

Regular paper

Photosynthetic enhancement and conductance to water vapor of field-grown *Solanum tuberosum* (L.) in response to CO₂ enrichment

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Abstract

The photosynthetic responses of potato [*Solanum tuberosum* (L.)] to CO₂ enrichment were studied in open-topped field chambers. Plants were raised in 2.4 m² plastic enclosures over three growing seasons from 1996 to 1998. Plots were continuously fertilized with 1, 1.5 and 2 times ambient daytime CO₂. These were the low (L), medium (M) and high (H) CO₂ treatments, respectively. Tuber dry matter yields were increased 9 and 40%, respectively, in the M and H treatments compared to the L CO₂ treatment. Net photosynthesis (P_n) and conductance to water vapor (g_s) of upper canopy leaves were measured at 1 or 2-week intervals at the growth CO₂ partial pressure and then P_n of plants in the L treatment was determined at 70 Pa CO₂ (L₇₀). Leaflet P_n rates averaged over all measurement dates were 28, 49 and 84% greater, respectively, in the M, H and L₇₀ CO₂ treatments, compared to plants in the L treatment. Changes of P_n in response to the L, M and H CO₂ treatments were proportional to increases of internal CO₂ (C_i) and at low leaf-to-air vapor pressure deficits mid-day g_s was inversely related to growth CO₂. The ratio of P_n at H compared to L₇₀ was 0.81 when averaged over all measurement dates. Leaf soluble protein, Rubisco protein and chlorophyll ($a + b$) levels were unaffected by CO₂ treatment. Total Rubisco activity was decreased by CO₂ enrichment in 1998, but percent activation was similar in the L, M and H plots. Leaf starch was increased but sucrose, glucose and fructose were unaffected by CO₂ treatment. The above findings indicated that a down regulation of P_n in response to elevated CO₂ was consistently observed in field-grown potato. This was attributed to a decrease of total Rubisco activity that was potentially due to the presence of inhibitory compounds bound to the active site of the enzyme. The amount of photosynthetic acclimation observed here did not preclude a persistent enhancement of P_n under the elevated CO₂ growth conditions.

Abbreviations: P_n – net photosynthesis; C_i – internal CO₂ concentration; Rubisco – ribulose 1,5-bisphosphate carboxylase/oxygenase; g_s – stomatal conductance; PAR – photosynthetically active radiation; L, M, H and L₇₀ – low (35 Pa), medium (53 Pa), high (70 Pa) and low at high CO₂ treatments, respectively; DOY – day of year; Chl ($a + b$), chlorophyll a plus b

Introduction

The degree of photosynthetic acclimation that occurs during growth in elevated CO₂ is variable among terrestrial plants. Campbell et al. (1988) reported that leaflet P_n rates of *Glycine max* were ca. 150% greater after 56 to 60 d of growth at 66 compared to 33 Pa

CO₂. Conversely, Raper and Peedin (1978) observed that P_n rates of rapidly expanding *Nicotiana tabacum* leaves did not differ significantly after 28 to 35 d treatment with 40 and 100 Pa CO₂. The majority of elevated CO₂ studies reported in the literature have been performed under greenhouse or controlled environment conditions. These studies are difficult to

interpret because the quality and quantity of light, the rooting volume and nutrient levels are major complicating factors. It has been suggested that the differing photosynthetic responses of crop plants to CO₂ enrichment in greenhouse and controlled environment studies could be influenced by these extrinsic variables (Arp 1991). Much less is known about the responses of crop plants to elevated CO₂ under field conditions. The objectives of the current study were to determine the effects of CO₂ enrichment on leaflet gas exchange rates of *Solanum tuberosum* (potato) in the field.

