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Effects of CO_2 Enrichment and Carbohydrate Content on the Dark Respiration of Soybeans[1]

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ABSTRACT

During the period of most active leaf expansion, the foliar dark respiration rate of soybeans (*Glycine max* cv Williams), grown for 2 weeks in 1000 microliters CO_2 per liter air, was 1.45 milligrams CO_2 evolved per hour leaf density thickness, and this was twice the rate displayed by leaves of control plants (350 microliters CO_2 per liter air). There was a higher foliar nonstructural carbohydrate level (*e.g.* sucrose and starch) in the CO_2 enriched compared with CO_2 normal plants. For example, leaves of enriched plants displayed levels of nonstructural carbohydrate equivalent to 174 milligrams glucose per gram dry weight compared to the 84 milligrams glucose per gram dry weight found in control plant leaves. As the leaves of CO_2 enriched plants approached full expansion, both the foliar respiration rate and carbohydrate content of the CO_2 enriched leaves decreased until they were equivalent with those same parameters in the leaves of control plants. A strong positive correlation between respiration rate and carbohydrate content was seen in high CO_2 adapted plants, but not in the control plants.

Mitochondria, isolated simultaneously from the leaves of CO_2 enriched and control plants, showed no difference in NADH or malate-glutamate dependent O_2 uptake, and there were no observed differences in the specific activities of NAD^+ linked isocitrate dehydrogenase and cytochrome *c* oxidase. Since the mitochondrial O_2 uptake and total enzyme activities were not greater in young enriched leaves, the increase in leaf respiration rate was not caused by metabolic adaptations in the leaf mitochondria as a response to long term CO_2 enrichment. It was concluded, that the higher respiration rate in the enriched plant's foliage was attributable, in part, to a higher carbohydrate status.

Plants grown in elevated CO_2 environments have higher photosynthetic rates, greater foliar starch accumulation, and greater biomass production than plants grown in ambient levels of CO_2 (11, 17). Carbon assimilation is enhanced at high CO_2 partial pressures by providing more substrate, by overcoming the O_2 inhibition of photosynthesis, and by diminishing glycolate pathway activity (28). A high CO_2 environment also affects the morphology of a plant by increasing the thickness and Fw[2] of leaves. These increases, however, are due to an increase in the volume and water content of the cells, and not an increase in the actual number of cells (15).

Recently, attention has been given to the role of carbohydrate status in regulating carbon metabolism (9). It has been suggested that high carbohydrate levels inhibit photosynthesis (3, 25). Azcon-Bieto (3) postulates that above a certain critical level of carbohydrate, CO_2 assimilation would decrease due to carbohydrate accumulation impairing the production or consumption of ATP/NADPH in photosynthesis. However, Robinson (22) has reported higher photosynthetic activities associated with high starch and sucrose levels.

Carbohydrate status may also have an effect on dark respiration. A high carbohydrate status has been associated with elevated respiration rates in a number of studies (1, 2, 5, 20, 26). Dark respiration is thought to have two components, growth and maintenance. Growth respiration occurs in those parts of a plant which are actively dividing or expanding (21). The rate of growth respiration is thought to be related to the rate of photosynthesis. Maintenance respiration occurs throughout the life of the plant and is proportional to the Dw of the plant (18, 21). During plant growth both types of respiration occur, but growth respiration would decrease as the leaf matures. Both are thought to involve carbohydrate oxidation through glycolysis, the pentose phosphate pathway and the tricarboxylic acid cycle (2).

In this study, long-term CO_2 enrichment was used to increase carbohydrate levels in order to investigate the relationship between dark respiration and carbohydrate status. The effect of increased carbohydrate status on dark respiration was studied by monitoring dark CO_2 release from whole leaves. Subcellular fractionation of enriched and control leaves was performed to determine if there were increases in the activity or number of mitochondria as a consequence of a high CO_2 level.

