Interactions in stomatal function

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Abstract

The modelling of stomatal responses is hindered by gaps in our knowledge of the interactions between the effects of different environmental variables, and of the mechanistic basis for correlations between physiological variables. The objective of this thesis was to fill some of these gaps by studying short term stomatal responses to the environment, and by contrasting some current models against this new information. Four questions were addressed through simulation and gas-exchange experiments on *Hedera helix* subsp. *canariensis* (Willd.) Coutinho.

(1) **What is the relationship between stomatal responses and the rate of photosynthesis?** The CO$_2$ flux density and stomatal conductance are closely correlated, but there is not a simple causal link between them. This relationship is complex, and depends on both parallel but independent responses to light of stomata and photosynthesis, and indirect response of stomata mediated by photosynthesis. This indirect response occurs through CO$_2$ depletion in the air spaces of the mesophyll and stomatal response to CO$_2$. No evidence was found in favour of the proposed effect of photosynthesis on stomata through an unknown messenger.

(2) **What is the nature of the interaction between stomatal responses to humidity and temperature?** The hypothesis that these responses are brought about by a single response to relative humidity at the leaf surface was tested, and shown to be incompatible with the responses of *Hedera helix*. It is suggested that the most appropriate variable for expressing humidity is, in this context, the water vapour deficit at the leaf surface.

(3) **What is the role of the boundary layer in the control of stomatal opening?** Real world and simulation experiments were used to show that responses to bulk air water vapour and CO$_2$ mol fractions are both dependent on stomatal responses to CO$_2$ and humidity. It is also shown that a feedforward response to humidity requires feedback through another variable for stability under natural conditions. Response to wind speed is due to changes in humidity and CO$_2$ mol fraction at the leaf surface.

(4) **Are our current knowledge, and the resulting models, good enough for predicting**
short-term stomatal responses to changes in the environment? The need for a careful analysis of simulation models is stressed. Ball’s empirical model of stomatal conductance was analysed. The original interpretation was found to be flawed, and a new one was proposed. The new interpretation views the model as a description of the relationship between CO$_2$ flux rate and stomatal conductance, rather than of stomatal conductance alone. It is shown that this model is useful for describing the behaviour of the intercellular CO$_2$ concentration. The model was tested against data from the experiments. It was found that the responses to temperature and humidity are not treated in a satisfactory way. The response of the model to other variables is realistic. A modification to the model is described and tested. It is concluded that the model is a good starting point for the development of simulation models to be used as submodels in canopy and regional models.
Declaration

This thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported within was executed by myself, unless otherwise stated.

June 1991
Preface

The aim of ecophysiological research in a changing global environment

In the past, ecophysiological research has disregarded changes in the global environment, except when considering long term processes such as species evolution. In the last few decades the rate of change has markedly increased as a result of the impact on the environment of mankind through technology. This has made the prediction of both (1) the future change in the global physical environment, and (2) the consequences of this change for living organisms, become urgent matters. Surface properties and gas exchange of vegetation may affect the global environment through modification of the energy balance, the carbon cycle and the hydrological cycle. The recognition that vegetational change (a transition from one surface type to another) has an effect on the physical behaviour of the environment at a global scale is novel within ecophysiology, and is important as a justification for the development of this discipline. Changes in the biosphere that feed back into changes in the physical environment are very important, but they are not the only important ones. A knowledge of the effects of environmental change on the ecosystem and all its components —either functional, structural, population, or genetic— is also needed to predict changes in the biosphere, and ultimately their consequences for the future of mankind. The ultimate objective should be to predict both the physical and biological environment that future generations will encounter, and to assess the risks of following contrasting strategies in the use of resources.

Predicting the future change of world vegetation, and its effect on global climate and environment is a taxing task. It makes necessary the integration of events over a wide expanse of time and space. This complexity requires the use of adequate conceptual tools such as general systems theory and hierarchy theory. The physiological response of individual plants and animals propagate throughout the system in several different ways. There is evidence that the effect of an increased CO₂ concentration in the
atmosphere can be different in different ecosystems, and on different plant species (e.g. Morison, 1990). A physiological response does not need to affect the short term spatial integral of a response to have an effect on future worldwide changes. In the long-run, effects on competition and species survival, or population biology can be as important as more direct effects.

The crucial question is: Given a long enough expanse of time, can physiological responses of organisms significantly affect the global system? The answer is clearly “yes” when these responses are being driven by changes in the global environment and as a consequence of this they occur simultaneously on different parts of the earth’s surface. The situation is different when responses are driven by local disturbances happening at random, in which case they would tend to cancel out upon integration in a larger spatial scale. The most dangerous situation would be for the whole system to enter into a loop with positive feedback, in which changes in the climate and in the vegetation would go in the same direction and reinforce each other. This would happen if an increase in CO$_2$ in the atmosphere, or a change in temperature, were to lead to a decrease in the flow of CO$_2$ towards sinks (e.g. forests biomass and oceans). At the present time, there are indications of negative feedback, but it is possible that in the foreseeable future the speed of change may accelerate over a threshold above which instability will ensue.

It is true that in the past much ecophysiological research has been done at the organ or single plant level and that whole canopy and global effects have usually been neglected. However, we must not now make the opposite mistake by blindly swinging towards a whole canopy-centered approach. To obtain an understanding of the whole system, we must establish relationships between the behaviour of the system at different spatial and temporal scales, taking into account both physical and biotic components, and using alternative viewpoints —i.e. ecophysiology, ecosystem analysis, population biology.

**Why study stomatal responses?**

The effect of changes in stomatal conductance on the flow rates of water vapour and CO$_2$ depends on the spatial scale considered. At larger scales the stomatal control of the fluxes decreases, and the magnitude of this decrease depends on the value of other conductances, mainly the boundary layer conductance between the leaf and the reference level in the atmosphere were molar fractions remain unchanged. Although stomatal conductance does not have a great effect on whole canopy or regional water exchange in many situations (Jarvis & McNaughton, 1986), it is a necessary variable
for the understanding of how plants interact with their environment (Cowan, 1988). That stomatal responses have a smaller effect on flow rates through entities at larger spatial scales than individual leaves, does not mean that the stability and evolution of the whole system are independent of these responses.

The consideration of a simple example can help. Even in a situation where a change in the integrated stomatal conductance of all the leaves of the plants in the canopy has no effect on the total water flux per unit of ground area, a change in stomatal conductance of one genotype or species will have an effect on the partitioning of water resources between individuals of the same, or different, species. In many situations this can be of the greatest importance: for example it can alter the amount of water used by a crop competing with weeds; it can drive evolution through natural selection; it can even determine the survival of the vegetation cover.

So, even though in many situations we would not expect that changes in stomatal responses resulting from changes in the environment would lead in the short term to big changes in global CO$_2$ and water fluxes, such changes could have, for example, important effects on the species composition of vegetation by altering competitive relationships, and on the economic productivity of forest and agricultural systems.

Independently of changes in the global environment, an understanding of stomatal behaviour is important to applied fields such as forestry, agriculture, horticulture, and irrigation. Hence, in most situations where water supply to crops is limited, yield depends on the efficient use of this supply. Water use efficiency depends on stomatal behaviour through its effects on the rates of transpiration and photosynthesis.

**Scope of this project**

To predict the effect of long term changes in climate on the short term responses of stomata, it is first necessary to have an adequate knowledge of these responses in plants grown under normal conditions. To have a model of short-term stomatal responses based on a simplified but realistic representation of the mechanism involved, the first unavoidable step is to study this mechanism. For this, it is *not* necessary to grow plants under future environmental conditions, and it is easier not to do so. Longer term effects, such as changes in stomatal dimensions and frequency, cannot affect the nature of the *mechanism* of short term responses. Once a satisfactory model is available it can be reparameterized for plants grown under different conditions with less exhaustive experimentation.

Although research on stomata started long ago and has been intense (Meidner,
1987), the complexity of their responses are still a challenge to our understanding. Interactions between responses to different stimuli, and the dynamics of the responses need further study. It is also important to assess whether current knowledge is good enough for the prediction of steady-state stomatal responses, as is required in mechanistic models of CO$_2$ and water fluxes at larger spatial scales, for example forest canopies. Climatic variables that are expected to change significantly in the near future are CO$_2$ molar fraction of the air, temperature, rainfall and humidity. Stomatal responses to light and CO$_2$ are closely linked. These are the variables on which the emphasis has been placed.

**Objective**

The modelling of stomatal responses to the environment is hindered by gaps in our knowledge of the interactions between the effects of different environmental variables, and of the mechanistic basis for correlations between physiological variables. The general objective of this project was to fill some of these gaps by studying short term stomatal responses to the environment, and by contrasting some current models against this new information. Specific objectives are defined in detail by the following questions:

1. What is the relationship between stomatal action and the rate of photosynthesis?

2. What is the nature of the interaction between stomatal responses to humidity and temperature?

3. What is the role of the boundary layer in the control of stomatal opening?

4. Is our current knowledge, and are the resulting models, good enough for predicting short-term responses of stomata to changes in the environment?

The responses of *Hedera helix* plants were studied under controlled conditions in the laboratory. A computer controlled gas-exchange system was used to measure the flows of water vapour and CO$_2$ between a leaf and the air in an enclosing chamber. From these measurements conductances and molar fractions were computed. The effect of the boundary layer was both measured and modelled. A recently developed model of $g_w$ and some of its derivatives was contrasted with the observed response, and as a result, changes to this model were proposed.
Dedication

A Tarja y el osito Pú
Acknowledgements

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# List of symbols

## Basic symbols

In equations only S.I. units without scale factors are used, and are not indicated. For displaying data, scale factors are frequently used with these same basic units, and are given along with the data. The units given here apply to the equations.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>Molar flux density of CO$_2$ at the leaf surface (mol m$^{-2}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$b$</td>
<td>Thickness of the boundary layer (m).</td>
</tr>
<tr>
<td>$D$</td>
<td>Air water vapour deficit at position indicated (mol mol$^{-1}$).</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusivity in air (mol m$^{-3}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$E$</td>
<td>Molar flux density of water vapour at the leaf surface (mol m$^{-2}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$E$</td>
<td>Molar flux density of water vapour per unit land area (mol m$^{-2}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$g$</td>
<td>Conductance per unit leaf area (mol m$^{-2}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$G$</td>
<td>Conductance per unit land area (mol m$^{-2}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$h$</td>
<td>Relative humidity (fraction).</td>
</tr>
<tr>
<td>$I$</td>
<td>Quantum flux density (mol m$^{-2}$ s$^{-1}$).</td>
</tr>
<tr>
<td>$J$</td>
<td>Molar flow rate (mol s$^{-1}$).</td>
</tr>
<tr>
<td>$l$</td>
<td>Length or dimension of a leaf in the wind direction (m).</td>
</tr>
<tr>
<td>$\ell$</td>
<td>Proportional length of short section of a split IRGA cell (fraction).</td>
</tr>
<tr>
<td>$P_{\text{atm}}$</td>
<td>Atmospheric pressure (Pa).</td>
</tr>
<tr>
<td>PAR</td>
<td>Radiation within the wavelength range 400–700 nm.</td>
</tr>
<tr>
<td>$s$</td>
<td>Signal from instrument (V).</td>
</tr>
<tr>
<td>$S$</td>
<td>Leaf area (m$^2$).</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature ($^\circ$C).</td>
</tr>
<tr>
<td>$u$</td>
<td>Wind speed (m s$^{-1}$).</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Sensitivity of IRGA (V mol$^{-1}$).</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength of light (nm).</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Molar fraction of gas indicated by superscript in air (mol mol$^{-1}$).</td>
</tr>
</tbody>
</table>
Subscripts

Subscripts denote location in the leaf or gas system.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Air outside the boundary layer of the leaf.</td>
</tr>
<tr>
<td>b</td>
<td>Boundary layer.</td>
</tr>
<tr>
<td>cyl</td>
<td>Compressed air cylinder, used as reference.</td>
</tr>
<tr>
<td>hum</td>
<td>Humidifier inlet.</td>
</tr>
<tr>
<td>i</td>
<td>Air in the intercellular space of the leaf.</td>
</tr>
<tr>
<td>in</td>
<td>Leaf chamber inlet.</td>
</tr>
<tr>
<td>in,IRGA</td>
<td>IRGA reference cell.</td>
</tr>
<tr>
<td>l</td>
<td>Leaf.</td>
</tr>
<tr>
<td>out</td>
<td>Leaf chamber outlet.</td>
</tr>
<tr>
<td>s</td>
<td>Leaf surface, or air at the leaf surface.</td>
</tr>
<tr>
<td>std</td>
<td>Standard concentration.</td>
</tr>
<tr>
<td>t</td>
<td>Total (for conductances).</td>
</tr>
<tr>
<td>tot</td>
<td>Mixing tray outlet.</td>
</tr>
<tr>
<td>trap</td>
<td>Water trap outlet.</td>
</tr>
</tbody>
</table>

Superscripts

Superscripts denote the substance, entity, or property to which the base symbol refers.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>air</td>
<td>Air.</td>
</tr>
<tr>
<td>c</td>
<td>CO₂.</td>
</tr>
<tr>
<td>dew</td>
<td>Dew point.</td>
</tr>
<tr>
<td>dry</td>
<td>Dry, CO₂-free air.</td>
</tr>
<tr>
<td>w</td>
<td>Water vapour.</td>
</tr>
<tr>
<td>w*</td>
<td>Saturating water vapour.</td>
</tr>
</tbody>
</table>
Accents

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>~</td>
<td>Standardized to $\chi_{\text{in,IRGA}}^c = 350 , \mu\text{mol mol}^{-1}$.</td>
</tr>
<tr>
<td>~</td>
<td>Measured with the IRGA.</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

This chapter is a brief review of current ideas about stomatal responses, as observed from different viewpoints and at various scales. Both real world experimentation and simulation modelling are reviewed. The purpose of the chapter is to create the context for a detailed discussion of the responses at the leaf scale later in this thesis. The next two chapters describe the gas-exchange apparatus, and plant material used in the experiments. The four chapters that follow address individual objectives, and discuss the relevance of the results obtained in view of current knowledge. The last chapter is a summary discussion.

1.1 Stomatal responses in the real world

1.1.1 What variables do stomata sense? And where?

Stomata are sensitive to light, CO$_2$, humidity, and temperature. They are also sensitive to chemical signals, and through them to other environment variables such as soil water content, photoassimilate demand and stress events such as drought. CO$_2$ is thought to be sensed on the inner side of the guard cells (Meidner & Mansfield, 1968, page 76), and so the concentration seen by them is $\chi^c_i$ (Mott, 1988). It has been proposed that air water vapour content is sensed at or near the outer surface of the leaf through localized transpiration from guard cells (Lange et al., 1971; Mansfield, 1986) or through sensing of relative humidity by the guard cells (Ball, 1988). However, it has also been suggested that cuticular transpiration from the outer leaf surface is negligible and that the response to humidity depends on a restriction of water supply to guard cells through subsidiary cells (Nonami et al., 1990). In a whole leaf, the response of $g^w$ to light depends on both the response to light of the guard cells, and an indirect effect of mesophyll photosynthesis through $\chi^c_i$, and it has been suggested that it also acts
CHAPTER 1. INTRODUCTION

through another unknown messenger. Stomata respond indirectly to the soil water content. A chemical signal, most probably abscisic acid, synthesized in the roots and carried to the shoots by the xylem sap flow decreases stomatal aperture when soil dries, even if the shoot water status is not affected (Jones, 1990; Davies et al., 1990; Zhang & Davies, 1991).

1.1.2 The basis of stomatal movements

The stomatal pore opens and closes as a consequence of change in shape of the guard cells. The driving forces for shape changes are the absolute turgor pressure of the guard cells and the difference in turgor pressure between them and the epidermal cells that surround them (Cowan, 1977). The change in shape is dependent on the elasticity of the cell walls in different directions, which is a consequence of the orientation of microfibrils (Weyers & Meidner, 1990), and on wall thickness in different parts of the cell. There have been reports of walls stiffening as stomata open (Weyers & Meidner, 1990), and suggestions of an effect of abscisic acid on the elastic modulus of guard cell walls (Kondo, 1989). However, the active movement of stomata depends on the build up and release of osmotic potential in the guard cells by transport and synthesis of solutes. Fujino (1967) and Fischer (1968) discovered the central role of K$^+$ in stomatal movements. The balancing anion can be organic (e.g. malate) or inorganic (e.g. Cl$^-$). Sugars can also be osmotically significant (Zeiger, 1990).

Zeiger et al. (1977) have proposed a chemiosmotic hypothesis for solute transport leading to stomatal opening in which the primary motive force for solute accumulation is an H$^+$ gradient (Zeiger, 1983; Zeiger, 1986, give an account of this model). Different opening stimuli contribute to an H$^+$ gradient by H$^+$ extrusion, and this gradient drives the uptake of K$^+$. More recent evidence of the dynamics of solute fluxes during opening and closing points to a more complex mechanism. Both a proton pump and membrane potential-sensitive K$^+$ channels play a role in solute accumulation and release (MacRobbie, 1988; Raschke et al., 1988). Blue light effects on stomata could be mediated by a plasma membrane redox system distinct from the proton translocating ATPase (Raghavendra, 1990), however there is not enough data available to establish whether this is the case or not (Zeiger, 1990). Starch hydrolysis and CO$_2$ fixation are additional sources of osmotica (Zeiger, 1990). Stomatal closure is not just brought about by stopping the opening mechanism, but rather by a closing mechanism—a transient increase in solute efflux (MacRobbie, 1988). Closure is affected by respiratory inhibitors and hypoxia (Weyers et al., 1982; Pemadasa, 1981). The effect of CN$^-$ and DCMU on stomatal opening is different under blue and red light, indicating
that the response to light is dependent on more than one source of energy and that different mechanisms are involved depending on the wavelength of light (Schwartz & Zeiger, 1984; Shimazaki, 1989). Respiration is not always the source of energy: under red light photophosphorylation can play this role. However, there is uncertainty on the importance of guard cell chloroplasts as a source of energy for stomatal opening under white light illumination (Zeiger, 1990; Dahse et al., 1990).

Stomata respond to light both directly and indirectly. Direct responses are those in which light is sensed in the guard cells, indirect ones are those in which light is sensed in other cells of the leaf. There are three photosystems involved in direct responses to light: guard cell photosynthesis and a blue light absorbing system are the primary systems (e.g. Sharkey & Ogawa, 1987; Zeiger, 1990), and phytochrome has a regulatory role (Holmes & Klein, 1985). The role of phytochrome is considered by Zeiger (1990) to be limited to its effect on circadian rhythms. According to Holmes (1989) phytochrome plays a more important role by regulating the speed with which stomata open and close.

The mechanism of response to CO$_2$ is not known (Mott, 1990), and most hypotheses are as undetailed as stating that it “...acts at some point in the ion accumulation mechanism” (Morison, 1987), or “...the main action of CO$_2$ is upon ion transport processes in the cell membranes” (Mansfield, 1983). Edwards & Bowling (1985) explained their experimental results by postulating an electrogenic proton pump in the plasmalemma which is inhibited by CO$_2$. Mansfield et al. (1990) have recently reviewed the literature on the action of CO$_2$ on guard cells. These authors suggest that there are two opposing actions of CO$_2$ on stomata: (1) stomatal aperture through enhanced malate synthesis, and the usually prevailing action, (2) stomatal closure through one or more of the following mechanisms: modulation of photophosphorylation, modulation of oxidative phosphorylation, a direct action on the plasma membrane, and/or an unknown mechanism.

Responses to water vapour pressure are not a simple passive effect on guard cell and epidermal water relations. When subjected to step changes in air humidity stomata display a response that has two phases (Grantz, 1990). Initially there is a passive phase during which the response is opposite to that in the succeeding active phase. During the passive phase there is no movement of solutes. In the later phase closure is concurrent with the decrease of solute content of the guard cells (Grantz, 1990). It was not known until recently which way of expressing air humidity (e.g. $h$ vs. $\chi^w$) was most appropriate because the sensing mechanism is unknown. However the experiments reported in Chapter 5 and information from experiments comparing stomatal behaviour in a helium-oxygen mix with that in air (Mott & Parkhurst, 1991) show that $D^w$ drives
this response through transpiration (either the total flow or a component of it).

No temperature sensor has been postulated in guard cells and the effect of tempera-
ture is most probably the result of a balance between its effects on the different
metabolic pathways of the cells, but this is still an open question (Zeiger, 1983). In
whole leaves some of these effects could be indirect through $\chi_c$ because temperature
affects $A$ through its effects on the rates of respiration and photosynthesis. Stomatal
aperture usually has an optimum temperature not far from the growth temperature.
Temperature not only affects steady-state stomatal aperture but also the rate of ap-
erture change. Meidner & Heath (1959) observed a $Q_{10}$ of 2.2 for rate of opening in
response to a dark to light transition in onion.

There are two main methods in use to study the responses of isolated guard cells:
protoplasts, which are cells devoid of walls, and “isolated” stomata. Guard cell pro-
toplasts are produced from peeled epidermis, by enzymatic digestion, and separation
from epidermal cell protoplasts (Zeiger, 1983; Weyers & Meidner, 1990). “Isolated”
stomata are used in situ in peeled epidermis, in which epidermal cells have been selec-
tively killed, usually by low pH (Squire & Mansfield, 1972; Weyers & Meidner, 1990).
Although the physical characteristics of these cells and their normal environment is
lost, they allow study of certain aspects of their functioning without the difficulties of
interpretation brought about by the presence nearby of several dissimilar kinds of cells,
as in a whole leaf. Guard cell protoplasts respond to light by swelling and by changing
the pH of the medium in which they are suspended (Zeiger & Hepler, 1977). There is
a concurrent flow of solutes and changes in membrane potential (Zeiger, 1990).

Before the availability of the techniques described in the previous paragraph, most
metabolic studies were done on epidermal peels. Information on the effect of having
K$^+$ salts of different anions in the medium, or substances that generate artificial ion
channels in membranes, and their interactions with responses to light and CO$_2$ was
obtained in this way (e.g. Wardle & Short, 1981). Epidermal peels with living epidermal
cells have also been used in many experiments on responses of stomatal aperture to
hormones. Stomata normally close in response to abscisic acid, and open in response
to cytokinins and indol-3-ylacetic acid (e.g. Mansfield, 1983). Interactions between
hormones are complex, and affect the sensitivity to CO$_2$ (Snaith & Mansfield, 1982).

The role of the subsidiary cells is both mechanical and as a source and sink of
solute. In grasses K$^+$ and Cl$^-$ shuttle between guard cells and subsidiary cells con-
currently with stomatal movements (Pallaghy, 1971; Raschke & Fellows, 1971). In
dicotyledoneae the role of the adjacent epidermal cells is not so clear. Not all species
have morphologically distinct subsidiary cells. Penny & Bowling (1974) have suggested
from data for Commelina communis that K$^+$ moves between guard cells and epidermal
cells through the subsidiary cells, and that active transport is involved. In the same species, Penny et al. (1976) observed a similar pattern of change in Cl$^-$ concentrations across the stomatal complex. In both dicotyledoneae and monocotyledoneae there are no plasmodesmata connecting mature guard cells with neighbouring cells, so solutes transported between them must go through the apoplast (Weyers & Meidner, 1990).

Although in the last few years our knowledge of the mechanism of solute transport in guard cells has advanced quickly, there is still no clear picture of its regulation in any plant species. As discussed above, stomatal responses to environmental stimuli are mainly transduced into a solute potential and its concomitant turgor potential. Any hypothesis about the intermediate steps leading from the presence of a stimulus to the accumulation of solutes is, at this time, very dependent on our preconceptions. It has to be based on what is known to happen, or assumed to happen in other organisms and on the kind of system within these organisms that we take as a model for stomata. We can boldly divide the possible mechanisms by which stimuli interact into three groups as follows.

1. Mechanisms based on what is known about energy transduction and solute transport. In this case environmental signals would be transduced into a proton gradient. This gradient being the common step unifying the different responses, this is the model originally proposed by Zeiger (1983). Although there is experimental evidence showing the important role of proton extrusion in stomatal opening, there is no evidence that the generation of this proton gradient is the step at which the interactions occur —i.e. the stage where transduction paths for different stimuli converge.

2. Mechanisms based on what is known about action of hormones, transmission of nerve impulses, and other regulatory systems in animals in which Ca$^{2+}$ plays a very important role as a messenger. This model was recently suggested by MacRobbie (1988) but the evidence is scanty. It is known that there are Ca$^{2+}$ channels in the plasma membrane and tonoplast of plants, and most probably also Ca$^{2+}$ ATPase in the plasma membrane (Sussman & Surowy, 1987; Marmè, 1988). A few Ca$^{2+}$, calmodulin regulated enzymes have also been found in plants (Marmè, 1988). Ca-dependent protein kinase activity has been detected in guard cell protoplasts, and calmodulin is also present in these cells (Mansfield et al., 1990). Cytosolic calcium regulates ion channels in the plasma membrane of Vicia faba (Schroeder & Hagiwara, 1989). In Commelina communis abscisic acid induces an increase in cytosolic free Ca$^{2+}$ that precedes stomatal closure (McAinsh et al., 1990). Abscisic acid, darkness, and cytokinins might employ
Ca\textsuperscript{2+} as second messenger (Mansfield et al., 1990).

3. Mechanisms based on what is known about other sensory systems like chemiotaxis in *Escherichia coli* or vision in humans. These systems sense changes in time of the level of the stimuli. In *Escherichia coli* this sensory adaptation (range adjustment) is effected by methylation of the receptor protein, and this allows the bacterium to sense the change in concentration by comparing the concentration to which it was exposed during the last second to that which it was exposed during the last three seconds (Stryer, 1988). The phosphorylation—and activation—status of several plant enzymes has been shown to be altered by light (Budde & Randall, 1990). Chlorophyll, phytochrome and a blue light photoreceptor seem to be involved with different enzymes (Budde & Randall, 1990). There is no evidence of which I am aware that shows cross-adaptation of a receptor protein in plant cells—i.e. change in the sensitivity to one stimulus caused by a different one. However, there is evidence of adaptation of light sensors allowing them to function over several orders of magnitude of $I$ (Galland, 1989). The overshoot many times observed in stomatal responses to step increases in the quantum flux density of blue light could be caused by partial adaptation.

Thus interactions between responses to different environmental stimuli could happen by transduction into a proton gradient, a pool of osmotica, the release of a common messenger like Ca\textsuperscript{2+}, or by cross-adaptation of the sensitivity of receptors. A direct effect on the proton gradient could be either through proton pumps or through ion channels or ports (e.g. in the human eye light closes Na\textsuperscript{+} channels causing the hyperpolarization of the membrane, but this response depends on the basal light level).

From a systems viewpoint, the sensory mechanism of guard cells can follow one of two contrasting hypothetical models. I am going to call them the balance model and the set-point model. In the balance model the effects of different stimuli contribute to an intermediate pool of a chemical species or to a potential gradient. In contrast, in the set-point model stimuli affect the ‘setting’ of a control system. There is evidence in favour of the idea that stomatal sensing of environmental variables is carried out by a system that follows the set-point model. There seems to be a mechanism for building up osmotic potential that can use different osmotica according to their availability. In particular, anions can be substituted one for another (Mansfield, 1983).

Based on control engineering common sense, one might think that a system that follows the set-point model would be more reliable because it would be able to sense one variable independently of changes in other state or environmental variables. However, there is little evidence available that could allow us to distinguish between these two
hypotheses.

1.1.3 Conductance of leaves to water vapour and \( \text{CO}_2 \)

The total conductance of a leaf is the result of the stomatal, cuticular, and boundary layer conductances. For a given set of conditions \( g^w_t \) depends not only on the density and size of stomatal pores, but also on the shape and size of the leaf. The conductance of a leaf surface and its boundary layer is not based on a totally diffusive process. It depends on wind speed and the aerodynamic characteristics of the leaf. This is mainly due to their effects on the thickness of the boundary layer. However, it has also been proposed that there can be mass flow of air through the leaf due to differences in pressure at different points of its surface (Vogel, 1978). These local pressure differences depend on the local wind speed (e.g. the wind profile near the edges of a leaf is different to that at its center).

Leaf surface conductances are measured as spatial averages. \( g^w_s \) is the result of the conductance of individual stomata, and their distribution. \( g^w_b \) is the average conductance of a boundary layer that is of non-uniform thickness (e.g. Grace & Wilson, 1976). \( g^w_c \) is a property of the cuticle, and depends on its integrity, but it has been also proposed that it could depend on \( \chi^w_s \) (Grace, 1977). They are related as follows:

\[
 g^w_t = \frac{1}{1/g^w_s + 1/g^w_b},
\]

where

\[
 g^w_l = g^w_s + g^w_c.
\]

The boundary layer affects responses of \( g^w_t \) to bulk air concentrations of \( \text{CO}_2 \) and water vapour by altering these concentrations at the place where they are sensed by stomata, and also by being a component of \( g^w_t \) (see Equation 1.1 above). Although \( g^w_s \) is a property of the leaf, it is brought about by the responses of individual stomata. Most environmental variables are sensed directly by the guard cells, and this has to be taken into account in any analysis of the responses of \( g^w_s \), \( g^w_l \), or \( g^w_c \). Driving variables must be defined at the leaf surface to be meaningful. The value of these variables at the leaf surface (e.g. \( \chi^w_s \) and \( \chi^w_c \)) depends, for a given value of the corresponding variables in the bulk air (e.g. \( \chi^w_a \) and \( \chi^w_c \)), on the thickness of the boundary layer. The boundary layer affects both the total conductance, the effective conductance controlling flow rates of water, \( \text{CO}_2 \), and sensible heat, and the concentrations at the leaf surface where they affect stomata. In natural conditions the boundary layer conductance also affects the energy balance of the leaf, and so its temperature which then affects stomata. (See
also Chapter 6.)

As pointed out above, \( g_w^b \) and \( g_w^s \) are spatial averages. The thickness of the boundary layer depends on the local wind speed, which changes across the leaf surface as a function of the distance to the leaf edge and wind direction (e.g. Nobel, 1983). Aperture of stomata varies both randomly (Laisk et al., 1980; van Gardingen et al., 1989), and systematically through the leaf surface (Smith et al., 1989): the aperture of individual stomata varies around a local mean value, this mean value being usually higher at the center of a leaf than at the edges.

Leaf conductance and CO\(_2\) assimilation rate are usually correlated under naturally occurring conditions. This correlation is not mechanistic as it can be readily broken (Jarvis & Morison, 1981). For example, light is sensed both directly by guard cells and indirectly through mesophyll photosynthesis. (See also Chapter 4.) However some authors do not accept the practical validity of this view and interpret the operational link that is frequently observed between \( g_w^b \) and \( A \) in mechanistic terms (e.g. Wong et al., 1979).

1.1.4 Conductance of canopies

If we move our reference level from the air immediately outside the leaf boundary layer, to some plane in the turbulent layer of air above the canopy where the driving variables are once again independent of the fluxes, we add new sources of resistance to the flow of water vapour and CO\(_2\) between the leaf mesophyll and this more distant reference level. This additional resistance is represented as an aerodynamic conductance across the canopy boundary layer to the base of the mixed layer above. It is very important to realize that by changing the reference level we are also changing what we assume to remain unchanged. When our reference is just outside the leaf boundary layer we assume all the conditions in the rest of the canopy, including \( g_w^s \) of other leaves, to remain constant.

Heterogeneity of surface properties also occurs at the canopy scale (Grace, 1991), and it depends on the type of vegetation — e.g. crops are usually homogeneous, but natural vegetation such as savannas and open woodlands can be patchy.

Stomatal control of canopy transpiration compared to leaf transpiration has been analysed in recent reviews (Jarvis & McNaughton, 1986; Finnigan & Raupach, 1987; McNaughton & Jarvis, 1991). The effect of a change in stomatal conductance is larger on the transpiration of an individual leaf than on the transpiration of a canopy because of the shorter path length. This shorter path has a higher conductance of which \( g_w^b \) is a more important component. When analysing a canopy, conductances and flow
densities are expressed per unit land area, and they represent the spatial integral of the conductances and flow densities at the leaf surfaces that make up the canopy.

