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Role of changing environmental parameters in leaf gas exchange of *Arbutus unedo* L. assessed by field and laboratory measurements

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Abstract

In the frame of the foreseen climate global changes we analysed the physiological responses of *Arbutus unedo* L. to the variations of carbon dioxide concentration, leaf temperature, and irradiance by measurements of leaf gas exchange and leaf water potential performed both in field and in the laboratory. Stomatal conductance was not affected by increase of leaf temperature. The growth conditions of potted plants likely made stomata more sensitive to the variation of external parameters than naturally growing plants. The interaction between high CO_2 concentration and temperature involved important down-regulation mechanisms in the metabolic pathway of the carbon fixation. From an ecological point of view, the ability of *A. unedo* to adapt to the field stress makes it highly competitive in the Mediterranean plant community.

Additional key words: climate change; CO_2 concentration; Mediterranean-type ecosystems; mesophyll conductance; net photosynthetic rate; physiological adaptation to stresses; respiration rates; ribulose-1,5-bisphosphate carboxylase/oxygenase; stomatal conductance; transpiration rate.

Introduction

Plants growing in Mediterranean-type ecosystems are adapted to the seasonal variation of environmental factors (high irradiance and temperature, water availability in the soil, *etc.*) through morpho-structural and physiological adjustments (Pereira and Chaves 1995). Moreover, Mediterranean plants show strong reductions of leaf gas exchange rates during drought periods, avoiding thus water losses and concurrently keep a positive water and carbon balance (Beyschlag *et al.* 1987, Tenhunen *et al.* 1987, 1990, Manes *et al.* 1997b). The so-called midday stomatal closure could be due to different causes such as high values of air temperature (Schulze *et al.* 1974), high difference of water vapour pressure between the leaf and external air (Raschke and Resemann 1986) and to water availability in the soil (Lösch *et al.* 1982). As a consequence, the reduction of photosynthesis can be either related to a stomatal limitation or caused by a reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) in quantity and activity (Vu and Yelenosky 1988).

The increase of ambient CO_2 concentration (C_a), strongly due to anthropogenic emissions, and the following increase of temperature (greenhouse effect) could affect in different way the physiological adjustments mentioned above (Woodward 2002). In this regard, many studies at different scales have been carried out on natural plants and crops at different C_a, leaf temperature (T_L), and air humidity, singly or in combination (Wheeler *et al.* 1994, Rawson 1995, Carter and Saarikko 1996, Scarascia-Mugnozza *et al.* 1996, Matthews *et al.* 1997, Paoletti *et al.* 1997, Crawford and Wolfe 1999, Smith *et al.* 2000, Fuhrer 2003). These studies assumed great importance when they were carried out on plants growing in the Mediterranean climate-type ecosystems, inferring on their

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Abbreviations: C_a – external CO_2; C_c – chloroplast CO_2 concentration; C_i – sub-stomatal CO_2 concentration; CE – carboxylation efficiency; E – leaf transpiration; g_s – stomatal conductance to water vapour; g_{CO2} – stomatal conductance to carbon dioxide; g_m – mesophyll conductance; J_c – carboxylative electron flow; J_o – oxygenative electron flow; J_r – rates of formation and consumption of RuBP; J_t – total light-driven electron flow; J_{ccc} – carboxylative electron flow calculated by using C_c; J_{occ} – oxygenative electron flow calculated by using C_c; J_{tcc} – total light-driven electron flow calculated by using C_c; P_N – net photosynthetic rate; Q_{10} – the proportional increase of a parameter value for a ten degrees increase in temperature; R_d – day respiration; R_n – dark respiration; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; T_d – leaf temperature during the R_d measurement; T_L – leaf temperature; T_n – leaf temperature during R_n measurement; v_c – velocity of carboxylation; v_{ccc} – velocity of carboxylation calculated by using C_c; v_o – velocity of oxygenation; v_{occ} – velocity of oxygenation calculated by using C_c; VPD – vapour pressure deficit between air and internal leaf; Γ – CO_2 compensation concentration in absence of dark respiration; Ψ – leaf water potential.

abilities to endure the seasonal variation of environmental parameters, and the shifting of plant species composition driven by the foreseen global climate change.

Aiming to elucidate the physiological abilities of Mediterranean plants to endure environmental stresses, this paper is focused on: (*a*) evaluating the response of gas exchanges of a Mediterranean sclerophyllous species such as *Arbutus unedo* L. to the variation of the environmental parameters such as temperature, irradiance, and CO_2 concentration in a controlled leaf chamber; (*b*) to compare the results obtained in controlled conditions with physiological measurements carried out directly in field.

