Acclimation to High CO₂ in Monoecious Cucumbers

II. CARBON EXCHANGE RATES, ENZYME ACTIVITIES, AND STARCH AND NUTRIENT CONCENTRATIONS

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ABSTRACT

Carbon exchange capacity of cucumber (Cucumis sativus L.) germinated and grown in controlled environment chambers at 1000 microliters per liter CO₂ decreased from the vegetative growth stage to the fruiting stage, during which time capacity of plants grown at 350 microliters per liter increased. Carbon exchange rates (CERs) measured under growth conditions during the fruiting period were, in fact, lower in plants grown at 1000 microliters per liter CO₂ than those grown at 350. Progressive decreases in CERs in 1000 microliters per liter plants were associated with decreasing stomatal conductances and activities of ribulose bisphosphate carboxylase and carbonic anhydrase. Leaf starch concentrations were higher in 1000 microliters per liter CO₂ grown-plants than in 350 microliters per liter grown plants but calcium and nitrogen concentrations were lower, the greatest difference occurring at flowering. Sucrose synthase and sucrose-P-synthase activities were similar in 1000 microliters per liter compared to 350 microliters per liter plants during vegetative growth and flowering but higher in 350 microliters per liter plants at fruiting. The decreased carbon exchange rates observed in this cultivar at 1000 microliters per liter CO₂ could explain the lack of any yield increase (MM Peet 1986 Plant Physiol 80: 59-62) when compared with plants grown at 350 microliters per liter.

Growth rates and yield of most plants including gynoecious, seedless cucumbers (Cucumis sativus L.) grown commercially in greenhouses, increase with an increase in CO₂ concentration (see summary in Kimball [15]). There have been no previous studies, however, on CO₂ enrichment effects on field-type monoecious, seeded cucumbers. These cucumbers differ from greenhouse cucumbers in that only one or two fruit generally develop at a time, apparently because the first-pollinated fruit prevents subsequent fruit from developing. This inhibition was reported as early as 1928 (31), and was described in 1934 (19), and subsequently but has still not been explained (see citations in Uzcategui and Baker [32]). Pharr et al. (24) suggested that carbohydrate production limits fruit development in the seeded outdoor

cultivar Calypso, as they estimated 40% of the canopy would be required to support growth of one fruit.

In a recent study (22) on the monoecious seeded cultivar, Chipper, however, CO₂ enrichment (1000 μL L⁻¹) did not increase dry weight beyond the first 16 d after planting compared to control plants grown at 350 μL L⁻¹ CO₂ and final fruit weight and number did not differ. Similar results were seen in a preliminary study with the same cultivar grown at 675 and 350 μL L⁻¹ CO₂. In both studies considerable leaf necrosis appeared in the high-CO₂-grown plants.

We report here on the effects of high CO₂ concentration during growth of a field-type monoecious cucumber on gas exchange, starch, and nutrient concentrations and the activities of several enzymes possibly rate-limiting to carbon fixation and partitioning (SPS, SS, RubPCase, and CA).

MATERIALS AND METHODS

Seeds of cucumbers (Cucumis sativus cv Chipper) were germinated and grown in the controlled environment chambers of the Southeastern Plant Environmental Laboratory at Duke University. From the time of planting, a 12 h, 26/20°C thermoperiod, 65 to 70% RH, and 300 μE m⁻² s⁻¹ irradiance at pot level were maintained. CO₂ concentration was 350 or 1000 μL L⁻¹ CO₂. Plant density was 97 m⁻² before transplanting (15 d) and 10.8 m⁻² thereafter. Growth media was a 1:1:1 (w:w:v) mixture of gravel, vermiculite, and calcined clay, which is a heated, pulverized soil conditioner prepared by International Minerals Corporation.

Pots were watered to the drip point once daily with half strength Hoagland solution until 33 d after seeding at which time plants were sprayed with iron chelate because of their light green leaf color. After d 33, watering was increased to 3 times daily.

