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# Adjustments of net photosynthesis in *Solanum tuberosum* in response to reciprocal changes in ambient and elevated growth $CO_2$ partial pressures

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**Single leaf photosynthetic rates and various leaf components of potato (*Solanum tuberosum* L.) were studied 1–3 days after reciprocally transferring plants between the ambient and elevated growth $CO_2$ treatments. Plants were raised from individual tuber sections in controlled environment chambers at either ambient (36 Pa) or elevated (72 Pa) $CO_2$. One half of the plants in each growth $CO_2$ treatment were transferred to the opposite $CO_2$ treatment 34 days after sowing (DAS). Net photosynthesis ($P_n$) rates and various leaf components were then measured 34, 35 and 37 DAS at both 36 and 72 Pa $CO_2$. Three-day means of single leaf $P_n$ rates, leaf starch, glucose, initial and total Rubisco activity, Rubisco protein, chlorophyll ($a+b$), chlorophyll ($a/b$), α-amino N, and nitrate levels differed significantly in the continuous ambient and elevated $CO_2$ treatments. Acclimation of single leaf $P_n$ rates was partially to completely reversed 3 days after elevated $CO_2$-grown plants were shifted to ambient $CO_2$, whereas there was little evidence of photosynthetic acclimation 3 days after ambient $CO_2$-grown plants were shifted to elevated $CO_2$. In a four-way comparison of the 36, 72, 36 to 72 (shifted up) and 72 to 36 (shifted down) Pa $CO_2$ treatments 37 DAS, leaf starch, soluble carbohydrates, Rubisco protein and nitrate were the only photosynthetic factors that differed significantly. Simple and multiple regression analyses suggested that negative changes of $P_n$ in response to growth $CO_2$ treatment were most closely correlated with increased leaf starch levels.**

## Introduction

Prior studies have established that growth in elevated atmospheric $CO_2$ usually resulted in increased rates of $P_n$ and of biomass production (Bowes 1991, Stitt 1991). Diminished photosynthetic capacity that often occurred during long-term growth in elevated $CO_2$ was associated with modifications in both plant growth and in the chemical components of leaves and other plant parts. Among the many reported physiological and constitutive changes of plants in response to elevated growth $CO_2$ were a build-up of leaf starch (Poorter et al. 1997), decreased soluble proteins and total foliar N (Wong 1979, Nie et al. 1995), chlorosis and other forms of visible leaf damage (Tripp et al. 1991), premature senescence (Sicher and Bunce 1997, 1998) and decreased Rubisco activity and Rubisco protein levels (Wong 1979, Van Oosten and Besford 1995). There was also evidence that temporarily enhanced plant growth rates under elevated $CO_2$ could result in various nutrient limitations, particularly N and P insufficiencies (Arp 1991). Nutrient deficiencies would adversely affect rates of protein synthesis and thereby inhibit $P_n$ indirectly (Stitt and Krapp 1998).

Large numbers of studies have investigated acclimation of $P_n$ in response to $CO_2$ enrichment (e.g. Stitt and Krapp 1998). Photosynthetic acclimation has been defined as differing rates of $P_n$ for ambient and elevated $CO_2$-grown plants measured at the same $CO_2$ partial pressure (Long 1991). Direct comparisons of plants grown continuously in ambient and elevated $CO_2$ have generated diverse and often conflicting observations. This inherent complexity has made it difficult to identify mechanisms responsible for photosynthetic acclimation. An alternative and less frequent approach to studying growth $CO_2$ effects on $P_n$ was to examine the induction and/or the reversibility of photosynthetic acclimation after transferring plants between atmospheres having either enriched or ambient $CO_2$. Prior studies

*Abbreviations* – Chl ($a+b$), chlorophyll *a* plus *b*; Chl ($a/b$), chlorophyll *a* to *b* ratio.; PAR, photosynthetically active radiation; $p_i(36)$ and $p_i(72)$, estimated internal leaf $CO_2$ partial pressures measured at 36 and 72 Pa external $CO_2$, respectively; $P_n(36)$ and $P_n(72)$, net photosynthesis rates measured at 36 and 72 Pa external $CO_2$, respectively; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase.

using this approach have been performed with cotton (Sasek et al. 1985), rice (Gesch et al. 1998), clover (Morin et al. 1992), tobacco (Sicher et al. 1994) and soybean (Sicher et al. 1995). Most of these earlier investigations showed that photosynthetic acclimation was almost completely reversed within 1–3 days after plants were transferred from elevated to ambient $CO_2$. Leaf starch levels (Sasek et al. 1985, Sicher et al. 1995), Rubisco activity, and Rubisco protein and soluble carbohydrate concentrations (Gesch et al. 1998) also were altered within days after plants were transferred from elevated to ambient $CO_2$.