McNaughton & Jarvis (1991) use the concept of feedback loops in control systems to describe the effect of stomatal conductance and other variables on leaf and canopy transpiration. They drew block diagrams of the control systems that operate at the leaf and canopy scales, and from these diagrams derived control equations. Starting with a very small area of a leaf they build by stages a description of transpiration of a canopy by nesting control structures that describe the different sources of feedback at each level. The control of transpiration by stomatal conductance decreases as new sources of feedback are included by scaling up. The previously proposed concept of de-coupling between leaf transpiration and the environment (Jarvis & McNaughton, 1986) is represented by the feedback caused by boundary layer conductance ($G_{w}^{b}$) through its effects on temperature and humidity (McNaughton & Jarvis, 1991).

Depending on the gain of the different feedback loops, brought about mainly by differences in $G_{w}^{b}$, the dependence of $E$ on $G_{s}^{w}$ varies. If $G_{w}^{b}$ is high as in some tree canopies, then $E$ depends strongly on $G_{s}^{w}$. In contrast, in short vegetation canopies, $G_{w}^{b}$ is small and $E$ is controlled mainly by radiation.

1.2 Models of stomatal response

1.2.1 Classification of models

Simulation models can be either mechanistic or empirical. Empirical models are also called descriptive because they simply describe the relationship between two or more variables while mechanistic models include indications of causality (Hall & Day, 1977). Other criteria can be used for a classification of models: (1) spatial scale, (2) time scale, (3) whether they are goal oriented or not, (4) whether they are static or dynamic.

1. Spatial scale differentiates models by the size of the object whose behaviour is modelled —e.g. a single stoma, a leaf, a plant, a canopy, or a region.

2. The time scale is related to the time lapse during which the behaviour happens —e.g. from minutes, days, and growth season to centuries or millennia.

3. I will call those models that are based on the idea that the system modelled —the plant, or one of its processes— tends to towards a goal, goal oriented. They can be seen as based on teleological ideas —e.g. stomata respond to light so as to keep $\chi_i^c$ constant. Both mechanistic and empirical models can be goal oriented.
In the first case the goal arises from assumptions about a mechanism, or causal chain of events, in the second case the apparent goal comes from observation.

4. Static models are used to simulate steady-state responses. Dynamic models simulate the changes in time of a state variable in response to changes in the value of driving variables.

Which kind of model is to be preferred? It depends on the objective, but in general mechanistic models are better than empirical models when used for extrapolation. Another advantage of mechanistic models is that they summarize the knowledge about a system in a testable way, thus helping our understanding of the system. This is balanced by the need for a much better understanding of the functioning of a system to be able to build a mechanistic model. Whether to construct dynamic or static models depends entirely on their intended use—e.g. In the case of stomata, if we are interested in responses to sunflecks, we need a dynamic model. Empirical goal oriented models provide insights about the results of a process, but not about the causal mechanism involved.

In the next two sections I shall consider only models at the scale of a single leaf. As the mechanism of stomatal response is not well known, few attempts have been done to build mechanistic models. Empirical models are much more common.

1.2.2 Empirical models

Several authors have developed static empirical models of $g_w^s$ responses (e.g. Jarvis, 1976; Thorpe et al., 1980; Lösch & Tenhunen, 1981; Avissar et al., 1985). Thorpe et al. (1980) developed a simple model of stomatal conductance of an apple leaf that includes only the effects of light and water vapour deficit. Jarvis (1976) proposed a more comprehensive model that takes into account responses to temperature, CO$_2$ and leaf water potential, as well as light and water vapour deficit. The model was fitted to field data for *Picea sitchensis* and *Pseudotsuga menziesii*, and also to measurements done in the laboratory. Avissar et al. (1985) developed a model for a tobacco leaf that includes the same variables except that soil water potential replaces leaf water potential. These three models include only multiplicative factors for the effects of the different variables. The functions used to empirically describe the individual responses are not the same in all the models. Lösch & Tenhunen (1981) designed a model to describe the responses of *Polypodium vulgare*. They used data measured in epidermal strips as a basis, to generate an intermediate result of the degree of aperture of a single stoma which was used to compute $g_w^s$, so although it is an empirical model of stomatal responses, it could be considered a semi-mechanistic model of leaf responses.
This model includes interactions between the effects of temperature and $D^w_n$, and water potential and $D^w_a$. Aphalo (1988) used a dynamic empirical model of $g^w_s$ as a submodel in a model of water vapour, CO$_2$, and energy flux densities between a leaf and the atmosphere. Kirschbaum et al. (1988) developed a dynamic model that simulates stomatal responses to lightflecks.

Some static empirical models use the correlation between $g^w_s$ and another plant response ($A$) to achieve a simpler mathematical description, but they do not include any causal relationship, so they are in no way mechanistic (e.g. Ball et al., 1987). Ball’s model uses one variable as a surrogate for others, it is in other words an indirect description. It could be called “operational” in the sense that it makes use of a relationship that seems to be an operational goal of the plant mechanism. This apparent goal comes from empirical observation, not from a causal mechanism. (See Chapter 7 for a detailed discussion of Ball’s model and some of its derivatives).

### 1.2.3 Mechanistic models

A dynamic mechanistic model of stomatal action was developed by Penning de Vries (1972). This model includes, as part of the mechanism, the water relations of the guard cells. Stomatal aperture is calculated from pressure potentials which depend on the effect of environmental variables on the water potential and its components. The author made many assumptions about the mechanism because not enough data was available. This model was used to describe the stomatal behaviour of turnip.

Optimization models search for an explanation in a much longer time scale. The question is why has certain behaviour been selected during evolution and not how it is implemented by the physiology of the plant. They are goal oriented, but this goal has a mechanistic basis in natural selection. The most popular of these models was proposed by Cowan & Farquhar (1977), and it applies to a leaf in an individual plant (Cowan, 1988). It treats transpiration as a cost and photosynthesis as a benefit and assumes that the plant behaves so that the daily integral of $A$ is maximum for a given daily integral of $E$. Solving the model under this assumption leads to constant marginal cost relative to marginal benefit with respect to changes in $g^w_s$ throughout the day, i.e.

$$
\frac{\partial E}{\partial g^w_s} = \frac{\partial E}{\partial A} = \lambda.
$$

(1.3)

This hypothesis has been tested by measuring $\lambda$ to see whether it does remain constant throughout a day under natural or controlled conditions. There are data bearing out this model (e.g. Farquhar et al., 1980), but also data opposing it (Fites &
Teskey, 1988). The assumption of constant $\lambda$ is readily broken in controlled conditions—by changing environmental variables in ways not usually occurring in nature. In the field $\lambda$ has been shown not to be constant but at the same time $A$ and $E$ were close to the values expected had $\lambda$ been constant (Williams, 1983). The departure of $\lambda$ and $g_s^w$ from their modelled optima was largest in the early morning and late afternoon. The assumed objective of using water resources with maximum efficiency is probably not always valid. Then, it is not surprising that the model fails to describe the behaviour of some species.
Chapter 2

Gas exchange system

A gas exchange system previously developed by A. P. Sandford and P. G. Jarvis was used in the experiments. The hardware has undergone only minor changes, but the software has been completely rewritten. Because of this, the emphasis in the discussion that follows will be on the program and algorithms used to control the system. However, a description of the system hardware is given because it is required for understanding the algorithms. Some data on its performance is also provided.

The system is designed to be capable of controlling the molar fractions of CO$_2$ and water vapour in the bulk air in the chamber. By doing all the calculations in real-time, it can also control both molar fractions at the leaf surface and the CO$_2$ molar fraction in the intercellular spaces. Leaf or air temperature is controlled. Values of CO$_2$ flux density, transpiration and conductance are computed and displayed in real-time.

2.1 Hardware

The gas-exchange system is configured as an open differential system. Its air circuit diagram can be divided into three main blocks: air-conditioning gear, leaf-chamber, and measuring instruments (Fig. 2.1). In the following sections each of these blocks, as well as ancillary equipment, are described.

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{Fig2_1.png}
  \caption{Simple block diagram of the air circuit of the gas-exchange system.}
\end{figure}
2.1.1 Conditioning of ingoing air

The molar fractions of CO$_2$, and water vapour of the air going into the chamber can be adjusted by means of mass-flow controllers driven by electrical signals. The flow rate of air at the chamber inlet can also be controlled. A diagram of this ‘air-conditioning’ part of the system is given in Fig. 2.2.

Room air is pumped through columns of ‘soda-lime’ (to remove CO$_2$), and silica-gel and ‘drierite’ (to remove water vapour). Part of this flow of dry, CO$_2$-free air bubbles through water kept at 35.0 °C. By mixing these two flows of air, moist CO$_2$-free air is obtained. This moist air is then mixed with pure CO$_2$ coming from a cylinder. The molar fractions of CO$_2$ and water vapour depend on the electrically controlled throughputs of the mass-flow controllers (FC 261, and FC 260; Tylan (UK) Ltd., Swindon, U.K.). A diaphragm pump (B100SE, Charles-Austen Pumps Ltd, Byfleet, Surrey, U.K.) is then used to push the air at a slight over-pressure through another mass-flow controller (Tylan FC 260) and the leaf chamber.

2.1.2 Leaf chamber

The chamber used has a volume of 1250 cm$^3$. It has a double glazed glass window at the top and is made of nickel-plated brass. The details of its construction and dimensions can be seen in the diagram in Fig. 2.3. The temperature of the leaf chamber is controlled by means of a Peltier unit (14 V, 8 A; ‘Cambion’ Part No. 803-1008-01, Cambridge Thermionic Corp., Cambridge, Mass., U.S.A.) on which it sits. This unit is driven by a temperature controller (type 070, Eurotherm Ltd., Durrington, West Sussex, U.K.) that
Figure 2.3. Spatial distribution of photon flux density on a horizontal plane situated 50 cm below a vertically positioned metal-halide reflector lamp (Wotan ‘power-star’; HQI-R 250 W/NDL; Wotan Lamps Ltd., London). Measured with a Li-Cor cosine corrected PAR sensor (type Li-190SB, Li-Cor Ltd., Lincoln, Nebraska). A diagram of a lengthwise cross-section of the leaf chamber is overlaid. L: leaf, S: light sensor, W: window, F: fan, Fn: fins for heat exchange. Drawn to scale, width of the chamber: 12.4 cm.

uses a thermojunction as sensor. Depending on whether this thermojunction is attached to the enclosed leaf, or left free inside the chamber, either leaf or air temperature is kept constant at a preset value. Ventilation inside the chamber is achieved by means of an axial fan located at the same end as the air inlet and outlet. The speed of the fan is controlled by a variable voltage source.

A silicon photo-diode (type BPW21, R.S. Components Ltd., Corby, Northants, U.K.) is used as a light sensor. This diode is sensitive to ‘visible’ light but its spectral response is not flat for photon flux density of PAR. It was calibrated for different light sources against a recently calibrated PAR quantum sensor (type Li-190SB, Li-Cor Ltd., Lincoln, Nebraska).

Leaf and air temperatures are measured with small thermojunctions made from 0.1 mm diameter copper and constantan wires. The thermojunctions were kept in contact with the shaded surface of the leaf. An ice-point reference unit (Zeref, Hanovia Ltd., Slough, Berkshire, U.K.) is used for the reference junctions.
2.1.3 Light source

A metal-halide reflector lamp was used as a light source (Wotan ‘power-star’ HQI-R 250 W/NDL; driven by a choke for 400 W HQI lamps, type IZL; Wotan Lamps Ltd., London). Its spectrum, as seen through the chamber window and the different filters used, is given in Fig. 2.4. Photon flux density at the chamber was controlled by means of reflecting neutral density filters. A heat mirror was always used to prevent overheating of the leaf chamber. The spatial distribution of light under the source was such that the photon flux density at any point within the useful area of the chamber was within ±8% of that measured using the photo-diode permanently affixed inside the chamber (Fig. 2.3).

2.1.4 Measurement of ingoing and outgoing flows

The water-vapour contents of air at the chamber inlet and outlet are measured by means of two dew-point meters (series 3000, Michell Instruments Ltd., Cambridge, U.K.). The molar concentration of CO$_2$ is measured with an infra-red gas analyser (URAS 3E, Hartmann & Braun AG, Frankfurt, Germany). This analyser can be used in either a differential mode, or an absolute mode. The pressure difference between the inlets to the IRGA cells is monitored with a differential electronic pressure meter (M-10, Mercury Electronics, Glasgow, U.K.). A double water vapour trap (MGK 1 gas cooler, Waltz Mess- und Regeltechnik, Effeltrich, Germany; type 815 temperature controller, Eurotherm Ltd., Durrington, West Sussex, U.K.; and a custom built d.c. power amplifier) is installed on the sample and reference air lines between the dew-point meters and the IRGA$^1$. The flow rate of air through these instruments is controlled by means of manually set rotameters (KDG Flowmeters, Burgess Hill, Sussex, U.K.).

2.1.5 Calibration of the IRGA

The sample and reference cells of the IRGA are split into two sections of 0.95 and 0.05 of the total length. These sections are independent with respect to air flow but constitute a single path for the infra-red radiation. This allows calibration of the differential sensitivity of the IRGA with air of normal CO$_2$ concentration independently of the background CO$_2$ concentration. Any change in concentration of CO$_2$ in the short section of the cell is equivalent to a change of 5% of its magnitude over the whole path. Compressed air from an aluminium cylinder, and dry CO$_2$-free air pumped through columns filled with ‘soda-lime’ and silica gel are used as calibration standards.

$^1$This cold trap was added after some of the experiments had been done.
Figure 2.4. Photon spectra of the light sources used in the gas exchange experiments. (a) HQI-R lamp with a heat filter, as seen through the chamber window; (b) with a red filter (---); and with a blue filter (--.--). Measured with an optical spectrum analyser (model 6800; with a 6100 monochromator, with a 0.9 mm slit installed; and a 6118 photo-tube detector. Monolight Instruments Ltd., Weybridge, Surrey, U.K.).
Figure 2.5. Functional diagram of the air circuit used for measurement and calibration. VM: group of valves working as a manifold; P: diaphragm pump; RT: needle valve and rotameter; DPM: dew-point meter; WVC: water vapour condenser; LC1, LC2: long cells of the IRGA; SC1, SC2: short cells of the IRGA. The differential pressure meter, individual solenoid valves, and blow-offs have not been included in the diagram to simplify it. Detailed diagrams are given by Sandford (1987).

The inlets to the cells are switched between the different air sources by means of solenoid valves (Fig. 2.5).

The linearity, sensitivity, and zero offset are checked and adjusted using air of known volume fractions of CO\textsubscript{2}. Air of different CO\textsubscript{2} concentrations is obtained by mixing pure CO\textsubscript{2} and dry, CO\textsubscript{2}-free air with a set of three gas mixing pumps connected in a cascade (Digamix G27/3F, SA27/3F, and SA18/3F; H. Wösthoff GmbH, Bochum, Germany).

2.1.6 Data-logging and control

A dedicated personal computer (IBM PC-ATX, IBM United Kingdom International Products Ltd., North Harbour, Portsmouth, U.K.) controls the gas-exchange system using a datalogger (3530B Orion Data Logging System, Solartron Instrumentation Group, Farnborough, Hampshire, U.K.) as an input and output front-end. The datalogger handles both analogue and digital signals, and communicates with the computer through a serial bidirectional data link. Part of the data processing is done on the datalogger (mean of repeated measurements, offset compensation and scaling). The rest of the data processing is done in real-time on the computer. Calculations needed for control also take place in the computer, which sets the outputs of the datalogger that control the mass-flow controllers, valves and pumps.
2.2 Software

I redesigned and rewrote the program that controls the hardware described above with the aim of making it easy to maintain and change. Alterations to the program could be needed to adapt it to changes in the hardware or to the requirements of new experiments. In its use it had to be reliable, fault-tolerant, and provide diagnosis for the most common error conditions arising from hardware faults, software errors and user mistakes. Initially I had no intention of changing data processing or control algorithms. However, during the course of this project I found it necessary to add calculations giving the molar fractions of CO$_2$ and water vapour at the leaf surface, and the commands for controlling them during experiments. It was also necessary to improve or replace many of the existing control and measurement algorithms.

Two versions of the program exist, one for systems with a water trap before the IRGA, the other for systems in which moist air goes through the gas analyser. The version for systems that include a water trap assumes that when the dew point of the air going through the trap is below the temperature of the trap no humidification occurs. This would be true only if the water trap was bypassed under this condition. In reality what happens is that the air is moistened, but its water content varies depending on the quantity of water remaining inside the trap. When changing from moist to dry air going through the trap, the humidity of the air at its outlet keeps changing for several hours. However, as the saturated molar fraction at the temperature normally used for the trap (1.0 $^\circ$C) is low, and as this temperature is not far from the maximum cooling of the dew-point meters the error in total molar flow rate is small (<0.5%), even if a bypass is not used.

2.2.1 Algorithms

The design of the program was based on the flow of data. There are two ‘kinds’ of data: raw data obtained from the sensors, and processed data giving the state of the system and plant leaf. The steps required are (1) acquisition of the raw data from the datalogger, (2) processing of these data to obtain the state of the system, (3) checking the validity of these data, and (4) displaying, printing and storing these data.

In parallel with data acquisition and processing the system must be controlled and calibrated. A calibration is again a data transformation: several sets of raw data acquired after switching valves in different configurations are processed into a set of calibration data expressed at a standard condition. The steps required are then: (1) set valves, (2) acquire raw data, (3) check that data are valid, repeat steps (1) to (3) until all necessary data are available, (4) process the raw data sets into calibration
data, and (5) display, print, and save it.

Control consists of (1) computing the values required for the controlled variables necessary to obtain the requested value of a dependent variable, (2) checking that it is safe to set the values requested, (3) sending the commands to the datalogger. The control algorithm does not use a feedback loop—except through the operator. This decision was made because the response time is long and includes both the response of the measuring system and the measured leaf. Effective control requires intelligence, provided by the human operator.

It is also necessary to prevent conditions dangerous to the integrity of the system or that would affect the validity of data not yet acquired. This is achieved by (1) predicting the danger of an undesirable happening, and by (2) altering the state of the system so as to avert this danger without operator intervention.

Calibration of the dew-point meters

The sensitivity of the dew-point meters is very stable, so only the offset is routinely measured. This calibration is done on its own or concurrently with the IRGA calibration. It is assumed that the offset is a temperature error$^2$.

The procedure is as follows:

- **Zero offset:**
  1. Set valves so that both reference and sample DPMs are connected to the reference air stream.
  2. *Rebalance the dew-point meters. (Optional.)*
  3. Wait long enough to flush air through them and get a steady output.
  4. Take a set of readings.
  5. The difference between the recorded dew-points gives the zero offset.

During a calibration, when reference air is flowing through both dew point-meters, the mean of the dew-points measured by them is used as the reference to calculate a zero offset for each of them (i.e., half of the total zero offset is attributed to each dew-point meter). When using the calibration data, these offset corrections are applied to $T_{\text{dew}}^{\text{in}}$ and $T_{\text{dew}}^{\text{out}}$. These dew-point temperatures and current atmospheric pressure are used to compute $\chi_w^{\text{in}}$ and $\chi_w^{\text{out}}$.

$^2$In the previous version of the program, written by A. P. Sandford, it was assumed to be a water vapour molar fraction error.
Differential IRGA calibration

The following algorithm and equations can only be used with infra-red gas analysers having a split sample cell. This ‘short-cell calibration’ method is based on that described by Thorpe (1978). Calibration consists of two main steps: the calculation of the zero offset, and the calculation of the sensitivity or gain. As the differential offset and sensitivity of an IRGA depend on the background $\chi_{in}$ it is necessary either to recalibrate each time $\chi_{in}$ is altered, or to correct calibration data for this change in $\chi_{in}$. To achieve this second option, calibration data are stored expressed at a standard $\chi_{in}=350 \mu$mol mol$^{-1}$, and when used, corrected for the actual value of $\chi_{in}$. The actual procedure is:

**Zero offset:**
1. Set valves so that the long and short sections of the reference and sample cells of the IRGA are connected to the reference air stream.
2. Wait long enough to flush the full length of the sample cell.
3. Take a set of readings.
4. The IRGA output is its zero offset ($s_{null}$).

**Sensitivity:**
5. Set valves so that the short sample cell is connected to the CO$_2$-free air source.
6. Wait long enough to flush the short sample cell.
7. Take a set of readings, getting $s_{free}$.
8. Set valves so that the short sample cell is connected to a source of air of known CO$_2$ molar fraction.
9. Wait long enough to flush the short sample cell.
10. Take a set of readings, getting $s_{in}$.
11. Do sensitivity calculations using equations 2.1 or 2.2–2.4.
12. Compute error of mass flow meters using equation 2.5.

**Standardize calibration:**
13. Calculate offset at $\chi_{in}=350 \mu$mol mol$^{-1}$ using equation 2.7.
14. Calculate sensitivity at $\chi_{in}=350 \mu$mol mol$^{-1}$ using equation 2.9.

If sensitivity is computed from ‘standard’ air from a cylinder then it is given by:

$$\beta = \frac{s_{in} - s_{free}}{\ell \chi_{cyl}}.$$  \hspace{1cm} (2.1)

However, to compute the sensitivity of the IRGA using the reference air stream as a standard, it is first necessary to calculate the water vapour content of the reference air
at the IRGA:

\[ \chi_{w, \text{IRGA}}^w = \begin{cases} \chi_{w, \text{trap}}^w, & \text{with water trap if } \chi_{w, \text{trap}}^w < \chi_{w, \text{in}}^w, \\ \chi_{w, \text{in}}^w, & \text{otherwise,} \end{cases} \tag{2.2} \]

and then to get an estimate of the CO\(_2\) concentration from the measured flow rates:

\[ \chi_{c, \text{IRGA}}^c = \frac{J_{\text{tot}}^c}{(J_{\text{dry, tot}}^c + J_{\text{tot}}^c)} (1 - \chi_{w, \text{in}, \text{IRGA}}^w) \tag{2.3} \]

and finally to obtain the differential sensitivity as:

\[ \beta = \frac{s_{\text{in}} - s_{\text{free}}}{\ell \chi_{c, \text{IRGA}}^c}. \tag{2.4} \]

In this case \( s_{\text{in}} = s_{\text{null}} \), and steps 8 to 10 of the algorithm are redundant.

If the sensitivity is computed using air from a cylinder then it is possible to measure the error in the mixing ratio of the mass-flow controllers that are used to mix the air going into the chamber:

\[ \chi_{c, \text{error}}^c = \frac{s_{\text{null}} - s_{\text{free}}}{\ell \beta} - \chi_{c, \text{IRGA}}^c, \tag{2.5} \]

for which \( \chi_{\text{in,IRGA}}^c \) is calculated using equations 2.3 and 2.4. If standard air from a cylinder is not used, step 12 of the algorithm must be skipped.

In this case it is also possible to get an absolute value for \( \chi_{\text{in}}^c \):

\[ \chi_{\text{in}}^c = \frac{s_{\text{null}} - s_{\text{free}}}{\ell \beta}. \tag{2.6} \]

The dependence of \( s_{\text{null}} \) on \( \chi_{\text{in}}^c \) is taken into account by means of an empirically measured linear relationship. The following equation is used to standardize the measured offset to \( \chi_{\text{in}}^c = \chi_{\text{std}}^c \), where \( \chi_{\text{std}}^c = 350 \, \mu\text{mol mol}^{-1} \):

\[ \tilde{s}_{\text{null}} = s_{\text{null}} + k_o (\chi_{\text{std}}^c - \chi_{\text{in}}^c), \tag{2.7} \]

and when it is used it is adjusted for the current value of \( \chi_{\text{in}}^c \):

\[ s_{\text{null}} = \tilde{s}_{\text{null}} + k_o (\chi_{\text{in}}^c - \chi_{\text{std}}^c). \tag{2.8} \]

The differential sensitivity or gain of the IRGA also depends on the background \( \chi_{\text{in}}^c \) and can be corrected using a method adapted from that proposed by Thorpe (1978).
Standardization to $\chi_{in}^c=350\mu\text{mol mol}^{-1}$ is done as:

$$\tilde{\beta} = \beta \frac{\chi_{in}^c + k_s}{\chi_{std}^c + k}$$

(2.9)

where $k_s$ and $k$ are empirical constants obtained by measuring the sensitivity at different values of $\chi_{in}^c$. When used, this standardized sensitivity is first adjusted to the current value of $\chi_{in}^c$:

$$\beta = \tilde{\beta} \frac{\chi_{std}^c + k_s}{\chi_{in}^c + k}.$$  

(2.10)

- **Using a calibration**
  1. Compute offset at current $\chi_{in}^c$.
  2. Compute sensitivity at current $\chi_{in}^c$.
  3. Compute $\Delta\chi_{IRGA}$ from raw voltage reading using equation 2.11.

A differential reading in $\mu\text{mol mol}^{-1}$ is computed as:

$$\Delta\chi_{IRGA} = \frac{s_{samp} - s_{nul}}{\beta},$$

(2.11)

where $s_{samp}$ is the current signal from the IRGA, and $s_{nul}$ and $\beta$ have been computed for the current value of $\chi_{in,IRGA}$ from stored calibration data using equation 2.26 below.

**Calculations of the leaf and chamber states**

The equations used in the calculations in sections 2.2.1 and 2.2.1 are based on those given by von Caemmerer & Farquhar (1981). Some of the equations have been rearranged for computational reasons. In what follows their presentation follows the program listing and not the original sources.

There are several assumptions involved in the use of these equations:

- The system (leaf + chamber) is in a steady-state.

- Flux densities of water vapour and CO$_2$ are the same over the leaf surface(s) involved in gas exchange.

- Gradients of water vapour and CO$_2$ molar fractions are the same over the leaf surface(s) involved in gas exchange.

*These assumptions imply that:*

- Stomatal conductance is the same throughout the leaf surface(s).
o Boundary layer conductance is the same throughout the leaf surface(s).

o Temperature is the same in all the leaf.

All the results depend on the first assumption being valid. Non steady-state conditions require more complicated calculations than those described here. Calculations for non steady-state conditions must take into account the dynamic characteristics of the gas-exchange system (time constant, and lag). For a given $A$ and $E$, these characteristics affect the observed concentration differences, and their rate of change. Using steady-state assumptions under non steady-state conditions leads to the underestimation of the magnitude and speed of changes in $A$ and $E$.

The time constant of a system depends on the volume of the chamber and the flow rate of air through it, the lag depends on the volume of air in the tubing between the chamber and the measuring instruments and the flow rate through this tubing. Further complications are added by adsorption of water and CO$_2$ on the walls of the chamber and tubing. In practice, before a gas-exchange system can be used under non steady-state conditions, its dynamic behaviour must be measured.

The second and third assumptions only affect the calculated conductances, and the concentrations internal to the boundary layer. Although these equations assume a single gas exchange surface, they can also be applied to amphistomatous leaves if stomatal and boundary layer conductances are the same on both sides. In hyper- or hypostomatous leaves the boundary layer conductance to be used in the calculations is that of the single surface over which gas exchange is taking place. In symmetrical amphistomatous leaves the boundary layer conductance to be used is that of both leaf faces in parallel. These calculations are not rigorous for asymmetrical amphistomatous leaves, or leaves having a patchy distribution of stomatal aperture. In practice, these assumptions are seldom true, and so the calculated conductances and molar fractions are only approximations to their mean values over the surface of the leaf. They differ from the true mean because non-linear relationships are involved in their computation.

**Flow rates.** The total flow rate of dry, CO$_2$-free air ($J_{\text{tot}}^{\text{dry}}$), the dry air flow rate at the humidifier inlet ($J_{\text{hum}}^{\text{dry}}$), the CO$_2$ flow rate ($J_{\text{tot}}^{\text{c}}$), and the moist air flow rate at the chamber inlet ($J_{\text{in}}^{\text{air}}$) are measured. The diagram in Fig. 2.2 shows the places in the system where the mass-flow controllers used to measure these flow rates are located. The water vapour flow rate evaporating from the humidifier is computed as

$$J_{\text{hum}}^{\text{w}} = J_{\text{hum}}^{\text{dry}} \frac{\chi_{\text{hum}}^{\text{w}}}{1 - \chi_{\text{hum}}^{\text{w}}}$$

(2.12)
and the total moist air flow rate at the outlet of the ‘mixing tray’ \( J_{\text{tot}}^{\text{air}} \) is calculated from the other flow rates as

\[
J_{\text{tot}}^{\text{air}} = J_{\text{tot}}^{\text{dry}} + J_{\text{tot}}^{c} + J_{\text{hum}}^{w}.
\] (2.13)

**Water molar fractions, conductance and molar flux density.** The molar fractions of water vapour in the air at the chamber inlet \( \chi_{\text{in}}^{w} \), and outlet \( \chi_{\text{out}}^{w} \) are directly calculated from the measured dew-points and ambient pressure. The molar fraction of water vapour in the intercellular air space is computed assuming that it is saturated at the leaf temperature,

\[
\chi_{i}^{w} = \chi_{i}^{w*}.
\] (2.14)

The air in the chamber is assumed to be well stirred so,

\[
\chi_{a}^{w} = \chi_{\text{out}}^{w}.
\] (2.15)

The apparent molar fraction of water vapour in the ‘dry CO\(_2\)-free’ air is computed as:

\[
\chi_{\text{dry}}^{w} = \frac{\chi_{\text{in}}^{w} J_{\text{tot}}^{\text{air}} - J_{\text{hum}}^{w}}{J_{\text{tot}}^{\text{dry}}},
\] (2.16)

where no correction is needed for \( \chi_{\text{dry}}^{w} \) because this flow of water vapour is included in the measured \( J_{\text{tot}}^{\text{dry}} \). \( \chi_{\text{dry}}^{w} \) reflects both the water vapour content in the dry air, the efficiency of the humidifier, and errors in the calibration of the mass-flow controller used to set \( J_{\text{hum}}^{\text{dry}} \). The normal procedure is to calibrate this mass-flow meter for an apparent humidifier efficiency of 100%.

Relative humidity of the bulk air in the chamber is

\[
h_{a} = \frac{\chi_{a}^{w}}{\chi_{a}^{w*}}
\] (2.17)

and the bulk air to leaf water deficit is

\[
D_{a}^{w} = \chi_{l}^{w*} - \chi_{a}^{w}.
\] (2.18)

The calculation of the water vapour flux density from the leaf to the air in the chamber takes into account the difference between \( J_{\text{in}}^{\text{air}} \) and \( J_{\text{out}}^{\text{air}} \) due to the added water vapour:

\[
E = \frac{J_{\text{in}}^{\text{air}} (\chi_{\text{out}}^{w} - \chi_{\text{in}}^{w})}{(1 - \chi_{\text{out}}^{w}) S}.
\] (2.19)
The total conductance to water vapour is

$$g_w^t = \frac{E[1 - (\chi_a^w + \chi_i^w)/2]}{\chi_i^w - \chi_a^w},$$  \hspace{1cm} \text{(2.20)}$$

where the factor $1 - (\chi_a^w + \chi_i^w)/2$ is a correction for the effect of mass flow.

Leaf conductance to water vapour (stomatal and cuticular conductances in parallel) is computed as

$$g_w^l = g_w^t g_w^b g_w^b - g_w^t,$$  \hspace{1cm} \text{(2.21)}$$

where $g_w^b$ is the boundary layer conductance to water vapour previously measured for a replica of the leaf.

The molar fraction of water vapour at the leaf surface is

$$\chi_s^w = \chi_a^w + (\chi_i^w - \chi_a^w)\frac{g_w^t}{g_w^b},$$  \hspace{1cm} \text{(2.22)}$$

the water vapour deficit at the leaf surface is

$$D_w^s = \chi_i^w - \chi_s^w,$$  \hspace{1cm} \text{(2.23)}$$

and the relative humidity at the leaf surface is

$$h_s = \frac{\chi_s^w}{\chi_i^w},$$  \hspace{1cm} \text{(2.24)}$$

assuming that the air at the leaf surface is at the same temperature as the leaf.

**CO₂ molar fractions, conductance and molar flux density.** The molar fraction of CO₂ in the air at the chamber inlet ($\chi_{in}^c$) is measured only during calibrations\(^3\), otherwise it is computed from the flow rates and the error observed during the calibration as

$$\chi_{in}^c = \frac{J_{in}^c}{J_{tot}} + \chi_{error}^c.$$  \hspace{1cm} \text{(2.25)}$$

Any change in water vapour content affects the molar fraction of the other components of the air, so if a water trap is used, the molar fraction of CO₂ at the IRGA is different.

