DOI: 10.1007/s11099-011-0007-1 PHOTOSYNTHETICA 49 (1): 65-74, 2011

Photosynthetic and leaf respiration activity of *Malcolmia littorea* (L.) R. Br. in response to air temperature

L. GRATANI[+], R. CATONI, and L. VARONE

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

Abstract

Plant traits of *Malcolmia littorea* growing at the Botanic Garden of Rome and transplanted from the wild population developing along the *Latium* coast (Italy) were analyzed. The highest photosynthetic rates [P_N, 22.5 ± 0.5 μmol(CO_2) m^{-2} s^{-1}], associated to the highest chlorophyll content (Chl, 60 ± 5 SPAD units), and respiration rates [R, 11.1 ± 0.2 μmol(CO_2) m^{-2} s^{-1}] were reached in spring, when mean air temperature (T_m) was in the range 17°C to 23°C. P_N, Chl, and R decreased by 86, 38, and 59% in summer when mean maximum air temperature (T_{max}) was 30.3 ± 2.6°C. Leaf water potential decreased by 34% in summer compared to the spring value, and it was associated to a relative water content (RWC) of 74 ± 4%, and to a water-use efficiency (WUE) of 2.15 ± 0.81 μmol(CO_2) $mmol^{-1}$(H_2O). Moreover, also low air temperatures determined a significant P_N and R decreases (by 52 and 40% compared to the maximum, respectively). Responsiveness of gross photosynthetic rate (P_g) to R was higher than that to P_N as underlined by the slope of the regression line between the two variables. The results underlined a low tolerance to both high- and low air temperatures of *M. littorea*. The selected key traits (R, WUE, Chl) by the discriminant analysis might be used to monitor the *M. littorea* wild population in the long time. The *ex situ* cultivated plants could be propagated and used to increase the individuals number of the wild population.

Additional key words: air temperature; leaf respiration; leaf water potential; photosynthesis.

Introduction

Coastal sand dunes are characterized by a high plant diversity, including rare or endemic species, as a result of the environmental heterogeneity and climatic setting (Martinez and Psuty 2004, Barrett-Mold and Burningham 2009). In the past, coastal regions have often been inappropriately managed, largely due to the lack of adequate planning. The most important factors causing disturbance were cattle grazing, farming, reforestation, and urbanization which have eliminated many stretches of the internal vegetation types, while tourism and coastal erosion endangered beach and embryo-dune communities (O'Shea and Kirkpatrick 2000, Brown and McLachlan 2002, Taveira Pinto 2004, Clarke and Na Ayutthaya 2010).

Human pressure on coastal zones around the world has increased dramatically in the last 50 years, and this phenomenon is particularly striking in the Mediterranean Basin (Anthony 1997, Cori 1999, Curr *et al*. 2000, Kutiel *et al*. 2000, Comor *et al*. 2008). The concept of coastal zone management has nowadays become generally accepted (Taveira Pinto 2004, Rooney 2010). It is now recognized that healthy vegetated sandy ecosystems can provide fundamental ecosystem services to nearby urban areas, effectively acting in the long-term planning efforts, as the best possible protective buffer against infrastructure damages due to coastal erosion (Jolicoeur and O'Carrol 2007, Thompson and Schlocher 2008, Lucrezi *et al*. 2009, Mattheus *et al*. 2010). Moreover, plant diversity conservation has received special emphasis in recent years because of dune stabilization and land-use changes are resulting in the disappearance of a remarkable number of endemic or rare species (Ernst *et al*. 1996, Lammerts *et al*. 1999, Grootjans *et al*. 2002, Arens and Geelen 2006, Ma and Liu 2008).

Received 29 July 2010, *accepted* 25 January 2011.

[+] Corresponding author: fax: +39 06 49912358, e-mail: loretta.gratani@uniroma1.it

Abbreviations: Chl – chlorophyll content; DM – leaf dry mass; E – transpiration rate; g_s – stomatal conductance; L – leaf thickness; LA – leaf area; LMA – leaf mass per unit of leaf area; LTD – leaf tissue density; P_g – gross photosynthetic rate; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; R – leaf respiration rate; RWC – relative leaf water content; T_c – air temperature into the leaf chamber; T_l – leaf temperature; TLA – total leaf area per plants; WP – whole-plant photosynthetic rate; WR – whole-plant respiration rate; WUE – instantaneous water-use efficiency; Ψ_{pd} – predawn leaf water potential.

Acknowledgments: This paper was supported by the grants PRIN 2007 from Ministry for University and Scientific Research (MIUR).

It is therefore essential to monitor these environments at local level, and in particular those areas that still retain relatively well-preserved habitats (Grunewald and Schubert 2007), in order to protect and manage their biodiversity, thus maintaining the ecosystem services they provide (Provoost *et al*. 2004, Carboni *et al*. 2009, Gratani *et al*. 2009). Knowledge of life history traits of sand dune species may be important to assess their future presence, in particular when wild populations number and size are small, and habitat is fragmented (Münzbergová *et al*. 2005, Gratani *et al*. 2009).

Malcolmia littorea (L.) R. Br. is a perennial species growing along coastal sand dunes (Pignatti 1982). In Europe it is distributed in France, Spain, Italy, and Portugal (Tutin 1993). It is included in the Red List of the Italian Flora as a vulnerable (VU) species for the *Latium* (Central Italy) because it grows exclusively on the coast between San Felice Circeo and Terracina (Conti *et al*. 1997, Conti *et al*. 2005). A significant threat to small and isolated populations is their sensitivity to environmental, genetic, and demographic stochasticity (Morgan 2000).

The main objective of this research was to analyze morphological, anatomical and physiological traits of *M. littorea* plants growing at the Botanic Garden of Rome. The importance of conserving rare and endangered species has been emphasized through *ex situ* conservation (Mills and Schwartz 2005, Pearman and Weber 2005, Gratani *et al*. 2008, Acosta *et al*. 2009). Considering the likely increase in risks to *in situ* populations, *ex situ* conservation is an indispensable component of the conservation tool box (Swarts and Dixon 2009). Because the geographic distribution of rare species is likely to shift under global climate change, reintroduction/translocation of such species to the original habitats might become necessary for effective conservation (Dormann 2007).

Thus, we analyzed the key traits which might be used to monitor the *M. littorea* wild population in the long time. Moreover, the *ex situ* cultivated plants might be used to increase the individuals number of the wild population.

Material and methods

Study site: Experiments were carried out in the period January–December 2009 on ten representative *M. littorea* plants growing at the Botanical Garden of Rome (41°53’N, 12°28’E; 53 m a.s.l.). These plants were transplanted from the wild population growing along the coast between San Felice Circeo and Terracina (41°14’N;13°05’E), and cultivated outdoors. At the moment, the wild population was represented by a limited number of plants which were threatened by the building expansion of the coastal area.

