

STOMATAL RESPONSE TO ENVIRONMENTAL CONDITIONS

by

PETER INNES

BSc (University of London)

A thesis presented in part fulfilment of the
requirements for the degree of Doctor of Philosophy in
the Faculty of Science of the University of London,
and for the Diploma of Imperial College

Department of Botany

Imperial College of Science and Technology

Silwood Park

Sunninghill

Ascot

Berkshire

1981

ABSTRACT

An investigation was made of the stomatal responses to environmental factors by enclosing leaves in sealed assimilation chambers, partially controlling the conditions inside the chambers, and measuring the gas exchange rates of the leaves. Stomatal resistance was then estimated from these rates. The main species used in the investigation was Quercus petraea (Mattuschka) Leiblein, and measurements were also made on Fraxinus excelsior L., Acer pseudoplatanus L., Nicotiana tabacum L., and Phaseolus vulgaris L. The stomata of all of the species investigated were found to respond to air humidity, expressed in this study as the water potential of leaf chamber air, $-\psi_{\text{air}}$. This direct response to $-\psi_{\text{air}}$, independent of bulk leaf water potential and also independent of transpirational flux through the stomata, was the most important single factor in determining stomatal resistance; an increase in $-\psi_{\text{air}}$ (i.e. air becoming drier) causing an increase in stomatal resistance. The stomatal response to sub-stomatal CO_2 concentration, C_i , was dependent upon the level of $-\psi_{\text{air}}$: at low levels of $-\psi_{\text{air}}$ (humid air) there was little response to C_i over a natural range, but at higher levels of $-\psi_{\text{air}}$, stomatal resistance increased directly with C_i . The combination and interaction of the $-\psi_{\text{air}}$ and C_i responses provided a balanced regulation of production against water loss according to the prevailing conditions. A simple multiple regression was used to calculate stomatal resistance in terms of the responses to $-\psi_{\text{air}}$ and C_i , and there was good correspondence between measured and calculated values. No direct effect light on stomatal resistance was detected, but there was some evidence to suggest a blue-light requirement for stomatal opening. In general, lack of O_2 had an opening effect on stomata, but did not prevent stomatal closure in the dark, nor would lack of O_2 open stomata which were closed. No consistent pattern of direct stomatal response to leaf temperature was evident. The role of stomatal response to environmental conditions in the control of gas exchange is discussed.

	<u>LIST OF CONTENTS</u>	Page
Title page		1
Abstract		2
List of contents		3
List of tables		7

CHAPTER 1 Introduction and literature review

1.1 Introduction	10
1.2 Light and carbon dioxide	12
1.3 Light effects independent of carbon dioxide	14
1.4 Leaf water potential	16
1.5 Direct response to humidity	20
1.6 Temperature	23
1.7 Oxygen	25

CHAPTER 2 Materials and methods

2.1 The gas exchange system and methods of measurement	29
2.1/1 Mixing of gases for IRGA calibration and gas exchange measurements	29
2.1/2 The gas flow system	38
2.1/3 The data logging system	41
2.1/4 Experimental rationale	44

LIST OF CONTENTS (continued)

CHAPTER 2 Materials and methods (continued)

2.2	The assimilation chamber	46
2.2/1	Chamber design	46
2.2/2	Temperature measurement	51
2.2/3	Mixing of air in the assimilation chamber	52
2.2/4	Measurement of radiant flux density of photosynthetically active radiation .	55
2.3	Calibration of equipment	60
2.3/1	Calibration of the IRGA	60
2.3/2	Calibration of the dewpoint hygrometer	69
2.3/3	Calibration of the flowmeters	76
2.3/4	Calculation of the pressure conversion factors for different parts of the gas circuit	81
2.3/5	Calibration of the photocells	86
2.4	Experimental material and procedures	90
2.4/1	Plant material	90
2.4/2	Experimental procedures	90
2.5	Methods of calculation	94
2.5/1	List of extensively used symbols	94
2.5/2	Theory of synchronous CO ₂ and H ₂ O exchange measurements	95
2.5/3	Calculation of the IRGA calibration intercept, 'a'	97
2.5/4	Determination of the water vapour concentration in the leaf chamber	99
2.5/5	Determination of the CO ₂ concentration in the leaf chamber	99
2.5/6	Calculation of gas exchange rates	100

LIST OF CONTENTS (continued)

CHAPTER 2 Materials and methods (continued)

2.5 Methods of calculation (continued)

2.5/7	Determination of the water vapour concentration at the liquid-air interface inside the leaf	103
2.5/8	Calculation of the resistances to gaseous transfer	104
2.5/9	Determination of the leaf boundary layer resistance	107
2.5/10	Determination of the stomatal resistances to water vapour and CO ₂ transfer	110
2.5/11	Determination of the sub-stomatal CO ₂ concentration	111
2.5/12	Determination of the water potential of the leaf chamber air	111

CHAPTER 3 Results 115

Introduction 116

3.1 Diurnal behavior of stomata 123

3.2 Responses to individual environmental variables 126

3.2/1 Light and carbon dioxide 126

3.2/2 Water potential of leaf chamber air 133

3.2/3 Interaction of the C_i and - Ψ_{air} responses 144

3.2/4 Leaf temperature 148

3.3 Hypotheses of stomatal behavior 154

3.3/1 Regression results 160

3.3/2 Effects of C_i, - Ψ_{air} , and light 1693.3/3 Effects of ambient O₂ concentration 178

LIST OF CONTENTS (continued)

CHAPTER 3 Results (continued)

3.4 Effects of the $-\psi_{\text{air}}$ response on transpiration, E_T 187

CHAPTER 4 Discussion 193

Summary	209
Acknowledgements	211
References	212
Appendix 1 - Marquardt's algorithm	226
Appendix 2 - Raw data	microfiche
Appendix 3 - Results of regression comparisons	microfiche

LIST OF TABLES

CHAPTER 2	page
Table 2A - Dewpoint hygrometer calibration: the relationship between thermistor resistance (R_T) and output voltage (X).	71
Table 2B - Dewpoint hygrometer calibration: values of calibration constants.	71
Table 2C - Dewpoint hygrometer calibration: the relationship between absolute temperature (t) and thermistor resistance (R_T).	73
Table 2D - Dewpoint hygrometer calibration: comparison of two calibrations.	77
Table 2E - Results of flowmeter calibrations.	80
Table 2F - Results of pressure ^N conversion factor determination.84	
Table 2G - Details of <i>Quercus petraea</i> Experiments 1 and 2, and <i>Fraxinus excelsior</i> Experiment 1.	93
Table 2I - Typical values of leaf boundary layer resistance to water vapour transfer ($r_{a(H_2O)}$).	109
Table 2J - Conversion table for $-\psi_{air}$ ($J. mol^{-1}$) and ΔH (mbar).	114
CHAPTER 3	
Table 3A - Sample set of data to illustrate the format of appendix 2.	117
Table 3B - Results for a leaf of <i>Quercus petraea</i> collected over a nine day period.	121
Table 3C - Prevention of stomatal closure in the dark at low ambient CO_2 concentrations for two tobacco leaves.	126
Table 3D - Sample data set to show the format of appendix 3.156	
Table 3E - The sources of data for the pooled regressions. 161	
continued.....	

LIST OF TABLES (continued)

CHAPTER 3 (continued)

Table 3F	- Regression coefficients for pooled data fitted to regression 2.	166
Table 3G	- Results for a leaf of <i>Quercus petraea</i> at low levels of red light.	174

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The process of photosynthesis, probably the most important set of chemical reactions in the world, is crucial to the existence of almost all living things, both as the source of all food and for the replenishment of oxygen (O_2). However, loss of water is an inevitable consequence of the assimilation of carbon dioxide (CO_2), because evolution has not produced a membrane which allows the passage of CO_2 from the gaseous phase to an aqueous phase, but at the same time does not allow the transfer of water vapour. The distribution of green plants on the earth's surface is largely determined by there being sufficient soil water to replace that lost by transpiration. Rates of photosynthesis and transpiration are dependent upon how quickly CO_2 and water vapour are transferred between the atmosphere and the leaf, which are controlled to a large extent by the stomatal pores occurring on the aerial parts of most terrestrial plants. These pores are situated between two specialized epidermal cells, the guard cells, which bring about the opening and closing movements of stomata by changes in their dimensions.

Much is known about the morphology and anatomy of stomata, and about the physical aspects of diffusion of gas through stomata, but there has not been general agreement about which environmental variables control stomatal behaviour. If this could be resolved it would clearly be of great benefit in trying to elicit the biochemical changes which lie behind the mechanisms of stomatal movements. In addition, the ability to predict the performance of stomata under a given set of environmental conditions could be of great value in finding ways of improving the water use efficiency of commercial crops, without seriously reducing photosynthetic yield.

The broad aim of this study was to investigate the stomatal response to various environmental factors, and to examine how such

responses interact to control the gas exchange of leaves. This was carried out by enclosing leaves in sealed assimilation chambers, partially controlling the environmental conditions inside the chambers, and measuring the gas exchange rates of the leaves: the stomatal response was then estimated from these rates. The main species used in the investigation was Quercus petraea (Mattuschka) Leiblein, the dominant oak of northern and western Britain, and of considerable ecological and amenity value. Some measurements were made on two other deciduous tree species Fraxinus excelsior L. and Acer pseudoplatanus L.. In addition, when the tree species were not in leaf, measurements were made on Nicotiana tabacum L. cv. Bel-W3 and Phaseolus vulgaris L.. The most important factors known to affect stomata were varied both singly (as far as was possible) and in combination, and the responses of the stomata were estimated by careful measurement of gas exchange rates. An empirical model was derived to help identify how the various responses to individual factors interact in the control of stomata.

Stomatal behaviour as affected by environmental conditions has been widely investigated experimentally and has been comprehensively reviewed several times: Meidner & Mansfield (1968); Raschke (1975); Hall, Schulze & Lange (1976); Cowan (1977); Sheriff (1979). In the remainder of this chapter some of the most important work investigating stomatal responses to environmental conditions is summarized.

1.2 Light and carbon dioxide

According to Meidner & Mansfield (1968) "Light is probably more important in determining the course of stomatal behaviour than any other component of the environment". This is undoubtedly the case, borne out by the fact that, in general, stomata tend to open during the day and close at night. However, it has been suggested (Raschke, 1975) that the major effect of light on stomatal movement is indirect, operating via the effect of the sub-stomatal carbon dioxide concentration (C_i). Scarth (1932) first made the suggestion that stomata respond to light indirectly by responding to changes in C_i brought about by changes in rate of assimilation with change in light intensity. Freudenberger (1940) showed that increases in ambient CO_2 caused stomatal closure, and decreases caused opening, in illuminated or darkened or etiolated leaves. Similar results were obtained by Heath and co-workers (1948, 1950, 1954). Such results are strongly supported by the available evidence reviewed by Meidner & Mansfield (1968) and by Raschke (1975). Raschke (1975) concluded that stomata respond to light indirectly by responding to the reductions in CO_2 in the mesophyll and in the guard cells, and that these responses close feedback loops which synchronize stomatal opening with the demand of the mesophyll for CO_2 .

More recently, Raschke et al (1978) illuminated attached leaves of Zea mays L. with monochromatic light, with either the upper or lower epidermis facing the light source. An inversion of the light direction caused at least a 200-fold change in the quantum flux into each epidermis. This variation in quantum flux did not affect stomatal conductance in the individual epidermes. Stomatal conductance was, however, correlated with C_i and the relationship between conductance and C_i remained the same if changes were brought about by changes in ambient CO_2 concentration instead of light.

Clearly, there is abundant evidence that stomata can be made to close with increase in C_i , but it is not clear whether natural variations in C_i have a substantial effect on stomatal behaviour of plants growing in the field. Many data have been presented which indicate only small, or no, effects of C_i on stomatal aperture over a natural range, for example, Heath & Milthorpe (1950), Jones & Mansfield (1970), Ludlow & Jarvis (1971), Akita & Moss (1972), Hall & Kaufmann (1975a), Ng (1978). It is possible that some seemingly conflicting results may be due to species differences in stomatal response to CO_2 as suggested by Pallas (1965) and Akita & Moss (1972). Controversy concerning the role of CO_2 in stomatal response may be partially resolved by the idea that the CO_2 control system can be overruled by other environmental and internal factors, as indicated by Turner (1970). Schulze *et al* (1975a) found that leaf resistance of Prunus armeniaca L., under natural conditions, was not consistently related to C_i and they concluded that "for the regulation of diffusion resistance in apricot under natural conditions, the effects of plant internal control mechanisms are overruled or modified by the external climatic factors of air humidity and temperature". Hall & Kaufmann (1975a) showed that stomatal resistance of Sesamum indicum L. responded little to variations in C_i over a natural range in humid air, but it did show moderate response to C_i when the humidity difference between leaf and air was increased. Raschke (1973) found that stomata of Xanthium strumarium well supplied with water were not sensitive to CO_2 , but they became sensitized by endogenous Abscissic acid (ABA) produced in strongly transpiring leaves.

1.3 Light effects independent of carbon dioxide

If stomatal opening depended only upon a reduction of C_i brought about by photosynthesis, then red and blue light should not differ greatly in the efficiency with which they produce opening (Meidner & Mansfield, 1968). The observations of Kuiper (1964), and Meidner & Mansfield (1966) showed greater opening in blue light suggesting a response not dependent upon C_i . A reaction to light independent of CO_2 concentration had been postulated by Heath & Russell (1954) to explain opening due to increased light intensity which occurred while the intercellular space CO_2 concentration was maintained at a low level. Meidner & Mansfield (1966) showed with Xanthium strumarium that although the CO_2 compensation point differed very little between red and blue light, stomatal opening was very much greater in blue, and the energy of red light required to produce "unit opening" of stomata was about seven times that in blue. Raschke (1967a) obtained similar results with Zea mays L.. Hsiao (1973) determined the action spectrum of stomatal aperture with Vicia faba leaves which were insensitive to CO_2 , and showed a peak of action in the blue region with no action in the red region at similar quantum flux. Raschke (1975) observed that blue light is known to enhance respiration (after Kowallik, 1971) and speculated that the energy supply to the turgor mechanism was also enhanced. He concluded that blue light may thus enhance stomatal opening, but was not essential.

More recently, Nelson & Mayo (1975) have shown a response to blue light in guard cells of Paphiopedilum spp. devoid of chloroplasts, and Zeiger & Hepler (1977) a similar response in isolated guard cell protoplasts of onion cotyledons. Raschke et al (1978) examined the interaction of light and CO_2 responses in Zea mays and found a small increase in conductance in stomata of the upper surface in response to blue light which was superimposed on the dominating response to C_i .

It may be concluded from the available evidence that the removal of CO_2 from intercellular spaces, and blue light are both capable of affecting stomata, and that their effects are independent to some degree.

1.4 Leaf water potential

Hsiao (1973) has reviewed data which demonstrate that stomata are insensitive to reductions in leaf water potential until a threshold level is passed, with various agricultural plants. Above this threshold level, leaf resistance remains constant and below it, leaf resistance increases rapidly with further decrease in potential. The position of this threshold varies widely between different experiments and species (see reviews by Turner, 1974 and McCree, 1974), but is generally thought to be between approximately -7 and -18 bars (Raschke, 1975). In field conditions leaf water potentials may drop to very low levels without reaching the threshold value for stomatal closure, for example Schulze et al (1975a) reported water potentials as low as -43.5 bar in Prunus armeniaca L. with a high transpiration rate without stomatal closure.

Conversely, increased leaf water stress has been observed to increase stomatal resistance gradually by some workers, e.g. Biscoe (1972) working with Beta vulgaris, and Redshaw & Meidner (1972) working with tobacco. It has been suggested (Hall et al, 1976) that the threshold concept may be partially an artifact due to the use of the "resistance" parameter, because the relation between conductance and leaf pressure potential appears to be linear over a range of values (Gardner, 1973; McCree, 1974), while resistance responses are curvilinear increasing almost asymptotically at the "threshold" value.

However, Schulze et al (1975a) support the view that variations in bulk leaf water status above some critical value have little effect on leaf resistance and that the leaf water status is, in fact, itself determined by the influence of stomatal resistance on transpiration

. . . Most observations are consistent with the view that there is a (species dependent) range of high water potentials over which there is no direct influence on stomatal aperture.

Stalfelt (1929, 1955) was the first to distinguish hydropassive and hydroactive stomatal movement (active and passive movement in Stalfelt's own terminology). The hydropassive movements are due to rapid local turgor changes in the leaf, and can be demonstrated when a turgid leaf is detached and left without a water supply, (Meidner & Mansfield, 1968; Heath, 1975). The stomata open widely for a few minutes before closure and it is thought that transpiration from the mesophyll cells at first continues and as they lose turgor they withdraw water from the epidermis and subsidiary cells (if present). The loss of turgor from the latter allows the guard cells to "bulge out" and open the pore. Later, as the subsidiary cells take water from the guard cells, the pore closes. Such a response is possible because the guard cells have no direct connection with the mesophyll, and liquid water can only be transferred between guard cells and mesophyll cells via epidermal cells (Meidner & Mansfield, 1968; Delwiche & Cooke, 1977). The converse processes occur when a detached leaf under water stress, with part open stomata, is suddenly supplied with water: the stomata close rapidly and later re-open (Heath, 1975). Such rapid hydropassive movements are less often observed in intact plants (partly because of the initiation of a hydroactive process; see below), but the same tendencies presumably occur with every change in the rate of transpiration. Hydropassive movements are described by Raschke (1975) as resulting from positive hydropassive feedback, because changes in epidermal turgor may lead to stomatal responses in the "wrong" direction. However, these responses are transient, and also the assumption that transpiration only takes place from the mesophyll cells may be an oversimplification; water is lost through the cuticle and from the guard cells themselves (Lange et al, 1971; Raschke, 1975).

Stalfelt's (1929) active movement was so called because the guard cells played an active part in producing it, and he suspected the

involvement of an active response. The mechanism of this movement, now called hydroactive movement, evaded identification until fairly recently, with the work begun by Little & Eidt (1968), and Mittleheuser & Van Steveninck (1969), who demonstrated that exogenous abscisic acid (ABA) reduced transpiration and closed stomata. Wright & Hiron (1969), Hiron & Wright (1973) showed that ABA was formed in wilting leaves, and stomata closed as a consequence. Turgor was regained and ABA synthesis ceased in the recovering turgescient leaf after this regulation. More detailed investigations by Cummins (1971), Raschke (1974) and Raschke et al (1974) suggested that the effect of ABA on stomata depends upon the concentration of ABA in the transpiration stream together with the rate of transpiration, rather than on the absolute amounts in the leaf.

Thus, ABA is synthesised in response to decrease in turgor in wilting leaves and causes decrease in turgor associated with loss of solute in the guard cells. Cowan (1977) speculates that loss of turgor in the guard cells could be reinforced by synthesis of ABA (or related compounds) within the guard cells themselves. This effect is equivalent to what Raschke (1975) called "negative hydroactive feedback in guard cells". It is not clear whether ABA synthesis is triggered by the reduction in water potential or turgor or the increase in the concentration of a triggering substance in a cell compartment, but there is evidence that it is synthesized in the chloroplasts (Milborrow, 1974). Raschke (1975) reports that the hydroactive feedback within guard cells appears to be absent when leaves are subjected to very rapid water loss, the ^tstomata failing to close at a threshold value of leaf water potential. He speculates that this is because ABA does not form rapidly enough in sufficient quantity.

There is no evidence as yet that ABA synthesis is caused by small changes of water potential in turgid tissue. It does appear that ABA

and related compounds play a major role in stomatal closure in wilting plants, but their involvement in stomatal movement in turgid plants is speculative (Cowan, 1977).

1.5 Direct response to humidity

Until fairly recently, it was thought that stomatal regulation of leaf water relations was achieved entirely by a feedback process, whereby increased transpirational water loss caused increased water deficits in the bulk leaf which led to stomatal closure e.g. Drake et al., (1970). It was suggested that atmospheric humidity influenced stomatal movement only indirectly via transpiration. However, more recent work has suggested that stomata can also respond directly to ambient humidity in a way which is independent of bulk leaf water status.

Porometric measurements by Paschke (1970) showed that detached leaves of Zea mays L. of equal water contents had much higher transpirational resistances in dry air than in moist air, and he concluded that direct evaporation from the epidermis (in particular, the guard cells) had a modifying effect on stomatal aperture. Lange et al (1971) demonstrated a direct effect of change in ambient humidity on the aperture of stomatal strips of Valeriana^{ella} locusta and Polypodium vulgare. The studies of Schulze et al (1972), with Prunus armeniaca growing in the field, clearly demonstrated that stomata responded to humidity independently of effects on bulk leaf water status. In their studies stomatal response was inferred from gas exchange measurements, and closure in dry air was so pronounced that transpiration was less than in humid air, and the relative water content of the leaves actually increased with drier air. Schulze and co-workers (1972, 1974, 1975a, 1975b) have worked extensively on the way in which stomata respond directly to various environmental parameters which affect transpiration rate, and they have shown that the phenomenon of midday closure which commonly occurs in P. armeniaca, is not attributable to changes in leaf water status or intercellular CO₂ or to an intrinsic endogenous rhythm, but is in fact a response to the difference in humidity between leaf

and air and leaf temperature. Similar results were obtained with the xeromorphic stems of Hammada scoparia, and the succulent leaves of Zygophyllum dumosum.

Camacho-B, Hall & Kaufmann (1974) and Hall & Kaufmann (1975a) showed that humidity induced changes in leaf resistance were not accompanied by measurable changes in bulk leaf water status in leaves of Sesamum indicum L.: these changes in resistance were not mediated by a stomatal CO₂ control system because the substomatal CO₂ concentration was kept constant. Hall & Kaufmann (1975b) have also found similar results with Helianthus annuus and Beta vulgaris, as did Watts, Jarvis et al (1974) with Picea sitchensis. Lawlor & Milford (1975) discuss evidence of the effects of humidity on stomatal aperture in Beta vulgaris and various pasture plants such as Trifolium repens and Lolium perenne. Aston (1976), working with Helianthus annuus, showed that stomatal resistance increased linearly as the water vapour concentration difference between leaf and air increased, even though leaf water content did not change. Lösch (1977) has shown a direct response of stomata to humidity gradients with isolated epidermal strips of Polypodium vulgare. Several other investigations with intact plants point to the existence of a direct humidity effect, and these are cited in Hall et al (1976) and Cowan (1977).

Clearly, indirect measurements on whole leaves and direct observations on isolated epidermal strips have proved that there is a direct response of stomata to change in ambient humidity, a response which is independent of the temperature responses considered in the next section. It seems fair to assume that such a response must act by influencing the water relations of the guard cells. Lange et al (1971) have proposed that the mechanism of the response may involve peristomatal transpiration (after Seybold, 1961/2) which is water loss from the guard cells through the cuticle and cuticular ledges to the atmosphere. If, under high

evaporative conditions, the guard cells lose more water per unit time than do the neighbouring epidermal cells, then the guard cells will lose turgor with a consequent decrease in stomatal aperture. If this were so, then the guard cells are acting as humidity sensors and the difference in humidity between the leaf and surrounding air represents the driving force for water vapour loss through the guard cell cuticle. Maercker (1965) using autoradiography after feeding tritiated water to leaves, provided some evidence that the rate of cuticular transpiration may be higher from guard cells relative to ordinary epidermal cells. Similar results have been obtained by Aston & Jones (1976) with oats. The implication of such a mechanism for direct response to humidity is that there is a loss of water which is not under stomatal control, but which can take place at a sufficient rate to affect the turgor of the stomatal apparatus.

Cowan (1977) has formalized the argument for peristomatal control in terms of a "feedforward" mechanism using models based on control theory, and has concluded that "the case that the physiological mechanism (of direct response to humidity) is very strong", but adding "all one might strictly conclude is that there is a loss of water from the leaf to which the stomata are sensitive, but which is not modulated by the reaction of the stomata to it". What is certain is that such a response mechanism would provide an adaptive advantage especially in arid environments because it would prevent the development of water stress in the metabolically active cells within the leaf (Schulze et al, 1972) and would also improve water-use efficiency (Hall & Kaufmann, 1975; Cowan, 1977).

1.6 Temperature

The close coupling between leaf temperature and humidity difference from leaf to air has made it difficult to assess the effects of temperature independently. There have been divergent views in the literature concerning the influence of temperature on guard cell behaviour. Some reports have suggested that increasing temperature favours opening, e.g. Drake et al (1970) working with Xanthium strumarium. Other work has indicated closure at high temperatures e.g. Wuenscher & Kozlowski (1971), working with several species of Quercus and Acer saccharum. Intermediate temperatures favouring stomatal opening have also been reported e.g. by Whiteman & Koller (1964) with Pinus halepensis. Recently, since more attention has been paid to the responses of stomata to changes in air humidity, some of the more seemingly contradictory data relating to temperature responses have been reconciled. Under natural conditions the humidity difference between leaf and air is normally large at high temperatures, and its influence may have been erroneously interpreted as a temperature effect. For example, Wuenscher & Kozlowski (1971) reported increasing stomatal closure over the range 20°C to 40°C. They maintained a constant vapour concentration in the assimilation chamber over the range of temperatures explored, but since the intercellular spaces of the leaf are always assumed to be saturated at leaf temperature, this meant that the humidity difference between leaf and air was increasing as the temperature increased.

Schulze et al (1973) conducted experiments with Prunus armeniaca under constant evaporative conditions and showed that stomata opened with increase in leaf temperature providing water stress was low. If, however, the leaf was under substantial stress, then the stomata closed as temperature increased irrespective of the humidity difference. Similar results were obtained with Sesamum indicum by Hall & Kaufmann

(1975a) who reported that, with constant small humidity differences, stomatal resistance decreased with increasing temperature and also that resistance responded more to difference in humidity at lower temperatures (1975b). Lössch (1977) has reported that temperature exerts a strong influence on stomatal aperture using epidermal strips of Polypodium vulgare. Working with a constant leaf to air humidity difference, Lössch found that low temperatures caused closure, that maximal opening occurred at 20°C to 28°C, and that higher temperatures led to a slight reduction in aperture. Lössch (1977) also showed a humidity response independent of temperature and went on to conclude that temperature and humidity difference induce guard cell movements by means of two independent mechanisms. Response to temperature is further complicated by the fact that Ludlow & Wilson (1971) have reported that temperature preconditioning is important.

The findings reported in the literature are not necessarily in total conflict, as it would be hardly surprising if different species have different temperature optima, for example an opening response would be of great benefit to a well watered, broad leaved plant, reducing the danger of overheating (by enhancing evaporative cooling) and maintaining net photosynthesis, providing sufficient water was available. Inevitably, there are also indirect effects of temperature exerted through the water relations of the guard cells, and temperature induced net photosynthetic responses altering the sub-stomatal CO₂ concentration of the leaf. It may be more relevant to discuss the interactions of leaf water status, ambient humidity, sub-stomatal CO₂ concentration and temperature in terms of the adaptive advantages such interactions would seem to offer in specific situations, and this will be considered in the discussion section.

1.7 Oxygen

Raschke (1975) has summarised the events which take place in the guard cells of opening stomata, from the available evidence. Briefly, malate is formed (possibly from starch) via phosphoenolpyruvate (PEP), and is transported into the vacuole. Hydrogen ions (H^+) are expelled from the guard cells, and potassium (K^+) and chloride (Cl^-) ions are taken up. While the formation of malate is exergonic, all of the other associated processes are energy requiring and will require ATP or reducing equivalents. Thus, it appears that there is a net energy requirement for opening. The guard cells have two possible sources of energy: oxidative phosphorylation and photosynthetic phosphorylation (Meidner & Mansfield, 1968), oxygen being required for the former but not the latter. The fact that stomata can open in response to CO_2 -free air in the dark (Freudenberger, 1940; Heath, 1948; Raschke, 1975) shows that photosynthetic phosphorylation is not essential for the supply of energy to the stomatal mechanism, so it seems that the energy required for stomatal opening can come from oxidative phosphorylation alone.

There is strong evidence that oxygen (O_2) is necessary for stomatal opening to occur, in light or dark, from a closed position, e.g. Heath & Orchard (1956), Zelitch (1965), Meidner & Mansfield (1968), Burrows & Milthorpe (1975). However, the effect of lack of ambient O_2 on open stomata in an otherwise unaltered environment, has given variable responses. There has not been general agreement with Heath & Orchard's (1956) finding that lack of O_2 actually accelerated stomatal closure with wheat. Fujino (1967) came to the conclusion that both opening and closing were energy requiring processes, and this was supported by Louget (1968) ^(REFERRED TO BY HEATH, 1975) who found that in Pelargonium sp., anaerobic conditions completely inhibited closure due to darkness or high CO_2 , and also inhibited opening in response to light or low CO_2 . Raschke (1975)

states that the subsidiary cells of maize contain numerous mitochondria which may indicate an active reabsorption of K^+ by subsidiary cells during closure. This seems a little strange when one remembers that the K^+ ions have to be actively transported into the guard cells during opening. One might expect that transport of K^+ out of the guard cells, where they have been held against a concentration gradient, would be passive, unless membranes prevent such passive movement. Since there is a net energy requirement during opening one might also expect a net energy release during closure. However, this assumes that the processes which occur during closure, are the opposite of those which occur during opening.

Meidner & Mansfield (1968) speculate that the total energy requirements for the production of opening might be greater than for its maintenance, and since photophosphorylation can go on in the absence of O_2 this might provide enough energy to keep stomata open, whereas additional energy from oxidative phosphorylation may be required to produce opening from an initially closed condition. This is supported by the work of Walker & Zelitch (1963) who found an O_2 requirement for the opening movement in response to light, but that lack of O_2 in light did not induce closure in stomata which were already open. Hall (1975) reported rapid closure of stomata with Capsicum sp., but not with bean or sunflower on exposure to O_2 -free air. Akita & Moss (1972) have shown that stomata of some species fail to close in an O_2 -free atmosphere.

Levitt (1976) states that stomata permit the entrance of O_2 into the leaf at a rapid enough rate to support aerobic respiration sufficient to provide for the metabolic needs of the leaf. Also that stomata have become adapted to this function by opening in response to low O_2 concentration inside the leaf. Raschke's (1967) observation that a short exposure of Vicia faba leaves to anaerobic conditions led to

increased opening might tend to support this view. However, it is possible that an opening response to lower O_2 concentrations could, in fact, be a response to a consequent lowering of internal CO_2 concentration: O_2 is known to inhibit photosynthesis (Warburg, 1920; Bjorkman, 1966), so a reduction in ambient O_2 could result in increased photosynthesis, a reduction in internal CO_2 concentration, and an opening response of the stomata. The energy for such a response is thus provided by photophosphoryllation. This idea is, however, purely speculative.

As can be seen, the effects of anaerobic conditions on stomatal behaviour are variable, and a further consideration of the O_2 requirement of stomatal movement will be given in the discussion.

CHAPTER 2: MATERIALS AND METHODS

2.1 The gas exchange system and methods of measurement

The gas exchange system used was an open system whereby a gas stream of known properties was passed through an assimilation chamber at a measured flow rate, then through a dewpoint hygrometer (Cambridge Systems Inc., Model 880) and an Infra-Red Gas Analyser (IRGA) (Sir Howard Grubb Parson & Co Ltd, Model SB2) before exhausting from the system to the atmosphere.

2.1/1 Mixing of gases for IRGA calibration and gas exchange measurements.

Standard gases of known carbon dioxide (CO_2) concentration were prepared for calibration of the IRGA and also for gas exchange experiments involving varying oxygen (O_2) concentrations. The gases were prepared by the volume method using Wösthoff gas mixing pumps (Gasmischpumpe, H Wösthoff, Bochum, BRD). The basic gas mixing apparatus, consisting of two pumps (type SA27 or SA18) cascaded in series, is shown in figure 2.1. This formed the basis for all gas mixing operations.

Pressure regulating valves (PR1 and PR2) were placed in the gas lines directly after the CO_2 and nitrogen (N_2) cylinders in order that the inlet pressures to the first gas mixing pump (GM1) could be balanced. A manometer (M1) provided a visual means of checking that these inlet pressures were equal. The CO_2/N_2 mixture was then passed, via a mixing vessel (MV1), into one of the inlets on the second gas mixing pump (GM2). An overflow relief vessel (O1) between the outlet of GM1 and the inlet of GM2, prevented the entry of outside air into the second pump during the suction cycle of the first pump, and also affected a means of balancing the inlet pressures to the second pump (by adjusting the head of pressure and balancing the fluid in the manometer M2). The resistances on the cylinder sides of M1 and M2 were short pieces of capillary tubing. These made it more difficult to empty the manometers of fluid by injudicious handling of the pressure regulators PR1 and PR2.

FIGURE 2.1 Basic gas mixing apparatus.

D1 } D2 }	silica gel drying towers
GM1 } GM2 }	gas mixing pumps
M1 } M2 }	manometers
MV1	mixing vessel
O1	overflow relief vessel
PR1 } PR2 }	pressure regulating valves
R1 } R2 }	resistances (capillary tubing)
RS4	reservoir
S1 } S2 }	pressure stabilizing vessels
SL1 } SL2 }	soda lime towers

Basic gas mixing apparatus

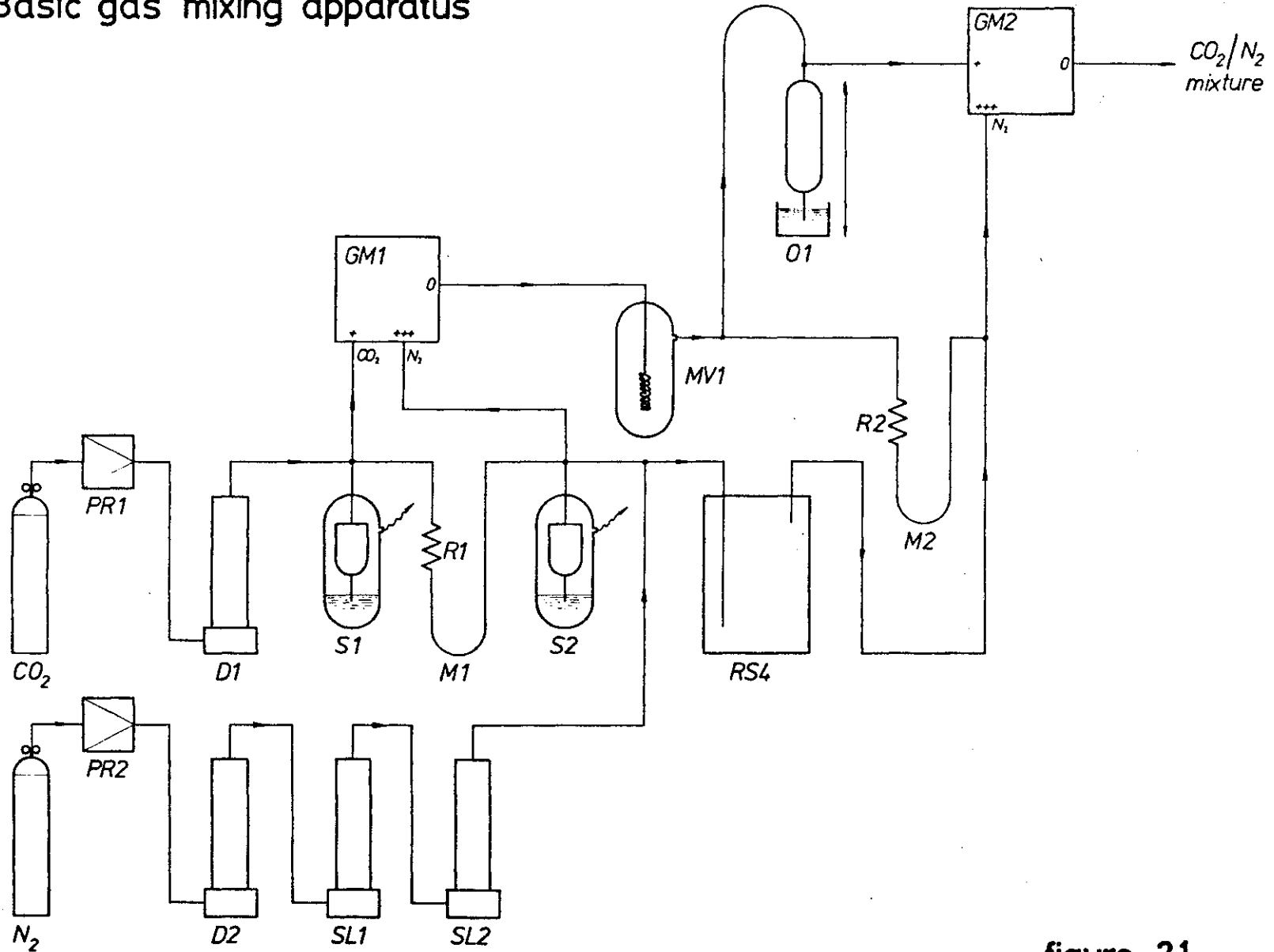


figure 2.1

. FIGURE 2.2 Apparatus for IRGA calibration.

F3	flowmeter
M4	} manometers
M5	
PR4	} pressure regulating valves
PR5	
R2	reservoir
S3	pressure stabilizing vessel
V3	flow regulating (needle) valve

Input from basic
gas mixing
apparatus fig.2.1

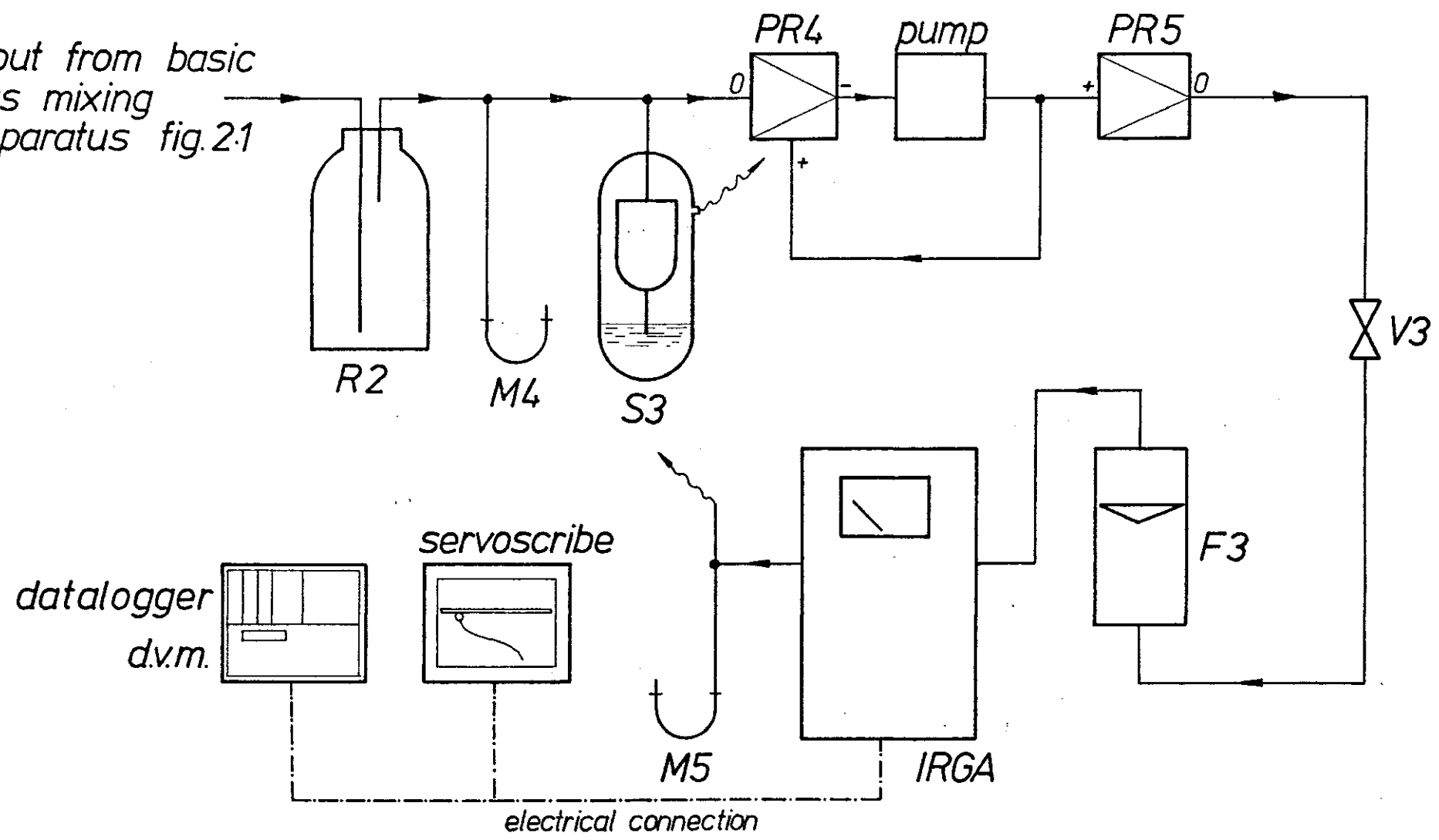


FIGURE 2.2

The additional apparatus needed for IRGA calibration is shown in figure 2.2. The gas mixing pumps required that pressure at their outlet be less than 100 mm. water gauge (above atmospheric). This presented a problem because pressures in excess of this figure could occur due to the resistance of the tubing required to pass the gas through the rest of the system. A simple pressure regulating system was designed to keep the outlet pressure of GM2 at, or close to, atmospheric pressure. This system is shown in figure 2.2 after the gas mixing reservoir R2.

A vacuum was applied to the -ve outlet of the pressure regulating valve PR4. Pressure at the regulated inlet of PR4 (labelled '0' in figure 2.2) was adjusted until the manometer M4 was balanced. This meant that the pressure at the outlet of the gas mixing reservoir R2 and hence at the outlet of GM2 was equal to atmospheric pressure. Any excess gas produced by the gas mixing pumps was lost to the atmosphere via the pressure stabilizing vessel S3. A loop was provided after the pump back to PR4 so that if the vacuum applied at the -ve outlet of PR4 exceeded the supply of gas from the gas mixing pumps, then gas at the correct CO₂ concentration was drawn into PR4 and not outside air.

The additional apparatus for the generation of CO₂/N₂ mixtures at zero O₂ concentration is shown in figure 2.3. Again the outlet pressure regulating apparatus was used.

The generation of gas mixtures with varying O₂ concentration involved a third gas mixing pump cascaded in series, and the additional apparatus is shown in figure 2.4. The O₂ inlet pressure to GM3 could be adjusted by means of a pressure regulating valve (PR3), and the inlet pressure of the CO₂/N₂ mixture from the basic gas mixing apparatus could be adjusted by means of an overflow relief vessel (O2), which also prevented the entry of outside air into GM3 during the suction

FIGURE 2.3 Apparatus for the generation of $\text{CO}_2:\text{N}_2$ mixtures for gas exchange measurements.

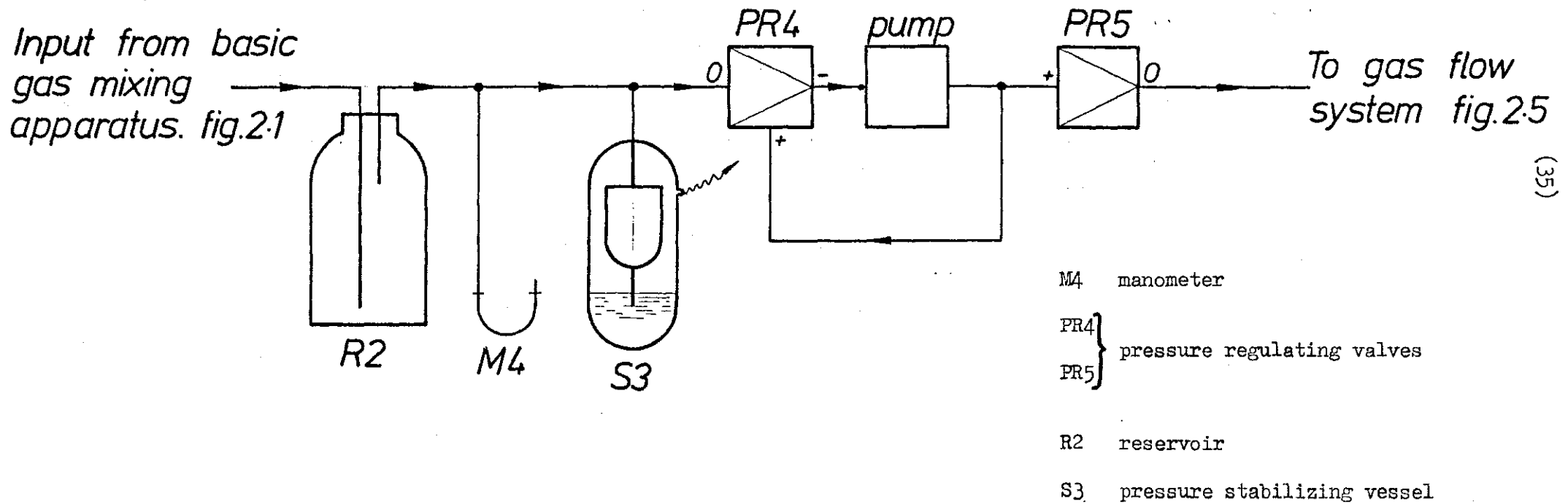


FIGURE 2.4 Apparatus for the generation of $\text{CO}_2:\text{N}_2:\text{O}_2$ mixtures for gas exchange measurements.

GM3	gas mixing pump
M3	} manometers
M4	
LV2	mixing vessel
O2	overflow relief vessel
PR3	} pressure regulating valves
PR4	
PR5	
R2	resistance (capillary tubing)
R3	reservoir
S3	pressure stabilizing vessel

Input from basic
gas mixing
apparatus.
fig. 2.1

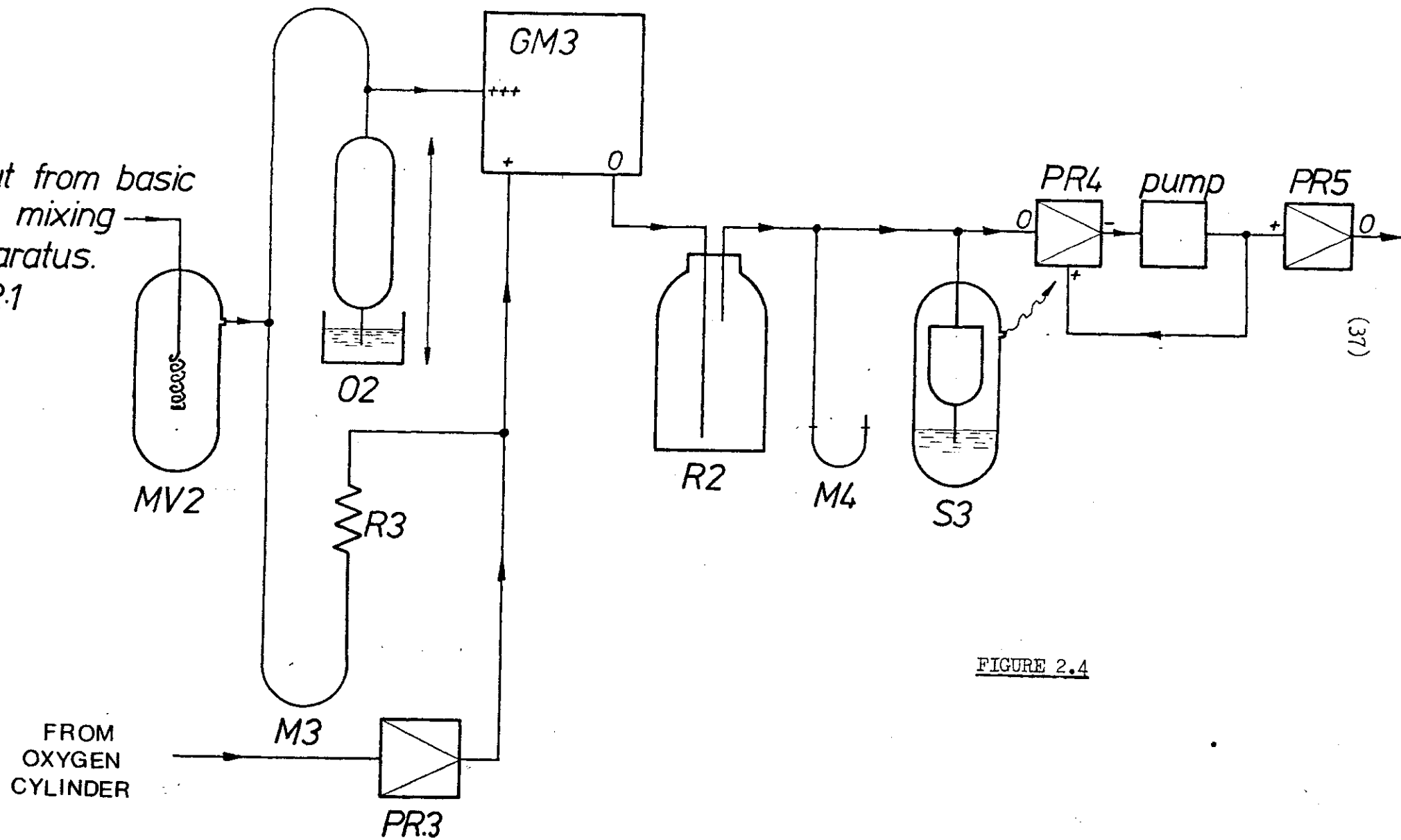


FIGURE 2.4

cycle of the previous pump (GM2). The outlet pressure regulating apparatus was again used, to keep the outlet pressure of GM3 at atmospheric.

2.1/2 The gas flow system.

The gas flow system is shown in figure 2.5. The dewpoint temperature of the gas entering the chamber could be controlled accurately by means of the humidification apparatus shown in figure 2.5. The gas supply was first saturated by passage over distilled water contained in a perspex tube. It then passed through a condenser immersed in a water bath, the temperature of which was precisely controlled by circulating coolant and a thermostatically controlled heater. The dewpoint temperature of the air emerging from the humidification apparatus was thus equal to the temperature of the water in the water bath. This was confirmed by measuring the dewpoint temperature with the hygrometer. The dilution of the CO_2 content of the air caused by humidification was taken into account in the calculation of gas exchange rates (see Section 2.5/6).

The humidified gas was then passed through a sintered bronze filter (Norgren Ltd, Warwicks, UK) to remove any solid particles. This was necessary as the IRGA and the dewpoint hygrometer, being photometric devices, would have been sensitive to such particles. The gas was then split according to the number of chambers in use, up to seven streams being possible. The flow rate of each stream was adjusted by means of needle valves (Simplifix Couplings Ltd, Berks, UK) and measured with flowmeters (G A Platon Ltd, UK and Rotameter Manufacturing Co, UK). One stream went directly from flowmeter to solenoid valve unit (stream 3). This stream provided a reference humidity and CO_2 concentration. The other streams passed to the solenoid valve unit via leaf chambers.

The solenoid valve unit (designed and constructed by Dr P W Mueller, Imperial College, London) allowed any one stream to be directed through

Gas flow system

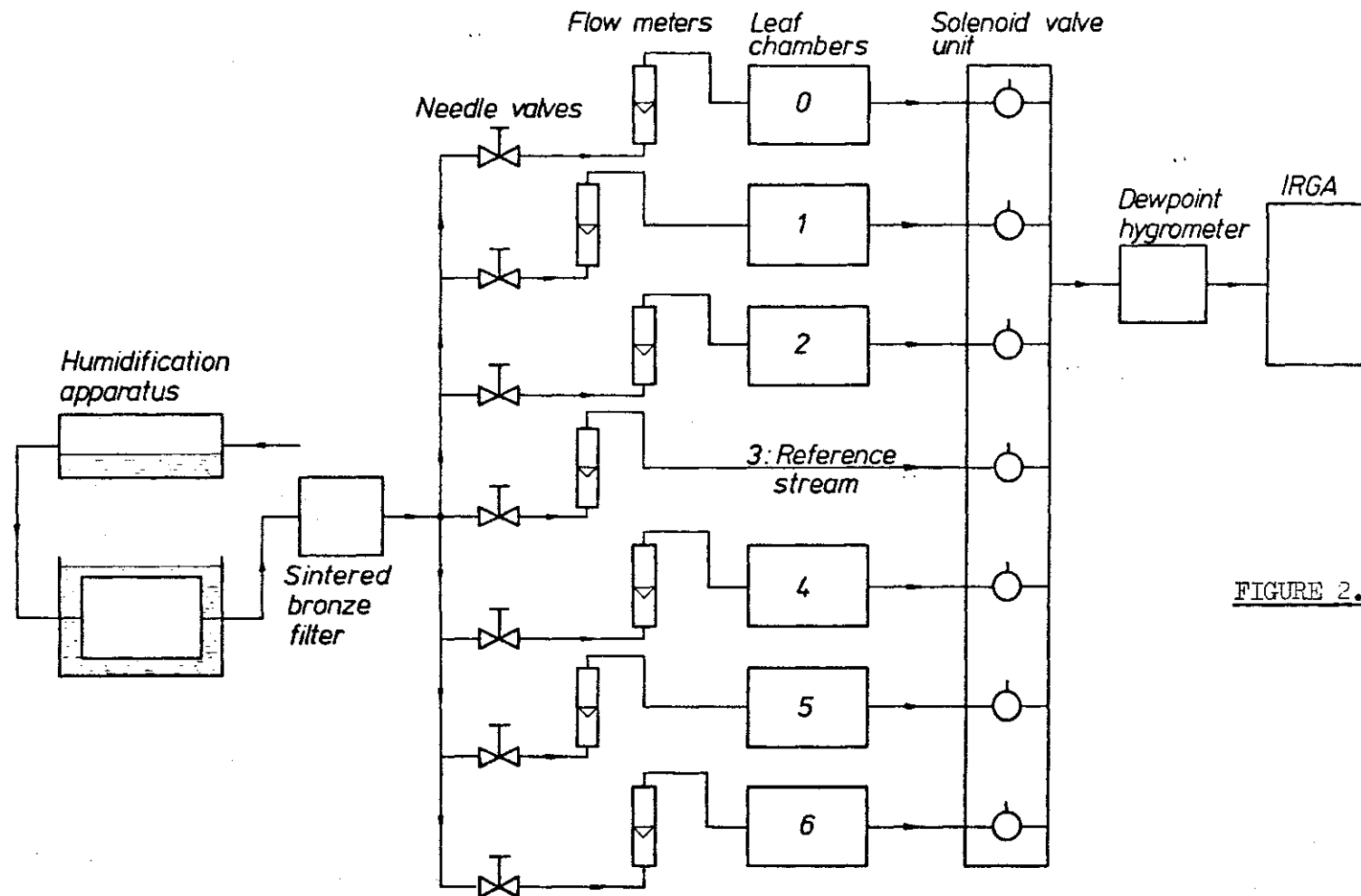


FIGURE 2.5

the dewpoint hygrometer and IRGA at any one time. Other streams ran to waste and care was taken to ensure that flow rate was not affected by switching. This was achieved by varying the resistance in the outlet to waste in each stream with Hoffman clips so that it equalled the resistance when that particular stream was passed through the rest of the system.

