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Photosynthetic characteristics of sun and shade leaves in the canopy of *Arbutus unedo* L. trees exposed to *in situ* long-term elevated CO₂

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Abstract

Photosynthetic parameters were studied in *Arbutus unedo* L. trees growing at either ambient (AC) or elevated EC (mean 465 μmol mol⁻¹) CO_2 concentration near a natural CO_2 vent in Orciatico, Italy. Diurnal courses of net photosynthetic rate (P_N), ratio of variable to maximum chlorophyll fluorescence (F_v/F_m), and quantum yield of electron transport through photosystem 2 (Φ_2) were measured on sun and shade leaves. The contents of N, C, Ca, K, P, and chlorophyll (Chl) and specific leaf area (SLA) in these leaf categories were also determined. A morning peak and midday depression of P_N were found for both AC and EC sun leaves. Long-term EC caused little or no down-acclimation of P_N in sun leaves. The estimate of total daily CO_2 uptake was lower in AC leaves than in EC leaves. In shade leaves, it reached up to 70 % of the value of sun leaves. The F_v/F_m ratio showed decreasing trend in the morning, reached a minimum at midday (90 % of dawn value), and then increased in the afternoon. The EC had no effect on F_v/F_m either in sun or shade leaves. Plants grown near the CO_2 spring had lower Chl content, higher SLA, and higher Ca and K contents than plants grown under AC.

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Abbreviations: AC - trees or their leaves grown under ambient atmospheric CO_2 concentration; C_i - intracellular CO_2 concentration; Chl - chlorophyll; DM - dry mass; E - transpiration rate; EC - trees or their leaves grown under elevated atmospheric CO_2 concentration; g_s - stomatal conductance; I_c - compensation irradiance; LA - leaf area; LST - local summer time; OTC - open top chamber; PPFD - photosynthetic photon flux density; PS - photosystem; R_D - dark respiration rate; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase; SLA - specific leaf area; WUE - water use efficiency; Φ_{CO2} - quantum yield of CO_2 fixation; Φ_2 - quantum yield of electron transport through photosystem 2 calculated as $\Delta F/F_m'$.

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Additional key words: chlorophyll fluorescence; CO_2 spring; compensation irradiance; global change; intercellular CO_2 concentration; quantum yield of photosystem 2; respiration rate; specific leaf area; stomatal conductance; transpiration rate; water use efficiency.

Introduction

Responses of tree species photosynthesis to EC have been studied intensively over the last two decades (*see* Ceulemans and Mousseau 1994 and literature cited therein). Unfortunately, the results of experiments with trees conducted so far do not enable a firm hypothesis to be made of future behaviour of trees under EC. Many studies have been carried out on seedlings exposed to EC for several days (short-term experiments) or weeks (long-term experiments) in laboratories (Ceulemans and Mousseau 1994). These studies report enhanced photosynthesis and biomass production. However, in some long-term studies, a phenomenon of down-acclimation of photosynthesis has been observed (Jarvis 1989, Ceulemans and Mousseau 1994). Other authors found no evidence of down-acclimation of photosynthesis in open-top chamber (OTC) grown plants (Idso and Kimball 1991, Long and Drake 1991, Wullschleger *et al.* 1992).

The results of laboratory experiments are not very predictive due to the age of the trees, short period of exposure to EC, and lack of natural variation of environmental factors (*e.g.*, air temperature and humidity). In this respect, more valuable are studies of field-grown trees exposed to EC in OTCs over several seasons (Scarascia-Mugnozza *et al.* 1996, Wang *et al.* 1996). This approach, however, has some limitations that make the interpretation of the results difficult. Internal OTCs environment does not always match the external environment due, *e.g.*, to reduction of incident PPFD by absorption in the OTCs cover, shifted air temperature inside the OTCs, and artificially directed air flow (obviously from tree base upwards). In addition, the studies were done only on young trees because it would be extremely expensive to construct and run OTCs over trees of large dimensions. These facts and contradictions led to free air CO_2 enrichment (FACE) experiments that, generally speaking, excluded the difference in environmental factors between AC and EC plants. Unfortunately, only a limited number of FACE studies have so far involved trees (Amthor 1995, Bucher *et al.* 1998).

It is not certain whether even exposure over several seasons is long enough for tree acclimation. An alternative approach for the exposure of trees to *in situ* EC has therefore been introduced using natural CO_2 enrichment near CO_2 vents (Miglietta *et al.* 1993b). This approach has similar advantages to the FACE projects and furthermore it is less expensive. The trees are surely long-term acclimated because they have experienced EC throughout their life cycle. There is a network of field experimental plots with natural CO_2 enrichment in Italy used for research on effects of EC on trees. We chose one of these plots to study the effect of EC on diurnal courses of photosynthetic parameters in *Arbutus unedo* L. leaves. *A. unedo* is a shallow-rooted evergreen laurophyllous shrub, or a tree characteristic of Mediterranean coastal scrub vegetation that grows mainly on silicic soils. The aim of this study was to distinguish the photosynthetic response of sun and shade foliage on *A. unedo* to EC, estimate their daily CO_2 uptake, and evaluate the diurnal variation in effectivity of photosystem 2 (PS2).