In spite of the obvious agronomic importance of potato, we are aware of only a few studies (Sage et al. 1989; Wheeler and Tibbits 1997; Ludewig et al. 1998; Miglietta et al. 1998) dealing with the effects of elevated CO₂ on the growth and photosynthetic properties of this plant. Sage et al. (1989) observed little or no photosynthetic acclimation of *S. tuberosum* at low C_i, although a positive stimulation of P_n occurred when C_i exceed 60 Pa CO₂. These authors also reported that Rubisco activation state in this species decreased from 85% to 66% when the growth CO₂ partial pressure was increased from 30 to 95 Pa. Ludewig et al. (1998) observed a slight negative acclimation of P_n for 8 week-old wild type *S. tuberosum* grown at elevated CO₂. This effect of elevated CO₂ on P_n did not occur in 3 week-old plants, and Rubisco activity was unaffected by CO₂ enrichment. These authors attributed the CO₂-dependent adjustment of P_n in potato to an imbalance between rates of end product synthesis and rates of CO₂ assimilation. However, it was not obvious how the 3 and 8 week-old wild type plants differed with respect to rates of starch and sucrose synthesis. Wheeler and Tibbits (1997) reported that total biomass was increased but tuber dry matter yield was unaffected by enrichment with 100 Pa CO₂ during growth of potato in controlled environments. Miglietta et al. (1998) found that tuber yield was 40% greater at 66 Pa compared to ambient CO₂ in a free-air CO₂ enrichment study. Additional information on the acclimation of P_n in *S. tuberosum* in response to CO₂ enrichment is needed to resolve these conflicting observations. Because most of these preceding studies were performed in either glasshouses or in controlled environment chambers, a field study on the photosynthetic responses of potato to growth in elevated CO₂ could be insightful.

The inhibition of P_n during growth in elevated CO₂ is often correlated with excessive starch and sucrose accumulation. We postulated that developing tubers would mitigate the acclimation response of P_n

in this species by providing a strong sink for current photosynthate.

Materials and methods

Plant materials

Certified disease-free potatoes (*Solanum tuberosum* L. cv. Atlantic) were planted in the field at Beltsville, MD, USA, in a well-fertilized and limed, uniform Codorus silt loam. Seed tuber sections with two to three buds each were planted in hills about 30 cm apart using a 30 cm row spacing. Plants were grown during the 1996 through 1998 growing seasons in acrylic open-topped chambers covering 2.4 m² land area (Sicher and Bunce 1997). Plots were sown the first week of June in 1996 and 1997 and during the last week of March in 1998. Three CO₂ treatments were planted in replicate chambers using a completely randomized design. The CO₂ treatments were maintained continuously from planting and were either ambient (35 ± 2 Pa), 1.5 times ambient (53 ± 5 Pa) or twice ambient (70 ± 5 Pa), when measured during the day. These are referred to as the low (L), medium (M) and high (H) CO₂ treatments, respectively. Chamber CO₂ concentrations were monitored continuously with a WMA-1 infrared gas analyzer (PP-Systems, Haverhill, MA, USA)¹ and pure, supplemental CO₂ (MGI Industries, Baltimore, MD, USA) was injected, as needed, from a 6 ton cryogenic storage tank. Constant air flow (12 m³ min⁻¹) through each chamber was maintained with exhaust blowers and adjustable height mixing fans produced an air speed of 1.5 m s⁻¹ near the top of the canopy. Chamber air temperatures were ca. 1 °C greater on average and PAR was reduced ca. 10% in comparison to the external environment. Other growth conditions have been described elsewhere (Sicher and Bunce 1997).

Gas-exchange measurements

Single leaf P_n was determined on 7–9 dates each year at ca. 1–2-week intervals. Measurements of P_n were initiated about 1 month after planting and were terminated in August of 1996 and 1997 and in July of 1998. Gas exchange rates were measured with a portable, open photosynthesis system equipped with CO₂ control (CIRAS-1, PP Systems, Haverhill, MA). Measurements were performed using the terminal leaflet of the most recently, fully expanded leaf in the upper canopy within 2 h of solar noon on sunny days when

the incident irradiation exceeded $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Leaf temperatures during the P_n measurements were 24–33 °C, but on any given measurement date leaf temperatures did not vary by more than 1 °C between CO₂ treatments. Gas exchange rates were determined at the respective chamber CO₂ partial pressure used for plant growth (i.e. L, M and H) and then P_n of leaflets in the low CO₂ treatment was measured at 70 Pa CO₂ (L₇₀). Net CO₂ exchange rates, stomatal conductances to H₂O vapor (g_s) and intercellular CO₂ concentrations (C_i) were obtained from the output of the photosynthesis system. Leaf-to-air vapor pressure difference was calculated from the ratio of transpiration to stomatal conductance and the vapor pressure of air inside the leaf boundary layer was used as a reference (Bunce 1998). Gas exchange values for each CO₂ treatment were based on measurements of three different leaflets from two chambers ($n = 6$) and 3 year means were for $n = 96$.