The IRGA was used in absolute mode i.e. the absolute amount of CO_2 in the analysis tube was measured whilst the reference tube was kept sealed during calibration and measurement. Thus, although gas flowed only in the analysis tube, a reference CO_2 concentration was provided by stream 3 which did not pass through a chamber. By measuring the difference in absolute CO_2 concentration between the reference stream and all other streams (the analysis streams), the CO_2 exchange of the leaves in the leaf chambers could be calculated (see Section 2.5/6).

The gas sample lines of the system consisted basically of nylon tubing, but short lengths of PVC tubing were used immediately around the chambers to provide flexibility in the line. All tubing was of 5/16" outside diameter, which was considered small enough to minimise the response time of the system, but not small enough for there to be a very large pressure drop along the lines.

Care was taken to ensure that the temperatures of all of the apparatus, particularly the tubing and the heat exchanger mounted inside the leaf chamber, were kept above the dewpoint temperature of the gas flowing in the system. This was achieved by monitoring the various temperatures and adjusting the flow rates and incoming dewpoint, to avoid condensation in the system, which would have invalidated transpiration measurements.

2.1/3 The Data Logging system

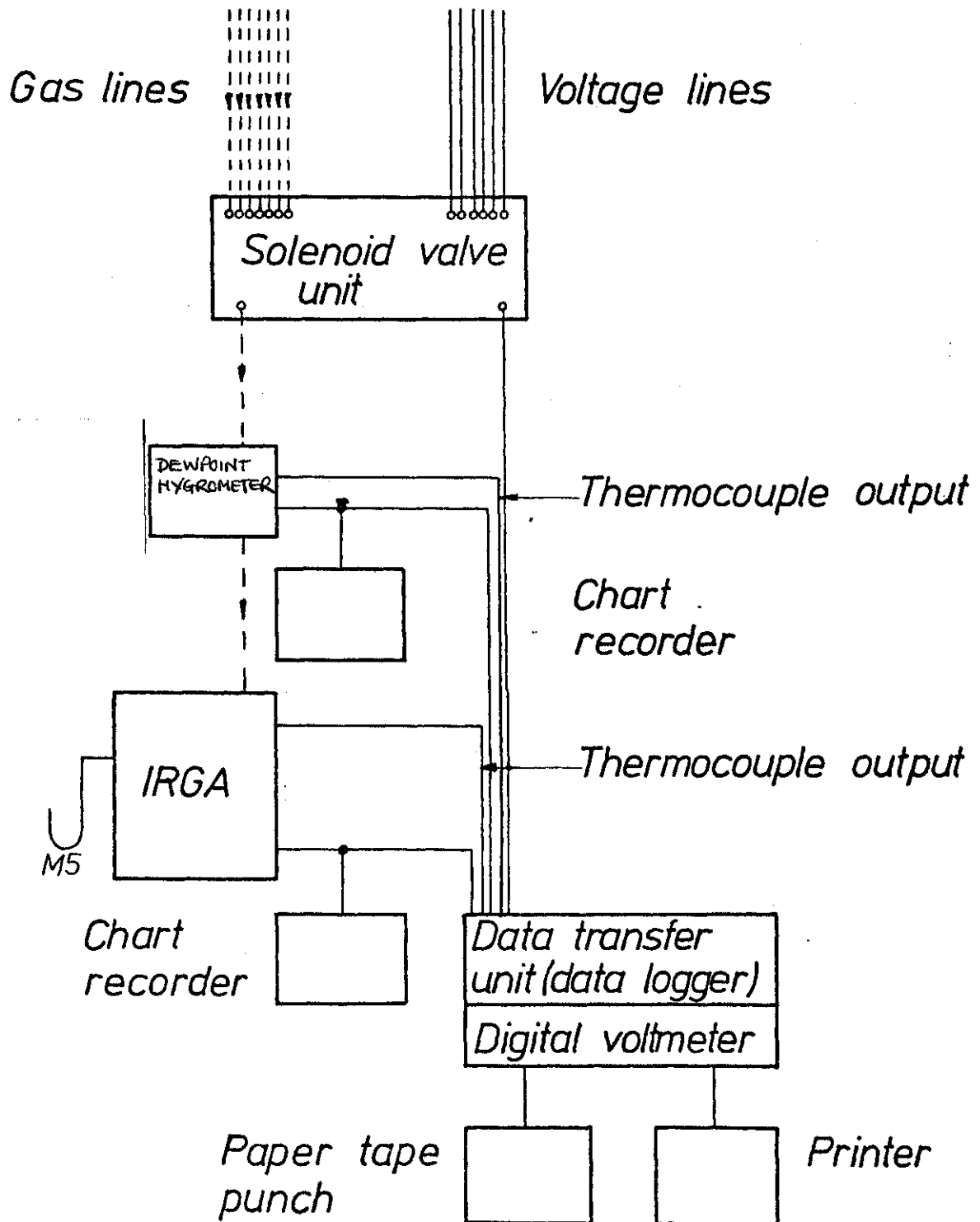
The calculation of gas exchange rates and transfer resistances involved the measurement of many parameters, and it was desirable that the data be collected automatically and in a form suitable for subsequent processing by digital computer. For this reason a data logging system was installed consisting of: a Solartron LM1604 DC digital voltmeter (Solartron Electronic Group Ltd, UK); a Solartron 3230 Data Transfer Unit (data logger); a Facit 4070 paper tape punch (Facit Ltd, UK); and an Addmaster Model 35 serial entry printer (Addmaster Corp, USA).

In addition, chart recorders (Servoscribe 1s, Model RE541.20, Smiths Industries Ltd, UK) were connected to the outputs of the dew-point hygrometer and the IRGA. Thus, the data was collected in analogue form on the chart recorders and in digital form on the tape punch and printer. This combination of display and punched tape record had several advantages:

1. The recorder charts and printer provided a permanent visual display so it was possible to follow what was happening during the course of an experiment.
2. The punched paper tape could be fed into a computer for data processing without further manipulation. In this way, errors associated with transference of data from charts were avoided.
3. When non steady-state phenomena were being followed, measurements could be made as frequently as required.
4. When step functions were being employed, attainment of a steady-state could be followed on the chart recorders and digital records taken only when desired.

A block diagram of the data logging system is shown in figure 2.6. As shown in the gas flow section, there were a maximum of seven streams

FIGURE 2.6 The data logging system.



to be analysed (one reference stream and up to six analysis streams). The solenoid valve unit enabled any one gas stream to be directed through the dewpoint hygrometer and IRGA, and a switch unit incorporated into the solenoid valve unit enabled the corresponding transducers for that stream to be connected to the data logger. The switching could be done manually or automatically, controlled by a clock incorporated into the solenoid valve unit. Streams not being used could be skipped.

When a data logger scan was initiated, the signals from the solenoid valve unit, dewpoint hygrometer and IRGA were switched sequentially, measured with the digital voltmeter and recorded on the paper tape and printer. Triggering of the data logger could be initiated manually, automatically with a clock incorporated into the data logger, or automatically with the clock in the solenoid valve unit. Each time the data logger was triggered, records of the following were obtained:

1. The time of measurement, from the clock in the data logger.
2. The IRGA output voltage.
3. The dewpoint hygrometer output voltage.
4. The IRGA thermocouple voltage (see Section 2.5).
5. The dewpoint meter thermocouple voltage (see Section 2.5).
6. The chamber air temperature thermocouple voltage (analysis only).
7. The leaf temperature thermocouple voltage (analysis streams only).
8. The chamber photocell voltage (analysis streams only).

In addition, the flow rate of the gas stream being sampled was noted, together with the reading on the manometer M5 (this enabled the pressure in different parts of the system to be calculated as shown in Section 2.3/4).

2.1/4 Experimental rationale

When the reference stream was sampled, the following quantities were obtained:

1. The IRGA output voltage, x_r (volts); measured with the digital voltmeter.
2. The water vapour pressure, E_{hr} (mbar); calculated using the dewpoint hygrometer output voltage as shown in Section 2.3/2.
3. The pressure in the IRGA analysis tube, P_{ar} (mbar); estimated from barometric pressure plus the pressure across the manometer (M5) between the IRGA outlet and ambient.
4. The pressures in the flowmeter, P_{fr} (mbar), and hygrometer, P_{hr} (mbar); estimated as linear functions of P_{ar} according to the calibration shown in Section 2.3/4.
5. The IRGA analysis tube temperature, T_{ar} ($^{\circ}\text{C}$); measured with a thermocouple placed in the IRGA analysis tube.
6. The dewpoint hygrometer temperature, T_{hr} ($^{\circ}\text{C}$); measured with a thermocouple placed in the hygrometer inlet.
7. The flowmeter temperature, T_{fr} ($^{\circ}\text{C}$).

When an analysis stream was sampled, the following quantities were obtained:

1. The IRGA output voltage, x_a (volts).
2. The water vapour pressure, E_{ha} (mbar). (See Section 2.3/2).
3. The pressure in the IRGA analysis tube, P_{aa} (mbar).
4. The pressures in the flowmeter, P_{fa} (mbar); chamber, P_{ca} (mbar), and hygrometer, P_{ha} (mbar); estimated as linear functions of P_{aa} .
5. The IRGA analysis tube temperature, T_{aa} ($^{\circ}\text{C}$).
6. The dewpoint hygrometer temperature, T_{ha} ($^{\circ}\text{C}$).
7. The leaf temperature, T_l ($^{\circ}\text{C}$), and chamber air temperature, T_{ca} ($^{\circ}\text{C}$); measured with thermocouples inside the chamber.

8. The flowmeter temperature, T_{fa} ($^{\circ}\text{C}$); assumed equal to T_{ca} .

Atmospheric pressure was read from a mercury barometer.

2.2 The assimilation chamber

2.2/1 Chamber design

All of the experiments were to be carried out using an open gas exchange system with attached leaves enclosed in an assimilation chamber. The main design criteria of the chamber were (after Jarvis & Catsky, 1971):

1. that it would assist in the continuous measurement of water vapour and CO_2 exchange between the leaf and its predetermined microclimate.
2. that leaf temperature and air temperature in the chamber could be accurately monitored, and to some extent controlled.
3. that gradients of temperature and gas concentration inside the chamber should be eliminated by adequate mixing.
4. that the thickness of the leaf boundary layer should be maintained small and constant.
5. that the flux density of photosynthetically active radiation incident on the leaf surface could be accurately and continuously monitored.

One of the assimilation chambers is shown in figures 2.7 and 2.8. The basic shell of the chamber was a die-cast aluminium box (Eddystone Radio Ltd, type 6357D). The aluminium lid of the box was discarded and the top of the box was milled flat so that a 'Perspex' lid could be fitted. A butyl rubber gasket placed between the perspex and the body of the chamber ensured a gas tight fit when the lid was screwed into position.

A simple heat exchanger was incorporated into the chambers, made up of a U-shaped piece of tubular copper with a flat plate of copper welded onto it. Either tap water or water at constant temperature (controlled by a thermocirculator) was passed through the copper pipe. Care was taken to ensure that the temperature of the circulating water

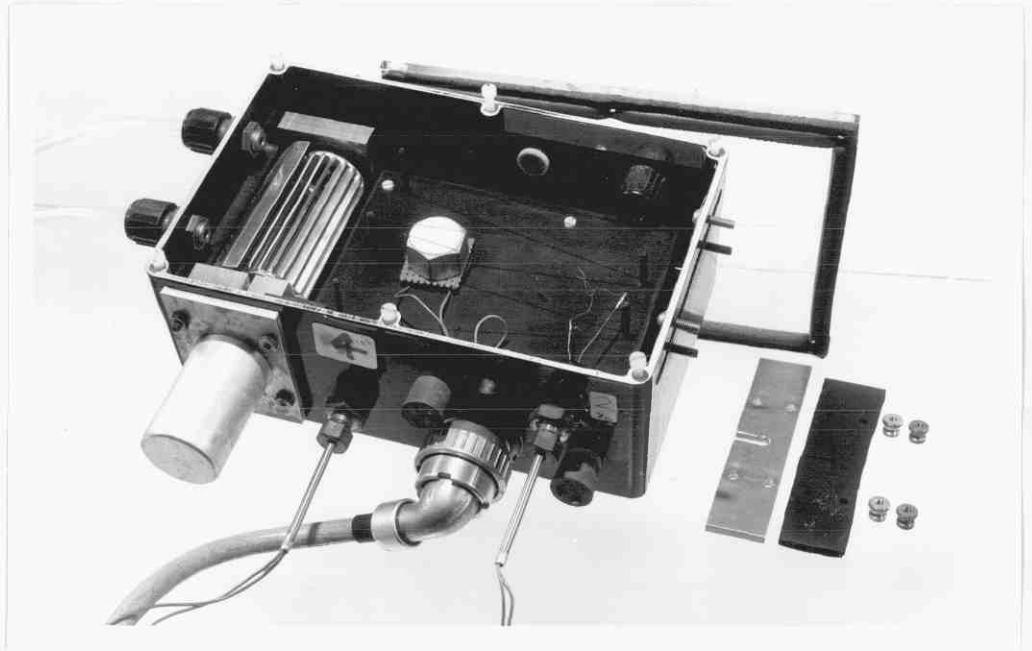


FIGURE 2.7 The assimilation chamber

FIGURE 2.8 The assimilation chamber.

Key:

- | | | |
|----|--|-----------------|
| 1 | Aluminium sealing plate | (Section 2.2/1) |
| 2 | Neoprene gasket | (" ") |
| 3 | Gas-tight connector: supplies power to fan and carries electrical connections from photocell | (Section 2.2/1) |
| 4 | Air temperature thermocouple | (" 2.2/2) |
| 5 | Leaf temperature thermocouple | (" ") |
| 6 | Gas inlet | (" 2.2/3) |
| 7 | Gas outlet | (" ") |
| 8 | Squirrel ~~cage~~ fan | (" ") |
| 9 | Fan motor housing | |
| 10 | Photocell assembly | (" 2.2/4) |
| 11 | ~~PERS~~ ex plate covering heat exchanger | |
| 12 | Cotton thread to support leaf | |

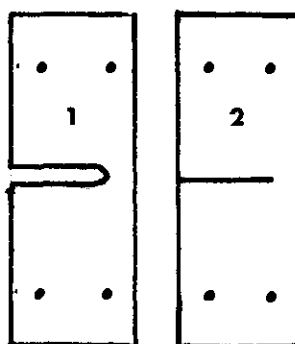
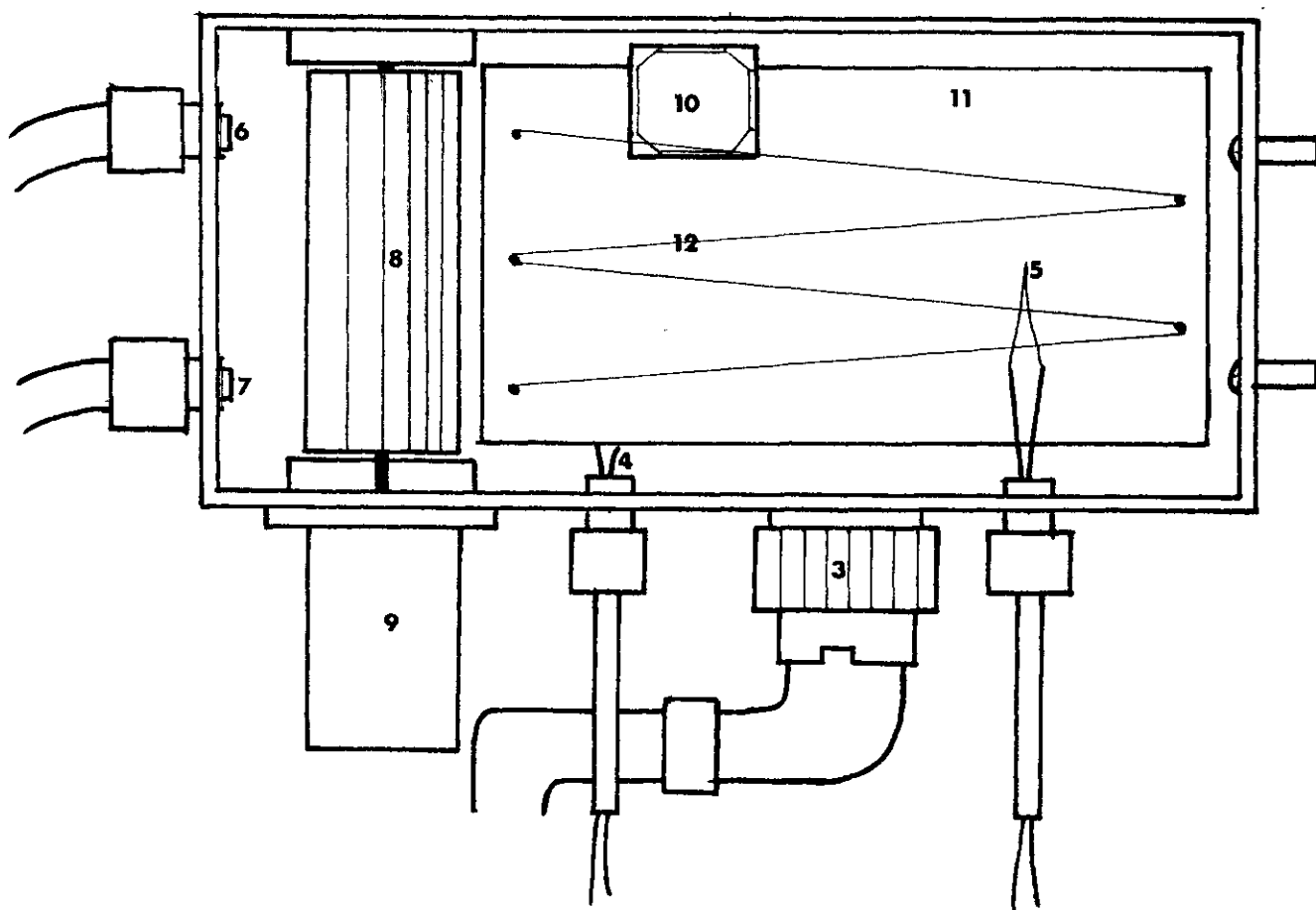
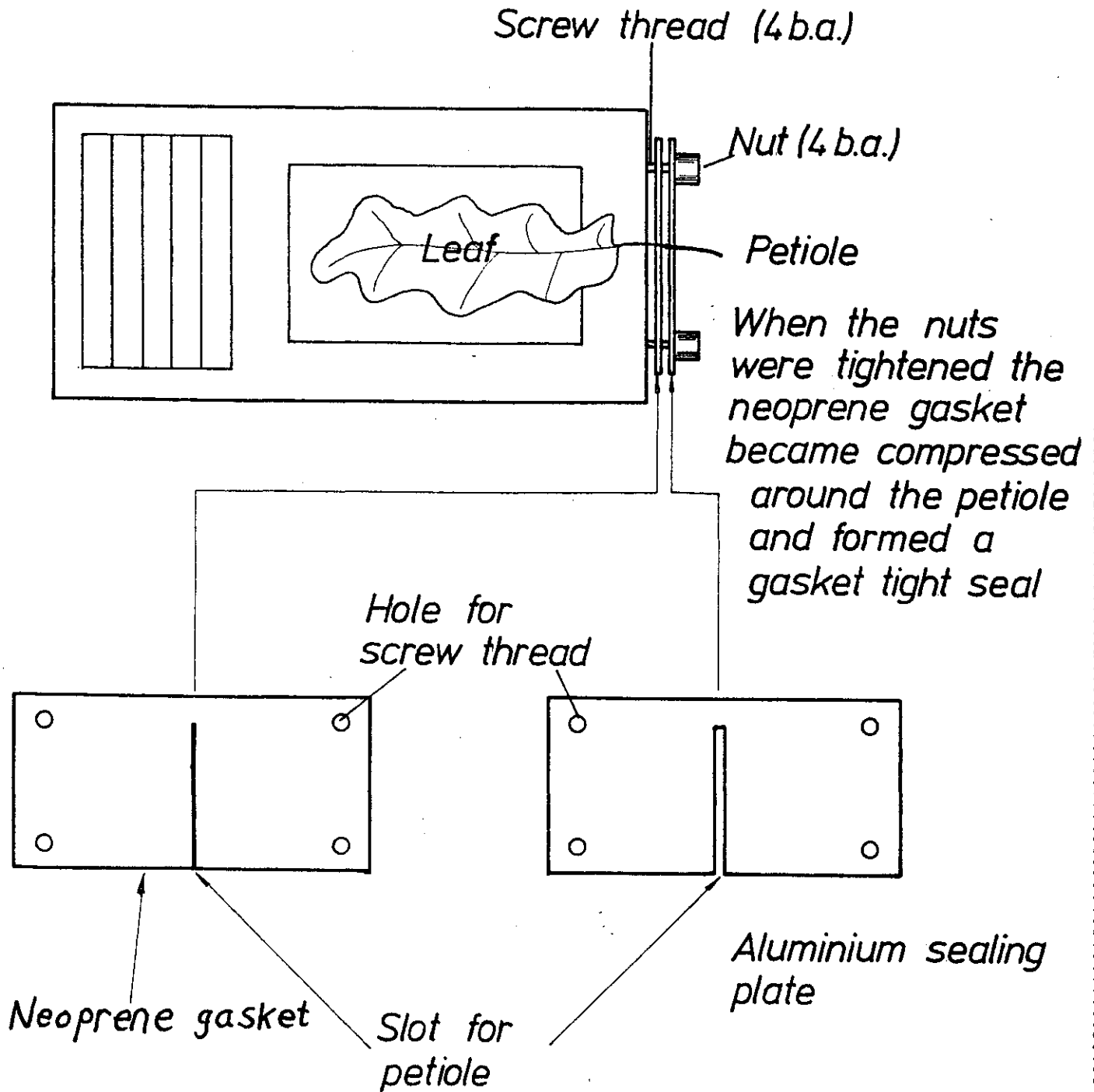


FIGURE 2.9 Method of sealing around leaf petiole.



was kept above the dewpoint temperature of the air in the chamber to avoid condensation occurring on the heat exchanger.

The interior of the chamber was painted matt black with catalysed polyurethane paint. All irradiance was therefore incident on the upper surface of leaves placed in the chamber. Gas-tight electrical connectors were provided for fan power supply (to the chamber) and photocell output (from the chamber). A leaf was inserted through a slot in the end of the chamber opposite the fan, and was supported by nylon threads. A gas-tight seal was obtained around the leaf petiole by compression of a neoprene cellular foam gasket as shown in figure 2.9.

2.2/2 Temperature measurement

For the purpose of calculating rates of gas exchange of the leaf, the assumption was made that the water vapour concentration at the liquid-air interface within the leaf was equivalent to the concentration at the saturation vapour pressure at leaf temperature. Because of the strong dependence of the saturation vapour pressure on temperature, the leaf temperature needed to be determined with considerable accuracy. Thermocouples were used to measure the surface temperature of the leaf. However, the temperature required is that of the liquid-air interface within the leaf. Because of the low thermal capacity and high thermal conductivity of leaves, for most leaves it can be shown (Perrier, 1971) that temperature gradients across the pathway leading to the interface are small and non-significant.

The chamber air and leaf temperature thermocouples were made from 30 swg chromel-constantan and 42 swg chromel-constantan respectively. These were made to conform with British Standard 4937 part 6 in the range 0° - 200° C. The reference junctions were kept in a constant temperature water bath. The thermocouples were independently calibrated and found to give an output of $61.1 \mu\text{V}$ per $^{\circ}\text{C}$ difference between junctions.

The output was measured with the digital voltmeter (see Section 2.1/3) which was capable of resolving 1 μV . Allowing for an electrical "noise" of 6 μV , it was possible to measure leaf temperature to within 0.1°C, the accuracy suggested by Jarvis (1971).

When a leaf was placed in a chamber, the leaf temperature thermocouple was sprung against the undersurface of the leaf and care was taken to ensure that there was always a good contact between thermocouple and leaf.

2.2/3 Mixing of air within the assimilation chamber

The leaf boundary layer consists of a thin layer of air close to the leaf surface in which movement of air is by laminar flow, plus a transition region to the fully turbulent conditions of the ambient air. The thickness of this layer determines the boundary layer resistance, and is a function of windspeed, leaf dimension, anatomy and orientation. The boundary layer resistance is in series with the other resistances to the transfer of CO_2 , O_2 , water vapour, heat and momentum, these other resistances being under physiological control. Clearly, in the leaf chamber studies reported here, of the response of leaves to environmental conditions, it was vital that:

1. The boundary layer should be maintained small relative to the total leaf resistance.
2. The boundary layer should be maintained constant throughout any one experiment and reproducible between experiments.

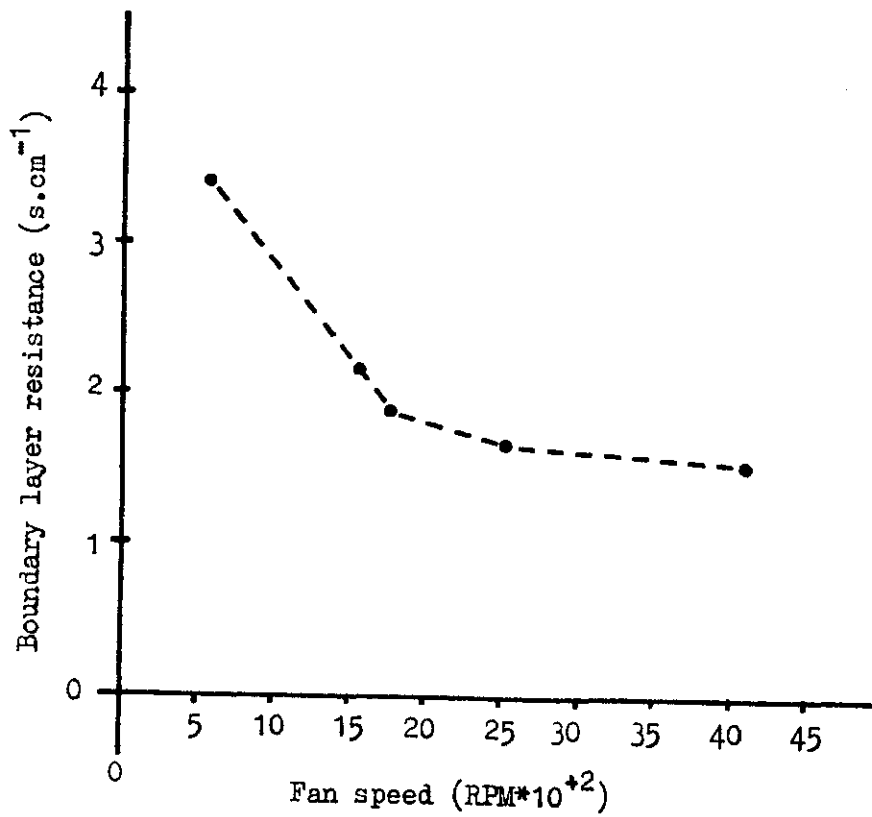
These criteria were achieved by introducing stirring and circulation of the air within the chamber by the use of an internal fan (see figures 2.7 & 2.8). The inlet and outlet gas tubes were placed directly above the fan to facilitate rapid mixing and ensure that eddies of unmixed air were not swept into the exhaust port. This meant that gradients of CO_2 and water vapour across the chamber were eliminated

and that the outlet air was representative of conditions inside the chamber. "Squirrel cage" fans were used, each powered by a low voltage D.C. motor (Mitsumi, Japan) fed by a variable voltage D.C. power supply.

It was necessary to examine how the fan speed affected boundary layer resistance value so that the two criteria stated above could be satisfied. Since all the fans were operated from a single power supply, the supply voltage reading could be used as an indicator of fan speed after a suitable calibration had been carried out.

One of the fans (that in chamber 2) was chosen at random and values of boundary layer resistance, at different fan speeds, were calculated. Fan speed was measured with a transistor strobotorch model 1202D (Dawe Instruments Ltd, London). The boundary layer resistance assessed was that of a water saturated leaf replica. The techniques used in boundary layer assessment are given in Section 2.5/9. The results are shown in figure 2.10. It can be seen that the boundary layer resistance became minimized at a fan speed of about 2500 rpm, above which there was no further decrease in resistance with increase in speed. This corresponded to a reading of 2.0 volts. All other fan speeds were then measured at this voltage and it was found that they were all within 6% of the 2520 rpm of the chamber 2 fan. It was thus concluded that in all subsequent gas exchange experiments the fan supply voltage could be set at 2.0 volts in order to minimize the boundary layer resistance of the leaves. It should be noted, however, that even in a chamber with adequate mixing of air, fully developed turbulence is not usually induced (Monteith, 1965). The actual values of boundary layer resistance depended upon the species under consideration, and full details are given in Section 2.5/9.

FIGURE 2.10 The effect of leaf chamber fan speed on boundary layer resistance.



2.2/4 Measurement of the Radiant Flux Density of Photosynthetically Active Radiation

Photosynthetically Active Radiation (PAR) has been defined as that part of the electromagnetic spectrum which induces photosynthesis on absorption by pigments in the plant (Nichiporovitch, 1954; Gaastra, 1959; Kubin & Hladek, 1963). The approximate limits of this region are defined by wavelengths of 400-700 nm. Over this range little wavelength specificity is shown, a simple integral relationship existing between the number of molecules changed photosynthetically and the number of quanta absorbed, regardless of quantum energy (e.g. Rabinowitch, 1951).

To determine the response of net photosynthesis to irradiance, the radiant flux density incident on the leaf must be measured precisely. Clearly, the detector must transform the absorbed radiant energy into a measurable form, which is proportional to the quantum content of the radiant flux. Since the perspex lid of the leaf chamber was bound to reduce the radiant flux density incident on the leaf, a measuring circuit was developed which measured this flux density with a sensor mounted in the chamber and in the plane of the leaf.

A schematic diagram of the components of the measuring circuit are shown in figure 2.11. The photodiode used was a Ferranti silicon photovoltaic cell (type MS1BE). This cell was very sensitive to near infra-red radiation with a peak sensitivity around 800-900 nm, which is outside the PAR range, so an interference filter (KG Glass Calflex ^{BALZERS HIGH VACUUM, LICHTENSTEIN}) Bl/Kl₃ was fixed to the top of the cell. This filter produced a sharp cut-off in transmission at about 800 nm, near infra-red and infra-red being reflected.

The photocells used had a current output that was approximately linear with incident radiation when a low external resistance was used.

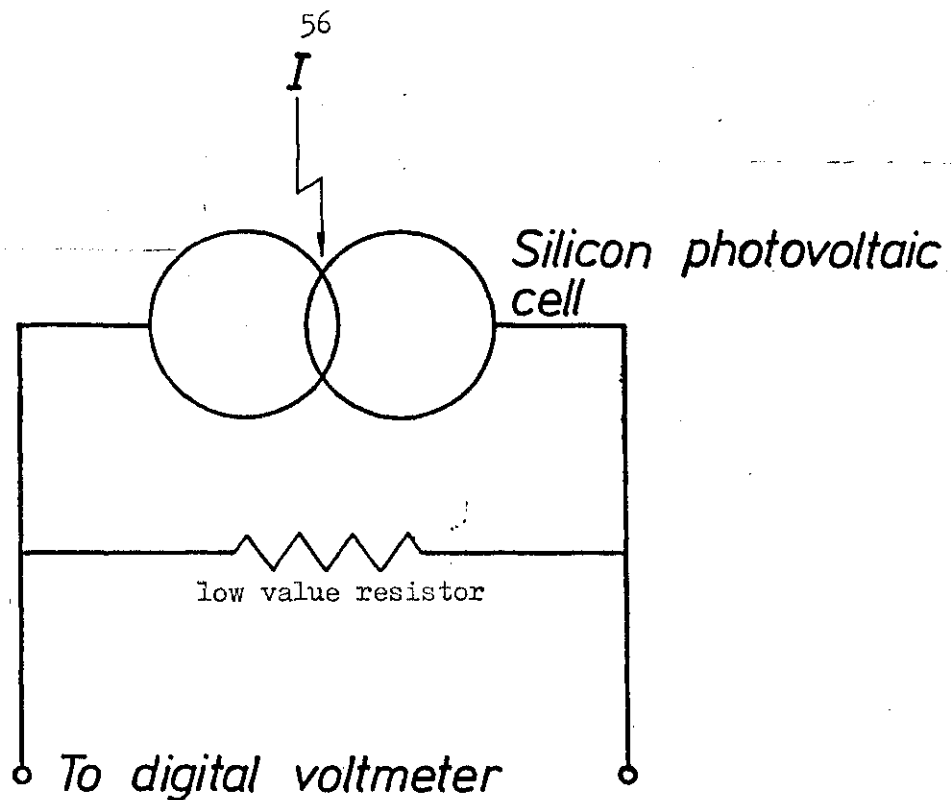
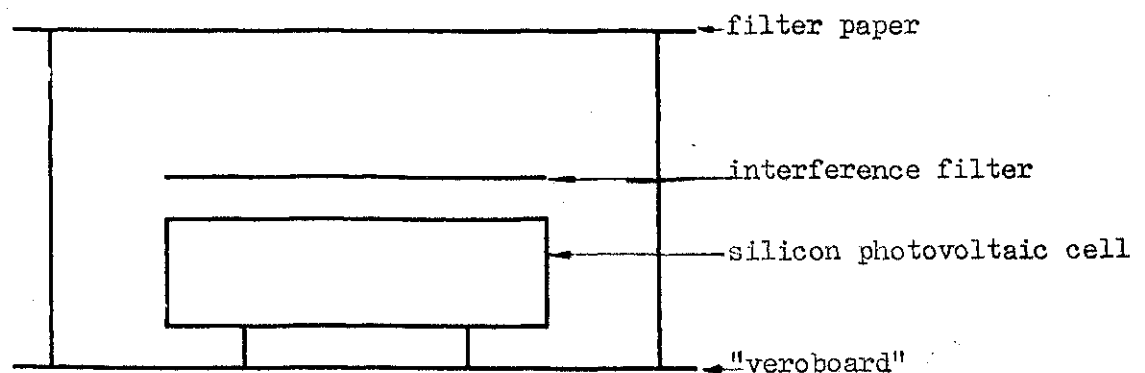


FIGURE 2.11 Schematic diagram of the circuit for measuring incident radiant flux density in the leaf chamber.

FIGURE 2.12 The photocell assembly.



The data logging equipment used in the study (Section 2.1/3) incorporated a high impedance voltmeter. Each photocell was shunted by a low value resistor (100 ohm), and the voltage developed across this resistor was approximately linear with incident irradiance.

The photocell/filter combination and the resistor were mounted on a piece of "veroboard" to which the leads to the DVM were attached. The photocell/filter assembly was placed inside a brass casing, with a piece of Whatman's No. 1 filter paper over the surface. This kept the photocell free from dust etc (figure 2.12).

Such measuring circuits were placed in each of the chambers and these were calibrated against a Lambda Ll-1905 quantum sensor (Lambda Instruments Corp) as outlined in Section 2.3/5.

Uniform irradiation of the leaf in the assimilation chamber was achieved by ensuring an even distribution of irradiance in the direct beam from a light source. The method employed was to use light sources which irradiated a relatively large area homogeneously. Two types of lamp were used:

1. A bank of two 500 W tungsten incandescent "High Bay" reflector lamps (Atlas Lighting Ltd, St Martin's Lane, London).
2. A 1000 W Metal Halide discharge lamp; Thorn Colorarc type MB1F with fluorescent coating (Thorn Lighting Ltd, UK). The spectral energy distribution of this lamp consisted of a continuous spectrum of closely spaced lines produced by a combination of the line emissions from its metal halide additives. The fluorescent coating increased the emission in the red wavelength region. Unfortunately, this lamp was acquired in 1977 and was only available for later parts of this work.

The flux density of PAR incident on the leaf was dependent on the distance from the source, but at an equivalent distance, the flux

density from the metal halide lamp was very much greater than that from the tungsten lamps. Maximum irradiance was about $300 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ from the tungsten lamps and $650 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ from the metal halide lamp.

Because of the possibility of a "glasshouse effect" of the assimilation chamber increasing leaf temperature, it was necessary to absorb much of the flux of thermal radiation before it reached the chamber. This was achieved by the use of a water filter made up of 16 cm of distilled water contained in a 'perspex' tank placed between the lamp and the chamber. This was particularly important under the tungsten lamp which had a high infra-red energy output per input watt of energy.

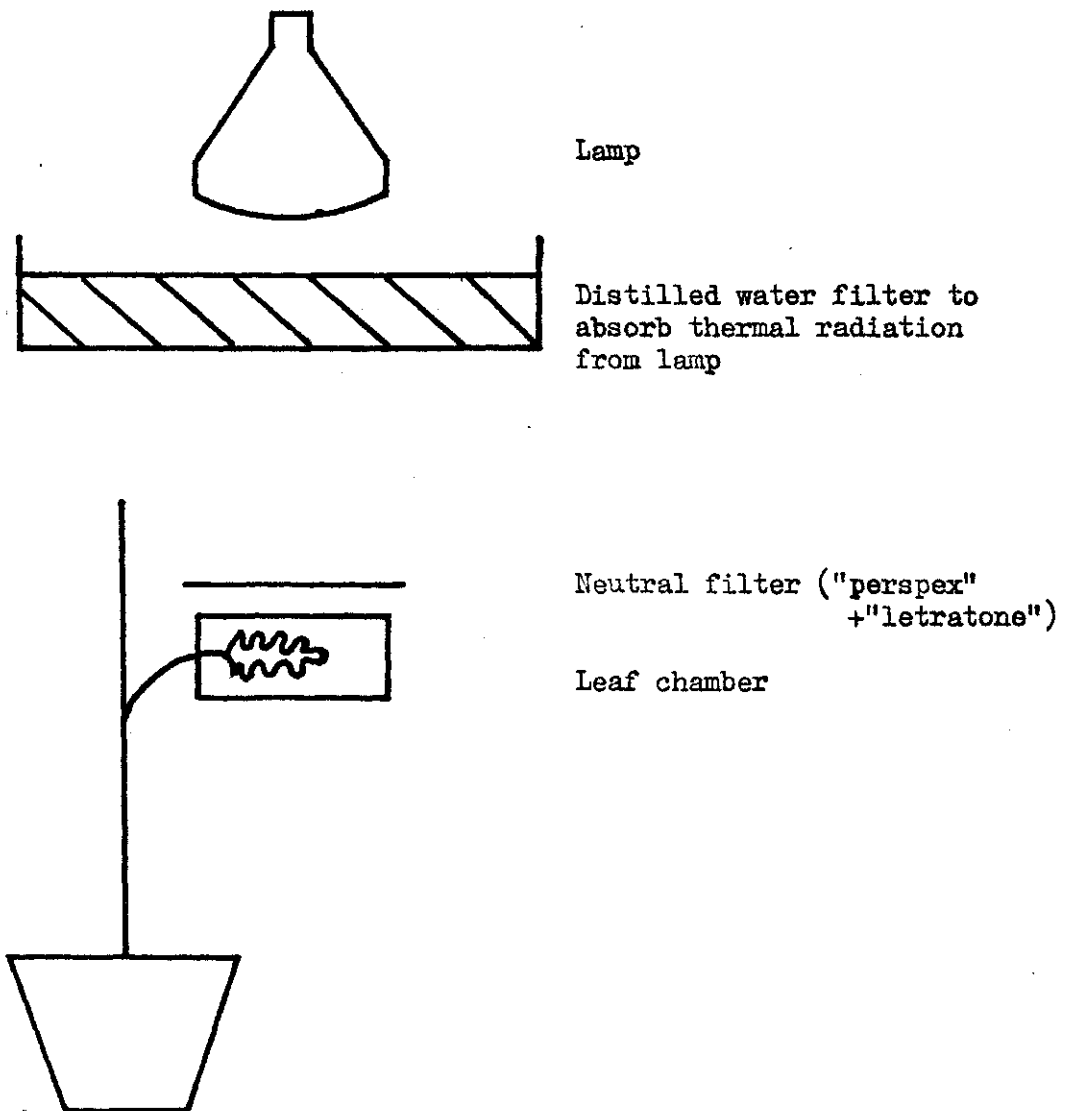
The flux density of PAR at the leaf was controlled by interposing neutral filters which reduced the flux density without alteration of its spectral composition. These filters were made up of a thin piece of 'perspex' (2 mm) backed with sheets of 'Letratone'. The number of layers of 'Letratone' controlled the amount of radiation transmitted. Where more than one sheet of 'Letratone' was present, different dot spacings were used to avoid Moiré effects. Nine such filters were made up as follows: (expressed as a nominal percentage of the transmission through 2 mm clear perspex):

100%; 81%; 64%; 49%; 36%; 25%; 16%; 9%; 2.7%

The filters could simply be laid over the 'perspex' lid of the assimilation chamber (figure 2.13). Zero irradiance at the leaf surface was achieved by laying a piece of thin plywood, painted matt black, over the leaf chamber and covering this with a piece of black velvet curtain material.

These filters were used for the control of the flux density of PAR incident upon the leaf, and were also used in photocell calibration as outlined in Section 2.3/5.

FIGURE 2.13 Control of the flux density of photosynthetically active radiation incident on the leaf.



2.3 Calibration of equipment

2.3/1 Calibration of the infra-red gas analyser

The following technique was devised for the calibration of a Grubb-Parsons SB2 Infra-Red Gas Analyser (IRGA) when used in an open circuit measuring system. The IRGA was fitted with optical filters which transmit only radiation between 3.8 and 5.7 μm so that the main absorption bands of water vapour had no effect on the instrument. The procedure outlined describes the calculations required to convert analyser readings into gas concentrations, and also how compressed air cylinders were calibrated so that their CO_2 content was accurately determined.

For the purpose of using IRGA readings for the calculation of gas exchange rates in terms of mass flux of CO_2 , it was necessary to express carbon dioxide concentration in terms of mass per unit volume, e.g. kg.m^{-3} . For IRGA calibration it was assumed that the analyser output voltage was a function of the mass of absorbing material (i.e. CO_2) present in the analysis tube if the mass of absorbing material present in the reference light path was held constant.

Method:

The IRGA was calibrated by using standard gases with known CO_2 concentration. The gas mixtures were prepared by the volume method using Wüsthoff gas mixing pumps as described in Section 2.1/1 (Fig. 2.2). Dry Gas of known CO_2 concentration was generated by the gas mixing pumps and this gas was passed into the IRGA analysis tube. When a steady trace on the servoscribe had been obtained, several readings of IRGA output voltage were measured with the digital voltmeter, together with the analysis tube temperature and pressure. Gas temperature in the analysis tube was measured by means of a chromel-constantan thermocouple inserted via the outlet tube, and pressure in the tube

was measured against known atmospheric pressure by means of a manometer (M5) connected to the analysis tube. This procedure was repeated for several gas concentrations by changing the gear ratios of the pumps to produce the desired concentration.

e.g. Pump GM1 with 1% gears produces 1% CO_2 in N_2

this goes into Pump GM2 with 2.7% gears.

$\therefore$ CO_2 concentration in the output of GM2 is:

$$2.7\% \text{ of } 1\% = 0.027\% = 270 \text{ ppm } \text{CO}_2$$

Similarly fitting 2.9% gears to GM2 produces 290 ppm CO_2

3.0% " " " " 300 ppm CO_2

3.2% " " " " 320 ppm CO_2

3.5% " " " " 350 ppm CO_2

The above represents a typical range of CO_2 concentrations for calibration purposes for IRGA range 6, which generally had a full scale deflection of about 80 ppm. The Grubb-Parsons SB2 IRGA has six sensitivity ranges, each range having approximately double the sensitivity of the previous range. In practice, it was found that only ranges 4,5,6 were of sufficient sensitivity to use.

After calibration had been completed, air from cylinders which had to be calibrated for CO_2 content, was passed into the IRGA. Several readings of IRGA output voltage, analysis tube temperature and pressure were recorded for each cylinder. Cylinder CO_2 content could then be calculated as outlined in the following section.

Gas analyser output voltage/ CO_2 concentration relationship

The CO_2 concentration in the IRGA analysis tube for each calibration gas was calculated as follows:

$$C = \frac{(Y)(273.15)(44.01)(Pa)}{(1013.2)(22.4136)(Ta)} \quad \text{kg.m}^{-3} \quad (2.1)$$

where: Y is the CO₂ concentration in volumes per volume

Pa is the IRGA analysis tube pressure (mbar)

Ta is the IRGA analysis tube temperature (°K)

44.01 is the molecular weight of CO₂

1013.2 mbar is standard pressure

273.15 °K is standard temperature

22.4136 is the volume (l) of one gram mole of a gas at STP

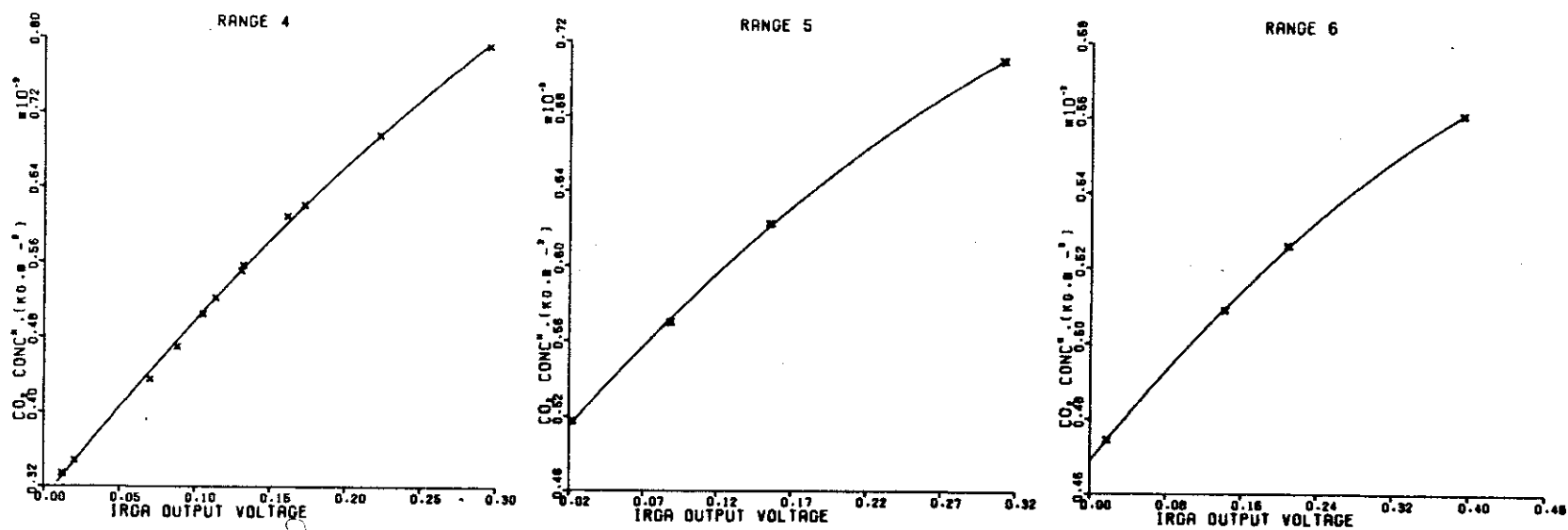
When C(kg.m⁻³) was plotted against IRGA output voltage, X (volts), it was clear that the analyser output was not a linear function of CO₂ concentration. Examples of typical calibration curves can be seen in figure 2.14. A variety of non-linear regressions were fitted to calibration data and the best fits were given by a quadratic equation of the form:

$$C = a + bX + cX^2 \quad (2.2)$$

A program was written which fitted such a quadratic regression to the calibration data and computed the values of 'a', 'b' and 'c', along with the standard error of the regression equation. The values of IRGA output voltage obtained from the cylinder calibrations were then substituted into the regression equation, to give cylinder CO₂ concentrations in kg.m⁻³. This was converted into volumes per volume using equation 2.1.

The assumption was made that adjustment of the instrument zero control (a mechanical shutter which intercepts some of the reference beam) was equivalent to adding or subtracting a mass of CO₂ to the reference tube, and that such adjustment affected only the position of the intercept 'a' and not the shape of the calibration curve (determined by the values of the calibration constants 'b' and 'c'). This assumption was tested by carrying out two calibrations sequentially, over the same range, with the zero control altered between calibrations. The two regression equations obtained were:

FIGURE 2.14 Examples of typical IRGA calibration curves.



THIS JOB WAS EXECUTED ON 78/09/27. AT 12.37.33.

	S.E.
$C = .00044826 + (.00064327)(X) - (.00056337)(X^2)$	.0000013683
$C = .00056240 + (.00062183)(X) - (.00023593)(X^2)$	.00000076286

Although the values of the calibration constants (b and c) obtained from the two calibrations differed, when the regressions were plotted without the intercept, the two graphs obtained were similar in shape (figure 2.15). At the point where the regressions are most divergent (an IRGA output voltage value of 0.28), the two regressions give CO_2 concentrations in the tube which differ by only $0.1 \times 10^{-4} \text{ kg.m}^{-3}$ (approximately 6 ppm). Thus, there seemed little error involved in assuming that alteration of the zero setting merely altered the value of the intercept 'a'.

It was necessary to establish whether a calibration remained valid independently of the absolute amount of CO_2 in the analysis tube. This was particularly important if an experiment was being carried out at elevated levels of ambient CO_2 whereas the calibration of the instrument had been performed in the normal 250-350 ppm range. To test this, two calibrations were carried out sequentially, the first in the range 250-350 ppm CO_2 , and the second (after adjustment of the zero) in the range 320-400 ppm CO_2 . The regression equations obtained were:

	S.E.
$C = .0005624 + (.00062183)(X) - (.00023593)(X^2)$	.76286 $\times 10^{-6}$
$C = .00046628 + (.00076336)(X) - (.00084247)(X^2)$	.69844 $\times 10^{-7}$

When the regressions were plotted without the intercepts (figure 2.16), the two graphs obtained were very similar in shape. At the point where the graphs are most divergent (an IRGA output voltage of 0.14 volts), the two regressions give CO_2 concentrations which differ by $0.085 \times 10^{-4} \text{ kg.m}^{-3}$ (approximately 5 ppm). Thus, there was little error involved in assuming the calibration to be independent of the

FIGURE 2.15 Results of two sequential IRGA calibrations, plotted without their intercepts.

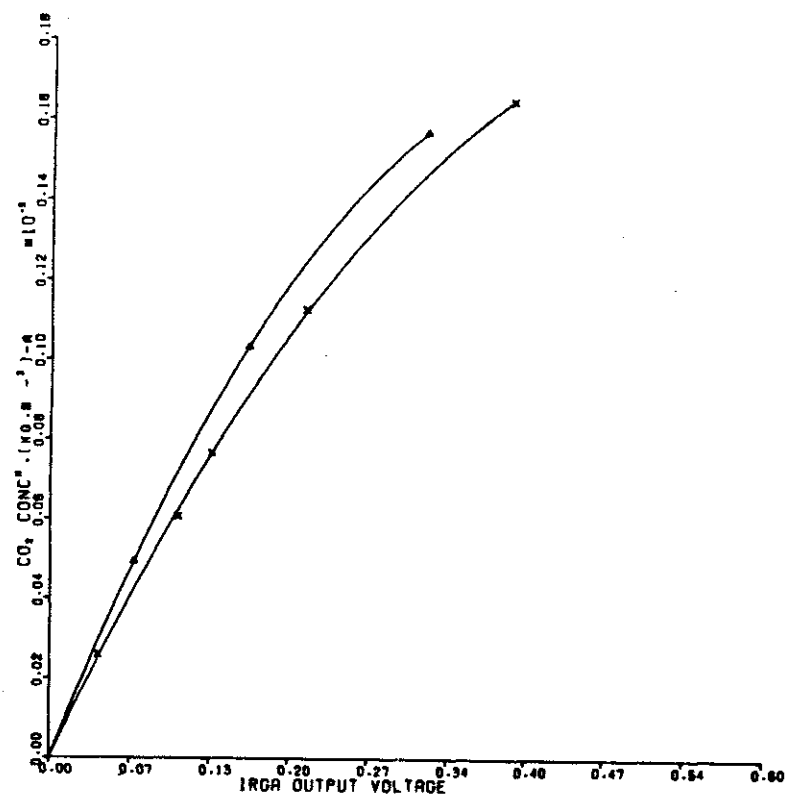


FIGURE 2.16 Results of two sequential IRGA calibrations, one in the range 250-350ppm CO₂ and the other in the range 320-400ppm CO₂, plotted without their intercepts.

