THE EFFECTS OF INCREASED ATMOSPHERIC CARBON DIOXIDE ON GROWTH AND MORPHOLOGY OF SEEDLINGS OF SILVER BIRCH (*BETULA PENDULA* ROTH.) AND SITKA SPRUCE (*PICEA SITCHENSIS* (BONG.) CARR.)

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ABSTRACT

The concentration of CO_2 is increasing at a rate of *ca* 1.8μmol mol-1 per year. If the current trend continues, atmospheric CO_2 concentration will reach double the 1980's ambient concentration by about 2080. An increase in atmospheric CO_2 concentration will have direct effects on plants as a result of the role CO_2 plays in determining rate of photosynthesis and transpiration. It may also have secondary effects on plant growth. The objective of this study was to determine the effects of long-term exposure to elevated CO_2 on the growth and morphology of seedlings of Sitka spruce and silver birch, with a focus on the effects of elevated CO_2 on the expansion, surface characteristics, specific leaf area, anatomy and chlorophyll content of leaves of silver birch. Seedlings of Sitka spruce and silver birch were placed in pots in open top chambers receiving ambient or elevated CO_2 . After one growing season there was a significant increase in biomass production in seedlings of Sitka spruce grown in elevated CO_2 compared to those grown in ambient CO_2, but after eighteen months this effect had disappeared. The initial increase in biomass was a result of a significant increase in root mass. At the end of the second growing season there was no effect of elevated CO_2 on allocation of dry mass. There was no effect of elevated CO_2 on total number of branches produced at the end of the second growing season but there was an increase in the rate of branch production. Nitrogen, phosphorus and potassium concentration of leaves was reduced in plants grown in elevated CO_2.

After one growing season biomass was increased in elevated CO_2 plants by *ca* 20%, rates of photosynthesis were also higher in elevated CO_2 plants. There was no effect of CO_2 treatment on root: shoot ratio. There was a significant increase in branch production in plants grown in elevated CO_2, but despite an increase in leaf area production early on in the growing season, there was no difference in total tree leaf area at the end of the growing season. Nitrogen, phosphorus and potassium concentration of leaves was reduced in plants grown in elevated CO_2 and leaves on elevated CO_2 plants senesced *ca* one week earlier than leaves of plants grown in ambient CO_2.

Leaf extension rates were higher in elevated CO_2 plants during the morning but there were no consistent effects of CO_2 treatment on leaf water potential or osmotic potential.

Leaf thickness increased in elevated CO_2 plants and was accompanied by an increase in the number of layers of palisade cells. Chlorophyll content was reduced in plants grown in elevated CO_2. Stomatal conductance was reduced in plants grown and measured in elevated CO_2 as a result of both a reduction in the width of apertures, and a reduction in stomatal density. There was no effect of elevated CO_2 treatment on stomatal index and changes in stomatal density were a result of changes in leaf expansion.

The implications of these results for the future functioning of Sitka spruce and silver birch, and the relevance of this study in relation to future predictions of tree growth in elevated CO_2 are discussed.

DECLARATION

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.

Lynn Evans June 1994

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To D.W and P.A.J.W.

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PREFACE

Fluctuations in concentrations of gases in the atmosphere are not new phenomena; the composition of the atmosphere is dynamic and is intrinsically linked to geochemical and biological processes. Emission of volcanic gases, formation of oceans and sedimentary carbonate rocks and the evolution of living organisms have all had significant effects on the composition of the atmosphere (Levine, 1985). Such chemical evolution continues in the present with anthropogenic activities resulting in ever increasing inputs of gases into the atmosphere. The increase in the atmospheric concentrations of NO_x, SO_2, CFCs, CH_4, CO and CO_2, has become a major cause for concern as increasing levels of pollution, acid precipitation, holes in the ozone layer and the threat of the 'greenhouse effect' concentrate the attention of environmentalists, scientists and the general public alike.

The increase in atmospheric concentration of CO_2 has received much attention because of the role CO_2 molecules play in the greenhouse effect (Houghton *et al.*, 1990). Data from the Vostok and Greenland ice cores have shown that concentration of atmospheric CO_2 has increased from approximately 280 μmol mol^{-1} two hundred years ago to its present day value of *ca* 360 μmol mol^{-1} (Barnola *et al.*, 1987; Lorius *et al*, 1990). The onset of the industrial revolution marked the beginning of this trend as a result of increasing manufacturing and industrial processes. The main causes of today's emissions of CO_2 are deforestation and the burning of fossil fuels. The concentration of CO_2 is currently increasing at *ca* 1.8 μmol mol^{-1} (0.5%) per year (although this has been reduced in 1992 and 1993 by almost half, possibly as a result of the eruption of Mount Pinatubo). If the current trend continues atmospheric CO_2 concentration will reach double the 1980's ambient concentration by about 2080 (Houghton *et al.*, 1990).

Increase in concentration of atmospheric CO_2 may have indirect effects on ecosystems *via* climatic warming and direct effects on plants resulting from the role CO_2 plays in controlling rates of photosynthesis and transpiration.

Changes in net primary production and water use result in changes in ecosystem structure and metabolism. This will have wide ranging implications.

Conversely, ecosystems affect climate. Vegetation affects regional hydrology *via* the process of evapotranspiration and the effects that vegetative cover have on reducing surface runoff (Melillo *et al.*, 1990). Large scale changes in land use and the latitudinal and altitudinal displacement of ecosystems are likely to change the extent and

composition of vegetative cover and, as a result, may change the albedo of the earth's surface.

Terrestrial ecosystems, and forests in particular, play an important role in the terrestrial carbon cycle. Forests cover approximately one third of the land area of the earth and are responsible for approximately two thirds of the total amount of photosynthesis. Measurements of atmospheric CO_2 concentration from 'flask' networks show seasonal variations in CO_2 concentration (Keeling, Bacastow and Whorf, 1982; Keeling *et al.*, 1989). The amplitude of these oscillations varies with latitude, small seasonal differences in CO_2 concentration occurring near the equator and in the southern hemisphere and large differences in northern latitudes (Keeling, 1983). The larger amplitudes of the seasonal oscillations in northern compared with southern latitudes has been attributed to the large expanses of vegetation, and in particular, the northern, temperate and boreal forests (Jarvis, 1989). If CO_2 does stimulate the growth of plants and trees in particular, forests could in the future represent an important long term net storage of terrestrial carbon in trees and soil (Jarvis, 1989).

Thus an understanding of the ways in which trees will respond to a rising CO_2 concentration is of increasing importance in the rapidly changing environment, particularly if information is to be provided to enable predictions concerning the future functioning of forest ecosystems are to be made and well informed management decisions to be taken.

Horticulturists have been aware of the 'fertilising' effect of increased concentrations of CO_2 for many years (Wittwer, 1988; Hand, 1988), and for many years it has been a standard horticultural practice for growers to raise the ambient CO_2 concentration within their glasshouses to maximise the yield of crops such as tomato and lettuce (Small and White, 1930; Calvert and Slack, 1975; Hand, 1988). With the current rise in atmospheric CO_2 concentration, effects of increased atmospheric concentrations now begin to have implications for plant growth and functioning outside the glasshouse environment of the horticulturalist.

Growth of plants may be stimulated by an increase in the concentration of atmospheric CO_2 as a result of increase in photosynthesis and reduction in stomatal conductance (g_S). Increase in CO_2 concentration may also have secondary effects on plant growth (Bowes, 1991); of particular relevance to this study is the effect of elevated CO_2 on leaf growth. Leaf growth may be increased in elevated CO_2 as a result of increase in substrate availability, i.e. carbohydrates and amino acids, enhancing the growth of leaves, or through more direct effects on leaf initiation (Morison and Gifford, 1984; Tolley and Strain, 1984a). The effects of elevated CO_2 on leaf growth will have long

term effects on growth and functioning of plants. Leaves are the main sites for photosynthesis and transpiration: an increase in leaf area increases the potential for the interception of radiation and therefore primary production, but may offset the effect of a reduction in g_S on water loss. Leaf surface characteristics (stomatal density, size of stomata) and anatomical organisation of leaves alter the flux of CO_2 and H_2O molecules into and out of leaves and are therefore intrinsically linked to the processes of photosynthesis and transpiration. Changes in leaf phenology, for example accelerated budburst or senescence, will change the length of the foliated growing season and the potential for biomass production.

Kramer and Sionit (1987) stated that an increase in leaf area has been shown to be the reason for increased productivity in elevated CO_2 plants over fairly long time periods. At present the effects of elevated CO_2 on leaf growth in trees are not well understood (Jarvis, 1994). Because trees are long lived, ideally the response of trees to elevated CO_2 should be investigated over long term periods. Juvenility in trees also raises a problem with respect to short term experiments. However, within the three year timescale of a PhD., long term experiments are not possible. None the less small changes in rates of growth during the early stages of the life of a tree may have major consequences for biomass a few years later. With fast growing species some of the problems of juvenility may be avoided. This thesis aims to make a start by investigating the early growth of silver birch and Sitka spruce with particular emphasis on the growth and properties of the leaves of silver birch, over as long a period as possible within the prescribed Research Council framework.

CHAPTER 1 INTRODUCTION

1.1 AIMS

The aim of the research described in this thesis was to determine the effect of long-term exposure to an elevated concentration of CO_2 on the growth and morphology of seedlings of silver birch (*Betula pendula* Roth.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.), with a focus on the effects of elevated CO_2 on the expansion, surface characteristics, specific leaf area, anatomy and chlorophyll content of leaves of silver birch.

Specific objectives

The following questions were addressed:

- What are the effects of long-term growth in elevated CO_2 on production of:
  a) total biomass;
  b) branches;
  c) leaves; and
  d) roots
  in silver birch and Sitka spruce? Do the nature and magnitude of responses differ between species?

- a) What is the effect of elevated CO_2 on leaf expansion in silver birch, and b) which biophysical parameters controlling leaf expansion are affected?

- Is stomatal conductance reduced in silver birch in elevated CO_2 as a result of an increase in the degree of stomatal closure and/or a reduction in stomatal density?

- How are a) specific leaf area, b) leaf anatomy and c) chlorophyll content in silver birch affected by growth in elevated CO_2 ?

- What implications can be drawn from these findings for the future functioning of silver birch and Sitka spruce in an elevated CO_2 environment?

Methodological approach

In order to address these questions it was decided that it was necessary to grow the trees in conditions as close as possible to their *natural* environment, and an open top

chamber experiment was initiated in June 1990. Sitka spruce and silver birch were chosen in this work as they are both important constituents of Scottish forests. They have several contrasting physiological, morphological and ecological characteristics: for example seedling silver birch are faster growing and initiate leaves throughout the season, whereas in Sitka spruce, needle primordia are laid down the previous year (Chapter 2) and may, therefore, be expected to respond differently to an increase in CO_2 concentration (Oberbauer *et al.*, 1985; Norby *et al.*, 1994). The silver birch trees used in this study were grown from seed in elevated CO_2 to reduce the problems of acclimation: the Sitka spruce plants, however, were purchased as two-year-old seedlings from the Economic Forestry Group (EFG). The plants were placed in pots (Chapter 2) outside in open top chambers under natural solar radiation. Open top chambers track the dynamic changes in temperature, radiation and rainfall experienced by unenclosed plants (Leadley and Drake, 1993) and were considered to provide the closest approximation to the *natural* environment experienced by trees in a forest, available within the constraints of using elevated CO_2. The number of plants that could be placed within each chamber and the number of chambers available (four elevated and four ambient) provided both sufficient plant material and an acceptable number of replicates. Although they allow strict control of radiation, temperature and humidity, controlled environment chambers were rejected as they are unsuitable for long-term experiments, especially with potentially large plants such as trees, putting serious constraints on the duration of experiments, pot size the amount of plant material available and replication. There is also a degree of scepticism concerning the use of data from controlled environments to make predictions about how plants behave in their natural surroundings (Mousseau and Saugier, 1992).

1.2 BACKGROUND

1.2.1 The role of CO_2 in photosynthesis in C3 plants

CO_2 is a substrate for photosynthesis. In photosynthesis CO_2 is converted to sugar phosphates in the chloroplast via the reductive pentose phosphate, or Calvin cycle (Calvin and Benson, 1948).

CO_2 reacts with ribulose bisphosphate, RuBP (a pentose) to form two molecules of the three carbon compound, phosphoglyceric acid (PGA), a reaction catalysed by ribulose bisphosphate carboxylase/ oxygenase (rubisco). PGA then undergoes phosphorylation and reduction in reactions using ATP and NAD(P)H, produced from the electron transport process, to form glyceraldehyde-3-phosphate (GAP). Molecules of triose phosphate can be converted to starch or exported from the chloroplast to the cytoplasm

to be converted to sucrose. However, for the cycle to continue, a proportion of the triose phosphate must undergo reactions which eventually result in the regeneration of RuBP.

In addition to catalysing the reaction by which CO_2 is incorporated into the RuBP molecule, rubisco also catalyses the reaction of RuBP with O_2, the first step of the oxidative glycollate cycle. Rubisco thus acts as a central link between photosynthesis and photorespiration (Figure 1.1).

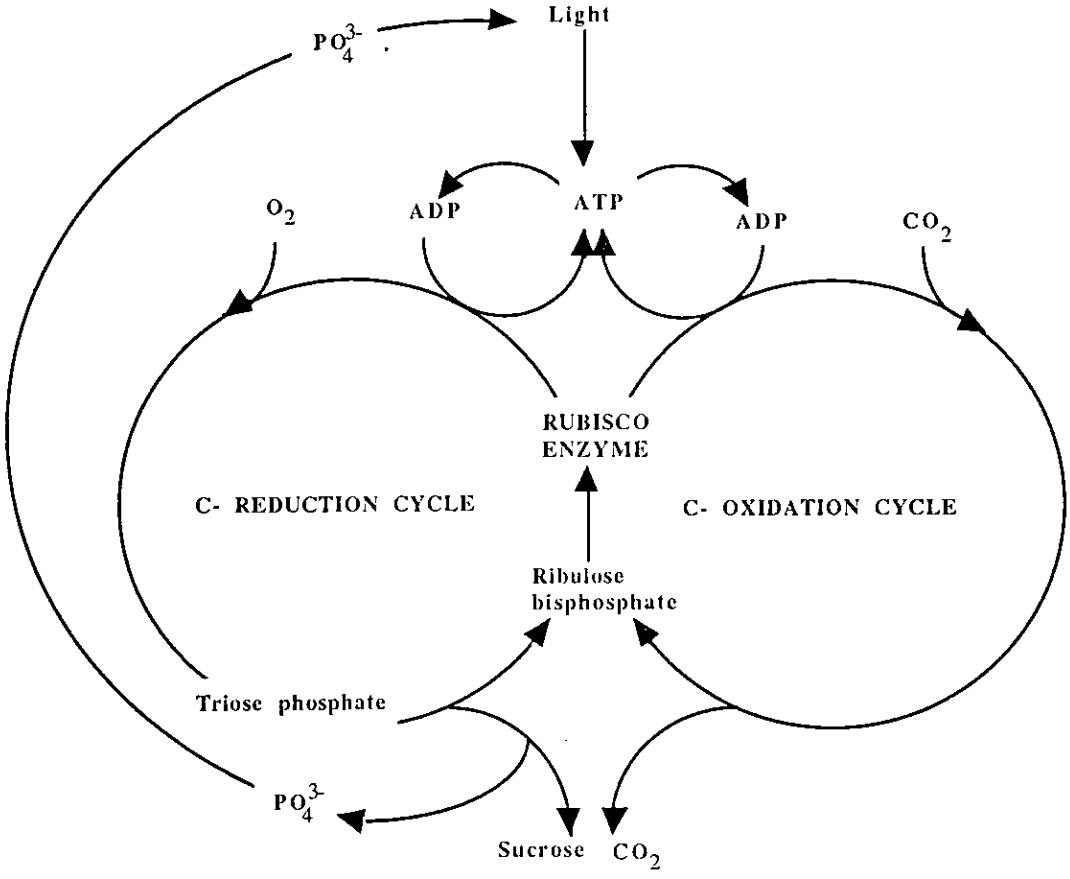


Figure 1.1 A simplified diagram showing the carbon reduction and carbon oxidation cycles (adapted from Andrews and Lorimer, 1987). Molecules of CO_2 and O_2 compete for places on the active sites of the rubisco molecule. An increase in the number of CO_2 molecules increases carbon reduction and suppresses carbon oxidation by competitive inhibition.

Rubisco has a relatively low affinity for CO_2 and under present atmospheric concentrations of CO_2 and O_2 is substrate limited (Stitt, 1991). An increase in CO_2 concentration increases the rate of photosynthesis of non-acclimated C3 plants, at least in the short term, by stimulating the carboxylation of ribulose bisphosphate and suppressing photorespiration (Stitt, 1991). For the rate of photosynthesis to be

sustained one molecule of Ru1,5bisP must be regenerated for every molecule used in carboxylation or oxygenation and this requires:

- an increase in activity of the carbon reduction cycle and an increase in NADPH and ATP production from the light reactions of photosynthesis; and
- an increase in the end products of photosynthesis (predominantly starch and sucrose).

NADPH and ATP are used in the regeneration of Ru1,5bisP in the carbon oxidation cycle. When the concentration of CO_2 is increased, more of this NADPH and ATP is available for use in the carbon reduction cycle.

The major end products of photosynthesis are starch and sucrose. Sucrose is synthesised from triose phosphate in the cytosol (Stitt, Huber and Kerr, 1987a) and starch in the chloroplast (Ap Rees, 1992). Sucrose synthesis and CO_2 assimilation are intrinsically linked. During photosynthesis CO_2 and P_i are converted to triose phosphate in chloroplasts; triose phosphate is then released from chloroplasts in exchange for P_i *via* the phosphate translocator (Heldt *et al.*, 1977). Synthesis of sucrose from triose phosphate in the cytosol results in release of P_i, which is returned to the chloroplast; triose phosphate is released and the process continues. When sink demand for photosynthate is reduced the processes involved in synthesising these products are reduced and this leads to build up of phosphorylated intermediates (triose phosphate and PGA) and the pool of P_i in the cytosol and chloroplasts is depleted. This leads to reduction in the rate of photosynthesis as a result of P_i limitation (Walker and Sivak, 1986).

The majority of reports in the literature that describe the effects of elevated CO_2 on photosynthesis of acclimating plants show an initial increase in photosynthetic rate (Kramer, 1981; Tolley and Strain, 1984a,b); Conroy *et al.*, 1986b); Ziska *et al.*, 1991; Idso *et al.*, 1991a,b); Radoglou, Aphalo and Jarvis, 1992). Kimball (1983), in a review of 430 experiments on the effects of an increase in CO_2 concentration calculated a mean stimulation in photosynthetic rate of 33±6% and Cure and Acock (1986) in a review of 10 crop plants (8 of which exhibited C3 metabolism) calculated a mean stimulation in photosynthetic rate of 52%. Similar results have been obtained for trees: Eamus and Jarvis (1989) compiled increases in CO_2 assimilation rate of between 30 and 200% for young trees, and in a recent review of 73 tree species, Luxmoore, Wullschleger and Hansen (1993) calculated a mean increase in photosynthetic rate of 46%. That such figures are varied and often fall below expected values calculated on the assumption that rubisco is limiting (Farquhar and von Caemmerer, 1982) is not

surprising as the response of photosynthetic rates to a doubling of CO_2 concentration depends on the distribution of control of photosynthesis (i.e. between rubisco, RuBP regeneration and end product synthesis) which in turn is dependent on:

- species;
- conditions;
- the length of the experiment; and
- degree of acclimation of the plants involved (Stitt, 1991).

Several workers have reported reductions in photosynthetic rate after long-term exposure to elevated CO_2 (Wulff and Strain, 1982; Tolley and Strain, 1984a, 1985a; De Lucia *et al.*, 1985; Oberbauer *et al.*, 1985; Sionit *et al.*, 1985; Garbutt *et al.*, 1990), although the phenomenon is not universal and some workers have found no reduction in photosynthesis after 3 years (Idso and Kimball, 1991). Down regulation of photosynthesis has been attributed to the lack of sustainable sinks for photosynthate and this may cause direct inhibition of photosynthesis as a result of feedback regulation of sucrose synthesis (Sasek, *et al.*, 1985; Stitt, 1991) or starch accumulation (Cave *et al.*, 1981; DeLucia *et al.*, 1985; Eamus and Jarvis, 1989) resulting in physical damage to the grana of the chloroplast (Wulff and Strain, 1982), although so far the evidence supporting the inhibition of photosynthesis by starch accumulation is purely correlative (Stitt, 1991).

Accumulation of carbohydrates may have an indirect long-term effect on the regulation of photosynthesis involving reduction in the expression of genes coding for key proteins required for photosynthesis (Stitt, 1991; Farrar, 1992). Such coarse control may represent a long-term response to source-sink imbalance (Krapp, Quick and Stitt, 1991).

1.2.2 Response of stomatal conductance to an increase in CO_2 concentration

The epidermis and cuticle of most higher plants are relatively impermeable to CO_2 and water vapour. Nearly all CO_2 entering, and water vapour leaving, the leaf does so through stomatal pores. As a result, stomata play a key role in regulating the amount of carbon assimilated and water transpired, and therefore have a significant impact on competitive ability, survival capacity and productivity. Opening and closure of stomatal apertures is controlled by the shape of the guard cells. Changes in cell shape are brought about by changes in turgor pressure of the guard cells (Meidner and Heath,

1959; Cowan, 1977) and are dependent on differences in thickness and elasticity of the cell wall in different parts of the cell (Weyers and Meidner, 1990). There is much conjecture concerning the biochemical mechanisms controlling stomatal movement (Mott, 1990; Mansfield *et al.*, 1990). Stomata are sensitive to both environmental and internal variables affecting the leaf, including solar radiation, water vapour pressure, temperature, plant water status and intercerllular CO_2 concentration (C_i). It has been hypothesised that stomatal response to these variables is integrated to maximise water use while minimising any reduction in photosynthesis (Farquhar and Sharkey, 1982). It is thought that this is accomplished by both feedback and feedforward processes and that as the conditions plants experience vary so too does the degree to which these are employed (Raschke, 1979). Stomatal movements result in changes in both partial pressures of CO_2 at the sites of carboxylation and rates of transpiration. Changes in rates of transpiration alter both temperature and water potential of leaves (Farquhar and Sharkey, 1982).

Short term response of stomatal aperture to CO_2 concentration in non-acclimated plants

In general, stomatal aperture shows a *short-term* decreases in response to an increase in the concentration of CO_2 (Figure 1.2). Although the sensitivity of stomata to CO_2 varies between species, some plants, particularly tree species, have been found to be relatively insensitive (e.g. Beadle, Jarvis and Neilson, 1979; Tolley and Strain, 1985).

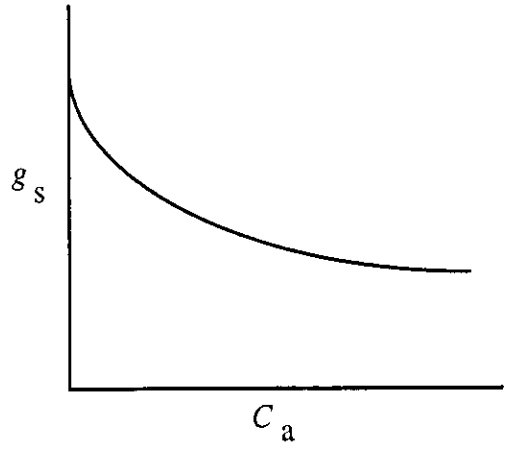


Figure 1.2 Generalised response of stomatal conductance (g_S) to ambient CO_2 concentration (C_a) (Adapted from Jarvis, 1989).

The mean intercellular space CO_2 concentration (C_i) is the effective CO_2 concentration determining stomatal aperture (Mott, 1988.) The mechanism for this response is not understood (Mott, 1990), but the site of perception of CO_2 appears to be the internal

surface of the guard cells. Mansfield *et al.* (1990) have proposed that guard cells respond to CO_2 concentration in two opposing ways:

- guard cell turgor increases with CO_2 concentration as a result of increased malate synthesis, and
- guard cell turgor is reduced as CO_2 concentration increases as a result of modulation of photophosphorylation, and/or modulation of oxidative phosphorylation and/or an unknown mechanism.

Response of g_s to elevated CO_2 in acclimating plants

In general, stomatal conductance is reduced when plants are grown in elevated CO_2 (Oberbauer *et al.*, 1986; Hollinger, 1987; Fetcher *et al.*, 1988; Bunce, 1992), although there are several reports of no such response (Conroy *et al.*, 1986b); Surano *et al.*, 1986; Bunce, 1992; Wullschlege, Norby and Hendrix, 1992; Gunderson *et al.*, 1993). Eamus and Jarvis (1989) reviewed the data then available for tree species and calculated that the general response of g_s to elevated CO_2 concentration was a decrease of between 10 and 60%. Morison (1985) compiled data from 16 C3, mainly crop species and calculated a mean reduction in g_s of 40% in plants grown in elevated CO_2. He concluded that reduction in stomatal conductance was the normal response but that sensitivity of stomata to CO_2 was affected by the conditions during growth and measurement.

The response of stomata to CO_2 concentration is complicated by interactions with other environmental variables; sensitivity to C_i increases with increasing photon flux density (PFD) at moderate values but may decrease in some species at high PFD (Wong *et al.*, 1985; Morison and Jarvis, 1983). An increase in (abscisic acid) concentrations as a result of water stress reduces g_s but increases sensitivity to C_i (Raschke, Pierce and Popiela, 1976; Raschke, 1987). Application of IAA (indole acetic acid) decreases stomatal sensitivity to C_i (Snaith and Mansfield, 1982) and application of CKK (cytokinin) opens stomata closed by exposure to elevated CO_2 concentration (Blackman and Davies, 1984). As yet, little work has been done especially on trees to determine effects of elevated CO_2 on sensitivity of stomata to PFD or water status.

Acclimation of g_s to CO_2 concentration with long-term exposure to elevated CO_2 has been shown to occur resulting in either an increase (Jarvis, 1989; Petterson and MacDonald, 1992) or a decrease (Hollinger, 1987) in g_s at the same C_i.

Determination of long-term effects of elevated CO_2 on stomatal conductance has usually depended on porometers which measure leaf conductance but do not give any information on whether differences in leaf conductance between two samples are a result of physiological responses affecting stomatal aperture, or changes in the structure of the leaf epidermis. The latter effects of elevated CO_2 are discussed below.

1.2.3 Effects of elevated CO_2 on stomatal density

In addition to stomatal aperture, stomatal conductance also depends on the size and frequency of stomata. Any changes in size or frequency of stomata with CO_2 treatment represent a longer-term developmental response to elevated CO_2.

The number and size of stomata varies within and between species and reflects the conditions under which leaves have developed. Irradiance, drought, nutrient availability, salinity and CO_2 concentration may all affect stomatal density either directly *via* an effect on the number of stomata initiated, or indirectly *via* an effect on leaf expansion (Jones, 1985; Terry *et al.*, 1983). Evidence for a developmental response to elevated CO_2 affecting stomatal density has been obtained from three sources:

- from study of herbarium specimens and leaves preserved in peat;
- from controlled experiments; and
- from plants growing adjacent to natural sources of CO_2 emission.

Evidence from herbarium specimens and leaves preserved in peat

Woodward and Bazzaz (1988) examined herbarium specimens collected over the last two hundred years for eight temperate tree species and, having determined the approximate concentrations of atmospheric CO_2 that the plants had grown in from ice core data, concluded that stomatal density had decreased by approximately 40% in response to a doubling of the atmospheric CO_2 concentration. It is not clear whether this was the result of developmental acclimation or genetic adaptation (Jarvis, 1989). Körner (1988) found no such effect when he compared measurements of stomatal density of specimens of alpine plants collected in 1890 and 1918, with stomatal density measurements from plants recently collected from the same area. Doubt was cast on the validity of these results by Woodward (1993) who pointed out that Körner had failed to take into account the environmental history of the leaves and the area on the leaves where the measurements were made. In a comparison of herbarium specimens of 14 trees and shrubs collected two hundred and forty years ago with present day tissue,

Penuelas and Matamala (1990) reported an average decrease in stomatal density of approximately 20% .

Beerling and Chaloner (1993b) examined leaf material of *Olea europaea* collected on four dates over a three thousand year period between 1327 BC and 1978 AD. Again using CO_2 data from ice cores it was shown that stomatal density was lower in plants that developed in conditions of higher CO_2 concentration. As the material was collected from a Mediterranean type climate that was considered not to have altered significantly over the period, the reduction in stomatal density was attributed directly to the changing CO_2 concentration. Investigation of herbarium leaf material of *Quercus robur* also showed a decline in stomatal density with increasing CO_2 concentration (Beerling and Chaloner, 1993a). However, investigation of the relationship between atmospheric CO_2 concentration and stomatal density of leaves preserved in peat of the arctic-alpine shrub *Salix herbacea* using fossil leaves, showed that rising CO_2 concentration over a time span of 11 500 years has been accompanied by an increase in stomatal density. This was attributed to temperature and water availability of the early post-glacial environment counteracting the effect of lower CO_2 concentration and/or natural selection favouring a different response over the last 200 years (Beerling *et al.*, 1992).

Evidence from controlled experiments

Several experiments have been done to investigate the effects of CO_2 treatment on stomatal density and the results so far have varied greatly amongst species. Woodward and Bazzaz (1988) grew plants in controlled environments at different CO_2 concentrations and reported a slight reduction in both abaxial and adaxial stomatal density and index in *Setaria, Amaranthus,* and *Ambrosia* at CO_2 concentrations above 350 $\mu mol\ mol^{-1}$, although the effect was not as large as in a previous experiment with plants grown at CO_2 concentrations below 350 $\mu mol\ mol^{-1}$ (Woodward, 1987). Thomas and Harvey (1983) found no significant effect of growth in elevated CO_2 on the stomatal density or index of soybean or sweetgum and Radoglou and Jarvis (1990b) found no effect on four poplar clones. However, Gaudillerie and Mousseau (1989) did find significant effects of growth in elevated CO_2 on stomatal density of *Populus euramericana*. Oberbauer *et al.*, (1985) found no effect on *Ochroma lagopus* but described a decrease in the abaxial stomatal density of *Pentaclethra macroloba* grown in elevated CO_2.

Evidence from natural sources

Stomatal and epidermal cell density, stomatal index and guard cell size have been measured on leaves of trees of *Quercus pubescens* growing adjacent to, and remote from, natural CO_2 vents in Italy, but no differences in stomatal density or index were found (Miglietta and Raschi, 1993).

Evidence from the above three sources suggests that stomatal density is affected by the CO_2 concentration in which the plants are grown but that the response is larger at subambient CO_2 concentrations and varies between species. Salisbury (1928) emphasised the importance of measurements of stomatal index

$$\text{Stomatal index} = \left(\frac{\text{no. of stomata}}{\text{no. of stomata + no. of subsidiary and epidermal cells}} \right) \times 100 \quad (1.1)$$

to separate effects of environmental variables affecting leaf area from direct effects of CO_2 on initiation of stomata.

The effect of a decrease in g_s resulting from either a reduction in stomatal aperture and/or a reduction in stomatal density, is to lower transpirational losses on a leaf area basis, which, together with increases in photosynthetic rate, results in an increase in instantaneous water use efficiency (WUE) (Eamus, 1991). An increase in instantaneous water use efficiency has been reported for many species grown in elevated CO2: *Ochroma lagopus* and *Pentaclethra macroloba* (Oberbauer*et al.*, 1985); *Populus euramericana* (Gaudillere and Mousseau, 1989); *Liquidamber stryaciflua* (Tolley and Strain, 1985); *Pinus echinata* (Norby *et al*, 1987); *Nothofagus fusca* and *Pseudotsuga menziesii* (Hollinger, 1987).

1.2.4 Biomass production

Increase in photosynthesis and reduction of transpirational losses may positively enhance biomass production. An increase in photosynthesis potentially provides more carbohydrate for plant growth, and improved water relations may enhance cell extension via a positive effect on turgor pressure (Chapter 4). Reports of an increase in biomass of plants grown in elevated CO_2 are widespread e.g. (Thomas and Harvey, 1983; Oberbauer *et al.*, 1985; Sionit *et al.*, 1985; Conroy *et al.*, 1986a); Luxmoore *et al.*, 1986; Brown and Higginbotham, 1987; Hollinger *et al.*, 1987; Norby *et al.*, 1987; O'Neil *et al.*, 1987a; Ziska *et al.*, 1991; Norby *et al.*, 1992) and extensive reviews have been made of effects of increased CO_2 concentration on growth of crops (Kimball,

1983; Cure and Acock, 1986) and young trees (Eamus and Jarvis, 1989). In a recent review, Luxmoore *et al.* (1993) compiled data from 73 tree species, growth response varied from 5% to 250%, with a mean response of 32%.

1.2.5 Resource allocation

Rate of photosynthesis determines the amount of carbohydrate produced by source leaves but the way in which that carbohydrate is used has important effects on future productivity, competitive ability, economic viability and survival of that plant. Changes in allocation patterns may represent important feedback interactions determining the long-term utilisation of resources of plants in an elevated CO_2 environment. Growth in elevated CO_2 has been reported to result in increased root production, particularly of fine roots (Idso and Kimball, 1991; Norby, Wullschleger and Gunderson, 1994), increased branch production (Kramer 1981; Idso and Kimball, 1991), leaf area (Kramer, 1981; Higginbotham *et al.*, 1985; Tolley and Strain, 1985; Ziska *et al.*, 1991), leaf mass (Oberbauer *et al.*, 1985; Pettersson *et al.*, 1993), although the reported responses are variable amongst species. The responses of allocation patterns of species of trees are discussed in Chapter 3.

Increase in allocation of dry mass to roots increases the potential for nutrient absorption and water uptake. Increase in number of branches produced affects canopy structure, absorption of radiation and speed of canopy closure. Increase in leaf area increases the potential for radiation absorption and water loss: the potential effects of elevated CO_2 on leaf area production are discussed in the next section (§1.2.6.). A reduction in specific leaf area (SLA) may be a result of an increase in leaf thickness and alterations in anatomical organisation: the potential effects of elevated CO_2 on leaf characteristics, including SLA and leaf thickness, are discussed in (§1.2.6.).

1.2.6 Leaf area production

There have been many reports in the literature of elevated CO_2 concentrations stimulating leaf area development (Goudriaan and de Ruiter, 1983; Higginbotham *et al.*, 1985; Tolley and Strain, 1984a; Leith *et al.*, 1986; Leadley and Reynolds, 1988; Caporn, 1989; Eamus and Jarvis, 1989; Wong, 1990; Ziska *et al.*, 1991), both in terms of an increase in number of leaves produced (Tolley and Strain, 1984a) and size of the individual leaves (Sionit, 1983; Rogers *et al.*, 1983; Oberbauer *et al.*, 1985). However, results vary with species and some workers have found no response (Mousseau and Enoch, 1989), or a reduction in leaf area (Bazzaz *et al*, 1990) in elevated CO_2 plants.

Leaf area of a plant is an important determinant of its biomass production (Milthorpe and Moorby, 1974). Leaves are the main sites of photosynthesis. Increase in leaf area increases interception of solar radiation and growth rate (Monteith and Elston, 1983). In addition to this role in radiation interception and biomass production, leaf area also plays a key role in determining the amount of water transpired from a plant or canopy. The leaf area available for radiation interception and transpiration is determined by the number of leaves present on the plant at any time, the individual size of those leaves and their arrangement on the plant.

Extension of the lamina

Surface areas of individual leaves are determined by the number of primordial cells, the rate of cell division, the duration of cell division, the final number of cells and the degree to which those cells enlarge.

Plant cell walls give strength and structure to cells and rigidity to the plant as a whole. Cell walls play important roles in defence against pathogens, protection from physical stresses, including osmotic swelling in hypotonic environments, cell to cell communication, water movement and control of cell growth (Nobel, 1985).

Cell walls are deposited as a series of layers. The layers consist of a microfibrillar phase and a matrix phase. The microfibrillar phase consists of chains of cellulose (β1,4-glucan) molecules arranged to form microfibrills. The matrix phase of cell walls consists of polysaccharides (pectins, hemicelluloses), proteins, glycoproteins and phenolic compounds. Covalent and non-covalent bonds formed between matrix molecules hold the microfibrils in position forming a set of interlinked networks. A number of models have been proposed to describe the precise arrangement of networks forming the cell wall (Preston, 1974; Passioura and Fry,1992)

Water moving into cells along osmotic gradients, causes the plasmalemma to push against the cell wall: this outward pressure is termed turgor pressure. Cell walls have a degree of elasticity and this "buffers" cells size to some extent against changes in turgor pressure. Irreversible plant cell enlargement occurs in actively growing leaves when turgor pressure builds up within cells to such an extent that it results in plastic, i.e. non-reversible, extension of cell walls. This hydrostatic pressure or turgor pressure is the driving force for growth. The pressure necessary to cause cell walls to undergo this plastic extension is termed "the yield turgor." Turgor pressure exerts stress on load-bearing bonds within the cell wall and growth of the cell occurs when these bonds are broken, resulting in 'relaxation' of elastically stretched wall elements. Relaxation of the cell wall reduces the water potential in the cell and water flows into the cell (Cosgrove,

1986). This influx of water reduces the concentration of solutes within the cell (i.e. the osmotic potential). In order for the cell to continue to grow, the osmotic concentration must be maintained by production or uptake of solutes into the cell. The two processes of cell wall yielding and water uptake occur simultaneously in a number of cells allowing cells and leaf to expand at a constant rate (Cleland, 1971, 1977; Taiz, 1984).

The process of cell wall extension is not a passive process. Early work done on *Nitella*, and oat coleoptiles (Cleland, 1971) showed that if a respiration inhibitor (e.g.sodium or potassium vanadate), or uncoupler (e.g. DCMU, dichlorophenyldimethylurea) is applied, growth stops irrespective of turgor pressure. This led to the conclusion that a metabolic process is required to sustain growth by maintaining turgor and mediating bond breakage and synthesis (Taiz, 1984; Brett and Waldron, 1990).

Extension of leaves has been shown to respond to increase in PFD (Van Volkenburgh and Cleland, 1980, 1981) and this was not associated with an increase in leaf turgor but was thought to result from cell wall acidification leading to wall loosening.

The nature of this process is described by the *acid growth hypothesis* which was first put forward as a result of studies on *Nitella* of the action of auxin in stimulating growth (Cleland, 1971) and has since been backed up by work on PFD-stimulated growth in *Nitella* and *Atriplex* (Cleland, 1986) and *Phaseolus vularis* (Van Volkenburgh and Cleland, 1980; 1981). The hypothesis is that the effects of auxin and PFD on cell walls is mediated by acidification of the cell walls caused by an increase in the action of proton-extruding ATPase enzymes in the plasmalemma. It is suggested that certain bonds within the wall may be weakened directly by acid and/or the increased activity of bond-breaking enzymes with an acid pH optimum. PFD is thought to act indirectly by increasing intracellular ATP *via* some part of the photosynthetic process and that this increase in ATP activates the proton pump (Van Volkenburgh and Cleland, 1980; Van Volkenburgh, Cleland and Schmidt, 1985).

Lockhart (1965) proposed a mathematical model to describe the biophysical control of steady-state growth of individual cells. In a simplified form the Lockhart equation states that under certain conditions (e.g. isothermal conditions, elastic equilibrium and linear viscoelastic cell wall) increase in cell volume is proportional to turgor pressure in excess of a critical turgor (yield turgor) and extensibility of the cell walls, i.e.

$$\frac{dv}{dt} = m\,(P - Y), \tag{1.2}$$

dv/dt is increase in cell volume with time, P is turgor pressure, Y is the yield threshold (i.e. the value of turgor below which the cell wall does not extend) and m is the extensibility of the cell wall.

The increase in cell volume can also be written as:

$$\frac{dv}{dt} = k\,(\psi_o - \psi_i) = k\,(\psi_o - P + \pi), \tag{1.3}$$

where k is hydraulic conductance of the cell membrane, ψ_o is water potential outside the cell, ψ_i is water potential inside the cell and π is osmotic potential within the cell. Combining equations (1.1) and (1.2) gives:

$$\frac{dv}{dt} = \frac{mk}{m+k}(\psi_o + \pi - Y) \tag{1.4}$$

If k is assumed to be much larger than m (Cosgrove, 1986) then equation (1.4) can be assumed to be equivalent to equation (1.2) in most cases, (Passioura and Fry, 1992).

From equation (1.2) it can be seen that any environmental variable affecting either m or $(P\text{-}Y)$ may affect cell extension and by, implication, leaf extension. The effects of both temperature and radiation on leaf extension are well established (Terry, Waldron and Taylor, 1983). Both radiation quantity and quality affect leaf extension (Baker and Enoch, 1983). CO_2 concentration also affects leaf growth. There are several possible ways in which elevated CO_2 concentration may affect leaf growth:

- increased photosynthetic rates result in increase in production of carbohydrates, increasing osmotic potential, and turgor pressure (Arp, 1991; Morison, 1993);

- increased photosynthetic rates result in increase in amount of carbohydrate available for incorporation into new plant material and for respiration;
- reductions in stomatal conductance (g_S) lead to a decrease in water loss per unit leaf area resulting in less negative water potentials and therefore increasing turgor pressure (Terry *et al.*, 1983);
- increased photosynthetic rates result in increase in intracellular ATP amounts which, in turn, activate excretion of protons by an ATPase mediated proton pump, causing increase in the PFD-activated acidification of plant cell walls; and
- direct or indirect effect on hormone concentrations.

Although there are many reports of the effects of CO_2 on production of leaf area, little has been done to investigate the mechanisms producing these effects and it is not yet clear whether increase in leaf area in plants in elevated CO_2 is the result of an increase in substrate availability or whether there is a more direct effect (Eamus and Jarvis, 1989).

1.2.7 Leaf characteristics

In addition to leaf area, other characteristics of leaves, for example specific leaf area (SLA), leaf thickness, anatomical organisation, chlorophyll content and chlorophyll *a*:*b* ratio are important in determining photosynthetic capacity and, therefore, under certain circumstances biomass production. All of these properties have been shown to be affected by elevated CO_2 concentration. Changes in leaf characteristics may have important long-term effects on photosynthetic capacity of plants grown in elevated CO_2.

Specific leaf area and leaf thickness

Growth in elevated CO_2 results in increase in leaf thickness and reduction in SLA in many tree species: *Pinus taeda, Liquidambar stryaciflua* (Tolley and Strain, 1984); *Ochrama lagopous, Pentaclethra macroloba* (Oberbauer *et al.*, 1985); *Silver birch* (Pettersson and McDonald, 1992); *Populus* clones (Thomas and Harvey, 1983; Leadley *et al.*, 1987; Leadley and Reynolds, 1988; Radoglou and Jarvis, 1990a). Reduction in SLA may be a result of increase in storage of end products of photosynthesis, and/or an increase in structural content of leaves. An increase in accumulation of carbohydrates in source leaves has been reported for many plants

grown in elevated CO_2 (Huber, Rogers and Israel, 1984; Farrar, 1992; Pettersson and McDonald, 1992; Norby *et al.* 1991, 1992).

Leaf anatomy

Increases in leaf thickness may be accompanied by alterations in leaf anatomy, either via changes in size of cells or number or type of cells and volume and distribution of intercellular spaces. Internal cell surface area per unit leaf area is closely linked to photosynthesis and water use efficiency (Nobel, 1985); increase in internal cell surface area increases the area available for absorption of CO_2 molecules at sites of carboxylation.

The initiation of an additional layer of palisade cells has been reported in response to elevated CO_2 in *Glycine max* (Hofstra and Hesketh, 1975; Thomas and Harvey, 1983) and *Castanea sativa* (Mousseau and Enoch, 1989). An increase in the proportion of spongy parenchyma tissue has been reported for poplar (Radoglou and Jarvis, 1990a) and loblolly pine (Thomas and Harvey, 1983), whereas the increase in leaf thickness in sweetgum was concomitant with increase in size of cells in all cell layers (Thomas and Harvey, 1983).

Chlorophyll content

Chlorophyll *a* and *b* form a central part of the light harvesting apparatus, the function of which is to utilise quanta to produce ATP. Reductions in total chlorophyll content have been reported in several species grown in elevated CO_2: yellow poplar (*Liriodendron tulipifera*) and white oak (*Quercus alba*) (Wullschleger, Norby and Hendrix, 1992), *Liquidambar stryaciflua* (Tolley and Strain 1984a) and *Desmodium paniculatum* (Wulff and Strain, 1982). Reduction in chlorophyll *a* :chlorophyll *b* ratio have also been reported e.g. in tomato (Madsen, 1968) and *Trifolium subterraneum* (Tolley and Strain, 1985).

