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Effect of long term exposure to high CO_2 concentrations on photosynthetic characteristics of *Prunus avium* L. plants

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Abstract

The effect of two elevated carbon dioxide concentrations, 700 μmol(CO_2) mol^{-1} (C700) and 1 400 μmol(CO_2) mol^{-1} (C1400), on photosynthetic performances of 1-year-old *Prunus avium* L. plant was studied. Plants grown at C700 were characterised by increased net photosynthetic rate (P_N) as compared to those grown at C1400. Plant photosynthetic adjustment to C1400 resulted in 27 % higher P_N than in control at atmospheric CO_2 concentration (C_a) at the beginning of the experiment (3-4 weeks) with a consequent decline to the end of the experiment. Thus, 1 400 μmol(CO_2) mol^{-1} had short-term stimulatory effect on plant P_N. Both chlorophyll (Chl) *a* and *b* concentrations dramatically decreased during exposure to C1400. Compensation irradiance was increased by 57 % in C700 and by 87 % in C1400. Photochemical efficiency (φ) was affected by balloon environment, however, a clear stimulatory effect of C700 was detected. Opposite influence of both elevated CO_2 concentrations on P_{Nmax} was established: slight increase by C700 (2.7 % at C_a), but considerable decrease by C1400 (63 % at C_a). Exposure to C700 enhanced compensation irradiance by 42 %, while C1400 by only 21 %. Either C700 or C1400 did not reduce stomatal conductance (g_s). Leaf area per plant (LAR) was more stimulated by C700 than by C1400. High unit area leaf mass, specific leaf area, and dry matter accumulation in roots without affecting tissue density characterised plants grown in C1400. However, when considering the root : shoot ratio, these plants allocated less carbon to the roots than plants from others treatments.

Additional key words: chlorophyll fluorescence; compensation irradiance; dry mass; growth; photochemical efficiency; stomatal conductance; sweet cherry.

Introduction

Plant structure and metabolism have been adapted for million years to a steady concentration of 300 μmol(CO_2) mol^{-1}. Nowadays, due to anthropogenic activities (emission from industrial and technical sources), the mean increase in atmospheric CO_2 concentration is 3-4 % per year (Crane 1985). From many predictions, the time for doubling of the CO_2 concentration has been put at around the third quarter of the 21st century. In spite of the enormous number of publications dedicated to this problem at present, the effect of high CO_2 concentration on plant metabolism is not yet clear. Plant species are probably specific in physiological response to high CO_2 concentrations (Zhang and Nobel 1996). Plant metabolism modifications depend also on the duration of exposure to elevated CO_2. In short term exposure (hours–days) switch from ambient to elevated CO_2 results in an increase of physiological activity, and thus high CO_2 concentration may have beneficial effect on plant growth and development (Long 1991, Harley *et al.* 1992). In long-term exposure experiments, plant response to high CO_2 is less clear. Some authors have reported increase in P_N (Barnes *et al.* 1995), while other studies show that the stimulation is not sustained over long period (Kramer 1981, Webber *et al.* 1994, Urban and Marek 1999). The magnitude of

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Abbreviations: C_a – control at atmospheric CO_2; C_{ab} – control at atmospheric CO_2 in the balloon; C700 – 700 μmol mol^{-1} CO_2 concentration; C_i – internal CO_2 concentration; C1400 – 1 400 μmol mol^{-1} CO_2 concentration; Chl – chlorophyll; g_s – stomatal conductance; K – carboxylation efficiency; lg – stomatal limitation; LAR – leaf area ratio; LMR – leaf mass ratio, P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; R:S – root:shoot ratio; UALM – unit area leaf mass; Γ – carbon dioxide compensation concentration; φ – photochemical efficiency.
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photosynthetic enhancement or inhibition varies with plasticity and maximum of photosynthetic characteristics of a species (Fordham *et al.* 1997).

Plant response to high CO_2 depends not only on time but also on concentration. A doubtful moment is as to what extent increased CO_2 concentration will enhance photosynthesis and what is the limiting factor for physiological processes. The objective of the present study was to determine long-term (60 d) effect of high CO_2 concentrations C700 and C1400 on growth and photosynthetic activity of sweet cherry trees (*Prunus avium* L.).

Materials and methods

Fifty 1-year-old sweet cherry trees (*Prunus avium* L.) cv. Winsdor grafted on Mazzard rootstocks were grown in 38 000 cm^3 plastic pots with sterilised greenhouse soil mix (5 sandy loam : 3 sphagnum peat : 2 torpedo sand). The trees were cut to an active bud and trained to 3 shoots. Soluble 20N-20P-20K fertiliser (500 g kg^{-1}) was applied every 2 weeks and the trees were watered as necessary. Five potted plants were placed in a woody ring with 122-cm diameter. Six similar balloons were made from clear polyethylene and put on the rings. Their heights were adjusted to plant growth. The plants were grown in controlled environment balloons supplied with CO_2 from a CO_2 tank. The concentration of CO_2 in the balloons was monitored and maintained at necessary concentration (depending on treatment) during experiment. Air enriched with CO_2 was blown into the balloons by blower fans and exited through holes at the top of the balloons. CO_2 concentration was measured at the centre of the balloon. The plants were grown at the ambient concentration [370 μmol(CO_2) mol^{-1}] and high-CO_2 conditions [700 and 1 400 μmol(CO_2) mol^{-1}] at natural irradiance. The control plants were grown outside. In order to detect the growth-balloon effect, ten plants (five per balloon) from two similar balloons with airflow but no added CO_2 were used as control. The experiments were conducted from 10 June to 10 August 1998, that is a period of over 60 d. After two weeks of acclimation to CO_2 concentrations, photosynthetic parameters were measured during the subsequent 46 d.