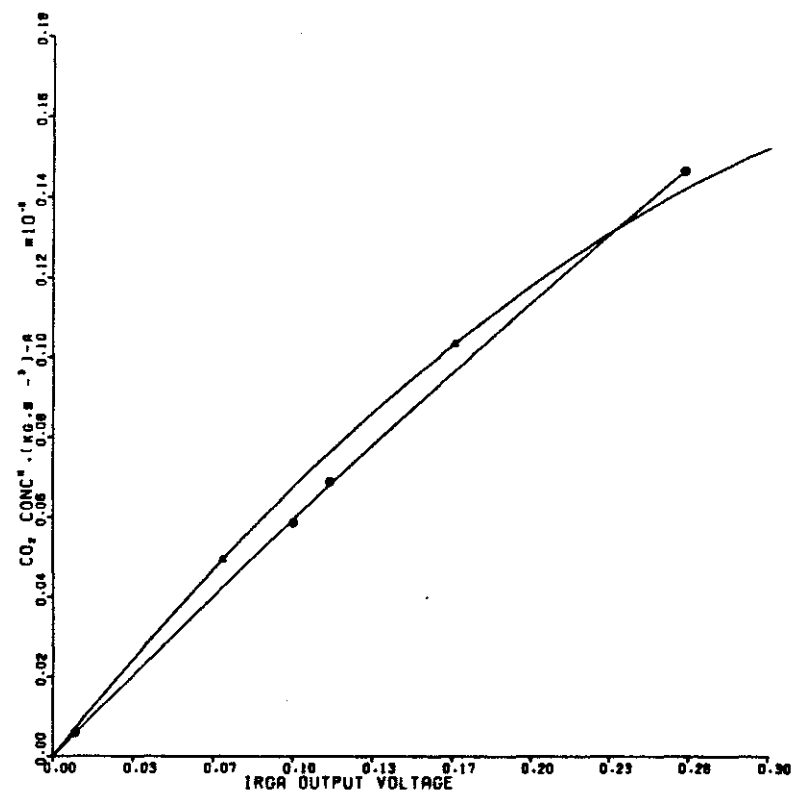
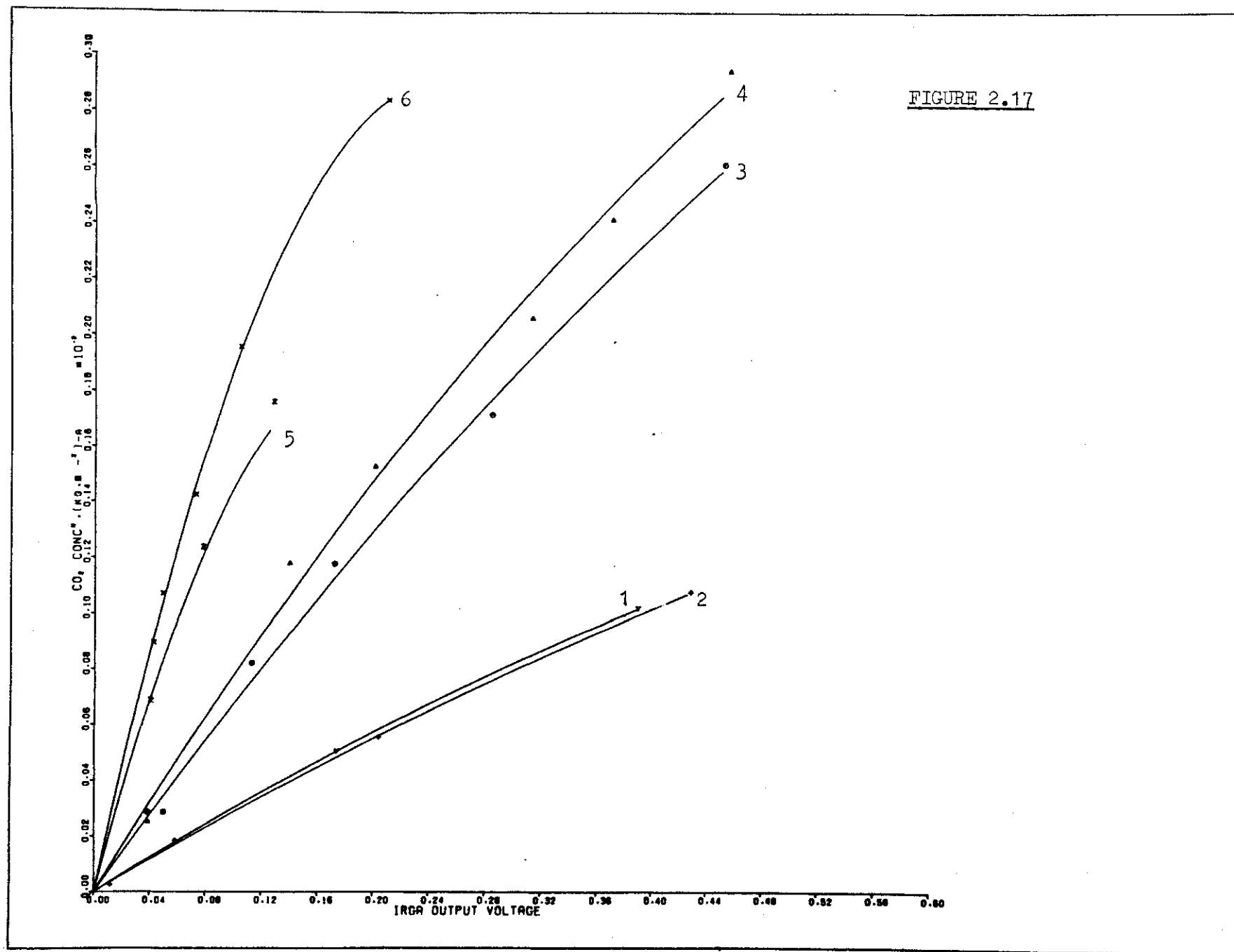


FIGURE 2.17 A selection of IRGA calibrations taken over a seven month period:

1	Calibrated on	20	January	1977
2	"	"	7	February "
3	"	"	28	April "
4	"	"	31	May "
5	"	"	30	July "
6	"	"	16	August "



absolute amount of CO_2 in the analysis tube.

The value of the calibration intercept 'a' had to be determined during the course of an experiment as it was dependent on IRGA zero setting (and was also thought to vary with IRGA amplifier drift). The value of 'a' relevant to a particular zero setting in use, could be calculated every time reference gas passed through the IRGA during operation, by the procedure outlined in Section 2.5/3. Once 'a' had been calculated, any value of IRGA output voltage, such as values obtained during gas exchange measurements, could be converted into CO_2 concentrations (kg.m^{-3}) according to equation 2.2.

It was necessary to establish for how long a calibration remained valid. Figure 2.17 shows a selection of calibrations (drawn without the intercept) taken over a seven month period. As can be seen, the sensitivity of the IRGA detector decreases with time, but calibrations taken within approximately three weeks of each other produce very similar graphs. In other words, the calibration did not appear to alter very much within a three week period, so three weeks was chosen to be the maximum time between calibrations. In fact, calibrations were often taken more frequently than this especially as the detector aged.

2.3/2 Calibration of the dewpoint hygrometer

The following technique was devised for calibration of the dewpoint hygrometer (Cambridge Systems Model 880, EG & G Inc) when used in an open circuit measuring system. This instrument utilizes the dewpoint condensation principle to determine the water vapour concentration in a gas mixture.

A cooled metal surface (or "mirror") is presented to the gas sample so that the temperature of the gas at the metal surface is the same as the temperature of the metal. When the metal surface temperature is exactly at the dewpoint temperature a state of equilibrium exists

whereby the rate at which water molecules evaporate from the surface is exactly equal to the rate at which other water molecules condense on the surface. In this condition, the amount of water on the metal surface is unchanging, and this is the temperature at which the gas is saturated with respect to water, viz the dewpoint temperature. The dewpoint hygrometer senses, optically, when this state of dynamic equilibrium exists, and measures the temperature at the metal surface.

The temperature sensing element of the instrument is a precision thermistor embedded in the cooled metal surface. An analysis of the hygrometer circuit by Dr P W Mueller showed that the resistance of the thermistor (R_T , ohms) was related to the output voltage of the instrument (X , millivolts) according to:

$$R_T = \frac{a - bX}{c + dX} \quad (2.3)$$

The values of the calibration constants a , b , c , d were calculated as follows:

Terminals were provided on the instrument for the connection of a remote thermistor. A resistor whose value had been measured precisely was connected to these terminals and several readings of output voltage were measured with the digital voltmeter. This procedure was repeated for twelve resistance values. Thus, known resistances were substituted for R_T and readings of X were taken. Marquardt's algorithm (see Appendix 1) was used to give the best least squares fit to the relationship between R_T and X shown in equation 2.3. Tables 2A and 2B show typical sets of results.

Thus, for any value of output voltage the resistance of the thermistor could be calculated. These values of resistance then needed to be translated into measurements of dewpoint temperature. Thermistor resistance, R_T , is normally thought to be related to absolute

TABLE 2A Marquardt's algorithm was used to give the best least squares fit to the relationship:

$$R_T = \frac{a - bX}{c + dX} \text{ where } a, b, c, d \text{ are constants (see text for details).}$$

Output voltages(X,mV) are means of 7 values each.

Resistance value $R_T(\text{ohms})$	Measured 22-7-75 $X(\text{mV})$	Measured 23-9-77 $X(\text{mV})$
1003.0	37.04	37.14
1204.0	34.15	34.28
1405.0	31.61	31.75
1606.0	29.37	29.52
1807.0	27.39	27.56
2008.0	25.63	25.78
2208.5	24.04	24.17
2409.5	22.60	22.74
2611.0	21.29	21.45
2812.0	20.09	20.24
3817.0	15.43	15.59
4820.0	12.20	12.29

TABLE 2B Values of calibration constants.

	<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	<u>S.E.</u>
22-7-75:	.18062893E14	.29383832E12	.96704510E9	.16699769E9	.5232218
23-9-77:	.18110581E14	.29672339E12	.98537232E9	.16383873E9	.33576818E1

temperature, $t(^{\circ}\text{K})$, in the following way:

$$R_T = e^{\frac{1+m}{t}} \quad (2.4)$$

where 1 and m are constants.

However, preliminary examination of resistance values for different temperatures, provided in the instrument manual, suggested that this may not be very precise over a wide temperature range. A better fit to the data was obtained by modification of equation 2.4 to:

$$R_T = e^{\frac{1+m}{t+c}} \quad (2.5)$$

Least squares estimates of the parameters 1 , m , c , were obtained, again using Marquardt's algorithm, for the data given in the instrument manual (see table 2C).

$$\text{From equation 2.5: } R_T = e^{1 + \left(\frac{m}{t+c}\right)}$$

$$\text{taking natural logs: } \ln(R_T) = 1 + \left(\frac{m}{t+c}\right)$$

$$\therefore \ln(R_T) - 1 = \frac{m}{t+c}$$

$$\therefore \frac{1}{\ln(R_T) - 1} = \frac{t+c}{m}$$

$$\therefore t = \frac{m}{\ln(R_T) - 1} - c \quad (2.6)$$

Thus, values of output voltage measured during an analysis could be converted into corresponding thermistor resistance values according to equation 2.3. These resistance values could then be used to calculate the temperature at the mirror surface (and hence dewpoint temperature) according to equation 2.6.

At mirror temperatures below 0°C (32°F) the water on the mirror surface was assumed to be in the form of ice (see sources of error) and hence the frost point temperature was measured. The dewpoint and frost

TABLE 2C Marquardt's algorithm was used to give the best least squares fit to the relationship between R_t and t (see equation 2.5):

$R_t = e^{1+m/(t+c)}$ where $1, m, c$ are constants. Data are taken from Cambridge Systems Model 880 Dewpoint Hygrometer Manual.

R_t (ohms)	t (°K)	R_t (ohms)	t (°K)	R_t (ohms)	t (°K)
19710.0	232.05	4172.0	263.16	1155.0	294.27
18550.0	233.16	3968.0	264.27	1108.0	295.38
17460.0	234.27	3776.0	265.38	1063.0	296.49
16450.0	235.38	3594.0	266.49	1021.0	297.60
15490.0	236.49	3422.0	267.60	979.7	298.72
14600.0	237.60	3260.0	268.72	940.4	299.83
13760.0	238.72	3106.0	269.83	903.2	300.94
12990.0	239.83	2960.0	270.74	867.5	302.04
12250.0	240.94	2822.0	272.04	833.7	303.16
11570.0	242.04	2691.0	273.16	801.4	304.27
10920.0	243.16	2567.0	274.27	770.6	305.38
10323.0	244.27	2449.0	275.38	741.0	306.49
9758.0	245.38	2338.0	276.49	712.8	307.60
9228.0	246.49	2232.0	277.60	685.8	308.72
8730.0	247.60	2133.0	278.72	660.0	309.83
8261.0	248.72	2037.0	279.83	635.3	310.94
7821.0	249.83	1946.0	280.94	611.6	312.04
7407.0	250.94	1860.0	282.04	589.0	313.16
7017.0	252.04	1779.0	283.16	567.4	314.27
6649.0	253.16	1702.0	284.27	546.7	315.38
6305.0	254.27	1628.0	285.38	526.9	316.49
5981.0	255.38	1558.0	286.49	507.9	317.60
5676.0	256.49	1492.0	287.60	489.7	318.72
5387.0	257.60	1428.0	288.72	472.2	319.83
5115.0	258.72	1369.0	289.83	455.4	320.94
4858.0	259.83	1311.0	290.94	439.4	322.04
4617.0	260.94	1256.0	292.04	423.9	323.16
4388.0	262.04	1204.0	293.16	409.2	324.27

Values of calibration constants:

1	m	c	S.E. (ohms)
-6.5126041	4879.5200	65.448250	2.1252345

point are not the same for a gas sample of fixed water vapour content, as the temperature at which a surface of ice must be maintained to be in equilibrium with water vapour is slightly higher than that for water (liquid). By definition (from List, 1958):

The dew point (frost point) temperature is the temperature to which the air must be cooled in order that it shall be saturated with respect to water (ice). The dew point or frost point defines the partial pressure of the water vapour in the gas sample, from the Smithsonian Meteorological Tables (List, 1958) using the Goff-Gratch formulation:

$$\begin{aligned} \log_{10} E_w = & -7.90298 (T_s/T - 1) + 5.02808 \log_{10} (T_s/T) \\ & - (1.3816)(10^{-7})(10^{11.344(1 - T/T_s)} - 1) \\ & + (8.1328)(10^{-3})(10^{-3.40149(T_s/T - 1)} - 1) + \log_{10} E_{ws} \end{aligned} \quad (2.7)$$

and:

$$\begin{aligned} \log_{10} E_i = & -9.09718 (T_o/T - 1) - 3.56654 \log_{10} (T_o/T) \\ & + 0.876793 (1 - T/T_o) + \log_{10} E_{io} \end{aligned} \quad (2.8)$$

where:

E_w = saturation vapour pressure over a plane surface of pure liquid water (mbar)

E_i = saturation vapour pressure over a plane surface of pure water ice (mbar)

T = absolute (thermodynamic) temperature at which saturation occurs ($^{\circ}\text{K}$)

T_s = steam point temperature (373.16 $^{\circ}\text{K}$)

T_o = ice point temperature (273.16 $^{\circ}\text{K}$)

E_{ws} = saturation pressure of pure liquid water at steam point temperature (1 standard atmosphere = 1013.246 mbar)

E_{io} = saturation pressure of pure water ice at ice point temperature (.0060273 standard atmospheres = 6.1071 mbar)

Equation 2.6 gives the temperature at which saturation occurs, and this value can be substituted for T in equation 2.7 or 2.8. The total

pressure, P_p , (mbar) in the hygrometer is known, and the partial pressure of the water vapour is given by equation 2.7 (for temperatures above 273.16°K) or equation 2.8 (for temperatures below 273.16°K).

Therefore:

$\frac{E_w}{P_p}$ gives water vapour concentration in volumes per volume ($\text{m}^3.\text{m}^{-3}$)
for dew point temperatures

and

$\frac{E_i}{P_p}$ gives water vapour concentration for frost point temperatures.

(See Section 2.5)

Sources of error:

Bulletin DP-2 (1972) produced by manufacturers of the instrument contained an error analysis which suggested that in the worst case, the hygrometer would measure dewpoint temperature $\pm 0.78^\circ\text{C}$ (1.4°F). In addition, there were two further possible sources of error when measurements were taken in the present system.

1. At mirror temperatures below 0°C (32°F) the water on the mirror surface could be ice or super cooled liquid. According to the instrument manual, super cooled liquid is formed for a short time when the temperature is not far below freezing point, but this changes to ice fairly quickly. In the present analysis, it was assumed that ice was formed for all readings below 0°C for two reasons:
 - i) Readings taken were generally steady-state readings and it was assumed that any super cooled liquid formed initially would have had time to turn to ice.
 - ii) The mirror could be force cooled by over-riding the automatic control system. In this manner, the deposit could be positively frozen ensuring the measurements taken were frost point determinations. This system was used when it was thought that not enough time had elapsed for any liquid to turn to ice.

In fact, relatively few of the readings taken were below 0°C . This only occurred when the gas supply to the chambers had not been humidified. For the reasons outlined, the problem of super cooled liquid was not thought to be significant.

2. A second possible extra source of error could have arisen if the calibration changed appreciably with time. As previously shown, the values of the parameters l , m , c in equation 2.5 were obtained by regression analysis using the temperature against resistance data provided in the instrument manual. This part of the calibration is constant assuming that the data given in the manual is correct. Thus, the part of the calibration which could vary is confined to the relationship between thermistor resistance and output voltage (equation 2.3). Tables 2A and 2B show resistance against output voltage for calibrations performed on 22 July 1975 and 25 September 1977 respectively. The calculated calibration constants a , b , c , d were used to calculate dewpoint temperature using equations 2.3 and 2.6 for a range of typical output voltages. The results are shown in table 2D. It can be seen that although the values for the calibration constants vary slightly for the two calibrations, the actual dewpoint temperatures produced are very similar; mean difference being 0.188°C (standard error of differences 0.045). This difference is well within the range quoted by the manufacturers for errors associated with measuring dewpoint with this instrument.

2.3/3 Calibration of the flowmeters

It was necessary to the accuracy of measurement of gas exchange that the flow rates through the assimilation chambers be accurately known. The accuracy of the flowmeter manufacturer's calibration marks are dependent upon the pressure at the flowmeter outlet. For this reason, the flowmeters used in the present system were calibrated

TABLE 2D Using equations 2.3 & 2.6, dewpoint temperatures were calculated for a range of typical output voltages in order to compare two calibrations.

<u>Calibration date</u>	<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	<u>S.E.</u>
22-7-75	.18062893E14	.29383832E12	.9670451E9	.16699769E9	.52326218
23-9-77	.18110581E14	.29672339E12	.9853723E9	.16383873E9	.33576818E1

<u>Output voltage (mV)</u>	<u>Dewpoint temperature 22-7-75 (°C)</u>	<u>Dewpoint temperature 23-9-77 (°C)</u>
9.036	-18.610	-18.726
10.542	-15.946	-16.008
12.048	-13.404	-13.567
13.554	-10.958	-11.139
15.060	-8.590	-8.785
16.566	-6.281	-6.487
18.072	-4.018	-4.234
19.578	-1.787	-2.012
21.084	0.418	0.190
22.590	2.611	2.380
24.096	4.802	4.568
25.602	6.998	6.765
27.108	9.209	8.978
28.614	11.444	11.218
30.120	13.712	13.493
31.626	16.025	15.815
33.132	18.392	18.194
34.638	20.826	20.643
36.144	23.341	23.176
37.650	25.953	25.811
39.156	28.680	28.566
40.662	31.545	31.465

Mean difference between dewpoint temperatures = 0.188 °C

Standard error of difference = 0.045 °C

in situ by independent means. For flow rates up to 2.0 l.min^{-1} the soap bubble method described by Levy (1964) is satisfactory. Briefly, a simple bubble meter is constructed consisting of a glass tube mounted vertically, and scribed with two marks delimiting a known volume. A soap film is introduced into the tube and any gas flow through the tube urges its movement up the tube. Hence, the gas flow is made visible by the soap film which acts as a gas-tight piston of negligible weight and low friction. Using an accurate stopwatch the soap film bubble is timed over the known volume between the two marks and the flow rate can be found.

The flowmeters used were of the variable area glass tube and float type, and measured volume flow. They were of two commercial types: G A Platon Ltd, Hants, UK and Rotameter Manufacturing Co, Surrey, UK. Care was taken to ensure that the flowmeters were mounted vertically. The calibration was performed in situ and so the apparatus was as shown in figure 2.5 with the addition of the bubble meter shown in figure 2.18 which was connected to the IRGA outlet. The leaf chambers were by-passed by connecting their inlet and outlet gas lines together.

Method:

Each flowmeter was calibrated individually as follows: The needle valves were adjusted so that a constant flow rate reading was obtained on the flowmeter. Soap films were then introduced into the bubble meter and accurately timed between the two marks. The time used in calculations was the mean of ten readings. This was repeated at five different flow rates and for each flowmeter.

Results:

If the volume of the bubble meter between the marks is $V(\text{l})$ and the "sweep time" is $t(\text{s})$, then the true volume flow rate is given by

$$\frac{V}{t} \times 60 \text{ l.min}^{-1}$$

FIGURE 2.18 Bubble meter used in calibration of flowmeter.

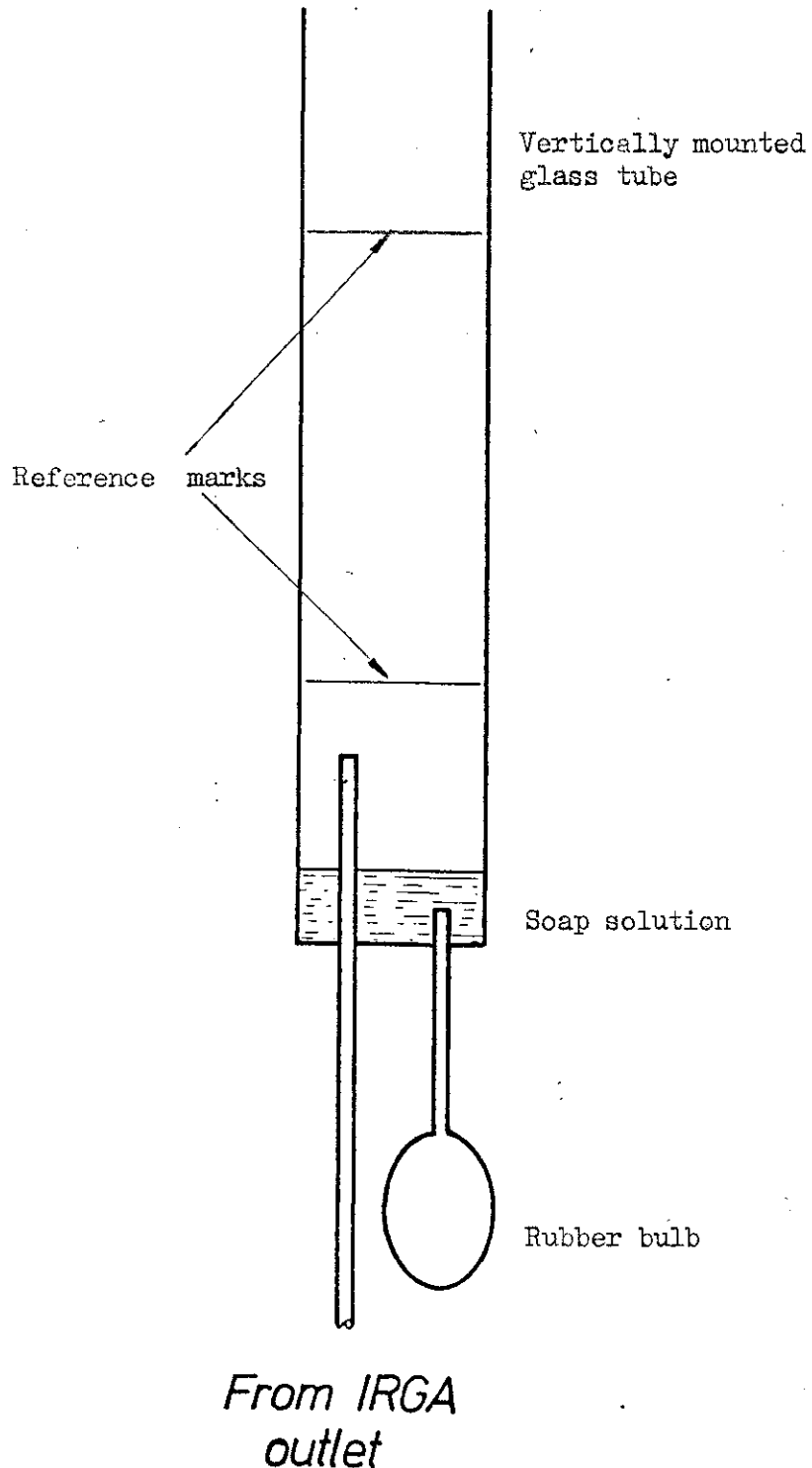


TABLE 2E Results of flowmeter calibration.

Volume of bubble = 0.314021 l.

Stream	Time of sweep (s)	Indicated flow rate (l.min ⁻¹)	Actual flow rate (l.min ⁻¹)
0	68.2	0.5	0.28
0	54.7	0.6	0.35
0	44.3	0.7	0.44
0	39.2	0.8	0.48
0	29.5	1.0	0.64
1	28.8	0.5	0.65
1	24.9	0.6	0.76
1	22.1	0.7	0.85
1	19.7	0.8	0.96
1	16.1	1.0	1.17
2	36.1	0.5	0.52
2	29.8	0.6	0.63
2	27.2	0.7	0.69
2	23.0	0.8	0.81
2	18.0	1.0	1.04
3	102.0	0.2	0.18
3	75.4	0.3	0.25
3	43.0	0.5	0.44
3	35.1	0.6	0.54
4	41.2	0.5	0.46
4	34.3	0.6	0.55
4	29.0	0.7	0.65
4	25.0	0.8	0.75
4	19.7	1.0	0.96
5	37.4	0.5	0.50
5	32.2	0.6	0.59
5	27.1	0.7	0.70
5	23.8	0.8	0.80
5	18.9	1.0	1.00
6	37.4	0.5	0.50
6	31.0	0.6	0.60
6	26.5	0.7	0.70
6	23.4	0.8	0.80
6	18.5	1.0	1.00

The results are shown in table 2E. Calibration graphs were drawn for each flowmeter, and the data fitted to linear regressions. Thus, flowmeter readings taken during subsequent gas exchange measurements could be converted to true flow rates using the calibration appropriate for that stream.

2.3/4 Calculation of the pressure conversion factors for different parts of the gas circuit

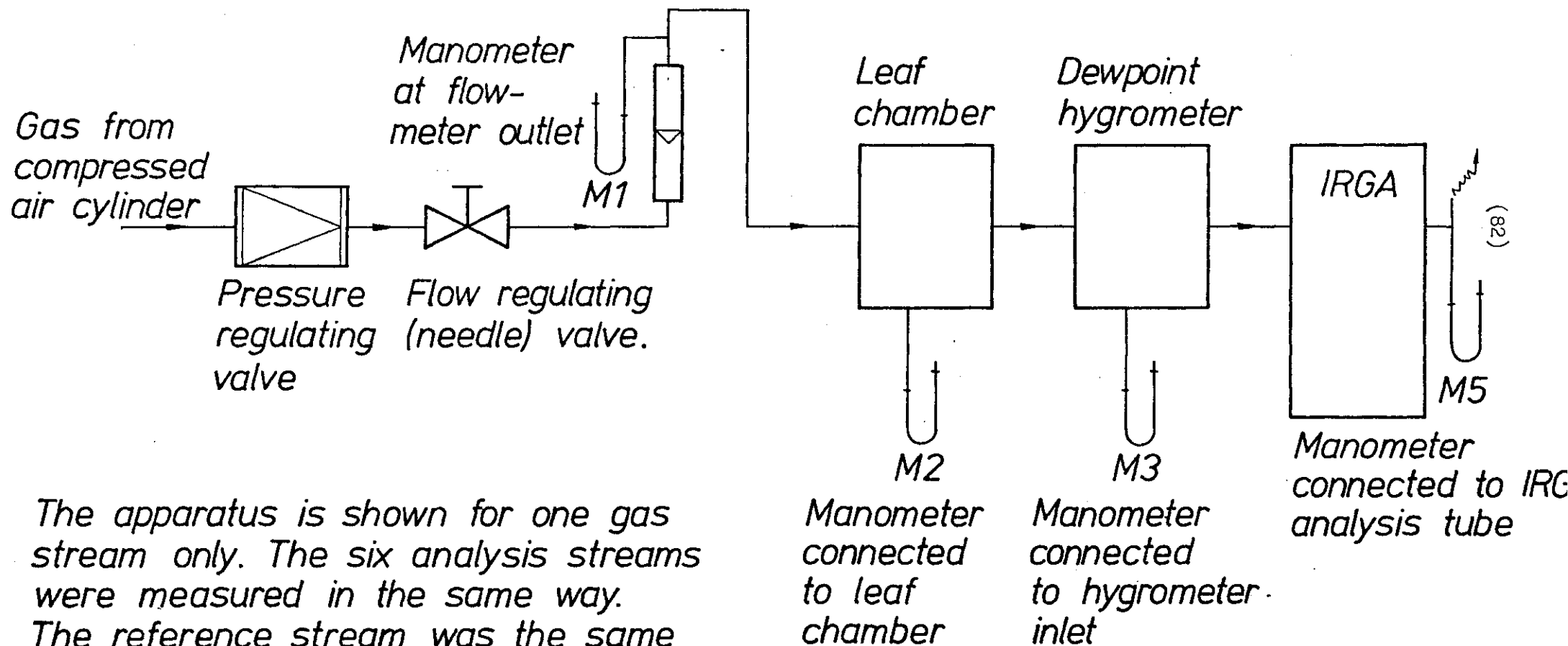
The calculation of gas exchange rates and transfer resistances necessitated the measurement of the pressure in different parts of the gas handling circuit, in particular:

- 1) The pressure in the IRGA analysis tube.
- 2) The pressure in the dewpoint hygrometer.
- 3) The pressure in the leaf chamber.
- 4) The pressure at the flowmeter outlet.

The apparatus shown in figure 2.19 was assembled in order to determine the relationship between the pressures in different parts of the system. Gas from a compressed air cylinder was passed through the system at a constant flow rate. Simultaneous readings were taken on each of the manometers M1, M2, M3 and M5. This was repeated for four different flow rates and for each gas stream (six analysis and one reference).

The manometer connected to the IRGA analysis tube (M5) was kept in position for all subsequent gas exchange measurements, and so the other manometers were calibrated against M5. The results for the individual gas streams were pooled to give three sets of data relating readings on M1, M2, M3 to readings on M5. The nature of the variables made it clear that if there was a zero reading on any of the manometers M1, M2, M3, then there would also be a zero reading on M5, as there was

FIGURE 2.19 Apparatus for determination of pressure conversion factors.



The apparatus is shown for one gas stream only. The six analysis streams were measured in the same way. The reference stream was the same except for the absence of the leaf chamber

no pressure in the system. Thus, linear regressions were fitted to the data with the lines forced to go through the origin (0.0, 0.0). For example, table 2F shows the results for readings recorded on M3 against simultaneous readings recorded on M5. For a linear regression forced through the origin, the fitted line gives a least squares estimate of the values of Y according to:

$$Y = bX$$

where b is the slope of the regression (the regression coefficient)

and X is, in this case, reading on M5

Y is, in this case, reading on M3

b is given by $\frac{\sum XY}{\sum X^2}$ (Snedecor & Cochran, 1971)

Similar regressions were carried out on the other two sets of data, and the complete results are shown in figure 2.20. As can be seen, all three sets of data are good fits to a linear regression forced through the origin, showing that the pressures in the flowmeter, chamber and hygrometer can be regarded as linear functions of the pressure in the IRGA analysis tube.

It was next necessary to convert readings on the manometers to actual pressures in millibars:

The density of mercury (H_g) at 25°C is 13.534 g.ml⁻¹ (or g.cm⁻³)

∴ in a column of H_g of height 1 cm, there are 13.534 g.cm⁻² (grams-force.
cm⁻²)

Standard pressure is 76 cm H_g .

∴ in a column of H_g of height 76 cm, there are:

$$(13.534)(76) = 1028.58 \text{ g.cm}^{-2}$$

This is equivalent to 1013.246 mbar (also standard pressure)

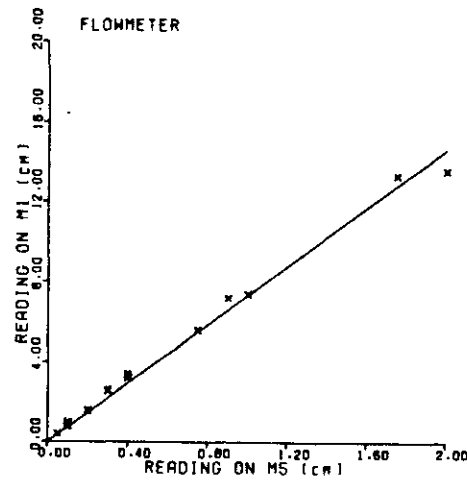
$$\therefore 1028.58 \text{ g.cm}^{-2} \equiv 1013.246 \text{ mbar}$$

$$1 \text{ g.cm}^{-2} \equiv \frac{1013.246}{1028.580} = 0.9851 \text{ mbar} \quad (2.9)$$

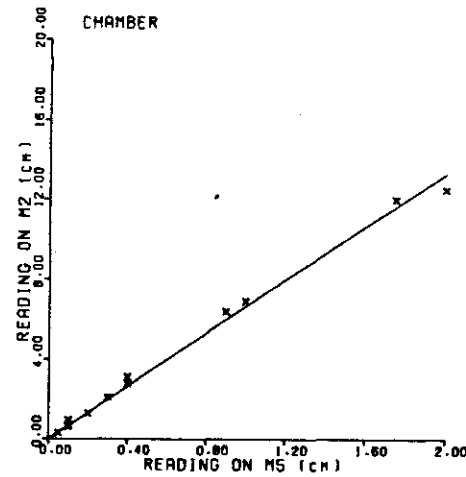
TABLE 2F Pressure conversion factor determination. Readings recorded on manometer M5 against simultaneous readings recorded on manometer M3 (see figure 2.19).

<u>Stream number</u>	<u>Reading on M5 (cm)</u>	<u>Reading on M3 (cm)</u>
0	2.00	0.45
0	4.90	1.10
0	1.20	0.20
0	0.30	0.05
1	1.30	0.30
1	4.00	0.95
1	7.20	1.75
1	10.10	2.60
2	0.55	0.10
2	2.20	0.50
2	5.00	1.20
2	10.60	2.90
3	0.45	0.10
3	3.20	0.75
3	1.80	0.40
3	0.90	0.20
4	7.80	2.00
4	4.15	1.00
4	1.70	0.40
4	0.40	0.10
5	0.55	0.10
5	7.10	1.75
5	3.70	0.90
5	1.70	0.40
6	1.30	0.30
6	0.80	0.20
6	0.40	0.10
6	0.20	0.05

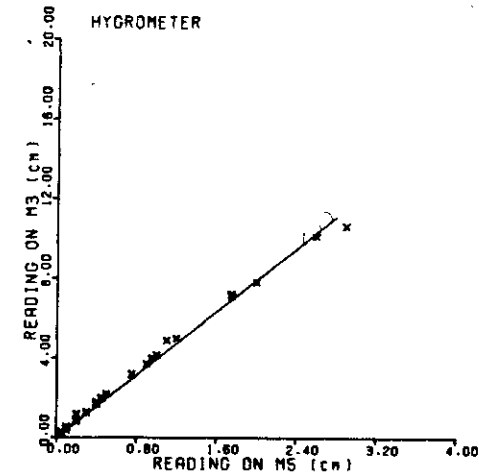
FIGURE 2.20 Results for the calculation of the pressure conversion factors for different parts of the gas flow system.



$$\begin{aligned}\Sigma XY &= 74.3825 \\ \Sigma X^2 &= 10.1275 \\ \frac{\Sigma XY}{\Sigma X^2} &= 7.3446 \\ \text{s.e.} &= 0.4211 \\ Y &= 7.3446X\end{aligned}$$



$$\begin{aligned}\Sigma XY &= 62.1500 \\ \Sigma X^2 &= 9.3550 \\ \frac{\Sigma XY}{\Sigma X^2} &= 6.6435 \\ \text{s.e.} &= 0.3632 \\ Y &= 6.6435X\end{aligned}$$



$$\begin{aligned}\Sigma XY &= 128.3950 \\ \Sigma X^2 &= 32.5075 \\ \frac{\Sigma XY}{\Sigma X^2} &= 3.9497 \\ \text{s.e.} &= 0.2695 \\ Y &= 3.9497X\end{aligned}$$

The density of the manometer fluid in M1, M2, M3, M5 was measured using a specific gravity bottle and found to be 0.7893 g.ml^{-1} .

$\therefore$ in a column of height 1 cm, there are 0.7893 g.cm^{-2} (grams-force. cm^{-2})
but, from 2.9: 1 g cm^{-2} (grams-force. cm^{-2}) $\equiv 0.9851 \text{ mbar}$

$\therefore$ in a column of manometer fluid of height 1 cm, there are:

$$(0.7893)(0.9851) = 0.7775 \text{ mbar.} \approx 0.78 \text{ mbar. pressure.}$$

Thus, a 1 cm height difference in the arms of the manometer M5 represents a difference in pressure of 0.78 mbar between the IRGA analysis tube and ambient. From the regression analyses calculated earlier, the pressure in other parts of the system could be calculated. If ambient barometric pressure is P_{mbar} and h is the height difference recorded on M5 then:

$$\text{Pressure in the IRGA analysis tube} = P + (0.78)(h)$$

$$\text{" " " flowmeter} = P + (0.78)(h)(7.3446)$$

$$\text{" " " chamber} = P + (0.78)(h)(6.6435)$$

$$\text{" " " hygrometer} = P + (0.78)(h)(3.0808)$$

During subsequent gas exchange measurements only M5 was in position. When measurements were taken, the reading on M5 was noted, enabling the pressures elsewhere in the gas circuit to be calculated by the methods outlined.

2.3/5 Calibration of the photocells

The photocells (Section 2.2/4) were to be used exclusively for laboratory studies under two different types of lamp source, and at different distances from the lamps. Obviously, the source emission spectra of the lamps was different, but relatively constant for a given lamp. It was thus preferable that calibrations be carried out in situ for each combination of source and distance from source to be

used in the course of the experiments. This was achieved by simultaneous measurement of irradiance with the photocell and with another independent precisely calibrated detector.

The independent detector used was a Lambda LI-1905 quantum sensor connected to a Lambda LI-185 photometer amplifier (both Lambda Instruments Corp). This sensor, which was mounted in a cosine corrected head, had a spectral response which was very close to the ideal quantum response in the 400-700 nm waveband, and measured irradiance in units of $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR. It had itself been calibrated against working quartz-halogen lamp standards, traceable to American National Bureau of Standards records.

Method of Calibration:

The quantum sensor was placed inside a leaf chamber alongside, and at the same level as, the photocell to be calibrated and at the level of a leaf surface. Simultaneous readings of photocell output voltage, measured with the digital voltmeter, and irradiance measured by the quantum sensor ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR) were taken. This was repeated at ten levels of irradiance (including zero irradiance). All photocells were calibrated in this way under both light sources, and at different distances from the sources.

Photocell output voltage was then plotted against irradiance, and the data were fitted to a number of regressions. The nature of the variables made it clear that if there was zero irradiance then there would also be zero output voltage, thus the regressions were forced through the origin (i.e. 0.0, 0.0). It was found that a quadratic regression generally gave the line of best fit, i.e.

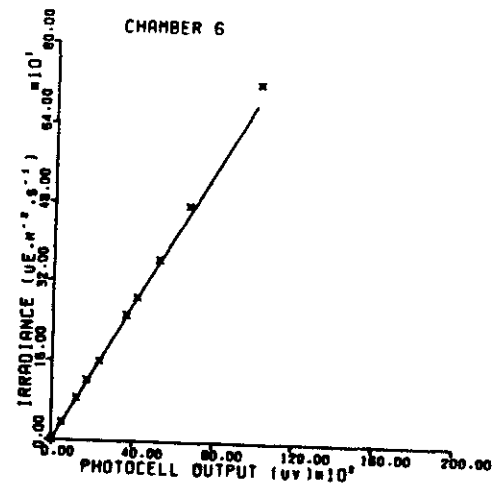
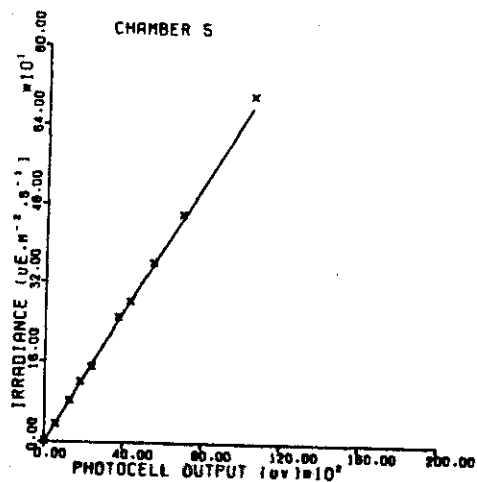
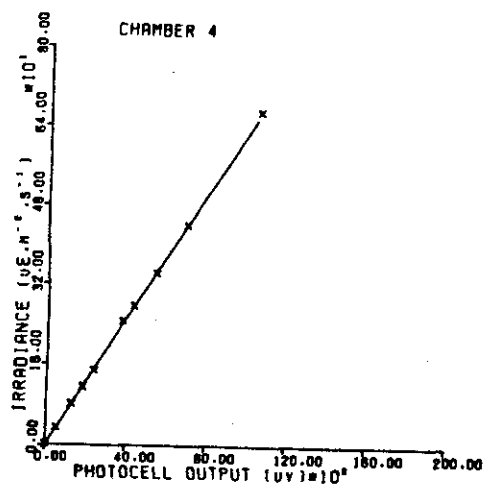
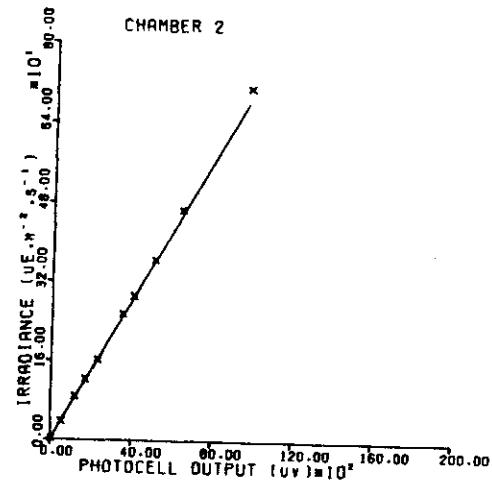
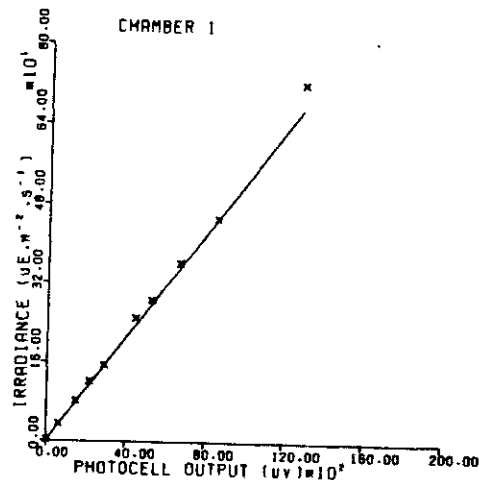
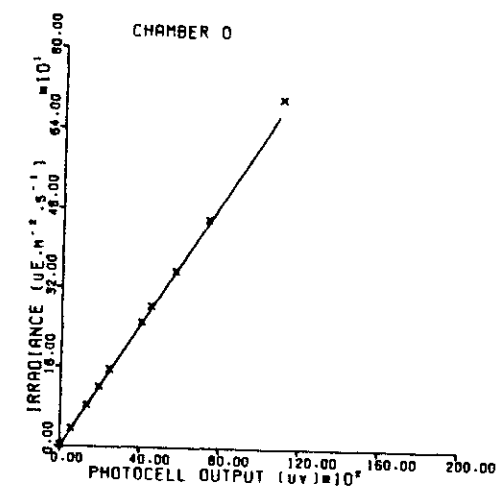
$$Y = bX + cX^2 \quad (2.10)$$

where: Y = Irradiance ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR)
 X = photocell output voltage (μV)
 b, c are calibration constants found by regression analysis (Snedecor & Cochran, 1971)

Some typical calibrations are shown in figure 2.21. The lines look fairly linear in the calibration range because of the quadratic co-efficient ('c' in equation 2.10) being very small compared to 'b'.

Thus, values of photocell output voltage taken during gas exchange measurements could be converted into measurements of chamber irradiance using the appropriate calibration constants and equation 2.10.

FIGURE 2.21 Calibrations of the photocells.



2.4 Experimental material and procedures

2.4/1 Plant material

Two year old seedlings of Quercus petraea (Mattuschka) Leiblein, Fraxinus excelsior L., and Acer pseudoplatanus L. were planted in a peat-loam (1:3) mixture in 25 cm plastic pots and grown in a glasshouse for six months before measurements were made. The other two species used in this study, Nicotiana tabacum L.cv.Bel-W3 and Phaseolus vulgaris L. were sown in 10 cm plastic pots in a peat-loam (1:3) mixture, and were used when the deciduous tree species were not in leaf. All plants were kept in a glasshouse; day temperature was maintained at 20°C, and night temperature at 14°C, with a 15 hour photoperiod. Natural radiant flux density was supplemented by a bank of fluorescent tubes. The plants were watered twice weekly, fertilized monthly, and were kept free from foliar disease by spraying with appropriate chemicals.

Leaves of the tree species showed intermittent growth (alternation of flushes of growth with bud formation) under the long days, and all gas exchange measurements were performed on young, but fully expanded, leaves.

2.4/2 Experimental procedure

Plants to be used for gas exchange measurements were transferred from the glasshouse to the laboratory, and leaves were placed in the leaf chambers during the afternoon of the day before measurements were planned to begin. The lamps in the laboratory (Section 2.2/4) were switched to coincide with the photoperiod of the glasshouse. Leaves were given a minimum of 2.5 hours after the lights had been switched on as a further acclimatisation period at the radiant flux density which was highest for that day's measurements, before any gas exchange measurements were made (see Section 3.1). After any subsequent change in environmental conditions, measurements were not made until a steady-

state had again been reached as indicated by the dewpoint hygrometer and IRGA servoscribe traces (Section 2.3/3), except when the time course of stomatal movements was being followed. The time required to reach a steady-state after a change in environmental conditions was generally between 30 minutes to 2 hours depending upon the species and the nature of the change.

Attempts to measure leaf water potential (Ψ_L) non-destructively using a leaf psychrometer (Wescor Ltd, Logan, Utah, USA) were unsuccessful since they involved removing the leaf from the chamber, making a measurement, then replacing the leaf. A further stabilisation period had then to be allowed and it was found that the whole procedure was too lengthy to be practical. To keep values of Ψ_L above the threshold value at which stomata are affected (see Section 1.3), plants in the laboratory were watered daily. The restricted measurements with the leaf psychrometer referred to above did indicate that Ψ_L values were high (c. 0.5 MPa) and varied little throughout the day. Thus it was assumed that changes in Ψ_L were small throughout the period of measurement.

Preliminary experiments showed that the CO_2 concentration of atmospheric air drawn from outside the laboratory fluctuated considerably. Because of the dependence of rate of photosynthesis and stomatal resistance upon ambient CO_2 concentration, it was decided to use gas of known CO_2 concentration. This gas was prepared in a number of ways depending upon the particular experiment in progress:

- 1) The most extensively used source of gas was from a compressed air cylinder which had been calibrated for CO_2 content according to the procedure described in Section 2.3/1.
- 2) Gas from a cylinder of oxygen-free nitrogen was used when it was desired to supply the leaf with zero CO_2 and zero O_2 .

- 3) For experiments involving a supply to the leaf with zero CO_2 at normal O_2 levels, air drawn from outside the laboratory was passed through two soda-lime towers.
- 4) For experiments involving varying O_2 concentration, gas was prepared using gas mixing pumps, as described in Section 2.1/1. The main drawback of using these gas mixing pumps for gas exchange measurements was that a maximum delivery of only about 0.8 l.min^{-1} was possible with the present system, which meant that only one leaf could be supplied at a time.

To determine whether endogenous rhythms in stomatal movement were occurring which would confound the effects of changes in environmental conditions, several preliminary experiments were carried out which monitored the stomatal resistance under constant conditions throughout the photoperiod. The results are outlined in Section 3.1.

To provide basic information necessary for the construction of the model of stomatal behaviour (Section 3.3), three experiments were carried out over a wide range of levels of radiant flux density, leaf chamber inlet humidity, ambient CO_2 concentration and ambient O_2 concentration. Details of these experiments are given in Table 2G.

TABLE 2G Details of Quercus experiments 1&2, Fraxinus experiment 1.

Experiment	Levels of radiant flux density ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Levels of inlet air dewpoint temperature ($^{\circ}\text{C}$)	Levels of ambient CO_2 (ppm)	Levels of ambient O_2 (%)
QEXPT1	200	-9	97	20
	100	+8	307	4
	35	+11	480	
	0			
QEXPT2	230	-9	97	20
	130	+10	307	4
	45		480	
	0			
FEXPT1	110	-9	97	20
	60	+10	307	
	20		480	
	0			

On consecutive days, a leaf was subjected to one of the possible combinations of humidity, CO_2 , O_2 , and its steady-state gas exchange monitored at the four levels of radiant flux density. The combinations were allocated randomly to consecutive days.

2.5 Methods of calculation

2.5/1 List of extensively used symbols

Most of the symbols following have a second subscript in the text, which will be either 'r' to signify measurements made on the reference stream, or 'a' to signify measurements made on an analysis stream.

A	- the leaf area (m^2)
C_a	- the concentration of CO_2 in the IRGA analysis tube ($kg.m^{-3}$)
C_c	- " " " " " leaf chamber "
C_f	- " " " " " at the flowmeter outlet ($kg.m^{-3}$)
C_h	- " " " " " in the hygrometer "
C_i	- the sub-stomatal CO_2 concentration
D_{CO_2}	- the molecular diffusivity of CO_2 ($m^2.s^{-1}$)
D_{H_2O}	- " " " " " water vapour ($m^2.s^{-1}$)
E_{ca}	- the partial pressure of water vapour in the leaf chamber (mbar)
E_h	- " " " " " " " hygrometer "
E_L	- the saturation vapour pressure at leaf temperature (mbar)
F	- the flow rate of gas into the leaf chamber ($m^3.s^{-1}$)
P_a	- the total pressure in the IRGA analysis tube (mbar)
P_c	- " " " " " leaf chamber "
P_f	- " " " " at the flowmeter outlet "
P_h	- " " " " in the hygrometer "
q_{CO_2}	- the CO_2 exchange rate of the leaf ($kg.m^{-2}.s^{-1}$)
q_{H_2O}	- the water vapour exchange rate of the leaf ($kg.m^{-2}.s^{-1}$)
$r_{a(H_2O)}$	- the boundary layer resistance to water vapour transfer ($s.m^{-1}$)
r_a	- " " " " " CO_2 " "
$r_{s(H_2O)}$	- the stomatal resistance to water vapour transfer "
r_s	- " " " " " CO_2 " "
T_a	- the temperature of gas in the IRGA analysis tube ($^{\circ}K$)
T_c	- " " " " " leaf chamber "

T_f	- the temperature of gas at the flowmeter outlet ($^{\circ}\text{K}$)
T_h	- " " " " in the hygrometer "
T_L	- the leaf temperature
W_a	- the concentration of water vapour in the IRGA analysis tube (kg.m^{-3})
W_c	- " " " " " " leaf chamber "
W_{cL}	- the concentration of water vapour at the liquid-air interface in the leaf (kg.m^{-3})
W_f	- " " " " " in the flowmeter outlet "
W_h	- " " " " " in the hygrometer "
W_R	- " " " " " at the replica -air interface "
X_a	- the IRGA output voltage for an analysis stream (v)
X_r	- " " " " " a reference stream (v)
ψ_{air}	- the chemical potential of water vapour in the leaf chamber (water potential of leaf chamber air) J.mol^{-1} .

