LIGHT AND CO₂ EFFECTS ON STOMATA

by

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The effects of light and carbon dioxide on the stomata of Scots pine, Sitka spruce and dayflower were investigated. Gas exchange methods were used to measure the response of stomatal conductance of whole shoots to changes in spectral composition, quantum flux density and intercellular CO₂ concentration.

The response of the stomata to CO₂ differed between the species. In the light, low intercellular CO₂ concentrations caused larger stomatal conductances in all three species, although the effect was small in Sitka spruce. High intercellular CO₂ concentrations had little effect in the conifers but inhibited stomatal opening in dayflower. In the dark, intercellular CO₂ concentration only affected stomata in dayflower shoots.

Evidence was found for two direct effects of light on stomata; in all three species blue light caused larger increases in stomatal conductance than red light, particularly at low quantum flux densities. The opposite was true for net assimilation; the quantum yield in blue light was between 50 and 70% that in red light. Stomata responded to both blue and red light when intercellular CO₂ concentrations were maintained at or below the compensation point when net assimilation was negative. In dayflower, stomatal conductance increased in response to white light while the intercellular CO₂ concentration was experimentally maintained constant at 466, 302 and 46 cm⁻³ m⁻³. There was an interaction of light and CO₂; the largest increase in stomatal conductance occurred at the lowest intercellular CO₂ concentration and the inhibitory effect of CO₂ increased with increasing quantum flux density.
While dayflower stomata showed high sensitivity to intercellular CO$_2$ concentration, an analysis using optimal control theory methods indicated that stomata were not effective in controlling leaf gas exchange in well-watered humid conditions. In these conditions the direct responses of stomata to light were dominant and not the indirect effect of intercellular CO$_2$ concentration.
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# CONTENTS

| TITLE PAGE | i |
| ABSTRACT | ii-iii |
| DECLARATION | iv |
| ACKNOWLEDGEMENTS | v |
| CONTENTS | vi-ii |

## CHAPTER 1 INTRODUCTION

1

## CHAPTER 2 MATERIALS AND METHODS

2.1 Introduction 4
2.2 Plant Material 5
2.3 Growth Room Conditions 5
2.4 Water Vapour and CO₂ exchange 6
   2.4.1 Theory 6
   2.4.2 Gas exchange equipment 9
   2.4.3 Data acquisition and computation 13
   2.4.4 Additional equipment 15
   2.4.5 Error analysis 16
2.5 Experimental Procedure 18
   2.5.1 Conifers 18
   2.5.2 Dayflower 19

## CHAPTER 3 CO₂ EFFECTS ON STOMATA

3.1 The response of Stomata to CO₂ 21
   3.1.1 Introduction 21
   3.1.2 The range of responses 22
3.2 Experiments 33
3.3 Results 37
   3.3.1 Response of gₛ to Cᵣ in conifers 37
   3.3.2 Response of gₛ to Cᵣ in dayflower 40
3.4 Discussion 43
3.5 Conclusions 54

## CHAPTER 4 LIGHT EFFECTS ON STOMATA

4.1 The Response of Stomata to Light 55
   4.1.1 Introduction 55
   4.1.2 Stomatal responses to light 56
4.2 Experiments 67
4.3 Results
   4.3.1 The response of gₛ and A to blue and red light in Scots pine 72
   4.3.2 Wavelength sensitivity of gₛ in Scots pine 77
   4.3.3 The response of gₛ and A to CO₂ and O₂ in 81
Stomata occur in the epidermes of practically all aerial parts of terrestrial plants above the evolutionary level of the Anthocerotae. They occur in leaves, herbaceous stems and petioles, in parts of the angiosperm flower and in many fruits (Meidner and Mansfield 1968).

In the leaf the stomata are situated in the one position where they are most effectively placed to control the efflux of water vapour and the influx of carbon dioxide to the interior of the leaf. However, stomatal aperture is dependent on several variables which are dependent upon the rates of transpiration and carbon dioxide assimilation; for example, bulk leaf water potential and intercellular $\text{CO}_2$ concentration. The question then arises as to what extent do stomata control transpiration and photosynthesis, or are controlled by transpiration and photosynthesis (Jarvis and Morison 1980)?

To answer this question we must define the factors that affect stomata, transpiration and photosynthesis and investigate the mechanisms linking transpiration, photosynthesis and stomatal aperture.

Clearly, it is possible to physically isolate stomata in epidermes from the rest of the leaf and to investigate the responses of such isolated stomata to environmental variables (e.g. Lösch 1976). While such methods have been used with undoubted success to elucidate the mechanisms operating at the cellular level (e.g. Fischer 1968), results thus obtained should be interpreted with caution when considering whole leaves. Moreover, investigations on isolated
stomata obviously will not reveal links with transpiration and photosynthesis. Where the emphasis is, as here, on the role of stomata in the gas exchange of leaves then studies on whole leaves are appropriate. Again the method is not without problems in that gas exchange methods necessarily integrate the responses of a population of stomata (see Laisk, Oja and Kull 1980).

The volume of literature available on stomatal physiology indicates that it is obviously impracticable to investigate all the factors affecting stomata, transpiration and photosynthesis. Using controlled experimental conditions it is possible to limit the consideration to single factors; though this is not without dangers in that it limits the possible variety of the response of biological systems to environmental variables.

I have limited this study to the effects of light and carbon dioxide on stomata. Light and carbon dioxide are conveniently considered together as both appear to make substantial contributions to the daily cycle of stomatal movement, though their relative importance is disputed. While carbon dioxide is rarely an important environmental variable in natural conditions, many workers have suggested that the intercellular CO$_2$ concentration forms the link between mesophyll assimilation and stomatal aperture, permitting CO$_2$ assimilation to control the stomata. However, there is growing evidence that stomata can respond to light independently of the response of mesophyll assimilation to light (e.g. Mansfield and Meidner 1966).

The objectives of this study were a) to investigate the effects of CO$_2$ concentration and light on stomata, b) to experimentally separate the two effects, thereby indicating the extent of the control by
mesophyll photosynthesis of stomata, and c) to assess the relative contribution of the responses to CO$_2$ and light in stomatal opening.

The study has used two techniques to separate the effects of CO$_2$ and light on stomatal aperture. The first has relied on the experimental manipulation of light quality, quantum flux density and CO$_2$ concentration. The second technique has used a previous finding that the stomata of Scots pine (Pinus sylvestris L.) and Sitka spruce (Picea sitchensis [Bong.] Carr.) were apparently insensitive to CO$_2$ but responded to light (e.g. Ng 1978, Beadle 1977) as a system to separate the effects of light and carbon dioxide and to compare that system with a species which is known to be more "typical" in its response to CO$_2$ and light; dayflower (Commelina communis L.).
2.1 Introduction

The data in this thesis were collected from water vapour and carbon dioxide flux measurements with single, enclosed shoots of potted plants of *Pinus sylvestris*, L., *Picea sitchensis* (Bong.) Carr. and *Commelina communis* L.. The essential details of the materials and methods used in that work between January 1978 and May 1980 are presented below; more detailed descriptions are placed in the appendices as indicated.

This study is concerned with the effects of only two factors, light and CO$_2$, on stomata. Such an approach requires that other factors affecting stomata are recognised and, further, that their effects are minimised and standardised, so that any given experimental treatment is not confounded by changes in other variables. For that purpose, plant material of each of the three species was standardised with respect to age and physiological condition and was preconditioned to provide well-watered plants with responsive stomata over a long photoperiod.

Temperature and humidity conditions were chosen to permit the maximum range of stomatal conductance; small leaf-to-air vapour pressure differences (VPD) reduced the possible control of stomatal conductance by transpiration through changes in leaf water potential to a minimum. Care was taken to remove the largely artificial interaction between increased conductance and reduced VPD that can occur when leaves are enclosed in chambers of small volume and with
incoming air of constant vapour pressure.

2.2 Plant Material

Two year old (1+1) plants of _P. sylvestris_ (Scots pine, provenance NT 10) and _P. sitchensis_ (Sitka spruce, Queen Charlotte Islands provenance) were grown on for a year in a peat:sand mixture (University of California 2Cd mixture) in plastic pots. Over the two year period of experiments groups of plants were grown outside, in a cool frame and in a glasshouse to produce a supply of vigorous shoots between 4 and 7 months old, irrespective of season. Plants were brought in to a controlled environment growth room for preconditioning at least 6 weeks before use. Shoots used in the experiments were always the upper lateral shoots with current year needles only.

Seed of _C. communis_ (dayflower), supplied by Dr. Penny, University of Edinburgh, was germinated on a mist bench in a peat:sand mixture covered with coarse gravel, and transferred at approximately 3 cm height into individual pots in the growth room. The top two fully-expanded leaves and the uppermost, unfurled leaf of 4-7 week old plants were used.

2.3 Growth Room Conditions

Scots pine and Sitka spruce plants were preconditioned in a 14/10 hour day/night cycle, with 18/14 °C air temperature and 0.5/0.4 kPa VPD.

Dayflower seedlings were grown in a 16/8 hour day/night cycle with
20/15 °C air temperature and 0.6/0.4 kPa VPD.

Light sources in the room were 400W metal halide lamps, supplemented with 60W tungsten bulbs (see Appendix 3). The average photosynthetically active radiation (PhAR) at the height of the conifer shoots used was 340 μE m⁻² s⁻¹ and 390 μE m⁻² s⁻¹ at the height of the dayflower shoots used.

The soil was kept near field capacity and fertilised once a week with a commercial mixture (Bio no. 5 N:P:K 19.1:19.1:22.3 W/W %).

2.4 Water Vapour and CO₂ Exchange

2.4.1 Theory

The relevant theory has been presented in detail in Šesták, Čatský and Jarvis (1971); its application here follows that of Ludlow and Jarvis (1971).

The diffusive flux of water vapour and carbon dioxide can be described by equations analogous to Ohm’s Law:

\[ \text{Flux} = \frac{\text{gradient}}{\text{resistance}} \]  

(2.4.1a)

For transpiration, \( E \):

\[ E = \frac{(X_i - X_a)}{R} \]  

(2.4.1b)

where \( E \) is in g m⁻² s⁻¹, \( X_i \) and \( X_a \) are water vapour concentrations (g m⁻³) at the vapour/liquid interface and in the ambient air respectively; and \( R \) is the total diffusive resistance for water vapour, (s m⁻¹).

Vapour pressure, \( e \), in kPa is related to \( X \) by:

\[ X = 2167 e / T \]  

(2.4.1c)

where \( T \) is temperature in degrees Kelvin (Monteith 1973).

Combining 2.4.1c and 2.4.1b above:
\[ E = \frac{2167 \left( e_i - e_a \right)}{\left( T \cdot R \right)} \quad \text{(2.4.1d)} \]

In a leaf chamber vapour flux is determined from vapour pressures of incoming \( e_{\text{in}} \) and outgoing \( e_{\text{out}} \) air, the flow rate \( f, \text{m}^3 \text{s}^{-1} \) of the air through the chamber, and expressed on a leaf area basis \( a, \text{m}^2 \).

\[ E = \frac{2167 \left( e_{\text{out}} - e_{\text{in}} \right)}{a \cdot T} \quad \text{(2.4.1e)} \]

Combining equations 2.4.1d and 2.4.1e, and rearranging for \( R \):

\[ R = \frac{a}{f} \cdot \frac{\left( e_i - e_a \right)}{\left( e_{\text{out}} - e_{\text{in}} \right)} \quad \text{(2.4.1f)} \]

In a stirred chamber \( e_{\text{a}} = e_{\text{out}} \cdot e_{\text{in}} \) is assumed to be the saturated vapour pressure at leaf temperature.

Total diffusive resistance, \( R \), is partitioned into stomatal \( (r_s) \) and cuticular \( (r_c) \) resistances in parallel, with an aerodynamic resistance \( (r_a) \) in series, (all in \( s \text{ cm}^{-1} \)):

\[ R/100 = \left( \frac{1}{r_s} + \frac{1}{r_c} \right)^{-1} + r_a \quad \text{(2.4.1g)} \]

\( r_c \) is normally very large compared to \( r_s \) and can be ignored except at high stomatal resistances (e.g. Beadle 1977) so:

\[ r_s = \frac{R}{100} - r_a \quad \text{(2.4.1h)} \]

While use of resistance is more convenient computationally in the analysis of series and parallel components below, conductance expresses the direct proportionality between flux and concentration difference, (Burrows and Milthorpe 1976). The conductances to water vapour are the reciprocals of the resistances (\( \text{cm s}^{-1} \)) and equations 2.4.1g and h become:

\[ g = \frac{g_c + g_s \cdot g_a}{g_c + g_s + g_a} \quad \text{(2.4.1i)} \]

\[ g_s = \frac{g \cdot g_a}{g_a - g} \quad \text{(2.4.1j)} \]

For net CO\(_2\) assimilation, \( A \):

\[ A = \frac{\left( C_{\text{a}} - C_{\text{i}} \right)}{R'} \quad \text{(2.4.1k)} \]
where \( Q_m \) and \( Q_i \) are mass CO\(_2\) concentrations (g m\(^{-3}\)), with the same subscripts as before and \( R' \) is the resistance to CO\(_2\) diffusion (s m\(^{-1}\)).

\( R' \) can be partitioned in a similar way to \( R \):

\[
R'/100 = (1/r_s' + 1/r_c')^{-1} + r_a'
\]

(2.4.11)

where the prime ' denotes the resistance for CO\(_2\) diffusion. The stomatal resistances to water vapour and CO\(_2\) are related by the ratio of the molecular diffusivities of the gases in air, assuming that only diffusion takes place in the pore (Jarvis 1971, p. 569):

\[
D/D' = 1.605
\]

(2.4.1m)

In the boundary layer where conditions change from molecular diffusion to turbulent transfer the relationship is (Thom, 1968):

\[
(D/D')^{2/3} = 1.37
\]

(2.4.1n)

Equation 2.4.1k becomes:

\[
A = (Q_m - Q_i) / (1.605r_s + 1.37r_a)
\]

(2.4.1o)

Using the molecular weight and molar volume, corrected from S.T.P. to 20 °C:

\[
A = 1.829 (C_a - C_i)/(1.605r_s + 1.37r_a)
\]

(2.4.1p)

where \( C_a \) and \( C_i \) are volume concentrations (cm\(^3\) m\(^{-3}\)). In a leaf chamber, CO\(_2\) flux is determined from the difference between the volume concentration entering and leaving the chamber \((C_{in} - C_{out})\), the flow rate, and the leaf area enclosed:

\[
A = 1.829 (C_{in} - C_{out}) \cdot f/a
\]

(2.4.1q)

In a well stirred chamber \( C_a = C_{out} \), so from equation 2.4.1p:

\[
C_i = C_a - (A/1.829)(1.605r_s + 1.37r_a)
\]

(2.4.1r)
2.4.2 Gas exchange equipment

General

An "open" gas exchange system was used, (Jarvis 1971). The system was essentially a development of that used by Ludlow and Jarvis (1971), and has been described by Leverenz (1978) and Ng (1978).

The equipment allowed control and measurement of temperature, CO₂ concentration, VPD, spectral composition and quantum flux density within a single, stirred shoot chamber. The system was characterised by a large degree of precision in measurement, and stability and flexibility in environmental conditions imposed.

Figure 2.4.2a is a diagram of the main elements of the system and their pneumatic relations. Details of the equipment (manufacturers, model numbers, etc.) are given in Appendix 2, together with detailed diagrams.

Pneumatic Layout

Air, pumped from outside the building (c. 25 m above ground level), either bypassed or entered the CO₂ or water vapour conditioning units before division into reference and chamber air streams.

In the CO₂ conditioning unit CO₂-free air from CO₂ absorbent columns and pure cylinder CO₂ were mixed using three gas mixing pumps cascaded in series to give air containing CO₂ concentrations from zero to 5%.

In the water vapour conditioning unit air was humidified and mixed with silica gel-dried air with precision flow meters. Manual vapour pressure control was better than 0.03 kPa.
Figure 2.4.2a Diagram of the main elements in the gas exchange system. Key: 1 hemispheric reflector, 2 metal halide lamps, 3 Fresnel lenses, 4 glass filters, DPM dew-point hygrometer, IRGA infra-red gas analyser, TMF thermal mass flow meter, RHS relative humidity sensors, M10 electronic manometer. (not to scale)
Air was delivered to the shoot chamber through a thermal mass flow controller at rates between 10 and 45 cm$^3$ s$^{-1}$, with negligible fluctuations.

Chamber

The chamber was cast in brass with two vertical perspex windows and a removable door, through which the shoots were inserted. The internal volume was c. $10^3$ cm$^3$. The chamber rested on a thermoelectric Peltier device; thyristor drive current regulation ensured leaf temperature control at 20 °C was better than 0.2 °C. Air in the chamber was rapidly mixed with a fan over the inlet; linear air speeds were between 0.5 and 2.5 m s$^{-1}$ (measured with a hot-wire anemometer), fluctuating in space and time.

Measurement Apparatus

Reference air and air from the chamber was pumped at flow rates of 8 cm$^3$ s$^{-1}$ through a solenoid valve switching complex to the humidity and CO$_2$ measurement apparatus. Pressures were maintained equal and less than 30 Pa above atmospheric values, monitored with an electronic manometer (see Appendix 4.5).

Reference and chamber air streams each passed over a thin film capacitance humidity sensor (Salasmaa and Kostamo 1975). Adjacent to each sensor was a Cu-Co soldered thermocouple junction. The sensors were mounted in Teflon plugs inserted in an aluminium block. The drilled air passages for reference and sample air streams were c. 30 cm in length to minimise temperature fluctuations. The block was thermally insulated, and maintained at high temperatures (29-33 °C) giving low relative humidities (RH) of 20-45% for VPD in the
range 0.3–10 kPa and eliminating the sensor hysteresis which occurred at relative humidities above 60%. In addition, the same air stream could be passed over both sensors to give a zero differential check; in practice this was always c. 6% of the difference in RH between sample and reference air streams. Sensor output could be individually recorded with the thermocouple readings, together with the differential output from both sensors which was used for a continuous trace of transpiration over time.

The dew point of either air stream was also measured with a mirror dew-point hygrometer. In experiments this was used to monitor the chamber vapour pressure which was kept constant as stomatal conductance changed by altering the vapour pressure of the air entering the chamber.

The dew-point meter and the RH sensors were calibrated in situ with saturated air from a water bath, (see Appendix 4.1).

Air streams passed finally into the sample and reference cells of an infra-red gas analyser (IRGA), equipped with H₂O absorption band interference filters. The IRGA was operated in the differential mode and calibrated by the "path length" method using CO₂-free air and cylinder air of known CO₂ concentrations, (Parkinson and Legg 1971, see Appendix 4.5).

All flow switching for reference and sample air stream conditioning and measurement and for all transducer calibrations was effected by solenoid valves, electronically activated, together with all pumps, from a central panel.

Thermocouples for leaf, chamber air and RH sensor temperature measurement and leaf temperature control consisted of soldered 38 and 42 SWG Cu-Co wire measurement junctions. The reference junctions
were kept in an electronic ice-point unit, at 0.0 ± 0.1 °C.

A zirconium oxide ceramic cell oxygen meter monitored chamber oxygen levels in low O₂ experiments, (Björkman and Gauhl 1970). The logarithmic output was calibrated in 20 steps from 20% to 0.5% O₂ in N₂ using a single gas mixing pump.

Illumination

Plant material in the chamber was illuminated by one metal halide lamp in a hemispheric reflector each side of the chamber. Near parallel, horizontal beams (see Leverenz and Jarvis 1979) were produced through Fresnel lenses with focal lengths of 20 cm. Infra-red wavelengths were removed by heat mirrors. Quantum flux density was changed in steps with neutral glass filters. Broad band illumination with red (600-750 nm) or blue (400-500 nm) was effected by coloured glass filters. The measured spectrum is shown in Figure 2.4.2b, further details of the filters, sources and measurement devices can be found in Appendix 3.

Quantum flux density incident on the shoot was measured with one photodiode facing each window, positioned in the plane of the shoot. These were calibrated in situ for each waveband used against a PhAR quantum sensor and a quanta spectrophotometer. The incident quantum flux density each side was combined for the total, (Q₁).

2.4.3 Data acquisition and computation

All transducer outputs were displayed on a high resolution digital voltmeter, interfaced with an analogue scanner and data transfer unit; signals were also displayed on a 12-point chart recorder. Readings were manually noted and entered immediately on a computer.
Figure 2.4.2b  Measured quantum flux density spectral distribution in the leaf chamber with filters KG2+GG400 'white light', BG12 'blue light' and RG610 'red light'. See Appendix 3.
terminal for the calculation of results. From May 1979 the data
transfer unit was interfaced with a paper tape punch; data sets could
be transferred to the tape at time intervals down to 10 seconds. All
subsequent computation used FORTRAN programs.

2.4.4 Additional equipment

All E, g_s and A measurements are expressed on a projected leaf
area basis measured with an optical planimeter, accurate to 0.1 mm^2.
The factor for conversion to total leaf area is c. 2.5 for Scots pine
needles (Körner, Scheel and Bauer 1979), between 2.5 and 2.7 for Sitka
spruce needles (Ludlow and Jarvis 1971) and 2 for dayflower leaves.
Assimilation rates on a projected leaf area basis can be converted to
a plan area basis (the needle area actually receiving light in situ
in the chamber) by a factor of 2.2 for Scots pine shoots, determined
photographically.

Stomata occur in longitudinal rows on the abaxial and adaxial
surfaces of Scots pine needles (Ng 1978) and in parallel rows either
side of the midrib on the adaxial (but lower) side of Sitka spruce
needles (Jeffree, Johnson and Jarvis 1971). Dayflower leaves are
amphistomatous.

Xylem pressure potentials were measured using the pressure chamber
method. Pairs of pine needles were inserted in a small volume
pressure chamber; spruce and dayflower shoots were measured in a
larger pressure bomb.
2.4.5 Error Analysis

Combining equations 2.4.1f and 2.4.1j, stomatal conductance is calculated from:

\[
g_s = \frac{1}{a} \frac{e_i - e_{out}}{f (e_{out} - e_{in})} - r_a
\]  

(2.4.5a)

The determination of \( g_s \) is therefore subject to error in air and leaf vapour pressure, flow rate, leaf area and aerodynamic resistance measurement. The latter was empirically determined from water vapour flux measurements and is also subject to errors in the other measurements (see Appendix 4.3). However, \( r_a \) is low in conifer shoots due to the small dimensions of individual needles and the dissected shoot structure. In a well stirred chamber, with high air speeds, \( r_a \) is small (c. 0.03 s cm\(^{-1}\)). Total shoot resistances vary between 1.3 and 30 s cm\(^{-1}\). Errors in aerodynamic resistance are therefore of small importance in measurements with conifers as \( r_a \) varies from 0.1\% of the total resistance at high \( r_s \) (e.g. in the dark), to 2.4\% at low resistances (e.g. at the highest quantum flux densities).

Errors in aerodynamic resistance become more important with the wider, laminar leaves of dayflower with a larger \( r_a \) (0.14 s cm\(^{-1}\)). Total shoot resistances ranged between 0.7 and 11.0 s cm\(^{-1}\) and \( r_a \) varies between 1.3\% of the total resistance in the dark, to 20.0\% at the lowest \( r_s \) measured.

Net \( \text{CO}_2 \) assimilation, \( A \), calculated from equation 2.4.1p is also subject to errors in the flow rate and leaf area, and in the \( \text{CO}_2 \) concentration measurement. The errors in photosynthetic measurements
with a differential IRGA have been discussed in detail by Jančič (1970).

Intercellular space CO$_2$ concentration, $C_i$, is calculated from simultaneous A and $g_s$ measurements. Combining equations 2.4.1f, 2.4.1q and 2.4.1r:

$$C_i = C_a - \left( \frac{(C_{in} - C_{out}) \cdot 1.605}{(e_{out} - e_{in})} + 0.37 r_a \right)$$  \hspace{1cm} (2.4.5b)

where $C_a$ and $C_{in}$ are determined from the IRGA differential and sensitivity measurements. The aerodynamic resistance is less important and $C_i$ is largely independent of flow rate and leaf area.

Calculation of the error involved in equations 2.4.5a, 2.4.1p and 2.4.5b is complex. In particular, the errors in vapour pressure measurements, $e_{in}$ and $e_{out}$, are not independent as Ng (1978) has assumed. The same is true of the CO$_2$ concentration measurements $C_a$ and $C_{in}$.

It should be noted that large relative errors can result from the difference of numbers that are nearly equal. This has two implications in the above equations; errors were larger at a small VPD (where $e_{out}$ tends to $e_{in}$) and when the differentials $(e_{out} - e_{in})$ and $(C_{in} - C_{out})$ were small.

This latter source of error was minimised by maintaining large differentials using low flow rates and large leaf areas wherever possible. Sources of error in all the measurements and techniques used to minimise them are discussed in Appendix 4.

Random error was reduced by using constant flow rates in any one set of experiments (except where stated). The digital voltmeter used for all transducer readings results in a large degree of precision. In addition, in all experiments since May 1979 (experiments with...
Sitka spruce and dayflower), a data acquisition system was used in conjunction with the RH sensors. This allowed the averaging of 6-10 sets of readings at each measurement period, reducing random error considerably. In experiments with Scots pine data points were based on single measurements of the difference between the dew-points of reference and chamber air streams when readings were stable.

2.5 Experimental Procedure

2.5.1 Conifers

Attached top lateral shoots were placed in the chamber the preceding night in darkness and in the VPD and temperature conditions that were subsequently to be used. No experimental treatments were imposed outside the photoperiods of the growth room from which the plants were taken. Three days prior to the experiment, pine shoots were thinned by removal of about 1/4 of the total pairs of needles, mainly towards the tip of the shoot. Trimming reduced the quantum flux density for saturation of the light response (Ng and Jarvis, 1980), and in these experiments the available quantum flux density in separate spectral regions was limited. The remaining needle area on a shoot averaged 35 cm$^2$.

Sitka spruce shoots were trimmed to remove top and bottom needles that would otherwise point directly at the light sources (see Leverenz and Jarvis 1979). The shoots were arranged to display the remaining needles perpendicular to the beam. The average needle area left on the shoot was c. 15 cm$^2$.

