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Increase of photosynthesis and starch in potato under elevated CO_2 is dependent on leaf age

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Summary

Potato plants (*Solanum tuberosum* cv. Bintje) were grown in open top chambers under ambient (400 $\mu L\,L^{-1}$) and elevated CO_2 (720 $\mu L\,L^{-1}$). After 50 days one half of each group was transferred to the other CO_2 concentration and the effects were studied in relation to leaf age (old, middle-aged and young leaves) in each of the four groups. Under long-term exposure to elevated CO_2, photosynthesis increased between 10% and 40% compared to ambient CO_2. A subsequent shift of the same plants to ambient CO_2 caused a 20—40% decline in photosynthetic rate, which was most pronounced in young leaves. After shifting from long-term ambient to elevated CO_2, photosynthesis also increased most strongly in young leaves (90%); these experiments show that photosynthesis was downregulated in the upper young fully expanded leaves of potato growing long-term under elevated CO_2. Soluble sugar content in all leaf classes under long-term exposure was stable irrespective of the CO_2 treatment, however under elevated CO_2 young leaves showed a strongly increased starch accumulation (up to 400%). In all leaf classes starch levels dropped in response to the shift from 720 to 400 $\mu L\,L^{-1}$ approaching ambient CO_2 levels. After the shift to 720 $\mu L\,L^{-1}$, sucrose and starch levels increased, principally in young leaves. There is clear evidence that leaves of different age vary in their responses to changes in atmospheric CO_2 concentration.

Abbreviation: OTC, open top chamber

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Introduction

Earth's atmospheric CO_2 concentration (approx. 360 $\mu L\,L^{-1}$, Keeling et al., 1995) is rising mainly by burning of fossil fuels and land-use change in the tropics and may be doubled by the end of this century (Griffin and Seemann, 1996, Prentice et al., 2001). Such a drastic increase can be expected to have a strong impact on agriculture, silviculture and biomass production in general.

Under optimal conditions, an increase of 30–50% in biomass production has been observed during long-term exposure to a doubled CO_2-concentration as a result of enhanced photosynthesis (Cure and Acock, 1986). However, an initial boost of photosynthesis can subsequently be partially or fully down-regulated during long-term exposure to elevated CO_2 (Stitt, 1991). This photosynthetic acclimation has been defined as differing rates of photosynthesis for ambient and elevated CO_2-grown plants measured at the same CO_2 partial pressure (Long, 1991). Numerous reports postulate a link between down-regulation of photosynthesis and accumulation of leaf carbohydrates at elevated CO_2 (Stitt, 1991; Wolfe et al., 1998). It has been shown that accumulation of sugars in the mesophyll down-regulate the transcription of genes involved in photosynthesis (Moore et al., 1999), unbalance the triose phosphate/phosphate shuttle or impair carbohydrate processing in the cytosol by enzyme inhibition (Stitt, 1991).

Several recent studies of the effects of elevated atmospheric CO_2 on potato plants (Ludewig et al., 1998; Sicher and Bunce, 1999; Schapendonk et al., 2000)—like those of other crop plants—focus on photosynthesis acclimation and biomass production of fully expanded, just matured leaves in the upper canopy. We hypothesised that at elevated CO_2 acclimation of photosynthesis varies with leaf age and photosynthetic response could be related with the non-structural carbohydrate level of the leaf. In this assay, leaves of different age were studied, while an attempt was made to integrate several parameters of leaf carbohydrate management under short- and long-term exposure to elevated CO_2. We investigated effects of elevated CO_2 on photosynthesis rate and non-structural carbohydrate.

Material and methods

Plant material and growth conditions

Tubers of *Solanum tuberosum* L. cv. Bintje with single sprouts were planted at a depth of 5–10 cm in 15 L pots containing a mixture of sand and loam (1:1 vol) and were fertilised with NPK-potato fertiliser (180 $kg\,ha^{-1}$). Soil moisture was monitored daily using a portable TDR-tensiometer (TRIME-FM-2, IMKO, Ettlingen, Germany) and maintained above 70% of field capacity by manual irrigation. Standard procedures were used to control fungal infection and insect pests.

The plants were exposed during 5 weeks (May and June 1999) at the Institute of Plant Ecology, Justus-Liebig University Giessen, to either ambient CO_2 (approx. 400 $\mu L\,L^{-1}$) or elevated CO_2 (approx. 720 $\mu L\,L^{-1}$) in circular open top chambers (OTC, 5 chambers per treatment) of 3.15 m diameter and 2.40 m height (Fangmeier et al., 1992) (Fig. 1A). PAR conditions (Fig. 1B), air temperature (Fig. 1C) and relative humidity (Fig. 1D) were recorded inside the OTC continuously and stored as hourly means during the period of exposure.

After this 5-week period (called long-term exposure), half of the plants of either group were transferred (at 10:00 a.m.) from ambient to elevated CO_2, the other half vice versa; they were subsequently kept under the new conditions for 24 h (short-term exposure). Following gas exchange measurements, leaves from the four plant groups were collected 0, 1, 3, 6, and 24 h after transfer for biochemical analysis and dry matter determination. Whole plants were harvested at the same phenological stage of flowering and tuber formation (stages 6 and 4 according to Hack et al., 1993). Fully mature leaves of three different ages were taken for analysis from the same plant: "old" leaves, aged 5 weeks (5th leaf counted from the bottom), "middle-aged" leaves which were 3 weeks old (10th leaf from the bottom), and "young" leaves, $1\frac{1}{2}$ weeks of age (uppermost mature leaf). Leaf material was powdered in liquid N_2 and stored at $-80\,^{\circ}C$ for determination of non-structural carbohydrate content.

