

Regular paper

## Seasonal patterns of photosynthetic response and acclimation to elevated carbon dioxide in field-grown strawberry

James A. Bunce

*Climate Stress Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Maryland, USA;*  
*Current address: Plant Science Institute, ACSL, USDA-ARS, Beltsville Agricultural Research Center, 10300*  
*Baltimore Ave., Beltsville, MD 20705-2350, USA (e-mail: buncej@ba.ars.usda.gov; fax: +1-301-504-5823)*

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### Abstract

Strawberry (*Fragaria* × *ananassa*) plants were grown in field plots at the current ambient [CO<sub>2</sub>], and at ambient + 300 and ambient + 600 μmol mol<sup>-1</sup> [CO<sub>2</sub>]. Approximately weekly measurements were made of single leaf gas exchange of upper canopy leaves from early spring through fall of two years, in order to determine the temperature dependence of the stimulation of photosynthesis by elevated [CO<sub>2</sub>], whether growth at elevated [CO<sub>2</sub>] resulted in acclimation of photosynthesis, and whether any photosynthetic acclimation was reduced when fruiting created additional demand for the products of photosynthesis. Stimulation of photosynthetic CO<sub>2</sub> assimilation by short-term increases in [CO<sub>2</sub>] increased strongly with measurement temperature. The stimulation exceeded that predicted from the kinetic characteristics of ribulose-1,5-bisphosphate carboxylase at all temperatures. Acclimation of photosynthesis to growth at elevated [CO<sub>2</sub>] was evident from early spring through summer, including the fruiting period in early summer, with lower rates under standard measurement conditions in plants grown at elevated [CO<sub>2</sub>]. The degree of acclimation increased with growth [CO<sub>2</sub>]. However, there were no significant differences between [CO<sub>2</sub>] treatments in total nitrogen per leaf area, and photosynthetic acclimation was reversed one day after switching the [CO<sub>2</sub>] treatments. Tests showed that acclimation did not result from a limitation of photosynthesis by triose phosphate utilization rate at elevated [CO<sub>2</sub>]. Photosynthetic acclimation was not evident during dry periods in midsummer, when the elevated [CO<sub>2</sub>] treatments conserved soil water and photosynthesis declined more at ambient than at elevated [CO<sub>2</sub>]. Acclimation was also not evident during the fall, when plants were vegetative, despite wet conditions and continued higher leaf starch content at elevated [CO<sub>2</sub>]. Stomatal conductance responded little to short-term changes in [CO<sub>2</sub>] except during drought, and changed in parallel with photosynthetic acclimation through the seasons in response to the long-term [CO<sub>2</sub>] treatments. The data do not support the hypothesis that source-sink balance controls the seasonal occurrence of photosynthetic acclimation to elevated [CO<sub>2</sub>] in this species.

### Introduction

Rising atmospheric [CO<sub>2</sub>] is anticipated to stimulate photosynthesis of C<sub>3</sub> plants, possibly increasing growth and yield. However, quantitative predictions of effects of elevated [CO<sub>2</sub>] on photosynthesis remain uncertain for at least two reasons: variation in the temperature dependence of the short-term response

of photosynthesis to elevated [CO<sub>2</sub>], and our current inability to predict either the occurrence or the magnitude of photosynthetic acclimation to elevated [CO<sub>2</sub>].

Elevated [CO<sub>2</sub>] is expected to stimulate photosynthesis more at high than at low temperatures, based on the kinetic characteristics of ribulose-1,5-bisphosphate carboxylase (Long 1991; Kirschbaum 1994). While

this has been confirmed in many cases (e.g. Sage et al. 1995; Hikosaka et al. 1999), it has been found that the stimulation of photosynthesis by elevated  $[\text{CO}_2]$  at cool temperatures may substantially exceed that predicted by Rubisco kinetics in some species which retain photosynthetically active leaves at cold times of year, e.g. winter wheat and barley (Bunce 1998a), dandelion (Bunce 2000), loblolly pine (Teskey 1997), and several cool-season pasture species (Greer et al. 1995). We hypothesized that strawberry, which also has green leaves at cold times of the year, would also have greater than expected stimulation of photosynthesis by elevated  $[\text{CO}_2]$  at cool temperatures.

Acclimation of photosynthesis to elevated  $[\text{CO}_2]$ , such that rates of photosynthesis are lower for plants grown at elevated  $[\text{CO}_2]$  than at lower  $[\text{CO}_2]$ , when all plants are compared under the same measurement conditions, is a common but not universal result. Neither the occurrence nor the magnitude of acclimation seem to be predictable as yet. While photosynthetic acclimation to elevated  $[\text{CO}_2]$  sometimes occurs in crops in fertile soil (e.g. Nie et al. 1995; Sicher and Bunce 1997, 1998), it is more common at low fertility (e.g. Ziska et al. 1996; Isopp et al. 2000). Strawberry is a crop commonly grown with little or no applied fertilizer, and glasshouse experiments at low fertility indicated such severe photosynthetic acclimation to elevated  $[\text{CO}_2]$  that photosynthesis under the growth  $[\text{CO}_2]$  measurement conditions decreased at  $[\text{CO}_2]$ s above  $600 \mu\text{mol mol}^{-1}$  (Keutgen et al. 1997).

