RESPONSE OF STOMATA TO ENVIRONMENTAL VARIABLES IN *PINUS SYLVESTRIS* L.

by

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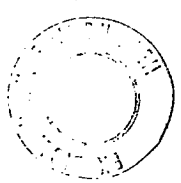

ABSTRACT

The response of stomata in Pinus sylvestris to environmental variables has been investigated in controlled environments in the laboratory, and in the field.

Stomatal conductance saturates at a lower light level in field trees than in growth room plants. Light saturation occurs at a lower photon flux density at high leaf-air vapour pressure differences, D_l, than at low D_l. Hysteresis in the response to light was shown to be caused by extreme sluggishness in stomatal response, and by a carry-over effect of the previous light level. This is particularly noticeable in fluctuating light.

The stomata were insensitive to intercellular CO_2 concentrations over the range 20 to 5500 $cm^3\ m^{-3}$ at high and low photon flux densities.

In growth room plants, the response to increasing D_l was exponential, but in field trees, this response tends towards linearity. Stomatal conductance is more sensitive to D_l at low temperatures than at high temperatures. The leaf temperature for maximal stomatal opening increases with increasing D_l.

Stomatal conductance declines in relation to decreasing leaf water potential, ψ, from a threshold value of ca -0.85 MPa in growth room plants. At high ψ of - 0.2 MPa, the stomata become insensitive to D_l.

It is suggested that increasing transpiration from the walls of the subsidiary cell into the antechamber and from the guard cell wall into the pore can explain the observed concurrent decrease in

stomatal conductance and transpiration. A high hypodermal resistance and a variable temperature sensitive endodermal resistance to liquid water transport have been postulated to support this hypothesis.

The application of the Höfler diagram to the guard cells, with certain assumptions, provides some explanations on stomatal response during a drying cycle, as well as on non-sensitivity to D_1 during high ψ.

An empirical model was derived, and when applied to laboratory data and field data, can account for 80% and 63% of the variation respectively.

DECLARATION

This thesis has been composed by myself and the work that goes into this thesis is my own except for the field experiments, where I was a member of a team. The model of stomatal conductance was developed in collaboration with Helen Talbot and Prof. P.G. Jarvis. I am entirely responsible for the data analyses, interpretations and conclusions.

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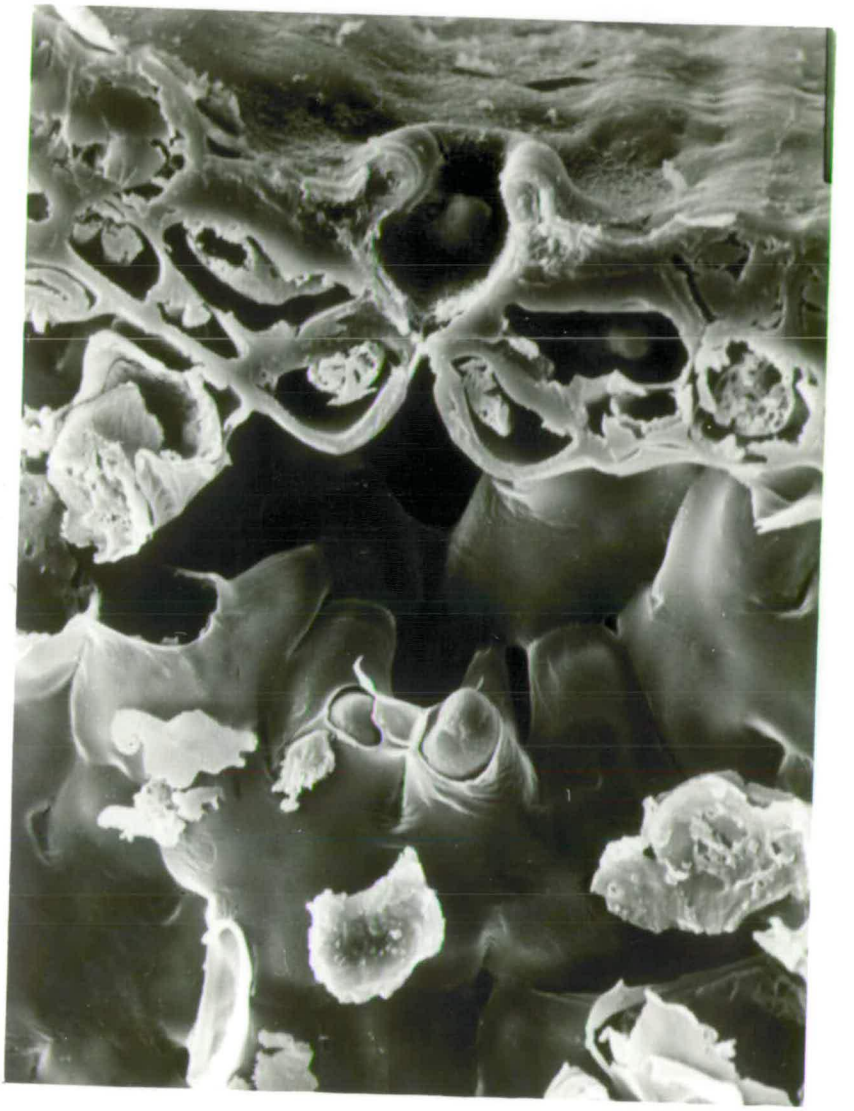


Frontispiece Stereoscan micrograph of cross section through a stoma of Pinus sylvestris, showing also subsidiary cells, antechamber, epidermis, hypodermis and mesophyll. Freeze fractured, mag. X 1000. See also Figure 4.2.2. (Reproduced with kind permission of C.E. Jeffree)

COMMON SYMBOLS USED IN THE TEXT

Symbol	Description	Unit
C_a	Ambient CO_2 concentration	$cm^3\ m^{-3}$
C_i	Intercellular CO_2 concentration	$cm^3\ m^{-3}$
D_l	Leaf-air vapour pressure difference	kPa
D_a	Vapour pressure deficit	kPa
E	Transpiration rate	$mg\ m^{-2}\ s^{-1}$
G	Relative stomatal conductance	dimensionless
g_s	Stomatal conductance	$cm\ s^{-1}$
Q	Photon flux density	$\mu E\ m^{-2}\ s^{-1}$
T_a	Ambient temperature	^oC
T_l	Leaf temperature	^oC
ψ	Leaf water potential	MPa

CHAPTER 1

INTRODUCTION

Pinus sylvestris L. (Scots pine) is the most widely distributed conifer in the world, and is found naturally from Britain, across Eurasia and south almost to the Mediterranean area. It is the only native conifer tree in Britain. Relict woods of native Pinus sylvestris occur in Scotland in addition to substantial areas of plantations throughout the United Kingdom. The forests are of economic value in the production of saleable timber, as well as of scenic and ecological value. In addition an extensive Scots pine plantation at Thetford Forest in Norfolk has been the site for the last 10 years of a fundamental micrometeorological study of processes in coniferous forest canopies.

The transpiration and evaporation of water from a forest canopy depends on the stomatal conductance of the leaves and on the duration of canopy wetness (Stewart 1977; Jarvis & Stewart 1978). On dry days the transpiratory flux from the stomata accounts for only 40% of the available energy derived from net radiation, and the Bowen ratio is often over 2. On wet days, because of the high canopy boundary layer conductance, the rate of evaporation exceeds the rate of input of available energy, and the Bowen ratio is negative. Hence, to arrive at a more precise estimate of the water yield from a forested catchment area, we need to know not only the rainfall pattern, but also, to have good information about the stomatal conductance of the forest canopy.

The broad aim of this thesis is, therefore, to obtain more precise information on the effects of environmental variables on stomatal conductance in a forest canopy. This information, in conjunction with other studies on interception losses, canopy wetness, etc., will enable us to arrive at a better estimate of the total water loss from, and hence water yield of the forested catchment areas.

The specific aim of this thesis, is to define the effects of environmental variables, singly and in certain combinations, and their interactions, if any, on stomatal conductance, so as to obtain a much better understanding of stomatal behaviour, and perhaps eventually to formulate a hypothesis on stomatal action.

The research to be described here has been treated as a physiological investigation. Although a proper understanding of the mechanism of stomatal action must involve the biochemistry of the guard cells, this is beyond the scope of this study. Attention has been focused on what could be measured physiologically from outside the leaf, i.e., the flux of water vapour in response to a range of variables and what could be seen with a light microscope, i.e., the anatomy and dimensions of the leaf.

Certain difficulties arise in investigating the stomatal physiology of Pinus sylvestris. Firstly, the stomata are hidden below a covering of wax tubes and cannot be observed directly in living leaves. Secondly, it is difficult to obtain epidermal strips, and thirdly the stomata respond to imposed treatments rather sluggishly. However, because of the economic and ecological importance of Pinus sylvestris, it is very desirable that we should seek to

obtain knowledge of its stomatal behaviour which may be useful in long-term management planning on water use, and in short-term gains in knowledge on tree and stomatal physiology.

Indeed, the physiology of such 'difficult' plants as conifers should not be avoided but should be investigated because novel and stimulating phenomena may be revealed as found by Haberlandt in the last century. I hope that the results obtained from this thesis will provide enlightenment on some aspects of stomatal physiology, especially of conifers and will stimulate further research.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Stomatal response to light

Stomata open in response to increasing photon flux density of visible radiation (0.4 - 0.7 μm) and close in declining photon flux density. There is a rapid increase in stomatal conductance, g_s, over low flux densities, followed by a gradual, but still substantial increase over higher levels. At a certain high photon flux density, g_s becomes independent of light. Such responses are typical and have been described for Quercus alba (white oak) (Hinckley, Schroeder et al. 1975), Pinus contorta (lodgepole pine) (Dykstra 1974), Tsuga heterophylla (western hemlock) (Keller & Tregunna 1976), numerous Quercus species (Wuenscher & Kozlowski 1971), and for annual crop species (Ehrler & van Eavel 1968; Burrows & Milthorpe 1976).

Both the highest g_s attained and the saturating photon flux density vary with the species (Holmgren, Jarvis & Jarvis 1965; Whiteman & Koller 1967; Turner 1969; Turner & Begg 1973; Turner 1974; Davies & Kozlowski 1974; Burrows & Milthorpe 1976; Tan, Black and Nnyamah 1977). In many cases, the saturating photon flux densities are the naturally occurring photon flux densities in which the plants were grown (Davies & Kozlowski 1974).

Turner (1974) observed that in certain amphistomatous C_3 species, g_s was consistently higher than in certain amphistomatous C_4 species. This was also found by Slatyer (1969) who compared a C_4 species, Atriplex spongiosa and a C_3 species, A. hastata, both

grown under the same environmental conditions. Burrows and Milthorpe (1976), however, pointed to the high conductances of two C_4 species of Pennisetum and warned against such generalisation, although they believed that conductances of hypostomatous leaves as a whole were often less than those of amphistomatous leaves.

The stomatal response to light for a particular species varies with leaf age (Turner 1969; Ludlow & Wilson 1971b; Kriedemann 1971; Rawson & Woodward 1976). For instance, in Quercus rubra (red oak) and Acer rubrum (maple), the stomata of freshly emerged leaves are closed and do not respond to changes in light. Thereafter, the enlarging leaves become increasingly sensitive to changes in light. Maximum conductance is achieved at about the final leaf area (Turner 1974). As senescence sets in, g_s declines rapidly (Turner 1969; Gee & Federer 1973; Turner & Begg 1973; Rawson & Woodward 1976). The failure of the stomata to open in senescent leaves may result from translocation of K^+ from ageing leaves.

The light regime in which the plants are grown affects the stomatal response to light. Ludlow and Wilson (1971a) measured g_s in leaves of Panicum maximum and Phaseolus atropurpureus, grown under 11, 33 and 100% sunlight. They recorded decreased g_s in plants grown under low sunlight, which they attributed to the observed low leaf chlorophyll content and reduced leaf thickness. Wood and Turner (1971) were able to link shade tolerance in four woodland species to rate of stomatal opening and closing in response to a large change in light, stomatal response to dim light and stomatal response to bright light of short duration. Sunflecks, which

consist of bright light of short duration, and dim light are normal conditions in the understorey of a mixed forest, and Fagus grandifolia (beech), a shade tolerant species, was shown to be very adapted to such conditions compared to Liriodendron tulipifera (yellow poplar), a shade intolerant species.

Pasternak and Wilson (1971) observed that the stomata of Sorghum vulgare (sorghum) were very responsive to changes in light, unlike those of Gossypium hirsutum (cotton). In a later paper Pasternak and Wilson (1973) found that the stomata of Sorghum vulgare closed rapidly with a sudden reduction in light, but the recovery of full opening after re-exposure was slow and dependent on the degree of shading and time of day. The greater the shading, the slower was the recovery. The duration of shading, however, did not affect the time for recovery. The slow recovery in the afternoon, according to Meidner and Mansfield (1968), results from an interaction between light and the diurnal endogenous movement of the stomata.

Interactions with other variables

Because the opening effect of light and the closing effect of darkness can be reversed by changing the CO_2 concentration of the air (see Section 2.2), it has been suggested that light changes stomatal aperture by changing the intercellular space concentration of CO_2, C_i, through the dependence of photosynthesis on photon flux density (Raschke 1975).

If stomata open in response to a lowering in C_i, the most favourable wavelengths for stomatal opening should be those most effective for photosynthetic CO_2 uptake, and therefore red and blue light should affect stomata similarly. However, Mouravieff (1958), with Veronica beccabunga and Kuiper (1964) with Senecio odoris,

demonstrated that, on a quantum basis, blue light was more effective than red in causing stomatal opening, suggesting an effect of light which does not depend upon a lowering of C_i. A reaction to light, independent of CO_2 concentration was postulated by Heath and Russell (1954) to explain opening caused by increased light which occurred while C_i was reduced from 100 to 0 $cm^3\ m^{-3}$. Their experiments were performed with white light and therefore the spectral region involved was unknown. Mansfield and Meidner (1966) were able to show an enormously larger stomatal opening in blue than in red light, when detached leaves of Xanthium pennsylvanicum were illuminated with equal quanta of blue and red light in a closed system maintained at the CO_2 compensation point. The CO_2 concentration was about the same in the blue and red light treatments. There was therefore little evidence that CO_2 uptake was larger in blue than in red light. Similar enhanced stomatal opening in blue light was reported in Zea mays in CO_2-free air (Raschke 1967), in guard cells devoid of chloroplasts in Paphiopedilum spp. (slipper orchid) (Nelson & Mayo 1975), in isolated guard cell protoplasts of Allium cepa (onion) (Zeigler & Hepler 1977) and in normal air in Aspidistra elatior (Keerberg, Keerberg & Parnik 1971).

Furthermore, Hsiao, Allaway and Evans (1973) showed that epidermal strips in CO_2-free air and devoid of mesophyll can respond to light in a manner similar to leaf disc in normal air. Also, Heath and Russell (1954a) had shown the occurrence of a light effect that was transmitted from one part of a leaf to another, while C_i was kept at 0 $cm^3\ m^{-3}$. These findings indicate a stomatal response to light which is not mediated through C_i. These findings also show that the guard cells can function independently of the mesophyll

tissue. In view of this evidence the suggestion that C_i determines stomatal aperture seems untenable. The way by which blue light causes a greater stomatal opening than red light is, however, not fully understood.

Turner (1974) found that stomata of field grown Zea mays, Sorghum bicolor and Nicotiana tabacum did not open in light when the plants were under water stress. This phenomenon was also pointed out by Glover (1959) for Zea mays and Sorghum vulgare. Landsberg, Beadle, Biscoe et al. (1975) suggested that in Malus sylvestris (apple) the stomata only respond to light at water potentials, ψ above -1.4 MPa. On the other hand, some early results (Pisek & Winkler 1953, cited in Stalfelt 1955) suggest that the threshold value of ψ was lower at high than at low light. Results from Beadle, Stevenson et al. (1973) showed that this probably occurred in Zea mays and Sorghum vulgare. Hansen (1971) with Beta vulgaris showed that the light level required to saturate stomatal opening increased with increasing ψ.

Studies on the adaxial and abaxial stomata led Kassam (1973) to suggest a light-water deficit interaction in stomatal behaviour. More recently, Ludlow and Ng (1976) recorded partial stomatal closure in stomata of Panicum maximum, as well as progressive saturation of g_s at lower photon flux density as ψ declined. Biscoe (1972) and Jordan, Brown and Thomas (1975) found a higher sensitivity to ψ as the light level was increased. The results from Beadle, Stevenson et al. (1973) on Zea mays do not support this postulate, although in their data on Sorghum vulgare, there were some indications of an interaction with light at the lower ψ levels. Another effect

of light was observed by Willis and Balasubramaniam (1968). They showed that stomatal opening in response to light was delayed, while closing in response to darkness was accelerated in water stressed plants of Pelargonium.

The light response of stomata appeared to be affected by preconditioning to a particular set of environmental conditions. Ludlow and Wilson (1971a) observed a higher g_s value for 4 leguminous species measured at 30°C between plants grown at 20°C and preconditioned to 30°C during the preceding 15 h dark period and plants grown at 20°C and not preconditioned. Vapour pressure deficit affects the light response in Picea sitchensis. Watts, Neilson and Jarvis (1976) found that there was larger stomatal response to light at lower vapour pressure deficits. Similar stomatal behaviour was also observed in Picea engelmannii (Kaufmann 1976). Watts, Neilson and Jarvis (1976) also noted a lower light saturation point for g_s at lower vapour pressure deficit.

Response to light in conifers

Turner (1974) compared the light responses for mature leaves of several crop and tree species and found that the values of g_s for Pinus resinosa (red pine) was one-eighth of that of Nicotiana tabacum (tobacco) and Helianthus annuus (sunflower) or one-quarter to one-fifth of that of Quercus rubra, Acer rubrum, Zea mays (maize) or Sorghum vulgare. The saturating photon flux density for Pinus resinosa was also very low compared to that of the other species.

The stomata of Picea sitchensis and Pinus contorta showed the characteristic light response described in other plant species

(Ludlow & Jarvis 1971; Dykstra 1974). Rutter (1978) investigated the stomatal response to light in 3 conifers. He found maximal stomatal aperture at photon flux density of 300 $\mu E\ m^{-2}\ s^{-1}$. Stomatal conductance was higher in Abies concolor than Libocedrus decurrens or Pinus ponderosa. Above 300 $\mu E\ m^{-s}\ s^{-1}$, g_s was stable in Pinus ponderosa but declined in both Libocedrus decurrens and Abies concolor. It has been shown that stomatal response to light was larger at low vapour pressure deficit, D_a than at high D_a, in both Picea sitchensis (Watts, Neilson & Jarvis 1976) and in Picea engelmannii (Kaufmann 1976).

Neilson (unpublished) found that forest shoots of Picea sitchensis obtained from the top of the canopy had higher values of g_s than shoots from the bottom of the canopy, when exposed to the same photon flux densities in the laboratory. Such results were obtained in spite of a lower stomatal density for needles at the top of the canopy compared to that for needles at the bottom of the canopy. Similar change of g_s with canopy height has also been reported by Turner and Begg (1973) for Zea mays (maize), Sorghum bicolor (sorghum) and Nicotiana tabacum (tobacco) during certain times of the day. Watts (1977) attributed the lower g_s in Picea sitchensis at the lower canopy level to adaptation of the stomata to low light.

In Pinus contorta, g_s in shade-adapted foliage saturates at lower light levels than in sun-adapted foliage (Hinckley, Schroeder et al. 1975). Keller and Tregunna (1976) found that the g_s in shade-adapted trees of Tsuga heterophylla (western hemlock) increased as the quantum flux density was increased, while the stomata of sun-adapted trees opened widest at an intermediate photon flux density, but declined

when the photon flux density was increased. The shade-adapted trees, when exposed to high light suffered desiccation due to increased transpiration.

2.2 Stomatal response to CO_2

Stomata response to low CO_2 concentration by opening and to high CO_2 concentration by closing. This type of response is seen in light and in darkness. It was demonstrated in a wide range of plant species: in Zea mays (maize) (Meidner 1962; Pallas 1965; Raschke 1972), in Helianthus annuus (sunflower) (Whiteman & Koller 1967) in Xanthium strumarium (Jones & Mansfield 1970; Raschke 1974) in Vicia faba (Raschke 1975) in Sorghum bicolor (sorghum), Gossypium hirsutum, Glycine max and Lycopersicon esculentum (Pallas 1965).

In a classical experiment, Heath and Russell (1954) forced air through the leaf as well as across the leaf surface in Triticum aestivum (wheat), to control C_i. They demonstrated that, in light, stomata closed when the CO_2 concentration was increased above ambient concentration (about 300 $cm^3 m^{-3}$) and the stomata opened when the CO_2 concentration was reduced from ambient concentration to the CO_2 compensation point (about 100 cm^3 m^{-3}). At any CO_2 concentration, further stomatal opening was induced by increasing the light level. Gaastra (1959) in experiments on transpiration in Brassica pusna (turnip), using CO_2 concentrations between 0 and 1500 $cm^3 m^{-3}$, also showed that stomata closed in high CO_2 concentration and opened in low CO_2 concentration. In the dark, Heath and Russell (1954) and Gaastra (1959) showed that CO_2-free air prevented stomatal closure.

Heath (1940) also showed that in darkness stomata were opened in low CO_2 concentration, provided that they had not been closed prior to the treatment of decreased CO_2 concentration, indicating the importance of C_i, rather than C_a in the control of stomatal aperture. The CO_2 dependent response was also shown to occur in both C_3 and C_4 plants. In general stomata of C_3 species were less responsive to CO_2 than were the stomata of C_4 species (Ludlow & Wilson 1971; Akita & Moss 1972). It was also seen in a CAM plant, Agave americana (Neales 1970) and in a fern, Phyllitis scolopendrium (hart's tongue fern) (Mansfield & Willmer 1969). The apparently universal occurrence of a response to C_i led Raschke to suggest a stomatal sensor for CO_2 located in the guard cell itself (Raschke 1975). However, a lack of stomatal response to CO_2 has been reported in several species, e.g. Vitis vinifera (vine) (Kriedemann, Sward & Downton 1976) for CO_2 range between 275 and 950 $cm^3\ m^{-3}$, Picca sitchensis (sitka spruce) (Ludlow & Jarvis 1971), Brassica oleracea (kale) (Parkinson 1968) both at CO_2 concentration above 100 $cm^3\ m^{-3}$, and in Gossypium hirsutum between 200 to 2000 $cm^3\ m^{-3}$ (Bierhuizen & Slatyer 1964). Pallas (1965) also reported of lack of CO_2 response in Sorghum bicolor and Zea mays between 500 to 4000 $cm^3\ m^{-3}$. An increase in g_s with increase in CO_2 concentration was found in Brassica oleracea and in Picea sitchensis between CO_2 concentrations of 0 to 100 $cm^3\ m^{-3}$ (Parkinson 1968; Ludlow & Jarvis 1971, respectively). These observations do not seem to agree either with the suggestion of the universal occurrence of a CO_2 sensor in the guard cells or with Raschke's suggestion that stomata response to light only indirectly by responding to changes in C_i brought about by light (Raschke 1975), since the stomata in all these species do respond normally to light.

Interactions with other variables

Gaastra (1959), with Brassica pusna, Downes (1971) with Sorghum sudanense, Akita and Moss (1972) with Triticum aestivum and Zea mays, and McPherson and Slatyer (1973) with Pennisetum typhoides, all showed a lower stomatal sensitivity to C_a at high light than at low light. Hall and Kaufmann (1975) had shown that stomata of Sesamum indicum (sesame) were more responsive to C_i in dry air than in humid air. They observed that in humid air, the stomata respond little to variation in C_i over a natural range. Similar results may have been obtained by Heath and Milthorpe (1950). They reported in Tritium aestivum no stomatal response to C_a with low air flow rates over the leaf and strong stomatal response with high air flow rates over the leaf, which very likely was air of larger leaf-air vapour pressure difference.

There are a few studies on the role of CO_2 in stomatal response to water stress. Heath and Meidner (1961) and Allaway and Mansfield (1970) showed that moderate to severe water stress caused an increase in the CO_2 compensation point, and possibly an increase in C_i, which can account for part of the stomatal closure during water stress. However, mild water stress has been shown to reverse the opening caused by CO_2-free air (Stalfelt 1961), indicating that at least a part of the closing process is independant of changes in CO_2 concentration. On the other hand, in Malus sylvestris (apple), it was reported that exposure to CO_2-free air extended the range for which g_s was independent of leaf water potential (West and Gaff 1976). This latter finding is in accordance with Raschke's suggestion that CO_2 is required for ABA to cause stomatal closure during water stress

(Raschke 1975a). However, objections were also raised against this postulation (Mansfield 1976).

Response to CO_2 in conifers

The stomata of Picea sitchensis do not respond to C_a between 30 and 6000 $cm^3 m^{-3}$ (Neilson & Jarvis 1975; Beadle 1977). Neilson and Jarvis (1975) used cut forest shoots and potted plants grown in growth cabinets, and varied C_i from 50 to 600 $cm^3 m^{-3}$ for temperatures between zero and 30°C. Beadle (1977) expanded these observations and found no evidence for the decrease in g_s at CO_2 concentrations below 100 $cm^3 m^{-3}$ observed by Ludlow and Jarvis (1971) and by Parkinson (1968) in Brassica oleracea. The lack of stomatal sensitivity to CO_2 reported by Beadle (1977) cannot be caused by high shoot water potential, as suggested by Raschke (1975) since similar results were obtained at shoot water potentials of -0.8 MPa as at -2.4 MPa.

2.3 Stomatal response to leaf water potential

Most observations on the relationships between stomatal conductance and leaf water status have clearly demonstrated that there is a threshold level of leaf water potential, ψ, above which g_s remains constant and high, and below which the stomata close dramatically. For Lycopersicon esculentum, Phaseolus vulgaris, Glycine max, Vitis vinifera and greenhouse-grown Gossypium hirsutum, the threshold value of ψ was between -0.7 to -1.6 MPa (Hsiao 1973). The leaves of many plant species may thus be subjected to moderate water stress before the stomata respond to changes in ψ. The

actual value of the threshold ψ is probably related to the drought tolerance of a species. For example, Sorghum vulgare (sorghum) long considered to be one of the most drought tolerant crop plants may have a threshold ψ as low as -2.0 MPa in the field (Turner 1974). Adaxial and abaxial stomata have been observed to differ in their threshold level of ψ in Phaseolus vulgaris (French bean) (Kanemasu & Tanner 1969) and Gossypium hirsutum (Jordan, Brown & Thomas 1975), slightly in Vicia faba (Kassam 1973) but not in Zea mays and Sorghum vulgare (Sanchez-Diaz & Kramer 1971).

Threshold type responses are not always observed; in some cases there was a gradual decrease in g_s as ψ declines (Raschke 1970; van den Driessche 1971; Biscoe 1972). Biscoe (1972) attributed this to the build-up in solute concentration during slow drying. A similar observation and explanation was put forward by Pasternak and Wilson (1974) in comparing stomatal responses in excised leaves of Sorghum vulgare (fast drying) and intact leaves (slow drying). Kassam (1973) put forward another explanation for the absence of a threshold ψ. He postulated that interaction with light could be responsible: that high light levels might result in a threshold response, whereas low light levels would result in g_s being more closely linked to ψ.

Stomatal response to ψ varies with stage of growth of the plant, leaf age and leaf position in the canopy. The stomata of Zea mays and Sorghum vulgare remained open and did not respond to decreasing ψ after initiation of reproductive growth (Ackerson & Kreg 1977). The threshold ψ required to induce stomatal closure in Triticum aestivum became progressively lower as the plant developed

from the tillering phase into the grain filling phase. As the flag leaf aged, stomatal closure occurred at progressively lower ψ (Frank, Power & Willis 1973). In Gossypium hirsutum, the younger leaves kept their stomata open at lower ψ than the older leaves (Jordan, Brown & Thomas 1975). Stomata of leaves in the upper canopy in Sorghum vulgare, Nicotiana tabacum and Zea mays closed at lower ψ than those in the lower canopy (Turner 1974a). Similar stomatal response was also seen in Glycine max and Triticum aestivum (Teare & Kanemasu 1972; Denmead & Millar 1976).

The growing environment influences stomatal response to water stress (Kanemasu & Tanner 1969; Jordan & Ritchie 1971). Stomata of Sorghum vulgare and Gossypium hirsutum become less sensitive to water stress if the plants were preconditioned by previous water stress (McCree 1974; Thomas, Brown & Jordan 1976; Brown, Jordan & Thomas 1976). In general, field grown plants are less responsive to water stress than glasshouse- or chamber-grown plants. Plants of Gossypium hirsutum grown in a growth room had threshold ψ of -1.6 MPa, while field-grown plants had threshold ψ of -2.7 MPa (Jordan & Ritchie 1971). Smaller differences of the same type have been observed for Allium cepa (Millar, Gardner & Goltz 1971), Zea mays (Boyer 1970; Sanchez-Diaz & Kramer 1971; Turner 1974) and Sorghum vulgare (Sanchez-Diaz & Kramer 1971; Turner 1974). Turner (1974) suggested two reasons for these differences. Plants in the field are subjected to much higher light levels than those in growth rooms. There is a larger soil volume for root growth. The solute potential of the leaf, therefore, adjusts to lower values as the soil water potential decreases. This evidence suggests a remarkable adaptation of the stomatal apparatus to the

growing conditions. Denmead and Millar (1976) observed that in conditions of mild water stress in the field, induced by high transpiration, g_s in Triticum aestivum decreased just sufficiently to maintain ψ at its threshold level, thereby imposing a limit on excessive transpiration.

Many workers have noted that the occurrence of a leaf water deficit partially inhibits stomatal opening for some time subsequent to the apparent recovery in plant water status (e.g. Stalfelt 1955; Glover 1959; Heath & Mansfield 1962; Meidner & Mansfield 1968). Glover observed that severe drought of longer than a week caused the stomata of Zea mays to be half closed permanently, although the leaves had recovered full turgidity. The new leaves that developed after the drought, however, had normal sized stomatal opening.

There is evidence to show that endogenous abscisic acid, ABA, rises as a plant is water stressed and falls when it is re-watered, and that the rise and fall in the endogenous ABA level is associated with the opening and closing of the stomata (Wright 1972; Beardsell & Cohen 1975). It has also been shown that exogenous ABA is a powerful inhibitor of stomatal opening and transpiration (Jones & Mansfield 1970; Cummins, Kende & Raschke 1971; Tal & Imber 1972). Consequently it has been suggested that water stress affects stomata via its effect on the level of ABA (Cummins, Kende & Raschke 1971; Kriedemann, Loveys, Fuller & Leopold 1972) or on the balance between ABA and cytokinins (Mizrahi, Blumenfeld & Richmond 1970; Tal & Imber 1971). Cummins, Kende and Raschke (1971) suggested that the rapidity and ready reversibility of the action of ABA on stomata

would make it a good modulator of stomatal behaviour. However, Hsiao (1973) pointed out that the relative rates of stomatal opening and closing and ABA accumulation and degradation were such as to render the possibility of direct modulation of stomatal response by ABA unlikely.

Response to leaf water potential in conifers

Many conifers can tolerate moderate water stress and their stomata are unaffected by declining ψ until a threshold value of -1.5 to -2.5 MPa is reached (Table 2.3.1). Pinus taeda is one exception; its stomata start to close at values of ψ lower than -0.4 MPa. Lopushinsky (1969) observed that pines and spruce, e.g. Pinus sylvestris and Picea sitchensis are less tolerant of water stress than firs, e.g. Abies grandis. Although the threshold type response has been observed for many conifers, a close coupling between declining ψ and g_s was found in Pinus taeda (Kaufmann 1968) and in Pinus contorta (Dykstra 1974). Kaufmann (1968) reported that after a drying cycle and subsequent to re-watering the stomata of Pinus taeda remain partially close for about 5 days, although the leaf water potential had recovered after a day. Blake and Ferrell (1977) observed in seedlings of Pseudotsuga menziesii a rapid build-up of endogenous ABA when ψ declined to -1.0 to -1.2 MPa, but the stomata started to close at ψ of -1.5 MPa, when the ABA level was 32 pmol cm^{-2}. This figure of 32 pmol cm^{-2} is at the high end of the range reported by Hiron and Wright (1973) and Kriedemann, Loveys et al. (1972). On re-watering recovery to a normal ABA

Table 2.3.1 Threshold[1] values of shoot water potential for different species of conifers.

Species	Threshold (MPa)	Sources
Picea sitchensis	-1.5	Beadle 1977
	-1.4	Watts and Neilson 1978
Picea abis	-3.7	Jarvis and Jarvis 1963
Picea engelmannii	-1.6	Lopushinsky 1969
	-1.5	Kaufmann 1976
Pinus resinosa	-1.5	Waggoner and Turner 1971
Pinus ponderosa	-1.7	Lopushinsky 1969
	-1.8	Running 1976
Pinus contorta	-1.6	Lopushinsky 1969
Pinus sylvestris	-1.5	Jarvis and Jarvis 1963
	-2.0	D. Whitehead (personal communication)
Pinus taeda	-0.4	Brix 1962
Pseudotsuga menziesii	-2.0	Running 1976
	2.1	Lopushinsky 1969
Abies grandis	-2.5	Lopushinsky 1969
Abies sp.	-0.8 to -1.0	Puritch 1973

[1] Threshold value of shoot water potential is defined as the value of above which g_s remains constant and below which g_s declines rapidly.

level and g_s values took 6 days, which is long compared to that observed for Zea mays (2 days) and Sorghum vulgare (1 day) (Beardsell & Cohen 1975). However, night-time g_s values for re-watered plants were found to be significantly higher than the controls at 6 days after re-watering.

2.4 Stomatal response to humidity and temperature

Many early researchers on stomatal behaviour ignored the importance of ambient humidity mainly because of reports of a small response (Wilson 1948; Meidner & Mansfield 1968). However, ambient humidity has recently been recognised as an environmental factor which is as important as light, CO_2, plant water status, and temperature in controlling stomatal behaviour (Raschke 1970; Lange, Losch et al. 1971; Schulze, Lange et al. 1972).

There have been observed stomatal response purportedly ascribed to ambient humidity which was really confounded with temperature variation (e.g. Camacho-B, Hall & Kaufmann 1974). There have also been many studies of temperature effects on stomata which were conducted without controlling or measuring vapour pressure deficit (e.g. Dykstra 1974; Wuenscher & Kozlowski 1971). Since vapour pressure deficit is a function of temperature, the reported stomatal effects could be caused by a humidity change, a temperature change or a combination of both. Such results are therefore likely to yield limited information and are to be treated with caution.

Response to ambient humidity

Numerous studies have shown that dry air causes stomata to close and humid air causes stomata to open both in whole plants, e.g. Zea mays (Raschke 1970); Citrus sinensis (Hall, Camacho-B

& Kaufmann 1975); Gossypium hirsutum and Sorghum vulgare (Ackerson & Krieg 1977) and in isolated epidermal strips of Polypodium vulgare (Lange, Losch et al. 1971; Losch 1977). However, other experimental results show no significant effects of leaf-air vapour pressure difference on stomatal aperture (Wilson 1948; Meidner & Mansfield 1968; Raschke & Kuhl 1969).

Leaf-air vapour pressure difference, D_l, is the driving force in transpiration and is a measure of the evaporative demand of the atmosphere. Previously, it was assumed that as D_l increased, transpiration increased, and ψ declined until the threshold ψ was reached, causing the stomata to close. However, in some cases it has been shown that stomata do respond to D_l, without concurrent changes in ψ (Macklon & Weatherley 1965; Watts, Jarvis et al. 1974). It is therefore possible that the effect of D_l is a direct effect on stomatal aperture not mediated through effect on ψ. There are also results showing a closing response to humidity with concomitant increase in relative water content of the leaves (Tinklin & Bowling 1969; Schulze et al. 1972). These data seem to indicate that stomata can respond to ambient humidity independently of changes in ψ. A recent study by Hall and Hoffman (1976) appeared to affirm this.

There is some evidence to show that growing plants in a dry air environment resulted in reduced transpiration, caused by lower g_s. This was shown in Glycine max (Beardsell, Mitchell & Thomas 1973) and in Zea mays (Slavik 1973). Kaufmann and Levy (1976), however, compared two groups of plants of Citrus jambhiri, one grown in a humid air environment, the other in a dry air environment, and found no significant difference in their response to D_a.

Interactions with other variables

There is some evidence to show that light levels affect the stomatal response to D_a. Davies and Kozlowski (1974) observed that a humidity change had more effect on the stomata of Fraxinus americana (ash) and Acer saccharum (maple) at low light levels than at high light levels. In Picea engelmanii (Engelmann spruce), Kaufmann (1976) reported more complete stomatal closure in response to D_l at low light than at high light. The water status of the leaf also affects the stomatal response to D_l. Schulze, Lange et al. (1972) showed a lower sensitivity to D_l in irrigated plants of Prunus armeniaca and Hammada scoparia than in non-irrigated plants. Hinckley, Schroeder et al. (1975) demonstrated that effects of D_a were negligible at values of ψ above 0.2 MPa, but increased at lower ψ. Rawson, Begg and Woodward (1977) suggested that stomatal response to humidity might be partly dependent on plant water status and on whether part or all of the plant was exposed to the humidity change. The plants in this study were all well watered. Where only a small part of the plant, i.e. a single leaf had been exposed to the changing humidity conditions within the cuvette, while the remainder of the plant remained under conditions of low evaporative demand, they found no response to D_a. In contrast, when whole plants were exposed to the changing humidity conditions, there was a stomatal response to D_a. Unfortunately, Rawson, Begg and Woodward (1977) did not provide data to support the statement that there was a higher ψ value in the single, attached whole leaf and a lower ψ value in the whole plant. Hall and Kaufmann (1975a) reported that

the response to D_l was more marked at low temperature than at high temperature. They showed this to occur in Sesamum indicum (sesame), Beta vulgaris (sugar beet) and Helianthus annuus (sunflower). Recently, Losch (1977) with isolated epidermal strips of Polypodium vulgare observed similar findings. Reduced stomatal response to humidity at higher temperatures was also apparent in the data of Drake, Raschke and Salisbury (1970).

Response to humidity in conifers

The response of stomata to humidity in most conifers is similar to the response seen in other species. For example, in intensive studies on Picea sitchensis (sitka spruce), it has been shown that g_s decreases in response to increasing D_a. Such a response has been observed in field studies on forest shoots (Watts, Neilson & Jarvis 1976), in laboratory studies on cut forest shoots (Neilson & Jarvis 1975) and in potted seedlings in a wind tunnel (Grace, Malcolm & Bradbury 1975). Concurrent measurements on Ψ showed that the response to humidity occurred with no significant change in the water status of the leaf (Watts, Jarvis et al. 1974; Grace, Malcolm & Bradbury 1975; Watts & Neilson 1978). Hodges (1967) studied the humidity response in four conifers in the field. Ambient temperature was uncontrolled and therefore only species differences in stomatal response to D_l can be inferred. Stomata of Abies grandis (grand fir) were less responsive to increasing D_l than those of Abies procera (noble fir). Stomata of Abies grandis and Pseudotsuga menziesii (Douglas fir) closed at a higher leaf-air vapour pressure difference than those of Abies procera. Hodges (1967) conducted a laboratory

experiment on Pinus sylvestris (Scots pine). Presumably leaf temperature was not controlled. He reported that, under conditions of decreasing relative humidity, the stomata of Pinus sylvestris closed and later opened again. Rutter (1978) conducted field studies on three conifers. He found stomatal responses to humidity in Pinus ponderosa (ponderosa pine) and in Libocedrus decurrens (incense-cedar), but none in Abies concolor (white fir). Bennett and Rook (1978) in laboratory studies, reported stomatal sensitivity to D_1 in two clones of Pinus radiata. It has already been reported that there was a more pronounced effect of humidity on stomata in Picea engelmanii in low light than in high light (Kaufmann 1976).

Response to temperature

Many recent reports indicate that stomatal aperture increases with rising temperatures (Wilson 1948; Stalfelt 1962; Hofstra & Hesketh 1969; Raschke 1970a; Schulze, Lange et al. 1973, 1974; Hall, Camacho-B & Kaufmann 1975; Hall & Kaufmann 1975). While there are some reports which indicate that stomatal aperture is largest at intermediate temperatures (Wilson 1948; Stalfelt 1962; Hofstra & Hesketh 1969; van den Driessche, Connor & Tunstall 1971; Sharpe 1973; Doley & Yates 1976), others have shown that stomata open wider at even higher temperatures (Schulze, Lange et al. 1973, 1974; Hall, Camacho-B & Kaufmann 1975; Hall & Kaufmann 1975). Zelitch (1969) pointed out that different methods could account for some of these differences and different responses by different species

could also be involved. Also, it is probable that at sufficiently high temperatures all stomata will close.

Schulze, Lange et al. (1973) found that, in four desert species and in Prunus armeniaca, g_s increased as the temperature increased, so long as the plants were not water stressed. When they were water stressed, the stomatal response was reversed, i.e., the stomata closed as temperature gradually increased. These results were obtained at constant D_l. They suggested that the stomatal response to temperature was independent of the stomatal response to D_l. Losch (1977) who measured stomatal width of groups of guard cells from epidermal strips of Polypodium vulgare, also concluded that the stomatal response to temperature was independent of the stomatal response to D_a. He observed maximal stomatal opening at 20 to 22°C, while at higher temperature he found a slight stomatal closure. Stalfelt (1962) who took pains to remove effects of water deficit, observed that steady state stomatal aperture in Vicia faba increased with increasing temperature between 5 and 40°C. At 45°C, there was stomatal closure, which Stalfelt ascribed to excess of respiratory CO_2. At constant D_l, as the leaf temperature increased from 20 to 34°C, g_s in Sesamum indicum increased with a concomitant increase in C_i and rate of photosynthesis (Hall & Kaufmann 1975). Hofstra and Hesketh (1969) concluded that the temperature response of the stomata of several species appeared to be correlated with the response of photosynthesis and photorespiration to temperature. On the other hand, Stalfelt's observation that there was a temperature induced opening in the dark and in CO_2-free air, indicated the lack of

a requirement for newly synthesised assimilates for stomatal opening (Stalfelt 1962). Similarly, Mansfield (1965) found that he could induce stomatal opening in the dark by increasing the temperature gradually from 27 to 36°C. The increase in g_s at very high temperature and zero net photosynthesis was also noted by El-Sharkawy and Hesketh (1964). Raschke (1970a) observed that between 15 and 35°C, stomatal aperture in Zea mays was proportional to net photosynthesis and appeared to be controlled by C_i. However, at temperatures below 15°C and above 35°C, stomatal aperture was insensitive to C_i and the stomata were widely open even at 50°C when there was high C_i because of respiration.