Thirty five, 42, and 58 d after seeding gas exchange was measured using a small, hand-held cuvette enclosing both upper and lower leaf surfaces Patterson et al. [21] for cuvette description and a Beckman 865 IR gas analyzer. All measurements were made inside the growth chambers, selecting the uppermost fully expanded leaf on each of eight plants. Measurements were made during the middle of the photoperiod (1000–1530 h) during which period CER for individual plants did not vary greatly.

Measurement conditions for determining rates of photosynthesis were as close as possible to the growth conditions in the

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2 Abbreviations: SPS, sucrose phosphate synthase; SS, sucrose synthase; RuBPCase, ribulose bisphosphate carboxylase; CA, carbonic anhydrase; CER, carbon exchange rate.
chamber. Although the cuvette was not cooled artificially, high flow rates (1–2 L min⁻¹) maintained leaf temperatures in the cuvette within ±0.5 to 1.0°C of ambient. The airstream was hydrated to a dew point equivalent to 62% RH. On d 42 and 58, the airstream consisted of 345 or 977 µL L⁻¹ CO₂ and 20% O₂ from cylinders. On d 35 bottled gas was not available and outdoor air (355 ± 20 µL L⁻¹ CO₂) was substituted. Photosynthesis was measured under chamber light conditions, which differed only slightly between measurement dates and between the two chambers. Measurement irradiances for the plants grown at 1000 µL L⁻¹ CO₂ averaged 544 ± 8.33, 525 ± 13, and 514 ± 17 µE m⁻² s⁻¹ for the three harvests, respectively. For the plants grown at 350 µL L⁻¹ CO₂, measurement irradiances averaged 560 ± 11, 568 ± 19, and 508 ± 16 µE m⁻² s⁻¹, respectively.

Stomatal conductances were measured with a LI-COR steady state porometer (LI-1600, LI-COR, Lincoln, NE) under the same irradiances as measurements of carbon exchange rates and more or less simultaneously. Total stomatal conductances (1/rₙ) for the leaves were calculated from resistances of the upper and lower leaf surfaces using the formula:

\[ 1/r = 1/r_\text{u} + 1/r_\text{l} \]

where \( r_\text{u} \) is total leaf resistance to diffusion of water vapor, \( r_\text{l} \) is the resistance of the upper leaf surface only, and \( r_\text{l} \) is the lower surface resistance only.

Stomatal conductances were used to correct photosynthetic rates for internal CO₂ concentration. Internal leaf CO₂ concentration (\( C_\text{i} \)) was calculated as:

\[ C_\text{i} = C_\text{e} - 1.6 \frac{\text{CER}}{g} \]

where \( C_\text{e} \) is the external CO₂ concentration, CER is net CO₂ uptake rate, and \( g \) is the stomatal conductance to water vapor.

By 0830 h of the morning after completion of gas exchange measurements, the leaves used for gas exchange measurements were detached for analysis of RuBPCase, SPS, SS, and CA activities. Chl and starch contents were also determined. Chl was measured by the procedure of Arnon (2). Extracts for determination of SPS and SS activity were prepared as described in Camp et al. (4) and assayed by measurement of sucrose-P (+sucrose) formation from UDP-glucose and fructose-6-P (SPS) or fructose (SS) as described by Huber (12). RuBPCase activity was determined by the method of Perchowericz et al. (23) with modifications as described in Camp et al. (5). For the starch assay, leaf samples were homogenized in 80% ethanol and starch was estimated as glucose released by amylglucosidase treatment of the ethanol-insoluble fraction of leaf samples (13). All data are expressed on a fresh weight basis.

Nutrient concentrations were determined from leaf tissue harvested on d 36, 43, and 60. N was assayed by Kjeldahl digestion, K by flame photometer, P by a colorimetric assay using ascorbic acid and sodium molybdate, and Ca and Mg by atomic absorption spectrometry. Analyses were conducted by the North Carolina Department of Agriculture Plant Analysis Laboratory, which also provided guidelines for acceptable nutrient concentrations.