Photosynthetic CO_2 uptake

Photosynthesis rate was determined by a closed gas-exchange system (LI-6200, Li-Cor, Lincoln, NE, USA). Instantaneous gas-exchange measurements were made in the morning (10:00–12:00) on three different leaf age classes for the four CO_2 treatments (three plants per treatment), that is at 320 $\mu L\,L^{-1}$ for ambient CO_2 and 600 $\mu L\,L^{-1}$ for elevated CO_2 in the leaf measuring chamber, closely matching the respective growth chamber CO_2 conditions. All measurements were performed at light saturation ($Q = 1000-1200\,\mu mol\,m^{-2}\,s^{-1}$) and between 23 and 25 °C.

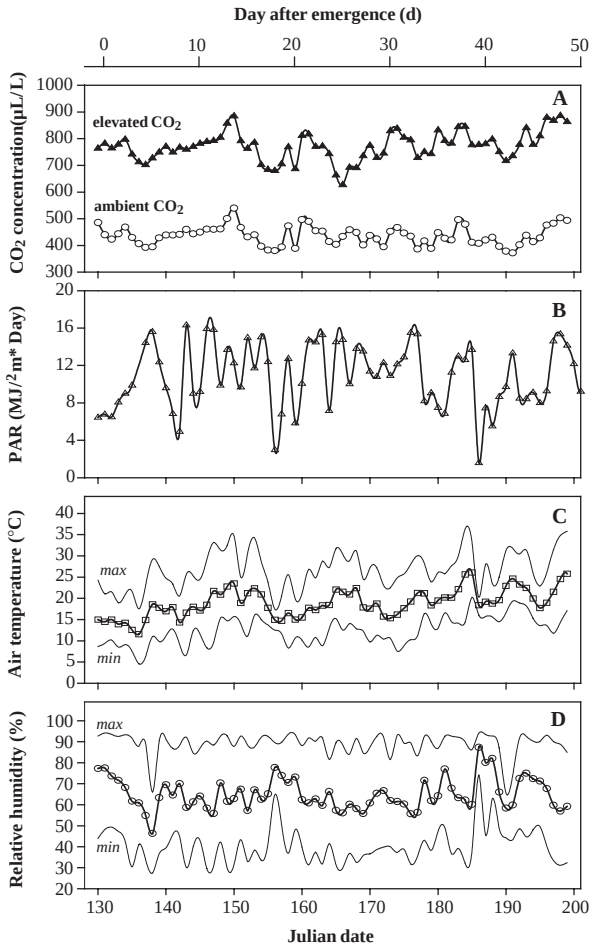

Figure 1. CO_2 concentration (A) in open top chambers as average of the five OTC of both CO_2 treatments (ambient or elevated); photosynthetic active radiation (PAR) (B), air temperature (C) and relative humidity (D) in the OTC during the experiment.

Leaf non-structural carbohydrate determination

Approximately 0.025 g of powdered frozen leaf material was weighed in 2 mL Eppendorf cups. After addition of Polyclar AT (Serva, Heidelberg, Germany), 1 mL of boiling millipore water was added. Samples were then immediately placed into boiling water for 10 min to stop enzyme activity, agitated for 10 min at room temperature on a table shaker (KL2, Bühler, Tübingen, Germany) at 420 oscillations per min, and then centrifuged for 2 min at 10,000*g* (Beckman microfuge TM). The supernatant was collected, and the pellet reextracted in 1 mL millipore water. After repeated centrifugation, the second supernatant was added to the first. The pellet was repeatedly washed with 2 mL millipore water and centrifuged, until it contained less than 2% of the soluble sugars. Soluble sugar content in the extracts was determined enzymatically using a Boehringer kit assay (Cat. No. 716260, Boehringer Mannheim, Germany) for sucrose, glucose and fructose. The amounts prescribed in the Boehringer manual were adapted to measurements of 200 µL in a microplate reader (Benchmark, Bio-Rad, Hercules, CA, USA) at 340 nm. The starch-containing pellets were digested overnight on a shaker in 0.5 mL amyloglucosidase-citrate/KOH buffer [0.2 mg amyloglucosidase (EC 3.2.1.3, Sigma, Deisenhofen, Germany) in 1 mL of 0.2 M citrate/KOH buffer, pH 4.6 (Sigma, Deisenhofen, Germany)]. The samples were centrifuged for 2 min at 10,000*g* (Beckman microfuge TM), and starch content was measured as glucose content in the supernatant which was analysed enzymatically using a D-glucose determination Kit (Cat. No. 207748 Boehringer, Mannheim, Germany) at 340 nm as described above.

Photosynthetic pigments

Approximately 0.025 g of powdered frozen leaf material was weighed in 2 mL Eppendorf cups and pigment extraction was carried out adding 2 mL of 80% cold acetone according to Lichtenhaler and Wellburn (1983). Chlorophyll *a*, *b* and carotenoids were spectrophotometrically measured at 663 and 646 nm wavelength.

