Plant Physiol. (1994) 106: 1163–1168

Acclimation of Respiratory O_2 Uptake in Green Tissues of Field-Grown Native Species after Long-Term Exposure to Elevated Atmospheric CO_2[1]

Joaquim Azcón-Bieto, Miquel A. Gonzalez-Meler, Wendy Doherty, and Bert G. Drake*

Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, Maryland 21037 (W.D., B.G.D.); and Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain (J.A.-B., M.A.G.-M.)

C_3 and C_4 plants were grown in open-top chambers in the field at two CO_2 concentrations, normal ambient (ambient) and normal ambient + 340 $\mu L\ L^{-1}$ (elevated). Dark oxygen uptake was measured in leaves and stems using a liquid-phase Clark-type oxygen electrode. High CO_2 treatment decreased dark oxygen uptake in stems of *Scirpus olneyi* (C_3) and leaves of *Lindera benzoin* (C_3) expressed on either a dry weight or area basis. Respiration of *Spartina patens* (C_4) leaves was unaffected by CO_2 treatment. Leaf dry weight per unit area was unchanged by CO_2, but respiration per unit of carbon or per unit of nitrogen was decreased in the C_3 species grown at high CO_2. The component of respiration in stems of *S. olneyi* and leaves of *L. benzoin* primarily affected by long-term exposure to the elevated CO_2 treatment was the activity of the cytochrome pathway. Elevated CO_2 had no effect on activity and capacity of the alternative pathway in *S. olneyi*. The cytochrome *c* oxidase activity, assayed in a cell-free extract, was strongly decreased by growth at high CO_2 in stems of *S. olneyi* but it was unaffected in *S. patens* leaves. The activity of cytochrome c oxidase and complex III extracted from mature leaves of *L. benzoin* was also decreased after one growing season of plant exposure to elevated CO_2 concentration. These results show that in some C_3 species respiration will be reduced when plants are grown in elevated atmospheric CO_2. The possible physiological causes and implications of these effects are discussed.

Rising atmospheric CO_2 concentrations can have important effects on plant physiological processes, particularly photosynthesis and transpiration. The global magnitude of these processes is so large that small changes in them can have serious consequences for the global carbon balance and global climate change (Gifford, 1988; Bazzaz, 1990; Houghton, 1991; Bowes, 1993; Goudriaan, 1993). However, the effects of rising CO_2 on dark respiration are much less known.

In contrast to photosynthesis, respiration releases CO_2 as a part of normal metabolic activity. In global terms, respiration must be approximately equal to global net primary production in the long term (Gifford, 1988). It has been suggested that increasing temperature could substantially increase respiration, thus causing terrestrial ecosystems to become a major source of CO_2 (Woodwell et al., 1978). Alternatively, direct effects of changes in atmospheric CO_2, which would cause a reduction of total CO_2 released by the earth's vegetation, might mitigate the rate of increase in atmospheric CO_2 (Gifford, 1994). Thus, it is important to know whether increased atmospheric CO_2 levels will result in increased, unchanged, or decreased plant respiration rates.

Changes in CO_2 concentrations can have short-term effects on respiratory rates. Several authors have found rapid inhibitory effects of increasing atmospheric CO_2 on dark CO_2 efflux rates (Bunce, 1990; Amthor et al., 1992) that could be related to inhibition of the activity of some respiratory enzymes (e.g. succinate dehydrogenase [Frenkel and Patterson, 1973; Shaish et al., 1989] or Cyt *c* oxidase [Palet et al., 1991, 1992]).

Studies involving CO_2 enrichment over short to medium time periods (days to weeks) have shown reductions mainly in respiratory CO_2 release in C_3 plants (Reuveni and Gale, 1985; Bunce, 1990; Bunce and Caulfield, 1991; El Kohen et al., 1991; Baker et al., 1992; Gary and Veyres, 1992; Wullschleger et al., 1992a; reviewed by Poorter et al., 1992, and Wullschleger et al., 1994). However, some reports found increases of O_2 uptake or CO_2 efflux from plants growing in enriched CO_2 concentrations coinciding with higher carbohydrate levels (Williams et al., 1992; Thomas et al., 1993; see also Poorter et al., 1992).