\(^3\)the program could be easily modified to take both absolute and differential measurements of the CO₂ concentration for every measurement, but this would increase the time necessary for getting each data point.
to that at the chamber inlet.

\[
\chi_{\text{in,IRGA}}^c = \begin{cases} 
\chi_{\text{in}}^c & \text{with water trap if } \chi_{\text{trap}}^w < \chi_{\text{in}}^w, \\
\chi_{\text{in}}^c & \text{otherwise.}
\end{cases} \tag{2.26}
\]

The calculation of the molar fraction at the chamber outlet also depends on whether a water trap is used or not.

\[
\chi_{\text{out}}^c = \begin{cases} 
(\chi_{\text{in,IRGA}}^c + \Delta \chi_{\text{IRGA}}^c) \frac{1 - \chi_{\text{out}}^w}{1 - \chi_{\text{trap}}^w}, & \text{with water trap if } \chi_{\text{trap}}^w < \chi_{\text{out}}^w, \\
\chi_{\text{in,IRGA}}^c + \Delta \chi_{\text{IRGA}}^c, & \text{otherwise.}
\end{cases} \tag{2.27}
\]

The difference in molar fraction of CO\(_2\) between the chamber inlet and the chamber outlet (\(\Delta \chi_{\text{in-out}}^c\)), is different to that measured at the IRGA (\(\Delta \chi_{\text{IRGA}}^c\)) when a water trap is used, but can always be calculated as

\[
\Delta \chi_{\text{in-out}}^c = \chi_{\text{out}}^c - \chi_{\text{in}}^c. \tag{2.28}
\]

The flux density of CO\(_2\) between the leaf and the air in the chamber is given by

\[
A = \frac{-J_{\text{air}}^c \Delta \chi_{\text{in-out}}^c (1 - \chi_{\text{in}}^w)}{(1 - \chi_{\text{out}}^w)S} - E \chi_{\text{in}}^c \tag{2.29}
\]

which includes corrections for both the difference between \(J_{\text{in}}^c\) and \(J_{\text{out}}^c\), and the dilution effect of the flow of water on the molar fraction of CO\(_2\).

The total conductance to CO\(_2\) is calculated from the conductances to water vapour taking into account the different diffusivities of water vapour and CO\(_2\), and the only partially diffusive process in the boundary layer:

\[
g_c^t = \left( \frac{1.60}{g_1^w} + \frac{1.37}{g_0^w} \right)^{-1}. \tag{2.30}
\]

The molar fraction of CO\(_2\) in the intercellular space of the leaf must be calculated taking into account the effect of the flow of water on the flow of CO\(_2\), a trace gas:

\[
\chi_i^c = \frac{(g_c^t - E/2) \chi_{\text{a}}^c - A}{g_c^t + E/2}. \tag{2.31}
\]

The molar fraction of CO\(_2\) at the leaf surface is similarly calculated, using the equation
proposed by Ball (1987):

\[
\chi_c = \frac{\left( g_b c - E/2 \right) \chi_c^b - A}{g_b c + E/2}.
\]

(2.32)

Control algorithms

As stated above, after using the system for a while it was realized that many of the control algorithms were not working as expected and they were modified to make them more robust with respect to various calibration and operator errors. The possibility of controlling several new variables was added. The algorithms were made as independent from the system hardware configuration as possible. The algorithms used are given below.

Controlling humidity Only the first algorithm from this group assumes an open gas-exchange system. The others are equally suitable for both closed loop and open gas-exchange systems.

- Control \( \chi_w^a \) by altering \( \chi_w^m \)
  1. Check requested \( \chi_w^a \) against chamber wall temperature and room temperature, and ignore requests that would lead to condensation.
  2. Compute minimum dew-point that can be measured as room temperature minus the maximum cooling that the dew-point meters can achieve.
  3. Check the requested \( \chi_w^a \) against minimum dew-point that can be measured, and if necessary adjust the requested \( \chi_w^a \) to keep the dew-point at least 5 \(^\circ\)C above this minimum value.
  4. Estimate \( E \) under the new condition, and from it, the difference in water vapour molar fraction between the air streams going into, and coming out of the chamber.
  5. Compute required \( \chi_w^m \) as the requested \( \chi_w^a \) minus the difference resulting from expected transpiration.
  6. Check the required \( \chi_w^m \) against the minimum dew-point that can be measured, and if necessary adjust the air flow rate through the chamber to keep \( \chi_w^m \) at a value that would keep the dew-point at least 2 \(^\circ\)C above this minimum.
  7. Compute required change in \( \chi_w^m \).
  8. If this change is small request a relative change in the value of \( \chi_w^m \), otherwise request an absolute value for \( \chi_w^m \).

- Control \( \chi_w^s \) by altering \( \chi_w^a \)
  1. Compute required \( \chi_w^a \) assuming that \( \chi_w^a / \chi_w^s \) remains unchanged.
  2. Request required \( \chi_w^a \).
• Control $h_a$ by altering $\chi_a^w$
  1. Compute required $\chi_a^w$ from requested $h_a$ and $\chi_a^{w*}$ at current $P_{atm}$ and $T_a$.
  2. Request required $\chi_a^w$.

• Control $h_s$ by altering $\chi_s^w$
  1. Compute required $\chi_s^w$ from requested $h_s$ and $\chi_s^{w*}$ at current $P_{atm}$ and $T_l$.
  2. Request required $\chi_s^w$.

• Control $D_a^w$ by altering $\chi_a^w$
  1. Compute required $\chi_a^w$ from requested $D_a^w$ and $\chi_l^{w*}$ at current $P_{atm}$ and $T_l$.
  2. Request required $\chi_a^w$.

• Control $D_s^w$ by altering $\chi_s^w$
  1. Compute required $\chi_s^w$ from requested $D_s^w$ and $\chi_l^{w*}$ at current $P_{atm}$ and $T_l$.
  2. Request required $\chi_s^w$.

• Control $E$ by altering $\chi_a^w$
  1. Compute required $\chi_a^w$ from requested $E$, $\chi_l^{w*}$ at current $P_{atm}$ and $T_l$, current $\chi_a^w - \chi_a^w_{out}$, and current $E$, i.e. assuming no change in $g^w$.
  2. Request required $\chi_a^w$.

Control of CO$_2$ molar fraction

• Control $\chi_a^c$ by changing $\chi_a^c$
  1. Guess what the value of $A$ will be after the change in $\chi_a^c$ takes place: if requested $\chi_a^c > 60$ µmol mol$^{-1}$ and $A > 0.5$ µmol m$^{-2}$ s$^{-1}$ then assume that $A$ is a linear function of $\chi_a^c$, otherwise assume that $A$ is not going to change.
  2. Check whether expected value of $A$ is negative, and if so set it to zero.
  3. Compute $J_{air}^{out}$ from $J_{air}^{out}$, expected $A$, current $E$, and leaf area.
  4. Compute required $\chi_a^c$ in to get the requested $\chi_a^c$ taking into account the change in total flow ($J_{air}^{out} - J_{air}^{in}$).
  5. If $\chi_a^c - \chi_a^c_{in}$ would be out of the IRGA sensitivity range, then adjust $J_{air}^{in}$ (NOT IMPLEMENTED).
  6. Compute required change in $\chi_a^c$.
  7. If this change is small request a relative change in the value of $\chi_a^c$, otherwise request an absolute value for $\chi_a^c$. 

• **Control \( \chi_i^c \) by changing \( \chi_{in}^c \)**
  1. Check whether current \( g_i^w \) is high enough to yield a reliable estimate of \( \chi_i^c \). If not ignore the request to change \( \chi_i^c \).
  2. Guess what the value of \( A \) will be after the change in \( \chi_i^c \) takes place: if \( 50 \mu\text{mol mol}^{-1} > \text{requested} \chi_i^c > 280 \mu\text{mol mol}^{-1} \) and \( A > 0 \) then assume that \( A \) is a linear function of \( \chi_i^c \), otherwise assume that \( A \) is not going to change.
  3. Compute total conductance to CO\(_2\).
  4. Compute required \( \chi_a^c \) taking into account the effect of \( E \) on \( \chi_i^c \).
  5. Check whether expected value of \( A \) is negative, and if so set it to zero.
  6. Compute \( J_{air}^{out} \) from \( J_{air}^{in} \), expected \( A \), current \( E \), and leaf area.
  7. Compute required \( \chi_{in}^c \) to get the requested \( \chi_a^c \) taking into account the change in total flow \( (J_{air}^{out} - J_{air}^{in}) \).
  8. *If \( \chi_{out}^c - \chi_{in}^c \) would be out of the IRGA sensitivity range, then adjust \( J_{air}^{in} \) (NOT IMPLEMENTED).*
  9. Compute required change in \( \chi_{in}^c \).
  10. If this change is small request a relative change in the value of \( \chi_{in}^c \), otherwise request an absolute value for \( \chi_{in}^c \).

• **Control \( \chi_s^c \) by changing \( \chi_a^c \)**
  1. Compute required \( \chi_a^c \), assuming that \( \chi_s^c/\chi_a^c \) is not going to change.
  2. Request required \( \chi_a^c \).

**Runtime error checking**

Data are checked for the following conditions: water condensation, wrong CO\(_2\) flow rate, wrong dry air flow rate, wrong air flow rate through the humidifier, wrong air flow rate through the chamber, humidifier temperature too low, chamber temperature too hot, pressure imbalance between IRGA cells, no wind in chamber, moist ‘dry air’, and data set not valid. Of these conditions the only one that is dealt with automatically is water condensation.

• **Test for error conditions**
  1. Check that the data set is valid (not marked as not valid because of problems during data acquisition or calculation).
  2. Compare dew-point at \( \chi_a^w \) with the temperature of the chamber wall and room temperature, using a safety margin of 2.5 mmol mol\(^{-1}\).
  3. Compare measured flow rates against those requested.
  4. Check that water temperature in the humidifier is close to the set point.
5. Check that chamber air temperature is not too high.
6. Check that air temperature inside the measuring rack is not too high.
7. Check that there is no pressure difference between the cells of the IRGA.
8. Infer whether the internal chamber fan is working or not by comparing the air temperature and wall temperature in the chamber.
9. Check that ‘dry air’ is not moist.

When danger of, or actual, condensation is detected the following algorithm is used to recover:

1. Select the lowest temperature from room or chamber wall temperature.
2. Use this temperature to find out whether there is a danger of water condensation, or that condensation has already occurred.
3. If there has been condensation then set the air flow rate through the humidifier to zero to get very dry air to remove liquid water from the system, otherwise decrease $\chi_w$ just enough to get a value of $\chi_w$ slightly less than that which would trigger a ‘danger of condensation’ state.

2.2.2 Implementation

The software includes two programs: \texttt{runexper} and \texttt{lookdata}. The first one is used to control the system, and acquire and process data in ‘real time’. The second one can be used to reprocess and look at data previously saved to a disk file. There are two programs because I decided that the best approach was to save the raw data instead of the processed data, in contrast to what was done in the program written by A. P. Sandford. The rationale is that doing so adds both flexibility and safety, without increasing the store space required. It is safer because it allows the errors in the calculations to be fixed. It is more flexible because it allows, in some experiments, the measurement of $g_w$ and leaf area after taking gas-exchange data. It also makes it possible to measure the sensitivity of the results to errors in the measurement of $g_w$ and leaf area, or in the calibrations.

The programs were written in Modula-2 (Logitech Modula-2 Development System, Version 3.0; Logitech Inc., Fremont, California, U.S.A.). This was done because it is a language closely related to Pascal which was used in the original program written by A. P. Sandford. The program was totally rewritten, and redesigned. The old program was badly structured and had too many global variables. The data variables in the new programs are structured according to the data flow, and the procedures are grouped in modules according to the position of their use in the data flow and their degree of independence from the hardware configuration of the system. All the code that depends
on the actual data-logger is in a few modules and is not spread all over the program listing. The same is true for those parts that depend on the gas-exchange system being of an open design. Output is handled independently from data processing. Having followed a modular design, all code common to both programs is not duplicated. In this way both programs are simultaneously updated when changes are introduced.

A very simple change that had a very important effect on the quality of the data obtained from the system was to use in the calculations the median of three consecutive raw data measurements, or of five measurements in the case of calibrations. This greatly reduced random errors, and, as data transmission rate between the computer and datalogger was increased, it had only a small effect on the total time required to get a set of measured values.

The listings were written using meaningful variable names, and further comments have been added when the text of the program was not clear enough. Each file has a header where a record of its history is kept. When several versions of the same module exist, they coexist in a single file as comments. These modules can be interconverted between different versions by means of the program M2VERS. Some versions are useful for debugging or testing, others reflect the changes necessary for different hardware, such as the presence or absence of a water trap before the IRGA.

The source code of the programs is provided in the diskette attached to this thesis. Table 2.1 gives a brief description of the contents of the modules specific to this program. The listings of these modules add up to more than 5500 lines of text. Several other ‘library’ modules were written or adapted to be used in these programs, but their use is not limited to them.

### 2.3 Performance

Having described the hardware and software of the gas-exchange system I am now going to give some data on its performance. The steady-state performance of the system is shown in Fig. 2.6. These data were measured with an empty gas-exchange chamber with the system running without intervention of an operator and they show the stability of $\chi_w^a$ and $\chi_c^a$. $\chi_c^a$ displayed oscillations with an amplitude of less than 1 $\mu$mol mol$^{-1}$, while $\chi_w^a$ drifted approximately 0.2 mmol mol$^{-1}$ in 5 h.

The dynamic response of the gas-exchange system was measured by following the time course of the IRGA output signal after a step change in concentration. As the volume of air in the reference and sample branches of the system’s air circuit is different, a step change in $\chi_{\text{in}}^c$ reaches the IRGA cells out of phase, and produces a huge swing in the differential output. In a test done by changing $\chi_{\text{in}}^c$ from 350 to 600 $\mu$mol mol$^{-1}$ a
Table 2.1. Partial list of modules from the program used to control the gas-exchange system (runexper), and from that used to reprocess data (lookdata).

<table>
<thead>
<tr>
<th>MODULE</th>
<th>Description</th>
<th>Versions</th>
</tr>
</thead>
<tbody>
<tr>
<td>RunExperiment</td>
<td>Main (program) module of program used to control the system during an experiment and to acquire data. Main time loop and menu.</td>
<td>—</td>
</tr>
<tr>
<td>LookData</td>
<td>Main (program) module of program used to reprocess raw data saved during an experiment.</td>
<td>—</td>
</tr>
<tr>
<td>DataTypes</td>
<td>Declarations of data TYPES used in more than one module.</td>
<td>—</td>
</tr>
<tr>
<td>Logger</td>
<td>Communications with the data-logger.</td>
<td>Normal, Testing</td>
</tr>
<tr>
<td>DataAcquisition</td>
<td>Measurement of raw data.</td>
<td>NoTrap, WaterTrap</td>
</tr>
<tr>
<td>DataProcessing</td>
<td>Computation of system state from raw data and calibration data.</td>
<td>NoTrap, WaterTrap</td>
</tr>
<tr>
<td>Calibration</td>
<td>Calibration of IRGA and dew-point meters.</td>
<td>NoTrap, WaterTrap</td>
</tr>
<tr>
<td>StandardizeIRGA</td>
<td>Correction of differential IRGA calibrations for differences in the background CO₂ concentration.</td>
<td>—</td>
</tr>
<tr>
<td>PressureBalancing</td>
<td>Rebalancing of air flow rate through IRGA cells to keep a null pressure difference between them.</td>
<td>—</td>
</tr>
<tr>
<td>SystemControl</td>
<td>Control of mass-flow controllers, valves, and pumps.</td>
<td>Normal, Testing</td>
</tr>
<tr>
<td>RefControl</td>
<td>Control of molar fractions of the air going into the chamber.</td>
<td>—</td>
</tr>
<tr>
<td>ExpControl</td>
<td>Control of derived variables.</td>
<td>—</td>
</tr>
<tr>
<td>Check</td>
<td>Test system state data for error conditions, and provide automatic recovery for some of them.</td>
<td>Run, Look</td>
</tr>
<tr>
<td>ErrorHandler</td>
<td>Display error messages and warnings, and emergency shut-down.</td>
<td>Run, Look</td>
</tr>
<tr>
<td>DataIO</td>
<td>Input and output of raw data to/from disk files.</td>
<td>—</td>
</tr>
<tr>
<td>CalibIO</td>
<td>Input and output of calibration data to/from disk files.</td>
<td>—</td>
</tr>
<tr>
<td>Screens</td>
<td>Data output to the CRT screen and printer.</td>
<td>—</td>
</tr>
</tbody>
</table>
Figure 2.6. Steady-state performance of the gas-exchange system running without intervention of an operator and with an empty leaf chamber. Time course of (a) water vapour molar fraction ($\chi^w_a$), and (b) CO$_2$ molar fraction ($\chi^c_a$).
new steady-state was reached 5 min after a step change in CO₂ concentration, and the
lag before any change was observed in the output was 23 s \( (J_{\text{air}} = 2 \text{ mmol s}^{-1}, J_{\text{tot}} = 4 \text{ mmol s}^{-1}) \). The raw output also displayed a slight change in the differential offset of the IRGA caused by the change in background CO₂ concentration.

The errors in the measurements of \( A \) and \( E \) depend on the area of the leaf or leaves enclosed in the chamber, and on the flow rate of air through the chamber \( (J_{\text{air}}) \). The short term noise, measured as the spread of 5 consecutive readings taken within 5 min, for a leaf with an area of 50 cm² and \( J_{\text{air}} = 2 \text{ mmol s}^{-1} \) was for \( A \) approximately 0.2 µmol m\(^{-2}\) s\(^{-1}\), and for \( E \) approximately 0.01 mmol m\(^{-2}\) s\(^{-1}\) \( (T_{i}=20 \, ^{\circ}\text{C}, D_{w}^{+} \approx 8 \text{ mmol mol}^{-1}, \chi_{c}^{e} \approx 350 \mu\text{mol mol}^{-1}) \). This short term random noise can be easily removed from the data by smoothing it using the median of 3 or 5 measurements instead of individual data points. In contrast, measurement errors caused by errors in the calibrations of the IRGA and dew-point meters cannot be eliminated in this way.

The coefficients of variability, from a set of 19 IRGA calibrations done during a single day and under constant background CO₂ concentrations, were 0.64 % for the sensitivity or gain, and 1.18 % for the offset. But a closer look at the data as displayed in the box diagrams in Fig. 2.7 shows that errors are not normally distributed — there were outliers, and the distribution for the sensitivity was skewed. This variability includes the drift of the IRGA throughout a day and measurement errors during calibration.

When altering \( \chi_{c}^{e} \), whether we have to recalibrate the IRGA or not depends on how well the corrections incorporated in the program are able to compensate for the effects of the background CO₂ molar fraction. For a set of 27 calibrations measured at different values of \( \chi_{c}^{e} \), the standardized differential gain of the IRGA \( (\tilde{\beta}) \) was insensitive to \( \chi_{c}^{e} \), and the standardized differential offset \( (\tilde{s}_{\text{nul}}) \) decreased less than 2 % of the full-scale signal for a change in \( \chi_{c}^{e} \) of 600 µmol mol\(^{-1}\) (Fig. 2.8). These results were obtained using values measured more than four years earlier for the coefficients in Equation 2.9, and for that in Equation 2.7 a value measured 18 months earlier. The decrease of \( \tilde{s}_{\text{nul}} \) with \( \chi_{c}^{e} \) can be corrected by updating the value of the constant used in the calculations. However, the error observed represents an error of only 0.33 µmol mol\(^{-1}\) per 100 µmol mol\(^{-1}\) of change in \( \chi_{c}^{e} \).

During the course of the experiments, the IRGA was recalibrated when \( \chi_{c}^{e} \) was altered by more than 50 µmol mol\(^{-1}\) because, in this case, the standardization procedure would not be able to completely correct for the sensitivity of the IRGA to \( \chi_{c}^{e} \). The IRGA was also recalibrated whenever the room temperature changed by more than 5 °C. The dew-point meters were recalibrated (offset only) when \( T_{\text{dew}} \) changed by more than 5 °C. If \( \chi_{c}^{e} \) and \( \chi_{c}^{w} \) were not altered significantly, calibrations were repeated at least every six hours to compensate for the usually very small drift of the IRGA and
Figure 2.7. Box diagrams for the IRGA calibration data measured during a single day, and under the same reference CO$_2$ molar fraction ($\chi_{in}^c=365$ µmol mol$^{-1}$). (a) standardized differential offset ($\tilde{s}_{null}$), and (b) standardized differential gain ($\tilde{\beta}$) of the IRGA. In a box diagram the crossbar at the center of the box is the median, the length of the box is the fourth spread, the lines extending from the end of the box give the tail length, and * or o indicate the location of outliers (see Emerson & Strenio, 1983).

dew-point meters.

The performance of the gas-exchange system is satisfactory but it could be further improved. The dynamic response of the system to changes in the molar fractions in the reference air could be improved by putting a flask in the reference branch of the air circuit to balance the volume of the chamber, and by keeping a fixed ratio between the air flow rates through the chamber and this flask. This would make differential measurements insensitive to step changes in $\chi_{in}^c$ and $\chi_{in}^w$ which would allow much easier control of the system, and with some limitations would also allow the measurement of the dynamics of plant response to these changes. This change would also improve the rejection of noise in $\chi_{in}^c$ by making the whole gas-exchange system truly differential.

Another significant improvement would be to have an environmental chamber to control the conditions ($I, \chi_{in}^w, \chi_{in}^c$, and $T_a$) in which the whole plant is kept independently of those in the room were the measuring instruments are located. This would make it possible to use extreme environmental conditions without affecting the instruments, and what is more important, to keep the whole plant in a homogeneous and known environment.
Figure 2.8. Sensitivity of the standardized IRGA calibration to the background CO$_2$ concentration. Plots of (a) the differential offset ($\tilde{s}_{\text{null}}$), and (b) the differential gain or sensitivity ($\tilde{\beta}$) of the IRGA vs. the reference molar fraction of CO$_2$ ($\chi_{\text{in}}^c$). Values shown were standardized to $\chi_{\text{in}}^c = 350$ µmol mol$^{-1}$. Data from 27 calibrations done on three different days (indicated with different symbols).
Chapter 3

Plants

3.1 Taxonomy and plant culture

The experiments were carried out on ivy \( \textit{Hedera helix} \) subsp. \textit{canariensis} (Willd.) Coutinho plants. Ivy has two different phases: adult and juvenile. Only the juvenile phase was used. Ivy is a common garden plant with numerous horticultural forms, both with normal and variegated leaves. Plants from a non-variegated clone were used in the gas-exchange experiments. Plants were identified using Rose (1980) as a guide, but for the Latin name Tutin \emph{et al.} (1968) was followed. \textit{Hedera helix} has a wide distribution—from Norway to Southern Europe and N. Iran (Tutin \emph{et al.}, 1968). Grime \emph{et al.} (1988) describe it as a long-lived evergreen woody species, most characteristic of shaded habitats, and commonly occurring in woodlands and hedgerows, either carpeting the ground or growing vertically up the trunks of trees. They classify ivy, according to its established strategy, as a stress-tolerant competitor. The subspecies used has large flat leaves with long petioles which makes it suitable for gas exchange experiments. Leaves are long lived (i.e. several years).

The plants were grown in a heated greenhouse from cuttings collected in the gardens of the University of Edinburgh at King’s Buildings, Edinburgh, U.K. They were grown in 12, 16 or 18 cm diameter plastic pots filled with a peat-perlite-vermiculite mix. Plants were repotted at least once a year, and when they became too big to handle (branches longer than 1.5m) they were cut back. Before the beginning of the experiments the plants were transferred to growth chambers. At this time they were fertilized with slow release granules (Fisons plc, Ipswich, U.K.; N=14 %, P=6.1 %, and K=11.6 %, w/w) at 2.5 g per pot. Afterwards they were fertilized weekly with liquid fertilizer (Liquinure, Fisons plc, Ipswich, U.K.; N=8 %, P=1.7 %, K=11.6 %, w/w, and micronutrients) at 0.5 cm\(^3\) per pot. Further details about plant growth conditions are given in later
3.2 Microscopic description of the leaves

Leaves similar to those used in the experiments were observed microscopically. Both the surface of the leaves and their internal structure were observed. In the first case the samples were gold sputtered, and then observed at 3 kv with a S-90 B scanning electron microscope (SEM) (Cambridge Instruments Ltd., Cambridge, U.K.). In the second case the samples were cryofixed: they were first glued to stubs with an embedding medium (Tissue Tek II O.C.T. compound, Emscope Laboratories, Ashford, Kent, U.K.), they were then frozen in liquid N\textsubscript{2}, once frozen the specimens were fractured under vacuum, gold coated in an argon atmosphere, and finally transferred under vacuum to the SEM. The fixation procedure was carried out in a cryo-preparation system (Emscope SP2000, Emscope Laboratories, Ashford, Kent, U.K.), and the observations done with a SEM fitted with a cold stage (Stereoscan 250, Cambridge Instruments Ltd., Cambridge, U.K.). In both cases photomicrographs were taken on T-Max 100 film (Kodak Limited, Hemel Hempstead, U.K.). Additional observations of imprints of the leaf surface were done with an optical microscope. The imprints were made with Loctite super glue 3 (Loctite UK, Welwyn Garden City, Herts., U.K.) using the method of Wilson \textit{et al.} (1981).

The surface of the leaves, as seen with the SEM, was smooth, with stomata in the abaxial epidermis, and the location of the anticlinal walls of epidermal cells just visible in the adaxial epidermis (Figs. 3.1 and 3.2). Imprints, observed through a light microscope, confirmed these results, but the image had a shallower depth of focus than with the SEM. Stomatal frequency in Fig. 3.2 is 188 stomata mm\textsuperscript{-2}, which is similar to the 150 stomata mm\textsuperscript{-2} observed by Aphalo & Sánchez (1986) in this species. The length of the stomata was approximately 30 \mu m, and their width 28 \mu m.

A thick cuticle covers the outer walls of the epidermis and a ridge borders the antechamber of the stomatal pore (Figs. 3.3 & 3.4). In the lengthwise fracture of the guard cell the outer walls are very thick, but this could be because the fracture is close to the anticlinal walls of the guard cell. The walls of the guard cells are lignified (Ziegler, 1987), something that is not frequent in angiosperms.

Ivy leaves have a clearly defined palisade parenchyma adjacent to the adaxial epidermis (Fig. 3.5). In the section shown in this figure there were two layers of well differentiated palisade cells, and a third layer with less elongated cells. Other leaves, used as replicates, had either two or three layers of palisade parenchyma. The spongy parenchyma had a compact honeycomb structure (Fig. 3.5). In ivy the thickness of
the palisade parenchyma depends on the quantum flux density during growth (Bauer & Thöni, 1988) and on the growth phase (Bauer & Bauer, 1980).
Figure 3.1. SEM photograph of the surface of the adaxial epidermis of an ivy leaf. The arrowhead points to one of the shallow groves on the surface, that show the position of anticlinal walls underneath.
Figure 3.2. SEM photograph of the surface of the abaxial epidermis of an ivy leaf.
Figure 3.3. SEM photograph of the abaxial epidermis of an ivy leaf showing one stoma. The throat of the stoma is indicated by an arrowhead.
Figure 3.4. Lengthwise transverse fracture of an ivy stoma. Cy: cytoplasm, W: cell wall, Cu: cuticle, r: ridge.
Figure 3.5. Transverse fracture of an ivy leaf. The numbers indicate the cell layers in the palisade parenchyma, and the arrowhead with an $s$ indicates a stoma.
3.3 Optical characteristics of the leaves

The optical properties of ivy leaves similar to those used in the gas exchange experiments were studied by means of a spectroradiometer fitted with an integrating sphere (Optical spectrum analyser model 6800; with a 6100 monochromator, with a 0.9 nm slit installed; a 6118 photo-tube detector; and a 6190 integrating sphere. Monolight Instruments Ltd., Weybridge, Surrey, U.K.). Transmittance (normal/diffuse)\(^1\) and reflectance (normal/diffuse) were measured for both sides of three replicate green leaves and one white leaf, absorptance was calculated from these measurements.

A typical spectrum showing the proportions of the incident radiation that are absorbed, reflected, and transmitted is given in Fig. 3.6, and the values integrated over PAR are given in Table 3.1. The adaxial surface had very low values of reflectance and transmittance in the photosynthetically active part of the spectrum, even in the green region —to the eye this surface of the leaves looked almost black. PAR absorptance for this surface was 95 % (Table 3.1). In the far-red and near infrared region (\(\lambda \geq 750\) nm) the transmittance and reflectance each increased to nearly 50 %. The transmittance, and especially the reflectance, were higher for the abaxial surface, with a shallow peak of reflectance in the green —to the eye this surface looked green. PAR absorptance of the abaxial surface was 87 % which is 8 % lower than that of the adaxial surface. In the far-red (\(\lambda = 700–750\) nm) region the increase in reflectance of the abaxial surface started at a shorter wavelength than for the adaxial surface. Except for the very low reflectance and transmittance in the green region of the spectrum for the adaxial surface, and the lower transmittance over the whole visible part of the spectrum in both surfaces, these spectra did not differ much from those reported for soybean (Woolley, 1971, Figs. 14 and 16).

The different reflectance of the abaxial and adaxial epidermes can be explained by the structure of the underlying mesophyll tissue. Ivy leaves are dorsiventral with clearly differentiated palisade and spongy regions (Fig. 3.5), and it has been observed that spongy mesophyll scatters light more effectively than the palisade mesophyll (Knapp \textit{et al.}, 1988; Vogelman \textit{et al.}, 1988). That the main effect is internal scattering at the air-water interface can be easily demonstrated by infiltrating albino portions of variegated leaves with water: they become almost clear. The transmittance and reflectance of these white parts are nearly 50 % for most of the visible region of the spectrum (Fig. 3.7).

\(^1\)As defined in Commission Internationale de L’Eclairage (1982).
Figure 3.6. Transmittance and reflectance spectra of a typical green ivy leaf. (a) Adaxial surface, (b) abaxial surface. T: transmittance, R: reflectance, A: absorbance.
Figure 3.7. Transmittance and reflectance spectra of a white ivy leaf. (a) Adaxial surface, (b) abaxial surface. T: transmittance, R: reflectance, A: absorptance.
Table 3.1. Absorptance, reflectance, and transmittance of photosynthetically active radiation (λ =400–700 nm) for the abaxial and adaxial surfaces of ivy leaves. Values are means of measurements from three green leaves, with the standard error of the mean in brackets, and data from one white leaf.

<table>
<thead>
<tr>
<th></th>
<th>green leaves</th>
<th>white leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>abaxial</td>
<td>adaxial</td>
</tr>
<tr>
<td>absorptance (%)</td>
<td>87.3 (0.10)</td>
<td>94.7 (0.17)</td>
</tr>
<tr>
<td>reflectance (%)</td>
<td>12.3 (0.06)</td>
<td>4.9 (0.17)</td>
</tr>
<tr>
<td>transmittance (%)</td>
<td>0.4 (0.11)</td>
<td>0.5 (0.22)</td>
</tr>
</tbody>
</table>

3.4 Test of assumptions concerning $g^w_s$ and $g^w_c$

An upper limit to $g^w_c$ was obtained by measuring the water exchange of a detached leaf kept in darkness. The same gas-exchange system was used as in other experiments\(^2\), but to increase the sensitivity a very low air flow rate was used (0.5 mmol s\(^{-1}\)). From Equation 1.2 it follows that if $g^w_s \approx 0$ then $g^w_l \approx g^w_c$. The lowest value of $g^w_l$ observed during 6 h in darkness was assumed to be equal to $g^w_c$. In leaves similar to those used in the stomatal conductance experiments, $g^w_c$, for both epidermes in parallel, and expressed per unit of projected leaf area, was less than 2 mmol m\(^{-2}\) s\(^{-1}\) (n=3; $T_i=20$ °C, $D^w_s \approx 10$ mmol mol\(^{-1}\)). A $g^w_c$ of this order of magnitude is common in xerophytes (Weyers & Meidner, 1990, Table 2.3). The $g^w_c$ observed in *Hedera helix* was so low that in discussions elsewhere in this thesis $g^w_l$ measurements were considered equivalent to $g^w_s$ values.

Another important issue is to prevent circadian rhythms from affecting the results when other variables are under study. The approach taken was to measure $g^w_s$ under two different constant sets of conditions throughout a day: darkness, and non-saturating light. An example of the results under light is given in Figure 3.8. No stomatal opening was observed in darkness, even during the daytime. From the results obtained under illumination it was assumed that the safe working period was from 2.5 h after the start of the normal photoperiod to 1 h before its end. Measurements in all other experiments were restricted to this period.