Plant parts outside the gas exchange chamber were enclosed in a
humid, plastic bag to minimise water loss.

Previous study on conifers has shown considerable sluggishness in the response of stomatal conductance to light (e.g. Watts and Neilson 1978). Ng and Jarvis (1980) have described "hysteresis" in the response of $g_s$ in Scots pine to quantum flux density. At 10 °C only 66.7% of the increase in $g_s$ had taken place after 110 minutes, whereas at 20 °C no hysteresis was apparent after 2.5 hours equilibration time. For this reason all work with Scots pine and Sitka spruce reported here was carried out at 20 °C and with 110 minutes allowed for equilibration, unless otherwise stated. Readings were taken, and the IRGA recalibrated at 60 and 110 minute intervals after a change in conditions.

All experiments with Scots pine were carried out at 20 °C and a constant VPD of 0.7 kPa. Ng (1978) has shown these conditions to result in maximum $g_s$ in plants with identical preconditioning.

In Sitka spruce in a growth room, Watts and Neilson (1978) found a linear decrease in $g_s$ with VPD increase from 0.05 to 1.20 kPa. All Sitka spruce experiments were therefore performed at a low VPD of 0.5 kPa at 20 °C leaf temperature.

2.5.2 Dayflower

Stomatal conductance changed rapidly in dayflower with changes in conditions. Treatment periods were reduced to between 45 and 75 minutes, which was still sufficient to ensure equilibrium conditions. Initial experiments showed that $g_s$ was sensitive to VPD in these plants; maximum $g_s$ occurred at the smallest VPD. Experiments were all carried out at a VPD of 0.33kPa with a leaf temperature of 20 °C. This VPD is close to the lower technical limit in the chamber,
especially at large quantum flux densities when the chamber walls can be 2 °C lower than leaf temperature. Lower VPD conditions caused condensation and experiments were curtailed. Experiments in which enclosed shoots showed guttation were also curtailed.

Preliminary experiments also indicated that $g_s$ declined markedly eight hours after the growth room "lights on" time. No experiments were extended beyond this time. Stomata in cut shoots in water closed even earlier; $g_s$ did not respond to CO$_2$-free air or light after being in the chamber overnight. Attached shoots were therefore used, and inserted into the chamber approximately 3 hours before the growth room "lights on" for at least one hour equilibration in darkness, before experiments commenced.
3.1 The Response of Stomata to CO$_2$

3.1.1 Introduction

Most plant species investigated show an inverse relationship between the degree of stomatal opening and the concentration of carbon dioxide in the leaf. Linsbauer (1916) first suggested that the cause of stomatal opening was the photosynthetic removal of intercellular CO$_2$. Early experimental evidence came from the work of Freudenberger (1940) studying the trends of stomatal movement on exposure to 0, 30, 150, 300 and 25,000 cm$^3$ m$^{-3}$ CO$_2$ in illuminated Canna leaves: she observed that changes to a lower CO$_2$ concentration stimulated opening and changes to a higher CO$_2$ initiated closure. This pattern of stomatal responses to CO$_2$ was also found in extensive studies by Heath and coworkers (e.g. Heath 1948, 1950, Heath and Russell 1954a and b). The early observations and hypotheses have been extensively reviewed (e.g. Heath 1959, Meidner and Mansfield 1968). Since that time, advances in techniques in physiological and biochemical studies have greatly broadened the understanding of the stomatal mechanism. However, the role of CO$_2$ in stomatal movement is still a subject of much controversy. The reviews of Raschke (1975a, 1979) cover many of the recent publications; however, there is still no one unifying hypothesis to embrace all the observations.

This chapter assesses the range of reported responses of stomata to CO$_2$ (3.1.2). Contrasting results of experiments (3.2) for Scots pine, Sitka spruce and dayflower are presented (3.3) and discussed.
3.1.2 The range of responses

A wide range of responses to CO₂ have been reported, varying from complete insensitivity in cotton (*Gossypium hirsutum* L.) between 200 and 2000 cm⁻³ ambient CO₂ concentration (Bierhuizen and Slatyer 1964), to absolute control of stomatal aperture by CO₂ concentration in maize (Raschke 1965). The differences are not restricted to inter-specific contrasts. Within one species response to CO₂ varies with environmental conditions, (e.g. Farquhar, Dubbe and Raschke 1978, Lüschen 1976). These variations in the control of stomata exercised by CO₂ with different conditions is not unexpected because environmental variables interact together and with CO₂ in the control of stomatal movements. Some of the earlier work on CO₂ responses overlooked the importance of such interactions with the result that there are conflicting reports.

The work of Heath and Milthorpe (1950) on wheat stomata (*Triticum aestivum* L.) first demonstrated the additional effects of water stress on the response to CO₂. Changing the flow rate of dry air across a wheat leaf in the light changed the response of stomata to CO₂. At low flow rates there was no effect of CO₂ between 300 and 0 cm⁻³ but at high flow rates there was a large change in resistance, attributed to increasing water stress. Heath and Russell (1954a and b) illustrated the interaction between light intensity and CO₂ in experiments in which air was passed both across and through a wheat leaf. Stomata were more open at any given external CO₂ concentration at higher light intensities. Heath and Russell considered that light changed the effective CO₂ concentration in the
guard cells through photosynthesis, and changed the apparent response of stomata to CO₂.

Stomata do not respond directly to the CO₂ concentration around the leaf. The CO₂ sensor for stomatal action is located in the guard cells (Mouravieff 1956, Pallaghy 1971, Raschke 1972). The inner lateral walls of the guard cells are permeable to CO₂ (Meidner and Mansfield 1965) and the majority of workers since Scarth and Shaw (1951) and Williams (1954) have assumed that the concentration in the guard cells is dependent on the concentration in the leaf intercellular spaces (Cᵢ) not the external CO₂ concentration. The intercellular CO₂ concentration depends on the flux of CO₂ through the pore, and is determined by the rate of net assimilation (A), the ambient CO₂ concentration (Cₐ) and the stomatal conductance (gₛ). It has been suggested that Cᵢ acts as the signal in a feedback control loop of A, gₛ and Cₐ (Raschke 1975a); for example, changes in illumination change A and therefore Cᵢ, causing changes in gₛ. The intercellular CO₂ concentration cannot normally be independently measured and is calculated from determinations of A, gₛ and Cₐ. This results in a general correlation of gₛ and Cᵢ when, for example, stomata open in response to increasing quantum flux density. Only experiments in which changing Cₐ is used to manipulate Cᵢ permit firm conclusions on the response of stomata to CO₂.

In addition, results from experiments in different conditions are only comparable when expressed as the response of stomata to the intercellular CO₂ concentration rather than the ambient CO₂ concentration, as A will change with different light intensities, temperatures and the photosynthetic capacities of the plants.

However, some reservations on the assumption that Cᵢ is the CO₂
concentration to which stomata respond should be noted. Allaway and Milthorpe (1976) suggest that the CO$_2$ concentration at the guard cell inner lateral walls may be closer to $C_a$ than to mean $C_i$ when stomata are open. Raschke (1975b) suggests that the stomata "sense" a CO$_2$ concentration somewhere between the average in the intercellular spaces ($C_i$) and in the atmosphere outside the leaf, and he defines a CO$_2$ concentration in the pore at the midpoint of the concentration drop from the ambient CO$_2$ to the intercellular CO$_2$. The point may be valid but it is unjustified to assume a particular concentration difference from a profile which is not known. Moreover, the work of Tyree and Yianoulis (1980) indicates that between 50 and 75% of the evaporation through a stoma comes from the guard cell and subsidiary cell inner lateral walls; the calculated intercellular CO$_2$ is probably a close approximation to the CO$_2$ concentration affecting the guard cells.

Maize (Zea mays L.)

A large stomatal response to $C_i$ has been noted in maize in a range of conditions and by a number of workers (e.g. Meidner 1962, Pallas 1965, Raschke 1965, 1972, Farquhar et al 1978, Raschke, Hanebuth and Farquhar 1978).

Stomata in maize can open in response to lowered $C_i$, down to zero $C_i$ (Meidner 1962, Raschke 1979, Fig. 17) or show a maximum opening at 100 cm$^3$ m$^{-3}$ $C_i$ (Raschke 1976). This changing sensitivity to low $C_i$ may have caused the hysteresis noted by Dubbe, Farquhar and Raschke (1978). This latter study showed maximum sensitivity to intercellular CO$_2$ concentration between 100 and 200 cm$^3$ m$^{-3}$.

Maize stomata closed at external concentrations of 2000 cm$^3$ m$^{-3}$.
The closing response to increased CO$_2$ showed the same lag times, velocities and dependence on concentrations in light and in darkness (Raschke 1972), suggesting that the same mechanism is involved in the response to CO$_2$ in the light and in the dark. In particular manipulation of C$_1$ experimentally between 0 and 350 cm$^{-3}$ m$^{-3}$ controlled stomatal conductance whether in the light or in the dark (Pallaghy 1971, Raschke et al 1978).

Raschke (1975a) has concluded that stomatal aperture in maize is controlled by a light-independent response to C$_1$. The direct effects of light are superimposed on this CO$_2$ response (Karvé 1961, Raschke 1966, 1967a, 1979, Raschke et al 1978). This will be discussed in detail in Chapter 4.

Sensitivity to CO$_2$ differed between field-grown and greenhouse-grown maize plants (Farquhar et al 1978, Raschke 1979, Fig 17), as has been found in many stomatal responses to environmental variables (e.g. Burrows and Milthorpe 1976). Different responses of abaxial and adaxial stomata in the same leaves to environmental variables are also well known (see reviews of Turner 1979, Pospíšilová and Solárová 1980). In maize leaves adaxial stomata were more sensitive to increased C$_a$ than the abaxial stomata (Domes 1971, Gifford and Musgrave 1973).

The observation that light and CO$_2$ effects can be independent has long been recognised; stomata can be maintained open in the dark in CO$_2$-free air and opening can be initiated in the dark if the intercellular spaces are swept with CO$_2$-free air (see Heath 1959, Mouravieff 1956, Mansfield 1965, Allaway and Mansfield 1967).
The most obvious example of nocturnal opening is in plants possessing the Crassulacean acid photosynthetic pathway (CAM plants). These plants, when under water stress, show "an inverted stomatal rhythm" (Nishida 1963). High intercellular CO₂ concentrations, sometimes higher than 2.0% have been found in a range of taxonomically widely separated CAM plants during deacidification in the daytime (Neales 1970, Cockburn, Ting and Sternberg 1979, Spalding et al 1979). Stomatal resistances in these conditions were very high (larger than 150 s cm⁻¹ in Opuntia ficus-indica L., Cockburn et al 1979). In the dark Cᵢ decreased to 270-280 cm⁻³ m⁻³ (Spalding et al 1979) when stomatal resistance decreased greatly. Neales (1970) and Cockburn et al (1979) tested the hypothesis of control of stomata by intercellular CO₂ concentration in CAM plants: external concentrations of up to 2% were applied to cladodes of O. ficus-indica and leaves of Agave desertii Engelm. and A. americana and stimulated stomatal closure in the dark. There is a further example of Cᵢ control in O. inermis in the data of Osmond and colleagues (1979a and b) when stomatal opening occurred in the light in conditions of rapid CO₂ fixation and low Cᵢ. Osmond et al (1979b) note that cladode resistance was much smaller in the light in these conditions of daytime fixation than at night, and this may indicate an additional direct response of stomata to light.

C₃ and C₄ plants

Pallas (1965) and Akita and Moss (1972) have suggested that stomata in C₃ plants are less sensitive to external CO₂ concentrations than stomata in C₄ plants. However, such a comparison is difficult to make as the photosynthetic capacities of the two
groups of plants differ. Changes in $C_a$ will cause different changes in $C_i$, depending on the response of net assimilation to CO$_2$; measuring different stomatal responses to $C_a$ does not necessarily indicate different stomatal responses to CO$_2$.

The experiments of Akita and Moss (1972) are not conclusive; the decline in transpiration rate of wheat, barley (Hordeum vulgare L.) and dandelion (Taraxacum officinale L.) with increasing $C_a$ from 0 to 0.08% was smaller than that in maize, foxtail (Setaria viridis L.) and pigweed (Amaranthus retroflexus L.). However, the VPD increased from 0.2 to 1.7 kPa during the experiments as transpiration declined and the different response to CO$_2$ between species may be confounded by different responses to humidity. It should be emphasised that the effect of $g_s$ on the local evaporative conditions in a leaf chamber is an artificial situation which rarely prevails in natural conditions.

Pallas (1965, no specific names given) demonstrated a decline in transpiration in sorghum and maize to a constant low level at 3000 and 2000 cm$^3$m$^{-3}$ respectively, indicative of stomatal closure (verified microscopically). Transpiration in soybean and cotton decreased with increasing $C_a$ up to 2000 cm$^3$m$^{-3}$ and increased again at 4000 cm$^3$m$^{-3}$. Stomata of tomato did not close at those concentrations.

The discussion above on maize, and data on Sorghum album Parodi (Ludlow and Wilson 1971), Pennisetum typhoides [Burm.] S.E. H. (McPherson and Slatyer 1973), Spartina townsendii s. l. (Long and Woolhouse 1978), Amaranthus powelli S. Wats. (Dubbe et al 1978) and Hilaria rigida [Thurb.] Benth. ex Scribn. (Nobel 1980) also indicate that stomata in C$_4$ species are sensitive to CO$_2$, though a general comparison with the sensitivity of stomata in C$_3$ species is not
possible.

High CO₂

Knowledge of the response of stomata to high ambient CO₂ concentration is limited. Spray carnation plants (Dianthus caryophyllus L.) showed increased stomatal resistance up to an intercellular CO₂ concentration of 3000 cm³ m⁻³, particularly at higher light intensities (Enoch and Hurd 1977). Stomatal resistance doubled in plants of vine (Vitis vinifera L.) in an enriched atmosphere of 1200-1300 cm³ m⁻³ (Kriedmann, Sward and Downton 1976). It should be noted that the apparent insensitivity of stomata to CO₂ in subsequent gas exchange experiments may be due to the low (2%) oxygen levels used in those experiments. Stomata of vine leaves closed when C_a increased from 0 to 1.0-1.6% (Loveys, Kriedmann and Törökfalvy 1973). The effect was not fully reversible as the authors claim; gₛ changed from 0.82 (CO₂-free) to 0.03 (high CO₂) to 0.40 cm s⁻¹ (CO₂-free).

Sunflower (Helianthus annuus L.)

Some species appear particularly variable in the response of stomata to CO₂. Coudriaan and van Laar (1978) report that "stomata were wide open" in sunflower between 200 and 1200 cm³ m⁻³, at four light levels. Stomata remained open in the dark and in high CO₂. The implications from their presentation are unclear, however, as they report an 8-fold change in gₛ during the experiment, from 0.63 to 5.0 cm s⁻¹. Data of Whiteman and Koller (1967a) on sunflower have been cited as evidence of stomatal sensitivity to high CO₂ (Raschke 1975a) and as demonstrating a "negligible response of stomatal
aperture to $C_1$ (Goudriaan and van Laar 1978). Figure 2 in Whiteman and Koller shows a doubling of stomatal resistance with increases in $C_1$ from 50 to 350 cm$^3$ m$^{-3}$. However, the implications are clouded by similar increases in resistance with change from low to high light, which may reflect inadequate temperature control, or increased water stress.

**Abscisic Acid and CO$_2$ effects**

Differences in the sensitivity of stomata to CO$_2$ may reflect "sensitisation" of stomata to CO$_2$ (Raschke 1975a). Stomatal conductance showed no response to changes in ambient CO$_2$ from 0 to 600 cm$^3$ m$^{-3}$ in well-watered Xanthium strumarium L. plants. The stomata were sensitised to CO$_2$ by increasing the levels of endogenous abscisic acid (ABA) in the leaves by water-stress (Raschke 1974, 1976) or chilling (Drake and Raschke 1974). Adding ABA to the transpiration stream was equally effective in inducing a response to CO$_2$ (Raschke 1975b, Dubbe et al. 1978). Raschke (1975b) suggested that, in this species, CO$_2$ is only effective in inducing closure in the presence of ABA, and ABA administered in the transpiration stream only causes closure if CO$_2$ is present. However, Mansfield (1976) found that stomata in the same species responded to both CO$_2$ and ABA separately; together, the effects were only additive.

Other species that show stomatal sensitivity to CO$_2$ in normal conditions showed increased sensitivity to CO$_2$ after addition of ABA (Dubbe et al. 1978) and an interaction of CO$_2$ and ABA has been noted in dayflower and cotton (G. hirsutum, Raschke 1975b). However, ABA causes stomatal closure in dayflower without CO$_2$ involvement: one bioassay for ABA uses dayflower epidermal strips in a CO$_2$-free
<table>
<thead>
<tr>
<th>Species</th>
<th>Range</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fagus sylvatica</td>
<td>100-800</td>
<td>Koch 1969</td>
</tr>
<tr>
<td>Malus sylvestris</td>
<td>100-300</td>
<td>Warrict 1977</td>
</tr>
<tr>
<td>Eucalyptus pauciflora</td>
<td>100-300</td>
<td>Wong et al. 1978</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>atm, 1300</td>
<td>Kriedemann et al. 1976</td>
</tr>
<tr>
<td>Pelargonium zonale</td>
<td>atm, 1360</td>
<td>Koch 1969</td>
</tr>
<tr>
<td>Bacopa caroliniana</td>
<td>atm, 1340</td>
<td>Mouravieff 1958</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>atm, 1200</td>
<td>Fischer 1968</td>
</tr>
<tr>
<td>Vigna luteola</td>
<td>atm, 1300</td>
<td>Ludlow &amp; Wilson 1971</td>
</tr>
<tr>
<td>Sesamum indicum</td>
<td>50-300</td>
<td>Hall &amp; Kaufmann 1975</td>
</tr>
<tr>
<td>Canna sp.</td>
<td>30-25000</td>
<td>Freudenberg 1940</td>
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<td>Sansevieria sp.</td>
<td>300, 1150</td>
<td>Nelson &amp; Mayo 1975</td>
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<td>Rhamnus frangula</td>
<td>100-400</td>
<td>Allaway &amp; Mansfield 1967</td>
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<td>Brassica pusana</td>
<td>0-1340</td>
<td>Gaeta 1959</td>
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<td>Coleus spicatus</td>
<td>0-450</td>
<td>Dubbe et al. 1978</td>
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<td>Glycine max</td>
<td>0-4000</td>
<td>Pallas 1965</td>
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<td>Lycopersicon esculentum</td>
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<td>Nelson &amp; Mayo 1975</td>
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<tr>
<td>Helianthus annuus</td>
<td>100-400</td>
<td>Whitman &amp; Koller 1967a</td>
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<td>Kochia indica</td>
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<td>Whitman &amp; Koller 1967b</td>
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<tr>
<td>Commelina communis</td>
<td>0-375</td>
<td>Sachs 1979</td>
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<tr>
<td>Triticum aestivum</td>
<td>0-840</td>
<td>Heath &amp; Russell 1954a &amp; b</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>72-1530</td>
<td>Laik, Oja &amp; Koll 1980</td>
</tr>
<tr>
<td>Avena sativa</td>
<td>0, atm</td>
<td>e.g. Brogden &amp; Johnson 1975</td>
</tr>
<tr>
<td>Allium cepa</td>
<td>0, atm</td>
<td>Heath &amp; Heidner 1957</td>
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**CAM plants**

<table>
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<tr>
<th>Species</th>
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<th>Author(s)</th>
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<tr>
<td>Agave americana</td>
<td>200-1000</td>
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<tr>
<td>A. deserti</td>
<td>atm, 22</td>
<td>Cockburn et al. 1979</td>
</tr>
<tr>
<td>Opuntia ficus-indica</td>
<td>atm, 22</td>
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**Ferns**

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<th>Author(s)</th>
</tr>
</thead>
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<tr>
<td>Phyllitis scoatopendrium</td>
<td>0, 880, 10000</td>
<td>Mansfield &amp; Willmer 1969</td>
</tr>
<tr>
<td>Polypodium vulgare</td>
<td>0-400</td>
<td>Lisch 1976</td>
</tr>
</tbody>
</table>

where: $s_g$ is stomatal conductance, $E$ is transpiration, $r_s$ is stomatal resistance, $v_P$ is viscous flow porometer and $um$ is aperture measurement. $C_s$, $C_a$ and $atm$ are intercellular, ambient and normal atmospheric CO₂ concentrations, respectively, (cm² m⁻³).
atmosphere (Ogunkamni, Tucker and Mansfield 1973). Stomata in both epidermal strips (Travis and Mansfield 1977, 1979b) and whole leaves (Saxe 1979) of dayflower are normally sensitive to CO₂.

It is apparent that the role of ABA in the CO₂ response of stomata has not been resolved. There is no doubt that ABA is important in particular cases. An extreme example is the reversion of the wiltly tomato (*Lycopersicon esculentum* Mill.) mutant *flacca* to normal stomatal control of water loss on application of ABA (Imber and Tal 1970). This, and other work on the mutant, has prompted Tal and his coworkers to conclude that "ABA, in the intact plant, ensures normally regulated stomatal behaviour".

**Other species**

Table 3.1.2 lists many of the species in which a stomatal response to CO₂ has been found. Many of the results have limited value, being single tests between CO₂-free air and normal atmospheric concentration; there are relatively few complete response curves of $g_s$, $r_s$ or aperture to intercellular CO₂. As mentioned before, results where a response to C has been measured are confounded by changes in net assimilation resulting in changing $C_i$. It is clear from the literature that lowered CO₂ concentrations generally enhance stomatal opening.

**Insensitivity to CO₂**

However, contrary responses to CO₂ have noted: stomata in tomato and cotton did not close in CO₂ concentrations up to 4000 cm³ m⁻³, as mentioned above (Bierhuizen and Slatyer 1964, Pallas 1965). Stomatal resistance in *Brassica oleracea* L. var. *acephala* (Parkinson 1968) and
Sitka spruce (Ludlow and Jarvis 1971) increased in ambient CO$_2$ concentrations lower than 150 cm$^3$ m$^{-3}$. Sitka spruce showed no stomatal closure at higher C$_a$ concentrations. An inhibitory effect of low concentrations (lower than 100 cm$^3$ m$^{-3}$) has been noted in *X. strumarium*, *G. hirsutum*, maize (Raschke 1975a), *Sorghum alnum* (Ludlow and Wilson 1971), *Spartina townsendii* (Long and Woolhouse 1978) and *Hilaria rigida* (Nobel 1980). Insensitivity to CO$_2$ at high water potentials and thus low endogenous ABA levels has been discussed above; however, the insensitivity of Sitka spruce stomata to CO$_2$ persists at low water potentials (lower than -2.4 MPa, Beadle 1977).

Beadle concludes that "at any water potential, the response [to C$_a$ in Sitka spruce] can be described by a parallel line horizontal to the C$_a$ axis". Stomatal conductance in Scots pine was insensitive to CO$_2$ from 0 to 5000 cm$^3$ m$^{-3}$ at three quantum flux densities and in darkness (Ng 1978).

These latter results with Sitka spruce and Scots pine are at variance with every other result reported. Furthermore, they suggest that stomatal conductance in these two species is independent of mesophyll assimilation and hence that the feedback loop of $A$, $g_s$, and C$_i$ is inoperative (Raschke 1975a). This has prompted further investigation of the response of stomata to CO$_2$ in these two species and a comparison with dayflower which is known to show stomatal sensitivity to CO$_2$. 
3.2 Experiments

The response of stomatal conductance to changing intercellular CO$_2$ in whole shoots of Scots pine, Sitka spruce and dayflower was examined using the methods described in Chapter 2.

Response of $g_s$ to $C_a$ in Scots pine and Sitka spruce

Time courses

Stomata of Scots pine and Sitka spruce take up to three hours to reach a new steady-state conductance after changes in quantum flux density (see Chapter 2.5.1). The time course of the response of $g_s$ to large changes in $C_a$ was investigated.

Shoots of Scots pine were illuminated with a low quantum flux density of white light (230 μE m$^{-2}$ s$^{-1}$) in normal atmospheric CO$_2$ concentrations. When $g_s$ reached a steady state after three hours, CO$_2$ was removed from the airstream. After a further three hours shoots were exposed to high $C_a$ (1000 cm$^3$ m$^{-3}$). (Experiment 3.1)

The time course of $g_s$ in Sitka spruce in response to large changes in $C_a$ (0 to 700 cm$^3$ m$^{-3}$) in darkness and in light was examined. Previous experiments had demonstrated that broad-band blue light was more effective than red light in opening stomata in Sitka spruce and Scots pine (see Chapter 4); one shoot was illuminated with low and medium quantum flux densities of blue light on one day and red light on a subsequent day. (Experiment 3.2)
Steady-state response

Shoots of Scots pine were illuminated in alternating two hour periods of red and blue light of equal quantum flux density in air of atmospheric CO$_2$ concentration or in CO$_2$-free air. The experiment is described in detail in Chapter 4.2; here only the response to CO$_2$-free air is relevant and the results are replotted from Figures 4.3.3a and b.

The results from the above experiments indicate that stomatal conductance in Scots pine responded to CO$_2$-free air but do not describe the sensitivity of $g_s$ across the physiological range; the response of $g_s$ to intercellular CO$_2$ concentrations between 0 and 600 cm$^3$ m$^{-3}$ was examined. Shoots of Sitka spruce and Scots pine were illuminated with red or blue light of equal quantum flux density; the ambient CO$_2$ concentration was changed in steps in the following sequence:

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>CO$_2$ (cm$^3$ m$^{-3}$)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Scots pine</td>
<td>Sitka spruce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>c. 350</td>
<td>c. 350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>500</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>200</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>150,120</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>120,100</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>100,60</td>
<td>600,700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.5</td>
<td>0</td>
<td>230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.5</td>
<td>600,700</td>
<td>230</td>
<td></td>
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</table>

The higher CO$_2$ figure in a time period refers to the concentration used in red light; single figures indicate the same concentration was used in red and blue light. All CO$_2$ figures refer to the CO$_2$ concentration entering the chamber, not the concentration around the leaf.
No steady-state stomatal conductance was established in the last treatment as $g_s$ was still declining slowly as the photoperiod was exceeded. (Experiment 3.3)

Response of $g_s$ to $C_{i}$ in dayflower

In contrast to conifers, stomatal conductance in dayflower responded rapidly to changes in $C_a$. The response of $g_s$ to large changes in $C_a$ was too rapid to measure because of the changing humidity conditions in the chamber; humidity of the inflowing air had to be adjusted to maintain a constant VPD. This necessitated a delay of 5-10 minutes before a new equilibrium in the chamber and reference air streams permitted renewed measurement. Preliminary experiments indicated that stomatal conductance started to change within 5-8 minutes and reached a new equilibrium within a further 10 minutes.

