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Responses of two olive tree (*Olea europaea* L.) cultivars to elevated CO_2 concentration in the field

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Abstract

Five-year-old plants of two olive cultivars (Frantoio and Moraiolo) grown in large pots were exposed for 7 to 8 months to ambient (AC) or elevated (EC) CO_2 concentration in a free-air CO_2 enrichment (FACE) facility. Exposure to EC enhanced net photosynthetic rate (P_N) and decreased stomatal conductance, leading to greater instantaneous transpiration efficiency. Stomata density also decreased under EC, while the ratio of intercellular (C_i) to atmospheric CO_2 concentration and chlorophyll content did not differ, except for the cv. Moraiolo after seven months of exposure to EC. Analysis of the relationship between photosynthesis and C_i indicated no significant change in carboxylation efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase after five months of exposure to EC. Based on estimates derived from the P_N-C_i relationship, there were no apparent treatment differences in daytime respiration, CO_2 compensation concentration, CO_2-saturated photosynthetic rate, or photosynthetic rate at the mean C_i, but there was a reduction in stomata limitation to P_N at EC. Thus 5-year-old olive trees did not exhibit down regulation of leaf-level photosynthesis in their response to EC, though some indication of adjustment was evident for the cv. Frantoio with respect to the cv. Moraiolo.

Additional key words: chlorophyll; FACE; gas exchange; global change; intercellular CO_2 concentration; net photosynthetic rate; photorespiration; ribulose-1,5-bisphosphate carboxylase/oxygenase; stomata density; stomatal conductance; transpiration efficiency.

Introduction

The projected accumulation of CO_2 and other greenhouse gases in the atmosphere may cause an increase in mean global temperature at the surface of the earth (*e.g.*, Kattenburg *et al.* 1996). Due to the increase in potential evapotranspiration linked to these changes and to a concurrent decrease in precipitation at Mediterranean latitudes forecast by general circulation models, plant communities of Mediterranean environments will probably face more severe drought in the future (Houghton *et al.* 1995). However, studies incorporated into global change models have been conducted predominantly on temperate forest or crop species. In order to implement global models with direct effects of elevated [CO_2] (EC) on vegetation, experiments must be done on all the important components of the biosphere, including Mediterranean-type systems.

Responses of plants to increasing atmospheric concentration of CO_2 generally take the form of increases in carbon assimilation and sequestration, with concurrent reductions in water vapour fluxes (Eamus and Jarvis 1989). Increasing [CO_2] overcomes biochemical limitations by enhancing [CO_2] at the site of carboxylation in C_3 species and by reducing the oxygenase activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBPCO), resulting in initially increased rates of carbon fixation. However, prolonged growth at EC eventually may result

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Abbreviations: AC, ambient CO_2 concentration; C_a, CO_2 concentration in air; C_i, intercellular CO_2 concentration; Chl, chlorophyll; cv., cultivar; E, leaf transpiration rate; EC, elevated CO_2 concentration; FACE, free-air CO_2 enrichment; g_s, stomatal conductance; ITE, instantaneous transpiration efficiency, L_s, relative limitation of g_s on photosynthesis; P_N, net photosynthetic rate; PAR, photosynthetically active radiation; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; S_D, stomata density; VPD, vapour pressure deficit; Γ, CO_2 compensation concentration.

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in a down-regulation response of the photosynthetic apparatus (Sage 1994). Although anatomical or morphological and not physiological, stomata adjustment in the long-term realised by changes in stomata frequency may have equally significant impact on leaf conductance, thus on gas exchange, and particularly on water use efficiency (Morison 1998).

Woody perennials are significant in the global carbon cycle and their long life spans provide them with the potential to be considerable sinks for long-term storage of carbon (Atkinson 2000). The stomata response to EC in evergreen trees may be less strong than in woody deciduous and herbaceous vegetation (Saxe *et al.* 1998). Intraspecific variation in the responsiveness of woody plants to EC has been identified (Radoglou and Jarvis 1990, Wayne and Bazzaz 1995, Tognetti *et al.* 1999). Olive (*Olea europaea* L.) is an important widely cultivated evergreen tree of the Mediterranean climate, but its response to EC has never been studied. In this study, we examined the effects of exposure to EC on early photosynthetic acclimation potential and stomata behaviour of olive leaves. We hypothesised that CO_2-induced changes in gas exchange and stomata characters would be small in comparison to typical responses of deciduous trees or herbs. We compared leaves of two olive cultivars (Frantoio and Moraiolo) which have shown physiological and morphological differences in response to ozone fumigation (Minnocci *et al.* 1999). Plants were grown in conditions close to their natural environments, in large pots to limit restrictions on rooting volumes. The use of container was chosen to allow the transfer of plants from a natural CO_2 degassing vent to a nearby free-air CO_2 enrichment (FACE) facility, which was already in use, and *vice versa*. The rationale for transferring plants from the CO_2 vent to the FACE facility was to use the former as a pre-treatment.