Biochemical measurements

Samples were harvested on five dates during the 1998 growing season for leaf tissue analysis. Four of these sampling dates corresponded to dates used for measuring photosynthesis. Leaf discs totaling 3.5 cm² leaf area were removed from either the terminal or subtending leaflets of upper canopy leaves. Three pairs of discs were harvested from each plant and two plants were sampled from each chamber on the specified dates. Leaf discs were rapidly frozen with liquid N₂ and stored in Al foil bags at –80 °C until use. Tuber numbers per chamber and dry weight were determined at maturity in 1997 and 1998.

Initial and total Rubisco activities were determined radiochemically before and after activation of the enzyme with CO₂ and Mg²⁺ according to Perchorowicz et al. (1981). Leaf tissue was extracted in ground glass tissue homogenizers at 0 °C in 1 ml buffer containing 50 mM Bicine-NaOH, pH 8.2, 10 mM MgCl₂, 10% (v/v) glycerol, 5 mM dithiothreitol, 2% BSA (w/v) and 0.01% Triton-X-100. Extracts were centrifuged 3 min at 4 °C in a microfuge (model B, Beckman, Fullerton, CA) and supernatants were immediately frozen in liquid N₂. Enzyme extracts were preincubated for 0 (initial activity) or 20 min (total activity) at 0 °C in stoppered vials containing 40 mM Bicine-NaOH, pH 8.2, 8 mM MgCl₂, 10 mM NaHCO₃, 5 mM dithiothreitol and 1 mM 6-phosphogluconate. Assays were initiated by injecting 25 μl enzyme into 0.2 ml reaction mixtures containing 40 mM Bicine-

NaOH, pH 8.2, 8 mM MgCl₂, 0.6 mM Ru1,5bisP (Sigma Chemical Co., St. Louis, MO) and 10 mM [¹⁴C]NaHCO₃ (0.35 Ci mol⁻¹). Assays were terminated after 30 s at 25 °C with 0.2 ml of 0.5 N HCl. Rubisco proteins were separated by denaturing gel electrophoresis, stained with Comassie brilliant blue-R and quantified according to the procedure of Makino et al. (1986). Total soluble protein was measured with a dye-binding method using standard curves prepared with BSA (Bradford 1976). Solvent extracts were used to measure both pigment levels and various carbohydrate fractions (Sicher and Bunce 1997). Leaf Chl ($a + b$) concentrations were determined in acetone according to Lichtenthaler (1987). Leaf starch was recovered from the pellet fraction and was digested for 4 h at 45 °C with a mixture of amyloglucosidase (A-7255, Sigma Chem. Co., St. Louis, MO) and α -amylase (Mylase 100, GB Fermentation Ind., Charlotte, NC) in 0.1 M acetate buffer, pH 4.5. The alcohol fraction was evaporated under N₂ at 37 °C. Sucrose was hydrolyzed for 4 h at 37 °C with acid invertase (5 mg ml⁻¹, I-4504, Sigma Chem. Co., St. Louis, MO) in 0.2 M citrate buffer, pH 5.0. Free glucose and fructose, as well as glucose liberated from starch and sucrose, were measured spectrophotometrically in coupled enzyme assays (Bergmeyer et al. 1965).

Statistical treatments

Significant differences were calculated by a one-way analysis of variance procedure using $P \leq 0.05$ (SuperANOVA, Abacus Concepts, Berkeley, CA). Gas-exchange data for all 3 years were combined after determining that the treatment by date interaction was nonsignificant.

Results

Tuber yield

Potato plants in the L CO₂ plots produced an average of 40 tubers per chamber, with a mean yield of 410 g for the 1997 and 1998 harvests (data not shown). Tuber number per chamber was 14 and 44% greater on average in the M and H treatments compared to the L CO₂ treatment, respectively. Similarly, tuber dry matter was increased 9 and 40%, respectively, in the M and H treatments. Results for the L and H treatments were significantly different at $P \leq 0.05$.