Soluble protein content

Powdered frozen leaf material of 0.04–0.05 g was weighed in 2 mL Eppendorf cups. After extraction with 2 mL of 100 mM Hepes buffer pH 8.0, samples were boiled in water for 5 min and subsequently cooled down in ice. Soluble proteins were precipitated with 70% acetone, centrifuged at 10,000*g* (Beckman microfuge TM) and redisolved in 6 M urea in 5% acetic acid. Determination of soluble protein content was carried out spectrophotometrically at 595 nm according to Bradford (1976).

Statistical analysis

Significance was calculated by a Univariance analysis (SPSS 10.1 version). Mean differences between the factors were tested by LSD at $P<0.05(^{*})$, $P<0.01(^{**})$, or $P<0.001(^{***})$. Factors were tested on the effect of long-term exposure to ambient and elevated CO_2-concentration and short-term exposure with reference to leaf age.

Results

Growth features and photosynthesis rates of potato plants under ambient or elevated CO_2

Potato plants (cv. Bintje) were exposed to either ambient (approx. 400 $\mu L L^{-1}$) or elevated (approx. 720 $\mu L L^{-1}$) CO_2 for 5 weeks. Growth analysis data were obtained during harvest (Casanova Katny, 2002). Dry weight of leaves, stems and tubers were in the proportion of 2:1:8. Thus most of the photoassimilates were directed to tubers, which showed a significant increase in weight of approx. 25% ($P \leqslant 0.01$) in plants growing under elevated CO_2-concentration. No significant differences were observed for the leaf area and shoot length of plants under both CO_2 conditions (Casanova Katny, 2002).

At ambient CO_2 photosynthesis rate decreased with increase of leaf age, being about 40% higher in young leaves compared to old leaves. A strong increase of approx. 40% in photosynthesis rate under long-term exposure to elevated CO_2 was observed principally in old- and middle-aged leaves compared to the same leaf age classes at ambient CO_2 (Fig. 2). The univariance showed that different leaf classes and the CO_2-regime had a highly significant effect on photosynthesis rate ($P \leqslant 0.001$).

During the first hour after the shift from elevated to ambient CO_2, a significant ($P \leqslant 0.001$) decrease of 20–40% of photosynthesis rate was observed (measured at ambient CO_2) in all leaf age classes of plants growing long term under elevated CO_2; this lower level was still maintained after one day (Fig. 2). Contrarily, photosynthesis significantly ($P \leqslant 0.001$) increased after a shift from ambient to elevated CO_2 (Fig. 2). One hour after exposure to the new regime the increase was more pronounced in young (ca. 90%) than in old or middle-aged leaves (43% and 54%). After 1 day, the effects of transfer to another regime had become largely attenuated principally in the young leaves (Figs. 2D–F).

Soluble sugar content under long- or short-term exposure to elevated CO_2

Soluble carbohydrate content showed no marked differences between both CO_2 regimes in the respective leaf classes under long-term exposure (Table 1). Glucose and fructose content remained stable and similar between the different classes of leaf age under both CO_2 treatments (Table 1). Sucrose tended to be higher in middle-aged and young leaves under elevated CO_2 (Table 1). Our results indicate that pools of soluble sugar are not increased by long-term exposure to elevated CO_2.