Materials and methods

Area of study: The measurements were carried out in the Mediterranean costal dune maquis of Grotta di Piastra site located in the Presidential Estate of Castelporziano (41°42'N, 12°21'E) near Rome (Central Italy). This area is characterised by mean annual precipitation of 740 mm occurring in autumn (November–December) and spring (March–April), whereas high air temperature and irradiance occur during the summer drought period lasting from May to August. The climate is of a Mediterranean type (Manes *et al.* 1997a,b).

Sampling period and potted plant management: Field campaigns were performed on May, June, July, August, and October 1994, July and August 1996, and June and July 1998. Four-years-old *A. unedo* rooted plants coming from Castelporziano estate were potted in pots of 20 000 cm^3 and grown in the Botanical Garden of University of Rome for three months in open air, prior to the laboratory measurements of gas exchange performed during June and July 1998. All plants were held at full water field capacity. In two potted plants the CO_2 response curves at different irradiances, T_L, and C_a were determined.

Gas exchange and leaf water potential: In the laboratory, measurements of net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], leaf transpiration rate, E [mmol(H_2O) m^{-2} s^{-1}], stomatal conductance to water, g_s [mol(H_2O) m^{-2} s^{-1}] and to CO_2, g_{CO_2} [mol(CO_2) m^{-2} s^{-1}], and sub-stomatal concentration of CO_2, C_i [μmol mol^{-1}] were carried out by a portable infrared gas analyser, *CIRAS-I* (*PP Systems*, Hitchin, UK). The carboxylation efficiency, CE [μmol(CO_2) (μmol $mol^{-1})^{-1}$] was estimated by linear fit on the initial slope of the P_N *vs.* C_i curve (Caemmerer and Farquhar 1981) at T_L of 25 °C. CO_2 compensation concentration in absence of dark respiration, Γ^* [μmol mol^{-1}] was calculated using the P_N *vs.* C_i curve obtained at two non-saturating irradiances, 200 and 75 μmol(photon) m^{-2} s^{-1} and at 25 °C. For T_L higher than 25 °C, Γ^* was estimated by a polynomial regression reported by Brooks and Farquhar (1985):

$$\Gamma^* = \mathrm{P}\,[42.7 + 1.68\,(T_L - 25) + 0.012\,(T_L - 25)^2] \quad (1)$$

where P is the atmospheric pressure [MPa].

Day respiration, R_d [μmol(CO_2) m^{-2} s^{-1}] was calculated by Q_{10} response function:

$$R_d = R_n\, Q_{10}^{(Td - Tn)/10} \quad (2)$$

where R_n is the net CO_2 assimilation measured in the dark [μmol(CO_2) m^{-2} s^{-1}], T_n [°C] is the leaf temperature at which R_N was measured, T_d [°C] is the leaf temperature at which R_D was measured, and Q_{10} is the proportional increase of a parameter value for a 10 °C increase in temperature (Berry and Raison 1981, Larcher 1983). For Mediterranean climate, Q_{10} is around 2.2 (Larcher 1983). The velocity of carboxylation, v_c and of oxygenation, v_o [μmol(CO_2) m^{-2} s^{-1}] and the rates of formation and consumption for RuBP, J_r [μmol(CO_2) m^{-2} s^{-1}] were calculated according to Brooks and Farquhar (1985) and Sharkey *et al.* (1988), at different T_L:

$$v_c = (P_N + R_d)\,(1 - \Gamma^*) \quad (3)$$

$$v_o = 2\,(v_c - P_N - R_d) \quad (4)$$

$$J_r = v_c\,(1 + \Phi) \quad (5)$$

$$\Phi = v_o\, v_c^{-1} = 2\,\Gamma^*\, C_i^{-1} \quad (6)$$

The total light-driven electron flow (J_t) and its partitioning into carboxylative (J_c) and oxygenative (J_o) electron flows [μmol m^{-2} s^{-1}] were calculated according to Valentini *et al.* (1995):

$$J_c = 4\,(P_N + R_D + v_o) \quad (7)$$

assuming the use of four electrons per CO_2 molecule fixed:

$$J_o = 4\,(2\,v_o) \quad (8)$$

and assuming the typical stoichiometry of photorespiratory pathway: two glycollate-P molecules into one phosphoglyceric acid molecules and the release of one CO_2 (from decarboxylation of one of the two glycines during glycine-serine conversion in the mitochondrion):

$$J_t = J_c + J_o = 4\,(P_N + R_d + 3\,v_o) \quad (9)$$

by re-arrangement of Eqs. (7) and (8).

v_c, v_o, R_d, J_t, J_c, and J_o were also calculated at two C_a (350 and 720 μmol mol^{-1}). The experimental design considered three steps (350, 720, and 350 μmol mol^{-1}, respectively), where each leaf temperature was changed by 5 °C steps (25, 30, 35, and 40 °C).