A reduction in chlorophyll may reduce light harvesting efficiency in plants grown in elevated CO_2 (Oberbauer *et al.*, 1985; Houpis *et al.*, 1988). However, despite a reduction in chlorophyll content, Wulff and Strain (1982) and Wullschleger, Norby and Hendrix (1992) reported an increase in light harvesting efficiency in plants grown in elevated CO_2. This was attributable in part, to changes in leaf anatomy.

1.3 OUTLINE OF THESIS

The species used in this study, silver birch (*Betula pendula* Roth.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.), conditions of growth and open top chambers are described in Chapter 2.

Growth response of Sitka spruce and silver birch to elevated CO_2 concentration, in terms of biomass accumulation, patterns of allocation, effects on phenology and total leaf production are described in Chapter 3.

Growth response of individual leaves of silver birch and effects of elevated CO_2 on the biophysical processes that control leaf extension are described in Chapter 4.

Effects of elevated CO_2 on leaf characteristics are described in Chapters 5 and 6: leaf conductance, stomatal density and stomatal index measurements and direct measurements of stomatal apertures obtained using a scanning electron microscope and an image analyser are presented in Chapter 5.

Effects of growth in elevated CO_2 on SLA, leaf thickness, anatomical organisation, chlorophyll and carbohydrate content are described in Chapter 6.

In Chapter 7 these findings are drawn together in a discussion of their implications for the future functioning of trees, along with an assessment of the approach and techniques used and suggestions for further research.

This study forms part of a much larger, series of experiments currently being undertaken at the University of Edinburgh and the Institute of Terrestrial Ecology to investigate effects of long-term exposure to elevated CO_2 concentration on temperate tree species as part of an European initiative. The trees remaining at the end of this study were transferred to purpose-built individual tree chambers where experiments on them continue.

CHAPTER 2 PLANT MATERIAL AND OPEN TOP CHAMBERS

2.1 PLANT MATERIAL

Forests form an important part of the terrestrial carbon cycle (Solomon and Cramer, 1993). If an increase in atmospheric concentration of CO_2 stimulates plant growth, trees may represent an important additional sink for carbon. Most of the studies made on the response of trees to elevated CO_2 concentration have been done on American species that have very little relevance to European forests. The work in this study was done on two species, silver birch *(Betula pendula* Roth.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.), both of which are important constituents of Scottish forests. silver birch is native to Britain and Sitka spruce is an introduced species that is now an important British crop.

2.1.1 Sitka spruce

Sitka spruce is native to the west coast of North America where it occurs from Alaska to California, mainly on coastal sites. It was introduced into Britain in 1831 and is now the most important species in upland forestry in Britain, comprising over 50% of current planting (Rook, 1992). It thrives on most of the sites available for afforestation in this country, exhibiting a high degree of vigour. In 1980 the total area of Sitka spruce plantations in Scotland covered more than 364 000 ha or 48% of the total coniferous upland forest area and 28% of the total forest area (Rook, 1992). Although low strength properties make it unsuitable for constructional timber, it is a highly versatile soft-wood timber used, for example, for manufacturing particle board and fibreboard and it is ideal for pulp for the manufacture of paper (Rook, 1992). Spruce seedlings grow slowly compared with many broadleaves as a result of relatively low photosynthetic rates and slower rates of leaf area production but mature canopies of Sitka may be very productive. Cannell (1987) outlined three reasons for their high productivity:

- needles persist for six to eight years, leading to deep canopies which can intercept almost all solar radiation;
- needles and shoots are structured to allow good penetration of light into the canopy; and

- ability of photosynthetic apparatus to adapt to low light and low temperature, so that there is net carbon gain throughout almost the whole year.

In contrast to silver birch, Sitka spruce exhibits a 'fixed' growth pattern; the length of new shoots is predetermined during bud formation the previous year. Buds contain the preformed initials (internodes and leaves) and expand in the following year, although under certain conditions juvenile spruce may also exhibit growth from buds produced in the same year (lammas, or 'free' growth) (Kozlowski, Kramer and Pollardy, 1991). It has been suggested that different growth patterns of species will alter the nature and magnitude of their response to elevated CO_2 (Oberbauer *et al.*, 1985). The 'free' growth pattern exhibited by silver birch allows repeated initiation of sinks which, together with its relatively faster growth rate, may be expected to increase the size of its response to elevated CO_2, whereas the response of Sitka spruce may be more constrained as a result of the determinate nature of its growth pattern.

2.1.2 Silver birch

Silver birch is a fast growing deciduous tree, common throughout northern and central Europe and locally on mountains southwards to Spain. It is native to Britain and in conjunction with *Betula pubescens* with which it readily hybridises, is the second most abundant broad leaf tree in Britain, covering approximately 21% of the total area of woodland (Steele and Peterken, 1982). It is the most abundant broadleaf species in Scotland, a pioneer species of open ground, burnt areas and forest clearance, and has been categorised by Grime, Hodgson and Hunt (1988) as intermediate between a competitor and a stress-tolerant competitor. It regenerates readily from seed but has a relatively short life span (60-70 years) and is often superceded by oak. The use of silver birch in land restoration and conservation is currently being encouraged (Patterson, 1993) *via* a series of grant schemes (e.g. the Woodland Grant Scheme). It is not an important tree for timber production in Britain, although it is grown extensively in Sweden where it is used for the manufacture of particleboard, fibreboard, floors and furniture.

Silver birch exhibits 'free' growth, which involves elongation of shoots by simultaneous initiation and elongation of new shoot components as well as extension from buds laid down in the previous year (Kozlowski *et al.*, 1991).

2.2 GROWING CONDITIONS

2.2.1 Sitka spruce

In May 1990 two-year old seedlings of Sitka spruce (*Picea sitchensis* (Bong.) Carr., QCI provenance 20 (identification number 83(2015)s Lot 2) were purchased from EFG (Economic Forestry Group) . The seedlings were bare rooted and had previously been in cold storage to delay budburst. Seedlings were placed in individual (1.5 dm^3) pots containing a standard sand:peat:loam mixture (1:5:3). The pots containing the plants were then transferred to eight open top chambers (OTCs) located at the Institute of Terrestrial Ecology, Bush Estate, near Edinburgh (55° 31' N, 3° 12'W). Ambient air was passed through four of the OTCs; air entering the remaining four chambers was supplemented with CO_2 to give a concentration of 250 µmol mol^{-1} above ambient (§2.4). Thirty plants were placed in each chamber. The pots were placed on capillary matting and watered to field capacity daily. The seedlings did not receive any additional fertilizer.

In March 1991 the Sitka spruce seedlings were repotted into 5 dm^3 pots containing sand:peat:loam (2:5:3). Two extra chambers were utilised in 1991, one receiving supplementary CO_2, the other receiving ambient air, making a total of four per treatment. Twenty five plants were placed randomly in each chamber. All the plants were placed in chambers with the same CO_2 regime that they had been growing under the previous year but the concentration of CO_2 entering the elevated CO_2 chambers was increased to 350 µmol mol^{-1} above ambient. The plants were watered as before. From March 1991 the plants were fertilised monthly with a liquid fertiliser (Chempak no.3., Chempak Ltd, Hoddesdon, Herts).

2.2.2 Silver birch

In March 1991 seeds of silver birch (*Betula pendula* Roth.) (provenance FC 87/20) were sown in seed trays containing a mixture of sand, peat and grit and placed in polythene germination tunnels. Air entering one of the tunnels was supplemented with CO_2 to a concentration of ambient plus 350 µmol mol^{-1}. The concentration of CO_2 was monitored at weekly intervals using a portable infra-red gas analyser (IRGA), (LCA-2, ADC Hoddesdon, Herts, UK). The seedlings were watered with an overhead irrigation system.

At the three leaf stage the seedlings were transplanted into 1.5 dm^3 pots containing a standard potting compost (John Innes No.2, Bowers plc, Cambridge) and transferred

to eight of the open top chambers with the same CO_2 regime as previously outlined for the Sitka spruce seedlings. The pots containing the plants were placed on capillary matting and watered twice daily and plants were fertilised monthly with a liquid fertiliser (Chempak no.3., Chempak Ltd, Hoddesdon, Herts).

In March 1992 the silver birch seedlings were repotted into 5 dm^3 pots containing sand:peat:loam (2:5:3). Ten plants were placed in each chamber in randomised blocks of five plants. All plants were placed in chambers with the same CO_2 regime they had been growing in the previous year. The plants were watered as before and were fertilised monthly with a liquid fertiliser (Chempak no.3., Chempak Ltd, Hoddesdon, Herts).

2.3 OPEN TOP CHAMBERS

The open top chambers (OTCs) used in the experiments described in this thesis are octagonal in shape, 3 m in diameter and 2.4 m high. They are constructed of a light-weight aluminium frame, with 3 mm horticultural glass. A frustum at the top of the chamber and a glass shelf situated 0.5 m below the frustum, serve to deflect air and reduce incursions of ambient air which may dilute the CO_2 concentration within the chamber. Air was filtered and drawn via a fan (EK31, radial and axial fan, Cold Harbour Lane, Harpenden,Herts) and entered the chamber through a duct connected to a perforated polythene sleeve (plenum) 1.5 m above the floor of the chamber. There were two air changes per minute. The CO_2 monitoring and control system was initially controlled by a datalogger (21X, Campbell Scientific Ltd, Loughborough) but was replaced with a 286 personal computer and interface card system in 1991 (Barton, 1993). A diaphragm pump (Charles Austin Ltd, Weybridge, Surrey) drew air from the chambers through 4 mm nylon sample lines, each of which contained a two-way solenoid valve which, when activated, diverted air to an IRGA (ADC Mark 2, Analytical Development Co.Ltd, Hoddeston, Herts). The solenoid valves were switched sequentially every two minutes: residual air was flushed through the IRGA during the first minute, to allow the IRGA to stabilise and a mean of readings taken at fifteen second intervals during the second minute was stored by the data logger or personal computer. The CO_2 entering the chambers was supplied from a 16 tonne bulk liquid tank *via* a vaporiser (Distillers M.G., UK.) along 4 mm nylon supply lines which injected the CO_2 into the air inlet duct, downstream of the fan. The flow of CO_2 along the supply lines was controlled by mass flow controllers (Tylan FC280, Torrance CA, USA). Each elevated CO_2 chamber received ambient air plus 250 $\mu mol\ mol^{-1}$ CO_2

in 1990. The concentration of CO_2 was increased to 350 μmol mol^{-1} above ambient in 1991. Ambient CO_2 concentrations vary diurnally, so that injecting a constant amount of CO_2 into the chambers allowed the concentration of CO_2 in the elevated CO_2 chambers to vary similarly.

The chambers were washed and disinfected at the beginning of each season to maximise light transmission and limit the incidence of disease .

The air temperature within the chambers was measured at a height of 1.5 m above the chamber floor using a ventilated, radiation-shielded thermistor (RS Ltd, Loughborough). Outside air temperature and solar radiation were measured using a platinum resistance thermometer (Delta-T devices Ltd, Burwell, Cambridge) and a solarimeter (CM3, Kipp and Zenon Ltd, Delft, Holland) respectively, mounted on the roof of a *portacabin* adjacent to the OTCs. Data loggers (21X, Campbell Scientific Ltd, Loughborough) were used to collect and store the data. The median daytime temperature within the chambers was approximately 2 °C above the outside temperature but reached 4 °C above for short periods during a few hot summer days. Figures (2.1a-d) show the temperatures in one ambient and one elevated CO_2 chamber, compared to the outside temperature measured during four days in the summer of 1991. The quality of photosynthetically active radiation reaching the plants in the chambers was not affected by the glass. Transmittance of solar radiation was reduced by less than 10% (Lee and Barton, 1993).

2.4 DISTRIBUTION OF OPEN TOP CHAMBERS

Plant position within each chamber wasrandomised. The open top chambers used in this study were situated on a gradual slope. Because of possible gradients in environmental conditions along the slope,four blocks each containing one ambient and one elevated CO_2 chamber, were created along the gradient of the slope. This approach allowed for comparison between treatments as well as blocks. The effects of elevated CO_2 could therefore be corrected for a possible block effect, caused by different environmental conditions along the slope.

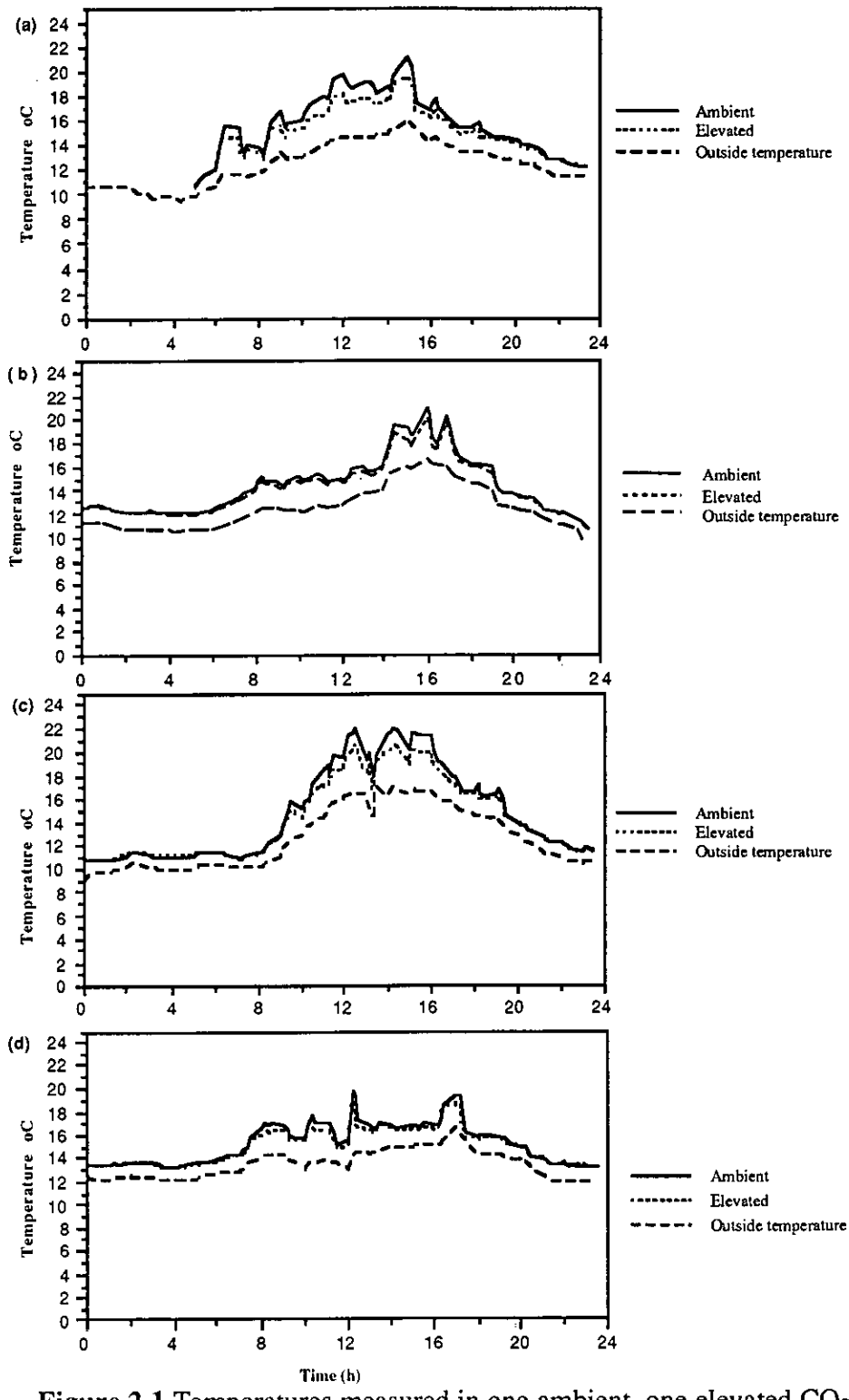


Figure 2.1 Temperatures measured in one ambient, one elevated CO_2 chamber and outside on a)DOY 195, b)DOY 196, c)DOY 197, d)DOY 200. Values represent means of measurements taken at 5 minute intervals over a 30 minute period. Time is in GMT.

CHAPTER 3 THE EFFECT OF ELEVATED CARBON DIOXIDE ON THE GROWTH AND MORPHOLOGY OF SITKA SPRUCE AND SILVER BIRCH

3.1 INTRODUCTION

In the previous chapter the plants, growing conditions and open top chambers were described. This chapter describes the effect of an elevated concentration of CO_2 on the growth and morphology of Sitka spruce and silver birch. The aim of the study reported here was to determine the effects of elevated CO_2 on the production and morphological allocation of biomass in Sitka spruce and silver birch.

Biomass production

Both increase in rate of photosynthesis and reduction of transpirational losses may enhance biomass production. Reports of an increase in biomass of trees grown in elevated CO_2, compared to ambient CO_2, are widespread: *Castanea sativa* (Mousseau and Enoch, 1989; El Kohen, Rouhier and Mousseau, 1992), *Citrus aurantium* (Idso and Kimball, 1991; Idso, Kimball and Allen, 1991a,b), *Quercus alba* (Norby, Wullschleger and Gunderson, 1994), *Fagus grandifolia, Betula papyrifera, Prunus serotina, Acer saccharum* and *Tsuga canadensis* (Bazzaz, Coleman and Morse, 1990), *Picea abies* (Mortenson, 1982), *Pinus contorta* (Higginbotham *et al.*, 1984), *Ochroma lagopus* and *Pentaclethra macroloba* (Oberbauer *et al.*, 1985), *Pinus radiata* (Conroy *et al.*, 1990). Eamus and Jarvis (1989) estimated that the median increase in biomass production with doubling of CO_2 concentration was about 40% in young trees, and in a recent review Luxmoore *et al.* (1993) calculated a mean growth response of 32% for 73 tree species grown in elevated CO_2.

Morphological allocation

In addition to increasing biomass production, elevated CO_2 may alter the structure of plants as a result of changes in the pattern of carbon allocation.

Increases in root to shoot ratios have been reported in response to growth in elevated CO_2 (Sionit *et al.*, 1981; Higginbotham *et al.*, 1985; Luxmoore *et al.*, 1986; Norby *et al.*, 1987; Oberbauer, *et al.*, 1986; Norby *et al.*, 1994), although the majority of reports of trees grown in elevated CO_2 under well-watered conditions with adequate nutrition showed no effect, or a slight decrease in root: shoot ratio: *Fagus grandifolia, Betula*

papyrifera, Prunus serotina, Acer saccharum, Acer rubrum, Pinus strobus and *Tsuga canadensis* (Bazzaz, Coleman and Morse, 1990), *Liriodendron tulipifera* (Norby, Wullschleger and Gunderson, 1994). Jarvis and Eamus (1989) summarised no significant changes in root:shoot ratio in young tree seedlings grown under non-nutrient limiting conditions. The allometric constant (*k*) determined for Sitka spruce did not change (D. Jones, pers. comm.), suggesting that whole plant C allocation was not affected by growth in elevated CO_2.

An increase in production of branches has been reported in elevated CO_2 concentration (Mortensen, 1987; Idso *et al.*, 1991; Samuelson and Seilor, 1993), although again results vary and there was no increase in branch production in *Castanea sativa* (Mousseau and Enoch, 1989) or *Populus* clones (Radoglou and Jarvis, 1990a). An increase in total leaf area has been reported in *Liquidambar stryaciflua* (Sionit *et al.*, 1985), *Ochroma lagopus, Pentaclethra macroloba* (Oberbauer *et al.*, 1985), *Pinus contorta* (Higginbotham *et al.*, 1985), *Liquidambar stryaciflua, Pinus taeda* (Tolley and Strain, 1984), *Fagus grandiflora* and *Acer saccharum* (Bazzaz *et al.*, 1990).

Morphological changes may be attributable to increases in supply of photosynthate increasing growth of existing sinks or initiating the development of new ones (Stitt, 1991), although direct effects of elevated CO_2 on leaf or root production cannot be ruled out (Jarvis, 1989). The extent and manner in which species respond to elevated concentrations of atmospheric CO_2 is expected to vary as a result of differences in growth strategies that are themselves under genetic control (Kozlowski *et al.*, 1991). Fast growing plants and those exhibiting indeterminate growth patterns are expected to show larger response to CO_2 treatment than slower growing plants or those exhibiting determinate growth patterns, as a result of their increased sink capacity. Inability to provide adequate sinks for photosynthate, resulting in a source-sink imbalance, has been associated with reduction in photosynthesis (§1.2.1.) and, therefore, biomass production. Oberbauer *et al.* (1985) reported a larger response of growth in elevated CO_2 in the pioneer species *Ochroma lagopus* compared to the climax species *Pentaclethra macroloba.*

The different growth patterns of silver birch and Sitka spruce were described in Chapter 2. It is suggested that such differences will affect the nature and magnitude of the responses of these species to elevated CO_2. Differences in responses of tree species to elevated CO_2 have implications for the future composition and structure of forests.

3.2 MATERIAL AND METHODS - SITKA SPRUCE

In May 1990, two-year-old seedlings of Sitka spruce were placed in individual (1.5 dm^3) pots containing a standard sand:peat:loam mixture (1:5:3), and transferred to eight open top chambers (OTC's). Thirty plants were placed in each chamber. Ambient air was passed through four of the OTCs, the air entering the remaining four chambers was supplemented with CO_2 to a concentration of 250 $\mu mol\ mol^{-1}$ above ambient. In March 1991 the seedlings were repotted into 5 dm^3 pots containing sand:peat:loam (2:5:3). Twenty five plants were placed in each chamber. All the plants were placed in chambers with the same CO_2 regime that they had been growing under the previous year but the concentration of CO_2 entering the elevated CO_2 chambers was increased to 350 $\mu mol\ mol^{-1}$ above ambient. Full details of growth conditions and open top chambers are given in Chapter 2.

3.2.1 Non-destructive growth measurements

Measurements of height, leader length and basal diameter were made at weekly intervals. Measurements were made on 40 plants per treatment (ten plants from each chamber) in 1990 and 45 plants per treatment (15 from each of three chambers per treatment) in 1991.

3.2.2 Harvest

Twenty plants were harvested in June 1990 before they were transferred to the OTCs. Measurements of height and basal diameter were made and each plant was separated into its constituent parts (roots, shoots, needles), the parts oven dried at 70 ^oC and weighed using an electronic balance (Sauter, model RE1E14, Fisons Scientific Equipment, Loughborough).

Three subsequent harvests were made. In January 1991, at the end of the first growing season, 20 plants per CO_2 treatment (five from each of four chambers per CO_2 treatment) were harvested; in July 1991, midway through the second growing season 45 plants were harvested per CO_2 treatment (fifteen from each of three chambers per CO_2 treatment); and in January 1992, at the end of the second growing season 15 plants were harvested per CO_2 treatment (taken randomly from five chambers per treatment). Measurements of height and basal diameter were made and each plant was separated into its constituent parts (roots, shoots, needles), the parts oven dried at 70

^{o}C and weighed using an electronic balance (Sauter, model RE1E14, Fisons Scientific Equipment, Loughborough). Sub-samples of needles were taken for nutrient analysis.

3.2.3 Bud characteristics

Number of primordia

In January 1991, a terminal bud was removed from two first order lateral shoots on each seedling harvested. After excision the bud scales were removed. One bud was used to count the number of primordia in a bud and the other used to measure bud dimensions. Once the bud scales had been removed, the bud was viewed under a binocular microscope. In Sitka spruce, needle primordia are laid down sequentially in phyllotactic spirals (Figure 3.1). The divergence angle formed between centres of two successive primordia and the apical dome is close to the Fibonacci angle of 137.5°, giving contact parastichies (spirals of primordia) numbering in the series 1,2,3,5,8,13...(Cannell, 1978). This regular arrangement allows estimation of the number of primordia per bud as the product of the number of primordia in each phyllatactic spiral and the number of spirals (Chandler, 1989).

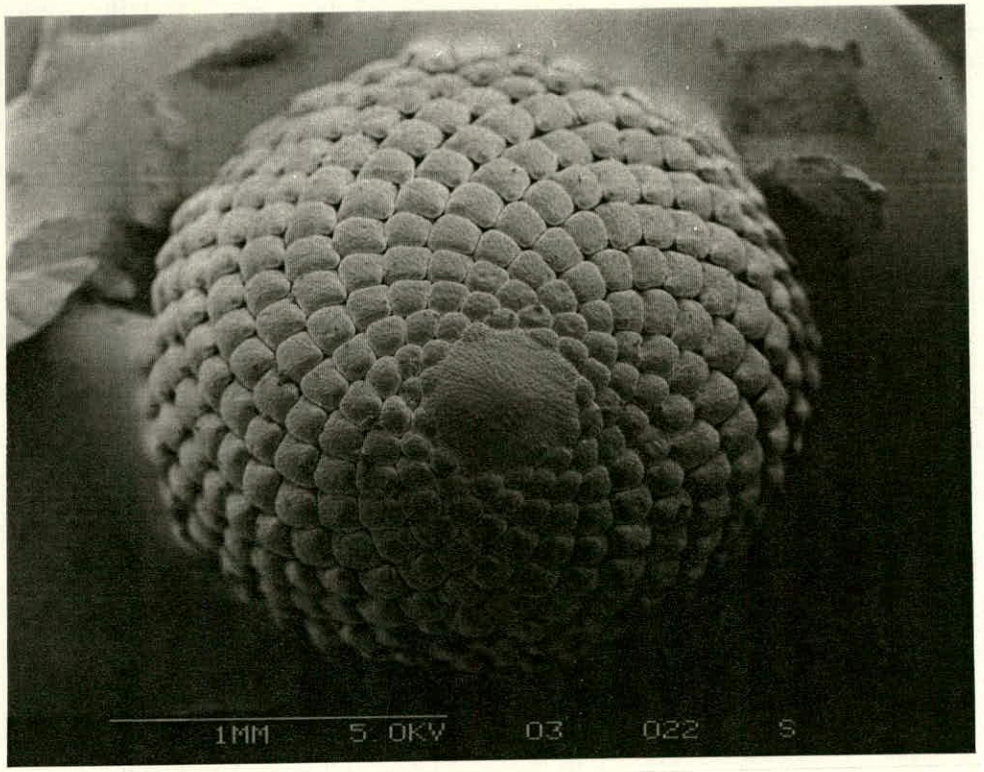


Figure 3.1 Scanning electron micrograph showing needle primordia arranged in spiral phyllatactic patterns in a bud.

Bud dimensions

Two methods were used to prepare material for determination of bud dimensions.

In the first method, the bud scales were removed from freshly excised dormant buds and the buds attached to a modified cryostub with a thin layer of tissue cryo-adhesive (Tissuetek, Lab-Tek division, Miles laboratories Inc,. Naperville, Illonois, USA). An additional amount of cryo adhesive was then used to cover the sample and the stub and sample plunged into a dewar of liquid nitrogen. The stub and sample were then inserted into a cryostat (Reichert-Jung, Leica, Cambridge) to equilibrate prior to sectioning. The temperature within the cryochamber was set at 18.7 ^{o}C and the angle of the knife at 8^{o}. Sections bisecting the bud were cut, transferred onto microscope slides, stained with safranin, rinsed in methanol and dehydrated in ethanol for one hour before mounting in Canada balsalm.

In the second method, buds were cut in half longitudinally and thin sections cut by hand under a binocular microscope (Chandler, 1989). These were then stained and mounted as before. All sections were viewed under a light microscope (Ortholux, Leitz Ltd., Luton) and photographed. Measurements were made from prints of the dimensions of the bud (Figure 3.2).

Comparisons between fresh and prepared tissue were made. Shrinkage of the linear dimensions of fresh tissue, as a result of the staining preparation used was found to be approximately 15% for both procedures, in agreement with the findings of Chandler (1989).

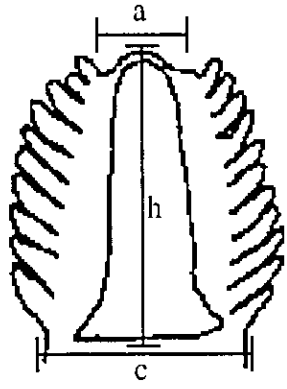


Figure 3.2 Diagram of a longitudinal section through the centre of a dormant bud.
a = Diameter of apical dome
h = Bud height (axial distance from base to vertex of the dome)
c = Basal diameter of the bud core.

3.2.4 Needle area

At each end of season harvest (January 1991 and 1992) six needles were removed from midway down the leader of each seedling harvested and projected individual needle area (A) determined from measurements of width (w) and length (l) i.e:

$$A = b \,.\, w.\, l \tag{3.1}$$

Steele (1987) determined projected area of needles from photographs from which he calculated b from the above relationship as:

$$b = A/\ (w.l) \tag{3.2}$$

Measurements of needle dimensions were made using an image analyser (Quantimet 970, Cambridge Instruments Ltd, Cambridge, UK).

3.2.5 Nutrient analysis

In July 1991 macro-nutrient (nitrogen, potassium and phosphorus) contents of current year leaf tissue from eight trees per CO_2 treatment (two trees from each of four chambers per treatment) were analysed by the Forestry Commission Research Division using HPLC (Dionex, Camberly,Surrey).

3.2.6 Statistics

A two way ANOVA was used to determine effects of the two CO_2 concentrations (treatment effect) and effects of the blocks (inter-chamber effect) on the dependent variables. Data were tested for normality and met the assumptions of the parametric analysis. An un-paired t-test was performed on harvest data of Sitka spruce collected in January 1992. Geometric mean regression analysis was used to calculate the allometric constant k (Richer, 1984).

3.3 MATERIAL AND METHODS -SILVER BIRCH

Seeds of silver birch were germinated under ambient (unsupplemented) or elevated (ambient + 350 μmol mol^{-1}) CO_2 concentration. At the three-leaf stage the seedlings were transplanted into 1.5 dm^3 pots and placed in eight OTCs with the same CO_2 regime. Details of establishment and growth conditions are given in Chapter 2.

3.3.1 Harvest

In May 1991 ten plants were harvested prior to transplantation. Measurements of height, number of main stem leaves, number of side shoot leaves and leaf area were made. Leaf area was measured using a leaf area meter (model ICI-201, CID Inc. Seattle, USA). Plants were then split up into their constituent parts (root, stem, main stem leaves, side shoots and side shoot leaves) and these oven dried at 70 ^{o}C and weighed using an electric balance (Sauter, model RE1E14, Fisons Scientific, Loughborough). A second harvest was made in late August. Ten plants were removed from each of three chambers per CO_2 treatment and harvested as before. Samples of leaves were taken for nutrient analysis.

3.3.2 Non-destructive growth measurements

Measurements of height, number of main stem leaves, number of side shoots and number of side shoot leaves were made on ten plants from three chambers per treatment at weekly intervals throughout the growing season. Leaf area of main stem and side shoot leaves was measured on one occasion in June, using a portable leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). The youngest fully developed main stem and side shoot leaf were measured on thirty plants per CO_2 treatment, (ten plants from each of three chambers). An estimate of total plant leaf area was calculated from area number of leaves.

3.3.3 Gas Exchange

Measurements of photosynthesis and stomatal conductance were made using a LCA-3 leaf chamber open analysis system (Analytical Development Co Ltd., Hoddesdon, Herts). The cuvette was clamped onto a leaf and left until a steady reading of photosynthesis was obtained (usually around two minutes) before a reading was taken. Measurements were made between 11.00 and 13.00 BST on three occasions in July 1991, forty plants were measured on each occassion (ten from each of four chambers).

3.3.4 Nutrient analysis

Macro-nutrient (nitrogen, potassium and phosphorus) contents of current year leaf tissue from eight trees per treatment (two trees from each of four chambers per CO_2 treatment) was analysed by the Forestry Commission Research Division using HPLC (Dionex, Camberly, Surrey).

3.3.5 Statistics

A two way ANOVA was used to determine effects of the two CO_2 concentrations (treatment effect) and effects of the blocks (inter-chamber effect) on the dependent variables. Data were tested for normality and met the assumptions of the parametric analysis.

3.4 RESULTS - SITKA SPRUCE

3.4.1 Leader extension

The leader length of Sitka spruce seedlings over the 1990 growing season is shown in Figure 3.3. Plants were measured at weekly intervals. There was a slight increase in length of elevated CO_2 plants compared to ambient CO_2 plants between day of year (DOY) 210 and 250 but this was only significant at $P<0.05$ on one occasion (DOY 208). There was no significant difference in leader length of plants measured in September. The rate of leader extension is shown in Figure 3.4. Rate of leader extension was significantly increased ($P<0.05$) in plants grown in elevated CO_2 on two occasions midway through the season (DOY 192 and 208), although there was also significant interchamber variation between plants measured on DOY 192, 200 and 208. The increased rate of leader extension of elevated CO_2 plants was not sustained and fell significantly below that of the ambient CO_2 plants by September (DOY 258). Although there was no significant difference between the leader lengths of plants measured in September, of the plants harvested in January 1991 those grown in elevated CO_2 had significantly longer leaders than the ambient CO_2 grown plants (Table 3.2, $P<0.01$). There was no significant difference in leader length amongst plants grown in different chambers.

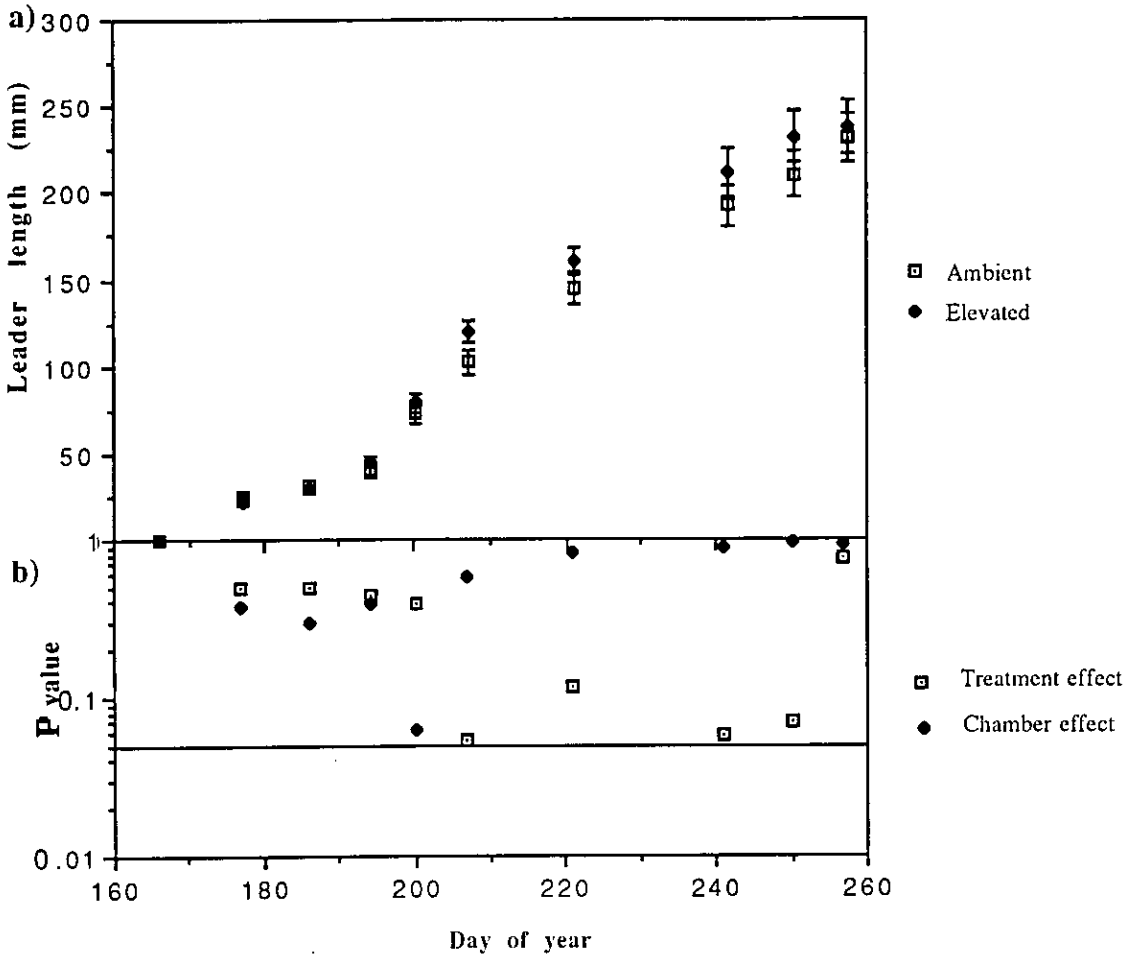


Figure 3.3 Leader length of Sitka spruce in 1990

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1}). Each value is the mean of 40 plants (ten plants from each of four chambers); error bars depict two standard errors of the means. Measurements were made at weekly intervals between early June, prior to budburst, and late September 1990.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

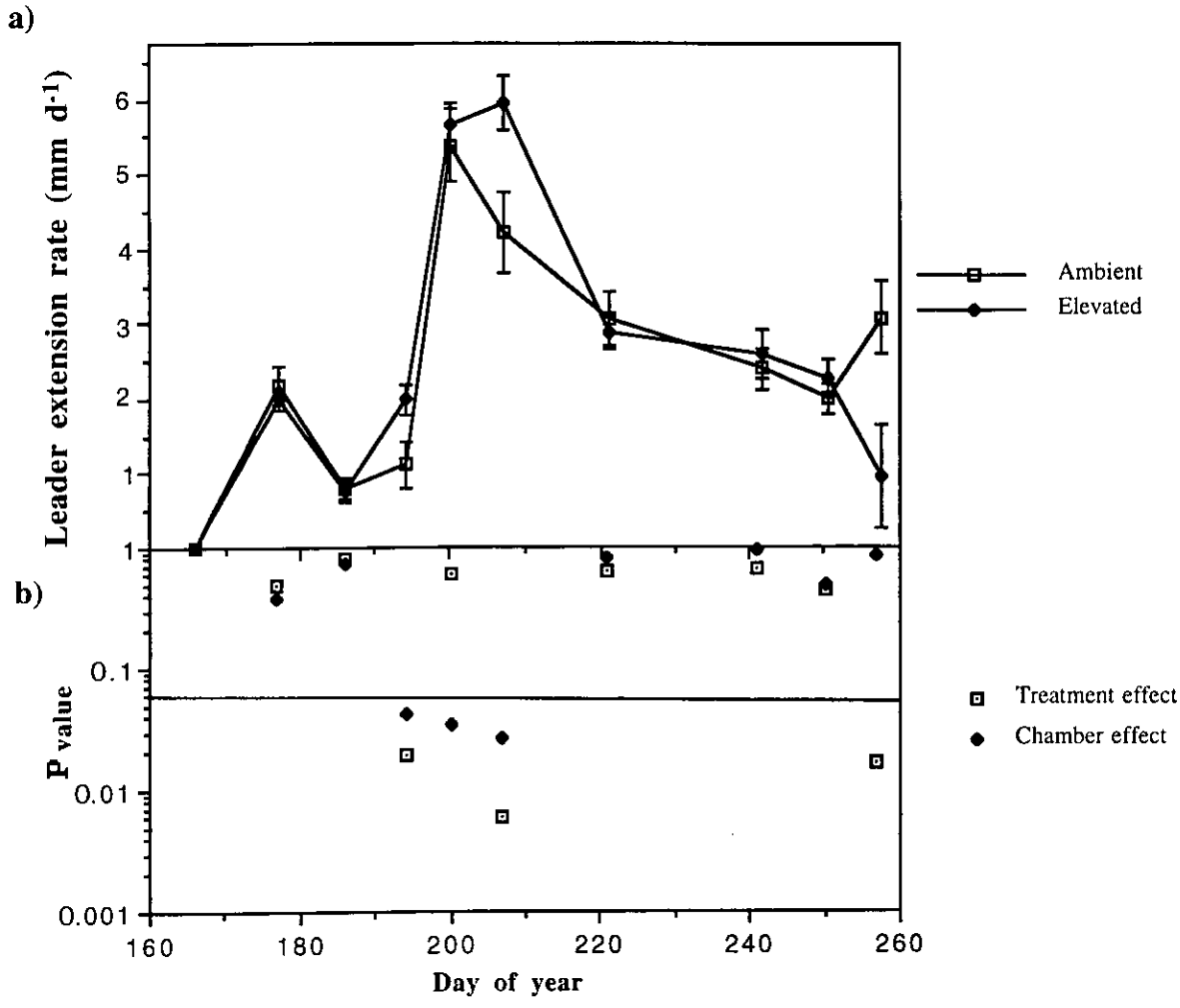


Figure 3.4 Rate of leader extension of Sitka spruce in 1990

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1}). Each value is the mean of 40 plants (ten plants from each of four chambers), error bars depict two standard errors of the means. Measurements were made at weekly intervals between early June, prior to budburst, and late September 1990.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

Figures 3.5 and 3.6 show leader length and rate of leader extension, respectively, of seedlings measured over the 1991 growing season. Plants were measured at weekly intervals commencing one week before budburst. There was no effect of CO_2 treatment on rate of leader extension during the growing season (April to late September), although rate of leader extension was significantly increased in ambient, compared to elevated CO_2 plants on one occasion (DOY 242). There was a slight reduction in the leader length of plants grown in elevated CO_2 compared with ambient

CO_2 plants harvested in January 1992 (Table 3.4), but this was not significant at $P<0.05$.