The climate of the San Felice Circeo and Terracina area was of the Mediterranean type, and most of the total annual rainfall (689 mm) occurred in autumn and winter (Lazio Regional Agency for Development and Agricultural Innovation; Metereological Station of Pontinia, Latina, data of the period 2004 to 2009). The mean minimum air temperature (T_{min}) of the coldest month (February) was 2.5 ± 1.3°C, the mean maximum air temperature (T_{max}) of the hottest month (July) 31.9 ± 1.6°C, and the mean annual air temperature (T_{m}) 16.0 ± 5.6°C.

The climate of Rome is of the Mediterranean type, and most of the total annual rainfall (681 mm) occurred in autumn and winter. T_{min} of the coldest month (January) was 5.3 ± 1.7°C, T_{max} of the hottest month (July) was 30.9 ± 1.2°C, and T_{m} 16.8 ± 6.4°C. (Meteorological Station of the Collegio Romano, data for the period 1995 to 2009). During the study period T_{min} was 4.1 ± 3.9°C, T_{max} 32.4±1.6°C, and T_{m} 16.7±6.8°C. Total annual rainfall was 675 mm.

Leaf morphology and anatomy: Measurements of leaf morphology included leaf area (LA, cm^2), obtained by the *Image Analysis System (Delta-T Devices*, UK), and leaf dry mass (DM, mg), when oven-dried at 80°C to constant mass, using two fully expanded leaves per each of the considered plants. Leaf mass per unit of leaf area (LMA, mg cm^{-2}) was calculated by the ratio of leaf DM and LA. Leaf thickness (L, µm) was measured by leaf sections from five fresh, fully expanded leaves of the selected plants and measured by light microscope. Leaf tissue density (LTD, mg cm^{-3}) was calculated by the ratio of LMA and total leaf thickness (Wright and Westoby 2002).

Plant traits were measured on the cultivated plants including the total number of leaves per plant, and the total leaf area per plant (TLA, cm^2). TLA was calculated by multiplying the total leaf number per plant and the mean LA.

Gas exchange measurements were carried out by an infrared gas analyser *ADC-LCA4* (*ADC BioScientific Ltd.*, Hoddesdon, UK), equipped with a leaf chamber (*PLC, Parkinson Leaf Chamber*). Measurements were made weekly (3 days per month) on fully expanded leaves (two leaves per each of the considered plant, per each sampling day) during the study period.

Net photosynthetic rate (P_{N}), stomatal conductance (g_{s}), transpiration rate (E), photosynthetically active radiation (PAR), air temperature in the leaf chamber (T_{c}), leaf temperature (T_{l}), and leaf respiration rates (R) were carried out under natural conditions, on cloud-free days, in the morning, from 8.00 to 12.00 h to ensure that maximum P_{N} and R were measured (Reich *et al*. 1995; Varone and Gratani 2007). During measurements PAR was ≥ 1,500 µmol(photon) m^{-2} s^{-1}. Environmental controls within the leaf chamber were maintained to match the outdoor environmental conditions (Ow *et al.* 2008).

The instantaneous water-use efficiency (WUE) was calculated as the ratio of net photosynthetic rate and transpiration rate (Niu *et al.* 2006).

On each sampling occasion, R measurements were carried out after P_N ones (on the same leaves), as CO_2 efflux, darkening leaf chamber by a black paper, according to Cai *et al.* (2005), for 30 min prior to each measurement, to avoid transient post-illumination bursts of CO_2 releasing (Atkin *et al.* 1998a,b). The shown R and P_N data represented the mean of the maximum collected values of the three measurement days in the month.

Whole-plant photosynthesis and respiration: The whole-plant photosynthetic rates (WP) and the whole-plant respiration rates (WR) were estimated multiplying TLA by the mean photosynthetic and respiration rates, respectively (Handons *et al.* 1988, Anderson and Tomlinson 1998). WP and WR were calculated using the mean value of winter, spring, and summer R and P_N, respectively.

Gross photosynthesis: Gross photosynthetic rate (P_g) was calculated using net photosynthetic rate and leaf respiration rate as: $P_g = P_N + R$.

Water relations: Predawn leaf water potential (Ψ_{pd}, MPa) was measured on five fully expanded leaves, with a pressure chamber (*SKPM 1400 Skye Instruments*, UK). Measurements were carried out in May and August.

Relative leaf water content at predawn (RWC_{pd}, %) was calculated, at the same time and on the same leaves used for Ψ_{pd} measurements, as: RWC = (FM – DM)/(TM – DM) × 100, where FM was 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.

Chlorophyll content (Chl) was measured by a *SPAD-502* meter (*Konica Minolta Sensing, Inc.*, Osaka, Japan). Chl measurements were carried out on fully expanded leaves (two leaves per plant in each sampling occasion) after cleaning the surface dust from each leaf, and on various points on the surface of each leaf sample (the same leaves used for gas-exchange measurements) (Gratani 1992).

Statistics: Differences in the considered variables were determined by the analysis of variance (*ANOVA*), and *Tukey* test for multiple comparisons.

The relationships between net photosynthesis and leaf temperature (photosynthetic thermal window *sensu* Larcher 1994) were tested by the regression between these two variables. Leaf temperature enabling 100%, 100 to 90%, and 90 to 50% of the highest P_N ($T_{100\%}$; $T_{100–90\%}$, and $T_{90–50\%}$, respectively) were calculated.

The regression analysis was carried out to evaluate the correlations among the considered physiological traits.

A stepwise discriminant analysis was carried out using the physiological variables (P_N, R, E, P_g, g_s, WUE, and Chl) as predictors, in order to evaluate which variables discriminated between the different seasons of the year. At each step, all traits were reviewed in order to evaluate those contributing most to the discrimination, according to Nevo *et al.* (2000), and Menalled and Kelty (2001).

The discriminatory power was evaluated considering the partial Wilks'lambda (*i.e.* a lower Wilks' lambda meant a higher discriminatory power).

All statistical tests were performed using a statistical software package (*Statistica*, *Statsoft*, USA).

Results

Plant and leaf traits: The total number of leaves per plant was 952 ± 200. TLA was 1,383 ± 890 cm^2. *M. littorea* fully expanded leaves were characterised by LA of 1.1 ± 0.1 cm^2, and DM of 9.6 ± 1.3 mg. LMA and LTD were 9.0 ± 0.6 mg cm^{-2} and 284 ± 24 mg cm^{-3}, respectively. Leaf thickness was 317 ± 53 μm.

Gas exchange: PAR and T trends during gas-exchange measurements are shown in Fig. 1. The highest P_N [22.5 ± 0.5 μmol(CO_2) m^{-2} s^{-1}] and R [11.1 ± 0.2 μmol(CO_2) m^{-2} s^{-1}] were measured in May, when the mean daily air temperature was in the range 17.0 to 23.1°C (Fig. 2). P_N and R decreased significantly ($p \leq 0.05$) in winter (by 52 and 40%, respectively) and in summer (by 86 and 59%, respectively).

g_s had the same P_N trend with the highest values in spring [0.206 ± 0.03 mol(H_2O) m^{-2} s^{-1}], decreasing in winter [0.108 ± 0.03 mol(H_2O) m^{-2} s^{-1}] and summer [0.058 ± 0.02 mol(H_2O) m^{-2} s^{-1}] (Fig. 2). There was a significant ($p \leq 0.05$) relationship between g_s and P_N (Table 1).