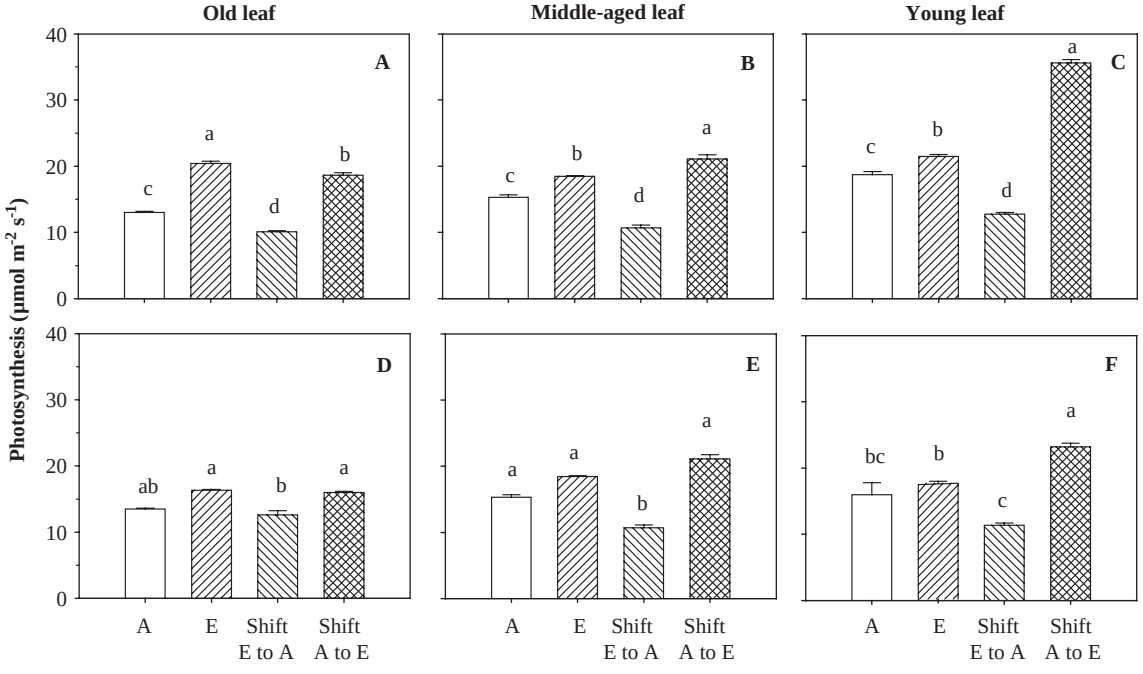

Figure 2. Photosynthesis rate under four different CO_2-regimes for three age classes of potato leaves. CO_2 effects on photosynthesis in plants at ambient CO_2 (A) or elevated (E) and after transfer from elevated to ambient (shift E to A) or from ambient to elevated CO_2 (shift A to E) after 1 h (A–C) and after 1 day (D–F); results are means ($\pm$SE) of three different plants of each CO_2-regime. Duncan test letters indicate significant differences between the means ($P \leqslant 0.05$).

Table 1. Non-structural carbohydrates content of three different classes of leaf age of potato plants growing under long-term exposure to ambient and elevated CO_2-concentration

	Long-term exposure to	
	Ambient CO_2	Elevated CO_2
Starch (mg g^{-1} DW)		
Old	23±4.8	30±4.7 ns
Middle-aged	34±6.7	145±13***
Young	38±6.4	137±11***
Sucrose (mg g^{-1} DW)		
Old	17±1.9	14±1.2 ns
Middle-aged	19±2.3	22±1.7 ns
Young	19±1.6	20±1.8 ns
Glucose (mg g^{-1} DW)		
Old	2±0.5	2±0.3 ns
Middle-aged	2±0.2	3±0.3 ns
Young	3±0.3	3±0.4 ns
Fructose (mg g^{-1} DW)		
Old	2±0.5	2±0.4 ns
Middle-aged	3±0.3	3±0.5 ns
Young	3±0.3	3±0.4 ns

Values are means±SE ($n = 3$). Samples were collected at 10:00 h. *P*-values show significance levels for the treatments, *** = $P \leqslant 0.001$; ns=not significant.

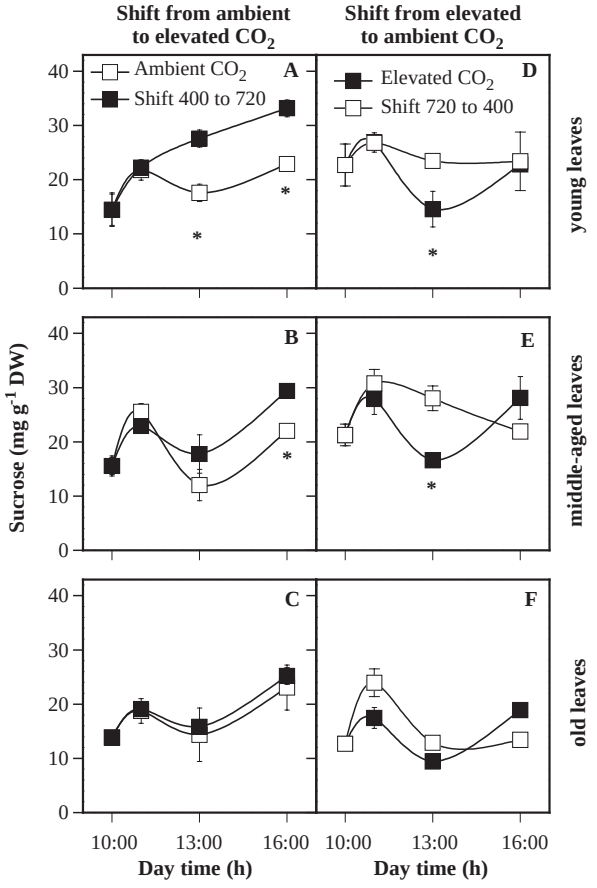

Figure 3. Short-term effects of shift from ambient to elevated CO_2 (400–720 μL L^{-1}) and from elevated to ambient CO_2 (720–400 μL L^{-1}) on sucrose content in three leaf age classes from plants grown under long-term exposure to ambient or elevated CO_2. Leaf samples were harvested at given time intervals; results are averages of three different plants ±SE. LSD *P* values show significant differences for $P \leqslant 0.05$(*).

In plants transferred from ambient to elevated CO_2, old leaves did not show a response to elevated CO_2 concentration for sucrose (Fig. 3C). Only the two younger leaf classes exhibited a pronounced reaction to the modified CO_2 regime: 3 h after transfer, sucrose content had increased by 47% and 57% in middle-aged and young leaves, respectively (Figs. 3A and B), but after 1 day the effect only persisted in the young leaves (Table 2). No significant increase was observed for glucose and fructose content during the day after the shift (Casanova Katny, 2002). One day after transfer to elevated CO_2, the levels of fructose and glucose had risen markedly only in young leaves (Table 2). Three hours after plant transfer from elevated to ambient CO_2 the sucrose level had increased (between 30% and 60%) in young and middle-aged leaves (Figs. 3D and E), but the responses disappeared after 6 h. A slight decrease of soluble sugars was observed for all age classes 1 day after transfer from elevated to ambient CO_2 (Table 2).