Gas exchange was measured using a portable photosynthesis system *LI-6400* (*CIRAS*, U.S.A.). The effect of CO_2 concentration on net photosynthetic rate (P_N) over time was determined at each measurement time for 2 plants from each balloon (4 replicates per treatment) and from outside as well. The measurements were done between 08:00 and 12:00 at natural irradiance and temperature.

The response of P_N to internal CO_2 concentration (C_i) was determined on days 46-57 following CO_2 treatment by increasing stepwise (10 min acclimation for each step) to the following concentrations: 0, 70, 100, 150, 200, 300, 500, 700, 900, 1 200, and 1 400 μmol(CO_2) mol^{-1}. The P_N *vs.* C_i response curves were measured on a fully expanded leaf by placing it into environmentally controlled cuvette under the following conditions: flow rate 3.42 cm^3 s^{-1}, vapour pressure deficit (VPD) –3 kPa, 370 μmol(CO_2) mol^{-1}, photosynthetic photon flux density active radiation (PPFD) 800 μmol m^{-2} s^{-1}, leaf temperature 28 °C, the whole plant being at treatment conditions. Gas exchange pa-rameters were calculated and non-linear regression model was fitted to each P_N response curve (Layne and Flore 1992):

$$y = b(1)\left[1 - b(2)e^{-b(3)x}\right]$$

The best-fit curves for the pooled P_N-C_i derived data at each CO_2 concentration are shown in Fig. 4. The gas exchange parameters were calculated using the *BASIC* computer program of Moon and Flore (1986). Stomata limitation to CO_2 assimilation was calculated from the individual P_N-C_i curves by the differential method (Jones 1985). The ratio of the slope of the demand and supply curve at the point where they intersect (the operating point) was also calculated. The carbon dioxide compensation concentration (Γ) was extrapolated from the P_N-C_i curve at the C_i at which P_N is zero. Carboxylation efficiency (K) was calculated from raw data in the linear portion of the slope of the P_N-C_i curve. The response of P_N to PPFD was measured on days 40 to 57 by increasing PPFD stepwise (5 min acclimation for each step) to the following levels: 0, 30, 70, 130, 180, 260, 500, 600, 800, 1 200, and 1 400 μmol m^{-2} s^{-1}. Photochemical efficiency (φ) was calculated from values in the linear portion between 0-200 μmol m^{-2} s^{-1} from the P_N *vs.* PPFD curve. The compensation and saturation irradiances were calculated from the irradiance response curves.

Leaves used for measuring gas exchange were collected for chlorophyll (Chl) analysis. Four discs (0.385 cm^2 each) were punched from the middle of the lamina of fresh leaf. The disc mass was recorded and Chl was extracted in 10 cm^3 of DMF (*N,N*-dimethylformamide) in darkness at 5 °C for 48 h. Absorbance of extract was read at 664, 647, and 625 nm on a UV/Vis spectrophotometer (*U-3110, Hitachi*, Tokyo, Japan). Calculation for Chl *a* and *b* was made according to Moran (1982).

Chl fluorescence was measured with portable fluorometer (*Hansatech Instruments*, Norfolk, UK) on 4 leaves of each treatment. Leaves were dark acclimated for 15 min by using dark acclimation cuvettes and then irradiated with 1 000 μmol m^{-2} s^{-1}.

At the end of the experiment, leaves were harvested and the leaf area per plant was determined destructively. In order to determine biomass increment during the experiment, five trees of each treatment were harvested after 60 d of experiment. The plant parts were dried for 48 h at 105 °C for dry mass determination. The density of woody tissue is its dry mass per fresh volume [g cm^{-3}].

Results

P_N of individual leaves showed significant differences depending on treatment. During first two weeks of the experiment, plants grown at C_{ab} showed lower P_N than plants at C_a (perhaps due to the acclimation period), thereafter, they showed similar trend with close values. Plants under the C1400 treatment exhibited the highest P_N (27 % higher than C_a) from the beginning of the experiment, but this level could not be maintained for more than 4 weeks and consequently it declined to a lower rate with little fluctuations to the end of the experiment (Fig. 1).