RESULTS

Gas Exchange Rates. CERs of all plants were measured both at 977 and 345 µL L⁻¹ CO₂ except for the initial (vegetative) sampling date when only CER at 345 µL L⁻¹ CO₂ was measured. CER of 1000 µL L⁻¹ CO₂ grown plants measured at a CO₂ concentration near growth levels (977 µL L⁻¹) declined from the flowering to the fruiting stage while CER of 350-grown plants measured near growth levels (345 µL L⁻¹) increased 20% from the vegetative to the fruiting stage (Fig. 1). Thus, under growth conditions CERs in the two treatments were similar at flowering but actually lower in the 1000 µL L⁻¹-grown plants at fruiting. During vegetative growth, CERs under growth conditions were probably higher in 1000 µL L⁻¹-grown than 350 µL L⁻¹-grown plants since CER at 345 µL L⁻¹ CO₂ in 1000 µL L⁻¹-grown plants was only slightly (23%) lower than CER at 345 µL L⁻¹ in 350 µL L⁻¹ CO₂-grown plants (Fig. 2). Differences in photosynthetic capacity when plants from both treatments were measured at 345 µL L⁻¹ increased with time. By flowering, CERs of 1000 µL L⁻¹-grown plants were 39% and by fruiting they were 56% lower than CERs in 350 µL L⁻¹-grown plants. Similar trends were seen when CERs were compared at 977 µL L⁻¹ (data for 350-grown plants measured at 977 µL L⁻¹ not shown).

On all sampling dates, stomatal conductances (Fig. 1) were lower in 1000 µL L⁻¹-grown plants than in 350 µL L⁻¹ plants. In 1000 µL L⁻¹-grown plants, conductances showed the same pattern of decline from the vegetative to fruiting (third) sampling date as did photosynthetic rates measured at 345 µL L⁻¹ (Fig. 2). In the
350 μL L⁻¹ plants, conductances and CER at 345 both increased slightly from the vegetative to flowering sampling periods. While CER continued to increase in the fruiting period, conductances declined drastically.

Intercellular CO₂ levels (Table I) in the 350 μL L⁻¹-grown plants were similar during vegetative growth and flowering, but declined at fruiting because of lower conductances. Intercellular CO₂ level (C₅) cannot be calculated for the 1000 μL L⁻¹ grown plants for the vegetative period, but Cₛ were similar at flowering and fruiting. In this case, proportional declines in conductance and photosynthesis resulted in similar intercellular CO₂ levels.

**Enzyme Activities.** Carbonic anhydrase (Fig. 2) was measured only at flowering and fruiting. On both dates, activities were much higher in 350 μL L⁻¹-grown plants than in those from the 1000 μL L⁻¹ CO₂ treatment. For both treatments, activities declined from flowering to fruiting, but the decline was greater in the 1000 μL L⁻¹ CO₂ plants than in the 350 μL L⁻¹-grown plants.

RuBPCase activities (Fig. 2) were higher in 1000 compared to 350 μL L⁻¹-grown plants during vegetative growth, but were much lower thereafter. RuBPCase activities, like CER at 345 μL L⁻¹ and conductances, declined in the 1000 μL L⁻¹ plants from vegetative growth to flowering. In the 350 μL L⁻¹-grown plants, RuBPCase activities were similar during vegetative growth and flowering, but then declined during fruiting.

SPS activity (Fig. 3) was higher in the 1000 μL L⁻¹ plants than in the 350 μL L⁻¹-grown plants during vegetative growth, similar at flowering, and lower during fruiting. In both treatments, SPS activities were higher during vegetative growth than at flowering. In the 1000 μL L⁻¹ treatment, activities then declined slightly at the time of flowering, while in the 350 μL L⁻¹ treatment they increased again, although not to the level found in the leaves during vegetative growth.

