Acclimation of Two Tomato Species to High Atmospheric CO₂

I. Sugar and Starch Concentrations

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ABSTRACT

Lycopersicon esculentum Mill. cv Vedetos and Lycopersicon chmielewskii Rick, LA1028, were exposed to two CO₂ concentrations (330 or 900 microliters per liter) for 10 weeks. Tomato plants grown at 900 microliters per liter contained more starch and more sugars than the control. However, we found no significant accumulation of starch and sugars in the young leaves of L. esculentum exposed to high CO₂. Carbon exchange rates were significantly higher in CO₂-enriched plants for the first few weeks of treatment but thereafter decreased as tomato plants acclimated to high atmospheric CO₂. This indicates that the long-term decline of photosynthetic efficiency of leaf 5 cannot be attributed to an accumulation of sugar and/or starch. The average concentration of starch in leaves 5 and 9 was always higher in L. esculentum than in L. chmielewskii (151.7% higher). A higher proportion of photosynthates was directed into starch for L. esculentum than for L. chmielewskii. However, these characteristics did not improve the long-term photosynthetic efficiency of L. chmielewskii grown at high CO₂ when compared with L. esculentum. The chloroplasts of tomato plants exposed to the higher CO₂ concentration exhibited a marked accumulation of starch. The results reported here suggest that starch and/or sugar accumulation under high CO₂ cannot entirely explain the loss of photosynthetic efficiency of high CO₂-grown plants.

The enhancement of growth and yields by increasing the level of CO₂ in the atmosphere has been reported for many species (6). However, the long-term effects of high CO₂ levels on the physiological and biochemical behaviors of tomato plants need to be studied further. Many species lose their photosynthetic efficiency when subjected for an extended period to high concentrations of CO₂ in the atmosphere (5, 9, 12), and become gradually less efficient in the use of the added CO₂. In a recent study, Yelle et al. (21) showed that the beneficial effects of CO₂ enrichment on the relative growth and photosynthetic rates were not maintained as tomato plants matured. Photosynthesis of leaf 5 was 37% higher than

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2 Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; CER, carbon exchange rate; RGR, relative growth rate.
metabolism. However, *L. chmielewskii* and *L. esculentum* responded similarly to high CO₂. The first objective of this study was to assess whether the particular sink metabolism and carbohydrate assimilation of *L. chmielewskii* could modify the long-term response of tomato plants to high CO₂. The second objective was to verify whether the long-term decline in photosynthesis of greenhouse tomato results from starch/sugar accumulation and/or chloroplast deterioration.

**Table I. Carbon Exchange Rates of Two Tomato Species Grown at 330 and 900 µL L⁻¹ CO₂ for a 10-Week Period**

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf Number</th>
<th>Week of Enrichment</th>
<th>Carbon Exchange Rate (µmol CO₂ m⁻² s⁻¹)</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>330</td>
<td>900</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>5</td>
<td>2</td>
<td>0.54 ± 0.02</td>
<td>0.70 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>0.42 ± 0.03</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.43 ± 0.02</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>0.47 ± 0.03</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2</td>
<td>0.41 ± 0.03</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>0.38 ± 0.03</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.41 ± 0.02</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>0.48 ± 0.01</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td><em>L. chmielewskii</em></td>
<td>5</td>
<td>2</td>
<td>0.57 ± 0.03</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>0.47 ± 0.03</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.44 ± 0.04</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>0.47 ± 0.03</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2</td>
<td>0.46 ± 0.03</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>0.50 ± 0.03</td>
<td>0.53 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>0.40 ± 0.02</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>0.46 ± 0.03</td>
<td>0.50 ± 0.04</td>
</tr>
</tbody>
</table>

* Each value is the mean of four values ± se and the average of 2 weeks of experiment. Measurements were taken weekly.