Meidner and Mansfield (1968) explained the occurrence of midday closure in Allium cepa (onion) which was first observed by Loftfield (1921) as caused by high C_i produced at the high midday temperature. They cited evidence from the work of Heath and Orchard (1957), Heath and Meidner (1957) and Meidner and Heath (1959). However, the concomitant increase in D_a at high temperature was not taken into account. There was also no actual measurement of C_i. Data from Hall and Kaufmann (1975) seemed to indicate that, when D_l was not maintained constant, as temperature increased, the increase in D_l could be the cause of stomatal closure and not the change in C_i, since C_i was found to actually decrease. This latter observation contradicted the findings of Heath and Orchard (1957). When the leaf cavity in Allium cepa was swept with CO_2-free air, stomatal closure at high temperature was prevented (Meidner & Heath 1959). Hall and Kaufmann (1975) pointed out that, if D_l was not controlled,

at high temperature, the increased sensitivity to CO_2-free air at high D_l or even the effect of CO_2-free air could separately have prevented stomatal closure. Obviously, more work could be done to establish the true cause of midday closure.

The temperature in which the plant is grown affects the stomatal response to temperature. For example, Doley and Yates (1976) found that, in Astrebla lappacea (Mitchell grass) the temperature for maximal stomatal opening increased with the growth temperature, although the two temperatures were not exactly similar. Further, they found that when the plants were transferred from one temperature regime to another, the stomata adapted to the changed temperature conditions after about one thermoperiod. In Xanthium pennsylvanicum, high temperature treatment had no significant effect on stomatal aperture, but chilling caused a large but variable increase in diffusive resistance to water vapour (Drake & Salisbury 1972). Pasternak and Wilson (1972) found that exposure of whole plants of Sorghum vulgare to cold ($5^{\circ}C$) or hot ($30^{\circ}C$) nights, or exposure of shoots only to a range of low night temperatures (5 - $15^{\circ}C$) reduced both the rate and size of stomatal aperture during the succeeding day. They attributed the slow rate of stomatal opening to the development of water deficits in the leaves. Night temperature also has an effect on stomatal opening. Zelitch (1963) found that plants of Nicotiana tabacum (tobacco) kept for 5 to 7 nights at $16^{\circ}C$ had reduced stomatal aperture compared to those kept at $27^{\circ}C$.

Response to temperature in conifers

Not much is known about the effect of temperature on stomatal response in conifers. Whiteman and Koller (1964) with Pinus halepensis

found that stomatal aperture was largest at intermediate temperatures of 24 - 27°C. Neilson and Jarvis (1975) with forest shoots of Picea sitchensis also found similar behaviour with maximum g_s at 15°C with constant D_l. Rutter (1978) found that g_s in Pinus ponderosa increased with temperature between 10 and 15°C, reached a maximum at 15°C and thereafter declined. A similar response was seen in Abies concolor, except that above 15°C temperature had less effect on g_s, and the stomata remained partially opened even at 35°C. Stomatal aperture in Libocedrus decurrens was maximum at 10°C, slowly decreased between 10 and 25°C, and subsequently at higher temperatures, a rapid decline was observed.

The stomatal response of Picea sitchensis was affected by the growth temperature (Neilson & Jarvis 1975). At temperatures between 5 and 20°C young plants of Picea sitchensis, grown for 3 months at day/night temperature of 18/15°C had higher values of g_s than those grown at 13/10°C or 7/4°C. Below 5°C, there was little difference in g_s amongst the three treatments. Further, g_s was considerably reduced on the day following a 1 hour exposure to -5°C in summer and autumn, but not in winter. Neilson and Jarvis (1975) could not attribute this finding to a rise in C_i or in D_l. They suggested ice formation as a result of freezing and a possible rise in the level of abscisic acid.

2.5 Mechanism of stomatal movements and current hypotheses

Stomatal opening is the result of solute accumulation in guard cells. The consequent water influx causes turgor pressure in the guard cells to increase above that in the surrounding epidermal

cells, stretching the guard cell pair so that they separate in the middle to form the stomatal pore. Stomatal closing is accomplished by the reverse process: a decline in guard-cell solutes and consequent loss of guard-cell turgor. Recent experimental findings implicate K^+ as the major solute acting as an osmoticum in guard cells (Fischer& Hsiao 1968; Humble & Raschke 1971; Fischer 1972; Penny & Bowling 1974; Hsiao 1976). Influx and efflux of K^+ between the surrounding epidermal cells and the guard cells accompanied stomatal movements (Humble & Raschke 1971; Penny & Bowling 1974). Electroneutrality is maintained in the guard cells by organic anions, mainly malate (Allaway 1973) and/or by Cl^- (Raschke & Fellows 1971; Penny, Kelday & Bowling 1976) and/or by exchange of H^+ for K^+ (Raschke & Humble 1973).

Effect of light and CO_2

As discussed earlier, stomata open in the light and close in darkness, and stomata open in CO_2-free air and close in air of high CO_2 concentration. How light and CO_2 stimulate stomatal movements is still far from clear. Von Mohl in 1856 put forward the hypothesis that light and CO_2 affected stomata via guard cell photosynthesis and that the resulting assimilates were responsible for turgor increases in guard cells. This hypothesis fell out of favour when it was found that stomata can open in the dark in response to low CO_2 concentrations, and more recently that K^+ was the main osmoticum in stomatal movements. Recently it has been shown that guard cells cannot convert CO_2 into sugars through the Calvin cycle (Raschke & Dittrich 1977).

It seems that guard cells possess PEP carboxylase (Willmer, Pallas & Black 1973), and can fix endogenous or exogenous CO_2 (Raschke & Dittrich 1977) or $-HCO_3$ (Willmer & Dittrich 1974) into malate in light or dark, whether the stomata are open or closed (Raschke & Dittrich 1977). Such a mechanism allows the guard cells to sense changes in the CO_2 concentration in the immediate vicinity (Raschke 1975; Raschke & Dittrich 1977). Dittrich and Raschke (1977b) showed, however, that the guard cells were not able to reduce photosynthetically the fixed CO_2 and therefore had to rely on imported sugars from the mesophyll to satisfy their metabolic carbohydrate requirements. They found that the imported sugars could be converted into malate or stored as starch or serve as an energy source through oxidative phosphorylation (Dittrich & Raschke 1977b). They also found that during stomatal closure, rapid mobilisation of malate was achieved by three methods: rapid movement out of the guard cells, together with K^+ and Cl^-, oxidation in the tricarboxylic acids cycle or starch formation through gluconeogenesis.

Raschke (1975) proposed the following hypothesis to link C_i to the turgor changes in guard cells via malate. By opening in respond to low C_i during high photosynthetic uptake of CO_2 or low C_a and by closing in response to high C_a or C_i the stomata function to attain an eventual stable C_i. The effect of light is indirect, and occurs as the result of changes in the intercellular and intracellular CO_2 concentrations because of changing rates of photosynthesis. The presence of ABA is required to make stomata sensitive to CO_2 and stomata respond to ABA only if CO_2 is available. During water stress

ABA overrides the CO_2 response and causes stomatal closure by inhibition of H^+ expulsion. Malate serves as an anion in the vacuole of the inflated guard cell, and the concentration of malic acid in the cytoplasm determines direction and magnitude of the ion movements across plasmalemma and tonoplast. The concentration of malic acid depends in turn on the relative rates of its production and removal. When more malate is produced in response to a high C_a than can be moved into the vacuole, and more hydrogen ions are released than can be exchanged for K^+ from the environment of the guard cells, the cytoplasm will be filled with malate and acidify, resulting in stoppage of malate formation and consequently a prevention of stomatal opening or, if stomata are open, in stomatal closure.

The proportion of malate acting as counter ion for K^+ will be one factor determining the amount of malate formed in the guard cells if electroneutrality is to be maintained. According to Raschke's hypothesis, the plant species that use a lower proportion of malate as counter ion will be more sensitive to high CO_2 concentration than the plant species that utilises more malate as counter ions. In Zea mays, Cl^- forms 40% of the anion (Raschke & Fellows 1971) while in Vicia faba Cl^- forms 5% (Humble & Raschke 1971). Zea mays is a C_4 species (Akita & Moss 1972) and therefore there is a possibility that it is more sensitive to high CO_2 concentration than Vicia faba.

However, the real problem in this hypothesis is that it does not relate the size of the stomatal aperture to the CO_2 concentration. According to this hypothesis, the largest stomatal aperture is not at zero CO_2 concentration. In CO_2-free air, there would be less

malate formation than in normal air as the guard cells have to depend on imported sugars from the mesophyll and their own store of starch. There would be a lower influx of K^+ and therefore a smaller stomatal aperture than in normal air. If there is such a light reliance on exogenous CO_2, the guard cell is less effective as a CO_2 sensor for C_i than hypothesised. Another problem is that in the dark stomata can be opened by CO_2-free air (Heath & Russell 1954). Raschke's hypothesis has difficulty explaining this finding since there is no photosynthetic production of sugars in the mesophyll in the dark in CO_2-free air. Finally, it could not explain the lack of response to CO_2 in some plant species, e.g. Picea sitchensis. Mansfield (1976) recently tried to affirm Raschke's finding that there is an interrelation between ABA and CO_2 (Raschke 1975a) and found no evidence for this. Finally, Raschke's hypothesis is incompatible with findings of CO_2 independent light responses, e.g. effect of blue light and light transmission effects (Heath & Russell 1954a) since the hypothesis revolves around changes in C_i and these effects can take place without changes in C_i or in absence of CO_2.

Effect of water stress

The finding that water flow through the epidermis can exist with a certain degree of isolation from the rest of the leaf (La Rue 1930; Thoday 1938; William 1950; Sheriff & Meidner 1974; Meidner 1975) supports the existence of a high hydraulic resistance between the epidermis and the mesophyll. Such a relationship would explain the threshold type stomatal response in which the stomata remain

open and unaffected by a declining ψ, until the threshold ψ is reached, when most probably, the differential in ψ between the mesophyll and the epidermis has increased to the point where the hydraulic resistance between them is overcome, and the consequent withdrawal of water from the guard cells result in rapid stomatal closure. However, this is not the case for all species and Raschke's observation on Zea mays indicates for that species (Raschke 1970) a linear decline in g_s with decreasing ψ. According to Raschke, the guard cells can sense changes in ψ between the bundle sheath and the mesophyll or within the mesophyll. He suggests that a change in ψ in the mesophyll will induce a change in the stomatal aperture since they are hydraulically linked (Raschke 1970).

The manner with which ABA causes stomatal closure, its reversibility in action, and the fact that it is produced in increasing amount during water stress makes it the most likely hormone involved in stomatal closure during water stress. The only problem is whether ABA can be produced fast enough or in sufficient quantity to explain the rapidity of stomatal closure (Hsiao 1973).

Effect of humidity

When stomata open in humid air and close in dry air, they appear to function as a humidity sensor. How humidity causes a change in stomatal aperture is still not clear. It seems, however, likely that the response is mediated through a feedback mechanism linking evaporation sites, e.g. wet cell walls lining the intercellular

spaces and substomatal cavity, to the guard cells. Also, it involves fluxes of water. The important question is which is the site of largest evaporation and what is the hydraulic pathway linking it to the guard cells.

The classical hypothesis is that the mesophyll with its convoluted walls and tortuosity offers the largest surface for evaporation. By being sited nearest the water carrying vein endings, its walls are continuously wetted and the air in the intercellular spaces is at the saturated vapour pressure. During increasing D_l, the increase in transpiration affects the mesophyll Ψ which in turn affects the guard cell turgor, since the guard cell obtains its water via the mesophyll. Indirect evidence for such a feedback mechanism was shown by Stalfelt (1955) who found that stomata of Vicia faba, Rheum rhaponticum and Rumex acetosa closed with increasing water deficit. Raschke (1970) found that the stomata of Zea mays were hydraulically linked to the water supply to the leaf, and responded to changes in hydrostatic pressure in the water supply to the leaf. Recent data from Aston and Jones (1976) showed that the most intense evaporation came from the mesophyll cell-walls, lining the substomatal cavity and intercellular spaces.

That this view did not represent the whole picture became evident when it was found that fluorescent dye in the transpiration stream accumulated in the anticlinal walls of epidermal cells, especially those surrounding the guard cells (Strugger 1939), and when tritiated water in the transpiration stream was found to blacken stripping film above the guard cells (Maercker 1965). Seybold

(1961) formulated the hypothesis that direct cuticular water loss from the guard cells and subsidiary cells, the so called peristomatal transpiration influences the aperture of the stomata. The findings of Lange, Losch et al. (1971) and Losch (1977) were explained in terms of peristomatal transpiration. Lange, Losch et al. (1971) had already shown that adjacent stomata on the same epidermal strip in different air streams may assume different apertures. The primary site of action seems to be at the stomata themselves and is not the result of feedback from the leaf as a whole. Data from whole leaves had shown that the relation of g_s to D_l was clearly independent of ψ (Macklon & Weatherley 1965; Watts, Jarvis et al. 1974). It was also observed that water flow through the epidermis can occur with a certain degree of isolation from the remainder of the leaf (La Rue 1930; Thoday 1938; Williams 1950; Sheriff & Meidner 1974; Meidner 1975). Also, lateral flow of water through the epidermis was found sufficient to maintain the high rate of evaporation from the subsidiary and guard cells (Meidner 1976). These findings were consistent with the concept of peristomatal transpiration.

Recently, Aston and Jones (1976), using monosilicic acid as a tracer, demonstrated intense evaporation from the inner periclinal walls of the subsidiary cells and the inner periclinal walls and anticlinal walls, adjacent to the stomatal pore of guard cells, and moderate evaporation from the outer periclinal walls of guard and subsidiary cells. This evidence gave support to the existence of peristomatal transpiration.

In principle, therefore, this hypothesis seems to be most firmly based. Reservations are, however, based on the reliability

of the techniques employed. Some of the solutes used were subject to preferential adsorption on cell wall material especially the pectin-rich regions of the cuticle and anticlinal walls. Similar objection must be raised against stripping films in contact with a portion of the stomatal apparatus.

Meidner (1975) suggested that evaporation from the internal guard cell and subsidiary cell walls should be higher than from the mesophyll cell walls. Simple consideration of the evaporation pathway, and vicinity of the possible sources to the external air suggested that evaporation should be higher from the cells close to the pore. Using a simple scale model of the substomatal cavity, he showed that the rate of evaporation inside the cavity was higher near the pore than from farther away. The results of Aston and Jones (1976) however, do not support this concept.

Aston and Jones (1976) indicated that internal cuticle is no barrier to evaporation as was suggested by Jarvis and Slatyer (1970). Consequently, peristomatal transpiration is possible in conifers, e.g. Picea sitchensis, which possesses heavily cuticularised guard cells (Jeffree, Johnson & Jarvis 1971; Jeffree 1974). Thus, from all evidence the existence of peristomatal transpiration seems the most likely of the three hypotheses proposed. However, further discussion must await more decisive experimentation.

Effect of temperature

Stomatal movements involve K^+ influx and efflux, anion formation and other biochemical processes including mobilisation of photosynthates. All these biochemical processes would be accelerated

by higher temperatures. For example, Raschke (1970a) found a good correlation between increasing g_s with increasing temperature and increase in net assimilation rate. He suggested that C_i controlled stomatal aperture between 15 to 35°C. At higher temperatures enzyme denaturation may block the metabolic events required for stomatal opening, resulting in smaller stomatal aperture. Alternatively, high water deficits or large D_l at high temperatures could also cause stomatal closure (Schulze, Lange et al. 1973). At temperatures around 0°C, ice formation, or possibly ABA formation, may be the reasons for the small stomatal aperture (as suggested by Watts, Neilson & Jarvis 1976).

CHAPTER 3

MATERIAL AND METHOD

3.1 Introduction

This thesis contains experimental data collected in the laboratory at the Department of Forestry and Natural Resources and in the field from an experimental site at Thetford Forest, Norfolk, South-East England. The author was a member of a team working on a field project at Thetford Forest, and the field data were collected in conjunction with others in the team. The experimental work was done in 1976 and 1977.

3.2 Plant material

Two-year old (1 + 1) seedlings of Pinus sylvestris were planted in University of California peat-sand (3:1) mixture in 15 cm diameter plastic pots and grown in a glasshouse for 6 months before the start of the experiment. At least 4 weeks before the experiment, the seedlings were transferred from the glasshouse to a 1.39 m^2 controlled environment growth chamber and kept in the following conditions: day temperature 18°C and night temperature 14°C, day length 14 - 17 h, vapour pressure deficit 0.46 - 0.52 kPa, and photon flux density of 260 $\mu E\ m^{-2}\ s^{-1}$ at the height of the sampled shoots. Light was provided by a mixture of tungsten and Kolararc metal halide lamps (ATLAS 400 W MBIF 21330 - 1). The plants were watered and fertilised twice weekly with a commercial fertiliser mixture (Bio No. 5 nitrogen 19.1%, phosphoric acid 19.1%, potash 22.3% w/w).

3.3 Measurement of stomatal conductance

Stomatal conductance, g_s was measured by use of a porometer and by gas exchange.

Porometer

The null balance porometer (Beardsell, Jarvis & Davidson 1972) was employed for stomatal conductance measurement in the field, wind tunnel and in the growth room. It had a leaf chamber with an internal volume of c. 600 cm^3 and can enclose a 6 cm length of shoot. Photon flux density was measured by a quantum sensor (Lambda Instrument Co., Model LI-190S) mounted on the side of the porometer cup, and temperature within the chamber was measured with a thermistor.

Stomatal conductance was calculated from the leaf area and the flow rate of dry air into the chamber, as described by Beardsell et al. (1972), with the addition of a temperature correction factor for the PCRC-11 sulphonated polystyrene humidity sensor.

Gas exchange system

The block diagram of the open circuit gas exchange system is presented in Fig. 3.3.1 to show the interrelationships of the main components. A detailed description of the system can be found in Leverenz (1978). The system used is a development of that used by Ludlow and Jarvis (1971).

The four 10 litre mixing tanks removed short term changes in CO_2 and water vapour concentration. Outside air of normal CO_2 concentration

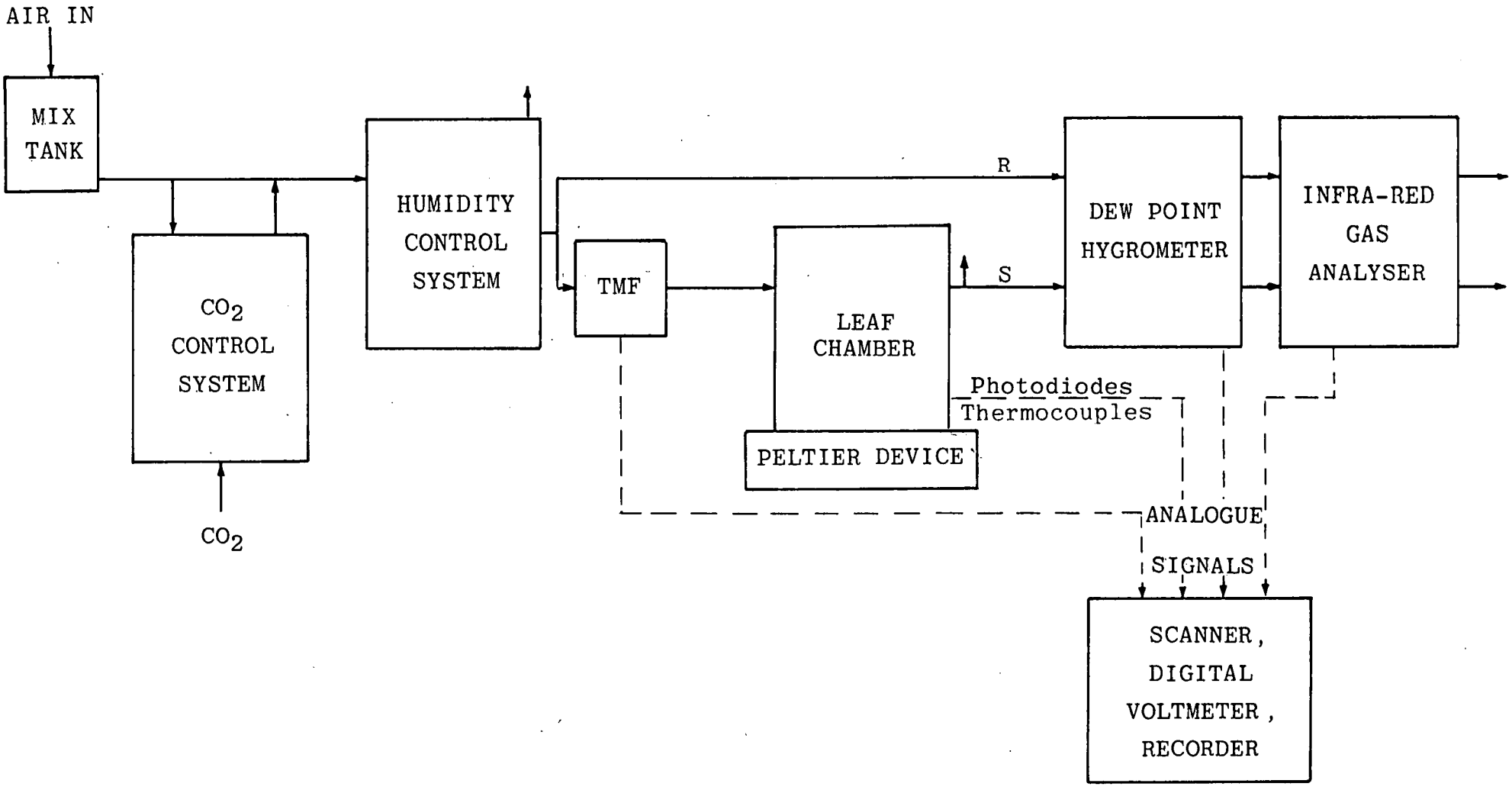


Figure 3.3.1 Flow diagram of open gas exchange system. R = reference air; S = sample air; ↑ = blow-off; TMF = Brook's thermal mass flowmeter and flow controller. See text for details.

of c. 350 cm^{-3} m^{-3} was used. Alternatively, when air of a range of CO_2 concentrations was required, the air flow was ducted into the CO_2 control system wherein pure CO_2 and CO_2-free air were mixed accurately by three gas mixing pumps (H. Wostoff oHG., Type SA18, SA27 and G27) arranged in cascade.

The air with prescribed CO_2 concentration and humidity was then pumped into the leaf chamber at a known flow rate. On leaving the chamber the air was analysed for water vapour and CO_2 concentration. Water vapour concentration was measured as dew point temperature by a dew point hygrometer (Cambridge System Inc. Model 880) and CO_2 concentration was measured with an infra-red gas analyser (Hartmann & Braun URAS 2T). Before entering the leaf chamber, a part of the air flow was passed direct to the dew point hydrometer. This air flow, called the reference air, provided measurements of the water vapour concentration of the air entering the leaf chamber. A system of flowmeters (GEC - Elliot Rotameter 1100) (regulated manually) and three-way solenoid valves (Shraeder M454 SA) (activated electronically by push button switches in a control panel) provided control of flow rate and direction of flow respectively. One double headed and six single headed pumps (Charles Austin models F65 D E and Dymax Mk. 2) maintained flow and positive pressure in the entire gas circuit. Two blow-offs operating at two points, one downstream from the humidity system and the other immediately after the leaf-chamber indicated positive pressure. Voltage signals from various measuring instruments including two photodiodes, two copper-constantan thermocouples, (all found in the leaf chamber) the thermal mass flowmeter (Brooks Inst. Model 5810

sensor), dew point hygrometer and infra-red gas analyser were displayed on a digital voltmeter (Solartron Schlumberger A200) and monitored on a potentiometric multipoint, strip-chart recorder (Philips PM 8235/01) or individually on a single channel flatbed chart recorder (Servoscribe RE 541.20).

Humidity control system

The humidity control system is illustrated in Fig. 3.3.2. Dry air was produced by passing outside air through two silica gel columns, S1 and S2, and water vapour saturated air was produced by passing outside air through two wash bottles, H1 and H2 which contained loosely arranged "Miracloth" wetted with distilled water placed at the bottom of the bottles. Both airstreams were subsequently mixed in five mixing bottles, M1 to M5, arranged in series. The volumetric proportion of wet to dry air was controlled manually by regulating the flowmeters F1 and F2. Beyond the mixing bottles the flow branched into two, one the reference air, flowed direct to the dew point hygrometer and the other, the sample air, was passed through the thermal mass flowmeter and controller before entering the leaf chamber. The entire humidity control system was kept at a constant temperature of 40^{o}C by a 100 W lamp bulb which was switched on or off by a contact mercury thermometer wired to a relay (Jumo).

Leaf Chamber

With the exception of the two 1.2 cm thick removable perspex windows on the two vertical sides, the removable brass back and front

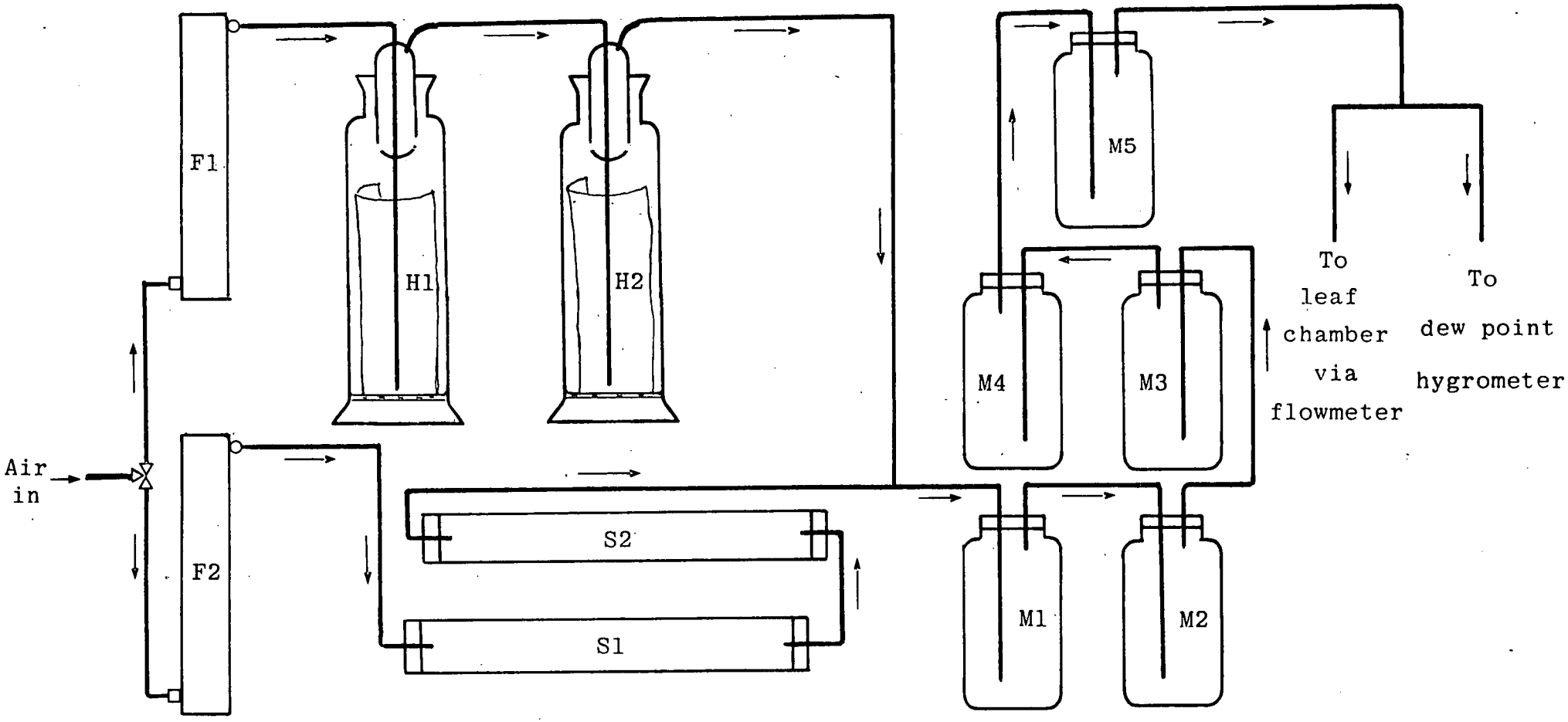


Figure 3.3.2 Diagram of humidity control system. See text for details.

lids, the leaf chamber was made from a brass casting. The walls were on average, 1.2 cm thick and the internal volume was 440 cm^3. These features allowed good conduction of heat, resulting in good even temperature distribution within the chamber. The internal walls were painted matt black to reduce unwanted light reflections. Two photodiodes (Radio Spares 305-462) facing the perspex windows measured the incident photon flux density (Fig. 3.3.3). The photodiodes were calibrated *in situ* against a quantum sensor placed in position to measure the average photon flux density received by the experimental shoot. The quantum sensor is sensitive only to radiation in the wavelength 400-700 μm and therefore the calibrated readings from the photodiode are a measure of the photosynthetic useful quantum flux. Leaf temperature was measured by a copper-constantan thermocouple with a soldered thermojunction of 40 SWG wire (0.01143 cm diameter). The thermojunction was soldered, boiled in distilled water, filed to a point, and varnished. The thermojunction was embedded in the leaf tissue, to provide an accurate measure of leaf temperature. The reference temperature was provided by an electronic thermocouple ice-point reference unit (Mectron, Zeref). The air temperature thermocouple was made in the same manner. A check with an accurate mercury thermometer showed that the error in temperature measurement by the thermocouples was $\pm$ 0.1°C. A 3 cm radius twisted bladed fan, powered by a 1.5 volt d.c. micromotor (Portescap 15 C 11-110-0) stirred the chamber air, resulting in a constant and low boundary layer conductance of 30.303 cm s^{-1} and an even mix of gases within the leaf chamber. The leaf chamber sat on an air cooled

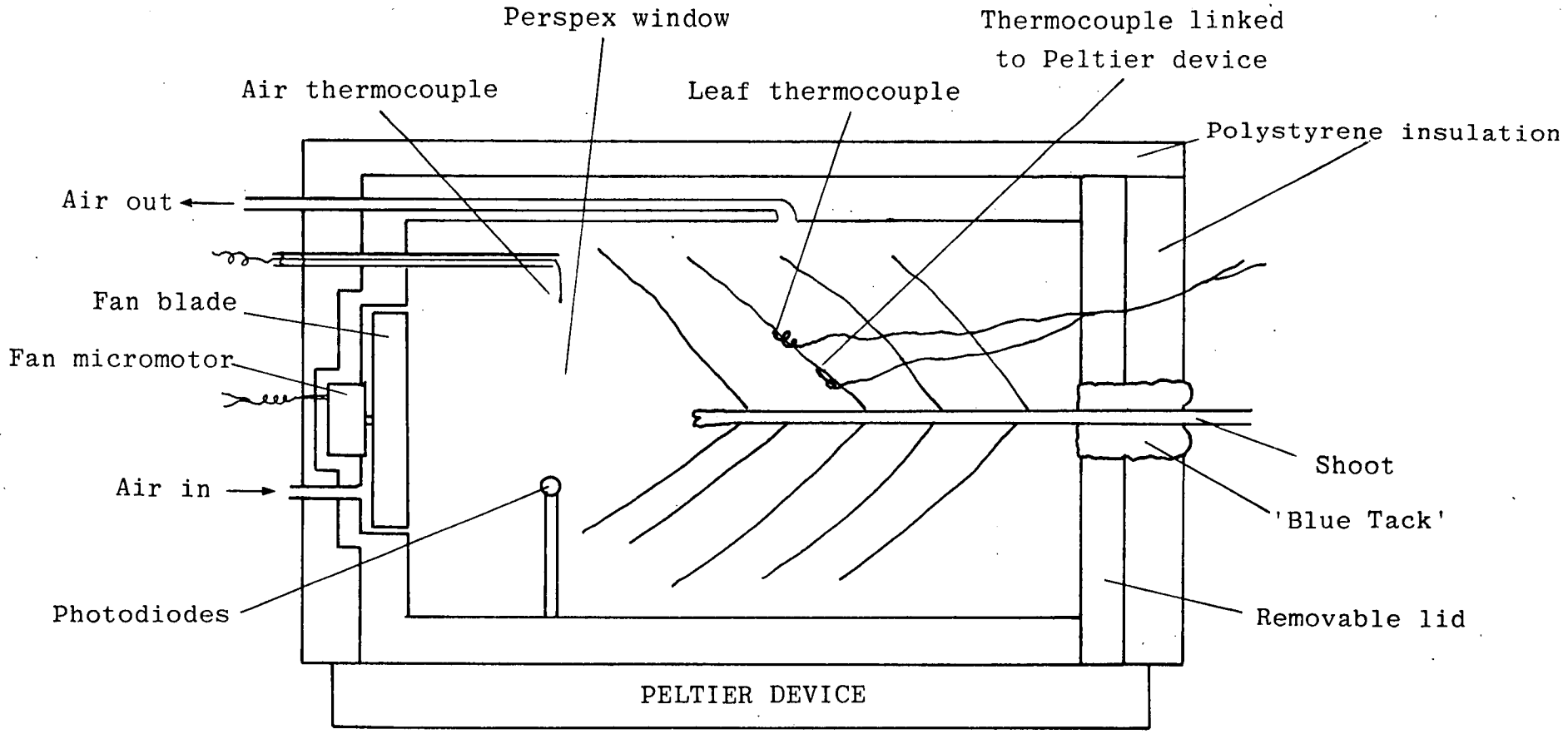


Figure 3.3.3 Diagram of side-view of leaf chamber. See text for details.

thermoelectric Peltier device (Cambion Model 803-1010-01) with current flow regulated by a thryristor controller (Eurotherm Type No. 070, 071W-2). This device was capable of maintaining a constant leaf temperature of between 5 to 50°C within the leaf chamber. A Dow Corning heat sink compound provided efficient heat transfer between the two metallic surfaces. All other external metallic surfaces of the leaf chamber were covered with a 2 cm thickness of polystyrene insulation. Laminated tubing lined with polyethylene (Samuel Moore & Co. "Dekabon" 1300) was used to duct the humidified air into the leaf chamber and from the leaf chamber to the analytical instruments. The polyethylene lining and aluminium laminated wall prevented CO_2 loss and water vapour absorption. The tubing was insulated by a tubular foam rubber jacket and the entire conduit was heated to an even 35°C by an electric heater tape running alongside the tubing. An air conditioner provided control of room temperature. Hence, problems of condensation of water vapour in the tubing and leaf chamber was reduced to a minimum.

Optical bench

The optical bench is illustrated in Fig. 3.3.4. The two 400 W metal halide lamps (Wotan HQl-T) were each placed in position at the focal point of an aluminium foil lined metallic hemisphere and provided in combination a total photon flux density of 1600-1800 $\mu E\ m^{-2}\ s^{-1}$. The fan-cooled acrylic Fresnal lenses acted as collimators, and provided parallel beams of light. The photon flux density was varied

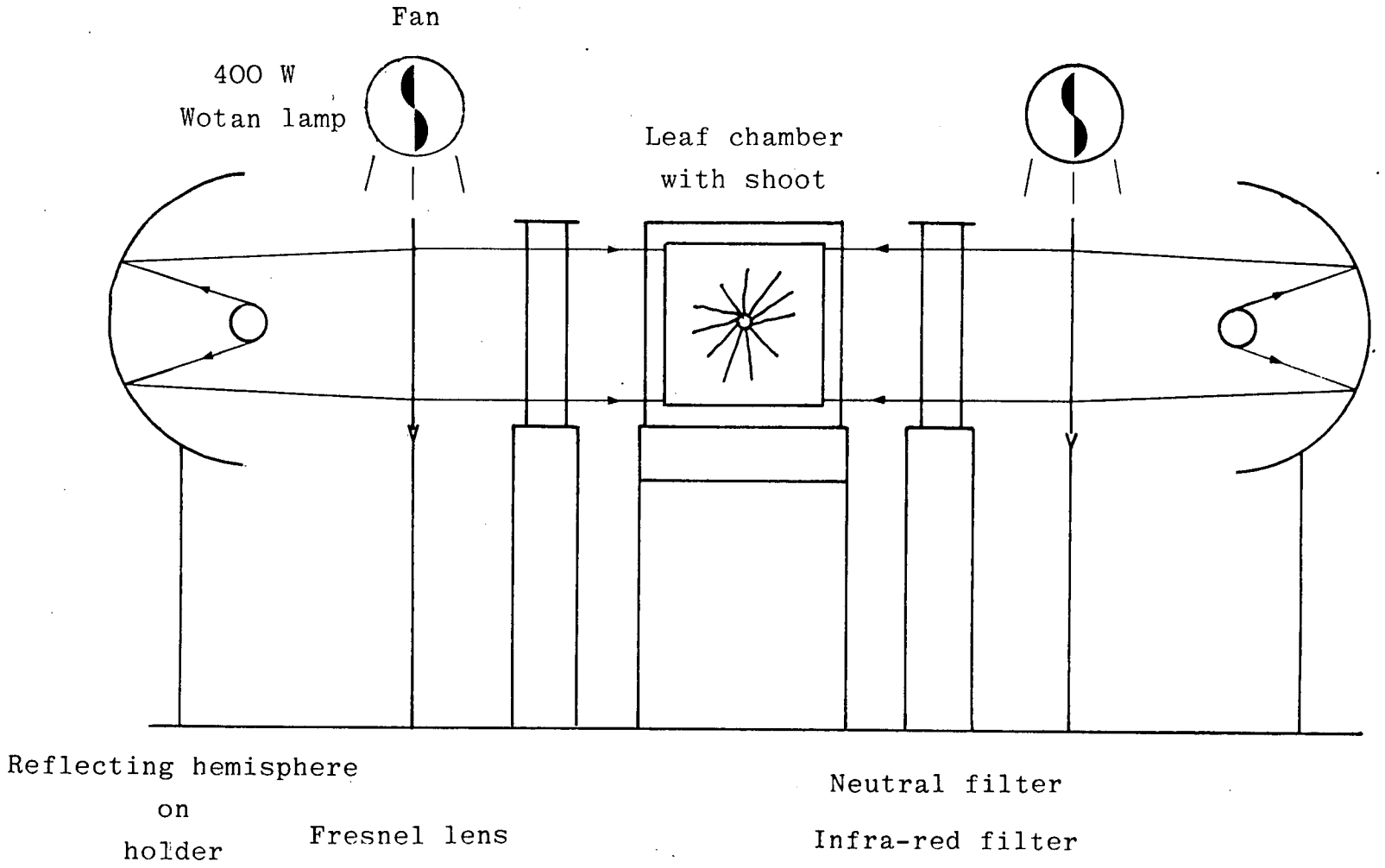


Figure 3.3.4 Diagram of optical bench. See text for details.

by use of neutral density filters (H.V. Skan Ltd.). Also positioned in each filter holder was an infra-red filter (Schott KG2) to cut out substantial amounts of heat from each lamp.

Theory of water vapour transfer

The diffusion of water vapour out of a leaf, under steady state conditions and over a wide range of leaf-air vapour pressure differences can be described by Fick's law as

$$E = g_t (W_i - W_a) \qquad 3.1$$

Where E is the transpiratory flux ($g\ cm^{-2}\ s^{-1}$); W_i and W_a ($g\ cm^{-3}$) are the water vapour concentration at the sites of evaporation in the cell walls and in the ambient air, respectively; g_t ($cm\ s^{-1}$) is the total conductance to water vapour transfer. Vapour pressure, e in mbar and vapour concentration, W in $g\ cm^{-3}$ can be related by

$$W = \frac{217e}{T} . 10^6 \qquad 3.2$$

assumed $T_a = T_l$

where T is the absolute temperature in degrees Kelvin (°K) (Monteith 1973). Hence

$$E = 10^6 . 217 g_t (e_i - e_a) / T \qquad 3.3$$

where e_i and e_a are the corresponding vapour pressures in mbar and $(e_i - e_a)$ is the leaf-air vapour pressure difference, D_l in mbar.

In transpiration, water evaporates from the walls of the mesophyll and epidermal cells, within the leaf, diffuses across the substomatal cavity and through the stomatal pore and is then transferred across the external boundary layer. The boundary layer consists of a thin layer of air close to the leaf surface in which air movement is laminar and a transition zone to the fully turbulent conditions in the ambient air (Jarvis 1971 p. 571). A small amount of evaporation also occurs from the outer walls of the epidermal cells through the cuticle and across the boundary layer. The total conductance g_t can therefore be split up into three components. The stomatal pore, substomatal cavity and any cell wall resistance are together included in the stomatal conductance g_s, which is considered to be in parallel with the cuticular conductance g_c. Both conductances are in series with the boundary layer conductance g_a. These relationships can be written as:

$$g_t = \frac{(g_c + g_s)\, g_a}{g_c + g_s + g_a} \qquad 3.4$$

Since g_c is usually very small compared to g_s, g_c may vary considerably without appreciably affecting the estimate of g_s. g_s can therefore be considered as

$$g_s = \frac{g_t\, g_a}{g_a - g_t} \qquad 3.5$$

In the determination of stomatal conductance from water vapour

exchange in a leaf chamber, under steady state conditions, the flux of water transpired per unit leaf area, $E(g\ cm^{-2}\ s^{-1})$ is expressed as

$$E = f(W_2 - W_1) / A \qquad 3.6$$

where W_2 and W_1 $(g\ cm^{-3})$ are the water vapour concentrations of the air leaving the leaf chamber and entering the leaf chamber, respectively; f $(cm^3\ s^{-1})$ is the flow rate of the air entering the chamber, and A (cm^2) is the leaf area. Converting W in $g\ cm^{-3}$ to e in mbar using equation 3.2

$$E = 10^6 .217f\ (e_2 - e_1) / AT \qquad 3.7$$

From equation 3.3

$$g_t = \frac{e_2 - e_1}{e_i - e_a} \cdot \frac{f}{A} \qquad 3.8$$

The boundary layer conductance, g_a, can be measured and is maintained large and constant in the leaf chamber by internal stirring with a high speed fan.

For the same reason of the thoroughly mixed gases within the leaf chamber, the humidity of the air leaving the leaf chamber, e_2 can be taken as equal to the humidity of the air close to the leaf, e_a.

Hence

$$g_t = \frac{e_2 - e_1}{e_i - e_2} \cdot \frac{f}{A} \qquad 3.9$$

e_i is the saturated vapour pressure at the leaf temperature. To convert dew point temperatures and leaf temperatures, T(^{o}C) to the corresponding saturation vapour pressures, e (mbar), the Tetens equation was used:

$$e = 6.108 \times 10^{(7.5T/T + 237.3)} \qquad 3.10$$

(1 mbar is taken as equal to 0.1 kPa)

Values of g_t are therefore obtained, and g_s subsequently solved from equation 3.5. A Fortran program previously used in the University of Aberdeen was modified for use with the gas exchange data. All conductance values are based on projected leaf area.