2.5/2 Theory of synchronous CO_2 and H_2O exchange measurements

Infra-Red Gas Analyser

In Section 2.3/1 it was shown that the output voltage of a Grubb-Parsons SB2 IRGA, with a sealed reference tube was a function of the mass of CO_2 per unit volume of air.

$$C_a = a + bX_a + cX_a^2 \quad (2.11)$$

where C_a is CO_2 concentration (kg.m^{-3}) and X_a is IRGA output voltage.

C_a depended on air temperature and pressure in the analysis tube, therefore these quantities had to be measured. The parameters b and c are calibration constants determined during instrument calibration (see Section 2.3/1). The intercept, a , had to be measured during operation since it was dependent on instrument zero setting and varied with amplifier drift.

Dewpoint Hygrometer:

In Section 2.3/2 it was shown that the Cambridge Systems Model 880 Dewpoint Hygrometer was capable of indicating water vapour pressure at the temperature and pressure present in the instrument. If water vapour pressure was E_p , and P_p was the total pressure in the hygrometer, then:

$$\frac{E_p}{P_p} \quad (2.12)$$

gave water vapour concentration in volumes per volume (e.g. $m^3.m^{-3}$).

Flowmeters:

In Section 2.3/3 it was shown that the flowmeters used indicated flow in $m^3.s^{-1}$ at the flowmeter temperature and pressure (T_f and P_f). The flow rate was accurately known in order to calculate gas exchange rates (see Section 2.3/3).

Gas Exchange:

In the technique used, the carbon dioxide content of the air was known by prior analysis (from a calibrated cylinder or generated by gas mixing pumps; see Section 2.4/2). The CO_2 content of the gas in an analysis stream was increased or decreased by the CO_2 exchange of the leaf, diluted by water evaporation in the humidification apparatus, and may have been further diluted by evaporation of water from the leaf. The gas in the reference stream was diluted only by water evaporating in the humidifier.

Interconversion of gas concentrations

If: Y is the volume ratio of a gas in a mixture ($m^3.m^{-3}$)

C is the concentration of the gas as mass per unit volume ($kg.m^{-3}$)

M is the molecular weight of the gas

P is the pressure of the gas (mbar)

T is the temperature of the gas ($^{\circ}K$)

1013.246 mbar is standard pressure

273.16°K is standard temperature

22.4136 is the volume (litres) of one gram mole of a gas at STP

$$\text{then: } C = \frac{(Y)(273.16)(P)(M)}{(T)(1013.246)(22.4136)} \quad \text{kg.m}^{-3}$$

$$\text{and: } Y = \frac{(C)(T)(1013.246)(22.4136)}{(273.16)(P)(M)} \quad \text{m}^3.\text{m}^{-3}$$

For Carbon dioxide, M_{CO_2} is 44.01

$$\therefore C_{\text{CO}_2} = \frac{(Y_{\text{CO}_2})(273.16)(44.01)(P)}{(T)(1013.246)(22.4136)} \quad \text{kg.m}^{-3}$$

$$C_{\text{CO}_2} = \frac{(Y_{\text{CO}_2})(P)(\eta)}{(T)} \quad \text{where } \eta = 0.52934883 \quad (2.13)$$

$$\text{and } Y_{\text{CO}_2} = \frac{(C_{\text{CO}_2})(T)}{(P)(\eta)} \quad \text{m}^3.\text{m}^{-3} \quad (2.14)$$

For water vapour, $M_{\text{H}_2\text{O}} = 18.018$

$$\therefore C_{\text{H}_2\text{O}} = \frac{(Y_{\text{H}_2\text{O}})(273.16)(18.018)(P)}{(T)(1013.246)(22.4136)} \quad \text{kg.m}^{-3}$$

$$\therefore C_{\text{H}_2\text{O}} = \frac{(Y_{\text{H}_2\text{O}})(P)(\phi)}{(T)} \quad \text{kg.m}^{-3} \quad (2.15)$$

where $\phi = 0.21671909$

$$\text{and } Y_{\text{H}_2\text{O}} = \frac{(C_{\text{H}_2\text{O}})(T)}{(P)(\phi)} \quad \text{m}^3.\text{m}^{-3} \quad (2.16)$$

2.5/3 Calculation of the IRGA calibration intercept 'a'

For reference gas flowing in the system, the hygrometer gave vapour pressure, E_{hr} (mbar). Also known were: P_{fr} , P_{hr} , P_{ar} , T_{fr} , T_{hr} , T_{ar} , and X_r , the gas analyser voltage.

The hygrometer indicated that the vapour pressure in the reference stream (after humidification) was E_{hr} , when the total pressure was P_{hr} . The partial pressures of the other gases present, including CO_2 , sum to $(P_{\text{hr}} - E_{\text{hr}})$ mbar. If V_d was the volume ratio of the CO_2 before humidification ($\text{m}^3.\text{m}^{-3}$), then the volume ratio of CO_2 after humidification $V_{\text{wr}}(\text{m}^3.\text{m}^{-3})$ was given by:

$$V_{wr} = \frac{(Vd)(P_{hr} - E_{hr})}{P_{hr}} \quad m^3.m^{-3}$$

$$V_{wr} = (Vd)(1 - H_r) \quad m^3.m^{-3} \quad \text{where } H_r = \frac{E_{hr}}{P_{hr}} \quad (2.17)$$

This was also the volume ratio of CO_2 present in the IRGA analysis tube for the reference stream. The CO_2 concentration, C_{ar} ($kg.m^{-3}$) in the IRGA analysis tube at P_{ar} , T_{ar} , was given by (using equation 2.13):

$$C_{ar} = \frac{(Vd)(1 - H_r)(P_{ar})(\eta)}{(T_{ar})} \quad kg.m^{-3} \quad (2.18)$$

but, from equation 2.11:

$$C_{ar} = a + bX_r + cX_r^2 \quad kg.m^{-3}$$

$$\therefore a = C_{ar} - bX_r - cX_r^2$$

substituting for C_{ar} , using equation 2.18:

$$a = \left[\frac{(Vd)(1 - H_r)(P_{ar})(\eta)}{(T_{ar})} \right] - bX_r - cX_r^2 \quad (2.19)$$

Thus the intercept, 'a', of the calibration equation could be calculated from instrument readings every time the reference stream was sampled. The reference air also provided an estimate of the volume ratio ($m^3.m^{-3}$) of water vapour, before it entered a leaf chamber:

$$H_r = \frac{E_{hr}}{P_{hr}} \quad m^3.m^{-3} \quad (2.20)$$

' H_r ' and 'a' were found to vary with time during measurement, so reference readings were taken at frequent intervals (every 30-45 minutes). Interpolation between successive reference readings was used to provide numerical values for 'a' and ' H_r ' at intermediate times, when analysis stream measurements were taken.

For an analysis stream flowing in the system, the hygrometer gave vapour pressure, E_{ha} (mbar). Also known were:

$P_{fa}, P_{ca}, P_{ha}, P_{aa}, T_{fa}, T_{ca}, T_{ha}, T_{aa}$ and X_a .

2.5/4 Determination of the water vapour concentration in the leaf chamber

The volume ratio of water vapour in the hygrometer when analysis stream gas was passing through was given by H_a ($m^3.m^{-3}$)

where:

$$H_a = \frac{E_{ha}}{P_{ha}} \quad m^3.m^{-3} \quad (2.21)$$

This was also the volume ratio of water vapour in the leaf chamber.

The water vapour concentration, W_{ca} ($kg.m^{-3}$), in the leaf chamber, at P_{ca}, T_{ca} , was given by (cf. equation 2.15):

$$W_{ca} = \frac{(H_a)(P_{ca})(\phi)}{(T_{ca})} \quad (2.22)$$

2.5/5 Determination of the CO_2 concentration in the leaf chamber

The CO_2 concentration of analysis stream gas when in the IRGA analyser tube (at P_{aa}, T_{aa}) was given by C_{aa} ($kg.m^{-3}$)

$$C_{aa} = a + bX_a + cX_a^2 \quad kg.m^{-3} \quad (2.11)$$

Thus, the volume ratio of CO_2 in the IRGA analysis tube, Y_a ($m^3.m^{-3}$), was given by: (cf. equation 2.14)

$$Y_a = \frac{(C_{aa})(T_{aa})}{(P_{aa})(\eta)} \quad m^3.m^{-3}$$

This was also the volume ratio of CO_2 in the leaf chamber. The CO_2 concentration in the leaf chamber, C_{ca} ($kg.m^{-3}$) at P_{ca}, T_{ca} , was given by (cf. equation 2.13):

$$C_{ca} = \frac{(Y_a)(P_{ca})(\eta)}{(T_{ca})} \quad kg.m^{-3}$$

Substituting for Y_a :

$$C_{ca} = \frac{(C_{aa})(T_{aa})(P_{ca})(\eta)}{(P_{aa})(\eta)(T_{ca})} \quad kg.m^{-3}$$

substituting for C_{aa} (using equation 2.11)

$$C_{ca} = \frac{(a + bX_a + cX_a^2)(T_{aa})(P_{ca})}{(P_{aa})(T_{ca})} \quad \text{kg.m}^{-3} \quad (2.23)$$

2.5/6 Calculation of gas exchange rates

The exchange rates were calculated as the product of concentration change, ΔD (kg.m^{-3}) and flow rate, F ($\text{m}^3.\text{s}^{-1}$), divided by the projected leaf area, A (m^2). Since the flowmeters measured flow rate at P_f and T_f , it followed that if the concentration change was corrected to the temperature and pressure at the flowmeter outlet (T_f and P_f), then the gas exchange rate, q , was given by:

$$q = \frac{(\Delta D)(F)}{(A)} \quad \frac{(\text{kg.m}^{-3})(\text{m}^3.\text{s}^{-1})}{\text{m}^2} \quad \text{kg.m}^{-2}.\text{s}^{-1}$$

Determination of water vapour exchange

When reference air was sampled, the volume ratio of water vapour before it entered the chamber was given (from equation 2.20):

$$H_r = \frac{E_{hr}}{P_{hr}} \quad \text{m}^3.\text{m}^{-3}$$

When analysis air was sampled, the volume ratio of water vapour after it left the chamber was given (from equation 2.21):

$$H_a = \frac{E_{ha}}{P_{ha}} \quad \text{m}^3.\text{m}^{-3}$$

The water vapour exchange by the leaf in the chamber resulted in a volume change (cf. CO_2 exchange) which had to be taken into account.

Let Z (m^3) be the volume of water vapour that was added to 1 m^3 of air as it passes over the leaf (the 1 m^3 being measured at the same temperature and pressure as Z). The total volume was $1 + Z$ (m^3), and $H_r + Z$ (m^3) of this was water vapour:

$$\therefore \frac{H_r + Z}{1 + Z} = H_a \quad m^3.m^{-3}$$

$$\therefore Z = \frac{H_a - H_r}{1 - H_a} \quad m^3.m^{-3}$$

Z was thus the volume of water vapour that was added to unit volume of air as it passed over the leaf. This was converted to a weight per unit volume, ΔD_{fa} ($kg.m^{-3}$) at the temperature and pressure of the analysis flowmeter (T_{fa} , P_{fa}) using equation 2.15:

$$\Delta D_{fa} = \frac{(Z)(P_{fa})(\phi)}{(T_{fa})} \quad kg.m^{-3}$$

substituting for Z:

$$\Delta D_{fa} = \frac{(H_a - H_r)(P_{fa})(\phi)}{(1 - H_a)(T_{fa})} \quad kg.m^{-3}$$

This, ΔD_{fa} , was the concentration change, corrected for the temperature and pressure of the flowmeter, necessary to calculate water vapour exchange, q_{H_2O} ($kg.m^{-2}.s^{-1}$)

$$q_{H_2O} = \frac{(D_{fa})(F)}{(A)} \quad kg.m^{-2}.s^{-1}$$

$$q_{H_2O} = \frac{(H_a - H_r)(P_{fa})(\phi)(F)}{(1 - H_a)(T_{fa})(A)} \quad kg.m^{-2}.s^{-1} \quad (2.24)$$

Determination of the net CO_2 exchange

Any volume change resulting from CO_2 exchange in the leaf chamber was assumed to be negligible, since the photosynthetic quotient* is generally equal to unity (Sestak et al, 1971).

The volume ratio of CO_2 before humidification was V_d ($m^3.m^{-3}$). It has been shown (by equation 2.17) that the volume ratio of CO_2 after humidification was V_{wr} ($m^3.m^{-3}$)

Where $V_{wr} = V_d(1 - H_r) \quad m^3.m^{-3}$

This was the volume ratio of CO_2 in an analysis stream before it

* Photosynthetic quotient is equal to the number of oxygen molecules liberated divided by the number of CO_2 molecules absorbed.

entered the leaf chamber. Therefore, at the temperature and pressure of an analysis flowmeter (T_{fa} , P_{fa}) the CO_2 concentration C_{fr} ($kg.m^{-3}$) would have been:

$$C_{fr} = \frac{(V_d(1 - H_r)(P_{fa})(\eta))}{(T_{fa})} \quad kg.m^{-3} \quad (2.25)$$

Suppose that CO_2 exchange by the leaf changed the CO_2 volume ratio V_d ($m^3.m^{-3}$) to a new value V_{da} ($m^3.m^{-3}$). V_{da} is uncorrected for the dilution caused by humidification and transpiration. The dilution caused by humidification was known to be $(V_{da})(1 - H_r)$ (equation 2.17) $m^3.m^{-3}$. Therefore, at the temperature and pressure of the flowmeters (T_{fa} , P_{fa}), the carbon dioxide concentration, C_{fa} ($kg.m^{-3}$) was given by (using equation 2.13):

$$C_{fa} = \frac{(V_{da})(1 - H_r)(P_{fa})(\eta)}{T_{fa}} \quad kg.m^{-3} \quad (2.26)$$

The difference between C_{fa} and C_{fr} ($kg.m^{-3}$) was the concentration change at the temperature and pressure of the flowmeter, allowing for the dilution caused by humidification. The net CO_2 exchange rate q_{CO_2} ($kg.m^{-2}.s^{-1}$) was thus given by:

$$q_{CO_2} = \frac{(C_{fa} - C_{fr})(F)}{A} \quad kg.m^{-2}.s^{-1}$$

substituting for C_{fa} and C_{fr} (equations 2.25 and 2.26):

$$q_{CO_2} = \left(\frac{F}{A}\right) \left[\frac{(V_{da})(1 - H_r)(P_{fa})(\eta)}{T_{fa}} - \frac{(V_d)(1 - H_r)(P_{fa})(\eta)}{T_{fa}} \right]$$

$$q_{CO_2} = \frac{(F)(1 - H_r)(P_{fa})(\eta)}{(A)(T_{fa})} [V_{da} - V_d] \quad (2.27)$$

The only unknown on the right hand side of equation 2.27 was V_{da} ($m^3.m^{-3}$) and this was calculated as follows:

V_{da} was the volume ratio of CO_2 after gas exchange had occurred, uncorrected for the dilution caused by humidification and transpiration.

After correction of V_{da} for humidification and transpiration, let the resultant CO_2 volume ratio be V_{wa} ($m^3.m^{-3}$). The hygrometer indicated that the water vapour pressure of the air after it emerged from the chamber was E_{ha} when the total pressure was P_{ha} . The partial pressures of other gases present must sum to $(P_{ha} - E_{ha})$ mbar.

$$\therefore V_{wa} = \frac{(V_{da})(P_{ha} - E_{ha})}{P_{ha}} \quad m^3.m^{-3}$$

$$\therefore V_{wa} = (V_{da})(1 - H_a) \quad m^3.m^{-3} \quad \text{where } H_a = \frac{E_{ha}}{P_{ha}}$$

This was also the volume ratio of CO_2 in the IRGA analysis tube when analysis gas was flowing. The CO_2 concentration, C_{aa} ($kg.m^{-3}$), in the IRGA analysis tube (at T_{aa} , P_{aa}) was thus given by (equation 2.13):

$$C_{aa} = \frac{(V_{wa})(P_{aa})(\eta)}{T_{aa}} = \frac{(V_{da})(1 - H_a)(P_{aa})(\eta)}{T_{aa}} \quad kg.m^{-3} \quad (2.28)$$

However, C_{aa} ($kg.m^{-3}$) was also given by:

$$C_{aa} = a + bX_a + cX_a^2 \quad kg.m^{-3} \quad (2.29)$$

equating equations 2.28 and 2.29:

$$\frac{(V_{da})(1 - H_a)(P_{aa})(\eta)}{T_{aa}} = a + bX_a + cX_a^2 \quad kg.m^{-3}$$

$$\therefore V_{da} = \frac{(a + bX_a + cX_a^2)(T_{aa})}{(1 - H_a)(P_{aa})(\eta)} \quad m^3.m^{-3}$$

The value of V_{da} was substituted into equation 2.27 to give the net CO_2 exchange rate:

$$q_{CO_2} = \frac{(F)(1 - H_r)(P_{fa})(\eta)}{(A)(T_{fa})} \left[\frac{(a + bX_a + cX_a^2)(T_{aa})}{(1 - H_a)(P_{aa})(\eta)} - V_d \right] kg.m^{-2}.s^{-1} \quad (2.30)$$

2.5/7 Determination of the water vapour concentration at the liquid-air interface inside the leaf

The partial pressure of water vapour at the liquid-air interface within the leaf was assumed to be equal to saturation vapour pressure

at leaf temperature. The implications of this assumption are discussed by Jarvis (1971). Care was taken to ensure a precise value of leaf temperature to within 0.1°C (as discussed in Section 2.2/2). Substitution of leaf temperature into the Goff-Gratch Formulation (given in Section 2.3/2) gave the saturation vapour pressure at the liquid-air interface, E_L (mbar). If the total pressure inside the leaf chamber was P_{ca} (mbar), then the volume ratio of water vapour at the liquid-air interface was given by:

$$\frac{E_L}{P_{ca}} \text{ m}^3 \cdot \text{m}^{-3}$$

which could be converted to the concentration of water vapour at the liquid-air interface, W_{cL} ($\text{kg} \cdot \text{m}^{-3}$) as follows:

$$W_{cL} = \frac{E_L}{P_{ca}} \cdot \frac{(273.16)}{T_L} \cdot \frac{(18.018)}{(22.4136)} \quad (2.31)$$

where T_L is leaf temperature ($^{\circ}\text{K}$).

2.5/8 Calculation of resistances to gaseous transfer

When the stomata of a leaf are open, CO_2 moves from a source in the ambient air to a sink at the sites of carboxylation in the chloroplast. Simultaneously, water vapour moves in the opposite direction from a source at the liquid-air interface within the leaf to a sink in the ambient air. Along the transfer pathways several more or less discrete sections can be identified (figure 2.22), and certain segments of the pathways are common to both water vapour and CO_2 . Use of this was made in calculating resistance to CO_2 transfer from resistance to water vapour transfer, which was more easily measured.

In the leaf chamber studies reported here, the total resistance to water vapour transfer from the leaf was estimated. This total resistance could be partitioned into several components, but in this

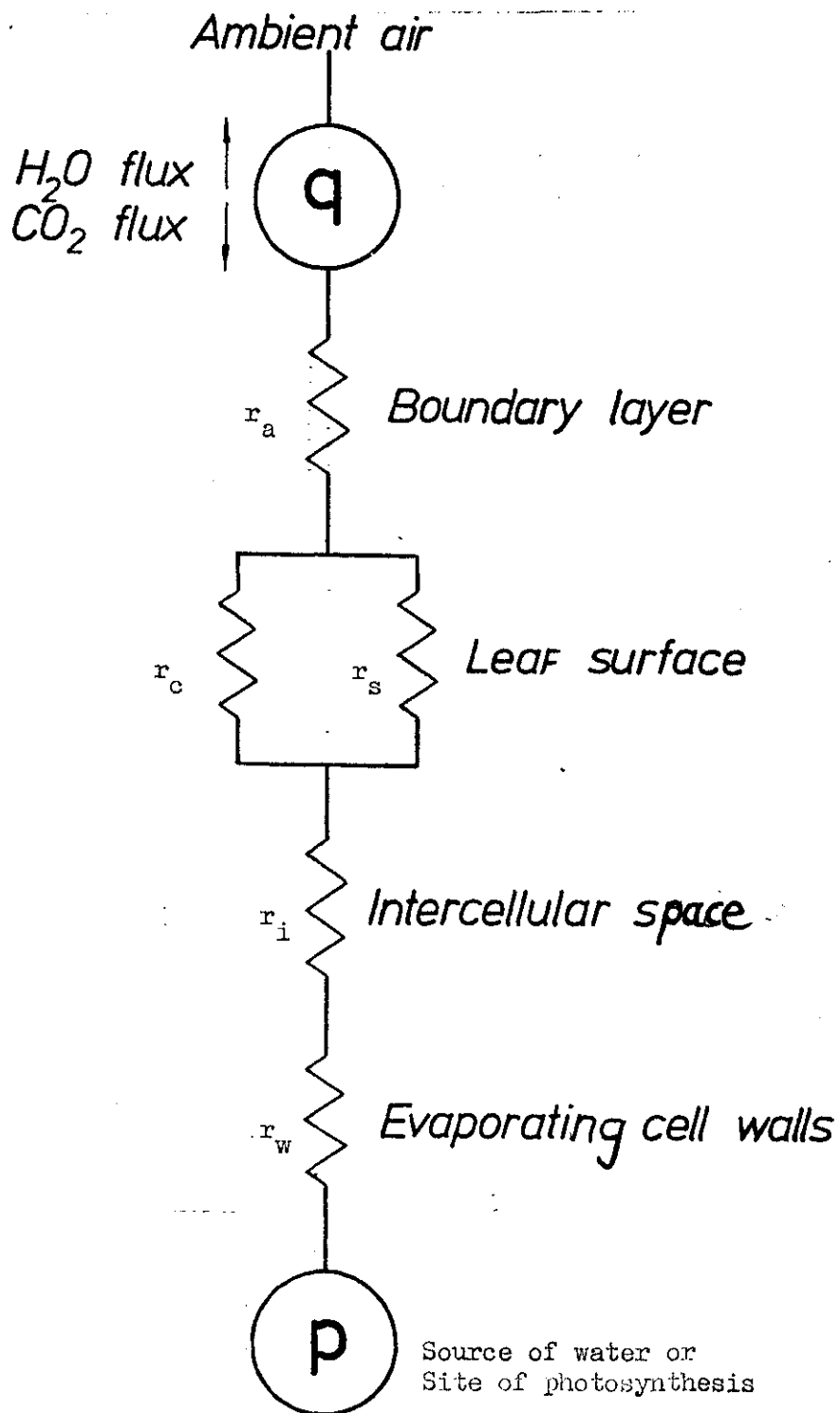
study it was decided to separate only two components in the transfer pathway: the boundary layer resistance, and a stomatal resistance (r_s) which was principally a resistance to diffusion through the stomatal pore and intercellular spaces, with a component due to the properties of the liquid-air interface. This was thought to be justified in the following terms.

- 1) An assessment of the boundary layer resistance could be obtained fairly easily by the leaf replica method (see next section).
- 2) The leaves used in these experiments were kept well watered to avoid problems of measuring leaf water status accurately in situ. Thus changes in the leaf water status were assumed to be small and hence the cuticular resistance was assumed to be constant (Schönherr & Bucovac, cited in Schulze et al, 1974), and large relative to the stomatal resistance, r_s . Water loss through the cuticle was assumed constant and small.
- 3) The studies involved rapid and reversible changes in total diffusion resistance and this was attributed to changes in stomatal aperture. This was important bearing in mind that the main objective of the study was to investigate how the stomata control gas exchange, and the fact that the other components of the transfer pathway of water vapour are not easily separated.
- 4) Most of the experimental work was conducted using broad leaved deciduous tree species (principally Quercus petraea) which are hypostomatous, thus a differing response of stomata on upper and lower surfaces (e.g. Koller, 1970) could not occur.

A generalised transfer equation can be written for the transfer of water vapour or CO_2 in air (Jarvis, 1971):

$$q_v = - K_v \cdot \frac{dc}{dz} \quad (2.32)$$

FIGURE 2.22 Resistances to gaseous transfer (after Lake, 1967).



where: q_v = the net flux of mass per unit volume and time ($\text{kg.m}^{-2}.\text{s}^{-1}$)

K_v = a transfer coefficient ($\text{m}^2.\text{s}^{-1}$)

$\frac{dc}{dz}$ = the gradient of partial pressure in the transfer pathway

When transfer is by molecular diffusion $K_v = D_v$, the molecular diffusivity of the property being transferred. Hence, over the same pathway when transfer is by molecular diffusion, the transfer resistances of water vapour and CO_2 are related by their molecular diffusivities:

$$r_{\text{CO}_2} = r_{\text{H}_2\text{O}} \cdot \frac{D_{\text{H}_2\text{O}}}{D_{\text{CO}_2}}$$

However, in the leaf boundary layer transport of water vapour and CO_2 is not wholly diffusive and Thom (1968) has demonstrated the validity of the following equation in this region:

$$r_{\text{CO}_2} = r_{\text{H}_2\text{O}} \left\{ \frac{D_{\text{H}_2\text{O}}}{D_{\text{CO}_2}} \right\}^{2/3}$$

Considerable uncertainty exists as to the precise values of $D_{\text{H}_2\text{O}}$ and D_{CO_2} , but Jarvis (1971) reported work by Fuller, Schettler & Griddings (1966) which gives a ratio for $D_{\text{H}_2\text{O}}/D_{\text{CO}_2}$ of 1.605, independent of temperature and pressure. This value was used in these studies. To summarise, using the symbols of figure 2.22:

$$\text{In the boundary layer: } r_a = r_{a(\text{H}_2\text{O})}(1.605)^{2/3} = r_a (1.37) \quad (2.34)$$

$$\text{In the diffusion pathway: } r_s = r_{s(\text{H}_2\text{O})}(1.605) \quad (2.35)$$

2.5/9 Determination of the leaf boundary layer resistance

The technique used to assess leaf boundary layer resistance was to expose a water saturated replica in the leaf chamber in the same position as the leaf. Powell (1940) showed that evaporation from a coarsely structured, inert matrix such as blotting paper was similar

to evaporation from a free water surface. For the boundary layer resistance assessment of a leaf from a hypostomatous species (Quercus petraea, Fraxinus excelsior, Acer pseudoplatanus) the replica was made from "Benchkote" (Whatman Ltd) which is a blotting paper with a waterproof polyethylene coating on one side. Thus, evaporation was from one surface only, simulating the hypostomatous leaf. For the boundary layer assessment of a leaf from an amphistomatous species (Nicotiana tabacum, Phaseolus vulgaris) Whatman No. 1 filter paper was used. The replicas were made by tracing around the leaf to be assessed onto the replica material, and then cutting out the replica to the exact shape of the leaf. A limitation of this method is that although leaf size, shape and exposure can be simulated accurately, the surface roughness of the leaf caused by the presence of hairs etc, can not. This is likely to lead to a slight error in boundary layer resistance assessment because Woolley (1964) has demonstrated that hairs contribute significantly to r_a .

It was assumed that the only resistance to evaporation from the replica-air interface was the boundary layer resistance to water vapour transfer $r_{a(H_2O)}$ ($s.m^{-1}$) which was calculated from the following:

$$q_{H_2O} = \frac{W_R - W_{ca}}{r_{a(H_2O)}} \quad kg.m^{-2}.s^{-1} \quad (2.36)$$

$$\therefore r_{a(H_2O)} = \frac{W_R - W_{ca}}{q_{H_2O}} \quad (2.37)$$

where q_{H_2O} is the flux of water vapour from the replica ($kg.m^{-2}.s^{-1}$) calculated from gas exchange measurements using equation 2.24.

W_{ca} is the concentration of water vapour in the leaf chamber ($kg.m^{-3}$) calculated using equation 2.22.

W_R is the water vapour concentration at the replica-air interface assuming saturation at replica temperature (see equation 2.31).

TABLE 2I Typical values of leaf boundary layer resistance to water vapour transfer $\left[r_{a(H_2O)} \right]$, measured according to the method outlined in Section 2.5/9.

	Leaf identifier	Area (cm. ²)	$r_{a(H_2O)}$ (s.m ⁻¹)
Oak	Q29/6L1	36	105
	Q9/5L0	39	133
	Q29/6L0	42	135
	Q13/4L1	44	141
	Q7/3L2	45	192
	Q17/3L0	47	116
	Q28/2L1	53	140
	Q17/3L1	60	200
Tobacco	N27/3L3	49	83
French bean	P17/2L0	43	42

Boundary layer resistance to CO_2 transfer, r_a (s.m^{-1}) could be calculated from $r_a(\text{H}_2\text{O})$ using equation 2.35:

$$r_a = 1.37 r_{a(\text{H}_2\text{O})}$$

Obviously boundary layer resistances varied according to leaf size and shape, and some typical values are given in table 2I. It was found to be unnecessary to assess every leaf for boundary layer resistance, the same value could be used for leaves from the same species of similar area.

2.5/10 Determination of stomatal resistance to water vapour transfer and stomatal resistance to CO_2 transfer

The stomatal resistance to water vapour efflux from the leaf, $r_{s(\text{H}_2\text{O})}$ (s.m^{-1}) was estimated from the following:

$$q_{\text{H}_2\text{O}} = \frac{W_{\text{cL}} - W_{\text{ca}}}{(r_{s(\text{H}_2\text{O})} + r_{a(\text{H}_2\text{O})})} \quad \text{kg.m}^{-2}.\text{s}^{-1} \quad (2.37)$$

$$\therefore r_{s(\text{H}_2\text{O})} + r_{a(\text{H}_2\text{O})} = \frac{W_{\text{cL}} - W_{\text{ca}}}{q_{\text{H}_2\text{O}}} \quad \text{s.m}^{-1}$$

where $q_{\text{H}_2\text{O}}$ is the total flux of water vapour ($\text{kg.m}^{-2}.\text{s}^{-1}$) calculated from equation 2.24.

W_{ca} is the concentration of water vapour in the leaf chamber (kg.m^{-3}) calculated from equation 2.22.

W_{cL} is the concentration of water vapour at the liquid-air interface within the leaf calculated from equation 2.31.

$r_{a(\text{H}_2\text{O})}$ is the boundary layer resistance to water vapour transfer (given by equation 2.37).

Stomatal resistance to CO_2 transfer, r_s (s.m^{-1}) could then be calculated according to equation 2.35:

$$r_s = 1.605 r_{s(\text{H}_2\text{O})} \quad \text{s.m}^{-1}$$

2.5/11 Determination of the sub-stomatal CO_2 concentration

The sub-stomatal CO_2 concentration, C_i (kg.m^{-3}) was calculated as follows: for the transport of CO_2 between the air and the leaf:

$$q_{\text{CO}_2} = \frac{C_{\text{ca}} - C_i}{(r_s + r_a)} \quad \text{kg.m}^{-2}.\text{s}^{-1}$$

$$\therefore C_i = C_{\text{ca}} - q_{\text{CO}_2} (r_s + r_a) \quad \text{kg.m}^{-3}$$

where q_{CO_2} is the net flux of CO_2 calculated using equation 2.30.

C_{ca} is the concentration of CO_2 in the leaf chamber (kg.m^{-3}) calculated using equation 2.23.

r_s is the stomatal resistance to CO_2 transfer (from equation 2.35).

r_a is the boundary layer resistance to CO_2 transfer (from equation 2.34).

2.5/12 Determination of the water potential of the leaf chamber air, Ψ_{air}

Diffusion depends upon a difference of chemical potential between any two points in a system. Water potential (Ψ) is a term which is used to express the difference between the chemical potential of water at any point in a system (μ_w) either in the liquid or vapour phase, and that of pure water under standard conditions (μ°_w). Following Slatyer (1967), water potential is given by:

$$\Psi_m = \mu_w - \mu^{\circ}_w = RT \ln (e/e_o) \quad \text{J.mol}^{-1}$$

where Ψ_m is the difference in the chemical potential (of unit mass) of water at a particular point in the system and that of pure water under standard conditions (J.mol^{-1})

R is the ideal gas constant ($\text{J.mol}^{-1}.\text{K}^{-1}$)

T is the absolute temperature ($^{\circ}\text{K}$)

e is the vapour pressure of water in the system under study (mbar)

e_o is the saturation vapour pressure of pure water at temperature T (mbar).

The relative humidity term (e/e_o) is generally less than 1, hence $\ln (e/e_o)$ is negative and Ψ_m is negative. It follows that the chemical

potential of a free water datum is zero, since $\ln (e/e_o) = 0$. The units of Ψ_m are $J.mol^{-1}$, but they can be converted to units dimensionally equivalent to pressures by dividing by the partial molal volume of water, V_m :

$$\Psi_v = \frac{RT \ln (e/e_o)}{V_m} = \frac{J.mol^{-1}}{m^3.mol^{-1}} = J.m^{-3} = N.m^{-2}$$

where Ψ_v now refers to unit volume of water.

Since V_m is also a function of temperature, it was decided to retain the units of chemical potential, $J.mol^{-1}$, in the present study. The water potential of the leaf chamber air, Ψ_{air} , was thus given by:

$$\Psi_{air} = RT_{ca} \ln (e_{ha}/e_o)$$

where T_{ca} is the leaf chamber air temperature

e_{ha} is the vapour pressure of the air in the leaf chamber

e_o is the saturation vapour pressure at T_{ca} °K.

Since Ψ_{air} was always negative quantity, it was considered convenient to change its sign and express it as $-\Psi_{air}$. The principal reason was that stomatal resistance has been found to vary directly with $-\Psi_{air}$ (see Chapter 1) and this made modelling simpler (see Section 3.3). Thus an increase in $-\Psi_{air}$ refers to air becoming drier and a decrease in $-\Psi_{air}$ refers to air becoming wetter (i.e. $-\Psi_{air}$ is equivalent to vapour pressure deficit, but expressed as a potential rather than a pressure).

In the assessment of humidity effects on stomata, humidity is commonly expressed in the literature in terms of a difference in vapour pressure between the leaf and the air (ΔH). (assuming saturation vapour pressure at leaf temperature), in units of mbar. Although these units take into account differences between leaf and air temperatures, they do not contain a relative humidity term and hence are not directly related to the evaporating power of the air. Since the proposed

mechanism of the direct humidity effect (outlined later) depends upon the evaporating power of the air, it was decided that $-\Psi_{\text{air}}$ (J.mol^{-1}) was the most appropriate measure of air humidity. Table 2J provides a simple comparison of $-\Psi_{\text{air}}$ and ΔH terms.

TABLE 2J Conversion table for $-\psi_{\text{air}}$ (J.mol^{-1}) and ΔH (mbar.). The calculations of ΔH assume that leaf and air temperatures are the same.

Relative humidity (%)	Temperature ($^{\circ}\text{C}$)					
	15		20		25	
	$-\psi_{\text{air}}$	ΔH	$-\psi_{\text{air}}$	ΔH	$-\psi_{\text{air}}$	ΔH
40	2195	10.20	2233	14.00	2271	18.97
50	1660	8.50	1689	11.66	1718	15.81
60	1224	6.81	1246	9.34	1266	12.66
70	856	5.11	870	7.01	884	9.49
80	534	3.40	544	4.67	553	6.33
90	252	1.70	257	2.33	261	3.16
100	0	0.00	0	0.00	0	0.00

	30		35	
	$-\psi_{\text{air}}$	ΔH	$-\psi_{\text{air}}$	ΔH
40	2310	26.43	2348	33.71
50	1747	22.19	1776	28.09
60	1288	17.94	1309	22.47
70	899	13.72	914	16.86
80	562	9.47	572	11.24
90	266	5.24	270	5.62
100	0	0.00	0	0.00

CHAPTER 3: RESULTS

Introduction

The raw data comprising 125 individual data sets are given in appendix 2 (microfiche) and the format of the data presentation is shown in table 3A. Each page of the microfiche displays data obtained from a single leaf, recorded during the course of one day. Each data set is identified by a letter denoting the species under study (see table 3A), followed by six numbers which show the date of measurement. The final symbol is used to distinguish between leaves measured on the same day.

As outlined in Chapter 1, changes in stomatal aperture are the result of a combination of responses to many environmental variables. As a first step in understanding this complex interaction of mechanisms it was important to know the responses to individual variables, one at a time, with the other important factors held constant and non-limiting. Although leaf chamber inlet concentrations of humidity and CO_2 could be controlled, it was difficult to control $-\psi_{\text{air}}$ and C_i accurately. Maintaining a constant CO_2 concentration at the chamber inlet did not ensure a constant C_i , which was also dependent upon rate of CO_2 exchange, r_s and other factors. Further complications arose in attempting to control $-\psi_{\text{air}}$, since any variable which caused a change in $r_{s(\text{H}_2\text{O})}$ also caused a consequent change in E_p , which affected water vapour concentration in the leaf chamber, thus altering $-\psi_{\text{air}}$. This meant that the important environmental variables had to be measured accurately rather than controlled accurately. The large body of data collected was studied in detail and the responses to individual variables was assessed as follows:

In most cases the effects of varying one factor were resolved by selecting the points at which all other environmental factors were

TABLE 3A Sample data set to illustrate the format of appendix 2.

Each of the four sheets of appendix 2 has the following first page:

EACH PAGE OF THIS MICROFICHE DISPLAYS DATA OBTAINED FROM A SINGLE LEAF AND WERE RECORDED DURING THE COURSE OF ONE DAY.

EACH LINE CONTAINS A NUMBER OF ITEMS DERIVED FROM A SINGLE SET OF SIMULTANEOUS MEASUREMENTS AND ARE (READING FROM LEFT TO RIGHT) -

- | | |
|---|-------------|
| 1. CARBON DIOXIDE EXCHANGE RATE. | SEE NOTE 1. |
| 2. IRRADIANCE. | SEE NOTE 2. |
| 3. AMBIENT CARBON DIOXIDE CONCENTRATION. | SEE NOTE 3. |
| 4. LEAF TEMPERATURE. | SEE NOTE 4. |
| 5. AMBIENT WATER VAPOUR CONCENTRATION. | SEE NOTE 3. |
| 6. STOMATAL DIFFUSION RESISTANCE TO WATER VAPOUR. | SEE NOTE 5. |
| 7. WATER POTENTIAL DIFFERENCE BETWEEN LEAF AND AIR. | SEE NOTE 6. |

- NOTE 1. KILOGRAMS PER SQUARE METER PER SECOND.
NOTE 2. MICROEINSTEINS PER SQUARE METER PER SECOND.
NOTE 3. KILOGRAMS PER CUBIC METER.
NOTE 4. DEGREES CENTIGRADE.
NOTE 5. SECONDS PER METER.
NOTE 6. JOULES PER MOLE (EXPRESSED AS AN ABSOLUTE VALUE, WITHOUT THE MINUS SIGN).

continued.....

TABLE 3A (continued).....

Species identifiers:

Q Quercus petraea

F Fraxinus excelsior

A Acer pseudoplatanus

N Nicotiana tabacum

P Phaseolus vulgaris

Each subsequent page contains a single data set:

15 Q1106760 - - -15=page number; Q=species identifier; 110676=date of measurement; 0=leaf identifier.

CO ₂ exchange rate	I	Ambient CO ₂ concentration	T _L	Ambient H ₂ O vapour conc.	r _s (H ₂ O)	-ψ _{air}
.4113E-06	240.4	.4730E-03	27.5	.1312E-01	339.5	1863.
.4093E-06	240.4	.4734E-03	27.6	.1336E-01	327.6	1825.
.3363E-06	176.1	.4934E-03	27.5	.1296E-01	349.3	1909.
.3344E-06	176.1	.4962E-03	27.5	.1297E-01	346.8	1892.
.2781E-06	138.0	.5132E-03	27.6	.1243E-01	383.8	2014.
.2770E-06	138.0	.5135E-03	27.7	.1232E-01	398.4	2042.
.2550E-06	109.5	.5206E-03	27.5	.1178E-01	426.5	2130.
.2572E-06	109.5	.5202E-03	27.4	.1193E-01	413.9	2087.
.2245E-06	95.2	.5303E-03	27.4	.1158E-01	440.1	2160.
.2248E-06	95.2	.5301E-03	27.6	.1150E-01	450.8	2190.
.1439E-06	59.5	.5539E-03	28.2	.1072E-01	579.6	2445.
.1445E-06	59.5	.5535E-03	28.3	.1075E-01	583.1	2455.
.9250E-07	48.8	.5677E-03	29.0	.1079E-01	624.8	2547.
.9498E-07	48.8	.5670E-03	29.0	.1076E-01	627.7	2547.
.4785E-07	34.5	.5814E-03	29.0	.1060E-01	649.3	2585.
.4639E-07	34.5	.5815E-03	29.1	.1063E-01	656.9	2599.
.8970E-08	16.2	.5930E-03	29.1	.1031E-01	692.9	2679.
.1080E-07	16.2	.5921E-03	29.3	.1034E-01	705.7	2692.
-.2732E-07	0	.6053E-03	28.5	.9876E-02	720.6	2716.
-.3009E-07	0	.6060E-03	28.6	.9785E-02	739.9	2751.

continued.....

• TABLE 3A (continued).....

The final page of each microfiche sheet contains an index showing which data sets are contained on that sheet. e.g.

INDEX.

1	01105760
2	01105761
3	018057602
4	018057611
5	018057612
6	01805761B
7	019057601
8	019057602
9	019057611
10	019057612
11	025057601
12	025057602
13	026057601
14	026057602
15	01106760
16	01506760
17	01606764
18	01706764
19	02306761
20	02406761
21	00607760
22	00707760
23	00807760
24	00907760
25	01009761
26	00305771
27	01706771
28	03006770
29	00407770
30	00807770
31	01804781
32	01904781
33	02004781
34	02704781
35	02804781
36	00505781
37	01005781
38	01105781
39	01205781
40	01605781
41	01705781
42	02705781
43	01109782
44	01209782
45	01309782
46	0DRYING1
47	0DRYING2
48	0DRYING3
49	0DRYING4
50	0DRYING5

constant within a defined range within an individual data set. In the other cases sets of data for the same leaf were combined, even though these data may have been collected over several days, and again the effects of varying one factor were resolved by selecting points at which all other environmental factors were constant within a defined range. For example, table 3B gives some results obtained with a single oak leaf over a nine day period. The points marked (*) are at equivalent values of C_i ($0.40 - 0.44 \times 10^{-3} \text{ kg.m}^{-3}$), I ($213 - 243 \mu\text{m.m}^{-2} \text{ s}^{-1}$) and T_L ($29.0 - 32.0^\circ\text{C}$), and can be used to examine the relationship between $r_{s(\text{H}_2\text{O})}$ and $-\psi_{\text{air}}$ (these particular points are incorporated into figure 3.24).

All of the graphs contained in the results section have a data identifier shown to enable the raw data to be located in appendix 2. When only one identifier is given, the measurements were made during the course of one day. When data have been combined using the technique outlined above, two data identifiers are given which define the period over which the measurements were made. The defined range of the constant environmental parameters is given for each graph, and in all cases the units are as follows:

$$\begin{array}{ll} C_i & \text{kg.m}^{-3} \times 10^3 \\ -\psi_{\text{air}} & \text{J.mol}^{-1} \\ I & \mu\text{E.m}^{-2}.\text{s}^{-1} \\ T_L & ^\circ\text{C} \end{array}$$

The environmental factors important in the control of stomata can be strongly correlated and in addition it is a combination of various responses which cause stomatal movements. In order to aid the analysis and interpretation of the observations obtained in the work reported, and also to aid the assessment of the interaction of stomatal responses, it was decided to formulate an empirical mathematical hypothesis. The

TABLE 3B Results for a leaf of Quercus petraea collected over a nine day period.

Date	$-\Psi_{\text{air}}$ J.mol ⁻¹	I $\mu\text{E.m}^{-2}\text{s}^{-1}$	C_i $\text{kg.m}^{-3} \times 10^6$	T_L °C	$r_s(\text{H}_2\text{O})$ s.m^{-1}	
30.6.77	1546	231	.43	29.7	718	*
	1529	233	.43	29.7	703	*
	1547	242	.43	29.9	719	*
	1560	243	.42	29.8	736	*
	2320	240	.39	30.7	742	
	2373	239	.39	30.8	770	
	2350	242	.40	30.6	759	
	2370	243	.39	30.7	769	
	2339	239	.39	30.6	755	
	2328	240	.39	30.5	749	
4.7.77	2384	241	.41	31.0	776	*
	2415	241	.41	31.5	789	*
	2425	242	.41	31.5	794	*
	2489	240	.40	31.6	831	
	1095	230	.38	34.4	928	
	985	231	.38	34.1	883	
	1079	230	.38	34.5	923	
	1014	229	.38	34.4	896	
8.7.77	404	214	.44	29.7	435	*
	364	214	.44	29.6	417	*
	299	214	.44	29.5	399	*
	357	213	.44	29.7	412	*

basic strategy was:

1. To construct a hypothesis from functional relationships based on the knowledge of the responses to individual variables.
2. To use the hypothesis to identify the relative importance of any one variable, and to show how the responses to various factors interact in the control of stomatal responses.
3. To construct the hypothesis in the form of a simple multiple regression which could be used in a predictive capacity.

In section 3.1, the diurnal behavior of stomata is examined. In section 3.2, the responses to individual factors are considered, in order to show the relationships on which the regression hypothesis was based. The formulation, testing and results of the hypothesis are described in section 3.3.

The common symbols used throughout the results section are (see section 2.5 for definitions and methods of estimation):

- C_i - the sub-stomatal CO_2 concentration.
- ψ_{air} - the water potential of air in the leaf chamber.
- E_T - the rate of transpiration.
- I - the radiant flux density of photosynthetically active radiation.
- $r_{s(H_2O)}$ - the stomatal resistance to water vapour transfer.
- r_s - the stomatal resistance to CO_2 transfer.
- T_L - the leaf temperature.

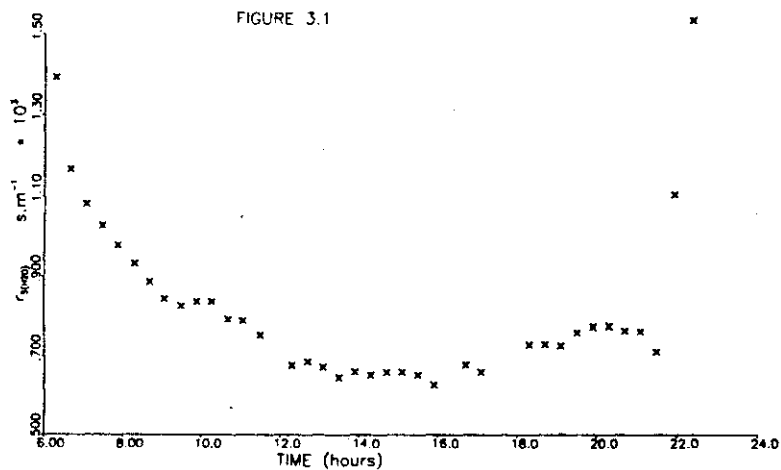
In all of the following graphs stomatal resistance is plotted as $r_{s(H_2O)}$ unless C_i is an independent variable, in which case r_s is plotted. The conversion between the two is given by (see section 2.5):

$$r_s = 1.605(r_{s(H_2O)})$$

3.1 Diurnal behavior of stomata

Examples of the time course of $r_{s(H_2O)}$ under constant conditions throughout the photoperiod are shown in figures 3.1 to 3.3 for oak and figure 3.4 for ash. On changing from dark to light (06.00 hours), $r_{s(H_2O)}$ decreased rapidly in the first hour and at a slower rate for a further two hours. There was a stable period between 09.00 and 18.00 hours over which time $r_{s(H_2O)}$ varied little and so gas exchange measurements were confined to this period. After 18.00 hours, $r_{s(H_2O)}$ began to increase slowly until the lights were switched off at 21.00 hours, after which $r_{s(H_2O)}$ increased rapidly, doubling in value within one hour of lights out. Maximum values of $r_{s(H_2O)}$ (not shown on the graphs) occurred about five hours after the lights had been switched off and $r_{s(H_2O)}$ remained at this value until the beginning of the next photoperiod.

FIGURES 3.1 & 3.2 The time course of $r_s(H_2O)$ under constant conditions throughout the photoperiod.



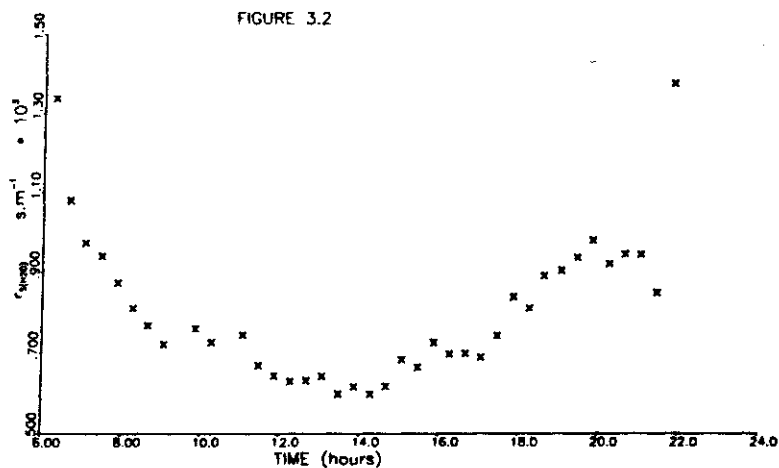
Q260776

$$C_i = 0.38 - 0.42$$

$$-\Psi_{air} = 850 - 950$$

$$T_L = 26 - 32$$

$$I = 205 - 215$$



Q270776

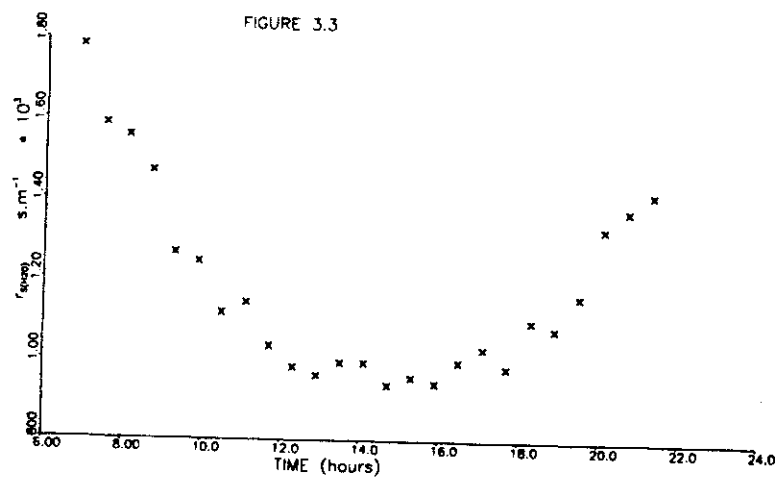
$$C_i = 0.39 - 0.43$$

$$-\Psi_{air} = 450 - 550$$

$$T_L = 29 - 33$$

$$I = 170 - 200$$

FIGURES 3.3 & 3.4 The time course of $r_{s(H_2O)}$ under constant conditions throughout the photoperiod.



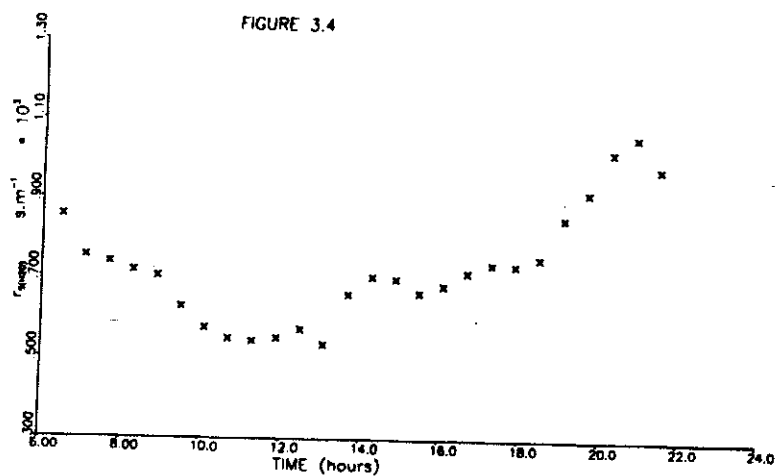
2030876

$C_i = 0.48 - 0.50$

$-\Psi_{air} = 600 - 700$

$T_L = 28 - 34$

$I = 100 - 120$



F030876

$C_i = 0.48 - 0.50$

$-\Psi_{air} = 350 - 450$

$T_L = 28 - 34$

$I = 55 - 65$

3.2 Responses to individual environmental variables

3.2/1 Light and carbon dioxide

As outlined in chapter 1, there are believed to be two mechanisms which can act to cause stomata to open with increasing radiant flux density: an indirect response brought about by changes in C_i , and a direct response predominantly to blue light. Although the direct response has been detected in many species, its importance in the regulation of stomatal aperture has not been clearly established.

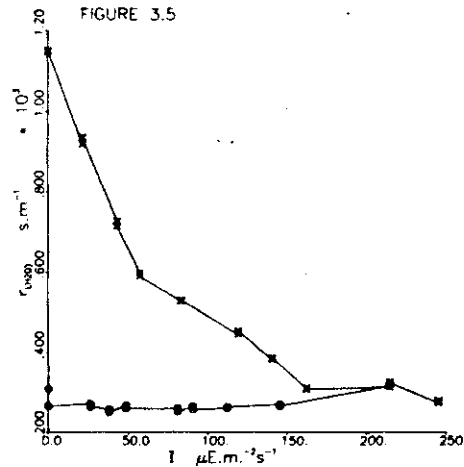
The relationship between $r_{s(H_2O)}$ and I is shown in figure 3.5 for an oak leaf: at normal ambient CO_2 concentrations $r_{s(H_2O)}$ increased as I decreased. However, when the same leaf was supplied with a low ambient CO_2 concentration, $r_{s(H_2O)}$ was independent of I : C_i was at $0.66 \times 10^{-4} \text{ kg.m}^{-3}$ ($\approx 37 \text{ ppm}$) and the stomata remained open, even in the dark. The same effect is shown for another oak leaf (figure 3.6) and a sycamore leaf (figure 3.7) and was also obtained with tobacco (table 3C).