Materials and methods

Plants: The study site is located in a Mediterranean forest near Orciatico, central Italy (43°26'N, 0°40'E) at 270 m a.s.l. The forest on the site was coppiced about 30 years ago, and since then it has been growing undisturbed and unmanaged; it is dominated by deciduous tree species *Quercus pubescens* Willd., *Q. cerris* L., and evergreen tree species *Q. ilex* L. and *A. unedo*. The site is characterized by a mean annual temperature of 23.5 °C, and annual precipitation of 938.8 mm (30-year average). The CO_2 emissions from vents located along a narrow stream have been described in Hättenschwiler *et al.* (1997). At the study site, the trees grow on a NW-facing gentle slope and reach a height of 4-8 m. Two groups of *A. unedo* trees in the forest stand were selected for ecophysiological measurements: (*1*) situated 15 m from a natural CO_2 vent (EC), (*2*) situated 120 m S of the vent where no effect of the vent on atmospheric CO_2 concentration was apparent (AC). Both EC and AC trees were chosen on the basis that sun leaves forming the upper canopy would be accessible from a 4 m tall ladder. Throughout the day, no tree-to-tree or ladder-to-tree shading effects took place. The measurements were made during the summer season (June/July 1996) after full expansion of new leaves. Several rainfalls preceeded the period of measurements, therefore the trees were not under water stress, which is common for Mediterranean vegetation in midsummer.

Environment: During the ecophysiological measurements, the daily fluctuations in CO_2 concentration were monitored at the study site using an absolute IRGA *EGM-1* (*PP-System*, Hitchin, U.K.). The analyser was placed 4 m above ground level in the middle part of the tree canopy. The CO_2 concentration was measured continuously from 07:00 to 15:00 h local summer time (LST) and the values were stored at 1 min intervals. Additionally, a full set of microclimatological values was collected by another research group operating at the site (C. Stylinski, unpublished) by a complete field weather station (*Campbell*, U.K.).

Gas exchange mesurements were made on intact leaves of the upper (sun leaves) and lower canopy (shade leaves) using a portable system (*CIRAS-1*, *PP-System*, Hitchin, U.K.). The measurements started in the early morning (07:30 LST) and lasted until evening (20:00 LST) in order to establish diurnal courses of P_N, transpiration rate (E), and leaf stomatal conductance (g_s) in both sun and shade leaves. During the daytime, the measurements were made at 1 h intervals. All gas exchange measurements were taken under natural PPFD. Measured leaves were placed in the leaf chamber in their natural orientation. In several cases, however, due to leaf chamber characteristics the natural leaf inclination was disturbed. To evaluate short-term effects of EC in sun and shade leaves at the AC and EC sites, gas exchange was measured under two CO_2 concentrations. After placing the leaf in the leaf chamber, it was supplied with 355 μmol(CO_2) mol^{-1}. The value of steady-state P_N was recorded after equilibrium, when intercellular CO_2 concentration (C_i) reached constant value. The CO_2 concentration in the chamber was then increased to 465 μmol(CO_2) mol^{-1}, and steady-state P_N recorded again. The value of 465 μmol(CO_2) mol^{-1} was found to be representative for the EC site.

Irradiance response curves of P_N: Vigorous shoots of *A. unedo* bearing sun/shade leaves were cut from the canopy early in the morning (07:00-07:30 LST), their cut ends were placed into water, and transported to the field laboratory. Before measurements (09:00 LST), the shoots were adapted for 1 h to the PPFD of 200 μmol m^{-2} s^{-1} and air temperature of 27.5 °C. Steady state P_N was measured either at 350 or 700 μmol(CO_2) mol^{-1} in response to descending PPFD (500, 380, 255, 120, 51, 29, 18, 0 μmol m^{-2} s^{-1}) using an *HQI-R* 250W lamp (*Osram*, Germany) and an open IR gasometric system *CIRAS-01* (*PP Systems*, England) combined with a leaf chamber *PLC-C* (*PP Systems*, England). P_N values were fitted by an exponential. Mean values of dark respiration rate (R_D), compensation irradiance (I_c), apparent quantum yield of CO_2 fixation (Φ_{CO2}), and maximum net photosynthesis (P_{Nmax}) were then evaluated. Φ_{CO2} was determined from the linear part of irradiance response curves between 18 and 29 μmol m^{-2} s^{-1}. I_c was calculated from the linear regression, when $P_N = 0$. P_{Nmax} was determined from an exponential regression fitted to the P_N values as an asymptote.

Chl fluorescence measurements: Diurnal courses of variable to maximum Chl fluorescence ratio (F_v/F_m) were measured in sun and shade leaves at both AC and EC sites. A portable fluorometer (*OS-500, OptiSciences*, USA) was used at the following setting: modulated radiation (λ = 660 nm) used for F_0 determination of 0.1 μmol m^{-2} s^{-1}, saturation pulses (λ = 655 nm) of 5000 μmol m^{-2} s^{-1}, and pulse duration 0.7 s. Prior to measurement, the leaves were dark-adapted for 30 min. F_v/F_m was measured at approximately 1 h intervals between 07:30 and 21:00 LST. Single measurements were repeated on at least 5 leaves each time.