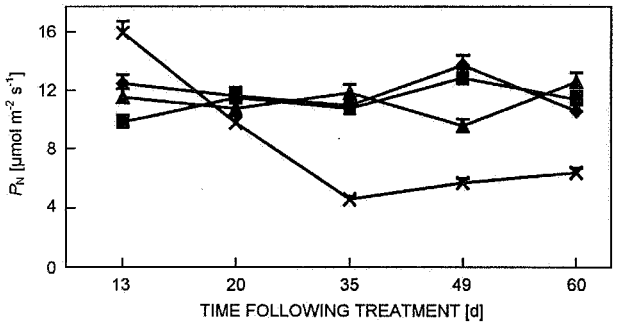

Fig. 1. The effect of CO_2 concentration on leaf net photosynthetic rate (P_N) of 1-year-old sweet cherry trees grown at different CO_2 concentrations. Means ± SE of at least 4 measurements. ◆ control, 370 μmol(CO_2) mol^{-1} (outside); ■ control, 370 μmol(CO_2) mol^{-1} (balloon); ▲ 700 μmol(CO_2) mol^{-1}; × 1 400 μmol(CO_2) mol^{-1}.

Plants at C700 showed increased P_N as compared with plants from the C1400 treatment (Fig. 1).

P_N *vs.* PPFD response curves were affected more by C1400 than by C700 and showed differences in calculated parameters depending on treatment (Fig. 2, Table 1). Plants grown in double atmospheric CO_2 behaved similar to C_a plants in compensation irradiance, however, considering balloon effect (34 % decreased), compensation irradiance of plants at C700 was increased by 57 %. C1400 increased plant compensation irradiance by 87 % as compared to the C_{ab}, while their φ was decreased by 26 % (Table 1). This parameter was affected by balloon environment (20 % decreased), however, a clear stimulatory influence of C700 was detected (21 % higher C_{ab}). Saturation irradiance was decreased by C1400 concentration (Table 1). Opposite influence of elevated CO_2 concentrations on P_{Nmax} was recorded: slightly increased by C700 (2.7 % at C_{ab}), but considerably decreased by C1400 (63 % at C_{ab}).

For all treatments, the dependence of P_N on C_i was a simple hyperbola (Fig. 3). The values fit well the model equation used to predict the response of P_N to CO_2 enrichment.

Plants grown at elevated CO_2 exhibited significant increase in Γ (Table 2), with differences between C700 (42 % higher C_{ab}) and C1400 (21 % higher C_{ab}) treatments.

Table 1. The effect of elevated CO_2 concentrations on parameters derived from P_N *vs.* PPFD curves of 1-year-old sweet cherry trees after 47-50 d of exposure.

Treatment	Compensation irradiance [μmol(quantum) m^{-2} s^{-1}]	φ [μmol(CO_2) mol^{-1}]	P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	Saturation irradiance [μmol(quantum) m^{-2} s^{-1}]
C_a	46.60±5.59	0.049±0.004	14.11±3.213	800
C_{ab}	30.51±2.75	0.039±0.004	14.44±3.783	800
C700	47.95±5.28	0.047±0.005	14.83±5.091	800
C1400	56.94±7.97	0.029±0.004	9.10±4.223	500

Considering Γ an indicator of photorespiratory activity (Šesták 1985), plants treated with elevated CO_2 concentration had higher photorespiration rates than plants from ambient CO_2. P_N *vs.* C_i curves of plants grown at high CO_2 showed lower initial slope as compared with both controls, thus indicating a reduced efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) carboxylation (Table 2). Ratios of intercellular to ambient CO_2 concentration were more reduced in the leaves grown at ambient than high CO_2. However, CO_2 assimilation at C_a was decreased by the treatment: compared to C_a, P_{Nmax} was 22 % reduced by C700 and 24 % reduced by C1400. With increasing C_i (above 450 μmol mol^{-1}) plants in the control reduced their P_N. In contrast, plants

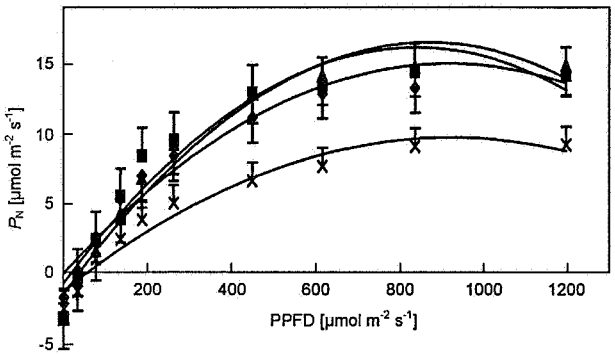

Fig. 2. Effect of CO_2 concentration on the response of net photosynthetic rate (P_N) to photosynthetic photon flux density (PPFD) of 1-year-old sweet cherry trees grown at different CO_2 concentrations. Means ± SE of at least 4 measurements. ◆ control, 370 μmol(CO_2) mol^{-1} (outside);■ control, 370 μmol(CO_2) mol^{-1} (balloon); ▲ 700 μmol(CO_2) mol^{-1}; × 1 400 μmol(CO_2) mol^{-1}.

at high CO_2, particularly at C1400, increased P_N up to 1 200 μmol mol^{-1}, indicating that in enriched CO_2 environment plants have relatively greater P_i regeneration capacity.

