Bangor University

DOCTOR OF PHILOSOPHY

Growth of Urtica urens in elevated CO

Marriott, David

Award date:
1999

Awarding institution:
University of Wales, Bangor

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 19. Oct. 2024

Growth of *Urtica urens* in elevated CO_2

A thesis submitted to the University of Wales
for the degree of Doctor of Philosophy

by

David Marriott B.Sc.

School of Biological Sciences and Institute of Terrestrial Ecology
Bangor, 1995 - 1998

Submitted March 1999

I'W DDEFNYDDIO YN Y
LLYFRGELL YN UNIG

TO BE CONSULTED IN THE
LIBRARY ONLY

P.C.B LLYFRGELL
U.W.B LIBRARY

CONTENTS

SUMMARY

The current literature indicates that the stimulation of relative growth rate (RGR) by an elevated atmospheric CO_2 concentration (C_a) is transient. The effects of a twice-ambient C_a on the RGR and related processes of *Urtica urens* L. were investigated to better understand the mechanisms behind the growth response. Plants were grown hydroponically without nutrient limitation in controlled-environment cabinets. Consistent with studies of other C_3 species , the initial CO_2-stimulation of RGR of *U. urens* was not sustained, declining within days of exposure to elevated C_a. This decline in RGR was caused mainly by a decreased leaf area ratio (LAR) due to a decreased specific leaf area (SLA); a reduction in the CO_2-stimulation of net assimilation rate (NAR) made a relatively small contribution. The effects of elevated C_a on RGR, LAR, SLA and NAR were not attributable to interactions between accelerated plant size and ontogenetic drift or the earlier onset of environmental constraints. The early changes in NAR and LAR could not be explained by reductions in tissue N concentration or by current models of plant growth which propose a central role for soluble sugars in down-regulating growth by signalling the inability of sinks to use increased assimilate supply. A direct effect of elevated C_a on respiration was found, the causes and consequences of which were not clear. It was suggested that the mechanisms behind growth responses to elevated C_a are still poorly understood, but might be improved if future research concentrated more on morphological responses.

ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisors, John Farrar (University of Wales, Bangor) and Clare Stirling (Institute of Terrestrial Ecology, Bangor), for their support throughout my work and during the writing of this thesis. I give particular thanks also to Gwynfor Williams, for his technical expertise and enthusiasm in the construction of the cuvette used in the measurements of CO_2 exchange in whole plants.

I would like to express my appreciation for the constructive discussions that took place with many people working in the Plant Sciences at both the University of Wales and at I.T.E. in Bangor, particularly Harry Harmens and Paul Richardson. I thank also the many people who gave up their time to share their knowledge of analytical techniques and equipment, particularly Gareth Williams and Louise Thurlow. Finally, I acknowledge N.E.R.C. and I.T.E. who provided the funding for this work.

ABBREVIATIONS

A	Net photosynthesis (CO_2 assimilation rate)
A_G	Gross photosynthesis
A_{max}	The maximum attainable rate of net photosynthesis at saturating PPFD and C_i
A_{sat}	The PPFD-saturated rate of net photosynthesis
α	The maximum efficiency of light conversion
ANOVA	Analysis of variance
C_a	Atmospheric carbon dioxide concentration
C_i	Intercellular carbon dioxide concentration
C_3	Referring to any plant that produces the 3-carbon intermediate phosphoglyceric acid in the first step in photosynthesis
C_4	Referring to any plant that produces the 4-carbon intermediate oxaloacetic acid in the first step in photosynthesis
CAM	Crassulacean acid metabolism, referring to any plant that stores CO_2 in a 4-carbon intermediate during darkness
CER	Net CO_2 exchange rate
CIRAS	Combined infra-red analysis system (PP Systems, Herts., UK)
DW	Dry weight
E_T	Transpiration rate
GMR	Geometric mean regression
g_s	Stomatal conductance to water vapour
HPLC	High performance liquid chromatography
IRGA	Infra-red gas analyzer
J	The potential rate of electron transport

J_{max}	The maximum potential rate of electron transport
K_c	The Michaelis-Menten constant of rubisco for CO_2
K_o	The Michaelis-Menten constant of rubisco for O_2
K_M	The Michaelis-Menten constant
k	Allometric coefficient derived from the slope of a simple linear regression
L_A	Leaf area
L_W	Leaf weight
LAR	Leaf area ratio
$\overline{L}AR$	Mean leaf area ratio, the average LAR over a specified interval of time
Log_e	Natural logarithm
LWR	Leaf weight ratio
$\overline{L}WR$	Mean leaf weight ratio, the average LWR over a specified interval of time
M	Molarity (mol l^{-1})
MES	2-[N-Morpholino]ethane-sulphonic acid
N_L	Leaf organic nitrogen concentration
N_P	Plant organic nitrogen concentration
N_{RT}	Root organic nitrogen concentration
N_{ST}	Stem organic nitrogen concentration
N_{SHT}	Shoot organic nitrogen concentration
NAR	Net assimilation rate
$\overline{N}AR$	Mean net assimilation rate, the average NAR over a specified interval of time
$NO_3^-{}_A$	Assimilation rate of nitrate
$NO_3^-{}_U$	Uptake rate of nitrate

PAGE	Polyacrylamide gel electrophoresis
PPFD	Photosynthetic photon flux density, equivalent to photosynthetically active radiation (PAR) occurring at wavelengths between 400 and 700 nm
r	The coefficient of correlation
r^2	The coefficient of determination
r_b	Boundary layer resistance to water vapour
r_c	Cuticular resistance to water vapour
r_a	Stomatal resistance to water vapour
R_d	Dark respiration (respiration occurring in the dark)
R_l	Day respiration (non-photorespiratory respiration occurring in the light)
RT_W	Root weight
RGR	Relative growth rate
$\overline{RGR}$	Mean relative growth rate, the average RGR over a specified interval of time
RH	Relative humidity
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RuBP	Ribulose-1,5-bisphosphate
SAR_N	Specific absorption rate of nitrogen by roots
$\overline{SAR}_N$	Mean specific absorption rate of nitrogen by roots, the average SAR_N over a specified interval of time
SDW	Structural dry weight, the total dry weight minus the weight of non-structural carbohydrates (starch and/or soluble sugars)
SLA	Specific leaf area
$\overline{SLA}$	Mean SLA, the average SLA over a specified interval of time
S/R	Ratio of shoot dry weight to root dry weight
TNC	Total non-structural carbohydrates (soluble sugars and starch)

T	Temperature
t	Time
Γ^*	CO_2 compensation point in the absence of day respiration
θ	Convexity of a non-rectangular hyperbola
v	Allometric coefficient derived by the slope of a geometric mean regression
$V_{c,max}$	The maximum rate of carboxylation limited by the amount and/or activity of rubisco
$V_{o,max}$	The maximum rate of oxygenation limited by the amount and/or activity of rubisco
VPD	(water) Vapour pressure deficit
W_c	The RuBP-saturated rate of carboxylation
W_j	The RuBP-limited rate of carboxylation
Y_G	Growth conversion efficiency

CHAPTER 1

General introduction

1.1. Rising C_a and climate change

Atmospheric CO_2 (C_a), as well as other greenhouse gases such as water vapour and methane, is largely transparent to what is predominantly short-wave direct solar radiation but absorbs the subsequent long-wave radiation radiated or reflected from the earth's surface. In turn, these gases re-radiate this energy both to space and to the earth, thereby in the latter case raising the temperature of the ground and lower atmosphere to values conducive to life. Measurements of the deuterium content of water in Antarctic ice cores, and of the CO_2 concentration of air bubbles trapped within, indicate that the fluctuations in mean global temperature over the last 160,000 years closely parallel the fluctuations in C_a (Barnola *et al.*, 1987). It is probable, therefore, that C_a is a major component driving global temperatures, and hence temperature-dependent climatic variables such as wind and rain.

Since the 19th century, C_a has been rising in a manner coincident with industrial activity. Barnola *et al.* (1987) estimate the pre-industrial C_a to have been in the region of 270 μmol mol^{-1}. Direct measurements of C_a starting in 1958 at Hawaii show that concentrations have increased logarithmically from 315 μmol mol^{-1} in 1958 to 354 μmol mol^{-1} in 1989 (Keeling *et al.*, 1989), leading to concern that the past and present anthropogenic inputs of CO_2 into the atmosphere are rapidly changing the earth's climate. By the end of the next century, some models predict a 2-fold rise in C_a to about 700 μmol mol^{-1}, with a 2.5 °C rise in mean global surface temperature that will not be spatially homogenous (IPCC, 1990). Of particular concern is that such climatic change is likely to include alterations in the quantity and distribution of rainfall, and a greater frequency of severe weather events (IPCC, 1990).

1.2. Rising C_a and plants

During their evolutionary history, terrestrial plants have experienced C_as far in excess of those predicted for the next century and beyond. Carbonate concentrations and $\delta^{13}C$ values in geothite suggest that the advance of plants onto land about 420 million years ago took place in an atmosphere containing CO_2 in excess of 5000 $\mu mol\ mol^{-1}$ (Yapp and Poths, 1992). From this point forward, C_a declined gradually, embracing the appearance of flowering plants in the fossil record about 130 million years ago, and then stabilized so that for the last 25 - 65 million years plants have experienced a C_a comparable to that of the present day and at a concentration perhaps sufficiently low to induce the evolution of terrestrial C_4 species for the first time (Ehleringer *et al.*, 1991). Whilst the magnitude of any future C_a is therefore unlikely to be a novel event for plants, it is arguable whether plants have the genetic capacity needed to adapt to the unusually rapid rise in C_a that is now taking place, or even whether present-day plants have sufficient genetic memory to do so.

Rising C_a could affect plants indirectly through the effects of temperature and of climatic changes otherwise driven by temperature, or directly through its effects on major plant physiological processes, particularly increased photosynthesis (Bowes, 1991), decreased transpiration (Mott, 1991), and perhaps also decreased dark respiration (Wullschleger *et al.*, 1994). Both temperature-mediated and direct effects of C_a will affect plant growth, with important implications for both natural and artificial ecosystems. However, unlike the projected increases in temperature, rising C_a is inevitable, spatially homogenous, and predictable (Jones and Wigley, 1990), and thus of particular research value. Moreover, any direct effects of C_a on the balance between photosynthesis and respiration will clearly have an impact on the global cycling of carbon, and hence on the global climate itself. For these reasons at least, an understanding of plant responses to elevated C_a is needed if we are to predict the ecological, agricultural and socio-economic impacts of climate change.

1.3. Rising C_a and plant growth

Elevated C_a increases the biochemical efficiency of photosynthesis in C_3 plants and hence their potential for growth. Accordingly, the dry weight of C_3 plants grown in elevated C_a is nearly always increased when compared at a common point of time to that of plants grown in ambient C_a (Kimball, 1883; Cure and Acock, 1986; Bazazz, 1990; Hunt *et al.*, 1991; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Stirling *et al.*, 1998). Net photosynthesis is increased by CO_2 because carboxylation by rubisco is limited by the current C_a, and because CO_2 competes with O_2 for the same binding sites on rubisco and hence reduces oxygenation and subsequent photorespiratory carbon loss (Bowes, 1991). Theoretical expectations of the initial increase in photosynthesis in individual leaves exposed to twice-ambient C_a range from 20 % if photosynthesis is limited by RuBP supply to 70 % if photosynthesis is limited by the amount and/or activity of rubisco (Stitt, 1991). In practice, the net CO_2 gain by both individual leaves and entire canopies will probably lie somewhere between these two extremes, depending on species, environmental conditions such as the supply of light and nutrients, and on the extent to which elevated C_a reduces the stomatal conductance.

By reducing stomatal conductance, elevated C_a could in principle stimulate growth by increasing the ratio of photosynthesis to transpiration (water use efficiency) (Bowes, 1993). However, improvements in water use efficiency are likely to promote growth only in conditions of drought stress (Bazazz, 1990), and the widely reported increases in dry weight in elevated C_a can be attributed primarily to increases in photosynthesis (Wong, 1979; Poorter *et al.*, 1988). The link from photosynthesis to growth involves a number of potentially limiting steps in the partitioning and use of photoassimilate. In the simplest terms, a positive growth response to elevated C_a could result from the accumulation of additional soluble sugar as non-structural carbon, for example in specialized storage organs, or it could result from the deposition of structural material following use of soluble sugar in dark respiration (R_d). Clearly, the accumulation of non-structural carbon will limit the immediate productive potential of a plant since such carbon is not used to generate new resource-acquiring structure (Wong, 1990). In contrast, an increase in structural material may or may not increase the productive potential, depending on the way fixed carbon is partitioned and used within the plant.

The central equation in plant growth analysis recognizes that an index of efficiency is needed to compare the growth of plants independent of both their size and their duration of growth (Evans, 1972):

Relative growth rate (RGR) = Net assimilation rate (NAR) * Leaf area ratio (LAR)

where RGR is the dry weight gain per unit time per unit dry weight ($g\ g^{-1}\ d^{-1}$), NAR is the dry weight gain per unit time per unit leaf area ($g\ m^{-2}\ d^{-1}$), and LAR is the ratio of leaf area to plant dry weight ($m^2\ g^{-1}$).

Despite the long and established history of use of this equation in studies of plant growth, CO_2-enrichment studies and literature reviews of them have often persisted in trying to quantify and compare the effects of elevated C_a on growth in terms of the dry weight gain after widely-different periods of time (Cure and Acock, 1986; Poorter, 1993). Nevertheless, it is readily apparent from such studies that the increases in dry weight due to elevated C_a are not only highly variable, both between and within species, but also surprisingly moderate and unlikely to reflect even the more conservative estimates of whole-plant net carbon gain. Accordingly, studies that recognize the importance of RGR as an index of growth make it clear that the stimulation of RGR in elevated C_a is transient and occurs for only a short duration in the early stages of exposure (Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Fonseca *et al.*, 1996; Stirling *et al.*, 1998).

1.4. Research aims and outline of thesis

The principal aim of this thesis is to reach a better understanding of how and why C_3 plants in elevated C_a are unable to sustain a higher RGR in time. This will not only help to predict plant responses in a future high CO_2 world, but may also target physiological mechanisms that currently limit crop productivity. Studies which have analyzed RGR in elevated C_a in terms of its components often find limitations related to photosynthetic capacity (NAR) as well as those that directly quantify the partitioning between leaf area and dry weight (LAR) (Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Fonseca *et al.*, 1996; Stirling *et al.*, 1998).

However, the mechanistic bases that underlie the response of RGR to elevated C_a are still far from clear, partly because of the many problems encountered when trying to compare plants grown in ambient C_a with plants grown in elevated C_a. A particular problem is that any stimulation of RGR by elevated C_a leads inevitably to larger plants when compared at any common point of time. Hence, it is necessary to distinguish what could be direct responses to elevated C_a from changes associated with ontogeny or the earlier onset of some environmental constraint to growth. This issue is extensively addressed in this thesis, partly by analytical techniques such as allometric analysis, and partly by careful control and monitoring of the conditions of growth. A similar general concern is extended to finding suitable and comparable bases for the expression of data, since changes in tissue composition commonly occur in elevated C_a.

To make what are sometimes lengthy experimental chapters more manageable for the reader, a separate chapter is included giving a detailed account of materials and methods (Chapter 2). Also in Chapter 2, there is a brief description of *Urtica urens* and reasons for its selection as a subject for study. Chapters 3 - 6 examine established and novel hypotheses that may explain why plants in elevated C_a are unable to sustain a higher RGR. Chapter 3 describes the growth response of the plant to elevated C_a within an experimental system typical of many CO_2-enrichment studies, so that the behaviour of *U. urens* can be compared to other, more intensively-studied species.

The timing and nature of the responses observed in Chapter 3 largely determine the experimental design common to Chapters 4, 5 and 6, where the early responses to elevated C_a are examined with regard to a more rigorous investigation of their mechanistic bases. The early growth responses to elevated C_a are described in Chapter 4. With reference to these results, the roles of tissue N, soluble sugars and respiration are examined in Chapters 4, 5 and 6, respectively.

The role of soluble sugars is investigated in context of the extended hypothesis that the decline in RGR in elevated C_a is caused by the repression of photosynthesis due to the accumulation of soluble sugars in leaves, resulting from an inability of sinks to utilize the additional fixed carbon that is available from an increased activity of source leaves (Stitt, 1991; Van Oosten *et al.*, 1994; Pollock and Farrar, 1996). As such, this hypothesis currently stands as the only comprehensive explanation for a decline in RGR in elevated C_a. The concept that plants can be divided into regions that produce and export assimilate (sources) and those that import assimilate for growth or storage (sinks) has a long and established history as a working model of plant growth (Farrar, 1996; Pollock and Farrar, 1996).

In Chapter 7, investigation of the hypothesis is extended to an alternative experimental system that similarly perturbs the sink-source balance, namely the imposition of N deficiency, in order to assess the broader significance of sugar-represssion of photosynthesis as a mechanism regulating plant growth in changing environments. The thesis ends with a general discussion in Chapter 8.

CHAPTER 2

General materials and methods

2.1. *Urtica urens* as a subject for study

Urtica urens L. (the small or annual nettle) is one of four species in the dicotyledonous family Urticaceae to be found in the British Isles, where it is wide-spread but most common in eastern locations, around coasts and in cultivated ground with light soils (Clapham *et al.*, 1981). Grime *et al.* (1988) state that it is generally regarded as doubtfully native and that, despite its recent decline as an arable weed, its range may be expanding due to the creation of fertile disturbed habitats. These authors consider its functional type (Grime, 1977) as between Ruderal and Competitor-Ruderal. Like its close relative, *Urtica dioica, U. urens* is a C_3 species (Grime *et al.*, 1988) and possesses stinging hairs that inject histamine and serotonin which act synergistically to cause the well-known local odoema, itching and pain (Vickery and Vickery, 1981). Superficially, *U. urens* closely resembles *U. dioica*, having simple, toothed, ovate, pedunculate, decussate leaves and lateral spike-like inflorescences, but differs in many respects including its annual life-form, its capacity to branch freely, its monoecious flowers, and its reliance on seed as the sole means of regeneration. Also unlike the rhizomatous *U. dioica*, *U. urens* has a simple profusely-branched tap-root system. Although sometimes known as small nettle, in fertile conditions *U. urens* can assume the habit of a large bushy plant up to 60 cm in height.

U. urens has been the subject of some ecological studies (more recent examples include Cornelius and Markan, 1984; Boot *et al.*, 1986; Mutikainen and Walls, 1995; Jornsgard *et al.*, 1996), but none involving elevated C_a. Although its physiological characteristics are generally little known, a number of general characteristics make *U. urens* a suitable system for studying the responses of C_3 species to elevated C_a, including (1) small seeds (ca. 0.5 mg per seed) together with an annual life-form, conferring a relatively low capacity to store carbon reserves so that responses to changes in C_a are likely to be both immediate and direct, and (2) large leaves (up to 70 cm^2) borne in pairs, enabling many replicatable destructive measurements of leaves to be made.

2.2. Growth conditions

Plants of *U. urens* were grown in a hydroponic culture system in controlled-environment cabinets (Sanyo Gallenkamp, model SGC660/C/HQI) with a 16 h photoperiod, day/night temperatures of 20/16 °C respectively, day/night water vapour pressure deficits (VPD) of 0.7/0.54 kPa respectively (equivalent to 70 % relative humidity (RH)), and with a photosynthetic photon flux density (PPFD) incident on the tops of plants at between 350 and 800 $\mu mol\ m^{-2}\ s^{-1}$ according to plant height. Light was provided by 250 W metal halide lamps (Model HQI/NDL, FGL Lighting Ltd., Pinewood Studios, Bucks., UK) supplemented by 60 W tungsten lamps.

Seeds of *U. urens* (Herbiseed, Wokingham, UK) were placed on absorbent paper saturated with deionized water and incubated in darkness in ambient CO_2 at 20 °C for 6 d until plumule emergence, at which point seedlings were allowed light at 150 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. After a further 6 d, when cotyledons were approaching full expansion, seedlings were secured onto polystyrene floats and distributed between 10 l capacity plastic troughs, such that roots were suspended in a 13 cm deep aerated full-strength modified Long Ashton nutrient solution (Hewitt, 1966). The nutrient solution included 12 mM NO_3^- as the sole source of available mineral N, 4 mM K^+, and 1.35 mM PO_4^{2-}, and was modified with the addition of sodium metasilicate as a source of Si^{3+} (at 50 μM). To minimize risk of nutrient deficiency, restricted root growth and mutual shading, nutrient solutions were renewed twice to thrice-weekly according to plant size, and plants were positioned so that physical contact between plants was avoided. The largest ratio of root volume (measured by displacement of water) to nutrient solution volume was never more than 0.2. Aeration and stirring of the nutrient solution were achieved by pumping air through 30 cm long ‘air-stones’ (Aqua Air, Interpet, Dorking, UK) at a rate of 1 l min^{-1}.

The control of elevated C_a was achieved using a flow regulator and infra-red CO_2 analyzer (IRGA) (PP-Systems, Herts., UK), whereby the flow of CO_2 from an external cylinder containing the pure compressed gas (BOC Ltd., Manchester, UK) into the cabinet was adjusted automatically to match the measured cabinet C_a to a predetermined value. To reduce possible effects of cabinet variability on results, plants and CO_2 regimes were switched between cabinets twice-weekly. At the same time, the positions of individual plants within and between troughs were randomized.

2.3. Growth analysis

2.3.1. Classical growth analysis

From data obtained of dry weights and leaf area, the following components of plant growth were calculated as mean values over a specified harvest interval, having first paired replicates across harvests according to size (Evans, 1972; Hunt, 1978):

(1) Whole plant mean relative growth rate, $\overline{\text{RGR}}$, using the formula:

$$\overline{\text{RGR}} = (\log_e DW_2 - \log_e DW_1) \,/\, (t_2 - t_1)$$

where DW is the dry weight (g) per plant, $\log_e$ is the natural logarithm, t is time (d), and the subscripts $_1$ and $_2$ define DW and t at the beginning and end of each harvest interval.

(2) Mean net assimilation rate, $\overline{\text{NAR}}$, using the formula:

$$\overline{\text{NAR}} = [(DW_2 - DW_1) \,/\, (t_2 - t_1)] * [(\log_e L_{A2} - \log_e L_{A1}) / (L_{A2} - L_{A1})]$$

where L_A is the total leaf area (m^2) per plant, and the subscripts $_1$ and $_2$ define L_A as well as DW and t at the beginning and end of each harvest interval.

(3) Mean leaf area ratio, $\overline{\text{LAR}}$, using the formula:

$$\overline{\text{LAR}} = [(L_{A1}/DW_1) + (L_{A2}/DW_2)] / 2$$

(4) Mean leaf weight ratio, $\overline{\text{LWR}}$, using the formula:

$$\overline{\text{LWR}} = [(L_{W1}/DW_1) + (L_{W2}/DW_2)] / 2$$

where L_W is the leaf dry weight (g) per plant, and the subscripts $_1$ and $_2$ define L_W as well as DW at the beginning and end of each harvest interval.

(5) Mean specific leaf area, $\overline{\text{SLA}}$, using the formula:

$$\overline{\text{SLA}} = [(L_{A1}/L_{W1}) + (L_{A2}/L_{W2})] / 2$$

Using data obtained of organic nitrogen content per plant, the mean specific absorption rate of nitrogen by roots, $\overline{\text{SAR}}_N$, was also determined over specified harvest intervals using the formula of Welbank (1962):

$$\overline{\text{SAR}}_N = [(N_{P2}-N_{P1}) / (t_2-t_1)] * [(\log_e RT_{W2}-\log_e RT_{W1})/(RT_{W2}-RT_{W1})]$$

where N_P is the organic nitrogen content (mg) per plant, RT_W is the root dry weight (g) per plant, and the subscripts $_1$ and $_2$ define N_P, RT_W and t at the beginning and end of each harvest interval.

2.3.2. Functional growth analysis

From data obtained of dry weights and leaf area, instantaneous values of whole plant relative growth rate (RGR), and of its components net assimilation rate (NAR) and leaf area ratio (LAR), were calculated following the curve-fitting approach of Hughes and Freeman (1967). Their approach was modified to also derive instantaneous values of leaf weight ratio (LWR), specific leaf area (SLA) and the specific absorption rate of N by roots (SAR_N) using data of whole plant organic nitrogen content. The selection of polynomials to fit the time-course of logarithmically-transformed primary data (dry weight of whole plant, leaf and root, leaf area and N content) followed the stepwise method used by Hunt and Parsons (1974), whereby the chosen polynomial included the highest-order term to be significntly different from 0 when analyzed by t-test (Zar, 1989). Using this method, second-order polynomials (quadratic equations) were found to best fit primary data of total dry weight per plant, leaf area per plant, leaf dry weight per plant, and root dry weight per plant. A quadratic equation was also the best fit to data of organic N content per plant in plants grown in elevated C_a, but data of organic N content in plants grown in ambient C_a were best described by a linear equation. However, to enable practical comparisons of curves, a quadratic equation was nevertheless applied, so that in all cases:

$$\log_e DW = a + bt + ct^2 \qquad (eqn.\ 1)$$
$$\log_e L_A = d + et + ft^2 \qquad (eqn.\ 2)$$
$$\log_e L_W = g + ht + it^2 \qquad (eqn.\ 3)$$
$$\log_e N_P = j + kt + lt^2 \qquad (eqn.\ 4)$$
$$\log_e RT_W = m + nt + ot^2 \qquad (eqn.\ 5)$$

where $\boldsymbol{t}$ is time (d), the lower-case letters a - o are equation constants, $\log_e$ is the natural logarithm, DW is the dry weight (g) per plant, L_A is the leaf area (m^2) per plant, L_W is the leaf dry weight (g), N_P is the nitrogen content (mg) per plant and RT_W is the root dry weight (g). From equations 1, 2 and 3, RGR, NAR, LAR, LWR and SLA were derived as follows:

$$\text{RGR} = \delta(\log_e DW)/\delta t = b + 2ct \qquad (eqn.\ 6)$$
$$\text{LAR} = L_A/DW = \text{antilog}_e\ (eqn.\ 2 - eqn.\ 1) \qquad (eqn.\ 7)$$
$$\text{NAR} = \text{RGR/LAR} = eqn.\ 6\ /\ eqn.\ 7 \qquad (eqn.\ 8)$$
$$\text{LWR} = L_W/DW = \text{antilog}_e\ (eqn.\ 3 - eqn.\ 1) \qquad (eqn.\ 9)$$
$$\text{SLA} = \text{LAR/LWR} = eqn.\ 7\ /\ eqn.\ 9 \qquad (eqn.\ 10)$$

From equations 4 and 5, SAR_N was derived after calculating instantaneous values of the relative increase in N_P (RNR) and the N_P:root weight ratio (N_P/RT_W):

$$\text{RNR} = \delta(\log_e N_P)/\delta t = k + 2lt \qquad (eqn.\ 11)$$
$$N_P/RT_W = \text{antilog}_e\ (eqn.\ 5 - eqn.\ 4) \qquad (eqn.\ 12)$$
$$SAR_N = \text{RNR}/(N_P/RT_W) = eqn.\ 11\ /\ eqn.\ 12 \qquad (eqn.\ 13)$$

t-tests were used to determine whether differences due to elevated C_a between corresponding pairs of constants (describing linear and quadratic terms) were significantly different (Hughes and Freeman, 1967).

2.4. Allometric analysis

The growth and development of one part of a plant is often linearly related to the growth and development of another part, at least for substantial periods of growth in constant environmental conditions (Pearsall, 1927; Troughton, 1955). Such allometric relationships can be described by the formula:

$$y = bx^k$$

where y and x are any two plant variates, and b and k are constants. The allometric constant or coefficient (k) can be determined most practically as the slope of the linear regression relating $\log_e$ y to $\log_e$ x:

$$\log_e y = \log_e b + k \log_e x$$

where b expresses the regression intercept of y when x is zero. Because the plant variates are inevitably mutually-related, a geometric mean regression (GMR) will describe the relationship better than a regression based on the more usual method of least squares (Ricker, 1984). The slope (v) of a GMR is related to k by $v = k/r$ where r is the correlation coefficient (Ricker, 1984; Farrar and Gunn, 1996).

In many allometric relationships (for example shoot and root weights), v deviates from unity, indicating an ontogenetic drift. The deviation of v from unity could also occur in response to temporal environmental gradients, such as a progressive decline in nutrient availability either due to the depletion of a non-renewed nutrient pool or due to the more rapid utilization of replenished nutrients as plants increase in size. Comparisons at common points of time between the effects of treatments (e.g. elevated C_a) that also alter the rate of increase in plant size carry the risk of confusing the effects related to plant size with those of the treatments imposed. Allometric analysis therefore provides a means of distinguishing the genuine direct effects of the treatments that are under investigation. To analyze changes in allometric relationships due to elevated C_a, differences in v, the vertical placement of the GMR line (its elevation), and any deviation of v from unity were tested for statistical significance using Student's t-test as described by Zar (1989).

2.5. Measurement of gas exchange in individual leaves

2.5.1. Photosynthesis, respiration and stomatal conductance to water vapour

The rates of CO_2 and H_2O exchange in individual leaves were determined under laboratory or growth conditions using an open combined CO_2/H_2O infra-red analysis system and clamp-on leaf cuvette (CIRAS-1, PP Systems, Hitchin, Herts., UK). The cuvette enclosed 2.5 cm^2 of leaf area and the air temperature inside the cuvette was maintained at 21.5 ± 1 °C. Temperature control was generally achieved by circulating water via a temperature-controlled water-bath through a water-jacket surrounding the cuvette, but in cases where measurements were made in constant temperature environments, fan-assisted dissipation of any proximal heat source was sufficient for temperature control. The VPD in the air entering the cuvette was adjusted (using the CIRAS control system) to give a VPD in the air within the cuvette during measurement in the region of 1 - 1.2 kPa (50 - 60 % RH), depending on the rate of evapotranspiration. Before each set of measurements, leaks were tested for and detected by observing rapid fluctuations in R_d after blowing CO_2-enriched air through a tube around the gaskets sealing the leaf surface to the borders of the cuvette, and corrected by repositioning the cuvette on the leaf.

The calculations used by the CIRAS software to determine the net CO_2 exchange rate (CER) and the stomatal conductance to water vapour (g_s) have been fully described elsewhere (CIRAS operators manual, PP systems), and only relevant details will be given here.

Firstly, the transpiration rate (E_T) is calculated from the mass flow of air entering the cuvette per unit leaf area (W) and the water vapour pressure of air entering (e_{in}) and leaving (e_{out}) the cuvette:

$$E_T = [W*(e_{out} - e_{in})] / (P - e_{out})$$

where P is the atmospheric pressure. The net CER can then be calculated from the difference in CO_2 concentration in the air entering (C_{in}) and leaving (C_{out}) the cuvette with due account of the water vapour loading of C_{out}:

$$CER = C_{in}*W - C_{out}*(W + E_T)$$

The stomatal conductance to water vapour can also be calculated as the reciprocal of the stomatal resistance to water vapour (r_s) such that:

$$g_s = 1/r_s = [e_{leaf} - e_{out}) / (E_T*P)] - r_b$$

where e_{leaf} is the water vapour pressure within the leaf, which is assumed to be saturated at leaf temperature, and r_b is the boundary layer resistance to water vapour, which was given a value of 0.21 m^2 s mol^{-1} as specified by the CIRAS operators manual to match the type of cuvette.

The rate of net photosynthesis (A) was determined as the net CER at a defined PPFD using a clip-on light source and spectrally-neutral filters, and with the flow-rate of air entering the cuvette set to between 0.3 and 0.4 l min^{-1} to give CO_2 differentials in the region of 20 to 50 μmol mol^{-1}. The same conditions were used to determine g_s. Leaf dark respiration (R_d) was determined as net CER in darkness, with the flow-rate of air entering the cuvette set to 0.15 l min^{-1} to give CO_2 differentials in the region of 5 to 10 μmol mol^{-1} and within the recommended range for maximum sensitivity (10 - 50 μmol mol^{-1}). Given that the infra-red analysis cells of the CIRAS operate with a through-flow of air of 100 ml min^{-1}, a positive pressure within the cuvette was maintained during measurement of R_d due to a surplus inflow of air of 50 ml min^{-1} entering the cuvette.

2.5.2. Construction and analysis of A/C_i curves

The CIRAS software can calculate the intercellular CO_2 concentration (C_i) at a given C_a, which enables the construction of a curve describing the response of A to C_i, as illustrated in Figure 2.1. C_i is calculated using the equation of Von Caemmerer and Farquhar (1981):

$$C_i = (((1/((1.37*r_b+1.6*r_s)-0.5*E_T))*C_a))-CER)/((1/(1.37*r_b+1.6*r_s))+0.5*E_T)$$

where 1.37 defines r_b in terms of the ratio of diffusivity of CO_2 relative to water vapour in the boundary layer, and 1.6 defines r_s in terms of the ratio of diffusivity of CO_2 relative to water vapour in air. All parameters have been previously defined in Section 2.5.1.

At saturating PPFD, which in leaves of *U. urens* is approached at 2000 μmol m^{-2} s^{-1} (Fig. 2.2), the A/C_i response can be related to at least two component processes of photosynthesis (Sharkey, 1985). Firstly, the amount and/or activity of rubisco can be related to the slope of the initial, approximately linear region of the A/C_i response curve when A is saturated by RuBP but limited by C_i. Secondly, the capacity to regenerate RuBP, which may be determined by the light reactions of photosynthesis and/or by the recycling of inorganic phosphate, is related to the magnitude of the asymptote when A is saturated by both RuBP and C_i. These processes can be quantified, respectively, as the maximum rate of carboxylation limited by the amount and/or activity of rubisco ($V_{c,max}$) and the maximum attainable rate of photosynthesis at saturating light intensity and C_i (A_{max}). Using the approach of McMurtrie and Wang (1993), $V_{c,max}$ and A_{max} were derived as will now be described.

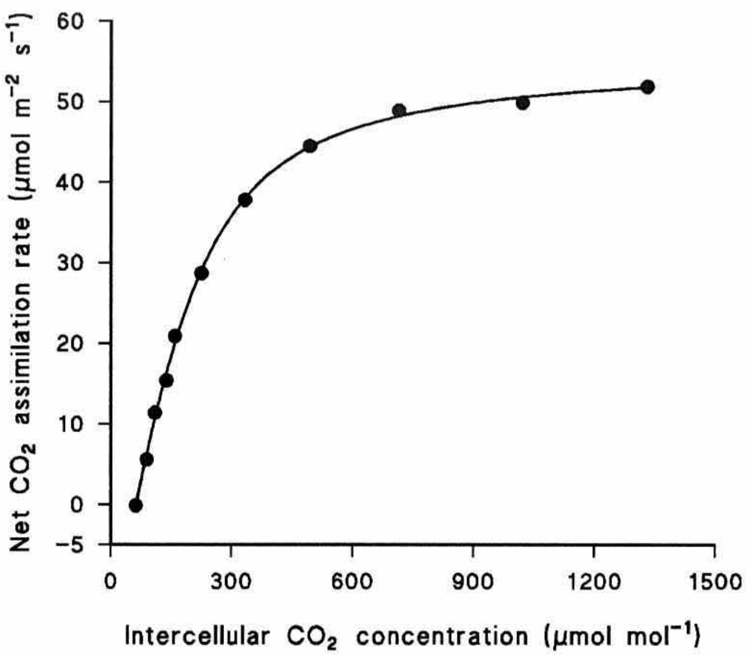

Figure 2.1. The relationship between net CO_2 assimilation rate (A) and intercellular CO_2 concentration (C_i) at 2000 μmol m^{-2} s^{-1} PPFD in a mature leaf of *U. urens*. C_i was calculated by a combined infra-red gas analysis system (CIRAS-1, PP systems, Herts., UK) according to the equation of Von Caemmerer and Farquhar (1981). The curve is fitted to an equation describing a non-rectangular hyperbola (Thornley and Johnson, 1990).

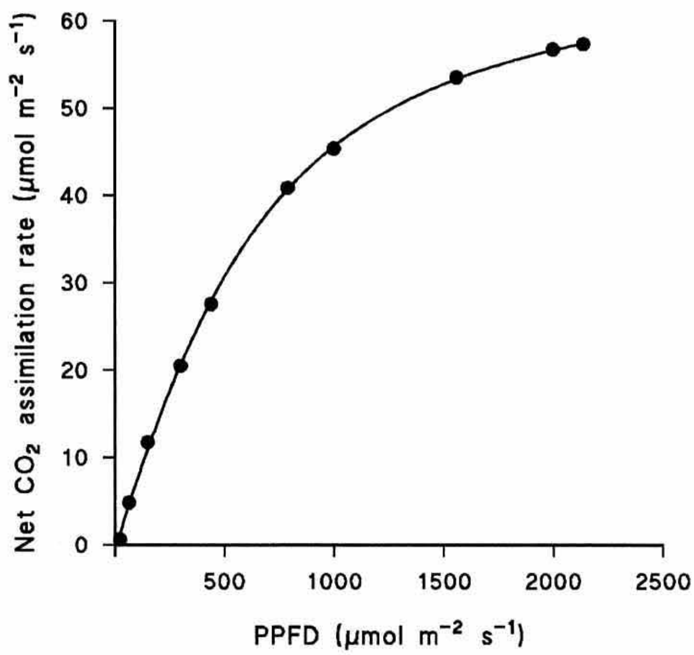

Figure 2.2. The relationship between net CO_2 assimilation rate (A) and photosynthetic photon flux density (PPFD) at 1200 μmol m^{-2} s^{-1} C_i in a mature leaf of *U. urens*. PPFD was varied using spectrally-neutral filters. The curve is fitted to an equation describing a non-rectangular hyperbola (Thornley and Johnson, 1990).

The rate of photosynthesis limited by the amount and/or activity of rubisco can be expressed as (Farquhar *et al.*, 1980):

$$A = W_c(1-\Gamma^{*}/C_i) - R_L$$

where Γ^{*} is the CO_2 compensation point in the absence of day respiration (μmol mol^{-1}) described by:

$$\Gamma^{*} = O(0.5V_{o,max}K_c/V_{c,max}K_c)$$

where $V_{o,max}$ is the maximum rate of oxygenation limited by the amount and/or activity of rubisco, K_c and K_o are the Michaelis constants of rubisco for CO_2 (μbar) and O_2 (mbar) respectively, and O is the partial pressure of O_2 (mbar), and where R_L is the day respiration rate (μmol m^{-2} s^{-1}). W_c is the RuBP-saturated rate of carboxylation (μmol m^{-2} s^{-1}) described by:

$$W_c = V_{c,max}\{[C_i / C_i+K_c(1+O/K_o)]\}$$

The rate of photosynthesis limited by the capacity to regenerate RuBP can be expressed as (Farquhar *et al.*, 1980):

$$A = W_j(1-\Gamma^{*}/C_i)-R_L$$

where W_j is is the RuBP-regeneration-limited rate of carboxylation (μmol m^{-2} s^{-1}) described by:

$$W_j = JC_i/(4.5C_i+7\Gamma^{*}/3)$$

where J is the potential rate of electron transport (μEq m^{-2} s^{-1}) and $4.5C_i$ assumes that 4.5 mol of electrons are needed to reduce 1 mol of CO_2.

J can be solved as the smaller and positive solution of the non-rectangular hyperbola relating J to PPFD (McMurtrie and Wang, 1993):

$$\theta J^2 - (\alpha I + J_{max})J + \alpha I j_{max} = 0$$

where θ is the convexity of the non-rectangular hyperbola, α is the maximum efficiency of light energy conversion (mmol mol^{-1}), I is PPFD (μmol m^{-2} s^{-1}), and J_{max} is the maximum potential rate of potential electron transport (μEq m^{-2} s^{-1}). The potential rate of photosynthesis at saturating PPFD and C_i (P_m) has been expressed as (Harley *et al.*, 1992):

$$P_m = J \text{ / mol electrons needed to reduce 1 mol } CO_2.$$

Therefore the maximum attainable rate of photosynthesis at saturating PPFD and C_i (A_{max}) can be expressed as:

$$A_{max} = J_{max} / 4.5$$

$V_{c,max}$ and A_{max} were estimated from the A/C_i curves using C_is between 50 and 150 μmol mol^{-1} for $V_{c,max}$, and between 250 and 1500 μmol mol^{-1} for A_{max}, following the approach of McMurtrie and Wang (1993) where the equations of Farquhar *et al.* (1980) are modified to include experimentally-derived values and/or temperature (T, °C) dependencies of photosynthetic processes. These parameters are: $\Gamma^* = 1.7T$ (Badger and Andrews, 1974; Farquhar, 1988), $K_c = 39.05\exp(0.086T)$ (Harley *et al.*, 1985; Wang *et al.*, 1991), $K_o = 506.52\exp(0.086T)/T$ (Badger and Andrews, 1974; Farquhar, 1988), and $\alpha = 0.385$ (Farquhar and Von Caemmerer, 1982).

Using the notation required by the software programme Fig.P (Biosoft, Cambridge), $V_{c,max}$ can therefore be solved by the maximum likelihood method in the equation relating A to C_i as:

$$A=\{(1-((1.7*T)/C_i))*((V_{c,max}*C_i)/(C_i+(39.05*exp(0.086*T))*(1+(210/((506.52*exp(0.086*T))/T))))\}-R_L$$

where R_L is solved as the y-intercept (Wullschleger, 1993).

A_{max} can be solved in the equation:

$$A=\{(1-((1.7*T)/C_i))*((((0.385*I+A_{max}-sqrt((0.385*I+A_{max})\hat{}2-4*\theta*I*0.385*A_{max}))/(2*\theta))*C_i)/(C_i+7*(1.7*T)/3)\}-R_L$$

where the value of R_L is derived from the equation for $V_{c,max}$ (Wullschleger, 1993), and θ is solved as a parameter. From the A/C_i response shown in Figure 2.1, the solutions to the equations for $V_{c,max}$ and A_{max} when T = 21.5 °C are shown in Figure 2.3, with values of 167.5 and 63.5 μmol m^{-2} s^{-1} respectively.

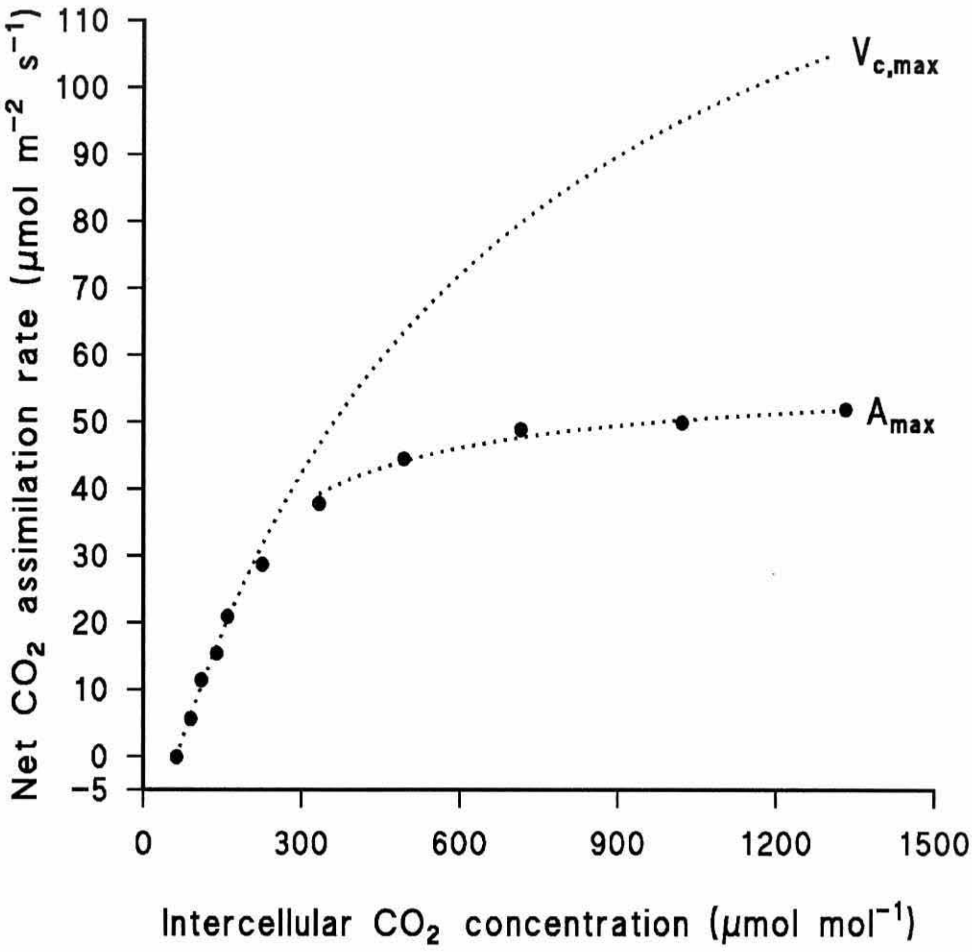

Figure 2.3. Curves fitted to the data shown in Figure 2.1 from equations that describe the relationship of net CO_2 assimilation rate (A) at saturating PPFD to both limiting and saturating intercellular CO_2 concentrations (C_i), allowing for solutions of the maximum rate of carboxylation limited by the amount and/or activity of rubisco ($V_{c,max}$) and the maximum attainable rate of photosynthesis (A_{max}) respectively (McMurtrie and Wang, 1993). The calculated values of $V_{c,max}$ and A_{max} are given in the text.

2.6. Measurement of gas exchange in intact shoots and roots

2.6.1. Basic design of system

Photosynthesis and respiration of intact shoots and roots were measured as net CO_2 exchange (CER) in a constant temperature laboratory (20 °C) using an open system, the basic layout of which is illustrated in Figure 2.4. Using a pump (Model B100 D/E, Charles Austin Pumps Ltd., Surrey, UK), ambient air was drawn from a point about 8 m above ground level, firstly through a 200 l mixing barrel to buffer any external fluctuations in C_a, and secondly through a CO_2 control system (Fig. 2.5) before being pushed through a humidity control system (Fig. 2.6) and into a combined shoot and root cuvette (Figs. 2.7 and 2.8) at a flow-rate determined by manually-adjustable flow-regulators with needle valves (KDG Mobrey Ltd., Sussex, UK). The CO_2 concentration of the air-stream leaving the cuvette was sampled and determined using a bench differential infra-red gas analyser (IRGA) (Series 225, ADC Ltd., Hoddesdon, UK) and multi-channel switching unit (ADC Ltd.) after removal of water vapour in drying columns containing layers of self-indicating $CaSO_4$ plus $CoCl_2$ ('DRIERITE', 8 mesh size, W.A. Hammond Drierite Company, Ohio, USA) and $CaCl_2$ (0-10 mm, BDH laboratory supplies, Dorset, UK). Air was transported within the system using PVC tubing (Portex Ltd., Kent, UK).

2.6.2. Control of elevated C_a

The basic layout of the CO_2 control system is illustrated in Figure 2.5. Ambient air was drawn through a water trap cooled to 5 °C in a water-bath so that air was at approximately constant temperature and water vapour pressure independent of atmospheric conditions. CO_2 was then introduced into the air-stream after diluting with N_2, with the CO_2/N_2 ratio determined by two model FC-260 4S mass flow controllers and a model RO-28 control unit (Tylan General, UK), and with the entry of the CO_2 mixture into the ambient air-stream controlled by a manually-adjustable flow-regulator. CO_2 and N_2 were supplied from cylinders containing the pure compressed gas (BOC Ltd., Manchester, UK). After controlled humidification of the air-stream (Section 2.6.3), during which water was held at pH 5.5 using HCl and 2 mM 2-[N-Morpholino]ethane-sulphonic acid (MES) to prevent absorption of CO_2 as HCO_3^-, the CO_2 concentration in the humidified air-stream was sampled and determined using a portable IRGA (Fuji), and adjustments made to the mass flow controllers and needle valve so that the CO_2 concentration was maintained at 700 ± 40 μmol mol^{-1} without acute fluctuations. For measurements made at ambient CO_2 concentrations, N_2 alone was introduced into the ambient air-stream to account for effects of possible contaminants.

2.6.3. Control of relative humidity

The basic layout of the relative humidity (RH) control system is illustrated in Figure 2.6. By extrapolation from the equation of Buck (1981) describing saturating water vapour pressure as a function of temperature, it was calculated that a RH of 70 % at 21.5 °C (the average air temperature of the shoot compartment during measurements of photosynthesis) would be achieved if air is first saturated with water vapour at 15.8 °C. Dried ambient or CO_2-enriched air at 5 °C (Section 2.6.2) was therefore saturated with water vapour by passing the air through water held at 16 °C. Humidified air was then allowed to warm to constant room temperature (20 °C) in an equilibration vessel and then to 21.5 °C inside the cuvette. This system of control resulted in 70 % RH in the empty shoot compartment, measured using a portable digital thermohygrometer package (Type THG-388, Fisher Scientific, UK).

2.6.4. Cuvette

A photograph of the cuvette used for measuring gas exchange of intact shoots and roots is shown in Figure 2.7, and a diagram of it is shown in Figure 2.8. The cuvette was constructed at the University of Wales, Bangor by Mr Gwynfor Williams, who also contributed greatly to its design. It consisted of a 25 l capacity shoot compartment made from 6 mm polycarbonate and a 2.3 l capacity perspex root compartment which were joined and supported by a free-standing perspex base plate. Both shoot and root compartments were secured to the base plate using thumb-screw clamps and closed-cell rubber gaskets (Foxon's Angling Supplies, St. Asaph, Clwyd, UK), but could be readily removed for access. PPFD was supplied at an intensity comparable to that in growth cabinets by a height-adjustable 250 W metal halide lamp (Model HQI/NDL, FGL Lighting Ltd., Pinewood Studios, Bucks., UK) positioned directly above the shoot compartment. Heat from the lamp was dissipated using water (at 32 mm depth) in a perspex bath with fan-assisted cooling. Air inside the shoot compartment was mixed by a vertically-aligned 25 cm long cross-flow fan with a maximum flow rate of 1.8 l s^{-1} (RS Components Ltd., Northants., UK) placed in one of the corners of the shoot compartment. During measurements, PPFD was measured intermittently using a portable quantum sensor package comprising a model SKP215 sensor and model SKP200 measuring unit (Skye Instruments Ltd., Powys, UK), and air temperature was monitored continually by a column-mounted shielded type-T thermocouple (RS Components Ltd., Northants., UK). Entry and exit of air to and from the shoot compartment were through bulk-head connectors in the base plate, such that there were two exit ports positioned at opposite sides of the shoot compartment, and a single entry port. The pressure in the shoot compartment was measured using a pressure gauge connected to a third port in the base plate, and was maintained at positive pressures at any flow-rate. Reference air for the shoot compartment was provided by splitting the air supply before entry into the cuvette.

The root compartment was filled with full-strength Long Ashton nutrient solution leaving a 250 ml air-space to allow the exit of air (one port), and the solution was stirred by the entry of air at the bottom (one port) through an 2.5 cm long 'air-stone' (Aqua Air, Interpet, Dorking, UK). A replicate root cuvette was set up to provide reference air for measuring root respiration. To avoid CO_2 absorption, the nutrient solutions were adjusted to pH 5.5 using 2 mM MES-HCl. The pH of solutions containing even the largest measured root systems (up to 0.9 l in volume) increased only slightly to pH 5.62 after 3 h.

Plants were fixed into position using a fixing plate (Fig. 2.9) that could be secured to the base plate using thumb-screw clamps and closed-cell rubber gaskets (Foxon's Angling Supplies, St. Asaph, Clwyd, UK). Any remaining gaps between shoot and root compartments (e.g. around the stem base) were sealed using a water-proofing putty (Electrician water-proofing compound, Centaure MFG, Worcs., UK).

2.6.5. Protocol for measurements

After fixing into the cuvette, plants were allowed 1 h to settle before measurements started. During the second 30 min, possible leaks into compartments were tested for by blowing CO_2-enriched air along the seals, and leaks between compartments were discounted by ensuring that differential CO_2 readings from the root compartment were stable before and after darkening the shoot. Photosynthesis was measured over a 30 min period, after which the shoot compartment was darkened. After a further 30 min equilibration, shoot and root respiration were measured also over a 30 min period. Using this protocol, it was possible to measure gas-exchange in one plant grown in ambient C_a and one plant grown in elevated C_a per day. Each plant received fresh nutrient solution, and drying columns were renewed at least daily.

Flow-rates were adjusted to keep CO_2 differentials between 10 and 50 $\mu mol\ mol^{-1}$. The net CER ($\mu mol\ min^{-1}$) was calculated from the flow-rates of air entering the shoot and root compartments of the cuvette, and from the difference between $[CO_2]$ of the air leaving each compartment and that of reference air for each compartment, by:

$$\text{CER} = \frac{CO_2 \text{ differential } (\mu mol\ mol^{-1}) * \text{flow-rate } (l\ min^{-1})}{\text{the gas contant } (= 22.4)}$$

The IRGA was calibrated using 700 $\mu mol\ mol^{-1} \pm 1$ % standard CO_2 (CryoService Ltd., Worcs., UK). Standardized depletions at both ambient and elevated C_a were conducted to account for the decreases in differential sensitivity with increasing reference $[CO_2]$.

Total respiration (R_d) d^{-1} was estimated as dark CER min^{-1} * 1440. With a 16 h photoperiod (Section 2.2), gross photosynthesis (A_G) d^{-1} was estimated as illuminated CER min^{-1} * 960 plus $R_d\ d^{-1}$. Consequently, net photosynthesis (A) d^{-1} was estimated as $A_G\ d^{-1}$ minus $R_d\ d^{-1}$.

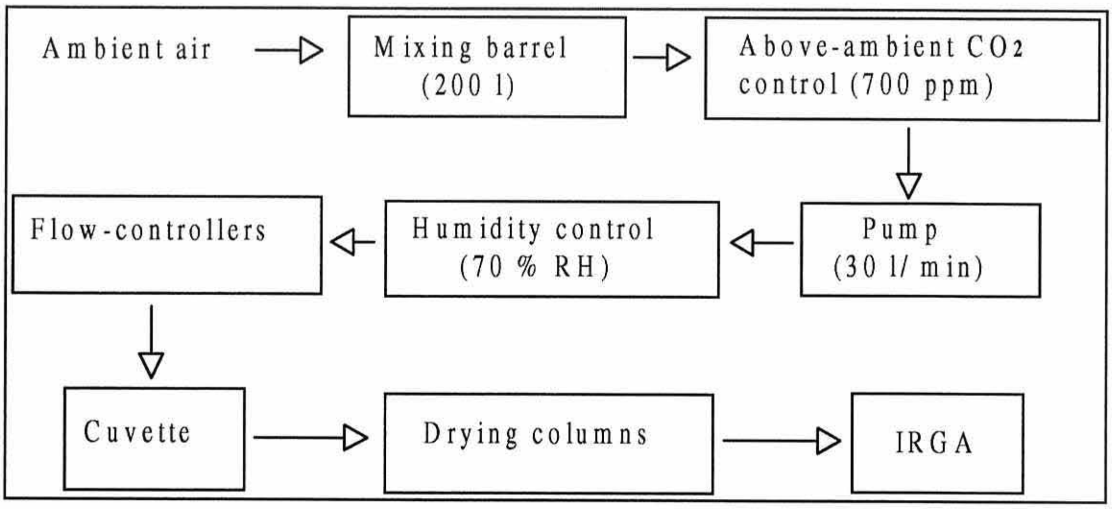

Figure 2.4. Layout of a system designed to measure gas exchange in whole plants at ambient or above-ambient C_a. Ambient air is drawn using a pump through a mixing barrel and then through a CO_2 control system before being pushed through a humidity control system and into a combined shoot and root cuvette at known flow-rates. The [CO_2] of the air-stream leaving the cuvette is sampled and determined using an infra-red gas analyser (IRGA) after removal of water vapour in drying columns.

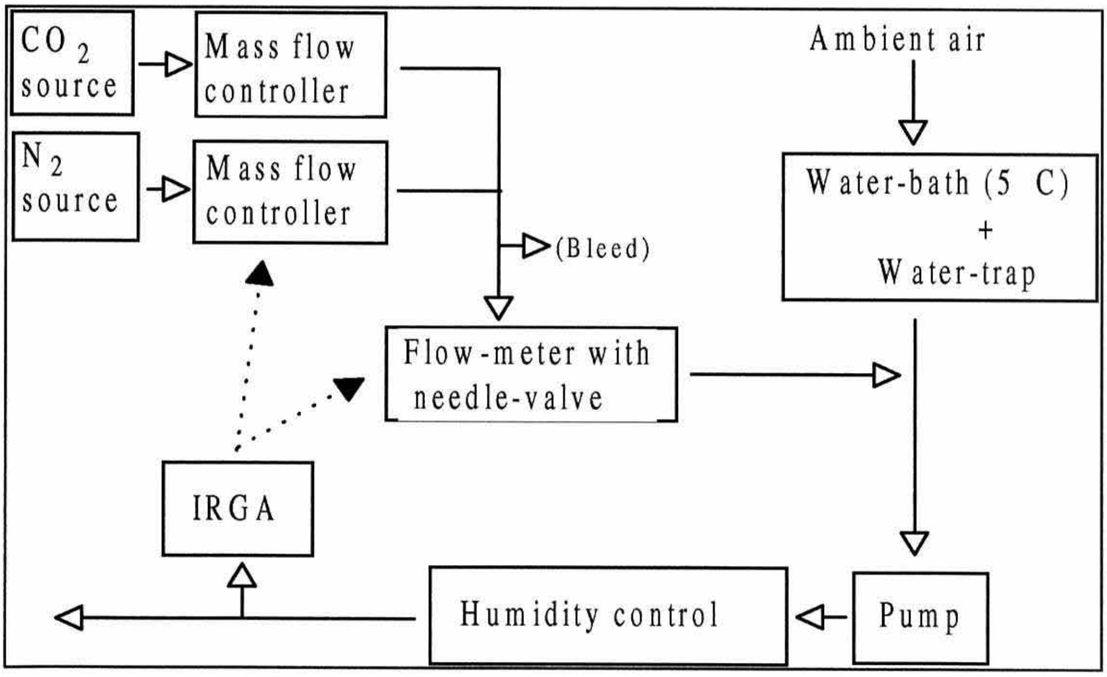

Figure 2.5. Layout of the CO_2 control in a system designed to measure gas exchange in whole plants at ambient or above-ambient C_a. Ambient air is drawn through a water trap cooled to 5 °C in a water-bath so that air is at approximately constant temperature and water vapour pressure independent of atmospheric conditions. After diluting with N_2, CO_2 is introduced into the air-stream at a controlled rate and the [CO_2] in the air-stream is determined using a portable infra red gas analyser (IRGA) following controlled humidification.