C_i was assumed equal to the chloroplast CO_2 concentration, C_c [μmol mol^{-1}]. In this regard, we calculated C_c to test this assumption, considering a mesophyll conductance, g_m of 1.61 mmol m^{-2} s^{-1} kPa^{-1} for *A. unedo* leaves (Loreto *et al.* 1992), that is

$$C_c = C_i\,(P_N\, g_m^{-1}) \quad (10)$$

Leaves were acclimated to different CO_2 concentrations and temperatures for approximately one hour before the measurements.

Field measurements of gas exchange, g_s and g_{CO2} and calculations of C_i were performed on the sunlit leaves placed at the top of the canopy (1.80 m in height) using two portable instruments *CIRAS-I* (*PP Systems*, Hitchin, UK) and *Li-Cor 6200* (*Li-Cor*, Lincoln, USA). T_a, air vapour pressure [hPa], and irradiance, PAR [μmol(photon) m^{-2} s^{-1}] were also measured, whereas relative humidity, RH [%] and vapour pressure deficit between air and internal leaf, VPD [hPa] were successively calculated. The average values of environmental and physiological parameters measured in the range 11:30 to 14:30 h (GMT + 1), were used for the statistical analysis of data. Projected leaf area was measured by an image analysis system (*Delta T*, Cambridge, UK).

Field measurements of leaf water potential, Ψ [MPa] were made by using a portable pressure chamber (*PMS Instrument Co.*, Corvallis, USA) on one year old leaves of *A. unedo*. The relative water content of leaves (RWC) was calculated as

$$\%RWC = 100\ (FM\ DM)\ (TM\ DM)^{-1} \tag{11}$$

where FM, DM, and TM were fresh, dry, and saturated mass, respectively. DM was measured after oven-drying at 70 °C for 48 h.

Statistical evaluations were carried out by the *SPSS* statistical software package (*SPSS*, Chicago, USA). Multiple regression analyses were done on physiological variables and the stepwise method was utilised to set up the multiple regression model. One-way analysis of variance (ANOVA) and the Student-Neumann-Keuls' test (at significance level of 0.05) were carried out for all eco-physiological data to evaluate the difference of means among months. The number of physiological measurements in the field was not lower than one hundred values for the monthly trends and fifty values for the monthly trends of Ψ.

Results

Physiological response of potted plants to the variations of T_L and C_a: The CO_2 response curve (Fig. 1*A*) showed asymptotic values of P_N approximately at 10–11 μmol(CO_2) m^{-2} s^{-1}, and CE of 0.0615 [μmol(CO_2) m^{-2} s^{-1}] (μmol mol^{-1})$^{-1}$. Values of Fig. 1*B* were used to calculate R_d and Γ^* giving values of 0.23 μmol(CO_2) m^{-2} s^{-1} and 54.9 μmol mol^{-1}, respectively. These values have been used for succeeding calculations.

g_s showed a decreasing trend in relation to the increase of T_L (Fig. 2*A*) in the 1st step of experiment (350 μmol mol^{-1}). In fact, g_s ranged 0.70±0.02 to 0.90±0.06 mol(H_2O) m^{-2} s^{-1} between 25 and 35 °C, whereas it reached very low values at 40 °C. A similar trend was observed for P_N, which decreased from 7.5 to 5.0 μmol(CO_2) m^{-2} s^{-1} between 25 and 35 °C, and peaked 0.4±0.2 μmol(CO_2) m^{-2} s^{-1} at 40 °C. *E* changed according to the variation of both leaf temperature and g_s until 35 °C, after that a decrease of values was observed.

