New Phytol. (1994), **128**, 443–450

Energy content, construction cost and phytomass accumulation of *Glycine max* (L.) Merr. and *Sorghum bicolor* (L.) Moench grown in elevated CO_2 in the field

BY JEFFREY S. AMTHOR[1], ROBERT J. MITCHELL[2], G. BRETT RUNION[3], HUGO H. ROGERS[3], STEPHEN A. PRIOR[3] AND C. WESLEY WOOD[4]

[1] *Health and Ecological Assessment Division and Global Climate Research Division, L-256, Lawrence Livermore National Laboratory, PO Box 808, Livermore, California 94550-9900, USA*
[2] *Joseph W. Jones Ecological Research Center, PO Box 2324, Newton, Georgia 31770, USA*
[3] *National Soil Dynamics Laboratory, USDA-ARS, PO Box 3439, Auburn, Alabama 36831, USA*
[4] *Department of Agronomy and Soils, Auburn University, Auburn, Alabama 36849, USA*

(*Received 1 March 1994; accepted 5 July 1994*)

SUMMARY

Grain sorghum [*Sorghum bicolor* (L.) Moench, a C_4 crop] and soybean [*Glycine max* (L.) Merr. cv. Stonewall, a C_3 crop] plants were grown in ambient (*c.* 360 $\mu l\ l^{-1}$) and twice-ambient (*c.* 720 $\mu l\ l^{-1}$) CO_2 levels in open-top chambers in soil without root constriction. Plant dry mass, energy content, composition and construction cost (i.e. amount of carbohydrate required to synthesize a unit of plant dry mass) were assessed at the end of the growing season. Elevated CO_2 (a) increased phytomass accumulation (kg per plant) in both species, (b) had little affect on energy concentration (MJ kg^{-1} plant) but caused large increases in the amount of plant energy per ground area (MJ m^{-2} ground), and (c) did not alter specific growth cost (kg carbohydrate kg^{-1} plant growth) but greatly increased growth cost per ground area (kg carbohydrate m^{-2} ground) because growth was enhanced. For soybean, twice-ambient CO_2 resulted in a 50% increase in the amount of nitrogen and energy in grain (seed plus pod) per ground area. This response to elevated CO_2 has important implications for agricultural productivity during the next century because the rate of human population growth is exceeding the rate of increase of land used for agriculture so that future food demands can only be met by greater production per ground area.

Key words: Carbon dioxide, construction cost, energy content, *Glycine max* (soybean), *Sorghum bicolor* (grain sorghum).

INTRODUCTION

Atmospheric CO_2 partial pressure affects higher-plant physiology, growth, and productivity in many, often complex, ways. For example, CO_2 is a substrate of photosynthesis and is also a competitive inhibitor of photorespiration, so an increase in CO_2 generally enhances leaf and canopy CO_2 assimilation in C_3 (95% of higher-plant species) plants (Drake & Leadley, 1991; Bowes, 1993). Elevated CO_2 may slow or increase plant respiration, depending in part on the basis of expressing respiration rate and whether short-term or long-term elevated CO_2 treatments are used (Amthor, 1991; Amthor, Koch & Bloom, 1992; Wullschleger, Ziska & Bunce, 1994). Stomatal aperture is often negatively related to CO_2 and elevated CO_2 generally increases the ratio of CO_2 assimilated to water transpired (Eamus, 1991; Beerling & Woodward, 1993; Gunderson, Norby & Wullschleger, 1993). An overall effect of elevated CO_2 is usually (Lawlor & Mitchell, 1991; Bowes, 1993), but not always (Hunt *et al.*, 1993), increased plant growth and productivity.

In addition to effects on photosynthesis, photorespiration, respiration, transpiration, and growth, CO_2 enrichment may also alter partitioning of

photoassimilate among plant organs (Rogers, Runion & Krupa, 1994) and can affect plant chemical composition. Often, nitrogen concentration is lower and the C/N ratio is higher in elevated CO_2 (Wong, 1979; Norby, O'Neill & Luxmoore, 1986; Curtis, Drake & Whigham, 1989; Curtis *et al.*, 1990; Hocking & Meyer, 1991; Kuehny *et al.*, 1991; Norby *et al.*, 1992; Coleman, McConnaughay & Bazzaz, 1993). The altered chemical composition of plants grown in elevated CO_2 may influence the metabolic costs associated with growth, i.e. the efficiency of converting photoassimilate into new plant structure and long-term storage. Altered composition may also affect insect herbivore behaviour and performance (e.g. Fajer, 1989) and has important implications for the human food supply.