In a preliminary experiment single excised dayflower shoots were illuminated with either red, blue or white light in steps of increasing quantum flux density. After maximum $Q_i$ was reached (120, 230 and 770 $\mu$E m$^{-2}$ s$^{-1}$ in blue, red and white light, respectively) $C_a$ was reduced in steps to zero; experiments finished with a high $C_a$ treatment ($c. 400$ cm$^3$ m$^{-2}$). Only limited confidence can be attached to these results as cut shoots, in water, were later found to exhibit pronounced decrease of $g_s$ early in the photoperiod, and most of the measurements presented here were made late in the day. In addition, the VPD used (0.5 kPa) was later found to reduce $g_s$, and instability of the infra-red gas analyser gave larger errors than usual for $C_i$.

(Experiment 3.4)

The quantum flux density available when illuminating dayflower shoots from one side only (see Chapter 2.5.2) in the red and blue
wavebands is limited. Only white light provided a large range of quantum flux densities. The response of \( g_s \) to \( C_1 \) was examined in a range of quantum flux densities in white light.

As stated above, excised shoots in water could not be used on more than one day precluding the use of individual shoots in a prolonged series of treatments. Single attached shoots were used in subsequent experiments, overcoming the difficulties with intershoot variability which would otherwise necessitate a large number of replicates.

The response of stomatal conductance to \( C_1 \) was measured on four consecutive days at a different quantum flux density with a single attached shoot. The experiments commenced at high \( C_a \) and decreased in steps, at c. 100 minute intervals. Ambient \( CO_2 \) concentrations lower than 100 \( cm^3 m^{-3} \) were not used to avoid deleterious effects on the shoot. (Experiment 3.5)
3.3 Results

3.3.1 Response of $g_s$ to $C_a$ in conifers

The response of stomatal conductance in Scots pine to large changes in ambient CO$_2$ concentrations at a low quantum flux density of white light is shown in Fig 3.3.1a. Intershoot variability was large and $g_s$ varied by 100% in any one treatment. The opening response to light reached a steady state in three hours in normal atmospheric CO$_2$ concentrations. The response to CO$_2$ changes was slow; stomatal conductance approached an equilibrium value in three hours on exposure to CO$_2$-free air with $g_s$ approximately 90% larger than in atmospheric ambient CO$_2$ concentrations. The response to high $C_a$ was more variable; over a 4-5 hour period $g_s$ decreased to a value only slightly smaller than that at normal $C_a$.

Fig. 3.3.1b shows the response of $g_s$ in Sitka spruce to step changes in light and $C_a$. After increasing in blue light in CO$_2$-free air, $g_s$ barely decreased over three hours in 700 cm$^3$ m$^{-3}$ $C_a$. Stomatal conductance increased with a ten-fold increase in quantum flux density in CO$_2$-free air; changing to 700 cm$^3$ m$^{-3}$ $C_a$ caused a slight decrease in $g_s$. However, the natural photoperiod was exceeded and the experiment terminated. The decrease of $g_s$ in high $C_a$ could not be distinguished from the decrease in $g_s$ at the same time of day in shoots maintained in darkness. A lack of response to high $C_a$ was also observed in a similar experiment using red light (data not shown).

The above results emphasise the importance of adequate equilibration times for stomatal responses in conifers.
Fig. 3.3.1a Time course of $g_s$ in three shoots of Scots pine exposed to three ambient CO$_2$ concentrations. $\bar{C}_a$ Illumination commenced at time $t = 0$, $Q_i = 230 \mu$E m$^{-2}$ s$^{-1}$, white light.

(Experiment 3.1)
Fig. 3.3.1b Time course of $g_s$ in one shoot of Sitka spruce exposed to different ambient CO$_2$ concentrations ($C_i$). Experiment commenced in darkness and shoot illuminated with blue light at $Q_{i1} = 25$ and $Q_{i2} = 230 \mu$E m$^{-2}$s$^{-1}$. (Experiment 3.2)
Fig 3.3.1c shows the increase of $g_s$ in Scots pine in CO$_2$-free air compared to that in normal CO$_2$ concentrations in red and blue light of equal $Q_i$; the final values in white light have been replotted from Fig. 3.3.1a for comparison. CO$_2$-free air resulted in a 80% and 50% increase of mean $g_s$ in red and blue light, respectively.

Figs. 3.3.1d and e show the response of $g_s$ to $C_i$ in Sitka spruce and Scots pine, respectively, at equal red and blue quantum flux density. There was a clear increase of $g_s$ at below-atmospheric $C_a$ in both species and wavebands (this latter point is considered in detail in Chapter 4). Stomatal conductance in CO$_2$-free air was nearly double that in normal $C_a$ in Scots pine shoots illuminated with blue light. The difference in red light was less, but is statistically significant. In Sitka spruce stomatal conductance only increased by a third at low $C_i$ in both blue and red light. In both species maximum sensitivity of stomatal conductance to $C_i$ was between 100 and 250 cm$^2$ m$^{-3}$; there was no reduction in $g_s$ at very low $C_i$. Conductance decreased slowly on changing to high $C_a$ from very low $C_a$ in Sitka spruce, but it was not possible to distinguish between the effects of endogenous stomatal rhythm and increased $C_a$ in this final treatment. Shoot or needle water potential was between -0.5 and -0.7 MPa in all plants and no changes greater than -0.2 MPa were measured over a day.

3.3.2 Response of $g_s$ to $C_i$ in dayflower.

The response of stomatal conductance to changes in intercellular CO$_2$ was large in red, white and blue light (Fig. 3.3.2a). Maximum sensitivity to $C_i$ was between 100 and 300 cm$^2$ m$^{-3}$; the response saturated at intercellular CO$_2$ concentrations lower than 70 cm$^2$ m$^{-3}$. 
Fig. 3.3.1c Mean \( g_s \) in Scots pine in atmospheric and zero ambient \( \text{CO}_2 \) concentrations in equal \( Q_1 \) of red and blue light (230 \( \mu \text{E m}^{-2}\text{s}^{-1}) \). \( g_s \) from Fig. 3.3.1a redrawn for comparison. Significance levels of the increase in zero \( C_a \) indicated: *** \( P<0.001 \), ** \( P<0.01 \), * \( P<0.05 \) derived from paired t-tests (Experiment 4.4)
Fig. 3.3.1d Relationship between $G_r$ and $C_i$ in Sitka spruce in equal $Q_i$ of red (o) and blue (●) light (230 μE m$^{-2}$ s$^{-1}$). Treatments imposed from atmospheric $C_a$ to low $C_a$ to high $C_a$. Points are means of 4 shoots ± 1 s.e.m., sin$^{-1}$ transformation. At $G_r = 1.0$ $g_s = 0.623 \pm 0.033$ cm s$^{-1}$ (Experiment 3.3).
Fig. 3.3.1e Relationship between $G_r$ and $C_i$ in Scots pine in equal $Q_1$ of red (○) and blue (●) light (230 µE m$^{-2}$s$^{-1}$). Treatments imposed from atmospheric $C_a$ to high $C_a$ to 200 cm$^3$ m$^{-3}$ to low $C_a$. Points are means of 4 shoots ± 1 s.e.m., sin$^{-1}$ transformation.

At $G_r = 1.0$, $g_s = 0.532 \pm 0.036$ cm s$^{-1}$ (Experiment 3.3)
Intershoot variability was large and no conclusion should be drawn from the different magnitudes of \( g_s \) in red, blue and white light (see Section 3.2 and Chapter 4).

The large effect of intercellular CO\(_2\) on \( g_s \) is also apparent in Fig. 3.3.2b. Stomata opened in the dark if \( C_i \) was lowered, and \( g_s \) was small at high \( C_i \), indicating that the stomata were not closed in atmospheric \( C_a \) in the dark. However, stomatal conductance in the dark at low \( C_i \) was only one fifth of the maximum measured at high quantum flux densities. When \( Q_i \) was low stomatal conductance increased with decreasing \( C_i \) from 600 to 100 cm\(^3\) m\(^{-3}\), but at higher quantum flux densities \( g_s \) approached saturation at intercellular CO\(_2\) concentrations lower than 150 cm\(^3\) m\(^{-3}\). The sensitivity of stomatal conductance to \( C_i \) at normal \( C_a \) (the slope of the curve at the points arrowed) increased with increasing quantum flux density. Intercellular CO\(_2\) concentrations higher than 450 cm\(^3\) m\(^{-3}\) resulted in small \( g_s \) at all quantum flux densities, though \( g_s \) was larger than in the dark. Results from a similar experiment are presented in Chapter 5.3.1.
Fig. 3.3.2a  Legend overleaf, facing page.
Fig. 3.3.2b Legend facing page. Note the small scale for $g_s$ in comparison with previous figures.
Figure 3.3.2a

Relationship between $g_s$ and $C_i$ in three individual excised shoots of dayflower in red (o) blue (●) and white (■) light (140, 120 and 770 μE m$^{-2}$s$^{-1}$; respectively). VPD = 0.50 kPa, leaf temperature = 20.0 °C. Points are means ±1 s.e.m. of 6-8 measurements over a 10 minute period after c. 70 minutes at each ambient CO$_2$ concentration, for a single shoot.

(Experiment 3.4)

Figure 3.3.2b

Relationship between $g_s$ and $C_i$ in a single shoot of dayflower at four quantum flux densities ($Q_i$). Points are means of 6-10 measurements over an 11 minute period at c. 70 and 90 minutes after a change of the ambient CO$_2$ concentration, one standard error is smaller than each point. $g_s$ at an ambient CO$_2$ concentration of 330 cm$^3$m$^{-3}$ shown arrowed.

(Experiment 3.5)
3.4 Discussion

The response of stomatal conductance to intercellular CO$_2$ in Scots pine and Sitka spruce described above is at odds with the results of Ludlow and Jarvis (1971), Beadle, Jarvis and Neilson (1979) and Ng (1978). No satisfactory explanation for this difference can be advanced. In particular the large response to CO$_2$-free air in Scots pine conflicts with the work of Ng. The same plant growing conditions, provenance material and methods have been used in this work. It is possible that the long time for the full response to CO$_2$ changes may have caused some changes of stomatal conductance to be overlooked. Ng used treatment times of 45 minutes to 1 hour for each ambient CO$_2$ concentration in a sequence from 400 cm$^3$ m$^{-3}$ to CO$_2$-free and from 400 to 5000 cm$^3$ m$^{-3}$, in a total of 8 steps. Some effect should have been apparent over this time period. No response was found by Ng in plants from the growth rooms at either 10.7 or 20.6 °C at 4 different quantum flux densities. Forest shoots showed small (10 to 30%) increases of $g_s$ at ambient CO$_2$ concentrations lower than 100 cm$^3$ m$^{-3}$; their significance was obscured by intershoot variability. Ng concluded from a large number of experiments that there was no response to $C_a$ between 0 and 5000 cm$^3$ m$^{-3}$. There is little doubt that the plants used here gave different results.

The increase of stomatal conductance at low intercellular CO$_2$ concentrations in Sitka spruce was smaller than that found in Scots pine. The result is the opposite of that noted by Ludlow and Jarvis (1971), and disagrees with that of Beadle et al (1979) who found no stomatal response to CO$_2$. Stomata in some species may be
"sensitised" to \( \text{CO}_2 \) by ABA (see Section 3.1.2). While no information is available on the ABA content in the shoots used here, plants used in this study were not water-stressed; at no time were water potentials lower than -0.7 MPa measured in shoots or needles outside the chamber. The VPD in the chamber was small (0.5 kPa for Sitka spruce and 0.7 kPa for Scots pine); transpiration losses from the shoots in the chamber were small and it is unlikely that \( \Psi \) was much lower than in the rest of the plant. Moreover, application of ABA stimulated stomatal closure in high \( C_a \), and decreased \( g_s \) at all \( C_i \) values in other species (e.g. Dubbe et al 1978, Raschke 1979 Fig. 17); ABA did not increase stomatal opening in low intercellular \( \text{CO}_2 \) concentrations. In this study, stomatal conductance in Scots pine and Sitka spruce increased in response to low \( C_i \) and there was no response to high \( C_i \). It is therefore unlikely that differences in ABA content can explain the difference between the results here and those reported previously. In addition, stomatal conductance in Sitka spruce was relatively insensitive to \( C_i \) between 20 and 400 \( \text{cm}^3 \text{m}^{-2} \), with \( \Psi \) between -0.5 and -2.5 MPa (Beadle et al 1979, Fig. 6). ABA levels are presumed to increase with lowered \( \Psi \), yet there was no large effect of \( C_i \); the response of \( g_s \) to increasing \( C_i \) was small (10% decrease) and this decrease at high \( \Psi \) was reversed at low \( \Psi \). Beadle et al concluded that the guard cells of Sitka spruce "may be inherently insensitive to \( \text{CO}_2 \)." The results of the present study, where \( g_s \) showed small increases to low intercellular \( \text{CO}_2 \) concentrations, indicate that this is not always the case.

The maximum stomatal conductance differed between these two studies. The maximum \( g_s \) measured by Beadle et al was low (0.20 \( \text{cm s}^{-1} \), 1000 \( \mu \text{E m}^{-2} \text{s}^{-1} \) white light, -0.5 MPa \( \Psi \), VPD of 0.5 kPa at 20
°C) compared to that measured in the present study (0.62 cm s⁻¹, 230 μE m⁻² s⁻¹ blue light, VPD of 0.6 kPa at 20 °C). In dayflower, large apertures were more sensitive to CO₂ than small apertures (Wilson, Ogunkanni and Mansfield 1978, Travis and Mansfield 1979b). This is discussed further in Chapter 6. No conclusion can be made on the different response to low Cᵢ here. However, the present study does support the view that stomata of Scots pine and Sitka spruce do not respond to ambient concentrations above atmospheric.

The response of stomatal conductance to intercellular CO₂ in dayflower shoots differs substantially from that of Scots pine and Sitka spruce. Stomatal conductance in dayflower was sensitive to Cᵢ between 70 and 500 cm⁻³ m⁻³; the response was similar to that reported by Saxe (1979) from microscopic measurements on individual stomata in intact leaves of dayflower. However, Saxe showed the opening response of gₛ to CO₂-free air in the dark was large; CO₂-free air in darkness caused opening to almost the same aperture as high light (10 kLUX). While stomata responded to low intercellular CO₂ concentrations in both light and darkness in the present study, the response of gₛ to Cᵢ in the dark was always much smaller than that in the light. This differs from the response of gₛ to Cᵢ in maize, which is almost independent of light (Raschke, Hanebuth and Farquhar 1978). Moreover, the response to light at any one Cᵢ was smaller at higher intercellular CO₂ concentrations higher than 450 cm⁻³ m⁻³ resulted in small gₛ at all Qᵢ values. There is clearly an interaction of CO₂ and light: at very high Cᵢ there would be no response to quantum flux density and at larger Qᵢ, stomatal conductance would be very sensitive to the intercellular CO₂ concentration. Travis and Mansfield (1979b) have come to the same
conclusion for stomata in epidermal strips of this species. Data from Saxe (1979, Fig. 10), when expressed as aperture against $C_i$, not $C_a$, show a small interaction of light and $CO_2$ in dayflower leaves. Results with *Eucalyptus pauciflora* Sieb. ex Spreng. (Wong, Cowan and Farquhar 1978, Fig. 4) are similar to the results in Fig. 3.3.2b here; the response of $g_s$ to $C_i$ shows the same two relationships at low and high quantum flux densities.

However, larger sensitivity to $C_i$ at high quantum flux density is contrary to evidence for *Sorghum sudanense* [Piper] Stapf. (Downes 1971), *Pennisetum typhoides* (McPherson and Slatyer 1973), *Spartina townsendii* (Long and Woolhouse 1978) and three $C_3$ and $C_4$ species (Akita and Moss 1972). This evidence is not unequivocal. McPherson and Slatyer's data have been misinterpreted (e.g. Hall, Schulze and Lange 1976); their figure 2 depicts the relationship between $r_s'$ and net assimilation against $C_a$ not $C_i$. Replotted as $g_s'$ against $C_i$ the evidence for decreasing sensitivity of $g_s'$ with increasing $Q_i$ is scant; the range of $C_i$ at different quantum flux densities varies. In addition, no mention is made of VPD control in the leaf chamber with changing $r_s'$ and there is evidence that $g_s$ in irrigated *P. typhoides* responds to VPD in the field (Squire 1979). These same criticisms can be levelled at the results of Downes (1971) and Akita and Moss (1972, see Introduction 3.1.2). Humidity control is important in such experiments; for example, transpiration will decrease as $g_s$ decreases in response to high $C_i$ resulting in larger VPD and reinforcing the closing response to $C_i$ in species with VPD-sensitive stomata. In addition, there is evidence of a more pronounced response of $g_s$ to VPD at lower quantum flux densities (Davies and Kozlowski 1974, Kaufmann 1976, Pallardy and Kozlowski...
1979); the response of stomata to CO₂ may be reinforced at low quantum flux density if there is inadequate VPD control.

However, stomata in *S. townsendii* showed a large sensitivity to CO₂ (Long and Woolhouse 1978) particularly at low quantum flux densities and there is no ready explanation for the differences between this response and the patterns found in dayflower and in *E. pauciflora*.

The classical experiment of Heath and Russell (1954a and b) demonstrated the interaction of light and CO₂ in wheat leaves through which air of known CO₂ concentrations (effectively C₁) was forced. These authors, and later Mansfield and Meidner (1968) and Raschke (1975a), suggested that light, acting through guard cell photosynthesis, affects the balance between inter- and intracellular CO₂. However, recent biochemical evidence on guard cell photosynthetic capacity suggests this is not necessarily true. This point is further discussed in Chapter 4.

Raschke (1975b) has suggested that there is a "simultaneous requirement" for CO₂ and ABA in *X. strumarium, G. hirsutum* and dayflower. The results reported here provide circumstantial support for the evidence (presented in 3.2.1) that ABA is not necessary for the response of gₛ to C₁. While no information is available on the ABA content of the dayflower shoots used, plants were not and had never been, water stressed. Shoots frequently showed guttation under experimental conditions and at no time were values of \( \psi \) lower than -0.35 MPa measured. The response to CO₂ shown in Fig 3.3.2a was measured in excised shoots, with the cut ends in water; \( \psi \) measured after the experiments was c. -0.1 MPa.

The response of dayflower stomata to C₁ parallels the "typical"
response in the literature exemplified by *Sesamum indicum* (Hall and Kaufmann 1975), apple (*Malus sylvestris* vars., Warrit 1977), *C. hirsutum* and *A. powelli* (Dubbe *et al* 1978). The response of $g_s$ to $C_i$ in Scots pine and Sitka spruce measured by similar methods differs radically. Only one response in the literature, that for tomato (Pallas 1965) appears similar. This difference in response suggests that either the mechanisms or the sensitivities of the mechanisms differ between the two conifers and dayflower. The very different effects of $CO_2$ in different species which exhibit similar responses to light suggests that the $CO_2$ response is secondary to the light response; this will be considered in detail in the next Chapter.
3.5 Conclusions

Scots pine and Sitka spruce stomatal conductance increased with decreases in intercellular \( \text{CO}_2 \) concentration below those in normal atmospheric \( C_a \) in the light. No change occurred in the dark. The maximum increase occurred in \( \text{CO}_2 \)-free air with a total increase of 50 to 90% in Scots pine and 30% in Sitka spruce.

Stomatal conductance in Scots pine and Sitka spruce did not respond to increases in \( C_1 \) above those in normal atmospheric \( C_a \) up to 700 cm\(^3\) m\(^{-3}\).

In dayflower stomatal conductance responded markedly to intercellular \( \text{CO}_2 \) concentration both in darkness and in the light. High \( C_1 \) resulted in small \( g_s \) at all quantum flux densities. Low \( C_1 \) below that at atmospheric ambient \( \text{CO}_2 \) concentrations resulted in large increases in stomatal conductance.

There was an interaction of the effects of intercellular \( \text{CO}_2 \) concentration and quantum flux density on stomatal conductance in dayflower. The response to \( C_1 \) was more marked at higher quantum flux densities.
4.1 The Response of Stomata to Light

4.1.1 Introduction

The earliest observations on stomata (e.g. von Mohl 1856, Darwin 1898) revealed that stomata open in the light and close in the dark. The subject has been reviewed extensively more recently (e.g. Meidner and Mansfield 1968, Raschke 1975). Exceptions to this pattern are found in CAM plants, in which the stomatal rhythm is inverted in water-stressed conditions, and in other species when other variables, for example water stress and endogenous rhythms, override the response to light. Indeed, stressed CAM plants can be regarded as a special case in which the light response has been overridden by the CO$_2$ response, as discussed in Chapter 3.1.2.

The mechanism of the response to light is unresolved. Raschke (1975a) contends that "stomata respond to light only indirectly by responding to changes in the CO$_2$ concentration brought about in the leaf by light". From the discussion in the previous chapter, it is evident that CO$_2$ can affect stomata independently of light, and that light, acting through mesophyll assimilation, can affect intercellular CO$_2$ and therefore indirectly affect stomata. However, there are observations in the literature and in the results presented here which suggest an effect of light that is "direct"; that is, independent of changes in the intercellular CO$_2$ concentration, and independent of mesophyll assimilation.

This chapter examines the responses of stomata to light and the
4.1.2 Stomatal responses to light

The opening response to light in the visible range is generally described as an hyperbolic function of quantum flux density, whether measured as conductance of leaves (e.g. Turner 1974) or as apertures of individual stomata (e.g. Saxe 1979). The hyperbolic relationship is similar to that between net assimilation rate and Q1 and the simplest explanation of the resulting correlation is that it reflects a mechanistic link through the intercellular CO2 concentration. Alternatively, both stomatal action and mesophyll assimilation could depend on light through completely independent mechanisms (Jarvis and Morison 1980).

The response to light varies between species, both in the "initial slope" (the near linear response at low quantum flux densities) and in the conductance or aperture attained at high, near-saturating quantum flux densities (see Burrows and Milthorpe 1976).

Speed of response

The time taken for the re-establishment of steady-state apertures after a change in quantum flux density varies between species. Much of the variation reported must be attributed to different methods as well as plant material. Gas exchange studies have frequently failed
to compensate for changing evaporative conditions in the leaf chambers as stomata open or close. Use of resistance measurements, although demonstrating a rapid decrease in $r_s$ following illumination, frequently fail to reveal longer term changes in stomatal aperture (e.g. Davies and Kozlowski 1974, Nelson and Mayo 1975).

The time course of opening depends on the lag time, the opening rates and the final apertures attained (Woods and Turner 1971). Measurements of mean aperture, conductance or resistance do not reveal the changes in individual stomata. Opening of individual stomata in dayflower leaves took from 2 to 4 hours in CO$_2$-free air at 10 klx; each stoma showed a three phase pattern of opening: increase, inactive period and further opening (Saxe 1979). This detail is lost in averaging as few as six stomata which produces a curvilinear increase of mean aperture with time. Individual stomata in leaves of barley (*Hordeum vulgare*) approached maximum aperture after 4 hours; the preliminary lag time in leaf conductance can be attributed to the variation in individual stomatal apertures over a leaf surface, rather than to a physiological effect (Laisk, Oja and Kull 1979).

The initial opening movement occurs within five minutes generally, but the length of time taken to reach a new steady-state aperture is commonly underestimated; taking up to three hours in *Vicia faba* (e.g. Hsiao, Allaway and Evans 1976), two hours in *Xanthium strumarium* (Mansfield and Heath 1963) and six hours in Sitka spruce (Watts and Neilson 1978). Such long periods are several orders of magnitude larger than the response of net assimilation to light, resulting in large changes in the intercellular CO$_2$ concentration as stomata open.

**CO$_2$-independent responses**
The steady-state response to light within a species depends upon preconditioning (e.g. Mansfield and Heath 1963), leaf age (e.g. McPherson and Slatyer 1973, Turner 1974) and other variables, for example humidity (Davies and Kozlowski 1974, Kaufmann 1976) and plant water stress (e.g. Davies and Kozlowski 1975, Lösch 1977, 1979). Individual stomata respond differently to light under apparently similar conditions (e.g. Saxe 1979). Adaxial and abaxial stomata differ in the sensitivity to light, even though under a given quantum flux density, both epidermes are presumably exposed to similar intercellular CO₂ concentrations (see reviews of Turner 1979, Pospíšilová and Solárová 1980). Abaxial stomata are more sensitive to the total quantum flux density received by the leaf than adaxial stomata, though abaxial stomata only receive 10-15% of the Qincident on the adaxial surface. Raschke, Hanebuth and Farquhar (1978) have suggested that both the adaxial and abaxial stomata of maize respond to light only indirectly through changes in Cᵢ. However, their data are not conclusive: a major part of the experimental evidence refers to leaves in CO₂-free air. The authors state that "only a small quantum flux density was required to open stomata in CO₂-free air maximally", while this response was correlated with small changes in Cᵢ (from 10 to 0.6 cm⁻³), it indicates a large response to light independent of large changes of Cᵢ. Other results in the paper also indicate that, at any given intercellular CO₂ concentration, illuminated stomata are more open than stomata in darkness. Pemadasa (1979) has found that the greater photosensitivity of adaxial stomata in other species cannot be overcome by flushing the intercellular spaces with CO₂-free air, again indicating a direct response to light.
Heath and Russell (1954) provided early evidence for a \( C_i \)-independent light response in wheat leaves in which the stomata responded to changing illumination while the intercellular spaces were swept with air of known \( CO_2 \) concentrations. More recent evidence in whole leaves of a response to light at constant \( C_i \) has been provided in *Eucalyptus pauciflora* (Wong, Cowan and Farquhar 1978) and maize (Raschke 1979, p. 430, Fig. 18).

