Effect of long term exposure to high CO₂ concentrations on photosynthetic characteristics of Prunus avium L. plants

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Abstract

The effect of two elevated carbon dioxide concentrations, 700 μmol(CO₂) mol⁻¹ (C700) and 1 400 μmol(CO₂) 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 (Pₙ) as compared to those grown at C1400. Plant photosynthetic adjustment to C1400 resulted in 27 % higher Pₙ than in control at atmospheric CO₂ concentration (Cₐ) at the beginning of the experiment (3-4 weeks) with a consequent decline to the end of the experiment. Thus, 1 400 μmol(CO₂) mol⁻¹ had short-term stimulatory effect on plant Pₙ. 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 CO₂ concentrations on Pₙₙ₉₉₉₉ was established: slight increase by C700 (2.7 % at Cₐ), but considerable decrease by C1400 (63 % at Cₐ). Exposure to C700 enhanced compensation irradiance by 42 %, while C1400 by only 21 %. Either C700 or C1400 did not reduce stomatal conductance (gₛ). 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(CO₂) mol⁻¹. Nowadays, due to anthropogenic activities (emission from industrial and technical sources), the mean increase in atmospheric CO₂ concentration is 3-4 % per year (Crane 1985). From many predictions, the time for doubling of the CO₂ 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 CO₂ concentration on plant metabolism is not yet clear. Plant species are probably specific in physiological response to high CO₂ concentra-

Received 1 March 2001, accepted 4 June 2001.
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Abbreviations: Cₐ - control at atmospheric CO₂; Cₐ - control at atmospheric CO₂ in the balloon; C700 - 700 μmol mol⁻¹ CO₂ concentration; Cₐ - internal CO₂ concentration; C1400 - 1 400 μmol mol⁻¹ CO₂ concentration; Chl - chlorophyll; gₛ - stomatal conductance; K - carboxylation efficiency; Iₛ - stomatal limitation; LAR - leaf area ratio; LMR - leaf mass ratio; Pₙ - net photosynthetic rate; PPDF - photosynthetic photon flux density; R:S - root:shoot ratio; UALM - unit area leaf mass; Φ - carbon dioxide compensation concentration; φ - photochemical efficiency.

Acknowledgements: This study was based upon work supported by Fulbright Program (U.S.A.). The author gratefully acknowledges Dr. James Flore and Dr. Sarah Breitckreut, Department of Horticulture, Michigan State University (U.S.A.), for assistance and help.
photosynthetic enhancement or inhibition varies with plasticity and maximum of photosynthetic characteristics of a species (Fordoam et al. 1997).

Plant response to high CO₂ depends not only on time but also on concentration. A doubtful moment is as to what extent increased CO₂ 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 CO₂ 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 cm² 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 wooden 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 CO₂ from a CO₂ tank. The concentration of CO₂ in the balloons was monitored and maintained at necessary concentration (depending on treatment) during experiment. Air enriched with CO₂ was blown into the balloons by blower fans and exited through holes at the top of the balloons. CO₂ concentration was measured at the centre of the balloon. The plants were grown at the ambient concentration [370 μmol(CO₂) mol⁻¹] and high-CO₂ conditions [700 and 1 400 μmol(CO₂) mol⁻¹] at natural irradiance. The control plants were grown outside. In order to detect the growth-ballooon effect, ten plants (five per balloon) from two similar balloons with airflow but no added CO₂ 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 CO₂ 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 CO₂ concentration on net photosynthetic rate (Pₚₙ) 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 Pₚₙ to internal CO₂ concentration (Cᵢ) was determined on days 46-57 following CO₂ 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(CO₂) mol⁻¹. The Pₚₙ vs. Cᵢ response curves were measured on a fully expanded leaf by placing it into environmentally controlled cuvette under the following conditions: flow rate 3.42 cm² s⁻¹, vapour pressure deficit (VPD) -3 kPa, 370 μmol(CO₂) 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 parameters were calculated and non-linear regression model was fitted to each Pₚₙ 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 Pₚₙ-Cᵢ derived data at each CO₂ 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 CO₂ assimilation was calculated from the individual Pₚₙ-Cᵢ 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 (Cᵣ) was extrapolated from the Pₚₙ-Cᵢ curve at the Cᵢ at which Pₚₙ is zero. Carboxylation efficiency (K) was calculated from raw data in the linear portion of the slope of the Pₚₙ-Cᵢ curve. The response of Pₚₙ 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 (qₚ) was calculated from values in the linear portion between 0-200 μmol m⁻² s⁻¹ from the Pₚₙ 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 cm² each) were punched from the middle of the lamina of fresh leaf. The disc mass was recorded and Chl was extracted in 10 cm³ 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 $C_4$ (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 $C_4$) 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 CO₂ concentration on leaf net photosynthetic rate ($P_N$) of 1-year-old sweet cherry trees grown at different CO₂ concentrations. Means ± SE of at least 4 measurements. ◆ control, 370 μmol(CO₂) mol⁻¹ (outside); ■ control, 370 μmol(CO₂) mol⁻¹ (balloon); ▲ 700 μmol(CO₂) mol⁻¹; × 1 400 μmol(CO₂) mol⁻¹.](image)