WUE had the highest value in spring [6.3 ± 1.1 μmol(CO_2) mmol(H_2O)$^{-1}$] decreasing by 66% in summer and by 43% in winter (Fig. 2).

The polynomial relationship between P_N and T_l indicated that P_N reached the maximum rates when T_l was 25°C and it dropped below half of its maximum when T_l was under 15°C and above 35°C, respectively (Fig. 3). Moreover, the correlation between R and air temperature was stronger (R^2 = 0.54) than that between P_N and air temperature (R^2 = 0.29) (Table 1).

Whole-plant photosynthesis and respiration: WP was 2.5 ± 0.5 [μmol(CO_2) s^{-1}] in spring, decreasing by 60% in

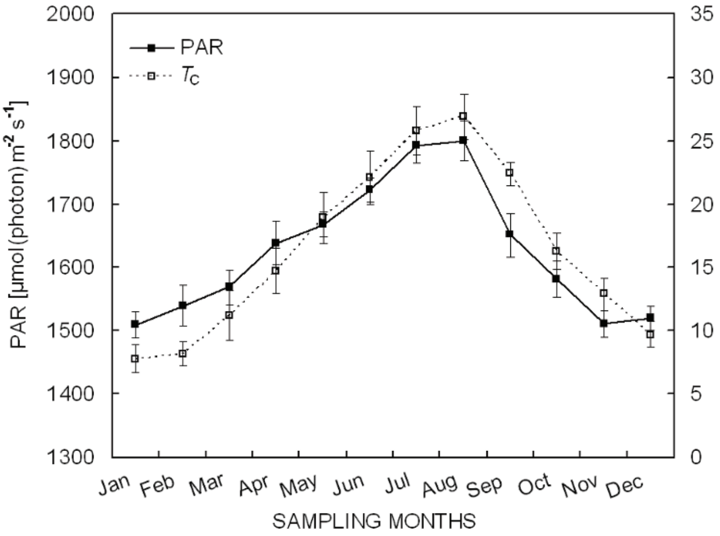

Fig. 1. Photosynthetically active radiation (PAR) and air temperature in the leaf chamber (T_c) carried out at the same time of the gas-exchange measurements. Each point is the mean value of three sampling days per month ($n = 60$). Mean values (± SD) are shown.

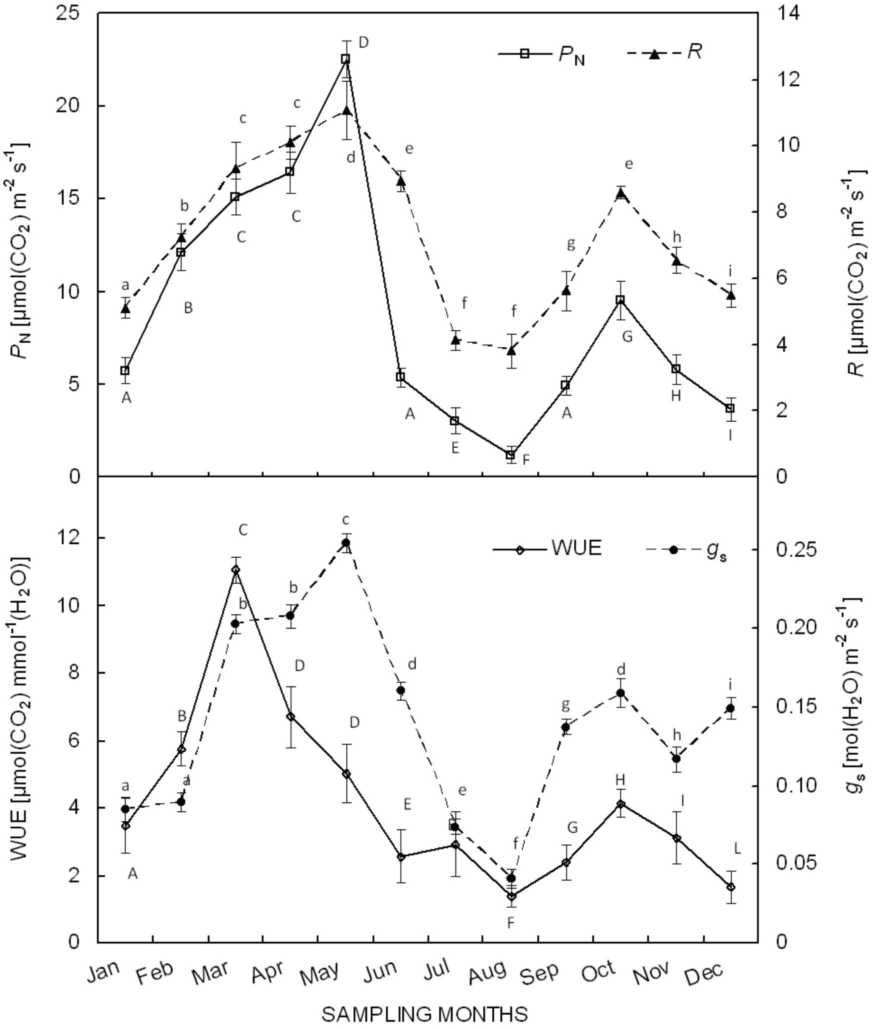

Fig. 2. Trends of net photosynthetic rate (P_N), leaf respiration rate (R), stomatal conductance (g_s), and water-use efficiency (WUE) of *M. littorea* during the study period. Each point is the mean value of three sampling days per month ($n = 60$). Mean values (± SD) are shown. For each trait mean values with the same *letters* are not significantly different (*ANOVA*, $p \leq 0.05$).

winter, and by 80% in summer. WR was 1.4 ± 0.6 µmol(CO$_2$) s^{-1} in spring, decreasing by 43% in winter and by 45% in summer.

Gross photosynthesis: The highest P_g [33.6 ± 0.7 µmol(CO$_2$) m^{-2} s^{-1}] was measured in May, decreasing by 82% in July and August (mean value), increasing more than 100% in October, and decreasing by 73% in December (Fig. 4). During the favorable periods (spring and autumn) the highest P_g was mostly justified by the highest P_N than by the R one, while during the stress periods (winter and summer) R contributed mostly to P_g than P_N, as confirmed by the lowest P_N/R ratio (Fig. 4). Moreover, the regression analysis between P_g and P_N, and between P_g and R, underlined that 96% of P_g variations depended on P_N (Fig. 5). The slope of the regression line between P_g and P_N was lower (1.32) than that between P_g and R (3.23).

Water relations: The Ψ_{pd} and RWC values in spring were –1.4 ± 0.5 MPa and 82 ± 3%, respectively. Ψ_{pd} decreased by 34% in summer associated to a RWC of 74 ± 4%.