**Table II. Effects of Two CO₂ Concentrations (330 and 900 µL L⁻¹) on Starch Content of the Leaves of *L. esculentum* and *L. chmielewskii***

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf Number</th>
<th>Time of Day</th>
<th>Starch Content (µmol glc/g fr wt)</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>330</td>
<td>900</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>5</td>
<td>0800</td>
<td>54.5 ± 13.9*</td>
<td>58.7 ± 11.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1600</td>
<td>106.9 ± 17.9</td>
<td>129.3 ± 22.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>96.0%</td>
<td>120.4%</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0800</td>
<td>52.8 ± 15.2</td>
<td>117.3 ± 38.8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1600</td>
<td>100.3 ± 24.7</td>
<td>201.7 ± 32.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>89.7%</td>
<td>71.9%</td>
</tr>
<tr>
<td><em>L. chmielewskii</em></td>
<td>5</td>
<td>0800</td>
<td>19.0 ± 5.4</td>
<td>35.4 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1600</td>
<td>67.0 ± 7.9</td>
<td>80.0 ± 8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>252.6%</td>
<td>125.8%</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0800</td>
<td>13.6 ± 2.4</td>
<td>26.5 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1600</td>
<td>38.6 ± 4.6</td>
<td>46.4 ± 4.6</td>
</tr>
</tbody>
</table>

* Each value is a mean of four replications ± se and the average of 10 weeks of experiment. Measurements were taken weekly. glc, glucose. * Percent increase between 0800 h and 1600 h.

**MATERIALS AND METHODS**

**Plant Material**

*Lycopersicon esculentum* Mill., cv Vedettos, and *Lycopersicon chmielewskii* Rick, LA 1028, were seeded in rockwool blocks (Grodania, Denmark) on December 15, 1987, and transplanted on January 17, 1988, into 16 independent hydroponic nutrient film technique systems (NFT). The plants...
were grown in four identical glasshouse compartments (24 m²). The carbon dioxide concentration within each compartment was maintained at 330 ± 50 (control, ambient) or 900 ± 50 μL of CO₂ L⁻¹. There were two repetitions of each CO₂ treatment, and two repetitions of each species within each compartment. Each experimental unit was randomly distributed in each compartment and consisted of 20 plants.

Carbon dioxide levels were monitored and controlled by an infrared gas analyzer (Priva Computers, No. APBA 251 E CO₂ Monitor). The CO₂ was supplied from a pure liquid source during the photoperiod. Day and night temperatures were maintained at a minimum of 22°C ± 2°C and 17°C ± 2°C, respectively. The ventilation temperature was set at 30°C. Temperatures were measured by six thermocouples in each compartment; four for the nutrient solutions and two for air temperatures. The thermocouples were connected to a Minivent 205 recorder (Doric Scientific). Supplemental lighting (150 μmol m⁻² s⁻¹ PAR) was supplied by high-pressure sodium lamps for a photoperiod of 16 h.

Nutrient solutions, which were renewed every 5 days, contained (μL L⁻¹) 176 N, 46 P, 284 K, 140 Ca, 47 Mg, 2 Fe, 1 Mn, 0.4 Zn, 0.29 B, 0.03 Cu, and 0.06 Mo. Solutions were adjusted daily with H₂SO₄ to a pH of 5.8 and to an electric conductivity of 220 dS·cm⁻¹ using a complete nutrient solution.

**Carbon Exchange Rates**

Gas exchange was measured on the fifth and ninth leaves with a portable photosynthesis apparatus (LI-6000 from LI-COR Inc.). Leaves were counted from the top of the plants, starting with the first leaf larger than 2 cm. Photosynthetic rates were measured at the same CO₂ concentration under which the plants were grown. The measurement period was 90 s, with an air flow inside the 1-L leaf chamber of 6 cm³ s⁻¹ and a leaf area of 28 cm². Each value reported is the average from 2 weeks of measurement. Each week measurement consists of four series of four measurements taken randomly during the middle of the day (1000 to 1400 h) in each experimental unit.