The changes in SS activity (Fig. 3) with time and the differences between the treatments were somewhat similar to those in SPS activities. For both enzymatic, activities were highest in 1000 μL L⁻¹-grown plants than the 350 μL L⁻¹-grown plants during vegetative growth and flowering, but the reverse was true at fruiting. For both enzymes, the activities of 350 μL L⁻¹-grown plants were lowest at flowering, while the activities of 1000 μL L⁻¹-grown plants were lowest at fruiting. Patterns of change in the two enzymes differed most conspicuously in the 350 μL L⁻¹-grown plants, where highest SPS activity was found during vegetative growth, while SS activity was highest at fruiting.

**Starch Levels.** Starch levels (Fig. 3) in 1000 μL L⁻¹-grown plants exceeded those in 350 μL L⁻¹ plants on all sampling dates. Changes in starch levels with time differed in the two treatments, however, and were not related in any obvious way to changes in rates of photosynthesis. In the 1000 μL L⁻¹ plants, starch levels peaked at the time of flowering (d 43) and were lowest at fruiting. In the 350 μL L⁻¹-grown plants, tissue starch levels were similar throughout the entire measurement period.

**Leaf Necrosis.** In both a preliminary study (spring 1980) and the study reported here, by d 44 CO₂ enriched plants appeared lighter green than controls and had a pronounced yellow margin on most leaves. In enriched treatments, older leaves in particular had a blotchy, light-green appearance with some interveinal necrosis. In both experiments, one or two of the oldest leaves had withered in the enriched treatment, but not in the control treatment. In the second experiment which incorporated higher levels of enrichment (1000 versus 675 μL L⁻¹) the tendency for lighter leaves in enriched plants was more pronounced, and plants appeared smaller and more spindly in some cases than 350 μL L⁻¹-grown plants. Chlorosis also appeared earlier, showing up by d 33 in the 1000 μL L⁻¹ plants. In the first experiment, although color was lighter, plant vigor was not noticeably decreased in the 675 μL L⁻¹ plants compared to 350 μL L⁻¹-grown plants.

**Nutrient Concentrations.** Nutrient concentrations (Fig. 4) in all the tissues analyzed were within or slightly above those considered normal (3.5-4.5% N, 0.35-0.65% P, 3.5-5.0% K, 1.5-4.0% Ca, and 0.2-0.4% Mg) by the North Carolina Department of Agriculture. During vegetative growth and flowering, concentrations of all elements, but particularly calcium, were considerably lower in the 1000 μL L⁻¹-grown compared to 350 μL L⁻¹-grown plants. By fruiting, however, they were similar in the two treatments. Starch concentrations above 20 μg g⁻¹ fresh

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**Table 1. Intercellular CO₂ Concentration for Cucumber Plants Grown at 350 and 1000 μL L⁻¹ CO₂**

<table>
<thead>
<tr>
<th>CO₂ Level</th>
<th>Intercellular CO₂ concentration (C₅) μL L⁻¹</th>
<th>Vegetative</th>
<th>Flowering</th>
<th>Fruiting</th>
</tr>
</thead>
<tbody>
<tr>
<td>350</td>
<td>321</td>
<td>325</td>
<td>313</td>
<td></td>
</tr>
<tr>
<td>± SE</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
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<tr>
<td>1000</td>
<td>979</td>
<td>982</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± SE</td>
<td>2.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 3.** Starch concentrations (mg g⁻¹ fresh wt), SS and SPS activities (μmol g⁻¹ fresh wt h⁻¹) in leaves from cucumber plants grown at 350 (Δ, ■, ●) and 1000 μL L⁻¹ CO₂ (Δ, □, ○).

**Fig. 4.** Nutrient concentrations (% dry weight) and starch concentration in leaves grown at 350 or 1000 μL L⁻¹ CO₂ and harvested during vegetative growth, flowering or fruiting.
weight were associated with lower nutrient levels, as seen during vegetative growth and flowering in the enriched plants. The percent change in starch was greater than the percentage change in dry weight, however.