MATERIALS AND METHODS

Plant Material. Soybeans (*Glycine max* cv Williams) were planted in 15-cm pots containing vermiculite and were grown in Environmental Growth Chambers No. M-2 at 25°C, with a 12-h photoperiod. The plants were illuminated with a mixture of fluorescent and incandescent bulbs at 600 $\mu E/m^2$ at pot height. They were flushed daily with a nutrient solution described previously (22). The plants were germinated in 350 $\mu l/L$ CO_2. At 10 d postemergence, half of the plants were placed in a separate growth chamber with identical conditions with the exception of an enriched CO_2 level of 1000 $\mu l/L$, while the other half (control) remained at 350 $\mu l/L$ CO_2.

Leaf Respiration Measurements. Data collection began when Tf 4 was approximately one-third of the fully expanded size—

[1] Sponsored jointly by the United States Department of Agriculture and the George Washington University through the Cooperative Education Program, and is part of Terry Hrubec's MS thesis.

[2] Abbreviations: Fw, fresh weight; Dw, dry weight; LDT, leaf density thickness, g fresh weight/dm^2; SHAM, salicylhydroxamic acid; SLW, specific leaf weight, g dry weight/dm^2; Tf, trifoliolate position-numbered acropetally.

large enough to fit into the gas exchange cuvette. Five enriched and five control plants were selected at random, and one at a time taken to the measuring chamber—a small growth chamber having the same conditions as in the primary growth chambers. The leaf to be measured, while still attached to the plant, was placed in a clear acrylic cuvette open to the chamber air. The plants were allowed to equilibrate for 10 min to recover from handling, whereupon the lights were turned off and the cuvette closed to the chamber air. Dark respiration was measured after the postillumination burst as described in Wolf *et al.* (27). CO_2 released into CO_2-free air was monitored by a Beckman 865 IR gas analyzer.[3]

The plants were carefully removed from the cuvette and either returned to the primary growth chamber if other leaves on the same plant were to be observed, or harvested. On days when more than one leaf position was measured, positions 4 and 5 were examined in that order. All plants of a given CO_2 concentration would be measured before changing the CO_2 level in the test chamber. All dark respiration measurements were conducted during the day between the hours of 10:00 and 16:00.

Leaf Photosynthesis Measurements. Carbon assimilation was measured during leaf expansion in both CO_2 enriched and control plants as described in Wolf *et al.* (27).

Leaf Sampling. All the leaves from a plant were harvested simultaneously to prevent the removal of one leaf from affecting dark respiration in subsequent leaves. The leaves were quickly removed and leaf area measured using a LI-COR model LI-3000 leaf area meter. The Fw was determined and each Tf placed in a small envelope which was then immersed in liquid N_2. The frozen tissue was then lyophilized, weighed for SLW determination, and stored desiccated at −20°C.

Carbohydrate Determination. All freeze-dried tissue was brought to room temperature and the primary and secondary veins removed. For each Tf, 100 mg of deveined tissue were pooled so that all normal or CO_2 enriched tissue from a single Tf position was mixed for each day. From this pool three samples, each weighing approximately 20 mg, were taken for each position measured in 1 d. The results from the three samples were then averaged to give the final values for amounts of sucrose, starch, and glucose as determined using the methods of Dickson (8). Glucose released from starch (by amyloglucosidase) or from sucrose (by invertase) was converted to glucose 6-P and quantified by the methods of Latzko and Gibbs (13).

Crude Mitochondria Isolation. Crude mitochondria were prepared by differential centrifugation. When the plants were 19 d old (postemergence), 10 to 15 g of Tf 4 leaf tissues from enriched and control plants were rinsed in distilled H_2O, dried, and the midveins were removed. The tissue was cut with scissors into 50 ml of cold grind medium described previously (10) contained in a glass bowl on ice. The tissue was chopped on ice for 4 min with razor blades and then ground in a Polytron homogenizer (setting 6) for 6 s. The brei was filtered through two layers of Miracloth and two layers of 20-μm mesh nylon netting to remove whole cells. The filtrate was spun for 10 min at 1500g on a Dupont Sorval RC-5 centrifuge. The postnuclear supernatant was spun for 2.5 min at 27,000g to pellet the mitochondria. The mitochondrial pellet was resuspended to a volume of 10 ml in a Potter-Elvehjem tissue homogenizer fitted with a Teflon plunger. The resuspending medium contained 0.3 M sucrose, 10 mM Hepes-KOH (pH 7.2), 10 mM KCl, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 0.1% BSA (w/v).