Most previous $CO_2$ enrichment studies have attributed observed decreases of photosynthetic capacity to changes of Rubisco activity or Rubisco protein concentration (Bowes 1991, Stitt 1991). Our earlier field $CO_2$ enrichment experiment with potato was consistent with this generalization (Sicher and Bunce 1999). However, it has not been established that reversible changes of $P_n$ in response to transitions between elevated and ambient $CO_2$ growth conditions were the result of altered Rubisco activity. The extent to which leaf constituents and other factors associated with photosynthetic acclimation are reversed upon changing growth $CO_2$ treatments is also uncertain. Our objective in the current study was to use quantitative statistical approaches to elucidate the role of Rubisco activity and possibly other leaf factors in altering $P_n$ in response to transitions between ambient and elevated growth $CO_2$.

The hypothesis in the present study, was that the reversible changes of $P_n$ in *Solanum tuberosum* L. within 1–3 days after changing the ambient and elevated growth $CO_2$ conditions would be attributable to altered Rubisco activity. We further assumed that leaf constituents affecting the inhibition of $P_n$ during growth in elevated $CO_2$ would be reversible concomitant with changes of $P_n$.

## Materials and methods

### Plant material

Potato plants (*Solanum tuberosum* L. cv. Atlantic) were grown from tuber sections planted in 3-l pots filled with vermiculite. Plants were raised in controlled environment chambers (model M-3, EGC Corporation, Chagrin Falls, OH, USA) with a 14-h/10-h light/dark photoperiod and at a 22°C/16°C day/night temperature. Relative humidity was never less than 50% and irradiance was 1.05 mmol $m^{-2}$ $s^{-1}$ PAR (Sicher and Bunce 1997). Between 10 and 20 plants were grown continuously at either 36 (ambient) or 72 (elevated) Pa $CO_2$. One-half of the ambient $CO_2$-grown plants were transferred to 72 Pa $CO_2$ (shifted up) beginning 34 days after sowing (DAS), and simultaneously, one-half of the elevated $CO_2$-grown plants were transferred to 36 Pa $CO_2$ (shifted down). Results are representative of 3 experiments using a total of 4 growth chambers. Gas-exchange and Rubisco activity data were combined from the results of two experiments. Data for all other measurements were from one experiment ($n = 4–5$) to reduce variability.

### Gas-exchange measurements

Net photosynthesis ($P_n$) rates were measured 3-h after the start of the light period with a portable infrared gas-analysis system (CIRAS-1, PP Systems, Haverhill, MA, USA) using the terminal leaflet of the uppermost fully expanded leaf (Bunce 1998). Gas-exchange measurements were performed 34, 35 and 37 DAS at both 36 and 72 Pa external $CO_2$ using plants in all 4 $CO_2$ treatments. These are referred to as the $P_n(36)$ and the $P_n(72)$ measurements, respectively. Other than $CO_2$, all measurement conditions were essentially as for plant growth. Measurements were repeated until consecutive values varied by less than 5%. Internal $CO_2$ partial pressures at both measurement $CO_2$ levels [$p_i(36)$ and $p_i(72)$] and $P_n$ rates were obtained following von Caemmerer and Farquhar (1981) and were expressed as means $\pm$ SE ($n = 6$).

### Leaf components

Immediately after performing the gas-exchange measurements, 3 pairs of leaf discs (3.6 $cm^2$) were removed from the penultimate leaflets of the same leaf used for gas-exchange analysis. Foliar samples were used to measure pigments, carbohydrates, protein levels, Rubisco activity and other leaf constituents. One-third of the samples were extracted with methanol:chloroform:water (5:3:1, v/v) and chlorophyll (Chl) *a* and *b* were determined in 80% acetone after partitioning the solvent extracts (Lichtenthaler 1987). Leaf starch was obtained from the pellet fraction and was quantified enzymically according to Hendrix (1993). The aqueous fraction was concentrated to a minimum volume and was diluted to 1.0 ml with deionized $H_2O$. Sucrose, glucose and fructose were measured in coupled enzyme assays according to Bergmeyer et al. (1974). The aqueous concentrates were diluted 1:4 and nitrate was measured by isocratic high-pressure liquid chromatography (HPLC) using an anion exchange column (Whatman Partisil-10 SAX, Clifton, NJ, USA) according to Thayer and Huffaker (1980). The column was eluted with 50 m*M* phosphate buffer pH 3.0, at 1.0 ml $min^{-1}$ and nitrate was detected at 210 nm using a multi-wavelength detector (Waters 490E, Milford, MA, USA). Recovery of standard nitrate was greater than 90%. $\alpha$-Amino N also was measured in the aqueous concentrates by a ninhydrin procedure using glycine as a standard (Sicher and Bunce 1998). Initial and total Rubisco activity was measured using a separate pair of leaf discs. Assays were performed before and after activating the enzyme with $CO_2$ and $Mg^{2+}$ using a radiometric procedure (Sicher and Bunce 1999). Rubisco protein was quantified by a dye-binding method after separation by denaturing gel electrophoresis (Sicher et al. 1994).