Information about long-term (months to years) CO_2 enrichment effects on plant respiration is rather scarce, but some results suggest inhibition of respiration (Bunce and Caulfield, 1991; Wullschleger et al., 1992a; Ziska and Bunce, 1993). In a field experiment of the effects of long-term CO_2 enrichment, a previous study has shown that the salt marsh C_3 plant *Scirpus olneyi* showed about 50% reduction in respiratory CO_2 release from individual shoots after growth in twice the present normal ambient CO_2 in open-top chambers (B.G. Drake, unpublished data).

In the present study we have further investigated the

[1] Supported by grants from the U.S. Department of Energy, the Comisión Interministerial de Ciencia y Tecnología, grant AGR89-580 (Spanish government), the scholarship from the Plan de Formación de Personal Investigador to M.A.G., and the Smithsonian Institution.

* Corresponding author; fax 1-301-261-7954.

Abbreviations: CCCP, carbonyl-cyanide-*m*-chlorophenylhydrazone; SHAM, salicylhydroxamic acid; V_{alt}, capacity of the alternative pathway; v_{alt}, activity of the alternative pathway; v_{cyt}, activity of the Cyt pathway; V_{max}, the maximum rate of oxygen uptake.

responses of respiration in these salt marsh plants to long-term CO_2 treatment as well as those in an understory shrub, *Lindera benzoin* (C_3), by measuring dark O_2 uptake (instead of CO_2 release) of intact and sliced green tissues acclimated to different CO_2 levels in the field. Dark O_2-uptake rates in a buffer solution equilibrated with ambient CO_2 reflect mostly the overall activity of the mitochondrial electron transport chain expressed in the tissue. The use of inhibitors in liquid-phase oxygen electrodes provides information about relative rates of the Cyt and alternative pathways. As such, these measurements indicate the acclimation of respiration to long-term exposure to elevated CO_2. The results obtained here confirm that growth in elevated CO_2 depresses respiration in green tissues and that the activity of the Cyt pathway is reduced, accounting for the effect of elevated CO_2 concentration on dark respiration in the C_3 sedge *S. olneyi*.

MATERIALS AND METHODS

Plant Growth

Open-top chambers were placed in *Scirpus olneyi* (C_3) and *Spartina patens* (C_4) brackish marsh communities on the Chesapeake Bay throughout the growing season from the spring of 1987 to the present. Two different CO_2 concentrations were used: normal ambient and elevated (normal ambient + 340 $\mu L\ L^{-1}$). The shrub *Lindera benzoin* (C_3) was exposed to ambient and elevated CO_2 concentrations from the spring of 1991 to the present using open-top chambers in the understory of an eastern deciduous forest. A review of the major findings of this long-term CO_2 project is found in Drake (1992). Results for the respiration study reported here were obtained on material collected in 1991 and 1992 during July and August when the net ecosystem carbon assimilation was at its maximum (Drake and Leadley, 1991).

Determination of Respiratory Activities

Dark O_2-uptake rates of intact and sliced tissues were measured in the dark at constant temperatures using a liquid-phase O_2 electrode (Clark-type, Rank Brothers, Cambridge, UK) in ambient air-equilibrated 20 mM Mes buffer (pH 6.0). Samples of mature tissues were placed in the electrode cuvette and depletion of oxygen was linear in the stirred solution of the closed cuvette, except at low concentrations of O_2. To avoid oxygen-limiting conditions inside the cuvette, all measurements were terminated when O_2 was consumed by about 50 to 60%. A nylon net separated the plant material from the stirring bar and the electrode. Inhibitors (cyanide, SHAM), substrates (Suc, Gly), and an uncoupler (CCCP) were added into the electrode cuvette from stock solutions with a Hamilton microsyringe. V_{max} was obtained after incubation of the plant material for 30 to 60 min in a medium that contained 20 mM Suc, 20 mM Gly, and 0.2 mM $CaCl_2$. Plant samples were collected during the light period and were kept in the dark before measurements. After measurements were completed, the plant samples were placed in an oven-drier at 60°C until constant dry weight was obtained. Dry samples were ground and their total carbon and nitrogen contents were determined using GC.