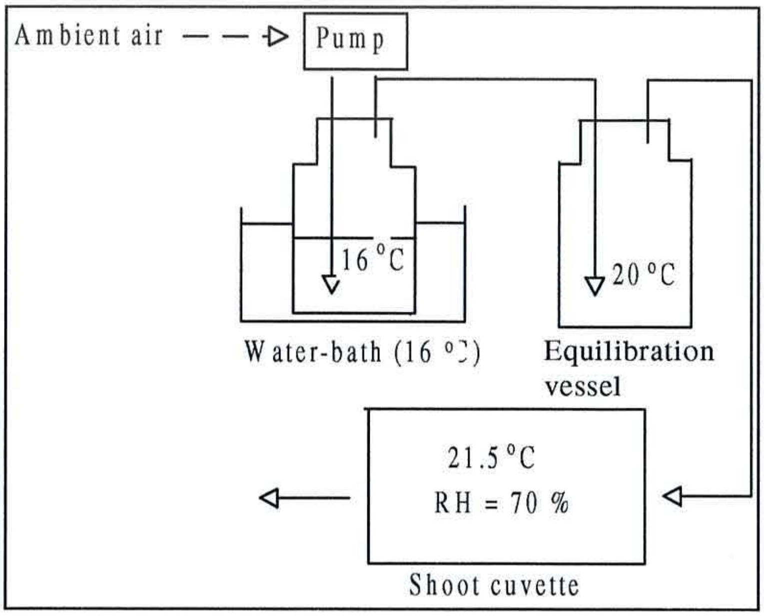

Figure 2.6. Layout of the relative humidity (RH) control in a system designed to measure gas exchange in whole plants at ambient or above-ambient C_a. Dried ambient or CO_2-enriched air at 5 °C is saturated with water vapour by passing the air through water held at 16 °C in a vessel placed in a water-bath. The humidified air is then allowed to warm to constant room temperature (20 °C) in an equilibration vessel and then to 21.5 °C inside the cuvette to give 70 % RH.

Figure 2.7. Cuvette used for measuring gas exchange of intact shoots and roots of *U. urens*.

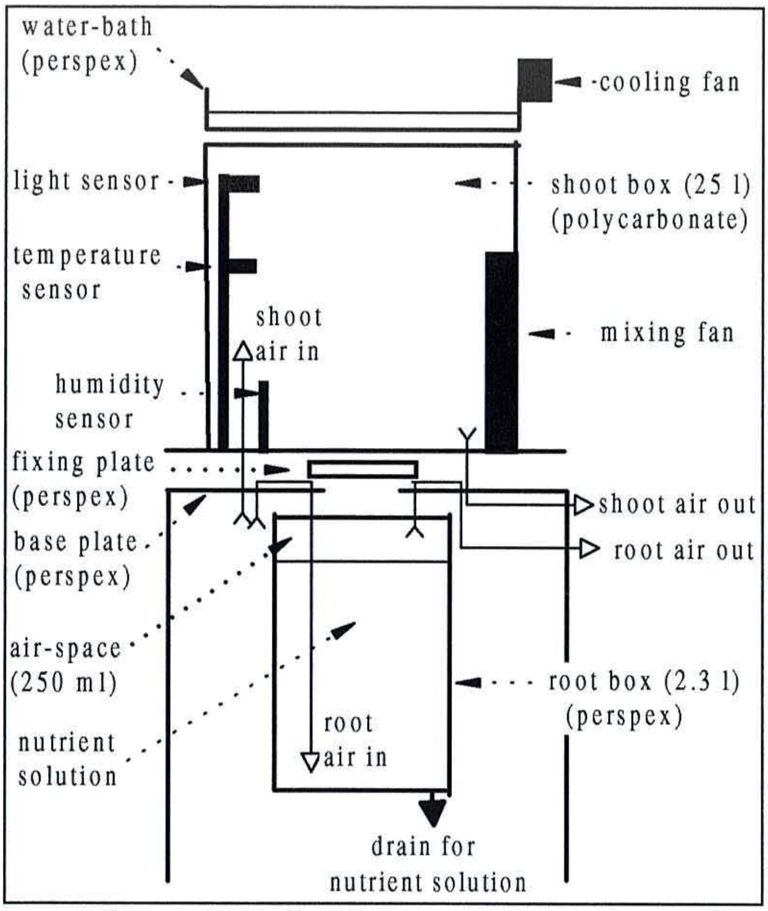

Figure 2.8. Diagram of a cuvette designed to measure gas exchange of intact shoots and roots. See text for description.

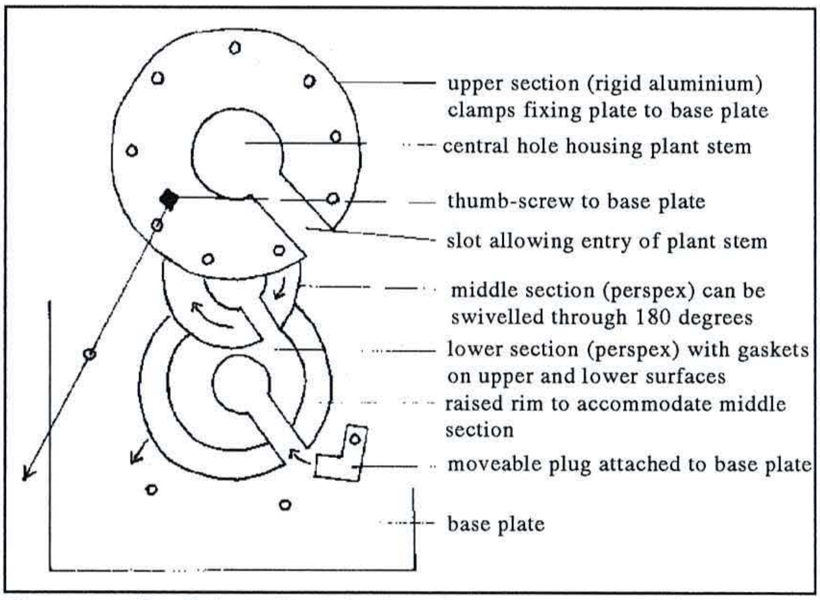

Figure 2.9. Diagram of a fixing plate designed to create a gas-tight seal between intact shoots and roots, thus enabling separate measurements of gas exchange in a cuvette previously illustrated (Figs. 2.7 and 2.8). The fixing plate has three sections that can be securely clamped to the base plate of the cuvette's shoot compartment. Closed-cell rubber gaskets effectively seal the fixing plate as a unit to the base plate. Each section has a slot and central hole to accomodate a plant stem, and gaps that remain around the stem are sealed with a suitable putty.

2.7. Measurement of non-structural carbohydrates

2.7.1. Extraction of soluble carbohydrates and starch

Soluble carbohydrates were extracted after Farrar and Farrar (1985). Plant material (up to 2 cm^2 leaf area and ca. 0.2 g dry weight) was incubated sequentially in 5 ml 95 % (v/v) ethanol for 1 h at 80 °C, 5 ml 95 % (v/v) ethanol for 19 h at 60 °C, and 5 ml deionized water for 5 h at 25 °C. Extracts were decanted, combined and made up to 20 ml with 50 % (v/v) ethanol. Later analysis of additional extractions (5 ml 50 % (v/v) ethanol for 6 h at 60 °C) of leaf and root material showed that 98 - 99 % of soluble carbohydrate was extracted during the previous extractions.

Starch was precipitated with iodine after extraction in perchloric acid according to the method of Lustinec *et al.* (1983). Leaf material remaining after extraction of soluble carbohydrates was homogenized in 2 ml 32 % (v/v) perchloric acid and the homogenate left to stand for 20 min at room temperature before vacuum filtration. 1 ml of the filtrate was added to 3 ml of a solution containing 0.196 % (w/v) iodine, 0.28 % KI and 3 % (w/v) NaCl, and the mixture left to stand for 30 min at 5 °C. The mixture was then filtered, the first 1 ml under gravity flow and the remainder under vacuum. The filter disk was washed sequentially with (1) a solution containing 0.84 % (w/v) iodine, 1.2 % (w/v) KI and 3.2 % (v/v) perchloric acid, (2) a solution containing 67.2 % (v/v) ethanol and 2 % (w/v) NaCl, and (3) a solution containing 67.2 % (v/v) ethanol and 1 % (w/v) NaOH. The washed disk was then placed in 5 ml 0.75 M H_2SO_4 and left for 30 min at 100 °C. The extract was decanted and made up to 10 ml with 0.75 M H_2SO_4.

2.7.2. Colorimetric measurement of total soluble carbohydrates and starch

Total soluble carbohydrates and starch were quantified spectrophotometrically according to the method of Dubois *et al.* (1956). 1 ml 5 % (w/v) phenol and 5 ml concentrated H_2SO_4 were mixed vigorously with a 1 ml aliquot (diluted if necessary) of carbohydrate extract, containing < 200 μg carbohydrate, in heat-resistant glass tubes. The mixture was allowed to cool for 30 min, after which absorbance was read at 490 nm against an ethanol, water or filter disk/0.75 M H_2SO_4 blank. Standard curves were prepared using sucrose (for ethanol and water extracts) or glucose (for starch extracts) at concentrations between 10 and 200 μg ml^{-1}. The amount of soluble carbohydrate was measured in sucrose equivalents, and the amount of starch was determined by multiplying the values obtained in glucose equivalents by 0.9.

2.7.3. Measurement of total and specific soluble carbohydrates by HPLC

Species of ethanol- and water-soluble carbohydrates were isolated and quantified by high performance liquid chromatography (HPLC) (Cairns and Pollock, 1986) using an Aminex HPX 87C ion-exchange column (Bio-Rad, UK) and Shimadzu refractive index detector. Inulin, stachyose, raffinose, sucrose, glucose, fructose and mannitol were used as standards, Measurements were made over a 20 min retention time and peaks were integrated using the Valuchrom software programme (Bio-Rad, UK).

Extracts of soluble carbohydrates, obtained as described in Section 2.7.1, were prepared for HPLC as follows: 3 ml of extract was dried down overnight in a centrifugal evaporator (Speed-Vac Concentrator, Savant) under vacuum supplied by a freeze-dryer (Modulyo, Edwards, Sussex, UK). The residue was resuspended in 200 μl deionized water and passed through an ion-exchange column consisting of two ca. 100 μl layers of ion-exchange resins (Amberlite cation and anion exchange resins, Sigma Chemical Co., Dorset, UK) suspended above a glass wool plug within a 1 ml capacity pipette tip. The column was flushed with 50 μl deionized water and any retained eluent expressed by centrifugation for 1 min at 6,500 rpm; this process was repeated with a further 500 μl water. After drying down the deionized eluent by centrifugal evaporation, the residue was resuspended in 100 μl (ethanol extracts) or 60 μl (water extracts) deionized water and passed, by means of centrifugation for 2 min at 6,500 rpm, through filter units with a 0.2 μm pore-size. An aliquot of the filtrate was then overloaded into a 20 μl capacity loop and allowed to pass through the Aminex exchange column. Degassed, deionized water at 85 °C was used as the carrier solvent at a flow rate of 0.6 ml min^{-1}.

2.8. Measurement of plant organic N

Whole-plant organic N was determined after conversion of organic N to ammonium by acid digestion as described by Allen (1989). Dried plant material was ground in a hammer mill through a 1.5 mm diameter sieve and mixed thoroughly. Dried ground apple leaves were used as internal standards (Standard Reference Material 1515, National Institute of Standards and Technology, Gaithersburg, USA). 50 - 250 mg of ground plant material was placed in Kjeldahl tubes with 4.4 ml of a solution containing 10 M H_2SO_4, 55 % (v/v) H_2O_2 (100 volume 'Analar' grade), 6.9 mM Se and 0.14 M Li_2SO_4 (hydrated 'Analar' grade), and heated using a block digester at 120 °C for 1 h, 180 °C for 2 h, 250 °C for 1 h and finally at 360 °C until the solution cleared. After cooling, each sample was diluted to 50 ml and then 1 ml further diluted to 100 ml.

The ammonium content of the samples was analyzed by cation chromatography using a Dionex 2000i/SP system comprising a CS12 analytical column (Dionex, Camberley, UK) with 20 mM methane sulphonic acid as eluent, and using conductivity detection with auto self-regenerating suppression. Standards were prepared with NH_4Cl. The recovery of organic nitrogen from internal standards was 97 % ± 6 standard deviations.

2.9. Measurement of soluble proteins

2.9.1. Extraction of soluble proteins

1.9 cm^2 of frozen leaf material was rapidly homogenized in 1 ml ice-cold extraction medium consisting of 50 mM HEPES-KOH (pH 7.4), 5 mM $MgCl_2$, 5 mM $NaHCO_3$, 1 mM ethylenediaminetetraacetic acid, 1 mM ethyleneglycol-bis(ß-amino-ethyl ether)N,N'-tetraacetic acid, 10 % (v/v) glycerol, 0.1 % (v/v) Triton X-100, 2 mM benzamidine, 2 mM ε-aminocaproic acid, 5.7 mM phenylmethylsulphonylfluoride, and 65 mM dithiothreitol (Quick *et al.*, 1991).

2.9.2. Measurement of total souble protein

Total soluble protein was quantified spectrophotometrically according the method of Bradford (1976). 50 μl of supernatant from the centrifuged crude extract was diluted with 50 μl extraction medium and mixed with 5 ml assay solution consisting of 0.01 % (w/v) Coomassie Brilliant Blue G, 4.75 % (v/v) ethanol, and 10 % (v/v) orthophosphoric acid. The reaction mixture was left for 10 min before reading absorbance at 595 nm against a blank containing 100 μl extraction medium (Model DMS 100 UV-visible Spectrophotometer, Varian Techtron Pty Ltd., Victoria, Australia). Standard curves were prepared using either bovine serum albumin or spinach rubisco (0.1 - 1 mg ml^{-1}).

2.9.3. Measurement of rubisco protein

Rubisco protein was determined after Hibberd *et al.* (1996a) using a densitometer and integrating recorder (Vitatron, Fisons, UK) to scan rubisco large subunit bands isolated by polyacrylamide gel electrophoresis (Laemmli, 1970). 150 μl crude extract was mixed with 50 μl loading buffer containing 1.88 M Tris-HCl (pH 6.8), 76.3 % (v/v) glycerol, 0.14 M sodium dodecyl sulphate, 10 % ß-mercaptoethanol, and 0.02 (w/v) bromophenol blue. The mixture was heated for 2 min at 100 °C before centrifugation for 2 min at 13,000 rpm. 10 μl of supernatant was loaded onto 1 mm thick polyacrylamide mini-gels and run for ca. 60 min at constant voltage (200 V) using a vertical dual-slab electrophoresis cell (Mini-Protean II, Bio-Rad, UK). The running buffer contained 24.8 mM Tris-KOH (pH 8.3), 1.4 % (w/v) glycine and 0.1 % (w/v) sodium dodecyl sulphate. Proteins were concentrated in ca. 1 cm depth of stacking gel containing 5 % (v/v) acrylamide/N,N'-methylene-bis-acrylamide, 0.1 M Tris-HCl (pH 6.8) and 0.01 % (w/v) sodium dodecyl sulphate, and separated in ca. 5 cm depth of resolving gel containing 12.5 % (v/v) acrylamide/N,N'-methylene-bis-acylamide, 0.37 M Tris-KOH (pH 8.8), 0.02 % (w/v) sodium dodecyl sulphate and 14.2 % (w/v) sucrose. The stacking gel was set with 0.07 % (w/v) ammonium persulphate (AMPS) and 0.07 % (v/v) tetramethylethylenediamine (TEMED), and the resolving gel with 0.055 % (w/v) AMPS and 0.055 % (v/v) TEMED.

Small and large sub-units of rubisco ran as the two major bands, and were identified initially by running purified spinach rubisco as a marker (Fig. 2.10). Bands were fixed and stained for 14 h in a solution containing 0.2 % (w/v) Coomassie Brilliant Blue R-250 (Sigma, Dorset), 50 % (v/v) ethanol and 10 % (v/v) acetic acid, and destained for ca. 2 h in a solution containing 20 % (v/v) ethanol and 10 % (v/v) acetic acid. Bands were scanned at 616 nm to determine the areas of the absorption peaks. Standard curves were prepared using spinach rubisco (1 - 5 $\mu g\ \mu l^{-1}$), and the rubisco content of the samples was quantified in terms of the rubisco large sub-unit, which ran as the more clearly-defined band (Fig. 2.10).

The values obtained by this method for rubisco content (Table 5.5) were highly variable, unusually high and almost certainly erroneous, since the amount of rubisco in leaves of C_3 species usually reaches 20 – 25 % of total leaf soluble protein (Evans, 1989). The sources of methodological error were difficult to locate, but may have resided in variablity between gels causing leakage after loading of the rubisco extracts.

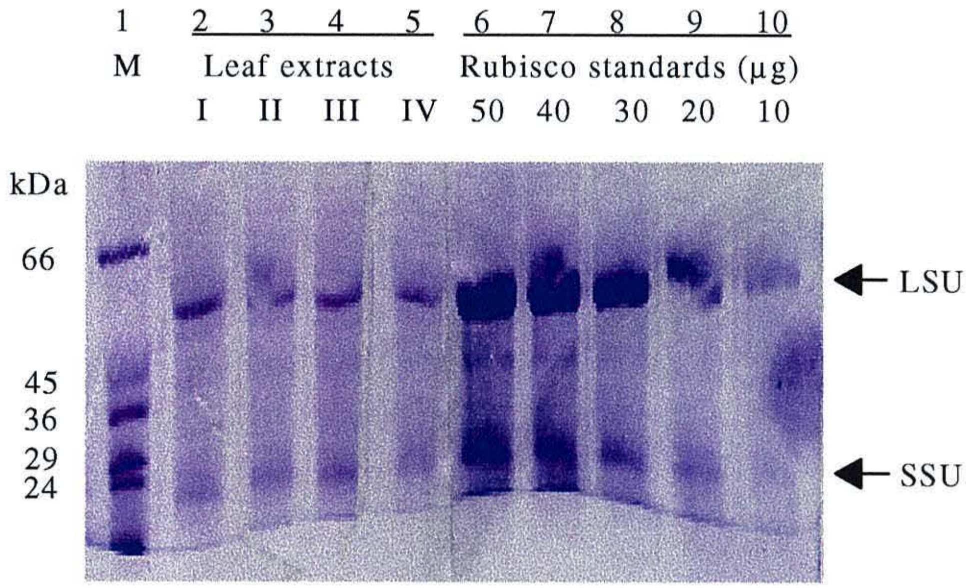

Figure 2.10. Polyacylamide gel showing the large (LSU) and small (SSU) subunits of rubisco protein extracted from leaves of *U. urens*, where each leaf extract (lanes 2 – 5) was taken from 1.9 cm^2 of leaf material. Bands are stained with Coomassie Brilliant Blue dye and can be compared to molecular weight markers (M) (lane 1) and spinach rubisco standards.

2.10. Measurement of chlorophylls and carotenoids

To extract photosynthetic pigments, 1.9 cm^2 of frozen leaf material was rapidly homogenized in 1 ml ice-cold 80 % (v/v) acetone (MacKinney, 1941). After vacuum filtration of the extract, the filtrate was diluted with 80 % (v/v) acetone to produce a final volume of 10 ml. To minimize degradation of pigments, extractions were performed on ice and in dim lighting. Immediately after preparation of the extracts, absorbances over a 1 cm pathlength were read against acetone blanks at 663, 646 and 470 nm (Model DMS 100 UV-visible Spectrophotometer, Varian Techtron Pty Ltd., Victoria, Australia), which allowed quantification ($\mu g\ ml^{-1}$) of chlorophyll *a*, chlorophyll *b* and total carotenoids (xanthophyll and carotenes) according to the equations of Lichtenthaler and Wellburn (1983):

$$\text{Chlorophyll } a = 12.21 * A_{663} - 2.81 * A_{646} \qquad (eqn.\ x)$$

$$\text{Chlorophyll } b = 20.13 * A_{646} - 5.03 * A_{663} \qquad (eqn.\ y)$$

$$\text{Total Carotenoids} = \{(1000 * A_{470}) - (3.27 * eqn.\ x) - (104 * eqn.\ y)\} / 229$$

where A is the absorbance of light at a wavelength specified by numbers in subscript.

CHAPTER 3

Physiological and morphological limitations to growth of *Urtica urens* in elevated CO_2. Distinguishing direct effects of CO_2 from effects of ontogeny and environmental constraint

3.1. INTRODUCTION

An elevated atmospheric CO_2 concentration (elevated C_a) increases the biochemical efficiency of photosynthesis in C_3 plants and hence their potential for growth. Net photosynthesis is increased by CO_2 because carboxylation by rubisco is limited by the current C_a, and because CO_2 competes with O_2 for the same binding sites on rubisco and hence reduces oxygenation and subsequent photorespiratory carbon loss (Bowes, 1991). Theoretical expectations of the initial increase in photosynthesis in individual leaves exposed to twice-ambient C_a range from 20 % if photosynthesis is limited by RuBP supply to 70 % if photosynthesis is limited by the amount and/or activity of rubisco (Stitt, 1991). In practice, the net CO_2 gain by both individual leaves and entire shoot systems will probably lie somewhere between these two extremes, depending on species, environmental conditions and on the extent to which elevated C_a reduces the stomatal conductance (Mott, 1991). By reducing stomatal conductance, elevated C_a could in principle stimulate growth by increasing the ratio of photosynthesis to transpiration (water use efficiency) (Bowes, 1993). Since improvements in water use efficiency probably only promote growth in conditions of drought stress (Bazazz, 1990), this effect of CO_2 will not be considered further here.

The final yield return is, however, invariably less than even the most conservative estimation of net CO_2 gain in twice-ambient C_a. In principle, a proportional and sustained increase in relative growth rate (RGR) of 20 % in a plant growing exponentially at, for example, 0.2 $g\ g^{-1}\ d^{-1}$ should result in an increase in dry weight of over 200 % after 4 weeks. This is considerably greater than the average 41 % increase collated by Poorter (1993) for 131 C_3 species grown in elevated C_a for periods of similar or greater duration.

From such observations, it can be deduced that the stimulation of RGR by elevated C_a is transient, and more detailed growth studies that have considered the time-course of RGR in elevated C_a make it clear that the stimulation of RGR is not only transient, but also occurs in the early stages of exposure (Poorter *et al.*, 1988; Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Fonseca *et al.*, 1996; Stirling *et al.*, 1998). Understanding how and why plants are unable to sustain a higher RGR will not only help to predict plant responses in a future high CO_2 world (IPCC, 1990), but may also target physiological mechanisms that currently limit crop productivity.

Plants grown for days, weeks or months in elevated C_a frequently show potentially growth-limiting changes in physiology and morphology compared with plants grown in ambient C_a when measured at the same point of time. These changes can include the down-regulation of photosynthetic capacity in individual leaves (Long and Drake, 1992; Sage, 1994; Van Oosten *et al.*, 1995; Hibberd *et al.*, 1996a), reductions in the components net assimilation rate (NAR), leaf area ratio (LAR) and specific leaf area (SLA) that partially describe RGR (Acock and Pasternak, 1986; Poorter, 1993; Stulen *et al.*, 1994; Gebauer *et al.*, 1996; Roumet *et al.*, 1996; Stirling *et al.*, 1998), and decreased tissue N concentrations (Garbutt *et al.*, 1990; Stulen *et al.*, 1994). However, because the decline in RGR in elevated C_a may reflect no more than the advancement in time of the usual ontogenetic drift (Poorter *et al.*, 1988; Poorter and Pothmann, 1992; Stirling *et al.*, 1998), there is now concern as to whether any of these responses are actually direct effects of CO_2 or whether they are artifacts arising from accelerated ontogeny or from interactions between accelerated plant size and environmental constraints such as the supply of nutrients, water, light and even physical space (Arp, 1991).

Elevated C_a has been shown to accelerate ontogeny including the development of leaves (Cure *et al.*, 1989) and the onset of flowering (Mortensen, 1987). Other developmental shifts in physiology and morphology will almost certainly be coupled to plant size, which is accelerated by the initial stimulation of RGR. Notably, RGR itself decreases with plant size via changes in both NAR and SLA (Poorter and Pothmann, 1992). The findings of the few studies that have looked specifically at the size-dependency of RGR in elevated C_a are not consistent, with some studies concluding that the decline in the CO_2-stimulation of RGR is at least partly independent of plant size (Fonseca *et al.*, 1996), and others concluding that the stimulation is entirely size-dependent (Poorter *et al.*, 1988; Stirling *et al.*, 1998). In a similar manner to RGR, tissue N concentrations generally decrease with plant size, reflecting either an ontogenetic drift or a constraint of mineral N supply brought about by a greater demand for N in larger plants in elevated C_a (Coleman *et al.*, 1993). Significantly perhaps, RGR is highly sensitive to N supply via effects on NAR, LAR and SLA (Poorter *et al.*, 1990; Pettersson *et al.*, 1993), as are other potentially growth-limiting morphological characteristics such as the shoot:root ratio (Gebauer *et al.*, 1996) which is most often reduced in elevated C_a (Hocking and Meyer, 1991; Rogers *et al.*, 1992).

Both direct effects of CO_2 and effects related to plant size may share a common mechanistic basis mediated by a shift in the balance between assimilate supply and use, which may explain why leaves of plants growing in elevated C_a typically accumulate non-structural carbohydrates (Farrar and Williams, 1991). This accumulation of non-structural carbohydrates may be responsible for photosynthetic down-regulation in elevated C_a via an inhibitory effect of soluble sugars on the expression of genes encoding rubisco and other proteins involved in photosynthesis (Van Oosten *at al.*, 1994), as has been shown in a number experimental systems, such as by feeding glucose to leaves in the transpiration stream (Krapp *et al.*, 1991). Stitt (1991) cites much circumstantial evidence to suggest that the demand by sinks for assimilate plays a central role in regulating growth in elevated C_a by determining the sugar status in leaves and hence the rate of photosynthesis. In this way, reductions in NAR have been explained (Poorter, 1993), although many studies find no evidence of photosynthetic down-regulation in elevated C_a (Stirling *et al.*, 1997). Once again, interactions between plant size and environmental constraints such as mineral N supply (Sage, 1994) or pot size (Arp, 1991) have been implicated in causing the photosynthetic response. An apparent down-regulation of photosynthesis may also be seen if comparisons are made using leaves of different developmental histories related to different plant sizes. For example, the same order leaf of a larger plant may be more prone to self-shading during development and may be at a different developmental stage, with concomitant differences in photosynthetic characteristics (Besford *et al.*, 1985).

In this study, *Urtica urens* L. was exposed to elevated C_a to determine which aspects of growth-related physiology and/or morphology are directly attributable to elevated C_a. Therefore, plants were grown in an experimental system aimed to ensure ample resource supply, and where possible data were analyzed allometrically to account for possible effects related to plant size. Single-leaf photosynthesis was compared using leaves of comparable developmental stages at the individual-leaf and canopy level.

3.2. MATERIALS AND METHODS

3.2.1. Growth conditions

Plants of *U. urens* were grown in a hydroponic culture system in controlled-environment cabinets as described in Section 2.2. Seeds were germinated in ambient C_a, and a proportion of the seedlings received elevated C_a (680 μmol mol^{-1}) immediately after suspension in nutrient solution (12 days after sowing and with a seedling dry weight of approximately 0.8 mg). Exposure to elevated C_a continued for 26 d. After 5 d of growth in nutrient solution, plants were selected for uniformity of shoot height and leaf development within each CO_2 treatment, and marked for use in time-dependent measurements. A number of plants falling outside this selection criterion were also left to provide additional material for allometric analyses. Henceforth, the day at which exposure to elevated C_a began will be designated as day 0.

3.2.2. Growth analysis

Classical growth analysis was used to describe and quantify growth over the 26 d period. Four plants were harvested at day 0, and four per treatment at days 10, 21 and 26. Plants were separated into main-stem leaves and branch-stem leaves (unfolded leaves > 1 cm length), main-stem and branch stems (including folded leaves, leaves ≤ 1 cm length and inflorescences if present), and roots. Dry weights were determined after oven-drying at 60 °C for at least 48 h. Total leaf areas of main-stem and branch-stems (all leaves > 1 cm length) were measured using a digital image analysis system (Delta-T Ltd, Cambridge, UK).

From the data of dry weight and leaf area the following components of plant growth were calculated for each harvest interval (days 0 - 10, 10 - 21, and 21 - 26) from replicates paired across harvests according to size (Evans, 1972; Hunt, 1978): (1) Whole plant mean relative growth rate, $\overline{RGR}$, (2) Mean net assimilation rate, $\overline{NAR}$, (3) Mean leaf area ratio, $\overline{LAR}$, (4) Mean leaf weight ratio, $\overline{LWR}$, and (5) Mean specific leaf area, $\overline{SLA}$. Also, using data obtained for organic N content per plant, the mean specific absorption rate of N by roots, $\overline{S}AR_N$, was determined (Wellbank, 1962) for the harvest intervals spanning days 10 - 21 and 21 - 26. The respective formulae used for the calculation of these parameters are given in Section 2.3.1.

3.2.3. Photosynthesis in individual leaves

Measurements of net photosynthesis (A) were made at day 26 in the second-youngest fully-expanded main-stem leaf. The approximate stage of leaf expansion was determined using sequential measurements of leaf length and width. The light-saturated rate of photosynthesis (A_{sat}) was measured at 2000 μmol m^{-2} s^{-1} PPFD (approaching light saturation of A; Fig. 2.2), using a combined CO_2/H_2O analysis system and clamp-on leaf cuvette (CIRAS-1, PP Systems, Hitchin, Herts., UK) as described in Section 2.5.1. Regardless of the C_a experienced by plants during growth, measurements of A_{sat} were made at both ambient and 680 μmol mol^{-1} C_a to give A_{sat} at the C_a of growth and also allow calculation of the percent change in A_{sat} due to elevated C_a during measurement. A_{sat} was also measured at a range of intercellular CO_2 concentrations (C_i) between 50 and 1300 μmol mol^{-1} for the construction of A/C_i curves (Section 2.5.2). The maximum rate of carboxylation limited by the amount and/or activity of rubisco ($V_{c,max}$) and the maximum attainable rate of photosynthesis at saturating light intensity and C_i (A_{max}) were derived from these curves following the approach of McMurtrie and Wang (1993), as described fully in Section 2.5.2.

3.2.4. Organic N

Whole-plant organic N concentration was determined after conversion of organic N to ammonium by acid digestion (Allen, 1989), as described in Section 2.8. Dried, ground leaf, stem and root material from harvests made at days 10, 21 and 26 were combined and mixed thoroughly to ensure even representation in sub-samples.

3.2.5. Non-structural carbohydrates (soluble sugars and starch)

Four 0.5 cm^2 leaf disks were cut from a range of fully-expanded main-stem leaves and four from a range of furthest-expanded branch-stem leaves (where present) at days 10, 21 and 26. Leaf disks were rapidly frozen in liquid N_2 and stored at -20 °C for subsequent determination of soluble sugars and starch, as described in Section 2.7. Soluble sugars were extracted (Farrar and Farrar, 1985) from frozen leaf material (total area per sample = 2 cm^2), having first been dried for 24 h in a vacuum desiccator (Modulyo, Edwards, Sussex, UK) for dry weight determination. Starch was precipitated with iodine after extraction in perchloric acid (Lustinec *et al.*, 1983). Total soluble sugars and starch were then quantified spectrophotometrically (Dubois *et al.*, 1956).

3.2.6. Allometric analysis

Allometric analysis (Pearsall, 1927; Troughton, 1955) was used to distinguish between direct effects of elevated CO_2 and effects related to plant size, as described in Section 2.4. Geometric mean regressions (GMRs) were fitted to logarithmically-transformed data:

$$\log_e \mathrm{y} = \log_e b + v \log_e \mathrm{x}$$

where y and x were data of dry weight (including and excluding TNC in the case of leaves), leaf area and organic N content. The GMR slope (v) was taken to represent the allometric coefficient (Ricker, 1984; Farrar and Gunn, 1996) and the constant b expresses the regression intercept of y when x is zero. To account for possible shifts in allometric relations, a number of plants growing in ambient C_a were left for harvesting after day 26 at a size roughly comparable to that of plants growing in elevated C_a at that time.

3.2.7. Statistical analyses

For allometric analyses, differences in v, displacement of the GMR line (elevation), and deviation of v from unity were tested for statistical significance using Student's t-test as described by Zar (1989). Two-way (CO_2 and time) analysis of variance followed by Tukey-tests were used to test for significant differences in $\overline{R}GR$ and its components, non-structural carbohydrate concentration and organic N concentration. Significant differences in photosynthetic responses were tested by Student's t-test. All statistical analyses were performed using the computer software package SPSS (Prentice Hall, New Jersey).

3.3. RESULTS

3.3.1. Plant growth

An increase in the dry weight per plant was evident in elevated C_a after 3 weeks, with an increase of about 100 % occurring primarily between 10 and 21 d; the increase was of a similar magnitude 5 d later at final harvest (Fig. 3.1.A). Hence, $\overline{RGR}$ was increased transiently, over only the 10 - 21 d harvest interval (Table 3.1). The leaf area per plant was also increased by elevated C_a, but the increase was only significant at day 26 (Fig. 3.B). Table 3.1 shows that $\overline{RGR}$ in elevated C_a was augmented by increases in $\overline{NAR}$ but constrained by reductions in $\overline{LAR}$ due to reductions in $\overline{SLA}$ rather than $\overline{LWR}$. The 30 % stimulation of $\overline{RGR}$ in elevated C_a resulted from a 100 % stimulation of $\overline{NAR}$ offset by a 30 % reduction in $\overline{LAR}$, whilst the subsequent decline in $\overline{RGR}$ resulted from a much-diminished stimulation of $\overline{NAR}$ of 30 % with a 40 % reduction in $\overline{LAR}$. $\overline{SAR}_N$ was unchanged by in elevated C_a over the 10 - 21 day harvest interval, but was significantly reduced over the final harvest interval (Table 3.1).

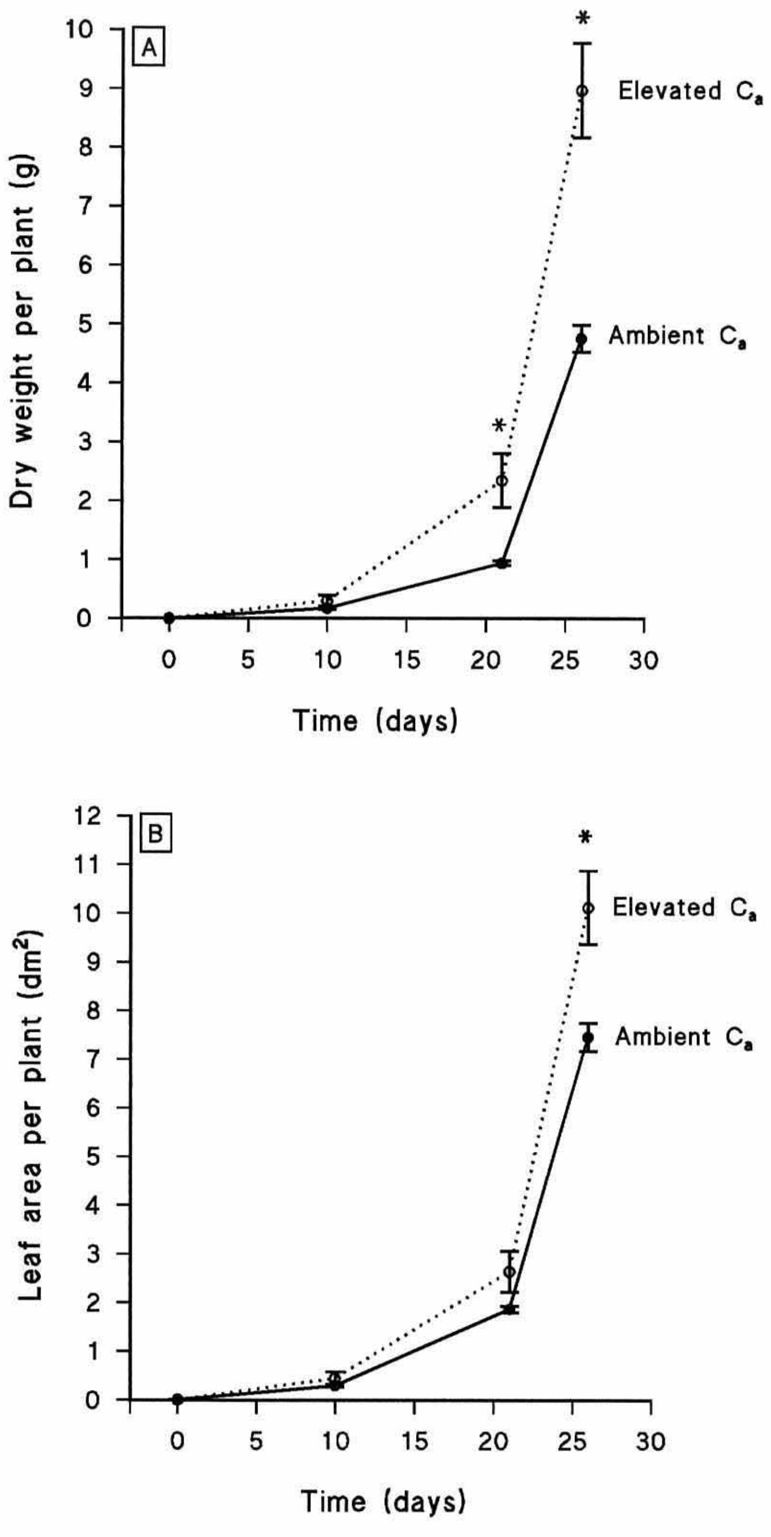

Figure 3.1. (A) Total dry weight and (B) Total leaf area of *U. urens* during 26 d of growth in ambient (ca. 350 μmol mol^{-1}) or elevated (680 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were 12 d old. Data are shown as means (n = 4) with standard error bars. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks.

Table 3.1. Classical analysis of growth of *U. urens* during 26 d in ambient (ca. 350 $\mu mol\ mol^{-1}$) or elevated (680 $\mu mol\ mol^{-1}$) C_a, showing $\overline{RGR}$ (mean relative growth rate), $\overline{NAR}$ (mean net assimilation rate), $\overline{LAR}$ (mean leaf area ratio), $\overline{LWR}$ (mean leaf weight ratio) and $\overline{SLA}$ (mean specific leaf area) over three harvest intervals, and also $\overline{SAR}_N$ (the mean specific absorption rate of N by roots) over two harvest intervals. Exposure to elevated C_a began at day 0 when plants were 12 d old. Data are shown as means (n = 4) ± standard errors. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks.

	HARVEST INTERVAL (DAYS)					
	0 - 10		**10 - 21**		**21 - 26**	
	Ambient C_a	Elevated C_a	Ambient C_a	Elevated C_a	Ambient C_a	Elevated C_a
$\overline{RGR}$ ($g\ g^{-1}\ d^{-1}$)	0.534 (±0.015)	0.584 (±0.027)	0.155 (±0.010)	0.203 (±0.014) *	0.324 (±0.004)	0.277 (±0.023)
$\overline{NAR}$ ($g\ m^{-2}\ d^{-1}$)	24.0 (±0.91)	29.4 (±2.12)	8.19 (±0.37)	15.8 (±1.01) *	18.9 (±0.42)	24.3 (±1.73) *
$\overline{LAR}$ ($m^2\ g^{-1}$)	0.046 (±0.0025)	0.039 (±0.0027)	0.019 (±0.0004)	0.013 (±0.0002) *	0.018 (±0.0003)	0.011 (±0.0003)*
$\overline{LWR}$ ($g\ g^{-1}$)	0.541 (±0.009)	0.551 (±0.006)	0.592 (±0.007)	0.611 (±0.003)	0.573 (±0.005)	0.568 (±0.004)
$\overline{SLA}$ ($m^2\ g^{-1}$)	0.081 (±0.0067)	0.079 (±0.0066)	0.031 (±0.0009)	0.022 (±0.0067) *	0.031 (±0.0006)	0.020 (±0.0003)*
$\overline{SAR}_N$ ($mg\ g^{-1}\ d^{-1}$)	-	-	35.6 (±2.8)	38.2 (±2.6)	71.3 (±2.4)	57.4 (±4.9) *

3.3.2. Photosynthesis in individual leaves

Measurements of photosynthesis in the second-youngest fully-expanded main-stem leaf are shown in Table 3.2. After 26 d in elevated C_a, the light-saturated rate of photosynthesis (A_{sat}) was increased by about 60 %. Although the percent increase in photosynthesis when photosynthesis measured at ambient C_a was compared with photosynthesis measured at elevated C_a was significantly greater in plants grown in elevated C_a, neither $V_{c,max}$ or A_{max} were significantly affected by elevated C_a.

3.3.3. Organic N

Organic N concentration per plant per unit dry weight (N_P) was consistently reduced in plants grown in elevated C_a (Fig. 3.2), with reductions in the region of 20 % and 10 % statistically significant at days 21 and 26 respectively. The significant reduction in N_P at day 21 but not at day 26 persisted after the subtraction from whole plant dry weight of the weight of leaf total non-structural carbohydrates.

3.3.4. Non-structural carbohydrates

The concentrations per unit area of soluble sugars, starch and total non-structural carbohydrates (TNC) are shown for main-stem leaves, branch-stem leaves and all leaves in Figure 3.3. At day 10, there were no significant differences in the concentrations of soluble sugars, starch or TNC in main-stem leaves. This was the case also for all leaves since branch-stem leaves at day 10 were less than 1 cm in length and therefore not included in the analysis. In leaves of plants grown in ambient C_a, starch was present at day 10 only, and at a concentration of about 2 g m^{-2}, but starch was present at all points of harvest in elevated C_a in both main-stem leaves and branch-stem leaves at respective concentrations of about 8 and 3 g m^{-2} at day 21, and 4 and 1 g m^{-2} at day 26.

At days 21 and 26, the concentrations of soluble sugars in elevated C_a were significantly higher in all leaves. The accumulation of soluble sugars was greater in main-stem leaves than in branch-stem leaves in both ambient and elevated C_a, but the increases due to elevated C_a were most pronounced in main-stem leaves, amounting to more than 100 %.

The increases in starch and soluble sugars so far described meant that TNC concentrations in elevated C_a were significantly higher at days 21 and 26 in main-stem leaves, branch-stem leaves and all leaves.

3.3.5. Specific leaf area at individual harvests

SLA calculated at individual harvests was consistently reduced in elevated C_a, and the decrease was statistically significant at days 21 and 26 both before and after subtraction of leaf total non-structural carbohydrates (TNC) from leaf dry weight (Fig. 3.4). The reduction in total leaf SLA in elevated C_a (Fig. 3.4.C) was caused by reductions in both main-stem leaf SLA (Fig. 3.4.A) and branch-stem leaf SLA (Fig. 3.4.B), but reduced SLA was most pronounced in branch-stem leaves. In both ambient and elevated C_a, SLA was lower in main-stem leaves than in branch-stem leaves.

Table 3.2. Measurements of photosynthesis in the second-youngest fully-expanded main-stem leaf of *U. urens* after 26 d of growth in ambient (ca. 350 μmol mol^{-1}) or elevated (680 μmol mol^{-1}) C_a, showing the light-saturated rate of photosynthesis (A_{sat}), the percent increase in A_{sat} when measured at both ambient and elevated C_a, the maximum rate of carboxylation ($V_{c,max}$), and the maximum attainable rate of light and CO_2-saturated photosynthesis (A_{max}). Data are shown as means (n = 4) ± standard errors. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks.

	Ambient C_a	Elevated C_a
A_{sat} (μmol CO_2 m^{-2} s^{-1})	27.1 (±0.6)	43.6 (±0.9) *
% increase in A_{sat} measured at elevated C_a	58.9 (±2.1)	67.7 (±2.3) *
$V_{c,max}$ (μmol CO_2 m^{-2} s^{-1})	115.6 (±4.0)	100.8 (±5.4)
A_{max} (μmol CO_2 m^{-2} s^{-1})	59.2 (±1.4)	60.4 (±1.8)

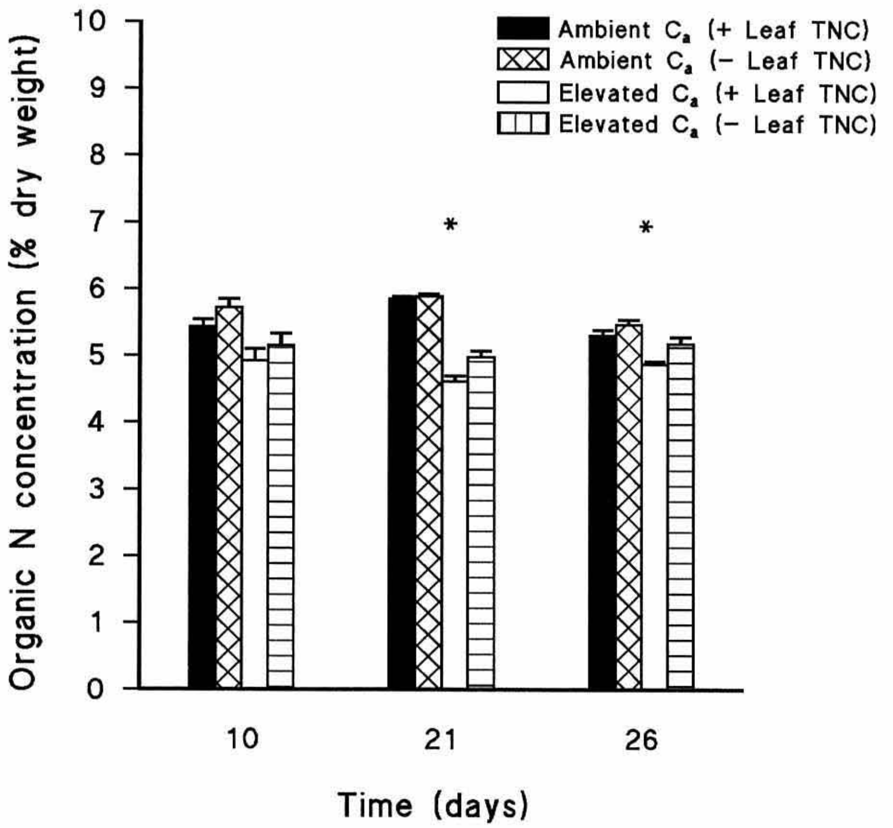

Figure 3.2. Organic N concentration in whole plants of *U. urens* after 10, 21 and 26 d of growth in ambient (ca. 350 μmol mol^{-1}) or elevated (680 μmol mol^{-1}) C_a. N concentration was calculated as a percentage of the dry weight including and excluding the weight of leaf total non-structural carbohydrates (+/- leaf TNC). Data are shown as means (n = 4) ± standard error bars. Significant differences ($p < 0.05$) due to C_a (+ leaf TNC only) are indicated by asterisks. See text for further statistical comparisons.

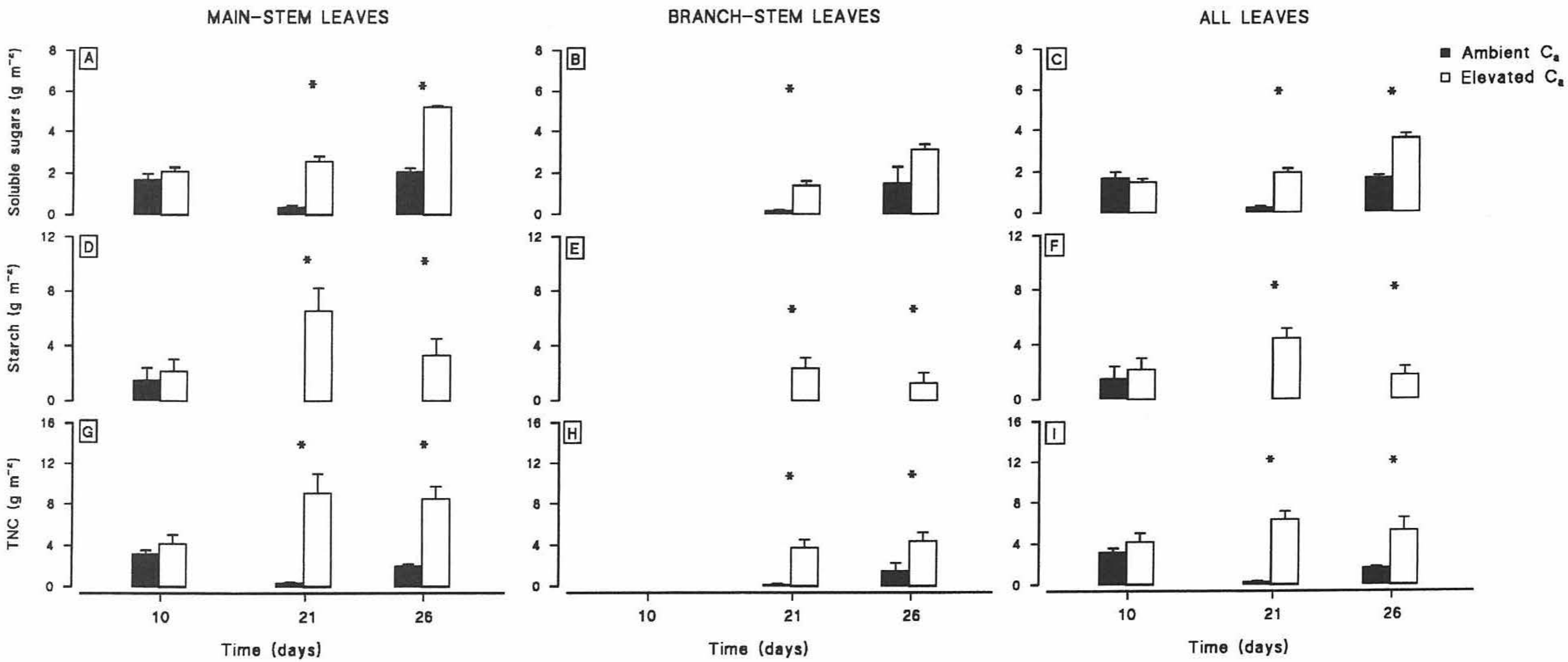

Figure 3.3. Concentrations of (A,B,C) Soluble sugars, (D,E,F) Starch and (G,H,I) Total non-structural carbohydrates (TNC) in (A,B,C) Main-stem leaves, (D,E,F) Branch-stem leaves and (G,H,I) All leaves of *U. urens* after 10, 21 and 26 d of growth in ambient (ca. 350 µmol mol^{-1}) or elevated (680 µmol mol^{-1}) C_a. Data are shown as means (n = 4) ± standard error bars. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks.

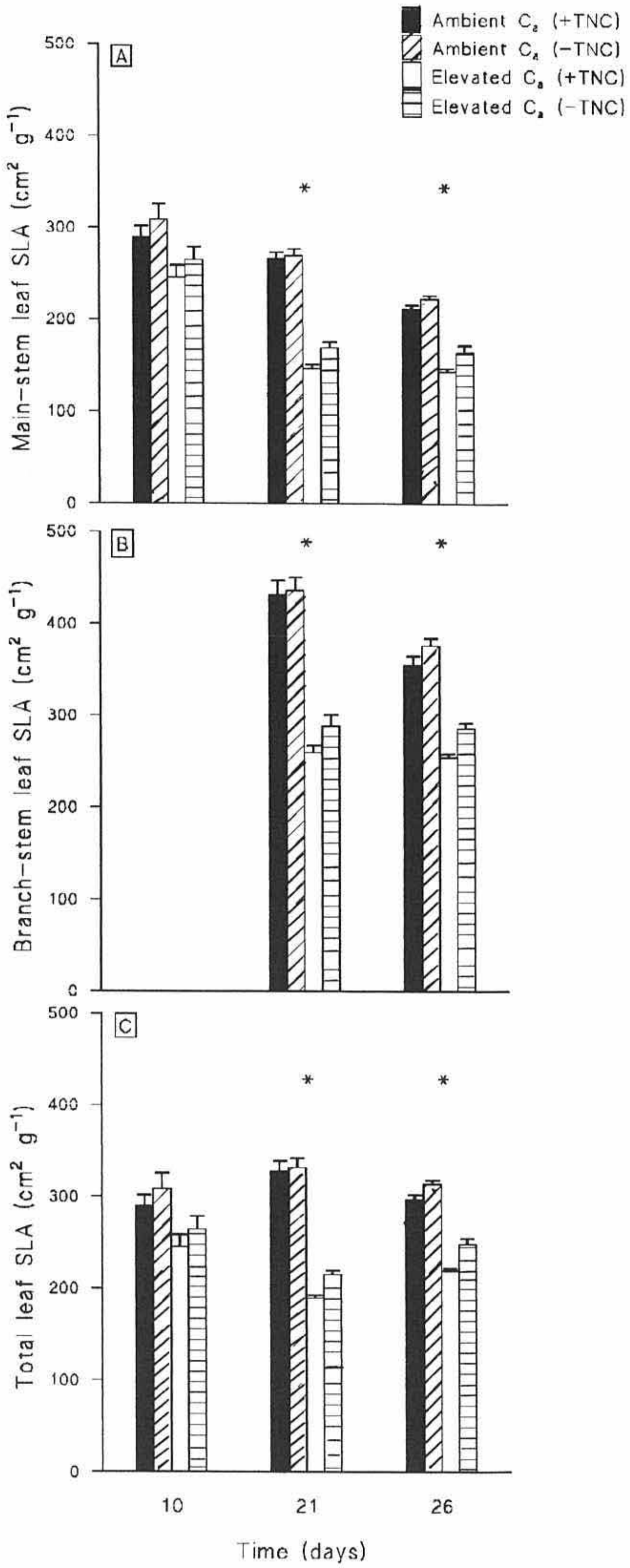

Figure 3.4. Specific leaf area (SLA) in (A) Main-stem leaves, (B) Branch-stem leaves and (C) All leaves of *U. urens* after 10, 21 and 26 d of growth in ambient (ca. 350 µmol mol^{-1}) or elevated (680 µmol mol^{-1}) C_a. SLA is calculated including and excluding the weight of total non-structural carbohydrates (+/- TNC). Significant differences ($p < 0.05$) due to C_a are indicated by asterisks. See text for futher statistical comparisons.

3.3.6. Allometric relationships

Values of the allometric coefficient (v), the relative displacement of the geometric mean regression (GMR) line (elevation), and the significance of any deviation of v from unity are shown in Table 3.3. Elevated C_a resulted in significant reductions in the elevations of total leaf area against plant dry weight (allometric LAR), leaf area against leaf dry weight *and* leaf structural dry weight (allometric SLA), and organic N content against plant dry weight (allometric tissue N concentration). The actual GMR lines describing SLA and N concentration are illustrated in Figures 3.5 and 3.6 respectively. However, elevations were unchanged in regressions of total leaf dry weight against plant dry weight (allometric LWR), branch-stem leaf area *against* main-stem leaf area, and shoot dry weight against root dry weight (allometric S/R). Changes in v occurred in some allometric SLA relations, and in the allometric S/R where v was significantly reduced in elevated C_a. The GMR line describing S/R can be seen in Figure 3.7. Ontogenetic shifts, as indicated by deviations of v from unity, were evident as size-dependent reductions in LAR, LWR and S/R, but not in total leaf SLA or tissue N concentration .

Table 3.3. Allometric relations in *U. urens* grown in ambient (ca. 350 µmol mol^{-1}) or elevated (680 µmol mol^{-1}) C_a for 26 d, showing the results of analysis of geometric mean regressions (GMRs) describing logarithmically-transformed variables (x, y) of leaf area, dry weight (DW), structural dry weight (SDW) and organic N content. The table shows the GMR slope (*v*, the allometric coefficient) with the coefficient of determination (r^2) for each GMR in parenthesis, the relative elevation of the GMR line (↑, ↓ and = denoting an upwards, downwards and no significant displacement of the GMR line), and whether *v* deviates significantly from unity (slope ≠ 1). SDW is DW minus the weight of total non-structural carbohydrate (TNC). *, ** and *** indicate significant differences due to C_a at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively; ns indicates that differences are not significant.

Variable		GMR slope (*v*)		Elevation		Slope ≠ 1	
$\log_e$ y	$\log_e$ x	350	680	350	680	350	680
Total leaf area (cm^2)	Plant DW (mg)	0.956 (0.995)	0.912 (0.997) ns	↑	↓ ***	*	***
Total leaf area (cm^2)	Total leaf DW (mg)	1.000 (0.998)	0.956 (0.995) ns	↑	↓ ***	ns	ns
Main-stem leaf area (cm^2)	Main-stem leaf DW (mg)	0.861 (0.996)	0.762 (0.988) **	↑	↓ ***	***	***
Branch-stem leaf area (cm^2)	Branch-stem leaf DW (mg)	0.902 (0.998)	0.987 (0.998) **	↑	↓ ***	***	ns
Total leaf area (cm^2)	Total leaf SDW (mg)	0.986 (0.999)	0.971 (0.995) ns	↑	↓ ***	ns	ns
Main-stem leaf area (cm^2)	Main-stem leaf SDW (mg)	0.847 (0.997)	0.785 (0.986) ns	↑	↓ ***	ns	ns
Branch-stem leaf area (cm^2)	Branch-stem leaf SDW (mg)	0.921 (0.998)	0.994 (0.996) *	↑	↓ ***	***	ns
Branch-stem leaf area (cm^2)	Main-stem leaf area (cm^2)	1.999 (0.981)	2.077 (0.914) ns	=	=	***	***
Total leaf DW (mg)	Plant DW (mg)	0.956 (0.999)	0.955 (0.999) ns	=	=	**	**
Shoot DW (mg)	Root DW (mg)	1.082 (0.998)	0.982 (0.997) ***	=	=	***	ns
Plant organic N (mg)	Plant DW (mg)	0.994 (0.999)	0.991 (0.999) ns	↑	↓ ***	ns	ns
Plant organic N (mg)	Plant DW minus leaf TNC (mg)	0.986 (0.999)	0.997 (0.999) ns	↑	↓ ***	ns	ns

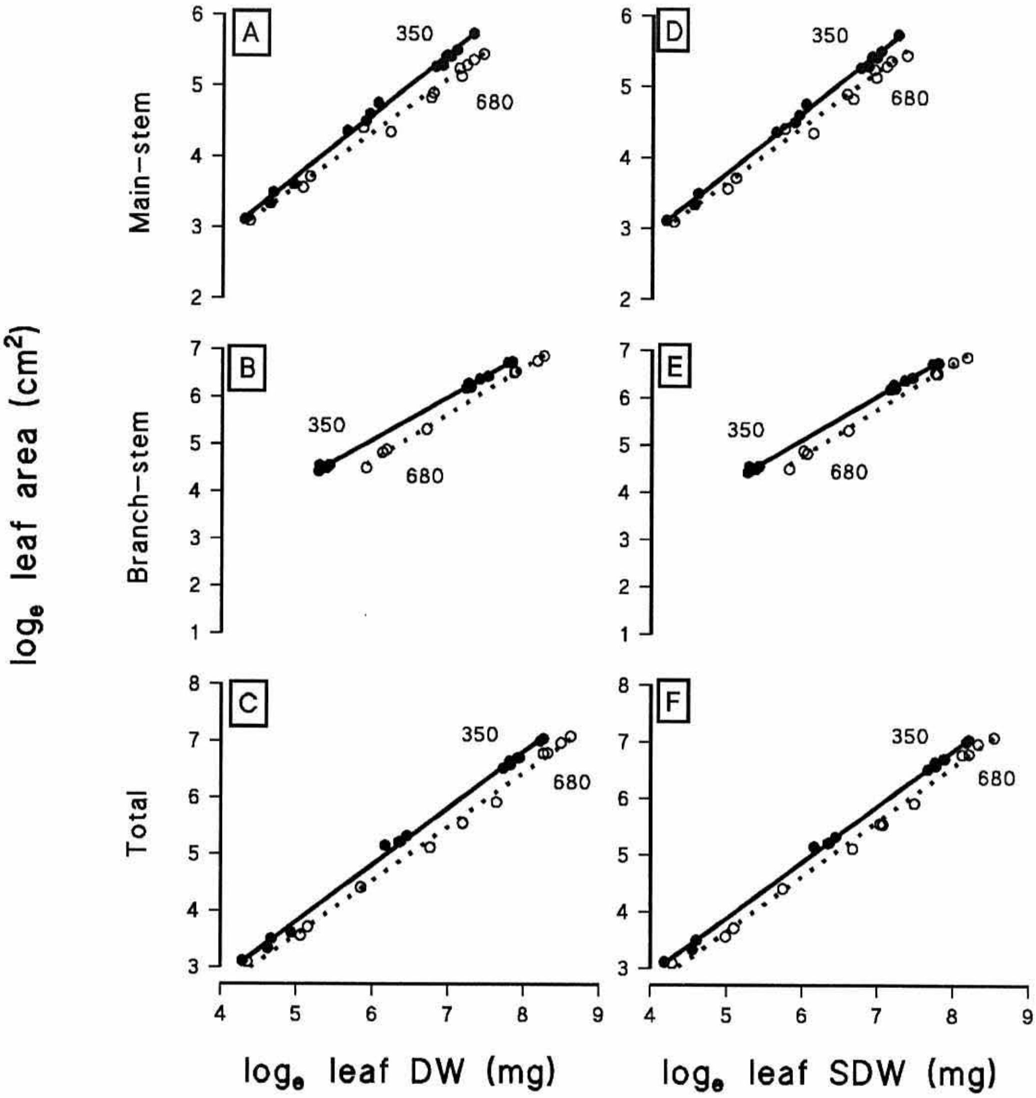

Figure 3.5. Geometric mean regressions (GMRs) of logarithmically-transformed data of leaf area and leaf dry weight (A,B,C) Including (denoted as DW) and (D,E,F) excluding (denoted as SDW) total non-structural carbohydrates in leaves of *U. urens* grown for 26 d in ambient (ca. 350 μmol mol^{-1}) or elevated (680 μmol mol^{-1}) C_a. Relationships are shown for main-stem leaves (A,D), branch-stem leaves (B,E) and all leaves (C,F). The GMR slope (v), its relative elevation, and its coefficient of determination (r^2) are given in Table 3.3.