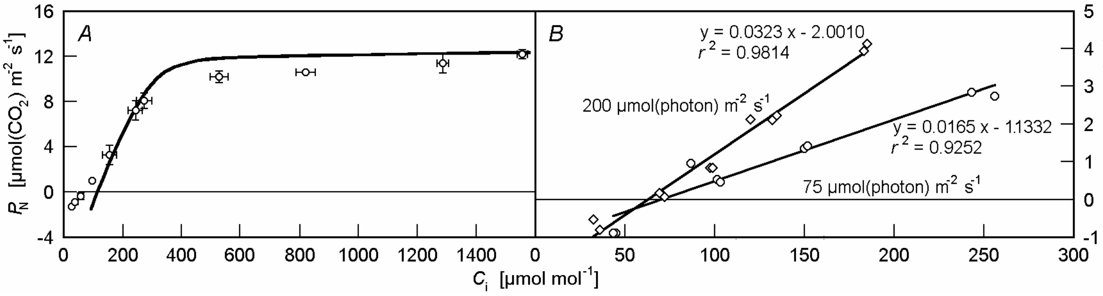

Fig. 1. *A*: CO_2 response curve measured on current leaves of *Arbutus unedo* at constant values of leaf temperature (25 °C), relative humidity (54 %), and irradiance [1 500 μmol(photon) m^{-2} s^{-1}]. *B*: Initial slope of CO_2 response curves performed at two different irradiances and at 20 °C, according to Brooks and Farquhar (1985), to calculate the day respiration (R_d) and the CO_2 compensation concentration in absence of dark respiration (Γ^*). Carboxylation efficiencies were 0.0323 [μmol(CO_2) m^{-2} s^{-1}] (μmol mol^{-1})$^{-1}$ at 200 μmol(photon) m^{-2} s^{-1} and 0.0165 [μmol(CO_2) m^{-2} s^{-1}] (μmol mol^{-1})$^{-1}$ at 75 μmol(photon) m^{-2} s^{-1}.

C_i ranged between 160–180 μmol mol^{-1} with a deep raise at 40 °C (Fig. 2*B*). R_d exhibited a progressive increase of values following the T_L increase (Fig. 2*B*). At twofold CO_2 concentration (2nd step), P_N showed a recovery of values joined to the decrease of T_L (Fig. 2*A*), whereas g_s did not recover and, as consequence, *E* showed lower values [on average 0.34 mmol(H_2O) m^{-2} s^{-1}] than those measured in the 1st step (Fig. 2*A*). Under these conditions R_d rapidly decreased (Fig. 2*B*). At increasing T_L and external CO_2 concentration of 350 μmol mol^{-1} (3rd step), a decrease in P_N and the insensitivity to these variations of g_s was evident. The increase of both R_d and C_i values was concurrently observed.

The value of v_c was almost constant at increasing T_L ranging 11.6–9.3 μmol(CO_2) m^{-2} s^{-1} in the 1st step (Fig. 2*C*), although a reduction of values of 35 % was observed in the 2nd and 3rd steps. Values of v_o pointed out the apparent insensitivity of the RuBP oxygenation rate to the increase of T_L in the 2nd and 3rd steps (Fig. 2*C*). However, at 40 °C both the carboxylation and oxygenation rates dropped, indicating a reduced ability of RuBPCO to operate at high T_L (Jordan and Ogren 1984). As a consequence, the formation and consumption rates of RuBP (J_r) were also limited (Fig. 2*D*). RuBPCO activity was partially restored in the 2nd step and at moderate T_L, as highlighted by v_c values [7.3 μmol(CO_2) m^{-2} s^{-1} at 20 °C in the 2nd step] (Fig. 2*C*).