Purified Mitochondria Isolation. Mitochondria from enriched and control soybean leaves were separated from chloroplasts using discontinuous Percoll gradients as recently developed by Hrubec *et al.* (10).

Mitochondrial O_2 Uptake. Mitochondrial fractions from the Percoll gradients and resuspended crude mitochondrial pellets were used for O_2 uptake studies in a Clark-type O_2 electrode at 25°C. For both types, 800 μl of mitochondria were added to 200 μl of resuspension medium (described above). The following additions were made: 2.5 mM NADH, 10 mM malate in 10 mM Hepes-KOH (pH 7.2), 10 mM glutamate in 10 mM Hepes-KOH (pH 7.2), 0.03 mM ADP. The final volume after additions was 1.15 ml.

Chlorophyll Determination. Chl was measured to determine chloroplast contamination of mitochondrial pellets and gradient fractions. Chl was extracted in 100% methanol using the method of Cosio *et al.* (6).

Enzyme Assays. NAD^+ isocitrate dehydrogenase was measured by the method of Cox (7), modified by the addition of 0.5 mM KCN and 0.01% Triton X-100. Hydroxypyruvate reductase was measured by the method of Laing *et al.* (12), modified by the addition of 0.5 mM KCN. Cyt *c* oxidase was assayed by the method of Simon (24), and catalase by the method of Lück (14).

Protein. Mitochondria from both isolation techniques were diluted in an isotonic solution and pelleted using a Beckman Microfuge-12 to remove the BSA. The supernatant was discarded and the pellet rinsed, resuspended, and repelleted. This final pellet was resuspended in a known volume and the protein measured by the method of Bradford (4).

RESULTS

Growth Data. Since CO_2 enrichment affects the morphology of leaves, plant growth was monitored to better understand the morphological differences between the two groups of plants used in this study. Fw, Dw, leaf area, LDT, and SLW were measured over a 7-d period during leaf expansion (Tables I and II). The leaf area increased for the first 3 to 5 d of measurement and then remained constant. Leaf area was consistently greater in enriched plants; however, this difference was not significant at $\alpha = 0.05$. Fw increased in a manner similar to the leaf area with no significant difference between enriched and control plants. Fw increased very little after maximal area was reached.

LDT increased as the leaf developed (Table I). Because CO_2 enrichment increased the thickness of the leaf, the LDT has a greater value in the enriched plants. This measurement is a good indicator of leaf volume as the changes in weight due to starch content are negligible compared to the water weight. SLW is not an appropriate measurement of growth in CO_2 enriched soybeans as it varies greatly with the starch content of the leaves. Figure 1 shows the change in SLW and LDT of Tf 4 over a 24-h period at 18 d postemergence. Presumably, the 60% increase in SLW seen in enriched plants during the time of illumination is due to starch accumulation. The change in SLW over the 24-h period is greater than the change in SLW over the week of leaf maturation (Table I). The LDT remained constant over the 24 h and so was the preferred basis of expression for respiration rates.

Dark Respiration. The dark respiration rate was elevated in the enriched plants, but only during the period of leaf expansion. The dark respiration of expanding leaves decreased as the mature size is approached, as was seen in both control and enriched plants (Fig. 2). However, the dark respiration in the enriched plants decreased at a greater rate, resulting in equivalent respiration rates in both control and enriched plants by the last day of measurement. The same trend was seen in leaves 4 and 5, which suggests that CO_2 enrichment may initially increase the respiration rate in all leaves.

CO_2 enrichment alters growth rate and morphology, thus it is difficult to select comparable plants from experimental and

[3] Mention of a trademark, proprietary product, or vendor does not constitute guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table I. *Fw, Leaf Area, Specific Leaf Weight, and Leaf Density Thickness of Tf 4 and 5 in CO_2 Enriched and Control Plants over the 7 d of Leaf Expansion*

Fw and leaf area are the average of five plants ± SD, while SLW and LDT are calculated from those averages.