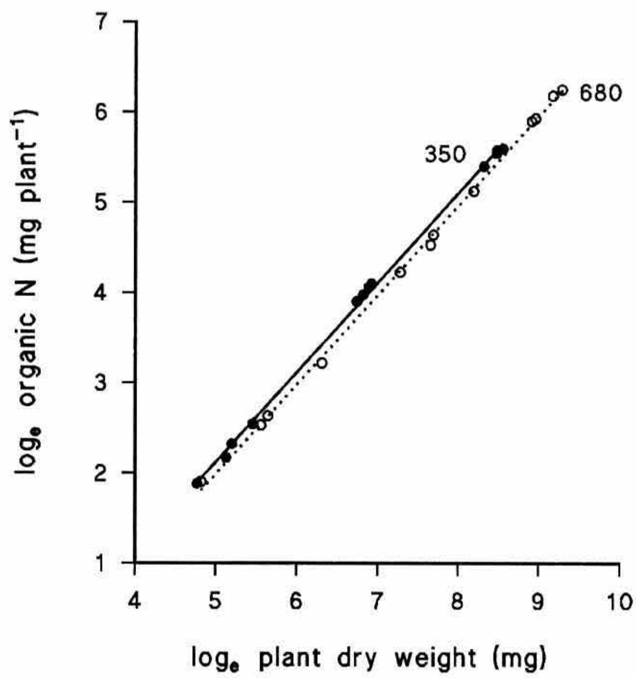

Figure 3.6. Geometric mean regression (GMR) of logarithmically-transformed data of organic N content of whole plants of *U. urens* grown for 26 d in ambient (ca. 350 µmol mol^{-1}) or elevated (680 µmol mol^{-1}) C_a. The GMR slope (v), its relative elevation, and its coefficient of determination (r^2) are given in Table 3.3.

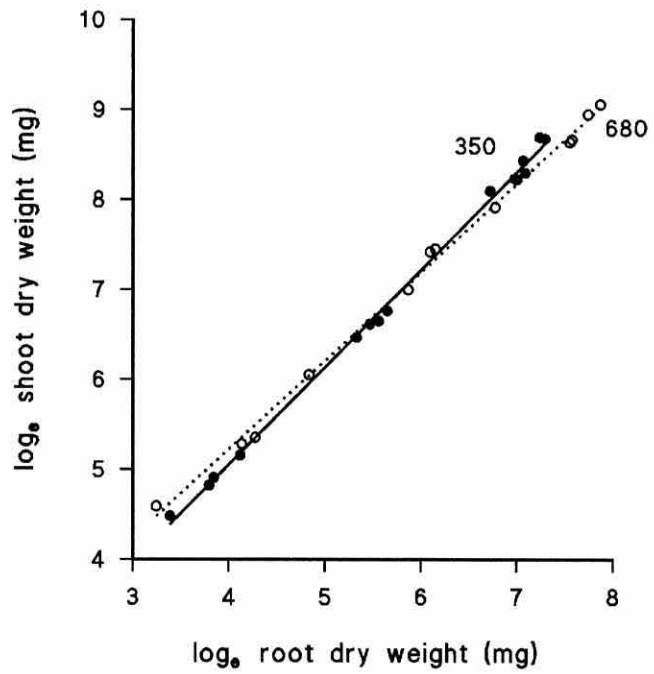

Figure 3.7. Geometric mean regression (GMR) of logarithmically-transformed data of the dry weight of shoots and roots of *U. urens* grown for 26 d in ambient (ca. 350 µmol mol^{-1}) or elevated (680 µmol mol^{-1}) C_a. The GMR slope (v), its relative elevation, and its coefficient of determination (r^2) are given in Table 3.3.

3.4. DISCUSSION

3.4.1. Plant growth

Consistent with studies of other C_3 species (Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Fonseca *et al.*, 1996; Stirling *et al.*, 1998), elevated C_a resulted in only a transient stimulation of RGR in *U. urens*, occurring for an undefined duration at some time between 10 and 21 d (Table 3.1). The increase in dry weight of about 100 % (Fig. 3.1.A) was considerably greater than the average increase of 40 % collated by Poorter (1993) for a range of CO_2-enrichment studies of similar duration involving native herbaceous C_3 species, and contradicts the conclusion of Hunt *et al.* (1991) that native fast-growing annuals are amongst the least responsive to elevated C_a. The differences in the growth responses between these earlier studies and the present study may reflect differences specific to phylogeny or perhaps even soil *against* hydroponic culture systems, since dry weight of the closely related *Urtica dioica* in 700 μmol mol^{-1} C_a was increased by 90 % after 3 weeks of growth in a comparable hydroponic culture system (Den Hertog *et al.*, 1996).

In elevated C_a, RGR was driven by increased NAR but constrained by decreased LAR (Table 3.1). Similar opposing effects of NAR and LAR on RGR have been found before (Gebauer *et al.*, 1996; Roumet *et al.*, 1996; Stirling *et al.*, 1998). More unusually, the stimulation of growth by elevated C_a has been linked primarily to increased LAR rather than NAR (Kramer, 1981; Körner, 1991). In the present study, the early reduction in LAR constrained the initial stimulation of RGR in elevated C_a, and the subsequent decline in RGR was more obviously linked to a reduction in NAR. A similar decline in the magnitude of the CO_2-stimulation of NAR has been noted in studies of other species (Poorter, 1993; Stulen *et al.*, 1994), including *U. dioica* (Stulen *et al.*, 1994). In the present study, however, plants grown in both ambient and elevated C_a showed time- and size-dependent increases in RGR and NAR, at least after the very early high values, which represent a reversal of the trend usually observed in studies of similar duration (Poorter, 1993; Stulen *et al.*, 1994).

3.4.2. Relationship between NAR and photosynthesis

An evaluation of the size-dependency of NAR in elevated C_a requires a higher resolution in time than was available here. Hence, it is not known if the decline in the CO_2-stimulation of NAR here reflected the advancement in time of the usual ontogenetic drift, as has been the conclusion of the few studies that have addressed this question (Poorter *et al.*, 1988). Nevertheless, the decline in the CO_2-stimulation of NAR between 21 and 26 d could not be linked to down-regulation of photosynthetic capacity, at least when measured in the second-youngest fully-expanded main-stem leaf at day 26. The point is clearly demonstrated because the stimulation of photosynthesis due to elevated C_a was not decreased in leaves of plants grown in elevated C_a compared with those grown in ambient C_a (Table 3.2). The cause of the decline in NAR is not clear, but was unlikely to be due to accelerated mutual shading by larger plants in elevated C_a since all plants were managed so that their canopies never overlapped. From observations of plants *in situ*, a possible explanation lies in alterations in the angles of petioles such that light interception is reduced per unit leaf area. In support of these observations, Poorter *et al.* (1988) found an increased total leaf area relative to the projected total leaf area in *Plantago major* grown in elevated C_a. Similar alterations in canopy architecture may also explain the findings of Smart *et al.* (1994), where the attenuation of light through wheat canopies was less rapid in elevated compared with ambient C_a.

The absence of photosynthetic down-regulation here, where care was taken to encourage unrestricted plant growth (Section 2.2) and to compare leaves of similar developmental histories, is consistent with the findings of other studies and the view that its occurrence may be a product of environmental constraints (Arp, 1991; Sage, 1994; Stirling *et al.*, 1997; Harmens *et al.*, 1998) and/or perhaps ontogenetic differences. Nitrogen-limitation in particular has been blamed for many cases of photosynthetic down-regulation (Sage, 1994), and it is perhaps of significance that the present study clearly achieved non-limiting N nutrition. That this was achieved is implicit in the allometric analysis of whole-plant tissue organic N concentration (N_P), whereby the allometric coefficient (v) relating organic N content to dry weight was close to unity in plants grown in both ambient and elevated C_a (Fig. 3.6; Table 3.3). The stimulation of photosynthesis due to elevated C_a was in fact greater in leaves of plants grown in elevated C_a (Table 3.2), but $V_{c,max}$ and

A_{max} did not change in a way which could, in principle, explain the acclimation of photosynthesis (Table 3.2).

3.4.3. Direct and indirect effects of CO_2 on morphology

Consistent with other studies (Acock and Pasternak, 1986; Gebauer *et al.*, 1996; Roumet *et al.*, 1996; Stirling *et al.*, 1998), the decrease in LAR in elevated C_a was entirely due to reductions in SLA rather than LWR (Table 3.1; Fig. 3.4). The reduction in LAR indicates that the fixed carbon available in elevated C_a may not be not used as in ambient C_a to generate the equivalent leaf area. The observed balance between SLA and LWR in elevated C_a suggests that a proportion at least of the fixed carbon is exported to the sites of leaf development, but is subsequently partitioned into weight rather than area. Moreover, the evidence here suggests that fixed carbon is used in leaves for the production of structural weight, since the reductions in SLA persisted after the removal of total non-structural carbohydrates (TNC) (Fig. 3.4). Other studies have also found that TNC can not account entirely for the reduction in SLA in elevated C_a (Acock and Pasternak, 1986; Den Hertog *et al.*, 1996). Accordingly perhaps, Thomas and Harvey (1983) found that decreased structural SLA in elevated C_a was associated with increases in leaf thickness and in the number of cell layers in the leaf. However, there are also cases where the reductions in SLA in elevated C_a can be attributed entirely to the accumulation of TNC (Wong, 1990; Poorter, 1993; Baxter *et al.*, 1994a; Roumet *et al.*, 1996).

The few studies that have looked at SLA allometrically (Gebauer *et al.*, 1996; Stirling *et al.*, 1998) concluded that the reductions in SLA were either entirely ontogenetic, or could be explained by interactions between plant size and environmental constraints, particularly mineral N supply. Other environmental factors perhaps worthy of consideration include the supply of light, which is well-known as a potent variable in affecting SLA (Hart, 1988). For example, the light environment external to the canopies of larger plants may be altered by the steep gradients of light typical within controlled-environment growth cabinets (Chapter 4, Fig.4.1). Similarly, changes in SLA might also reflect alterations in the distribution of light within a larger canopy. On an individual plant level, the internal distribution of light may also explain why SLA in branch-stem leaves was consistently higher than that in main-stem leaves, in both ambient and elevated C_a (Fig. 3.4).

Instances where ontogeny was implicated in the SLA response were based on the finding that the allometric coefficient (*k*, in these cases) was unaltered by elevated C_a. In agreement with those results, the allometric coefficient (*v*, essentially the same as *k* with high correlation coefficients) for the regression of $\log_e$ total leaf area *against* $\log_e$ total leaf dry weight was not significantly affected by elevated C_a (Table 3.3; Fig. 3.5). This insensitivity of *v* describing SLA for all leaves was the outcome of a significantly reduced *v* describing SLA for main-stem leaves but a significantly increased *v* describing SLA for branch-stem leaves. However, the regression describing SLA for all leaves in elevated C_a was significantly displaced downwards (Table 3.3; Fig. 3.5.), which must therefore indicate an undetected reduction in *v* early in the experiment. Previous attempts to analyze early changes in allometric relations have looked for differences in the regression intercept *b* (Troughton, 1955), but this approach has been accorded little or no biological significance because the intercept of a log-log regression is sensitive to units of expression and is inevitably extrapolated beyond the available data set (Kavanagh and Richards, 1941; Stirling *et al.*, 1998). Since in the present study the elevations of regressions were tested for displacement without recourse to their intercepts (Zar, 1989), there is a strong case for concluding that elevated C_a caused a reduction in both non-structural and structural SLA at some very early stage of growth, and, moreover, that elevated C_a continued to affect SLA even after this initial alteration in allometric relations, since a subsequent increase in *v* might be expected otherwise to have restored the regression line to the equivalent elevation found in ambient C_a.

Stirling *et al.* (1998) have drawn attention to the fact that changes in allometric coefficients due to CO_2 are generally seen only in larger plants and species. Such changes may conceivably arise from shifts in allometric relations either inherent in the ontogeny of the species, such as can be caused by the onset of flowering (Troughton, 1956), or indicative that an environmental constraint, such as mineral N supply, has surpassed a critical threshold. As in the present sudy, CO_2-enrichment studies using allometric analysis need to compare data sets that include plants of similar sizes, especially of the largest plants, to properly account for these allometric shifts.

3.4.4. Organic N

Elevated C_a decreased the N concentration in whole plants (N_P) by about 10 - 20 % (Fig. 3.2). Similar reductions in N_P have been widely and consistently reported in other studies (Luo *et al.*, 1994; Stulen *et al.*, 1994; Den Hertog *et al.*, 1996). As was the case for SLA, the regression relating $\log_e$ N content to $\log_e$ plant dry weight in elevated C_a had an unaltered allometric coefficient but a significantly decreased elevation (Table 3.3; Fig. 3.6), again indicating an early decrease in *v* followed by a sustained, albeit smaller, effect on decreasing N_P. This observation contradicts the suggestion of Coleman *et al.* (1993) that decreased N_P in elevated C_a is size-dependent, reflecting either a usual ontogenetic drift or the earlier onset of N limitation. Interestingly, no evidence was found even for an ontogenetic shift in N_P, since the slopes of these regressions did not differ from unity (Table 3.3). This may be a feature of fast-growing herbaceous species during exponential phases of growth, or it could point to general and potentially serious problems with the inadvertent imposition of nutrient limitation under many experimental culture systems.

In some studies, the decrease in tissue N concentrations can be accounted for entirely by dilution by total non-structural carbohydrates (TNC) (Kuehny *et al.*, 1991; Chu *et al.*, 1992), but in others the decrease persists on a structural dry weight basis (Wong, 1990; Den Hertog *et al.*, 1996). Although in the present study it was not known if stems and roots accumulated TNC in elevated C_a, the decrease in tissue N concentration found at single time-points persisted after subtraction of leaf TNC at day 21, but was no longer statistically significant at day 26 (Fig. 3.2). However, allometric analysis of the same sets of data support a conclusion that the reduction in N_P was independent of leaf TNC, since regression elevations remained significantly different after correcting for them (Table 3.3).

There are reasons to expect a reduction in N_P in elevated C_a, based on an increasing understanding of the interdependence of C and N metabolism. Two lines of evidence may be of particular significance, since together they address the primary processes that determine N_P, namely the uptake and assimilation of mineral N. Firstly, Gastal and Saugier (1989) showed that the ratio of nitrate uptake rate to CO_2 assimilation rate ($NO_3^-{}_U$/A) decreases when A is increased above a certain threshold. In their experiment,

$NO_3^-{}_U/A$ remained constant at both 200 and 440 μmol m^{-2} s^{-1} PPFD, but was markedly decreased at 875 μmol m^{-2} s^{-1} PPFD. Further increases in A due to an elevation of C_a had no effect on N_U. These response characteristics held for a period of days until the imposition of the next increment in A, which suggests that longer-term alterations in the C/N ratio due to elevated C_a are at least feasable. Secondly, De Cires *et al.* (1993) demonstrated a negative relationship between the rate of nitrate assimilation rate ($NO_3^-{}_A$) and A in leaves when A was varied by changing both PPFD and C_a. Their measurements of $NO_3^-{}_A$ were based on the extractable nitrate reductase activity immediately after measurements of A, so whether an altered $NO_3^-{}_A/A$ could persist with longer-term exposure to elevated C_a is unclear. Any reduction in $NO_3^-{}_A/A$ when A is increased by C_a would be broadly consistent with the hypothesis and increasing evidence that nitrate and CO_2 assimilatory processes compete for photosynthetically generated reductant (Le Van Quy, 1991).

The physiological significance of decreased tissue N concentrations in elevated C_a is unclear, but it is known that whole plant organic N concentration is strongly and linearly correlated to RGR through its components NAR, LAR and SLA (Poorter *et al.*, 1990; Pettersson *et al.*, 1993). From the study of Petersson *et al.* (1993), where plant N concentration was varied by different relative addidion rates of mineral N using the method of Ingestad and Lund (1986), it can be estimated that a 20 % reduction in N_P was associated with reductions in RGR, NAR and SLA of 35 %, 27 % and 20 % respectively. In particular, leaf expansion is strongly inhibited by an internal N deficiency, and is notably more inhibited than is root extension (James *et al.*, 1993). Changes in N_P may therefore explain the reductions in LAR in elevated C_a, as well as reductions in the shoot:root ratio (S/R) demonstrated here using an allometric approach to its analysis (Table 3.3; Fig. 3.7) and in other studies where ratios have been compared at common points of time (Hocking and Meyer, 1991).

Further evidence for a role of N in the growth responses to elevated C_a can be found in measurements of SAR_N (a crude estimation of the specific uptake rate of mineral N by roots), which is often closely coupled to RGR (Stulen *et al.*, 1994). However, in the present study, SAR_N was not increased in parallel with the stimulation of RGR between days 10 and 21 as might be expected, and was markedly decreased between days 21 and

26 where only a small reduction in RGR occurred, if at all (Table 3.1). These responses of SAR_N to elevated C_a can be explained by the progressively decreasing S/R (Table 3.3; Fig. 3.7), which was unaffected in the experiments reported by Stulen *et al.* (1994), along with a relatively constant N_P over time (Fig. 3.2) and with size (Table 3.3; Fig. 3.6). Reductions in S/R in elevated C_a could therefore represent potentially growth-limiting responses if the specific uptake activity of nutrients by roots is consequently reduced, but further studies are needed that address more directly the effects of elevated C_a on nutrient uptake characteristics.

3.4.5. Non-structural carbohydrates

The accumulation of soluble sugars and starch found in leaves in elevated C_a (Fig. 3.3) is in agreement with most CO_2-enrichment studies (Farrar and Williams, 1991). The accumulation of total non-structural carbohydrate (TNC) occurred only at the beginning and end of the 21 - 26 d harvest interval during which RGR was in decline, which is consistent with the view that the TNC status of leaves reflects the status of assimilate use by sinks and hence a sink-source (Section 1.4, p. 6) imbalance (Stitt, 1991; Pollock and Farrar, 1996). Whilst the reduction in the CO_2-stimulation of NAR at this time is broadly consistent with the view that the accumulation of soluble sugars in leaves causes a down-regulation of photosynthetic capacity and hence growth (Stitt, 1991; Poorter, 1993; Van Oosten *et al.*, 1994; Pollock and Farrar, 1996), the accumulation of soluble sugars in main-stem leaves (Fig. 3.3.A) was not associated with reduced photosynthetic capacity, $V_{c,max}$ or A_{max} measured in a representative main-stem leaf (Table 3.2). Possible explanations for the insensitivity of photosynthesis to soluble sugar status will be discussed in Chapter 5, and explanations for the apparent independency of NAR on photosynthetic capacity were suggested earlier (Section 3.4.2). Pollock and Farrar (1996) also raise the possibility that soluble sugars play wider roles in the regulation of plant growth, such as in morphogenesis.

3.4.6. Conclusions

The results of this study indicate that the reductions in specific leaf area (SLA) and tissue N concentration are directly attributable to elevated C_a, rather than to any effect of ontogeny or interactions between plant size and environmental constraints. Through its effect on leaf area ratio (LAR), the reduction in SLA seems particularly important in limiting even the early stimulation of relative growth rate (RGR) in elevated C_a, whilst the subsequent decline in the stimulation was more obviously linked to a reduction in the CO_2-stimulation of the net assimilation rate (NAR). The design of future CO_2-enrichment experiments may need more resolution to account for the very early onset of CO_2 effects, as well as for the possible size-dependency of all measured responses. With such a design, Chapters 4 and 5 investigate more fully the possible roles of organic N and soluble sugars in mediating these early changes in growth in elevated C_a.

CHAPTER 4

Growth of *Urtica urens* in elevated CO_2: I. Early changes and the role of organic N

4.1. INTRODUCTION

An elevated atmospheric CO_2 concentration (elevated C_a) increases the biochemical efficiency of photosynthesis in C_3 plants and hence their potential for growth (Bowes, 1991). The growth responses of *Urtica urens* to elevated C_a over a period of 26 d (Chapter 3) are broadly consistent with those of other herbaceous C_3 species for periods of similar or longer duration (Poorter *et al.*, 1988; Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Stirling *et al.*, 1998). It is now clear that elevated C_a probably affects the growth of C_3 species in a highly characteristic manner, whereby an early stimulation of relative growth rate (RGR), lasting for perhaps no more than a few days, is followed by a decline so that RGR eventually approaches that found in ambient C_a.

The initial, transient stimulation of RGR means that the dry weight of a plant in elevated C_a will always be greater compared with that of a plant in ambient C_a at any common point of time. For both agricultural and ecological reasons, it is important to know whether the increase in dry weight actually represents the full growth potential of a species in elevated C_a, as might be the case if the decline in RGR simply reflects the size-dependent advancement in time of the usual ontogenetic drift (Evans, 1972; Givnish, 1986; Poorter *et al.*, 1988), or of an environmental constraint on growth such as N limitation. It follows that the mechanisms underlying the decline in RGR will also be affected by ontogeny and environmental constraint, but only a few CO_2-enrichment experiments have investigated the possible size-dependency of responses (Chapter 3; Poorter *et al.*, 1988; Farrar and Williams, 1991; Coleman *et al.*, 1993; Stirling *et al.*, 1998; Fonseca *et al.*, 1996; Gunn *et al.*, 1998), or have shown unambiguously that resources did not become limiting during the experiment (Chapter 3).

Similarly, few CO_2-enrichment experiments have been designed in a way that allows investigation of what are clearly early and dynamic changes in growth and growth-related physiology (Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Van Oosten *et al.*, 1994; Fonseca *et al.*, 1996), or have routinely attempted to establish comparable bases for the expression of data, which can be affected, for example, by an accumulation of non-structural carbon in elevated C_a (Wong, 1990; Chu *et al.*, 1991; Kuehny *et al.*, 1991; Baxter *et al.*, 1994a,b; Wullschleger *et al.*, 1994; Baxter *et al.*, 1995; Den Hertog *et al.*, 1996). Moreover, the extent to which the increase in dry weight in elevated C_a is caused by an increase in structural material relative to non-structural carbon has received little attention (Den Hertog *et al.*, 1996), but may have major implications for future growth potential (Wong, 1990). For example, an accumulation of the additional fixed carbon available in elevated C_a as non-structural carbon may reflect its under-utilization to drive growth-related processes such as nutrient uptake and assimilation. For all of these reasons, the questions as to whether and, if so, why the potential for growth in elevated C_a is not fully-realized remain largely unanswered, despite a large and expanding body of literature on the effects of elevated C_a on plant growth.

From the evidence available, a number of potentially growth-limiting changes in physiology and morphology of plants grown in elevated C_a can be identified. These can include (1) the down-regulation of photosynthetic capacity in individual leaves (Cure and Acock, 1986; Long and Drake, 1992; Sage, 1994; Van Oosten *et al.*, 1995), (2) a decline in the stimulation of net assimilation rate (NAR) (Chapter 3; Poorter *et al.*, 1988; Poorter, 1993; Stulen *et al.*, 1994), (3) an inhibition of the rate of dark respiration (R_d) (Gifford *et al.*, 1985; Spencer and Bowes, 1986; Amthor *et al.*, 1992; Poorter *et al.*, 1992; Ziska and Bunce, 1993; Bunce, 1994; Thomas and Griffin, 1994; Wullschleger *et al.*, 1994), (4) changes in the partitioning of leaf area and dry weight causing reductions in leaf area ratio (LAR) and specific leaf area (SLA) (Chapter 3; Acock and Pasternak, 1986; Poorter, 1993; Stulen *et al.*, 1994; Gebauer *et al.*, 1996; Roumet *et al.*, 1996; Stirling *et al.*, 1998), and (5) decreased tissue N concentrations (Chapter 3; Garbutt *et al.*, 1990; Stulen *et al.*, 1994). Of these changes, only the reductions in LAR, SLA and tissue N concentration are reported with real consistency.

In Chapter 3, allometric analysis was used to demonstrate for the first time that the widely-reported reduction in LAR, which is driven by a reduction in SLA, can limit RGR in elevated C_a independently of both ontogeny and interactions between plant size and environmental constraints. A decline in the stimulation of NAR was also found, but this study was unable to address the possible size-dependencies of either NAR or RGR itself due to their unusually discontinuous time-courses during the earlier phases of growth. In the present study, plants were switched from ambient to elevated C_a at a more mature stage of growth when RGR and NAR may be more probably linear or declining, to enable an investigation of the size-dependencies of their responses to elevated C_a. By monitoring growth responses more frequently and over a shorter period of time, the timing and duration of these early changes in RGR, NAR and LAR were determined with more precision, with a view to investigating their mechanistic bases. In this respect, the present chapter investigates the extent to which changes in RGR can be attributed to an increase in structural material relative to non-structural carbon, and addresses the hypothesis (stated and justified in the previous chapter) that the reductions in RGR, NAR and LAR are closeley linked to a reduction in the concentration of organic N in plant tissues. Chapters 5 and 6 will then investigate, respectively, the roles soluble sugars and respiration in the early growth responses to elevated C_a that are described here.

4.2. MATERIALS AND METHODS

4.2.1. Growth conditions

Plants of *U. urens* were grown in a hydroponic culture system in controlled-environment cabinets, as described in Section 2.2, but with the further modifications that the pH of the nutrient solution was adjusted to 5.5 using NaOH and buffered with 2 mM MES, and that the air bubbled through the nutrient solution was drawn from within the cabinets. In order to reduce variability, plants were switched from ambient to elevated C_a (700 μmol mol^{-1}) at a defined fresh weight of 8 g (± a maximum of 0.75 g), having a shoot height of 7 - 8 cm and a dry weight of ca. 0.8 g. Plants varied as to the period of time taken to attain this designated size, but were approximately 30 d (± a maximum of 4 d) old from sowing, with $\overline{R}$GRs of 0.161 g g^{-1} fresh weight d^{-1} (±0.006 standard errors) over a single time interval spanning periods of 6 - 9 d preceding the switch. Plants were exposed to elevated C_a for 10 d. Henceforth, the day at which exposure to elevated C_a began will be designated as day 0.

4.2.2. Growth analysis

At each harvest, plants were separated into leaves (unfolded leaves > 1 cm length), stems (including folded leaves, leaves ≤ 1 cm length and inflorescences if present), and roots. Dry weight (DW) was determined after oven-drying at 60 °C for at least 48 h. To determine the structural dry weight (SDW), the weight of total non-structural carbohydrates (TNC) (soluble sugars and starch) was subtracted from the total DW. TNC was measured as described in Chapter 5, Section 5.2.5, and the actual data of TNC concentrations are also shown in Chapter 5 (Figs. 5.3 and 5.4). Total leaf area (all leaves > 1 cm length) was measured using a digital image analysis system (Delta-T Ltd., Cambridge, UK).

Classical growth analysis (average values over distinct time intervals) was used to describe and quantify crude changes in growth over the 10 d period. Five plants were harvested at day 0, five plants per treatment at day 4, and five plants per treatment at day

10. From the data of DW, SDW and leaf area, the following components of plant growth were calculated for each harvest interval (days 0 – 4 and 4 - 10) from replicates paired across harvests according to size (Evans, 1972; Hunt, 1978): (1) Whole plant mean relative growth rate, $\overline{RGR}$, (2) Mean net assimilation rate, $\overline{NAR}$, (3) Mean leaf area ratio, $\overline{LAR}$, (4) Mean leaf weight ratio, $\overline{LWR}$, and (5) Mean specific leaf area, $\overline{SLA}$. Using data obtained for organic N content per plant (Section 4.2.3), the mean specific absorption rate of N by roots, $\overline{SAR}_N$, was determined (Wellbank, 1962) for each harvest interval. The respective formulae used for the calculation of these parameters are given in Section 2.3.1.

Functional growth analysis (instantaneous values derived by curve-fitting) was used to describe dynamic changes in growth over the 10 d period. From the harvests described above and from additional harvests (one plant per treatment) made at days 1, 2, 3, 5, 6, 7, 8 and 9, instantaneous values of whole plant relative growth rate (RGR), and of its components net assimilation rate (NAR) and leaf area ratio (LAR), were calculated following the curve-fitting approach of Hughes and Freeman (1967). Their approach was modified to also derive instantaneous values of leaf weight ratio (LWR), specific leaf area (SLA) and the specific absorption rate of N by roots (SAR_N) using data of whole plant organic N content. The functional approach used here is described fully in Section 2.3.2. Second-order polynomials (quadratic equations) were used to describe the primary logarithmically-transformed data of dry weight, leaf area and organic N content. The choice of polynomial followed the method of Hunt and Parsons (1974) as described in Section 2.3.2. To assess the size-dependencies of RGR and NAR, RGR and NAR were plotted against data of dry weight and leaf area.

4.2.3. Organic N

Organic N concentration in leaves, roots, stems and whole plants was determined by measuring the ammonium concentration of acid digests of 50 - 250 mg of well-mixed oven-dried material (Section 4.2.2) as described by Allen (1989) and in Section 2.8.

4.2.4. Allometric analysis

Allometric analysis (Pearsall, 1927; Troughton, 1955) was used to distinguish between direct effects of elevated CO_2 and effects related to plant size, as described in Section 2.4. Geometric mean regressions (GMRs) were fitted to logarithmically-transformed data:

$$\log_e y = \log_e b + v \log_e x$$

where y and x were data of dry weight (including and excluding TNC), leaf area and organic N content. The GMR slope (v) represents the allometric coefficient (Ricker, 1984; Farrar and Gunn, 1996) and the constant b expresses the regression intercept of y when x is zero.To account for possible shifts in allometric relations, a number of plants growing in ambient C_a were left for harvesting after day 26 at a size roughly comparable to that of plants growing in elevated C_a at that time.

4.2.5. Statistical analyses

Two-way (CO_2 and time) analysis of variance followed by Tukey tests were used to test for significant differences in $\overline{R}$GR and components thereof (days 0 - 4 and 4 -10), and in organic N concentration at times when replication of data was available (days 0, 4 and 10). For functional analysis of growth, significant differences between corresponding pairs of constants in the fitted equations were analyzed by t-test as described in Section 2.3.2. For allometric analyses, differences in v, the displacement of the GMR line (elevation), and deviation of v from unity were tested for statistical significance using Student's t-test as described by Zar (1989). All statistical analyses were performed using the software package SPSS (Prentice Hall, New Jersey).

4.3. RESULTS

4.3.1. Plant growth

A vertical gradient of PPFD exists in controlled-environment cabinets. Elevated C_a did not affect the increase in height of plants over 10 d, so the increase in PPFD incident at the shoot apex over time was similar for plants grown in both ambient and elevated C_a (Fig. 4.1).

Classical growth analysis

In elevated C_a, the mean dry weight per plant, including and excluding total non-structural carbohydrates (TNC), was about 20 % and 30 % greater at days 4 and 10 respectively (Fig. 4.2). $\overline{\text{RGR}}$ was significantly greater in elevated C_a over both harvest intervals, but the stimulation over the 0 - 4 d interval (about 14 %) was larger than that over the 4 - 10 d interval (about 8 %) (Table 4.1). The mean leaf area per plant was significantly increased by elevated C_a at day 10 but not at day 4 (Fig. 4.2). Over both harvest intervals, the increases in $\overline{\text{RGR}}$ due to elevated C_a were driven by increases in $\overline{\text{NAR}}$ but constrained by reductions in $\overline{\text{LAR}}$ (Table 4.1). Reductions in $\overline{\text{SLA}}$ were responsible for the reductions in $\overline{\text{LAR}}$, but the limiting effect of $\overline{\text{SLA}}$ on $\overline{\text{RGR}}$ and $\overline{\text{LAR}}$ was counteracted by increases in $\overline{\text{LWR}}$ (Table 4.1). The 14 % stimulation of $\overline{\text{RGR}}$ by elevated C_a over the 0 - 4 d harvest interval resulted from a 22 % stimulation of $\overline{\text{NAR}}$ offset by a 5 % reduction in $\overline{\text{LAR}}$, whilst the subsequent decline in the CO_2-stimulation of $\overline{\text{RGR}}$ over the 4 - 10 d harvest interval resulted from a small reduction in the size of stimulation of $\overline{\text{NAR}}$ (20 %) offset by a larger decrease in the size of reduction in $\overline{\text{LAR}}$ (10 %) (Table 4.1). $\overline{\text{SAR}}_N$ was significantly greater in elevated C_a over the 0 - 4 d harvest interval, but was unchanged over the 4 - 10 d harvest interval (Table 4.1).

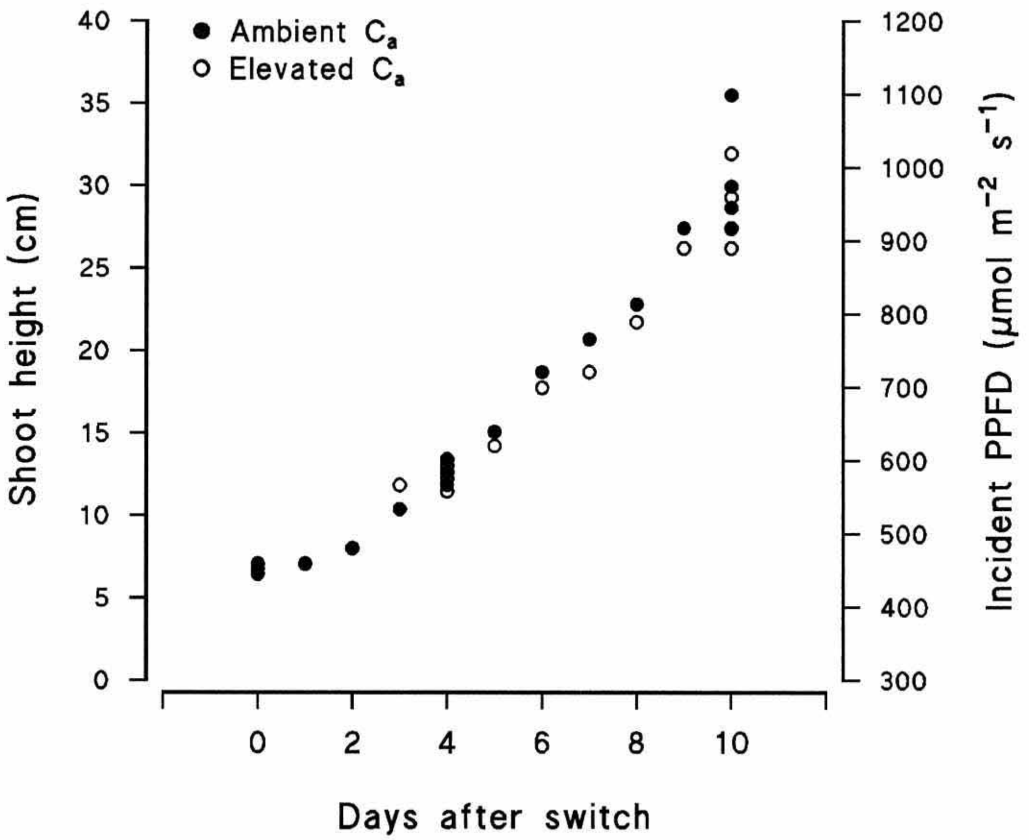

Figure 4.1. Shoot height of and photosynthetic photon flux density (PPFD) incident on *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. PPFD was measured at the shoot apex using a portable quantum sensor (Skye Instruments Ltd., UK).

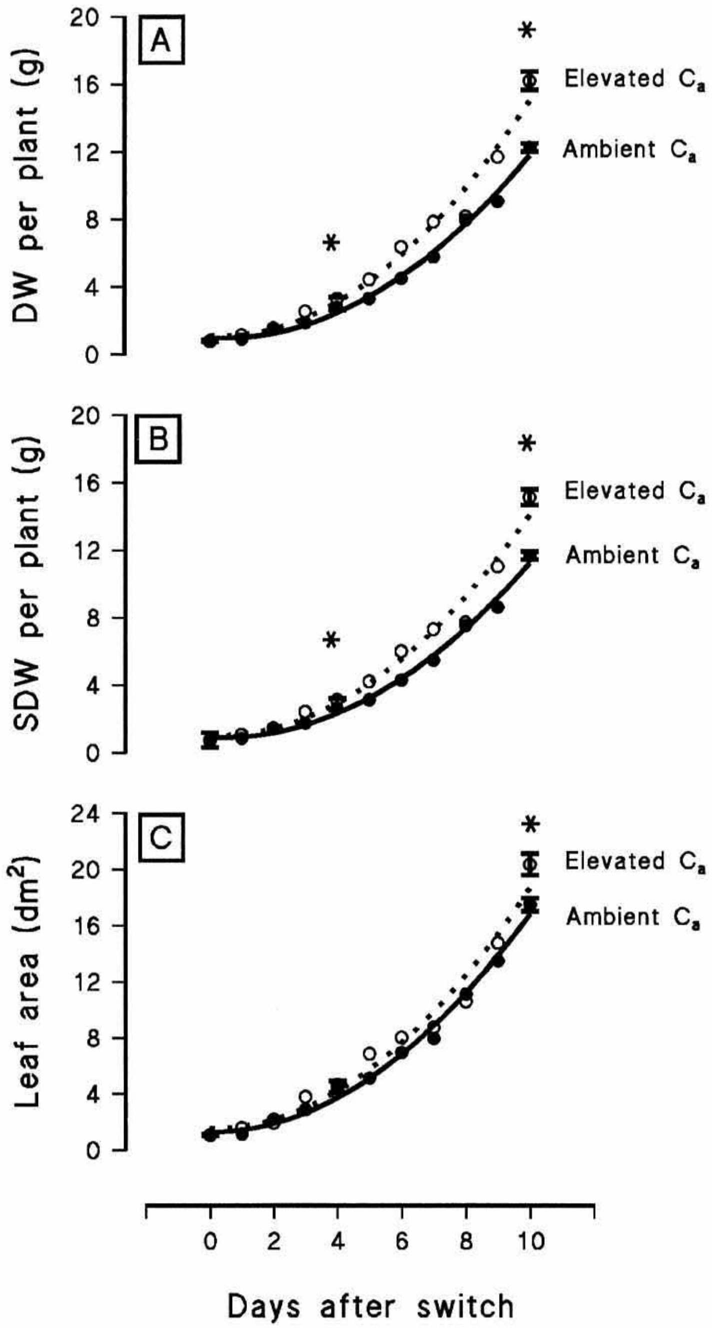

Figure 4.2. (A) Total dry weight (DW), (B) Total structural dry weight (SDW) and (C) Total leaf area of *U. urens* during 10 d after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. SDW is the total DW minus the weight of total non-structural carbohydrates (soluble carbohydrate and starch). Data are shown individually (days 1, 2, 3, 5, 6, 7, 8, and 9), or as means (n = 4 - 5) (days 0, 4, and 10) with standard error bars, and are fitted by quadratic curves. Significant differences ($p < 0.05$) due to C_a at days 4 and 10 are indicated by asterisks.

Table 4.1. Classical analysis of growth of *U. urens* after switching plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a for 10 d at approximately 30 d of age (day 0). The table shows $\overline{RGR}$ (mean relative growth rate), $\overline{NAR}$ (mean net assimilation rate), $\overline{LAR}$ (mean leaf area ratio), $\overline{LWR}$ (mean leaf weight ratio), $\overline{SLA}$ (mean specific leaf area), and $\overline{SAR_N}$ (the mean specific absorption rate of N by roots) over two harvest intervals (days 0 - 4 and 4 - 10). Values are calculated including and excluding the weight of total non-structural carbohydrates (+/- TNC) in tissues. Data are shown as means (n = 4) ± standard errors. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks.

	HARVEST INTERVAL (DAYS)			
	0 - 4		4 - 10	
	Ambient C_a	Elevated C_a	Ambient C_a	Elevated C_a
+ TNC				
$\overline{RGR}$ (g g^{-1} d^{-1})	0.313 (±0.004)	0.358 (±0.009) ***	0.242 (±0.004)	0.262 (±0.001) *
$\overline{NAR}$ (g m^{-2} d^{-1})	21.0 (±0.28)	25.6 (±1.0) **	16.4 (±0.46)	19.8 (±0.09) **
$\overline{LAR}$ (m^2 g^{-1})	0.0147 (±0.0003)	0.0139 (±0.0003) *	0.0149 (±0.0002)	0.0135 (±0.0002) **
$\overline{LWR}$ (g g^{-1})	0.568 (±0.012)	0.582 (±0.008)	0.532 (±0.003)	0.573 (±0.004) **
$\overline{SLA}$ (m^2 g^{-1})	0.0260 (±0.0003)	0.0240 (±0.0005) **	0.0280 (±0.0003)	0.0235 (±0.0003) ***
$\overline{SAR_N}$ (mg g^{-1} d^{-1})	60.9 (±5.6)	85.1 (±5.4) **	68.1 (±2.2)	66.6 (±3.1)
- TNC				
$\overline{RGR}$ (g g^{-1} d^{-1})	0.318 (±0.004)	0.361 (±0.010) ***	0.242 (±0.004)	0.260 (±0.001) *
$\overline{NAR}$ (g m^{-2} d^{-1})	20.2 (±0.26)	24.4 (±0.94) **	15.6 (±0.43)	18.5 (±0.19) **
$\overline{LAR}$ (m^2 g^{-1})	0.0156 (±0.0003)	0.0148 (±0.0002) *	0.0157 (±0.0002)	0.0143 (±0.0002) **
$\overline{LWR}$ (g g^{-1})	0.563 (±0.011)	0.575 (±0.007)	0.528 (±0.003)	0.567 (±0.004) **
$\overline{SLA}$ (m^2 g^{-1})	0.0278 (±0.0004)	0.0258 (±0.0005) **	0.0296 (±0.0003)	0.0253 (±0.0004) ***
$\overline{SAR_N}$ (mg g^{-1} d^{-1})	63.2 (±5.8)	88.3 (±5.6) **	70.5 (±2.3)	69.0 (±3.1)

Functional growth analysis

The quadratic equations fitted to logarithmically-transformed primary data of plant dry weight, leaf area and leaf dry weight are shown in Figure 4.3, and of root dry weight and organic N per plant in Figure 4.4. With the exception of organic N in plants grown in ambient C_a, where the equation of best fit was linear, all logarithmically-transformed primary data were best described by quadratic equations. The patterns were the same when the dry weights excluded TNC (data not shown). The linear and quadratic terms of all fitted equations are given in Table 4.2. In elevated C_a, the linear term was significantly higher in equations describing the dry weight (including and excluding TNC) of whole plants and leaves, and in the equation describing organic N content per plant. Of the quadratic terms, only that in the equation describing organic N content per plant was significantly affected by CO_2, where a reduction was found in elevated C_a.

The instantaneous values of relative growth rate and its components which were derived from the terms given in Table 4.2 are shown, including and excluding TNC, in Figure 4.5 (RGR, NAR and LAR), Figure 4.6 (LWR and SLA), and Figure 4.7 (SAR_N). In both ambient and elevated C_a, RGR and NAR declined over the 10 d period. Compared with ambient C_a, RGR in elevated C_a was stimulated for only the first 7 d because the magnitude of the initial stimulation of RGR was not sustained. However, the magnitude of the initial stimulation of NAR in elevated C_a was sustained for about the first 4 - 5 d, and thereafter declined. The early decline in the CO_2-stimulation of RGR was therefore driven an by an early reduction in LAR in relation to an increasing LAR in ambient C_a over the first 4 - 5 d, whilst from this time onwards the continued decline in the stimulation of RGR was more obviously driven by a decline in the stimulation of NAR.

The early reduction in LAR during the first 4 - 5 d in elevated compared with ambient C_a was driven by a reduction in SLA over the same period of time (Fig. 4.6), but this constraint on RGR was moderated to a small degree by an increasing LWR in elevated C_a. SAR_N was stimulated in elevated compared with ambient C_a for the first 8 d, but the magnitude of the initial stimulation of SAR_N was sustained for no more than the first 1 - 2 d and thereafter declined (Fig. 4.7).

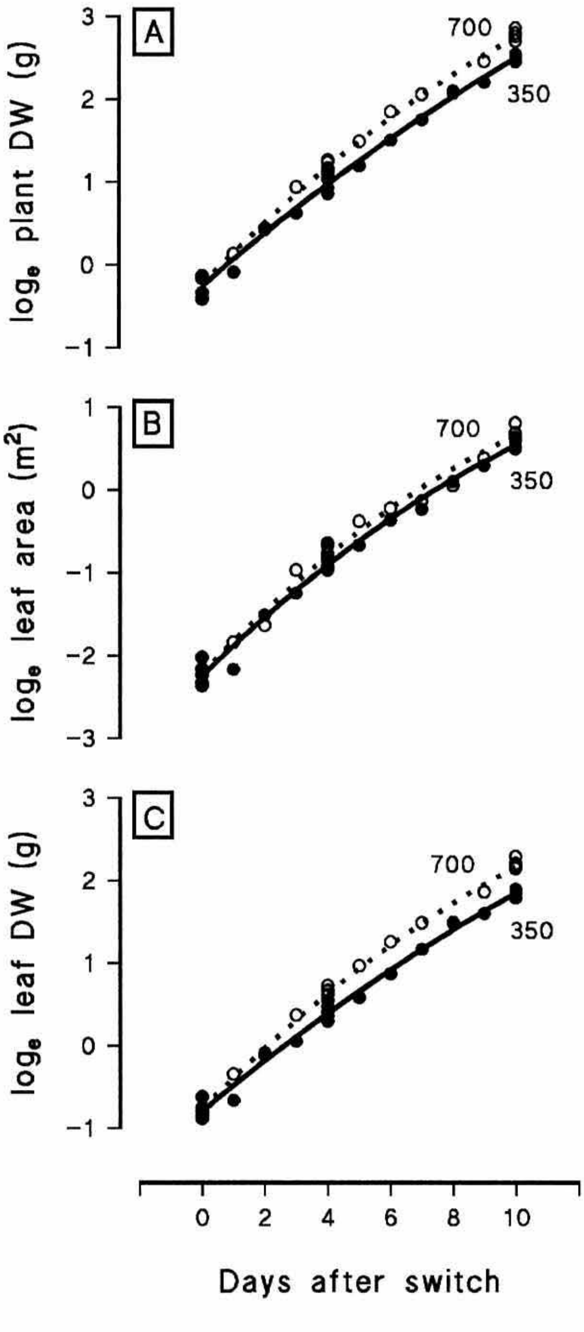

Figure 4.3. (A) Log_e plant dry weight (DW), (B) Log_e total leaf area and (C) Log_e leaf DW of *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown individually, and are fitted to quadratic curves. The coefficient of determination (r^2) for each curve, and the constants (± standard errors) describing the linear and quadratic terms in each equation are given in Table 4.2.

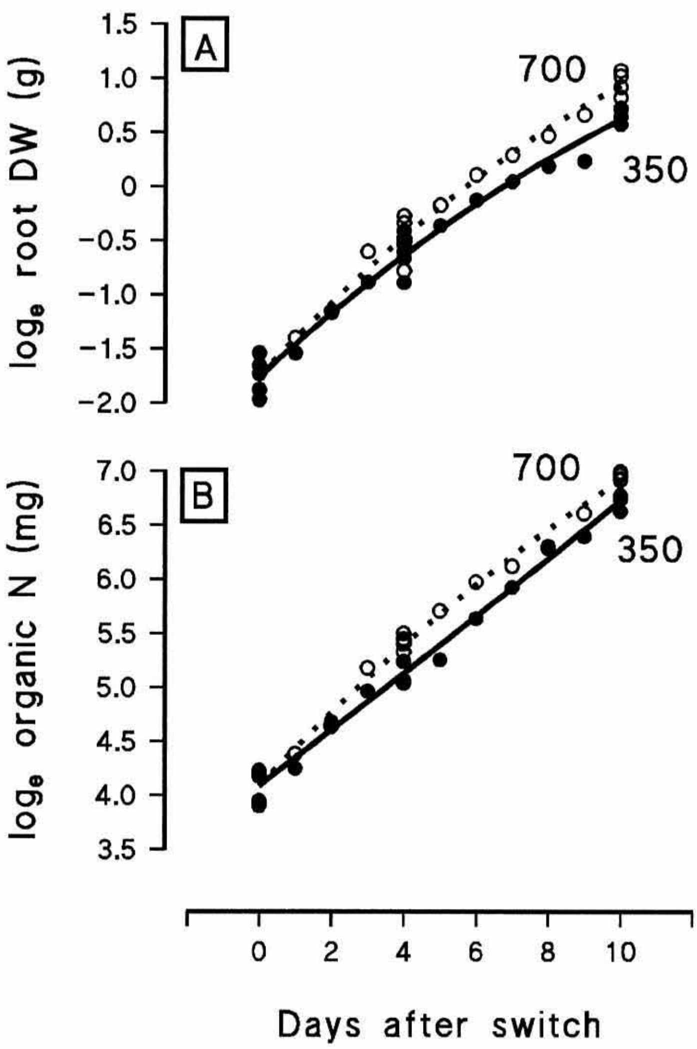

Figure 4.4. (A) Log$_e$ root dry weight (DW) and (B) Log$_e$ plant organic N content of *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown individually, and are fitted to quadratic curves. The coefficient of determination (r^2) for each curve, and the constants (± standard errors) describing the linear and quadratic terms in each equation are given in Table 4.2.

Table 4.2. Functional analysis of growth of *U. urens* after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d at approximately 30 d of age. The table shows the constants *b* and *c* that describe the linear and quadratic terms, respectively, in equations of the form: $y = a + b + ct^2$ (Figs. 4.3 and 4.4) where y is data of dry weight (including and excluding total non-structural carbohydrates (TNC)), leaf area and plant organic N content, and *t* is time (d). Standard errors for *b* and *c* are shown in parentheses. r^2 is the coefficient of determination for each equation. Significant differences ($p < 0.05$) due to C_a between corresponding pairs of constants are indicated by asterisks.

		Including TNC			Excluding TNC		
y	C_a	*b*	*c*	r^2	*b*	*c*	r^2
$\log_e$ plant DW (g)	350	0.329 (±0.0197)	-0.0054 (±0.0019)	0.992	0.335 (±0.0205)	-0.0058 (±0.0020)	0.992
	700	0.392 (±0.0189) *	-0.0094 (±0.0018)	0.994	0.397 (±0.0194) *	-0.0098 (±0.0019)	0.993
$\log_e$ leaf area (m^2)	350	0.374 (±0.0252)	-0.0095 (±0.0024)	0.988	NOT APPLICABLE		
	700	0.399 (±0.0260)	-0.0112 (±0.0025)	0.987	NOT APPLICABLE		
$\log_e$ leaf DW (g)	350	0.317 (±0.0185)	-0.0058 (±0.0018)	0.992	0.325 (±0.0187)	-0.0058 (±0.0018)	0.992
	700	0.386 (±0.0189) *	-0.0094 (±0.0018)	0.994	0.392 (±0.0190) *	-0.0099 (±0.0018)	0.994
$\log_e$ root DW (g)	350	0.308 (±0.0269)	-0.0071 (±0.0026)	0.981	0.310 (±0.0273)	-0.0071 (±0.0026)	0.980
	700	0.356 (±0.0282)	-0.0088 (±0.0027)	0.983	0.358 (±0.0283)	-0.0090 (±0.0027)	0.983
$\log_e$ organic N (mg)	350	0.257 (±0.0216)	-0.0006 (±0.0021)	0.990	NOT APPLICABLE		
	700	0.351 (±0.0209)**	-0.0070 (±0.0020) *	0.992	NOT APPLICABLE		

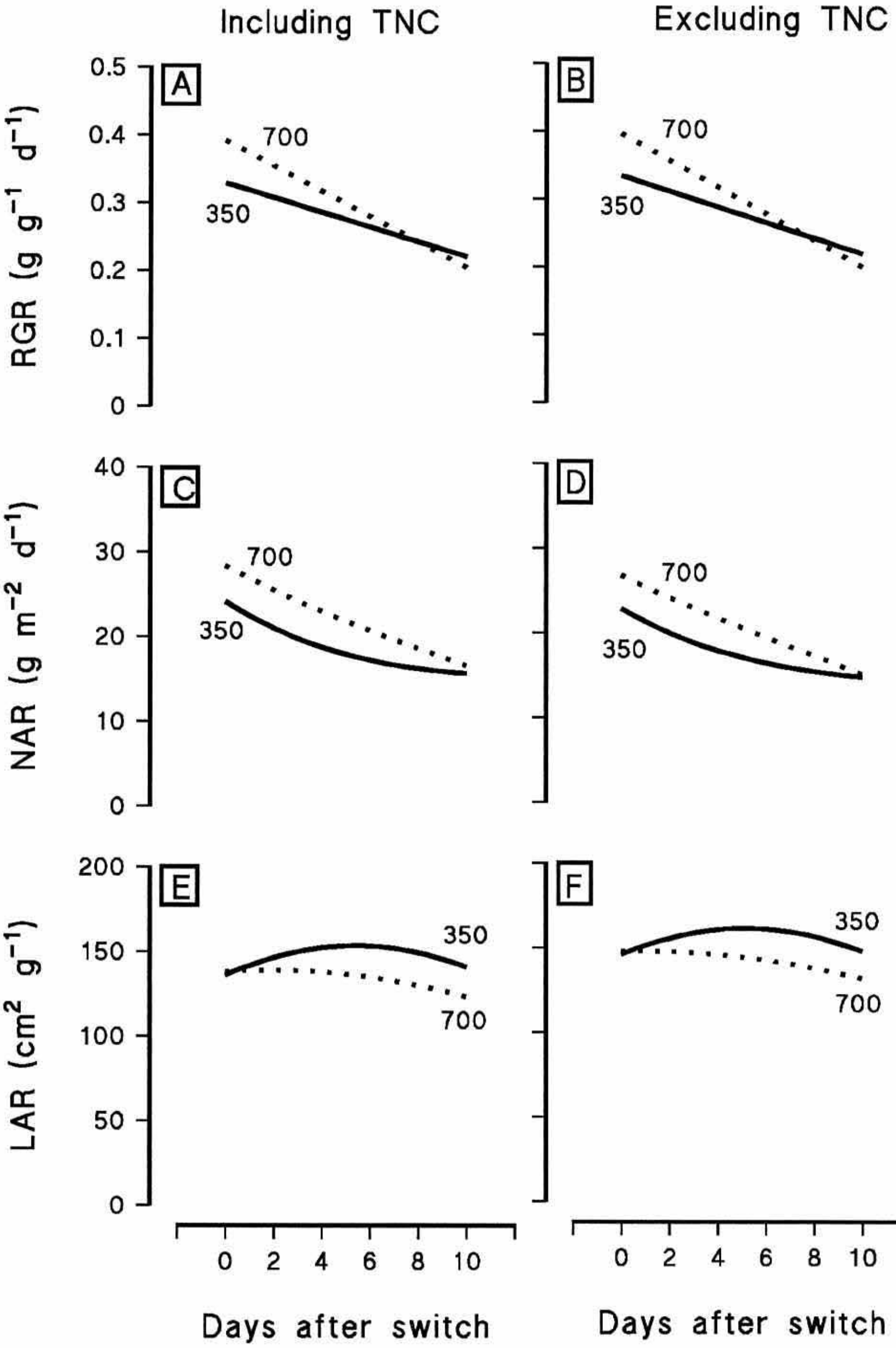

Figure 4.5. Functional analysis of growth including (A,C,E) and excluding (B,D,F) the weight of total non-structural carbohydrates (TNC) of *U. urens* during a 10 d period after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Curves show (A,B) Instantaneous relative growth rate (RGR), (C,D) Instantaneous net assimilation rate (NAR), and (E,F) Instantaneous leaf area ratio (LAR), and are solutions of the quadratic equations fitted to logarithmically-transformed primary data (Fig. 4.3; Table 4.2) as described in Section 2.3.2.

