Impact of elevated CO_2 concentration on growth and development of clones of Sitka spruce and water stressed cherry

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Abstract

The atmospheric [CO_2] has been rising steadily for the last 150 years, largely as a result of land-use change and anthropogenic emissions from the burning of fossil fuels. Models predict that the current concentration of atmospheric [CO_2] will double within the next century and that temperatures will increase. Predicted increases in [CO_2] and temperature are likely to affect plant growth, yield, biomass allocation, and bud phenology. The likely increase in the evapotranspiration potential caused by an increase in air and soil temperature could have a negative effect in particular in areas with limited water resources.

The present experiments were designed to study the effects of rising [CO_2] on the long-term growth and carbon allocation of four clones of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) taken from two provenances, and the long-term interactive effects of elevated [CO_2] and water stress on growth, plant water use and plant water relations of cherry seedlings (*Prunus avium* L.).

Two-year-old saplings of four clones of Sitka spruce were grown in ten open top chambers (OTCs) at two CO_2 concentrations (~350 and ~700 μmol mol^{-1}) for two growing seasons at the Institute of Terrestrial Ecology, near Edinburgh (UK). The saplings in elevated [CO_2] were significantly larger in all respects than those grown in ambient [CO_2]. Each clone showed a positive growth response to elevated [CO_2] over the whole duration of the experiment. Only a few studies have been made to date on responses of clonal plant to elevated [CO_2].

Cherry plants were grown at two CO_2 concentrations (~350 and ~700 μmol mol^{-1}) for two years from seed in six OTCs within an unheated glasshouse at the University of Edinburgh. The experiment was designed to mimic the effects of natural water stress on the growth of young cherry seedlings. Elevated [CO_2] significantly increased total dry mass production in both water regimes. Since water uptake did not differ in either well-watered or water-stressed seedlings between elevated and ambient [CO_2], the growth increase brought about in elevated [CO_2] led to significantly higher plant water use efficiency. However, the interaction between elevated [CO_2] and water stress was not significant, and elevated [CO_2] did not ameliorate the depression in growth of cherry seedlings subjected to two subsequent drying cycles. Consequently, with the future scenario of global change with higher temperature and evapotranspiration, cultivation of cherry trees may be cut back in regions which will experience an increased frequency and intensity of drought.

Measurements of A/C_i curves and of Rubisco activity in Sitka spruce showed a certain degree of down-regulation of photosynthesis in elevated [CO_2] in the third year. Whereas in cherry, measurements of A/C_i curves made during both growing seasons, showed no down-regulation of photosynthesis per unit leaf area grown in elevated [CO_2], although Rubisco activity was reduced.

In both species, long-term relative growth rates were significantly higher in elevated [CO_2]. However, the differences in plant dry mass at the end of the experiments were

a consequence of the more rapid growth in the early phase of exposure to elevated [CO_2]. After this initial phase current relative growth rates were similar in elevated [CO_2] than in ambient [CO_2]. When both elevated and ambient [CO_2]-grown Sitka spruce and cherry saplings were compared when the same size, the trees were similar in the two [CO_2] treatments with respect to the characteristics measured (basal area, component dry mass). Thus, one of the main effects of elevated [CO_2] on long-term tree growth is to speed-up of development in all aspects.

Declaration

I declare that this thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported within was carried out by myself, unless otherwise stated.

To Valentina, Francesco, and Lorenzo

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Contents

Abbreviations

A	Net photosynthetic rate
A_{BS}	Absorbance
A_L	Leaf area
C_i	Intercellular space CO_2 concentration
$[CO_2]$	CO_2 concentration
dae	Days after emergence
FACE	Free air carbon dioxide enrichment
g_s	Stomatal conductance
IRGA	Infra-red gas analyser
ITE	Instantaneous transpiration efficiency
L_A	Leaf area
LAR	Leaf area ratio
LMR	Leaf mass ratio
ln	Natural logarithm
M	Dry mass
N	Nitrogen
NAR	Net assimilation rate
NUE	Nitrogen use efficiency
OTC	Open top chamber
P	Phosphate
P_i	Phosphate inorganic
PPFD	Photosynthetic photon flux density
RGR	Relative growth rate
R_A	Current leaf area relative growth rate
R_L	Current leaf dry mass relative growth rate
R/S	Root to shoot ratio
R_T	Current total dry mass relative growth rate
R^t	Long-term relative growth rate
R^t_L	Long-term leaf dry mass relative growth rate
R^t_T	Long-term total dry mass relative growth rate
Rubisco	Ribulose 1,5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose 1,5-bisphosphate
SEM	Standard error of the mean
SLA	Specific leaf area
S^2_M	Variance of dry mass
S^2_R	Variance of RGR
t	Time
V_{FLASK}	Volumetric flask
VPD	Vapour Pressure Deficit
WUE	Water Use Efficiency

CHAPTER 1
Introduction

1.1 Introduction

Ice core data from USSR Vostok Station have shown that the concentration of CO_2 in the atmosphere has varied considerably over geological time (Barnola *et al.*, 1987). The atmospheric [CO_2] ranged from 180 to 200 μmol mol^{-1} during the last two glacial maxima (130,000 to 160,000 and 13,000 to 40,000 years ago), but following the last glacial melting, the [CO_2] rose to about 260 to 270 μmol mol^{-1} and remained stable until the beginning of the industrial revolution. However, over the last 150 years, the atmospheric [CO_2] has been rising steadily, largely as a result of land-use change and anthropogenic emissions from the burning of fossil fuels.

About one third of the Earth's land area is covered by forest (Kozlowski *et al.*, 1991). Many forest tree species are long-lived, and likely to experience atmospheric [CO_2] double the pre-industrial level in the years between 2050 and 2100 (Hidore, 1996). The steady build-up of atmospheric CO_2 concentration may affect plant growth and productivity in different ways. Models also predict that over some period temperatures will increase by about 3 °C (IPCC, 1996). Consequently, fossil-fuel-driven increases in [CO_2] may modify the composition of plant communities, through forest migration (Bradshaw and McNeilly, 1991; Huntley, 1991). The potential rapid temperature changes are also likely to shift thermal limits which will raise both the latitudinal and the altitudinal limits of agricultural crop cultivation (Parry, 1992; Parry *et al.*, 1992; Lee *et al.*, 1994), and there may also be changes in climatic extremes, such as the magnitude and frequency of drought, storms, heat waves, and severe frost, rising sea levels and changes in the frequency and distribution of precipitation (Hougton, 1994). The combination of elevated temperatures and floods, and especially the increased incidence of droughts in some

areas, probably constitutes the greatest risk to the biosphere of global climate change (Kozlowski *et al.*, 1991; Woodward *et al.*, 1991; Houghton, 1994; Hidore, 1996).

However, carbon dioxide is essential for photosynthesis, which sustains plant life (the basis of the entire food chain), and, thus, increasing levels of [CO_2] will directly influence plant physiology. Drake *et al.* (1997) have recently pointed out that photosynthesis, transpiration and respiration seem to be the only processes by which elevated [CO_2] can be sensed directly by plants and ecosystems. Therefore, study of the effects of elevated [CO_2] on these processes is crucial to understanding the effects of rising CO_2 concentration on plant production and resource use efficiency.

Because of the dependence of photosynthetic rate (A) on [CO_2], the question whether plants possess physiological and morphological mechanisms for directly sensing changes of atmospheric [CO_2] has frequently been raised (Mott, 1990). Morison (1985) by analysing the response of A to photon flux density, found that C_i reached a plateau value at low irradiance while A was still increasing, and suggested that stomatal conductance (g_s) is controlled in order to maintain a quasi-constant C_i in changing environment. Mott (1988) showed that stomatal aperture responds to the intercellular CO_2 concentrations, such that the ratio of ambient to intercellular [CO_2] remains approximately constant. A conservative value of the ratio C_i to ambient [CO_2] indicates that changes in ambient [CO_2], by causing proportional changes in C_i, make responses to C_i effective sensors of changes of atmospheric [CO_2] (C_a) (Mott, 1990). Decreased g_s associated with high C_i is an adaptive response to C_a, by which diffusional limitations to A are adjusted in response to changes in mesophyll demand for CO_2 (i.e. the biochemical limitations to A) resulting in an increase in water use efficiency (WUE).

Analysis of the effects of elevated [CO_2] on g_s in woody plants shows that responses are highly variable. Eamus & Jarvis (1989) reported an average decrease between 0 to 70% in g_s, but recent studies have demonstrated that there can be lack of response of g_s (Curtis, 1996; Murthy *et al.*, 1997) or even increase of g_s (Heath & Kerstiens,

1997) in elevated [CO_2]. However, decrease of stomatal conductance does not necessary imply that the diffusional limitations to A are increased in elevated [CO_2] (Long, 1991). Indeed Drake *et al.* (1997) have shown that the ratio of C_i/C_a is almost identical in ambient and elevated [CO_2] and that the maintenance of this constant ratio ias coupled with decreased g_s. Thus, decrease in g_s can explain reduction in leaf transpiration (Morison, 1993), and, because g_s is not strongly limiting A, will improve WUE per unit of leaf area in elevated [CO_2].

In C_3 species, short-term response of A to changes in intercellular CO_2 concentrations (C_i) are well known (Von Caemmerer & Farquhar, 1981). Carbon dioxide is in competition with oxygen for the active sites of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco), thus increases in CO_2 will shift the balance towards carboxylation and reduce photorespiratory loss (Stitt, 1991; 1996). However, the greenhouse effect brought about by anthropogenic emissions of CO_2 includes correlated increases in temperature. Increase in [CO_2] and temperature will have contrasting effects on the ratio of photorespiration to photosynthesis. Rising temperature will increase the solubility of O_2 and especially the specificity of Rubisco for O_2, relative to CO_2, and this will decrease the RuBP-saturated and the RuBP-limited rates of carboxylation, favouring oxygenation and thus increasing the proportion of photosynthesis lost to photorespiration (Jordan & Ogren, 1984). However, as the atmospheric CO_2 concentration increases, carboxylation by Rubisco will be favoured. Furthermore, depression of the rate of oxygenation relative to carboxylation by elevated [CO_2] will produce an upward shift in the temperature optimum of photosynthesis (Long, 1991). Moreover, Ehleringer & Björkman (1977) have shown that the maximum quantum yield (ϕ) of C_3 species decreases with increase in temperature, since increasing amounts of the NADPH and ATP produced by electron transport are diverted into photorespiration. However, by decreasing photorespiration elevated [CO_2] will reduce the decline in ϕ at all temperatures (Ehleringer & Björkman, 1977). Consequently, also the compensation photon flux density of photosynthesis is depressed at all temperatures by elevated [CO_2], and, as for photosynthesis and ϕ, the effect will be largest at higher temperatures (Long &

Drake, 1992). This decrease is of considerable significance for the ground-flora and understorey plants in forest (Long, 1991).

In short-term experiments elevated [CO_2] stimulates photosynthesis of woody plants (about 40% in conifers and 61% in broad leaves), and the evidence for this is overwhelming (Ceulemans & Mousseau, 1994) (see also reviews by Strain & Cure, 1985; Eamus & Jarvis 1989; Luxmoore *et al.*, 1993; Amthor, 1995). Nonetheless, a consistent feature of many long-term studies has been a decline in Rubisco activity and pigments of the light harvesting system, resulting in downward acclimation of photosynthesis process. However, this down-regulation of photosynthesis rarely makes up completely for the stimulation of A in elevated [CO_2]. Both inadequate potting volume (Arp, 1991) and nutrition (Linder & McDonald, 1993), by altering the source/sink balance, can contribute to acclimation in the photosynthetic apparatus. In plants well-supplied with nutrients, acclimation of assimilation rate has commonly not been found in elevated [CO_2] (Ziska *et al.*, 1990; Pettersson & McDonald, 1992). When trees growing in elevated [CO_2] are rooted in the ground, and adequate sinks are available, down-regulation of photosynthesis does not generally occur (Long & Drake, 1991; Idso and Kimball, 1992c; Teskey, 1997).

Masle *et al.* (1993) showed that growth in elevated [CO_2] of transgenic plants of *Nicotiana tabacum* transformed to produce 13 - 18% less small subunit of Rubisco was similar to that of the wild type. Long & Drake (1992) calculated that about 35% of Rubisco content can be lost in elevated [CO_2] before Rubisco will co-limit A. This suggests that acclimation may represent an optimisation of the distribution of the resources within the chloroplast to avoid the situation that either Rubisco or the apparatus for the regeneration of RuBP are in excess (Sage *et al.*, 1989). Following reduction in Rubisco, pigments of the light-harvesting complexes are usually decreased by elevated [CO_2]. Moreover, since protein turn-over is energetically costly, this down-ward acclimation in photosynthetic capacity leads to reduced maintenance respiration in elevated [CO_2] through reduced amounts of tissue protein (Ziska & Bunce, 1994; Wullschleger *et al.*, 1994; Amthor, 1995). Nitrogen

redistribution away from non-limiting components may greatly increase nitrogen use efficiency, since more carbon is assimilated per unit of leaf nitrogen, irrespective of the availability of nitrogen in the soil (Drake *et al.*, 1997).

Forests are important in the global carbon budget because they are the ecosystems which hold the largest pool of carbon. Kauppi *et al.* (1992) studied the variation of forest resources in Western Europe (i.e. Austria, Finland, France, Germany, Sweden and Switzerland) and found that the standing biomass built-up in the 1970s and 1980s as result of both increase in afforested area and enhanced plant growth. During this period, European forests accumulated 85 to 120 million tons of carbon per year, which could account for 8 to 10% of the 'missing sink' for CO_2 in the global carbon budget (Moffat, 1997). This increase in forest resources was caused both by growth of the existing forest and reversion of agricultural land to forest. In the eastern and southern parts of USA 9 to 11 millions hectares of agricultural land have been reverted to forest in the last 100 years. The increased biomass of forest ecosystems in USA has offset about 25% of U.S. greenhouse gas emission in the last 40 years (Moffat, 1997). The important role that forests can play in the global carbon cycle has encouraged a very large number of studies on the effects of increasing [CO_2] on tree growth and physiology.

There is increasing evidence that tree growth in elevated [CO_2] is increased. However, different experimental conditions and age of the species have often produced contrasting results, and there are still major uncertainties about the effects of elevated [CO_2] on tree growth. Poor control of adequate nutrient supply and limited rooting volume have resulted in artifacts, and it is important to separate the effect of elevated [CO_2] *per se* from the effects of sink and nutrient limitation. In addition, there is little information available on growth of different genotypes in elevated [CO_2]. It is very likely that the genetic source of plants will affect growth as global change occurs, and this may have major consequence for selection of genotypes of forest species, i.e. there is considerable variation within, as well as between, species in response to elevated [CO_2]. Finally, there is need for research on

the interaction between elevated [CO_2] and water stress, which is the major factor limiting plant productivity over large areas of the globe, the Mediterranean region in particular.

1.2 Aim of the study

The following aims are addressed:

- To study the effects of elevated [CO_2] on the long-term growth and carbon allocation of clonal saplings of two different provenance and of seedlings when both nutrient and soil volume are not limiting.

- To determine whether the growth increase in elevated [CO_2] is a long-term or a transient effect.

- To investigate the occurrence of acclimation of the photosynthetic capacity in stress-free (adequate nutrition, water, rooting volume) plants.

- To assess whether elevated [CO_2] alleviates the inhibition of tree growth induced by water stress, and whether the higher instantaneous transpiration efficiency (ITE) per unit of leaf area in elevated [CO_2], can be offset (at the plant level) by an increase in leaf area.

- To determine if elevated [CO_2], by improving plant water relations, increases tolerance to water stress.

- To test the hypothesis that the rapid onset of water stress imposed at the height of the growing season (increasing evaporative demand and decreasing soil water availability), when seedlings growing in elevated [CO_2] have already developed maximum leaf area and reached a larger size (both above and below the ground),

results in faster consumption of the available water in elevated [CO_2] than in ambient [CO_2].

1.3 Outline of the thesis

Chapter 2 describes the two tree species studied, Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and cherry (*Prunus avium* L.), the experimental sites, growth conditions and some methods of chemical analysis used.

Chapter 3 focuses on the effects of elevated CO_2 concentration and provenance on biomass production and allocation, phenology and ontogeny of four clones of Sitka spruce over two growing seasons.

Chapter 4 deals with the long-term effects of stress-free growth in elevated [CO_2] on leaf gas exchange, photosynthetic capacity, carbon and nitrogen relationship of four clones of Sitka spruce.

Chapters 5 reports the long-term interactive effects of elevated [CO_2] and water stress on the growth, dry mass allocation and whole plant WUE of young cherry seedlings over two growing seasons.

Chapter 6 presents the interactions between elevated [CO_2] and water stress on the gas exchange, photosynthetic capacity, water relations, carbon and nitrogen concentrations, and the relationship between plant WUE and leaf ITE of young cherry seedlings over two growing seasons.

Chapter 7 addresses the question as to whether the increase in total dry mass in response to elevated [CO_2] is a long-term or a transient effect in stress-free (adequate nutrition, water, pot space) growth conditions.

Chapter 8 summarises the main conclusions from the Sitka spruce and cherry studies, discusses their implications for the future scenario of global change and puts forward suggestions for further research.

CHAPTER 2

Carbon Dioxide Treatment Facilities, Trees, and Methods of Chemical Analysis

2.1 Trees

Two tree species, Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and cherry (*Prunus avium* L.), were studied. Both species are important in Europe: Sitka spruce is very important as a British forest crop, whereas cherry is a major tree crop in Italy. Both species were grown in open top chambers (OTCs), but at two different experimental sites. The studies on clonal Sitka spruce were done in OTCs located at the Institute of Terrestrial Ecology (ITE), Bush Estate, near Edinburgh, (55° 51' N, 3° 12' W, 198 m altitude). Cherry plants were grown in OTCs installed inside a glasshouse at the University of Edinburgh (55° 34' N, 3° 12' W, 185 m altitude).

2.1.1 Sitka spruce

Sitka spruce is a conifer in the Pinaceae of the Gymnospermae. Conifers are the most abundant trees of the Northern temperate forests and rank amongst the world's most important natural resources. The Pinaceae is a medium-size, worldwide family, with members primarily in Europe, Asia, and North America. The genus *Picea* contains approximately 40 species largely occurring in the temperate and boreal regions of the Northern Hemisphere, usually to be found on wet sites with poor, shallow soils. Sitka spruce grows up to 60 m in height when mature, with a cylindrical trunk, and a short, often open crown. It is a native of North America where it grows in a narrow zone, about 80 km wide, from sea level to 1,000 m, along the Pacific Coastal region from southern Alaska to northern California. It has been introduced to Europe where it is widely planted, and today is a major forest crop. The wood of Sitka spruce is of low density (330 kg m^{-3}), but is relatively strong and resilient. It is used in general

construction and for manufacturing boxes, crates, sounding boards for musical instruments, plywood and paper.

Four clones of Sitka spruce from two provenances, at 53.2° N (Skidegate a and Skidegate b) and at 41.3° N (North Bend a and North Bend b), taken from 5-year-old trees, were propagated in March 1990 from physiologically mature trees growing in a clonal provenance trial in Scotland. When the cuttings had rooted (July 1990) they were potted and grown in a glasshouse until 1 March 1991, when 240 saplings (60 per clone) were transferred to six OTCs at the ITE site. There were three OTCs per [CO_2] treatment and 10 saplings per clone per chamber. The present study started at the end of winter 1992, when 24 saplings (6 per clone) per [CO_2] treatment were harvested. The clones were repotted before budburst at the end of March 1992 and then randomly placed in ten OTCs, five replicates per [CO_2] treatment.

2.1.2 Cherry

Cherry is in the Rosaceae (Dicotyledonae) which contains approximately 120 *genera* and 3,600 species distributed worldwide. The genus *Prunus* is especially abundant in the temperate regions of Europe, North America, and Asia, where members of this genus are widely cultivated for their edible fruit. *Prunus avium*, commonly known as mazzard cherry, sweet cherry or gean, has a wide crown and can reach 24 m in height. It is native to southeastern Europe, North Africa and Western Asia, and has long been cultivated in Southern Europe for its commercially important fruits (principally as dessert fruits, but also for jams and liqueurs and in the food industry). Cherry trees are also important as a timber crop throughout Europe.

In southern Europe drought dramatically influences tree growth and productivity. Growth in elevated atmospheric CO_2 concentrations is expected to be less affected by water stress (Eamus & Jarvis, 1989; Eamus, 1991; Chaves & Pereira, 1992) and, as a consequence, the trees may have improved WUE over those grown in ambient

[CO_2] conditions. However, increase in overall biomass of trees grown in elevated [CO_2] may result in more rapid consumption of the available water. This study was designed to examine the effects of water stress on the growth of young *P. avium* seedlings, which could be used as rootstocks for cultivated sweet cherry, or as a timber crop.

Plants of cherry were grown from seed in six cylindrical OTCs contained within an unheated glasshouse. In April 1993 seeds of cherry, from a commercial orchard in Italy were kept for a month in a cold room at 4 °C, then sown in cellular seed trays containing a mixture of sand and peat, and placed in two OTCs, one with ambient (~350 μmol mol^{-1}) and one with elevated (ambient + ~350 μmol mol^{-1}) CO_2 concentration. After emergence, which occurred on May 20 when at least one seedling had emerged in most tray cells, the seedlings were thinned, leaving one plant per cell. Thirty three days after emergence, at the three-four leaf stage, 60 seedlings germinated in elevated [CO_2] and 120 seedlings in ambient [CO_2] were selected and transplanted into soil columns (6.6 dm^3). The elevated [CO_2] seedlings were then randomly placed in three elevated [CO_2] OTCs, while half of the ambient CO_2 seedlings were placed in three ambient [CO_2] OTCs, and half were placed in three different blocks alongside the OTCs to be the unchambered control in the glasshouse.

2.2 Experimental sites and carbon dioxide exposure facilities

OTCs were originally employed for air pollution studies (Jäger & Weigel, 1993), but since the start of [CO_2] research they have become the most widely used, controlled environment technique for long-term [CO_2] enrichment studies on young trees (Ceulemans & Mousseau, 1994). The characteristics of OTCs have been extensively described (Allen *et al.*, 1992; Colls *et al.*, 1993; Leadley & Drake, 1993; Lee & Barton, 1993; Pontailler *et al.*, 1995). Environmental variables within the chambers, i.e. ventilation rate, vapour pressure deficit (VPD), air and leaf temperature, and

radiation, differ slightly from outside. However, their relatively simple design, low construction and maintenance cost (relative to phytotrons and glasshouses), along with the low cost of CO_2 (relative to free air carbon dioxide enrichment - FACE), make OTCs suitable CO_2 exposure facilities for studies on small to medium stature trees. Moreover, FACE experiments on field-grown cotton have shown that data on the effects of elevated [CO_2] on potted plants grown in OTCs can be "transferred to open field situations" (Mauney *et al.*, 1994).

At the ITE site there were 24 OTCs, situated on a gentle slope. They were made of a lightweight aluminium frame with 3 mm glass panels and were designed to ensure uniform distribution of [CO_2]-enriched air throughout the tree canopy and to minimise air incursion through the open top. The OTCs were 3 m in diameter with a floor area of 7 m^2, a volume of 22.4 m^3, and a height of 2.3 m to the base of a frustrum, which deflected air and reduced the size of the top opening; reducing the incursion of external air and consequent dilution of the CO_2 concentration inside the elevated [CO_2] chambers. Air was injected from a perforated polyethylene sleeve, 1.5 m above ground level, placed underneath a glass shelf situated 0.5 m below the frustrum. At the beginning of each growing season the OTCs were washed, to minimise the attenuation of solar radiation, and disinfected, to limit the occurrence of disease. Air temperature data were measured over the whole experimental period from one elevated and one ambient OTC at a height of 1.5 m using a ventilated, radiation-shielded thermistor (RS Ltd., Loughborough, UK); external air temperature was measured by a thermistor positioned on the roof of a "Portacabin" placed nearby the chambers. The data were taken every 20 seconds, averaged over five minute intervals, and the mean recorded by a data logger (21X, Campbell Scientific Ltd., Loughborough, UK). The mean daily air temperature in the OTCs was 1.7 (± 0.57 SEM) °C higher than outside, with a maximum deviation of 5 °C, for short periods during the hottest summer days (Figure 2.1). Approximately 15% of the photosynthetic photon flux density (PPFD) was intercepted by the lightweight frame and glass panels (a detailed description of the physical and environmental characteristics of the OTCs is given by Fowler *et al.*, 1989).

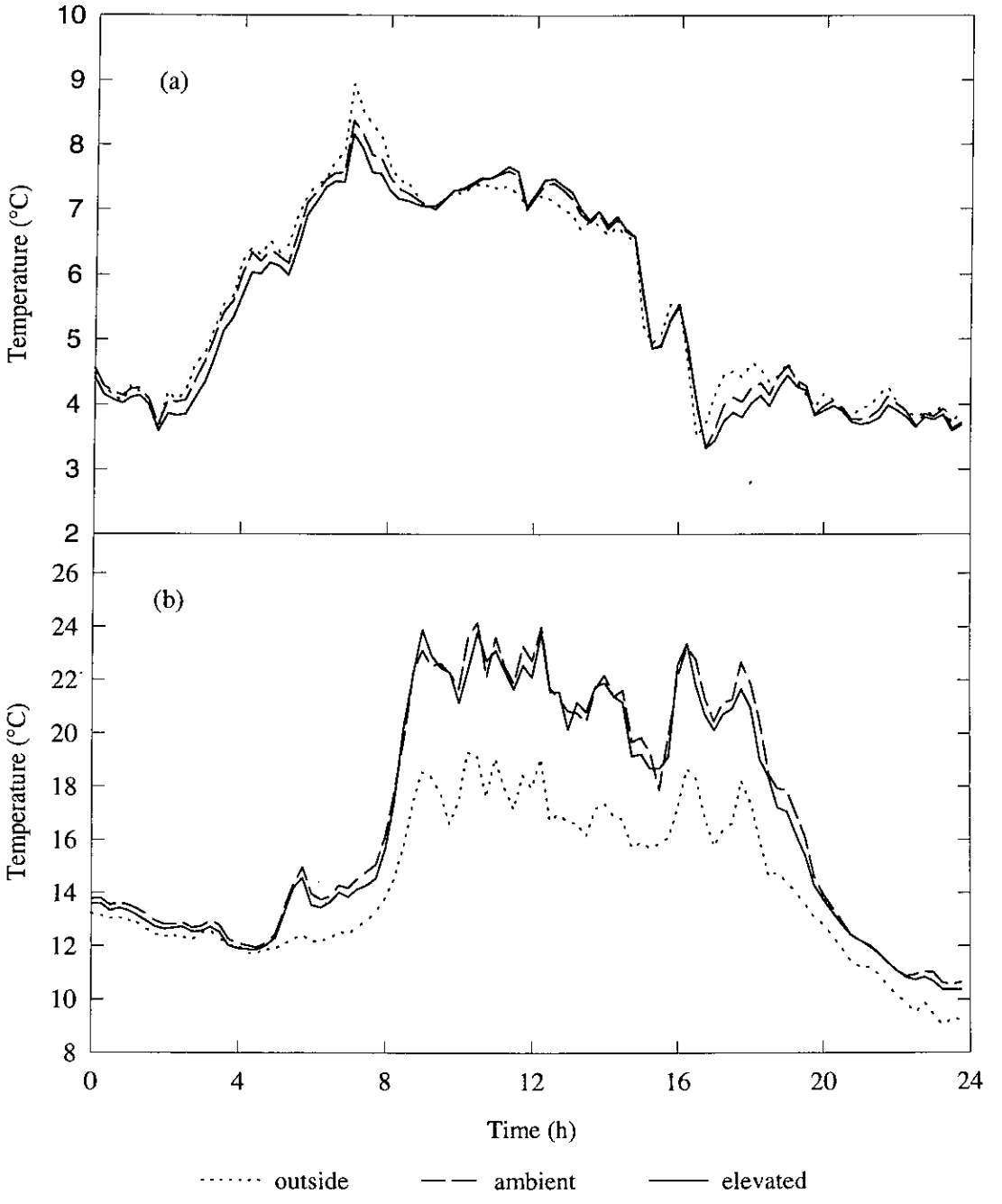

Figure 2.1. Diurnal air temperature fluctuation at the ITE site on (a) January 5 1993, and (b) July 28 1993, measured inside one elevated [CO_2] and one ambient [CO_2] OTC, and outside.

The OTCs at the Edinburgh site were made of three aluminium hoops covered with sheets of transparent acrylic plastic. They were 1.25 m in diameter; and 1.5 and 2.3 m in height in the first and second year of the experiment, respectively, to the base of a frustrum, which was 0.2 m high. Air, blown from outside the glasshouse, was injected through a perforated polyethylene cushion situated underneath a 0.3 m high wire base, on which the pots were placed. Air temperature was measured inside each chamber and near the three outside blocks during the growth seasons 1993-94, using fine wire (36 swg) copper-constantan thermojunctions positioned above the plants. The environmental data were taken every 20 seconds, averaged over five minute intervals and stored in a data logger (Delta-logger, Delta-T Devices Ltd, Cambridge, UK). The mean air temperature within the OTCs (Figure 2.2) was about 0.86 (± 0.06 SEM) °C higher than the outside temperature, with a maximum deviation of 3 °C, for short periods during the hottest summer days). The transmittance of the OTC acrylic sheet was 85-90% between 350 and 850 nm.

The transmittance and the quality of the PPFD reaching the plants inside the chamber was similar at both sites (Lee & Barton, 1993). However, since the OTCs at Edinburgh University were installed inside a glasshouse the mean air temperatures at this site were higher than those at the ITE site. During the summer 1993, the mean outside air temperature at the Edinburgh site was 3.7 (± 0.05 SEM) °C higher than the mean outside air temperature at the ITE site, whereas the mean air temperature inside the chambers at Edinburgh side was 2 (± 0.03 SEM) °C higher than that at the ITE site, with a maximum deviation of 4 °C, for short periods during the hottest days - Figures 2.1b and 2.2a.

To reduce differences between the internal and external OTC microclimates at both ITE and Edinburgh sites, air, from intakes positioned near the ground, was filtered and supplied via a fan (EK31, radial and axial fan, Cold Harbour Lane, Harpenden, Herts, UK) to each chamber at a high flow rate, providing air changes of three volumes per minute. To double the present day ambient CO_2 concentration, pure CO_2 was added to the air stream entering three of the OTCs. The CO_2 from a tank

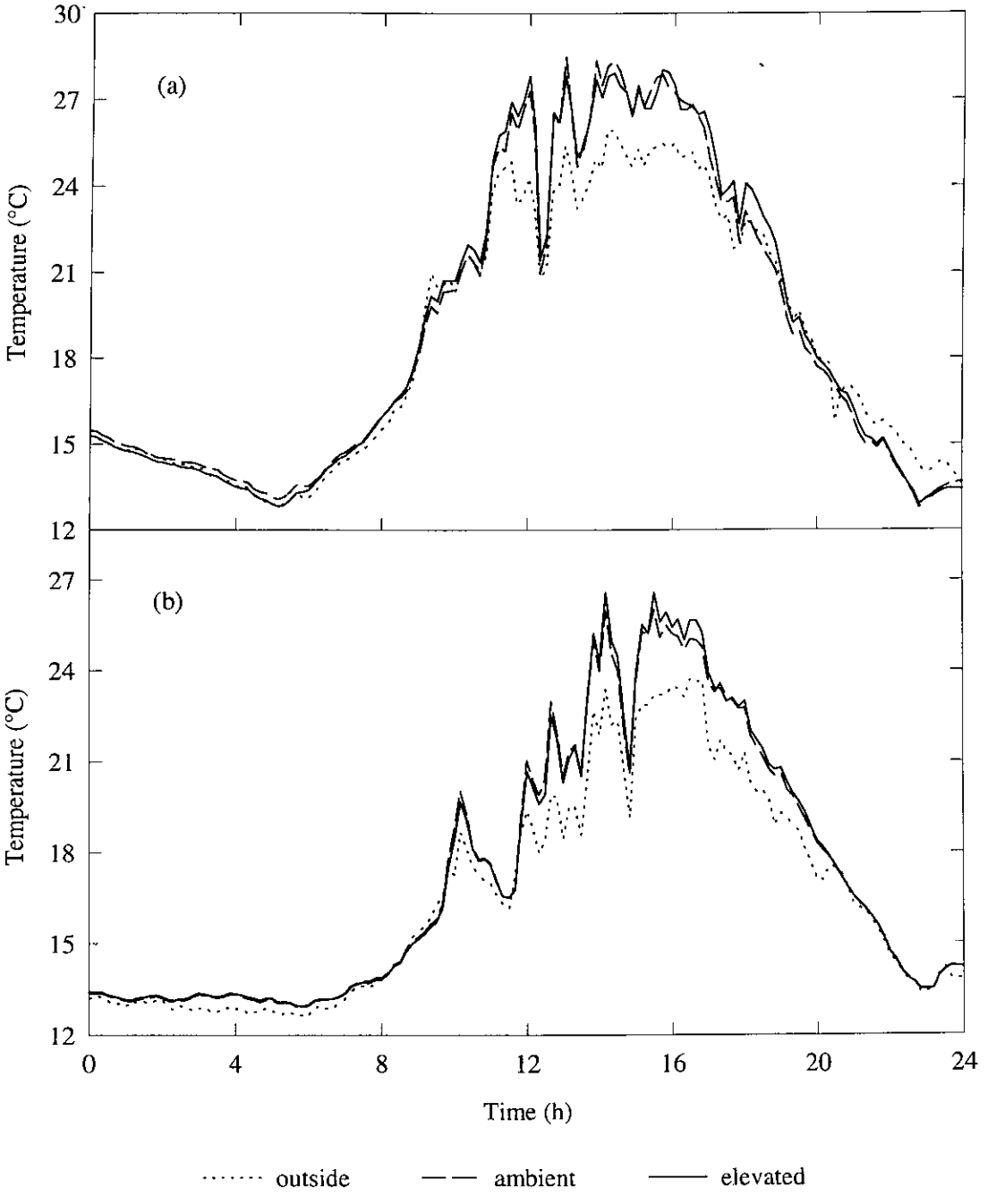

Figure 2.2. Diurnal air temperature fluctuation measured on (a) July 28 1993 and (b) August 15 1994 at the Edinburgh site. Data are means of the elevated and ambient [CO_2] OTCs, and of the three outside blocks.

(ITE) or cylinders (Edinburgh University) was vaporised, passed through a pressure reducing valve, and then injected into the air inlet duct, downstream of the fan units where it was mixed thoroughly before being released into the OTCs. Thus, the CO_2 concentration was maintained at ~350 μmol mol^{-1} in the ambient [CO_2] chambers and at ambient + ~350 μmol mol^{-1} in the elevated [CO_2] chambers, i.e. at approximately 700 μmol mol^{-1} depending on the fluctuations in the ambient air. The [CO_2] monitoring and control system used, comprised sample and injection sub-units that were controlled continuously day and night throughout the experimental periods, by a personal computer *via* an interface card (Barton *et al.*, 1993).

Air from all the OTCs was continuously drawn by a diaphragm pump (B100SE, Charles Austin Ltd, Weybridge, UK) through 4 mm diameter nylon sample lines. Each sample line was fitted with a needle valve flow controller to balance the flow rates through the lines, and contained a three-way solenoid valve which was controlled by the computer *via* a multiplexer and relays. When activated, the solenoid valve diverted air to an infra-red gas analyser (IRGA). The IRGA was regularly calibrated using air containing known concentrations of CO_2. The solenoid valves were switched sequentially every minute; during the first 35 seconds the system was flushed to expel residual air and to allow the IRGA to stabilise on the new readings, which were taken every two seconds during the remaining 25 seconds, averaged and the mean recorded by the personal computer for later examination and display.

Trees at both sites were exposed to elevated [CO_2] for 24 h d^{-1} for 365 d per annum. At the ITE site, pure CO_2 was supplied from a 16 tonne bulk liquid tank (Distillers MG, Glasgow, UK), into the ambient air stream to the elevated [CO_2] chambers to increase the ambient CO_2 concentration by 350 μmol mol^{-1}, and this was continuously monitored by an IRGA (Mark 2, Analytical Development Co. Ltd., Hoddesdon, Herts, UK). The CO_2 consumption was about 9.6 tonne per chamber per year.

At the Edinburgh site, the three elevated [CO_2] OTCs, were supplied with pure CO_2 from packs of eight cylinders (Distillers Co. Ltd., Glasgow, UK) to increase the ambient CO_2 concentration by 350 μmol mol^{-1}, and this was continuously monitored by an IRGA (WMA-2 CO_2 Analyser/Controller, PP Systems, Hitchin, UK). The CO_2 consumption was about 1 tonne per chamber per year. Every two weeks, the glasshouse CO_2 concentration was monitored for 24 hours. The glasshouse side windows remained open to prevent a build-up of [CO_2], and thus the diurnal cycle in [CO_2] was maintained close to that of the ambient [CO_2] chambers.

2.3 Determination of nutrient, sugar, and starch concentrations

Samples were collected from leaves and roots of both Sitka spruce and cherry at each harvest to determine nutrient, sugar, and starch concentrations. These samples were first plunged into liquid nitrogen, subsequently kept in small plastic vials in a freezer at -25 °C, and than transferred to a freeze-drier (Edwards High Vacuum Ltd., Crawley, Sussex, UK). The freeze-dried tissue was ground to fine powder using a ceramic grinding vessel. Details of number of samples analysed at each stage and for each of these analyses are given in Chapter 4 for Sitka spruce and Chapter 6 for cherry.

2.3.1 Nutrient analysis

Nutrient concentrations (N, P, K, Mg, Ca) were measured following the wet digestion procedure for plant material (Allen *et al.*, 1974). Freeze-dried, ground tissue, ranging in mass (*M*) between 0.095 and 0.105 g, was accurately weighed into "Pyrex" test-tubes. First 2 cm^3 of concentrated H_2SO_4 was added to each test tube, shaken to avoid clumping of the material. Subsequently 0.75 cm^3 of H_2O_2 was added twice, shaking the tube again and controlling the vigorous reaction. The tubes were then placed for six hours in a dry-block heater at 320 °C until a colourless solution

was obtained. The solution was allowed to cool to room temperature and after having added (very carefully) distilled water it was transferred to a 50 cm^3 volumetric flask (V_{FLASK}) where 0.25 cm^3 of a 10% solution of lanthanum was then added before adjusting to volume.

The cations K, Mg, Ca were determined by atomic absorption spectroscopy (UNICAM 919, A.A. Cambridge, UK). Standard solutions were made in the range of 10-40 g m^{-3} for K, 0-4 g m^{-3} for Mg, and 10-30 g m^{-3} for Ca, N and P were measured using a flow-injection analyser (FLOW 3000, Perstorp, Oregon, USA). Nitrogen was measured by a gas diffusion system (as described in application note ASN 50-03/84, Perstorp Analytical Ltd, Maidenhead, UK). The sample was injected into a stream of NaOH 2 M 8 %, where the N present as NH_4^+ was converted to $NH_{3(gas)}$ which then migrated through a gas-permeable membrane into a stream of acid-base indicator. The colour change was measured at 590 nm. Standard solutions of N were prepared in the range of 0-60 g m^{-3} N, adding H_2SO_4 (concentrated) so that the values of the samples lay within concentrations of the standard solutions but not at their extremes. Phosphorus was measured colorimetrically by the ammonium molybdate-ascorbic acid method at 690 nm (as described in application note ASN 60-04/83, Perstorp Analytical Ltd, Maidenhead, UK). Standard solutions of P were prepared in the range of 0-5 g m^{-3} P, adding H_2SO_4 (concentrated) so that the values of the samples lay within concentrations of the standard solutions but not at their extremes.

Three replicate analyses were made on the same sample, and the averaged optical density was used by the instrumentation software to calculate the concentration (g m^{-3}) of each element (N_C) in the solutions. The percentage of each element in a sample was then worked out as follows:

$$\% \text{ element} = (N_C \cdot V_{FLASK}) / (M \cdot 10^4)$$

2.3.2 Soluble carbohydrate analysis

Soluble sugar pool were measured by high pressure anion-exchange liquid chromatography (HPLC) coupled with pulsed amperometric detection (Corradini *et al.*, 1993). In order to extract the soluble sugars, 5 cm^3 of double distilled water was added to freeze-dried, ground tissue of the cherry samples ranging in D_M between 0.049-0.051 g. The Sitka spruce samples were particularly acid, thus instead of adding double-distilled water, 5 cm^3 of NaOH solution 0.0025 mol m^{-3} was added to ~0.050 g of ground tissue. The samples were shaken to avoid clumping of the material and incubated for 15 min. in a water bath at 30 °C. The solutions were then centrifuged for 15 minutes at 5000 *rpm*, and the supernatant was poured off and vacuum filtered using a 0.2 μm nitrocellulose filter (Whatman International Ltd, Maidstone, UK). The filtered supernatants of root samples were diluted 1:50, whereas those of leaf samples were diluted 1:100, adding double distilled water. The diluted solutions were then assayed using a Dionex DX 500 (Dionex Corporation, Sunnyvale, California, USA) equipped with a ED40 electrochemical detector in pulsed amperometric mode. A CarboPac PA1 (250 x 4 I.D) pellicular anion exchange column, equipped with a CarboPac PA1 guard column (50 x 4 mm I.D.), was used. The chromatographic data were collected and plotted using the Dionex Auto Ion 450 Chromatography Workstation. The Dionex DX 500 was calibrated using standard solutions of 100 g m^{-3} sugars. Details of the type and amounts of the soluble sugars used in the standard solutions are given in Appendix I. The samples were eluted under isocratic conditions with mobile phases containing sodium hydroxide 60 mM. The sample loop volume was 10 μl, and the eluent flow rate 0.8 ml/min. The percentage of soluble sugars in a sample was calculated as follows:

$$\% \text{ soluble sugars} = ((\text{HPLC reading} \cdot \text{dilution factor} / 1000) / M) \cdot 100$$

2.3.3 Starch analysis

Starch concentration was determined by the iodometric method (Allen, 1989). Starch was extracted adding 5 cm^3 of 32% $HClO_4$ (by volume) to samples of freeze-dried, ground tissue ranging in mass between 0.049-0.051 g. The solution was shaken for 30 minutes at room temperature, and then centrifuged at 5000 *rpm* for 15 minutes. A volume (1 cm^3) of the supernatant was poured into a 25 cm^3 volumetric flask containing about 15 cm^3 of distilled water, to which were added first 150 mm^3 of HCl 10^3 mol m^{-3} and immediately after 0.25 cm^3 of iodine solution (0.2 % I_2 in 2% KI), before adjusting to volume with distilled water.

A sample of ~0.05 g of pure starch was treated following the same procedure as for the starch extracted described above. Volumes of pure starch solution were added in the range of 0-0.24 cm^3 to the volumetric flasks and made up to 25 cm^3 to obtain the standard solutions used for the calibration curve. The absorbance of the iodine was read at 610 nm using a spectrophotometer, using the standard solution without starch as a blank for the readings. The concentration of starch (S_C) in each standard solution was calculated as follows:

$$S_C = (M_{PS} \cdot V_{SS})/(V_{HClO4} \cdot V_{FLASK})$$

where, M_{PS} is mass of pure starch, and V_{SS} is volume of pure starch solution, V_{HClO4} is volume of $HClO_4$, and V_{FLASK} is the volume of the volumetric flasks (i.e., 25 cm^3). A linear regression between the absorbance and pure starch concentration of the standard solutions was then determined (R^2 = 0.997). The parameters of the regression of the calibration curve were used to transform the absorbance of tissue samples into known concentrations of starch. The percentage of starch in the tissue samples was then worked out as follows:

$$\% \text{ starch} = ((S_C \cdot V_{HClO4} \cdot V_{FLASK})/(V_{SN} \cdot M)) \cdot 100$$

where V_{SN} is volume of the supernatant.

2.4 Chlorophyll analysis

The concentration of chlorophylls *a* and *b*, and *a+b* were measured in intact leaf tissues by immersion in N,N-dimethylformamide (DMF) following the techniques described by Porra *et al.* (1989). For Sitka spruce, three needles from one clone per chamber were sampled from the middle part of a current-year branch, and immediately plunged into liquid nitrogen. After measuring the leaf area (A_L) and fresh mass, the needles were put in glass vials containing 5 cm^3 DMF (V_{DMF}) and immediately placed in darkness. The vials were kept in darkness for seven to eight days before absorbance (A_{BS}) of the solution was read on a spectrophotometer at 647 nm, 664 nm, and 750 nm, using DMF as a blank

For cherry, three leaf discs were taken from leaves of the same age from one seedling per chamber of each treatment. The discs were taken from the central portion of the leaves, avoiding large veins, and were immediately plunged into liquid nitrogen. Subsequently, the leaf discs, which had a total area of 2.40 cm^{-2}, were immersed in 5 cm^3 DMF and immediately placed in darkness for four to five days before absorbance was read. Chlorophyll concentration in mg cm^{-2} was calculated according to the following equations:

$$\text{chlorophyll } a = (12.00 \cdot (A_{BS}^{(664)} - A_{BS}^{(750)}) - 3.11 \cdot (A_{BS}^{(647)} - A_{BS}^{(750)}) / (V_{DMF}/A_L)$$

$$\text{chlorophyll } b = (20.78 \cdot (A_{BS}^{(647)} - A_{BS}^{(750)}) - 4.88 \cdot (A_{BS}^{(664)} - A_{BS}^{(750)}) / (V_{DMF}/A_L)$$

$$\text{chlorophyll } a+b = (17.67 \cdot (A_{BS}^{(647)} - A_{BS}^{(750)}) + 7.12 \cdot (A_{BS}^{(664)} - A_{BS}^{(750)}) / (V_{DMF}/A_L)$$

2.5 Nutrient stock solutions

Trees were fertilized once a week throughout each growing season, following Ingestad principles (Ingestad & Ågren, 1992, 1995). These involve nutrient supply at exponentially-increasing amounts to the rooting medium, so that plant nutrient concentrations assume steady-state values (i.e. the time derivative of the nutrient

concentration is zero). At steady-state internal nutrient status, the relative nutrient uptake rate must equal the plant relative growth rate, and the different plant parts have an almost identical relative growth rate. To maintain plant nutrient concentrations at steady-state after exponential growth, nutrients must be supplied at free access (i.e. nutrient addition rate higher than the requirement). At free access 'maximal' plant growth rate is maintained.

Full details of the amounts of the hydrated compounds and of the elements used in the stock solutions are given in Appendix II.

CHAPTER 3

Long Term Growth of Four Clones of Sitka Spruce Under Elevated Carbon Dioxide Concentrations

3.1 Introduction

Carbon dioxide concentration in the atmosphere may double during the next century (IPCC, 1996). Forest growth is likely to be beneficial to the global carbon balance: as trees grow they store carbon as wood in stems, branches and roots, preventing it from returning to the atmosphere. If forest growth, and consequent storage of carbon, creates a net sink of CO_2 this will lead to negative feedbacks, off-setting, to some extent, the [CO_2] build-up in the atmosphere. Thus, forest tree growth may be important for the global carbon cycle and can contribute to the mitigation of global warming (Jarvis, 1995). However, as trees mature the net carbon stored will tend towards a steady state value, unless further forests are planted.

The increasing attention and concern about the likely impact of the greenhouse effect on terrestrial ecosystems, and forests in particular, has encouraged a very large number of studies. Plants grown in elevated [CO_2] often show an increase in biomass production (Eamus & Jarvis, 1989). There is now an extensive literature and some will be summarised below, starting with those studies that showed a positive response of plant growth to enhanced [CO_2].

The total dry mass of four *Populus* clones was significantly increased by an average of 45% in elevated [CO_2]; the increase in growth response ranging from a minimum of 22% for Columbia River to a maximum of 90% for Robusta (Radoglou & Jarvis, 1990). Dry mass production of sweetgum (*Liquidambar styraciflua*) grown for 112 days in both high and low irradiance was significantly increased in elevated CO_2 (Tolley & Strain, 1984a). The dry mass of small birch seedlings grown with optimum nutrition, was enhanced after 70 days in elevated [CO_2] (Pettersson & McDonald,

1992). High rates of nutrient supply stimulated growth of *Salix phylicifolia* by about 100% after four months of growth in enhanced [CO_2] (Silvola & Ahlholm, 1993). The extent of the increase in biomass production brought about by enhanced [CO_2] differed between *Ochroma lagopus*, a fast-growing tropical tree species, and *Pentaclethra macroloba*, a slow-growing tropical tree species (Oberbauer *et al.*, 1985); the increase in growth of *O. lagopus* was twice as high as that of *P. macroloba*. The dry mass of *Pinus radiata* and *Nothofagus fusca* was significantly larger in elevated [CO_2] (Hollinger, 1987); dry mass production of *Pinus radiata* was enhanced by 30-40% when the present CO_2 concentration was doubled and phosphorus was not deficient. Moreover, the growth response to elevated [CO_2] was larger at lower water potentials (Conroy *et al.*, 1986a,b; Conroy *et al.*, 1988). Similar results were found in two advanced selections (families 20010 and 20062) of *P. radiata* grown for two years in elevated [CO_2] (Conroy *et al.*, 1990), but this was in contrast to the growth response shown by phosphorus deficient *Eucalyptus grandis* seedlings grown for six weeks in elevated [CO_2] (Conroy *et al.*, 1992). Dry mass of *E. grandis* seedlings was significantly increased by elevated [CO_2] at each rate of nitrogen or phosphorus supply, but the highest relative increase in plant dry mass was obtained at low rates of phosphorus supply. Growth of *Castanea sativa* was increased by about 20% in response to doubled [CO_2] (Mousseau & Enoch, 1989; El Kohen *et al.*, 1992; El Kohen & Mousseau, 1994), and this did not differ significantly between plants grown on fertilized or unfertilized soil (El Kohen *et al.*, 1992; El Kohen & Mousseau, 1994). Similarly, the increase in whole plant dry mass of yellow-poplar (*Liriodendron tulipifera*) in elevated [CO_2] after 24 weeks was slightly more in unfertilized than in fertilized seedlings (Norby & O'Neill, 1991). Positive growth responses to elevated [CO_2], although not always significant, were obtained, despite nitrogen deficiency, in one-year-old seedlings of *Quercus alba* (Norby *et al.*, 1986; Norby & O'Neill, 1989). Johnson *et al.* (1995) found no effect of elevated [CO_2] on *Pinus ponderosa* seedlings grown for 58 weeks in conditions of extreme nitrogen deficiency, whereas in conditions of higher (but still suboptimal), nitrogen supply, the total biomass of ponderosa pine was increased by about 100% in elevated [CO_2], and the increment was larger at a CO_2 concentration of 525 µmol

mol^{-1} than at 700 μmol mol^{-1}. Similar results were found by Silvola & Ahlholm (1995) on birch (*Betula pendula*) seedlings grown for four months at four concentrations of CO_2 and at three nutrient supply rates. A far larger increase in growth was found in sour orange trees planted directly into the ground and grown for three years in elevated CO_2 concentrations compared with control trees (Idso & Kimball, 1992a,b).

In general, the increased growth response to elevated [CO_2] seems to be the consequence of more rapid early growth, and the response is variable amongst species and clones (Radoglou & Jarvis, 1990; Ceulemans *et al.* 1994). Over a range of 156 species there was an increase of 37% in the vegetative biomass of plants exposed to elevated [CO_2] (Poorter, 1993). Tree biomass increased by about 40% in elevated [CO_2] compared to ambient [CO_2] (Eamus & Jarvis, 1989; Jarvis, 1989; Lee & Jarvis, 1996). However, there are many conflicting reports in the literature on growth responses to elevated [CO_2]. Ceulemans *et al.* (1994) reported that elevated [CO_2] increased whole plant dry mass in only one out of three poplar clones studied; in the other two clones total dry mass was actually reduced in response to elevated [CO_2]. The dry mass of *Pseudotsuga menziesii* was not affected by enhanced [CO_2] (Hollinger, 1987). No significant effect of elevated [CO_2] was detected on dry mass production of *Pinus taeda* seedlings grown for 84 days in both high and low irradiance (Tolley & Strain, 1984a), of *Liriodendron tulipifera* after three growing seasons in field conditions (without supply of fertiliser or supplementary water) (Norby *et al.*, 1992), and of *Salix phylicifolia* when grown in a pure peat-sand mixture (Silvola & Ahlholm, 1993). Elevated [CO_2] increased dry mass of birch seedlings after four months (Silvola & Ahlholm, 1995), and after one year of growth (Evans, 1994). However, during the second year the growth the response was reversed and the seedlings accumulated about 20% less total biomass in elevated [CO_2] than in ambient [CO_2] (Lee *et al.*, 1993).

Many of the results reported in the literature are affected by low rates of nutrient supply, along with inadequate rooting volume (Ceulemans & Mousseau, 1994).

Because of limiting nutrients and interaction with other stresses, these results can not necessarily be applied to trees in the natural environment. In addition, many of these observations were obtained in short-term studies on trees not acclimated to elevated [CO_2], and hence potentially generating misinformation and confusion. Thus, many uncertainties and assumptions still exist in predicting the long-term effect of elevated [CO_2] on tree growth.

Shifts in biomass allocation driven by temperature increase may also reduce the effect of elevated [CO_2] on tree growth. DeLucia *et al.* (1994) investigated biomass allocation in *Pinus ponderosa* trees of equivalent height or diameter growing in montane or desert stands on the same soil type. The differences in climatic conditions between the two sites were comparable to those predicted by general circulation models over the next 50 to 100 years at these particular locations. Calculations made using allometric relationships showed that considerably more dry mass was allocated into sapwood at the expense of foliage in the desert trees compared to the montane trees. The authors concluded that this shift in dry mass allocation, brought about by temperature and water stress may offset the growth stimulation of ponderosa pine induced by future [CO_2] elevation.

Changes in allocation patterns may have large repercussions on productivity and competitive ability. For instance, affects on the rate of branch production may change canopy structure and influence the absorption of radiation, whereas affects on fine root production improves the potential for nutrient absorption and water uptake. It has frequently been reported that growth in elevated [CO_2] causes changes in dry mass allocation between plant components, although this appears to be species-specific. However, ontogenic changes in allocation usually occur during growth, so this does not necessarily imply changes in the allometric relationship between plant components when plants are the same size. In a number of the studies, an increase in elevated [CO_2] resulted in increase in fine-root dry mass and turnover (Idso & Kimball, 1992b; Norby *et al.*, 1992; Rogers *et al.*, 1994; Norby *et al.*, 1996). Conroy *et al.* (1990) studying the response of two advanced selections of *Pinus radiata* to

elevated [CO_2] found that one allocated extra dry mass to the trunk, whereas the second allocated more dry mass to roots and branches. Conflicting results have been obtained for the root to shoot mass ratio of plants grown in elevated [CO_2] (Norby & O'Neill, 1991).

The tap root and live fine root mass of *Liriodendron tulipifera* saplings grown in the field were the only plant components that were significantly increased after three years of growth in elevated [CO_2] (Norby *et al.*, 1992). A significant increase in the root:shoot ratio was found for *Castanea sativa* (El Kohen *et al.*, 1992; El Kohen & Mousseau, 1994) grown on unfertilized soils. However, in plants grown on fertilized soils the root:shoot ratio was unchanged in *Pinus taeda* (seedlings grown for 84 days in high irradiance) (Tolley & Strain, 1984a), in *Pinus radiata*, *Nothofagus fusca*, and *Pseudotsuga menziesii* after 120 days of growth in elevated [CO_2] (Hollinger, 1987), and in *Betula pendula* grown for 70 days in Ingestad units (Pettersson & McDonald, 1992). There was no change also in the root to shoot ratio of Sitka spruce in response to elevated [CO_2] (Townend, 1993). Moreover, the root to shoot ratio was decreased in *Pinus taeda* seedlings grown for 84 days in low irradiance (Tolley & Strain, 1984a), in *Castanea sativa* (El Kohen *et al.*, 1992; El Kohen & Mousseau, 1994), in *Eucalyptus grandis* (Conroy *et al.*, 1992), and in *Liquidambar styraciflua* (grown for 112 days in both high and low irradiance) (Tolley & Strain, 1984b).

The present experiments were designed to study the effects of rising [CO_2] on long-term growth and carbon allocation in four clones of Sitka spruce taken from two provenances. Despite being a non-native species, originating from North-western America (see Chapter 2), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is a major forest crop in Europe. Predicted increases in [CO_2] and temperature are likely to affect plant growth, yield, biomass allocation, and bud phenology. Timing and duration of bud dormancy is likely to be affected by future changes in air temperature (Hänninen, 1991). A pronounced effect of enhanced [CO_2] on bud dormancy of Sitka spruce was found by Murray *et al.* (1994). Seedlings grown in elevated [CO_2] had a shorter growing season than ambient [CO_2]-grown seedlings, which flushed

earlier in the spring and delayed dormancy in autumn. However, this effect was found to be a consequence of the interaction between elevated [CO_2] and low nutrient supply, since with a high nutrient supply bud dormancy was unaffected by elevated CO_2 concentrations. The authors concluded that rising [CO_2] along with global warming is likely to reduce the risk of frost damage on Sitka spruce, and that this effect will be larger on plants growing on nutrient-poor soil than on fertile soil. Shifts in thermal limits may change the degree to which the phenological characteristics of Sitka spruce plants are coupled with local climatic conditions, and this can affect the likelihood of frost damage (Hänninen, 1991; Murray *et al.*, 1994). It is very likely that the magnitude of such responses may vary amongst clones.

3.2 Materials and Methods

In March 1991 four clones of Sitka spruce (North Bend a, North Bend b, Skidegate a, and Skidegate b), taken from 5-year-old trees, were potted into standard potting compost (sand:peat:loam mixture 1:5:3) and grown in OTCs for three years in elevated (~700 μmol mol^{-1}) or ambient (~350 μmol mol^{-1}) CO_2 (120 saplings per [CO_2] treatment; 30 per clone). The study started in 1992, at the onset of the second growing season (see Chapter 2). The plants were repotted into 4 dm^3 pots in spring 1992 (96 saplings per [CO_2] treatment, 24 per clone), and into 10 dm^3 pots in spring 1993 (28 saplings per [CO_2] treatment, 7 per clone), while the plants were dormant. The saplings were regularly watered to pot water capacity and fertilized in order to supply mineral nutrients at free access rates (details of the growth conditions are given in Chapter 2).