	Fresh Wt	Leaf Area	SLW	LDT
	g	*cm^2*	*g Dw/dm^2*	*g Fw/dm^2*
Enriched				
Tf 4				
18 d	2.14 ± 0.48	120 ± 22	0.343	1.78
20 d	3.31 ± 0.22	175 ± 7	0.389	1.89
23 d	3.67 ± 0.34	193 ± 13	0.333	1.90
25 d	3.50 ± 0.56	184 ± 23	0.350	1.90
Tf 5				
20 d	1.97 ± 0.21	115 ± 14	0.375	1.71
23 d	3.47 ± 0.37	197 ± 15	0.315	1.76
25 d	3.77 ± 0.70	202 ± 22	0.309	1.86
27 d	3.78 ± 0.20	213 ± 7	0.345	1.86
Control				
Tf 4				
18 d	1.47 ± 0.22	102 ± 17	0.270	1.44
20 d	2.80 ± 0.23	176 ± 15	0.290	1.59
23 d	2.91 ± 0.28	173 ± 12	0.287	1.68
25 d	2.96 ± 0.10	179 ± 9	0.353	1.65
Tf 5				
20 d	1.53 ± 0.26	101 ± 18	0.272	1.52
23 d	2.29 ± 0.28	147 ± 15	0.275	1.56
25 d	2.69 ± 0.21	166 ± 9	0.291	1.63
27 d	2.74 ± 0.27	170 ± 13	0.345	1.60

Table II. *Carbohydrate Content and Dw of Leaves 4 and 5 in CO_2 Enriched and Control Plants over the 7-d Period of Leaf Expansion*

Carbohydrate values are averages of three samples while Dw are averages of five plants. Starch and sucrose are expressed as glucose equivalents.

	Glucose	Sucrose	Starch	Dw
	mg/g Dw	*mg/g Dw*	*mg/g Dw*	*g*
Enriched				
Tf 4				
18 d	2.87	9.53	161.78	0.41 ± 0.09
20 d	0.55	10.46	208.88	0.69 ± 0.12
23 d	0.70	12.77	57.49	0.64 ± 0.06
25 d	0.14	7.45	44.41	0.65 ± 0.13
Tf 5				
20 d	0.36	7.44	209.71	0.43 ± 0.03
23 d	0.98	15.01	108.64	0.62 ± 0.08
25 d	0.54	9.05	106.74	0.78 ± 0.21
27 d	0.11	5.42	50.37	0.70 ± 0.13
Control				
Tf 4				
18 d	0.86	5.20	78.78	0.25 ± 0.03
20 d	1.61	8.63	97.86	0.47 ± 0.04
23 d	0.14	5.87	88.26	0.50 ± 0.06
25 d	0.73	6.07	37.60	0.51 ± 0.04
Tf 5				
20 d	0.53	5.66	75.54	0.27 ± 0.04
23 d	0.14	4.96	92.96	0.40 ± 0.04
25 d	0.98	6.31	60.53	0.48 ± 0.03
27 d	0.16	5.47	95.83	0.60 ± 0.09

control conditions. To compensate for this, the dark respiration was expressed in a variety of ways, *e.g.* based on LDT, leaf area, Fw, Dw, and SLW. In all instances, the enriched plants had respiration rates approximately 50% greater than controls during the time of leaf expansion but diminished to an equivalent rate

