Journal of Experimental Botany, Vol. 50, No. 334, pp. 645–653, May 1999

Journal of Experimental Botany

Photosynthetic acclimation to elevated CO_2 in a sunflower canopy

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Received 31 July 1998; Accepted 15 January 1999

Abstract

Sunflower canopies were grown in mesocosom gas exchange chambers at ambient and elevated CO_2 concentrations (360 and 700 ppm) and leaf photosynthetic capacities measured at several depths within each canopy. Elevated [CO_2] had little effect on whole-canopy photosynthetic capacity and total leaf area, but had marked effects on the distribution of photosynthetic capacity and leaf area within the canopy. Elevated [CO_2] did not significantly reduce the photosynthetic capacities per unit leaf area of young leaves at the top of the canopy, but it did reduce the photosynthetic capacities of older leaves by as much as 40%. This effect was not dependent on the canopy light environment since elevated [CO_2] also reduced the photosynthetic capacities of older leaves exposed to full sun on the south edge of the canopy. In addition to the effects on leaf photosynthetic capacity, elevated [CO_2] shifted the distribution of leaf area within the canopy so that more leaf area was concentrated near the top of the canopy. This change resulted in as much as a 50% reduction in photon flux density in the upper portions of the elevated [CO_2] canopy relative to the ambient [CO_2] canopy, even though there was no significant difference in the total canopy leaf area. This reduction in PFD appeared to account for leaf carbohydrate contents that were actually lower for many of the shaded leaves in the elevated as opposed to the ambient [CO_2] canopy. Photosynthetic capacities were not significantly correlated with any of the individual leaf carbohydrate contents. However, there was a strong negative correlation between photosynthetic capacity and the ratio of hexose sugars to sucrose, consistent with the hypothesis that sucrose cycling is a component of the biochemical signalling pathway controlling photosynthetic acclimation to elevated [CO_2].

Key words: *Helianthus annuus*, carbohydrates, carbon dioxide, CO_2, light, nitrogen, photosynthesis, Rubisco.

Introduction

Increasing atmospheric CO_2 concentrations are predicted to increase photosynthesis and plant growth (Griffin and Seemann, 1996). The extent of this increase will depend not only on the short-term stimulation of photosynthetic rate but also on longer-term acclimation responses of photosynthetic capacity. Growth in elevated [CO_2] for periods longer than a few days often results in reductions in photosynthetic capacities per unit leaf area (Van Oosten and Besford, 1996; Drake *et al*., 1997; Moore *et al*., 1999). This down-regulation of photosynthesis is thought to be a response to changes in cellular sugar levels resulting from increases in carbohydrate production relative to the rates of carbohydrate export and utilization. It has recently been proposed that the mechanism for down-regulation of photosynthesis at elevated [CO_2] involves hexokinase (Jang and Sheen, 1994; Koch, 1996) and sucrose cycling through invertase (Goldschmidt and Huber, 1992; Moore *et al*., 1998).

Many studies have examined the effects of elevated atmospheric [CO_2] on photosynthetic capacities of individual plants grown in pots. However, few studies have examined the response of plants grown in dense canopies.

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At the whole plant and canopy levels, photosynthetic capacity depends not only on photosynthetic capacity per unit leaf area but also on total leaf area. Elevated [CO_2] often increases leaf area (Taylor *et al.*, 1994), but the extent of this stimulation depends on species and other environmental variables (Ackerly *et al.*, 1992; Gay and Hauck, 1994; Gardner *et al.*, 1995; Sims *et al.*, 1998*a*). Much of the data on long-term plant responses to elevated [CO_2] have been gathered from single plants grown in pots. However, plants growing in the field are usually in competition with neighbouring plants. In particular, competition will affect the light environment of leaves below the top of the canopy. Plants grown entirely in low light tend not to down-regulate photosynthesis at elevated [CO_2] as much as high-light-grown plants (Ehret and Jolliffe, 1985; Sims *et al.*, 1998*b*). However, there are relatively few studies of acclimation to elevated [CO_2] for herbaceous plant canopy leaves that initially develop in full sun and are subsequently shaded as the canopy continues to grow. In addition to the effects of [CO_2] and photon flux density (PFD) on these leaves, photosynthetic capacities may be influenced by increasing leaf age. It has been suggested that elevated [CO_2] increases the rate of leaf development and senescence and thus the apparent down-regulation of photosynthetic capacity by elevated [CO_2] may increase with leaf age (Besford *et al.*, 1990). However, even in isolated plants it is difficult to separate the effects of increasing leaf age from the effects of self-shading. Studies with vines where self-shading can be eliminated suggest that decreasing PFD is more important than increasing leaf age in regulating the decline in photosynthetic capacity of older leaves at ambient [CO_2] concentrations (Hikosaka, 1996).