Non-destructive growth measurements (height, leader extension, number of main branches, basal diameter) of all plants in each chamber (96 saplings per [CO_2] treatment, 24 per clone in 1992, and 28 saplings per [CO_2] treatment, 7 per clone in 1993) were followed throughout the growing seasons. The basal diameter (d), measured at the plant collar, was used to calculate basal area ($(d/2)^2 \cdot \pi$), where π =

3.14).

After the baseline harvest made in March 1991 (day 1), four other harvests were made to determine growth: in March 1992, day 381 (24 saplings per [CO_2] treatment, 6 per clone), in September 1992, day 551 (40 saplings per [CO_2] treatment, 10 per clone), in February 1993, day 719 (20 saplings per [CO_2] treatment, 5 per clone), and in October 1993, day 972 (20 saplings per [CO_2] treatment, 5 per clone). Each plant was divided in to leaf, main stem, side stems, and roots, which were separated from soil by washing carefully by hand to minimise the loss of fine roots. Plant component parts were then oven dried for 48 h at 70 °C and weighed, using an electronic balance (Sauter, model RE1E14, Fisons Scientific Equipment, Loughborough), to give dry mass (DM).

Data were tested using factorial ANOVA (four-way maximum interactions) to determine the main effects of [CO_2], clone, time, and chamber on all dependent variables. Where appropriate, the treatment means were compared using Duncan's multiple range test.

3.3 Results

The results presented in this study relate to the second (1992) and third (1993) growing season during the exposure of Sitka spruce saplings in ambient or elevated [CO_2]. No significant inter-chamber effect on any of the growth parameters measured on the four clones of Sitka spruce was found, and thus the interactions with 'chamber' are not shown.

Plants grown in elevated [CO_2] were significantly taller ($P < 0.001$) in both 1992 (Figure 3.1a) and 1993 (Figure 3.1b), but leader extension showed a consistent large positive response to elevated [CO_2] only during the 1992 growing season ($P < 0.001$). No significant differences were found in leader extension throughout the

third growing season between the [CO_2] treatments. In 1993 leader extension was slightly larger in elevated [CO_2] than in ambient [CO_2] for most of the growing season, but because of lammas growth of the ambient [CO_2] saplings the overall mean leader extension of the saplings in the two [CO_2] treatments became equal at the end of the growing season.

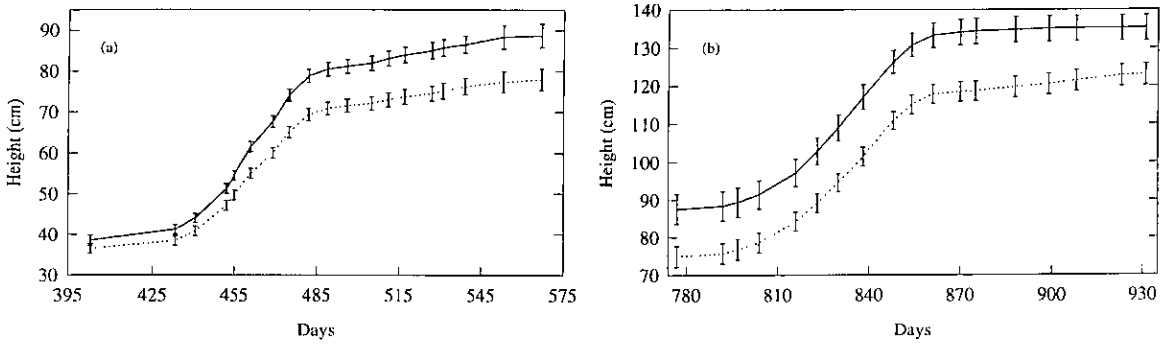

Figure 3.1. Height of the Sitka spruce saplings grown in ambient (·····) or elevated (——)[CO_2], shown as days from the beginning of the experiment (1 March 1991), in the growing seasons a) 1992 (data are means of 96 plants per treatment ± 1 SEM) and b) 1993 (data are means of 28 plants per treatment ± 1 SEM).

Clone provenance had a strong influence on sapling height. In 1992 the North Bend b clone was the tallest irrespective of [CO_2] treatment, although differences in height were significant ($P < 0.01$) only with respect to the Skidegate clones (Table 3.1). In the following growing season the North Bend a clone was tallest ($P < 0.05$) in both [CO_2] treatments (Table 3.1). Thus, the two more southerly clones were significantly taller ($P < 0.001$) than the two more northerly clones in both [CO_2] treatments and in both growing seasons. Elevated [CO_2] significantly affected the height of the Skidegate clones ($P < 0.01$) but not that of the North Bend clones in the 1992 growing season. In 1993, however, only the Skidegate b clone showed a significant positive response of height ($P < 0.01$) to elevated [CO_2].

Table 3.1. Summary of the effects of the two [CO_2] treatments on some growth characteristics of the four clones of Sitka spruce, measured at the end of the growing seasons 1992 (data are means of 24 plants per treatment ± 1 SEM) and 1993 (data are means of 7 plants per treatment ± 1 SEM); elv = elevated [CO_2], amb = ambient [CO_2], Sk.a=Skidegate a, Sk.b=Skidegate b, N.B.a=North Bend a, and N.B.b=North Bend b, no. = number.

Treat.	clone	height (cm) 1992	height (cm) 1993	leader (cm) 1992	leader (cm) 1993	basal area (cm^2) 1992	basal area (cm^2) 1993	branch (no.) 1992	branch (no.) 1993
elv	Sk.a	78.0 ± 2.04	124.5 ± 2.14	44.9 ± 1.93	48.4 ± 2.81	2.41 ± 0.14	5.39 ± 0.41	50.9 ± 4.19	186.2 ± 20.46
amb	Sk.a	68.2 ± 2.97	118.4 ± 4.04	38.2 ± 1.44	50.6 ± 3.61	1.98 ± 0.14	4.73 ± 0.22	47.9 ± 4.19	172.0 ± 28.07
elv	Sk.b	83.7 ± 4.53	133.0 ± 4.89	54.3 ± 3.10	53.0 ± 3.97	2.43 ± 0.14	5.66 ± 0.29	42.9 ± 3.13	177.6 ± 18.10
amb	Sk.b	68.8 ± 3.82	113.3 ± 2.39	38.6 ± 3.30	48.2 ± 4.67	2.05 ± 0.12	4.96 ± 0.32	41.5 ± 3.03	150.8 ± 15.78
elv	N.B.a	93.6 ± 5.79	149.2 ± 6.02	50.5 ± 3.01	52.4 ± 5.47	2.48 ± 0.24	6.25 ± 0.18	48.4 ± 3.57	167.2 ± 0.89
amb	N.B.a	84.2 ± 4.59	132.7 ± 6.22	42.7 ± 2.50	50.9 ± 6.28	1.77 ± 0.14	4.40 ± 0.25	43.7 ± 4.30	129.6 ± 13.76
elv	N.B.b	99.2 ± 6.97	139.6 ± 7.39	51.2 ± 3.55	39.0 ± 6.45	2.52 ± 0.22	5.58 ± 0.33	80.7 ± 7.51	226.8 ± 26.30
amb	N.B.b	88.9 ± 6.09	126.3 ± 5.23	44.3 ± 2.63	42.7 ± 6.80	2.19 ± 0.20	4.48 ± 0.15	67.7 ± 8.16	211.4 ± 24.79

The magnitude and pattern of the response to elevated [CO_2] differed among clones over the two growing seasons. In 1992 elevated [CO_2] significantly affected leader extension in all the clones ($P < 0.001$) (Figure 3.2a-d). Elevated [CO_2] had a pronounced effect on leader extension of the Skidegate b clone (Figure 3.2b), resulting in about 41% ($P < 0.01$) increase in leader extension compared with ambient [CO_2]-grown trees by the end of the season (Table 3.1). The Skidegate a clone also had a significant height increase in elevated [CO_2] ($P < 0.05$). The Skidegate b clone showed slightly larger leader extension than the North Bend b and North Bend a clones, and grew 10 cm more than the Skidegate a clone in elevated [CO_2] (Table 3.1), but the difference was not significant at the 5% level. The North Bend a clone was the only clone which did not have free (lammas) growth during the growing season of 1992 (Figure 3.2c), resulting in a period of shoot extension about 40 days shorter than that of the other clones. However, the [CO_2] treatments did not significantly affect length of the growing season of any of the four clones (Figure 3.2a-d).

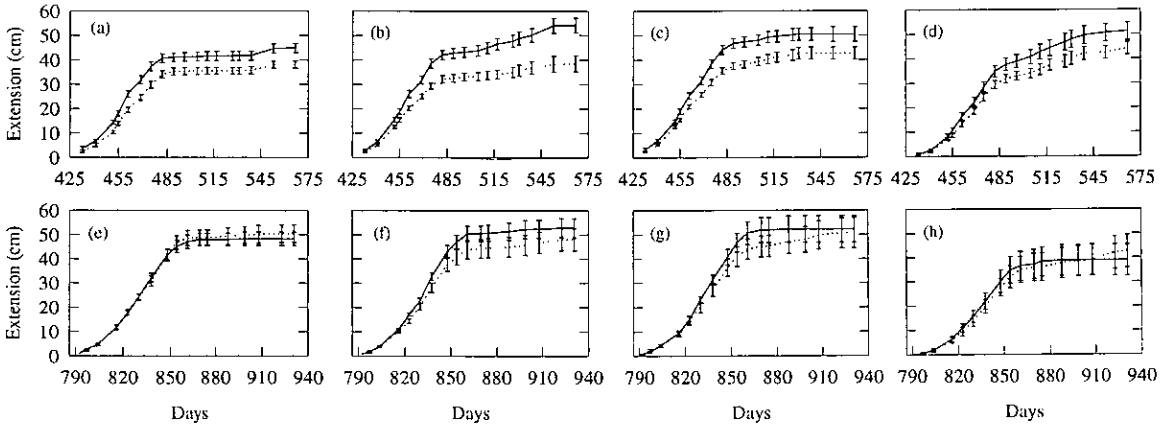

Figure 3.2. Leader extension of the clones Skidegate a (*a*,*e*), Skidegate b (*b*,*f*), North Bend a (*c*,*g*), and North Bend b (*d*,*h*) grown in ambient (·····) or elevated (——) [CO_2], shown as days from the beginning of the experiment (1 March 1991) in the growing seasons 1992 (*a*,*b*,*c*,*d*) and 1993 (*e*,*f*,*g*,*h*). Data are means of 24 plants in 1992 and 7 plants in 1993 per treatment ± 1 SEM.

In contrast to the results in 1992, no significant difference in leader extension of each clone was found between the two [CO_2] treatments throughout the third growing season (Figure 3.2 e-h), although the more northerly clones showed, on average, slightly larger leader extension than the North Bend clones in both ambient and elevated [CO_2] in 1993. Elevated [CO_2] did, however, affect the timing of bud set, leading to dormancy (around the end of July) about 43 to 53 days earlier in three of the four clones, with the exception of Skidegate b which showed, along with all the ambient [CO_2]-grown clones, lammas growth late in the summer. By the

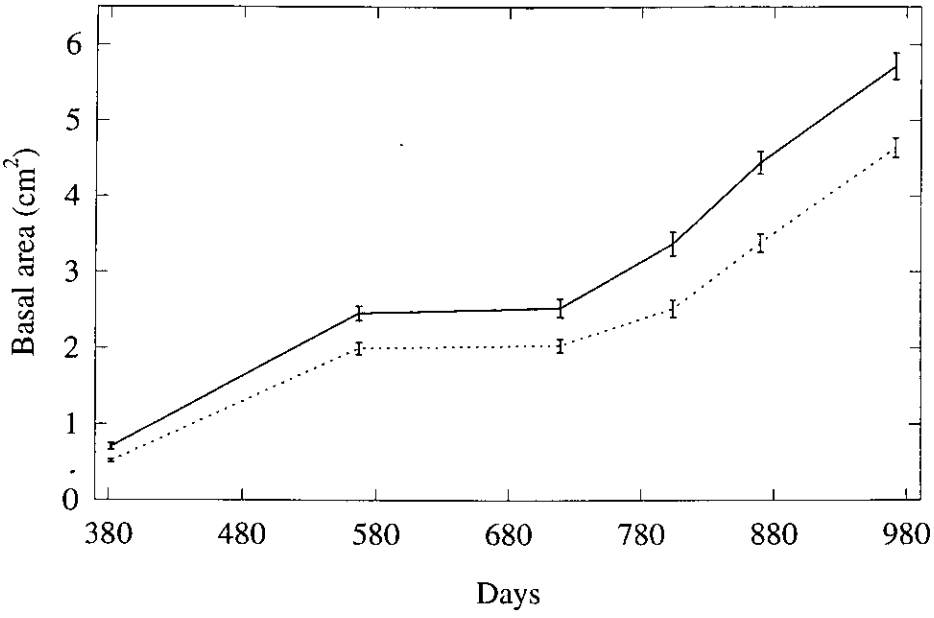

Figure 3.3. Basal area of the Sitka spruce saplings grown in ambient (·····) or elevated [CO_2] (——) shown as days from the beginning of the experiment (1 March 1991), in the growing seasons 1992 (data are means of 96 plants per treatment ± 1 SEM) and 1993 (data are means of 28 plants per treatment ± 1 SEM).

end of the growing season this resulted in the Skidegate a and North Bend b clones (Figure 3.2e,h) having slightly larger leader extension in ambient [CO_2] than in elevated [CO_2]. The Skidegate b, North Bend a, and, to some extent, Skidegate a

clones showed leader extension similar to that of the previous year in elevated [CO_2], whereas the North Bend b clone showed a large decrease in extension in elevated [CO_2] by comparison with the previous year. Thus, the Skidegate b and the North Bend a clones had the largest, and the North Bend b clone the smallest, leader extension in elevated [CO_2] in 1993 (Table 3.1).

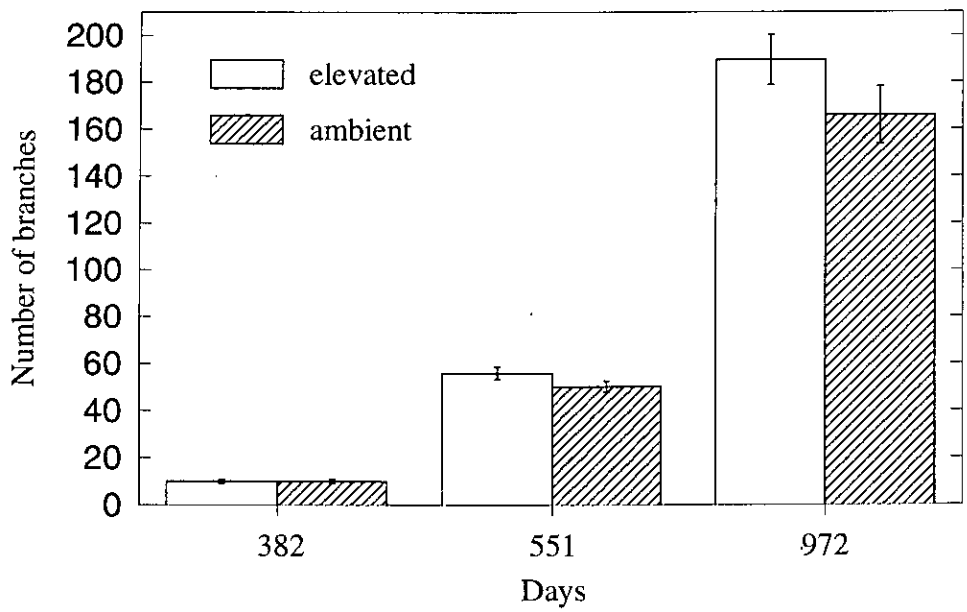

Figure 3.4. Number of branches of the Sitka spruce saplings grown in ambient or elevated [CO_2] shown as days from the beginning of the experiment (1 March 1991), in the growing seasons 1992 (data are means of 96 plants per treatment ± 1 SEM) and 1993 (data are means of 28 plants per treatment ± 1 SEM).

The saplings grown in elevated [CO_2] had significantly ($P < 0.001$) larger basal area throughout both the growing seasons 1992 and 1993 (Figure 3.3). Towards the end of the second growing season (days 567) basal area had increased by about 23% in elevated [CO_2] compared to ambient [CO_2], and this effect persisted to the end of the third growing season (days 972). Basal area showed a positive response to elevated

[CO_2] in all the clones, although the increase was not significant in the North Bend b clone in 1992 and in the Skidegate clones in 1993 ($P < 0.10$) (Table 3.1). The relative stimulation in basal area was largest in the North Bend a clone: about 40% at the end of the second, and 42% at the end of the third growing season; whereas it was least for North Bend b in 1992 (about 15%), and for the two Skidegate clones in 1993 (about 14%). On average, at the end of the experiment, the southerly clones had a larger, although not significant, increase in basal area in response to elevated [CO_2] than either of the Skidegate clones.

More branches were produced in elevated [CO_2] in both 1992 and 1993 in each clone, but the overall difference was not significant at the 5% level (Figure 3.4). The increase was significant only for the North Bend a clone in 1993, which produced about 29% more branches in elevated [CO_2] than in ambient [CO_2] (Table 3.1).

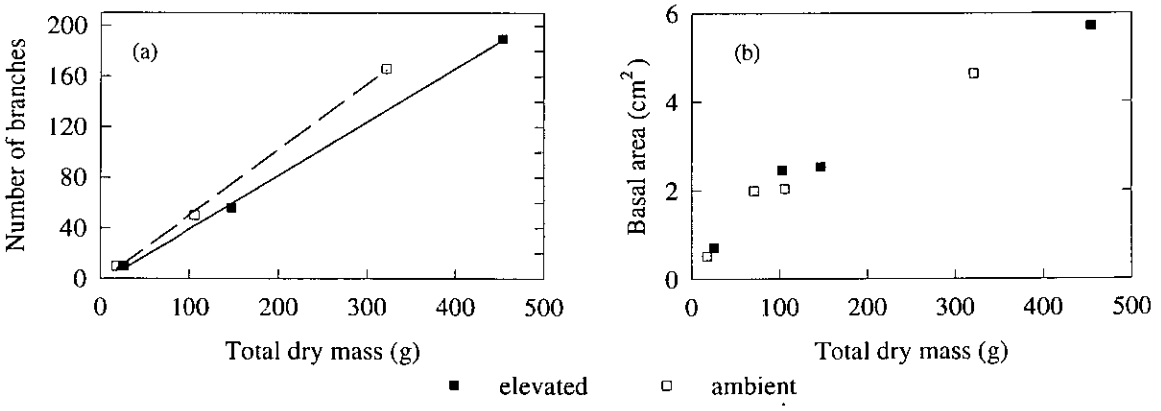

Figure 3.5. Linear relationships between combined mean (a) number of branches, and (b) basal area (R^2 = 0.961) and the mean total dry mass of the Sitka spruce saplings. Coefficients of determination (R^2) of the linear relationships:

component	elevated	ambient
number of branches	0.999	0.999
basal area	0.967	0.962

However when ambient and elevated [CO_2] saplings were compared at the same size,

there was evidence that the number of branches was larger in ambient [CO_2] than in elevated [CO_2] (Figure 3.5a), whereas a linear relationships was found between basal area and the mean total dry mass of the Sitka spruce saplings (Figure 3.5b; R^2 = 0.961).

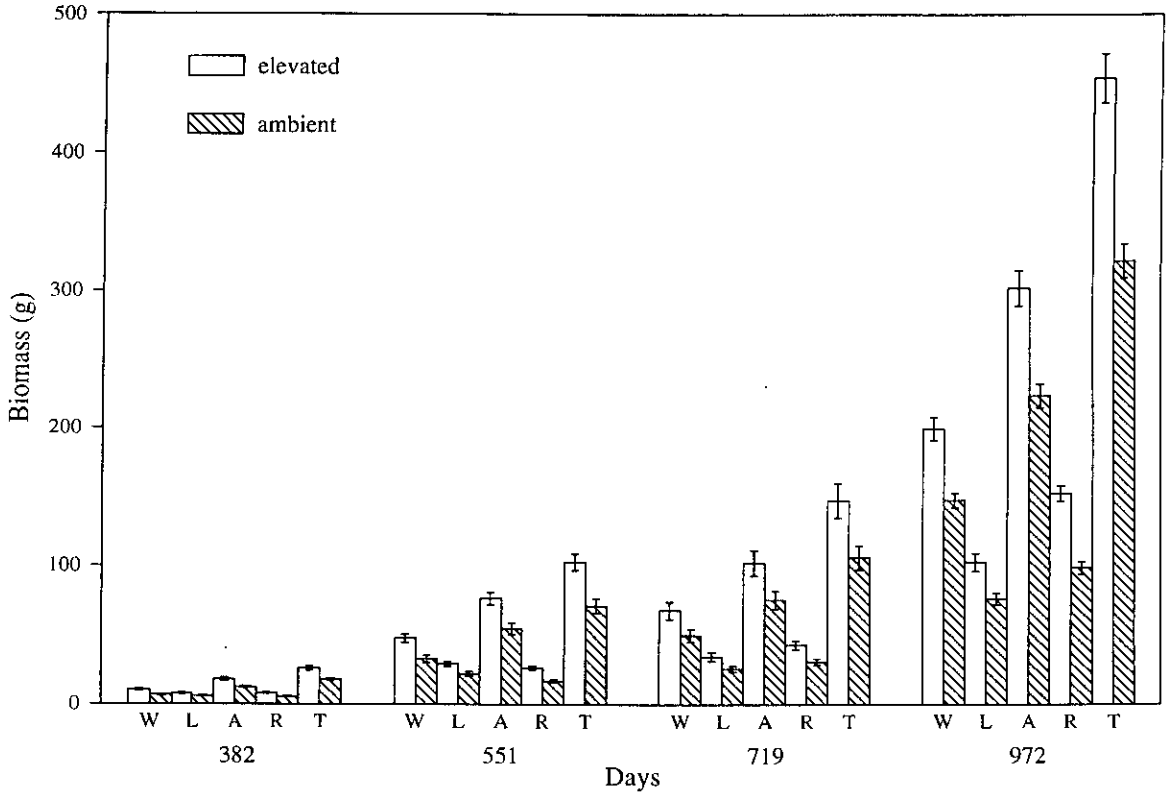

Figure 3.6. Dry mass allocation in Sitka spruce saplings grown in ambient or elevated [CO_2] in the growing seasons 1992-1993, shown as days from the beginning of the experiment (1 March 1991). Data are means of 20 to 40 plants per [CO_2] treatment ± 1 SEM; W = wood dry mass, L = leaf dry mass, A = above ground dry mass, R = root dry mass, T = total dry mass.

Figure 3.6 presents the results of four harvests made in March 1992 (day 382), September 1992 (day 551), February 1993 (day 719), and October 1993 (day 972) where the data from the four clones have been combined. The overall response to elevated [CO_2], in terms of total and component dry mass, was similar throughout the three growing seasons. The saplings grown in elevated [CO_2] were approximately

42% larger than those grown in ambient [CO_2] (Table 3.2). Enhancement of leaf dry mass by elevated [CO_2] was remarkably constant during the second and the third year of growth, whereas the relative increase in wood dry mass declined during the second and third growing seasons. Relatively more dry mass was allocated below-ground than above-ground mass in both years, and the relative increase was larger at the end of each growing season (day 551 and 972) than at the onset (day 382 and 719).

Table 3.2. Percentage increase (calculated as: $100 \cdot (E_{LV}-A_{MB})/A_{MB}$, where E_{LV} is mass in elevated [CO_2], and A_{MB} is mass in ambient [CO_2]) in total and component dry mass of Sitka spruce saplings in response to elevated [CO_2], shown as days from the beginning of the [CO_2] exposure. Data are means of 20 to 40 plants per [CO_2] treatment.

days	leaf	wood	above-ground	root	total biomass
382	33 $P < 0.01$	55 $P < 0.001$	44 $P < 0.001$	45 $P < 0.001$	45 $P < 0.001$
551	33 $P < 0.01$	45 $P < 0.001$	40 $P < 0.001$	57 $P < 0.001$	44 $P < 0.001$
719	34 $P < 0.05$	35 $P < 0.05$	35 $P < 0.05$	39 $P < 0.01$	37 $P < 0.05$
972	35 $P < 0.001$	35 $P < 0.001$	35 $P < 0.001$	54 $P < 0.001$	41 $P < 0.001$

Figure 3.7 shows the total dry mass produced in the four clones grown in ambient or elevated [CO_2] over the growing seasons 1992-93. There were no significant differences in sapling dry mass among the clones grown in ambient [CO_2] throughout the duration of the experiment. At each harvest the North Bend b clone showed the highest dry mass production at both CO_2 concentrations. However, total dry mass produced by the North Bend b clone was significantly larger ($P < 0.05$) than that produced by the Skidegate a clone on day 551 and at the end of the experiment (day 972) in elevated [CO_2]. With the exception of the Skidegate b clone, which showed

Table 3.3. Percentage increase in component dry mass of four clones of Sitka spruce in response to elevated [CO_2], after three years of growth (day 972). Data are means of 5 plants per [CO_2] treatment.

clone	leaf	wood	above ground	root
Skidegate a	18.2 ns	15.5 ns	16.4 ns	38.5 $P < 0.05$
Skidegate b	24.0 $P < 0.05$	35.9 $P < 0.05$	31.8 $P < 0.05$	37.0 $P < 0.05$
North Bend a	47.4 $P < 0.01$	50.1 $P < 0.01$	49.4 $P < 0.01$	81.5 $P < 0.001$
North Bend b	47.9 $P < 0.05$	38.8 $P < 0.05$	42.3 $P < 0.05$	63.9 $P < 0.01$

significant differences in total dry mass between the two [CO_2] treatments from day 551, significant differences in biomass of each of the other three clones in response to CO_2 concentration, became evident only at the end of the third growing season.

The more northerly clones were significantly less responsive to CO_2 enrichment than the North Bend clones in total dry mass ($P < 0.01$), leaf ($P < 0.05$), wood ($P < 0.01$), and root ($P < 0.05$). On day 972, the increase in total dry mass related to growth in

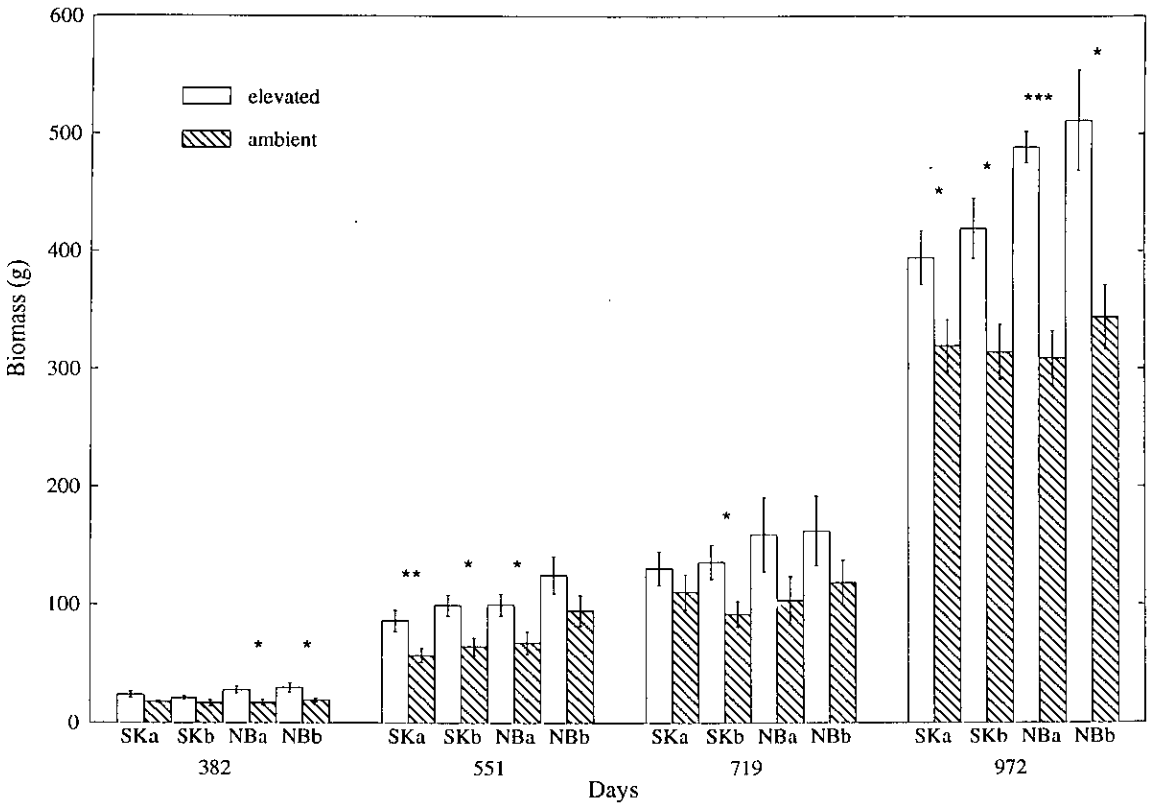

Figure 3.7. Total dry mass production of the four Sitka spruce clones in ambient and elevated [CO_2] in the growing seasons 1992-1993, shown as days from the beginning of the experiment (1 March 1991); the significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$) indicate the difference in total dry mass of each clone in response to the [CO_2] treatments. Data are means of 5 to 10 plants per [CO_2] treatment ± 1 SEM; SK a = Skidegate a, SK b = Skidegate b, NB a = North Bend a, and NB b = North Bend b.

elevated [CO_2] was about 58% in North Bend a, 49% in North Bend b, 34% in Skidegate b, and 24% in Skidegate a. Both the North Bend clones and the Skidegate b clone showed a significant increase in dry mass of each plant component part in response to CO_2 fertilisation (Table 3.3). On the other hand, the Skidegate a clone showed a significant increase only in root dry mass. A large increase was observed in the root dry mass of the North Bend a and b clones, and in wood dry mass of the North Bend a clone.

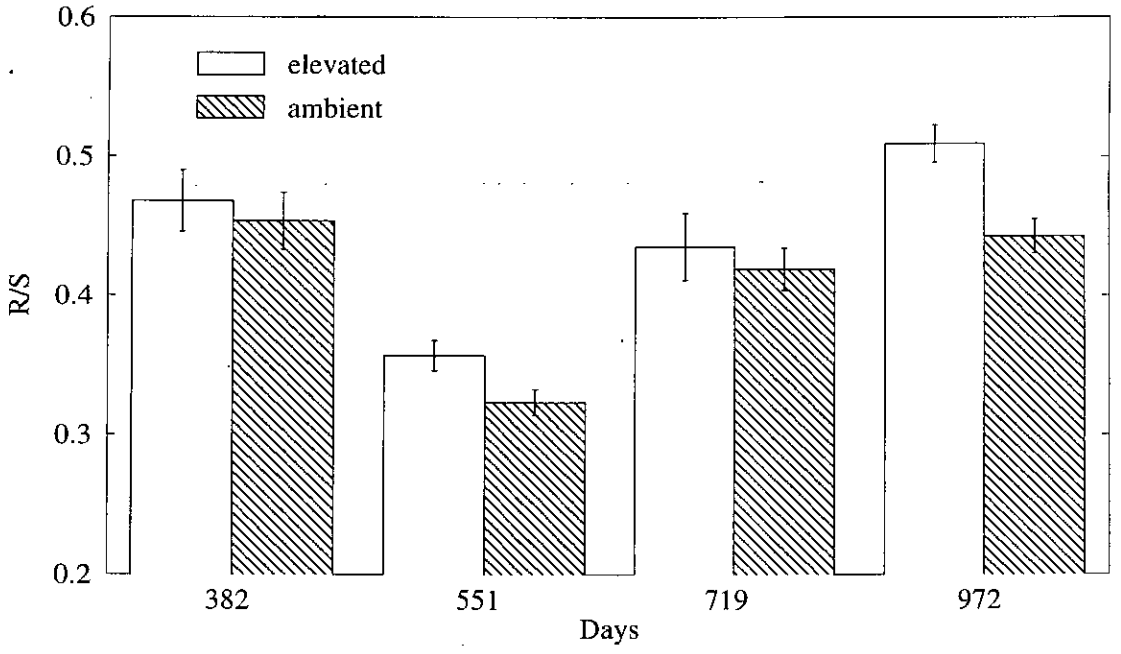

Figure 3.8. Root to shoot mass ratio (R/S) of the Sitka spruce saplings grown in ambient and elevated [CO_2] in the growing seasons 1992-1993, shown as days from the beginning of the experiment (1 March 1991). Data are means of 20 to 40 plants per [CO_2] treatment ± 1 SEM.

The dry mass of each plant component part was not statistically different among the clones grown in ambient [CO_2] (Table 3.4). Elevated [CO_2] significantly affected the production of leaf dry mass in the North Bend b clone, and wood dry mass in the North Bend a clone. This led to an increase in above-ground dry mass produced in

Table 3.4. Component dry mass (g) allocation in the four Sitka spruce clones after three years of growth (day 972) in ambient or elevated [CO_2]. Data are means of 5 plants per [CO_2] treatment ± 1 SEM; letters (a,b) are used to indicate significant differences at $P < 0.05$ in the same column.

	ambient CO_2 concentration				elevated CO_2 concentration			
clone	leaf	wood	above ground	root	leaf	wood	above ground	root
Skidegate a	73.7 ± 6.7 a	143.0 ± 6.9 a	216.7 ± 12.2 a	102.8 ± 10.7 a	87.1 ± 6.8 a	165.2 ± 9.2 a	252.3 ± 15.6 a	142.4 ± 9.4 a
Skidegate b	72.5 ± 6.2 a	139.6 ± 9.0 a	212.1 ± 15.0 a	102.1 ± 9.2 a	89.9 ± 5.1 a	189.7 ± 12.4 a	279.6 ± 17.4 ab	139.9 ± 8.4 a
North Bend a	65.8 ± 6.0 a	157.0 ± 13.8 a	222.7 ± 19.6 a	86.1 ± 3.9 a	97.0 ± 4.1 a	235.6 ± 10.0 b	332.6 ± 11.5 b	156.3 ± 6.6 a
North Bend b	92.0 ± 10.5 a	148.5 ± 8.9 a	240.5 ± 19.0 a	103.4 ± 8.7 a	136.1 ± 16.6 b	206.1 ± 18.0 ab	342.2 ± 28.9 b	169.5 ± 14.6 a

both the North Bend clones, although it was only significantly different from that of the Skidegate a clone.

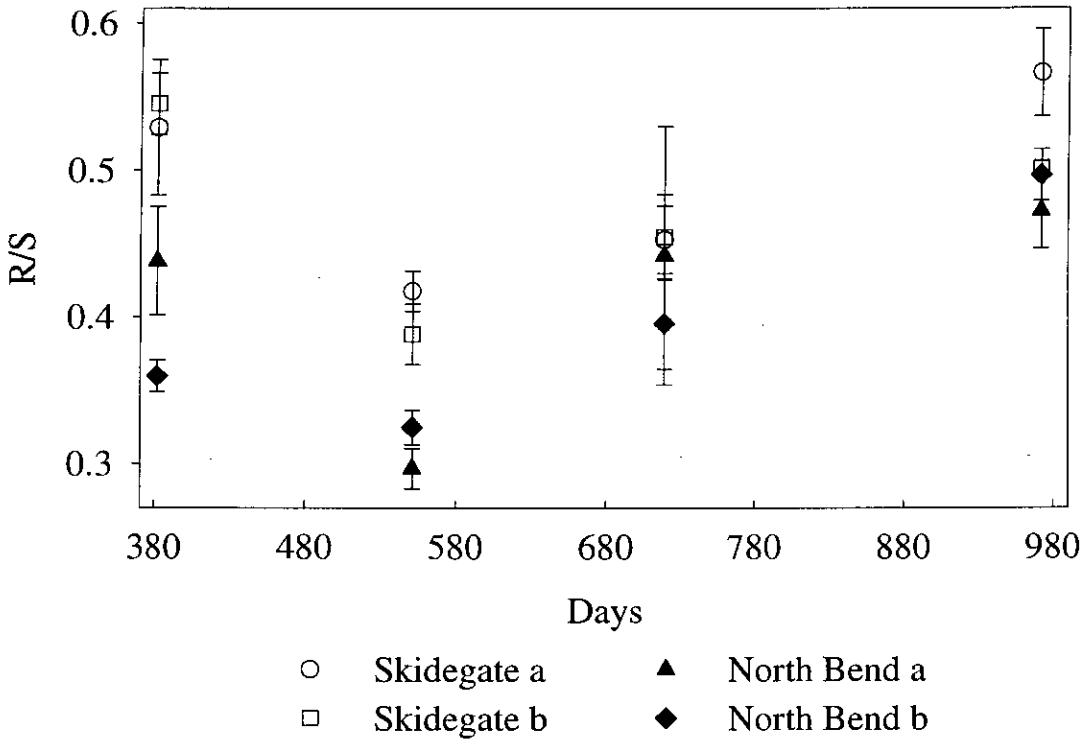

Figure 3.9. Root to shoot mass ratio (R/S) of four clones of Sitka spruce grown in elevated CO_2 in the growing seasons 1992 and 1993, shown as days from the beginning of the experiment (1 March 1991). Data are means of 5 to 10 plants per CO_2 treatment ± 1 SEM.

The combined mean root to shoot mass ratio (R/S) was influenced by the time of harvest (Figure 3.8). The ratio R/S of saplings harvested at the beginning of the growing season (days 382 and 719) did not differ between treatments, whereas saplings harvested at the end of the growing season (days 551 and 972) had larger ($P < 0.001$) R/S in elevated [CO_2]. On day 972, CO_2 fertilisation significantly increased ($P < 0.05$) the ratio R/S of both North Bend clones, and the Skidegate a clone, but not the Skidegate b clone. However, the Skidegate clones allocated proportionally

more dry mass to the root than the North Bend clones in elevated [CO_2] (Figure 3.9).

The relative distribution of the mean total dry mass amongst the components showed no effects in allocation following growth in elevated [CO_2].

Figure 3.10 shows the relationships between the dry mass allocation and the mean total dry mass produced in both ambient and elevated [CO_2]. Growth in different CO_2 concentrations did not affect the proportion of dry mass allocated to the various organs, as demonstrated by the linear relationships between the mean total dry mass and mass allocated to leaves (Figure 3.10a), wood (Figure 3.10b), above-ground organs (Figure 3.10c), and roots (Figure 3.10d).

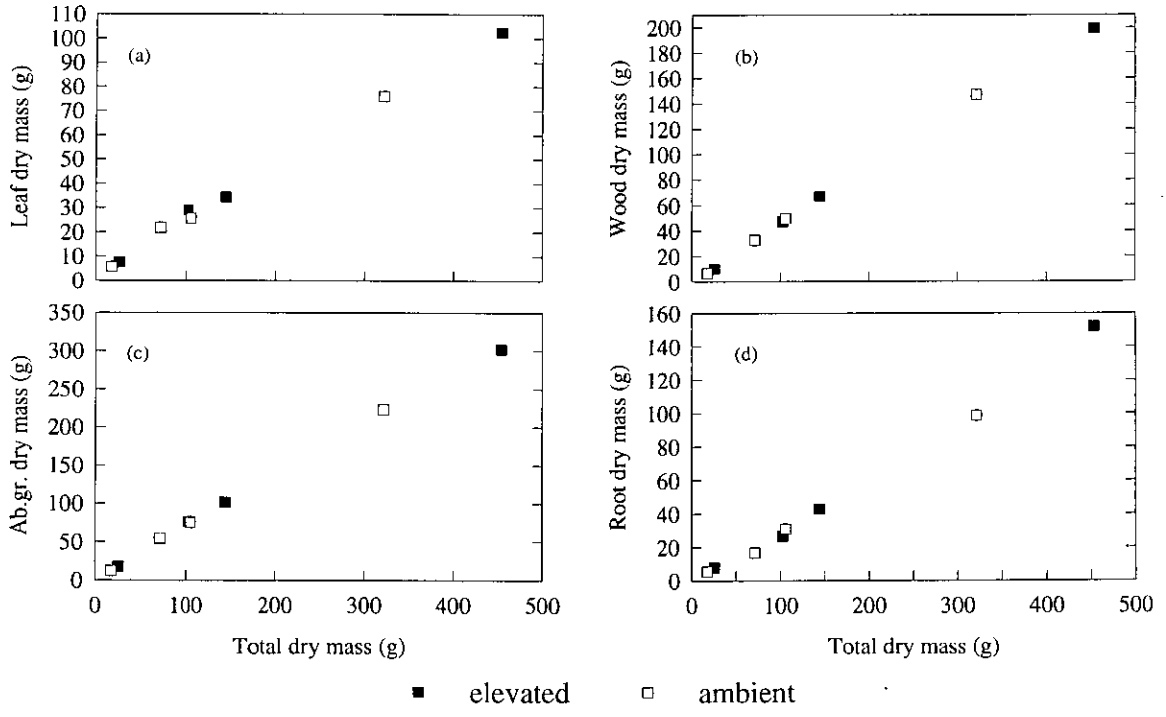

Figure 3.10. Linear relationships between combined mean (a) leaf dry mass (R^2 = 0.997), (b) wood dry mass (R^2 = 0.999), (c) above ground (Ab.gr.) dry mass (R^2 = 0.999), and (d) root dry mass (R^2 = 0.995) and the mean total dry mass of the Sitka spruce saplings.

3.4 Discussion

Saplings in elevated [CO_2] were significantly larger in all respects than those grown in ambient [CO_2] (Table 3.2, Figure 3.6). The basal area (a measure of the sapwood area) of the elevated [CO_2]-grown saplings was significantly increased (Figure 3.3). However, the plot of basal area (Figure 3.5b) *versus* total sapling dry mass shows that there were no effect of [CO_2] treatment when plants were the same size, but elevated [CO_2] enhanced the rate of plant development. More branches were produced in response to CO_2 enrichment, although this was not statistically significant (Figure 3.4), and the percentage increase in branch production in elevated CO_2 concentrations became larger at the end of each growing season, from about 2% in 1991 to 14% in 1993. Evans (1994) found that the number of current year branches of Sitka spruce seedlings grown for two growing seasons in elevated or ambient [CO_2] did not differ significantly. Furthermore, exposure to elevated [CO_2] did not increase branch production in seedlings of *Castanea sativa* (Mousseau & Enoch, 1989), or in bagged branches of mature Sitka spruce trees (Barton, 1997). In contrast, Evans (1994) and Rey (1997) found that branching was increased in seedlings of silver birch grown from seed in elevated [CO_2]. Branching in poplar clones was either not affected (Radoglou & Jarvis, 1990) or there were significantly fewer branches (Ceulemans *et al.*, 1994) in elevated [CO_2].

The growth responses of the four clones of Sitka spruce were well within the ranges reported in the literature for other clonal saplings (Radoglou & Jarvis, 1990; Ceulemans *et al.*, 1994; Ceulemans *et al.*, 1995) or seedlings (Eamus & Jarvis, 1989; Luxmoore *et al.*, 1993; Ceulemans & Mousseau, 1994; Amthor, 1995; Lee & Jarvis, 1996). Each clone showed a positive growth response to elevated [CO_2] over the three-year duration of the experiment, but only at the end of the third growing season was the increase in dry mass statistically significant in all four clones (Figure 3.7).

Increases in total dry mass production of Sitka spruce in response to elevated [CO_2] were also found by Townend (1993; 1995). After six months of exposure to elevated [CO_2], he found that the relative growth rate was increased by 9.8% in well-watered

saplings and 6.9% in droughted saplings (Townend, 1993). When Sitka spruce plants were germinated and grown for two years in elevated [CO_2], without nutrient and water limitations, total dry mass was increased by about 52% (Townend, 1995). The increase was, thus, slightly larger than that observed in the present study and much larger than that observed in other experiments on Sitka spruce (e.g. branches on mature clonal trees and long-term experiments with seedlings). Nutrition may be one of the reasons. Townend (1995) found that total dry mass was only increased by 19% in response to elevated [CO_2] in unfertilized seedlings. Murray *et al.* (1996), on the other hand, did not find any increase in total dry mass in Sitka spruce saplings after three years of growth in elevated [CO_2], in an experiment in which pot volume was not limiting, but the saplings were supplied with nutrients only once a year (i.e. at the beginning of each growing season). In Evans' experiment (1994) on Sitka spruce, performed the same site and using the same carbon dioxide exposure facilities, seedlings were about 40% larger than those grown in ambient [CO_2] after one growing season, but at the end of the second season the effect of elevated [CO_2] on total dry mass had completely disappeared and there were no significant differences in total plant dry mass between plants grown in ambient or elevated [CO_2]. Root restriction may have occurred since the seedlings were repotted into small pots, 1.5 dm^3 and 5 dm^3 pots before the beginning of the first and second growing seasons, respectively. After repotting prior to the beginning of the experiment, the seedlings did not receive any additional fertilizer, and they were only fertilized monthly with a commercial liquid fertiliser during the second growing season. Nonetheless, at the beginning of the third growing season, the seedlings were repotted into 15 dm^3 pots and fertilized following the Ingestad approach (Ingestad & Ågren, 1992), to provide a supply of mineral nutrients at free access, however at the end of the third year, elevated [CO_2] increased the total dry mass by only about 10% (Lee & Jarvis, 1996).

Juvenile and mature trees have developmental and physiological differences which lead to different sensitivities to CO_2 fertilisation (Jarvis, 1995; Lee & Jarvis, 1996). Branch bags were used to study growth and physiological processes in elevated [CO_2] in a stand of a 19-year-old clone of Sitka spruce (Barton *et al.*, 1993). At the

end of the four-year experiment dry mass of branches in elevated [CO_2] was about 15% larger than in ambient [CO_2] but this difference was not statistically significant ($P > 0.05$) (Barton, 1997). Thus, age of the Sitka spruce plant material used in experiments simulating environmental changes is another factor which must be taken into account, since young plants can compound small changes in growth rate over a long period of time (see Chapter 7). However, the four clones used here may have been particularly responsive to elevated [CO_2] because they had been selected for their forestry potential to give a good yield of timber in the shortest possible time.

In ambient [CO_2] clonal provenance did not affect dry mass production, which was similar throughout the experiment in terms of both total (Figure 3.7) and component dry mass (Table 3.4). However, in elevated [CO_2] the North Bend b clone produced significantly more dry mass than the Skidegate a clone, and in general, the more southerly clones significantly out-performed the Skidegate clones. Differences amongst clones in response to doubling the concentration of [CO_2] have been reported previously (Ceulemans *et al.*, 1994). Elevated [CO_2] enhanced the above-ground dry mass of Beaupré by about 38% and Robusta by about 55%, which was thus relatively more stimulated (Ceulemans *et al.*, 1995), and in the second year of growth, elevated [CO_2] further increased above-ground dry mass of both poplar clones (Lee & Overdieck, 1997). This increase was proportionally larger in the slower-growing clone Robusta. In yet another study on the same poplar clones (Radoglou & Jarvis, 1990), the clones Beaupré, Columbia River, Robusta and Raspalje were grown for three-months in OTCs in Scotland with elevated or ambient [CO_2]. As in the Sitka spruce clones, the total dry mass of all four poplar clones responded positively to doubling the concentration of [CO_2], ranging from 22% for Columbia River to 90% for the clone Robusta. Similar to the findings of Ceulemans *et al.* (1995), the fast-growing clone Beaupré was the most productive and the slow-growing clone Robusta was relatively more stimulated by elevated [CO_2].

Environmental changes affecting both interspecific and intraspecific growth responses may result in changed wood structure and composition as CO_2

concentration rises. Three out of the four Sitka spruce clones significantly increased wood dry mass in elevated [CO_2] (Table 3.3). The North Bend a clone responded particularly strongly to [CO_2], showing the highest relative (Table 3.3) and absolute (Table 3.4) increases in wood production and basal area (Table 3.1). Differences in wood production were also found in two advanced selections of *P. radiata* grown for two years in elevated [CO_2] (Conroy *et al.*, 1990). One family (20010) allocated extra dry mass to the trunk, whereas the high ranking (for commercial wood production) family (20062) allocated more dry mass to roots and branches. According to the authors this family may be outstripped as CO_2 concentration rises. Similarly, the present study would suggest that the more southerly clones of Sitka spruce, in particular North Bend a, could turn out to be more productive in elevated [CO_2] and, therefore, more widely cultivated in the future. Unfortunately, nothing is known about the effect of elevated [CO_2] on wood quality of the different clones, although wood density of *Pinus radiata* increased in elevated [CO_2], and this is an indicator of timber quality (Conroy *et al.*, 1990). In a recent study (Hättenschwiler *et al.*, 1996), enhanced CO_2 concentrations significantly increased the wood densities of six genotypes of *Picea abies* after three-year growth in model ecosystems by about 12%. Conversely, wood density was decreased by wet deposition of N which could offset the effect of rising CO_2 concentration on wood density. As well as being important from the immediate economic point of view, wood production can be converted into long-lived products, which can lock-up large amounts of carbon, effectively removing some of the anthropogenic [CO_2] from the atmosphere.

Height and leader extension are important characteristics that affect competitiveness and survival and thus may allow more rapid establishment of young seedlings and exploitation of gaps within forests. The elevated [CO_2]-grown saplings were significantly taller than the ambient [CO_2]-grown saplings at the end of the experiment (Figure 3.1). However, this was the result of more growth in the earlier years of [CO_2] exposure, since no differences were found in leader extension in the final year (Figure 3.2). The more southerly clones were significantly taller than the Skidegate clones in both ambient and elevated [CO_2]. However, after three years

growth in elevated [CO_2] only the Skidegate b clone was significantly taller than the other clones (Table 3.1). Again, this was a consequence of the larger growth seen in the earlier years

The ambient [CO_2]-grown clones had a period of free-growth late in the summer (Figure 3.2e-h), producing further buds and shoots, whereas free-growth was apparently inhibited in elevated CO_2. This led to a reduction in the growth difference between saplings in the two [CO_2] treatments, and gave the ambient [CO_2] saplings a growth advantage which could have been compounded in the following years, since each parental shoot of a young tree can produce a number of branches in proportion to its length (Cannell, 1987). The loss of lammas growth may result from the effect of elevated [CO_2] on development, i.e. earlier transition from indeterminate to determinate growth pattern. Contrasting height responses to elevated [CO_2] over time have been reported previously. For example, height of Sitka spruce saplings was not affected by three-years growth in elevated [CO_2] (Murray *et al.*, 1996). In birch, less stem elongation during the second year of growth in elevated [CO_2] was found to allow seedlings grown at the ambient [CO_2] to grown taller than their counterparts raised in elevated [CO_2] (Lee *et al.*, personal communication). Mousseau & Enoch (1989) also found an unusual early cessation of stem elongation in *Castanea sativa* seedlings grown in elevated [CO_2].

The four clones of Sitka spruce showed different patterns of bud phenology between the two seasons (Figure 3.2). In 1992 elevated [CO_2] did not affect the timing of bud-set (Figure 3.2a-d), and only the North Bend a clone had a shorter growing season (by about 40 days) (Figure 3.2c). However, in the following year, elevated [CO_2] significantly advanced the timing of cessation of growth of the two North Bend clones (Figure 3.2g,h) and of the Skidegate a clone by about 43 to 53 days (Figure 3.2e). Murray *et al.* (1994) reported that during the first year of growth (1991) of these four clones in elevated [CO_2], the length of the growing season of three out of the four clones was significantly affected. Enhanced [CO_2] delayed the timing of bud-burst and brought forward bud-set of the two North Bend clones and the

Skidegate b clone. The length of the growing season of the Skidegate a clone was also reduced, but not significantly. However, the reduction in length of the growing season found by Murray *et al.* (1994) ranged from a minimum of seven days in the Skidegate a clone to a maximum of 20 days in the North Bend b clone, and thus was much less than in the 1993 growing season.

Phenological characteristics affecting the length of the growing season are important not only for growth, but also for escaping the likelihood of frost damage, especially for non-native trees (such as Sitka spruce in Europe, where bud phenology may not be well coupled to the local climate) (Murray *et al.*, 1994). Repo *et al.* (1996) reported that frost hardening of 25-year-old Scots pine saplings was enhanced in the first, but not in the second, year of growth in elevated [CO_2]. In contrast to the results found by Murray *et al.* (1994), dehardening of the Scots pine saplings proceeded faster in elevated [CO_2] than in ambient [CO_2] in the spring of both years.

The potential usefulness of experiments in artificial conditions on the effects of elevated [CO_2] on long term growth of trees has frequently been raised (e.g. Eamus & Jarvis, 1989; Ceulemans & Mousseau, 1994; Amthor, 1995). Arp (1991) claimed that pot volume could restrict root growth and affect root to shoot ratio as well as photosynthetic capacity. Using data compiled from the available literature, he found a significant, high correlation between pot size and change in the root to shoot mass ratio of plants grown in elevated [CO_2]. Apparently, small pot volumes (less than ~3.5 dm^3) strongly increased the R/S ratio, compared with plants grown in large containers or in the field, and pot volumes of ~3.5 to ~12.5 dm^3 led to an intermediate response. However, McConnaughay *et al.* (1993a,b) argued that small pots do not necessarily reduce growth response to elevated [CO_2], since [CO_2]-induced growth enhancement of *Abutilon theophrasti*, a C_3 dicotyledon shrub, and *Setaria fabereii*, a C_4 monocotyledon grass, was highly stimulated by nutrient concentration, regardless of pot volume, and fine root length densities of both species were similar to those found in the field (Berntson *et al.*, 1993). Other studies (Pettersson & McDonald, 1992; Linder & McDonald, 1993) have emphasised the

importance of controlling plant nutrition in order to interpret the impact of elevated [CO_2] on plant growth and allocation, since nutrient availability (primarily nitrogen) can determine both growth rate and the pattern of dry mass allocation amongst plant organs (Ericsson, 1995; McDonald *et al.*, 1996). Moreover, the literature shows no effect of elevated [CO_2] on R/S ratio if there is no change in the C:N ratio and water is not limiting (Stulen & den Hertog, 1993; Jarvis, 1995).

The rooting volume of the pots used in this study was increased as the saplings grew from 4 dm^3 at the start of the 1992 growing season to 10 dm^3 at the start of the 1993 growing season. Thus according to Arp (1991), the pot volumes used for the clonal saplings were adequate for plants with an intermediate response by the root : shoot ratio to elevated [CO_2]. In addition, the saplings were also free of water stress and supplied with free access to nutrients and so avoided the occurrence of constrained rooting which could lead to anomalous results. In fact, the linear relationships found between total plant dry mass and basal area (Figure 3.5b), and dry mass allocated to each plant component (Figure 3.10) indicate that there were no overall differences between the [CO_2] treatments in the proportion of dry mass allocated to the shoot (Figure 3.10c) or root (Figure 3.10d) in plants at an equivalent growth stage, and this suggests that the saplings were not pot limited. During growth and development changes in allocation pattern occur. Consequently, the significant shift in the R/S ratio (Figure 3.8) in response to elevated [CO_2], may be a developmental response, since there were no differences in allocation between [CO_2] treatments when the saplings were the same size. Similarly, Evans (1994) found that after one growing season in elevated [CO_2], Sitka spruce seedlings were about 40% larger and had an increased root to shoot ratio. However, at the end of the second growing season there was no differences between the two [CO_2] treatments in both biomass production and allocation. Similar results were found by Murray *et al.* (1996). Both total dry mass and R/S ratio of Sitka spruce seedlings were not affected after three years growth in elevated [CO_2], and the allometric relationship between shoot and root dry mass did not change throughout the duration of the experiment.

Only a few studies have been made to date on clonal plant response to elevated [CO_2], and the differential growth of different genotypes may prove to be important for forest species, particularly for forest crops. The results obtained with the Sitka spruce saplings indicate that some clones, for instance the North Bend clones, may grow better in lowland Scotland as climate change occurs. This may be exploited in assessment of nursery stock for future forest planting, although it may be questionable whether results obtained with potted clonal saplings, without nutrient limitation, and with little competition can be applied to growth in the field (Amthor, 1995). Townend (1995) and Murray *et al.* (1996) have shown, for instance, that when nutrition was limiting Sitka spruce growth was little affected by elevated [CO_2]. However, we deliberately chose in this study to grow the saplings without any nutrient limitation to rule out any natural environmental constraint that might have interfered with growth. Indeed, growth was stimulated by elevated [CO_2], and this resulted from an initial higher growth rate (Chapter 7), but at the same size the trees were similar in the two [CO_2] treatments. This indicates the one of the main effect of elevated [CO_2] on long-term tree growth is to speed-up development in all aspects.

CHAPTER 4

The Effect of Elevated Carbon Dioxide Concentrations on the Physiology of Four Clones of Sitka Spruce

4.1 Introduction

The accuracy of predictions of future atmospheric [CO_2] is limited by the uncertainties in the size of the individual sources and sinks. However, the natural biogeochemical movement of carbon into and out of terrestrial vegetation and oceans is much larger than that from anthropogenic activities (fossil-fuel use and deforestation) (Hidore, 1996). About 100 gigatons of carbon per year (~15% of the atmospheric pool of carbon) is exchanged between the atmosphere and terrestrial vegetation (removed from the atmosphere through photosynthesis, and returned to the atmosphere by plant respiration and organic mass decomposition) (Houghton, 1994; Amthor, 1995). Thus, any increase in plant photosynthesis and forest tree growth brought about by elevated [CO_2] is of particular importance in regulating the global carbon cycle. The task of forecasting the magnitude of the rate of carbon gain and water loss by forest trees in response to rising [CO_2] is limited by the lack of information on the long-term impact of elevated [CO_2] on tree physiology.

Plants using the C_3 pathway of photosynthesis constitute about 95% of terrestrial species. Since CO_2 is in competition with O_2 for the active sites of Rubisco, which is a bifunctional enzyme having both carboxylase and oxygenase activity (Leegood, 1993), an increase of CO_2 concentration shifts the balance towards carboxylation. Thus, C_3 plants are more sensitive than C_4 and CAM plants to variations in CO_2 concentration in the atmosphere. Predicted changes in atmospheric [CO_2] are expected to increase the photosynthetic rate in C_3 plants both by increasing the rate of carbon fixation and by reducing photorespiratory loss of carbon. At the present CO_2 concentration, the ratio of photorespiratory loss of carbon to photosynthetic gain is estimated in the range 0.10-0.30 for C_3 plants (Amthor, 1995). Generally, the

photosynthetic rate of woody plants is increased by a doubling in CO_2 concentration (Eamus & Jarvis, 1989). Furthermore, elevated [CO_2] can also affect the rate of increase in assimilation rate from winter to early spring (Murthy *et al.*, 1997). Gunderson & Wullschleger (1994), in surveying studies of 39 tree species, showed an average gain in photosynthetic rate of 44% as a result of doubling [CO_2].