TABLE 3C: Prevention of stomatal closure in the dark at low ambient CO_2 concentrations for two tobacco leaves.

TOBACCO 6.3.78

Leaf 0: at $200 \mu\text{E.m}^{-2}.\text{s}^{-1}$, $r_{s(H_2O)} = 1385 \text{ m.s}^{-1}$	$\left\{ \begin{array}{l} -\psi_{\text{air}} = 300-1100 \text{ J.mol}^{-1} \\ T_{\text{air}} = 27-29^\circ\text{C} \\ C_i^L = 0.05 \times 10^{-3} \text{ kg.m}^{-3} \end{array} \right.$
at $0 \mu\text{E.m}^{-2}.\text{s}^{-1}$, $r_{s(H_2O)} = 1316 \text{ m.s}^{-1}$	
Leaf 10: at $231 \mu\text{E.m}^{-2}.\text{s}^{-1}$, $r_{s(H_2O)} = 1094 \text{ m.s}^{-1}$	$\left\{ \begin{array}{l} -\psi_{\text{air}} = 500-1100 \text{ J.mol}^{-1} \\ T_{\text{air}} = 27-29^\circ\text{C} \\ C_i^L = 0.06 \times 10^{-3} \text{ kg.m}^{-3} \end{array} \right.$
at $0 \mu\text{E.m}^{-2}.\text{s}^{-1}$, $r_{s(H_2O)} = 1005 \text{ m.s}^{-1}$	

FIGURES 3.5, 3.6 & 3.7 The relationship between I and $r_s(H_2O)$ at normal ambient CO_2 concentrations and in CO_2 -free air.



Q110576

x - in normal CO_2 concⁿs.

$$-\psi_{air} = 2321 - 2921$$

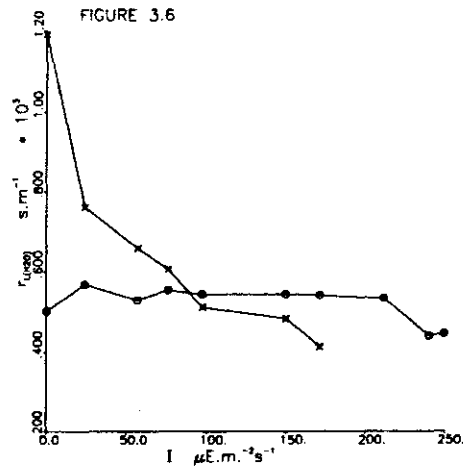
$$T_L = 22.3 - 22.7$$

Q080676 (same leaf as above)

o - in CO_2 -free air

$$-\psi_{air} = 2300 - 2900$$

$$T_L = 31.0 - 32.0$$



Q230676

x - in normal CO_2 concⁿ.

$$-\psi_{air} = 2500 - 3300$$

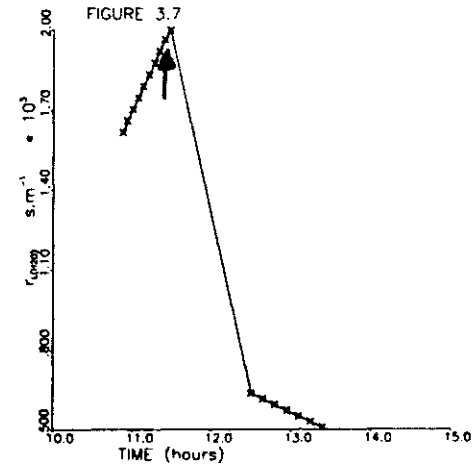
$$T_L = 28.8 - 30.2$$

Q240676 (same leaf as above)

o - in CO_2 -free air

$$-\psi_{air} = 2300 - 2500$$

$$T_L = 29.5 - 30.5$$



A111076

$$T_L = 23.0 - 24.0$$

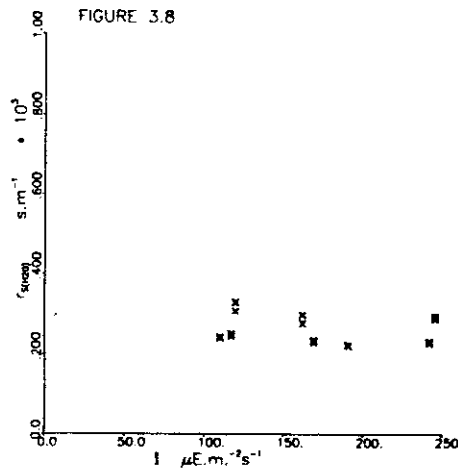
$$I = 0.0$$

$$-\psi_{air} = 1470 \text{ (closed stomata)}$$

$$= 95 \text{ (open stomata)}$$

↑ indicates when CO_2 -free air was supplied

FIGURES 3.8, 3.9 & 3.10 The relationship between I and $r_{s(H_2O)}$ at constant C_i .

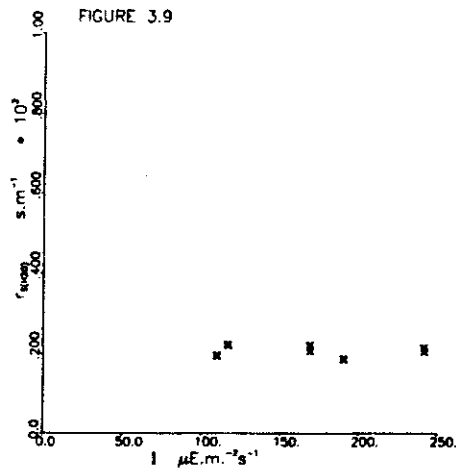


Q250576-Q260576 (leaf 0)

$$C_i = 0.39 - 0.45$$

$$-\psi_{air} = 1330 - 1850$$

$$T_L = 21.4 - 25.4$$

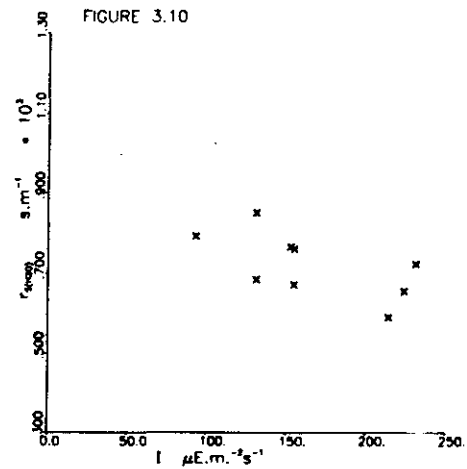


Q250576-Q260576 (leaf 1)

$$C_i = 0.41 - 0.49$$

$$-\psi_{air} = 800 - 960$$

$$T_L = 23.2 - 23.4$$



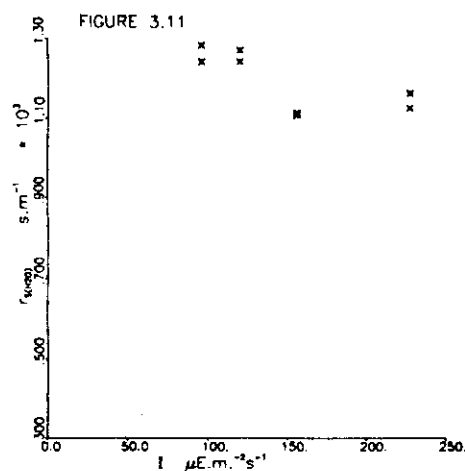
Q060776-Q090776

$$C_i = 0.17 - 0.23$$

$$-\psi_{air} = 1460 - 1860$$

$$T_L = 30.0 - 36.0$$

FIGURES 3.11, 3.12 & 3.13 The relationship between I and $r_{s(H_2O)}$ at constant C_i .

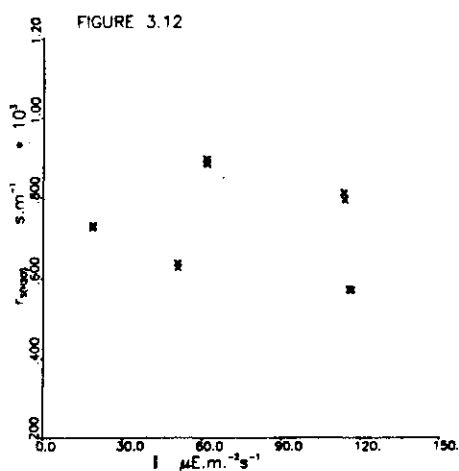


Q180576-Q190576

$$C_i = 0.46 - 0.54$$

$$-\psi_{air} = 1820 - 2900$$

$$T_L = 22.3 - 24.3$$

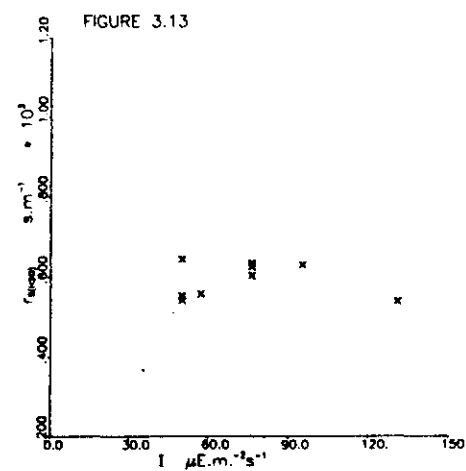


F010977-F090977

$$C_i = 0.21 - 0.27$$

$$-\psi_{air} = 1704 - 1904$$

$$T_L = 29.4 - 31.2$$



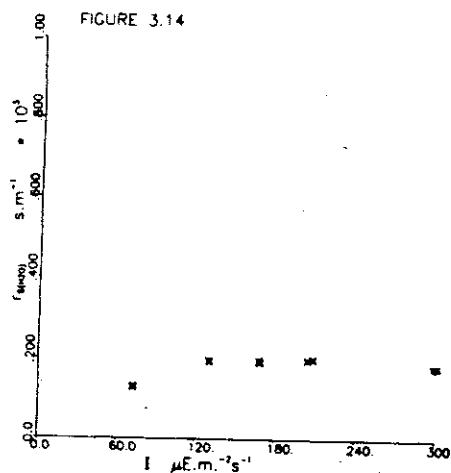
A071076-A191076

$$C_i = 0.38 - 0.46$$

$$-\psi_{air} = 50 - 150$$

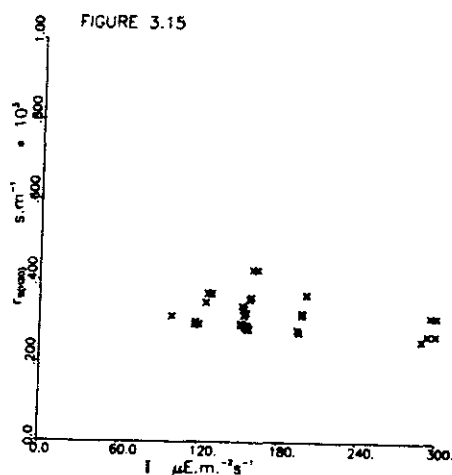
$$T_L = 22.0 - 26.0$$

FIGURES 3.14, 3.15 & 3.16 The relationship between I and $r_s(\text{H}_2\text{O})$ at constant C_i .



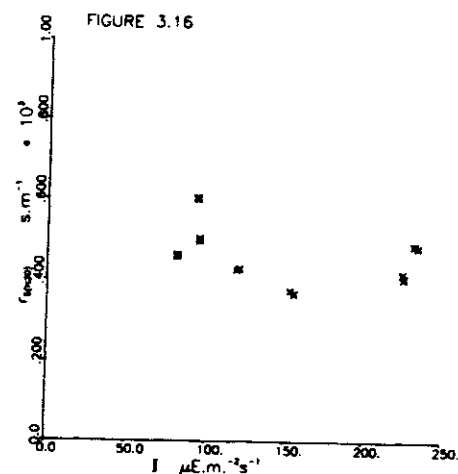
P100277-P180277

$C_i = 0.30 - 0.40$
 $-\psi_{\text{air}} = 760 - 1100$
 $T_L = 26.1 - 27.1$



N270178-N020278

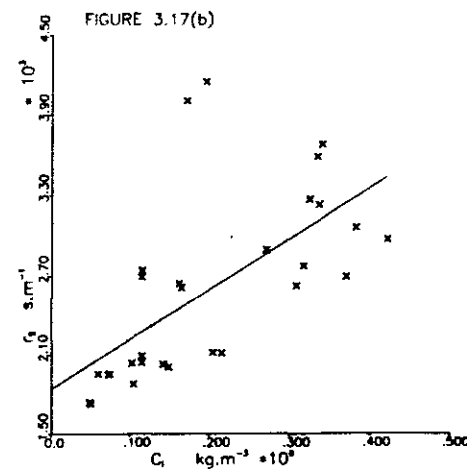
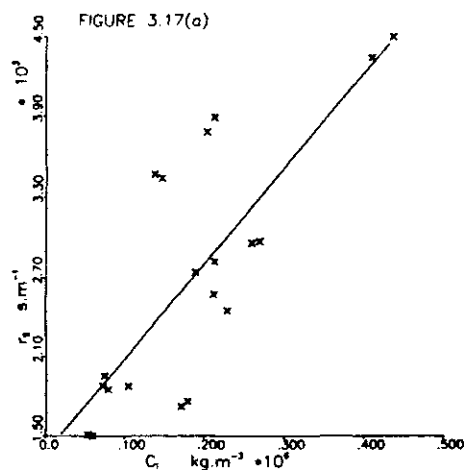
$C_i = 0.32 - 0.38$
 $-\psi_{\text{air}} = 1560 - 1716$
 $T_L = 26.4 - 30.4$



N110278-N150278

$C_i = 0.38 - 0.42$
 $-\psi_{\text{air}} = 1890 - 2470$
 $T_L = 26.6 - 28.6$

FIGURE 3.17 The response of r_s to C_i : a) at constant I b) over a wide range of values of I .



Results from Quercus Experiment 1.

a) I constant

$$-\psi_{\text{air}} = 550 - 950$$

$$T_L = 30.5 - 33.5$$

$$I = 181 - 219$$

Regression coefficients:

$$Y = a + bX$$

$$a = 1373.1$$

$$b = 71.53 * 10^8$$

correlation coefficient:

$$r = 0.7867$$

b) I variable

$$-\psi_{\text{air}} = 730 - 1030$$

$$T_L = 29.0 - 33.0$$

$$I = 0 - 200$$

Regression coefficients:

$$Y = a + bX$$

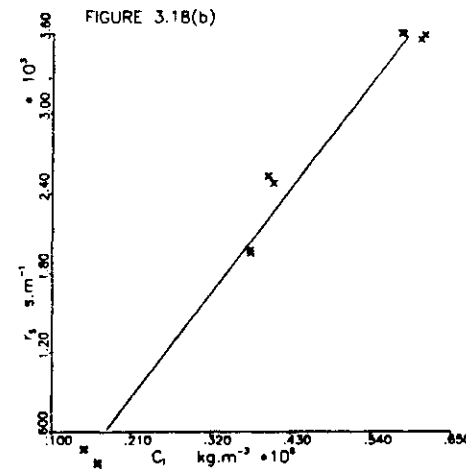
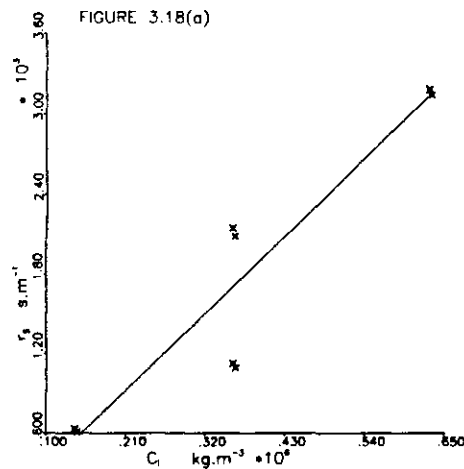
$$a = 1840.9$$

$$b = 37.83 * 10^8$$

correlation coefficient:

$$r = 0.6333$$

FIGURE 3.18 The response of r_s to C_i : a) at constant I b) over a wide range of values of I .



Results from Quercus Experiment 2.

a) I constant

$$-\psi_{\text{air}} = 172 - 572$$

$$T_L = 29.6 - 35.6$$

$$I = 246 - 278$$

Regression coefficients:

$$Y = a + bX$$

$$a = -188.0$$

$$b = 53.0 \cdot 10^8$$

correlation coefficient:

$$r = 0.9463$$

b) I variable

$$-\psi_{\text{air}} = 90 - 490$$

$$T_L = 28.1 - 34.1$$

$$I = 0 - 250$$

Regression coefficients:

$$Y = a + bX$$

$$a = -651.1$$

$$b = 71.8 \cdot 10^8$$

correlation coefficient:

$$r = 0.9923$$

Prevention of stomatal closure in the dark by CO_2 -free air has been reported many times (eg Heath 1950; Scarth and Shaw, 1951) and the above results show that this can occur in oak, sycamore and tobacco. Clearly, there is a CO_2 dependent response which is independent of light.

An examination of the relationship between $r_{s(\text{H}_2\text{O})}$ and I , at constant C_i was possible by pooling data for the same leaf and using the method outlined earlier. Some results are shown in figures 3.8 to 3.16 for all five species used in this study. In some cases there is an apparent decrease in $r_{s(\text{H}_2\text{O})}$ at the higher values of I , but this may have been because values of $-\psi_{\text{air}}$ and C_i were within the range stated, but both tended to be lower at the high I values. These results provide no evidence of an independent effect of I , although possible effects at very low levels of I could not be investigated by this method because of high levels of C_i . Figure 3.17 is drawn from results of *Quercus* experiment 1 (see table 2G) in which a wide range of C_i was obtained by varying ambient CO_2 . The response of r_s to C_i is shown; (a) with I constant, and (b) with I variable. When linear regressions of r_s and I on C_i were calculated for the two graphs, the slopes of the lines were not significantly different. A similar result was obtained with data from *Quercus* experiment 2 (figure 3.18). Again an independent effect of I could not be detected.

The results so far have shown that there is an effect of C_i on r_s , but have not shown that I has a detectable direct effect in white light. Further consideration is given in section 3.2/2.

3.2/2 Water potential of leaf chamber air, $-\psi_{\text{air}}$

Until recently it was widely assumed that ambient humidity affected $r_{s(\text{H}_2\text{O})}$ via a negative feedback system involving the bulk

FIGURES 3.19 & 3.20 Changes in $r_{s(H_2O)}$ and E_T in response to changes in $-\psi_{air}$.

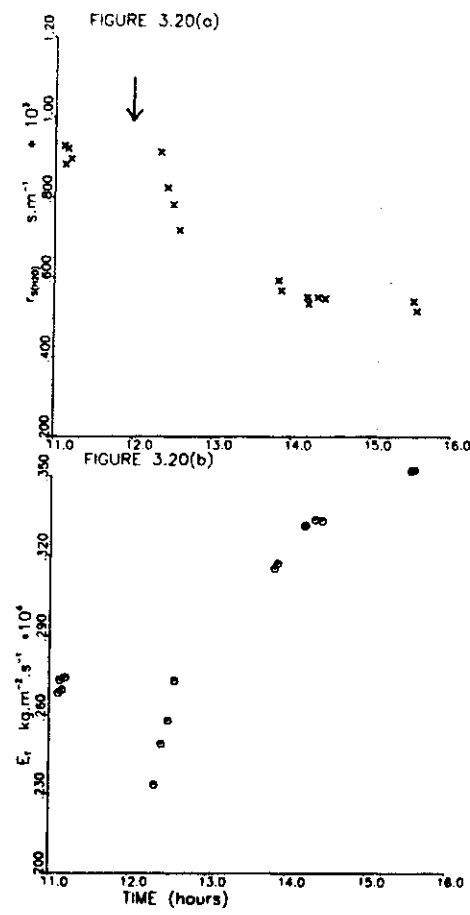
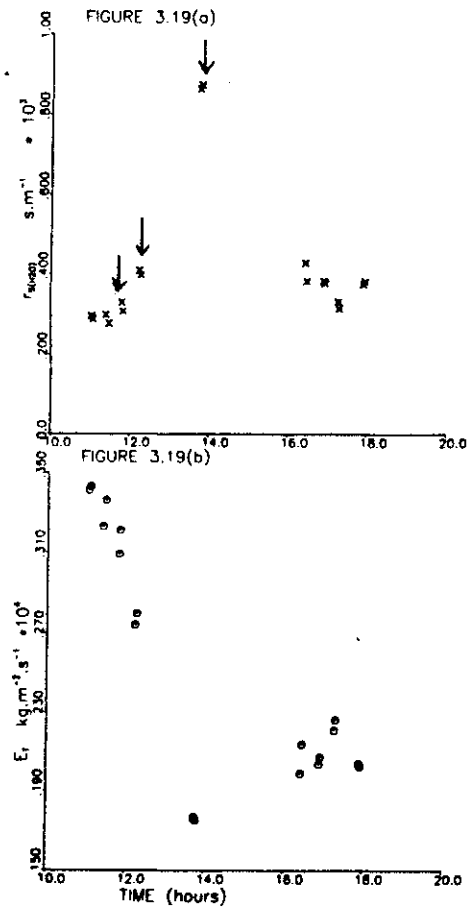
Arrows ($\downarrow$) indicate when changes in $-\psi_{air}$ occurred.

FIGURE 3.19: Q180576

$C_i = 0.45 - 0.55$
 $T_L = 25.0 - 26.0$
 $I = 154$
 Initially, $-\psi_{air} = 1280$
 1st. change " = 1536
 2nd. change " = 2400
 3rd. change " = 860

FIGURE 3.20: Q040777

$C_i = 0.33 - 0.39$
 $T_L = 34.7 - 36.3$
 $I = 232$
 Initially, $-\psi_{air} = 1047$
 1st. change " = 107



FIGURES 3.21 & 3.22 Changes in $r_s(\text{H}_2\text{O})$ and E_T in response to changes in $-\psi_{\text{air}}$.

Arrows ($\downarrow$) indicate when changes in $-\psi_{\text{air}}$ occurred.

FIGURE 3.21: N160278

$$C_i = 0.36 - 0.40$$

$$T_L = 28.4 - 28.8$$

$$I = 100$$

$$\text{Initially, } -\psi_{\text{air}} = 1250$$

$$\text{1st. change " } = 654$$

$$\text{2nd. change " } = 1665$$

FIGURE 3.22: P110277

$$C_i = 0.22 - 0.42$$

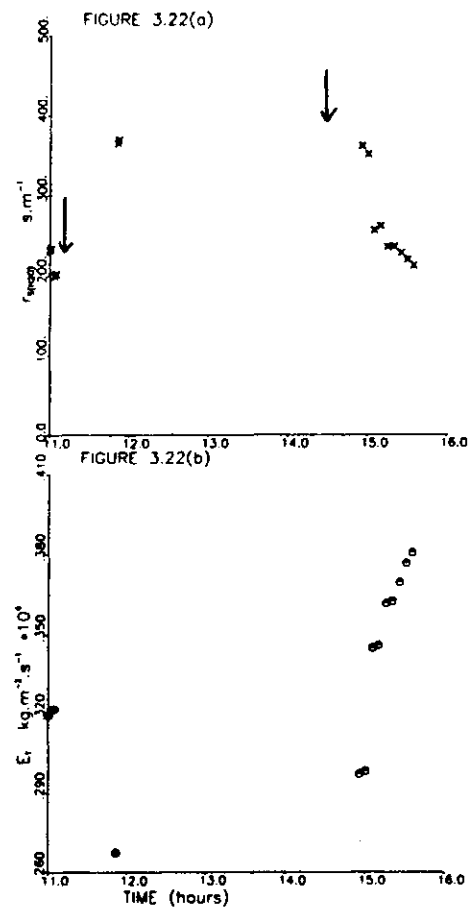
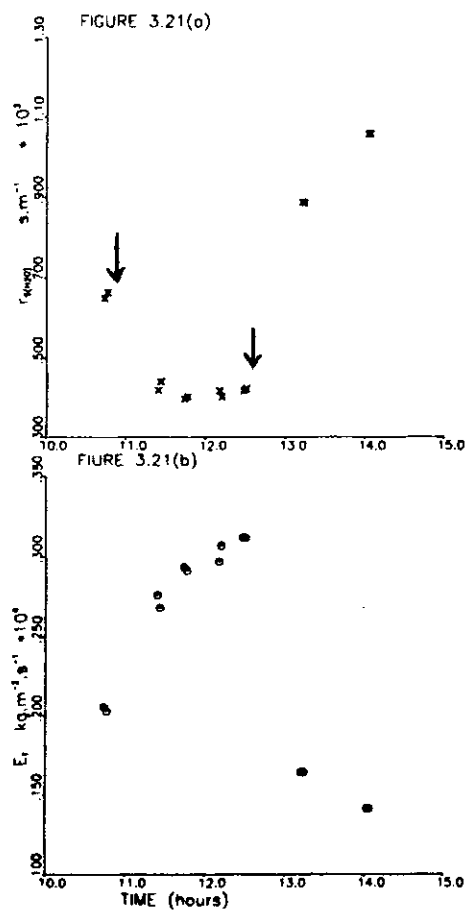
$$T_L = 25.5 - 26.5$$

$$I = 298$$

$$\text{Initially, } -\psi_{\text{air}} = 1074$$

$$\text{1st. change " } = 1246$$

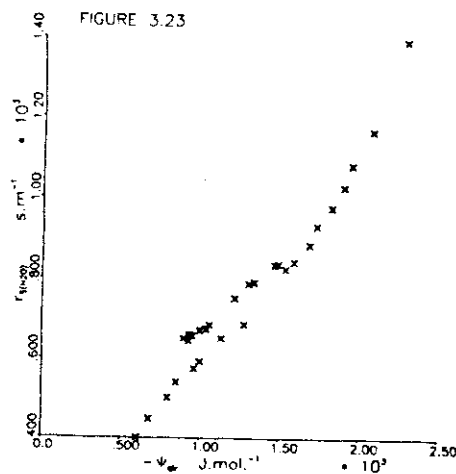
$$\text{2nd. change " } = 1168$$



leaf water status as follows: E_T increases with $-\psi_{air}$ until a critical level of water stress is reached in the leaf whereupon stomatal closure is rapid and E_T is reduced (Hsiao, 1973). The evidence for an additional control mechanism involving a direct effect of $-\psi_{air}$ on stomata was outlined in section 1.4. Clearly, a reduction in E_T via such a negative feedback system will always involve a decrease in leaf water potential, whereas a direct effect of $-\psi_{air}$ could result in a relative increase in leaf water potential (as in Schulze *et al.*, 1972). Unfortunately, non-destructive estimates of leaf water status *in situ* are difficult to make in leaf chamber studies of this type and were not attempted in this work. However, the results do show considerable evidence of the existence of such a direct response to $-\psi_{air}$. In all cases, the plants were kept well supplied with water and it was assumed that acute levels of stress in the leaf are unlikely to have developed.

Strong evidence for a direct effect of $-\psi_{air}$ was provided by the fact that stomatal movements brought about by changes in ambient humidity were readily reversible. Figures 3.19 and 3.20 show how $r_{s(H_2O)}$ varied according to $-\psi_{air}$ which was varied by adjustment of the chamber inlet humidity and the flow rate. As $-\psi_{air}$ was increased, $r_{s(H_2O)}$ also increased; and when $-\psi_{air}$ was decreased again, $r_{s(H_2O)}$ also decreased within a short time. The corresponding effects on E_T confirmed that the response was stomatal, since E_T decreased with increase in evaporative demand and *vica versa*. Similar responses are shown for tobacco and french bean in figures 3.21 and 3.22 respectively. Whilst the negative feedback theory can account for stomatal closure in response to increase in E_T , the rapid decrease in $r_{s(H_2O)}$ when $-\psi_{air}$ was lowered, shown in figures 3.19 to 3.22, cannot be explained as a reversal of such a mechanism. In figures 3.19 to 3.22 a sudden decrease in $-\psi_{air}$ caused a rapid increase in E_T which must have been

FIGURES 3.23, 3.24 & 3.25 The relationship between $r_{s(H_2O)}$ and $-\psi_{air}$ at constant C_i and constant I .

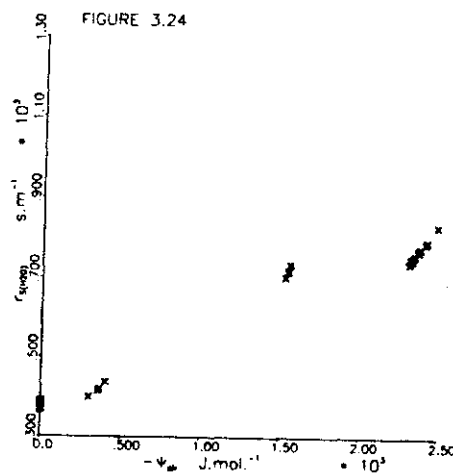


Q250777-Q260777

$$C_i = 0.39 - 0.45$$

$$T_L = 29.0 - 31.0$$

$$I = 206 - 218$$

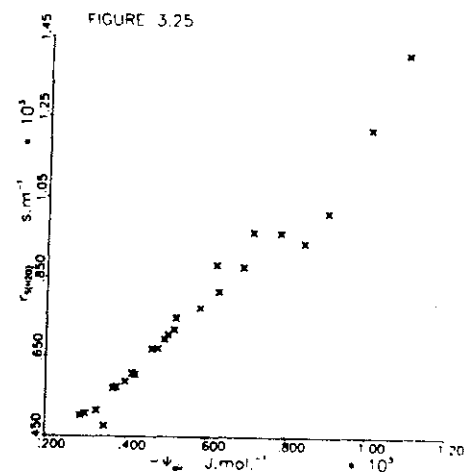


Q300677-Q080777

$$C_i = 0.39 - 0.43$$

$$T_L = 30.0 - 31.6$$

$$I = 218 - 244$$



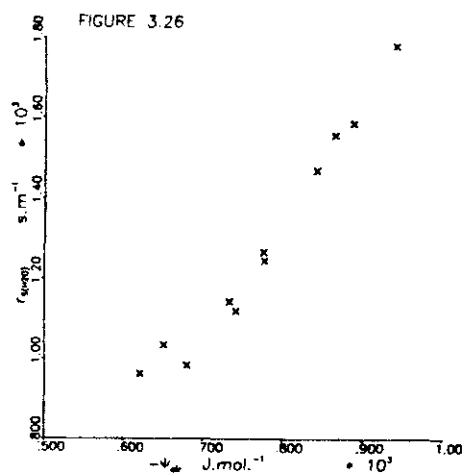
Q050877

$$C_i = 0.33 - 0.43$$

$$T_L = 26.6 - 30.5$$

$$I = 188 - 200$$

FIGURES 3.26, 3.27 & 3.28 The relationship between $r_s(\text{H}_2\text{O})$ and $-\psi_{\text{air}}$ at constant C_i and constant I .

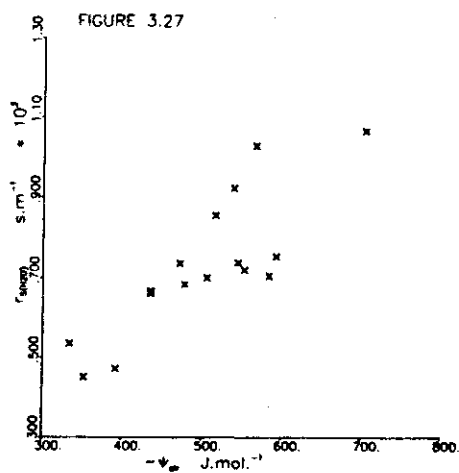


Q030877

$$C_i = 0.44 - 0.52$$

$$T_L = 27.9 - 29.9$$

$$I = 108 - 124$$

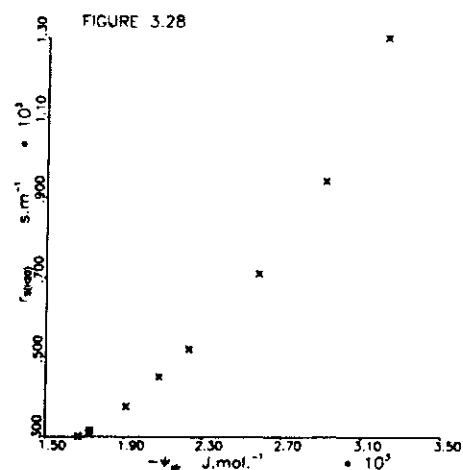


F030877

$$C_i = 0.47 - 0.53$$

$$T_L = 30.9 - 34.9$$

$$I = 55 - 61$$



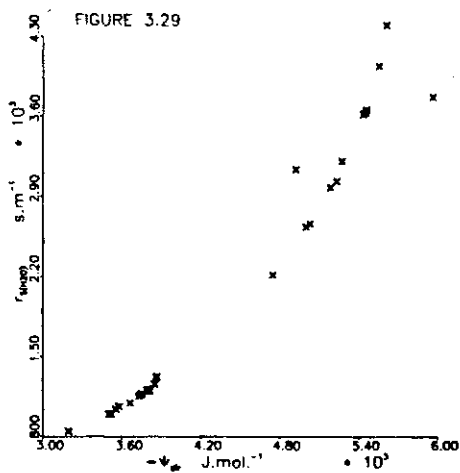
P170277

$$C_i = 0.34 - 0.38$$

$$T_L = 23.3 - 29.1$$

$$I = 241$$

FIGURES 3.29 & 3.30 The relationship between $r_{s(H_2O)}$ and $-\psi_{air}$ at constant C_i and constant I .

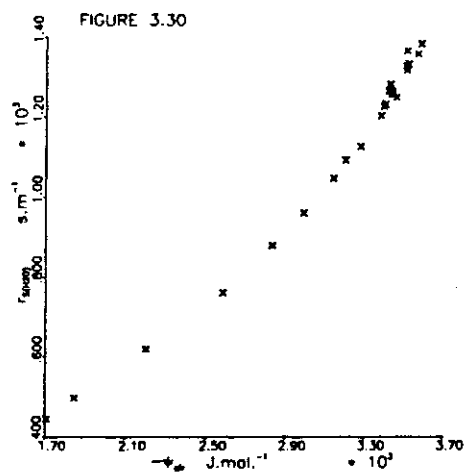


NO20278

$$C_i = 0.44 - 0.50$$

$$T_L = 30.0 - 32.0$$

$$I = 30 - 32$$



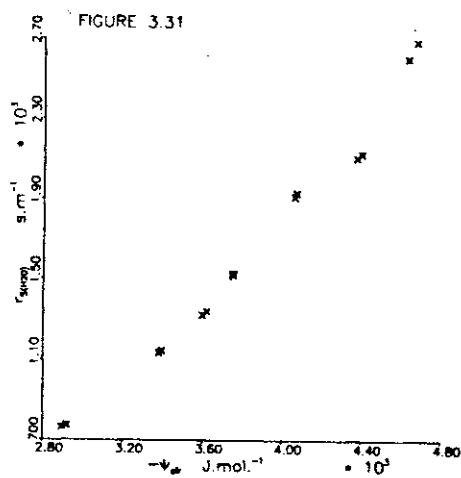
N140278

$$C_i = 0.60 - 0.66$$

$$T_L = 27.5 - 28.5$$

$$I = 27 - 29$$

FIGURES 3.31, 3.32 & 3.33 The relationship between $r_{s(H_2O)}$ and $-\psi_{air}$ at constant C_i , but over a wide range of values of I .

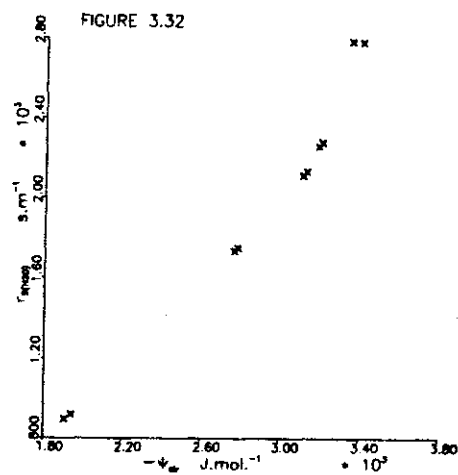


Q160676

$$C_i = 0.22 - 0.28$$

$$T_L = 28.1 - 28.9$$

$$I = 48 - 238$$

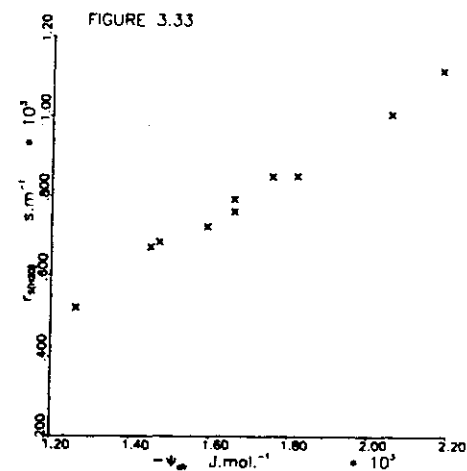


Q270478

$$C_i = 0.14 - 0.24$$

$$T_L = 29.6 - 33.6$$

$$I = 116 - 343$$



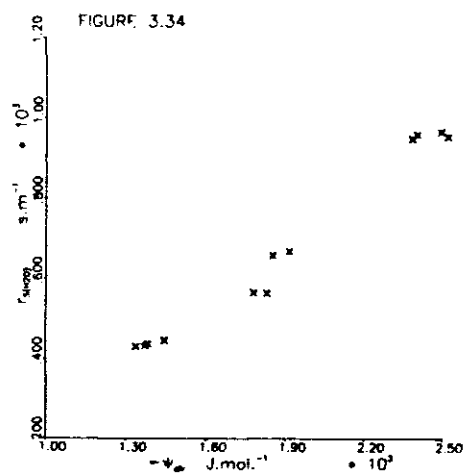
Q060777-Q070777

$$C_i = 0.17 - 0.23$$

$$T_L = 34.1 - 35.3$$

$$I = 86 - 230$$

FIGURES 3.34, 3.35 & 3.36 The relationship between $r_{s(H_2O)}$ and $-\psi_{air}$ at constant C_i , but over a wide range of values of I .

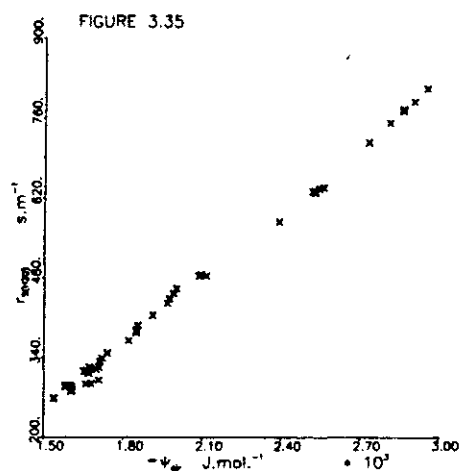


F180877-F190877

$C_i = 0.20 - 0.26$

$T_L = 29.5 - 30.5$

$I = 21 - 114$

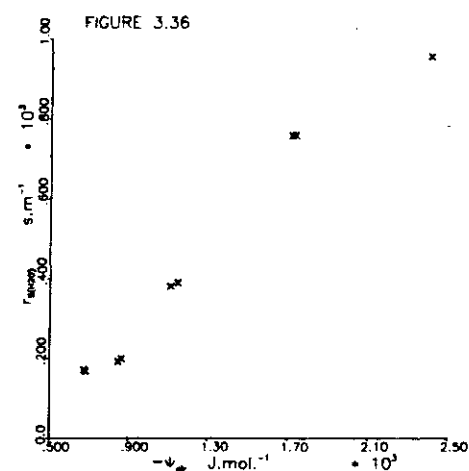


N310178-N010278

$C_i = 0.34 - 0.36$

$T_L = 27.5 - 29.5$

$I = 55 - 295$



P100277-P170277

$C_i = 0.48 - 0.54$

$T_L = 26.4 - 27.6$

$I = 39 - 291$

caused by stomatal opening.

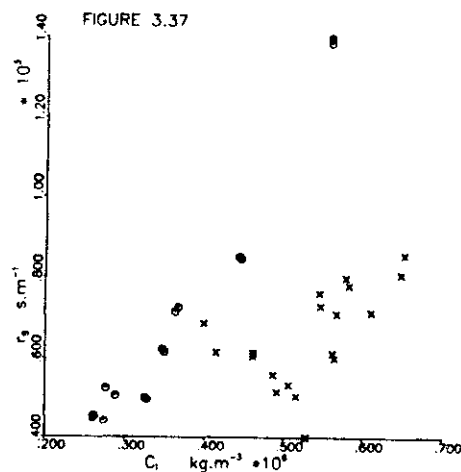
The nature of the $-\psi_{\text{air}}$ response at constant C_i , I and T_L is shown in figures 3.23 to 3.30 for oak, ash, tobacco and french bean. If the stomatal response to $-\psi_{\text{air}}$ was operating via the bulk leaf water status one would expect, on increasing $-\psi_{\text{air}}$, that there would be a wide range of $-\psi_{\text{air}}$ over which $r_{s(\text{H}_2\text{O})}$ would not change very much (E_T would increase), then there would be a sudden rapid rise in $r_{s(\text{H}_2\text{O})}$ corresponding to the "switch-like" closure of stomata when the threshold value of leaf water potential was reached. However, figures 3.23 to 3.30 show that a progressive increase in $-\psi_{\text{air}}$ always resulted in a progressive increase in $r_{s(\text{H}_2\text{O})}$ with no evidence of sudden stomatal closure.

Figures 3.23 to 3.30 showed the relationship between $r_{s(\text{H}_2\text{O})}$ and $-\psi_{\text{air}}$ at constant C_i and constant I . However, even when values of I were not constant, there was still a linear relationship between $r_{s(\text{H}_2\text{O})}$ and $-\psi_{\text{air}}$ with little scatter (figures 3.31 to 3.36). These data provided further evidence that the response of $r_{s(\text{H}_2\text{O})}$ to white light was principally a direct response to C_i . Thus, in the formulation of the hypothesis, the response to I was described by a C_i response only.

3.2/3 Interaction of C_i and $-\psi_{\text{air}}$ responses.

The previous two sections have shown the existence of two independent mechanisms which in many ways are antagonistic. For the purposes of constructing the hypothesis, the nature of the interaction between the $-\psi_{\text{air}}$ and C_i responses needed to be investigated. The response of r_s to C_i at different values of $-\psi_{\text{air}}$ for the same leaf is shown in figures 3.37 to 3.43 for all five species. At a particular level of $-\psi_{\text{air}}$ there was a linear relationship between r_s and C_i , but

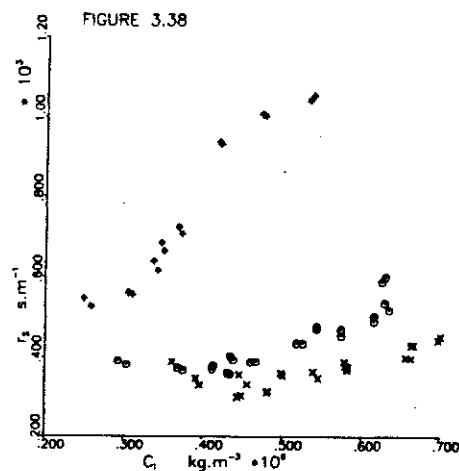
FIGURES 3.37, 3.38 & 3.39 The relationship between r_s and C_i at different values of $-\psi_{air}$.



Q110576-Q190576

x $-\psi_{air} = 710 - 970$
 $T_L = 22.2 - 26.2$

o $-\psi_{air} = 2026 - 2526$
 $T_L = 22.7 - 24.7$

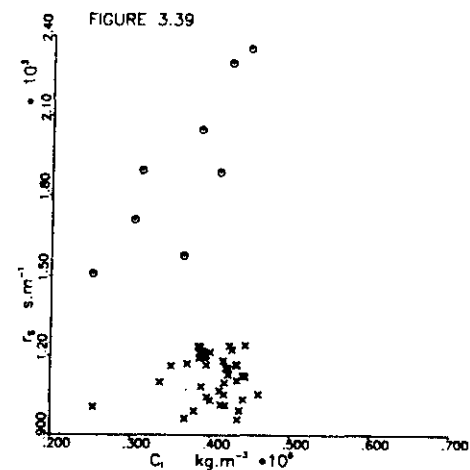


Q250576-Q110676

x $-\psi_{air} = 826 - 1002$
 $T_L = 22.2 - 24.2$

o $-\psi_{air} = 1770 - 2090$
 $T_L = 21.0 - 23.0$

↑ $-\psi_{air} = 1925 - 2485$
 $T_L = 27.4 - 28.6$

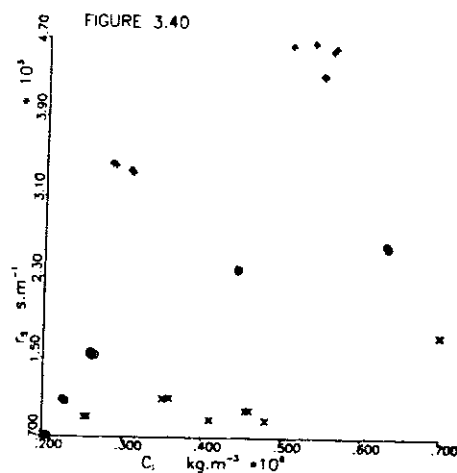


Q300676-Q280776

x $-\psi_{air} = 490 - 890$
 $T_L = 30.9 - 33.9$
 $I = 161 - 171$

o $-\psi_{air} = 1027 - 1227$
 $T_L = 30.0 - 33.0$
 $I = 180 - 230$

FIGURES 3.40 & 3.41 The relationship between r_s and C_i at different values of $-\psi_{\text{air}}$.

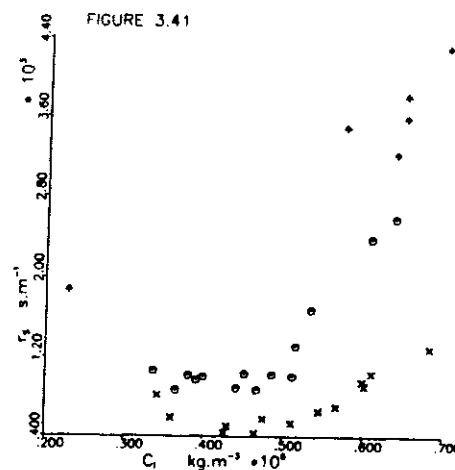


F110877-F190877

x $-\psi_{\text{air}} = 1100 - 1700$
 $T_L = 29.7 - 31.7$

o $-\psi_{\text{air}} = 2000 - 2400$
 $T_L = 30.5 - 32.5$

↑ $-\psi_{\text{air}} = 2700 - 3300$
 $T_L = 31.0 - 33.0$



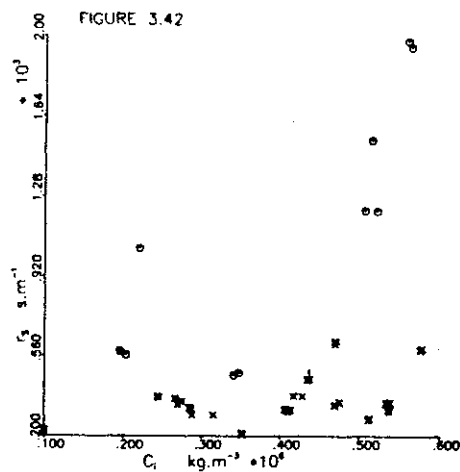
A071076-A191076

x $-\psi_{\text{air}} = 45 - 55$
 $T_L = 21.3 - 25.3$

o $-\psi_{\text{air}} = 260 - 380$
 $T_L = 22.7 - 24.1$

↑ $-\psi_{\text{air}} = 1290 - 1690$
 $T_L = 22.1 - 23.9$

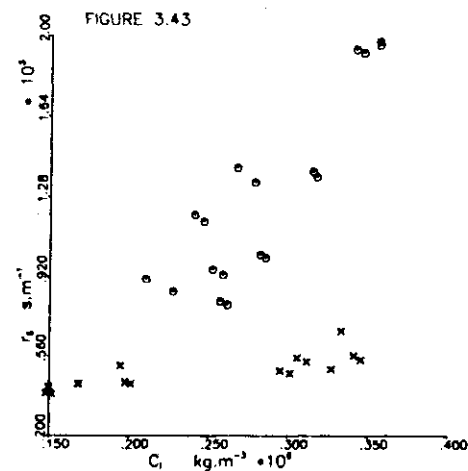
FIGURES 3.42 & 3.43 The relationship between r_s and C_i at different values of $-\psi_{air}$.



P100277-P180277

x $-\psi_{air} = 780 - 1180$
 $T_L = 26.0 - 28.0$

o $-\psi_{air} = 1534 - 2134$
 $T_L = 26.4 - 28.4$



N280278-N020378

x $-\psi_{air} = 350 - 550$
 $T_L = 27.9 - 28.9$

o $-\psi_{air} = 1420 - 1920$
 $T_L = 28.4 - 30.4$

the slope of this relationship was a function of $-\psi_{\text{air}}$: as the value of $-\psi_{\text{air}}$ increased, the slope also increased. This was the basic relationship used in the construction of the hypothesis (section 3.3).

3.2/4 Leaf temperature (T_L)

Studies of stomatal response to temperature have often yielded contradictory results as evidenced by the fact that four different responses have been reported by various authors (see Sheriff, 1979). This is no doubt indicative of the indirect role of temperature in the principal processes known to affect stomata which have already been discussed. Net rates of photosynthesis are maximum at an optimum T_L which varies according to species and growing conditions (eg Treharne and Nelson, 1975). Below the optimum, an increase in T_L , would lower C_i thereby tending to increase stomatal aperture via the C_i feedback loop. However, as T_L increased above the optimum, C_i would increase and may cause the stomata to close. Thus T_L has a major indirect role in determining stomatal response. In the present study, systematic assessment of a direct response to T_L was not possible for the following reasons :

1. To examine possible direct effects at high temperature it is necessary to have high levels of chamber inlet humidity (otherwise the $-\psi_{\text{air}}$ response would close the stomata). However, this carries an inherent risk of condensation occurring in other parts of the gas flow circuit.
2. To examine possible direct effects at low temperatures it is necessary to have cold water circulating through the heat exchanger in the leaf chamber in order to offset the heating effect of the lamps. This produces a risk of water vapour in the chambers (as a result of transpiration) condensing on the heat exchanger.

3. It was found to be very difficult with the present system to maintain a constant $-\psi_{\text{air}}$ whilst varying T_L because of the close coupling between the two.

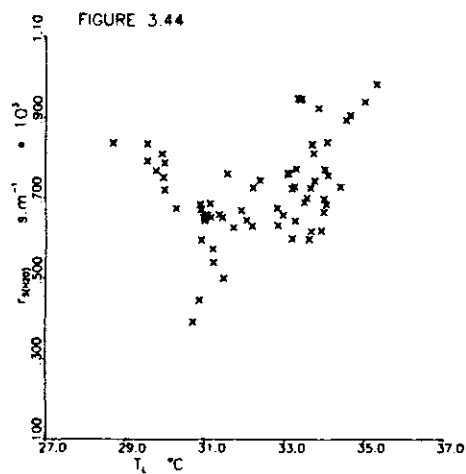
Although it was not possible to assess the effects of T_L systematically over a wide range, the data did provide some indications of such effects, independent of $-\psi_{\text{air}}$ and C_i . The response to T_L in the range 27° to 37°C with $-\psi_{\text{air}}$ kept constant as far as possible, is shown for oak in figures 3.44 to 3.46. In all cases there was some evidence of an optimum T_L for stomatal opening between 30 and 32°C . A similar response is shown for ash in figures 3.47 and 3.48, with an apparent minimum $r_{s(\text{H}_2\text{O})}$ in the range 29 to 31°C . However, the apparent optimum value of T_L may not have been a true reflection of a direct response for the following reasons :

1. Although the values plotted are at approximately equivalent levels of C_i and $-\psi_{\text{air}}$, below 30°C the values of C_i tended to be slightly higher than above this temperature, and the higher values of $r_{s(\text{H}_2\text{O})}$ below 30°C may have had a component due to the C_i response.
2. Similarly, the values of $-\psi_{\text{air}}$ above the apparent optimum temperature tended to be higher and the increase in $r_{s(\text{H}_2\text{O})}$ above 30°C may have had a component due to increasing $-\psi_{\text{air}}$.

The minimum values of $r_{s(\text{H}_2\text{O})}$ for oak and ash may thus have been caused by an optimum combination of the antagonistic C_i and $-\psi_{\text{air}}$ responses. Figure 3.49 shows $r_{s(\text{H}_2\text{O})}$ plotted against T_L for a sycamore leaf, but there was no evidence of an optimum T_L . For leaves of tobacco, a range of graphs were produced which indicated either a slight increase in $r_{s(\text{H}_2\text{O})}$ with T_L (figures 3.50 and 3.51) or no response to T_L (figure 3.52).

In this study, little variation in leaf chamber air temperature was possible and consequently T_L was confined to a fairly narrow range.