Another difficulty with experiments on plants grown in small pots is that root volume limitations may increase the down-regulation of photosynthesis (Thomas and Strain, 1991; Arp, 1991; Curtis and Wang, 1998). For example, elevated [CO_2] can result in substantial decreases in photosynthetic capacity of soybeans grown in pots in a greenhouse (Sims *et al.*, 1998*b*), but Campbell *et al.* (1988) reported that elevated [CO_2] actually increased photosynthetic capacities of soybeans when they were grown in the field. This may be related to changes in cellular sugar contents since restricted rooting volume can reduce growth of roots and thus demand for carbohydrate export from leaves (Arp, 1991).

To test the effects of elevated [CO_2] on photosynthetic capacities of whole canopies, use was made of the EcoCELLs at the Desert Research Institute (Reno, NV, USA). These large, whole-system gas-exchange chambers allowed the control of the [CO_2] around whole sunflower canopies as well as allowing the measurement both of individual leaf and whole canopy photosynthetic responses. In addition, the large (6.7 m^3) pots reduced the likelihood of small-pot-size effects and better simulated field conditions. The whole-system carbon balance results will be reported elsewhere. The effects of elevated [CO_2] on the distribution of light and photosynthetic capacity with increasing depth in the canopy are reported here.

Materials and methods

Plant material and growth conditions

Sunflowers (*Helianthus annuus* var. Mammoth) were planted in early July in two large controlled-environment chambers (EcoCELLs, Desert Research Institute, Reno, Nevada, USA; see Griffin *et al.* (1996) for a complete technical description). In each chamber there were three 6.7 m^3 pots filled, in layers from the bottom, with 1 m pea gravel, 0.4 m washed river sand and 0.4 m of a 1 : 1 (v : v) mixture of washed river sand and top soil from a tallgrass prairie (Konza Prairie Long-term Ecological Research Site, Manhattan, KS, USA). The three pots were positioned side by side so that the sunflowers developed a continuous canopy measuring 2.85×3.9 m (long axis oriented north/south) and was considered to be a single unit for sampling purposes. There were 108 plants per cell (36 per pot with 0.33 m spacing between plants).

Pots were watered as needed with tap water to maintain soil water content near field capacity. No fertilizer was added. Daytime air temperature was controlled at $28 \pm 0.5\,^{\circ}C$ and night-time at $13 \pm 0.5\,^{\circ}C$. Daytime relative humidity was controlled at $30 \pm 5\%$ and night-time at $60 \pm 5\%$. The chambers received sunlight, which had passed through the greenhouse roof (a two-layer clear acrylic) and the thin plastic tops of the EcoCELLs (low-density polyethylene). Photon flux density (PFD) in the EcoCELLs was approximately 85% of that incident on the greenhouse and averaged 31.6 ± 5.7 mol m^{-2} d^{-1} with a mean maximum instantaneous PFD of 1545 ± 107 μmol m^{-2} s^{-1} over the course of the experiment. More than 90% of the days during the experiment were cloudless. One EcoCELL received ambient [CO_2] (360 to 390 ppm) while the other received ambient air plus 350 ppm CO_2.

Measurements of one young leaf (60–80% full expansion, exposed to full sun at the top of the canopy) on each of six randomly selected plants per EcoCELL were made on a weekly basis as soon as the plants had leaves large enough to fill the gas exchange cuvette. Measurements of leaves at different heights in the canopy were made 48 d after planting when the canopy was fully closed. For these measurements, three plants of similar size were selected in the centre of each canopy and another three on the south edge of each canopy. Six leaves, in leaf positions 6, 9, 12, 15, 18, and 21 counting from oldest to youngest, were selected on each plant. In some cases, leaves one position above or below the given number were selected on the canopy edge plants to ensure that fully sunlit leaves were used.

Gas exchange measurements

CO_2 assimilation was measured with an open-system gas-exchange apparatus (Li-Cor 6400, Li-Cor Inc, Lincoln, NE, USA) equipped with the standard leaf chamber (encloses 6 cm^2 leaf area) and the CO_2 injector system (model 6400–01, Li-Cor Inc, Lincoln, NE, USA) for control of [CO_2]. PFD for all measurements was 1500 μmol m^{-2} s^{-1} provided by a red LED light source (model 6400–02, Li-Cor Inc, Lincoln, NE, USA). Leaf temperature was controlled at 28 °C and water vapour concentration was 30 ± 2 mmol mol^{-1}. Leaf sections free of

major veins were enclosed in the chamber. Photosynthetic rates varied by less than 10% across the lamina for mature leaves. After enclosing the leaves in the chamber, 5–10 min were allowed for the photosynthetic rate to stabilize. Then the photosynthetic rate was measured first at the growth [CO_2] (360 or 700 ppm CO_2) and then at the opposite [CO_2]. The weekly young leaf measurements were made between 11.00 h and 13.00 h PST. The canopy gradient measurements were made between 08.00 h and 16.00 h PST with alternation between the EcoCELLs between measurements.

