# Photosynthetic response of geranium to elevated $CO_2$ as affected by leaf age and time of $CO_2$ exposure

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Received November 1, 1990

KELLY, D. W., HICKLENTON, P. R., and REEKIE, E. G. 1991. Photosynthetic response of geranium to elevated $CO_2$ as affected by leaf age and time of $CO_2$ exposure. Can. J. Bot. **69**: 2482–2488.

Geranium plants were grown from seed in chambers maintained at 350 or 1000 $\mu L \cdot L^{-1}$ $CO_2$. Phtopsynthesis as affected by leaf age and by leaf position was determined. Elevated $CO_2$ enhanced photosynthesis to the greatest extent in middle-aged leaves; very young leaves exhibited little enhancement, and net photosynthesis in the oldest leaves was depressed by elevated $CO_2$. Temporary increases in net photosynthesis (relative to leaves developed at high $CO_2$) resulted when young leaves grown at 350 $\mu L \cdot L^{-1}$ $CO_2$ were switched to 1000 $\mu L \cdot L^{-1}$ $CO_2$. Leaves switched later in development exhibited permanent enhancement. Middle-aged leaves exhibited a temporary depression followed by permanent enhancement. Leaves developed at high $CO_2$ and switched to low $CO_2$ did not exhibit any photosynthetic depression relative to plants grown continuously at low $CO_2$. Similarly, leaves developed at low $CO_2$, switched to high $CO_2$ for various lengths of time, and returned to low $CO_2$ showed no photosynthetic depression. Leaves developed at low $CO_2$ and switched to high $CO_2$ exhibited increases in specific leaf weight and leaf thickness. The increase in leaf thickness was proportional to length of time spent at high $CO_2$. High $CO_2$ depressed the rate at which stomata developed but did not affect final stomatal density. Results suggest that photosynthesis at low $CO_2$ was limited by $CO_2$ regardless of developmental environment, whereas photosynthesis at high $CO_2$ was limited by the developmental characteristics of the leaf. Further, both biochemical and structural modifications appear to be involved in this response. Because of the very different responses of young versus old leaves, future studies should be careful to consider leaf age in assessing response to elevated $CO_2$.

*Key words*: carbon dioxide, elevated $CO_2$, photosynthesis, geranium.

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Des plants de géranium ont été cultivés à partir de graines dans des chambres où la teneur en $CO_2$ à 350 ou 1000 $\mu L \cdot L^{-1}$ de $CO_2$. L'activité photosynthétique a été mesurée en fonction de l'âge et de la position des feuilles. Une teneur élevée en $CO_2$ augmente le plus fortement la photosynthèse chez les feuilles d'âge moyen; les très jeunes feuilles ne montrent que peu d'augmentation, et la photosynthèse nette chez les feuilles les plus âgées diminue en présence de teneur élevée en $CO_2$. On observe une agumentation temporair. de la photosynthèse nette (comparativement aux feuilles qui se sont développées en présence de $CO_2$ élevé) chez les jeunes feuilles formées en présence de 350 et transposées à 1000 $\mu L \cdot L^{-1}$ de $CO_2$. Les feuilles transposées plus tard au cours de leur développement montrent une stimulation permanente. Les feuilles formées en présence de $CO_2$ élevées et transposées à la teneur plus faible en $CO_2$ ne montrent aucune diminution photosynthétique comparativement à celle des plantes continuellement maintenues en présence de $CO_2$ faible. De la même façon les feuilles qui se sont développées en présence de faible teneur en $CO_2$, lorsqu'elles sont transposées à la forte teneur en $CO_2$ pendant des durées différentes et retournées à la faible teneur, ne montrent aucune diminution de la photosynthèse. Les feuilles formées avec peu de $CO_2$ et transposées avec beaucoup de $CO_2$ montrent une augmentation en épaisseur et en poids. L'augmentation de l'épaisseur de la feuille est proportionnelle à la durée passée en présence du $CO_2$ élevé. Le $CO_2$ élevé diminue le taux de développement des stomates, mais n'affecte pas la densité finale de stomates. Les résultats suggèrent que la photosynthèse en présence de peu de $CO_2$ est limité par la $CO_2$ indépendamment du milieu de développement, alors que la photosynthèse en présence d'une forte teneur en $CO_2$ est limitée par les caractéristiques du développement foliaire. De plus, les modifications biochimiques aussi bien que structurales semblent impliquées dans cette réaction. Compte tenu des réactions très différentes des jeunes feuilles par rapport à celles qui sont plus âgées, les études ultérieures devraient prendre en considération l'âge des feuilles lorsqu'on évalue la réaction des plantes aux teneurs accrues en $CO_2$.

*Mots clés* : bioxyde de carbone, $CO_2$ accru, photosynthèse, géranium.

[Traduit par la rédaction]

## Introduction

One of the more striking conclusions of recent research has been the transient nature of $CO_2$-induced photosynthetic enhancement. While short-term $CO_2$ enrichment typically promotes net photosynthesis, long-term enrichment, lasting days to weeks, can result in a subsequent decline in net carbon assimilation (Kramer 1981). This response is correlated with a number of changes in plant biochemistry and morphology, including decreases in stomatal density (Woodward 1987), the formation of large starch granules in chloroplasts (Cave *et al.* 1981; Wulff and Strain 1982), increases in leaf thickness due to the formation of additional mesophyll cells (Thomas and

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Printed in Canada / Imprimé au Canada

Harvey 1983), a reduction in ribulose-1,5-bisphosphate carboxylase (RuBPCase) activity (Wong 1979), and increases in allocation to nonphotosynthetic structures (Acock and Allen 1985). Though all or any of these changes may contribute to the long-term decrease in photosynthetic response to elevated $CO_2$ the relative importance of each has not been determined.