In this study a C_3 crop [soybean, *Glycine max* (L.) Merr.] and a C_4 crop [grain sorghum, *Sorghum bicolor* (L.) Moench] were grown out-of-doors without root constriction in ambient and twice-ambient atmospheric CO_2 levels. The purpose of the experiment was to quantify effects of CO_2 on the growth, composition, and energy content of these crops. Construction (growth) costs of the crops and their individual organs were then estimated from the measurements of tissue composition and energy content. If construction cost is smaller in elevated CO_2 the efficiency of photoassimilate use is increased with respect to growth whereas if construction cost is larger photoassimilate is used less efficiently. Ambient and twice-ambient CO_2 levels were chosen because atmospheric CO_2 may exceed 700 μl l^{-1} within the next 100 yr (Houghton, Callander & Varney, 1992).

MATERIALS AND METHODS

Plant material and carbon dioxide treatments

During 1992, soybean cv. Stonewall (30 plants m^{-2} of ground) and grain sorghum (26 plants m^{-2} of ground) were grown from seeds in open-top chambers (Rogers, Heck & Heagle, 1983*b*; Rogers *et al.*, 1983*a*; Rogers *et al.*, 1984) in Blanton loamy sand without volume constriction (2 m deep × 7 m wide × 76 m long soil bins) out-of-doors at Auburn, Alabama, USA. Both soybean and grain sorghum received 3·4 g N m^{-2} (as ammonium nitrate added to the soil surface) 2 d after planting and the grain sorghum received an additional 6·7 g N m^{-2} ground 30 d after planting. Both crops were irrigated to avoid visible signs of significant water stress: during the experiment *c.* 0·482 m of rain fell and *c.* 0·115 m of irrigation water was applied.

Soybean and grain sorghum were each grown in three chambers containing ambient (*c.* 360 μl l^{-1} [*c.* 36 Pa], 24 h d^{-1}) and another three chambers containing twice-ambient (*c.* 720 μl l^{-1} [*c.* 72 Pa], 24 h d^{-1}) CO_2 concentrations. Unchambered plots of each species were used to examine effects of chambers *per se*. Experimental plot edge effects were negligible both inside and outside the chambers.

Plant and organ harvesting and chemical analysis

Plants were harvested at maturity and separated into roots, stems, leaves, seeds, pods (for soybeans, by removal of seeds), and chaff (for grain sorghum). Plant tissues were dried (55 °C), weighed, and ground in a Retsch grinder to pass through a 0·2 mm sieve. Energy content of all tissues was determined using bomb calorimetry following the methods of Lieth (1975). Nonstructural carbohydrates (hexoses, sucrose and starch) were determined using a modification (Green, 1993) of the enzymatic procedure developed by Jones, Outlaw & Lowry (1977). Percent ash for each tissue was determined by combustion (500 °C for 4 h) in a muffle furnace. Organic nitrogen was determined using a LECO CHN-600 analyzer. Sulphur content of the plant tissues was determined by inductively coupled plasma spectroscopy after extraction with a double-acid solution (Hue & Evans, 1986).

Calculation of construction cost

Construction (growth) cost represents the amount of photosynthate required to provide carbon skeletons, reductant, and ATP used during the synthesis of plant tissue (Williams *et al.*, 1987); it does not include the costs of maintaining existing phytomass (i.e. maintenance respiration; Penning de Vries *et al.*, 1989). Construction costs (C, kg glucose kg^{-1} dry mass grown) for the harvested soybean and grain sorghum individual organs and whole plants were calculated according to Williams *et al.* (1987)

$$C = [(0{\cdot}06968\,\Delta H_C - 0{\cdot}065)(1-A) + 0{\cdot}5359\,kN]/E_G,$$

where ΔH_C is the tissue ash-free heat of combustion or energy concentration (MJ kg^{-1}), A is the ash concentration (kg ash kg^{-1} tissue), k is the oxidation state of the substrate nitrogen (−3 for ammonium and +5 for nitrate), N is the organic nitrogen concentration of the tissue (kg N kg^{-1} tissue), and E_G is the growth efficiency as defined by McDermitt & Loomis (1981). In this study, k was set to +1 and E_G was set to 0·85 with measured values used for all other parameters. The chosen value of E_G, i.e. 0·85, was smaller than the value derived by McDermitt & Loomis (1981), i.e. about 0·88, in order to account for somewhat larger respiratory costs of photoassimilate translocation compared with the values used in the analysis of McDermitt & Loomis (1981), (see Amthor 1994*a*). The assumption that E_G was a constant may have been invalid, but this had no more than a minor impact on the conclusions of the study. Other methods can be used to assess construction costs (see, e.g., Lafitte & Loomis, 1988);

Table 1. *Mean dry mass (g per plant) and harvest index of* Sorghum bicolor *and* Glycine max *grown in open-top chambers containing ambient (1 × −c) or twice ambient (2 × −c) CO_2 or grown outside chambers (1 × −o) in ambient air. All plants were harvested at maturity*