### Statistical treatments

Significant differences for mean values ($n = 13–18$) of photosynthetic factors measured 34, 35 and 37 DAS were compared using a repeated measures analysis of variance (ANOVA) procedure (Statview 5.0, SAS Inst., Raleigh, NC, USA) with date and treatment as conditionals. Mean differences were tested at $P \leq 0.05$ (*) or $P \leq 0.001$ (**). Factors

that differed significantly were tested for reversibility in reciprocal transfer experiments based on measurements performed 34 and 37 DAS (n = 4–9). An overall $CO_2$ treatment effect was identified using a one-way ANOVA procedure and individual treatment means that differed significantly were assigned probabilities using Fisher's protected LSD test. In addition, correlation coefficients and probability values for photosynthetic responses measured at 36 and 72 Pa $CO_2$ and various leaf constituents measured 34, 35 and 37 DAS were calculated by linear and multiple regression analyses using the same computer program.

## Results

### Physiological responses to $CO_2$ enrichment

Prior to examining the reversibility of $P_n$ upon switching $CO_2$ treatments, we first sought to establish which photosynthetic parameters in *S. tuberosum* responded to continuous $CO_2$ enrichment. Three-day means measured 34, 35 and 37 DAS and corresponding significance levels of the various factors measured in this study are shown in Table 1. Mean single leaf $P_n$ rates determined at both 36 and 72 Pa $CO_2$ were 37 and 25% lower for plants grown in the continuous elevated compared with the continuous ambient $CO_2$ treatments, respectively. Differences between means for both $P_n(36)$ and $P_n(72)$ were highly significant. Similarly, leaflet starch levels were almost threefold greater in elevated compared with ambient $CO_2$-grown plants. The only other factor that was highly significantly different between the continuous ambient and continuous elevated $CO_2$ treatments was total Rubisco activity, which was about 21% lower in elevated compared with ambient $CO_2$-grown plants. In contrast to the above, $p_i(36)$, $p_i(72)$, sucrose, fructose, soluble protein and percent Rubisco activation did not differ between the ambient and elevated $CO_2$ treatments. Rubisco activation also did not differ between $CO_2$ treatments after the data were transformed with a trigonometric function prior to statistical analysis. Leaflet sucrose levels were unaffected but glucose was decreased by about 32% in response to $CO_2$ enrichment. Leaflet Chl $(a+b)$ levels, the Chl $(a/b)$ ratio, Rubisco protein, $\alpha$-amino N, nitrate, and initial Rubisco activity were all lower in elevated compared with ambient $CO_2$-grown plants ($P \leq 0.05$). Averaged over $CO_2$ treatments, the Chl $(a/b)$ ratio increased and $\alpha$-amino N levels decreased significantly with leaf age. Soluble protein differed by date because leaf concentrations were slightly greater 35 DAS compared with 34 and 37 DAS (data not shown). A $CO_2$ by date interaction was detected for $P_n(36)$ and $p_i(72)$. However, these were minor and did not affect the overall differences in photosynthetic rates because of the growth $CO_2$ treatments.

### Reversibility of photosynthetic gas-exchange rates

The responses of net leaflet $P_n$ rates of ambient and elevated $CO_2$-grown potato plants to reciprocal transfers in $CO_2$ treatment were dependent upon the direction of transfer and on the measurement $CO_2$ partial pressure (Fig. 1A,B). Leaflet $P_n$ rates of elevated $CO_2$-grown plants, determined 72 h after the plants were shifted down (72 to 36 Pa), were either partially or completely de-acclimated under the $P_n(36)$ and $P_n(72)$ measurement conditions, respectively. Comparable leaflet $P_n(72)$ rates measured 37 DAS of plants that were shifted up (36 to 72 Pa) were unchanged in comparison with the ambient controls.