Quantum yield of electron transport through PS2 (Φ_2) was measured on light-adapted leaves when natural direct sunlight was used as actinic radiation. Instrumental setting was: modulated radiation used for F_0 determination (λ = 660 nm) of 0.1 μmol m^{-2} s^{-1}, saturation pulses (λ = 655 nm) of 7 000 μmol m^{-2} s^{-1}, and pulse duration 0.7 s. Leaves were placed on a special window-frame clip assuring a 60° angle of fluorescence probe axis from leaf surface and given 10 min adaptation to natural PPFD. A saturation pulse was then applied and Φ_2 was calculated as $\Delta F/F_m'$, where ΔF is the difference between the maximum fluorescence under saturating irradiance and steady-state fluorescence under actinic radiation. F_m' represents maximum fluorescence under saturating PPFD. Diurnal trends of Φ_2 were recorded for sun and shade leaves at AC and EC sites.

Pigment and element analysis: Samples of sun and shade leaves were collected from the AC and EC trees, transferred to the laboratory, and oven-dried at 85 °C to a constant mass. Before drying, the leaf area (LA) of each sample was measured using a *ScanJet II P* table scanner (*Hewlett Packard*, U.K.), and LA determining software (30 leaves *per* sample). SLA was then determined as the ratio of LA to the dry mass (DM) of a leaf. The contents of selected elements were detemined in 3 sub-samples taken from each leaf category. Total nitrogen (N) and carbon (C) contents were measured by using an *EA-1106* gas chromatograph (*Carlo Erba*, Milano, Italy). The contents of phosphorus (P), calcium (Ca), and potassium (K) were determined in leaf samples mineralised in H_2SO_4 and H_2O_2 using a *Flapho* flame spectrometer (Germany). Analysis of Chl (*a*+*b*) content in leaves was done using an extract in 80 % acetone. For each leaf category, the extract absorbance was measured with a spectrophotometer (*Specol*, Germany) in λ = 645 and 663 nm, and pigment contents were calculated using Arnon's procedure (1949).

Statistical analysis of values was done using Student's *t*-test, one-way ANOVA, and Student-Newman-Keuls test. The differences are understood as statistically significant at $p<0.05$, if not stated differently.

Results

Local CO_2 environment: The concentration of CO_2 at the EC site showed a wide variation depending mostly on wind speed and direction. On calm days, with no or only a light breeze, the CO_2 concentration was close to the normal atmospheric values. On windy days, when the forest near the CO_2 vent was well ventilated, maximum daily mean CO_2 concentration reached the highest values. Diurnal courses of CO_2 concentration at the EC site had an irregular series of rapid fluctuations upwards from more or less ambient baseline. For gas exchange measurements simulating the short-term effect of EC, we found the value of 465 $\mu mol(CO_2)\ mol^{-1}$ as representative of the EC site.

Leaf characteristics: There was no difference in SLA between EC and AC sun leaves. On the other hand, SLA was significantly higher in EC than AC shade leaves (+22.4 %). Higher SLA values were found in shade leaves, that were thinner than sun leaves (Table 1).

Table 1. Specific leaf area (SLA) and chlorophyll (Chl) content in leaf categories (mean ± standard deviation) of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration. Different characters in upper index represent statistically different values tested by one-way ANOVA and Student-Newman-Keuls test ($p<0.05$).

	SLA [$m^2\ kg^{-1}$]	Chl (*a*+*b*) [$g\ m^{-2}$]	 [$g\ kg^{-1}$]
AC shade	10.74 ± 2.20^a	0.41 ± 0.02^a	4.41 ± 0.05^a
AC sun	7.10 ± 0.96^b	0.32 ± 0.01^b	2.26 ± 0.01^b
EC shade	13.15 ± 2.10^c	0.35 ± 0.03^c	4.59 ± 0.06^a
EC sun	7.20 ± 0.60^b	0.29 ± 0.03^b	2.08 ± 0.02^b

Both sun and shade leaves grown at the EC site showed lower Chl content expressed on a LA basis (Table 1). In general, Chl contents were higher in shade leaves than in sun leaves. Contents of K and Ca expressed on a DM basis differed significantly between leaf categories, being always higher in shade than in sun leaves, and, with the exception of EC shade leaves, lower in EC than AC leaves (Table 2). However, on a LA basis, similar values were obtained for all leaf categories (values not shown). Contents of N and P were about one third higher in AC sun and EC shade leaves than in AC shade and EC sun leaves.

Table 2. Element content [g kg^{-1}(DM)] and carbon to nitrogen ratio in leaf categories (mean ± standard deviation) of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration. Different characters in upper index represent statistically different values tested by one-way ANOVA and Student-Newman-Keuls test ($p<0.05$).

