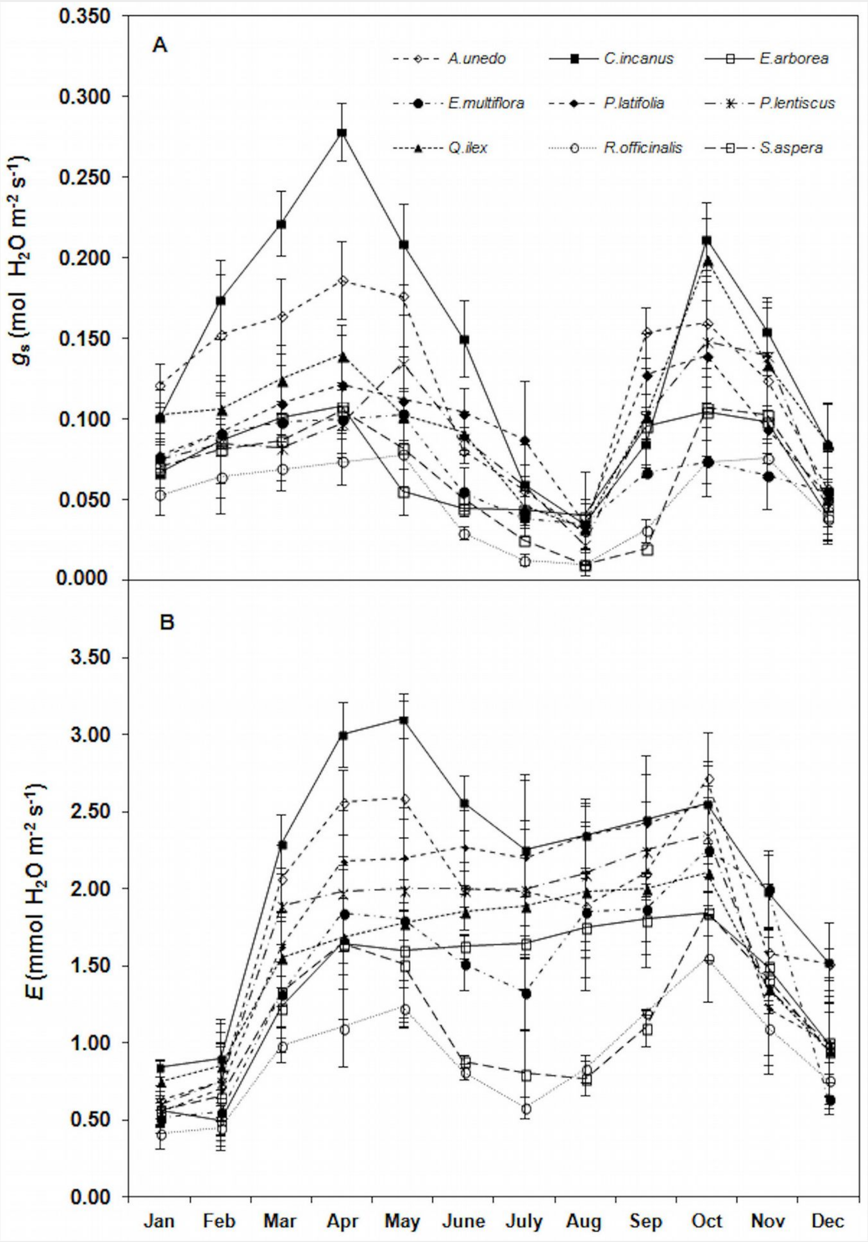

Fig. 6 - Trend of (A) stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and (B) transpiration rates (E, mmol H_2O m^{-2} s^{-1}) during the study. Each point is the mean value of four sampling days per months (n = 24). Error bars represent the standard deviation.

Tab. 4 - Weights of each structural trait in the component 1 and 2 extracted by the Partial Least Squares Regression (PLSR), and variable influence on projection (VIP) values for traits in the component 1. PLSR was carried out with structural traits as predictor variables and physiological traits as response variables. (*LMA*): leaf mass per unit leaf area; (*LTD*): leaf tissue density; (*PCI*): potential conductance index; (f_{ias}): fraction of mesophyll volume occupied by the intercellular air spaces; (*LT*): total leaf lamina thickness; (*CET*): thickness of both upper and lower cuticle and epidermis.