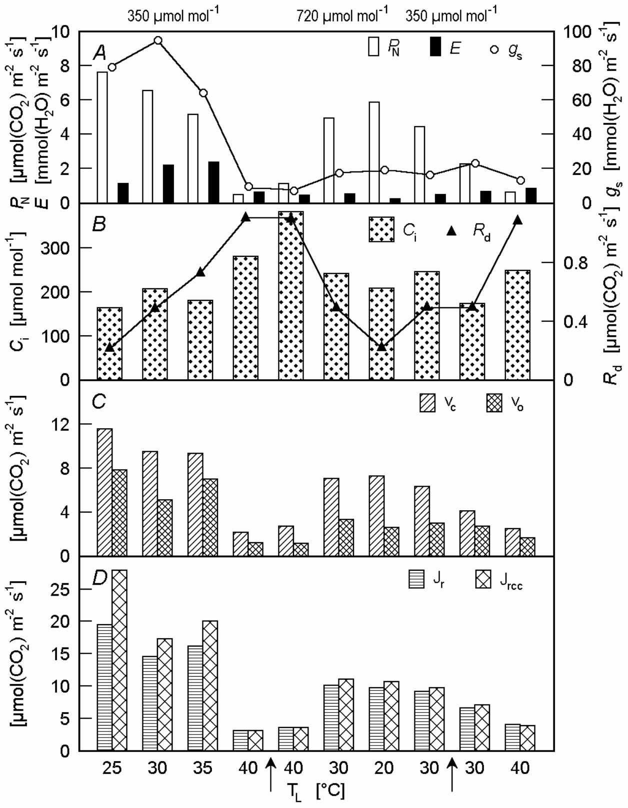

Fig. 2. Trends of (*A*) gas exchange and stomatal conductance (g_s) values, (*B*) CO_2 sub-stomatal concentration, C_i [μmol mol^{-1}] and day respiration, R_d [μmol(CO_2) m^{-2} s^{-1}] values, (*C*) v_c and v_o values [μmol(CO_2) m^{-2} s^{-1}], and (*D*) rates of formation and consumption for RuBP calculated by using C_i, J_r [μmol(CO_2) m^{-2} s^{-1}] and C_c, J_{rcc} [μmol(CO_2) m^{-2} s^{-1}] values are reported in function of leaf temperature and external CO_2. *Arrows* indicate the variations of external CO_2 concentration.

The total electron flow (J_t) and its partitioning into RuBP carboxylative and RuBP oxygenative electron flows (J_c and J_o, respectively) are depicted in Fig. 3*A*. J_c and J_o were similar in the 1st and 3rd steps of moderate T_L regimes, whereas low values were calculated at 350 and 720 μmol mol^{-1} and 40 °C. Lowering of T_L in the 2nd step allowed a partial recovery of J_c but only for 70 % of the 1st step values (Fig. 3*A*). The fraction of total electron flow devoted to carboxylation, J_c/J_t ratio (Fig. 3*B*), was higher than that devoted to oxygenation (J_o/J_t ratio). J_c/J_t and J_o/J_t values were similar at the end of the experiment, pointing out a similar partition of total electron flow in the RuBP carboxylation and oxygenation processes.

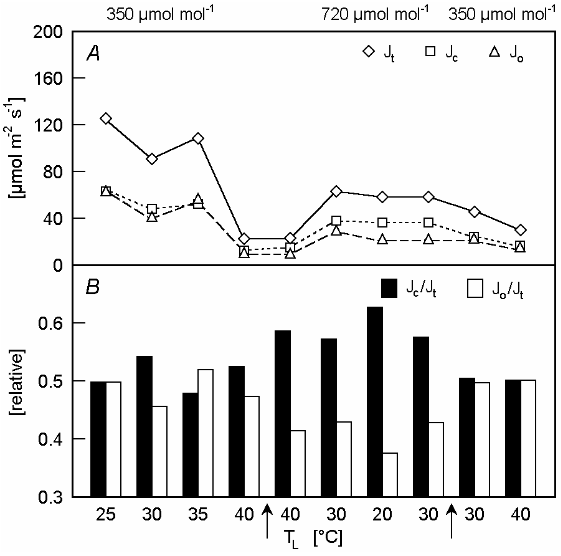

Fig. 3. (*A*) Total light-driven flow, J_t [μmol m^{-2} s^{-1}] and its partitioning into carboxylative, J_c [μmol m^{-2} s^{-1}] and oxygenative, J_o [μmol m^{-2} s^{-1}] electron flows, and (*B*) fractions of total electron flow devoted to carboxylation (J_c/J_t) and to oxygenation (J_o/J_t) are all reported in function of leaf temperature and external CO_2. The variations of external CO_2 concentrations are indicated by *arrows*.