FIGURES 3.44, 3.45 & 3.46 The stomatal response to leaf temperature.

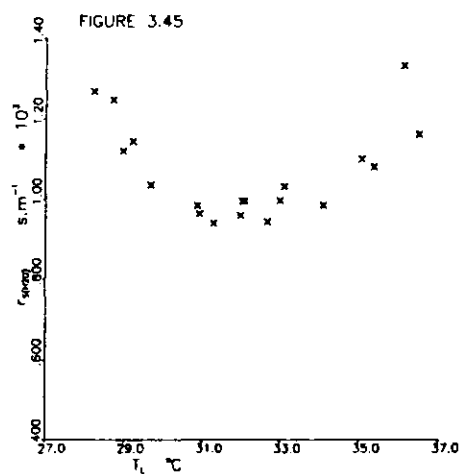


Q250777-Q280777

$$C_i = 0.37 - 0.43$$

$$-\psi_{\text{air}} = 662 - 1122$$

$$I = 185 - 215$$

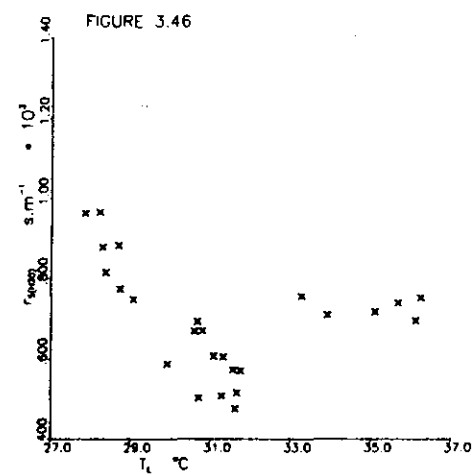


Q020877-Q040877

$$C_i = 0.47 - 0.51$$

$$-\psi_{\text{air}} = 660 - 900$$

$$I = 91 - 109$$



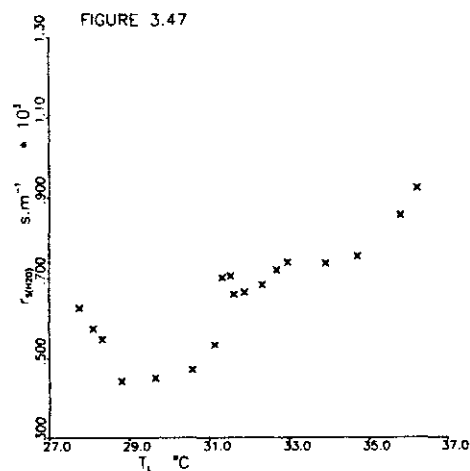
Q040877-Q050877

$$C_i = 0.32 - 0.42$$

$$-\psi_{\text{air}} = 370 - 690$$

$$I = 191 - 199$$

FIGURES 3.47, 3.48 & 3.49 The stomatal response to leaf temperature.

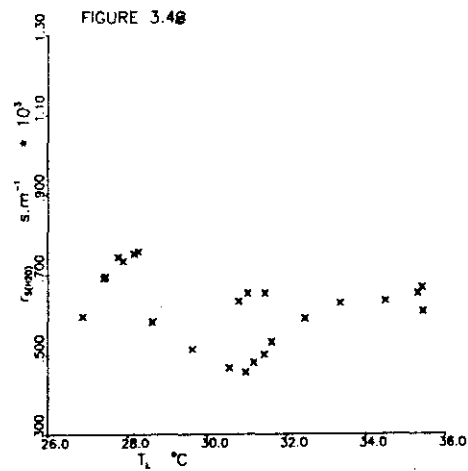


F030877-F040877

$C_i = 0.49 - 0.51$

$-\psi_{air} = 400 - 490$

$I = 55 - 65$

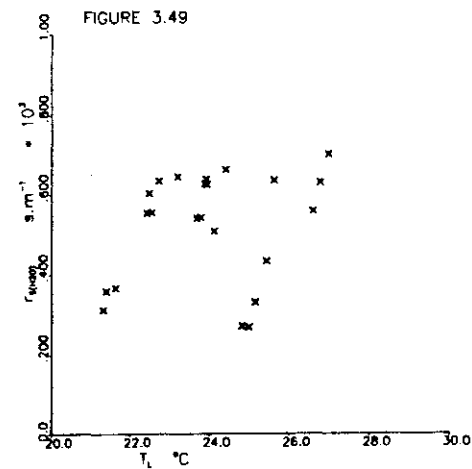


F040877-F050877

$C_i = 0.34 - 0.42$

$-\psi_{air} = 385 - 745$

$I = 108 - 112$



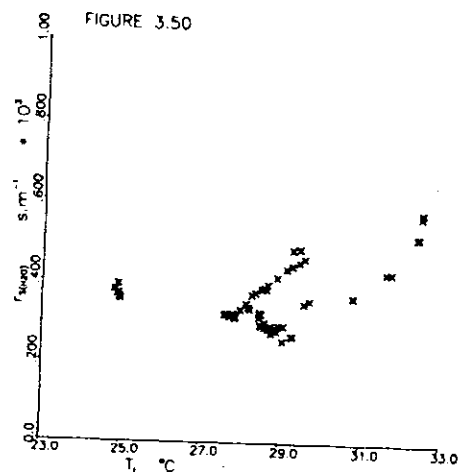
A071077-A191077

$C_i = 0.36 - 0.50$

$-\psi_{air} = 0 - 94$

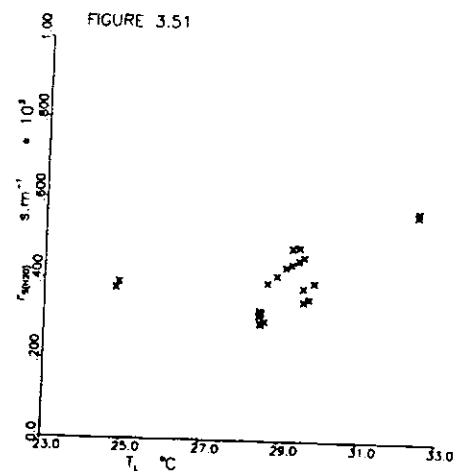
$I = 9 - 131$

FIGURES 3.50, 3.51 & 3.52 The stomatal response to leaf temperature.



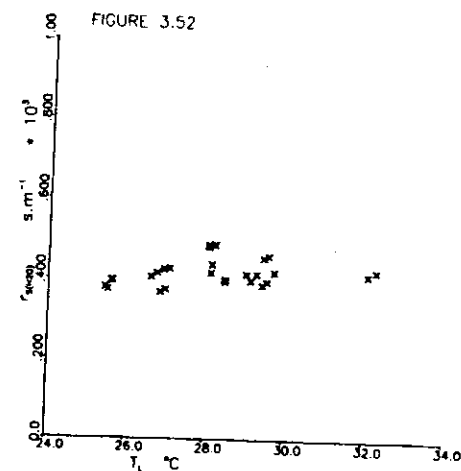
N270178-N020278

$C_i = 0.33 - 0.39$
 $-\psi_{air} = 1580 - 1920$
 $I = 95 - 205$



N270178-N020278

$C_i = 0.33 - 0.39$
 $-\psi_{air} = 1772 - 2062$
 $I = 100 - 116$



N110278-N160278

$C_i = 0.34 - 0.44$
 $-\psi_{air} = 1300 - 2200$
 $I = 100 - 200$

There was insufficient data to perform a full statistical analysis in order to show whether the optimum temperature was a real effect. Within the range investigated, T_L had no consistent direct effect on $r_{s(H_2O)}$ and thus no direct T_L response was incorporated in the hypothesis.

3.3 Hypotheses of stomatal behaviour

In section 3.2 it was shown that the relationship between r_s and C_i could be described by a straight line, the slope of which varied directly with $-\psi_{air}$. Four simple regressions were examined in order to determine which was the most appropriate for describing r_s as a function of C_i and $-\psi_{air}$.

REGRESSION 1 : A simple linear regression with r_s as a function of C_i , independent of $-\psi_{air}$:

$$r_s = a_1 + b_1 X \quad 3.1$$

where $X = C_i$

The remainder were multiple regressions with either 2 or 3 independent variables.

REGRESSION 2 :

$$r_s = a_2 + b_2 X_1 + c_2 X_2 \quad 3.2$$

where $X_1 = (C_i)(-\psi_{air})$

$$X_2 = -\psi_{air}$$

REGRESSION 3 :

$$r_s = a_3 + b_3 X_1 + c_3 X_2 \quad 3.3$$

where $X_1 = C_i$

$$X_2 = -\psi_{air}$$

REGRESSION 4 :

$$r_s = a_4 + b_4 X_1 + c_4 X_2 + d_4 X_3 \quad 3.4$$

where $X_1 = (C_i)(-\psi_{air})$

$$X_2 = -\psi_{air}$$

$$X_3 = C_i$$

The majority of the data, comprising 113 individual data sets, were fitted to the above four equations and an analysis of variance was

performed for each data set for each hypothesis; a sample set of data is given in table 3D, in order to illustrate the format of the analysis of variance. The variance ratios, given as the last line of each analysis, were calculated in order to compare the four equations. In all cases the null hypothesis was made that they were of equal precision and the significance of the ratio of their variances was judged by comparison with the theoretical F distribution. For example; analysis of variance: regression 1 vs regression 2 (Q11067670, 20points):

<u>Variance due to</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Regression 1	1	999888.00	999888.00	
Component due to $-\psi_{\text{air}}$ term in regression 2	1	56502.22	56502.22	241.11
<u>residual</u>	<u>17</u>	<u>3983.78</u>	234.34	
Total variation in r_s	19	1060370.00		

Calculated from: residual SS from regression 1 fit = 60486.00
 residual SS from regression 2 fit = 3983.78 } see table 3D

Therefore component due to $-\psi_{\text{air}}$ term in regression 2 = 60486.00 - 3983.78 = 56502.22 . The value of F in this case was highly significant ($p < 0.001$) hence the improvement in fit due to using regression 2 as opposed to regression 1 was unlikely to be due to chance.

Four comparisons were made using the F test as follows (see table 3D) :

1. The improvement due to using equation 3.2 compared to equation 3.1
2. The improvement due to using equation 3.3 compared to equation 3.1
3. The improvement due to using equation 3.4 compared to equation 3.2
4. The improvement due to using equation 3.4 compared to equation 3.3

TABLE 3D Sample data set to show the format of the analyses of variance for the four regression comparison. The complete analyses are given in appendix 3.

Q1106760

$$r_s \quad (-\Psi_{\text{air}})(C_i) \quad -\Psi_{\text{air}} \quad C_i$$

Actual data

MATRIX

X Y vector
n

Basic sums of squares of X matrix

REGRESSION NO _ _ 1 _

MATRIX

- Matrix sub-set

REGRESSION COEFFICIENTS

$$\text{i.e. } r_s = 44.669 + .1864E+7 (C_i)$$

ANALYSIS OF VARIANCE

REGRESSION NO _ _ 2 _

MATRIX

Matrix sub-set

REGRESSION COEFFICIENTS

$$\text{i.e. } r_s = -597.04 + 123.44(C_i)(-\Psi_{\text{air}}) + .56812(-\Psi_{\text{air}})$$

ANALYSIS OF VARIANCE

01106760

1	544.90	.46365	1862.9	.24888E-03
2	525.80	.47111	1824.7	.25819E-03
3	560.63	.58212	1909.5	.30486E-03
4	556.61	.58657	1891.7	.31007E-03
5	616.00	.68862	2014.2	.34189E-03
6	639.43	.68695	2042.2	.33638E-03
7	684.53	.73708	2130.0	.34604E-03
8	664.31	.72899	2086.8	.34934E-03
9	706.36	.80288	2159.9	.37172E-03
10	723.53	.80482	2190.3	.36745E-03
11	930.26	1.0269	2444.7	.42004E-03
12	935.88	1.0269	2455.1	.41827E-03
13	1002.8	1.2098	2547.4	.47494E-03
14	1007.5	1.2005	2547.1	.47131E-03
15	1042.1	1.3739	2584.8	.53153E-03
16	1054.3	1.3845	2599.5	.53259E-03
17	1112.1	1.5621	2679.3	.58302E-03
18	1132.6	1.5612	2692.4	.57987E-03
19	1156.6	1.7300	2716.2	.63690E-03
20	1187.5	1.7655	2751.2	.64173E-03

Actual
data

MATRIX

1060373.622

= SS(Y) sums of squares of r_s

1874.
.1452E+07
.5364

3.4427
2550.3
.99351E-03

2550.3
.20023E+07
.73117

.99351E-03
.73117
.28779E-06

REGRESSION NO 1

MATRIX

.5364

.28779E-06 matrix sub-set

REGRESSION COEFFICIENTS

44.669

.18640E+07

 $R(S) = A + B(C(i))$

ANALYSIS OF VARIANCE SS

MS

F

REGRESSION	1.	999888.	999888.	297.56
RESIDUAL	18.	60486.0	3360.34	

S.E. = 57.97

TOTAL 19. .106037E+07

REGRESSION NO 2

MATRIX

1874.
.1452E+07

3.4427
2550.3

2550.3
.20023E+07

REGRESSION COEFFICIENTS

-597.04

123.44

.56812

 $R(S) = A + B(C(i)*PSI) + C(PSI)$

ANALYSIS OF VARIANCE

REGRESSION	2.	.105639E+07	528195.	2253.97
RESIDUAL	17.	3983.78	234.340	

S.E. = 15.31

TOTAL 19. .106037E+07

continued....

TABLE 3D continued

REGRESSION NO 3

MATRIX

.5364 .1452E+07	.28779E-06 .73117	.73117 .20023E+07
--------------------	----------------------	----------------------

REGRESSION COEFFICIENTS
-712.10 .29255E+06 .61851

R(S) = A + B(C(i)) + C(PSI)

ANALYSIS OF VARIANCE

REGRESSION	2.	.105521E+07	527603.	1735.40	S.E. = 17.44
RESIDUAL	17.	5168.41	304.024		
TOTAL	19.	.106037E+07			

REGRESSION NO 4

MATRIX

1874. .1452E+07 .5364	3.4427 2550.3 .99351E-03	2550.3 .20023E+07 .73117	.99351E-03 .73117 .28779E-06
-----------------------------	--------------------------------	--------------------------------	------------------------------------

REGRESSION COEFFICIENTS
-236.31 707.42 .49088 -.18254E+07

R(S) = A + B(C(i)*PSI) + C(PSI) + D(C(i))

ANALYSIS OF VARIANCE

REGRESSION	3.	.105931E+07	353104.	5317.35	S.E. = 8.15
RESIDUAL	16.	1062.50	66.4060		
TOTAL	19.	.106037E+07			

VARIANCE RATIOS 241.11 181.95 43.99 61.83
(1) (2) (3) (4)

The results of the comparisons were as follows:

Probability level	Improvement of eqn.3.2 over eqn.3.1	Improvement of eqn.3.3 over eqn.3.1	Improvement of eqn.3.4 over eqn.3.2	Improvement of eqn.3.4 over eqn.3.3
No significant improvement	13	20	54	48
p 0.05	9	12	11	11
p 0.01	18	22	18	13
p 0.001	73	59	30	41

The variance ratio tests showed equation 3.2 to be more appropriate than equations 3.1 and 3.3, and the latter two were rejected. Further comparison of equations 3.2 and 3.4 was carried out by comparing the results of predictions for pooled data as described in the following section.

3.3/1 Regression results.

In this section results are given for pooled data sets for four species (oak, ash, tobacco and bean); within a pooled set, data is for the same leaf. The sources of data used in each of the pooled sets, together with their locations in appendix 2, are given in table 3E. The results are shown as three-dimensional plots (eg figure 3.55) as follows:

1. The x-axis(abscissa) is C_i (kg.m^{-3})
2. The y-axis(ordinate) is $-\Psi_{\text{air}}$ (J.mol^{-1})
3. The z-axis is r_s (s.m^{-1})
4. The grid drawn is the fitted regression. Each observed data point is drawn as a circle (o) and is connected to a cross (x) which is its associated expected (ie regression prediction) value in the plane of the grid.
5. The symbol 'i' beneath a plot indicates that those points falling outside the values defined by the axes have been omitted.

Further comparison of equations 3.2 and 3.4 was made by examining

TABLE 3E The sources of data for the pooled regressions.

<u>Data set</u>	<u>Figure number(s)</u>	<u>Pooled data</u>	<u>Location of raw data</u>
QSET1	3.53,3.54	Q1105760 Q1805761 Q1805760 Q1905760 Q1905761 Q2505760P1 Q2505760P2 Q2605760 Q2605761 Q1106760 Q0607760 Q0707760 Q0807760 Q0907760	Appendix 2,sheet 2
QSET2	3.53,3.55	Q1105761 Q1805761P1 Q1805761P2 Q1905761P1 Q1905761P2	Appendix 2,sheet 2
QSET5	3.57	QDRYING1 QDRYING2 QDRYING3 QDRYING4 QDRYING5	Appendix 2,sheet 2
QSET9	3.58	Q1109782 Q1209782 Q1309782	Appendix 2,sheet 2
NSET1	3.59	N2701780 N3001780 N3101780 N0102780 N0202780	Appendix 2,sheet 3
NSET2	3.60	H1102783 N1302783 N1402783 N1502783 N1602783	Appendix 2,sheet 3
NSET3	3.61	N2802780 N0103780 N0203780 N0603780	Appendix 2,sheet 3

continued.....

TABLE 3E (continued)....

<u>Data set</u>	<u>Figure number</u>	<u>Pooled data</u>	<u>Location of raw data</u>
FSET1	3.62	F1108771 F1208771 F1808771 F1908771	Appendix2, sheet 4
QSET8(BLUE LIGHT)	3.63	Q0505781 Q1105781	Appendix2, sheet 2
NSET5(RED LIGHT)	3.64	N300378E N040478E N060478E N100478E	Appendix2, sheet 3
NSET5(BLUE LIGHT)	3.65	N310378E	Appendix2, sheet 3
QUERCUS EXPT1	3.68	QEXPT1P2-P18	Appendix2, sheet 1
QUERCUS EXPT2	3.69	QEXPT2P1-P12	Appendix2, sheet 1
PSET2(O ₂ PRESENT)	3.74	P1002770 P1102770 P1702770 P1802770	Appendix2, sheet 4
PSET2(O ₂ ABSENT)	3.74	P1002770A P1402770 P1002770B	Appendix2, sheet 4

SQIYCAM

$$z = a + bxy + cy + dx + \psi \quad y = R_s \quad z = R_s \quad x = C_b \quad (4)$$

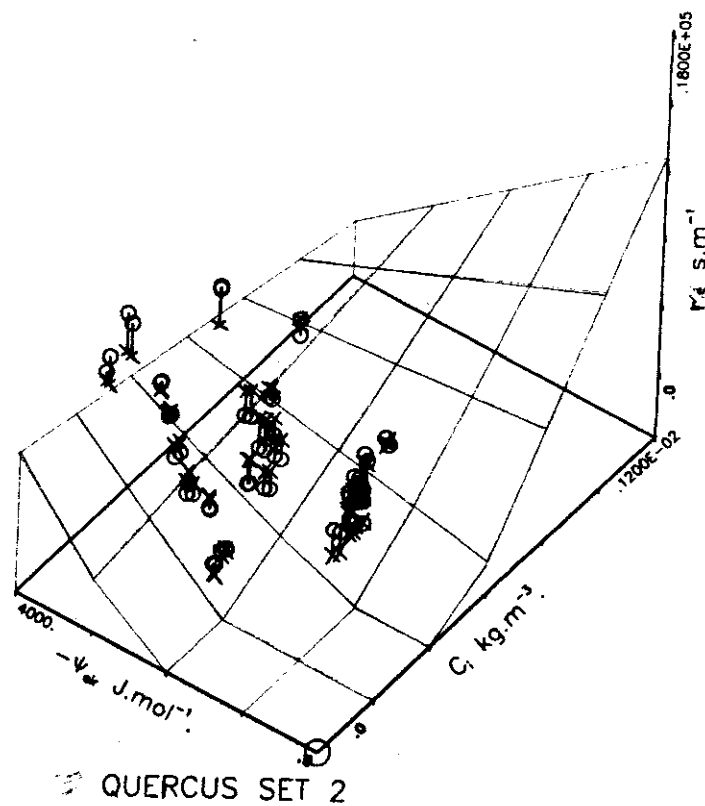
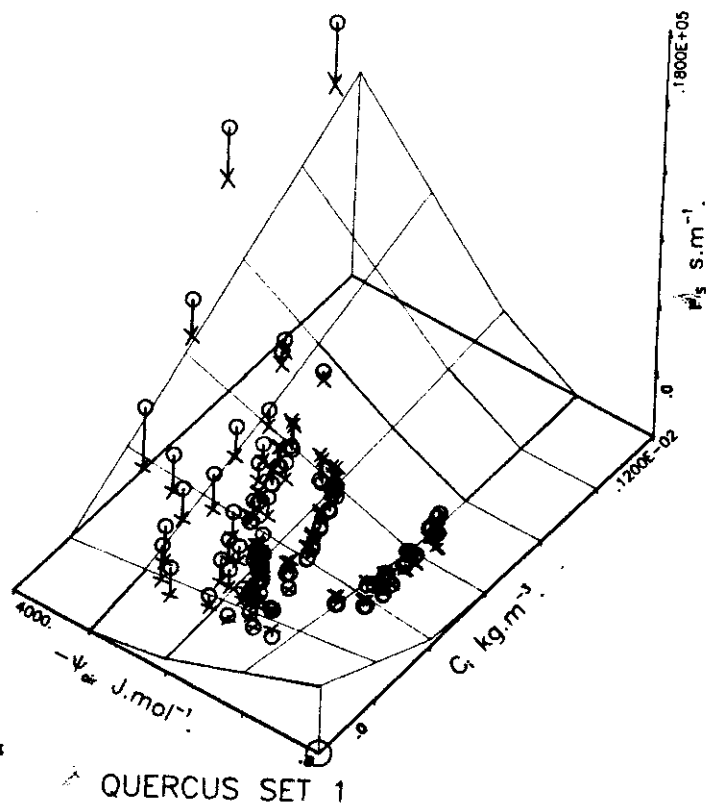


FIGURE 3.53 Typical results using regression 4.

FIGURES 3.54 & 3.55 Results for two Quercus data sets using regression 2.

Figure 3.54

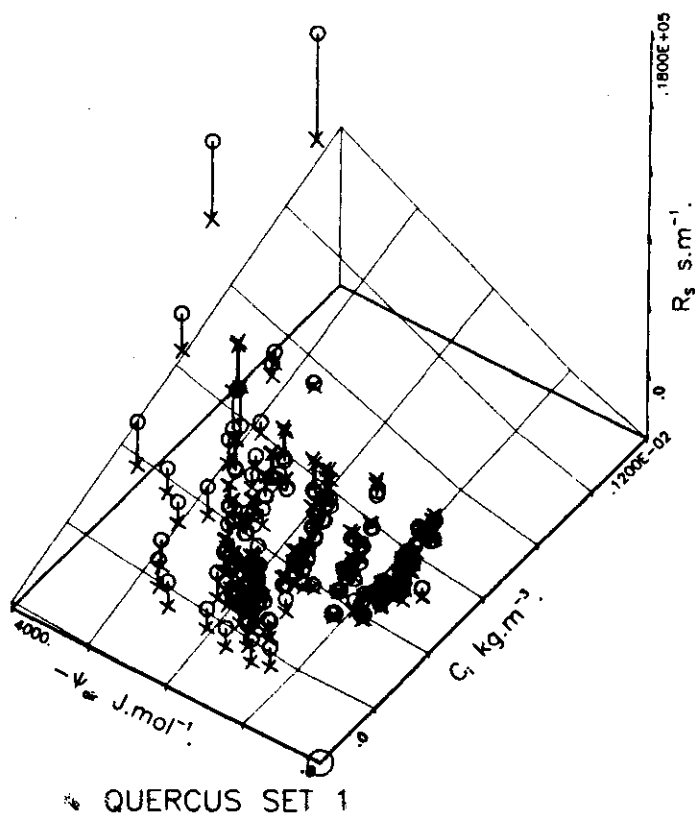
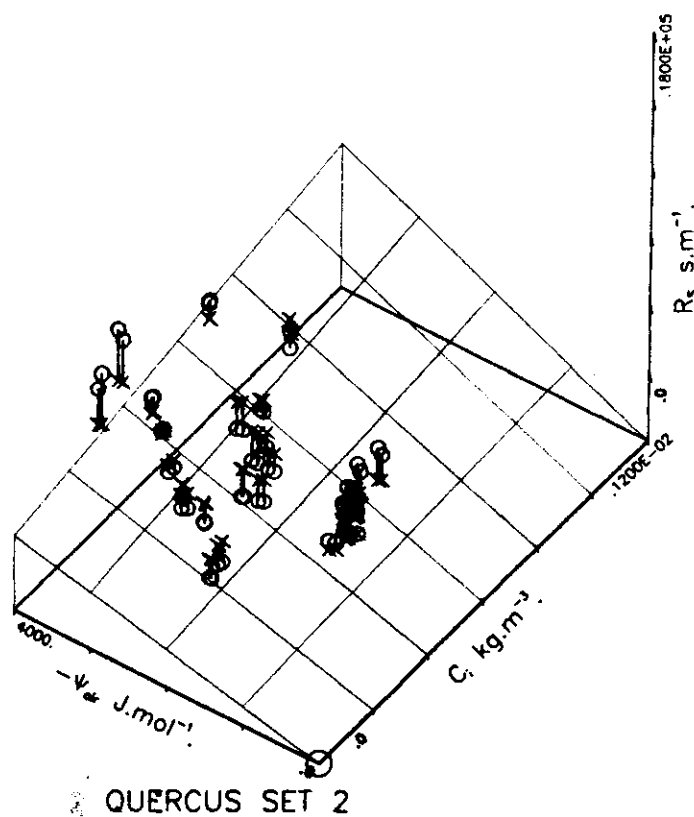


Figure 3.55



plots of the results: the same data were fitted to regression 4 (figure 3.53) and regression 2 (figures 3.54 and 3.55) and a comparison was made of the regression surfaces produced. Equation 3.4 produced smaller standard errors for the pooled data, however, as can be seen (figures 3.53 - 3.55) the extra parameter of the equation 3.4 regressions provided more flexibility to this model and produced surfaces which were distorted to pass close to individual data points, and these distortions were in different directions for different data sets. The regressions produced by equation 3.2 were less flexible and this tended to 'average out' individual points and produce smoother regression surfaces. This pattern was consistent over all the pooled data set examined and it was decided that regression 2 (equation 3.2) gave a more realistic and consistent prediction of r_s in accordance with the results outlined in section 3.2. In addition, regression 2 predicted that at zero ($-\psi_{air}$), r_s is constant whereas regression 4 (equation 3.4) predicted that at zero ($-\psi_{air}$), r_s is proportional to C_i . Figures 3.28 - 3.43 tended to indicate that as ($-\psi_{air}$) approached zero, r_s was constant and independent of C_i .

In the light of the evidence outlined above, it was decided that the increased complexity of regression 4 was not justified and that regression 2 was the most appropriate, and results will now refer to this regression.

The table of regression coefficients for pooled data sets (table 3F) shows that there were basically 3 combinations of parameters 'a', 'b', 'c' produced and these represent the relationships shown conceptually in figure 3.56 (i-iii) :

- i. 'a', 'b', 'c' all positive: the r_s intercept is always positive, but increases with $-\psi_{air}$. The slope of the line increases with $-\psi_{air}$. At a particular value of C_i , r_s increases with $-\psi_{air}$.

TABLE 3F Regression coefficients for pooled data fitted to regression 2.

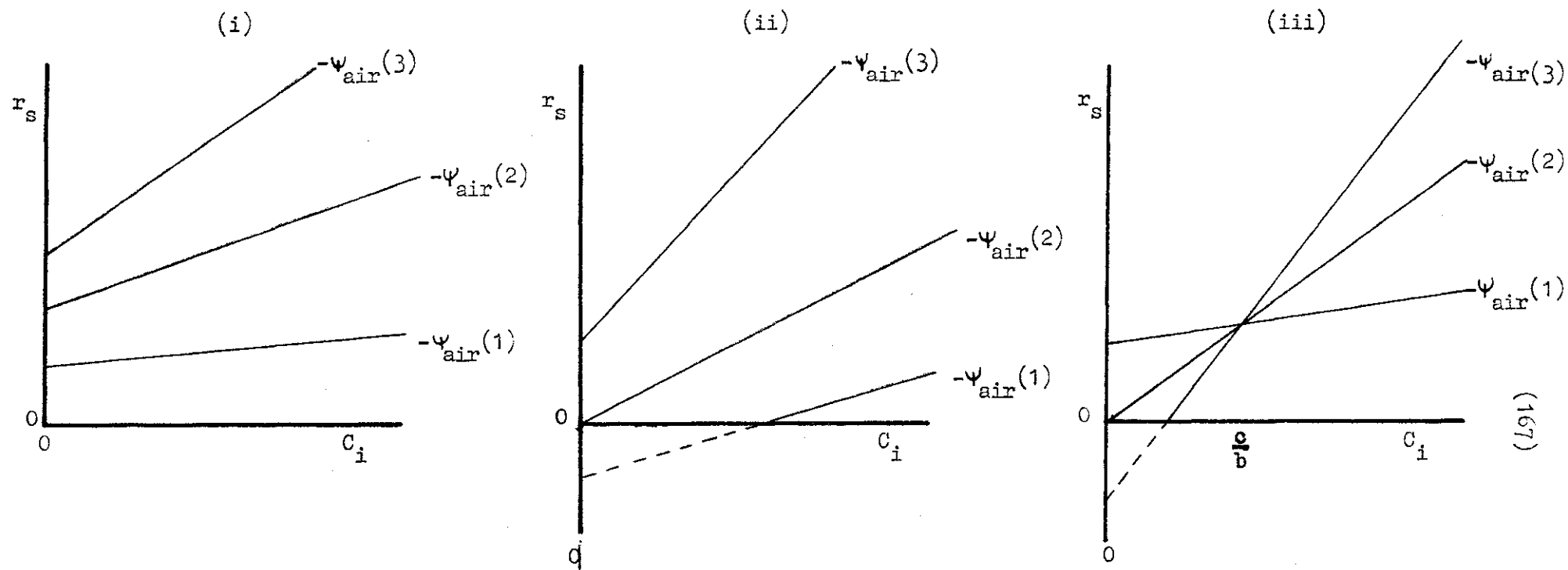
Within a pooled set, data is for the same leaf (except *).

$$Y = a + bX_1 + cX_2$$

where: $Y=r_s$; $X_1=(C_i)(-\Psi_{\text{air}})$; $X_2=-\Psi_{\text{air}}$ see equation 3.2

Data set ident.	O ₂ level (%)	a	b	c	s.e. of fit	Figure number	Variance accounted for(number of points)
QEXPT1	20	2368.5	2126.7	.10638	665.0	3.68	84.2 (55)
QEXPT1	4	2543.0	732.8	.04181	1377.0	3.68	17.2 (38)
QEXPT2	20	2415.1	1691.7	-.44936	1476.0	3.69	56.6 (60)
QEXPT2	4	568.4	1087.4	.92435	1900.0	3.69	41.5 (43)
QSET1	20	-549.9	1409.0	.21189	858.8	3.54	59.1 (169)
QSET2	20	-4.3	635.3	.83660	937.6	3.55	54.2 (58)
QSET5	20	-1283.8	1636.2	.67481	1360.0	3.57	91.1 (36)
QSET8 BLUE	20	1282.0	4309.0	-1.3140	2776.0	3.63	70.1 (102)
QSET9	20	3461.5	2003.6	-1.0572	1155.0	3.58	19.8 (39)
*Q NORMAL O ₂	20	365.2	1595.0	.49530		3.66	
*Q LOW O ₂	0-4	2045.0	1102.0	.09743		3.70	
NSET1	20	33.1	3784.5	-1.0439	836.2	3.59	89.5 (145)
NSET2	20	521.4	2302.4	-.63952	1186.0	3.60	70.4 (73)
NSET3	20	1342.2	3827.8	-1.2513	867.9	3.61	84.8 (55)
NSET5 RED	20	3761.0	-1144.0	3.85500	7989.0	3.64	44.2 (58)
NSET5 BLUE	20	-1737.0	40.5	2.87500	2269.0	3.65	80.0 (54)
*NICOTIANA	20	-614.8	1827.0	.17340		3.67	
PSET2							
O2 PRESENT	20	-365.8	1289.0	.21360		3.74	
PSET2							
O2 ABSENT	0	375.2	81.3	.78070		3.74	
PSET1	20	-4551.9	5794.9	.90282	4601.0	3.62	86.4 (42)

FIGURE 3.56: Conceptual diagrams of Hypothesis 2 results according to the parameters a , b , c . At a particular value of $-\psi_{\text{air}}$, the relationship is a straight line.



$-\psi_{\text{air}}(1) < -\psi_{\text{air}}(2) < -\psi_{\text{air}}(3)$. Negative r_s shown as dashed line.

- ii. 'a' negative, 'b' and 'c' positive: the r_s intercept is negative if $a > c (-\psi_{\text{air}})$, zero if $a = c (-\psi_{\text{air}})$ and positive if $a < c (-\psi_{\text{air}})$. The slope of the line increases with $-\psi_{\text{air}}$, and at a particular value of C_i , r_s increases with $-\psi_{\text{air}}$.
- iii. 'a' and 'b' positive, 'c' negative: the r_s intercept is positive if $a > c (-\psi_{\text{air}})$, zero if $a = c (-\psi_{\text{air}})$ and negative if $a < c (-\psi_{\text{air}})$. The slope of the line increases with $-\psi_{\text{air}}$ and r_s increases with $-\psi_{\text{air}}$ when $C_i > \frac{c}{b}$, the point at which the lines intercept.

In addition, one data set (NICOTIANA SET 5, RED LIGHT) had 'a' and 'c' positive and 'b' negative. However, this data set was for tobacco at very low levels of red light and the regression produced a very poor fit to the data (see below).

The relationships shown in figure 3.56 are of a purely conceptual nature and, of course, negative values of r_s cannot occur in practice. The three dimensional plots discussed below show that when the regression produced was of the type shown in figure 3.56 (i) with 'a' negative, then $c (-\psi_{\text{air}})$ was always greater than the absolute value of 'a', and negative values of r_s were not produced. Conversely, when the regression was of the type shown in figure 3.56 (iii), 'a' was always greater than the absolute value of $c (-\psi_{\text{air}})$ and, again, negative values of r_s did not occur.

The variance accounted for by the regression was assessed by expressing the regression sums of squares as a percentage of the total sums of squares (table 3F). For oak, the range of values produced was 17.2% (QEXPT1, 4%O₂; 38 points) to 91.1% (QUERCUS SET5; 36 points) with an overall weighted mean of 58%. Thus, over all conditions, the regression accounted for 58% of the variation in r_s . However, it can be seen that the poorest fits occurred at reduced levels of ambient oxygen (there being no direct O₂ effect incorporated in the model), and when the

low O_2 sets were removed, the regression accounted for 65.8% of the variation in r_s . For other species the weighted means were (all at normal O_2 levels): 77.1% for tobacco, 70.0% for bean and 86.4% for ash.

3.3/2 Effects of C_i , $-\psi_{air}$ and light.

The nature of the interaction between the $-\psi_{air}$ and C_i responses illustrated in figures 3.54, 3.55, 3.57 - 3.62 for oak, tobacco and ash at normal ambient levels of O_2 . At low values of $-\psi_{air}$ there is little response to C_i , but the slope of the relationship between C_i and r_s increases with $-\psi_{air}$. The biggest differences between observed and expected values occur at the highest observed values of r_s , ie in the dark. Such points are particularly evident in figures 3.54 and 3.57 - 3.59. At zero irradiance the observed values of r_s always exceed the predicted value. The regression thus gives a good fit to the data, particularly in the light, although there is no independent effect of I built in. This may be because of the inherent difficulty of measuring the high values of r_s which occurred in the dark due mainly to the difficulty in measuring low values of dewpoint temperature accurately, or it could be evidence of an independent effect of light.

The results of limited experiments at low levels of red and blue light with leaves of oak and tobacco are shown in figures 3.63 - 3.65. A plot was not possible with oak at low levels of red light because of the very high values of r_s :- the stomata were never open (the data are given in table 3G). For both species, the observed values of r_s were lower in the blue light than in red light at equivalent values of $-\psi_{air}$ and C_i . In addition, the regression gave much better fits to the blue light data for both species (see also table 3F).

Data for nine individual data sets (nine different leaves) for oak were pooled further to give an overall relationship between r_s ,

FIGURES 3.57 & 3.58 Regression results for two Quercus data sets.

Figure 3.57

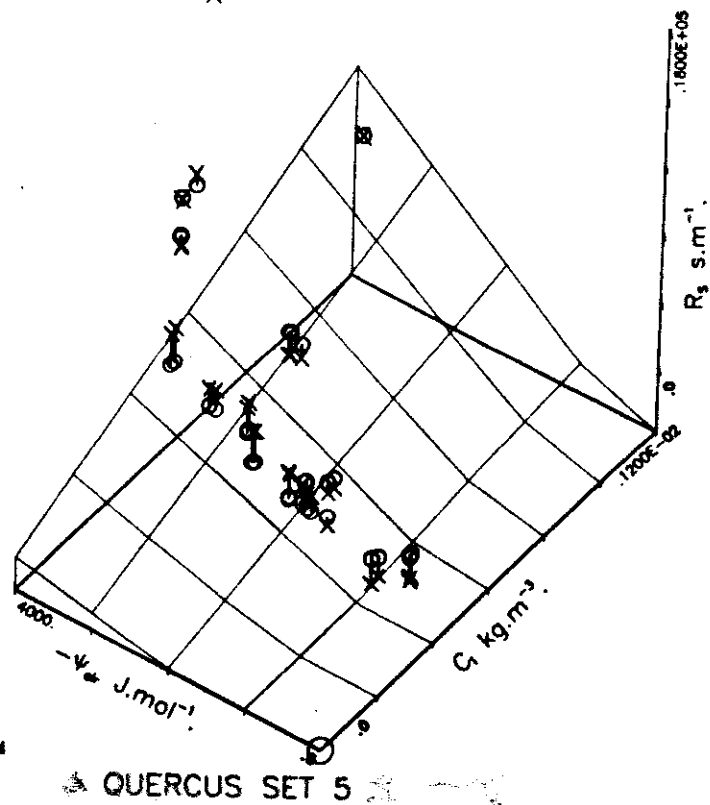
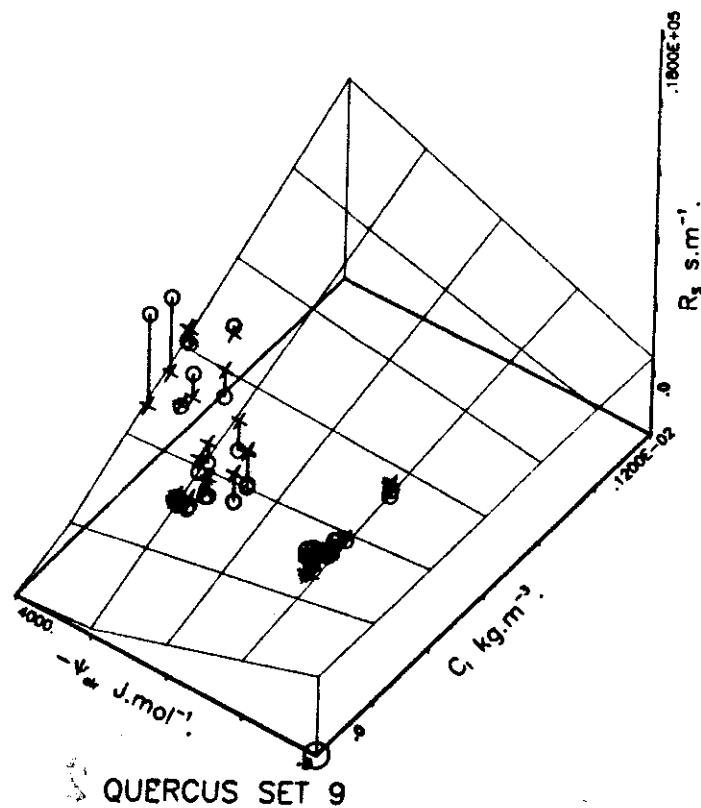


Figure 3.58



FIGURES 3.59 & 3.60 Regression results for two Nicotiana data sets.

Figure 3.59

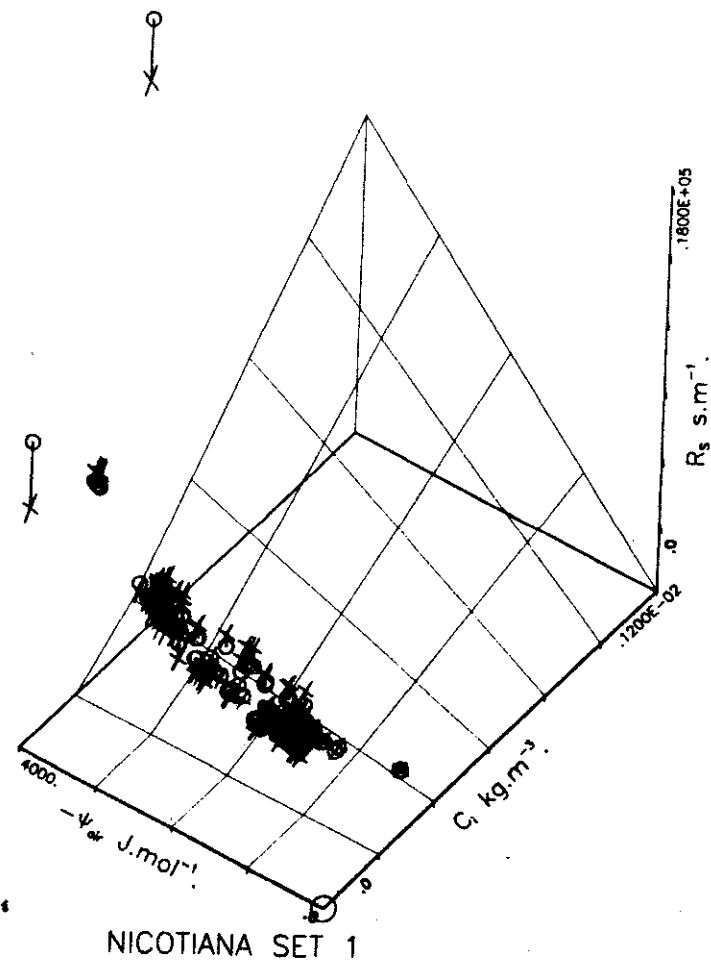
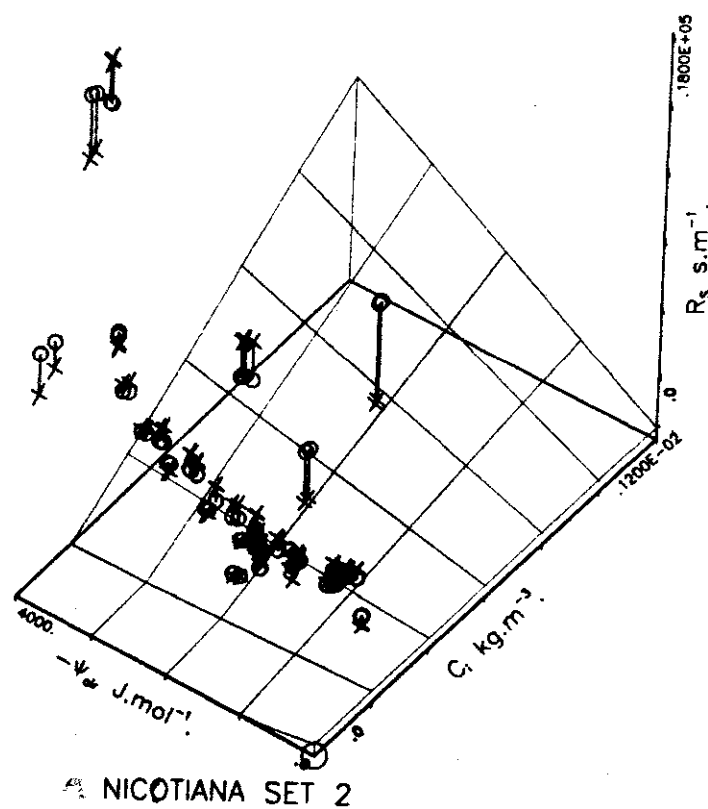


Figure 3.60



FIGURES 3.61 & 3.62 Regression results for one Nicotiana data set and one Fraxinus data set.

Figure 3.61

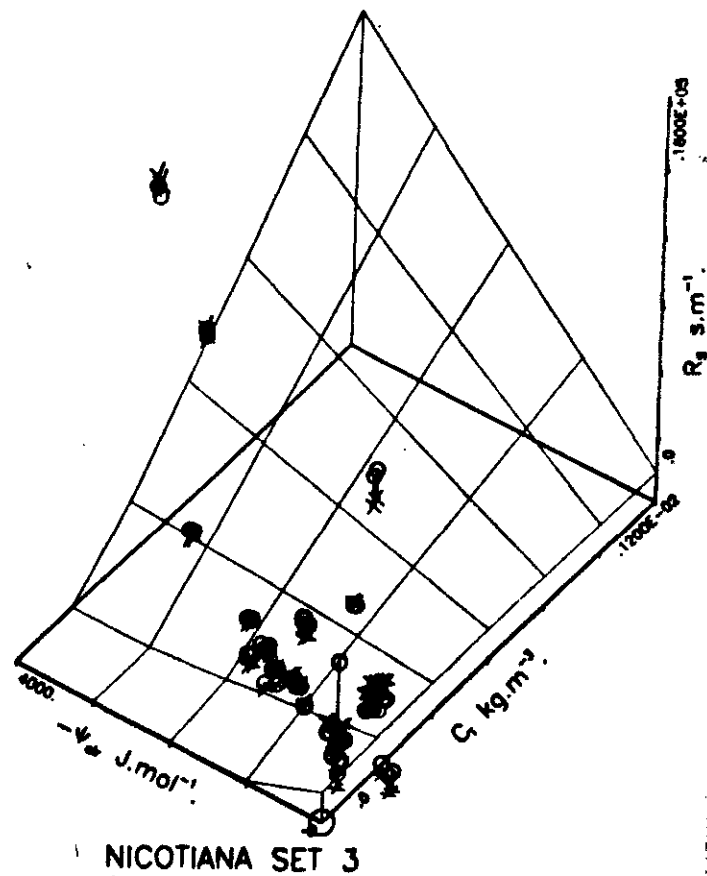


Figure 3.62

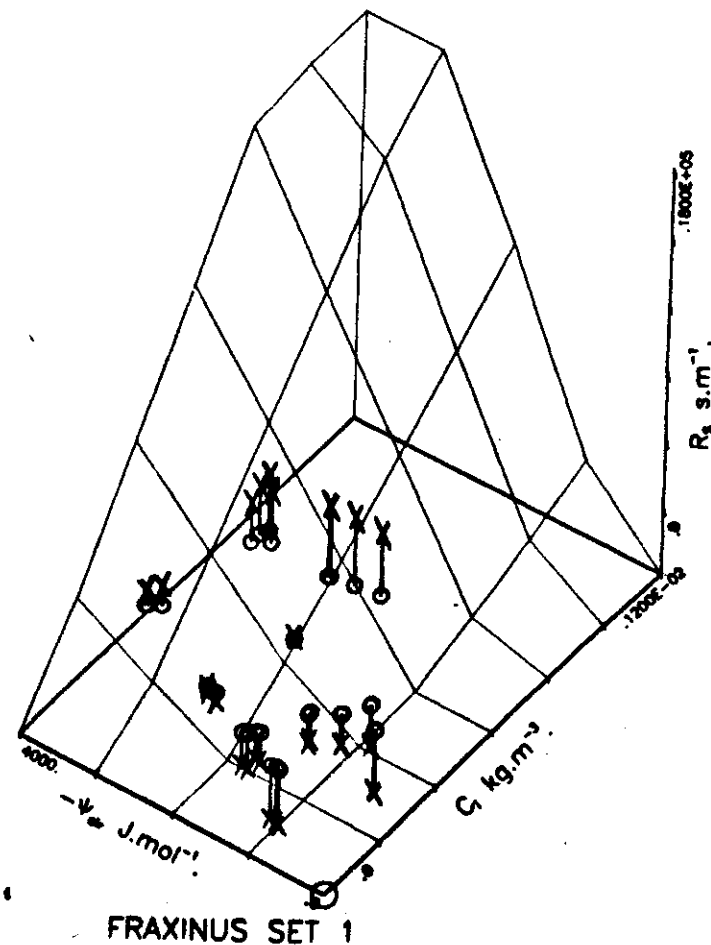


FIGURE 3.63 Regression results for a Quercus data set at low levels of blue light.

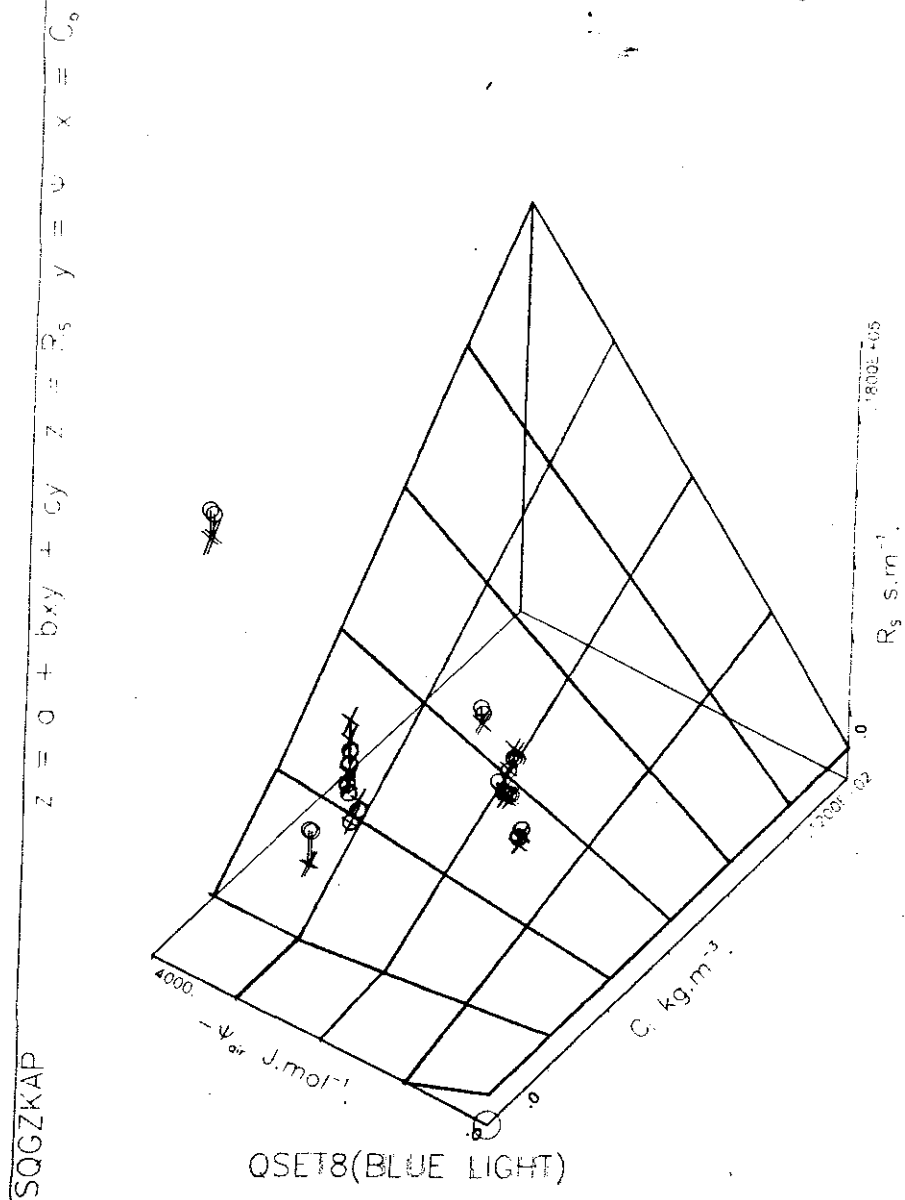


TABLE 3G Results for a leaf of Quercus petraea at low levels of red light.