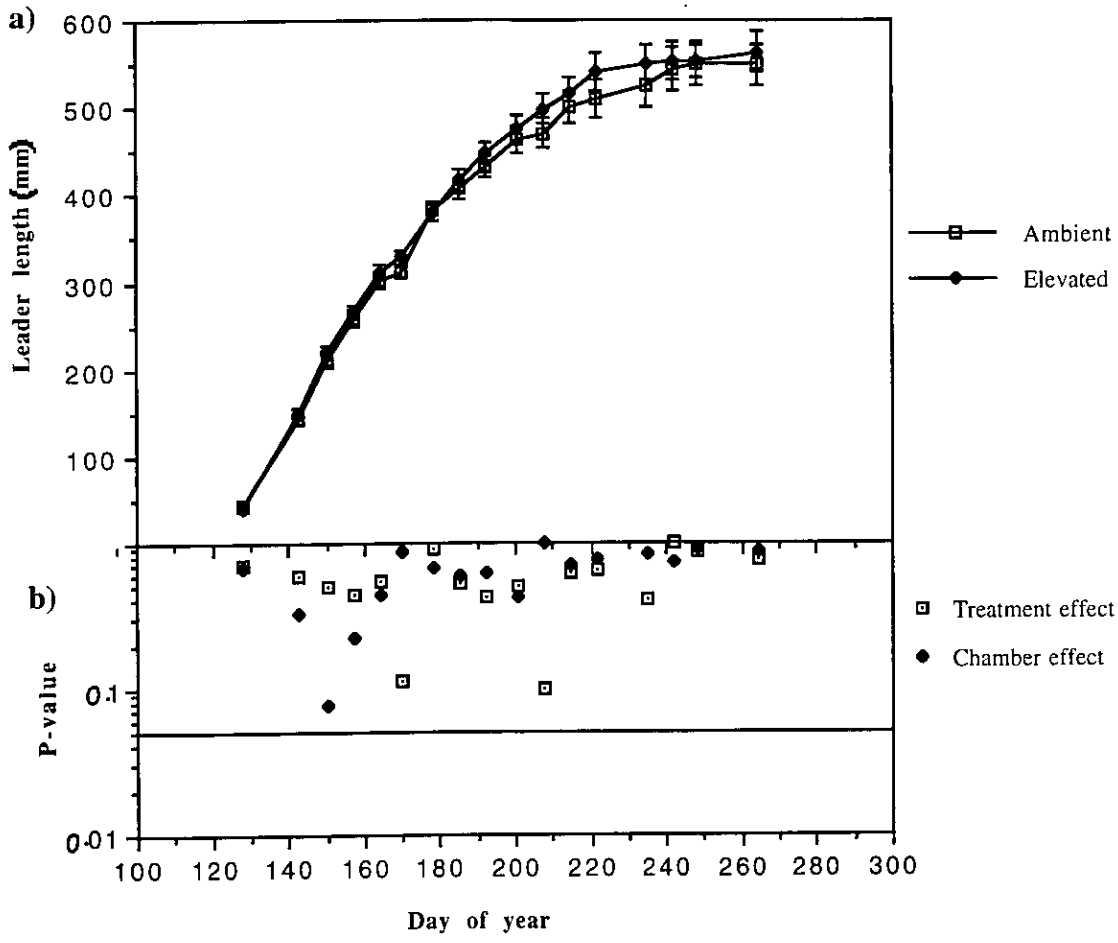


Figure 3.5 Leader length of Sitka spruce in 1991

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 350 μmol mol^{-1}). Each value is the mean of 40 plants per treatment (ten plants from each of four chambers), error bars depict two standard errors of the means. Measurements were made at weekly intervals between April, prior to budburst and late September 1991.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

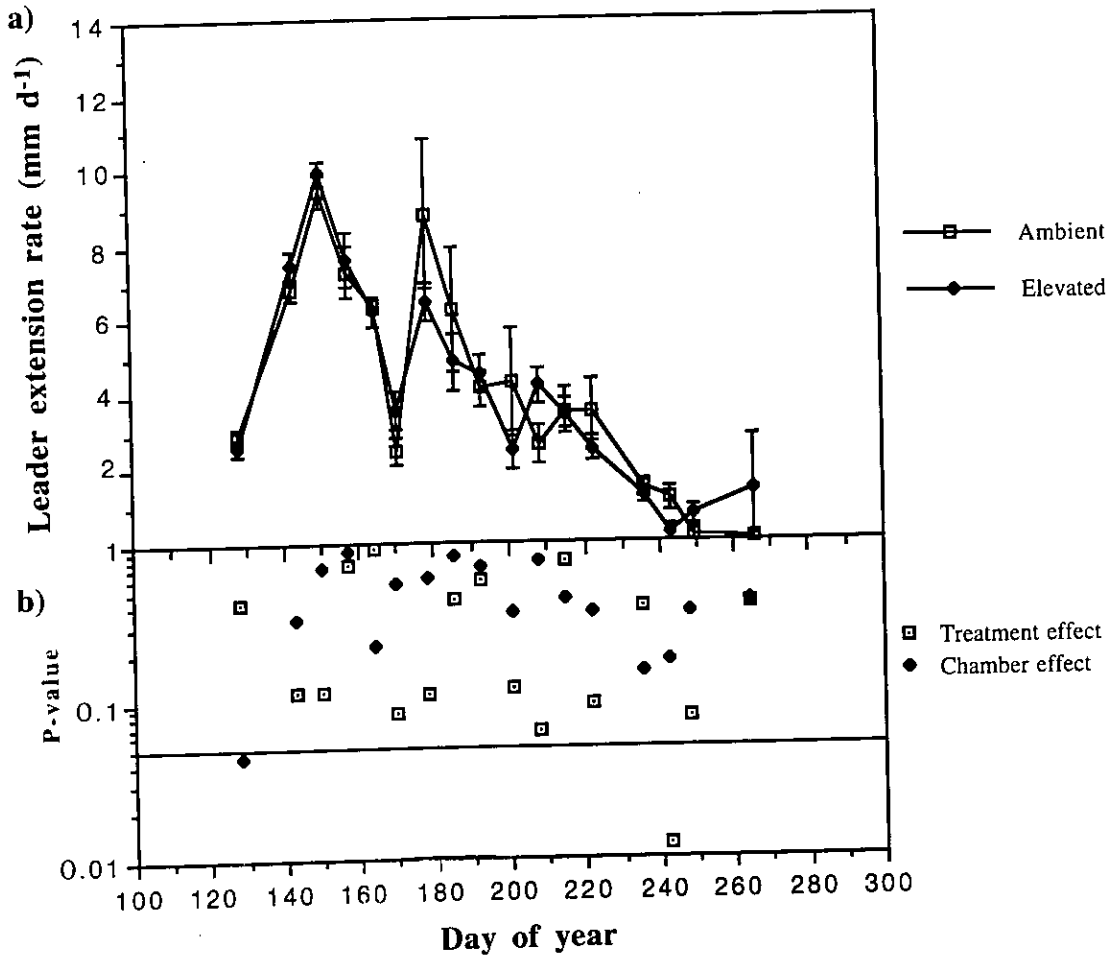


Figure 3.6 Rate of leader extension of Sitka spruce in 1991

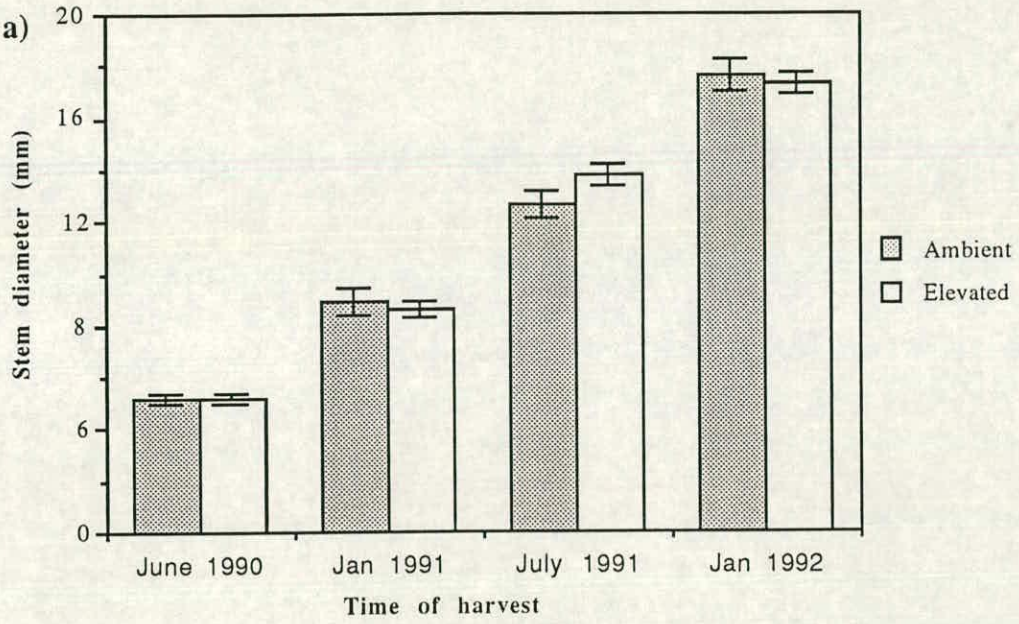
(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 350 μmol mol^{-1}). Each value is the mean of forty plants per treatment (ten plants from each of four chambers), error bars depict two standard errors of the means. Measurements were made at weekly intervals between April, prior to budburst and late September 1991.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

3.4.2 Stem diameter

Figure 3.7 shows the mean stem diameters of plants grown in ambient and elevated CO_2 and measured over an eighteen month period. No significant effect of CO_2 treatment on stem diameter was observed and there was no significant difference between plants grown in different chambers.

a)



(b)

	June 1990	Jan 1991	July 1991		Jan 1992
Treatment effect P<	-	0.668	0.094	P<	0.629
Chamber effect P<	-	0.759	0.483		
Interaction P<	-	0.604	0.501		

Figure 3.7 Stem diameter of Sitka spruce

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1} in 1990 and + 350 μmol mol^{-1} in 1991). Values are the means of between 15 and 45 plants per treatment: errors bars depict two standard errors of the means. Full details of the numbers of plants harvested at each time interval are given in §3.2.2. Measurements were made 2 cm above the root collar.

(b) Results of a two-way ANOVA on data from January 1991 and July 1991, and results of an unpaired t-test on data from January 1992 are also presented.

3.4.3 Characteristics of dormant buds

Table 3.1 shows the dimensions of dormant buds grown in ambient and elevated CO_2 and the number of primordia per bud. There were no significant differences between the characteristics of dormant buds laid down during the 1990 season in ambient or elevated CO_2. There was a slight increase in number of primordia per bud in elevated CO_2 plants compared with plants grown in ambient CO_2 but this effect was not significant ($p > 0.05$).

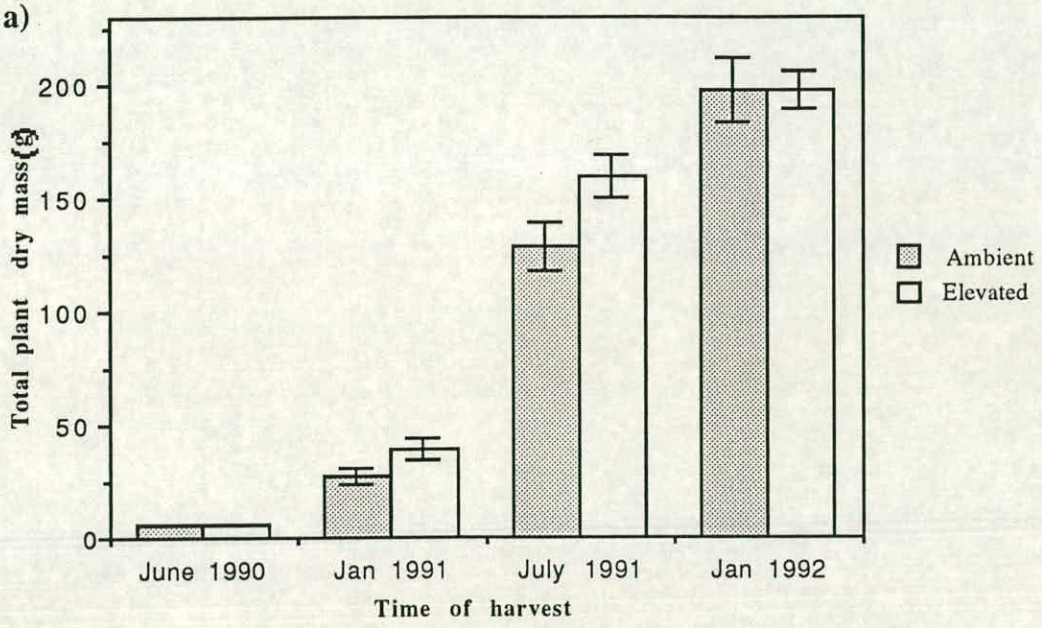
Table 3.1 Bud dimensions of apical buds excised from first order lateral shoots of Sitka spruce in January 1991. A=diameter of apical bud, B=bud height, C=basal diameter of bud core. Each value is the mean ± one standard error, of 20 buds (one from each of five plants per chamber). Results from a two-way ANOVA are also presented. None of the parameters measured were significant at the 5% level ($P>0.05$).

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
A mm	0.26 ± 0.01	0.27 ± 0.01	0.135	0.563	0.470
B mm	2.01 ± 0.04	2.08 ± 0.05	0.117	0.327	0.415
C mm	1.21 ± 0.05	1.32 ± 0.04	0.079	0.636	0.904
Number of primordia per bud	160 ±3.82	170 ±7.10	0.133	0.323	0.122

3.4.4 Biomass production

Figure 3.8 shows the total plant dry mass of plants grown in ambient or elevated CO_2 over two growing seasons. The total plant dry mass was slightly increased in plants growing in elevated CO_2 compared to those grown in ambient CO_2 in January 1991 and this increase had become significant by July 1991 ($P<0.05$). As the experiment continued the effect of the CO_2 treatment disappeared. There was no significant difference in total plant dry mass between plants grown in ambient or elevated CO_2 harvested at the end of the second growing season (January 1992).

a)



(b)

	June 1990	Jan 1991	July 1991		Jan 1992
Treatment effect P<	-	0.077	0.025*	P<	0.987
Chamber effect P<	-	0.453	0.371		
Interaction P<	-	0.578	0.501		

Figure 3.8 Total dry mass of Sitka spruce

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1} in 1990 and + 350 μmol mol^{-1} in 1991). Values are the means of between 15 and 45 plants per treatment: error bars depict two standard errors of the means. Full details of the numbers of plants harvested at each time interval are given in §3.2.2.

(b) Results of a two-way ANOVA on data in January 1991 and July 1991, and results of an unpaired t-test on data collected in January 1992 are also presented.

3.4.5 Growth characteristics

Table 3.2 shows the growth characteristics of seedlings harvested in January 1991. The increase in total plant dry mass of seedlings harvested in January 1991 was almost entirely a result of significant increase in the allocation of carbon to roots in plants grown in elevated CO_2. There was no significant difference in total amount of shoot biomass of plants harvested at this time, despite a significant increase in leader length of elevated CO_2 seedlings. There was no significant difference in number of branches or dry mass of needles on plants grown in elevated CO_2 compared to ambient CO_2 ($P>0.05$) .

Table 3.3 shows the growth characteristics of seedlings harvested in July 1991. Total dry mass of plants harvested in July 1991 was significantly larger in elevated CO_2, as a result of significant ($P<0.05$) increase in both root and shoot dry mass. Significantly more branches were produced in elevated CO_2 and the total mass of needles was significantly increased ($P<0.05$) in the plants grown in elevated compared to ambient CO_2.

Table 3.4 shows the growth characteristics of seedlings harvested in January 1992. By January 1992 the treatment effect on total plant dry mass had disappeared (Figure 3.8). No significant differences in biomass allocation were seen; there were no significant differences in total needle dry mass, number of branches or allocation of carbon to roots between plants grown in ambient or elevated CO_2.

Table 3.2 Growth characteristics of Sitka spruce grown in ambient and elevated CO_2, harvested in January 1991. Values are means ± one standard error of 20 plants per treatment (five from each of four chambers). Results from a two-way ANOVA are also presented. * indicates differences significant at $P<0.05$.

Parameter	Ambient CO_2	Elevated CO_2	Treatment effect P<	Interchamber effect P<	Interaction P<
Leader length mm	223.8±24.2	323.4±19.5	0.088	0.161	0.686
Total shoot d.m. g	16.25±1.74	18.58±1.85	0.374	0.167	0.803
Needle d.m. g	7.79±0.78	9.32±0.88	0.135	0.371	0.699
Root d.m. g	11.49±1.40	20.25±3.32	0.029*	0.793	0.432

Table 3.3 Growth characteristics of Sitka spruce grown in ambient and elevated CO_2 harvested in July 1991. Values are means ± one standard error of 45 plants per treatment (fifteen from each of three chambers). Results from a two-way ANOVA are also presented. *= significant at P<0.05. n=current years growth, n-1=previous years growth.

Parameter	Ambient CO_2	Elevated CO_2	Treatment effect P<	Interchamber effect P<	Interaction P<
Leader length mm	457.7±31.60	423.2±21.4	0.414	0.524	0.491
Total shoot d.m. g	103.9±7.65	127.2±6.68	0.045*	0.359	0.515
needle d.m. g	37.82±3.62	54.56±4.52	0.013*	0.576	0.625
Root d.m. g	25.31±2.02	33.54±1.71	0.007**	0.498	0.500
No.of branches (n)	107.30±9.07	113.80±9.30	0.623	0.249	0.294
No. of branches (n-1)	33.90±2.60	37.30±3.70	0.479	0.995	0.205

Table 3.4 Growth characteristics of Sitka spruce grown in ambient and elevated CO_2 harvested in January 1992. Values are means ± one standard error of 15 plants per treatment (taken randomly from five chambers). Results from an unpaired t-test are also presented. *= significant at P<0.05. n=current years growth, n-1=previous years growth.

Parameter	Ambient CO_2	Elevated CO_2	P<
Leader length mm	668.50±42.10	600.00±42.00	0.289
Total shoot d.m. g	142.54±9.91	136.66±5.81	0.663
needle d.m. g	117.81±8.08	114.55±5.43	0.754
Root d.m. g	54.98±4.86	61.10±3.70	0.349
No. branches (n)	128.80±18.40	112.70±11.54	0.491
No. branches (n-1)	33.60±4.27	35.20±3.29	0.781

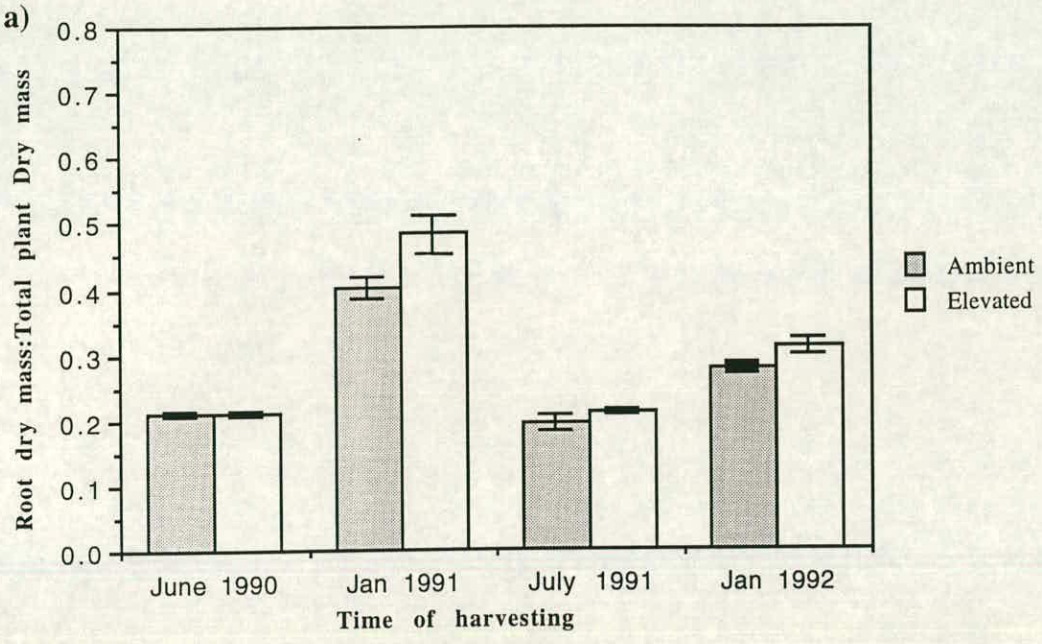
Table 3.5 shows the area of individual needles of Sitka spruce. Growth in elevated CO_2 resulted in a significant increase in area of individual needles produced in 1990 but there was no effect of elevated CO_2 treatment on the area of needles produced in 1991. SLA of needles was not affected by growth in elevated CO_2.

Table 3.5 Individual needle area and SLA of needles of Sitka spruce. Needles were removed from midway down the leader. Values are the means ± one standard error of six needles from 20 plants per treatment. Results from a two-way ANOVA are also presented. *represents significance at P<0.05.

		Ambient	Elevated	Treatment effect P<	Chamber effect P<	Interaction
Area mm^2	1990	12.27±0.86	17.78±0.62	0.001**	0.895	0.635
	1991	24.79±0.83	25.87±0.94	0.376	0.112	0.375
SLA cm^2g^{-1}	1990	44.8±6.04	49.3 ±5.9	0.313	0.405	0.168
	1991	58.6 ±4.3	64.2±5.6	0.400	0.424	0.391

Figure 3.9 shows the root to total plant dry mass ratio of plants harvested between January 1991 and January 1992. There was a significant increase in root:total plant dry mass of seedlings grown in elevated CO_2 and harvested in January 1991. Root:total plant dry mass had decreased in all plants harvested in July 1991. The increase in root:total plant dry mass in elevated CO_2 plants had disappeared by July 1991, although there was a slight increase in elevated CO_2 plants harvested in January 1992. Figure 3.10 shows the shoot to total plant dry mass ratios. This figure shows a converse trend to that of root:total plant dry mass in Figure 3.9. The needle to total dry mass ratios are shown in Figure 3.11. There was a significant increase in the needle:total plant dry mass of plants grown in elevated, compared to ambient CO_2 and harvested in July 1991 but there was no significant difference between plants harvested at any other time. The increase in needle:total plant dry mass in elevated CO_2 plants may be a result of increase in the dry mass of individual needles and/or an increase in the rate of needle production.

a)



(b)

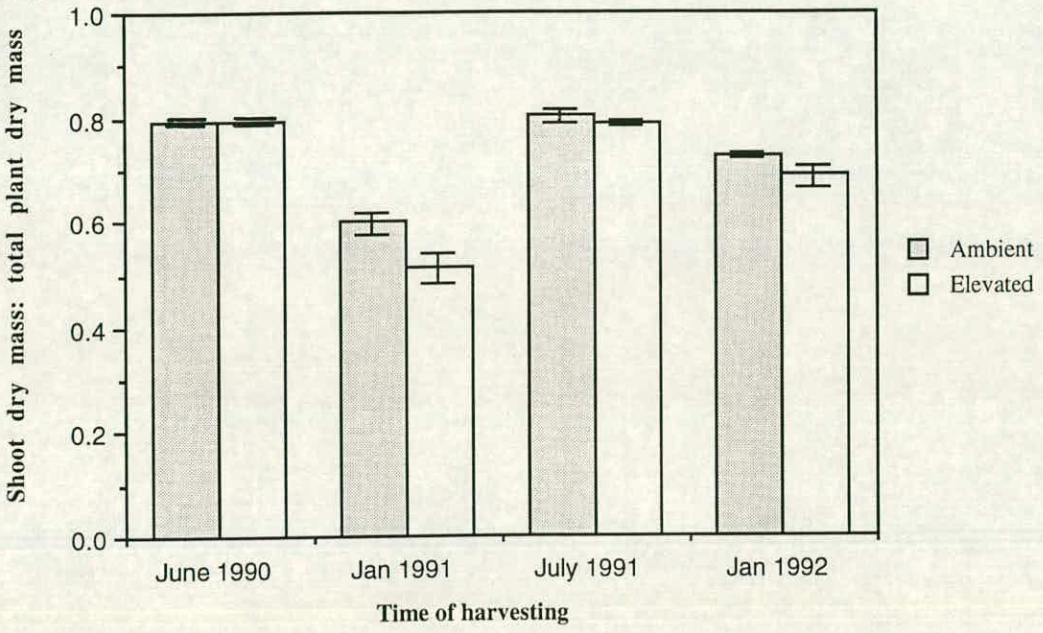
	June 1990	Jan 1991	July 1991		Jan 1992
Treatment effect P<	-	0.007**	0.143	P<	0.088
Chamber effect P<	-	0.917	0.506		
Interaction P<	-	0.075	0.756		

Figure 3.9 Ratio of root dry mass to total plant dry mass of Sitka spruce

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1} in 1990 and + 350 μmol mol^{-1} in 1991 and 1992). Values are the means of between 15 and 45 plants per treatment; error bars depict two standard errors of the means. Full details of the numbers of plants harvested at each time interval are given in §3.2.2.

(b) Results of a two-way ANOVA on data from January 1991 and July 1991, and results of an unpaired t-test on data from January 1992 are also presented.

(a)



(b)

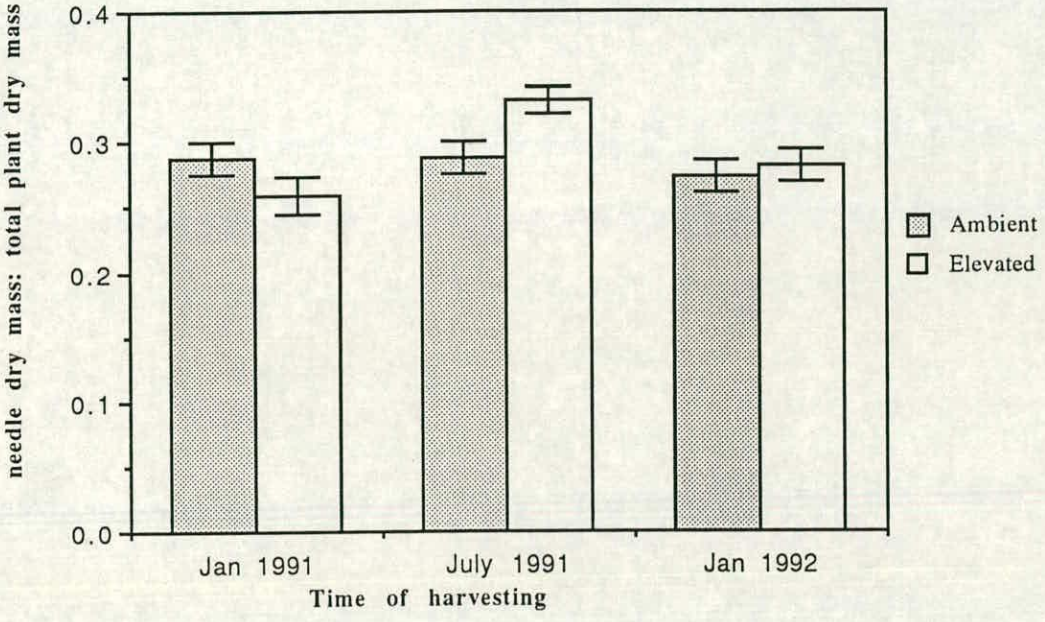
	June 1990	Jan 1991	July 1991		Jan 1992
Treatment effect P<	-	0.007**	0.143	P<	0.088
Chamber effect P<	-	0.917	0.506		
Interaction P<	-	0.075	0.756		

Figure 3.10 Ratio of shoot dry mass to total plant dry mass of Sitka spruce

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1} in 1990 and + 350 μmol mol^{-1} in 1991 and 1992). Values are the means of between 15 and 45 plants per treatment; error bars depict two standard errors of the means. Full details of the numbers of plants harvested at each time interval are given in 3.2.2.

(b) Results of a two-way ANOVA on data from January 1991 and July 1991, and results of an unpaired t-test on data from January 1992 are also presented.

a)



(b)

	Jan 1991	July 1991		Jan 1992
Treatment effect P<	0.110	0.026*	P<	0.672
Chamber effect P<	0.920	0.759		
Interaction P<	0.415	0.831		

Figure 3.11 Ratio of needle dry mass to total plant dry mass of Sitka spruce.

(a) Plants grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 250 μmol mol^{-1} in 1990 and + 350 μmol mol^{-1} in 1991 and 1992). Values are the means of between 15 and 45 plants per treatment; error bars depict two standard errors of the means. Full details of the numbers of plants harvested at each time interval are given in 3.2.2.

(b) Results of a two-way ANOVA on data fromJanuary 1991 and July 1991, and results of an unpaired t-test on data from January 1992 are also presented.

Figure 3.12 shows the relationship between root and shoot dry mass of plants grown in ambient and elevated CO_2. There was no significant difference in the allometric constant k for ambient and elevated CO_2 plants (1.09 *cf.* 0.85) suggesting that partitioning of dry mass was not significantly affected by growth in elevated CO_2.

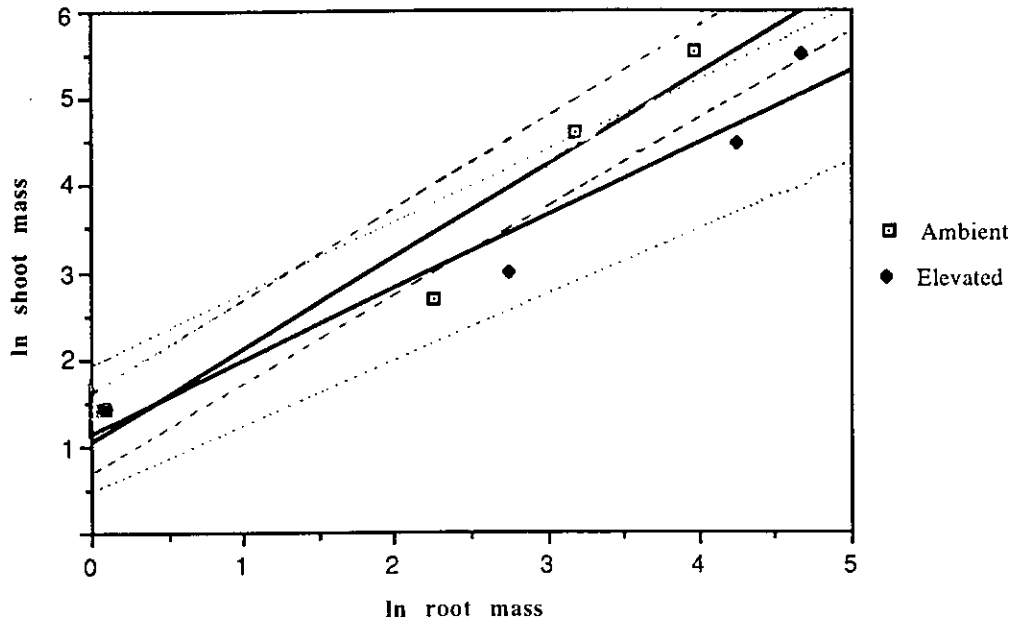


Figure 3.12 Allometric relationship of shoot to root dry mass of Sitka spruce. Plants were grown at two concentrations of atmospheric CO_2 (ambient and elevated); values are the means of between 15 and 45 plants per treatment. Full details of the number of plants harvested at each time interval are given in §3.2.2. Solid lines represent the geometric mean regression; dotted lines represent 95% confidence intervals.

Table 3.6 shows results from nutrient analysis conducted at the end of the 1991 growing season. All nutrients were lower in elevated CO_2 plants and nitrogen was significantly lower ($P<0.05$). Elevated CO_2 plants harvested at this time were found to have received less than adequate nutrition (<1 mass % nitrogen) (Ingestad, 1971; Binns, Mayhead and MacKenzie, 1986). Lower nutrient concentrations in elevated CO_2 plants may have resulted from dilution by increased growth rates or by increased dry mass in leaves because of starch accumulation, or may represent a real shortage.

Table 3.6 Nutrient analysis for needles of Sitka spruce July 1991. Values are means (± one standard error) of 18 samples per treatment (six samples from each of three chambers).

	Ambient CO_2	Elevated CO_2
Nitrogen mass %	1.26±0.09	0.89±0.10
Phosphorus mass %	0.22±0.02	0.20±0.02
Potassium mass %	0.56±0.06	0.46±0.05

3.5 RESULTS - SILVER BIRCH

Results are presented here for shoot extension, main stem leaf and side shoot production, dry mass allocation, growth characteristics and nutrient analysis of silver birch for the 1991 growing season.

3.5.1 Shoot extension

The height and shoot extension rate of silver birch seedlings measured over the 1991 growing season are shown in Figures 3.13 and 3.14, respectively. There was a slight increase in shoot extension rate of seedlings grown in elevated CO_2 over the first half of the season but this was not significant at $P<0.05$ and fell below that of plants grown in ambient CO_2 by the end of the season, with the result that by the end of the September the elevated CO_2 seedlings were slightly shorter than those grown in ambient CO_2 (not significant at $P<0.05$).

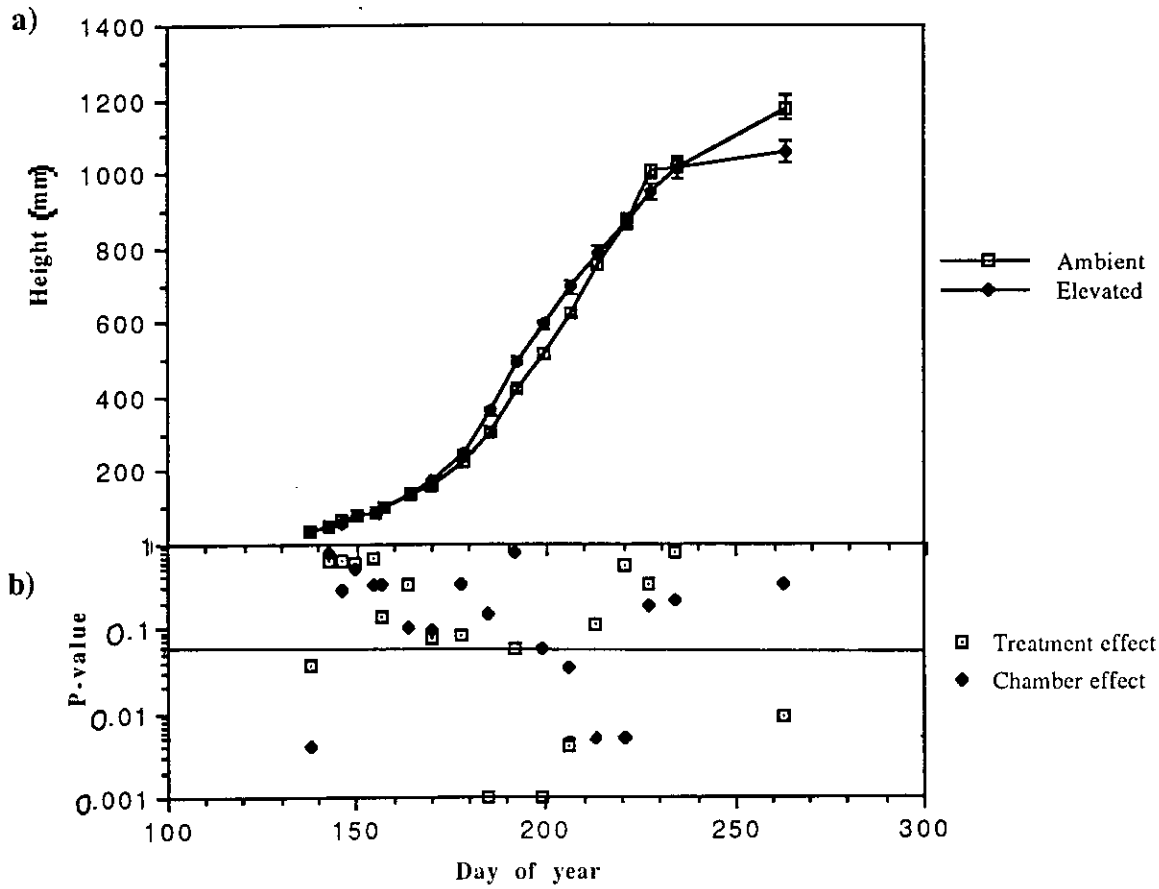


Figure 3.13 Height of silver birch seedlings

(a) Plants were grown at two concentrations of atmospheric CO_2 ambient (unsupplemented) and elevated (ambient + 350 μmol mol^{-1}). Each value is the mean of 30 plants per treatment (ten plants from each of four chambers per treatment); error bars depict two standard errors of the means. Measurements were made at weekly intervals between April and late September 1991.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

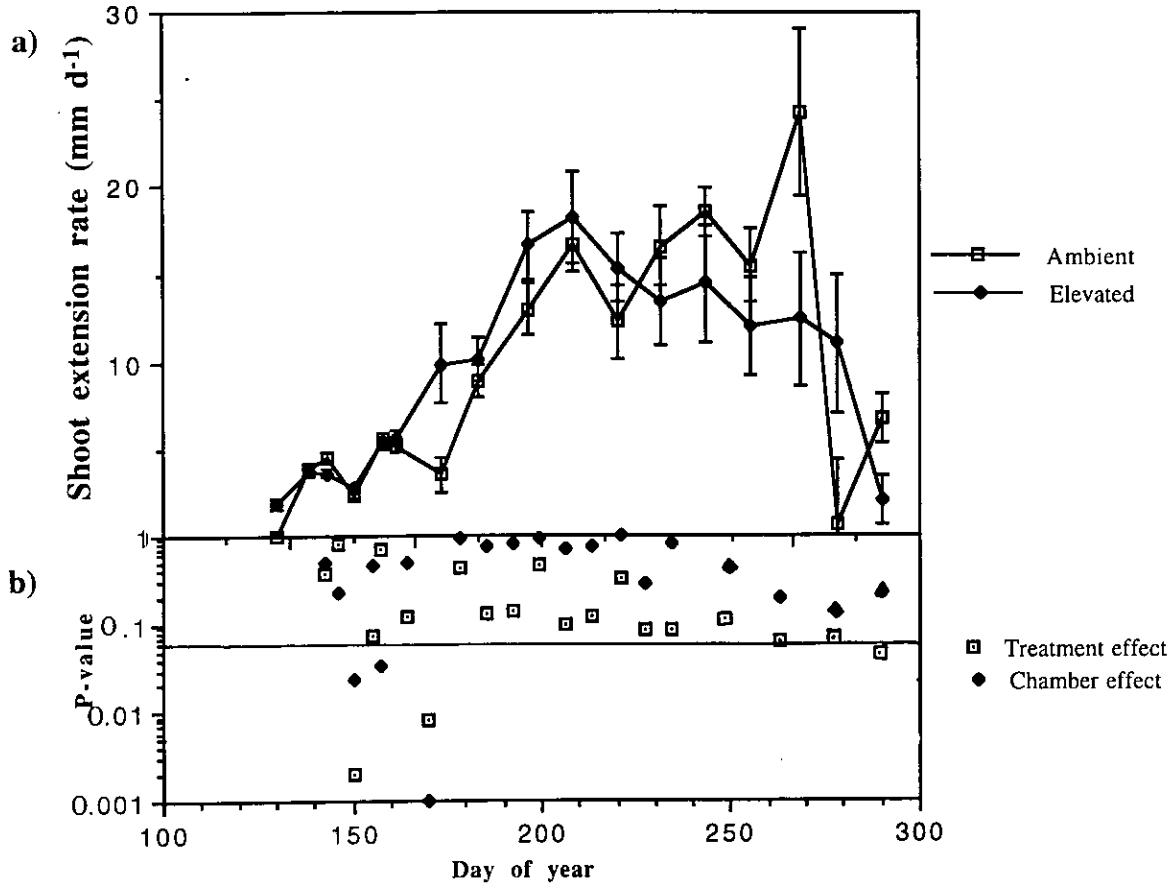


Figure 3.14 Shoot extension rate of silver birch seedlings

(a) Plants were grown at two concentrations of atmospheric CO_2 ambient (unsupplemented) and elevated (ambient + 350 μmol mol^{-1}). Each value is the mean of 30 plants per treatment (ten plants from each of four chambers per treatment); error bars depict two standard errors of the means. Measurements were made at weekly intervals between April and late September 1991.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

3.5.2 Main stem leaf production

There was no effect of growth in elevated CO_2 on rates or duration of main stem leaf production. No significant differences were seen in final number of main stem leaves per plant (Figure 3.15). The number of main stem leaves produced by plants grown in elevated CO_2 was only significantly above that of ambient CO_2 plants on one occasion (DOY 200): throughtout the rest of the season there was no significant difference

between the plants. There was a significant inter-chamber effect on only one occasion (DOY 207).

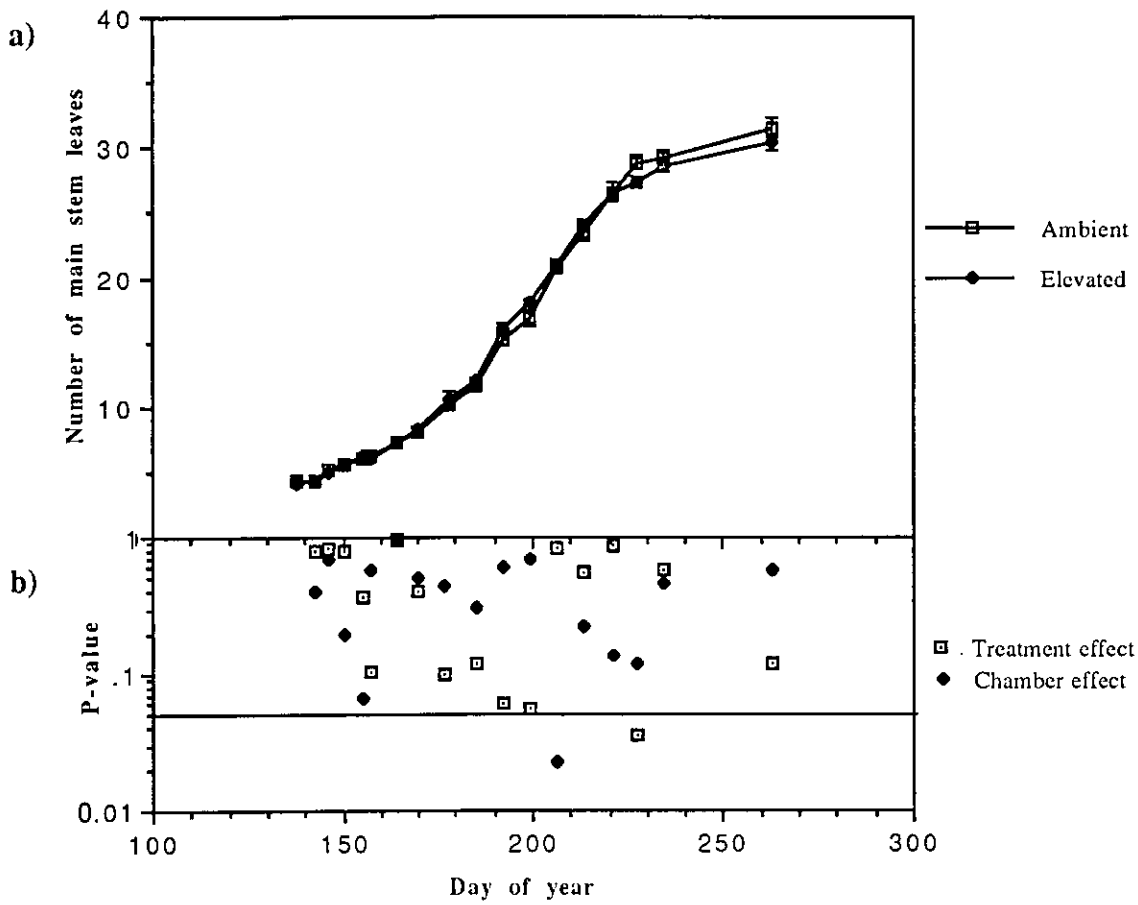


Figure 3.15 Number of leaves produced from the mainstem of silver birch seedlings

(a) Plants were grown at two concentrations of atmospheric CO_2; ambient (unsupplemented) and elevated (ambient + 350 μmol mol^{-1}). Each value is the mean of 30 plants per treatment (ten plants from each of four chambers per treatment); error bars depict two standard errors of the means. Measurements were made at weekly intervals between April and late September 1991.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

3.5.3 Side shoot production

The increase in mean number of side shoots per plant with time is shown in Figure 3.16. Rate of side shoot production was increased by growth in elevated CO_2 and from DOY 200 the difference in the number of side shoots produced was significantly larger

in seedlings grown in elevated, compared to ambient CO_2 ($P<0.05$). Rate of side shoot production reached a maximum at approximately DOY 220 and then declined with time in all seedlings measured, but the difference between treatments was maintained.

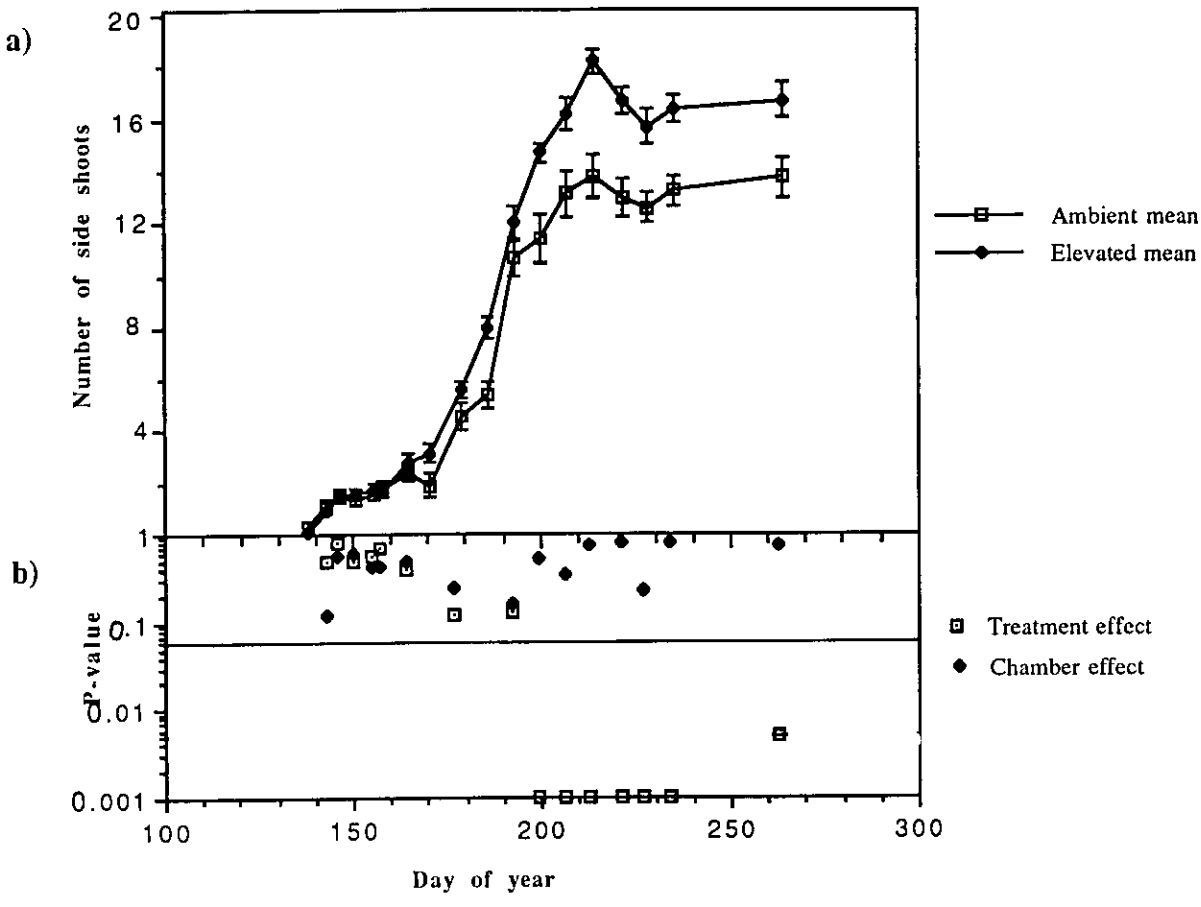


Figure 3.16 Number of side shoots produced on silver birch seedlings

(a) Plants were grown at two concentrations of atmospheric CO_2, ambient (unsupplemented) and elevated (ambient + 350 μmol mol^{-1}).Each value is the mean of 30 plants per treatment (ten plants from each of four chambers per treatment); error bars depict two standard errors of the means. Measurements were made at weekly intervals between April and late September 1991.