First determination of Chl was done after 13 d of experiment and only small differences in Chl *a* contents were apparent between C700 and C_a treatments at this time. Exposure of plants to C1400 decreased Chl concentration per dry mass and this trend persisted during the experiment (Fig. 4). At the final determination the concentration of Chl *a* was 51 % and of Chl *b* 54 % less than in C_a, being the lowest Chl content recorded during the experiment. The Chl *a/b* ratio was not affected much by treatments, however, plants raised at C1400 exhibited reduced value of this index at the end of the experiment, indicating Chl *a* degradation in leaves grown at this treatment.

Chl fluorescence parameters fluctuated and not all differences between treatments were significant (Fig. 5).

Table 2. The effect of high CO_2 concentrations on gas exchange parameters P_N at 37 Pa and P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}], Γ and C_i [μmol(CO_2) mol^{-1}], K , g_s, and lg at 37 Pa [mmol(CO_2) m^{-2} s^{-1}], derived from P_N *vs.* C_i response curves of fully expanded leaves of 1-year-old *Prunus avium* plants.

Treatment	Γ	K	P_N at 37 Pa	g_s at 37 Pa	$P_{N\,max}$	lg at 37 Pa	C_i at 37 Pa	C_i/C_a [%]
C_a	63.395+10.94	152.5	27.41	255.532	32.00	0.483	262	70.80
C_{ab}	93.091+5.38	264.0	25.23	181.814	30,50	0.291	270	72.97
C700	132.703+7.58	73.4	13.25	527.102	23.80	0.020	360	97.20
C1400	112.872+8.14	44.2	12.20	286.027	23.21	0.090	301	81.35

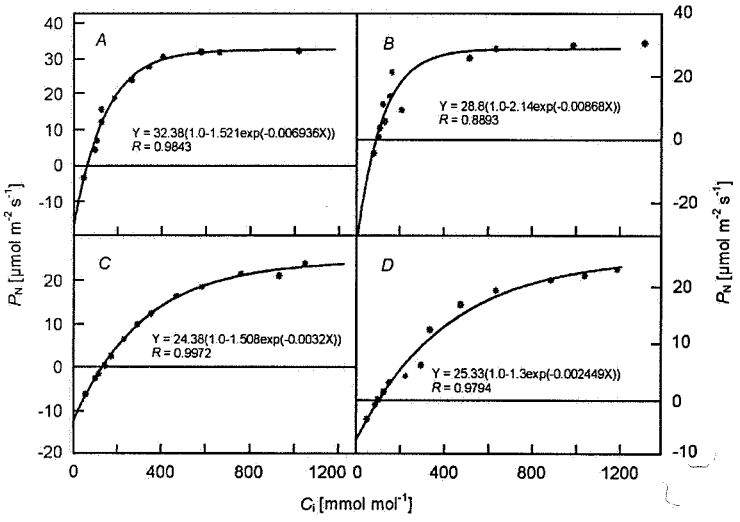

Fig. 3. The response of net photosynthetic rate (P_N) to internal CO_2 (C_i) of 1-year-old sweet cherry trees grown at different CO_2 concentrations. *A* – control, 370 μmol(CO_2) mol^{-1} (outside); *B* – control, 370 μmol(CO_2) mol^{-1} (balloon); C – 700 μmol(CO_2) mol^{-1}; D – 1 400 μmol(CO_2) mol^{-1}.

However, F_0 was highest in the leaves grown at C1400 at all sampling data. Plants at C700 exhibited lower F_0 than plants at C1400, but higher than the controls, particularly at the beginning and end of the experiment (Fig. 5). Plants under both elevated CO_2 concentrations showed a tendency to decreased F_v/F_m ratio.

Stomata conductance was influenced by the balloon effect, and plants from C_{ab} had higher transpiration rate (values not shown) than plants grown outside. There are no records of increased leaf temperature in the balloons,