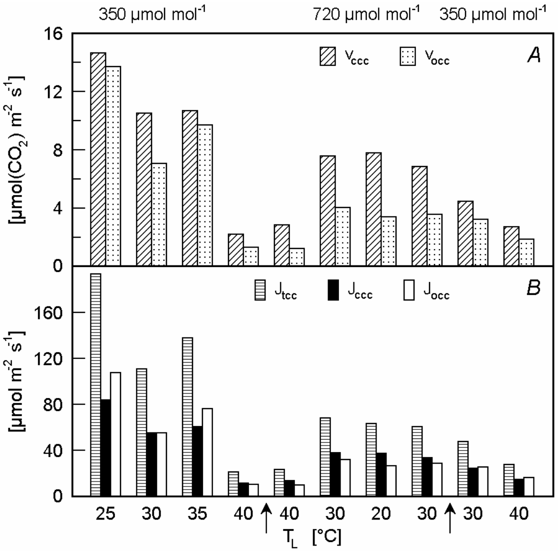

Fig. 4. (*A*) Velocity of carboxylation, v_c [μmol(CO_2) m^{-2} s^{-1}] and oxygenation, v_o [μmol(CO_2) m^{-2} s^{-1}], and (*B*) total light-driven flow, J_t [μmol m^{-2} s^{-1}] and its partitioning into carboxylative, J_c [μmol m^{-2} s^{-1}] and oxygenative, J_o [μmol m^{-2} s^{-1}] electron flows, calculated by using C_c values [μmol mol^{-1}]. *Arrows* indicate the variations of external CO_2 concentrations.

The values of C_c were used to re-calculate the response of physiological parameters at different T_L and C_a, highlighting higher values of v_c at 350 and 720 μmol mol^{-1} (16 and 4 %, respectively) than those calculated by C_i values (Fig. 4*A*). Similarly, v_o showed higher values of

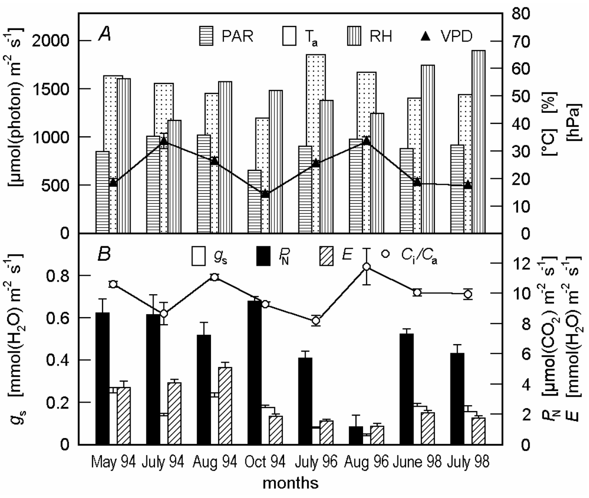

Fig. 5. (*A*) Overall trends of environmental parameters measured in the test site of Grotta di Piastra during the measuring campaigns, and (*b*) of physiological parameters measured on current leaves of *Arbutus unedo* plants. *Vertical bars* are standard errors. For abbreviations see the text.

about 49 and 21 % at 350 and 720 µmol mol^{-1}, respectively. As a consequence, J_{ccc} and J_{occ} were also affected by these differences (Fig. 4*B*); an increase of mean values of J_{occ} (80 and 28 % at 350 and 720 µmol mol^{-1}, respectively) was found. Hence the assumption that C_i and C_c are the same should be rejected.

Field measurements of gas exchange and leaf water potential (Fig. 5): Photosynthetic activity measured in the 1994 campaign showed a rather constant values ranging from 7.2±0.9 to 8.8±0.8 µmol(CO$_2$) m^{-2} s^{-1}, and peaking in October [9.5±0.3 µmol(CO$_2$) m^{-2} s^{-1}]. Wide range of g_s and E was found [150±10 to 250±20 mmol(H$_2$O) m^{-2} s^{-1} in spring and 3.8±0.4 to 5.1±0.3 mmol(H$_2$O) m^{-2} s^{-1} in summer], following the high values of T_a and VPD, although relative humidity was higher than 50 % (except in July). Ψ determined in May 1994 showed rather negative value in the morning (-1.45 MPa), beholding such values during the hottest hours of the day. In the following summer months, Ψ progressively reached low values (–1.9 and –3.1 MPa, respectively, for July and August 1994), whereas in October the Ψ values were similar to those measured in May.

The summer months of 1996 and 1998 were more arid than those of 1994, as can be deduced by high values of the Mitrakos' Monthly Drought Index (Table 1; Mitrakos 1980). g_s showed values lower than those of 1994 (70±10 and 40±10 mmol(H$_2$O) m^{-2} s^{-1} in July and August 1996), affecting thus P_N and E (Fig. 5*B*).

Table 1. Monthly mean values of temperature [°C] and rainfalls [mm] based on data of Castelporziano meteorological station, and Mitrakos' Monthly Drought Index (stress units) calculated on the rainfall basis and ranging between 0 (no stress) and 100 (high stress). For abbreviations see the text.