Starch content under long or short-term exposure to elevated CO_2

Leaves of plants growing permanently at ambient CO_2 showed a daily increase in starch content, with a midday depression and a maximum at the early evening (Figs. 4A–C). A similar starch profile was also found for old leaves at elevated CO_2 with a significant (35%) increase (Table 1). The two younger classes of leaf age in plants growing permanently under elevated CO_2 exhibited an atypical pattern with an approx. four-fold higher starch level than that at ambient CO_2 (significant, $P \leqslant 0.001$) (Table 1).

After transfer from ambient to elevated CO_2, starch had risen drastically in old (77%), middle-aged (115%) and young leaves (67%) in the early evening (Figs. 4A and B), with a typical daily pattern. One day after transfer, the increase had been maintained in all leaves being higher in the middle-aged (95%) and young leaves (110%) (Table 2). Three hours after transfer from elevated to ambient CO_2, the young and middle-aged leaves exhibited an increase in starch of 32% and

Table 2. Non-structural carbohydrates content in potato leaves of different age classes under long-term exposure compared with leaves after 1 day of short-term exposure either to elevated (shift from 400 to 720) or ambient CO_2-concentrations (shift from 720 to 400)

	Long-term exposure to		Short-term exposure	
	A 400	E 720	Shift 400–720	Shift 720–400
Starch (mg g^{-1} DW)				
Old	15±4.4	19±1.2 ns	24±6.2 ns	26±10 ns
Middle-aged	20±3.1	150±16***	38±5.8*	110±33 ns
Young	22±2.0	123±26***	47±2.1**	90±17 ns
Sucrose (mg g^{-1} DW)				
Old	15±1.5	13±0.7 ns	15±1.0 ns	11±2.0 ns
Middle-aged	16±2.7	19±1.0 ns	13±0.8 ns	14±2.0 ns
Young	16±1.2	19±1.9**	21±1.5*	16±0.2 ns
Glucose (mg g^{-1} DW)				
Old	1±0.3	1±0.3 ns	2±0.4 *	2±0.4 ns
Middle-aged	2±0.0	2±0.3 ns	3±0.3 ns	2±0.6 ns
Young	2±0.3	3±0.3 ns	4±1.0 ns	3±0.1 ns
Fructose (mg g^{-1} DW)				
Old	2±0.3	2±0.4 ns	3±0.7 ns	2±1.0 ns
Middle-aged	3±0.6	3±0.3 ns	3±0.5 ns	3±0.9 ns
Young	3±0.0	4±1.6 ns	4±1.0 ns	2±0.0 ns

Samples were taken at 10:00 h. Values are means±SE ($n = 3$). *P*-values show significance levels for the treatments, $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$; $^{***}P \leqslant 0.001$; ns=not significant.

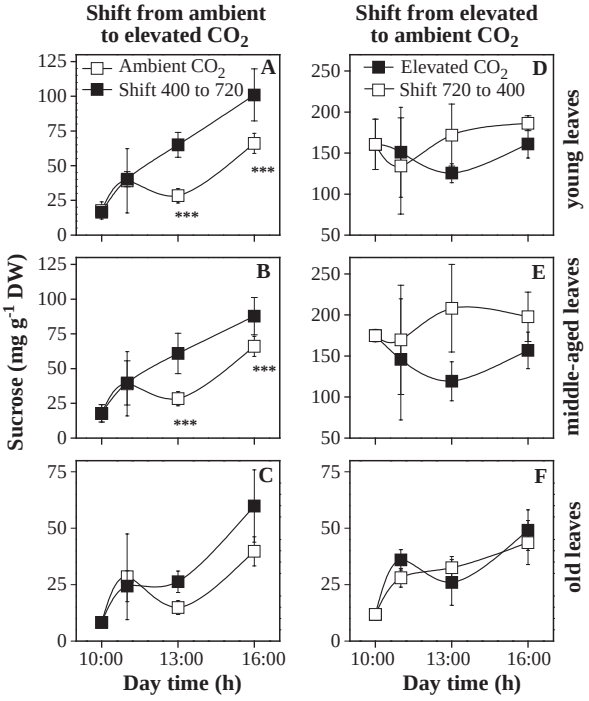

Figure 4. Short-term effects of a shift from elevated to ambient (720–400 μL L^{-1}), or from ambient to elevated CO_2 (400–720 μL L^{-1}) on starch content in three leaf age classes. Leaf samples were harvested at given time intervals. Results are averages of three different plants ±SE. LSD *P* values show significant differences for $P \leqslant 0.001$(***).

37%, respectively (Figs. 4D and E). The increase turned into a fractional decrease (26%) after 1 day. The starch content of old leaves did not show any significant reaction (Figs. 4C and F, Table 2).

Soluble protein and total chlorophyll content under long- and short-term exposure to elevated CO_2

No significant differences in soluble protein content between the treatments (long- and short-term exposure to elevated CO_2) were found, however, independently of the treatments, it could be observed that the differences between leaf age classes (middle-aged and young leaves versus old leaves) were markedly significant ($P \leqslant 0.01$). Values reached between 30 and 40 mg g^{-1} FW in all leaf classes, being highest in the young leaves (Table 3).