Canopy structure and light environment

Leaf areas were calculated from measurements of leaf length and width using allometric relationships developed from a subset of similar leaves. Leaf areas of all leaves on the six randomly selected plants in each chamber were measured each week at the time of the photosynthesis measurements and used to calculate total leaf area index for the canopy. For the canopy-gradient plants, leaf area and leaf height above the ground at the mid-point of the blade were measured for each leaf used for the photosynthetic measurements. Also at this time, PFD was measured at six heights (15, 30, 45, 60, 75, and 90 cm) and six random positions within the centre of each canopy. Since the leaves on the south edge of the canopy were not shaded by other leaves, the PFDs they received were similar to those above the canopy (except for the lowest leaf position at which the leaf was much smaller and consequently partially shaded in some cases).

Leaf composition

For the canopy gradient measurements, leaf samples were collected the day following the completion of the photosynthesis measurements. As much as possible, these leaf samples were collected from the same area of the leaf used for gas exchange measurements. Small leaf punches (0.33 cm^2) were collected at six times throughout the day (pre-dawn 06.00 h, post-dawn 08.00 h, noon 12.00 h, pre-sunset 16.00 h, post-sunset 20.00 h, and 23.00 h), immediately frozen in liquid nitrogen and stored at −80 °C until they were analysed for carbohydrates by the technique of Hendrix (1983). Larger leaf discs (3.3 cm^2) were collected in the early afternoon, immediately frozen in liquid nitrogen and stored at −80 °C until they were analysed for total Rubisco active sites by a CABP binding technique (Evans and Seemann, 1984). Additional small discs were collected at the same time as the Rubisco samples, dried at 60 °C for 48 h and weighed to determine leaf dry mass. These samples were later used to measure total leaf nitrogen content (model 2400 CHN analyser, Perkin Elmer, Norwalk, CT, USA).

Statistical analysis

The results were analysed using ANOVA and linear regression in the computer package StatView (SAS Institute Inc, Cary, NC, USA). Measurements of several leaves at different heights on the same plant or measurements of the same leaf or plant over time were treated as repeated measures.

Results

Elevated [CO_2] increased photosynthetic rates per unit area at high PFD for leaves at the top of the canopy by about 50% throughout canopy development (Fig. 1a). However, when both ambient and elevated [CO_2] grown leaves were measured at the same [CO_2], there were no

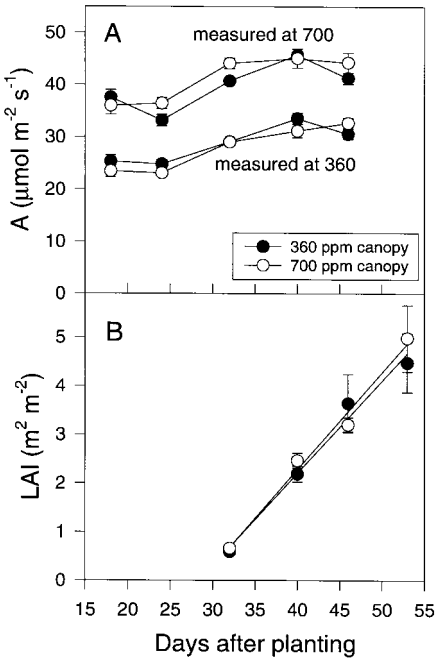

Fig. 1. Total canopy leaf area index (LAI) and photosynthetic rates (A), measured at light saturation and the indicated [CO_2] over the course of canopy development, for young, upper canopy sunflower leaves grown in ambient [CO_2] (filled symbols) or ambient + 350 ppm [CO_2] (open symbols). Sample size was 6 for each treatment and measurement time. Error bars represent standard error.

significant differences in their photosynthetic capacities per unit leaf area. Elevated [CO_2] also did not have any significant effect on total leaf area in the canopy (Fig. 1b). There was a significant ($P<0.001$) increase in high PFD photosynthetic rates at the top of both the 360 and 700 ppm canopies after canopy closure (at an LAI of around 2) (Fig. 1a). However, photosynthetic capacities of the older shaded leaves declined (Fig. 2a, b). This decline occurred more rapidly for shaded leaves on plants in the centre of the canopies (Fig. 2a) than for those on the south edge which all received full sun (Fig. 2b) (significant leaf position (edge/centre)/leaf number (height) interaction, $P<0.001$). Elevated [CO_2] also appeared to increase the rate of decline in photosynthetic capacity with increasing leaf age ([CO_2]/leaf number interaction, $P=0.065$). This [CO_2] effect was observed both for leaves in the centre of the canopy and on the south edge of the canopy and did not interact significantly with edge/centre position. In Fig. 2c, d, photosynthetic capacities are expressed as a percentage of the photosynthetic capacity when the leaves were young. These relative values differ slightly from the actual photosynthetic capacities since the photosynthetic capacities of the upper young leaves increased following canopy closure (Fig. 1a). With increasing depth in the canopy, and thus leaf age, photosynthetic capacity initially increased by about 10%, in all but the elevated [CO_2] edge plants, before declining.