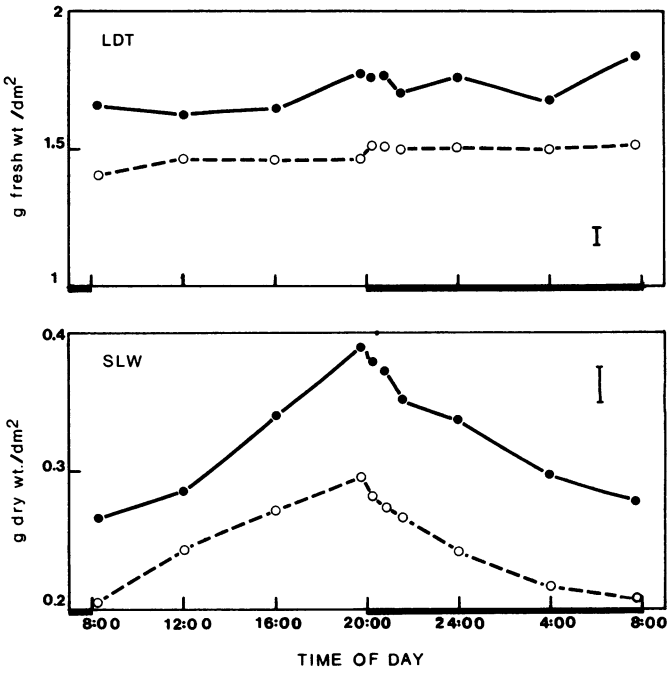

FIG. 1. LDT (g Fw/dm^2) and SLW (g Dw/dm^2) over a 24-h period in 1000 μl/L CO_2 enriched (●) and control 350 μl/L (○) plants. Each point represents the mean of four plants. The bold ordinate line represents the dark portion of the 24 h. The plants were grown for 10 d at 350 μl/L whereupon half the plants were transferred to 1000 μ/L CO_2. After 8 d of enrichment the growth of Tf 4 was monitored over the 24-h period. The tissue was processed as described in "Materials and Methods". The error bar represents the largest SE.

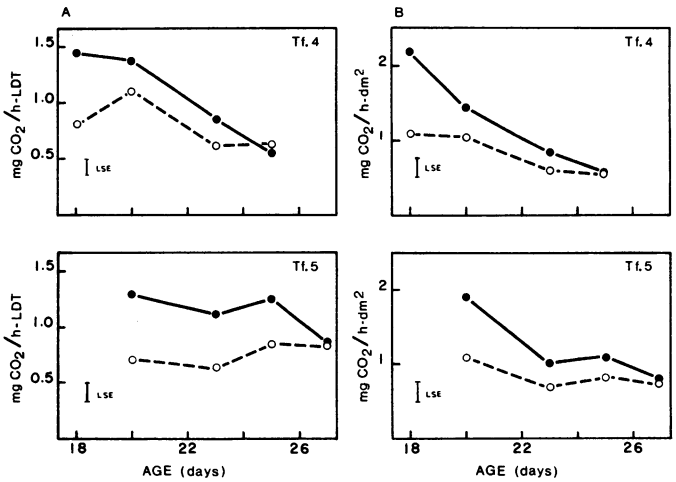

FIG. 2. Dark respiration of Tf 4 and 5 in 1000 μ/L (●) and 350 μ/L CO_2 (○) over the time of leaf expansion. The data are expressed (A) on a LDT (g Fw/dm^2) basis, and (B) on a leaf area basis (dm^2). Each point represents a mean of five plants. The error bar represents the largest SE in both enriched and control respiration rates.

as the leaf matured. Respiration rates expressed on a LDT and leaf area basis are shown in Figure 2. Rates based on the other parameters exhibited the same pattern. For example, on a SLW basis, Tf 4 initially had a rate of 8.21 mg CO_2/SLW in the enriched plant as compared to a rate of 4.47 in the control (data not shown). At the end of the measurement period, the respiration rate of the enriched plant had decreased steadily to 3.53 mg CO_2/SLW while the control rate did not change significantly (final rate 4.23 mg CO_2/SLW) (data not shown). When the

respiration rates are expressed on a Fw, Dw, or leaf area basis both enriched and control respiration rates decrease as the leaf matures. For example, the rate of the enriched Tf 4 decreased from 6.29 mg CO_2/h·g Dw to 1.73 mg CO_2/h·g Dw; whereas the rate of the control decreased from 4.43 to 2.08 mg CO_2/h·g Dw (data not shown).

Leaf Carbohydrate Content. During the period of leaf expansion, the total carbohydrate (starch, sucrose, and glucose) content of the CO_2 enriched leaves decreased from a high initial value to a much lower value. The control leaves had fairly constant levels of carbohydrate that were comparable to the lowest levels for the enriched plants (Fig. 3). Table II shows the individual carbohydrate components and the Dw. The glucose levels varied with the time of day and were lower in leaves harvested in the afternoon. The sucrose and starch content of the leaves was much higher in enriched plants during leaf expansion and the first few days after maximum size was reached. The starch content of the enriched plants then decreased rapidly to a level similar to that of controls (Table II).