In C_3 species, short-term responses of photosynthetic rate to stepwise changes in intercellular CO_2 concentration (C_i) are well characterised (Von Caemmerer & Farquhar, 1981). A/C_i relationships at saturating PPFDs show an initial linear response which is related to the Rubisco limited rate of carboxylation. As C_i increases further, the curve becomes curvilinear, and A is believed to be related to the rate of ribulose 1,5-bisphosphate (RuBP) regeneration, which may be limited by both electron transport capacity and inorganic phosphate turnover (triose phosphate use).

Long-term growth in elevated [CO_2] often results in a variable decrease (depending on the species) in the amount of the photosynthetic pigments and enzymes, for instance in the amount and activation state of Rubisco. This has commonly been referred to as 'photosynthetic acclimation' (Long & Drake, 1992; Amthor, 1995). Apparently, this decrease occurs even when the supply of nitrogen is adequate and rooting volume large (Long, 1991). Another consistent feature of long-term studies is an enhanced accumulation of nonstructural carbohydrates in leaves of plants exposed to elevated [CO_2] (Sage, 1994; Bowes, 1996). The increased content of nonstructural carbohydrates, and particularly hexoses (i.e. fructose and glucose), may act end-product repressors of photosynthetic gene expression, triggering a cascade of reactions which lead to acclimation of the photosynthetic apparatus (Sheen, 1994; Bowes, 1996; Van Oosten & Besford, 1996). Induced down-regulation of Rubisco in response to enhanced [CO_2] was mimicked when tomato leaf tissues were fed with soluble sugars and was increased by reducing sink demand (Van Oosten & Besford, 1994; Van Oosten *et al.*, 1994). Transgenic plants of tomato, potato, tobacco and *Arabidopsis thaliana* have been shown to adapt the rate of photosynthesis to the demand for photoassimilates, showing that photosynthesis is sink-regulated (Sonnewald & Willmitzer, 1992).

Stomatal conductance of trees has either been decreased by elevated [CO_2] (by approximately 30-40%; see reviews by Eamus & Jarvis, 1989; Jarvis, 1989; Mott, 1990), remained unaffected (Hollinger 1987; Conroy *et al.*, 1988; Ellsworth *et al.*, 1995; Murthy *et al.*, 1997) or even increased (Heath & Kerstiens, 1997). However, in a recent review Drake *et al.* (1997), using data compiled from the available literature, have shown that photosynthesis in elevated [CO_2] appears to be less limited by stomata than in ambient [CO_2], since the ratio of C_i/C_a (the intercellular CO_2 concentration to atmospheric CO_2 concentration ratio) is not affected by elevated [CO_2]. Since stomatal resistance ($1/g_s$) is the only important limitation to water vapour loss, partial closure of the stomata may reduce transpiration, which, coupled with increased A, will necessarily improve WUE at the leaf level in elevated [CO_2].

This study reports the long-term effects of stress-free (adequate nutrition, water, pot space) growth in elevated [CO_2] on gas exchange, and carbon and nitrogen relationships of four clones of Sitka spruce after three years of CO_2 exposure. No work has previously been done to compare the effects of elevated [CO_2] on clonal plants originating from provenance at different latitudes propagated from parent trees evolved in different climates. It has been shown in Chapter 3 that the dry mass of all four clones was significantly increased by elevated [CO_2], but the more northerly clones were significantly less responsive than the more southerly clones. To understand whether these growth differences resulted from a direct effect of elevated [CO_2] on photosynthesis, the photosynthetic capacity of the clones was studied. Physiological responses to elevated [CO_2] may differ amongst clones, and it is fundamental to understand how these processes are influenced. This may have major consequences for the choice of different genotypes used for plant breeding and reforestation programmes, since the selection of genotypes at current atmospheric [CO_2] is likely to provide an inadequate guide to the derivation of parameters for scaling-up models and enabling predictions to be made on the likely effects of elevated [CO_2] on forests.

4.2 Materials and Methods

Saplings of four clones of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) from two provenances, at 53.2° N (Skidegate a and Skidegate b) and at 41.3° N (North Bend a and North Bend b), were grown for three growing seasons in OTCs with ambient [CO_2] (~350 μmol mol^{-1}) or elevated [CO_2] (ambient + ~350 μmol mol^{-1}). The OTCs were located at the Institute of Terrestrial Ecology (ITE), Bush Estate, near Edinburgh. The present study was made during the second and third growing season. The saplings were potted into standard potting compost, watered every other day to pot water capacity and regularly fertilized in both growing seasons following Ingestad principles (Ingestad & Ågren, 1992, 1995). Full details of the four clones of Sitka spruce, growth conditions, number of harvests made, and of the statistical analyses used to test the data are given in Chapters 2 and 3.

Gas exchange measurements were made inside a glasshouse at the University of Edinburgh, on the central section of current-year branches using a portable gas exchange system (ADC-LCA-3, Analytical Development Co. Ltd., Hoddesdon, UK) equipped with a Parkinson leaf chamber (conifer PLC-3). To ensure a gas-tight seal for the conifer leaf cuvette, needles were removed from each side of the central section of the branches at the beginning of July. To enable measurements of PPFD-saturated photosynthetic rates, illumination of the leaf cuvette by natural sunlight was supplemented with artificial light (provided by a white fluorescent lamp) to maintain the PPFD at the level of the needles above 1700 μmol $m^{-2}s^{-1}$. The projected area of needles was determined by removing all the needles enclosed in the leaf cuvette and passing them through a leaf area meter (LI 3000, LI-COR Inc., Lincoln, NE, USA). Instantaneous leaf CO_2 assimilation rates (A) and stomatal conductance (g_s) were measured between 11.00 and 13.00 h at the end of July 1993 on 20 saplings (five plants per clone) per [CO_2] treatment, at the growth CO_2 concentrations. Instantaneous water use efficiency (WUE_i) was than calculated as the rate of CO_2 assimilation per unit of water transpired. Short-term measurements (~10 minutes) of PPFD-saturated CO_2 assimilation rate in relation to changes in leaf internal CO_2 concentration (A/C_i) were made between 10.00 and 17.00 h in August 1993, over a

range of CO_2 concentrations between 40 and 1200 μmol mol^{-1} on twelve saplings (three per clone) per [CO_2] treatment. The initial slope of the A/C_i curves is an estimate of the carboxylation efficiency (RuBP-saturated rate of Rubisco), whereas the maximum rate of assimilation (A_{MAX}) (the net CO_2 assimilation rate under conditions of PPFD and CO_2 saturation) is indicative of the role of RuBP regulation. Relative stomatal limitation (l) to photosynthetic rates was estimated from A/C_i curves, according to Long & Hällgren (1993), as follows:

$$l = (A_o - A) / A_o$$

where A is the photosynthetic rate measured at the growth CO_2 concentrations and A_o is the rate of photosynthesis which would occur with C_i equal to the growth CO_2 concentrations.

Rubisco activity was analysed *in vitro* by Dr. R. Besford at Horticulture Research International Institute, Littlehampton (UK); 'final' Rubisco activity was assayed spectrophotometrically by a coupled enzyme method (determining 3PGA phosphokinase activity and NADP-G3P dehydrogenase) after pre-incubation at 20 °C in extraction medium containing 25 mM $MgCl_2$ (Besford, 1984, Van Oosten *et al.*, 1995). Five needles from one clone per chamber (i.e. 100 needles per [CO_2] treatment) were sampled in July 1993 for Rubisco activity assays. The needles, removed from mid-way along current-year branches (upper-most whorl), were rapidly weighed before plunging them in to liquid nitrogen. Before measuring Rubisco activity, the projected area of needles was measured using a leaf area meter.

Needle concentration of chlorophylls *a*, *b*, and *a+b* was measured on different needles sampled in the same way as above. The saplings were sampled once a month from March to October in 1992, and from February to October in 1993. Three needles from one clone per chamber were removed from mid-way down a current-year branch (upper-most whorl) and immediately plunged in to liquid nitrogen. The method for extraction and measurement of chlorophyll (Porra *et al.*, 1989) has been described in Chapter 2. Before measuring the concentration of chlorophylls the projected area of needles was measured using a leaf area meter.

Samples for macro-nutrient (nitrogen, phosphorus, potassium, calcium and manganese), sugar and starch concentrations of roots and current year needles (from midway down a current-year branch of the upper-most whorl) were taken at each harvest made during the 1992 and 1993 growing seasons. The numbers of root and leaf samples taken were 24 per [CO_2] treatment (6 per clone) on day 381, 40 per [CO_2] treatment (10 per clone) on day 551, and 20 per [CO_2] treatment (5 per clone) on day 719 and 972. Full details of the methods of nutrient, sugar, and starch analysis are given in Chapter 2.

4.3 Results

As found in Chapter 3, there were no significant inter-chamber effects on any of the physiological parameters measured on the four clones of Sitka spruce, and, thus, the interactions 'chamber' are not shown.

CO_2 assimilation rate

Elevated CO_2 significantly ($P < 0.001$) increased CO_2 assimilation rate of Sitka spruce saplings by about 62% in summer 1993, when measured at the growth CO_2 concentrations (Table 4.1). This stimulation occurred in all the clones, but was higher in the Skidegate a and b clones (about 95% and 76%, respectively) than in the North Bend clones (about 43%) (Table 4.2). Thus, photosynthesis of the more northerly clones was more responsive to elevated CO_2 concentration, although not significantly so, than photosynthesis of the North Bend clones. The relationship between PPFD-saturated CO_2 assimilation rate and leaf internal CO_2 concentration was used to ascertain the biochemical limitation to photosynthesis. These A/C_i measurements showed a certain degree of down-regulation of photosynthesis in the saplings grown in elevated [CO_2] (Figure 4.1). The decrease in A_{MAX} in the elevated [CO_2] treatment was of the order of -25% ($P < 0.001$). This downward acclimation of A_{MAX} was observed in all four clones grown in elevated [CO_2] (Figure 4.2). It was significant at

$P < 0.05$ in the North Bend a (about -28%, the largest difference), North Bend b, and Skidegate b clones, and at $P < 0.10$ in the Skidegate a (about -21%, the least difference).

Table 4.1. Rubisco activity *in vitro* (on leaf area basis), assimilation rate (A), stomatal conductance (g_s), and WUE_I of the Sitka spruce saplings (all clones) in ambient or elevated [CO_2]. Data are means of 20 plants per [CO_2] treatment ± 1 SEM. A, g_s, and WUE_I were measured inside a glasshouse at the University of Edinburgh at the end of July 1993. Assimilation measurements were made at the growth CO_2 concentration, with a mean temperature of 24.9 ± 0.28 °C (1 SEM) at saturating PPFD (> 1700 μmol $m^{-2}s^{-1}$) between 11.00 and 13.00 h.

	Rubisco (μmol $m^{-2}s^{-1}$)	A (μmol $m^{-2}s^{-1}$)	g_s (mol $m^{-2}s^{-1}$)	WUE_I (mmol mol^{-1})
elevated	27.88 ± 1.54	16.83 ± 0.44	0.17 ± 0.004	6.78 ± 0.29
ambient	43.72 ± 3.13	10.09 ± 0.37	0.21 ± 0.005	4.34 ± 0.23
Statistical significance:				
[CO_2]	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
clone				
[CO_2] x clone				

Rubisco activity

The A/C_i response curves also showed that ambient [CO_2]-grown saplings had higher carboxylation efficiency than elevated [CO_2]-grown saplings (Figure 4.1). Carboxylation efficiency is generally interpreted as a limitation by Rubisco activity, which was significantly reduced *in vitro* by 36% in elevated [CO_2] (Table 4.1). Figure 4.2 shows that all elevated [CO_2] clones had a depression in carboxylation efficiency *in vivo*. In parallel, Rubisco activity *in vitro* significantly decreased by about -22% in Skidegate b, -36% in North Bend b, -39% in North Bend a, and -43% in Skidegate a in response to long-term growth in elevated [CO_2] (Table 4.2). However, there were no significant differences in Rubisco activity among the clones, or in the extent of the change in Rubisco activity induced by elevated [CO_2].

Needle chlorophyll concentration

Elevated [CO_2] did not affect the chlorophyll *a/b* ratio, in both growing seasons (Tables 4.3 and 4.4). However, chlorophyll concentrations (*a*, *b*, and, total) were

statistically affected in both growing seasons, and were lower at all sampling times in the saplings grown in elevated [CO_2] compared with the ambient [CO_2]-grown saplings.

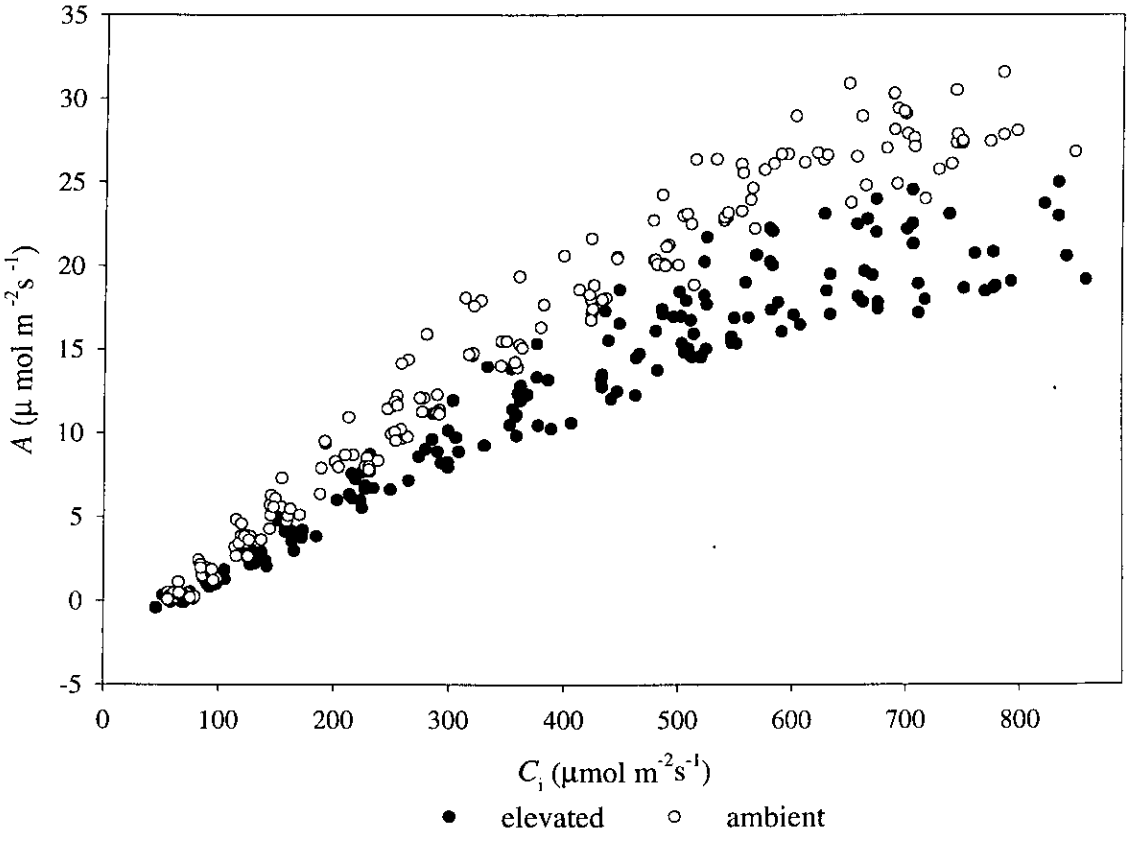

Figure 4.1. The relationship between net CO_2 assimilation rate (A) of Sitka spruce saplings of the four clones and intercellular CO_2 concentration (C_i) in conditions of PPFD saturation. The measurements were made between August and October 1993 on shoots of twelve saplings per [CO_2] treatment. Mean A_{MAX}, averaged across the four clones, was statistically different between treatments ($P < 0.001$).

Stomatal conductance

Stomatal conductance was strongly affected by growth in elevated [CO_2] ($P < 0.001$). Stomatal conductance of saplings grown and measured in elevated [CO_2] was about 20% less than that of plants grown and measured in ambient [CO_2] (Table 4.1).

However, relative stomatal limitation to photosynthesis was decreased from about 36% in ambient [CO_2] to about 18% in elevated [CO_2]-grown saplings. All the four clones showed a significant decrease in g_s in elevated [CO_2], when measured at the growth CO_2 concentrations (Table 4.2). This decrease in g_s was largest in the North Bend a clone (about -29%), and least for Skidegate a (about -11%). Short-term response of stomatal conductance to stepwise changes in intercellular CO_2 concentrations was relatively high in both elevated- and ambient [CO_2]-grown saplings (Figure 4.3). The North Bend a clone was the only clone in which elevated [CO_2]-grown plants showed significant reduction in g_s over range of C_i's, since there were no differences in the g_s/C_i relationship in the other three clones between the [CO_2] treatments ($P < 0.05$).

Instantaneous water use efficiency

An estimate of the instantaneous leaf water use efficiency was calculated as the ratio of net assimilation rate to water lost by transpiration. WUE_I increased significantly (about +56%) in saplings grown and measured in elevated [CO_2] compared to saplings grown and measured in ambient [CO_2] (Table 4.1). All the four clones had a significant increase in instantaneous WUE_I in response to elevated [CO_2], when measured at the growth CO_2 concentrations (Table 4.2). This increase was about 40% in North Bend a, 59% in Skidegate a, 61% in North Bend b, and 65% in Skidegate b. However, there were no significant differences ($P < 0.05$) in WUE_I among the clones in both the [CO_2] treatments.

Sugar and starch concentrations

Sugar concentration (sucrose, fructose, glucose, etc. percentages are reported in Appendix 3 Table 1) per unit of dry mass in both needle and root (Figure 4.4) were not significantly increased by elevated [CO_2], other than at the first harvest (382 d). There was no clear trend in the sugar concentrations among clones, and all responded similarly to elevated [CO_2] (Appendix 3 Figure 1).

Table 4.2. Rubisco activity *in vitro* (on leaf area basis), assimilation rate (A), stomatal conductance (g_s), and WUE_I of the four Sitka spruce clones. A, g_s, and WUE_I were measured at the growth CO_2 concentrations. Data represent the means of 5 plants per treatment ± 1 SEM. The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$) apply to the difference in response to the [CO_2] treatments of each clone.

	Rubisco (µmol $m^{-2}s^{-1}$)		A (µmol $m^{-2}s^{-1}$)		g_s (mol $m^{-2}s^{-1}$)		WUE_I (mmol mol^{-1})	
	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient
Skidegate a	29.97 ± 1.99	52.41 ± 7.48 **	18.33 ± 0.99	9.39 ± 0.52 ***	0.184 ± 0.006	0.206 ± 0.010 *	7.27 ± 0.91	4.56 ± 0.58 *
Skidegate b	28.10 ± 2.13	36.23 ± 2.77 *	16.33 ± 0.72	9.27 ± 0.11 **	0.175 ± 0.007	0.219 ± 0.002 ***	6.61 ± 0.37	4.01 ± 0.35 **
North Bend a	27.51 ± 4.41	45.48 ± 6.35 *	15.88 ± 0.49	11.15 ± 0.23 ***	0.155 ± 0.002	0.217 ± 0.002 ***	6.12 ± 0.65	4.38 ± 0.59 *
North Bend b	25.94 ± 2.90	40.75 ± 5.33 *	16.19 ± 0.67	11.29 ± 1.28 **	0.166 ± 0.009	0.205 ± 0.018 *	7.11 ± 0.24	4.42 ± 0.54 **

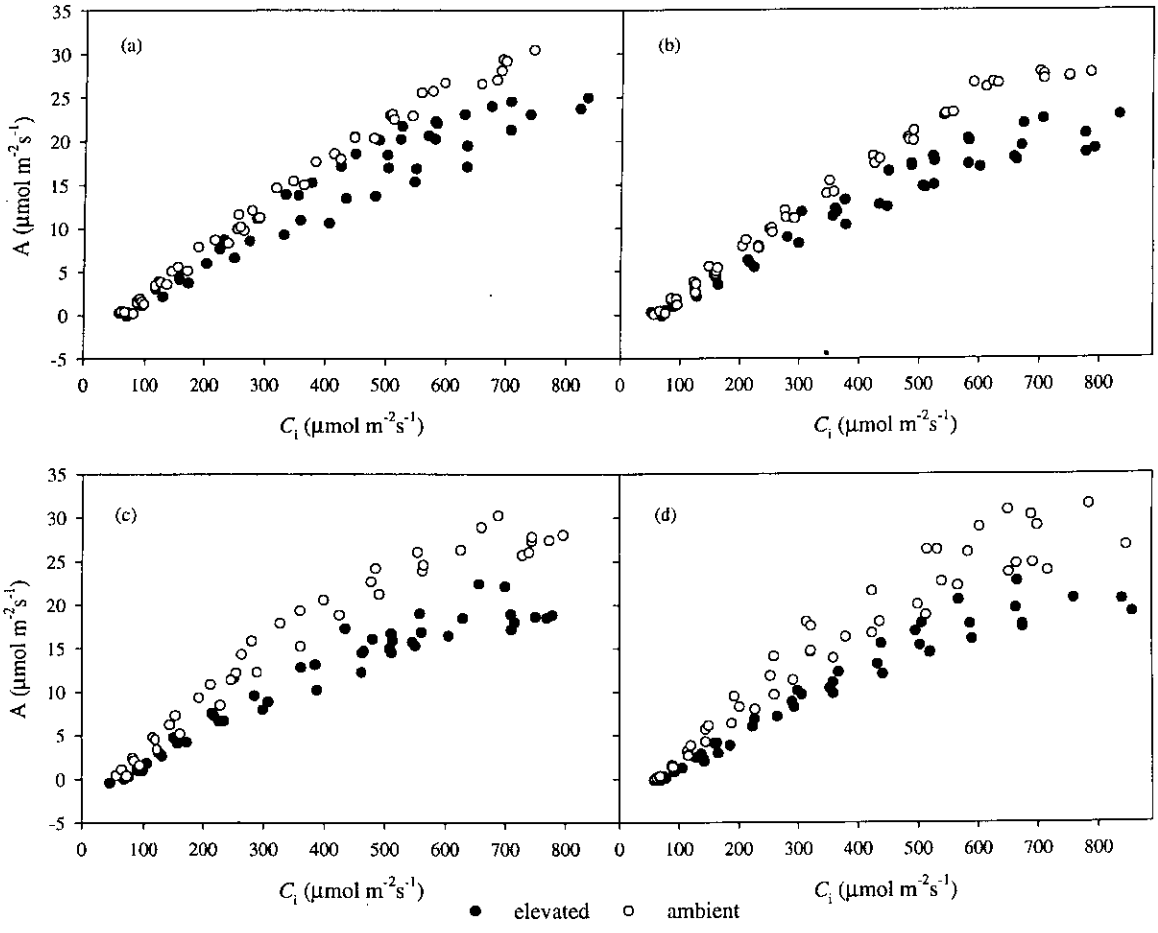

Figure 4.2. The relationship between net CO_2 assimilation rate (A) of the four Sitka spruce clones and intercellular CO_2 concentration (C_i) in conditions of PPFD saturation; (a) Skidegate a, (b) Skidegate b, (c) North Bend a, and (d) North Bend b. The measurements were made between August and October 1993 on shoots of three plants per clone per [CO_2] treatment.

There was a significant increase in starch concentration at the end of both the 1992 and 1993 growing season (days 551 and 972, respectively) in the needles (Figure 4.5a) and roots (Figure 4.5b) of saplings grown in elevated [CO_2] compared with ambient [CO_2]-grown plants. The amount of starch accumulated in the needles of the four clones of Sitka spruce grown in elevated [CO_2] was much larger on day 972,

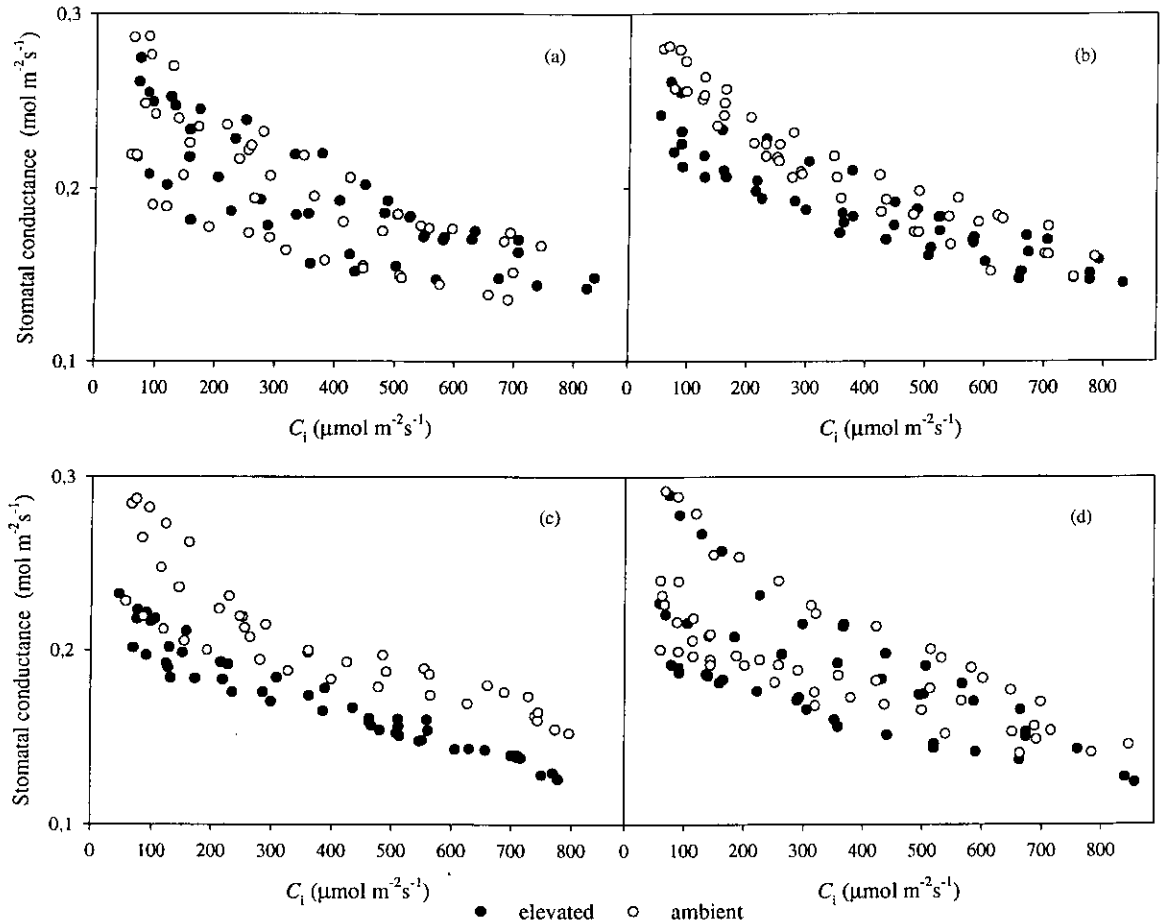

Figure 4.3. The relationship between stomatal conductance (g_s) of the four Sitka spruce clones and leaf internal CO_2 concentration (C_i) in conditions of PPFD saturation; (a) Skidegate a, (b) Skidegate b, (c) North Bend a, and (d) North Bend b. The measurements were made between August and October 1993 on shoots of three plants per clone per [CO_2] treatment.

compared with day 551 (Figure 4.6a). Starch concentrations of the roots of all the clones were also increased at the end of the 1992 and 1993 growing season by elevated [CO_2] (Figure 4.6b). This increase was significant in each clone on day 551, but on day 972 root starch concentration increased significantly only in the North Bend clones grown in elevated [CO_2].

Table 4.3. Chlorophyll concentration (mg cm^{-2}) measured at monthly intervals from March to October 1992 on needles of Sitka spruce saplings grown in ambient or elevated [CO_2]. One-year-old foliage was analysed from March to May while the later assays were on current year foliage. Data are means of 20 plants per treatment ± 1 SEM.

	chlorophyll *a/b*		chlorophyll *a*		chlorophyll *b*		total chlorophyll	
	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient
March	2.67 ± 0.04	2.71 ± 0.04	56.32 ± 2.14	64.14 ± 2.72	21.22 ± 0.84	23.84 ± 1.14	77.53 ± 2.93	87.98 ± 3.83
April	2.58 ± 0.04	2.64 ± 0.04	50.84 ± 2.14	58.75 ± 2.72	19.67 ± 0.84	22.23 ± 1.14	70.53 ± 2.93	80.98 ± 3.83
May	2.59 ± 0.07	2.51 ± 0.03	37.44 ± 1.10	41.19 ± 1.46	14.64 ± 0.57	16.49 ± 0.67	52.09 ± 1.61	57.68 ± 2.10
June	2.25 ± 0.06	2.33 ± 0.08	50.45 ± 2.75	58.87 ± 2.80	22.83 ± 1.36	25.59 ± 1.52	73.28 ± 3.99	84.46 ± 4.08
July	3.53 ± 0.15	3.63 ± 0.15	50.38 ± 2.90	59.66 ± 3.02	15.01 ± 0.74	17.09 ± 1.00	66.44 ± 3.05	76.76 ± 3.53
August	3.06 ± 0.23	2.91 ± 0.11	55.09 ± 6.41	70.81 ± 5.05	20.61 ± 2.98	25.19 ± 2.19	75.70 ± 9.36	96.01 ± 7.14
September	2.56 ± 0.10	2.68 ± 0.07	57.29 ± 2.88	62.11 ± 3.31	22.85 ± 1.50	23.43 ± 1.34	80.14 ± 4.07	85.54 ± 4.52
October	3.34 ± 0.18	3.63 ± 0.20	60.51 ± 3.81	66.99 ± 2.43	19.24 ± 1.71	19.50 ± 1.39	79.75 ± 5.46	86.49 ± 3.66
Statistical significance:								
[CO_2]	ns		$P < 0.001$		$P < 0.001$		$P < 0.001$	
Time	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$	
Interaction	ns		ns		ns		ns	

Table 4.4. Chlorophyll concentration (mg cm^{-2}) measured at monthly intervals from February to October 1993 on needles of Sitka spruce saplings grown in ambient or elevated [CO_2]. One-year-old foliage was analysed from February to May while the later assays were on current year foliage. Data are means of 20 plants per treatment ± 1 SEM.

	chlorophyll *a/b*		chlorophyll *a*		chlorophyll *b*		total chlorophyll	
	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient
February	2.08 ± 0.04	2.06 ± 0.05	61.27 ± 4.23	72.39 ± 3.55	30.00 ± 2.43	35.51 ± 2.10	91.27 ± 6.62	107.90 ± 5.51
March	2.38 ± 0.07	2.54 ± 0.16	55.06 ± 4.42	59.05 ± 3.13	23.61 ± 2.15	24.90 ± 1.84	78.66 ± 6.51	83.95 ± 4.88
April	2.29 ± 0.05	2.20 ± 0.06	73.49 ± 4.96	76.05 ± 4.81	32.04 ± 2.08	34.99 ± 2.49	105.53 ± 6.96	111.04 ± 7.16
May	2.65 ± 0.16	2.47 ± 0.04	80.57 ± 4.64	87.21 ± 5.18	31.33 ± 2.15	35.52 ± 2.25	111.90 ± 6.43	122.73 ± 7.39
June	2.61 ± 0.04	2.51 ± 0.04	23.89 ± 1.79	25.83 ± 1.25	9.29 ± 0.78	10.30 ± 0.49	33.18 ± 2.56	36.14 ± 1.72
July	2.66 ± 0.10	2.61 ± 0.02	22.47 ± 2.05	29.77 ± 2.08	8.68 ± 0.88	11.44 ± 0.84	31.15 ± 2.91	41.21 ± 2.92
August	2.58 ± 0.06	2.55 ± 0.11	28.97 ± 2.76	35.98 ± 2.59	11.47 ± 1.21	14.73 ± 1.27	40.44 ± 3.95	50.71 ± 3.73
September	2.44 ± 0.03	2.37 ± 0.05	23.66 ± 2.25	36.01 ± 3.05	9.78 ± 0.97	15.19 ± 1.28	33.45 ± 3.22	51.20 ± 4.31
October	2.96 ± 0.14	2.57 ± 0.04	27.89 ± 2.47	36.38 ± 2.03	9.91 ± 1.10	14.32 ± 0.96	37.80 ± 3.50	50.70 ± 2.97
Statistical significance:								
[CO_2]	ns		$P < 0.001$		$P < 0.001$		$P < 0.001$	
Time	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$	
Interaction	ns		ns		ns		ns	

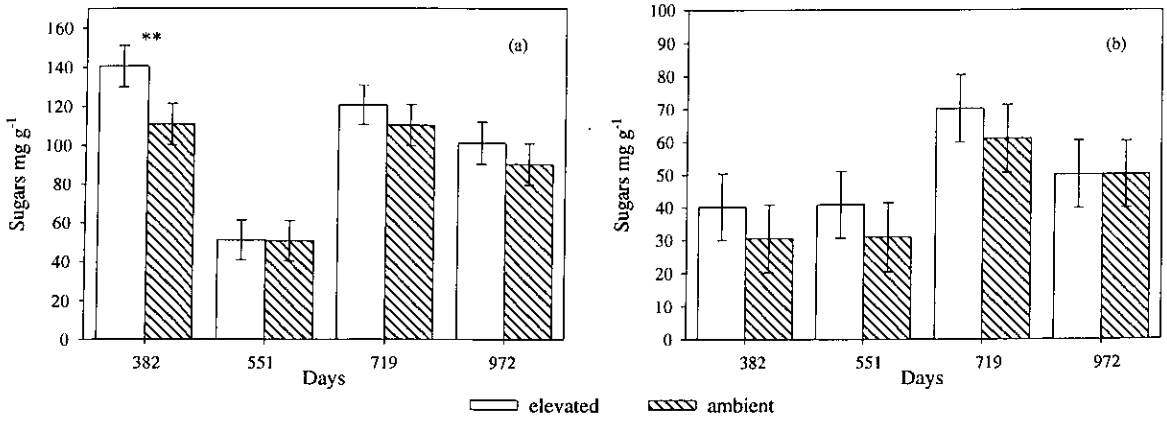

Figure 4.4. Needle (a) and root (b) soluble sugar concentrations per unit dry mass in the Sitka spruce saplings grown in ambient or elevated [CO_2] in the growing seasons 1992 and 1993, shown as days from the beginning of the experiment (1 March 1991). Data are means of 20 to 40 plants per treatment ± 1 SEM. The significance level (** = $P < 0.01$) shows the difference in sugar concentration in response to the [CO_2] treatments.

Nutrient concentrations

Although the saplings were supplied with non-limiting nutrients to ensure free access (details are given in Chapters 2 and 3), differences were found in the nutrient concentrations of needles and roots between the [CO_2] treatments. In elevated [CO_2] there was a significant reduction in needle (Figure 4.7a) and root (Figure 4.7b) N concentration of the Sitka spruce saplings harvested at the beginning and end of both the 1992 and 1993 growing seasons. The decrease in N concentrations ranged from -16% (on day 551) to -38% (on day 972) in the needles, whereas in roots it was remarkably constant at each harvest (about -15%). In addition, N concentration of both needles and roots (Table 4.5) was significantly reduced in each clone grown in elevated [CO_2] at the end of the third growing season (day 972). Figure 4.8 shows the relationships between leaf N concentration and mean total dry mass of the saplings in both ambient and elevated [CO_2]. Growth in different CO_2 concentrations affected the nitrogen concentrations when the saplings were the same size, as demonstrated by the linear relationships between the mean total dry mass and nitrogen

concentration of leaves (R^2 = 0.926 for the elevated [CO_2] saplings, and R^2 = 0.993 for the ambient [CO_2] saplings).

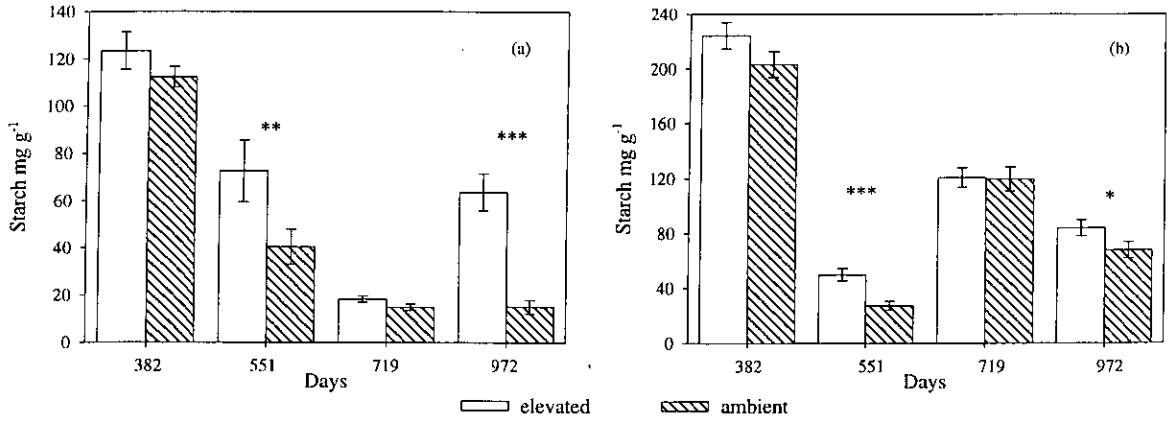

Figure 4.5. Needle (a) and root (b) starch concentrations per unit of dry mass in the Sitka spruce saplings grown in ambient or elevated [CO_2], shown as days from the beginning of the experiment. Data are means of 20 to 40 plants per treatment ± 1 SEM. The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$) show the difference in starch concentration in response to the [CO_2] treatments.

At each harvest, needle (Figure 4.9a) and root (Figure 4.9b) P concentrations of the saplings in elevated [CO_2] decreased, but the differences were significant only on day 972 in needles and on day 551 in roots. There were no significant differences in P concentration of needles and roots among clones in the two [CO_2] treatments after three years of growth (Table 4.5). Significantly different K concentrations were found in needles (Figure 4.9c) and roots (Figure 4.9d) of the saplings between the two [CO_2] treatments at the end of both 1992 and 1993 growing seasons. At the end of the third growing season, only the Skidegate a clone and the North Bend clones showed significant reductions in needle and root K concentration (Table 4.5).

Table 4.5. Nutrient concentration (mg g^{-1}) in the four clones of Sitka spruce after three years of growth (day 972) in ambient or elevated [CO_2]. Data are means of 5 plants per treatment ± 1 SEM. The significance levels (* = $P < 0.05$, ** = $P < 0.01$) show the difference in each clone in response to the [CO_2] treatments.

	Skidegate a		Skidegate b		North Bend a		North Bend b	
	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient
needle nutrient content:								
N	0.89 ± 0.117	1.44 ± 0.168 *	1.03 ± 0.103	1.54 ± 0.141 *	0.94 ± 0.024	1.31 ± 0.115 *	0.87 ± 0.095	1.57 ± 0.069 **
P	0.30 ± 0.020	0.35 ± 0.022 ns	0.21 ± 0.009	0.21 ± 0.009 ns	0.26 ± 0.006	0.29 ± 0.016 ns	0.24 ± 0.023	0.30 ± 0.012 ns
K	1.25 ± 0.047	1.49 ± 0.023 **	1.22 ± 0.051	1.24 ± 0.045 ns	1.53 ± 0.033	1.59 ± 0.026 ns	1.43 ± 0.048	1.55 ± 0.029 ns
K	1.25 ± 0.047	1.49 ± 0.023 **	1.22 ± 0.051	1.24 ± 0.045 ns	1.53 ± 0.033	1.59 ± 0.026 ns	1.43 ± 0.048	1.55 ± 0.029 ns
Ca	0.76 ± 0.030	0.83 ± 0.016 ns	0.65 ± 0.025	0.74 ± 0.034 ns	0.27 ± 0.015	0.31 ± 0.037 ns	0.19 ± 0.026	0.36 v 0.047 *
Mg	0.12 ± 0.002	0.14 ± 0.009 ns	0.09 ± 0.007	0.10 ± 0.006 ns	0.06 ± 0.002	0.07 ± 0.003 ns	0.07 ± 0.011	0.10 ± 0.010 ns
root nutrient content:								
N	1.00 ± 0.024	1.24 ± 0.058 *	1.29 ± 0.039	1.14 ± 0.038 *	1.05 ± 0.052	1.54 ± 0.124 *	1.11 ± 0.032	1.31 ± 0.019 **
P	0.22 ± 0.031	0.24 ± 0.014 ns	0.27 ± 0.012	0.23 ± 0.015 ns	0.20 ± 0.010	0.25 ± 0.019 ns	0.22 ± 0.005	0.27 ± 0.023 ns
K	0.88 ± 0.024	1.05 ± 0.084 ns	0.95 ± 0.042	0.96 ± 0.048 ns	1.13 ± 0.060	1.46 ± 0.074 *	1.08 ± 0.051	1.31 ± 0.038 *
Ca	0.34 ± 0.016	0.36 ± 0.016 ns	0.39 ± 0.044	0.45 ± 0.020 ns	0.44 ± 0.033	0.52 ± 0.026 ns	0.39 ± 0.022	0.48 ± 0.018 *
Mg	0.18 ± 0.005	0.17 ± 0.008 ns	0.18 ± 0.002	0.14 ± 0.011 *	0.16 ± 0.006	0.18 ± 0.005 ns	0.17 ± 0.002	0.20 ± 0.016 ns

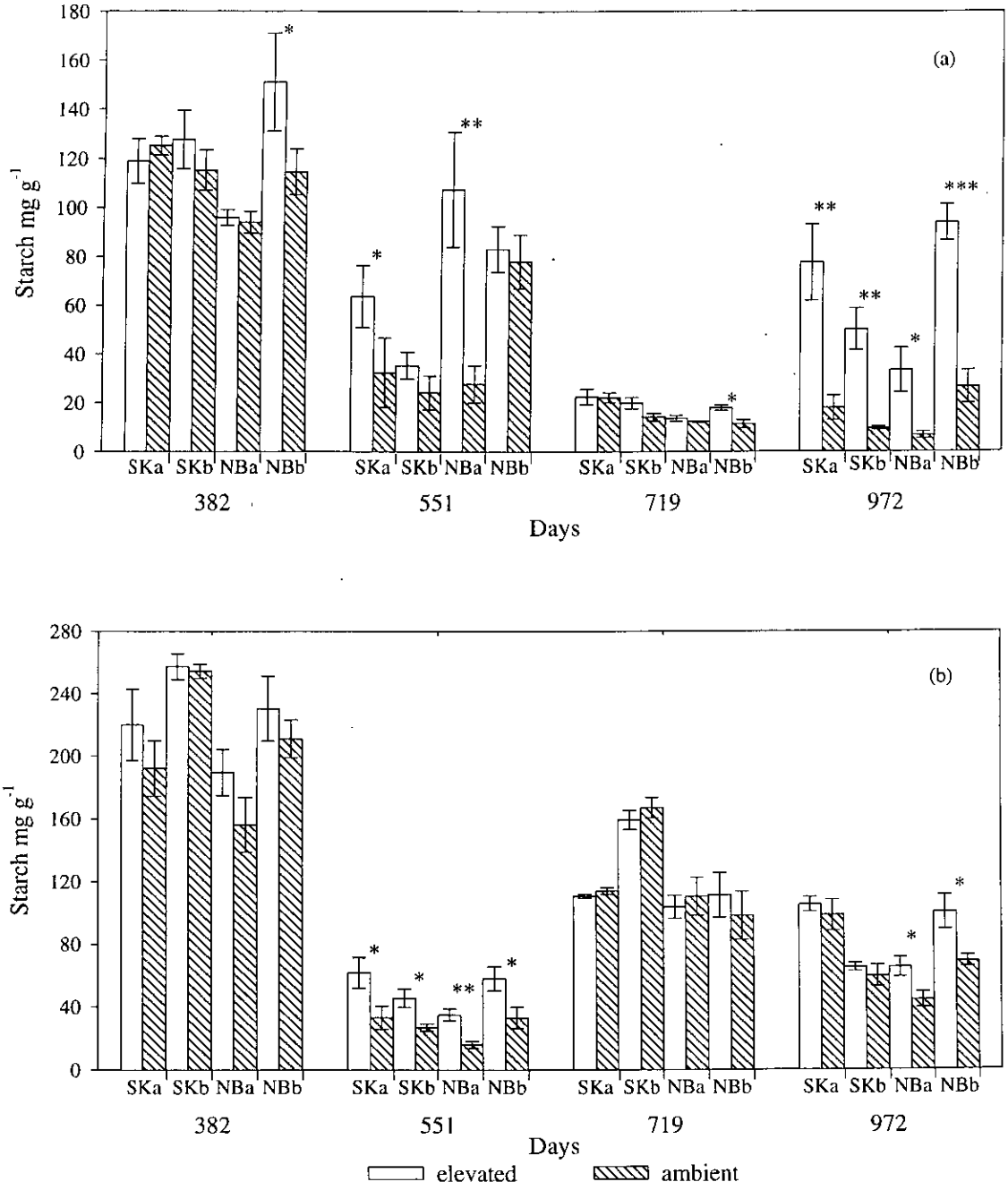

Figure 4.6. Needle (a) and root (b) starch concentrations per unit dry mass in the four Sitka spruce clones grown in ambient or elevated [CO_2], shown as days from the beginning of the experiment. Data are means of 5 to 10 plants per treatment ± 1 SEM. The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$) show the difference in starch concentration in each clone in response to the [CO_2] treatment. SK a = Skidegate a, SK b = Skidegate b, NB a = North Bend a, and NB b = North Bend b.

Calcium and magnesium concentrations were also reduced in elevated [CO_2]. However, needle calcium concentration was significantly affected only on day 382 (Figure 4.9e), and root calcium concentration was significantly decreased only on day 972 (Figure 4.9f) in saplings grown in elevated [CO_2]. With the exception of the North Bend b clone, which showed significant differences in both needle and root calcium concentrations between the two [CO_2] treatments at the end of the third growing season, there were no significant differences in calcium concentration of each of the other three clones in response to elevated [CO_2] (Table 4.5). Needle magnesium concentration of the elevated [CO_2] saplings was significantly reduced at the beginning of both the 1992 and 1993 growing seasons compared to that of the ambient [CO_2]-grown plants (Figure 4.9g). Accordingly, the needle magnesium concentrations of each clone were not statistically different between the two [CO_2] treatments on day 972 (Table 4.5). There were no differences in root magnesium concentration of saplings between the two [CO_2] treatments at each harvest (Figure 4.9h). However, root magnesium concentration of the Skidegate b clone grown in elevated [CO_2] was significantly affected at the end of the third growing season.

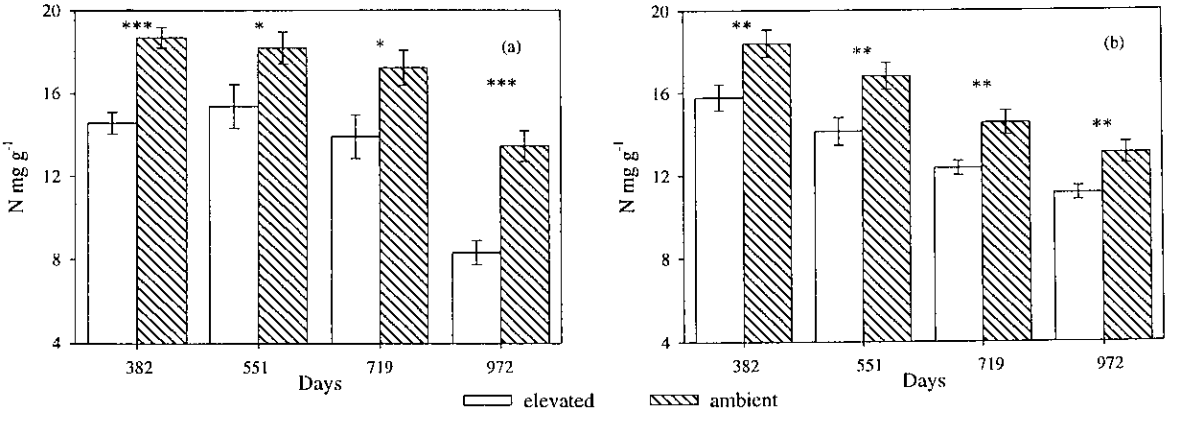

Figure 4.7. Needle (a) and root (b) nitrogen concentrations per unit of dry mass in the Sitka spruce saplings grown in ambient or elevated [CO_2], shown as days from the beginning of the experiment. Data are means of 20 to 40 plants per treatment ± 1 SEM. The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$) show the difference in nitrogen concentration in response to the [CO_2] treatments.

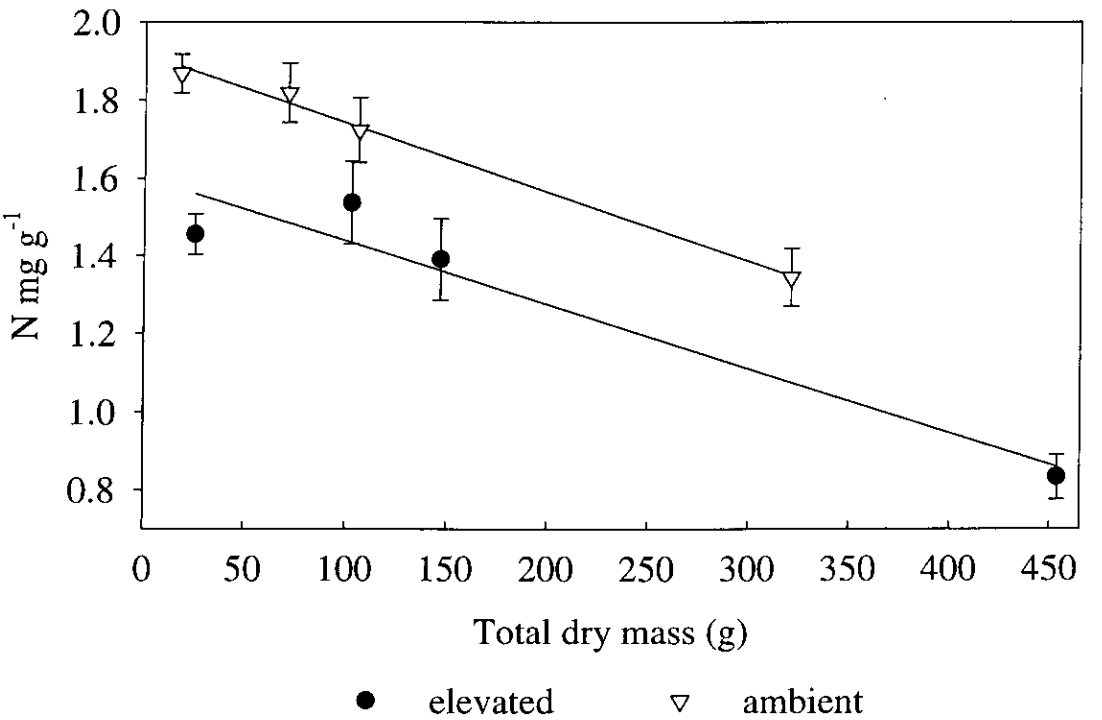

Figure 4.8. Linear relationships between of the combined mean foliar nitrogen concentration and total dry mass of the Sitka spruce saplings grown in ambient or elevated [CO_2]. Data are means of 20 to 40 plants per treatment ± 1 SEM.

4.4 Discussion

The four clones of Sitka spruce were grown in stress-free conditions (adequate nutrition and water) to assess the effect of elevated [CO_2] on tree physiology *per se*, thus ruling out any other effects that insufficient water or nutrient supply might have caused. Some species, for instance *Pinus radiata* (Conroy *et al.*, 1986b) and *Pinus taeda* (Thomas *et al.*, 1994), have shown a lack of response of *A* to elevated CO_2 concentration in low nutrient conditions. Recently, Drake *et al.*, (1997) have stressed the importance of available nutrients in determining the extent of the stimulation of *A* in elevated [CO_2]: in a review of eight experiments the average stimulation dropped

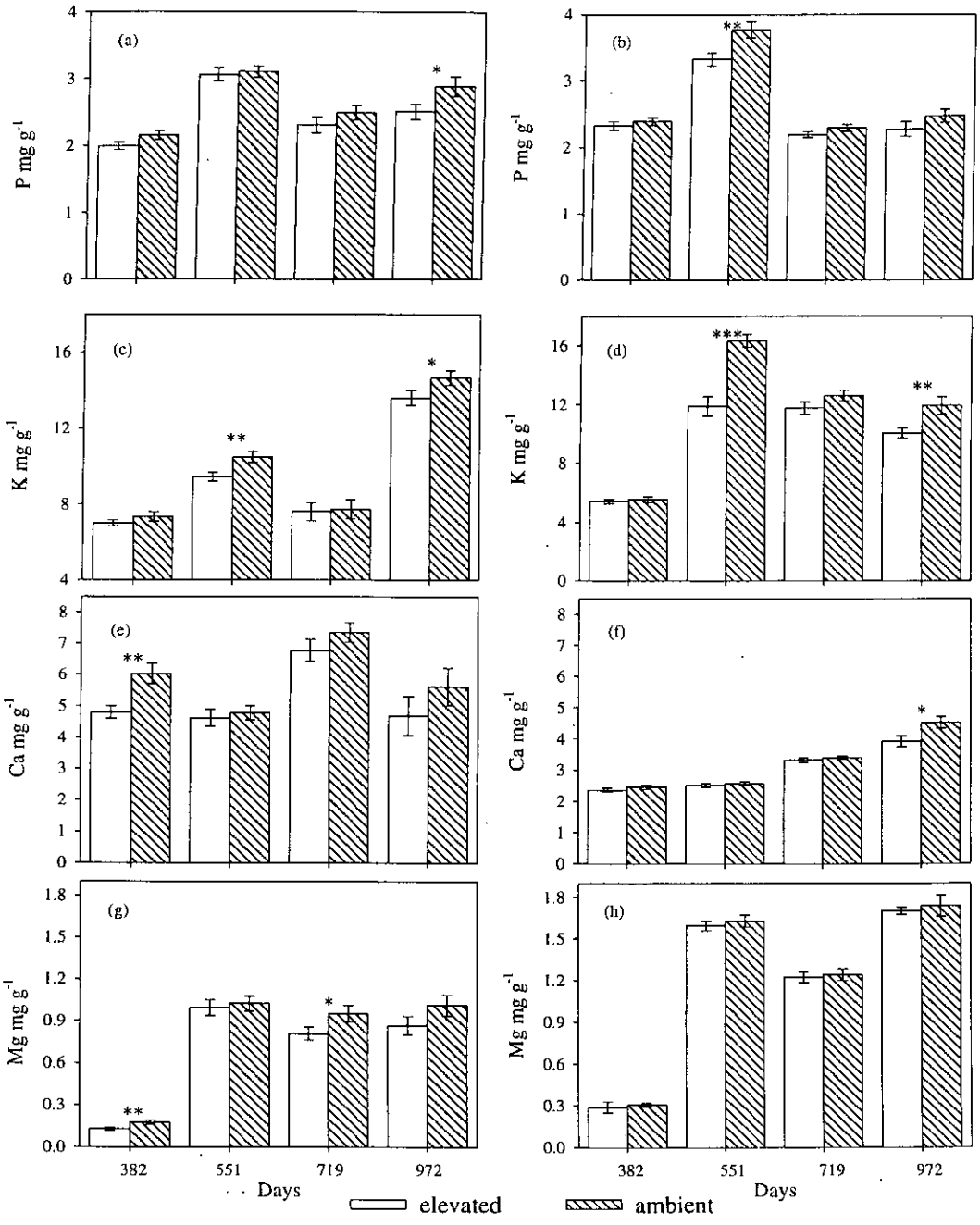

Figure 4.9. Needle (a,c,e,g) and root (b,d,f,h) phosphorus (a,b), potassium (c,d), calcium (e,f), and magnesium (g,h) concentrations per unit of dry mass in the Sitka spruce saplings grown in ambient or elevated [CO_2], shown as days from the beginning of the experiment. Data are means of 20 to 40 plants per treatment ± 1 SEM. The significance levels (* = $P < 0.05$, ** = $P < 0.01$) show the difference in nutrient concentration in response to the [CO_2] treatments.

from 57% at high nitrogen supply to 23% with low availability of nitrogen. Long-term growth in elevated [CO_2] significantly increased instantaneous photosynthetic rates of the clonal Sitka spruce saplings by about 62% (Table 4.1). Similar increases in assimilation rate were found, on average over the entire study period, in juvenile foliage of nine-year-old *Pinus taeda* trees exposed for the second year to doubling of the CO_2 concentration (Murthy *et al.*, 1997). There are not many studies available on the effects of growth in elevated [CO_2] on the photosynthetic rates of Sitka spruce. Townend (1993) found a significant effect of elevated [CO_2] on the response of A to PPFD in two-year old Sitka spruce clones, but he did not quantify this increase. A of both current year and one-year-old shoots of mature Sitka spruce was doubled in elevated [CO_2] in a branch bag experiment (Barton, 1997). Similar results have been found in other coniferous species. The relative increase in photosynthetic rate of foliage of branches of 22-year-old *Pinus taeda* trees in elevated [CO_2] ($A_{amb+330}/A_{amb}$) was slightly more than twice that in ambient [CO_2] (Teskey, 1997). Similar results were observed on mature trees of *P. taeda* exposed to FACE (Ellsworth *et al.*, 1995) and on *Pinus eldarica* (Garcia *et al.*, 1994).

Instantaneous photosynthetic rates of the two different provenance of the four clones of Sitka spruce responded differently to elevated [CO_2] (Table 4.2). The more southerly clones (North Bend a and b) showed an increase in assimilation rate of about 43%, which is very close to the average increase reported in the survey by Gunderson & Wullschleger (1994), whereas the increases in photosynthetic rates of the more northerly clones (Skidegate a and b) were well above the average. However, the Skidegate a and b clones produced significantly less total dry mass than the North Bend clones in elevated [CO_2] at the end of the third growing season (see Chapter 3). Either the increase in instantaneous [CO_2] assimilation rates of each clone measured at the beginning of August 1993 were not representative of the average increase over the entire growth period studied, or there were larger losses of carbon in respiration, volatilization, root exudation and fine root turnover in the more northerly clones. Unfortunately, these processes were not studied in this work, but they can account for a large proportion of the assimilates lost (Lynch & Whipps, 1990; Amthor, 1995).