**DISCUSSION**

When photosynthetic rates of high and low CO₂-grown plants have been measured at the same CO₂ concentration, lower rates of enriched plants have been recorded in many crops (34, 36) including cucumbers (1, 8). In most of these studies in situ (i.e., measured at growth CO₂ concentration) CERs were still higher in enriched plants, but in beans (*Phaseolus vulgaris* L.) (34), in situ CER in high CO₂ grown plants was similar to or less than that of 'normal' CO₂ grown plants even when measured at high and low CO₂ concentrations, respectively, a result similar to that seen in the present study at flowering and fruiting. In soybeans (7) and cucumbers (14), as in the present study, differences between photosynthetic rates under ambient CO₂ of high and low CO₂ grown plants increased with time.

Photosynthetic reductions with time in CO₂ enriched plants in this study were associated with decreased CA and RuBPCase activities (Fig. 2). Lower CA activities in CO₂ enriched plants have been reported in bean (25) and cotton (6). Lower RuBPCase activities have been reported in CO₂ enriched bean (25), cotton, maize (36), and soybean (35). There are also reports, however, of only slightly lower photosynthetic capacity and similar RuBPCase activities in wheat (9) and of higher RuBPCase and photosynthesis in CO₂-enriched tomatoes (10, 11).

Variations in photosynthetic response to high CO₂ levels exists not only between species, but between experimental treatments with a single species. Container-grown soybeans showed a much greater photosynthetic response to CO₂ level than did field-grown plants (30). In low nitrogen-grown cotton, ambient plants had higher photosynthetic rates at all intercellular CO₂ partial pressures, but in high nitrogen-grown plants this occurred only at low partial pressures (36).

A direct relation between increases above a critical level in carbohydrate concentration and declines in CO₂ assimilation was recently shown in wheat (3). High starch levels were associated with lowered CER compared to controls in CO₂ enriched soybeans (20) and defruited cucumber (24). Higher starch concentrations in CO₂-enriched plants compared to 350 μL-L⁻¹ plants were associated with reduced CER during vegetative growth and flowering in the present study. At fruiting, however, starch concentrations were similar in the two treatments, but CER was greatly reduced in enriched plants compared to 350 μL-L⁻¹-grown plants.

SPS activities are negatively correlated with starch levels in soybeans (13). We did not see this correlation, possibly because in the present study, leaf starch concentration was measured in the early morning rather than at the end of the photoperiod. Changes in starch reported here strongly reflect differences in the extent of starch mobilization at night and thus might not parallel SPS changes as closely as daytime starch changes. There were, however, some similarities between SPS activities and in situ CER. At flowering, both photosynthetic rates in situ and SPS activities were similar in the two treatments and at flowering, in situ photosynthetic rates and SPS activities in 1000 μL-L⁻¹ plants were both only a third of that of 350 μL-L⁻¹ CO₂ plants. SS activities showed somewhat the same pattern as SPS activities. They were 32% higher initially in the 1000 μL-L⁻¹ treatments, but more than 3 times as high in the 350 μL-L⁻¹ treatments by the last sampling (at fruiting). The patterns of change in SPS and SS activities with time are similar to those reported by Huber and Israel in soybeans (13).

In addition to having depressed enzyme activities, CO₂-enriched plants had lower stomatal conductances as has been observed in studies with CO₂-enriched soybeans (27). Lower conductances at fruiting reduced intercellular CO₂ levels in 350 μL-L⁻¹ plants, but did not reduce C in 1000 μL-L⁻¹ plants because of the proportional decline in CER. Since intercellular CO₂ was not reduced in the high CO₂ grown plants at fruiting, the decreased stomatal conductances did not account for the decreases in photosynthesis seen in these plants from flowering to fruiting.