(b) Level of significance between plants grown in elevated or ambient CO_2 (treatment effect) and between plants grown in different chambers (inter-chamber effect). The bold line represents the 0.05 level of significance.

Table 3.7 shows the total mean area of main-stem and side-shoot leaves in June 1991. There was a slight increase in main-stem leaf area and total tree leaf area in elevated CO_2 plants compared with ambient CO_2 plants, but this was not significant at $P<0.05$. There was no significant difference in area of side-shoot leaves between plants.

Table 3.7 Area of individual main stem and side shoot leaves and estimated total tree leaf area of silver birch seedlings measured in June 1991. Values are means ± one standard error of 30 plants per CO_2 treatment (ten plants from each of three chambers). * = data statistically significant at P<0.05. **data statistically significant at (P<0.01).

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
Main stem leaf cm^2	17.14±0.68	19.51±0.82	0.07	0.674	0.963
Side shoot leaf cm^2	7.41±0.09	7.61±0.08	0.119	0.583	0.501
Total plant leaf area cm^2	183.15±7.01	208.1±9.79	0.087	0.777	0.819

3.5.4 Biomass production

Table 3.8 shows the results of an initial harvest conducted on seedlings, at the time of transplanting, prior to their transfer to the open top chambers. There were no significant difference between the seedlings germinated in ambient or elevated CO_2 (P<0.05), although there was a slight increase in root dry mass of seedlings germinated in ambient CO_2 compared to those germinated in elevated CO_2.

Table 3.8 Initial harvest of seedlings of silver birch grown in ambient and elevated CO_2. Seedlings were harvested in May 1991 prior to seedlings being placed in open top chambers. Data are means of ten seedlings ± one standard error. Results from an unpaired t-test are also presented. * = data statistically significant at P<0.05. **data statistically significant at (P<0.01). d.m.=dry biomass.

Parameter	Elevated CO_2	Ambient CO_2	t-test
Total d.m. mg	233.0±3.3	288.0±4.2	0.316
Total shoot d.m. mg	189.0±8.7	207.0±8.5	0.658
Main stem leaves d.m.mg	141.0±7.1	167.0±7.1	0.415
Root d.m. mg	4.4±1.9	7.6±5.4	0.098
Shoot:root d.m.	3.41±0.49	4.76±0.65	0.116

Table 3.9 shows the allocation of dry matter within seedlings 24 weeks after germination. Total plant biomass was significantly increased in seedlings grown in elevated CO_2 compared to ambient CO_2 seedlings (P<0.05). Total shoot dry mass was significantly increased in elevated CO_2 seedlings, as a result of increase in allocation of carbon to side shoots. There were significant increases(P<0.05) in dry mass of both side shoots and side shoot leaves. There was no significant effect of growth in elevated CO_2 on allocation of carbon to roots.

Table 3.10 shows the growth characteristics of seedlings after 24 weeks. Growth in elevated CO_2 resulted in plants that tended to be significantly thicker than those grown in ambient CO_2, although there was no significant difference in height between seedlings. There was no treatment effect on number or area of main stem leaves. Side shoot production was enhanced in elevated CO_2 and there was a slight increase (P<0.05) in the number of side shoot leaves produced but there was no increase in side-shoot leaf area, suggesting that the mean area of individual leaves produced from side shoots was reduced in elevated CO_2 plants. There was no effect of growth in elevated CO_2 on total plant leaf area in August 1991.

Leaf mass ratio (LMR, the fraction of plant dry mass comprising leaves) was significantly increased (P<0.05), in plants grown in elevated CO_2 and average specific leaf area (SLA, projected leaf area per leaf dry mass) was slightly reduced. Leaf area ratio (LAR, total projected leaf area per plant dry mass) was significantly reduced

($P<0.05$) by growth in elevated CO_2, although there was a significant interaction between CO_2 treatment and chamber, indicating that the magnitude of the CO_2 response was different in plants from different chambers. There was no significant effect of elevated CO_2 on root mass which was slightly increased and, despite the increase in above ground biomass, no significant effect of CO_2 treatment on shoot: root ratio was observed. There was a significant interaction between CO_2 treatment and chamber, suggesting that the magnitude of the CO_2 response was different in plants from different chambers.

Table 3.9 Allocation of dry mass in seedlings of silver birch in August 1991. Measurements represent the mean ± one standard error of 30 plants per treatment (except roots, where n=13). Results from a two-way analysis of variance are also presented * = data statistically significant at $P<0.05$. **data statistically significant at ($P<0.01$)

Dry mass g	Elevated CO_2	Ambient CO_2	Treat. effect P<	Chamber effect P<	Interaction P<
Total shoot d.m.	28.39±1.42	23.22±1.19	*0.008	0.204	0.601
Root d.m.	10.72±1.55	9.09±1.31	0.417	0.131	0.153
Total leaf d.m.	10.73±0.61	7.71±0.49	*0.000	0.226	0.807
Main stem leaf d.m.	3.82±0.22	3.11±0.17	0.012	0.257	0.599
Side shoot leaves	6.91±0.48	4.93±0.35	*0.002	0.151	0.720
Main stem leaf d.m.	12.34±0.55	11.01±0.52	0.080	0.396	0.633
Side stem d.m.	5.73±0.42	4.49±0.35	*0.031	0.0369	0.368

Table 3.10 Growth characteristics of seedlings of silver birch in August 1991. Measurements represent the mean ± one standard error of 30 plants per treatment (except shoot:root ratio, where n=13). Results from a two-way analysis of variance are also presented * = data statistically significant at P<0.05. **data statistically significant at (P<0.01)

	Elevated CO_2	Ambient CO_2	Treatment effect P<	Inter-chamber effect P<	Interaction P<
No.side shoot leaves	116.96 ±7.16	100.4 ±7.11	0.100	0.598	0.928
No.main stem leaves	24.97 ±1.24	24.33 ±1.27	0.713	*0.026	0.276
No. side shoots	15.93 ±0.74	13.63 ±0.71	*0.044	0.589	0.847
Height mm	1058.77 ±33.95	1071.30 ±26.91	0.768	0.071	0.097
Stem diameter mm	12.83 ±0.37	11.80 ±0.32	*0.050	0.310	0.859
Total leaf area cm^2	1907.41 ±111.39	1881.65 ±99.18	0.864	0.137	0.349
Side shoot leaf area cm^2	1359.37 ±89.27	1319.07 ±83.77	0.746	0.137	0.750
Main stem leaf area cm^2	567.62 ±35.74	606.55 ±33.80	0.406	*0.009	0.291
Shoot:root	3.24 ±0.250	2.99 ±0.37	0.529	0.395	0.020*
SLA cm^2g^{-1}	186.49 ±9.11	260.29 ±16.44	0.085	0.243	0.666
LMR gcm^{-2}	0.00581 ±0.00034	0.00424 ±0.00026	0.041*	0.977	0.065
LAR $cm^2\ g^{-1}$	60.27 ±3.27	73.99 ±3.27	0.005*	0.210	0.001**

3.5.5 Photosynthesis

Table 3.11 shows the effect of growth in elevated CO_2 on stomatal conductance and assimilation rate of silver birch seedlings. Stomatal conductance was not significantly reduced in plants grown and measured in elevated CO_2 ($P>0.05$). There was a significant variation in stomatal conductance between plants growing in the different chambers. Variation in g_S may have been a result of the differences in the PFD experienced by plants in the different chambers at the time that measurements were taken, but variation in the water status of seedlings as a result of growing the plants in pots cannot be ruled out. Assimilation rate was significantly increased by growth in elevated CO_2 ($P<0.05$), but again variation between chambers was also large, although not significantly so.

Table 3.11 Stomatal conductance, g_S (mmol m^{-2} s^{-1}) and assimilation rate, A (μmol CO_2 m^{-2} s^{-1}) of silver birch grown at two concentrations of atmospheric CO_2 (ambient and elevated). Measurements were made between 11.00 and 13.00 BST on three occasions in July 1991, forty plants were measured on each occassion (ten from each of four chambers). Values are the means of 120 measurements per treatment ± one standard error. PFD = 869±69.3, leaf temperature = 23.15±0.30.

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
g_S	212.31 ±32.94	164.38 ±30.72	0.125	0.048*	0.094
A	8.73 ± 1.10	12.35 ± 1.65	0.037*	0.052	0.030*

3.5.6 Nutrient analysis

Table 3.12 shows the nutrient analysis data for leaves of silver birch harvested in August 1991. Neither ambient nor elevated CO_2 plants were found to be significantly nutrient deficient at this time (Ingestad, 1971).

Table 3.12 Nutrient concentration of leaves of silver birch harvested in August 1991. Each value is the mean ± one standard error of eight samples (two samples from each of four chambers per treatment).

	Ambient CO_2	Elevated CO_2
Nitrogen mass %	1.90±0.03	1.52±0.10
Phosphorus mass %	0.27±0.04	0.33±0.02
Potassium mass %	0.88±0.15	0.88±0.07

3.5.7 Summary of results

(i) Sitka spruce

After one growing season there was a 40% increase in biomass production in seeedlings of Sitka spruce grown in elevated CO_2 compared to those grown in ambient CO_2, but this fell to 24 % midway through second season and there was no effect on biomass production after eighteen months. The initial increase in biomass production was a result of a significant increase in root mass. After the second growing season there was no effect of elevated CO_2 on the allocation of dry matter. There was no difference in the number of branches produced in ambient or elevated CO_2 seedlings after eighteen months, although the rate of branch production was increased in plants grown in elevated CO_2. The area of individual needles was increased in 1991 but there was no significant effect of growth in elevated CO_2 on the area of needles produced in 1992. SLA was unaffected, by growth in elevated CO_2. Total nitrogen, phosphorus and potassium concentration of leaves were reduced in plants grown in elevated CO_2.

(ii) Silver birch

After one growing season total plant biomass was increased in seedlings of silver birch grown in elevated compared to ambient CO_2. Biomass production was increased in elevated CO_2 plants by approximately 20%. Rates of photosynthesis were also higher in plants grown in elevated CO_2. The increase in biomass was a result of an increase in stem diameter and an increase in the allocation of dry mass to leaves and branches. The number of branches produced and the rate of branch production was increased in elevated CO_2 plants, but the number of leaves produced was only slightly increased and there was no effect of elevated CO_2 treatment on total tree leaf area. LMR decreased

slightly in plants grown in elevated CO_2. Leaves grown in elevated CO_2 senesced approximately one week to ten days earlier than leaves on ambient CO_2 plants. Total nitrogen, phosphorus and potassium concentration of leaves was reduced in plants grown in elevated CO_2.

3.6. DISCUSSION

The increase in biomass production after one growing season in Sitka spruce seedlings grown in elevated CO_2 compared to those grown in ambient CO_2 was mainly a result of an increase in allocation of dry matter to the roots. These plants did not receive any additional nutrient treatment during the 1990 growing season and nutrient concentrations were lower in elevated compared to ambient CO_2 seedlings harvested in July 1991. The initial enhanced allocation of resources to the roots may be a response to nutrient limitation. These findings are very similar to those of El Kohen, Rouhier and Mousseau (1992) who observed an increase in root: shoot ratios of unfertilised seedlings of *Castaneae sativa* grown in elevated CO_2 but no effect of growth in elevated CO_2 on carbon allocation of well fertilised seedlings. There was no effect of elevated CO_2 on allocation of biomass in Sitka spruce plants grown in elevated CO_2 in January 1992.

The effect of elevated CO_2 on total biomass production had disappeared by the end of the second growing season, despite higher rates of photosynthesis in elevated CO_2-grown plants and no evidence of down-regulation (Lee *et al.* 1993). Similar results for other species have been reported. Norby, Wullschleger and Gunderson (1994) found no significant effect of elevated CO_2 concentration on biomass production of *Liriodendron tulipifera* despite a sustained increase in photosynthesis per unit leaf area and reduced foliar respiration. This was attributed to a reduction in leaf area and a possible increase in fine root production. In this study with Sitka spruce there was only a slight reduction in leaf area production, estimated from measurements of total needle mass and the SLA of individual needles. No data are available for fine root production or root exudation in this experiment, but the results highlight the need for more comprehensive study of plant respiration and the below-ground processes of Sitka spruce grown in elevated CO_2. An increase in fine root mass would potentially improve water relations and the uptake of nutrients and may stimulate mycorrhizal activity. An increase in root exudation may stimulate microbial activity in soil resulting in an increase in the rate of nitrogen mineralisation.

In this study biomass increased in silver birch seedlings grown in elevated CO_2 by *ca* 20% after one growing season. These findings are similar to those of Pettersson and

McDonald (1992) who observed an increase in biomass production in silver birch seedlings grown in elevated CO_2. Rochefort and Bazzaz (1992) also found an increase in biomass production in four birch species grown in elevated CO_2 (*Betula alleghaniensis, Betula populifolia, Betula papyrifera and Betula lenta*). The increase in biomass production in elevated CO_2-grown silver birch seedlings was a result of an increase in both photosynthetic rate and leaf area production early in the growing season. Rate of branch production and the total number and mass of branches produced increased leading to a larger tree area per tree. Increased branching in silver birch may increase area of leaves available for the interception of solar radiation early on in the growing season but may also speed the onset of self-shading. No effect of elevated CO_2 on branch production of silver birch was found by Pettersson and McDonald (1992), but this may have been a result of the short duration of their experiment. Despite an initial stimulation of leaf area production, there was no effect of elevated CO_2 treatment on the total area of leaves produced at the end of the growing season. The relative reduction in rate of leaf area production in elevated CO_2 seedlings towards the end of the season may have partially offset the increase in photosynthetic rate of these seedlings and reduced the net effect of growth in elevated CO_2. A reduction in rate of leaf area production was reported by Norby, Wullschleger and Gunderson, (1994) for *Liriodendron tulipifera* and Pettersson and McDonald, (1992) for silver birch. The reduction in rate of leaf area production during the latter part of the season in this study may have been in response to inadequate nitrogen supply. There was no effect elevated CO_2 on root:shoot ratio of silver birch seedlings in this study, although more thorough studies are required to determine the effects of growth in elevated CO_2 on fine root and exudate production in silver birch seedlings.

Rate of photosynthesis increased and g_S was reduced in silver birch plants in this study. This results in an increase in instantaneous WUE. Instantaneous WUE has been found to increase in many plants in elevated CO_2 (e.g. Hollinger, 1987; Conroy *et al.* 1986b); Norby and O'Neill, 1991, Eamus, 1991; Ziska *et al.*, 1991; Bunce, 1992). Whether WUE integrated over time increases in young silver birch trees remains to be tested. The effects of growth in elevated CO_2 on stomatal conductance and the implications for plant growth are discussed more fully in Chapter 5.

Although there was no evidence of down regulation of photosynthesis of these plants in July 1991, down regulation may have occurred later in the season as a result of source-sink imbalance caused by inadequate pot size and/or nutrient limitation. Inadequate rooting volume has been associated with a reduction in photosynthesis (Arp 1991), although these studies failed to separate the effects of rooting volume and nutrient supply. In a recent paper, Nicotra *et al.* (1994) found no effect of rooting volume but

did find a positive correlation between nutrient supply and growth enhancement of plants grown in elevated CO_2. Early senescence is often linked to nitrogen limitation, and in this study leaves on seedlings grown in elevated CO_2 senesced between one week and ten days earlier than those grown in ambient CO_2. This may have been a result of nutrient limitation, restrictive pot size (Tschaplinski, Norby and Wullschleger, 1993) or a direct effect of elevated CO_2 on plant phenology.

No direct comparisons can be made between the growth of the two species in elevated CO_2 as the Sitka spruce were placed in elevated CO_2 as two-year-old seedlings and may have exhibited a larger acclimation response than the silver birch which were grown from seed in elevated CO_2. However, there are indications that the allocation strategies differed between the species; there was no increase in branching in Sitka spruce whereas the number of branches produced in silver birch was significantly increased in elevated CO_2.

This study, along with many other studies using pot-grown plants in open top chambers, fails adequately to separate the effects on growth and development of elevated CO_2 treatment from the effects of nutrition and pots. In many of the experiments reported in the literature, nutrient supply was found to have a more pronounced effect on biomass partitioning than CO_2 concentration (eg El Kohen, Rouhier and Mousseau, 1992). The importance of controlling plant nutrition in experiments investigating plant response to any environmental variable has been emphasised by Ingestad and Lund (1986) and more recently with respect to elevated CO_2 by Pettersson and McDonald (1992) and Linder and McDonald (1994). In addition small pot size *per se* may limit growth and affect source-sink relations (Arp, 1991). Stulen and Hertog (1993) have also pointed out the difficulty in maintaining even nutrient and water distribution within pots. Pots may absorb solar radiation resulting in uneven root temperature and therefore root growth. Limitations of these experiments serve to highlight the need for studies into interactions between elevated CO_2 and water and nutrition, and the need for long-term experiments to determine whether the effects of elevated CO_2 observed on seedlings in this study are sustained as the trees mature.

CHAPTER 4 THE EFFECT OF ELEVATED CARBON DIOXIDE ON LEAF EXTENSION IN SILVER BIRCH

4.1 INTRODUCTION

The aim of the experiment described in this chapter was to determine the effect of growth in elevated CO_2 on leaf extension in individual leaves of silver birch and to establish which of the cellular processes involved in the control of leaf extension were affected.

An increase in leaf area increases the potential for the interception of solar radiation and therefore biomass production (Milthorpe and Moorby, 1974; Monteith and Elston, 1983). Leaf area also plays a key role in determining the amount of water transpired from a plant or canopy (Jarvis and McNaughton, 1986). Leaf area may be increased as a result of increase in the number of leaves and/or increase in the area of individual leaves.

The area of individual leaves has been found to increase in both crop and tree species in elevated CO_2, for example *Glycine max* (Sionit *et al.*, 1981), *Ochroma lagopus* and *Pentaclethra macroloba* (Oberbauer *et al.*, 1985). However, response of leaf growth to elevated CO_2 varies between species and some workers have found a reduction in area of individual leaves in plants grown in elevated CO_2 (eg. Mousseau and Enoch, 1989).

Extension of individual leaves can be described by the model of steady state growth of individual cells, developed by Lockhart (1965) (§1.2.6). Although this model describes the growth of individual cells it has been used to investigate the growth of leaves (Dale, 1988) and roots (Pritchard *et al.*, 1991).

In a simplified form the Lockhart equation states that under certain conditions, e.g. isothermal conditions, elastic equilibrium and linear viscoelastic walls, the increase in cell volume defined below is proportional to the turgor pressure in excess of a critical turgor (yield turgor) and the extensibility of the cell walls, i.e.

$$\frac{dv}{dt} = m\,(P - Y) \qquad (4.1)$$

where dv/dt is increase in cell volume with time, P is turgor pressure and Y is yield threshold (i.e. the value of turgor below which the cell wall does not extend) and m is the extensibility of the cell wall.

From equation 4.1 it can be seen that any environmental variable affecting either m or $(P\text{-}Y)$ may affect cell extension and, by implication, leaf extension (Frost,Taylor and Davies, 1991). Although there are many reports of the effects of CO_2 on the production of leaf area, there have been few investigations of mechanisms producing these effects and it is not yet clear whether the increase in leaf area in plants in elevated CO_2 is a result of an increase in substrate availability or whether there are more direct effects (Jarvis, 1989). The need to investigate the effects of elevated CO_2 on the biophysical parameters controlling leaf expansion has recently been emphasised by Jarvis (1993).

The experiment described in this chapter was designed to measure leaf expansion between emergence and full expansion in silver birch, to determine whether growth in elevated CO_2 increased leaf extension rate and final leaf area. The diurnal pattern of leaf extension was also investigated and compared to measurements of m, P and Y measured over the same period.

4.2 MATERIALS AND METHODS

4.2.1 Establishment

Seeds of silver birch Roth (provenance FC 87/20) were germinated under ambient (unsupplemented) or elevated (ambient + 350 μmol mol^{-1}) CO_2 concentration. At the three-leaf stage the seedlings were transplanted into 1.5 dm^3 pots and placed into eight open top chambers with the same CO_2 regime (four chambers per CO_2 treatment). Details of establishment and growth conditions are given in Chapter 2.

Leaves on main stems were numbered sequentially in order of appearance with the first leaf to emerge being numbered one. Leaves on side shoots were not considered in the numbering process. All measurements were made on the main stem leaves of the same number from each plant.

4.2.2 Leaf area measurements.

The maximum linear dimensions (length and width) of each leaf under investigation were measured. The leaf area of individual leaves was calculated from the maximum linear dimensions according to the relationship;

$$A = k.l.w. \qquad (4.2)$$

where A is the area of the leaf, l is the length of the leaf from tip to base of the petiole, w is the width across the widest point of the leaf and k is a constant.

The value of k was obtained from the regression line of A on $l.w.$ for a sample of leaves (n=100). Measurements of the area of each leaf were made indirectly by photocopying each leaf onto paper of known specific area, cutting out the image and weighing the paper on an electronic balance (Sauter, model RE1E14, Fisons Scientific Equipment, Loughborough).

4.2.3 Turgor pressure

Turgor pressure can be calculated as the difference between water potential and osmotic potential of cells:

$$P=\psi-\pi \tag{4.3}$$

where ψ is water potential, P is turgor pressure and π is osmotic potential (Dainty, 1976).

Although equation 4.3 has been expressed in terms of individual cells, it can be applied to bulk tissue if ψ, P and π are regarded as volume or weight-averaged values for all compartments of all cells in the tissue (Tyree and Jarvis, 1982).

4.2.4 Water potential

Xylem pressure potential of leaves was measured using the pressure chamber technique (Scholander, 1965). The leaf under investigation was excised midway down the petiole using a sharp razor blade. The leaf was then inserted in the pressure chamber, the petiole being held in place and sealed air tight with a rubber bung with the cut end of the petiole protruding to allow observation with a binocular microscope.

When the leaf was severed the water in the xylem vessels receded as a result of the higher pressure present outside the leaf and the breakage of the water columns held under tension in the whole plant. The pressure chamber was attached to a nitrogen cylinder and gas pressure was applied until the water forced through the xylem vessels just began to exude from the cut end of the petiole. At this point the pressure applied was considered to equal the negative xylem pressure present within the leaf at the time of excision (Scholander, 1965). It was assumed that the osmotic potential of the xylem sap was negligible so that the value of xylem pressure potential obtained represented an average value of the water potential of the tissue in the chamber (Nobel, 1985).

On removal from the pressure chamber the leaf was cut into two equal sections. One section was stored in 100% methanol to be used for the measurement of cell wall extensibility (§4.2.7): the other section was placed in a labelled plastic syringe, and plunged into a dewar of liquid nitrogen, and used for the measurement of osmotic potential (§4.2.5).

4.2.5 Osmotic potential

An indirect measure of osmotic potential was obtained using a vapour pressure psychrometer (5100C, Wescor Inc. Logan UT, USA). Vapour pressure is a colligative solution property: compared with pure solvent (water) the vapour pressure of a solution is depressed in proportion to the number of particles dissolved in each kg of water. A measurement of vapour pressure depression, therefore, is an indirect measurement of solution concentration or osmolality (Turner, 1981).

The syringe containing the leaf material was subsequently removed from the liquid nitrogen and the leaf section allowed to thaw. The plunger was then depressed within the syringe to force sap from the tissue. A sample of 8 μm^3 of exuded sap was pippetted onto a solute-free paper disc in a circular sample holder. The holder was then conveyed into the measuring chamber by means of a slide assembly and the chamber closed and locked. After an equilibration period of approximately two minutes, a fine wire thermocouple, situated in the upper part of the sample chamber, was first used to measure the temperature of the sample chamber (reference temperature), and then the dew point temperature within the chamber. The difference between these two temperatures is the dew point temperature depression, itself a colligative property of the solution and a direct function of solution vapour pressure. The measurement procedure is controlled automatically and at the end of the process a value of osmolality is read off the machine. The psychrometer was calibrated using standard solutions of NaCl. No correction was made for apoplastic water content. The values obtained may be overestimates either as a result of dilution of the sap by apoplastic water or if some of the cells were undamaged by the freeze-thaw and crushing process (Tyree and Jarvis, 1982).

4.2.6 Yield turgor

Estimates of yield turgor were made using the pressure chamber technique (see Sands, McDonald and Stadenberg, 1992 for evaluation of techniques of measuring yield turgor) on the leaf immediately below the leaf under investigation for ψ, π and P. The leaf was wrapped in aluminium foil while still intact and then excised close to the point where the petiole was attached to the stem using a sharp razor blade. The assumption was made that Y was similar in both leaves. The validity of this assumption had been verified earlier on a similar sample of paired leaves. The leaf was wrapped in another layer of aluminium foil, care being taken to remove air from around the leaf. The foil-wrapped leaf was then placed in a polythene bag for 16 hours at room temperature in the dark. Measurements of leaf xylem water potential and osmotic potential were made in the same way as outlined in §4.2.4 and §4.2.5 and Y calculated using the following equation,

$$Y = \psi - \pi \tag{4.4}$$

where ψ is water potential, Y is yield turgor pressure and π is osmotic potential.

The theory behind this and other stress relaxation techniques is that if a cell is deprived of an external water supply, loosening of the cell wall (stress relaxation) continues, and reduction in water potential and turgor pressure occurs until the cell reaches a value of P equal to Y, the yield turgor of the cell (Cosgrove, Van Volkenburgh and Cleland, 1984; Cosgrove, 1985,1986; Sands, McDonald and Stadenberg, 1992, Cosgrove, 1993).

4.2.7 Cell wall extensibility

Cell wall extensibility was measured as percentage plasticity over elasticity determined by analysis of extension under mechanical loading using an Instron type extensiometer attached to a chart recorder (Van Volkenburgh, Hunt and Davies, 1983; Cleland 1984). The extensionmeter used in this study is shown in Figure 4.1.

The portion of leaf that had previously (§4.2.4) been placed in a vial containing 100% methanol to eliminate metabolic activity and remove proteins (Cleland, 1967) was rehydrated by soaking in distilled water for two hours. A strip 10 x 5 mm was cut in the lamina between the second and third major lateral veins and inserted between the clamps of the extensiometer which were initially set 5 mm apart. To prevent dehydration during measurement a drop of water was placed on the tissue between the clamps. The leaf strip was extended by moving the lower clamp downwards at a

known constant speed until a predetermined stress equivalent to a load of 20 g was obtained. The clamps were then returned to their original position and the tissue extended in the same way for a second time. In this way two load-extension relationship curves were obtained. Figure 4.2 shows an example of a load-extension curve. Following Van Volkenburgh, Hunt and Davies (1983), extensibility was calculated as the reciprocal slope of each load-extension curve;

$$\left(\frac{l_f - l_i}{l_f}\right) \times \frac{100}{2} = \% \text{ change in length per 10 g load} \quad (4.5)$$

where lf and l_i are the final and initial lengths of the sample, respectively.

The first load-extension curve gives a value of total extensibility and the second a value of elastic extensibility. Plastic extensibility is calculated from the difference between the total and elastic extensibilities. Cell wall extensibility is expressed as percentage plasticity per 10 g load. Data were corrected for variations in leaf thickness (Cleland, 1967); prior to extension, sections were weighed, measured and mass per unit length calculated for each section. Values of extensibility were corrected for variation in leaf thickness by multiplying values of extensibility by m/l (m=mass, l= length).

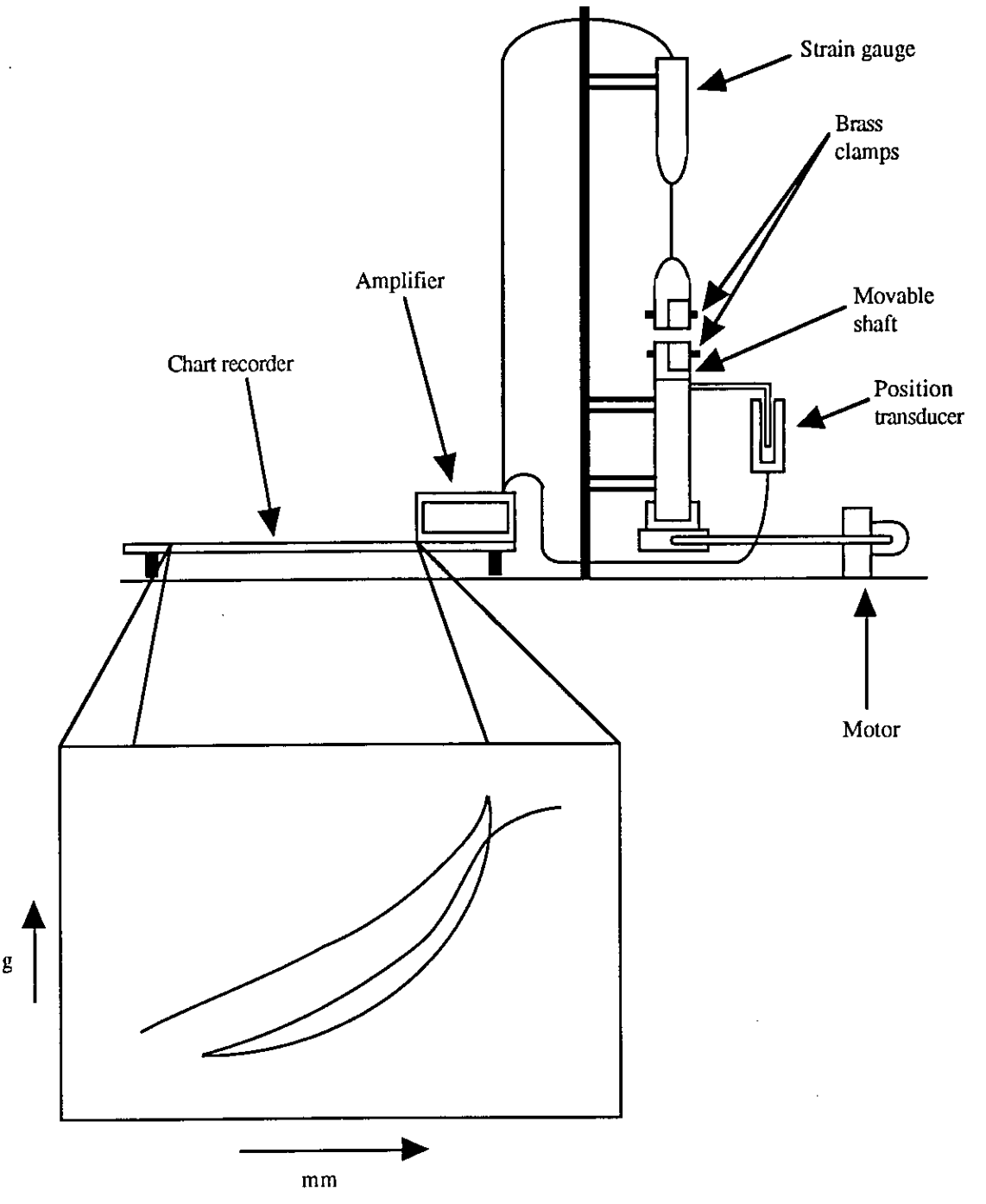


Figure 4.1 Extensionmeter used to estimate cell wall extensibility showing a typical load-extension curve obtained using the extensiometer. Sample is fixed between clamps and lower clamp is depressed at a fixed rate of 2 mm/minute. The load acting on the tissue is measured by a position transducer attached to the immobile clamp. Extension and load are plotted by a chart recorder.

4.2.8 Experimental procedure

(i) Final leaf area

The sixth leaf from 40 plants per CO_2 treatment (ten from each of four chambers) was tagged and its area measured at regular intervals over a period of 27 days, when all the leaves were fully expanded.

(ii) Diurnal pattern of leaf extension

Leaf extension rates were calculated from measurements of the maximum linear dimensions (length and width) made at four hourly intervals over a 24 hour period on four occasions between May and July 1991. All measurements were made on leaves of the same age from 40 leaves per CO_2 treatment (ten from each of four chambers). Times are BST unless otherwise stated.

(iii) Biophysics of leaf extension

On two occasions measurements of xylem pressure potential, osmotic potential and cell wall extensibility were made in conjunction with the measurements of leaf extension rate. Measurements were made at four hourly intervals between 6.00 BST and 18.00 BST. Because of the limited number of plants available for destructive sampling and the constraints of time, ten leaves per CO_2 treatment (five from each of two chambers) were sampled on each occasion.

4.2.9 Environmental variables

(i) Temperature

Air temperature was measured inside the open top chambers 1.5 m above the plants with calibrated ventilated thermistors surounded by radiation shields (RS Ltd. Loughborough) attached to a data logger (CR7, Campbell Scientific (UK) Ltd., Loughborough).

(i) Solar radiation

Solar radiation was measured using a solarimeter (CM3, Kipp and Zenon Ltd, Delft, Holland) placed on the roof of the control cabin adjacent to the open top chambers. The

reduction in solar radiation within the chambers caused by the glass was estimated to be approximately 10 % with no effects on photosynthetically active radiation (PAR) (Lee and Barton, 1993).

4.2.10 Statistics

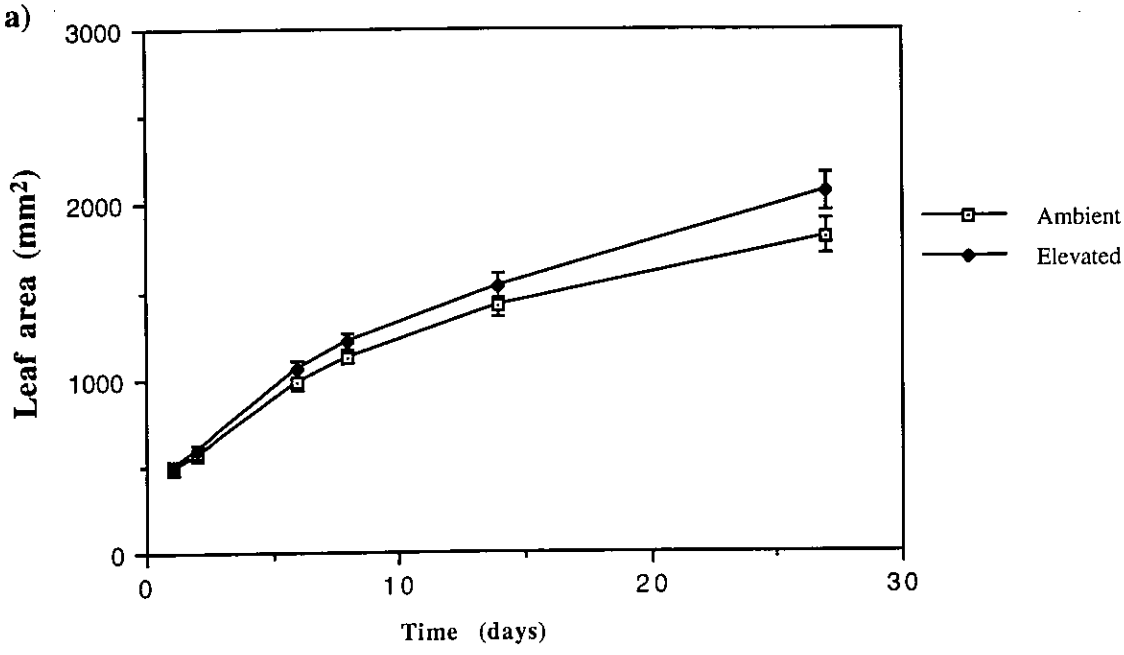
A two-way ANOVA was used to determine effects of the two CO_2 concentrations (treatment effect) and effects of the blocks (inter-chamber effect) on the dependent variables at each measurement interval. Data were tested for normality and met the assumptions of the parametric analysis.

4.3 RESULTS

4.3.1 Area of individual leaves

Figure 4.2 shows the increase in the area of the sixth leaf of plants measured over a 28 day period, beginning approximately three days after the leaves emerged. After 28 days the leaves were all fully expanded. The growth of leaves was increased in silver birch plants grown under increased concentrations of atmospheric CO_2 compared with those grown under ambient CO_2 conditions. Growth in elevated CO_2 resulted in an increase in leaf area of approximately 12%, which was significant at $P<0.05$. There was no significant difference between plants grown in the same CO_2 regime but in different chambers. Leaf area was calculated from measurements of length and width as described in §4.2.2. Elevated CO_2 concentration did not affect the shape of leaves (Figure 4.3).

a)



b)

Time (days)	1	3	7	9	14	27
Treatment effect P<	0.895	0.609	0.112	0.121	0.080*	0.026*
Inter-chamber effect P<	0.371	0.489	0.576	0.713	0.493	0.841
Interaction P<	0.775	0.529	0.320	0.337	0.315	0.876

Figure 4.2 Area of the fourth leaf of plants of silver birch grown from seed under two concentrations of atmospheric CO_2; ambient (unsupplemented) or elevated (ambient + 350 µmol mol^{-1}). All the leaves were fully expanded after 28 days.

a) Each value is the mean of 40 leaves per CO_2 treatment (one leaf from ten plants per chamber, four chambers per treatment); error bars depict two standard errors of the means.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, ** significant at P= 0.01.

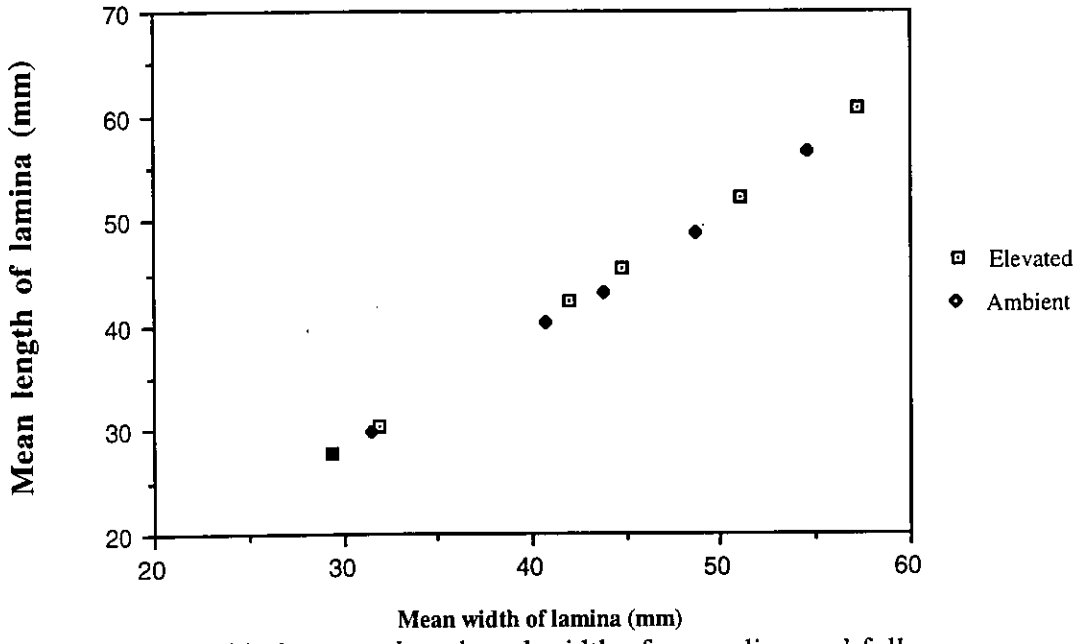
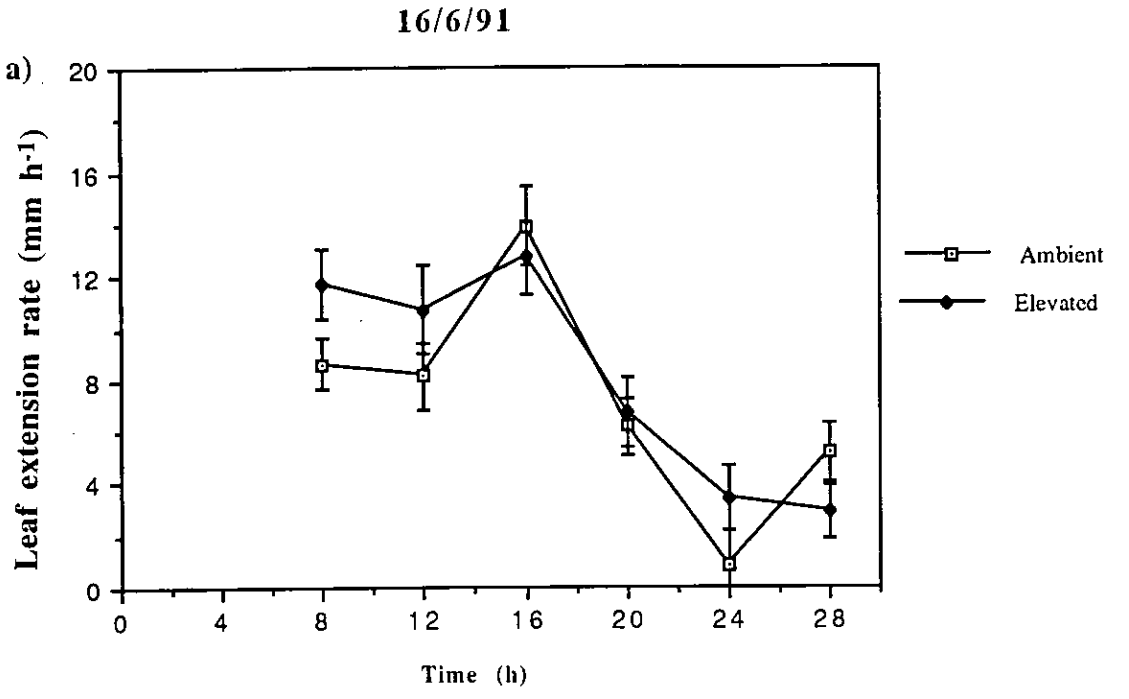


Figure 4.3 Relationship between length and width of expanding and fully developed sixth leaf of plants of silver birch grown in ambient and elevated concentrations of atmospheric CO_2.