	1994			1996			1998		
	June	July	August	June	July	August	June	July	August
T_a	20.5	25.3	26.3	22.0	23.2	23.9	21.4	24.0	25.3
$T_{a,max}$	26.4	31.8	32.7	27.7	29.2	30.1	27.3	30.4	31.7
$T_{a,min}$	14.6	18.7	19.9	16.4	17.3	17.7	15.5	17.7	18.8
Rain	70.3	17.2	0.4	11.4	0.8	12.2	6.6	12.2	17.2
Mitrakos Drought Index	0.0	65.6	99.2	77.2	98.4	72.0	86.8	75.6	65.6

P_N, E, and g_s measured in June 1998 showed average values of 7.2±0.4 µmol(CO$_2$) m^{-2} s^{-1}, 2.04±0.09 mmol (H$_2$O) m^{-2} s^{-1}, and 173±10 mmol(H$_2$O) m^{-2} s^{-1}, respectively. In July, high values of P_N were measured during the mid-day hours [6.9±0.9 µmol(CO$_2$) m^{-2} s^{-1} at noon], and average values of g_s and E were 149±21 mmol(H$_2$O) m^{-2} s^{-1} and 1.59±0.15 mmol(H$_2$O) m^{-2} s^{-1}, respectively. The good hydration of leaves in July was indicated by their average relative water content, RWC [%] of 87.00±1.00, whereas we found 91.00±0.87 in June. Ψ measured during summer in the hottest hours of 1996 and 1998 showed similar values ranging from –1.8 to –2.5 MPa.

Multiple regression analysis performed of overall environmental and physiological field data (1994–1998; $n = 369$), and assuming g_s as a dependent variable, showed a strong dependence of g_s mainly on P_N ($\beta = 0.73$, $t = 16.89$, $p << 0.01$), E ($\beta = 0.33$, $t = 9.10$, $p << 0.01$), and C_i ($\beta = 0.29$, $t = 9.33$, $p << 0.01$), and on other variables with lower β such as relative humidity ($\beta = 0.27$). Thus neither T_a nor TL did affect g_s.

Discussion

Field data highlighted a progressive reduction of g_s and P_N in *A. unedo* on monthly basis and a slight correlation between g_s and C_i, whereas other authors found much more limiting values of g_s and P_N likely due to the more stressing environmental conditions (Tenhunen *et al.* 1981, 1987, Harley *et al.* 1986). *A. unedo* modulates g_s and P_N in order to keep constant values of C_i; this behaviour is the result of an equilibrium state between stomatal responses to CO_2 and P_N (Jarvis *et al.* 1999). An increase in C_i, due to a reduction of the photosynthetic activity in the mesophyll and/or to increase of photorespiration process, was not observed during the summer field campaigns (data not shown). This indicates that the existence of patches with mainly two stomatal states, wide open and closed, enables photoinhibition to occur in the "closed" parts of the *A. unedo* leaves (Beyschlag *et al.* 1992), thus measured photosynthetic characteristics are not affected.

This mutual modulation of g_s and P_N allowed also to increase the water use efficiency (Morison 1993) by decreasing E. Reduction of E in mature leaves can cause an increase of T_L but, however, can result in an improved soil moisture availability when integrated over time (Field *et al.* 1995), maintaining thus g_s and hence the leaf gas exchange.

The beholding of appreciable gas exchange rates could also be due to the steep angles of leaves in the upper canopy. Phototaxis is a common feature of Mediterranean evergreen vegetation, which maintains its leaves for more than one growing season. Werner *et al.* (1999) reported for *A. unedo* an average value of 66±16° of leaf inclination during summer period. Although leaf inclination did not seem to have an important effect on the photochemical efficiency of photosystem 2, it could have had it for the g_s functioning and, in turn, for P_N, especially in summer.

Low values of Ψ measured in the summer campaigns allowed an adequate replenishment of water and, as a consequence, leaf gas exchange activity was beholden. The rather swift response of Ψ to the variations of environmental parameters pointed out the great ability of *A. unedo* to match its physiological demands and changing water availability. This was also due to the deep and well-developed root system (Mooney and Dunn 1970, Werner *et al.* 1999) which could reach the low deep water-table of the Castelporziano's coastal dune system and it could shorten or eliminate the duration of the drought stress.