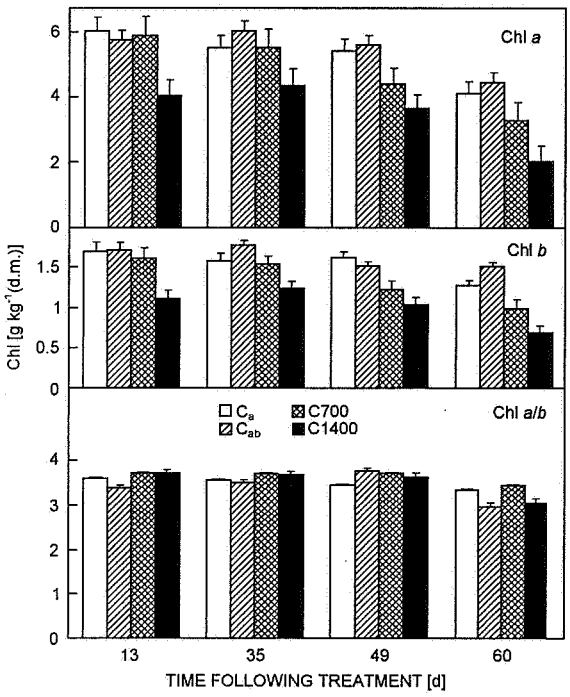

Fig. 4. Changes in chlorophyll (Chl) content of 1-year-old *Prunus avium* plants during 60-d exposure to different CO_2 concentrations. Means ± SE of at least 4 measurements. C_a – control, 370 μmol(CO_2) mol^{-1} (outside); C_{ab} – control, 370 μmol(CO_2) mol^{-1} (balloon); C700 – 700 μmol(CO_2) mol^{-1}; C1400 – 1 400 μmol(CO_2) mol^{-1}.

thus the high transpiration rate could be caused not by high temperature, but by permanent loss of water due to the airflow, which might have led to the increased g_s. Leaves grown in enriched CO_2, particularly at C1400, exhibited unstable g_s, however, a tendency of increasing it was evident. This trend was obvious in plants at C700, with the highest values of g_s. Plants grown at C1400 exhibited reduced g_s as compared with C700, but higher g_s compared to the control (Fig. 6).

Leaf g_s assessed from P_N-C_i curves showed that both controls increased g_s until $C_i = C_a$, then decreased it with increasing CO_2 (Fig. 7). Plants grown at C700 exhibited a higher g_s, however, the pattern was close to the controls: increased g_s up to 200 μmol mol^{-1} and consequent decline to C_i = C700. Concentrations higher than 600 μmol(CO_2) mol^{-1} had no effect on g_s of plants from this treatment. Plants grown under C1400 have enhanced g_s up to 1 000 μmol mol^{-1} with slow decrease at subsequent concentrations. Stomata limitation of plants grown in elevated carbon dioxide concentration was significantly reduced, thus increasing g_s (Table 2). Nonstomatal limitation was high, as was estimated from the reduction of K (27 % at C_{ab} for C700 and 17 % at C_{ab} for C1400).

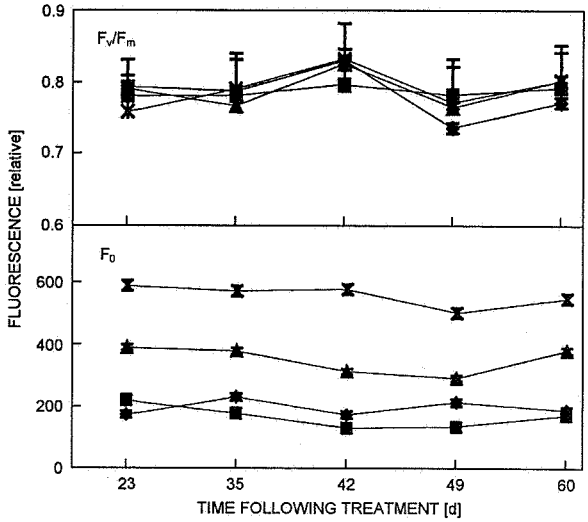

Fig. 5. Influence of 60-d growth at different CO_2 concentrations on F_0 and F_v/F_m chlorophyll fluorescence of 1-year old *Prunus avium* plants. Means ± SE of at least 5 measurements. ◆ control, 370 μmol(CO_2) mol^{-1} (outside); ■ control, 370 μmol(CO_2) mol^{-1} (balloon); ▲ 700 μmol(CO_2) mol^{-1}; × 1 400 μmol(CO_2) mol^{-1}.

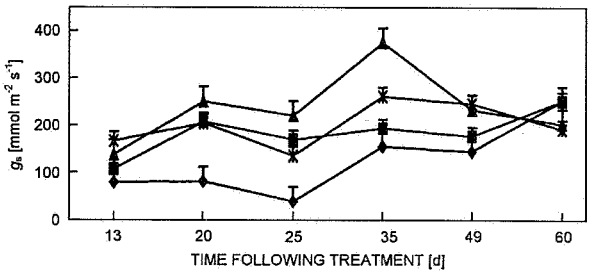

Fig. 6. Changes in stomatal conductance of 1-year-old *Prunus avium* plants during 60-d exposure to different CO_2 concentrations. Means ± SE of at least 3 measurements. ◆ control, 370 μmol(CO_2) mol^{-1} (outside); ■ control, 370 μmol(CO_2) mol^{-1} (balloon); ▲ 700 μmol(CO_2) mol^{-1}; × 1 400 μmol(CO_2) mol^{-1}.

Growth analysis revealed significant differences between plants depending on treatment. Plants treated with high CO_2 produced significantly more branches of second degree at the final sampling (Table 3). The lamina area showed no changes among treatments (values not shown), but the total leaf number differed between treatments due to differences in branch proliferation. Thus, leaf area per plant varied markedly with treatment, with the highest value for plants at C700.

The effect of C1400 was less apparent, but still stimulatory as compared to C_a (67 % higher) and C_{ab} (23 % higher).