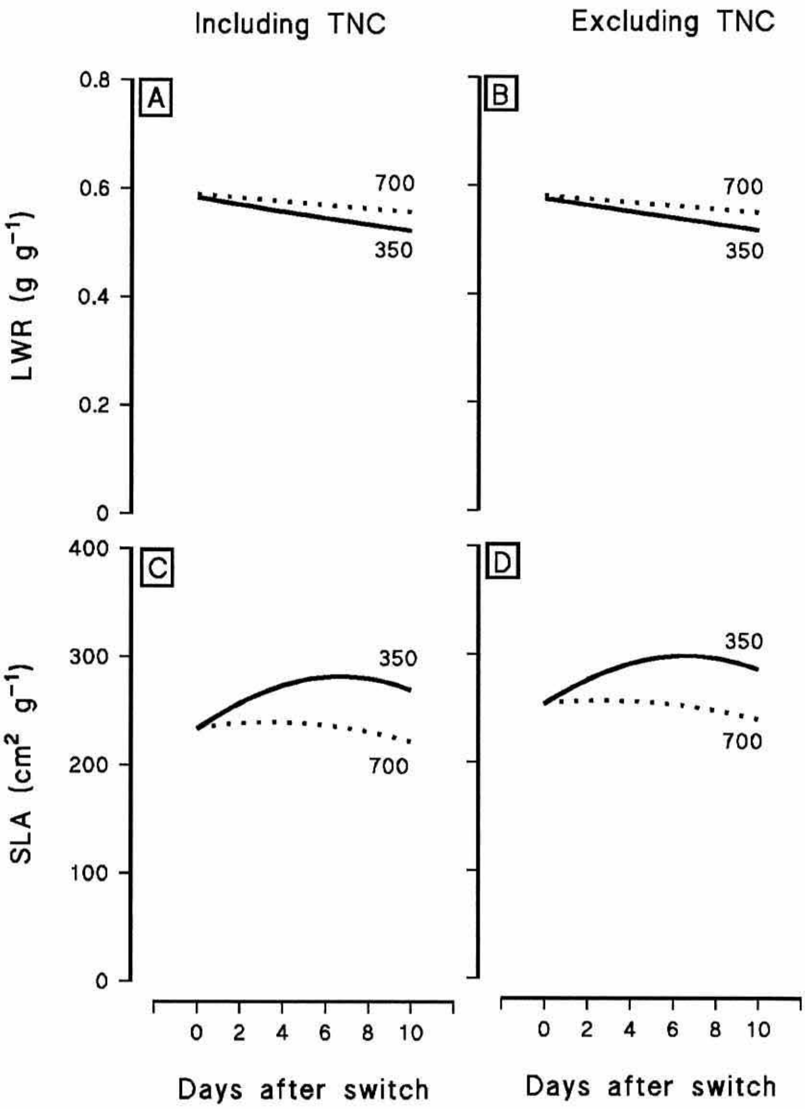

Figure 4.6. Functional analysis of growth including (A,C) and excluding (B,D) the weight of total non-structural carbohydrates (TNC) of *U. urens* during a 10 d period after switching plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Curves show (A,B) Instantaneous leaf weight ratio (LWR) and (C,D) Instantaneous specific leaf area (SLA), and are solutions of the quadratic equations fitted to logarithmically-transformed primary data (Fig. 4.3; Table 4.2) as described in Section 2.3.2.

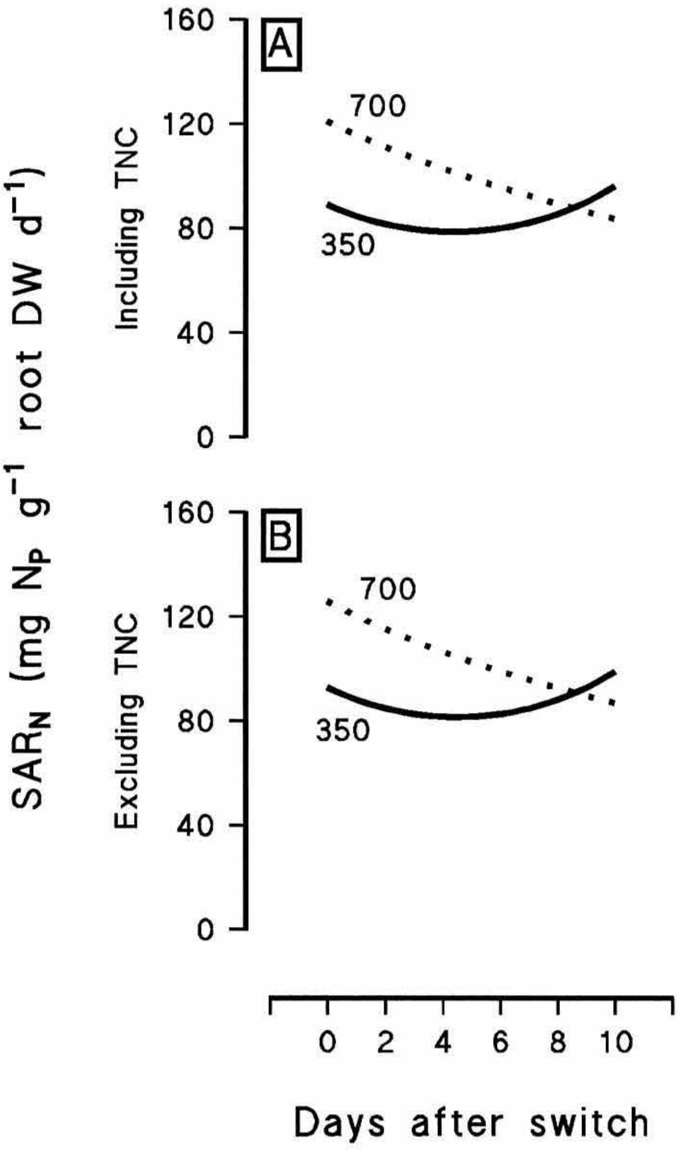

Figure 4.7. Instantaneous specific absorption rate of N by roots (SAR_N), including (A) and excluding (B) the weight of total non-structural carbohydrates (TNC), of *U. urens* during a 10 d period after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Curves are solutions of the quadratic equations fitted to logarithmically-transformed primary data (Fig. 4.4; Table 4.2) as described in Section 2.3.2.

4.3.2. Organic N

The concentrations of organic N per unit area in leaves (N_{LA}) are shown in Figure 4.8, and per unit structural dry weight in leaves (N_L), stems (N_{ST}), roots (N_{RT}) and whole plants (N_P) in Figure 4.9. In general, N_{LA} and N_L declined over the first 4 d in both ambient and elevated C_a, but the decline was more pronounced in ambient C_a, resulting in a significantly higher N_{LA} at day 4 in elevated C_a (Fig. 4.8). However, elevated C_a did not significantly affect N_L at day 4 (Fig. 4.9.A). At day 10, elevated C_a did not significantly affect N_{LA}, but resulted in a significant reduction in N_L. Small and statistically insignificant increases in mean N_{ST} and mean N_{RT} at day 10 contributed to the lack of any significant effect of elevated C_a on N_P at this time. Elevated C_a did not significantly affect N_P during the 10 d period (Fig. 4.9.D).

4.3.3. Size-dependency of responses

In general, the declines over time in the CO_2-stimulations of RGR and NAR (Fig. 4.5.A and C) were less steep when RGR and NAR were plotted against the total dry weight per plant and against the total leaf area per plant, but persited nevertheless (Fig. 4.10). A summary of the allometric analyses describing the partitioning of dry weight, leaf area and organic N is given in Table 4.3. The allometric coefficient (v) was affected by C_a only in the geometric mean regression (GMR) relating leaf area to leaf dry weight (allometric SLA), where a decrease in v was found in elevated C_a. The decrease in v describing SLA was no longer significant when leaf dry weight excluded the weight of TNC. Significantly decreased elevations were found in elevated C_a in the GMRs describing SLA and LAR (leaf area against whole plant dry weight). Significantly increased elevations were found in elevated C_a in the GMRs describing LWR (leaf dry weight against whole plant dry weight) and N_L per unit area (leaf N content against leaf area). All other allometric relationships describing organic N concentration were not significantly affected by C_a. Neither the slope or elevation of the GMR relating shoot to root dry weight (allometric S/R) was affected by elevated C_a, regardless of whether or not the dry weight included TNC.

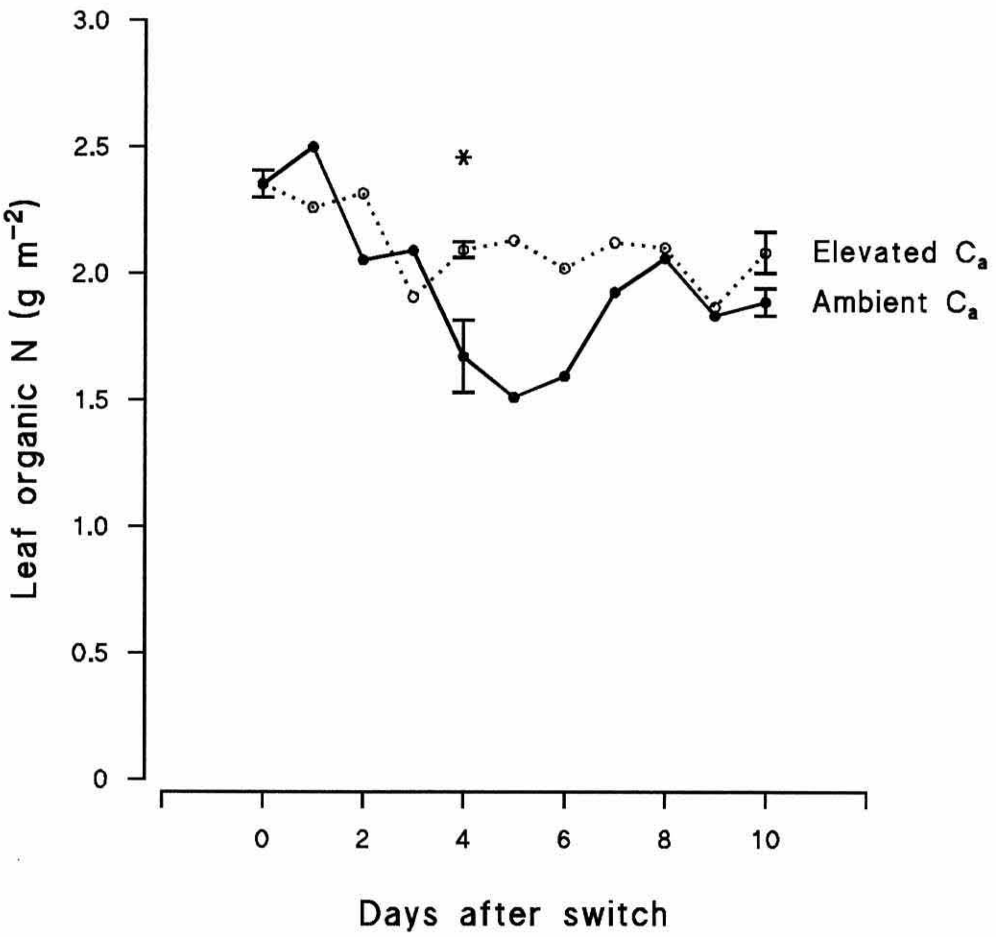

Figure 4.8. Organic N concentration per unit area in leaves of *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown individually (days 1, 2, 3, 5, 6, 7, 8 and 9), or as means (n = 4 - 5) (days 0, 4 and 10) with standard error bars. Significant differences ($p < 0.05$) due to C_a at days 4 and 10 are indicated by asterisks.

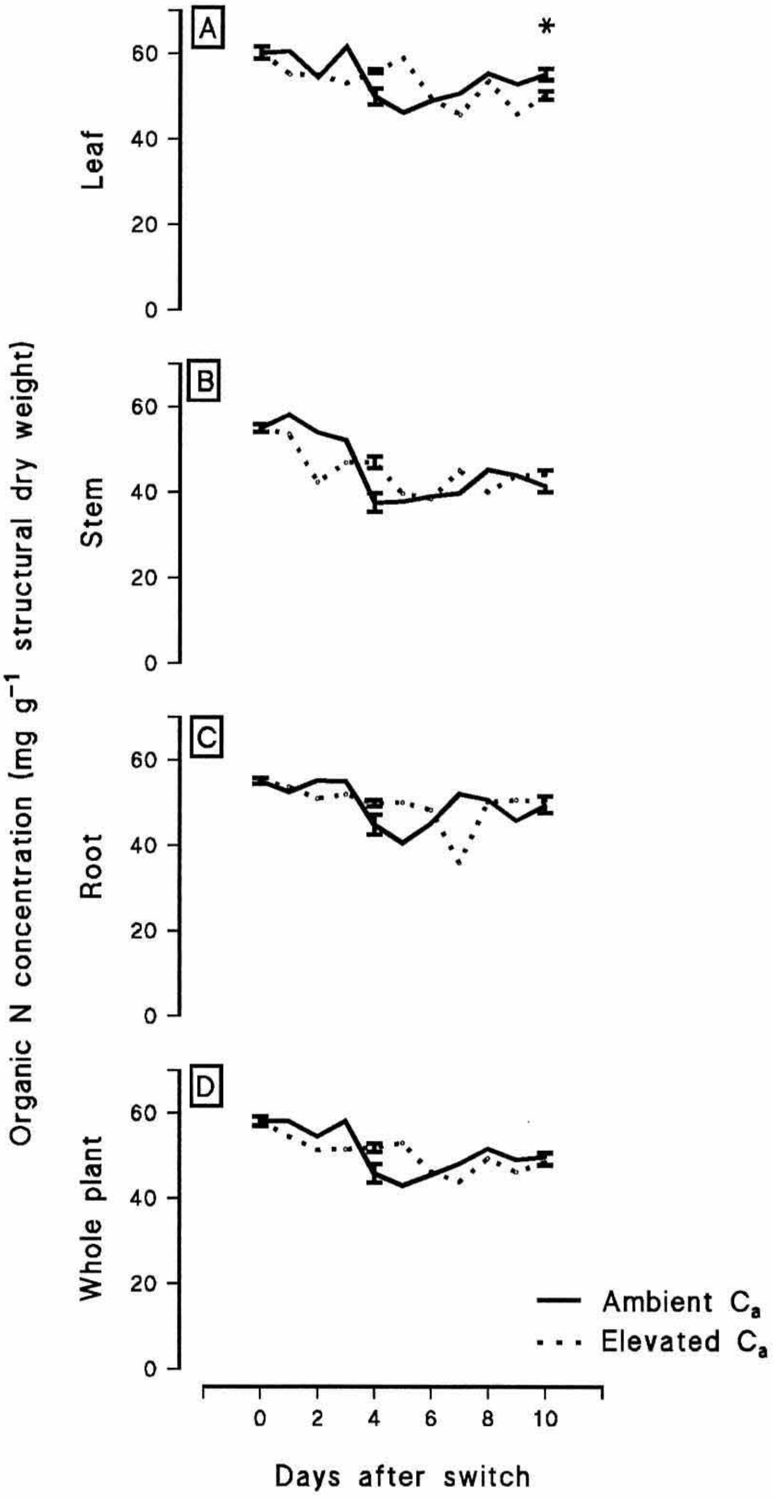

Figure 4.9. Organic N concentration, expressed per unit structural dry weight, in (A) Leaves, (B) Stems, (C) Roots and (D) Whole plants of *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Structural dry weight is the total dry weight minus the weight of total non-structural carbohydrates (soluble carbohydrate and starch). Data are shown individually (days 1, 2, 3, 5, 6, 7, 8 and 9), or as means (n = 4 - 5) (days 0, 4 and 10) with standard error bars. Significant differences ($p < 0.05$) due to C_a at days 4 and 10 are indicated by asterisks.

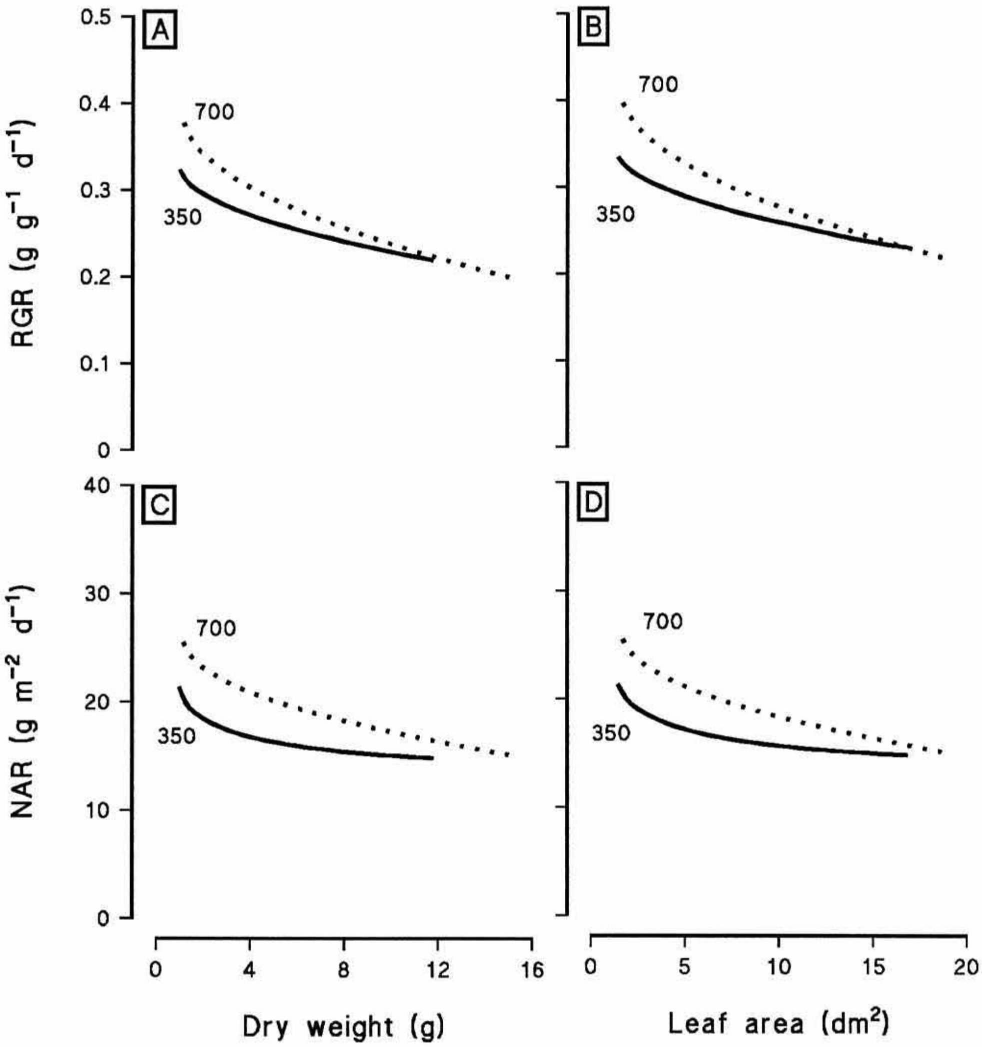

Figure 4.10. (A,B) Instantaneous relative growth rate (RGR) and (C,D) Instantaneous net assimilation rate (NAR) as dependent on total plant dry weight (A,C) and total leaf area (B,D) of *U. urens* during a 10 d period after switching plants from ambient (350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Values of RGR and NAR and data of dry weight and leaf area were derived from the quadratic curves shown in Figure 4.3.

Table 4.3. Allometric relations in *U. urens* switched at approximately 30 d of age from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d. The table gives a summary of analyses of geometric mean regressions (GMRs) describing logarithmically-transformed variables (x, y) of leaf area, dry weight (DW), structural dry weight (SDW) and organic N content. The table shows the GMR slope (v, the allometric coefficient) with the coefficient of determination (r^2) for each GMR in parenthesis, the relative elevation of the GMR line (↑, ↓ and = denoting an upwards, downwards and no significant displacement of the GMR line), and whether v deviates significantly from unity (slope ≠ 1). SDW is DW minus the weight of total non-structural carbohydrates (TNC). *, ** and *** indicate significant differences due to C_a at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively; ns indicates that differences are not significant.

Variable		GMR slope (v)		Elevation		Slope ≠ 1	
$\log_e$ y	$\log_e$ x	350	700	350	700	350	700
Leaf area (m^2)	Plant DW (g)	1.005 (0.994)	0.968 (0.993) ns	↑	↓ *	ns	ns
Leaf area (m^2)	Leaf DW (g)	1.045 (0.994)	0.987 (0.994) *	↑	↓ ***	*	ns
Leaf area (m^2)	Leaf SDW (g)	1.031 (0.994)	0.985 (0.995) ns	↑	↓ ***	ns	ns
Leaf DW (g)	Plant DW (g)	0.967 (0.998)	0.981 (0.984) ns	↓	↑ **	**	*
Shoot DW (g)	Root DW (g)	1.185 (0.990)	1.129 (0.988) ns	=	=	***	***
Shoot SDW (g)	Root SDW (g)	1.191 (0.990)	1.131 (0.989) ns	=	=	***	***
Plant organic N (g)	Plant DW (g)	0.934 (0.986)	0.921 (0.994) ns	=	=	**	***
Plant organic N (g)	Plant SDW (g)	0.927 (0.994)	0.920 (0.994) ns	=	=	***	**
Leaf organic N (g)	Leaf area (m^2)	0.944 (0.981)	0.968 (0.996) ns	↓	↑ *	*	*
Leaf organic N (g)	Leaf DW (g)	0.982 (0.991)	0.955 (0.997) ns	=	=	ns	**
Leaf organic N (g)	Leaf SDW (g)	0.973 (0.991)	0.953 (0.997) ns	=	=	ns	**
Stem organic N (g)	Stem DW (g)	0.929 (0.987)	0.933 (0.996) ns	=	=	**	***
Stem organic N (g)	Stem SDW (g)	0.923 (0.986)	0.932 (0.996) ns	=	=	**	**
Root organic N (g)	Root DW (g)	0.962 (0.988)	0.960 (0.993) ns	=	=	*	*
Root organic N (g)	Root DW (g)	0.959 (0.988)	0.959 (0.993) ns	=	=	ns	*

4.4. DISCUSSION

4.4.1. Plant growth

Declining RGR

From 30 d of age onwards, both RGR and NAR declined progressively over time and with increasing size, in both ambient and elevated C_a (Table 4.1; Figs. 4.5 and Fig. 4.10). The declines in RGR and NAR found here are consistent with the experimental evidence in general (Evans, 1972; Poorter, 1993), unlike the unusually discontinuous time-courses of them found in the previous chapter (Table 3.1). Workers including Givnish (1986) and Poorter (1993) support the established view that increases in size involve an increasing investment of fixed carbon in supportive tissues relative to productive tissues. Here, the finding that the decline in RGR was essentially unaffected by the removal of TNC from the total dry weight (Table 4.1; Fig. 4.5) is consistent with a decline in RGR that is mediated by structural changes. However, the finding that these structural changes involved a decline in NAR rather than LAR (Fig. 4.5) suggests that the decline in RGR may be driven by changes in the way fixed carbon is used within productive tissues, rather than by changes in the partitioning of fixed carbon between productive and supportive tissues.

The effects of elevated C_a on growth

The results reported in Chapter 3 (Table 3.1) indicated that the initial stimulation of RGR in elevated C_a was driven by increased NAR but constrained by an early reduction in LAR, which in turn was due to a reduction in SLA without a significant alteration in LWR. This pattern was confirmed here for mean values over the 0 - 4 d harvest interval (Table 4.1) as well as for instantaneous values during this period (Figs. 4.5 and 4.6). Consistent also with the results of the previous chapter, the CO_2-stimulation of RGR declined over time. Thus, although the $\overline{RGR}$ of plants in elevated C_a was stimulated over both harvest intervals during the 10 d period, the stimulation over days 0 - 4 was larger than that over days 4 - 10 (Table 4.1). Similarly, the functional approach to growth analysis described (inevitably) linear declines in RGR over time (Section 2.3.2), such that

RGR in elevated C_a declined more rapidly than RGR in ambient C_a, and the initial stimulation of RGR in elevated C_a was sustained for no more than about 8 d (Fig. 4.5). Very similar durations of the CO_2-stimulation of RGR have been reported in studies of similar design using other herbaceous C_3 species (Den Hertog *et al.*, 1993, 1996; Stulen *et al.*, 1994; Fonseca *et al.*, 1996).

A difference between the results here and those of Chapter 3 lies in the response of $\overline{NAR}$ in terms of its impact on $\overline{RGR}$. In the previous chapter, the decline in the CO_2-stimulation of $\overline{RGR}$ was linked specifically to a decline in the stimulation of $\overline{NAR}$ without a further reduction in the early reduction in $\overline{LAR}$ (Table 3.1). In the present chapter, however, no significant reduction in the CO_2-stimulation of $\overline{NAR}$ could be linked to the reduction in the CO_2-stimulation of $\overline{RGR}$ over the 4 - 10 d harvest interval, and instead $\overline{RGR}$ was constrained by a further reduction in $\overline{LAR}$ due to a reduction in $\overline{SLA}$ (Table 4.1). A further difference between the results here and in Chapter 3 is that $\overline{LWR}$ was significantly greater in elevated C_a here (Table 4.1).

However, the functional analysis of growth provided a description of RGR and its components more consistent with that reported in the previous chapter. Thus, a close examination of the curves of RGR, NAR and LAR between days 4 and 10 (Fig. 4.5) suggests that the reduction in the CO_2-stimulation of RGR over this time may in fact be coupled more closely to a reduction in the CO_2-stimulation of NAR than to a further decrease in the CO_2-diminution of LAR (Fig. 4.5). It is possible, therefore, that a reduction in the CO_2-stimulation of NAR did occur, but only towards the end of the 10 d period. Possible explanations for the discrepancies between the descriptions of growth provided by classical and functional approaches will be discussed later in the present chapter.

Specific absorption rate of N by roots

The mean specific absorption rate of N by roots, $\overline{SAR_N}$, increased in elevated C_a over days 0 - 4, in parallel with the initial CO_2-stimulation of $\overline{RGR}$. However, the CO_2-stimulation of $\overline{RGR}$ over days 4 -10 was not coupled to any increase in $\overline{SAR_N}$ (Table

4.1). Whilst this finding is inconsistent with the results of Chapter 3 where $\overline{SAR}_N$ was never stimulated by elevated C_a (Table 3.1), it is nevertheless consistent with the conclusion reached in Chapter 3 that the mismatching of SAR_N to RGR in elevated C_a conflicts both with the close coupling that has been found in other CO_2-enrichment studies (Stulen *et al.*, 1994), and with expectations of an up-regulation of N uptake in response to increased assimilate availability (Gastal and Saugier, 1989). Small, statistically insignificant decreases in N_P (Fig. 4.9.D) and shoot:root ratio (S/R) (implicit in the lower allometric coefficient describing it; Table 4.3) may explain this reduction in SAR_N by elevated C_a, as was concluded in Chapter 3 after a longer period of exposure when the changes in N_P and S/R were more pronounced and statistically significant (Section 3.4.4). However, the changes in SAR_N, N_P and S/R in elevated C_a that are suggested in the present chapter occur too late as to be involved in the early constraint on growth imposed by decreased LAR, and are otherwise poorly correlated to the later reduction, if any, of the CO_2-stimulation of NAR.

Structural growth

The effects of elevated C_a on RGR and its components were essentially unaffected by the removal of TNC from the total dry weight (Table 4.1; Figs. 4.5, 4.6 and 4.7). Whilst a number of CO_2-enrichment studies have accounted for TNC in the expression or presentation of selected data of growth-related parameters, often with conflicting results (Chapter 3; Acock and Pasternak, 1986; Wong, 1990; Poorter, 1993; Baxter *et al.*, 1994a; Den Hertog *et al.*, 1996; Roumet *et al.*, 1996), the present study is the first to determine the overall impact of TNC accumulation on growth. As such, it is clear that the CO_2-stimulation of RGR, as well as its decline, can reflect not only the way fixed carbon is partitioned, but more particularly the way fixed carbon is used in respiratory processes. This information allows for a more precise targeting of CO_2-sensitive processes. For example, it was proposed in Chapter 3 that the CO_2-diminution of LAR indicates that the fixed carbon available in elevated C_a may not be used as in ambient C_a to generate the equivalent leaf area, whilst the strong CO_2-diminution of SLA relative to LWR suggests that a proportion at least of the fixed carbon is exported to the sites of leaf development, but is subsequently partitioned into weight rather than area. It is now possible to deduce at least one key area limiting growth in elevated C_a, namely the use and partitioning of fixed carbon in developing leaves. Clearly therefore, respiratory use of carbon in elevated C_a represents a target for future investigation, and will be dealt with in Chapter 6.

Evaluation of the classical vs. functional approach

In general, there was close agreement between the descriptions of growth provided by classical and functional analysis. However, the functional approach did suggest a late reduction in the CO_2-stimulation of NAR (Fig. 4.5), a reduction which was not found using the classical approach (Table 4.1). A late response of NAR would be readily detectable (but perhaps also exaggerated) by functional analysis by means of the quadratic terms describing the relevant primary data of dry weight and leaf area (Fig. 4.3, Table 4.2). In contrast, crude averages of $\overline{N}AR$ over days 4 - 10 obtained by classical analysis will be relatively insensitive to short-term changes. More importantly perhaps, the calculation of $\overline{N}AR$ by the classical approach assumes a fixed relationship between leaf area and dry weight (Evans, 1972). Thus, major advantages of this and similar curve-fitting functional approaches over the classical approach are that dynamic changes in growth are more describable, and no such assumption is made in the calculation of NAR. On the other hand, major disadvantages of the functional approach are that its descriptions of growth are constrained entirely by the order of polynomial selected, and that it is less amenable to statistical analysis due to the difficulties encountered when attempting to compare fitted curves (Hunt, 1982; Poorter, 1989).

The first obstacle to an objective comparison of fitted curves arises if the primary data is fitted best by polynomials of different order. Some workers have argued on biological grounds for the fitting of a common order of polynomial regardless of the statistical significance of the fit (Hurd, 1977). Others have argued that the statistical significance of the fit stands as the only valid description of growth (Hunt and Parsons, 1974). Here, this problem was largely avoided since second order polynomials were the best fit to the primary data in both ambient and elevated C_a, with single exception of organic N content over time in ambient C_a (Fig. 4.4). The next obstacle arises due to difficulties in interpreting the significance of differences in the terms of non-linear polynomials, especially concerning quadratic and higher-order terms, since their variances do not stand independently of one another (Hunt, 1982).

Because of these difficulties, little or no biological significance may therefore be implied by the statistical insignificance of the quadratic terms (Table 4.2), which determine the changes over time in RGR and its components. Coupled with the generally high coefficients of determination describing the fits of the regressions to the primary data (Table 4.2), there is some justification for considering that the time-courses of RGR and its components calculated here using the functional approach are essentially correct descriptions. However, it may also be prudent to acknowledge the argument of Wickens and Cheeseman (1988) and Poorter (1989) that the functional approach used in the description of short-term environmental changes carries the risk that dynamic, short-term changes in growth will be undetected. Poorter (1989) recommends an analytical approach that combines many of the advantages of the classical approach with those of the functional approach. Whilst this synthetic approach has been used successfully in similar studies of growth in elevated C_a (Fonseca *et al.*, 1996), the short-term responses in that and the present study were very similar.

The size-dependency of RGR and its components

The results strongly suggest that the growth responses to elevated C_a are genuine, direct effects of CO_2, rather than indirect effects of plant size. Thus, the time-dependent declines in the CO_2-stimulation of RGR and NAR persisted when RGR and NAR were plotted against indices of size (Fig. 4.10). The same conclusion was reached by Fonseca *et al.* (1996) for RGR (on a fresh weight basis) of *Plantago major*, but conflicts with that of Poorter *et al.* (1988), for the same species, who found that the declines in both RGR (on a dry weight basis) and NAR were negated when plotted against leaf area as a index of size. An allometric approach to the analysis of LAR confirmed the findings and conclusions of Chapter 3, such that the reductions in LAR and SLA in elevated C_a also occurred independently of size, since the GMR lines describing their allometric relations were both significantly displaced downwards (Table 4.3).

The effects of elevated C_a on allometric relations persisted after the removal of TNC, indicating that the reductions in LAR and SLA (for example), cannot be attributed either to accelerated plant size or to an accumulation of TNC. This conclusion agrees with that of Chapter 3, but conflicts with the few studies which have also attempted to account for interference by both ontogeny and TNC within the same set of data (Den Hertog *et al.*, 1996), and with others which have investigated these factors in isolation to conclude that the reduction in SLA can be explained entirely by ontogeny (Gebauer *et al.*, 1996; Stirling *et al.*, 1998) or entirely by TNC (Wong, 1990; Poorter, 1993; Baxter *et al.*, 1994a; Roumet *et al.*, 1996). Such conflicting evidence may arise because of differences in analytical procedures. For example, Den Hertog *et al.* (1996) based their conclusions by plotting SLA against plant dry weight (with all dry weight components corrected for TNC), but offered no statistical analysis. Other studies have used the established allometric approach to allow a more objective analysis of the size-dependency of SLA (Gebauer *et al.*, 1996; Stirling *et al.*, 1998), but it was argued in Chapter 3 that the way such workers have interpreted the results of allometric analysis may be flawed.

The argument proposed was that differences in GMR elevations indicated early changes in v (Section 3.4.3). This view is partly substantiated here, since v describing SLA was significantly decreased in elevated C_a during the first 10 d of exposure, although, contrarily, v describing LAR was not significantly decreased (Table 4.3). Whilst it is likely that significant changes in the allometric relations describing LWR influenced the allometric analysis of LAR, the usual allometric approach that fits only linear regressions through data-sets is likely to miss what could be early, dynamic changes in allometric relations. There may be a case for refining the allometric approach to address these early changes, particularly in the description of the effects of environmental discontinuities. A similar argument has already been discussed for the analysis of growth (Wickens and Cheeseman, 1988; Poorter, 1989).

4.4.2. The role of organic N

The concentration of organic N in whole plants (N_P) was never significantly lower in elevated C_a during the 10 d of exposure (Fig. 4.9). In plant parts (Fig. 4.9), only the organic N concentration in leaves per unit structural dry weight (N_L) was significantly reduced by elevated C_a, and only at day 10 after the early CO_2-diminution of LAR but possibly coincident with the decline in the CO_2-stimulation of NAR. It is possible that the reduction in N_P found in Chapter 3 and frequently reported in studies of similar or greater duration (Luo *et al.*, 1994; Stulen *et al.*, 1994; Den Hertog *et al.*, 1996) requires a more prolonged exposure to elevated C_a, but the results of the present chapter make it clear that reductions in N_P have no role in causing the changes in NAR and LAR that constrain growth in elevated C_a. It is also clear that reductions in N_L have no obvious role in causing the changes in LAR, but a role for N_L in the response of NAR cannot be precluded.

The significant increase in the organic N concentration in leaves per unit area (N_{LA}) at day 4 in elevated C_a (Fig. 4.8) could perhaps be related to changes in the photorespiratory cycling of N that will inevitably occur through the CO_2-suppression of photorespiration. Within seconds or minutes after exposure to elevated C_a, lower rates of photorespiration (Bowes, 1991) will mean that less organic N within the leaf is committed to support photorespiration through the recycling of photorespiratory-generated NH_4^+ via glutamate using the same pathway involved in NO_3^- assimilation (Keys *et al.*, 1978; Somerville and Ogren, 1980). The commitment of N to photorespiration is considerable, such that the flux of NH_4^+ through the pathway may be as much as 10 times the rate of NO_3^- assimilation (Wallsgrove *et al.*, 1983).

From my own calculations, a flux of photorespiratory NH_4^+ through the pathway about 5 times greater than the rate of NO_3^- assimilation can be estimated with a few basic assumptions. Thus, a final C/N ratio of 8 after 50 % of the initially available fixed carbon has been released in the processes of dark respiration (Chapters 5 and 6; Amthor, 1989) implies the assimilation of 0.8 mol NO_3^- for every 24 mol CO_2 fixed in primary carboxylation. In turn, for every 24 mol CO_2 fixed, 4 mol NH_4^+ and 4 mol CO_2 would be released in photorespiration given a typical stoichiometry of 3 for the ratio of carboxylation to oxygenation at ambient C_a (Ogren, 1984). The CO_2-suppression of photorespiration is such that ratios of carboxylation to oxygenation could increase to as much as 8 in twice-ambient C_a depending on temperature (Laing *et al.*, 1974; Ogren, 1984). Continuing the example given, this would reduce the photorespiratory release of NH_4^+ to 1.5 mol for every 24 mol CO_2 fixed, such that the rate of photorespiratory NH_4^+ cycling would be reduced to less than twice the rate of NO_3^- assimilation.

One possible consequence of reduced photorespiration in elevated C_a may therefore be an early, sizeable, but transient increase in the availability of amino-N in leaves for use in processes other than photorespiration. After export, it is not inconceivable that the additional N could even drive a transient stimulation of growth. Moreover, a lower demand for N to support photorespiration may also explain why N_L frequently declines in the longer term in elevated C_a. However, this view is clearly not consistent with the increase in leaf organic N found at day 4 in elevated C_a (Fig. 4.8). Perhaps the CO_2-suppression of photorespiration reduces N loss by NH_4^+ volatilization from a cycle more leaky than has been supposed (Wallsgrove *et al.*, 1983), but clearly there may be a case for future investigations into the possibility that early changes in processes linked to N metabolism may be perturbed by elevated C_a with significant physiological consequences.

4.4.3. Conclusions

The initial CO_2-stimulation of RGR declined within 10 d of exposure to elevated C_a, due primarily to an early and sustained reduction in LAR, but probably also to a reduction in the CO_2-stimulation of NAR in the late stages of exposure. The changes in RGR, NAR and LAR were direct effects of elevated C_a, rather than indirect effects due to ontogeny or interactions between plant size and environmental constraints. A possible role for organic N in the initial CO_2-stimulation of RGR has been suggested, but no convincing evidence was found to support the hypothesis that the reductions in RGR, NAR and LAR are caused by a reduction in the concentration of organic N in plant tissues. The next chapter will investigate the role of soluble sugars in mediating these early changes in growth.

CHAPTER 5

Growth of *Urtica urens* in elevated CO_2: II. Early changes and the role of soluble sugars in signalling a sink-source imbalance

5.1. INTRODUCTION

In Chapter 4, it was concluded that within 10 d of exposure to an elevated atmospheric CO_2 concentration (elevated C_a) a reduction in the CO_2-stimulation of net assimilation rate (NAR) and a reduction in leaf area ratio (LAR) were both responsible for the decline in the initial CO_2-stimulation of relative growth rate (RGR) of *Urtica urens*. This transient stimulation of RGR in elevated C_a is consistent with other studies (Poorter *et al.*, 1988; Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Fonseca *et al.*, 1996; Stirling *et al.*, 1998). In agreement with Fonseca *et al.* (1996), the decline in the stimulation of RGR occurred at least partly independently of plant size and therefore involved direct effects of elevated C_a on processes limiting growth. Since the independence of size-effects extended to both NAR and LAR (Chapter 4), elevated C_a may directly affect these components of RGR by the same or by different mechanisms. In Chapter 4, no evidence was found to indicate a role of organic N concentration as a signal in the decline in the initial CO_2-stimulation of RGR. The present chapter investigates the role of soluble sugars in causing the early changes in growth described in Chapter 4, a hypothesis for which there is a large body of circumstantial evidence (Stitt, 1991; Van Oosten *et al.*, 1994; Pollock and Farrar, 1996), but remains unproved.

In an extensive review of CO_2-enrichment studies, Stitt (1991) argued that the growth response to elevated C_a is determined by the way in which the plant responds to a change in the balance between the activities of sinks and sources (concepts defined in Section 1.4.3, p. 6), which is signalled by an increased amount of soluble sugar available due to the stimulation of photosynthesis. There are three principal ways in which a plant could respond to this additional soluble sugar. Firstly, the additional sugar could accumulate as non-structural carbon in existing, new, specialized or non-specialized sites of storage (storage sinks), without consequence for growth other than the maintenance in time of a CO_2-stimulation of RGR. Such use of fixed carbon does not carry the potential to increase the immediate stimulation of RGR further (Wong, 1990), but may nevertheless be important to species which have evolved longer-term strategies to maximize their survival and competitive ability. In this respect, Chapter 4 showed conclusively that storage of non-structural carbon was not an important consideration in describing the growth of *U. urens* in elevated C_a.

Secondly, the additional sugar could be used in the processes of dark respiration (R_d) and deposited as structural material in regions of active cell division and expansion (meristematic sinks). Such use probably underlies the stimulation of RGR in *U. urens*. Unless elevated C_a fundamentally alters the way in which both additional and existing soluble sugar is used in R_d, such use should result at the very least in the maintenance in time of a CO_2-stimulation of RGR, but will carry the potential to increase the stimulation of RGR further if it increases the production of new resource-aquiring structure such as leaf area (Kramer, 1981; Körner, 1991). Any increase in structure will require a proportional increase in R_d dedicated to its biosynthesis (Farrar and Williams, 1991). Since there is now strong evidence that soluble sugars act widely at the level of gene expression not only in the up-regulation of R_d to enable their use as respiratory substrate, but also in the initiation of additional new sinks for their use (Farrar and Williams, 1991; Pollock and Farrar, 1996), a transient accumulation of soluble sugars at the sites of active growth could be expected to precede any CO_2-stimulation of RGR. However, a decline in the stimulation of RGR could occur if the accumulation of soluble sugars affects morphogenesis, resulting perhaps in a reduction in LAR.

Thirdly, an inability to use the additional soluble sugars in either meristematic or storage sinks could feed-back to down-regulate the rate of photosynthesis, thence NAR and hence RGR (Poorter, 1993). The current view is that the down-regulation of photosynthesis in elevated C_a is caused by the accumulation in leaves of soluble sugars, acting to repress the expression of genes encoding photosynthetic proteins such as rubisco and chlorophyll-binding protein (Van Oosten *et al.*, 1994; Krapp and Stitt, 1995; Pollock and Farrar, 1996). Figure 5.0 shows a model that incorporates a number of core hypotheses to investigate the role of sink capacity in causing the decline in the CO_2-stimulation of RGR. The reasoning behind this model can be summarized as follows.

From the evidence and arguments presented by Pollock and Farrar (1996), a clearly-defined sequence of events can be expected linking reduced NAR to an inability to utilize all the additional assimilate available in elevated C_a. In the first place, the rate of assimilate use by a growing plant or plant part will be proportional to its rate of respiration (Farrar and Williams, 1991). Therefore, an inability to utilize all the additional assimilate available in elevated C_a should manifest as a reduction in R_d relative to photosynthesis, coupled closely to an accumulation of soluble sugars in sinks. It is arguable that the more-or-less simultaneous measurement of all these parameters provides one of the few quantitative approaches to the concept of sink capacity. Any accumulation of soluble sugars in sinks in elevated C_a may also have implications for morphogenesis (Pollock and Farrar, 1996), including perhaps the determination of LAR (Fig. 5.0).

With the assumption that movement of solutes in the phloem is by bulk flow driven by turgor-pressure gradients (Munch, 1930), an accumulation of soluble sugars in sinks may then result in the transmission in the phloem of an increased sugar concentration and turgor pressure from the sites of unloading to the sites of loading. In turn, phloem-loading may be inhibited in some way by the increased sucrose concentration and/or turgor to cause an accumulation of soluble sugars in source leaves. Alternatively, an increased sugar concentration in leaves could occur independently of any reduction in sink capacity, if the sugars are needed to drive a higher flux of solutes in the phloem. Sugar accumulation in leaves is widely and consistently reported in CO_2-enrichment studies (Chapter 3; Farrar and Williams, 1991), and may be responsible for the down-regulation of photosynthesis due to repression of genes as described earlier (Van Oosten *et al.*, 1994; Krapp and Stitt, 1995). It is perhaps significant that such a role of sugars is now firmly established in other experimental systems (Sawada *et al.*, 1987; Krapp *et al.*, 1991; Paul and Driscoll, 1997). The consequent down-regulation of photosynthetic capacity of individual leaves may then be manifest as a reduction in NAR and hence RGR (Poorter, 1993). In this chapter, attempts are made to quantify the various components of the model described above and illustrated in Figure 5.0. Together with the analysis of growth presented in Chapter 4, this information is used to try to answer the fundamental question: Does sink capacity limit growth in elevated C_a?

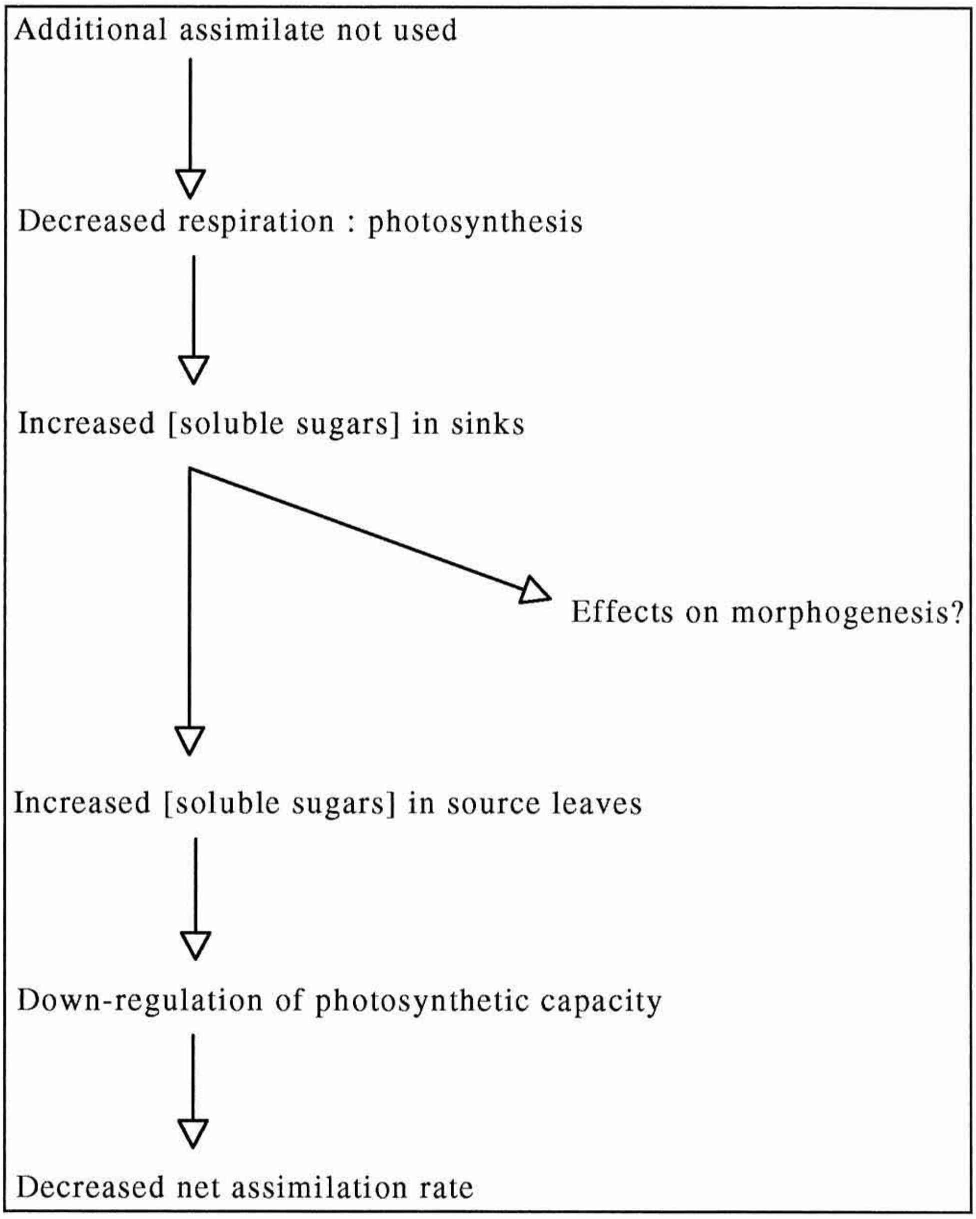

Figure 5.0. Hypothetical model to explain the consequence for net assimilation rate in a plant unable to use additional assimilate available in elevated C_a. The accumulation of soluble sugars in sinks may perhaps also affect morphogenesis.

5.2. MATERIALS AND METHODS

5.2.1. Growth conditions

Plants of *U. urens* were grown in a hydroponic culture system in controlled-environment cabinets. This chapter combines data from different but comparable experiments. Data of responses at the individual-leaf level were obtained from plants grown strictly as described in Section 2.2. These plants were switched from ambient to elevated C_a (680 μmol mol^{-1}) for 11 d at a defined shoot height of 7.5 cm (± 0.5 cm), approximately 30 d after sowing. Data on responses at the whole-plant level were obtained using a sub-set of plants grown as described in Chapter 4 (Section 4.2.1). As was described in Section 4.2.1, these plants were switched from ambient to elevated C_a (700 μmol mol^{-1}) for 10 d at a defined fresh weight of 8 g (± 0.75 g) (with a shoot height of 7 - 8 cm) approximately 30 d after sowing. Henceforth, the day at which exposure to elevated C_a began will be designated as day 0.

5.2.2. CO_2 exchange in whole plants

Net photosynthesis (A) and dark respiration (R_d) of intact shoots and roots were measured as CO_2 exchange rate (CER) using an open system (Section 2.6.1). Measurements were made in the laboratory, but under conditions of CO_2, humidity, PPFD, temperature and nutrient supply equivalent to those in the controlled-environment cabinet (Sections 2.6.2,3 and 4). A and R_d of one plant grown in ambient C_a and one plant grown in elevated C_a were measured each day as CER min^{-1} (Section 2.6.5). The calculation of gross photosynthesis (A_G) and the extrapolation of CER min^{-1} to give daily rates of A, A_G and R_d are described in Section 2.6.5. Other bases for the expression of CER (e.g. s^{-1} and as carbon flux) should be self-explanatory. Data were integrated over the two time intervals used for classical growth analysis as described in the previous chapter (Section 4.2.2), to give n = 4 (days 1 - 4) and n = 6 (days 5 - 10). To relate more closely CO_2 exchange and growth, $\overline{RGR}$ of the sub-set of plants used for measuring gas exchange was calculated for each time interval as the slope of the linear regression fitted to logarithmically-transformed data of their structural dry weights.

5.2.3. CO_2 exchange in individual leaves

Measurements of net photosynthesis (A) were made at days 0, 2, 6 and 10 on one of the two paired leaves borne at the 5th node on the main stem (leaf number 5), using a combined CO_2/H_2O analysis system and clamp-on leaf cuvette (CIRAS-1, PP Systems, Hitchin, Herts., UK), as described in Section 2.5.1. The stage of leaf expansion was monitored by measuring the area (Digital image analysis system, Delta-T Ltd., Cambridge, UK) of paper leaf replicates. A was measured at the approximate PPFD incident on the leaf under growth conditions (520 μmol m^{-2} s^{-1}) to give A_{sat} at the C_a of growth and also allow calculation of the percent change in A due to elevated C_a during measurement. A was also measured at near-saturating PPFD (1000 μmol m^{-2} s^{-1}) at range of intercellular CO_2 concentrations (C_i) between 50 and 1300 μmol mol^{-1} for the construction of A/C_i curves (Section 2.5.2). The maximum rate of carboxylation limited by the amount and/or activity of rubisco ($V_{c,max}$) and the maximum attainable rate of photosynthesis at saturating light intensity and C_i (A_{max}) were derived from these curves following the approach of McMurtrie and Wang (1993), as described fully in Section 2.5.2.

5.2.4. Leaf total soluble protein, rubisco and photosynthetic pigments

Leaf disks were cut from leaf number 5 at day 11 and rapidly frozen in liquid N_2. Soluble proteins were extracted from 1.9 cm^2 frozen leaf material as described in Section 2.9.1 (Quick *et al.*, 1991). Total soluble protein was quantified according to the method of Bradford (1976) (Section 2.9.2). Rubisco protein was determined in the same extracts (Hibberd *et al.*, 1996a) using a densitometer to scan rubisco large subunit bands isolated by polyacrylamide gel electrophoresis (Laemmli, 1970) and stained with Coomassie Brilliant Blue dye (Section 2.9.3; Fig. 2.10).

Photosynthetic pigments (chlorophylls *a* and *b*, and carotenoids) were extracted in 80 % (v/v) acetone from 1.9 cm^2 of frozen leaf material (MacKinney, 1941) and quantified spectrophotometrically according to Lichtenthaler and Wellburn (1983). The procedure is described fully in Section 2.10.

5.2.5. Non-structural carbohydrates

Plants were harvested as described in the previous chapter (Section 4.2.2). Soluble sugars were extracted from oven-dried and frozen plant material, sequentially in ethanol and then in deionized water (Farrar and Farrar, 1985) (Section 2.7.1). Starch was extracted from the material remaining after extraction of soluble sugars and converted to glucose (Lustinec *et al.*, 1983) (Section 2.7.1). For bulk determination of non-structural carbohydrate concentrations in plant tissues (leaves, roots, stems and whole plants), extractions of 30 - 40 mg well-mixed oven-dried material were analyzed according to Dubois *et al.* (1956) (Section 2.7.2).

Non-structural carbohydrates and starch were also determined in defined leaf (1.9 cm^2 of leaf number 5) and root (1 - 2 g fresh weight of root tips < 1 cm in length) tissues taken at day 11 and rapidly frozen in liquid N_2. For theses tissues, soluble carbohydrates were analyzed using high performance liquid chromatography (HPLC) (Cairns and Pollock, 1986) (Section 2.7.3) to allow quantification of particular carbohydrate species.

5.2.6. Organic N

Both the method of determination and the actual data of the organic N concentration in plant tissues have been described (Section 4.2.3) and shown (Figs. 4.8 and 4.9) in the previous chapter. In the present chapter, measurements of organic N are included only in the determination of the ratio of soluble sugars to organic N, and as a basis for the expression of other data.

5.2.7. Allometric analysis

Allometric analysis (Pearsall, 1927; Troughton, 1955) was used to distinguish between effects of elevated CO_2 and size on photosynthesis and R_d, as described in Section 2.4. Geometric mean regressions (GMRs) were fitted to logarithmically-transformed data:

$$\log_e y = \log_e b + v \log_e x$$

where y and x were data of dry weight (including and excluding TNC), leaf area and whole-plant CO_2 exchange. The GMR slope (v) represents the allometric coefficient (Ricker, 1984; Farrar and Gunn, 1996) and the constant b expresses the regression intercept of y when x is zero.

5.2.8. Source-sink differentiation

Within the broad definition of sources as regions that produce and export assimilate and of sinks as those that import assimilate for growth or storage (Farrar, 1996), sources were considered as leaves (unfolded leaves > 1 cm length) and sinks as stems (including shoot apices, folded leaves, leaves $\leq$ 1 cm length and inflorescences) and as entire root systems.

5.2.9. Statistical analyses

Student's t-test or two-way (CO_2 and time) analysis of variance followed by Tukey-tests were used to test for significant differences in non-structural carbohydrates, in the integrated data of whole-plant photosynthesis and respiration (Section 5.2.2), and in photosynthesis in individual leaves. For functional analysis of growth, significant differences between corresponding pairs of constants in the fitted equations were analyzed as described in Section 2.3.2. For allometric analyses, differences in v, the displacement of the GMR line (elevation), and deviation of v from unity were tested for statistical significance using Student's t-test as described by Zar (1989). Differences in the slopes of linear regressions used in the analysis of data other than by allometry were tested using the same approach (Zar, 1989). All statistical analyses were performed using the computer software package SPSS (Prentice Hall, New Jersey).

5.3. RESULTS

5.3.1. CO_2 exchange in whole plants

In both ambient and elevated C_a, net photosynthesis (A) in entire shoot systems and whole plants declined over the experimental period (Fig. 5.1). Similar declines over time were found for the rate of dark respiration (R_d) in shoots, roots and whole plants (Fig. 5.2). The data of photosynthesis expressed as shown in Figure 5.1, and expressed on different bases, are shown in Table 5.1 as means derived by pooling the individual measurements within two time intervals (days 1 - 4; days 5 - 10). Similarly integrated data of R_d expressed as shown in Figure 5.2, and expressed on different bases, are shown in Table 5.2. The declining values of both A (Fig. 5.1) and R_d (Fig. 5.2) contributed to the large standard errors of the mean values derived by integration, and no significant effects of elevated C_a were found on either A (Table 5.1) or R_d (Table 5.2) in any plant part or using any basis of expression.

Mean $\overline{R}$GRs over each time interval of whole plants, shoots and roots of plants in the sub-set on which measurements of CO_2 exchange were made are also shown in Table 5.1. Differences in mean values due to elevated C_a were not statistically significant, even over the 0 - 4 d interval.

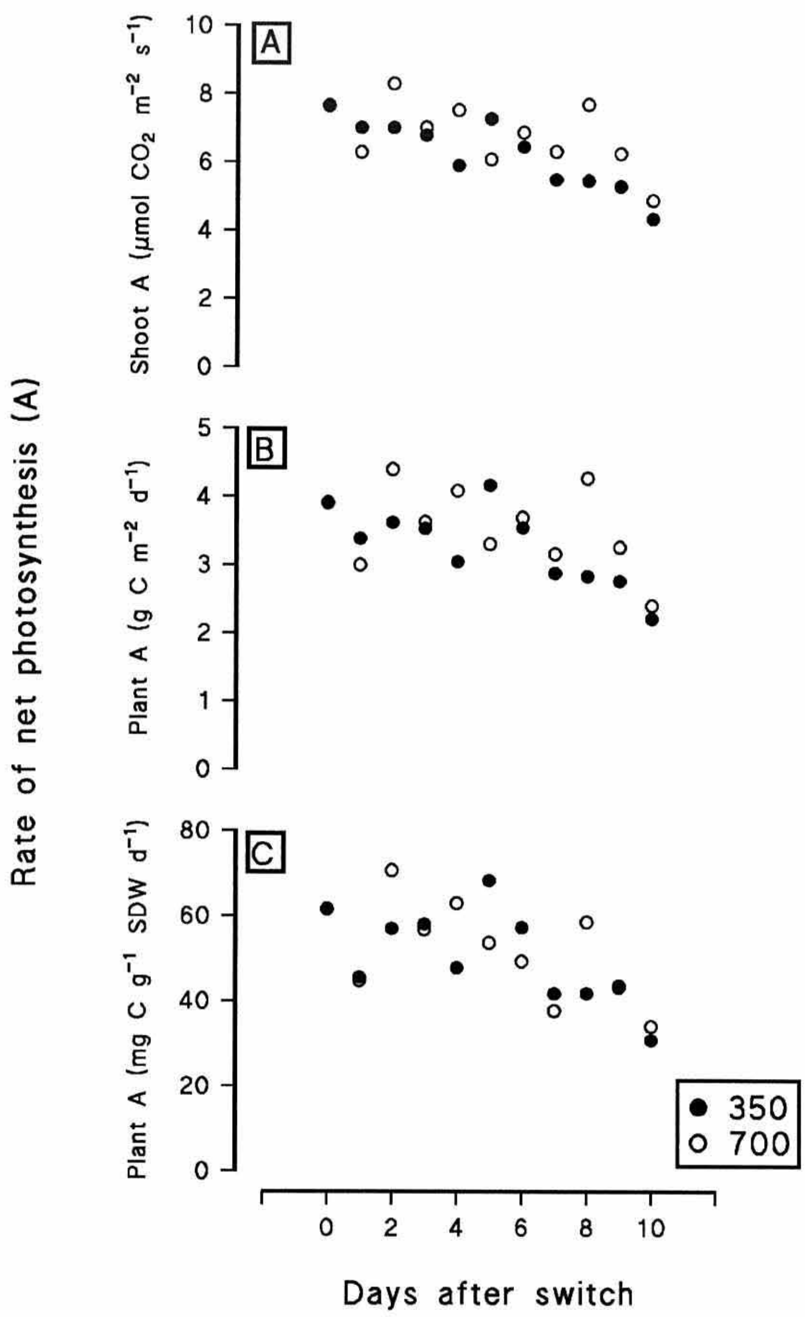

Figure 5.1. Net photosynthesis in entire shoots and whole plants of *U. urens* switched from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. (A) Net photosynthesis in shoots during measurement in the light, expressed per unit leaf area (and excluding root respiration), (B) Net photosynthesis in whole plants, expressed per unit leaf area and calculated as the projected daily rate of net C uptake (including total plant respiration), and (C) Net photosynthesis in whole plants, expressed per unit plant structural dry weight (SDW) and calculated as in (B). Data are shown as individual points. Data integrated as means over days 1 - 4 and over days 5 - 10 are shown in Table 5.1.