The fundamental cause of acclimation of photosynthesis to elevated  $[\text{CO}_2]$  is thought to be inability of plants to fully utilize the extra photosynthate produced (cf. Stitt and Krapp 1999). Lack of sink capacity may result from nutrient deficiency, low temperature, restricted rooting volume, or lack of a reproductive sink. In wheat, for example, acclimation of photosynthesis was more severe near the end of the season, when seed filling was complete (Sicher and Bunce 1997; Garcia et al. 1998), and in sugar beet initial photosynthetic acclimation disappeared when taproot expansion provided a larger sink (Ziska et al. 1995). One form of feedback inhibition of photosynthesis results from slow triose phosphate utilization (Sharkey 1985), and has been found to cause photosynthetic acclimation to elevated  $[\text{CO}_2]$  in beans (Socias et al. 1993) and wheat (McKee and Woodward 1994). We hypothesized that in strawberry, photosynthetic acclimation to elevated  $[\text{CO}_2]$  would be less during fruiting than at other times of the year, and tested for the oc-

currence of triose phosphate utilization limitation of photosynthesis in plants grown at elevated  $[\text{CO}_2]$ .

## Materials and methods

*Fragaria × ananassa* Duchesne cv. Honeoye obtained as rooted runners from Miller Nurseries, Canandaigua, New York, were planted into field plots in March 1998. Sixteen plants were transplanted into each of six open-topped clear acrylic chambers each of which covered  $1.1 \text{ m}^2$  of ground. Chambers were 1.8 m in height. A blower pulled air out of each chamber at the base at a rate of  $6 \text{ m}^3 \text{ min}^{-1}$ . Carbon dioxide was introduced into four of the chambers at the inlets of mixing fans positioned above the canopies. Flow rates of  $\text{CO}_2$  were such that two chambers had a  $[\text{CO}_2]$  of  $300 \pm 50 \mu\text{mol mol}^{-1}$  above that of outside air, and two chambers had a  $[\text{CO}_2]$  of  $600 \pm 50 \mu\text{mol mol}^{-1}$  above that of outside air, while two chambers received no supplemental  $\text{CO}_2$ . Samples of air from each  $[\text{CO}_2]$  treatment were pumped sequentially through an absolute infrared analyzer in an adjacent air-conditioned shelter, and  $[\text{CO}_2]$ , air temperatures, and PPFD were logged every 5 min. The chambers transmitted 90% of the PPFD, and had air temperatures which averaged  $1^\circ\text{C}$  above those of outside air. The mean daytime  $[\text{CO}_2]$  of ambient air was  $353 \mu\text{mol mol}^{-1}$ , with concentrations at night of  $400 - 600 \mu\text{mol mol}^{-1}$ .

The soil in the plots had received no lime or fertilizer for four years prior to the start of the experiment, and was not fertilized during the 28-month duration of the experiment. This was a deliberate attempt to have nutrients limiting to plant growth. To establish the fact that nutrients were limiting to plant growth, extra plants were transplanted into pots filled with soil from the field plot, and watered either with a complete Hoagland's solution or with water. Plants were grown outdoors at ambient  $[\text{CO}_2]$  for two months, and the final dry mass of the fertilized plants was 1.5 times that of the unfertilized plants. During the 28 month course of the field experiment, the soil pH decreased from 5.5 to 4.9. Flower buds were removed the first spring after planting, but plants were allowed to flower and fruit normally the next two springs, and fruits were picked by hand when ripe. Vegetative reproduction was allowed to proceed unimpeded, and occurred primarily during the few weeks immediately after fruiting, which ended in early June.

Rates of photosynthetic  $\text{CO}_2$  assimilation and stomatal conductances of single leaves were measured

about weekly from early spring through late fall in 1998 and 1999, continuing through the end of fruiting in June, 2000. On each measurement date, several leaves on the same plants were measured to determine the position of the leaf with the highest rates of gas exchange, and leaves at similar positions were used for all plants measured on that date. Gas exchange measurements were made near midday on clear days, with a photon flux density of photosynthetically active radiation of at least  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . A CIRAS-1 portable photosynthesis system (PP Systems, Haverhill, Massachusetts) was utilized, and incorporated automatic  $[\text{CO}_2]$  control, and a broad leaf cuvette which kept leaf temperature within  $3^\circ\text{C}$  of outside air temperature even in full sunlight. For plants grown at elevated  $[\text{CO}_2]$ , leaf gas exchange rates were determined at the daytime growth  $[\text{CO}_2]$ s of 650 or 950  $\mu\text{mol mol}^{-1}$ . Leaves of plants grown without supplemental  $\text{CO}_2$  were measured at  $[\text{CO}_2]$ s of 350, 650 and 950  $\mu\text{mol mol}^{-1}$ . Real-time display of gas exchange data was utilized to insure that steady-state rates of gas exchange were achieved at each measurement condition. On each measurement date, three leaves were measured from each chamber. Leaf discs were collected for determination of leaf water potential from one leaf per chamber immediately after gas exchange was measured. Water potentials were measured using insulated C-52 samples chambers and an HR-33 dew point meter (Wescor, Inc., Logan, Utah) which were kept in an air conditioned shelter adjacent to the site. Soil water potential was measured at depth of 25 cm, using Decagon matric potential sensors and readout system (Decagon Devices, Pulman, Washington).