Structural trait	Component 1	Component 2	VIP
LMA	-0.227	-0.169	0.230
LTD	-0.094	-0.617	0.369
PCI	0.583	0.370	0.520
f_{ias}	0.676	-0.340	0.548
LT	-0.356	0.237	0.364
CET	-0.125	0.470	0.326
Eingenvalues	1.44	1.23	-
% of explained variance	24.1	20.5	-

Partial least squares regression

PLSR extracted two significant components which explained 24.1 % (component 1) and 20.5 % (component 2) of the original variance in the physiological variables, respectively (Tab. 4). In particular, component 1 was mainly associated to f_{ias} and *PCI*, while component 2 was mainly associated to *LTD* and *CET*. f_{ias} and *PCI* had the highest VIP (0.548 and 0.520, respectively), followed by *LTD* (0.370), *LT* (0.364), *CET* (0.326) and *LMA* (0.260).

Moreover, since a high VIP was found for *PCI*, a simple linear regression analysis was carried out to analyze the relationship between *PCI* and its components (*i.e.*, L_A and *SD*). The results showed that *PCI* was significantly correlated with *SD* ($PCI = 0.0688\ SD - 2.3879$; $R^2 = 0.68$; $P \leq 0.05$) while any significant relationship was found between *PCI* and L_A ($PCI = 0.9423\ L_A - 8.5206$; $R^2 = 0.33$; $P > 0.05$)

Discussion

Drought resistance in Mediterranean plant species is realized by different traits or combination of traits (Gratani & Varone 2004). Our results highlight that leaf structural traits can be considered driving factors for physiological traits. This is confirmed by PLS which showed that structural and physiological traits co-vary. Structural trait variations explained 44.61 % of the total variance in P_N, R_L, R_L/P_G, Ψ and *RWC*. The most important structural variables in the physiological trait projection are f_{ias} and *PCI*, from which the internal CO_2 diffusion pattern mainly depends (Puglielli et al. 2015). Photosynthesis and respiration are the most fundamental physiological processes which affect the carbon cycle on a scale ranging from the leaf to the globe (Cavaleri et al. 2008). Moreover, it is recognized that leaf respiration changes are related to physiological factors (Atkin et al. 1997) as well as environmental factors, in particular temperature and water availability (Crous et al. 2011). To date, the R_L behavior during drought is still unclear, since R_L has been found to either increase or decrease depending on the severity of drought stress and the species (Loka et al. 2011).