As developmental stage of a leaf is likely to influence the extent to which biochemical and morphological changes may take place, it is expected that leaf age will have a marked effect upon response to photosynthesis to elevated $CO_2$. This study investigates three aspects of the relationship between leaf age and response to elevated $CO_2$. First, we examine how the relationship between photosynthesis and leaf age (Mooney *et al.* 1981) is influenced by development in a high-$CO_2$ environment. Although many studies have examined the response of recently expanded leaves to $CO_2$, there are few studies of how young expanding leaves or older mature leaves respond. Second, we examine the extent to which leaf age at time of exposure to elevated $CO_2$ may influence the response of photosynthesis. Given an understanding of developmental changes with leaf age, such information is potentially useful in interpreting the long-term response of photosynthesis to elevated $CO_2$. Third, we determine to what extent exposure to elevated $CO_2$ for various lengths of time may inhibit photosynthesis upon return to a low-$CO_2$ environment. The length of high-$CO_2$ exposure necessary to cause inhibition at low $CO_2$ and the duration of any inhibition provide information on the nature of these inhibitory effects.

## Materials and methods

*Plant culture*

Seeds of geranium (*Pelargonium* ×*hortorum* Baily cv. Ringo Rose) (Vesey's Seeds Ltd., York, P.E.I.) were planted in 10-cm diameter plastic pots containing 0.4 L potting soil (Nova Mix 300-S, Annapolis Valley Peat Co., Aylesford, N.S.). Soil was fertilized with controlled-release Nutricote 14:14:14, type 100 (4.5 kg/m$^3$), and Nutritrace a micronutrient supplement (0.5 kg/m$^3$) (Chisso-Ashai Fertilizer Co., Tokyo). Plants were held in controlled-environment growth chambers (model GR-36, Enconaire Systems Ltd., Winnipeg, Man.) under a 12-h photoperiod and a germinating temperature of 20°C. Seven days after planting, the night temperature was lowered to 15°C for the remainder of the experiment. Irradiance (25% input wattage incandescent – 75% cool white fluorescent) was initially maintained at 245 ± 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ incident photosynthetic photon flux (PPF). Thirty-two days after planting, PPF was increased to 330 ± 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During light periods, $CO_2$ levels were maintained at 350 $\mu L \cdot L^{-1}$ (low $CO_2$) or 1000 $\mu L \cdot L^{-1}$ (high $CO_2$). Concentrations were monitored by an infrared $CO_2$ analyzer (Lira 3000) drawing 30-s samples on a 180-s cycle (Lander Control Systems Inc., Orangeville, Ont.) Supplemental $CO_2$ was injected in to the chamber from a liquid $CO_2$ source. To minimize spatial effects within a chamber, plants were randomized weekly. Plants and $CO_2$ treatments were alternated between chambers each week to avoid confounding chamber effects with $CO_2$ treatments. All pots were watered daily as required.

*Experimental design*

Plants were culled to uniform size (based on height and number of leaves) to 108 plants in the low-$CO_2$ chamber and 12 plants in the high-$CO_2$ chamber. Plants were grown under these conditions for a period of 55 days, at which point switching treatments were initiated (designated day 0). Plants were randomly assigned to 1 of 20 $CO_2$ exposure treatments (Table 1), with six plants (replications) per treatment. One treatment at each $CO_2$ level served as controls (i.e., plants were grown continuously at either 350 or 1000 $\mu L \cdot L^{-1}$ $CO_2$). To examine the effect of development at high $CO_2$ on photosynthesis at low $CO_2$, a third treatment was switched from the high-$CO_2$ regime to low $CO_2$ on day 0. To study photosynthetic acclimation to elevated $CO_2$ and the effect of leaf age on this acclimation, additional treatment groups were switched from low to high $CO_2$ on days 0, 4, 7, 11, 14, 21, 28, 35, and 42. The final eight treatments groups were switched from low to high $CO_2$ on day 0. Each treatment day (see below) one of these groups was returned to low $CO_2$. By examining the subsequent performance of these plants at low $CO_2$ we could determine the effect of varying lengths of exposure to high $CO_2$ on leaves developed at low $CO_2$.

TABLE 1. Summary of $CO_2$ exposure treatments

| $CO_2$ treatments | Day of switch |
|---|---|
| Ccontrol 1000 | — |
| Control 350 | — |
| 1000 moved to 350 | 0 |
| 350 moved to 1000 | 0, 4, 7, 11, 14, 21, 28, 35, 42 |
| 1000 return to 350 | 4, 7, 11, 14, 21, 28, 35, 42 |

NOTE: Plants were grown continuously at either 350 or 1000 $\mu L \cdot L^{-1}$ $CO_2$ for the first 55 days after seeding. At that point (designated day 0), the 20 $CO_2$ exposure treatments were initiated.