	1 × −c	2 × −c	$P > F$ (df = 5) (2 × −c *vs.* 1 × −c)	1 × −o	$P > F$ (df = 5) (1 × −o *vs.* 1 × −c)
Sorghum bicolor					
Leaves	4·24	6·00	(0·007)	4·54	(0·563)
Stem	13·18	17·56	(0·009)	15·03	(0·187)
Roots	0·88	1·52	(0·003)	1·24	(0·040)
Grain	18·18	20·88	(0·099)	19·77	(0·305)
Whole plant	36·48	45·96	(0·018)	40·58	(0·236)
Harvest index	0·510	0·470	(0·001)	0·504	(0·500)
Glycine max					
Leaves	4·96	6·23	(0·030)	5·73	(0·152)
Stem	8·14	12·39	(0·011)	9·84	(0·220)
Below-ground	1·70	2·32	(0·003)	1·72	(0·914)
Pod	4·25	5·96	(0·010)	4·37	(0·770)
Seed	4·76	7·11	(0·145)	5·72	(0·530)
Whole plant	23·81	34·02	(0·013)	27·37	(0·298)
Harvest index	0·216	0·224	(0·368)	0·222	(0·443)

For *Glycine max*, below-ground dry mass was roots plus nodules and pod dry mass excludes seeds. Harvest index is grain or seed dry mass divided by total above-ground dry mass.

we chose the method of Williams *et al.* (1987) because of its simplicity. Assumptions and procedures used to calculate construction costs are discussed in detail by, e.g. McDermitt & Loomis (1981), Williams *et al.* (1987) and Lafitte & Loomis (1988). Conclusions of our study are probably independent of the methods used to calculate C.

We also calculated construction cost on a ground area basis (C_A, kg glucose m^{-2} ground) for individual organs and whole plants.

Study design and data analysis

The study was arranged in a split-plot design with plant species (grain sorghum or soybean) constituting the main treatment effect and CO_2 exposure concentration (360 $\mu l\ l^{-1}$ or 720 $\mu l\ l^{-1}$) the split-plot treatment. Whole-plot and split-plot treatments were assigned at random within three replicate blocks. All data analyses were performed using the general linear models procedure (PROC GLM) of the Statistical Analysis System (SAS Institute, Cary, NC) and appropriate error terms were specified for each treatment. CONTRAST statements were used to determine the statistical significance between interacting main effects variables. All statistical comparisons of treatment means were planned prior to the collection of data.

RESULTS AND DISCUSSION

Growth and energy content

Grain sorghum dry mass accumulation was significantly ($P \leqslant 0{\cdot}05$) greater in elevated compared with ambient CO_2 for all plant components but grain (Table 1). Whole-plant dry mass was nearly 26% greater ($P \approx 0{\cdot}018$) in twice-ambient compared with ambient CO_2. Although enhanced dry mass accumulation by C_4 species grown in elevated CO_2 has been previously reported (Rogers *et al.*, 1983*a*; Loomis & Lafitte, 1987; Lawlor & Mitchell, 1991; Bowes, 1993), it is not thought to be due to greatly increased leaf-level photosynthesis because C_4-photosynthesis is nearly CO_2-saturated at ambient CO_2 (Bowes, 1993); increased water-use efficiency may be responsible for enhanced C_4-plant growth in elevated CO_2 (Rogers *et al.*, 1983*a*). The harvest index of grain sorghum was about 8% lower ($P \approx 0{\cdot}001$) in elevated compared with ambient CO_2 (Table 1), which was due to proportionally greater positive response to elevated CO_2 in leaves and stems compared with grain, rather than to less grain. Chambers did not significantly affect grain sorghum dry mass accumulation, except for a reduction in root mass, but there was an overall tendency toward smaller plants inside the chambers (Table 1).

Soybean dry mass accumulation was significantly greater in elevated compared with ambient CO_2 for all plant components but seed (Table 1). Whole-plant dry mass was nearly 43% greater ($P \approx 0{\cdot}013$) in twice-ambient compared with ambient CO_2 in the open-top chambers. This was probably due in part to increased leaf-level photosynthesis in elevated CO_2 because soybean is a C_3 species, although water-use efficiency was likely improved as well (Lawlor & Mitchell, 1991). Harvest index of soybean was unaffected by CO_2 (Table 1); in previous CO_2 enrichment experiments, no change or a small decline in soybean harvest index has been observed (Rogers, Cure & Smith, 1986). As with grain sorghum, chambers did not significantly affect soybean dry mass accumulation, although the tend-

Table 2. *Mean organ and whole-plant energy concentration (MJ kg^{-1}) at harvest of* Sorghum bicolor *and* Glycine max *grown in open-top chambers containing ambient (1 × −c) or twice ambient (2 × −c) CO_2 or grown outside chambers (1 × −o) in ambient air*