Long-term growth in elevated [CO_2] often results in reduced amounts of photosynthetic pigments and enzymes (Eamus & Jarvis, 1989; Bowes, 1996). However, Arp (1991) showed that acclimation of photosynthetic capacity and size of pot were highly correlated, and that constrained rooting caused by inadequate pot volume may down-regulate photosynthetic capacity in elevated [CO_2]. In contrast, plants rooted into the ground did not show any degree of acclimation of photosynthetic capability (Arp & Drake, 1991; Idso & Kimball, 1992c; Liu & Teskey, 1995; Scarascia-Mugnozza *et al.*, 1996). Exposure to elevated [CO_2] of branches of mature Sitka spruce in a stand did not cause any acclimation of A in current year shoots, but it did result in some degree of down-regulation in one-year-old shoots (Barton, 1997). There were no significant changes in the A/C_i relationship measured in branches exposed to elevated [CO_2] of 22-year-old *Pinus taeda* trees in all the three years of growth, indicating that elevated [CO_2] did not alter the photosynthetic capacity of the foliage when adequate sinks are available (Teskey, 1997). Low rate of supply of nutrients can affect the size and activity of the photosynthetic system in elevated [CO_2] plants. Miglietta *et al.* (1996) showed that photosynthetic capacity of wheat was significantly decreased in plants grown with nitrogen deficiency in FACE, whereas A/C_i response curves did not reveal any significant effects of elevated [CO_2] on the photosynthetic capacity of well-fertilized plants. Similar results were found with *Pinus taeda* (Tissue *et al.*, 1993; Thomas *et al.*, 1994). There were no significant differences in A/C_i curves between the elevated and ambient [CO_2] plants of *Betula pendula* grown in Ingestad units with steady-state nutrition (Pettersson & McDonald, 1992). As emphasized above, the Sitka spruce saplings in this study were supplied with free access to nutrients. Moreover, dry mass allocation was identical when the plants were the same size suggesting that they were not pot limited (see Chapter 3). Yet nonetheless, acclimation of photosynthetic capacity occurred (Figure 4.1) in each clone (Figure 4.2). Acclimation of A was also found by Barton (1997) in two-year-old seedlings of Sitka spruce. He found that whole plant A was strongly down-regulated in elevated [CO_2] during the second year of growth, and it was not affected by different nutrient treatments.

A/C_i response curves showed both a decrease in A_{max}, which is biochemically related to the rate of regeneration of RuBP, and in carboxylation efficiency, which is limited by Rubisco activity. The latter is consistent with the measurements of Rubisco activity *in vitro* which decreased in elevated [CO_2]-grown saplings (Table 4.1). This decline of photosynthesis was in agreement with a down-regulation of Rubisco (Van Oosten & Besford, 1995), which may result from both lowered enzyme activation or a reduced amount of the enzyme (Van Oosten *et al.*, 1992; Tissue *et al.*, 1993; Vu *et al.*, 1997). Tomato plants in elevated [CO_2] showed more rapid ontogenetic decrease in the transcription of the Rubisco small-subunit than in ambient [CO_2] (Van Oosten & Besford, 1994; Van Oosten *et al.*, 1994). Acclimation of PPFD-saturated photosynthesis to CO_2 enrichment started before the tomato leaves were fully expanded (presumably when these leaves changed their "status" from sinks to photosynthetic active source organs). This decline of carboxylation capacity in tomato plants in elevated [CO_2] was followed after about 10 days by acclimation of the electron transport capacity of the light-harvesting complexes (i.e. the thylakoid proteins and chlorophyll of both photosystems, and cytochrome *f*) of mature leaves (Van Oosten & Besford, 1995; Van Oosten *et al.*, 1995).

The decline in chlorophyll concentration, which has been found in a number of experiments on plants grown in elevated [CO_2] (De Lucia *et al.*, 1985; Mousseau & Enoch, 1989; Radoglou & Jarvis, 1992; Wullschleger *et al.*, 1992), may also affect the assimilation capacity. Thus, the down-regulation of A_{max} shown by the elevated [CO_2]-grown clonal Sitka spruce saplings in this study may have been due, at least in part, to a significant reduction in chlorophyll concentration found during both the 1992 and 1993 growing seasons (Tables 4.3 and 4.4).

Nitrogen concentration was affected by growth in elevated [CO_2] in the Sitka spruce clones in the present study (Figure 4.7). Reduction in leaf N concentration in elevated [CO_2] was only partially caused by starch dilution, since starch concentration was not always affected (Figure 4.5a). There are many processes that if affected by elevated [CO_2] can lead to a decreased leaf N concentration. Rubisco is the largest pool of nitrogen in leaves (Stitt & Schulze, 1994; Drake *et al.*, 1997), and

its content can be reduced by about 35% in elevated [CO_2] before resulting in co-limitation of *A* (Long & Drake, 1992). Since less Rubisco is required in elevated [CO_2], the more carbon assimilated per unit of leaf N leads to increased nitrogen use efficiency (NUE). Inhibition of photorespiration in elevated [CO_2] plants may also reduce the amount of nitrogen required per unit dry mass produced (Conroy & Hocking, 1993). For example, Van Oosten *et al.* (1992) showed that the activity of two photorespiratory enzymes (glycolate oxidase and hydroxypyruvate) were decreased in *Picea abies* grown for two years in elevated [CO_2]. Chlorophyll concentration also constitutes a major pool of N in leaves (Evans, 1989), and pigments of the light-harvesting complexes are usually decreased by elevated [CO_2] (Ceulemans & Mousseau, 1994). Moreover, respiration associated with protein turn-over accounts for a considerable fraction of maintenance respiration (Amthor, 1995). Thus, changes in plant biochemistry in response to elevated [CO_2] may reduce specific maintenance respiration rates and hence the N requirement. Changes in the biochemistry of photosynthesis and photorespiration usually occur when N uptake does not keep pace with carbon uptake (Conroy & Hocking, 1993; Sage, 1994; Jarvis, 1995). This may be regarded as an optimisation process which involves reallocation of nitrogen away from non-limiting components into more limiting processes or organs (i.e. additional or larger sinks for the extra-carbon assimilated), leading to increased NUE.

The lower N requirement per unit of leaf area in elevated [CO_2] may account for the acclimation of the photosynthetic capability of the four clones, despite free access to nitrogen and the large rooting volume (10 dm^3), although, as Drake *et al.* (1997) pointed out, acclimation of *A* is the exception, rather than the rule, when the rooting volume exceeds 10 dm^3. However, a recent study on the interactive effect of elevated [CO_2] and N supply in field grown rice, has shown that reduced foliage concentration of N (even if calculated as a percentage of *structural* dry mass) was always associated with increased [CO_2] (Ziska *et al.*, 1996). In the Sitka spruce saplings leaf N concentration was different when the plants were the same size (Figure 4.8), indicating that growth in elevated [CO_2] increased the dry mass produced per unit of nitrogen taken up. Increased growth per unit of plant nitrogen and phosphorus was

also noted with seedlings of *Pinus ponderosa* grown in elevated [CO_2] (DeLucia *et al.*, 1997).

In a recent paper Van Oosten & Besford (1996) have described a molecular model for photosynthetic acclimation. The model invokes metabolite regulation of gene expression which probably occurs when the production of new assimilates is higher than the capacity to handle them. This can involve a source-sink imbalance leading to feedback effects on photosynthesis *via* end-product accumulation (Stitt, 1991). The cytoplasmatic pool of glucose may provide a regulatory signal for coarse control, which determines the amount of photosynthetic systems, by repressing the transcription of photosynthetic genes (Farrar, 1992). Yet, sugar concentration in needles of the Sitka spruce saplings was not increased significantly at the beginning, and end, of the 1993 growing season (Figure 4.4), at a time when photosynthetic acclimation in elevated [CO_2] was detected (Figure 4.1). This finding seems to conflict with the model put forward by Van Oosten & Besford (1996). However, Paul & Driscoll (1997) have recently shown that loss of photosynthetic activity is more correlated to the C/N ratio than to carbohydrate status *per se*. Accordingly, the increased leaf carbon/nitrogen ratio found on days 719 and 972 (i.e. equal sugar concentration, Figure 4.4a, and lower nitrogen concentration, Figure 4.7a) may be consistent with the photosynthetic acclimation in the clonal saplings grown in elevated [CO_2]. This result is consistent with the findings of a number of other studies. For instance, A of two full-sib families of *Pinus ponderosa* seedlings was decreased in elevated [CO_2] after about 39 days from germination; after 112 days from the beginning of the experiment elevated and ambient [CO_2] seedlings maintained similar A rates (Grulke *et al.*, 1993). However, the C/N ratio of needle tissues had been significantly increased in elevated [CO_2] since day 22.

Short-term responses of g_s/C_i relationship showed that stomatal conductance decreased with increasing CO_2 concentrations in both the elevated and ambient [CO_2] clonal saplings (Figure 4.3). A similar trend was observed in all four clones (Figure 4.3), but this was not necessarily associated with an increase in the gas-phase limitations to CO_2 uptake (Long & Drake, 1992). Elevated CO_2 concentration

frequently leads to a decline in g_s in C_3 plants (approximately 30-40%; see reviews by Eamus & Jarvis, 1989; Jarvis, 1989; Mott, 1990), but g_s in Sitka spruce is relatively insensitive to elevated [CO_2] (Ludlow *et al.*, 1971; Beadle *et al.*, 1979; Barton, 1997). Other, more recent, studies on conifers have shown that g_s of mature trees of *Pinus taeda* was insensitive to elevated [CO_2] (Ellsworth *et al.*, 1995; Liu & Teskey, 1995; Murthy *et al.*, 1997). In the present study, the overall mean g_s measured over growth [CO_2] conditions was decreased by approximately 20% in elevated [CO_2] (Table 4.1) and stomatal limitation to photosynthesis was halved. The combined mean WUE_i of the Sitka spruce clonal saplings was increased in response to elevated [CO_2] and this resulted from both increased photosynthetic rate and decreased stomatal conductance (Table 4.1). This result was consistent within the four clones (Table 4.2).

This study has highlighted acclimation of Rubisco and chlorophyll concentration as a means of improving nitrogen use efficiency in elevated [CO_2]. This is likely to result in an advantage for plants growing in nitrogen-limited environments, since the amount of N needed for growth was less when Sitka spruce saplings growing in elevated [CO_2] were the same size as saplings in ambient [CO_2]. Murray *et al.* (1996) found that total dry mass of Sitka spruce seedlings was marginally affected after three years of growth in elevated [CO_2] in nutritional conditions which resulted in ~1.7% leaf N concentration. Our experiment also lasted three years and the sapling total dry mass was significantly increased in elevated [CO_2] (see Chapter 3), however nutritional conditions resulted in ~0.8% leaf N concentration. It is important to appreciate that both these experiments were done on the same site, with the same carbon dioxide exposure facilities. Pot volume was not limiting in either study. The main differences were the age of the plant material (seedlings in Murray *et al.*'s experiment and clonal saplings in this work) and the nutrient conditions. The four clones had been selected for their forestry potential and were also genetically more uniform than the seedlings, but they were taken from five-year-old trees (see Chapter 2) and consequently had a higher degree of tissue maturity than the seedlings. However, one might expect the seedlings, being more plastic and having a higher degree of totipotency, to be more responsive to elevated

[CO_2] than the clonal saplings. When Sitka spruce seedlings were grown without nutrient limitation with a weekly supply of nutrients (Townend, 1995), the increase in total dry mass in response to elevated [CO_2] was larger than that seen here in the clonal saplings. In the experiment of Murray *et al.* the seedlings were top dressed with a *slow release* fertiliser at the start of each growing season. That apparently ensured an adequate supply of N, but there is no information available on the concentration of other macronutrients. The clonal saplings instead were fertilized weekly to ensure *immediate* free access to *all* nutrients. There is increasing evidence that elevated [CO_2] stimulates an early higher growth rate which is then magnified over time (see Chapter 7). It is not possible to see this initial growth stimulation in the experiment of Murray *et al.* so perhaps the *slow release* of fertiliser may have been inadequate to keep up with the initial higher growth stimulation in elevated [CO_2]. This once again raises the importance of supply and timing of nutrients.

Grulke *et al.* (1993) found that elevated [CO_2] had a much larger impact on growth of two full-sib families of *Pinus ponderosa* than the genetic source of the plants. However, the experiment was done on seedlings germinated and grown for four months in elevated or ambient [CO_2], and there are no data available either on total dry mass or on growth rates of the two full-sib families seedlings. Moreover, in our clonal Sitka spruce saplings, provenance did not significantly influence the photosynthetic capability (Figure 4.2 and Table 4.2), A and WUE_I measured at the growth CO_2 concentrations, although, as we have shown in Chapter 3, the more northerly clones were significantly less responsive to elevated [CO_2]. In our experiment genetic differences in growth response to elevated [CO_2] were already evident after the first year of growth, and they were magnified over time becoming significant after three full growing seasons (Figure 3.7). Many factors may have caused the initial larger growth stimulation in the North Bend clones than in the Skidegate clones in response to elevated [CO_2]. Oleksyn *et al.* (1992) found that in *Pinus sylvestris* root respiration accounted for about two-thirds of the total respiratory cost. Unfortunately, we were not able to measure the specific respiration rates of the four clones. However, the Skidegate clones allocated initially

proportionally more dry mass to the roots than the North Bend clones did (Figure 3.9), and this might have increased their whole-plant respiratory losses. Moreover, the more southerly clones had higher initial NUE than the more northerly clones (data not shown), showing that they had more N available for those processes or organs which were most limiting to growth at that particular time. Clonal provenance did affect growth in elevated [CO_2] and plant allocation and nitrogen use efficiency may have played an important role. This is particularly important for the northern countries where N is the most limiting resource and, therefore, will affect Sitka spruce growth as the atmospheric CO_2 concentration rises.

CHAPTER 5

Long-Term Interactive Effects of Elevated [CO_2] and Water Stress on Growth and Plant Water Use of Cherry (*Prunus avium*) Seedlings

5.1 Introduction

Water is the main factor limiting plant productivity in the Mediterranean region and thus the interaction between water availability and rising CO_2 concentrations is a major concern with respect to its effects on plant growth. If the emission of greenhouse gases continues to increase as expected, the average surface temperature of the Earth will increase by 0.2 - 0.4 °C per decade throughout the next century, as compared to an increase in temperature of 0.6 °C recorded during this century (Houghton *et al.*, 1990; Houghton, 1994; IPCC, 1996). Seasonal distribution and frequency of rainfall will also be greatly affected. The temperature increase will cause an increase in evaporative demand and water holding capacity of the air, and this could have a negative effect on plants in areas with limited water resources (Parry, 1990; Parry & Jiachen, 1991; Parry, 1992; Parry *et al.*, 1992; Jarvis, 1993). Rising [CO_2]-driven reduced soil water availability and the higher potential evapotranspiration will probably have the most important consequences for agriculture and for forest vegetation in which competition determines species composition (Tolley & Strain, 1984b; Rogers & Dahlman, 1993; Lee *et al.*, 1994; Jarvis, 1995).

It has been suggested that the beneficial effects of elevated [CO_2] on plant growth may depend upon plant water status (Idso, 1988): elevated [CO_2] would have less effect on plants in the well-watered optimum growth phase, more effect under non-lethal water-stressed conditions, and be most beneficial to severely water-stressed plants, resulting in an appreciable increase in growth. Several studies have shown that growth responses to elevated [CO_2] were larger in water-stressed plants than in

well-watered plants (Sionit *et al.*, 1980; Morison & Gifford, 1984a,b; Tolley & Strain, 1984b; Conroy *et al.*, 1986a, 1988; Marks & Strain, 1989; Kimball *et al.*, 1995). Clifford *et al.* (1993) found that plants of *Arachis hypogaea* grown in elevated [CO_2] in droughted conditions produced more than double the above ground dry mass of plants grown in ambient [CO_2], but that elevated [CO_2] also increased the harvest index of the droughted plants resulting in a six-fold increase in yield. However, quite variable results have been reported in studies on *Quercus petraea* and *Pinus pinaster* grown for one growing season in elevated [CO_2] in well-watered and water-stressed conditions (Guehl *et al.*, 1994). Total dry mass increase brought about by elevated [CO_2] was 138% in well-watered and 47% in droughted *Q. petraea* plants, whereas the total dry mass increase was 63% in well-watered *P. pinaster* plants but there was no increase in droughted conditions. The overall mean relative growth rate of two-year-old *Picea sitchensis* saplings was increased by 9.8% in well-watered saplings and 6.9% in droughted saplings after six months of growth in elevated [CO_2] (Townend, 1993). In a different experiment on Sitka spruce seedlings, Townend (1995) found that the growth response to the interaction between water stress and elevated [CO_2] was dependent on nutrient supply: elevated [CO_2] increased the percentage of dry mass produced by droughted seedlings compared to well-watered seedlings in the unfertilized treatment, but the increase was reduced in the fertilized treatment. The increase in relative growth rate of *Acer saccharum* in response to elevated [CO_2] was also dramatically reduced in droughted conditions compared to well-watered conditions, but conversely the percentage increase in dry mass of both *Liquidambar styraciflua* and *Platanus occidentalis* was larger in droughted than in well-watered conditions (Tschaplinski *et al.*, 1995).

In elevated CO_2 concentrations water use efficiency (WUE) per unit of leaf area has been shown to increase as a consequence of reduced transpiration rate (brought about by a decrease in stomatal conductance) and/or an increased assimilation rate (see reviews: Eamus & Jarvis, 1989; Jarvis, 1989; Eamus, 1991; Chaves & Pereira, 1992; Morison, 1993). Stomatal closure, reducing the amount of water loss through transpiration, is a mechanism of drought avoidance and thus the onset of water

stress may be delayed in plants growing in elevated [CO_2] because of reductions in stomatal conductance (Bhattacharya *et al.*, 1990). However, conflicting results were found by Heath & Kerstiens (1997) in *Fagus sylvatica* after two growing seasons in elevated [CO_2]. Stomatal conductance was not only increased by elevated [CO_2], but as drought developed stomatal closure was significantly delayed in beech plants growing with low nutrient supply. This resulted in a faster rate of soil drying even though elevated [CO_2] did not significantly increase leaf area. Thus, there is less certainty about the effects of high concentrations of [CO_2] on total water use per unit of ground area (Mousseau & Saugier, 1992).

Additionally, growth in elevated [CO_2] usually leads to faster growth of both leaf area and fine roots. This may increase total water consumption which in turn could offset higher instantaneous transpiration efficiency per unit of leaf surface (ITE) in elevated [CO_2] (Kerstiens *et al.*, 1995; Heath & Kerstiens, 1997), resulting in lower whole plant water use efficiency (Gaudillere & Mousseau, 1989; Melillo *et al.*, 1990). Thus, a measure of WUE based on the ratio of whole plant dry mass to total water transpired over the period of measurements may provide a more realistic description of WUE (Marks & Strain, 1989; Norby & O'Neill, 1989). Although Norby & O'Neill (1989) found a close relationship between ITE and whole plant WUE, the two indices do not contain identical information and generally increase of ITE in elevated [CO_2] is larger than increases in whole plant WUE (Morison, 1985). Thus, increased ITE does not necessarily imply that elevated [CO_2] improves resistance to water stress (Tschaplinski *et al.*, 1995; Beerling *et al.*, 1996). Drought resistance involves cellular and metabolic adaptations which affect plant water relations, and, in turn, photosynthesis and transpiration, root length and surface area, fine root turnover, soil exploration by roots, and xylem hydraulic conductivity (Tyree & Alexander, 1993).

Research on interactions between water stress and elevated [CO_2] has mostly been done on crops (Morison & Gifford, 1984a,b; Bhattacharya *et al.*, 1990; Chaudhuri *et al.*, 1990; Clifford *et al.*, 1993; Mauney *et al.*, 1994; Samarakoon *et al.*, 1995) or to study competition between C_3 - C_4 species (Marks & Strain, 1989). Little

information is available on the impact of water stress and elevated [CO_2] on tree growth and plant WUE. Moreover, no experimental work has been done on the effects of long-term interactions between elevated [CO_2] and drought on tree growth. Elevated [CO_2] increased whole plant WUE of *Aster pilosus* (C_3) over a 13 day-period by about 154% in well-watered conditions and 111% in droughted conditions, and in water-stressed plants of *Andropogon virgunicus* (C_4), by about 66%, although there was no increase in well-watered plants (Marks & Strain, 1989). However, Morison & Gifford (1984b) observed similar relative increases in WUE in response to elevated [CO_2] in C_3 crop species (average increase of 57%) and C_4 crop species (average increase of 50%). A constant, higher WUE, of almost 50%, was found during two years growth of *Lolium perenne* swards in elevated [CO_2] than in ambient [CO_2] (Schapendonk *et al.*, 1997). WUE of *Pinus radiata* plants grown for 22 weeks in elevated [CO_2] was increased by about 34% when the seedlings were adequately supplied with phosphorus, but was not significantly increased when the supply of phosphorus was limiting (Conroy *et al.*, 1988). Norby & O'Neill (1989) found an increase in whole plant WUE of 82% in *Quercus alba* over a 36-week experiment in elevated [CO_2]. A similar increase was found in *Quercus petraea* (80%, average of well-watered and droughted treatments), whereas in *Pinus pinaster*, the increase in WUE was about 50% (average of well-watered and droughted seedlings) in elevated [CO_2] (Guehl *et al.*, 1994).

Cherry (*Prunus avium*) is an important and valuable agricultural and timber crop throughout Europe. Cherry fruit production is economically important in Southern Europe, where droughts generally occur in the summer, at the time of maximum leaf and fruit growth, and influence tree growth and productivity. The current series of experiments was designed to mimic the effects of natural water stress on the growth of young cherry seedlings. In the first growing season, gradual water stress was imposed on rapidly growing cherry saplings. In the second growing season, rapid onset of water stress was imposed at the height of the growing season, when the cherry seedlings had already developed maximum leaf area. Our hypothesis was that rapidly growing young trees, out-performing in elevated [CO_2] those in ambient [CO_2] when the evaporative demand was rapidly increasing and while soil water

availability was rapidly decreasing, could have used up the available water more rapidly. In this Chapter we report the long-term interactive effects of elevated [CO_2] and water stress on growth and whole plant WUE of cherry saplings.

5.2 Materials and Methods

Cherry seeds were germinated in spring 1993 and grown for two years in ambient (~350 μmol mol^{-1}) or elevated (ambient + ~350 μmol mol^{-1}) CO_2 concentrations in six open-top chambers located inside a glasshouse at the University of Edinburgh. A further set of trees was maintained outside the chambers in three different blocks as a control - i.e. to separate the carbon dioxide effect from any temperature increase or other changes related to growth in the OTCs (details of the growth conditions are given in Chapter 2). The pots were frequently moved within the OTCs and outside blocks to overcome position effects. The seedlings were potted into potting compost (gravel:sand:peat:loam mixture 1:1:1.5:3) and regularly fertilized in both growing seasons in order to supply mineral nutrients at free access rates (details of the growth conditions are given in Chapter 2).

After the baseline harvest, made 33 days after emergence (dae), 180 seedlings (60 seedlings per [CO_2] treatment, 20 per OTC/outside block) were transplanted into 40 cm-long soil columns (6.6 dm^3), contained in black polythene tubes 45 cm long and 14.5 cm in diameter. Because of their volume and form, the plant roots in the soil columns were not pot bound, and allowed unrestricted root growth and gradual soil drying (Khalil & Grace, 1992). The walls and the base of the polythene tubes were perforated (at ~10 cm intervals) to allow free aeration of the soil and flow of excess water to the bottom of the column.

The seedlings were irrigated every other day to pot water capacity for the first five weeks until 69 dae. Then half of the seedlings (eight per OTC and outside block) were water-stressed by with-holding water for a six-week drying cycle (until 115 dae when the seedlings showed symptoms of severe water stress); simulating a

progressive decrease in water availability, while the remaining seedlings continued to be well-watered (pot capacity). At the end of the drying cycle the water-stressed seedlings were re-watered to pot capacity until the end of the first growing season (3 October, 135 dae). In 1994 the seedlings were transplanted before budburst into 15 dm^3 pots. The second growing season started on 1 April 1994 which is shown, for convenience, consecutively on the figures as 136 dae. The seedlings (nine for each water, [CO_2] treatment) were kept at pot water capacity for the first eleven weeks until 212 dae. Then the seedlings which had already experienced water stress in the first growing season were water-stressed again until 251 dae, while the remaining seedlings continued to be grown in well-watered conditions (pot capacity). In order to prevent a rapid decrease in water availability, the water-stressed seedlings were watered with 2 dm^3 of tap water after 10, 20 and 30 days, from the beginning of the drying cycle.

After the baseline harvest (when six plants for each [CO_2] treatment were harvested), six other harvests were made to determine growth during the 1993 growing season: on 60 and 69 dae (six seedlings for each [CO_2] treatment), on 80, 90, 103, and 115 dae (three for each water and [CO_2] treatment). There were two harvests during the second growing season: at the beginning of the water stress cycle, 212 dae (three seedlings for each water and [CO_2] treatment), and at the end of the drying cycle, 251 dae (final harvest - six seedlings for each water and [CO_2] treatment). To measure dry mass (DM) production, each plant was divided into leaf, main stem, side stems, and roots, which were separated from soil by washing and sieving carefully to minimise the loss of fine roots. Plant component parts were then oven dried for 48 h at 70 °C and weighed, using an electronic balance (Sauter, model RE1E14, Fisons Scientific Equipment, Loughborough). Leaf area (LA) was measured using a leaf area meter (LI 3100, LI-COR Inc., Lincoln, NB, USA).
Non-destructive growth measurements (height, leader extension, number of branches, basal diameter) of all seedlings in each chamber (ranging from 12 to 30 seedlings for each water and [CO_2] treatment in 1993, and from six to nine seedlings for each water and [CO_2] treatment in 1994) were followed throughout the growing seasons. The basal diameter (d), measured at the plant collar, was used to calculate

basal sapwood area as follows: $((d/2)^2 \cdot \pi)$, where $\pi = 3.14$.

Plant water use efficiency (the ratio of dry mass produced to total amount of water taken up in the same period of growth) was estimated in water-stressed plants in the first growing season and on well-watered and water-stressed plants in the second growing season. At the onset of the first drying cycle (69 dae), the water-stressed seedlings were first irrigated to pot water capacity and after the excess water had been drained the seedlings were weighed to 1 g on a digital balance (model QS32A, Sartorius Instrumentation Ltd, Germany). On each following harvest date, the droughted pots and seedlings were weighed before being harvested. Mean relative growth rates (R) of total dry mass was calculated between the harvest made on 80, 90, 103 and 115 dae and the harvests made on 69 dae in each [CO_2] treatment (details of R are given in Chapter 7). R was used to calculate the total dry mass that seedlings harvested on 80, 90, 103 and 115 dae, had had on 69 dae in each [CO_2] treatment, as follows:

$$M_{69}^{c} = e^{\ln (M_t^c) - R_t^c (t - 69)}$$

where M_{69} is the total dry mass of each seedling on 69 dae, c is the [CO_2] treatment, e is the base of Napierian logarithms (2.718), M_t is the total dry mass of each seedling harvested on t dae (i.e. 80, 90, 103 and 115), R_t is the mean relative growth rate during the time interval of day 69 to t (Table 5.1). Thus, the dry mass increase (C_t^c) of each seedling in each [CO_2] treatment and at each harvest date was then calculated as

$$C_t^c = M_t^c - M_{69}^c$$

and the total water uptake (U_t^c) of each seedling in each [CO_2] treatment and at each harvest date was calculated as

$$U_t^c = P_t^c - (P_{69}^c + C_t^c)$$

where P_t is the pot weight of each seedling harvested on t dae (i.e. 80, 90, 103 and 115), P_{69} is the pot weight that the same seedlings had on 69 dae. Plant water use efficiency ($g_{DM}\ kg_{H_2O}^{-1}$) was then estimated as the ratio of C_t^c to U_t^c. In 1994, during the whole second drying cycle (212 - 251 dae), all the well-watered seedlings (six

per [CO_2] treatment) were irrigated in excess with a known volume of water every other day (to ensure pot water capacity) and the drained water measured. Also the water-stressed seedlings (six per [CO_2] treatment) were irrigated in excess with a known volume of water on 212 dae, and then the amount of water which drained was measured. At the end of the drought period (just before the last harvest, 251 dae), the water-stressed seedlings were rewatered in excess with a known volume of water (to ensure pot capacity) and the drained water measured. R of total dry mass, calculated between 212 - 251 dae in each water regime (w) per [CO_2] treatment (Table 5.1), was used to calculate the total dry mass that the seedlings harvested on 251 dae had had on 212 dae in each [CO_2] treatment, as follows:

$$M^{c}_{w212} = e^{\ln (M^{c}_{w251}) - R^{c}_{w} (251 - 212)}$$

where M^{c}_{w212} and M^{c}_{w251} are the total dry mass of each seedling in each water regime per [CO_2] treatment on 212 and 251 dae, respectively. Consequently, plant biomass increase over the period 212 to 251 dae (C^{c}_{w}) was calculated as M^{c}_{w251} - M^{c}_{w212}. Thus, the water uptake by each seedling in each water regime per [CO_2] treatment (U^{c}_{w}) was calculated by subtracting the water drained (D^{c}_{w}) and plant biomass increase from the water supplied (S^{c}_{w}) as follows:

$$U^{c}_{w} = S^{c}_{w} - (D^{c}_{w} + C^{c}_{w})$$

and plant WUE was then estimated as the ratio of C^{c}_{w} to U^{c}_{w}.

Table 5.1. Mean relative growth rate (d^{-1}) of total dry mass of cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, used to calculate M^{c}_{69} and M^{c}_{w212}. Data are means of 3 to 6 plants per treatment; dae = days after emergence.

	well-watered			water-stressed		
dae	elevated	ambient	outside	elevated	ambient	outside
69-80	-----	-----	- ----	0.0649	0.0543	0.0417
69-90	-----	-----	- ----	0.0417	0.0365	0.0412
69-103	-----	-----	- ----	0.0308	0.0333	0.0378
69-115	-----	-----	- ----	0.0255	0.0267	0.0302
212-129	0.0150	0.0150	0.0213	0.0109	0.0114	0.0171

Data were tested using a factorial ANOVA (four-way maximum interactions) to determine the main effects of [CO_2] treatment, water treatment, time, and chamber on all dependent variables. Where appropriate, the treatment means were compared using Duncan's multiple range test.

5. 3 Results

No significant inter-chamber effect on any of the growth parameters measured on the cherry seedlings was found in each [CO_2] treatment, and, thus, the interactions chamber–day–[CO_2]– water regime are not shown.

Less than 30 days after germination, there was already a significant difference in height of the well-watered seedlings amongst the [CO_2] treatments (Figure 5.1a). Leader extension of both well-watered and water-stressed elevated [CO_2] seedlings showed consistently positive responses throughout the first growing season, whereas no significant differences were found between the elevated and ambient [CO_2] seedlings throughout the second growing season. Water regime significantly affected the height of the cherry seedlings in each of the [CO_2] treatments in the first, but not in second growing season (Table 5.2).

The [CO_2] treatments significantly affected the number of branches produced by the well-watered seedlings, but not those produced by the droughted plants (Appendix 4, Figure 1). There was no effect of water stress in any [CO_2] treatment (Table 5.2). The seedlings grown in elevated [CO_2] had significantly larger basal area throughout the duration of the experiment in both water treatments (Appendix 4, Table 1; Figure 5.2). Water stress had a dramatic effect in each [CO_2] treatment, but the relative stimulation was larger in ambient than in elevated [CO_2] at the end of both growing seasons (Table 5.2). Figure 5.3 shows the relationship between number of branches (Figure 5.3a,b) and basal diameter (Figure 5.3c,d) and mean total dry mass produced. The linear relationships found between mean total dry mass and both number of branches and basal area of well-watered and water-

stressed seedlings (Figure 5.3), indicates that when plants were the same size there was no differences in number of branches or basal area.

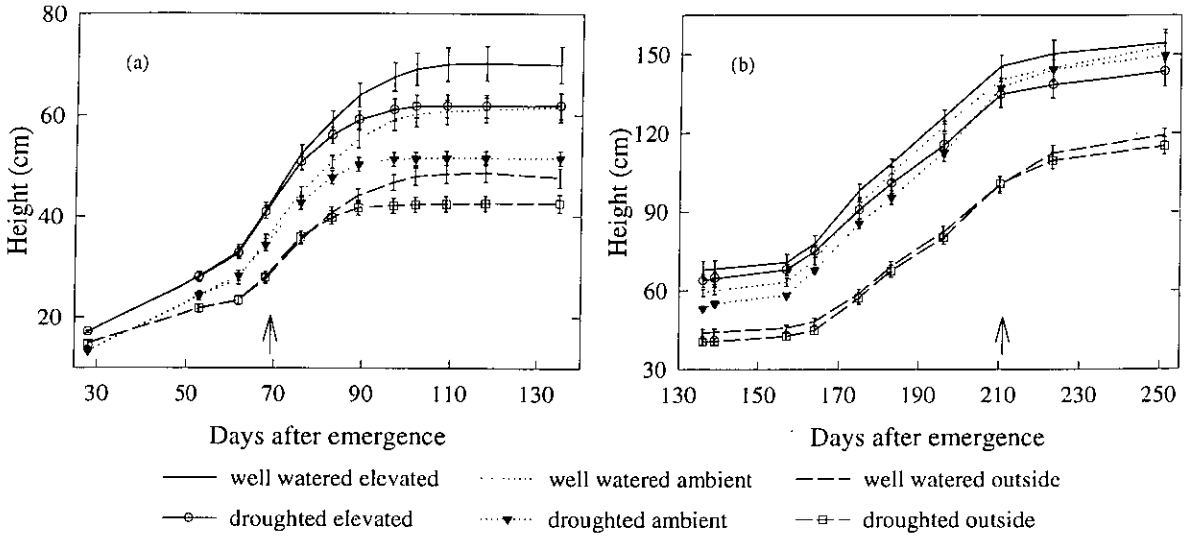

Figure 5.1. Height of the cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control shown as days after emergence, in the growing season a) 1993 (data are means of 12 to 30 plants per treatment ± 1 SEM) and b) 1994 (data are means of 6 to 12 plants per treatment ± 1 SEM). ↑ = onset of the water stress cycle; dae = days after emergence. The letters (a, b, c) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments.

	well-watered		water-stressed	
	1993	1994	1993	1994
[CO_2]	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	$P < 0.001$	ns	$P < 0.001$
dae	135	251	136	251
elevated	70.00 c	154.31 b	61.86 c	143.63 b
ambient	61.53 b	153.00 b	51.53 b	149.63 b
outside	47.71 a	119.31 a	42.60 a	115.25 a

[CO_2] treatments did not have any overall effect on number of leaves (Appendix 4, Table 1 and Figure 2). Also water stress did not have any significant effect, but by the end of the first growing season the relative stimulation in the number of leaves in the well-watered seedlings was large (Table 5.2). In contrast, leaf area was significantly affected by [CO_2] treatment (Appendix 4, Table 1), but was significantly larger in elevated [CO_2] than in ambient [CO_2] only in the first growing

Table 5.2. Percentage increase (calculated as: 100 [*W* - *D*] / *D*) of some growth characteristics of well-watered (*W*) cherry seedlings in response to water stress (*D*), measured at the end of the growing seasons 1993 and 1994. The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) show the difference in response to water stress.

	height		basal area		branch number		leaf number		leaf area	
treatment	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
elevated	14 *	7 ns	50 ***	46 ***	-36 ns	38 ns	37 ns	7 ns	45 **	8 ns
ambient	19 **	2 ns	93 ***	103 ***	-12 ns	12 ns	34 ns	2 ns	62 **	9 ns
outside	14 *	- 2 ns	73 ***	74 ***	-46 ns	18 ns	28 ns	6 ns	75 **	23 **

season (Figure 5.4). Water stress induced a significant reduction of leaf area in both [CO_2] treatments in the first growing season, but the differences between the two water regimes were drastically reduced 251 dae (Table 5.2). The relationship between the leaf area and basal area (a measure of the sapwood area) of the seedlings was affected by elevated [CO_2] in the water-stressed treatment, but not in the well-watered treatment (Appendix 4, Figure 3a).

Table 5.3. Percentage increase in total dry mass (calculated as in Table 5.2) of well-watered cherry seedlings in response to water stress (data are means of 3 to 6 plants per [CO_2] treatment). The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) show the difference in response to water stress; dae = days after emergence.

dae	elevated	ambient	outside
80	0.16 ns	5.54 ns	13.68 ns
90	10.51 ns	33.15 ns	12.02 ns
103	67.91 **	86.26 **	44.69 *
115	65.72 **	86.81 **	72.50 ***
212	22.08 ns	27.99 ns	24.69 ns
251	42.93 **	47.18 ***	46.41 **

The overall response of well-watered cherry seedlings in terms of total and component dry mass was significantly affected by [CO_2] treatments (Appendix 4, Table 1). With the exception of leaf dry mass (Figure 5.5a), the other component dry mass, i.e. wood dry mass (Figure 5.5b) and root dry mass (Figure 5.5c), were significantly increased in response to elevated [CO_2] at the end of the experiment. Consequently, both above-ground dry mass (Appendix 4, Figure 4a) and total dry

mass (Figure 5.5d) of well-watered seedlings were significantly larger in elevated [CO_2] than in ambient [CO_2] (Table 5.5). It is worth noting that, with the sole exception of root dry mass, significant differences in total and component dry mass between elevated [CO_2] and ambient [CO_2] were already evident after 33 days of growth. Also the stressed seedlings were larger in all respects, i.e. leaf dry mass (Figure 5.5e), wood dry mass (Figure 5.5f) and root dry mass (Figure 5.5g) and thus total dry mass (Figure 5.5h), in elevated [CO_2] than in ambient [CO_2] (Table 5.5).

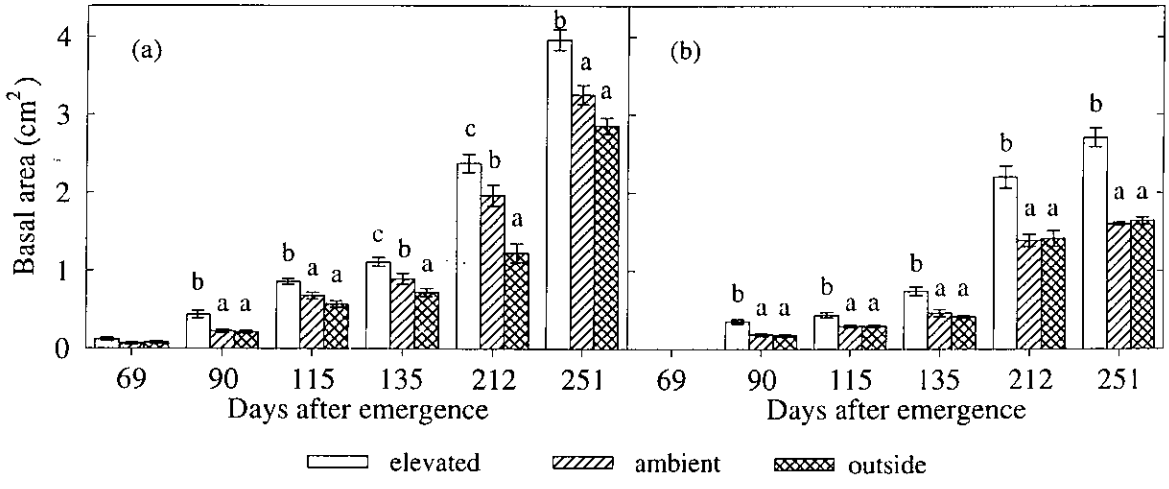

Figure 5.2. Basal area of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 6 to 30 plants per treatment ± 1 SEM. Letters (a, b, c) indicate significant differences at $P < 0.05$. Statistical significance:

	well-watered	water-stressed
[CO_2]	$P < 0.001$	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	$P < 0.001$

Table 5.3 shows that differences in total biomass between the water treatments were not significant until 103 dae, i.e. more than 30 days into water stress. At the end of the 1993 season, dry mass production in ambient [CO_2] was more affected by water stress, mainly because of depressed root growth of the water-stressed seedlings

(Table 5.4). In the second growing season, the differences in total dry mass between watered and the water-stressed seedlings were not significant at the beginning of the drying cycle (212 dae). However, at the end of the water stress cycle dry mass of drought seedlings was significantly depressed to the same extent in all [CO_2] treatments. Leaf dry mass in elevated and ambient [CO_2] was the only component of dry mass which was not significantly affected by drought (Table 5.4).

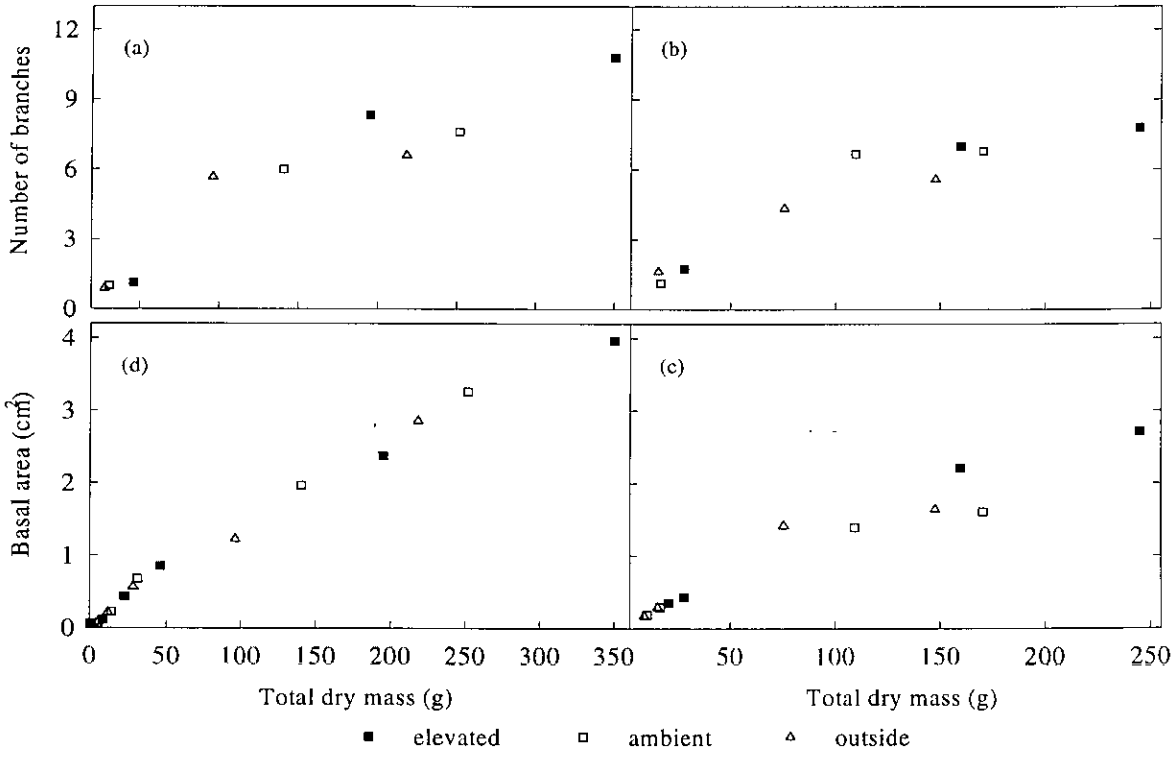

Figure 5.3. Linear relationships between the number of branches of (a) well-watered (R^2 = 0.889), and (b) water-stressed (R^2 = 0.877) plants, and all the basal area of (c) well-watered (R^2 = 0.988), and (d) water-stressed (R^2 = 0.942) plants, *versus* total dry mass of cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control in each water regime.

There was a similar pattern in growth responses, expressed as percentage increase induced by elevated [CO_2], in the well-watered and the water-stressed seedlings, particularly as plants grew larger (Figure 5.6). The percentage increase in leaf area

over time was remarkably similar over both growing seasons (Figure 5.6a), whereas the percentage increase in root dry mass became similar only in the second growing season (Figure 5.6b). However, the percentage increase in above-ground dry mass (Figure 5.6c) and total dry mass (Figure 5.6d) showed that growth in elevated CO_2 concentrations did not reduce the effects of water stress. In general, it is possible to see in Figure 5.6 that after an initial stimulation growth increase in response to elevated [CO_2] began declining. Growth decline in elevated [CO_2] is analysed in Chapter 7.

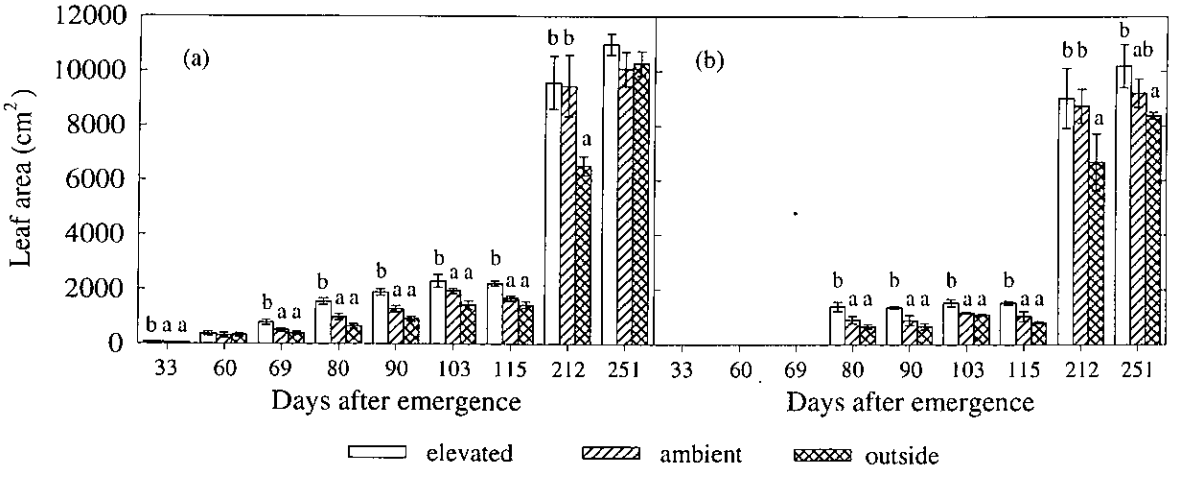

Figure 5.4. Leaf area of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 to 6 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$. Statistical significance:

	well-watered	water-stressed
[CO_2]	$P < 0.001$	$P < 0.05$
Time	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.05$	ns

The root to shoot mass ratio of well-watered seedlings was not significantly affected by elevated CO_2 throughout the duration of the experiment, with the exception of results 251 dae; when more dry mass was allocated to roots in elevated [CO_2] than ambient [CO_2] (Appendix 4, Figure 5a). Conversely, the overall allocation of dry

mass between root and shoot of the water-stressed plants was significantly affected by the [CO_2] treatments, although only on 80 and 251 dae was R/S ratio significantly higher in elevated [CO_2] than in ambient [CO_2] (Appendix 4, Figure 5b).

To compare dry mass allocation patterns amongst the [CO_2] treatments when plants were the same size, each component of dry mass was related to mean total dry mass produced in all [CO_2] treatments in both well-watered (Figure 5.7) and water-stressed seedlings (Figure 5.8). Linear relationships were found between mean total dry mass and combined leaf, wood, above-ground, and root dry mass in both water regimes, indicating that the proportion of dry mass allocated to the various organs was not affected by growth in different CO_2 concentrations.

Table 5.4. Percentage increase in component dry mass of well-watered cherry seedlings in response to water stress, measured at the end of the 1993 and 1994 water stress cycles. The significance levels (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) show the difference in response to water stress.

	leaf		wood		above-ground		root	
treatment	1993	1994	1993	1994	1993	1994	1993	1994
elevated	61 *	17 ns	82 *	46 ***	71 *	35 **	61 *	54 **
ambient	53 *	15 ns	97 ***	53 ***	71 **	37 ***	108 **	65 ***
outside	82 ***	26 *	78 **	47 **	81 ***	38 **	63 *	61 **

The total amount of water transpired by the water-stressed seedlings did not differ at each harvest date amongst the [CO_2] treatments (Figure 5.9a; Table 5.6). Similarly, water uptake by the well-watered seedlings was similar in elevated and ambient [CO_2] (Table 5.6). However, plant WUE was highly affected by elevated [CO_2] at each harvest date in both the water-stressed and well-watered seedlings (Figure 5.9a, Table 5.6). The relative increase in WUE ranged between 56 - 103% in the water-stressed plants during the first growing season and was similar for the seedlings grown in the two water treatments (about 47% in well-watered plants and about 52% in droughted plants) in the second growing season. It is noteworthy that WUE of the water-stressed seedlings was remarkably similar during both drying

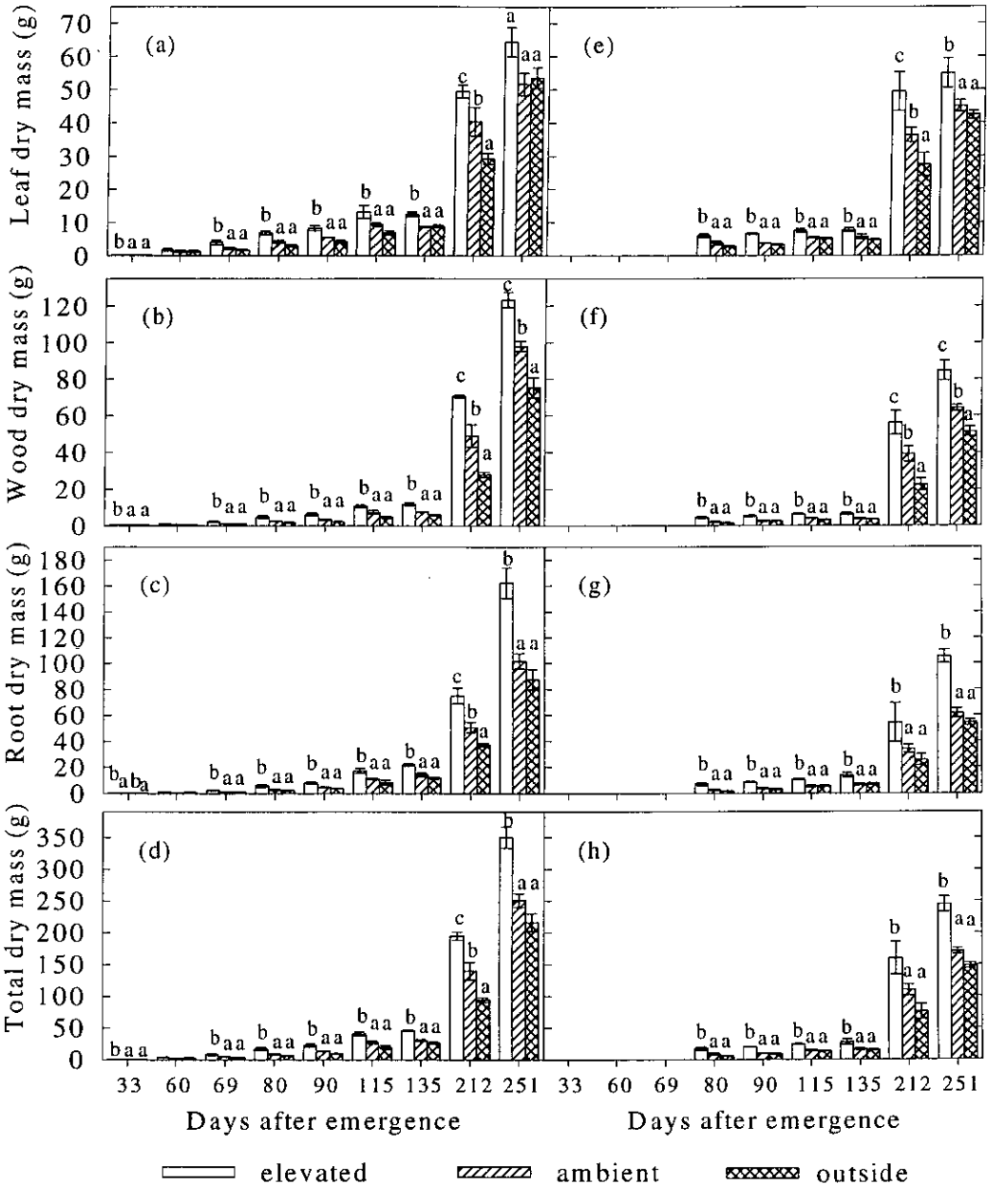

Figure 5.5. Leaf dry mass of (a,e), wood dry mass (b,f), root dry mass (c,g), and total dry mass (d,h) of well-watered (a-d) and water-stressed (e-h) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 to 6 plants per treatment ± 1 SEM). Letters (a, b, c) indicate significant differences at $P < 0.05$. The statistical significance is reported in Table 5.5.

Table 5.5. Statistics for total and component dry mass shown in Figure 5.5. Significance level of P (*** = $P < 0.001$, ns = not significant) from two-way ANOVA for the whole duration of the experiment; DM = dry mass.

	well-watered			
	leaf DM	wood DM	root DM	total DM
$[CO_2]$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	water-stressed			
	leaf DM	wood DM	root DM	total DM
$[CO_2]$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Interaction	ns	$P < 0.001$	$P < 0.001$	$P < 0.001$

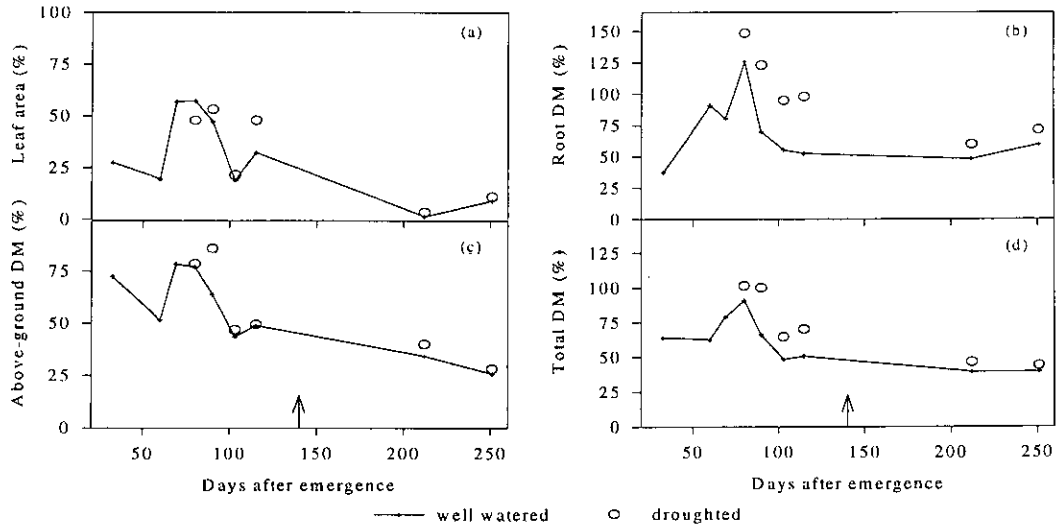

Figure 5.6. Percentage increase in a) leaf area, b) root dry mass, c) above-ground dry mass, and d) total dry mass of both well-watered and water-stressed seedlings in response to elevated $[CO_2]$. ↑ = onset of the second growing season (dae 136).

cycles. However, the relative increase attributable to elevated $[CO_2]$ was about 19% larger in WUE than in total plant dry mass at the end of the first drying cycle, whereas at the end of the second drying cycle it was about 8% larger in both water treatments.

Significant, lasting differences in growth between seedlings grown in ambient [CO_2] in the OTCs and in the outside control blocks were found only in height (Figure 5.1), since the significant differences seen in both well-watered and droughted seedlings in basal area (Figure 5.2a), number of leaves (Appendix 4, Figure 2), leaf area (Figure 5.4), and in total and component dry mass (Figure 5.5) were transient, and disappeared by the end of the experiment. Total water uptake was similar in ambient [CO_2] in the OTCs and in the outside control blocks in both water treatment (Figure 5.9a, Table 5.6). Also plant WUE of water-stressed seedlings was similar in the first growing season (Figure 5.9b), but was significantly reduced in the outside control seedlings in both water treatments during the second growing season (Table 5.6).

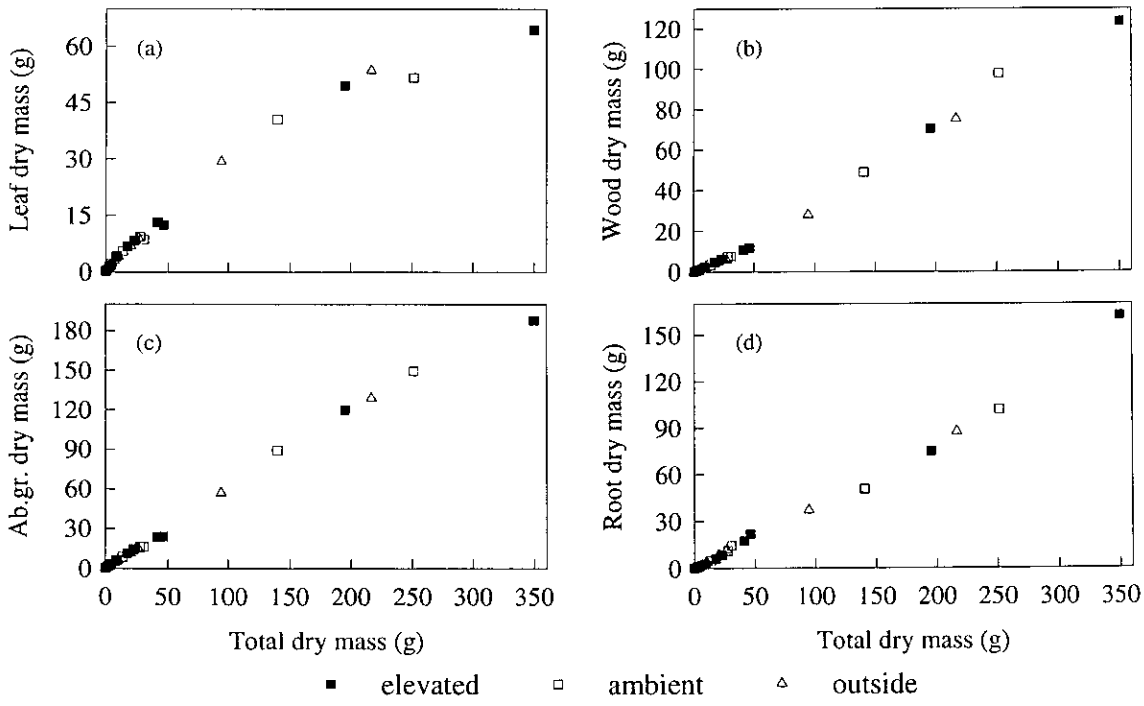

Figure 5.7. Linear relationships between combined mean (a) leaf dry mass (R^2 = 0.962), (b) wood dry mass (R^2 = 0.995), (c) above ground (Ab.gr.) dry mass (R^2 = 0.995), and (d) root dry mass (R^2 = 0.991) and mean total dry mass of well-watered cherry seedlings grown in elevated [CO_2], ambient [CO_2], or outside control.