The initial starch content of Tf 5 after 10 d of enrichment was 48.7% of the Dw, whereas the control starch content was only 27.6%. After 17 d of enrichment (27 d postemergence), the starch levels in the high CO_2 plants had decreased to 7.1% of the Dw while the levels in the control plants decreased to 15.9% over this time. The Dw of the enriched Tf 5 (0.7 g) was greater than that of the control (0.6 g), even though the starch comprised a smaller percentage of the Dw. This indicates that carbon was partitioned into structural elements rather than carbohydrate reserves in the expanded leaf of enriched plants.

Relationship between Dark Respiration and Carbohydrate Status. The total carbohydrate level of the enriched leaves (Fig. 3) followed the dark respiration rate (Fig. 2) fairly closely, especially when the respiration rate was expressed on a LDT basis. Least squares linear regression analysis of carbohydrate content and dark respiration rate in the enriched plants showed a strong relationship ($r^2 = 0.62$) (Fig. 4). Total carbohydrate and respiration were strongly correlated ($r = 0.79$, $P < 0.001$) in the enriched plants, but not correlated in the controls ($r = 0.19$, $P < 0.05$). This lack of correlation indicates that respiration rate cannot be predicted from the carbohydrate content in the control plants.

Crude Mitochondrial O_2 Uptake. Pelleted mitochondria from 19-d-old enriched and control Tf 4 showed no statistical difference at $\alpha = 0.05$ in either malate and glutamate dependent or NADH dependent O_2 uptake (Table III). State three rates (*i.e.* rates not limited by ADP or substrate) for malate and glutamate dependent O_2 uptake were 6.39 nmol O_2/min·g Fw in mitochondria from enriched plants and 6.62 in control mitochondria. State three rates for NADH dependent O_2 uptake were 21.70

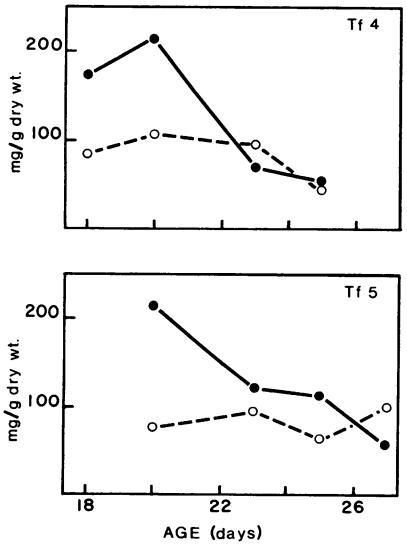

FIG. 3. Total carbohydrate content (starch, sucrose, and glucose) of Tf 4 and 5 in 1000 μ/L (●) and 350 μ/L (○) CO_2 over the period of leaf expansion. The values are the sums of the separate carbohydrate components (Table II) and are expressed as mg glucose equivalents/g Dw.

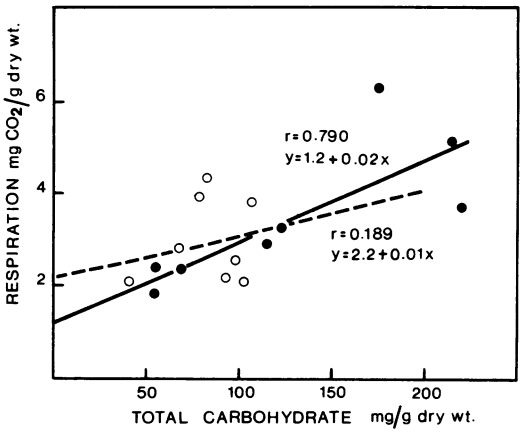

FIG. 4. Relationship between total carbohydrate content and respiration rate in 1000 μl/L (●) and 350 μl/L (○) CO_2. Data points represent averages of Tf 4 and 5 over the period of leaf expansion. Least squares linear regression analysis showed a strong relationship for enriched plants ($r^2 = 0.62$). The lack of correlation in the control plants indicates that respiration cannot be predicted from carbohydrate content.