**Starch and Sugar Determination**

Tissue from leaves 5 and 9 of *L. esculentum* and *L. chmielewskii* was harvested at 0800 h and 1600 h every week during 10 weeks. The tissue was ground with a mortar and pestle in 80% ethanol and boiled twice for 20 min in a water bath. Starch (ethanol-insoluble) and sugar (ethanol-soluble) fractions were obtained as outlined by Ozburn et al. (11). Starch was hydrolyzed overnight with 0.64 ng of amylloglucosidase and 11 units of α-amylase. The resulting glucose was determined by coupling the oxidation of glucose to the reduction of NAD⁺ with hexokinase and glucose-6-phosphate dehydrogenase. Absorbance was measured at 340 nm. Sugars (sucrose, glucose, and fructose) were separated and quantified by HPLC (Model LKB, Bromma). Samples were purified through a C₁₈ column and 50 μL was injected into a 4.6 mm (i.d.) x 250 mm column packed with 10 μm of P/10 carbohydrate (Whatman, N. 4221-001). The mobile phase was composed of 79% CH₃CN, 20% H₂O, 1% NH₄OH. The flow rate was 1.5 cm/min. Sugar concentrations were determined by a refractive index detector (Model LKB 2142).

**Tissue Processing for Electron Microscope Investigation**

Leaf samples were collected at weeks 4 and 10 from plants grown at either 330 or 900 μL of CO₂ L⁻¹. The samples were fixed with 3% glutaraldehyde in 0.1 m sodium cacodylate buffer, pH 7.2, dehydrated in a graded series of ethanol, and embedded in Epon 812. Ultrathin sections collected on Formvar-coated copper grids were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskope 102 electron microscope.
RESULTS AND DISCUSSION

Carbon Exchange Rates

The carbon exchange rates (CER) were higher in CO₂-enriched plants throughout the experiment. After 2 weeks of treatment, CO₂ enrichment increased the CER of leaf 5 by 29.6% and 38.6% for *L. esculentum* and *L. chmielewskii*, respectively (Table I). However, tomato leaves gradually acclimated throughout plant development, resulting in diminished CER. After 8 weeks of treatment, the CER was only 8.5% and 2.1% higher for leaf 5 of *L. esculentum* and *L. chmielewskii* respectively (Table I). Leaf 9 also showed a transient increase of CER under 900 μL L⁻¹ CO₂ followed by a decrease in photosynthetic efficiency compared to nonenriched plants (Table I). The decrease of photosynthesis was faster and more pronounced for *L. chmielewskii* than for *L. esculentum*. The low sink size of the wild specie may have caused the faster decline.

Starch and Sugars Levels

The concentrations of starch in leaves 5 and 9 were always higher in *L. esculentum* than in *L. chmielewskii* (average of 151.7% higher; Table II). In a previous study, Yelle et al. (21) showed that fruits of *L. chmielewskii* accumulated less starch than those of *L. esculentum* as a result of reduced concentrations of ADP-glucose pyrophosphorylase and a higher level of phosphorylase.

In general, tomato plants grown at 900 μL L⁻¹ CO₂ contained more starch than the control. The accumulation was similar for both species. Starch concentrations were always higher in leaves sampled at 1600 h than at 0800 h (Table I). The largest relative increases in starch accumulation during the day were found for leaves 5 and 9 of *L. chmielewskii* grown under ambient CO₂ (average of 252.6% and 183.5% higher, respectively). The plants of *L. chmielewskii* exposed to 900 μL of CO₂ L⁻¹ accumulated a lower percentage of starch during the day than the control. This was attributed to the relatively high starch content already present in CO₂-enriched leaves in the mornings. Due to high rates of fruit abortion and small fruit size, *L. chmielewskii* had a smaller sink than *L. esculentum*. A low sink demand and/or a deficient translocation of assimilates overnight would result in high starch levels found in the morning.