Determination of Cyt *c* Oxidase and Complex III

Green tissues were homogenized with a Polytron in a medium containing 25 mM Hepes buffer (pH 7.5), 1 mM EDTA, 1% (w/v) PVP, 0.2% (w/v) BSA fraction V, and 15 mM Na-ascorbate. The homogenate was filtered and centrifuged (3000–4000*g*, 5 min) and the final volume of the extract was calculated. For the Cyt *c* oxidase assay, 0.2 mL of the extract was added to 1.8 mL of reaction medium (pH 6.5) containing 50 mM KH_2PO_4, 0.1 mM EDTA, and 0.1% (w/v) BSA free fatty acid and were placed in the oxygen electrode cuvette. Then 8 mM Na-ascorbate, 0.5 mM *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride, 30 μM Cyt *c*, and 1 mM lauryl-maltoside were added to the closed cuvette to start the oxygen uptake reaction at 25°C. Sodium azide (1 mM) was added at the end of each measurement, and the residual rate was subtracted from the total rate. For complex III assay, 0.1 mL of homogenate was mixed with 0.9 mL of reaction medium containing 50 mM Tes buffer (pH 7.0), 1 mM EDTA, 1 mM potassium cyanide, 157 μM lauryl-maltoside, 0.5 mM duroquinol, and 25 μM Cyt *c*. Cyt *c* reduction was monitored at 550 nm (extinction coefficient 19.8 $mM^{-1}\ cm^{-1}$) using a Lambda 3B spectrophotometer (Perkin-Elmer) at room temperature. To account for nonenzymatic Cyt *c* reduction, separate assays were run with the same extract in the absence and in the presence of 5 μM antimycin A and the difference was calculated.

RESULTS AND DISCUSSION

The rate of dark O_2 uptake of green stems of *S. olneyi* (C_3) was significantly lower (on either an area or a dry weight basis) in plants grown at elevated than in those grown at normal ambient CO_2 (Tables I and II). Plants from unchambered control sites had respiration rates similar to plants

Table I. *Dark respiration rates of mature green tissues from S. olneyi (C_3), S. patens (C_4), and L. benzoin (C_3) plants grown at normal ambient or elevated (normal ambient + 340 $\mu L\ L^{-1}$) atmospheric CO_2 concentrations, inside open-top chambers in the field, during the summer of 1991*

Tissue segments (0.5–0.8 cm^2) were used in an O_2 electrode cuvette. Values shown are means ± SE of 4 to 11 replicates. The statistical significance levels (Student's *t* test) are: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$.

[CO_2] during Growth	Dark O_2 Uptake	
	25°C	35°C
$\mu L\ L^{-1}$	$\mu mol\ m^{-2}\ s^{-1}$	
Stems of *S. olneyi*		
350	0.40 ± 0.04	0.74 ± 0.05
700	0.20 ± 0.02c	0.45 ± 0.05b
Leaves of *S. patens*		
350	0.34 ± 0.03	0.58 ± 0.02
700	0.37 ± 0.01	0.56 ± 0.03
	25°C	30°C
Leaves of *L. benzoin*		
350	0.26 ± 0.02	0.47 ± 0.02
700	0.21 ± 0.03	0.30 ± 0.03b

Table II. *Dark respiration rates of mature green tissues normalized on dry weight, or carbon or nitrogen concentration*

Temperature during measurements was 25°C. Values shown are means ± SE of 4 to 11 replicates. For other details, see Table I.