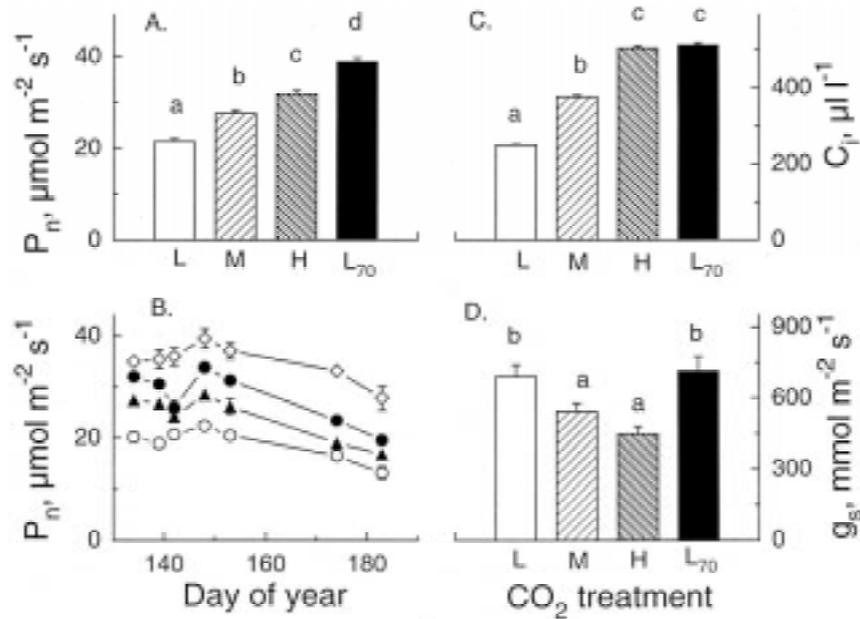


Figure 1. Gas exchange results for field-grown *Solanum tuberosum* in response to CO₂ enrichment. Mean values \pm SE ($n = 96$) of all measurement dates from 1996 to 1998 are for (A) net photosynthesis, P_n ; (B) Mean values \pm SE ($n = 12$) for measurements of P_n by date for the 1998 growing season. Measured leaf temperatures by date were 26,32,24,29,30,33 and 32 °C; (C) internal CO₂ concentration, C_i ; and (D) stomatal conductance to H₂O vapor, g_s . Measurements are for the L (35 Pa), M (53 Pa), H (70 Pa) and L₇₀ (grown at 35 Pa and measured at 70 Pa) CO₂ treatments. Letters above vertical bars denote significant differences at $P \leq 0.05$ and were determined using a one-way ANOVA. L (○), M (▲), H (●) and L₇₀ (◇).

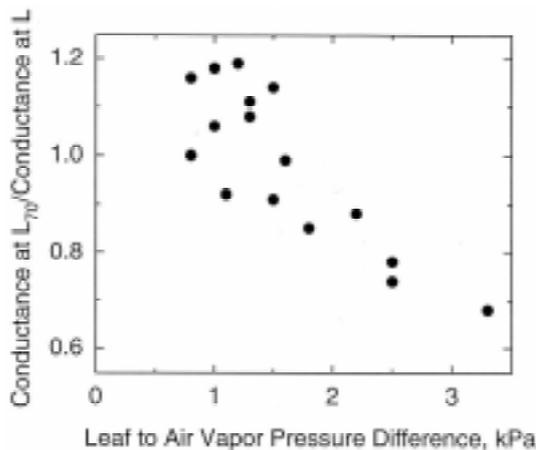


Figure 2. Effects of leaf-to-air vapor pressure difference on the short-term response of leaf conductance to increased carbon dioxide. Plants were grown in the field at ambient (L) CO₂ and PAR was ca. 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. When results were fitted by linear regression the y intercept was 1.29 and the slope was -0.19 ($r^2 = 0.70$).