The effects of high CO₂ levels on starch accumulation were greater for leaf 9 than for leaf 5 of *L. esculentum*. Compared with plants grown at 330 μL L⁻¹ CO₂, the concentration of starch in leaf 9 sampled at 0800 h and 1600 h was 121.9% and 101.1% higher under the high CO₂ concentration (Table II), whereas the corresponding differences for leaf 5 were only 7.6% and 20.9%. Leaf 9 of *L. chmielewskii* accumulated less starch than leaf 5 at both CO₂ concentrations. Starch concentration of leaves 5 and 9 of *L. chmielewskii* sampled at 0800 h were 86.4% and 94.6% higher under high CO₂, respectively. The difference in the afternoon samplings were only 19.4% and 20.3% (Table II). The smaller difference in the afternoon may be attributed to the lower sink demand of *L. chmielewskii*. Leaves of plants grown at 330 μL L⁻¹ CO₂ and sampled at 1600 h had a starch content approaching saturation.

Tomato plants grown at 900 μL L⁻¹ CO₂ contained more sucrose and glucose+fructose than the control. The highest levels of sugars for high CO₂-grown plants were found for leaf 5 of *L. chmielewskii*. The concentration of sucrose in the leaves was similar for both species under normal CO₂ levels (Table III). Under CO₂ enrichment, the sucrose concentration was significantly higher in *L. chmielewskii* than in *L. esculentum* for leaf 5 and slightly higher for leaf 9. The concentration of glucose+fructose of plants exposed to high CO₂ was higher in *L. chmielewskii* than in *L. esculentum* for leaf 5, but was lower for leaf 9. In all cases, CO₂ enrichment resulted in a larger increase of sugars in *L. chmielewskii* (average of 96.5% higher) than *L. esculentum* (average of 53.8% higher).

In a similar manner to the starch concentration, the concentrations of sucrose and glucose+fructose were always higher at the afternoon than at the morning samplings (Table III). The plants grown at ambient atmospheric CO₂ had the greatest increase in sugar accumulation during the day. In all cases, the concentrations of the sugars were already high at 0800 h for plants exposed to 900 μL L⁻¹, which explains the proportionally lower accumulation throughout the day.

Leaf 9 of *L. esculentum* accumulated more starch during the day than leaf 5 at high CO₂ levels. This difference in
ACCLIMATION OF TOMATO PLANTS TO HIGH CO₂

Figure 2. Sucrose content of two tomato species grown at 330 and 900 μL L⁻¹ for a 10-week period. (A, E) Leaf 5 and (B, F) leaf 9 of L. chmielewskii at 0800 h and 1600 h, respectively; (C, G) leaf 5 and (D, H) leaf 9 of L. esculentum at 0800 h and 1600 h, respectively. Each point represents the mean of four values ± SE.

Accumulation increased throughout plants development at both sampling times (Fig. 1, C, D, G, and H). A large accumulation of starch occurred in leaf 9 during weeks 8 through 10. The starch levels of CO₂-enriched and nonenriched plants were already very high at 0800 h during these weeks, suggesting that overnight translocation of photosynthates was limited. As a result of fruit harvesting, the increase of the source/sink ratio in the last 3 weeks may have caused the buildup of starch in L. esculentum leaves. The higher starch content for leaves of enriched plants was significant for both leaves of L. chmielewskii for most of the sampling times (Fig. 1, A, B, E, and F). A slight starch buildup was observed over the course of the experiment for leaf 5 of L. chmielewskii (Fig. 1, A and E), whereas the concentration remained more constant for leaf 9 throughout plant growth (Fig. 1, B and F). Starch concentrations of L. chmielewskii leaves were always higher at the afternoon than the morning samplings. These results suggest that, even if the sink size of L. chmielewskii was very small, a substantial translocation of assimilates occurred overnight.

Sucrose concentration was higher at 900 μL L⁻¹ than at 330 μL L⁻¹ during most of the sampling times for L. chmielewskii (Fig. 2, A, B, E, and F). For leaves 5 and 9 of L. esculentum, the high CO₂ level caused a buildup of sucrose at weeks 1 and/or 2 (Fig. 2, C, D, and H). This transient buildup just prior to flowering (week 1 and/or 2) was also observed for starch (Fig. 1, D, G, and H) and the two reducing sugars (Fig. 3, C, D, G, and H). A depletion of sugar and starch was measured after the beginning of anthesis (week 2 to 3) corresponding to very high sink demands.