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 CO₂ behaved similar to $C_4$ 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 $\varphi$ 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 CO₂ concentrations on $P_{\text{Nmax}}$ 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 $C_4$ was a simple hyperbola (Fig. 3). The values fit well the model equation used to predict the response of $P_N$ to CO₂ enrichment.

Plants grown at elevated CO₂ exhibited significant increase in $\Gamma$ (Table 2), with differences between C700 (42 % higher $C_{ab}$) and C1400 (21 % higher $C_{ab}$) treatments.

Table 1. The effect of elevated CO₂ concentrations on parameters derived from $P_N$ vs. PPFD curves of 1-year-old sweet cherry trees after 47-50 d of exposure.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Compensation irradiance [μmol(quantum) m⁻² s⁻¹]</th>
<th>$\varphi$ [μmol(CO₂) mol⁻¹]</th>
<th>$P_{\text{Nmax}}$ [μmol(CO₂) m⁻² s⁻¹]</th>
<th>Saturation irradiance [μmol(quantum) m⁻² s⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_4$</td>
<td>46.60±5.59</td>
<td>0.049±0.004</td>
<td>14.11±3.213</td>
<td>800</td>
</tr>
<tr>
<td>$C_{ab}$</td>
<td>30.51±2.75</td>
<td>0.039±0.004</td>
<td>14.44±3.783</td>
<td>800</td>
</tr>
<tr>
<td>C700</td>
<td>47.95±5.28</td>
<td>0.047±0.005</td>
<td>14.83±5.091</td>
<td>800</td>
</tr>
<tr>
<td>C1400</td>
<td>56.94±7.97</td>
<td>0.029±0.004</td>
<td>9.10±4.223</td>
<td>500</td>
</tr>
</tbody>
</table>

Considering $\Gamma$ an indicator of photorespiratory activity (Šesták 1985), plants treated with elevated CO₂ concentration had higher photorespiration rates than plants from ambient CO₂. $P_N$ vs. $C_4$ curves of plants grown at high CO₂ 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 CO₂ concentration were more reduced in the leaves grown at ambient than high CO₂. However, CO₂ assimilation at $C_4$ was decreased by the treatment; compared to $C_{ab}$, $P_{\text{Nmax}}$ was 22 % reduced by C700 and 24 % reduced by C1400. With increasing $C_4$ (above 450 μmol mol⁻¹) plants in the control reduced their $P_N$. In contrast, plants
Fig. 2. Effect of CO₂ 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 CO₂ concentrations. Means ± SE of at least 4 measurements. † control, 370 μmol(CO₂) mol⁻¹ (outside); ▲ control, 370 μmol(CO₂) mol⁻¹ (balloon); ▲ 700 μmol(CO₂) mol⁻¹; × 1400 μmol(CO₂) mol⁻¹.

Table 2. The effect of high CO₂ concentrations on gas exchange parameters $P_N$ at 37 Pa and $P_{max}$ [μmol(CO₂) m⁻² s⁻¹], $Γ$ and $C_1$ [μmol(CO₂) mol⁻¹], $K$, $g_s$, and $I$ at 37 Pa [mmol(CO₂) m⁻² s⁻¹], derived from $P_N$ vs. $C_1$ response curves of fully expanded leaves of 1-year-old Prunus avium plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$Γ$</th>
<th>$K$</th>
<th>$P_N$ at 37 Pa</th>
<th>$g_s$ at 37 Pa</th>
<th>$P_{max}$</th>
<th>$I$ at 37 Pa</th>
<th>$C_1$ at 37 Pa</th>
<th>$C_1/C_0$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₐ</td>
<td>63.395 ± 10.94</td>
<td>152.5</td>
<td>27.41</td>
<td>255.532</td>
<td>32.00</td>
<td>0.483</td>
<td>262</td>
<td>70.80</td>
</tr>
<tr>
<td>C₂b</td>
<td>93.091 ± 5.38</td>
<td>264.0</td>
<td>25.23</td>
<td>181.814</td>
<td>30.50</td>
<td>0.291</td>
<td>270</td>
<td>72.97</td>
</tr>
<tr>
<td>C700</td>
<td>132.703 ± 7.58</td>
<td>73.4</td>
<td>13.25</td>
<td>527.102</td>
<td>23.80</td>
<td>0.020</td>
<td>360</td>
<td>97.20</td>
</tr>
<tr>
<td>C1400</td>
<td>112.872 ± 8.14</td>
<td>44.2</td>
<td>12.20</td>
<td>286.027</td>
<td>23.21</td>
<td>0.090</td>
<td>301</td>
<td>81.35</td>
</tr>
</tbody>
</table>