A transient oscillation of $g^w_s$ was observed at the start of the day (Fig. 3.8). This kind of transient response has been described more frequently for grasses than non-grasses (e.g. Johnsson *et al.*, 1976). In the time course of $g^w_s$ in Fig. 3.8 environmental

\(^2\)The gas-exchange system is described in Chapter 2.
Figure 3.8. Leaf conductance of an ivy leaf throughout a day under constant conditions. Typical response under 340 μmol m$^{-2}$ s$^{-1}$ of white light. $D_w^*=10$ mmol mol$^{-1}$, $T_l=20$ °C, $\chi^c_a=350$ μmol mol$^{-1}$. The photoperiod was from 9:00 to 21:00.

conditions were not completely stable in the gas-exchange chamber between 9:00 and 11:00 because $E$ and $A$ were changing very fast, and this instability could have reinforced this effect. However, under background red illumination this fast transient has been shown to be a blue light response (Karlsson & Assmann, 1990). *Hedera helix* is the only known dicotyledon capable of fast stomatal opening$^3$ (Karlsson & Assmann, 1990), a type of response previously thought to be restricted to plants with grass-like stomata (Johnsson *et al.*, 1976).

$^3$As defined by the rise time and the delay time, and the ratio of their values under red and blue light (see Johnsson *et al.*, 1976).
Chapter 4

Stomatal responses to light

4.1 Introduction

Stomatal responses to light are complex: several photoreceptors and transduction chains are involved. The responses are called either direct or indirect according to the location of the light receptor. In direct responses light is sensed in the guard cells, in indirect ones in other cells (i.e. in the mesophyll). Direct responses have been postulated to take place through (1) an unidentified, blue absorbing photoreceptor, (2) chlorophyll in guard cell chloroplasts, and (3) phytochrome(?); the indirect response takes place mainly through mesophyll chlorophyll. Light absorbed in the mesophyll drives photosynthesis which, by altering the internal environment of the leaf, indirectly affects stomata. The three different receptors involved in direct responses to light differ in their spectral sensitivity. They also differ in their sensitivity to photon flux density. The blue light response is direct, that to PAR can be direct and/or indirect. It will not be fruitful to discuss further the poorly understood response through phytochrome.

Experimenters have used various procedures to distinguish between the different responses: monochromatic light (e.g. Johnsson et al., 1976; Aphalo & Sánchez, 1986), variegated chimeras (e.g. Virgin, 1957; Aphalo & Sánchez, 1986) or chlorophyll deficient mutants (e.g. Virgin, 1957; Skaar & Johnsson, 1980), species with uncommon characteristics (e.g. Nelson & Mayo, 1975) and chemicals affecting chlorophyll content (e.g. Karlsson et al., 1983). To separate direct from indirect light responses in whole leaves both chimeras (e.g. Aphalo & Sánchez, 1986), and leaf inversion experiments (e.g. Turner, 1970; Raschke et al., 1978; Aphalo & Sánchez, 1986) have been used.

Stomata are usually more sensitive to blue than to red light (Kuiper, 1964; Sharkey & Raschke, 1981a). However, not all species share the same high sensitivity to blue light: in Fuchsia magellanica $g_s$ is equally sensitive to blue and red light (Aphalo et al.,
while in *Hedera helix* $g_s^w$ is nearly 100 times more sensitive to blue than to red light (Aphalo & Sánchez, 1986), and in *Pinus sylvestris* it is approximately 10 times more sensitive to blue than to red light (Morison & Jarvis, 1983a). In some species responses to blue light are also faster (Johnsson *et al*., 1976).

The role proposed for the blue light-dependent system is to provide the plant with a means for opening stomata in the early morning and to respond quickly to sunflecks (Meidner & Mansfield, 1968; Zeiger *et al*., 1981; Aphalo & Sánchez, 1986; Zeiger, 1990). It has also been proposed that by modulating the sensitivity of this photosystem, plants tune stomatal behaviour to prevailing environmental conditions such as drought stress (Aphalo & Sánchez, 1986). It is still not clear which are the roles fulfilled by direct and indirect responses to PAR. Some species, such as *Petunia axillaris* and *Petunia hybrida*, seem to rely on endogenous rhythms regulating aperture in darkness and modulating sensitivity to light, for early morning aperture and midday closure (P. J. Aphalo, unpublished). In many species the speed with which stomata open in response to light depends on the phase of the circadian rhythm (Weyers & Meidner, 1990, give examples and primary references). It has also been shown that in *Avena sativa* the maximum amplitude of rapid (blue light-dependent) and slow (PAR-dependent) stomatal responses occur during opposite phases of the circadian rhythm (Brogårdh & Johnsson, 1975). In *Hedera helix* the effect of endogenous rhythms on $g_s^w$ is very small during the normal photoperiod (see Section 3.4), and response to blue light is rapid (Karlsson & Assmann, 1990).

Scarth (1932) was the first to suggest that light-induced stomatal opening was caused by photosynthetic removal of CO$_2$ from the intercellular spaces. Stomata are sensitive to CO$_2$ in light and darkness, and in whole leaves and epidermal strips (Heath, 1950; Heath & Milthorpe, 1950; Meidner & Mansfield, 1968; Morison, 1987). In whole leaves stomata are sensitive to $\chi_f$ (Mott, 1988). As $A$ is dependent on, but also affects, $\chi_f$, a feedback loop is generated between both processes. In some species or conditions stomata can be insensitive to CO$_2$ (Morison, 1987), and in many situations light responses independent of $\chi_f$ make a larger contribution to the total response to light than those dependent on $\chi_f$ (Dubbe *et al*., 1978; Sharkey & Raschke, 1981b).

Aphalo & Sánchez (1986) have suggested, based on the results of leaf inversion experiments, that in *Hedera helix* the blue light-dependent response of $g_s^w$ is direct, and the PAR-dependent one is indirect. This is in contrast to what Sharkey & Raschke (1981a) observed in *Xanthium strumarium*, a species in which both blue light and PAR-dependent responses were found to be mainly direct.

When $I$ is changed $A$ and $g_s^w$ are usually linearly correlated, if $\chi_\delta$ is kept constant
(e.g. Wong et al., 1979; Louwerse, 1980; Ramos & Hall, 1982). However this relationship cannot always be explained by the response of $g_w^w$ to $\chi_i^c$ (Wong et al., 1979). Even though this correlation can be experimentally broken, Wong et al. (1979) have proposed that it could depend on metabolites other than CO$_2$ conveying to the stomata information about the rate of photosynthesis in the mesophyll. Cowan et al. (1982) proposed that abscisic acid coordinates $A$ and $g_w^w$ even in responses to light. Although it has been shown that these hypotheses are not the main basis for this correlation, they could in some species be part of a more complex mechanism, and so need to be further investigated.

Two different experiments were done with the objective of elucidating the mechanism behind the coordination of changes in $A$ and $g_w^w$. In the first experiment, the responses of $A$ and $g_w^w$ to $I$ were measured under constant $\chi_i^c$ to describe the correlation between the effects of $I$ on $A$ and $g_w^w$. In the second experiment, irradiation with light of different wavelengths, and of either the abaxial or adaxial epidermis, was used to alter $A$ and $g_w^w$. Leaf inversion increases the $I$ received by the guard cells, and also affects the distribution of light within the leaf mesophyll. By keeping $\chi_i^c$ constant any effect of CO$_2$ on either $A$ or $g_w^w$ was prevented. This was intended to make any CO$_2$-independent correlation between $A$ and $g_w^w$ observable.

### 4.2 Materials and methods

#### 4.2.1 Plant material

Ivy plants were grown in a heated greenhouse. Three different sets of plants were used, two in two replicates of one experiment, and the third one in a second experiment. The plants were grown in 12 or 18 cm diameter plastic pots filled with a peat-perlite-vermiculite mix, watered every other day, and fertilized weekly (See Chapter 3 for details).

One set of plants —henceforth called set A— was kept for 4 months in a growth chamber at 20 °C, $h=30$–60 %, and a photoperiod of 12 h at 400 $\mu$mol m$^{-2}$ s$^{-1}$ at leaf level from metal halide lamps (Wotan ‘Power Star’ HQI-R 250 W/NDL, Wotan Lamps Ltd., London).

The second set of plants —set B— was kept in the same chamber and under similar conditions for 3 months.

The third set of plants —set C— was kept for more than 26 d in a growth room at 20 °C, $h=50$–70 %, and a photoperiod of 12 h at 500 $\mu$mol m$^{-2}$ s$^{-1}$ at leaf level from metal halide lamps (Kolorarc 400W MBIF/BU, Thorn Lighting Ltd., London, U.K.).
4.2.2 Gas exchange measurements

The computer-controlled gas-exchange system described in Chapter 2 was used. Boundary layer conductance was measured by means of leaf replicas of Whatman No. 3 filter paper covered with aluminium foil on the upper or lower side, according to the position of the leaf, and wetted with distilled water. $g^w_b$ was 650-700 mmol m$^{-2}$ s$^{-1}$ for the leaves used, and not affected by the position of the evaporating surface. The temperature of leaves and leaf replicas was measured with thermojunctions in contact with their shaded face. Steady-state measurements were made, and no data taken during the first hour after a change in conditions were used. However, the data were checked to see whether a steady state had been reached and this period was extended if necessary. The leaves to be measured the next day were placed overnight in the gas-exchange chamber in darkness with $\chi^c_s=350 \mu$mol mol$^{-1}$, $D^w_s=10$ mmol mol$^{-1}$, and $T_l=20$ $^\circ$C. Attached non-senescent fully expanded leaves were used in the experiments.

4.2.3 Experiments

The first experiment consisted of measuring the response of $g^w_s$, $A$, and $\chi^i_c$ to $I$ of white light under constant conditions of $\chi^c_s=350 \mu$mol mol$^{-1}$, $D^w_s=10$ mmol mol$^{-1}$, and $T_l=20$ $^\circ$C. This experiment was done using three plants from set A and was then replicated with another two plants from set B.

The second experiment consisted of measuring $g^w_s$ and $A$ under constant conditions of $\chi^c_i=220 \mu$mol mol$^{-1}$ and $I=500 \mu$mol m$^{-2}$ s$^{-1}$ of white light, $I=18 \mu$mol m$^{-2}$ s$^{-1}$ of blue light, or $I=120 \mu$mol m$^{-2}$ s$^{-1}$ of red light, in leaves in an inverted position as compared to the same leaves in normal position. The photon flux densities of red and blue light were selected so as to give approximately the same $g^w_s$. The plants were kept in darkness for 1 h after changing the position of the leaf only when blue or red light was used. These treatments were applied in a random order. Three plants from set C were used.

4.3 Results

4.3.1 Responses of $g^w_s$ and $A$ to quantum flux density

In most of the plants from both sets, the response of $g^w_s$ to $I$ did not saturate in the range of values tested (Fig. 4.1). The threshold for stomatal opening in white light was approximately 2 $\mu$mol m$^{-2}$ s$^{-1}$ in set A, and 7 $\mu$mol m$^{-2}$ s$^{-1}$ in set B. CO$_2$ flux density saturated at a lower $I$ than stomatal aperture, and light compensation occurred at 5 $\mu$mol m$^{-2}$ s$^{-1}$ (Fig. 4.2). In both sets of plants the initial slope was 0.05 mol of CO$_2$
per mol of photons. $\chi_i^c$ showed a minimum at 300 $\mu$mol m$^{-2}$s$^{-1}$ in plants from set A, and at 200 $\mu$mol m$^{-2}$s$^{-1}$ in those from set B (Fig. 4.3). $g_w^w$, $A$ and $\chi_i^c$ were higher in plants from set A than in plants from set B (Figs. 4.1, 4.2 & 4.3).

If $A$ is plotted vs. $g_w^w$ a good linear fit is achieved, except for the data measured at very low or very high irradiances (Fig. 4.4, Table 4.1). The slopes ($P=0.047$) and intercepts ($P=0.073$) were slightly different in the two sets of plants. When $g_w^w$ is plotted vs. $\chi_i^c$ the relationship is not as clear as with light, especially for data from set B (Fig. 4.5), and the relationship is not monotonic — i.e. there is more than one value of $g_w^w$ for a given $\chi_i^c$.

### 4.3.2 Leaf inversion experiment

The effects of leaf inversion on $g_w^w$ and $A$ were very different. Under 500 $\mu$mol mol$^{-1}$ of white light $g_w^w$ did not change, and $A$ decreased to 0.58 of its original value (Table 4.2). The effect on $A$ was readily reversible (data not shown). Increasing $I$ in inverted leaves under these conditions did not alter the steady-state $g_w^w$ even though $A$ increased somewhat; decreasing $\chi_i^c$ decreased $A$ and increased $g_w^w$ (Fig. 4.6). Under non-saturating red light $g_w^w$ more than doubled in response to leaf inversion, while $A$ decreased to 0.58 of that before inversion (Table 4.2). Under low $I$ of blue light $g_w^w$ doubled with leaf inversion, while $A$ remained almost unchanged and near zero (Table 4.2). As
Figure 4.2. CO₂ flux density vs. photon flux density of white light. \( D_w^s = 10 \text{ mmol mol}^{-1} \), \( \chi_c^s = 350 \text{ µmol mol}^{-1} \), \( T_i = 20 \degree \text{C} \). (a) Three plants from set A, (b) two plants from set B. Symbols indicate data from different plants.

Figure 4.3. Intercellular CO₂ concentration vs. photon flux density of white light. \( D_w^s = 10 \text{ mmol mol}^{-1} \), \( \chi_c^s = 350 \text{ µmol mol}^{-1} \), \( T_i = 20 \degree \text{C} \). Data from the same experiment as that in Fig. 4.2. (a) Three plants from set A, (b) two plants from set B. Symbols indicate data from different plants.
Figure 4.4. CO$_2$ flux density vs. stomatal conductance, measured under changing photon flux densities. Same data as in Figs. 4.2 & 4.1. $D_w^w=10$ mmol mol$^{-1}$, $\chi_s^c=350$ µmol mol$^{-1}$, $T_i=20$ °C. (a) Three plants from set A, (b) two plants from set B. Symbols indicate data from different plants.

Table 4.1. Regression of CO$_2$ flux density on stomatal conductance in leaves of *Hedera helix*. $I=35–500$ µmol m$^{-2}$s$^{-1}$ (white light), $D_w^w=10$ mmol mol$^{-1}$, $\chi_s^c=350$ µmol mol$^{-1}$, $T_i=20$ °C. A subset of the data in Fig. 4.4 was used in the calculations, and regression lines were fitted to data from single leaves for a restricted range of $I$.

<table>
<thead>
<tr>
<th>Plant</th>
<th>intercept (µmol m$^{-2}$ s$^{-1}$)</th>
<th>slope (µmol mol$^{-1}$)</th>
<th>$R^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>-2.59</td>
<td>89</td>
<td>0.998</td>
<td>4</td>
</tr>
<tr>
<td>A2</td>
<td>-1.47</td>
<td>83</td>
<td>0.999</td>
<td>4</td>
</tr>
<tr>
<td>A3</td>
<td>-1.28</td>
<td>83</td>
<td>0.986</td>
<td>4</td>
</tr>
<tr>
<td>B1</td>
<td>0.43</td>
<td>97</td>
<td>0.974</td>
<td>10</td>
</tr>
<tr>
<td>B2</td>
<td>-0.53</td>
<td>111</td>
<td>0.997</td>
<td>8</td>
</tr>
</tbody>
</table>
previously stated, the $I$ values of blue and red light were selected so that $g_w^w$ was similar, and this resulted in very different values of $A$. Leaf inversion had a significant effect on $\chi_c^i/\chi_c^s$ under both white and red light (Table 4.2).

4.4 Discussion

An important, and unsolved, question in plant physiology is: What is the mechanism behind the correlation between $A$ and $g_w^w$? This correlation has been observed in several experiments when $A$ and $g_w^w$ changed in response to different variables including light (Wong et al., 1979, 1985a, 1985b, 1985c; Louwerse, 1980; Ramos & Hall, 1982). It has also been observed that, in the case of responses to light, this correlation can be broken experimentally (Jarvis & Morison, 1981; Aphalo & Sánchez, 1986). The competing hypotheses to explain this correlation are (1) feedback through $\chi_c^i$, (2) feedback through another metabolite of $A$, and (3) parallel, but independent, responses to light of $g_w^w$ and $A$. Different researchers, using different species and conditions have found evidence bearing out hypotheses (1) and (3): the gain of the feedback loop through $\chi_c^i$ has been measured (Farquhar et al., 1978; Dubbe et al., 1978), and direct responses of stomata to light have been observed (e.g. Jarvis & Morison, 1981; Aphalo & Sánchez, 1986). Evidence in favour of hypothesis (2) is weak: Wong et al. (1979, 1985b, 1985c)
Table 4.2. Stomatal conductance ($g_{sw}^w$), CO$_2$ flux density ($A$), and ratio of intercellular to surface CO$_2$ molar fractions ($\chi_i^c/\chi_s^c$) in leaves of *Hedera helix* in inverted and normal positions. $D_w^w$=7 mmol mol$^{-1}$, $\chi_i^c$=220 µmol mol$^{-1}$, $T_i$=20 °C. Data from three plants from set C. **Part A**: means and standard errors of the means (in brackets). W: white light, R: red light, B: blue light. **Part B**: summary table of analysis of variance. A complete randomized blocks design was used, the plants being the blocks. Orthogonal contrasts were done to find out the origin of significant interactions (e.g. position(white) is the effect of normal vs. inverted position under white light). M.S.: mean square, $P$: probability.

**Part A**: means and standard errors

<table>
<thead>
<tr>
<th>Position</th>
<th>$I$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$g_{sw}^w$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>$A$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$\chi_i^c/\chi_s^c$ (mol mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>500 (W)</td>
<td>122 (5.3)</td>
<td>8.8 (0.43)</td>
<td>0.65 (0.01)</td>
</tr>
<tr>
<td>Inverted</td>
<td>500 (W)</td>
<td>129 (9.6)</td>
<td>5.1 (0.21)</td>
<td>0.77 (0.01)</td>
</tr>
<tr>
<td>Normal</td>
<td>120 (R)</td>
<td>52 (6.9)</td>
<td>5.3 (0.46)</td>
<td>0.55 (0.07)</td>
</tr>
<tr>
<td>Inverted</td>
<td>120 (R)</td>
<td>117 (8.5)</td>
<td>3.1 (0.07)</td>
<td>0.82 (0.02)</td>
</tr>
<tr>
<td>Normal</td>
<td>18 (B)</td>
<td>60 (11.9)</td>
<td>0.5 (0.05)</td>
<td>0.93 (0.02)</td>
</tr>
<tr>
<td>Inverted</td>
<td>18 (B)</td>
<td>118 (22.6)</td>
<td>0.5 (0.13)</td>
<td>0.96 (0.01)</td>
</tr>
</tbody>
</table>

**Part B**: analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>$g_{sw}^w$</th>
<th>$A$</th>
<th>$\chi_i^c/\chi_s^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M.S.</td>
<td>$P$</td>
<td>M.S.</td>
</tr>
<tr>
<td>Light</td>
<td>2</td>
<td>3210</td>
<td>&lt;0.001</td>
<td>62.33</td>
</tr>
<tr>
<td>red−blue</td>
<td>1</td>
<td>136</td>
<td>0.396</td>
<td>41.40</td>
</tr>
<tr>
<td>Position</td>
<td>1</td>
<td>7904</td>
<td>&lt;0.001</td>
<td>17.15</td>
</tr>
<tr>
<td>position(white)</td>
<td>1</td>
<td>74</td>
<td>0.529</td>
<td>20.54</td>
</tr>
<tr>
<td>position(red)</td>
<td>1</td>
<td>5436</td>
<td>&lt;0.001</td>
<td>7.37</td>
</tr>
<tr>
<td>position(blue)</td>
<td>1</td>
<td>5139</td>
<td>&lt;0.001</td>
<td>0.874</td>
</tr>
<tr>
<td>Light × position</td>
<td>2</td>
<td>1372</td>
<td>0.009</td>
<td>5.38</td>
</tr>
<tr>
<td>(red−blue) × pos.</td>
<td>1</td>
<td>2</td>
<td>0.915</td>
<td>3.89</td>
</tr>
<tr>
<td>Plants</td>
<td>2</td>
<td>2052</td>
<td>0.002</td>
<td>0.354</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>173</td>
<td>—</td>
<td>0.205</td>
</tr>
</tbody>
</table>
Figure 4.6. Responses of stomatal conductance (●) and CO₂ flux density (○) to increased photon flux density (I) and decreased intercellular CO₂ concentration (χᵢᵣ) in a leaf in inverted position. Starting conditions: χᵢᵣ = 220 µmol mol⁻¹, I = 500 µmol m⁻² s⁻¹ (white light), Dₛ = 7 mmol mol⁻¹, Tₑ = 20 °C. C⁻: χᵢᵣ reduced to 120 µmol mol⁻¹; PFD+: I increased to 750 µmol m⁻² s⁻¹.

provided some evidence suggesting that there is something else that is conveying information about A to the stomata, their main argument being that the highly constant proportionality between A and gₛₘ cannot be explained by feedback through χᵢᵣ. Most of the evidence supporting hypotheses (1) and (3) does not rule out hypothesis (2).

In Hedera helix A and gₛₘ were linearly correlated under constant χₛᵣ, but, having used a very wide range of I (2–760 µmol m⁻² s⁻¹), this correlation tended to break down at low and high I — high for a shade loving species. For I in the range 35–500 µmol m⁻² s⁻¹ correlations for individual leaves were very high but the slopes and intercepts differed slightly between the two sets of plants.

This correlation can be easily broken through manipulation of the experimental conditions. Stomatal conductance was almost the same in inverted leaves under white, red and blue light, and in leaves in a normal position under white light. In these same treatments A varied between 0.5 to 8.8 µmol m⁻² s⁻¹. In leaves in a normal position gₛₘ was similar under 120 µmol m⁻² s⁻¹ of red and 18 µmol m⁻² s⁻¹ of blue light, while A was 10 times higher under red light than under blue light.

At high I stomata continued to open with increasing I even though A was almost light saturated, leading to an increase in χᵢᵣ (Figs. 4.1, 4.2 & 4.5), as also observed in Phaseolus vulgaris plants grown at low I (Wong et al., 1985b). Under constant χₛᵣ,
this behaviour was reflected in a poor relationship between $g_w^w$ and $\chi_i^c$, indicating that the main effect of light on stomata is not through $\chi_i^c$. However, as stomata of ivy are sensitive to $\chi_i^c$ (see Chapter 6 and Fig. 4.6) part of the effect of light under constant $\chi_s^c$ must occur indirectly through $\chi_i^c$.

Under constant $\chi_i^c$ and saturating $I$, $g_w^w$ did not differ when stomata were directly illuminated, or shaded by the mesophyll, even though $A$ was higher in the latter than in the former case (Table 4.2). Under non-saturating blue or red light, inverting the leaves, and thus increasing $I$ on the guard cells, increased $g_w^w$, and either did not affect or decreased $A$, indicating that ivy stomata respond directly to both red and blue light.

Under red light $\chi_i^c/\chi_s^c$ increased from 0.56 to 0.82 in response to leaf inversion. Had $\chi_s^c$ not been decreased to keep $\chi_i^c$ constant, $\chi_i^c$ would have increased. Under red light Aphalo & Sánchez (1986) did not find an effect of leaf inversion on $g_w^w$. However, as they did not control $\chi_i^c$, a possible explanation for their results is that the direct effect of red light was masked by the increase in $\chi_i^c$ caused by the decrease in $A$.

Under blue light there was almost no effect of leaf inversion on $\chi_i^c/\chi_s^c$ because, as $A$ was very low, the change in $g_w^w$ had little effect on $\chi_i^c$. Aphalo & Sánchez (1986) did observe, under blue light, a big effect of leaf inversion on $g_w^w$, probably because under low $I$ and high $g_w^w$ there was no masking effect through $\chi_i^c$. A response of $g_w^w$ to blue light has been observed in the white portions of variegated leaves of *Hedera helix* (Aphalo & Sánchez, 1986).

In inverted leaves $g_w^w$ was light saturated at 500 $\mu$mol m$^{-2}$ s$^{-1}$ of white light (Fig. 4.6), which explains the lack of an effect of leaf inversion on $g_w^w$ under this condition. Even though $g_w^w$ was light saturated, it was not at its maximum, as under this value of $I$ it increased in response to a decrease in $\chi_i^c$. This indicates that aperture was not mechanically limited, it was limited by the capacity of the photosensors or by the transduction chain.

If the correlation between $g_w^w$ and $A$ was caused by a metabolite of photosynthesis different to CO$_2$, then it would not be possible to break this correlation by experimentally manipulating $\chi_i^c$—i.e. If the messenger is not affected then the relationship between $A$ and $g_w^w$ should not change. However, an increase in $\chi_i^c$ under white light led to an increase in $A$, and to a decrease in $g_w^w$, an effect opposite to what would be expected from the relationship between $A$ and $g_w^w$ under changing $I$ (Fig. 4.6). This information could be consistent with the hypothesis that this messenger is, or is dependent on, the surplus electron transport capacity in the mesophyll, but this hypothesis has to be rejected because it has been observed that stomata are sensitive to $\chi_i^c$ in darkness. So it can be concluded that CO$_2$ is the main ‘messenger’ for the indirect response of $g_w^w$ to light.
An opposite effect of CO$_2$ on $A$ and $g^w_s$, as observed in ivy, has been seen in other species, together with a lack of response of $g^w_s$ to CO$_2$, e.g. *Pinus sylvestris* (Jarvis & Morison, 1981). The degree of control of $g^w_s$ by the $\chi^c_i$ feedback loop varies with species and conditions (Dubbe *et al*., 1978; Sharkey & Raschke, 1981b).

In white and red light, $A$ was lower in inverted leaves than in those in a normal position, even though $\chi^c_i$ was kept constant. The decrease in $A$ is probably due to the dorsiventral structure of the leaves (described in Section 3.2), which, when a leaf is inverted, leads to a different distribution of light within the mesophyll. Only a small part of the decrease in $A$ can be explained by the difference in light absorptance of the two leaf surfaces (see Section 3.3). A similar effect of leaf inversion on $A$ has been observed in *Calopogonium mucunoides*, a legume (Ludlow & Wilson, 1971), and in *Picea sitchensis* (Leverenz & Jarvis, 1979), but not in *Pennisetum purpureum*, a grass (Ludlow & Wilson, 1971). In blue light, $A$ was very low, and no effect of leaf inversion on $A$ was observed probably because of a proportionally larger experimental error.

The results presented here are a confirmation of previous results that have indicated that most of the effect of light on stomata is direct (e.g. Sharkey & Raschke, 1981b; Morison & Jarvis, 1983a; Morison & Jarvis, 1983b). In ivy, if there is a messenger other than CO$_2$ involved in the coordination of $g^w_s$ with $A$, any effect of such a messenger must be quantitatively very small. The direct responses plus the response through $\chi^c_i$ are able to explain all the observed stomatal responses to light, even the apparent inconsistency between leaf inversion experiments done under constant $\chi^c_i$ and constant $\chi^a_i$. Not only it is unnecessary to postulate that some unknown messenger conveys information to the stomata about the rate of CO$_2$ assimilation in the mesophyll, but what is more important, such a messenger would be incompatible with the experimental results.
Chapter 5

Stomatal responses to humidity and temperature

5.1 Introduction

Humidity includes information on both the water vapour and energy content of air. A difficult and important question in biology is selecting an appropriate measure of humidity for studying a response because the relation between different ways of expressing humidity is not linear. Hall et al. (1976) have said that the mechanism for “direct” stomatal response to humidity is not known, and that the use of $D_a^w$ as the driving force, rather than other variables such as relative humidity, should be examined. According to Grantz (1990), this question is still open. It has been said both that ‘...stomata respond to relative humidity’ (Ball et al., 1987), and that ‘...a fall in humidity increases evaporation from the epidermis, and that stomata respond to the consequent fall in water potential’ (Sheriff, 1984). The assertion that stomata respond to relative humidity was mainly based on the good fit of data to the empirical model proposed by Ball et al. (1987), $g^{w}_s = kAh_s/\chi_c^s$, but there are two big problems in arriving at this conclusion. Firstly, correlation is being equated with causation, and secondly, any combined response of $A$ and $g^{w}_s$ to temperature that keeps $\chi_c^c/\chi_c^s$ constant under constant $h_s$ can fit this model. (See chapter 7.) It must also be stressed that a mechanistic interpretation of this model implies the lack of any direct response of $g^{w}_s$ to temperature.

I start by considering the question ‘Do stomata respond to relative humidity?’ In some respects, this is a misleading question simply because $h_s$ reflects simultaneously

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1This chapter is based on the article: Aphalo, P. J., & Jarvis, P. G. 1991. Do stomata respond to relative humidity? Plant Cell and Environment 14, 127–132.
change in the two variables, temperature and air water vapour content. $D_s^w$ and $h_s$ are related according to $h_s = 1 - (D_s^w/\chi_s^w)$, where $\chi_s^w$ is a function of $T_l$. At any particular $T_l$ this relationship is linear. Two, more explicit questions which define the problem are:

1. Do stomata respond to both air water vapour content and temperature?

2. Do the responses of stomata to air water vapour content and temperature interact in such a way that $h_s$ is a more appropriate variable than $\chi_s^w$ and yields a simpler description of the compound response?

What is known about stomatal responses to humidity and temperature? In a large number of species it has been observed that there is a response of $g_s^w$ to both temperature and humidity (e.g. L"osch & Tenhunen, 1981). In such studies the ‘humidity driving variable’ has usually been described as the difference between the water vapour concentration or partial pressure in the air outside the boundary layer and the saturated vapour pressure at the temperature of the leaf, and often expressed as a vapour pressure or an absolute humidity difference. This difference is the driving force for transpiration and consequently expression in this form implies that the response to humidity is a response to transpiration rate, i.e. evaporation of water in the cell walls of the leaf and its diffusion to the atmosphere.

However, it has been proposed that humidity is sensed at the leaf surface, and not through the rate of evaporation from the mesophyll. Lange et al. (1971) observed that in epidermal strips taken from Polypodium vulgare leaves, stomata responded to the water vapour content of the air at the leaf surface. By manipulating boundary layer thickness it has been shown that $g_s^w$ is dependent on $D_s^w$ (Bunce, 1985). The information available on the time course of the relationships between $g_s^w$, or transpiration, and leaf water status (epidermal cell turgor, and xylem water potential) induced by changes in $D_s^w$ (Shackel & Brinckmann, 1985), is also consistent with this hypothesis.

In gas-exchange experiments comparing stomatal response to humidity in air and helox\(^2\) it was found that stomatal aperture was related to the rate of transpiration, rather than to the molar fraction or relative humidity (Mott & Parkhurst, 1991). These experiments with helox give information about the process involved in sensing humidity, but not about the place where sensing takes place.

The relationship between $g_s^w$ and temperature that is observed under constant $D_s^w$ usually shows an optimum (e.g. Neilson & Jarvis, 1975; Osonubi & Davies, 1980). This

\(^2\)Helox is a mix of helium and oxygen, that has different physical properties to those of air because of the lower molecular weight of helium compared to nitrogen. The higher diffusivity of water vapour in helox than in air was used as a tool to increase conductances.
optimum can be broad-topped, especially under low $I$ (Osonubi & Davies, 1980). The response of $g^\text{w}_s$ to $T_l$ is thought to be mainly the result of the effects of temperature on the energy metabolism of the guard cells, but the question of whether there is a specific temperature sensor in guard cells remains open (e.g. Zeiger, 1983).

Why is it important to know whether stomata respond to $h_s$ or $D^\text{w}_s$? From a practical point of view it is essential to control the correct variable in experimentation, especially in controlled environments. Keeping the wrong humidity variable constant in an experiment to study the response of $g^\text{w}_s$ to temperature would result in almost useless data that would show the confounded effects of temperature and humidity. Secondly, using the wrong variable in a model to interpret values of $g^\text{w}_s$ measured in the field, must ultimately lead to the model breaking down. From a conceptual point of view, appreciation of the correct variable has a strong influence on hypotheses about the mechanism of stomatal action, and, in this case, has led to the development of the “feed-forward” hypothesis (Cowan, 1977).