Leaf gas-exchange

Effects of CO₂ enrichment on the gas-exchange responses of *S. tuberosum* were summarized for the

Table 1. Fisher's protected LSD probabilities for single leaf gas-exchange determinations of *Solanum tuberosum* averaged over years 1996–1998. Treatments generated a significant F -value for each parameter based on a one-way analysis of variance procedure and $n = 96$

Comparison	P_n	C_i	g_s
L vs. M	0.001	0.001	0.020
L vs. H	0.001	0.001	0.001
L vs. L ₇₀	0.001	0.001	0.735
M vs. H	0.001	0.001	0.129
M vs. L ₇₀	0.001	0.001	0.008
H vs. L ₇₀	0.001	0.274	0.001

1996 through 1998 growing seasons (Figure 1). Mean leaflet P_n rates over the 3 year investigation were 28, 49 and 84% greater, respectively, in the M, H and L₇₀ CO₂ treatments, when compared to data for plants in the ambient (L) CO₂ plots (Figure 1A). Treatment effects of CO₂ enrichment on rates of P_n were observed for each measurement date during the 1998 growing season (Figure 1B). Rates of P_n were max-

Table 2. Mean values for the 1998 planting and CO₂ treatment effects on various leaf constituents and Rubisco activity in field-grown *Solanum tuberosum*. Means are for $n = 20$ and P was obtained by a one-way ANOVA procedure

Measurement	L	M	H	P
Starch, mmol m ⁻²	35.1	57.2	76.7	0.001
Sucrose, mmol m ⁻²	5.1	5.5	5.8	0.28
Glucose, mmol m ⁻²	0.9	1.0	1.0	0.78
Fructose, mmol m ⁻²	1.1	1.4	1.4	0.56
Soluble protein, g m ⁻²	5.4	5.4	5.1	0.62
Rubisco protein, g m ⁻²	1.3	1.3	1.2	0.69
Chl ($a + b$), g m ⁻²	0.19	0.18	0.18	0.51
Chl (a/b),	4.8	4.8	4.6	0.72
Initial Rubisco activity, $\mu\text{mol m}^{-2} \text{s}^{-1}$	34.2	30.2	27.2	0.06
Total Rubisco activity, $\mu\text{mol m}^{-2} \text{s}^{-1}$	40.0	34.7	31.7	0.02
Percent Rubisco activation	84.7	87.7	83.5	0.25

imal between DOY 142 and 153, and then decreased as the plants grew older. Despite later sowing dates in 1996 and 1997, responses of P_n to CO₂ enrichment were similar during all three growing seasons (data not shown). There were also corresponding increases in C_i for the M and H, compared to the L CO₂ treatment (Figure 1C). However, mean C_i concentrations in the H and L₇₀ CO₂ treatments were 504 and 511 $\mu\text{l l}^{-1}$, respectively, and these values were not significantly different (Table 1). As anticipated, average rates of g_s measured at midday were decreased by CO₂ enrichment (Figure 1D), although measurements of g_s were similar in the L and L₇₀ treatments. As observed for other species (Bunce 1998), the short-term response of g_s to elevated CO₂ was dependent upon leaf-to-air water vapor pressure difference (Figure 2). This relationship was essentially linear ($r^2 = 0.70$) and lower conductance at elevated measurement CO₂ did not occur at values of leaf-to-air water vapor pressure difference less than ca. 1.5 kPa.

Leaf carbohydrates

Whole leaf starch levels were between 24 and 44 mmol m⁻² in the ambient (L) CO₂ treatments on five measurement dates during 1998 (Figure 3A). Mean leaf starch concentrations were greater in the M and H compared to the L CO₂ treatment (Table 2). Leaf starch in the M and H treatments was maximal on day

of year (DOY) 142 and 148, and decreased on subsequent sampling dates. Whole leaf concentrations of the three soluble carbohydrates measured in this study, sucrose, glucose and fructose, were unaffected by the M and H CO₂ treatments (Figures 3B–D).

Leaf components and Rubisco activity

Soluble protein levels were between 5.1 and 5.8 g m⁻² in the three CO₂ treatments, and these means were not significantly different (Figure 4A). Rubisco protein and total Chl ($a + b$) levels also did not differ in the the L, M and H treatments (Figure 4B, C). The Chl (a/b) ratio was between 4.6 and 4.8 in all three CO₂ treatments and these values were not significantly different. Initial and total Rubisco activities in the ambient (L) CO₂ treatment were 34.2 and 40.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, when data from all five sampling dates were averaged (Figure 4D). Initial Rubisco activity was similar in all three CO₂ treatments ($P \geq 0.05$), but total Rubisco activity was 13% and 21% lower in the M and H treatments, respectively, compared to the L CO₂ treatment. Rubisco in *S. tuberosum* was between 84 and 88% activated in the L, M and H plots, and no CO₂ treatment effects on percent activation were detected.