Fig. 3. The response of net photosynthetic rate ($P_N$) to internal CO₂ ($C_1$) of 1-year-old sweet cherry trees grown at different CO₂ concentrations. A – control, 370 μmol(CO₂) mol⁻¹ (outside); B – control, 370 μmol(CO₂) mol⁻¹ (balloon); C – 700 μmol(CO₂) mol⁻¹; D – 1400 μmol(CO₂) mol⁻¹.

However, $F_o$ was highest in the leaves grown at C1400 at all sampling data. Plants at C700 exhibited lower $F_o$ than plants at C1400, but higher than the controls, particularly at the beginning and end of the experiment (Fig. 5). Plants under both elevated CO₂ concentrations showed a tendency to decreased $F_o/F_m$ ratio.
Stomata conductance was influenced by the balloon effect, and plants from $C_{st}$ had higher transpiration rate (values not shown) than plants grown outside. There are no records of increased leaf temperature in the balloons, 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 $C_{i} = C_{st}$, 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 mol mol$^{-1}$ and consequent decline to $C_{i} = 700$. Concentrations higher than 600 mol 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 mol 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 $C_{st}$ for C700 and 17 % at $C_{st}$ 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 mol mol$^{-1}$ (outside); ■ control, 370 mol mol$^{-1}$ (balloon); ▲ 700 mol mol$^{-1}$; × 1 400 mol 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 $C_{st}$ (67 % higher) and $C_{st}$ (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. $C_700$ 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 $C_700$ 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 accumu-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$C_a$</th>
<th>$C_{ab}$</th>
<th>$C_700$</th>
<th>C1400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch number (1st degree) [cm]</td>
<td>2.00 ± 0.38</td>
<td>2.00 ± 0.41</td>
<td>2.00 ± 0.19</td>
<td>2.00 ± 0.47</td>
</tr>
<tr>
<td>Branch length (1st degree) [cm]</td>
<td>43.90 ± 3.44</td>
<td>58.31 ± 3.04</td>
<td>58.60 ± 5.27</td>
<td>59.10 ± 6.50</td>
</tr>
<tr>
<td>Branch number (2nd degree) [cm]</td>
<td>2.00 ± 0.14</td>
<td>1.00 ± 0.15</td>
<td>4.25 ± 0.71</td>
<td>3.50 ± 0.55</td>
</tr>
<tr>
<td>Branch length (2nd degree) [cm]</td>
<td>18.41 ± 2.14</td>
<td>26.49 ± 3.96</td>
<td>29.30 ± 2.73</td>
<td>26.20 ± 2.48</td>
</tr>
<tr>
<td>Leaf number</td>
<td>44.81 ± 9.09</td>
<td>69.25 ± 10.46</td>
<td>110.60 ± 6.76</td>
<td>113.00 ± 12.35</td>
</tr>
<tr>
<td>Root/shoot ratio</td>
<td>1.02</td>
<td>0.78</td>
<td>2.48</td>
<td>0.96</td>
</tr>
<tr>
<td>Above part/root ratio</td>
<td>1.476</td>
<td>2.073</td>
<td>1.832</td>
<td>1.670</td>
</tr>
<tr>
<td>Total biomass [g (dry mass)]</td>
<td>144.57 ± 13.25</td>
<td>150.99 ± 8.49</td>
<td>173.48 ± 21.14</td>
<td>205.22 ± 16.98</td>
</tr>
<tr>
<td>LAR [m$^2$ kg$^{-1}$ (plant)]</td>
<td>2.78 ± 0.14</td>
<td>3.41 ± 0.14</td>
<td>3.86 ± 0.21</td>
<td>2.94 ± 0.18</td>
</tr>
<tr>
<td>SLA [m$^2$ kg$^{-1}$ (leaf)]</td>
<td>136.72 ± 8.20</td>
<td>135.83 ± 9.51</td>
<td>136.11 ± 12.25</td>
<td>123.31 ± 13.56</td>
</tr>
<tr>
<td>LMR [kg (leaf) kg$^{-1}$ (plant)]</td>
<td>0.20 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Trunk cross section [mm$^2$]</td>
<td>59.69 ± 3.01</td>
<td>74.47 ± 4.23</td>
<td>86.54 ± 7.18</td>
<td>102.74 ± 5.98</td>
</tr>
<tr>
<td>Trunk tissue density [g cm$^{-1}$]</td>
<td>0.48 ± 0.02</td>
<td>0.43 ± 0.03</td>
<td>0.41 ± 0.03</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>Root tissue density [g cm$^{-1}$]</td>
<td>0.26 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.24 ± 0.02</td>
</tr>
</tbody>
</table>