I have carried out experiments to test (a) whether $g^\text{w}_s$ responds linearly to $D^\text{w}_s$ and $h_s$ at a fixed temperature, and (b) whether $g^\text{w}_s$ changes with $T_l$, and thus whether $h_s$ is a more appropriate measure of humidity than $D^\text{w}_s$. This was done by altering leaf temperature and ambient air humidity so as to maintain either $h_s$ or $D^\text{w}_s$ constant, whilst observing $g^\text{w}_s$.

### 5.2 Materials and methods

#### 5.2.1 Plant material

*Hedera helix* subsp. *canariensis* (Willd.) Coutinho plants were grown in a heated greenhouse. Two different sets of plants were used, in two replicates of the whole experiment. The plants were grown in 12 cm diameter plastic pots filled with a peat-perlite-vermiculite mix, watered every other day, and fertilized weekly (See Chapter 3 for details).

One set of plants —henceforth called set A— was moved 10 days before the beginning of the experiments from the greenhouse to a growth cabinet at 20 °C, with no humidity control ($h \approx 50\%$), and a photoperiod of 12 h at 200 μmol m$^{-2}$ s$^{-1}$ at leaf level from fluorescent tubes (Sylvania ‘Powertube’ F48T12-CW-VHO).

The second set of plants —set B— was kept for 2.5 months in a growth chamber at 20 °C, $h=30-60\%$, and a photoperiod of 12 h at 400 μmol m$^{-2}$ s$^{-1}$ at leaf level from metal halide lamps (Wotan ‘Power Star’ HQI-R 250 W/NDL, Wotan Lamps Ltd., London).
5.2.2 Gas exchange measurements

We used the computer-controlled, open path gas-exchange system described in Chapter 2. The equations used assume a single transpiring surface with uniform spatial distribution of temperature and conductance (see Section 2.2.1). By using a wind speed that gave a $g_w^b$ at least six times the maximum $g_w^s$ and a species with hypostomatous leaves, we attempted to keep the conditions of measurement close to those assumed in the calculations. $g_w^b$ was measured by means of leaf replicas of Whatman No. 3 filter paper covered on the upper side with aluminium foil and wetted with distilled water, and was within the range 650 to 750 mmol m$^{-2}$s$^{-1}$ for the different leaves used.

Steady-state measurements were made. A new steady value of $g_w^s$ was reached sooner after a change in humidity than after a change in temperature. In the first case no data taken during the first hour after a change in conditions were used; in the second case this time was doubled. However, the data were checked to see whether a steady state had been reached and these periods were extended if necessary.

The leaves to be measured the next day were placed overnight in the gas-exchange chamber in darkness with $\chi_c^s = 350 \mu$mol mol$^{-1}$, $D_w^s = 10$ mmol mol$^{-1}$, $T_l = 20$ °C for humidity response experiments, and $T_l = 15$ °C for temperature response experiments.

5.2.3 Experiments

We measured the response of $g_w^s$ to either $h_s$ or $D_w^s$ at a constant $T_l$ of 20 °C, and to increasing temperature at either a constant $h_s$ of 60 % or a constant $D_w^s$ of 10 mmol mol$^{-1}$. Humidity response was measured by changing the humidity in the gas-exchange chamber so that $D_w^s$ varied over the range 4–17 mmol mol$^{-1}$, but the environment of the rest of the plant was kept unchanged. In the temperature response experiment, the temperature of the leaf inside the chamber and room air temperature were increased simultaneously over the range 15–29 °C, and in one case 10–29 °C, keeping room air temperature within ±2 °C of $T_l$. Changing temperature at constant $h_s$ inevitably results in a change in $D_w^s$; conversely, changing $T_l$ at constant $D_w^s$ results in a change in $h_s$. Three plants, in each of the two sets, were used as replicates. The different treatments were applied to the same leaf from each plant on different days and in random order. This makes comparison between the effects of temperature at constant $h_s$ and at constant $D_w^s$ very sensitive.

All the experiments were carried out at a $\chi_c^s$ of 350 μmol mol$^{-1}$. A complete whole set of experiments was done at quantum flux densities of 200 and 340 μmol m$^{-2}$s$^{-1}$ on set A and set B plants, respectively. These quantum flux densities gave approximately 70–80 %, of the light-saturated rate of CO$_2$ assimilation for each set of plants.
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Figure 5.1. Relationship between stomatal conductance \( (g_w) \) and water saturation deficit at the leaf surface \( (D_w) \), or relative humidity at the leaf surface \( (h_s) \). (a) Data from three *Hedera helix* plants from set A, \( I=200 \, \mu\text{mol m}^{-2}\text{s}^{-1} \). \( R^2 \) for the linear regressions are 0.97 (□), 0.98 (●), and 0.99 (▽). (b) Data from one plant from set B, \( I=340 \, \mu\text{mol m}^{-2}\text{s}^{-1} \). \( R^2 \) for the linear regression is 0.98. Measured at \( T_l=20 \, ^\circ\text{C} \), and \( \chi_s=350 \, \mu\text{mol mol}^{-1} \). The numbers beside the symbols show the order in which measurements were taken.

5.3 Results and discussion

5.3.1 Response of \( g_w \) to humidity at constant temperature

In *Hedera helix* we observed a response of \( g_w \) to humidity that, under constant \( T_l \) and \( I \), was a linear function of both \( D_w \), and \( h_s \) (Fig. 5.1). For the individual plants, the proportion of the variation in \( g_w \) that was explained by a linear regression model was 90 % or more. This response showed no hysteresis.

Under constant \( \chi_s \), Ball (1988, Fig. 2.2.C) measured a linear response to \( D_w \) at \( I=250 \, \mu\text{mol m}^{-2}\text{s}^{-1} \), and a very slightly curved response at \( I=525 \), and 1375 \( \mu\text{mol m}^{-2}\text{s}^{-1} \). A curvilinear response of \( g_w \) to \( D_w \) has been previously reported by Bunce (1985) in *Glycine max*, *Abutilon theophrasti*, and *Datura stramonium*. In that set of experiments, carried out under \( I=1500 \, \mu\text{mol m}^{-2}\text{s}^{-1} \), the curvature seemed to be linked to high maximum values of \( g_w \), and could have been an artifact derived from the calculation procedures used, i.e. a linear regression was first fitted to the relation between total conductance and the leaf-to-air water vapour partial pressure difference, and then \( g_w \) and \( D_w \) were computed from this regression. Alternatively
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Figure 5.2. Relationship between stomatal conductance ($g_w^*$) and leaf temperature ($T_l$) under constant $D_w^*=10$ mmol mol$^{-1}$ ($\triangle$), and under constant $h_s=0.60$ (●). (a) Mean of three plants from set A, $I=200$ μmol m$^{-2}$ s$^{-1}$. (b) Typical plant from set B, $I=340$ μmol m$^{-2}$ s$^{-1}$. The arrow above the temperature axis shows the point at which humidity is identical for both treatments. Both treatments were applied to the same leaves of the same plants. All measurements were taken at $\chi^c_s=350$ μmol mol$^{-1}$.

feedback through $\chi^c_i$ could have led to the curvature. In Bunce’s experiments, CO$_2$ concentration was not altered to compensate for the effects of the changing $g_w^*$ on $\chi^c_s$ or $\chi^c_i$. Reversibility of the response to humidity in whole, attached leaves has been previously reported (Bunce, 1985), but no data were given.

5.3.2 Response of $g_w^*$ to temperature at constant $D_w^*$ or $h_s$

The response to temperature at constant $D_w^*$ was different to that at constant $h_s$. In plants from set A there was no response to $T_l$ in the range 15–28 °C under constant $D_w^*$ ($P>0.5$, Fig. 5.2.a), but when $h_s$ was kept constant, $g_w^*$ decreased with increasing $T_l$ —and consequently increasing $D_w^*$— ($P=0.003$, Fig. 5.2.a). In plants from set B, there was a different and significant effect of $T_l$ under both humidity treatments (Fig. 5.2.b), and the effect of $T_l$ was such that $g_w^*$ was higher at lower temperatures. Under constant $D_w^*$ the effect of an increase in temperature resulted in $g_w^*$ being inversely proportional to $h_s$ (Fig. 5.3.b) (i.e. the opposite to that consistent with the model of Ball et al.).

The different response to $T_l$ of the two sets of plants was not totally unexpected as they differed in both growth and measurement conditions. Since stomatal sensitivity
Figure 5.3. Relationship between stomatal conductance ($g_w^s$) and relative humidity at the leaf surface ($h_s$), measured under changing leaf temperature ($T_l$) in the range 10–29 °C. Symbols as in Fig. 5.2. (a) Mean of three plants from set A, $R^2=0.01$, $P>0.5$. (b) Typical plant from set B, $R^2=0.26$, $P=0.13$.

to $T_l$ has been shown to increase with increasing $I$ under constant $D_{aw}^w$ (Osonubi & Davies, 1980), a likely explanation is that the different response was largely the result of the lower $I$ used with set A than with set B.

The optimum temperature for $g_w^s$ varies widely between species and/or growth conditions. Ball (1988, Fig. 2.3.A) observed, in Glycine max at constant $D_{aw}^w$, an approximately linear increase in $g_w^s$ in response to $T_l$ in the range 20–35 °C. In contrast, in Picea sitchensis Neilson & Jarvis (1975) observed a $T_l$ response curve having an optimum at 15 °C under constant $D_{aw}^w \approx 5$ mmol mol$^{-1}$, and in these plants $g_w^s$ was insensitive to $\chi_{ci}^l$ and to $D_{aw}^w < 10$ mmol mol$^{-1}$.

Decreasing $g_w^s$ in response to increasing $T_l$ has been reported in many cases for constant air water vapour content, and consequently decreasing $h_s$ and increasing $D_{aw}^w$ (e.g. Wuenscher & Kozlowski, 1971). Although this is similar to what may happen outdoors during the daily time course of air temperature change, such results shed little light on the nature of the driving variable.

### 5.3.3 Interaction between humidity and temperature

When the pooled data from both humidity treatments of the temperature-response experiment with plants of set A are plotted against $h_s$ no clear pattern of response
Figure 5.4. Relationship between stomatal conductance ($g_w$) and water vapour deficit at the leaf surface ($D_{ws}$), measured under changing leaf temperature ($T_l$) in the range 10–29 °C. Symbols as in Fig. 5.2. (a) Mean of three plants from set A, $R^2=0.77$, $P=0.004$. (b) Typical plant from set B, $R^2=0.75$, $P=0.001$. The triangle with an underscore represents three overlapping data points.

appears ($R^2=0.01$, $P>0.5$), and the data from each treatment show a different pattern of change (Fig. 5.3.a). When these same data are plotted against $D_{ws}$ a clear linear decrease in $g_w$ in response to increasing $D_{ws}$ appears ($R^2=0.77$, $P=0.004$; Fig. 5.4.a): data from both treatments collapse into a single relationship only when expressed as a function of $D_{ws}$. In set B, where there is an effect of both temperature and humidity, the variation in the data cannot be described as a function of only $D_{ws}$ or $h_s$ (Figs. 5.3.b & 5.4.b). However, for a typical plant from this set, $D_{ws}$ explains 75 % of the variation while $h_s$ explains only 26 %.

Stronger evidence can be obtained by comparing the behaviour of $g_w$ under constant $T_l$ with that under constant $D_{ws}$. Changing $h_s$ by altering $T_l$ led to no response of $g_w$ (Fig. 5.3.a), or to the opposite response to that observed when changing $h_s$ under constant $T_l$ (Fig. 5.1.b vs. Fig. 5.3.b). $g_w$ decreased with increasing $h_s$ at constant $D_{ws}$ in set B (Fig. 5.3.b). Although there was a response to $T_l$ at constant $D_{ws}$ only in set B, the response to humidity did not differ between the two sets of plants in a way that would make both responses compatible with a single mechanism based solely on the sensing of $h_s$, thus reinforcing our argument. Even Ball (1988, Figs. 2.3.B, 2.3.C & 2.4), observed an effect of $T_l$ on $g_w$ at constant $h_s$, in a setting such that $g_w$ increased with
increasing $T_1$ at constant $D_s^w$, and this effect only disappeared when $g_s^w$ was substituted by $g_s^w/A$.

5.4 Conclusions

Based on these experiments, the answers, for *Hedera helix*, to the two questions stated in the introduction to this chapter are:

1. Stomata do respond to humidity, and sometimes respond to temperature as well. An inversely proportional response of $g_s^w$ to $D_s^w$ was consistently obtained. The response to $T_1$ at constant $D_s^w$ was sometimes absent, but when present this response was a decrease in $g_s^w$ with increasing $T_1$.

2. These responses do *not* interact in a way that makes $h_s$ a more appropriate way of expressing humidity than $\chi_s^w$. The apparent relation between $g_s^w$ and $h_s$ at constant $D_s^w$ was different to that at constant $T_1$, and so $h_s$ was unable to explain the responses of $g_s^w$ to both humidity and temperature.

$D_s^w$, together with $T_1$, give a more general and simpler description of the response of $g_s^w$ than $h_s$. The experiments provide no evidence in favour of a mechanism of humidity sensing based on $h_s$. There is no means by which the correlation between $T_1$, and the relationship between $D_s^w$ and $h_s$ can be broken experimentally. However, by using helox, it is possible to test whether the response depends on diffusional flow of water vapour or on sensing water vapour concentration directly. This test, done by Mott & Parkhurst (1991), showed that stomatal response to humidity depends on a diffusional flux, supporting my finding that $D_s^w$ is the preferred expression.
Chapter 6
The boundary layer and stomatal function

6.1 Introduction

In previous chapters I have considered the effect on stomata of the condition of the air at the leaf surface and in the intercellular spaces. However, because between the bulk air and the outermost parts of the leaf there is a boundary layer of air, in this chapter I will analyse stomatal function within a framework that includes the boundary layer. There are two different aspects to the problem: (1) the role of the boundary layer in the mechanism of stomatal response to the condition of the bulk air, and (2) the role of the boundary layer in stomatal responses to $\chi_a^w$, $\chi_a^c$, and wind speed under natural conditions.

Stomatal conductance changes with wind speed when $D_a^w$ and $\chi_a^c$ are kept constant (Caldwell, 1970; Grace et al., 1975; van Gardingen & Grace, 1991). However, although the boundary layer has been taken into account in descriptions of the soil-plant-atmosphere water continuum, in the calculation of $g_a^c$, or in analyses of the control of CO$_2$ fixation (e.g. Woodrow et al., 1987), its role as a component of the mechanism of stomatal response has remained unexplored, except for the experiments of Bunce (1985) [e.g. the effect of the boundary layer was not included in the feedback analysis made by Farquhar et al. (1978)].

In most studies of stomatal responses to humidity and CO$_2$ the experimentally controlled variables have been those describing bulk air properties. Responses to CO$_2$ have been studied by controlling $\chi_a^c$ and responses to air humidity by altering $D_a^w$ or $\chi_a^w$. In most gas exchange chambers wind speed is kept high so as to reduce the thickness of the boundary layer and make the difference between $\chi_a^c$ and $\chi_a^w$, and $D_a^w$ and $D_a^s$.
small, but this is not the case in the real world. Although $\chi_a^c$ and $D_w^a$ are variables of ecological interest, it is impossible for stomata to sense them directly. Both direct responses —those occurring within the guard cells— and indirect responses —those depending on events happening in other cells of the leaf— can only depend on the state of system variables inside the boundary layer. For this reason the analysis of stomatal responses to changes in bulk air properties must include the boundary layer as a component of the response mechanism. In nature the state of the air at the leaf surface cannot be considered as an independent variable—it strongly depends on $g_{wb}$ for a given state of the bulk air (Jarvis & McNaughton, 1986). The boundary layer is a source of feedback, and so it can alter the apparent behaviour of stomata.

The apparent responses of $g_w^s$ to $\chi_w^a$ and $\chi_a^c$ depend on the effects of these two variables, $g_b^w$ and $g_s^w$, on $D_s^w$ and $\chi_1^c$. Control diagrams are useful for visualizing interactions, and I have adapted that given by McNaughton & Jarvis (1991, Fig. 6) by including the effect of changes in $g_b^w$ and assuming constant $T_1$ (Fig. 6.1). A control diagram allows one to trace the propagation of a change in one variable (e.g. $d\chi_a^w$) through the system, and also shows the feedback loops.

Under natural conditions $g_b^w$ can be an important component of $g_w^s$. The thickness of the boundary layer, and hence the magnitude of $g_b^w$, varies widely according to leaf size and wind speed. For big leaves the boundary layer can be a few millimeters thick even under moderate wind speed. For ivy leaves of the size of those used in my experiments, thicknesses between 1.0 and 3.3 mm could be expected under natural conditions (assuming wind speeds between 0.1 and 1 m s$^{-1}$). For one side of the leaf, these represent $g_b^w \approx 290$ mmol m$^{-2}$ s$^{-1}$ and $g_b^w \approx 970$ mmol m$^{-2}$ s$^{-1}$, respectively$^1$. Some species such as Helianthus annuus (Körner et al., 1979), and Tectona grandis and Gmelina arborea (Grace et al., 1982) have high stomatal conductances and their leaves are several times the size of leaves of ivy, thus having thicker boundary layers at the same wind speed. In a rain forest canopy, it was found that $g_b^w$ increased with height, from 240 mmol m$^{-2}$ s$^{-1}$, for both leaf surfaces in parallel, at the forest floor to 1400 mmol m$^{-2}$ s$^{-1}$ at the top of the canopy (35 m) (Roberts et al., 1990).

Experiments were done to describe the effect of the boundary layer on stomatal response to change in the molar fractions of CO$_2$ and water vapour in the bulk air. Both actual and simulation experiments were done. The actual experiments included measurements to obtain the data needed to drive the simulations, and measurements of the response of $g_w^s$ to changes in the thickness of the boundary layer. The simulation experiments were done to derive stomatal responses to $\chi_a^c$ and $D_w^a$ and their interactions.

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$^1$These values arise from calculations based on equations given by Nobel (1983, pages 358, 391–392).
Figure 6.1. Control diagram showing the response of stomatal conductance (\(g^w_s\)) to changes in bulk air water vapour molar fraction (\(\chi^w_a\)), bulk air CO\(_2\) molar fraction (\(\chi^c_a\)), and wind speed (\(u\)), for a hypostomatous leaf. The changes in intercellular CO\(_2\) molar fraction (\(\chi^c_i\)), leaf surface water vapour deficit (\(D^w_s\)), boundary layer conductance (\(g^w_s\)), CO\(_2\) flux density (\(A\)) and transpiration (\(E\)), are also indicated. The top half of the diagram represents \(E\), the bottom half represents \(A\). The circles represent summation points and the boxes represent gain elements, with functions shown as partial derivatives.
6.2 Materials and methods

6.2.1 Plant material

Ivy plants were grown in a heated greenhouse. The plants were grown in 16 or 18 cm diameter plastic pots filled with a peat-perlite-vermiculite mix, watered every other day, and fertilized weekly (See Chapter 3 for details).

Two sets of plants were used, in three experiments. The plants were moved more than 75 days before the beginning of the experiments from the greenhouse to a growth room at 20/15 °C day/night, with no humidity control ($h \approx 60\%$), and a photoperiod of 12 h at 500 µmol m$^{-2}$ s$^{-1}$ at leaf level from metal halide lamps (Kolorarc 400W MBIF/BU, Thorn Lighting Ltd., London, U.K.).

6.2.2 Gas exchange measurements

The computer-controlled, open differential gas-exchange system described in Chapter 2 was used. Wind speed was measured with a hot wire anemometer (AVM501, Prosser Scientific Instruments Ltd., Hadleigh, Suffolk, U.K.). $g_{wb}$, for one side of the leaves, was measured at the two wind speeds used in the experiments by means of leaf replicas of Whatman No. 3 filter paper covered on the upper side with aluminium foil and wetted with distilled water.

Steady-state measurements of $A$ and $E$ were made, and no data taken during the first hour after a change in conditions were used. However, the data were checked to see whether a steady state had been reached and this period was extended if necessary. The temperature of leaves and leaf replicas was measured with a thermojunction in contact with their shaded face near the centre of the blade. The leaves to be measured the next day were placed overnight in the gas-exchange chamber in darkness with $\chi_{w}^{i}=350$ µmol mol$^{-1}$, $D_{w}^{s}=10$ mmol mol$^{-1}$, $T_{l}=20$ °C. Attached non-senescent fully expanded leaves were used in the experiments, the projected area of individual leaves being between 50 and 64 cm$^2$.

6.2.3 Simulation model

A simple model was developed to compute the apparent steady-state response of stomata to $\chi_{a}^{w}$ and $\chi_{a}^{c}$. Given a known response of $g_{s}^{w}$ to $D_{s}^{w}$ and $\chi_{a}^{c}$, and an $A$ vs. $\chi_{a}^{c}$ curve, the model computes $g_{s}^{w}$ for given $g_{wb}$, $\chi_{a}^{w}$ and $\chi_{a}^{c}$. This model simulates the effect of the boundary layer on the apparent response of stomata given a known stomatal response with $g_{w}^{b}$.
to $D^w_s$ and $\chi^c_i$. It is not a model of stomatal responses to CO$_2$ and humidity, it is instead a model of how these responses are modified by the boundary layer. The MWEB listing of the computer program is given in Appendix A.

The model is represented by a system of two simultaneous non-linear equations in two unknowns:

$$f(D^w_s, \chi^c_i) = 0,$$

(6.1a)

$$ff(D^w_s, \chi^c_i) = 0.$$  

(6.1b)

This system of equations embodies the conditions fulfilled by $E$ and $A$ when both flows are in steady-state. As both $A$ and $E$ depend on $g^w_s$ both equations are functions of $D^w_s$ and $\chi^c_i$. The use of $D^w_s$ and $\chi^c_i$ in these equations reflects the fact that these are the variables sensed by the guard cells. As $A$ affects $\chi^c_i$, and $E$ affects $D^w_s$, the two equations have to be solved simultaneously.

Equation 6.1a defines the equilibrium condition for $g^w_s$ with respect to $D^w_s$, and is:

$$D^w_s \left(1 - \frac{g^w_t}{g^w_b}\right) - D^w_s = 0,$$

(6.2)

or, in words, the value of $D^w_s$ calculated from $g^w_s$ must be the same as that used to compute $g^w_s$. Equation 6.2 was derived from Equation 2.22, assuming that $T_l$ remains constant.

Equation 6.1b is

$$\chi^c_a - \frac{A}{g^c_t} - \chi^c_i = 0,$$

(6.3)

and defines the steady-state condition for $g^w_s$ with respect to $\chi^c_i$. This equation could have been derived from Equation 2.31, but instead a simpler expression, without a correction for the mass flow of water, was used in the model.

In the equations $A$ is calculated as a function of $\chi^c_i$ using spline interpolation from tabulated data, and $g^c_t = (1/g^w_s + 1/g^w_b)^{-1}$ and $g^c_t = (1.60/g^w_s + 1.37/g^w_b)^{-1}$. $g^w_s$ is computed as the product of the conductance observed under standard conditions and scaling factors obtained by spline interpolation from tabulated data:

$$g^w_s = k f_0(D^w_s) f_1(\chi^c_i),$$

(6.4)

where $k$ is $g^w_s$ at a standard condition, and is a parameter of the model, $f_0$ and $f_1$ are spline functions giving the relative effect of $D^w_s$ and $\chi^c_i$ on $g^w_s$. Computing the compound effect of changes in CO$_2$ and water vapour molar fractions on $g^w_s$ as the
product of \( f_0 \) and \( f_1 \) assumes that these effects are multiplicative.

The equation given by Nobel (1983)\(^2\) was used to relate mean boundary layer thickness (\( b \)) to leaf dimension — i.e. the spatial average of leaf length in the wind direction, not the equivalent dimension — (\( l \)) and wind speed (\( u \)):

\[
b = 0.004 \sqrt{\frac{l}{u}},
\]

(6.5)

This equation gives only an approximation to the mean value of \( b \), because \( b \) varies across the leaf surface (see also Section 1.1.3), and because air flow in the field is not laminar. The value of 0.004 for the factor in Equation 6.5 was derived by Nobel from field measurements done by Pearman \textit{et al.} (1972). The conductance of the boundary layer to water vapour is related to its thickness by the molar diffusivity of water vapour in air (\( D^w \)), i.e. \( g_w^b = D^w / b \).

The system of two simultaneous non-linear equations is solved by an iterative procedure based on a quasi-Newton algorithm using finite differences to approximate the derivatives (Johnston, 1982; Press \textit{et al.}, 1986, were used as a guide). Simulations are driven by four text files containing the data:

1. **Relationship between** \( A \) **and** \( \chi_i^c \). Data pairs of \( \chi_i^c \), in mol mol\(^{-1} \), and \( A \), in mol m\(^{-2} \) s\(^{-1} \), give the points that are used for interpolation.

2. **Relationship between** \( g_s^w \) **and** \( D_s^w \). Data pairs of \( D_s^w \), in mol mol\(^{-1} \), and \( g_s^w \), as a proportion of that in standard conditions, give the points that are used for interpolation.

3. **Relationship between** \( g_s^w \) **and** \( \chi_i^c \). Data pairs of \( \chi_i^c \), in mol mol\(^{-1} \), and \( g_s^w \), as a proportion of that in standard conditions, give the points that are used for interpolation.

4. **Input file with values for the driving variables.** Each line of this file contains data for the simulation of the steady-state of \( g_s^w \), and \( A \) and \( E \), at a particular environmental condition. The driving variables are \( \chi_a^w \), \( \chi_a^c \), \( I \), \( T_i \), and \( g_b^w \). (\( I \) is not used in the current version of the model, and is assumed constant).

The output from the program is another text file, with one line for each line in the input file (4 in the list above). The state variables in the output file are \( g_s^w \), \( A \), \( E \),

---

\(^2\)This equation can be derived from that given by Monteith & Unsworth (1990, Equation 7.1) for a laminar boundary layer.
$D_w^s$, and $\chi_i^c$. The output also includes the the minimization errors for $D_w^s$ and $\chi_i^c$ and a text string that indicates whether the numerical algorithm has succeeded or not in solving the system of equations. $g_w^s$ was converted to $u$ for a given leaf size by means of a simple program written in the programming language AWK.

6.2.4 Experiments

Real world experiments

In one experiment —henceforth experiment I— the response to a change in $g_w^s$ was measured under both constant $\chi_a^c$ and $\chi_a^w$ (and so changing $\chi_s^c$ and $D_w^s$), and under constant $\chi_s^c$ and $D_w^s$. The value of $g_w^s$ was altered by changing wind speed in the leaf chamber. $g_w^s$, for one surface of the leaf, was 750 mmol m$^{-2}$ s$^{-1}$ for the ‘high’ wind speed treatment (0.8 m s$^{-1}$), and 360 mmol m$^{-2}$ s$^{-1}$ for the ‘low’ wind speed (0.2 m s$^{-1}$) treatment. The lowest $g_w^s$ was 2.5 times the highest value of $g_w^s$ observed, and the small errors in its measurement should not have caused significant errors in the estimation of $g_w^s$. The same sequence of treatments was applied to each of three plants from set B.

In a second experiment —experiment II— response curves of $g_w^s$ and $A$ to $D_w^s$ and $\chi_i^c$ were measured. The response to $D_w^s$ was measured at constant $\chi_i^c$$\approx$ 200 µmol mol$^{-1}$, and that to $\chi_i^c$ at constant $D_w^s$=7 mmol mol$^{-1}$. The response to $D_w^s$ in the range 5–16 mmol mol$^{-1}$ was measured, $D_w^s$ being changed in random order because there is no hysteresis in the humidity response of ivy stomata under these conditions (See Chapter 5). For measuring the response to CO$_2$, $\chi_i^c$ was first decreased to approximately 120 µmol mol$^{-1}$ and then increased in 5–7 steps to 300–350 µmol mol$^{-1}$. Three plants from set A were used.

In a third experiment —experiment III— the interaction between the responses of $g_w^s$ and $A$ to $\chi_i^c$ and $D_w^s$ was studied in a 2 x 2 factorial arrangement ($\chi_i^c$= 200 and 290 µmol mol$^{-1}$, $D_w^s$= 6 and 12 mmol mol$^{-1}$). The four treatments were applied to each plant in a fixed sequence: (1) low $D_w^s$ and low $\chi_i^c$, (2) high $D_w^s$ and low $\chi_i^c$, (3) low $D_w^s$ and high $\chi_i^c$, and (4) high $D_w^s$ and high $\chi_i^c$. This sequence was selected to obtain a decrease, or no change, in $g_w^s$ with successive treatments, and in this way preventing hysteresis from affecting the results. This is valid only because there is no effect of the time of day on $g_w^s$ (See Section 3.4). Three plants from set B were used.

Simulation experiments

Simulations were done driving the model with the $g_w^s$ and $A$ response curves to $\chi_i^c$ measured at constant $D_w^s$, and the $g_w^s$ response curve to $D_w^s$ measured at constant
The apparent responses of \( g_w \) to changes in \( \chi_a \) and \( \chi_i \) (from experiment II above). The apparent responses of \( g_w \) to changes in \( \chi_w \) and \( \chi_a \) were calculated for \( g_w = 100–1000 \text{ mmol m}^{-2}\text{s}^{-1} \). The response to wind speed was also computed. To assess how much of this response is dependent on changes in \( \chi_w \) and how much on changes in \( \chi_a \), simulations were also done with hypothetical stomata insensitive to \( D_w \).

### 6.3 Results and discussion

#### 6.3.1 Responses to \( D_w \) and \( \chi_i \)

**Experiment I**

Changing wind speed caused a change in \( g_w \) (Fig. 6.2), as previously observed in other species (Grace et al., 1975; Bunce, 1985). Decreasing \( g_w \) under constant \( \chi_w \) and \( D_w \) caused an increase in \( g_w \), but restoring \( \chi_w \) and \( D_w \) to their initial values caused \( g_w \) to decrease as much as it had increased. Subsequently, increasing \( g_b \) to its original value keeping \( \chi_w \) and \( D_w \) constant at their new values caused a decrease in \( g_w \) that once more reverted when \( \chi_w \) and \( D_w \) were restored to their initial state. This sequence of treatments was repeated in three plants with almost identical results, a typical time course is shown in Fig. 6.2 and the means in Table 6.1. In treatments 1 and 3, which had different wind speeds but the same \( \chi_w \) and \( D_w \), \( g_w \) and \( A \) were not significantly different. The differences in \( g_w \) and \( A \) between treatments 1 and 2 shows the effects of a decrease in wind speed, and between 3 and 4 the effects of an increase in wind speed, in both cases under constant \( \chi_w \) and \( D_w \) but with changing \( \chi_w \) and \( D_w \).

In an experiment where CO\(_2\) concentration was not controlled, Bunce (1985) attributed all the effect of wind speed to its effect on \( D_w \). The data presented here show that in ivy there are two effects, one through water vapour and another through CO\(_2\) (Table 6.1). Whether there is an effect through CO\(_2\) or not depends on stomatal sensitivity to CO\(_2\). In ivy there was also a small effect of wind speed on \( A \), caused by its effect on \( \chi_w \) (Table 6.1). A similar effect was also previously observed in other species by Bunce (1988a).

**Experiment II**

In this experiment, responses of \( g_w \) and \( A \) to CO\(_2\) and water vapour were measured one at a time, keeping the other variable constant at the place where it is sensed by stomata. \( g_w \) decreased linearly with increasing \( \chi_i \) under constant \( D_w \) (Fig. 6.4), and \( g_w \) decreased linearly with increasing \( D_w \) under constant \( \chi_i \) (Fig. 6.3). To the best of my knowledge, there are no previous reports of a \( g_w \) response to \( D_w \) measured under
CHAPTER 6. THE BOUNDARY LAYER AND STOMATAL FUNCTION

Figure 6.2. Effect of boundary layer conductance \( g_{wb} \) on stomatal conductance \( g_{ws} \) in a typical leaf. \( g_{wb} \) was altered by changing the wind speed. Five different treatments were applied in sequence: (1) \( g_{wb} = 750 \) mmol m\(^{-2}\) s\(^{-1}\), \( \chi_{ci} = 200 \) µmol mol\(^{-1}\), \( D_{ws} = 7 \) mmol mol\(^{-1}\), \( D_{wa} = 8.1 \) mmol mol\(^{-1}\); (2) \( g_{wb} = 360 \) mmol m\(^{-2}\) s\(^{-1}\), bulk air mol fractions as in (1); (3) \( g_{wb} = 360 \) mmol m\(^{-2}\) s\(^{-1}\), \( \chi_{ci} = 200 \) µmol mol\(^{-1}\), \( D_{ws} = 7 \) mmol mol\(^{-1}\); (4) \( g_{wb} = 750 \) mmol m\(^{-2}\) s\(^{-1}\) and bulk air mol fraction as in (3); (5) restored to \( g_{wb} = 750 \) mmol m\(^{-2}\) s\(^{-1}\), \( \chi_{ci} = 200 \) µmol mol\(^{-1}\), \( D_{ws} = 7 \) mmol mol\(^{-1}\). \( T = 20 \) °C, and \( I = 500 \) µmol m\(^{-2}\) s\(^{-1}\)). The vertical bars indicate the times when conditions were changed.