Discussion

Mechanisms of photosynthetic enhancement in response to short-term (minutes to hours) CO₂ enrichment are well understood and have been attributed to the catalytic properties of Rubisco (Bowes 1991). By comparison, photosynthetic adjustments that occur during long-term (days to weeks) plant growth in elevated CO₂ are more variable and are not as well explained (Stitt 1991; Sage 1994). We have quantified the extent of photosynthetic enhancement that occurred during growth of *Solanum tuberosum* at elevated CO₂ and have shown that this enhancement of P_n persisted throughout the growing season. Present results also demonstrate that, except for the L₇₀ measurement, changes of P_n in response to CO₂ enrichment were proportional to elevated CO₂-dependent increases of C_i . In addition, there was an inverse relationship between average midday g_s and the growth CO₂ treatment. A reduction of g_s in response to CO₂ enrichment is commonly observed in terrestrial plants (Sage 1994). In agreement with Farquhar and Sharkey (1982), changes of g_s and P_n in *S. tuberosum* maintained a ratio of C_i to ambient CO₂ of ca. 0.71 in

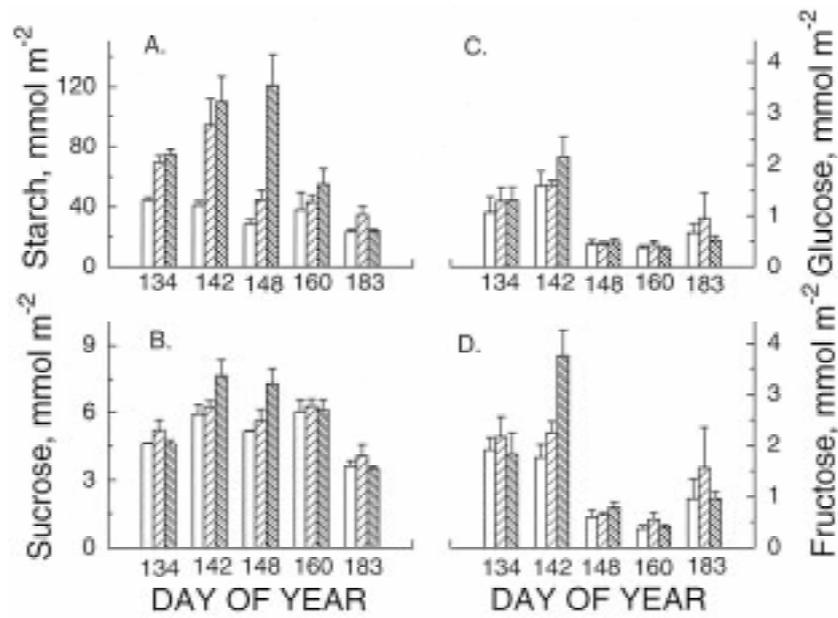


Figure 3. Effects of CO₂ enrichment on whole leaf concentrations of nonstructural carbohydrates in field-grown *Solanum tuberosum* during 1998. Means ± SE (*n* = 4) are shown for (A) Starch; (B) Sucrose; (C) Glucose; and (D) Fructose. Vertical bars represent L (open), M (single hatch) and H (cross hatch) CO₂ treatments, respectively.