Chl: The highest Chl was measured in May (60 ± 5 SPAD units), decreasing by 38% in summer, and by 24% in winter (Fig. 6). There was a significant ($p \leq 0.05$) correlation ($R^2 = 0.64$) between Chl and P_N (Table 1).

Table 1. Summary of regression analysis between net photosynthetic rate (P_N) and stomatal leaf conductance (g_s), P_N and air temperature (T, °C), leaf respiration rate (R) and T and between P_N and chlorophyll content (Chl). Regression equation and determination's coefficient (R^2) are shown. The correlation were significant at $p \leq 0.05$ ($n = 60$).

Regression equation	R^2
$P_N = 538.63\ g_s^2 - 37.97\ g_s + 8.83$	0.79
$P_N = -0.0916\ T^2 + 2.9073\ T - 10.474$	0.29
$R = -0.0511\ T^2 + 1.6972\ T - 4.8576$	0.54
$P_N = 0.77\ Chl - 26.86$	0.64

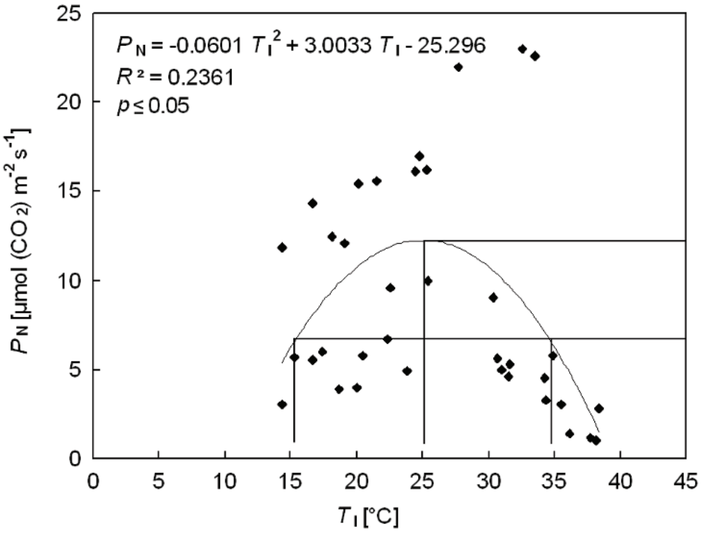

Fig. 3. Regression analysis between net photosynthetic rate (P_N) and leaf temperature (T_l) of *M. littorea*. Regression equation and determination coefficient (R^2) are shown. The relative photosynthetic capacity and T_l range outside of which P_N drops below 50% of the maximum ("photosynthetic thermal window" *sensu* Larcher 1994) are also shown.

Discriminant analysis: The results of the stepwise discriminant analysis showed that among the considered physiological parameters, R, WUE and Chl were included in the model, while P_N, P_g, g_s were excluded. Moreover, the values of Wilks' lambda indicated that R was the most discriminant variable among the different seasons of the year (Table 2).

Discussion

Plant species growing in the Mediterranean region are exposed to different environmental stresses, in particular high evaporative demands and high temperatures during summer, and low temperatures during winter (Gimeno *et al.* 2008, Varone and Gratani 2007, Rubio-Casal *et al.* 2010). High and low air temperature extremes in the Mediterranean climate can be important constraining factors for plants because they restrict primary productivity and influence their distribution, also in consideration of global change (Larcher 2000, Gratani *et al.* 2000, Awada *et al.* 2003, Baquedano and Castillo 2007).

Groom *et al.* (1991) underlined that any factor which reduces photosynthetic carbon fixation also reduces the species ability to compete in its habitat. As leaf respiration is related to photosynthesis by its dependence on substrate supply (Atkin *et al.* 2006), it appears to be an important determinant of plant productivity (Maseyk *et al.* 2008). Moreover, at the moment, only few reports focus on leaf respiration under reduced water supply (Flexas *et al.* 2005, Ribas-Carbo *et al.* 2005, Galmés *et al.* 2007). Water-stress experiments show that R generally decreases under water deficit, although additional stress may increase respiration rates (Melzack *et al.* 1985, Amthor 1989, Maseyk *et al.* 2008). Moreover, Vassileva *et al.* (2010) show that the effects of dehydration on leaf respiration depend on the duration and severity of drought stress.

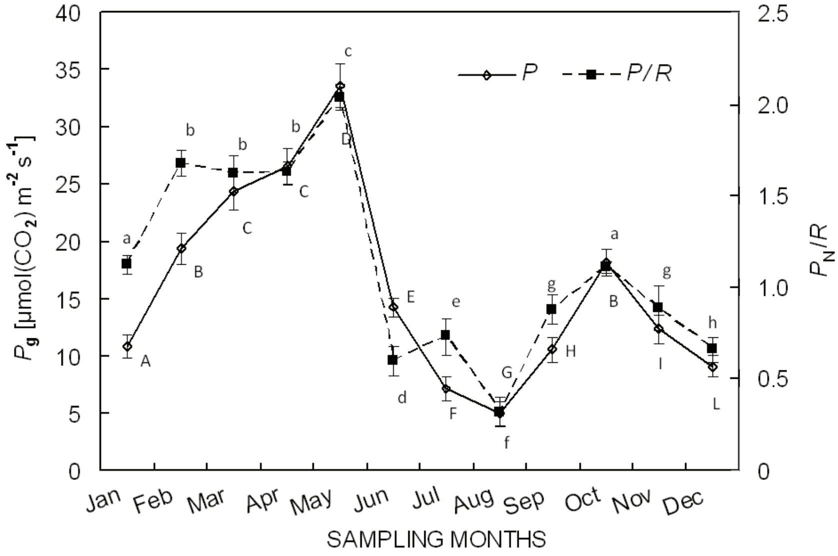

Fig. 4. Trend of gross photosynthesis (P_g) and the ratio between net photosynthesis and leaf respiration (P_N/R) of *M. littorea* during the study period. Each point is the mean value of three sampling days per month (n = 60). Mean values (± SD) are shown. For each trait mean values with the same *letters* are not significantly different (*ANOVA*, $p \leq 0.05$).

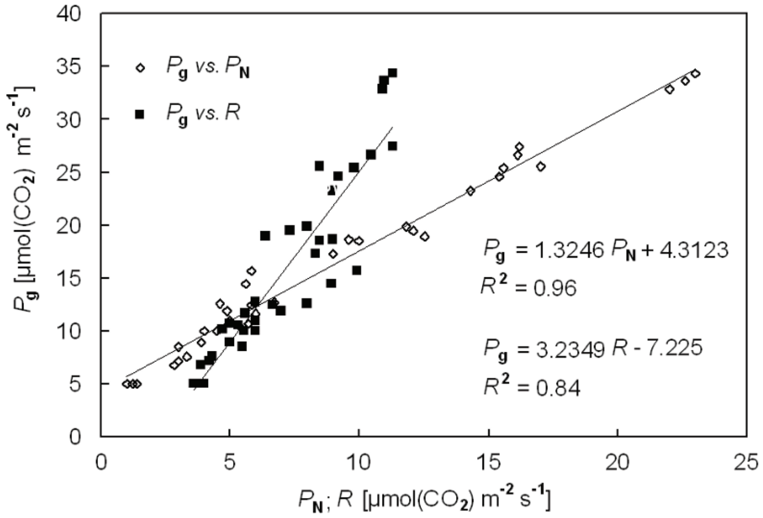

Fig. 5. Regression analysis between gross photosynthesis (P_g) and leaf respiration rate (R), and between P_g and net photosynthetic rate (P_N) of *M. littorea*. Regression equation and determination coefficient (R^2) are shown (n = 60).