Table III. *Substrate Dependent O_2 Uptake and Mitochondrial Enzyme Activity of Isolated Mitochondria*

Purified mitochondria were isolated on discontinuous Percoll gradients from plants grown in 1000 μl/L and 350 μl/L CO_2. Crude mitochondria were prepared by differential centrifugation. O_2 uptake was monitored after the following additions: 2.5 mM NADH, 10 mM malate, 10 mM glutamate, and 0.03 mM ADP. Total activity and specific activity of Cyt *c* oxidase and NAD^+ isocitrate dehydrogenase in purified mitochondria were assayed spectrophotometrically.

	Crude Mitochondria		Purified Mitochondria	
	Enriched	Control	Enriched	Control
	nmol O_2/min·g Fw			
Oxygen uptake				
NADH				
State 3	21.70	26.50	1.45	2.56
State 4	9.98	13.43	1.07	1.72
Malate and glutamate				
State 3	6.39	6.62	1.47	2.57
State 4	3.90	3.38	1.25	2.06
			nmol/min·ml	
Enzyme activity				
Total activity				
Cyt *c* oxidase			220.3	231.6
Isocitrate dehydrogenase			38.7	40.9
			nmol/min·mg protein	
Specific activity				
Cyt *c* oxidase			2771	2664
Isocitrate dehydrogenase			809	778

and 26.50 in enriched and control plants, respectively. The data are expressed as nmol O_2 uptake per min·g of original Fw, as it is difficult to correct for the chloroplast component in a protein measurement.

In both the enriched and control plants a ratio of Chl to protein was found to be 4:1 in thylakoid fragments from purified plastids in Tf 5 at 20 d postemergence. When chloroplast protein was subtracted from the protein of the mitochondrial fraction, malate and glutamate dependent activity was approximately 19 nmol O_2/min·mg protein for both the enriched mitochondria and controls. However, the standard deviation was quite large (40% of the mean) due to great variation in the corrected protein content measured in different preparations.

Purified Mitochondrial O_2 Uptake. The isolation technique (10) resulted in a clean band of mitochondria contaminated by only 4% of the total Chl in the gradient. This contamination occurred during fractionation when broken thylakoid fragments clinging to the walls of the polyallomer tube became mixed with the mitochondrial band as it moved up the tube. Purified mitochondria from 20-d-old Tf 5 of enriched plants exhibited a slightly lower rate of malate and glutamate dependent and NADH dependent O_2 uptake than controls (Table III). This difference, however, is not significant at $\alpha = 0.05$. Again the data are expressed on a Fw basis. The amount of O_2 uptake was less than that found for pelleted mitochondria. This was due to a shorter grinding time for the purified mitochondria, which greatly reduced the yield of mitochondria but also reduced the subsequent thylakoid contamination of the mitochondrial fraction.

Purified Mitochondrial Enzyme Assays. There was no difference between enriched and control plants in the specific activities of NAD^+ dependent isocitrate dehydrogenase or Cyt *c* oxidase (Table III). Also, no difference was seen between enriched and control plants in the total activities of these two enzymes.

Effect of CO_2 Enrichment on Photosynthesis. CO_2 assimilation was measured in fully expanded leaves from Tf 5 and 6 in control plants, and 6 and 7 in enriched plants grown under the same conditions as described in "Materials and Methods". Net photosynthetic rates for control Tf 5 and Tf 6 (measured at 350 μl CO_2/L) were 31.43 and 32.09 mg $CO_2/dm^2 \cdot h$, respectively. Rates for enriched Tf 6 and 7 (measured at 1000 μl CO_2/L) were 47.30 and 45.75 mg $CO_2/dm^2 \cdot h$, respectively. As expected, the elevated CO_2 concentration increased the photosynthetic rate.

Effect of CO_2 Enrichment on Peroxisomal Activity. The activities of peroxisomal enzymes hydroxypyruvate reductase and catalase were measured to determine if differences in activity occurred due to CO_2 enrichment. As the photorespiratory pathway is inhibited in high CO_2 environments, there could have been a difference in the activity of hydroxypyruvate reductase, an enzyme of this pathway. There was, however, no difference in the activity of hydroxypyruvate or catalase between enriched and control plants (data not shown).