**Light responses in epidermal strips**

The common observation that stomata isolated from the mesophyll respond to light (e.g. Fischer 1968, Raghavendra and Das 1972) even in \( CO_2 \)-free air (e.g. Travis and Mansfield 1979b) indicates that there is a direct effect of light. This has previously been attributed to the "scrubbing" action of guard cell chloroplasts in lowering the intracellular \( CO_2 \) concentration (Raschke 1975). However, there is increasing evidence that the Calvin cycle is absent from guard cells (Milthorpe, Thorpe and Willmer 1979). Guard cell chloroplasts have not been shown to be able to reduce \( CO_2 \) in *Tulipa gesneriana* L. (Shaw and MacLachlan 1954), *C. diffusa* L. (Willmer and Dittrich 1974), *C. communis* (Raschke and Dittrich 1977) and *Allium cepa* L. (Schnabl 1977). Outlaw et al (1980) have concluded that the photosynthetic carbon reduction pathway is absent from guard cells of *V. faba*. However, Lurie (1977) has reported rates of \( CO_2 \) fixation in illuminated epidermes of this species ten times those of non-illuminated epidermes. Lurie (1979) concludes that there is light enhanced \( CO_2 \)-fixation but not through the Calvin cycle. Part of the conflict in reports may be the result of contamination of epidermal tissues by undetected mesophyll chloroplasts (Zeiger,
Armond and Melis 1980). The question is still unanswered but if guard cells do not show pronounced light-enhanced CO₂-fixation then light must act directly on the stomatal mechanism and not be mediated by intracellular CO₂ depletion.

Red and blue light effects

a) blue light enhanced stomatal opening

A major part of the evidence for a direct light effect on stomata comes from the observation that blue wavelengths are more effective in causing stomatal opening than red wavelengths, while red light is more effective in net assimilation than blue light. The early work has been reviewed by Heath (1959) and Meidner and Mansfield (1968). Some early conflicting reports that red light is more effective than blue light (Darwin 1898, Paetz 1930) can be attributed to inadequate spectral quality control and the use of energy units rather than quantum flux densities for comparison of different spectral regions. Action spectra should be expressed on an isoquantum basis (see Gaudillère and Costes 1971). The maximum quantum yield of assimilation in blue light at 440 nm is between 40 and 70% of the maximum in red light at 620-670 nm, depending on the species (e.g. McCree 1972, Clark and Lister 1975, Inada 1976). Blue light (peak wavelengths 430-460 nm) is between 2 and 10 times more effective in opening stomata than red light (peak 655-680 nm, Mouravieff 1958, Kuiper 1964, Mansfield and Meidner 1966, Raschke 1967a). In V. faba the action spectra for stomatal aperture in epidermal strips in CO₂-free air, and for conductance in leaf discs in normal air, showed peaks in blue light (maxima at λ 440 nm)/little effect of red light at low quantum flux densities (13 μE m⁻² s⁻¹, Hsiao, Allaway and Evans
1973). At higher quantum flux densities (62 µE m$^{-2}$ s$^{-1}$) red light was half as effective as blue light in both rolled strips and leaf discs. These action spectra for opening were paralleled by $^{86}$Rb$^+$ uptake by guard cells. Hsiao and coworkers suggest that there is a light-driven mechanism which is independent of CO$_2$ and which occurs in both isolated guard cells and in whole leaves. In addition, in whole leaves there is a CO$_2$ response effected by mesophyll assimilation. Kana and Miller (1977) found that the opening rates (rather than the final apertures studied by Hsiao et al) of V. faba stomata were the same in broad band blue and red light. Light saturation of the opening rate occurred below the light compensation point for net assimilation. Maximum opening rate occurred while there was still a net efflux of CO$_2$ from the leaf, indicating a direct effect of light on the stomata.

Ogawa et al (1978) found that the action spectrum for steady-state malate formation in "isolated" guard cells of V. faba closely matched that of $^{86}$Rb$^+$ uptake and stomatal opening found by Hsiao et al (1973). There was a large peak in the blue and a smaller peak in the red regions of the spectrum at $\lambda$ 430 and 675 nm, respectively. The action spectrum did not match the absorption spectra for either mesophyll chloroplasts or epidermal strips; these exhibited larger peaks in the red than in the blue regions. Background red light had a strong synergistic effect on malate formation in blue light; Ogawa et al suggest that red light is absorbed by guard cell chlorophyll and supplies energy for the influx of K$^+$ while blue light activates PEP carboxylase. However, no malate was formed without K$^+$ in the incubation medium of the strips and it is not possible to differentiate between the effects of blue light on malate metabolism.
directly through enzyme stimulation or through increased rates of ion transport. This will be discussed in Chapter 6.

b) rapid blue light transpiration response

Pulses of blue light induced both a rapid and a slow increase in transpiration rate in *Avena sativa*, while red light induced only the slow response (Brogårdh 1975). The rapid blue light response (blr) was enhanced by pretreatment with CO$_2$-free air and was restored after long sequences of blue pulses by pre-illumination with red light (Skaar and Johnsson 1978). The blr was only induced by wavelengths shorter than 524 nm (peak $\lambda$ 420 nm) and was enhanced by illumination with background red light (Skaar and Johnsson 1978), a result similar to that mentioned above for malate formation in guard cells of *V. faba* (Ogawa et al 1978). However, the blr was restricted to species with graminaceous-type stomata (Johnsson et al 1976). Skaar and Johnsson (1978) point out that, as well as inducing a rapid transpiration response, blue light also caused a higher steady-state transpiration rate than red light in graminaceous plants and they suggest that the blue light-sensitive mechanism involved in the blr is also responsible for the enhanced stomatal opening in blue light in other plants.

The slow response present in red, blue and white light was eliminated in CO$_2$-free air (Brogårdh and Johnsson 1975, Brogårdh 1975) suggesting that the biphasic response in blue light resulted from the addition of a CO$_2$-independent process to a CO$_2$ response effected by the mesophyll. Support for this comes from the study of the Xan-U$^{21}$ mutant of *Hordeum vulgare* (Skaar and Johnsson 1980): achlorophyllous plants of the mutant exhibited a transpiration
response to blue light but not to red light, while transpiration rate increased in both red and blue light in green plants. Aclorophyllous plants were sensitive to CO$_2$, indicating that the CO$_2$ sensitive mechanism was not impaired in the mutant, and suggesting that the red light response in green plants acts through chlorophyll and the reduction of the intercellular CO$_2$ concentration by mesophyll assimilation.

c) blue light-enhanced net assimilation

V. Resenskaya (1972) has reviewed the evidence for non-stomatal effects of blue light on carbon metabolism, but there are also reports that, in some species, blue light enhances net assimilation at light saturation through effects on stomata. Gol'd (1966) suggested that the higher assimilation rate at saturating quantum flux densities in blue light, compared to red light, in *Aspidistra elatior* Bl. was "due to certain reactions taking place which assist photosynthesis even under light saturation". However, Begonia sp., *Ruscus aculeatus* L., and *Paris quadrifolia* L. did not show a blue light enhancement of assimilation in saturating red light (Gol'd 1969). The blue light enhancement of assimilation was only found in the shade-tolerant *A. elatior* and shade-grown *Hedera helix* L., *A. sativa* and *Convallaria majus* L. (Gol'd 1969). Keerberg et al (1971) demonstrated that the blue light effect in *A. elatior* could be attributed to increased stomatal opening: red light was ineffective in opening stomata in this species and at high $Q_1$ net assimilation was limited by CO$_2$ diffusion through the stomata. No data for the intercellular CO$_2$ concentration are given in the paper by Keerberg et al but $C_1$ must drop to low values in red light indicating that the
stomata of *A. elatior* are insensitive to $C_i$. However, Keerberg et al. (1971) note that blue and red light were equally effective in opening the stomata of bean (*Phaseolus vulgaris* L.). In *C. majus*, only wavelengths shorter than 520 nm (peak $\lambda$ 460 nm) enhanced net assimilation at saturating quantum flux densities of red light and this action spectrum coincided with the action spectrum for transpiration (Vo resenskaya and Polyakov 1975). Again, red light was ineffective in opening stomata either directly or through lowering the intercellular $CO_2$ concentration. Blue light opened stomata and in both *A. elatior* and *C. majus* resulted in higher intercellular $CO_2$ concentrations.

d) specific blue light response in *Allium* guard cells

Isolated protoplasts from guard cells of *Allium cepa* have been shown to swell when illuminated with broad band blue light (Zeiger and Hepler 1977), whereas no swelling was observed even at high quantum flux densities of red light. In contrast, red light caused guard cell swelling in paradermal sections where a mesophyll was present, agreeing with previous work on whole leaves of *A. cepa* (Meidner 1968). Guard cell protoplasts from *A. cepa* showed an intrinsic, vacuolar, green fluorescence when irradiated with broad band blue light, while epidermal cells did not (Zeiger and Hepler 1979). This autofluorescence may be a further manifestation of the blue light-sensitive mechanism in stomata; the authors suggest that the fluorescence was localised on the tonoplast membrane and indicated a flavin or flavoprotein blue light receptor acting directly on an ion pump. However, autofluorescence was restricted to *Allium* species and was not observed in several monocotyledons and
dicotyledons known to possess blue light enhanced stomatal opening, e.g. *V. faba* (Zeiger and Hepler 1979). Results with maize and barley were unclear because of the compressed vacuole of the guard cells and the fluorescence of guard cell wall compounds. Zeiger (1980) has attributed the restriction of autofluorescence to *Allium* to the differences in the cellular properties of guard cells which exclude fluorescence in normal conditions. Guard cells in epidermal strips of *V. faba* fluoresce in blue light in solutions that promote higher intracellular pH (Zeiger 1980). However, vacuoles of epidermal cells of *V. faba* also showed blue light-stimulated fluorescence in the same solutions and the fluorescing pigment may not be a specific feature of guard cells (Zeiger 1980). Further work is necessary to elucidate the significance of the autofluorescence in cells.

Summary

Stomata respond to light in a manner that loosely parallels net CO$_2$ assimilation in the underlying mesophyll tissue. This has suggested a link between the two processes through changes in intercellular CO$_2$. However, there is extensive evidence of a CO$_2$-independent response of stomata to light:

1) Stomatal conductance has been observed to change with light at constant intercellular CO$_2$ concentrations.

2) Stomata in the two epidermes show different responses to light, when presumably exposed to a similar intercellular CO$_2$ concentration.

3) Isolated stomata in epidermal strips respond to light even in CO$_2$-free air.

4a) In some species, blue light is more effective on a per quantum
basis than red light in opening stomata in whole leaves and epidermal strips. This occurs in both CO₂-containing and CO₂-free air. In some cases red light is ineffective in opening stomata, or its effect can be eliminated in CO₂-free air. In other cases red light is effective in CO₂-free air even in epidermal strips.

4b) A specific rapid, blue light-stimulated transpiration response has been found in graminaceous-type stomata. The response is not eliminated in CO₂-free air, unlike the slow response in red light.

4c) Isolated guard cell protoplasts of Allium spp. swell in blue light but not in red light.

Sitka spruce and Scots pine show a hyperbolic opening response of stomatal conductance to increasing quantum flux density (e.g. Beadle 1977, Ng and Jarvis 1980). However, in the previous chapter it was demonstrated that the stomata of these two species are partially insensitive (and in some cases completely insensitive) to changes in Cᵢ. This prompted an investigation of the stomatal response to light independent of Cᵢ in these two species and, in particular, a study of the response of gs to red and blue light. For comparison, the stomatal responses of dayflower, a plant which shows sensitivity to both Cᵢ and light, were investigated.
4.2 Experiments

The response of \( g_s \) to light in whole shoots of Scots pine, Sitka spruce and dayflower was examined together with the dependence of the light responses on mesophyll assimilation.

The methods have been described in Chapter 2.

Response of \( g_s \) and \( A \) to blue and red light in Scots pine and Sitka spruce

Individual shoots of Scots pine and Sitka spruce were illuminated in a sequence of increasing \( Q_1 \) of broad band blue and red light, commencing in darkness (see Appendix 3). The response to blue and red light was measured on different days in each of four shoots on different plants. Two shoots received the red light treatment first, and two the blue light treatment first. Plants were returned to the growth room for at least one day between the two treatments.

\( A \) and \( g_s \) were measured after 70 and 110 minutes in each \( Q_1 \) to confirm that equilibrium values were attained. Atmospheric CO\(_2\) concentrations were used. \( (\text{Experiment 4.1}) \)

The experiment was repeated using CO\(_2\)-free air with three shoots of Scots pine and Sitka spruce. \( (\text{Experiment 4.2}) \)

Wavelength sensitivity in Scots pine

The response of \( g_s \) in shoots of Scots pine to narrow wavebands was investigated. A series of eight interference filters were used (see Appendix 3); the quantum flux density was limited to 25 \( \mu \text{E m}^{-2}\text{s}^{-1} \) in each waveband. Shoots were illuminated from one side only for c. 80 minutes in each waveband. The filters were small \((25 \text{ cm}^2)\) and shoots
were trimmed to leave only c. 15 cm² projected needle area for illumination, with all the needles arranged normal to the light beam. Two shoots were each illuminated in 0 and 330 cm³ m⁻³ ambient CO₂ on different days and two shoots were each illuminated in a red to blue waveband sequence on one day and in a blue to red sequence on another day, in 330 cm³ m⁻³ Cₐ. (Experiment 4.3)

Response of gₛ and A to CO₂ and O₂ in Scots pine in red and blue light

Eight shoots of Scots pine were illuminated in alternating, two hour long treatments of red and blue light (230 μE m⁻² s⁻¹). Ambient CO₂ and O₂ concentrations were changed in steps in the following treatment sequence:

<table>
<thead>
<tr>
<th>DAY 1</th>
<th>DAY 3</th>
<th>DAY 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (hours)</td>
<td>Qᵢ (cm³ m⁻³)</td>
<td>CO₂ (cm³ m⁻³)</td>
</tr>
<tr>
<td>2</td>
<td>R 350</td>
<td>R 350</td>
</tr>
<tr>
<td>4</td>
<td>B 350</td>
<td>B 350</td>
</tr>
<tr>
<td>6</td>
<td>R 350</td>
<td>R 0</td>
</tr>
<tr>
<td>8</td>
<td>B 350</td>
<td>B 0</td>
</tr>
<tr>
<td>10</td>
<td>R 350</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(B) 350</td>
<td></td>
</tr>
</tbody>
</table>

Four shoots commenced with a red light treatment (as above), four with blue light. (Experiment 4.4)

The response of stomatal conductance and net assimilation in Scots pine to red and blue light in a range of CO₂ concentrations in 21% O₂ has been described in Chapter 3.2, Experiment 3.3. The same procedure was used with CO₂ mixtures in oxygen-free nitrogen, omitting the second CO₂ treatment (500 or 600 cm³ m⁻³) to shorten the experiment, reducing the length of time of exposure of shoots to low oxygen concentrations. The oxygen concentration in the chamber
was c. 0.1%; dark respiration rates of whole shoots and bare twigs remained unchanged during long periods of exposure (up to five hours) in this concentration.

Prolonged exposure (up to 14 hours in any one experiment) to light in low O$_2$ concentrations, including CO$_2$-free treatments, inhibited stomatal opening on subsequent days. No change in stomatal conductance in response to light was found in plants returned to the growth room for up to six days after low O$_2$ treatment. It was therefore inappropriate to compare the response of single shoots to red and blue light with and without oxygen. Only three shoots were used in red light and three different shoots in blue light.

(Experiment 4.5)

The time course of $g_s$ in response to red and blue light in Sitka spruce

Comparisons of the effect of blue light and red light on stomatal conductance are valid if steady-state $g_s$ is measured at each quantum flux density. If stomatal conductance has not reached steady-state at the measurement period, the same conclusion is only valid if the response time of $g_s$ to red and blue light is the same. In experiments 4.1 and 4.2 above $g_s$ was measured at c. 70 and 110 minutes intervals after a change in quantum flux density and measurement periods were extended if these two values were markedly different.

A further check was provided by measurements of the time course of $g_s$ in response to a single large increase in quantum flux density (0 to 230 μE m$^{-2}$s$^{-1}$) in both blue and red light in atmospheric concentrations of CO$_2$. Stomatal conductance was measured continually.
throughout the experiment with only short interruptions as adjustments were made to maintain a constant VPD in the chamber as transpiration rate changed.

The response of $g_s$ and $A$ in dayflower to light

The response of $g_s$ and $A$ to red and blue light in shoots of dayflower was determined as in Experiment 4.1, except that shoots were arranged to receive direct illumination on the adaxial surface only (see Section 2.5.2). However, to avoid decreases in the intercellular CO$_2$ concentration with increasing quantum flux density, background CO$_2$ concentrations were increased to maintain $C_i$ approximately constant. Net assimilation and stomatal conductance were calculated as often as steady-state conditions in the equipment allowed. As intercellular CO$_2$ concentration is independent of leaf area (see Section 2.4.5, equation 2.4.5b) $C_i$ could be calculated without the prior measurement of leaf area. Large increases in the chamber CO$_2$ concentration were effected with the gas mixing pumps, finer adjustments were made by altering the rate of air flow through the chamber. Inevitably, $C_i$ fluctuated during quasi-steady state conditions because of the almost instantaneous change of net assimilation with changes in quantum flux density, while $C_a$ took c. 15 minutes for a large change (c. 20 cm$^3$ m$^{-3}$). However, once the selected intercellular CO$_2$ concentration was attained at each $Q_i$, it was maintained for at least 60 minutes before a further change in quantum flux density. (Experiment 4.7)

A full range of quantum flux density was only available in white light. The response of $g_s$ and $A$ to white light in a single shoot of dayflower at three intercellular CO$_2$ concentrations was measured
using the methods described in Experiment 4.7. The response to $Q_1$ in one shoot was measured on three consecutive days at high $C_1$ (466 cm$^3$ m$^{-3}$, Day 1), medium $C_1$ (302 cm$^3$ m$^{-3}$, Day 2) and low $C_1$ (46 cm$^3$ m$^{-3}$ Day 3). The $C_1$ values chosen were taken from three regions of Figure 3.3.2b: high $C_1$, where there was smallest sensitivity to light; medium $C_1$, where there was largest sensitivity to CO$_2$ and low $C_1$, where there was largest sensitivity to light and smallest sensitivity to CO$_2$. Measurements were kept within the first nine hours of the growth room photoperiod, and the plant returned to the growth room for the remainder of the day and night. (Experiment 4.8)
4.3 Results

4.3.1 The response of $g_s$ and $A$ to blue and red light in Scots pine

Stomatal conductance increased in response to broad band blue light (400-500 nm) particularly at quantum flux densities lower than 100 $\mu$E m$^{-2}$s$^{-1}$ (Fig. 4.3.1a). The 400 watt light sources used at this time were inadequate to produce saturation of the response of $g_s$ to light. Broad band red light (610-750 nm) was ineffective until $Q_i$ exceeded 70 $\mu$E m$^{-2}$s$^{-1}$. Even at a quantum flux density of red light three times that of blue, $g_s$ was only 75% of the maximum.

While blue light was more effective on an incident quantum basis than red light in opening stomata the reverse was true for net CO$_2$ assimilation ($A$, Fig. 4.3.1b). The initial slopes (apparent quantum yields) were estimated from linear regressions (Table 4.3.1); in blue light the slope was less than half that in red light and the light compensation point in blue light was more than double that in red light. The self-shading of the needles in the chamber illuminated with parallel beam light resulted in very low apparent quantum yields.

The intercellular CO$_2$ concentration was always higher in blue light than in red light because of the contrasting effects of blue and red light on $A$ and $g_s$ (Fig. 4.3.1c). There is no overlap of the points for red and blue light; there is no single relationship of $g_s$ with $C_i$. It should be emphasised that this is a correlative exercise and $C_i$ is calculated from $A$, $C_a$ and $g_s$. The change in $g_s$ for an individual shoot in red, blue and white light is shown by the connected points in Fig. 4.3.1c, this emphasises the independence of $g_s$ from $C_i$; at larger stomatal conductance $C_i$ was c. 100 cm$^3$ m$^{-3}$
Fig. 4.3.1a Relationship between $G_r$ and $Q_i$ in Scots pine in red (o) and blue (●) light. 4 shoots in both red and blue light; normal atmospheric $C_a$.

At $G_r = 1.0$ $g_s = 0.278 \pm 0.048$ cm s$^{-1}$.

(Experiment 4.1)
Fig. 4.3.1b Relationship between A and $Q_i$ in Scots pine in red (o) and blue (●) light. (as Fig. 4.3.1a) $n = 4$ (Experiment 4.1)
### Table 4.3.1
Response of A in 4 shoots of Scots pine to broad band red and blue light of increasing quantum flux densities

<table>
<thead>
<tr>
<th></th>
<th>Red</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial points &lt; 300 μE m⁻² s⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>slope (x 10⁻⁴) mg μE⁻¹</td>
<td>5.79</td>
<td>3.09</td>
</tr>
<tr>
<td>intercept mg m⁻² s⁻¹</td>
<td>-0.027</td>
<td>-0.042</td>
</tr>
<tr>
<td>r²</td>
<td>0.99</td>
<td>0.95</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>s² (x 10⁻⁵)</td>
<td>3.034</td>
<td>5.412</td>
</tr>
<tr>
<td>s² not sig. diff. F = 1.784</td>
<td></td>
<td></td>
</tr>
<tr>
<td>slopes sig. diff. t = 11.02 P &lt;&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>light compensation point μE m⁻² s⁻¹</td>
<td>47</td>
<td>126 *</td>
</tr>
<tr>
<td>apparent quantum yield mol E⁻¹</td>
<td>0.013</td>
<td>0.007</td>
</tr>
<tr>
<td>quantum requirement E mol⁻¹</td>
<td>76</td>
<td>142</td>
</tr>
</tbody>
</table>

* P << 0.001

---

* P << 0.001
Fig. 4.3.1c  Relationship between $g_s$ and $C_i$ in Scots pine in red (o) and blue (●) light, (as Figs. 4.3.1a and b) and in white light (+) for a single shoot. Points have been joined for one shoot in each waveband.  (Experiment 4.1)  
($\Delta$ = darkness)
higher in blue light than in red light for any $g_s$.

Comparing Figs. 4.3.1a and b, it is evident that the opening response in blue light was independent of mesophyll assimilation: stomatal conductance increased from c. 0.05 cm s$^{-1}$ in darkness, to c. 0.23 cm s$^{-1}$ at 110 $\mu$E m$^{-2}$ s$^{-1}$, while $A$ remained negative, and there was still a net efflux of CO$_2$ from the leaf. In red light the threshold value of $Q_1$ for the response of $g_s$ coincided with the light compensation point for $A$, suggesting an assimilation-mediated response. However, exactly the same response of $g_s$ to blue and red light occurred in CO$_2$-free air (Fig. 4.3.1d). The response to red light still showed a threshold value though this was diminished to c. 40 $\mu$E m$^{-2}$ s$^{-1}$. The intercellular CO$_2$ concentration was c. 15 cm$^3$ m$^{-3}$ throughout all experiments, net assimilation was always negative, yet there was a response to both blue and red light. The use of $G_1$ conceals that the maximum $g_s$ was almost double that in normal air. However, these data are from different shoots and no firm conclusion can be drawn on the effect of CO$_2$-free air from this alone (see Section 3.3.1).

### 4.3.2 Wavelength sensitivity of $g_s$ in Scots pine

The quantum flux density available in narrow wavebands was limited to 25 $\mu$E m$^{-2}$ s$^{-1}$ which is below the threshold in broad band red light previously noted limiting the value of the results. However, even at this $Q_1$, blue wavelengths caused increases in $g_s$ with a peak at $\lambda$ 435 nm, although $g_s$ was small (Fig. 4.3.2). Red light was ineffective. The response did not differ between treatments where wavelengths were progressively increased or decreased (Fig. 4.3.2(a)), in atmospheric CO$_2$ concentrations or in CO$_2$-free air (Fig.
Fig. 4.3.1d Relationship between $G_r$ and $Q_1$ in red (o) and blue (●) light. Three shoots in both red and blue light; zero ambient $CO_2$ concentration.

At $G_r = 1.0$, $g_s = 0.532 \pm 0.154$ cm s$^{-1}$  

(Experiment 4.2)
Fig. 4.3.2  (Legend on facing page)
Figure 4.3.2

Action spectra of $\Delta G_r$ in Scots pine, where:

$$\Delta G_r = \frac{g_s - g_{s\text{dark}}}{g_{s\text{max}}}$$

Quantum flux density = 25 $\mu$E m$^{-2}$s$^{-1}$ at all wavelengths. Points connected are for an individual shoot in each treatment.

(a) Two shoots illuminated in progressively shorter wavelengths (o) and two in the opposite direction (●). At $\Delta G_r = 1.0$, $g_s = 0.154 \pm 0.014$ cm s$^{-1}$.

(b) Two shoots illuminated in CO$_2$-free air (o) and in 330 cm$^3$ m$^{-3}$ ambient CO$_2$ concentration (●). At $\Delta G_r = 1.0$, $g_s = 0.137 \pm 0.033$ cm s$^{-1}$.

(Experiment 4.3)
4.3.2(b)). Net assimilation remained negative throughout all experiments (because of the low \( Q_1 \)), and \( C_i \) was between 350 and 400 cm\(^3\) m\(^{-3}\) at all times. No differences in \( A \) were found in light of different wavelengths.

4.3.3 The response of \( g_s \) and \( A \) in Scots pine to \( \text{CO}_2 \) and \( O_2 \) in red and blue light

The response of stomatal conductance to blue and red light was reversible and repeatable (Figs. 4.3.3 a and b). The response of \( g_s \) to blue light was 6-8 times the response to the same quantum flux density of red light. Pre-illumination with red light enhanced the subsequent response to blue light: stomatal conductance was significantly larger in the first blue treatment in normal air on the three days with a prior red treatment (60% larger, \( P < 0.01 \), t-test), than in blue light preceded by darkness.