On some of the dates when leaf gas exchange measurements indicated the existence of photosynthetic acclimation to elevated  $[\text{CO}_2]$ , photosynthetic  $\text{CO}_2$  assimilation rates of plants grown at the highest  $[\text{CO}_2]$  were also measured at a  $[\text{CO}_2]$  of 1200  $\mu\text{mol mol}^{-1}$  to determine if photosynthetic rates were saturated for  $\text{CO}_2$  under the growth  $[\text{CO}_2]$  of 950  $\mu\text{mol mol}^{-1}$ . An increase in rate at the highest  $[\text{CO}_2]$  was taken as evidence that the rate at the growth  $[\text{CO}_2]$  was not limited by triose phosphate utilization rate.

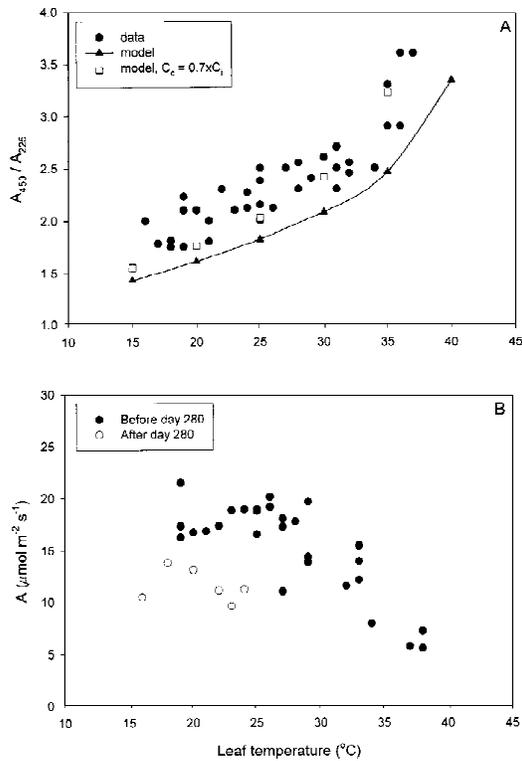
Starch and total nitrogen per unit of leaf area were determined on leaf samples on several of the dates when leaf gas exchange rates were determined. For starch analysis, leaf punches of known area were frozen in liquid  $\text{N}_2$  and stored at  $-80^\circ\text{C}$  until analyzed by the methods described in Sicher and Bunce (1999). For N analysis, whole leaflets were removed and their area determined with a portable photoelectric leaf area

meter. Leaflets were then frozen in liquid  $\text{N}_2$ , freeze-dried, and % N was determined by CHN analysis at the State of Maryland Soil Testing Laboratory. Specific leaf area for each leaflet was used with % N to determine the mass of nitrogen per unit of leaf area.

At the end of the experiment, the  $[\text{CO}_2]$  treatments were switched between one ambient and one + 600  $\mu\text{mol mol}^{-1}$  chamber, and midday photosynthetic  $\text{CO}_2$  assimilation rates were determined 0, 1 and 3 days after switching the treatments and compared with rates in chambers which had not been switched. Three leaves were measured per chamber, both at an external  $[\text{CO}_2]$  ( $C_a$ ) of 350 and 950  $\mu\text{mol mol}^{-1}$ .

The short-term response of photosynthetic  $\text{CO}_2$  assimilation rate to substomatal  $[\text{CO}_2]$  ( $C_i$ ) of the plants grown without supplemental  $\text{CO}_2$  was summarized as the relative increase in photosynthesis from a  $C_i = 225 \mu\text{mol mol}^{-1}$  to  $C_i = 450 \mu\text{mol mol}^{-1}$ . These values were chosen based on the fact that leaves measured at the current ambient  $[\text{CO}_2]$  had  $C_i$  values of  $225 \pm 15 \mu\text{mol mol}^{-1}$ , and these leaves had  $C_i$  values of  $450 \pm 30 \mu\text{mol mol}^{-1}$  when measured at 650  $\mu\text{mol mol}^{-1}$ . Photosynthesis rates at precisely  $C_i = 225$  and 450  $\mu\text{mol mol}^{-1}$  were estimated from a cubic spline curve fit. The observed relative increase in photosynthesis on a given measurement date was compared with the relative increase predicted from the kinetic characteristics of Rubisco and the measurement temperature (Bunce 2000), using a Farquhar-type  $\text{C}_3$  photosynthesis model (Long 1991). The maximum carboxylation rate in the model was set at a value consistent with assimilation rates measured at  $C_i = 225 \mu\text{mol mol}^{-1}$ , and the maximum rate of electron transport was set large enough to not be limiting. The model was also run using internal diffusive resistances to carbon dioxide of  $4-8 \text{ m}^2 \text{ s mol}^{-1}$  (varying with maximum carboxylation rate) such that the  $[\text{CO}_2]$  in the chloroplast ( $C_c$ ) was  $0.7 * C_i$  at  $C_i = 225 \mu\text{mol mol}^{-1}$ .