[CO_2] during Growth	Dark O_2 Uptake		
	Dry weight	Carbon	Nitrogen
$\mu L\ L^{-1}$	$\mu mol\ kg^{-1}$ dry weight s^{-1}	$\mu mol\ kg^{-1}\ C\ s^{-1}$	$\mu mol\ kg^{-1}\ N\ s^{-1}$
Stems of *S. olneyi*			
350	2.6 ± 0.2	6.0 ± 0.5	136 ± 11
700	1.5 ± 0.1c	2.9 ± 0.3c	80 ± 6b
Leaves of *S. patens*			
350	3.5 ± 0.4	7.2 ± 0.5	296 ± 17
700	3.7 ± 0.2	8.6 ± 0.3	383 ± 15b
Leaves of *L. benzoin*			
350	10.3 ± 0.2	21.5 ± 0.5	295 ± 21
700	7.0 ± 0.6b	14.5 ± 1.2b	225 ± 19a

enclosed in open-top chambers maintained at normal ambient CO_2 levels (data not shown). Mature leaves of *S. patens* (C_4) showed no reduction of leaf O_2 uptake rate (Tables I and II). These results are consistent with earlier observations made by measuring CO_2 efflux in these species (B.G. Drake, unpublished data).

The understory shrub *L. benzoin* exposed to elevated CO_2 for only 4 months in the forest also showed reduction of leaf respiration per unit leaf area compared to plants grown in ambient CO_2, especially when measured at high temperature (Tables I and II; results from summer 1991).

Data on carbon and nitrogen composition of the green tissues grown at different CO_2 regimes show little effect of the CO_2 treatment on specific dry weight and carbon content (Table III). The nitrogen content was significantly lower in stems of *S. olneyi* plants grown at elevated CO_2 than at ambient CO_2 but was similar in leaves of *S. patens* and *L. benzoin* from both CO_2 treatments (Table III; data obtained during summer 1991). Total soluble protein and Rubisco content in the C_3 species was significantly reduced when plants were grown at high CO_2 (J. Jacob and B.G. Drake, unpublished results), which could partly account for the reduction in tissue nitrogen concentration. When respiration was expressed either on the basis of leaf carbon or leaf nitrogen concentration, the rates were significantly lower in stems of *S. olneyi* and leaves of *L. benzoin* (both C_3 plants) in elevated CO_2 than in plants grown in normal ambient CO_2, but they were unaffected or increased in the C_4 species *S. patens* (Table II). This suggests that the decrease in respiration in both C_3 species studied at elevated CO_2 was not entirely due to a reduction in the nitrogen concentration (see Griffin et al., 1993). Although the reduced nitrogen concentration could have contributed to the observed changes in the respiratory rates according to the results of Wullschleger et al. (1992a), Ziska and Bunce (1993) showed a decrease in dark respiration of plants grown at elevated CO_2 in which there was no reduction in the protein content compared to plants grown in normal ambient CO_2 concentration.

The use of specific inhibitors of the two terminal oxidases of mitochondrial electron transport (cyanide for Cyt *c* oxidase and SHAM for the alternative oxidase) showed that the normal activity of the Cyt path (v_{cyt}) was very much reduced in *S. olneyi* stems grown at elevated CO_2. The activity of the alternative path was insignificant at both CO_2 treatments (Table IV). When the stem slices were incubated with substrates (e.g. Suc and also Gly) and the uncoupler CCCP (to avoid RC by adenylates), the maximum respiration rate (V_{max}) measured was similar for plants grown at the two CO_2 levels

Table III. *Dry weight per unit area and carbon and nitrogen concentrations of adult green tissues from plants grown at either ambient or elevated CO_2 concentrations*

Values shown are means ± SE of 4 to 11 replicates. For other details, see Table I.