In contrast, net assimilation was significantly smaller (by 20%, \( P < 0.01 \), t-test) in blue light, than in red light. This negative correlation of \( A \) and \( g_s \) in red and blue light resulted in intercellular \( \text{CO}_2 \) concentrations 40-60 cm\(^3\) m\(^{-3}\) higher in blue light than in red light. Changes in quantum flux waveband caused \( g_s \) to decrease while \( C_i \) decreased and to increase while \( C_i \) increased.

Red and blue light both caused increased opening in \( \text{CO}_2 \)-free air above that in normal air (discussed in Chapter 3), but \( g_s \) in red light was still only 30 to 40% that in blue light.

Low oxygen concentrations did not affect the opening response in blue light, whether from "red, 21% \( O_2 \)" or "red, 0.1% \( O_2 \)" treatments. However, the response in red light changed with pre-treatment: \( g_s \) was larger if preceded by "blue, 0.1% \( O_2 \)" than
Fig. 4.3.3a  A, C, and G, in Scots pine after alternating 2 hour treatments in red or blue light of equal intensity in atmospheric CO₂, zero CO₂ or in 0.1% O₂ with 330 cm⁻³ CO₂. Means of 4 shoots except where indicated. "Red light first" treatment. Significance levels of G change from value on the first day shown; paired t-tests. At Gᵣ = 1.0, gₛ = 0.40 ± 0.07 cm s⁻¹. (Experiment 4.4)
Figure 4.3.3b as Fig. 4.3.3a, but "blue light first" treatment.

At $G_r = 1.0$, $g_s = 0.36 \pm 0.02$ cm s$^{-1}$.

(Experiment 4.4)
by "blue, 21% \text{O}_2". This was exactly paralleled by the response of net assimilation to 0.1% \text{O}_2 in red light: \text{A} increased 2-3 fold. This is larger than expected by the inhibition of \text{PR} alone, and part of the stimulation can be attributed to the larger $g_s$ and higher intercellular CO$_2$ concentration. Net assimilation was not markedly stimulated by 0.1% \text{O}_2 in blue light; suggesting that the low assimilation efficiency of blue light is not due to a stimulation of PR.

Net assimilation was inhibited in blue light on returning to 21% \text{O}_2 but was stimulated in red light. Some of the increase in red light can be attributed to the increase in stomatal conductance; intercellular CO$_2$ concentrations were 25-40 cm$^3$ m$^{-3}$ higher than in normal air. In blue light, $g_s$ was larger than in any other treatment (c. 0.41 cm s$^{-1}$).

The response of stomatal conductance to C$_i$ in 21% \text{O}_2 has been presented in Fig. 3.3.1e. The response of net assimilation to C$_i$ at equal quantum flux densities of red and blue light is shown in Fig. 4.3.3c. Net assimilation was always larger at any C$_i$ in red light than in blue light, yet stomatal conductance was smaller. Net assimilation was limited by CO$_2$ diffusion through the stomata in atmospheric C$_a$ (denoted 'a') in red light (see also Fig. 4.3.1c). In blue light at atmospheric ambient CO$_2$ concentrations A was light-limited (see Fig. 4.3.1b). Linear regressions were fitted to the initial slopes (Table 4.3.3a). The slope was significantly lower in blue light than in red; the residual resistance to CO$_2$ diffusion, $r_r'$, was larger. CO$_2$ efflux into CO$_2$-free air was larger in blue light than in red light (0.050 compared to 0.082 mg m$^{-2}$ s$^{-1}$) and the CO$_2$ compensation point, $\Gamma$, was almost double the value in red light.
These latter two observations suggest that blue light stimulated PR or dark respiration. However, the pronounced saturation of the A/C\textsubscript{1} curve at intercellular CO\textsubscript{2} concentrations lower than 300 cm\textsuperscript{3} m\textsuperscript{-3} suggests that the major limitation to net assimilation in blue light is a photochemical process not a CO\textsubscript{2}-dependent reaction.

Net assimilation was stimulated in 0.1% O\textsubscript{2} in both red and blue light; the CO\textsubscript{2} compensation point was reduced to 25% of that in 21% O\textsubscript{2} in both red and blue light (Fig. 4.3.3d). However, γ was still higher in blue light than in red light (Table 4.3.3b) and the curves did not coincide. Again, A saturated in blue light at intercellular CO\textsubscript{2} concentrations above 300 cm\textsuperscript{3} m\textsuperscript{-3}.

Figure 4.3.3e presents the response of stomatal conductance to intercellular CO\textsubscript{2} in red and blue light in 0.1% O\textsubscript{2}. There is a large scatter of the data and the curves are not extended to g\textsubscript{s} at high C\textsubscript{l} (measured at the end of each experiment, after the CO\textsubscript{2}-free air treatment), and which was still declining after two hours. In comparison to Figure 3.3.1e, there is little difference between the response in red and blue light.

Figure 4.3.3f summarises the response of stomatal conductance to red and blue light in normal air, CO\textsubscript{2}-free air, 0.1% O\textsubscript{2} with 330 cm\textsuperscript{3} m\textsuperscript{-3} CO\textsubscript{2}, and CO\textsubscript{2}-free air with 0.1% O\textsubscript{2}. Data points have been taken from the results of 16 experiments plotted in Figs. 3.3.1e and 4.3.3e. Both red and blue light caused g\textsubscript{s} to increase above that in darkness in all the treatments, even in a nitrogen atmosphere; mean stomatal conductance was larger in blue than in red light in all treatments, though the difference was not statistically significant in 0.1% O\textsubscript{2}. Stomatal conductance was larger in CO\textsubscript{2}-free air in red and blue light in 21% O\textsubscript{2} (as demonstrated in Section 3.3.1), and also
Figure 4.3.3c

Relationship between A and \( C_1 \) in red (o) and blue (●) light in Scots pine; 21% \( O_2 \), five shoots illuminated in both wavebands. Curves hand fitted through the points for one shoot. 'a' indicates net assimilation rate at atmospheric ambient \( CO_2 \) concentrations.

(Experiment 3.3)

Figure 4.3.3d

Relationship between A and \( C_1 \) in red (o) and blue (●) light in Scots pine as above, but in 0.1% \( O_2 \) concentration. Only three shoots in either red or blue light, curve hand fitted through the points for one shoot in each waveband.

(Experiment 4.5)
Table 4.3.3a  
Response of A in 5 shoots of Scots pine to $C_i$ in broad band red and blue light of equal quantum flux densities, 230 $\mu$E m$^{-2}$ s$^{-1}$.

21% oxygen, initial points < 175 cm$^3$ m$^{-3}$ $C_i$

<table>
<thead>
<tr>
<th>Red</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>slope ($x 10^{-3}$) mg m$^{-2}$ s$^{-1}$</td>
<td>0.74</td>
</tr>
<tr>
<td>intercept mg m$^{-2}$ s$^{-1}$</td>
<td>-0.050</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.94</td>
</tr>
<tr>
<td>$n$</td>
<td>30</td>
</tr>
<tr>
<td>$s^2 (x 10^{-5})$</td>
<td>9.67</td>
</tr>
<tr>
<td>$s^2$ not sig. diff.</td>
<td>$F = 1.187$</td>
</tr>
</tbody>
</table>

slopes sig. diff. $t = 1.835$ 0.05 > $P < 0.025$

$CO_2$ compensation point, cm$^3$ m$^{-3}$ | 68 | 138 * |
residual resistance, $r_{r'}$, s cm$^{-1}$ | 26.6 | 31.8 |

* $P < 0.010$

Table 4.3.3b  
Response of A in 3 shoots of Scots pine to $C_i$ in broad band red and blue light of equal quantum flux densities, 230 $\mu$E m$^{-2}$ s$^{-1}$.

0.1% oxygen, initial points < 75 cm$^3$ m$^{-3}$ $C_i$

<table>
<thead>
<tr>
<th>Red</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>slope ($x 10^{-3}$) mg m$^{-2}$ s$^{-1}$</td>
<td>2.27</td>
</tr>
<tr>
<td>intercept mg m$^{-2}$ s$^{-1}$</td>
<td>-0.031</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.94</td>
</tr>
<tr>
<td>$n$</td>
<td>15</td>
</tr>
<tr>
<td>$s^2 (x 10^{-5})$</td>
<td>26.10</td>
</tr>
<tr>
<td>$s^2$ sig. diff.</td>
<td>$F = 10.86$</td>
</tr>
</tbody>
</table>

slopes sig. diff. $d = 4.482$ $P << 0.001$

$CO_2$ compensation point, cm$^3$ m$^{-3}$ | 17 | 34 * |
residual resistance, $r_{r'}$, s cm$^{-1}$ | 8.6 | 19.1 |

* $P < 0.05$
Fig. 4.3.3e  Relationship between $G_r$ and $C_i$ in Scots pine in 0.1% O$_2$ in red (o) and blue (●) light of equal $Q_i$ (230 μE m$^{-2}$s$^{-1}$) as Fig. 3.3.1e. arrows indicate the trend of the last points. mean of three shoots in either waveband ±1 s.e.m. shown where larger than the points.

At $G_r = 1.0$  $g_s = 0.508 \pm 0.041$ cm s$^{-1}$.

(Experiment 4.5)
Figure 4.3.3f (Legend on facing page)
Mean, steady-state $g_s$ in Scots pine in blue and red light of equal $Q_1$ (230 $\mu$E m$^{-2}$s$^{-1}$). in either atmospheric CO$_2$ concentrations ($C_a$), CO$_2$-free air, 0.1% O$_2$ with 330 cm$^3$ m$^{-3}$ CO$_2$ or in a nitrogen atmosphere. Points have been taken from Figures 3.3.1e and 4.3.3e and the $g_s$ prior to the start of the experiments (in darkness) is also shown. Percentage significance levels of the differences between treatments are shown from t-tests; shaded values are red/blue comparisons.
in 0.1% O₂. Stomatal conductance was not affected in 0.1% O₂ in blue light, but \( g_s \) was significantly larger in red light (\( P < 0.02 \), t-test).

4.3.4 The response of \( g_s \) and \( A \) to blue and red light in Sitka spruce.

The response of \( g_s \) in Sitka spruce to red and blue light in normal air (Fig. 4.3.4a) differed from that in Scots pine (Fig. 4.3.1a). While blue light remained more effective than red light the difference was not as marked as in Scots pine. The response to both red and blue light appeared to be reaching saturation at 620 and 320 \( \mu \text{E m}^{-2}\text{s}^{-1} \), respectively, although at different \( g_s \); red light would not produce the same maximum \( g_s \) as blue light even at large \( Q_l \).

There was no threshold quantum flux density for the effect of red light on \( g_s \). The "dark \( g_s \)" prior to the light treatments was large (mean 0.20 cm s⁻¹) in comparison to Scots pine (mean 0.050 cm s⁻¹); stomata in these shoots of Sitka spruce were not completely closed in darkness in the morning.

The differences in the effects of red and blue light in net assimilation were also less marked than in Scots pine (Fig. 4.3.4b, compared with Fig. 4.3.1b). The apparent quantum yield in blue light was only slightly lower than in red light (Table 4.3.4). Apparent quantum yields are higher than in Scots pine as trimming the needles into a single dorso-ventral plane eliminated self-shading. There was no difference in the light compensation points.

The intercellular CO₂ concentration was always higher in blue light than in red light at the same stomatal conductance, as in Scots pine, because of the contrasting effects of blue and red light on \( A \) and \( g_s \) (Fig. 4.3.4c). Again, there is no overlap of the points for
Fig. 4.3.4a Relationship between $G_r$ and $Q_i$ in Sitka spruce in red (o) and blue (●) light. Four shoots illuminated in both wavebands; atmospheric $C_a$. At $G_r = 1.0$, $g_s = 0.681 \pm 0.0377 \text{ cm s}^{-1}$. (Experiment 4.1)
Fig. 4.3.4b Relationship between A and $Q_i$ in Sitka spruce in red (o) and blue (●) light. Corresponding to Fig. 4.3.4a (Experiment 4.1)
Table 4.3.4

Response of A in 4 shoots of Sitka spruce to broad band red and blue light of increasing quantum flux densities

<table>
<thead>
<tr>
<th></th>
<th>Red</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial points</td>
<td>&lt; 120 $\mu E \text{ m}^{-2} \text{s}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>slope ($x 10^{-3}$ mg $\mu E^{-1}$)</td>
<td>1.87</td>
<td>1.55</td>
</tr>
<tr>
<td>intercept mg $\text{ m}^{-2} \text{s}^{-1}$</td>
<td>-0.060</td>
<td>-0.055</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.99</td>
<td>0.97</td>
</tr>
<tr>
<td>$n$</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>$s^2$ ($x 10^{-4}$)</td>
<td>1.69</td>
<td>1.52</td>
</tr>
<tr>
<td>$s^2$ not sig. diff.</td>
<td>$F = 1.112$</td>
<td></td>
</tr>
<tr>
<td>slopes sig. diff.</td>
<td>$t = 3.247 \ P &lt; 0.005$</td>
<td></td>
</tr>
<tr>
<td>light compensation point $\mu E \text{ m}^{-2} \text{s}^{-1}$</td>
<td>31</td>
<td>35 *</td>
</tr>
<tr>
<td>apparent quantum yield mol E$^{-1}$</td>
<td>0.043</td>
<td>0.035</td>
</tr>
<tr>
<td>quantum requirement E mol$^{-1}$</td>
<td>23.5</td>
<td>28.4</td>
</tr>
</tbody>
</table>

* not significant
Fig. 4.3.4c Relationship between $g_s$ and $C_i$ in Sitka spruce in red (o) and blue (●) light (as in Figs. 4.3.4a and b). Points have been joined for one shoot in both wavebands. (Experiment 4.1) ($\Delta$ = darkness)
red and blue light; there is no single relationship of \( g_s \) with \( C_1 \).

Again, as in Scots pine, the response of \( g_s \) to light is independent of mesophyll assimilation: similar opening response curves to red and blue light were found in CO\(_2\)-free air (Fig. 4.3.4d). Blue light remained more effective than red light in CO\(_2\)-free air; \( g_s \) showed larger increases at low quantum flux densities in both red and blue light and reached saturation at lower \( Q_i \) than in normal air (c. 250 and 100 \( \mu \)E m\(^{-2}\) s\(^{-1}\), respectively). Intershoot variability concealed any effect of CO\(_2\)-free air on absolute \( g_s \), but a final 2 hour period at low \( Q_i \) in normal CO\(_2\) concentrations only caused a slight decrease in \( g_s \) in blue light (see also Fig. 3.3.1b).

4.3.5 The time course of \( g_s \) in response to red and blue light in 

**Sitka spruce**

The change of stomatal conductance after a large increase in quantum flux density in both blue and red light was slow, taking up to four hours to reach maximum \( g_s \) (Figs. 4.3.5a and b). During the preceding night \( g_s \) declined to a minimum and then increased; there was a pronounced increase before "lights on". The change in stomatal conductance following illumination is described by the exponential equation:

\[
-g_s = g_{sm} (1 - e^{-t/k})
\]

(4.3.5a)

where \( g_{sm} \) is the asymptotic value of \( g_s \), \( t \) is time and \( k \) is the time constant. When \( t/k = 1 \), \( g_s = 0.63 \ g_{sm} \), and when \( t/k = 3 \), \( g_s = 0.95 \ g_{sm} \) (Landsberg 1977). The data from 3 shoots were fitted to equation 4.3.5a using a non-linear least squares optimisation procedure.
Fig. 4.3.4d Relationship between $G_r$ and $Q_j$ in Sitka spruce in red (o) and blue (●) light. CO$_2$-free air, three shoots illuminated in both wavebands. 
At $G_r=1.0$ $g_s = 0.681\pm0.037$ cm s$^{-1}$ (Experiment 4.2)
(H. Talbot, pers. comm.); the parameters are given in Table 4.3.5 below:

Table 4.3.5

Values of the parameters in equation 4.3.5a derived from fitting $g_s$ to time: $g_{sm}$ is the asymptotic value of $g_s$, $g_{s0}$ is the value of $g_s$ at time $t = 0$, and $k$ is the time constant, in min. $g_{sm}$ and $g_{s0}$ are in cm s\(^{-1}\).

<table>
<thead>
<tr>
<th>Shoot</th>
<th>RED</th>
<th></th>
<th>BLUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$g_{sm}$</td>
<td>$g_{s0}$</td>
<td>$k$</td>
</tr>
<tr>
<td>a</td>
<td>0.683</td>
<td>0.324</td>
<td>76</td>
</tr>
<tr>
<td>b</td>
<td>0.589</td>
<td>0.201</td>
<td>69</td>
</tr>
<tr>
<td>c</td>
<td>0.392</td>
<td>0.115</td>
<td>70</td>
</tr>
</tbody>
</table>

There is no marked difference in the time constant, $k$, in red and blue light; $k$ is slightly smaller in red light than in blue because the overall change in $g_s$ is smaller. The time constants are large ($k = 69-84$ min) because of the large change in $g_s$ in response to a change in quantum flux density from 0 to 230 $\mu$E m\(^{-2}\)s\(^{-1}\). In an opening response curve (as in Fig. 4.3.4a), changes in $Q_i$ are much smaller and $k$ is smaller (of the order of 30 minutes). Treatment periods 110 minutes long at each quantum flux density then covered approximately 0.95 of the total change in stomatal conductance.
Figure 4.3.5a  Time course of $g_s$ in a single shoot of Sitka spruce in darkness and when illuminated with blue light, $Q_i = 230 \mu E \ m^{-2} \ s^{-1}$. Average VPD and $C_i$ indicated where large changes took place. *Curves drawn by hand.* (Experiment 4.6)
Figure 4.3.5b Time course of $g_s$ in a single shoot of Sitka spruce in darkness and when illuminated with red light, $Q_i = 230 \mu E \text{ m}^{-2} \text{s}^{-1}$. Same shoot as Fig. 4.3.5a, (shoot 'a' in Table 4.3.5). (Experiment 4.6)
4.3.6 The response of $g_s$ and $A$ to red and blue light in dayflower

Experiments in which $g_s$ increases in response to increasing $Q_1$ inevitably result in declining intercellular CO$_2$ concentrations as net assimilation increases (e.g. Figs. 4.3.1c and 4.3.4c). In a species in which the stomata are sensitive to intercellular CO$_2$, changes in $C_1$ will therefore confound the measurement of any "direct" responses of $g_s$ to light. The sensitivity of dayflower stomata to $C_1$ has been demonstrated in Chapter 3.3. Preliminary experiments with dayflower showed a decrease in $C_1$ from 360 to 280 cm$^3$ m$^{-3}$ in an opening response curve in red light (data not shown). In blue light the decline was smaller (360 to 330 cm$^3$ m$^{-3}$); the difference in $C_1$ in red and blue light altered the response of $g_s$ to quantum flux density alone. To overcome this problem, background CO$_2$ was increased as $Q_1$ was increased and $C_1$ maintained approximately constant between 290 and 320 cm$^3$ m$^{-3}$ or at the CO$_2$ compensation point throughout the experiments (for details see Section 4.2, Experiment 4.7).

Stomatal conductance responded to increasing quantum flux density even though $C_1$ remained near 306 cm$^3$ m$^{-3}$ (Fig. 4.3.6a). Blue light was more effective than red light, especially at low quantum flux densities. Only the adaxial surface of shoots was illuminated and the available quantum flux density was low; in spite of this, $g_s$ tended towards saturation at 100 and 300 μE m$^{-2}$ s$^{-1}$ in blue and red light respectively.

Net assimilation was lower in blue light than in red light (Fig. 4.3.6b). The apparent quantum efficiency was significantly lower in blue light than in red light (Table 4.3.6). The response of
Figure 4.3.6a  Relationship between $G_r$ and $Q_i$ in dayflower in red (o) and blue (●) light. Two shoots in both wavebands; $C_i$ maintained constant at 306 cm$^3$ m$^{-3}$. At $G_r = 1.0$, $g_s = 0.913 \pm 0.062$ cm s$^{-1}$.  (Experiment 4.7)
Figure 4.3.6b  Relationship between $A$ and $Q_i$ in dayflower in red (o) and blue (●) light, corresponding to Fig. 4.3.6a. Linear regressions drawn through all point lower than 120 $\mu$E m$^{-2}$s$^{-1}$, see Table 4.3.6. (Experiment 4.7)
Table 4.3.6

Response of A in 2 shoots of Dayflower to broad band red and blue light of increasing quantum flux densities at a constant $C_l$ of 306 cm$^3$ m$^{-3}$

<table>
<thead>
<tr>
<th></th>
<th>Red</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial points &lt; 200 $\mu$E m$^{-2}$s$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>slope ($x 10^{-3}$) mg $\mu$E$^{-1}$</td>
<td>1.87</td>
<td>1.32</td>
</tr>
<tr>
<td>intercept mg m$^{-2}$ s$^{-1}$</td>
<td>-0.031</td>
<td>-0.048</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>$n$</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>$s^2 (x 10^{-5})$</td>
<td>3.67</td>
<td>6.16</td>
</tr>
</tbody>
</table>

$s^2$ not sig. diff.  $F = 1.68$

slopes sig. diff.  $t = 6.768$  $P << 0.001$

light compensation point  $\mu$E m$^{-2}$s$^{-1}$ | 17   | 37 * |

apparent quantum yield mol E$^{-1}$ | 0.043 | 0.030 |

quantum requirement E mol$^{-1}$ | 23.5 | 33.3 |

* $P << 0.010$
Figure 4.3.6c  Relationship between $g_s$, $A$ and $Q_i$ in dayflower in red (o) and blue (●) light; $C_i$ maintained nearly constant at 50 cm$^3$ m$^{-3}$.

One single shoot.

(Experiment 4.7)
Figure 4.3.6d  Relationship between $g_s$ and $C_i$ in dayflower as quantum flux density increased in red (o) and blue (●) light; $C_i$ maintained nearly constant corresponding to Figs. 4.3.6a, b & c.  
(Experiment 4.7)
net assimilation to quantum flux density did not show saturation even at high \( Q_1 \) because \( C_i \) was maintained high.

Stomatal conductance also increased in response to red and blue light when the intercellular \( \text{CO}_2 \) concentration remained at \( \approx 50 \text{ cm}^3 \text{ m}^{-3} \) (Fig. 4.3.1c). The data from Figures 4.3.6a and c are replotted as \( g_s \) against intercellular \( \text{CO}_2 \) concentration in Figure 4.3.6d; there was no directional change in \( C_i \) in any experiment while \( g_s \) increased from 0.2 to 1.0 cm s\(^{-1}\). There was no relationship of \( g_s \) and \( C_i \) in either red and blue light; the effects of blue and red light are independent of changes in \( C_i \).

4.3.7 The response of \( g_s \) and \( A \) to white light in dayflower at constant \( C_i \)

Stomatal conductance increased in response to increasing quantum flux density in white light at three constant intercellular \( \text{CO}_2 \) concentrations (Eq. 4.3.7a). \( C_i \) was almost constant throughout the experiments (even increasing slightly, Fig. 4.3.7b) yet there was a large response of stomatal conductance to quantum flux density. The response differed at each \( C_i \): the initial \( g_s \), the initial slope and the final \( g_s \) at large \( Q_1 \) all increased as \( C_i \) decreased. The response at intercellular \( \text{CO}_2 \) concentrations of 302 and 466 \( \text{cm}^3 \text{ m}^{-3} \) approached saturation at large \( Q_1 \); with \( C_i = 46 \text{ cm}^3 \text{ m}^{-3} \) \( g_s \) increased at quantum flux densities up to 700 \( \mu \text{E m}^{-2} \text{s}^{-1} \). The absence of any response to light at \( Q_1 \) higher than 300 \( \mu \text{E m}^{-2} \text{s}^{-1} \) at 466 \( \text{cm}^3 \text{ m}^{-3} \) \( C_i \) agrees with the results in Fig. 3.3.2b.

The response of \( g_s \) to light in Figure 4.3.6a above was independent of mesophyll assimilation: the largest response to light was at a constant intercellular \( \text{CO}_2 \) of 46 \( \text{cm}^3 \text{ m}^{-3} \) when \( A \) was zero or negative (Fig. 4.3.7c).

109
Figure 4.3.7a  Relationship between $g_s$ and $Q_i$ in dayflower in white light, intercellular CO$_2$ concentration maintained constant at three values ($C_i$). Single shoot.  (Experiment 4.8)
Figure 4.3.7b Relationship between $g_s$ and $C_i$ as quantum flux density of white light increased. Single shoot of dayflower; $C_i$ maintained nearly constant, vertical lines indicate mean concentration, symbols as in Fig. 4.3.7a (Experiment 4.8)
Figure 4.3.7c Relationship between $A$ and $Q_1$ in dayflower in white light, intercellular CO$_2$ concentration maintained constant at three values, corresponding to Fig. 4.3.7a (Experiment 4.7)
4.4 Discussion

Broad band blue light was more effective than broad band red light in increasing stomatal conductance in Scots pine, Sitka spruce and dayflower; a result in agreement with the blue light-enhanced stomatal opening widely reported.

While blue light was always more effective than red light in increasing stomatal conductance, the opposite was true for net assimilation. The greater efficiency of red light in CO₂ assimilation in Scots pine, Sitka spruce and Dayflower is in agreement with the photosynthetic action spectra of whole leaves (Gaudillère and Costes 1971, McCree 1972, Inada 1976). Poskuta (1968), citing the evidence for blue light-stimulated respiration in Chlorella of Kowallik (1967) and Pickett and French (1967), suggested that the lower efficiency of blue light in shoots of Picea glauca Moench/Voss. was caused by blue light-stimulated photorespiration. However, several other workers have not found any evidence for blue light-stimulated photorespiration in a wide range of species (e.g. Bulley, Tregunna and Nelson 1969, Voskresenskaya et al. 1970, Linder 1971, Clark and Lister 1975). The results presented here for Scots pine do not support the stimulation of PR by blue light. While the CO₂ compensation point in blue light was twice the value in red light in 21% O₂, 0.1% O₂ did not result in a larger percentage decrease of PR in blue light than in red light. The two curves of net assimilation against intercellular CO₂ in red and blue light do not coincide in low O₂, when PR should be completely inhibited.