	Ca	K	N	P	C/N
AC shade	6.49 ± 1.15^a	8.21 ± 1.47^a	17.68 ± 4.22^a	0.73 ± 0.12^a	49.00 ± 0.97^a
AC sun	4.84 ± 0.58^a	7.41 ± 0.52^a	22.75 ± 1.78^b	0.94 ± 0.05^b	29.16 ± 0.81^b
EC shade	4.94 ± 0.51^a	10.20 ± 0.29^b	22.56 ± 0.71^b	1.06 ± 0.01^b	37.52 ± 0.45^b
EC sun	3.81 ± 0.30^a	7.17 ± 0.53^a	17.74 ± 1.89^a	0.73 ± 0.02^a	50.44 ± 0.17^a

Gas exchange: The diurnal courses of P_N in sun leaves showed the midday depression that has often been reported for Mediterranean species growing in the open. A maximum P_N was reached in sun leaves at about 10:00 LST. For all sun leaves, minimum P_N values were found in betweeen 14:00 and 15:00 LST. The second, much lower, P_N daytime maximum was found between 17:00 and 18:00 LST, when slightly lower P_N values were found in EC than AC leaves (Fig. 1). For shade leaves, diurnal P_N courses showed only one daily maximum, the timing of which depended on the time of maximum PPFD. In AC leaves, the maxima of P_N were obtained at 11:00 LST and in EC leaves at 12:30 LST. Afternoon P_N values in both AC and EC leaves gradually decreased towards sunset.

The diurnal course of g_s in sun leaves was similar to that of P_N, with two maxima, the higher one of about 120 mmol m^{-2} s^{-1} at 10:00 LST, and the lower one of about 15 mmol m^{-2} s^{-1} between 14:00 and 16:00 LST (Fig. 1). No significant difference was found in g_s when measured in 355 compared to 465 $\mu mol(CO_2)$ m^{-2} s^{-1} and no difference was apparent between AC and EC sun leaves. In shade leaves, g_s showed only one daily maximum, the timing of which depended on maximum PPPFD penetrating to the lower canopy.

No effect of the two CO_2 concentrations, under which the gas exchange measurements were made, was found on E. On the other hand, a significant reduction in E throughout the day was apparent in EC compared to AC sun leaves. This difference was greater in the morning than in the afternoon (Fig. 1). The diurnal course of E showed a typical shape with morning (10:00-10:30 LST) and late afternoon (17:00 LST) maxima, and a midday depression (14:00-15:00 LST) for both AC and EC sun of leaves. Absolute maximum of 2.5 $mmol(H_2O)$ m^{-2} s^{-1} was found in AC sun leaves in the morning and minimum of 0.5 $mmol(H_2O)$ m^{-2} s^{-1} in EC sun leaves in late afternoon, respectively. For shade leaves, the different diurnal course for AC and EC leaves depended mostly on when the foliage obtained a short-term flash of direct sunlight. In general, the shade leaves had lower *E* [less than 0.7 mmol(H_2O) m^{-2} s^{-1}] throughout the day than sun leaves.