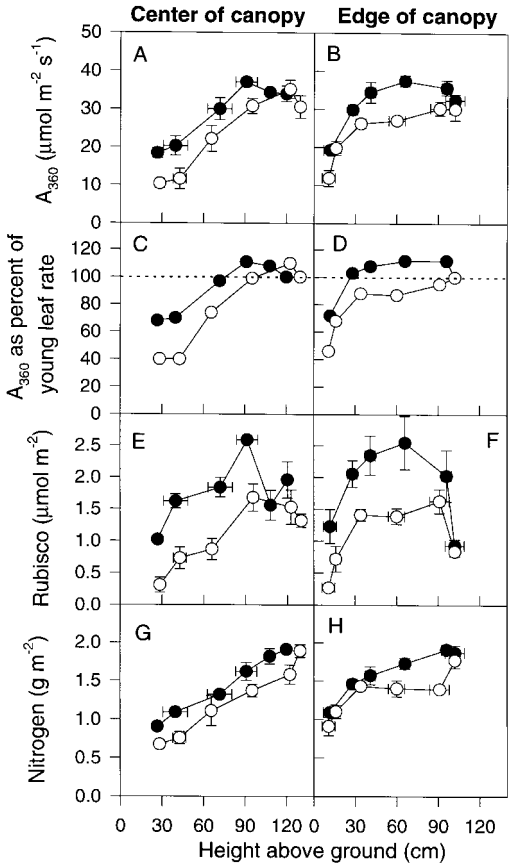

Fig. 2. Photosynthetic rate at light saturation and 360 ppm [CO_2] (A_{360}), A_{360} as a percentage of the mean rate for the same cohort of leaves measured when they were young, Rubisco content per unit leaf area, and nitrogen content per unit leaf area for leaves at six heights in the ambient (filled symbols) and ambient + 350 ppm [CO_2] (open symbols) canopies 48 d after planting. Centre canopy plants were entirely surrounded by other plants whereas the leaves measured on the canopy edge plants were exposed to full sun on the south edge of the canopy. Sample size was 3 for each treatment and canopy height. Error bars represent standard error.

For leaves on the south edge of the canopy this decline occurred only in the oldest leaves.

Rubisco protein levels responded in a manner similar to photosynthetic capacity in that there was no [CO_2] effect at the top of the canopies, but Rubisco content was reduced by elevated [CO_2] at lower levels of the canopies (Fig. 2e, f). However, Rubisco content and photosynthetic capacity were not entirely proportional. Rubisco content was reduced proportionally more by elevated [CO_2] than was photosynthetic capacity. In addition, there was a large increase in Rubisco content from the top of the canopy to mid-canopy without much change in the photosynthetic capacity. Reductions in photosynthetic capacity and Rubisco contents were accompanied by reductions in leaf nitrogen contents (Fig. 2g, h). A_{360} was well correlated with leaf nitrogen content ($A_{360} = 19.1 \times N + 0.32$, $r^2 = 0.73$, $P < 0.001$, data not shown) and this relationship was not affected by [CO_2] treatment.

Some of these changes in photosynthetic capacity and Rubisco content may have been driven by changes in PFD. PFD decreased rapidly with increasing depth in the canopy and this decrease was more rapid in the elevated [CO_2] canopy (Fig. 3a). However, there was no difference in PFD at the very bottom of the canopies. The difference between the [CO_2] treatments was not simply an effect of increased total leaf area since leaf area index (LAI) was only 11% higher in the elevated [CO_2] canopy at the final harvest and LAI was not significantly different at any point during canopy development (Fig. 1b). The difference in number of leaves per plant was also small; only 18% more leaves in the elevated [CO_2] canopy. However, there was a substantial difference in the distribution of leaf area, which had consequences for the light environment within the canopy. Leaves exposed to full sun on the edge of the canopy reached the same maximal leaf areas in the ambient and elevated chambers (Fig. 3c). However, elevated [CO_2] leaves reached that maximum sooner (note the 30–40% higher leaf areas for the uppermost two measured leaves in the elevated [CO_2] canopy, Fig. 3c). For shaded positions in the centre of the canopy, leaves on the ambient [CO_2] plants did not reach the same maximal leaf areas as the sunlit leaves on the edge of the canopy (compare Fig. 3b, c) whereas the elevated [CO_2] shaded leaves did reach the same leaf areas as the