*Experimental measurements*

Photosynthetic measurements were initiated on day 0 (i.e., 55 days after seeding). On day 0, the youngest leaf on which it was practical to make gas-exchange measurements was selected as the experimental leaf (single-leaf series). The leaf plastochron index (LPI) (Erickson and Michelini 1957) of experimental leaves was between 1 and 2 using a reference length of 30 mm. Gas-exchange measurements of experimental leaves were made on days 0, 4, 7, 11, 14, 21, 35, and 42 (i.e., each day plants were switched between $CO_2$ levels, with the exception of day 28 owing to technical difficulties).

At the end of the experiment (day 48), photosynthetic measurements of all main-stem leaves on a single plant (single-plant series) were made at final growth concentrations. Treatments measured were continuous low-$CO_2$ control, continuous high-$CO_2$ control, switched from low to high $CO_2$ on day 14, and switched from low to high $CO_2$ on day 28.

Net photosynthesis was measured with a portable gas exchange system (model LCA-2, Analytical Development Co. Ltd., Hoddesdon, England) under chamber conditions. Measurements on a given day were initiated 2 h after the start of the light period and were completed within a 4-h period. The system was calibrated daily using a primary standard calibration gas (395 $\mu L \cdot L^{-1}$ $CO_2$). For photosynthetic measurements at 1000 $\mu L \cdot L^{-1}$ $CO_2$, a mixture of compressed ambient air and 1200 $\mu L \cdot L^{-1}$ $CO_2$ in air was supplied to the unit through a gas proportioner. Water vapour was removed from supply gases before mixing by passage through magnesium perchlorate. The area contained within the leaf chamber ws 6.25 cm$^2$.

Early in the experiment (day 4) leaf tissue from the two $CO_2$ controls was prepared for observation with the scanning electron microsope according to the procedure of Tanaka (1981) as modified by Barnes and Blackmore (1984). At the end of the experiment (day 50) tissue from the two controls, in addition to treatments switched from low to high $CO_2$ throughout the experiment, was prepared in identical fashion. All prepared tissue from a single treatment was pooled. Stomatal density and leaf density and leaf thickness on three subsamples from each treatment were measured under a Jeol field emission scanning electron microscope at an accelerating voltage of 15 kV.

To determine differences in developmental rate among $CO_2$ treatments, leaf plastochron values of experimental leaves were remeasured on days 9, 23, 33, and 40.

Plants were harvested on day 50. Leaf area was measured using a LICOR leaf area meter (model Li-3000), and plant material was dried at 70°C for 48 h. Specific leaf weight (SLW; leaf biomass per unit leaf area, g/cm$^2$) was calculated for each treatment.