Table 6.1. Effect of boundary layer conductance \( g_{wb} \) on stomatal conductance \( g_{ws} \), CO\(_2\) flux density \( A \), and leaf surface CO\(_2\) molar fraction \( \chi_{cs} \). \( g_{wb} \) was altered by changing the wind speed. The sequence of treatments is indicated in Fig. 6.2. Means, and standard errors (in brackets) are given. Tukey’s hsd test for multiple comparisons was used. Significance was calculated using the error mean square from an ANOVA for a randomized complete blocks design, each one of the three plants used being a block. Different letters indicate \( P < 0.06 \), according to this test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( g_{ws} ) (mmol m(^{-2}) s(^{-1}))</th>
<th>( A ) (µmol m(^{-2}) s(^{-1}))</th>
<th>( \chi_{cs} ) (µmol mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>116(3.1) b</td>
<td>8.5(0.31) a</td>
<td>327(3.5) b</td>
</tr>
<tr>
<td>2</td>
<td>129(3.8) a</td>
<td>8.0(0.19) a</td>
<td>314(4.7) a</td>
</tr>
<tr>
<td>3</td>
<td>116(2.0) b</td>
<td>8.3(0.32) a</td>
<td>326(3.2) b</td>
</tr>
<tr>
<td>4</td>
<td>105(3.6) c</td>
<td>8.6(0.35) a</td>
<td>341(3.3) c</td>
</tr>
<tr>
<td>5</td>
<td>113(1.2) b</td>
<td>8.3(0.39) a</td>
<td>329(3.2) b</td>
</tr>
</tbody>
</table>
constant $\chi_i^c$, or of a $g_w^s$ response to $\chi_i^c$ measured under constant $D_w^s$. The response to $D_w^s$ was similar to that measured under constant $\chi_c^s$ and high $g_w^b$ (Fig. 5.1). A slightly curved response of $g_w^s$ to $D_w^s$ has been observed under constant $\chi_a^c$ in Picea sitchensis, but a linear response in Pinus sylvestris (Sandford, 1984, Figs. 7.1 & 5.1). Under constant $D_a^w$, the response of $g_w^s$ to $\chi_i^c$ in other species have been found to be variable and usually not linear, and to depend on $I$ and $D_w^a$ (Jarvis & Morison, 1981; Morison & Gifford, 1983; Morison, 1987).

$A$ increased with $\chi_i^c$ (Fig. 6.5), and the $A$ vs. $\chi_i^c$ curve was similar to that reported for low light grown ivy plants (Bauer & Thöni, 1988, Fig. 5). The CO$_2$ compensation concentration calculated by extrapolation was 42 $\mu$mol mol$^{-1}$ (S.E.=8.9 $\mu$mol mol$^{-1}$). This is very close to the value of 38 $\mu$mol mol$^{-1}$ that has been measured in Hedera helix at 20 $^\circ$C and under saturating $I$ (Bauer & Bauer, 1980).

No effect of $D_a^w$ on $A$ was observed under constant $\chi_i^c$, for $D_a^w \leq 15$ mmol mol$^{-1}$ (Fig. 6.6). However, in some plants there was a slight decrease in $A$ at $D_a^w > 15$ mmol mol$^{-1}$, but this was not a consistent response (data not shown). It is usually assumed that $A$ is not affected by $D_a^w$ under constant $\chi_i^c$, but there have been reports of a decrease of $A$ in response to increase in $D_a^w$ and $E$ independent of stomatal response (e.g. Sharkey, 1984; Bunce, 1988b). Our data do not rule out such an effect in ivy at high values of constant $\chi_i^c$. 

Figure 6.3. Relationship between stomatal conductance ($g_w^s$) and leaf surface water vapour deficit ($D_w^s$) measured at constant leaf temperature and intercellular CO$_2$ mol fraction ($T_l=20$ °C, $\chi_i^c \approx 200$ $\mu$mol mol$^{-1}$, and $I= 490$ $\mu$mol m$^{-2}$ s$^{-1}$). The different symbols indicate data from different plants, the dashed line is the relationship used in the model.
Figure 6.4. Relationship between stomatal conductance ($g_{sw}$) and intercellular CO$_2$ mol fraction ($\chi^c_i$) measured at constant leaf temperature and leaf surface water vapour deficit ($T_l= 20$ °C, $D_{sw}= 7$ mmol mol$^{-1}$, and $I= 490$ µmol m$^{-2}$s$^{-1}$). The different symbols indicate data from different plants, the dashed line is the relationship used in the model.

$D_{sw}$ and $E$.

The ratio $\chi^c_i/\chi^c_s$ decreased with increasing $D_{sw}$ (Fig. 6.7), and with increasing $\chi^c_i$ (Fig. 6.8). However, the slopes were not significantly different from zero at $P=0.05$ ($P=0.12$ for $D_{sw}$, and $P=0.07$ for $\chi^c_i$).

**Experiment III**

In the factorial experiment there were effects of both $D_{sw}$ and $\chi^c_i$ on $g_{sw}$ in agreement with experiment II, but in the factorial experiment $g_{sw}$ was higher than in the previous experiment. The ANOVA of the untransformed $g_{sw}$ data yielded a significant interaction term ($P=0.03$), indicating that the effects of $\chi^c_i$ and $D_{sw}$ are not additive. Using logarithms to transform these same data before computing the ANOVA, yielded a non-significant interaction (Table 6.2). That the effects of $\chi^c_i$ and $D_{sw}$ were additive in the log-transformed data indicates that the raw effects of $\chi^c_i$ and $D_{sw}$ on $g_{sw}$ were multiplicative, as assumed in the model. This kind of interaction has been assumed in models for other species (Jarvis, 1976; Avissar et al., 1985). As expected the effect of $\chi^c_i$ on $A$ was highly significant, but no effect of $D_{sw}$ on $A$ or interaction between $D_{sw}$ and $\chi^c_i$ was observed (Table 6.2).

The ratio $\chi^c_i/\chi^c_s$ was affected by $D_{sw}$ and $\chi^c_i$ (Table 6.2), decreasing with increase
**Figure 6.5.** Relationship between CO\(_2\) flux density \((A)\) and intercellular CO\(_2\) mol fraction \((\chi^c_i)\) measured at constant leaf temperature and leaf surface water vapour deficit \((T_l=20 \, ^\circ \text{C}, D_{ws}^w = 7 \, \text{mmol mol}^{-1}, \text{and } I= 490 \, \mu \text{mol m}^{-2}\text{s}^{-1})\). The different symbols indicate data from different plants, the dashed line is the relationship used in the model.

**Figure 6.6.** Relationship between CO\(_2\) flux density \((A)\) and leaf surface water vapour deficit \((D_{ws}^w)\) measured at constant leaf temperature and intercellular CO\(_2\) mol fraction \((T_l=20 \, ^\circ \text{C}, \chi^c_i \approx 200 \, \mu \text{mol mol}^{-1}, \text{and } I= 490 \, \mu \text{mol m}^{-2}\text{s}^{-1})\). The different symbols indicate data from different plants.
Figure 6.7. Relationship between the $\chi_i^c/\chi_s^c$ ratio and leaf surface water vapour deficit ($D_w^s$) measured at constant leaf temperature and intercellular CO$_2$ mol fraction ($T_l=20 \, ^\circ C$, $\chi_i^c \approx 200 \, \mu$mol mol$^{-1}$, and $I=490 \, \mu$mol m$^{-2}$ s$^{-1}$). The different symbols indicate data from different plants.

Figure 6.8. Relationship between the $\chi_i^c/\chi_s^c$ ratio and intercellular CO$_2$ mol fraction ($\chi_i^c$) measured at constant leaf temperature and leaf surface water vapour deficit ($T_l=20 \, ^\circ C$, $D_w^s=7 \, \text{mmol mol}^{-1}$, and $I=490 \, \mu$mol m$^{-2}$ s$^{-1}$). The different symbols indicate data from different plants.
Table 6.2. Effects of leaf surface water vapour deficit ($D^w_s$) and intercellular CO$_2$ concentration ($\chi^c_i$) on stomatal conductance ($g^w_s$), CO$_2$ flux density ($A$), and the $\chi^c_i/\chi^c_s$ ratio. $T_i=20$ °C, $I=500$ µmol m$^{-2}$ s$^{-1}$. **Part A:** means and standard errors of the means (in brackets). **Part B:** summary table of analysis of variance. A complete randomized blocks design was used, the plants being the blocks. M.S.: mean square, $P$: probability.

**Part A: means and standard errors**

<table>
<thead>
<tr>
<th>$\chi^c_i$</th>
<th>$D^w_s$</th>
<th>$g^w_s$</th>
<th>$A$</th>
<th>$\chi^c_i/\chi^c_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µmol mol$^{-1}$)</td>
<td>(mmol mol$^{-1}$)</td>
<td>(mmol m$^{-2}$ s$^{-1}$)</td>
<td>(µmol m$^{-2}$ s$^{-1}$)</td>
<td>(mol mol$^{-1}$)</td>
</tr>
<tr>
<td>200</td>
<td>6</td>
<td>127(17.5)</td>
<td>8.5(0.30)</td>
<td>0.64(0.023)</td>
</tr>
<tr>
<td>200</td>
<td>12</td>
<td>73(12.6)</td>
<td>8.5(0.39)</td>
<td>0.51(0.048)</td>
</tr>
<tr>
<td>290</td>
<td>6</td>
<td>66(18.4)</td>
<td>12.1(0.59)</td>
<td>0.48(0.079)</td>
</tr>
<tr>
<td>290</td>
<td>12</td>
<td>44(9.0)</td>
<td>12.4(0.90)</td>
<td>0.38(0.055)</td>
</tr>
</tbody>
</table>

**Part B: analysis of variance**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>$\log(g^w_s)$</th>
<th>$A$</th>
<th>$\chi^c_i/\chi^c_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M.S.</td>
<td>$P$</td>
<td>M.S.</td>
<td>$P$</td>
</tr>
<tr>
<td>$\chi^c_i$</td>
<td>1</td>
<td>1.126</td>
<td>&lt;0.001</td>
<td>41.11</td>
</tr>
<tr>
<td>$D^w_s$</td>
<td>1</td>
<td>0.650</td>
<td>0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>interac.</td>
<td>1</td>
<td>0.025</td>
<td>0.233</td>
<td>0.08</td>
</tr>
<tr>
<td>plants</td>
<td>2</td>
<td>0.408</td>
<td>0.001</td>
<td>2.95</td>
</tr>
<tr>
<td>error</td>
<td>6</td>
<td>0.014</td>
<td>—</td>
<td>0.42</td>
</tr>
</tbody>
</table>

In both $D^w_s$ and $\chi^c_i$. The measurements at different $\chi^c_i$ were made by changing $\chi^c_s$, and so are equivalent to those reported in the literature, except that I kept $D^w_s$ constant. However, in contrast to previous reports that $\chi^c_i/\chi^c_s$ (or $\chi^c_i/\chi^c_a$) is not affected by change in $\chi^c_a$ (e.g. Louwerse, 1980), in ivy there was a significant, although small, effect. In experiment II this effect was also observed, although not significant. There was no interaction between the effects of $\chi^c_i$ and $D^w_s$ on $\chi^c_i/\chi^c_s$ (Table 6.2).

In other species it has been observed that the effects of changes in $\chi^c_i$ and $D^w_s$ on $g^w_s$ are proportional to the current value of $g^w_s$: $\frac{dg^w_s}{dD^w_s}$ and $\frac{dg^w_s}{d\chi^c_i}$ were linearly correlated with $g^w_s$ in four grass species (Morison & Gifford, 1983). It is difficult to assess whether this is also true for ivy from the response curves to $\chi^c_i$ and $D^w_s$ (Figs. 6.4 & 5.1), but the fact that the effects of $\chi^c_i$ and $D^w_s$ on $\log(g^w_s)$ do not interact, i.e. are additive, seems to indicate that the effects of $\chi^c_i$ and $D^w_s$ on $g^w_s$ are proportional to $g^w_s$ (Table 6.2).

The data in Table 6.1 show that the effect of wind speed on $g^w_s$ is fully explained.
Figure 6.9. Simulated relationship between stomatal conductance \( g_{ws} \) and bulk air water vapour molar fraction \( \chi_{wa} \) for \( \square g_{bw}^w = 100 \), \( \diamondsuit g_{bw}^w = 200 \), \( \circ g_{bw}^w = 500 \), and \( \triangle g_{bw}^w = 1000 \) mmol m\(^{-2}\) s\(^{-1}\), under constant leaf temperature and bulk air CO\(_2\) mol fraction \( T_l = 20 \) °C, \( \chi_{ca} = 350 \) µmol mol\(^{-1}\), and \( I = 500 \) µmol m\(^{-2}\) s\(^{-1}\). Simulation based on response data in Figs. 6.3, 6.4, & 6.5.

by its effect on the CO\(_2\) and water vapour molar fractions at the leaf surface, and the data in Table 6.2 show that the effects of CO\(_2\) and humidity affect \( g_{ws} \) multiplicatively, thus bearing out the two main assumptions of the model.

### 6.3.2 Simulated responses of \( g_{ws} \) and \( A \) to bulk air state variables

**Water vapour molar fraction**

The model was used to calculate the responses of \( g_{ws} \) and \( A \) to \( \chi_{wa} \). Using as input the relationships indicated with dashed lines in Figs. 6.3, 6.4 & 6.5, the model yields the results in Figs. 6.9, 6.10, 6.11 & 6.12. As expected, \( g_{ws} \) increased with \( \chi_{wa} \), the slope being steeper at higher values of \( g_{bw} \) (Fig. 6.9). The response to \( g_{bw} \) was larger at lower ambient humidity, and the stomata partially compensated for the decrease in \( g_{bw} \)—i.e. \( g_{ws} \) was higher at lower values of \( g_{bw} \).

Because of the change in \( g_{bw} \), \( \chi_{ci} \) changed in response to both \( \chi_{wa} \) and \( g_{bw} \) (Fig. 6.10), and so \( A \) also changed (Fig. 6.11). The magnitude of the effect of \( \chi_{wa} \) on \( A \) depended on the value of \( g_{bw} \), this being a reflection of the effect of \( \chi_{wa} \) on \( \chi_{ci} \). The simulated response of \( \chi_{ci} \) to \( \chi_{wa} \), lower \( \chi_{ci} \) values at lower \( \chi_{wa} \), is similar to that observed in real experiments (Sandford, 1984, Fig. 7.8). The slope of this response was sensitive to \( g_{bw} \),
**Figure 6.10.** Simulated relationship between intercellular CO$_2$ mol fraction ($\chi_{ci}$) and bulk air water vapour molar fraction ($\chi_{wa}$) for (□) $g_b^w = 100$, (◇) $g_b^w = 200$, (○) $g_b^w = 500$, and (△) $g_b^w = 1000$ mmol m$^{-2}$ s$^{-1}$, under constant leaf temperature and bulk air CO$_2$ mol fraction ($T_l = 20$ °C, $\chi_{ca}^c = 350$ µmol mol$^{-1}$, and $I = 500$ µmol m$^{-2}$ s$^{-1}$). Simulation based on response data in Figs. 6.3, 6.4, & 6.5.

**Figure 6.11.** Simulated relationship between CO$_2$ flux density ($A$) and bulk air water vapour molar fraction ($\chi_{wa}$) for (□) $g_b^w = 100$, (◇) $g_b^w = 200$, (○) $g_b^w = 500$, and (△) $g_b^w = 1000$ mmol m$^{-2}$ s$^{-1}$, under constant leaf temperature and bulk air CO$_2$ mol fraction ($T_l = 20$ °C, $\chi_{ca}^c = 350$ µmol mol$^{-1}$, and $I = 500$ µmol m$^{-2}$ s$^{-1}$). Simulation based on response data in Figs. 6.3, 6.4, & 6.5.
being steeper at higher values of $g_w^b$. The simulated response of $A$ to $g_w^b$ was small, as it also was in the ‘wind speed’ experiment (Fig. 6.11 vs. Table 6.1).

The response of $g_w^s$ is both a reflection and a cause of the changes in $\chi_c^i$ and $D_w^s$ (Figs. 6.10 & 6.12). As expected, $D_w^s$ increased with decreasing $\chi_w^a$, but the relationship between $D_w^s$ and $\chi_w^a$ was different at different values of $g_w^b$. As a consequence of this, both the slope and the intercept of the response of $g_w^s$ to $\chi_w^a$ changed with $g_w^b$. At high values of $g_w^b$, the response was steeper, and $g_w^s$ was lower than at low values of $g_w^b$.

Part of the effect of $\chi_w^a$ on $g_w^s$ was through $\text{CO}_2$. This seems paradoxical, but is an unavoidable effect on $g_w^s$ of the decrease in $\chi_c^i$ that occurs in response to a decrease in $\chi_w^a$. This indirect effect of $\chi_w^a$ on $g_w^s$ can be seen in the control diagram in Fig. 6.1 by following the path that starts at $d\chi_w^a$, and goes through $\partial D_w^s/\partial \chi_w^a$, $dD_w^s$, $\partial g_w^s/\partial D_w^s$, $dg_w^s$, $\partial A/\partial g_w^s$, $dA$, $\partial \chi_c^i/\partial A$, $d\chi_c^i$, $\partial g_w^s/\partial \chi_c^i$, and ends at $dg_w^s$. Because a decrease in $\chi_c^i$ normally leads to higher $g_w^s$ ($\partial g_w^s/\partial \chi_c^i < 0$), this effect is a source of negative feedback on $g_w^s$.

The boundary layer is also a source of positive feedback. If $\chi_w^a$ remains unchanged, an increase in $g_w^s$ causes a decrease in $D_w^s$, and this decrease in $D_w^s$ would lead to further increase in $g_w^s$. Negative feedback through $\text{CO}_2$ stabilizes the response to $D_w^s$ because an increase in $\chi_w^a$ leads to an increase in both $g_w^s$ and $\chi_c^i$. In the model $\chi_c^i$ is
the only source of negative feedback, but in the real world other sources of feedback could be present.

If the response of stomata to $D_{ws}$ is a direct effect — i.e. feedforward —, and not an indirect effect of leaf water status, then a source of negative feedback is required for stability. This is so because, as explained above, the boundary layer is a source of positive feedback on $g_{ws}$. In the absence of negative feedback, the response of $g_{ws}$ to $D_{ws}$ would have only two stable states: fully open, and fully closed stomata. In a ‘noisy’ environment the state of an individual stoma would be unpredictable.

**Carbon dioxide molar fraction**

The model was also used to calculate the responses of $g_{ws}$ and $A$ to $\chi_c$. Using as input the relationships indicated by dashed lines in Figs. 6.3, 6.4, & 6.5, the model yields the results in Figs. 6.13, 6.14, 6.15 & 6.16. $g_{ws}$ decreased with $\chi_c$, the slope being similar at the different values of $g_{wb}$ (Fig. 6.13). $\chi_c$ followed the change in $\chi_c$, and had a large effect on $A$ (Figs. 6.14 & 6.15).

In contrast to the response of $g_{ws}$ to $D_{ws}$, the response of $\chi_c$ is inherently stable because there is negative feedback between $g_{ws}$ and $\chi_c$. The variable sensed by stomata is $\chi_f$, and its value is affected by $g_{ws}$ ($g_{wb}$ and $g_{ws}$ in series). However, positive feedback through $D_{ws}$ partly cancels the negative feedback attributable to $\chi_c$. This feedback
Figure 6.14. Simulated relationship between CO$_2$ flux density ($A$) and bulk air CO$_2$ molar fraction ($\chi^c_a$) for (□) $g^w_b = 100$, (◇) $g^w_b = 200$, (○) $g^w_b = 500$, and (△) $g^w_b = 1000$ mmol m$^{-2}$ s$^{-1}$, under constant leaf temperature and water vapour molar fraction in the bulk air ($T_l = 20$ °C, $\chi^w_a = 15$ mmol mol$^{-1}$, and $I = 500$ µmol m$^{-2}$ s$^{-1}$). Simulation based on stomatal response data in Figs. 6.4, 6.4, & 6.5.

Figure 6.15. Simulated relationship between intercellular CO$_2$ mol fraction ($\chi^i_c$) and bulk air CO$_2$ molar fraction ($\chi^c_a$) for (□) $g^w_b = 100$, (◇) $g^w_b = 200$, (○) $g^w_b = 500$, and (△) $g^w_b = 1000$ mmol m$^{-2}$ s$^{-1}$, under constant leaf temperature and water vapour molar fraction in the bulk air ($T_l = 20$ °C, $\chi^w_a = 15$ mmol mol$^{-1}$, and $I = 500$ µmol m$^{-2}$ s$^{-1}$). Simulation based on response data in Figs. 6.4, 6.4, & 6.5.
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Figure 6.16. Simulated relationship between leaf surface water vapour deficit ($D_w^s$) and bulk air CO$_2$ molar fraction ($\chi_c^a$) for $\square$ $g_w^b=100$, $\Diamond$ $g_w^b=200$, $\bigodot$ $g_w^b=500$, and $\triangle$ $g_w^b=1000$ mmol m$^{-2}$ s$^{-1}$, under constant leaf temperature and water vapour molar fraction in the bulk air ($T_l=20^\circ C$, $\chi_w^a=15$ mmol mol$^{-1}$, and $I=500$ µmol m$^{-2}$ s$^{-1}$). Simulation based on response data in Figs. 6.4, 6.4, & 6.5.

Through $D_w^s$ is a consequence of the change in $D_w^s$ in response to $\chi_c^a$ (Fig. 6.16). This source of feedback can be seen in the control diagram (Fig. 6.1) by following the path that starts at $d\chi_c^a$, and goes through $dg_w^w$, $dE$, and $dD_w^s$, ending at $dg_w^s$. The simulated effect of $g_w^w$ on the response of $g_w^s$ to CO$_2$ is not the same as that on the response of $g_w^s$ to humidity. The apparent sensitivity of $g_w^s$ to $\chi_c^a$ is higher at larger $g_w^b$. In contrast, the apparent sensitivity of $g_w^s$ to $\chi_c^a$ is not much affected by $g_w^b$: only the intercept changes (Figs. 6.9 & 6.13).

The response to CO$_2$ is affected by the gain of the humidity response loop ($\partial g_w^s / \partial D_w^s$), and by the gain of the CO$_2$ response loop ($\partial g_w^w / \partial \chi_c^a$). If the total gain of this loop is > 0 the boundary layer behaves as an amplifier. By running the model with data adjusted to make the stomata insensitive to $D_w^s$ ($\partial g_w^w / \partial D_w^s = 0$; in practice $f_0(D_w^w) = 1$, for any value of $D_w^w$, in Equation 6.4), the apparent sensitivity of $g_w^w$ to changes in $\chi_c^a$ is reduced at low values of $g_w^b$ (Fig. 6.17). The normal response to $D_w^s$ amplifies the response to $\chi_c^a$ under constant $D_w^s$, the gain depending on $g_w^w$.

At a given $\chi_c^a$, $g_w^w$ is smallest at $D_w^s=D_w^a$ i.e. $g_w^b=\infty$. The magnitude of the effect on $g_w^w$ of a change in $g_w^b$ depends on the relation between $g_w^w$ and $g_w^s$, i.e. when $g_w^b/g_w^s \approx 10$ this effect is very small. However, under low wind speed, when this ratio is smaller,
Figure 6.17. Simulated relationship between stomatal conductance \( (g_w^s) \) and bulk air CO\(_2\) molar fraction \( (\chi_c^a) \) for \((\square)\) \( g_w^b = 100 \), \((\diamondsuit)\) \( g_w^b = 200 \), \((\circ)\) \( g_w^b = 500 \), and \((\triangle)\) \( g_w^b = 1000 \) mmol m\(^{-2}\) s\(^{-1}\), under constant leaf temperature and water vapour molar fraction in the bulk air \((T_l= 20 \, ^\circ\text{C}, \chi_w^a = 15 \text{ mmol mol}^{-1}, \text{and} \ I = 500 \mu\text{mol m}^{-2}\text{s}^{-1})\). The stomata were assumed to be insensitive to humidity. Simulation based on response data in Figs. 6.4, & 6.5.

the effect of the boundary layer on \( g_w^s \) is larger.

**Wind speed**

The profile of water vapour mol fraction across the boundary layer has been measured for single leaves, and it depends on \( E \) and wind speed (Kitano & Eguchi, 1987b; Kitano & Eguchi, 1987a). Based on data for *Picea sitchensis* it has been proposed that reversible responses of stomata to wind depend on a response to humidity at the leaf surface (Grace *et al.*, 1975). This hypothesis seemed to be confirmed by the results of Bunce (1985). However, although a change in humidity at the leaf surface is the most obvious effect of the boundary layer, as suggested by Meidner & Mansfield (1968, page 100), CO\(_2\) must also be involved in the stomatal response to wind speed in those species and conditions in which stomata are sensitive to CO\(_2\). The model takes into account the effects of both \( D_w^s \) and \( \chi_i^c \) on \( g_w^s \).

Wind speed alters \( g_w^b \), so it affects the apparent response of stomata to \( \chi_w^a \) and \( \chi_w^c \) (Figs. 6.9 & 6.13). The results generated by the model for different values of \( g_w^b \), given above, can be plotted against wind speed for a leaf of a given dimension, obtaining in this way a response curve of \( g_w^s \) to wind speed (solid line in Fig. 6.18). The relationship
between wind speed and the thickness of the boundary layer is not linear. Most of the effect of wind on $g_w^s$ occurs at low wind speeds ($<0.5$ m s$^{-1}$).

Working with a model makes it easy to answer the question: how important is the part of the effect of wind speed on $g_w^s$ that is mediated by the response of $g_w^s$ to CO$_2$? By running the model with stomata insensitive to $D_w^s$ the effect through $\chi_i^c$ can be isolated from that through humidity. In ivy, at $\chi_a^w=15$ mmol mol$^{-1}$ and $T_i=20$ °C, the effect mediated by $\chi_i^c$ is roughly one third of the total effect of wind speed (broken line in Fig. 6.18).

The thickness of the boundary layer depends on the dimension of the leaf. At a given $\chi_a^w$ and $\chi_a^c$, a large and a small leaf with identical responses of $g_w^s$ to $D_w^s$ and $\chi_i^c$ would show different values of $g_w^s$ at the same wind speed. This has methodological implications for the measurement of $A$, $E$, and $g_w^s$ in the field. Data measured with a diffusion porometer by briefly enclosing a leaf is not comparable to data measured in a gas-exchange system. In the field, the wind speed prevailing at the time of the porometric measurement, as well as leaf size, affects the observed $g_w^s$. Field experiments with gas-exchange systems that track environmental conditions (e.g. Koch et al., 1971), give results that are biased whenever the wind speed inside the cuvette is different to that outside. Some of the species in which very high $g_w^s$ have been observed have large leaves (Körner et al., 1979; Grace et al., 1982), and it would be interesting to know whether this very high $g_w^s$ results from differences in stomatal sensitivity to $\chi_i^c$ and $D_s^w$ or whether it is caused by the thicker boundary layer of large leaves.

### 6.3.3 Caveat

The experiments discussed above show the effects of the boundary layer on stomatal responses in leaves artificially kept at a constant temperature. This is a simplification that helps us understand the responses to CO$_2$ and humidity, but is unrealistic because it does not take into account the effect of $E$ and $g_w^s$ on the temperature of the leaf. Evaporative cooling is a source of negative feedback on $D_w^s$, and so indirectly on $g_w^s$, and of either positive or negative feedback on $g_w^s$ through $T_i$, depending on the sign of the response of stomata to $T_i$. Keeping leaf temperature constant makes these feedback loops ineffective. In nature the feedback through $T_i$ could help to stabilize $g_w^s$, preventing oscillation, as demonstrated by Farquhar and Cowan (1974), but as $g_w^s$ in *Hedera helix* was stable under constant $T_i$ this simplification does not invalidate our argument. When $E$ is high, feedback can also occur through the bulk water status of the leaf.
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Figure 6.18. Simulated relationship between stomatal conductance \( (g_w^w) \) and wind speed \( (u) \) under constant leaf temperature, bulk air water vapour mol fraction and bulk air CO\(_2\) mol fraction \( (T_l = 20^\circ\text{C}, \chi_c^w = 350 \mu\text{mol mol}^{-1}, \chi_a^w = 15 \text{ mmol mol}^{-1}, \text{ and } I = 500 \mu\text{mol m}^{-2}\text{s}^{-1}) \). A leaf mean dimension of 7 cm was assumed in the calculations. The solid line is the simulated response for a normal ivy leaf, the broken line is the simulated response for a leaf with stomata insensitive to \( D_w^w \). Simulation based on response data in Figs. 6.3, 6.4, & 6.5.

6.4 Conclusions

As stomata of ivy are sensitive to both CO\(_2\) and humidity, the effect of the boundary layer conductance and wind speed on \( g_w^w \), is mediated by both CO\(_2\) and water vapour mol fractions at the leaf surface. The effect of the boundary layer on \( \chi_c^c \) and \( D_w^w \) also modifies the apparent responses of stomata to \( \chi_c^c \) and \( \chi_a^w \). The apparent response to \( \chi_c^c \) depends on the stomatal sensitivity to both CO\(_2\) and humidity, as the apparent response to \( \chi_a^w \) also does. A decrease in \( g_b^w \) causes a small increase in \( g_w^w \) that reduces its impact on \( g_t^w \). A feedforward response to humidity would need compensatory negative feedback through another variable for stability.
Chapter 7

Models of stomatal responses to the environment

7.1 Introduction

In this chapter I will discuss the problems that arise when the difference between empirical and mechanistic models is not taken into consideration when interpreting their behaviour. First I will discuss these problems in general, and afterwards in relation to a model of stomatal behaviour developed recently (Ball et al., 1987; Ball, 1988). In the discussion below, I will follow Hall & Day (1977) and Ören (1984) in the use of terms referring to models and modelling.¹

Models must be tested for their agreement with both agreed theory and experimental data. A theoretical analysis of a model includes the identification of all the assumptions involved and a check of the consistency of its logic structure. The validation of a model is a test of its agreement with the object modelled.

¹Definitions, following Hall & Day (1977) and Ören (1984).

**System.** A system is any object whose behaviour is of interest.

**Model.** A model is any abstraction or simplification of a system.

**State variable.** State variables are quantitative representations of the entities of the system that change (e.g. with time, in dynamic models).

**Driving variable.** Inputs from outside the system of interest are called forcing functions, or driving variables.

**Simulation.** A simulation is an experiment done with a model.

**Structure.** The structure of a model is given by the functional relation, without specification of values for the parameters.

**Behaviour.** The behaviour of a model is defined by the value of the state variables.
The empirical validation of a model’s behaviour does not constitute a validation of its structure or assumptions, and least of all, of the way in which the results of the simulation are being interpreted. There are different kinds of validation: (1) validation of model behaviour, (2) validation of model structure, and (3) validation of the interpretation of the results.

As discussed in Section 1.2.1, empirical models are also called descriptive because they simply describe the relationship between two or more variables while mechanistic models include indications of causality (Hall & Day, 1977). The behaviour of an empirical model can be valid or invalid, depending on whether it agrees with experimental data or not, but the structure of an empirical model is assumed a priori to be invalid —i.e. the functional relation is of no interest, as in curve fitting. The aim for mechanistic models is to mimic the structure of a real system —i.e. the functional structure of the model is expected to be a reflection of that of the real system. However, valid behaviour does not guarantee a valid structure. In a mechanistic model it is assumed that its structure can be validated, but its validation needs much more support than the simple agreement of observed and predicted final behaviour. The validation of the structure requires the validation of the internal behaviour of the model —i.e. causal relationships must be experimentally demonstrated. For the interpretation of the results of a simulation to be valid it is also necessary to prove the validity of all the assumptions, explicit and implicit, involved in the interpretation.