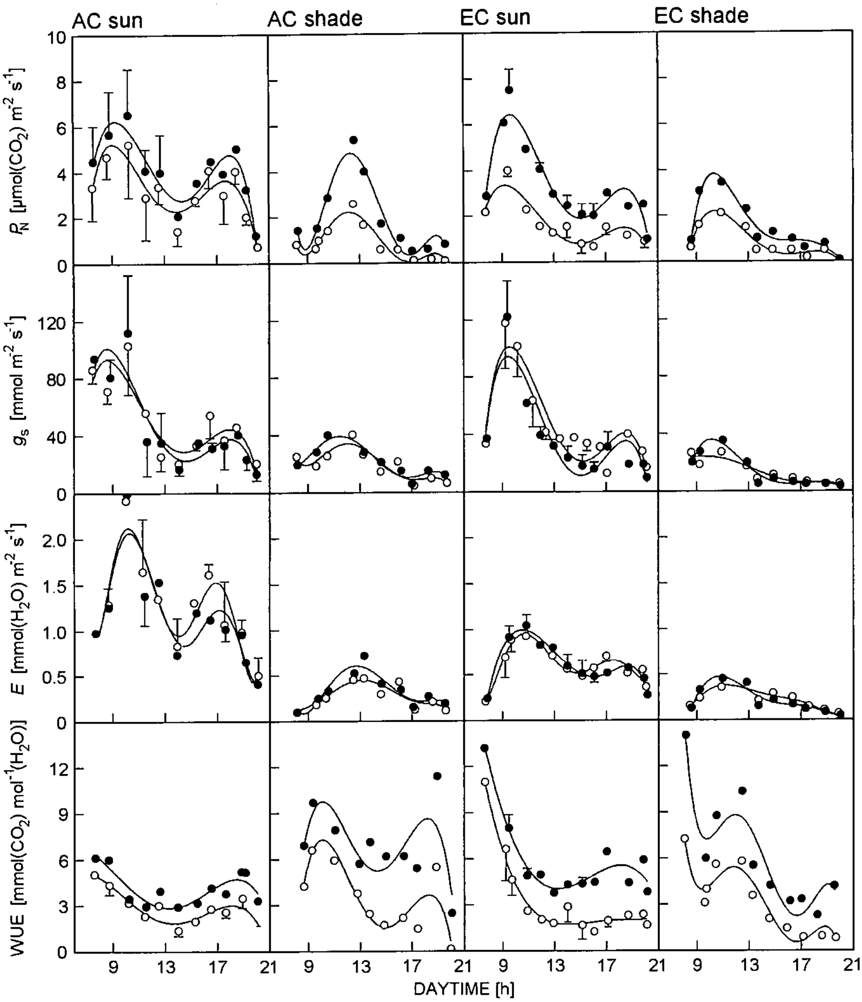

Fig. 1. Diurnal courses of net photosynthetic rate (P_N), stomatal conductance (g_s), leaf transpiration rate (*E*), and water use efficiency (WUE) in sun and shade leaves of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration and measured at 355 (*open symbols*) or 465 (*full symbols*) µmol(CO_2) mol^{-1}. Error bars are standard deviations. The fits are polynomials of 4th or 5th order.

In EC sun leaves, the diurnal course of water use efficiency (WUE) had a maximum in the early morning [11.0-13.0 mmol(CO_2) mol^{-1}(H_2O) at 08:00 LST] followed by a rapid decrease to about 2.0-5.0 mmol(CO_2) mol^{-1}(H_2O) at 12:00 LST. WUE then was a more or less constant with a slight increment at about 18:00 to 20:00 LST (Fig. 1). When measured under 465 µmol(CO_2) m^{-2} s^{-1}, the WUE was greater than that measured under 355 µmol(CO_2) m^{-2} s^{-1}. This difference was higher in EC sun leaves [2.0-3.0 mmol(CO_2) $mol^{-1}(H_2O)$] than in AC sun leaves [1.0-1.5 mmol(CO_2) $mol^{-1}(H_2O)$]. For shade leaves, the WUE diurnal course was irregular, with one or two peaks.

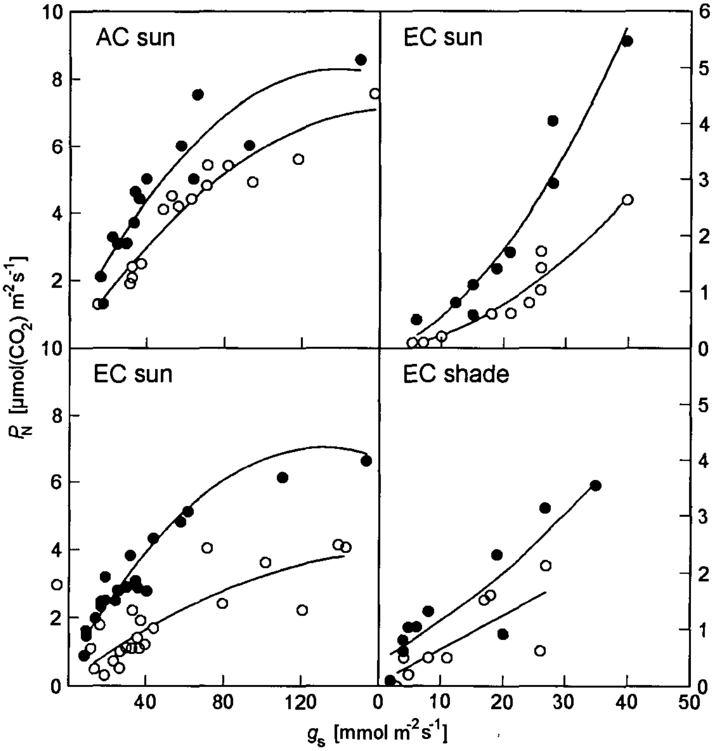

Fig. 2. Changes in net photosynthetic rate (P_N) plotted against stomatal conductance (g_s) in sun and shade leaves of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration and measured at 355 (*open symbols*) or 465 (*full symbols*) µmol(CO_2) mol^{-1}.

No photosynthetic parameter derived from irradiance response curves (Fig. 3) was significantly affected by long-term exposure to EC (Table 1). There was, however, the effect of irradiance since shade leaves showed significant decrease in I_c, R_D, and slight increase in Φ_{CO2}.

Chl fluorescence parameters: Predawn values of F_v/F_m, which indicate maximal photochemical efficiency of PS2, were similar in AC and EC sun leaves and AC shade leaves, and higher in EC shade leaves. Sun leaves on both AC and EC trees showed a decrease (steeper in AC leaves) in F_v/F_m from dawn (0.84) towards midday (approx. 0.78) and then a recovery in late afternoon (Fig. 4). For shade leaves, however, the rate and extent of the midday decrease was not so pronounced, reaching a minimum value of about 0.82. Throughout the day, the EC showed no difference in F_v/F_m values compared to AC leaves.