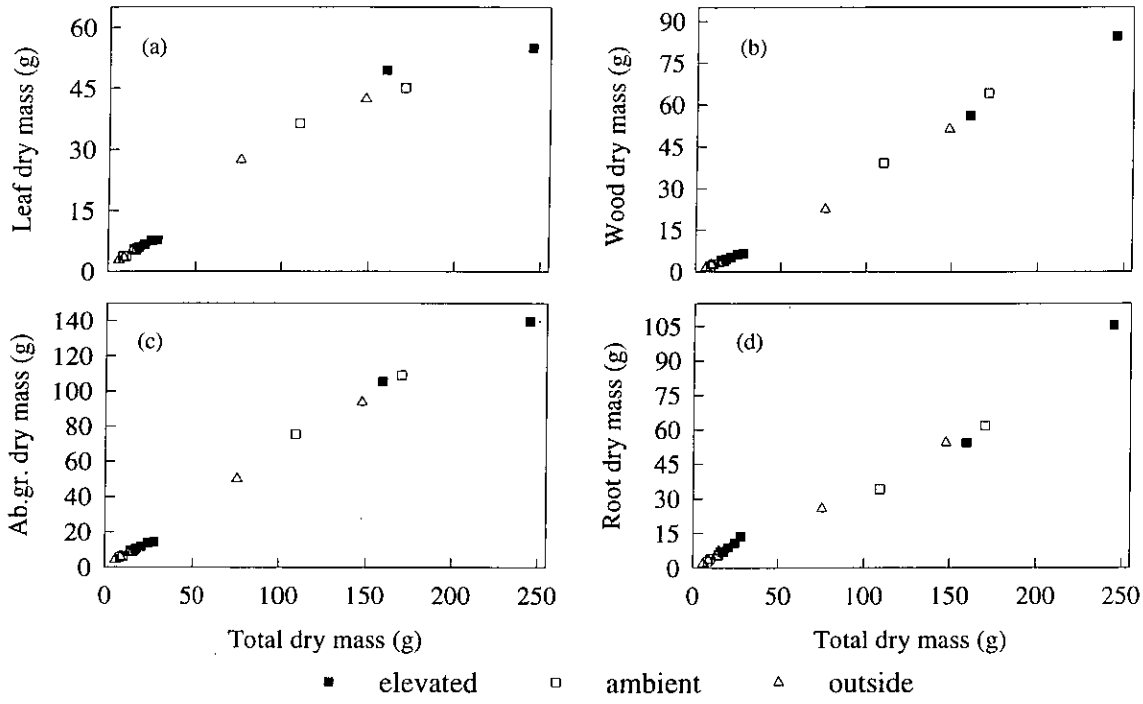

Figure 5.8. Linear relationships between combined mean (a) leaf dry mass (R^2 = 0.963), (b) wood dry mass (R^2 = 0.997), (c) above ground (Ab.gr.) dry mass (R^2 = 0.992), and (d) root dry mass (R^2 = 0.979) and mean total dry mass of water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control.

5. 4 Discussion

Elevated [CO_2] significantly increased total dry mass production in both water regimes (Figure 5.5d,h). Since water uptake did not differ in either well-watered or water-stressed seedlings between elevated and ambient [CO_2], the growth increase brought about in elevated [CO_2] led to significantly higher WUE (Figure 5.9, Table 5.6). However, the interaction between elevated [CO_2] and water stress was not significant (Appendix 4, Table 1), and thus elevated [CO_2] did not ameliorate the growth response of cherry seedlings subjected to two sequential drying cycles (Figure 5.6).

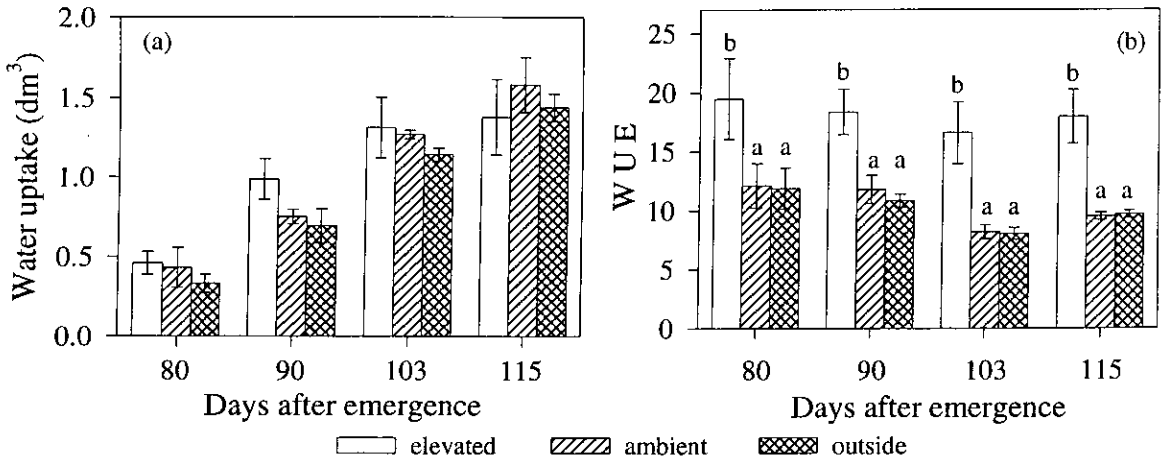

Figure 5.9. Total water uptake (a) and plant WUE (b) of water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, during the 1993 water stress cycle. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$. Statistical significance:

	Water uptake	WUE
[CO_2]	ns	$P < 0.001$
Time	$P < 0.001$	ns
Interaction	ns	ns

Table 5.6. Total water uptake and plant WUE of well-watered and water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, during the 1994 water stress cycle. Data are means of 6 plants per treatment ± 1 SEM; letters (a, b, c) indicate significant differences at $P < 0.05$ in the same column.

	water uptake		water use efficency	
treatment	well watered	water stressed	well watered	water stressed
elevated	16.97 ± 0.46 a	8.45 ± 0.16 a	11.48 ± 0.44 c	18.88 ± 0.89 c
ambient	17.99 ± 0.54 ab	8.88 ± 0.21 ab	7.79 ± 0.22 b	12.40 ± 0.62 b
outside	18.69 ± 0.54 b	9.24 ± 0.16 b	5.04 ± 0.28 a	8.21 ± 0.33 a
Statistical significance:				
[CO_2]	$P < 0.05$		$P < 0.001$	
water treatment	$P < 0.001$		$P < 0.001$	
[CO_2] x water	ns		$P < 0.01$	

Long-term growth in elevated [CO_2] increased total dry mass of well-watered

seedlings of about 39%. This growth response is well within the ranges reported in the literature for tree biomass (Eamus & Jarvis, 1989; Jarvis, 1989; Ceulemans & Mousseau, 1994; Lee & Jarvis, 1996). Wilkins *et al.* (1994) also studied the growth response of *Prunus avium* to elevated [CO_2]. They grew two-year-old seedlings and clonal rooted cuttings taken from mature trees, for two years with high or low nutrient supply. At the end of the second season of treatment there were no differences in growth response between the plants in elevated and ambient [CO_2] in the low nutrient supply. Whereas, in the high nutrient supply total dry mass increase in response to elevated [CO_2] was about 81% and 57% for seedlings and clones, respectively. However, both seedlings and clones were pruned at the beginning of the second growing season (half of the previous season's growth was removed), and thus their results were obtained in totally different conditions with respect to our study. A lower increase (24%) in dry mass production of one-year-old cherry seedlings grown in solardromes was found by Kerstiens & Hawes (1994) in an experiment set-up to evaluate to what extent root restriction could reduce growth response to elevated CO_2 concentrations.

The differences in wood dry mass (Figure 5.5b,f) between elevated and ambient [CO_2] were not accompanied by an increase in height (Figure 5.1) at the end of the second growing season. Also water stress did not decrease height-growth during the second drying cycle in any [CO_2] treatment (Table 5.2). However, this lack of effect may have been caused by the late occurrence of the drying cycle, which started when the seasonal height-growth was already slowing down. The absence of a lasting stimulation of elevated [CO_2] on height has been found several times. Leader extension of four-year-old Sitka spruce saplings was no different in elevated and ambient [CO_2] in the last year of growth (see Chapter 3). Height of *Pinus radiata* seedlings was affected by water stress, but not by elevated [CO_2], over a period of 22 weeks (Conroy *et al.*, 1986a). Height-growth of birch seedlings grown in pots in ambient [CO_2] exceeded that of the elevated [CO_2]-grown seedlings during the second year of growth in elevated [CO_2] (Lee *et al.*, 1993). Tree height of four-year-old birch seedlings rooted in the ground did not differ in elevated and ambient [CO_2] (Rey, 1997). Mousseau & Enoch (1989) also found an unusually early

cessation of stem elongation in *Castanea sativa* seedlings grown in elevated [CO_2].

The significant, transient differences in growth in ambient [CO_2] (i.e. advanced leaf development of the trees in the chambers) seedlings in the OTCs and in the outside control blocks could be a consequence of slower phenological development of the outside seedlings (i.e. a temperature effect).

The hypothesis that increased leaf area may offset the higher transpiration efficiency per unit of leaf surface, leading to an increased total water uptake in elevated [CO_2] is in conflict with the results. The area of individual leaves of the cherry seedlings was larger in elevated [CO_2] in the first growing season and the number of leaves did not differ among the [CO_2] treatments (Appendix 4, Figure 2), so that the total leaf area was larger. Yet, the total amount of water uptake was similar in the elevated and ambient [CO_2] droughted seedlings in 1993 (Figure 5.9). Consequently, daily evapotranspiration rate per unit of leaf area, the ratio of total daily water loss to leaf area, was reduced in elevated [CO_2]. Also in *Pinus pinaster* the total amount of water uptake was not affected by elevated [CO_2] in both well-watered and water stress conditions (Guehl *et al.*, 1994). Morison & Gifford (1984a,b) found that elevated [CO_2] increased leaf area in 14 out of 16 crop species by an average of 40%. In their experiments, the daily water loss by elevated [CO_2] plants had a similar time course to that in ambient [CO_2], indicating that the effect of increased leaf area in elevated [CO_2] nullified the effect of reduction of stomatal conductance on daily transpiration rate. Similar results were found in seedlings of *Acacia smallii* (Polley *et al.*, 1997), in *Lolium perenne* swards (Schapendonk *et al.*, 1997), and droughted plants of *Glycine max* (Prior *et al.*, 1991) grown in elevated [CO_2].

However, cherry leaf area was not affected by elevated [CO_2] in the second growing season (Figure 5.4), but still the total amount of water uptake was similar in elevated and ambient [CO_2] in both water treatments (Table 5.6). Therefore, total daily water loss and daily evapotranspiration rate per unit of leaf area of both well-watered and droughted plants were similar in elevated and ambient [CO_2]. Similarly, leaf area

and total water consumption of water-stressed seedlings of *Quercus petraea* were not affected by elevated [CO_2], whereas in well-watered conditions both leaf area and water uptake were increased by about 34% and 38%, respectively (Guehl *et al.*, 1994). In a recent paper Heath & Kerstiens (1997) observed that *Fagus sylvatica* seedlings grown with high nutrient supply in elevated [CO_2] had larger rates of soil water depletion, which resulted from both stimulation of leaf area development and lack of effect on stomatal conductance.

Water use efficiency of droughted seedlings in elevated [CO_2] was very similar in both the drying cycles. However, increase in WUE was affected differently by the increased total dry mass production and by the amount of evapotranspiration per unit leaf area. Droughted plants in elevated [CO_2] had both larger total dry mass and reduced evapotranspiration rate per unit of leaf area at the end of the first drying cycle, thus both factors contributed to increase WUE. Similarly, Norby & O'Neill (1989) in *Quercus alba*, and Morison & Gifford (1984b) in 16 crop species, observed that in elevated [CO_2] the increase in WUE exceeded the decrease in transpiration rate, indicating that the increase in dry mass production also contributed to enhance WUE.

It has frequently been reported that elevated [CO_2] may increase the root to shoot ratio (Tolley & Strain, 1984a; Norby & O'Neill, 1991; El Kohen *et al.*, 1992; Guehl *et al.*, 1994: El Kohen & Mousseau, 1994). Growth is extremely sensitive to water stress and hence is strongly influenced by the ability with which roots grow in drying soil and maintain an optimal water status (Tyree & Alexander, 1993). Bottomley *et al.* (1993) observed, by using proton nuclear magnetic resonance imaging, that 24-day-old seedlings of *Vicia faba* grown in elevated [CO_2] showed significantly increased hydration of upper roots and below ground stem after a drying cycle, whereas the ambient [CO_2] plants were water depleted from the entire root system. Any shift in biomass allocation driven by elevated [CO_2] may thus influence plant growth in droughted conditions. Carbon allocation of cherry seedlings between root and shoot was significantly affected in droughted conditions by elevated [CO_2] (Appendix 4, Figure 5). However, this was neither a consequence

of unbalanced nutrition since the seedlings were supplied with free access to nutrients, nor the occurrence of constrained rooting, since large pot volumes were used. It seems to be more a consequence of faster growth in elevated [CO_2] and thus ontogeny, since biomass allocation was similar when the seedlings were the same size in either the water-stressed (Figure 5.8) or well-watered (Figure 5.7) treatments. Similarly, the linear relationship between combined basal area and total seedling dry mass is additional evidence of faster growth and consequently a speeding-up of development in elevated [CO_2] (Figure 5.3). By plotting root dry mass *versus* shoot dry mass Kerstiens & Hawes (1994) showed that allocation was not affected by elevated [CO_2] when their cherry seedlings were the same size. Seedlings of *Pinus radiata* also showed a more advanced stage of development brought about by elevated [CO_2] (Conroy *et al.*, 1986a). Morison & Gifford (1984b) also found that annual plant matured more rapidly in elevated [CO_2]. Similar results were found in *Ipomoea batatas* (Bhattacharya *et al.*, 1985). Similarly, there were no significant effects on the percentage of dry mass allocated to the roots in response to elevated [CO_2] in clones of Sitka spruce grown under droughted conditions (Townend, 1993).

The hypothesis that plants growing in elevated [CO_2], which reach a larger size (both above and below the ground) when the evaporative demand is increasing while soil water availability is decreasing, may consume water faster was not demonstrated. Water uptake did not differ between the elevated and ambient [CO_2]-grown plants, and the increased water use efficiency of droughted seedlings in elevated [CO_2] did not result from a less severe than average influence of water stress on cherry plant dry mass production in long-term growth (Figure 5.6). This finding leads to two main considerations. Firstly, the main cultivation area of cherry in Europe is the Mediterranean basin which is characterised by summer droughts which heavily influence tree growth and productivity. With the future scenario of global change, with higher temperatures and potentially higher rates of evapotranspiration, cherry plants may undergo major drawbacks in regions which will experience an increased frequency and intensity of drought. Secondly, small changes in water stress tolerance may lead to growth decline in the long-run. In

order to assess whether elevated [CO_2] alleviates growth responses of trees to water stress, interactive studies need to be prolonged for several growth seasons, in order to take into account plant acclimation and the differential decline of growth which occurs in elevated and ambient [CO_2] (Figure 5.6).

CHAPTER 6

The Effects of Elevated [CO_2] and Water Stress on the Physiology of Cherry (*Prunus avium*) Seedlings

6.1 Introduction

Water stress is a major factor limiting plant productivity over large areas of the globe, where it affects growth of both agricultural and forestry species and also influencing vegetation distribution and composition. Because of the steady increase in greenhouse gases leading to the scenario of future higher temperatures and evaporative demand, drought occurrences will be increased in frequency, intensity, and erratic patterns, and will possibly affect regions that are not currently hit by drought. This raises important issues as to how growth in elevated [CO_2] is related to evaporative demand, water supply and water stress, and whether increased growth in elevated [CO_2] could be offset by the adverse influence of water stress on the soil-plant-atmosphere-continuum.

Instantaneous WUE_I may be affected directly in response to elevated [CO_2] or indirectly through interacting feedback pathways with leaf growth, leaf gas exchange, Rubisco and temperature (Jarvis, 1993). Elevated CO_2 concentration frequently leads to a decline in g_s of C_3 plants (approximately 30-40%; see reviews by Eamus & Jarvis, 1989; Jarvis, 1989; Mott, 1990; Drake *et al.*, 1997). Mott (1990) pointed out that stomata respond to intercellular CO_2 concentration (C_i) to adjust the diffusive limitations to the biochemical limitations on A, so improving WUE_I. Thus, with all else equal, decreased stomatal conductance is associated with elevated C_i. The apparent reduction in stomatal density since the beginning of the industrial revolution has been interpreted as an ecological evolution which may compensate for the increasing [CO_2] (Woodward, 1987; Körner, 1988; Beerling & Chaloner, 1993; Paoletti & Gellini, 1993). However, conflicting results have been found. For example, Bunce (1992) with *Malus domestica*, *Quercus prinus*, and *Q. robur* and

Overdieck & Forstreuter (1994) with *Fagus sylvatica* failed to find a decrease in g_s in response to elevated [CO_2]. Seedlings of *F. sylvatica* grown in elevated [CO_2] had faster rates of soil drying, resulting from a combination of higher g_s with no change in leaf area at low nutrient supply, and no change in g_s with increased leaf area at high nutrient supply (Heath & Kerstiens, 1997). Transpiration rate per unit of leaf area was also not affected in a different experiment on *F. sylvatica* using the branch bag technique (Dufrêne *et al.*, 1993). However, evapotranspiration on a ground area basis was reduced and leaf area index increased in a mini-stand of *F. sylvatica* grown in elevated [CO_2] (Overdieck & Forstreuter, 1994).

Hence, by reducing g_s, and thus reducing E and increasing A, elevated [CO_2] causes increased instantaneous transpiration efficiency (ITE) per unit of leaf area (Morison, 1985; Jarvis, 1989; Tyree & Alexander, 1993). Theoretically, improved ITE in drought-prone habitats should be relatively more beneficial to plant growth than in well-watered environments, because with the same amount of water available more biomass can be produced. However, the problem lies in scaling up ITE to whole trees, and then to forests over longer periods, to provide reliable estimates of long-term water use efficiency (WUE, dry mass produced/water consumed).

The ability of plants to function in droughted conditions involves a range of physiological mechanisms which affect plant water relations. Stomatal closure reducing the amount of water loss through transpiration is a mechanism of drought avoidance (Bhattacharya *et al.*, 1990). Hence, elevated [CO_2], by causing a decline in g_s, may reduce transpiration, leading to increases in plant water potential and a delayed onset of water stress. Moreover, it is reasonable to suppose that less negative plant water potentials in drying soils, enable plants to remain turgid and functional for a longer period. For instance, elevated [CO_2] may directly affect leaf expansion by increasing turgor (Morison, 1993). Thus, whether elevated [CO_2] ameliorates water stress depends on its interactions with plant water relations, g_s, and the biophysical and biochemical processes affecting photosynthesis that increase resistance to drought (Tyree & Alexander, 1993). The present study focused on the long-term interactions between elevated [CO_2] and water stress, and their effects on

leaf gas exchange, water relations, carbon and nitrogen concentrations, and the relationship between plant WUE and leaf ITE, in cherry seedlings.

6.2 Materials and Methods

Cherry seedlings (*Prunus avium* L.) were grown from seed for two growing seasons in three ambient [CO_2] OTCs (~350 μmol mol^{-1}), three elevated [CO_2] OTCs (ambient + ~350 μmol mol^{-1}), and in three outside blocks, all located inside a glasshouse at the University of Edinburgh. The seedlings were regularly fertilised in both growing seasons following Ingestad principles (Ingestad & Ågren, 1992, 1995). During the first growing season, the cherry seedlings were grown in 40 cm-long soil columns (6.6 dm^3), contained in black polythene tubes 45 cm long and 14.5 cm in diameter. The first growing season ended on 135 dae; the beginning of the second growing season is indicated consecutively as 136 dae. In the second growing season, the seedlings were transplanted before budburst into 15 dm^3 pots. Two water treatments were imposed on the seedlings: well-watered (seedlings irrigated every other day to pot water capacity throughout both growing seasons) and droughted. A drying cycle was imposed in both the growing seasons to half of the seedlings per OTC and outside block: from 69 to 115 dae in the first growing season, and in the second growing season from 212 to 251 dae on the same seedlings which had already experienced water stress. Until 69 dae, and from 115 to 212 dae, the droughted seedlings were watered every other day to pot water capacity. Full details of the growth conditions, number of harvests made, and of the statistical analyses used to test the data are given in Chapters 2 and 5.

Gas exchange measurements were made inside the glasshouse at the University of Edinburgh, on the central section of a newly-expanded leaf using a portable gas exchange system (ADC-LCA-3, Analytical Development Co. Ltd., Hoddesdon, Herts, UK) equipped with a Parkinson leaf chamber (narrow PLC-2). To enable measurements of PPFD-saturated photosynthetic rates, illumination of the leaf cuvette by natural sunlight was supplemented with artificial light (provided by a

white fluorescent lamp) to maintain a PPFD at the leaf surface of > 1700 µmol $m^{-2}s^{-1}$. Leaf CO_2 assimilation rates (A), stomatal conductance (g_s), and instantaneous transpiration efficiency (ITE), calculated as the rate of CO_2 assimilation per unit of water transpired, were measured between 11.00 and 14.00 h on three plants per [CO_2]-water treatment, on 69, 80, 90, 103, 115, 123 and 133 dae in the first growing season, and on 212, 235, and 251 dae in the second growing season. Gas exchange measurements were made at the growth CO_2 concentration and at the opposite CO_2 concentration (i.e. those seedlings growing in 700 µmol mol^{-1} [CO_2] were first measured at that concentration, and after exposure for at least an hour of the whole plants to ambient [CO_2] gas exchange was re-measured on the same leaf at this concentration, and *vice versa* with respect to seedlings growing in ambient [CO_2] both within and outside the OTCs). A daily course of gas exchange was measured on 103 dae, at 8:30, 13:30, 17:00, and 19:00 h, on three seedlings per [CO_2]-water treatment at the growth CO_2 concentration.

Short-term measurements (~10 minutes) of PPFD-saturated CO_2 assimilation rate in relation to leaf internal CO_2 concentration (A/C_i) were made between 10.00 and 17.00 h over a range of cuvette CO_2 concentrations between 40 and 1200 µmol mol^{-1}, on three to four seedlings per [CO_2]-water treatment. Measurements of the A/C_i response curves were made between 80-103 dae in the first growing season, and between 235-251 dae in the second growing season on the well-watered seedlings, whereas on droughted seedlings the measurements were made in the first growing season between 85-87 dae. The initial slope of the A/C_i curves is an estimate of the carboxylation efficiency (RuBP-saturated rate of Rubisco), whereas the maximum rate of assimilation (A_{MAX}) (the net CO_2 assimilation rate under conditions of PPFD and CO_2 saturation) is indicative of the role of RuBP regeneration and P_i limitation.

Stomatal density (mm^{-2}) was determined after the first drying cycle by making impressions of the abaxial surface of the leaves (Weyers & Johansen, 1985; Weyers & Meidner, 1990). Negative replicas of seven mature leaves per plant (six plants per [CO_2]-water treatment) were made by applying a silicon rubber matrix (Xantropren,

Bayer AG, Leverkusen, Germany) to the abaxial surface of leaves. To make positive impressions, the negative replicas were imprinted on microscope slides coated with clear finger-nail varnish. Three fields per positive imprint were randomly selected under a light microscope (Ortholux, Leitz Ltd., Luton, Beds) with the camera attachment at x 250 magnification, and photographed. Then, the stomatal density was calculated as the number of stomata per mm^2 of leaf area.

Gravimetric soil water content at pot water capacity was determined on a group of five soil columns. These were watered and sealed at the top to prevent loss of water by evaporation. After the excess water had drained for 24 h, the five soil columns were divided into four horizontal layers of 10 cm each (volume of ~1650 cm^3). Then, soil water content, as the volumetric fraction of each layer, was calculated by multiplying the mass fraction (percentage of oven-dry mass determined at 105°C for 48 h) by the soil bulk density (the ratio between oven-dry mass to sample volume) (Beadle *et al.*, 1993). Soil cores of 2 cm diameter were collected from the mid-point of each 10 cm soil column layer, soon after the gas exchange measurements on the same seedlings. The volumetric water content of each soil layer was estimated by multiplying its soil bulk density by the moisture mass fraction of the soil core collected from the same soil layer. Then, mean volumetric water content of each soil column was obtained as the averaged over the four layers.

Immediately after the gas exchange measurements, one newly expanded leaf per plant (three plants per [CO_2]-water treatment) was detached to determine bulk leaf water potential (Ψ), using a pressure chamber (SKPM 1400, Skye Instruments, Llandrindod Wells, UK). A daily course of Ψ was measured on 103 dae, at 4:30 (pre-dawn Ψ), 8:30, 13:30, 17:00, and 19:30 h, on three seedlings per [CO_2]-water treatment. Leaf osmotic potential (Ψ_s) was measured on the same leaves used for determining Ψ. Following removal from the pressure chamber, each leaf was rapidly placed in a 1 cm^3 plastic syringe and plunged into liquid nitrogen and stored in a freezer at -25 °C. Subsequently, the material collected was allowed to thaw, and the leaf inside the syringe was squeezed to force sap from the tissue. In this way, 8 mm^3

of exuded sap were collected for measuring the osmotic potential using a vapour pressure osmometer (5100C, Wescor Inc., Logan, USA).

Tissue water relations parameters of cherry leaves were derived from pressure-volume analysis (Tyree & Hammel, 1972). A newly-expanded leaf was detached from three plants per [CO_2]-water treatment around the end of the first drying cycle: in the late evening of 114-115 dae (droughted seedlings) and 116-117 dae (well-watered seedlings). Each leaf was recut under water, covered with a plastic bag, and allowed to rehydrate with the cut-end under water in a dark cold room at 5 °C for 12 h prior to measurement of pressure-volume curves. Immediately after rehydration, each leaf was weighed to determine the saturated mass (S_M). The pressure-volume curves were made employing the method described by Wilson *et al.* (1979). The hydrated leaf was placed in a Scholander pressure bomb, and increasing pressure was applied. The water exuded by the cut end was weighed to determine the leaf fresh mass (F_M) at the balancing pressure. At the end of the analyses each leaf was oven-dried at 80 °C for 48 hours to determine dry mass (D_M). The relative water content (R^*) was then calculated as follows:

$$R^* = (F_M - D_M)/(S_M - D_M)$$

The data obtained were used to calculate the osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_0), bulk modulus of elasticity of the cell (ε_B), and leaf dry mass to leaf turgid mass ratio (DM/TM ratio).

Samples for biochemical analysis (Rubisco activity and chlorophyll concentration) were taken simultaneously with the gas exchange measurements. A leaf disc (0.8 cm^2) was taken from the central section of three newly-expanded leaves on each plant (three plants per [CO_2]-water treatment) for Rubisco activity assays on 69, 80, 103, 115, 212, and 251 dae. The leaf discs were rapidly plunged into liquid nitrogen. Rubisco activity was analysed *in vitro* by Dr. R. Besford at Horticulture Research International Institute, Littlehampton (UK); 'final' Rubisco activity was assayed spectrophotometrically by a coupled enzyme method (determining 3PGA

phosphokinase activity and NADP-G3P dehydrogenase) after pre-incubation at 20 °C in extraction medium containing 25 mM $MgCl_2$ (Besford, 1984, Van Oosten *et al.*, 1995). Similarly, a leaf disk was taken from each of three leaves of the same age per seedling (three plants per [CO_2]-water treatment) to measure the chlorophyll *a*, *b*, and *a+b* concentrations on 69, 80, 90, 103, 115, 212, and 251 dae. The method for extraction and measurement of chlorophyll (Porra *et al.*, 1989) has been described in Chapter 2. Chlorophyll concentration was also measured *in situ* in well-watered seedlings on 251 dae using a hand-held chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd, Osaka, Japan), which measures the leaf transmittance at two wavelengths of ~430 nm and ~750 nm. The measurements were made on the same leaf section which was later used for biochemical assay of chlorophyll concentration and for determination of macro-nutrient concentrations. *In situ* measurements of chlorophyll concentration made on the upper-most fully grown leaves of field grown *Solanum tuberosum* were shown by Vos & Bom (1993) to be well correlated ($R^2 > 0.95$) with both leaf nitrogen concentration and leaf chlorophyll concentration assayed by biochemical analysis.

Samples (three plants per [CO_2]-water treatment) for soluble sugar and starch concentrations of roots and leaves were taken at each harvest made during the first growing season. Three samples per [CO_2]-water treatment were also taken to determine macro-nutrient concentrations (nitrogen, phosphorus, potassium, calcium and manganese) at harvests made during both the growing seasons. Full details of the methods of nutrient, sugar, and starch analysis are given in Chapter 2.

6.3 Results

As found in Chapter 5, there were no significant inter-chamber effects on any of the physiological parameters measured on the cherry seedlings, and thus the interactions with 'chamber' are not shown.

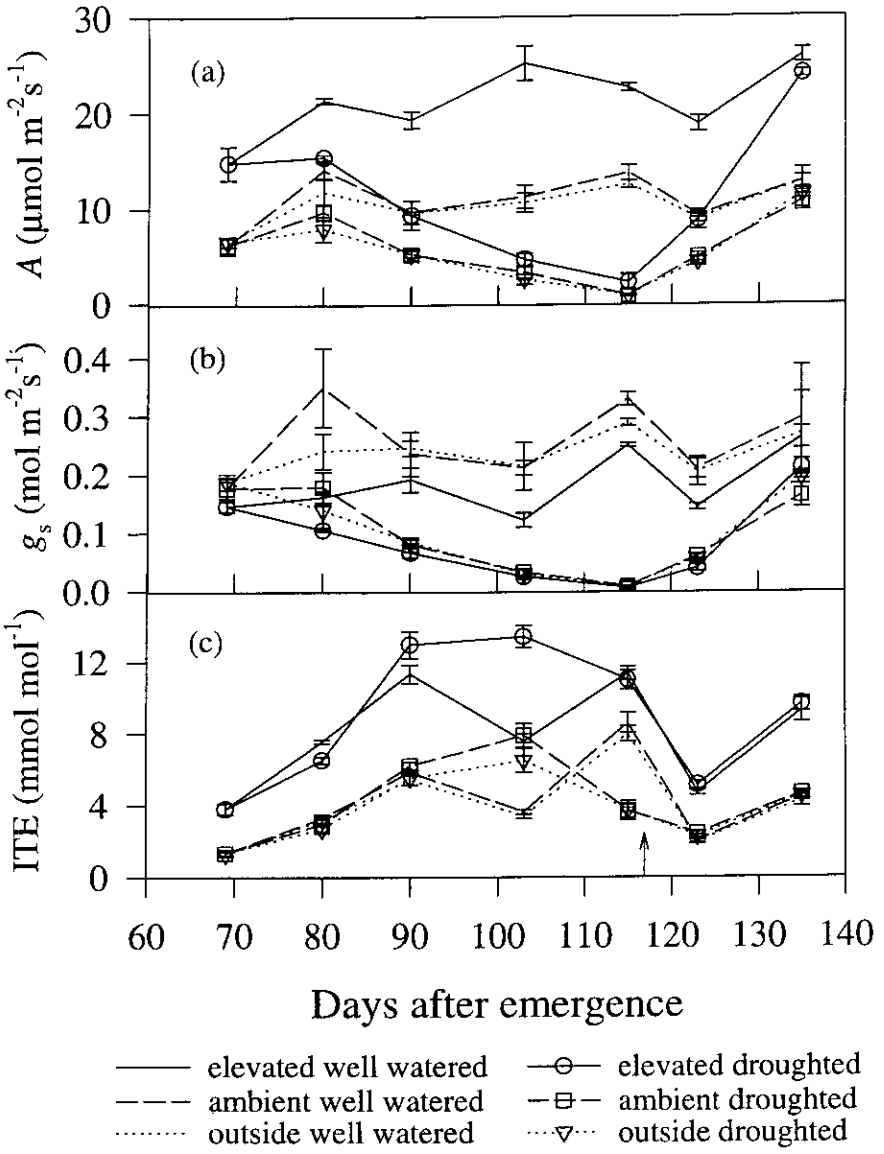

Figure 6.1. Time course of PPFD saturated a) assimilation rate (A), b) stomatal conductance (g_s), and c) ITE measured at the growth CO_2 concentration of well-watered and water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, during and after the first drought cycle (69-115 c). ↑ = end of the drying cycle. Data are mean of 3 plants per treatment ± 1 SEM. Statistical significance:

	well-watered			water-stress		
	A	g_s	ITE	A	g_s	ITE
Time	***	***	***	***	***	***
[CO_2]	***	**	***	***	ns	***
Time x [CO_2]	ns	ns	**	***	ns	***

CO_2 assimilation rate

The overall effect of elevated [CO_2] and water regime on *A* measured at the growth [CO_2] condition was significant in both the first (Appendix 5, Table 1) and second (Appendix 5, Table 2) growing seasons. Figure 6.1a illustrates the effect of [CO_2] and water treatment on *A* over the first growing season. Assimilation rate of well-watered cherry seedlings was significantly increased by elevated [CO_2] throughout the growing season. A similar pattern of changes in *A* measured in the growth [CO_2] conditions on well-watered seedlings was found during the second growing season (Figure 6.2a). Acclimation of *A* was not found in elevated [CO_2]-grown seedlings when measured at the same CO_2 concentrations in both the first (Appendix 5, Figure 1) and second (Appendix 5, Figure 2) growing seasons. This is consistent with A/C_i measurements (Figures 6.3a and 6.4a), and A_{MAX} was not significantly different ($P < 0.05$) between the [CO_2] treatments. However, A_{MAX} was higher in the first growing season (37.2, 35.4, and 33.4 μmol $m^{-2}s^{-1}$ in elevated [CO_2], in the ambient [CO_2] OTCs and outside-control, respectively) than in the second growing season (22.2, 24.6, and 24.6 μmol $m^{-2}s^{-1}$ in the elevated [CO_2] OTCs, and in the ambient [CO_2] OTCs and outside-control, respectively).

Water stress dramatically affected *A* of the droughted seedlings even during the early phase of the drying cycle in the first growing season (Figure 6.1a). *A* was significantly increased in response to elevated [CO_2] over the whole growing season, but water stress was so severe that by the end of the first drying cycle (115 dae) there were no differences between seedlings raised in elevated and ambient [CO_2]. Similar results were found during the second drying cycle (Figure 6.2a). It is worth noticing the "after-effect" following rewatering in the first growing season: *A* did not recover to well-watered rates for eight days (123 dae) in both [CO_2] treatments, but had recovered by the following measurement occasion, i.e. after 18 days (133 dae). Short-term PPFD-saturated A/C_i measurements made on 85-87 dae (i.e., after 16-18 days from the onset of the drying cycle), showed that A_{MAX} of droughted seedlings (17.9, 17.0, and 20.1 μmol $m^{-2}s^{-1}$ in the elevated [CO_2] OTCs, and in the ambient [CO_2] OTCs and outside-control, respectively) was halved in

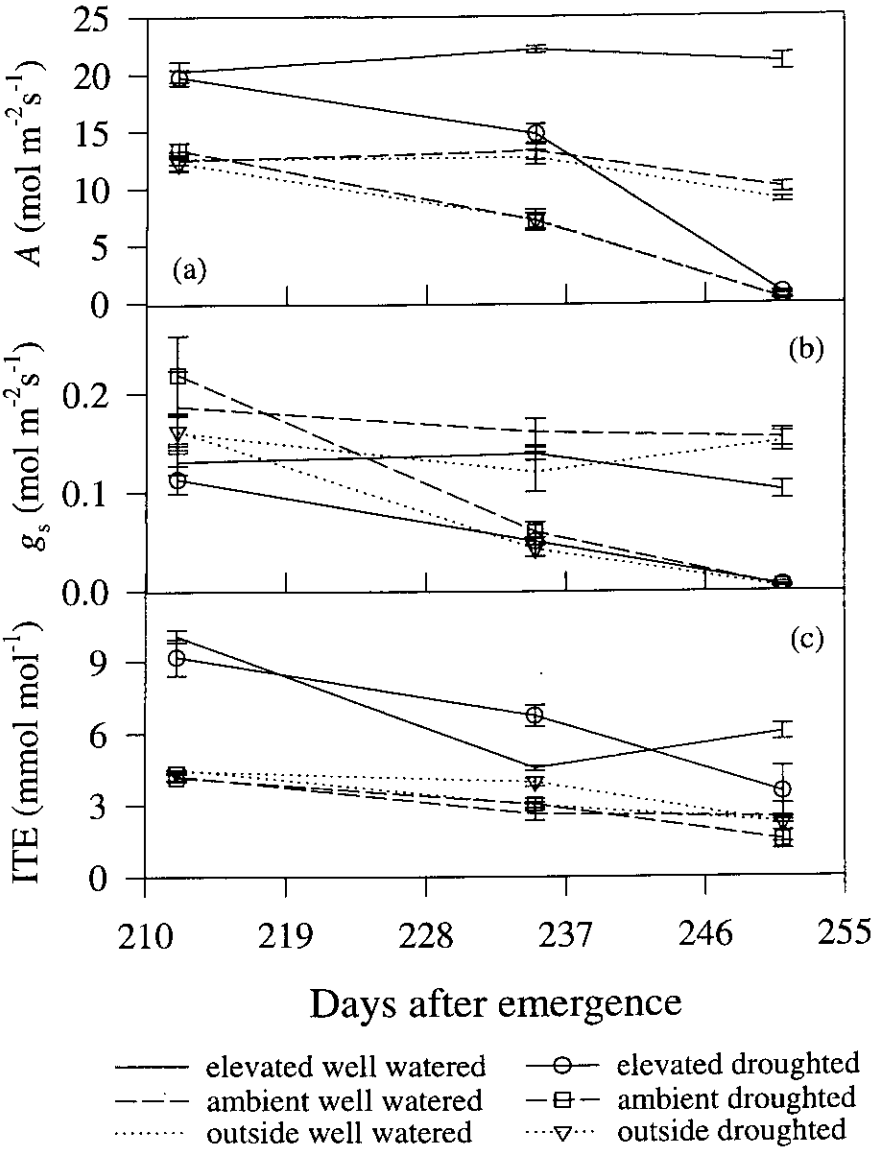

Figure 6.2. Time course of PPFD saturated a) assimilation rate (A), b) stomatal conductance (g_s), and c) ITE of well-watered and water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, over the second drought cycle (212-251 dae). Data are mean of 3 plants per treatment ± 1 SEM. Statistical significance:

	well-watered			water-stress		
	A	g_s	ITE	A	g_s	ITE
Time	***	ns	***	***	***	***
[CO_2]	***	$P < 0.10$	***	***	ns	***
Time x [CO_2]	$P < 0.10$	ns	***	***	ns	ns

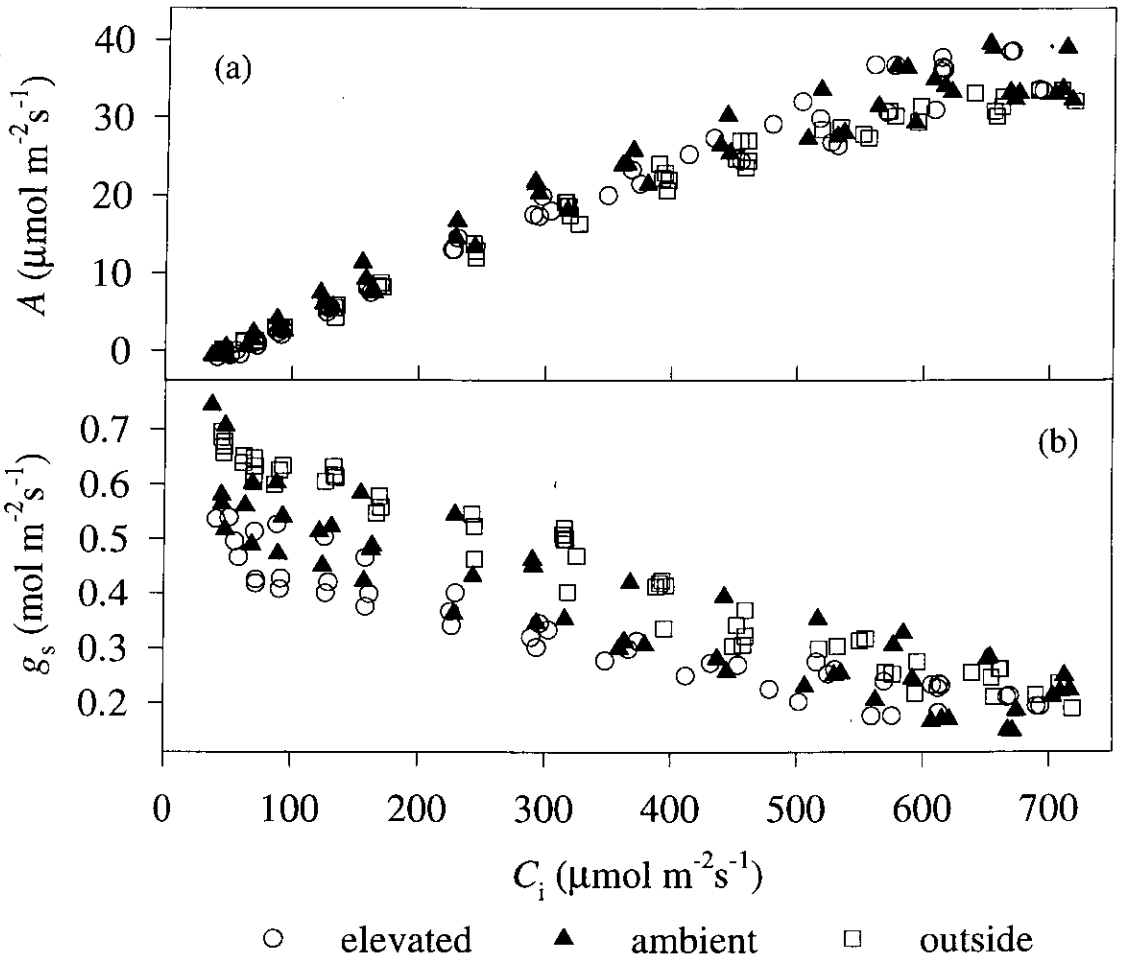

Figure 6.3. The relationship between PPFD saturated a) assimilation rate (A) and b) stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of well-watered cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control in the growing season 1993. Data are means of 3 plants.

both elevated and ambient [CO_2] (Figure 6.5a) compared to that of the well-watered seedlings (Figure 6.3a).

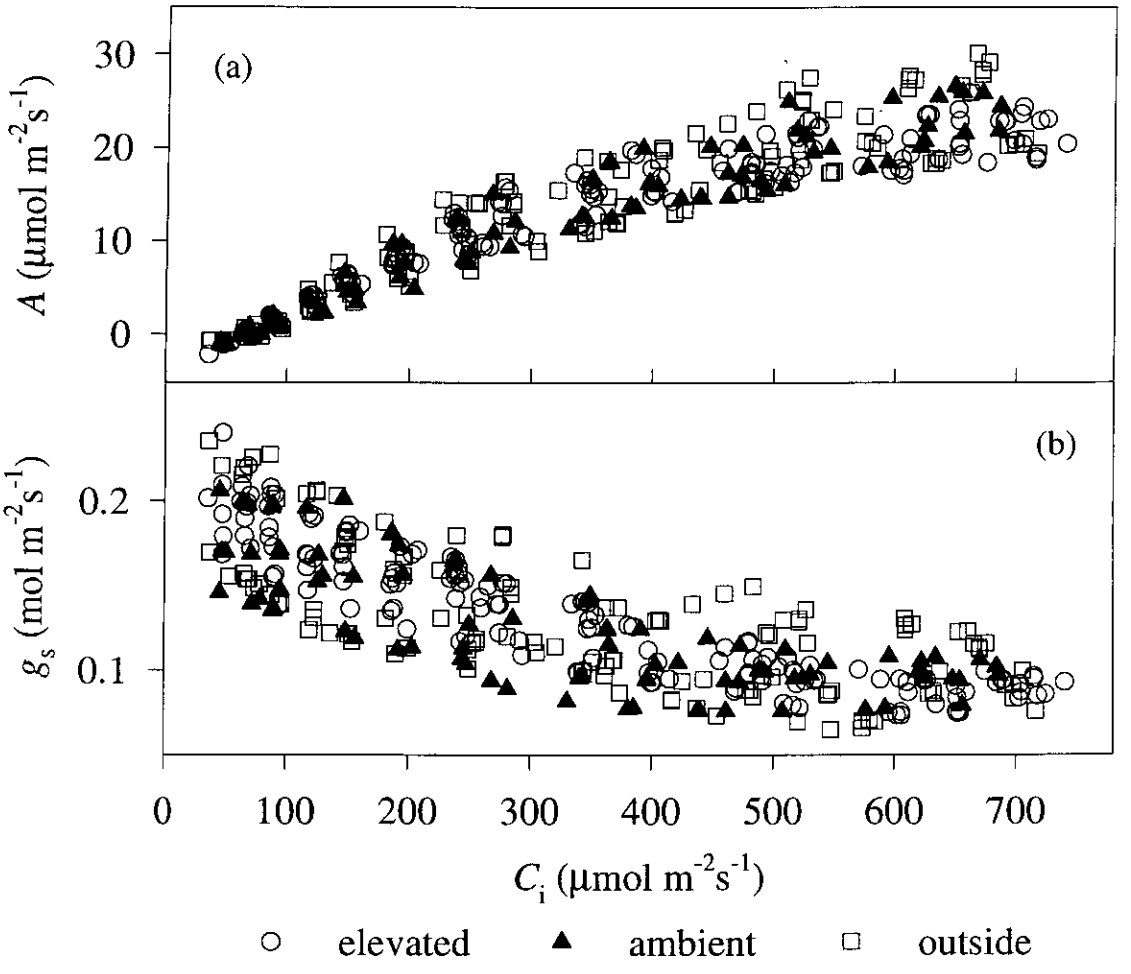

Figure 6.4. The relationship between PPFD saturated a) assimilation rate (A) and b) stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of well-watered cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control in the growing season 1994. Data are means of 3 to 5 plants.

Stomatal conductance and density

Growth in elevated [CO_2] and water stress significantly affected g_s measured at the growth [CO_2] condition throughout the duration of the experiment, although the interaction between time-[CO_2] and treatment-water regime were not significant (Appendix 5, Tables 1 and 2). Stomatal conductance of well-watered seedlings was

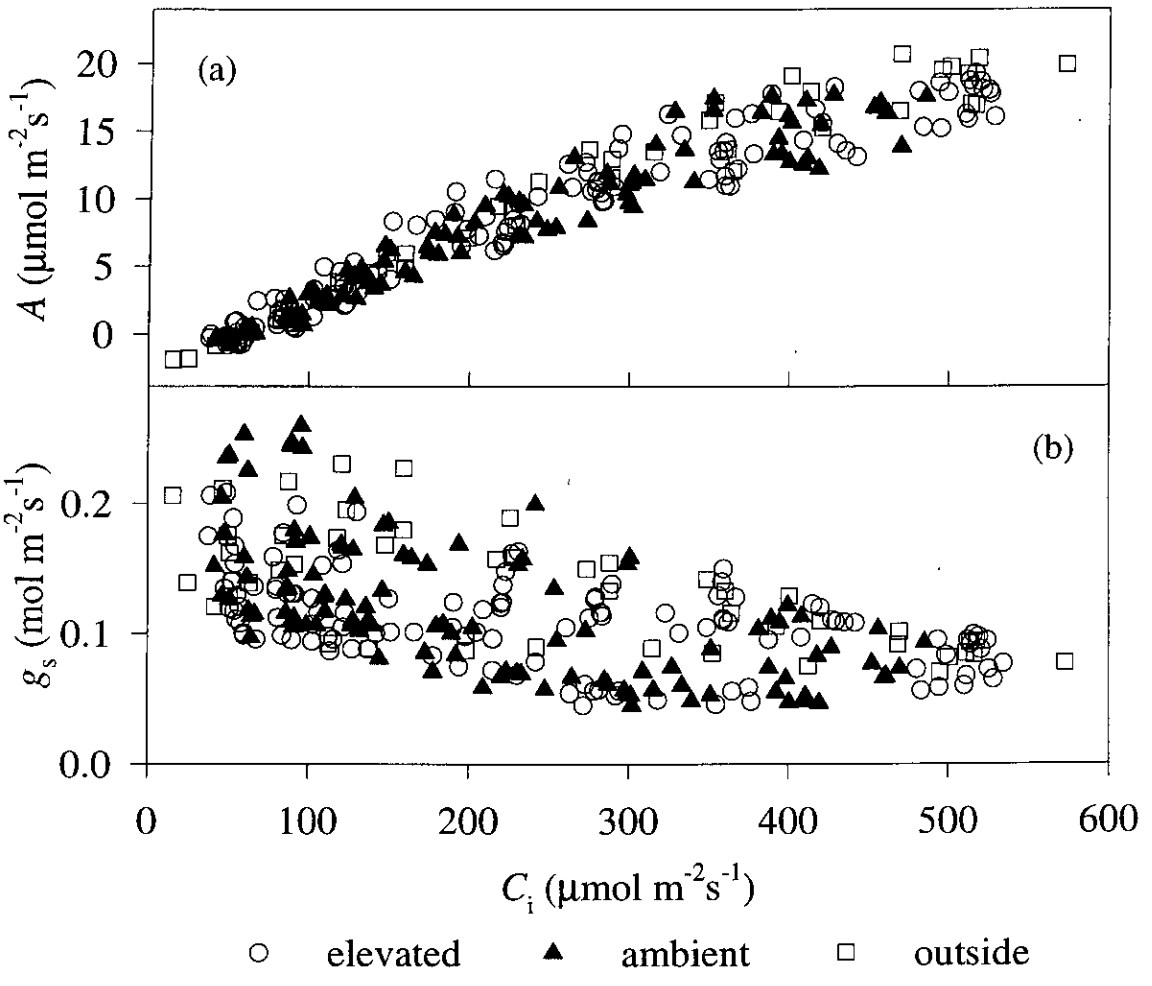

Figure 6.5. The relationship between PPFD-saturated a) assimilation rate (A) and b) stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control in the growing season 1993. Data are means of 3 plants.

strongly decreased by elevated [CO_2] ($P < 0.01$) during the first growing season, whereas for the droughted seedlings there were no significant differences in g_s between [CO_2] treatments (Figure 6.1b). Following rewatering, g_s of droughted seedlings did not immediately recover to well-watered in both [CO_2] treatments, and after 18 days it was still lower, although not significantly so, in droughted seedlings

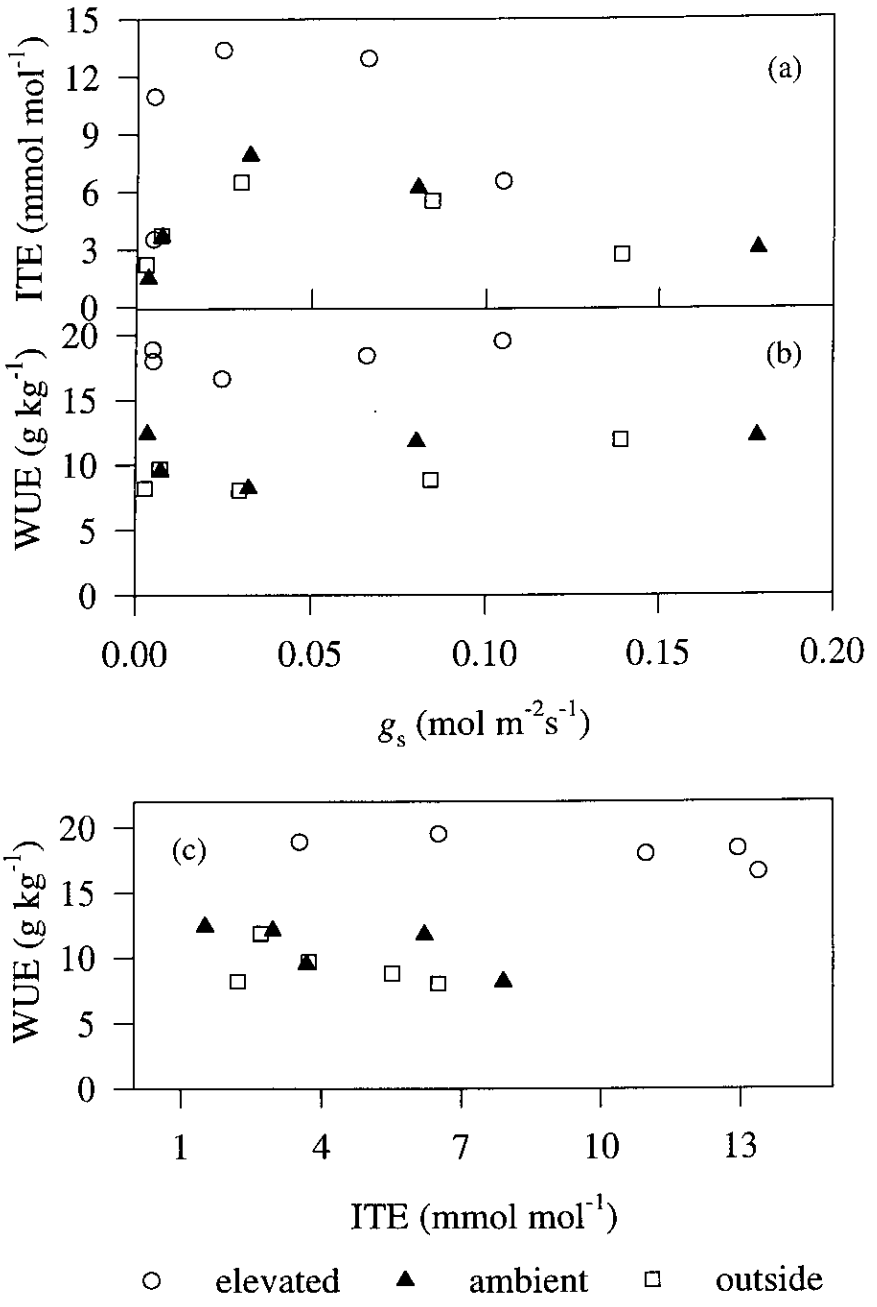

Figure 6.6. Relationship between (a) instantaneous transpiration efficiency (ITE), (b) plant water use efficiency (WUE) and stomatal conductance (g_s), and between (c) plant WUE and ITE of water-stressed cherry seedlings during both drying cycles.

than in well-watered seedlings. Short-term response of g_s to step-wise changes in C_i showed a slight trend for lower stomatal conductance in elevated [CO_2] seedlings compared to ambient [CO_2] seedlings in both well-watered (Figure 6.3b) and droughted (Figure 6.5b) conditions. However, g_s of droughted seedlings was relatively insensitive to C_i. Abaxial stomatal density measured at the end of the first drying cycle was not influenced by either [CO_2] treatment or water regime (Table 6.1). In the second growing season, the response of g_s to elevated [CO_2] and water stress was similar to that in the previous year: elevated CO_2 significantly ($P < 0.10$) decreased g_s of well-watered seedlings but not that of droughted seedlings (Figure 6.2b). However, g_s of well-watered seedlings remained relatively insensitive to intercellular CO_2 concentrations in the second growing season (Figure 6.4b).