Total chlorophyll content (*a*+*b*) varied between 1.4 and 1.9 mg g^{-1} FW in all leaves ages, the differences after long-term exposure and short-term exposure to ambient and elevated CO_2 were not significant (Table 3). No changes were found between the different leaf age classes.

Table 3. Total chlorophyll and soluble protein content in potato leaves of different age classes under long- and short-term exposure either to elevated (shift from 400 to 720) or ambient CO_2-concentrations (shift from 720 to 400)

	Long-term exposure to		Short-term exposure	
	A 400	E 720	Shift 400–720	Shift 720–400
Total chlorophyll content (mg g^{-1} FW)				
Old	1.4±0.0	1.7±0.1 ns	1.7±0.1 ns	1.6±0.1 ns
Middle-aged	1.7±0.0	1.6±0.1 ns	1.9±0.1 ns	1.8±0.1 ns
Young	1.7±0.1	1.7±0.1 ns	1.8±0.1 ns	1.8±0.1 ns
Soluble protein content (mg g^{-1} FW)				
Old	31±0.8	31±5.5 ns	29±2.3 ns	28±2.4 ns
Middle-aged	37±2.6	28±2.6 ns	37±5.5 ns	28±2.4 ns
Young	41±1.4	35±2.7 ns	37±2.8 ns	37±4.7 ns

Samples were taken at 10:00 h. Values are means±SE ($n = 3$). *P*-values show significance levels for the treatments, ns=not significant.

Discussion

Increase of photosynthesis under elevated CO_2 has previously been reported for other C_3 crop species (Cure and Acock, 1986; Sicher and Bunce, 1999; Vandermeiren et al., 2002) but only few studies were focussed on differences between leaves of the same plant (Osborne et al., 1998; Turnbull et al., 1998; Backhausen and Scheibe, 1999). We observed the highest increase of photosynthesis rate in middle-aged and old leaves of plants under long-term exposure to elevated CO_2. Although these leaves may be shaded by the uppermost, young leaves, they assimilate the excessive CO_2 more efficiently than the latter. This effect might be attributed to the stimulation of light-limited photosynthesis due to the shading, according to the observations in old wheat leaves under 550 μL L^{-1} CO_2 (Osborne et al. 1998); these authors suggest that an increased carbon gain in the 7th and 8th leaf at elevated CO_2 under light limitation was the result of a lower light compensation point, allowing a net *C* gain during a longer period in the day (Long and Drake, 1991; Osborne et al., 1998).

The decrease in photosynthesis rate at ambient CO_2 measured during the first hour after transferring plants previously grown under long-term elevated CO_2 conditions, demonstrates a strong down regulation in the youngest mature leaves in the upper canopy (Fig. 2C); this acclimation effect of long-term exposure to high CO_2 persisted after 24 h, suggesting that these leaves cannot recover their normal activity level at ambient CO_2 within this period, photosynthetic deacclimation may need more time. Sicher and Bunce (2001) found in potato (cv. Atlantic) growing long-term under high CO_2 (720 μL L^{-1}) that the leaves need about 3 days after change to ambient CO_2 (360 μL L^{-1}) to remove the acclimation effect on photosynthesis. Dependency of photosynthetic acclimation on leaf age was also observed in *Pinus radiata* (Turnbull et al., 1998) and *Nicotiana tabacum* (Backhausen and Scheibe, 1999).

Changes in photosynthetic rates after moving plants from ambient to elevated CO_2 suggest that photosynthesis reaches a CO_2-related equilibrium after an overshoot reaction (Fig. 3). Clearly, the stimulation of photosynthetic rate after 1 h of exposure to elevated CO_2 was highest in the young leaves, decreasing after 24 h (Fig. 3F). Shift of plants from ambient to elevated CO_2 confirms the hypothesis of different responses of the three-leaf age's classes to the high CO_2-concentration. These differences cannot be attributed to enhanced leaf senescence, because photosynthetic pigments and soluble protein content were similar in both CO_2-regimes and between the leaf age's classes (Table 3). Sicher and Bunce (1999) observed photosynthetic acclimation in the upper expanded leaves of potato, together with a decrease of Rubisco activity during long-term exposure to elevated CO_2; in short-term experiments, the same authors found no significant changes in nitrogen content or Rubisco activity in potato at elevated CO_2 (Sicher and Bunce, 2001).

Long-term experiments show that homeostatic control on sugar metabolism is very strict in potato leaves of various age classes (Table 1), soluble sugar concentrations being similar at ambient and elevated CO_2, despite permanently higher photosynthesis rates under elevated CO_2. The effects of a change from ambient to elevated CO_2 were more dramatic than those after the reverse treatment. An increased CO_2 concentration resulted in a temporary rise of sucrose level in younger leaves, which was maintained for at least 1 day (Fig. 3,

Table 2). The effect of the reverse change (from elevated to ambient CO_2 concentration) on the sucrose content had disappeared after 1 day. The diurnal starch cycle (Fig. 4) showed an unusual slight midday depression, principally in leaves under ambient CO_2 which we cannot explain properly with our results. As has been documented extensively (Farrar and Williams, 1991; Moore et al., 1997; Allen et al., 1998), starch content is markedly higher in leaves under elevated CO_2 (Fig. 4). In our study, large differences, however, appeared between leaves of different age on the same plant: old leaves contained much less starch than both younger leaf classes, also differences between the CO_2 regimes were less distinct in old leaves. Contrarily in *Lycopersicum esculentum* (Yelle et al., 1989), *Ricinus communis* (Grimmer and Komor, 1999) and *N. tabacum* (Backhausen and Scheibe, 1999) starch content in older leaves was higher than in young ones at high CO_2.