Materials and methods

Plants and growth conditions: Plants of two olive (*Olea europaea L.*) cultivars, Frantoio and Moraiolo, were raised in 30 000 cm^3 pots for five years. On 1 March 1999 (before bud break), 10 plants per cv. (120-150 cm in height and 2-3 cm in diameter), were transported to the natural CO_2 degassing vent of Bossoleto (*Rapolano Terme*, Siena, Italy), where they were constantly exposed to EC. For a detailed description of this natural laboratory see Körner and Miglietta (1994): CO_2 of mineral origin is emitted at this experimental site from a series of vents located on the flanks of a circular crater (about 80 m in diameter) and increases the $[CO_2]$ of surrounding air (500-1 000 μmol mol^{-1}). Control plants (10 per cv.) were placed nearby. After three months, plants were randomly assigned to four FACE-system rings (two EC, 560 μmol mol^{-1}, and two AC, 360 μmol mol^{-1}) at Rapolano Terme. The distance between the rings was 30 m. All pots were manually weeded, and all plants were daily drip irrigated throughout the experiment. Because nutrient conditions were near optimal at the start of the experiment, slow-release fertiliser was only applied once during the growing season.

The FACE-system consists of a perforated rectangular plenum (8×3 m), CO_2-supply and $[CO_2]$-monitoring components, and PC-based control program. Multiple vertical pipes (spacing 75 cm) were inserted into the distribution PVC plenum with internal diameter of 20 cm. The vertical pipes (PVC, internal diameter of 6.5 cm and 2 m height) had triple-jet gas emission ports, which can be opened or closed to adjust the height of injection into the rectangular plot. This permitted to follow the growth of plants and allowed for CO_2 fumigation of the plant canopy. A high volume blower injects air into the plenum. Pure CO_2 was mixed with ambient air by placing the outlet immediately after the blower at the level of a flexible pipe, which connects the blower to the plenum, and supplied 24 h per day. A motorised metering valve (*Zonemaster, Satchwell Control System*, Milano, Italy) controlled the CO_2 injection rate. A detailed description of the FACE-system can be found in Miglietta *et al.* (1997).

Gas exchange measurements were made on two leaves from four plants per cv. from each of the two treatments using a portable, open-system gas analyser (*CIRAS, PP-Systems*, Hitchin, UK), on intact attached leaves. Current-year whole leaves (fully expanded, of the same stage of development) were used; individual leaf area was previously measured as 0.717x – 0.095, where x is the product of leaf length by leaf width (Tattini *et al.* 1997).

The P_N-C_i response of olive leaves was determined during warm sunny (and windy to avoid overheating) days of July 1999 (after 5-6 months of exposure) at ambient conditions between 10:00 and 17:00 h solar time at saturating sunlight (PAR 1 500-1 600 μmol m^{-2} s^{-1}). The conditions inside the cuvette (6 cm^2) were leaf temperature of 30 °C and vapour pressure deficit (VPD) of 1.4 kPa. On average, leaf temperatures and VPD in the cuvette increased by ≤ 2 °C over the course of a complete sequence of measurements. The $[CO_2]$ of the cuvette was initially maintained at 200 μmol mol^{-1} for 5 min to stimulate stomata opening and then reduced to 75 μmol mol^{-1}. The $[CO_2]$ of the cuvette was incrementally increased in 10 steps up to 2 000 μmol mol^{-1}, and gas exchange properties were logged at each measurement $[CO_2]$ once the system had reached steady equilibrium.

The P_N-C_i response curves were made by fitting an empirical nonlinear model:

$$P_N = P_{Nmax}\,[1-(1-R_L/P_{Nmax})^{(1-C_i/\Gamma)}] \qquad (1)$$

where P_{Nmax}, the asymptotic level, represents the predicted CO_2-saturated P_N, R_L is the rate of daytime respiration at zero $[CO_2]$ (photorespiration), and Γ is the CO_2 compensation concentration (in the absence of daytime respiration). The fitting was carried out by Marquardt-Levenberg iterative least-square method available in *Sigma-Plot* software (*Jandel Scientific*, San Rafael, CA, USA). The initial slopes of the P_N-C_i curves (dP_N/dC_i) were calculated by linear regression of the first several points (C_i below 200-250 μmol mol^{-1}). Relative limitation of stomatal conductance (g_s) on photosynthesis (L_s) was estimated according to the equation:

$$L_s = (1 - P_{Ni}/P_{N0})\ 100 \qquad (2)$$

where P_{Ni} is the estimated P_N at C_i corresponding to the $[CO_2]$ in a specific ambient air (C_a), and P_{N0} is the estimated P_N at a C_a resulting in a C_i equal to the growth C_a, the rate at which stomatal resistance to CO_2 diffusion is assumed to be zero (Farquhar and Sharkey 1982). Both P_{Ni} and P_{N0} were derived using Eq. 1 (Liu and Teskey 1995).