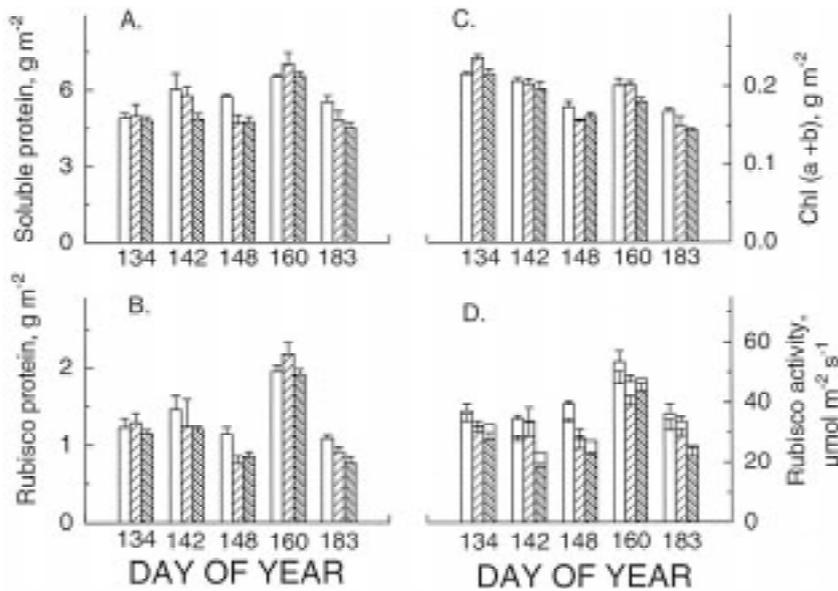


Figure 4. Effects of CO₂ enrichment on concentrations of N-containing leaf constituents and Rubisco activities of field-grown *Solanum tuberosum* during 1998. Means ± SE (*n* = 4) are shown for (A) Soluble protein; (B) Rubisco protein; (C) Chl (*a* + *b*); and (D) Initial (foreground) and total Rubisco (background) activities. Vertical bars are described in Figure 3.

the L, M and H plots. Consequently, there was no evidence for stomatal acclimation to elevated CO₂ that was separate from photosynthetic acclimation in this study. The dependence of short-term changes in g_s by elevated CO₂ on vapor pressure difference was similar in potato to that observed for other species (Bunce 1998). It should be noted that rates of P_n were lower in the H treatment than in the L₇₀ measurements. This difference occurred at the same C_i , thereby showing that long-term CO₂ enrichment negatively affected P_n in the H plots and that this decrease was attributable to nonstomatal factors.

Photosynthetic acclimation has been defined as differing rates of P_n for ambient and elevated CO₂-grown plants measured at the same CO₂ partial pressure (Long 1991). Using this criterion, we concluded that photosynthetic acclimation to the growth CO₂ treatment was evident when the H and L₇₀ P_n measurements were compared. Mean rates of P_n from 1996 through 1998 were 18% lower on average in the H compared to the L₇₀ measurements.

Photosynthetic acclimation was observed on every measurement date, except for two dates in 1997 that were affected by a soil moisture deficit (data not shown). Huxman et al. (1998) similarly observed that drought eliminated evidence of photosynthetic acclimation to elevated CO₂ in *Larrea tridentata*. These authors postulated that water deficits reduced carbohydrate formation to the point that feed back inhibition of P_n was undetectable. The above findings with potato differ in many respects from those of previous investigations (Sage et al. 1989; Ludewig et al. 1998) and document the value of multi-year field studies.

Unlike earlier investigations with wheat (Garcia et al. 1998; Sicher and Bunce 1998), photosynthetic acclimation in *S. tuberosum* did not increase progressively with leaf age. Acclimation of P_n increased with age in wheat flag leaves, so that all evidence of photosynthetic enhancement in response to CO₂ enrichment was abolished during the late stages of grain-filling. Leaf constituents, such as Chl ($a + b$), soluble protein and Rubisco protein, were significantly lower in flag leaves of wheat grown at elevated compared to ambient CO₂ (Nie et al. 1995; Sicher and Bunce 1998), and the elevated CO₂-dependent decline of P_n in wheat flag leaves was attributed to early maturation and accelerated senescence. Consistent with changes in P_n , effects of CO₂ enrichment on N-containing leaf constituents were greater in older than in newly expanded flag leaves. In the present study, Chl ($a + b$), soluble protein and Rubisco protein in potato leaflets were not

significantly altered by CO₂ enrichment. These results are in agreement with prior reports (Sage et al. 1989; Ludewig et al. 1998). Therefore, the more moderate amounts of photosynthetic acclimation observed in *S. tuberosum* were not associated with protein mobilization, and it was unlikely that accelerated senescence occurred in *S. tuberosum* grown in elevated CO₂. It was also probable that the mechanism of photosynthetic acclimation differed in wheat and potato leaves, at least during the latter stages of ontogeny. Unlike the reproductive stage in small-grain cereals, tuber formation in *S. tuberosum* likely precluded the possibility that P_n was sink limited. This conclusion assumes that phloem loading did not impose a restriction on C export independent of sink strength.