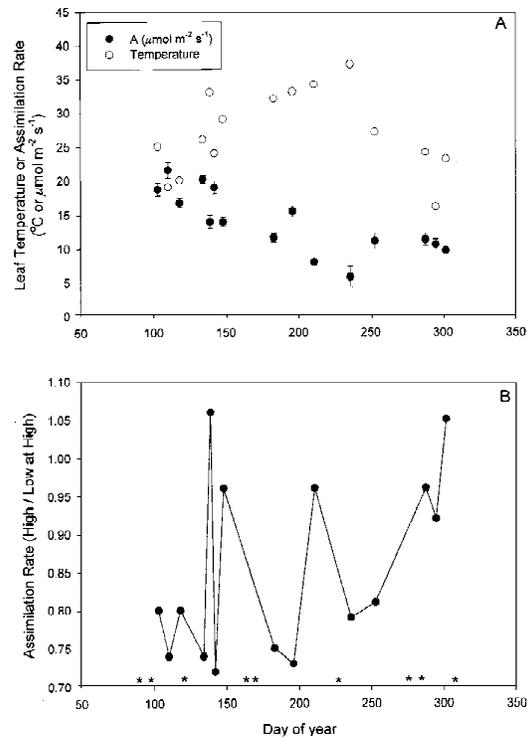
In testing for photosynthetic acclimation to elevated  $[\text{CO}_2]$ , average photosynthetic  $\text{CO}_2$  assimilation rates on each measurement date were calculated for each chamber, and  $[\text{CO}_2]$  treatments were compared using analysis of variance, with two chambers per treatment. Rates for plants grown and measured at + 300  $\mu\text{mol mol}^{-1}$  were compared with rates of plants grown at ambient  $[\text{CO}_2]$  but measured at + 300, and rates for plants grown and measured at + 600  $\mu\text{mol mol}^{-1}$  were compared with rates of plants grown at ambient  $[\text{CO}_2]$  but measured at + 600  $\mu\text{mol mol}^{-1}$



**Figure 1.** The temperature dependence of the short-term stimulation of photosynthesis by increasing the measurement  $C_i$  from 225 to 450  $\mu\text{mol mol}^{-1}$  for strawberry leaves grown at the current ambient  $[\text{CO}_2]$ , and the temperature dependencies predicted from Rubisco kinetics assuming  $C_c = C_i$ , and  $C_c = 0.7 * C_i$  (A), and the  $\text{CO}_2$  assimilation rate at  $C_i = 225$  (B). Each data point represents a mean ratio for a total of six leaves measured on a given date. Measurements were made at midday in the field at the ambient air temperature and in full sunlight.

## Results

The relative stimulation of photosynthetic  $\text{CO}_2$  assimilation rate from  $C_i = 225$  to  $C_i = 450 \mu\text{mol mol}^{-1}$  increased with measurement temperature, and at all temperatures exceeded that predicted from Rubisco kinetics (Figure 1A). When internal diffusive resistance was added to the model, a closer fit with experimental data occurred at the higher temperatures, but most of the experimental data still exceeded the relative stimulation predicted by the model at the lower temperatures (Figure 1A). For plants grown and measured at the current ambient  $[\text{CO}_2]$ , assimilation rates decreased at temperatures above about 25 °C (Figure 1B). In this data, the leaf to air water vapor pressure difference increased exponentially with temperature



**Figure 2.** The seasonal pattern for 1998 of midday photosynthetic  $\text{CO}_2$  assimilation rate of plants grown and measured at the current ambient  $[\text{CO}_2]$  of 350  $\mu\text{mol mol}^{-1}$  (A), and the ratio of assimilation rates of plants grown and measured at ambient + 600  $\mu\text{mol mol}^{-1}$  to those of plants grown at ambient  $[\text{CO}_2]$  but measured at + 600  $\mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  (B). Each point represents a mean based on measurements on three leaves for each of two replicate chambers per  $[\text{CO}_2]$  treatment. Bars represent SE for  $n = 2$  chambers. \* indicates days with precipitation.

from about 0.8 kPa at 18 °C to about 4.0 kPa at 38 °C. It was noticed that assimilation rates were lower at a given temperature for leaves measured after day 280 (Figure 1B), when photosynthetic acclimation to growth at elevated  $[\text{CO}_2]$  was not evident (see later).