However, plants that were shifted up (36 to 72 Pa) in the $P_n(36)$ measurement were partially acclimated to elevated $CO_2$ after 72 h of treatment. A $CO_2$ treatment effect on $p_i(36)$ was detected ($P \leq 0.001$) in the shifted experiments 37 DAS (Fig. 1C). However, the changes in $p_i(36)$ were too small to fundamentally alter the response of $P_n(36)$ to the switched treatments discussed above. No significant $CO_2$ treatment effects were detected for $p_i(72)$ when measured 37 DAS.

Table 1. Three-day means and significance levels of gas-exchange parameters and leaf components of ambient (36 Pa) and elevated (72 Pa) $CO_2$-grown *Solanum tuberosum*. Values are means for samples measured 34, 35 and 37 DAS. Probabilities are for $P \leq 0.05$ (*), $P \leq 0.001$ (**) and $P \geq 0.05$ (NS) based on a repeated measures analysis of variance procedure.

| Measurement | Growth $CO_2$ | | Probability | | |
|---|---|---|---|---|---|
| | 36 Pa | 72 Pa | $CO_2$ | Date | $CO_2 \times$ date |
| $P_n(36)$, μmol $m^{-2}$ $s^{-1}$ | 27.2 | 17.5 | ** | NS | * |
| $P_n$ (72), μmol $m^{-2}$ $s^{-1}$ | 50.2 | 37.6 | ** | NS | NS |
| $p_i(36)$, Pa | 23.8 | 23.2 | NS | NS | NS |
| $p_i(72)$, Pa | 47.1 | 45.2 | NS | NS | * |
| Starch, g $m^{-2}$ | 70.1 | 208.1 | ** | NS | NS |
| Sucrose, g $m^{-2}$ | 6.3 | 8.3 | NS | NS | NS |
| Glucose, g $m^{-2}$ | 0.44 | 0.24 | * | NS | NS |
| Fructose, g $m^{-2}$ | 0.52 | 0.34 | NS | NS | NS |
| Chl $(a+b)$, g $m^{-2}$ | 0.44 | 0.41 | * | NS | NS |
| Chl $(a/b)$, ratio | 4.8 | 4.6 | * | ** | NS |
| Soluble protein, g $m^{-2}$ | 11.3 | 11.2 | NS | * | NS |
| Rubisco protein, g $m^{-2}$ | 3.5 | 3.1 | * | NS | NS |
| $\alpha$-amino N, mmol $m^{-2}$ | 7.2 | 6.3 | * | * | NS |
| Nitrate, g $m^{-2}$ | 0.69 | 0.46 | * | NS | NS |
| Initial Rubisco activity, μmol $m^{-2}$ $s^{-1}$ | 53.2 | 43.1 | * | NS | NS |
| Total Rubisco activity, μmol $m^{-2}$ $s^{-1}$ | 73.6 | 58.2 | ** | NS | NS |
| Percent activation | 74 | 73 | NS | NS | NS |