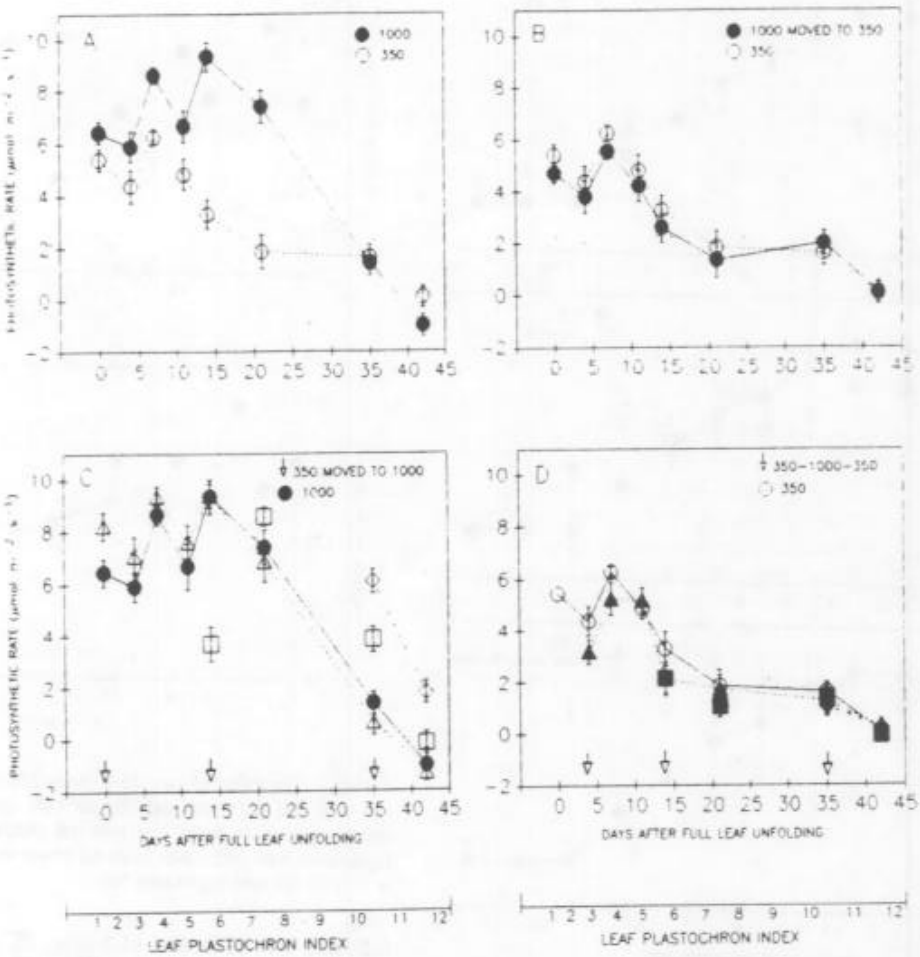


FIG. 1. Net photosynthesis of a leaf (A) grown at 350 (○) or 1000 (●) $\mu L \cdot L^{-1}$ $CO_2$ (photosynthesis measurd at growth concentration); (B) grown continuously at 350 $\mu L \cdot L^{-1}$ $CO_2$ (○), or switched from 1000 to 350 $\mu L \cdot L^{-1}$ $CO_2$ (●) at leaf expansion (photosynthesis measured at 350 $\mu L \cdot L^{-1}$ $CO_2$); (C) grown continuously at 1000 $\mu L \cdot L^{-1}$ $CO_2$ (●) or switched 0 (△), 14 (□), or 35 (◇) days after leaf expansion from 350 to 1000 $\mu L \cdot L^{-1}$ $CO_2$ (photosynthesis measured at 1000 $\mu L \cdot L^{-1}$ $CO_2$); and (D) grown continuously at 350 $\mu L \cdot L^{-1}$ $CO_2$ (○) or returned to 350 following 4 (▲), 14 (■), or 35 (◆) days at 1000 $\mu L \cdot L^{-1}$ $CO_2$ (photosynthesis measured at 350 $\mu L \cdot L^{-1}$ $CO_2$). Arrows on *x*-axis indicate day of switch. Stage of leaf development is given by leaf plastochron index scale at bottom. Mean values ± SE ($n$ = 6).

*Data analysis*

Data were analyzed by analysis of variance using the General Linear Models (GLM) procedure of SAS (version 6, for personal computers). The experimental design was completely random, and $CO_2$ treatment was the single factor in the analysis. Separate analyses were conducted for each measurement day. For the single-plant series, separate analyses were conducted for each plastochron value. Leaf plastochron indices were expressed to the nearest 0.5 plastochron. All tests of significance were based on the 0.05 level of probability. Anatomical measurements obtained with the scanning electron microscope were analysed by linear regression analysis. Regression was used to describe the relationship between time at low $CO_2$ and leaf thickness or stomatal density.

## Results

*Leaf gas exchange*

Photosynthetic rate of plants grown in continuous high and continuous low $CO_2$ did not differ early or late in the experiment, but continuous high $CO_2$ had significantly greater photosynthetic rates from day 7 until 21 (Fig. 1A). Net $CO_2$ exchange was negative in both treatments by day 42. In the single-plant series, the two oldest leaves measured (LPI = 10.5 and 11.5) in both control regimes had negative $CO_2$ exchange rates (Fig. 2A). In these leaves, net carbon loss was greater in those grown continuously at high $CO_2$. Only the four youngest leaves (LPI = 1.5, 2.5, 3.5, and 4.5) of plants continuously grown at 1000 $\mu L \cdot L^{-1}$ $CO_2$ possessed enhanced photosynthetic rates.

Leaf development at high $CO_2$ did not result in photosynthetic inhibition upon transfer to low $CO_2$, whether measured on day 0 or subsequently (Fig. 1B). In contrast, the complementary switch from low to high $CO_2$ on day 0 did reveal significant differences (Fig. 1C). Subsequent switches to elevated $CO_2$ revealed that the response to high $CO_2$ was dependent upon developmental stage. Plants switched to 1000 $\mu L \cdot L^{-1}$ $CO_2$ on day 7 responded identically to those switched on day 0 (data not shown for switch on day 7). That is, both switched treatments had significantly greater photosynthetic rates only on the day plants were switched. Plants switched from low to high $CO_2$ on day 14 and 21 experienced lower photosynthetic rates than the high-$CO_2$ control (data not