When comparing the photosynthetic  $\text{CO}_2$  assimilation rates of plants grown at elevated  $[\text{CO}_2]$  with those of plants grown without supplemental  $[\text{CO}_2]$  but measured at elevated  $[\text{CO}_2]$ , analysis of variance indicated that significant ( $P < 0.001$ ) effects of growth  $[\text{CO}_2]$ , measurement date, and a significant interaction between growth  $[\text{CO}_2]$  and measurement date. This interaction is illustrated for the 1998 data in Figure 2, for the plants grown at + 600  $\mu\text{mol mol}^{-1}$ . Evidence of acclimation (i.e. an assimilation ratio less than 1 in Figure 2) was not related to the temperature or the assimilation rate of the plants grown at the current

*Table 1.* Midday CO<sub>2</sub> assimilation rates (A) of leaves of strawberry plants grown at the current ambient [CO<sub>2</sub>], and ambient + 300 and ambient + 600 μmol mol<sup>-1</sup> [CO<sub>2</sub>]. The data set was divided into three classes, measurement dates after day of year 280, measurement dates when the leaf water potential (LWP) of ambient plants was below -1.9 MPa, and all other measurement dates. This subdivision eliminated a significant growth [CO<sub>2</sub>] by measurement date interaction. \* indicates a significant effect of growth [CO<sub>2</sub>] at *P* = 0.05. Values in parentheses are SE for *n* = 2 chambers per [CO<sub>2</sub>] treatment

Growth/measurement [CO <sub>2</sub> ] (μmol mol <sup>-1</sup> )	After day 280 A (± SE) (μmol m <sup>-2</sup> s <sup>-1</sup> )	Low LWP	All other data
+ 300 / + 300	22.8 (0.7)	24.0 (1.0)	27.2 (0.5)
+ 600 / + 600	28.1 (0.9)	27.8 (1.2)	31.4 (0.6)
Ambient / ambient	11.5 (0.5)	9.7 (0.7)	16.6 (0.5)
Ambient / +300	22.9 (0.8)	22.0 (1.1)	31.6 (0.6)*
Ambient / +600	29.3 (1.0)	28.3 (1.5)	40.9 (0.7)*
Number of measurement dates	6	5	23

ambient [CO<sub>2</sub>] in any obvious way (Figure 2). It was found that the data for all years could be subdivided into three sets in which the growth [CO<sub>2</sub>] by measurement date interaction terms were non-significant at *P* = 0.05. The three subsets were: all data after day of year 280, all data when leaf water potentials were below -1.9 MPa in plants grown at ambient [CO<sub>2</sub>], and all other data. Acclimation of photosynthesis to growth at elevated [CO<sub>2</sub>] was significant in the main data set, but not in the two restricted sets of dry conditions and during the fall (Table 1). There were no dates when photosynthetic acclimation to elevated [CO<sub>2</sub>] was large enough to eliminate the stimulation of photosynthesis at the growth [CO<sub>2</sub>]. Averaged over all dates, midday photosynthetic rates measured at the daytime growth [CO<sub>2</sub>] were 14.7, 26.0 and 30.3 μmol m<sup>-2</sup> s<sup>-1</sup> for the ambient, ambient + 300, and ambient + 600 μmol mol<sup>-1</sup> [CO<sub>2</sub>] treatments, respectively.

Acclimation of photosynthesis to growth at elevated [CO<sub>2</sub>] was evident even when measurements days followed several cloudy days. For example, in Figure 2, day of year 134 was preceded by 10 days with daily integrals of photosynthetically active radiation of less than 9 mol m<sup>-2</sup> d<sup>-1</sup> (compared with a clear day total of about 45 mol m<sup>-2</sup> d<sup>-1</sup> at this time of year). There were three additional measurement days which followed several days of cloudy weather, yet still indicated photosynthetic acclimation to elevated [CO<sub>2</sub>].

Stomatal conductance of plants grown at the current ambient [CO<sub>2</sub>] was unresponsive to measurement [CO<sub>2</sub>] except when plants had low leaf water poten-

tials (Table 2). Substomatal [CO<sub>2</sub>] was affected by growth [CO<sub>2</sub>] only under dry conditions (Table 2). The usually constant C<sub>i</sub> across growth [CO<sub>2</sub>] treatments reflected proportional changes in conductance and photosynthesis.

For each of four dates tested, photosynthetic rates of plants grown at the highest [CO<sub>2</sub>] were not saturated for CO<sub>2</sub> at the C<sub>i</sub> occurring under the growth conditions. Averaged over four measurement dates photosynthetic CO<sub>2</sub> assimilation rates of plants grown at + 600 μmol mol<sup>-1</sup> [CO<sub>2</sub>] were 35.1 and 41.8 μmol m<sup>-2</sup> s<sup>-1</sup> when measured at 950 and 1200 μmol mol<sup>-1</sup> [CO<sub>2</sub>], respectively, compared with a rate of 42 μmol mol<sup>-1</sup> at 950 μmol mol<sup>-1</sup> [CO<sub>2</sub>] for plant grown without supplemental [CO<sub>2</sub>].

Midday leaf water potentials on the 'dry' measurement dates averaged -2.2, -2.2, and -2.3 MPa for plants at the current ambient [CO<sub>2</sub>], ambient + 300 and ambient + 600, respectively. Soil water potentials at a depth of 25 cm averaged -1.13, -0.83, and -0.64 for the ambient, + 300 and + 600 [CO<sub>2</sub>] treatments, respectively, on these dates. Midday leaf water potentials averaged over all other measurement dates were -1.6 MPa for all [CO<sub>2</sub>] treatments.