Leaf starch was the only nonstructural carbohydrate that was altered by CO₂ enrichment in *S. tuberosum* (Figure 3). Our carbohydrate measurements were in broad agreement with results from a controlled environment investigation (Ludewig et al. 1998). This finding suggested that photosynthetic acclimation probably was not mediated by carbohydrate regulated suppression of gene expression. According to this hypothesis (Jang and Sheen 1997), intracellular sugar-sensing mechanisms respond to excess carbohydrate by decreasing transcripts for photosynthetically associated genes, such as the small subunit of Rubisco. Rubisco protein and total Chl were unaffected by CO₂ enrichment in this study. These results virtually eliminate the possibility that changes of major leaf proteins were responsible for photosynthetic acclimation to elevated CO₂ in *S. tuberosum*.

Following the initial observations of Wong (1979), there has been considerable interest in determining the effects of decreased Rubisco activity on photosynthetic acclimation to elevated CO₂. Rates of total Rubisco activity reported here were similar to those in an earlier study using potato leaf extracts (Ludewig et al. 1998). In the present study, total Rubisco activity was 13–21% lower at elevated than at ambient CO₂, but initial Rubisco activity was similar in all three CO₂ treatments. Unlike the current study, these investigators did not detect changes of total Rubisco activity in response to CO₂ enrichment. The elevated CO₂ effects on total Rubisco activity reported here were not the result of changes in leaf Rubisco protein content. Our preliminary attempts to measure Rubisco activity in these samples were unsuccessful, and it was necessary to add 2% BSA to the extraction medium and 1 mM 6-phosphogluconate to the activation medium to attain reliable measurements. This suggested that inhibitory

substances were present in the tissue preparations and their presence may have affected the enzyme measurements by blocking the active site (Paul et al. 1996). Inhibitory compounds that bind to the active site of Rubisco potentially may act *in vivo* (Eichelmann and Laisk 1999). It was interesting to note that the H/L ratio for total Rubisco activity was 0.79. This closely resembled photosynthetic acclimation in *S. tuberosum*, measured as the H/L₇₀ ratio of P_n, which had a mean value of 0.81.

A number of prior investigators (Socias et al 1993; McKee and Woodward 1994; Ludewig et al. 1998) have suggested that an end product synthesis limitation of P_n could explain the progressive decline of photosynthetic enhancement in elevated CO₂-grown plants. The problem with this explanation is that an end product synthesis limitation of P_n is not normally encountered at ambient temperatures and ambient partial pressures of CO₂. Moreover, Sage et al. (1989) reported that the O₂ sensitivity of P_n was the same for ambient and elevated CO₂-grown *S. tuberosum*, except at low temperatures. Although temperatures were variable in the field, i.e. 23–33 °C in 1998, our P_n measurements were performed at midday, when leaf temperature was unlikely to confer an end product synthesis limitation of P_n. However, the effects of starch and sucrose synthesis on P_n should be investigated more carefully in *S. tuberosum* and in other plants that exhibit photosynthetic acclimation but not elevated CO₂-dependent changes in major leaf proteins.

In summary, the enhancement of P_n by *S. tuberosum* in response to CO₂ enrichment in field chambers persisted over the entire growing season. In agreement with Miglietta et al. (1998), twice ambient CO₂ increased tuber yield up to 40%. The extent of photosynthetic acclimation under elevated CO₂ was 0.81 on average, when measured as the H/L₇₀ ratio of P_n. Acclimation of P_n was observed over 3 years of study and was attributed to nonstomatal factors. Total Rubisco activity was lower in the elevated compared to ambient CO₂ plots, and the decrease in total Rubisco activity was proportional to the amount of photosynthetic acclimation measured in this study. Changes of Rubisco activity in response to CO₂ enrichment were not the result of decreased Rubisco protein or of decreased percent activation and may have been the result of inhibitory compounds present in leaf extracts (Paul et al. 1996).

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Note

¹Mention of a trademark, proprietary product or vendor neither implies and endorsement nor constitutes a warranty of the product by the US Department of Agriculture.

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