Low values of LAR for plants grown at C1400 were

not due to reduced leaf area (50 % higher than at C_a), but to increased biomass, which makes leaf activity efficient. C700 stimulated more leaf area (67 % higher than at C_a) than total biomass (20 % higher than at C_a) and LAR of this treatment was larger (Table 3).

For better understanding of production process in elevated CO_2 concentration, we studied dry matter accumulation and partitioning between organs. The pattern of leaf dry matter accumulation was modified by high CO_2, thereby increasing the rate over the experiment (Fig. 8). The influence of C700 on unit area leaf mass (UALM) was apparent at the first determination (after 2 weeks), followed by a decrease preserved toward the end of the experiment. The largest increase in UALM was observed after 4 weeks of C1400, with a consequent decline, but it was still high as compared to the control (Fig. 8).

The highest accumulation of root dry matter was recorded in plants under C1400 treatment. They accumulated 56 % more biomass than C_a and C_{ab} plants, however, when considering carbon balance of plants by the R : S ratio, plants grown at C1400 allocated less carbon to the roots than plants from C_a and C700 treatments. Root tissue density was not affected by this treatment (Table 3). Plants grown in C700 exhibited no significant difference in total biomass accumulation as compared with C_a, but higher than at C_{ab}. This treatment did not increase tissue density of sweet cherry trees neither for trunk and roots.

Table 3. Growth and biomass parameters of 1-year-old sweet cherry trees grown at different CO_2 concentrations.

Parameter	C_a	C_{ab}	C700	C1400
Branch number (1st degree)	2.00 ± 0.38	2.00 ± 0.41	2.00 ± 0.19	2.00 ± 0.47
Branch length (1st degree) [cm]	43.90 ± 3.44	58.31 ± 3.04	58.60 ± 5.27	59.10 ± 6.50
Branch number (2nd degree)	2.00 ± 0.14	1.00 ± 0.15	4.25 ± 0.71	3.50 ± 0.55
Branch length (2nd degree) [cm]	18.41 ± 2.14	26.49 ± 3.96	29.30 ± 2.73	26.20 ± 2.48
Leaf number	44.81 ± 9.09	69.25 ± 10.46	110.60 ± 6.76	113.00 ± 12.35
Root/shoot ratio	1.02	0.78	2.48	0.96
Above part/root ratio	1.476	2.073	1.832	1.670
Total biomass [g(dry mass)]	144.57 ± 13.25	150.99 ± 8.49	173.48 ± 21.14	205.22 ± 16.98
LAR [m^2 kg^{-1}(plant)]	2.78 ± 0.14	3.41 ± 0.14	3.86 ± 0.21	2.94 ± 0.18
SLA [m^2 kg^{-1}(leaf)]	136.72 ± 8.20	135.83 ± 9.51	136.11 ± 12.25	123.31 ± 13.56
LMR [kg(leaf) kg^{-1}(plant)]	0.20 ± 0.01	0.27 ± 0.01	0.28 ± 0.02	0.24 ± 0.02
Trunk cross section [mm^2]	59.69 ± 3.01	74.47 ± 4.23	86.54 + 7.18	102.74 ± 5.98
Trunk tissue density [g cm^{-3}]	0.48 ± 0.02	0.43 ± 0.03	0.41 ± 0.03	0.45 ± 0.03
Root tissue density [g cm^{-3}]	0.26 ± 0.01	0.20 ± 0.01	0.19 ± 0.02	0.24 ± 0.02

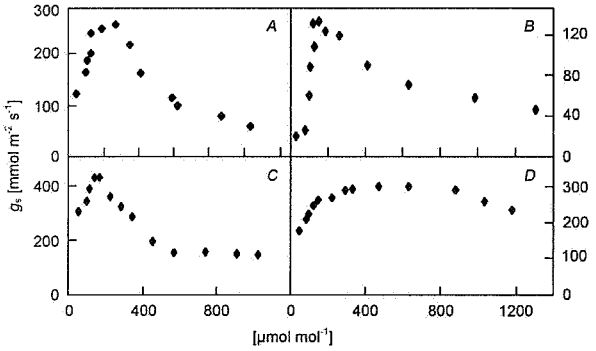

Fig. 7. Stomatal conductance (g_s) of 1-year-old *Prunus avium* leaves exposed to different CO_2 concentrations. *A* – control, 370 μmol(CO_2) mol^{-1} (outside); *B* – control, 370 μmol(CO_2) mol^{-1} (balloon); *C* – 700 μmol(CO_2) mol^{-1}; *D* – 1 400 μmol(CO_2) mol^{-1}.

The R : S ratio was significantly higher than for all other treatments and indicated that plants grown at this concentration allocated more carbon to the root at the expenses of shoots. Plants at C1400 had the highest cross-section area (71 % more than C_a and 37 % more than C_{ab}). C700 concentration also contributed to increment of trunk cross-section area (44 % more than C_a and 16 % more than C_{ab}). The relationships between treatments differed when considering trunk tissue density. The lowest density was recorded at C700 (85 % of that at C_a), at C1400 the trunk tissue density was increased, yet less when considering tissue density of plants grown outside (94 %).