4.3.2 Diurnal pattern of leaf extension rate

Figures 4.4-4.7 show diurnal variation in leaf extension rates. Leaf extension rate was highest during the period of daylight dropping to its lowest value during the night in leaves of both treatments. The pattern of leaf extension rate varied from day to day both within and between treatments, but the CO_2 treatment consistently resulted in an increase in leaf extension rate during the early part of the day. This increase was significant at the 5% level ($f<0.05$) on two occasions (27th June, Figure 4.5, and 5th July, Figure 4.6). Significant differences in the extension rates of leaves from different chambers also occured on two occasions (Figure 4.4). There was a significant interaction between CO_2 treatment effect and inter-chamber effect on leaf extension rate of plants measured at 8.00 on 16 May 1991, indicating that the size of the CO_2 response varied between plants from different chambers.

a)



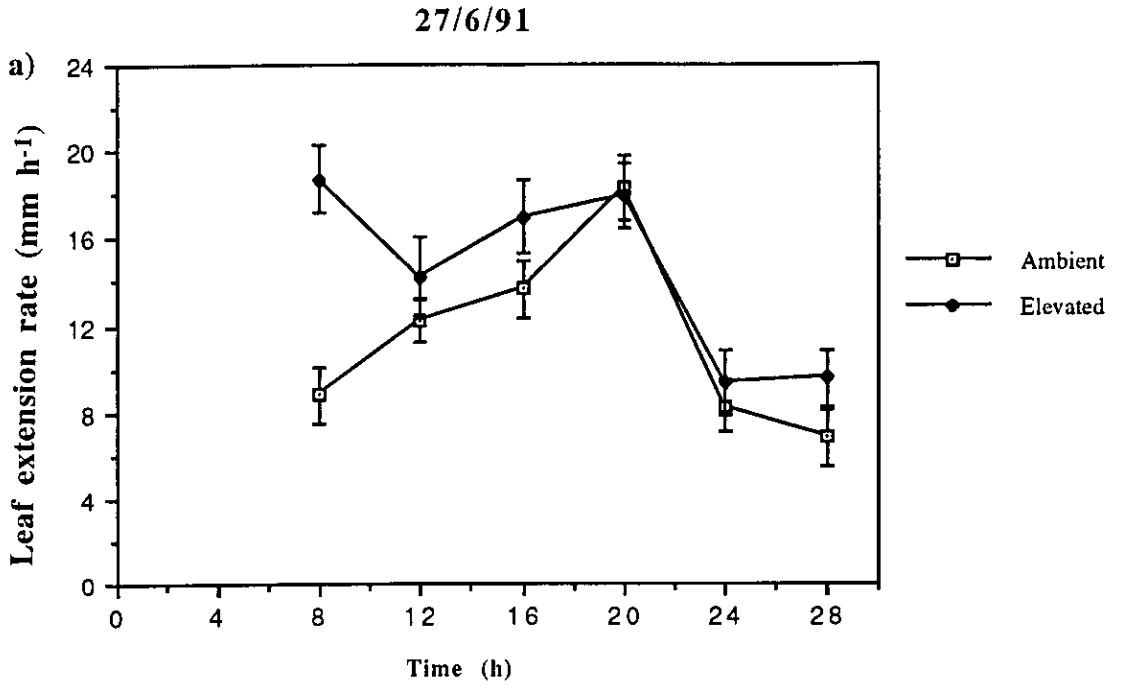
b)

Time (h)	8.00	12.00	16.00	20.00	24.00	04.00
Treatment effect P<	0.082	0.119	0.567	0.743	0.110	0.110
Inter-chamber effect P<	0.004**	0.411	0.215	0.057	0.030*	0.370
Interaction P<	0.005*	0.956	0.464	0.992	0.957	0.271

Figure 4.4 Diurnal variation in leaf extension rate (LER) from plants of silver birch grown in two concentrations of atmospheric CO_2. Measurements were made at four-hourly intervals.

a) Values are plotted at the midpoint of the sampling interval. Each value represents the mean of one leaf from 40 plants (ten from each of four chambers per CO_2 treatment); error bars depict two standard errors of the means. Measurements were made on 16th June 1991. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, ** significant at P= 0.01.



b)

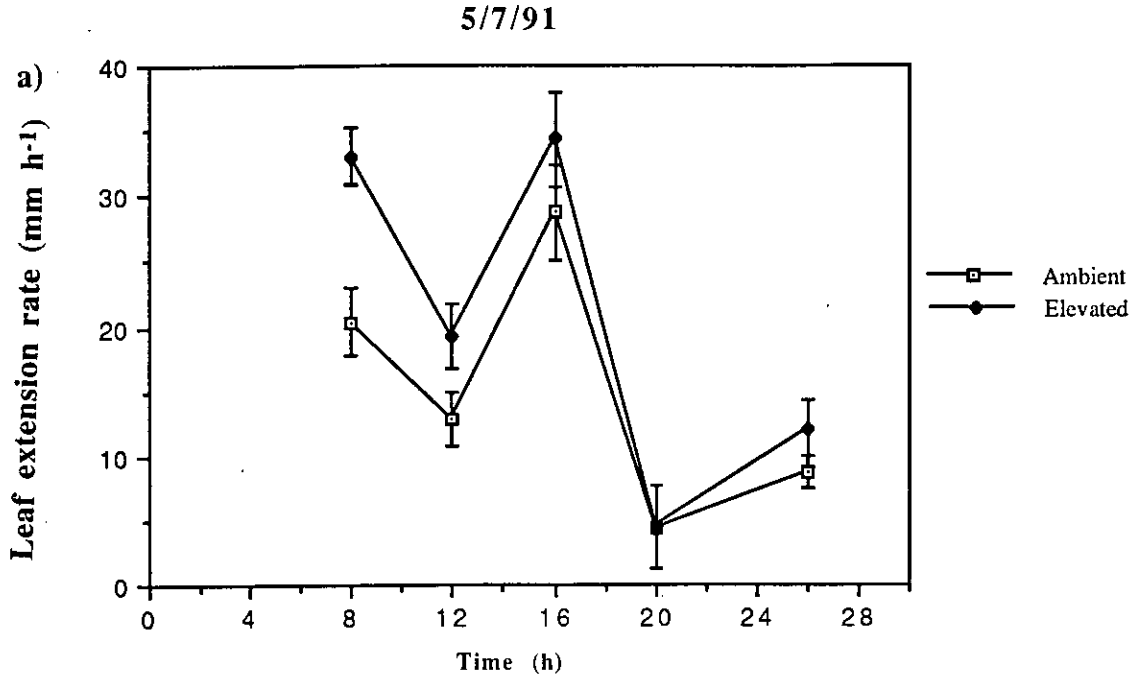
Time (h)	8.00	12.00	16.00	20.00	24.00	04.00
Treatment effect P<	0.001**	0.357	0.102	0.849	0.608	0.112
Inter-chamber effect P<	0.640	0.417	0.645	0.064	0.300	0.338
Interaction P<	0.252	0.371	0.997	0.592	0.655	0.679

Figure 4.5 Diurnal variation in leaf extension rate (LER) from plants of silver birch grown in two concentrations of atmospheric CO_2. Measurements were made at four-hourly intervals.

a) Values are plotted at the midpoint of the sampling interval. Each value represents the mean of one leaf from 40 plants (ten from each of four chambers per CO_2 treatment); error bars depict two standard errors of the means. Measurements were made on 27th June 1991. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, ** significant at P= 0.01.

a)



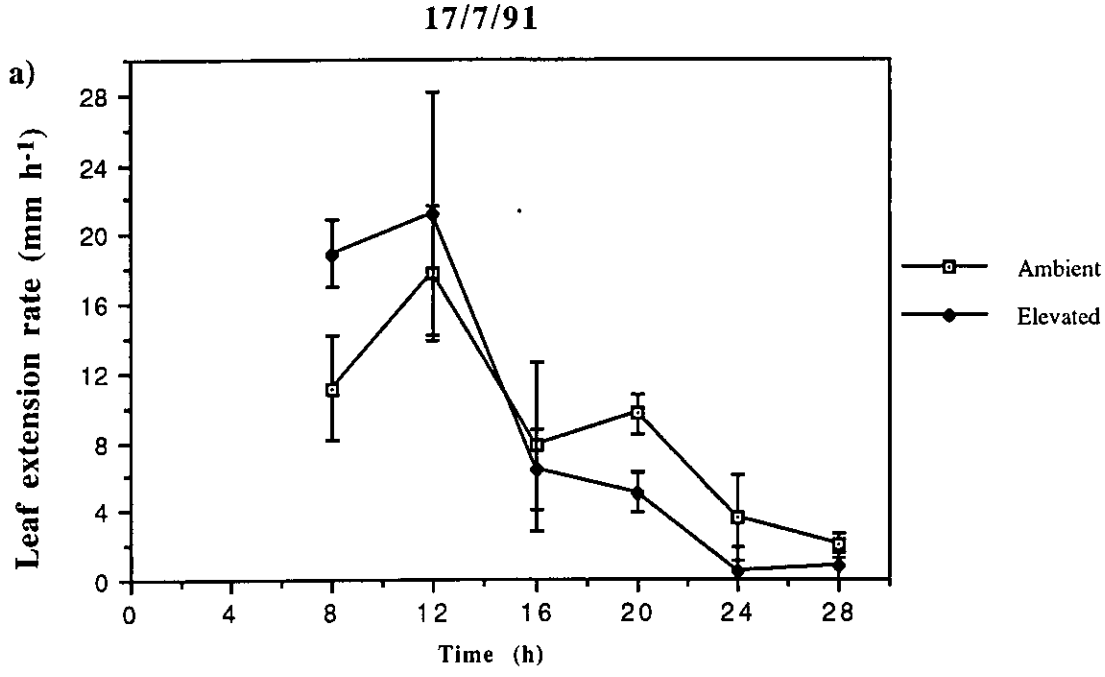
b)

Time (h)	8.00	12.00	16.00	20.00	02.00
Treatment effect P<	0.002**	0.059	0.093	0.980	0.133
Inter-chamber effect P<	0.892	0.239	0.721	0.941	0.571
Interaction P<	0.677	0.642	0.367	0.187	0.523

Figure 4.6 Diurnal variation in leaf extension rate (LER) from plants of silver birch grown in two concentrations of atmospheric CO_2. Measurements were made at four-hourly intervals.

a) Values are plotted at the midpoint of the sampling interval. Each value represents the mean of one leaf from 40 plants (ten from each of four chambers per CO_2 treatment); error bars depict two standard errors of the means. Measurements were made on 5th July. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, ** significant at P= 0.01.



b)

Time (h)	8.00	12.00	16.00	20.00	02.00
Treatment effect p<	0.073	0.459	0.857	0.006**	0.087
Inter-chamber effect p<	0.188	0.112	0.530	0.115	0.568
Interaction p<	0.566	0.551	0.298	0.376	0.776

Figure 4.7 Diurnal variation in leaf extension rate (LER) from plants of silver birch grown in two concentrations of atmospheric CO_2. Measurements were made at four-hourly intervals.

a) Values are plotted at the midpoint of the sampling interval. Each value represents the mean of one leaf from 40 plants (ten from each of four chambers per CO_2 treatment); error bars depict two standard error of the means. Measurements were made on 17th July. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, ** significant at P= 0.01.

4.3.3 Solar radiation and temperature

Figures 4.8 and 4.9 show diurnal variation in (i) solar radiation measured outside and (ii) air temperature measured within the open top chambers on two occasions during July 1991: (a) 5th July and (b) 17th July, respectively. As expected, solar radiation and air temperature follow the same diurnal pattern, increasing during the morning to reach maxima during the middle part of the day and then declining towards evening, although maxima were attained later in the day on the 17th July. Leaf extension rates were low for leaves from both treatments during the dark period when temperatures were also low. Lower night-time temperatures on 17th July resulted in slightly lower minimum night-time leaf extension rates. However, the pattern of leaf extension rates during daylight hours did not correlate with either temperature or light.

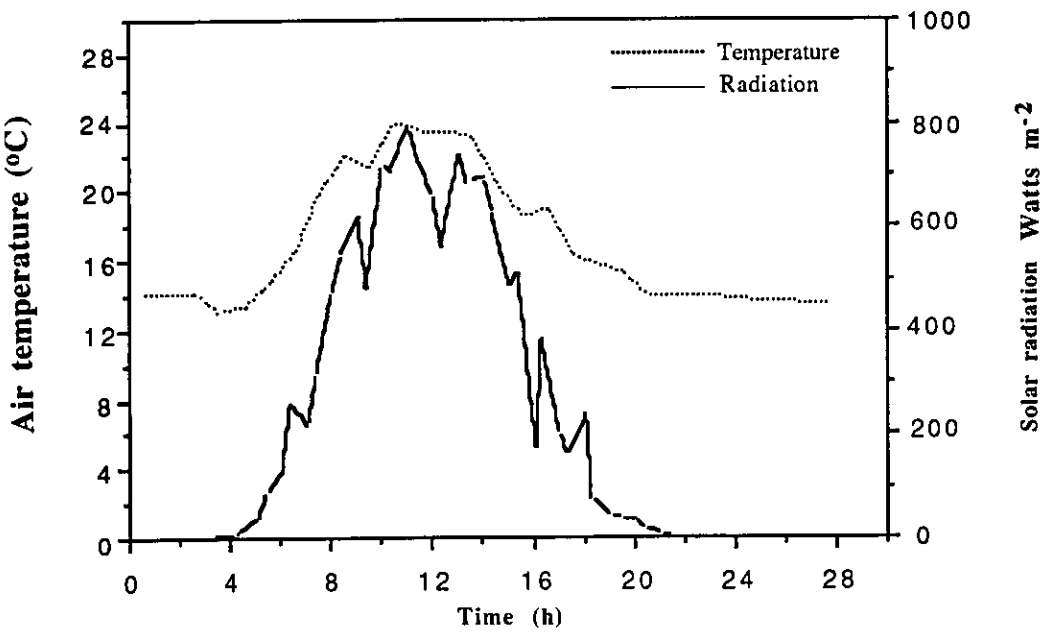


Figure 4.8 Diurnal variation in solar radiation and air temperature on 5th July 1991. solar radiation was measured on the roof of a portacabin adjacent to the open top chambers and air temperature was measured 1.5 m above the floor of one of the open top chambers. Measurements were made at five minute intervals, values are hourly means. Time is GMT.

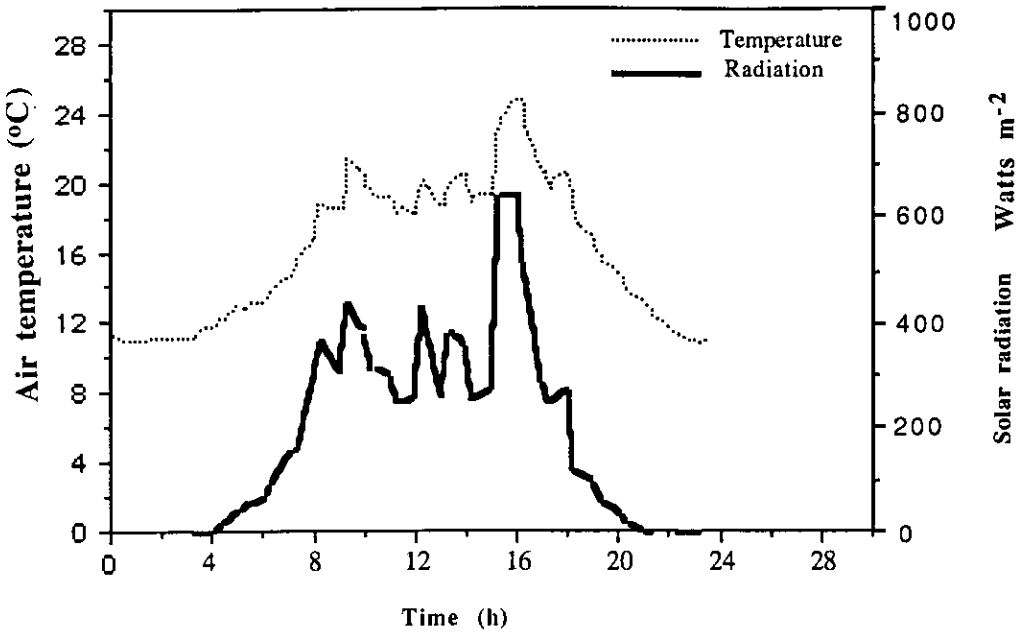


Figure 4.9 Diurnal variation in solar radiation and air temperature on 17th July 1991. solar radiation was measured on the roof of a portacabin adjacent to the open top chambers and air temperature was measured 1.5m above the floor of one of the open top chambers. Measurements were made at five minute intervals, values are hourly means. Time is GMT.

4.3.4 Water potential, osmotic potential and calculated turgor

Figures 4.10 to 4.12 show the variation in water potential (4.10), osmotic potential (4.11) and calculated turgor (4.12) of leaves measured on 5th July, 1991. Figures 4.13 to 4.15 show the variation in water potential (4.13), osmotic potential (4.14) and calculated turgor (4.15) of leaves measured on 17th July, 1991.

5th July 1991

Figure 4.10 shows that leaf water potential declined from its early morning value towards midday and then rose again to reach its highest value towards evening. CO_2 treatment resulted in higher water potentials during the day but by the evening (20.00) this difference had disappeared. There was little variation in leaf osmotic potential (Figure 4.11) over the course of the day but the osmotic potential of ambient CO_2 leaves was significantly higher at 12.00 ($p<0.05$) and there was also a significant inter-chamber difference in plants measured at 16.00. Leaf turgor pressure dropped slightly towards midmorning (12.00) and then increased to reach a maximum towards 20.00 in both treatments (Figure 4.12). Turgor pressures were slightly higher throughout the day in leaves of plants growing in elevated CO_2 compared to those of plants in ambient

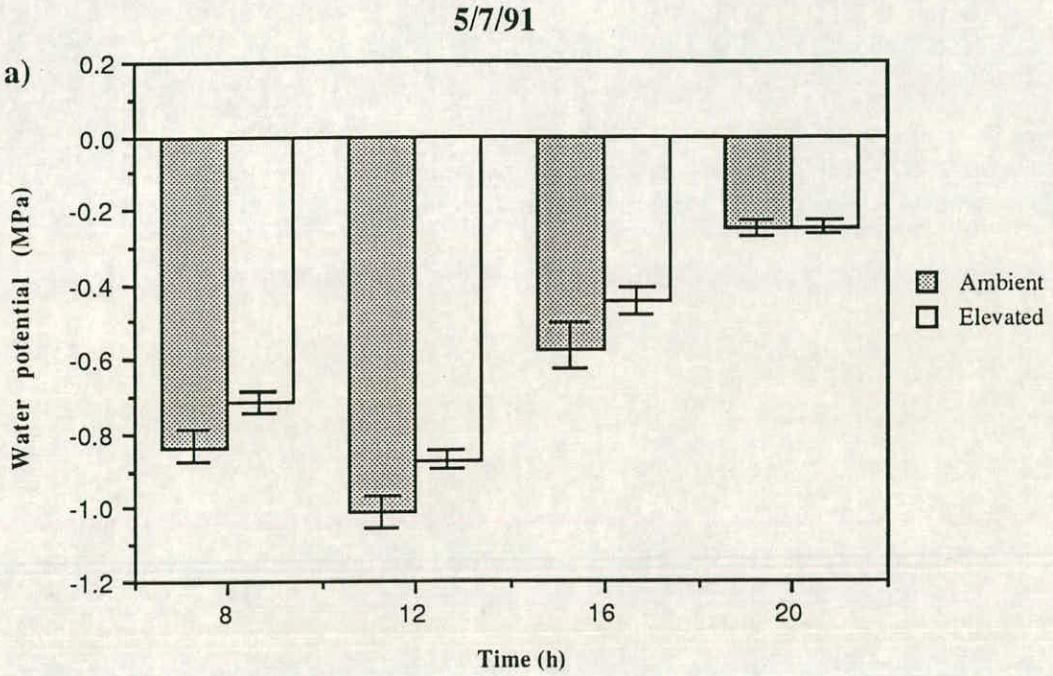
CO_2, although this only reached significance at 16.00 ($P<0.05$). The pattern of turgor pressure followed that of LER during the daylight period.

17th July 1991

Leaf water potentials changed more slowly than on the previous occasion and were more negative later in the day (16.00), recovering more slowly towards evening (Figure 4.13), although there was a trend of lower leaf water potentials in elevated CO_2. There were no significant differences between treatments, but inter-chamber variation was significant on two sampling occasions ($P<0.05$). There was little variation in leaf osmotic potential over the day (Figure 4.14). Elevated CO_2 treatment resulted in a significantly lower osmotic potential in the morning (8.00) but any treatment effect disappeared during the course of the day, but there was a significant inter-chamber difference between plants measured at 20.00.

The pattern of turgor pressure varied over the course of the day between treatments, as did LER. Turgor was high in the morning, dropped towards mid afternoon (16.00), and then recovered towards evening (20.00) (Figure 4.15). By contrast with 5th July, turgor pressure was higher in leaves of ambient CO_2 plants than in leaves of elevated CO_2 plants for most of the day, apart from 8.00 when P in the elevated CO_2 plants exceeded P in ambient CO_2. LER were high in elevated CO_2 plants in the morning when turgor pressure was high but fell below that of ambient plants in the afternoon when turgor pressure was higher in ambient CO_2 plants.

a)



b)

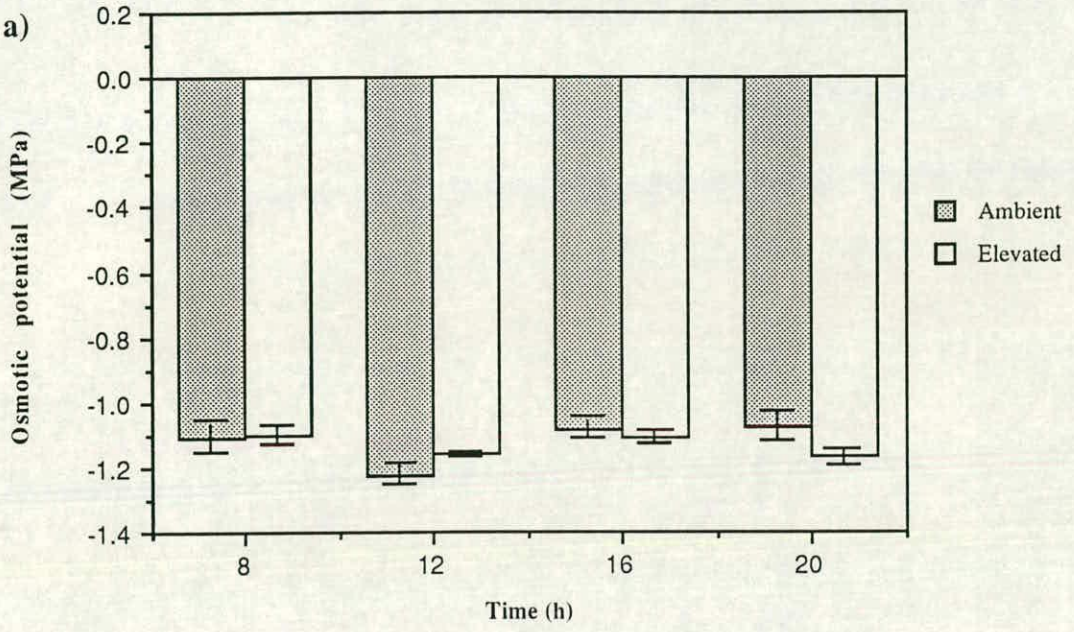
Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.018*	0.007*	0.054*	0.949
Inter-chamber effect P<	0.664	0.146	0.360	0.946
Interaction P<	0.664	0.363	0.134	0.837

Figure 4.10 Water potential of leaves of silver birch measured at four-hourly intervals on 5th July 1991. Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

a)



b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.764	0.033*	0.150	0.082†
Inter-chamber effect P<	0.411	0.910	0.007*	0.511
Interaction P<	0.170	0.618	0.688	0.851

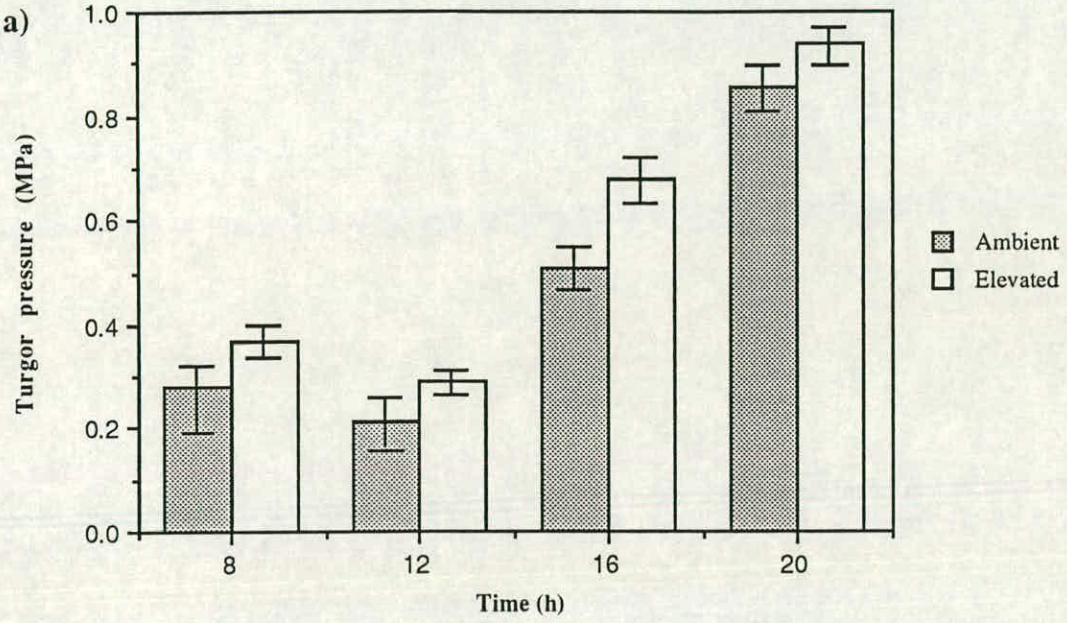
Figure 4.11 Osmotic potential of leaves of silver birch measured at four-hourly intervals on 5th July 1991. Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

5/7/91

a)



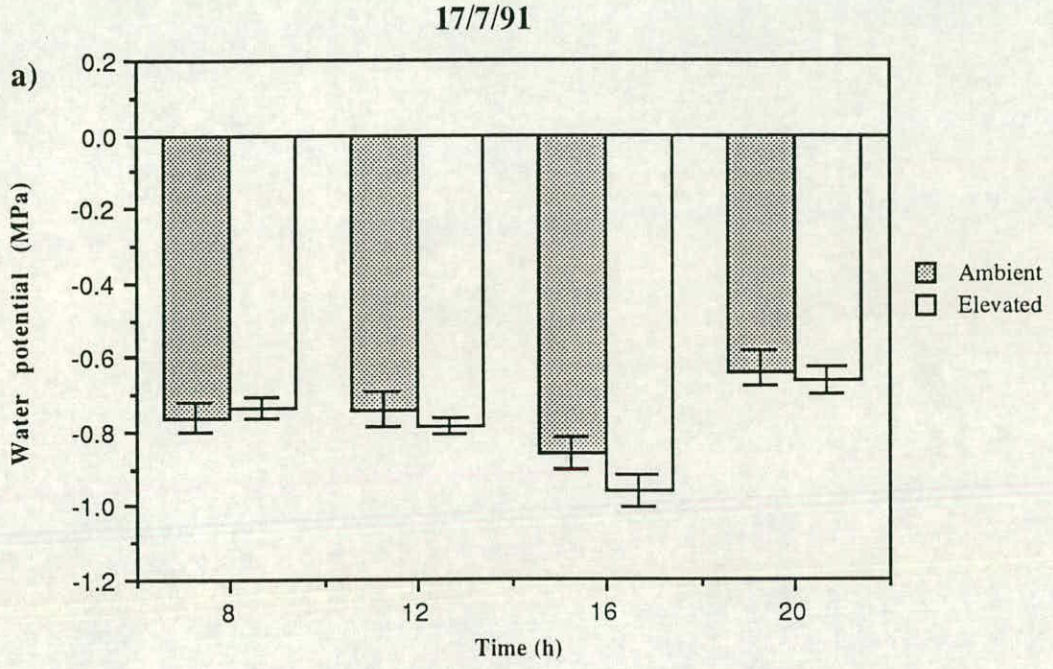
b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.078*	0.132	0.019*	0.111
Inter-chamber effect P<	0.401	0.160	0.807	0.648
Interaction P<	0.091	0.978	0.129	0.615

Figure 4.12 Calculated turgor pressure of leaves of silver birch measured at four-hourly intervals on 5th July 1991. Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.



b)

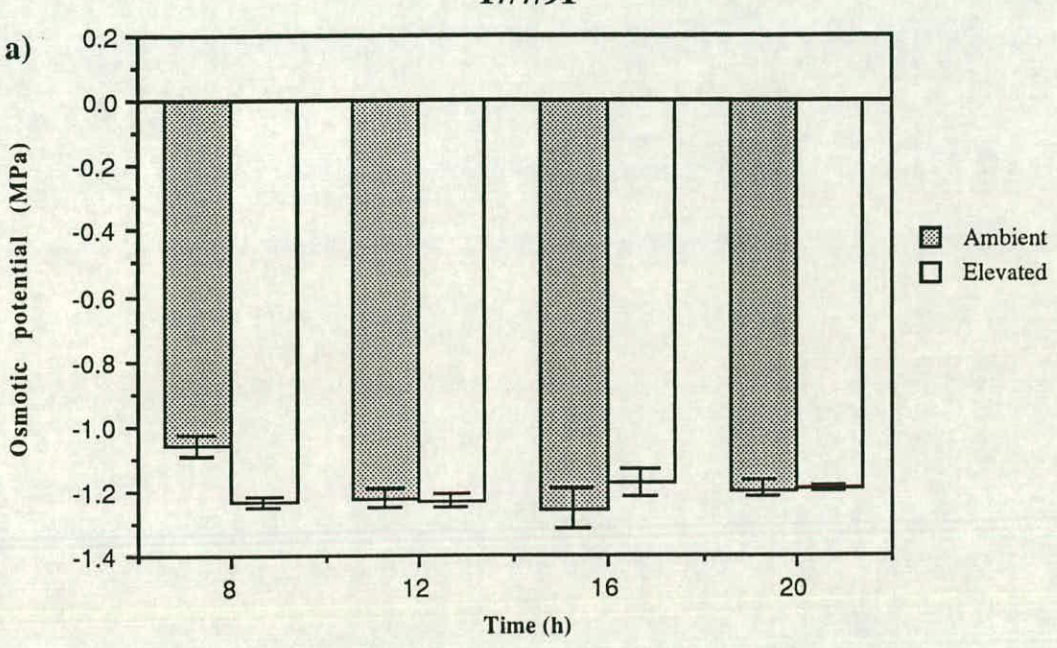
Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.546	0.474	0.154	0.741
Inter-chamber effect P<	0.034*	0.931	0.045*	0.123
Interaction P<	0.133	0.934	0.424	0.134

Figure 4.13 Water potential of leaves of silver birch measured at four hourly intervals on 17th July 1991. Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

a)



b)

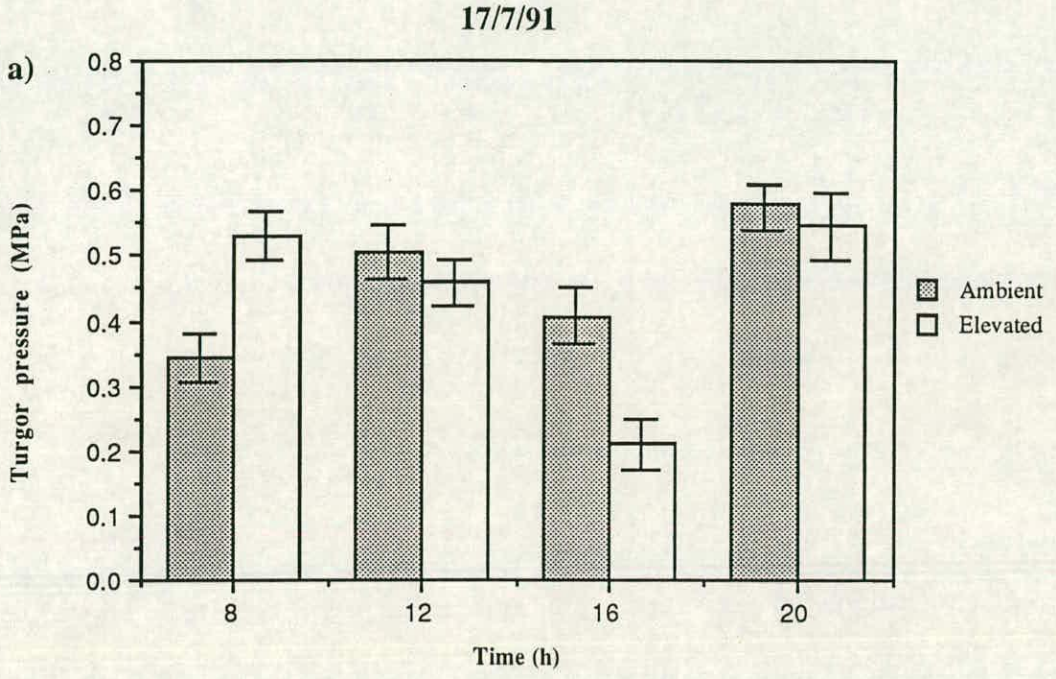
Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.002**	0.861	0.168	0.765
Inter-chamber effect P<	0.130	0.969	0.140	0.031*
Interaction P<	0.011*	0.350	0.557	0.454

Figure 4.14 Osmotic potential of leaves of silver birch measured at four-hourly intervals on 17th July 1991. Measurements were made on the ninth leaf from each plant.

a)Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

a)



b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.098†	0.429	0.035*	0.518
Inter-chamber effect P<	0.148	0.343	0.388	0.755
Interaction P<	0.367	0.112	0.875	0.566

Figure 4.15 Calculated turgor pressure of leaves of silver birch measured at four-hourly intervals on 17th July 1991. Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

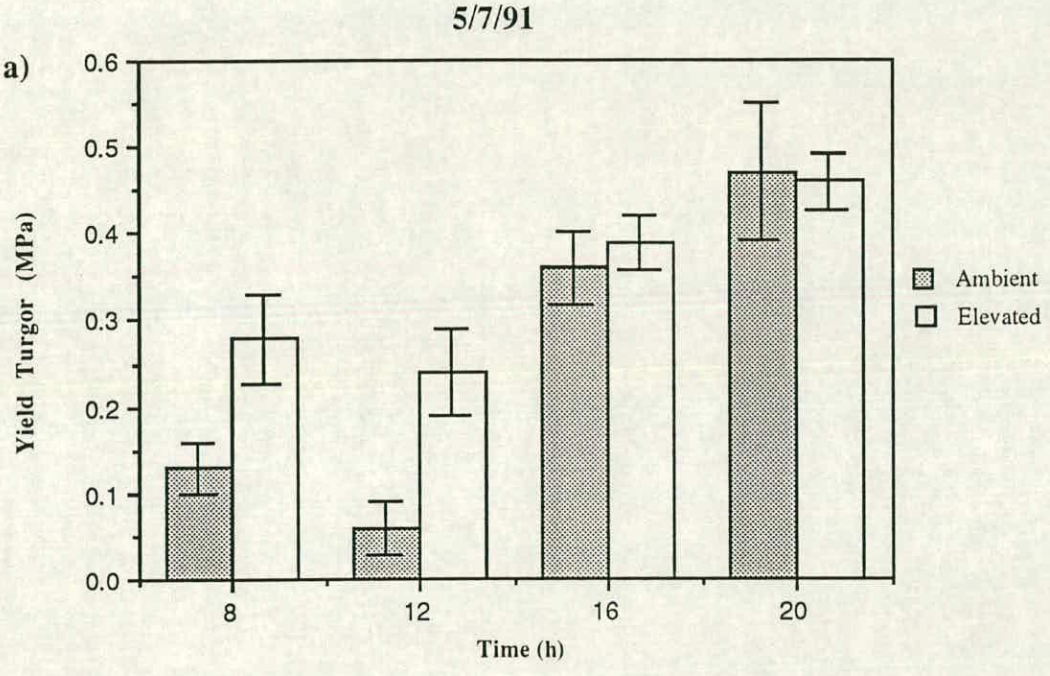
b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

4.3.5 Yield turgor

Figures 4.16 and 4.17 show that calculated yield turgor varied with time, on the 5th and 17th July 1991, respectively. Figure 4.16 shows that the amplitude of this variation was larger on 5th July and that growth in elevated CO_2 resulted in a

significant increase in the value of yield turgor during the morning (8.00-12.00) but that this difference disappeared towards evening. The values for yield turgor on 17th July were much lower throughout the day, and whilst yield turgor was higher in elevated CO_2 on three of the four occasions, the differences between treatments were not significant at P<0.1.

a)



b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.023*	0.013*	0.579	0.929
Inter-chamber effect P<	0.167	0.752	0.094†	0.479
Interaction P<	0.163	0.804	0.123	0.585

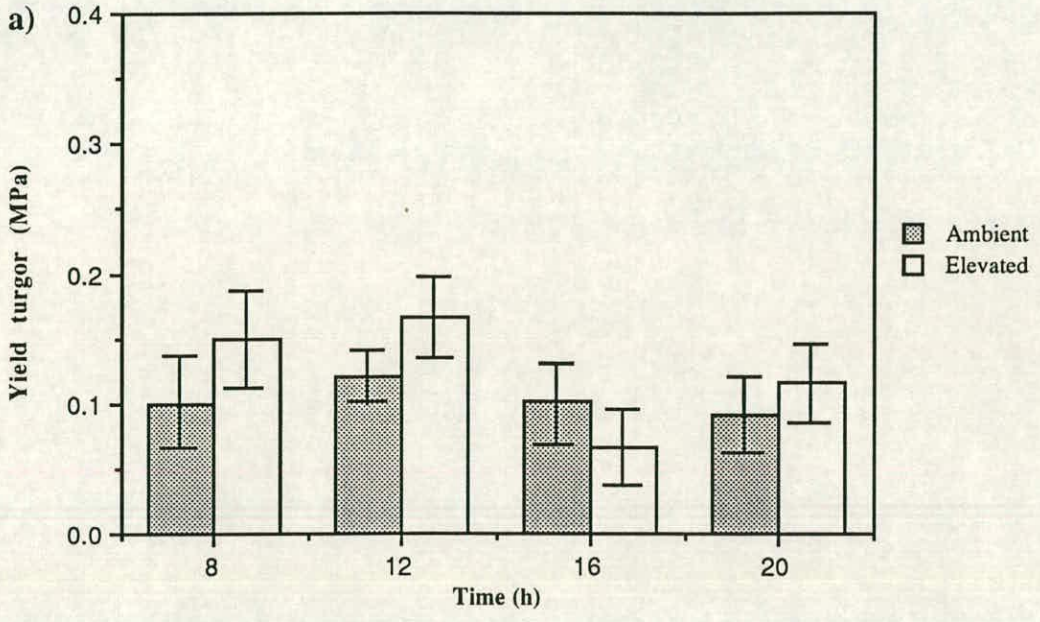
Figure 4.16 Calculated yield turgor of leaves of silver birch measured at four-hourly intervals on 5th July 1991. Measurements were made on the eighth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

17/7/91

a)



b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.123	0.129	0.700	0.163
Inter-chamber effect P<	0.154	0.710	0.652	0.678
Interaction P<	0.085	0.766	0.430	0.511

Figure 4.17 Calculated yield turgor of leaves of silver birch measured at four-hourly intervals on 17th July 1991. Measurements were made on the eighth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean, of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

4.3.6 Effective turgor Pressure

The effective turgor [calculated turgor (*P*) - yield turgor (*Y*)] of leaves measured at four hourly intervals are presented in Figure 4.18 for the 5th July and 4.19 for the 17th July. There was no consistent effect of CO_2 treatment on effective turgor.

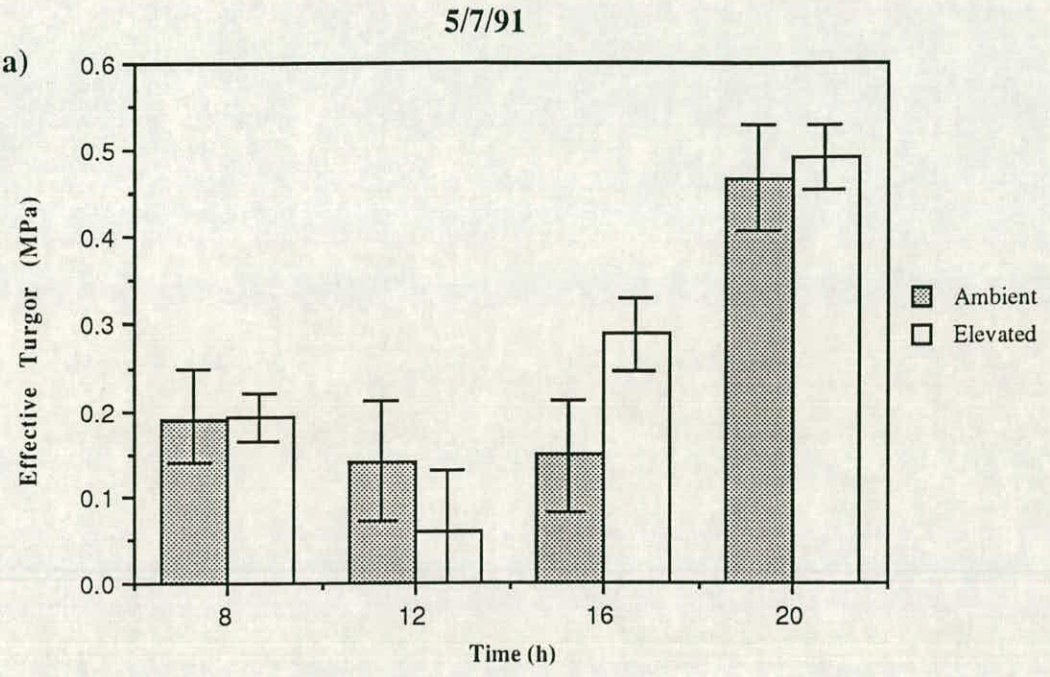
Figure 4.18 shows that the pattern of variation in (*P-Y*) was similar in leaves from both CO_2 treatments on 5^{th} July. The amplitude of the variation was slightly larger in leaves from elevated CO_2 plants, but this was not significant at P= 0.05. There was no significant difference in effective turgor of leaves measured at 8.00, although LER was significantly higher in elevated compared with ambient CO_2 plants measured at that time.

On 17th July effective turgor in elevated CO_2 was slightly (but not significantly) higher in the morning (8.00), but subsequently dropped slightly below the value of leaves in ambient CO_2 by the late afternoon (16.00) (Figure 4.19), following a similar pattern to LER.

On both days and in both CO_2 treatments effective turgor followed the same pattern as values for turgor measured on leaves at the same time, except at 12.00 on 5th (*cf* Figures 4.15 and 4.19).

Effective turgor increased in both treatments towards evening reaching a maximum at approximately the same value by 20.00.

a)



b)

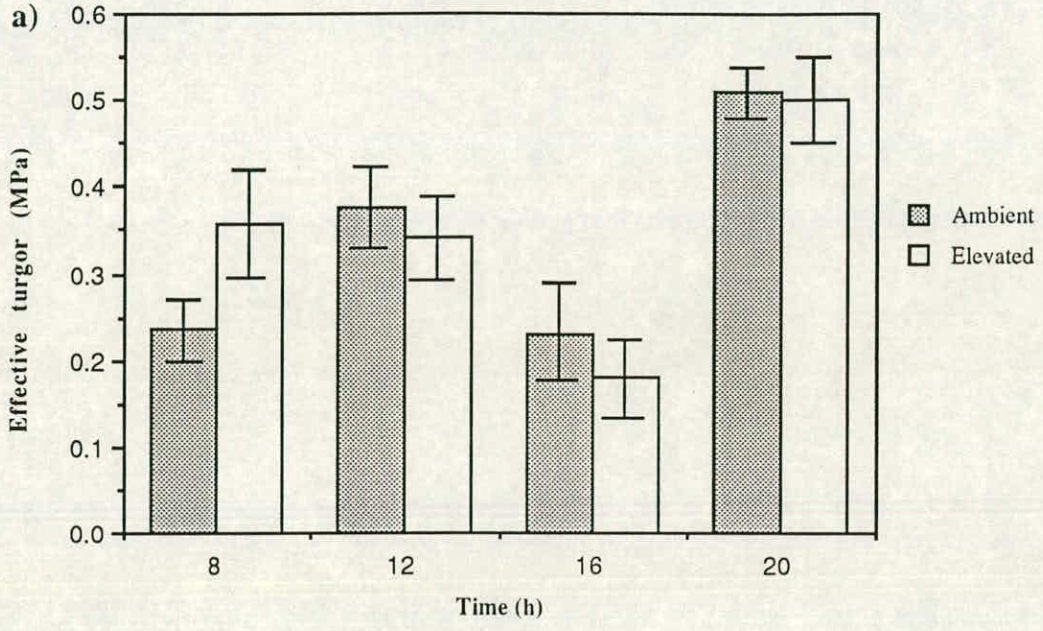
Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.580	0.502	0.063†	0.405
Inter-chamber effect P<	0.556	0.156	0.126	0.543
Interaction P<	0.186	0.820	0.589	0.427

Figure 4.18 Effective turgor (*P-Y*) of leaves of silver birch measured at four-hourly intervals on 5th July 1991. Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

a)



b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.104	0.636	0.647	0.878
Inter-chamber effect P<	0.026*	0.482	0.925	0.831
Interaction P<	0.025*	0.450	0.145	0.547

Figure 4.19 Effective turgor (*P-Y*) of leaves of silver birch measured at four-hourly intervals on 17th July 1991 Measurements were made on the ninth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

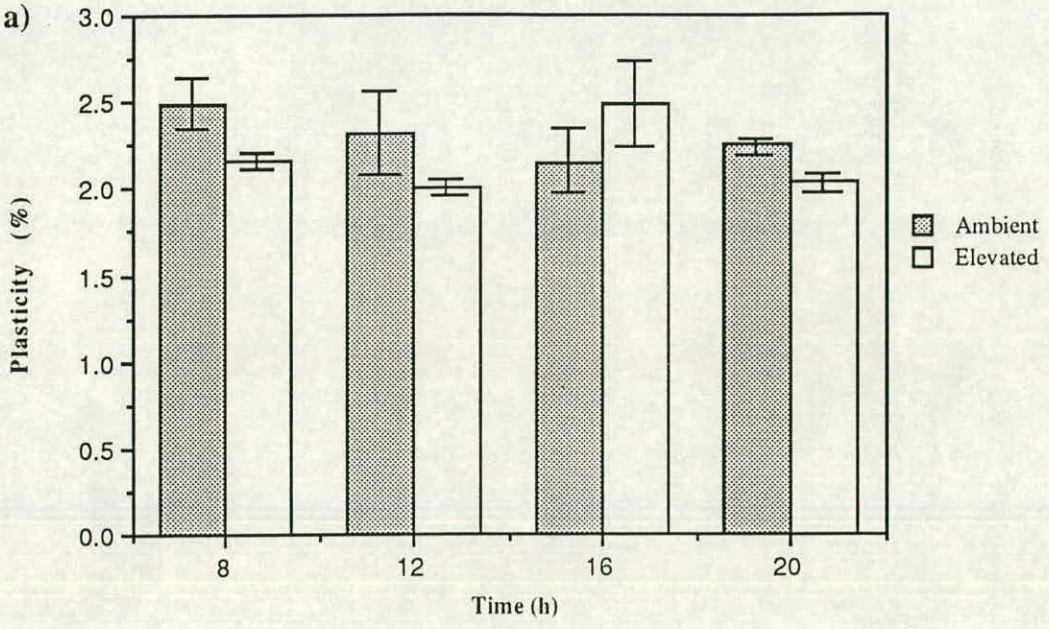
4.3.7 Plastic extensibility

Plastic extensibility (%plasticity/elasticity) of the leaves is shown in Figures 4.20 for the 5th July and 4.21 for the 17th July. The variation in plasticity over the course of the day was small on both the days that measurements were taken.