In short-term experiments, leaf responses to elevated CO_2 can be totally different. In contrast to the study of Sicher and Bunce (2001), in our experiments elevated CO_2 considerably affected starch content in potato leaves after 1 day (Table 2). In coincidence with our observations (Table 2), starch decreased after 1 day, respectively 2 days in *Gossypium hirsutum* (Farrar and Williams, 1991) and *Plantago major* (Fonseca and Stulen, 2000) following a shift from elevated to ambient CO_2 concentration. Apparently, the response of starch level to the CO_2 shift strongly depends on plant species and developmental stage. Interestingly, several authors suggest a strong relation between starch accumulation and acclimation of photosynthesis: Sasek et al. (1985) and Sicher and Bunce (2001) observed that a decrease in leaf starch was correlated with the deacclimation of photosynthesis. Increase in leaf starch has been attributed to a feedback mechanism, which diminishes sucrose synthesis in the cytosol, and hence export of triose phosphate units from the chloroplast, stimulating starch accumulation in the chloroplast (Wolfe et al., 1998). The corresponding decrease of P_i in the chloroplast increases the ratio of 3PGA/P_i, which can activate the enzyme ADPglucose pyrophosphorylase (ADPGase) and thereby starch synthesis. Starch synthesis may have adaptative value as released P_i prevents initial ''end product inhibition'' of photosynthesis (Stitt, 1991).

Another possible explanation of photosynthetic acclimation was proposed after studies on leaf ontogeny under elevated CO_2 performed on *N. tabacum*: Miller et al. (1997) suggested a model, where the decrease of photosynthesis rate is achieved by a shift in the timing rather than in the magnitude of the photosynthesis. They found that photosynthesis rate under elevated and ambient CO_2 were similar, but in leaves under elevated CO_2 the maximal photosynthesis rate was reached several days before compared with leaves under ambient CO_2 concentration. These authors indicate that as the phase of photosynthesis decline is associated with progressive leaf senescence, the shift in timing of this photosynthetic phase may represent a temporal shift in the leaf senescence development pattern and that this process may be regulated with the sink/source balance, where the photosynthetic decline must be dependent on sink limitation. However, if photosynthetic acclimation to high CO_2 is a phenomen linked to leaf senescence which is genetically regulated (Quirino et al., 2000), the acclimation of photosynthesis should not be reversible; this is contrasted by reports on deacclimation of photosynthesis in several plant species after change from elevated to ambient CO_2 concentration (Sasek et al., 1985; Sicher and Bunce, 2001).

Experiments about the response of single, attached leaflets of soybean exposed to different CO_2 concentrations independently of the whole plant, showed strong effects on the carbohydrate content of the leaflets (Sims et al., 1998). However, the authors found that photosynthetic capacity and Rubisco content were unaffected by the individual leaflet treatment and instead was regulated on the whole plant level. Our results indicate that the responses to elevated CO_2 are dependent on leaf age, suggesting that acclimation of photosynthesis is stronger in younger leaves and that this can be related to a higher accumulation of starch. Lake et al. (2001) reported on evidence for long-distance signals permitting *Arabidopsis thaliana* to sense the CO_2 concentration around the old leaf and to signal young leaves to reduce the number of stomata at elevated CO_2. The recognition of differential effects on different classes of leaf ages at the plant is of paramount importance to understand responses to elevated CO_2. Clearly, the response to high CO_2 strongly depends on the developmental stage of the leaf and the plant as a whole.

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References

Allen LH, Bisbal EC, Boote KJ. Nonstructural carbohydrates of soybean plants grown in subambient and superambient levels of CO_2. Photosynth Res 1998;56:143–55.

Backhausen JE, Scheibe R. Adaptation of tobacco plants to elevated CO_2: influence of leaf age on changes in physiology, redox states and NADP-malate dehydrogenase activity. J Exp Bot 1999;50:665–75.

Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

Casanova Katny MA. Auswirkungen von erhöhter CO_2-Konzentration auf Photosynthese, Kohlenhydratmetabolismus und Zuckerexport in Kartoffelpflanzen (*Solanum tuberosum* L.). Dissertation Justus Liebig Universität Gießen, 2002. 210pp.

Cure JD, Acock B. Crop responses to carbon dioxide doubling: a literature survey. Agr For Meteorol 1986;38:127–45.

Fangmeier A, Stein W, Jäger H-J. Advantages of an open-top chamber plant exposure system to assess the impact of atmospheric trace gases on vegetation. Angew Bot 1992;66:97–105.

Farrar JF, Williams ML. The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source–sink relations and respiration. Plant Cell Environ 1991;14:819–30.

Fonseca F, Stulen I. Effect of a switch from elevated to ambient CO_2 on growth and carbohydrate allocation of *Plantago major* ssp. *pleiosperma*. Phyton 2000;40:45–50.