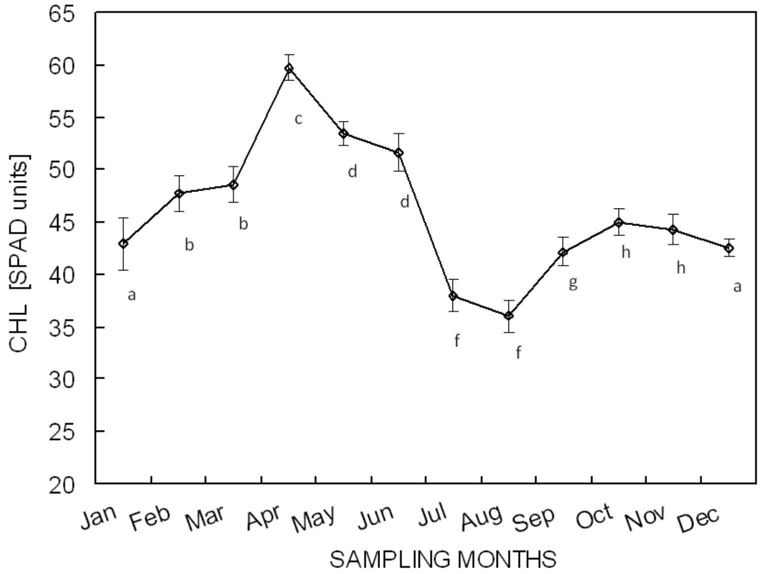

Fig. 6. Trend of chlorophyll content (Chl) of *M. littorea* during the study period. Each point is the mean value of three sampling days per month (n = 60). Mean values (± SD) are shown. For each trait mean values with the same *letters* are not significantly different (*ANOVA*, $p \leq 0.05$).

Table 2. Summarizing table obtained by stepwise discriminant analysis based on the following physiological variable: net photosynthetic rate (P_N), leaf respiration rate (R), leaf transpiration rate (E), gross photosynthesis (P_g), stomatal conductance (g_s), water-use efficiency (WUE) and chlorophyll content (Chl). Only the variables included in the model are shown. N – number in the model.

Variable	N	Wilk'lambda	R^2	F
R	1	0.42	0.55	13.7
WUE	2	0.50	0.57	9.7
Chl	3	0.74	0.17	3.3

The photosynthetic response to air temperature is an important focus of recent studies (Gratani *et al.* 2000, Medlyn *et al.* 2002, Gratani *et al.* 2009). Our results underline that the polynomial relationship between P_N and T_l is representative of the influence of temperature on *M. littorea* photosynthetic activity. The favorable T_l enabling 100–50% of *M. littorea* P_N is between 15 and 35°C. The highest P_N rates monitored in spring [22.5 ± 0.5 μmol(CO_2) m^{-2} s^{-1}] are associated to the highest R [11.1 ± 0.2 μmol(CO_2) m^{-2} s^{-1}] and Chl (60 ± 5 SPAD units).

P_N, R, and Chl significantly ($p \leq 0.05$) decrease during drought (by 86, 59 and 38%, respectively; T_{max} = 30.3 ± 2.6°C; 60 mm total rainfall of the period) attested by the low P_N/R (0.55 ± 0.21). At the beginning of summer (June) R decrease is lower than that of P_N, resulting in an altered balance between substrate and supply, according to Gálmes *et al.* (2007) for other Mediterranean species. The stronger relationship between R and T ($R^2 = 0.54$, $p \leq 0.05$) than that between P_N and T ($R^2 = 0.29$, $p \leq 0.05$) underlines that the factors involved in P_N variations are more than those involved in R variations. Chapin *et al.* (2000), Flanagan *et al.* (2002), and Ow *et al.* (2010) underline that factors such as soil water content, leaf water status, and leaf internal CO_2 concentration have a larger influence on P_N than on R.

g_s has the same P_N trend, as confirmed by the significant ($p \leq 0.05$) correlation between the two variables; nevertheless, during the stress periods (winter and summer) g_s has a lower decrease (by 47% and 71%, respectively) than P_N (by 52% and 86%, respectively) which underlines possible nonstomatal limitations of photosynthesis, according to the results of Flexas *et al.* (2001). In fact, during stress periods the photosynthetic rates could be constrained by metabolic alterations (Lawlor and Cornic 2002). Ohashi *et al.* (2006) underline that, at metabolic level, limitations to CO_2 assimilation cause an imbalance between the photochemical activity of the photosystem II and the electron requirement for photosynthesis, which leads to an increased susceptibility to leaf photodamage (He *et al.* 1995, Flagella *et al.* 1998).

It has been shown that in Mediterranean plant species chlorophyll content decrease, combined with an increased reflectance, may act as a supplementary defence against photodestruction (Kyparissis and Manetas 1993, Gratani and Varone 2004), lowering the intercepted light and, consequently, increasing the capacity to dissipate the excess of excitation energy (Munné-Bosch and Alegre 2000). Munné-Bosch and Alegre (2000), Gratani and Varone (2004), and Zunzunegui *et al.* (2010) show that in Mediterranean species the Chl decrease in response to drought may be more than 50% compared to the maximum. Our results suggest that *M. littorea* Chl decrease during the stress periods is lower than in other Mediterranean species; this result underlines that in *M. littorea* this mechanism does not contribute significantly to limit photoinhibitory effects, thus, explaining the high P_N decrease in summer and the week relationship between P_N and T_l ($R^2 = 0.23$, $p \leq 0.05$). In addition, our results underline that also cold temperatures have a great effect on P_N and R decreasing by 52% and 40%, respectively, compared to the maximum. This significant P_N and R decrease of *M. littorea* in winter suggests that episodic freezing or frosting events may severely affect its growth efficiency.

Leaf water relations data may provide a useful indication of different species capability to maintain relatively high photosynthetic rates during drought (White *et al.* 2000). *M. littorea* WUE [4.1 ± 2.8 μmol(CO_2) $mmol^{-1}$(H_2O)], mean value of the study period] is in the range of other dune species of the *Latium* coast [3.6 to 5.2 μmol(CO_2) $mmol^{-1}$(H_2O); Gratani *et al.* 2009]. The highest WUE values (6.3 ± 1.1 μmol(CO_2) $mmol^{-1}$(H_2O)] are reached in spring and they are associated to a Ψ_{pd} of –1.4 ± 0.5 MPa and a RWC of 82 ± 3%. WUE decreases by 82% during drought and it is associated to a Ψ_{pd} of –2.1 ± 0.1 MPa and to a RWC of 74 ± 4%. These results suggest that the cumulative carbon assimilation of *M. littorea* available for growth might be drastically reduced as drought stress is going to increase.