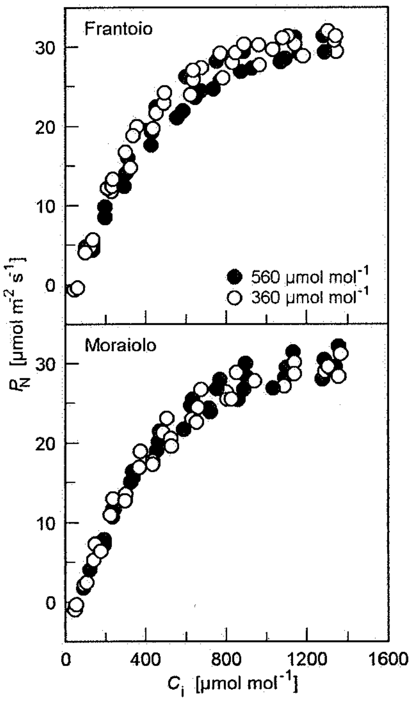

Fig. 1. Response curves (means of two leaves from four plants per cv. from each of the two treatments) of net photosynthetic rate (P_N) to intercellular $[CO_2]$ (C_i) for cultivars Moraiolo and Frantoio of olive grown under CO_2-enriched atmosphere of 560 μmol mol^{-1} (*closed circles*) or at ambient $[CO_2]$ of 360 μmol mol^{-1} (*open circles*) in a FACE system. Current-year fully expanded leaves of 5-year-old trees measured in July 1999 at ambient temperature and saturating PPFD. The statistical comparison of gas exchange parameters derived using modelled responses from five replicate P_N-C_i curves in each treatment-cv. combination is given in Table 1.

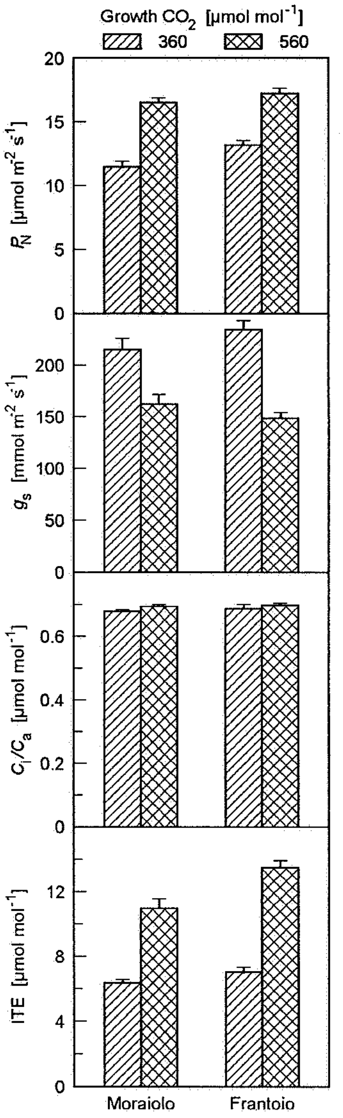

Fig. 2. Net photosynthetic rate (P_N), stomatal conductance (g_s), the ratio of intercellular to atmospheric $[CO_2]$ (C_i/C_a), and instantaneous transpiration efficiency (ITE) for cultivars Moraiolo and Frantoio of olive grown under CO_2-enriched atmosphere of 560 μmol mol^{-1} (*cross-hatched columns*) or at ambient $[CO_2]$ of 360 μmol mol^{-1} (*hatched columns*) in a FACE system. Current-year fully expanded leaves of 5-year-old trees measured during September 1999 at saturating PPFD and respective growth $[CO_2]$. Values are means ± SE (n = 5 individuals, two leaves from five plants per cv. from each of the two treatments). Differences between $[CO_2]$ treatments were always highly significant (p = 0.0001 for P_N, g_s, and ITE) except for C_i/C_a (p = n.s.). Differences between cultivars were significant for P_N and ITE (p = 0.0492 and 0.0057, respectively), while interactions between effects were never significant (ITE, p = 0.0582).

During September-October gas exchange measurements (irradiance-saturated photosynthetic rate, P_N, g_s, leaf transpiration rate E, and C_i) were made on two current-year leaves of the same stage of development from five plants per cv. from each of the two treatments. Maximum P_N, g_s, and ITE (instantaneous transpiration efficiency, P_N/E) were measured during sunny days from 09:00 to 14:00 h (25-27 °C), under saturating PAR (> 1 000 µmol m^{-2} s^{-1}). The same open gas exchange system was used for reciprocal P_N determination. The reference [CO_2] was set at 360 and 560 µmol mol^{-1}, and measurements were performed in the rings (measuring the two treatments at both reference-[CO_2], alternatively). In October mature, 5-7 fully expanded leaves of 5-year-old trees carrying or not carrying fruits were also sampled (only two plants per cv. and per treatment were carrying fruits and thus used for comparison).