[CO_2] during Growth	Dry Weight per Unit Area	Carbon Content		Nitrogen Content	
$\mu L\ L^{-1}$	g dry weight m^{-2}	g C m^{-2}	% C of dry wt	g N m^{-2}	% N of dry wt
Stems of *S. olneyi*					
350	150.2 ± 8.6	74.3 ± 5.7	44.0 ± 0.2	3.2 ± 0.2	1.9 ± 0.1
700	133.6 ± 4.0	65.0 ± 3.0	43.7 ± 0.3	2.3 ± 0.1b	1.5 ± 0.1b
Leaves of *S. patens*					
350	100.3 ± 1.9	46.3 ± 1.3	45.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.04
700	95.8 ± 3.3	44.4 ± 2.1	45.5 ± 0.2	1.0 ± 0.1	1.0 ± 0.01a
Leaves of *L. benzoin*					
350	25.3 ± 1.8	12.2 ± 1.0	48.1 ± 0.4	0.9 ± 0.1	3.5 ± 0.2
700	30.1 ± 1.7	14.5 ± 0.8	48.3 ± 0.1	0.9 ± 0.1	3.1 ± 0.1

Table IV. *Estimates of respiratory electron transport pathways in stem slices (about 1 mm thick) of S. olneyi*

Dark O_2 uptake rates were measured at 30°C either in the absence, or just after the addition of, substrates (10 mM Suc + 10 mM Gly) plus uncoupler (0.95 μM CCCP). When substrates and CCCP were used, the slices were incubated with 20 mM Suc + 20 mM Gly for 30 to 60 min at room temperature prior to measurement. The maximum uncoupled respiration rate is called V_{max}. Inhibitors of electron transport (12.5 mM SHAM; 1.1 mM KCN) were used to determine the coupled and uncoupled "activity" of both the cytochrome (v_{cyt}) and alternative (v_{alt}) pathways, and also the "capacity" of the alternative pathway (V_{alt}). The parameter ρ is the ratio v_{alt}/V_{alt} in uncoupled slices. Values shown are means ± SE of 3- to 5 replicates, except in the case of V_{max}, where the number of replicates was 6 to 10. For other details, see Table I.

[CO_2] during Growth	Dark O_2 Uptake						
	−Substrates −CCCP		+Substrates +CCCP				
	v_{cyt}	v_{alt}	V_{max}	v_{cyt}	v_{alt}	V_{alt}	ρ
μL L^{-1}	μmol kg^{-1} dry weight s^{-1}						
350	6.4 ± 0.3	0	15.3 ± 0.3	10.3 ± 0.3	3.6 ± 0.3	13.3 ± 0.3	0.3
700	3.6 ± 0.3c	0	14.7 ± 1.1	4.7 ± 0.6c	9.2 ± 1.9a	13.1 ± 1.4	0.7

in this study (Table IV). Under these conditions, the uncoupled activity of the Cyt pathway (which may reflect the capacity of this pathway) was greatly reduced under high CO_2 treatment. This correlates well with the activity normally expressed in the absence of uncoupler, but the expression of the alternative oxidase was greatly enhanced in uncoupled stems of high-CO_2-grown *S. olneyi* plants in the absence of substrate limitation (Table IV). The capacity of the alternative pathway (V_{alt}), estimated by cyanide resistance, was unchanged by the CO_2 treatment (Table IV).

The results showed that maximum respiratory rates (estimated with CCCP in the absence of substrate limitation) were similar under different CO_2 treatments. Under these experimental conditions the changes in the rates of the Cyt and alternative electron transport pathways were such that they were mutually compensated (Table IV). Our results also suggest that the limitations of respiration in high-CO_2-grown plants seem to be related more to changes in electron transport than to a limitation of substrate utilization pathways (e.g. carbon metabolism).

To confirm this idea, we measured the level of two enzyme complexes from the Cyt pathway: Cyt *c* oxidase and complex III (Cyt bc_1 complex; Tables V and VI). The results clearly indicated that high-CO_2-grown *S. olneyi* plants had lower Cyt *c* oxidase activities (Table V), and these activities were correlated with the intact tissue respiration (Fig. 1), suggesting that intact stem respiration of *S. olneyi* was tightly coupled to production of ATP in both CO_2 treatments. The correspondence between the estimates of the Cyt pathway obtained using either inhibitors or enzyme activities was remarkably good. In the case of *L. benzoin* grown at different CO_2 levels for more than 1 year, the activity of the intact tissue uncoupled Cyt pathway (estimated with inhibitors) and the Cyt *c* oxidase and the complex III activities were significantly reduced in elevated CO_2, and all three activities were correlated (Table VI; results of summer 1992). Interestingly, it has also been reported that elevated CO_2 directly inhibits Cyt *c* oxidase activity (Palet et al., 1991, 1992).