Calibration of dew point hygrometer

Water vapour saturated air flowing from the humidity system described above was ducted through two copper coils immersed in a stirred thermostatic water bath and cooler units (Grants Instruments, Cambridge, Model SX35 and CC20 respectively) that provided temperatures ranging from 5^{o}C to above 30^{o}C. The air flowing out of the copper coils had a dew point temperature similar to the water temperature of the bath. Water vapour that had condensed collected in reservoirs that formed the lower ends of the copper coils. Temperature of the bath was measured to within $\pm$ 0.1^{o}C with a calibrated mercury thermometer immersed in a central position in the bath. From the copper coils the air of known dew point temperature was passed to the dew point hygrometer. Output from the dew point hygrometer was monitored on a strip chart recorder and the reading was taken, when stable, with

a digital voltmeter. Linear regressions of voltage derived from the dew point hygrometer against dew point temperature were computed using a Fortran program.

Determination of boundary layer conductance

Boundary layer conductance was calculated from the measured rate of evaporation from a thin anhydrous calcium sulphate cast of a detached Pinus sylvestris shoot as described by Landsberg and Ludlow (1970). The evaporation measurement was done in the leaf chamber. It was found unnecessary to remove the leaf wax if care was taken in dipping the shoot repeatedly into the suspension of calcium sulphate. Prior to positioning the calcium sulphate covered shoot a constant flow rate of air of fixed humidity was maintained in the chamber. Surface temperature was measured by a thermocouple bound to the shoot in the same position as a fascicle and covered in the same manner with a thin layer of calcium sulphate. During each run, the shoot was wetted with deionised water, allowed to drip and then immediately placed in position in the leaf chamber. Readings were taken every 30 s and conductance values calculated for a set of results which showed stable evaporation rate and constant leaf temperature. The projected leaf area of the needles and area of the stem covered by the anhydrous calcium sulphate measured on the optical planimeter (Lambda Instr. Corp. Model LI-3100) was taken as the area of evaporation. The boundary layer conductance was calculated in the same way as the shoot conductance.

3.4 Measurement of leaf water potential

Leaf water potential was measured in the field and laboratory using an 18 cm long, 6.7 cm radius brass cylindrical pressure chamber. Fasciclesof two needles were removed from the plant, at the same level, or from the same branch as the shoot on which g_s was measured. A neat cut was made at the base of the fascicles with a sharp razor blade. The pointed end of the paired needles was pushed through a rubber washer and a brass washer in the screw head lid of the chamber until it was flush with the lid. The centre of the lid was screwed down and this compressed the rubber washer to form a tight seal around the cut end. Compressed air from a gas cylinder was used to pressurise the chamber. The pressure on the meter scale was noted when the meniscus of the xylem sap first appeared at the cut end of the shoot. This was observed using a low power binocular microscope. The pressure chamber gave reproducible measurements accurate to within $\pm$ 0.02 MPa if the same person made the measurements and the precautions outlined by Ritchie and Hinckley (1975) were followed. Pressure was applied at the same slow rate each time so as not to cause significant temperature increases within the chamber; the true end point was taken when sap was observed to exude from the cut xylem vessels and was not confused with bubbling from the cortical or pith tissues; the leaves were cut and stored in a plastic container with a piece of wet filter paper at the base to prevent drying out between collection and measurement.

Hellkvist, Richards and Jarvis (1974) showed that keeping

needles of Picea sitchensis in cool, damp conditions for up to 6 h caused no significant changes in the water potential. For replicate measurements three fascicles of about the same size and position in the canopy were chosen.

3.5 Performance of experiment

Laboratory experiment

The shoot for g_s measurement was transferred from the growth room and placed in position in the leaf chamber on the evening of the day before the experiment was planned to begin. The shoot was allowed to acclimatise overnight to the humidity conditions appropriate to the following day's experiment. On the day of the experiment the lamps were switched on to coincide with the beginning of the photoperiod in the growth room. The shoot was given two hours or more stabilisation period (see Section on diurnal rhythm) at a photon flux density which was normally the first light treatment or the photon flux density required for the day's experiment. After the stabilisation period, subsequent treatments, whether they were light levels, CO_2 concentration, leaf-air vapour pressure difference or temperature were given in steps. Each treatment was maintained for $\frac{3}{4}$ to 1 h, by which time a stable response was observed on the strip chart recorder.

Field experiment

Field measurements were made on five trees of Pinus sylvestris about 16 m tall in the centre of a 70 km^2 block of forest, located

at Thetford Forest, Norfolk, south-east England. The girth of the trees at breast height was approximately 70 cm and the density of the stand 775 trees ha^{-1} (Roberts 1977). Porometer measurements were made at three well-defined levels in the canopy. Level 1 was considered as the topmost three nodes of the canopy which received close to maximum irradiation. The central part of the canopy, nodes 4 to 6, where leaf area distribution was most dense was considered as Level 2. Level 3 was taken as the base of the canopy where irradiation was so low that few new shoots appeared (Nodes 7 to 8). Measurements were made out during a period of 8 months, from the end of March to mid-September 1977 at two hourly intervals, at times when the shoots were dry. The time of commencement of measurements and time of completion depended on the prevailing day length and weather conditions. At the end of a series of measurements the shoots were cut off, frozen in liquid nitrogen, and transferred in a thermos flask to the laboratory for projected leaf area measurement with an optical planimeter. Water potential measurements were made with a needle pressure bomb after each set of porometer measurements.

Wind tunnel experiment

Two experiments were done in the wind tunnel on the effect of leaf-air vapour pressure difference. A detailed description of the wind tunnel is given by Grace (1974). The tunnel is a close-circuit, low-speed type (Pankhurst & Holder 1965) and produces near laminar air-flow over a 1.6 x 0.8 m^2 experimental stage on which were arranged six potted plants. The plants were subjected to a 14 h photo-

period with the same photon flux density at shoot height from similar lamps as in the growth chamber. The plants were watered every evening during the 10 day experiment and soil evaporation reduced to a minimum by wrapping the pots in transparent plastic bags, tied around the base of the stems. The desired leaf-air vapour pressure difference was set the previous evening. Measurements were made 5 h after the lights came on, on one selected shoot per plant. Stomatal conductance was measured, using the null-balance porometer (Beardsell et al. 1972) and needle water potential was measured with a needle pressure bomb. Leaf temperatures were recorded on a strip chart recorder and four copper-constantan thermocouples with thermo-junctions embedded in the tissue of needles located at the same level as the selected shoots. A mercury thermometer positioned amongst the plant was used to provide a check on the leaf temperatures recorded by the thermocouples. The ambient humidity was measured using a dew point hygrometer on air extracted from a point downstream of the plants at the same level as the shoots. Occasional checks on the humidity were made with an Assmann psychrometer. Air flow was maintained at 2.75 m s^{-1} throughout the experiment.

3.6 Anatomical studies on leaf with a light microscope

Transverse, longitudinal and paradermal sections were prepared by hand-sectioning with a sharp razor blade, while the plant tissues were embedded in pith. Cut lengths of needles were previously shaken in chloroform to remove the wax. Thin sections were stained in potassium iodide solutions or with phloroglucinol

plus concentrated hydrochloric acid (to stain the lignin pink) and were mounted in 50% glycerol. For stomata density count on the adaxial and abaxial epidermis, and for measurements on the guard cells, stomatal slit and antechamber aperture, epidermal strips were prepared. A thin slice of the surface tissues were removed from a needle and the underlying mesophyll cells were scraped off with the edge of a razor blade, until only the epidermis and possibly the hypodermal layer remained. This was easiest done after the slice of leaf tissue was stained with potassium iodide, since the mesophyll was stained black or brown and can be easily distinguished from the lightly stained epidermis. The epidermal strip can withstand some rough scraping because of the external cuticularised layer, as well as the sclerified hypodermis. Density counts were made on preserved fascicles obtained from three well-defined levels in the canopy of mature trees planted in Thetford Forest. The preservative used was Formalin-acetic acid-alcohol (90 cm^3 50% Ethanol, 5 cm^3 Acetic acid and 5 cm^3 formalin) which was found not to significantly alter the shape or dimension of the plant tissues.

3.7 Analysis of error

Introduction

Stomatal conductance, g_s, as measured in the leaf chamber, is computed from the formulae:

$$g_s = 1/(A/f((e_i - e_2)/(e_2 - e_1)) - r_a) \qquad 3.11$$

where A is the leaf area in cm^2, f is the flow rate in $cm^3\ s^{-1}$ and

e_i, e_2 and e_1 are the saturated vapour pressures derived from leaf temperature, dew point temperature of the air leaving the leaf chamber, the sample air, and dew point temperature of the air entering the leaf chamber, the reference air, respectively (Fig. 3.3.1). r_a is the boundary layer resistance.

Each component in equation 3.11 contributes towards the overall error in the estimation of g_s.

A brief description of the errors involved is given below and the total error is subsequently estimated.

Flow rate

The flow meter was calibrated over the full range of flow rates by the displacement of water from inverted volumetric flasks. Care was taken to maintain atmospheric pressure and constant temperature during the procedure of measuring the volume of water displaced by the air flow.

Error in calibration was attributed to: 1) timing the flow, and 2) reading the volume of water displaced by the air flow. Random error was reduced by taking the mean of several readings at each of ten flow rates. A linear regression was fitted to the relationship between calibrated flow rate and output from the flow meter as measured on the digital voltmeter. The standard error of the mean slope of the regression line was noted since it is a measure of the calibration error. A calibration error of 0.5% was observed. Error in measurement of flow rate was also small. Taking this into account, the overall error was increased to 1%.

Leaf area measurement

Leaf area was measured with an optical planimeter (Lambda Instr. Corp. Model LI-1300). Random error in measurement was reduced by repeating each measurement twenty or more times and by taking the mean of ten readings closest to each other. The instrument was calibrated at intervals, using graph paper strips of length 5 cm and width 2 mm (approximate length and width of needles). Before carrying out a set of measurements, area measurement was checked against a 50 cm^2 reference. An overall error of 1.5% was considered to be a fair estimate.

Vapour pressures

Saturated vapour pressure at the relevant temperatures were derived from the measurement of dew point temperatures. In calibrating the dew point hygrometer (see relevant section), systematic error was attributed to: 1) parallax error in reading the temperatures of the water bath, and 2) the possibility that the air leaving the water bath was not saturated with water vapour at the temperature of the water bath. The parallax error was normally small.

The second error could be more serious and appropriate measures had been taken to reduce it. The two wash bottles in the humidity control system were checked regularly to ensure that they contained sufficient distilled water to thoroughly wet the "Miracloth". At regular time intervals air was led out from the two reservoirs of the copper coils and checked to ensure presence of condensed liquid water.

During performance of an experiment, the dew point hygrometer

was rebalanced before a set of measurements. Although the entire gas exchange system was heated to about 35°C, well above the normal running temperatures, an added precaution to reduce chances of hidden condensation in the valves and tubings, was by switching on the main pumps and letting the air flow through the main valves and tubings during the night prior to the experiment. The following morning, during the two or more hours stabilisation period, the other valves were activated to clear any hidden condensate. Condensation within the leaf chamber was normally detected by fluctuating dew point hygrometer readings.

When the value of g_s was expected to be low, the flow rate, f, was substantially reduced in order to maintain a high differential in dew point temperatures between the reference air and the sample air. This measure reduced error in the term $(e_2 - e_1)$ in equation 3.11.

From the standard error of the mean slope of the fitted regression line, an error of 0.1°C was ascribed to calibration. Another o.2°C error was ascribed to actual measurement procedures, giving in all a total error of 0.3°C.

Leaf temperature

The saturated vapour pressure, e_i was computed from measurement of leaf temperature. Error in e_i was therefore caused by error in measuring leaf temperatures. Thin thermocouple wires of 0.01143 cm diameter had been used. Attempts were made to ensure maximal contact between the leaf and the wires before the thermojunction was implanted in the leaf tissue. To minimise error caused

by radiation absorbed by the wires, the thermojunction and thermocouple wires were shielded as much as possible by the needles within the shoot. The ratio $(T_s - T)/(T_s - T_a)$ was therefore kept low to avoid large error (T_s, real surface temperature; T, thermocouple temperature; T_a, air temperature) (Perrier 1971). With conifers, because of the low boundary layer resistance as a result of the needle-shape leaves, there is a limit to $(T_s - T_a)$ (see Jarvis, James & Landsberg 1976). Hence the error in $(T_s - T)$ must be very small and was estimated to be $0.1^{o}C$.

The leaf thermocouple had been calibrated by adpressing the thermojunction against the bulb of an accurate mercury thermometer and using them as a stirrer in a 1000 ml beaker of water at various water temperatures. The error in calibration could not be more than $0.1^{o}C$. Total error was therefore within $\pm$ $0.2^{o}C$.

Boundary layer resistance

As in calculating g_s, the error in boundary layer resistance, r_a, is made up of the errors in flow rate, leaf area measurement, vapour pressure measurement and measurement of surface temperature of the plaster cast. The measurements of these involved similar techniques. An error of 10% is estimated for r_a. As the boundary layer resistance is usually very low, the error in r_a in absolute terms is also very low.

Estimation of error in stomatal conductance.

Table 3.7.1 shows the total error in each of the various components that go into the computation of g_s.

Table 3.7.1 Summary of error in the computation of stomatal conductance as measured by gas exchange

Measurement	Method	Error
Flow rate	Thermal mass flowmeter	1%
Leaf area	Optical planimeter	1.5%
Dew point temperature	Dew point hygrometer	0.3°C
$(e_2 - e_1)$	Calculated from dew point temperatures	2%
Leaf-air vapour pressure difference $(e_i - e_2)$	Calculated from dew point temperatures	0.2 to 0.9%
Leaf temperature	Copper-constantan thermocouple	0.2°C
Boundary layer resistance	Plaster cast technique	10%

When $Z = (A + B)$ or $(A - B)$ and the error on A and B are ΔA and ΔB, then the error, ΔZ in Z is

$$\Delta Z = \left[(\Delta A)^2 + (\Delta B)^2 \right]^{\frac{1}{2}} \qquad 3.12$$

When $Z = A \times B$ or A/B

$$\frac{\Delta Z}{Z} = \left[\left(\frac{\Delta A}{A}\right)^2 + \left(\frac{\Delta B}{B}\right)^2 \right]^{\frac{1}{2}} \qquad 3.13$$

By use of equations 3.12 and 3.13 the error in g_s, Δg_s, can be computed from equation 3.11.

Hence:

$$\frac{\Delta g_s}{g_s} = \left[\left(\frac{\Delta f}{f}\right)^2 + \left(\frac{\Delta A}{A}\right)^2 + \left(\frac{\Delta e_i}{e_i - e_2}\right)^2 + \left(\frac{\Delta e_2}{e_i - e_2}\right)^2 + \left(\frac{\Delta e_2}{e_2 - e_1}\right)^2 + \left(\frac{\Delta e_1}{e_2 - e_1}\right)^2 + (\Delta r_a)^2 \right]^{\frac{1}{2}} \qquad 3.14$$

The errors for a high g_s value and for a low g_s value were calculated using the conditions given in actual experiments.

Case I. High g_s.

At a leaf temperature, T_1 of 10.5°C, and a leaf-air vapour pressure difference of 0.523 kPa, the calculated g_s was 0.477 cm s^{-1}. Other component values and their error based on Table 3.7.1 are given as:

Flow rate, f : 47.83 ± 0.478 cm3 s^{-1}

Leaf area, A : 59.72 ± 0.896 cm2

Leaf temperature, T_1 : 10.05 ± 0.2°C

Dew point temperature for sample air : $2.08 \pm 0.3^{o}C$

Dew point temperature for reference air : $-5.35 \pm 0.3^{o}C$

Hence,

saturated vapour pressure at the site of evaporation,

$$e_i : 1.232 \pm 0.025 \text{ kPa}$$

vapour pressure of sample air, $e_2 : 0.710 \pm 0.015$ kPa

vapour pressure of reference air, $e_1 : 0.410 \pm 0.10$ kPa

Boundary layer resistance, $r_a : 0.0330 \pm 0.0035 \text{ cm}^{-1}$

Substituting into equation 3.14, the calculated error was found to be 8.4%.

Case II. Low g_s

At leaf temperature, T_1 of $25.49^{o}C$, and leaf-air vapour pressure difference of 2.362 kPa, the calculated g_s was 0.0486 cm s^{-1}. Other component values and their error based on Table 3.7.1 are given as:

Flow rate, $f : 30.17 \pm 0.3017 \text{ cm}^3 \text{ s}^{-1}$

Leaf area, $A : 42.39 \pm 0.6359 \text{ cm}^2$

Leaf temperature, $T_1 : 25.49 \pm 0.2^{o}C$

Dew point temperature for sample air : $5.43 \pm 0.3^{o}C$

Dew point temperature for reference air : $2.61 \pm 0.3^{o}C$

Hence,

saturated vapour pressure at the site of evaporation,

$$e_i : 3.261 \pm 0.039 \text{ kPa}$$

vapour pressure of sample air, $e_2 : 0.899 \pm 0.019$ kPa

vapour pressure of reference air, $e_1 : 0.737 \pm 0.016$ kPa

Boundary layer resistance, r_a : 0.0330 ± 0.003 s cm^{-1}

Substituting into equation 3.14 the calculated error was found to be 15.5%.

The figures 8.4 to 15.5% in the computation of g_s is considered as a fair estimate of the error involved in measuring g_s by gaseous exchange. A major portion of the error is systematic error. It is, however, rare that all the systematic errors of the component measurements come together at any one time and it is expected that in actual experimentation, the error is substantially lower. The reproducibility of results from one experiment to another on the same shoot suggests that in practice, the error is about $\pm$ 5%. From the point of view of interpreting the results, a far more significant source of variation is the difference between shoots. This can lead to standard errors of up to 50% as shown by the vertical bars in the figures.

CHAPTER 4

RESULTS

4.1 Introduction

Microscopic examination of the internal structures of needles of Pinus sylvestris was done with the following objectives in view:

(1) to define the diffusion resistance pathway for water vapour from the mesophyll cells to the outside air.

(2) to understand how the environmental variables affect the stomata via water movements.

(3) to find out how variation in g_s between canopy level is affected by stomatal frequency.

Because of the high variability in values of g_s amongst the plants most data have been normalised with respect to either the highest g_s value or to a convenient g_s value in order to obtain functional relationships. Wherever possible and meaningful, curves have been fitted using a non-linear least squares method. The functions used and the values of the parameters are given in tables referred to in the appropriate sections.

4.2 Anatomy of leaf

The stomata are arranged in longitudinal rows, 12 to 14 rows for the entire width of the adaxial or abaxial surface. Tube-like and plate-like wax secretions cover the external epidermis, particularly in broad, longitudinal bands in between the stomatal rows (Figure 4.2.1). There are more such bands on the adaxial than on the abaxial surface, which has more stomatal rows.

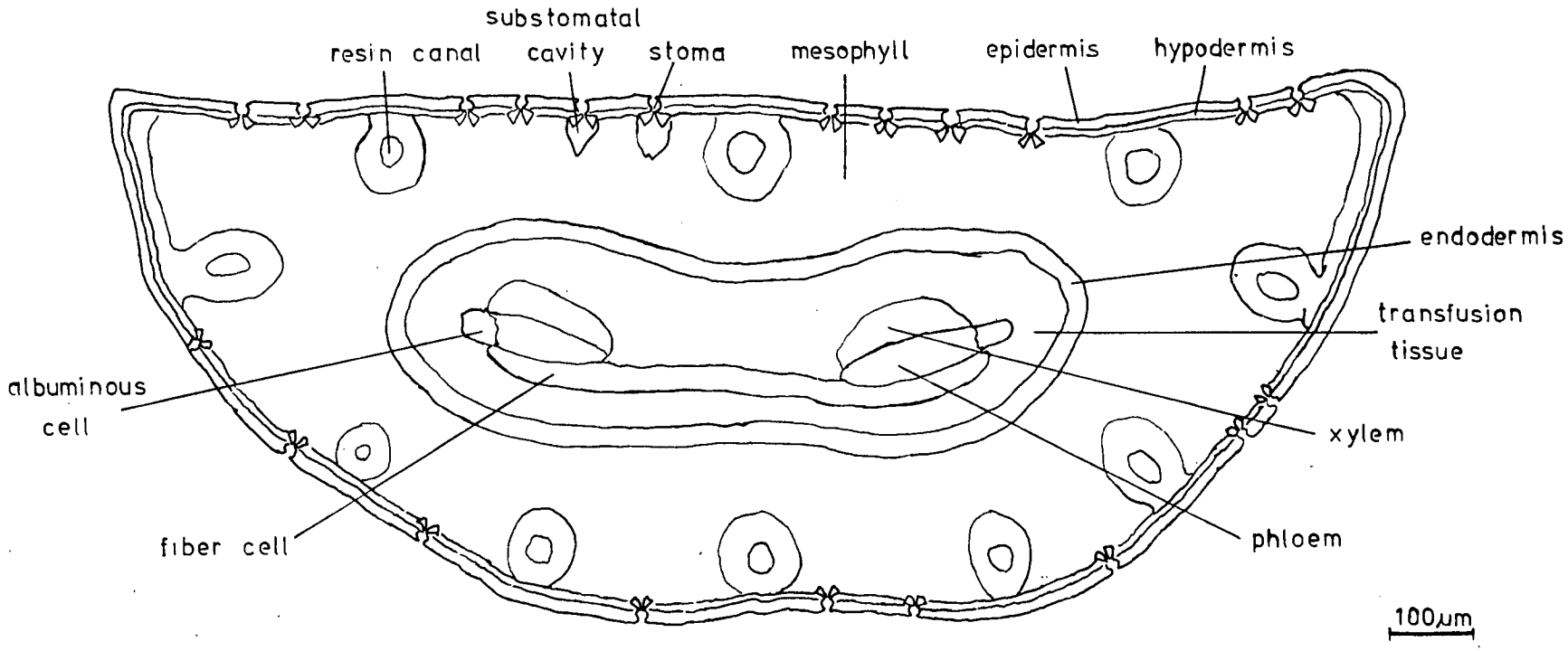


Figure 4.2.1 Transverse sections, showing layout of various tissues. The stomata are arranged in longitudinal rows, with relative position as shown. The epidermal surface is covered with a whitish extruded wax. The xylem points towards the adaxial side, the phloem toward the abaxial side.

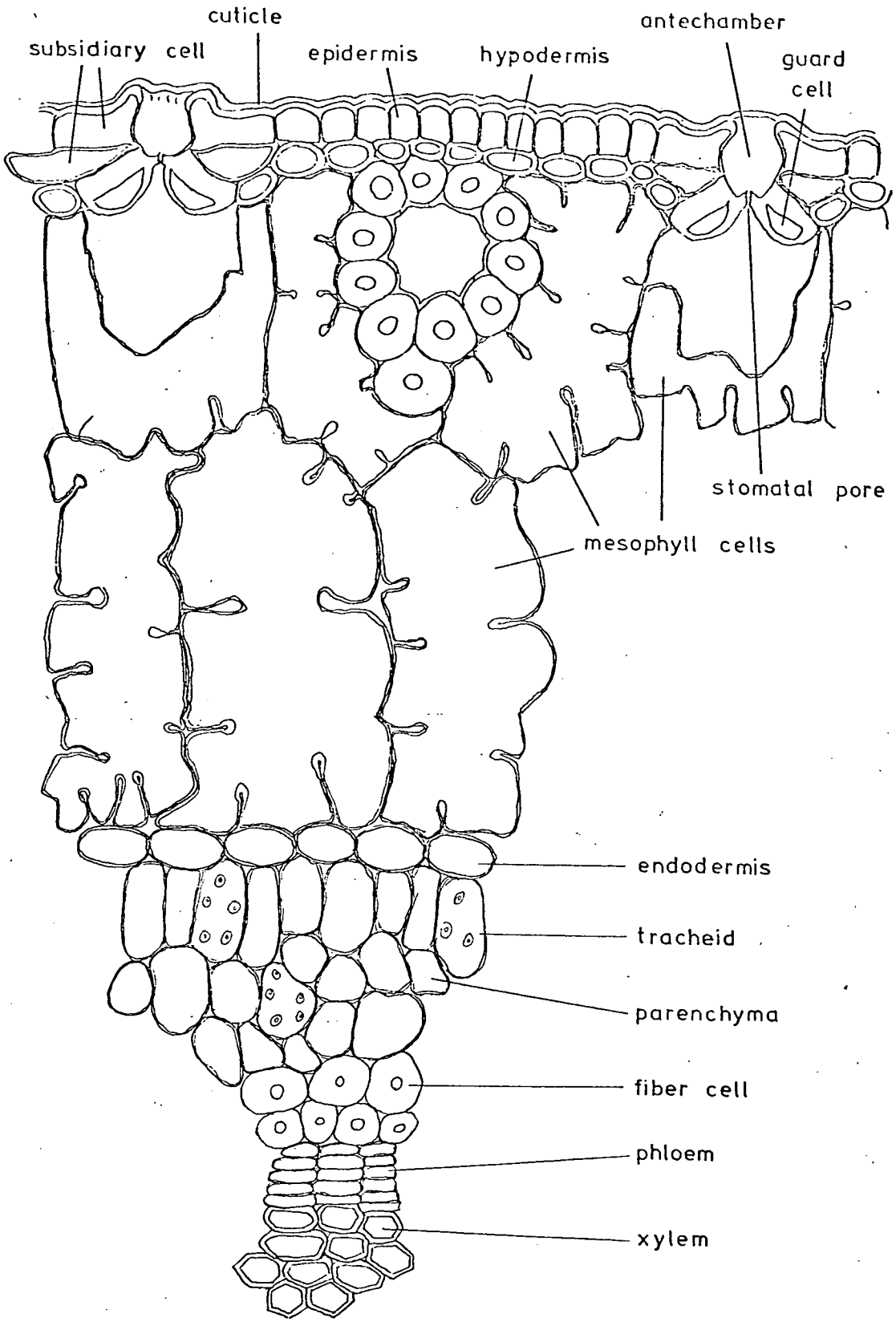


Figure 4.2.2 Details of tissues in transverse section. See text for description.

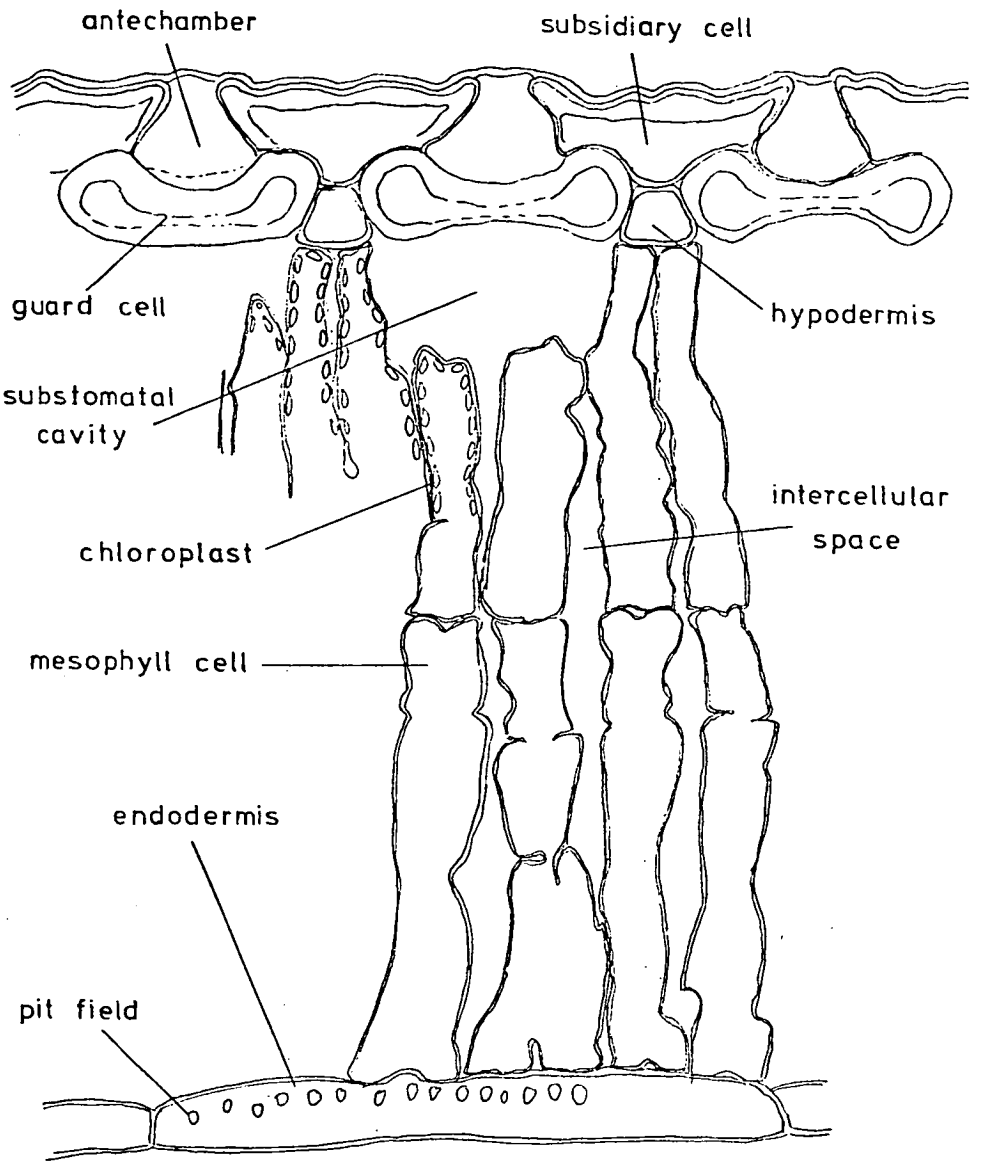


Figure 4.2.3 Longitudinal section, showing details of the guard cells. Note the lack of cuticle and thinner cutinized wall of the antechamber. In its natural state, the antechamber is filled with wax tubes intermeshed together to form a wax plug. The outside surface of the epidermis is also covered with a layer of wax.

The stomata are sunken and appear as though suspended from the subsidiary cells (Figures 4.2.2 and 4.2.3). The stomatal pore is overlaid by a pot-like, funnel shaped antechamber which is normally filled with a wax plug consisting of intermeshed wax tubes (Jeffree, Johnson & Jarvis 1971). The antechamber opens out into the external air by an opening in the epidermis of shape varying from hexagonal to circular.

A ring of subsidiary cells sit on top of each of the guard cells pair. The guard cells have a prominent ledge that borders on the stomatal pore which appeared as a long slender slit with pointed ends. The walls of the guard cells are of uneven thickness. The dorsal wall is thicker than the ventral wall, which faces the intercellular air spaces. The cuticularised layer of the epidermis is fairly thick.

A thick-walled sclerified hypodermis occurs beneath the epidermis except where the stomata are located.

The large, lobated mesophyll cells are thin-walled and are densely filled with chloroplast. They look compacted in transverse section (Figure 4.2.2), but in longitudinal section (Figure 4.2.3) they appear as loosely arranged irregular columns, separated from each other by corridor-like intercellular air spaces of non-uniform width. The mesophyll cells have typical invaginated folds derived from one wall layer to one side of the middle lamella (Harris 1971) (Figure 4.2.2). The larger of these invaginated folds do connect up with the intercellular spaces. Measurements in paradermal sections showed that the mesophyll has about 23 - 25% air spaces in the plane

mid-way between the guard cells and the endodermis, and increase to 60% at the level of the substomatal cavity. There are normally two mesophyll cells bridging the hypodermis to the endodermis. The mesophyll is typically U-shaped beneath the guard cells.

The two vascular bundles are surrounded by a transfusion tissue that is separated from the mesophyll by an endodermal sheath (Figure 4.2.1). The transfusion tissues consist of thin-walled tracheids and large parenchymatous cells. The xylem is located towards the adaxial side and consists of rows of tracheids and a few rays. The bundle tracheids have thicker secondary walls and are not deformed like the transfusion tracheids.

A fusiform cambium separates the xylem from the phloem.

The phloem is located towards the abaxial side and is adjacent to a sclerenchymatous layer of thick-walled fiber cells. Some fiber cells are also found between the two vascular bundles amongst the transfusion tissue. Adjacent to the outer edge of the phloem is a group of specialized parenchyma cells known as albuminous cells.

Frequency of stomata by level in the canopy

Field measurements show that g_s is highest in level 1, followed by level 2 and level 3. The value of g_s at level 2 and 3 are respectively 77 and 65% of that of level 1. The stomatal frequency is therefore consistent with decreasing g_s in progressing from level 1 to level 2 (see Table 4.2.1). However, level 3 shows the highest stomatal frequency whereas it has the lowest g_s. This suggests that while stomatal frequency does give an indication of the relative magnitude of g_s, other factors are also involved. One

Table 4.2.1 Stomatal frequency assessment on three canopy levels. Anatomical measurements done on 1-year-old needles at level 1 in the canopy of field grown trees, at Thetford Forest. Average of about 40 measurements.

	Adaxial Surface (μm)	Abaxial Surface (μm)
Stomatal frequency (mm^{-2})		
Level 1	105.6 ± 7.7	94.2 ± 5.2
Level 2	79.9 ± 7.2	79.1 ± 3.9
Level 3	119.6 ± 4.6	104.9 ± 2.1
Size of stomata		
Length	68.0 ± 0.77	66.3 ± 0.81
Breadth	46.3 ± 0.46	45.3 ± 0.72
Length of stomatal pore*	31.7 ± 0.54	30.4 ± 0.47
Width of stomatal pore*	1.5	1.5
Diameter of antechamber aperture	17.1 ± 0.33	15.7 ± 0.27
Size of substomatal cavity		
Length	18.5 ± 0.36	16.8 ± 0.32
Breadth	15.6 ± 0.31	14.6 ± 0.26
Depth of mesophyll tissue	230.0 ± 7.5	202.0 ± 10.8

* with stomata partially closed.

factor not considered here in the relative size of the stomatal pore. Needles in level 3 may have smaller stomatal pores; this could account for their lower g_s, in spite of the high stomatal frequency.

Comparison of the stomatal frequencies (Table 4.2.1) shows that the adaxial surface has a higher stomatal frequency than the abaxial surface for level 3, but not for level 1 and 2. Measurement of the length of the stomatal pore (mean of seventy measurements) shows that it is significantly longer in the adaxial surface (31.7 ± 0.5 μm) than in the abaxial surface (30.4 ± 0.5 μm) at level 1. Measurements of the width of the stomatal pore indicate no difference between the two surfaces. However, this is mainly because of the small width (1.5 μm) involved which made minute differences indistinguishable under the light microscope. Existing laboratory facilities do not allow separate measurements of g_s for the adaxial and abaxial surfaces in needles of most conifers. Nevertheless, based on the stomatal frequency as well as the measured length of the stomatal pore, it is possible that g_s in the adaxial surface is larger than g_s in the abaxial surface in Pinus sylvestris, particularly in level 3.

Derivation of stomatal diffusive resistances from anatomical measurements

Water vapour molecules diffuse out from the moist walls of the mesophyll, into the substomatal cavity, pass the stomatal pore, traverse the spaces between the wax tubes lining the antechamber walls and out through the antechamber aperture into the outside air (Figure 4.2.2). Along the way, the water vapour molecules encounter various diffusive resistances. The total resistance, r_T consists of

each of those resistances in series.

Hence,

$$r_T = r_p + r_i + r_w \quad (s\ cm^{-1}) \qquad 4.1$$

where r_p is the stomatal pore resistance, r_i the resistance attributable to the substomatal cavity, and r_w the resistance attributed to the wax-filled antechamber.

From Fick's law, the volume flux of water vapour, q, through unit area of leaf containing n stomatal pores of area A (cm^2) and depth k (cm) is:

$$q = n\ D\ A\ \frac{\Delta W}{k} \quad (cm^3\ cm^{-2}\ s^{-1})$$

$$= \frac{\Delta W}{r_p} \qquad 4.2$$

where $\Delta W/k$ is the concentration gradient in water vapour and D is the diffusion coefficient of water vapour in air ($\approx 0.25\ cm^2\ s^{-1}$). The area, A of the slit-shaped pore is w x b, where w is the mean width and b is the mean length of the stomatal pore (see Figure 4.2.4). From equation 4.2 the diffusive resistance of the stomatal pores per unit area of leaf surface, r_p is defined by:

$$r_p = \frac{k}{D\ w\ b\ n} \quad (s\ cm^{-1}) \qquad 4.3$$

The average length of the partially closed stomatal pore is 31.1 μm. It is expected that when the pore is open there is a reduction of this length to about 27 μm (Meidner & Mansfield 1968). This figure has been substituted for b in calculating r_p. Measurements of partially open stomatal pores show that w is about 2 μm. The figure of 3 μm has therefore been taken as the width of the

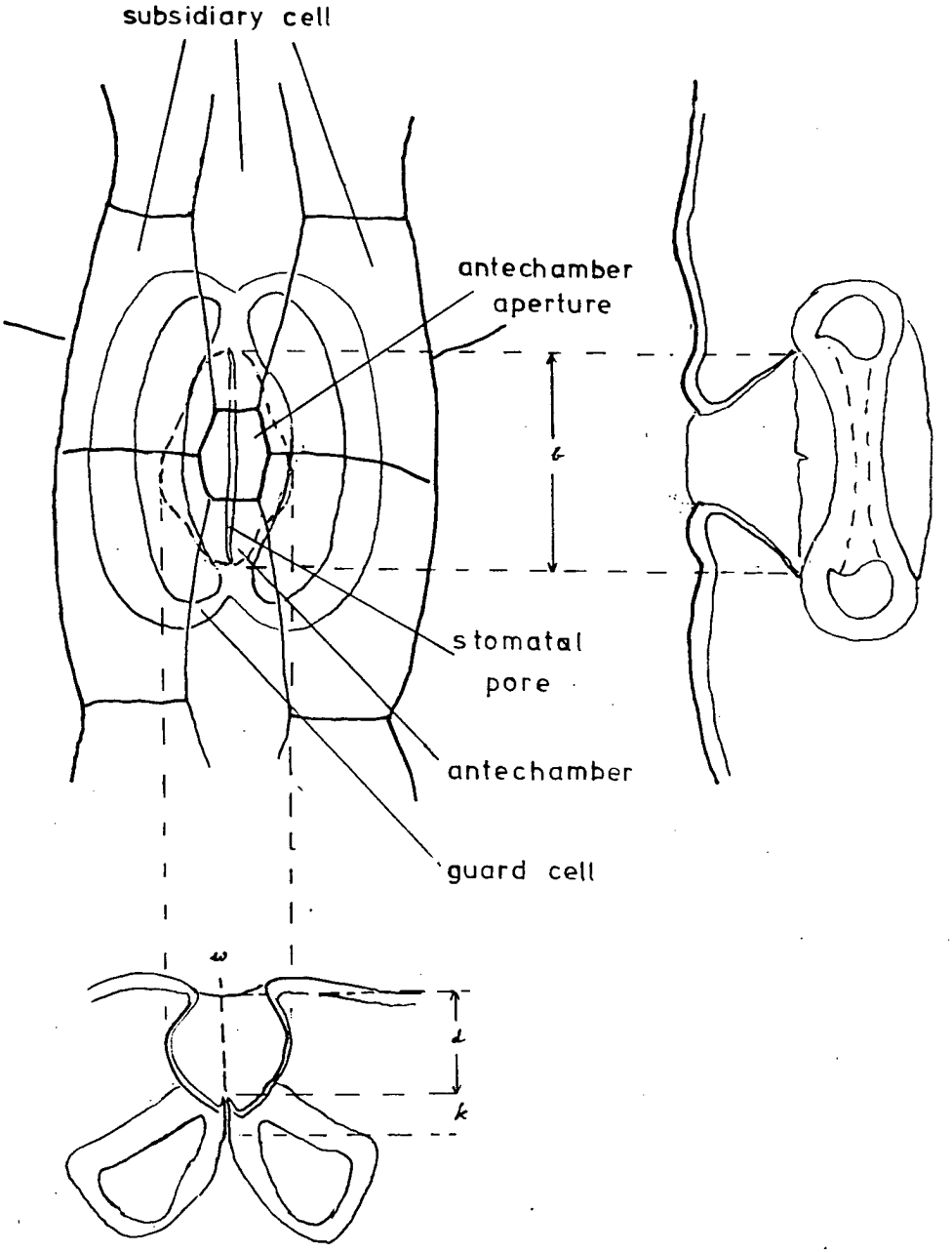


Figure 4.2.4 Surface view of the subsidiary cells and antechamber aperture, overlying the guard cells and stomatal pore. Outline of the antechamber and its projection in transverse section (bottom) and longitudinal section (right) is shown. The width of the pore is denoted as w, its depth, k, and its length, b; d is the depth of the antechamber.

open pore, w. The depth of the pore, k, was taken as 6 μm. An average figure of 10^4 cm^{-2} was taken for the stomatal frequency, n.

Substituting these figures (all are compiled in Table 4.2.2) into equation 4.3 yields a minimum estimate of

$$r_p = 0.296 \text{ s cm}^{-1}.$$

The mesophyll of needles in canopy level 1 forms a relatively thick layer between the hypodermis and substomatal cavity, and the endodermis. It is ca 216 μm from endodermis to hypodermis. The walls of the mesophyll cells adjacent to the air 'corridors' that form the intercellular spaces, are generally gently rounded. The average width of the air corridors is ca 7.8 μm. The substomatal cavity is relatively large and well-defined. Because of its close proximity to the stomatal pore, it is likely that a major proportion of the water vapour molecules which eventually diffuse out of the stomatal pore, will come from the walls of the mesophyll cells which line the substomatal cavity, rather than from the deeply placed underlying mesophyll cell walls.

The substomatal cavity can be regarded as a cylinder with a flat circular base of radius, a_i equivalent to half the mean width of the substomatal cavity, and depth, k_i equivalent to the maximum depth. This assumption approximates closely to the actual shape of the substomatal cavity, which very often has straight parallel walls, where it joins the hypodermis. By making use of the maximum depth, a maximum estimate of the diffusive resistance of the cavity is obtained. Any over estimation compensates for the irregular and rounded base of the substomatal cavity and probably also for the

Table 4.2.2 Values used in the calculation of diffusive resistances.