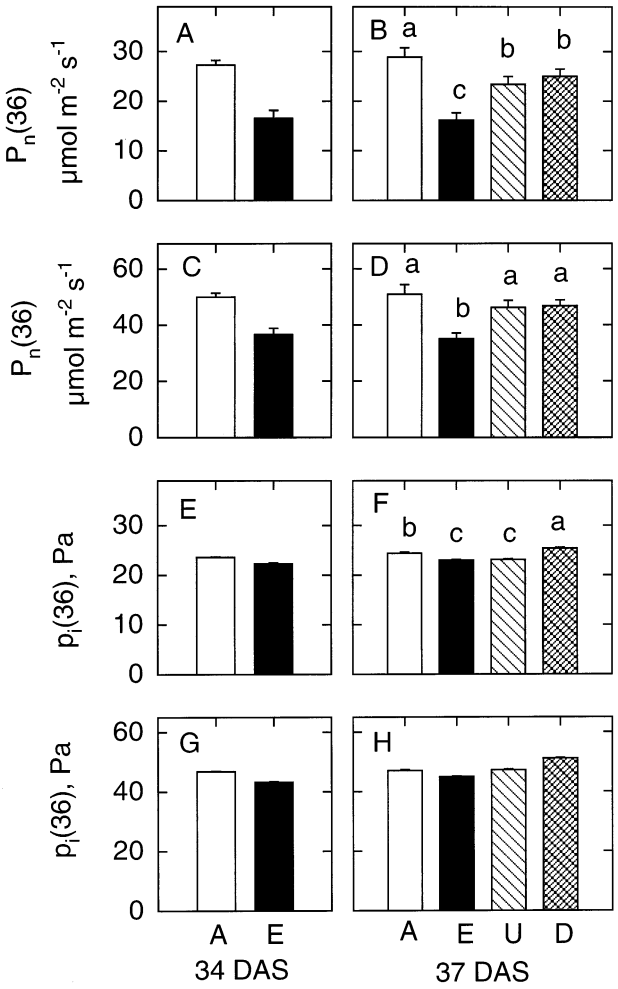


Fig. 1. Effects of a reciprocal transfer in growth $CO_2$ treatments on gas-exchange rates of *Solanum tuberosum* (L.). (A–D) Net rates of photosynthesis ($P_n$) and (E–H) internal leaf $CO_2$ partial pressures ($p_i$) were measured at 36 Pa (A,B,E,F) and 72 Pa (C,D,G,H) $CO_2$. Values are means ± SE for n = 4 for measurements performed 34 and 37 DAS. Separate letters designate significant differences at $P \leq 0.05$. Specific $CO_2$ treatments are the following: A, continuous ambient (open bars); E, continuous elevated (filled bars); U, shifted up 36 to > 72 Pa $CO_2$ (single hatch); and D, shifted down 72 to > 36 Pa $CO_2$ 34 (cross hatch).

### Reversibility of leaf carbohydrate levels

Starch levels in the shifted up and shifted down $CO_2$ treatments measured 37 DAS were not significantly different from the respective elevated and ambient $CO_2$ controls (Fig. 2A). Therefore, leaflet starch concentrations were fully acclimated and fully de-acclimated 72-h after a reciprocal shift in the ambient and elevated $CO_2$ treatments. The response of soluble carbohydrate concentrations to reciprocal shifts in the $CO_2$ treatment differed from the observations for starch (Fig. 2B–D). Sucrose and fructose, but not glucose, exhibited increased leaflet concentrations 72 h after ambient $CO_2$-grown plants were shifted up. Increased fructose and sucrose levels in the shifted up treatment measured 37 DAS were probably transitory. None of the three soluble carbohydrates measured in potato leaflets responded significantly to the shifted down treatment when determined 37 DAS.

### Reversibility of leaflet N-constituents

There were no changes of leaflet Rubisco protein levels measured 37 DAS when ambient $CO_2$-grown plants were shifted up (Fig. 3A). However, Rubisco protein increased 11.5% in the shifted down treatment when compared with elevated $CO_2$-grown plants 37 DAS. Rubisco protein levels were fully de-acclimated in potato leaflets 3-days after plants were transferred from elevated to ambient $CO_2$. A $CO_2$ treatment effect on leaflet nitrate concentrations also was observed (Fig. 3B). Values for nitrate in the shifted up plants were intermediate between the ambient and elevated $CO_2$ treatments. However, no significant differences were detected in leaflet nitrate levels in the elevated and the shifted down plants 37 DAS. Changes of Chl $(a+b)$, Chl $(a/b)$ ratio and $\alpha$-amino N were all non-significant with respect to all 4 of the $CO_2$ treatments 37 DAS (data not shown). The shifted up and shifted down $CO_2$ treatments also had no effect on initial or total Rubisco activity or on percent Rubisco activation (data not shown).

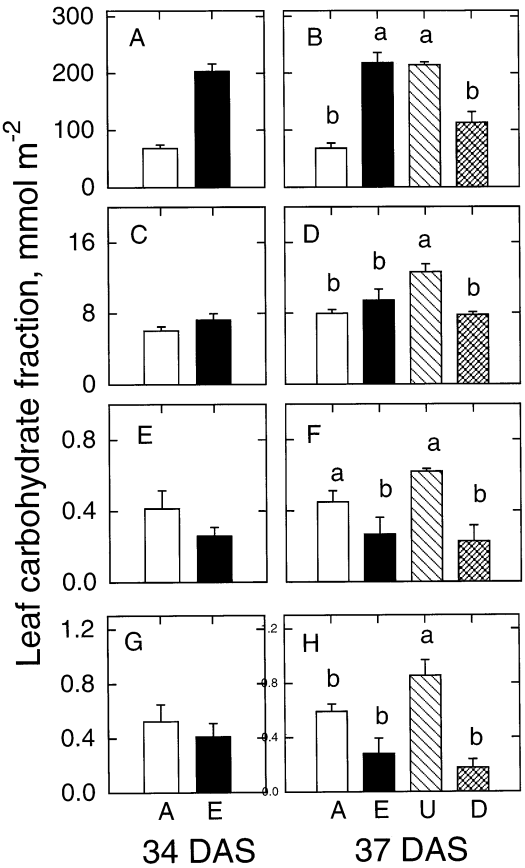


Fig. 2. Effects of a reciprocal transfer in growth $CO_2$ treatments on leaflet non-structural carbohydrate levels of *Solanum tuberosum* (L.). (A,B) Leaf starch, (C,D) sucrose, (E,F) glucose, and (G,H) fructose concentrations in leaflets of potato plants were measured 34 and 37 DAS. $CO_2$ treatments and other conditions were as in Fig. 1.