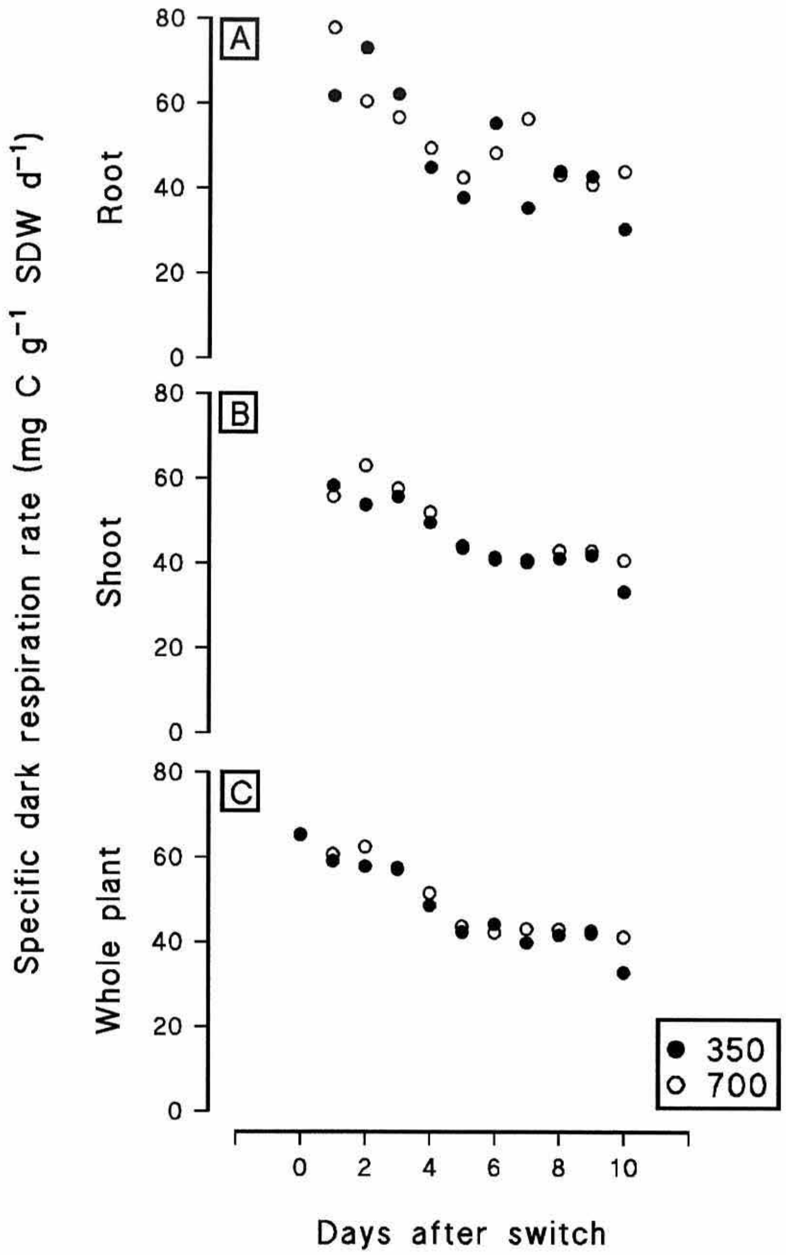

Figure 5.2. Dark respiration in (A) Roots, (B) Shoots and (C) Whole plants of *U. urens* switched from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Respiration is expressed per unit structural dry weight (SDW) and calculated as the projected daily rate of C efflux. Data are shown as individual points. Data integrated as means over days 1 - 4 and over days 5 - 10 are shown in Table 5.2.

Table 5.1. The rate of net photosynthesis (A) of intact shoots of *U. urens* switched from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a for 10 d at approximately 30 d of age. A was measured as CO_2 uptake and is expressed as uptake occurring during measurement in the light (µmol m^{-2} s^{-1}) including and excluding root respiration (+/- RTR_d), and as the projected daily rate of C uptake (g d^{-1}) including total plant respiration, per unit leaf area, dry weight (DW), structural dry weight (SDW) (= DW minus the weight of total non-structural carbohydrates), and organic N. Data are shown as means ± standard errors, with means derived by pooling individual measurements made over two periods of time (n = 4 at days 1 - 4; n = 6 at days 5 - 10). Also shown are mean relative growth rates, $\overline{R}GR$, over days 0 - 4 and days 4 - 10, of whole-plants, shoots and roots. $\overline{R}GR$ was calculated as the slope of linear regressions of $\log_e$ structural dry weight (SDW) over the appropriate time intervals, using only the plants on which measurements of gas exchange were made. Data of $\overline{R}GR$ are shown with the coefficient of determination (r^2) for each regression in parenthesis.

	Days 1 - 4		**Days 5 - 10**	
	Ambient C_a	**Elevated C_a**	**Ambient C_a**	**Elevated C_a**
Net photosynthesis (A)				
µmol CO_2 m^{-2} s^{-1} (-RTR_d)	6.7 (±0.26)	7.9 (±0.97)	5.7 (±0.41)	6.3 (±0.38)
µmol CO_2 m^{-2} s^{-1} (+RTR_d)	5.8 (±0.17)	7.0 (±0.98)	5.3 (±0.39)	5.8 (±0.36)
g C m^{-2} d^{-1}	3.4 (±0.12)	4.1 (±0.61)	3.1 (±0.28)	3.4 (±0.25)
mg C g^{-1} Shoot DW d^{-1}	61.9 (±3.5)	71.9 (±8.0)	54.5 (±7.0)	53.0 (±4.9)
mg C g^{-1} Shoot SDW d^{-1}	66.9 (±4.0)	77.0 (±8.7)	57.7 (±7.4)	56.4 (±5.1)
mg C g^{-1} Plant DW d^{-1}	48.6 (±2.8)	57.2 (6.6)	44.8 (±5.2)	43.6 (±3.7)
mg C g^{-1} Plant SDW d^{-1}	52.1 (±3.2)	60.9 (±7.1)	47.2 (±5.4)	46.2 (±3.9)
g C g^{-1} Leaf N d^{-1}	1.57 (±0.09)	1.93 (±0.24)	1.74 (±0.25)	1.64 (±0.12)
g C g^{-1} Shoot N d^{-1}	1.20 (±0.07)	1.47 (±0.18)	1.23 (±0.19)	1.17 (±0.10)
g C g^{-1} Plant N d^{-1}	0.96 (±0.06)	1.16 (±0.15)	1.00 (±0.14)	0.96 (±0.07)

	Days 0 - 4		**Days 4 - 10**	
$\overline{R}GR$ (mg SDW g^{-1} d^{-1})				
Whole plant	345 (0.985)	400 (0.983)	265 (0.995)	243 (0.976)
Shoot	350 (0.983)	407 (0.987)	279 (0.995)	248 (0.972)
Root	327 (0.985)	373 (0.960)	202 (0.956)	215 (0.982)

Table 5.2. The rate of dark respiration (R_d) in intact shoots and roots of *U. urens* switched from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a for 10 d at approximately 30 d of age. R_d was measured as CO_2 evolution in the dark, and is expressed as evolution occurring during measurement in the dark (µmol m^{-2} s^{-1}), and as the projected daily rate of C evolution (g d^{-1}) per unit dry weight (DW), structural dry weight (SDW) (= DW minus the weight of total non-structural carbohydrates), and organic N. Data are shown as means ± standard errors, with means derived by pooling individual measurements made over two periods of time (n = 4 at days 1 - 4; n = 6 at days 5 - 10).

	Days 1 - 4		**Days 5 - 10**	
Dark respiration (R_d)	**Ambient C_a**	**Elevated C_a**	**Ambient C_a**	**Elevated C_a**
Shoot R_d				
µmol CO_2 m^{-2} s^{-1}	2.7 (±0.17)	2.9 (±0.21)	2.1 (±0.06)	2.4 (±0.08)
mg C g^{-1} Shoot DW d^{-1}	50.3 (±1.5)	53.3 (±1.9)	38.2 (±1.4)	40.2 (±0.4)
mg C g^{-1} Shoot SDW d^{-1}	54.3 (±1.8)	57.1 (±2.3)	40.4 (±1.5)	41.9 (±0.6)
g C g^{-1} Shoot N d^{-1}	0.97 (±0.01)	1.09 (±0.06)	0.85 (±0.04)	0.87 (±0.02)
g C g^{-1} Leaf N d^{-1}	1.28 (±0.02)	1.42 (±0.08)	1.21 (±0.06)	1.23 (±0.05)
Root R_d				
mg C g^{-1} Root DW d^{-1}	58.0 (±5.5)	59.0 (±5.7)	39.6 (±3.4)	44.3 (±2.2)
mg C g^{-1} Root SDW d^{-1}	60.5 (±5.8)	61.2 (±6.0)	41.0 (±3.5)	45.9 (±2.3)
g C g^{-1} Root N d^{-1}	1.20 (±0.04)	1.18 (±0.10)	0.88 (±0.09)	0.99 (±0.12)
Whole plant R_d				
mg C g^{-1} Plant DW d^{-1}	52.0 (±1.9)	54.6 (±2.0)	38.4 (±8.2)	40.2 (±0.4)
mg C g^{-1} Plant SDW d^{-1}	55.7 (±±2.4)	58.1 (±2.4)	40.5 (±1.6)	42.7 (±0.3)
g C g^{-1} Plant N d^{-1}	1.02 (±0.02)	1.14 (±0.05)	0.85 (±0.05)	0.89 (±0.03)
R_d as % of gross daily A				
Whole plant R_d	51.7 (±1.6)	49.3 (±3.1)	46.9 (±2.0)	48.5 (±2.0)
Shoot R_d	39.4 (±1.3)	38.1 (±1.3)	38.7 (±2.2)	39.2 (±1.7)
Root R_d	12.4 (±1.0)	11.1 (±1.8)	8.2 (±0.5)	9.3 (±0.6)

5.3.2. Non-structural carbohydrates at the whole-plant level

The concentrations of non-structural carbohydrates in leaves, stems, roots and whole plants per unit structural dry weight over the 10 d period are shown in Figure 5.3. The concentration of soluble sugars in leaves was significantly increased by elevated C_a at day 10 only, with the increase amounting to about 30 % (Fig. 5.3.A). Elevated C_a did not at any time significantly increase the concentrations of soluble sugars, starch or TNC in either stems or roots. Starch was present in stems for only the first 4 d, and was never detected in roots. The significant increase in whole-plant TNC at day 10 in elevated C_a was therefore due principally to the accumulation of soluble sugars in leaves. Figure 5.3 also shows the ratios of the amount of soluble sugars to organic N in leaves, stems, roots and whole plants. The ratios were not affected by elevated C_a except in leaves at day 10, where the ratio was about 40 % greater.

The amount of non-structural carbohydrates per unit area of leaf over the 10 d period is shown in Figure 5.4. In leaves, the patterns of the amounts of soluble sugar, starch and TNC over time were essentially the same as those described previously on a structural dry weight basis, with a significant increase in soluble sugars at day 10 of about 50 % The amount of starch in leaves was highly variable, but in general starch persisted over time in elevated C_a in contrast to its progressive depletion in leaves of plants grown in ambient C_a. The amount of starch was never more than about 20 % of the amount of soluble sugars, so that the time-courses of the amount of total non-structural carbohydrate (TNC) in leaves reflected that of soluble sugars.

Elevated C_a did not significantly affect the concentration of TNC (comprised solely of soluble sugars) measured at day 11 in root tips (Table 5.3), although the TNC concentration was greater in root tips than that at day 10 in whole root systems (Fig. 5.12.K). Inulin, stachyose, raffinose and mannitol were not detected in root tips, nor was the carbohydrate species with a retention time coincident with that of glycerol, as was detected in some leaf material (Section 5.3.5).

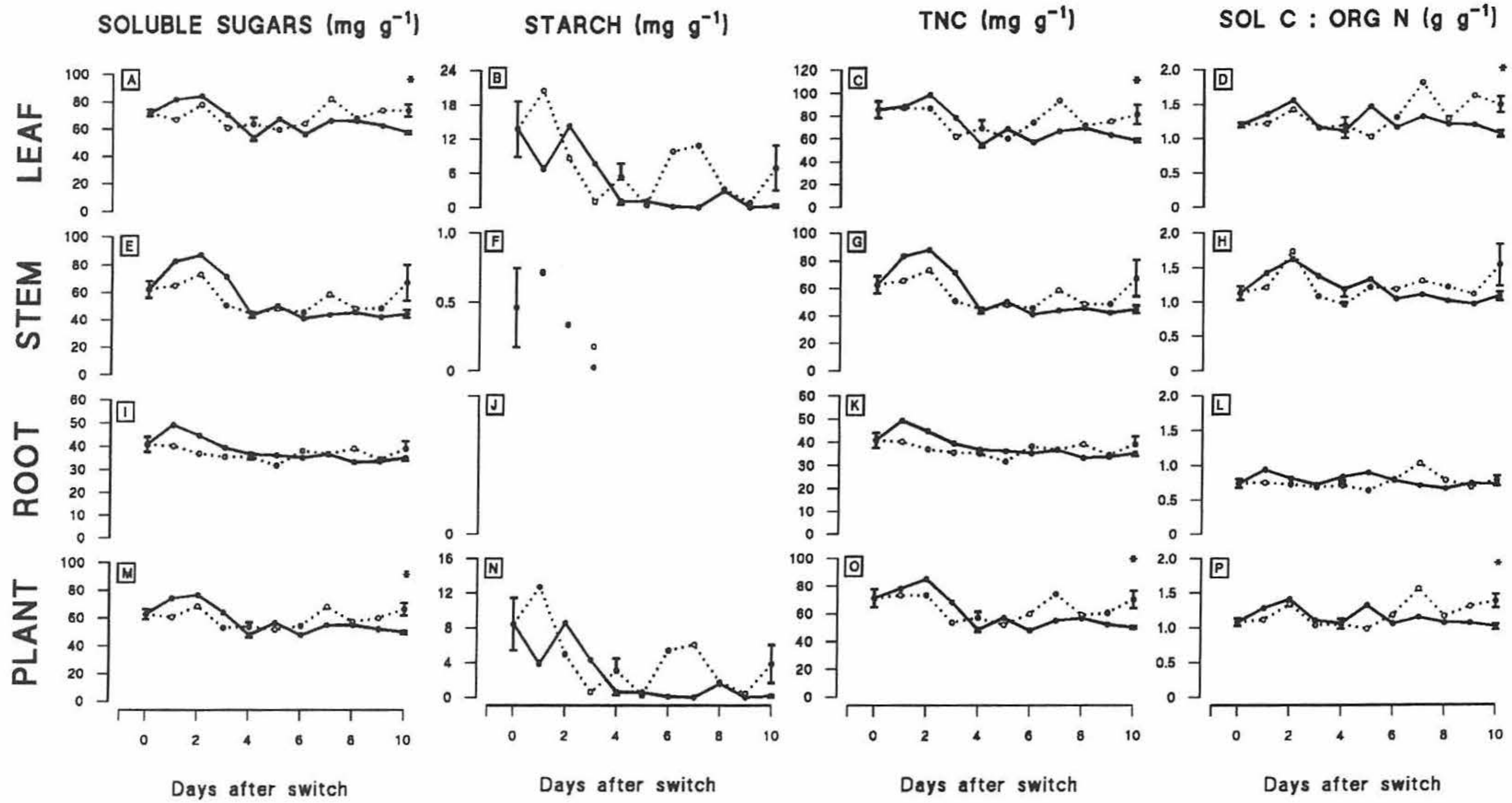

Figure 5.3. Concentration per unit structural dry weight (SDW) of soluble sugars (A,E,I,M), starch (B,F,J,N) and total non-structural carbohydrate (C,G,K,O), and the ratio of soluble sugars to organic nitrogen (D,H,L,P) in leaves (A,B,C,D), stems (E,F,G,H), roots (I,J,K,L) and whole plants (M,N,O,P) of *U. urens* during 10 d of growth after switching plants from ca. 350 µmol mol^{-1} (solid lines, filled circles) to 700 µmol mol^{-1} (dotted lines, open circles) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown individually (days 1, 2, 3, 5, 6, 7, 8 and 9), or as means (n = 4 - 5) (days 0, 4 and 10) with standard error bars. Significant differences ($p < 0.05$) due to C_a at days 4 and 10 are indicated by asterisks. No starch was detected in stems after day 3 (F) or in roots at any time (J).

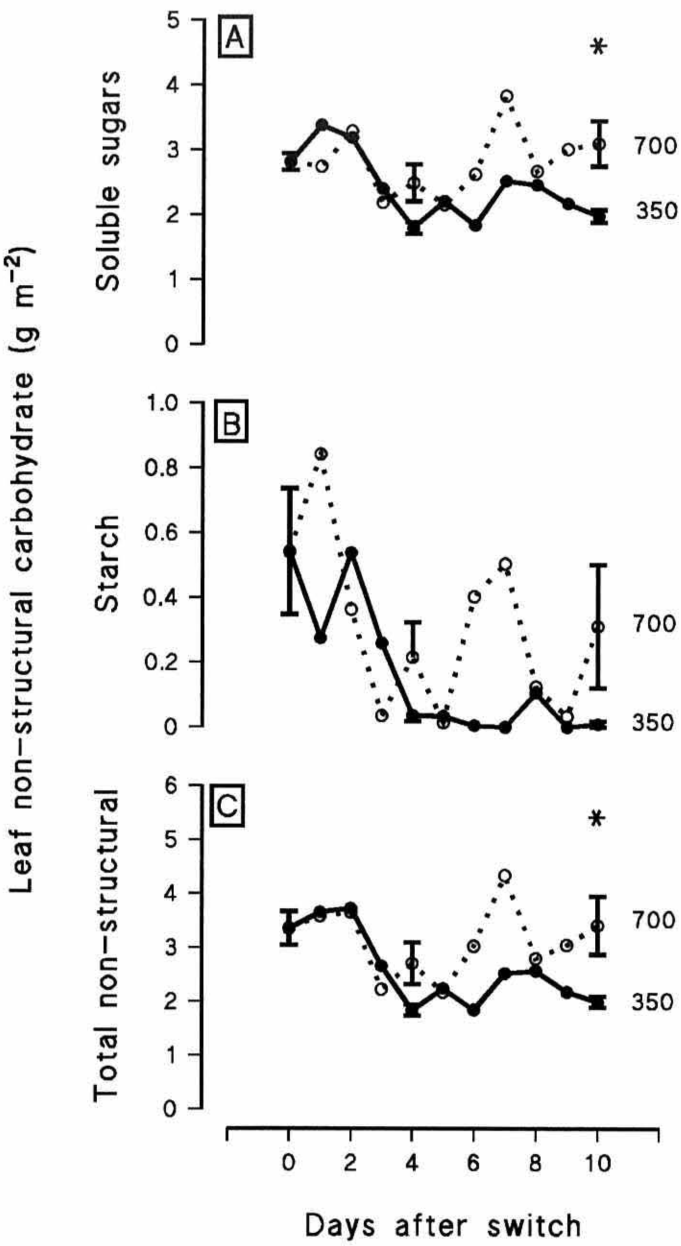

Figure 5.4. Concentration per unit area of (A) Soluble sugars, (B) Starch and (C) Total non-structural carbohydrates in leaves of *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown individually (days 1, 2, 3, 5, 6, 7, 8 and 9), or as means (n = 4 - 5) (days 0, 4 and 10) with standard error bars. Significant differences ($p < 0.05$) due to C_a at days 4 and 10 are indicated by asterisks.

Table 5.3. Concentrations of non-structural carbohydrates in roots of *U. urens* 11 d after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (680 µmol mol^{-1}) C_a. Data are shown as means (n = 5) ± standard errors. Soluble carbohydrates were identified and quantified by high performance liquid chromatography (HPLC).

FRACTION	CARBOHYDRATE	CONCENTRATION (mg g^{-1} SDW)	
		Ambient C_a	Elevated C_a
Ethanol-soluble	Sucrose	1.5 (±0.2)	3.5 (±1.5)
	Glucose	19.5 (±3.7)	33.4 (±9.3)
	Fructose	17.3 (±2.6)	31.4 (±8.1)
	Unidentified	8.5 (±0.9)	8.3 (±2.0)
	Total	44.3 (±12.0)	70.3 (±16.0)
Water-soluble	Unidentified	23.9 (±3.0)	19.5 (±6.8)
Total soluble		68.1 (±13.1)	89.7 (±19.3)
Insoluble	Starch	0	0
Total non-structural		68.1 (±13.1)	89.7 (±19.3)

5.3.3. Allometric analysis

A summary of the allometric analyses describing whole-plant CO_2 exchange is given in Table 5.4, and examples of geometric regressions fitted to the primary data are shown in Figures 5.5 and 5.6. No significant effects of elevated C_a on the allometric coefficient (v) were found, but in elevated C_a the elevations were higher in the GMRs relating both A and shoot R_d to leaf area (Fig. 5.5), and in those relating R_d in roots, shoots and whole plants to their respective structural dry weights (Fig. 5.6).

Table 5.4. Allometric relations describing specific rates of photosynthesis (A) and respiration (R_d) in intact shoots and roots of *U. urens* switched at approximately 30 d of age from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d. The table gives a summary of analyses of geometric mean regressions (GMRs) describing logarithmically-transformed variables (x, y) of A, R_d, leaf area, dry weight (DW), structural dry weight (SDW) and organic N content. Table shows the GMR slope (*v*, the allometric coefficient) with the coefficient of determination (r^2) for each GMR in parenthesis, the relative elevation of the GMR line (↑, ↓ and = denoting an upwards, downwards and no significant displacement of the GMR line), and whether ***v*** deviates significantly from unity (slope ≠ 1). SDW is DW minus the weight of total non-structural carbohydrate (TNC). *, ** and *** indicate significant differences due to C_a at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively; ns indicates that differences are not significant.

Variable		**GMR slope (*v*)**		**Elevation**		**Slope ≠ 1**	
$\log_e$ y	**$\log_e$ x**	**350**	**700**	**350**	**700**	**350**	**700**
A (g C d^{-1})	Leaf area (m^2)	0.865 (R-sq = 0.977)	0.955 (R-sq = 0.947) ns	↓	↑ **	**	ns
A (μmol CO_2 min^{-1})	Leaf area (m^2)	0.848 (R-sq = 0.988)	0.882 (R-sq = 0.966) ns	↓	↑ **	***	*
Plant R_d (μmol CO_2 min^{-1})	Plant DW (g)	0.805 (R-sq = 0.993)	0.833 (R-sq = 0.995) ns	↓	↑ **	***	***
Plant R_d (μmol CO_2 min^{-1})	Plant SDW (g)	0.797 (R-sq = 0.993)	0.829 (R-sq = 0.995) ns	↓	↑ **	***	***
Shoot R_d (μmol CO_2 min^{-1})	Shoot DW (g)	0.856 (R-sq = 0.994)	0.876 (R-sq = 0.990) ns	-	-	***	**
Shoot R_d (μmol CO_2 min^{-1})	Shoot SDW (g)	0.846 (R-sq = 0.993)	0.873 (R-sq = 0.990) ns	-	-	***	**
Shoot R_d (μmol CO_2 min^{-1})	Total leaf area (m^2)	0.883 (R-sq = 0.990)	0.936 (R-sq = 0.984) ns	↓	↑ *	**	ns
Root R_d (μmol CO_2 min^{-1})	Root DW (g)	0.646 (R-sq = 0.895)	0.725 (R-sq = 0.956) ns	↓	↑ *	***	***
Root R_d (μmol CO_2 min^{-1})	Root SDW (g)	0.643 (R-sq = 0.896)	0.724 (R-sq = 0.956) ns	↓	↑ *	***	***

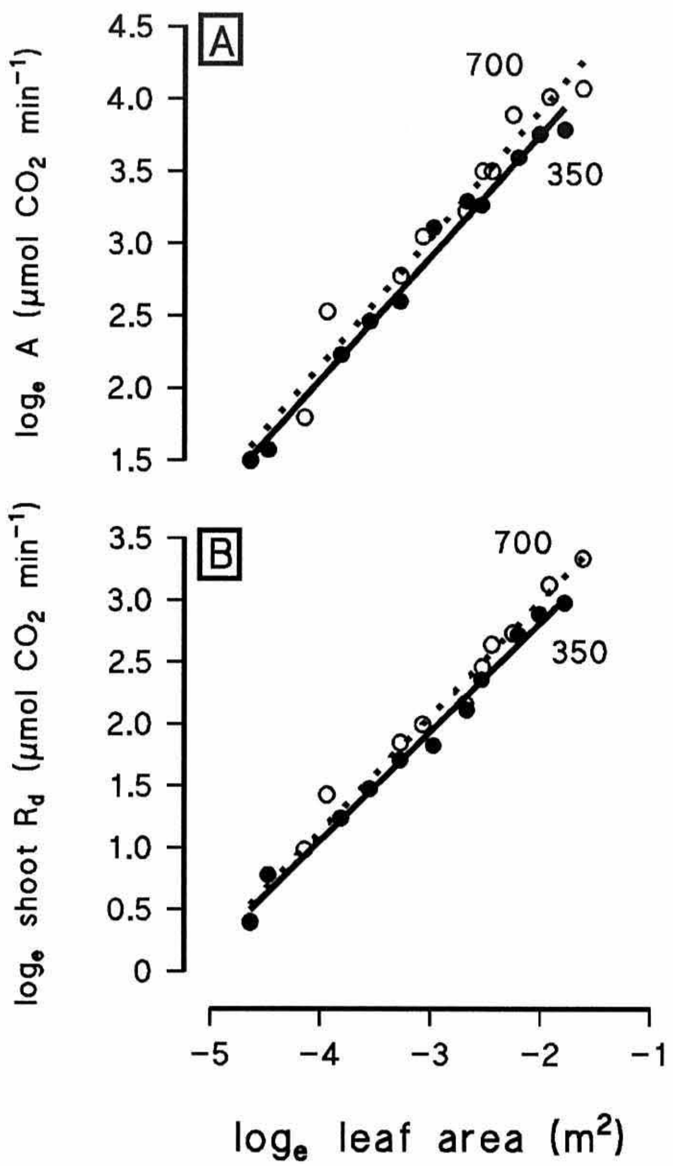

Figure 5.5. Geometric mean regressions (GMRs) of logarithmically-transformed data of leaf area against (A) Net photosynthesis, A, excluding root respiration, and (B) shoot dark respiration (R_d) in *U. urens* switched from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d when approximately 30 d of age. The GMR slope (v), its relative elevation, and its coefficient of determination (r^2) are given in Table 5.4.

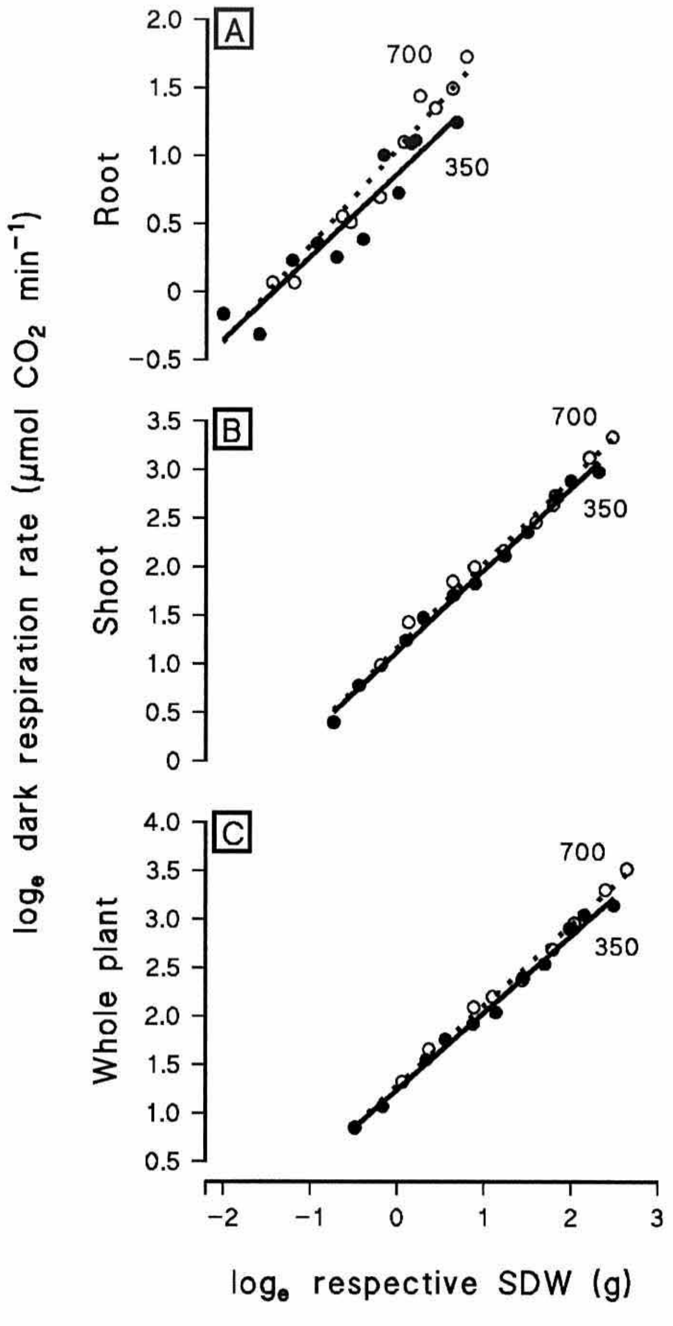

Figure 5.6. Geometric mean regressions (GMRs) of logarithmically-transformed data of structural dry weight (SDW) against the rate of respiration in (A) Roots, (B) Shoots and (C) Whole plants of *U. urens* switched from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a for 10 d when approximately 30 d of age. The GMR slope (v), its relative elevation, and its coefficient of determination (r^2) are given in Table 5.4.

5.3.4. CO_2 exchange in individual leaves

Neither the rate of expansion or final area of leaf number 5 was significantly affected by elevated C_a, and full expansion of this leaf was attained 6 - 7 d after switching (Fig. 5.7). The rate of net photosynthesis (A) in leaf number 5 in elevated C_a was significantly greater by about 30 % throughout the experimental period, when measured at the PPFD incident on the leaf under the conditions of growth (Fig. 5.8.A). In both ambient and elevated C_a, A declined after full expansion of the leaf, and the stimulation of A due to elevated C_a increased over time. The mean percent increase due to elevated C_a was consistently higher in leaves of plants grown in elevated C_a, but this was significant only at day 3 (Fig. 5.8.B). Neither $V_{c,max}$ or A_{max}, measured at days 6 and 10, was affected by elevated C_a, but the rates of both declined after full expansion of the leaf in both ambient and elevated C_a (Fig. 5.9).

5.3.5. Tissue composition at the single-leaf level

Total soluble protein, rubisco and photosynthetic pigments

The concentrations per unit area in leaf number 5 at day 11 of total soluble protein, rubisco protein, chlorophylls and carotenoids are shown in Table 5.5. Neither total soluble or rubisco protein was affected by elevated C_a. The proportion of soluble protein that is rubisco was unusually high, so that actual values of rubisco content are probably erroneous (Section 2.9.3). Although the increases in mean chlorophyll *a* and *b* contents in elevated C_a were individually insignificant, their combination meant that the total chlorophyll content was significantly greater in elevated C_a by about 15 %. Elevated C_a did not affect the ratio of chlorophylls *a* : *b*, or the amount of carotenoid pigments.

Non-structural carbohydrates

The concentration per unit area of total non-structural carbohydrates (TNC) in leaf number 5 at day 11 was significantly greater in elevated C_a (Table 5.6), but the accumulation of TNC could not account for the reduction in specific leaf area of the same leaf (Table 5.7). The accumulation of TNC in elevated C_a was due principally to an increase in the amount of sucrose, whilst the amounts of glucose, fructose and starch were not significantly affected. Inulin, stachyose, raffinose and mannitol were not detected, but about half of samples analyzed by HPLC contained a carbohydrate species with a retention time coincident with that of glycerol (data not shown or included).

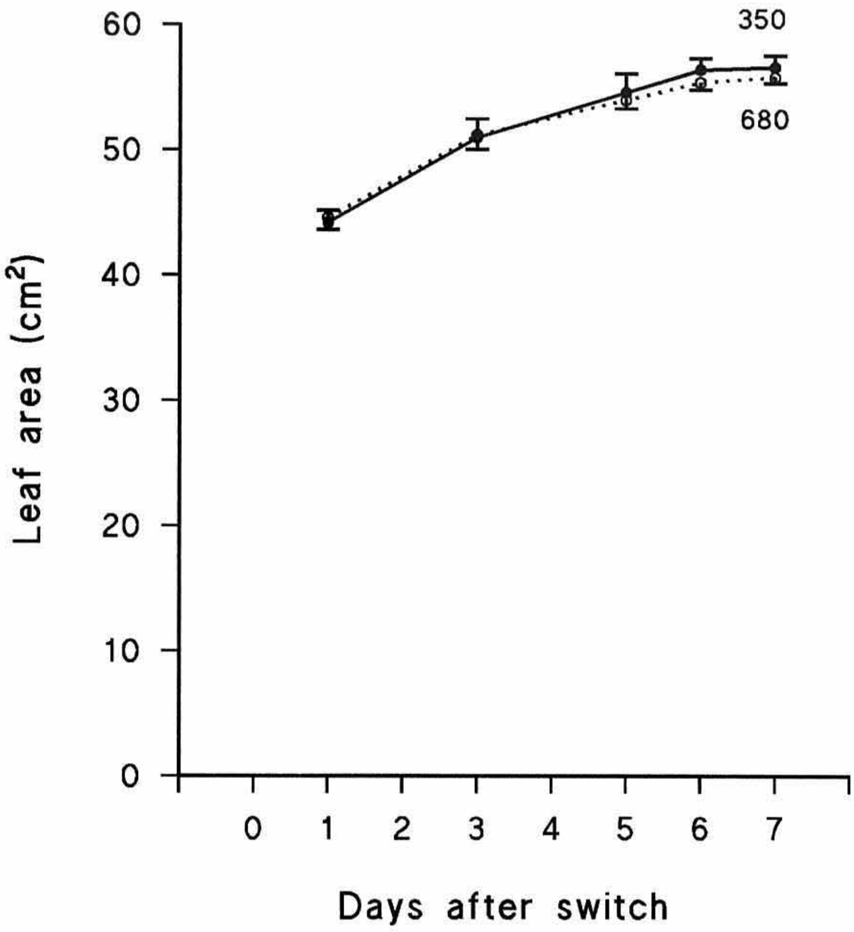

Figure 5.7. Leaf area expansion of one of the two paired leaves borne at the 5th node on the main stem (leaf number 5) of *U. urens* during 7 d of growth after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (680 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown as means (n = 5) ± standard error bars. At full expansion (days 6 - 7), leaf number 5 was the youngest fully-expanded leaf.

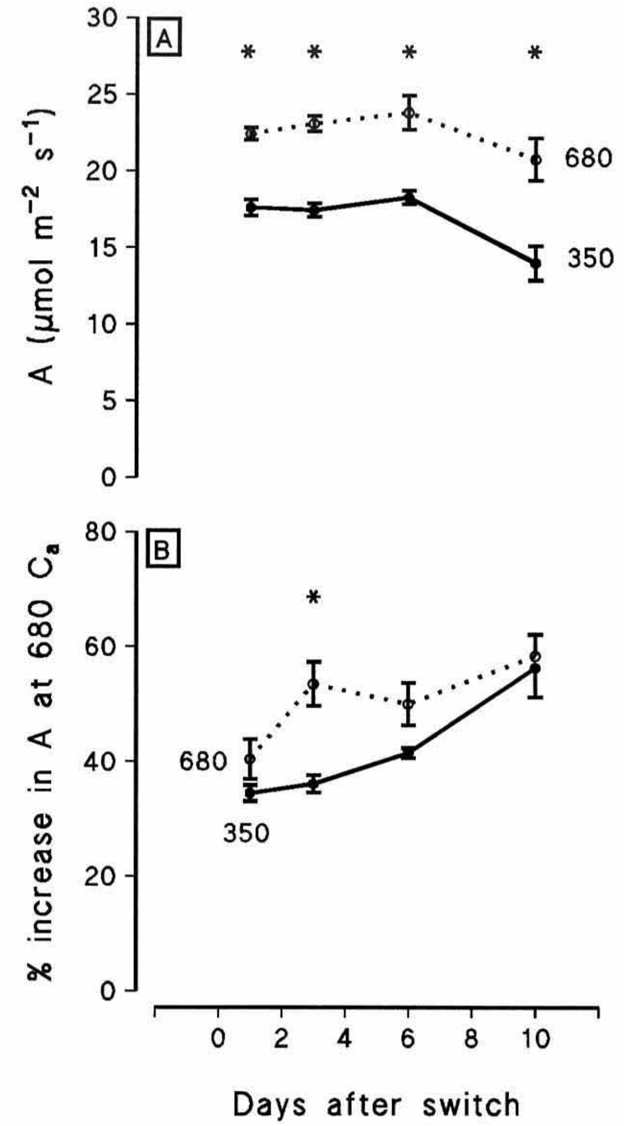

Figure 5.8. (A) The rate of net photosynthesis, A and (B) The percent stimulation of A by elevated C_a during measurement in a main-stem leaf (leaf number 5) of *U. urens* during 10 d of growth after switching plants from ambient (ca. 350 μmol mol^{-1}) to elevated (680 μmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown as means (n = 5) ± standard error bars. PPFD was incident on the leaf during measurement at 520 μmol m^{-2} s^{-1}, a rate equivalent to that under the conditions of growth. Full expansion of the leaf was attained at days 6 - 7. Significant differences ($p < 0.05$) due to C_a are indicated by aterisks.

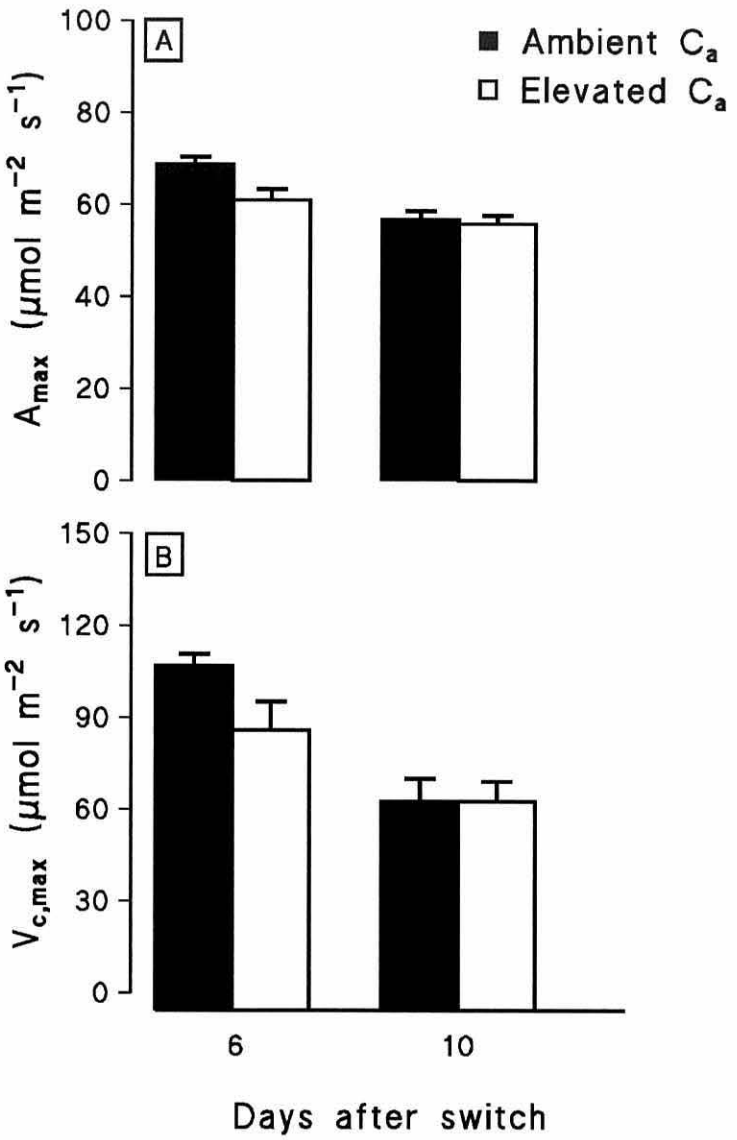

Figure 5.9. (A) The maximum rate of carboxylation ($V_{c,max}$) and (B) The maximum attainable rate of photosynthesis at saturating light intensity and C_i (A_{max}) in a main-stem leaf (leaf number 5) of *U. urens* at days 6 and 10 after switching plants from ambient (ca. 350 µmol mol^{-1}) to elevated (680 µmol mol^{-1}) C_a. Exposure to elevated C_a began at day 0 when plants were approximately 30 d old. Data are shown as means (n = 3 - 4) ± standard error bars. Incident PPFD was 1000 µmol m^{-2} s^{-1}. Full expansion of the leaf was attained at days 6 - 7.

Table 5.5. Concentrations per unit area of total soluble protein, rubisco protein, chlorophylls *a* and *b*, and total carotenoids in a main-stem leaf (leaf number 5) of *U. urens* 11 d after switching 30 d old plants from ambient (ca. 350 μmol mol^{-1}) to elevated (680 μmol mol^{-1}) C_a. Data are shown as means (n = 5) ± standard error bars. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks. An example of a polyacrylamide gel showing stained bands of rubisco protein after electrophoresis has been shown previously (Fig. 2.10).

	Ambient C_a		Elevated C_a	
Total soluble protein (g m^{-2})	9.0	(±0.4)	8.0	(±0.3)
Rubisco protein (g m^{-2})	6.3	(±1.0)	5.1	(±0.6)
Rubisco as % of soluble protein	72	(±13)	63	(±8)
Total chlorophyll (mg m^{-2})	350	(±10)	410	(±20) *
Chlorophyll *a* (mg m^{-2})	270	(±10)	320	(±20)
Chlorophyll *b* (mg m^{-2})	81	(±5)	95	(±5)
Ratio of chlorophyll *a* : *b*	3.4	(±0.3)	3.3	(±0.1)
Total carotenoids (mg m^{-2})	56	(±3.3)	63	(±5.1)

Table 5.6. Concentrations of non-structural carbohydrates in a main-stem leaf (leaf number 5) of *U. urens* 11 d after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (680 µmol mol^{-1}) C_a. Data are shown as means (n = 5) ± standard errors. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks. Soluble carbohydrates were identified and quantified by high performance liquid chromatography (HPLC).

FRACTION	CARBOHYDRATE	CONCENTRATION ($g\ m^{-2}$)	
		Ambient C_a	Elevated C_a
Ethanol-soluble	Sucrose	2.5 (±0.2)	5.1 (±0.9) *
	Glucose	2.7 (±0.1)	3.4 (±0.3)
	Fructose	4.5 (±0.4)	5.4 (±0.4)
	Unidentified	0.7 (±0.1)	0.5 (±0.1)
	Total	10.4 (±0.7)	14.4 (±1.4) *
Water-soluble	Unidentified	3.7 (±0.5)	3.3 (±0.4)
Total soluble		14.1 (±0.8)	17.7 (±1.5) *
Insoluble	Starch	1.1 (±0.2)	2.4 (±1.0)
Total non-structural		15.2 (±1.0)	20.1 (±1.7) *

Table 5.7. Specific leaf area of a main-stem leaf (leaf number 5) of *U. urens* 11 d after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (680 µmol mol^{-1}) C_a. Specific leaf area is calculated including and excluding the weight of total non-structural carbohydrate (TNC) (Tab. 5.3). Data are shown as means (n = 5) ± standard errors. Significant differences ($p < 0.05$) due to C_a are indicated by asterisks.

	SPECIFIC LEAF AREA ($cm^2\ g^{-1}$)	
	Ambient C_a	Elevated C_a
Including TNC	165.4 (± 4.1)	130.2 (± 9.1) *
Excluding TNC	215.1 (± 3.1)	172.5 (±12.1) *

5.4. DISCUSSION

5.4.1. Whole-plant photosynthesis and RGR

Relative growth rates in both ambient and elevated C_a were higher than the measurements of net photosynthesis of whole plants might predict. The concentration of C in plants typically ranges from about 35 - 46 % of the total dry weight (Rooney, 1994). Assuming that the concentration of C in *Urtica urens* is in the region of 40 %, a representative RGR of 300 mg g^{-1} DW d^{-1} (Table 5.1) requires a net C gain of 120 mg C g^{-1} DW d^{-1}. Clearly, the net C gain measured at approximately 50 mg C g^{-1} DW d^{-1} (Table 5.1) is too low to account for the RGRs observed. One possible explanation for this discrepancy may be differences in PPFD received by plants during the measurement of CO_2 exchange and that received by plants during growth in controlled-environment cabinets. Whilst care was taken during measurement to ensure an equivalent PPFD incident at the shoot apex, similar attention was not given to giving an equivalent amount of reflected PPFD, which may be considerable in the cabinet environment due to reflective walls. A similar discrepancy between net C gain and RGR could have arisen if the RGR of the sub-set of plants used in the measurement of CO_2 exchange differed significantly from the RGR calculated for the total set. However, this explanation is unlikely to apply here, since $\overline{R}GR$ of the sub-set (Table 5.1) was not lower than that of the total set over comparable time intervals (Chapter 4, Table 4.1).

5.4.2. The CO_2-stimulation of RGR

In contrast to the findings in Chapter 4, no significant CO_2-stimulation of RGR was found over days 0 - 4 (Table 5.1). Again, this discrepancy could be explained if the sub-set of plants used in the measurement of CO_2 exchange differed from the total set. Alternatively, the discrepancy may have arisen due to the different way in which $\overline{R}GR$ was calculated (Sections 4.2.2 and 5.2.2), a difference in approach considered most complimentary to the integrative analysis of whole-plant CO_2 exchange (Section 5.2.2), and otherwise necessitated by the lack of replication in the present chapter at days 0, 4 and 10. In the next chapter, a functional analysis allowing calculation of instantaneous RGR of the sub-set (Fig. 6.2; Table 6.2) shows a significant early CO_2-stimulation of

RGR similar to that found in Chapter 4 for the total set (Fig. 4.5; Table 4.2). Figure 6.2 also shows that the time-course of $\log_e$ structural dry weight of plants in the sub-set was not linear, with the result that values of r^2 for the linear regressions used to calculate $\overline{\mathrm{RGR}}$ in the present chapter were inevitably low. For these reasons, the different way in which $\overline{\mathrm{RGR}}$ was calculated, coupled to lower orders of replication, probably accounts for the absence of any statistically significant CO_2-stimulation of $\overline{\mathrm{RGR}}$. Similarly perhaps, the integration of non-replicated data of photosynthesis and repiration that were declining over time (Figs. 5.1 and 5.2) may have resulted in mean standard errors sufficiently large to render small CO_2 effects statistically insignificant (Tables 5.1 and 5.2). For these reasons, and respecting the statistical probability that elevated C_a did not affect either $\overline{\mathrm{RGR}}$ or whole-plant CO_2-exchange, the cautious accordance of biological significance to what appear to be small effects of elevated C_a may be useful, at least for the composition of hypotheses that could be tested in future experiments.

Following this line of argument, the responses of $\overline{\mathrm{RGR}}$, photosynthesis and respiration to elevated C_a can be described as follows. Over days 0 - 4, elevated C_a increased whole-plant $\overline{\mathrm{RGR}}$ by about 15 %, due to a 16 % increase in $\overline{\mathrm{RGR}}$ of shoots and a 14 % increase in that of roots (Table 5.1). Regardless of the units of expression, net photosynthesis of whole plants, integrated over days 1 - 4, was stimulated by elevated C_a by about 15 % (Table 5.1). Compared with the stimulation of $\overline{\mathrm{RGR}}$ and photosynthesis, elevated C_a resulted in only a small stimulation (about 4 %) of dark respiration (R_d) over the same time interval (Table 5.2). Consequently, whole-plant R_d as a percentage of gross photosynthesis was lower in elevated C_a over days 1 - 4 (Table 5.2).

Described in this way, the results invite a number of observations. Firstly, a CO_2-stimulation of RGR over the first time interval of about 14 % would be entirely consistent with a 15 % stimulation by elevated C_a of net C gain per unit dry weight (Table 5.1). As can be expected from the positive effects of elevated C_a on photosynthesis (Bowes, 1991), the rate of net photosynthesis (A) per unit area was increased in whole shoot systems (Table 5.1) and in individual leaves (Fig. 5.8). The larger and significant CO_2-stimulation of A found in individual leaves (30 %) compared with the stimulation found in entire shoot systems (15 %, if any) probably reflects the marked impact of the early reduction in LAR (Chapter 4) on constraining net C gain and RGR in elevated C_a.

Other issues are raised if the suggested uncouplings between gross photosynthesis and R_d and between R_d and structural RGR in elevated C_a are genuine. The simplest explanation for a reduction in R_d relative to photosynthesis is that a proportion of the additional assimilate in elevated C_a accumulates without respiratory use, perhaps reflecting a sink incapacity (Section 5.4.3). However, other explanations are needed here, since no accumulation of non-structural carbon occurred in plant tissues between days 1 - 4 in elevated C_a (Fig. 5.3). A reduction in R_d relative to photosynthesis could be a manifestation of the respiratory inhibition of R_d by CO_2 that has been frequently reported (Amthor *et al.*, 1992; Bunce, 1990; 1992; 1995; Bunce and Caulfield, 1991; Gifford *et al.*, 1985; Spencer and Bowes, 1986; Thomas and Griffin, 1994; Ziska and Bunce, 1993;). This possibility will be investigated further in Chapter 6.

A disproportionately large CO_2-stimulation of structural RGR relative to the CO_2-stimulation of R_d (Table 5.2) conflicts with the expectation that R_d will always be tightly coupled to RGR (Farrar and Williams, 1991). However, other reports exist indicating that the growth conversion efficiency of R_d is in some way increased by elevated C_a (Bunce and Caulfield, 1991; Ziska and Bunce, 1993). The possible effects of elevated C_a on functional components of R_d that relate to RGR will be examined in Chapter 6.

5.4.3. The decline in the CO_2-stimulation of RGR

It was found in Chapter 4 that the reduction in the CO_2-stimulation of NAR that was partly responsible for the decline in RGR occurred towards the end of the 10 d period (Fig. 4.5). As such, the sequence of events that would precede the decline in NAR as predicted by the model given previously (Fig. 5.0) should be readily detectable within the experimental window. Many components of the model were investigated using the total set of plants (Chapter 4), and are not, therefore, subject to the problems relating to the sub-set used in the measurements of whole-plant CO_2 exchange as discussed in Section 5.4.2. What evidence was found to support the hypothesis implicit in this model that the decline in the CO_2-stimulation of RGR occurs due to the signaling by soluble sugars of a sink-source imbalance?

Sink capacity and soluble sugar status in elevated C_a

The first event predicted by the model (Fig. 5.0) is a reduction in R_d relative to photosynthesis, coupled to an accumulation of soluble sugars in the actively respiring sites. Notwithstanding the arguments presented in Section 5.4.2, there was no convincing evidence to indicate that shoot, root or whole-plant R_d were reduced over days 1 - 4 as a percentage of gross photosynthesis (Table 5.2). Consistent with this, no accumulation of soluble sugars was found during or at the end of this time in shoots, roots or whole plants (Fig. 5.3). Together, these measurements of CO_2 exchange and sugar status in the early stages of exposure to elevated C_a argue strongly against any role of sink capacity in causing the later decline in the CO_2-stimulation of NAR. Moreover, sink capacity has no clear role in causing the early reduction in LAR in elevated C_a.

Measurements of CO_2 exchange and sugar status in the later stages of exposure to elevated C_a (days 5 - 10), which should arguably be more relevant to the later decline in the CO_2-stimulation of NAR, also indicated that the ability of sinks to utilize additional assimilate is not the factor responsible for the NAR response. Thus, R_d as a percentage of gross photosynthesis over this time interval was unaltered by elevated C_a (Table 5.2), and the small increases in the concentrations of soluble sugars in roots and stems at days 10 - 11 (Fig. 5.3; Table 5.3) were not only statistically insignificant, but were also evident after or at least coincident with the decline in the CO_2-stimulation of NAR.

Photosynthetic capacity and soluble sugar status in elevated C_a

By day 10, leaves in elevated C_a accumulated soluble sugars despite the absence of prior accumulation in sinks (Figs. 5.3 and 5.4). This suggests that the accumulation of sugars may be mediated by processes occurring within (e.g. export) rather than outside (e.g. sink capacity) the source leaf, but does not necessarily preclude the possibility that sugars are causal in the reduction in the CO_2-stimulation of NAR via the down-regulation of the photosynthetic capacity of individual leaves. However, the accumulation of soluble sugars in a representative main-stem leaf (Table 5.6) could not be linked to the down-regulation of photosynthetic capacity in this leaf, as indicated by a sustained increase in the rate of photosynthesis of about 30 % (Fig. 5.8). Also, elevated C_a did not significantly affect either $V_{c,max}$ or A_{max} in this leaf (Fig. 5.9), processes which are likely to respond to changes in the amounts of photosynthetic proteins that are sensitive to sugars through gene repression (Sharkey, 1985; Van Oosten *et al.*, 1994). Indeed, no significant effect of elevated C_a on the amounts of soluble protein and rubisco protein was found at day 11 (Table 5.5). These observations are consistent with those reported in Chapter 3, where an accumulation of soluble sugars in leaves also occurred without a reduction in photosynthetic capacity.

There are a number of possible explanations for the insensitivity of photosynthesis to soluble sugar status, an observation that contradicts other CO_2-enrichment studies (Van Oosten *et al.*, 1995) as well as studies using alternative means to increase the leaf soluble sugar concentration (Sawada *et al.*, 1987; Krapp *et al.*, 1991; Van Oosten and Besford, 1994). In the first place, the response is probably threshold-dependent (Pollock and Farrar, 1996; Hibberd *et al.*, 1996b), and the threshold sugar concentration may not be generally attained in CO_2-enrichment studies where exposure to no more than twice-ambient C_a is standard practice. Secondly, chemical and spatial partitioning of sugars both within and between cells and cell-types (Koroleva *et al.*, 1997) can mean that the additional sugars are effectively isolated from sensitive sites. In this respect, hexose may be the principle chemical species active in the repression of photosynthesis (Van Oosten *et al.*, 1994; Koch, 1996), or it may be involved in a more complex signaling mechanism such as the hexose:amino acid ratio (Paul and Driscoll, 1977). Similarly, only sugars accumulating in the cytosol of mesophyll cells can be expected to act on photosynthesis genes which are as yet known only to be nuclear (Van Oosten and Besford, 1994; Krapp and Stitt, 1995). With regard to at least some of these explanations for the apparent insensitivity of photosynthesis to soluble sugar status, it is perhaps significant that the concentration of hexose sugars was not significantly increased by elevated C_a (Table 5.6).

Although the stimulation of photosynthesis in elevated C_a was sustained at a similar magnitude over time (Fig. 5.8.A), and although neither $V_{c,max}$ or A_{max} were significantly affected by elevated C_a (Fig. 5.9), the percent stimulation of photosynthesis by elevated C_a during measurement was consistently greater in leaves of plants grown in elevated C_a, at least for the first 6 d (Fig. 5.8.B). This increase, which was particularly marked at day 3, suggests early changes in the physiology of photosynthesis independent of soluble sugars (Figs. 5.3 and 5.4). It is possible that relatively large early reductions in $V_{c,max}$ and/or increases in A_{max}, which could in principle underlie the observed response, were not detected due to the timing of measurements. Similarly, elevated C_a may have caused early alterations in stomatal behaviour.

To conclude this subsection, no evidence has been found here to indicate that the decline in the CO_2-stimulation of RGR occurs due to the signalling by soluble sugars of a sink-source imbalance. The results of Chapter 4 indicated no clear role for organic N in the response. What other explanations can be proposed?

Alternative explanations for the decline in the CO_2-stimulation of RGR

The CO_2-stimulation of photosynthesis per unit area in individual leaves was sustained in time, and was increased by about 30 % (Fig. 5.8). In contrast, the CO_2-stimulation of photosynthesis per unit area in entire shoot systems declined over time, and was never increased by more than about 15 % (Table 5.1), if at all. Because the incidence of PPFD per unit area was controlled during the measurement of photosynthesis at the single-leaf level, but was not similarly controlled during the measurement of photosynthesis at the whole-plant level, these observations strongly support the hypothesis, originally proposed in Chapter 3, that changes in canopy architecture occur in elevated C_a that reduce the amount of PPFD intercepted by shoot systems and hence reduce the CO_2-stimulation of NAR. In support of the hypothesis, Poorter *et al.* (1988) found an increased total leaf area relative to the projected total leaf area in *Plantago major* grown in elevated C_a. Similar alterations in canopy architecture may also explain the findings of Smart *et al.* (1994), where the attenuation of light through wheat canopies was less rapid in elevated compared with ambient C_a, although a decreased attenuation could also be caused by a reduction in SLA. Changes in canopy architecture could represent an important factor limiting the growth of plants in elevated C_a. As such, the hypothesis clearly demands a more direct investigation, and has received only limited attention in the literature (Poorter *et al.*, 1988).

5.4.4. Conclusions

From the evidence here, and from Chapter 4, it should now be clear that investigation of the decline in the CO_2-stimulation of RGR due to changes in LAR needs to be directed, not at whether the additional fixed carbon is used in elevated C_a, but at where and how it is used. Whilst it is possible that an increased flux of soluble sugars through respiratory processes in elevated C_a could in some way alter the leaf morphology, the remarkable finding that changes in R_d can be associated with reductions in LAR, even when plants are exposed to elevated C_a only during hours of darkness (Bunce, 1995), means that sugars once again have no clear role in the LAR response. Rather, it seems that some functional component of R_d is actually responsible for the partitioning of structural carbon subsequent to the respiratory use of fixed carbon. The next chapter investigates more fully the effects of elevated C_a on R_d, using the measurements of respiration presented here to analyze the responses of growth and maintenance respiration and presenting new data for respiration of individual leaves.

CHAPTER 6

Growth of *Urtica urens* in elevated CO_2: III. Early changes and the role of respiration

6.1. INTRODUCTION

Plant dark respiration (R_d), the net non-photorespiratory efflux of CO_2, is the final result of diverse physiological processes (Farrar, 1985). Predominant amongst these processes is the evolution of CO_2 arising directly or indirectly from the network of reactions involved in biosynthesis and energy production, which can be grouped into glycolysis, the pentose phosphate pathway, the tricarboxylic acid cycle, and mitochondrial electron transport. Energy generated by respiration can be used to drive nutrient uptake and to maintain existing structure, whilst the new structure built from the products of respiration is directly responsible for structural growth. However, other processes contribute to the influx or efflux of CO_2 in darkness, including the dark fixation of CO_2 by phosphoenol pyruvate carboxylase (PEPc) (Latzko and Kelly, 1983) and the evolution of CO_2 during the formation of ethylene (Lieberman, 1979).

Many studies have found that R_d is affected in some way by elevated C_a, with probably the commonest response being decreased R_d. This inhibition of R_d can be a direct, short-term effect in which R_d is reversibly decreased within minutes of exposing a plant or plant parts to elevated C_a (Bunce, 1990, 1994; Amthor *et al.*, 1992; Ziska and Bunce, 1993; Thomas and Griffin, 1994), as well as an indirect, longer-term effect in which R_d is irreversibly decreased within weeks or months of exposure to elevated C_a (Gifford *et al.*, 1985; Spencer and Bowes, 1986; Bunce, 1990, 1994; Bunce and Caulfield, 1991; Ziska and Bunce, 1993; Wullschleger *et al.*, 1994). From the limited evidence available, it appears that a longer period of growth in elevated C_a can interact with the short-term effect, so that the direct inhibition of R_d by elevated C_a is either moderated (Thomas and Griffin, 1994) or completely annulled (Bunce, 1990).