Symbol	Description		Value
r_p	stomatal resistance	(s cm^{-1})	0.296
k	depth of stomatal pore	(μm)	6
w	width of stomatal pore	(μm)	3
b	length of stomatal pore	(μm)	27
n	number of stomata	(cm^{-2})	10000
r_i	substomatal resistance	(s cm^{-1})	0.144
k_i	depth, substomatal cavity	(μm)	38.4
a_i	radius, substomatal cavity	(μm)	18.4
r_y	antechamber resistance without wax	(s cm^{-1})	0.529
r_w	antechamber resistance with wax	(s cm^{-1})	1.163
d	depth, antechamber	(μm)	18
A_o	area, antechamber aperture	(μm^2)	214
A	area, stomatal pore	(μm^2)	80
D	diffusion coefficients of water	(cm^2 s^{-1})	0.25

additional resistance encountered by those molecules which derive from the adjacent intercellular spaces.

It follows from equation 4.3 that:

$$r_i = \frac{k_i}{D \pi (a_i)^2 n} \quad (s\ cm^{-1}) \qquad 4.4$$

Substituting the figures given in Table 4.2.2 gives the diffusive resistance attributable to the substomatal cavity as:

$$r_i = 0.144 \quad s\ cm^{-1}$$

The wax tubes in the antechamber increase the tortuosity of the diffusion pathway and reduce the cross-sectional area through which diffusion can occur. For simplicity in calculating its diffusive resistance, the antechamber is assumed to be funnel-shaped, although in transverse section (Figure 4.2.2) it appears as pot-shaped and in longitudinal section (Figure 4.2.3) it appears as an inverted funnel. The water vapour molecules therefore are assumed to diffuse out from the stomatal pore, of area A, and move in the general direction of the circular antechamber aperture of area A_o, through a distance equivalent to the depth of the antechamber, d (Figure 4.2.4). The cross-sectional area through which the water vapour molecules diffuse is taken as increasing linearly with increasing distance from the stomatal pore.

Therefore the cross-sectional area, A_z, at a distance z (cm) from the stomatal pore is given by:

$$A_z = A + \frac{z}{d} (A_o - A) \quad (cm^2) \qquad 4.5$$

This assumption is independent of and far more important than the shape of the antechamber.

The diffusive resistance of the antechamber per unit area of leaf surface without the presence of wax tubes, r_y is:

$$r_y = \frac{1}{nD} \int_{z=0}^{z=d} \frac{dz}{A_z} \quad (s\ cm^{-1})$$

$$= \frac{d}{n\ D\ (A_o - A)} \quad (\frac{A_o}{A}) \qquad 4.6$$

Substituting the figures given in Table 4.2.2

$$r_y = 0.529 \quad s\ cm^{-1}$$

It is difficult, using a light microscope to estimate accurately, the cross-sectional area occluded by the wax tubes. Further, hand-sectioning inevitably disturbs and disorientates the wax tubes. In view of the lack of information, it is assumed that the length of the diffusion pathway is increased by 1.5 times, and the cross-sectional area, A_o, is reduced by half. Substituting these values into equation 4.6 gives the diffusive resistance of the antechamber with waxes present as:

$$r_w = 1.163 \quad s\ cm^{-1}$$

Thus, the calculated total resistance, r_T, is 0.296 + 0.144 + 1.163 = 1.603 s cm^{-1}. The corresponding conductance, g_s, is 0.624 cm s^{-1}, a figure which agrees well with the highest values of g_s, calculated from gas exchange measurements. The wax tubes contribute a resistance of 1.163 - 0.529 = 0.634 s cm^{-1} to the total resistance, i.e. 40% of the total, a figure agreeing well with the figure of 37%, calculated for Picea sitchensis by Jeffree, Johnson and Jarvis (1971).

Summary of results

(1) The sunken stoma is separated from the outside air by an antechamber, filled with wax tubes. Its position above a well-defined substomatal cavity, which acts as a reservoir for the moist air evaporating from the mesophyll via the intercellular spaces, places it strategically to sense the ambient humidity, as well as to act as control for transpiration and photosynthesis.

(2) A hypodermal layer separates the moist walls of the mesophyll from the subsidiary cells and guard cells.

(3) The guard cells of Pinus sylvestris are relatively large (67.2 x 45.8 μm), compared to other plants.

(4) A comparison of stomatal frequency on 1-year-old needles shows canopy level 1 having the highest stomatal frequency, followed by that of level 3 and level 2.

(5) The wax-filled antechamber and stomatal pore form respectively 75% and 18% of the total diffusive resistance from the substomatal cavity to the outside air (excluding boundary layer resistance) when the stomata pore is open.

4.3 Diurnal behaviour of stomata

Preliminary experiments were done to find out whether diurnal rhythms in stomatal conductance and leaf water potential might interfere with the effects of changes in the ambient environmental conditions in the leaf chamber during an experiment.

Figure 4.3.1 illustrates results of an experiment carried out in the growth room with potted plants. Stomatal conductance was measured in situ with a null-balance diffusion porometer

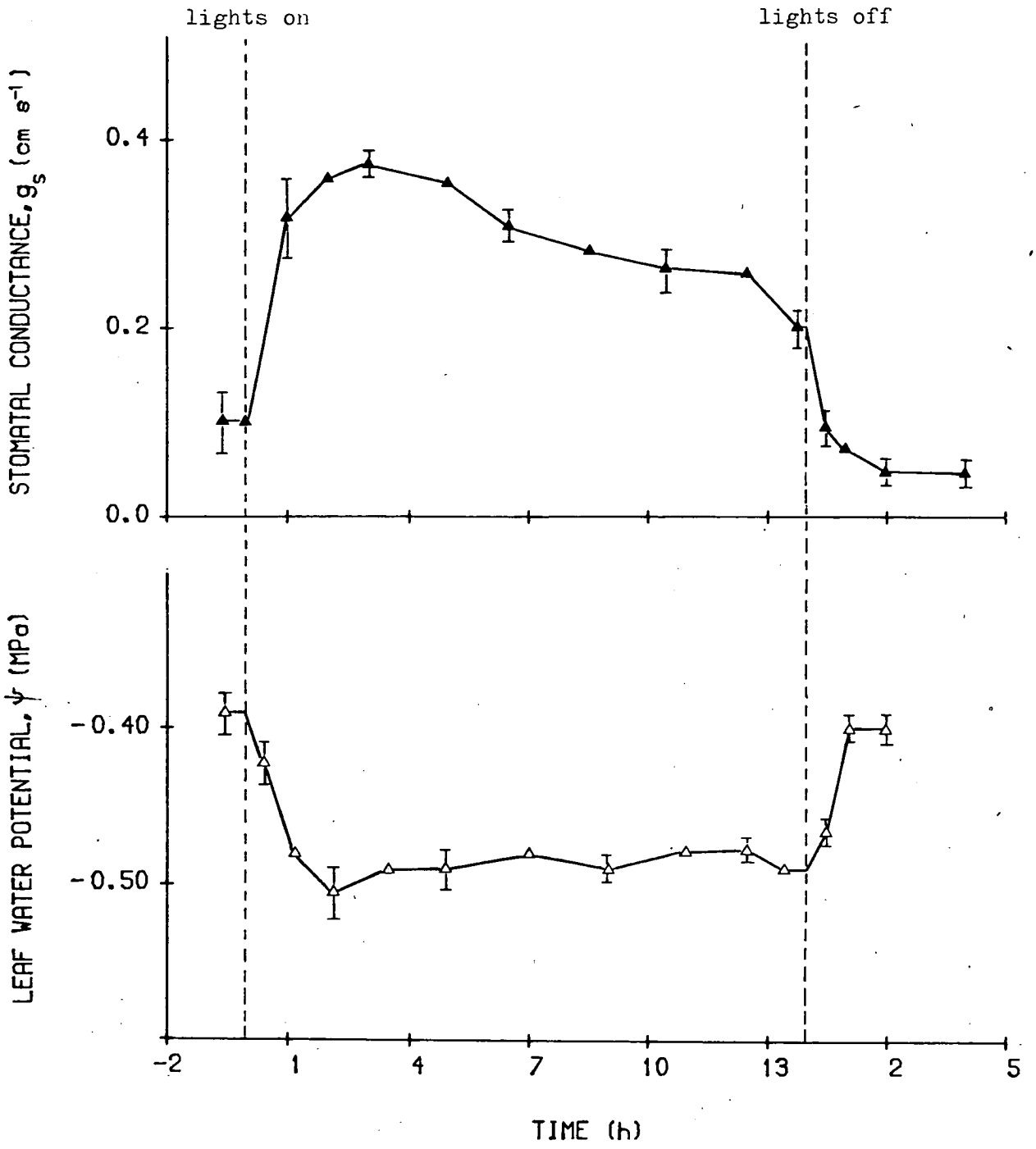


Figure 4.3.1 The change in stomatal conductance (g_s, ▲) and leaf water potential (ψ, △) with time at the beginning and end of illumination in a growth room. Photon flux density 255 μE m^{-2} s^{-1}; leaf temperature 19.0°C and leaf-air vapour pressure difference 0.52 kPa during the day and 14°C and 0.48 kPa respectively, during the night. All points are means of six measurements on six different potted plants with two standard errors shown.

(Beardsell, Jarvis & Davidson 1972). Leaf water potential, ψ was also measured. On changing from dark to light, g_s increased rapidly in the first hour, and then continued to increase at a slower rate until a maximum was reached after 3 h. g_s was essentially stable between 2 and 5 h after lights on. Subsequently there was a slow decrease in g_s, until the lights were turned off.

The changes in g_s were reflected by concurrent changes in ψ, which initially decreased, reaching a minimum of -0.5 MPa after 2 h. ψ subsequently fluctuated within narrow limits in a very slow climb for the remaining part of the light period.

On changing from light to dark, g_s decreased rapidly to a stabilised minimum after 2 h. This change was reflected in a rapid increase in ψ which reached a steady state value of -0.4 MPa after 1 h.

Two similar experiments were done with intact shoots in the leaf chamber. The first experiment was done with a photon flux density of 1580 μE m^{-2} s^{-1}. The results (Figure 4.3.2a) showed a rapid increase in g_s during the first 2.5 h after lights on, followed by a slow increase. The maximum value of g_s was reached after 3.5 h, and was maintained for the following 3 h, after which g_s slowly declined. The second experiment was done at a lower photon flux density of 160 μE m^{-2} s^{-1}. Other conditions were as in the previous experiment. On changing from dark to light, there was a slow increase in g_s during the first 0.5 h (Figure 4.3.2b), followed by a rapid increase to a maximum after 2.5 h. Subsequently, there was a slow decrease. A stable g_s value was reached 4.5 h

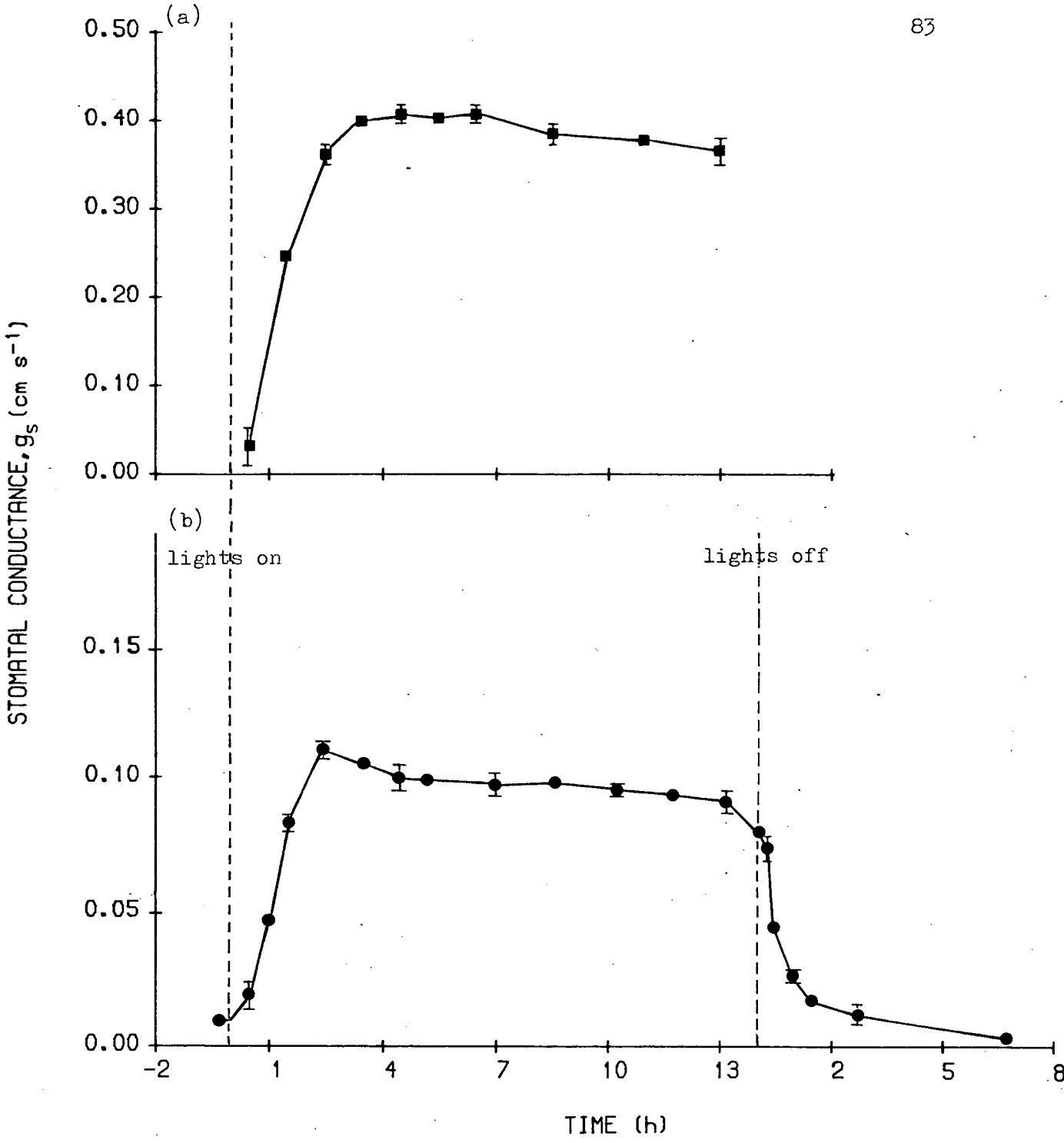


Figure 4.3.2 The change in stomatal conductance with time at the beginning and end of illumination (a) 1580 μE m^{-2} s^{-1}, and (b) 160 μE m^{-2} s^{-1} in the leaf chamber. Leaf temperature 10.2°C and leaf-air vapour pressure difference 0.53 kPa throughout. Bilateral illumination. All points are means of three measurements with two standard errors shown.

after lights on and lasted for the next 4 h, before a small reduction in g_s was recorded. An hour before the lights went off the decline in g_s accelerated.

On changing from light to dark, g_s declined rapidly for about 1 h. Subsequently, stomatal closure slowed down and complete closure was observed only after 7 h of darkness. There was no change in leaf temperature or leaf-air vapour pressure difference for the duration of the experiment. The response time for 66.7% change in g_s in response to a change in photon flux density from 0 to 160 $\mu E\ m^{-2}\ s^{-1}$ (stomatal opening) and from 160 to 0 $\mu E\ m^{-2}\ s^{-1}$ (stomatal closing) was estimated to be 110 and 60 min respectively.

In all three sets of results, after a period of stabilisation, there was a slow decline in g_s value. When the light level was 1580 $\mu E\ m^{-2}\ s^{-1}$ or 160 $\mu E\ m^{-2}\ s^{-1}$, stable g_s value was reached after 3.5 to 4.5 h. Difference in light level did not have much effect on the time for a stable g_s value to be obtained. On the other hand, when the leaf temperature was 19.0°C, instead of 10.2°C, the time to reach stabilisation was 2 h. This indicated that the higher leaf temperature had shortened the time to reach stable g_s value to 2 h from an average of 4 h.

In view of the fact that a 14 h photoperiod was used for the growth room plants, the 4 to 4.5 h initial stabilisation period was found to be too lengthy to be practical, whenever long experiments, involving leaf temperature at around 10.2°C was done. From Figure 4.3.2b it was estimated that the value of g_s at 2 h was similar to the value of g_s at 4.5 h. A stabilisation period of 2 h was

therefore considered as suitable before commencement of actual experiments and was subsequently used. In experiments where the light level was closer to 1580 $\mu E\ m^{-2}\ s^{-1}$, either as the first light treatment or as the prevailing light level for the day's experiment, a longer period of stabilisation had to be used. For experiments at leaf temperature of 19°C or higher, a 2 h stabilisation period was used.

Summary of results

(1) The diurnal rhythm in g_s consists of an initial stomatal opening stage, followed by a stabilisation stage and ended in a period of slow decline.

(2) The time for stabilised g_s value to be reached was affected by leaf temperature but not by light level. At 19°C this time period was 2 h, while at 10.2°C it was about 4 h.

(3) The diurnal change in g_s were reflected by concurrent changes in Ψ.

(4) The time for complete stomatal closure to occur was estimated to be about 7 h after the lights were turned off.

(5) The response time for 66.7% change in g_s in response to a change in photon flux density from 0 to 160 $\mu E\ m^{-2}\ s^{-1}$ and from 160 to 0 $\mu E\ m^{-2}\ s^{-1}$ was estimated to be 110 and 60 min respectively.

4.4 Stomatal response to light

Light induced opening and closing

In a number of experiments, photon flux density was increased in progressive steps, starting from darkness. An equilibration time of 0.75 to 1.0 h was used for each light level. Unilateral light was used. Relative stomatal conductance, G as a function of photon flux density, is shown in Figure 4.4.1. The relationship is hyperbolic in form. Stomatal conductance was not light saturated at the maximum photon flux density of 1400 $\mu E\ m^{-2}\ s^{-1}$. There was a measurable g_s in the dark, indicating that the stomata were not completely closed. When photon flux density was decreased in progressive steps, starting from a high photon flux density, a different relationship was found (Figure 4.4.2). A 0.75 to 1.0 h equilibration period and unilateral light was again used. The fitted rectangular hyperbola showed a pronounced convexity (Figure 4.4.2). Values of G were higher for all levels of photon flux density including darkness, than when photon flux density was progressively increased.

The experiments illustrated in Figures 4.4.1 and 4.4.2 were done separately. When both experiments were done, one after the other within a day, and with bilateral illumination, similar relationships between G and photon flux density were found (Figures 4.4.3a and 4.4.3b). Again the main variation depended on whether the experiment was done in step sequence of increasing photon flux density or in step sequence of decreasing photon flux density. There was a close similarity in the form of the light response curves

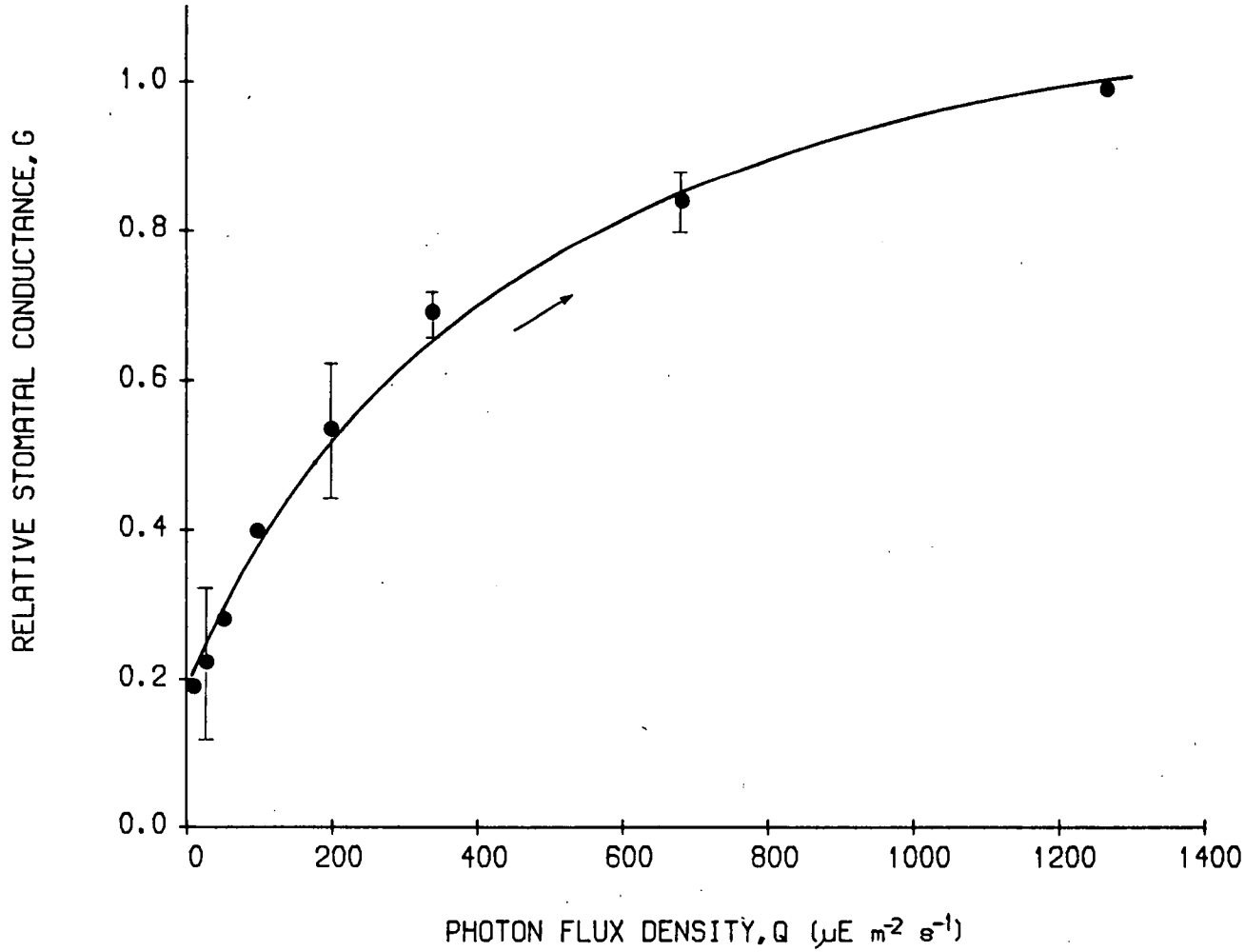


Figure 4.4.1 Relative stomatal conductance as a function of increasing photon flux density. Values of stomatal conductance, g_s have been normalised with respect to the value of g_s at the highest photon flux density. Fitted curves (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.147 cm s^{-1}. Unilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.54 kPa. Two standard errors are shown on representative points.

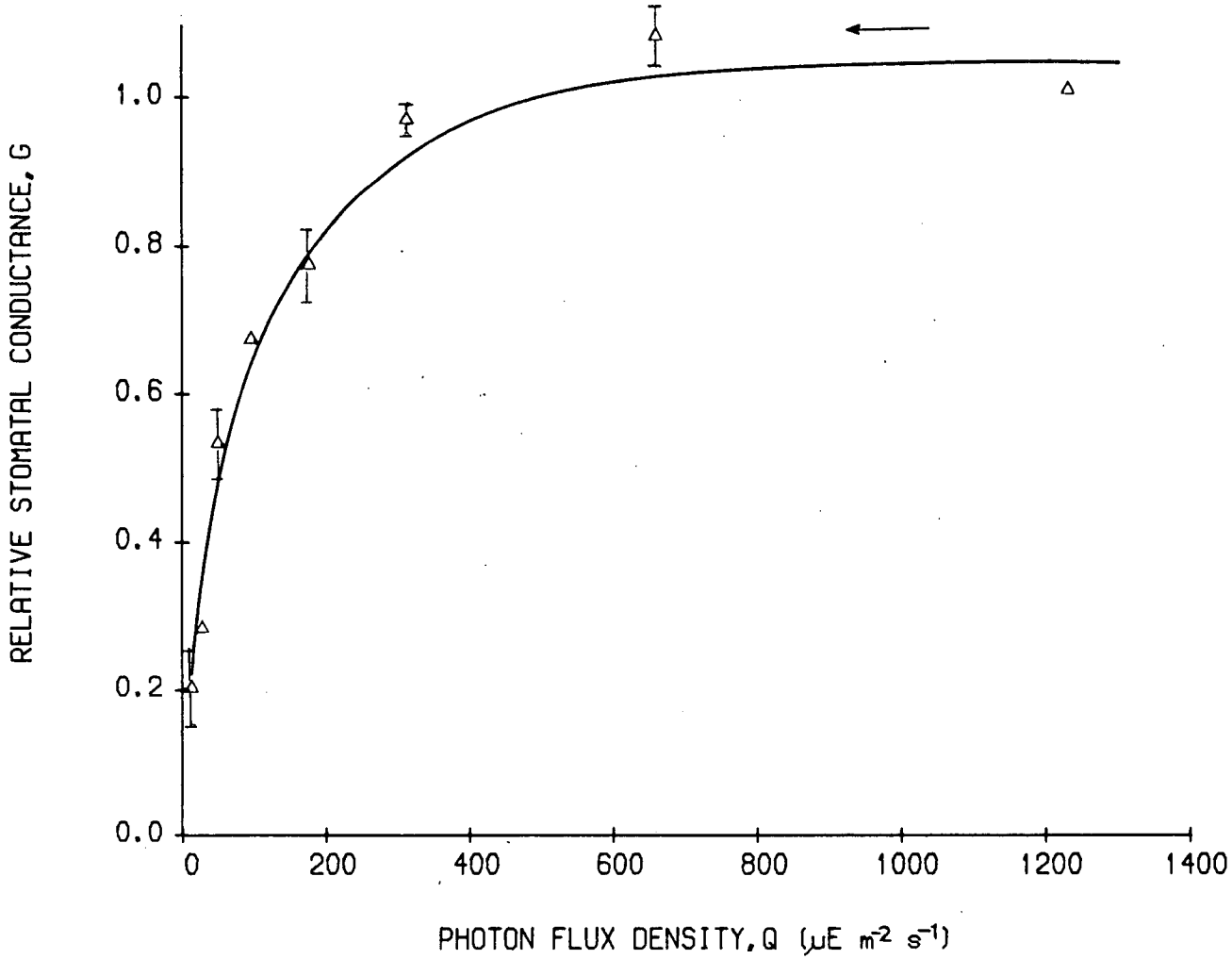


Figure 4.4.2 Relative stomatal conductance as a function of decreasing photon flux density. Normalised data; average g_s for three shoots at G = 1.0 is 0.138 cm s^{-1}. Fitted curve (see Table 4.7.1). Unilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.54 kPa. Two standard errors are shown on representative points.

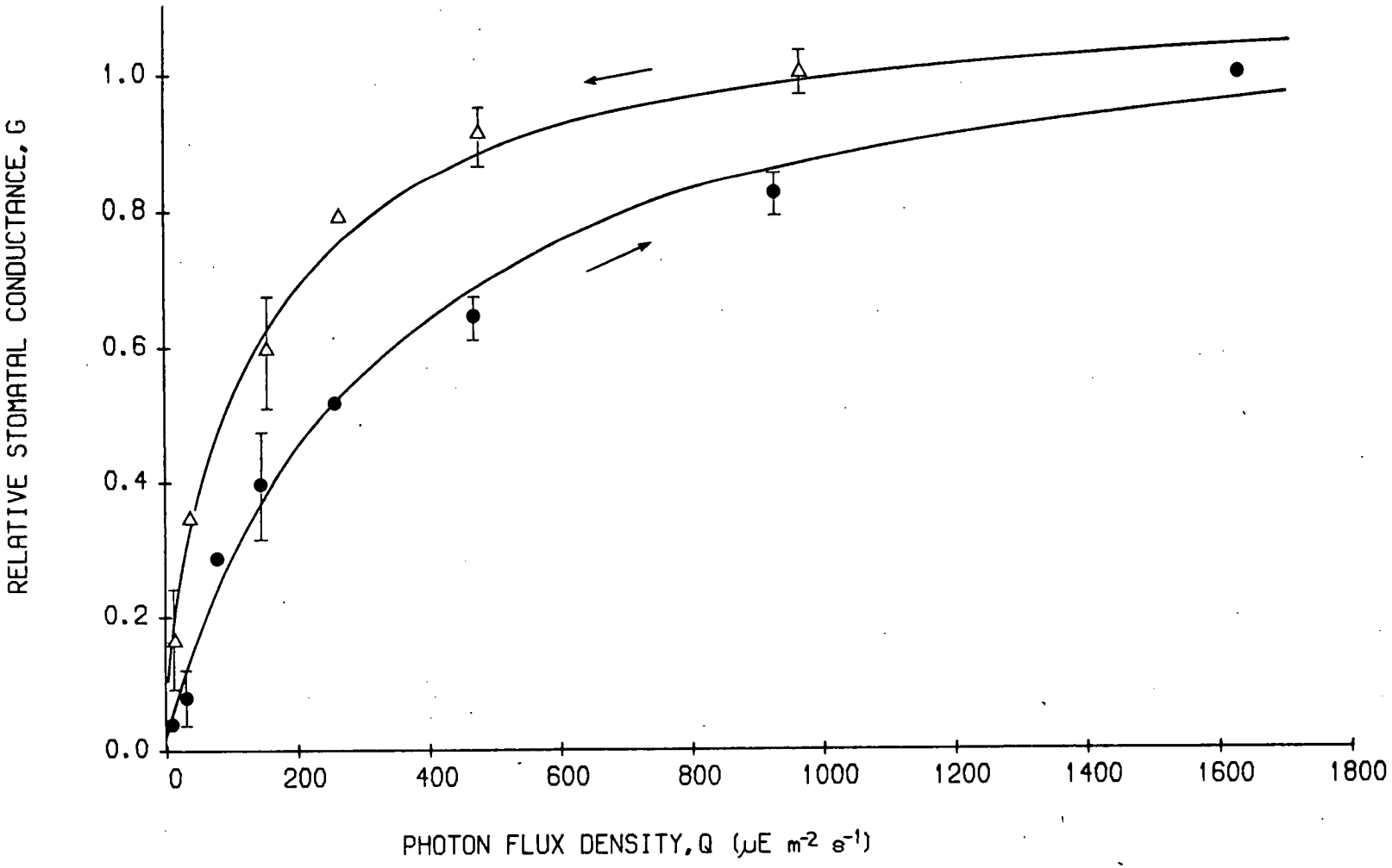


Figure 4.4.3a Relative stomatal conductance as a function of increasing (●) and decreasing photon flux density (△) in a sequence of increasing light steps followed by decreasing light steps. Fitted curves (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.214 cm s^{-1}. Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.54 kPa. Two standard errors are shown on representative points.

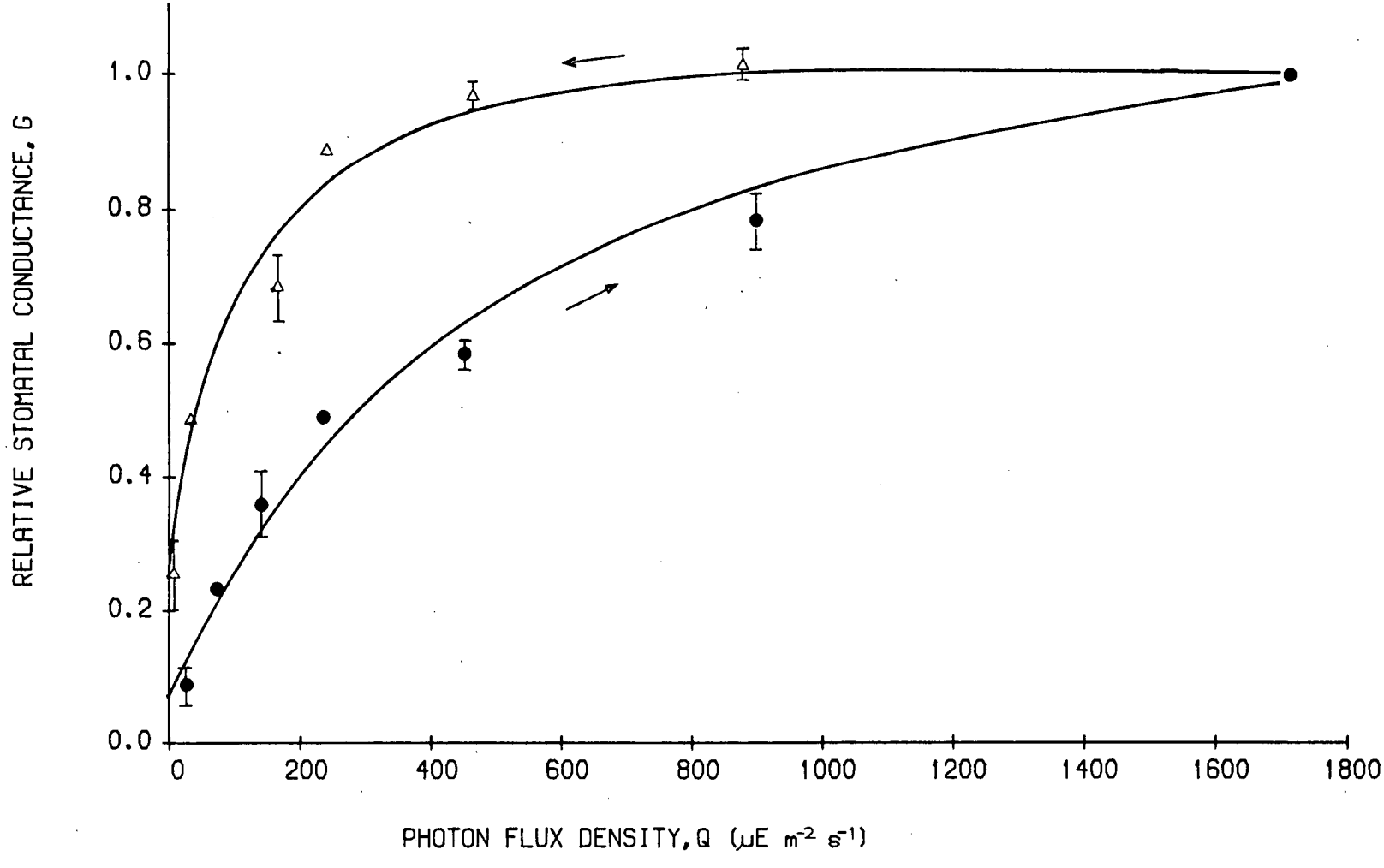


Figure 4.4.3b Relative stomatal conductance as a function of increasing (●) and decreasing photon flux density (△) in a sequence of increasing light steps followed by decreasing light steps. Fitted curves (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.449 cm s^{-1}. Forest shoots; bilateral illumination; leaf temperature 10.1°C; leaf-air vapour pressure difference 0.53 kPa. Two standard errors are shown on representative points.

between intact shoots of growth room plants (Figure 4.4.3a) and cut shoots of forest trees (Figure 4.4.3b). There was, however, a more pronounced hysteresis in the cut forest shoot, resulting from an unchanged stomatal aperture as photon flux density was decreased from 1700 $\mu E\ m^{-2}\ s^{-1}$ to 450 $\mu E\ m^{-2}\ s^{-1}$. Stomatal closure only began when the photon flux density was decreased below 450 $\mu E\ m^{-2}\ s^{-1}$. The more pronounced hysteresis in the cut forest shoots affected the value of G in the dark. There was a significantly larger stomatal opening in the dark after the sequence of reduced light treatments. In the growth room plants, a similar effect was less noticeable, presumably because of the less pronounced hysteresis.

Another experiment was performed with the sequence of light steps reversed, i.e. decreasing photon flux density in the morning and increasing photon flux density in the afternoon (Figure 4.4.4). Growth room plants were used. There was an even more pronounced hysteresis, mainly because of lower G during the sequence of increasing photon flux density. Following on from the sequence of decreasing photon flux density, the stomata did not respond to light. Instead the stomata closed further, although the photon flux density was progressively increased from darkness to 100 $\mu E\ m^{-2}\ s^{-1}$. There was therefore a carry-over effect, resulting from the influence of decreasing light steps and dark treatment.

The carry-over effect, resulting from changing from one sequence to another, whether from one of increasing to one of decreasing light steps or vice versa was clearly demonstrated in Table 4.4.1. The data in this table have been compiled from Figures 4.4.3a, 4.4.3b and 4.4.4. There was a continuation of the response of the

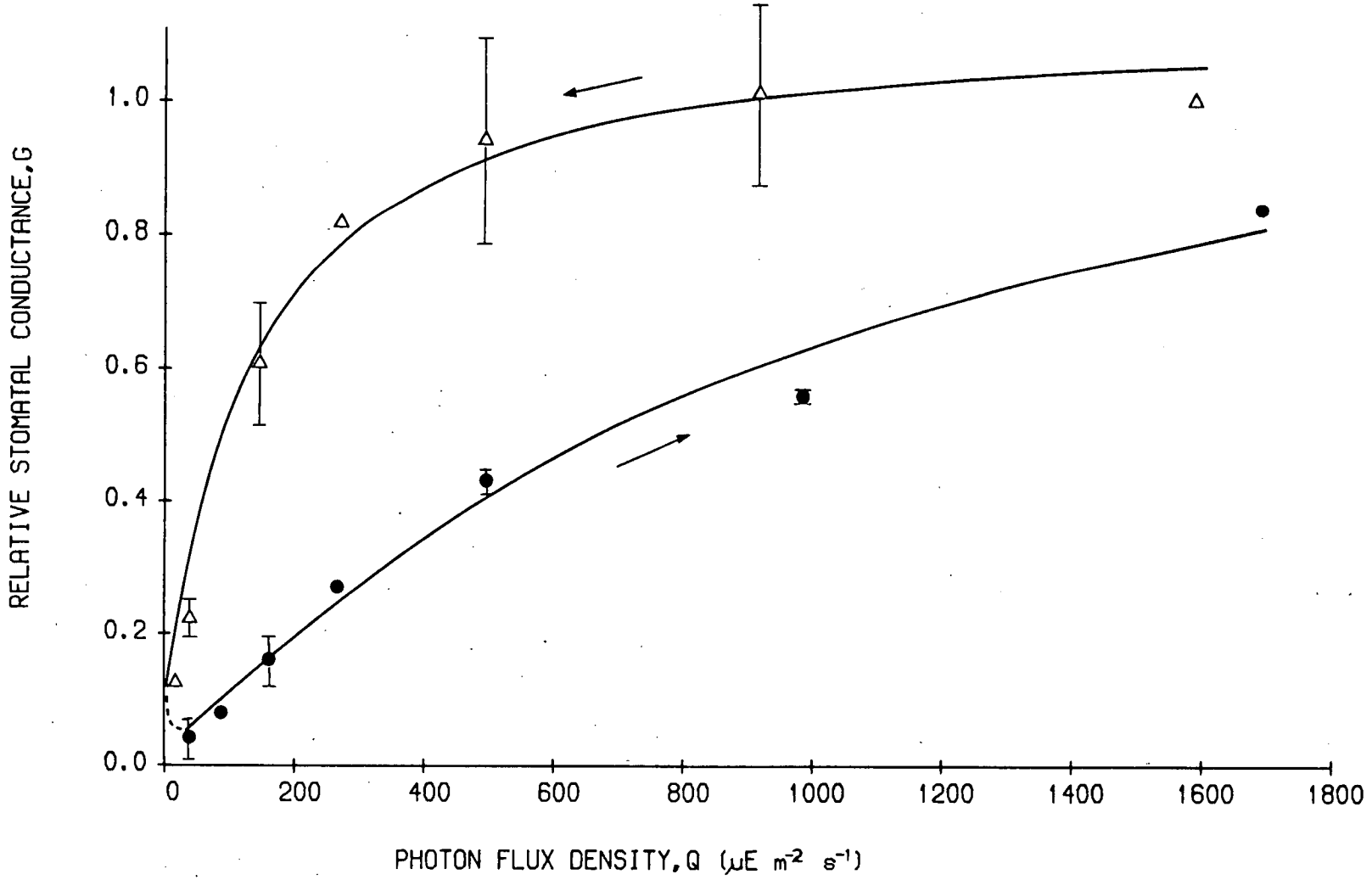


Figure 4.4.4 Relative stomatal conductance as a function of decreasing (△) and increasing photon flux density (●) in a sequence of decreasing light steps followed by increasing light steps. Fitted curves (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.278 cm s^{-1}. Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.53 kPa. Two standard errors are shown on representative points. The dotted line joining the two curves is handdrawn.

Table 4.4.1

a. Stomatal conductance (cm s^{-1}) on changing from medium light (650 - 900 $\mu E\ m^{-2}\ s^{-1}$) to high light (1200 - 1700 $\mu E\ m^{-2}\ s^{-1}$) and back again.

Plant	Medium light	High light	Medium light	Shoot Type
1	0.223	0.272	0.288	Growth room
2	0.238	0.270	0.284	potted plants
3	0.210	0.253	0.264	
mean	0.224	0.265	0.279	
s.e.	0.008	0.006	0.007	
4	0.313	0.399	0.417	Cut
5	0.404	0.472	0.475	forest
6	0.355	0.477	0.476	shoot
mean	0.357	0.449	0.456	
s.e.	0.026	0.025	0.020	
combined mean	0.291	0.357	0.367	
s.e.	0.032	0.043	0.041	

b. Stomatal conductance (cm s^{-1}) on changing from low light (30 $\mu E\ m^{-2}\ s^{-1}$) to dark, then low light, and finally medium low light (100 $\mu E\ u^{-2}\ s^{-1}$) in growth room plants

Plant	Low light	Dark	Low light	Medium low light
1	0.132	0.087	0.082	0.107
2	0.067	0.035	0.028	0.039
3	0.075	0.045	0.002	0.018
mean	0.091	0.056	0.037	0.055
s.e.	0.020	0.016	0.024	0.027

previous light treatments. It was only after one or two further step changes that the increasing or decreasing light steps had the expected effects of changing stomatal conductance.

Light and CO_2

A similar experiment was done in CO_2-free air, using growth room plants. The sequence of steps of increasing photon flux density was carried out before the sequence of steps of decreasing photon flux density. The light response (Figure 4.4.5a) was similar in form to that obtained in the earlier comparable experiment in normal air (Figure 4.4.3a). The light responses appeared to be free of any interacting effect caused by CO_2-free air and hysteresis was also observed. This experiment was repeated, using cut forest shoots (Figure 4.4.5b). The corresponding results in normal air are shown in Figure 4.4.3b. In this case there was less pronounced hysteresis, and stomatal conductance in the dark was enhanced in CO_2-free air. However, this finding was not confirmed in later experiments and could be caused by preconditioning in the forest to humid air.

The same experiment was done in air with CO_2 concentration corresponding to the CO_2 compensation point at the respective light level. The results clearly show similar pattern of hysteresis, as was seen in normal air or in CO_2-free air (Figure 4.4.6).