In conclusion, the lower efficiency of blue light in net assimilation must be a result of absorption of blue quanta by
photosynthetically-inactive pigments such as carotenoids and polyphenolics as has been widely concluded.

The response of stomatal conductance to red and blue light in the three species investigated was similar to that found in *V. faba* by Hsiao et al (1973). In both leaf discs in normal air and in epidermal strips in CO₂-free air, blue light (λ 440 nm) was more effective in opening stomata than red light (λ 660 nm). The response of stomata of *V. faba* to Q₁ saturated at different values of conductance or aperture in blue and red light as was found in Sitka spruce and in dayflower. Red light never stimulated stomatal opening up to the maximum observed in blue light, suggesting that the two wavebands act through different mechanisms on stomatal opening. At small Q₁ (13 μE m⁻² s⁻¹) stomata in leaf discs or in epidermal strips only responded to blue wavelengths, but at larger quantum flux densities (63 μE m⁻² s⁻¹) they responded to both blue and red wavelengths. This corresponds closely to the threshold value of Q₁ found in Scots pine (c. 70 μE m⁻² s⁻¹). However, no threshold for the response to red light was found in Sitka spruce or dayflower. Hsiao et al also reported that stomata in epidermal strips of dayflower responded markedly to blue light of a low quantum flux density (7 μE m⁻² s⁻¹) with a peak at the same wavelength as in *V. faba*. This is similar to the opening response to broad band blue light found for dayflower shoots in this study at a quantum flux density as low as 12 μE m⁻² s⁻¹.

The limited information on the action spectrum for gₛ in Scots pine indicates a peak near λ 432 nm. This agrees with the peak aperture and maximum ⁸⁶Rb⁺ uptake at λ 440 nm (Hsiao et al 1973) and the peak rate of malate formation at λ 433 nm (without background red
light) in *V. faba* stomata (Ogawa *et al.* 1978), and with the peak of stomatal opening in maize leaves at 439 nm (Karvén 1961). Other action spectra, measured with background red light, showed peaks between 460 and 470 nm, for the enhancement of CO₂ uptake in leaves of *Convallaria majus* (Voskresenskaya and Polyakov 1975), for the rapid blue light-induced transpiration response in *A. sativa* (Skaar and Johnsson 1978) and for malate formation in epidermal strips of *V. faba* (Ogawa *et al.* 1978). These authors have all concluded that there is a synergistic effect of red and blue light on stomatal movement. A similar synergistic effect was evident in the present study in Scots pine, where the increase in *gₛ* in blue light was enhanced by pre-illumination with red light. Brogårth (1975) has reported a similar effect of red light pre-illumination on the blr in *A. sativa* and he suggested that pre-illumination lowered *Cᵢ* and "sensitised" the stomata to the blr. This is unlikely in Scots pine in view of the relative insensitivity of stomatal conductance to intercellular CO₂; moreover, while *Cᵢ* decreased in red light, the subsequent blue light-induced increase in *gₛ* caused *Cᵢ* to increase. Skaar and Johnsson (1978) and Ogawa *et al.* (1978) considered that the synergistic effect results from an improved energy supply from red light-stimulated photosynthesis. However, Ogawa *et al.* proposed that the blue light effect was through activation of malate synthesis, probably by enhanced PEP carboxylase activity, while Skaar and Johnsson contend that blue light acts through a specific, membrane-bound pigment directly on the ion-transport processes. The mechanism of the blue light effect will be discussed further in Chapter 6.

In all three species investigated in this study, large increases
of stomatal conductance occurred at low quantum flux densities of blue light, while net assimilation was negative and $C_i$ was high, indicating that the response to blue light was not mediated by mesophyll assimilation. Moreover, the stomatal response to both blue and red light in CO$_2$-free air when net assimilation was negative and $C_i$ remained very low was similar to that in atmospheric CO$_2$ concentrations. Ng and Jarvis (1980) have previously found the same opening and closing responses to white light in Scots pine shoots in normal air, in air with CO$_2$ at the CO$_2$-compensation point, and in CO$_2$-free air.

The results with dayflower are similarly compelling evidence of a direct response to light; stomatal conductance increased in red and blue light while $C_i$ remained constant at 306 cm$^3$ m$^{-3}$ and at c. 50 cm$^3$ m$^{-3}$ when net assimilation remained near zero. In white light, stomatal conductance increased with increasing $Q_i$ while the intercellular CO$_2$ remained at 466, 302 or 46 cm$^3$ m$^{-3}$; again light was not acting through $C_i$ changes, or through mesophyll CO$_2$ assimilation as the maximum response to $Q_i$ occurred at 46 cm$^3$ m$^{-3}$ $C_i$ when A remained at or near zero at all quantum flux densities. The saturation of the response at each $C_i$ at different stomatal conductance indicates that even at infinite quantum flux density, $C_i$ will still have an inhibitory effect on $g_s$. The increasing divergence of the curves as $Q_i$ increases suggests increasing sensitivity to intercellular CO$_2$ as quantum flux density increases and is a further indication of the interaction of CO$_2$ and light noted in Chapter 3 and reported by Travis and Mansfield (1979) in epidermal strips of the same species. Mansfield et al (1980) have suggested that this interaction is a result of the ultimate control by both
light and CO$_2$ of the process of ion accumulation in the guard cells. This will be further discussed in Chapter 6.

Similar responses to light at constant $C_i$ have been described by Heath and Russell (1954a and b, discussed in the Introduction 4.1.2) in wheat leaves and by Raschke (1979, Fig. 18) in maize leaves. Raschke has presented a response curve of $g_s$ to $Q_i$ at a constant $C_i$ of 100 cm$^3$ m$^{-3}$; the response was large, conflicting with the absence of a major direct response to light in maize stomata proposed by Raschke, Hanebuth and Farquhar (1978). However, their results are not unequivocal (see Introduction 4.1.2) and in addition show a blue light-enhanced stomatal opening as found by Karvé (1961) and Raschke (1967a).

Several workers, in concluding that the blue light effect is CO$_2$-independent, have considered that the red light effect is CO$_2$-dependent (e.g. Skaar and Johnsson 1978). The finding that both blue and red light caused large increases in $g_s$ in Scots pine, Sitka spruce and dayflower at low and constant $C_i$ (10-20, 50 cm$^3$ m$^{-3}$) is important in the interpretation of the effect of light on stomata. In addition, a limited study with an albino shoot of Sitka spruce (arising from a periclinal chimaera) found increases in both blue and red light when there was no mesophyll assimilation. The conclusion that both blue and red light can cause "direct" effects on guard cells independent of $C_i$ and mesophyll assimilation is then inescapable. Similar results have been found in epidermal strips of V. faba (Hsiao et al 1973) and of dayflower (Mansfield et al 1980) but this contrasts with the CO$_2$-dependent transpiration response to red light in A. sativa (Brogårdh 1975) and in the Xan-$U^{21}$ mutant of Hordeum vulgare (Skaar and Johnsson 1980). Plants with
graminaceous-type stomata are known to differ in that they possess the bir which has not been found in other species (Johnsson et al 1976). Certainly, there is no evidence of any different response time to blue and red light in Sitka spruce (Section 4.3.5). It may be that graminaceous-type stomata also differ in the effect of red light.

Raschke (1975a) has previously suggested that the "direct" effect of light on guard cells is via the CO$_2$-scrubbing action of guard cell chloroplasts in the light. Red light could be acting in this way but there is evidence that guard cell chloroplasts are incapable of CO$_2$ reduction (see Introduction 4.1.2). Citing the findings of Raschke and Dittrich (1977) that epidermal strips of dayflower are unable to fix CO$_2$ photosynthetically, Mansfield et al (1980) concluded that the effect of red light on stomata in epidermal strips was through cyclic electron flow and ATP production in the guard cells resulting in a stimulation of ion transport. Further discussion of the mechanism of light effects on stomata is deferred to Chapter 6.

In all three species investigated the effects of CO$_2$ overlaid the effects of light. In the two conifer species the effect was small (Chapter 3) and only in dayflower was it possible to define an interaction of light and CO$_2$. The increase of stomatal conductance in low intercellular CO$_2$ concentrations persisted in low O$_2$ conditions in Scots pine. While a number of studies on the effects of low O$_2$ on stomatal movement have been carried out, the results are conflicting because of the wide range of low O$_2$ concentrations used and the presence or absence of CO$_2$ (e.g. Walker and Zelitch 1963, Humble and Hsiao 1970). The work of Saxe (1979) has indicated a further drawback; the aperture of individual stomata in a nitrogen
atmosphere changes markedly with time, showing a first "locked" phase and then closing and opening movements. As with many studies using metabolic inhibitors results can be selected to fit any hypothesis as Hsiao (1976) has emphasised.

None of the reports using white light can explain the different effects of low \( O_2 \) on \( g_s \) in red and blue light. Only two, contradictory reports exist on the effects of blue and red light in low \( O_2 \) conditions: Raschke (1967b) reported a large increase in conductance in \( V. \ faba \) leaves in blue light on replacing \( CO_2 \)-free air with \( N_2 \). Using epidermal strips of the same species, Lurie (1978) found that the mean stomatal aperture after 2.5 hours in blue light in normal air, 10 \( \mu m \), was reduced in \( N_2 \) to 6.0 \( \mu m \) while the aperture in red light remained unchanged at 7.5 \( \mu m \). The results with Scots pine differ completely; there was little effect of low \( O_2 \) on stomatal conductance in blue light, but a marked increase in \( g_s \) in red light. The stimulation in red light is not readily explicable. In so far as it parallels the increased mesophyll assimilation the stimulation suggests that a similar enhancement of \( CO_2 \) fixation may take place in the guard cells leading to a larger formation of organic acids (as counterions for \( K^+ \)) and increasing stomatal conductance. However, if guard cells do not possess the Calvin cycle of \( CO_2 \) fixation there would be no \( O_2 \)-dependent FR and another \( O_2 \)-inhibited process in the guard cells must be invoked.

Another possible explanation is suggested by the work of Dittrich and Raschke (1977) and Willmer et al (1978) on the translocation of assimilatory intermediates to the epidermis from the mesophyll. If such a process occurs in Scots pine, the increased net assimilation in red light, but not in blue light, in low \( O_2 \) may have increased the
availability of such compounds to the guard cells and thus stimulated opening. However, this does not account for the large increase of $g_s$ in blue light on return to 21% $O_2$ which must result from direct effects of $O_2$ on the guard cells. It is unprofitable to speculate further on the effect of low $O_2$ on stomata until more details of the biochemistry of guard cells is available and oxygen-sensitive processes can be elucidated.
4.5 Conclusions

a) Blue light was more effective on a per quantum basis in opening stomata than red light in Scots pine, Sitka spruce and dayflower.

b) The opposite was true for net assimilation: red light was more effective than blue light. The low efficiency of blue light was not a result of blue light-enhanced photorespiration but was caused by absorption of blue wavelengths by photosynthetically inactive pigments.

c) There were direct effects of both blue and red light on stomata independent of mesophyll CO$_2$ assimilation and C$_4$ in all three species. There were large increases in stomatal conductance in dayflower in response to increases in quantum flux density of white light when the intercellular CO$_2$ was maintained constant at low, medium and high concentrations. Stomatal conductance was more sensitive to quantum flux density at low intercellular CO$_2$ concentrations.

d) The greater effectiveness of blue light in opening stomata and the different effect of low O$_2$ on stomatal conductance in blue and red light suggest that blue and red light work through different mechanisms on stomatal opening.
5.1 Introduction

The preceding chapters have described the experimental separation of light effects from CO₂ effects on stomata. It is now necessary to assess the relative importance of light and CO₂ responses in circumstances in which the separation of light and CO₂ as variables does not occur.

So far I have considered what responses occur and how they occur. An alternative approach is to assume a purpose for stomata as "moderators, regulators and optimisers of gas exchange" (Raschke 1979). The basic assumption is a teleological one: the role of stomata is to effect a compromise between water loss and CO₂ uptake. Plant internal surfaces are effectively wet and the water vapour gradient to the atmosphere is two orders of magnitude larger than the reverse gradient of CO₂ concentration to the sites of carboxylation; several hundred more water molecules are lost than CO₂ molecules taken up per unit time.

The essential requirement to minimise water loss with respect to assimilation is to synchronise stomatal opening with mesophyll assimilatory activity. Broadly, this is the case since stomata open in daylight facilitating CO₂ uptake but if stomata are to reconcile the conflict between water loss and CO₂ uptake effectively they must be able to respond to the two processes directly (Raschke 1979).

Cowan, Farquhar and their coworkers have adopted control theory to analyse the interrelationships between transpiration, net
Figure 5.1 Block diagram showing the interrelationship between assimilation rate, A, leaf conductance, g, and intercellular \( \text{CO}_2 \) concentration. See text for details.
assimilation and leaf conductance (where leaf conductance, g, is related to stomatal conductance by \( \frac{1}{g} = \frac{1}{g_s} + \frac{1}{g_a} \), see equation 2.4.1j). Their approach expresses the interrelationships of these three processes and the effects of environmental variables on them as a series of "loops". A loop exists when, for example, a change in net assimilation rate after a change in quantum flux density alters the intercellular \( \text{CO}_2 \) concentration which then affects leaf conductance (Fig. 5.1). The subsequent change in g will affect \( C_i \), which then affects net assimilation. The loop of \( C_i \rightarrow g \rightarrow C_i \) (where \( \rightarrow \) is read as "affects") is a feedback loop; the effect of the response of g is to reduce the total change in \( C_i \). The total change taking place in one loop in response to a small change is a measure of the gain of the loop which has two components. In the example in Fig. 5.1 there are physiological gains; the direct response of A and g to changes in the intercellular \( \text{CO}_2 \) concentration which are dependent on physiological processes, and physical gains; the effect changes in net assimilation or a change in conductance have on \( C_i \). In feedback loops the gains are negative and the size of the gain is a measure of the degree of homeostasis (Farquhar, Dubbe and Raschke 1978). The closed loop gain is the ratio between the change that would occur without feedback and the change that occurs with feedback; it expresses the control exercised by the stomata on the two processes of assimilation and transpiration.

Cowan and Farquhar (1977) have expressed the control of transpiration and assimilation by leaf conductance, g, in the two loop gains \( \frac{\partial E}{\partial g} \) and \( \frac{\partial A}{\partial g} \). \( \frac{\partial E}{\partial g} \) expresses the sensitivity of transpiration rate to changes in leaf conductance; \( \frac{\partial A}{\partial g} \) expresses the sensitivity of net assimilation to changes in leaf conductance.
The ratio of the two loop gains, $\delta E/\delta A$ is the "gain ratio" (Farquhar 1979); when the ratio remains constant with changing environmental conditions stomatal behaviour is "optimal", i.e. transpiration losses are minimal for a given assimilation rate. However, while Farquhar, Schulze and Küppers (1980) have presented evidence that the gain ratio remained constant in *Nicotiana glauca* L. and *Corylus avellana* L., it is difficult to understand how stomata accomplish this; the ratio itself cannot be the signal that the stomata respond to. In particular, the approach does not assist the understanding of the processes involved in stomatal movement, although it does serve to integrate observed responses to single variables measured in experimental conditions.

While it is possible to separate CO$_2$ and light effects on stomata experimentally, it is difficult to assess their relative contribution when both are acting together. In the example in Figure 5.1 the change in leaf conductance caused by changes in the intercellular CO$_2$ concentration may be additional to any direct response of the stomata to changes in quantum flux density. In an attempt to quantify the contributions of each of these responses of stomata, Wong, Cowan and Farquhar (1978) have used control theory (as outlined above) in a study with *Eucalyptus pauciflora*. The intercellular CO$_2$ concentration changed little over an eight-fold increase in quantum flux density, indicating that stomata maintained a "constant C$_i$"; a suggestion that has been made by many workers (e.g. Raschke 1975a, Warritt 1977, Coudriaan and van Laar 1978, Louwerse 1980). However, the intercellular CO$_2$ concentration was not kept constant as the ambient CO$_2$ concentration was changed, but was dependent on the ambient CO$_2$ concentration; the relationship between C$_i$ and C$_a$ was
linear with a slope of 0.7. The gains of the loops involving assimilation ($G_A$) or conductance ($G_g$) and $C_i$ were small; the closed-loop gains were large (c. 0.8). Wong et al concluded that the direct responses of $g$ and $A$ to light were responsible for the near-constant $C_i$ at any one $C_a$ and not the response of the stomata to intercellular CO$_2$ concentration. This indicates that stomata were not responding to CO$_2$ uptake and were not "optimising" gas exchange.

The significance of the plot of $C_i$ against $C_a$, although used by many authors, has been misunderstood. Whiteman and Koller (1967a) used a simplified model of net assimilation, assuming that the CO$_2$ concentration at the site of carboxylation was zero, to express the slope of the $C_i/C_a$ relationship:

$$A = \xi_c (C_i / r'_r)$$  

$$A = \xi_c (C_a - C_i) / (r'_a + r'_s)$$

where $\xi_c$ is the density of CO$_2$ and the residual resistance, $r'_r >> r'_s > r'_a$. Solving the two simultaneous equations:

$$C_i \quad r'_r$$  

$$C_a \quad r'_r + r'_s + r'_a$$

if $r'_r >> (r'_s + r'_a)$ then the graph is linear with a slope near unity. However, the line will pass through the origin and this is not the case in C$_3$ plants. Strictly, with photorespiration, Equation 5.1a becomes:

$$A = \xi_c (C_i - \gamma) / r'_r$$

where $\gamma$ is the CO$_2$ compensation point (Lake 1967) and equation 5.1c becomes:

$$C_i \quad r'_r + (\gamma / C_a)(r'_a + r'_s)$$  

$$C_a \quad r'_r + r'_s + r'_a$$

126
<table>
<thead>
<tr>
<th>Species</th>
<th>conditions</th>
<th>slope</th>
<th>C range</th>
<th>author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helianthus annuus</em></td>
<td>H.L.</td>
<td>0.87</td>
<td>0-800</td>
<td>Goudriaan &amp; van Laar 1978</td>
</tr>
<tr>
<td></td>
<td>L.L. to H.L.</td>
<td>0.87-0.92</td>
<td>100-400</td>
<td>Whitteman &amp; Koller 1965a</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td>field H.L.</td>
<td>0.60</td>
<td>180-500</td>
<td>Flis 1980</td>
</tr>
<tr>
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<td>field H.L.</td>
<td>0.60</td>
<td>180-500</td>
<td></td>
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<tr>
<td></td>
<td>field-grown H.L.</td>
<td>0.41</td>
<td>60-600</td>
<td></td>
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<td></td>
<td>+ABA H.L.</td>
<td>0.40</td>
<td>60-600</td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus pauciflora</em></td>
<td>L.L. to H.L.</td>
<td>0.59-0.62</td>
<td>100-400</td>
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<tr>
<td><em>Xanthium strumarium</em></td>
<td>H.L.</td>
<td>0.92</td>
<td>0-550</td>
<td>Dubbe et al 1978</td>
</tr>
<tr>
<td></td>
<td>H.L.</td>
<td>0.90</td>
<td>0-500</td>
<td></td>
</tr>
<tr>
<td><em>Cossyphium hircutum</em></td>
<td>H.L.</td>
<td>0.92</td>
<td>0-500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ABA H.L.</td>
<td>0.65</td>
<td>0-500</td>
<td></td>
</tr>
<tr>
<td><em>Avena sativa</em></td>
<td>H.L.</td>
<td>0.90</td>
<td>0-500</td>
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<tr>
<td><em>Amaranthus powelli</em></td>
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<td>0.70</td>
<td>0-550</td>
<td></td>
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<tr>
<td></td>
<td>+ABA H.L.</td>
<td>0.36</td>
<td>0-500</td>
<td></td>
</tr>
<tr>
<td><em>Walus sylvestris</em></td>
<td>H.L.</td>
<td>0.83-0.58</td>
<td>0-600</td>
<td>Warril 1977</td>
</tr>
<tr>
<td><em>Plantas sylvestris</em></td>
<td>L.L. + H.L.</td>
<td>0.78-0.54</td>
<td>0-900</td>
<td>Ny 1978 &amp; pers. comm.</td>
</tr>
</tbody>
</table>

**Non-linear saturation functions**

<table>
<thead>
<tr>
<th>Species</th>
<th>conditions</th>
<th>C range</th>
<th>author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phascolus vulgaris</em></td>
<td>H.L.</td>
<td>C &gt; 210 if C &gt; 300</td>
<td>0-500</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>H.L.</td>
<td>C &gt; 120 if C &gt; 300</td>
<td>0-1200</td>
</tr>
<tr>
<td></td>
<td>H.L.</td>
<td>C &gt; 200 if C &gt; 300</td>
<td>0-600</td>
</tr>
<tr>
<td><em>Xanthium strumarium</em></td>
<td>+ABA curvilinear</td>
<td>0-600</td>
<td></td>
</tr>
</tbody>
</table>

where: L.L. and H.L. are low and high quantum flux densities, respectively, G.H. is green-house,
+ABA is addition of (+) abscisic acid to the transpiration stream,
N-fert is plants grown with a range of Nitrogen fertilisation regimes.
The line intersects the line of unit slope at the CO$_2$ compensation point when $A = 0$ and $C_i = C_a = \gamma$; at high ambient CO$_2$ concentrations and high assimilation rates the equation simplifies to 5.1c. Between these two extremes the interpretation is complicated by the interdependence of the variables; changing quantum flux density will affect $r_r', r_s'$ and $\gamma$.

The expression is analogous to that derived by Farquhar et al (1978) for the influence of the conductance ($G_g$) and assimilation loop ($G_A$) gains on the intercellular CO$_2$ concentration, or the combined loop gain:

$$\frac{dC_i}{dC_a} = \frac{\partial C_i/\partial C_a}{1 - G_g - G_A}$$ 5.1f

i.e. a change in $C_i$ expected through a change in $C_a$ at constant $g$ and $A$ will be modified by $1/(1-G_g-G_A)$. Since the loop gains are normally negative then the change in the intercellular CO$_2$ concentration expected from a change in the ambient CO$_2$ concentration will be reduced. It is evident that a slope of unity indicates no feedback, and a horizontal line where $C_i$ remains unchanged as $C_a$ changes indicates infinite gain of the feedback loops.

Table 5.1 presents data calculated from the literature for the slope of the $C_i/C_a$ line. Lower slopes indicate greater feedback (homeostasis) in either the conductance or the assimilation loops or both. The range for individual species is wide e.g. $Z$. mays 0.41-0.09. Different cultivars of apple (*Malus sylvestris* L.) showed differences in slope (Warritt 1977). The physiological condition of the plants influences the relationship: ABA supplied to leaves of $G$. 

128
hirsutum, X. strumarium and A. powellii decreased the slope, and
collapsed the response in Z. mays from a function showing saturation
(similar to that found by Farquhar et al 1978 and Goudriaan and van
Laar 1978) to a linear function (Dubbe et al 1978).

Environmental conditions also altered the combined loop gain
(defined in Equation 5.1f); increasing quantum flux density decreased
the slope in H. annuus (Whiteman and Koller 1967a), E. pauciflora
(Wong et al 1978) and P. sylvestris (Ng pers. comm.). The change in
slope in E. pauciflora over an eight-fold increase in quantum flux
density can be partitioned into a decrease in $G$ from -0.14 to -0.2
and a larger decrease in $G_A$ from -0.13 to -0.37. In sunflower the
results are less clear as stomatal conductance decreased as quantum
flux density increased, possibly because of changes in temperature
and VPD. In Scots pine the reduction in slope can only be attributed
to changes in the gain of the assimilation loop as stomata were
insensitive to changes in the intercellular CO$_2$ concentration (Ng
1978). It is not surprising that $G_A$ should increase (in absolute
terms) at higher quantum flux densities because $r'_r$ decreases (e.g.
Ludlow and Jarvis 1971) and net assimilation changes from a
light-limited to a CO$_2$-limited condition. The explanation for
increased $G$ at higher quantum flux densities is less evident but is
related to the interaction of light and CO$_2$ effects on stomata
discussed in Chapter 3 and 4.

In the following section the relative roles of the light and CO$_2$
effects on stomata in controlling gas exchange are examined in
daylight shoots.
5.2 Experiments

Stomata of dayflower have been shown to be sensitive to both intercellular CO$_2$ concentration and quantum flux density in the previous chapters. The response of stomatal conductance and net assimilation to changing $C_i$ at four different quantum flux densities was determined.

The response of $g_s$ and $A$ to $C_i$ and $Q_i$ in dayflower

The experimental method was similar to that in Experiment 3.5. The response of $g_s$ to $C_i$ was measured on four consecutive days at a different quantum flux density with a single attached shoot. The experiments commenced at high $C_a$ and decreased in steps, at c. 100 minute intervals. Measurements were taken at c. 70 and 90 minute intervals to check that equilibrium conditions had been reached at each ambient CO$_2$ concentration. Ambient CO$_2$ concentrations below 100 cm$^3$ m$^{-3}$ were not used to avoid photoinhibition of net assimilation at high quantum flux densities. (Experiment 5.1)

From these measurements the response curves of both stomatal conductance and net assimilation to $C_i$ were calculated at each quantum flux density $Q_i$. The response of stomatal conductance and net assimilation to $Q_i$ at constant $C_i$ were measured directly in Experiment 4.8 in a single shoot of dayflower.

The change in the intercellular CO$_2$ concentration as quantum flux density increased was calculated from a preliminary experiment in white light with one excised shoot of dayflower. The data for one shoot of Scots pine in white light is also shown, calculated from unpublished data of Ng.
5.3 Results and Analysis

5.3.1 Response of \( g_s \) and \( A \) in dayflower to \( Q_i \) and \( C_i \)

The effect of intercellular \( \text{CO}_2 \) concentration on stomatal conductance varied with quantum flux density (Fig. 5.3.1a): maximum sensitivity to \( C_i \) at 950 and 490 \( \text{uE m}^{-2} \text{s}^{-1} \) occurred between 300 and 500 \( \text{cm}^3 \text{m}^{-3} \) and the effect of low \( C_i \) saturated below 200 \( \text{cm}^3 \text{m}^{-3} \). At lower quantum flux densities stomatal conductance responded to intercellular \( \text{CO}_2 \) concentration throughout the range investigated. The stomatal conductances corresponding to \( C_a = 320 \text{ cm}^3 \text{m}^{-3} \) are indicated; these have been calculated from linear regressions of \( C_i \) against \( C_a \) at each \( Q_i \) for all points lower than 400 \( \text{cm}^3 \text{m}^{-3} \) (Fig. 5.3.1b).