Not all studies find decreased R_d in elevated C_a. In the longer term, both unaltered (Baxter *et al.*, 1995) and increased (Azcon-Bieto and Osmond, 1983; Hubec *et al.*, 1985; Poorter *et al.*, 1988) R_d have been reported. To add to these conflicting respiratory responses, no convincing evidence was found in Chapter 5 that R_d was significantly affected by elevated C_a, when R_d of intact shoots and roots grown in elevated C_a for 10 d was compared to R_d of those grown in ambient C_a.

As yet, both the mechanistic bases for and the physiological significances of these different respiratory responses to elevated C_a are unclear. In principle, it is possible to reconcile the conflicting reports of the indirect, longer-term effects of elevated C_a on R_d in terms of our current understanding of respiratory function and control (Farrar and Williams, 1991), such that, firstly, R_d is stoichiometrically linked to structural growth rate, and secondly, R_d is up-regulated by substrate supply in a manner suggestive of coarse control of respiratory enzymes by sugars. Hence, the different indirect effects of elevated C_a on R_d may simply reflect the characteristic time- and size-dependent changes in relative growth rate (RGR) and carbon assimilatory capacity (Chapter 3; Chapter 4; Poorter *et al.*, 1988; Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Fonseca *et al.*, 1996; Stirling *et al.*, 1998). Indeed, studies by Azcon-Bieto and Osmond (1983) and Hubec *et al.* (1985) demonstrate clearly that increased rates of leaf expansion and photosynthesis in elevated C_a are closely coupled to increases in R_d and leaf carbohydrate content.

However, the results of Chapter 5 suggested that R_d of shoots, roots and whole plants was not significantly increased by elevated C_a, despite significant increases in their respective structural RGRs. Similar alterations to the relationship between R_d and RGR were noted by Bunce and Caulfield (1991), who found that both decreased and unaltered whole plant R_d can underlie CO_2-stimulations of RGR. Because the results of Chapter 5 indicated that the proportion of carbon lost via respiration relative to carbon gained via photosynthesis was probably unaltered by elevated C_a, improvements in respiratory efficiency may be an important component of any CO_2-stimulation of RGR. In theory, such improvements could arise if there are reductions in either or both the growth and maintenance components of R_d (Thornley, 1970; Penning de Vries, 1975; McCree, 1982). Indeed, decreases in both growth and maintenance R_d have been reported in CO_2-

enrichment studies (Wullschleger *et al.*, 1992; Wullschleger and Norby, 1992; Ziska and Bunce, 1993), although different responses have also been found, including no change in the growth component but an increased maintenance R_d (Thomas and Griffin, 1994).

Following the arguments of some workers (Amthor, 1991; Ryan, 1991; Wullschleger *et al.*, 1994), reductions in growth and maintenance R_d could be direct consequences of decreased tissue N concentrations in elevated C_a, which would lower the respiratory costs associated with protein synthesis and turn-over. Although decreased tissue N concentrations are frequently reported in CO_2-enrichment studies (Chapter 3; Garbutt *et al.*, 1990; Stulen *et al.*, 1994), no such decrease was found (Chapter 4) that could explain the apparent perturbation in the relationship between RGR and R_d (Chapter 5).

Little is known of either the mechanistic basis for or the functional significance of the direct effect. Recent work by Gonzalez-Meyer *et al.* (1996), using isolated soybean mitochondria exposed to twice-ambient CO_2 concentrations, suggests that CO_2 acts directly to inhibit the activity of key enzymes of the tricarboxylic acid cycle and mitochondrial electron transport chain. Other explanations have included changes in intracellular pH affecting respiratory enzyme activity, and increased dark fixation of CO_2 by PEPc (Amthor, 1991). However, the existence of a direct effect of CO_2 on R_d can be challenged on numerous grounds. Firstly, there are theoretical difficulties in accepting that the respiratory apparatus in higher plants would have evolved a sensitivity to CO_2-repression in a soil environment used for their establishment which is characterized by CO_2 concentrations between 3000 and 17,000 μmol mol^{-1} (Lamborg *et al.*, 1983). Secondly, the CO_2-inhibition of R_d has not been routinely reported despite an extensive history of measuring respiration using oxygen electrodes and other systems where the CO_2 concentration around tissues is not controlled and often increases dramatically during measurement (J.F. Farrar, pers.comm.). Thirdly, any CO_2-repression of R_d should result in a reduction in the proportion of carbon lost via respiration relative to carbon gained via photosynthesis; for this, the results of Chapter 5 provided little convincing supportive evidence. Finally, the credibility of some reports of a direct effect has been widely challenged due to probable flaws in experimental design and interpretation (J.F. Farrar, pers.comm.).

Notwithstanding the reservations expressed above, robust reports of a direct inhibition of R_d by CO_2 clearly exist (Bunce, 1990, 1994; Ziska and Bunce, 1993; Thomas and Griffin, 1994). The remarkable finding of Bunce (1995), that a reduction in leaf area ratio (LAR) can occur in response to elevated C_a even when plants are exposed to elevated C_a only during hours of darkness, prompted the author to suggest a link between the CO_2-inhibition of R_d found in previous studies (Bunce, 1990, 1994) and the LAR response. This could represent an important observation, since not only is a reduction in LAR a widely and consistently reported effect of elevated C_a (Chapter 3; Chapter 4; Acock and Pasternak, 1986; Poorter, 1993; Stulen *et al.*, 1994; Gebauer *et al.*, 1996; Roumet *et al.*, 1996; Stirling *et al.*, 1998), but now emerges as perhaps the principal limitation to RGR in elevated C_a (Chapter 4).

The principal aim of the present chapter is to gain further insight as to if and how elevated C_a affects R_d. Following on from the observation made in Chapter 5 that a CO_2-stimulation of structural RGR occurred without a proportional increase in R_d, the data (obtained as described in that chapter) are further analyzed here to distinguish growth and maintenance R_d, and so determine the relative contribution of each functional component to the apparent discrepancy between R_d and RGR. In order to determine whether or not elevated C_a inhibits R_d, a different analytical approach than that used in Chapter 5 (Table 5.2) is taken to quantify the relationship between R_d and gross photosynthesis, and new data is presented of measurements of R_d made on individual leaves, which target specifically the direct response of R_d to elevated C_a.

6.2. MATERIALS AND METHODS

6.2.1. Growth conditions

Plants of *Urtica urens* were grown in a hydroponic culture system in controlled-environment cabinets, and were switched from ambient to elevated C_a (700 μmol mol^{-1}) for 10 d at a defined fresh weight of 8 g (± 0.75 g) (with a shoot height of 7 - 8 cm and a dry weight of ca. 0.8 g), approximately 30 d after sowing (as described in Chapter 4, Section 4.2.1). Henceforth, the day at which exposure to elevated C_a began will be designated as day 0.

6.2.2. CO_2 exchange in whole plants and individual leaves

Data of gross photosynthesis per plant (A_G) and R_d of intact shoots, roots and whole plants were taken from the measurements made and described in Chapter 5, and were expressed in units of mg C d^{-1} calculated by extrapolation of CO_2 exchange min^{-1} (Sections 2.6.5 and 5.2.2; Tables 5.1 and 5.2).

Measurements of R_d of individual leaves were made both under laboratory conditions and inside controlled-environment cabinets during the conditions of growth, using a combined CO_2/H_2O analysis system and clamp-on leaf cuvette (CIRAS-1, PP Systems, Hitchin, Herts., UK), as described in Section 2.5.1. To investigate the direct effect of CO_2 on R_d, measurements were made in the laboratory during the usual photoperiod of growth, but with conditions of darkness imposed Before measurements started, an equilibration period of 5 min was allowed after switching the portion of the leaf enclosed by the cuvette from light (2000 μmol m^{-2} s^{-1} PPFD) to darkness. R_d was measured 4 and 10 d after switching in a range of main-stem leaves (numbered 4, 5, 6 and 8 according to their order of appearance on the stem during growth). Measurements were made at ambient and 700 μmol mol^{-1} C_a to give values of R_d at the C_a of growth, and to allow calculation of the percent change in R_d due to elevated C_a occurring during measurement in any given leaf. Stable step changes in C_a were achieved within approximately 90 s, after which an equilibration period of 5 min was allowed before R_d was recorded. To test for reversibility of any CO_2 effect, the initial C_a imposed on a leaf was different from one set of measurements to the next. Leaks were tested for and detected by observing rapid fluctuations in R_d after blowing CO_2-enriched air through a tube around the gaskets sealing the leaf surface to the borders of the cuvette, and corrected by repositioning the cuvette on the leaf.

Unreplicated measurements of R_d inside the controlled-environment cabinets were made on leaf number 5 on the 8th d after switching, and were taken repeatedly at 15 min intervals throughout the usual 8 h of darkness using one CIRAS system operating under ambient C_a and another under elevated C_a. The C_a inside the cuvette was controlled by the CIRAS CO_2 control system to avoid the fluctuations in C_a generated by the cabinet control system (Section 2.2). Stomatal conductance to water vapour (g_s) and the intercellular CO_2 concentration (C_i) were also recorded at the same times (Section 2.5.1).

6.2.3. Growth and maintenance respiration

Linear regressions of whole-plant, shoot and root R_d (per unit structural dry weight) on their respective structural relative growth rates (RGRs) were used to estimate growth and maintenance respiration after Thornley (1976):

$$y = a + bx$$

where y is R_d, x is RGR, a is the y-intercept when x = 0 and represents the rate of maintenance R_d, and b is the slope and represents the coefficient of growth R_d. Instantaneous RGR was calculated using the functional approach described in Section 4.2.2, but using only the sub-set of plants used in the measurements of gas exchange (Chapter 5).

6.2.4. Statistical analyses

Analysis of variance followed by Tukey-tests were used to test for significant differences in single-leaf R_d. Differences in the slopes of linear regressions used in the analysis of whole-plant R_d over time were tested for statistical significance using Student's t-test as described by Zar (1989). For the functional analysis of growth, significant differences between corresponding pairs of constants in the fitted equations were analyzed as described in Section 2.3.2. All statistical analyses were performed using the computer software package SPSS (Prentice Hall, New Jersey).

6.3. RESULTS

6.3.1. Relationships between respiration and gross photosynthesis

Linear regressions of R_d of intact roots, shoots and whole plants against gross photosynthesis per plant (A_G) are shown in Figure 6.1. Elevated C_a did not significantly affect the slopes of the regressions (Table 6.1) and so did not alter the amount of carbon lost via R_d relative to the total carbon fixed by photosynthesis. As a proportion of A_G, about 50 % of carbon fixed was lost via R_d, with shoot R_d accounting for about 43 % and root R_d accounting for about 7 % (Table 6.1).

6.3.2. Growth and maintenance respiration

Figure 6.2 shows the instantaneous structural RGRs of roots, shoots and whole plants derived from the quadratic curves fitted to primary data of their logarithmically-transformed structural dry weights (SDWs). The constants describing the linear and quadratic terms of each quadratic equation are given in Table 6.2. For roots, shoots and whole plants, the linear term was significantly increased by elevated C_a. Elevated C_a also led to significant reductions in the quadratic term for shoots and whole plants, but not for roots.

Linear regressions of R_d against RGR so derived are shown in Figure 6.3. Values of the extrapolated y-intercept (*a*, representing the rate of maintenance R_d) and of the regression slope (*b*, representing the coefficient of growth R_d) are given in Table 6.3. Neither term was significantly affected by elevated C_a. For shoots and whole plants grown in ambient C_a, negative, clearly spurious, values of maintenance R_d were obtained (Table 6.3).

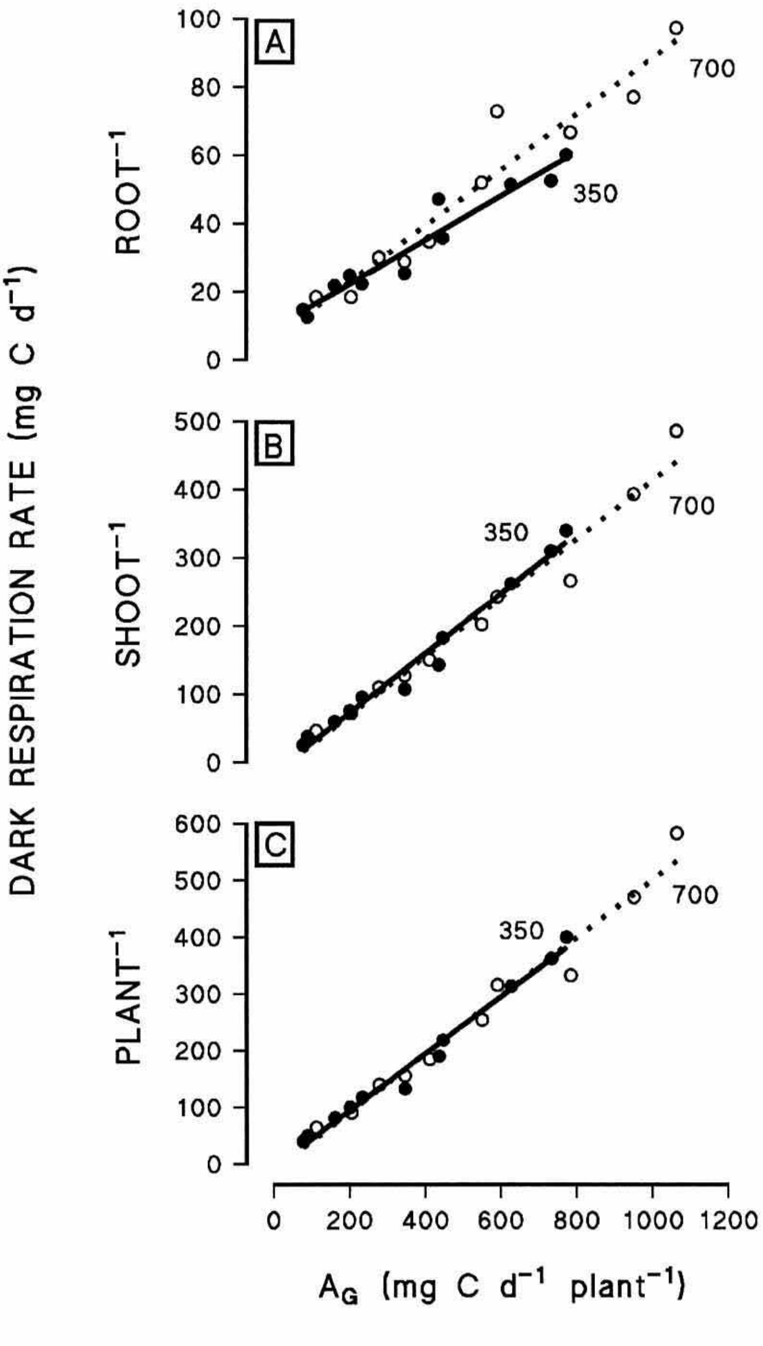

Figure 6.1. Relationship between the rate of dark respiration and gross photosynthesis (A_G) in (A) Intact roots, (B) Intact shoots and (C) Whole plants of *U. urens* during 10 d of growth after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Data are calculated as the projected daily rate of C exchange and are fitted by linear regressions. The slope and coefficient of determination (r^2) for each regression are shown in Table 6.1.

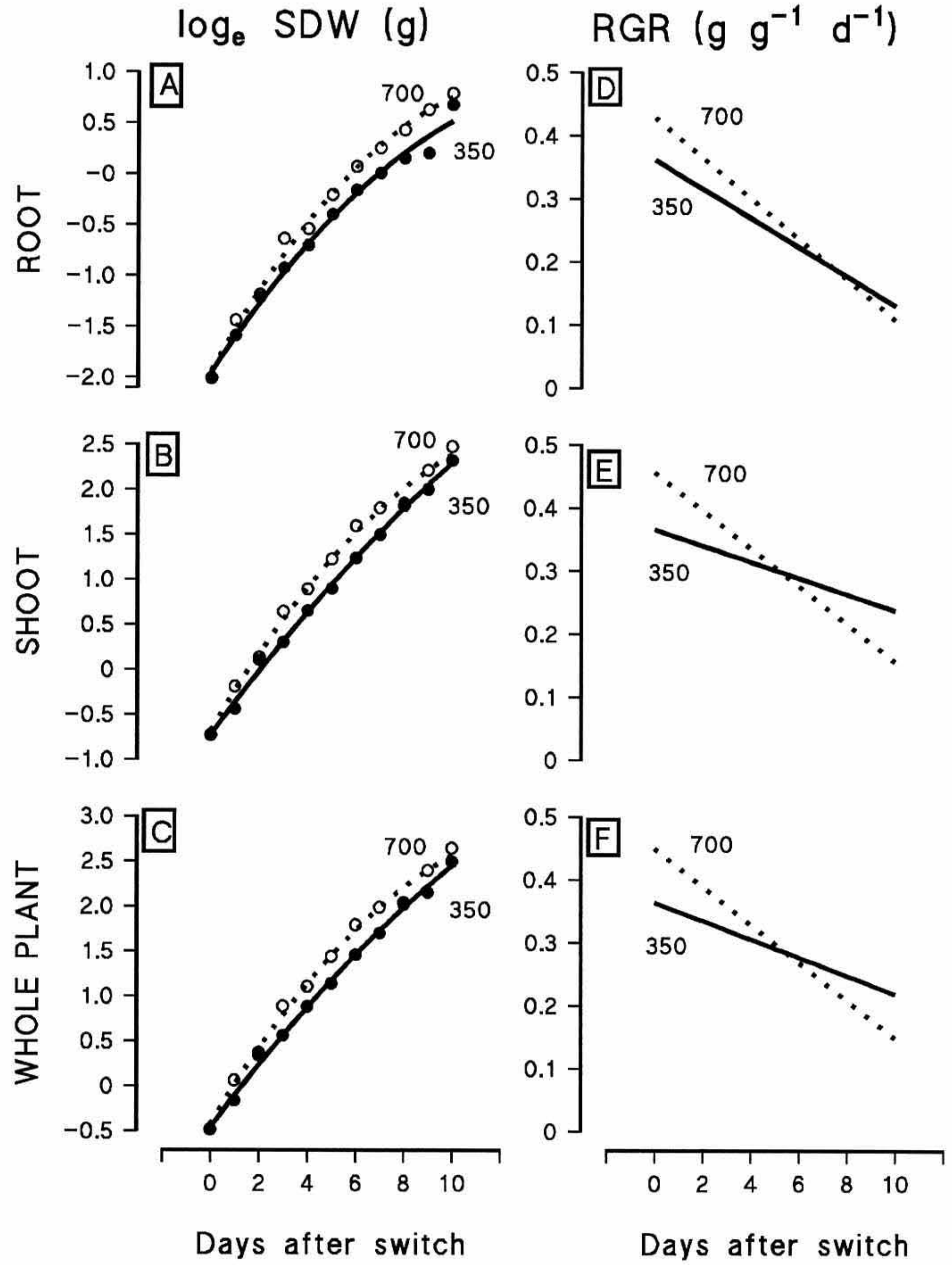

Figure 6.2. Quadratic curves fitted to (A) Log$_e$ root structural dry weight (SDW), (B) Log$_e$ shoot SDW and (C) Log$_e$ whole plant SDW over time, from which were derived their respective instantaneous relative growth rates (RGRs) (D,E,F) for use in Figure 6.3. Data are of *U. urens* during 10 d of growth after switching 30 d old plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a, and were obtained from plants used exclusively in the measurement of gas exchange of intact shoots and roots. SDW was calculated as dry weight minus the weight of total non-structural carbohydrates. The coefficient of determination (r^2) for each regression, and the constants (± standard errors) describing the linear and quadratic terms in each equation are given in Table 6.2.

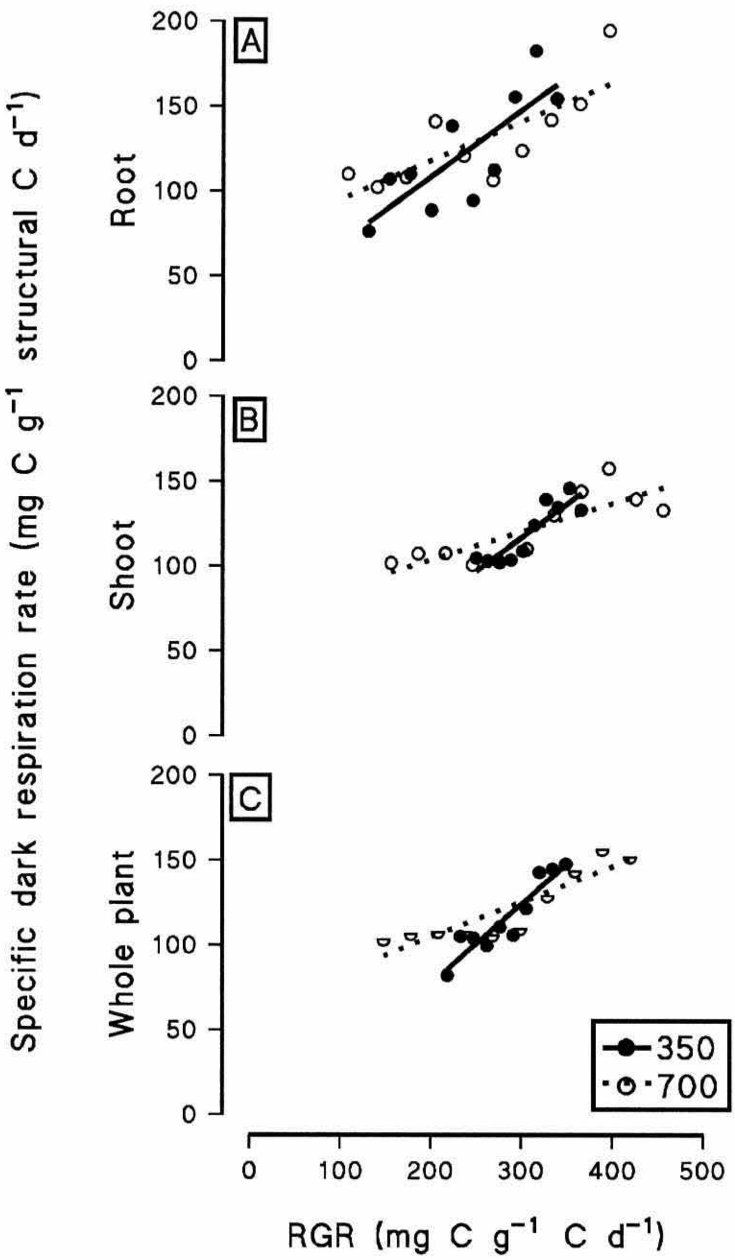

Figure 6.3. Relationship between specific dark respiration rate and instantaneous relative growth rate (RGR) in (A) Roots, (B) Shoots and (C) Whole plants of *U. urens* grown in ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. Plants were switched from ambient to elevated C_a for 10 d when approximately 30 d old. Respiration is expressed per unit structural C with the assumption that structural C is 40 % of the total structural dry weight (SDW). RGR was determined from quadratic equations fitted to primary data of SDW over time (Fig. 6.2; Table 6.2). Data are fitted by linear regressions. The y-intercept, slope and coefficient of determination (r^2) for each regression are shown in Table 6.3.

Table 6.1. Slopes of the linear regressions describing the relationship between the rate of dark respiration and gross photosynthesis (Fig. 6.1) in intact roots, intact shoots and whole plants of *U. urens* during 10 d of growth after switching 30 d old plants from ambient (ca. 350 μmol mol^{-1}) to elevated (700 μmol mol^{-1}) C_a. The coefficient of determination (r^2) is given in parentheses.

	Slope (dimensionless)	
	Ambient C_a	Elevated C_a
ROOTS	0.064 (0.935)	0.081 (0.936)
SHOOTS	0.435 (0.979)	0.430 (0.972)
WHOLE PLANT	0.500 (0.985)	0.512 (0.975)

Table 6.2. Summary of selected terms of the quadratic equations fitted to loarithmically-transformed primary data of root, shoot and whole plant structural dry weight (SDW) over time from which instantaneous relative growth rates were derived for plants used exclusively in the measurement of gas exchange of intact shoots and roots (Fig. 6.2). The table shows the constants *b* and *c* describing, respectively, the linear and quadratic terms in equations of the form $y = a + bt + ct^2$, where *y* is data of SDW, *t* is time (d), and *a* is the term describing *y* when $t = 0$ (not shown). The coefficient of determination (r^2) is given in parentheses for each equation. Significant differences ($p < 0.05$) between corresponding pairs of constants due to C_a are indicated by asterisks.

$\log_e$ SDW (g)	C_a	*b*	*c*	r^2
ROOT	Ambient	0.362 (±0.033)	-0.011 (±0.0032)	0.990
	Elevated	0.429 (±0.027)	-0.016 (±0.0026)	0.994
SHOOT	Ambient	0.366 (±0.021)	-0.006 (±0.0021)	0.997
	Elevated	0.456 (±0.032) *	-0.015 (±0.0031) *	0.994
PLANT	Ambient	0.364 (±0.020)	-0.007 (±0.0020)	0.997
	Elevated	0.450 (±0.030) *	-0.015 (±0.0029) *	0.994

Table 6.3. The y-intercepts (*a*) and slopes (*b*) of the linear regressions describing the relationship between the rate of dark respiration and relative growth rate (Fig. 6.3) in intact roots, intact shoots and whole plants of *U. urens* during 10 d of growth after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. The coefficient of determination (r^2) is given in parentheses.

	C_a	*a* (mg C g^{-1} C d^{-1})	*b* (dimensionless)	r^2
ROOT	Ambient	30.3	0.390	0.636
	Elevated	72.3	0.229	0.612
SHOOT	Ambient	-2.6	0.397	0.801
	Elevated	70.2	0.168	0.677
PLANT	Ambient	-17.3	0.470	0.871
	Elevated	63.0	0.208	0.808

6.3.3. Respiration of individual leaves

Measurements, made on plants growing in ambient or elevated C_a inside controlled-environment cabinets during the hours of darkness, of stomatal conductance to water vapour (g_s), intercellular CO_2 concentration (C_i) and the rate of dark respiration (R_d) in leaf number 5 are shown in Figure 6.4. Stomatal conductance to water vapour was higher during darkness than during the day (data not shown), and was consistently suppressed by elevated C_a (Fig. 6.4.A). The two marked depressions in g_s common to both CO_2 treatments may relate to defrosting events in the normal workings of the controlled-environment cabinet, or to auto-calibration events in the normal workings of the CIRAS system. Throughout the hours of darkness, elevated C_a resulted in a higher C_i and a lower R_d (Fig. 6.4).

Measurements of R_d under laboratory conditions at days 4 and 10 in leaf number 5, as well as in a range of other main-stem leaves, showed no significant reductions in R_d in elevated C_a (Table 6.4). However, irrespective of the C_a experienced during growth, R_d measured at both ambient and elevated C_a in any given leaf was consistently and significantly reduced by measurement at elevated C_a (Table 6.5). For any leaf, the percent reduction of R_d due to elevated C_a during measurement was always greater at day 10 (about 25 %) than at day 4 (about 15 %), and was not significantly affected by the C_a experienced during growth at any time (Table 6.5).

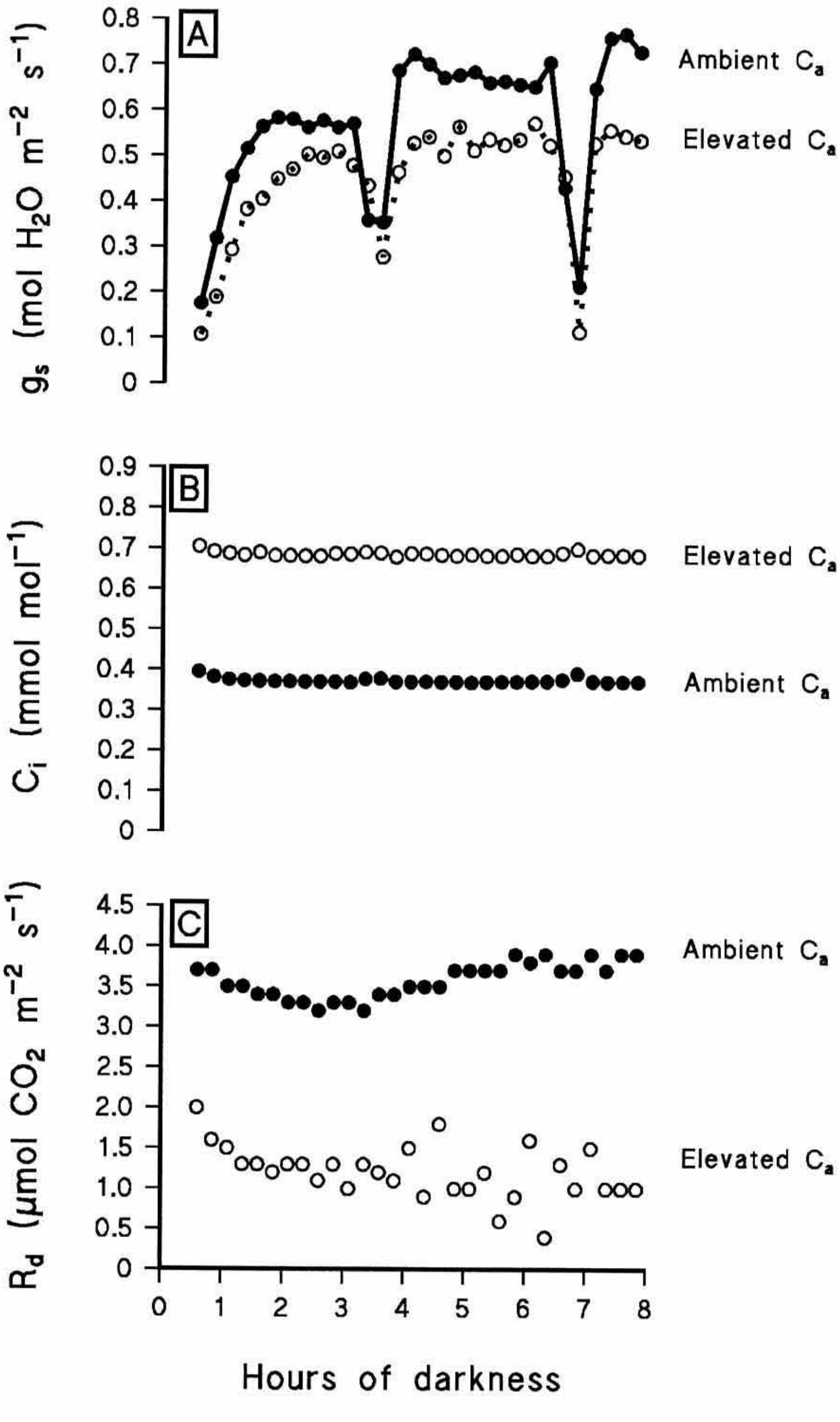

Figure 6.4. (A) Stomatal conductance to water vapour (g_s), (B) The intercellular CO_2 concentration (C_i), and (C) The rate of dark respiration (R_d) in a main-stem leaf (leaf number 5) of *U. urens* during the hours of darkness on the 8th d after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Data are shown as individual points.

Table 6.4. The rate of dark respiration in a range of main-stem leaves of *U. urens* 4 and 10 days after switching 30 d old plants from ambient (ca. 350 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) C_a. Data are shown as means (n = 3) ± standard errors. The leaf position number refers to its order of appearance on the main stem during growth. At day 4, leaf number 4 was the youngest fully-expanded leaf. At day 10, leaf number 5 was the youngest fully-expanded leaf.

	DARK RESPIRATION (µmol CO_2 m^{-2} s^{-1})			
	4 days after switch		10 days after switch	
Position on main stem	Ambient C_a	Elevated C_a	Ambient C_a	Elevated C_a
4	4.0 (±1.0)	3.7 (±1.3)	2.6 (±0.8)	2.3 (±0.6)
5	4.4 (±0.9)	3.8 (±0.4)	3.6 (±0.6)	2.8 (±0.8)
6	4.4 (±0.9)	4.2 (±1.0)	4.3 (±0.7)	3.4 (±1.0)
8	-	-	4.1 (±0.9)	3.1 (±0.4)

Table 6.5. The percent reduction in the rate of dark respiration (R_d) when R_d measured at elevated C_a (700 µmol mol^{-1}) is compared to R_d measured at ambient C_a (350 µmol mol^{-1}). R_d was measured in a range of main-stem leaves of *U. urens* 4 and 10 days after switching 30 d old from ambient (ca. 350 µmol mol^{-1}) to elevated C_a (700 µmol mol^{-1}) in growth conditions. Data are shown as means (n = 3) ± standard errors. Significant differences ($p < 0.05$) from a 0 % reduction are indicated by asterisks. The leaf position number refers to its order of appearance on the main stem during growth. The developmental status of leaves is as described in Table 6.4.

	% reduction of R_d when measured at elevated C_a			
	4 days after switch		10 days after switch	
Position on main stem	Ambient C_a	Elevated C_a	Ambient C_a	Elevated C_a
4	18 (±3.8) *	15 (±4.5) *	31 (±2.0) *	29 (±2.7) *
5	17 (±6.7)	16 (±4.5) *	20 (±3.5) *	22 (±2.5) *
6	18 (±1.0) *	11 (±7.0)	28 (±1.6) *	26 (±1.8)
8	-	-	13 (±2.9) *	32 (±2.1)

6.4. DISCUSSION

6.4.1. Growth and maintenance respiration in elevated C_a

Possible alterations due to elevated C_a in the usually tightly-coupled relationship between R_d and structural RGR (Farrar and Williams, 1991) were suggested in Chapter 5. Similar to the conclusions of other workers (Bunce and Caulfield, 1991; Ziska and Bunce, 1993), the CO_2-stimulation of R_d was less than the CO_2-stimulation of RGR might predict, suggesting reductions in either or both the growth and maintenance components of R_d. To my knowledge, growth and maintenance R_d in elevated C_a have so far been determined only for leaves. Such studies have reached the conflicting conclusions that both components are decreased in leaves of *Liriodendron tulipifera* (Wullschleger *et al.*, 1992) and white oak (Wullschleger and Norby, 1992), or that maintenance R_d is increased without alteration to growth R_d in leaves of soybean (Thomas and Griffin, 1994). The present study represents the first attempt to measure these components in entire roots, shoots and whole plants exposed to elevated C_a. Furthermore, the measurement and subtraction of total non-structural carbohydrate from all tissues (Chapter 5) allows for an estimation of structural RGR (Fig. 6.2) and thus, in principle, for a more precise investigation of growth R_d than is generally achieved in similar studies where the imposition of an environmental variable can result in the dilution of dry weight with non-structural carbon (Szaniawski and Kielkiewicz, 1982; Sawada *et al.*, 1987).

As with the studies of Wullschleger *et al.* (1992), Wullschleger and Norby (1992) and Thomas and Griffin (1994), estimations of growth and maintenance R_d were determined, respectively, as the slope (*b*) and y-intercept (*a*) of linear regressions of R_d against RGR (Thornley, 1976; Section 6.2.3; Fig. 6.3; Table 6.3). The single major difference lies in the basis of expression for R_d and RGR, such that in the present study the basis is structural dry weight (SDW), whilst a leaf area basis was used in the studies previously cited of growth and maintenance R_d in leaves. However, the present study clearly failed in its attempt to provide accurate or reliable values to compare growth and maintenance R_d in ambient and elevated C_a. Negative rates of *a* were extrapolated from the regressions for shoots and whole plants (Fig. 6.3; Table 6.3), and the positive rates for roots of approximately 30 and 70 mg C g^{-1} C d^{-1} (Table 6.3) are outside the range of 1 -

10 mg C g^{-1} C d^{-1} collated by Penning de Vries (1975) for a wide range of species and reports.

Linear regressions provided poor fits to relationships between R_d and RGR (Table 6.3), but the data points were also widely scattered (Fig. 6.3). These results could have arisen due to the combination of generally high variability in the measurements of R_d (Chapter 5; Fig. 5.2) and the possibility that instantaneous RGR was poorly described by the curve-fitting approach used here, particularly with regard to the inevitable description of RGR as a linear function of time (Section 6.2.3; Fig. 6.2). Moreover, there could be more fundamental problems with some of the basic assumptions underlying the use of regression analysis of R_d and RGR to determine growth and maintenance R_d. One assumption is that maintenance R_d is independent of RGR, whilst there is good evidence that maintenance R_d actually increases with RGR (McCree, 1982; Hay and Walker, 1989). Another potentially spurious assumption is that the relationship between R_d and RGR is linear over time or with ontogeny. The relationships shown in Figure 6.3 suggest the possibility that quadratic curves may provide better descriptions, although when analyzed as such in the present study, extremely unrealistic rates of *a* were obtained, particularly at elevated C_a (data not shown). Other approaches to the determination of growth and maintenance R_d (e.g. McCree, 1970) employ the same basic assumptions.

Notwithstanding the problems discussed in the previous two paragraphs, the present study found no significant effect of elevated C_a on either the growth or maintenance component of R_d (Fig. 6.3; Table 6.3). However, the data do point to the possibility that elevated C_a may result in an increased maintenance R_d and a decreased growth R_d, particularly in shoots, of *U. urens*. This observation perhaps merits future investigation in the same or different species. Any decrease in the coefficient of growth R_d means a corresponding increase in the ratio of the rate of dry weight (or leaf area) production to the rate of substrate supplied (the conversion efficiency, Y_G, as defined by Thornley, 1970). Since the synthesis of proteins and other nitrogen-rich organic compounds confer a high respiratory cost compared with the synthesis of carbohydrates (Hay and Walker, 1989), increased Y_G in elevated C_a has been linked to reductions in the amount of tissue N relative to structural and/or non-structural carbon (Ziska and Bunce, 1993; Wullschleger *et al.*, 1994). However, no significant reduction in the concentration of organic N in shoots was found in Chapter 4, and there may perhaps be a case for seeking alternative explanations for the apparent increases in Y_G explicit and implicit in many CO_2-enrichment studies (Chapter 5; Bunce and Caulfield, 1991; Wullschleger *et al.*, 1992; Wullschleger and Norby, 1992; Ziska and Bunce, 1993; Wullschleger *et al.*, 1994).

6.4.2. Evidence for a direct effect of elevated C_a on respiration

The findings presented in Table 6.5 add to existing reports of a direct, reversible inhibition of R_d by elevated C_a during measurement (Bunce, 1990, 1994; Amthor *et al.*, 1992; Ziska and Bunce, 1993; Thomas and Griffin, 1994). The 15 - 25 % reduction of leaf R_d found here (Table 6.5) is consistent with the magnitude of respiratory inhibition reported for leaves of *Rumex crispus* (Amthor *et al.*, 1992) and soybean (Thomas and Griffin, 1994). Although absolute values of leaf R_d of *U. urens* were high compared with many species (J.F. Farrar, pers. comm.), they are credible, and equivalent rates have been reported for other fast-growing C_3 herbaceous species such as soybean (Thomas and Griffin, 1994). Also, measurements of shoot R_d made using a different analysis system (Chapter 5) were very similar when expressed per unit leaf area (Table 5.2).

How can the direct inhibition of R_d by elevated C_a be explained? The possibility of measurement error must be a prime consideration, and this point is only reinforced by the general acceptance amongst workers that a substantial portion of the existing evidence (Amthor *et al.*, 1992) is no longer credible because of leaks into and out of the system used to measure CO_2 exchange (J.F. Farrar, pers. comm.). Leaks can be a particular problem in differential analysis systems because the low flow-rates of air entering a cuvette, which are needed to generate accurate and measurable CO_2 differentials from the low rates of R_d characteristic of plant tissues, reduce the positive pressure within the cuvette relative to the surrounding atmosphere. In this respect, the relatively high rates of R_d found in leaves of *U. urens* (Table 6.4) meant that adequate differentials were achieved together with a positive pressure due to a surplus inflow of air of 50 ml min^{-1} entering the cuvette (Section 2.5.1). Nevertheless, leaks were tested for by blowing CO_2-enriched air around the gaskets sealing the leaf surface to the borders of the cuvette (K.J. Parkinson, pers. comm.). With this method, any leak can be readily detected as a subsequent increase in the apparent CO_2 assimilation rate measured by the analysis system. When measuring R_d in leaves of *U. urens*, leaks were occasionally detected, and could always be excluded simply by a slight repositioning of the cuvette on the leaf.

Other problems that could arise using differential analysis systems to measure R_d at different C_as include changes in the CO_2-sensitivity of the infra-red analysis component with increasing CO_2, and changes in the extent to which CO_2 in the air destined for analysis is diluted by water vapour from evapotranspiration. Both eventualities have been carefully considered in the design of the CIRAS used in this study to measure R_d (K.J. Parkinson, pers. comm.), and at present there seem no reasons to doubt that CO_2 in some way inhibits leaf R_d during measurement (Table 6.5).

From the current literature, two mechanisms emerge as prime candidates to explain the direct effect. The first of these is the direct inhibition of respiratory enzymes by CO_2 (Amthor, 1991; Gonzalez-Meler *et al.*, 1996). Whilst extremely high C_as in the region of 20 - 150 mmol mol^{-1} are known to inhibit respiratory enzymes in various fruits (Knee, 1973; Monning, 1983; Kerbel *et al.*, 1988), the recent study of Gonzalez-Meler *et al.* (1996) is the first to show that respiratory inhibition can occur over C_as (360 - 720 μmol mol^{-1}) pertinent to studies of global climate change, and also well within the CO_2 concentrations of 3 - 17 mmol mol^{-1} found in soils (Lamborg *et al.*, 1983). From the study of Gonzalez-Meler *et al.* (1996), it would appear that elevated C_a inhibits the rate of O_2 uptake by isolated soybean mitochondria, and inhibits the activities of at least two respiratory enzymes, cytochrome *c* oxidase and succinate dehydrogenase. However, their study did not indicate whether the inhibition of O_2 uptake and enzyme activity manifests as a decreased efflux of CO_2.

The second possible mechanism is the increased dark fixation of CO_2 by phosphoenol pyruvate carboxylase (PEPc) (Amthor, 1991), an enzyme for which a number of roles have been suggested in C_3 plants, including the replenishment of intermediates of the Krebs cycle destined for biosynthesis and the refixation of respiratory-generated CO_2 (Latzko and Kelly, 1983). To my knowledge, no direct evidence yet exists to corroborate or refute this hypothesis, although Thomas and Griifin (1994) reported unpublished data suggesting that increased dark CO_2 fixation by PEPc may account for some, but not all, of the respiratory inhibition by elevated C_a.

For either mechanism to have physiological relevance during conditions of growth, elevated C_a must be shown to result in an elevated intracellular CO_2 concentration, or, at the very least, in an elevated intercellular CO_2 concentration (C_i). Consistent with this, the C_i calculated by CIRAS was always increased in elevated C_a both during the measurement of R_d in the laboratory (data not shown) and throughout the hours of darkness within the controlled-environment cabinet (Fig. 6.4.B). Concern has been raised (K.J. Parkinson, pers. comm.) as to whether CIRAS can reliably calculate C_i in darkness, due to the possible violation of some basic assumptions of the equation (Von Caemmerer and Farquhar, 1981; Section 2.5.2) used for its calculation. Briefly, the basic premise relevant to this concern is that the total transfer of CO_2 and water vapour between the atmosphere and intercellular spaces within the leaf is governed only by the resistances to their transfer imposed by the boundary layer of relatively still air surrounding the leaf (r_b) and the stomatal aperture (r_s). Implicitly here, the resistance to their transfer imposed by the cuticle (r_c), which in most plants is large and in the region of 50 - 200 m^2 s mol^{-1} (Fitter and Hay, 1987), is ignored. To calculate C_i, a knowledge of r_b and r_s specific to the transfer of CO_2 is needed, and to achieve this, fixed ratios of the diffusivity of CO_2 relative to water vapour through the boundary layer (1.37) and through the stomatal pore (1.6) are applied to values previously obtained for r_b and r_s specific to the transfer of water vapour (transpiration). In the light, when stomata are generally open, r_c represents a negligible fraction of the total resistance to transpiration because the stomatal and cuticular pathways run in parallel. However, in darkness when stomata are generally closed, any resistance to transpiration that is not due to r_b will be largely or entirely due to r_c. Because the equation for calculating C_i does not include a parameter to model the ratio of the diffusivity of CO_2 relative to water vapour through the cuticle, measurements of both C_i and r_s in darkness by CIRAS are therefore unlikely to be reliable.

This view is certainly justified, but only so far as it can be substantiated by the perceived wisdom that stomata close in the dark. In my attempts to measure R_d in leaves of *U. urens*, the apparent stomatal conductance to water vapour (g_s) is usually unaltered and often increases when leaves are suddenly transferred from light to darkness for periods of up to 30 min (data not shown). Since these measurements of R_d were generally conducted during the normal photoperiod, one possible explanation here is that stomatal behaviour in this species is governed by endogenous circadian rhythms, as is the case for perhaps the majority of plants (Meidner and Mansfield, 1968; Willmer, 1983). However, there are also reasons to believe that stomata in *U. urens* remain open throughout the normal dark period, a characteristic found in certain other non-CAM plants such as potato (Mansfield, 1976) and perhaps even a common and unique response of plants to certain exigencies of hydroponic culture (e.g. excess water or lack of root restriction).

Figure 6.4.A shows values of apparent g_s between 0.2 and 0.7 mol m^{-2} s^{-1}, several orders of magnitude higher than the values for cuticular conductance between 5 and 50 mmol m^{-2} s^{-1} that can be extrapolated from the data collated by Fitter and Hay (1987). Moreover, apparent g_s was consistently higher during the night than during the day (day-time data not shown), and increased markedly after lights were switched off (Fig. 6.4.A). Coupled with the fact that the lower g_s in elevated C_a (Fig. 6.4.A) is entirely consistent with the well-known effect of elevated C_a to reduce stomatal aperture (Mott, 1991), these observations argue strongly for significant stomatal opening in darkness, and thus indicate that the values of C_i calculated by CIRAS in darkness are probably true reflections of the actual C_i (Figure 6.4.B). It would appear, therefore, that the inhibition of apparent R_d by CO_2 under physiologically real conditions (Table 6.5) could be due, at least in principle, to a direct inhibition of respiratory enzyme activity by CO_2 (Amthor, 1991; Gonzalez-Meler *et al.*, 1996). Nevertheless, the very real theoretical difficulties in accepting such an effect (Section 6.1, p.129) remain.

6.4.3. Evidence for an indirect effect of elevated C_a on respiration

Some studies indicate that a period of growth in elevated C_a can diminish (Thomas and Griffin, 1994) or even annul (Bunce, 1990) the direct inhibition of R_d by elevated C_a. Here, however, no evidence was found for such a moderating interaction, since the percent reduction of R_d by elevated C_a during measurement was never significantly different in leaves grown in elevated C_a compared with those grown in ambient C_a (Table 6.5). Unreplicated measurements of leaf R_d made overnight under the conditions of growth suggested that the direct effect of CO_2 during measurement might persist to cause respiratory inhibition during growth in elevated C_a (Fig. 6.4.C). However, the significant direct inhibition of leaf R_d by elevated C_a during replicated measurement in the laboratory (Table 6.5) was not reflected in a significantly decreased R_d of leaves grown in elevated C_a (Table 6.4). Similarly, decreased R_d was not found in entire shoot systems expressed per unit leaf area (Chapter 5, Table 5.2), and shoot R_d was not reduced as a proportion of gross photosynthesis (Fig. 6.1; Table 6.1). Whilst it is possible that the true respiratory response to elevated C_a was in some way corrupted by measurement of R_d in the laboratory, or that the occurrence of any CO_2-inhibition of R_d at the C_a of growth may have gone undetected due to a highly variable base-line R_d and generally low orders of replication (Fig. 6.1; Tables 5.2, 6.1 and 6.4), it is also possible that growth in elevated C_a indirectly increases R_d to counter the direct inhibitory effect.

The latter view is consistent with the findings of Thomas and Griffin (1994), where leaf R_d was unchanged or even increased by elevated C_a despite a persistent direct respiratory inhibition during measurement. The conclusion reached by these workers was that leaf R_d was increased by elevated C_a due to an increase in the maintenance component of R_d, which they attributed to higher respiratory costs associated with the synthesis, turn-over and export of additional non-structural carbohydrates. Strong correlations between C_a, leaf R_d and leaf non-structural carbohydrate status are firmly established in the literature, in both fully expanded (Azcon-Bieto and Osmond, 1983) and expanding (Hrubec *at al.*, 1985) leaves. Such correlations are also consistent with our current understanding of respiratory control, such that R_d is up-regulated by substrate supply in a manner suggestive of coarse control of respiratory enzymes by sugars (Farrar and Williams, 1991). Notwithstanding the problems discussed in Section 6.4.1, the present study at least argues for further investigation into the possibility that maintenance R_d in leaves and shoots is increased by elevated C_a (Table 6.3). Signalling agents other than sugars could also contribute to a stimulation of R_d in elevated C_a. One possible candidate here is ethylene, production of which is stimulated by CO_2 (Horton, 1985; Philosoph-Hadas *et al.*, 1986) and which in turn generally stimulates R_d (Amthor, 1991). Moreover, the CO_2 evolution that is coupled to the formation of ethylene (Lieberman, 1979) could add significantly to the stimulation of apparent R_d. The possible wider role of ethylene in plant responses to elevated C_a is discussed in Chapter 8.

6.4.4. Conclusions

This study argues strongly for a direct and sustained inhibition of R_d by CO_2 at C_as pertinent to studies of climate change, and at concentrations perhaps also relevant to studies of below-ground processes such as root growth and seedling establishment. The study also suggests that elevated C_a might affect R_d in other, highly complex ways, reflecting the different physiological processes which contribute to the net efflux of CO_2 in darkness. Because it is often difficult to quantify each process independently, and because the functional significance of some of the processes are not fully understood, the implications of CO_2-effects on R_d for plant growth are unclear. However. further investigation into respiratory responses to elevated C_a may carry the potential to reveal mechanisms behind the characteristic growth-limiting changes in physiology and morphology that cannot be satisfactorily explained by current models (Chapters 3 - 5).

CHAPTER 7

The role of sugars in the down-regulation of photosynthesis and growth following the withdrawal of mineral nitrogen

7.1. INTRODUCTION

Accumulation of soluble sugars in leaves can often be associated with reductions in their photosynthetic capacity. This has been demonstrated or implied in diverse experimental systems that apparently perturb the sink-source balance (Neales and Incoll, 1968; Herold, 1989; Stitt, 1991; Pollock and Farrar, 1996), including cooling roots (Sawada *et al.*, 1987), feeding glucose directly to leaves via the transpiration stream (Krapp *et al.*, 1991), infecting leaves with biotrophic fungi (Scholes *et al.*, 1994), depriving plants of mineral N (Paul and Driscoll, 1997), and raising the atmospheric CO_2 concentration (C_a) (Van Oosten and Besford, 1995).

In principle, the response of photosynthesis to sugar status can be explained by an inhibitory effect of sugars on the expression of genes encoding key photosynthetic proteins. Hence, the accumulation of sugars in leaves due to cold-girdling of petioles decreased transcript levels of the small rubisco subunit, chlorophyll *a* binding protein, and thylakoid ATP synthase (Krapp and Stitt, 1995). Similarly, feeding sugar to detached leaves and leaf disks decreased transcript levels of the rubisco small subunit (Van Oosten and Besford, 1994). It is now clear that photosynthetic genes are just some of many that are sugar-responsive (Koch, 1996), such that sugars may play a key role in the regulation and coordination of plant growth in general (Pollock and Farrar, 1996). The sugar-repression of photosynthesis may perhaps represent a sensitive and common mechanism to prevent harmful consequences of unlimited accumulation of both structural and non-structural carbon, whenever the rate of assimilate supply exceeds its use in sinks. Moreover, it may play a critical role in the mobilization of organic N reserves (stored, for example, as rubisco protein in source leaves) following N deficiency (Paul and Stitt, 1993).

However, the results reported in previous chapters of this thesis for plants exposed to elevated C_a suggest that an accumulation of sugars in leaves need have no effect on photosynthetic capacity, and also that the down-regulation of growth may be related more to morphological changes such as in the leaf area ratio (LAR), than to indices of photosynthetic efficiency approximated by the net assimilation rate (NAR). Furthermore, the accumulation of sugars in leaves was not preceded by an accumulation of sugars in sinks, which has been predicted in theory (Pollock and Farrar, 1996; Chapter 5, Section 5.1), and which has also been found in practice using a model experimental system in which the roots of single-rooted soybean leaves were cooled (Sawada *et al.*, 1987).

It is possible that larger perturbations in the sink-source balance are needed to induce the repression of photosynthesis by sugars, than are caused by exposure to the approximately twice-ambient elevations of C_a typical of CO_2-enrichment studies. Nitrogen-deficiency is a common occurrence in both natural and agricultural systems, and may conceivably limit sink capacity by, for example, strongly inhibiting the expansion of leaves (James *et al.*, 1993). Accordingly, Paul and Driscoll (1997) found that the accumulation of soluble sugars in tobacco leaves, induced by depriving previously N-sufficient plants of mineral N, was closely followed by reductions in photosynthetic capacity and rubisco content. Their observations provide evidence that the sugar-repression of photosynthesis may be important in physiologically real systems.

Plants that have acclimated in the longer-term to elevated C_a almost invariably accumulate soluble sugars in leaves (Farrar and Williams, 1991), even when grown with a non-limiting nutrient supply and without other environmental constraints (Chapters 3 and 5). Because this accumulation of soluble sugar can persist in time (Chapter 3), the photosynthetic capacity of plants grown in elevated C_a may be more susceptible to down-regulation by sugars following the imposition of an environmental constraint that increases the soluble sugar concentration further. In the present chapter, this hypothesis is tested by withdrawing the mineral N supply of previously N-sufficient plants that have acclimated to ambient and elevated C_a. The questions are also asked as to whether the down-regulation of photosynthetic capacity is preceded by an accumulation of sugars in sinks as current models predict (Sawada *et al.*, 1987; Pollock and Farrar, 1996; Chapter

5, Section 5.1), and as to whether the sugar-repression of photosynthesis is indeed a sensitive and common mechanism to regulate the growth of plants according to the supply of resources.

7.2. MATERIALS AND METHODS

7.2.1. Growth conditions

Plants of *U. urens* were grown in a hydroponic culture system in controlled-environment cabinets as described in Section 2.2. Seedlings received ambient or elevated C_a (680 μmol mol^{-1}) immediately after suspension in nutrient solution 12 d after sowing. The nutrient solution contained 12 mM nitrate, the sole source of available mineral N. After 5 d, plants were selected for uniformity of shoot height and leaf development within each CO_2 treatment. Plants grown in ambient and elevated C_a were then switched to a nitrate-free solution for 6 d at 36 d after sowing, following immersion of root systems in deionized water for 3 min. In the nitrate-free nutrient solution, KNO_3 and $Ca(NO_3)_2$ were replaced with K_2SO_4 and $CaCl_2$ in amounts sufficient to keep the concentrations of K^+ and Ca^{2+} the same, but with the consequence of raising the concentrations of SO_4^{2-} and Cl^- from 1.5 mM and 0.1 mM to 2.75 mM and 5.01 mM respectively.

Four plants grown in either ambient or elevated C_a were harvested immediately before switching to the nitrate-free solution (day 0). Four replicates from each of the four treatments (ambient C_a with and without N; elevated C_a with and without N) were then harvested at day 6, and a number of additional harvests from each treatment were made during the days between. At each harvest, plants were separated into leaves (unfolded leaves > 1 cm length), stems (including folded leaves, leaves ≤ 1 cm length and inflorescences if present), and roots. Immediately after harvesting, roots were rinsed in 2 l deionized water for 2 min to dilute nutrients adhering to root surfaces. Dry weights were determined after oven-drying at 60 °C for at least 48 h. Total leaf area (all leaves > 1 cm length) were measured using a digital image analysis system (Delta-T Ltd, Cambridge, UK).

7.2.2. Growth analysis

Growth was analyzed using the classical approach, using the plants harvested at days 0 and 6. From the data of dry weight and leaf area, the following components of plant growth were calculated for the single harvest interval from replicates paired according to size (Evans, 1972; Hunt, 1978): (1) Whole plant mean relative growth rate, $\overline{RGR}$, (2) Mean net assimilation rate, $\overline{NAR}$, (3) Mean leaf area ratio, $\overline{LAR}$, (4) Mean leaf weight ratio, $\overline{LWR}$, and (5) Mean specific leaf area, $\overline{SLA}$. The formulae used for the calculation of these parameters are given in Section 2.3.1.

7.2.3. Gas exchange in individual leaves

Measurements of net photosynthesis (A) and stomatal conductance to water vapour (g_s) were made at days 3 and 6 in the youngest fully-expanded leaf of plants that were subsequently harvested at day 6. The approximate stage of leaf expansion was determined using sequential measurements of leaf length and width. The light-saturated rate of photosynthesis (A_{sat}) and g_s were measured at 2000 μmol m^{-2} s^{-1} PPFD (approaching light saturation of A; Fig. 2.2), using a combined CO_2/H_2O analysis system and clamp-on leaf cuvette (CIRAS-1, PP Systems, Hitchin, Herts., UK) as described in Section 2.5.1. Measurements were made at ambient and 680 μmol mol^{-1} C_a to give A_{sat} and g_s at the C_a of growth. A_{sat} was also measured at a range of intercellular CO_2 concentrations (C_i) between 50 and 1300 μmol mol^{-1} for the construction of A/C_i curves (Section 2.5.2). The maximum rate of carboxylation limited by the amount and/or activity of rubisco ($V_{c,max}$) and the maximum attainable rate of photosynthesis at saturating light intensity and C_i (A_{max}) were derived from these curves following the approach of McMurtrie and Wang (1993), as described in Section 2.5.2.

7.2.4. Total soluble carbohydrates

Soluble carbohydrates were extracted from oven-dried plant material sequentially in ethanol and then in deionized water (Farrar and Farrar, 1985) (Section 2.7.1). Extractions of 30 - 40 mg sub-samples taken from well-mixed oven-dried leaf, stem and root materials were analyzed according to Dubois *et al.* (1956) (Section 2.7.2).

7.2.5. Tissue nitrate

Nitrate was extracted after Cataldo *et al.* (1975) and measured using a model 93-3079 nitrate-selective electrode and a model 90-0029 reference electrode (Russel pH Ltd., Fife, Scotland). Leaves, stems and roots were ground in a hammer mill through a 1.5 mm diameter sieve after oven-drying at 60 °C for 48 h. 100 - 150 mg of ground plant material was incubated in 5 ml of water at 45 °C for 1 h, and centrifuged for 10 min at 3000 rpm. The supernatant was then decanted and made up to 10 ml for nitrate analysis after the addition of 0.2 ml of 2 M $(NH_4)_2SO_4$ to maintain a background ionic strength of 0.12 M. A standard curve was prepared using KNO_3 at concentrations between 0.1 and 100 mM. Measurements of standards and samples were stable after adding Cl^-, the principle anion that can interfere with the detection of NO_3^- by electrode, at concentrations of up to 0.6 mM. A significant proportion of the tissues harvested at day 6 of plants grown in both ambient and elevated C_a with a continued nitrate supply were lost prior to nitrate analysis, and no data of tissue nitrate concentration were obtained for these plants at this time.