Bilateral illumination on needles

Figure 4.4.7 shows the results of an experiment in which a sequence of increasing photon flux density was followed by a sequence of decreasing photon flux density on attached shoots of growth room

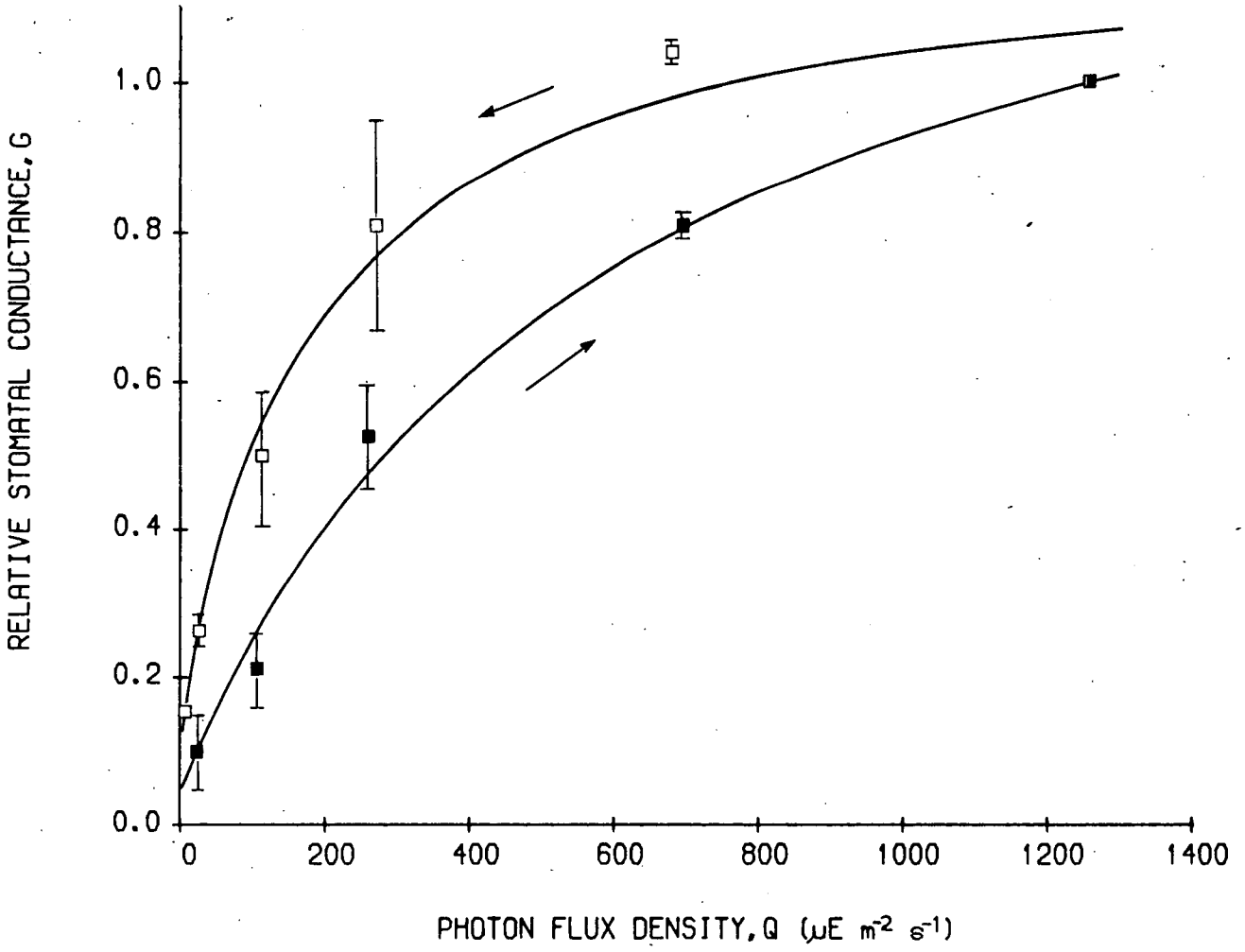


Figure 4.4.5a Relative stomatal as a function of increasing (■) and decreasing photon flux density (□) in a sequence of increasing light steps followed by decreasing light steps in CO_2 free air. Fitted curves (see Table 4.7.1) Average g_s for three shoots at G = 1.0 is 0.166 cm s^{-1}. Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.54 kPa. Two standard errors are shown on representative points.

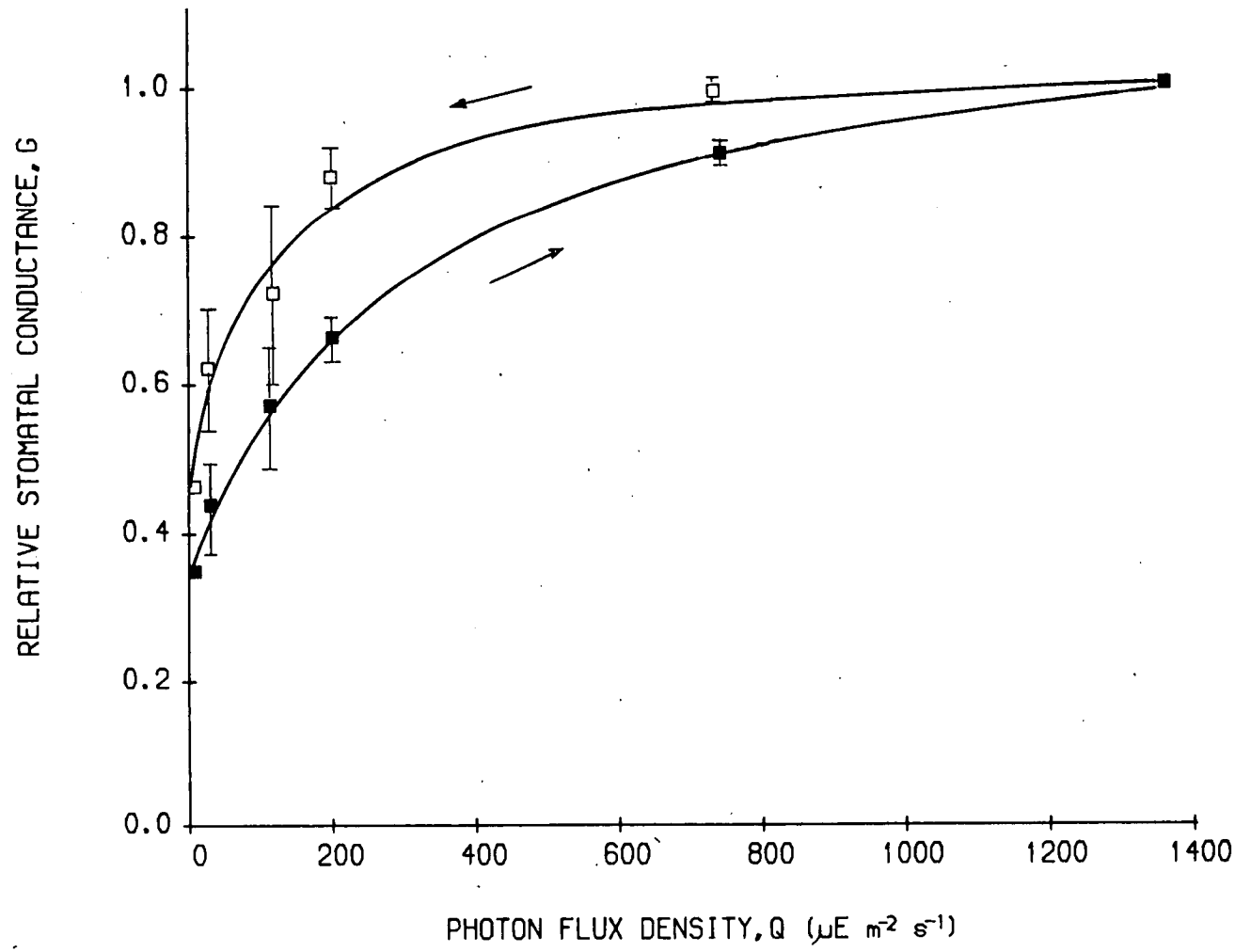


Figure 4.4.5b Relative stomatal conductance as a function of increasing (■) and decreasing (□) photon flux density in a sequence of increasing light steps followed by decreasing light steps in CO_2 free air. Fitted curves (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.650 cm s^{-1}. Forest shoots; bilateral illumination; leaf temperature 10.3°C; leaf-air vapour pressure difference 0.50 kPa. Two standard errors are shown on representative points.

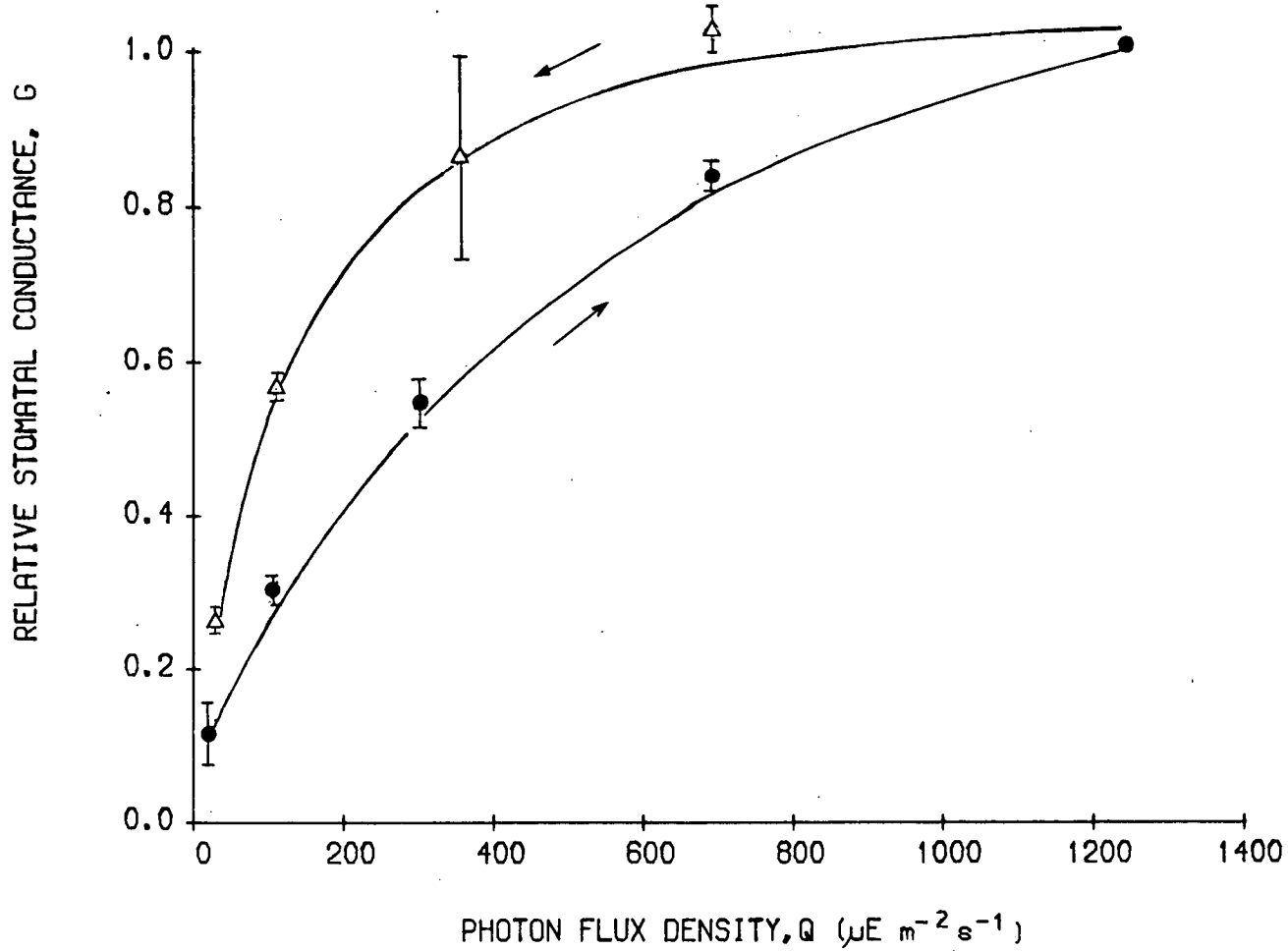


Figure 4.4.6 Relative stomatal conductance as a function of increasing (●) and decreasing (Δ) photon flux density in sequence of increasing light steps, followed by decreasing light steps in air with CO_2 concentrations equivalent to the CO_2 compensation point (29.75 cm^3 m^{-3}). Fitted curve (see Table 4.7.1). Average g_s for two shoots at G = 1 is 0.182 cm s^{-1}. Bilateral illumination; leaf temperature 10.3°C; leaf-air vapour pressure difference 0.56 kPa. Two standard errors shown.

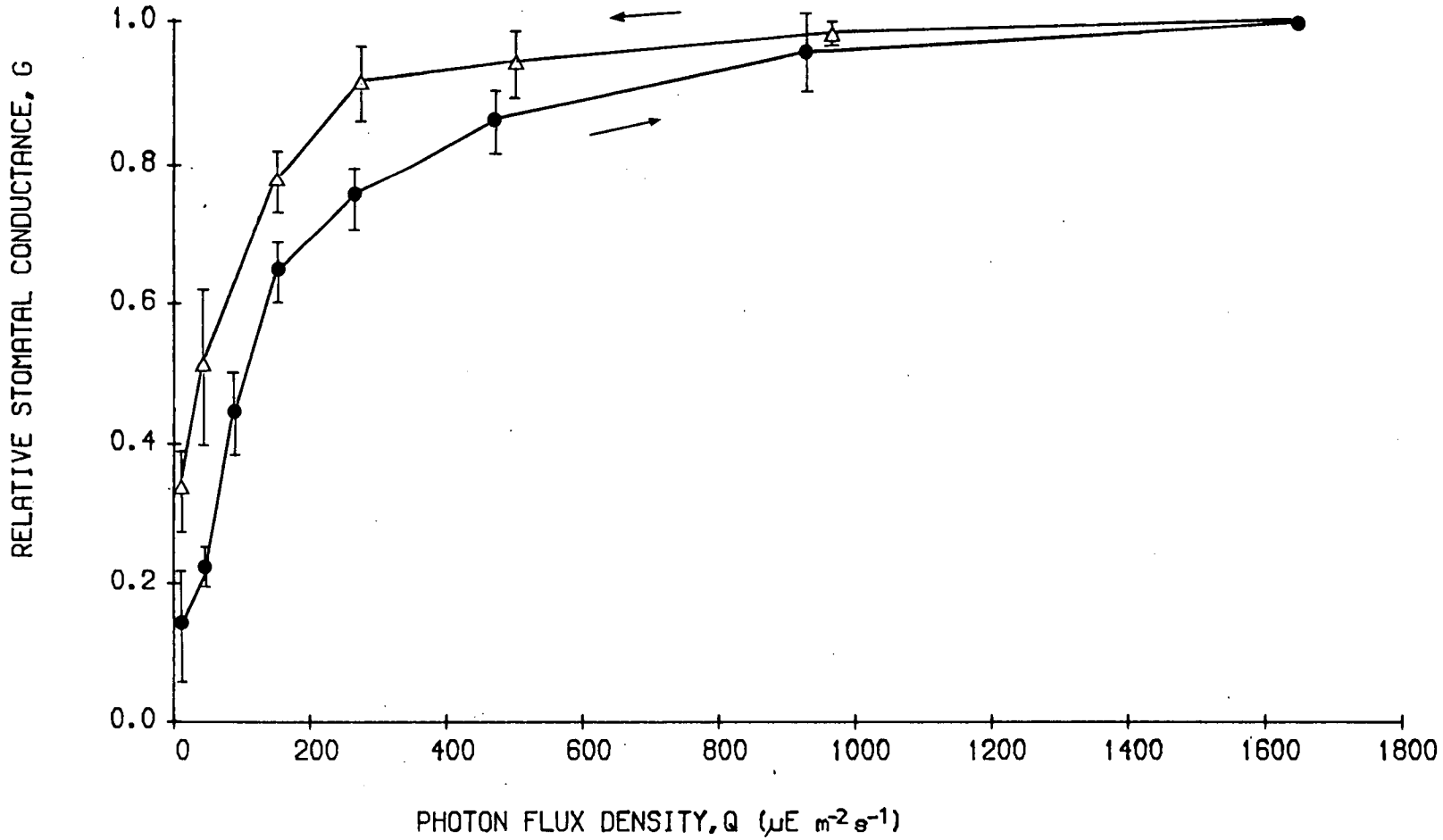


Figure 4.4.7 Relative stomatal conductance as a function of increasing (●) and decreasing photon flux density (Δ) in a sequence of increasing light steps followed by decreasing light steps. Needles arranged in a vertical plane on a shoot are exposed to bilateral illumination. Average g_s for three shoots at G = 1.0 is 0.296 cm s^{-1}. Leaf temperature 10.1°C; leaf-air vapour pressure difference 0.53 kPa. Two standard errors are shown on representative points.

plants. All the fascicles, except those in a single plane were removed. The fascicles attaching to the shoot were positioned vertically in the leaf chamber with the assistance of fine wires and were exposed to bilateral illumination perpendicular to the plane of the needles. Hysteresis disappeared at photon flux densities above 500 $\mu E\ m^{-2}\ s^{-1}$ and light saturation occurred at photon flux densities above 500 $\mu E\ m^{-2}\ s^{-1}$. G was also significantly larger in the dark for the sequence of decreasing light steps. Hysteresis is therefore still apparent, but only at the lower light levels, while it is absent at the higher light levels.

Fluctuating light

It has been seen that in changing from medium light (900 $\mu E\ m^{-2}\ s^{-1}$) to high light (1200 - 1700 $\mu E\ m^{-2}\ s^{-1}$) and back again there was an increase in G in spite of the lower photon flux density (Table 4.4.1). It is of interest, therefore, to know whether by increasing the photon flux density from one level to the next and back again to the first level g_s can be increased progressively to higher values. With this in mind, a series of experiments were carried out on three cut forest shoots. Figures 4.4.8a and 4.4.8b show the results of an experiment in which the photon flux density was changed between 450 to 1700 $\mu E\ m^{-2}\ s^{-1}$. There was an increase in g_s on changing the photon flux density level back to the high value (c.f. points numbered 13 and 1 in Figure 4.4.8a) after which a further cycle of such changes did not change g_s appreciably. The hysteresis was clearly present. The g_s value was maintained in spite of a four-fold reduction in photon flux density, which was

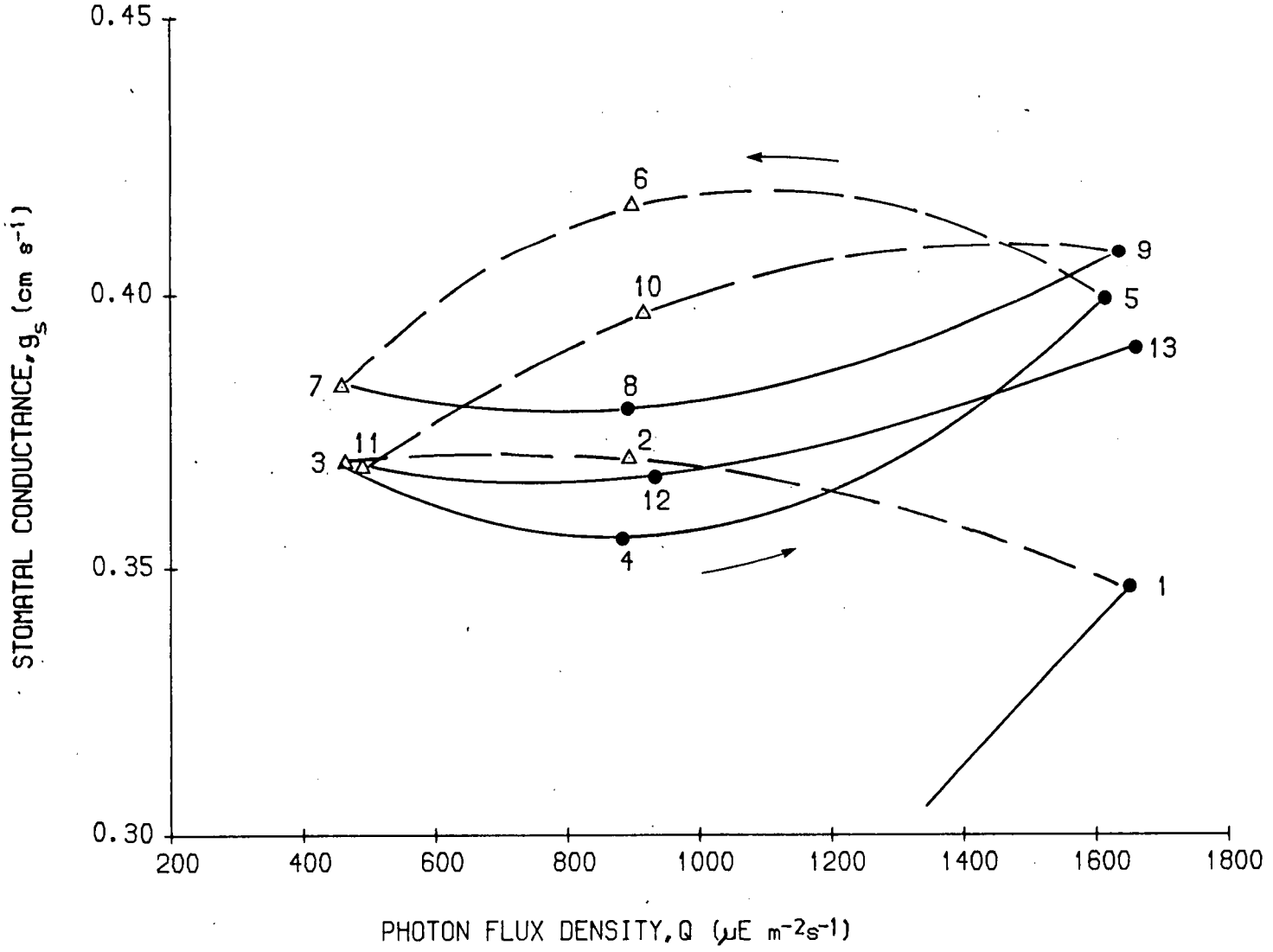


Figure 4.4.8a Forest shoot 1. Stomatal conductance as a function of changing photon flux density between 400 and 1700 μE m^{-2} s^{-1}. The light levels have been changed in the order of 1 to 12. Arrows indicate direction of increasing photon flux density (●——●) and decreasing photon flux density (Δ— —Δ). Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.52 kPa.

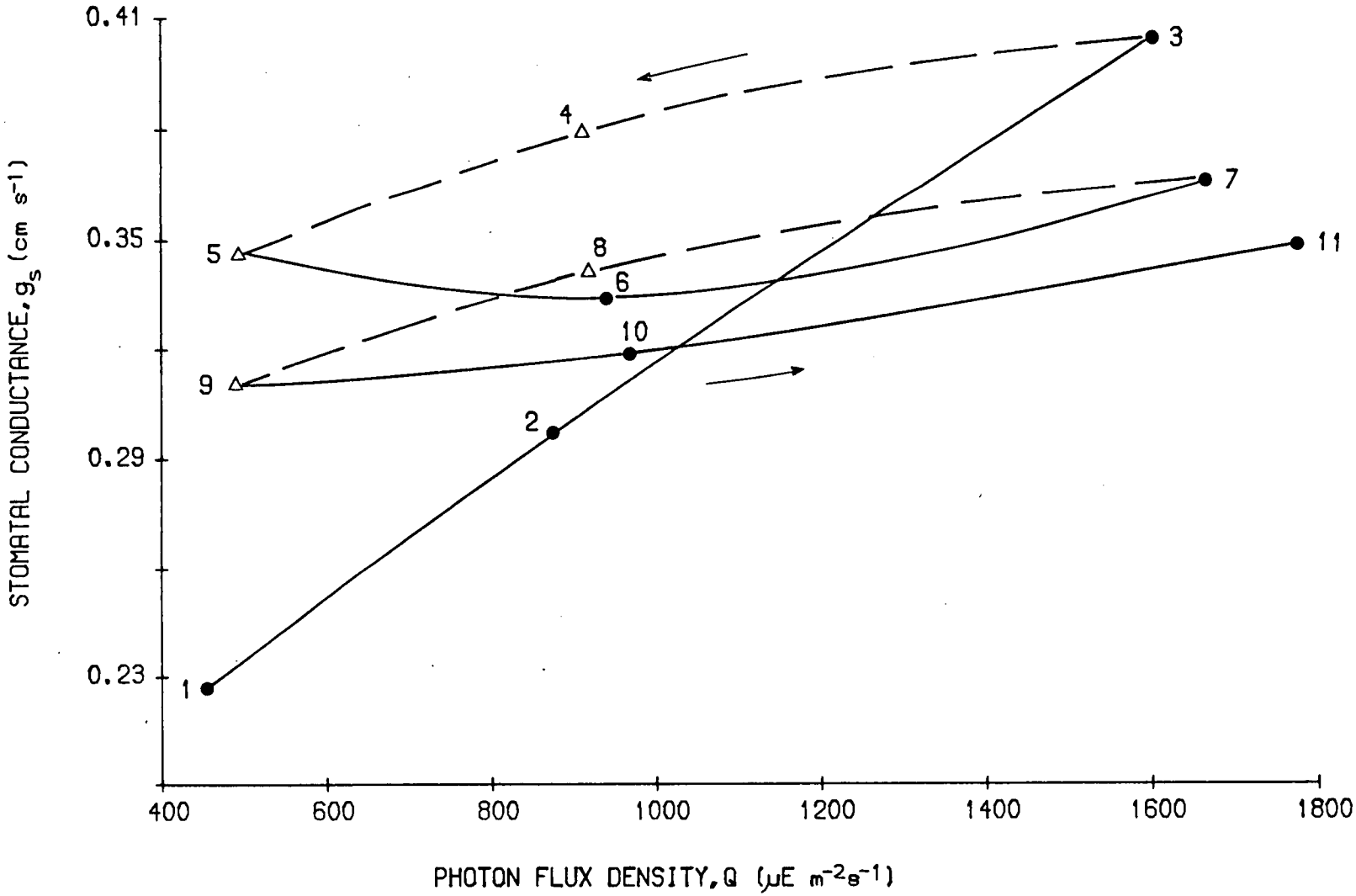


Figure 4.4.8b Forest shoot 2. Stomatal conductance as a function of changing photon flux density between 400 and 1800 $\mu E\ m^{-2}\ s^{-1}$. The light levels have been changed in the order of 1 to 11. Arrows indicate direction of increasing photon flux density (●——●) and decreasing photon flux density (Δ— —Δ). Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.54 kPa.

consistent with the stomatal response to a sequence of decreasing photon flux density. In Plant 2 (Figure 4.4.8b) the g_s value at 900 μE m^{-2} s^{-1} did not exceed the g_s value at the highest photon flux density of 1600 μE m^{-2} s^{-1}. There was a decline in g_s as the cycling in photon flux density progressed. This might have been caused by the longer delay before Plant 2 was exposed initially to 1600 μE m^{-2} s^{-1} and the slow decline in g_s was part of the diurnal change (see Figure 4.3.1). This delay was much shorter with Plant 1. Plant 3 was exposed to photon flux densities varying from 470 to 150 μE m^{-2} s^{-1} (Figure 4.4.8c). Essentially, the results obtained were similar to those found for Plant 1. Thus hysteresis can occur at any level of photon flux density, whenever changes in light levels occur.

Equilibration time

A 2.5 h equilibration period at each step change in light was tried in a further experiment (Figure 4.4.9). Growth room plants were used. The same sequential changes in photon flux density were made. Hysteresis did not appear and a single curve adequately described the light response. From these results, it appears that the hysteresis which showed up during short term (less than 1 h) changes in photon flux density is probably caused by a carry-over effect of step changes in light, as well as by sluggishness of the stomata in response to light, under the environmental conditions given.

Effect of temperature

An experiment was done on growth room plants at a leaf

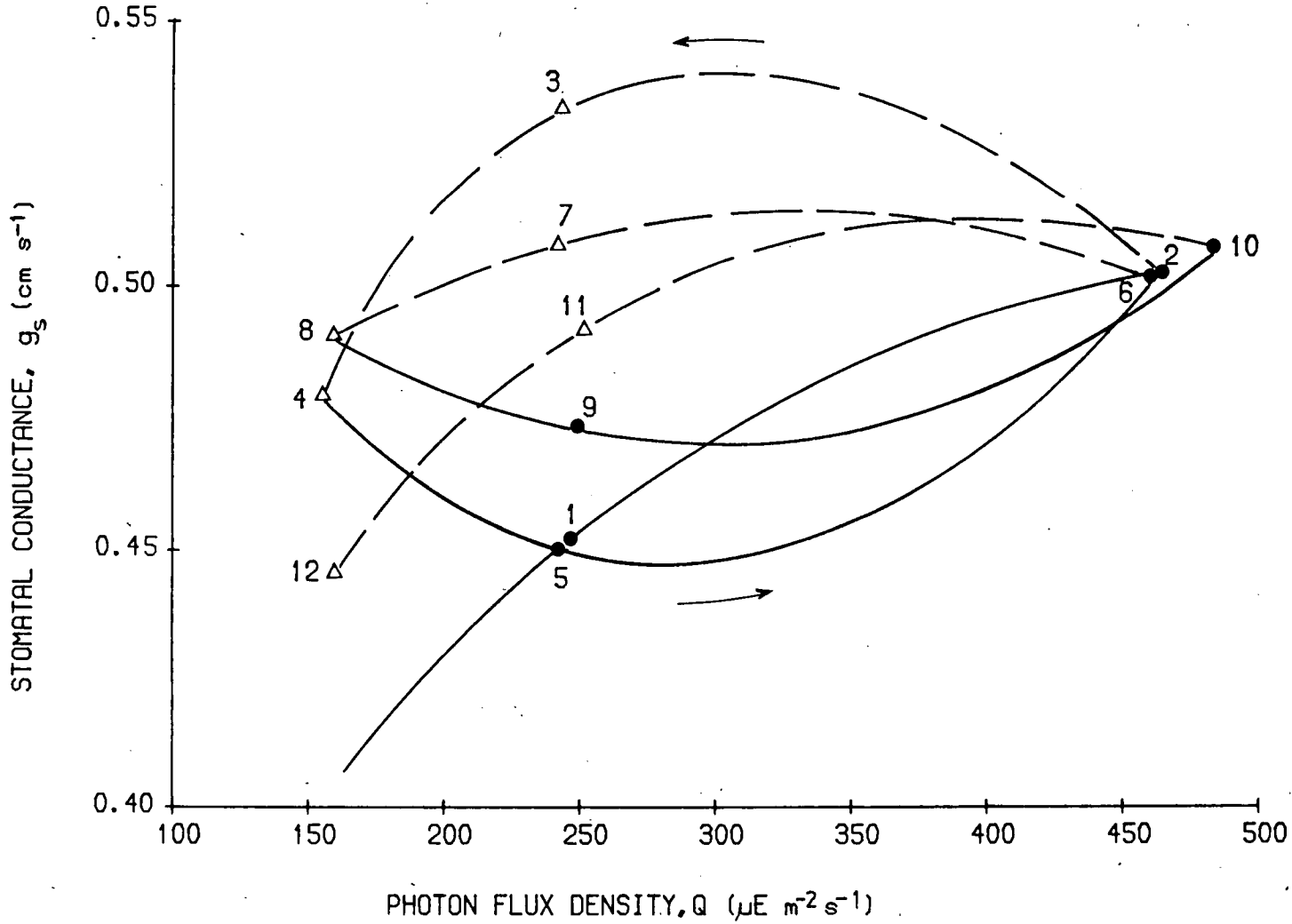


Figure 4.4.8c Forest shoot 3. Stomatal conductance as a function of changing photon flux density between 150 and 500 μE m^{-2} s^{-1}. The light levels have been changed in the order of 1 to 12. Arrows indicate direction of increasing photon flux density (●——●) and decreasing photon flux density (Δ— —Δ). Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.52 kPa.

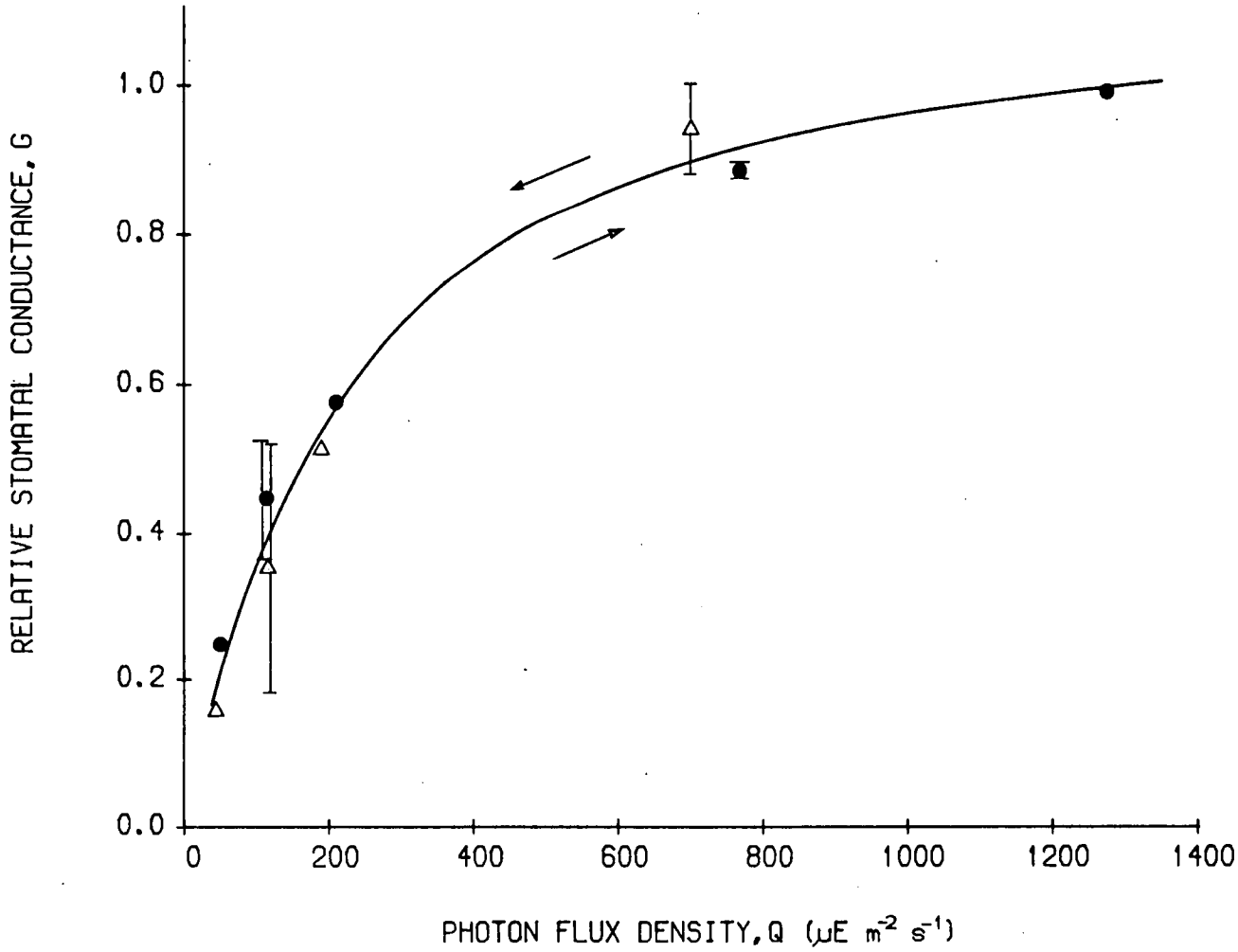


Figure 4.4.9 Relative stomatal conductance as a function of increasing (●) and decreasing photon flux density (△), with an equilibration period of 2.5 h. Fitted curve (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.272 cm s^{-1}. Bilateral illumination; leaf temperature 10.2°C; leaf-air vapour pressure difference 0.54 kPa. Two standard errors are shown on representative points.

temperature of 20°C, instead of at the 10°C previously used. All the other environmental conditions remained much the same. The results (Figure 4.4.10) show that hysteresis did not appear at the lower photon flux densities but was still apparent at higher photon flux densities. These results indicate that at 20°C the stomata respond to light more rapidly than at 10°C. The carry-over effect, however, remained.

Light and leaf-air vapour pressure difference

The shoots of two plants were subjected to a sequence of increasing photon flux density followed by a single step reduction in photon flux density at constant leaf-air vapour pressure difference and temperature. The experiment was repeated at three different leaf-air vapour pressure differences. The response of g_s to light was similar at photon flux densities below 150 μE m^{-2} s^{-1} (Figure 4.4.11) at all three leaf-air vapour pressure differences, D_l. Clear effects of D_l on the response to light were only seen at photon flux densities above 350 μE m^{-2} s^{-1}. The value of G was consistently higher the smaller the level of D_l at these light levels, and these differences increased as the photon flux density increased, from 350 to 1200 μE m^{-2} s^{-1}. At D_l of 2.32 kPa, with increasing photon flux density, G increased, reached a maximum value and then decreased. At D_l of 1.84 kPa, there was some indications of a lower increase in G above 700 μE m^{-2} s^{-1}. Hysteresis caused by carry-over effect was still evident at each of the three levels of D_l. However, because of the difference in response at photon flux

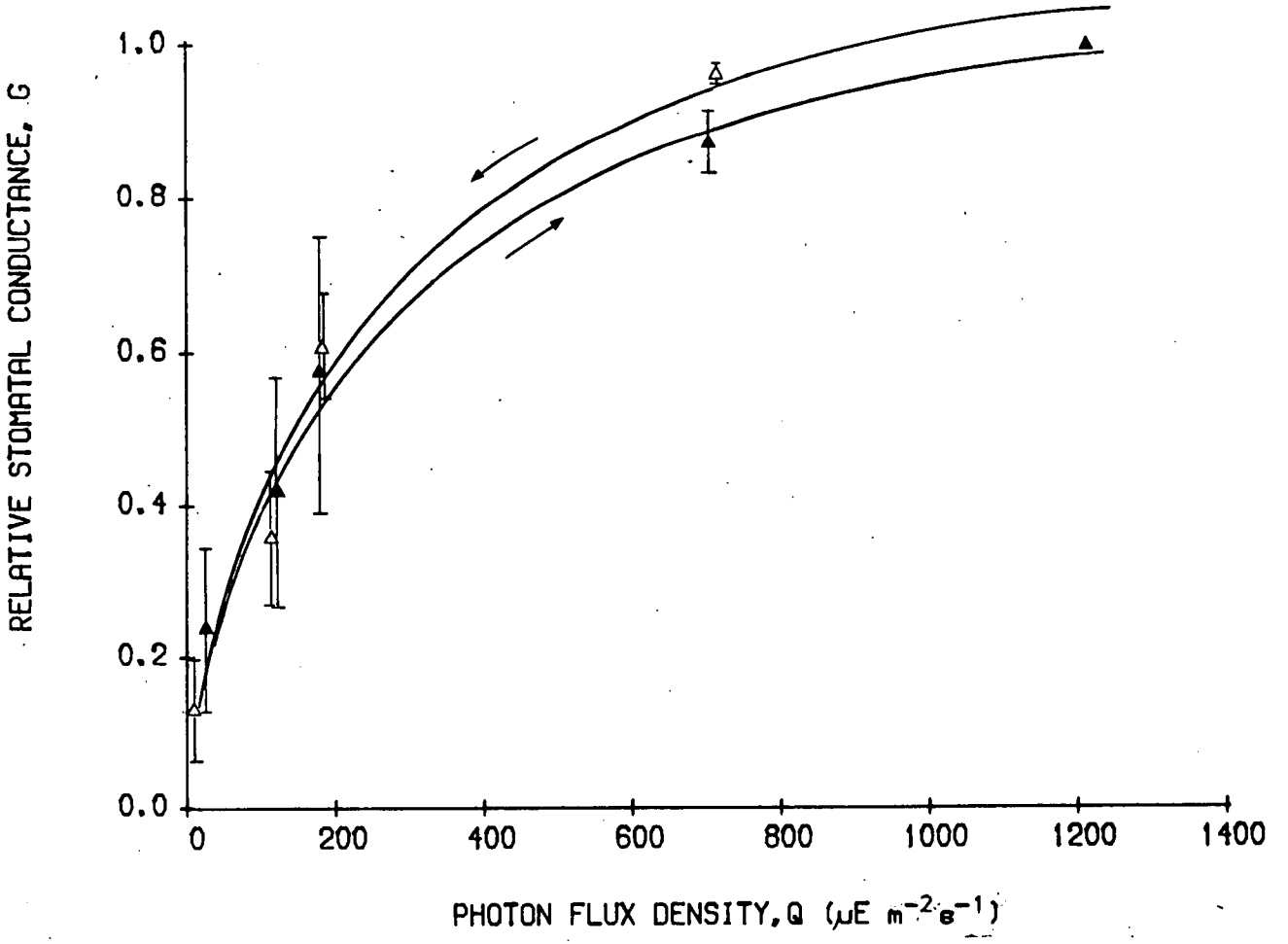


Figure 4.4.10 Relative stomatal conductance as a function of increasing (▲) and decreasing photon flux density (△) in a sequence of increasing light steps followed by decreasing light steps. Fitted curves (see Table 4.7.1). Average g_s for three shoots at G = 1.0 is 0.200 cm s^{-1}. Leaf temperature 20.2°C; leaf-air vapour pressure difference 0.59 kPa. Two standard errors are shown.