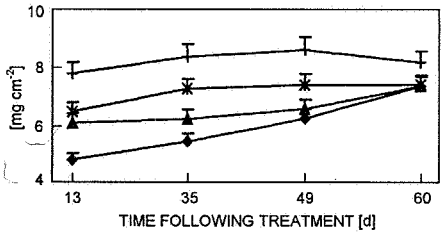

Fig. 8. The effect of 60-d growth at different CO_2 concentrations on unit area leaf mass (UALM) of 1-year-old *Prunus avium* of fully expanded leaf. Means ± SE of at least 4 measurements. ◆ control, 370 μmol(CO_2) mol^{-1} (outside); ■ control, 370 μmol(CO_2) mol^{-1} (balloon); ▲ 700 μmol(CO_2) mol^{-1}; × 1 400 μmol(CO_2) mol^{-1}.

Discussion

Our results show high sensitivity of 1-year-old sweet cherry trees to CO_2 concentration. Depending on treatment, plant response differed and varied during the experiment. During the first 3-4 weeks after transfer to enriched CO_2, both C700 and C1400 concentrations activated plant growth and photosynthesis. P_N rapidly increased with leaves operating at their maximum capacity after 3 weeks of treatment, particularly for plants at C1400. This time coincided with the period of photosynthetic apparatus formation, characterised by high photosynthetic activity as could be seen in the control plants. Chmora and Mokronosov (1994) relate such activation to intensification of nitrogen metabolism in the leaves. Especially C1400 markedly increased the rate of synthesis of some amino acids. Long-term enhanced CO_2 concentration declined N content in pine needles (Gielen *et al.* 2000). During the next 30 d, P_N varied considerably with CO_2 exposure. P_N for plants grown at C1400 declined to a lower constant rate per unit leaf area. Considering this situation, increased P_N during 3-4 weeks could be interpreted in terms of high CO_2 induced transient activation of photosynthesis as a stress response (Lichtenthaler 1996), while long-term exposure of sweet cherry trees resulted in a significant decrease of P_N. Plant growth at C700 did not show depressed P_N. Moreover, at several measurements a slight increase of P_N was recorded.

According to Harley *et al.* (1992), g_s decreases in elevated CO_2. Of course, these effects depend on water supply (Palanisamy 1999). In our experiments there were no depressing effects on g_s either by C700 or C1400 concentrations, however, there were fluctuations during the experiment. Unstable behaviour of physiological parameters could be explained by transitory state of plant organism under high CO_2 preceding another stable level of plant metabolism. The degree of responsiveness of g_s in two high CO_2 treatments differed. C700 stimulated g_s more than C1400. Established g_s values tended to be preserved during the experiment and at many measuring data, enhanced g_s was associated with high P_N. Differences in plant growth conditions led to a different stomata response when comparing g_s *vs.* C_i. Plants from ambient CO_2 (both controls) exhibited a typical response to increasing CO_2 concentrations (high g_s up to the operating point, high P_N followed by reduced g_s with increasing CO_2, and no influence of CO_2 concentration on P_N after saturation concentration was achieved). Plants grown in C700 exhibited maximum g_s at C_i = 200-300 μmol mol^{-1}, however, P_N was low. With increasing CO_2 concentration g_s decreased (still being higher than for controls), but P_N showed a pronounced stimulation by C_i = 700-1 000 μmol mol^{-1}, at which concentrations plants from atmospheric CO_2 did not respond. g_s of plants grown in C1400 positively responded to C_i = 0-1 200 μmol mol^{-1}, however, it was reduced when compared with controls. Low initial slope of P_N-C_i curve for plants grown under high CO_2 indicates a lower activation of RuBPCO (Sage *et al.* 1989). Plants at this treatment did not reach saturation, indicating that P_i regeneration capacity increased relative to RuBP-regeneration. Sage *et al.* (1988) suggested that this pattern might not reflect the acclimation, but excess of starch accumulation and subsequent distortion of the chloroplasts that causes a stress response.

Stomata limitation of plants raised in high CO_2 was low and thus P_N was reduced by mesophyll limitation mainly due to RuBPCO activity, as its carboxylation efficiency calculated from P_N-C_i curves was low compared with both controls. Adam *et al.* (1997) interpreted this situation as an indicator of down regulation in which the amount/activity of RuBPCO is decreased in response to greater concentration of carbon dioxide. Sage *et al.* (1988) consider that RuBPCO is partially deactivated in plants not fully acclimated to high CO_2. RuBPCO deactivation can occur under elevated CO_2 as a result of Mg^{2+} binding to starch grains (Plaut *et al.* 1987). According to van Oosten *et al.* (1994) and Cheng *et al.* (1998) RuBPCO synthesis requires coordinated expression between the nucleus and chloroplast genomes and growth at high CO_2 may disrupt the homeostatic control of RuBPCO protein transcript expression.