Many studies have assessed the seasonality of photosynthesis to improve estimates of global carbon budget (Ow *et al.* 2010). Moreover, because there is greater effect of air temperature and endogenous metabolism on respiration *vs.* the photosynthetic process, gross photosynthesis is believed to be a better measure than net photosynthesis for comparing carbon acquisition in the long time (Grulke 2010). *M. littorea* P_g variations during the year reflect net photosynthetic and respiration rates changing; the highest P_g measured in spring [33.6 ± 0.7 μmol(CO_2) m^{-2} s^{-1}, May] and in autumn [13.7 ± 3.9 μmol(CO_2) m^{-2} s^{-1}, mean value from September to November] decreases by 82% in drought (July and August) and by 73% in winter (December).

At morphological and anatomical levels, LMA may be considered an adaptive trait of plants to stress factors (Gamage and Jesson 2007, Jullien *et al.* 2009). Niinemets (2001) and Gratani and Varone (2006) underline the LMA increase in drought conditions, which is due to a higher biomass allocation to leaf structural components (Reich *et al.* 1999). Our results underline that *M. littorea*

LMA is at the borderline of other sand dune species developing along the *Latium* coast (6.8 to 30.6 mg cm^{-2}, Gratani
et al. 2009).

In order to conserve coastal dune ecosystems the need for adaptive management has become more widely recognized (*e.g.* French 2004, Van der Meulen and Udo de Haes 1996, Milligan *et al.* 2006, Saye and Pye 2007, Martinez and Psuty 2004, Barrett-Mold and Burningham 2009), and a better understanding of morphological, anatomical and physiological traits of plant species may provide a significant contribution to the conservation and protection of coastal habitats, and in particular of threatened and rare sand species.

Our results on the whole underline that *M. littorea* is characterized by a low tolerance to high- and low air temperatures (attested by a high significant P_N and R decrease during drought and in winter) which may be indicative of its low competitive ability relative to the other sand dune species coexisting along the *Latium* coast. The *ex situ M. littorea* cultivated plants may be used to increase the number of the wild population growing along the *Latium* coast. Moreover, our results underline the importance of monitoring the functional state of *M. littorea* wild population by the selected key traits (R, WUE, and Chl) in the long time.

References

Acosta, A., Carranza, M.L., Izzi, C.F.: Are there habitats that contribute best to plant species diversity in coastal dunes? – Biodivers. Conserv. **18**: 1087-1098, 2009.

Amthor, J.S.: Respiration and Crop Productivity. – Springer-Verlag, New York – Berlin – Heidelberg – London – Paris – Tokyo 1989.

Anderson, P.D., Tomlinson, P.T.: Ontogeny affect response of northern red oak seedlings to elevated CO_2 and water stress. I. Carbon assimilation and biomass production. – New Phytol. **140**: 477-491, 1998.

Anthony, E.J.: The status of beaches and shoreline development options on the French Riviera: A perspective and a prognosis. – J. Coast Conserv. **3**: 169-178, 1997.

Arens, S.M., Geelen, L.H.W.T.: Dune landscape rejuvenation by intended destabilisation in the Amsterdam water supply dunes. – J. Coastal Res. **22**: 1094-1107, 2006.

Atkin, O.K., Evans. J.R., Ball, M.C., Siebke, K.: Relationship between the inhibition of leaf respiration by light and enhancement of leaf dark respiration following light treatment. – Aust. J. Plant Physiol. **25**: 437-443, 1998a.

Atkin, O.K., Evans, J.R., Ball, M.C., Siebke, K., Pons, T.L., Lambers, H.: Light inhibition of leaf respiration: the role of irradiance and temperature. – In: Moller, I.M., Gardestrom, P., Gliminius, K., Glaser, E. (ed.): Plant Mitochondria: from Gene to Function. Pp. 567-574. Backhuys Publishers, Leiden 1998b.

Atkin, O.K., Scheurwater, I., Pons, T.L.: High thermal acclimatation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. – Global Change Biol. **12**: 500-515, 2006.

Awada, T., Radoglou, K., Fotelli, M.N., Constantinidou, H.I.A.: Ecophysiology of seedlings of three Mediterranean pine species in contrasting light regimes. – Tree Physiol. **23**: 33-41, 2003.

Baquedano, F.J., Castillo, F.J.: Drought tolerance in the Mediterranean species *Quercus coccifera, Quercus ilex*, *Pinus halepensis*, and *Juniperus phoenicea*. – Photosynthetica **45**: 229-238, 2007.

Barret-Mold, C., Burningham, H.: Contrasting ecology of prograding coastal dunes on the northwest coast of Ireland. – J. Coast Conserv. **14**: 81-90, 2009.

Brown, A.C., McLachlan, A.: Sandy shore ecosystems and the threats facing them: some predictions for the year 2025. – Environ. Conserv. **29**: 62-77, 2002.

Cai, Z.-Q., Slot, M., Fan, Z.-X.: Leaf development and photosynthetic properties of three tropical tree species with delayed greening. – Photosynthetica **43**: 91-98, 2005.

Carboni, M., Carranza, M.L., Acosta, A.: Assessing conservation status on coastal dunes: A multiscale approach. – Landsc. Urban Plan. **91**: 17-25, 2009.

Chapin F.S., III, McGuire A.D., Roderson, J. *et al.*: Arctic and boreal ecosystems of western North America as components of climate system. – Glob. Change Biol. **6**: 211-223, 2000.

Clarke, D., Na Ayutthaya, S.S.: Predicted effects of climate change, vegetation and tree cover on dune slack habitats at Ainsdale on the Sefton Coast, UK. – J. Coast Conserv. **14**: 115-125, 2010.

Comor, V., Orgeas, J., Ponel, P., Rolando, C., Delettre, Y.R.: Impact of anthropogenic disturbances on beetle communities of French Mediterranean coastal dunes. – Biodivers. Conserv. **17**: 1837-1852, 2008.

Conti, F., Manzi, A., Pedrotti, F.: [Regional Red Lists of the Italian Flora.] – TIPAR, Rome 1997. [In Italian.]

Conti, F., Abbate, G., Alessandrini, A., Blasi, C.: [An Annotated Checklist of the Italian Vascular Flora.] – Palombi Editori, Roma 2005. [In Italian.]

Cori, B.: Spatial dynamics of Mediterranean coastal regions. – J. Coast Conserv. **5**: 105-112, 1999.

Curr, R.H.F., Koh, A., Edwards, E., Williams, A.T., Davies, P.: Assessing anthropogenic impact on Mediterranean sand dunes from aerial digital photography. – J. Coast Conserv. **6**: 15-22, 2000.