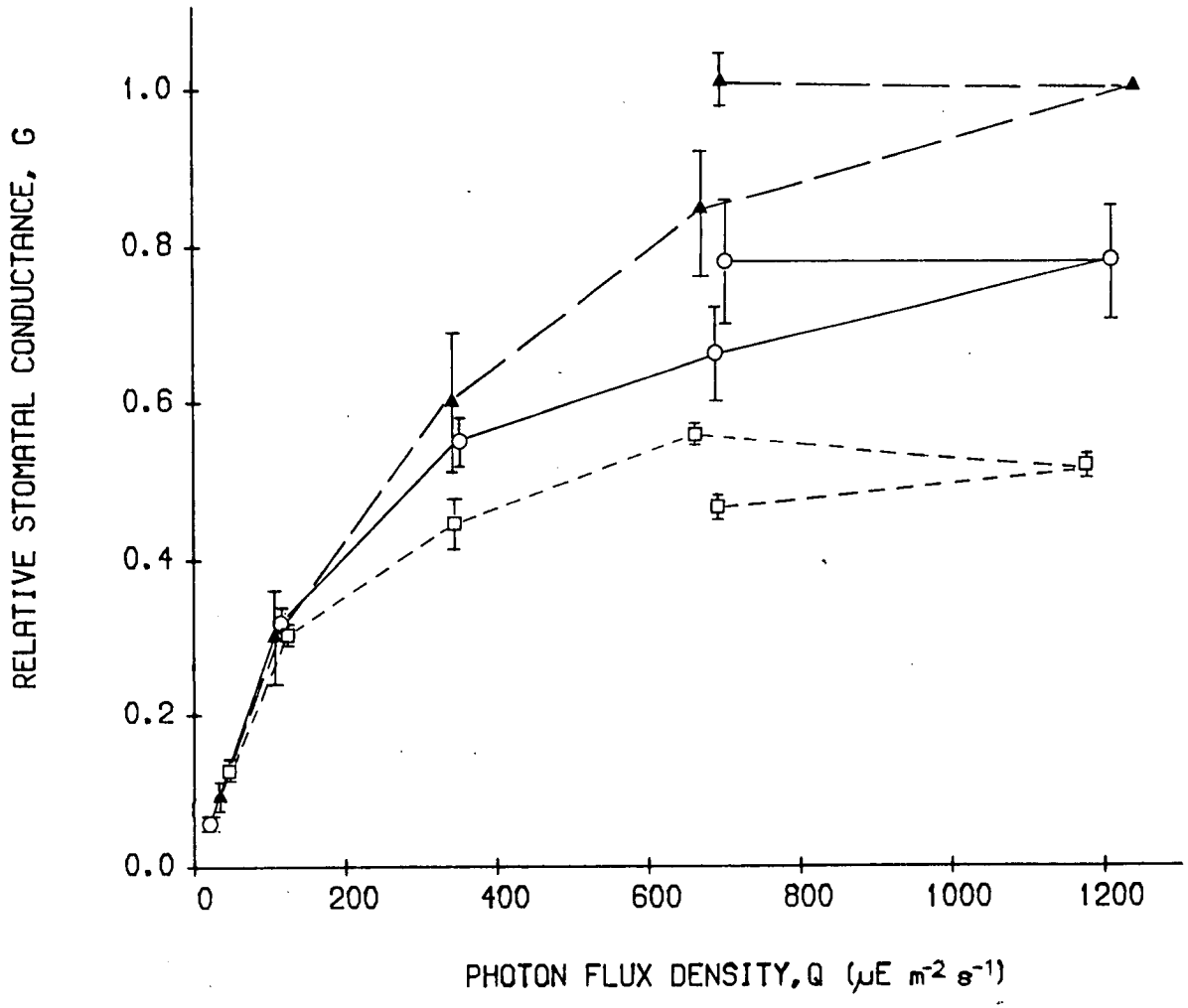


Figure 4.4.11 Relative stomatal conductance as a function of increasing photon flux density and a single step reduction in photon flux density, at three leaf-air vapour pressure differences, 0.96 kPa (▲——▲), 1.84 kPa (○——○) and 2.32 kPa (□- - -□). Average g_s for two shoots at G = 1.0 is 0.339 cm s^{-1}. Bilateral illumination; leaf temperature 25.5°C. Two standard errors shown.

densities above 700 $\mu E\ m^{-2}\ s^{-1}$, hysteresis was shown to be present at 0.96 kPa, disappearing at 1.84 kPa and appearing as a suppression of G at 2.32 kPa.

Summary of results

(1) The stomatal response to a sequence of progressive light steps showed hysteresis, when the direction of the sequence was reversed.

(2) This hysteresis was evident with equilibration periods of 0.75 to 1.0 h, at leaf temperatures of 10 to 26°C, leaf-air vapour pressure difference of 0.5 to 2.3 kPa. Hysteresis was observed in normal air, CO_2-free air, or in air with CO_2 concentration equivalent to the CO_2 compensation point, in intact shoots of growth room plants, or in detached shoots of forest trees and in needles illuminated unilaterally or bilaterally.

(3) One probable cause was a carry-over effect of the previous next higher or next lower light level. Another was the general sluggishness of the stomata in response to light, since hysteresis disappeared completely with an equilibration period of 2.5 h, and at the lower light levels at 20°C.

4.5 Response of stomata to CO_2 and leaf water potential

Response of stomata to CO_2

The ambient CO_2 concentration, C_a was varied to find out how C_a affects stomatal conductance. In the first experiment with growth

room plants, four levels of photon flux density were used; leaf temperature and leaf-air vapour pressure difference were maintained at 10.7°C and 0.52 kPa respectively. Figure 4.5.1 shows the lack of response of G to C_a over a range of 0 to 5000 $cm^3\ m^{-3}$, at all light levels. This experiment was repeated at a leaf temperature of 20.6°C (Figure 4.5.2). Except for difference in value of G in response to about similar level of photon flux density, essentially similar response to C_a were obtained. In another experiment at 10.6°C, this time with detached forest shoots (Figure 4.5.3), a lack of response of G to varying CO_2 concentration from 0 to 5000 $cm^3\ m^{-3}$ was again observed in darkness and at 180 $\mu E\ m^{-2}\ s^{-1}$. At the photon flux density of 1300 $\mu E\ m^{-2}\ s^{-1}$, there appeared to be a small increase in G at very low CO_2 concentrations, particularly 0 $cm^3\ m^{-3}$. This was, however, not confirmed in later experiments.

The shoots of two plants were subjected to varying levels of leaf-air vapour pressure difference, in normal air, with a CO_2 concentration of about 350 $cm^3\ m^{-3}$ and in CO_2-free air on alternate days. Stomatal conductance declined in relation to D_l similarly in both normal and CO_2-free air (Figure 4.5.4). A single curve can be fitted to the data points of each plant, irrespective of whether they were obtained in air of normal CO_2 concentration or in CO_2-free air. The response to leaf-air vapour pressure difference was similar to that shown in Figure 4.6.1 for a leaf temperature of 25.6°C.

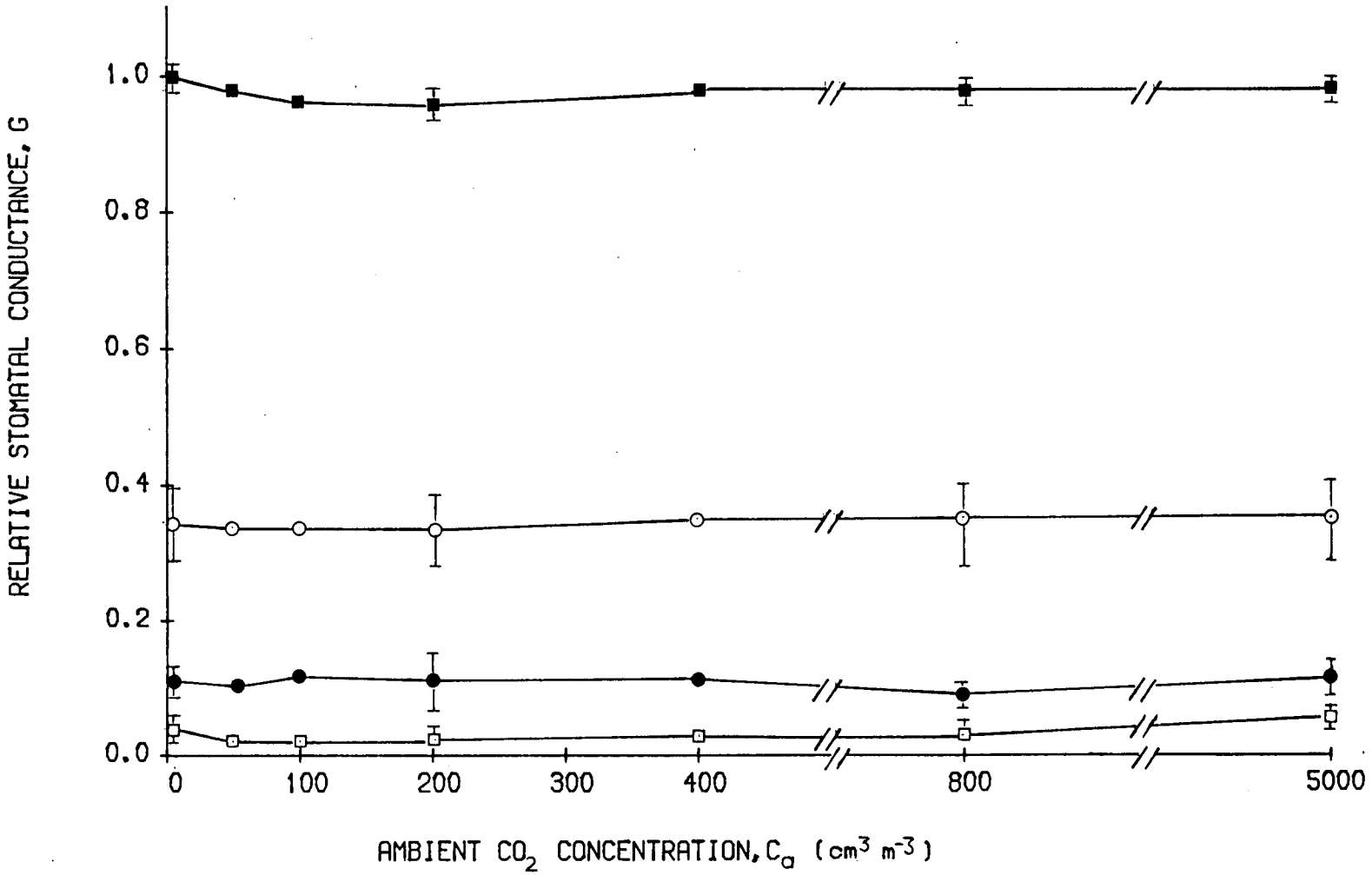


Figure 4.5.1 The relation between relative stomatal conductance and ambient CO_2 concentration at four levels of photon flux density: 1300 $\mu E\ m^{-2}\ s^{-1}$ (■), 180 $\mu E\ m^{-2} s^{-1}$ (○), 30 $\mu E\ m^{-2}\ s^{-1}$ (●), 0 $\mu E\ m^{-2}\ s^{-1}$ (□). Growth room plants. Average g_s for two shoots at G = 1.0 is 0.259 cm s^{-1}. Leaf temperature 10.7°C; leaf-air vapour pressure difference 0.52 kPa. Two standard errors are shown on representative points.

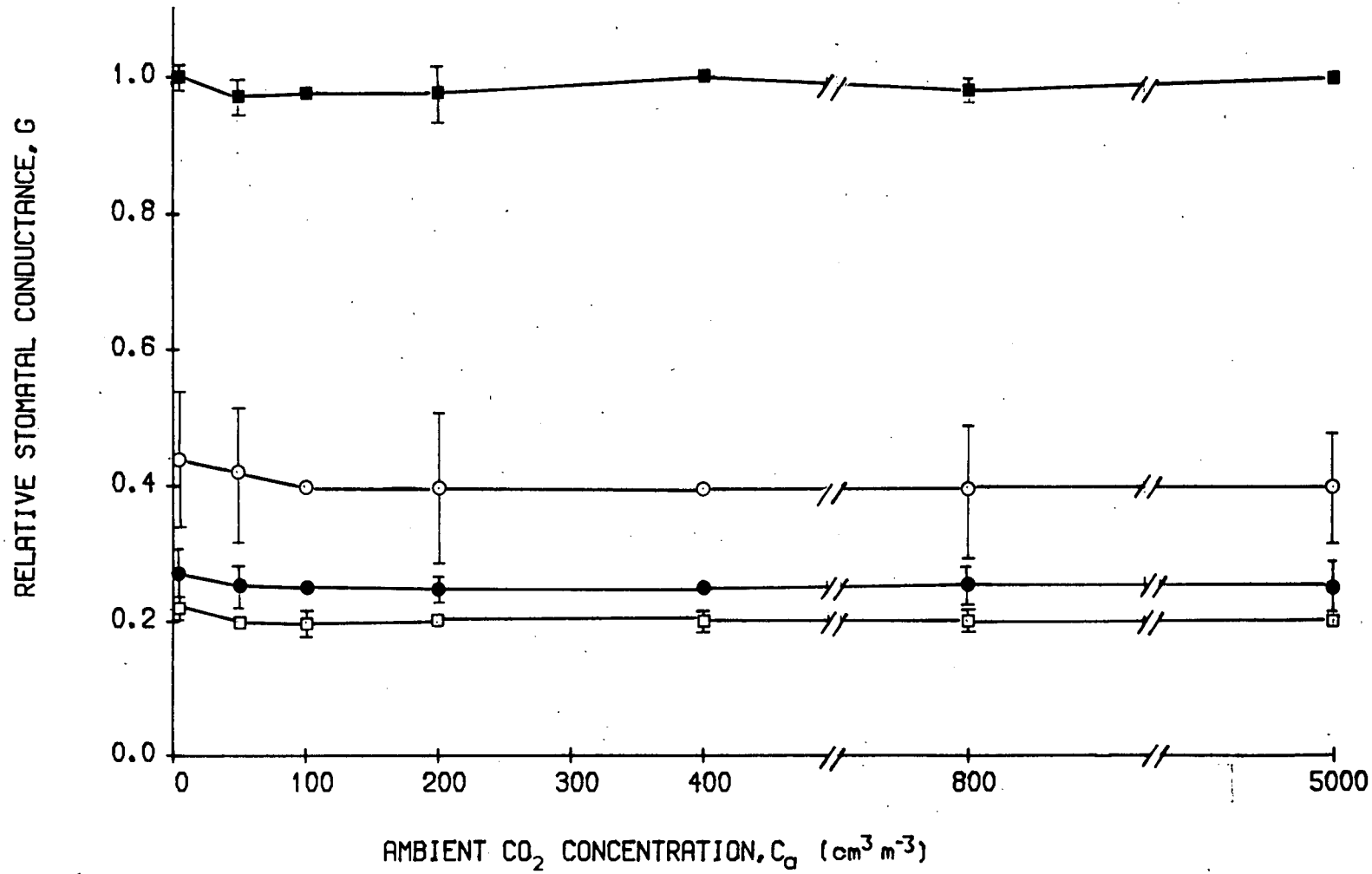


Figure 4.5.2 The relation between relative stomatal conductance and ambient CO_2 concentration at four levels of photon flux density: 1300 $\mu E\ m^{-2}\ s^{-1}$ (■), 170 $\mu E\ m^{-2}\ s^{-1}$ (○), 25 $\mu E\ m^{-2}\ s^{-1}$ (●), 0 $\mu E\ m^{-2}\ s^{-1}$ (□). Growth room plants. Average g_s for three shoots at G = 1.0 is 0.328 cm s^{-1}. Leaf temperature 20.6°C; leaf-air vapour pressure difference 0.58 kPa. Two standard errors are shown on representative points.

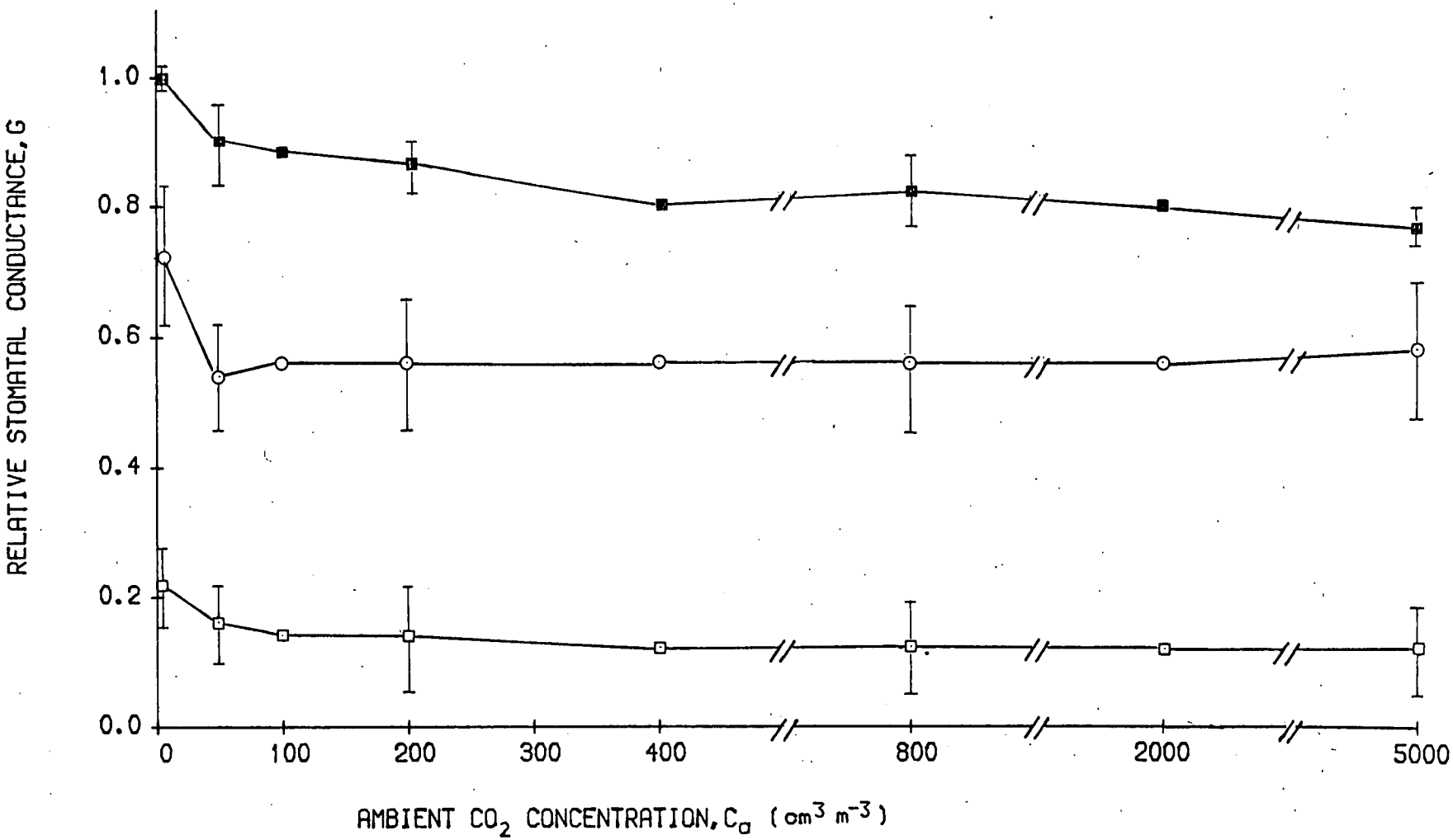


Figure 4.5.3 Relationship between relative stomatal conductance and ambient CO_2 concentration at three levels of photon flux density: 1300 μE m^{-2} s^{-1} (■), 180 μE m^{-2} s^{-1} (○), 0 μE m^{-2} s^{-1} (□). Cut forest shoots. Average g_s for two shoots at G = 1.0 is 0.602 cm s^{-1}. Leaf temperature 10.6°C; leaf-air vapour pressure difference 0.35 kPa. Two standard errors are shown on representative points.

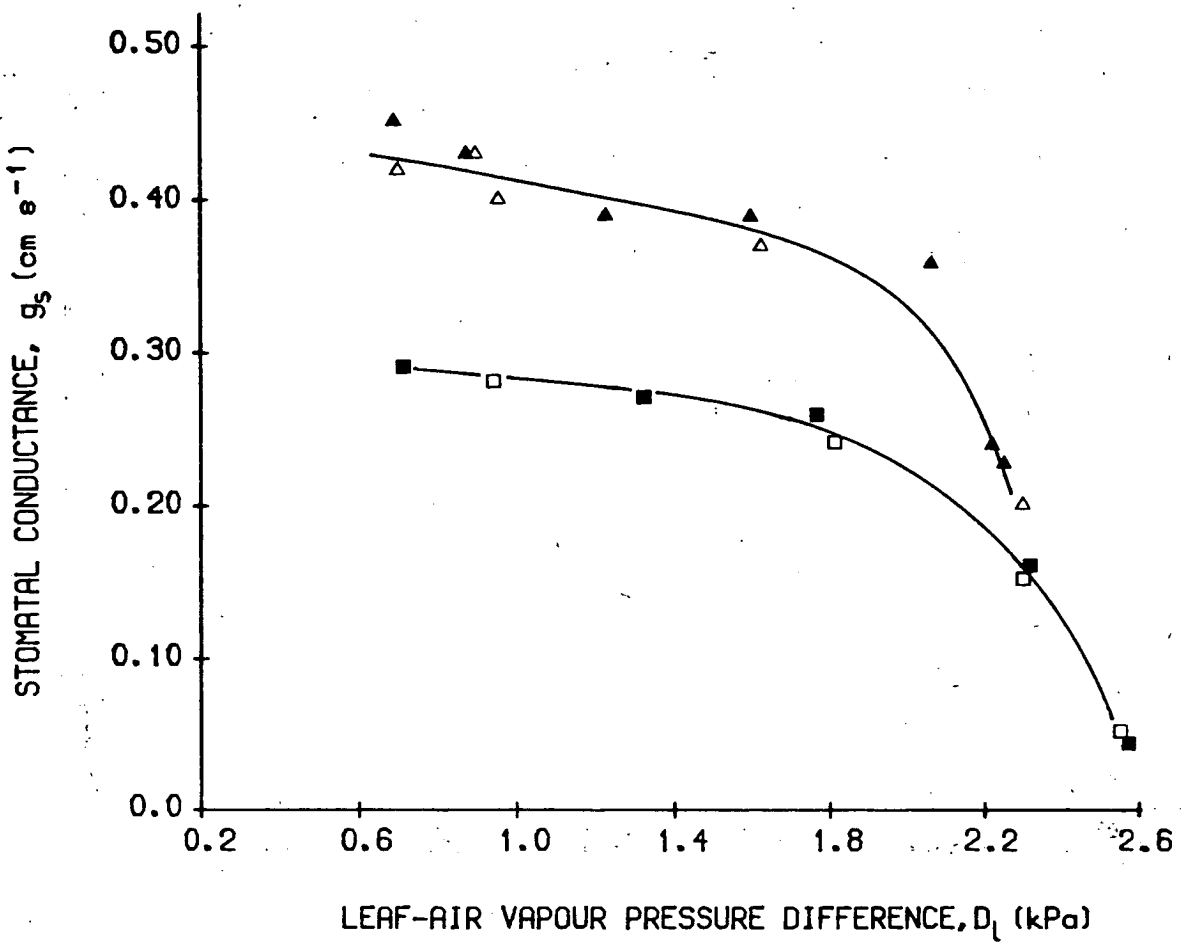


Figure 4.5.4 The relationship between stomatal conductance and leaf-air vapour pressure difference for two growth room plants: Plant 1 (▲,△) and Plant 2 (■,□) in normal air (▲,■) and CO_2-free air (△,□). Eye-drawn curves. Leaf chamber experiment. Photon flux density 1320 $\mu E\ m^{-2}\ s^{-1}$; leaf temperature 25.6°C. Each point is a single measurement.

Response of stomata to leaf water potential

Watering of five potted seedlings growing in the growth room was stopped and they were allowed to dry out in the growth room for 14 days. Stomatal conductance and leaf water potential were measured daily at about 5 h after lights on. A null balance porometer (Beardsell, Jarvis & Davidson 1972) was used to measure g_s. Water potential was measured with a needle pressure chamber.

There were differences in the rate of drying out between different plants (Figure 4.5.5) and there were small fluctuations in g_s at values of ψ higher than -0.85 MPa. However, in general, there was a progressive decline in g_s with decreasing ψ. At ψ higher than -0.85 MPa, the decline in g_s was small. At ψ less than -0.85 MPa, the decline was larger, until stomatal closure occurred at -1.4 MPa. At ψ lower than -1.4 MPa, the minute change in g_s could be a reflection of decline in cuticular conductance. The threshold value of ψ for growth room grown plants was therefore at around -0.85 MPa, compared to a threshold value of -1.2 MPa for field grown trees (Whitehead, D. personal communication 1977). The higher threshold value probably results from preconditioning by a better watering regime (McCree 1974; Davies 1977), and possibly a lower leaf-air vapour pressure difference. The progressive decline in g_s with decreasing ψ had also been noted in field grown trees (Roberts, J. personal communication 1978).

Summary of results

(1) There was a complete lack of stomatal response to ambient CO_2 concentration, C_a, between 0 and 5000 $cm^3\ m^{-3}$.

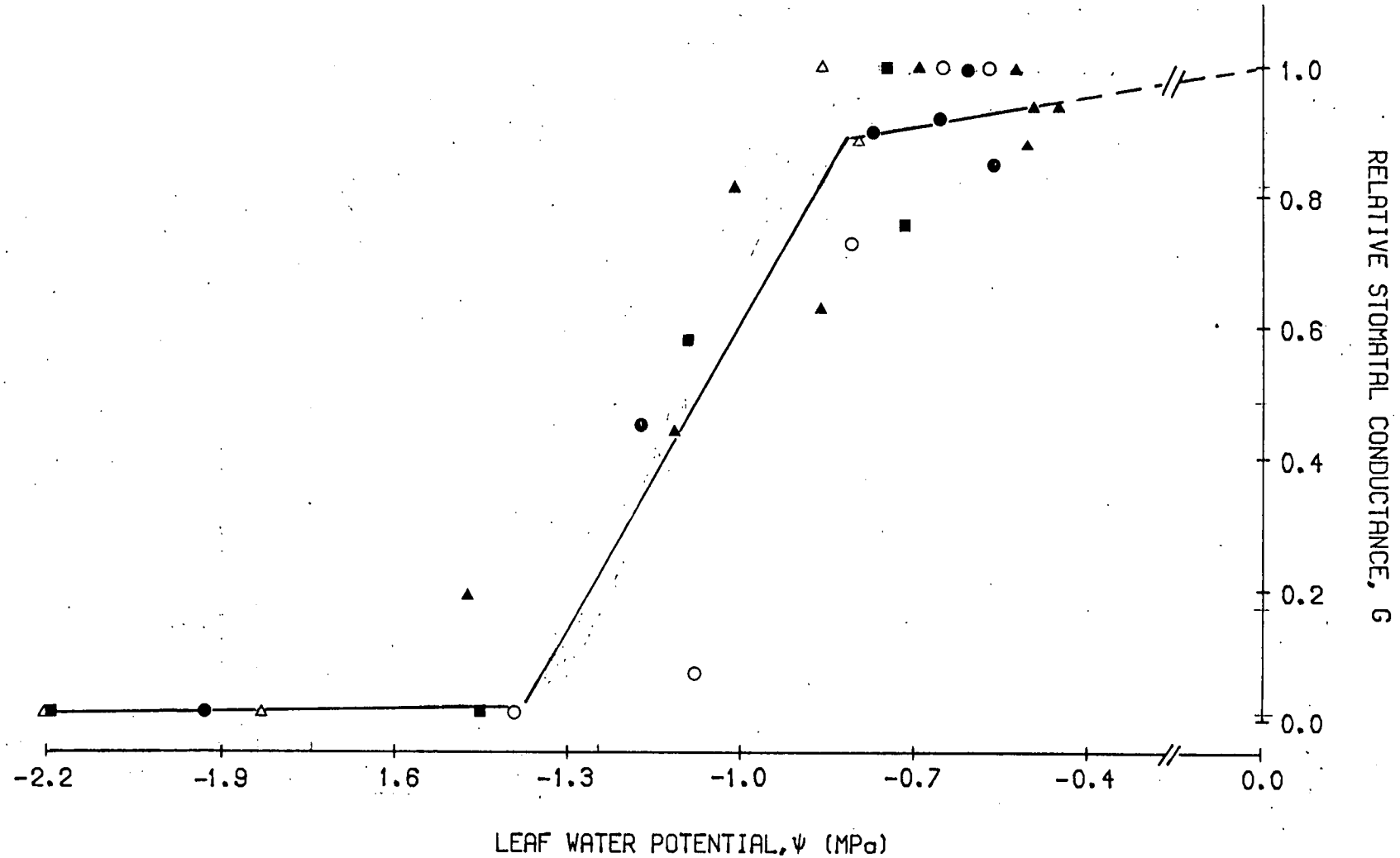


Figure 4.5.5 The relation between relative stomatal conductance and leaf water potential. The different symbols represent different plants. Eye-drawn straight lines have been fitted. Average g_s for five plants at G = 1.0 is 0.174 cm s^{-1}. Photon flux density at shoot level 255 μE m^{-2} s^{-1}; ambient temperature 20.2°C and leaf-air vapour pressure deficit 0.55 kPa.

(2) This was shown to occur at 10°C in detached forest shoots and in growth room plants, and at 20°C in growth room plants.

(3) The lack of response to C_a was observed in photon flux densities ranging from 0 to 1300 $\mu E\ m^{-2}\ s^{-1}$.

(4) At 25°C, there were no differences between stomatal response to leaf-air vapour pressure difference (0.6 to 2.6 kPa) in normal air and in CO_2-free air.

(5) There was a progressive decline in g_s with decreasing ψ. At ψ lower than -0.85 MPa, decline in g_s was rapid.

4.6 Response of stomata to humidity and temperature

Response of stomata to humidity

The stomatal response to increasing leaf-air vapour pressure difference was investigated for constant temperatures of 25.4, 20.2 and 10.2°C. Photon flux density was maintained at 1320 $\mu E\ m^{-2}\ s^{-1}$.

There was a larger g_s at low D_l than at high D_l (Figure 4.6.1). At 20.2 and 25.4°C the stomatal response to increasing D_l was exponential, with small changes in G at low D_l and larger changes at high D_l. The sensitivity of the stomata to increasing D_l was therefore low at low levels of D_l and high at high levels of D_l. At 10.2°C, the response of G to increasing D_l tended towards linearity. In general, there was increased sensitivity to increasing D_l at low temperature than at high temperature. This was apparent at 25.4°C, where there was little response to change in D_l from 0.6 to 1.7 kPa. At 10.2°C however, there was a two-fold decrease in G as D_l was increased from 0.5 to 0.8 kPa. When the leaf temperature was reduced, complete stomatal closure, i.e. G = 0, occurred at a lower D_l, as evidenced

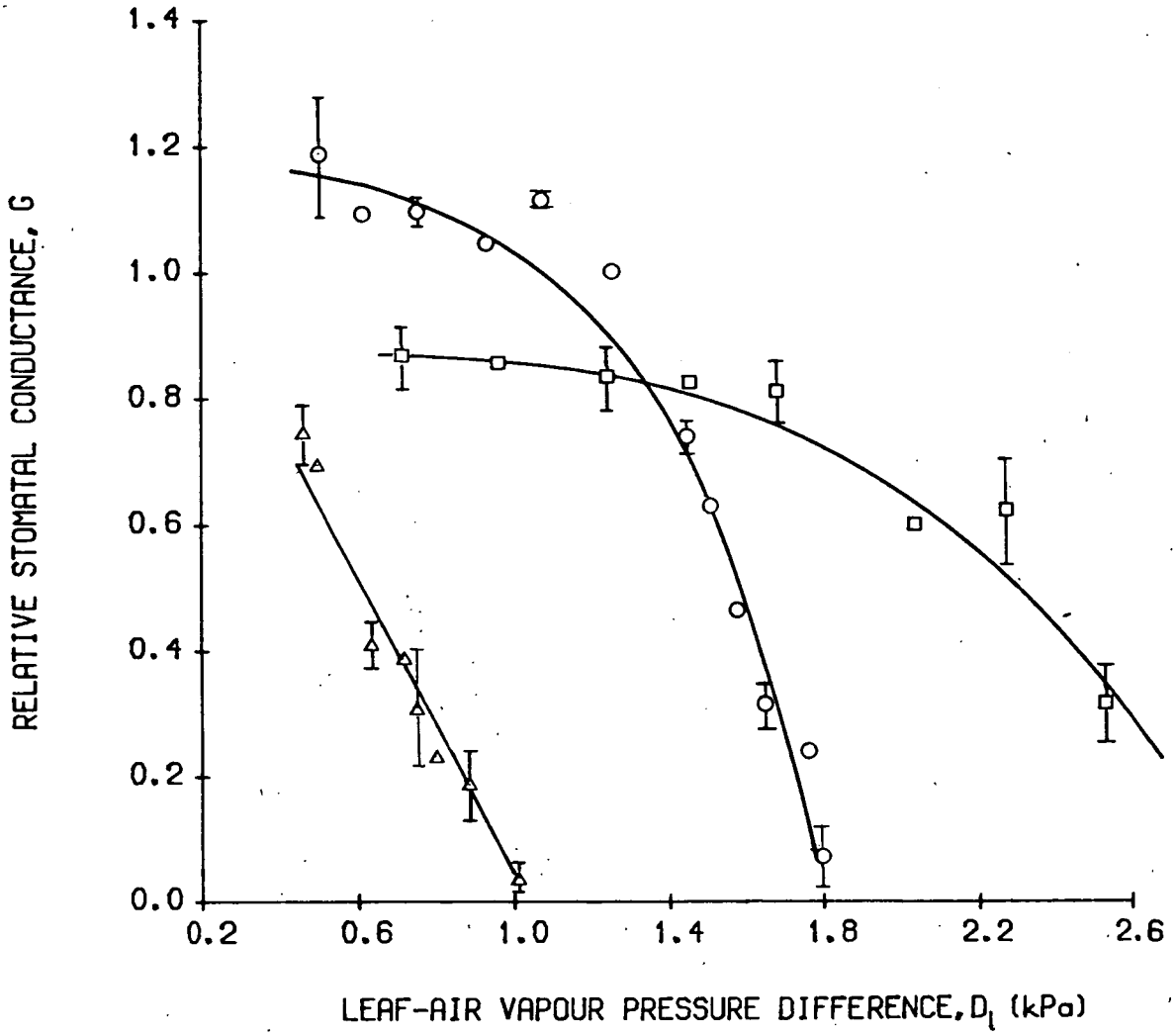


Figure 4.6.1 The relation between relative stomatal conductance and increasing leaf-air vapour pressure difference at constant temperatures of 25.4°C (□), 20.2°C (○) and 10.2°C (△). Fitted curves (see Table 4.7.2). Leaf chamber experiment. Average g_s at G = 1.0 is 0.430 cm s^{-1}. Photon flux density 1320 μE m^{-2} s^{-1}. All points are means of five measurements with two standard errors shown on representative points.

from 1.0 kPa for 10.2°C, 1.8 kPa for 20.2°C and around 2.8 kPa for 25.4°C. The large stomatal conductance attained at low D_l was larger at 20.2°C than at 25.4°C or at 10.2°C.

Transpiration rate, E, was calculated from gas exchange data for the above results. At 20.2°C and 25.4°C, transpiration rate increased with increasing D_l, reached a maximum value and then decreased with further increase in D_l (Figure 4.6.2). At 10.2°C, only the decrease in E is apparent because of lack of data at low values of D_l.

Six potted plants were exposed on successive days to different leaf-air vapour pressure differences, D_l, in the wind tunnel. Each level of D_l was maintained for a duration of about 20 hours, and recording done at a fixed time each day. All other conditions were maintained constant. The leaf temperature was 23.2°C. Figure 4.6.3 shows that g_s declined linearly in relation to increasing D_l, between 0.6 to 1.2 kPa. Between 0.3 kPa and 0.6 kPa and between 1.2 to 1.65 kPa, there was little change in g_s. Needle water potential fluctuated between -0.7 and -0.9 MPa, but essentially there was little significant change in ψ, as D_l was varied from 0.3 to 1.65 kPa.

Another wind tunnel experiment was done under similar environmental conditions except for the leaf temperature which was kept constant at 15.0°C (Figure 4.6.4). Shoots were cut from four potted plants and subsequently re-cut under water. The cut ends were immersed in beakers of water and the shoots and the plants from which they were obtained subsequently treated as pairs of plant material. These paired plant materials were arranged in the experimental

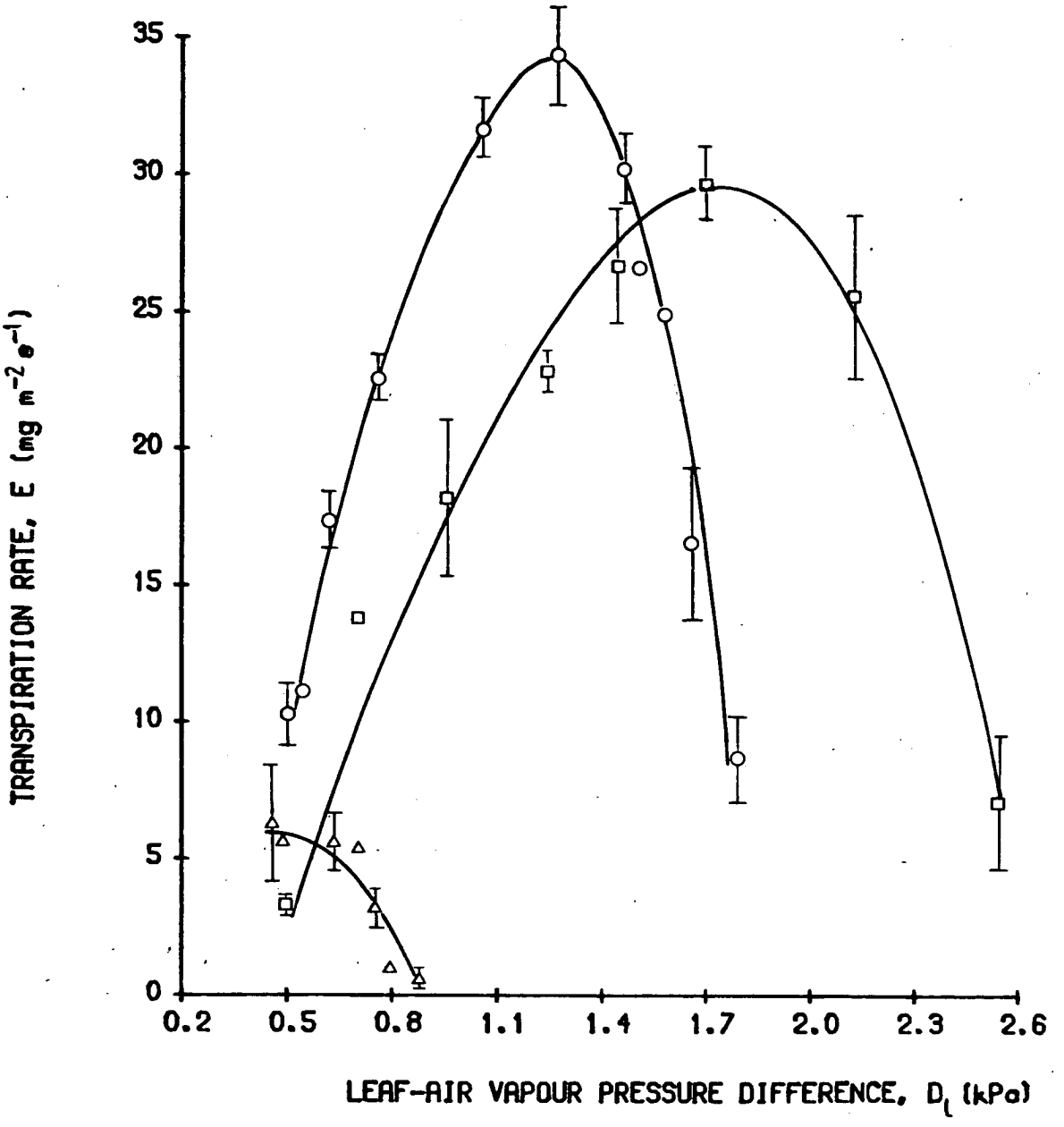


Figure 4.6.2 Transpiration rate as a function of increasing leaf-air vapour pressure difference at leaf temperatures of 25.4°C (□——□), 20.2°C (○—○) and at 10.2°C (△—△). The transpiration rates have been calculated for the results given in Figure 4.6.1. Other details are also given in that figure.

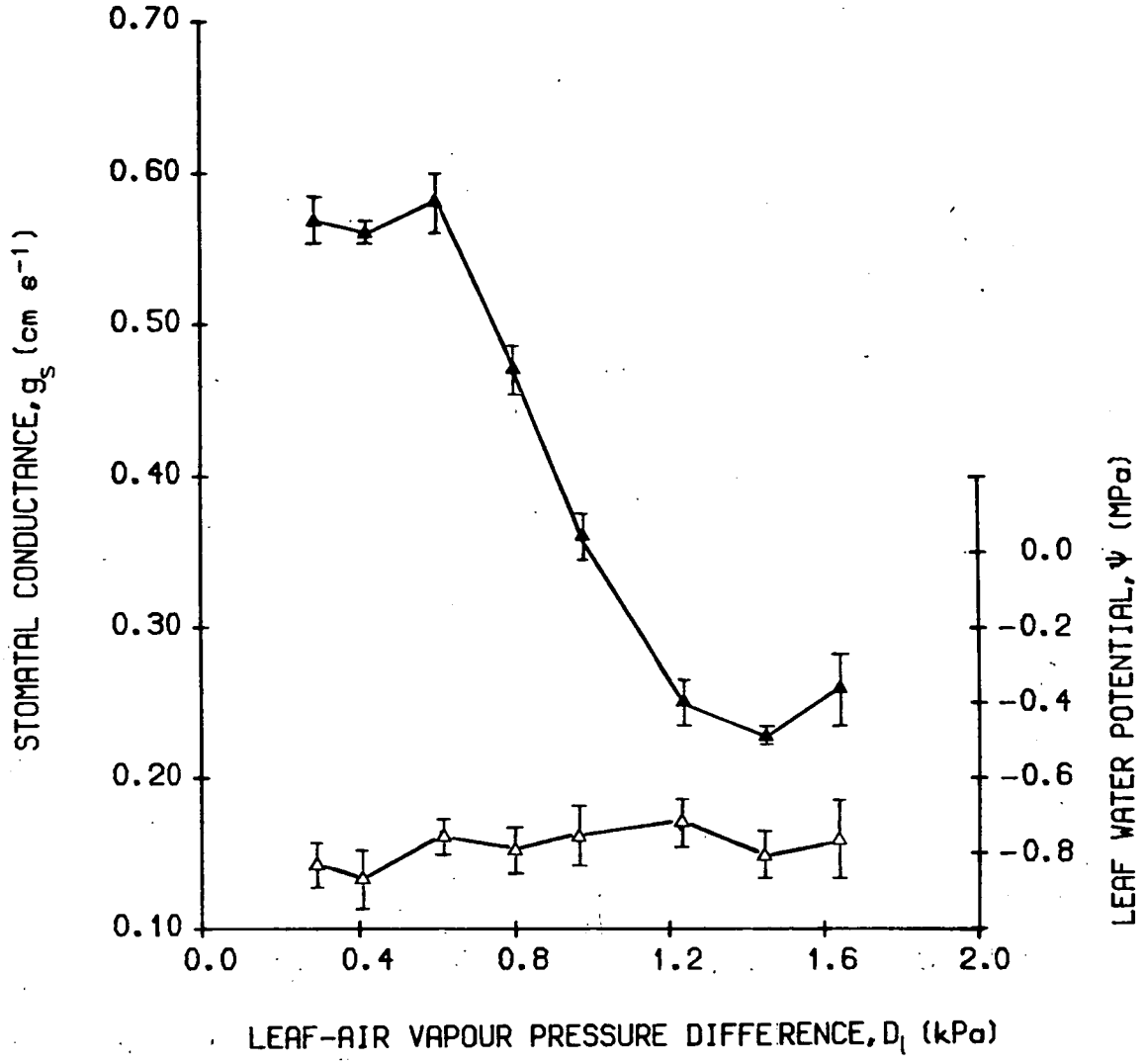


Figure 4.6.3 The response of stomatal conductance (▲) and leaf water potential (△) to increasing leaf-air vapour pressure difference. Wind tunnel experiment on six potted plants. Photon flux density at sampled shoot level 320 μE m^{-2} s^{-1}; leaf temperature 23.2°C and wind speed 2.75 m s^{-1}. Means with two standard errors are shown.