Table 6.1. Abaxial stomatal density (mm^{-2}) determined at the end of the first drying cycle on leaves of cherry seedlings, grown in ambient [CO_2], elevated [CO_2], or outside control. Data are means of 6 plants (nine leaves per plant) ± 1 SEM; ns = not significant level of P from two-way ANOVA.

	well-watered	water-stressed
elevated	81.0 ± 1.3	75.4 ± 5.1
ambient	84.4 ± 3.9	83.3 ± 3.5
outside	84.8 ± 1.6	85.1 ± 2.3
[CO_2]	ns	
water	ns	
[CO_2] x water	ns	

Instantaneous water use efficiency

Instantaneous transpiration use efficiency (ITE) (the ratio of leaf assimilation rate to transpiration rate) was strongly affected by growth in elevated [CO_2] ($P < 0.001$) but not by water regime in both the growing seasons (Appendix 5, Tables 1 and 2): ITE was significantly higher in elevated [CO_2] than in ambient [CO_2] (Figures 6.1c and 6.2c). The increase in ITE of well-watered seedlings ranged from ~94% to ~186% in the first growing season (with the only exception of 115 dae when the increase was

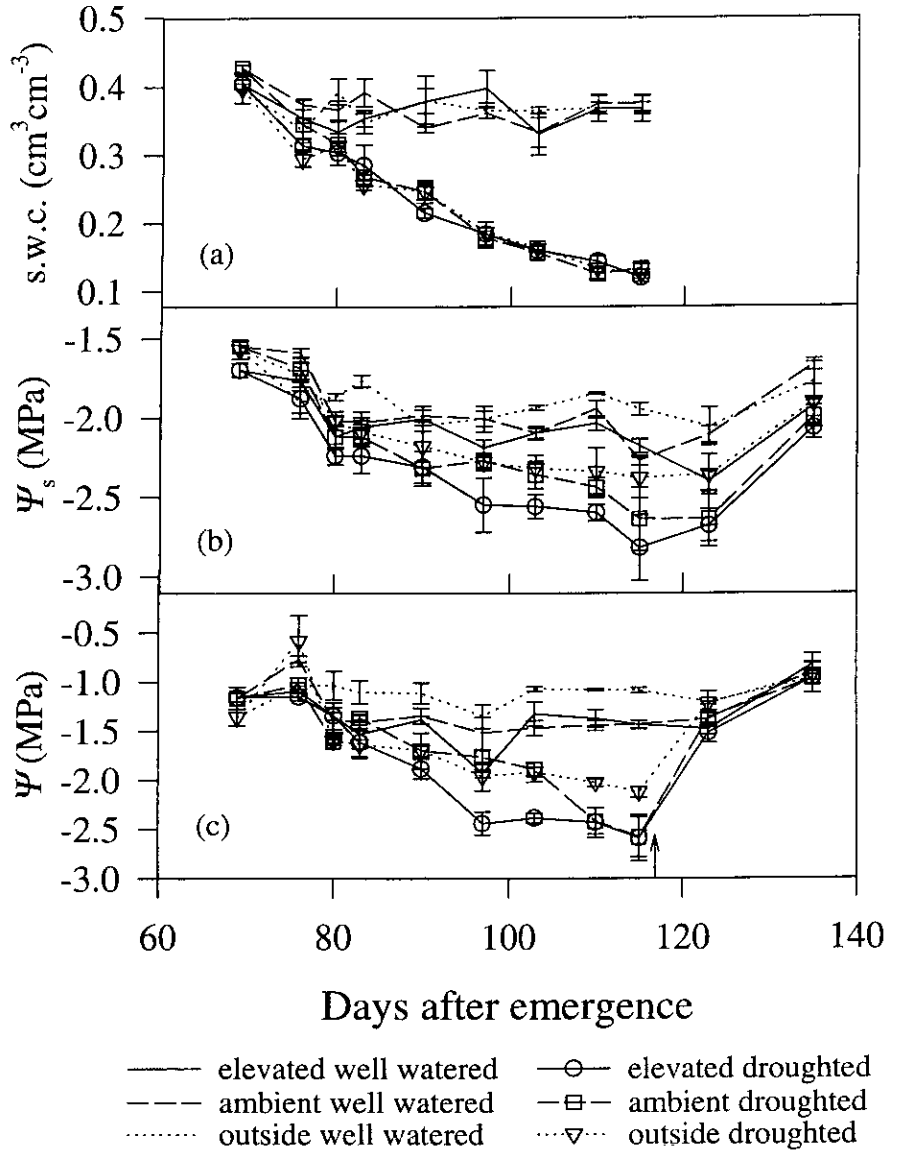

Figure 6.7. Time course of a) soil water capacity (s.w.c.) b) osmotic potential (Ψ_s), and c) bulk leaf water potential (Ψ) of well-watered and water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, during and after the first drought cycle (69-115 dae). ↑ = end of the drying cycle. Data are mean of 3 plants per treatment ± 1 SEM. Statistical significance:

	well-watered			water-stressed		
	s.w.c.	Ψ	Ψ_s	s.w.c.	Ψ	Ψ_s
time	*	***	***	***	***	***
[CO_2]	ns	***	***	ns	**	***
time x [CO_2]	ns	*	***	ns	*	ns

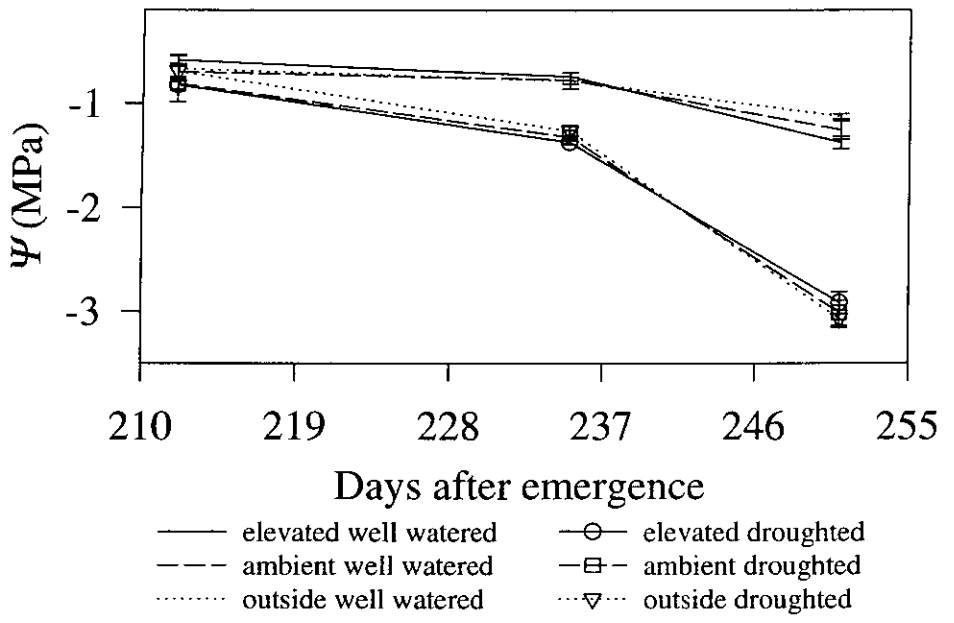

Figure 6.8. Time course of osmotic potential (Ψ_s) of well-watered and water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, over the second drought cycle (dae 212-251). Data are means of 3 plants per treatment ± 1 SEM. Statistical significance:

	well watered	water stress
time	***	***
[CO_2]	ns	ns
time x [CO_2]	ns	$P < 0.10$

only about 32%) and from ~74% to ~144% in the second growing season. In the droughted seedlings the increase in ITE brought about by elevated [CO_2] was ~70% on dae 103 and ranged from ~108% to ~198% during the rest of the first growing season, whereas in the second year of growth the increase ranged from ~122% to ~133%.

There were no significant differences in any of the gas exchange parameters measured between the ambient [CO_2] seedlings in OTCs and outside in both water treatments.

Instantaneous transpiration efficiency of droughted seedlings measured during both drying cycles was depressed by high (incipient water stress) and very low (maximum drought developed) values of g_s in all [CO_2] treatments (Figures 6.6a). However, plant WUE (see Chapter 5) varied very little as both g_s (Figures 6.6b) and ITE (Figures 6.6c) increased. Consequently, there was no relationship between plant WUE (dry mass produced to water lost ratio) and ITE (assimilation rate to transpiration rate ratio).

Soil water content and water relations

Figure 6.7 shows changes in soil water content, osmotic potential, and bulk leaf water potential over the first growing season. As expected, soil water content was strongly affected by water regime (Appendix 5, Table 3), but there were no differences in the rate of soil drying between the [CO_2] treatments between well-watered and droughted ssedlings (Figure 6.7a). Both [CO_2] and water treatments had a significant overall influence on midday osmotic potential, Ψ_s, and Ψ (Appendix 5, Table 4). Ψ_s (Figure 6.7b) and Ψ (Figure 6.7c) of well-watered plants fluctuated throughout the season. However, the time course of Ψ_s in elevated [CO_2] was significantly lower than in ambient [CO_2] in both the well-watered ($P < 0.001$) and droughted ($P < 0.05$) plants. There were no significant differences in Ψ of well-watered seedlings ($P < 0.10$) between elevated and ambient [CO_2] (Figure 6.7c). With increasing water stress, Ψ started declining after about a week. Elevated [CO_2] did not delay the onset of the decline in Ψ, which was faster in elevated [CO_2] than in ambient [CO_2], resulting in significant differences over the whole season at $P < 0.05$. However, on 110 dae (41 days after the onset of water stress) Ψ in ambient [CO_2] reached the same negative value as in elevated [CO_2], and from then on there were no longer significant differences between the treatments.

Table 6.2. Effects of the interaction between elevated [CO_2] and water stress on tissue water relations parameters derived from pressure-volume curves of cherry leaves. Data are means of 3 plants ± 1 SEM ; letters (a, b, c) indicate significant differences at $P < 0.05$ in the same line; π_{100} = osmotic potential at full turgor, π_0 = osmotic potential at zero turgor, R_0 relative water content at zero turgor, ε_B bulk modulus of elasticity of the cell, DM / TM ratio of leaf dry mass to leaf turgid mass.

Water relations characteristics	well-watered			water-stressed		
	elevated	ambient	outside	elevated	ambient	outside
π_{100} (MPa)	1.413 ± 0.070 c	1.609 ± 0.115 bc	1.570 ± 0.127 bc	2.008 ± 0.023 a	1.935 ± 0.071 ab	1.832 ± 0.128 ab
π_0 (MPa)	1.797 ± 0.021 c	1.880 ± 0.087 c	1.978 ± 0.116 bc	2.484 ± 0.085 a	2.445 ± 0.066 a	2.257 ± 0.079 ab
R_0 (%)	91.877 ± 2.040 ab	93.660 ± 0.050 b	87.353 ± 2.036 a	89.340 ± 0.893 ab	89.157 ± 0.290 ab	90.553 ± 0.645 ab
ε_B (MPa)	14.103 ± 2.452 a	18.700 ± 1.415 a	12.437 ± 2.062 a	17.367 ± 1.293 a	15.257 ± 0.321 a	17.430 ± 0.559 a
DM / TM	0.288 ± 0.008 abc	0.280 ± 0.004 ab	0.271 ± 0.003 a	0.303 ± 0.003 c	0.293 ± 0.005 bc	0.299 ± 0.003 c

During the second growing season, midday Ψ was affected only by the water treatments but not by [CO_2] treatments (Appendix 5, Table 2). Ψ of droughted seedlings declined rapidly from 235 dae (23 days after the onset of water stress) to the end of the experiment, and fell below -3 MPa to the same minimum value in all [CO_2] treatments (Figure 6.8).

In the first growing season there were significant differences between Ψ_s in the ambient [CO_2] OTCs and outside control treatments in both well-watered ($P < 0.10$) and droughted ($P < 0.05$) seedlings. Significant differences were also found in Ψ of well-watered plants ($P < 0.01$), but not in droughted seedlings, between the ambient [CO_2] OTCs and outside control treatments. However, there were no differences in Ψ between plants in ambient [CO_2] OTCs- and outside control-grown seedlings in both water treatments during the second growing season.

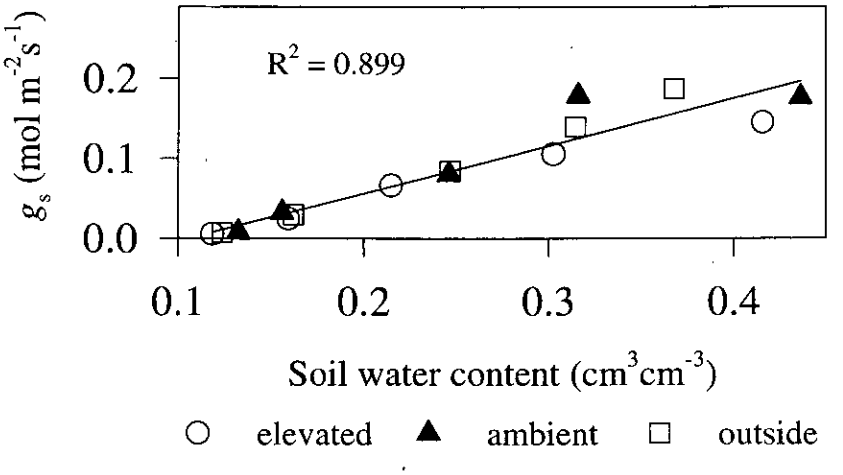

Figure 6.9. Linear relationship between all stomatal conductance (g_s) and all soil water content, measured during the first drought cycle (dae 69-115), of water-stressed cherry seedlings grown in elevated [CO_2], ambient [CO_2], or outside control.

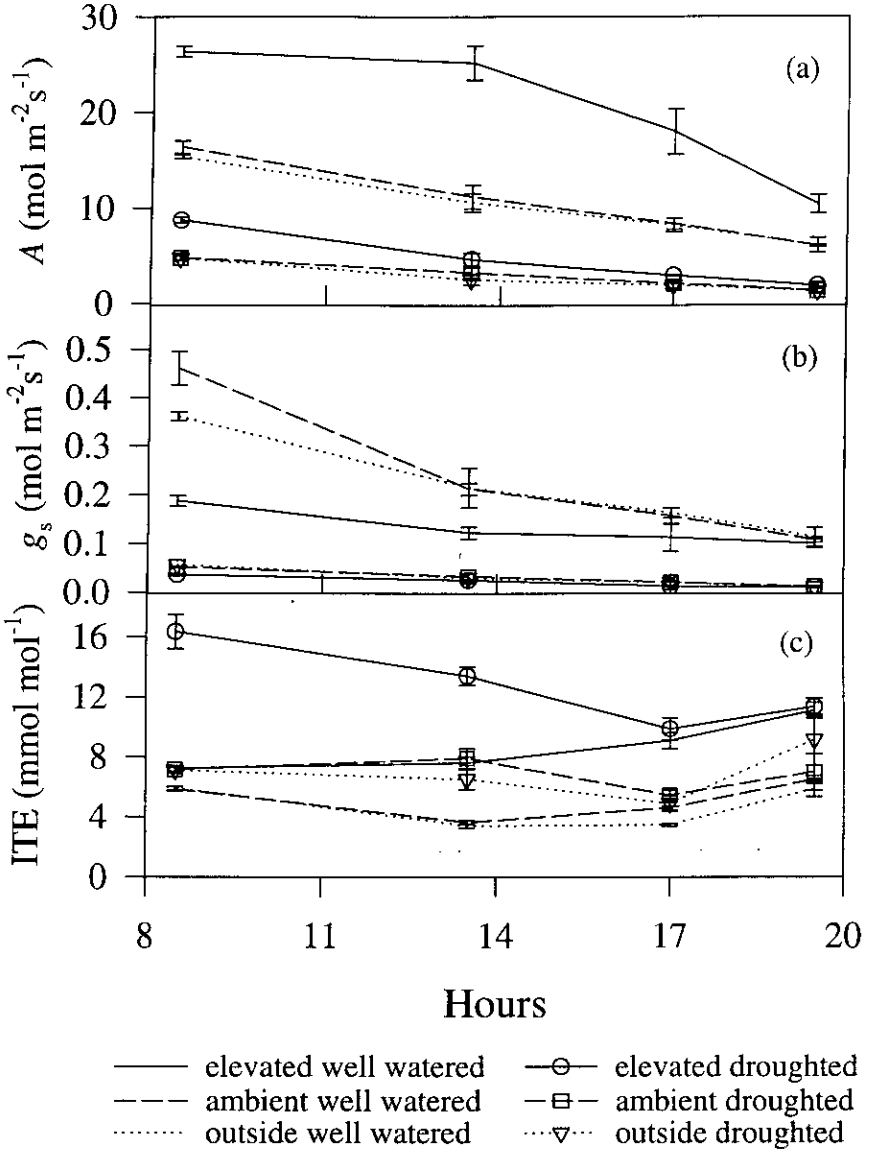

Figure 6.10. Time course on 103 dae of PPFD saturated a) assimilation rate (A), b) stomatal conductance (g_s), and c) ITE measured at the growth CO_2 concentration of well-watered and well-watered cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control. Data are mean of 3 plants per treatment ± 1 SEM. Statistical significance:

	well-watered			water-stressed		
	A	g_s	ITE	A	g_s	ITE
hour	***	***	***	***	***	**
[CO_2]	***	***	***	***	**	***
hour x [CO_2]	*	***	***	*	ns	*

All of the stomatal conductance data of droughted seedlings were re-plotted versus the mean soil water content, Ψ_s, Ψ, and turgor potential (Ψ_p) measured over the first drying cycle, and *versus* mean Ψ measured over the second drying cycle. A stronger linear relationship was found between g_s and soil water content (Figure 6.9) than between g_s and either Ψ_s or Ψ (Appendix 5, Figure 3a,b) during the first drying cycle, whereas g_s was not correlated with Ψ_p (Appendix 5, Figure 3c). A low R^2 of the linear relationship between g_s and Ψ was also found during the second drying cycle (Appendix 5, Figure 4).

Water stress significantly affected osmotic potentials at full (π_{100}) and zero turgor (π_0), but they were not influenced by [CO_2] treatment (Appendix 5, Table 4): both π_{100} and π_0 were significantly lower in well-watered plants than in droughted plants in all [CO_2] treatments (Table 6.2). However, all other tissue water relations parameters derived from pressure-volume curve analysis (i.e., R_0, ε_B, and DM/TM ratio) were not significantly affected by water or [CO_2] treatments, with the only exception of the DM/TM ratio which was significantly ($P < 0.05$) increased by water stress in the outside control seedlings.

Diurnal cycle

The overall diurnal fluctuation of gas exchange and water relations parameters measured on 103 dae (i.e. after 34 into the drought) was significantly affected by both the [CO_2] and water treatments (Appendix 5, Table 5). The diurnal trend of A and g_s measured in the growth [CO_2] conditions was typically higher in the morning and steadily declined during the afternoon in all treatments (Figure 6.10). Both A and g_s were significantly influenced by elevated [CO_2] in both water treatments, although the increase in A and the decrease in g_s disappeared in droughted seedlings by the end of the diurnal cycle. Stomatal conductance was very low throughout the day in the water-stressed seedlings of [CO_2] treatments, and was practically zero by the end of the day. ITE was significantly increased throughout the day in both water treatments in response to elevated [CO_2]. This increase was higher during the afternoon in the well-watered seedlings (between ~98 and ~110%), and in the

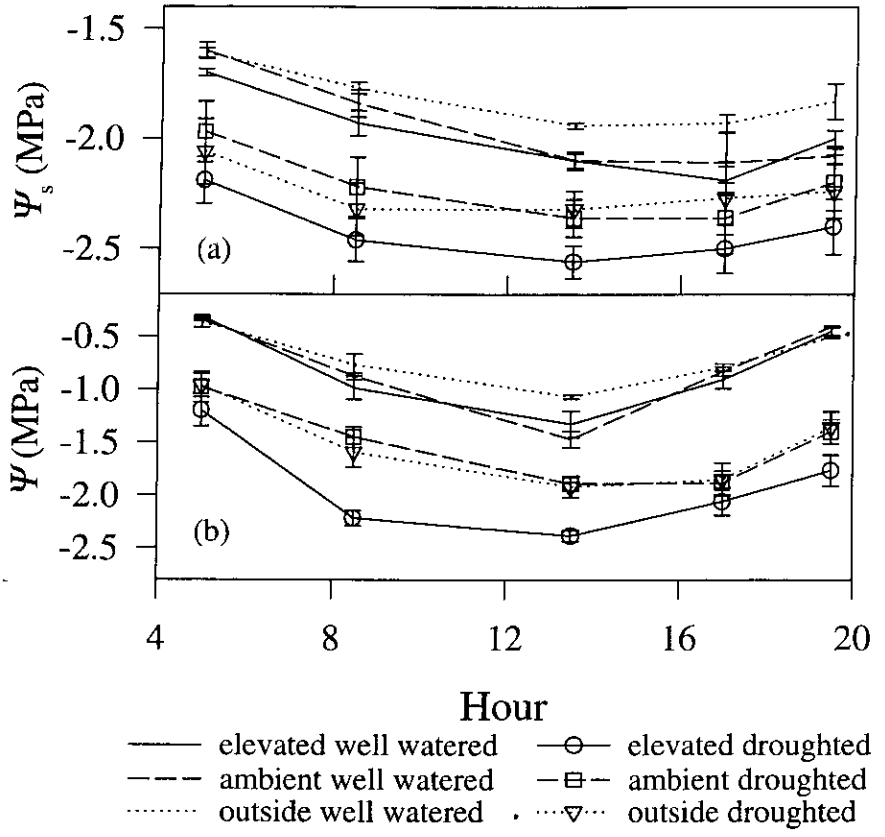

Figure 6.11. Time course on dae 103 of a) osmotic potential (Ψ_s), and b) bulk leaf water potential (Ψ) of well-watered and water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, during and after the first drought cycle (dae 69-115). Data are means of 3 plants per treatment ± 1 SEM. Statistical significance:

	well-watered		water-stressed	
	Ψ	Ψ_s	Ψ	Ψ_s
hour	***	***	***	*
[CO_2]	ns	***	***	*
hour x [CO_2]	$P < 0.10$	ns	ns	ns

morning in the stressed seedlings (~129%). Diurnal fluctuations of Ψ_s and Ψ were overall affected by both [CO_2] and water treatments (Appendix 5, Table 5). However, there were no significant differences ($P < 0.05$) in the diurnal cycle of Ψ_s

of the well-watered seedlings in elevated and ambient [CO_2], whereas Ψ_s was significantly decreased in the water-stressed plants in response to elevated [CO_2] (Figure 6.11). Pre-dawn Ψ was not affected by elevated [CO_2] in both water treatments, but Ψ of droughted plants was significantly lower ($P < 0.001$) in elevated [CO_2] than in ambient [CO_2] for the rest of the day, whereas there were no differences in well-watered seedlings between [CO_2] treatments.

There were no significant differences in any of the gas exchange parameters measured on seedlings in ambient [CO_2] in the OTCs and outside in both water treatments. The diurnal fluctuations of Ψ_s and Ψ, instead, were significantly lower in the well-watered seedlings in ambient [CO_2] in the OTCs than outside but not in the stressed seedlings.

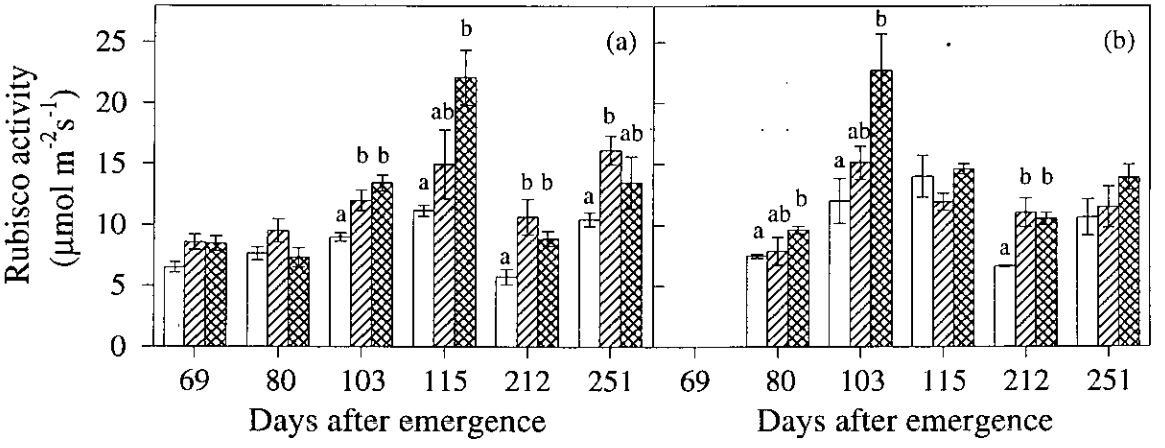

Figure 6.12. Rubisco activity of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

	well-watered		water-stressed	
	1993	1994	1993	1994
time	***	**	***	*
[CO_2]	**	*	**	$P < 0.10$
time x [CO_2]	*	ns	$P < 0.10$	ns

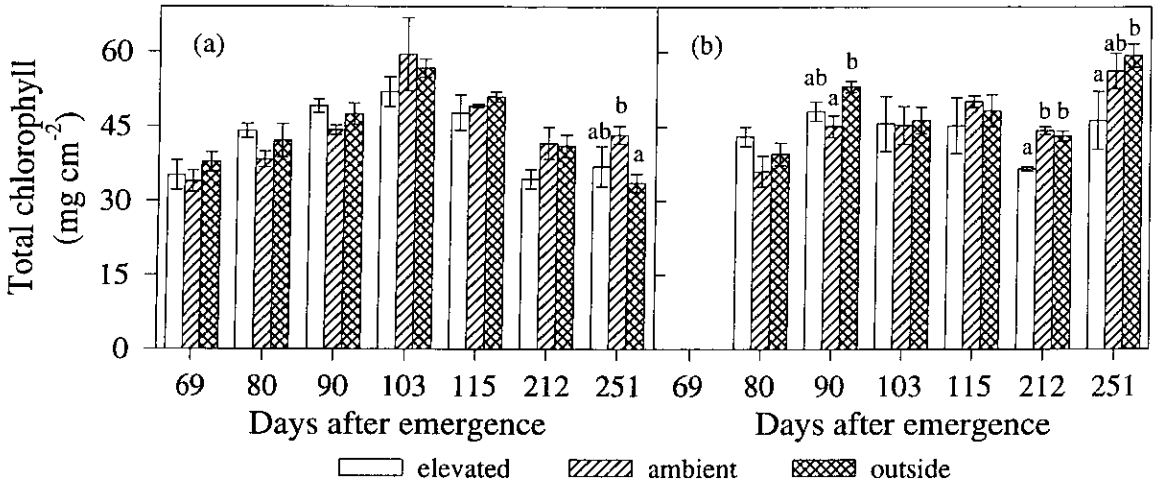

Figure 6.13. Total chlorophyll of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

	well-watered		water-stressed	
	1993	1994	1993	1994
time	***	ns	**	***
[CO_2]	ns	*	ns	*
time x [CO_2]	ns	ns	ns	ns

Rubisco activity

Rubisco activity was significantly affected by [CO_2] treatment but not by water regime in both the first (Appendix 5, Table 1) and second (Appendix 5, Table 2) growing seasons. Thus, there were no significant differences ($P < 0.10$) between well-watered and droughted seedlings in both elevated and ambient [CO_2] on each measuring date (Figure 6.12). However, Rubisco activity was slightly higher in water-stressed than in well-watered plants in elevated [CO_2] (up to 25% on 103 dae), but there was no clear pattern in ambient [CO_2]. Rubisco activity of the well-watered seedlings was reduced throughout the duration of the experiment in response to elevated [CO_2], although significant differences between elevated and ambient [CO_2] were restricted to 103 dae (during the first growing season), and on both sampling dates in the second growing season. Rubisco activity the of well-

watered seedlings was linearly related to leaf nitrogen concentration on 251 dae (Appendix 5, Figure 5). In stressed plants Rubisco activity was also decreased by elevated [CO_2], but the reduction was significant only on 212 dae.

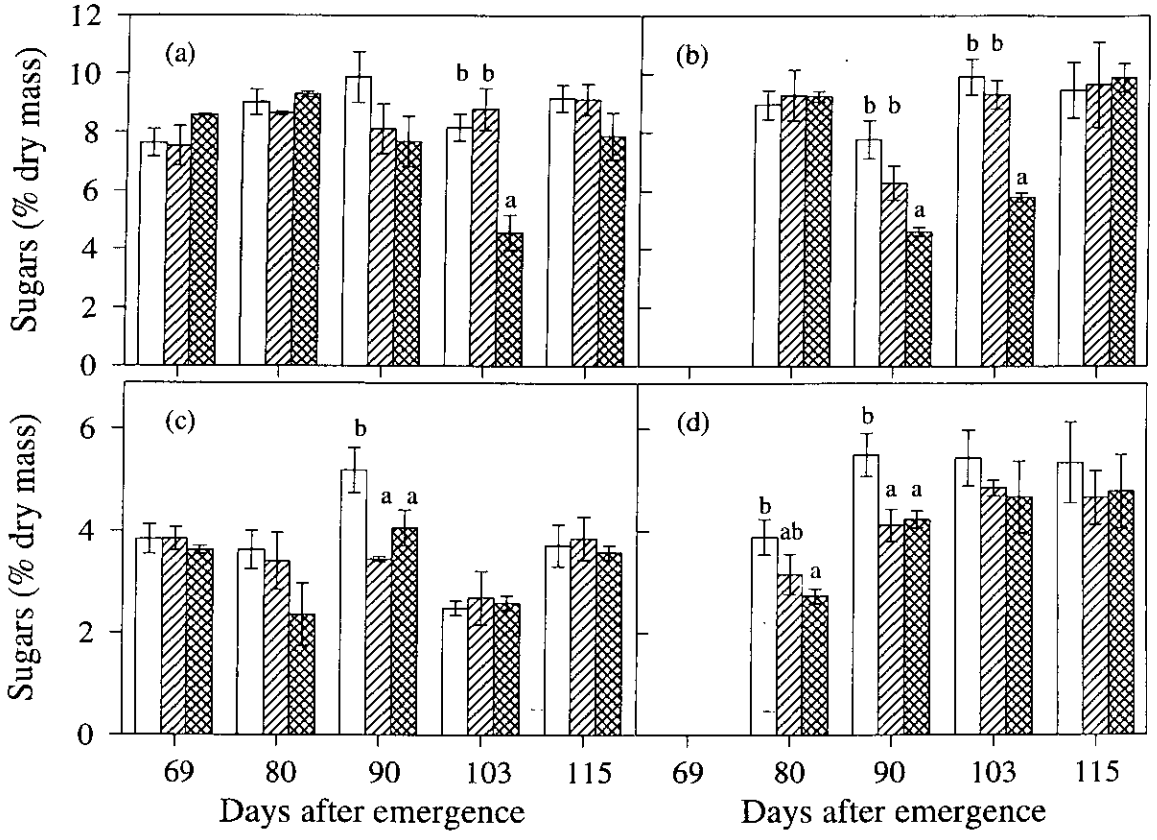

Figure 6.14. Leaf (a,b) and root (c,d) soluble sugars concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

	well-watered		water-stressed	
	leaf	root	leaf	root
time	**	***	***	***
[CO_2]	**	$P < 0.10$	*	*
time x [CO_2]	***	$P < 0.10$	**	ns

There were no significant differences in Rubisco activity between seedlings in ambient [CO_2] in the OTCs and outside control in both water treatments. However,

Rubisco activity was significantly increased on dae 80 and 90 and significantly decreased on dae 115 at $P < 0.10$ by water stress in the outside control treatment.

Chlorophyll concentration

The overall effect of elevated [CO_2] on leaf total chlorophyll concentration was significant in the second growing season (Appendix 5, Table 2) but not in the first growing season (Appendix 5, Table 1), whereas the water treatment had a significant effect in both years. Moreover, the chlorophyll a/b ratio was not affected by either [CO_2] or water treatments in the first growing season, whereas it was influenced by the water treatment in the second growing season. Figure 6.13 shows total chlorophyll concentration at each sampling time. There were no differences in the well-watered seedlings between elevated and ambient [CO_2], whereas in the water-stressed seedlings total chlorophyll concentration was significantly decreased by elevated [CO_2] only on 212 dae. Water stress did not influence total chlorophyll concentration in elevated [CO_2] on any sampling date, whereas in ambient [CO_2] it significantly increased chlorophyll concentration in the water-stressed seedlings on 251 dae.

Total chlorophyll concentration assayed spectrophotometrically in solution was not correlated with *in situ* measurements of chlorophyll concentration made using a leaf reflectance meter (Appendix 5, Figure 6a). Moreover, *in situ* measurements of chlorophyll concentration were not correlated with leaf nitrogen concentration (Appendix 5, Figure 6b).

Total chlorophyll concentration was similar in ambient [CO_2] in the OTCs and in the outside control in both well-watered and droughted seedlings. Significant differences were found only on 251 dae in well-watered plants and on 90 dae in water-stressed plants. Water stress had an irregular influence on total chlorophyll concentration of the outside control seedlings: the chlorophyll concentration of stressed plants significantly increased, on 90 dae ($P < 0.10$) and 251 dae ($P < 0.001$), and significantly decreased, on 103 dae ($P < 0.05$).

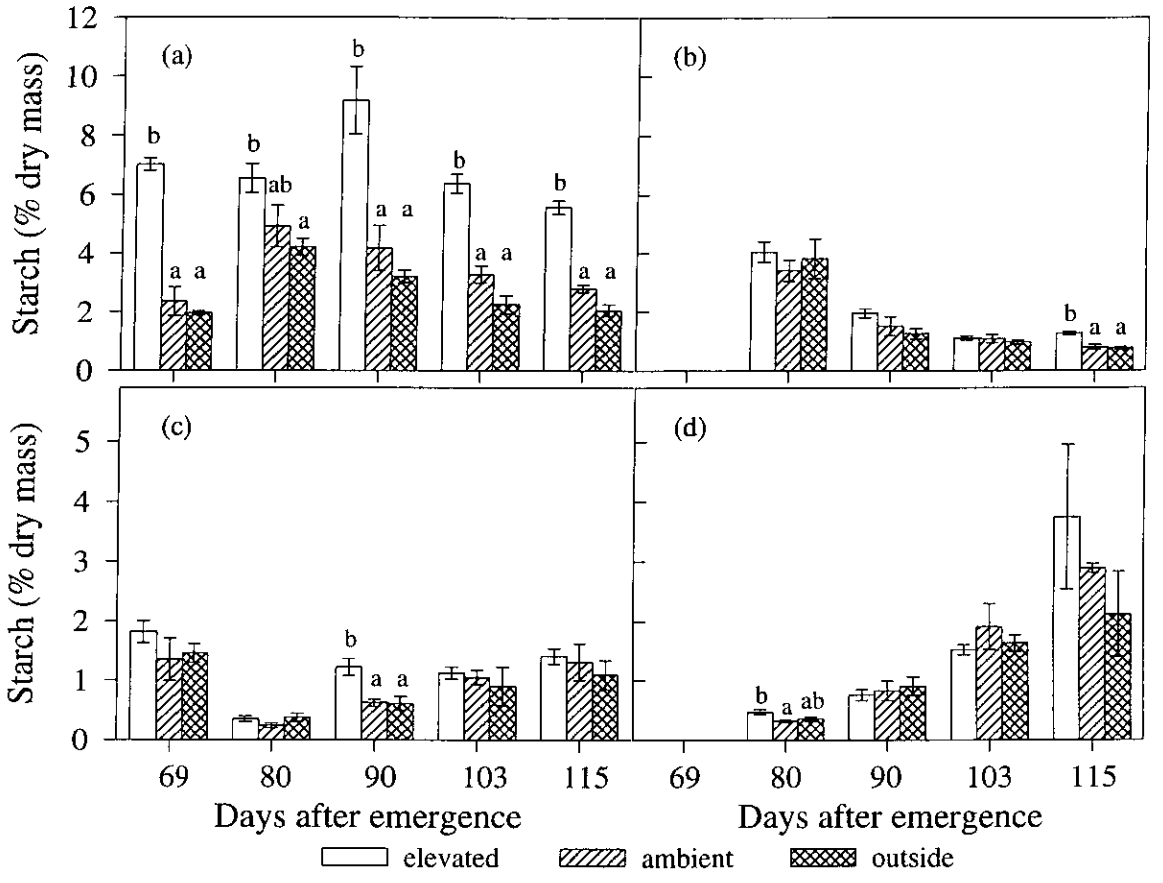

Figure 6.15. Leaf (a,b) and root (c,d) starch concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

	well-watered		water-stressed	
	leaf	root	leaf	root
time	***	**	***	***
[CO_2]	***	$P < 0.10$	***	ns
time x [CO_2]	$P < 0.10$	ns	***	ns

Sugar and starch concentration

[CO_2] treatment influenced overall leaf and root soluble sugar concentration (sorbitol, sucrose, fructose, glucose, etc.) per unit of dry mass of the cherry seedlings harvested before and during the first drying cycle (Appendix 5, Table 3). However, the soluble carbohydrate concentration was not increased in leaves of both

well-watered and droughted seedlings in response to elevated [CO_2], whereas in roots significant increases in elevated [CO_2] were found only 90 dae in both well-watered and stressed seedlings (Figure 6.14). Leaf sugar content was, in general, not affected by water stress (Appendix 5, Table 3). However, significant differences between water treatments were found, although without a clear trend, 103 dae in elevated [CO_2] ($P < 0.10$) and 90 dae in ambient [CO_2] ($P < 0.05$). There was a clear increase in soluble sugar concentration of roots of the water-stressed plants, although this was significant only 103 dae in both elevated ($P < 0.01$) and ambient ($P < 0.05$) [CO_2].

Differences in soluble sugar concentration in ambient [CO_2] between the seedlings in OTCs and outside were seen 103 dae in leaves and 90 dae in roots of well-watered seedlings, whereas in the water-stressed plants significant reductions were found 90 dae and 103 dae in leaf sugar concentration of the outside control seedlings. There was no clear trend in the outside control in response to water stress in leaf sugar concentration, which was significantly decreased 90 dae ($P < 0.01$) and significantly increased 115 dae ($P < 0.10$) in water-stressed plants. Conversely, root sugar concentration was increased by water stress, although this was significant only 103 dae ($P < 0.05$).

Leaf starch concentration was strongly increased by [CO_2] and water treatments in the cherry seedlings harvested before and during the first drying cycle (Appendix 5, Table 3). The increase in [CO_2] in the well-watered seedlings ranged from ~33% (80 dae) to ~198% (69 dae), whereas in the water-stressed seedlings the increase was significant only on 115 dae (~61%) (Figure 6.15). The amount of starch accumulated in the roots was, in general, not affected by elevated [CO_2] (Appendix 5, Table 3): it was always higher in elevated than in ambient [CO_2] in the well-watered seedlings, but the increase was significant only 90 dae (~128%). In contrast, there was no clear trend in starch concentration between elevated and ambient [CO_2] in the water-stressed seedlings, but a significant increase was found in elevated [CO_2] on 80 dae (~51%). Water stress reduced significantly leaf starch

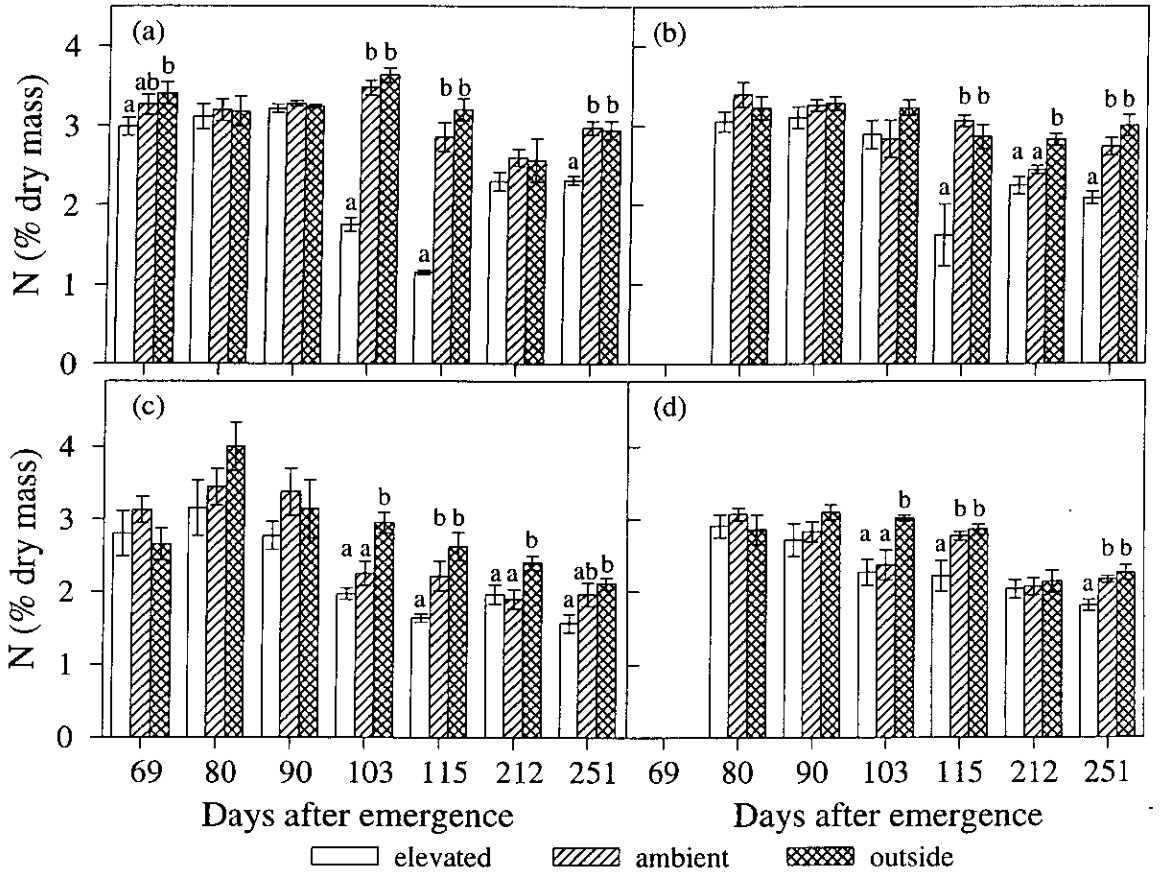

Figure 6.16. Leaf (a,b) and root (c,d) nitrogen (N) concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

1993	well-watered		water-stressed	
	leaf	root	leaf	root
time	***	***	***	ns
[CO_2]	***	**	***	$P < 0.10$
time x [CO_2]	*	ns	***	ns
1994	**well-watered**		**water-stressed**	
	leaf	root	leaf	root
time	*	ns	ns	ns
[CO_2]	**	*	***	$P < 0.10$
time x [CO_2]	ns	ns	ns	ns

concentration per unit of dry mass in all the [CO_2] treatments. The natural ontogenetic reduction in leaf starch over time which started 80 dae, was amplified as water stress increased. Conversely, water stress had the opposite effect on root starch, where the increasing ontogenetic trend was enhanced as soil moisture decreased.

There were no significant differences in leaf and root starch concentration between ambient [CO_2] seedlings in the OTCs and outside in either water treatment.

Nutrient concentration

Figure 6.16 shows leaf and root nitrogen concentration per unit of dry mass over the whole experimental period. [CO_2] treatment affected N concentrations of both leaves and roots in the first growing season (Appendix 5, Table 6), whereas only leaf nitrogen concentration was affected in the second growing season (Appendix 5, Table 7). Significant decreases in leaf and root N concentration were evident in both water treatments as the two growing seasons proceeded (115 and 251 dae, respectively). Water stress, in general, did not influence N concentration in any [CO_2] treatment.

In general, [CO_2] treatment did not affect P concentration of leaves in either growing season, although this was significantly reduced in elevated [CO_2] on 251 dae in both water regimes (Appendix 5, Figure 7). Root P concentration was decreased in the first, but not in the second growing season. However, P concentrations of both leaves and roots were significantly reduced by water stress (Appendix 5, Tables 6 and 7). The response of K (Appendix 5, Figure 8), Ca (Appendix 5, Figure 9), and Mg (Appendix 5, Figure 10) concentration of leaves and roots to the [CO_2] and water treatments was not uniform in the two growing seasons (Appendix 5, Tables 6 and 7).

6.4 Discussion

Stomatal conductance was significantly reduced in elevated [CO_2]-grown, unstressed seedlings in both the first (Figure 6.1) and second (Figure 6.2) growing seasons, whereas water-stressed seedlings showed little or no reduction in g_s. In contrast, A was significantly increased in response to elevated [CO_2] in both water regimes leading to improved ITE over the whole duration of the experiment. However, elevated [CO_2] did not increase water stress tolerance and the rate of water uptake was independent of [CO_2] treatment (Figure 6.7). Consequently, there was no relationship between ITE and plant WUE (Figure 6.6).

Higher A in elevated [CO_2] was evident in both growing seasons (Figures 6.1a and 2a), and the increase was larger in magnitude than the average increase of photosynthetic rates of 44% found by Gunderson & Wullschleger (1994) in survey of studies on 39 tree species. Moreover, long-term growth in elevated [CO_2] did not cause acclimation of photosynthesis of well-watered seedlings (Figures 6.3 and 6.4). Also, in water stress conditions A_{max} was unaffected by treatment [CO_2] (Figure 6.5). Absence of acclimation of A_{max} was found in leaves of *Quercus petraea* grown in elevated or ambient [CO_2] (Epron *et al.*, 1994), whereas acclimation was found in four clones of Sitka spruce (see Chapter 4). The cherry seedlings were grown in pots of large volume (10 dm^3 and 15 dm^3 in the first and second growing season, respectively) and were supplied with free access of nutrients to avoid the occurrence of unbalanced nutrition and constrained rooting. Arp (1991), surveying experiments done in elevated [CO_2], found that acclimation of photosynthesis and size of pot were highly correlated. In a recent paper Drake *et al.*, (1997) pointed out that when there is no limitation of rooting-volume, i.e. pot volume exceeding 10 dm^3, down-regulation of photosynthesis is an exception. The results obtained with cherry seedlings are in agreement with this view.

However, Rubisco activity of well-watered seedlings was decreased by elevated [CO_2] (Figure 6.12), and consistent with the lower leaf nitrogen concentration found at the end of the two growing seasons (Figure 6.16). Lower Rubisco activity in

elevated [CO_2] than in ambient [CO_2] was also found in cherry by Wilkins *et al.* (1994). Rubisco is the most abundant protein in the biosphere and may account for over 25% of the leaf nitrogen in C_3 species (Drake *et al.*, 1997), and is the most important form of nitrogen storage in vegetative tissues (Stitt & Schulze, 1994). Long-term growth in elevated [CO_2] often results in decrease in amounts of the photosynthetic pigments and enzymes (Long & Drake, 1992; Amthor, 1995). Apparently, this decrease may occur even when the supply of nitrogen is adequate and rooting volume large (Long, 1991). Long & Drake (1992) calculated that in elevated [CO_2] Rubisco can be reduced by about 35% before resulting in co-limitation of *A*. However, chlorophyll concentration of well-watered seedlings was not affected by elevated [CO_2] (Figure 6.13), confirming that the photosynthetic antennae pigments are less sensitive than Rubisco content to elevated [CO_2] (Van Oosten & Besford, 1995; Van Oosten *et al.*, 1995).

It has been proposed that increase in mass of sugars would regulate gene expression of the photosynthetic apparatus, and hence Rubisco activity and amount (Bowes, 1996; Van Oosten & Besford, 1996). However, in the cherry seedlings total soluble sugar concentrations were not different in the elevated and ambient [CO_2] treatments (Figure 6.14). Leaf starch concentration, however, did increase in response to elevated [CO_2] (Figure 6.15). Starch accumulation in leaves, by maintaining stroma P_i cycling, allows *A* to continue (Stitt, 1991; 1996), and by lowering the amount of soluble sugar in the cytosol reduces the source of the regulatory signal that, according to Van Oosten & Besford (1996), may effect coarse control of the photosynthetic genes. Paul & Driscoll (1997), by manipulating the source-sink ratio of *Nicotiana tabacum*, found that the loss of photosynthetic activity was more correlated to the hexose/amino acid ratio than to sugar concentration *per se*, and concluded that the regulatory signal causing repression of *A* depends more crucially on the carbon-nitrogen ratio than on the carbohydrate status of leaves. Accordingly, the increased leaf carbon/nitrogen ratio (i.e. equal soluble sugar concentration and lower nitrogen concentration - Figures 6.14 and 6.16) found on 103 and 105 dae may account for the loss of about 25% in Rubisco activity of the leaves of the cherry seedlings grown in elevated [CO_2].

It has been suggested that elevated [CO_2] may increase tolerance to drought by lowering osmotic potential, through the direct effect of elevated [CO_2] on *A* and consequently on soluble sugar concentration, thereby maintaining high Ψ (Tyree & Alexander, 1993). The osmotic potential of plants of *Triticum aestivum* grown in elevated [CO_2] declined more rapidly than in ambient [CO_2], resulting in the maintenance of turgor pressure and permitting growth to continue as water deficits develop (Sionit *et al.*, 1980). Conroy *et al.* (1988) found that droughted plants of *Helianthus annuus* grown in elevated [CO_2] maintained Ψ_s at lower values. The osmotic adjustment, by increasing turgor pressure and relative water content, prevented total stomatal closure in the water-stressed plants, and allowed the maintenance of *A*: similar results were found by Paez *et al.* (1984). In contrast, Ψ_s of well-watered, tropical species was not affected in elevated [CO_2] (Reekie & Bazzaz, 1989).

Water-stressed plants of *Ipomoea batatas* showed a more rapid decrease in leaf water potential in ambient [CO_2] than in elevated [CO_2] (Bhattacharya *et al.*, 1990). Stressed plants of *H. annuus* maintained Ψ at a more negative level in elevated [CO_2] than in ambient [CO_2] (Conroy *et al.*, 1988). In *Triticum aestivum* Ψ of the flag leaf declined more rapidly and reached a lower value in ambient CO_2 than in elevated [CO_2] (Sionit *et al.*, 1980). Leaf water potentials of droughted *Triticum aestivum* were often significantly less negative in the FACE than in the control treatment (Pinter *et al.*, 1996). However, in *Gossypium hirsutum* Ψ was less negative in the FACE treatment only towards the end of the season. Similarly, higher values of Ψ were found in plants of *Arachis hypogaea* grown in elevated [CO_2] in both irrigated and droughted conditions (Clifford *et al.*, 1993), as was observed by Rogers *et al.* (1984) on *Glycine max*. Other studies have shown that even in well-watered plants Ψ was less negative (Sionit & Patterson, 1985) or unchanged (Paez *et al.*, 1983, 1984) in elevated [CO_2] than in ambient [CO_2].

However, tolerance to water stress of cherry seedlings was not improved in response to long-term growth in elevated [CO_2]. Values of Ψ_s of elevated [CO_2]-grown

cherry seedlings were decreased in both water regimes (Figure 6.7), but no differences were found between [CO_2] treatments in any of the other physiological parameters (i.e., π_{100}, π_0, R_0, ε_B, and DM/TM ratio) which contribute to confer increased water stress tolerance (Table 6.2). Moreover, midday Ψ of stressed seedlings declined more rapidly in elevated [CO_2] than in ambient [CO_2] during the first drying cycle. It can be argued that midday Ψ depends strongly on environmental variables and consequently is subjected to much short-term variability, whereas pre-dawn Ψ, by approaching equilibrium with the effective soil water potential over night, is a more appropriate measure of plant water status (Jones, 1992). When pre-dawn Ψ was measured, on 103 (34 dae days from the onset of drought), there were no significant differences between [CO_2] treatments (Figure 6.11b).

The reduction in g_s in well-watered seedlings grown in elevated [CO_2] was not caused by a decrease in stomatal density, which was also unaffected by water regime (Table 6.1). Similarly, stomatal density of *Quercus petraea* and *Pinus pinaster* seedlings was not altered by either elevated [CO_2] or water stress (Guehl *et al.*, 1994). Elevated [CO_2] did not significantly reduce values of g_s in droughted seedlings, which were, on the contrary, highly correlated with soil water status (Figure 6.9), more so than with plant water status (Appendix 5, Figures 3 and 4). Moreover, following rewatering of the water-stressed plants, leaf water potential recovered after eight days to that of the unstressed seedlings, whereas g_s did not (Figure 6.7). Recently it has been widely shown that g_s is more closely related to soil water content than Ψ or Ψ_s (e.g. Jones, 1992; Khalil & Grace, 1992). That stomata respond more closely to root than to shoot events may result from a root-sourced signal (e.g. ABA) being transported in the transpiration stream to the shoot and causing stomatal closure (Davies & Zhang, 1991; Khalil & Grace, 1993; Tardieu & Davies, 1993).

Increased A in elevated [CO_2] resulted in considerable enhancement of ITE over both growing seasons. In general, a higher ITE may help plant growth in dry

environments where water availability is scarce. However, as has been put forward several times (e.g. Jarvis, 1993; Morison, 1993; Drake *et al.*, 1997), higher ITE per unit of leaf area may be partly offset on a plant scale in elevated [CO_2], because of the increase in leaf area. Moreover, if decrease in g_s reduces E, this will change the leaf energy balance leading to higher leaf temperature (e.g. Pinter *et al.*, 1996), which will in turn increase the vapour pressure difference through the leaf surface, and will partially offset the impact of stomatal closure on leaf transpiration. Because several feedbacks contribute to control whole-plant transpiration, it is possibly not surprising that soil water content was not affected by elevated [CO_2], and that plant WUE was almost unchanged, although ITE increased (Figure 6.6).

In Chapter 5 it was shown that plant WUE was highly affected by elevated [CO_2] at each harvest date, but that the total amount of water loss did not differ between ambient and elevated [CO_2], and consequently that elevated [CO_2] did not ameliorate the long-term growth response of the cherry seedlings to water stress. This is in keeping with the finding that elevated [CO_2] did not improve plant water relations through any of the parameters measured (Ψ, and the parameters derived from the pressure-curve analysis - Table 6.2) other than Ψ_s. However, Ψ_s by increasing turgor affects leaf expansion (Morison, 1993), and thus may concur in increasing leaf area development in elevated [CO_2] from the very early stages of growth. Increased leaf area, in turn, affects the amount of water consumption. Indeed, leaf area of the cherry seedlings was increased by elevated [CO_2] in the first growing season (Figure 5.6), and this could explain to some extent the lack of effect of elevated [CO_2] on total water taken-up by the water-stressed seedlings during the first drying cycle (Figure 6.7 and Figure 5.16). However, g_s was not significantly reduced by elevated [CO_2] in the water-stressed plants, and leaf area was not affected by [CO_2] treatment in the second growing season.

Water flux through the soil-plant-atmosphere-continuum is proportional to the drop in water potential across the plant and the hydraulic conductance, and depends on leaf area, stomatal and aerodynamic conductance of the leaves, and on the radiation absorbed and local atmospheric vapour pressure deficit (Monteith & Unsworth,

1990). The degree of soil exploration by roots affects water uptake, but there was no difference in soil water status, expressed gravimetrically as soil water content, between the ambient and elevated [CO_2] treatments (Figure 6.7). Consequently, it is likely that the degree of exploitation of soil volume by the plants was similar, and consequently root and soil resistances in the soil-plant-atmosphere pathway are unlikely to have been different in ambient and elevated [CO_2] treatments.

However, the slope (S) of the relationship between leaf area and cross-sectional area of sapwood of the water-stressed seedlings was less steep in elevated [CO_2], indicating that less leaf area was supported per unit area of conducting tissue (Appendix 4, Figure 3b). As Whitehead & Jarvis (1981) pointed out S is affected by the properties of the wood (i.e. hydraulic conductance), plant height, gradient of Ψ, and transpiration rate which in turn is related to the ratios of stomatal to boundary-layer conductances and to properties of the climate expressed as a climatological resistance (i.e. the ratio of VPD to net radiation). All else equal, the higher the transpiration rate, the smaller S. However, in Figure 3b (Appendix 4) two different patterns can be seen: the basal area less than 1 cm^2, i.e. growth during the first growing season, and the basal area larger than 1 cm^2, i.e. growth in the second growing season. In the first growing season S does not change, the reduced transpiration rates per unit of leaf area in elevated [CO_2] were counterbalanced by the increased leaf area and height. Therefore, the hydraulic conductance is likely to have been similar in the different [CO_2] treatments so that the size and diameter of the xylem vessels was not affected by elevated [CO_2].

However, in the second year of growth S was affected by elevated [CO_2], but height, leaf area, and g_s were not (see Chapter 5). Since there were no systematic differences in air temperature inside the OTCs and air was supplied to the OTCs at the same flow rate (three air changes per minute, see Chapter 2), one might expect that there were also no differences in VPD inside the OTCs, or in the boundary-layer conductances and in the climatological resistances. Therefore, the hydraulic conductance was reduced by elevated [CO_2] in water stress conditions. Thus, the size and diameter of the xylem vessels may have been influenced by the

combination of [CO_2] and water stress. In the recent paper Heath *et al.* (1997) have measured the hydraulic conductance of *Fagus sylvatica* and *Quercus robur* after three years of growth in elevated [CO_2]. They found that the whole-shoot hydraulic conductance was reduced, although not significantly, in *Q. robur* but not in *F. sylvatica*. The authors explained these results in term of coordination between maximum transpiration rate and hydraulic conductance, since during the previous year of growth, g_s of *Q. robur* was decreased in elevated [CO_2] during drought, whereas on the contrary g_s of *F. sylvatica* was increased.

Temperature and humidity gradients through the leaf boundary layers and atmospheric surface layer depend on the ratios of leaf stomatal to boundary-layer conductances and canopy to bulk air transfer conductances (McNaughton & Jarvis, 1991). These ratios are expressed as the degree of 'coupling' between the plants and the atmosphere, i.e. the effectiveness of vapour water and energy transfer, which is inversely related to the convective decoupling coefficient, the Ω factor (Jarvis, 1985; Jarvis & McNaughton, 1986). The Ω factor in OTCs (with three air changes per minute) is large (~0.8 for the large leaves in the watered treatment, and ~0.6 in the water-stressed treatment), and therefore the net radiation absorbed is the major driving force for transpiration (Jarvis, 1985). Thus VPD at the leaf surface does not change much with stomatal movement and transpiration is rather insensitive to small changes in stomatal conductance (McNaughton & Jarvis, 1991; Jarvis, 1993). This may explain the lack of effect of elevated [CO_2] on total plant transpiration, which was similar at each harvest in ambient and elevated [CO_2] in both water treatments (Figure 6.7; Figure 5.16 and Table 5.6), although in the well-watered plants leaf area was not affected and stomatal conductance was significantly reduced by elevated [CO_2].

Elevated [CO_2] neither increased water stress tolerance of cherry seedlings, nor had any particular beneficial effect on long-term growth in drought conditions (Chapter 5). Recent studies have shown that some trees may undergo higher risk of damage in drying soils in elevated [CO_2] than ambient [CO_2] (Kerstiens *et al.*, 1995; Beerling *et al.*, 1996; Heath & Kerstiens, 1997). Deciduous trees in woodland have a

relatively large value of Ω as in the OTCs (Jarvis & McNaughton, 1986) with a relatively large ratio of canopy to surface-layer conductances at canopy scale. As a result transpiration here also is likely to depend more on net radiation than on VPD and decrease in stomatal conductance brought about by elevated [CO_2] (McNaughton & Jarvis, 1991; Jarvis, 1993). Considering the predicted increase in temperature associated with [CO_2] build-up in the atmosphere, and that elevated [CO_2] may have a little impact upon transpiration of cherry, it is possible to conclude that in dry environments elevated [CO_2] may have a far lower impact on plant WUE of cherry than one may deduce from the literature.

CHAPTER 7

Growth in Elevated CO_2 Concentrations: a Long-term Effect or a Short-term Response?

7.1 Introduction

A doubling of the atmospheric CO_2 concentration increases C_3 plant growth by an average of 30 to 40%; in terms of agricultural marketable yield there is a 33% increase (Kimball, 1983); over a range of 156 species there is an increase of 37% of the vegetative biomass (Poorter, 1993) and tree biomass increases by about 40% (Eamus & Jarvis, 1989; Jarvis, 1989; Lee & Jarvis, 1996). However, the increase in CO_2 assimilation rate brought about by elevated atmospheric [CO_2] is often in the range of 60 to 80% (Luxmoore *et al.*, 1993; Ceulemans & Mousseau, 1994, Lee & Jarvis, 1996). Thus, even taking into account losses of assimilated CO_2 through respiration, the increase in photosynthesis exceeds the increase that would be needed to support the average growth increment found in the above reports.

During the phase of exponential growth plants are generally source-limited, since only part of the incident radiation is intercepted by the plant canopy. Thus, at this stage because of the 'compound interest law' (Blackman, 1919), even small differences in the rate of leaf growth, and hence in the size of the assimilatory system, can have large repercussions on the production of total biomass over time (Hsiao, 1982, 1994). According to Hsiao's model (1982), the phase of exponential growth ends when canopy closure is reached, and almost all the available photon flux reaching the canopy is intercepted. At this time, the compound interest of growth is lost and as a result biomass production is no longer determined by relative growth rate (RGR) of the leaves, but becomes proportional to canopy assimilation rate. Therefore, when plants are in the phase of exponential growth more emphasis should be placed on RGR of the leaves than on photosynthesis, but after canopy closure more emphasis should be placed on photosynthetic capacity.