Reductions in tissue nutrient concentrations of CO₂ enriched plants have been reported in greenhouse cucumbers (14), lettuce (16), and beans (25). Lower stomatal conductances in CO₂ enriched plants may reduce transpiration sufficiently to affect nutrient uptake as suggested by Madsen (18) for tomatoes. This linkage between transpiration and nutrient uptake is not important at natural soil fertility levels (29), but at high nutrient levels such as those we used, low transpiration may be a factor in reducing nutrient uptake (28).

The physiological significance of lower nutrient concentrations in CO₂ enriched plants is unclear. In studies using greenhouse cucumber (14), lettuce (16), beans (25), and in our study, all nutrients were within the adequate range, although the necrosis we observed resembled K deficiency. In other studies where cucumbers were CO₂ enriched under bright conditions for extended periods of time, similar necroses have been reported (14).

van Berkel (33) reported that 'glassiness' in cucumbers was promoted by high root pressure which causes air to be driven from the intercellular spaces. Madsen (17) attributed progressive deformation of CO₂-enriched tomato plants to the accumulation of starch in the leaves.

**Conclusions.** Lack of a sustained growth or a yield increase in this cultivar when grown at 1000 μL-L⁻¹ CO₂ was caused by an increasing depression of carbon exchange capacity such that the time plants were flowering, in situ photosynthetic rates were similar in both 350 and 1000 μL-L⁻¹ plants and by fruiting CER was actually higher in the 350 μL-L⁻¹ plants than in the 1000 μL-L⁻¹ plants. We expected that development of fruits between d 43 and 58 would stimulate photosynthetic rates in enriched plants.

This was not the case, however, even though leaf starch did decline. This reduction in starch (or other carbohydrates) might have eventually led to a recovery in CER as it was associated with an increase in relative growth rates on an area basis (22), but the lack of successful pollination after d 51 in both treatments indicated plants were past a 'window' in fruit set. Such a window was also seen in a similar cultivar given flower removal treatments in the greenhouse (26).

The implications of this study relative to the cause of first fruit inhibition are unclear since at the time of flowering, photosynthetic rates were similar in the two treatments. Possibly if the plants had not been exposed to higher CO₂ until fruit set, the response would have been greater. In lettuce (16) and tomatoes (18) restricting enrichment to the period of most rapid growth has also been suggested as a means of increasing response. Fruit number per plant in monococious seeded cucumber appears inelastic, however, and may be controlled internally rather than by carbohydrate supply. When we raised carbohydrate supply by CO₂ enrichment, leaf area/fruit increased from 11.3 to 12.7 dm² fruit rather than remaining the same or decreasing. Similarly, when Ramirez (26) removed fruit for 21 d before allowing fruit set, the area per fruit increased to 18.5 dm² compared to 13 in controls.

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**LITERATURE CITED**

1. Aoki, M., K. Yabuki 1977 Studies on the carbon dioxide enrichment for plant growth. VII. Changes in dry matter production and photosynthetic rates of...
cucumber during carbon dioxide enrichment. Agric Meteorol 18: 475-485
4. CAMP PJ, SC HUBER, DE MORELAND 1982 Biochemical changes that occur during senescence of wheat. I. Basis for the reduction of photosynthesis. Plant Physiol 70: 1641-1646
8. FRYDОРЖ 1976 Photosynthetic characteristics of cucumber seedlings grown under two levels of carbon dioxide. Photosynthetica 10: 335-338
11. HINKLETON PR, PA JOLLIFFE 1980 Alterations in the physiology of CO2 exchange in tomato plants grown in CO2-enriched atmospheres. Can J Bot 58: 2181-2189
14. ITO T 1978 Physiological aspects of carbon dioxide enrichment to cucumber plants grown in greenhouses. Acta Hortic 87: 139-146
18. MADSEN E 1975 Effect of CO2 enrichment on growth, development, fruit production and fruit quality of tomato from a physiological viewpoint. Phytotronics III: 318-330