7.2.6. Source-sink differentiation

Within the broad definition of sources as regions that produce and export assimilate and of sinks as those that import assimilate for growth or storage (Farrar, 1996), sources were considered as leaves (unfolded leaves > 1 cm length) and sinks as stems (including shoot apices, folded leaves, leaves ≤ 1 cm length and inflorescences) and as entire root systems.

7.2.7. Statistical analyses

Significant differences between mean values obtained at days 0 and 6 were analyzed by ANOVA and Tukey-tests with C_a, NO_3^- and time as factors (SPSS, Prentice Hall, New Jersey). The choice of polynomial to fit the time-course of data of soluble sugar and nitrate concentration following nitrate withdrawal followed the method of Hunt and Parsons (1974) (Section 2.3.2), and significant differences between corresponding pairs of constants in the fitted equations were analyzed after Hughes and Freeman (1967) (Section 2.3.2). All curves otherwise fitted to data were used only to indicate visual trends, and were accorded no biological or statistical significance.

7.3. RESULTS

7.3.1. Plant growth

Six d after the withdrawal of nitrate, both the total dry weight and total leaf area were significantly decreased compared with those of plants grown with a continued nitrate supply (Fig. 7.1). For nitrate-deprived plants, the reduction in dry weight (about 40 %) was less than the reduction in leaf area (about 65 %), and independent of C_a. Compared with nitrate-sufficient plants grown in ambient C_a, the dry weight and leaf area of plants grown in elevated C_a and similarly sufficent in N were not significantly different at day 0, but both were significantly increased at day 6 by 80 % and 40 %, respectively (Fig. 7.1).

Mean relative growth rates and components thereof, averaged over the 6 d period, are shown in Table 7.1. In nitrate-sufficient plants, whole-plant $\overline{RGR}$ in elevated C_a was significantly increased by about 70 % due to large stimulation of $\overline{NAR}$ (120 %), which was partly offset by a smaller but still significant reduction in $\overline{LAR}$ (22 %). The reduction in $\overline{LAR}$ due to elevated C_a was due to a significant reduction in $\overline{SLA}$ without any effect on $\overline{LWR}$. The relative growth rates of shoots, leaves and stems, but not of roots, were significantly increased by elevated C_a, with the largest component of the increase in shoot $\overline{RGR}$ being a 83 % stimulation of leaf $\overline{RGR}$.

The withdrawal of nitrate from plants grown in both ambient and elevated C_a resulted in significant reductions in whole-plant $\overline{RGR}$, but the reduction in $\overline{RGR}$ in elevated C_a (36 %) was less pronounced than that in ambient C_a (50 %). In both ambient and elevated C_a, the reductions in whole-plant $\overline{RGR}$ were caused primarily by reductions in $\overline{NAR}$, but were exacerbated by smaller, but significant, reductions in $\overline{LAR}$. In turn, the reductions in $\overline{LAR}$ following withdrawal of nitrate were caused primarily by reductions in $\overline{SLA}$, but were exacerbated by smaller, but significant, reductions in $\overline{LWR}$. The magnitude of these reductions in $\overline{LAR}$, $\overline{SLA}$ and $\overline{LWR}$ were similar in both ambient and elevated C_a, amounting to about 15 %, 10 % and 5 % respectively. Therefore, the relatively moderate reduction in $\overline{RGR}$ following withdrawal of nitrate in elevated C_a was due to a smaller reduction in $\overline{NAR}$ in elevated C_a (24 %) compared with ambient C_a (42 %).

In both ambient and elevated C_a, a reduction in the $\overline{RGR}$ of leaves was the largest single component responsible for the reduction in whole-plant $\overline{RGR}$ following the withdrawal of nitrate, amounting to 70 % in ambient C_a and 46 % in elevated C_a. In contrast, withdrawal of nitrate did not significantly affect root $\overline{RGR}$, and caused relatively small reductions in stem $\overline{RGR}$. The leaf area $\overline{RGR}$ was markedly reduced following withdrawal of nitrate, by 124 % in ambient C_a and by 80 % in elevated C_a. The loss of leaf area implicit in the negative value obtained for leaf area $\overline{RGR}$ in ambient C_a probably reflects observations of leaf senescence and abscission that were not apparent in elevated C_a during the 6 d period.

C_a did not significantly affect the shoot:root dry weight ratio (S/R) either at day 0 or at day 6 (Fig. 7.2).The S/R in nitrate-deprived plants was not significantly different at day 6 compared with that of nitrate-sufficient plants grown in either ambient or elevated C_a (Fig. 7.2.A,C). However, data from additional plants harvested between days 0 and 6 indicate that transient decreases S/R did occur at an earlier stage in plants grown in ambient C_a, although not in plants grown in elevated C_a (Fig. 7.2). Accounting for the weight of soluble sugars had no significant effects on S/R (Fig. 7.2.B,D).

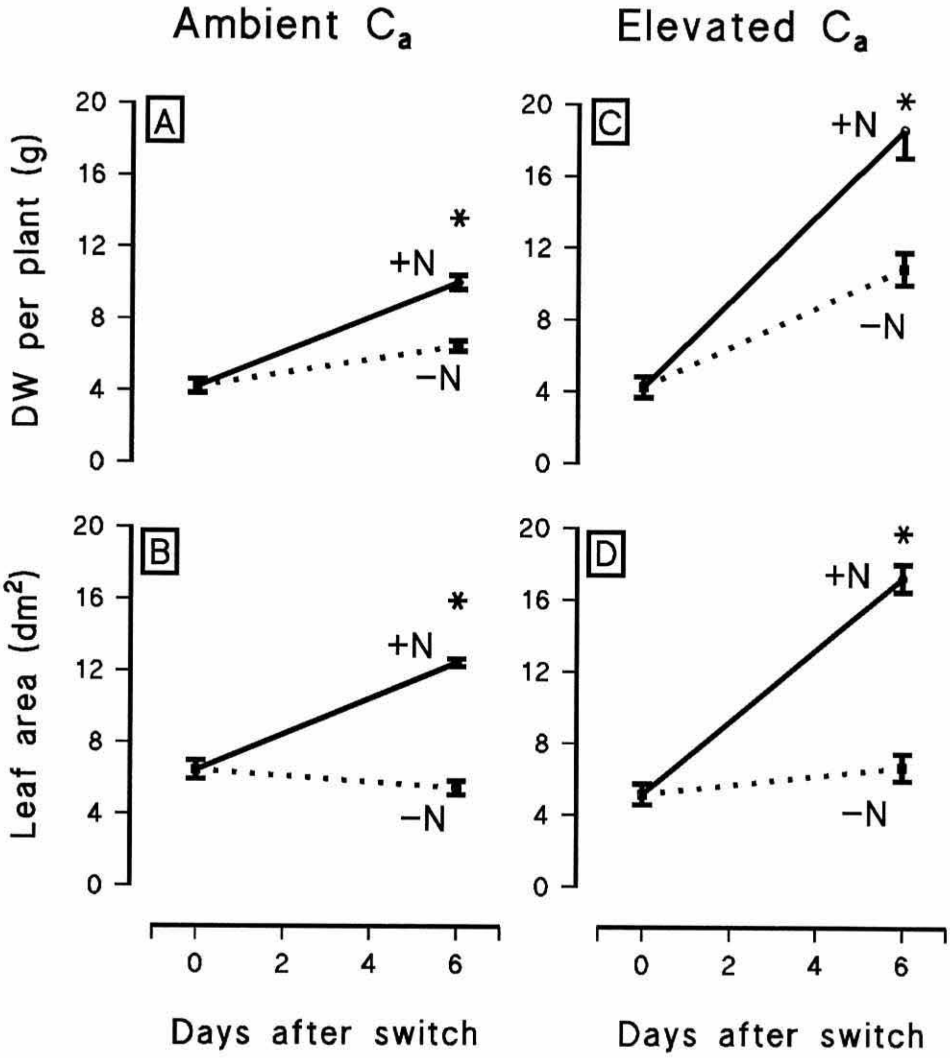

Figure 7.1. (A,C) Total dry weight (DW) per plant and (B,D) Total leaf area per plant of *U. urens* grown in (A,B) ambient (ca. 350 μmol mol^{-1}) or (C,D) elevated (680 μmol mol^{-1}) C_a. A number of plants were switched at 36 d of age (day 0) from a nutrient solution containing 12 mM nitrate (+N) to one without nitrate (-N), the sole source of mineral N, for 6 d. Data are shown as means with standard error bars (n = 4). Significant differences ($p < 0.05$) due to nitrate are indicated by asterisks. The results of statistical analyses for differences due to C_a are given in the text.

Table 7.1. Classical growth analysis of *U.urens* grown in ambient (ca. 350 μmol mol^{-1}) or elevated (680 μmol mol^{-1}) C_a and switched at 36 d of age (day 0) from a nutrient solution containing 12 mM nitrate to one without nitrate, the sole source of mineral N, for 6 d. The table shows mean values over 6 d of $\overline{RGR}$ (mean relative growth rate), $\overline{NAR}$ (mean net assimilation rate), $\overline{LAR}$ (mean leaf area ratio), $\overline{LWR}$ (mean leaf weight ratio) and $\overline{SLA}$ (mean specific leaf area). Data are shown as means (n = 4) ± standard errors. Significant differences ($p < 0.05$) are indicated by letters and numbers in superscript, where $^a/^b$ and $^c/^d$ show differences between nitrate treatments in ambient and elevated C_a respectively, and where $^1/^2$ and $^3/^4$ show differences due to elevated C_a with and without nitrate respectively.

		AMBIENT C_a		**ELEVATED C_a**	
		+ NITRATE	**- NITRATE**	**+ NITRATE**	**- NITRATE**
Plant $\overline{RGR}$	($g\ g^{-1}\ d^{-1}$)	0.145 (±0.019) ^{a1}	0.073 (±0.017) ^{b3}	0.249 (±0.015) ^{c2}	0.160 (±0.012) ^{d4}
$\overline{NAR}$	($g\ m^{-2}\ d^{-1}$)	10.5 (±0.62) ^{a1}	6.1 (±0.75) ^{b3}	23.3 (±0.22) ^{c2}	17.6 (±0.20) ^{d4}
$\overline{LAR}$	($cm^2\ g^{-1}$)	138.6 (±2.7) ^{a1}	118.5 (±1.1) ^{b3}	108.3 (±4.3) ^{c2}	92.1 (±3.6) ^{d4}
$\overline{SLA}$	($cm^2\ g^{-1}$)	266.4 (±2.6) ^{a1}	235.9 (±1.9) ^{b3}	210.4 (±7.9) ^{c2}	188.2 (±6.2) ^{c4}
$\overline{LWR}$	($g\ g^{-1}$)	0.519 (±0.008) ^{a1}	0.493 (±0.002) ^{b3}	0.513 (±0.005) ^{c1}	0.483 (±0.006) ^{d3}
Root $\overline{RGR}$	($g\ g^{-1}\ d^{-1}$)	0.129 (±0.018) ^{a1}	0.062 (±0.019) ^{a3}	0.179 (±0.010) ^{c1}	0.127 (±0.013) ^{c3}
Shoot $\overline{RGR}$	($g\ g^{-1}\ d^{-1}$)	0.148 (±0.009) ^{a1}	0.075 (±0.006) ^{b3}	0.262 (±0.017) ^{c2}	0.167 (±0.015) ^{d4}
Leaf $\overline{RGR}$	($g\ g^{-1}\ d^{-1}$)	0.131 (±0.007) ^{a1}	0.039 (±0.001) ^{b3}	0.240 (±0.017) ^{c2}	0.129 (±0.014) ^{d4}
Stem $\overline{RGR}$	($g\ g^{-1}\ d^{-1}$)	0.175 (±0.007) ^{a1}	0.123 (±0.012) ^{a3}	0.298 (±0.018) ^{c2}	0.222 (±0.019) ^{d4}
Leaf area $\overline{RGR}$	($m^2\ m^{-2}\ d^{-1}$)	0.111 (±0.011) ^{a1}	-0.027 (±0.005) ^{b3}	0.204 (±0.015) ^{c2}	0.044 (±0.013) ^{d4}

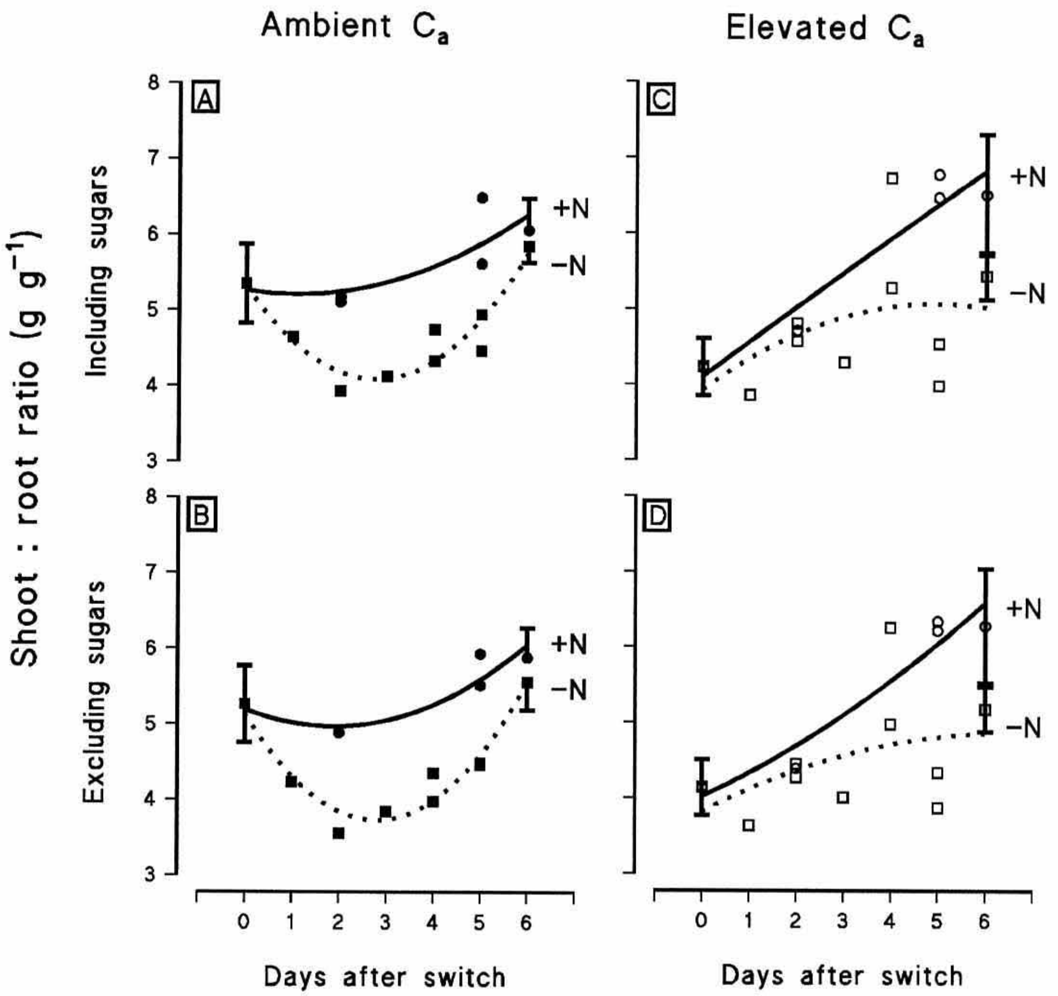

Figure 7.2. The ratio of shoot to root dry weight before (A,C) and after (B,D) subtraction of soluble sugars in *U. urens* grown in (A,B) ambient (ca. 350 μmol mol^{-1}) or (C,D) elevated (680 μmol mol^{-1}) C_a over 6 d following withdrawal of nitrate (-N) from a nutrient solution containing 12 mM nitrate (+N) as the sole source of mineral N. Quadratic curves were fitted to the data only to indicate trends. Data are shown as means (n = 4) with standard error bars, or as individual data points.

7.3.2. Tissue nitrate, soluble sugars and gas exchange

Following withdrawal of external nitrate, plants grown in both ambient and elevated C_a became rapidly depleted of tissue nitrate (Fig. 7.3.A,D). For reasons given in Section 7.2.5, no data were available to show a comparable time-course of nitrate in plants grown with a continued supply of external nitrate. C_a did not significantly affect the rate of nitrate depletion in any type of tissue, since the corresponding linear and quadratic terms of the equations fitted to logarithmically-transformed primary data (not shown) were not significantly different from one another (Table 7.2). The rate of nitrate depletion in stems was noticeably slower compared with that in leaves and roots, (Fig. 7.6; Table 7.2). Both the initial (day 0) and final (day 6) mean nitrate concentration was lower in elevated C_a, but differences were significant ($p < 0.05$) only in leaves and only when expressed on a leaf area basis (Fig. 7.3.A,D).

On a leaf area basis, the soluble sugar concentration in leaves of plants grown in elevated C_a compared with those in ambient C_a was significantly higher at day 6 but was not significantly different at day 0 (Fig. 7.3.B,E). In plants deprived of nitrate, only those grown in ambient C_a had significantly higher concentrations of soluble sugar in leaves at day 6, compared with the corresponding nitrate-sufficient plants (Fig. 7.3.B,E). However, data from additional plants harvested between days 0 and 6 indicated that a transient increase in soluble sugar concentration did occur at an earlier stage in leaves of plants grown in elevated C_a (Fig. 7.3.E). Similar early increases in soluble sugar concentration also occurred in leaves of plants grown in ambient C_a (Fig. 7.3.B).

In nitrate-sufficient plants, the light-saturated rate of photosynthesis (A_{sat}) in the youngest fully-expanded leaf was not significantly different at day 3 compared with that at day 6, but A_{sat} was stimulated by about 50 % at elevated compared with ambient C_a (Fig. 7.3.C,F). The withdrawal of nitrate resulted in progressive reductions in A_{sat} at both ambient and elevated C_a, but the reductions in A_{sat} at elevated C_a (45 % at day 3; 80 % at day 6) were slightly more pronounced than the reductions in A_{sat} in ambient C_a (38 % at day 3; 70 % at day 6) (Fig. 7.3.C,F).

Following withdrawal of nitrate, the changes in leaf soluble sugar concentrations per unit structural dry weight (SDW) (Fig. 7.4.A,D) paralleled those previously described per unit leaf area (Fig. 7.3.B,E). However, in nitrate-sufficient plants, expression of leaf sugars per unit SDW meant that the effect of elevated C_a at day 6 on leaf soluble sugars on a leaf area basis (Fig. 7.3.A,D) was no longer statistically significant (Fig. 7.4.A,D). At day 6, nitrate withdrawal resulted in significant increases in the soluble sugar concentration in both stems and roots of plants in elevated C_a (Fig. 7.4.E,F), but in stems only of plants in ambient C_a (Fig. 7.4.B,C). As was observed in leaves, data of soluble sugars in stems and roots of additional plants, grown in both ambient and elevated C_a and harvested between days 0 and 6, indicated that larger, transient increases in soluble sugars occurred in these tissues before day 6, particularly in stems (Fig. 7.4). The quadratic curves fitted to the time-course of logarithmically-transformed data of soluble sugar and nitrate concentration in leaves, stems and roots following withdrawal of nitrate are shown in Figure 7.6, and the constants describing the linear and quadratic terms are shown in Table 7.2. None of the constants were significantly affected by C_a.

The reductions in A_{sat} three d after withdrawal of nitrate (Fig. 7.3.C,F) were closely coupled to reductions in $V_{c,max}$ and A_{max}, but not to g_s, which was not significantly affected at this time (Fig. 7.5). However, large and significant reductions in g_s were found at day 6, amounting to about 80 % in both ambient and elevated C_a. The time-course of the reduction in $V_{c,max}$ paralleled that found for A_{sat} (Fig. 7.3.C,F), so that withdrawal of nitrate from plants grown in elevated C_a resulted in slightly earlier, larger reductions in $V_{c,max}$ compared with plants grown in ambient C_a (Fig. 7.5). Thus, the reduction in $V_{c,max}$ at day 3 in elevated C_a was by 41 % and was statistically significant, and in ambient C_a at the same point of time was by 36 % and statistically insignificant. Likewise, the reduction in $V_{c,max}$ at day 6 in elevated C_a was by 67 %, and in ambient C_a was by 57 %. In contrast, the reductions in A_{max} over time were similar in both ambient and elevated C_a. No significant effect of elevated C_a on g_s, $V_{c,max}$ or A_{max} was found in plants supplied continually with nitrate.

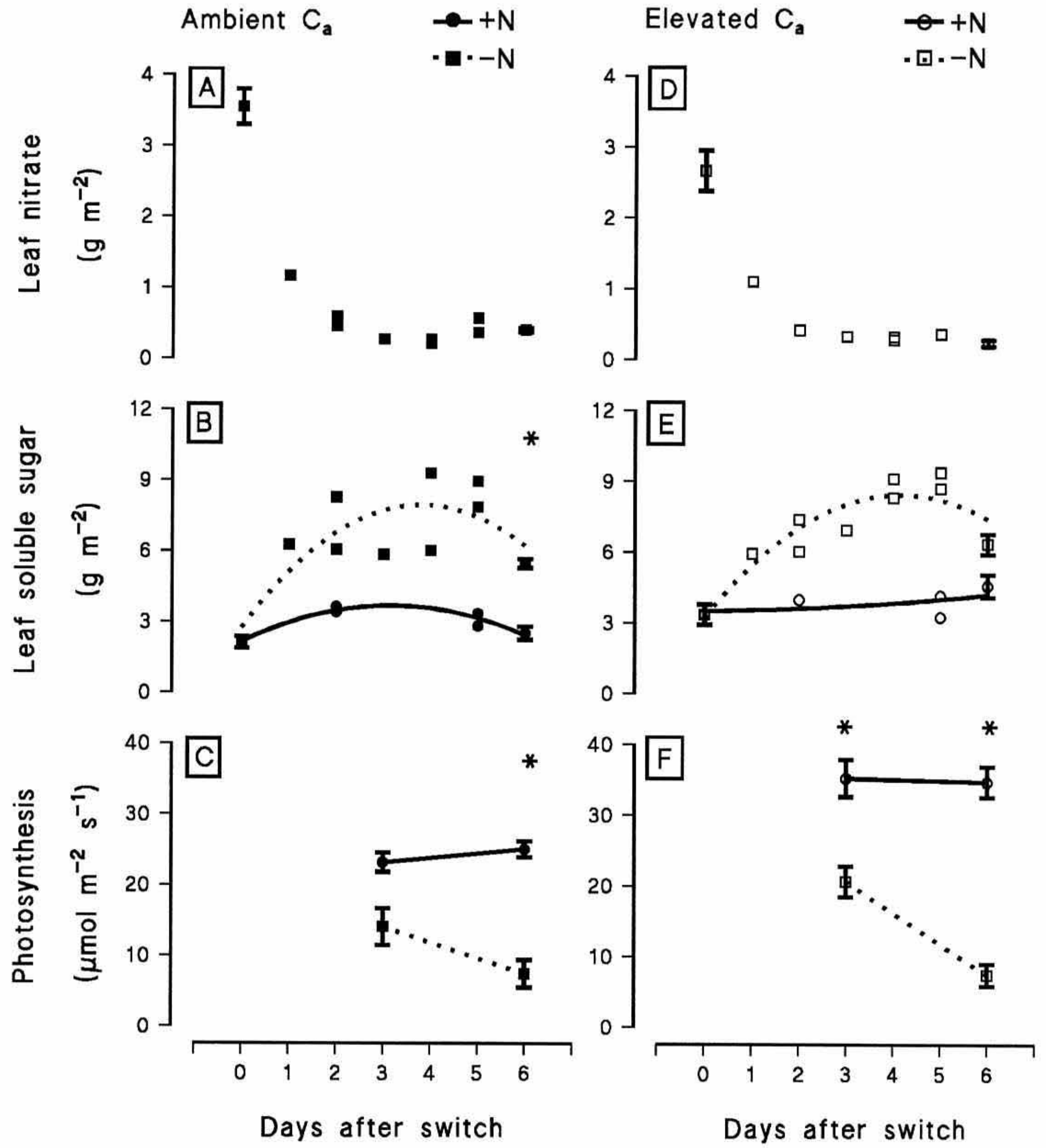

Figure 7.3. (A,D) Nitrate concentration in all leaves, (B,E) Soluble sugar concentration in all leaves and (C,F) The light-saturated rate of photosynthesis in the youngest fully-expanded leaf of *U. urens* grown in (A,B,C) ambient (ca. 350 μmol mol^{-1}) or (D,E,F) elevated (680 μmol mol^{-1}) C_a over 6 d following withdrawal of nitrate (-N) from a nutrient solution containing 12 mM nitrate (+N) as the sole source of mineral N. Data of nitrate concentration are shown as means with standard error bars (n = 4) or as individual data points. No data were available for nitrate concentration in leaves of plants sufficient in nitrate. Quadratic curves were fitted to data of leaf sugar concentration only to indicate trends, and these data are shown as means with standard error bars (n = 4) or as individual data points. Photosynthesis data are shown as means with standard error bars (n = 3). Significant differences ($p < 0.05$) due to nitrate are indicated by asterisks. The results of statistical analyses for differences due to C_a and time are given in the text.

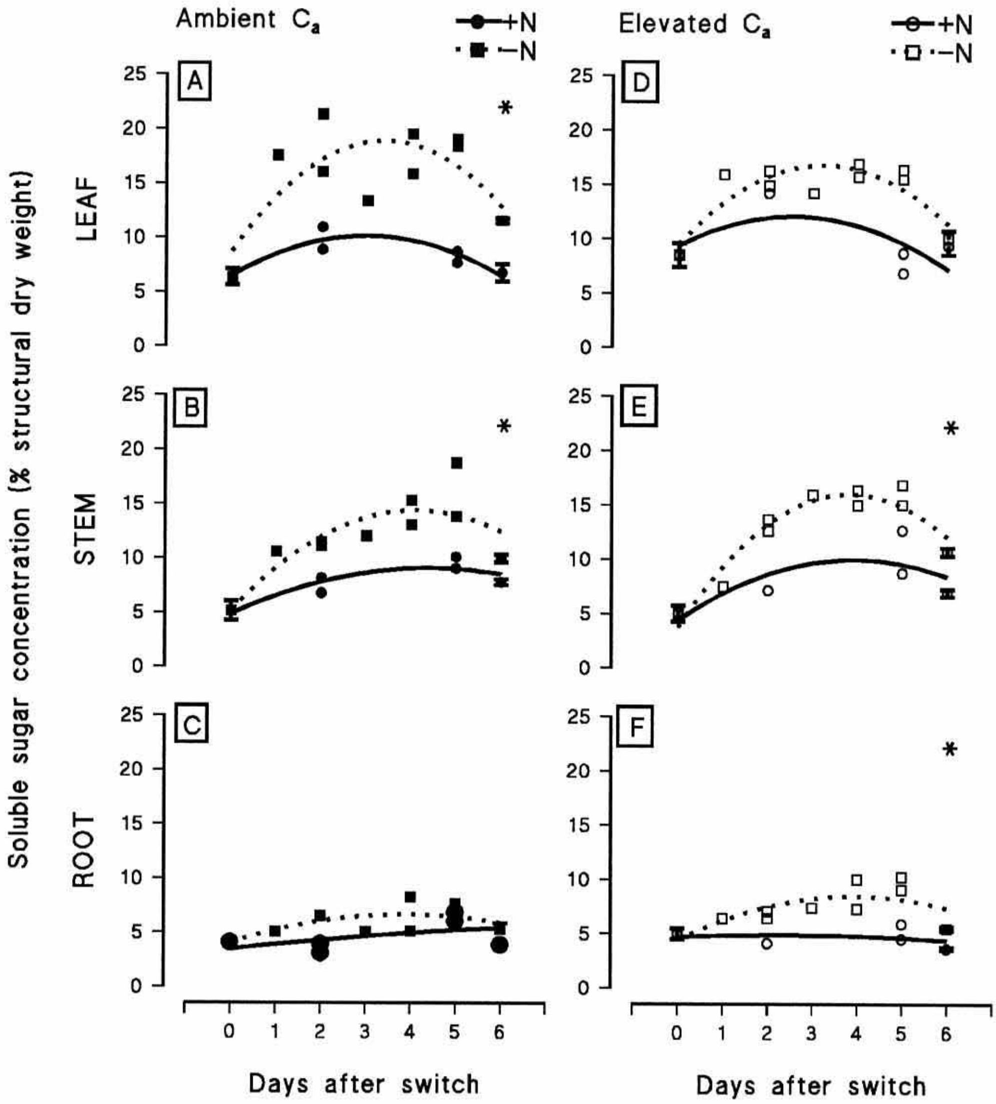

Figure 7.4. Soluble sugar concentration per unit structural dry weight in (A,D) All leaves, (B,E) Stems and (C,F) Roots of *U. urens* grown in (A,B,C) ambient (ca. 350 µmol mol^{-1})or (D,E,F) elevated (680 µmol mol^{-1}) C_a over 6 d following withdrawal of nitrate (-N) from a nutrient solution containing 12 mM nitrate (+N) as the sole source of mineral N. Structural dry weight is the total dry weight minus the weight of soluble sugars. Quadratic curves were fitted to the data only to indicate trends. Data are shown as means with standard error bars (n = 4) or as individual data points. Significant differences ($p < 0.05$) due to nitrate are indicated by asterisks. The results of statistical analyses for differences due to C_a and time are given in the text.

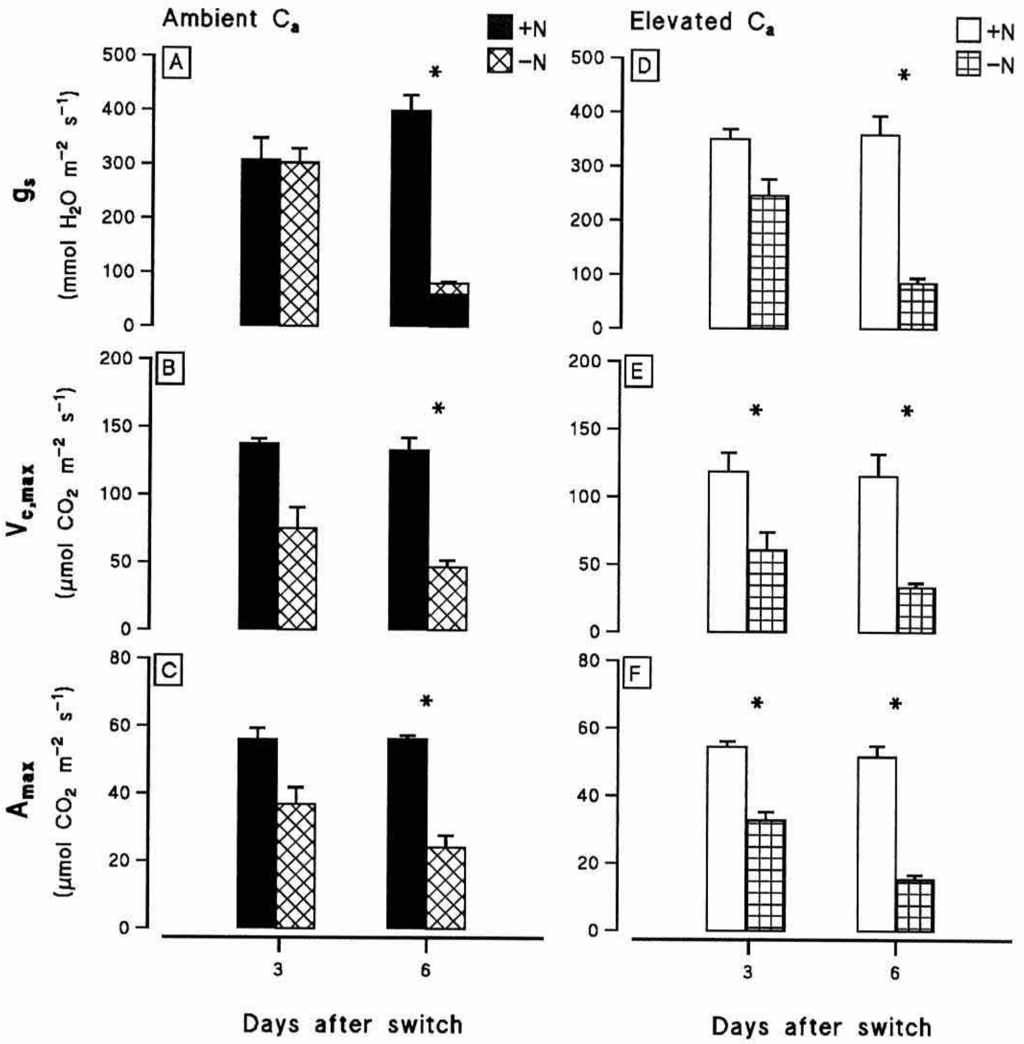

Figure 7.5. (A,D) Stomatal conductance to water vapour (g_s), (B,E), The maximum rate of carboxylation limited by the amount and/or activity of rubisco ($V_{c,max}$) and (C,F) The maximum rate of light/CO_2-saturated photosynthesis (A_{max}). Measurements were made in the youngest fully-expanded leaf of *U. urens* grown in (A,B,C) (ca. 350 μmol mol^{-1}) or elevated (D,E,F) (680 μmol mol^{-1}) C_a over 6 d following withdrawal of nitrate (-N) from a nutrient solution containing 12 mM nitrate (+N) as the sole source of mineral N. Data are shown as means (n = 3) with standard error bars. Significant differences ($p < 0.05$) due to nitrate indicated by asterisks. The results of statistical analyses for differences due to C_a and time are given in the text.

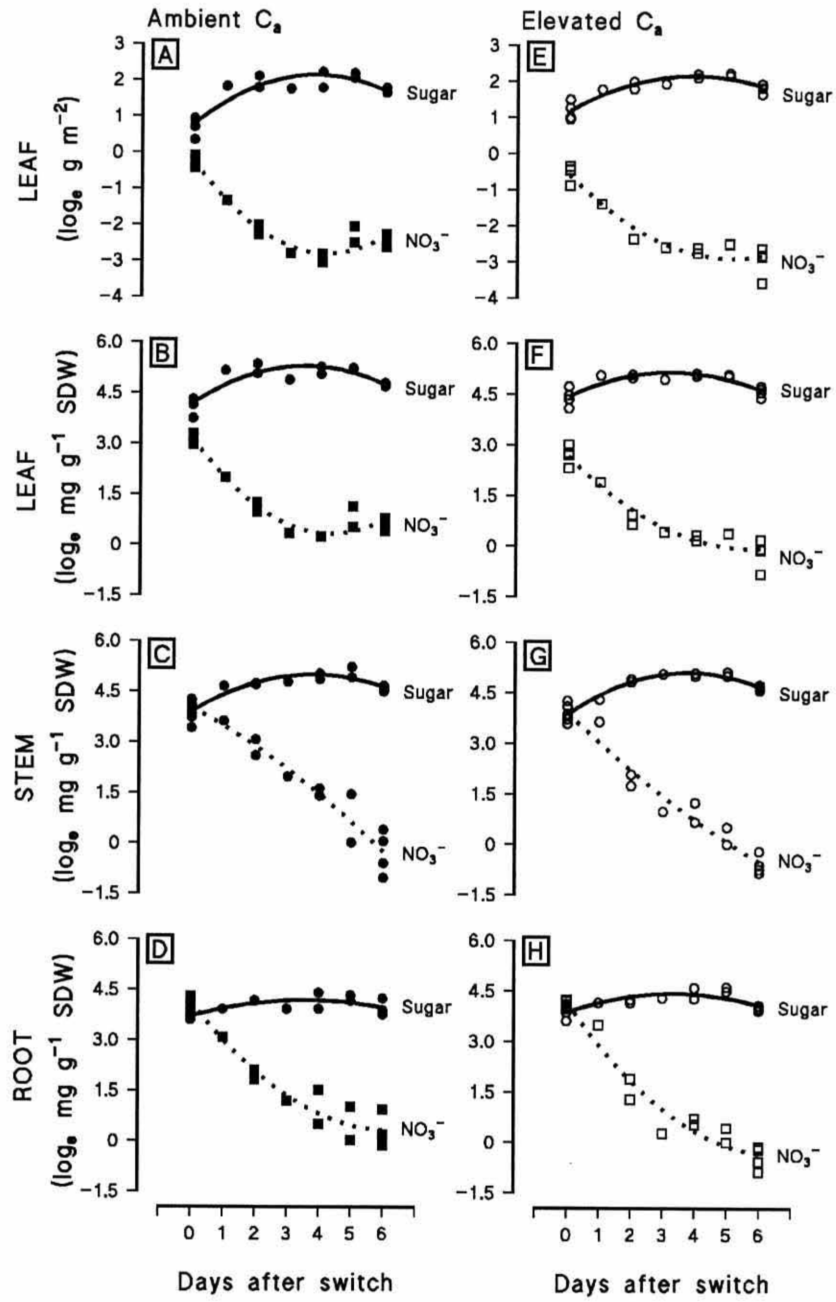

Figure 7.6. Quadratic curves fitted to logarithmically-transformed data of soluble sugar and nitrate concentration in (A,B,E,F) All leaves, (C,G) Stems and (D,H) Roots of *U. urens* grown in (A,B,C,D) ambient (ca. 350 µmol mol^{-1}) or (E,F,G,H) elevated (680 µmol mol^{-1}) C_a over 6 d following withdrawal of nitrate (-N) from a nutrient solution containing 12 mM nitrate (+N) as the sole source of mineral N. Data are expressed per unit structural dry weight (SDW, being the total dry weight minus the weight of soluble sugars), and also, in the case of leaves, per unit leaf area (A,E). The coefficient of determination (r^2) for the fitted equations and the constants (± standard errors) describing the linear and quadratic terms are shown in Table 7.2.

Table 7.2. Analysis of the curves fitted to the time-course of logarithmically-transformed data of soluble sugar and nitrate concentrations in tissues of *U. urens* following the withdrawal of external nitrate from plants grown in ambient (ca. 350 μmol mol^{-1}) or elevated (680 μmol mol^{-1}) C_a and previously sufficient in nitrate (Fig. 7.4). The table shows the constants *b* and *c* describing, respectively, the linear and quadratic terms in equations of the form $y = a + bt + ct^2$, where *y* is data of soluble sugar or nitrate concentration, *t* is time (d), and *a* is the term describing *y* when $t = 0$ (not shown). The coefficient of determination (r^2) is also given for each equation.

		Soluble sugar concentration			Tissue nitrate concentration		
	C_a	*b*	*c*	r^2	*b*	*c*	r^2
LEAF (g m^{-2})	Ambient	0.710 (±0.102)	-0.0932 (±0.016)	0.837	-1.231 (±0.103)	0.148 (±0.017)	0.951
	Elevated	0.494 (±0.070)	-0.0635 (±0.011)	0.851	-0.902 (±0.136)	0.089 (±0.022)	0.912
LEAF (mg g^{-1})	Ambient	0.628 (±0.098)	-0.0893 (±0.016)	0.780	-1.314 (±0.111)	0.152 (±0.018)	0.955
	Elevated	0.447 (±0.076)	-0.0694 (±0.012)	0.727	-0.949 (±0.142)	0.083 (±0.023)	0.930
STEM (mg g^{-1})	Ambient	0.579 (±0.096)	-0.0761 (±0.015)	0.793	-0.507 (±0.188)	0.034 (±0.019)	0.968
	Elevated	0.659 (±0.064)	-0.0869 (±0.010)	0.917	-0.913 (±0.148)	0.029 (±0.024)	0.952
ROOT (mg g^{-1})	Ambient	0.267 (±0.072)	-0.0370 (±0.012)	0.557	-1.217 (±0.161)	0.097 (±0.018)	0.952
	Elevated	0.337 (±0.077)	-0.0500 (±0.012)	0.604	-1.369 (±0.167)	0.103 (±0.027)	0.962

7.4. DISCUSSION

7.4.1. Plant growth

The reduction in whole-plant $\overline{RGR}$ following the withdrawal of nitrate was due primarily to a reduction in $\overline{NAR}$ and, to a lesser extent, in $\overline{LAR}$ (Table 7.1). This pattern is markedly different from the reduction in the initial stimulation of $\overline{RGR}$ which occurs in response to elevated C_a (Chapters 3 and 4), where the impact of $\overline{NAR}$ was marginal compared to that of $\overline{LAR}$ (Tables 3.1 and 4.1). These different responses of growth to C_a and N strongly suggest that they do not share a common mechanistic basis.

The results suggest that elevated C_a may moderate the limitation to growth imposed by N deficiency, since the reduction in whole-plant $\overline{RGR}$ due to withdrawal of nitrate was less in elevated compared with ambient C_a (Table 7.1). This moderation was apparently achieved by the ability of plants in elevated C_a to sustain a higher $\overline{NAR}$ following withdrawal of nitrate (Table 7.1). Superficially, this maintenance of $\overline{NAR}$ in elevated C_a is not consistent with the earlier, larger reductions in A_{sat} and $V_{c,max}$ in leaves of nitrate-deprived plants grown in elevated compared with ambient C_a (Figs. 7.3 and 7.5). However, the point has been made (Chapters 3 and 4) that measurements of photosynthetic capacity of single leaves need not be strongly correlated with $\overline{NAR}$. In many cases, canopy photosynthesis and $\overline{NAR}$ may be determined primarily by factors such as quantum yield (Baker *et al.*, 1994) or even perhaps canopy architecture (discussed in Section 3.4.2).

The absence of any significant decrease in shoot:root ratio (S/R) at day 6 following withdrawal of nitrate conflicts with the extensive literature demonstrating unequivocally that N deficiency causes a reduction in S/R (Brouwer, 1962; Wilson, 1988). This decrease usually occurs due to a decreased shoot RGR coupled with an increased root RGR, soon after the onset of N deprivation (Kuiper, 1988). Studies that impose a limiting step-decrease in the external N concentration are likely to find a progressively decreasing S/R with time, due to an increasing internal N deficiency linked to increasing plant size (H. Harmens, pers. comm.). In contrast, studies that impose a steady-state tissue N depletion by decreasing the external relative addition rate of N (using the technique of Ingestad and Lund, 1986) generally find that S/R eventually stabilizes at a lower value as shoot and root RGR equalize (McDonald *et al*, 1986; Van der Werf *et al.*, 1993). In the present study, where mineral N was completely withdrawn from plants previously sufficient in N, early reductions in S/R occurred that were no longer evident at day 6 (Fig. 7.2.A), indicating a late reduction in root RGR relative to shoot RGR. One possible explanation for this unusual response is that the withdrawal of external nitrate, rapidly followed by the depletion of internal nitrate reserves to sustain growth, generated an internal N deficiency so severe as to effectively halt growth within 6 d. Consequently, existing root structure may have been mobilized to shoots to drive one final reproductive effort before death.

Whole-plant $\overline{\text{RGR}}$ was found to be stimulated by elevated C_a over a 6 d period when plants were between 36 and 42 d old (Table 7.1). The timing of this stimulation was not consistent with that reported in Chapter 3 for plants similarly supplied with nitrate, where a CO_2-stimulation of $\overline{\text{RGR}}$ occurred only over the 10 - 21 d harvest interval when plants were between 22 and 33 d old (Table 3.1). Comparisons between the values of $\overline{\text{RGR}}$ shown in Table 7.1 and those shown in Table 3.1 for the 21 - 26 d harvest interval, suggest that the discrepancy can be ascribed to a particularly low $\overline{\text{RGR}}$ of plants grown in ambient C_a found in the present chapter. It is difficult to explain this inconsistency except in terms of species variability, low replication, and sampling error inherent in the classical approach to growth analysis, where variability at the beginning and end of each harvest interval can be greatly amplified in the calculation of $\overline{\text{RGR}}$ and its components. Variability and low replication may also explain why the S/R in nitrate-sufficient plants was not significantly affected by elevated C_a (Fig. 7.2). This result conflicts with the prediction implicit in the allometric analysis of S/R presented in Chapter 3, that a decreased allometric coefficient (v) describing the relationship between logarithmically-transformed shoot and root dry weight (Fig. 3.7; Table 3.3) should manifest as a reduction in S/R.

7.4.2. Evidence for the sink-regulation of photosynthesis during N deficiency

Consistent with other studies where mineral N has been withdrawn from previously N-sufficient plants, soluble sugars accumulated in leaves within a matter of days (Thornsteinsson *et al.*, 1987; Henry and Raper, 1991; Paul and Driscoll. 1997), and the rate of photosynthesis declined in close association (Thornsteinsson *et al.*, 1987; Paul and Driscoll. 1997) (Fig. 7.3). The decline in photosynthesis could be related to reductions in both $V_{c,max}$ and A_{max} (Fig. 7.5), and therefore to reductions in the activities and or/amounts of photosynthetic proteins (Sharkey, 1985); this is entirely consistent with a sugar-repression of photosynthesis mediated by an inhibition of gene expression. Moreover, the reductions in $V_{c,max}$ and A_{max} before any significant reduction in g_s (Fig. 7.5) suggest that sugar-repression may be particularly important in the regulation of photosynthesis during N deficiency. As was also indicated in the study by Henry and Raper (1991), the results in the present study suggest that the accumulation of sugars in plant tissues following a major disturbance to the sink-source balance is transient (Fig. 7.4), which supports the view that the sugar-repression of photosynthesis could play a role in restoring equilibrium between the activity of sources and sinks (Pollock and Farrar, 1996). However, the accumulation of sugars in leaves following withdrawal of nitrate was surprisingly small (Fig. 7.3), particularly in relation to the rapid depletion of tissue nitrate (Fig. 7.6) and the marked alterations in the components limiting plant growth (Table 7.1). Thus, whilst the sugar-repression of photosynthesis may indeed play a role in optimizing certain processes in response to perturbations likely to result in death (Section 7.4.1), it is probably not a sensitive and commonly-employed mechanism by which plants achieve sustainable growth in environments where the supply of resources change more gradually in time and space, as has been proposed by some workers (Neales and Incoll, 1968; Herold, 1989; Stitt, 1991; Pollock and Farrar, 1996).

If the accumulation of sugars in leaves occurs because the withdrawal of N in some way reduces the capacity of sinks, theoretical considerations (Pollock and Farrar, 1996), as well as observations in different experimental systems (Sawada *et al.*, 1987), predict that the accumulation of sugars in source leaves should be preceded by an accumulation of sugars in sinks. Here, the early and probably dynamic changes in the sugar concentration in tissues following withdrawal of nitrate may have meant that there were insufficient data to properly address the time-sequence of events, such that the accumulation of sugars in leaves, stems and roots appeared only to proceed in parallel (Fig. 7.4). Consistent with this view, the sequential changes in root and leaf sugars following root cooling occurred within at least 4 d in single-rooted soybean leaves (Sawada *et al.*, 1987). Other N-deprivation studies have also lacked the necessary resolution in time to address this question (Henry and Raper, 1991). As was concluded when plants were switched to elevated C_a (Chapter 5), investigations of the physiological consequences of environmental perturbations to the sink-source balance may require a much smaller experimental window, perhaps with a resolution of hours rather than days.

Although the time-sequence of events could not be determined here, the accumulation of sugars in leaves was always greater than in stems and roots, and the accumulation in roots was particularly small (Fig. 7.4). This pattern of sugar accumulation completely contradicts that found by Henry and Raper (1991), where the sugar concentration in tobacco tissue 5 d after withdrawal of N was highest in roots, second-highest in stems, and lowest by far in leaves. Superficially at least, the pattern of accumulation found by these workers also argues against the view that sink capacity limits photosynthesis during N deficiency. However, taking a more mechanistic approach to sinks and their capacity (Farrar, 1996), it becomes readily apparent that source leaves themselves represent major sinks for fixed carbon. In this respect, the sink capacity of source leaves may be especially sensitive to N deficiency, since they represent the principal sites for the incorporation of carbon skeletons into amino-N (Smirnoff and Stewart, 1985; Huppe and Turpin, 1994). An understanding of specific targets for N within the broader concept of sink capacity can also suggest explanations for the patterns of sugar accumulation in stems and roots. For example, James *et al.* (1993) demonstrated clearly that N deficiency inhibits leaf expansion more than it inhibits root extension. Similarly, studies by Van der Werf *et al.* (1992; 1994) indicate that, unlike shoots, root respiration and ATP production are only marginally reduced when the rate of nitrate uptake is reduced by as much as 90 %. Accordingly, in the present study, the reductions in shoot, stem and leaf $\overline{R}GR$ following N withdrawal were more pronounced than the reduction in root $\overline{R}GR$ (Table 7.1). To conclude this section, there is evidence which supports the case for a role of sugars in the sink-regulation of photosynthesis in severe N deficiency.

7.4.3. Interactions between nitrate deprivation and elevated C_a

The accumulation of soluble sugars in leaves due to elevated C_a alone was not large, with increases from about 2 to 3 g m^{-2} at day 0, and from about 2.5 to 4.5 g m^{-2} at day 6 (Fig. 7.3). Similarly small, often approximately 2-fold increases in the soluble sugar concentration in leaves due to elevated C_a have been reported previously, both when averaged for all the leaves on a plant as here (Chapter 3, Fig. 3.5; Chapter 5, Fig. 5.4), and when measured at the single-leaf level (Chapter 5, Table 5.6; Van Oosten and Besford, 1995; Hibberd *et al.*, 1996b; Harmens *et al.*, 1998). Consistent at least with some of these studies (Chapter 3; Chapter 5; Harmens *et al.*, 1998), such increases in soluble sugar concentration were not associated with the down-regulation of photosynthetic capacity (Fig. 7.5), although clearly this conclusion based on the present study alone would carry more weight if the soluble sugar concentration had been measured in the same single leaf on which measurements of photosynthesis were made.

Whilst there are a number of possible explanations for the apparent insensitivity of photosynthesis to the soluble sugar status of source leaves (Chapter 5, Section 5.4.3), the generally moderate accumulation of sugars in leaves in elevated C_a suggests that a critical threshold sugar concentration is rarely attained in CO_2-enrichment studies. Consistent with this view, reductions in the carboxylation efficiency of a source-leaf of barley grown in elevated C_a were evident only when the soluble sugar concentration exceeded 10 g m^{-2} (Hibberd *et al.*, 1996b). In the present study, the down-regulation of photosynthesis following the withdrawal of nitrate (Figs. 7.3 and 7.5) required a concentration of at least 6 g m^{-2} (Fig. 7.3). However, this value obtained as an average for all leaves significantly underestimates the actual concentration of sugars in the main-stem leaf on which measurements of photosynthesis were made, since it is evident from results reported in Chapter 3 that branch-stem leaves, of which there were quantitatively many more (data not shown), had lower sugar concentrations (Fig. 3.3).

Despite the higher leaf soluble sugar concentration in plants grown in elevated C_a, there was no convincing evidence to indicate that the accumulation of sugars in leaves following the withdrawal of nitrate was larger or more rapid in elevated C_a (Figs. 7.3 and 7.6). Rather, the lower linear term, in the quadratic equations describing the relationship between sugar concentration and time in elevated C_a (Table 7.2) suggests that the rate of sugar accumulation may even have been higher in ambient C_a, although a high variability in the data meant that differences were not statistically significant ($p = 0.07$, in the case of sugar concentration per unit area). One possible explanation for this unexpectedly moderate response in elevated C_a could be that a greater proportion of soluble sugar was used in growth, which was less reduced in elevated compared with ambient C_a following the withdrawal of nitrate (Table 7.1).

In the absence of any acceleration or increase in sugar accumulation following withdrawal of nitrate in elevated C_a, the results nevertheless indicated earlier, larger reductions in photosynthesis in these plants compared with plants grown in ambient C_a (Fig. 7.3). Moreover, the CO_2-mediated response to withdrawal of nitrate could be linked more specifically to earlier, larger reductions in $V_{c,max}$ (Fig. 7.5), rather than to other processes that can limit photosynthesis, such as A_{max} and g_s. Given that $V_{c,max}$ is closely related to the amount of active rubisco protein (Sharkey, 1985), and that, amongst photosynthetic proteins studied so far, rubisco is particularly sensitive to sugar repression (Van Oosten and Besford, 1994; Krapp and Stitt, 1995), signals other than sugars may be involved here that at least contribute to the down-regulation of photosynthesis.

Certainly, photosynthetic down-regulation mediated by rubisco can be induced without the obvious involvement of soluble sugars in other experimental systems, such as when barley leaves are infected with a fungal pathogen (Hibberd *et al.*, 1996b) and when *Dactylis glomerata* plants are grown continually with low external supplies of N (Harmens *et al.*, 1998). Similarly, the reduction in photosynthesis in soybean leaves following root cooling was more clearly related to large increases in starch (up to about 15 g m^{-2}) than to increases in soluble sugars, which were never higher than about 3.5 g m^{-2} (Sawada *et al.*, 1987). Cave *et al.* (1981) suggested that physical disruption of the photosynthetic apparatus by the accumulation of starch in *Trifolium subterraneum* leaves was responsible for photosynthetic down-regulation in elevated C_a in this, and perhaps other, starch-storing species.

Paul and Driscoll (1997) also questioned whether the generally moderate increases in soluble sugars reported in both CO_2-enrichment and nitrate-deprivation studies are sufficiently large to induce a photosynthetic response without the action of some additional factor. Following nitrate withdrawal, stronger correlations were found between photosynthetic capacity and the hexose:amino acid ratio than between photosynthetic capacity and hexose alone, suggesting that some component of N metabolism may contribute significantly to the response. Indeed, it is becoming increasingly clear that C and N metabolic pathways are intimately linked at many levels of physiological organization (Roy and Garnier, 1994 and papers therein). Major roles have emerged for nitrate and amino-N in the coarse control of nitrate uptake (Touraine *et al.*, 1994), nitrate assimilation (Oaks, 1993), and C_4 photosynthesis (Yamazaki *et al.*, 1986). Whether there is a role for N metabolites in the regulation of C_3 photosynthesis remains to be seen, but the results here suggest at the very least that the changes in tissue nitrate following its withdrawal externally are much greater than the changes in soluble sugars (Figs. 7.3 and 7.6). Moreover, the initially lower leaf nitrate concentration in elevated C_a (Fig. 7.3) followed by a decline in nitrate at a rate similar to that of plants grown in ambient C_a (Table 7.2) may have resulted in the earlier onset of a threshold nitrate concentration below which a down-regulation of photosynthesis will occur. However, it is also clear that the differences in the timing and magnitude of the photosynthetic response, following withdrawal of nitrate in elevated compared with ambient C_a, were perhaps too small to allow any convincing elucidation of a mechanistic basis mediated by either nitrate or sugars.

7.4.4. Conclusions

Following the imposition of a severe N limitation, evidence was found for the sugar-repression of photosynthetic capacity which was coupled with a reduced NAR and RGR. These findings suggest that the sugar-repression of photosynthetic genes can play a role in down-regulating growth in physiologically-real experimental systems. However, the severity of N limitation resulted in a surprisingly moderate accumulation of soluble sugars in leaves, relative to the depletion of tissue nitrate at least. Consistent with the views of Paul and Driscoll (1997), sugars may not be the only, or even principal, agent involved in signalling N deficiency or other environmental variables that perturb the sink-source balance. Moreover, it is possible that the sugar-repression of photosynthesis is in fact a drastic response to impending death, rather than a sensitive means of adaptation to less severe, but often more ecologically-real, changes in environmental conditions such as a gradual decline in mineral N availability or exposure to twice-ambient C_a.

CHAPTER 8

General discussion

8.1. Implications of the findings for existing climate change research and beyond

As primary producers, plants largely determine the structure of ecosystems through their type, abundance and distribution. Understanding the likely effects of anthropogenic or natural climate change on the structure of plant communities holds more than just an ecological interest, since it allows for the implementation of strategies now that might prevent or mitigate future loss of biodiversity from a biosphere whose present structure is unprecedented in the earth's history due to human activity, and probably unstable (Naeem *et al.*, 1994).

Various lines of research can contribute to the prediction of plant community structure in a future changed climate. Perhaps foremost of these is the correlation of past climatic changes with changes in the palaeoecological record (Huntley and Birks, 1983; Davis *et al.*, 1986). A similarly correlative approach, but without the added dimension of time, can be found in attempts to match existing climatic and geographical zones with structural (Raunkiaer, 1934; Holdridge, 1947), morphological (Box, 1981) and physiological (Woodward, 1987) characteristics. However, none of these approaches can account for the probability that the rapidity of the currently rising C_a and temperature (IPCC, 1990) is unprecedented in the evolutionary history and genetic memory of plants (Bowes, 1993). It is therefore direct experimental investigation that is likely to provide the approach of most predictive value.

Only a limited number of studies have had the facilities and resources to investigate the long-term effects of climate change over years in realistic physical environments, and on realistic model systems such as plant assemblages (e.g. Hebeisen *et al.*, 1997) and entire microcosms (e.g. Jones *et al.*, 1998), and can therefore predict with any real certainty how climate change will alter community and ecosystem structure. However, the findings of these studies indicate that the impact of climate change could be considerable. For example, Hebeisen *et al.* (1997) found that prolonged exposure to elevated C_a under FACE (free air CO_2 enrichment) favoured growth of *Trifolium repens* over *Lolium perenne* in a mixed sward, suggesting a future increase in the abundance and spread of leguminous species. Similarly, Jones *et al.* (1998) reported that long-term exposure of model terrestrial microcosms to elevated C_a resulted in a major alteration in the composition of soil fauna, which was attributed to the below-ground allocation of increased photosynthetically-fixed carbon.

There have been relatively few studies investigating the effects of elevated temperature as well as C_a. A short-term study by Stirling *et al.* (1998) on their effects on five native annual species in semi-natural environments concluded that a variable but positive growth response to elevated C_a alone was generally increased at elevated C_a and temperature in combination. However, the predictive value of experiments that elevate temperature are limited somewhat by the difficulties, unlike is the case for C_a, in making reliable models to predict either the magnitude or distribution of future increases in temperature (Jones and Wigley, 1990).

Given the considerable financial costs involved in running long-term, large-scale CO_2-enrichment studies, it is perhaps inevitable that predictions of plant community structure in a future changed climate have been and will be based on the cautious extrapolation from a wide range of shorter-term, smaller-scale experiments such as are reported in this thesis. As a predictive tool, the thesis has concentrated on plant relative growth rate (RGR), which is an important component of competitive ability particularly in environments relatively rich in resources (Tilman, 1988; Van der Verf *et al.*, 1998). Moreover, the capacity of plants to use CO_2 for growth represents an important factor potentially mediating the rate and eventual magnitude of the currently rising C_a, and hence the earth's future climate (IPCC, 1990).

As with studies of the growth of other herbaceous C_3 species exposed to approximately twice-ambient C_a from seed or from an early age (Poorter *et al.*, 1988; Bazazz, 1990; Den Hertog *et al.*, 1993, 1996; Poorter, 1993; Baxter *et al.*, 1994a; Stirling *et al.*, 1998), it was found in Chapter 3 that an initial CO_2-stimulation of the RGR of *Urtica urens* declined at some ill-defined stage early upon exposure, so that the stimulation of RGR was lost after about 21 d (Table 3.1). Together with the extensive literature in which a down-regulation of RGR in elevated C_a is implied from more rudimentary approaches to plant growth (Kimball, 1983; Cure and Acock, 1986; Hunt *et al.*, 1991; Poorter, 1993), these observations succeed in indicating that temperate herbaceous C_3 plants may have only limited impact on increasing community productivity or on mitigating rising C_a and future climate, but are otherwise so inconsistent between experiments (Arp, 1991) and between and within species (Poorter, 1993) as to fail in any convincing prediction of future community structure.