Leaf nitrogen per unit of area was measured on four dates in the spring and early summer, when photosynthetic acclimation was evident. The plants grown at the two elevated [CO<sub>2</sub>]s had mean values which were about 9% lower than plants grown at the current ambient [CO<sub>2</sub>], i.e. 2.0 g m<sup>-2</sup> compared with 2.2 g m<sup>-2</sup>, but these differences were not statistically significant at *P* = 0.05. Gas exchange measurements on these same dates indicated 15 and 26% lower photosynthetic CO<sub>2</sub> assimilation rates for plants grown at + 300 and + 600 μmol mol<sup>-1</sup> [CO<sub>2</sub>], respectively, compared with plants grown at the current ambient [CO<sub>2</sub>], when measurements were made at + 300 and + 600 μmol mol<sup>-1</sup>.

Leaf starch content was measured on three dates in the spring, when photosynthetic acclimation to elevated [CO<sub>2</sub>] was evident, and one date in the fall, when no acclimation occurred. Similar leaf starch values occurred on all dates for the ambient and ambient + 300 treatments, while for the ambient + 600 [CO<sub>2</sub>] treatment, higher starch values occurred in the spring than in the fall (Figure 3).

When the lowest and highest [CO<sub>2</sub>] treatments were switched, at the end of the experiment, CO<sub>2</sub> assimilation rates responded to the switch within one day, and by day three, were completely reversed (Table 3). The same patterns were obtained for photosyn-

Table 2. Midday stomatal conductances ( $g$ , in  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and substomatal  $[\text{CO}_2]$  ( $C_i$ , in  $\mu\text{mol mol}^{-1}$ ) of leaves of strawberry plants grown at the current ambient  $[\text{CO}_2]$ , and ambient + 300 and ambient + 600  $\mu\text{mol mol}^{-1}$   $[\text{CO}_2]$ . The data set was divided into three classes, measurement dates after day of year 280, measurement dates when the leaf water potential (LWP) of ambient plants was below  $-1.9$  MPa, and all other measurement dates. \* indicates a significant effect at  $P = 0.05$ , and ns indicates non-significance at  $P = 0.05$ . Values in parentheses are SE for  $n = 2$  chambers per  $[\text{CO}_2]$  treatment

Growth/measurement $[\text{CO}_2]$ ( $\mu\text{mol mol}^{-1}$ )	After day 280		Low LWP		All other data	
	$g$	$C_i$	$g$	$C_i$	$g$	$C_i$
+ 300 / + 300	196 (9)	421 (7)	251 (15)	474 (12)	270 (8)	447 (4)
+ 600 / + 600	172 (9)	641 (15)	190 (17)	678 (21)	214 (7)	675 (7)
Ambient / ambient	204 (13)	227 (4)	250 (23)	228 (4)	350 (11)	225 (5)
Ambient / + 300	205 (13)	439 (9)	200 (21)	455 (9)	348 (11)	449 (4)
Ambient / + 600	192 (14)	647 (17)	157 (19)	582 (20)	322 (11)	686 (7)
<i>Effect:</i>			<i>Significance of effect:</i>			
	$g$	$C_i$	$g$	$C_i$	$g$	$C_i$
Measurement at + 300	ns	*	*	*	ns	*
Measurement at + 600	ns	*	*	*	ns	*
Growth at + 300	ns	ns	ns	ns	*	ns
Growth at + 600	ns	ns	ns	*	*	ns

Table 3. Time course of  $\text{CO}_2$  assimilation rates ( $A$ ) measured at 350 and 950  $\mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  following switching  $[\text{CO}_2]$  treatments from ambient to ambient + 600  $\mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  and from ambient + 600 to ambient  $[\text{CO}_2]$  for strawberry plants in July 2000. Values are means ( $\pm$  SE) for  $n = 3$  leaves. Within a measurement date, means followed by different letters were significantly different using ANOVA and Fischer's protected LSD tests

Time since switching (d)	$[\text{CO}_2]$ treatment ( $\mu\text{mol mol}^{-1}$ )	$A$ at 350 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$A$ at 950 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
0	Ambient	13.4 (1.2) a	34.4 (1.2) a
0	+ 600	10.3 (0.6) b	29.3 (1.1) b
1	Ambient	13.9 (0.9) a	35.5 (1.1) a
1	+ 600	12.0 (0.1) b	28.0 (0.9) b
1	Ambient switched to + 600	12.3 (0.4) b	31.4 (0.5) b
1	+ 600 switched to Ambient	13.1 (0.4) a	33.6 (0.5) a
3	Ambient	13.9 (0.3) a	32.4 (2.1) a
3	+ 600	11.2 (0.9) b	24.6 (0.7) b
3	Ambient switched to + 600	10.9 (0.4) b	23.7 (1.9) b
3	+ 600 switched to Ambient	13.9 (0.9) a	33.1 (1.3) a

thetic rates measured at  $C_a = 350$  and  $950 \mu\text{mol mol}^{-1}$ .