Multiple regression analysis carried out on field data demonstrated that T_L did not affect g_s, in accord with Raschke and Resemann (1986) and Le Roux *et al.* (1999) but in contrast to other authors (Morison 1987, Drake *et al.* 1997). The insensitivity of g_s to the variation of environmental parameters was reported also by Barták *et al.* (1999): they showed that *A. unedo* plants, growing near CO_2 emitting vents throughout thirty years, did not evidence the photosynthetic acclimation to high CO_2 (465 µmol mol^{-1}), whereas g_s was similar to that of control trees. Moreover, Lange *et al.* (1982) placed *A. unedo* in an intermediate position among all Mediterranean plant species with regard to the stomatal sensitivity to atmospheric stresses.

Gas exchange measurements of *A. unedo* potted plants showed that g_s had low values at the highest temperatures, during the change of both T_L and CO_2 concentrations, without any recovery when T_L was lowered. The decrease of g_s values can not be due to a decrease in water vapour pressure since it was hold constant (1.30±0.04 kPa) inside the leaf chamber.

Potted and field plants experience different growth conditions, especially with regard to root constrains. The latter could modify the sensitivity of stomata opening mechanism to high temperatures (>35 °C) in potted plants, apart from CO_2 concentrations, precluding its ability to respond to limiting environmental conditions. In this regard, Jackson *et al.* (2000) reported that every factor influencing the hydraulic conductance of the *continuum* soil-plant-atmosphere also influences g_s and E. As a consequence, the occurrence of water status heterogeneity in different parts of the leaf elicits the occurrence of stomatal patchiness (Beyschlag and Eckstein 2001), causing the g_s insensitivity. All these findings were likely concurring to explain the observed relative insensitivity of g_s to changes of C_a and T_L (see Fig. 2*A*, 2nd and 3rd steps).

P_N measured on potted plants at increasing T_L showed constant values in the 20–35 °C range, supporting the evidences of Raschke and Resemann (1986) who postulated that *A. unedo* evolved a mechanism inside the mesophyll that is able to compensate T_a variations within a range of 20–37 °C. In this temperature range the carboxylation velocity, v_c, was rather constant, whereas the oxygenation velocity, v_o, slightly increased. This phenomenon is attributed, at least in part, to CO_2 solubility decreasing faster than that O_2 with increasing temperature (Ku and Edwards 1977). At twofold CO_2 concentration a variation of the carboxylase and oxygenase balance was observed, shifting toward the carboxylation activity, as indicated by higher values of v_c/v_o ratio at 720 µmol mol^{-1} than at 350 µmol mol^{-1}. These evidences demonstrated that *A. unedo* has a fine modulation of the photosynthetic process to the changing of T_L regime, both for short and long time (Barták *et al.* 1999), preventing damage due to the over-reduction of the photosynthetic apparatus and the inhibitory acidification of the thylakoids (Kobayashi *et al.* 1982). The reduction of P_N at high T_L (40 °C) might be due to the reversible and partial heat-inactivation of the Calvin-Benson cycle by a temperature-dependent mechanism (Weis 1981). In such way, the reduction of P_N was related to the slow-down of the Calvin-Benson cycle, eliciting the observed decrease of both the electron transport rate and RuBP formation and consumption.

On the other hand, Chaves and Pereira (1992) reported that under water stress and high temperature the increase of C_a resulted in an increased susceptibility to photoinhibition, although the latter could be instead due to the occurrence of stomatal patchiness when environmental stresses increased.

In conclusion, this paper mainly highlights that: (*a*) g_s is not clearly affected by increase of leaf temperature. (*b*) The interaction between high CO_2 and temperature involves important down-regulation mechanisms in the metabolic pathway of the carbon fixation in the leaf. In high

CO_2 the v_c/v_o ratio increases, although high temperatures may counteract this effect. (*c*) The different physiological response to increasing environmental stresses could be affected by different growth conditions, especially with regard to root constraints. The latter should be taken into consideration in the evaluation of the limiting effects on physiological performance, during the variation of environmental parameters.

Although warmer temperatures generally increase the respiration to photosynthesis ratio whereas the increasing CO_2 concentrations decrease it, there is still great uncertainty about the balance of these processes and about the extent and direction of acclimation to new combinations of temperature and CO_2 concentration, either at leaf or community level. Nevertheless, from an ecological point of view the adaptation ability of *A. unedo* to field stresses makes it highly competitive in the Mediterranean plant community. The root constraints experienced by potted plants affect the sensitivity of stomata to high temperature. This should be taken into consideration when laboratory gas exchange results are compared to field measurements performed in naturally growing plants. This paper also supports the evidence that confirm not only the importance of *A. unedo* in the costal dune system of the Mediterranean plant community, but also its potential key-role in the shifts in vegetation distribution induced by climate change.

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