	1 × −c	2 × −c	$P > F$ (df = 5) (2 × −c *vs.* 1 × −c)	1 × −o	$P > F$ (df = 5) (1 × −o *vs.* 1 × −c)
Sorghum bicolor					
Leaves	18·3	18·2	(0·474)	17·7	(0·006)
Stem	17·0	17·1	(0·210)	17·5	(0·003)
Roots	16·7	16·6	(0·500)	16·7	(0·851)
Grain	17·0	16·9	(0·385)	17·0	(0·844)
Whole plant	17·1	17·2	(0·803)	17·2	(0·196)
Glycine max					
Leaves	18·9	18·6	(0·079)	19·0	(0·747)
Stem	17·2	17·2	(0·667)	17·2	(0·904)
Below-ground	18·2	18·1	(0·463)	18·3	(0·819)
Grain	22·7	22·9	(0·113)	22·8	(0·407)
Whole plant	19·1	19·0	(0·464)	19·1	(0·642)

For *Glycine max*, below-ground dry mass was roots plus nodules and grain was pod plus seed.

Table 3. *Mean organ and whole-plant energy per ground area (MJ m^{-2} ground) at harvest of* Sorghum bicolor *and* Glycine max *grown in open-top chambers containing ambient (1 × −c) or twice ambient (2 × −c) CO_2 or grown outside chambers (1 × −o) in ambient air*

	1 × −c	2 × −c	$P > F$ (df = 5) (2 × −c *vs.* 1 × −c)	1 × −o	$P > F$ (df = 5) (1 × −o *vs.* 1 × −c)
Sorghum bicolor					
Leaves	2·01	2·82	(0·012)	2·08	(0·791)
Stem	5·78	7·76	(0·010)	6·77	(0·134)
Roots	0·38	0·65	(0·002)	0·54	(0·026)
Grain	7·98	9·13	(0·121)	8·69	(0·316)
Whole plant	16·14	20·36	(0·019)	18·06	(0·217)
Glycine max					
Leaves	2·82	3·49	(0·030)	3·27	(0·116)
Stem	4·23	6·41	(0·006)	5·11	(0·174)
Below-ground	0·68	0·97	(0·001)	0·73	(0·485)
Grain	3·26	4·89	(0·039)	3·92	(0·347)
Whole plant	10·99	15·77	(0·010)	13·03	(0·193)

For *Glycine max*, below-ground dry mass was roots plus nodules and grain was pod plus seed.

ency was toward smaller plants inside the chambers (Table 1).

Elevated CO_2 had insignificant effects on grain sorghum and soybean individual organ or whole-plant energy concentration (MJ kg^{-1}) at the time of harvest, with the exception of a small but marginally significant ($P \approx 0{\cdot}08$) decrease in soybean leaf energy concentration (Table 2). The chambers *per se* had few effects on plant energy concentration; the exceptions were for grain sorghum leaves and stems (Table 2). The large differences in energy concentration of grain sorghum grain, *c.* 17 MJ kg^{-1}, compared with soybean grain, *c.* 23 MJ kg^{-1} (Table 2), was expected. It reflects the larger fraction of carbohydrates in grain sorghum seeds compared to the higher levels of protein and lipid (both contain more energy per mass than storage carbohydrates) in soybean seeds and pods (Penning de Vries *et al.*, 1989).

Whole-plant and organ energy per ground area (MJ m^{-2}) was significantly greater in elevated compared to ambient CO_2, with the single exception of grain sorghum grain (Table 3). This greater energy per ground area was a result of the stimulation of dry mass accumulation by CO_2 enrichment because the energy concentration (MJ kg^{-1}) of the plants was not affected by CO_2 (Table 2). Except for grain sorghum roots, the chambers had insignificant effects on plant energy per ground area (Table 3).

On the whole, elevated CO_2 resulted in enhanced dry mass accumulation and significantly increased plant energy per ground area in both the C_3 and C_4 plants. The large increase in energy per ground area that was observed has not been explicitly reported prior to this study, although it might have been surmised from commonly observed effects of elevated CO_2 on dry mass accumulation and tissue composition. For example, soybean seed protein,

lipid and fibre concentration (kg kg^{-1}) were unaffected by CO_2 enrichment in similar experiments (Rogers *et al.*, 1986), although seed protein concentration was negatively related to CO_2 in potted plants (Rogers *et al.*, 1984). In the C_4 plant maize (*Zea mays* L.) seed protein, lipid and fibre concentration were unaffected by elevated CO_2 whereas growth was enhanced (Rogers *et al.*, 1983*a*).