Classical growth analysis, as elaborated by the British school (e.g. Kvèt *et al.*, 1971; Evans, 1972), can be regarded as an established standard method for analysing the effects of environmental variables on plant production, and, therefore, can be applied to estimate the impact of climate change on plant growth and biomass allocation (Bazzaz, 1993). The most appropriate growth characteristic enabling analysis of net photosynthetic production of plants in relation to environmental variables is RGR, the rate of dry matter increase per unit of dry mass (M) present per unit of time, which is independent of the number of growing plants per unit of ground area. Mean RGR over a time interval from t_1 to t_2 of two consecutive harvests (M_1 and M_2) is traditionally given by: $R = (\ln M_2 - \ln M_1) / (t_2 - t_1)$, where RGR is a proportionality factor which, using the 'monetary analogy' (Evans, 1972), corresponds to the rate of interest at which the initial 'capital' has to be invested to obtain the final 'capital' gain (Kvèt *et al.*, 1971).

Reviews have reported that elevated [CO_2] increases plant growth (e.g. Cure & Acock, 1986; Mortensen, 1987; Eamus & Jarvis, 1989; Rogers & Dahlman, 1993; Idso & Idso, 1994), and consequently mean RGR over the experimental period (i.e. between the initial and the final harvest) must be higher in elevated [CO_2] than in ambient [CO_2]. However, it is widely believed that the increased growth in elevated CO_2 concentrations results mainly from an initial stimulation, which may decline and even disappear over time. Enoch (1990), reviewing crop response to increasing [CO_2], hypothesised the existence of either a trigger or a threshold effect on growth. Furthermore, evidence of a faster decline in mean RGR (measured between two consecutive harvests as the experiment proceeds) in elevated [CO_2] than in ambient [CO_2] has been reported (e.g., Bazzaz, 1990; Bazzaz, 1993; Poorter, 1993).

A differential decline in mean RGR was also found when processing data from the FACE (free-air carbon dioxide enrichment) cotton project (Mauney *et al.*, 1994). The relative growth rates of cotton plants in the FACE and control treatments became similar 53 days after the beginning of CO_2 enrichment (81 days after seedling emergence) in 1989, 11 days (12 days after seedling emergence) in 1990 and 20 days (20 days after seedling emergence) in 1991.

Poorter (1993) factored R_T into NAR (net assimilation rate) and LAR (leaf area ratio), and then factored LAR into SLA (specific leaf area) and LMR (leaf mass ratio), and found that a decrease in SLA in elevated [CO_2] partly offset the increase in NAR. Lambers & Poorter (1992), reviewing the inherent variation of R_T between fast-growing and slow-growing plants, found that SLA is highly correlated with LAR, and that the latter outweighed NAR in explaining inherent variation in R_T. They concluded that LAR, the amount of plant biomass needed in order to produce the same LA (leaf area), is an important factor determining the potential growth of a plant, as found much earlier (e.g., Blackman & Wilson, 1951; Hughes & Evans, 1962).

In general, higher values of LAR occur in fast-growing plants than in slow-growing plants. However, plants grown in elevated [CO_2] usually have a lower SLA than in ambient [CO_2] mainly because of accumulation of starch in leaves, whereas NAR is always higher. The rate of CO_2 assimilation per unit of LA is also higher, but proportionally less biomass is invested in leaf area and therefore in new photosynthetic systems (e.g. in relation to a doubling of photosynthesis, there is only 40% more LA). However, accumulation of starch in leaves can be seen as storage of assimilates which occurs when the flux of new assimilate production exceeds the flux of assimilates loaded into the phloem (Körner *et al.*, 1995) and exported for growth and formation of reserve and defence compounds, and therefore should not be regarded as in competition with growth (Chapin *et al.*, 1990; Stitt & Schulze, 1994; Schulze & Stitt, 1995). In addition, starch accumulation reducing the size of the cytoplasmatic pool of sugars, can to some extent decrease the fine control (end-product feedback), which regulates metabolism, and the coarse control (gene expression), which determines the amount of photosynthetic systems (Farrar, 1992). Thus, starch accumulation in chloroplasts, as long as it does not reach levels which may damage thylakoid membranes, cannot be a major factor in the down-regulation of photosynthesis occurring in elevated [CO_2] conditions.

Elevated [CO_2]-grown plants have traits of both fast- and slow-growing plants. These traits include: higher NAR, thicker leaves (extra layers of mesophyll cells in some

species), higher photosynthetic nitrogen use efficiency, reduced chlorophyll content per unit area, and lower SLA and, therefore, LAR (Lambers & Poorter, 1992; Ceulemans & Mousseau, 1994). Thus, less organic nitrogen is invested in photosynthetic proteins (mainly Rubisco) and in light-harvesting complexes in heavier leaves as a consequence of acclimation to more favourable growth conditions (Lambers & Poorter, 1992), and, therefore, the specific maintenance respiration resulting from protein turnover of the photosynthetic system should be reduced (Amthor, 1995).

Elevated [CO_2]-grown plants also have similar traits to plants grown under high photosynthetic photon flux density (Fichtner *et al.*, 1994) including: higher photosynthetic rates, which lead to increased carbohydrate production, more starch accumulation, higher NAR, thicker leaves, and a shift in the allocation of biomass from shoot to root. These similar traits are presumably the common result of enhanced CO_2 assimilation in high photosynthetic photon flux density and atmospheric CO_2 concentration. All these traits would allow more rapid exploitation of gaps within forests and establishment of young seedlings. Conversely, more rapid growth will lead to more rapid canopy closure thus limiting growth and establishment of new tree seedlings. In other words the process of ecological succession may be accelerated and those plants best able to use the extra CO_2 will come to dominate the ecosystem.

The question that this study addresses is whether the increase in total biomass brought about by enhanced [CO_2] is a long-term or a transient effect under non-limiting conditions, i.e. whether the differential compound interest of growth between elevated and ambient [CO_2] plants that occurs during early exponential growth is maintained or declines. The present study used classic growth analysis to examine the effect of long-term exposure to ambient (~350 $\mu mol\ mol^{-1}$) or elevated (ambient + ~350 $\mu mol\ mol^{-1}$) CO_2 concentrations on four clones of Sitka spruce and cherry saplings grown in open top chambers.

7.2 Materials and Methods

Full details of the four clones of Sitka spruce and cherry seedlings, growth conditions, number of harvests made, and the statistical analyses used to test the data are given in Chapter 2, 3, and 5.

The number of Sitka spruce harvested varied between 20 to 40 per [CO_2] treatment depending on harvest date. In cherry, the number of plants harvested was six per [CO_2] treatment for the baseline and final harvests, and three per [CO_2] treatment for all the intermediate harvests. Each plant was divided into leaf, stem (including branches), and root and then oven dried for 48 h at 70 °C to give dry mass (M). Leaf area (LA) of cherry was measured using a leaf area meter (LI 3100, LI-COR Inc., Lincoln, NE, USA).

Classical growth analysis (Kvèt *et. al.*, 1971; Evans, 1972) was applied to both species and current mean relative growth rates were calculated for total dry mass (R_T) and leaf dry mass (R_L) between two consecutive harvests, and long term mean relative growth rates for total dry mass and leaf dry mass (R_T^t and R_L^t, respectively) between each harvest and the baseline harvest. Cherry R_T^t was broken down into NAR (amount of biomass produced per unit of LA), and LAR (amount of LA per unit of plant dry mass, i.e. the size of assimilatory apparatus per unit of plant dry mass). LAR can be equated with the product of SLA and LMR, where SLA (LA per unit of leaf dry mass) is a measure of leaf density and thickness, and LMR (leaf dry mass per unit of plant dry mass) is the proportion of the total dry mass in the leaves. NAR is regarded as being the functional component and LAR the morphological component of R_T (Evans, 1972), but, as Lambers & Poorter (1992) pointed out, the two growth characteristics are not completely independent but, on the contrary, are to some extent mutually dependent.

The NAR of cherry saplings was calculated, according to Kvèt *et. al.*, (1971), as follows:

$[(M_2 - M_1) / (t_2 - t_1)] \cdot [(L_{A2}^{\alpha-1} - L_{A1}^{\alpha-1}) / (L_{A2}^{\alpha} - L_{A1}^{\alpha})] \cdot [\alpha / (\alpha - 1)]$

where L_{A1} and L_{A2} are the values of mean leaf area at the harvest carried out at times t_1 and t_2, respectively, α is equal to the ratio R_T to R_A (mean relative growth rate of leaf area), and was calculated as the slope of the linear regression plotted between $\ln D_M$ and $\ln L_A$ of the elevated ($R^2 = 0.995$, $\alpha = 1.11$) and ambient ($R^2 = 0.994$, $\alpha = 1.09$) [CO_2] treatments, and of the outside control ($R^2 = 0.997$, $\alpha = 1.08$).

Since no significant inter-chamber effect on growth of both clonal Sitka spruce and cherry saplings was found in each [CO_2] treatment (see Chapter 3 and 5), a two way analysis of variance (ANOVA) was used to determine effects of the [CO_2] treatments and time on the cherry LAR and SLA, and on both current and long term RGR of the Sitka spruce saplings, averaged across the four clones mean, i.e. R_T, R_L, R_T^t, and R_L^t. No replicates of current and long term RGR were available for cherry and for the four clones of Sitka spruce. Thus, in order to determine differences of these growth characteristics in response to the [CO_2] treatments, the variance of mean current and long term RGRs (S_R^2) was calculated according to Kvèt *et. al.*, (1971) as follows:

$S_R^2 = [(S_{M1}^2 / M_1^2) + (S_{M2}^2 / M_2^2)] / (t_2 - t_1)^2$,

where S_{M1}^2 and S_{M2}^2 are the variances of the primary values M_1 and M_2, respectively. The treatment means of total and leaf dry mass of both cherry and Sitka spruce were compared using Duncan's multiple range test.

7.3 Results

Total dry mass and leaf dry mass of Sitka spruce saplings were significantly higher in elevated [CO_2] (*ca* 40% for total dry mass and *ca* 34% for leaf dry mass) than in ambient [CO_2] at each harvest throughout the experimental period (Table 7.1). However, the logarithmic plots in Figure 7.1, the slopes of which are RGRs, show that during the first year the relative growth rates of both total (Figure 7.1a) and leaf (Figure 7.1b) dry mass were larger in elevated [CO_2] than in ambient [CO_2], but that subsequently the distance between the two curves remained constant (i.e. with the

same slope) indicating that both R_T and R_L for the two treatments did not differ. The long term relative growth rates, R_T^t (Figure 7.2a) and R_L^t (Figure 7.2b), were always significantly higher at each harvest in elevated [CO_2]-grown than in ambient [CO_2]-grown plants. By contrast, after the first interval of growth (days 1-382), both the current R_T and R_L were not significantly different between CO_2 treatments and remained unchanged for the rest of the experimental period, even when calculated over long periods, i.e. the period 382-972 (Table 7.2). This trend was consistent for both current and long term relative growth rate in each of the four clones of Sitka spruce (Table 7.3).

Table 7.1 Percentage increase in leaf and total dry mass of the Sitka spruce saplings in response to elevated [CO_2], shown as days from the beginning of the [CO_2] exposure. Data are means of 20 to 40 plants per treatment.

days	leaf	total
382	33 $P < 0.01$	45 $P < 0.001$
551	33 $P < 0.01$	44 $P < 0.001$
719	34 $P < 0.05$	37 $P < 0.05$
972	35 $P < 0.001$	41 $P < 0.001$

Total dry mass of cherry saplings was almost always significantly higher in elevated [CO_2] than in ambient [CO_2] or in the outside control (Table 7.4). However, after an initial stimulation, the increase in total dry mass began declining relative to the ambient [CO_2] saplings. The increase in total biomass in elevated [CO_2] with respect to outside control, showed a similar pattern during the first growing season, but another remarkable increase was recorded on 212 dae in the second growing season. At the end of the experiment, enhanced [CO_2] significantly increased the total dry mass produced of about 39% and 62% compared to that of the ambient [CO_2] and the outside control treatments, respectively. A similar trend was seen in leaf dry mass

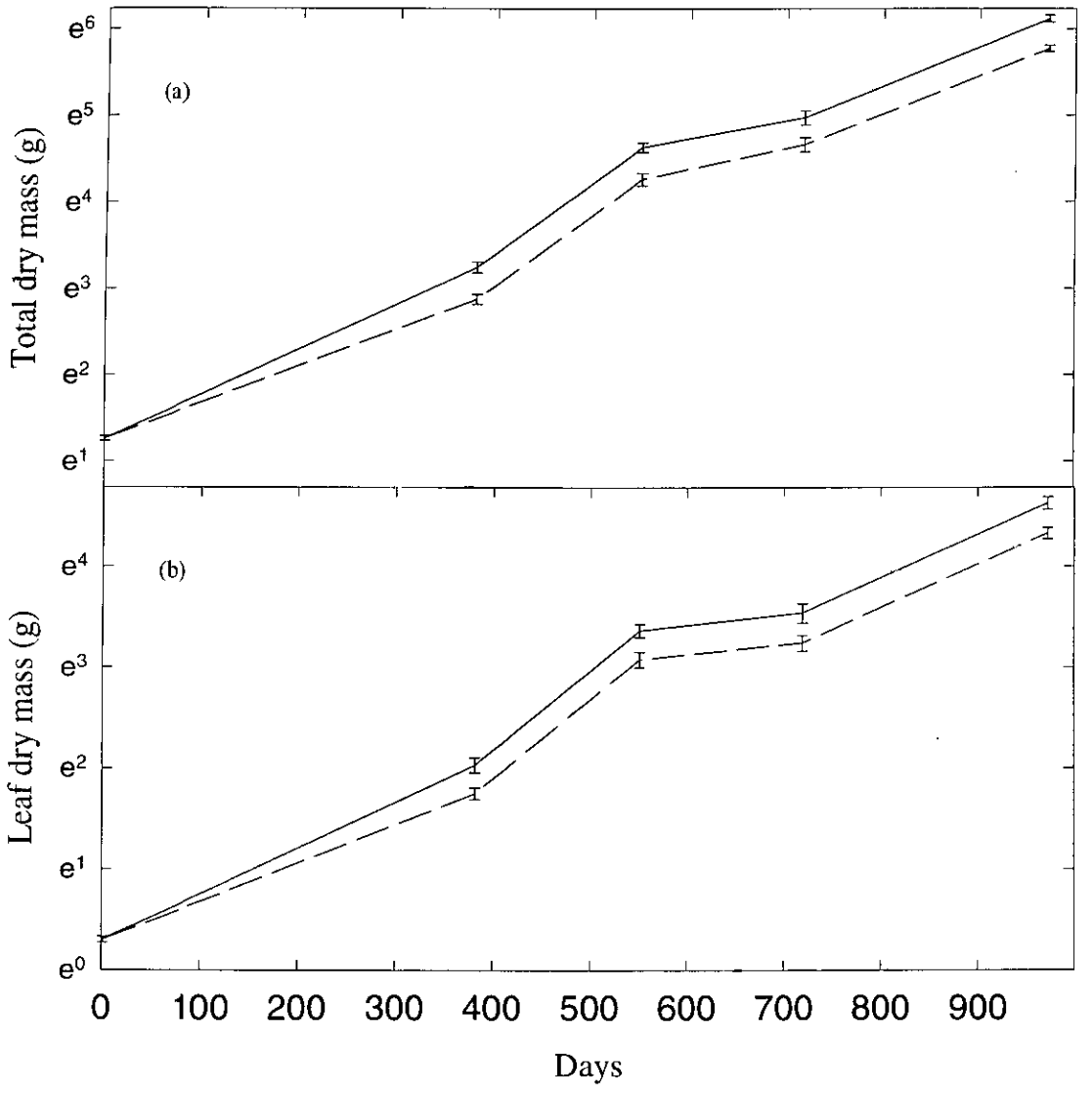

Figure 7.1 Combined exponential total dry mass (a) and leaf dry mass (b) of the Sitka spruce saplings *versus* days of exposure to ambient [CO_2] (---) or elevated [CO_2] (—); the dry mass data are plotted on a logarithmic scale so that the slopes of the lines show the relative growth rates. Data are means of 20 to 40 plants per treatment ± 1 SEM.

production in response to elevated [CO_2] (Table 7.4). However, in general, the relative increase in leaf dry mass in elevated [CO_2] was lower than that of total

biomass after 33 dae. In addition, the percentage increases in leaf dry mass resulting from CO_2 enrichment were not significant at the end of the experiment. The larger difference in total and leaf dry mass during, rather than at the end of, the growing season between the trees in the chambers and those of the outside control may be a consequence of slower phenological development in the outside control (i.e. a temperature effect).

Table 7.2 Mean current relative growth rate (mg $g^{-1}d^{-1}$) of total (R_T) and leaf (R_L) dry mass of the Sitka spruce saplings grown in ambient (~350 µmol mol^{-1} - amb) or elevated (~700 µmol mol^{-1} - elv) [CO_2], for three periods from the beginning of the [CO_2] exposure. Data are means of four plants per treatment ± 1 SEM (calculated across the four clones), ns = not significant.

	total dry mass RGR			leaf dry mass RGR		
days	elv	amb		elv	amb	
382-551	8.22 ± 0.38	8.12 ± 0.53		7.88 ± 0.34	7.87 ± 0.36	
551-719	2.24 ± 0.28	2.28 ± 0.55		1.02 ± 0.36	0.96 ± 0.14	
719-972	4.37 ± 0.03	4.50 ± 0.16		4.32 ± 0.14	4.30 ± 0.33	
Statistical significance ($P > F$)						
CO_2		ns			ns	
Time		< 0.001			< 0.001	
Interaction		ns			ns	
382-972	4.86 ± 0.07	ns	4.91 ± 0.02	4.40 ± 0.05	ns	4.37 ± 0.08

Since there were several sequential harvests in the first year after seedling emergence, it is possible to see that the differences between treatments of R_T (Figure 7.3a) and R_L (Figure 7.3b) were set at a very early stage during the first month after emergence (i.e. by 33 dae), when the trees were less than 20 cm high and had only six or seven leaves. At this time radiation was not attenuated by overlapping leaves and, therefore, shading was not a factor influencing growth in either treatment. Biomass accumulation of cherry (Figure 7.3a,b) and, therefore, R_T and R_L (Table 7.5), then followed the same parallel pattern as did Sitka spruce for the remainder of the first and for the whole of the second growing season. When calculated on a long-term basis, i.e. the period 33-251 dae, R_T did not differ among the treatments, whereas R_L was even significantly lower in elevated [CO_2] than in ambient [CO_2] (Table 7.5). On the other hand, mean long-term relative growth rates of cherry

saplings, R_T^t and R_L^t, were significantly higher in elevated [CO_2] than in ambient [CO_2] both in the OTCs and in the outside control (Figure 7.4). Current and long term NAR did respond positively to doubling the concentrations of CO_2 (Table 7.6) whereas, by contrast, LAR and SLA were negatively affected ($P < 0.001$) (Table 7.7).

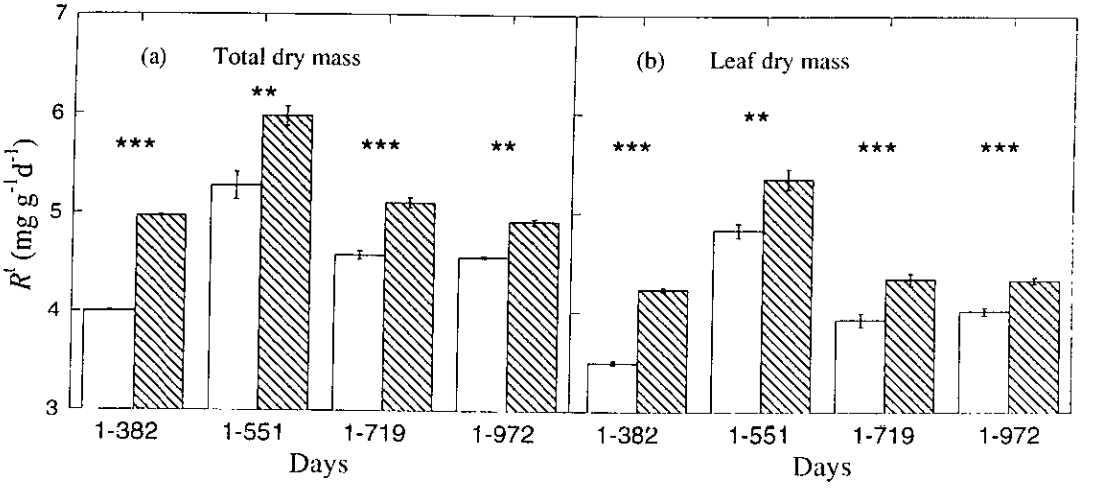

Figure 7.2 Combined mean long term relative growth rates of (a) total dry mass (R_T^t) and (b) leaf dry mass (R_L^t) of the Sitka spruce saplings *versus* days of exposure (period between each harvest date and the baseline harvest made on day 1) to ambient (▭) or elevated [CO_2] (▨). Data are means of the long term relative growth rate of the 4 clones per [CO_2] treatment ± 1 SEM, and the significance levels (** = P<0.01, *** = P<0.001) show the difference in response to the [CO_2] treatments.

Although the Sitka spruce and cherry trees were large, well-branched and leafed at the end of the experiment, they only experienced a small amount of self-shading and did not experience light-limitation because the experimental design and chamber design allowed unlimited side-light to reach the plants. However, there was an ontogenetic decline in growth by the end of each growing season in both species. Sitka spruce and cherry sapling R_T was affected by size of the plants only when the saplings were small: R_T plotted *versus* total dry mass was higher in elevated [CO_2] than in ambient [CO_2] only when less than about 20 to 25 g of total biomass had been produced (Figure 7.5).

Table 7.3 Mean current (R_T) and long term (R_T^t) relative growth rates of total dry mass (mg $g^{-1}d^{-1}$) of the four Sitka spruce clones grown in ambient (amb) or elevated (elv) [CO_2], as a function of days from the beginning of the [CO_2] exposure. Data are means of 5 to 10 plants per treatment ± S_R^2.

	Skidegate a		Skidegate b		North Bend a		North Bend b	
days	elv	amb	elv	amb	elv	amb	elv	amb
R_T								
551	7.6 ± 0.62	6.8 ± 0.41	9.1 ± 0.40	8.0 ± 0.97	7.5 ± 0.52	8.1 ± 1.06	8.5 ± 0.92	9.4 ± 0.77
719	2.5 ± 0.64	3.9 ± 0.75	1.9 ± 0.48	2.1 ± 0.75	2.8 ± 1.00	2.5 ± 1.51	1.6 ± 1.25	1.3 ± 1.19
972	4.4 ± 0.14	4.2 ± 0.21	4.5 ± 0.12	4.9 ± 0.17	4.4 ± 0.31	4.3 ± 0.41	4.5 ± 0.37	4.2 ± 0.29
382-972	4.7 ± 0.02	4.9 ± 0.01	5.1 ± 0.05	5.0 ± 0.05	4.9 ± 0.02	4.9 ± 0.04	4.8 ± 0.04	4.9 ± 0.02
R_T^t								
1-382	4.8 ± 0.17	4.0 ± 0.13	4.5 ± 0.18	3.9 ± 0.26	5.2 ± 0.17	3.9 ± 0.20	5.4 ± 0.21	4.2 ± 0.16
1-551	5.7 ± 0.09	4.9 ± 0.09	5.9 ± 0.10	5.1 ± 0.12	5.9 ± 0.09	5.2 ± 0.12	6.3 ± 0.12	5.8 ± 0.12
1-719	4.9 ± 0.05	4.7 ± 0.05	5.0 ± 0.06	4.4 ± 0.06	5.2 ± 0.07	4.6 ± 0.08	5.2 ± 0.08	4.8 ± 0.07
1-972	4.8 ± 0.02	4.6 ± 0.02	4.8 ± 0.03	4.5 ± 0.03	5.0 ± 0.02	4.5 ± 0.02	5.0 ± 0.02	4.6 ± 0.02

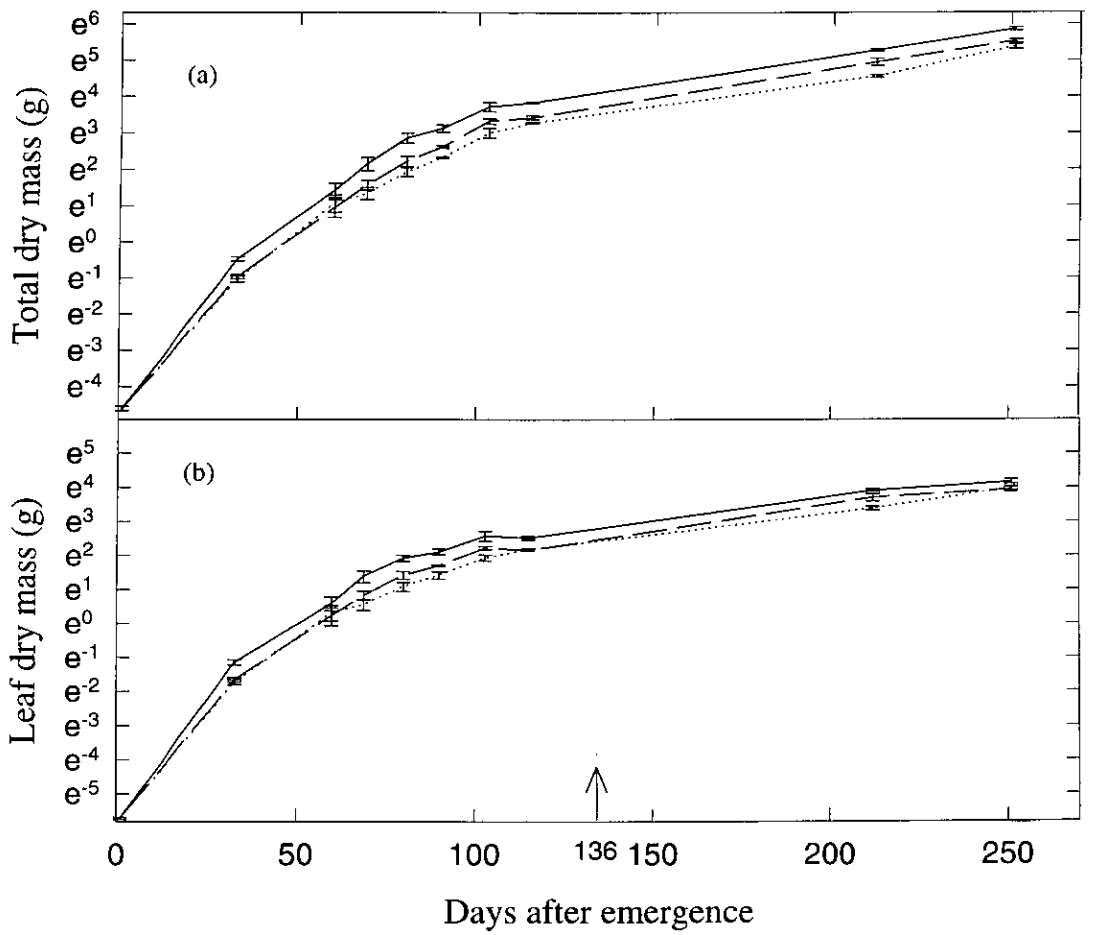

Figure 7.3 Exponential total dry mass (a) and leaf dry mass (b) of cherry saplings *versus* days after emergence in ambient [CO_2] (---), elevated [CO_2] (—), or outside control (····). The dry mass data are plotted on a logarithmic scale so that the slopes of the lines show relative growth rates. The beginning of the second growing season is shown, for convenience, consecutively as dae 136; Data are means of 3 to 6 plants per treatment ± 1 SEM.

7.4 Discussion

No overall differences amongst the treatments were found in the proportion of dry mass allocated to the roots when plants are the same size (Chapter 3 and 5), and,

therefore, the saplings were not pot limited, regardless of [CO_2] treatment. Thus, both tree species were stress-free (adequate nutrition, water, pot space), but with growth departing from initial exponentiality as a result of ontogenenetic loss of totipotency.

Table 7.4 Percentage increase in leaf and total dry mass of the cherry saplings in response to elevated [CO_2] with respect to ambient [CO_2] (amb) and outside control (out) saplings, as a function of days after emergence (dae). Data are means of 3 to 6 plants per treatment, ns = not significant.

	amb		out	
dae	leaf	total	leaf	total
33	67 $P < 0.001$	64 $P < 0.001$	79 $P < 0.001$	75 $P < 0.001$
60	44 ns	62 ns	32 ns	42 ns
69	75 $P < 0.05$	79 $P < 0.05$	129 $P < 0.05$	124 $P < 0.05$
80	65 $P < 0.01$	91 $P < 0.05$	132 $P < 0.01$	155 $P < 0.05$
90	50 $P < 0.01$	66 $P < 0.01$	98 $P < 0.01$	124 $P < 0.01$
103	42 $P < 0.05$	48 $P < 0.05$	90 $P < 0.05$	105 $P < 0.05$
115	44 $P < 0.01$	50 $P < 0.001$	40 $P < 0.01$	74 $P < 0.001$
212	22 $P < 0.05$	39 $P < 0.05$	69 $P < 0.05$	107 $P < 0.05$
251	25 ns	39 $P < 0.001$	20 ns	62 $P < 0.001$

The saplings of Sitka spruce (Figure 7.1a) and cherry (Figure 7.3a) showed positive growth responses to elevated CO_2 during the experiment. The overall response to elevated [CO_2], in terms of total plant dry mass, increased throughout the experiments: at the end of the experiments both species were approximately 40% larger in elevated [CO_2] than in ambient [CO_2] (Table 7.1 and 4). As a result, the long term mean R_T^t and R_L^t were significantly higher in elevated [CO_2] in the saplings of Sitka spruce (Figure 7.2) and cherry (Figure 7.4). However, because of the differential decline in RGR with time, R_T and R_L of Sitka spruce (Table 7.2 and 7.3) and cherry (Table 7.5) saplings in elevated [CO_2] became the same as in ambient [CO_2]. As a result, in both Sitka spruce and cherry the large differences in total biomass at the end of the experiments were a consequence of the more rapid growth in the early phase of exposure to elevated [CO_2]. In fact, after this initial phase (namely the period 1-382 for Sitka spruce and 1-33 for cherry), mean R_T and R_L

measured on a long-term basis, i.e. until the end of the experiment (period 382-972 for Sitka spruce and 33-251 for cherry), were similar, or even lower in elevated [CO_2] than in ambient [CO_2] (Table 7.2 and 7.5). This pattern of growth was seen also in each of the four clones of Sitka spruce (Table 7.3).

Table 7.5 Mean current relative growth rate (mg $g^{-1}d^{-1}$) of total and leaf dry mass of the cherry saplings grown in ambient [CO_2] (~350 µmol mol^{-1}, - amb), in elevated (~700 µmol mol^{-1}, - elv) [CO_2], or as outside control (out) as a function of days after emergence (dae). 212 and 251 dae are the harvest days in the second growth season, which started on dae 136. Data are means of 3 to 6 plants per treatment $\pm S_R^2$.

	total dry mass R_T			leaf dry mass R_L		
dae	elv	amb	out	elv	amb	out
60	70.6 ± 0.2	70.9 ± 0.3	78.2 ± 0.3	64.3 ± 0.2	69.9 ± 0.3	75.5 ± 0.2
69	79.4 ± 2.6	70.0 ± 2.8	49.3 ± 3.1	88.9 ± 2.7	69.7 ± 3.0	47.4 ± 2.9
80	65.0 ± 1.3	59.8 ± 1.9	53.4 ± 1.0	48.7 ± 1.0	54.2 ± 0.8	47.8 ± 1.1
90	26.1 ± 0.9	40.1 ± 0.6	39.1 ± 0.5	18.4 ± 0.5	28.2 ± 0.4	34.1 ± 0.9
103	45.2 ± 0.5	53.9 ± 0.1	52.1 ± 0.3	36.1 ± 0.5	40.3 ± 0.1	39.2 ± 0.3
115	9.5 ± 0.3	8.9 ± 0.2	23.4 ± 0.4	4.9 ± 0.5	5.2 ± 0.1	20.7 ± 0.2
212	14.8 ± 0.1	15.6 ± 0.1	13.0 ± 0.1	13.0 ± 0.1	14.5 ± 0.1	12.3 ± 0.1
251	15.0 ± 0.1	15.0 ± 0.2	21.3 ± 0.2	6.8 ± 0.1	6.3 ± 0.1	15.4 ± 0.1
33-251	29.0 ± 0.1	29.8 ± 0.1	29.4 ± 0.1	24.4 ± 0.3	25.7 ± 0.1	26.2 ± 0.1

The decline of RGR in elevated [CO_2] to equal that in ambient [CO_2] with time, found for both cherry and Sitka spruce, raises an important question. Although both total plant growth and net assimilation rate remained higher in elevated than in ambient [CO_2], what combination of compensatory process leads to subsequent equality of relative growth rate in stress-free conditions?

The net photosynthetic rate (A) per unit leaf area of Sitka spruce grown and measured in elevated [CO_2] was about 67% higher during the second year, and about 62% higher during the third year, than for plants grown and measured in ambient CO_2. Measurements of A/C_i (C_i is the mean intercellular space CO_2 concentration) curves and of Rubisco activity showed a certain degree of down-regulation of photosynthesis in elevated [CO_2] in the third year (Chapter 4). Whereas, for cherry the net photosynthetic rate of elevated [CO_2]-grown plants was in the range of 40 to 140% higher throughout the first growing season and 79% higher during the second

140% higher throughout the first growing season and 79% higher during the second year of growth, than for the ambient [CO_2] plants. Measurements of A/C_i curves made during the second growing season, showed no down-regulation of photosynthesis per unit leaf area in plants grown in elevated [CO_2], although Rubisco activity was significantly reduced (Chapter 6).

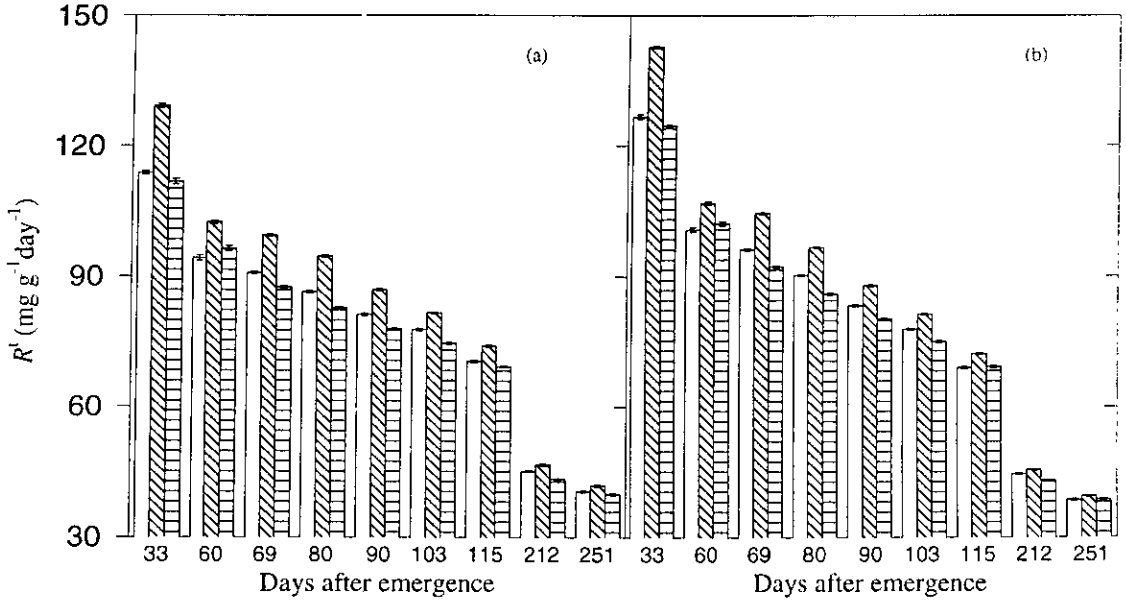

Figure 7.4 Mean long term relative growth rates of (a) total dry mass (R_T^t) and (b) leaf dry mass (R_L^t) of cherry saplings *versus* days after emergence (period between each harvest date and the baseline harvest made on day 1) to ambient [CO_2] (▭) and elevated [CO_2] (▨), or outside control (▭). 212 and 251 dae are the harvest days in the second growth season, which started on dae 136. Data are means of 3 to 6 plants per treatment ± S_R^2.

That increasing growth in elevated atmospheric CO_2 concentrations is less than the increase in photosynthesis has frequently been observed, as was found for both cherry and Sitka spruce in the present work. Evans (1994) found that at the end of the second year of growth of Sitka spruce seedlings in elevated [CO_2], differences in biomass production between the treatments had disappeared, although no evidence of down-regulation was shown by the elevated [CO_2]-grown plants. However (see reviews by Eamus and Jarvis, 1989; Luxmoore *et al.*, 1993; Ceulemans & Mousseau,

increase in respiration rate, change in starch accumulation, change in size of the cytoplasmic pool of sugars, increase in volatilisation and root exudation losses, fine root turnover, and nitrogen deficiency.

Table 7.6 Current and long term net assimilation rate, NAR (g $m^{-2}d^{-1}$) of the cherry saplings grown in ambient [CO_2] (amb), elevated (elv) [CO_2], or outside control (out) as a function of days after emergence (dae). Data are means of 3 to 6 plants per treatment.

dae	elv	amb	out
Current NAR			
60	7.13	5.39	6.87
69	8.84	6.22	2.85
80	7.27	5.60	5.60
90	3.07	4.05	4.30
103	6.75	6.79	6.59
115	1.86	1.35	3.80
212	3.00	2.46	2.05
251	3.87	2.91	3.78
33-251	6.33	5.18	4.65
Long term NAR			
1-33	10.32	7.73	9.10
1-60	9.33	6.89	7.89
1-69	8.56	7.35	7.64
1-80	8.16	6.66	7.49
1-90	7.84	6.97	7.15
1-103	10.27	8.44	8.25
1-115	10.72	9.54	9.75
1-212	6.27	4.78	4.70
1-251	8.36	6.82	5.98

Rhizodeposition products and respiratory CO_2 from the roots and associated micro-organisms can account for a large proportion of lost assimilates, even more than 40% of the dry matter produced (Lynch & Whipps, 1990). Norby *et al.* (1992) found that CO_2 efflux from the soil in elevated [CO_2] chambers, as a result of yellow-poplar fine root turnover, was about 24% higher than that from ambient [CO_2] chambers. Acceleration of some of these processes in elevated [CO_2] may be the cause of convergence of the relative growth rates in in elevated and ambient [CO_2]. However,

some of the processes mentioned above may not be the cause of the limitation to growth of stress-free plants in elevated [CO_2], but may in fact be the result of a 'limited' capacity to increase growth.

Table 7.7 Leaf area ratio (LAR) and specific leaf area (SLA) of the cherry saplings grown in ambient [CO_2], elevated [CO_2], or outside control (out); dae = days after emergence. Data are means of 3 to 6 plants per treatment ± 1 SEM.

	LAR ($cm^2\ g^{-1}$)			SLA ($cm^2\ g^{-1}$)		
dae	elv	amb	out	elv	amb	out
33	114.7 ± 4.4	147.4 ± 6.4	118.8 ± 5.5	227.9 ± 13.1	296.2 ± 11.3	237.8 ± 12.3
60	89.9 ± 4.8	118.1 ± 6.7	114.2 ± 7.2	208.8 ± 4.9	244.8 ± 7.9	236.7 ± 6.3
69	89.7 ± 4.8	104.4 ± 2.7	99.1 ± 6.9	192.8 ± 5.5	222.1 ± 6.1	216.6 ± 5.1
80	91.8 ± 10.7	107.9 ± 3.5	93.7 ± 9.1	227.3 ± 10.6	237.0 ± 8.1	216.3 ± 6.6
90	84.7 ± 8.5	92.7 ± 6.7	89.1 ± 6.5	231.7 ± 14.1	232.7 ± 16.0	223.1 ± 16.7
103	55.6 ± 6.3	70.0 ± 2.4	72.1 ± 4.5	177.8 ± 23.7	208.4 ± 4.4	205.1 ± 4.0
115	47.7 ± 1.1	55.1 ± 5.2	54.0 ± 5.5	177.6 ± 6.8	193.4 ± 12.3	160.7 ± 9.9
212	49.0 ± 4.3	67.1 ± 1.6	68.9 ± 1.3	192.5 ± 12.6	233.0 ± 6.8	222.7 ± 2.9
251	31.6 ± 1.4	40.1 ± 1.3	47.8 ± 1.3	172.8 ± 6.8	196.7 ± 5.3	193.1 ± 4.7
Statistical significance ($P > F$)						
CO_2		< 0.001			< 0.001	
Time		< 0.001			< 0.001	
Interaction		ns			0.104	

The convergence of RGR in elevated [CO_2] with that in ambient [CO_2] occurred when both Sitka spruce and cherry plants were not limited by self-shading. Although in cherry NAR (Table 7.6) was much higher in elevated [CO_2] throughout the experimental period, both LAR and SLA showed the opposite trend (Table 7). Even during the first period of rapid growth of cherry when R^t in elevated [CO_2] was much higher than in ambient [CO_2], the reductions in LAR and SLA amounted to about 30%. In general, RGR is highly correlated with LAR and SLA (Evans, 1972; Lambers & Poorter, 1992). Higher LAR indicates that a larger size of assimilatory apparatus is maintained per unit of plant dry mass. Similarly, higher SLA indicates an increase in the leaf area maintained per unit of leaf dry mass. Thus, higher LAR and SLA occur when the construction costs of the assimilatory apparatus is reduced. This is a typical compensatory response for declining growth with reduced carbon

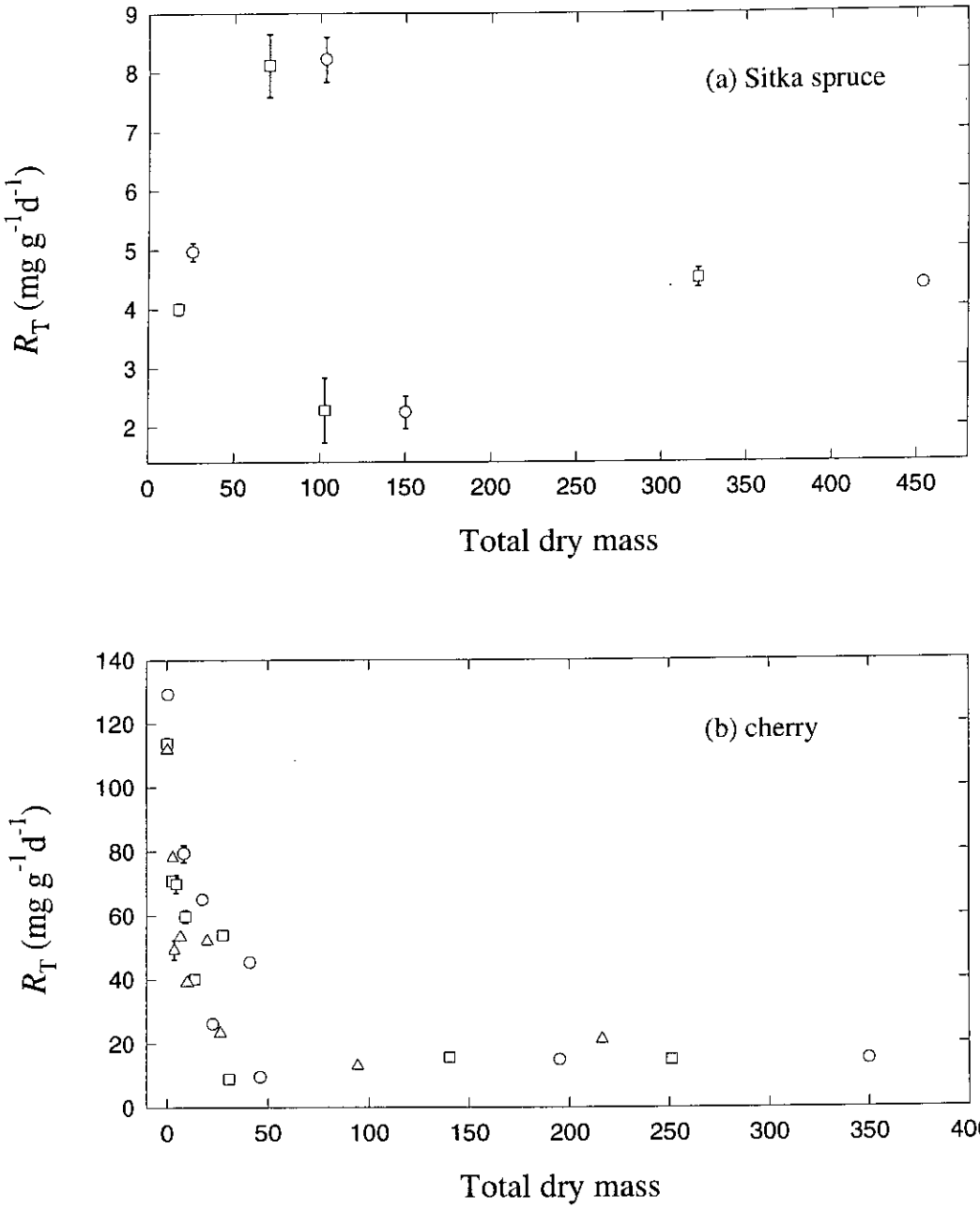

Figure 7.5 Mean current relative growth rate of total dry mass (R_T) of (a) the Sitka spruce and (b) the cherry saplings grown in ambient [CO_2] (□), elevated CO_2 (O), or outside control (only cherry - Δ), *versus* total dry mass. For Sitka spruce saplings data are means of 20 to 40 plants ± 1 SEM per treatment, whereas for cherry data are means of 3 to 6 plants per treatment ± S_R^2.

gain that results a from lower rate of photosynthesis (Fichtner *et al.*, 1994). The larger LAR and SLA observed in ambient [CO_2] saplings was a compensation by which they maintained RGR similar to that of the elevated [CO_2] saplings despite a lower NAR. Similar results were found by DeLucia *et al.* (1994): NAR of *Pinus ponderosa* seedlings was significantly increased by elevated [CO_2] but this was offset by a reduced LAR, resulting in similar RGRs of seedlings grown in ambient or elevated [CO_2].

Poorter (1993) pointed out that increase in plant size, with more biomass invested in support tissue, increases self-shading, which together with acclimation and size constraints, can totally offset, with time, the stimulation in growth rate of elevated [CO_2]-grown plants. Goudriaan (1994), using the expolinear growth equation investigated the apparent down-regulation of growth in elevated [CO_2] and concluded that the primary explanation for the differential decline in RGR is more mechanistic than functional: growth and hence shading, is so strongly accelerated that the reduction of RGR sets in much earlier. In other words, elevated [CO_2] leads to plants getting bigger quicker, with all the associated regulation that results progressively from increase in size.

However, our results demonstrate that the primary effect of elevated [CO_2] is an increase in the initial relative growth rate for a restricted period beyond which RGR is essentially similar for all treatments, the enhancement in elevated [CO_2] being lost. The explanation for this may be the faster decline in elevated [CO_2] of R_L (i.e., the relative growth rate of the size of the assimilatory apparatus). This was only detected after 382 days in Sitka spruce (Figure 7.1b), because of the lack of intermediate harvests, but became evident after only 33 dae in cherry saplings (Figure 7.3b) where more sequential harvests were carried out. Because cherry plants were still growing exponentially after 33 dae, leaf growth rate could well be the major factor determining final biomass accumulation (Hsiao, 1982, 1994).

For ontogenetic reasons, RGR declines as plants get larger and this is particularly so with young woody plants (Jarvis & Jarvis, 1964). The tissue are not all totipotent

with respect to cell division and in young trees in particular, mass becomes sequestered in dead structural and conducting tissues so that RGR falls dramatically as trees age (e.g. Rutter, 1957). Thus if young trees get bigger more quickly in elevated [CO_2], it is to be expected that this decline in RGR will be brought forward. In other words, to some extent there is a certain 'inevitability' (Gifford *et al.*, 1996) that RGR will be similar in young trees growing in elevated [CO_2] and in ambient [CO_2], when compared at the same time (Table 7.2-7.5). Conversely, it is possible that no difference in RGR will be evident if the comparison is made between plants of the same size (Evans, 1972), so long as allocation and developmental processes are not affected by the treatment. In fact, only during the early phase of exposure to CO_2 fertilisation, did elevated [CO_2]-grown saplings have a higher R_T, despite the much larger total dry mass (Figure 7.5). In contrast, R_T plotted *versus* total dry mass was similar in elevated [CO_2] and ambient [CO_2] for both Sitka spruce (Figure 7.5a) and cherry (Figure 7.5b) as the saplings grew larger. However, even when the relative growth rate of plants of similar dry mass is similar amongst treatments, the same question still arises: what are the reasons that the RGR is no longer affected by the treatments?

Since the maximum potential growth of a plant is genetically fixed, it is possible that in both the genetically identical clones (Sitka spruce) and the open-pollinated seedling cherry, after the initial phase when RGR was higher in elevated [CO_2] than in ambient [CO_2], the total plant photosynthetic production exceeded the potential needs of plant growth processes in elevated [CO_2]. Both Sitka spruce and cherry were supplied with adequate nutrition and water, and did nor suffer from root restriction. If there were other environmental limitations, they were natural constraints which all trees in this study were exposed to. The potential for growth may be the result of either natural selection made over evolutionary time scales or genetic manipulation made by plant breeders, both resulting in plants optimised for the environments in which they are grown. That is to say, the trade-offs amongst the current physiological processes of growth may not be balanced as climate change occurs. As a result, the sink constraints that are generally put forward to account for growth acclimation to elevated [CO_2] may actually be genetic growth limitations

which determine the maximum effect of elevated [CO_2]. Thus, as Amthor (1995) has pointed out, scientific improvements, namely molecular engineering, plant breeding and management practice, are the most important driving forces (much more than elevated [CO_2] concentrations) in improving plant productivity, and could be employed to balance better the different processes affecting growth and to optimise the internal constraints limiting plant response to rising [CO_2].

The reliability of [CO_2] studies performed in enclosed environments and on potted plants as a basis for conclusions on the effect of elevated [CO_2] on the long term growth of trees has been frequently raised (e.g. Eamus & Jarvis, 1989; Ceulemans & Mousseau, 1994; Amthor, 1995). However, following the conclusions of Mauney *et al.* (1994), drawn from a FACE experiment with field-grown cotton, it may be possible to claim that data on the effects of elevated [CO_2] on growth obtained from glasshouse and OTC experiments that were done mainly on potted plants can be "transferred to open field situations". Moreover, a recent study (Hättenschwiler *et al.*, 1997) found that annual stimulation of stem diameter of mature Mediterranean oaks, belonging to a natural forest stand grown for their entire life around natural CO_2 vents, declined from 80% when the oaks were five-years old to 20% when they were 30-years old, thus confirming the declining effect of elevated [CO_2] with tree age. Thus, the differential decline of RGR highlighted in this paper may be relevant for models predicting the magnitude of tree response to rising [CO_2].

CHAPTER 8

Concluding Remarks

Forest tree species can live from decades to centuries and will experience climate change, from rising [CO_2] and changed patterns of precipitation and evaporation, during their life-span. Because of the importance of trees as carbon sinks, their positive influence on the water cycle and feedbacks on local climate, many studies in the last 15 years have been undertaken on the effects of climate change on tree growth and physiology. However, the majority of these studies have focused on agricultural crops, and when trees were studied the results were found to be influenced by the length of the experiments, and inadequate nutrient supply and rooting volume. When the experiments described here were started these three 'factors' were taken into account. In the previous Chapters we have analysed the effects of elevated [CO_2] in conjunction with provenance of clonal Sitka spruce and with subsequent drying cycles in cherry. In Chapter 7 we have focused on the comparative growth of the two species, and have emphasised the importance of an initial stimulus which, compounded over time, magnifies the effect of elevated [CO_2] on growth. The following discussion places the results of the thesis into context.

In the first analysis, the photosynthetic capability of the two species was differently affected by elevated [CO_2]. A_{max} (Figure 4.1), Rubisco activity (Table 4.1), and total chlorophyll concentration (Tables 4.4) of Sitka spruce saplings were significantly decreased in elevated [CO_2] in the third growing season, whereas cherry showed downward acclimation of Rubisco activity only in both growing seasons (Figures 6.12a). The decline in Rubisco activity in cherry did not affect A_{max} (Figures 6.3 and 6.4), but in the second year in elevated [CO_2], Rubisco activity decreased from the beginning of the growing season. Thus, one might speculate that if the experiment had lasted longer (as for the Sitka spruce saplings, for instance), the inhibition of Rubisco activity may well have resulted in down-regulation of A.

However, as reported in Chapter 7, the percentage increase in *A* was higher than the percentage increase in growth in response to elevated [CO_2] in both Sitka spruce and cherry. This is another common trait between the two species, but it is also the usual response to growth in elevated [CO_2] (Luxmoore *et al.*, 1993; Norby *et al.*, 1996). Increased *A*, lower Rubisco content, and inhibition of photorespiration are probably the main reasons for the changes in chemical properties of the leaves in elevated [CO_2]. Higher *A* in elevated [CO_2] affects carbohydrate concentration of foliage, while decreased Rubisco and photorespiration, and in turn maintenance respiration, reduce leaf nitrogen concentration (Koch & Mooney, 1996). Because both leaf starch and N concentration were significantly affected during the growing seasons in Sitka spruce and cherry, both species had a higher NUE in elevated [CO_2] than in ambient [CO_2].

The experiments on the two species were totally different in all respect other than the supply and timing of nutrients (i.e. genetics, tissue age, provenance, soil, and, especially, temperature, see Chapter 2). However, having ruled out any nutrient and rooting volume limitations, which would have caused several artifacts, it is possible to conclude that long-term exposure to elevated [CO_2] had similar impacts on the physiology of Sitka spruce and cherry. Moreover, growth of both species, clonal Sitka spruce and cherry, was similarly affected by elevated [CO_2] at the end of the experiments. The total plant dry mass increase in elevated [CO_2] relative to ambient [CO_2] was approximately 40% (Table 7.1 and 7.4). However, the increase in total dry mass in Sitka spruce was the average of the increase in all four clones in response to elevated [CO_2], and the genetic differences in the growth response to elevated [CO_2] were significant. The more southerly clones were significantly larger than the more northerly in elevated [CO_2]. Since the clones originated from different latitudinal provenances, and were acclimated to different temperature and daylength climates, it is possibly not surprising that they had different abilities to adapt to their new environment, even if all the four clones had been selected for their forestry potential and, thus, were fast-growing genotypes. Since there is variation amongst Sitka spruce clones in response to elevated [CO_2] it may be possible that the similarities in mean Sitka spruce response and cherry response are coincidental.

However, there were two further traits common to both Sitka spruce and cherry. Firstly, allocation was not affected by elevated [CO_2]: when plants of both species were grown in elevated and ambient [CO_2] and compared when they were the same size, the allocation of biomass to each plant component was similar (see Chapters 3 and 5). Secondly, after an early higher growth stimulation in elevated [CO_2], the current relative growth rate of both Sitka spruce and cherry was similar between [CO_2] treatments (see Chapters 7). The unchanged pattern of dry mass allocation and the differential decline of mean R are among the most interesting results of growth in elevated [CO_2]. According to Ingestad's principles (Ingestad & Lund, 1986; Ingestad & Ågren, 1992; 1995), by suppling plants with exponentially-increasing amount of nutrients, the internal nutrient concentration remains stable over time (steady-state). When plants are in steady-state conditions, the relative uptake rate is equal to the plant relative growth rate, and in theory, in this condition R should be equal for each plant organ. All plants in this study were supplied with free access to nutrients and this allowed both Sitka spruce saplings and cherry seedlings to maintain a constant ratio amongst the different plant components. This emphasises once again the importance of adequate nutrition in the elevated [CO_2] experiments.

The similar R between the [CO_2] treatments after a more rapid growth in the early phase of exposure to elevated [CO_2] and the similar allocation pattern suggests that elevated [CO_2] accelerates ontogeny. Thus one of the main effects of elevated [CO_2] on long-term tree growth is to speed-up ontogenic development in all aspects, so that, for instance, a three-year-old tree has all the characteristics of a five-year-old tree. The findings by Hättenschwiler *et al.* (1997a,b) seem to be in keeping with the acceleration in ontogenic development. They found that mature Mediterranean oaks from a natural forest stand grown for their entire life around natural CO_2 springs had a shift of about three years in biomass increment compared to control trees.

After initial stimulation, the fast decline of R in elevated [CO_2] plants over time to values similar to those measured in ambient [CO_2] plants does not imply that both agricultural and forest ecosystems are unresponsive to rising [CO_2]. When plants are the same size R is higher in elevated [CO_2] than in ambient [CO_2] and consequently absolute growth is stimulated by elevated [CO_2]. However, even in the absence of stress high absolute growth

can not be sustained indefinitely: there is a high probability that canopy closure will occur earlier in elevated [CO_2] thus limiting growth rates (Norby *et al.*, 1996) - eventually ambient [CO_2] plants will catch up. Moreover, the increased amounts of assimilates that may be allocated below ground in elevated [CO_2] plants by means of enhanced fine-root turnover and rhizo-deposition of exudates is potentially important ecologically and can have strong indirect effects on plant production as well as contributing as a stable sink to CO_2 sequestration. There is increasing evidence that the amount of carbon stored in soil as peat or as other forms of organic matter can account for about two-thirds of the total carbon sequestrated by the high-latitude forests (Dixon *et al.*, 1994).

Forest ecosystems hold the largest pool of carbon, and, thus forest growth can, to some extent, offset rising greenhouse gas emissions and global warming (Dixon *et al.*, 1994). There is evidence that the carbon stocks of boreal forests are expanding and are, therefore, withdrawing CO_2 from the atmosphere and locking it away in organic matter. Kauppi *et al.* (1992) have demonstrated that in Western Europe, for instance, forest resources increased in the 1970s and 1980s. However, in the 1980s deforestation in the tropics was about 0.8% per year of the area of the tropical forest, and the carbon storage capacity of the tropical forest averages from 1.5 to 2.5 times the carbon storage capacity of mid- and high- latitude forests (Houghton, 1995). Thus, the potential role that boreal forests can play as a sink in the global carbon cycle may be limited in the face of increased deforestation in the Tropics.