Dormann, C.F.: Promising the future? Global change projections of species distributions. – Basic Appl. Ecol. **8**: 387-397, 2007.

Ernst, W.H.O., Slings, Q.L., Nelisssen, H.J.M.: Pedogenesis in coastal wet dune slacks after sod-cutting in relation to regeneration. – Plant Soil **180**: 219-230, 1996.

Flagella, Z., Campanile, R.G., Stoppelli, M.C., De Caro, A., Di Fonzo, N.: Drought tolerance of photosynthetic electron transport under CO_2-enriched and normal air in cereal species. – Physiol. Plant. **104**: 753-759, 1998.

Flanagan, L.B., Wever, L.A., Carlson, P.J.: Seasonal and interannual variation in carbon dioxide exchange and carbon balance in a northern temperate grassland. – Glob. Change Biol. **8**: 599-615, 2002.

Flexas, J., Gulías, J., Jonasson, S., Medrano, H., Mus, M.: Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. – Acta Oecol. **22**: 33-43, 2001.

Flexas, J., Galmés, J., Ribas-Carbo, M., Medrano, H.: The effects of water stress on plant respiration. – In: Lambers, H., Ribas-Carbo. M. (ed.): Plant Respiration: From Cell to Ecosystem. Advances in photosynthesis and respiration series, Vol. 18. Pp. 85-94. Springer, Dordrecht 2005.

French, P.W.: The changing nature of, and approaches to, UK coastal management at the start of the twenty-first century. – Geogr. J. **170**:116-125, 2004.

Galmés, J., Ribas-Carbó, M., Medrano, H., Flexas, J.: Response of leaf respiration to water stress in Mediterranean species with different growth forms. J. Arid Environ. **68**: 206-222, 2007.

Gamage, H.K., Jesson, L.: Leaf heteroblasty is not an adaptation to shade: seedling anatomical and physiological responses to light. – New Zealand J. Ecol. **31**: 245-254, 2007.

Gimeno, T.E., Pías, B., Lemos-Filho, J.P., Valladares, F.: Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedling to drought and cold. – Tree Physiol. **29**: 87-98, 2008.

Gratani, L.: A non-destructive method to determine chlorophyll content of leaves. – Photosynthetica **26**: 469-473, 1992.

Gratani, L., Crescente, M.F., Fabrini, G., Varone, L.: Growth pattern of *Bidens cernua* L.: relationships between relative growth rate and its physiological and morphological components. – Photosynthetica **46**: 179-184, 2008.

Gratani, L., Pesoli, P., Crescente, M.F., Aichner, K., Larcher, W.: Photosynthesis as a temperature indicator in *Quercus ilex* L. – Global Planet. Change **24**: 153-163, 2000.

Gratani, L., Varone, L.: Leaf key traits of *Erica arborea* L., *Erica multiflora* L. and *Rosmarinus officinalis* L. co-occurring in the Mediterranean maquis. – Flora **199**: 58-69, 2004.

Gratani, L., Varone, L.: Long-time variations in leaf mass and area of Mediterranean evergreen broad-leaf and narrow-leaf maquis species. – Photosynthetica **44**: 161-168, 2006.

Gratani, L., Varone, L., Crescente, M.F.: Photosynthetic activity and water use efficiency of dune species: the influence of air temperature on functioning. – Photosynthetica **47**: 575-585, 2009.

Groom, Q.J., Baker, N.R., Long, S.P.: Photoinhibition of holly (*Ilex aquifolium*) in the field during the winter. – Physiol. Plant. **83**: 585-590, 1991.

Grootjans, A.P., Geelen, H.W.T., Jansen, A.J.M., Lammerts, E.J.: Restoration of coastal dune slacks in the Netherlands. – Hydrobiologia **478**: 181-203, 2002.

Grulke, N.E.: Plasticity in physiological traits in conifers: Implications for response to climate change in the western U.S. – Environ. Poll. **158**: 2032-2042, 2010.

Grunewald, R., Schubert, H.: The definition of a new plant diversity index "H'_{dune}" for assessing human damage on coastal dunes—Derived from the Shannon index of entropy H'. – Ecol. Indic. **7**: 1-21, 2007.

Hanson, P.J., Isebrands, J.G., Dickson, R.E., Dixon, R.K.: Ontogenetic patterns of CO_2 of *Quercus rubra* L. leaves during three flushes on shoot growth. II. Insertion gradients of leaf photosynthesis. – Forest Sci. **34**: 69-76, 1988.

He, J.X., Wang, J., Liang, H.G.: Effects of water-stress on photochemical function and protein-metabolism of photosystem II in wheat leaves. – Physiol. Plant. **93**: 771-777, 1995.

Jolicoeur, S., O' Carroll, S.: Sandy barriers, climate change and long-term planning of strategic coastal infrastructures, Îles-de-la-Madeleine, Gulf of St. Lawrence (Québec, Canada). – Landsc. Urban Plan. **81**: 287-298, 2007.

Jullien, A., Allirand, J.M., Mathieu, A., Andrieu, B., Ney, B.: Variations in leaf mass per area according to N nutrition, plant age, and leaf position reflect ontogenetic plasticity in winter oilseed rape (Brassica napus L.). – Field Crop Res. **114**: 188-197, 2009.

Kutiel, P., Kutiel, H., Lavee, H.: Vegetation response to possible scenarios of rainfall variations along a Mediterranean–extreme arid climatic transect. – J. Arid Environ. **44**: 277-290, 2000.

Kyparissis, A., Manetas, Y.: Seasonal leaf dimorphism in a semideciduous Mediterranean shrubs: ecophysiological comparisons between winter and summer leaves. – Acta Oecol. **14**: 23-32, 1993.

Lammerts, E.J., Pegtel, D.M., Grootjans, A.P., van der Veen, A.: Nutrient limitation and vegetation change in a coastal dune slack. – J. Veg. Sci. **10**: 111-122, 1999.

Larcher, W.: Photosynthesis as a tool for indicating temperature stress events. – In: Schulze, E.D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 261-277. Springer-Verlag, Berlin – Heidelberg 1994.

Larcher, W.: Temperature stress and survival ability of Mediterranean sclerophyllous plants. – Plant Biosystems **134**: 279-295, 2000.

Lawlor, D.W., Cornic, G.: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. – Plant Cell Environ. **25**: 275-294, 2002.

Lucrezi, S., Schlacher, T.A., Walker, S.: Monitoring human impacts on sandy shore ecosystems: a test of ghost crabs (*Ocypode* spp.) as biological indicators on an urban beach. – Environ. Monit. Asses. **152**: 413-424, 2009.

Ma, J., Liu, Z.: Spatiotemporal pattern of seed bank in the annual psammophyte *Agriophyllum squarrosum* Moq. (Chenopodiaceae) on the active sand dunes of northeastern Inner Mongolia, China. – Plant Soil **311**: 97-107, 2008.

Martinez, M.L., Psuty, N.P.: Coastal Dunes: Ecology and Conservation. Ecological Studies. – Springer-Verlag, Berlin 2004.