Plant composition

In addition to increased dry mass accumulation (kg per plant), and in spite of little influence on energy concentration (MJ kg^{-1}), elevated CO_2 had some effects on tissue composition. For example, we observed a tendency toward lower organ and whole-plant nitrogen concentration (kg N kg^{-1} dry mass) but greater amounts of organ and whole-plant nitrogen per ground area (kg N m^{-2} ground) in elevated compared with ambient CO_2 (data not shown). For soybean specifically, elevated compared with ambient CO_2 greatly increased the nitrogen in stems [0·20 *vs.* 0·14 g N per plant ($P \approx 0{\cdot}002$) and 5·9 *vs.* 4·2 g N m^{-2} ground ($P < 0{\cdot}001$), respectively], roots [0·040 *vs.* 0·029 g N per plant ($P \approx 0{\cdot}044$) and 1·2 *vs.* 0·87 g N m^{-2} ground ($P \approx 0{\cdot}044$), respectively], grains [0·47 *vs.* 0·31 g N per plant ($P < 0{\cdot}001$) and 14·3 *vs.* 9·4 g N m^{-2} ground ($P < 0{\cdot}001$), respectively], and whole plants [0·92 *vs.* 0·67 g N per plant ($P < 0{\cdot}001$) and 28 *vs.* 20 g N m^{-2} ground ($P < 0{\cdot}001$), respectively]. We assume that the increase in nitrogen in soybean was associated with a comparable increase in protein.

The increase in soybean nitrogen content was probably related to greater N_2 fixation (only 3·4 g N m^{-2} were added to the soil as fertilizer in the soybean plots) in root nodules of soybean (a leguminous species that can form a symbiotic relationship with N_2-assimilating bacteria) growing in elevated CO_2. The fixation of N_2 carries with it a large carbohydrate cost (Pate & Layzell, 1990) and elevated CO_2 almost certainly resulted in greater soybean carbohydrate production. Soybean leaves, however, had lower nitrogen concentrations in elevated compared with ambient CO_2 [0·034 *vs.* 0·038 kg kg^{-1} ($P \approx 0{\cdot}005$), respectively], which is consistent with earlier reports of lower tissue nitrogen concentrations (Wong, 1979; Norby *et al.*, 1986; Curtis *et al.*, 1989, 1990; Hocking & Meyer 1991; Kuehny *et al.*, 1991; Norby *et al.*, 1992; Coleman *et al.*, 1993). In a previous field study, there was little effect of CO_2 enrichment on the elemental composition (C, H, N, O and S) or energy concentration (MJ kg^{-1}) of above-ground phytomass (grain was not analyzed separately) in maize grown in open-top chambers, although there was a 'tendency for a slight dilution of N' (Loomis & Lafitte, 1987). Elevated CO_2 did increase maize water-use efficiency in that study, however, and tended to increase above-ground phytomass accumulation, particularly for water-stressed plants. The nitrogen concentration of wheat (*Triticum aestivum* L., a C_3 species) seeds, leaves, chaff and stems was unaffected by elevated CO_2 (Havelka, Wittenbach & Boyle, 1984). In the C_3 N_2-fixing legume cowpea [*Vigna unguiculata* (L.) Walp.], elevated CO_2 resulted in an increase in seed nitrogen concentration (cited in Lawlor & Mitchell, 1991).

There were few significant effects of CO_2 treatment on total non-structural carbohydrate (hexoses plus sucrose plus starch; each was measured separately) concentration (kg kg^{-1}) at the time of harvest. An exception was a higher concentration in leaves of soybean grown in elevated compared with ambient CO_2 [0·126 *vs.* 0·092 kg kg^{-1} ($P \approx 0{\cdot}018$), respectively]. The higher non-structural carbohydrate concentration of soybean leaves in elevated CO_2 might have been the cause of the marginally lower energy concentration of soybean leaves (Table 2) because the energy concentration of carbohydrates (about 15–16 MJ kg^{-1}) is lower than the energy concentration of total plant dry mass (cf. values in Table 2). Total non-structural carbohydrate per ground area (kg m^{-2}) in grain sorghum roots and soybean leaves was greater in elevated compared with ambient CO_2 at the time of harvest (data not shown). Increases in non-structural carbohydrate concentration of plants growing in elevated CO_2 have been frequently observed, but the nonstructural carbohydrate concentration (kg kg^{-1}) of rice (*Oryza sativa* L., a C_3 species) grain (grain only, without chaff) was not affected by CO_2 level during growth while grain production (kg m^{-2}) was positively related to CO_2 level (Rowland-Bamford *et al.*, 1990). Whole-shoot nonstructural carbohydrate concentration, however, was positively related to CO_2 (from 16 to 50 Pa CO_2) in rice (Rowland-Bamford *et al.*, 1990).

Construction cost

Elevated CO_2 did not affect calculated construction cost C in these experiments with the possible exception of a small reduction (*c.* 2%, $P \approx 0{\cdot}05$) in soybean leaf C (Table 4). Thus, the apparent efficiency of converting photosynthate into new plant structure and long-term storage was not affected by elevated CO_2. Chambers, however, significantly increased (*c.* 4%) grain sorghum leaf C and decreased (*c.* 3%) grain sorghum stem C, but they did not affect any other construction costs (Table 4). Our derived values of C for grain sorghum and soybean organs (Table 4) were only slightly smaller than theoretical calculations given in Penning de Vries *et al.* (1989: table 11) which suggests that our methods were sound.