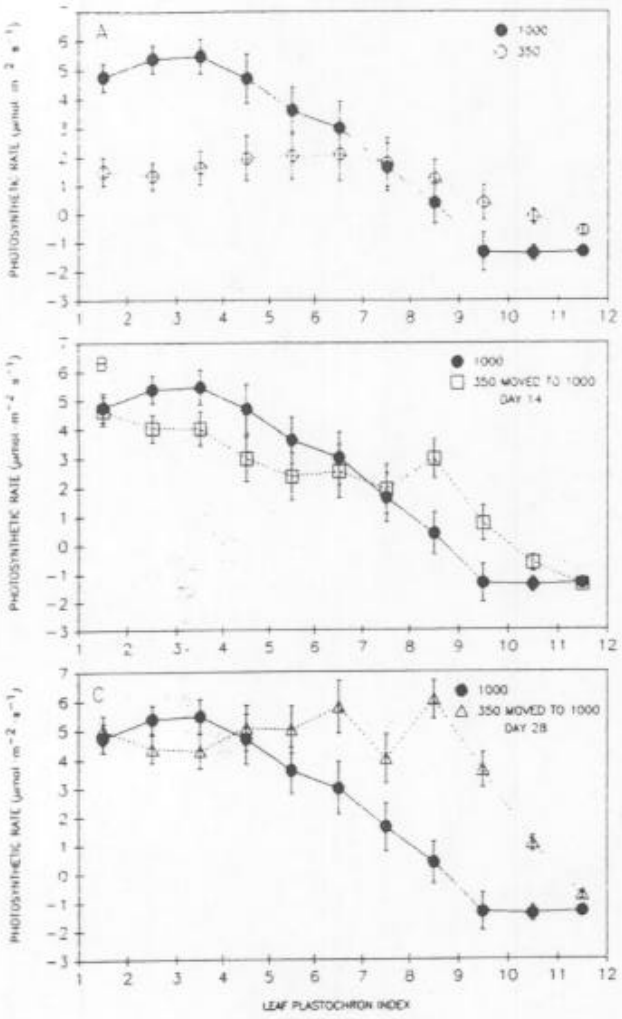


FIG. 2. Photosynthetic rates of leaf on the main stem of mature plants grown continuously at 1000 $\mu L \cdot L^{-1}$ $CO_2$ (●) or (A) grown continuously at 350 $\mu L \cdot L^{-1}$ $CO_2$ (○); (B) grown until day 14 at 350 $\mu L \cdot L^{-1}$ $CO_2$ before removal to 1000 $\mu L \cdot L^{-1}$ $CO_2$ (□); or (C) grown until day 28 at 350 $\mu L \cdot L^{-1}$ $CO_2$ before removal to 1000 $\mu L \cdot L^{-1}$ $CO_2$ (△). Photosynthetic measurements were made at final growth concentration. Mean values ± SE ($n = 6$).

shown for switch on day 21). Nevertheless, by the following measurement day, rates for these two switched treatments were significantly greater than the continuous high-$CO_2$ treatment. Unlike the photosynthetic response of younger leaves, these rates remained greater than the high-$CO_2$ control over the entire period of photosynthetic gain. After the rapid photosynthetic decline in the high-$CO_2$ control, subsequent switches from low to high $CO_2$ (days 35 and 42) possessed greater photosynthetic rates immediately, retaining this advantage until senescence (data not shown for switch on day 42).

The single-plant series revealed similar patterns. On plants switched to high $CO_2$ on day 14, leaves with a final LPI of 8.5 and 9.5 had higher photosynthetic rates than plants grown continuously at high $CO_2$ (Fig. 2B). At the time of switch these leaves had an approximate LPI of 3 and 4, respectively (data not shown). Likewise, plants switched to high $CO_2$ on day 28 had higher photosynthetic rates on leaves with a final LPI of

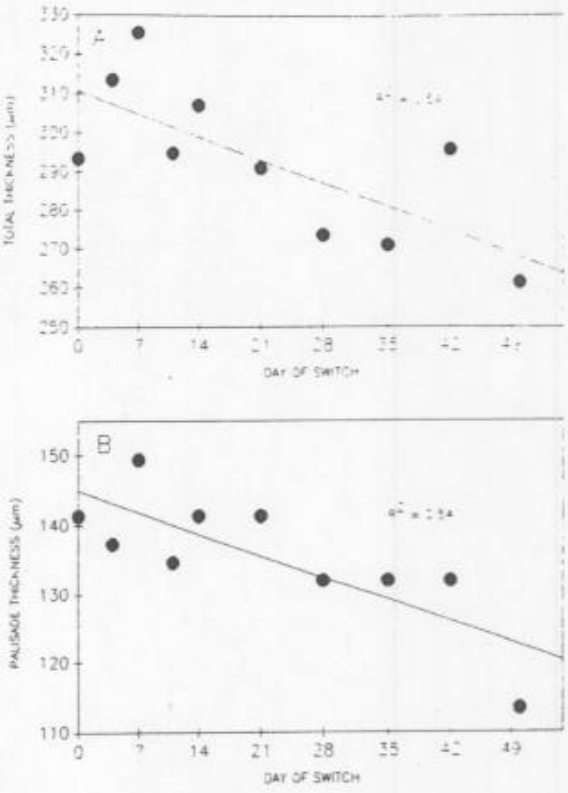


FIG. 3. The effect of switching from 350 to 1000 $\mu L \cdot L^{-1}$ $CO_2$ on (A) total leaf thickness and (B) palisade layer thickness. Measurements were taken from leaf material prepared on day 50. Linear regressions were calculated from the treatment means. $R^2 = 0.54$ and $\alpha < 0.05$ for both regression lines.

6.5, 8.5, 9.5, 10.5, and 11.5 (Fig. 2C). On day 28 the LPIs of these leaves were 4, 6, 7, 8, and 9, respectively (data not shown). In both circumstances, only leaves that had developed at least three plastochrons at low $CO_2$ possessed enhanced photosynthetic rates when switched from 350 to 1000 $\mu L \cdot L^{-1}$ $CO_2$. This enhancement was retained until the end of the experiment.

There was no reduction in photosynthetic potential in low-$CO_2$ developed leaves temporarily exposed to high $CO_2$ (Fig. 1D). Photosynthetic rates upon return to low $CO_2$, after high $CO_2$ exposures of 4 to 35 days, did not differ from ambient controls. The treatment returned to low $CO_2$ on day 42 had a lower photosynthetic rate than the control (data not shown); however, net photosynthesis was negative.

*Leaf development*

By design, experimental leaves initially had similar leaf plastochron indices ($1 < LPI \leq 2$). Subsequent comparisons of LPI among treatments revealed that elevated $CO_2$ exposure had no effect on the rate of leaf unfolding (data not shown). Continuous $CO_2$ enrichment did, however, significantly increase specific leaf weight from $7.80 \times 10^{-3}$ g/cm$^2$ at low $CO_2$ to $9.40 \times 10^{-3}$ g/cm$^2$ at high $CO_2$.