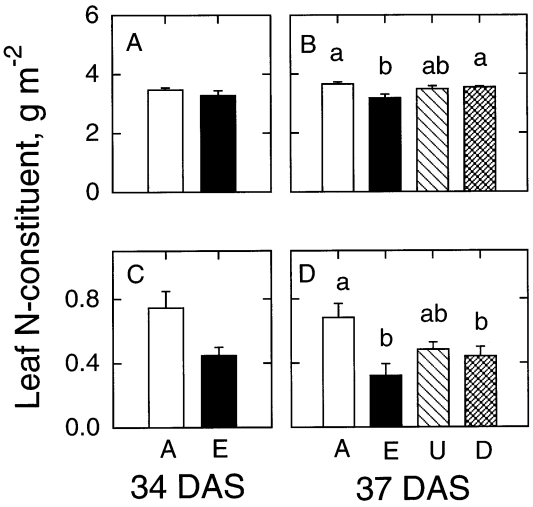


Fig. 3. Effects of a reciprocal transfer in growth $CO_2$ treatments on nitrate and Rubisco protein levels in leaflets of *Solanum tuberosum* (L.). (A,B) Rubisco protein and (C,D) nitrate concentrations in leaflets of potato plants were measured 34 and 37 DAS. $CO_2$ treatments and other conditions were as in Fig. 1.

### Regression analyses of $CO_2$-dependent acclimation responses

Relationships between $P_n$ measured 34, 35 and 37 DAS versus various potato leaf components in response to reciprocal transfers between ambient and elevated $CO_2$ growth conditions were analyzed by linear regression (Fig. 4a–c). Three leaf factors, leaf starch, initial Rubisco activity and nitrate concentration, generated statistically significant ($P < 0.05$) regressions versus both $P_n(36)$ and $P_n(72)$. Leaf starch was negatively correlated with $P_n(36)$, whereas both initial Rubisco activity and leaf nitrate levels were positively correlated with $P_n(36)$. There was a better correlation between $P_n(36)$ and leaf starch [$y = -0.059(x) + 31.84$ ($R^2 = 0.701$, $P \leq 0.003$)] than there was for initial Rubisco activity [$y = 0.789(x) + 29.01$ ($R^2 = 0.517$, $P \leq 0.019$)] or for leaf nitrate concentration [$y = 0.248(x) + 9.25$ ($R^2 = 0.562$, $P \leq 0.013$)]. Multiple regression analysis for $P_n(36)$ versus leaf starch and initial Rubisco activity also was significant ($R^2 = 0.756$, $P \leq 0.006$), and indicated a significant $P$-value for starch ($P = 0.048$) but not for initial Rubisco activity ($P = 0.381$). A comparable analysis for $P_n(36)$ versus leaf starch and nitrate also was statistically significant ($R^2 = 0.765$, $P \leq 0.006$). The $P$-value for starch was significant ($P = 0.044$), whereas that for nitrate was not ($P = 0.210$).

## Discussion

The physiological responses of *S. tuberosum* to $CO_2$ enrichment were consistent with prior reports for a number of other $C_3$ crop species, including tomato (Van Oosten and Besford 1995), wheat (Nie et al. 1995, Sicher and Bunce 1997, 1998), rice (Gesch et al. 1998), tobacco (Sicher et al. 1994) and cotton (Sasek et al. 1985). Single leaf rates of $P_n$ in potato were inhibited 25–37% on average when compared at the same measurement $CO_2$ partial pressure. In spite of this observed photosynthetic acclimation, rates of $P_n$ measured at the same growth $CO_2$ concentration were always greater under $CO_2$ enrichment. Leaf starch was almost threefold greater in leaflets of elevated compared with ambient $CO_2$, whereas total and initial Rubisco activity, Rubisco protein, Chl ($a+b$), the Chl ($a/b$) ratio and nitrate levels were all decreased in leaflets of potato plants grown at