After the first week, the high CO₂ concentration caused a significant increase in the fructose+glucose concentration in leaf 5 of L. chmielewskii. This accumulation remained throughout the experiment (Fig. 3, A and E). Leaf 9 of L. chmielewskii exposed to high CO₂ accumulated fructose+glucose at a higher rate than the control after 4 weeks and maintained this rate for the rest of the experiment (Fig. 3, B and F). Leaf 5 of L. esculentum sampled at 0800 h accumulated a similar concentration of fructose+glucose at both CO₂ concentrations (Fig. 3C) while for leaf 9, weeks 2, 8, and 10 had higher concentrations of fructose+glucose at 900 than at 330 μL L⁻¹ (Fig. 3D). For the afternoon samplings, the concentration of reducing sugars in leaf 5 of L. esculentum was similar at 330 and 900 μL L⁻¹ for the first 5 weeks of sampling. Thereafter, the accumulation was significantly higher at 900 μL L⁻¹ (Fig. 3G). For leaf 9 (Fig. 3H), the concentration of reducing sugar was significantly higher at 900 μL L⁻¹ than at 330 μL L⁻¹ at weeks 1, 6, 8, and 10.

The high starch and sugar accumulations found in leaves grown at 900 μL of CO₂ L⁻¹ (Figs. 1, 2, and 3) were related to a reduction of relative growth rate (RGR) and photosynthesis. Yelle et al. (21) previously showed that the RGR of
CO₂-enriched plants of *L. esculentum* and *L. chmielewskii* were increased by 18.1% and 33.3%, respectively after 2 weeks of treatment. Thereafter, the RGRs of CO₂-enriched plants declined faster than the control, resulting in RGR 17.7% and 34.7% lower than the control for *L. esculentum* and *L. chmielewskii*, respectively, after 10 weeks. Carbon exchange rates of high CO₂-grown plants were initially significantly higher than the control for *L. esculentum* and *L. chmielewskii* (Table I). However, after 8 weeks of treatment, the CER of CO₂ enriched plants were not significantly different than the 330 μL L⁻¹ CO₂-grown plants. High starch content in both leaves of *L. chmielewskii* and in leaf 9 of *L. esculentum* (Table II) suggests that the buildup of starch caused the acclimation of high CO₂-grown plants (21). This hypothesis has been previously suggested by many authors (7, 8, 13, 16). However, the differences in starch content resulting from high CO₂ were never significantly different for leaf 5 of *L. esculentum* at 0800 h (Fig. 1C) and were only significant at 1600 h for weeks 1 and 2 (Fig. 1G). The starch levels were much higher in leaf 9, and the differences between treatments were significant at most of the sampling dates. Yet, the decline of photosynthesis over the course of the experiment was very similar in both leaves. These results, taken with those of the sugars, indicate that the decreases in the photosynthetic rates cannot be solely explained by changes in the starch and sugar concentrations in the leaves. Based on a percentage of dry weight, the average starch concentration ranged from 4% to 17% for the first 5 weeks (weeks corresponding to an important decline of photosynthesis) of sampling (Fig. 1). Nafziger and Koller (10) found a direct correlation between starch accumulation under high atmospheric CO₂ and the decline of photosynthesis. However, they found no significant decline in photosynthesis associated with starch concentration between 5% and 17%.

Azcon-Bieto (2) reported that the accumulation of soluble sugars in wheat leaves was correlated to the inhibition of photosynthesis. Except for leaf 5 of *L. esculentum* sampled at 0800 h at week one, our study found no substantial accumulation of sugars during the first 5 weeks of treatment for *L. esculentum* (Figs. 2, C and G, 3, C and G), suggesting that the rapid decline of the relative growth rate and photosynthesis could not be attributed to sugar accumulation. Thus, we conclude that the buildup of starch and sugar under our conditions was not high enough to cause feedback inhibition of photosynthesis.