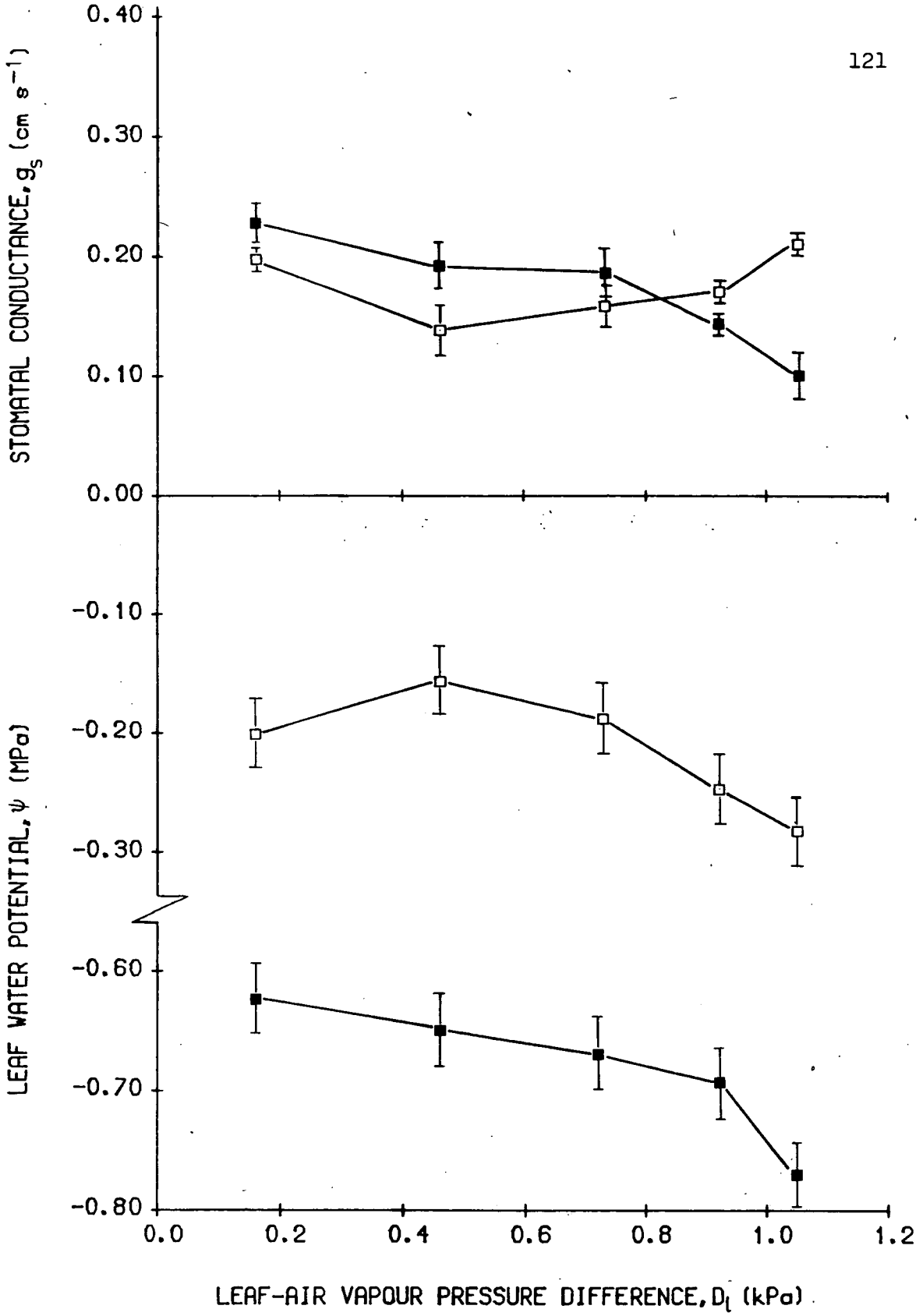


Figure 4.6.4 The response of stomatal conductance and leaf water potential to increasing leaf-air vapour pressure difference in whole plants (■) and cut shoots standing in water (□). Wind tunnel experiment on four pairs of potted plants and cut shoots. Photon flux density at sampled shoot level 320 μE m^{-2} s^{-1}, leaf temperature 15.0°C and wind speed 2.75 m s^{-1}. Means with two standard errors are shown.

stage of the wind tunnel and exposed to the similar level of photon flux density. At the end of each day's recording, the ends of the cut shoots were recut underwater, the water changed and the potted plants watered and re-bagged at soil level. The cut shoots were adjusted in height so as to receive similar level of photon flux density as previously.

In the whole plants there was an exponential decrease in g_s with increasing D_l. The cut shoots, however, showed no definite trend and were apparently insensitive to increasing D_l. Except for the value of ψ at D_l of 0.15 kPa there was a similar declining ψ as D_l was increased in both plant materials. The cut shoots, however, had a mean ψ value which was more than three fold higher than that of the whole plants. Hence the insensitivity to increasing D_l in the cut shoots appeared to be caused by the high values of ψ.

The data in Figure 4.4.11 were replotted against D_l at four levels of photon flux density (Figure 4.6.5). It is apparent that the sensitivity of G to changes in D_l is less at lower photon flux density. At 110 μE m^{-2} s^{-1}, there was practically little change in G as D_l was reduced from 0.95 to 2.3 kPa, while at 1200 μE m^{-2} s^{-1} for the same change in D_l, the value of G was reduced by half.

Response of stomata to temperature

Leaf-air vapour pressure difference was controlled as far as possible, while leaf temperature was varied between 5 and 31°C. Two levels of leaf-air vapour pressure difference were investigated at a high photon flux density.

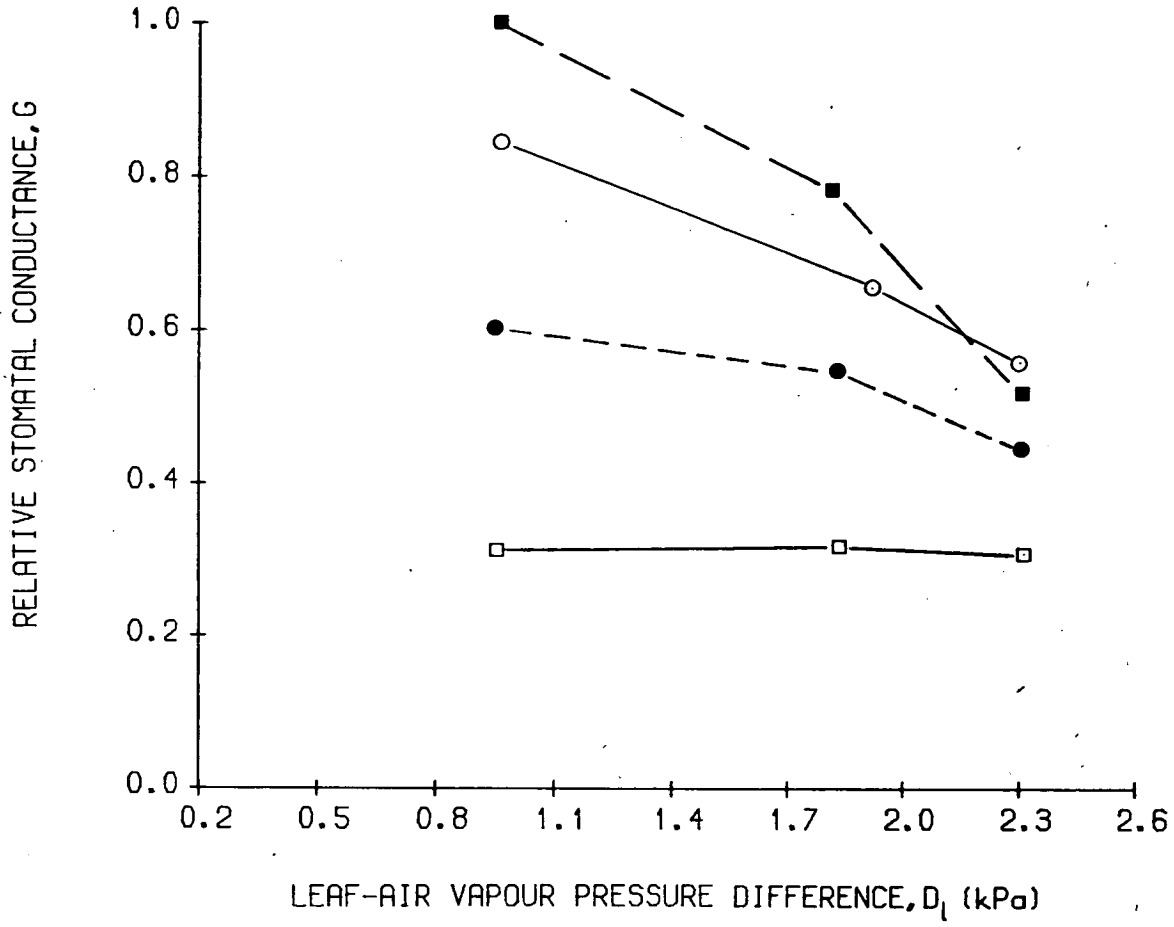


Figure 4.6.5 The relation between relative stomatal conductance and leaf-air vapour pressure difference at four levels of photon flux density: 1200 μE m^{-2} s^{-1} (■—■), 680 μE m^{-2} s^{-1} (○——○) 340 μE m^{-2} s^{-1} (●- -●) and 110 μE m^{-2} s^{-1} (□——□). Replotted from Figure 4.4.11 with data derived from a sequence of increasing photon flux density.

Relative stomatal conductance, G, increased with increasing leaf temperature up to around 20°C (Figure 4.6.6), after which it decreased with further increase in temperature. The optimum temperature at D_l of 0.62 kPa was about 20°C. There appeared to be a slightly higher optimum temperature at D_l of 0.95 kPa. It is of note, however, that at around 30°C, G at D_l of 0.62 kPa was not significantly different from that at D_l of 0.95 kPa. However, at lower temperatures G attained at 0.62 kPa became increasingly larger than that attained at 0.95 kPa. This can be interpreted as an increase in sensitivity to D_l at lower leaf temperatures as had already been shown in Figure 4.6.1.

Summary of results

(1) Stomatal conductance decreased with increase in leaf-air vapour pressure difference, D_l. At 20°C and 25°C, the decrease in g_s was exponential above a certain threshold value of D_l. Under similar conditions but at 10°C, g_s decreased linearly with increasing D_l.

(2) Over the full range of D_l, g_s was more sensitive to D_l at lower leaf temperatures than at higher leaf temperatures.

(3) Complete stomatal closure occurred at a lower value of D_l at lower temperatures than at higher temperatures.

(4) g_s was more sensitive to D_l at higher photon flux density than at lower photon flux density.

(5) Transpiration rate, at constant leaf temperature, increased to a maximum with increasing leaf-air vapour pressure

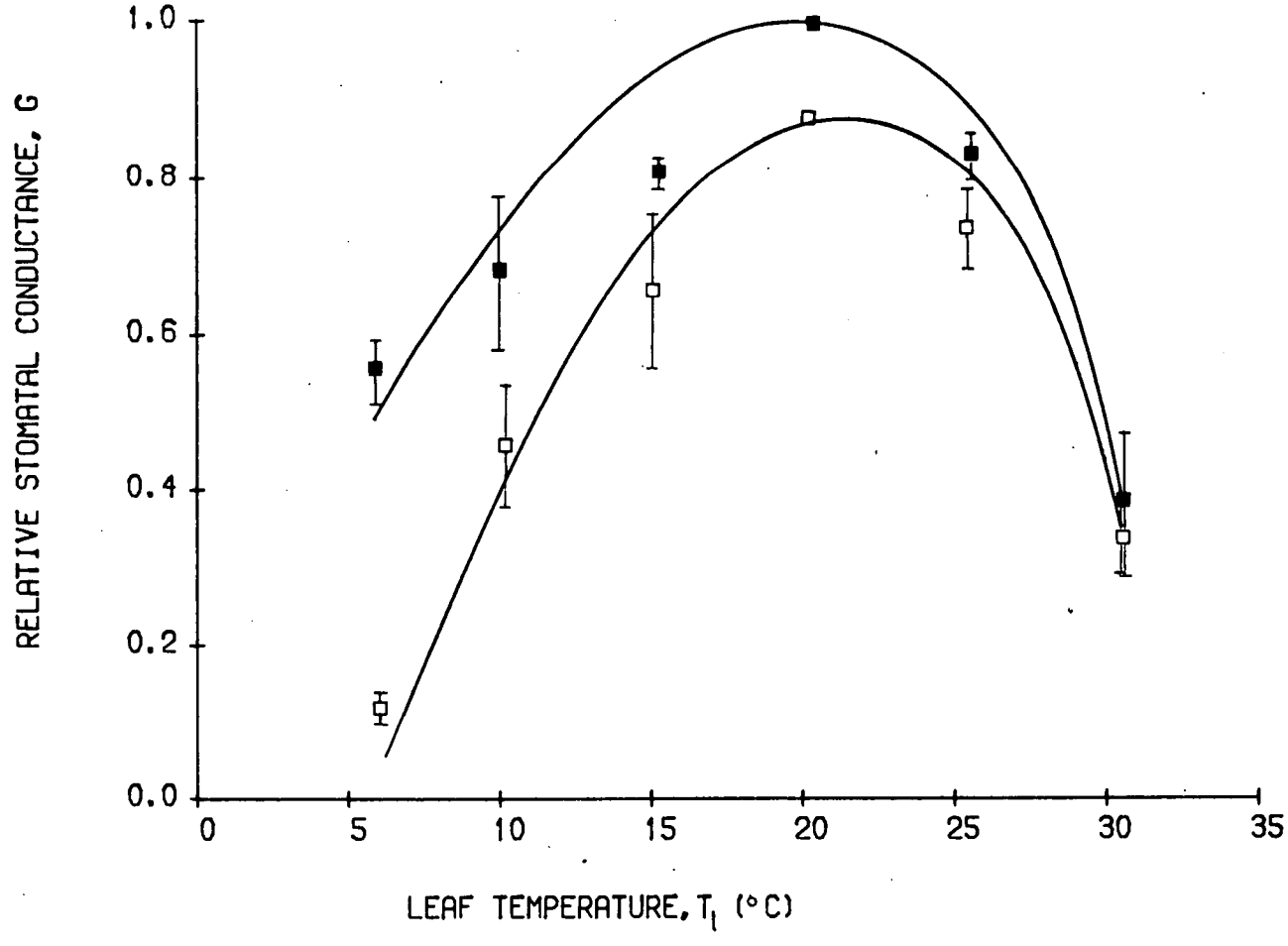


Figure 4.6.6 The relation between relative stomatal conductance and leaf temperature at leaf-air vapour pressure differences of 0.62 ± 0.02 kPa (■) and 0.95 ± 0.02 kPa (□). Fitted curves see Table 4.7.2). Average g_s at G = 1.0 is 0.442 cm s^{-1}. Photon flux density 1260 μE m^{-2} s^{-1}. All points are means of five measurements with two standard errors shown on representative points.

difference and subsequently decreased at higher levels of leaf-air vapour pressure differences.

(6) Where the entire plant was exposed to each level of D_l for a period of about 20 h in the wind tunnel at 23°C the response of g_s to increasing D_l was sigmoidal.

(7) At 23°C leaf water potential, ψ, remained more or less constant while g_s varied in relation to changing D_l. At 15°C, however, stomatal closure in response to increasing D_l was accompanied by a gradual decline in ψ.

(8) g_s was insensitive to D_l in cut shoots standing in water in the wind tunnel; in these shoots ψ varied between 0.2 to 0.3 MPa.

(9) The optimum leaf temperature for stomatal conductance at constant D_l of 0.62 kPa was 20°C. This optimum was higher at higher D_l.

(10) At a leaf temperature of ca 31°C, stomatal conductance was almost zero and independent of D_l. At lower leaf temperatures, there was an increasingly larger difference between stomatal conductance at the different values of D_l. Stomatal closure occurred at -0.6 and 5.2°C at D_l of 0.62 and 0.95 kPa respectively.

4.7 Modelling stomatal behaviour

Introduction

The response of stomata to step function changes in quantum flux density, leaf-air vapour pressure difference, leaf temperature, leaf water potential, ambient CO_2 concentration, one variable or two variables at a time, while the other variables are held constant, has been established in the laboratory. However, to understand fully stomatal behaviour, the effect on the stomata of other combinations of environmental variables, or the simultaneous influence of three or more environmental variables has to be known. To fully obtain the latter experimentally in the laboratory would be extremely time-consuming and laborious, and may not be necessary if there are no interactions between variables in their effects.

In the field many environmental variables are strongly correlated one with another and tend to fluctuate together. Great difficulty exists in the interpretation of the effect of any single environmental variable, unless the field data are numerous and adequately spread uniformly over the entire variable space. When that occurs, the upper limit of a scatter diagram of stomatal conductance against any one variable indicates the response to that particular independent variable, when the other variables are not limiting (Jarvis 1976). This objective is difficult to achieve in the field and the resulting upper limit is usually discontinuous and irregular.

A reason for the difficulty in achieving an even spread of data point over the entire variable space is the strong association between certain of the environmental variables. This is particularly so in the

case of ambient temperature, T_a, and vapour pressure deficit, D_a. At high T_a, D_a tends to be high and at low T_a, D_a tends to be low. It is rare to obtain at high T_a, a low value of D_a or at low T_a, a high value of D_a. Another example is the association between low values of ψ and high T_a. Hence difficulty is usually encountered in the interpretation of field data involving these environmental variables.

A first step towards a synthesis of the results obtained and the definition of future objectives is the formulation of an empirical model, which provides a convenient means of summarising a large quantity of data in terms of a few parameters. Such a summary is also valuable for calculating the response of the stomata to a range of variables and may also be of value in extrapolation. It may also be important in the assistance it will give to the interpretation of field data.

In this study the steady state stomatal conductance of Pinus sylvestris has been shown to be strongly influenced by current levels of quantum flux density, leaf temperature and leaf-air vapour pressure difference. It has also been shown not to be affected by leaf water potential above a threshold value of ψ. Further, varying ambient concentration of CO_2 has no effect on stomatal aperture. In the laboratory experiments described, ψ was kept constant at values above threshold ψ. Therefore ψ is not likely to have any effect on g_s, and is not therefore considered in the models to be described. Besides, in the field, the effect of ψ was found to be negligible, presumably because of the low threshold value of ψ, which was rarely encountered. A relation between g_s and C_a is also not included because of the lack of response of g_s to C_a.

The parameters which describe the response of stomata to the various environmental variables have been estimated by the method of nonlinear least squares regression (Webb 1972). The data set consisted of observations of simultaneously measured values of photon flux density, leaf-air vapour pressure difference and leaf temperature, in addition to the corresponding values of stomatal conductance.

Model 1. Light.

The relation between g_s and photon flux density, Q, has been shown to be appropriately described by a rectangular hyperbola (Figure 4.7.1). In the dark, g_s has a finite value, P_k. This value is not to be confused with cuticular conductance, which has not been measured. The mathematical expression for a rectangular hyperbola is:

$$g_s\ (Q) = P_m\ S_c\ (Q + q)\ /\ (P_m + S_c\ (Q + q)) \qquad 4.7$$

when $$q = P_k\ /\ S_c \qquad 4.8$$

P_m is the asymototic value of g_s at finite Q, and S_c is $\Delta g_s / \Delta Q$ at the point where the hyperbola meets the abscissa.

Model 2. Leaf temperature.

The relation between stomatal conductance and leaf temperature can be described by the following:

$$r = (T_p - T_o)\ /\ (T_o - T_n) \qquad 4.9$$

$$B = 1\ /\ (T_o - T_n)\ (T_p - T_o)^r \qquad 4.10$$

and $$g_s\ (T_l) = B\ (T_l - T_n)\ (T_p - T_l)^r \qquad 4.11$$

where $g_s\ (T_l)$ is the value of g_s varying between temperatures, T_n and

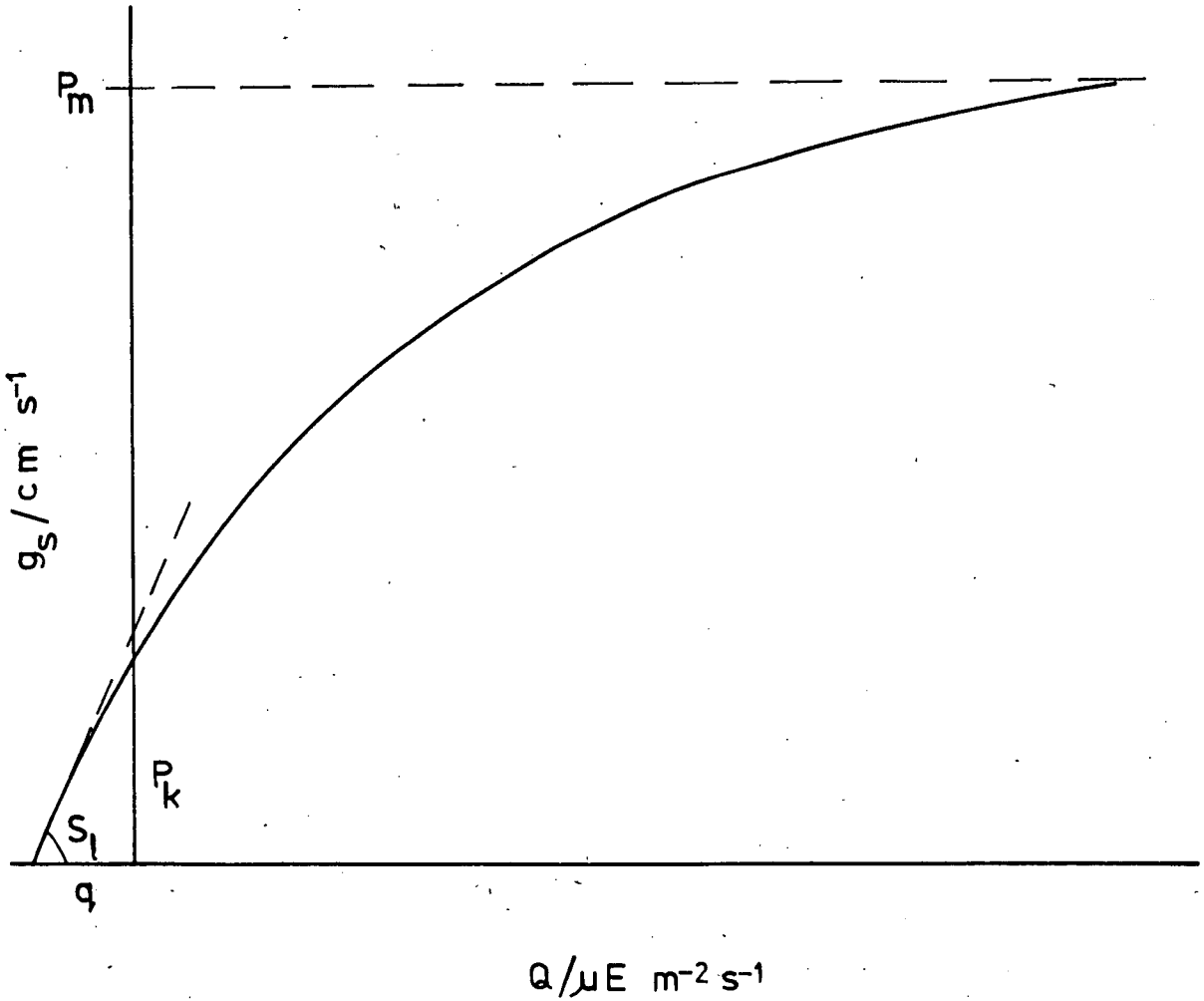


Figure 4.7.1 Model 1. The relation between stomatal conductance, g_s and photon flux density, Q, as given by Equations 4.7 and 4.8 . Redrawn from Figure 4.4.2. Leaf temperature, T_l and leaf-air vapour pressure difference, D_l have been kept constant. The three parameters, P_m, P_k and S_l in the equations are shown.

T_p, the low and high leaf temperatures, respectively, at which g_s is zero and T_o is the temperature at which g_s is highest (Figure 4.7.2). r is the temperature ratio that describes whether the optimum temperature is biased towards T_n or towards T_p.

Model 3. Leaf-air vapour pressure difference.

Stomatal conductance decreases exponentially with increasing leaf-air vapour pressure difference. The following exponential equation describes this relationship.

$$g_s (D_l) = P_r (1 - e^{s_r (D_l - V_h)}) \qquad 4.12$$

where $g_s (D_l)$ is the value of g_s as D_l varies between 0 and V_h. The maximum value of g_s at $D_l = 0$ is P_r; the value of D_l at $g_s = 0$ is V_h (Figure 4.7.3); S_r is the exponent or the slope of the exponential curve at $D_l = V_h$.

Complete model

If there is no snergistic interaction amongst the various variables

$$g_s (Q, T_l, D_l) = g_s (Q) q_s (T_l) g_s (D_l) \qquad 4.13$$

The influence of photon flux density on g_s is largely independent of the effects of both temperature and leaf-air vapour pressure difference (see Figure 4.4.11). The effect of photon flux density is therefore assumed to be completely independent of the effects of the other environmental variables. Thus:

$$g_s (Q, D_l) = g_s (Q) g_s (D_l) \qquad 4.14$$

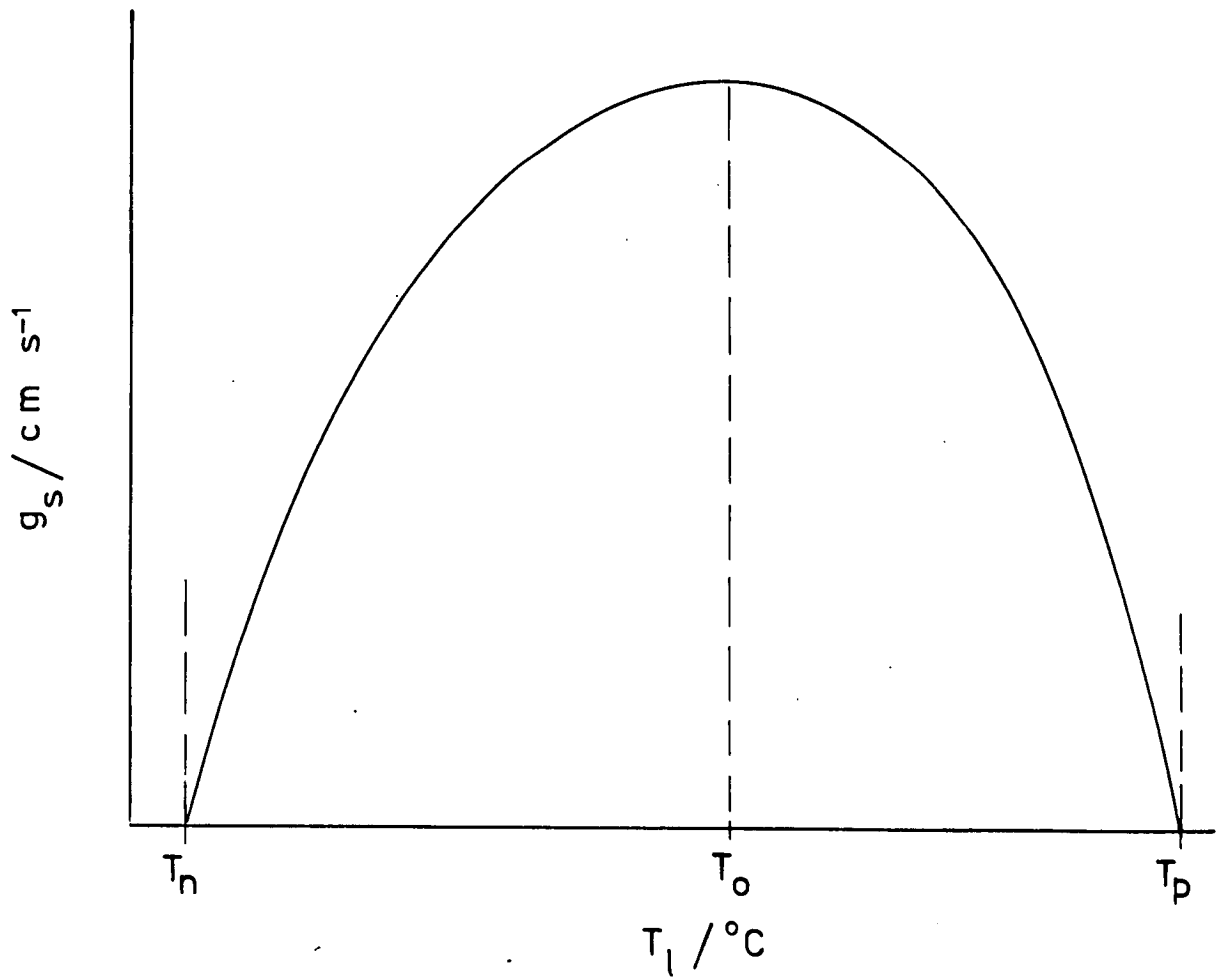


Figure 4.7.2 Model 2. The relation between stomatal conductance, g_s and leaf temperature, T_l, as given by Equations 4.9 , 4.10 and 4.11. Modified from Figure 4.6.6. Leaf air vapour pressure difference, D_l and photon flux density, Q have been kept constant. The three parameters in the equations are shown.

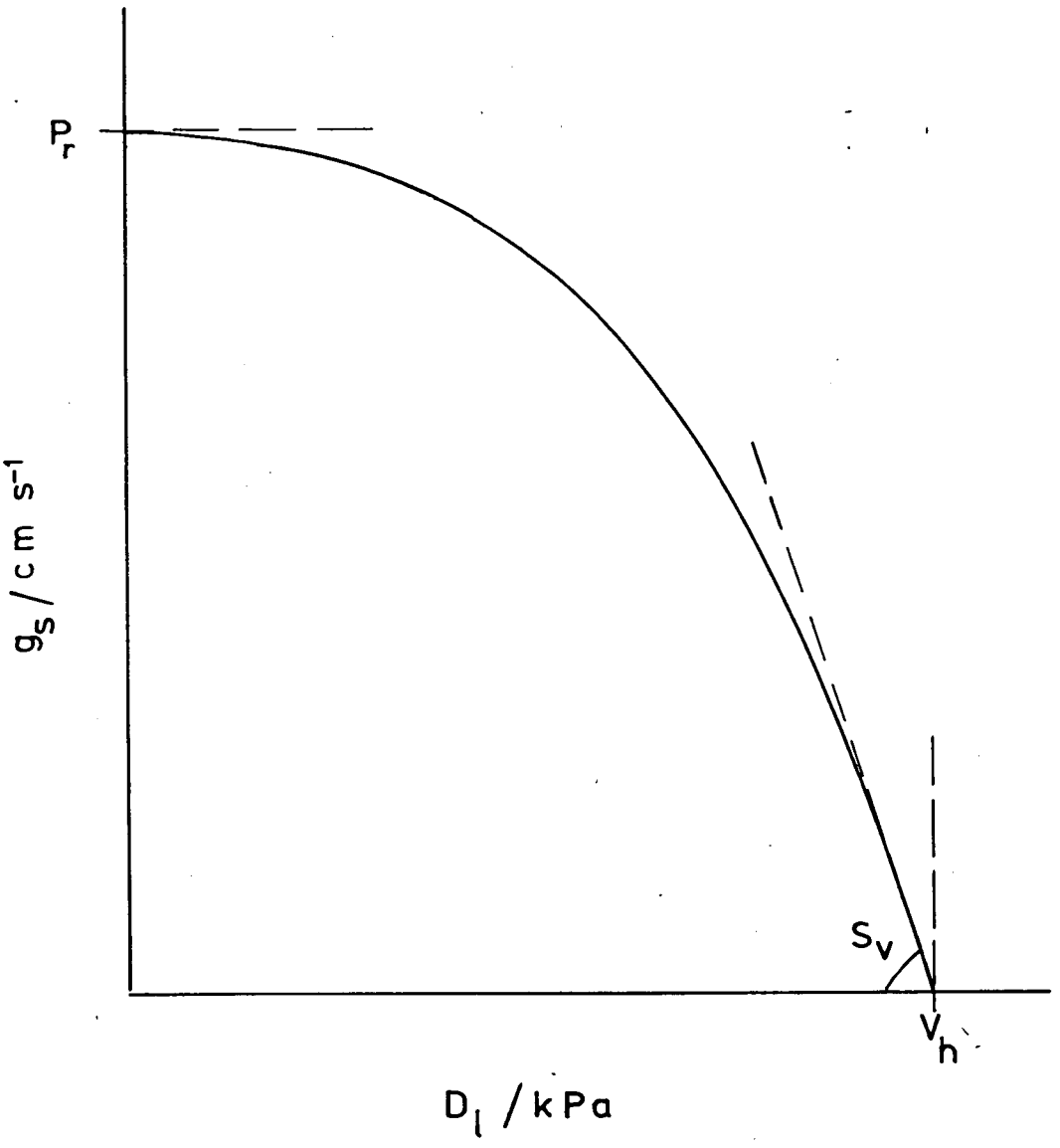


Figure 4.7.3 Model 3. The relation between stomatal conductance, g_s and leaf-air vapour pressure difference, D_l, as given by Equation 4.12. Redrawn from Figure 4.6.1. Leaf temperature, T_l and photon flux density, Q are kept constant. The three parameters in the equation are shown.

Figure 4.7.3 shows that the relation between g_s and D_l can be described by an exponential equation, involving the three parameters, P_r, V_h and S_r. All three parameters appear to be functions of temperature. The functions employed in Model 3 have therefore been utilised in conjunction with additional expressions relating V_h and P_r to temperature. The exponent S_v is assumed to be independent of temperature as the model is not very sensitive to its exact value. V_h is assumed to increase linearly with temperature from a value of zero at the base temperature, T_q, which is the low temperature at which both g_s and D_l are zero.

Hence,

$$V_h = S_x (T_l - T_q) \qquad 4.15$$

where S_x is the slope of this relationship. P_r is obtained as a function of temperature from the curve relating g_s to temperature at zero D_l. At saturating light $P_r = P_m$.

The complete model is based on Equation 4.13, but includes modifications to Model 3 to allow for the interaction between T_l and D_l. The complete model is therefore given as:

$$q = P_k / S_l$$

$$g_s (Q) = S_l (Q + q) / (P_m + S_l (Q + q))$$

$$r = (T_p - T_o) / (T_o - T_q)$$

$$B = 1 / (T_o - T_q) (T_p - T_o)^r$$

$$g_s (T_l) = B (T_l - T_q) (T_p - T_l)^r$$

$$V_h = S_x (T_l - T_q)$$

$$g_s\ (D_1) = 1 - e^{S_v\ (D_1 - V_h)}$$

$$g_s\ (Q,\ T_1,\ D_1) = P_m\ g_s\ (Q)\ g_s\ (T_1)\ g_s\ (D_1)$$

Application of the models

Models 1, 2 and 3 were applied to experimental results obtained in the laboratory, where individual variables were varied, while keeping the other variables constant. The derived parameters, as well as the goodness of fit, R^2, for various experiments illustrated in earlier figures, are listed in Table 4.7.1 and 4.7.2. Subsequently, all the experimental data relating g_s to Q, T_1, and D_1 were pooled together and the combined model was applied to the data set.

It is to be noted that the laboratory data had been normalised to a particular value of g_s, so that the relative stomatal conductance, G, has a maximum value of 1.0 in the case of experiments on photon flux density and leaf temperature, 1.2 in the case of experiments on leaf-air vapour pressure difference (see Figure 4.6.1). Application of the model remains the same, g_s being replaced by G.

Finally, the field data, collected from March to October 1976 were used. The results of the application of the model on the pooled laboratory data and the field data are given in Table 4.7.3 together with a summary description of the various parameters used in the combined model. The field data were obtained from three canopy levels. Adjustment factors were therefore used to relate g_s values derived from level 2 and level 3 to level 1. These two adjustment

Table 4.7.1 Value of the parameters derived from application of Model 1 to the normalised responses of g_s to photon flux density. Values of g_s have been normalised with respect to the value at highest photon flux density in each experiment.

Figure	Sequence of increasing light steps				Sequence of decreasing light steps			
	P_m (dimensionless)	S_1 (μE^{-1} m^2 s)	P_k (dimensionless)	R^2	P_m (dimensionless)	S_1 (μE^{-1} m^2 s)	P_k (dimensionless)	R^2
4.4.1	1.308	0.003	0.221	0.82	-	-	-	-
4.4.2	-	-	-	-	1.156	0.014	0.145	0.93
4.4.3a	1.147	0.004	0.025	0.94	1.130	0.008	0.123	0.89
4.4.3b	1.247	0.003	0.077	0.97	1.090	0.014	0.352	0.95
4.4.4	1.367	0.001	0.002	0.97	1.128	0.010	0.054	0.89
4.4.5a	1.425	0.003	0.053	0.97	1.190	0.008	0.151	0.96
4.4.5b	1.133	0.006	0.505	0.95	1.043	0.021	0.600	0.86
4.4.6	1.500	0.002	0.084	0.97	1.124	0.011	0.001	0.95
4.4.9	1.154	0.006	0.004	0.90	1.193	0.005	0.030	0.89
4.4.10	1.193	0.005	0.162	0.86	1.245	0.005	0.085	0.93

Table 4.7.2 Values of the parameter derived from application of Model 2 and 3 to the normalised response of stomatal conductance to temperature, T_l and leaf-air vapour pressure difference, D_l. The values of stomatal conductance have been normalised with respect to the value at 20°C at 1.3 kPa (Figure 4.6.1) and with respect to the maximum value of g_s at 20°C (Figure 4.6.6).

Figure	T_l	D_l	Parameters			R^2
			P_r (dimensionless)	S_v (kPa^{-1})	V_h (kPa)	
4.6.1	25°C	variable	0.91	1.98	2.83	0.78
	20°C	variable	1.23	2.74	1.80	0.86
	10°C	variable	1.23	1.20	1.03	0.81
			T_n (°C)	T_o (°C)	T_p (°C)	
4.6.6	variable	0.6 kPa	-0.6	20.0	32.0	0.70
	variable	0.95 kPa	-5.2	21.4	31.9	0.65

Table 4.7.3 Values of the parameters derived from application of the complete model

Quantity	Symbol	Laboratory data	Field data	Units
maximum g_s	P_m	0.674	3.00	cm s^{-1}
g_s; Q = 0	P_k	0.028	0.432	cm s^{-1}
dg_s/dQ; Q = 0	S_1	30	760	m^3 E^{-1}
low temperature for g_s = 0	T_q	-1.20	-6.99	^oC
bias ratio	r	0.634	2.48	-
high temperature for g_s = 0	T_p	31.2	45.0	^oC
$dD_1/d(T_1 - T_q)$	S_x	0.100	0.796	kPa $^oC^{-1}$
dg_s/dD_1	S_v	1.277	0.016	cm s^{-1} kPa^{-1}
adjustment factor level 2	a_1		0.775	
adjustment factor level 3	a_2		0.646	
	R^2	0.795	0.632	

factors were included as two additional parameters in the complete model.

The model accounted for 80% and 63% of the variation in the laboratory data and field data respectively (Table 4.7.3). Both data sets indicated a measurable stomatal opening at zero photon flux density. The steeper slope of the light curve in the field indicates g_s saturating at a lower photon flux density than in laboratory plants. The lower temperature limit as well as the higher temperature limit at zero D_l and zero g_s for the field data compared to the laboratory data suggest a certain amount of acclimatisation to the wider range of temperature to which the field trees are exposed. The steeper slope of the D_l response curve in the laboratory data is an indication of the higher sensitivity of g_s to high D_l. However, it is to be noted that the response of g_s to increasing D_l in the laboratory is strongly curvilinear but in the field it tends towards linearity. There is therefore a more marked sensitivity to increasing D_l in the field at low D_l compared to that in the growth room-grown plants.

The stomatal conductance of needles in canopy level 2 is 78% of that in level 1, while that in level 3 is only 65% of that in level 1.

Because of the tendency towards linearity in the stomatal response to D_l in the field, replacement of the exponential equation by a straight line relationship, as well as exclusion of the interaction expression, appears to give an equally good fit. However, the inclusion of the interaction factor in the model, gives a better description of the response to T_l and to D_l. For example, the linear

relationship indicates zero g_s at high D_l to be independent of temperature, so that at a low temperature, there would be measurable stomatal opening at D_l as high as 2.4 kPa.

An examination of the distribution of the residuals (predicted g_s - measured g_s) with respect to the three variables shows an even distribution of the negative and positive errors on either side of the zero line. This suggests that the model is satisfactory.

The low R^2 may therefore result from inadequacies in the distribution of the data. The fact that the complete model accounts for 80% of the variation in the laboratory data but only 63% in the field data suggests a more uneven distribution of the data over the variable space in the field data than in the laboratory data (Jarvis 1976). This suggestion is borne out by the strong correlation between T_a and D_l which occurs in the field, resulting in high D_l at high temperatures and low D_l at low temperatures.

Summary of results

(1) Mathematical models were derived to describe the stomatal response to photon flux density, leaf temperature and leaf-air vapour pressure difference. Models 1, 2 and 3 were successfully used on laboratory data in which a single variable was changed at a time.

(2) Subsequently, these mathematical expressions were combined, together with an interaction function into a complete model which was used on pooled laboratory data consisting of the response of stomatal conductance to three variables. The complete model contained eight parameters. This model was

successfully run on the laboratory data as well as on field data, and the values of the parameters derived. When used on field data two additional parameters were used to accommodate systematic difference in the values of stomatal conductance of needles at different levels in the canopy but in the same environmental conditions.