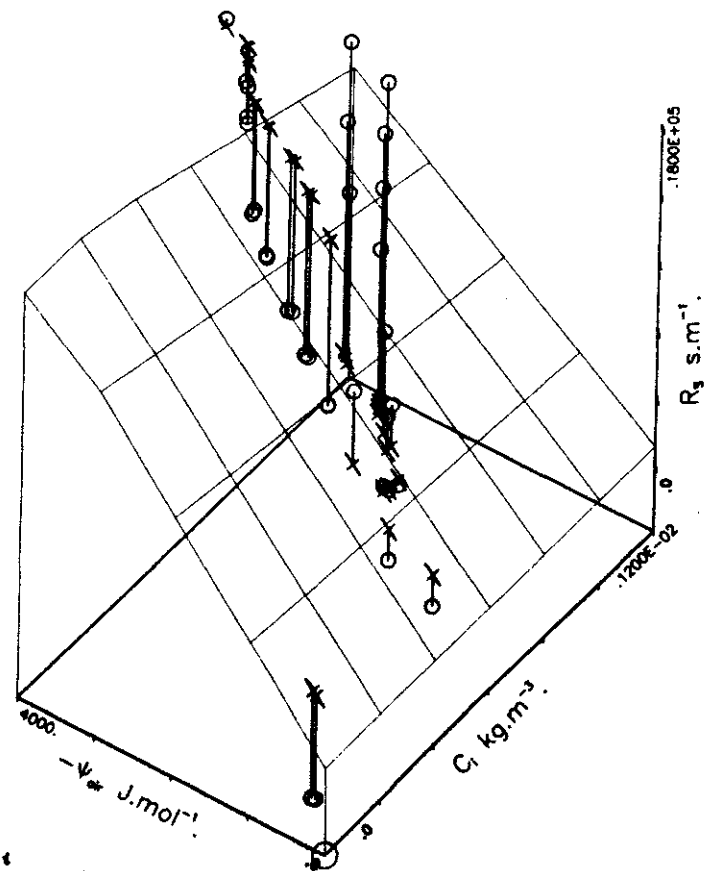
See figure 3.63 for results for the same leaf at low levels of blue light. A plot was not possible the results in red light because of the high values of r_s involved.

I $\mu\text{E.m}^{-2}\text{s}^{-1}$	T_L °C	C_i $\text{kg.m}^{-3}\times 10^6$	$-\Psi_{\text{air}}$ J.mol^{-1}	Observed r_s s.m^{-1}	Predicted r_s s.m^{-1}
41	29.6	.36	5128	8364	7535
41	29.7	.36	5136	8460	7629
30	30.5	.45	5582	11490	11550
29	30.5	.45	5614	11010	10680
23	31.4	.69	5353	11170	10870
23	31.3	.71	5369	12880	13300
18	31.8	.94	5544	12930	13430
18	31.9	.96	5551	14280	14730
16	32.0	1.07	5648	14570	15080
16	32.0	1.09	5681	14595	*
11	32.0	1.25	5818	16122	*
11	33.0	1.27	5855	16520	*
8	32.0	1.35	5923	17631	*
8	32.1	1.39	5954	18103	*
6	32.7	1.72	6265	24221	*
6	32.7	1.70	6273	24500	*

* not predicted due to constraints in the model.

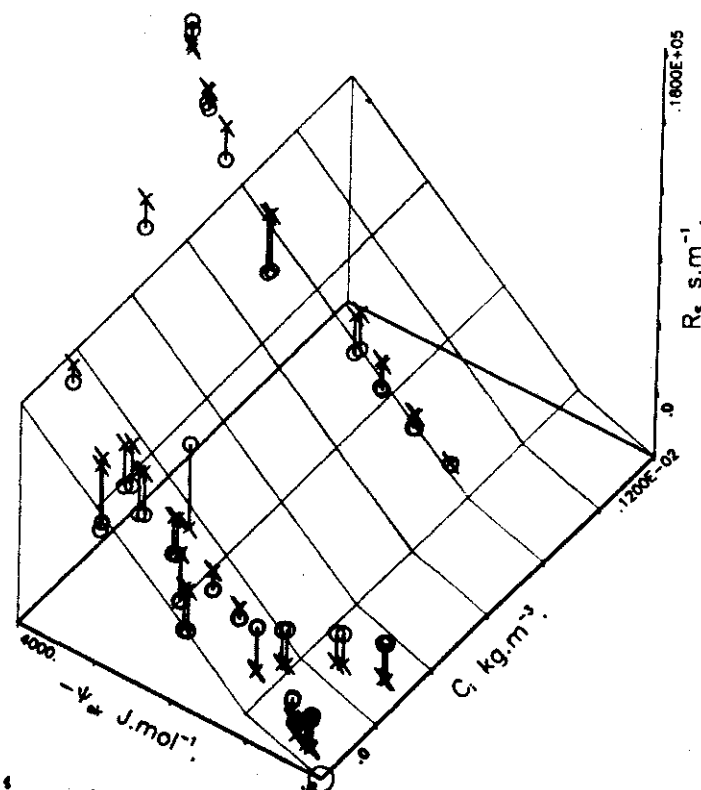
FIGURES 3.64 & 3.65 Regression results for a Nicotiana leaf at low levels of red and blue light.

Figure 3.64



NICOTIANA SET 5 (RED LIGHT)

Figure 3.65



NICOTIANA SET 5 (BLUE LIGHT)

FIGURE 3.66 Regression results for combined data from nine individual Quercus data sets.

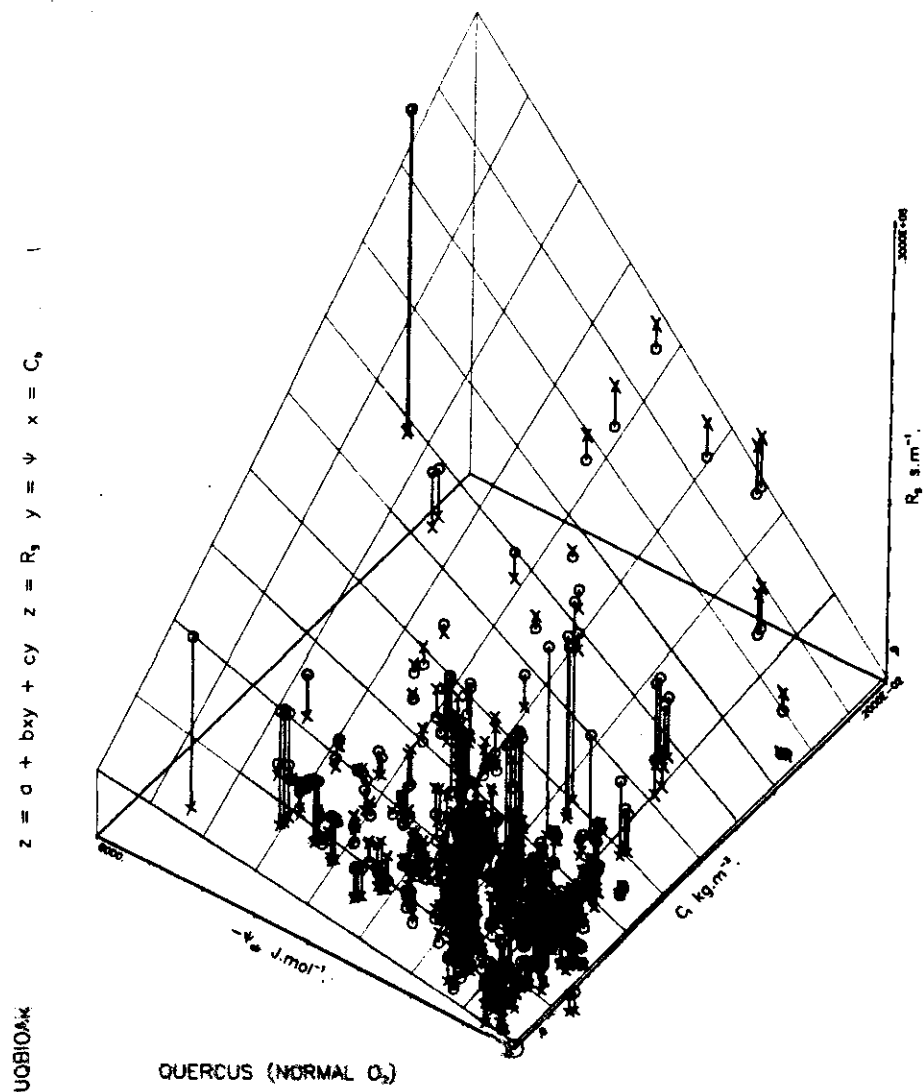
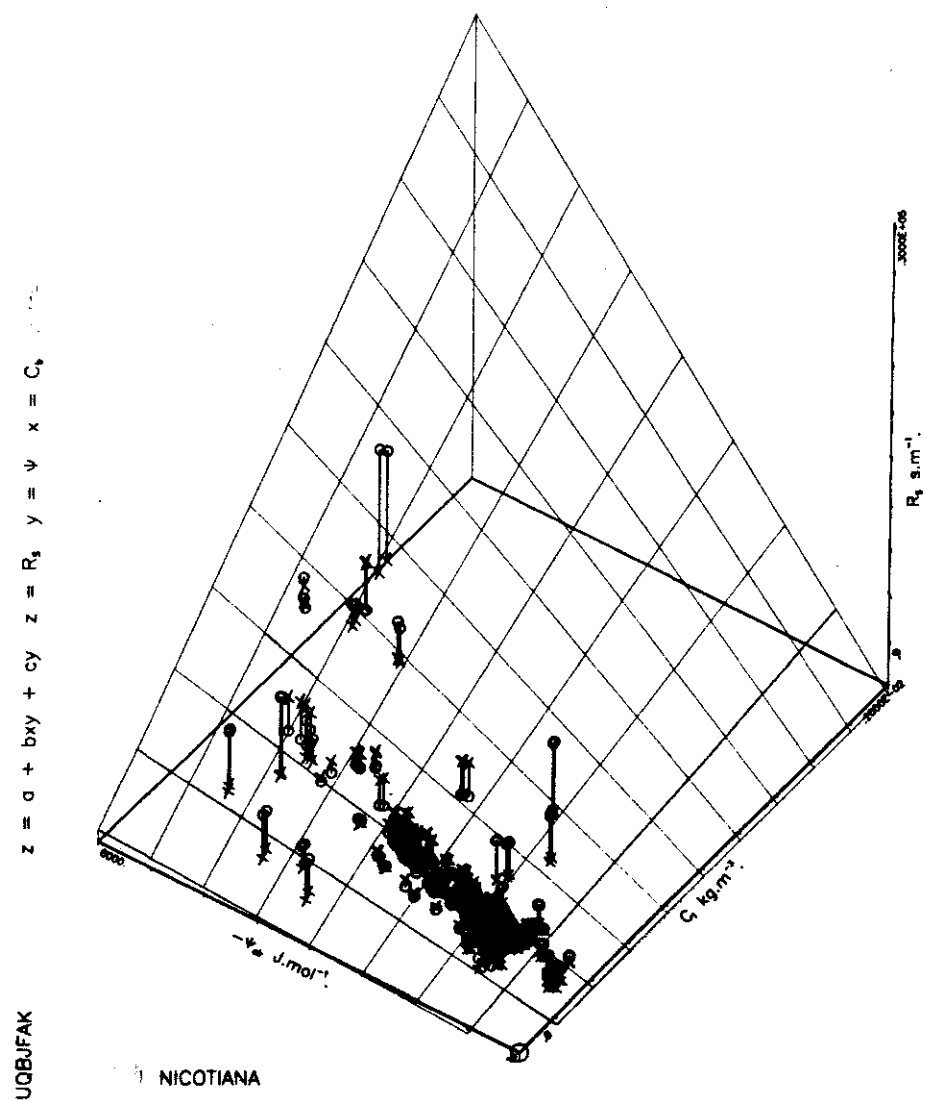


FIGURE 3.67 Regression results for combined data from five individual Nicotiana data sets.



$-\psi_{\text{air}}$ and C_i ; the results are shown in figure 3.66. Similarly five data sets were pooled to show the same relationship for ^atobacco^{leaf} (figure 3.67). The grids showing the regression values are very similar for the two species, but tobacco appears to show less response to $-\psi_{\text{air}}$ at very low C_i values than oak.

3.3/3 Effects of ambient O_2 concentration.

The effects of varying O_2 concentration were investigated with three species: oak, tobacco and bean. For oak, the regression gave the poorest fits at reduced ambient O_2 concentrations (see table 3F), and since there was no direct response to O_2 incorporated in the model, this suggested that O_2 was having an influence on r_s . Figure 3.68 shows results for *Quercus* experiment 1 at 4% and 20% O_2 indicating that r_s tends to be lower at equivalent values of $-\psi_{\text{air}}$ and C_i , at the lower O_2 concentration. This difference is not so apparent, however, in the results for *Quercus* experiment 2 (figure 3.69). However, when data obtained at low ambient O_2 (0 - 4%) were pooled, for the same nine leaves used for the overall regression at normal O_2 shown in figure 3.66, the results confirmed that values of r_s were lower at the reduced O_2 concentrations (cf. figures 3.66 and 3.70). Figure 3.71 shows data for an oak leaf in O_2 -free air: there was very little stomatal closure until the leaf was in darkness, when closure occurred. Figure 3.72 shows data for the same leaf, but with the stomata closed at the beginning of the experiment (this was achieved by keeping the leaf at normal O_2 , but in the dark prior to the experiment). In this case the closed stomata would not re-open at any level of I when exposed to O_2 -free air.

Figure 3.73 shows the difference in light response curves for tobacco leaves in normal and O_2 -free ambient air. As with the oak, r_s values were lower in O_2 -free air, but closure was not prevented in

FIGURE 3.68 Regression results for Quercus Experiment 1 at 4% and 20% ambient O_2 concentrations.

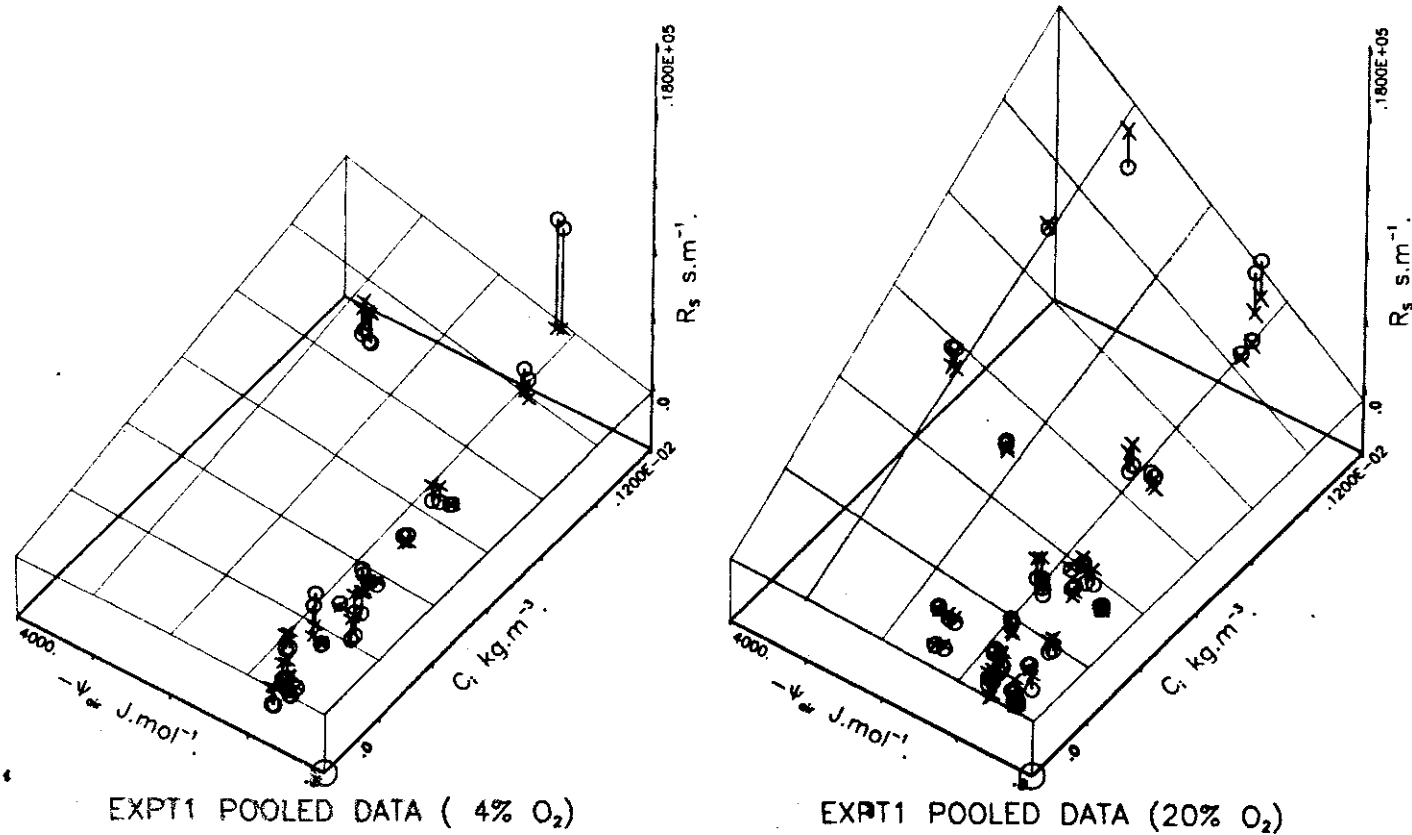
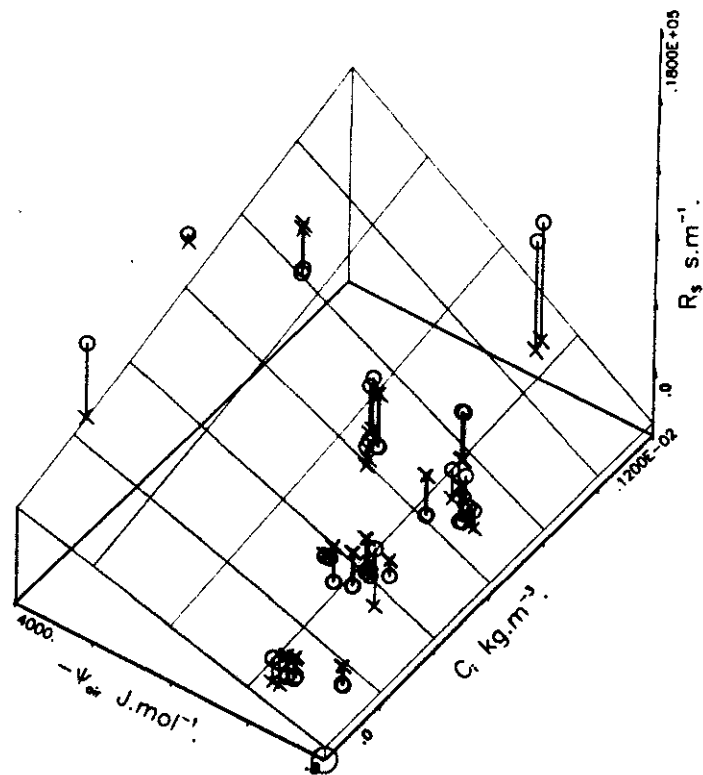
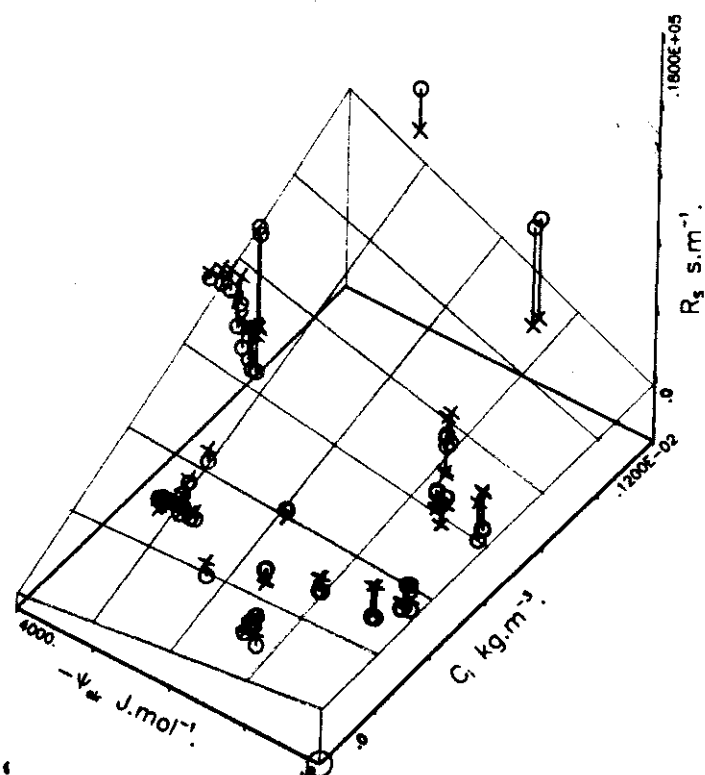


FIGURE 3.69 Regression results for Quercus Experiment 2 at 4% and 20% ambient O_2 concentrations.

SBIXDAM $z = a + bxy + cy$ $z = R_s$ $y = \psi$ $x = C_b$

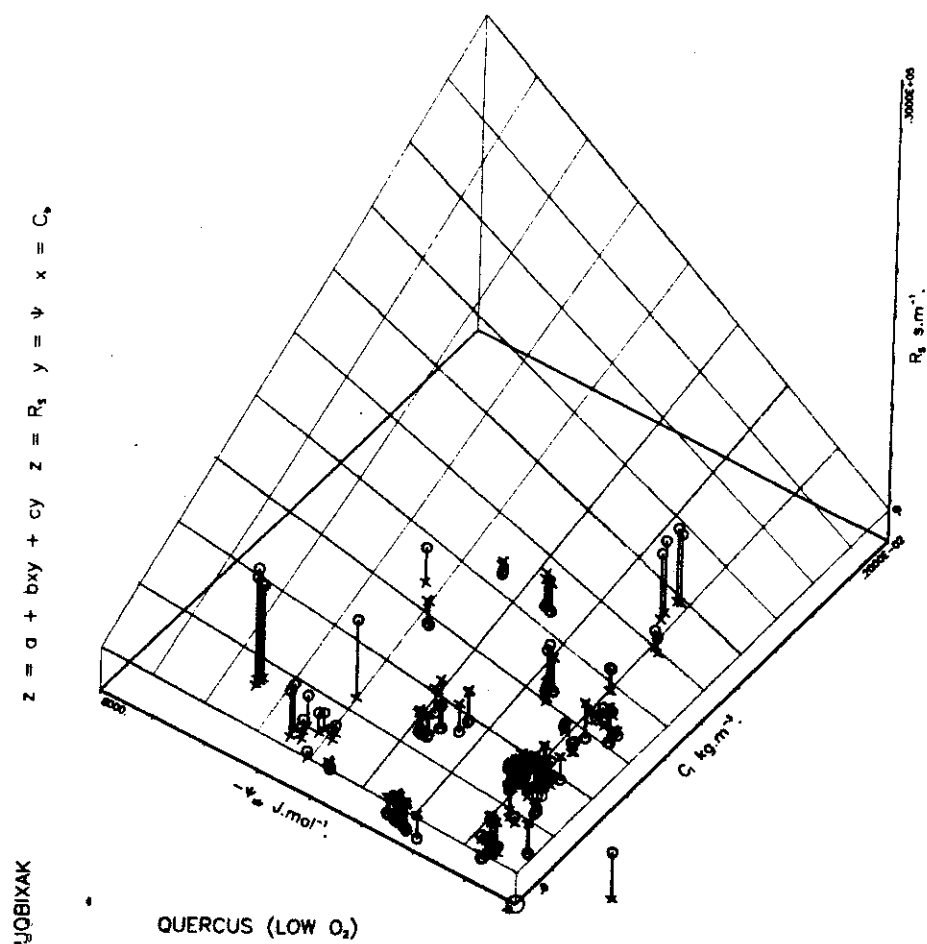


EXPT2 POOLED DATA (4% O_2)

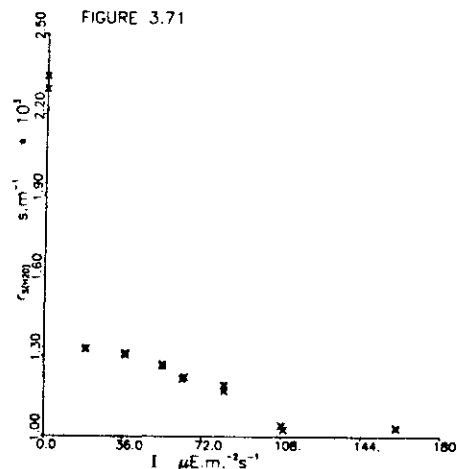


EXPT2 POOLED DATA (20% O_2)

FIGURE 3.70 Regression results for combined data from nine individual Quercus data sets at reduced (0 - 4%) ambient O_2 concentrations.



FIGURES 3.71, 3.72 & 3.73 Effects of reduced ambient O_2 concentration on $r_s(H_2O)$.

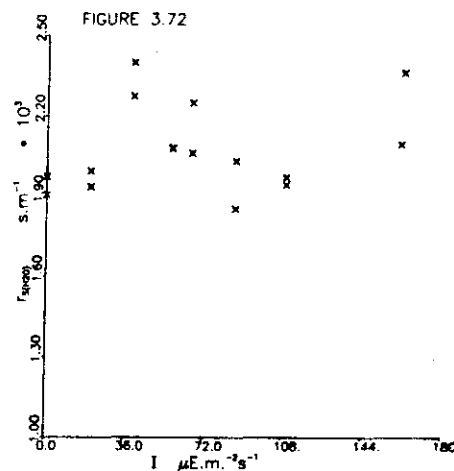


Q130978 (O_2 -free air)

$$C_i = 0.30 - 0.50$$

$$-\psi_{air} = 2600 - 3400$$

$$T_L = 23.0 - 25.5$$

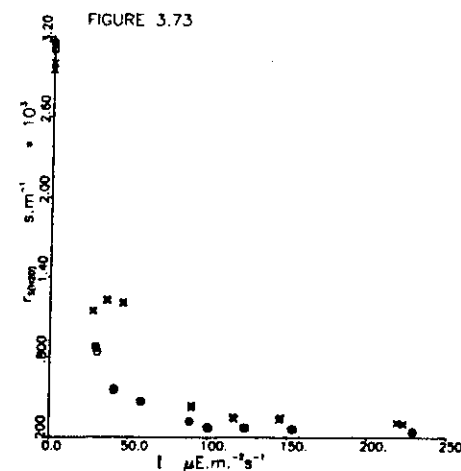


Q120978 (O_2 -free air)

$$C_i = 0.26 - 0.34$$

$$-\psi_{air} = 1070 - 1130$$

$$T_L = 25.0 - 25.8$$



x N010378 (normal O_2 concⁿ.)

$$C_i = 0.30 - 0.38$$

$$-\psi_{air} = 350 - 1150$$

$$T_L = 27.4 - 30.4$$

o N020778 (O_2 -free air)

$$C_i = 0.17 - 0.23$$

$$-\psi_{air} = 310 - 970$$

$$T_L = 28.2 - 29.0$$

$z = a + bxy + cy$ $z = R_s$ $y = \psi$ $x = C_i$

FIGURE 3.74 Regression results for a Phaseolus leaf at normal ambient O_2 concentration and in O_2 -free air.

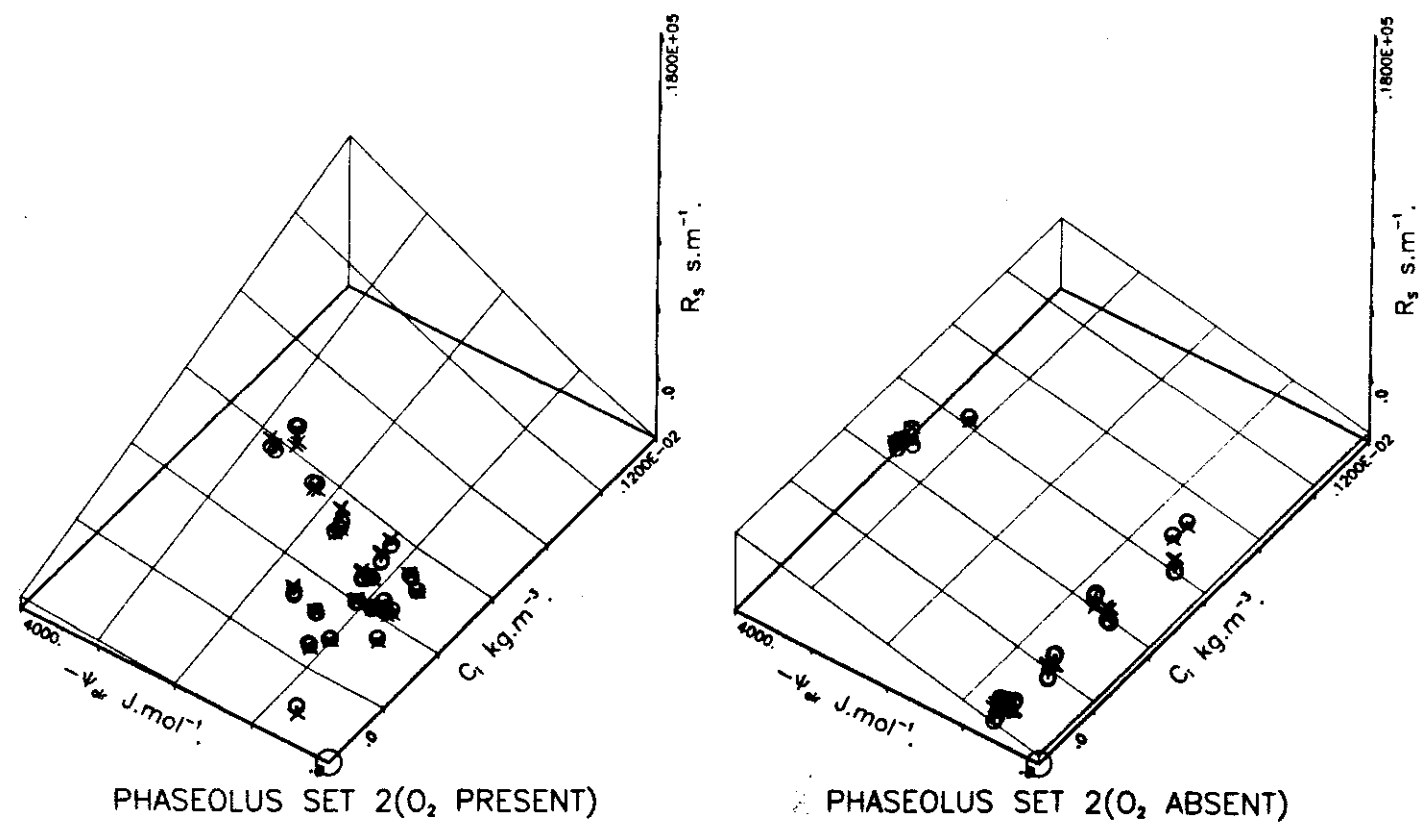
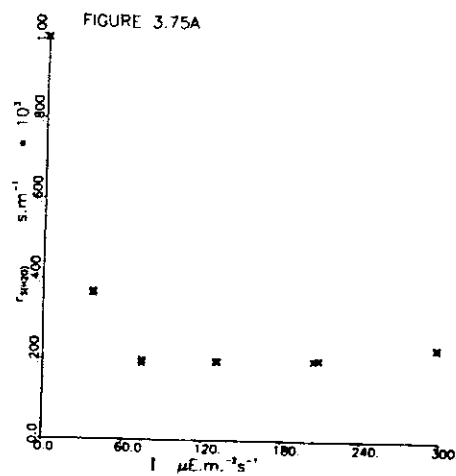


FIGURE 3.75 Effects of varying ambient O_2 concentration on stomatal resistance of a Phaseolus leaf.

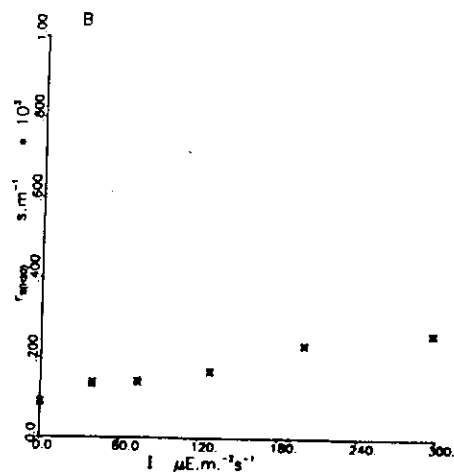


P110277 (O_2 -free air)

$$C_i = 0.31 - 0.51$$

$$-\psi_{air} = 850 - 1150$$

$$T_L = 25.5 - 26.3$$



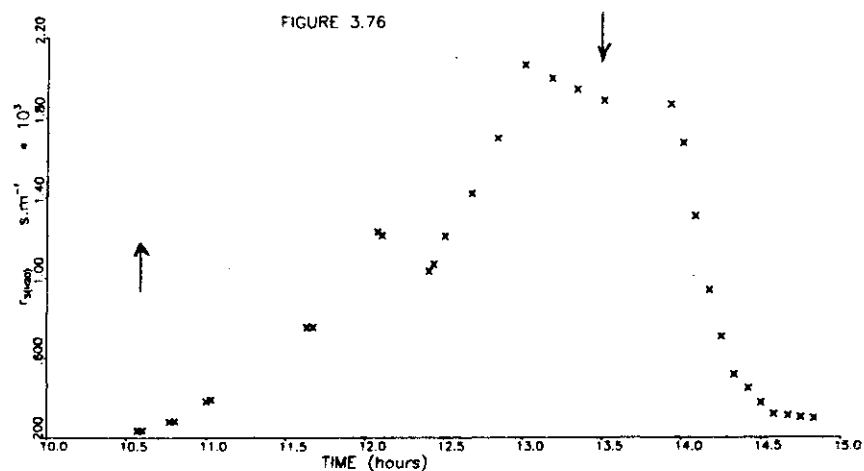
P100277 (O_2 -free air)

$$C_i = 0.13$$

$$-\psi_{air} = 515 - 685$$

$$T_L = 26.3 - 27.9$$

FIGURES 3.76 & 3.77 Failure of O_2 -free ambient air to re-open the closed stomata of a Phaseolus leaf.



P170277

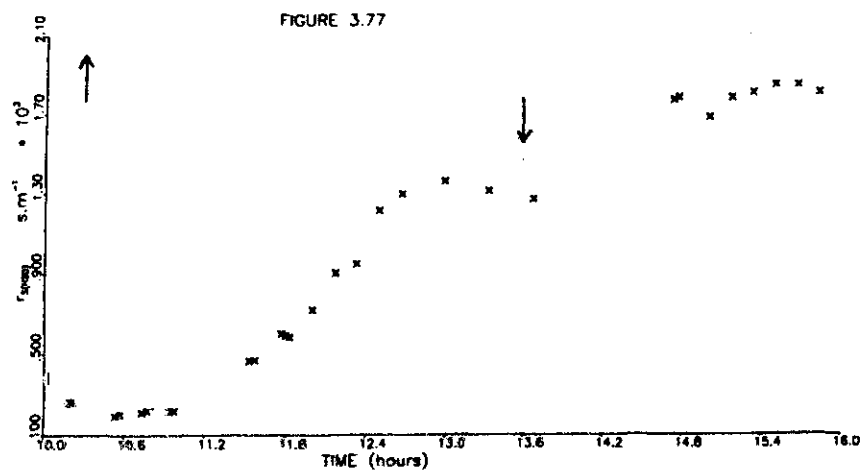
↑ I reduced progressively from 255 to 0

↓ Lights switched on, I = 245

C_i = variable as I altered

$-\psi_{air}$ = 2010 - 2190

T_L = 25.0 - 29.0



P180277

↑ I reduced progressively from 250 to 0

↓ Leaf supplied with O_2 -free air

C_i = variable as I altered

$-\psi_{air}$ = 1120 - 1500

T_L = 25.4 - 28.0

the dark. In figure 3.74 results are shown for a bean leaf in normal and O_2 -free ambient air. Again, values of r_s were lower in O_2 -free air at equivalent values of $-\psi_{air}$ and C_i . The effect of O_2 -free air on light response curves for another bean leaf are shown in figure 3.75. In contrast to the results for oak and tobacco, there was no stomatal closure in the dark in O_2 -free air when $-\psi_{air}$ values were low (figure 3.75 b); r_s values were measured for two hours at zero I but no closure occurred. At a slightly higher level of $-\psi_{air}$, however, stomatal closure did occur in the dark (figure 3.75a). Figure 3.76 shows the time course of stomatal closure of a bean leaf in normal air, caused by decreasing I; when the lights were switched on again, the stomata re-opened. Figure 3.77 shows stomatal closure of the same leaf, caused by decreasing I. The leaf was then supplied with O_2 -free air, but re-opening did not occur.

The results thus indicated that, for all three species, lack of O_2 produced an opening effect in the light, but would not prevent closure in the dark, nor could closed stomata be re-opened in response to O_2 -free air.

3.4 Effects of the $-\psi_{\text{air}}$ response on transpiration, E_T

The results of simultaneous measurements of E_T as oak stomata closed in response to $-\psi_{\text{air}}$ are shown in figures 3.78-3.81. As $-\psi_{\text{air}}$ increased, $r_{s(\text{H}_2\text{O})}$ began to increase tending to reduce E_T . However, the increase in $-\psi_{\text{air}}$ also caused an increase in evaporative demand tending to increase E_T . The net result of these two antagonistic demands was that at low values of $-\psi_{\text{air}}$ the increase in E_T due to increased evaporative demand more than offset the restriction of E_T imposed by closing stomata. As $-\psi_{\text{air}}$ continued to increase, E_T reached a maximum and then began to decline with further increase in $-\psi_{\text{air}}$ (figures 3.78 and 3.79). Figures 3.80 and 3.81 show only the closing response because initial values of $-\psi_{\text{air}}$ were not low enough to cause the initial increase in E_T . Similar responses are shown for ash (figure 3.82); bean (figure 3.83); and tobacco (figures 3.84 and 3.85). The reductions in E_T after a maximum has been reached, in response to increased $-\psi_{\text{air}}$, is crucial to the proposed mechanism of the $-\psi_{\text{air}}$ response outlined later.

FIGURES 3.78 - 3.85 Effects of the stomatal response to $-\psi_{\text{air}}$ on the rate of transpiration, E_T .

Figure 3.78 Q110576

$$C_i = 0.38 - 0.46$$

$$T_L = 22.0 - 26.0$$

$$I = 160 - 240$$

Figure 3.79 Q300677

$$C_i = 0.38 - 0.42$$

$$T_L = 31.5 - 33.5$$

$$I = 235 - 245$$

Figure 3.80 Q030877

$$C_i = 0.44 - 0.52$$

$$T_L = 27.9 - 29.9$$

$$I = 108 - 124$$

Figure 3.81 Q050877

$$C_i = 0.33 - 0.43$$

$$T_L = 26.5 - 30.5$$

$$I = 188 - 200$$

Figure 3.82 F180877-F190877

$$C_i = 0.20 - 0.26$$

$$T_L = 29.5 - 30.5$$

$$I = 21 - 114$$

Figure 3.83 P110277

$$C_i = 0.20 - 0.36$$

$$T_L = 26.5 - 27.5$$

$$I = 296 - 300$$

Figure 3.84 N110278-N150278

$$C_i = 0.41 - 0.45$$

$$T_L = 26.7 - 28.7$$

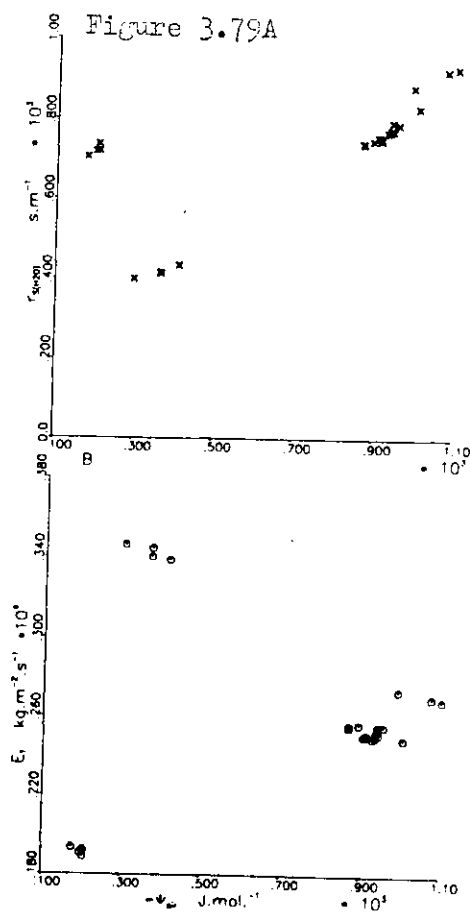
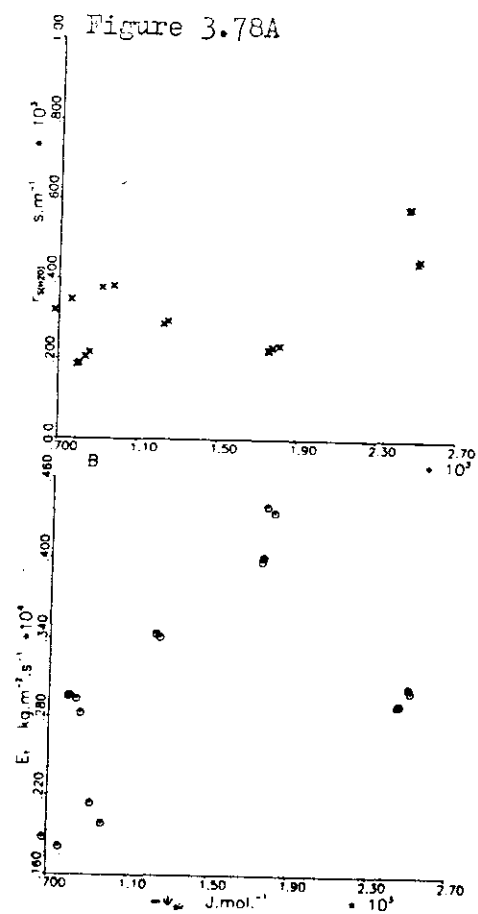
$$I = 29 - 231$$

Figure 3.85 N160278

$$C_i = 0.35 - 0.41$$

$$T_L = 27.0 - 29.0$$

$$I = 38 - 226$$



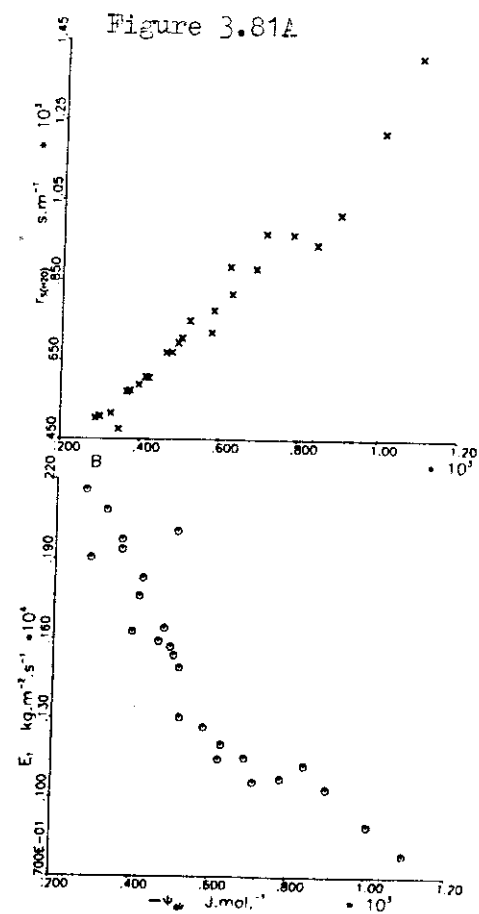
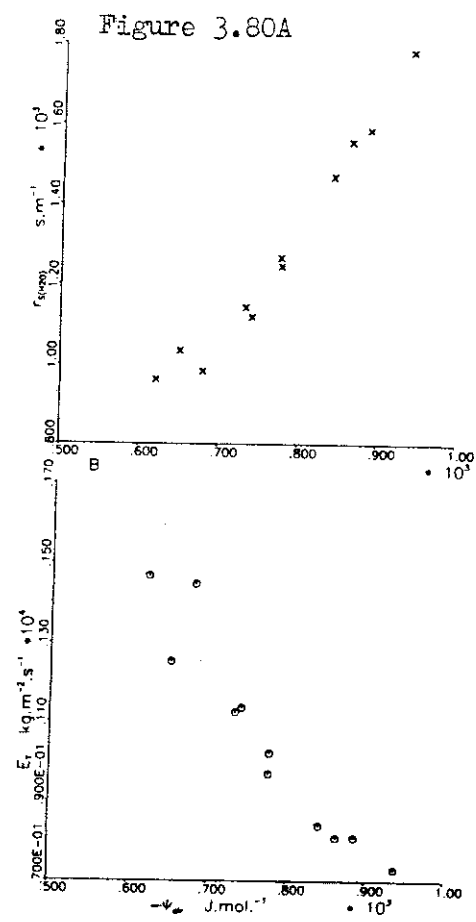


Figure 3.82A

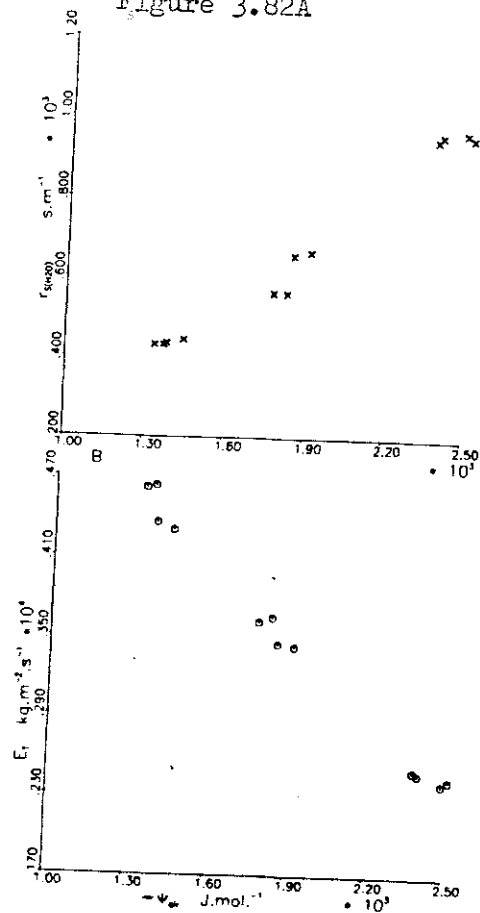
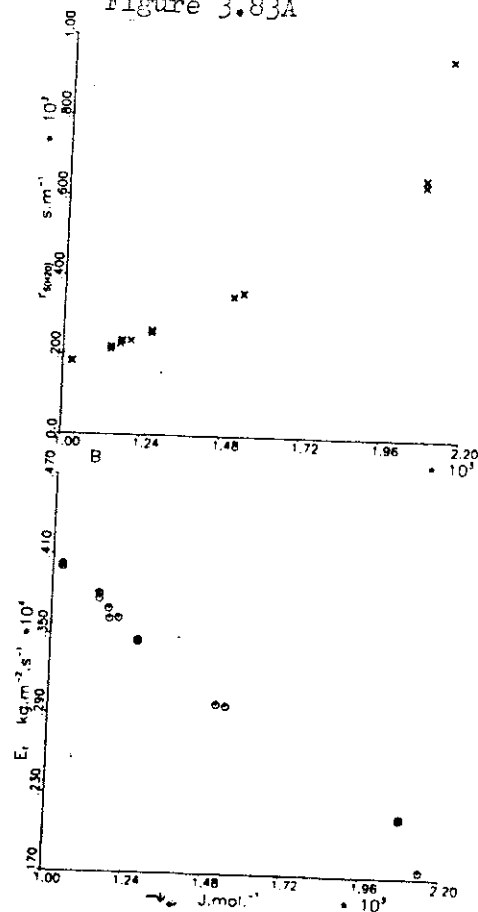
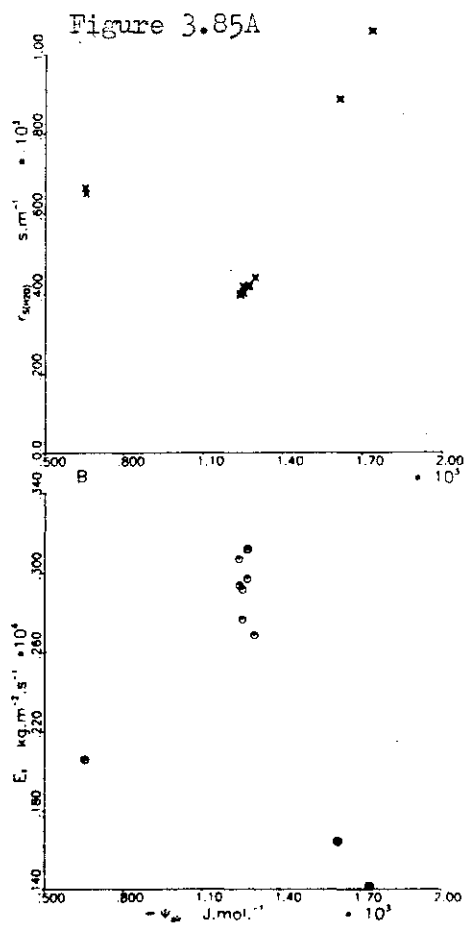
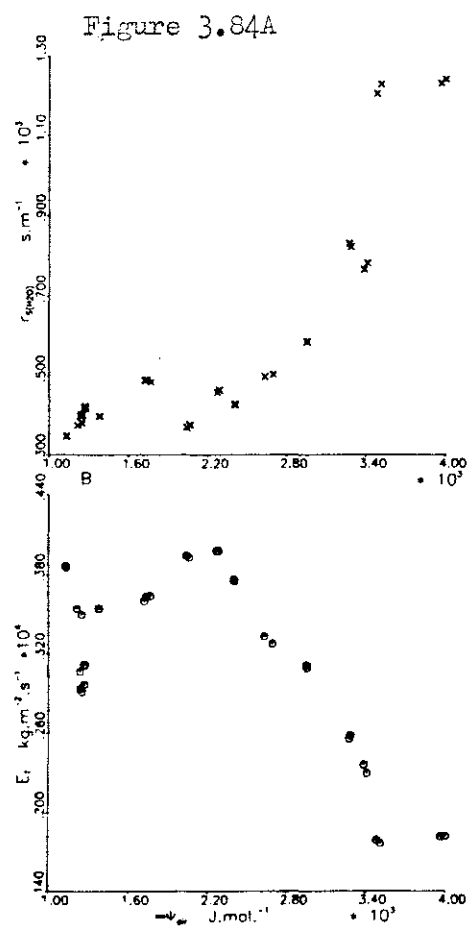


Figure 3.83A





CHAPTER 4: DISCUSSION

DISCUSSION

The work reported has indicated that two important mechanisms in the control of stomatal aperture of all five species studied were the responses to $-\psi_{\text{air}}$ and C_i , and that most of the movements of open stomata are attributable to one or other, or a combination of these responses. Many other reports, covering a wide range of species, have also indicated that stomata respond directly to humidity, e.g. Lange et al (1971), Schulze et al (1972 & 1975a), Hall & Kaufmann (1975a), Neilson & Jarvis (1975), Sheriff (1979), Landsberg & Butler (1980). The response to intercellular CO_2 concentration has been considered an important factor in controlling stomatal aperture by some authors e.g. Ketellapper (1963), Meidner & Mansfield (1968), Raschke (1975). However, in some reports variations in C_i did not substantially affect stomatal aperture, e.g. Zelitch (1969), Neilson & Jarvis (1975), Ng (1978).

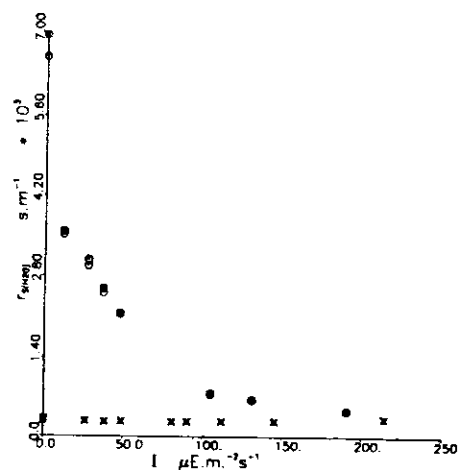
The way in which the $-\psi_{\text{air}}$ and C_i responses interact is indicated by the regression results (e.g. figure 3.66), and this interaction may explain some of the controversy regarding the responsiveness of stomata to C_i . Clearly, at low values of $-\psi_{\text{air}}$ (i.e. in humid air) there is very little response to C_i over a natural range, in any of the species studied (Section 3.2), but there is a pronounced effect over a natural range of C_i as $-\psi_{\text{air}}$ increases. This would agree with Hall & Kaufmann's (1975a) findings with Sesamum indicum that stomata responded little to C_i in humid air, but did exhibit a response to C_i when humidity decreased. These authors also reported that low C_i decreased, but did not eliminate the stomatal response to humidity gradient (difference between leaf and air humidities), and this too is consistent with the results shown in figure 3.66. The interaction of $-\psi_{\text{air}}$ and C_i responses could also account for the observation that

humidity effects are more pronounced in low light (presumably at high C_i) than at high light intensity (low C_i) reported by Davies & Kozlowski (1974) with Fraxinus americana and Acer saccharum, and by Kaufmann (1976) with Picea engelmannii. Some of the variability of the response to C_i in turgid leaves may also be attributed to differences between species (Mansfield, 1971); to a requirement for the presence of abscisic acid (ABA) for the stomata to be sensitive to C_i (Raschke, 1975) and to variations in the environments of experimental plants (Sheriff, 1979).