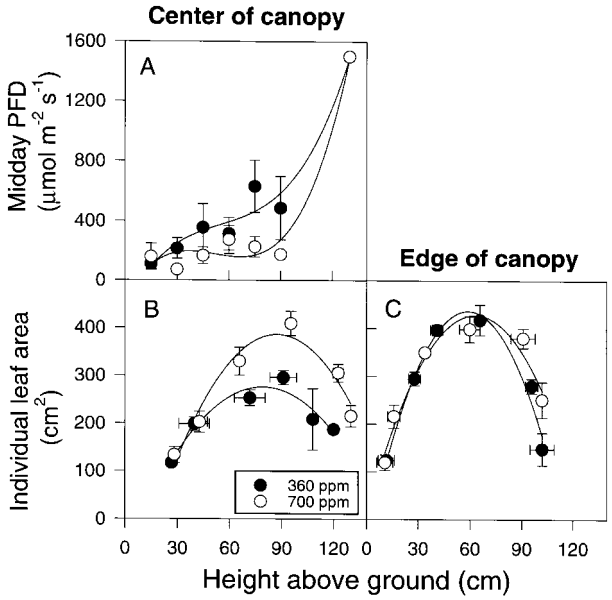

Fig. 3. Midday photon flux density (PFD) in the centre of the canopy and individual leaf areas of the leaves measured for photosynthetic capacity in the ambient (filled symbols) and ambient + 350 ppm [CO_2] (open symbols) canopies 48 d after planting. Centre canopy plants were entirely surrounded by other plants whereas the leaves measured on the canopy edge plants were exposed to full sun on the south edge of the canopy. Sample size for PFD was 6 for each treatment and canopy height. Sample size for leaf area was 3 for each treatment and canopy height. Error bars represent standard error. Lines were fitted with polynomial regression (3rd order in (A) and 2nd order in (B) and (C)).

sunlit edge leaves (significant $[CO_2]$/(edge/centre) interaction, $P<0.05$). Thus there was more leaf area in the upper portion of the elevated $[CO_2]$ canopy compared to the ambient $[CO_2]$ canopy, but not significantly different total LAI. This is consistent with the reduced PFDs in the upper portion of the elevated $[CO_2]$ canopy, but similar PFDs at the bottom of the canopies (Fig. 3a).

Since photosynthetic acclimation to elevated $[CO_2]$ has been proposed to be a response to increased carbohydrate concentrations, leaf carbohydrate concentrations were measured. Results are presented only for three leaf positions in the centre of the canopy that demonstrate the trends seen in the full data set. Due to the complexity of the observed responses and the limited power of the repeated measures design there was no significant $[CO_2]$ main effect. However, several trends are apparent. All carbohydrates appeared to be increased by elevated $[CO_2]$ at midday in leaves at the top and south edge of the canopies, i.e. the sunlit leaves (Fig. 4, note different scale for starch plots). Glucose contents were also increased by elevated $[CO_2]$ for old leaves near the bottom of the canopy (leaf 9). However, there was little effect on leaves at mid-levels of the canopy (leaf 15). Leaf fructose contents were generally proportional to the glucose contents (but averaging 34% lower, data not shown). In contrast to glucose and fructose, leaf sucrose and starch contents generally decreased with increasing depth in the canopy and this decline was more rapid in the elevated $[CO_2]$ canopy, resulting in sucrose and starch contents that were actually lower for elevated than for ambient $[CO_2]$ leaves near the bottom of the canopy (Fig. 4). Even for leaves at the top of the canopy, starch reserves were almost entirely utilized during the night resulting in low starch contents for both ambient and elevated leaves early in the morning.

PFD in the canopy appeared to explain part of the variation in leaf carbohydrate contents, but these relationships changed depending on the carbohydrate measured. Leaf glucose contents increased significantly ($P<0.05$) with PFD only for ambient $[CO_2]$ plants (Fig. 5a) whereas sucrose increased strongly ($P<0.01$) with PFD in both ambient and elevated $[CO_2]$ (Fig. 5b). Since this relationship was not affected by $[CO_2]$, the lower sucrose contents of mid-canopy leaves at elevated $[CO_2]$ appear to result from the lower PFDs in the elevated $[CO_2]$ canopy (Fig. 3a). Starch contents were also well correlated ($P<0.01$) with PFD but in contrast to sucrose, starch contents were higher at elevated than at ambient $[CO_2]$ for any given PFD (Fig. 5c).

Photosynthetic capacities and Rubisco contents were not significantly correlated with any of the individual carbohydrate contents. However, there was a strong negative correlation ($P<0.001$) between the ratio of hexoses (glucose + fructose) to sucrose and photosynthetic rate measured at 360 ppm $[CO_2]$ and light saturation (Fig. 6). This relationship was significant for all daily time points ($r^2=0.6$–0.8, $P<0.001$), but was strongest at 20.00 h. This relationship did not differ significantly between the $[CO_2]$ treatments or between leaves in the centre or on the edge of the canopy.