Leaves switched to high $CO_2$ shortly after expansion became thicker than treatments switched later in development (Fig. 3A). This was due, in part, to the response of the pali-

TABLE 2. Stomatal density (mm $^{-2}$) of leaves developed in ambient or $CO_2$-enriched atmospheres (350 or 1000 $\mu L \cdot L^{-1}$ $CO_2$) at an early and late stage of development

| $CO_2$ treatment ($\mu L \cdot L^{-1}$) | Leaf plastochron index | Stomatal density | |
|---|---|---|---|
| | | Adaxial | Abaxial |
| 350 | 2.5 | 34.50*a* | 114.93*ab* |
| 1000 | 2.5 | 17.23*b* | 86.20*a* |
| 350 | 12.5 | 23.00*ab* | 135.03*b* |
| 1000 | 12.5 | 30.20*ab* | 123.53*b* |

NOTE: Means within one column followed by different letters are significantly different at the 0.05 level of probability.

sade layer, which increased thickness with lengthening exposures to elevated $CO_2$ (Fig. 3B).

Final stomatal density was not dependent upon total exposure to elevated $CO_2$ (Table 2). However, tissue from the high-$CO_2$ control examined at the beginning of the experiment did have a lower stomatal density than either the low-$CO_2$ control examined at the same time or the high-$CO_2$ control examined at the experiment's completion.

## Discussion

*Leaf age response*

In both the single-leaf and the single-plant series, middle-aged leaves were the most responsive to $CO_2$ enrichment. Indeed, in the single-leaf series, $CO_2$ enrichment did not enhance photosynthesis of very young or very old leaves (Fig. 1A). In the single-plant series, net gas exchange of the oldest leaves of high-$CO_2$ plants was actually lower than that of low-$CO_2$ plants by day 48. Few previous studies have examined the effect of leaf age upon response to elevated $CO_2$, but Hicklenton and Jolliffe (1980) and Havelka *et al.* (1984) also report that older leaves do not respond as well as young, fully unfolded leaves.

*Acclimation to $CO_2$ concentration*

Plants switched from high to low $CO_2$ on day 0 failed to show any significant photosynthetic depression. On the other hand, the reciprocal switch from low to high $CO_2$ produced both rate enhancement and in the case of middle-aged leaves, short-term photosynthetic depression. These findings suggest, as others have (Tolbert and Zelitch 1983), that plants measured at 350 $\mu L \cdot L^{-1}$ $CO_2$ are limited by $CO_2$ concentration, regardless of developmental environment. Where photosynthetic enhancement is shown at 1000 $\mu L \cdot L^{-1}$ $CO_2$ by plants developed at low $CO_2$ it appears that leaves developed at high-$CO_2$ were limited by the photosynthetic apparatus of the leaf rather than by $CO_2$. It is possible that leaf physiology as well as biochemical and structural modifications of the photosynthetic apparatus plays a role in these responses. The rate depression in middle-aged leaves switched from low to high $CO_2$ may be due to short-term stomatal closure, although it is not clear why young and old leaves do not show a similar response. Where short-term photosynthetic enhancement was evident (e.g., plants moved to high $CO_2$ at day 0; Fig. 1), biochemical rather than structural adjustments are suggested, since structural modifications would take longer than 4 days (Mauney *et al.* 1979). On the other hand, long-term enhancement in older leaves (e.g., plants moved on days 14, 21, 35, and 42; Fig. 1) could also be due to permanent structural differences between leaves developed at low and high $CO_2$. The exact nature of these biochemical and structural modifications is beyond the scope of this study, but the literature and the data suggest several possibilities.

It has been suggested that reductions in RuBPCase levels with elevated $CO_2$ may be responsible for decreases in photosynthetic enhancement over time. Although three of five species recently studied provide little evidence that RuBPCase plays any role in the acclimation process, two weedy species in the same study do not dismiss the possibility (Sage *et al.* 1989). If RuBPCase is more abundant at low $CO_2$ than at high $CO_2$, a higher photosynthetic rate at elevated $CO_2$ may result. However, once a plant from low $CO_2$ is maintained at high $CO_2$ for a period of time, enzyme pools may reduce to the level of plants developed in that environment. Thus, a transient response of less than 4 days could be expected.

Many plant species accumulate starch in response to $CO_2$ enrichment (Madsen 1968; Hofstra and Hesketh 1975; Nafziger and Koller 1976; Cave *et al.* 1981; Wulff and Strain 1982; Ackerson *et al.* 1984; DeLucia *et al.* 1985; Ehret and Jolliffe 1985; Sasek *et al.* 1985). Several studies have concluded that excess starch is responsible for photosynthetic inhibition at elevated $CO_2$ via disruption of chloroplast membranes (Cave *et al.* 1981; Wulff and Strain 1982), end-product inhibition (Wulff and Strain 1982; Sasek *et al.* 1985), or increases in mesophyll resistance (Hofstra and Hesketh 1975). Although elevated starch levels in plants removed from $CO_2$ enrichment may deplete within 3 days (Sasek *et al.* 1985; Hofstra and Hesketh 1975), repair of chloroplast membrane damage would likely take much longer. For this reason, photosynthetic depression on return to low $CO_2$ was hypothesized to be proportional to the duration of elevated $CO_2$ exposure. This, however, was not observed (Fig. 1). At no point during active photosynthesis were rates of groups returned to low $CO_2$ comparatively lower. It is quite possible that during the 12-h dark period, starch that had accumulated during the light period was utilized, preventing a cumulative effect (DeLucia *et al.* 1985). If irradiance or photoperiod had been increased, quite different responses may have resulted.