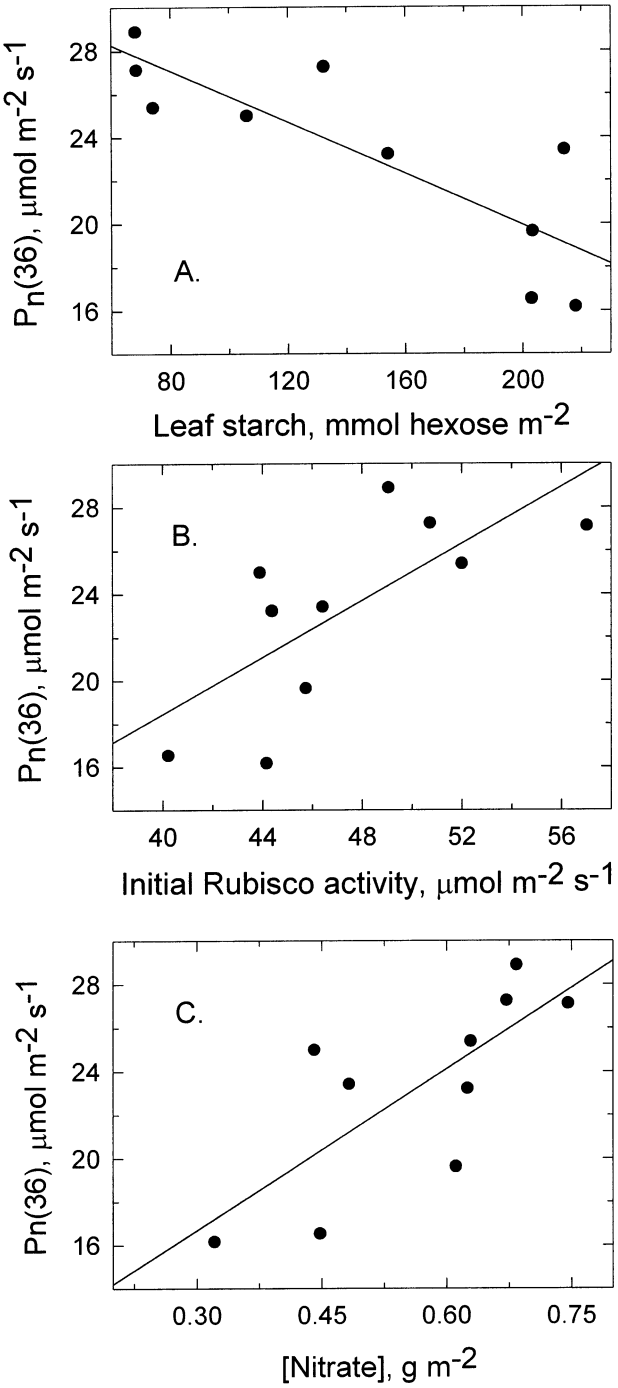


Fig. 4. Simple linear regression analyses of potato leaflet biochemical factors versus $P_n(36)$ in response to reciprocal transfers between ambient and elevated growth $CO_2$ treatments. Regressions were for (A) leaf starch, (B) initial Rubisco activity and (C) leaflet nitrate concentration. Individual values were means ($n = 4–6$) measured 34, 35 and 37 DAS.

elevated compared with ambient $CO_2$. Current results showing that total Rubisco activity was reduced by $CO_2$ enrichment also were in agreement with a prior field study using potato grown in open-topped field chambers (Sicher and Bunce 1999). Although some of the physiological responses of potato to elevated $CO_2$ were not as great as reports for other species, we concluded that photosynthetic acclimation in potato involved conventional changes of leaf components and of related photosynthetic factors.

Reciprocal changes of growth $CO_2$ concentrations demonstrated that the inhibition of $P_n$ in *S. tuberosum* under $CO_2$ enrichment was partially to completely reversed 3 days after plants were shifted down from elevated to ambient $CO_2$. This was consistent with prior reports for soybean (Sicher et al. 1995), cotton (Sasek et al. 1985) and mature rice leaves (Gesch et al. 1998). Conversely, little or no acclimation of $P_n$ was observed 3 days after potato plants were shifted up from ambient to elevated $CO_2$. Sicher et al. (1994) reported that $P_n$ rates of mature tobacco leaves required a week or more to acclimate to elevated $CO_2$ after being shifted up from ambient $CO_2$. In comparison, Morin et al. (1992) observed that $P_n$ rates of clover acclimated to elevated $CO_2$ within 24 h, although plants in this experiment were transferred to an atmosphere having a twelvefold increase in ambient $CO_2$. Gesch et al. (1998) observed an apparent 15% inhibition of $P_n$ (i.e. maximum acclimation) in mature rice leaves 3 days after plants were shifted up from ambient to elevated $CO_2$. Measurements of $p_i(36)$ and $p_i(72)$ in the current study suggested that changes of $P_n$ in response to shifting the ambient and elevated $CO_2$ treatments were the result of non-stomatal factors (cf. Sasek et al. 1985). At least for the species examined thus far, transfer studies using 2–3 times ambient $CO_2$ demonstrated that acclimation and de-acclimation of $P_n$ can occur at different rates. This finding may have potential implications for understanding the biochemical basis for photosynthetic acclimation to elevated $CO_2$.