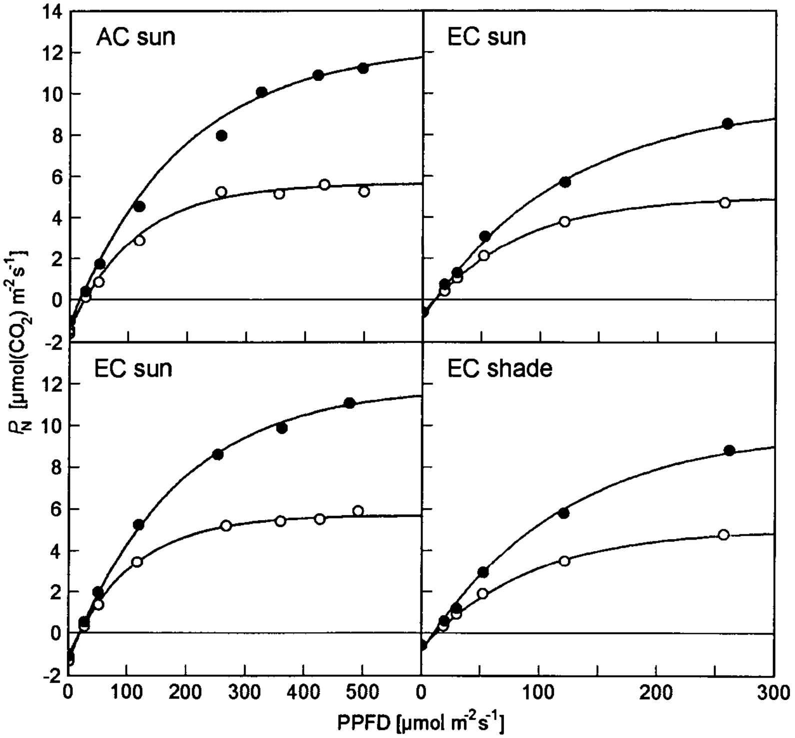

Fig. 3. Irradiance response curves of net photosynthetic rate (P_N) in sun and shade leaves of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration and measured at 355 (*open symbols*) or 700 (*full symbols*) µmol(CO_2) mol^{-1}. Standard deviations (not indicated) were less than 5 % of P_N values.

Quantum yield of electron transport through PS2 (Φ_2) showed a typical diurnal course with a marked minimum during the morning (10:00) followed by a gradual increase, with the second minimum recorded at about 18:00-19:00 (Fig. 4). In sun leaves, Φ_2 was lower at comparable times than in shade leaves, the difference being more pronounced during the early morning. In both AC and EC sun leaves, Φ_2 decreased from a dawn maximum value to the first minimum (about 0.30-0.37) observed at 10:00-11:00 LST, it then increased and was more or less constant around midday, followed by a second minimum at about 18:00-19:30 LST. It then increased again towards sunset showing values comparable to the dawn maximum. There was no difference in Φ_2 between AC and EC sun leaves. Shade leaves compared to sun ones showed higher Φ_2 values throughout the daytime, remaining almost constant with one or two slight decreases caused by penetration of direct sunlight into the lower canopy which enhanced P_N.

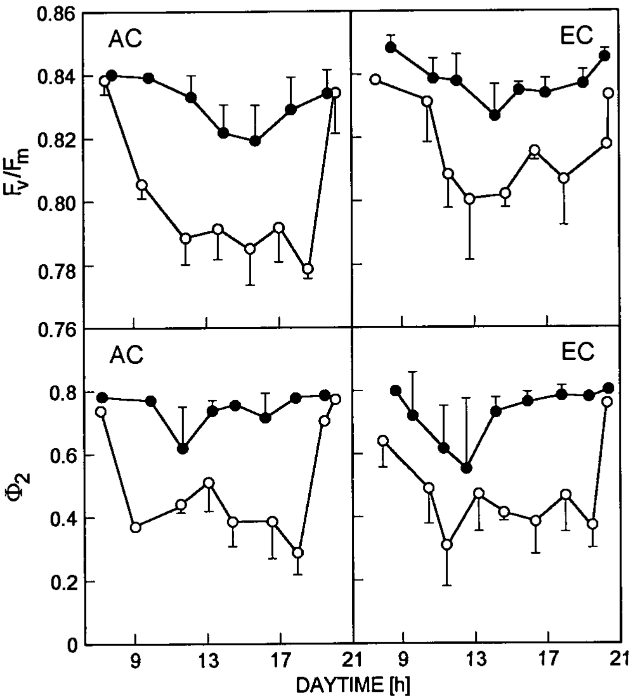

Fig. 4. Changes in chlorophyll fluorescence ratio F_v/F_m (*top*) and in quantum yield of electron transport through photosystem 2, Φ_2 (*bottom*) in sun (*open symbols*) and shade (*full symbols*) leaves of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration. Error bars are standard deviations.

Table 3. Photosynthetic parameters (means ± standard deviation) derived from irradiance response curve, compensation irradiance (I_c), apparent quantum yield of photosynthesis (Φ_{CO2}), and dark repiration rate (R_D) for leaves of *Arbutus unedo* grown at ambient (AC) or elevated (EC) CO_2 concentration and measured at 350 or 700 μmol(CO_2) mol^{-1}.