**Ultrastructural Observation of the Chloroplasts**

The chloroplasts of leaf 9 of tomato plants exposed to high atmospheric CO₂ concentrations exhibited a marked accu-

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**Figure 4.** Electron micrograph of chloroplasts of two tomato species grown at 330 and 900 μL L⁻¹ CO₂ for a 4-week period. (A, B) *L. esculentum* at 330 and 900 μL L⁻¹; (C, D) *L. chmielewskii* at 330 and 900 μL L⁻¹. S, starch; G, granum; T, thylakoid.
mulation of starch (Fig. 4). After 4 weeks of CO₂ enrichment, the accumulation was notable in both species, but no modification of the chloroplast integrity was noted (Fig. 4, B and D). At week 10, the starch content in some of the chloroplasts of CO₂-enriched plants distorted the thylakoids. However, only a small proportion of the chloroplasts mainly located in the phloem cells were so affected. The distortion was usually more pronounced in L. esculentum (Fig. 5B) than in L. chmielewskii (Fig. 5D). This could be attributed to lower levels of starch in L. chmielewskii than in L. esculentum leaves. Madsen (7) demonstrated that tomato plants grown under high CO₂ levels accumulated starch and had deformed thylakoids. Carmi and Shomer (3) and Wildman et al. (19) also reported that the accumulation of starch within the chloroplast damaged the thylakoids and grana. Troughton (17) hypothesized that such damage reduced photosynthetic rates.

The size and number of starch grains in the chloroplasts concur with the starch data. An accumulation of starch was detectable at week 4, but without visible modification of the chloroplast ultrastructure. This suggests that the decline of photosynthesis of high CO₂-grown plants during the first 4 weeks cannot be attributed directly to the damage of the chloroplasts. Also, the present data reveal that the chloroplast ultrastructure was more affected in L. esculentum than in L. chmielewskii under high CO₂, but L. chmielewskii showed a faster decline in photosynthesis. Thus, it can be concluded that modification of chloroplast ultrastructure was not the primary reason for acclimation to high CO₂ concentrations.

CONCLUSION

We measured a high concentration of starch in mature tomato leaves exposed to high CO₂. The starch accumulation was related to a decline in photosynthesis, suggesting that starch buildup acted as a feedback inhibitor of photosynthesis. However, leaf 5 showed the same decline in photosynthesis under high CO₂ but without any significantly higher accumulation of starch. This indicates that the long-term decline of photosynthetic efficiency of leaf 5 cannot be attributed to a buildup of starch. Similarly, the acclimation to high CO₂ concentration did not appear related to sugar accumulation in leaf 5 of L. esculentum.

Two species were studied here to verify whether their different sink metabolisms and carbohydrate assimilations could modify the long-term response to high CO₂. Both species

Figure 5. Electron micrograph of chloroplasts of two tomato species grown at 330 and 900 µL L⁻¹ CO₂ for a 10-week period. (A, B) L. esculentum at 330 and 900 µL L⁻¹; (C, D) L. chmielewskii at 330 and 900 µL L⁻¹. S, starch; G, granum; T, thylakoid, ST, stroma; CW, cell wall; Cy, cytoplasm.
accumulated starch and sugars when exposed to high CO₂ concentration, but the partitioning of photosynthates was more directed into starch for *L. esculentum* than for *L. chmielewskii*. Furthermore, *L. chmielewskii* had relatively lower concentrations of starch than *L. esculentum* at both CO₂ levels. However, these characteristics did not improve the long-term photosynthetic efficiency of *L. chmielewskii* grown at high CO₂ when compared with *L. esculentum*.

Our results suggest that and/or sugar accumulations under high CO₂ concentrations cannot entirely explain the loss of photosynthetic efficiency of high CO₂-grown plants. We previously showed that stomatal conductance declined significantly under high CO₂ (21). However, this decline alone could not explain the reduced photosynthetic rates since internal CO₂ concentrations were not modified throughout plant growth. Further research should be undertaken to determine the biochemical and physiological reasons underlying the efficiency loss. Research should be oriented to study the effects of high CO₂ on the mesophyll resistance.

ACKNOWLEDGMENTS

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LITERATURE CITED