An increase in leaf thickness with increasing $CO_2$ exposure has often been observed (Madsen 1968; Hofstra and Hesketh 1975; Downton *et al.* 1980; Thomas and Harvey 1983; Leadley *et al.* 1987), though it is not universal (Ehret and Jolliffe 1985). Downton *et al.* (1980) speculate that increased diffusional resistance, resulting from greater leaf thickness, is responsible for photosynthetic differences between plants grown at high and low $CO_2$. Until day 14, switches of the experimental leaf from low to high $CO_2$ produced, at most, a temporary photosynthetic stimulation. Switching older leaves resulted in permanent effects (Fig. 1). The response of plants switched in the single-leaf series was in accord with that of the single-plant series (Fig. 2). Only leaves that had developed at low $CO_2$ for a period of at least three plastochrons maintained photosynthetic stimulation at high $CO_2$. Although no causal relationship was demonstrated in this study, the duration of enhancement may have been dependent upon leaf thickness. As leaves that were switched later in development were not as thick as leaves switched earlier (Fig. 3), mesophyll resistance may have been reduced sufficiently to maintain a photosynthetic advantage.

Leaf stomatal density does not respond to $CO_2$ in a predictable fashion. O'Leary and Knecht (1981) showed a significantly lower stomatal density under high $CO_2$ on one leaf surface but not on the other. Woodward (1987) found that sto-

matal density did not respond to $CO_2$ levels exceeding the current level. Others have observed inconsistent effects (Ford and Thorne 1967). In this study, no dependence of final stomatal density upon exposure to high $CO_2$ was observed. Thus, stomatal density was not thought to contribute to the differential photosynthetic responses observed among control and switch treatments. There were, however, differences in stomatal density between controls at a young age. Previous studies show stomata continue to differentiate for some time as the leaf develops (Meidner and Mansfield 1968). Thus, elevated $CO_2$ may possibly have postponed stomatal development. A temporarily greater stomatal density at low $CO_2$ could possibly promote the short-term photosynthetic enhancement of young, low-$CO_2$ developed leaves moved to high $CO_2$, assuming that these leaves are limited by stomatal conductance at high $CO_2$.

## Conclusion

Our study reveals some important relationships between leaf age and the response to elevated $CO_2$. Under our growth conditions, chloroplast damage resulting from starch accumulation was probably not responsible for the long-term decrease in net photosynthetic enhancement. We suspect moderate irradiance prevented excessive starch buildup. The long-term decrease in photosynthetic enhancement at high $CO_2$ appears to be the result of acclimatory processes, as older leaves moved to high $CO_2$ do not show this decrease. It is not clear what these acclimatory changes are, but there are probably several judging from that fact that middle-aged leaves have a different response than both older leaves and younger leaves. Acclimatory changes with leaf age may involve both biochemical and morphological responses, such as leaf thickness, stomatal development, and RuBPCase levels. Because of the very different responses of young versus old leaves, future studies should be careful to consider leaf age in assessing response to elevated $CO_2$.

## Acknowledgements

This study was supported by Agriculture Canada, the Nova Scotia Greenhouse Growers Association, and the Natural Sciences and Engineering Research Council of Canada. We thank Julia Reekie for technical assistance.

ACKERSON, R. C., HAVELKA, U. D., and BOYLE, M. G. 1984. $CO_2$-enrichment effects on soybean physiology. II. Effects on stage-specific $CO_2$ exposure. Crop Sci. **24**: 1150–1154.

ACOCK, B., and ALLEN, L. H. 1985. Crop responses to elevated carbon dioxide concentrations. *In* Direct effects of increasing carbon dioxide on vegetation. *Edited by* B. R. Strain and J. D. Cure. U.S. Department of Energy, Washington, DC. pp. 53–97.

BARNES, A. J., and BLACKMORE, S. 1984. Scaning electron microscoy of chloroplast ultrastructure. Micron Microsc. Acta. **15**: 187–194.

CAVE, G., TOLLEY, L. C., and STRAIN, B. R. 1981. Effect of carbon dioxide enrichment on chlorophyll content, starch content, and starch grain structure in *Trifolium subterraneum* leaves. Physiol. Plant. **51**: 171–174.

DELUCIA, E. H., SASEK, T. W., and STRAIN, B. R. 1985. Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosynth. Res. **7**: 175–184.

DOWNTON, W. J., BJORKMAN, O. S., and PIKE, C. S. 1980. Consequences of increased atmospheric concentrations of carbon dioxide for growth and photosynthesis of higher plants. *In* Carbon dioxide and climate: Australian research. *Edited by* G. I. Pearman. Australia Academy of Science, Canberra. pp. 143–151.

EHRET, D. L., and JOLLIFFE, P. A. 1985. Photosynthetic carbon dioxide exchange of bean plants grown at elevated carbon dioxide concentrations. Can. J. Bot. **63**: 2026–2030.

ERICKSON, R. O., and MICHELINI, F. J. 1957. The plastochron index. Am. J. Bot. **44**: 297–305.

FORD, M. A., and THORNE, G. N. 1967. Effect of $CO_2$ concentration on growth of sugar-beet, barley, kale, and maize. Ann. Bot. (London), **31**: 629–644.