Leaf type		I_c [μmol m^{-2} s^{-1}]	Φ_{CO2} [mmol mol^{-1}]	R_D [μmol(CO_2) m^{-2} s^{-1}]
sun	EC 350	24.30 ± 3.90	51 ± 7	+.45 ± 0.05
	EC 700	19.60 ± 4.20	60 ± 7	1.25 ± 0.13
	AC 350	31.30 ± 6.36	44 ± 5	1.39 ± 0.18
	AC 700	20.10 ± 3.60	55 ± 3	1.10 ± 0.14
shade	EC 350	11.50 ± 3.30	55 ± 3	0.55 ± 0.07
	EC 700	9.42 ± 0.76	64 ± 7	0.60 ± 0.00
	AC 350	10.49 ± 1.86	58 ± 5	0.56 ± 0.12
	AC 700	8.39 ± 0.25	67 ± 12	0.53 ± 0.06

Discussion

Leaf characteristics: An increase in SLA has already been found in *A. unedo* growing in the CO_2 spring of Armaiolo in Italy (Jones *et al.* 1995). Such an increase is not common for plants grown under EC (Farrar and Williams 1991) and may be a species specific response.

A loss of Chl content in EC plants has been observed in several species and attributed to an inhibition of the transcription of *cab* genes by the accumulation of soluble sugars (Van Oosten *et al.* 1994). Dilution effect on the decrease in Chl content (DM basis) might be excluded because *A. unedo* leaves showed increase, not decrese in SLA. Real loss of Chl from cells as observed by Besford (1990) cannot, however, be ruled out. Increase of Chl content on DM basis in shade compared to sun leaves of *A. unedo* is comparable to the differences found generally between sun and shade leaves of tree species (*e.g.*, *Fagus sylvatica* - Lichtenthaler *et al.* 1981).

EC may either decrease leaf mineral concentrations due to decreased mineral uptake or increased carbon uptake, and cause no change if the mineral and carbon uptake are proportional (Eamus and Jarvis 1989, Peñuelas *et al.* 1997). In our plants, the leaf content of Ca decreased in EC plants, while K increased. The difference was particularly evident in shade leaves. This may be due to a different allocation to organs. Obviously, decrease in the leaf concentration per DM of both macronutrients and micronutrients is reported in other species (Overdieck 1993, Menconi *et al.* 1997). N concentration has often been found lower in EC than in AC plants. Such a reduction has previously been observed in other CO_2 spring species (Körner and Miglietta 1994; R. Tognetti and A. Raschi, unpublished) and attributed to a dilution by carbon present in the tissues (Sage *et al.* 1989). Another explanation could be dilution due to growth. In our experiment, shade leaves of EC plants had similar N concentration to sun leaves of AC plants, and a higher N concentration than sun leaves of EC plants and shade leaves of AC plants, similar to that found in the shade-intolerant *Betula papyrifera* Marsh. (Kubiske and Pregitzer 1996).

Diurnal course of gas exchange: Midday depression of P_N is common in Mediterranean tree species, *e.g.*, *Arbutus unedo* (Tenhunen *et al.* 1987), *Quercus pubescens* (Damesin and Rambal 1995), *Olea europea* (Angelopoulos *et al.* 1995) as well as central European broadlef species. The midday P_N depression observed was caused by decreasing g_s (Fig. 1). Dependence of P_N on g_s slightly differed between AC and EC leaves (Fig. 2) showing a lower P_N for a certain g_s in EC leaves. This might indicate limitation of P_N in EC plants caused probably by decreased ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity. The morning P_N maximum was well comparable to the ranges of 4.5-9.9 and 5.5-6.5 μmol m^{-2} s^{-1} reported for *A. unedo* by Fleck *et al.* (1995) and Castell and Terradas (1995), respectively. Despite the fact that g_s in EC sun leaves was reduced throughout the day much less than in AC leaves, *E* was significantly lower in EC than AC leaves. This would indicate increased WUE in EC trees, comparably to other tree species (Guehl *et al.* 1992).

The early morning maxima of E found in sun leaves agree with the range value of 2.0-4.0 mmol(H_2O) m^{-2} s^{-1} reported by Fleck *et al.* (1995) and Castell and Terradas (1995) for *A. unedo* at the same time of year. Our g_s values for AC and EC sun leaves are comparable to g_s values of about 10 mmol m^{-2} s^{-1} in sun leaves of *A. unedo* measured at midday in August by Jones *et al.* (1995).

Shade leaves of *A. unedo* might make an important contribution to whole-tree CO_2 uptake because their daily uptake *per* LA reached the value up to 70 % of sun leaves. This could be explained by extremely low g_s of sun leaves throughout most of the day, appart from early morning. P_N in sun leaves was therefore fairly low during the day despite sufficient PPFD. Shade leaves, being PPFD-limited for a substantial part of the day, reached fairly high P_N values when they received sufficient PPFD *via* direct sunlight through sunflecks. The total CO_2 uptake therefore depends only on the duration of direct sunlight.

The CO_2 concentration at the EC site fluctuates extremely rapidly during the day and does not maintain constant values. This may affect stomatal regulation, while the concentration of 465 μmol(CO_2) mol^{-1} in the cuvette may not be representative of the external condition. Moreover, for estimating the seasonal course of P_N, the variations of interacting factors, such as, *e.g.*, nutrient availability, precipitation, water stress, that might enhance or limit P_N more than EC, should be taken in account.