The above results suggest that Cyt *c* oxidase levels (and perhaps other components of the Cyt path) may contribute to the control of respiration rate in plants exposed to high levels of atmospheric CO_2 when grown under natural conditions. Thus, the levels of Cyt path components can be an important physiological limiting factor for coupled respiration. The fact that CO_2 inhibits activity of Cyt *c* oxidase in short-term and long-term studies suggests a relation between

Table V. *Effect of elevated CO_2 concentration during growth in the field on Cyt c oxidase activity of S. olneyi stems and of S. patens leaves*

Temperature during the assay was 25°C. Values shown are means ± SE of three to six determinations using different tissue samples. The assay was repeated twice for every sample and the results were averaged. For statistical details, see Table I.

[CO_2] during Growth	Cyt c Oxidase Activity	
μL L^{-1}	μmol O_2 kg^{-1} fresh weight s^{-1}	μmol O_2 kg^{-1} dry weight s^{-1}
Stems of *S. olneyi*		
350	3.3 ± 0.4	11.4 ± 1.4
700	1.4 ± 0.2b	5.0 ± 0.6b
Leaves of *S. patens*		
350	32.9 ± 2.7	69.4 ± 5.0
700	29.5 ± 2.2	65.3 ± 4.7

Table VI. *Effect of elevated CO_2 concentration during growth on the uncoupled (by CCCP) Cyt pathway, the Cyt c oxidase activity, and the complex III (Cyt bc_1) activity of L. benzoin leaves*

Measurements were made during the summer of 1992, the second year of exposure to elevated CO_2 within open-top chambers. Temperature during measurements was 25°C. Values shown are means ± SE of six replicates using different tissue samples. The assay was repeated twice for every sample and the results were averaged. For other statistical details, see Table I.

[CO_2] during Growth	Uncoupled Cyt Pathway	Cyt c Oxidase Activity	Cyt bc_1 Complex Activity
$\mu L\ L^{-1}$	$\mu mol\ O_2\ kg^{-1}\ dry\ weight\ s^{-1}$		$mmol\ Cyt\ c\ kg^{-1}\ dry\ weight\ s^{-1}$
350	14.4 ± 1.1	38.9 ± 3.6	40.6 ± 5.6
700	7.3 ± 1.3a	10.8 ± 1.7c	16.1 ± 1.7b

these effects. But plant respiration might also be enhanced over a short time period by CO_2 through changes in carbohydrate levels, due to increased photosynthesis rates (Azcón-Bieto et al., 1983; Azcón-Bieto and Osmond, 1983; Hrubec et al., 1985; Lambers, 1985; Amthor, 1991). Although these short-term CO_2 effects on respiration are apparently contradictory, they are independent and can occur simultaneously. Interestingly, Krömer et al. (1993) reported changes in mitochondrial respiration mediated by changes in photorespiration rates under different CO_2 conditions.

In summary, we present evidence to support the hypothesis that in some plants, respiration of green tissues will be reduced by rising atmospheric CO_2 concentration coincident with increased growth: elevated CO_2 stimulated photosynthetic CO_2 assimilation and growth of roots and shoots of *S. olneyi* in the field during 7 years of exposure to high CO_2 (B.G. Drake, unpublished data). Reductions in tissue nitrogen concentration are also a feature of the response to the CO_2 treatments in this plant and in other plants studied and reduced rates of respiration are commonly observed with lower nitrogen concentrations. But the reduction in tissue nitrogen, perhaps through reduction of the content of proteins, mainly Rubisco, explains only part of the CO_2-dependent variations in respiratory rate reported here. Reduction of the rate of respiration in *S. olneyi* and *L. benzoin* was shown to be due largely to reduction in some enzymatic complexes of the mitochondrial electron transport chain, resulting in the reduction in the activity of the Cyt pathway.