Figure 4.20 shows that there were no significant treatment differences in the plasticity of leaves at any of the sampling times on the 5th July. Significant variation existed in the plastic extensibility of leaves from different open top chambers measured at 8.00 and 20.00. However, no similar variation was recorded in leaf extension rates measured at that time (Figure 4.6).

On 17th July there was little variation in plasticity of leaves sampled between 8.00 and 16.00 in either treatment, but the plasticity of elevated CO_2 leaves was significantly below that of the ambient CO_2 leaves measured at 20.00 ($P<0.05$ Figure 4.21). At this time, the calculated values of turgor pressure were high in both treatments but the higher plastic extensibility in the leaves of ambient CO_2 plants were not mirrored by concurrent leaf extension rates (Figure 4.7). Air temperatures recorded at this time were similar to those recorded in the morning when extension rates were higher (Figure 4.9).

a)



b)

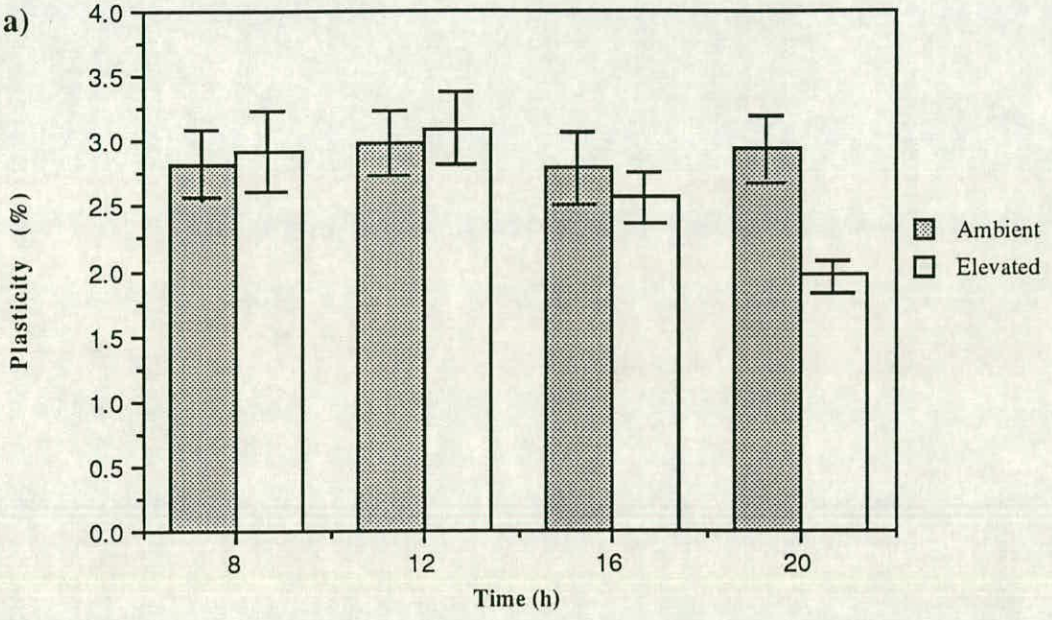
Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.101	0.137	0.138	0.125
Inter-chamber effect P<	0.012*	0.800	0.072†	0.001*
Interaction P<	0.578	0.797	0.138	0.071†

Figure 4.20 Plastic extensibility (plasticity) of leaves of silver birch measured at four-hourly intervals on 5th July 1991. Measurements were made on the eighth leaf from each plant.

Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

a)



b)

Time (h)	8.00	12.00	16.00	20.00
Treatment effect P<	0.798	0.873	0.840	0.008*
Inter-chamber effect P<	0.679	0.892	0.676	0.418
Interaction P<	0.160	0.163	0.953	0.170

Figure 4.21 Plastic extensibility (plasticity) of leaves of silver birch measured at four-hourly intervals on 17th July 1991. Measurements were made on the eighth leaf from each plant.

a) Values are shown at the midpoint of the sampling interval and represent the mean of ten leaves (five from each of two chambers per treatment); error bars depict two standard errors of the means. Time is BST.

b) Level of significance for the differences between plants grown in elevated or ambient CO_2 (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect). * significant at P= 0.05, † significant at P= 0.10.

4.3.8 Summary of results

Leaf extension rates were higher during the morning in plants grown in elevated CO_2 compared to those grown under ambient conditions, on all the days that measurements were made. Table 4.1 and 4.2 separate the results into two parts and summarises those obtained in the morning (8.00-12.00) (Table 4.1), and those obtained during the latter part of the day (16.00-20.00) (Table 4.2).

Table 4.1 Summary of the results obtained during the morning (8.00-12.00) of the two days measurements were taken. E represents elevated CO_2 and A ambient CO_2. * is significant at P=0.05, ** significant at P=0.001 and † represent a difference between CO_2 treatments that is significant at P=0.10, n.s = not significant.

	5th July		17th July	
Time	8.00	12.00	8.00	12.00
Leaf extension	E>A**	E>A†	E>A†	n.s
ψ	E>A*	E>A**	n.s	n.s
π	n.s	E<A*	E<A**	n.s
P	E>A†	n.s	E>A†	n.s
Y	E>A*	E>A*	n.s	n.s
P-Y	n.s	n.s	n.s	n.s
Plastic extensibility	n.s	n.s	n.s	n.s

Table 4.2 Summary of the results obtained during the latter part of the day (16.00-26.00) of the two days measurements were taken. E represents elevated CO_2 and A, ambient CO_2. * is significant at 0.05, ** significant at 0.001 and † represent a difference that is significant at 0.01, n.s = not significant.

	5th July			17th July		
Time	16.00	20.00	26.00	16.00	20.00	02.00
Leaf extension	E>A†	n.s	n.s	n.s	A>E**	A>E†
ψ	E>A†	n.s	n.s	n.s	n.s	
π	n.s	E>A†		n.s	n.s	
P	E>A*	E>A†		A>E*	n.s	
Y	n.s	n.s		n.s	n.s	
P-Y	E>A†	n.s		n.s	n.s	
Plastic extensibility	n.s	n.s		n.s	A>E**	

8.00-12.00

Leaf extension rates were higher in plants measured on the 5th compared to the 17th July. The increase in leaf extension rate during the morning in plants grown in elevated CO_2 was also higher on the 5th. This increase in leaf extension rate in elevated CO_2 plants was accompanied by significant ($P<0.05$) increases in ψ and Y on 5th. On the 17th the increase in leaf extension rate in elevated CO_2 plants in the morning was accompanied by a significant decrease in π. Both radiation and temperature were lower during the morning of 17th compared to 5th July.

16.00-20.00

The slight increase in leaf extension rates in elevated CO_2 plants observed during the latter part of the 5th July was accompanied by a significant increase in P (at 16.00) and a slight (non-significant at $P<0.05$) increase in ψ, π and $(P\text{-}Y)$. During the latter part of 17th July the leaf extension rate of plants grown in elevated CO_2 concentrations was significantly below that of the ambient CO_2 grown plants. The higher levels of leaf extension in ambient CO_2 grown plants was accompanied by a significant increase in P and in plasticity and a slight (non significant at $P=0.05$) increase in ψ, π and $(P\text{-}Y)$.

Both radiation and temperature levels rose sharply at 16.00 on 17th July and remained high for two hours, but both were already dropping by this time on 5th July.

There was no rain on either day that measurements were made. Night time temperature was higher on the 5th and day time temperature reached a maximum earlier than on 17th July. There was more cloud cover on the 17th with the result that PFD was lower than on the 5th July, although it increased sharply between 15.00 and 17.00, reaching higher values than recorded at the same time on the 5th July.

4.4 DISCUSSION

4.4.1 Differences in LER measured on 5th and 17th July

The reasons for the observed differences in the parameters measured between days are not readily explained by differences in weather and an alternative is suggested. An increase in leaf area of individual leaves of plants grown in elevated CO_2 was observed in May; however, when the seedlings were harvested at the end of August there was no increase in either the number of leaves produced from the main stem or the total area of those leaves (§3.5.4). This suggests that the leaves produced later in the season in elevated CO_2 plants were smaller than those produced by plants grown in ambient CO_2. Although rates of leaf extension were still slightly increased in elevated CO_2 plants, compared to ambient CO_2 plants measured on the 17th July, they fell below those of the ambient CO_2 plants in the afternoon, with the result that there was no difference in the expansion of leaf area in leaves from elevated or ambient CO_2 measured over the 24-hour period (246 mm^2 in elevated CO_2 leaves *cf.* 238 mm^2 in ambient CO_2 leaves, $P<0.05$). The different pattern of leaf extension observed between 16.00 and 20.00 in leaves measured on 17th July may be indicative of a reduction in the size of leaves produced in elevated CO_2.

4.4.2 Diurnal variation in leaf extension rate

Although the pattern of leaf extension varied over the diurnal period both between and within treatments, in all cases higher rates of leaf extension were recorded during the daylight period in accordance with results for silver birch of Taylor and Davies (1985, 1986b) and McDonald, Stadenberg and Sands (1992). Despite such variations, the extension rates of leaves in elevated CO_2 were consistently higher during the early morning than those in ambient CO_2. The following factors help to explain the high early morning increase in leaf extension rates.

4.4.3 Temperature

The process of cell wall loosening involves the breaking of bonds by enzymes and is therefore expected to be closely coupled to leaf temperature (Kemp and Blacklow, 1980; McDonald, Stadenberg and Sands, 1992). In the experiment described here, leaf extension rates were low during the night when temperatures were low and lower minimum extension rates were recorded when the minimum night-time temperature was lower, but during the daylight period leaf extension rates only correlated with temperature during the middle part of the day (12.00 - 16.00) with a lag of approximately two hours. Temperature effects on tissue extensibility alone could not account for the low rates of extension seen during the early evening as temperatures at this time were similar to those in the early morning when extension rates were high.

4.4.4 Photon flux density

Both radiation quantity and quality are known to affect leaf expansion. Rates of leaf extension were higher on 5th July compared to those measured on 17th July, when PFDs were lower. Increases in PFD enhance the rate of photosynthesis and the availability of carbohydrate for energy and the maintenance of osmotic potential, but if carbohydrate availability was a factor limiting leaf expansion, leaves from elevated CO_2 plants would be expected to show a consistent increase in leaf extension rate (Barlow, 1983)

4.4.5 Turgor pressure

CO_2 has been reported to reduce g_S resulting in lower rates of transpiration and higher water potentials which, in addition to an increase in osmotic potential because of increased carbohydrate production, may be expected to increase turgor. In this experiment there were no consistent effects of elevated CO_2 treatment on either osmotic potential, or on water potential, despite a reduction in g_S (§5.3.1), but turgor pressure was consistently higher in plants in elevated CO_2 on the 5th July.

The pattern of turgor varied both with time and treatment on the two days, mainly as a result of variation in leaf water potential. Leaf extension rates did correlate with turgor qualitatively over the middle part of the day (approximately 12.00 to 16.00) but the correlation was poor during the latter part of the day (20.00-26.00) when temperature and radiation levels were low. Bunce (1977) found a good correlation between turgor and leaf extension only when temperatures were high. In a controlled environment experiment on silver birch by Taylor and Davies (1986b) the main site of control

appeared to be the cell wall and no correlation between leaf extension and turgor pressure was found.

4.4.6 Yield turgor

Values obtained for yield turgor differed between the two days. The values obtained on the 5th July are similar to reported values of yield turgor for silver birch of 0.3-0.4 MPa (McDonald, Lohammar and Ingestad, 1992), whereas the values obtained during the 17th July were lower than expected. Yield turgor was found to vary over the course of the day but the pattern was not consistent between days. This variation in yield turgor is in apparent disagreement with the findings of McDonald, Sands and Stadenberg (1992) who found no diurnal variation in yield turgor for *Salix viminalis* using the psychrometer method. In a controlled environment experiment on silver birch, Taylor and Davies (1986b) found no difference in yield turgor between light and dark periods in plants growing at constant temperature but in a parallel experiment (Taylor and Davies, 1986a) they did find that yield turgor was affected by changes in the red: far red ratio of incident light. In the experiment described here the quality of light, including the red:far red ratio reaching the leaves of any plant varies with the angle of incidence of the sun relative to the chamber and the proximity of neighbouring plants, and this may account to some extent for the variation in yield turgor. If the red:far red ratio does indeed affect yield turgor the low values recorded on the 17th July may be explained by the increased cloud cover on this day resulting in an increase in the red: far red ratio of radiation reaching the leaves. The validity of the technique used to measure yield turgor in this experiment is discussed in §4.49.

4.4.7 Cell wall plasticity

Values for plastic extensibility of the tissue did not correlate with the values obtained for leaf extension rates. In a similar experiment on *Salix viminalis*, McDonald, Stadenberg and Sands (1992) suggested that the failure of plasticity measurements to correlate with leaf extension rates may relate to the technique used to measure plasticity: an assessment of the Instron approach to estimate plasticity is made in section §4.49. Despite inconclusive evidence of differential plasticity in this experiment, an increase in cell wall extensibility associated with a PFD-activated wall loosening process is considered to contribute to the high rates of leaf extension observed in elevated CO_2 plants in the early morning. Taylor and Davies (1986b) found higher values of plastic extensibility in silver birch during the photo period. They also reported a decrease in cell-surface pH in silver birch leaves exposed to PFD when extensibility and leaf growth rates were highest. Extension of leaves of *Phaseolus vulgaris* has been shown

to respond to an increase in PFD of 250 μmol^{-1} $m^{-2}s^{-1}$ (VanVolkenburgh and Cleland, 1980, 1981) and this was not associated with an increase in leaf turgor but was thought to result from cell wall acidification leading to wall loosening.

4.4.8 Control of leaf extension

The factors affecting leaf extension vary over the course of the day, such that at any time the limiting factor for cell extension may vary. From this experiment it can be suggested that cell wall extensibility was limiting leaf expansion during the early morning and evening in leaves from ambient CO_2 grown plants but this was partly ameliorated in the leaves of elevated CO_2 plants. During the middle part of the day when temperature and PFD were comparatively high, calculated turgor played a more prominent part and leaf extension rates followed the pattern of turgor pressure in the leaves of plants from both treatments. The low rates of extension measured at 20.00, when turgor pressures were relatively high may be the result of low PFD and low temperature reducing extensibility. The virtual cessation of growth during the night is attributable both to the darkness and to low temperatures reducing cell wall extensibility.

4.4.9 Assessment of the techniques used in this study

Turgor pressure

Bulk leaf turgor pressure was calculated from measurements of leaf water potential by the pressure chamber and osmotic potential on extracted sap according to equation 4.3. Both these measurements were average values for the leaf tissue sampled. It has been suggested that the rate of expansion of the epidermis controls leaf extension. The heterogeneity of cells within leaf tissue and the difference in numbers of cells of other tissues (§6.3.2) may mean that the bulk values obtained in this study do not accurately reflect those of the epidermis. No correction was made in the estimation of osmotic potential for apoplastic water content and the values of P obtained therefore, may be overestimates as a result of dilution of sap by apoplastic water. Time of sampling may also complicate the interpretation of results in this study. Values of water potential (and therefore turgor) are usually high in plants measured at dawn, and subsequently drop. In this study water potential may have already begun to drop by the time the first measurements were made: measurements made of dawn might have shown a better correlation to the early morning leaf extension rates.

Instron technique for estimating plasticity of cell walls

One problem of measurements made using the Instron technique is that the tissue is stored in methanol, which eliminates enzyme activity, and is then rehydrated prior to measurement. The rehydration process may lead to the formation of new covalent interactions within the cell wall thus changing some of the wall properties (Brett and Waldron, 1990)

Another potential problem is that the stress applied by the Instron technique is unidirectional whereas there is good evidence to suggest that the force experienced by an expanding cell wall is multi-directional (multi-axial) stress. The cellular heterogeneity of higher plant tissue also complicates interpretation of results as it is generally accepted that cell expansion of the epidermal layer controls leaf expansion (Cosgrove, 1993).

Some of the problems associated with the calculation of turgor and the use of the Instron technique could be removed by the use of a micro pressure probe, or turgor probe (Husken, Steudle and Zimmerman, 1978). Turgor probes enable direct measurements of individual cells to be made (Cosgrove *et al.*, 1984; Cosgrove, 1987) and, by simply comparing extension rate and turgor pressure, changes in wall rheology can be followed (Pritchard, *et al.*, 1991). The turgor probe technique has proved to be difficult with the small cells of silver birch (J. Pritchard and D.Tomas, pers. comm.).

Yield turgor

The pressure-chamber technique (based on the technique of Cosgrove, 1987 and modified by Sands *et al.* 1992) used for the evaluation of yield turgor in this study (§4.2.6) was evaluated for *Salix* by Sands, *et al.* (1992). A comparison of this method with the osmotic-solutions method (Van Volkenburgh and Cleland, 1981) and the psychrometer technique (Cosgrove *et al.*, 1984) showed good agreement between results and resulted in the recommendation of the pressure-chamber approach when a large number of simultaneous measurements of yield turgor are to be made. An equivalent evaluation for silver birch may be necessary to validate the use of this technique in the future.

When doing an experiment of this nature in the field it is inevitable that variations will occur in leaf temperature, photon quality and quantity reaching the leaves as a result of the position of plants within the chambers. Water and nutrient gradients within pots may further complicate results. This problem will have been compounded by the necessarily small sample size used in this study. It is suggested that in future similar

studies should be done with a larger sample size and in conjunction with a series of short-term experiments in controlled environments designed to investigate the interaction between elevated CO_2 and, for example, PFD, red:far red ratio, nutrient availability and water status, in determining leaf extension rate.

4.4.10 Conclusions

Increase in the atmospheric concentration of CO_2 results in increase in the area of individual leaves of silver birch seedlings, at least in the early part of the growing season. This increase in leaf area is a result of an increase in leaf extension rate during the early morning caused by an increase in turgor pressure. The role of PFD-activated acidification of cell walls in elevated CO_2 plants in contributing to leaf extension rates requires further investigation. There was no difference in the number of leaves produced from the main stem or in the total area of those leaves between plants grown in ambient or elevated CO_2 and harvested at the end of August 1991. This suggests that the increase in LER is not sustained and leaves produced later in the season were smaller in plants grown in elevated CO_2. The reduction in LER measured during the afternoon of 17th July provides some evidence for this. The increase in leaf area in plants grown in elevated CO_2 early in the season will have enabled them to grow faster and utilise resources faster than plants grown in ambient CO_2 but this advantage was not sustained and there was no significant difference in total leaf area of plants by the end of August 1991.

CHAPTER 5 THE EFFECTS OF ELEVATED CARBON DIOXIDE ON LEAF SURFACE CHARACTERISTICS OF SILVER BIRCH

5.1 INTRODUCTION

Chapter 4 described the effects of an increase in the atmospheric concentration of CO_2 on the extension of leaves of silver birch It has been established that in fully developed leaves photosynthetic activity and transpiration are closely linked to leaf structure (Nobel, 1985). Stomatal size and number, and the organisation of the internal structure of leaves play an important part in determining the rate of absorption of CO_2 at the sites of carboxylation, and the rate at which water is lost from leaves (Nobel, 1985). Chapters 5 and 6 of this thesis, describe the effects of an increase in the concentration of atmospheric CO_2 on leaf structure. In this chapter the effects of growth in elevated CO_2 on leaf surface properties are described.

Stomata show a short-term physiological response to an increase in ambient CO_2 concentration (§1.2.2), although some species may be relatively insensitive (Beadle *et al.*, 1979). In general, stomatal conductance has been found to be reduced in trees grown and measured in elevated CO_2, for example: *Pinus taeda, Liquidamber stryaciflua* (Fetcher *et al.*, 1988); *Ochroma lagopos, Pentaclethra macroloba* (Oberbauer et al, 1986); *Pinus radiata* (Hollinger, 1987); *Acer saccharinum* (Bunce, 1992). In a review Eamus and Jarvis (1989) indicated that the reduction in g_S in young trees was generally between 10 and 60%. Most of the determinations of the long-term effects of elevated CO_2 on stomata have relied on porometers. These measure leaf conductance but do not provide any information concerning changes in surface characteristics (Mansfield *et al.*, 1990). Evidence from herbarium specimens (Woodward and Bazzaz, 1988; Beerling and Chaloner 1992,1993a,b; Beerling *et al.*, 1993) and controlled environment studies (Woodward and Bazzaz, 1988) have shown that in some species stomatal density is sensitive to CO_2 concentration, and is less on leaves of plants grown in elevated CO_2 concentration.

The aim of this study was to determine the effect of elevated CO_2 on stomatal conductance and leaf surface characteristics of silver birch. Measurements of g_S were made using a porometer and stomatal density was estimated from replicates of the leaf surface. Measurements of the length, width and area of individual stomatal apertures were also made by direct observation using a scanning electron microscope and image

analyser to assess the relative contribution of the physiological response to elevated CO_2 and that of changes in leaf surface properties in determining leaf surface conductance.

5.2 MATERIALS AND METHODS

5.2.1 Establishment

Seeds of silver birch were germinated under ambient (unsupplemented) or elevated (ambient + 350 μmol mol $^{-1}$) CO_2 concentration. At the three-leaf stage the seedlings were transplanted into 1.5 dm^3 pots and placed into eight open top chambers with the same CO_2 regime. Twenty five plants were placed in each chamber. In March 1992 the silver birch seedlings were repotted into 5 dm^3 pots containing sand:peat:loam (2:5:3). Ten plants were placed in each chamber. All plants were placed in in chambers with the same CO_2 regime they had been growing in the previous year.

A full description of the plant material used in this experiment and the conditions under which the plants were germinated and grown is given in Chapter 2.

5.2.2 Porometer measurements

In 1991 stomatal conductance was measured using a null balance diffusion porometer (LI-1600, Li-Cor Instruments Inc., Lincoln, Nebraska, USA). The porometer head was clamped onto the leaf, and leaf temperature, air temperature, PFD and leaf surface conductance were recorded. The boundary layer conductance (g_a) in the cuvette is minimised by the fan and the value of surface conductance (g_{sur}) calculated by the porometer has been corrected for g_a using the manufacturers estimate of 2.7 mol m^{-2} s^{-1} to give g_s. Measurements were made between 11.00 and 13.00 BST on the abaxial surface of 40 fully expanded leaves per CO_2 treatment (ten leaves from each of four chambers per CO_2 treatment) on four occasions between July and September in 1991. In 1992 a transient porometer (AP4, Delta-T devices Ltd, Burwell, Cambridge) was used. Measurements were made between 11.00 and 13.00 on the abaxial surface of 40 fully expanded leaves per CO_2 treatment (ten leaves from each of four chambers per CO_2 treatment) on four occasions between April and June 1992.

5.2.3 Leaf surface impressions

Impressions of the abaxial surface of leaves were made non-destructively using "Xantropren" (Bajer, Germany) a silicon rubber material used primarily for dental impressions. This material was used to make replicas of 30 leaves per CO_2 treatment (one leaf from each of ten leaves per chamber, three chambers per CO_2 treatment). "Xantopren" is produced by mixing a silicon rubber base with an activator. The two compounds were mixed and a layer of "Xantopren" approximately 1-2 mm thick was spread over the leaf surface. The compound sets in approximately three minutes, depending on the amount of activator used. Once dry the replicas were peeled off the leaves and stored in labelled vials. Positive impressions were made by painting clear nail varnish onto a microscope slide and placing the Xantopren impression face down on the slide. Another slide was placed on top to add weight to ensure the replica remained flat against the nail varnish. Replicas were left for a minimum of one hour to dry in a desiccator but best results were obtained when they were left overnight. On removal of the imprint a positive impression was left in the nail varnish. Positive impressions were examined at 250x magnification under a light microscope (Ortholux, Leitz Ltd., Luton, Beds) with a camera attachment. Six fields per slide were selected at random and photographed. Numbers of stomata and epidermal cells were counted by viewing the negatives under a photographic enlarger. Calculations of stomatal frequency (number of stomata per mm^2), epidermal cell frequency (number of epidermal cells per mm^2) and stomatal index (1.1) were made.

Stomatal index enables the effects on stomatal density to be seperated into changes in the number of stomata initiated and changes in epidermal cell extension.

5.2.4 Determination of stomatal aperture

Direct measurements of stomatal apertures were made using the technique described by Van Gardingen, Jeffree and Grace (1989.)

Measurements of stomatal conductance were made on four leaves per CO_2 treatment (two from each of two chambers per CO_2 treatment) using a null balance diffusion porometer (Li-1600, LiCor inc. Lincoln Nebraska USA) §5.2.1.

As soon after conductance was measured as possible (and within 17 seconds) the portion of leaf under investigation was excised and mounted abaxial side up, on a cryo-specimen stub using a cryo-adhesive (Tissue tek OCT compound, Lab-tek products,

Illinois USA). One portion of leaf (or specimen) was taken from each of the leaves on which a measurement of g_S was made. (Prior to this experiment a sample of leaves was excised and the response of g_S measured using the porometer for one minute after excision. There was no change in g_S forty seconds after excision). The stubs were then placed in a dewar containing liquid nitrogen where they were stored until they were examined under a scanning electron microscope (S250 MK1, Cambridge Instruments, Cambridge, U.K.) and photographed using 120 and 35 mm film. Low magnification images were recorded to be used to measure stomatal density. High magnification images of approximately six stomatal apertures per frame were recorded to be used for the direct measurement of aperture dimensions using an image analysis system (Quantimet 970, Cambridge Instruments, Cambridge, UK.) The area, width, length and perimeter of each stoma were measured.

Values of stomatal conductance, g_S , were calculated for each specimen using equations 5.2) and 5.3) (Van Gardingen, Jeffree and Grace, 1989):

$$g_s = \frac{nDP_a}{RT\sum_{i=1}^{k}\left[\frac{d}{\pi a_i b_i'} + \frac{\ln\left(4a_i / b_i'\right)}{\pi a_i}\right] / k} \qquad (5.2)$$

$$b' = \frac{A}{\pi a} \qquad (5.3)$$

where A is Pore area (m^2),
a is Major axis radius (m),
b is Minor axis radius (m),
b' is Minor axis radius calculated from equation 5.3) (m),
D isDiffusive coefficient of water vapour in air (m^2s^{-1}),
d is Depth of pathway for diffusive gas exchange (m),
g_S is Stomatal conductance (mol $m^{-2}s^{-1}$),
k is Number of stomatal apertures measured per specimen,
n is number of stomata per m^2, i.e. stomatal density (m^{-2}),
P_a is Atmospheric pressure (J m^{-3}),
R is Universal gas constant (8.314 J mol^{-1} K^{-1}),
T is Mean of leaf and air temperature (K).

Measurements of *d* were made from scanning electron micrographs taken of freeze fractured leaves of the same age, and sampled at the same time, as those in the experiment. Measurements of *d* ±1 μm were made from eight stomata per CO_2 treatment (two stomata from two leaves per chamber, two chambers per CO_2 treatment).

5.3 RESULTS

5.3.1 Stomatal conductance

Tables 5.1 and 5.2, show the mean value of stomatal conductance for plants measured on four clear days during July 1991, and between the end of May and beginning of June 1992, respectively. On each occasion data were collected from leaves of the same age from ten plants from each of four chambers per treatment, all measurements were made between 11.00 and 13.00 hours. The same plants were measured in 1991 and 1992. Plants were measured at the same concentration of atmospheric CO_2 that they were grown in. Analysis of variance showed that stomatal conductance varied between the days of measurement ($P<0.01$) but the values of stomatal conductance were consistently lower in plants grown and measured in elevated CO_2, than in ambient CO_2 ($P<0.05$), although there was also a significant CO_2 and inter-chamber interaction, suggesting that the magnitude of the effect of elevated CO_2 varied between chambers. The reduction in stomatal conductance in elevated CO_2 compared to ambient CO_2 was approximately 15%, although the measurements were made with different porometers in the two years.

Table 5.1 Stomatal conductance of abaxial leaves of silver birch grown and measured in two concentrations of atmospheric CO_2 measured on four occasions during July 1991. On each occasion measurements were made on forty plants per treatment (ten from each of four chambers per CO_2 treatment). Leaf temperature = 21 ±2°C, RH = 50 ±8%. Values represent the mean (± one standard error) of one leaf from 160 plants. Results from a two-way ANOVA are also presented. * significant at P=0.05, ** significant at P=0.01.

	Ambient	Elevated	Treatment effect P<	Inter-chamber effect P<	Interaction P<
g_s (mmol m^{-2} s^{-1})	337.03 ±9.71	285.97 ±7.63	0.001**	0.848	0.435

Table 5.2 Stomatal conductance of abaxial leaves of silver birch grown and measured in two concentrations of atmospheric CO_2 measured on four occasions during May/June 1992 On each occasion measurements were made on forty plants per treatment (ten from each of four chambers per CO_2 treatment).. Leaf temperature = 22 ± 2.5 °C, RH = 40%. Values represent the mean (± one standard error) of one leaf from 160 plants. Results from a two-way ANOVA are also presented. * significant at P=0.05, ** significant at P=0.01.

	Ambient	Elevated	Treatment effect P<	Chamber effect P<	Interaction P<
g_S (mmol m^{-2} s^{-1})	255.34 ±11.76	216.44 ±10.69	0.013*	0.080	0.003**

5.3.2 Leaf surface impressions

Tables 5.3 and 5.4 show the effects of growth in two concentrations of atmospheric CO_2 on the frequency of stomata on the abaxial surface of leaves from plants measured midway through their first and second season in elevated CO_2 conditions. Stomatal density (the number of stomata per mm^2 of leaf surface) was slightly reduced in 1991 in plants grown in elevated CO_2, and the reduction in stomatal density was significant ($P<0.05$) in leaves grown and measured in elevated CO_2 in 1992. Epidermal cell density was slightly reduced in elevated CO_2 plants measured in 1991 and significantly reduced in elevated CO_2 plants measured in 1992. There was no significant effect of growth in elevated CO_2 on stomatal index. In 1991 there was a significant variation in epidermal cell density in leaves produced in different chambers and this resulted in variation in stomatal index. Results of stomatal index and epidermal cell density indicate that the reduction in the number of stomata per unit area is a result of an increase in the expansion of epidermal cells and not an increase in the initiation of stomata. Conditions during growth, for example PFD, nitrogen availability, temperature and water status are known to affect leaf development (Terry *et al.*, 1983) and changes in one or more of these environmental variables may be responsible for the variable response amongst chambers.

Table 5.3 Stomatal density and index, and epidermal cell density of the abaxial leaves of silver birch grown in two concentrations of atmospheric CO_2; measured in July 1991 Values represent the mean (± one standard error) of one leaf from 40 plants (ten from each of four chambers per CO_2 treatment.) Results from a two-way ANOVA are also presented. * significant at P=0.05, ** significant at P=0.01.

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Inter-chamber effect P<	Interaction P<
Stomatal index	11.16 ±0.24	11.43 ±0.21	0.401	0.042*	0.159
Stomatal density mm^{-2}	115.73 ±4.05	109.60 ±2.68	0.112	0.743	0.385
Epidermal cell density mm^{-2}	914.90 ±21.90	861.73 ±17.9	0.062	0.008**	0.553

Table 5.4 Stomatal density and index, and epidermal cell density of the abaxial leaves of silver birch grown in two concentrations of atmospheric CO_2, measured in June 1992 Values represent the mean (± one standard error) of one leaf from 40 plants (ten from each of four chambers per CO_2 treatment.) Results from a two-way ANOVA are also presented. * significant at P=0.05, ** significant at P=0.01.

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
Stomatal index	9.89 ±0.18	9.85 ±0.26	0.891	0.472	0.520
Stomatal density mm^{-2}	212.50 ±4.76	197.27 ±4.40	0.026*	0.679	0.495
Epidermal cell density mm^{-2}	1932.05 ±18.88	1824.77 ±36.84	0.015*	0.986	0.789

5.3.3 Stomatal aperture measurements

Figure 5.1 shows a scanning electron micrograph of the abaxial surface of a leaf of silver birch. There was no evidence of damage to the leaf surface caused by either the porometer or the procedure used to excise, store and prepare the specimens. A specimen is defined as the portion of leaf excised after porometer readings were made, attached to a cryo-stub and viewed using the SEM. Four specimens were taken per CO_2 treatment.

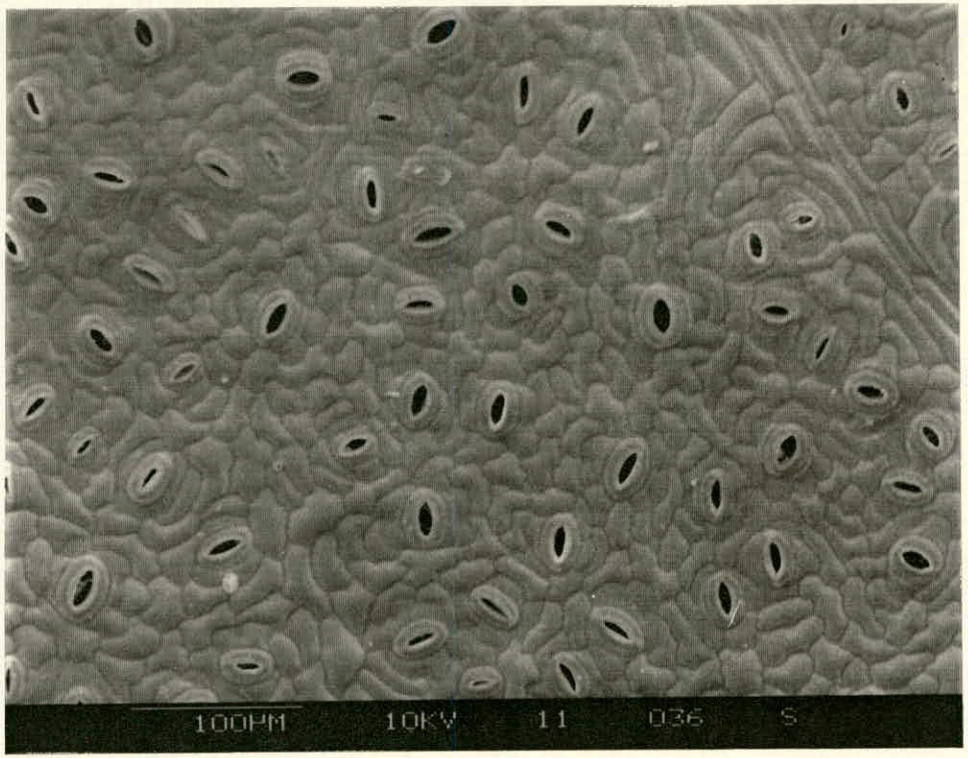


Figure 5.1 Scanning electron micrograph showing the abaxial surface of a leaf of silver birch.

The mean dimensions of stomatal apertures, obtained using image analysis are summarised in Table 5.5, along with the stomatal density and leaf surface conductance for each specimen. Stomatal density varied between specimens, but the variation was larger in leaves from ambient CO_2 grown plants. There was no significant difference in stomatal density with CO_2 treatment. Both mean aperture area and width tended to increase as g_S increased. The mean area and width measurements obtained for the Ambient 2 specimen were larger than those of Ambient 3 despite the lower value of surface conductance; this is attributable to the lower stomatal density of the second specimen. The mean area of apertures from leaves of elevated CO_2 plants was 49% smaller compared to those of ambient CO_2 plants and width of apertures from elevated CO_2 leaves was 43% smaller compared to those of leaves from ambient CO_2 plants. The mean length of apertures was 17% smaller in plants grown in elevated CO_2. The length to width ratio of apertures was significantly higher ($P< 0.001$) in elevated CO_2 plants compared with ambient CO_2 plants.

Table 5.5 Mean stomatal aperture dimensions (± one standard error), leaf surface conductances and stomatal density of leaves of silver birch grown in two concentrations of atmospheric CO_2. Measurements were made on four leaves from each treatment. The number of stomatal apertures measured on each sample is also given. Percentage change ([(ambient-elevated)/ambient]x100) is shown.

Treatment	No.	Area (μm^2)	Length (μm)	Width (μm)	Stomatal density (mm^{-2})	g_s (porometer) (mmol $m^{-2}s^{-1}$)	Number of apertures measured
Ambient	1	23.6 ±1.08	13.63 ±0.35	2.65 ±0.06	____	185	133
Ambient	2	112.95 ±3.47	20.96± 0.31	8.67 ±0.43	65.5 ±1.26	449	171
Ambient	3	62.56 ±1.82	13.3 ±0.17	6.9 ±0.12	179.65 ±6.91	514	148
Ambient	4	114.6 ±2.84	19.17 ±0.20	9.12 ±0.14	151.05 ±3.32	602	221
Ambient mean		78.43± 21.91	16.77± 1.94	6.84±1 .47	131.90± 34.45	437.50 ±89.82	
Elevated	1	26.83 ±2.21	11.26 ±0.50	3.1 ±0.14	161.00 ±5.15	302	133
Elevated	2	20.97 ±1.2	11.03 ±0.54	2.75 ±0.09	126.25 ±5.88	358	70
Elevated	3	49.57 ±2.35	16.83 ±0.23	4.44 ±0.16	186.75 ±3.64	449	122
Elevated	4	61.28 ±2.18	16.76 ±0.26	5.28 ±0.13	154.25 ±0.85	478	163
Elevated mean		36.95 ±9.48	13.97 ±1.63	3.89 ±0.59	157.06 ±12.43	396.75 ±40.63	
% change		-49%	-17%	-43%	19%	-9%	

Figures 5.2 and 5.3 show the size-class distributions of apertures from leaf specimens from ambient and elevated CO_2 plants, respectively. The distribution of aperture dimensions across the size classes varied for each specimen. Each specimen had a wide range in all the parameters measured.

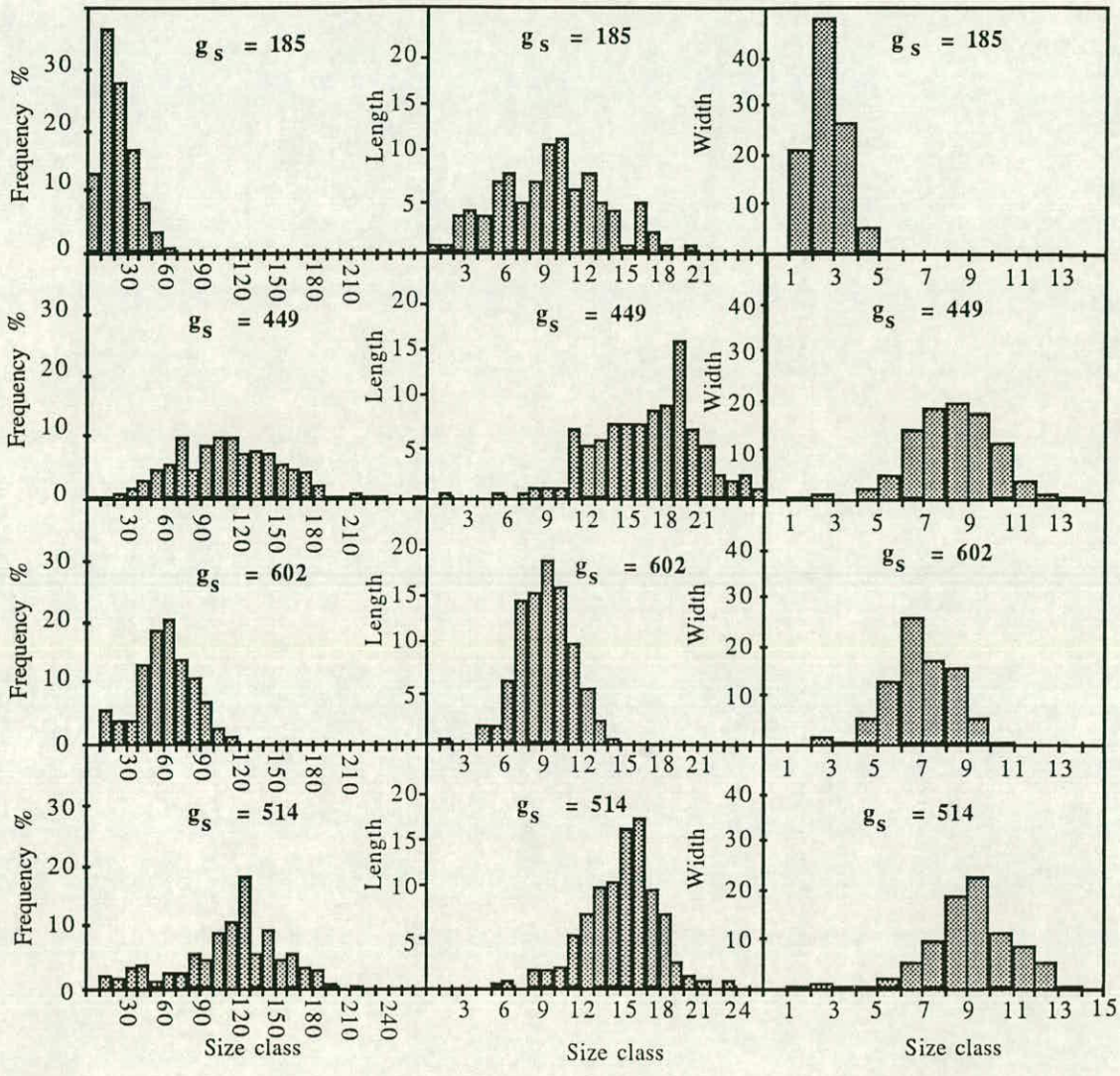


Figure 5.2 Size class frequency distributions for the dimensions of stomatal apertures of silver birch grown in ambient CO_2. The value of g_S of each leaf specimen measured by the porometer is given in units of mmol m^{-2} s^{-1}.

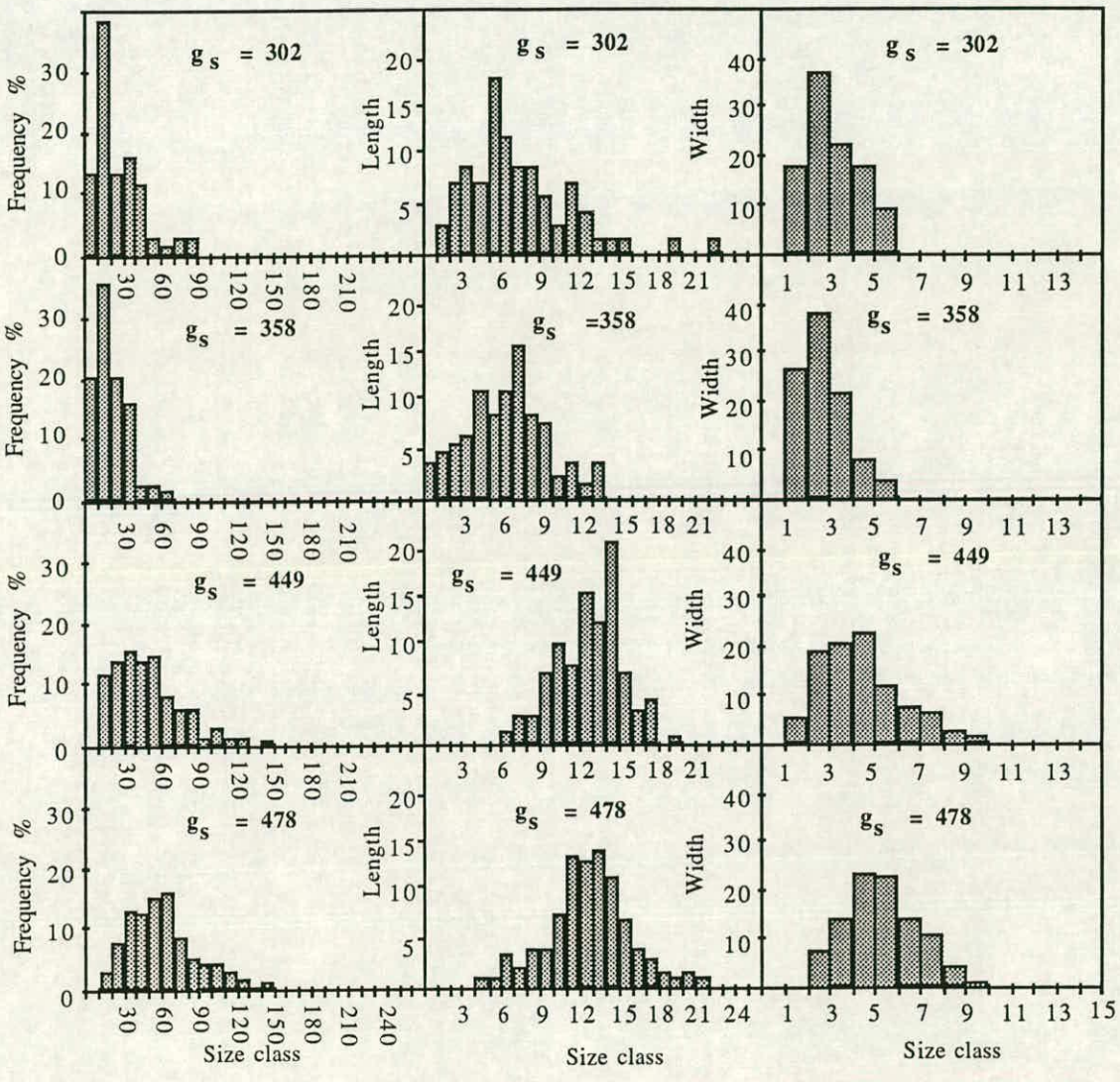


Figure 5.3 Relative size class frequency distributions for the dimensions of stomatal apertures of silver birch grown in elevated CO_2 (350+ 350 µmol m^2s^{-1}). The value of g_S for each leaf sample is given in units of mmol m^{-2} s^{-1}.