HAVELKA, U. D., ACKERSON, R. C., BOYLE, M. G., and WITTENBACH, V. A. 1984. $CO_2$-enrichment effects on soybean physiology. I. Effects of long-term $CO_2$ exposures. Crop Sci. **24**: 1146–1150.

HICKLENTON, P. R., and JOLLIFFE, P. A. 1980. Alterations in the physiology of $CO_2$ exchange in tomato plants grown in $CO_2$ enriched atmospheres. Can. J. Bot. **58**: 2181–2189.

HOFSTRA, G., and HESKETH, J. D. 1975. The effects of temperature and $CO_2$ enrichment on photosynthesis in soybean. *In* Environmental and biological control of photosynthesis. *Edited by* R. Marcelle. Dr. W. Junk Publishers, The Hague. pp. 71–80.

KRAMER, P. J. 1981. Carbon dioxide concentration, photosynthesis, and dry matter production. BioScience, **31**: 29–34.

LARIGUADERIE, A., HILBERT, D. W., and OECHEL, W. C. 1988. Effect of carbon dioxide enrichment and nitrogen availability on resource acquisition and resource allocation in a grass. *Bromus mollis*. Oecologia, **77**: 544–549.

LEADLEY, P. W., REYNOLDS, J. A., THOMAS, J. F., and REYNOLDS, J. F. 1987. Effects of $CO_2$ enrichment on internal leaf surface area in soybeans. Bot. Gaz. **148**: 137–140.

MADSEN, E. 1968. Effects of $CO_2$ concentration on the accumulation of starch and sugar in tomato leaves. Physiol. Plant. **21**: 168–175.

MAUNEY, J. R., QUINN, G., FRY, K. E., and HESKETH, J. D. 1979. Correlation of photosynthetic carbon dioxide uptake and carbohydrate accumulation in cotton, soybean, sunflower, and sorghum. Photosynthetica (Prague), **13**: 260–266.

MOONEY, H. A., FIELD, C., GULMON, S. L., and BAZZAZ, F. A. 1981. Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. Oecologia, **50**: 109–112.

MEIDNER, H., and MANSFIELD, T. A. 1968. Physiology of stomata. McGraw-Hill, London.

NAFZIGER, E. D., and KOLLER, H. R. 1976. Influence of leaf starch concentration on carbon dioxide assimilation in soybean. Plant Physiol. **57**: 560–563.

O'LEARY, J. W., and KNECHT, G. N. 1981. Elevated $CO_2$ concentration increases stomate numbers in *Phaseolus vulgaris* leaves. Bot. Gaz. **142**: 438–441.

OSMAN, A. M., and MILTHORPE, F. L. 1971. Photosynthesis of wheat leaves in relation to leaf age, illuminance, and nutrient supply. II. Results. Photosynthetica (Prague), **5**: 61–70.

PEET, M. M., HUBER, S. C., and PATTERSON, D. T. 1986. Acclimation to high $CO_2$ in monoecious cucumbers. Plant Physiol. **80**: 63–67.

PORTER, M. A., and GRODZINSKI, B. 1984. Acclimation to high $CO_2$ in bean. Plant Physiol. **74**: 413–416.

SAGE, R. F., PEARCY, R. W., and SEEMAN, J. R. 1987. The nitrogen use efficiency of $C_3$ and $C_4$ plants. Plant Physiol. **85**: 355–359.

SAGE, R. F., SHARKEY, T. D., and SEEMAN, J. R. 1988. The in-vivo response of the ribulose-1.5-bisphosphate carboxylase activation state and the pool sizes of the photosynthetic metabolites to elevated carbon dioxide in *Phaseolus vugaris*. Planta, **174**: 407–416.

——— 1989. Acclimation of photosynthesis to elevated $CO_2$ in five $C_3$ species. Plant Physiol. **89**: 590–596.

SASEK, T. W., DELUCIA, E. H., and STRAIN, B. R. 1985. Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated $CO_2$ concentrations. Plant Physiol. **78**: 619–622.

TANAKA, K. 1981. Demonstration of intracellular structures by high resolution scanning electron microscopy. Scanning Electron Microsc. **2**: 1–8.

THOMAS, J. F., and HARVEY, C. N. 1983. Leaf anatomy of four species grown under continuous $CO_2$ enrichment. Bot. Gaz. **144**: 303–309.

TOLBERT, N. E., and ZELITCH, I. 1983. Carbon Metabolism. *In* Carbon dioxide and plants: The response of plants to rising levels of carbon dioxide. *Edited by* E. R. Lemon. Westview Press, Inc., Boulder, CO. pp. 21–64.

VU, C. V., ALLEN, L. H., and BOWES, G. 1983. Effects of light and elevated $CO_2$ on the ribulose bisphosphate carboxylase activity and ribulose bisphosphate level of soybean leaves. Plant Physiol. **73**: 729–734.

WONG, S. C. 1979. Elevated atmospheric partial pressure of $CO_2$ and plant growth. Oecologia, **44**: 68–74.

WOODWARD, F. I. 1987. Stomatal numbers are sensitive to increases in $CO_2$ from pre-industrial levels. Nature (London), **327**: 617–618.

WULFF, R. D., and STRAIN, B. R. 1982. Effects of $CO_2$ enrichment on growth and photosynthesis in *Desmodium paniculatum*. Can. J. Bot. **60**: 1084–1091.