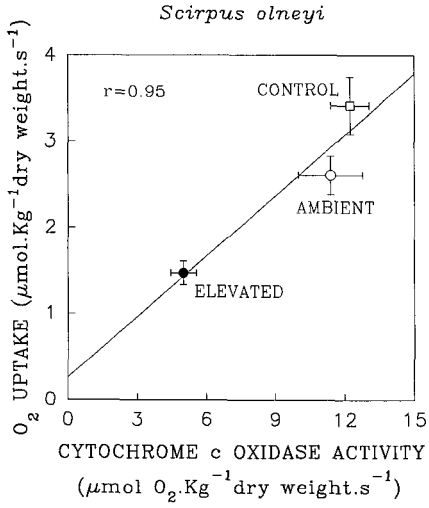

Figure 1. The dependence of the rate of intact tissue dark respiration from green stems of *S. olneyi* on the extracted activity of the enzyme Cyt c oxidase measured at 25°C. Plants were grown in the field inside open-top chambers (summer 1991) at either ambient or elevated CO_2 concentrations, as well as in unchambered control plots. Bars are 2 SE For other details, see Tables II and V.

ACKNOWLEDGMENTS

We thank J.N. Siedow and X. Aranda for useful comments and G. Peresta for technical support. This paper forms part of the Ph.D. thesis project of M.A.G.

Received March 21, 1994; accepted July 13, 1994.

LITERATURE CITED

Amthor JS (1991) Respiration in a future, higher-CO_2 world: opinion. Plant Cell Environ **14:** 13–20

Amthor JS, Koch GW, Bloom AJ (1992) CO_2 inhibits respiration in leaves of *Rumex crispus* L. Plant Physiol **98:** 757–760

Azcón-Bieto J, Lambers H, Day DA (1983) Effect of photosynthesis and carbohydrate status on respiratory rates and the involvement of the alternative pathway in leaf respiration. Plant Physiol **72:** 598–603

Azcón-Bieto J, Osmond CB (1983) Relationship between photosynthesis and respiration. The effect of carbohydrate status on the rate of CO_2 production by respiration in darkened and illuminated wheat leaves. Plant Physiol **71:** 574–581

Baker JT, Laugel F, Boote KJ, Allen LH Jr (1992) Effects of daytime carbon dioxide concentration on dark respiration in rice. Plant Cell Environ **15:** 231–239

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 levels. Annu Rev Ecol Syst **21:** 167–196

Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol **44:** 309–332

Bunce JA (1990) Short- and long-term inhibition of respiratory carbon dioxide efflux by elevated carbon dioxide. Ann Bot **65:** 637–642

Bunce JA, Caulfield F (1991) Reduced respiratory carbon dioxide efflux during growth at elevated carbon dioxide in three herbaceous perennial species. Ann Bot **67:** 325–330

Drake BG (1992) A field study of the effects of elevated CO_2 on ecosystem processes in a Chesapeake Bay wetland. Aust J Bot **40:** 579–595

Drake BG, Leadley PW (1991) Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO_2. Plant Cell Environ **14:** 853–860

El Kohen A, Pontailler J-Y, Mousseau M (1991) Effet d'un doublement du CO_2 atmosphérique sur la respiration à l'obscurité des

parties aeriennes de jeunes chataigniers (*Castanea sativa* Mill). C R Acad Sci Paris III **312:** 477–481

Frenkel C, Patterson ME (1973) Effect of carbon dioxide on activity of succinic dehydrogenase in 'Bartlett' pears during cold storage. HortScience **8:** 395–396

Gary C, Veyres P (1992) CO_2 concentration and the photosynthesis-respiration balance of vegetative tomato plants. *In* H Lambers, LHW van der Plas, eds, Molecular, Biochemical and Physiological Aspects of Plant Respiration. SPB Academic, The Hague, The Netherlands, pp 553–557

Gifford RM (1988) Direct effects of higher carbon dioxide concentrations on vegetation. *In* G Pearman, ed, Greenhouse: Planning for Climate Change. EJ Brill, Leiden, The Netherlands, pp 506–519