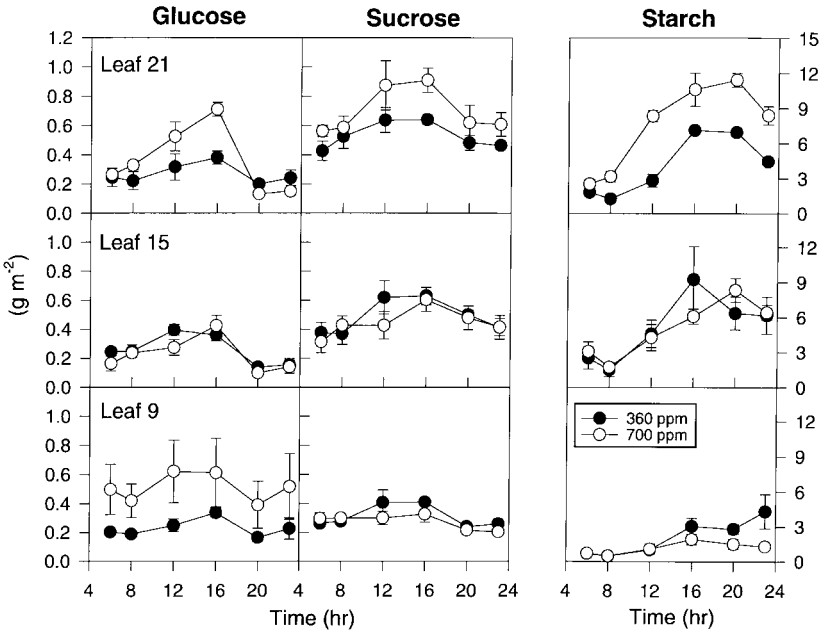

Fig. 4. Glucose, sucrose and starch contents per unit leaf area over a diurnal time-course for 3 of the leaves measured for photosynthetic capacity in the ambient (filled symbols) and ambient + 350 ppm $[CO_2]$ (open symbols) canopies 48 d after planting. Leaf numbers run from oldest to youngest. Data are presented only for centre canopy plants. Sample size was 3 for each treatment and canopy height. Error bars represent standard error.

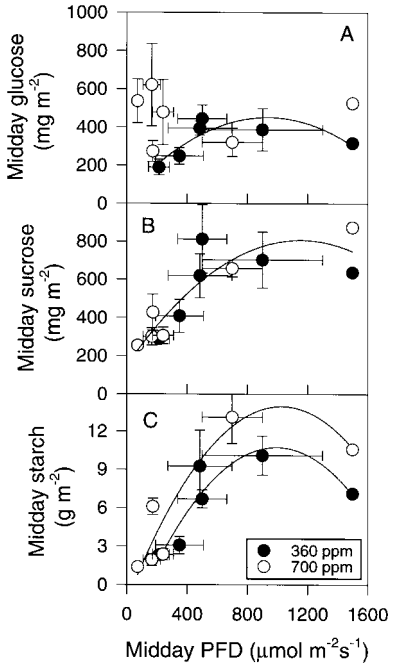

Fig. 5. Midday glucose, sucrose, and starch contents per unit leaf area as a function of midday PFD in the centre of the ambient [CO_2] canopy (filled symbols) and the ambient + 350 ppm [CO_2] canopy (open symbols) 48 d after planting. Sample size was 3 for each point and error bars represent standard error. Lines were fitted as second order polynomial regressions. No line is fitted to the elevated [CO_2] data in A since this relationship was not significant.

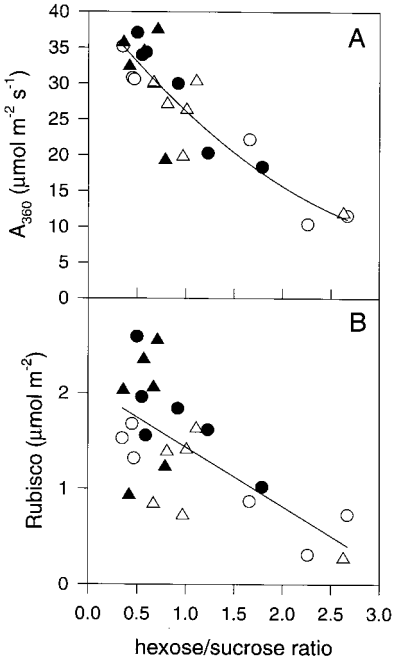

Fig. 6. Photosynthetic rate at light saturation and 360 ppm [CO_2] (A_{360}) and Rubisco content as a function of the ratio of hexose (glucose + fructose) to sucrose (all samples collected at 20.00 h) for ambient [CO_2] (filled symbols) or ambient + 350 ppm [CO_2] (open symbols) leaves and for centre (circles) or edge (triangles) leaves 48 d after planting. Lines were fitted as a second order polynomial regression in (A) and linear regression in (B).