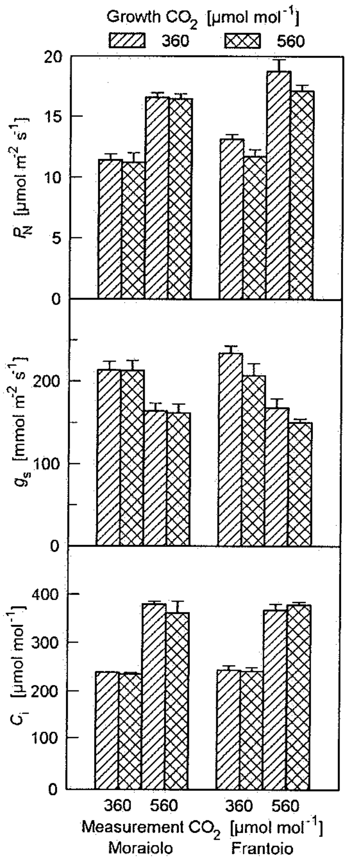

Fig. 3. Comparative rate of net photosynthesis (P_N), stomatal conductance (g_s), and intercellular [CO_2] (C_i) for cultivars Moraiolo and Frantoio of olive grown under CO_2-enriched atmosphere of 560 µmol mol^{-1} (*cross-hatched columns*) or at ambient [CO_2] of 360 µmol mol^{-1} (*hatched columns*) in a FACE system. Measured on current-year fully expanded leaves of 5-year-old trees in September 1999. For all plants gas exchange was reciprocally measured at [CO_2] of 360 or 560 µmol mol^{-1} at saturating PPFD. Means ± SE (n = 5 individuals, two leaves from five plants per cv. from each of the two treatments); differences between measurement [CO_2] were always significant ($p < 0.0001$), differences between cultivars were significant only for P_N ($p = 0.0258$), while differences between growth [CO_2] and interactions between effects were never significant.

Stomata density and chlorophyll analyses: Observations of stomata density were made on portions of leaves taken from the central area of fully expanded leaves collected in mid-July and in mid-October. Samples from two leaves of five plants per cv. from each of the two treatments were selected between second-order veins. They were immediately plunged in liquid nitrogen and there stored until observation. Frozen-hydrated leaf laminae were sputter-coated with 20 nm of gold (measured by quartz thin-film monitor) into the cryopreparation chamber of *SCU 020* (*BalTec*, Liechtenstein). They were observed at 8 kV in a scanning electron microscope (*SEM 515*, *Philips*, The Netherlands) maintained at –160 °C. Slow-scan images were digitised at a resolution of 768×576 pixels (256 grey levels) and analysed with *AnalySIS 2.0* (*Soft-Imaging Software*, Germany). Stomata density (S_D, number of stomata mm^{-2}) of three fields per sample (500 × magnification) was determined on the recorded digital images. Stomata overlapping the margins were excluded.

Chl *a*, Chl *b*, and Chl (*a*+*b*) concentrations were measured on two excised leaves (similar to those of gas exchange measurements) from five plants per cv. from each of the two treatments in mid-July and mid-October. About 0.5 g(fresh mass) of leaf tissue was macerated in 10 cm^3 *N*,*N*-dimethylformamide in the dark for 48 h at 4 °C; pigment concentrations were determined in the extracts spectrophotometrically (*Lambda 6 UV-VIS*, *Perkin-Elmer*, UK), and expressed on a leaf area basis (Moran 1982).

Statistical analysis: Values for single leaves were (two per plant) pooled on a plant basis and subjected to two- and/or three-way analysis of variance (ANOVA) to examine the effects of cv., growth [CO_2], and/or measurement [CO_2]. The percentage difference in a given variable between the EC and AC treatments was calculated as [(EC – AC)/AC] 100.

Results

The response of P_N to C_i in current-year leaves (July) differed only slightly between the two cultivars and for AC and EC treatments (Fig. 1). This was reflected in the photosynthetic parameters calculated from the P_N/C_i curves (Table 1); there were no significant effects of the $[CO_2]$ treatments on P_{Nmax}, R_L, Γ, and dP_N/dC_i. The estimated average P_N at a given C_i did not differ between $[CO_2]$ treatments. P_N at a C_a resulting in a C_i equal to the growth C_a was significantly enhanced in the EC treatment (23 and 36 %, respectively, for the cvs. Frantoio and Moraiolo). Relative stomata limitation of P_N in EC-leaves was significantly higher than in leaves grown at AC, but lower in leaves grown at 560 µmol mol^{-1}. When $[CO_2]$ treatments were compared for L_s at their respective growth C_a, the differences were consistently significant and values of L_s at growth AC were significantly reduced by EC in both cultivars similarly (about 20 %); the differences between cultivars where always significant, with generally higher values for the cv. Moraiolo.

P_N of current year foliage (September) responded positively to growth EC (Fig. 2). EC enhanced P_N by 44 % in the cv. Moraiolo and by 31 % in the cv. Frantoio. P_N was higher in the cv. Frantoio than in the cv. Moraiolo. g_s was also significantly affected by EC. Foliage of plants grown in EC had lower g_s than that of plants grown in AC, and the reduction was stronger in the cv. Frantoio. Comparison of g_s at the growth $[CO_2]$ shows that increase in AC from 360 to 560 µmol mol^{-1} led to a 24 and 37 %

Table 1. Foliar gas exchange parameters for two cultivars of olive tree grown under CO_2-enriched atmosphere of 560 µmol mol^{-1} (EC) or at ambient $[CO_2]$ of 360 µmol mol^{-1} (AC) in a FACE system, estimated from response curves of net photosynthetic rate (P_N) to internal $[CO_2]$ (C_i). Measured on current-year fully expanded leaves of 3-year-old trees in July 1999. Means ± SE for each treatment (n = 4 individuals, two leaves from four plants per cv. from each of the two treatments). Parameters and equations are defined in the text. Significance (p-level) of treatment effect for cultivar and CO_2 treatment and the interactions between effects are shown; ns, non-significant.