Gifford RM (1994) The global carbon cycle: a viewpoint on the missing sink. Aust J Plant Physiol **21:** 1–15

Goudriaan J (1993) Interaction of ocean and biosphere in their transient responses to increasing atmospheric CO_2. Vegetatio **104/105:** 329–337

Griffin KL, Thomas RB, Strain BR (1993) Effects of nitrogen supply and elevated carbon dioxide on construction cost in leaves of *Pinus taeda* (L.) seedlings. Oecologia **95:** 575–580

Houghton JT, Jenkins GJ, Euphraums JJ (1991) Climate Change: The IPCC Scientific Assessment. Cambridge University Press, Cambridge, UK

Hrubec TC, Robinson JM, Donaldson RP (1985) Effects of CO_2 enrichment and carbohydrate content on the dark respiration of soybeans. Plant Physiol **79:** 684–689

Krömer S, Malmberg G, Gardestrom P (1993) Mitochondrial contribution to photosynthetic metabolism. A study with barley (*Hordeum vulgare* L.) leaf protoplasts at different light intensities and CO_2 concentrations. Plant Physiol **102:** 947–955

Lambers H (1985) Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. *In* R Douce, DA Day, eds, Encyclopedia of Plant Physiology, Vol 18. Springer-Verlag, Berlin, pp 418–473

Palet A, Ribas-Carbó M, Argilés JM, Azcón-Bieto J (1991) Short-term effects of carbon dioxide on carnation callus cell respiration. Plant Physiol **96:** 467–472

Palet A, Ribas-Carbó M, González-Meler MA, Aranda X, Azcón-Bieto J (1992) Short-term effects of CO_2/bicarbonate on plant cell respiration. *In* H Lambers, LHW van der Plas, eds, Molecular, Biochemical and Physiological Aspects of Plant Respiration. SPB Academic, The Hague, The Netherlands, pp 597–601

Poorter H, Gifford RM, Kriedemann PE, Wong SC (1992) A quantitative analysis of dark respiration and carbon content as factors in the growth response of plants to elevated CO_2. Aust J Bot **40:** 501–513

Reuveni J, Gale J (1985) The effect of high levels of carbon dioxide on dark respiration and growth of plants. Plant Cell Environ **8:** 623–628

Shaish A, Roth-Bejarano N, Itai C (1989) The response of stomata to CO_2 relates to its effect on respiration and ATP level. Physiol Plant **76:** 107–111

Thomas RB, Reid CD, Ybema R, Strain BR (1993) Growth and maintenance components of leaf respiration of cotton grown in elevated carbon dioxide partial pressure. Plant Cell Environ **16:** 539–546

Williams ML, Jones DG, Baxter R, Farrar JF (1992) The effect of enhanced concentrations of atmospheric CO_2 on leaf respiration. *In* H Lambers, LHW van der Plas, eds, Molecular, Biochemical and Physiological Aspects of Plant Respiration. SPB Academic, The Hague, The Netherlands, pp 547–551

Woodwell GM, Whittaker RH, Reiners WA, Likens GE, Delwiche CC, Botkin DB (1978) The biota and the world carbon budget. Science **199:** 141–146

Wullschleger SD, Norby RJ, Gunderson CA (1992a) Growth and maintenance respiration in leaves of *Liriodendron tulipifera* L saplings exposed to long-term carbon dioxide enrichment in the field. New Phytol **121:** 515–523

Wullschleger SD, Norby RJ, Hendrix DL (1992b) Carbon exchange rates, chlorophyll contents, and carbohydrate status on two forest species exposed to carbon dioxide enrichment. Tree Physiol **10:** 21–31

Wullschleger SD, Ziska LH, Bunce JA (1994) Respiratory responses of higher plants to atmospheric CO_2 enrichment. Physiol Plant **90:** 221–229

Ziska LH, Bunce JA (1993) Inhibition of whole plant respiration by elevated CO_2 as modified by growth temperature. Physiol Plant **87:** 459–466