Figure 5.4 shows the comparison between the value of g_S obtained using a porometer and the value of g_S calculated from measurements of stomatal apertures, using equations (5.1) and (5.2). Specimen (1) from ambient CO_2 was not included in these calculations as no value of stomatal density was available. Linear regression of g_S (calculated) against g_S (porometer) gave a slope of 1.688 and an intercept of -399.63. The coefficient of determination, R^2, was 0.891 which was shown by analysis of variance to be significant ($p< 0.001$). d was 10 μm for ambient leaves and 11 μm for elevated leaves.

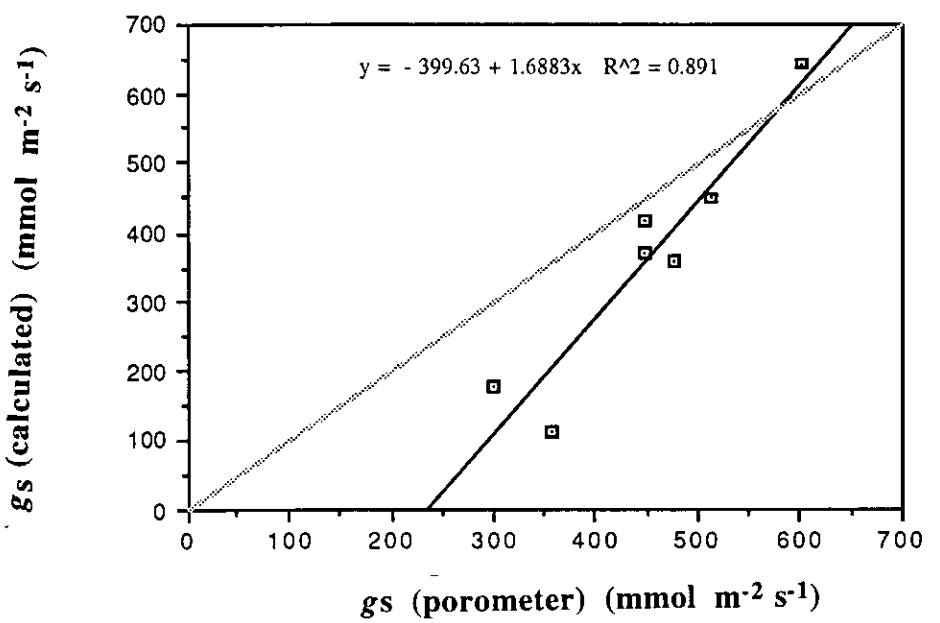


Figure 5.4 Values of g_S calculated using equations (5.1) and (5.2).against measurements of g_S obtained using a porometer. The solid line represents a regression line of g_S against g_S and has a slope of 1.688 and an intercept of -399.63. R^2 for the regression is 0.891. The dotted line represents a 1:1 relationship.

5.4 DISCUSSION

5.4.1 Reduction of g_S in increased CO_2.

This study has demonstrated that an increase in the concentration of atmospheric CO_2 during growth resulted in a decrease in stomatal conductance in silver birch. Lower stomatal conductance in plants grown in elevated atmospheric CO_2 compared to those

in ambient CO_2 has been reported for many tree species investigated including *Pinus ponderosa* (Surano *et al.*, 1986) and *Acer saccharum* (Bunce, 1992), but no means all.

The reduction in stomatal conductance of approximately 15% reported in this study is in broad agreement with the general estimate of 10 to 60% reviewed by Eamus and Jarvis (1989). Variation in the stomatal response to elevated CO_2 concentration occurs according to species, age and conditions during growth and measurement (Morison, 1985). Although few data are available to describe the effect of elevated CO_2 on the sensitivity of stomatal response to environmental variables, it has been suggested that variations in stomatal response to C_i occur in response to changes in water relations, which influence abscisic acid concentrations in leaves, and PFD. The plants under investigation in this experiment were watered to field capacity twice-daily and this may contribute to the relatively small reduction in g_S observed in elevated CO_2 plants.

An increase in g_S after long-term growth in elevated CO_2 has been reported for some other species (Brenner, Sandford and Jarvis, unpublished). Pettersson and McDonald (1992) reported an increase in g_S in silver birch plants grown in elevated CO_2: after 40 days g_S of elevated CO_2 leaves was higher than g_S of ambient CO_2 leaves. There was no evidence of down regulation in the response of stomatal conductance to C_i concentration in the plants in this study, measured in July of 1991 (Evans *et al.*, 1993) and there was no significant difference in g_S between plants measured after four months and those measured after fifteen months in elevated CO_2.

Since stomatal index was unchanged by treatment, the reduction in stomatal density observed in response to elevated CO_2 treatment was a result of an increase in epidermal cell expansion and not an increase in the number of stomata initiated. This is confirmed by the reductions in epidermal cell density. Thus reduction in stomatal density contributed to the lower stomatal conductance measured by the porometer in plants grown in elevated CO_2. Similar reductions in stomatal density have been reported for *Pentaclethra macroloba* (Oberbauer *et al.*, 1985) although no decrease in stomatal density was found in *Populus* clones (Radoglou and Jarvis, 1990b) or *Ochroma lagopus* (Oberbauer *et al.*, 1985). In elevated CO_2 individual stomata showed small reductions in width of the apertures and length of the stomatal complex and a slight increase in depth of the sub-stomatal cavity compared to plants grown in ambient CO_2, all of which also contribute to the reduction in stomatal conductance.

The correlation between the values of g_S obtained using the porometer and g_S calculated from direct observations of stomatal apertures using equation (5.2), was high (R^2=0.891), although the slope and intercept differed significantly from those found in a similar experiment on *Avena fatua* L. (R^2 = 0.96, intercept = -8±56, slope =

1.0±0.1, Van Gardingen *et al.*, 1989). In this study, calculated values of g_S were lower than those measured by the porometer at low conductances. The porometer was calibrated immediately prior to this experiment for measurement of humidity and temperature. There are however, several possible (and not mutually exclusive) sources of error in the calculated values of g_S:

- the mathematical model describing the shape of the substomatal cavity assumes a cylindrical shape, which may be an over-simplification of the true shape;
- there was evidence of a large degree of heterogeneity in the length and width of guard cell complexes in silver birch; this may indicate that such heterogeneity also exists in the depth of the substomatal cavity, and such heterogeneity cannot be discounted by measurements of d made in this experiment because of the small sample size; and
- large variations in stomatal density may exist across the leaf and measurements of stomatal density made from scanning electron micrographs may not be representative of the area of leaf measured by the porometer, as the area of specimen used to measure stomatal density amounted to less than 2% of the area of leaf used to measure g_S.

Oversimplification in the model describing the substomatal complex may contribute to the discrepancy in the correlation in Figure 5.4. Figure 5.5 shows the results of a sensitivity test to determine the effects of aperture width and depth of diffusive pathway on the calculated value of g_S. Calculated values of g_S would have been overestimated if d had been taken to be smaller and underestimated if d had been taken to be larger than the actual value for each specimen. Calculated values of g_S were very sensitive to changes in b'.

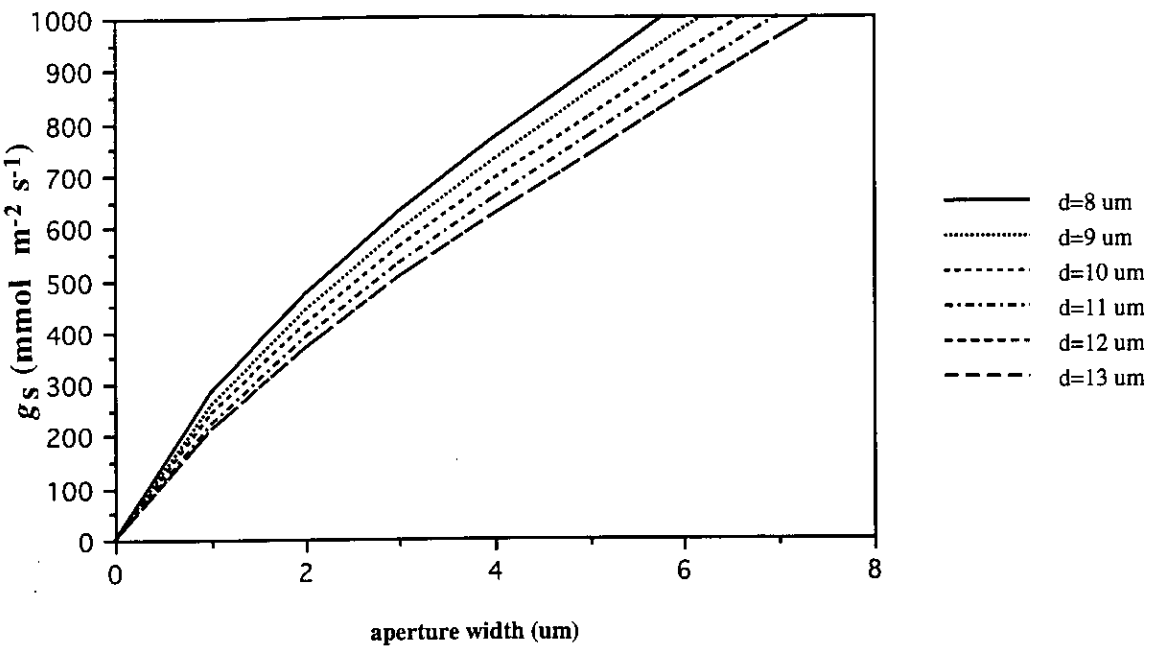


Figure 5.5 The effects of aperture width on the value of g_S calculated using equations (5.1) and (5.2) for a diffusive pathway depth (d) of 9-13 μm. Pore length was taken to be 15 μm and stomatal density 100 mm^{-2}.

The most likely explanation for the discrepencies between g_S calculated using equations 5.2 and 5.3, and g_S measured using the porometer is that the values of stomatal density are not representative of the larger area measured by the porometer. The presence of veins in the leaf sample can have a large effect on estimation of stomatal density and g_S. As only a small proportion of the area of leaf measured by the porometer was used to estimate stomatal density, the presence of veins may have led to stomatal density being overestimated. This will have been much less of a problem in the more homogenous tissue of *Avena fatua* used in the study of Van Gardingen, Jeffree and Grace (1989).

Figure 5.6 shows the results of a sensitivity test to determine the effects of aperture area and stomatal conductance on the calculated value of g_S. Calculated values of g_S would have been overestimated if n had been taken to be larger and underestimated if n had been taken to be smaller than the actual value for each specimen. g_S is sensitive to changes in aperture area.

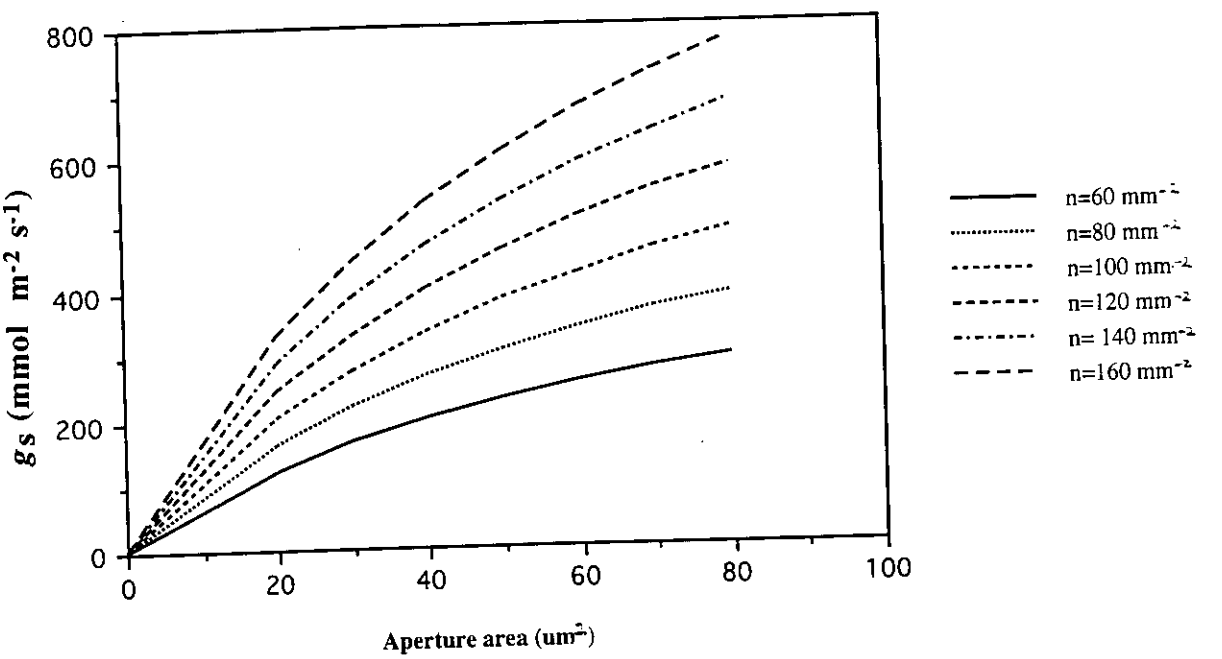


Figure 5.6 The effects of aperture area on the value of g_S calculated using equations (5.1) and (5.2) for a stomatal density (n) of 60-160 mm-2).

The discrepency in the correlation in Figure 5.4 serves to emphasis the potential problems of using this direct observation technique. In future studies of this nature, preliminary studies should be undertaken to assess the heterogeneity of the material and both the sample size and the area of each sample investigated increased.

Systems available to allow computer-enhancement of digitised video images and mouse-driven screen cursor systems can facilitate the recording of apertures and make an increase in the number of samples examined more feasible (Weyers and Meider, 1990).

The use of other techniques to measure stomatal apertures, for example, the use of epidermal peels or impressions of leaf surfaces was rejected. Leaves of silver birch do not readily yield epidermal strips and distortion of stomatal pore-dimensions that is likely to result from the preparation of such peels would introduce a different kind of inaccuracy in this study. The use of imprints or impressions made using Xantopren or similar material, has been found to be inaccurate at low conductances (Glinka and Meider, 1968; Weyers and Johansen, 1985) and often introduces involuntary errors because the imprint material forms the best replicas of the most open pores and this often leads to over-estimation of the degree of stomatal opening (Weyers and Meidner, 1990).

5.4.2 Implications of a reduction in g_S

A reduction in stomatal conductance will result in nearly proportional reduced rates of transpiration for tall trees, such as silver birch, that are well-coupled to the atmosphere (McNaughton and Jarvis, 1983,1991). Transpiration plays a central role in determining:

- plant water status;
- leaf energy balance; and
- together with the rate of photosynthesis, water use efficiency.

Water use efficiency

A reduction in stomatal conductance leads to a reduction in both photosynthesis and transpiration on a leaf area basis. As a result of the differential effect of stomatal conductance on the rates of photosynthesis and transpiration (Farqhuar and Sharkey, 1982), transpiration is reduced more than photosynthesis, resulting in an increase in *instantaneous* water use efficiency (WUE), even in the absence of an increase in photosynthesis. In this study, both increase in the rate of photosynthesis (§3.5.4) and reduction in g_S (§5.3.1) have been found to occur in leaves of elevated CO_2 plants, compared to ambient CO_2 plants, and this results in an increase in *instantaneous* WUE. An increase in water use efficiency in plants grown in elevated CO_2 has been reported for most species investigated including : *Ficus obtusifolia, Psychotria limonensis, Tabebuia rosea, Acacia mangium* (Ziska *et al.*, 1991), *Pinus ponderosa* (Surano *et al.*, 1986) and *Acer saccharum* (Bunce, 1992), see Morison (1993) for review of crop species and Eamus and Jarvis (1989) for a review of tree species. Although water use may be reduced on a leaf area basis as a result of reduction in g_S, this may be offset to some extent, on a whole plant basis, by an increase in leaf area production (Eamus and Jarvis, 1989). In the silver birch in this study, there was a slight (approximately 15%) increase in the area of individual leaves produced early in the season in plants grown in elevated CO_2 (May-June), but this was not sustained and there was no difference in total leaf area between ambient CO_2 and elevated CO_2 plants by the end of the growing season (§3.5.4). None the less, the smaller leaves produced late in the season (§3.5.4) in elevated CO_2 may have had higher stomatal density and, therefore, g_S. WUE of whole plants intergrated over time is not readily established from measurements of *instantaneous* WUE of leaves with a porometer.

A change in water use efficiency of one species will affect the distribution of water resources between species and, under conditions of limited water availability, may affect competitive balance and the species composition of an area.

Water potential and turgor pressure

The reduction in g_S and, therefore, transpiration rate may result in an increase in leaf turgor as a result of a decrease in water potential. Turgor pressure is the 'driving force' for growth. Increased turgor has been reported in several species grown in elevated CO_2 (Townend, 1993). The adverse affects of droughting were ameliorated in plants of *Liquidambar stryaciflua* in response to elevated CO_2 treatment: whole plant water potential remained higher over the course of a drying cycle, water stress was delayed and the plants maintained their photosynthetic rate at a higher level for longer (Tolley and Strain 1985). The onset of severe stress in *Glycine max* was delayed by the maintenance of water potential associated with a reduction in transpirational losses as a result of lower g_S (Cure *et al.*, 1987). Effects of an increase in atmospheric CO_2 concentration on water potential, osmotic potential and leaf turgor of the plants used in this study in relation to leaf extension are described in Chapter 4: no consistent effect on leaf water potential or turgor potential was seen in plants measured over two diurnal periods in July 1991. The absence of any consistent effect of CO_2 on leaf water potential may have been a result of the water status of the plants at the time measurements were taken. It has been suggested that any increase in leaf water potentials may not become apparent at high soil moisture content (Morison, 1985, 1987; Davies and Mansfield, 1987).

Leaf energy balance

Reduction in transpiration from stomatal closure may result in less heat being dissipated via evaporation. The resulting increase in leaf temperature will in turn raise the vapour pressure inside the leaf increasing the vapour pressure difference between leaf and air (Monteith and Unsworth, 1990) and partially compensating for closure of the stomata. Such an increase in leaf surface vapour pressure deficit is likely to result in further closure of the stomata which would lead to further increase in leaf temperature. Increases in leaf temperature as a result of reductions in transpiration may have important effects on photosynthesis at low wind speed or at high temperatures. Although the temperatures experienced by the plants were probably not sufficiently high in this study to affect them adversely, this has been the case in other studies. For example, *Pinus ponderosa* trees grown in elevated CO_2 showed signs of stress including accelerated needle abscission and chlorosis , and this was attributed, at least

in part, to elevated foliar temperatures resulting from CO_2-induced stomatal closure lowering transpirational cooling of needles (Surano *et al.*, 1986). Similarly, leaf temperature of *Acer saccharum* also exceeded the optimum for photosynthesis at midday in plants grown in elevated CO_2, and again this was attributed to a reduction in transpirational cooling (Bunce, 1992).

5.4.3 Conclusions

- g_S was reduced in young silver birch seedlings grown and measured in elevated CO_2, but only by 15%.
- Stomatal index of leaves was unaffected by growth in elevated CO_2
- Stomatal density was lower as a result of increase in epidermal cell expansion in elevated CO_2, and this contributed to the reduction in g_S. However, the reduction in stomatal density may not be sustained, as leaves produced later in the growing season were smaller (Chapter 3) and this may have led to increase in stomatal density and g_S.
- Slight reduction in width of the stomatal apertures and in length of the guard cell complex, and small increase in depth of the substomatal chambers contribute to the reduction in g_S in plants grown in elevated CO_2.
- Direct observation of apertures using a scanning electron microscope failed to assess adequately the relative effects of a reduction in stomatal aperture (i.e. physiological response of the stomata) and changes in leaf surface characteristics on the stomatal conductance of leaves grown in elevated CO_2. This failure was a result of the heterogeneous distribution and dimensions of stomata in leaf surfaces of silver birch tissue and the necessarily small number and area of samples that could be processed using this technique.
- Reduction in g_S is likely to contribute to an increase in WUE in young silver birch seedlings grown in elevated CO_2, but changes in leaf area may have a more significant effect on whole plant water use.

CHAPTER 6 THE EFFECTS OF ELEVATED CARBON DIOXIDE ON LEAF CHARACTERISTICS OF SILVER BIRCH

6.1 INTRODUCTION

In this chapter and the previous one, the effects of an increase in atmospheric CO_2 on leaf structure are examined. The effects of growth in elevated CO_2 on the characteristics of the leaf surface were described in Chapter 5. The aim of the study described in this chapter was to determine the effect of growth in elevated CO_2 on leaf characteristics such as specific leaf area (SLA), leaf thickness, anatomical organisation, chlorophyll content, chlorophyll *a*:*b* ratio of young silver birch trees, germinated and grown in elevated CO_2.

Variation in the photosynthetic capacity of plants has been shown in some cases to be associated with variations in leaf characteristics (Björkman *et al.*, 1972; Lewandowska and Jarvis, 1977). Leaf characteristics such as SLA, leaf thickness, anatomical organisation, chlorophyll content, chlorophyll *a*:*b* ratio are known to be affected by the radiation environment (Björkman and Holmgren, 1963; Björkman, 1975; Lichtenthaler, 1985; Caesar, 1989) and nitrogen availability (Terry *et al.*, 1983; Trewavas, 1985) experienced by developing leaves. Similar changes have also been reported for plants grown in elevated CO_2 (§1.2.7). Such characteristics of leaves contribute to our understanding of the implications of long term growth in elevated CO_2 on photosynthetic capacity of silver birch plants.

6.2 MATERIAL AND METHODS

Seeds of silver birch (provenance FC 87/20) were germinated under ambient (unsupplemented) or elevated (ambient + 350 μmol mol^{-1}) CO_2 concentration. At the three-leaf stage the seedlings were transplanted into 1.5 dm^3 pots and placed in eight OTCs with the same CO_2 regime. Details of establishment and growth conditions are given in Chapter 2.

6.2.1 Leaf thickness

The thickness of leaves was measured in August 1991 and May 1992, using electronic calipers (RS, Loughborough, UK). Forty leaves per CO_2 treatment were measured (one leaf from each of ten plants per chamber, four chambers per CO_2 treatment). On each occasion all measurements were made on leaves of the same age and between the second and third lateral vein.

6.2.2 Leaf mass to leaf area ratio

In August 1991 and May 1992 leaf discs of known area were taken from leaves of the same age from each of 40 plants per CO_2 treatment (six discs from each of ten plants per chamber, four chambers per CO_2 treatment.) The fresh mass of each sample was measured using an electronic balance with 0.1 mg resolution (Sauter, RE1E14, Fisons Scientific Equipment, Loughborough). The discs were then placed in paper bags and transferred to the oven at 70 ^{o}C overnight. Measurements of dry mass were made the following day.

6.2.3 Cell count

In May 1992, leaf discs were taken from leaves of the same age from 24 plants per CO_2 treatment (three discs from each of six plants per chamber, four chambers per CO_2 treatment). A parallel sample of leaf discs was taken for the determination of fresh and dry mass. The leaf discs were placed in vials containing 25 cm^3 of chloroform:methanol 1:1 for 48 hours to remove cuticular wax. After 48 hours the chloroform:methanol solution was removed and the discs treated with 5 mol m^{-3} NaOH to effect alkaline hydrolysis of the cuticle. Discs were then rinsed thoroughly and macerated for 24 hours in 5 cm^3 of 5% w/v chromium trioxide solution. The tissue was taken up into a pipette and gently expelled; this was done 10 times and then the procedure was repeated using a 5 cm^3 syringe attached to a hypodermic needle . This process was repeated 20 times to give an even cell suspension. The suspension was diluted to a known volume with water to give a suspension which, when placed on the haemocytometer, gave approximately one cell per square. A sample of the suspension was placed on a haemocytometer slide (Hawksley Crystaite, Modified Fuchs Rosenthal) and cell counts made for six replicate grids (Chandler, 1989).

6.2.4 Chlorophyll determination

In July and September 1991 and April 1992, leaf discs were taken from leaves of the same age from each of twenty plants per treatment (a sample of three discs from each of five plants per chamber, four chambers per CO_2 treatment). The leaf discs were placed in glass vials containing 5 cm^3 dimethyl formaldehyde (DMF). The vials were kept in the dark and transferred as soon as possible into a cold room. The samples were left in the cold room for 48 hours (they are stable for 10 days) before their "absorbance" (*A*) was read on a spectrophotometer at 663.8 nm and 646.8 nm using DMF as a blank (Porra, Thompson and Kriedemann, 1989).

Chlorophyll concentrations in $\mu g/cm^3$ were calculated according to the following equations:

$$\text{chll } a = 12.00\ A^{(663.8)} - 3.11\ A^{(646.8)}$$

$$\text{chll } b = 20.78\ A^{(646.8)} - 4.88\ A^{(663.8)}$$

$$\text{chlls } a+b = 17.67\ A^{(646.8)} + 7.12\ A^{(663.8)}$$

6.2.5 Determination of reducing sugars, starch and structural dry matter content

At the time of harvesting (August 1991, §3.5.4) three leaves were collected from 20 trees per CO_2 treatment (three leaves from each of five trees per chamber, four chambers per treatment) at midday, placed in plastic vials and immersed immediately in liquid nitrogen. This leaf tissue was subsequently transferred directly from the liquid nitrogen to a freeze dryer (Edwards High Vacuum Ltd. Crawley, Sussex).

The freeze dried tissue was then ground and 20 mg removed from each sample. These sub-samples were combined according to chamber, resulting in four 100 mg samples per treatment. 2 cm^3 of double distilled water was added to each 100 mg sample and the solutions stored overnight at 60 °C. The solutions were then centrifuged at 5000 for 20 minutes, the supernatant was poured off and stored, the pellet washed with 2 cm^3 distilled water and re-centrifuged. This process was repeated four times and the total supernatants stored. Figure 6.1 summarises the procedure followed. The supernatant (*S*, Figure 6.1) was assayed to determine the amount of reducing and non-reducing sugars present in the sample. The pellet (*P*, Figure 6.1) was treated to remove

protein and analysed to determine starch content and amount of structural dry matter (adapted from Hodge and Hofreiter, 1962).

Reducing sugars

2 cm^3 of DNS reagent (Appendix) were added to 3 cm^3 of supernatant (*S*) in a boiling tube. The solution was boiled for ten minutes in a water bath. After boiling, the "absorbance" coefficient of the solution was read at 540 nm using a spectrophotometer and compared against a standard curve obtained using glucose, with DNS reagent as a blank (Hodge and Hofreiter, 1962).

Total carbohydrate

1 cm^3 of Anthrone reagent (0.2% Anthrone in conc. H_2S0_4) was added to 0.5 cm^3 of supernatant and the solution boiled in a water bath for five minutes. After boiling, the absorbance coefficient of the solution was read at 620 nm using a spectrophotometer and compared against a standard curve obtained using glucose, with Anthrone reagent as a blank (Hodge and Hofreiter, 1962).

Non-reducing sugars (sucrose)

Sucrose content was calculated as the difference between total carbohydrate and reducing sugars.

Protein removal

The pellet (*P*) was treated to remove soluble protein, to enable the subsequent determination of structural dry matter content. 2 cm^3 protease in Tris buffer, pH 7.4 (Sigma, UK) was added to the pellet, which was then incubated at 30 °C for 24 hours. Following incubation the solution was centrifuged at 5000 for 20 minutes, the supernatant was poured off, the pellet washed with 2 cm^3 distilled water and the process repeated two more times. Soluble protein was removed in the supernatant (Peterson, 1977).

Starch

The remaining pellet was treated with α amylase (Termamyl, NovoNordisk, UK) and amyloglucosidase to break down starch to monosaccharides, which could then be assayed using Anthrone: 2 cm^3 of distilled water was added to the pellet followed by

60 nm^3 of α amylase. The solution was incubated at 90 °C for one hour, cooled and treated with 1 cm^3 amyloglucosidase for one hour at 55 °C. The resulting supernatant was assayed with Anthrone as above (Hodge and Hofreiter, 1962).

Structural dry matter

The residual pellet was dried and weighed to determine the mass of structural dry matter.

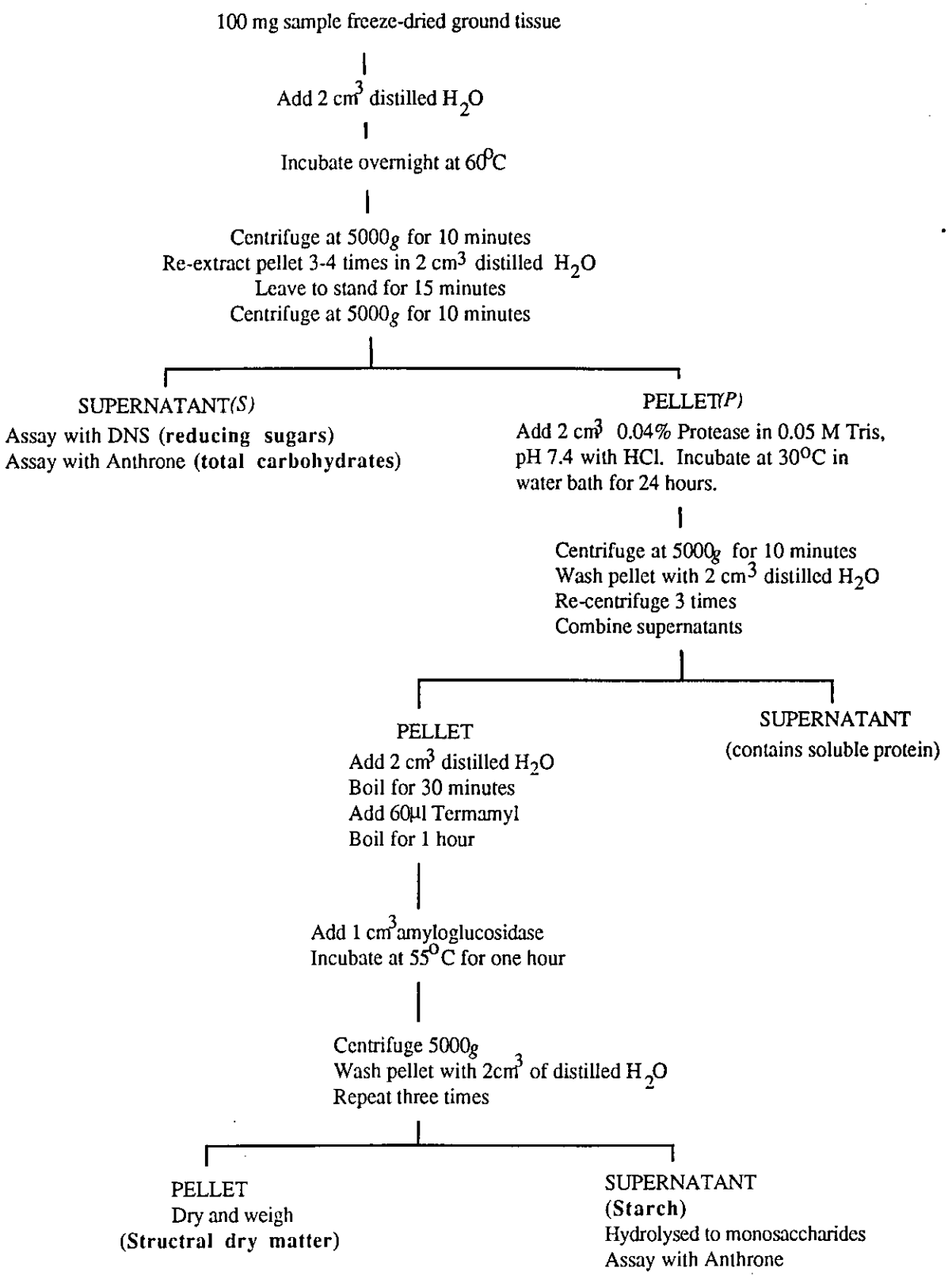


Figure 6.1 Procedure followed in the determination of reducing sugars, starch and structural dry matter content.

6.2.6 Scanning electron microscopy

In June 1991, at 12.00 BST one leaf from each of four plants per CO_2 treatment was excised and 2 cm^2 strips taken from between the second and third main lateral veins of each leaf were attached to copper cryo-stubs using a thin layer of tissue cryo-adhesive (Tissue-tek, Agar Scientific Ltd. Essex, UK). Cryo-fixation was carried out using the Emscope SP2000 cryogenic preparation system (Emscope Laboratories Ltd., Ashford, Kent). The stubs were placed into pre-cooled liquid nitrogen (-210 °C) in an atmosphere of dry argon. The specimens were then transferred to the main chamber of the SP2000 where they were freeze-fractured and gold-coated. The stub and specimen was then transferred under vacuum into a scanning electron microscope (Steroscan 250, Cambridge, UK). Photomicrographs of the internal anatomical organisation of the leaves were made using a 120 format camera back.

6.2.7 Preparation of resin embedded sections of leaf tissue

Resin embedding

Two newly developed but fully expanded leaves from eight plants per treatment (two plants from each of four chambers per treatment) were excised from the trees in May 1992 at 12.00 BST and placed in a shallow petri-dish containing sufficient solution of Karnovskys:Hepes (K/H,) 1:1 (Appendix) to submerge the leaf completely. Strips approximately 1 mm in width were cut from the leaf between the second and third lateral vein. The strips were placed in specimen handling capsules (Agar Scientific Ltd. Essex, UK) submerged in the solution. When sufficient strips were placed in the capsule, the capsule was labelled. Once labelled, the capsules were placed in glass soda vials (Agar Scientific Ltd. Essex, UK) containing K/H solution. Air bubbles were removed by drawing the liquid through the capsule using a pipette. The vials were then labelled and placed in a rotator for four hours to ensure complete penetration of the leaf tissue by the solution. After four hours the solution was pipetted out of the vials. The tissue was rinsed with Hepes (Appendix) to remove all trace of Karnovskys. The Hepes solution was pipetted into the vial and drawn through the capsule, again ensuring that no air bubbles were present in the vial. The vials were left for twenty minutes, this procedure was repeated three times. The capsules were subsequently left in Hepes solution overnight and a further rinse of Hepes was given in the morning.

The Hepes was removed and approximately one fifth of a vial of osmium tetroxide was added to each vial, to 'fix' the tissue. The osmium tetroxide was left for two hours, the capsules were then given three twenty minute rinses with distilled water.

The leaf tissue was then dehydrated by adding increasingly concentrated solutions of ethanol. The ethanol was then gradually replaced by polypropylene oxide (PPO) and finally the PPO by Agar 100 resin (epon 812 substitute) (Table 6.1). The capsules were then opened and the individual strips of leaf material placed in the slots of mould trays (Agar Scientific Ltd. Essex, UK) and the slots labelled. Resin was added to fill the slots and the mould trays placed in an oven at 60 °C in a fume cupboard for twelve hours (Glauert, 1974).

Table 6.1 Fixation Procedure

Reagent	Time
1:1 Karnovskys : Hepes	4 hours
Hepes	20 minutes
Hepes	20 minutes
Hepes	20 minutes
1% Osmium tetroxide	2 hours
Distilled H_20	20 minutes
Distilled H_20	20 minutes
Distilled H_20	20 minutes
20% Ethanol	45 minutes
40% Ethanol	45 minutes
60% Ethanol	45 minutes
80% Ethanol	45 minutes
90% Ethanol	90 minutes
100% Ethanol	3 hours
100% Ethanol	Overnight
2:1 Ethanol : PPO	30 minutes
1:1 Ethanol : PPO	30 minutes
1:2 Ethanol : PPO	30 minutes
1:3 Ethanol : PPO	30 minutes
PPO	45 minutes
PPO	45 minutes
2:1 PPO: Agar resin	45 minutes
1:1 PPO: Agar resin	120 minutes
1:2 PPO: Agar resin	4 hours
1:3 PPO: Agar resin	Overnight
Agar resin	Overnight

Sectioning for light microscopy

100 μm thick transverse sections of the embedded leaf tissue were made using an ultramicrotone (Reichert Jung Ultracut, Leica, Cambridge). Glass knives used in the ultramicrotone were made using a knifemaker (LKB, Leica, Cambridge). Sections of leaf tissue were flooded with a solution of 1% (w/v) toluidine blue in 0.3 mol m^{-3} sodium tetraborate and placed on a hot-plate at 60 °C for two minutes. Following three washes with distilled water, the sections were dried and mounted in resin (Agar Scientific Ltd., Essex,) (Reid, 1974). Leaf tissue was stained for chloroplast starch

using a Schiffs reagent test kit (Sigma, Poole, Dorset). Sections were viewed under a light microscope (Ortholux, Lietz Ltd., Luton, Beds.) at 200x magnification and photographs taken using a 35 mm SLR camera.

6.3 RESULTS

6.3.1 Leaf thickness

Table 6.2 shows the effect of growth in elevated CO_2 on the thickness of fully-developed leaves. Measurements were made in August of 1991. The leaves from plants grown in elevated CO_2 were slightly ($P<0.05$) thicker than those from plants grown under ambient CO_2 conditions.

Table 6.2 Thickness of leaves of silver birch (mm) grown in ambient (non-supplemented) or elevated (ambient + 350 μmol mol^{-1}) CO_2. Measurements were made on leaf number 20 (i.e the 20th mainstem leaf to emerge). Values are means (± one standard error) of 40 leaves per treatment (10 leaves from each of four chambers per CO_2 treatment). The levels of significance for the differences between elevated and ambient CO_2-grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. * significant at P=0.05, ** significant at P=0.01.

Ambient CO_2	Elevated CO_2	Treatment effect P<	Inter-chamber effect P<	Interaction P<
0.29±0.09	0.30±0.08	0.07	0.68	0.109

Measurements of the thickness of leaves on the same plants the following year (after 16 months in elevated CO_2), are shown in Table 6.3. The leaves of trees grown in elevated CO_2 were significantly thicker than those of ambient-grown plants. The leaves produced in the second year were thinner in both CO_2 treatments compared with the leaves produced the year before.

Table 6.3 Thickness of leaves of silver birch (mm) grown in ambient (non-supplemented) or elevated (ambient + 350 μmol mol^{-1}) CO_2.Measurements were made on fully expanded leaves of the same age. Values are means (± one standard error) of 40 leaves per treatment (10 leaves from each of 4 chambers per CO_2 treatment). The levels of significance for the differences between elevated and ambient CO_2-grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. * significant at the 0.05 level, ** significant at the 0.001 level

Ambient CO_2	Elevated CO_2	Treatment effect P<	Inter-chamber effect P<	Interaction P<
0.20±0.01	0.22±0.01	0.010*	0.196	0.191

6.3.2 Anatomical organisation

Figures 6.2 and 6.3 show scanning electron micrographs of freeze fractures through leaves of silver birch grown under elevated and ambient concentrations of atmospheric CO_2, respectively, taken in August 1991. Leaves from plants grown in elevated CO_2 (Figure 6.2) showed much heterogeneity in the density of mesophyll cells with limited air spaces and also regions of very spongy mesophyll with large intercellular spaces. Of particular interest was a double layer of palisade cells in elevated CO_2 plants which was not evident in leaves from ambient CO_2-grown plants. The layer immediately below the palisade layer was intermediate between mesophyll and palisade layers in some of the leaves from ambient plants (Figure 6.3) and this was more pronounced in areas of tissue adjacent to vascular tissue. Leaf sections through ambient CO_2 leaves were slightly thinner than those through elevated CO_2 leaves but did show a similar degree of heterogeneity in the density of mesophyll cells and the distribution of intercellular air spaces.

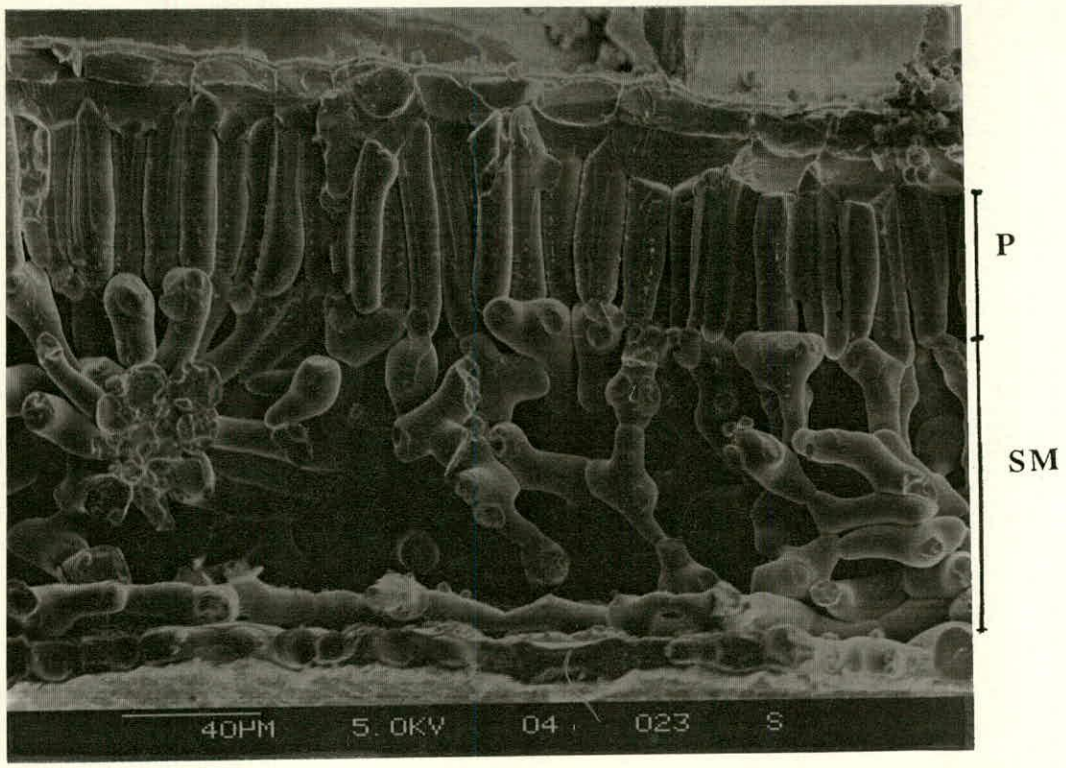


Figure 6.2 Scanning electron micrograph of freeze fracture through a leaf of silver birch grown in ambient CO_2 and excised in June 1991. Note that there is only one layer of palisade cells. The layer immediately below the palisade cell layer is intermediate between mesophyll and palisade layers. P-palisade, SM-spongy mesophyll.