The interpretation of the results of simulations often includes the inference of causal relationships. The distinction between causal relationship and correlation seems in practice to get blurred when complex models are involved. The process for establishing causal relationships cannot be reversed. The nature and existence of the causal links must be demonstrated a priori to the construction of a mechanistic model. Empirical models cannot be used to prove causal relationships. It is easy to recognize that a correlation between an arbitrary set of variables does not necessarily imply causation. However when these same variables are transformed by means of a complex model, correlations are in some cases erroneously used to infer causation.

### 7.2 Analysis of Ball’s empirical model

#### 7.2.1 The model

A simple, quantitative, empirical model of stomatal conductance has been recently developed (Ball et al., 1987; Ball, 1988). The model was based on data from a series of gas-exchange experiments in which the responses of $g^w_s$ to many variables and their
interactions were studied. This model provided a concise description of Ball’s data set, and has been successfully fitted to data from other species (Leuning, 1990).

Ball (1988, page 11) says: ‘The empirical approach which we have used in this work does not presuppose knowledge of the mechanistic bases of the responses described by the model. Nevertheless, the analysis may provide insights into the mechanistic basis of guard cell function.’ I agree with this possibility, with the caveat that it requires many a priori assumptions, but I completely disagree with his interpretation of the results.

Ball (1988, page 21) also says that ‘...normalizing stomatal conductance with respect to \( A \) is a means of separating the influence associated with photosynthesis from the presumably separate responses of stomata to \( \text{CO}_2 \) and humidity.’ and ‘The mechanistic basis of the linear conductance/assimilation relationship is not clear and we reiterate that this empirical analysis is not predicated upon any particular relationship.’ Nevertheless, as I show below, when Ball used the model to conclude that \( g^w_s \) responds to \( h_s \), he implicitly interpreted this ‘association’ between \( g^w_s \) and \( A \) as causation, and this is what I want to challenge.

### 7.2.2 Is Ball’s interpretation of the model valid?

The model in its simplest form\(^2\) is:

\[
g^w_s = k A h_s \chi_s \tag{7.1}
\]

and the good fit of some data sets to this model was used as a basis for stating that ‘...stomata respond to relative humidity’ (Ball et al., 1987). However by rearranging the equation above we obtain:

\[
A = k^{-1} g^w_s \frac{\chi_s}{h_s} \tag{7.2}
\]

so, this model could as well be used to conclude that assimilation rate responds to \( 1/h_s \). This counter-example demonstrates that implicit assumptions are more important to the outcome of this reasoning process than the actual data. Why is this so? The reason is that we are unconsciously assuming, when making these mechanistic interpretations of the model, that all the variables to the right of the equals sign can be treated as driving variables —i.e. we are assuming that these variables are exogenous to the system modelled. We are assuming a priori that \( A \) controls \( g^w_s \), or vice versa. The former is what the authors who developed the model have assumed and this reflected

\(^2\)for some species \( g^w_s = k_0 + k_1 A h_s / \chi_s \) was used
not only in their interpretation of the model but also in the way in which they have plotted the data.

It has been observed that a feedback loop links \( g_s^w \) and \( A \) (Farquhar et al., 1978), which means that \( A \) and \( g_s^w \) are interdependent (Fig. 6.1). It has also been shown that both \( A \) and \( g_s^w \) can respond independently of each other to environmental variables (Jarvis & Morison, 1981), including light (Meidner, 1968; Karlsson et al., 1983; Aphalo & Sánchez, 1986) and humidity (Bunce, 1988b) (See also discussions in Chapters 4 & 6). From this evidence it follows that neither of the two assumptions is correct — neither \( A \) controls \( g_s^w \), nor \( g_s^w \) controls \( A \). Neither \( A \) nor \( g_s^w \) can be considered to be driving variables in the real world. They are both state variables, and it is impossible to experimentally control them without altering any environmental variable.

Two main objections can be made to the original interpretation of the model. Firstly, it does not take into account that equation 7.1 is only partially determined because there are two unknowns in it: \( A \) and \( g_s^w \). This means that there are an infinite number of pairs of values of \( A \) and \( g_s^w \) that satisfy this equation. Secondly, a functional relationship has been taken as equivalent to a causal relationship. In this I follow Bunge (1959, pages 92–95) who raises several objections to a functional view of causation, some of which are as follows: ‘(a) Functions express constant relations . . . But functions are insufficient to state anything concerning the cause that produces the state or the phenomenon in question . . . (b) Functional relations are reversible whenever the functions in question are single valued . . . whereas genuine causal connections are essentially asymmetrical . . . The failure to account for the genetic connections is a shortcoming of the functional relation. But not all connections in the world are genetic; in many, perhaps in most, cases we are confronted with interdependence, as it is shown by the pervasiveness of the function concept in the sciences.’

### 7.2.3 An alternative interpretation

What it is possible to say is that there is a relationship between \( A \) and \( g_s^w \), and that they are not independent. This is not a simple one way relationship, \( A \) and \( g_s^w \) affect each other through \( \chi_c \), and the relationship also depends on their responses to other variables. The parallel responses of \( g_s^w \) and \( A \) to \( I \) and other variables contribute, under natural conditions, to the correlation between \( A \) and \( g_s^w \), but, as discussed in Chapter 4, this correlation can be experimentally broken.

By eliminating \( A \) and \( g_s^w \) from the model we obtain an expression showing what it is that remains constant when \( A \) and \( g_s^w \) change concurrently in response to \( I, D_s^w \),
and $T_l$. Assimilation rate is

$$A \approx g^c_s (\chi^c_s - \chi^c_i) \quad (7.3)$$

and

$$g^c_s = 0.63 g^w_s. \quad (7.4)$$

By substituting equations 7.3 and 7.4 in 7.1, and then rearranging we get

$$g^w_s = 0.63 k g^w_s \left(1 - \frac{\chi^c_i}{\chi^c_s}\right) h_s, \quad (7.5)$$

eliminating $g^w_s$ and rearranging we get:

$$\chi^c_i = \chi^c_s \left(1 - \frac{1.60}{kh_s}\right) \quad (7.6)$$

which is the solution for $\chi^c_i$ given by Ball (1988, Equation A2.3). This relationship remains true for all the data that fit the model, whatever the measurement conditions. It is invariant for changes in $T_l$, $I$, and $D^w_{ws}$. $\chi^c_i/\chi^c_s$ is a function only of $h_s$ — i.e. simultaneous changes of $A$ and $g^w_s$ in response to other variables do not affect this relationship.

To return to Equation 7.2, the dependence of $A$ on $1/h_s$, is somewhat puzzling. However, once we realize that $g^w_s$ is not an independent variable but an increasing function of $h_s$, it is easy to visualize $1/h_s$ as a ‘correction’ for the steeper increase of $g^w_s$ than of $A$ as $h_s$ increases. The increase in $A$, when $g^w_s$ increases in response to $h_s$, is less than proportional because (1) the increase in $\chi^c_i$ is less than proportional to the increase in $g^w_s$ because as $\chi^c_i$ increases, $A$ also increases, affecting $C_s - C_i$, and (2) because the relationship between $A$ and $\chi^c_i$ is not linear. In other words, ‘$1/h_s$’ corrects $A$ for the effect of the curvature of the $A$ vs. $\chi^c_i$ relationship, and for the dependence of $\chi^c_s - \chi^c_i$ on $A$. Of course, this is also valid as an explanation for the apparent response of $g^w_s$ to $h_s$ in equation 7.1. But because of our preconceptions it is not as easy to accept it for equation 7.1 as it is for equation 7.2. Ball’s model gives no evidence in favour, or against, a hypothetical response of stomata to $h_s$. Such evidence must come from experiments such as those discussed in Chapter 5, which indicate that stomata respond to $D^w_s$ — not $h_s$.

The confusion surrounding the interpretation of this model stems from the fact that it does not predict the state of a single state variable, but rather a relationship between the state of two variables — $A$ and $g^w_s$. To use it for predicting the state of one of these two variables we need a value for the other variable under the same state of the driving variables. With this model if we have an independent estimate or measurement of $A$
we can predict $g_w$, or if we have $g_w$ we can predict $A$. It is as much a model of CO$_2$ assimilation as it is a model of stomatal conductance. It could be stated as

$$\frac{g_w}{A} = k \frac{h_s}{\chi_c}.$$  

In the original statement of the model $A$ is a driving variable, and this is not a problem for its use as a predictive tool. However, when making a mechanistic interpretation it is necessary to take into account which variables are operationally independent in the real world, and which are not.

### 7.2.4 Is the behaviour of the model valid?

Having identified the assumptions and logic behind the model, we may still test it by contrasting its operational behaviour with experimental data. It has been observed that $g_w$ and $A$ are usually linearly correlated under constant $\chi_a$ or $\chi_s$ and $D_w$ (Wong et al., 1979; Louwerse, 1980). This was also the case in ivy (Fig. 4.4 & Table 4.1). If in Ball’s model we replace $\chi_a$ and $h_s$ with constants we obtain $g_w = k' A$, which agrees with what has been observed in the real world. However, although this correlation is consistent, most authors have been cautious not to take it as evidence of a causal link (e.g. Wong et al., 1985c). As discussed in Chapter 4, there is a link between $A$ and $g_w$ caused by feedback through $\chi_i$ and also parallel responses of $g_w$ and $A$ to $I$ (see also Chapter 6).

When $\chi_a$ is altered $\chi_i$ changes in such a way that the ratio $\chi_i/\chi_a$ remains roughly constant (Louwerse, 1980; Morison & Gifford, 1983). These authors found that in some species the linear regression of $\chi_i$ on $\chi_a$ did not go exactly through the origin, implying that the ratio is not truly constant. This is also the case in ivy (See Fig. 6.8 and discussion in Chapter 6). As the model, in its simplest form, assumes a fixed ratio, it only approximates reality, but as deviations from a constant ratio are not too big, its behaviour can be considered satisfactory in this respect.

When $D_w$ or $T_1$ change, $\chi_i$ generally changes. As discussed in Chapter 5, stomatal responses to water vapour mol fraction and temperature are not consistent with a single response through $h_s$. However, as we have seen, Ball’s model implies that $\chi_i$ changes linearly with $h_s$ under constant $\chi_s$. In four grasses $\chi_i/\chi_a$ changed almost linearly with $D_w$ (Morison & Gifford, 1983). In ivy $\chi_i/\chi_s$ changed very little in response to $D_w$, being none the less higher at low $D_w$ (Fig. 6.7). The response of $\chi_i/\chi_s$ to $h_s$ was different under constant $T_1$ from that under constant $D_w$, and the biggest effect was that of $T_1$ under constant $h_s$ (Fig. 7.1). In Ball’s model temperature and humidity are represented by a single input variable, $h_s$, so this model is very unrealistic in its
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Figure 7.1. Ratio between intercellular and leaf surface CO\textsubscript{2} mol fractions vs. leaf surface relative humidity. Measured (□) under constant temperature ($T_1=20$ °C), or changing temperature and (△) constant leaf surface water vapour deficit ($D_{w_s}=10$ mmol mol\textsuperscript{-1}), or (○) constant leaf surface relative humidity ($h_s=0.60$); $I=340$ µmol m\textsuperscript{-2}s\textsuperscript{-1}. Data from one typical ivy plant from the experiment described in Chapter 5 obtained at the same time as data in Figs. 5.1b & 5.4b.

At a single temperature $h_s$ and $D_{w_s}$ are linearly related, so problems appear only when a range of values of $T_1$ is considered. This causes the model eventually to break down, as in the case of $T_1$ and humidity data for ivy —for these data a linear regression of $A$ on $g_{w_s}$ gives a better fit than Ball’s model (Fig. 7.2). As these data were measured at constant $\chi_c$, the only difference between the linear regression and Ball’s model is in whether $h_s$ is taken into account or not. Lloyd (1991) also found that for his data alternative models gave a better fit than Ball’s. He found that models that used $D_{w_s}$ instead of $h_s$ gave better fits and more consistent results at different values of $I$ and $T_1$.

7.2.5 Related models

Ball (1988), for data from some species, observed an intercept different from zero, the model then becoming

\[ g_{w_s}^{w} = k_0 + k_1 A \frac{h_s}{\chi_c^w}. \]  \hspace{1cm} (7.8)
Figure 7.2. Scatter diagrams of stomatal conductance \( (g_w^s) \) and CO\(_2\) flux density \( (A) \) (left) and of \( g_w^s \) and Ball’s index \( (A h_s/\chi^c_s) \) (right) at a range of temperature and humidity. Data measured under constant temperature \( (T_l=20\, ^\circ\text{C}) \) (□), or changing temperature and constant leaf surface water vapour deficit \( (D_w^s=10\, \text{mmol mol}^{-1}) \) (△), or constant leaf surface relative humidity \( (h_s=0.60) \) (○); \( I=340\, \mu\text{mol m}^{-2}\text{s}^{-1} \). Data from one typical plant from the experiment described in Chapter 5 obtained at the same time as data in Figs. 5.1b, 5.4b & 7.1.

Leuning (1990) found a small improvement in the correlation with data from *Eucalyptus grandis* by replacing \( \chi^c_s \) with \( \chi^c_s - \Gamma \) where \( \Gamma \) is the CO\(_2\) compensation point, his model being

\[
g_w^s = k_0 + k_1 A \frac{h_s}{\chi^c_s - \Gamma}. \tag{7.9}
\]

Lloyd (1991) tested several models, including Equations 7.8 and 7.9, and found that the best fit of \( g_w^s \) response data to humidity and temperature for *Macadamia integrifolia* was to the model

\[
g_w^s = \frac{1 - k_1 (1 - |T_l/T_{opt}|)}{k_2 \sqrt{D_w^s}}, \tag{7.10}
\]

were \( T_{opt} \) is the optimal \( T_l \) for \( g_w^s \). This model does not include \( A \), or \( \chi^c_s \), and so is closer to models like that of Jarvis (1976), than to Ball’s model. That Lloyd found the best fit to this model is probably a consequence of his data sets not including responses to \( I \) and \( \chi^c_s \). In these data sets, measured in the laboratory, two values of \( I \) were used, but the models were fitted separately to data for each value of \( I \). Another of the
models tested by this author,

\[ g_w^s = k_0 + k_1 \frac{A}{D_w^s \chi_i^c}, \]  

(7.11)

also gave a better fit than Equation 7.9 to the data for *Macadamia integrifolia*. This model differs from Ball’s model in that \( h_s \) has been replaced by \( 1/D_w^s \), and \( \chi_s^c \) has been replaced by \( \chi_i^c \). This model includes \( D_w^s \), but not \( T_1 \), being inadequate because, as discussed in Chapter 5, \( g_w^s \) frequently responds to \( T_1 \). But, the main problem is that this model also includes the factor \( A/\chi_i^c \), that under constant \( \chi_s^c \), is a function of \( g_w^s \).

If we have \( \chi_s^c, \chi_i^c, \) and \( A \), then we can calculate \( g_s^c \approx A/(\chi_s^c - \chi_i^c) \), and we do not need a model.

### 7.3 A new model

Based on the insight gained from this analysis, I have developed a new, but related, model that is a more flexible option than the original one. It is more flexible because I took into account both my data for ivy and Ball’s data during its development. Only the treatment of temperature and humidity responses have been changed from Ball’s model. The new model includes as driving variables both \( T_1 \) and \( D_w^s \), instead of only \( h_s \). The equation

\[ g_w^s = \frac{A}{\chi_s^c \left[ k + f_1(D_s^w) + f_2(T_1) + f_3(D_s^w, T_1) \right]} \]  

(7.12)

defines a family of models in which the slope of the \( A \) vs. \( g_w^s \) relationship is a function of both \( D_s^w \) and \( T_1 \). \( \chi_i^c \) for this model is given by

\[ \chi_i^c = \chi_s^c \left[ 1 - \frac{1.60}{k + f_1(D_s^w) + f_2(T_1) + f_3(D_s^w, T_1)} \right]. \]  

(7.13)

I suggest the following expressions for \( f_i \):

\[ f_1 = k_1 D_s^w, \]  

(7.14)

\[ f_2 = k_2 T_1, \]  

(7.15)

and

\[ f_3 = k_3 D_s^w T_1. \]  

(7.16)

This new model was tested by fitting it to data from ivy, and comparing the residual sum of squares to that of the fit to other models (Table 7.1). As what was changed was the description of humidity and temperature responses, the data used was from the
Table 7.1. Comparison between models of stomatal response. Residual sums of squares from least squares fits to data for Hedera helix from the experiments described in Chapter 5. Data were the mean of three plants for set A \((T_l=15–28 \, ^\circ C, \, D_{ws}=6–15 \, \text{mmol mol}^{-1}, \, h_s=0.4–0.75, \, I=200 \, \mu\text{mol m}^{-2} \text{s}^{-1}, \, \chi_c=350 \, \mu\text{mol mol}^{-1})\), and that from a typical plant for set B \((T_l=10–28, \, D_{ws}=5–15, \, h_s=0.4–0.75, \, I=340 \, \mu\text{mol m}^{-2} \text{s}^{-1}, \, \chi_c=350 \, \mu\text{mol mol}^{-1})\). Values for the coefficients given in Table B.1, page 132.

<table>
<thead>
<tr>
<th>Model</th>
<th>Eq.</th>
<th>SS_{residual} set A (n=8)</th>
<th>SS_{residual} set B (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (g_{ws}^w = k)</td>
<td>—</td>
<td>184</td>
<td>2568</td>
</tr>
<tr>
<td>2. (g_{ws}^w = kA)</td>
<td>—</td>
<td>51</td>
<td>1359</td>
</tr>
<tr>
<td>3. (g_{ws}^w = k \frac{Ah}{\chi_s})</td>
<td>7.1</td>
<td>782</td>
<td>6168</td>
</tr>
<tr>
<td>4. (g_{ws}^w = \frac{A}{\chi_s} (k_0 + k_1 D_{ws} + k_2 T_l))</td>
<td>—</td>
<td>30</td>
<td>690</td>
</tr>
<tr>
<td>5. (g_{ws}^w = \frac{A}{\chi_s} (k_0 + k_1 D_{ws} + k_2 T_l + k_3 D_{ws} T_l))</td>
<td>7.12</td>
<td>25</td>
<td>564</td>
</tr>
<tr>
<td>6. (g_{ws}^w = k_0 + k_1 D_{ws})</td>
<td>—</td>
<td>43</td>
<td>851</td>
</tr>
<tr>
<td>7. (g_{ws}^w = k_0 + k_1 D_{ws} + k_2 T_l)</td>
<td>—</td>
<td>42</td>
<td>272</td>
</tr>
<tr>
<td>8. (g_{ws}^w = \frac{1-k_1(1-T_l/T_{opt})}{k_2 \sqrt{D_{ws}}})</td>
<td>7.10</td>
<td>90</td>
<td>1373</td>
</tr>
</tbody>
</table>

For the data from ivy, Ball’s model gave the worst fit, even worse than the fit to a constant —i.e. the mean value of \(g_{ws}^w\) (model 3 vs. model 1 in Table 7.1). The linear regression, through the origin, of \(g_{ws}^w\) on \(A\) (model 2 in Table 7.1) explained 72 % of the variation around the mean in set A and 47 % in set B. The new model was tested with and without an interaction term (Equation 7.16). Without an interaction term (model 4 in Table 7.1) it gave a good fit, that was only slightly improved by the addition of an interaction term (model 5 in Table 7.1). The interaction term could be important in other data sets. For data sets in which the response to \(T_l\) has an optimum, a more complicated function could be necessary to describe this response.

Models that do not include \(A\) as a driving variable were also tested. These models (6–8 in Table 7.1) are unable to describe responses to variables for which \(A\) is a surrogate, but give good fits to the \(D_{ws}\) and \(T_l\) response data for ivy. Even very simple models (6 & 7 in Table 7.1) give much better fits than Ball’s model —the residual sum of squares for these models was the smallest, or nearly so. The model that gave
the best fit with Lloyd’s data for *Macadamia integrifolia* (model 8 in Table 7.1), gives larger sums of squares than the simple models. As discussed in Chapter 5 regressions of $g^w_s$ on $h_s$ give very unsatisfactory fits to the responses of $g^w_s$ to $D^w_s$ and $T_1$ for ivy.

### 7.4 Conclusions

Ball’s model is not a model of $g^w_s$ but a model of the relationship between $A$ and $g^w_s$. Its original interpretation was flawed, but a different interpretation highlights many of the properties of the coordinated changes of $A$ and $g^w_s$. In this respect it is a useful empirical, operational tool for some predictive purposes but, as any empirical model, it is of no use for defining causal relationships.

Ball’s model, although not mechanistic, is fairly realistic in its treatment of responses to $I$ and other variables that do not affect the $A$ vs. $g^w_s$ relationship. It is also realistic in its treatment of the effect of $\chi^c_s$ on the apparent relationship between $A$ and $g^w_s$. Its behaviour in response to $I$ and $\chi^c_s$ is realistic only under ‘normal’ conditions, but is not satisfactory under some experimental conditions, such as monochromatic light. The behaviour of Ball’s model is not realistic with respect to the effect of water vapour and temperature on $g^w_s/A$, but works properly under restricted conditions such as when $g^w_s$ increases with temperature.

As a prediction tool its usefulness is limited to this restricted set of conditions, and hindered by the need of $A$ as an input variable. When combined with a model of $A$ it can be used in the prediction of $g^w_s$ (Leuning, 1990; Collatz *et al.*, 1991). When the model is used in this way, $A$ becomes a surrogate for variables that affect both $A$ and $g^w_s$, but not the relationship between them. One of the most important of these variables is $I$, but replacing $A$ with $f(I)$ would be of no use because Ball’s model, and the model I developed, are both models of the relationship between $A$ and $g^w_s$. A completely new model, that takes into account the effects on $g^w_s$ of $I$, $\chi^c_1$, $D^w_s$, and $T_1$ would have to be developed.

Including in Ball’s model a more realistic treatment of responses to $T_1$ and $D^w_s$ should make its use by extrapolation much safer, and its use with other species such as ivy possible. The new model proposed is a step in this direction.

“Stomatal conductance” models that use the correlation with $A$ look, at first sight, very attractive because of their simplicity (few parameters). However, this simplicity comes at a high price: a complicated model is needed to simulate $A$ if they are to be driven by environmental variables alone. Models that do not rely on $A$ as a surrogate for environmental variables, need more parameters, at least one for each variable considered.
Chapter 8

Discussion

8.1 The contribution of this thesis

In the preface I defined the objective of this thesis by four questions. In the sections that follow I shall answer these questions, and also comment on the methods used.

8.1.1 What is the relationship between stomatal action and the rate of photosynthesis?

As discussed in Chapter 4, the mechanism behind the correlation between $A$ and $g_w^v$ is complex. This mechanism includes parallel, but independent, responses of photosynthesis and stomata to $I$, and an indirect response of stomata to $A$ through CO$_2$.

The consequence of this correlation under normal environmental conditions is that $\chi_{ci}^c$ remains nearly constant. This value of $\chi_{ci}^c$ is dependent on some variables such as $D_w^v$ and $\chi_{ca}^c$, but it is almost independent of others such as $I$.

Although it was clear from previous work that stomata respond directly to light, it was not clear whether the only additional response was through CO$_2$, or whether there was some other metabolite involved in this response. The experiments discussed in Chapter 4 clearly show that there is no need to postulate the existence of a messenger other than CO$_2$ to explain the response of stomata to light.

The relationship between stomatal action and the rate of photosynthesis is not simply a cause-effect relationship between $A$ and $g_w^v$. Neither $g_w^v$ controls $A$, nor $A$ controls $g_w^v$, but instead, this relationship depends on coordinated, but in part independent, responses of $g_w^v$ and $A$ to the environment. This coordination is effected by information being passed between processes that take place in the mesophyll and in the guard cells at the time $g_w^v$ and $A$ are responding to the environment, and also by information acquired by the genetic code of the plant during evolution. The question of why the
responses of $g_w$ and $A$ are coordinated in a way that usually keeps $\chi^c_i$ constant, has to be answered by means of optimization hypotheses that go beyond the scope of this thesis.

8.1.2 What is the nature of the interaction between stomatal responses to humidity and temperature?

From experiments described in the literature it was impossible to know which was the best way of expressing humidity when studying stomatal responses. The experiments described in Chapter 5 together with those recently done by Mott & Parkhurst (1991) make an important contribution towards solving this problem, by showing that relative humidity is inadequate, and that $D_w$ should be preferred.

The experiments described in Chapter 5 clearly show that responses of $g_w$ to $T_l$ and $D_w$ are independent and that they cannot be explained by a single response to $h_s$. As the response to $T_l$ usually displays an optimum, the apparent response to $h_s$ changes with $T_l$. From my data it is clear that it is more appropriate to use $D_w$ than $h_s$ when describing stomatal responses to humidity. The good fit of some data sets to $h_s$ is a fortuitous consequence of using a range of $T_l$ within which $g_w$ increases with increasing temperature, and of scaling $g_w$ as $g_w/A$.

8.1.3 What is the role of the boundary layer in the control of stomatal opening?

Depending on leaf dimension and wind speed $g_b^w$ can significantly alter the apparent response of $g_w^w$ to $\chi^c_a$ and $\chi^w_a$. Because of the feedback loops involved, the responses of $g_w^w$ to $\chi^c_a$ and $\chi^w_a$ each include responses to both $\chi^c_i$ and $D_w$. The boundary layer alters the state of the variables sensed by the guard cells —i.e. $\chi^c_i$ and $D_w^w$— and so it is a source of feedback.

A feedforward, i.e. direct, response of guard cells to $D_w$ requires negative feedback through another variable for stability because positive feedback would otherwise lead to either completely open or completely closed stomata.

The experiments and simulations in Chapter 6 show that, as long as stomata are sensitive to both $\chi^c_i$ and $D_w$, responses of $g_w^w$ to wind speed have two components, one resulting from changes in $\chi^c_a$ —and $\chi^c_i$— and another from changes in $D_w^w$.

The effect of wind speed —and hence $g_b^w$— on stomata has received little attention from plant ecophysicologists. No previous analysis has been made of the involvement of both CO$_2$ and humidity in the responses of stomata to wind, or of the effect of $g_b$ on the apparent responses of stomata to changes in $\chi^c_a$ and $\chi^w_a$. The results given in Chapter 6
indicate that, for given responses of $g_w$ to $\chi^c$ and $D_w$, the apparent responses of $g_w$ to $D_a$ and $\chi^c$ depend on the size of the leaf and wind speed, showing that this effect of the boundary layer should be considered when comparing data measured under different conditions, or with different methods. When scaling up from responses of stomata to the response of $g_w$ for a whole leaf, the effect of the boundary layer must be considered, and the value of $g_w$ resulting from scaling up will depend on leaf dimension and wind speed.

8.1.4 Is our current knowledge, and are the resulting models, good enough for predicting short-term responses of stomata to changes in the environment?

No valid mechanistic model of stomatal responses is available. As discussed in Chapters 1 and 7, a distinction must be made between models that include $A$ as a driving variable and those that rely only on environmental variables. From the discussion in Chapter 4, it follows that any mechanistic model of $g_w$ should include $I$ and $\chi^c$ as driving variables. In empirical models $A$ has been used as a surrogate for these and other variables. This is safe as long as the correlation between $A$ and $g_w$ holds.

Several different empirical models have been found to give the best fit to different data sets. Ball’s model is apparently too simple, and treats the responses of $g_w/A$ to $T_l$ and $D_w$ inadequately. However it is a good starting point for developing more complex and flexible models with wider validity. For this purpose, it is necessary to understand the logic behind this type of model to be able to give a sound interpretation to them. Further development is necessary before we may have a model to use in canopy or regional scale models.

The discussion in Chapter 7 is a contribution to the understanding of why Ball’s model fits some data sets, and why it fails in other cases. This chapter also makes a contribution towards the interpretation of Ball’s model. I propose a modification to this model that is empirical, but based on current knowledge about stomatal responses to $D_w$ and $T_l$, and their correlation with changes in $A$. This model is not tailored to one data set, but it takes into account other information with the aim of obtaining a model of more general usefulness.

Our current knowledge is not good enough for developing models of responses of stomata to short term changes in the environment that are generally valid. Several different models are available, but they succeed in describing the responses of $g_w$ to the environment only for certain species or conditions, and as most of them are empirical, there is little in common in their mathematical structures.
8.1.5 Methods

Equipment

I rewrote the gas-exchange system software incorporating algorithms to calculate and control in real time the molar fractions of CO$_2$ and water vapour at the leaf surface, which makes this gas-exchange system one of the few with this capability. I hope this software is going to be useful to other people using this system in the future, and also to people writing programs for other gas-exchange systems.

Modelling technique

Modelling was used as a tool to explore the consequences of the effect of a physical part of the system—the boundary layer—on the response of stomata. The model was kept as simple as possible, and the computer program written using a style that has been called ‘literate programming’ (Bentley & Knuth, 1986) with the aim of making it as readable as possible. As far as I know, this technique has not been used before for simulation models, but could be very useful by making program listings understandable to non-programmers and in this way subject to the same peer review criteria as experimentation.

Experimental design

No attempt was made to measure response surfaces to two or more variables as a way of studying interactions. This was an experimental design decision based on the practical difficulties of such an approach. Experiments involving measurement of $g_s^w$ are complicated by hysteresis of some responses, such as the responses to CO$_2$ and light. This has two consequences: firstly, a random order of application of treatments leads to large experimental errors, and secondly, when many points are needed to build a response surface it is not possible to use a systematic approach without biasing the results—or at least limiting their validity to the particular sequence used.

To keep such apparent experimental errors small and to reduce the probability of bias in the results it is preferable to apply all the treatments, to each experimental unit (leaf or plant), in the shortest possible time. To reduce both error and bias, the number of treatments per experiment was kept low, and the hypotheses were tested by comparison of response curves rather than means. In some measure, the experiments in this thesis show how simple experiments can be designed to address complex questions, and how adequate statistical design can help to increase the sensitivity of the experiments without increasing the number of measurements.


8.2 Implications for the future of stomatal conductance modelling

8.2.1 Current knowledge and models

Knowledge about stomatal responses to single variables is more substantial than that about the interactions between them. In the literature there are some descriptions of interactions in different species (e.g. Lösch & Tenhunen, 1981; Ball, 1988), mostly from response surface experiments. A way of obtaining this information more efficiently would be to use simple factorial experiments (e.g. two variables at two or three levels) combined with the measurement of dose response curves for individual variables —i.e. the approach taken in Chapter 6.

Field measurements do not lead to a mechanistic explanation, because different environmental variables are correlated, and although this approach can be useful for deriving empirical functions to predict the response of $g_s^w$ in the field, it has many limitations if we want to identify which variables are driving stomatal action, and how (Jarvis, 1976). Field measurements are also useful for understanding how responses at the leaf or stomatal level are influenced by correlations between environmental variables, and by processes occurring at the canopy level (e.g. Grantz & Meinzer, 1991).

Mechanistic models of leaf $g_s^w$ should simulate responses of stomata to the variables defined at the place where they are sensed, and take interactions between variables into account. The large number of variables to which stomata respond make the number of possible interactions also large, and significant interactions need to be identified and measured before attempting to simulate stomatal responses in a complex environment [e.g. in Commelina communis the sensitivity of $g_s^w$ to $\chi_i^c$ depends on $I$ (Jarvis & Morrison, 1981)]. Many possible interactions remain unknown or poorly specified because even though they may have been measured, the state of variables not studied has not been kept constant at the place where these variables are sensed by the guard cells, e.g. constant $\chi_a^c$ instead of constant $\chi_i^c$ (cf. the experiments described in Chapter 6).

The models that have been developed reflect the state of our knowledge of stomatal function. Few models of $g_s^w$ are mechanistic (e.g. Penning de Vries, 1972), most are empirical, some are driven only by environmental variables (e.g. Jarvis, 1976), but others take advantage of the correlation between $A$ and $g_s^w$ (e.g. Ball, 1988).