Griffin KL, Seemann JR. Plants, CO_2 and photosynthesis in the 21st century. Chem Biol 1996;3:245–54.

Grimmer C, Komor E. Assimilate export by leaves of *Ricinus communis* L. growing under normal and elevated carbon dioxide concentrations: the same rate during the day, a different rate at night. Planta 1999;209:275–81.

Hack H, Gall H, Klemke Th, Klose R, Meier U, Stauss R, Witzenberger A. Phänologische Entwicklungsstadien der Kartoffel (*Solanum tuberosum* L.). Nachrichtenbl Dtsch Pflanzenschutzdienstes 1993;45:11–9.

Keeling CD, Whorf TP, Wahlen M, van der Plicht J. Interannual extremes in the rate of atmospheric carbon dioxide since 1980. Nature 1995;375:666–70.

Lake JA, Quick WP, Beerling DJ, Woodward FI. Signals from mature to new leaves. Nature 2001;411: 145–54.

Lichtenhaler HK, Wellburn AR. Determination of the total carotinoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans 1983;603:591–2.

Long SP. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated. Plant Cell Environ 1991;14:729–39.

Long SP, Drake BG. Effect of the long-term elevation of CO_2 concentration in the field on the quantum yield of photosynthesis of the C_3 sedge *Scirpus olneyi*. Plant Physiol 1991;96:221–6.

Ludewig F, Sonnewald U, Kauder F, Heineke D, Geiger M, Stitt M, Müller-Röber B, Gillissen B, Kühn C, Frommer W. The role of transient starch in acclimation to elevated atmospheric CO_2. FEBS Lett 1998;429:147–51.

Miller A, Tsai CH, Hemphill D, Endres M, Rodermel S, Spalding M. Elevated CO_2 effects during leaf ontogeny. A new perspective on acclimation. Plant Physiol 1997;115:1195–200.

Moore BD, Palmquist DE, Seemann JR. Influence of plant growth at high CO_2 concentrations on leaf content of ribulose-1,5-bisphosphate carboxylase/oxygenase and intracellular distribution on soluble carbohydrates in tobacco, snapdragon, and parsley. Plant Physiol 1997; 115:241–8.

Moore BD, Cheng SH, Sims D, Seemann JR. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. Plant Cell Environ 1999;22:567–82.

Osborne CP, Laroche J, Garcia RL, Kimball BA, Wall GW, Pinter PJ, Lamorte RL, Hendrey GR, Long SP. Does leaf position within a canopy affect acclimation of photosynthesis to elevated CO_2? Analysis of a wheat crop under free-air CO_2 enrichment. Plant Physiol 1998;117:1037–45.

Prentice IC, Farquhar GD, Fasham MJR, Goulden ML, Heimann M, Jaramillo VJ, Kheshgi HS, Le Quéré C, Scholes RJ, Wallace DWR. The carbon cycle and atmospheric carbon dioxide. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA editors. Climate change 2001. The scientific basis. Cambridge: Cambridge University Press; 2001. p. 183–237.

Quirino BF, Noh YS, Himelblau E, Amasino RM. Molecular aspects of leaf senescence. Trends Plant Sci 2000; 5:278–82.

Sasek TW, De Lucía EH, Strain BR. Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevetad CO_2 concentrations. Plant Physiol 1985;78:612–22.

Schapendonk AM, van Oijen M, Dijkstra P, Pot CS, Jordi WM, Stoopen GM. Effects of elevated CO_2 concentration on photosynthetic acclimation and productivity of two potato cultivars grown in open-top chambers. Aust J Plant Physiol 2000;27:1119–30.

Sicher RC, Bunce JA. Photosynthetic enhancement and conductance to water vapour of field-grown *Solanum tuberosum* L. in response to CO_2 enrichment. Photosynth Res 1999;62:155–63.

Sicher RC, Bunce JA. Adjustments of net photosynthesis in *Solanum tuberosum* in response to reciprocal changes in ambient and elevated growth CO_2 partial pressures. Physiol Plantarum 2001;112:55–61.

Sims DA, Luo Y, Seemann JR. Importance of leaf versus whole plant CO_2 environment for photosynthetic acclimation. Plant Cell Environ 1998;21: 1189–96.

Stitt M. Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ 1991;14:741–62.

Turnbull MH, Tissue DT, Griffin KL, Rogers GND, Whitehead D. Photosynthetic acclimation to long-term exposure to elevated CO_2 concentration in *Pinus radiata* D. Don. is related to age of needles. Plant Cell Environ 1998;21:1019–28.

Vandermeiren K, Black C, Lawson T, Casanova MA, Ojanperä K. Photosynthetic and stomatal responses of potatoes grown under elevated CO_2 and/or O_3— results from the European CHIP-program. Eur J Agron 2002;17:337–52.

Wolfe DW, Gifford RM, Hilbert D, Luo YQ. Integration of photosynthetic acclimation to CO_2 at the whole-plant level. Glob Change Biol 1998;4:879–93.

Yelle S, Beeson RC, Trudel MJ, Gosselin A. Acclimation of two tomato species to high atmospheric CO_2. I. Sugar and starch concentrations. Plant Physiol 1989;90: 1465–72.