## Discussion

Leaf gas exchange measurements in this study began in the spring as soon as new leaves finished area expansion (about day 100), and ended in the fall when severe freezes killed most leaves (about day 310).

Very few green leaves persisted during the winter. The gas exchange measurement periods were estimated to cover more than 90% of the yearly leaf area duration, and thus should be reasonably representative of this cultivar in this climate.

The short-term response of strawberry leaf photosynthesis to elevated  $[\text{CO}_2]$  did not have the insensitivity to seasonal temperature changes observed in some other cool climate species (Teskey 1997; Bunce 1998, 2000), but had a larger stimulation at all temperatures than predicted from Rubisco kinetic characteristics. The simplest explanation for a larger stimulation than expected, a significant internal diffusive resistance, could account for this discrepancy at warm temperatures, but probably not at low temperatures. We used assumed internal resistances such that  $C_c = 0.7 * C_i$  for measurements at  $C_a = 350 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$ , but these resistances were not large enough to explain the observed stimulation of photosynthesis by elevated  $[\text{CO}_2]$  at cool temperatures. Such resistances are on the high end of estimates of internal resistance for other species (Laisk and Loreto 1996; Gillon and Yakir 2000). Our simulations indicate that the stimulation of photosynthesis by elevated  $[\text{CO}_2]$  is not very sensitive to internal resistance at cool temperatures. In some of the other studies where the stimulation of photosynthesis by elevated  $[\text{CO}_2]$  was larger than predicted by Rubisco kinetics at cool temperatures, measurements indicated an unexpectedly high  $\text{CO}_2$  compensation point, which suggested a low apparent

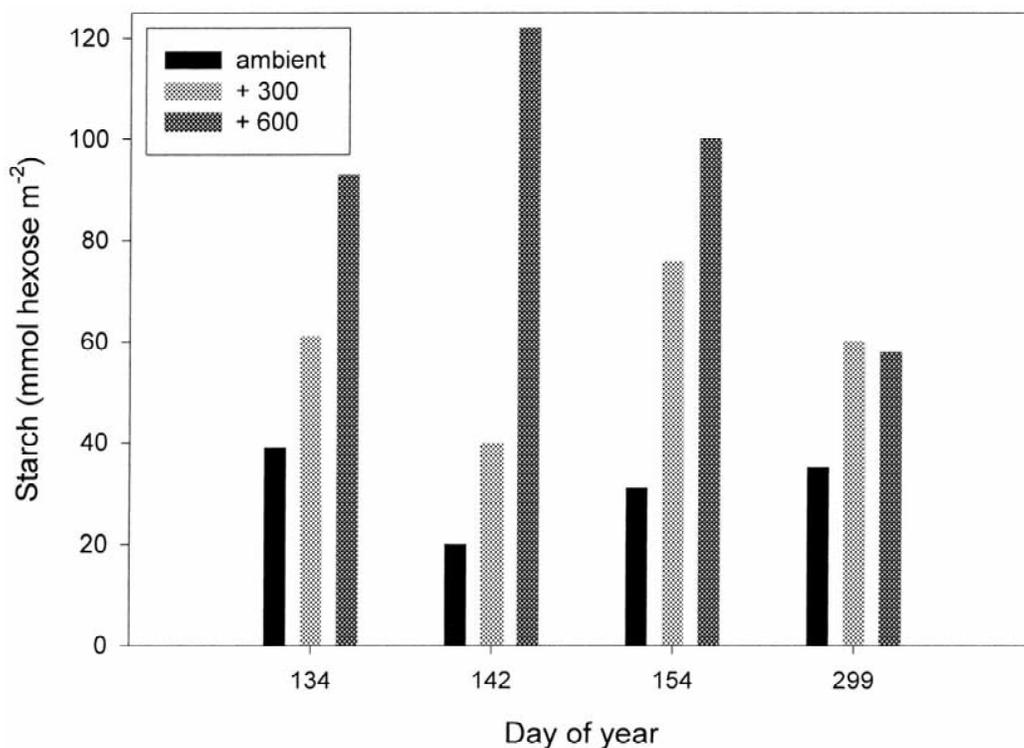


Figure 3. Leaf starch contents for strawberry leaves grown at the current ambient  $[\text{CO}_2]$ , ambient + 300 and ambient + 600  $\mu\text{mol mol}^{-1}$  on four measurement dates. Photosynthetic acclimation to elevated  $[\text{CO}_2]$  was evident on the first three dates, but not on the last date.

specificity of Rubisco (Bunce 1998, 2000). This aspect was not investigated in the present study. Our data indicate that, in the absence of photosynthetic acclimation to growth at elevated  $[\text{CO}_2]$ , strawberry leaf photosynthesis would be strongly stimulated by rising atmospheric  $[\text{CO}_2]$  even at cool times of the year.