The correlation between $A$ and $g_s^w$ is useful practically because it allows the use of $A$ as a surrogate for a range of environmental and plant variables. This is not an ideal approach, but is one that is within reach from our current knowledge of stomatal behaviour. However, it is very important to realize that, even though $A$ can be used as
a driving variable in the calculation of $g_s^w$, $A$ does not control $g_s^w$ in reality —i.e. there is no simple causal link between $A$ and $g_s^w$. $A$ and $g_s^w$ are interdependent. The need to simultaneously calculate $A$ and $g_s^w$ is a problem of models based on the correlation between $A$ and $g_s^w$, but it is a problem that mechanistic models also have —i.e. $\chi_i^c$ would be needed as a driving variable in any mechanistic model of $g_s^w$, and because of this, such a model would also require the simultaneous calculation of $g_s^w$ and $A$.

Mechanistic submodels of the responses of leaf $g_s^w$ to environmental variables are needed to build models at larger spatial and longer time scales, such as the scales of whole plants, canopies and stands. A mechanistic model of whole leaf $g_s^w$ could be based on an empirical model of stomatal responses, without including the complexity of the mechanism of solute transport and accumulation in guard cells (e.g. ion channels, ion pumps, membrane potentials, and second messengers).

But the response of $g_s^w$ should be scaled-up taking into account the effect of the boundary layer, instead of simply multiplying the leaf area by a value of $g_s^w$ calculated from $\chi_a^w$ and $\chi_a^c$. By changing the object studied, we also change the reference point —i.e. the position where molar fractions are not affected by the surface fluxes being measured or modelled. What we call boundary layer depends on this reference point, so depending on the spatial scale at which we work, we have a leaf boundary layer, a canopy boundary layer, or a planetary boundary layer. We can think of these boundary layers as being nested one inside the other.

### 8.2.2 Towards a mechanistic model of canopy conductance

Mechanistic, or at least partly mechanistic, models of leaf $g_s^w$ that take into account the direct responses of stomata to $CO_2$, light, temperature, water vapour deficit and the place where these variables are sensed, and also the responses to hormones, will be more robust than the empirical models currently in use. In many species responses of adaxial and abaxial stomata will have to be modelled separately. With the exception of the effects mediated through chemical signals, these direct responses have been taken into account in the empirical model proposed in Chapter 7.

At the scale of the whole leaf, the effects on the variables sensed by the stomata of boundary layer thickness, shading by the mesophyll, $CO_2$ flux density, and leaf energy balance should be taken into account. The effect of changes in the leaf water status occurring directly and through chemical signals also needs to be considered. Some of these effects —boundary layer, $CO_2$ flux density and leaf energy balance— have been taken into account in the model proposed by Collatz et al. (1991).
When scaling up to a whole plant, the difference in CO\textsubscript{2} molar fraction and water vapour molar fraction in different layers of the air volume occupied by the plant, leaf display and shading between leaves, soil water deficit and photoassimilate supply-demand balance need to be considered.

When scaling up from a single plant to a forest or field crop the effect of the canopy boundary layer and the concurrent response of the different plants making up the canopy will need to be included.

McNaughton & Jarvis (1991) have provided an analysis of the scaling up of water fluxes, and analogous equations could be developed for CO\textsubscript{2} from their diagrams. The model MAESTRO provides a description of light interception that could be used for computing the light regime in different layers of a canopy (Wang & Jarvis, 1990). The main limitation to the development of a mechanistic model of canopy conductance seems to be the unavailability of a mechanistic model of stomatal conductance. A model that explicitly does the scaling up from stomata to canopy will be computationally intensive, and probably impractical for predictive use, but will help to the understanding of the scaling up mechanism. Such a model could be used to find out when and why simpler models (e.g. ‘big leaf’ canopy models) break down (see the comparison of different canopy models in Finnigan & Raupach, 1987).

### 8.3 Possible practical applications of the results

#### 8.3.1 Forecasting the effects of global change

Submodels to calculate canopy conductance ($G_s^w$) are an important part of models that are used for predicting the behaviour of vegetation in response to global change and the influence of vegetation on the atmosphere, both under current and future conditions. There are two approaches to modelling $G_s^w$: (1) scaling from leaves to canopies, or (2) deriving $G_s^w$ from flux measurements (Baldocchi et al., 1991). The first approach involves scaling up and requires a knowledge of responses of $g_s^w$ to environmental variables, and of how $g_s^w$ is integrated in a canopy. The second approach depends on assumptions about the homogeneity and extension of the canopy, and about soil evaporation. Usually the estimates of $G_s^w$ obtained using this second approach differ from those obtained from integration of measurements or simulations at leaf level (Finnigan & Raupach, 1987; Baldocchi et al., 1991).

The results presented in this thesis are useful with respect to the first of these two approaches to the calculation of $G_s^w$ by providing information about which variables are involved, how they interact, and how the thickness of the boundary layer affects
stomatal action. The model proposed in Chapter 7 is simple enough to be useful in this context, and more flexible than that proposed by Ball et al. (1987).

8.3.2 Agriculture and forestry

In many parts of the world, a limited water resource is the most important constraint on agriculture and forest production. The ability to predict plant water use and CO$_2$ fixation under different environmental and management conditions is important for devising management strategies that will generate ecologically sustainable and economically viable production systems.

The prediction of water use and CO$_2$ fixation by plant canopies requires prediction of conductances, including stomatal conductance. A better knowledge of the mechanistic basis of stomatal function will help us understand the physiological basis of these processes and in this way make modelling and decision making more robust.

The results presented are also important for plant breeding because the ability to predict the performance of ideotypes of stomatal behaviour could be used to set the objectives of a selection programme based on an ecophysiological knowledge of plant function. In this context we need to select for plant characteristics that are important for the performance of the whole crop stand. Thus the use of physiological criteria in plant breeding requires both a knowledge of plant functioning and of how plants interact with each other and with the environment. To be able to predict the effect of plant characteristics on the performance of the crop or forest stand we need also to develop principles for scaling up.

8.4 The future

Many aspects of the response of $g_s^w$ and $G_s^w$ to the environment remain unknown. There is no consistent data set, measured on a single species, of the responses of $g_s^w$ to all the variables to which stomata are sensitive, measured taking into consideration the place where the variables are sensed. Until this kind of information is available for several species, including crops, weeds, trees, sun- and shade-loving plants, generalizations will be very difficult —we will not be able to recognize species specific idiosyncrasies from generally occurring features.

Attempts at developing dynamic models of $g_s^w$ have been empirical (Aphalo, 1988), or they have considered only the response to $I$ (e.g. Kirschbaum et al., 1988). The dynamics of stomatal responses to $I$ can be important in the lower strata of canopies, but probably does not have a big effect on $G_s^w$. 
Heterogeneity of stomatal aperture in different parts of a leaf affects calculations of $\chi_c^f$ and $D_s^W$ (see Section 2.2.1). van Kraalingen (1990) has done simulations with a model to assess the consequences of patchiness in stomatal aperture on the results derived from gas-exchange experiments. This heterogeneity of stomatal action across the leaf surface can cause measurement artifacts, and needs further investigation, especially with respect to its dynamics.

We are just beginning to be able to scale-up steady-state responses from the leaf scale up to the whole plant and canopy scales, but the models at the smaller scale are still crude and limit our progress. Although it is true that when scaling up we usually need less detail about the processes occurring and a smaller scale than when we are dealing directly with systems at this smaller scale, it is also true that we need to understand much of this detail, before being able to decide how much of this detail is needed.

When scaling-up, the heterogeneity of the canopy is usually dealt with by dividing the canopy into layers that are assumed to be homogeneous. More sophisticated methods of integration should be developed. Spatial and temporal integration is just one aspect of scaling-up, but should not be neglected because advances in integration methods could make models less computationally intensive.

When I started this project I received the comment ‘Why are you studying stomata? We already know all that can be learnt about them.’ After three years of research by me and by others, it is clear that this person was wrong. Stomatal physiology remains as fascinating and challenging as ever. We can expect quick advance in the next few years because the techniques for measurement are available, and because the development of the field is of increasing importance in a globally changing environment.
Appendix A

The BOUNDARY model

The listing of the computer program which implements the model described in Chapter 6 is included in this appendix. It was written in Modula-2 using the MWEB system. It makes use of modules from the M2Simul library of tools for simulation model program writing.

The MWEB system is an implementation of the WEB system of ‘literate’ programming for the language Modula-2. It consists in two preprocessors that are used to generate a Modula-2 file and a \TeX\ file. The first is compiled and executed, the second is used to generate the formatted output given here. This output is generated automatically from the MWEB source file and although nicely formatted is the ‘listing’ of the program.
1. Introduction. This program calculates the effect of the boundary layer on the apparent sensitivity of stomata to environmental variables. It was written by Pedro J. Aphalo in June and July, 1990.

It was written in Modula-2 (Wirth, 1985) using MWEB (Sewell, 1989) and TPX (Knuth, 1986). It makes imports from modules of the M2Simul library previously developed by the author (Aphalo, 1989).

define banner = 'This is BOUNDARY, Version 0.0'

2. The model simulates the effect of the boundary layer, based on a description of the response of the plant. This model does not simulate the response of stomata per se but rather the physical effect of the boundary layer conductance on the concentrations seen by the stomata and its effect on the apparent response when, as in natural conditions, these concentrations are not independent variables.

As we are not interested in the description of stomatal responses we do not want to assume any particular functional form for their response. For this reason we are going to use interpolated values from tabulated data.

The problem can be set up as a system of two simultaneous equations:

\[
\begin{align*}
D_s &= f(D_s, C_i) \\
C_i &= g(D_s, C_i)
\end{align*}
\]

where only state variables are shown. This system is equivalent to:

\[
\begin{align*}
0 &= f(D_s, C_i) - D_s \\
0 &= g(D_s, C_i) - C_i
\end{align*}
\]

or

\[
\begin{align*}
0 &= f_{\text{error}}(D_s, C_i) \\
0 &= g_{\text{error}}(D_s, C_i)
\end{align*}
\]

Both \(f_{\text{error}}\) and \(g_{\text{error}}\) are functions of \(g_s\) and environment variables. \(D_s\) and \(C_i\) are the only state variables of our model. An iterative procedure must be used to solve this system under each environmental condition of interest. \(g_s\) and the rates of CO₂ assimilation and of transpiration can be computed from the values of state and environment variables.
3. Structure of the program. The program reads environmental data from an input file, and saves the results to an output file. Following a top down design we lay down the general structure, whose components will be filled in later.

module boundary;

(Import list 4)

type (Types of the program 5)

var (Global variables of the program 6)

(Procedure definitions of the program 8)

begin

display_line(banner);

{Get file names 42;}

{Open files 37;}

{Setup the functions 14;}

{Setup the equations 7;}

{Compute behavior 25;}

{Close files 39;}

end boundary.

4. The system of equations. Taking advantage of Modula-2's procedure data type we are going to build a vector of function procedures to store the system of equations, and a vector of reals to store its state. As we are going to use the module Equations to solve this system, the vectors have to be compatible with those used there.

define solve_eqs ≡ [Solve] (* procedure *)

define behaviour ≡ [BehaviorType] (* type *)

define dummy_point ≡ [point]

define dummy_real ≡ 0.0

define equation ≡ [ModelFunc] (* type *)
define eq_vector ≡ [ModelFuncArray] (* type *)
define state_vector ≡ [ModelArray] (* type *)
define Ds ≡ 0 (* Index for Ds in state_vector *)
define C1 ≡ 1 (* Index for C1 in state_vector *)

(Import list 4)

from Equations, import behaviour, equation, state_vector, eq_vector, solve_eqs;

See also sections 9, 12, 34, 35, and 41.

This code is used in section 3.

5. (Types of the program 5)

environment_variables = (Wa, Ca, I, TI, gb); (* forcing variables *)

env_vector = array environment_variables of real;

This code is used in section 3.

6. (Global variables of the program 6)

guessed_state, steady_state: state_vector;

env_state: env_vector;

equations: eq_vector;

See also sections 13, 18, 29, and 36.

This code is used in section 3.
APPENDIX A. THE BOUNDARY MODEL

7. \( \langle \text{Setup the equations 7} \rangle \equiv \)
\[\text{equations}[Ds] \leftarrow Ds\text{-error}\_\text{proc};\]
\[\text{equations}[Ci] \leftarrow Ci\text{-error}\_\text{proc};\]

This code is used in section 3.

8. We use Tetten's equation (Murray, 1967) to calculate the saturated vapour pressure, and by dividing it by the total pressure we get a mol fraction. The temperature is in °C, and the atmospheric pressure in Pa is assumed constant. We also assume \( \text{temperature} > 0 \text{ °C} \).

\( \langle \text{Procedure definitions of the program 8} \rangle \equiv \)
procedure \( W\_\text{sat}(\text{temperature} : \text{real}) : \text{real} ; \)
    
    \text{const} \( P\_\text{atm} = 1.013 \times 10^{5}; \) \( \{ \text{ Pa } \} \)
    
    \text{var} \( P\_\text{water} : \text{real}; \)
    
    \text{begin}
    
    \( P\_\text{water} \leftarrow 6.1078 \times 10^{2} \times \exp((17.269 \times \text{temperature}/(237.3 + \text{temperature})));
    
    \text{return} \( P\_\text{water}/P\_\text{atm}; \) \( \{ \text{ PaPa}^{-1} \equiv \text{molmol}^{-1} \} \)
    
    \text{end} \ W\_\text{sat};

See also sections 15, 16, 20, 21, 22, 23, and 24.

9. This code is used in section 3.

9. Modula-2 has no exponentiation operator, so we have to import a procedure from a library module.

\( \langle \text{Import list 4} \rangle \equiv \)
from \( \text{MathLib0} \) import \( \text{exp} ; \)

10. We define \( Wl \) and \( Da \) as macros.

\( \langle \text{Define list 4} \rangle \equiv \)
\( \text{define} \ Wl \equiv W\_\text{sat}(\text{env\_state}[Tl]) \)
\( \text{define} \ Da \equiv (Wl - \text{env\_state}[Wa]) \)

11. We also define macros for computing the total conductances to water vapour and \( \text{CO}_2 \). The constant '1.60' is the ratio between the diffusivities of water vapour and \( \text{CO}_2 \) in air. For \( g_s \) a smaller value is used because the process is not fully diffusive.

\( \langle \text{Define list 4} \rangle \equiv \)
\( \text{define} \ gt(#) \equiv (1.0/(1.0/\text{env\_state}[gb] + 1.0/gs\_\text{proc}(#))) \)
\( \text{define} \ gt\_\text{CO}_2(#) \equiv (1.0/(1.37/\text{env\_state}[gb] + 1.00/gs\_\text{proc}(#))) \)
12. Stomatal response is calculated as the product of a conductance value for standard conditions and functions that describe the effect of individual variables as a proportion of this value. For these stomatal response functions we use interpolation from tables of data read from disk files. We use procedures and types imported from the module Splines from the M2Simul library. The structure of these data files is described in the M2Simul library. These files should contain \( g_s \) values in the range 0 to 1, as functions of \( I \) in \( \mu \text{mol m}^{-2} \text{s}^{-1} \), and \( D_s \) and \( C_i \) in \( \text{mol mol}^{-1} \).

```
define spline_handle \equiv \text{Spline}
define init_gs_gfd(#) \equiv \text{CreateSpline}(#, gs_gfd_spl, gs_gfd_ok);
define init_gs_Ds(#) \equiv \text{CreateSpline}(#, gs_Ds_spl, gs_Ds_ok);
define init_gs_Ci(#) \equiv \text{CreateSpline}(#, gs_Ci_spl, gs_Ci_ok);
define gs_gfd(#) \equiv \text{FunVal}(gs_gfd_spl, #)
define gs_Ds(#) \equiv \text{FunVal}(gs_Ds_spl, #)
define gs_Ci(#) \equiv \text{FunVal}(gs_Ci_spl, #)
define gs_max \equiv 80.0 \cdot 10^{-3} \quad (\text{mol m}^{-2} \text{s}^{-1})

(Import list 4) \equiv
from Splines import Spline, CreateSpline, FunVal;
```

13. \( \text{(Global variables of the program 6)} \equiv \)
\( gs_gfd\_spl, gs_Ds\_spl, gs_Ci\_spl: \text{spline\_handle}; \)
\( gs_gfd\_ok, gs_Ds\_ok, gs_Ci\_ok: \text{boolean}; \)

14. \( \langle \text{Setup the functions 14} \rangle \equiv \)
\( \text{init}\_gs\_gfd(gs\_gfd\_file); \)
\( \text{init}\_gs\_Ds(gs\_Ds\_file); \)
\( \text{init}\_gs\_Ci(gs\_Ci\_file); \)
See also section 19.
This code is used in section 3.

15. \( \langle \text{Procedure definitions of the program 8} \rangle \equiv \)
procedure gs_proc(sys\_state : state\_vector): real;
begin
  return gs_max * gs_gfd(env\_state[I]) * gs_Ds(sys\_state[Ds]) * gs_Ci(sys\_state[Ci]);
end gs_proc;

16. \( \langle \text{Procedure definitions of the program 8} \rangle \equiv \)
procedure Ds_proc(sys\_state : state\_vector): real;
begin
  return Da * (1.0 - gt(sys\_state)/env\_state[g]);
end Ds_proc;

17. We are also going to use interpolation from tabulated data for describing the rate of CO\(_2\) assimilation as a function of \( C_i \). Assimilation data must be in \( \text{mol m}^{-2} \text{s}^{-1} \) as a function of \( C_i \) in \( \text{mol mol}^{-1} \).

```
define init_A.Ci(#) \equiv \text{CreateSpline}(#, A.Ci\_spl, A.Ci\_ok);
define A.Ci(#) \equiv \text{FunVal}(A.Ci\_spl, #)
```
APPENDIX A. THE BOUNDARY MODEL

18. (Global variables of the program 6) +≡
   \( A_{Ci}.spl: \text{spline\_handle}; \)
   \( A_{Ci}.ok: \text{boolean}; \)

19. (Setup the functions 14) +≡
   \text{init}\_A_{Ci}(A_{Ci}\_file);

20. (Procedure definitions of the program 8) +≡
   \text{procedure} \ A_{proc}(\text{sys\_state} : \text{state\_vector}): \text{real};
   \begin{align*}
   & \text{begin} \\
   & \quad \text{return} \ A_{Ci}(\text{sys\_state}[Ci]); \\
   & \text{end} \ A_{proc};
   \end{align*}

21. (Procedure definitions of the program 8) +≡
   \text{procedure} \ E_{proc}(\text{sys\_state} : \text{state\_vector}): \text{real};
   \begin{align*}
   & \text{begin} \\
   & \quad \text{return} \ Da * \text{gt}(\text{sys\_state}); \\
   & \text{end} \ E_{proc};
   \end{align*}

22. We are going to use a rough approximation to compute \( C_i \). We are not going to correct it for the effects of the mass flow of water vapour.

23. (Procedure definitions of the program 8) +≡
   \text{procedure} \ Ci_{\text{proc}}(\text{sys\_state} : \text{state\_vector}): \text{real};
   \begin{align*}
   & \text{begin} \\
   & \quad \text{return} \ \text{env\_state}[Ca] - A_{\text{proc}}(\text{sys\_state})/\text{g\_CO2}(\text{sys\_state}); \\
   & \text{end} \ Ci_{\text{proc}};
   \end{align*}

24. (Procedure definitions of the program 8) +≡
   \text{procedure} \ Ds_{\text{error\_proc}}(\text{time} : \text{real}; \text{sys\_data} : \text{state\_vector}; \text{behav} : \text{behaviour}): \text{real};
   \begin{align*}
   & \text{begin} \\
   & \quad \text{return} \ Ds_{\text{proc}}(\text{sys\_data}) - \text{sys\_data}[Ds]; \\
   & \text{end} \ Ds_{\text{error\_proc}};
   \end{align*}

25. (Procedure definitions of the program 8) +≡
   \text{procedure} \ Ci_{\text{error\_proc}}(\text{time} : \text{real}; \text{sys\_data} : \text{state\_vector}; \text{behav} : \text{behaviour}): \text{real};
   \begin{align*}
   & \text{begin} \\
   & \quad \text{return} \ Ci_{\text{proc}}(\text{sys\_data}) - \text{sys\_data}[Ci]; \\
   & \text{end} \ Ci_{\text{error\_proc}};
   \end{align*}
25. **Running the simulation.** For each environmental condition we must repeat several steps. The details of each of these steps are going to be filled in in the following sections.

(Compute behavior 25) ≡
while ¬end_of_data do
  (Load environment data 26)
  if done then
    (Compute a guess 27)
    (Compute one data point 28)
    (Save state data 30)
    (Show vital signs 32)
  end ;
end ;

This code is used in section 3.

26. We read the environment data from a free format file. Each line is expected to contain five real values, one for each of the following variables: \( W_e \) (mol mol\(^{-1}\)), \( C_a \) (mol mol\(^{-1}\)), \( I \) (\( \mu \)mol m\(^{-2}\) s\(^{-1}\)), \( T_i \) (°C), and \( g_b \) (mol m\(^{-2}\) s\(^{-1}\)).

(Load environment data 26) ≡
read_real(in_file, env.state[Wa]);
read_real(in_file, env.state[Ca]);
read_real(in_file, env.state[I]);
read_real(in_file, env.state[Ti]);
read_real(in_file, env.state[gb]);

This code is used in section 26.

27. To solve the equations, we first need a guess for \( D_s \) and \( C_i \). We take the bold approach of using \( D_s = 0.75 D_a \) and \( C_i = 200.0 \mu \)mol mol\(^{-1}\) as the starting point for the minimisation. If environmental data were sorted by \( g_b \) it could be better to use as a guess the values of \( D_s \) and \( C_i \) calculated for the previous data point.

define num_eq ≡ 2
define max_iterations ≡ 100
(Compute a guess 27) ≡
guessed_state[Ds] ← 0.75 * Da;
guessed_state[Ci] ← 200.0 * 10\(^{-6}\);

This code is used in section 26.

28. (Compute one data point 28) ≡
iterations ← max_iterations;
solve_eqs(equations, num_eq, guessed_state, iterations, steady_state);
actual_iterations ← iterations;
Ds_error ← Ds.error_proc(dummy_real, steady_state, dummy_bhv);
Ci_error ← Ci.error_proc(dummy_real, steady_state, dummy_bhv);

This code is used in section 25.

29. (Global variables of the program 6) ≡
iterations, actual_iterations: cardinal;
Ds_error, Ci_error: real;
30. We save the state variables \( D \), and \( C_i \), and their 'errors'. We also save \( g_s \), \( A \) and \( E \) calculated for this condition. The output consists in seven real values per line: \( g_s \) (mol m\(^{-2}\) s\(^{-1}\)), \( A \) (mol m\(^{-2}\) s\(^{-1}\)), \( E \) (mol m\(^{-3}\) s\(^{-1}\)), \( D \) (mol mol\(^{-1}\)), \( C_i \) (mol mol\(^{-1}\)), \( D \) error (mol mol\(^{-1}\)), and \( C_i \) error (mol mol\(^{-1}\)).

\[
\text{Write: state data 30, etc.}
\]

\[
\text{E}^	ext{wnte-real(outfile, g_s_proc(steady-state)); write-real(outfile, A_proc(steady-state)); write-real(outfile, E_proc(steady-state)); write-real(outfile, steady_state[Ds]); write-real(outfile, steady_state[Ci]); write-real(outfile, Ds_error); write-real(outfile, Ci_error);}
\]

See also section 31.

This code is used in section 25.

31. We save the outcome of all computations, even if they are suspect, but mark them in the file as such. We add a text string at the end of each line that indicates whether the solution computed was "GOOD" or "BAD". "GOOD" means that the iterative algorithm has converged.

\[
\text{Write state data 30, etc.}
\]

\[
\text{if (actual_iterations < max.iterations) then write_str(outfile, 'GOOD'); new_line(outfile); else write_str(outfile, 'BAD'); new_line(outfile); end;}
\]

32. (Show vital signs 32) \equiv display_dot;

This code is used in section 25.

33. System dependent part. What follows is highly dependent on the compiler and library used. This is the part of the program that would need to be changed to be able to compile it in a different computer or with a different compiler. The program could also be modified to use the standard input and output when no filenames are supplied in the command line.

We used Logitech's Modula-2 compiler for MS-DOS, Version 3.0.

34. CRT screen output.

\[
\text{Define display_line(#) : IoInut.WriteString(#); IoInut.WriteLine;}
\]

\[
\text{Define display_dot : IoInut.Write(' .');}
\]

(Import list 4) \equiv import IoInut;
APPENDIX A. THE BOUNDARY MODEL

36. File input and output.

```define lookup ≡ [lookup]
define close ≡ [close]
define not_done(#) ≡ (#.res ≠ done)
define end_of_data ≡ (in_file.eof)
define done ≡ [done]
define write_str ≡ [WriteString]
define write_real ≡ [WriteReal]
define new_line ≡ [WriteLn]
define read_real ≡ [ReadReal]
define text_file ≡ [File]

from [FileSystem] import lookup, Response, text_file, close;
from [FileInOut] import write_str, write_real, read_real, done, new_line;
```

36. (Global variables of the program 6) +≡

```in_file, out_file: text_file;
in_filename, out_filename: array [0 .. 55] of char;
```

37. (Open files 37) ≡

```lookup(in_file, in_filename, false);
if not_done(in_file) then
    fatalError('unable to open input file');
end;
```

See also section 36.

This code is used in section 3.

38. (Open files 37) +≡

```lookup(out_file, out_filename, true);
if not_done(out_file) then
    fatalError('unable to open output file');
end;
```

39. (Close files 39) ≡

```close(in_file);
close(out_file);
```

This code is used in section 3.

40. File names for tabulated data files to be used to get stomatal and assimilation responses by spline interpolation.

```define gs_gfd_file ≡ 'gs_gfd.dat'
declare gs_Ds_file ≡ 'gs_ds.dat'
declare gs_ci_file ≡ 'gs_ci.dat'
declare A_ci_file ≡ 'a_ci.dat'
```
APPENDIX A. THE BOUNDARY MODEL

41. Reading file names from the command line.

\[
\text{define } \text{arg\_count} \equiv \text{ArgCount} \\
\text{define } \text{arg} \equiv \text{Arg} \\
\text{(Import list 4) +\equiv} \\
\text{from } \text{CommandLine; import arg\_count, arg;}
\]

42. (Get file names 42) \equiv

\[
\text{if } \text{arg\_count}() = 2 \text{ then} \\
\text{arg}(1, \text{in\_filename}); \\
\text{arg}(2, \text{out\_filename}); \\
\text{else} \\
\text{fatal\_error('usage: boundary\_inFile\_outFile');} \\
\text{end} ;
\]

This code is used in section 3.

43. Error handling;

\[
\text{define } \text{fatal\_error(#)} \equiv \text{display\_line('Fatal\_error!'); display\_line(#); HALT()}
\]

44. References.


45. Index.

\[
\text{A-Ci: 17, 20.} \\
\text{A-Ci\_file: 19, 40.} \\
\text{A-Ci\_ok: 17, 18.} \\
\text{A-Ci\_spl: 17, 18.} \\
\text{A\_proc: 20, 22, 30.} \\
\text{actual\_iterations: 28, 29, 31.} \\
\text{arg: 41, 42.} \\
\text{arg\_count: 41, 42.} \\
\text{banner: 1, 3.} \\
\text{behav: 23, 24.} \\
\text{behaviour: 4, 23, 24.} \\
\text{boolean: 13, 18.} \\
\text{boundary: 3.} \\
\text{Ca: 5, 22, 26.} \\
\text{cardinal: 29.} \\
\text{char: 36.} \\
\text{Ci: 4, 7, 15, 20, 24, 27, 30.} \\
\text{Ci\_error: 28, 29, 30.} \\
\text{Ci\_error\_proc: 7, 24, 28.} \\
\text{Ci\_proc: 22, 24.} \\
\text{close: 35, 39.} \\
\text{Da: 10, 16, 21, 27.} \\
\text{display\_dot: 32, 34.}
\]

\[
\text{display\_line: 3, 34, 43.} \\
\text{done: 25, 35.} \\
\text{Ds: 4, 7, 15, 23, 27, 30.} \\
\text{Ds\_error: 28, 29, 30.} \\
\text{Ds\_error\_proc: 7, 23, 28.} \\
\text{Ds\_proc: 16, 23.} \\
\text{dummy\_bhv: 4, 28.} \\
\text{dummy\_real: 4, 28.} \\
\text{E\_proc: 21, 30.} \\
\text{end\_of\_data: 25, 35.} \\
\text{env\_state: 6, 10, 11, 15, 16, 22, 26.} \\
\text{env\_vector: 5, 6.} \\
\text{environment\_variables: 5.} \\
\text{eq\_vector: 4, 6.} \\
\text{equation: 4.} \\
\text{equations: 6, 7, 28.} \\
\text{exp: 8, 9.} \\
\text{false: 37.} \\
\text{fatal\_error: 37, 38, 42, 43.} \\
\text{gb: 5, 11, 16, 26.} \\
\text{gs\_Ci: 12, 15.} \\
\text{gs\_Ci\_file: 14, 40.} \\
\text{gs\_Ci\_ok: 12, 13.}
\]
APPENDIX A. THE BOUNDARY MODEL

gs.Ds: 12, 15.
gs.Ds_file: 14, 40.
gs.Ds_ok: 12, 13.
gs.Ds_spl: 12, 13.
gs.max: 12, 16.
gs.proc: 11, 15, 30.
gs.qfd: 12, 15.
gs.qfd_file: 14, 40.
gs.qfd_ok: 12, 13.
gs.qfd_spl: 12, 13.
gt: 11, 15, 21.
gt.CO2: 11, 22.
guessed.state: 6, 27, 28.
I: 5.
in_file: 26, 35, 36, 37, 39.
in_filename: 36, 37, 42.
init_A_Ci: 17, 19.
init.gs.Ci: 12, 14.
init.gs.Ds: 12, 14.
init.gs_qfd: 12, 14.
iters: 28, 29.
lookup: 35, 37, 38.
new_line: 31, 35.
not.done: 35, 37, 38.
num_eq: 27, 28.
out_file: 30, 31, 36, 38, 39.
out_filename: 36, 38, 42.
P_atm: 8.
P_water: 8.
read.real: 26, 35.
real: 4, 5, 6, 15, 16, 20, 21, 22, 23, 24, 25, 29, 30.
solve_eqs: 4, 28.
spline.handle: 12, 13, 18.
state_vector: 4, 6, 15, 16, 20, 21, 22, 23, 24.
steady.state: 6, 28, 30.
sys.data: 23, 24.
sys.state: 15, 16, 20, 21, 22.
system_dependencies: 33.
temperature: 8.
text_file: 35, 36.
time: 23, 24.
Tl: 5, 10, 26.
true: 38.
W_sat: 8, 10.
Wa: 5, 10, 26.
Wl: 10.
write_real: 30, 35.
write_str: 31, 35.
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NAMES OF THE SECTIONS

Close files 39  Used in section 3.
Compute a guess 27  Used in section 25.
Compute behavior 25  Used in section 3.
Compute one data point 28  Used in section 25.
Get file names 42  Used in section 3.
Global variables of the program 6, 13, 18, 29, 36  Used in section 3.
Import list 4, 9, 12, 34, 35, 41  Used in section 3.
Load environment data 26  Used in section 25.
Open files 37, 38  Used in section 3.
Procedure definitions of the program 8, 15, 16, 20, 21, 22, 23, 24  Used in section 3.
Save state data 30, 31  Used in section 25.
Setup the equations 7  Used in section 3.
Setup the functions 14, 19  Used in section 3.
Show vital signs 32  Used in section 25.
Types of the program 5  Used in section 3.
Appendix B

Comparison of models

Table B.1. Comparison between models of stomatal response. Values of the coefficients from least squares regressions of different models to data for *Hedera helix* from the experiments described in Chapter 5. See Table 7.1 for equations and residual sums of squares. Data were the mean of three plants for set A (*T*$_l$=15–28 °C, *D*$_w$=6–15 mmol mol$^{-1}$, *h*$_s$=0.4–0.75, *I*=200 µmol m$^{-2}$ s$^{-1}$, *χ*$_s$=350 µmol mol$^{-1}$), and that from a typical plant for set B (*T*$_l$=10–28, *D*$_w$=5–15, *h*$_s$=0.4–0.75, *I*=340 µmol m$^{-2}$ s$^{-1}$, *χ*$_s$=350 µmol mol$^{-1}$). For model 8, *T*$_{opt}$ is under the heading *k*$_0$.

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<th><em>k</em>$_1$</th>
<th><em>k</em>$_2$</th>
<th><em>k</em>$_3$</th>
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References


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