The last hundred years have witnessed a reversion of agricultural land to forests in Western Europe and the USA. However, it is not clear whether such a trend can continue in the future. It is estimated that current world food production barely meets the present world food demand and the world population is steadily increasing (Houghton, 1994; Hidore, 1996). It is, therefore, likely that as global demand for energy and for agricultural land continues to increase, the reversion of agricultural land to forest in the developed countries will soon stop and deforestation in the tropics will continue - unless a new agricultural revolution takes place at the beginning of the 21st century.

The likely increase in frequency and intensity of drought as consequence of the rising temperature, will strongly affect forest growth in regions such as the Mediterranean basin

where water is already the main factor limiting plant productivity. Although, the impact of elevated [CO_2] on the regional scale may be minimal (Jarvis & McNaughton, 1986; Eamus, 1991; Jarvis, 1993), at the plant and stand scale elevated [CO_2] may result in increased WUE (Eamus, 1991). The cherry study has confirmed that elevated [CO_2] increased plant WUE, however, stomatal conductance and total water consumption of water-stressed seedlings were not reduced in elevated [CO_2]. In this study ambient [CO_2] plants and elevated [CO_2] plants were grown under similar temperatures. However, as temperature increases with rising atmospheric CO_2 the leaf-to-air VPD will also increase, and, thus, transpiration from vegetation will further increase leading to a decrease in plant WUE. Any gains to plant growth from an increase in WUE due to elevated [CO_2] may be offset by a reduction in WUE from temperature effects. As the frequency and intensity of drought will increase with global climate change water availability in soil will decrease. If drought tolerance is increased due to elevated [CO_2] then WUE may not be affected adversely by reduced water availability. However, no evidence was found to suggest that drought tolerance was increased in cherry with elevated [CO_2] and so WUE may be expected to decrease further under conditions of decreased water availability.

As global change progresses new technologies may be used to fully take advantage of the effects on plant growth of increased [CO_2]. Both molecular engineering and traditional plant breeding could be employed to produce plants better able to cope with the various processes limiting plant growth in elevated CO_2 environments. For instance, breeding of cultivars which can sustain a higher relative growth rate of leaf dry mass for longer periods during the exponential phase of growth may be very important since they could compound a higher "interest". Thus, the differential decline of plant R would be postponed to take full advantage, in terms of final plant productivity, of the more rapid initial growth rate.

APPENDIX 1

Sugar standard solutions

Stock solutions 100 g m^{-3} of inositol, sorbitol, fucose, rhamnose, arabinose, galactose, glucose, xylose, fructose and sucrose were made by dissolving the equivalent amount of each sugar in double-distilled water. 100 cm^3 of sugar standard solution were then prepared by adding:

5 cm^3 of inositol, sorbitol, fucose, rhamnose, arabinose stock solutions,

15 cm^3 of galactose stock solution,

10 cm^3 of glucose and xylose stock solutions,

20 cm^3 of fructose and sucrose stock solutions,

to obtain a final concentration of each sugar in the standard solution as follows:

Inositol	5 g m^{-3}
Sorbitol	5 g m^{-3}
Fucose	5 g m^{-3}
Rhamnose	5 g m^{-3}
Arabinose	5 g m^{-3}
Galactose	15 g m^{-3}
Glucose	10 g m^{-3}
Xylose	10 g m^{-3}
Fructose	20 g m^{-3}
Sucrose	20 g m^{-3}

Appendix II

Nutrient stock solutions

The amounts of the hydrated compounds in g dm^{-3} used in the stock solutions, diluted 1:1000, were as follows (from: Ingestad & Lund,1986):

Solution 1	
K_2SO_4	48.97
K_2HPO_4	33.62
KH_2PO_4	30.89
KNO_3	49.24
NH_4NO_3	221.6

Solution 2	
$Ca(NO_3)_2$	41.3386
$Mg(NO_3)_2$	89.76943
$Fe(NO_3)_3$	5.050418
$Mn(NO_3)_2$	1.827112
H_3BO_3	1.144
$Zn(NO_3)_2$	0.273283
$CuCl_2$	0.080487
Na_2MoO_4	0.017626

The amounts of elements in g dm^{-3} were as follows:

Macronutrients	
N	99.83789
P	13.0079
K	64.98384
S	9.007333
Ca	7.014611
Mg	8.509208

Micronutrients	
Fe	0.698145
Mn	0.399912
B	0.20001
Zn	0.060055
Cu	0.030001
Na	0.00335
Mo	0.006989

Table 1. Percentage of the known soluble sugar of Sitka spruce saplings grown in ambient or elevated CO_2.

a) needles soluble carbohydrate

	382		551		719		972	
sugar	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient
inositol	5.87	7.60	11.03	12.53	7.17	8.88	5.07	8.07
sorbitol	0.20	0.28	0.48	0.97	2.84	4.47	0.25	0.45
fucose	0.24	0.23	0.42	0.40	0.33	0.31	0.56	0.60
rhamnose	0.24	0.23	0.55	0.48	0.21	0.16	0.44	0.42
arabinose	0.93	1.98	1.58	0.15	2.55	1.69	1.60	0.67
glucose	7.59	7.75	33.51	37.19	13.43	11.04	20.01	23.39
fructose	1.99	1.11	22.76	26.47	1.40	0.64	13.78	18.92
sucrose	69.81	71.68	20.53	14.75	59.54	63.10	43.41	28.55
total known	86.87	90.86	90.87	92.93	87.47	90.30	85.10	81.06

b) root soluble carbohydrate

	382		551		719		972	
sugar	elevated	ambient	elevated	ambient	elevated	ambient	elevated	ambient
inositol	3.20	4.93	1.60	2.08	2.13	2.68	3.61	3.74
mannitol	1.06	1.21	1.40	1.54	1.80	2.18	3.82	3.16
glucose	5.32	4.66	19.60	11.27	7.09	8.03	23.61	23.70
fructose	7.68	5.83	23.29	21.51	21.02	15.29	28.66	32.33
sucrose	78.89	79.44	25.47	36.99	59.39	66.23	36.86	33.72
total known	96.16	96.07	71.36	73.39	91.43	94.42	96.57	96.65

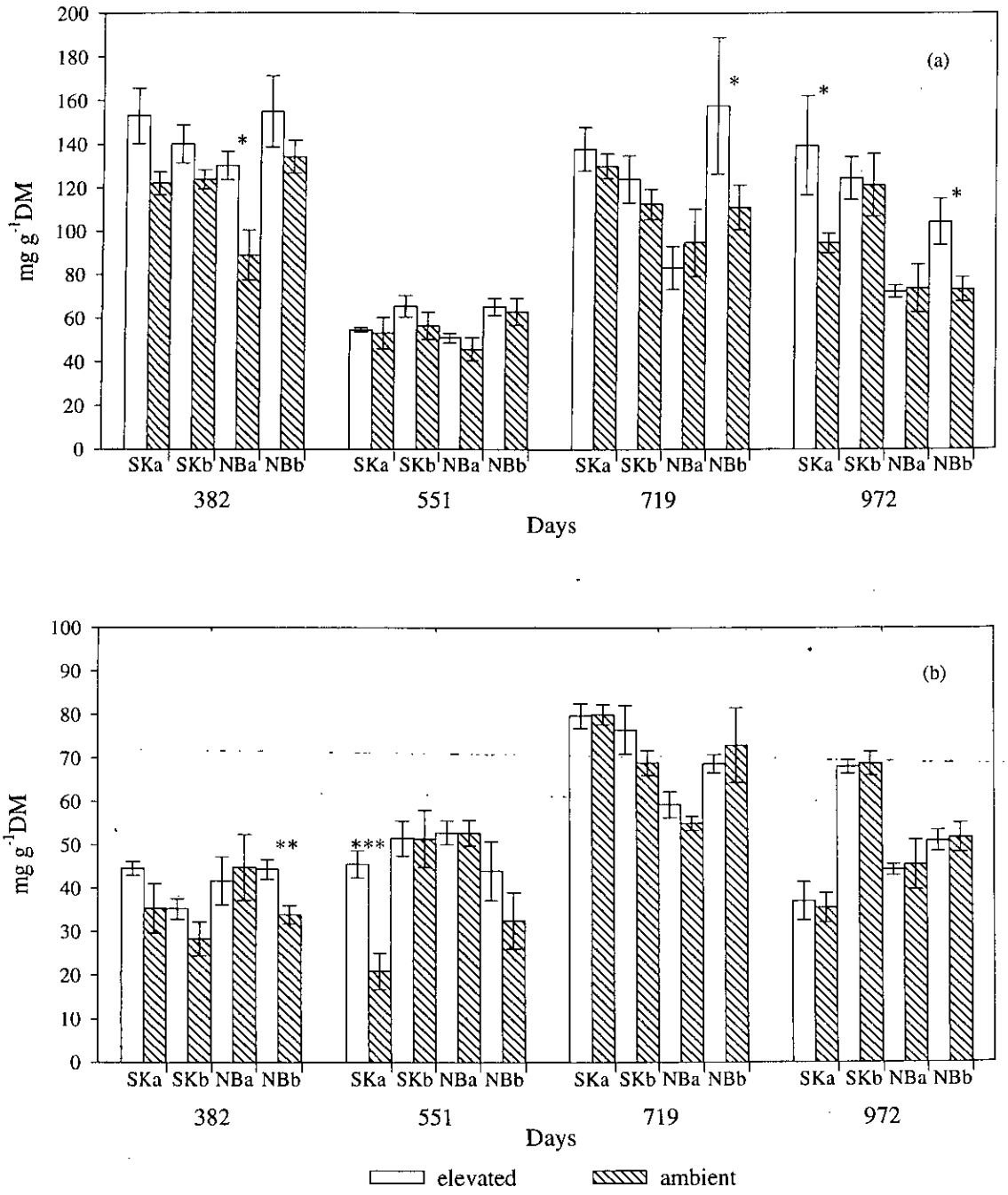

Figure 1. Neddle (a) and root (b) soluble sugar concentration per unit of dry mass (DM) of the four Sitka spruce clones grown in ambient or elevated CO_2, shown as days from the beginning of the experiment. Data are means of 5 plants per treatment ± SEM, the significance level (*= $P<0.05$, ** = $P<0.01$, *** = $P<0.001$) show the difference in soluble sugar content of each clone in response to the CO_2 treatments. SK a = Skidegate a, SK b = Skidegate b, NB a = North Bend a, and NB b = North Bend b.

APPENDIX 4

Table 1. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the whole duration of the experiment; no. = number, LA = leaf area, DM = dry mass, Ab-gr = above ground, R/S = root to shoot mass ratio.

	Height	Basal area	Leaf (no.)	LA	Wood DM	Leaf DM	Ab-gr DM	Root DM	Total DM	R/S
Time	***	***	***	***	***	***	***	***	***	***
$[CO_2]$	***	**	ns	***	***	***	***	***	***	***
Water treatment	***	***	ns	**	***	***	***	***	***	ns
Time x $[CO_2]$	***	**	*	**	***	***	***	***	***	ns
Time x water	***	***	ns	ns	***	***	***	***	***	ns
$[CO_2]$ x water	**	***	ns	ns	ns	ns	ns	ns	ns	ns
Time x $[CO_2]$ x water	ns	**	ns	ns	ns	ns	ns	ns	ns	ns

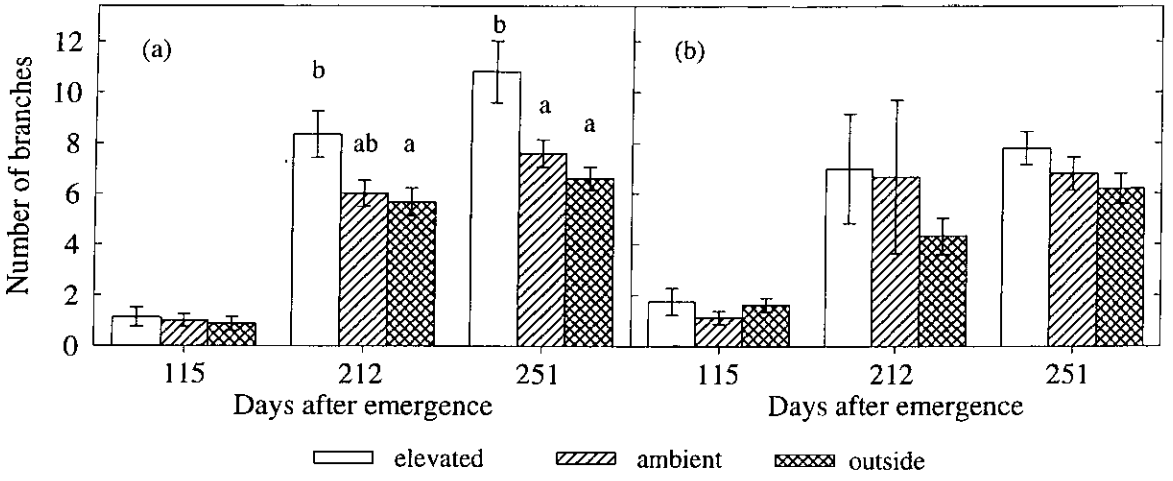

Figure 1. Number of branches of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 6 to 15 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$. Statistical significance:

	well-watered	water-stressed
[CO_2]	$P < 0.001$	ns
Time	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	ns

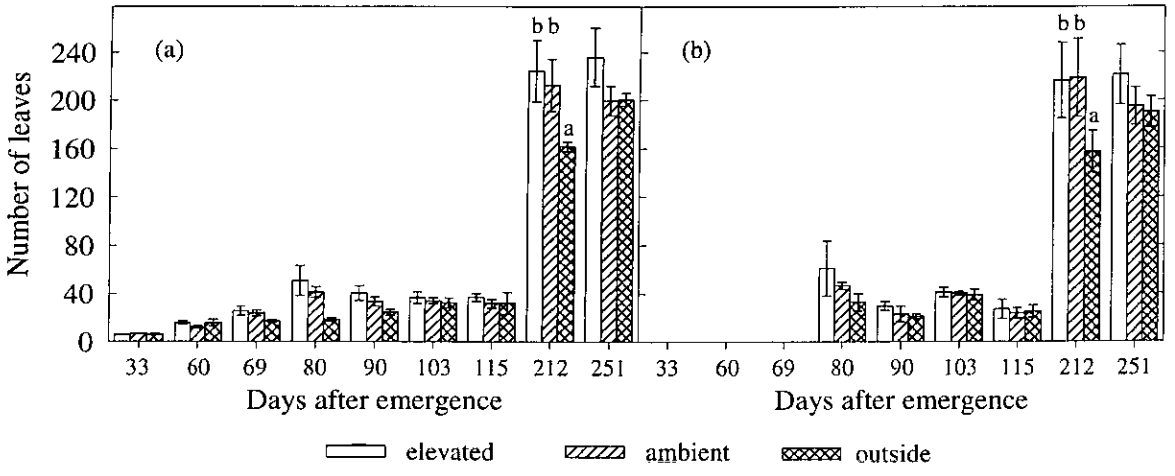

Figure 2. Number of leaves of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 to 6 plants per treatment ± 1 SEM. Letters (a, b,) indicate significant differences at $P < 0.05$. Statistical significance:

	well-watered	water-stressed
[CO_2]	ns	ns
Time	$P < 0.01$	$P < 0.001$
Interaction	ns	ns

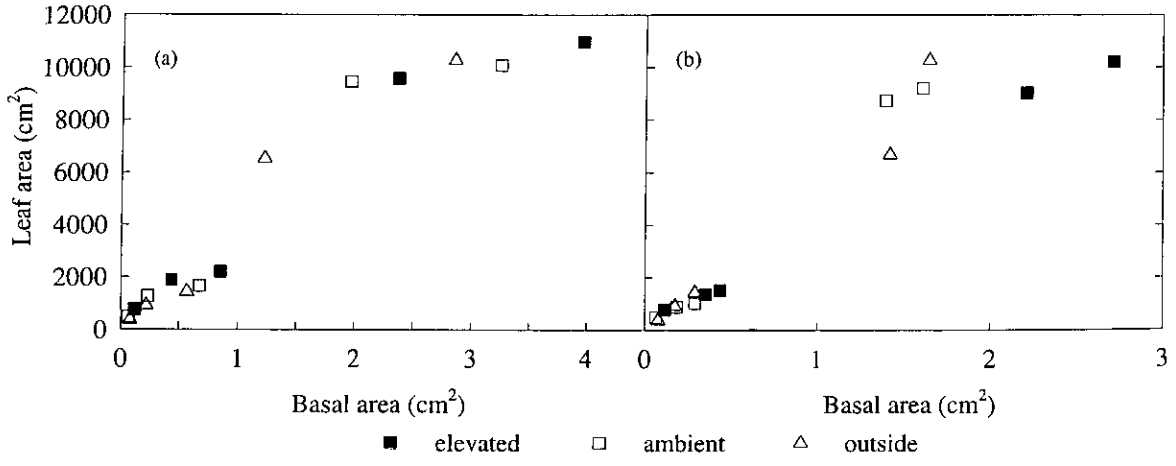

Figure 3. Linear relationships between leaf area and basal area of (a) well-watered and (b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control. The coefficients of determination (R^2) of the linear relationships are:

	well-watered	water-stressed
elevated	0.930	0.995
ambient	0.904	0.991
outside	0.944	0.964

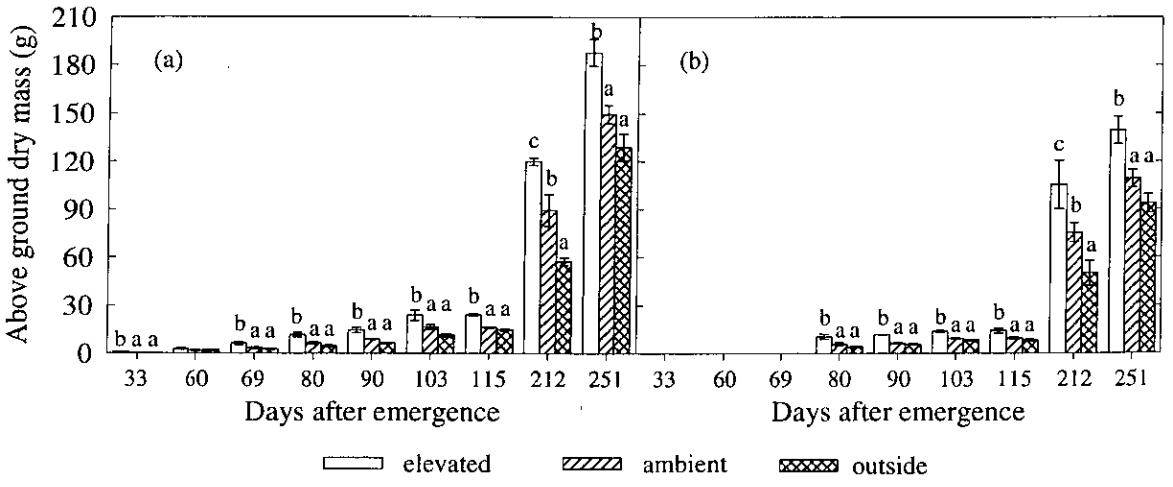

Figure 4. Above-ground dry mass of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 to 6 plants per treatment ± 1 SEM. Letters (a, b, c) indicate significant differences at $P < 0.05$. Statistical significance:

	well-watered	water-stressed
[CO_2]	$P < 0.001$	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	$P < 0.001$

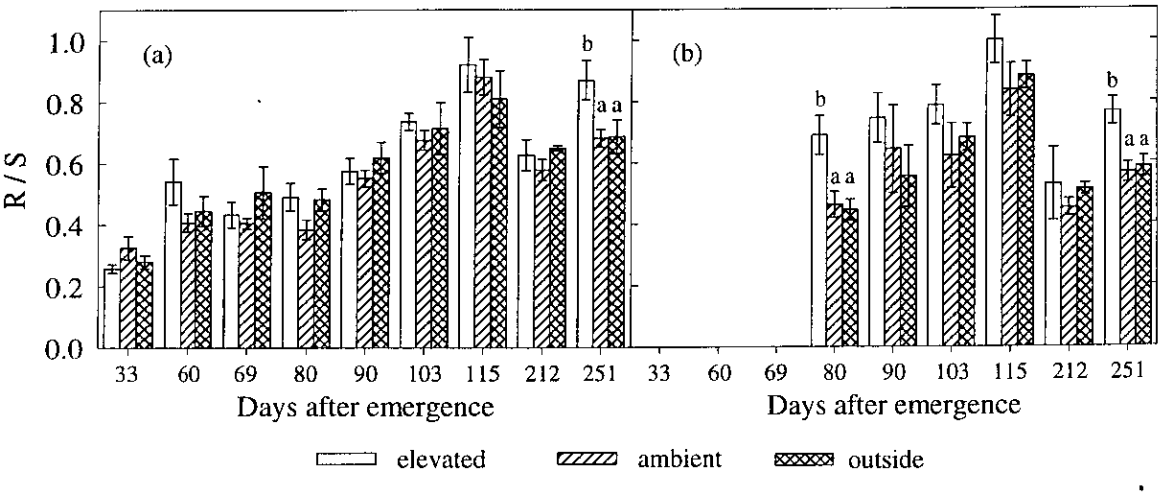

Figure 5. Root to shoot mass ratio (R/S) of a) well-watered and b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 to 6 plants per treatment ± 1 SEM. Letters (a, b) are used to indicate significant differences at $P < 0.05$. Statistical significance:

	well-watered	water-stressed
[CO_2]	ns	$P < 0.001$
Time	$P < 0.001$	$P < 0.001$
Interaction	ns	ns

APPENDIX 5

Table 1. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the first growing season; A = assimilation rate, g_s = stomatal conductance, ITE = instantaneous transpiration efficiency.

	A	g_s	ITE	Rubisco	total chlorophyll	chlorophyll a/b
time	***	***	***	***	***	***
$[CO_2]$	***	***	***	***	ns	ns
water	***	***	ns	ns	*	ns
time x $[CO_2]$	**	ns	**	*	ns	ns
$[CO_2]$ x water	***	*	$P < 0.10$	ns	ns	ns
time x water	***	**	***	***	*	ns
time x $[CO_2]$ x water	*	ns	***	*	ns	ns

Table 2. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the second growing season; A = assimilation rate, g_s = stomatal conductance, ITE = instantaneous transpiration efficiency; Ψ = bulk leaf water potential.

	A	g_s	ITE	Rubisco	Ψ	total chlorophyll	chlorophyll a/b
time	***	***	***	***	***	***	ns
$[CO_2]$	***	**	***	**	ns	***	ns
water	***	***	ns	ns	***	***	**
time x $[CO_2]$	ns	ns	***	ns	ns	ns	ns
$[CO_2]$ x water	***	ns	ns	ns	ns	ns	ns
time x water	***	***	***	ns	***	***	*
time x $[CO_2]$ x water	***	ns	$P < 0.10$	ns	*	$P < 0.10$	ns

Table 3. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the first growing season; SWC = soil water content, Ψ_s = osmotic potential, Ψ = bulk leaf water potential.

	SWC	Ψ	Ψ_s	leaf sugars	root sugars	leaf starch	root starch
time	***	***	***	***	***	***	***
$[CO_2]$	ns	***	***	***	**	***	ns
water	***	***	***	ns	***	***	*
time x $[CO_2]$	ns	***	ns	***	ns	***	ns
$[CO_2]$ x water	ns	ns	ns	ns	ns	***	ns
time x water	***	***	***	***	***	***	*
time x $[CO_2]$ x water	ns	ns	ns	ns	ns	*	ns

Table 4. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the first growing season; π_{100} = osmotic potential at full turgor, π_0 = osmotic potential at zero turgor, R_0 relative water content at zero turgor, ε_B bulk modulus of elasticity of the cell, DM / TM ratio of leaf dry mass to leaf turgid mass.

	π_{100}	π_0	R_0	ε_B	DM/TM
$[CO_2]$	ns	ns	ns	ns	ns
water	**	***	ns	ns	**
$[CO_2]$ x water	ns	ns	ns	ns	ns

Table 5. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the diurnal cycle on dae 103; A = assimilation rate, g_s = stomatal conductance, Ψ_s = osmotic potential, Ψ = bulk leaf water potential.

	A	g_s	ITE	Ψ	Ψ_s
hour	***	***	***	***	***
$[CO_2]$	***	***	***	***	***
water	***	***	***	***	***
hour x $[CO_2]$	**	***	ns	ns	ns
$[CO_2]$ x water	***	***	*	***	ns
hour x water	***	***	***	$P < 0.10$	ns
hour x $[CO_2]$ x water	$P < 0.10$	***	***	ns	ns

Table 6. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the first growing season; N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium.

	leaf N	leaf P	leaf K	leaf Ca	leaf Mg	root N	root P	root K	root Ca	root Mg
time	***	**	***	***	*	***	***	***	***	***
[CO_2]	***	ns	$P < 0.10$	ns	***	***	***	***	***	***
water	ns	***	**	ns	**	ns	***	***	ns	ns
time x [CO_2]	***	ns	**	ns	ns	*	***	**	ns	***
[CO_2] x water	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
time x water	ns	***	*	ns	**	*	$P < 0.10$	*	ns	*
time x [CO_2] x water	***	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 7. Significance level of P (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant) from three-way ANOVA for the second growing season; N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium.

	leaf N	leaf P	leaf K	leaf Ca	leaf Mg	root N	root P	root K	root Ca	root Mg
time	*	*	*	**	***	ns	***	***	***	*
[CO_2]	***	ns	**	***	ns	**	ns	*	***	**
water	ns	$P < 0.10$	ns	ns	$P < 0.10$	ns	*	ns	*	ns
time x [CO_2]	ns	*	ns	ns	ns	ns	ns	ns	*	ns
[CO_2] x water	ns	ns	ns	ns	$P < 0.10$	ns	ns	ns	ns	ns
time x water	ns	ns	ns	ns	ns	ns	$P < 0.10$	$P < 0.10$	*	ns
time x [CO_2] x water	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

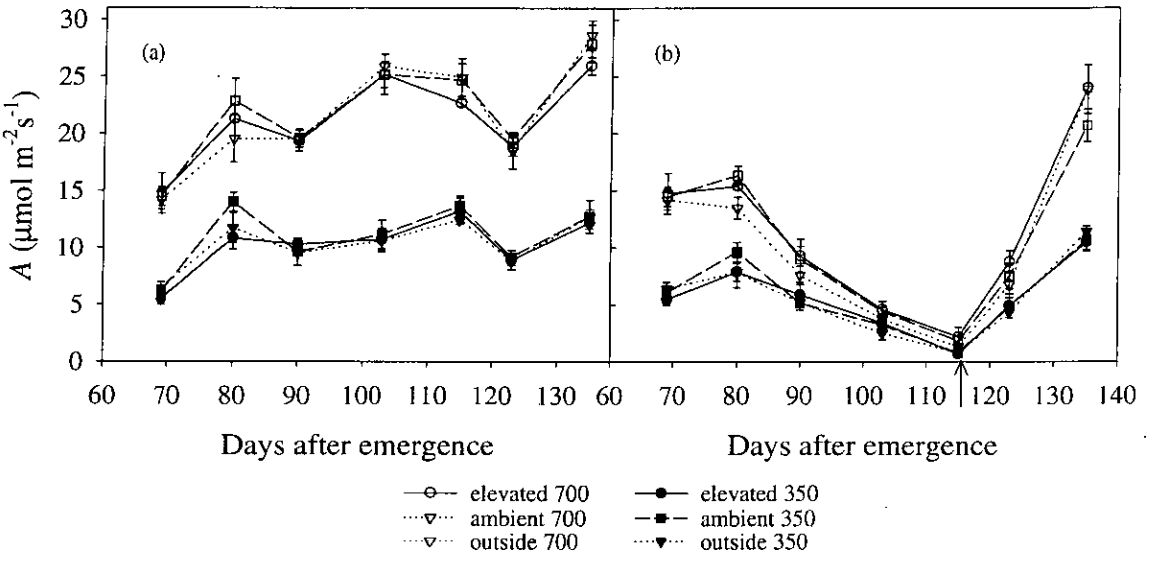

Figure 1. Time course of PPFD saturated assimilation rate (*A*) measured at 350 and 700 µmol $m^{-2}s^{-1}$ CO_2 concentration of (a) well-watered and (b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, during and after the first drought cycle (dae 69-115). ↑ = end of the drying cycle. Data are means of 3 plants per treatment ± 1 SEM.

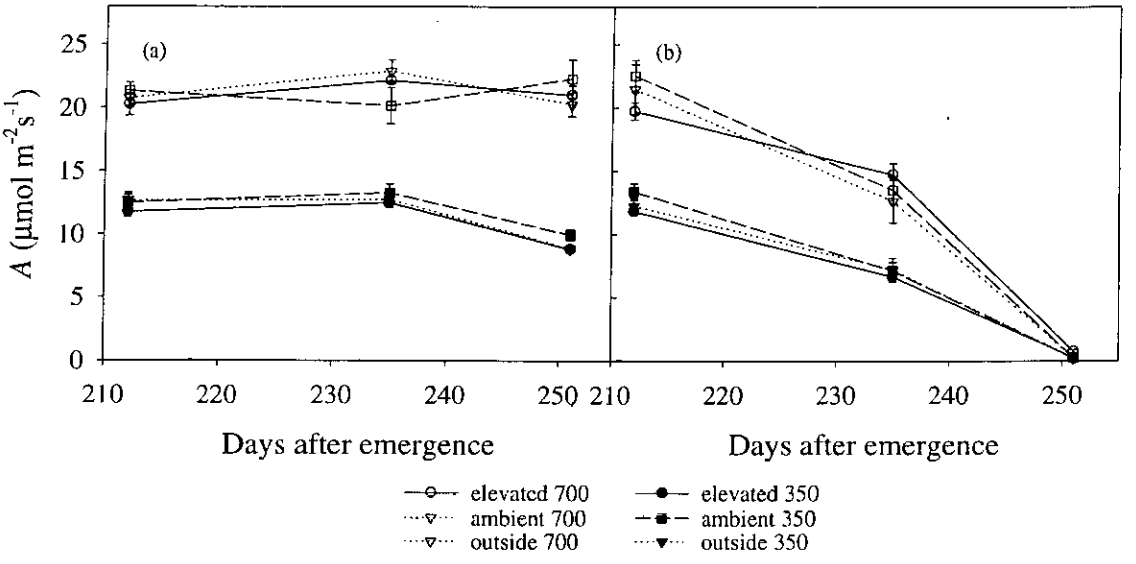

Figure 2. Time course of PPFD saturated assimilation rate (*A*) measured at 350 and 700 µmol $m^{-2}s^{-1}$ CO_2 concentration of (a) well-watered and (b) water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, over the second drought cycle (dae 212-251). Data are means of 3 plants per treatment ± 1 SEM.

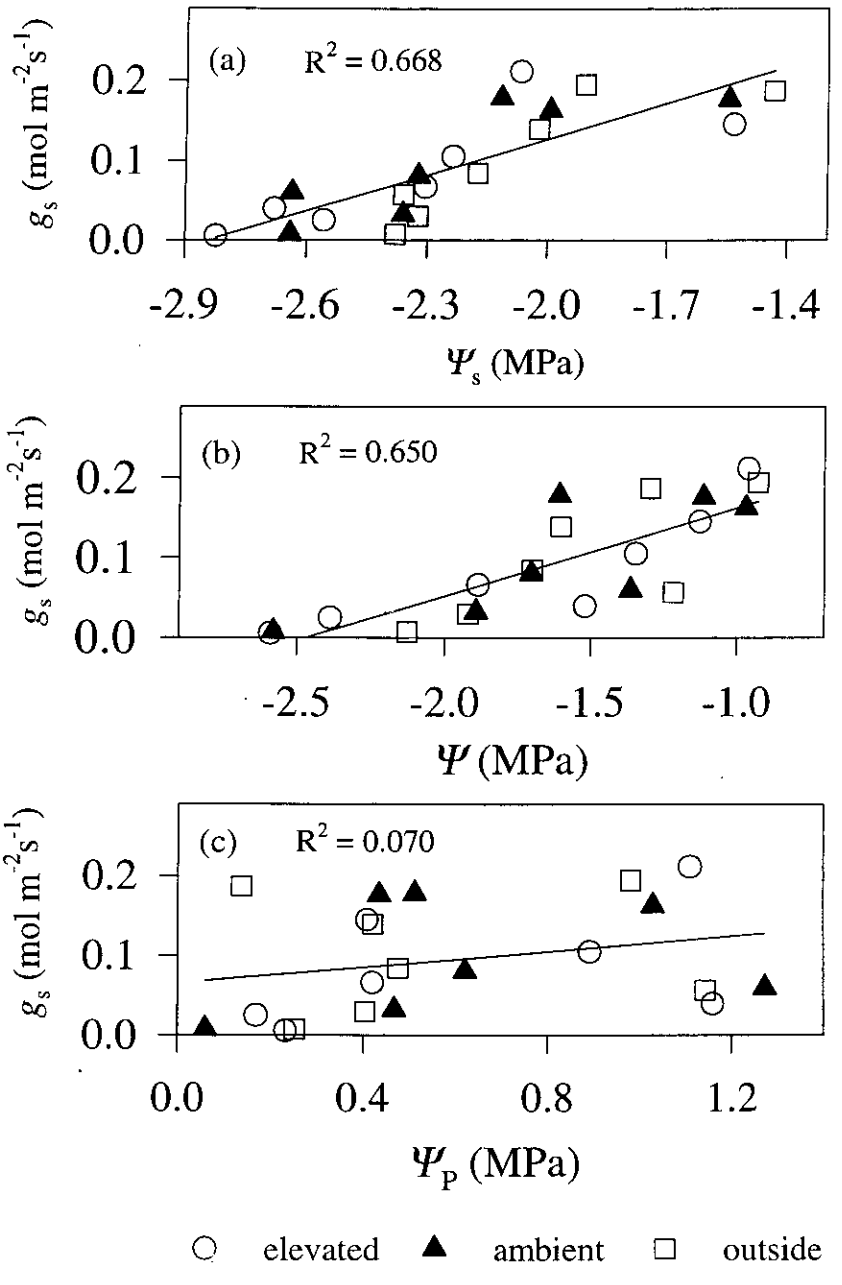

Figure 3. Linear relationship between all mean stomatal conductance (g_s) and all mean midday a) osmotic potential (Ψ_s), b) bulk leaf water potential (Ψ), and c) turgor potential (Ψ_p), measured during the first drought cycle (dae 69-115), of water-stressed cherry seedlings grown in elevated [CO_2], ambient [CO_2], or outside control.

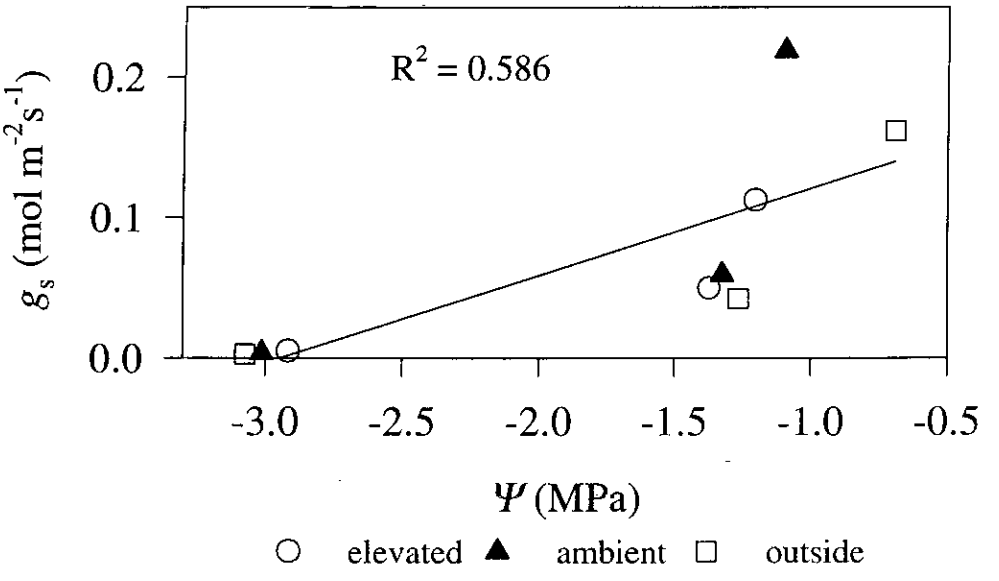

Figure 4. Linear relationship between all mean stomatal conductance (g_s) and all mean midday bulk leaf water potential (Ψ), measured during the second drought cycle (dae 212-251), of water-stressed cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control.

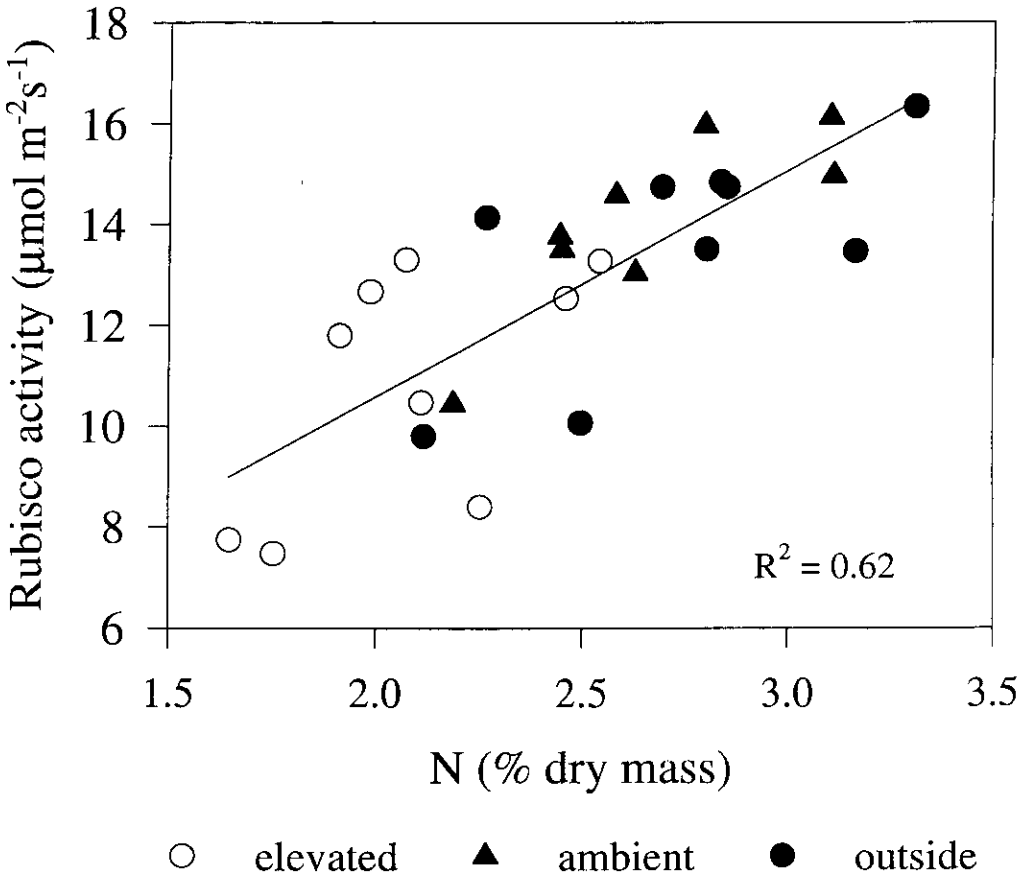

Figure 5. Linear relationship between all mean Rubisco activity and combined mean leaf nitrogen (N) concentration in well-watered cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control. Data are means of 3 plants per treatment ± 1 SEM.

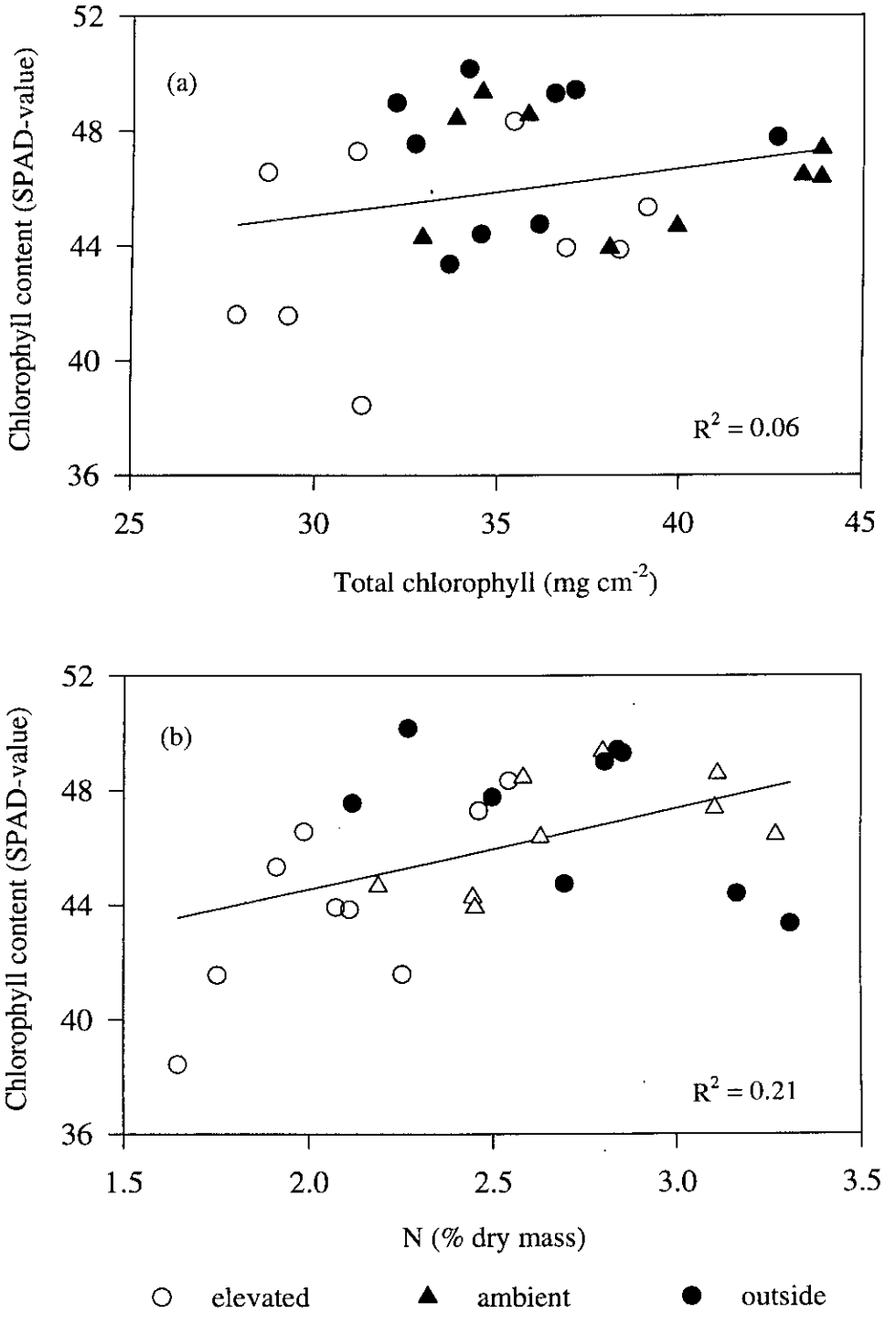

Figure 6. Linear relationship between all *in situ* chlorophyll concentration and all (a) total chlorophyll concentration and (b) leaf nitrogen (N) concentration in well-watered seedlings grown in ambient [CO_2], elevated [CO_2], or outside control. Data are means of 3 plants per treatment ± 1 SEM.

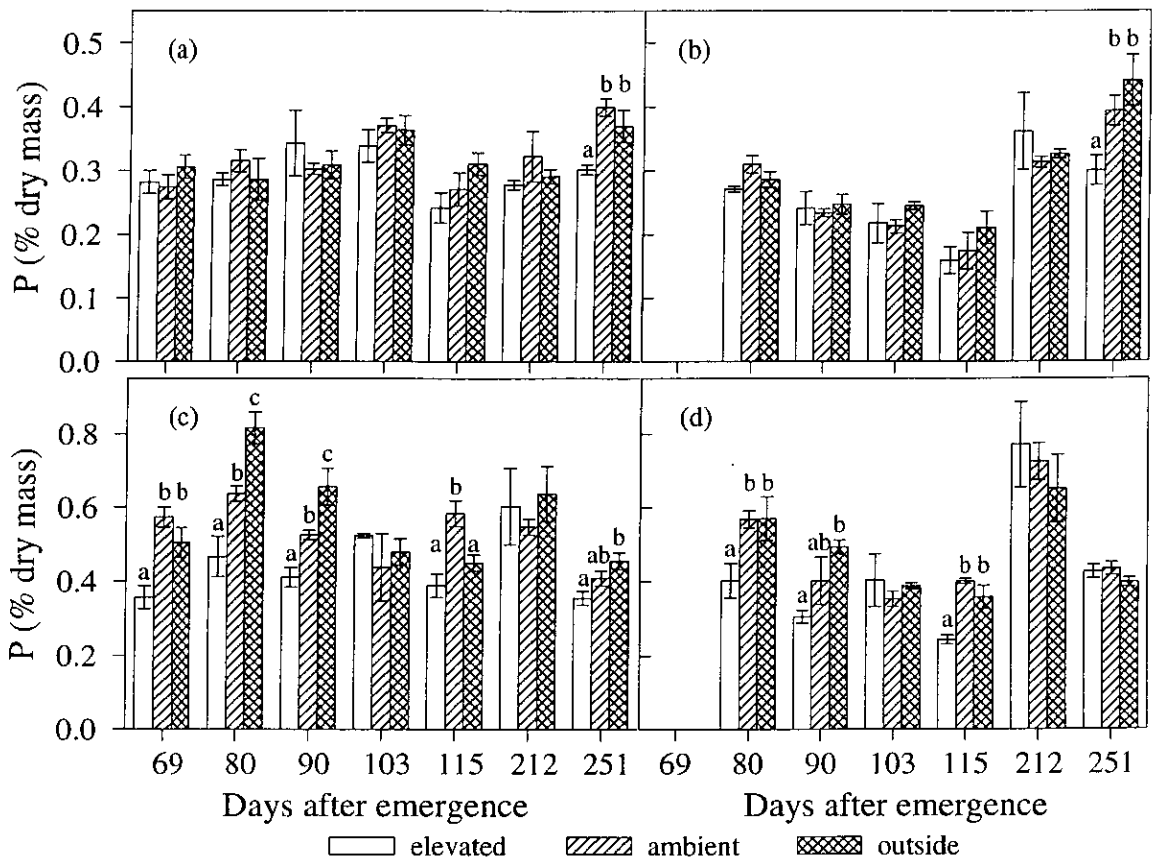

Figure 7. Leaf (a,b) and root (c,d) phosphorus (P) concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b, c) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

1993	well-watered		water-stressed	
	leaf	root	leaf	root
time	***	***	*	***
[CO_2]	ns	***	ns	***
time x [CO_2]	ns	**	ns	ns
1994	well-watered		water-stressed	
	leaf	root	leaf	root
time	**	***	ns	***
[CO_2]	ns	ns	ns	ns
time x [CO_2]	ns	ns	ns	ns

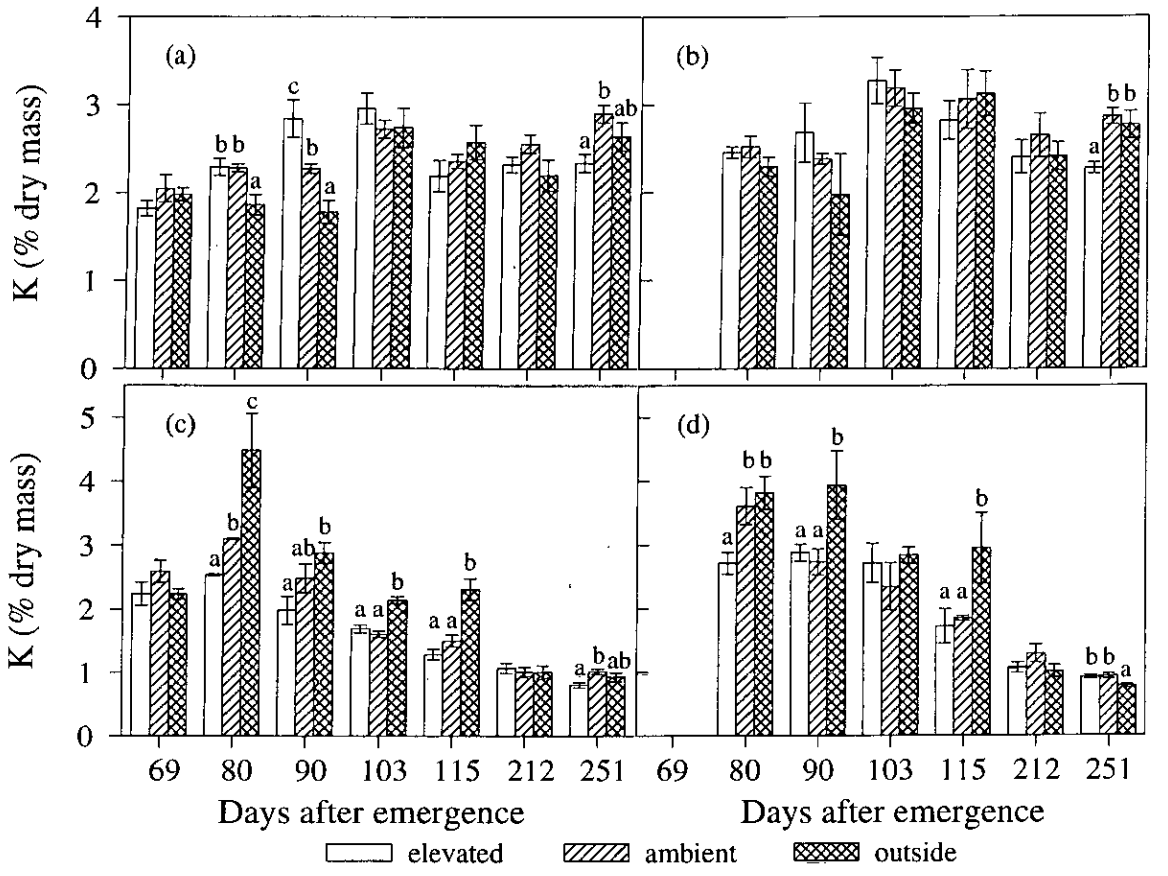

Figure 8. Leaf (a,b) and root (c,d) potassium (K) concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b, c) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

1993	well-watered		water-stressed	
	leaf	root	leaf	root
time	***	***	***	***
[CO_2]	ns	***	ns	**
time x [CO_2]	ns	*	**	ns
1994	well-watered		water-stressed	
	leaf	root	leaf	root
time	*	$P < 0.10$	ns	***
[CO_2]	*	ns	$P < 0.10$	*
time x [CO_2]	ns	ns	ns	ns

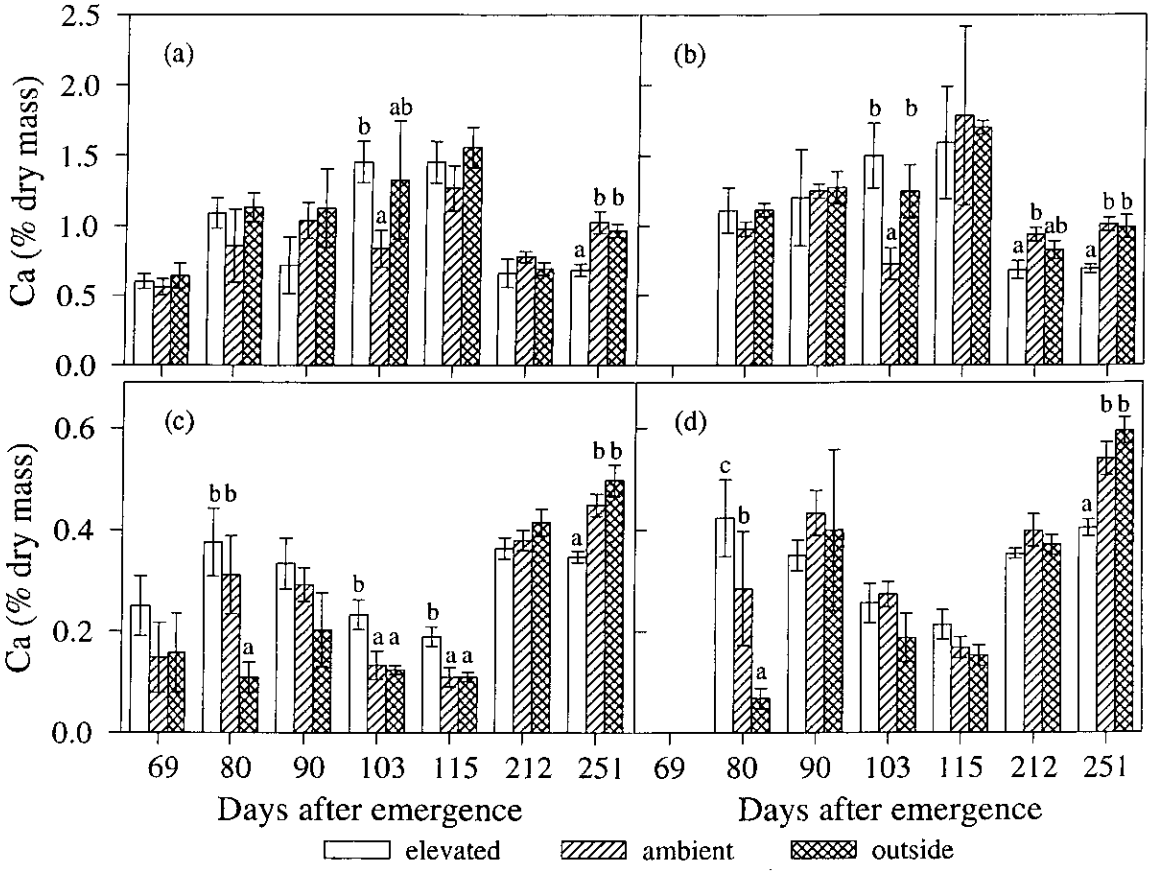

Figure 9. Leaf (a,b) and root (c,d) calcium (Ca) concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b, c) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

1993	well-watered		water-stressed	
	leaf	root	leaf	root
time	***	**	***	*
[CO_2]	ns	**	ns	$P < 0.10$
time x [CO_2]	ns	ns	ns	ns
1994	**well-watered**		**water-stressed**	
	leaf	root	leaf	root
time	**	*	ns	***
[CO_2]	*	**	**	**
time x [CO_2]	ns	ns	ns	*

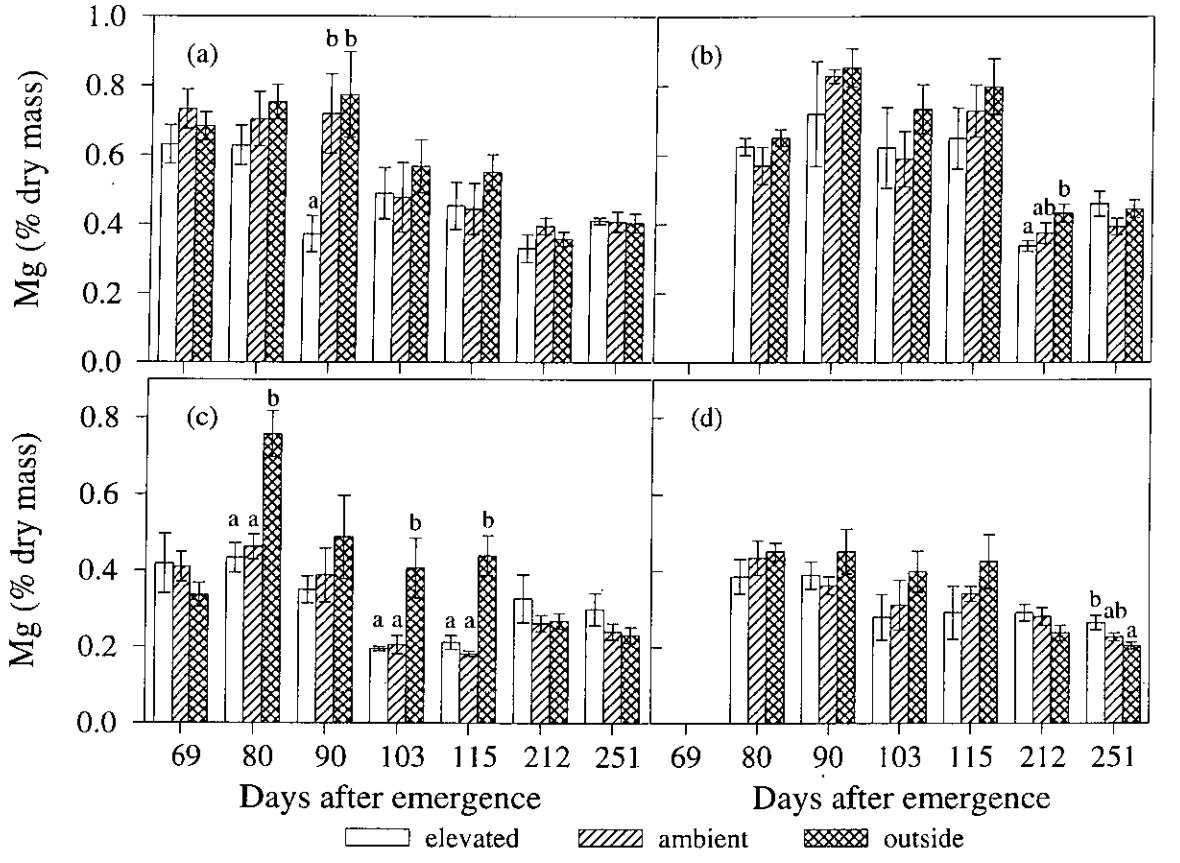

Figure 10. Leaf (a,b) and root (c,d) magnesium (Mg) concentrations in well-watered (a,c) and water-stressed (b,d) cherry seedlings grown in ambient [CO_2], elevated [CO_2], or outside control, shown as days after emergence. Data are means of 3 plants per treatment ± 1 SEM. Letters (a, b) indicate significant differences at $P < 0.05$ amongst the [CO_2] treatments. Statistical significance:

1993	well-watered		water-stressed	
	leaf	root	leaf	root
time	*	***	**	ns
[CO_2]	$P < 0.10$	**	**	ns
time x [CO_2]	ns	*	ns	ns
1994	**well-watered**		**water-stressed**	
	leaf	root	leaf	root
time	**	ns	*	*
[CO_2]	ns	ns	ns	*
time x [CO_2]	ns	ns	ns	ns

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