Parameter		cv. Frantoio		cv. Moraiolo		ANOVA		
		EC	AC	EC	AC	cv.	$[CO_2]$	cv.×$[CO_2]$
P_{Nmax}	[µmol m^{-2} s^{-1}]	31.46 (0.40)	31.57 (0.50)	31.79 (0.81)	30.95 (0.36)	ns	ns	ns
R_L	[µmol m^{-2} s^{-1}]	–5.42 (0.69)	–6.14 (0.48)	–5.41 (0.14)	–5.62 (0.32)	ns	ns	ns
Γ	[µmol mol^{-1}]	63.04 (3.85)	62.69 (2.55)	66.44 (0.65)	66.94 (0.87)	ns	ns	ns
dP_N/dC_i	[mol m^{-2} s^{-1}]	0.065 (0.002)	0.069 (0.001)	0.056 (0.003)	0.062 (0.005)	ns	ns	ns
P_N at C_i = 240	[µmol m^{-2} s^{-1}]	11.09 (0.41)	12.42 (0.36)	10.70 (0.31)	10.83 (0.56)	ns	ns	ns
P_N at C_i = 370	[µmol m^{-2} s^{-1}]	16.63 (0.60)	18.29 (0.50)	16.28 (0.44)	16.37 (0.71)	ns	ns	ns
P_{N0} at C_i = growth C_a	[µmol m^{-2} s^{-1}]	22.07 (0.68)	17.91 (0.49)	21.89 (0.56)	16.06 (0.70)	ns	0.0001	ns
L_s at C_a = 360	[%]	49.80 (0.32)	30.70 (0.29)	51.12 (0.25)	32.40 (0.53)	0.0037	0.0001	ns
L_s at C_a = 560	[%]	24.71 (0.42)	32.13 (0.30)	25.63 (0.22)	33.92 (0.56)	0.0118	0.0001	ns
L_s at growth C_a	[%]	24.71 (0.42)	30.70 (0.29)	25.63 (0.22)	32.40 (0.53)	0.0118	0.0001	ns

Table 2. Stomata density (S_D) and chlorophyll concentrations [Chl *a*, Chl *b*, and Chl (*a*+*b*)] of current-year fully developed leaves of olive cultivars Moraiolo and Frantoio grown under CO_2-enriched atmosphere of 560 µmol mol^{-1} (EC) or at ambient $[CO_2]$ of 360 µmol mol^{-1} (AC), in a FACE system. Means ± SE within brackets (n = 5 individuals, two leaves from five plants per cv. from each of the two treatments). Percent change, [(EC – AC)/AC] 100, and significance (ANOVA, p-level) are also reported; ${}^{*}p \le 0.05$, ${}^{**}p \le 0.01$, ${}^{***}p \le 0.001$, ${}^{****}p \le 0.0001$, n.s. $p > 0.05$ (not significant).

Parameter		cv. Frantoio			cv. Moraiolo			ANOVA		
		EC	AC	Δ [%]	EC	AC	Δ [%]	cv.	$[CO_2]$	cv.×$[CO_2]$
July										
S_D	[stomata mm^{-2}]	396 (10)	491 (9)	–19	519 (15)	527 (21)	–2	****	**	**
Chl *a*	[g m^{-2}]	0.406 (0.003)	0.487 (0.014)	–17	0.602 (0.024)	0.671 (0.010)	–10	***	n.s.	n.s.
Chl *b*	[g m^{-2}]	0.148 (0.016)	0.154 (0.002)	–4	0.186 (0.007)	0.222 (0.005)	–16	***	n.s.	n.s.
Chl (*a*+*b*)	[g m^{-2}]	0.608 (0.056)	0.640 (0.015)	–5	0.786 (0.031)	0.892 (0.005)	–12	***	n.s.	n.s.
October										
S_D	[stomata mm^{-2}]	448 (19)	503 (6)	–11	544 (5)	596 (19)	–9	****	***	
Chl *a*	[g m^{-2}]	0.304 (0.01)	0.335 (0.047)	–9	0.427 (0.02)	0.312 (0.018)	37	n.s.	n.s.	*
Chl *b*	[g m^{-2}]	0.094 (0.003)	0.103 (0.016)	–9	0.134 (0.006)	0.094 (0.008)	9	n.s.	n.s.	*
Chl (*a*+*b*)	[g m^{-2}]	0.398 (0.013)	0.438 (0.063)	–9	0.562 (0.026)	0.406 (0.025)	38	n.s.	n.s.	*

decrease in g_s for the cvs. Moraiolo and Frantoio, respectively. g_s was somewhat higher in the cv. Frantoio than in the cv. Moraiolo when plants were grown at AC. The ratio of C_i to C_a was unaffected by the [CO_2] enrichment in both cultivars, with a 2.3 % increase for the cv. Moraiolo and a 1.4 % decrease for the cv. Frantoio. Generally, as a result of increased P_N and decreased g_s, ITE of leaves increased by 73 % in the cv. Moraiolo and by 94 % in the cv. Frantoio when plants were grown at EC. The cv. Frantoio had higher ITE than the cv. Moraiolo, particularly when plants were grown in EC.

For the cv. Frantoio (September), P_N and g_s tended to be lower in leaves of plants grown at EC than AC when measured at the same [CO_2] (Fig. 3). Nevertheless, results of ANOVA indicated no statistically significant differences in P_N, g_s, and C_i between treatments when measured at the same [CO_2]. The two cultivars differed somewhat in P_N, with higher values measured in Frantoio grown at 360 μmol mol^{-1} and measured either at 360 μmol mol^{-1} or at 560 μmol mol^{-1}. The effect of measurement [CO_2] was always consistent. In October, P_N was higher in plants which were bearing fruits with respect to non-bearing plants, regardless of treatments (values not shown); growth [CO_2] was without effect.