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. 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$^{-1}$.

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 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$^{-1}$. 294
Discussion

Our results show high sensitivity of 1-year-old sweet cherry trees to CO₂ concentration. Depending on treatment, plant response differed and varied during the experiment. During the first 3-4 weeks after transfer to enriched CO₂, both C700 and C1400 concentrations activated plant growth and photosynthesis. Pₙ 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 CO₂ concentration declined N content in pine needles (Gislen et al. 2000). During the next 30 d, Pₙ varied considerably with CO₂ exposure. Pₙ for plants grown at C1400 declined to a lower constant rate per unit leaf area. Considering this situation, increased Pₙ during 3-4 weeks could be interpreted in terms of high CO₂ induced transient activation of photosynthesis as a stress response (Lichtenhaler 1996), while long-term exposure of sweet cherry trees resulted in a significant decrease of Pₙ. Plant growth at C700 did not show depressed Pₙ. Moreover, at several measurements a slight increase of Pₙ was recorded.

According to Harley et al. (1992), gₛ decreases in elevated CO₂. Of course, these effects depend on water supply (Palanisamy 1999). In our experiments there were no depressing effects on gₛ 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 CO₂ preceding another stable level of plant metabolism. The degree of responsiveness of gₛ in two high CO₂ treatments differed. C700 stimulated gₛ more than C1400. Established gₛ values tended to be preserved during the experiment and at many measuring data, enhanced gₛ is associated with high Pₙ. Differences in plant growth conditions lead to a different stomata response when comparing gₛ vs. Cₚ. Plants from ambient CO₂ (both controls) exhibited a typical response to increasing CO₂ concentrations (high gₛ up to the operating point, high Pₙ followed by reduced gₛ, with increasing CO₂, and no influence of CO₂ concentration on Pₙ after saturation concentration was achieved). Plants grown in C700 exhibited maximum gₛ at Cₚ = 200-300 μmol mol⁻¹, however, Pₙ was low. With increasing CO₂ concentration gₛ decreased (still being higher than for controls), but Pₙ showed a pronounced stimulation by Cₚ = 700-1 000 μmol mol⁻¹, at which concentrations plants from atmospheric CO₂ did not respond. gₛ of plants grown in C1400 positively responded to Cₚ = 0-1 200 μmol mol⁻¹, however, it was reduced when compared with controls. Low initial slope of Pₙ-Cₚ curve for plants grown under high CO₂ indicates a lower activation of RuBPSCO (Sage et al. 1989). Plants at this treatment did not reach saturation, indicating that Pₙ 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 CO₂ was low and thus Pₙ was reduced by mesophyll limitation mainly due to RuBPSCO activity, as its carboxylation efficiency calculated from Pₙ-Cₚ 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 RuBPSCO is decreased in response to greater concentration of carbon dioxide. Sage et al. (1988) consider that RuBPSCO is partially deactivated in plants not fully acclimated to high CO₂. RuBPSCO deactivation can occur under elevated CO₂ as a result of Mg²⁺ binding to starch grains (Plaut et al. 1987). According to van Oosten et al. (1994) and Cheng et al. (1998) RuBPSCO synthesis requires coordinated expression between the nucleus and chloroplast genomes and growth at high CO₂ may disrupt the homeostatic control of RuBPSCO 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 CO₂. In our experiment, 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 Pₙ and the drop in Chl and β-carotene contents are attributed to the reduction of eff-ectiveness of protective mechanism against oxidation due to the formation of active oxygen species that affect pho-to-synthetic 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 CO₂ in P. avium. In our experiment CO₂ enrichment led to the increase in Fₚ 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 $C_i = 250-600 \, \mu\text{mol mol}^{-1}$ is limited by the capacity for RuBP regeneration, and decreased $P_N$ for plants under high CO$_2$ was 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 $F_r/F_m$) decreased during the experiment. A partial inactivation of PS2 centres could protect the remaining PS2 centres from photodestruction (Lichtenenthaler 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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