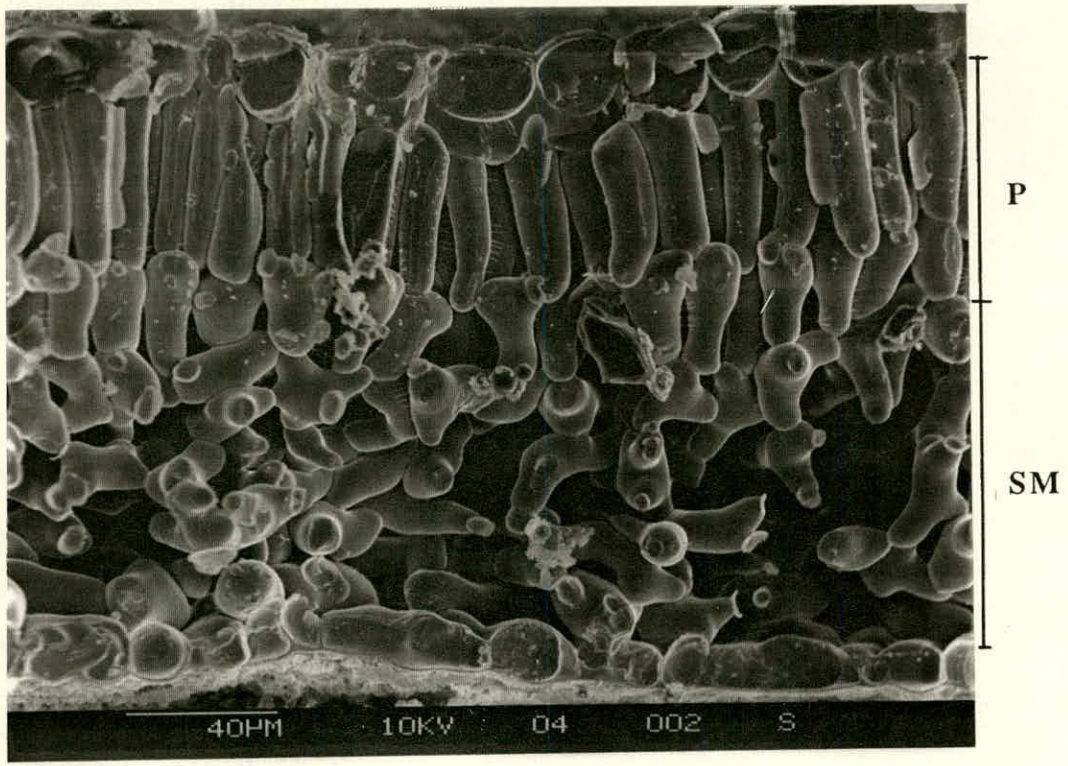


Figure 6.3 Scanning electron micrograph of freeze fracture through a leaf of birch grown in elevated CO_2.and excised in June 1991 Note the double palisade layer. P-palisade, SM-spongy mesophyll.

Transverse sections through ambient and elevated CO_2 leaves produced in 1992 are shown in Figures 6.4 (ambient CO_2) and 6.5 (elevated CO_2). Sections were stained with Schiffs reagent to show starch grains. Leaves from plants grown in elevated CO_2 had an extra layer of palisade cells. There were more, and larger starch grains in the chloroplasts of plants grown in elevated CO_2.

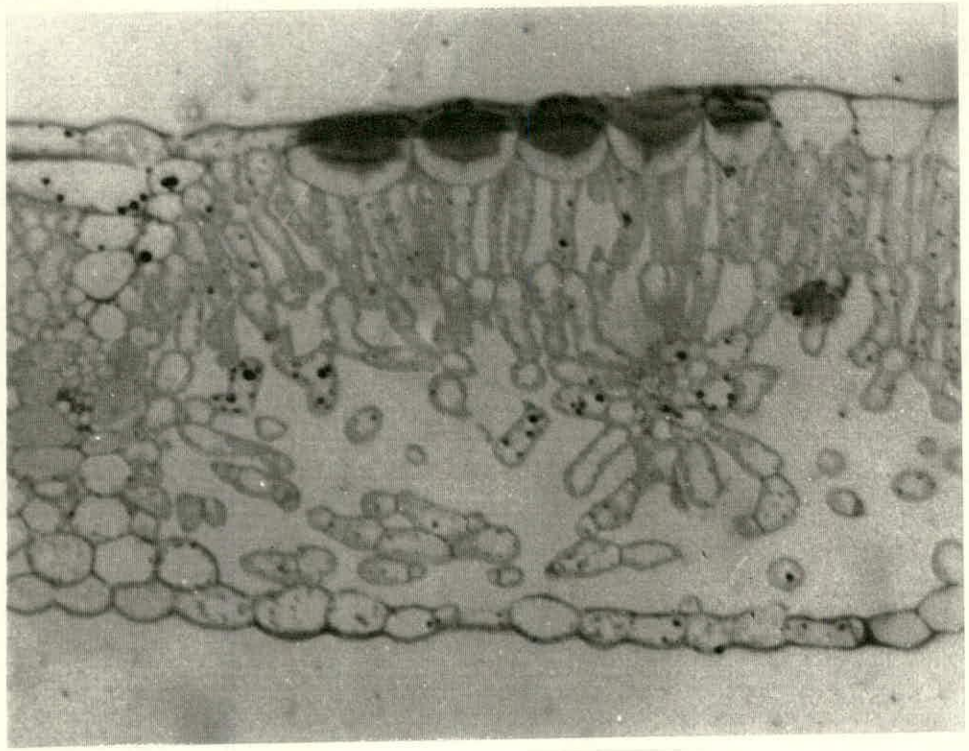


Figure 6.4 Transverse section through a leaf of silver birch grown in ambient CO_2 and excised in May 1992. P-palisade, SM-spongy mesophyll, ST-starch grains.

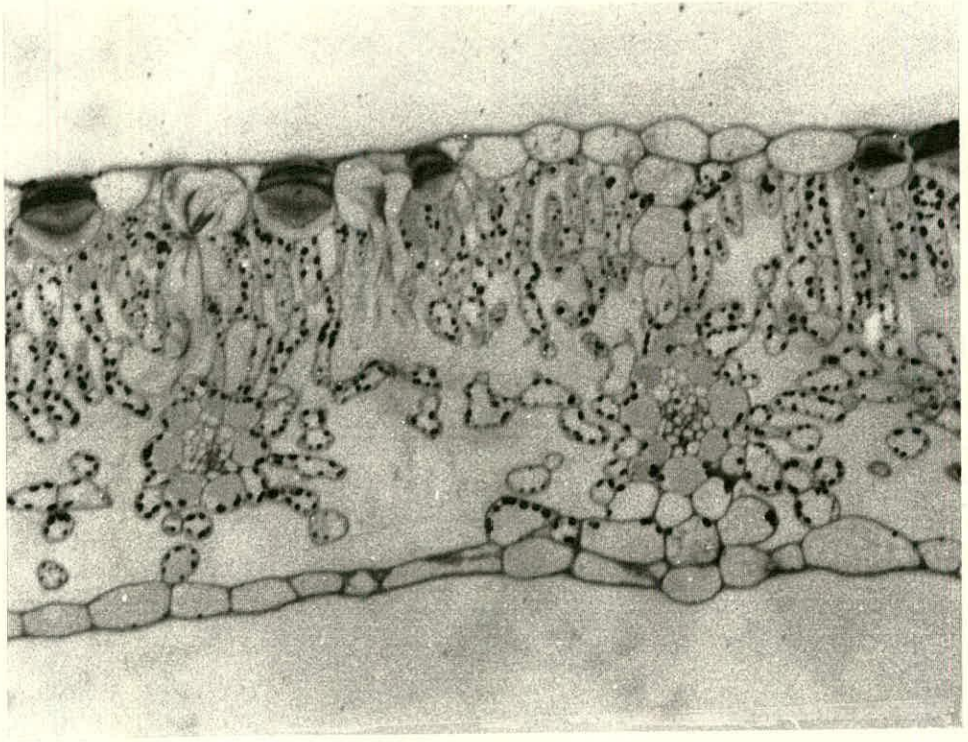


Figure 6.5 Transverse section through a leaf of silver birch grown in elevated CO_2.and excised in May 1992 Note the double palisade layer, P-palisade, SM-spongy mesophyll, ST-starch grains.

The increase in leaf thickness in elevated CO_2 plants was concomitant with an increase in the ratio of dry mass to area and also in the dry mass: fresh mass ratio in leaves produced in 1991 (Table 6.4) and in 1992 (Table 6.5).

Table 6.4 Ratios of dry mass to area (g cm^{-2}) and dry mass to fresh mass of leaves of silver birch grown in ambient and elevated CO_2 and measured in July 1991. Measurements were made on leaf number 20 (i.e the 20th mainstem leaf to emerge). Values are means ± one standard error of 40 leaves per CO_2 treatment (10 leaves from each of four chambers). Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P= 0.05, ** significant at P= 0.001.

1991	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
Dry mass/area	0.0051 ±0.0001	0.0062 ±0.00011	0.001**	0.818	0.412
Dry mass/fresh mass	0.386 ±0.007	0.430 ±0.008	0.001**	0.805	0.279

Table 6.5 Ratio of dry mass to area (g cm^{-2}) and dry mass to fresh mass ratio of leaves of silver birch grown in ambient and elevated CO_2 and measured in May 1992. Values are means ± one standard error of twenty leaves per CO_2 treatment (five leaves from each of four chambers per treatment) for each leaf number. Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P=0.05, ** significant at P= 0.001.

1992	Ambient CO_2	Elevated CO_2	Treatment effect P<	Inter-chamber effect P<	Interaction P<
Dry mass/area	0.0044 ± 0.0010	0.0051 ± 0.0011	0.001**	0.621	0.096
Dry mass/fresh mass	0.344 ± 0.005	0.350 ± 0.005	0.590	0.403	0.713

6.3.3 Carbohydrate content

Table 6.6 shows carbohydrate content per leaf surface area of leaves produced in 1991, and harvested in August of that year. Amounts of structural carbohydrate, starch and soluble carbohydrate content (sucrose, fructose etc.) were increased in leaves of plants grown in elevated CO_2. Structural carbohydrate was significantly increased in leaves of plants grown in elevated CO_2.

Table 6.6 Carbohydrate contents (mg m^{-2}) of leaves of silver birch grown in two CO_2 concentrations; ambient and elevated and measured in July 1991. Values are means (± one standard error) of four samples per CO_2 treatment (five samples were taken from each of four chambers per CO_2 treatment and combined by chamber). Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P= 0.05, ** significant at P=0.01.

	Ambient	Elevated	Treatment effect P<	Inter-chamber effect P<
Structural carbohydrate	2470±120.0	3742±510.0	0.021*	0.498
Starch	73.4±3.7	84.1±8.7	0.472	0.784
Soluble carbohydrate	29.5±11.0	33.3±15	0.190	0.519

The increased starch content of elevated CO_2 leaves is also apparent from the increased number of starch grains stained in figure 6.4 compared to figure 6.5.

Table 6.7 shows the effect of growth in elevated CO_2 on the number of cells produced per leaf sampled in May 1992. There was no CO_2 treatment effect on the number of cells expressed on an area basis; the number of cells in elevated CO_2 leaves was reduced, when expressed on a dry mass or fresh mass basis.

Table 6.7 Number of cells per leaf surface area, dry mass and fresh mass of leaves of silver birch grown in ambient and elevated CO_2 and measured in June 1992. Values are means ± one standard error of 24 leaves per CO_2 treatment (samples of leaf material were taken from six plants from each of four chambers per treatment). Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P=0.05, ** significant at P=0.01.

1992	Ambient CO_2	Elevated CO_2	Treatment effect P<	Inter-chamber effect P<	Interaction P<
Cells m^{-2}	$8.99x10^5$ $\pm 0.16x10^5$	$9.18x10^5$ $\pm 0.23x10^5$	0.511	0.354	0.599
Cells/g dry mass.	$6.80x10^7$ $\pm 0.08x10^7$	$6.08x10^7$ $\pm 0.14x10^7$	0.00**	0.677	0.439
Cells/g fresh mass	$2.32x10^7$ $\pm 0.03x10^7$	$2.14x10^7$ $\pm 0.04x10^7$	0.005**	0.734	0.454

6.3.4 Chlorophyll content

Tables 6.8, 6.9 and 6.10 show the chlorophyll content of leaves from plants grown in ambient or elevated CO_2 and sampled in July and September 1991 and April 1992. There was no effect of elevated CO_2 treatment on the total chlorophyll content of leaves sampled in July or September 1991, although the chlorophyll *a* to *b* was slightly reduced. The effect of chamber was, however, significant on leaves sampled in July. Total chlorophyll content was larger in leaves produced in 1992, in both CO_2 treatments compared to leaves produced the previous year. Total chlorophyll content was significantly smaller in the leaves of elevated CO_2 plants sampled in April 1992 but there was no effect of CO_2 treatment on chlorophyll *a* :*b* ratios, but chamber did have a significant effect on the amount of chlorophyll *b* .

Table 6.8 Chlorophyll content (mg cm^{-2}) of leaves of silver birch grown in ambient and elevated CO_2 and measured in July 1991. Values are means ± one standard error of twenty samples per CO_2 treatment (one sample was taken from each of five plants per chamber, four chambers per treatment). Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P=0.05, ** significant at P= 0.01.

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
chll *a*	22.01 ±0.88	21.73 ±0.73	0.778	0.001**	0.515
chll *b*	8.03 ±0.46	8.66 ±0.49	0.128	0.00**	0.394
a+b	30.03 ±1.29	30.39 ±1.14	0.797	0.00**	0.41
a/b	2.83 ±0.12	2.58 ±0.08	0.08	0.067	0.813

Table 6.9 Chlorophyll content (mg cm^{-2}) of leaves of silver birch grown in ambient and elevated CO_2 and measured in September 1991. Values are means ± one standard error of twenty samples per CO_2 treatment (one sample was taken from each of three plants per chamber, four chambers per treatment). Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P=0.05, ** significant at P=0.01.

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
chll *a*	21.13 ±1.35	20.10 ±2.21	0.681	0.097	0.366
chll *b*	6.91 ±0.44	6.71 ±0.66	0.798	0.138	0.474
a+b	28.04 ±1.79	26.81 ±2.8	0.707	0.103	0.393
a/b	3.05 ±0.03	2.97 ±0.07	0.156	0.616	0.126

Table 6.10 Chlorophyll content (mg cm^{-2}) of leaves of silver birch grown in ambient and elevated CO_2 and measured in April 1992. Values are means ± one standard error of 25 samples per CO_2 treatment (one sample was taken from each of five plants per chamber, five chambers per treatment). Levels of significance for the differences between elevated and ambient CO_2 grown leaves (treatment effect) and between leaves from plants grown in different chambers (inter-chamber effect) are also presented. *significant at P=0.05, ** significant at P= 0.01.

	Ambient CO_2	Elevated CO_2	Treatment effect P<	Chamber effect P<	Interaction P<
chll *a*	54.91 ±2.8	52.59 ±4.5	0.503	0.074	0.019
chll *b*	16.87 ±1.32	15.53 ±1.52	0.397	0.045*	0.622
a+b	71.78 ±3.54	61.02 ±3.31	0.004**	0.072	0.002**
a/b	3.50 ±0.39	3.69 ±0.51	0.772	0.096	0.942

6.3.5. Summary of results

Leaf thickness increased in plants in elevated CO_2, and this effect was more pronounced in younger leaves. This increase in leaf thickness was concomitant with increase in the leaf dry mass : area ratio. Leaf dry mass : fresh mass ratio increased in elevated CO_2 leaves produced during the first season, but there was no effect of CO_2 treatment on leaves produced during the second season. Leaves from elevated CO_2 plants had slightly higher soluble carbohydrates (sucrose, fructan etc.), but there was no increase of starch accumulation in leaves from elevated CO_2 plants measured in July 1991. However, transverse sections through leaves that developed in 1992 showed an increase in the number and size of starch grains when stained with Schiffs reagent. In plants grown in elevated CO_2 there was an extra layer of palisade cells. The increase in leaf thickness was not a result of an increase in the number of cells, but a slight increase in structural carbohydrate content of elevated CO_2 leaves suggested that the cells were larger. There was no significant effect on total chlorophyll content or chlorophyll *a*: chlorophyll *b* ratio of leaves of plants measured after four and six months growth in elevated CO_2, but after sixteen months total chlorophyll content measured on a leaf area basis was significantly lower in elevated CO_2 compared to leaves from plants grown in

ambient CO_2. In elevated CO_2 there was a reduction in both chlorophyll *a* and *b*, but there was no effect on chlorophyll *a:b* ratio.

6.4 DISCUSSION

6.4.1 Leaf anatomy

An increase in leaf thickness has been reported in several species grown in elevated CO_2 including: *Desmodium paniculatum* (Wulff and Strain, 1982), *Glycine max, Liquidambar stryaciflua and Pinus taeda* (Thomas and Harvey, 1983), *Castanea sativa* (Mousseau and Enoch, 1989), *Populus* clones (Radoglou and Jarvis, 1990a), *Phaseolus vulgaris* (Radoglou and Jarvis 1992). In this study, an increase in leaf thickness was concomitant with the production of an extra layer of palisade cells. Despite the presence of an extra palisade layer there was no evidence of an increase in the number of cells produced on a leaf area basis. This suggests that the extra palisade layer was produced at the expense of mesophyll cells. Analysis of structural carbohydrate content indicated that the cells were slightly larger in leaves of plants grown in elevated CO_2. The initiation of an additional layer of palisade cells in response to growth in elevated CO_2 has been reported for *Glycine max* (Hofstra and Hesketh, 1975; Thomas and Harvey, 1983) and *Castanea sativa* (Mousseau and Enoch, 1989). Although the spongy mesophyll region of a leaf generally has a larger volume of air, the palisade region has a larger total area of cell wall exposed to intercellular spaces, and therefore, a larger surface area available for the inward diffusion of CO_2 (Nobel, 1985). An increase in the size of the palisade region in leaves of plants grown in elevated CO_2 will increase A_{mes}/A (A_{mes} = total leaf area of cell walls of mesophyll cells exposed to intercellular air spaces, A=area of one side of same leaf, Nobel, 1985). Leaves of sun plants generally have a larger proportion of palisade cells compared to leaves of shade plants and A_{mes}/A is often two to four times larger for sun leaves than shade leaves. An increase in A_{mes}/A in leaves of plants grown in elevated CO_2 may increase total leaf conductance and therefore photosynthetic potential, and would therefore be worth measuring.

6.4.2 Chlorophyll content

There was no significant difference in total chlorophyll content on a leaf area basis in plants grown in elevated and ambient CO_2 measured in July or September 1991. There was a significant reduction in total chlorophyll content of leaves from elevated CO_2 plants in April 1992. A reduction in chlorophyll content on a dry mass and leaf area

basis as a result of growth in elevated CO_2 has been observed by several workers (Madsen, 1968; Tolley and Strain, 1985, Oberbauer, Strain and Fetcher, 1985; Delucia *et al.*, 1985 Wullschleger, Norby and Hendrix 1992, Radoglou and Jarvis, 1992). There was a small reduction in chlorophyll *a:b* ratio in leaves from elevated CO_2 plants measured in 1991, but there was no difference between elevated and ambient CO_2 plants in April 1992. Reduction in chlorophyll *a::b* has been reported in tomato (Madsen, 1968) and *Trifolium subterranean* (Cave *et al.*, 1981), although no reduction in chlorophyll *b* content was reported by Radoglou and Jarvis (1992) for *Phaseolus vulgaris*.

There were significant differences in chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of leaves from different chambers in July 1991, and in chlorophyll *b* content of leaves measured in April 1992. Both nitrogen supply (Ingestad, 1971; Linder and Rook, 1984; McDonald *et al.*, 1992) and radiation environment (Björkman *et al.*, 1972; Lichtenthaler, 1985) have significant effects on chlorophyll synthesis. The variation in chlorophyll content in this study suggests that there was significant variation in the radiation environment and/or nitrogen supply of leaves from different chambers. Each plant received the same amount of liquid fertiliser every month. The OTCs have been positioned in the field so that they do not shade each other and to minimise the possibility of variation in incoming radiation to each chamber. Plants were placed randomly within each chamber but position of plants in the chamber and proximity of neighbours must have resulted in variation in radiation incident on leaves of different plants within a chamber. A larger sample size might have reduced the impact of such variation. Each plant received the same amount of liquid fertiliser every month.

Reduction in chlorophyll content may imply a reduction in the size or number of reaction centres (Lewandowska and Jarvis, 1977) or disruption of the chloroplast and may reduce light harvesting capacity and therefore photosynthetic potential (Oberbauer *et al.* 1985). However it is more widely accepted that chlorophyll is usually non-limiting unless the plant is severely stressed (Linder and Rook, 1984).

Despite a reduction in chlorophyll *a* and chlorophyll *b* (and therefore total chlorophyll) content in *Desmodium paniculatum* (Wulff and Strain, 1982) and *Liriodendron tulipifera* and *Quercus alba* (Wullschleger *et al.*, 1992) there was an increase in light harvesting capacity of all three species. An increase in the initial slope of light -response curves (i.e. the apparent quantum yield) of these three species and an increase in photochemical quenching in *Liriodendron tulipifera* and *Quercus alba* measured using pulse-modulated fluorescence techniques led Wulff and Strain (1982) and Wullschleger *et al.* (1992) to suggest that plants grown in elevated CO_2 may have an increased capacity for radiation utilisation. Although, Long and Drake (1991) found

no change in quantum yield in *Scirpus olneyi* grown in elevated CO_2 measured using a transparent leaf chamber incorporated into an Ulbricht intergrating sphere.

In a field situation many leaves experience low PFD as a result of shading by other leaves, under such conditions CO_2 assimilation will be affected by quantum yield as much as by the light saturation rate (Long *et al.*, 1993). Whether changes in anatomical organisation and chlorophyll content in silver birch seedlings grown in elevated CO_2 affect light harvesting capacity and quantum yield should be tested.

6.4.3. Specific leaf area and carbohydrate content

The reduction in SLA in leaves of plants grown in elevated CO_2 in this study was concomitant with a slight increase in structural carbohydrate content but no increase in cell number on a leaf area basis. This suggests that the cells were slightly larger in elevated CO_2 leaves. There was also an increase in soluble carbohydrate content in leaves from elevated CO_2 plants, and this is consistent with the increased rate of photosynthesis in plants grown in elevated CO_2 recorded in this study (§3.5.5; Evans *et al., 1993*) and by other workers (Pettersson *et al.*, 1993). Both sucrose and starch accumulation have been reported for plants grown in elevated CO_2 (Farrar and Williams, 1991). The accumulation of large amounts of starch has been reported for many plants grown in elevated CO_2 and has been associated with a source-sink imbalance. When supply of the products of photosynthesis exceeds the demands of sinks, sucrose and other intermediate metabolites accumulates in the leaf, and sucrose synthesis is reduced by feedback control of sucrose phosphate synthetase (SPS) resulting in the diversion of more carbon to starch (Stitt, 1991). Accumulation of large amounts of starch resulting from source-sink imbalance has been implicated in the down-regulation of photosynthesis in plants grown in elevated CO_2 for long periods. In this study there was no increase in starch accumulation in elevated CO_2 compared to ambient CO_2 plants measured in July 1991, and no evidence of down regulation of photosynthesis (Evans *et al. 1993*). There was, however, visual evidence of starch accumulation in the chloroplasts of leaves produced in elevated CO_2 in May 1992 and this will have contributed to the reduction in SLA in elevated CO_2 plants.

6.4.4. Conclusions

Growth of silver birch in elevated CO_2 results in;

- an increase in leaf thickness and a reduction in SLA;
- an increase in structural dry matter content of leaves, but no increase in cell number on a leaf area basis;
- alterations in the anatomical organisation of leaves resulting in an additional layer of palisade cells; and
- a reduction in total chlorophyll content, but no effect on the chlorophyll *a* to *b* ratio.

The effect of such changes in leaf characteristics on the light harvesting efficiency and photosynthetic capacity of silver birch grown in elevated CO_2 need further investigation.

CHAPTER 7 GENERAL DISCUSSION

In most species, photosynthesis increases and g_s decreases, at least in the short term, in response to increase in atmospheric CO_2 concentration (Eamus and Jarvis, 1989; Stitt, 1991; Bowes, 1991; Luxmoore *et al.*, 1993). Whether these responses at leaf scale will be translated into long-term increases in whole plant growth will depend, to some extent, on changes in morphology and resource allocation of plants in elevated CO_2 (Woodward, 1993). The research described in this thesis examined the effects of growth in elevated CO_2 on the resource allocation patterns of two contrasting species of juvenile trees: Sitka spruce (a conifer with a determinate growth pattern) and silver birch (a broadleaf with an indeterminate growth pattern) (Chapter 3), and has focused on the effects of elevated CO_2 on leaf expansion of silver birch (Chapter 4), including leaf surface properties (Chapter 5) and leaf characteristics (Chapter 6). In the introduction to this thesis the questions addressed in this work were defined, in this chapter the answers to those questions will be discussed, along with comments on the methods used, suggestions for further research and the possible implications for longer term growth of more mature trees and forests that can be drawn from studies on seedlings.

What are the effects of long-term growth in elevated CO_2 on :
a) total biomass;
b) branch;
c) leaf; and
d) root production
in Sitka spruce and silver birch? Does the nature and magnitude of response differ between species?

Despite an initial increase in biomass production in elevated CO_2 above that of ambient CO_2 plants, no significant effect on total biomass of Sitka spruce was found after 16 months. Norby *et al.*, (1992) describe a similar effect on total biomass production in *Liriodendron tulipifera*, although sustained increases have been reported for other tree species (Sour orange, Idso and Kimball and Allen, 1991; Idso and Kimball, 1991, 1992; *Castaneae sativa* , El Kohen *et al.*, 1992; Mousseau and Saugier, 1992; *Pinus radiata*, Hollinger, 1987; Conroy *et al.* 1988, 1990). After six months in elevated CO_2 there was a significant increase in allocation of dry mass to roots in Sitka spruce but no effect of elevated CO_2 on above ground biomass production. By contrast, Townend (1993) reported no effect of growth in elevated CO_2 on root: shoot ratios in clones of

Sitka spruce. El Kohen *et al.*, (1992) showed that change in dry mass allocation of *Castaneae sativa* in relation to CO_2 was determined by nutrient availability. This is in accordance with the theory that allocation of dry mass between root and shoot is controlled by the balance between supply of photosynthate and rate of uptake of nitrogen (Agren and Ingestad, 1987). Although the Sitka spruce seedlings did receive additional fertiliser during the second year of this study, they were shown to be nutrient deficient and this may account for the increased proportion of root dry mass of plants harvested in January 1991 and July 1991. Bare-rooted seedlings invest a larger proportion of dry matter into roots during establishment (Kozlowski *et al.*, 1991), and this may also have been responsible for the large initial increase in root mass in plants grown in elevated CO_2. After eight months plants received additional nutrients at monthly intervals. Although elevated CO_2 plants harvested after thirteen months still had larger roots, after eighteen months there were no differences in allocation patterns between plants grown in ambient and elevated CO_2. Townend (1993) reported a reduction in ratio of needle to shoot dry mass of Sitka spruce seedlings grown in elevated CO_2. In this study, needle to shoot dry mass ratio was unaffected by growth in elevated CO_2.

Photosynthetic rate was higher in Sitka spruce seedlings grown in elevated CO_2 (H.J.S. Lee pers. comm.). This is in agreement with the findings of Townend (1993). Tolley and Strain (1984a,b) reported a decline in photosynthetic rate of *Liquidambar stryaciflua* and *Pinus taeda* as the duration of the experiment increased, and similar findings have been reported for other tree species (*Pentaclethra macroloba, Ochroma lagopus,* Oberbauer *et al.*, 1985 and *Castanae sativa*, Mousseau and Saugier, 1992). However, Idso and Kimball (1992) found no such reduction in *Citrus aurantium* after three years and Gunderson *et al.* (1993) and Wullschleger *et al.* (1994) found no reduction in photosynthetic rate in *Liriodendron tulipifera* or *Quercus alba* after two years of growth in elevated CO_2. Inadequate rooting volume in small pots may restrict uptake of nutrients or water and may reduce the potential for roots to act as sinks for photosynthate. In several field studies where root exploration was unconstrained the initial high rates of photosynthesis and biomass production have been sustained (eg. Arp and Drake, 1991; Idso *et al.*, 1991). Inadequate rooting volume resulting in source-sink imbalance and excess accumulation of carbohydrate has been correlated with a reduction in photosynthetic rate and, therefore, biomass production (Arp, 1991). Thomas and Strain (1991) showed that down-regulation of photosynthesis was more pronounced in small pots than in large pots, but studies of this kind fail to separate effects of rooting volume and nutrient supply. In a recent paper Nicotra *et al.* (1994) described work which found no effect of rooting volume but did find a positive correlation between nutrient supply and growth enhancement of plants grown in

elevated CO_2. The phenomenon is not universal and there was no acclimation of photosynthesis in *Liriodendron tulipifera* despite low leaf nitrogen content (Norby *et al.*, 1992; Curtis *et al.*, 1989). There was no evidence of photosynthetic down-regulation in Sitka spruce seedlings grown in this study and measured in July 1991 (Lee *et al.*, 1993, H.S.J. Lee pers. comm.) despite the low nitrogen status of the seedlings, indicating that there was still an adequate sink for photosynthate at the time measurements were taken.

The absense of an effect of elevated CO_2 on biomass production in Sitka spruce, despite increased photosynthetic rates suggests an increase in carbon cycling. A similar growth response was reported for *Liriodendron tulipifera* (Wullschleger *et al.*, 1994) and was attributed to partial compensation for increase in photosynthetic rate by a reduction in leaf area and respiration rate and an increase in root exudation and fine root production. In this study, there was no reduction in leaf area of Sitka spruce, suggesting that there may have been an increase in fine root production, fine root turnover, root exudation or respiration. A proportion of fine roots remain in the soil when plants are harvested and it is possible, therefore that the measurements of root dry mass obtained in this experiment underestimate total root production. No attempt to determine amount of root exudation was made in this study. Increased fine root production has been reported in plants grown in elevated CO_2 (Norby *et al.* 1987; O'Neill *et al.* 1987a,b); Idso and Kimball, 1992), root exudation increased in *Pinus echinata* grown in elevated CO_2 (Norby *et al.*, 1987). An increase in fine root production will increase the potential for absorption of nutrients and water uptake in plants grown in elevated CO_2 (Oechel and Strain, 1985). An increase in root exudation may stimulate microbial activity in soil and increase nitrogen availability (Norby, 1987). Further research is required to determine effects of elevated CO_2 on respiration rates and the hypothesis that elevated CO_2 increases fine root production and root exudation remains to be tested, long-term experiments are required to investigate the potential for positive and negative feedback on carbon and nitrogen dynamics in the soil (Zak, 1993).

After six months, biomass production was increased by approximately 20% in seedlings of silver birch grown in elevated CO_2 compared to ambient-grown plants. This is consistent with the findings for silver birch by Pettersson and McDonald (1992). Photosynthetic rate was higher in silver birch seedlings grown and measured in elevated CO_2, this is in agreement with the findings of Pettersson *et al.* (1992). There was no evidence of down regulation of photosynthesis in silver birch seedlings measured in July 1991, despite low nitrogen status of leaves. In birch the number of branches produced increased. This was the main factor contributing to increased

biomass in plants grown in elevated CO_2. There was no effect of elevated CO_2 on root mass, although the rate of production, amount and turnover of fine roots produced in elevated CO_2 was not examined. Pettersson *et al.* (1993) found no effect of elevated CO_2 on root to shoot ratio of seedlings of *Betula pendula* grown at optimum nutrition in Ingestad units. They did not find any effect of elevated CO_2 on branch production but this may be because of the short duration of their experiment (60 days). There are several reports of leaf area production increasing in response to elevated CO_2 (Higginbotham *et al.*, 1985; Radoglou and Jarvis, 1990a; Wong, 1990; Ziska *et al.*, 1990), but despite early indications of an increase in the area of individual leaves in response to elevated CO_2, there was no significant difference in leaf area between elevated and ambient CO_2 plants after six months. However, the initial increase in leaf area in birch grown in elevated CO_2 is likely to have contributed to the increase in biomass production and may have conferred an additional advantage for seedling establishment in elevated CO_2. Potential for long term biomass production as a result of increased rates of photosynthesis in elevated CO_2 may be partially offset by relative reduction in leaf area. Similar compensatory responses have been described for other species (Norby *et al.*, 1992; Pettersson *et al.*, 1993). Earlier onset of senescence will also reduce potential for biomass production, although both the relative reduction in leaf area production and early senescence may be attributable to the low nitrogen status of plants.

- **a) What is the effect of elevated CO_2 on leaf expansion in birch and b) which biophysical parameters controlling leaf expansion are affected?**

In this study, leaf expansion increased in birch in elevated CO_2 early in the foliated season (§4.3.1). There was no consistent CO_2 treatment effect on any of the biophysical parameters measured, despite consistent increase in LER in leaves from elevated CO_2 plants during early morning. Lack of evidence of effects of elevated CO_2 on the biophysical parameters measured in this study may be, in part, a result of experimental procedure and the techniques used (Chapter 4). The semi-controlled environment of OTCs in a field situation do not enable effects of other environmental variables (eg. PFD, temperature, water availability, nutrition) to be separated from those of elevated CO_2. Parallel studies in controlled environment chambers using the turgor probe technique are required to determine interactions between elevated CO_2 and the main environmental variables. Further research is also required to investigate effects of elevated CO_2 on PFD-activated acidification of cell walls.

- **Is stomatal conductance reduced in birch in elevated CO_2 as a result of an increase in the degree of stomatal closure and/or a reduction in stomatal density?**

In this study, a reduction in g_s of 15% was found in leaves from plants grown in elevated CO_2, Pettersson *et al.* (1992, 1993) also report a slight reduction in stomatal conductance of birch growing in elevated CO_2. Reduction in stomatal conductance in this study was produced by a combination of a reduction in degree of opening of stomata, a reduction in stomatal density and a slight reduction in size of guard cell complexes in plants grown in elevated CO_2. However, direct observation of apertures using an SEM failed to adequately assess the relative effects of a reduction in stomatal aperture and of changes on g_S of elevated CO_2 plants, because of the heterogeneity of the leaf surfaces and the neccessarily small sample size used (Chapter 5). There was no effect of elevated CO_2 on stomatal index and reduction in stomatal density was a result of increase in leaf expansion. Leaves that developed in elevated CO_2 later in the season were smaller than those produced in ambient CO_2 and stomatal density was therefore likely to have been increased. The reduction in size of leaves produced late in the season may have been a result of low nitrogen status of plants at this time.

Sensitivity of stomata to elevated CO_2 has been shown to be affected by other environmental variables including PFD (Morison and Gifford, 1983) and VPD (vapour pressure deficit) (Hollinger, 1987) in some species, although little work has been done on trees. The relatively low sensitivity of silver birch to elevated CO_2 in this study may be a result of high water status and relatively high PFD at the time measurements were made. Differences in g_S between plants grown and measured in elevated or ambient CO_2 may also vary with time of day. Sensitivity of stomata to CO_2 has been shown to increase with time in *Pinus radiata* (Hollinger, 1987) and decrease with time in Sitka spruce and silver birch (P.G. Jarvis, pers. comm.). There was no effect of growth in elevated CO_2 on sensitivity of stomata to CO_2 concentration in silver birch seedlings in this study measured in July 1991 (Evans *et al., 1993*). However, the time scale over which acclimation of stomatal sensitivity to CO_2 concentration occurs is not known and may extend over more than one growing season. Further research is required to determine the effect of growth in elevated CO_2 on sensitivity of stomata to CO_2 concentration and also to investigate the interaction between CO_2 concentration and other environmental variables (including PFD, ABA and VPD) in regulating g_S in seedlings of silver birch.

Elevated CO_2 has been shown to improve instantaneous water use efficiency as a result of increased photosynthetic rates and reduced stomatal conductance in many species (for example: *Liquidambar stryaciflua,* Tolley and Strain, 1984b; *Quercus prinus* and *Malus domestica,* Bunce, 1992; and see Morison, 1993). Reduction in instantaneous water use at the leaf scale is often offset by an increase in leaf area (Eamus and Jarvis, 1989). In this study there was no effect of elevated CO_2 on total plant leaf area after six months. Thus instantaneous water use efficiency may increase in birch in elevated CO_2 as a result of both reduction in stomatal conductance and increase in photosynthetic rate. Whether this will lead to an increase in integrated water use efficiency over days or seasons requires investigation.

Elevated CO_2 has been shown to reduce adverse effects of drought on growth in some species (Tolley and Strain, 1984a,b; Sionit *et al.* 1985; Conroy *et al.* 1986a); Hollinger, 1987; Johnsen, 1993; Townend, 1993). A reduction in g_s may maintain higher minimum water potentials during drought and this may reduce the incidence of photoinhibition and increase the duration of photosynthetic CO_2 uptake, before water stress occurs (Morison, 1993). Given a situation in which both root mass and leaf area are the same in both ambient and elevated CO_2, a reduction in g_s should lower leaf water potential (Morison, 1993). Although these conditions were satisfied in this study, no clear effects of elevated CO_2 on leaf water potential were found. There was no effect of elevated CO_2 on osmotic potential in seedlings of silver birch in this study, although osmotic potential was increased in seedlings of *Liquidambar stryaciflua* grown in elevated CO_2 compared to those grown in ambient CO_2 (Tolley and Strain 1984b). The hypothesis remains to be tested that elevated CO_2 will offset the adverse effects of drought on young birch trees.

- **How are a) specific leaf area, b) leaf anatomy and c) chlorophyll content in birch affected by growth in elevated CO_2 ?**

Specific leaf area decreased in birch in this study. SLA has been found to decrease in many species grown in elevated CO_2 (for example *Populus* clones, Radoglou and Jarvis, 1990a; *Betula pendula,* McDonald and Pettersson, 1992). This decrease may be a result of increase in starch accumulation and/or an increase in leaf thickness resulting from alterations in leaf anatomy. Accumulation of starch is dependent on source-sink status at the time of measuring. Starch accumulation was not significantly increased in leaves of elevated CO_2 plants measured in August 1991, but there was visual evidence of starch accumulation in elevated CO_2 leaves in May 1992 and this will have contributed to the reduction in SLA in elevated CO_2 plants. There was an increase in number of palisade cells in silver birch grown in elevated CO_2. Similar effects have been found in *Castaneae sativa* (Mousseau and Enoch, 1989) and *Glycine max*

(Thomas and Harvey, 1983). An increase in number of layers of palisade cells increases the surface area of cells available for the absorption of CO_2.

Chlorophyll content of leaves was reduced in silver birch seedlings grown in elevated CO_2. However, despite reduction in chlorophyll content, light harvesting efficiency of *Liriodendron tulipifera* has been reported to be improved by growth in elevated CO_2, and this was attributed, in part, to anatomical changes (Norby *et al.*, 1992). In the field, many leaves experience low photon flux density as a result of self shading or canopy closure, and in such circumstances the CO_2 assimilation rate will be affected as much by light harvesting efficiency as by the light saturation rate (Long, 1993). Whether the changes in leaf anatomy observed in this study would result in increased light harvesting capacity and increased quantum yield, or would be offset by reduction in chlorophyll requires investigation.

- **What implications can be drawn from these findings for the future functioning of silver birch and Sitka spruce in an elevated CO_2 environment?**

Small changes in rates of growth and allocation patterns during the early stages of the life of a tree may have major consequences for viability and biomass production later on. The initial increase in allocation of dry mass to roots of Sitka spruce may facilitate establishment and competition in nutrient-poor soils. An initial increase in the rate of growth and leaf area production in birch seedlings will increase the rate at which they can utilise available resourses and will thus aid competition. Although direct comparisons cannot be made between species because of the differences in growing conditions in this experiment, there was an indication that changes in pattern of resource allocation differed between Sitka spruce and birch. Initial increase in leaf area production in silver birch in elevated CO_2 may enable seedlings to exploit available resources and may aid establishment. It has been proposed that fine root production is increased in Sitka spruce seedlings in elevated CO_2, this may increase improve uptake of nitrogen and water.

The stimulatory effect of growth of elevated CO_2 of plants in a well managed system, may be large but in most wild plants this potential is not likely to be fully realised as a result of other limiting factors. The potential of elevated CO_2 to improve ability to withstand water and nutrient stress as a result of increased water use efficiency and increase in fine root production, increased mineralization of the soil and improved nitrogen use efficiency requires further investigation.

In the previous sections, some of the effects of growth in elevated CO_2 on Sitka spruce and silver birch have been discussed. The following sections discuss the role of studies on seedlings in providing information for predicting responses of trees and forests to elevated CO_2.

Because of the large size and longevity of trees direct studies on the response of mature trees and forests are not feasible, so that, instead we are reliant to a large degree on extrapolation of data obtained from studies on seedlings. Whilst information now abounds describing the effects of elevated CO_2 on tree seedlings, questions have been raised concerning the relevance and usefulness of extrapolating such information studies to determine the response of trees and forests to elevated CO_2 (Mousseau and Saugier, 1992). Extrapolating data from experiments on seedlings to predict the response of mature trees is dubious: mature tissue differs in size, morphology and anatomy compared to juvenile tissue and it cannot be assumed that mature tissue will respond in the same way as juvenile tissue (Hackett, 1985; Kozlowski *et al.*, 1991). Another complicating factor is the degree of acclimation of plants from which the data were obtained. Acclimation of different processes to elevated CO_2 may take minutes, or years and it has been suggested that full acclimation might require the growth of trees for more than one generation in elevated CO_2 (Eamus and Jarvis, 1989).

Long-term studies, starting with seed germinated in elevated CO_2 should be undertaken to determine whether the responses of seedlings to elevated CO_2 observed in this study are sustained over the long term. The differences in dry matter allocation in Sitka spruce at different times in this study emphasis this point. There are an increasing number of long-term experiments with trees: a long-term experiment is continuing on sour orange trees in Arizona and has already shown no down regulation of photosynthesis after 4 years (Idso, Wall and Kimball, 1993). Long term experiments are also being done on *Liriodendron tulipifera* and *Quercus alba* (Wullschleger *et al.*, 1994). At the end of this study the seedlings of birch were transferred to individual purpose-built tree chambers where they are rooted into the ground and experiments on them continue with the same CO_2 regimes. In a parallel experiment to obtain information on the functioning of mature tissue, branch bags are being used to fumigate entire branches of mature Sitka spruce trees (Barton, Lee and Jarvis, 1993), the assumption being made that branches are autonomous for carbon (Sprugel, Hinckley and Schnap, 1991).

These studies on young trees serve to highlight gaps in our understanding about the responses of trees and forests to elevated CO_2 . The interactions between CO_2 and PFD, nutrients and water highlighted by this study are likely to be of increasing importance in trees approaching canopy closure when environmental variables may

increasingly limit growth. Changes in branching and leaf area production in trees grown in elevated CO_2 affects leaf area density in tree crowns and this has been shown to have significant effects on radiation interception and biomass production (Wang, Jarvis and Taylor, 1991). The potential for biomass production by a closed canopy depends on photosynthetic rate on a leaf area basis so that acclimation of photosynthesis to elevated CO_2, if it occurs, will have a larger effect on biomass production than any stimulatory effect of elevated CO_2 on leaf area production.

An underlying objective of research investigating responses of trees to elevated CO_2 is to enable predictions to be made concerning the likely effects of elevated CO_2 on ecosystems, and forest ecosystems in particular. Despite significant progress in understanding the response of individual trees to growth in elevated CO_2, the complexity of forest ecosystems is such that we are still a long way off being able to predict their response to elevated CO_2. To date, only two long term experiments have been conducted on plant communities in the field, a study of Alaskan tundra (Tissue and Oechel, 1987; Oechel *et al.* 1993) and a study of salt marsh vegetation (Curtis *et al.* 1989). The practical difficulties of obtaining this kind of information for forests are immense, although the use of free air carbon dioxide exposure (FACE) systems has opened up possibilities for limited exposure and is currently being explored and developed (B. Strain pers. comm.).

The complex nature of ecosystems, with individuals with different life cycles, strategies and morphology competing for resources (radiation, nutrients, water) makes a systems approach the most feasible (Körner, 1993). However, a major disadvantage of a such an approach, utilising *top-down* models is that they cannot be credibly used to make predictions beyond the range of variables used in derivation of the parameters and functions. The incorporation and integration of process-based routines is necessary to provide a more flexible tool (Jarvis, 1993). Development and parameterisation of such integrated models, requires:

- an ecological approach to experimentation, using a wide range of key species, and taking into account the *natural* range of environmental variables experienced by each species, and the development of a representative ecological database;
- identification of key processes (above and below-ground) sensitive to elevated CO_2; and
- mechanistic understanding of these key processes and their interaction with the main environmental variables (solar radiation, temperature, nutrient availability, water availability).

Responses of trees (and forests) to elevated CO_2 are complex, acting on different processes and on different spatial and time scales, and interacting with other environmental variables, so that we are still a long way from being able to make reliable predictions about the effects of a sustained increase in atmospheric CO_2 on woodlands and forests. The problem is immense and large scale, long-term collaborative projects with good alignment of techniques are necessary to facilitate the development, parameterisation and application of suitable models.

APPENDIX

DNS reagent

150 g of NaKtartarate in 250 ml H_2O was added to 5 g of DNS (Dinitrosalicylic acid) and 100 ml of 2M NaOH. This solution was then made up to 400 ml with H_2O and filtered.

Karnovskys solution

10g of paraformaldehyde was dissolved in 200cm3 of distilled water and heated to 60 °C on a stirrer within a fume-cupboard. A few drops of NaOH were added to clear the solution, the heat was turned off as soon as the solution cleared. 50 cm^3 of stock gluteraldehyde solution were added to make the solution up to 250 cm^3.

Hepes solution

0.1M Hepes was prepared, 5.95g Hepes was dissolved in 250 cm3 of distilled water. NaOH was added until pH = 7.2

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