Griffin, Thomas & Strain (1993) reported reductions in leaf construction cost for loblolly pine (*Pinus*

Table 4. *Specific construction cost (C, kg glucose kg^{-1} dry phytomass grown) at harvest of* Sorghum bicolor *and* Glycine max *grown in open-top chambers containing ambient (1 × −c) or twice ambient (2 × −c) CO_2 or grown outside chambers (1 × −o) in ambient air*

	1 × −c	2 × −c	$P > F$ (df = 5) (2 × −c *vs.* 1 × −c)	1 × −o	$P > F$ (df = 5) (1 × −o *vs.* 1 × −c)
Sorghum bicolor					
Leaves	1·44	1·43	(0·459)	1·39	(0·008)
Stem	1·32	1·34	(0·249)	1·36	(0·004)
Roots	1·31	1·29	(0·476)	1·31	(0·948)
Grain	1·33	1·32	(0·287)	1·33	(0·885)
Whole plant	1·34	1·34	(0·973)	1·35	(0·266)
Glycine max					
Leaves	1·53	1·50	(0·051)	1·53	(0·926)
Stem	1·36	1·35	(0·539)	1·36	(0·933)
Below-ground	1·45	1·44	(0·439)	1·45	(0·836)
Grain	1·87	1·89	(0·100)	1·88	(0·564)
Whole plant	1·54	1·53	(0·327)	1·54	(0·878)

For *Glycine max*, below-ground dry mass was roots plus nodules and grain was pod plus seed.

Table 5. *Construction cost on a ground area basis (C_A, kg glucose m^{-2} ground) at harvest of* Sorghum bicolor *and* Glycine max *grown in open-top chambers containing ambient (1 × −c) or twice ambient (2 × −c) CO_2 or grown outside chambers (1 × −o) in ambient air*

	1 × −c	2 × −c	$P > F$ (df = 5) (2 × −c *vs.* 1 × −c)	1 × −o	$P > F$ (df = 5) (1 × −o *vs.* 1 × −c)
Sorghum bicolor					
Leaves	0·158	0·222	(0·014)	0·163	(0·805)
Stem	0·451	0·606	(0·011)	0·528	(0·137)
Roots	0·030	0·051	(0·002)	0·042	(0·029)
Grain	0·626	0·714	(0·126)	0·681	(0·319)
Whole plant	1·264	1·592	(0·020)	1·414	(0·222)
Glycine max					
Leaves	0·228	0·281	(0·032)	0·264	(0·118)
Stem	0·334	0·505	(0·006)	0·403	(0·174)
Below-ground	0·055	0·078	(0·001)	0·058	(0·492)
Grain	0·269	0·404	(0·032)	0·323	(0·328)
Whole plant	0·885	1·269	(0·009)	1·047	(0·188)

For *Glycine max*, below-ground dry mass was roots plus nodules and grain was pod plus seed.

taeda L., a C_3 species) seedlings grown in a glasshouse in elevated CO_2 using the method of Williams *et al.* (1987) and attributed that response to an accumulation of 'inexpensive' non-structural carbohydrates. Construction cost of plants grown in the field in elevated CO_2 has been estimated (by a different method) once before, but that was for total above-ground phytomass only (Loomis & Lafitte, 1987). In that study, a slight tendency toward increased construction cost with elevated CO_2 in maize shoots harvested near the end of the grain filling period was observed. Other experiments (reviewed in Amthor, 1994*b*) with elevated CO_2, using different methods to estimate C or a related parameter, have indicated no effect or a reduction in construction cost in individual leaves or potted plants.

Elevated CO_2 increased C_A significantly for all plant components measured with the exception of grain sorghum grain (Table 5); the mean value of grain sorghum grain C_A was increased *c.* 14% in elevated CO_2, but the level of significance was marginal ($P \approx 0{\cdot}126$, df = 5; Table 5). The chambers *per se* had little influence on C_A, except for grain sorghum roots for which the chambers reduced C_A (Table 5) because of a reduction in root dry mass (Table 1). On the whole, CO_2 enrichment significantly increased the photosynthate requirements for plant growth on a ground area basis because of increased plant size. The requirement for additional photosynthate was obviously met by increased photosynthesis (per ground area) because the plants were larger as a result of CO_2 enrichment.