The stomatal responses to $-\Psi_{\text{air}}$ and C_i are, in most instances, antagonistic: most plants, in the light, require a supply of CO_2 , but also need to minimize water loss. The availability of water is a limiting resource whereas there is no restriction to the availability of CO_2 in the atmosphere. It may thus be expected that the water conserving response (to $-\Psi_{\text{air}}$) would be able to over-ride the response to the depletion of CO_2 in the sub-stomatal cavities and intercellular spaces of the leaf. This strategy, which is of obvious advantage to the plant, is shown to occur in the three tree species in figures 3.38-3.41, and is also predicted by the regression results (figures 3.35-3.67): even at low values of C_i , high values of r_g (indicating at least partial stomatal closure) can occur if the air is relatively dry. Confirmation that the $-\Psi_{\text{air}}$ response can over-ride the C_i response with oak is given by data of the type shown in figure 4.1, which shows the stomatal response in CO_2 -free ambient air. As outlined in Section 3.2/1 (figures 3.5 and 3.6) oak stomata were prevented from closing in the dark by supplying CO_2 -free air at constant, relatively high humidity. However, when $-\Psi_{\text{air}}$ was increased as I fell, then stomatal closure occurred. Further, the stomata of both leaves shown in figure 4.1 remained closed in the dark in CO_2 -free air at high

FIGURE 4.1

Stomatal response to CO_2 -free air depends upon the level of $-\psi_{\text{air}}$.



x at constant $-\psi_{\text{air}}$ (Q080676)

$$C_i = 0.066$$

$$-\psi_{\text{air}} = 2600$$

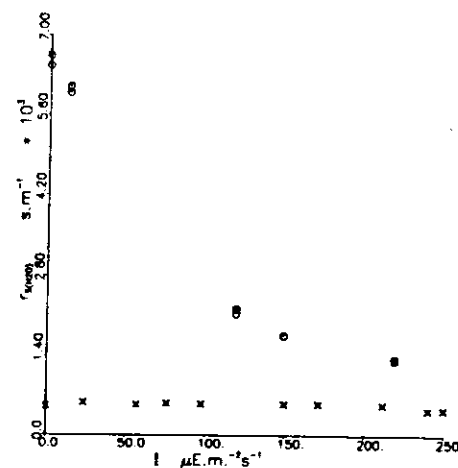
$$T_L = 30.5 - 31.5$$

o with increasing $-\psi_{\text{air}}$ (Q150676)

$$C_i = 0.051$$

$$-\psi_{\text{air}} = \begin{cases} 1950 & (\text{at } I = 200) \\ 5050 & (\text{at } I = 0) \end{cases}$$

$$T_L = 27.6 - 29.6$$



x at constant $-\psi_{\text{air}}$ (Q240676)

$$C_i = 0.030$$

$$-\psi_{\text{air}} = 2300 - 2500$$

$$T_L = 30.5 - 31.5$$

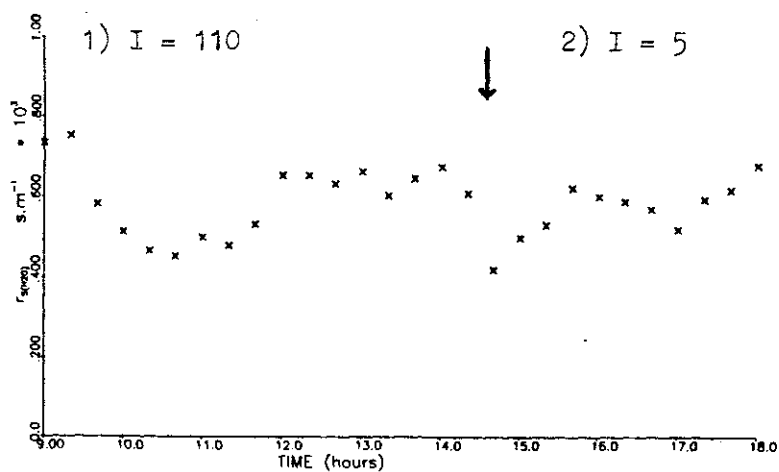
o with increasing $-\psi_{\text{air}}$ (Q170676)

$$C_i = 0.080$$

$$-\psi_{\text{air}} = \begin{cases} 3600 & (\text{at } I = 220) \\ 5500 & (\text{at } I = 0) \end{cases}$$

$$T_L = 27.6 - 28.0$$

FIGURE 4.2 An example of lack of response of $r_{s(H_2O)}$ to a change in C_i at a low value of $-\psi_{air}$.
The arrow indicates when the change in I occurred.



F050877

1) $C_i = 0.33 - 0.43$
 $-\psi_{air} = 400 - 700$
 $T_L = 29.0 - 31.0$

2) $C_i = 0.52 - 0.66$
 $-\psi_{air} = 550 - 950$
 $T_L = 25.0 - 27.0$

$-\psi_{\text{air}}$, implying that there is a mechanism to detect $-\psi_{\text{air}}$ when stomata are closed. Such a mechanism indicates a direct response to $-\psi_{\text{air}}$ which cannot be operating via the bulk leaf water status (see below). Figure 4.2 shows data for an ash leaf, which followed the time course of $r_{\text{s(H}_2\text{O)}}$ when I was changed from $110 \mu\text{E.m}^{-2}.\text{s}^{-1}$ to the very low value of $5 \mu\text{E.m}^{-2}.\text{s}^{-1}$, while $-\psi_{\text{air}}$ was maintained at a low level of approximately 600 J.mol^{-1} . The change in I brought about a change in C_i from $0.38\text{E-}3$ to $0.59\text{E-}3 \text{ kg.m}^{-3}$, but $r_{\text{s(H}_2\text{O)}}$ was not affected; C_i had no effect at this level of $-\psi_{\text{air}}$. At the other end of the C_i scale, the results shown in figures 3.38-3.43 and the regression results (e.g. figures 3.66, 3.67) indicated that stomata can remain open at elevated levels of C_i , if the ambient air is humid.

There is a fundamental difference between the C_i and $-\psi_{\text{air}}$ responses. The C_i response is equivalent to a negative feedback process which tends to maintain a level of C_i appropriate to the prevailing light conditions: decreased I would lead to a reduction in rate of photosynthesis and an increase in C_i with a consequent decrease in r_{s} . The response to $-\psi_{\text{air}}$, however, is different in the region where both r_{s} and E_{T} are decreasing as $-\psi_{\text{air}}$ increases (figures 3.78-3.85), since reduced r_{s} causes a decrease in E_{T} which tends to cause a further increase in $-\psi_{\text{air}}$. Because such a system would tend to lead to complete stomatal closure (given that the $-\psi_{\text{air}}$ response can over-ride the C_i response) as soon as $-\psi_{\text{air}}$ started to increase, the $-\psi_{\text{air}}$ response requires a sensor external to the stoma itself, otherwise an equilibrium would never be obtained.

The interaction of the $-\psi_{\text{air}}$ and C_i responses is of adaptive significance to the plant in that it provides a balanced regulation of dry matter production against water loss. From the point of view of the plant, it is essential that the stomata close before too

much water is lost, and that dry matter production is permitted as soon as conditions are favourable. The direct response to $-\psi_{\text{air}}$ is rapid and reversible in turgid leaves (figures 3.19-3.22) and allows stomata to respond to current conditions with minimum delay. At high light intensities the $-\psi_{\text{air}}$ and C_i responses are antagonistic since a closure in response to $-\psi_{\text{air}}$ would cause C_i to decrease. However, the response to $-\psi_{\text{air}}$ would be expected to over-ride the C_i response and stomatal aperture would thus be reduced, which will have the effect of increasing water use efficiency (w.u.e.) because the reduction in water loss will be proportionally greater than the reduction in CO_2 uptake (Hall & Kaufmann, 1975b; Cowan, 1977; Farquhar *et al.*, 1980). At low light intensities the stomatal response again depends upon the level of $-\psi_{\text{air}}$. In dry air the C_i and $-\psi_{\text{air}}$ responses combine to accelerate stomatal closure (e.g. figures 3.66 and 3.67). However, in humid air stomata remain at least partially open, even when C_i is large (figures 3.38-3.43 and 3.66, 3.67). There appears to be no penalty in stomata remaining open in humid air and it is possible that such a response would permit the entry of O_2 into the leaf to support aerobic respiration (there would be little inter- and intracellular O_2 derived from photosynthesis at low light levels).

Although no direct assessments of bulk leaf water potential, ψ_L , were made in this study, the data given in Section 3.2/2 suggested a direct humidity response independent of ψ_L , as found for a range of species by Schulze *et al.* (1972), Davies & Kozlowski (1974), Hall & Kaufmann (1975a, b), Lange (1975), Neilson & Jarvis (1975), Warrit *et al.* (1980), Jarvis (1980). Further evidence of the importance of $-\psi_{\text{air}}$ was provided by the data in figure 4.1 which showed that oak stomata would remain open in CO_2 -free air in the dark at constant, small

values of $-\psi_{\text{air}}$, but would close and remain closed if $-\psi_{\text{air}}$ was large, implying that there was a mechanism which detected $-\psi_{\text{air}}$ even when stomata were closed. This clearly indicates a " $-\psi_{\text{air}}$ detector" which is independent of the transpirational flux through the stomata, and not dependent on the negative feedback system involving ψ_L .

The data shown in figures 3.78-3.85 also shows that the $-\psi_{\text{air}}$ response is independent of the rate of transpiration: the increase in r_s in response to $-\psi_{\text{air}}$ resulted in a decrease in rate of transpiration despite an increase in the evaporating power of the air. Thus, the response to $-\psi_{\text{air}}$ is a response which cannot be a function of transpirational flux since if this were the case an initial closing response as transpiration began to increase would reduce the transpirational flux, preventing further stomatal closure (Schulze et al, 1972; Farquhar, 1978). Transpiration rate could not continue to decline as $-\psi_{\text{air}}$ increased further, but would level off at a maximum rate (Jarvis, 1980). The reduction in E_T with increasing $-\psi_{\text{air}}$ reported in the present work has also been shown by West & Gaff (1976) for seedlings of Malus sylvestris, Ng (1978) with Pinus sylvestris, and by Farquhar et al (1980) with Nicotiana glauca and Corylus avellana.

There is well documented evidence for several species that there is relatively high hydraulic resistance between the vascular tissue and the epidermis via the mesophyll (e.g. Sheriff & Meidner, 1974; Meidner, 1975; Sheriff, 1980; Warrit et al, 1980). Given that the water relations of the epidermis occur with a degree of isolation from the remainder of the leaf, and that the stomatal response to $-\psi_{\text{air}}$ must be brought about by changes in the turgor potentials of the guard cells and adjacent epidermal cells, then the $-\psi_{\text{air}}$ response must be due to water relations "local" to the epidermis. Two possibilities exist:

1. Evaporation from the inner surfaces of the epidermis (including guard cells), i.e. internal to the stomatal pore, cause the necessary changes in epidermal water status.
2. Evaporation from the outer surfaces of the epidermal cells, external to the stomatal pore, are responsible for the changes in epidermal water status which cause the response to $-\psi_{\text{air}}$.

Possibility (1) could not explain the response of r_s to $-\psi_{\text{air}}$ since a reduction in stomatal aperture would increase the humidity of the leaf air spaces, reducing evaporation from the epidermis into the sub-stomatal cavities, and producing a feedback response linked to the rate of transpirational flux through the stomata. Using possibility (2) above, the following hypothesis, shown schematically in figure 4.3, provides a possible mechanism of the stomatal response to $-\psi_{\text{air}}$.

The crucial assumption of this hypothesis is that there is a high hydraulic resistance to water flow between the epidermis and the mesophyll ($r_{h(H_2O)}$), and evidence in support of this assumption has been cited above. Since there are large effects of $-\psi_{\text{air}}$ on $r_{s(H_2O)}$ and E_T assumed to occur without consequent changes in ψ_L , the mesophyll resistance ($r_{m(H_2O)}$) must either be small or inversely proportional to E_T . The model operates as follows: when the stomata are open there is a transpirational flux through the stomata (E_T), the source of water being the wet cell walls of the mesophyll tissue and the cell walls of the epidermal cells lining the sub-stomatal cavities (Meidner, 1975; Aston & Jones, 1976). In addition, there is direct evaporation from the outer surfaces of the epidermal (including guard) cells (E_c , the cuticular transpiration). If the ambient air becomes drier, both E_T and E_c begin to increase with the increase in evaporating power of the air. However, since $r_{h(H_2O)}$ is large, a water stress develops in the epidermis which is independent of ψ_L . This is manifested as a decrease

FIGURE 4.3

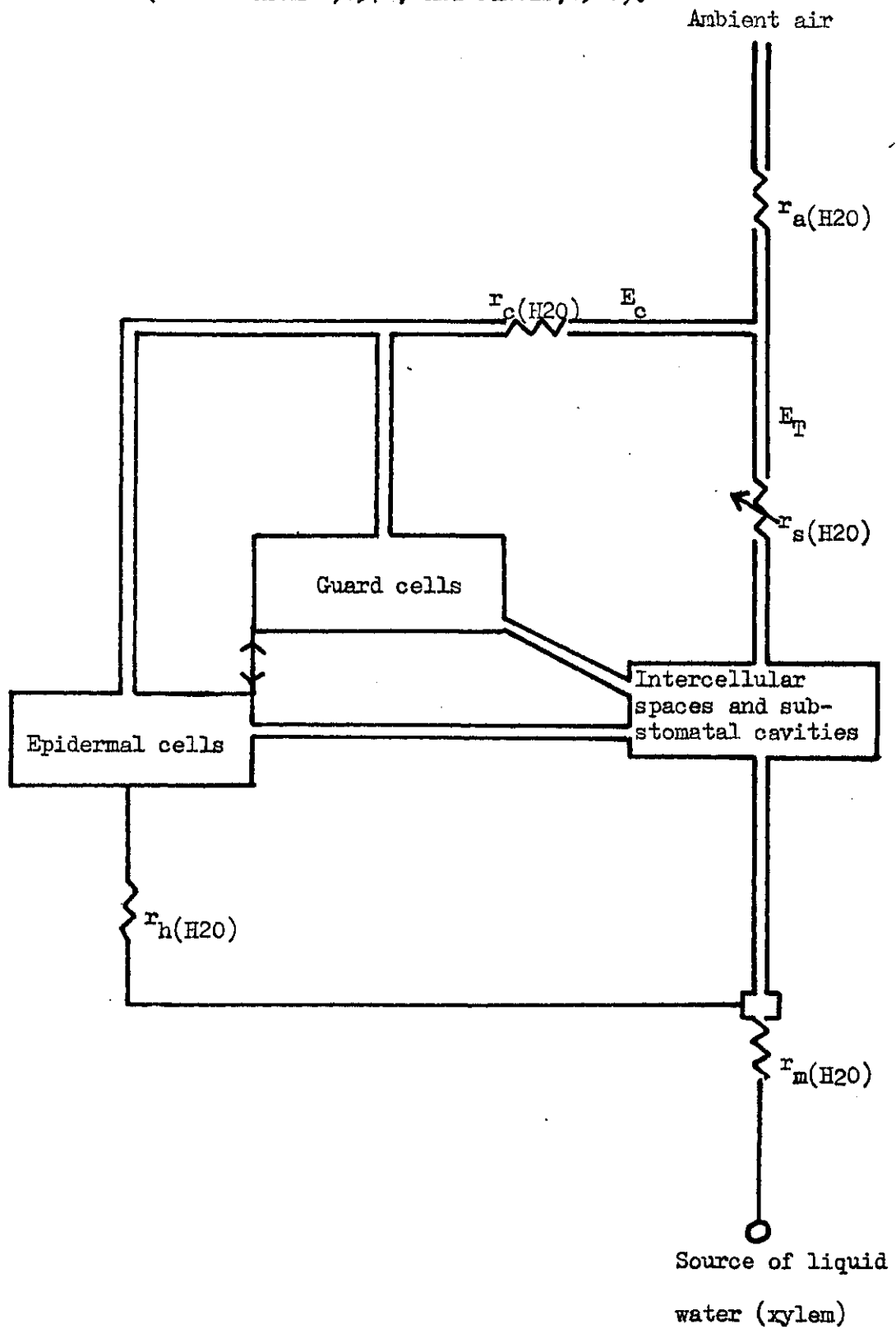
key:

$r_{a(H_2O)}$	boundary layer resistance to water vapour transfer				
$r_{c(H_2O)}$	cuticular	"	"	"	"
$r_{s(H_2O)}$	stomatal	"	"	"	"
$r_{h(H_2O)}$	resistance to liquid water flow between mesophyll and epidermis				
$r_m(H_2O)$	mesophyll resistance to liquid water flow				
E_T	transpirational flux through stomata				
E_c	direct evaporative flux from epidermal and guard cells				

Pathways of liquid water flow: _____

" " water vapour flow: =====

FIGURE 4.3 The mechanism of the stomatal response to $-\psi_{\text{air}}$
(after Raschke, 1970, and Jarvis, 1980).



in the water content and turgor pressure of the guard cells resulting in stomatal closure and a decrease in E_T . As long as E_c exceeds the rate at which water can flow from the mesophyll to the epidermis, the stomatal aperture will be inversely proportional to $-\Psi_{air}$. If $-\Psi_{air}$ decreases, E_c will decrease and the process will be reversed. The stomatal response would thus be controlled by $-\Psi_{air}$ via the control of E_c assuming the resistance to cuticular transpiration ($r_{c(H_2O)}$) is constant. Such a humidity sensor is buried below the boundary layer in which the humidity is likely to be higher than that of the ambient air. Strictly speaking, the humidity sensor would be sensing conditions in the boundary layer. However, the humidity of the boundary layer is itself a function of the humidity of the ambient air and the boundary layer resistance, $r_{a(H_2O)}$.

The threshold-type response in which stomata are independent of bulk leaf water status until a threshold value of Ψ_L is reached (Hsiao, 1973) can perhaps also be explained in terms of $r_{h(H_2O)}$. The sudden response at the threshold level could reflect the fact that the difference in water potential between the mesophyll and the epidermis has increased to such an extent as the leaf has dried out, that the resistance to flow, $r_{h(H_2O)}$, has been overcome causing a rapid withdrawal of water from the guard cells and resulting in stomatal closure.

The mechanism which causes the C_i effect on stomata is not known, but it is generally thought that the response is to CO_2 concentration in the guard cells (Katellaper, 1963), which is mediated by C_i (Raschke, 1975). The opening of stomata in CO_2 -free air (figures 3.5-3.7), and the fact that stomatal responses to CO_2 have been reported in species which do not have guard cell chloroplasts (Nelson & Mayo, 1975) suggests that photosynthesis in guard cells is not necessary for stomata to respond to CO_2 , and the response to C_i is probably due to

an effect on guard cell metabolism. Several theories have been suggested, e.g. Raschke (1975), Thorpe & Milthorpe (1977), Willmer & Rutter (1977), but these could not be tested in the current work since no attempt was made to measure the metabolic processes occurring in the guard cells.

The results of Mansfield (1965) suggested an independent effect of light on r_s , since stomata opened more widely in white light than they did in the dark in CO_2 -free air. The results shown in figures 3.5 and 3.6 for oak do not support this: at equivalent values of $-\psi_{\text{air}}$, there was little difference between r_s of illuminated leaves and those same leaves in the dark in CO_2 -free air. In the results outlined in Section 3.2/1 (figures 3.8-3.16), no direct effect of I was detectable, although effects at very low levels of I could not be investigated. The regression gave a good fit to data in the light, though no independent I effect was incorporated, but often underestimated r_s in the dark at normal levels of ambient CO_2 and O_2 . The results of limited experiments at low levels of blue and red light with oak and tobacco (Section 3.3/2) suggested that values of r_s were lower in blue light and that the regression gave a better fit to these data. A possible explanation is that there is a blue light requirement for stomatal opening at normal levels of ambient CO_2 , and that this requirement is qualitative and not quantitative. In this way, r_s would be independent of I as long as the blue light requirement was met, hence the regressions would give a good fit. This blue light requirement is not qualitative since the regressions gave an equally good fit over a wide range of I and presumably the blue light component increases with I . Unfortunately there is insufficient data in the present work for this hypothesis to be fully assessed, but it is consistent with the data of West & Gaff (1976) who showed that seedling leaves of Malus sylvestris had a

low light requirement for minimum r_s of $10-20 \text{ W.m}^{-2}$ (which represented only 3% of the full sunlight), but at values of I exceeding this, r_s was independent of I . The authors also reported that similar results were obtained with several other species by Gaastra (1959), Kuiper (1961), Kanemasu and Tanner (1969), Turner (1970). Meidner & Mansfield (1968) reported that blue light produces an opening at very low light intensity and they suggested that such a response would maintain opening and avoid large fluctuations in r_s in cloudy conditions when I was changing continuously.

The effects of varying ambient O_2 concentration were investigated with three species: oak, tobacco and bean, and the results indicated that lack of O_2 produced an opening effect, but did not prevent stomatal closure in the dark (figures 3.71-3.77). In none of the species did lack of O_2 open stomata which were closed, indicating that oxidative phosphorylation is necessary for stomatal opening to occur from a closed position, as found for a variety of species by Heath & Orchard (1956), Zelitch (1965), Meidner & Mansfield (1968), Burrows & Milthorpe (1975). The maintenance of stomatal opening in O_2 -free conditions agrees with Meidner & Mansfield's (1968) suggestion that the energy requirement of maintaining open stomata can be met by photophosphorylation in the absence of O_2 . This would explain the stomatal closure which occurred with oak and tobacco (and with bean at higher levels of $-\psi_{\text{air}}$) when leaves were placed in the dark in O_2 -free conditions. The failure of bean stomata to close in O_2 -free air in the dark is puzzling, but can perhaps be explained by the fact that this experiment (figures 3.75b) was carried out at very low values of $-\psi_{\text{air}}$, and opening could have been maintained through a $-\psi_{\text{air}}$ effect.

An important component of the maintenance of stomatal opening under conditions of reduced O_2 concentration is undoubtedly the reduction

in C_i caused by inhibition of photorespiration. Supplying a leaf with reduced O_2 will inhibit photorespiration and hence increase photosynthesis (Moore, 1981) resulting in lower C_i and an opening response. This opening effect is likely to be increased at higher temperatures since photorespiration is normally stimulated at high temperature. The role of O_2 in stomatal movement is further complicated by the fact that supplying a leaf with O_2 -free ambient air in the light does not guarantee intercellular anaerobic conditions. The stimulation of photosynthesis by inhibition of photorespiration may provide enough O_2 for the oxygen requirement for stomatal movement to be met. The position is thus complicated, but the regression results shown in figures 3.68, 3.70, 3.74 predict that at equivalent values of $-\Psi_{air}$ and C_i , lower O_2 concentrations promote stomatal opening.

A direct temperature response was not incorporated into the regressions since no consistent pattern of response was evident over the restricted range of temperatures investigated. The data shown in figures 3.44-3.52 did suggest that there may have been an optimum temperature for oak and ash in the range 29-32°C, but no such suggestion was evident in the results for sycamore or tobacco. Maximum stomatal opening at intermediate temperatures have been reported for several species by other authors, e.g. Hofstra & Hesketh (1969), Neilson & Jarvis (1975), Losch (1977), Ng (1978). Of course, possible direct effects of temperature are very difficult to disentangle because of the indirect role of T_L in all of the processes which affect stomatal movement. Sheriff (1979) has made the comment that since stomatal aperture depends upon guard cell turgor and hence on guard cell metabolism, an increase in T_L might be expected to cause stomatal opening by stimulating metabolic processes. However, the processes and enzymes in the guard cells which would determine such a response

have not been studied extensively. The involvement of temperature in both the $-\Psi_{\text{air}}$ and C_i responses is illustrated by Hofstra & Hesketh's (1969) observation that the T_L response of stomata appeared to be correlated with the responses of photosynthesis and photorespiration, and by Hall & Kaufmann's (1975a, b) data showing that the optimum temperature for net photosynthesis varied depending on humidity differences between leaf and air. Interpretation of possible T_L effects is further complicated by the result that the temperature at which the plant is grown affects its stomatal response (Ludlow & Wilson, 1971; Neilson & Jarvis, 1975). This last observation has further implications in that it could convey to the plant an optimum temperature mechanism which is relevant to local conditions. It is thus very likely that different species have different temperature optima.

SUMMARY

1. The most important responses of stomata appeared to be those to $-\psi_{\text{air}}$ and C_i , and most of the movements of open stomata were attributable to one or other, or a combination of these responses.
2. The response to $-\psi_{\text{air}}$ was a water conserving response whereby an increase in $-\psi_{\text{air}}$ (i.e. air becoming drier) caused an increase in stomatal resistance.
3. The C_i response was equivalent to a negative feedback process whereby an increase in C_i caused an increase in stomatal resistance, the extent of which depended upon the level of $-\psi_{\text{air}}$.
4. At low levels of $-\psi_{\text{air}}$ (i.e. in humid air) there was little response to C_i over a natural range, but there was a pronounced stomatal response to C_i at higher levels of $-\psi_{\text{air}}$.
5. The combination and interaction of the $-\psi_{\text{air}}$ and C_i responses seemed to be of adaptive advantage to the plant by providing a balanced regulation of production against water loss according to the prevailing conditions.
6. The evidence suggested that the $-\psi_{\text{air}}$ response was independent of the transpirational flux through the stomata. A simplified version of the models of Raschke (1970) and Jarvis (1980) was used to illustrate the proposed mechanism of the $-\psi_{\text{air}}$ response in terms of the cuticular transpiration and the resistance to water flow between the mesophyll and the epidermis.
7. A simple multiple regression was used to calculate stomatal resistance in terms of only two factors: $-\psi_{\text{air}}$ and C_i . Correspondence between measured and calculated values was good, particularly at normal O_2 .

concentrations in the light. The regression could be used in a predictive capacity, and could be further refined by the addition of other independent variables, possibly T_L and O_2 concentration, when the responses to these factors are better understood.

8. No direct effect of light was detected, but the results of limited experiments with blue and red light with Quercus petraea and Nicotiana tabacum, together with the regression results, suggested that there may be a blue-light requirement for stomatal opening at normal levels of CO_2 .
9. With Quercus petraea, Nicotiana tabacum, and Phaseolus vulgaris, lack of O_2 had an opening effect on stomata, but did not prevent stomatal closure in the dark (except for one set of Phaseolus results at very low levels of $-\psi_{air}$), and lack of O_2 would not open stomata which were closed.
10. No consistent pattern of direct stomatal response to T_L was evident over the restricted range of temperatures studied. However, data for Quercus petraea and Fraxinus excelsior suggested that there may have been an optimum temperature for these species in the range 29-32°C.

ACKNOWLEDGEMENTS

The work reported in this thesis was carried out under the supervision of Dr. P.W. Mueller whom I would like to thank for his encouragement of the work, his helpful comments on the manuscript, and particularly for his help with the computations. The assistance and advice of Professor A.J. Rutter is gratefully acknowledged, as is the help and patience of Janet Innes. I would also like to express my thanks to the following:

Mr. B.C. Allen

Mr. R.D. Blackwell

Mr. K.J. Collett

Mr. E.E. Green

Mr. C.J. Heijne

Mrs. S. Heijne

Mr. B.F. Scott

Financial support during the period of this work was provided by the Natural Environment Research Council.

The author wishes to acknowledge that the assimilation chambers and parts of the gas flow system and recording apparatus were constructed and assembled by Dr. P.W. Mueller and W.L. Mason Esq. before the work reported here commenced.

REFERENCES

- AKITA, S., & MOSS, D. (1972). Differential stomatal response between C_3 and C_4 species to atmospheric CO_2 concentration and light. Crop Science 12:789-793.
- ASTON, M.J. (1976). Variation of stomatal diffusive resistance with ambient humidity in sunflower (Helianthus annuus). Australian Journal of Plant Physiology 3:489-501.
- ASTON, M.J., & JONES, M.M. (1976). A study of the transpiration surfaces of Avena sterilis L. var. Algerian, using monosilic acid as a tracer for water movement. Planta (Berlin) 130:121-129.
- BARRS, H.D. (1970). Controlled environment studies of the effect of variable atmospheric water stress on photosynthesis, transpiration and water stress of Zea mays L. and other species. In: Proceedings of a symposium on Plant Response to Climatic Factors. Uppsala, Sweden. UNESCO. pp:249-258.
- BISCOE, P.V. (1972). The diffusion resistance and water status of leaves of Beta vulgaris. Journal of Experimental Botany 23: 930-940.
- BJORKMAN, O. (1966). The effect of oxygen concentration on photosynthesis in higher plants. Physiologia Plantarum 19:618-633.
- BURROWS, F.J. & MILTHORPE, F.L. (1975). Stomatal conductance in gas exchange control. In: Water Deficits and Plant Growth, Volume IV (Ed T.T. Kozlowski). Academic Press.
- CAMACHO-B, S.E., HALL, A.E., & KAUFMANN, M.R. (1974). Efficiency and regulation of water transport in some woody and herbaceous species. Plant Physiology 54:169-172.
- CHARTIER, P. (1970). A model of CO_2 assimilation in the leaf. In: prediction and Measurement of Photosynthetic Productivity (Ed. I. Setlik) Proceedings of IBP/PP Technical Meeting, Trebon, 1969. Pudoc. pp:307-316.

- CONWAY, G.R., GLASS, N.R., & WILCOX, J.C. (1970). Fitting non-linear models to biological data by Marquardt's algorithm. Ecology 51: 503-507.
- COWAN, I.R. (1977). Stomatal behavior and environment. Advances in Botanical Research 4:117-228.
- CULMINS, W.R. (1971). On the stomatal response to abscisic acid. Ph.D. Thesis. Michigan State University, East Lansing.
- DAVIES, W.J., & KOZLOWSKI, T.T. (1974). Stomatal responses of five woody angiosperms to light and humidity. Canadian Journal of Botany 52:1525-1534.
- DRAKE, B.G., RASCHKE, K., & SALISBURY, F.B. (1970). Temperatures and transpiration resistances of Xanthium leaves as affected by air temperature, humidity and windspeed. Plant Physiology 46: 324-330.
- FARQUHAR, G.D., SCHULZE, E.D., & KUPPERS, M. (1980). Responses to humidity by stomata of Nicotiana glauca L. and Corylus avellana L. are consistent with the optimisation of carbon dioxide uptake with respect to water loss. Australian Journal of Plant Physiology 7:315-327.
- FREUDENBERGER, H. (1940). Die reaktion der schliesszellen auf kohlen-saure und sauerstoff entzug. Protoplasma 35:15-54.
- FUJINO, M. (1967). Scientific Bulletin of the Faculty of Education, Nagasaki University 18:1-47.
- GAASTRA, P. (1959). Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. Meded. Landbouoges. Wageningen 59:1-68.
- GARDNER, W.R. (1973). Internal water status and plant response in relation to the external water regime. In: Proceedings of a symposium on Plant Response to Climatic Factors (Ed: R.O. Slatyer), Uppsala, Sweden. UNESCO. pp:221-225.

- HALL, A.E., & KAUFMANN, M.R. (1975a). Stomatal response to environment with Sesamum indicum L. Plant Physiology 55:455-459.
- HALL, A.E., & KAUFMANN, M.R. (1975b). Regulation of water transport in the soil-plant-atmosphere continuum. In: Perspectives of Biophysical Ecology (Eds. D.M. Gates & R.B. Schmerl). Ecological Studies Volume 12, Springer-Verlag, Berlin. pp:187-202.
- HALL, A.E., SCHULZE, E.D., & LANGE, O.L. (1976). Current perspectives of steady-state stomatal responses to environment. In: Water and Plant Life: Problems and Modern Approaches (Eds. O.L. Lange, L. Kappen, & E.D. Schulze). Ecological Studies Volume 19. Springer-Verlag, Berlin. pp:169-188.
- HALL, A.J. (1975). The influence of fruit excision and defloration on photosynthesis in Capsicum annuum L. Ph.D. Thesis, Macquarrie University, North Ryde, Australia.
- HEATH, O.V.S. (1948). Control of stomatal movement by a reduction in the normal carbon dioxide content of the air. Nature 161: 179-181.
- HEATH, O.V.S. (1950). The role of carbon dioxide in the light response of stomata I. Journal of Experimental Botany 1:29-62.
- HEATH, O.V.S. (1975). Stomata. Oxford Biology Reader 37.
- HEATH, O.V.S. & MILTHORPE, F.L. (1950). The role of carbon dioxide in the light response of stomata II. Journal of Experimental Botany 1: 227-243.
- HEATH, O.V.S., & ORCHARD, B. (1956). Effects of anaerobic conditions upon stomatal movements- a test of William's hypothesis of the stomatal mechanisms. Journal of Experimental Botany 7:313-325.
- HEATH, O.V.S., & RUSSELL, J. (1954). An investigation of the light responses of wheat stomata with the attempted elimination of control by the mesophyll II. Interactions with carbon dioxide. Journal of Experimental Botany 5:1-15.

- HIRON, R.W.P., & WRIGHT, S.T.C. (1973). The role of endogenous abscisic acid in the response of plants to stress. Journal of Experimental Botany 24:769-781.
- HOFSTRA, G., & HESKETH, J.D. (1969). The effect of temperature on stomatal aperture in different species. Canadian Journal of Botany 47:1307-1310.
- HSIAO, T.C. (1973). Plant response to water stress. Annual Review of Plant Physiology 24:519-570.
- HSIAO, T.C. (1976). Stomatal ion transport. Encyclopedia of Plant Physiology Volume 2, part B (Eds: U. Lüttge & M.G. Pitman). Springer-Verlag, Berlin. pp:195-221.
- HUMBLE, G.D., & RASCHKE, K. (1971). Stomatal opening quantitatively related to potassium transport. Evidence from electron probe analysis. Plant Physiology 48:447-453.
- JARVIS, P.G. (1971). The estimation of resistances to carbon dioxide transfer. In: Plant Photosynthetic Production: Manual of Methods (Eds: Z. Sestak, J. Catsky, P.G. Jarvis). Junk, The Hague.
- JARVIS, P.G. (1980). Stomatal response to water stress in conifers. In: Adaptation of Plants to Water and High Temperature Stress (Eds: N.C. Turner & P.J. Kramer). Wiley-Interscience, New York. pp105-122
- JARVIS, P.G., & CATSKY, J. (1971). General principles of gasometric methods and the main aspects of installation design. In: Plant Photosynthetic Production: Manual of Methods (Eds. Z. Sestak, J. Catsky, P.G. Jarvis). Junk, The Hague.
- JONES, R.J. & MANSFIELD, T.A. (1970). Increases in the diffusion resistances of leaves in carbon dioxide enriched atmosphere. Journal of Experimental Botany 21:951-958.
- KANELMASU, E.T., & TANNER, C.B. (1969). Stomatal diffusion resistance of snap beans II. Effect of light. Plant Physiology 44: 1542-1546.
- KAUFMANN, M.R. (1976). Stomatal response of Engelmann Spruce to humidity, light and water stress. Plant Physiology 57:898-901.

- KETELLAPPER, H.J. (1963). Stomatal physiology. Annual Review of Plant Physiology 14:249-270.
- KOLLER, D. (1970). The partitioning of resistance to photosynthetic CO₂ uptake in the leaf. New Phytologist 69:971-981.
- KOWALLIK, W. (1971). Photosynthesis and photorespiration. (Eds. M.D. Hatch, C.B. Osmond, R.O. Slatyer). Wiley-Interscience, New York. pp:514-522.
- KUBIN, S., & HLADK, L. (1963). An integrating recorder for photosynthetically active radiant energy with improved resolution. Plant and Cell Physiology 4:153ff.
- KUIPER, P.J.C. (1961). The effects of environmental factors on the transpiration of leaves with special reference to stomatal light response. Meded. Landbhoogeschool, Wageningen 61:1-49.
- KUIPER, P.J.C. (1964). Dependence upon wavelength of stomatal movement in epidermal tissue of Senecio odoris. Plant Physiology 39:952-955.
- LANDSBERG, J.J., & BUTLER, D.R. (1980). Stomatal response to humidity: Implications for transpiration. Plant, Cell and Environment 3: 29-33.
- LANGE, O.L., LOSCH, R., SCHULZE, E.D., & KAPPEN, L. (1971). Responses of stomata to changes in humidity. Planta (Berlin) 100:76-86.
- LAWLOR, D.W., & MILFORD, G.F.J. (1975). The control of water and carbon dioxide flux in water stressed sugar beet. Journal of Experimental Botany 26:657-665.
- LEVITT, J. (1976). Physiological basis of stomatal response. In: Water and Plant Life: Problems and Modern Approaches (Eds. O.L. Lange, L. Kappen, E.D. Schulze). Ecological Studies Volume 19. Springer-Verlag, Berlin. pp:160-168.

- LEVY, A. (1964). The accuracy of the bubble meter method for gas flow measurements. Journal of Scientific Instrumentation 41:449-453.
- LIST, R.J. (1958). Smithsonian meteorological tables (6th. edition). Smithsonian Institute, Washington.
- LITTLE, C.H.A., & EIDT, D.C. (1968). Effect of abscisic acid on budbreak and transpiration in woody species. Nature 220:498-499.
- LOMMEN, P.W., SCHWINTZER, C.R., YOCUM, C.S., & GATES, D.M. (1971). A model describing photosynthesis in terms of gas diffusion and enzyme kinetics. Planta (Berlin) 98:195-220.
- LOSCH, R. (1977). Responses of stomata to environmental factors: Experiments with isolated epidermal strips of Polypodium vulgare. I. Temperature and humidity. Oecologia (Berlin) 29:85-97.
- LUDLOW, M.M., & JARVIS, P.G. (1971). Photosynthesis in sitka spruce (Picea sitchensis (Bong) Carr.) I. General characteristics. Journal of Applied Ecology 8:925-953.
- LUDLOW, M.M., & WILSON, G.L. (1971). Photosynthesis of tropical pasture plants. I. Illuminance, carbon dioxide concentration and leaf-air vapour pressure difference. Australian Journal of Biological Science 24:449-470.
- MAERCKER, U. (1965). Zur kenntnis der transpiration der schließzellen. Protoplasma 60:61-78.
- MANFIELD, T.A. (1965). Glycollic acid metabolism and the movements of stomata. Nature 205:617-618.
- MARQUARDT, D.W. (1963). An algorithm for least-squares estimation of non-linear parameters. Journal of the Society of Independent Applied Mathematics 11:431-441.

- MARQUARDT, D.W. (1966). Least squares estimation of non-linear parameters. IBM SHARE Library Distribution No. 309401, MLIN 2, August 1966.
- McCREE, K.J. (1974). Changes in the stomatal response characteristics of grain sorghum produced by water stress during growth. Crop Science 14:273-278.
- MEIDNER, H. (1975). Water supply, evaporation and vapour diffusion in leaves. Journal of Experimental Botany 26:666-673.
- MEIDNER, H., & MANSFIELD, T.A. (1966). Stomatal responses to illumination. Biology Review 40:483-509.
- MEIDNER, H., & MANSFIELD, T.A. (1968). Physiology of stomata. McGraw-Hill, London.
- MILBORROW, B.V. (1974). The chemistry and physiology of abscisic acid. Annual Review of Plant Physiology 25:259-307.
- MITTELHEUSER, C.J., & Van STEVENINCK, R.F.M. (1969). Stomatal closure and inhibition of transpiration induced by (RS)-abscisic acid. Nature 221:281-282.
- MONTEITH, J.L. (1965). Evaporation and environment. Symposia of The Society of Experimental Biology 19:205-234.
- MOORE, P. (1981). The varied ways plants trap the sun. New Scientist 12:394-397.
- MOSS, D.N. (1964). Optimum lighting of leaves. Crop Science 4:131-136.
- NEILSON, R.E., & JARVIS, P.G. (1975). Photosynthesis in sitka spruce (Picea sitchensis (Bong) Carr). IV. Response of stomata to temperature. Journal of Applied Ecology 12:879-891.
- NELSON, S.D. & MAYO, M.M. (1975). The occurrence of functional non-chlorophyllous guard cells in Paphiopedilum spp. Canadian Journal of Botany 53:1-7.

- NG, P.A.P. (1978). Response of stomata to environmental variables in Pinus sylvestris L. Ph.D. Thesis, University of Edinburgh, Edinburgh, U.K.
- NICHIPOROVICH, A.A. (1954). Fotosintez i teoriya polucheniya vyokikh urozhaev. (Photosynthesis and theory of high yields). Timiryazevski Chteniya 15:1-93.
- PALLAS, J.E. (1965). Transpiration and stomatal opening with changes in carbon dioxide content of the air. Science 147:171-173.
- PENNY, M.G., & BOWLING, D.J.F. (1974). A study of potassium gradients in the epidermis of intact leaves of Commelina communis L. in relation to stomatal opening. Planta (Berlin) 119:17-25.
- PERRIER, A. (1971). Leaf temperature measurement. In: Plant Photosynthetic Production: Manual of Methods. (Eds. Z. Sestak, J. Catsky, P.G. Jarvis). Junk, The Hague. pp:632-671.
- POWELL, R.W. (1940). Further experiments on the evaporation of water from saturated surfaces. Transcripts of The Institute of Chemical Engineers 18:36-55.
- RABINOWITCH, E.E. (1951). Photosynthesis and related processes. Inter-Science, New York.
- RASCHKE, K. (1967a). Der einfluss von rot und blau licht auf die ofrnungs und schliess geschwindigkeit der stomata von Zea mays L. Naturwissenschaften 54:72-75.
- RASCHKE, K. (1967b). Ber. Deut. Bot. Ges. 80:138-144.
- RASCHKE, K. (1970). Stomatal responses to pressure changes and interruptions in the water supply of detached leaves of Zea mays L. Plant Physiology 45:415-423.

- RASCHKE, K. (1973). Absciscic acid sensitizes stomata to CO₂ in leaves of Xanthium strumarium L. Proceedings of the 8th International Conference on Plant Growth Regulators, Tokyo 1973. pp:1151-1158.
- RASCHKE, K. (1974). Plant Research '73, MSU/AEC Plant Research Laboratory, Michigan State University. pp:23-28.
- RASCHKE, K. (1975). Stomatal action. Annual Review of Plant Physiology 26:309-340.
- RASCHKE, K., & DITTRICH, P. (1977). ¹⁴C carbon dioxide fixation by isolated leaf epidermes with stomata open or closed. Planta (Berlin) 134:69-75.
- RASCHKE, K., & HUMBLE, G.D. (1973). No uptake of anions required by opening stomata of Vicia faba: Guard cells release hydrogen ions. Planta (Berlin) 115:47-57.
- RASCHKE, K., HANEBUTH, W.F., & FARQUHAR, G.D. (1978). Relationship between stomatal conductance and light intensity in leaves of Zea mays L., derived from experiments using the mesophyll as shade. Planta (Berlin) 139:73-77.
- RASCHKE, K., ZEEVAART, J.A.D., PIERCE, M., & POPIELA, C.C. (1974). Plant Research '73, MSU/AEC Plant Research Laboratory, Michigan State University. pp:28-29.
- REDSHAW, A.J., & MEIDNER, H. (1972). Effects of water stress on the resistance to uptake of carbon dioxide in tobacco. Journal of Experimental Botany 23:229-240.
- REED, K.L., HAMERLY, E.R., DINGER, B.E., & JARVIS, P.G. (1976). An analytical model for field measurement of photosynthesis. Journal of Applied Ecology 13:925-942.

- SCARTH, G.W. (1932). Mechanism of the action of light and other factors on stomatal movement. Plant Physiology 7:481-504.
- SCARTH, G.W., & SHAW, M. (1951). Stomatal movement and photosynthesis in Pelargonium I. Plant Physiology 26:207-225.
- SCHULZE, E.D., LANGE, O.L., BUSCHBOM, U., KAPPEN, L., & EVENARI, M. (1972). Stomatal responses to changes in humidity in plants growing in the desert. Planta (Berlin) 108:259-270.
- SCHULZE, E.D., LANGE, O.L., EVENARI, M., KAPPEN, L., & BUSCHBOM, U. (1974). The role of air humidity and leaf temperature in controlling stomatal resistance of Prunus armeniaca L. under desert conditions. I. A simulation of the daily course of stomatal resistance. Oecologia (Berlin) 17:159-170.
- SCHULZE, E.D., LANGE, O.L., EVENARI, M., KAPPEN, L., & BUSCHBOM, U. (1975b). The role of air humidity and leaf temperature in controlling stomatal resistance of Prunus armeniaca L. under desert conditions. III. The effect on water use efficiency. Oecologia (Berlin) 19:303-314.
- SCHULZE, E.D., LANGE, O.L., KAPPEN, L., BUSCHBOM, U., & EVENARI, M. (1973). Stomatal responses to changes in temperature at increasing water stress. Planta (Berlin) 110:29-42.
- SCHULZE, E.D., LANGE, O.L., KAPPEN, L., EVENARI, M., & BUSCHBOM, U. (1975a). The role of air humidity and leaf temperature in controlling stomatal resistance of Prunus armeniaca L. under desert conditions. II. The significance of leaf water status and internal CO₂ concentration. Oecologia (Berlin) 18:219-233.
- SESTAK, Z., CATSKY, J., & JARVIS, P.G. (1971). Plant Photosynthetic Production: Manual of Methods. Junk, The Hague.
- SEYBOLD, A. (1962). Ergebnisse und probleme pflanzlicher transpirationanalyse. Jh. Heidelberger Akad. Wiss. 1961/2:5-8.

SHERIFF, D.W. (1979). Stomatal aperture and the sensing of the environment by guard cells. Plant, Cell and Environment 2:15-22.

SHERIFF, D.W., & MEIDNER, H. (1974). Water pathways in leaves of Hedera helix L. and Tradescantia virginiana L. Journal of Experimental Botany 25:1147-1156.

SLATYER, R.O. (1967). Plant-water relationships. Academic Press, London.

SNEDECOR, G.W., & COCHRAN, W.G. (1971). Statistical Methods. Iowa State University Press. 7th. edition.

STALFELT, M.G. (1929). Pulsierende blattgewebe. Planta (Berlin) 7:720-734.

STALFELT, M.G. (1955). The stomata as a hydrophotic regulator of the water deficit of the plant. Physiologia Plantarum 8:572-593.

THOM, A.S. (1968). The exchange of momentum, mass and heat between an artificial leaf and airflow in a wind tunnel. Quarterly Journal of The Royal Meteorological Society 94:44-55.

THORPE, N., & MILTHORPE, F.L. (1977). Stomatal metabolism: CO₂ fixation and respiration. Australian Journal of Plant Physiology 4:611-621.

TREHARNE, K.J., & NELSON, C.J. (1975). Effect of growth temperature on photosynthetic and photorespiratory activity in tall fescue. In: Environmental and Biological Control of Photosynthesis (Ed. R. Marcelle). Junk, The Hague. pp:61-69.

TURNER, N.C. (1970). Responses of adaxial and abaxial stomata to light. New Phytologist 69:647-653.

TURNER, N.C. (1974). In: Mechanisms of Regulation of Plant Growth (Eds. R.L. Bielski, A.R. Ferguson, M.M. Cresswell). Bulletin 12, Royal Society of New Zealand, Wellington. pp:423-432.

WALKER, D.A., & ZELITCH, I. (1963). Some effects of metabolic inhibitors and anaerobic conditions on stomatal movement. Plant Physiology 38:390-396.

- WARBURG, O. (1920). Über die geschwindigkeit der photochemischen kohlen-
saurezersetzung in lebenden zellen. II. Biochem. Z. 103:188-217.
- WATTS, W.R., JARVIS, P.G., NEILSON, R.E., & BEADLE, C.L. (1974). Responses
of stomata of sitka spruce to environmental variables. Abstracts
of a meeting of The International Association of Plant Physiolog-
ists, Wurzburg 85.
- WEST, D.W., & GAFF, D.F. (1976). The effect of leaf water potential,
leaf temperature and light intensity on leaf diffusion resistance
and the transpiration of leaves of Malus sylvestris. Physiologia
Plantarum 38:98-104.
- WHITEMAN, P.C., & KOLLER, D. (1964). Environmental control of photosyn-
thesis and transpiration in Pinus halepensis. Israeli Journal
of Botany 13:116ff.
- WHITEMAN, P.C., & KOLLER, D. (1967). Interactions of carbon dioxide conc-
entration, light intensity and temperature on plant resistance to
water vapour and carbon dioxide diffusion. New Phytologist 66:
463-473.
- WILLMER, C.N., & RUTTER, J.C. (1977). Guard cell malic acid metabolism
during stomatal movements. Nature 269:327-328.
- WOOLLEY, J.T. (1964). Water relations of soybean leaf hairs. Agronomy
Journal 6:569-571.
- WRIGHT, S.T.C., & HIRON, R.W.P. (1969). (+)-abscisic acid, the growth
inhibitor induced in detached wheat leaves by a period of
wilting. Nature 224:719-720.
- WUENSCHER, J.E., & KOZLOWSKI, T.T. (1971). The response of transpiration
resistance to leaf temperature as a dessication resistance mech-
anism in tree seedlings. Physiologia Plantarum 24:254-259.

- ZEIGER, E., & HEPLER, P.K. (1977). Light and stomatal function: blue light stimulates swelling of guard cell protoplasts. Science 196:887-889.
- ZELITCH, I. (1965). Environmental and biochemical control of stomatal movements in leaves. Biological Review 40:463-482.
- ZELITCH, I. (1969). Stomatal control. Annual Review of Plant Physiology 20:329-350.

ADDITIONAL REFERENCES

- DELWICHE, M.J. & COOKE, J.R. (1977). An analytical model of the hydraulic aspects of stomatal dynamics. Journal of Theoretical Biology 69:113-141.
- FARQUHAR, G.D. (1978). Feedforward responses of stomata to humidity. Australian Journal of Plant Physiology 5:787-800.
- FULLER, E.N., SCHETTLER, P.D., & GIDDINGS, J.C. (1966). A new method for prediction of binary gas-phase diffusion coefficients. Industrial and Engineering Chemistry 58:18-27.
- LAKE, J.V. (1967). Respiration of leaves during photosynthesis. I. Estimates from an electrical analogue. Australian Journal of Biological Sciences 20:487-493.
- LANGE, O.L. (1975). Plant water relations. In: Fortschritte der Botanik 37 (Eds: H. Ellenberg, K. Esser, H. Merxmüller, E. Schnepf, H. Ziegler). Springer-Verlag, Berlin. pp78-97.
- MANSFIELD, T.A. (1971). Stomata: versatile sensory devices but difficult experimental subjects. Journal of Biological Education 5:115-123.
- WARRIT, B., LANDSBERG, J.J., & THORPE, M.R. (1980). Responses of apple leaf stomata to environmental factors. Plant, Cell & Environment 3: 13-22.

APPENDIX 1 Notes on Marquardt's algorithm for least-squares
estimation of non-linear parameters.

Marquardt's algorithm for least-squares estimation of non-linear parameters

This summary of Marquardt's algorithm is based largely on Conway et al (1970).

In fitting derived or empirical formulae to biological data the object is to minimize the residuals, R_i , for each value X_i between the actual values Y_i and the predicted values $\hat{Y}_i$ calculated from the function.

$$\begin{aligned} \text{i.e.} \quad Y_i &= f(X_i) \\ \text{and} \quad R_i &= Y_i - f(X_i) = Y_i - \hat{Y}_i \end{aligned}$$

Several approaches are available, but in general the Least-Squares Method whereby the sums of squares of the residuals R_i^2 is minimised is to be preferred.

Assuming a three parameter (a, b, c) model with one independent variable X_i with i varying between 1 and N:

$$\sum_{i=1}^N R_i^2 = \sum_{i=1}^N \left[Y_i - f(X_i; a, b, c) \right]^2$$

for $\sum R_i^2$ to be minimized, the partial derivatives of $\sum R_i^2$ with respect to each of the parameters must be equal to zero, thus giving a set of normal equations:

$$\frac{\partial \sum_{i=1}^N R_i^2}{\partial a} = 0 \quad \frac{\partial \sum_{i=1}^N R_i^2}{\partial b} = 0 \quad \frac{\partial \sum_{i=1}^N R_i^2}{\partial c} = 0$$

which are then solved for the best parameter values.

If these equations are linear the best parameter values are found by standard linear regression. However, when the descriptive function enters non-linearity a set of non-linear normal equations are produced

and the correct approach is to use an iterative method in which the minimum $\sum R_1^2$ is found by making suitable corrections to initial parameter estimates. At each iteration the set of parameter corrections are added to the estimates from the previous iteration so that $\sum R_1^2$ continues to decline. The procedure is halted when some arbitrary measure of convergence has been achieved. This can be a predetermined rate of decrease in $\sum R_1^2$ or when parameter corrections are smaller than some value. Methods using the rate of decrease in $\sum R_1^2$ (gradient methods) are able to converge on true parameter values even though the initial estimates may be far removed from the true values, but the convergence is slow. Methods achieving convergence when parameter corrections are small will converge very rapidly providing the initial estimates are good, but may not converge at all if the initial values are far removed from the true values. Marquardt's method (1963 & 1966) uses a combination of these two approaches. The closer the initial parameter estimates are to the true values, the faster the method will converge. The most important criterion is to get good estimates of those parameters to which the function is most sensitive.

Marquardt's algorithm was used for Least-Squares Estimation on non-linear Parameters in the present study:

- 1) To obtain estimates of the parameters a, b, c, d in the

$$\text{equation: } R_T = \frac{a - bX}{c + dX} \quad \text{used in the calibration of the dewpoint hygrometer}$$

(see Section 2.3/2)

where R_T is thermistor resistance and X is the output voltage

(4 parameters, one independent variable)

- 2) To obtain estimates of the parameters l, m and c in the equation:

$R_T = e^{1 + \frac{m}{T+c}}$ used in the calibration of the dewpoint hygrometer

(see Section 2.3/2).

where R_T is thermistor resistance and T is temperature ($^{\circ}\text{K}$)

(Three parameters and one independent variable).