Discussion

Photosynthetic acclimation to elevated [CO_2] in the sunflower canopy occurred only in older leaves. Photosynthetic capacities of older leaves in the elevated [CO_2] canopy were as much as 40% lower than those of similar aged leaves from the ambient [CO_2] canopy, but photosynthetic capacity of young leaves at the top of the canopy was not affected by [CO_2]. Similar results have been reported for wheat canopies (Nie *et al.*, 1995; Osborne *et al.*, 1998). These results also suggest that the [CO_2]/leaf age interaction was independent of the decline in PFD with increasing depth in the canopy. For both ambient and elevated [CO_2] canopies, exposure of older leaves to full sun on the south edge of the canopy maintained high photosynthetic capacities for a longer time compared to similar aged leaves in the centre of the canopy. However, down-regulation of photosynthetic capacity in elevated [CO_2] occurred to a similar extent with increasing leaf age regardless of the PFD to which the leaf was exposed. Consequently, these results for a plant canopy are consistent with those for isolated plants. Besford *et al.* (1990) reported that elevated [CO_2] resulted in more rapid attainment of maximum photosynthetic capacities in tomato leaves, but also resulted in more rapid loss of photosynthetic capacity with leaf age. Xu *et al.* (1994) also reported that photosynthetic acclimation to elevated [CO_2] occurred in old soybean leaves but not in young ones. However, they reported the opposite response for pea, i.e. more acclimation in young than in old leaves.

These data suggest that changes in photosynthetic capacity, both in response to elevated [CO_2] and leaf ageing, may be related to changes in leaf sugar metabolism. Although photosynthetic capacities were not correlated with any of the individual sugar concentrations, photosynthetic capacity was well correlated with leaf hexose/sucrose ratio and a single relationship was obtained for all of the treatment or leaf age conditions. Changes in hexose/sucrose ratio might have resulted from changes in acid invertase activity since hexose/sucrose ratio and acid invertase activity have been found to correlate in other studies (Zrenner *et al.*, 1996; Moore *et al.*, 1998). However, it is also possible that changes in production and utilization rates of sucrose and hexose interacted with a stable acid invertase capacity to result in the variable hexose/sucrose ratios. In either case, futile cycling of sucrose through acid invertase has been suggested to contribute to photosynthetic down-regulation under elevated [CO_2] (Moore *et al.*, 1998) and sink-limited conditions (Goldschmidt and Huber, 1992). Increased hydrolysis of sucrose by acid invertase would provide increased hexose substrates for hexokinase, which in turn plays a role in sugar repression of photosynthetic genes (Jang and Sheen, 1994; Koch, 1996).

Since photosynthetic capacities were maintained for a longer time in leaves exposed to full sun on the south edge of the canopy than in shaded leaves in the centre of the canopy, PFD also appears to be a factor in photosynthetic decline with leaf age. Similar conclusions have been drawn from studies of vines where self-shading was eliminated (Hikosaka, 1996). The mechanisms for this response are not entirely clear but may result from direct effects of PFD on gene transcription (Kloppstech, 1997). This decline in photosynthetic capacity with decreasing PFD in the canopy has been suggested to increase whole canopy photosynthetic efficiency since nitrogen can be reallocated from shaded leaves to leaves at the top of the canopy where PFDs and potential photosynthetic rates are higher. A steeper gradient in leaf nitrogen contents was found in the centre of the canopy rather than on the south edge and elevated [CO_2] further decreased nitrogen contents of lower leaves. However, since this [CO_2] effect was not accompanied by an increase in nitrogen contents or photosynthetic capacities of upper leaves, it is not clear whether there was a net benefit to the plants. Theoretical predictions suggest that elevated [CO_2] should not in fact change the optimal pattern of nitrogen distribution within a canopy (Hikosaka and Hirose, 1998). Alternatively, nitrogen saved by down-regulation of photosynthetic capacity might have been allocated to the roots.

No significant difference in LAI between the sunflower canopies grown at different [CO_2] was found in this study. This is in contrast to predictions by some authors that LAI would increase for elevated [CO_2] canopies because elevated [CO_2] reduces the light compensation point for photosynthesis and would thus stimulate leaf production and retention at lower levels in the canopy (Pearcy and Björkman, 1983; Long and Drake, 1991). However, actual results from other studies are inconsistent. Elevated [CO_2] resulted in increased LAI for canopies of perennial ryegrass (Nijs *et al*., 1988), soybean (Campbell *et al*., 1990) and rice (Rowland-Bamford *et al*., 1991) but had no effect on an artificial forest ecosystem (Körner and Arnone, 1992), rice stands (Ziska *et al*., 1996) and stands of *Abutilon* and *Ambrosia* (Hirose *et al*., 1996). The prediction that elevated [CO_2] would increase LAI because of reductions in the light compensation point depends on the assumption that elevated [CO_2] would result in greater leaf production and/or longer retention of leaves deep in the canopy. Since the variety of sunflower used in this study does not produce lateral branches and there was no leaf death during the course of this experiment, there may have been no possibility for this mechanism to operate in this experiment. An alternate hypothesis was presented by Hirose *et al*. (1996). They found that LAI was strongly correlated with above-ground nitrogen and proposed that elevated [CO_2] would only increase LAI when additional nitrogen uptake was possible. Similar results have been presented by Ziska *et al*. (1996) for rice. Total nitrogen uptake of the sunflowers in this study was the same in the ambient and elevated [CO_2] canopies (data not shown). Since no additional fertilizer was provided in this experiment, it is possible that the quantity of soil nitrogen limited leaf area production.