An important distinction concerning measures of organ and whole-plant energy, non-structural carbohydrate, and nitrogen concentrations (and therefore construction costs) must be made regarding the timing of tissue sampling. In this study we analyzed

plants at the end of the growing season so measures of dry mass amount and quality (composition) were related to whole-season integrations of plant activities. Mobilization of leaf proteins during grain filling may lead to an underestimation of true construction cost when leaves are sampled at the end of the growing season (indeed, at the end of a leaf's life whenever it occurs). In some other studies, tissues have been sampled in the middle of the growing season or throughout a growing season or life of an organ, so comparisons among studies should be made with caution. These considerations are probably insignificant to estimates of seed construction costs.

We expect (Amthor, 1991) that growth respiration (Penning de Vries *et al.*, 1989) increases roughly in proportion to increased growth for many plants exposed to elevated CO_2, which was apparently the case in this field experiment. Total respiration, i.e. the sum of growth respiration and maintenance respiration, may respond somewhat differently to elevated CO_2. For example, there is some evidence from field-grown plants that maintenance respiration requirements can be reduced by CO_2 enrichment, at least in leaves (Wullschleger & Norby, 1992; Wullschleger, Norby & Gunderson, 1992), although the opposite response has been reported for potted-plant leaves (Thomas *et al.*, 1993; discussed by Amthor, 1994*b*). The general picture emerging from recent experiments is that the efficiency of photosynthate use is not greatly altered by long-term CO_2 enrichment, and may even be increased (Amthor, 1994*b*), so that increased photosynthesis experienced by many plants in elevated CO_2 leads more or less directly to increased growth although various feedback mechanisms may cause a gradual decline in, or down regulation of, photosynthesis (Bowes, 1993).

With respect to agricultural productivity and food quality, growth of both the C_3 and C_4 crop species was stimulated by CO_2 enrichment whereas grain quality was not greatly affected. We conclude from available data (e.g. Table 2; Rogers *et al.*, 1983*a*, 1984, 1986) that grain quality is not significantly affected by elevated atmospheric CO_2, although it would be prudent to measure grain quality, as well as grain production, in additional CO_2-enrichment studies with crops in the field because present data are limited. The 50% increases in energy and nitrogen in soybean grain per ground area have important implications for agricultural productivity and human food supply in a future higher-CO_2 world. Namely, a doubling of atmospheric CO_2 may increase usable energy production by soybean crops by 50% on a ground area basis. This is especially important in view of the fact that human population growth rate is exceeding the rate of increase of land area used for agriculture so that food production per unit land area must increase to meet future food demands (Loomis & Connor, 1992).

ACKNOWLEDGEMENTS

This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. The Alabama Agricultural Experiment Station and Auburn University supported this research. Ms Trina Cagle, Ms Tammy Counts, and Mr Barry Dorman are thanked for technical support.

REFERENCES

Amthor JS. 1991. Respiration in a future, higher-CO_2 world. *Plant, Cell and Environment* **14**: 13–20.

Amthor JS. 1994*a*. Respiration and carbon assimilate use. In: Boote KJ, Bennett JM, Sinclair TR, Paulsen GM, eds. *Physiology and determination of crop yield.* Madison, WI: American Society of Agronomy, 221–250.

Amthor JS. 1994*b*. Plant respiratory responses to elevated CO_2 and their implications for growth enhancement with increasing atmospheric CO_2. In: Allen LH, ed. *Advances in CO_2 effects research.* Madison, WI: American Society of Agronomy (in the press).

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

Beerling DJ, Woodward FI. 1993. Ecophysiological responses of plants to global environmental change since the Last Glacial Maximum. *New Phytologist* **125**: 641–648.

Bowes G. 1993. Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**: 309–332.

Coleman JS, McConnaughay KDM, Bazzaz FA. 1993. Elevated CO_2 and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? *Oecologia* **93**: 195–200.

Curtis PS, Balduman LM, Drake BG, Whigham DF. 1990. Elevated atmospheric CO_2 effects on belowground processes in C_3 and C_4 estuarine marsh communities. *Ecology* **71**: 2001–2006.

Curtis PS, Drake BG, Whigham DF. 1989. Nitrogen and carbon dynamics in C_3 and C_4 estuarine marsh plants grown under elevated CO_2 in situ. *Oecologia* **78**: 297–301.

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

Eamus D. 1991. The interaction of rising CO_2 and temperatures with water use efficiency. *Plant, Cell and Environment* **14**: 843–852.

Fajer ED. 1989. The effects of enriched CO_2 atmospheres on plant-insect herbivore interactions: growth responses of larvae of the specialist butterfly, *Junonia coenia* (Lepidoptera: Nymphalidae). *Oecologia* **81**: 514–520.

Green TN. 1993. *Nitrogen and water stress interactions in loblolly pine* (Pinus taeda *L.*) Ph.D. thesis, Auburn University, USA.

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.

Gunderson CA, Norby RJ, Wullschleger SD. 1993. Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. *Plant, Cell and Environment* **16**: 797–807.