The above findings demonstrated that $P_n$ rates and various potato leaf constituents responded to reciprocal transfers between the ambient and elevated growth $CO_2$ treatments. Regression analyses were used to quantitatively assess which leaf constituents responded to $CO_2$-dependent changes of $P_n$. Leaf starch levels, initial Rubisco activity and leaf nitrate concentrations were most closely correlated with responses of $P_n$ to changes in growth $CO_2$. A build-up of leaf starch and soluble carbohydrates has been frequently associated with feedback-inhibited photosynthesis (Azcón-Bieto 1983). Recent efforts have identified an end product synthesis limitation (Ludewig et al. 1998), sucrose cycling (Moore et al. 1998) and carbohydrate regulated gene expression (Jang and Sheen 1997) as mechanisms that carbohydrates modify the photosynthetic capacity of leaves. Sasek et al. (1985) observed that excess leaf starch was completely mobilized 3 days after elevated $CO_2$-grown cotton was transferred to ambient $CO_2$. This closely coincided with the de-acclimation of $P_n$ in cotton and suggested to these authors that a $CO_2$-dependent build-up of starch was responsible for the inhibition of $P_n$. Rey and Jarvis (1998) similarly concluded that decreases of photosynthetic capacity in response to elevated growth $CO_2$ were associated with increased leaf starch levels in *Betula pendula*. Leaflet starch levels were threefold greater in elevated than in ambient $CO_2$-grown potato plants. Moreover, starch levels were completely acclimated and de-acclimated within 3 days after potato plants were shifted up or down. Not surprisingly, there was a close correlation between leaf starch levels and the acclimation of $P_n$ in *S. tuberosum* in the present study ($R^2 = 0.701$). The mechanism by which elevated leaf starch resulted in a decrease of $P_n$ in response to $CO_2$ enrichment is unknown. In contrast to starch, there was no direct correlation between changes on total foliar soluble carbohydrate pools and photosynthetic acclimation to elevated $CO_2$ in potato.

Decreased Rubisco activity and Rubisco protein levels frequently have been observed in association with photosynthetic acclimation to elevated $CO_2$ (Bowes 1991, Stitt 1991). Results with *S. tuberosum* grown continuously with ambient and elevated $CO_2$ both here and in open-topped field chambers (Sicher and Bunce 1999) were in general agreement with this conclusion. Initial and total Rubisco activities remained unchanged 3 days after potato plants were reciprocally transferred between the ambient and elevated $CO_2$ treatments. However, a 12% increase of Rubisco protein was observed 3 days after plants were shifted from elevated to ambient $CO_2$. As the changes of Rubisco protein and of Rubisco activity in the current study were small, it was not obvious that Rubisco was responsible for the 25–37% inhibition of photosynthesis that occurred in this species as a result of $CO_2$ enrichment.

Growth in elevated $CO_2$ can create an imbalance between nutrient supply and utilization in roots and leaves (Stitt and Krapp 1998, Geiger et al. 1999). In agreement with this observation, foliar nitrate levels were significantly lower in elevated than in ambient $CO_2$-grown potato plants both here and previously (Ludewig et al. 1998). This occurred in spite of the fact that the nutrient solution in this study was 12 m*M* in nitrate and 3.5 m*M* in ammonium. However, leaflet nitrate levels were unchanged 3 days after elevated $CO_2$-grown plants were shifted down to ambient $CO_2$. Nitrate concentrations in these plants were 10–25 times greater than those observed in potato raised in open-topped field chambers (Sicher and Bunce 1999). Consequently, it cannot be concluded that reduced leaflet nitrate levels during growth in elevated $CO_2$ created an N-limited condition. It was not obvious why nitrate levels were decreased by growth in elevated $CO_2$, although lower evapotranspiration rates and a greater dependence on reduced N sources could be factors.

In summary, reciprocal transfer experiments with *S. tuberosum* showed that the inhibition of $P_n$ during growth in elevated $CO_2$ was partially to completely reversed 3 days after plants were transferred to ambient $CO_2$. However, little or no evidence of photosynthetic acclimation was observed 3 days after plants were shifted up from ambient to elevated growth $CO_2$. Leaf starch, soluble carbohydrates, Rubisco activity, Rubisco protein, Chl $(a + b)$ and nitrate were all affected by $CO_2$ enrichment in this study. Simple and multiple regression analyses indicated that growth $CO_2$-dependent changes of $P_n$ in potato were most closely correlated with leaf starch levels. The mechanism whereby leaf starch affected $P_n$ has not been identified.

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