Leaves of the cv. Moraiolo showed generally higher S_D than those of the cv. Frantoio (Table 2). In July, growth at EC had caused a consistent reduction (19 %) in S_D in leaves of the cv. Frantoio, but this was not the case of the cv. Moraiolo (2 %). In October, instead, also the cv. Moraiolo showed such a reduction in S_D, similar (about 10 %) to the cv. Frantoio.

After five months of treatment, plants of both cultivars did not show significant changes in the amounts of Chl *a* and Chl *b* per unit leaf area when grown in EC (Table 2). However, after seven months of exposure to EC, leaves of the cv. Moraiolo showed a significant increase in Chl concentrations compared to control plants (total Chl, 38 %). Differences between cultivars were consistent in July but not later in the season, with the cv. Moraiolo showing higher values than the cv. Frantoio.

Discussion

Curves of P_N as a function of C_i have been used to assess photosynthetic acclimation and to identify the mechanisms contributing to acclimation (Gunderson and Wullschleger 1994). Changes in P_N are the result of changes in both g_s and mesophyll capacity for photosynthesis. The impact of EC on mesophyll capacity in turn depends on the carboxylation efficiency (activity, amount, and kinetic properties) of RuBPCO and the capacity for photosynthetic electron transport and ribulose-1,5-bisphosphate regeneration (*e.g.*, Radoglou *et al.* 1992, Bunce 2000). The activity of RuBPCO is related to the slope of the P_N/C_i curve, at low C_i (Caemmerer and Farquhar 1984). We observed no significant change in RuBPCO carboxylation efficiency in leaves grown at EC after five or six months. Also, there was no significant reduction in CO_2-saturated photosynthetic rate (P_{Nmax}) or photosynthetic rate at average C_i, further demonstrating that EC does not induce consistent down-regulation of photosynthetic capacity during the first season of exposure. Probably, olive plants were still in a state of transition from AC to EC, one growing season being not enough to achieve full acclimation. However, these results are in agreement with other studies of field-grown Mediterranean evergreen trees that indicate no change in photosynthetic capacity during long-term exposure to EC (Scarascia-Mugnozza *et al.* 1996), but contrast with responses in many experiments with potted plants (Sage 1994). Large pots may have not strongly restricted rooting volume, which represents a large sink for photosynthates.

Comparison of P_N at the growth [CO_2] shows that EC resulted in a persistent increase in photosynthesis of current-year leaves after seven months of exposure in the field. This is in agreement with the majority of reports in the literature where such an increase in [CO_2] has resulted in enhanced P_N in C_3 plants (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Saxe *et al.* 1998). We suggest that there was no reduction in the enhancement of photosynthetic capacity of the foliage grown at EC over one growing season, because there was no difference in P_N between [CO_2] treatments when measured at the same [CO_2], for the cv. Moraiolo. Nonetheless, some indication of early photosynthetic acclimation was evident for the cv. Frantoio. These results suggest that as the growing season progresses, downward acclimation of photosynthesis might occur depending on the genotype. This specific and time-related response is consistent with other CO_2-enrichment studies conducted in the field or in large pots (Sage 1994).

Stomata closure in response to EC is a common phenomenon (Morison 1998). In our study, the decline in g_s probably resulted from direct effects of CO_2 on the stomata guard cells, because plants were watered regularly and leaf water potential did not differ between treatments. The mechanisms by which CO_2 affects stomata aperture are not known and there may be inter- and intra-specific variation in CO_2 sensitivity of g_s (Eamus and Jarvis 1989). In our study, the cv. Frantoio showed a tendency for negative photosynthetic acclimation and reduced g_s sensitivity at high [CO_2] compared to the cv. Moraiolo, but this was not significant. The difference in g_s between EC- and AC-grown 5-year-old plants was mainly a result of the direct effect of short-term stomata response to CO_2. As these plants were relatively young, well watered, and supplied with nutrients this is consistent with the absence of evident photosynthetic acclimation to CO_2 (Drake *et al.* 1997). In plants grown at full irradiance and with an adequate supply of soil water, stomata sensitivity to CO_2 may be reduced (Eamus and Jarvis 1989). The tendency for g_s to be lower in 5-year-old plants grown in EC than in 5-year-old plants grown at AC when measured at the same $[CO_2]$ may diminish the opportunity for photosynthesis to fully respond to EC in the long-term in cv. Frantoio.

The close coupling of mesophyll and stomata was shown by the relative constancy of the ratio of C_i to C_a which was unchanged at high $[CO_2]$ (Morison 1998). Despite EC reduced g_s, the constancy of the ratio of C_i/C_a (approximately 0.7 μmol mol^{-1}) and of the C_i at corresponding $[CO_2]$ suggests that there was little acclimation to EC in 5-year-old olive plants (cf. Liu and Teskey 1995, Rey and Jarvis 1998). However, EC during growth decreased the stomata limitation to photosynthesis, reflecting unchanged rates of CO_2 diffusion and an increase in biochemical limitations with increasing C_i. Thus, despite the observed reduction in g_s in response to EC, stomata appear to be less limiting to photosynthesis in EC plants than in AC plants (Drake *et al.* 1997). The greater stomata limitation in both cultivars, when grown in EC and measured at normal ambient $[CO_2]$, might indicate some acclimation of g_s to high $[CO_2]$.