DISCUSSION

Long-term CO_2 enrichment elevated dark respiration rates during leaf expansion (Fig. 2). The respiration rates decreased, however, as the leaf developed, until equivalent with the control rate. A larger decrease in the respiration rate was observed in the enriched plants regardless of the basis of expression. However, in the control plants a decrease in respiration rate was seen only when expressed on a leaf area, Fw, or Dw basis. No decrease was seen when expressed on a LDT, or SLW basis (Fig. 2).

A decrease in respiration rate during leaf development has already been reported in soybeans (cv Amsoy 71) for Tf 2 by Silvius *et al.* (23). They reported a greater decrease in the dark respiration rate of developing leaves (on an area basis) than we found, but this is probably due to the different cultivar used. Similar decreases in respiration during leaf development have also been reported for other species (20, 22, 26). The data in all of these studies were expressed on a leaf area basis and may exhibit other trends if expressed differently. Leaf area, although a conventional basis of expression for dark respiration, does not take into account the thickness of the leaf and thus misrepresents the volume of tissue which is responsible for CO_2 release. McMillen and McClendon (19) demonstrated very clearly the importance of the basis of expression. In particular, when comparing leaves of different thicknesses, they found inversions of relative photosynthetic rates between thick and thin leaf plants depending on the basis of expression.

The decrease in the respiration rate during leaf development is generally explained as diminishing growth respiration. Growth respiration is thought to be proportional to the photosynthetic rate and thus to the carbohydrate level. Photosynthetic rate has been reported to decrease in an expanding leaf (23). Therefore, as the photosynthetic rate decreases so would the carbohydrate level and the growth respiration rate.

The dark respiration of leaves 4 and 5 was elevated by CO_2 enrichment while the leaf was expanding (Fig. 3; Table I). An increase in dark respiration with CO_2 enrichment has been reported previously (2); however, only short-term CO_2 enrichment (7 h) was used. In that study, as in this work (Fig. 3), the increase in CO_2 release was seen only when there was a corresponding high carbohydrate level. Others have also reported high respiration rates in conjunction with high carbohydrate levels in expanding leaves (5, 20, 26) and mature leaves (2). The correlation of respiration rate with carbohydrate content is seen in expanding leaves even though a variety of techniques such as CO_2 enrichment, partial defoliation, and shading have been employed to change the carbohydrate level.

One explanation for the increase in respiration rate seen in the enriched plants may be the excessively high starch levels (approximately 50% of the Dw). Madsen (16) reported that chloroplast structure and leaves of tomato plants were deformed by excessive starch accumulation. Therefore, the high respiration rate may be an attempt to 'burn up' excess starch and prevent damage to the chloroplasts.

The biochemical and physiological aspects of plant growth and leaf expansion need to be investigated further to fully understand the control of carbon metabolism in growing tissue. The direction of carbon flow still remains to be determined. It is not known if the mitochondria in the light are utilizing newly synthesized pyruvate derived from newly formed 3-P-glycerate, or whether they only use pyruvate derived from starch and sucrose degradation. This latter path of carbon flow would involve a great waste of metabolic energy, as the triose-P must first be converted to sucrose and starch and then degraded again through glycolysis to pyruvate.

It has been shown in this study and others that a correlation exists between carbohydrate content and rate of dark respiration (Fig. 4). The cause of the elevated respiration rate seen with high carbohydrate status is not known. We have shown in this study, however, that plants which have a high carbohydrate content induced by CO_2 enrichment have no difference in the specific activities of two mitochondrial respiratory enzymes (Table III). As isocitrate dehydrogenase is the rate limiting step of the tricarboxylic acid cycle, we felt this enzyme to be a good indicator of overall tricarboxylic acid cycle activity. Therefore, the elevated respiration rate in enriched plants is probably not due to a difference in the mitochondrial activity. There is also no difference in the recovery of mitochondrial enzyme activity from equal amounts (g) of normal and enriched tissue. Since this is a measure of total activity per g Fw of tissue, a higher activity would be expected if more mitochondria were present in enriched leaves. Therefore, the higher respiration rate is not due to a

greater mitochondrial number. This data strengthens the argument that dark respiration rate in green leaves is mediated, at least in part, by carbohydrate status.

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