Maseyk, K., Grünzweig, J.M., Rotenberg, E., Yakir, D.: Respiration acclimation contributes to high carbon-use efficiency in a seasonally dry pine forest. – Global Change Biol. **14**: 1553-1567, 2008.

Mattheus, C.R., Rodriguez, A.B., McKee, B.A., Currin, C.A.: Impact of land-use change and hard structures on the evolution of fringing marsh shorelines. – Estuar. Coast. Shelf Sci. **88**: 365-376, 2010.

Medlyn, B.E., Loustau, D., Delzon, S.: Temperature response of parameters of a biochemically based model of photosynthesis. I. Seasonal changes in mature maritime pine (*Pinus pinaster* Ait.). – Plant Cell Environ. **25**: 1155-1165, 2002.

Melzack, R.N., Bravdo, B., Riov, J.: The effect of water-stress on photosynthesis and related parameters in *Pinus halepensis*. – Physiol. Plant. **64**: 295-300, 1985.

Menalled, F.D., Kelty, M.J.: Crown structure and biomass allocation strategies of three juvenile tropical tree species. – Plant Ecol. **152**: 1-11, 2001.

Milligan, J., O'Riordan, T., Watkinson, A.: Designing coastlines fit for the future. – English Nature Res. Rep.: **702**: 52, 2006.

Mills, M.H., Schwartz, M.W.: Rare plants at the extremes of distribution: broadly and narrowly distributed rare species. – Biodivers. Conserv. **14**: 1401-1420, 2005.

Morgan, J.W.: Reproductive success in reestablished versus natural population of a threatened grassland daisy (*Rutidosis leptorrhynchoides*). – Conserv. Biol. **14**: 780-785, 2000.

Munné-Bosch, S., Alegre, L.: Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. – Planta **210**: 925-931, 2000.

Münzbergová, Z., Mildén, M., Ehrlén, J., Herben, T.: Population viability and reintroduction strategies: A spatially explicit landscape-level approach. – Ecol. Appl. **15**: 1377-1386, 2005.

Nevo, E., Ivanitskaya, E., Filippucci, M.G., Beiles, A.: Speciation and adaptive radiation of subterranean mole rats, *Spalax ehrenbergi* superspecies, in Jordan. – Biol. J. Linn. Soc. **69**: 263-281, 2000.

Niinemets, Ü.: Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. – Ecology **82**: 453-469, 2001.

Niu, S.L., Jiang, G.M., Wan, S.Q., Li, Y.G., Gao, L., Liu, M.Z.: A sand-fixing pioneer C_3 species in sandland displays characteristics of C_4 metabolism. – Environ. Exp. Bot. **57**: 123-130, 2006.

Ohashi, Y., Nakayama, N., Saneoka, H., Fujita, K.: Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. – Biol. Plant. **50**: 138-141, 2006.

O'Shea, E.M., Kirkpatrick, J.B.: The impact of suburbanization on remnant coastal vegetation in Hobart, Tasmania. – Appl. Veg Sci. **3**: 243-252, 2000.

Ow, L.F., Griffin, K.L., Whitehead, D., Walcroft, A.S., Turnbull, M.H: Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoids* x *nigra*. – New Phytol. **178**: 123-134, 2008.

Ow, L.F., Whitehead, D., Walcroft, A.S., Turnbull, M.H.: Seasonal variation in foliar carbon exchange in *Pinus* radiate and *Populus deltoids*: respiration acclimates fully to changes in temperature but photosynthesis does not. – Glob. Change Biol. **16**: 288-302, 2010.

Pearman, P.B., Weber, D.: Common species determine richness patterns in biodiversity indicator taxa. – Biol. Conserv. **138**: 109-119, 2007.

Pignatti, S.: [Flora of Italy.] – Edagricole, Bologna 1982. [In Italian.]

Provoost, S., Ampe, C., Bonte, D., Cosyns, E., Hoffmann, M.: Ecology, management and monitoring of grey dunes in Flanders. – J. Coast Conserv. **10**: 33-42, 2004.

Reich, P.B., Kloeppel, B.D., Ellsworth, D.S., Walters, M.B.: Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. – Oecologia **104**: 24-30, 1995.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C., Bowman, W.D.: Generality of leaf trait relationships: A test across six biomes. – Ecology **80**: 1955-1969, 1999.

Ribas-Carbo, M., Taylor, N.L., Giles, L. *et al.*: Effects of Water Stress on Respiration in Soybean Leaves. – Plant Physiol. **139**: 466-473, 2005.

Rooney, P.: Changing perspectives in coastal dune management. – J. Coast Conserv. **14**: 71-73, 2010.

Rubio-Casal, A.E., Leira-Docea, P.M., Figueroaa, M.E., Castilloa, J.M.: Contrasted tolerance to low and high temperatures of three tree taxa co-occurring on coastal dune forests under Mediterranean climate. – J. Arid Environ. **74**: 429-439, 2010.

Saye, S.E., Pye, K.: Implications of sea level rise for coastal dune habitat conservation in Wales, UK. – J. Coast Conserv. **11**: 31-52, 2007.

Swarts, N.D., Dixon, K.W.: Perspectives on orchid conservation in botanic gardens. – Trend in Plant Sci. **14**: 590-598, 2009.

Taveira Pinto, F.: Coastal management: Issues and tools. Introduction. – J. Coast Conserv. **10**: 3-4, 2004.

Thompson, L.M.C., Schlacher, T.A.: Physical damage to coastal dunes and ecological impacts caused by vehicle tracks associated with beach camping on sandy shores: a case study from Fraser Island, Australia. – J. Coast Conserv. **12**: 67-82, 2008.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A.: Flora Europaea. 1. (2nd Ed.) – Cambridge Univ. Press, Cambridge 1993.

van der Meulen, F., de Haes, H.A.U: Nature conservation and integrated coastal zone management in Europe: present and future. – Landsc. Urban Plan. **34**: 401-410, 1996.

Varone, L., Gratani, L.: Physiological response of eight Mediterranean maquis specie to low air temperatures during winter. – Photosynthetica **45**: 385-391, 2007.

Vassileva, V., Signarbieux, C., Anders, I., Feller, U.: Genotypic variation in drought stress response and subsequent recovery of wheat (*Triticum aestivum* L.). – J. Plant Res. DOI 10.1007/s10265-010-0340-7, 2010.

White, D.A., Turner, N.C., Galbraith, J.H.: Leaf water relations and stomatal behaviour of four allopatric Eucalyptus species planted in Mediterranean southwestern Australia. – Tree Physiol. **20**: 1157-1165, 2000.

Wright, I.J., Westoby, M.: Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. – New Phytol. **155**: 403-416, 2002.

Zunzunegui, M., Díaz-Barradas, M.C., Ain-Lhout, F., Alvarez-Cansino, L., Esquivias, M.P., García Novo, F.: Seasonal physiological plasticity and recovery capacity after summer stress in Mediterranean scrub communities. – Plant Ecol. DOI 10.1007/s11258-010-9809-7, 2010.