Photosynthetic parameters: Most evergreen tree species have, due to high degree of sclerophylly of leaves, lower P_{Nmax} than deciduous species (Prado and De Moraes 1997). For European evergreens, it obviously varies within the range of 3.0-10.0 (*see, e.g.*, Lange *et al.* 1982, Gratani 1997). Our results showed high coincidence for *A. unedo* leaves (Fig. 1, Table 3). In our experiment, I_c and R_D apparently decreased in shade compared to sun leaves which is in agreement to results for tropical (Sims and Pearcy 1994) or temperate (Tjoelker *et al.* 1995) forest species grown along a PPFD gradient. This shade acclimation provides the leaves advantage in an exploitation of small amounts of PPFD incident to lower canopy. It helps the leaves of *A. unedo* to maintain relatively high daily carbon uptake (up to 70 % of sun leaves).

Direct and long-term effects of EC on gas exchange: Enhancement of P_N in response to EC during P_N measurements was observed not only at saturating PPFD but throughout the whole PPFD interval (+110-140 %). It supports theoretical expectations coming from biochemical model of C_3 photosynthesis (Farquhar *et al.* 1982) in which P_N is related to actual proportion between oxygenation and reduction activities of RuBPCO. Since under CO_2 concentration doubled during measurements, decreased oxygenation is experienced, and if more substrate is available for photosynthesis, P_N is enhanced. Thus in sun leaves, less limited by amount of available RuBPCO than shade leaves, the direct effect of EC is more pronounced.

Exposure to long-term EC had only minor effect on P_N. Almost no difference in P_{Nmax} and P_N over whole PPFD range between AC and EC sun leaves in *A. unedo* indicates that the species does not show down-acclimation to long-term EC. This corresponds to the results of Jones *et al.* (1995) who found no difference in CO_2

response curves between AC and EC leaves. In other Mediterranean species growing near CO_2 vents, either evidence for tendentially negative acclimation or no down-acclimation of P_N was found (Miglietta *et al.* 1993a, Bettarini *et al.* 1997). The acclimation of Mediterranean trees to *in situ* EC is probably species-specific and varies within a wide range. As an extreme, a positive P_N acclimation (up to 100 % increase compared to control trees) is reported in OTC-grown *Q. ilex* (Scarascia-Mugnozza *et al.* 1996).

Shade leaves show some enhancement of P_{Nmax} of about 6 % which might be attributted to generally higher stomata opening of shade leaves throughout a day light period (Fig. 1). It might be hypothesised that sun leaves of *A. unedo* which show high degree of stomatal closure during a majority of a day (g_s about 15.0 mmol m^{-2} s^{-1}) and likely also relatively slow movements of stomatal guard cells following a change in environmental conditions, are less responsive to long-term EC than shade leaves. On the other hand, long-term EC slightly promoted R_D in sun leaves of *A. unedo* while no effect was observed in shade leaves. Increase in R_D is documented for some broadleaf tree species (*e.g.*, *Eucalyptus* sp. - Idso and Kimball 1993, *Alnus glutinosa* - Vogel and Curtis 1995) but a decrease in R_D is reported more frequently (*e.g.*, Wullschleger and Norby 1992, for review *see* Ceulemans and Mousseau 1994). Decreased R_D due to EC is associated with the decrease of maintenance respiration.

Diurnal course of Chl fluorescence parameters: The slight decrease of F_v/F_m around midday (maximum decrease 10 %) is associated with light-dependent inactivation of PS2. In a Mediterranean climate, high temperature effect on the decrease in F_v/F_m might also be considered. The greatest decrease in F_v/F_m occurred in sun leaves around midday when P_N reached the lowest value, as reported by Scarascia-Mugnozza *et al.* (1996) for *Q. ilex*. Increasing CO_2 concentrations resulted in an increase in the photosynthetic efficiency of *Scirpus olneyi* at low irradiances (Long and Drake 1991). This seems to agree with the higher F_v/F_m values recorded in EC shade leaves in early morning.

Diurnal courses of Φ_2 revealed the decline in P_N around midday (Φ_2 maximum) due to increasing proportion of closed PS2 reaction centres caused by high PPFD, a phenomenon commonly observed in many field grown tree species (*e.g.*, *Acer platanoides* - Schindler and Lichtenthaler 1996). Since no significant differences in Φ_2 courses were found between AC and EC sun leaves measured under both AC and EC, we conclude that the hyphothesis of no acclimation of *in situ* P_N to long-term EC is consistent. Shade leaves of *A. unedo* compared to sun leaves showed much higher Φ_2 values throughout the day which was caused by the much lower PPFD available to them. The comparison of Φ_2 between AC and EC shade leaves has no relevance because they experienced different PPFD in similar periods of the day.

Our results on *A. unedo* trees exposed to natural long-term EC indicated that although no significant downward acclimation on *in situ* P_N was evidenced, a reduction in *E* did take place; moreover, several points suggest that the role of shade leaves in a future CO_2 enriched world may acquire more relevance. This warrants further experiments in order to predict how global change may affect the leaf metabolism of this species.

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