Photosynthetic apparatus of sweet cherry leaves was severely affected by long-term exposure to C1400 and Chl synthesis evidenced this phenomenon. During the first stimulatory period of the experiment, Chl amount was increased, later it declined, with minimal values at the end of the experiment. Keutgen *et al.* (1997) relate Chl amount decline with nitrogen deficiency that limits the availability of amino acids and synthesis of the enzymes. On the other hand, Knee (1991) argues that Chl degrada-tion is a clear indication of leaf senescence processes during long-term exposure to high CO_2. In our experi-ment, after 50-60 d of plant growth at C1400, red pig-mentation appeared on leaves. Righetti *et al.* (1996) con-sider this phenomenon as a biochemical alteration in *P. avium* plants. The decrease in P_N and the drop in Chl and ß-carotene contents are attributed to the reduction of ef-fectiveness of protective mechanism against oxidation due to the formation of active oxygen species that affect pho-tosynthetic enzymes, including peroxidation of lipids and bleaching of pigments. C1400 concentration affected not only the total amount of Chl, but Chl *a/b* ratio as well. According to Keutgen *et al.* (1997) the decrease in Chl *a/b* ratio may reflect the damage of photosystem 2 (PS2) core complex. Wilkins *et al.* (1994) found a decrease of D1 and D2 in PS2 core complex during long-term exposure to high CO_2 in *P. avium*. In our experiment CO_2 enrichment led to the increase in F_0 that along with reduced

Chl amount is an indicator of structural damage of PS2 and not all reaction centres opened for primary chemistry.

According to Caemmerer and Farquhar (1981), P_N at C_i = 250-600 μmol mol^{-1} is limited by the capacity for RuBP regeneration, and decreased P_N for plants under high CO_2 is partly attributed to a lower Chl content that can reduce light harvesting. This situation was typical for plants grown in C1400, their quantum efficiency of radiant energy transduction was decreased by 41 % as compared to C_a and was accompanied by decreased capacity for PPFD-saturated photosynthesis. Photochemical conversion efficiency of PS2 (measured as F_v/F_m) decreased during the experiment. A partial inactivation of PS2 centres could protect the remaining PS2 centres from photodestruction (Lichtenthaler 1996). A slow increase of PPFD-saturated P_N of plants grown at C700 as compared with plants in the controls was found. The same effect with increasing CO_2 was established by Silvola and Ahlholm (1992) in willow plants.

Elevated concentration of CO_2 affected growth parameters, dry matter accumulation, and its partitioning between organs. C700 stimulated growth throughout the experiment, plants showed higher LAR. High CO_2 stimulated branch proliferation and the number of leaves per plant, respectively, however SLA of plants grown in C1400 was considerably decreased due to higher increase in total biomass. Poorter (1994) explained low SLA mainly in terms of higher contents of lignin organic N-compounds, total non-structural saccharides, hemicellulose, and organic acids expressed per unit leaf area. In our experiment, applied high CO_2 concentrations stimulated allocation of more biomass to leaves as was established by UALM. According to Poorter *et al.* (1997) this pronounced increase in UALM is due to changes in leaf chemical composition, mainly due to the accumulation of total non-structural saccharides. Despite low P_N during the second month of the experiment, plants in C1400 preserved the highest UALM and only at the final record date they showed decrease in UALM. This decline might have been caused by senescing of the leaf tissues. C1400 stimulated total dry biomass accumulation. Steady increase of dry matter when P_N declines after the initial growth is a common physiological response to high CO_2 concentration (Mott 1990, Righetti *et al.* 1996, Atkinson *et al.* 1997). Van der Werf (1996) considers that high carbon gain per plant is attributed not to high LMR or P_N, but to increased SLA, which is in accordance with our results. 60-d growth at different CO_2 concentrations led to a different biomass partitioning between organs. The highest accumulation of root dry mass was recorded for plants at C1400, however, the root/shoot ratio was lower than in plants at C700. Mousseau and Saugier (1992) relate the increase in root investment in trees at elevated CO_2 to a poor mineral status. In our experiments large pots were used to prevent root growth restriction, however, 60-d experiment under C1400 stimulated root growth and pot size could constrain their volume. This could shift C allocation from roots to trunk and branches. Plants from C1400 enlarged their trunk cross-section area, but preserved high tissue density. According to Keutgen *et al.* (1997) decreased concentration/activity of RuBPCO indicates reduced N content in plant. Considering the reduced RuBPCO activity for plants in high CO_2, our plants probably had a lack of N that could constrain root development even if plants were fertilised regularly.

We found that sweet cherry tree response to elevated CO_2 changed during the experiment and depended on CO_2 concentration. 60-d growth in high CO_2 may be a long-term exposure, however, in tree life scale this is not a long one. Many physiological parameters were not totally acclimated to high CO_2 and showed fluctuations. High standard deviations of some parameters of plants grown in C1400 could in this case evidence a transitory state of organisms, which reacted unbalanced. Fast recovery of physiology of photosynthetic apparatus after the experiment ended is another evidence of the necessity to increase exposure time for trees to get plants totally acclimated to high CO_2 concentration.

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