A possible mechanism of stomata acclimation and adjustment was through the reduction of stomata density in plants grown at EC (in the cv. Frantoio this happened first with respect to the cv. Moraiolo), which is in accordance with the hypothesis of adaptive modifications of stomata number (Woodward 1987). This change may have significant impact on conductance and hence on gas exchange, and particularly on water use efficiency (Heath and Mansfield 2000). We may hypothesise that a stronger reduction of stomata density in EC-grown plants of the cv. Frantoio was the cause of the somewhat lower g_s (and P_N) values when leaves were measured at the same $[CO_2]$ (Berryman *et al.* 1994). Comparisons of stomata density-CO_2 responses for olive as well as other species from different climates and over the different time scale of centuries and millennia indicate a general decline in density with increasing $[CO_2]$, with no evidence of consistent changes in CO_2 sensitivity (Woodward and Beerling 1997).

As a result of increase in P_N and decrease in g_s, instantaneous transpiration efficiency of leaves increased with increasing growth $[CO_2]$ in both cultivars, but the increase of the ratio between leaf P_N and transpiration rate was more pronounced in the cv. Frantoio. It seems that in the latter cultivar g_s decreases proportionately more in relation to photosynthesis in plants grown in EC with the important (for Mediterranean environments) consequence that water may be conserved.

There was not a significant reduction in Chl content (which with RuBPCO is the largest nitrogen pool in the leaf) under EC. This implies that leaves of plants grown at high $[CO_2]$ were similarly efficient in the capture of photons for photosynthesis as plants grown at AC. Wullschleger *et al.* (1992) showed that EC enhanced photosynthesis in *Liriodendron tulipifera* and *Quercus alba* despite significant reductions in Chl content. Leaves of the cv. Moraiolo even displayed an increase in Chl concentrations after seven months of exposure to EC, which may suggest an increase in efficiency of radiant energy capture through a shift in carbon allocation with time.

In conclusion, current-year leaves of 5-year-old olive plants exposed to EC in the field for one growing season had enhanced P_N and reduced g_s and stomata density compared with plants grown at AC, but similar Chl content (except for the cv. Moraiolo after seven months of exposure to EC). Reciprocal gas exchange measurements and the modelling of P_N-C_i curves indicated that there was only a tendency for reduction of photosynthetic capacity and acclimation of g_s in response to EC in 5-year-old olive plants potted in large containers. The process of down-regulation is a general response to EC in suboptimal environments (Curtis 1996). Indeed, our 5-year-old plants were well watered, fertilised, and in large pots. However, Huxman *et al.* (1998) found that down-regulation of photosynthesis to EC diminished following exposure to drought in the desert shrub *Larrea tridentata* Cav., which had been exposed to EC for five months. Conversely, down-regulation of photosynthesis to EC increased under low soil nutrient supply in other species (*e.g.*, Thomas *et al.* 1994). Considering the semiarid area (other than water, nitrogen becomes less available to plants in drought conditions) of distribution of olive (Mediterranean-type ecosystems), the hypothesis that down-regulation response of photosynthesis and acclimation of g_s may be seasonally transient requires further systematic evaluation.

References

Atkinson, C.J.: Effects of climate change on the behaviour of woody perennials. – In: Agrawal, S.B., Agrawal, M. (ed.): Environmental Pollution and Plant Responses. Pp. 19-32. Lewis Publishers, Boca Raton 2000.

Berryman, C.A., Eamus, D., Duff, G.A.: Stomatal responses to a range of variables in two tropical tree species grown with CO_2 enrichment. – J. exp. Bot. **45**: 539-546, 1994.

Bunce, J.A.: Contrasting effects of carbon dioxide and irradiance on the acclimation of photosynthesis in developing soybean leaves. – Photosynthetica **38**: 83-89, 2000.

Caemmerer, S. von, Farquhar, G.D.: Effects of partial defoliation, changes in irradiance during growth, short-term water stress and growth at enhanced p(CO_2) on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. – Planta **160**: 320-329, 1984.

Ceulemans, R., Mousseau, M.: Tansley Review No. 71. Effects of elevated atmospheric CO_2 on woody plants. – New Phytol. **127**: 425-446, 1994.

Curtis, P.S.: A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. – Plant Cell Environ. **19**: 127-137, 1996.

Drake, B.G., Gonzàlez-Meler, M.A., Long, S.P.: More efficient plants: a consequence of rising atmospheric CO_2? – Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 607-637, 1997.

Eamus, D., Jarvis, P.G.: The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. – Adv. ecol. Res. **19**: 1-55, 1989.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. – Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Gunderson, C.A., Wullschleger, S.D.: Photosynthetic acclimation in trees to rising CO_2: A broader perspective. – Photosynth. Res. **39**: 369-388, 1994.

Heath, J., Mansfield, T.A.: CO_2 enrichment of the atmosphere and the water economy of plants. – In: Agrawal, S.B., Agrawal, M. (ed.): Environmental Pollution and Plant Responses. Pp. 33-43. Lewis Publishers, Boca Raton 2000.