The response curves of assimilation to \( C_i \) are presented in Fig. 5.3.1c. The rates of assimilation corresponding to \( C_a = 320 \text{ cm}^3 \text{m}^{-3} \) are indicated, calculated as above. These points lie at or near the plateau of the curves indicating that \( A \) was not limited by \( \text{CO}_2 \) diffusion. The departure from linearity at higher quantum flux densities when the intercellular \( \text{CO}_2 \) concentration was higher than 450 \( \text{cm}^3 \text{m}^{-3} \) is a result of the considerable inhibition of stomatal opening at high \( C_i \).

Following the analysis of Farquhar et al (1978) the gains of the \( g/C_i \) and \( A/C_i \) loops were calculated for the four quantum flux densities at \( C_a = 320 \text{ cm}^3 \text{m}^{-3} \) (Table 5.3.1). The physical gains were calculated from expressions derived from Equation 2.4.1r rewritten as:

\[
C_i = C_a - 1.6 \frac{A}{g} 
\]

5.3.1b

with appropriate units: \( C_i, C_a \) in \( \mu\text{mol m}^{-1} \), \( A \) in \( \mu\text{mol m}^{-2} \text{s}^{-1} \) and \( g \)
Figure 5.3.1a Relationship between \( g_s \) and \( C_i \) in dayflower at four quantum flux densities of white light (\( Q_1 \)). Treatments imposed from high to low \( C_a \), lowest \( Q_1 \) first day, higher \( Q_1 \) on subsequent days. Arrows indicate \( C_i \) at \( C_a \) of 320 cm\(^3\) m\(^{-3}\).
Figure 5.3.1b  Relationship between $C_i$ and $C_a$ at four quantum flux densities in dayflower in white light. Symbols as in Fig. 5.3.1a. Linear regressions for $Q_1 = 240$ and $130$ uE m$^{-2}$s$^{-1}$ shown for points lower than $400$ cm$^3$ m$^{-3}$ $C_a$. Vertical bar indicates maximum spread of regression lines at $C_a = 320$ cm$^3$ m$^{-3}$. 

$C_i$: intercellular CO$_2$ (cm$^3$ m$^{-3}$)
Figure 5.3.1c Relationship between A and $C_i$ in dayflower at four quantum flux densities of white light. Single shoot as Fig. 5.3.1a (Experiment 5.1)

$A$ is the net assimilation, $Q_1$ is the quantum flux density in $\mu E m^{-2} s^{-1}$, and $C_i$ is the intercellular $CO_2$ concentration in $cm^3 m^{-3}$. The graph shows the relationship between net assimilation and intercellular $CO_2$ concentration at different quantum flux densities.
Differentiating 5.3.1b:

\[ \frac{\partial C_i}{\partial g} \bigg|_{A, Ca} = 1.6 \, \text{A} / \, \text{g}^2 \]
\[ \frac{\partial C_i}{\partial A} \bigg|_{Ca, g} = -1.6 \, / \, \text{g} \]
\[ \frac{\partial C_i}{\partial C_a} \bigg|_{A, g} = 1 \]

The physiological gains were derived graphically from the slopes of the response curves at the points marked for \( C_a = 320 \, \text{cm m}^{-3} \) (Figs. 5.3.1a and c), corrected for \( g_a \).

The open loop gains \( G_g \) and \( G_A \) (the combined effect of the physical and physiological gains on \( g \) and on \( A \), respectively,) were small; combined as \( 1/(1-G_A - G_g) \) the net effect was to reduce the changes in the intercellular \( CO_2 \) concentration resulting from ambient \( CO_2 \) concentration changes by only 5-10% irrespective of quantum flux density. As previously discussed, this combined loop gain is the slope of the \( C_i/C_a \) relationship at the point \( C_a = 320 \, \text{cm m}^{-3} \); it is evident from the linearity of the relationship in Figure 5.3.1b that \( 1/(1-G_A - G_g) \) remained constant and small throughout the range of ambient \( CO_2 \) concentrations from 100 to 400 \( \text{cm}^3 \, \text{m}^{-3} \), only increasing when quantum flux density was high and \( C_a \) was above 450 \( \text{cm}^3 \, \text{m}^{-3} \).

This analysis appears to contradict the experimental evidence; the calculated gains indicate a negligible "feedback" of \( C_i \rightarrow g \rightarrow C_i \) even though there is a large response of stomata to \( C_i \) (Fig. 5.3.1a). The contradiction is resolved if account is taken of the magnitude of \( g_s \) of dayflower in this experiment: the stomatal conductance measured at large quantum flux densities with an ambient \( CO_2 \) concentration of 320 \( \text{cm}^3 \, \text{m}^{-3} \) (2.17 \( \text{cm s}^{-1} \)) is among the largest values recorded (see the review by Körner, Scheel and Bauer 1979). The physical gains \( \frac{\partial C_i}{\partial g} \bigg|_{A, Ca} \) and \( \frac{\partial C_i}{\partial A} \bigg|_{Ca, g} \), (the change in \( C_i \) caused by changes in \( g \) and \( A \) respectively,) depend on the size of the leaf conductance.
Table 5.3.1

Closed and Open loop gains and their component values, for one shoot of dayflower at four quantum flux densities at an ambient CO₂ concentration of 320 μmol mol⁻¹

<table>
<thead>
<tr>
<th>quantity</th>
<th>dimension</th>
<th>$Q_0 μE m^{-2} s^{-1}$</th>
<th>130</th>
<th>210</th>
<th>490</th>
<th>950</th>
</tr>
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<tbody>
<tr>
<td>$C_i$</td>
<td>μmol mol⁻¹</td>
<td>307</td>
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<td>289</td>
<td>283</td>
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<tr>
<td>$A$</td>
<td>μmol m⁻²s⁻¹</td>
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<td>5.68</td>
<td>8.75</td>
<td>14.20</td>
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<tr>
<td>$g$</td>
<td>mol m⁻²s⁻¹</td>
<td>0.249</td>
<td>0.333</td>
<td>0.626</td>
<td>0.901</td>
<td></td>
</tr>
</tbody>
</table>

**physical gains**

\[ \left( \frac{\partial C_i}{\partial A} \right)_{C_a, g} m²s⁻¹mol⁻¹ \]

-6.426 \quad -4.812 \quad -2.558 \quad -1.776

\[ \left( \frac{\partial C_i}{\partial g} \right)_{C_a, A} m²s⁻¹mol⁻¹ \times 10^6 \]

43.87 \quad 81.06 \quad 35.73 \quad 27.99

**physiological gains**

\[ \left( \frac{\partial A}{\partial C_i} \right)_{Q_i} mmol m⁻²s⁻¹ \]

2.92 \quad 7.69 \quad 15.91 \quad 15.91

\[ \left( \frac{\partial g}{\partial C_i} \right)_{Q_i} mol m⁻²s⁻¹ \]

-404 \quad -646 \quad -1201 \quad -1299

**loop gains**

$G_g$ dimensionless

-0.018 \quad -0.059 \quad -0.043 \quad -0.036

$G_A$ "

-0.019 \quad -0.037 \quad -0.047 \quad -0.028

1/(1-$G_g$) "

0.982 \quad 0.950 \quad 0.959 \quad 0.965

1/(1-$G_A$) "

0.981 \quad 0.964 \quad 0.961 \quad 0.973

1/(1-$G_g$-$GA$) or (\(\frac{\partial C_i}{\partial C_a}\))

0.964 \quad 0.918 \quad 0.917 \quad 0.939

\[ \left( \frac{\partial g}{\partial Q_i} \right)_{C_i} mol E^{-1} \]

1056 \quad 659 \quad 392 \quad 100

\[ \left( \frac{\partial A}{\partial Q_i} \right)_{C_i} mol E^{-1} \]

31.8 \quad 27.3 \quad 17.8 \quad c. 0.0

\[ \frac{dC_i}{dQ_i} m²s E^{-1} \]

-0.152 \quad -0.072 \quad -0.029 \quad 0.003

\[ \frac{dg}{dQ_i} mol E^{-1} \]

1119 \quad 705 \quad 427 \quad 97

\[ \frac{dA}{dQ_i} mmol E^{-1} \]

31.3 \quad 26.8 \quad 17.3 \quad c. 0.0
While the large slopes (near unity) of the $C_i/C_a$ relationship indicate that the feedback loops relating conductance and assimilation to intercellular CO$_2$ concentration are largely ineffective at reducing changes in $C_i$ caused by changes in $C_a$ (Fig. 5.3.1c), $C_i$ remained approximately constant at any ambient CO$_2$ concentration in spite of a seven-fold increase in quantum flux density. This suggests that stomatal conductance and net assimilation are responding in parallel to changes in quantum flux density, and are not linked through the effects of intercellular CO$_2$ concentration on stomata.

It is possible to calculate the relative roles of the direct stomatal response to light and the indirect response through $C_i$, following the analysis of Wong et al (1978). However, the calculation is influenced by the curve fitting procedures used to derive response curves of net assimilation and leaf conductance to quantum flux density at constant $C_i$. Confidence in the calculations is limited by estimating slopes from four-point graphs (corresponding to the four $Q_i$ values used) to calculate the partial differentials $(\Delta g/\Delta Q_i)_{Cl}$ and $(\Delta A/\Delta Q_i)_{Cl}$.

To resolve this difficulty, the response of $g$ and $A$ to $Q_i$ was measured directly (see Section 4.3.7). The partial differentials were calculated from the slope of the curves of net assimilation and stomatal conductance at a constant intercellular CO$_2$ concentration of 302 cm$^{-3}$ m$^{-2}$ (Fig. 4.3.7c and a, respectively) at the four quantum flux densities previously used. However, the values derived are not exactly appropriate because the intercellular CO$_2$ concentrations

(Equations 5.3.1c and d); large values of $g$ reduce the gains considerably.
previously calculated at each $Q_i$ were those corresponding to $C_a = 320 \text{ cm}^3 \text{ m}^{-3}$, and are $5-19 \text{ cm}^3 \text{ m}^{-3}$ lower than the intercellular CO$_2$ concentration of $302 \text{ cm}^3 \text{ m}^{-3}$. The values of $(\partial g/\partial Q_i)_C$ and $(\partial A/\partial Q_i)_C$ will be slight under and over estimates, respectively; they are presented in Table 5.3.1, together with $dC_i/dQ_i$, the change in $C_i$ with increasing quantum flux density. This latter value is small as is evident from the narrow vertical spread of the points in Fig. 5.3.1b at each ambient CO$_2$ concentration. The value of $dC_i/dQ_i$ is largest (in absolute terms) at the smallest $Q_i$ and tends towards zero at large $Q_i$.

Formally, $dC_i/dQ_i$ is calculated from:

$$
\frac{dC_i}{dQ_i} = \frac{(\partial g/\partial A)_{C_i}(\partial g/\partial Q_i)_C + (\partial C_i/\partial A)_{C_i}(\partial A/\partial Q_i)_C}{1 - g_A - g_g}
$$

5.3.1f

The small size of the changes in the intercellular CO$_2$ concentration with increasing quantum flux density is, again, linked to the absolute magnitude of leaf conductance through the physical gains $(\partial g/\partial A)_{C_i}$ and $(\partial C_i/\partial A)_{C_i}$ in the numerator. The effect of the combined loop gain (the denominator) is to reduce the already small changes. The small increase in $C_i$ at the largest quantum flux density is attributable to errors involved in estimating the slope near the asymptote of the $A/Q_i$ curve. Again, from Fig. 5.3.1b it is evident that $dC_i/dQ_i$, calculated above for $C_a = 320 \text{ cm}^3 \text{ m}^{-3}$, changes little between ambient CO$_2$ concentrations from 100 to $400 \text{ cm}^3 \text{ m}^{-3}$, only increasing slightly above that.
The effect of quantum flux density on leaf conductance and net assimilation (if all other variables are constant) is a product of the direct responses to light and the indirect response through \( C_i \):

\[
\frac{d g}{d Q_i} = \left( \frac{\partial g}{\partial Q_i} \right)_{C_i} + \left( \frac{\partial g}{\partial C_i} \right) \frac{d C_i}{d Q_i} \quad 5.3.1g
\]

\[
\frac{d A}{d Q_i} = \left( \frac{\partial A}{\partial Q_i} \right)_{C_i} + \left( \frac{\partial A}{\partial C_i} \right) \frac{d C_i}{d Q_i} \quad 5.3.1h
\]

Figure 5.3.1d compares the calculated values \( \frac{d g}{d Q_i} \) and \( \frac{d A}{d Q_i} \) (Table 5.3.1) with the partial differentials at constant \( C_i \) (i.e. neglecting the second and third terms in equations 5.3.1g and h above). Between 92 and 100% of the increase in \( g \) associated with increased quantum flux density can be attributed to direct effects of light on the stomata. Conversely, only a small proportion of the light response of stomata was mediated through reductions in the intercellular \( CO_2 \) concentration.

Furthermore, it is possible to extrapolate from the responses at \( C_a = 320 \text{ cm}^3 \text{ m}^{-3} \) at the range of quantum flux densities used to consider the case of a transition from darkness to low quantum flux densities. In this case leaf conductance is small, net assimilation is negative and the intercellular \( CO_2 \) concentration is high. It is evident from the hyperbolic form of the \( A/Q_i \) and \( g_s/Q_i \) curves at constant \( C_i \) (Figs. 4.3.7a and c) that \( \left( \frac{\partial g}{\partial Q_i} \right)_{C_i} \) and \( \left( \frac{\partial A}{\partial Q_i} \right)_{C_i} \) will be large at low quantum flux densities. The high intercellular \( CO_2 \) concentration may decrease the response of conductance but the effect is not large at low quantum flux densities (compare the initial slopes at 302 and 466 \text{ cm}^3 \text{ m}^{-3} , Fig. 4.3.7a). The response to changes in the intercellular \( CO_2 \) concentration on illumination (the physiological
Figure 5.3.1d Proportion of the total change in A and g as a result of increasing quantum flux density attributable to the direct response to light \( \frac{\Delta A}{\Delta Q_i} \) and \( \frac{\Delta g}{\Delta Q_i} \).
gains $(\partial A/\partial C_i)_{Q_1}$ and $(\partial g/\partial C_i)_{Q_1}$ will be slight because the maximum sensitivity of net assimilation and leaf conductance to $C_i$ is at high quantum flux densities.

The changes in leaf conductance on illumination from darkness will be very largely a result of the response to $Q_1$ and not a result of the effect of $C_i$. However, intercellular CO$_2$ concentration will show large changes at small quantum flux densities because of the small leaf conductance and the large physical gains $(\partial C_i/\partial A)_{Q_1}$ and $(\partial C_i/\partial g)_{Q_1}$. This is the reason for the large initial changes in $C_i$ as stomata open in response to light (Fig. 5.3.1c). The slope at any point is only approximately $dC_i/dQ_1$ because of changes in chamber CO$_2$ concentration ($C_a$) with changing assimilation rate. The near constant intercellular CO$_2$ concentration at higher quantum flux densities is a result of the parallel responses of net assimilation and leaf conductance to $Q_1$. This is particularly obvious in Scots pine; Ng (1978) found the stomata were completely insensitive to changes in the intercellular CO$_2$ concentration.
Figure 5.3.1e Change in $C_1$ with increasing quantum flux density of white light occurring during an opening response curve of $q_s$ to $Q_1$. (o) dayflower (excised shoot) (•) Scots pine mean of four shoots, calculated from unpublished data of Ng.
5.4 Discussion

The results obtained with dayflower lead to the same conclusions as have been drawn by Wong et al (1978) for *Eucalyptus pauciflora*. Stomatal response to the intercellular CO$_2$ concentration did not maintain $C_i$ constant through a negative feedback loop. The stomatal response to quantum flux density was almost entirely a result of the direct response of the guard cells to light rather than through changes in $C_i$. The near-constant intercellular CO$_2$ concentration over a seven-fold range in quantum flux density is a result of parallel responses of net assimilation and leaf conductance to light acting directly on the distinct processes and not through a link via $C_i$.

The conclusions are only valid for the conditions used in this experiment. Attention has already been drawn to the importance of the magnitude of leaf conductance. It is readily apparent from Fig. 5.3.1c that $g$ was large when the ambient CO$_2$ concentration was 320 cm$^3$ m$^{-3}$; the diffusion of CO$_2$ into the leaf did not limit assimilation. The same is true for the results of Wong et al (1978) with *E. pauciflora*.

However, the magnitude of leaf conductance is under the control of other variables than quantum flux density and intercellular CO$_2$ concentration alone. In these experiments water potential, temperature and humidity were maintained at "optimal" levels as far as was possible resulting in large leaf conductances. This inevitably reduced the physical gains $(\partial C_i/\partial A)_{Ca,g}$ and $(\partial C_i/\partial g)_{Ca,A}$. In particular, the experiments with dayflower were carried out at the minimum practicable VPD (0.33 kPa) to remove the effect of
transpiration on stomata through leaf water potential. The response of stomata to VPD was large in these shoots (see Chapter 2.5.2) small VPD resulted in large leaf conductance. A decrease in VPD would lead to increases in leaf conductance and would increase the physical gains. Because leaf conductance was large the effect of the feedback loop \( C_\text{g} \rightarrow C_\text{g} \rightarrow C_\text{g} \) was removed; this is also evident in the results with \( E. \text{pauciflora} \). Experiments were performed at 1.0-1.6 kPa VPD and the authors report that stomata were not affected by VPD. Larger gains of the feedback loops involving A or g and intercellular CO\(_2\) concentration will occur at larger VPD giving greater apparent regulation of the intercellular CO\(_2\) concentration as the ambient CO\(_2\) concentration is changed. This simply expresses the larger physical component resulting from decreased leaf conductance; there are no a priori reasons why large VPD should change the physiological gains (processes within the guard cells). There is evidence for the larger physical gains of the \( g/C_\text{g} \) loop at lower humidity in \( S. \text{indicum} \) (Hall and Kaufmann 1975, Hall, Schulze and Lange 1976).

Other variables (such as water stress and temperature) may affect both the physical and physiological gains of the \( A/C_\text{g} \) and \( g/C_\text{g} \) loops. Dubbe, Farquhar and Raschke (1978) showed that the introduction of ABA into the transpiration stream of leaves of \( C. \text{hirsutum}, Z. \text{mays}, X. \text{strumarium} \) and \( A. \text{powellii} \) increased both the physical and physiological gains. Such changes in the physiological gains indicate real interactions between variables at the cellular level; the results with dayflower and \( E. \text{pauciflora} \) both show an interaction between quantum flux density and intercellular CO\(_2\) concentration. Both species showed increased stomatal sensitivity at higher quantum flux densities. At the same time, it is evident that the gains of
the loops vary between species; stomata in Sitka spruce show very little sensitivity to intercellular CO$_2$ concentration and an analysis of this kind is unnecessary.

The merit of the optimal control theory approach has been questioned. While it can reveal no more about the mechanisms underlying observed stomatal responses than an experimental approach where the variables are separated artificially, it does contribute to our understanding of the relative importance of particular responses when variables are acting together, as has been demonstrated above. However, in the above analysis one limitation of the approach is obvious; for practical expediency only one or two variables can be analysed at any one time. It can be readily appreciated that negative gains will be underestimated and positive gains overestimated if other loops are also operating (Farquhar, Dubbe and Raschke 1978). For example, an increase in intercellular CO$_2$ concentration will close stomata reducing transpiration and improving tissue water status. This leads to a reduction in the final change in leaf conductance expected from the increase in C$_4$. However, the problem of the analysis of the multiple factors affecting stomata is obviously not restricted to this type of analysis, but is also inherent in an approach where environmental factors are experimentally separated. Optimal control theory at least presents a theoretical framework to investigate the interaction of variables, even though at the moment a full analysis is too complex to undertake.
5.5 Conclusions

a) The intercellular CO$_2$ concentration in one dayflower shoot remained constant over a seven-fold increase of quantum flux density. The intercellular CO$_2$ concentration was linearly dependent on the ambient CO$_2$ concentration; stomata did not control C$_1$.

b) The near-constant intercellular CO$_2$ concentration as quantum flux increased was a result of the parallel response of leaf conductance and net assimilation to light.

c) The opening of stomata in response to light from darkness can be attributed to the direct effects of light on the guard cells; the change of intercellular CO$_2$ concentration as quantum flux increased was not the cause of stomatal opening.

d) The relative contribution of the direct effects of light on stomata and the indirect effect through changes in the intercellular CO$_2$ concentration alters with the other variables that affect stomata.
6.1 Introduction

The experimental evidence presented in this thesis clearly establishes the existence of both a CO₂-independent light response and a light-independent CO₂ response in stomata. In this general discussion these results are integrated with current hypotheses and ideas on the physiological functioning of stomatal guard cells.

6.2 Guard Cell Ion Transport

It is now generally accepted that stomatal opening is the result of the osmotic effect of accumulation of potassium salts in the guard cells (Hsiao 1976). Potassium accumulates in guard cells in response to light in pteridophytes, gymnosperms (Gingko biloba L., Pinus sylvestris and P. taeda L.) and angiosperms and is also associated with nocturnal stomatal opening in CAM plants (Willmer and Pallas 1973, Dayanandan and Kaufman 1975). The influx of K⁺ into the guard cells is associated with an efflux of protons (Raschke and Humble 1973). There is considerable evidence from other ion transport systems in plants (reviewed by Bellando et al. 1979) that the link between potassium influx and proton efflux is through an electrogenic H⁺ extrusion pump. As MacRobbie (1977) has pointed out, guard cell behaviour during opening is not inherently different from turgor control processes in other vacuolated plant cells; it is possible that an electrogenic proton pump works on the plasmalemma and
Proton extrusion has been shown to be stimulated by light in many plant cell types (e.g. Spanswick 1974). Moody and Zeiger (1978) have suggested that the membrane hyperpolarisation induced by light in Allium cepa guard cells is the result of the photoactivation of an electrogenic proton pump. Further, Zeiger and Hepler (1977) suggested that the swelling of guard cell protoplasts in blue light is brought about by a membrane-bound receptor which acts directly on a proton pump, thereby stimulating ion transport. Zeiger and Hepler (1979) have linked such a receptor with the green fluorescence observed in onion guard cell protoplasts. They suggest that the photoreceptor is a flavin or flavo-protein bound to the tonoplast. This would agree with current hypotheses about the nature of the blue light receptor involved in many biological processes (see reviews of Briggs 1976, Presti and Delbruck 1978, Senger 1980). Briggs (1976) suggested that the receptor flavoprotein, on activation by blue light, reduces a b-type cytochrome; the system is reoxidised in darkness. Such a system accounts for many aspects of the rapid transpiration response induced by blue light in Avena sativa reported by Skaar and Johnsson (1978). There are strong similarities between stomatal action and other ion transport processes showing a specific blue light effect. In particular, nyctinasty involves the redistribution of K⁺ in pulvinules and motor cells; such systems show enhanced response to blue light (e.g. Fondeville, Borthwick and Hendricks 1967, Evans and Allaway 1972).
However, several authors have suggested an alternative mechanism for the specific blue light effect on stomata (e.g. Meidner 1968, Lurie 1978) based on the extensive effects of blue light on carbon metabolism (see review by Woskresenskaya 1972). In colourless mutant Chlorella cells blue light stimulated oxidative respiration (Pickett and French 1967, Kowallik 1967), synthesis and activation of PEP carboxylase (Kamiya and Miyachi 1974) and fixation of CO$_2$ into aspartate (Kamiya and Miyachi 1975). Ogawa et al (1978) have concluded that the blue light effect on guard cells is effected by activation of PEP carboxylase, an enzyme which is highly active in guard cells (Willmer et al 1973, Outlaw and Kennedy 1978). This then stimulates the production of malate which acts as a counterion for potassium. However, the malate concentration in guard cells is not necessarily correlated with stomatal opening and, in epidermal strips, can be reduced by high concentrations of chloride in the bathing medium. Such reductions of malate concentration do not affect the response of stomata to light or CO$_2$ (Travis and Mansfield 1979b, Van Kirk and Raschke 1978). In addition, onion guard cells do not possess malate as an organic counterion (Schnabl and Zeigler 1977) yet blue light induced swelling of isolated guard cell protoplasts (Zeiger and Hepler 1977) and stimulated stomatal opening (Meidner 1968) in this species. It seems unlikely that blue light acts solely through malate metabolism.

An important feature of the blue light response is the low quantum flux density required for saturation: stomata in Scots pine, Sitka spruce and dayflower all showed large increases in conductance with low quantum flux densities suggesting that blue light acts directly on the ion transport systems. However, there is more than one
light-dependent process operating in guard cells; this is the only explanation for separate red and blue light effects independent of intercellular CO₂ concentration and mesophyll assimilation. Lurie (1978) has suggested that there is an interaction between various light-activated pathways to supply both energy and organic anions for stomatal opening in *V. faba*. In this scheme red and blue light both act through photosynthesis to produce ATP and organic acids; blue light, in addition, provides energy by stimulating oxidative phosphorylation and stimulates organic anion production from starch breakdown. This presumes a photosynthetic role for the guard cell chloroplasts which is open to debate (see Section 4.1.2). However, guard cells do contain chloroplasts; this is a remarkably consistent feature (Zeiger, Armond and Melis 1980). The only exceptions are species of *Paphiopedilum* (Nelson and Mayo, Rutter and Willmer 1979, Zeiger *et al* 1980) and *Juniperus x media* var. *Plumosa* Albovariegata (Morison, unpublished). This latter plant is a periclinal chimaera and showed no chlorophyll in the epidermis in either green or white portions of leaves. Nelson and Mayo reported that stomata of *Paphiopedilum leeanum* showed normal responses to light, including the blue light effect. However, as the results were obtained from measurements on whole leaves it is possible that the effect of red light was mediated by a reduction in the intercellular CO₂ concentration effected by mesophyll assimilation.