Overall, our results show a similar R_L response across the considered species, with the lowest rates in winter (0.95 ± 0.44 μmol m^{-2} s^{-1}, mean value) and the highest in the dry period (3.05 ± 0.96 μmol m^{-2} s^{-1}, mean value). This finding supports the hypothesis that R_L increases under drought, which may reflect an enhanced leaf senescence, solute accumulation and energy dissipation by the mitochondria to prevent oxidative damage by excess reductants from light reactions (Atkin & Macherel 2009). The metabolic damages under drought requires a surplus of respiratory products (*i.e.*, ATP and reducing equivalents – Varone & Gratani 2015). Thus, R_L increases to meet the demand for higher ATP levels (Atkin & Macherel 2009). Nevertheless, a different R_L impact on gross photosynthesis (*i.e.*, R_L/P_G) under drought was observed among the considered species. In particular, *C. incanus, E. multiflora, R. officinalis* and *S. aspera* show the highest R_L/P_G (0.54 ± 0.08, mean value) due to a higher R_L (3.31 ± 1.02 μmol m^{-2} s^{-1}, mean value) associated to the highest P_N decrease under drought (by 84%, mean value). Differences among the species in R_L/P_G are also related to a different P_N sensitivity to drought, which has a higher inhibitory effect on photosynthesis than on respiration (Flexas et al. 2005). The higher P_N sensitivity to drought is also confirmed by the thermal windows analysis which highlights that P_N drops below half of its maximum when leaf temperature is above 33.4 °C (mean value of the species – Gratani & Varone 2004, Catoni 2013). Moreover, Ψ_{pd} which can be considered a powerful indicator of drought stress (Jones 2007) varies from -1.30 ± 0.27 MPa in August to -0.11± 0.04 MPa in May (mean of the considered species) associated to a 10% RWC_{pd} decrease in August. *R. officinalis, C. incanus*, and *E. multiflora* show a lower capacity to adjust photosynthesis in drought also due to their shallow root system (Amato & Sarnataro 2001), which entails only the access to the superficial soil profile subjected to large changes in water content (Aubert 1978). The response of *R. officinalis* to drought may also be explained by 87% g_s decrease and the lowest PCI (5.5 ± 1.4) which depends on both stomatal density (SD = 168 ± 23 stomata mm^{-2}) and size (SPS_{LAWB}= 219 ± 47μm^2). This indicates a low capacity of *R. officinalis* to regulate stomatal conductance (Holland & Richardson 2009). Moreover, the higher C_E decrease (by 87%), indicating a lower Rubisco activity, suggests that in addition to a stomatal limitation, there are a non-stomatal limitation of photosynthesis according to Llorens et al. (2003). On the contrary, *Q. ilex, P. latifolia, P. lentiscus, A. unedo* and *E. arborea* are characterized by a lower R_L/P_G (0.22 ± 0.05, mean value) under drought, resulting from a lower R_L (2.55 ± 0.52 μmol m^{-2} s^{-1}, mean value) and a lower P_N decrease (62% of the maximum, mean value). The sclerophyllous species and *E. arborea* have a sufficiently higher Ψ_{pd} (-0.64 ± 0.21 MPa, mean value) and RWC_{pd} (92 ± 2%, mean value), reflecting their deep root system which accesses water from those parts of the soil profile subjected to narrow changes in water content (Gratani & Varone 2004). Moreover, the higher LMA and LTD of these species (17.4 ± 3.5 mg cm^{-2} and 523 ± 73 mg cm^{-3}, mean value, respectively) result in a higher leaf compactness which improves drought resistance. This behavior was also attested by the thermal windows analysis showing that P_N drops below half of its maximum when leaf temperature is above 37.0 °C (mean value of the species – Gratani & Varone 2004). *A. unedo* displays several mechanisms of drought stress resistance (Munné-Bosch & Peñuelas 2004). It is functionally adapted to cope with the summer drought by its stomatal regulation to which contributes a higher PCI (18.2 ± 2.8) and the steeper leaf inclination angle (Gratani & Ghia 2002), a mechanism which prevents the potential photo-inhibition of water-stressed leaves during drought (Werner et al. 1999).

With regards to P_{Gy} (*i.e.*, the daily photosynthetic and respiratory activity over the year), *C. incanus* had the highest P_{Gy} and *R. officinalis* and *E. multiflora* the lowest. In particular, *C. incanus* maintains a relatively high R_L in spring necessary to produce summer leaves (Catoni et al. 2012, Catoni & Gratani 2014). The higher P_N in *C. incanus* is reflected by a higher f_{ias} (38%) and a lower LMA (15.1 ± 0.7 mg cm^{-2}), which result from a shorter diffusion path from stomata to chloroplasts (Gratani & Varone 2004). Moreover, this is consistent with the high C_E (0.063 ± 0.034 mol m^{-2} s^{-1}, mean value of the study period) compared to the others species, according to the results of Arena et al. (2013), considering that the apparent carboxylation efficiency is usually correlates with Rubisco activity (Von Caemmerer 2000). On the contrary, the lowest P_{Gy} in *R. officialis* and in *E. multiflora* mainly reflects the low P_N during the year, as a consequence of the low C_E value (0.031 ± 0.007 mol m^{-2} s^{-1}, mean value) and the lower f_{ias} (26 ± 1%, mean value), associated to a higher LMA and LTD (23.6 ±3.9 mg cm^{-2} and 670 ±127 mg cm^{-3} mean value, respectively).

Conclusions

The results of this study revealed similar R_L trends across the considered species over the year. Nevertheless, large R_L/P_G variations among the species depend on the different sensitivity of both R_L and P_N to drought. Considering the increase of drought stress which is expected to occur in the Mediterranean basin and that the photosynthesis of Mediterranean evergreen species is frequently limited by suboptimal conditions (*i.e.*, water deficit, high light intensity and high temperature), improving the knowledge of leaf respiration variations and its effect on gross photosynthesis over time will result in a more accurate estimation of carbon balance.

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