Houghton, J.T., Meiro-Filho, L.G., Callander, B.A., Harris, N., Kattenburg, A., Maskell, K.: Climate Change 1995. The Science of Climate Change. – Second Assessment Report of the Intergovernmental Panel on Global Change. Cambridge University Press, Cambridge 1995.

Huxman, T.E., Hamerlynck, E.P., Moore, B.D., Smith, S.D., Jordan, D.N., Zitzer, S.F., Nowak, R.S., Coleman, J.S., Seeman, J.R.: Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO_2: interaction with drought under glasshouse and field (FACE) exposure. – Plant Cell Environ. **21**: 1153-1161, 1998.

Kattenburg, A., Giorgi, F., Grassil, H., Meehl, G.A., Mitchell, J.F.B., Stouffer, R.J., Tokioka, T., Weaver, A.J., Wigley, T.M.L.: Climate-models-projections of future climate. – In : Houghton, J.T., Meiro-Filho, L.G., Callander, B.A., Harris, N., Kattenburg, A., Maskell, K. (ed.): Climate Change 1995. The Science of Climate Change. Contribution of Working Group I to the Second Assessment Report of the Intergovernmental Panel on Climate Change. Pp. 285-357. Cambridge University Press, Cambridge 1996.

Körner, C., Miglietta, F.: Long term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. – Oecologia **99**: 343-351, 1994.

Liu, S., Teskey, R.O.: Responses of foliar gas exchange to long-term elevated CO_2 concentrations in mature loblolly pine trees. – Tree Physiol. **15**: 351-359, 1995.

Miglietta, F., Magliulo, V., Bindi, M., Cerio, L., Vaccari, F.P., Loduca, V., Peressotti, A.: Free air CO_2 enrichment of potato (*Solanum tuberosum* L.): development, growth and yield. – Global Change Biol. **4**: 163-172, 1998.

Minnocci, A., Panicucci, A., Sebastiani, L., Lorenzini, G., Vitagliano, C.: Physiological and morphological responses of olive plants to ozone exposure during a growing season. – Tree Physiol. **19**: 391-397, 1999.

Moran, R.: Formulae for determination of chlorophyllous pigments extracted with *N,N*-dimethylformamide. – Plant Physiol. **69**: 1376-1381, 1982.

Morison, J.I.L.: Stomatal response to increased CO_2 concentration. – J. exp. Bot. **49**: 443-452, 1998.

Radoglou, K.M., Aphalo, P., Jarvis, P.G.: Response of photosynthesis, stomatal conductance and water use efficiency to elevated CO_2 and nutrient supply in acclimated seedlings of *Phaseolus vulgaris* L. – Ann. Bot. **70**: 257-264, 1992.

Radoglou, K.M., Jarvis, P.G.: Effects of CO_2 enrichment on four poplar clones. I. Growth and leaf anatomy. – Ann. Bot. **65**: 617-626, 1990.

Rey, A., Jarvis, P.G.: Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. – Tree Physiol. **18**: 441-450, 1998.

Sage, R.F.: Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. – Photosynth. Res. **39**: 351-368, 1994.

Saxe, H., Ellsworth, D.S., Heath, J.: Tansley Review No. 98. Tree and forest functioning in an enriched CO_2 atmosphere. – New Phytol. **139**: 395-436, 1998.

Scarascia-Mugnozza, G., De Angelis, P., Matteucci, G., Valentini, R.: Long-term exposure to elevated [CO_2] in a natural *Quercus ilex* L. community: net photosynthesis and photochemical efficiency of PSII at different levels of water stress. – Plant Cell Environ. **19**: 643-654, 1996.

Tattini, M., Lombardini, L., Gucci, R.: The effect of NaCl stress and relief on gas exchange properties of two olive cultivars differing in tolerance to salinity. – Plant Soil **197**: 87-93, 1997.

Thomas, R.B., Lewis, J.D., Strain, B.R.: Effects of leaf nutrient status on photosynthetic capacity in loblolly pine (*Pinus taeda* L.) seedlings grown in elevated atmospheric CO_2. – Tree Physiol. **14**: 947-960, 1994.

Tognetti, R., Longobucco, A., Raschi, A., Miglietta, F., Fumagalli, I.: Responses of two *Populus* clones to elevated atmospheric CO_2 concentration in the field. – Ann. Forest Sci. **56**: 493-500, 1999.

Wayne, P.M., Bazzaz, F.A.: Yellow birch growth responses to elevated CO_2 atmospheres: density-dependent differences among maternal families. – Global Change Biol. **1**: 315-324, 1995.

Woodward, F.I.: Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. – Nature **327**: 617-618, 1987.

Woodward, F.I., Beerling, D.J.: Plant-CO_2 responses in the long term: plants from CO_2 springs in Florida and tombs in Egypt. – In : Raschi, A., Miglietta, F., Tognetti, R., van Gardingen, P.R. (ed.): Plant Responses to Carbon Dioxide: Evidence from Natural Springs. Pp. 103-113. Cambridge University Press, Cambridge 1997.

Wullschleger, S.D., Norby, R.J., Hendrix, D.L.: Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. – Tree Physiol. **10**: 21-31, 1992.