There is much circumstantial evidence from studies with metabolic inhibitors that guard cell chloroplasts are capable of cyclic photophosphorylation (Hsiao 1976, Lurie 1979). Zeiger *et al* (1980) have provided convincing evidence that chloroplasts in guard cells of *V. faba* and *Chlorophytum comosum* possess photosystem 1 and 2 and...
non-cyclic phosphorylation. It appears that guard cell chloroplasts are capable of generating photochemical energy; if they are incapable of photosynthetic CO₂ reduction the energy formed could become available for ion transport. However, as Zeiger et al have emphasised, nothing is known about the transfer of ATP and NADPH from guard cell chloroplasts to the sites of ion transport (the plasmalemma and the tonoplast).

Experimental results presented previously (Section 4.3) provide evidence for a synergistic effect of red and blue light. Similar results have been reported by Voskresenskaya and Polyakov (1975), Ogawa et al (1978) and Skaar and Johnsson (1978). Ion transport in guard cells may be stimulated by the action of light on a number of different pathways (ion transport, anion synthesis and energy supply). Red and blue light acting together would increase stomatal opening; synergistic effects may be important in natural illumination.

6.4 CO₂ Effects on Stomata

The mode of action of CO₂ on stomata is as yet undefined; any proposed mechanisms must remain speculative. It is unlikely, however, that the effects are confined to a single process, but as with the effect of light on stomata, operate through a number of pathways.

Light causes stomata to open by stimulating the net K⁺ accumulation in guard cells. Net accumulation depends on the balance between influx and efflux; K⁺ uptake is generally against a concentration gradient from inside to outside the cells, efflux of K⁺
will occur down this gradient. The response of stomata in epidermal strips to light and CO$_2$ is strongly dependent on the KCl concentration of the external medium (Travis and Mansfield 1979b). Whereas the stomatal opening stimulated by light was positively related to the KCl concentration of the bathing solution, the inhibitory effects of CO$_2$ on stomatal aperture were overridden at high external concentrations of KCl. Mansfield et al (1980) have suggested that this is evidence that light and CO$_2$ act on stomatal movement at the same level. Light stimulates K$^+$ uptake and will result in large K$^+$ accumulations at higher external KCl concentrations; the reduced inhibition of aperture by CO$_2$ at high external concentrations may be the result of reduced efflux of K$^+$ down the smaller gradient from the cell to the medium. Any factor affecting membrane permeability of K$^+$ will affect stomatal aperture: the potassium ionophore benzo-18-crown-6 causes stomatal closure, probably by increasing K$^+$ efflux from guard cells (Richardson et al 1979).

While the above discussion indicates that an effect of CO$_2$ on net ion accumulation is highly probable, it is doubtful whether such an effect could completely account for the large stomatal opening observed in the dark in CO$_2$-free air by many workers. This opening can be of similar magnitude to the opening in light if CO$_2$-free air is accompanied by a rise in temperature (e.g. Mansfield et al 1980). It is possible that further effects of CO$_2$ on stomata are mediated through guard cell carbon metabolism.
Carbon dioxide is undoubtedly involved in malate formation by the carboxylation of PEP formed, presumably, from starch (e.g. Outlaw and Manchester 1979). Raschke (1975a) suggested that the malate concentration in the guard cells is dependent on the CO$_2$ concentration in the guard cell cell environment and dependent on the relative activities of PEP carboxylase and malic enzyme. Epidermal strips will fix exogenous $^{14}$CO$_2$ in both darkness and light, and when stomata are open or closed (Willmer, Pallas and Black 1973, Willmer and Dittrich 1974), though it should be noted that these, and other 14C-incorporation experiments have used very high concentrations (c. 1.5%) and very different fixation rates and patterns may occur in vivo in natural atmospheres.

However, as many workers have pointed out, the major difficulty with the role of malate as a CO$_2$ sensor in guard cells is that stomatal aperture and malate concentration are generally positively correlated (Allaway 1973, Pearson and Milthorpe 1974, Travis and Mansfield 1977) whereas CO$_2$ concentration and stomatal aperture are generally negatively correlated. Maximum stomatal apertures and malate concentration occur at low CO$_2$ concentrations when PEP carboxylation and malate formation should be reduced. It may be that endogenous sources of CO$_2$ are sufficient for malate synthesis in low CO$_2$ concentrations (Raschke 1977); Travis and Mansfield (1977) reported that only 1% of the CO$_2$ incorporated into malate came from exogenous sources. It is, however, difficult to reconcile such a strong internal source of CO$_2$ with Raschke’s proposed intercellular
CO₂ concentration sensing control system. Raschke (1979) has suggested that CO₂ acts in a system regulating pH in the cytoplasm involving malate, PEP carboxylase and malic enzyme; the pH optima for malic enzyme is lower than the optimum for PEP carboxylase (Willmer, Pallas and Black 1973) and Raschke postulated that these two enzymes form a "push-pull" system. Increased CO₂ concentrations lead to higher rates of malate synthesis lowering the cytoplasmic pH; inhibiting PEP carboxylase and stimulating malate decarboxylation. This is the basis of the "biochemical pH-stat" of Raven and Smith (1976). While there is no direct experimental evidence of the ability of such a system to account for the effects of CO₂ on stomatal aperture, such a proposal can account for the effects of other stimuli on H⁺ extrusion and ion transport. For example, hyperpolarisation of the plasmalemma by fusicoccin will decrease the pH of the cytoplasm; PEP carboxylation will be stimulated and the malate concentration will increase. Stomatal aperture was positively correlated with the CO₂ concentration in the presence of fusicoccin (Travis and Mansfield 1979a). This may be the result of a role of CO₂ in the production of anions in guard cells; in this case, where H⁺ extrusion is stimulated, availability of anions may be limiting stomatal opening and increased CO₂ concentrations will stimulate malate synthesis.

However, as Mansfield et al (1980) have emphasised, it is difficult to accept that a malate formation system such as that outlined above can have a primary control function in stomatal movements when the presence of malate is not essential to the light and CO₂ responses of stomata.

The effect of CO₂ on stomatal movements needs further experimental
clarification before any definitive statements can be made.

6.6 Conclusion

There is strong experimental evidence that light and CO₂ exert separate effects on stomata. Stomata can respond to light directly; in addition, stomata will respond to changes in the CO₂ concentration around the guard cells. Therefore stomata can be influenced by the rate of CO₂ fixation in the mesophyll. The total effect of light on stomata in whole leaves is then composed of two direct responses and an indirect response:

1) A direct response to blue light, possibly acting through a flavin pigment directly on electron transport and the H⁺/K⁺ pump, or stimulating increased oxidative phosphorylation, ion transport and anion synthesis.

2) A direct response to white light where quanta are absorbed by chlorophyll resulting in photosynthetic ATP and NADPH production. These or derived products are transported to the cell membranes and drive the H⁺/K⁺ pump.

3) An indirect response effected by changes in the intercellular CO₂ concentration. Reduced C₁ either increases net K⁺ accumulation directly or acts through anion metabolism.

There are, however, undefined areas in this scheme, in particular, the possible role of light-enhanced CO₂ fixation in guard cells. It is important to recognise that while these responses may have
separate mechanisms at the cellular level there is undoubtedly a variable degree of interaction. It is also important to recognise that the relative contributions of each component will vary with species. Stomata of Sitka spruce showed almost complete insensitivity to high intercellular CO$_2$ concentrations while dayflower showed almost complete inhibition of stomatal opening at high CO$_2$ (Chapter 3). The contribution of each component will also vary with environmental variables and the physiological state of the plant material; for example, the effect of CO$_2$ increases with increasing VPD and increasing plant water stress in particular species.

In conclusion, the effects of light and CO$_2$ on stomatal movements have been experimentally separated in Scots pine, Sitka spruce and dayflower. The responses to light in the three species were consistent with the suggestions in the literature of a blue light effect, together with a direct effect of white light. All three species showed stomatal opening in response to low intercellular CO$_2$ concentrations although the difference between the conifer species and dayflower in the sensitivity of stomata to C$_1$ was marked. The comparative insensitivity of the conifer species indicates that there was little or no control of stomata by mesophyll assimilation through intercellular CO$_2$ concentration. Even in dayflower the direct response of stomata to light was large and, in unstressed humid conditions, almost completely accounted for the effect of light. This also suggests that mesophyll CO$_2$ assimilation in these conditions did not control stomata. While there is a general correlation between mesophyll assimilation, intercellular CO$_2$ concentration and stomatal conductance, such correlations can be
readily broken in experiments: intercellular CO$_2$ concentration is not a consistent link between assimilation and conductance, although in some conditions it can become an important variable affecting stomata and leaf gas exchange control.
REFERENCES


165


Raschke, K. & Dittrich, P. (1977) $^{14}$C carbon dioxide fixation by isolated leaf epidermis with stomata closed or open. Planta


### APPENDIX 1

**LIST OF SYMBOLS AND ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
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<tr>
<td>A</td>
<td>net CO₂ assimilation rate</td>
<td>mg m⁻²s⁻¹</td>
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<td>Cₐ</td>
<td>ambient CO₂ concentration</td>
<td>cm³ m⁻³</td>
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<td>Cᵢ</td>
<td>intercellular space CO₂ concentration</td>
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<td>vapour pressure</td>
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<tr>
<td>E</td>
<td>transpiration rate</td>
<td>g m⁻²s⁻¹</td>
</tr>
<tr>
<td>g, g'</td>
<td>leaf conductance</td>
<td>cm s⁻¹</td>
</tr>
<tr>
<td>gₛ, gₛ'</td>
<td>stomatal conductance</td>
<td>cm s⁻¹</td>
</tr>
<tr>
<td>gₐ, gₐ'</td>
<td>aerodynamic conductance</td>
<td>cm s⁻¹</td>
</tr>
<tr>
<td>gₖ, gₖ'</td>
<td>cuticular conductance</td>
<td>cm s⁻¹</td>
</tr>
<tr>
<td>Gᵣ</td>
<td>relative stomatal conductance, expressed with respect to the dimensionless maximum in an experiment</td>
<td></td>
</tr>
<tr>
<td>Gₐ</td>
<td>gain of the assimilation/C₁ loop</td>
<td></td>
</tr>
<tr>
<td>Gₔ</td>
<td>gain of the conductance/Cᵢ loop</td>
<td></td>
</tr>
<tr>
<td>Qᵢ</td>
<td>incident quantum flux density</td>
<td>µE m⁻²s⁻¹</td>
</tr>
<tr>
<td>rₐ, rₐ'</td>
<td>aerodynamic resistance</td>
<td>s cm⁻¹</td>
</tr>
<tr>
<td>rₛ, rₛ'</td>
<td>stomatal resistance</td>
<td>s cm⁻¹</td>
</tr>
<tr>
<td>rₖ, rₖ'</td>
<td>cuticular resistance</td>
<td>s cm⁻¹</td>
</tr>
<tr>
<td>rᵣ</td>
<td>residual resistance</td>
<td>s cm⁻¹</td>
</tr>
</tbody>
</table>

prime ' indicates resistance or conductance to CO₂.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Γ</td>
<td>CO₂ compensation concentration</td>
<td>cm³ m⁻³</td>
</tr>
<tr>
<td>Ψ</td>
<td>total water potential</td>
<td>-MPa</td>
</tr>
<tr>
<td>ABA</td>
<td>(+) Abscisic acid</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
<td></td>
</tr>
<tr>
<td>CAM</td>
<td>Crassulacean Acid Metabolism</td>
<td></td>
</tr>
<tr>
<td>PEP</td>
<td>phosphoenolpyruvate</td>
<td></td>
</tr>
<tr>
<td>NADPH</td>
<td>reduced form of nicotinamide adenine dinucleotide phosphate</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>photorespiration</td>
<td></td>
</tr>
<tr>
<td>VPD</td>
<td>leaf-air vapour pressure difference</td>
<td></td>
</tr>
</tbody>
</table>

181
Figure A2.1a  Pneumatic Layout of the gas exchange system prior to the leaf chamber. See following pages for Key.
Figure A2.1b  Pneumatic Layout of the components of the gas exchange system after the leaf chamber. See following pages for Key.
A2.1 Equipment Details

Diagrams A2.1a and A2.1b show the detailed arrangement of the components of the gas exchange system. The key to both diagrams is listed below.

KEY:

P pumps
1 Charles-Austen, double-ended diaphragm; model F85DE.
2, 3, 4 Wosthoff gas mixing pumps, oil separation; models SA18/3F, SA27/3F, G27/3F; end flow rate = 50 cm$^3$ s$^{-1}$.
5 2 x Charles-Austen Capex Mk. II diaphragm pumps in parallel.
6, 7, 8 Charles-Austen Capex Mk. III diaphragm pumps, flow rate normally 8 cm$^3$ s$^{-1}$.
9 Charles-Austen Dymax Mk. II diaphragm pump, flow rate normally 8 cm$^3$ s$^{-1}$.

V valves
⑩ Shrader 3-way solenoid valves (M454SA).
⑪ Shrader 2-way solenoid valves (M455SA).
"dummy" Shrader 3-way valve, not energised.

F flowmeters
all GEC-Elliot float flowmeters, model 1100, with needle valves.
1 with DU flow controller, range 0-250 cm$^3$ s$^{-1}$.
2 with DU flow controller, range 0-83 cm$^3$ s$^{-1}$.
3 with DU flow controller, range 0-42 cm$^3$ s$^{-1}$.
4, 5, 6, 7 no flow controller, range 0-42 cm$^3$ s$^{-1}$.

SG silica gel (coarse grade) columns, 1 m long.
CA CO$_2$ absorbent columns, "Carbosorb" (soda lime, 4-10 mesh, BDH), 1 metre long.
H humidifying bottles, 2 x 1 dm$^3$ Drescher flasks with "Miracloth" (Calbiochem) and distilled water.
M10 electronic manometer, model M10, capsule type A100, Mercury Instruments.
DPM dew-point hygrometer, model 440 or modified 880 EG & G.
RHS relative humidity sensors, model 6061 Vaisala, electronics built by J.E. Follan.
TMF thermal mass flow controller, controller 835A and meter 5810, Brooks.
IRGA infra-red gas analyser, URAS 2T, Hartmann and Brown.

A2.2 List of Manufacturers

The details of equipment models and makes that have not previously been listed in the text are listed below. Addresses refer to within the U.K. unless otherwise stated.

Air pumps: Charles-Austen, Byfleet, Surrey.
"Carbosorb": BDH, Poole, Dorset.
Chamber temperature controller: controller, 2 thyristor stacks, 2 thyristor drive units, Eurotherm, Worthing, Sussex.
Dew point hygrometer: Cambridge Systems, EG & G, from Auriema, Slough, Bucks.
Digital voltmeter: A200 with A204 fan-out unit, Series 3 Analogue Scanner and Data Transfer Unit, Solartron Electronics Group, Farnborough, Hampshire.
Flowmeters: GEC-Elliot, Margate, Kent.
Gas cylinders: aluminium, Rank-Hilger, Margate, Kent.
Gas mixing pumps: Wosthoff oHG, D463 Bochum, FRG.
Hot wire anemometer: Davimeter, Airflow Developments Ltd., High Wycombe, Bucks.
Infra-red gas analyser: Hartmann and Braun U.K., Moulton Park, Northampton.
Manometer: Mercury Instruments (Scotland), Glasgow.
"Miracloth": Calbiochem Ltd., Hereford.
Optical planimeter: LI-3100, Lambda Instrument Inc., Lincoln, Nebraska, U.S.A
Particle filters: Whatman, Maidstone, Kent.
Potentiometric chart recorder: model PM8235, Philips, Pye Unicam Ltd., Cambridge.
Relative humidity sensors: Vaisala OY, Helsinki, Finland.
Thermocouple ice-point reference: Zeref model 136, Mectron (Frigistor), Slough, Bucks.
Water bath: type SX35 and water circulator type FH15, Grant Instruments Ltd., Cambridge.
Figure A3.1 The internal transmittance of the coloured glass filters KG12 ('heat mirror'), BG12, GG400 and RG610, (from manufacturers specifications).
APPENDIX 3
LIGHT SOURCES FILTERS AND SPECTRAL DISTRIBUTIONS

Metal halide lamps ("Power Stars", Wotan Lamps Ltd., London, England) were used to illuminate the chamber. Up to May 1979, 400 W lamps (bulb HQI-400W/DH) were used. The optical arrangement and components used to produce a high flux density of near parallel beam radiation have been described in detail by Leverenz and Jarvis (1979). In May 1979 the two lamps were replaced with 1000 W units (bulb HQI-T 1000W/D) to increase $Q_i$. However, these bulbs have a longer arc length (5.6 compared to 2.5 cm) and the divergence angle of the beam was increased from 7.3° to 15.7° with a 20 cm focal length Fresnel lens (see Leverenz and Jarvis, 1979).

The spectral composition of the illumination was altered with 2 mm thick glass filters (Schott & General, Mainz, BRD). The internal transmittance of the filters is shown in Fig. A3.1. Filters KG2 (infra-red cut-off) and GG400 (< 400 nm cut-off) defined "white light" (400-750 nm) and filters BG12 or RG610 could be added for blue (400-500 nm) or red (600-750 nm) spectral regions, respectively.

$Q_i$ was altered with a set of 5 mm thick neutral density filters (Frew-Smith Ltd., Irvine, Scotland), transmitting approximately 1, 2.5, 5, 12.5, 25 and 50% of the incident light.

In experiments on the wavelength sensitivity of $g_o$ in Scots pine a single 400 W Wotan lamp was focussed with a parabolic reflector to produce a high $Q_i$ on single, small (25 cm$^2$) metal interference
filters (Balzers High Vacuum Ltd., Berkhamsted, England). Even with this arrangement the flux density transmitted in the narrow wavebands of the filters (wavelengths: 364, 382, 435, 491, 542, 616, 650, 680 nm, half band width 9-12 nm) was limited. Quantum flux density was measured with a thermopile (CM3, Kipp and Zonen, Delft) and equalised with neutral density filters.

The spectral distribution of red, white and blue $Q_\lambda$ in the leaf chamber (Fig. 2.4.2b) was measured with a quanta spectraphotometer (QSM 2500, Tectum Instruments, Umea, Sweden). The instrument used a wedge interference filter (half band width 13-15 nm) to scan automatically between 400 and 750 nm, each full scan lasting 63 seconds. The sensor head was fully cosine corrected and output calibration was better than 10% (manufacturers specifications). $Q_\lambda$ could be integrated over the scan length. Table A3 presents comparisons of the blue, red and total $Q_\lambda$ in the leaf chamber, in the growth room and in daylight.

<table>
<thead>
<tr>
<th>Illumination</th>
<th>blue/red</th>
<th>red/total</th>
<th>blue/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaf chamber</td>
<td>0.56</td>
<td>0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>growth room</td>
<td>0.37</td>
<td>0.26</td>
<td>0.12</td>
</tr>
<tr>
<td>daylight, cloudy</td>
<td>0.68</td>
<td>0.22</td>
<td>0.15</td>
</tr>
<tr>
<td>daylight, direct sun</td>
<td>0.24</td>
<td>0.41</td>
<td>0.10</td>
</tr>
</tbody>
</table>

where: blue and red refer to the wavelengths defined by the filters BG12 and RG610. Growth room light sources: 15 x 400 W metal halide (Thorn MBIF "Kolorarc") and 16 x 60 W tungsten bulbs.

Quantum flux density in the chamber was measured with silicon photodiodes (305-462, Radiospares, London, England) which have a
peak spectral sensitivity at 760 nm. The output of the diodes was small when used in conjunction with an 100 ohm resistor, especially in the blue region of the spectrum. From May 1979 a linear photometer circuit (built by J.E. Follan) was used to amplify the output of each photodiode to approximately 2.3 V at 1000 μE m⁻² s⁻¹ in white light, increasing the measurement precision.

The photodiodes were calibrated against a quantum sensor (LI-190SR, Lambda Instruments Co. Inc., Nebraska, U.S.A.) and with the quanta spectrophotometer. During calibration of each photodiode, the quantum sensor was placed in the plane of the shoot midway along the shoot, to approximate the average Q₁ incident on the shoot. The photodiodes were calibrated separately in red, blue and white light at 20 °C chamber temperature.
A4.1 Vapour Pressure Determination

Dew point meters (DPM) and relative humidity (RH) sensors were frequently calibrated in situ with wet air passed through 4.5 m of copper tubing in a temperature controlled water bath. Dew-points were varied between 2 and 22 °C, with control better than 0.05 °C, equivalent to saturated vapour pressures between 0.7 and 2.6 kPa. Bath temperature was measured with a mercury-in-glass thermometer with 0.05 °C readability. Flow rates were low (c. 17 cm³ s⁻¹). Least squares linear regressions related DPM output to dew-point; typical 99% confidence limits of regression coefficients at the mean were < ± 0.3%.

RH sensor outputs were calibrated against the true RH at the sensor surface, calculated from:

\[ \text{RH} = \frac{e_a}{e_{sat}} \]

where \( e_a \) is vapour pressure in the air stream and \( e_{sat} \) the saturated vapour pressure at the sensor temperature, measured with a thermocouple. Linear regressions related RH sensor outputs to RH; typical 99% limits of the regression coefficients at the mean were < ± 0.1%.

Systematic errors can be attributed to thermometer and thermocouple accuracy.

All temperatures (dew-point, RH sensors and leaf) were converted to saturated vapour pressure using Teten's equation (Murray 1967), rewritten in \( \log_{10} \) form:

\[ e_{sat} = 0.61078 \times \frac{[7.5 \times T]}{[237.3 + T]} \]
where \( e_{\text{sat}} \) is in kPa and \( T \) in degrees centigrade.

**A4.2 Leaf Temperature Measurement**

The plastic coated thermocouple wires were wrapped around the conifer shoots and the filed junction sprung against a needle surface. Insertion into needles caused undesirable damage when shoots were used on several days. In well stirred conditions, with shoots of low aerodynamic resistance, needle temperatures stayed closely coupled to air temperature (Jarvis, James and Landsberg 1976) especially at low to medium \( Q_1 \) such as used in the majority of the experiments in this study.

In the experiments with dayflower, wires were shaded by the leaves and pressed on to the unilluminated side of leaves, midway between tip and base and midway between midrib and leaf edge. Errors in leaf temperature were probably larger as a result of the higher aerodynamic resistance and the more uneven temperature distribution over laminar leaves. This is most important at high \( Q_1 \) and high transpiration rates, when conduction along the wires is likely and the leaf is cooled by evaporation. However, in practice, in these latter conditions leaf-air temperature differences up to 2.6 °C could be measured, indicating good thermal contact of the thermocouple with the leaf.

In all the experiments the chamber temperature controller used similar thermocouples arranged in an identical manner to the measurement thermocouples.

**A4.3 Aerodynamic Resistance**

The magnitude of the aerodynamic resistance to water vapour flux,
from the leaf is a function of wind speed, and the size and shape of leaves (Landsberg and Ludlow 1970). In the leaf chamber \( r_a \) was reduced to a small value by high air speeds. An equation analogous to 2.4.1f was used to calculate \( r_a \):

\[
r_a = \frac{(a/f)(e_{sat} - e_{out})}{(e_{out} - e_{in})}
\]

(A4.3a)

where \( e_{sat} \) is the saturated vapour pressure at the temperature of the leaf surface. For Scots pine, the figure for \( r_a \) determined by Ng (1978) for similar shoots in the same chamber was used. Using the plaster cast method (Landsberg and Ludlow 1970), Ng calculated \( r_a \) to be 0.033 s cm\(^{-1}\) (± 10% standard error).

The estimation of \( r_a \) for the Sitka spruce shoots was prevented by difficulties in obtaining a continuous thin coating of gypsum over the needles. However, Beadle (1977) and Leverenz (1978) assumed \( r_a \) was zero for Sitka spruce shoots in similar stirred chambers. Ludlow and Jarvis (1971) estimated \( r_a \) values of 0.02 and 0.03 s cm\(^{-1}\) for Sitka spruce, and Leverenz and Jarvis (1979) estimated an \( r_a \) of 0.08 s cm\(^{-1}\) for a densely foliated shoot. Shoots used here were not densely foliated and an upper limit of 0.04 s cm\(^{-1}\) was therefore used in the calculations.

The \( r_a \) of dayflower shoots was estimated with wet filter-paper replicas on copper wire frames. Six replicas with a range of leaf areas and leaf orientations gave an estimate of \( r_a \) of 0.14 s cm\(^{-1}\) (± 6% 99% confidence limits).

### 4.4 Flow Rate

The thermal mass flow meter and controller (TMF) were calibrated in situ, at 20 °C against a wet-type flow meter (model M75-INS,
Wright and Co., Westminster, England) and by an inverted volumetric flask in water. Care was taken to minimise changes in water level in the flask during filling which cause errors due to pressure changes. A linear regression related TMF output to flow rate over the range 5-50 cm$^3$ s$^{-1}$, with 99% confidence limits of ± 1.2% for the regression coefficient.

A4.5 CO$_2$ Concentrations

The sensitivity of the differential gas analyser (IRGA) was calibrated at every measurement period during experiments, using the "tube length" method, (Parkinson and Legg 1971). The sample short cell of the IRGA was purged with CO$_2$-free air flowing through a 1 m soda-lime column, while the remaining cells were flushed with air of known CO$_2$ concentrations from an aluminium cylinder. The ratio of the short to long cell length had previously been determined to be 0.04485 (± 5% 99% confidence limits, Ng, 1978).

The cylinder air CO$_2$ concentration was determined by comparison with a flowing reference CO$_2$ mixture in air from the three gas mixing pumps. These pumps were accurate to better than 2 cm$^3$ m$^{-3}$ at 300 cm$^3$ m$^{-3}$ (Bate, D'Aoust and Canvin 1969). In experiments when atmospheric air was used, background CO$_2$ concentrations (C$_a$) were determined at every measurement period with the IRGA by comparison with the cylinder air.

The case of the instrument was continuously flushed with CO$_2$-free air to reduce variability in the readings (Janáč 1970). The measurement cells vented to the laboratory air; pressure differences between reference and sample air streams were continually monitored with an electrical manometer and zeroed with needle valves.
Residual differences in pressure between the air streams were $< 3$ Pa and pressure was $< 30$ Pa above atmospheric pressure.