The decline in PFD at mid-levels of the elevated [CO_2] sunflower canopy resulted not from increases in total leaf area but rather from changes in the distribution of leaf area. There was more leaf area in the upper portions of the elevated [CO_2] canopy than in the ambient canopy. Similar trends can be seen in the data from other canopy studies. Wayne and Bazzaz (1997) found similar PFDs for ambient and elevated [CO_2]-treated birch seedling canopies at all levels except for the upper middle portion, where PFD was reduced in the elevated [CO_2] canopy. Hirose *et al*. (1996) found that the leaf area distribution of an Abutilon canopy was skewed toward the upper portion, but there was no significant difference in total leaf area or light penetration to the bottom of the canopy. It is suggested that the change in leaf area distribution resulted from a [CO_2] stimulation of leaf expansion and final leaf area development for shaded leaves. Several studies have found that elevated [CO_2] increases the rate of leaf expansion (Cure *et al*., 1989; Leadley and Reynolds, 1989; Ferris and Taylor, 1994; Gay and Hauck, 1994; Taylor *et al*., 1994; Gardner *et al*., 1995; Sims *et al*., 1998*a*). However, most studies report no effect of elevated [CO_2] on final leaf size (Leadley and Reynolds, 1989; Cure *et al*., 1989; Sims *et al*., 1998*a*). In the present experiment it appeared that there was a maximum leaf size, possibly determined by genetics, that was not attained when ambient [CO_2] leaves were shaded. Elevated [CO_2] may have provided the additional carbohydrate supply required for these leaves to attain their full size. This difference in leaf sizes was not observed for leaves at the bottom of the canopy that reached full expansion prior to canopy closure. The concept of a maximal leaf size is supported by the results of Allen *et al*. (1989) who reported a substantial decrease in leaf size when plants were grown at sub-ambient [CO_2], but little increase in leaf size for plants grown at elevated [CO_2]. Interestingly, a similar effect has been reported for whole birch seedlings growing in competition, i.e. elevated [CO_2] increases the size of the smaller individuals more than the larger ones resulting in a reduction in size variation within the population (Wayne and Bazzaz, 1997). They proposed that elevated [CO_2] resulted in greater growth stimulation of the smaller individuals because they were more shaded and thus carbon limited.

The differences in PFD within the canopy appeared to account for some unexpected carbohydrate results. Elevated [CO_2] generally increases sucrose and starch contents of leaves (Long and Drake, 1992). However, sucrose and starch contents were actually lower in many

of the older, shaded leaves in the elevated compared to the ambient [CO_2] canopy. This difference appeared to be a result of the lower PFDs at the mid-levels in the elevated [CO_2] canopy. Sucrose content was correlated with PFD and thus, presumably, to the photosynthetic rate of the leaves. Similar correlations between sucrose concentrations and photosynthetic rate/export have been reported for soybean (Thorne and Koller, 1974; Fader and Koller, 1983), tomato (Ho, 1976), *Salvia splendens* (Jiao and Grodzinski, 1996) and sugar beet (Servaites and Geiger, 1974). In addition, Grodzinski *et al*. (1998) measured 21 species and found a general correlation between photosynthetic rate and partitioning into total ethanol-soluble sugars. Starch content was a function of both the PFD and the [CO_2] concentration. The [CO_2] effect on starch content may have been a result of the reduced rate of photorespiration in leaves exposed to elevated [CO_2]. Morin *et al*. (1992), working with clover, found that starch accumulation increased when photosynthesis was increased by elevated [CO_2], but not when photosynthesis was increased a similar amount by an increase in light. The authors concluded that increased starch content at high [CO_2] was a result of reduced photorespiration and, consequently, reduced phosphate regeneration in the chloroplast rather than a limitation in carbohydrate utilization by the rest of the plant. Starch accumulation in leaves exposed to low [O_2], which reduces photorespiration, also supports this conclusion (Madore and Grodzinski, 1984).

Acknowledgements

We are indebted to the following people for development and maintenance of the EcoCELLs and the plants in this experiment: Dr Timothy Ball, Dr James Coleman, Dr Dale Johnson, David Schorran, Liz Sotoodeh, and Valerie Yturiaga. This work was supported by NSF-EPSCOR Cooperative Agreement No. EPS-9353227 and NSF Grant No. IBN 9420054 to JRS.

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