Havelka UD, Wittenbach VA, Boyle MG. 1984. CO_2-enrichment effects on wheat yield and physiology. *Crop Science* **24**: 1163–1168.

Hocking PJ, Meyer CP. 1991. Effects of CO_2 enrichment and nitrogen stress on growth and partitioning of dry matter and nitrogen in wheat and maize. *Australian Journal of Plant Physiology* **18**: 339–356.

Houghton JT, Callander BA, Varney SK. (Eds.). 1992. *Climate change 1992. The supplementary report to the IPCC scientific assessment.* Cambridge: Cambridge University Press.

Hue NV, Evans CE. 1986. Procedures used for soil and plant analysis by the Auburn University Soil Testing laboratory. Auburn University, Auburn, Alabama.

Hunt R, Hand DW, Hannah MA, Neal AM. 1993. Further responses to CO_2 enrichment in British herbaceous species. *Functional Ecology* **7**: 661–668.

Jones MGK, Outlaw WH, Lowry OL. 1977. Enzymatic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissue. *Plant Physiology* **60**: 379–383.

Kuehny JS, Peet MM, Nelson PV, Willits DH. 1991. Nutrient dilution by starch in CO_2-enriched Chrysanthemum. *Journal of Experimental Botany* **42**: 711–716.

Lafitte HR, Loomis RS. 1988. Calculation of growth yield, growth respiration and heat content of grain sorghum from elemental and proximal analyses. *Annals of Botany* **62**: 353–361.

Lawlor DW, Mitchell RAC. 1991. The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. *Plant, Cell and Environment* **14**: 807–818.

Lieth H. 1975. Measurement of caloric values. In Lieth H, Whittaker R, eds. *Primary productivity of the biosphere.* New York: Springer-Verlag, 119–129.

Loomis RS, Connor DJ. 1992. *Crop ecology: productivity and management in agricultural systems.* Cambridge: Cambridge University Press.

Loomis RS, Lafitte HR. 1987. The carbon economy of a maize crop exposed to elevated CO_2 concentrations and water stress, as determined from elemental analysis. *Field Crops Research* **17**: 63–74.

McDermitt DK, Loomis RS. 1981. Elemental composition of biomass and its relation to energy content, growth efficiency, and growth yield. *Annals of Botany* **48**: 275–290.

Norby RJ, Gunderson CA, Wullschleger SD, O'Neill EG, McCracken MK. 1992. Productivity and compensatory responses of yellow-poplar trees in elevated CO_2. *Nature* **357**: 322–324.

Norby RJ, O'Neill EG, Luxmoore RJ. 1986. Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. *Plant Physiology* **82**: 83–89.

Pate JS, Layzell DB. 1990. Energetics and biological costs of nitrogen assimilation. In: Miflin BJ, ed. *The biochemistry of plants*, vol. 16, *Intermediary nitrogen metabolism.* San Diego: Academic Press, 1–42.

Penning de Vries FWT, Jansen DM, ten Berge HFM, Bakema A. 1989. *Simulation of ecophysiological processes of growth in several annual crops.* Wageningen, The Netherlands: PUDOC.

Rogers HH, Bingham GE, Cure JD, Smith JM, Surano KA. 1983*a*. Responses of selected plant species to elevated carbon dioxide in the field. *Journal of Environmental Quality* **12**: 569–574.

Rogers HH, Cure JD, Smith JM. 1986. Soybean growth and yield response to elevated carbon dioxide. *Agriculture, Ecosystems and Environment* **16**: 113–128.

Rogers HH, Cure JD, Thomas JF, Smith JM. 1984. Influence of elevated CO_2 on growth of soybean plants. *Crop Science* **24**: 361–366.

Rogers HH, Heck WW, Heagle AS. 1983*b*. A field technique for the study of plant responses to elevated carbon dioxide concentrations. *Journal of the Air Pollution Control Association* **33**: 42–44.

Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**: 155–189.

Rowland-Bamford AJ, Allen LH Jr, Baker JT, Boote KJ. 1990. Carbon dioxide effects on carbohydrate status and partitioning in rice. *Journal of Experimental Botany* **41**: 1601–1608.

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 and Environment* **16**: 539–546.

Williams K, Percival F, Merino J, Mooney HA. 1987. Estimation of tissue construction cost from heat of combustion and organic nitrogen content. *Plant, Cell and Environment* **10**: 725–734.

Wong SC. 1979. Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. *Oecologia* **44**: 68–74.

Wullschleger SD, Norby RJ. 1992. Respiration cost of leaf growth and maintenance in white oak saplings exposed to atmospheric CO_2 enrichment. *Canadian Journal of Forest Research* **22**: 1717–1721.

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

Wullschleger SD, Ziska LH, Bunce JA. 1994. Respiration responses of higher plants to atmospheric CO_2 enrichment. *Physiologia Plantarum* **90**: 221–229.