A more powerful predictive tool must lie in taking more functional or mechanistic approaches to growth in elevated C_a. Recognizing this, Hunt *et al.* (1991, 1993) examined the growth responses to elevated C_a of a range of herbaceous species grouped into ecological classes according to their C-S-R functional type (Grime, 1977), and concluded that growth was stimulated by elevated C_a more in 'Competitors' than in 'Ruderals' or 'Stress tolerators'. However, other studies which have investigated growth and growth-related responses of annual species to C_a within the context of the C-R-S model have reached the conflicting conclusion that the model does not predict the direction of either the photosynthetic (Stirling *et al.*, 1997) or growth (Stirling *et al.*, 1998) responses. Likewise, the relatively large growth response to elevated C_a of *U. urens* (Section 3.4.1), which is classified as between 'Ruderal' and 'Competitor-Ruderal' (Grime *et al.*, 1988), conflicts with the conclusion of Hunt *et al.* (1991) that native fast-growing annuals are amongst the least responsive to elevated C_a.

A more robust model that can explain the differential growth responses of plants to elevated C_a was proposed by Stitt (1991), such that CO_2-responsiveness will be largest in those plants that have the greatest capacity (sink capacity) to use the additional soluble sugar available in elevated C_a due to the stimulation of photosynthesis in source leaves. Stitt (1991) discussed the extensive and convincing circumstantial evidence in the literature that argues for a central role of sink capacity, which may be determined by genotype or environment, in mediating the growth response to elevated C_a. This circumstantial evidence is supported by a well-developed mechanistic model, whereby plants having a low sink capacity will accumulate soluble sugars in tissues, and their accumulation in source leaves, a frequently-reported response to elevated C_a (Farrar and

Williams, 1991), will result in decreased growth due to photosynthetic down-regulation following the sugar-repression of genes encoding key photosynthetic proteins (Stitt, 1991; Van Oosten *et al.*, 1994; Pollock and Farrar, 1996).

A primary objective of this thesis was to investigate more rigorously the validity of this model as a predictor of plant growth in elevated C_a. No convincing supportive evidence was found. At a purely descriptive level, it was evident in Chapters 3 and 4 that the reduction in the CO_2-stimulation of RGR of *U. urens* was more strongly coupled to a decreased leaf area ratio (LAR) than to a reduction in the CO_2-stimulation of net assimilation rate (NAR), the component clearly related to photosynthetic capacity. At a more mechanistic level, sugars accumulated in leaves in elevated C_a but did not cause any down-regulation of photosynthetic capacity (Chapters 3 and 5). Similarly, the accumulation of sugars in leaves was not preceded by accumulation of sugars in sinks (Chapter 5; Fig. 5.3), as predicted by theory (Fig. 5.0; Pollock and Farrar, 1996) and by experiment using model systems (Sawada *et al.*, 1987); neither was it preceded by responses that might otherwise indicate a change in sink capacity, such as a reduction in the proportion of gross photosynthesis used in respiration (Chapters 5 and 6). Moreover, the reduction in LAR without an accumulation of sugars in sinks conflicted with the suggestion of Pollock and Farrar (1996) that sugars could also play a role in determining morphogenesis.

In Chapter 7, evidence was found for the applicability of the model in a different, but nevertheless physiologically-real, experimental system whereby the sink-source balance was perturbed by depriving plants of mineral N. However, the severity of the perturbation, coupled with a surprisingly small accumulation of sugars in leaves (Fig. 7.3), suggested that the sugar-repression of photosynthesis may in fact be just one component of a mechanism that, in annual plants at least, mobilizes reserves to drive one final reproductive effort before death, rather than a sensitive and commonly-employed mechanism by which plants achieve coordinated and sustainable growth in continuously changing environments (Neales and Incoll, 1968; Herold, 1989; Stitt, 1991; Pollock and Farrar, 1996).

Alternative explanations were therefore sought in this thesis to explain plant growth responses to elevated C_a. The inevitable acceleration of plant size caused by even a transient CO_2-stimulation of RGR raises the possibility that responses attributed to elevated C_a could in fact be due to ontogenetic drift or to the earlier onset of an environmental constraint on growth. However, only few experiments have been designed or analyzed in a way that can account for effects of plant size, or can directly investigate the early changes in RGR that are implied in longer-term studies such as in Chapter 3 (Poorter *et al.*, 1988; Fonseca *et al.*, 1996; Stirling *et al.*, 1998). Consequently, a mechanistic understanding of responses to elevated C_a has remained hypothetical, fuelled only by reports of a wide range of potentially unrelated changes in physiology and morphology (Sections 3.1 and 4.1).

In order to address these issues, a number of key methodological approaches were used in the experiments reported in this thesis. Firstly, plants were grown with a supply of nutrients intended to be non-limiting for growth at all plant sizes (Section 2.2). That this was achieved, at least for mineral N, is implicit in the allometric analysis of whole-plant tissue organic N concentration (N_P), whereby the allometric coefficient (v) relating $\log_e$ organic N content to $\log_e$ dry weight was close to unity in plants grown in both ambient and elevated C_a (Fig. 3.6; Table 3.3). Secondly, in Chapters 4, 5 and 6, relatively mature plants were switched from ambient to elevated C_a and their responses monitored on a daily basis for 10 d. Such an experimental design represents a definite improvement in terms of providing tighter correlative evidence between growth and physiology, but it should be emphasized that the findings can be seen principally as a much-needed step towards targeting rewarding areas for future climate change research. In agreement with the study of Fonseca *et al* (1996) on *Plantago major*, it was found in Chapter 4 that the CO_2-stimulation of RGR of *U. urens* persisted for no longer than about 4 - 8 d (Table 4.1; Fig. 4.5). Moreover, functional growth analysis suggested that the decline in the CO_2-stimulation of RGR started within a few days or perhaps even hours following exposure to elevated C_a (Fig. 4.5), and showed clearly that the principal component responsible for constraining RGR in elevated C_a was an early reduction in LAR (Fig. 4.5) due to decreased SLA (Fig. 4.6). Evidence was also found for a relatively minor reduction on the CO_2-stimulation of NAR, but at a later time (Fig. 4.5).

Thirdly, the possible size-dependencies of responses were routinely investigated using allometric analysis (Tables 3.3, 4.3 and 5.4) or by plotting responses against indices of size rather than against time (Fig. 4.10). From arguments presented in Section 3.4.3 and supported in Chapter 4 for according biological significance to the elevations of allometric regression slopes, the novel conclusion was reached that the reductions in LAR and SLA were in fact a genuine effects of elevated C_a rather than consequences of accelerated plant size, contrary to the suggestions of some workers (Stirling *et al.*, 1998). Similarly, and contrary to the conclusions of Poorter *et al.* (1988) and Poorter and Pothmann (1992), genuine effects of C_a on both RGR and NAR were suggested by the plots showing that the responses of RGR and NAR over time (Fig. 4.5) persisted when plotted against indices of size (Fig. 4.10).

Having established that accelerated plant size probably does not account for the effects of elevated C_a on RGR and its components, Chapter 4 investigated the possible role of plant organic N concentration (N_P) in causing these responses (Section 3.4.4), a hypothesis prompted by the finding that plants growing in elevated C_a in the longer term had a decreased N_P (Fig. 3.2) independent of their size (Fig. 3.6). However, a role for N_P here was refuted on the grounds that no reduction in N_P occurred during the early stages of exposure to elevated C_a (Fig. 4.9) when the initial changes in RGR, NAR and LAR occurred (Fig. 4.5). However, it should be emphasized that decreased N_P, a widely-reported response of plants to CO_2-enrichment (Luo *et al.*, 1994), is likely to have wider implications for plant growth and ecosystem functioning in general, for example through lowering the food value of plant tissues for insect herbivores (Bezemer and Jones, 1998) and retarding the rate of decomposition (Kampichler *et al.*, 1998).

Finally, given that the rate of dark respiration (R_d) is stoichiometrically linked to the rate of structural growth (Farrar and Williams, 1991) and that the responses of RGR, NAR and SLA to elevated C_a were unaltered by the removal of total non-structural carbohydrates (Figs. 4.5 and 4.6), Chapter 6 investigated the possibility that direct and/or indirect effects of elevated C_a on components of R_d could be involved in the growth responses. The results showed that elevated C_a can cause a direct and reversible inhibition of apparent R_d in both expanding and fully-expanded leaves (Table 6.5), a finding reported by other workers for both leaves and whole plants (Bunce, 1990; 1994;

Amthor *et al.*, 1992; Ziska and Bunce, 1993; Thomas and Griffin, 1994). However, there was some evidence, worthy perhaps of further investigation, that the direct inhibition of R_d may have been off-set by an increase in the maintenance component of R_d (Fig. 6.3; Table 6.3), and that the final consequence of respiration for growth in elevated C_a may in fact be a decrease in the coefficient of growth R_d (Fig. 6.3; Table 6.3).

Any decrease in the coefficient of growth R_d would mean an increase in the growth conversion efficiency (Y_G; Thornley, 1970). Increases in Y_G are implicit in a number of CO_2-enrichment studies, including Chapter 5, finding that the CO_2-stimulation of R_d is proportionately less than the CO_2-stimulation of RGR (e.g. Bunce and Caulfield, 1991). Current models of R_d (Amthor, 1986; Farrar and Williams, 1991) are probably inadequate to predict how the effects of elevated C_a on R_d will affect plant growth, but their elaboration could lead to significant improvements in understanding how climate change will affect the growth of plants and may even reveal novel aspects of the role of respiration in plant productivity, the importance of which is widely recognized (e.g. McCree, 1982).

8.2. Implications of the findings for future research

A logical progression of the findings of this thesis into future research would be an investigation as to whether the early responses of *U. urens* to elevated C_a are common to other species, and also whether they can occur in more ecologically-real conditions, such as when plants are grown in soil or under natural light. The potentially unusual stomatal opening during the hours of darkness reported in Chapter 6 (Fig. 6.4) raises the disturbing possibility that plants grown in hydroponics (or otherwise in controlled-environment cabinets) could develop morphological and physiological characteristics unique to these ecologically-unreal conditions. Moreover, investigations under such conditions which aim to assess what are likely to be major impacts of reduced stomatal conductance (g_s) in elevated C_a on plant growth and ecosystem functioning in xeric environments (Bazazz, 1990; Bowes, 1993), may have only limited predictive value.

It could also be argued that there is a case for conducting switching experiments, similar to those described in Chapters 4, 5 and 6, on a species with lower inherent variability and/or using higher replication to enable a more rigorous testing of the hypotheses presented here. Nevertheless, there is perhaps now room for renewed speculation as to how elevated C_a down-regulates RGR. In the first place, the experiments reported in Chapters 3 - 5 indicate that changes in morphological characteristics, namely the reductions in LAR and SLA which are also consistently reported in other CO_2-enrichment studies (Acock and Pasternack, 1986; Poorter, 1993; Stulen *et al.*, 1994; Roumet *et al.*, 1996; Stirling *et al.*, 1998), constrain RGR in elevated C_a more than physiological characteristics such as NAR and the photosynthetic capacity of individual leaves. Moreover, the changes in LAR and SLA reflected genuine alterations in structure, since they persisted after the removal of total non-structural carbohydrates (Chapters 3 and 4).

In view of these conclusions, the study of Bunce (1995) is remarkable in demonstrating that these reductions in LAR can occur following exposure to elevated C_a during the hours of darkness only. This finding is entirely consistent with the conclusion reached in this thesis that the immediate products of photosynthesis play no role in determining morphogenesis (Chapter 5). Bunce (1995) has proposed a causal link between the frequently-observed inhibition of R_d by elevated C_a (e.g. Chapter 6) and the reductions in LAR. It remains to be seen whether further investigations reveal a novel understanding of a direct regulation of plant growth by CO_2.

Further investigations of the reductions in SLA that underlie the reductions in LAR may also help to target key aspects of physiology that constrain growth in elevated C_a. Moreover, it is becoming increasingly clear that both genotypic and phenotypic variation in RGR is strongly linked to SLA within widely differing plant types and environments (Tilman, 1988; Cambridge and Lambers, 1998; Pyankov *et al.*, 1998; Van der Verf *et al.*, 1998). Some workers have reported increases in leaf thickness and in the number of cell layers in leaves of plants exposed to elevated C_a (Thomas and Harvey, 1983). Preliminary and cursory investigation of transverse sections of individual leaves of *U. urens* suggested increases in leaf thickness due to increases in the size of cells, particularly in the length (perpendicular to the leaf surface) and volume of those forming the palisade layer. The mechanics of leaf growth have been characterized mainly in terms of the effects of light (Dale, 1988; Hart, 1988), and the effects of CO_2 are not fully understood. At present, the effects of elevated C_a on leaf expansion have been investigated in terms of the generation of leaf surface area rather than thickness (Taylor *et al.*, 1994), although these authors did purport to show an effect of elevated C_a on increasing cell wall extensibility due to increased activity of xyloglucan endotransglycosylase. Interestingly perhaps, the reduction in SLA reported in Chapter 5 for a single main-stem leaf (Table 5.7) was measured 11 d after exposure to elevated C_a when at the start of exposure this leaf was already nearly 80 % of full expansion (Fig. 5.7). This suggests that at least some of the SLA response to elevated C_a occurs after the stage of cell-division, and could mean that a significant proportion of the additional assimilate available due to increased photosynthesis is not exported but deposited directly as structural material in (for example) cell walls.

Another observation from this thesis that perhaps merits future investigation is the possible relationship between the decline in the CO_2-stimulation of NAR and alterations in canopy architecture that reduce the amount of light intercepted by the canopy (Sections 3.4.2 and 5.4.3). Such alterations could include reductions in the angles of leaves relative to incident PPFD and physical distortion of the surfaces of leaves. From cursory observations of *U. urens* plants in elevated C_a, both possibilities perhaps deserve future quantitative investigation. Van der Werf *et al.* (1998) has suggested that NAR may be quantitatively more important than LAR in limiting RGR in dicotyledonous species, whilst the reverse may be the case for monocotyledonous species. Whilst the results reported in this thesis conflict with this view, they nevertheless indicate that variation in NAR, as well as in LAR, may need explaining in terms of structural and morphological characteristics.

The alterations in canopy architecture described above are reminiscent of the epinasty and hypertrophy characteristic of plant responses to ethylene (Abeles, 1973; Lieberman, 1979). Respiration of fruits and possibly also of other plant tissues is also strongly promoted by ethylene (Amthor, 1991). In fact, numerous lines of evidence support the possibility that ethylene could play a role in plant responses to elevated C_a. In the first place, there are reasons to expect that elevated C_a will result in an increased atmospheric ethylene concentration, since C_a is well-known as a promoter of ethylene synthesis and activity (Philosoph-Hadas *et al.*, 1986), within the range of C_a (0 - 1000 μmol mol^{-1})

relevant to climate change research (Horton, 1985). Significantly, this range of C_a is much too low to induce the equally well-known inhibition by CO_2 of ethylene action and ethylene-mediated responses in general (Lieberman, 1979; Sisler and Wood, 1988).

An increased atmospheric ethylene concentration could also arise from contamination of CO_2 cylinders from which an elevated C_a is usually maintained in experiments. This possibility was addressed by Morison and Gifford (1984), who found that the amount of ethylene in some 'food grade' CO_2 cylinders was sufficiently high as to completely annul the positive effect of elevated C_a (with ethylene removed) on growth. Although the concentrations of ethylene found by these workers in 'industrial grade' CO_2 cylinders, of the type used to maintain elevated C_a in this thesis (Section 2.2) and probably the majority of CO_2-enrichment studies, were lower by several orders of magnitude and therefore unlikely to have any significant effect, their results did indicate that plant growth is highly sensitive to atmospheric ethylene at concentrations between 0.02 and 0.06 $\mu mol\ mol^{-1}$. Moreover, their results showed that the ethylene-mediated reversal of the usual stimulation of plant growth by elevated C_a was associated with marked reductions in SLA, lending support for a role of ethylene in more usual CO_2 exposure systems.

8.3. Conclusions

The findings of this thesis add to an extensive literature pertaining to the effects of elevated C_a on plants. Perhaps their main contribution to this research area is to highlight the probability that our understanding of the mechanisms underlying plant responses to elevated C_a is still rudimentary. This lack of understanding persists despite intensive past and present research effort, and despite a common perception that the time has come to dedicate funding towards other areas of plant and climate change research. Previous studies that have investigated the mechanisms behind plant responses to elevated C_a have perhaps concentrated overmuch on molecular and physiological responses (e.g. Chapter 5). It now appears that changes in morphology may well provide a more rewarding research area, not only with regard to improving our understanding of the effects of C_a and climate change, but also to reach a better understanding of how plants adapt to a wide range of environmental variables. It is perhaps not without significance that the creation of plants with higher SLA is now considered a major goal within broad attempts to improve plant and crop productivity (Lambers, 1998).

REFERENCES

ABELES, F.B. (1973). 'Ethylene in Plant Biology'. Academic Press, London.

ACOCK, B. and PASTERNAK, D. (1986). Effects of CO_2 concentration on composition, anatomy and morphology of plants. *In* 'Carbon Dioxide Enrichment of Greenhouse Crops. Volume II. Physiology, Yield and Economics (Eds. H.Z. Enoch and B.A. Kimball), pp. 41-52. CRC Press Inc., Boca Raton. ISBN 0-8493-5612-1.

ALLEN, S.E. (1989). Analysis of vegetation and other organic materials. *In* 'Chemical Analysis of Ecological Materials' (Eds. S.E. Allen, H.M. Grimshaw, T.A. Parkinson and C. Quarmby), pp. 46-61. Blackwell Scientific, Oxford.

AMTHOR, J.S. (1986). Evolution and applicability of a whole plant respiration model. *Journal of Theoretical Biology* **122**, 473-490.

AMTHOR, J.S. (1989). 'Respiration and Crop Productivity'. Springer-Verlag, Berlin.

AMTHOR, J.S. (1991). Respiration in a future, higher-CO_2 world. *Plant, Cell and Environment* **14**, 13-20.

AMTHOR, J.S., KOCH, G.W. and BLOOM, A.J. (1992). CO_2 inhibits respiration in leaves of *Rumex crispus* L.. *Plant Physiology* **98**, 757-

AP REES, T. (1990). Carbon metabolism in mitochondria. *In* 'Plant Biochemistry, Physiology and Molecular Biology' (Eds. D.T. Dennis and D.H. Turpin), pp. 106-123. Longman Sci. Tech., Essex.

ARP, W.J. (1991). Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant, Cell and Environment* **14**, 869-876.

AZCON-BIETO, J. and OSMOND, C.B. (1983). Relationship between photosynthesis and respiration. *Plant Physiology* **71**, 574-581.

BADGER, M.R. and ANDREWS, T.J. (1974). Effects of carbon dioxide, oxygen and temperature on a high-affinity form of ribulose disphosphate carboxylase-oxygenase from spinach. *Biochemical and Biophysical Research Communications* **60**, 204-210.

BAKER, N.P., FARAGE, P.K., STIRLING, C.M. and LONG, S.P. (1994). Photoinhibition of crop photosynthesis in the field at low temperatures. *In* 'Photoinhibition of Photosynthesis: from Molecular Mechanisms to the Field' (Eds. N.R. Baker and J.R. Bowyer), pp. 349-363.

BARNOLA, J.M., RAYNAUD, D., KOROTKEVICH, Y.S. and LORIUS, C. (1987). Vostok ice core provides 160,000-year record of atmospheric CO_2. *Nature* **329**, 408-424.

BAXTER, R., ASHENDEN, T.W., SPARKS, T.H. and FARRAR, J.F. (1994a). Effects of elevated carbon dioxide on three montane grass species. I. Growth and dry matter partitioning. *Journal of Experimental Botany* **45**, 305-315.

BAXTER, R., ASHENDEN, T.W., SPARKS, T.H. and FARRAR, J.F. (1994b). Effects of elevated carbon dioxide on three montane grass species. II. Nutrient uptake, allocation and efficiency of use. *Journal of Experimental Botany* **45**, 1267-1278.

BAXTER, R., BELL, S.A., SPARKS, T.H., ASHENDEN, T.W. and FARRAR, J.F. (1995). Effects of elevated carbon dioxide on three montane grass species. III. Source leaf metabolism and whole plant carbon partitioning. *Journal of Experimental Botany* **46**, 917-929.

BAZZAZ, F.A. (1990). The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics* **21**, 167-196.

BESFORD, R.T., LUDWIG, L.J. and WITHERS, A.C. (1990). The greenhouse effect: Acclimation of tomato plants growing in high CO_2. Photosynthesis and ribulose-1,5-bisphosphate carboxylase protein. *Journal of Experimental Botany* **42**, 925-931.

BESFORD, R.T., WITHERS, A.C. and LUDWIG, L.J. (1985). Ribulose bisphosphate carboxylase activity during leaf development in tomato. *Journal of Experimental Botany* **36**, 1530-1541.

BEZEMER, T.M. and JONES, T.M. (1998). Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. *Oikos* **82**, 212-222.

BOOT, R., RAYNAL, D.J. and GRIME, J.P. (1986). Comparative study of the influence of drought stress on flowering in *Urtica dioica* and *Urtica urens*. *Journal of Ecology* **74**, 485-495.

BOWES, G. (1991). Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. *Plant, Cell and Environment* **14**, 795-806.

BOWES, G. (1993). Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 309-332.

BOX, E.O. (1981). 'Macroclimate and Plant Forms: An Introduction to Predictive Modelling in Phytogeography. Dr. W. Junk, The Hague.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgramme quantities of protein utilizing the principle of protein dye binding. *Annals of Biochemistry* **72**, 248-254.

BROOKS, A. and FARQUHAR, G.D. (1985). Effect of temperature on the CO_2/O_2 specificity of ribulose bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Planta* **165**, 397-406.

BROUWER, R. (1962). Distribution of dry matter in the plant. *Netherlands Journal of Agricultural Science* **31**, 335-348.

BUCK, A.L. (1981). New equations for computing vapour pressure. *Journal of Applied Meteorology* **20**, 1527-1532.

BUNCE, J.A. (1990). Short and long term inhibition of respiratory carbon dioxide efflux by elevated carbon dioxide *Annals of Botany* **65**, 637-642.

BUNCE, J.A. (1994). Responses of respiration to increasing atmospheric carbon dioxide concentrations. *Physiologia Plantarum* **90**, 427-430.

BUNCE, J.A. (1995). Effects of elevated carbon dioxide concentration in the dark on the growth of soybean seedlings. *Annals of Botany* **75**, 365-368.

BUNCE, J.A. and CAULFIELD, F. (1991). Reduced respiratory carbon dioxide efflux in three herbaceous perennial species. *Annals of Botany* **67**, 325-330.

CAIRNS, A.J. and POLLOCK, C.J. (1986). Fructan biosynthesis in excised leaves of *Lolium temulentum* L. I. Chromatographic characterization of oligofructans and their labeling pattern following ^{14}C feeding. *New Phytologist* **109**, 1661-1667.

CAMBRIDGE, M.L. and LAMBERS, H. (1998). Specific leaf area and functional leaf anatomy in Western Australian seagrasses. *In* 'Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences' (Eds. H. Lambers, H. Poorter and M.M.I. Van Vuren), pp.89-100. Backhuys Publishers, Leiden, The Netherlands. ISBN 90-73348-96-X.

CATALDO, D.A., HAROON, M., SCHRADER, L.E. and YOUNGS, V.L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* **6**, 71-80.

CAVE, G., TOLLEY, L.C. and STRAIN, B.R. (1981). Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. *Physiologia Plantarum* **51**, 171-174.

CHU, C.C., COLEMAN, J.S. and MOONEY, H.A. (1992). Controls of biomass partitioning between roots and shoots: Atmospheric CO_2 enrichment and the acquisition and allocation of carbon and nitrogen in wild radish. *Oecologia* **89**, 580-587.

CLAPHAM, A.R., TUTIN, T.G. and WARBURG, E.F. (1981). 'Excursion Flora of the British Isles'. Cambridge University Press.

COLEMAN, J.S., McCONNAUGHAY, K.D.M. and BAZZAZ, F.A. (1993). Elevated CO_2 and plant nitrogen use: is reduced tissue nitrogen concentration size-dependent? *Oecologia* **93**, 195-200.

CORNELIUS, R. and MARKAN, K. (1984). Effect of ozone on the interference of *Urtica urens* L. and *Chenopodium album* L. *Angewandte Botanik* **58**, 195-205.

CURE, J.D. and ACOCK, B. (1986). Crop responses to carbon dioxide doubling: a literature survey. *Agricultural and Forestry Meteorology* **38**, 127-145.

CURE, J.D., RUFTY, T.W. and ISRAEL, D.W. (1989). Alterations in soybean leaf development and photosynthesis in a CO_2-enriched atmosphere. *Botanical Gazette* **150**, 337-345.

DALE, J.E. (1988). The control of leaf expansion. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**, 267-295.

DAVIS, M.B., WOODS, K.D., WEBB, S.L. and FUTYMA, R.B. (1986). Dispersal versus climate: expansion of *Fagus* and *Tsuga* into the upper Great Lakes region. *Vegetatio* **67**, 93-103.

DE CIRES, A., DE LA TORRE, A., DELGADO, B. and LARA, C. (1993). Role of light and CO_2 fixation in the control of nitrate-reductase activity in barley leaves. *Planta* **190**, 277-283.

DEN HERTOG, J., STULEN, I. and LAMBERS, H. (1993). Assimilation, respiration and allocation of carbon in *Plantago major* as affected by atmospheric CO_2 levels. *Vegetatio* **104/105**, 369-378.

DEN HERTOG, J., STULEN, I., FONSECA, F. and DELEA, P. (1996). Modulation of carbon and nitrogen allocation in *Urtica dioica* and *Plantago major* by elevated CO_2: Impact of accumulation of nonstructural carbohydrates and ontogenetic drift. *Physiologia Plantarum* **98**, 77-88.

DUBOIS, M., GILES, K.A., HAMILTON, J.K., REBUS, P.A. and SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Annals of Chemistry*, **28**, 350-356.

EHLERINGER, J.R., SAGE, R.F., FLANAGAN, LB. and PEARCY, R.W. (1991). Climate change and the evolution of C_4 photosynthesis. *Trends in Ecological Evolution* **6**, 95-99.

EVANS, G.C. (1972). 'The Quantitative Analysis of Plant Growth'. Blackwell Scientific Publications, Oxford.

EVANS, J.R. (1989). Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* **78**, 9-19.

FARQUHAR, G.D. (1988). Models relating subcellular effects of temperature to whole plant responses. *In* 'Plants and Temperature, Symposia of the Society of Experimental Botany No. 42' (Eds. S.P. Long and F.I. Woodward), pp. 395-409. Company of Biologists Ltd., Cambridge.

FARQUHAR, G.D. and SHARKEY, T.D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* **33**, 317-345.

FARQUHAR, G.D. and VON CAEMMERER, S. (1982). Modelling of photosynthetic response to environmental conditions. *In* 'Encyclopedia of Plant Physiology Volume 12b: Physiological Plant Ecology II' (Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler), pp. 547-587. Springer, Berlin Heidelberg New York.

FARQUHAR, G.D., VON CAEMMERER, S. and BERRY, J.A. (1980). A biochemical model of photosynthetic (CO_2) assimilation in leaves of C_3 species. *Planta* **149**, 78-90.

FARRAR, J.F. (1985). The respiratory source of CO_2. *Plant, Cell and Environment* **8**, 427-438.

FARRAR, J.F. (1996). Sinks - integral parts of a whole plant. *Journal of Experimental Botany* **47**, 1273-1279.

FARRAR, S.C. and FARRAR, J.F. (1985). Carbon fluxes in leaf blades of barley. *New Phytologist* **100**, 271-283.

FARRAR, J.F. and GUNN, S. (1996). Effects of temperature and atmospheric carbon dioxide on source-sink relations in the context of climate change. *In* 'Photoassimilate Distribution in Plants and Crops' (Eds. E. Zamski and A.A. Schaffer), pp. 389-406. Marcel Dekker Inc., New York.

FARRAR, J.F. and WILLIAMS, M.L. (1991). The effects of increased carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. *Plant, Cell and Environment* **14**, 819-830.

FITTER, A.H. and HAY, R.K.M. (1987). 'Environmental Physiology of Plants'. Academic Press. ISBN 0-12-257763-9.

FONSECA, F., DEN HERTOG, J. and STULEN I. (1996). The response of *Plantago major* ssp. *pleiosperma* to elevated CO_2 is modulated by the formation of secondary shoots. *New Phytologist* **133**, 627-635.

GARBUTT, K., WILLIAMS, W.E. and BAZZAZ, F.A. (1990). Analysis of the differential response of five annuals to elevated CO_2 during growth. *Ecology* **71**, 1185-1194.

GASTAL, F. and SAUGIER, B. (1989). Relationships between nitrogen uptake and carbon assimilation in whole plants of tall fescue. *Plant, Cell and Environment* **12**, 407-418.

GEBAUER, R.L.E., REYNOLDS, J.F. and STRAIN, B.R. (1996). Allometric relations and growth in *Pinus taeda*: the effect of elevated CO_2 and changing N availability. *New Phytologist* **134**, 85-93.

GIFFORD, R.M., LAMBERS, H. and MORISON, J.I.L. (1985). Respiration of crop species under CO_2 enrichment. *Physiologia Plantarum* **63**, 351-356.

GIVNISH, T.J. (1986). Optimal stomatal conductance, allocation of energy between leaves and roots and the marginal cost of transpiration. *In* 'On the Economy of Plant Form' (Ed. T.J. Givnish), pp. 171-213. Cambridge University Press.

GONZALEZ-MELER, M.A., RIBAS-CARBO, M., SIEDOW, J.N. and DRAKE, B.G. (1996). Direct inhibition of plant mitochondrial respiration by elevated CO_2. *Plant Physiology* **112**, 1349-1355.

GRIME, J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* **111**, 1169-1193.

GRIME, J.P., HODGSON, J.G. and HUNT, R. (1988). 'Comparative Plant Ecology: a Functional Approach to Common British Species'. Unwin Hyman, London.

GUNN, S., BAILEY, S.J. and FARRAR, J.F. (in press). Partitioning of dry weight and leaf area within plants of three native species grown in elevated CO_2.

HARLEY, P.C., WEBER, J.A. and GATES, D.M. (1985).Interactive effects of light, leaf temperature, CO_2, and O_2 on photosynthesis in soybean. *Planta* **165**, 249-263.

HARMENS, H., STIRLING, C.M., MARSHALL, C. and FARRAR, J. (1998). Elevated CO_2 and N: Interactive effects on photosynthesis of *Dactylis glomerata* L. (Cocksfoot). *In* 'Responses of Plant Metabolism to Air Pollution' (Eds. L.J. De Kok and I. Stulen) pp. 319-322. Backhuys Publishers, Leiden, The Netherlands.

HART, J.W. (1988). Light and Plant Growth. *In* 'Topics in Plant Physiology I.'(Series Eds. M. Black and J. Chapman). Unwin Hyman, London. ISBN 0 04 581022 2 HB.

HAY, R.K.M. and WALKER, A.J. (1989). 'An Introduction to the Physiology of Crop Yield'. Longman Scientific and Technical, New York.

HEBEISEN, T., LUSCHER, A., ZANETTI, S., FISCHER, B.U., HARTWIG, U.A., FREHNER, M., HENDREY, G.R., BLUM, H. and NOSBERGER, J. (1997). Growth response of *Trifolium repens* L. and *Lolium perenne* L. as monocultures and bi-species mixture to free air CO_2 enrichment and management. *Global Change Biology* **3**, 149-160.

HENRY, L.T. and RAPER, C.D. (1991). Soluble carbohydrate allocation to roots, photosynthetic rate of leaves, and nitrate assimilation as affected by nitrogen stress and irradiance. *Botanical Gazette* **152**, 23-33.

HEROLD, A. (1980). Regulation of photosynthesis by sink activity: the missing link. *New Phytologist* **86**, 131-144.

HEWITT, E.J. (1966). Sand and Water Culture Methods used in the study of plant nutrition. 2nd Edition. *Commonwealth Agriculture Bureaux of Technical Communications* **22**.

HIBBERD, J.M., RICHARDSON, P., WHITBREAD, R. and FARRAR, J.F. (1996a). Effects of leaf age, basal meristem and infection with powdery mildew on photosynthesis in barley grown in 700 μmol mol^{-1} CO_2. *New Phytologist* **134**, 317-325.

HIBBERD, J.M., WHITBREAD, R. and FARRAR, J.F. (1996b). Carbohydrate metabolism in source leaves of barley grown in 700 ppm CO_2 and infected with powdery mildew. *New Phytologist* **133**, 659-671.

HOCKING, P.J. and MEYER, C.P. (1991). Carbon dioxide enrichment decreases critical nitrate and nitrogen concentrations in wheat. *Journal of Plant Nutrition* **14**, 571-584.

HOLDRIDGE, L.R. (1947). Determination of world plant formations from simple climatic data. *Science* **105**, 367-368.

HORTON. R.F. (1985). Carbon dioxide flux and ethylene production in leaves. *In* 'Ethylene and Plant Development' (Eds. J.A. Roberts and G.A. Tucker), pp.37-46. Butterworths, London.

HUBEC, T.C., ROBINSON, J.M. and DONALDSON, R.P. (1985). Effects of CO_2-enrichment and carbohydrate content on the dark respiration of soybeans. *Plant Physiology* **79**, 684-689.

HUGHES, A.P. and FREEMAN, P.R. (1967). Growth analysis using frequent small harvests. *Journal of Applied Ecology* **4**, 553-560.

HUNT, R. (1978). 'Plant Growth Analysis'. The Institute of Biology's Studies in Biology No. 96. Edward Arnold, London. ISBN 0-7131-2695-7.

HUNT, R. (1982). 'Plant Growth Curves. The Functional Approach to Plant Growth Analysis'. Edward Arnold, London. ISBN 0-7131-2844-5.

HUNT, R., HAND, D.W., HANNAH, M.A. and NEAL, A.M. (1991). Response to CO_2-enrichment in 27 herbaceous species. *Functional Ecology* **5**, 410-421.

HUNT, R., HAND, D.W., HANNAH, M.A. and NEAL, A.M. (1993). Further responses to CO_2 enrichment in British herbaceous species. *Functional Ecology* **7**, 661-668.

HUNT, R. and PARSONS, I.T. (1974). A computer program for deriving growth-functions in plant growth analysis. *Journal of Applied Ecology* **11**, 297-307.

HUNTLEY, B. and BIRKS, H.J.B. (1983). 'An Atlas of Past and Present Pollen Maps for Europe: 0 - 13,000 years ago'. Cambridge University Press, Cambridge.

HUPPE, H.C. and TURPIN, D.H. (1994). Integration of carbon and nitrogen assimilation in plant and algal cells. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**, 577-607.

HURD, R.G. (1977). Vegetative plant growth analysis in controlled environments. *Annals of Botany* **41**, 779-787.

INGESTAD, T. and LUND, A.B. (1986). Theory and techniques for steady-state mineral nutrition and growth of plants. *Scandinavian Journal of Forest Research* **1**, 439-453.

IPCC (1990).. 'Climate change: the Intergovernmental Panel on Climate Change Scientific Assessment' (Eds. J.T. Houghton, G.J. Jenkins and J.J. Ephraums). Cambridge University Press, Cambridge

JAMES, A., McDONALD, S. and STADENBERG, I. (1993). Nitrogen supply and the control of expansive growth and function in leaves and roots. *Plant and Soil* **155/156**, 195-198.

JONES, T.H., THOMPSON, I.J., LAWTON, J.H., BEZEMER, T.M., BARDGETT, R.D., BLACKBURN, T.M., BRUCE, K.D., CANNON, P.F., HALL, G.S., HARTLEY, S.E., HOWSON, G., JONES, C.G., KAMPICHLER, C., KANDELER, E. and RITCHIE, D.A. (1998). Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science* **280**, 441-443.

JONES, P.D. and WIGLEY, T.M.L. (1990). Global warming trends. *Scientific American* **263** No. 2, 66-73.

JORNSGARD, B., RASMUSSEN, K., HILL, J. and CHRISTIANSEN, J.L. (1996). Influence of nitrogen on competition between cereals and their natural weed populations. *Weed Research* **36**, 461-470.

KAMPICHLER, C., KANDELER, E., BARDGETT, R.D., JONES, T.H. and THOMPSON, I.J. (1998). Impact of elevated atmospheric CO_2 concentration on soil microbial biomass and activity in a complex, weedy field model ecosystem. *Global Change Biology* **4**, 335-346.

KAVANAGH, A.J. and RICHARDS, O.W. (1941). Mathematical analysis of the relative growth of organisms. *Proceedings of Rochester Academy of Science* **8**, 150-174.

KEELING, C.D., BACASTOW, R.B., CARTER, A.F., PIPER, S.C., WHORF, T.P., HEINMANN, M., MOOK, W.G. and ROULOFFZEN, H. (1989). A three-dimensional model of atmospheric CO_2 transport based on observed winds: 1. Analysis of observational data. *In* 'Aspects of Climate Variability in the Pacific and Western Americas' (Ed. D.H. Peterson), pp. 165-236. Geophysical Monograph, AGU, Washington DC.

KERBEL, E.L., KADER, A.A. and ROMANI, R.J. (1988). Effects of elevated CO_2 concentrations on glycolysis in intact 'Bartlett' pear fruit. *Plant Physiology* **86**, 1205-1209.

KEYS, A.J., BIRD, I.F., CORNELIUS, M.J., LEA, P.J., WALLSGROVE, R.M. and MIFLIN, B. (1978). Photorespiratory nitrogen cycle. *Nature* **275**, 741-743.

KIMBALL, B.A. (1983). Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal* **75**, 779-788.

KNEE, M. (1973). Effects of cold atmosphere storage on respiratory metabolism of apple fruit tissue. *Journal of the Science of Food and Agriculture* **24**, 1289-1298.

KOCH, K.E. (1996). Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 509-540.

KöRNER, C.H. (1991). Some often overlooked plant characteristics as determinants of plant growth: a reconsideration. *Functional Ecology* **5**, 162-173.

KOROLEVA, O.A., FARRAR, J.F., TOMOS, A.D. and POLLOCK, C.J. (1997). Patterns of solute in individual mesophyll, bundle sheaf and epidermal cells of barley leaves induced to accumulate carbohydrate. *New Phytologist* **136**, 97-104.

KRAMER, J. (1981). Carbon dioxide concentration, photosynthesis and dry matter production. *Bioscience* **31**, 29-33.

KRAPP, A., QUICK, W.P. and STITT, M. (1991). Ribulose-1,5-bisphosphate carboxylase-oxygenase, other photosynthetic enzymes and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. *Planta* **186**, 58-69.

KRAPP, A. and STITT, M. (1995). An evaluation of direct and indirect mechanisms for the 'sink-regulation' of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. *Planta* **195**, 313-323.

KUEHNY, J.S., PEET, M.M., NELSON, P.V. and WILLIS, D.H. (1991). Nutrient dilution by starch in CO_2-enriched chrysanthemum. *Journal of Experimental Botany* **42**, 711-716.

KUIPER, D. (1988). Growth responses of *Plantago major* L. ssp. *pleiosperma* (Pilger) to changes in mineral supply. *Plant Physiology* **87**, 555-557.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of a bacteriophage T4. *Nature* **227**, 680.

LAING, W.A., OGREN, W.L. and HAGEMAN, R.H. (1974).Regulation of soybean net photosynthetic CO_2 fixation by the interaction of CO_2, O_2, and ribulose-1,5-disphosphate carboxylase. *Plant Physiology* **54**, 678-685.

LAMBERS, H. (1998). Epilogue: Research on the control of plant growth - where do we go from here? *In* 'Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences' (Eds. H. Lambers, H. Poorter and M.M.I. Van Vuren), pp. 567-582. Backhuys Publishers, Leiden, The Netherlands. ISBN 90-73348-96-X.

LAMBORG, M.R., HARDY, R.W.F. and PAUL, E.A. (1983). Microbial effects. *In* 'CO_2 and Plants. The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide' (Ed. E.R. Lemon), pp. 131-176. Westview Press, Boulder, USA.

LATZKO, E. and KELLY, G.J. (1983). The many-faceted function of phosphoenolpyruvate carboxylase in C_3 plants. *Physiologie Vegetale* **21**, 805-815.

LE VAN QUY, LAMAZE, T. and CHAMPIGNY, M-L. (1991). Short-term effects of nitrate on sucrose synthesis in wheat leaves. *Planta* **185**, 53-57.

LICHTENTHALER, H.K. and WELLBURN, A.R. (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society. Transactions* **11**, 591-592.

LIEBERMAN, M. (1979). Biosynthesis and action of ethylene. *Annual Review of Plant Physiology* **30**, 533-591.

LONG, S.P. and DRAKE, B.G. (1992). Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. In 'Crop Photosynthesis: Spatial and Temporal Determinants (Eds. N.R. Baker and H. Thomas), pp. 69-103. Elsevier Science, New York.

LUO, Y., FIELD, C.B. and MOONEY, H.A. (1994). Predicting responses of photosynthesis and root fraction to elevated [CO_2]:: Interactions among carbon, nitrogen and growth. *Plant, Cell and Environment* **17**, 1195-1204.

LUSTINEC, J., HADACOVA, V., KAMINEK, M. and PROCHAZKA, Z. (1983). Quantitative determination of starch, amylose, and amylopectin in plant tissues using glass fiber paper. *Analytical Biochemistry* **132**, 265-271.

MACKINNEY, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* **140**, 315-322.

MANSFIELD, T.A. (1976). The role of stomata in determining the responses of plants to air pollutants. *In* 'Commentaries in Plant Science' (Ed. H. Smith), pp. 13-22. Pergamon Press, Oxford.

McCREE, K.J. (1970). An equation for the rate of respiration of white clover plants grown under controlled conditions. *In* 'Prediction and Measurement of Photosynthetic Productivity' (Ed. I. Setik), pp. 221-229. Pudoc, Wageningen.

McCREE, K.J. (1982). The role of respiration in crop production. *Iowa State Journal of Research* **56**, 291-306.

McDONALD, A.J.S., ERICSSON, A. and LOHAMMER, T. (1986). Growth response to step-decrease in nutrient availability in small birch (*Betula pendula* Roth). *Plant, Cell and Environment* **9**, 427-432.

McMURTRIE, R.E. and WANG, Y.-P. (1993). Mathematical models of the photosynthetic response of tree stands to rising CO_2 concentrations and temperatures. *Plant, Cell and Environment* **16**, 1-3.

MEIDNER, H. and MANSFIELD, T.A. (1968). 'The Physiology of Stomata'. McCraw-Hill, London.

MONNING, A. (1983). Studies on the reaction of Krebs cycle enzymes from apple tissue (cv. Cox Orange) to increased levels of CO_2. *Acta Horticulturalia (Peking)* **138**, 113-119.

MORISON, J.I.L. and GIFFORD, R.M. (1984). Ethylene contamination of CO_2 cylinders. *Plant Physiology* **75**, 275-277.

MORTENSEN, L.M. (1987). Review: CO_2 enrichment in glasshouses. Crop responses. *Scientia Horticulturae* **33**, 1-25.

MOTT, K.A. (1991). Sensing of atmospheric CO_2 by plants. *Plant, Cell and Environment* **13**, 731-737.

MÜNCH, E. (1930). 'Die Stoffbewegungen in der Pflanze'. Jena.

MUTIKAINEN, P. and WALLS, M. (1995). Growth, reproduction and defense in nettles - responses to herbivory modified by competition and fertilization. *Oecologia* **104**, 487-495.

NAEEM, S., THOMPSON, L.J., LAWLER, S.P., LAWTON, J.H. and WOODFIN, R.M. (1994). Declining biodiversity can alter the performance of ecosystems. *Nature* 368, 734-736.

NEALES, T.F. and INCOLL, L.D. (1968). The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf: a review of the hypothesis. *Botanical Review* **34**, 107-125.

OGREN, W.L. (1984). Photorespiration: pathways, regulation, and modification. *Annual Review of Plant Physiology* **35**, 415-442.

OAKS, A. (1994). Primary nitrogen assimilation in higher plants and its regulation. *Canadian Journal of Botany* **72**, 739-750.

PAUL, M.J. and DRISCOLL, S.P. (1997). Sugar repression of photosynthesis: the role of carbohydrates in signaling nitrogen deficiency through sink:source imbalance. *Plant, Cell and Environment* **20**, 110-116

PAUL, M.J. and STITT, M. (1993). Effects of nitrogen and phosphorus deficiencies on levels of carbohydrates, respiratory enzymes and metabolites in seedlings of tobacco and their response to exogenous sucrose. *Plant, Cell and Environment* **16**, 1047-1057.

PEARSALL, W.H. (1927). Growth studies. VI. On the relative sizes of growing plant organs. *Annals of Botany* **151**, 549-556.

PENNING DE VRIES, F.W.T. (1975). The cost of maintenance processes in plant cells. *Annals of Botany* **39**, 77-92.

PETTERSSON, R. McDONALD, A.J.S. and STADENBERG, I. (1993). Response of small birch plants (*Betula pendula* Roth.) to elevated CO_2 and nitrogen supply. *Plant, Cell and Environment* **16**, 1115-1121.

PHILOSOPH-HADAS, S., AHARONI, N. and YANG, S.F. (1986). Carbon dioxide enhances the development of ethylene forming enzyme in tobacco leaf discs. *Plant Physiology* **82**, 925-929.

POLLOCK, C.J. and FARRAR, J.F. (1996). Source-sink relations: the role of sucrose. *In* 'Photosynthesis and Environment' (Ed. N. Baker), pp. 261-279. Kluwer Academic Publisher, Dordrecht.

POORTER, H. (1989). Plant growth analysis: towards a synthesis of the classical and functional approach. *Physiologia Plantarum* **75**, 237-244.

POORTER, H. (1993). Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. *Vegetatio* **104/105**, 77-97.

POORTER, H., POT, S. and LAMBERS, H. (1988). The effect of an elevated atmospheric CO_2 concentration on growth, photosynthesis and respiration of *Plantago major*. *Physiologia Plantarum* **73**, 553-559.

POORTER, H. and POTHMANN, P. (1992). Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on ontogeny. *New Phytologist* **120**, 159-166.

POORTER, H., REMKES, C. and LAMBERS, H. (1990). Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology* **94**, 621-627.

PYANKOV, V.I., IVANOVA, L.A. and LAMBERS, H. (1998). Quantitative anatomy of photosynthetic tissues of plant species of different functional types in a boreal vegetation. *In* 'Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences' (Eds. H. Lambers, H. Poorter and M.M.I. Van Vuren), pp.71-88. Backhuys Publishers, Leiden, The Netherlands. ISBN 90-73348-96-X.

QUICK, W.P., SCHURR, U., SCHEIBE, R., SCHULZE, E-D., RODERMAL, S.R. and STITT, M. (1991). Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' rbcS. I. Impact on photosynthesis in ambient growth conditions. *Planta* **183**, 542-554.

RAUNKIAER, C. (1934). 'The Life Forms of Plants and Statistical Plant Geography'. Clarendon Press, Oxford.

RICKER, W.E. (1984). Computation and uses of central trend lines. *Canadian Journal of Zoology* **62**, 1897-1905.

ROGERS, G.S., PETERSON, C.M., McCRIMMON, J.N. and CURE, J.D. (1992). Response of plant roots to elevated atmospheric carbon dioxide. *Plant, Cell and Environment* **15**, 749-752.

ROONEY, J.M. (1994). The carbon and nitrogen dependence of plant development. *In* 'A Whole Plant Perspective on Carbon-Nitrogen Interactions' (Eds. J. Roy and E. Garnier), pp. 217-229. SPB Academic Publishing bv, The Hague, The Netherlands.

ROUMET, C., BEL, M.P., SONIE, L., JARDON, F. and ROY, J. (1996). Growth responses of grasses to elevated CO_2: a physiological plurispecific analysis. *New Phytologist* **133**, 595-603.

ROY, J. and GARNIER, E. (1994). 'A Whole Plant Perspective on Carbon-Nitrogen Interactions'. SPB Academic Publishing bv, The Hague, The Netherlands.

RYAN, M.G. (1991). Effects of climate change on plant respiration. *Ecological Applications* **1**, 157-167.

SAGE, R.F. (1994). Acclimation of photosynthesis to increasing CO_2: The gas exchange perspective. *Photosynthesis Research* **39**, 351-368.

SAWADA, S., KAWAMURA, H., HAYAKAWA, T. and KASAI, M. (1987). Regulation of photosynthetic metabolites by low-temperature treatments of roots of single-rooted soybean plants. *Plant and Cell Physiology* **28**, 235-241.
SCHOLES, J.D., LEE, P.J., HORTON, P. and LEWIS, D.H. (1994). Invertase: understanding the changes in photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. *New Phytologist* **126**, 213-222.

SHARKEY, T.D. (1985). Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. *Botanical Review* **51**, 53-105.

SISLER, E.C. and WOOD, C. (1988). Interaction of ethylene and CO_2. *Physiologia Plantarum* **73**, 440-444.

SMART, D.R., CHATTERTON, N.J. and BUGBEE, B. (1994). The influence of elevated CO_2 on non-structural carbohydrate and fructan distribution and fructan accumulation in wheat canopies. *Plant, Cell and Environment* **17**, 435-442.

SMIRNOFF, N. and STEWART, G.R. (1985). Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. *Physiologia plantarum* **64**, 133-140.

SOMERVILLE, C.R. and OGREN, W.L. (1983). An *Arabidopsis thaliana* mutant defective in chloroplast dicarboxylate transport. *Proceedings of the National Academy of Sciences USA* **80**, 1290-1294.

SPENCER, W. and BOWES, G. (1986). Photosynthesis and growth of water hyacinth under CO_2 enrichment. *Plant Physiology 82*, 528-533.

STIRLING, C.M., DAVEY, P.M., WILLIAMS, T.G. and LONG, S.P. (1997). Acclimation of photosynthesis to elevated CO_2 and temperature in five British native species of contrasting functional type. *Global Change Biology* **3**, 237-246.

STIRLING, C.M., HEDDELL-COWIE, M., JONES, M.L., ASHENDEN, T.W. and SPARKS, T.H. (1998). Effects of elevated CO_2 and temperature on growth and allometry of five native fast growing annual species. *New Phytologist* **140**, 343-354.

STITT, M. (1991). Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment* **14**, 741-762.

STULEN, I., Den HERTOG, J., DRELON, F. and ROY, J. (1994). An integrated approach to the influence of CO_2 on plant growth using data for three herbaceous species. *In* 'A Whole Plant Perspective on Carbon-Nitrogen Interactions' (Eds. J. Roy and E. Garnier), pp. 229-245. SPB Academic Publishing bv, The Hague, The Netherlands.

SZANIAWSKI, R.K. and KIELKIEWICZ, M. (1982). Maintenance and growth respiration in shoots and roots of sunflower plants grown at different root temperatures. *Physiologia Plantarum* **54**, 500-504.

TAYLOR, G., RANASINGHE, S., BOSAC, C., GARDNER, S.D.L. and FERRIS, R. (1994). Elevated CO_2 and plant growth: cellular mechanisms and responses of whole plants. *Journal of Experimental Botany* **45**, 1761-1774.

THOMAS, R.B. and GRIFFIN, K.L. (1994). Direct and indirect effects of atmospheric carbon dioxide enrichment on leaf respiration of *Glycine max* (L.) Merr.. *Plant Physiology* **104**, 355-361.

THOMAS, J.F. and HARVEY, C.N. (1983). Leaf anatomy of four species grown under continuous CO_2 enrichment. *Botanical Gazette* **144**, 303-309.

THORNLEY, J.H.M. (1970). Respiration, growth and maintenance in plants. *Nature* **227**, 304-305.

THORNLEY, J.H.M. (1976). 'Mathematical Models in Plant Physiology'. Academic Press.

THORNLEY, J.H.M. and JOHNSON, I.R. (1990). 'Plant and Crop Modelling: a Mathematical Approach to Plant and Crop Physiology'. Oxford Science Publications, Oxford.

THORNSTEINSSON, B., TILLBERG, J.E. and TILLBERG, E. (1987). Carbohydrate partitioning, photosynthesis and growth in *Lemna gibba* G. Effects of nitrogen limitation. *Physiologia Plantarum* **71**, 264-270.

TILMAN, D. (1988). 'Plant Strategies and the Structure and Dynamics of Plant Communities'. Princeton University, Princeton.

TOURAINE, B., CLARKSON, D.T. and MULLER, B. (1994). Regulation of nitrate uptake at the whole plant level. *In* 'A Whole Plant Perspective on Carbon-Nitrogen Interactions' (Eds. J. Roy and E. Garnier), pp. 11-30. SPB Academic Publishing bv, The Hague, The Netherlands.

TROUGHTON, A. (1955). The application of the allometric formula to the study of the relationship between the roots and shoots of young grass plants. *Agricultural Progress* **30**, 59-65.

TROUGHTON, A. (1956). Studies on the growth of young grass plants with special reference to the relationship between the shoot and root systems. *Journal of the British Grassland Society* **11**, 56-65.

VAN DER WERF, A., ENSERINK, T., SMIT, B. and BOOIJ, R. (1993). Allocation of carbon and nitrogen as a function of the internal nitrogen status of the plant: modelling allocation under non-steady-state conditions. *Plant and Soil* **155/156**, 183-186.

VAN DER WERF, A., GEERTS, R.H.E.M., JACOBS, F.H.H., KOREVAAR, H., OOMES, J.M. and DE VISSER, W. (1998). The importance of relative growth rate and associated traits: implications for growth at all life stages. *In* 'Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences' (Eds. H. Lambers, H. Poorter and M.M.I. Van Vuren), pp.71-88. Backhuys Publishers, Leiden, The Netherlands. ISBN 90-73348-96-X.

VAN DER WERF, A., POORTER, H. and LAMBERS, H. (1994). Respiration as dependent on a species' inherent growth rate and on the nitrogen supply to the plant. *In* 'A Whole Plant Perspective on Carbon-Nitrogen Interactions' (Eds. J. Roy and E. Garnier), pp. 11-30. SPB Academic Publishing bv, The Hague, The Netherlands.

VAN DER WERF, A., WELSCHEN, R. and LAMBERS, H. (1992). Respiratory losses increase with decreasing inherent growth rate of a species and with decreasing nitrate supply: a search for explanations for theses observations. *In* 'Molecular, Biochemical and Physiological Aspects of Plant Respiration' (Eds. H. Lambers and L.H.W. Van der Plas), pp. 483-492. SPB Academic Publishing bv, The Hague, The Netherlands.

VAN OOSTEN, J.-J. and BESFORD, R.T. (1994). Sugar feeding mimics the effect of acclimation to high CO_2-rapid down regulation of RuBisCO small subunit transcripts but not of the large subunit transcripts. *Journal of Plant Physiology* **143**, 306-312.

VAN OOSTEN, J.-J. and BESFORD, R.T. (1995). Some relationships between the gas exchange, biochemistry and molecular biology of photosynthesis during development of tomato leaves after transfer to different carbon dioxide concentrations. *Plant, Cell and Environment* **18**, 1253-1266.

VAN OOSTEN, J.-J., WILKINS, D. and BESFORD, R.T. (1994). Regulation of the expression of photosynthetic nuclear genes by CO_2 is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO_2? *Plant, Cell and Environment* **17**, 913-923.

VAN OOSTEN, J.-J., WILKINS, D. and BESFORD, R.T. (1995). Acclimation of tomato to different carbon dioxide concentrations. Relationships between biochemistry and gas exchange during leaf development. *New Phytologist* **130**, 357-367.

VICKERY, M.L. and VICKERY, B. (1981). Secondary Plant Metabolism. The Macmillan Press Ltd., London.

VON CAEMMERER, S. and EVANS, J.R. (1991). Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. *Australian Journal of Plant Physiology* **18**, 287-305.

VON CAEMMERER, S., and FARQUHAR, G.D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376-387.

WALLSGROVE, R.M., KEYS, A.J., LEA, P. and MIFLIN, B.J. (1983). Photosynthesis, photorespiration and nitrogen metabolism. *Plant, Cell and Environment* **6**, 301-309.

WANG, Y-P., GIFFORD, R.M., FARQUHAR, G.D. and WONG, S.C. (1991). Direct effect of elevated CO_2 on canopy development of a wheat crop from sowing to anthesis: a model assessment. *In* 'Climatic Variation and Change: Implications for Agriculture in the Pacific Rim' (Eds. S. Geng and C.W. Cady), pp. 19-26. University of California, Davis, CA

WELBANK, P.J. (1962). The effects of competition with *Agropyron repens* and of nitrogen- and water-supply on the nitrogen content of *Impatiens parviflora*. *Annals of Botany* **26**, 361-373.

WICKENS, L.K. and CHEESEMAN, J.M. (1988). Application of growth analysis to physiological studies involving environmental discontinuities. *Physiologia Plantarum* **73**, 271-277.

WILLMER, C. (1983). 'Stomata'. Longman, London.

WILSON, J.B. (1988). A review of evidence on the control of shoot:root ratio in relation to models. *Annals of Botany* **61**, 433-449.

WONG, S.C. (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.

WONG, S.C. (1990). Elevated atmospheric partial pressure of CO_2 and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. *Photosynthesis Research* **23**, 171-180.

WOODWARD, F.I. (1987). 'Climate and Plant Distribution'. Cambridge University Press, Cambridge.

WULLSCHLEGER, S.D. (1993). Biochemical limitations to carbon assimilation in C_3 plants – a retrospective analysis of the A/C_i curves from 109 species. *Journal of Experimental Botany* **44**, 907-920.

WULLSCHLEGER, S.D. and NORBY, R.J. (1992). Respiratory 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, S.D. NORBY, R.J. and GUNDERSON, C.A. (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, S.D., ZISKA, L.H. and BUNCE, J.A. (1994). Respiratory responses of higher plants to atmospheric CO_2 enrichment. *Physiologia Plantarum* **90**, 221-229.

YAMAZAKI, M., WATANABE, A. and SUGIYAMA, T. (1986). Nitrogen-regulated accumulation of mRNA and protein for photosynthetic carbon assimilating enzymes in maize. *Plant Cell Physiology* **27**, 443-452.

YAPP, C.J. and POTHS, H. (1992). Ancient atmospheric CO_2 pressures inferred from natural geothites. *Nature* **355**, 342-344.

YELLE, S., BEESON, R.C., TRUDEL, M.J. and GOSSELIN, A. (1989). Acclimation of two tomato species to high atmospheric CO_2. I. Sugar and starch concentrations. *Plant Physiology* **90**, 1465-1472.

ZAR, J.H. (1989). Biostatistical Analysis. 3rd Edition. Prentice Hall, New Jersey, USA.

ZISKA, L.H. and BUNCE, J.A. (1993). Inhibition of whole plant respiration by elevated CO_2 as modified by growth temperature. *Physiologia Plantarum* **87**, 459-466.