However, acclimation of photosynthesis to elevated  $[\text{CO}_2]$  was evident on about two thirds of the measurement days. The days when no acclimation occurred fit into two classes, occasions when plants grown at the current ambient  $[\text{CO}_2]$  were under water stress, and in the fall, after day 280. Other studies have also shown that acclimation of photosynthesis to elevated  $[\text{CO}_2]$  occurring under well watered conditions may not be evident during water stress (Huxman et al. 1998; Sicher and Bunce 1999). This probably occurred because plants grown at lower  $[\text{CO}_2]$  had higher stomatal conductance and depleted soil water faster than plants grown at elevated  $[\text{CO}_2]$ , as documented here for strawberries. The water deficit inhibited photosynthesis more for plants grown at the lower  $[\text{CO}_2]$ , and resulted in no photosynthetic differ-

ences between plants grown at ambient and elevated  $[\text{CO}_2]$ . The lack of evidence of photosynthetic acclimation to elevated  $[\text{CO}_2]$  in the fall may have a somewhat similar cause, greater inhibition of photosynthesis by environmental stress in plants at the lower  $[\text{CO}_2]$ . This is suggested by the lower rates of photosynthesis of plants grown at the current ambient  $[\text{CO}_2]$  at a given measurement temperature in the fall than at other times of year. There are reports for other species of elevated  $[\text{CO}_2]$  protecting against damage to photosynthesis caused by low temperatures (Sionit et al. 1981; Potvin and Strain 1985; Boese et al. 1997), but these studies have been for chilling sensitive species. In this study, the period in the fall when photosynthetic acclimation did not occur was after frosts had occurred.

The cause of the acclimation of photosynthesis to growth at elevated  $[\text{CO}_2]$  which occurred on the majority of measurement dates was not clear. The data presented make it unlikely that any of three commonly proposed causes were operating, triose phosphate utilization limitation at elevated  $[\text{CO}_2]$ , low leaf nitrogen

content and high source–sink ratio. Two observations ruled out triosphosphate utilization rate limitation. First, photosynthetic CO<sub>2</sub> assimilation rates of plants grown at the highest [CO<sub>2</sub>] were not saturated for CO<sub>2</sub> at the concentration used to assay for photosynthetic acclimation. Secondly, the switching experiment showed that acclimation was evident when photosynthesis was measured at the current ambient [CO<sub>2</sub>], which was severely limiting for all plants. Leaf nitrogen content per unit of leaf area was only slightly and not statistically significantly lower in plants grown at elevated [CO<sub>2</sub>]. The rapid reversibility of photosynthetic acclimation also argues against changes in major leaf nitrogen-containing constituents controlling photosynthetic acclimation. For example, in rice, transfer to lower [CO<sub>2</sub>] resulted in an increase in the transcripts for the small subunit of Rubisco within one day, but no change in Rubisco activity or soluble protein was detectable after three days (Gesch et al. 2000), although changes were detected at ten days. High source–sink ratio at elevated [CO<sub>2</sub>] as a cause of the photosynthetic acclimation is also unlikely, since acclimation did not decrease during fruiting, nor during long periods of cloudy weather, and leaf starch content remained high in the fall when no acclimation occurred.

Acclimation of photosynthetic CO<sub>2</sub> assimilation was accompanied by a parallel acclimation of stomatal conductance such that substomatal [CO<sub>2</sub>] was not affected by the growth [CO<sub>2</sub>] except when water stress occurred and photosynthetic acclimation was not evident. Proportional acclimation of assimilation rate and stomatal conductance to growth at elevated [CO<sub>2</sub>] has been reported for other species (e.g. Bunce 2001). Constant C<sub>i</sub> rules out an increase in stomatal limitation of photosynthesis as a cause of photosynthetic acclimation, unless patchy stomatal conductance occurred (Buckley et al. 1997). Patchy stomatal conductance has not been reported in studies of acclimation to elevated [CO<sub>2</sub>]. The observation that stomatal conductance was insensitive to measurement [CO<sub>2</sub>] except during water stress is consistent with other evidence that water stress or high abscisic acid content may increase stomatal sensitivity to [CO<sub>2</sub>] (Bunce 1998b; Leymarie et al. 1999).

Measurements of Rubisco activity and activation status might have been useful in identifying the causes of acclimation, and were attempted. However, the assays were unsuccessful in this tissue, presumably because of large amounts of inhibitory compounds which could not be overcome using the usual methods. Whatever the cause of photosynthetic acclimation in

the present study, consideration of seasonal changes in plant source–sink balance did not help to predict its occurrence.

While acclimation of photosynthesis to elevated [CO<sub>2</sub>] was frequently evident in these nutrient-limited, field-grown strawberries, the degree of acclimation was never enough to eliminate the substantial stimulation of photosynthesis by growth at elevated [CO<sub>2</sub>]. The more severe photosynthetic acclimation to elevated [CO<sub>2</sub>] observed by Keutgen et al. (1997) in sand culture in a glasshouse was accompanied by greatly reduced leaf nitrogen contents, which did not occur in the present study. It is unclear whether such extreme nutrient deficiencies are likely under normal cultural conditions in the field, or whether higher fertility than used in the present study could eliminate acclimation of photosynthesis to elevated [CO<sub>2</sub>].

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