(3) When used on laboratory and on field data, the complete model accounted for 80% and 63% of the variation, respectively. It was considered satisfactory in application.

4.8 Interpretation of stomatal response in the field

The complete model was applied to field data obtained over a period of 8 months at Thetford. The parameters obtained are listed in Table 4.7.3.

Response to photon flux density

Using these parameters, the relation between g_s and photon flux density is shown in Figure 4.8.1 at four values of vapour pressure deficits. The data showed a tendency for stomatal conductance to saturate at a lower photon flux density at higher D_a. This has already been shown to occur in growth room grown plants (Figure 4.4.11). The larger value of g_s at lower D_a is clearly shown. There was a small and measurable g_s in the dark. In most respects, the response to D_a and photon flux density is similar to that observed in the laboratory.

On the basis of the laboratory experiments, the response to photon flux density was assumed to be independent of the response to

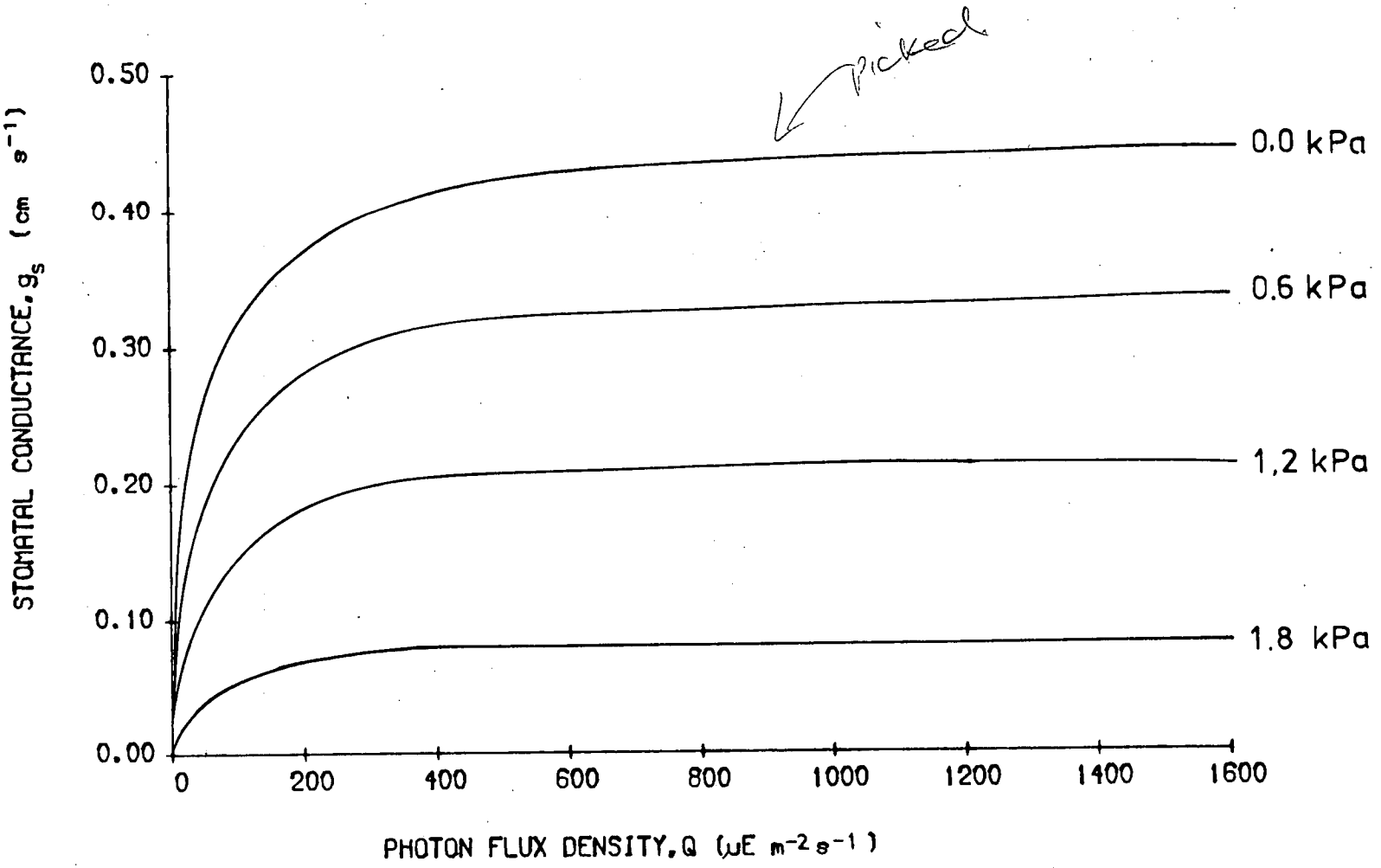


Figure 4.8.1 The relation between stomatal conductance of 1-year-old and current-year needles at level 2 in the canopy and photon flux density at four levels of vapour pressure deficits, and an ambient temperature of 20°C. The curves have been fitted using the parameters derived from the application of the complete model to the field data.

temperature. Using the appropriate parameters, the consequence of this assumption is evident in Figure 4.8.2. At a vapour pressure deficit of 0.5 kPa, g_s was largest between 15°C and 20°C, and smallest at 5°C. Values of g_s were similar at 15 and 20°C. Because no interaction was assumed, the relative values of g_s with respect to temperature remain the same at all levels of photon flux density.

Response to vapour pressure deficit

The field data clearly do not show an asymtote at the lower end of the D_a range in the same way as was exhibited by the results of the laboratory experiments (Figure 4.8.3). Although an exponential equation was fitted to the field data the derived parameters gave a response which is very close to linearity. The increased sensitivity to increasing D_a at lower temperatures is evident. At low D_a the highest g_s was at an ambient temperature of 15°C, suggesting that the optimum temperature for stomatal opening at D_a near to zero was 15°C. In other respects the response to D_a and ambient temperature is as expected from the laboratory experiments.

Response to ambient temperature

The response to ambient temperature at four levels of vapour pressure deficit, D_a is shown in Figure 4.8.4. At a D_a of 0.5 kPa, the optimum temperature was about 17.5°C, while at a D_a of 1.5 kPa and 2.5 kPa, it was 24°C and 32°C respectively. The upper temperature at which stomatal closure occurs was higher in the field data than in the laboratory experiments with growth room plants, suggesting a certain amount of adaptation to the wider range of temperature in

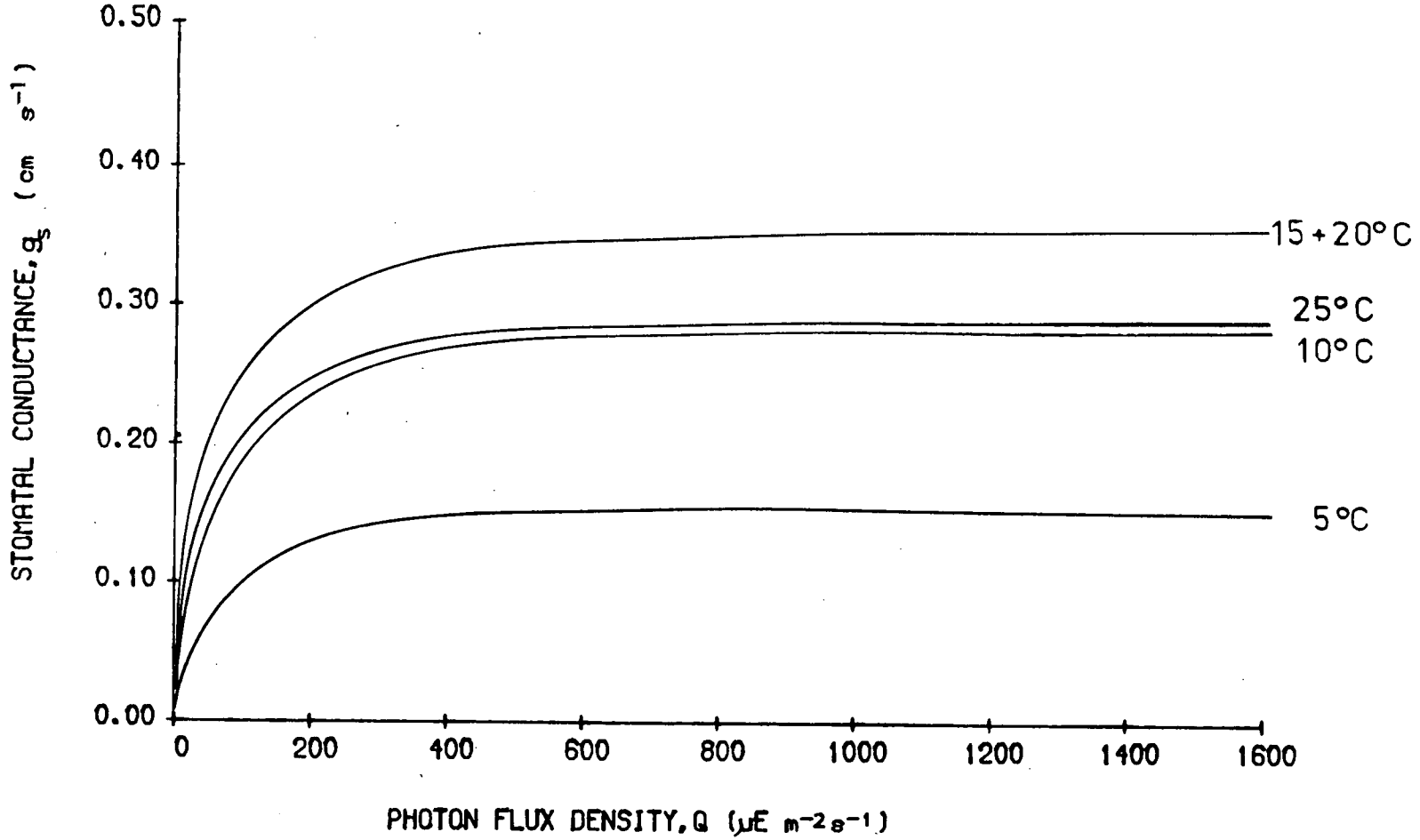


Figure 4.8.2 The relation between stomatal conductance of 1-year-old and current-year needles at level 2 in the canopy and photon flux density at five ambient temperatures. Vapour pressure deficit is 0.5 kPa. The curves have been fitted using the parameters derived from the application of the complete model to the field data.

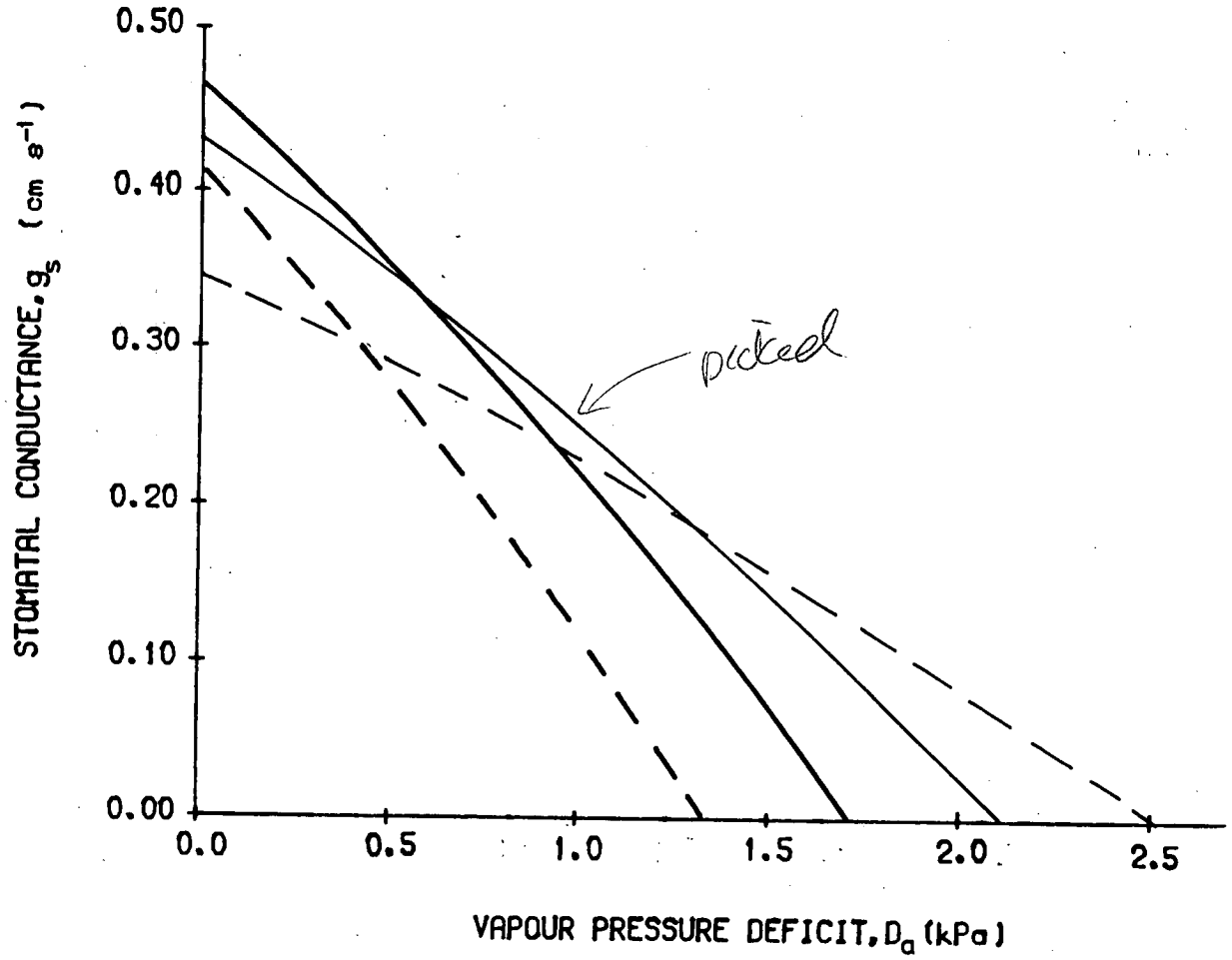


Figure 4.8.3 The relation between stomatal conductance of 1-year-old and current-year needles at level 2 in the canopy and vapour pressure deficit, at ambient temperature of 25°C (— —), 20°C (———) 15°C (———), and 10°C (— —). Photon flux density was 1000 μE m^{-2} s^{-1}. The curves have been fitted using the parameters derived from the application of the complete model to the field data.

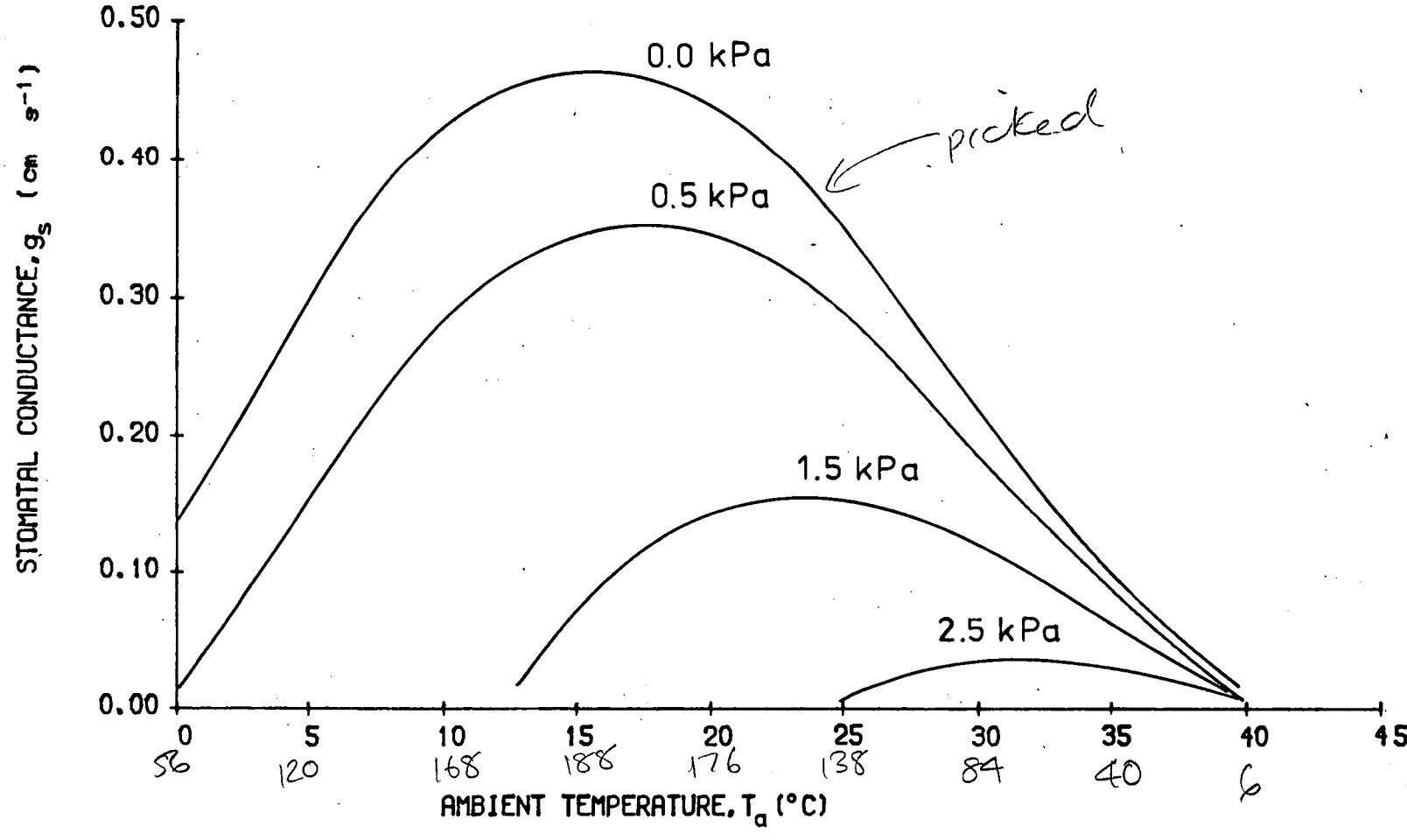


Figure 4.8.4 The relation between stomatal conductance of 1-year-old and current-year needles at level 2 in the canopy and ambient temperature, at four levels of vapour pressure deficits and a photon flux density of 1000 μE m^{-2} s^{-1}. The curves have been fitted using parameters derived from the application of the complete model to the field data.

the field. The lower temperature for stomatal closure was, however, comparable. The depression of g_s at high D_a was again evident.

Summary of results

(1) Stomatal conductance saturates at a lower photon flux density in field grown trees than in growth room plants.

(2) There is a tendency for stomatal conductance to saturate at a lower photon flux density at higher vapour pressure deficits than at lower vapour pressure deficits.

(3) Because of acclimatisation to the wider range of temperatures to which the trees in the field are exposed, the higher temperature at which the stomata close is at $40^{o}C$ compared to $31^{o}C$ in the growth room plants.

(4) The response to photon flux density is independent of the response to temperature.

(5) The response to vapour pressure deficit is curvilinear. There is a pronounced lack of an asymptote at low vapour pressure deficits, suggesting a higher sensitivity to low D_a than in growth room plants.

CHAPTER 5

DISCUSSION

5.1 Response to light

The results from the light experiments in which each step change in photon flux density was given an equilibration period of 2.5 h (Figure 4.4.9) showed the steady-state response of g_s to light. Stomatal conductance did not reach light saturation at 1400 μE m^{-2} s^{-1} and the response curve indicated an increasing g_s with increasing photon flux density. In most of the other light experiments with a sequence of increasing photon flux density, g_s also did not reach complete light saturation. Because of the geometrical arrangement of the needles on the shoot, and the parallel beam of light used, a certain proportion of the stomata will be in shadow and therefore receiving a lower photon flux density than that measured by the photodiodes, even though bilateral illumination was used. The experiment with all needles arranged in a vertical plane and illuminated bilaterally shows that when all the stomata receive the same photon flux density g_s saturates at a lower photon flux density of about 1000 μE m^{-2} s^{-1}.

In the field, the needles are exposed to diffused light emanating from many different directions (Norman & Jarvis 1975). Therefore there is less likelihood of a sharp reduction in light level caused by shadows. Hence, it is not unexpected to find that measurements made in the field show that g_s saturates at a low photon flux density. However, it is surprising to find that

this photon flux density is often very much lower (about 400 μE m^{-2} s^{-1}) than the saturating values observed in the laboratory. According to Hansen (1971) and Ludlow and Ng (1976) g_s saturates at a lower photon flux density when ψ is low than when ψ is high. This could provide a reason for the lower photon flux density at light saturation found in the field than in laboratory experiments on well watered growth room plants.

The disappearance of light hysterêsis after a 2.5 h equilibration period but not after a 0.75 to 1 h equilibration period indicates that the stomata were rather sluggish in responding to light and need a long period to reach a true steady state. Time response experiments (Figures 4.3.2a and 4.3.2b) showed that g_s takes about 4 h to reach stabilisation after an increase in photon flux density from 0 to 1580 μE m^{-2} s^{-1} or from 0 to 160 μE m^{-2} s^{-1}. This is long compared to 3 min (stomata opening) and 12 min (stomata closing) for Fagus grandifolia or 12 min (opening) and 20 min (closing) for Quercus rubra (Woods & Turner 1971). In another conifer, Picea sitchensis, it has been shown that it takes about 6 h for g_s to stabilise completely after a change in light from 0 to 600 μE m^{-2} s^{-1} (Watts & Neilson 1978).

When the leaf temperature was raised to about 20°C the stabilisation time was reduced to 2 h (Figure 4.3.1), and hysterêsis at the lower light levels disappeared (Figure 4.4.10). Such a reduction in time response resulting from an increase in temperature has been observed by other workers (Meidner & Heath 1959; Brun 1962; Mansfield 1965). These findings indicate that stomatal opening and closing depend strongly on a temperature dependent

process. The effect of temperature could be on membrane permeability, viscosity of water or a whole range of enzymatic processes in the guard cells.

The hysteresis caused by a carry-over effect was, however, not reduced by an increase in temperature up to 25°C or by an increase in D_l from 0.5 to 2.3 kPa. The carry-over effect gives certain ecological advantages to the plant since during short term (0.75 to 1 h) fluctuations in light levels, g_s is maintained or even increased as shown in Figures 4.4.8a, 4.4.8b and 4.4.8c. In an environment where other factors are not limiting, a higher g_s would result in an increase in photosynthesis. When there is a progressive decline in photon flux density, g_s is still maintained consistently higher than when there is a progressive increase in photon flux density (e.g. Figures 4.4.3a and 4.4.3b). This again would be expected to have a similar benefit of maintaining photosynthesis during declining photon flux densities.

Hysteresis in stomatal response to light had been noted previously (notably Burrows & Milthorpe 1976, and Hall, Schulze & Lange 1976), but as far as is known there is no detailed documented literature on it. Burrows and Milthorpe (1976) acknowledged it as an effect of light that caused some variation in the measurement of g_s. Hall *et al.* (1976) thought that hysteresis occurred only at low photon flux densities. Experiments on stomatal responses to light are frequently conducted with decreasing light only. Consequently, light hysteresis could not possibly be observed. Further, the plants on which such experiments are done are more

often than not, quicker responding than conifers (Turner 1974) and any hysteresis observed would be less pronounced. Measurements by Wood and Turner (1971) showed no hysteresis in g_s in Fagus grandifolia (beech) and Liriodendron tulipifera (yellow poplar) on changing from low to high photon flux density and then back again. They did, however, note a longer lag period in response in Liriodendron tulipifera compared to that in Fagus grandifolia. This is of relevance because the stomata of Liriodendron tulipifera are more sluggish in response to light than those of Fagus grandifolia. The reason why hysteresis was not noted in Liriodendron tulipifera but was noted in Pinus sylvestris is probably because the time to reach stabilisation for a change in light was only 20 to 35 min in Liriodendron tulipifera compared to the 4 h in Pinus sylvestris.

There was a measurable g_s value in the dark prior to illuminating the shoot. This could not be an artefact resulting from room light, or time of the day when recording g_s, because it was observed in time response experiment, with g_s measured in the growth room (Figure 4.3.1) as well as in assimilation chamber experiments (e.g. Figure 4.4.1). Similar stomatal behaviour has been observed in Picea sitchensis (Watt & Neilson 1978). The stomata closed after 7 h of darkness (Figure 4.3.2b) at 10.0°C and D_l of 0.55 kPa. But in the morning, minutes before the lights came on, for the commencement of the normal 14 h photoperiod, the measured g_s was low but measurable. This suggests that the stomata were closed only part of the night and opened to some extent again just before the lights came on the following morning.

Such pre-dawn stomatal opening has been previously noted in the literature (see Meidner & Mansfield 1968).

Although the response time calculated for a 66.7% change in g_s in response to a change in photon flux density from 0 to 160 μE m^{-2} s^{-1} was 110 min for stomatal opening and 260 min for stomatal closing, the time for complete stabilisation to occur was 4.5 h for opening and 7 h for closing (Figure 4.3.2b). This emphasises that on changing the photon flux density from 160 to 0 μE m^{-2} s^{-1}, there was initially a phase of rapid stomatal closure, followed by a phase of slow but steady decline in g_s until completion of stomatal closure. A similar occurrence was observed in Xanthium pennsylvanicum by Mansfield and Meidner (1966). This phenomenon led them to suggest two separate mechanisms in stomatal closure, one independent of CO_2 and the other, the second phase, dependent on the accumulation of respiratory CO_2. The fact that, in Pinus sylvestris, stomatal closure is not necessarily induced by high C_i casts some doubt on the occurrence of the second mechanism. Both opening and closure occurred with equal facility in air with or without CO_2 (Figures 4.4.3a and 4.4.5a). Woods and Turner (1971) have already shown that in Liriodendron tulipifera, the response time for a change in g_s in response to a change in photon flux density was independent of the magnitude of the change in photon flux density. In Pinus sylvestris g_s behaved similarly. Both plant species are considered as relatively sluggish in their response to light and both species are regarded as shade intolerant (Woods & Turner 1971; Zelawski & Kinelska 1967). Such similarities

in response to light are probably not coincidental but are features that allow them to adapt to their environment. Although no experiment was done on the effect of water stress on the response time in Pinus sylvestris, it is of note that, as another feature that allows plants to adapt to their environment, most plants will accelerate stomatal closure and delay stomatal opening in reaction to change in light levels when they are under water stress (Davies & Kozlowski 1975).

5.2 Response to CO_2

A lack of response to ambient CO_2 concentration between 0 and 5000 $cm^3\ m^{-3}$ at saturating light has been shown (Figures 4.5.1, 4.5.2 and 4.5.3). In addition the response to light was independent of C_a. The stomata opened and closed in response to light whether in CO_2-free air (Figures 4.4.5a and 4.4.5b) or in air with CO_2 concentration at the CO_2 compensation concentration (Figure 4.4.6), or in air of higher concentrations (Figures 4.5.1, 4.5.2 and 4.5.3). The variation of internal CO_2 concentration, C_i, with increasing C_a is shown in Figure 5.2.1 for four levels of photon flux density. When C_a was zero, the corresponding level of C_i was less than 20 $cm^3\ m^{-3}$ at a photon flux density of 1300 $\mu E\ m^{-2}\ s^{-1}$. Therefore, when there was a lack of response of g_s to CO_2-free air, C_i was at a level below that of the CO_2 compensation concentration of 42.0 $cm^3\ m^{-3}$. Even at that low CO_2 concentration, there was no response of g_s to CO_2. Figure 5.2.2 shows that as the photon flux density was reduced from 1300 to 170 $\mu E\ m^{-2}\ s^{-1}$, C_i remained unchanged at low values of C_a and increased

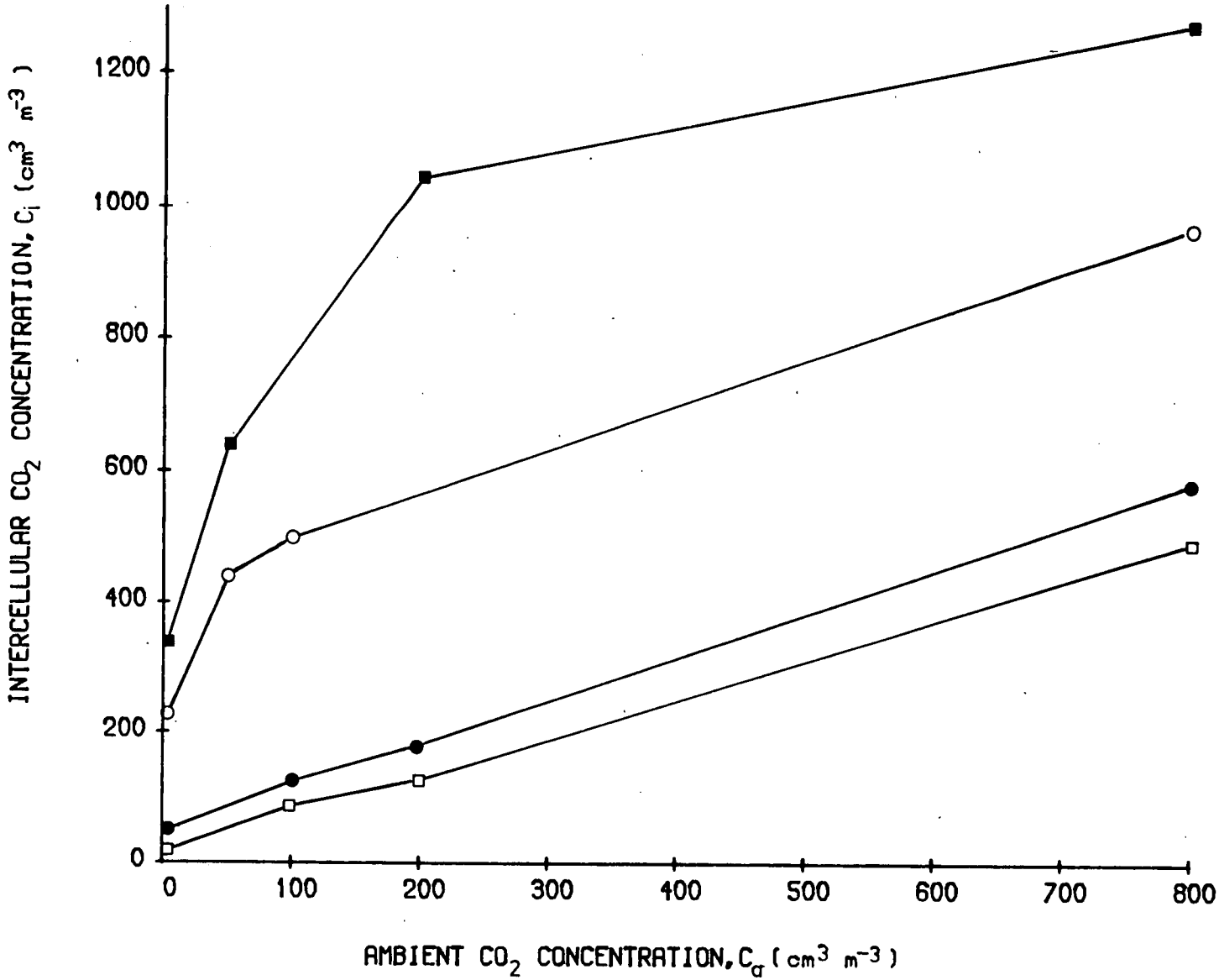


Figure 5.2.1 The relation between intercellular CO_2 concentration and ambient CO_2 concentration at four levels of photon flux density: 1300 μE m^{-2} s^{-1} (□), 170 μE m^{-2} s^{-1} (●), 25 μE m^{-2} s^{-1} (○), and 0 μE m^{-2} s^{-1} (■). Leaf temperature 20.6°C and leaf-air vapour pressure difference 0.56 kPa.

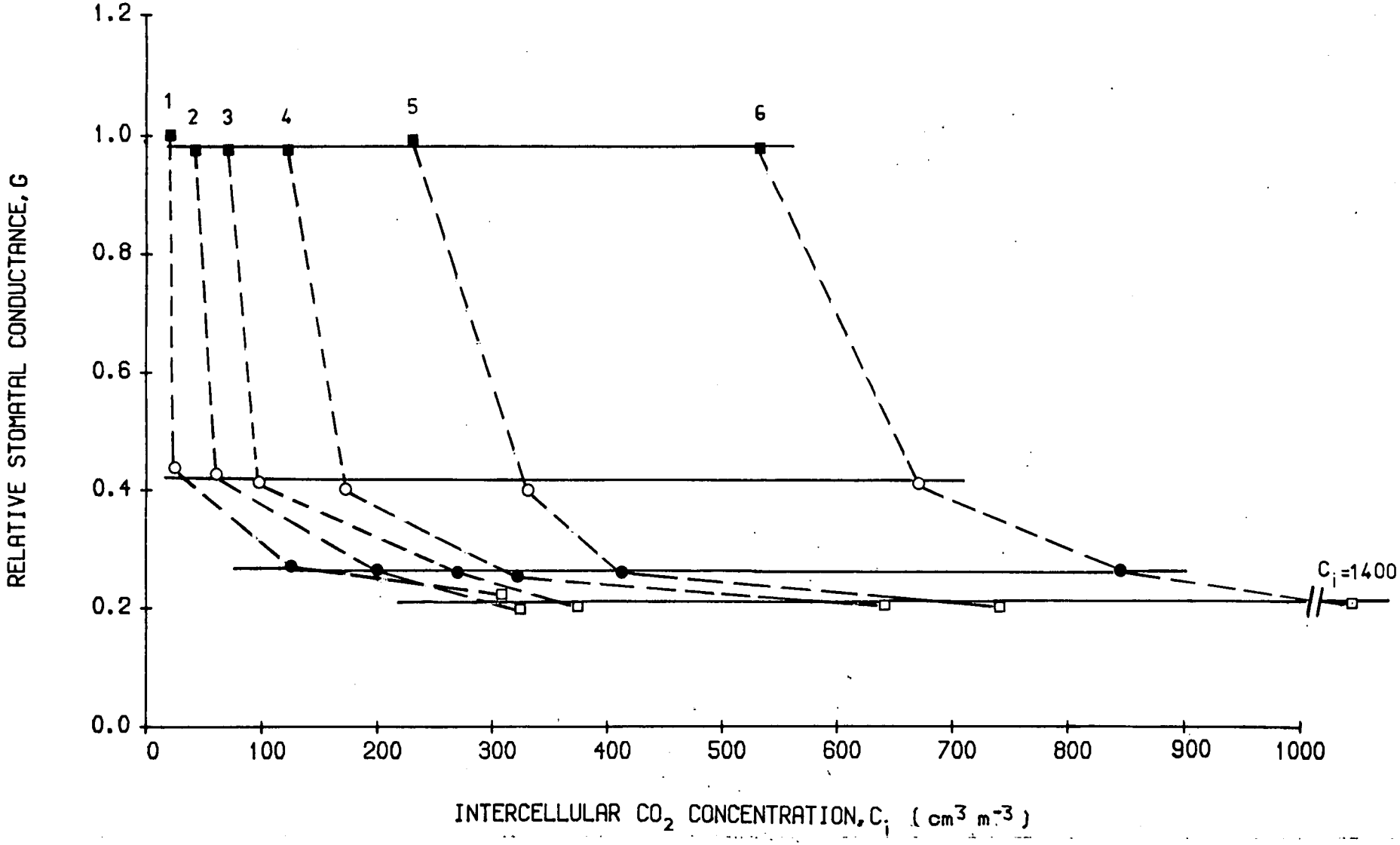


Figure 5.2.2 The relation between relative stomatal conductance and intercellular CO_2 concentration at four levels of photon flux density: 1300 μE m^{-2} s^{-1} (■), 170 μE m^{-2} s^{-1} (○), 25 μE m^{-2} s^{-1} (●), 0 μE m^{-2} s^{-1} (□), and the corresponding changes in ambient CO_2 concentration, C_a at 0(1), 50(2), 100 (3), 200(4), 400(5) and 800(6) cm^3 m^{-3}. Redrawn from Figure 4.5.2 where details are given.

at high values of C_a. A further decrease in photon flux density caused a larger increase in C_i until in darkness, a big increase in C_i resulted, with a very small decrease in g_s, in response to the lowering of photon flux density. The lack of response of g_s to changing C_i is apparent. A lack of response of g_s to various concentrations of CO_2 had already been shown to occur in Picea sitchensis (Neilson & Jarvis 1975; Beadle 1977). Other species in which g_s show no response to CO_2 include Vitis vinifera (Kriedemann, Sward & Downton 1976), Brassica oleracea (kale) (Parkinson 1968) and Gossypium hirsutum (Bierhuizen & Slatyer 1964). It had been postulated by many workers that the response to light is mediated through a change in C_i (Meidner & Mansfield 1965; Raschke 1975). The above results show that this postulate cannot be of universal application to all plant species.

The response to C_a seems to be independent of the response to D_l. The finding by Hall and Kaufmann (1975) that the stomata of Sesamum indicum responded to varying C_i in dry air but not in humid air does not hold in the case of Pinus sylvestris (Figure 4.5.4). Beadle (1977) has already shown that in Picea sitchensis the lack of response to C_a is not the result of high ψ and that a response cannot be induced by low ψ, as suggested by Raschke (1975). This is probably true also for Pinus sylvestris.

Although no information is available, it is probable that the flux of K^+ into and out of guard cells, which is responsible for stomatal opening and closing in other plants, also occurs in Pinus sylvestris. The stimulus for the K^+ flux could be brought about by the activity of phosphoenolpyruvate carboxylase in the

guard cells of Pinus sylvestris as in other plants. If malate concentration in the guard cells acts as an important controlling factor in stomatal movements (Raschke 1975) in Pinus sylvestris and supposing that CO_2 is required for malate formation, the question arises as to how it is possible for stomatal opening to occur in Pinus sylvestris in response to changes in light levels in an atmosphere free of CO_2. Raschke (1972) estimated that enough CO_2 would be available in solution in the guard cell cytoplasm for fixation by PEP carboxylase. Such an endogenous mitochondrial source of CO_2 might therefore be sufficient to meet the demand for CO_2 and the energy required could be supplied by ATP derived from cyclic photophosphorylation.

5.3 Response to leaf-air vapour pressure difference

Stomatal conductance declined in response to increasing D_l exponentially at 20 and 25°C and linearly at 10°C (Figure 4.6.1). Similar exponential responses have been reported in laboratory studies on Picea sitchensis (Beadle 1977) and on epidermal strips of Polypodium vulgare (Losch 1977). Field data, however, showed that the response to increasing D_l was slightly exponential (Figure 4.8.3). In contrast, in measurements on Picea sitchensis, in situ in forest canopies (Watts, Neilson & Jarvis 1976) and seedlings in controlled environment growth cabinets (Watts & Neilson 1978) and in wind tunnel experiments (Grace, Malcolm & Bradbury 1975), g_s was found to decrease linearly with increasing vapour pressure deficit, D_a over a range of temperatures. Similarly, in Pinus sylvestris there was a difference between the

shape of the response curve in the leaf chamber experiments (Figure 4.6.1) and in experiments on whole plants exposed to changing D_l in the wind tunnel (Figure 4.6.3). In the wind tunnel experiment the stomatal response to increasing D_l was sigmoidal.

In Figure 4.6.1 the difference in response between individual curves was caused by difference in leaf temperature since, all the other environmental variables were kept more or less constant. In comparison with these experiments, the wind tunnel experiment (Figure 4.6.3) was done at a lower photon flux density and with a somewhat different procedure. The lower photon flux density, however, could not account for the different shape of the response curves and other factors must be considered.

In the wind tunnel experiment each level of D_l was maintained for about 20 h, while in the leaf chamber experiment the period of exposure to a particular value of D_l was 0.75 to 1.0 h. The longer duration could result in some form of acclimatisation to each humidity level (Beardsell, Mitchell & Thomas 1973; Slavik 1973).

Rawson, Begg and Woodward (1977) reported that there was no response to D_a in several plant species when a shoot was exposed to changing humidity while the rest of the plant remained in conditions of low evaporative demand. This suggests that the response to D_l may depend upon the base level of water potential in the plant. While ψ remained more or less constant at -0.8 MPa in the wind tunnel experiments, unfortunately ψ was not recorded in the leaf chamber experiments. However, there is little reason to suspect that there was a drastic change in ψ during the course of these experiments, but it is possible that the base level in the plant was somewhat

higher than in the wind tunnel experiments. According to Hinckley, Schroeder et al. (1975), there are differences in the response of g_s to D_a at different levels of ψ. Schulze, Lange et al. (1972) observed a higher sensitivity of g_s to D_l in irrigated plants of Prunus armemiaca and Hamada scoparia than in the unirrigated plants. It is of note, therefore, that because of the absence of an asymtote in the response to increasing D_l, particularly at higher temperatures, there was a higher sensitivity in stomatal response to low values of D_l in the field (Figure 4.8.3) than in the laboratory experiments (Figure 4.6.1). There is a lower ψ value in the field than in well-watered growth chamber grown plants. The apparently higher sensitivity to increasing D_l with decreasing value of ψ is further supported by the findings of the wind tunnel experiments on cut shoots standing in water. Measured ψ was as high as -0.2 MPa, and there was complete insensitivity to increasing D_l.

Difference in level of photon flux density used during the course of the experiments could result in varying transpiration rate, with the consequent difference in base level water potential. As seen above, this could result in difference in sensitivity to increasing leaf-air vapour pressure difference. Alternatively, there could be a change in hydraulic resistance in the water supply pathway from the roots to the leaf, as a result of the changing transpiration rate in the leaf caused by increasing D_l. A lower hydraulic resistance would ensure a better water supply and a higher ψ. Consequently a lower sensitivity to D_l might occur (Schulze,

Lange et al. 1972). All these factors, whether individually or in concert could be involved in some of the differences in response to D_l observed. Similar reasons could be called upon to explain differences in response between the field experiments and the laboratory experiments or between responses found for part of a shoot and for the whole plant exposed to changing ambient humidity (Rawson, Begg & Woodward 1977). For responses to varying D_l to be really comparable, other environmental variables, particularly ψ and photon flux density must be recorded and standardised.

5.4 Hypothesis on stomatal response to leaf-air vapour pressure difference and temperature

Background to a hypothesis

(1) The decline in g_s with increasing D_l does not appear to be related to the leaf water potential (Figure 4.6.3), since ψ remains almost constant at about -0.8 MPa, while g_s declined linearly from 0.6 to 1.2 kPa. On the other hand, Figure 4.6.4 shows that at a lower temperature there is a decline in ψ with increasing D_l. Such a response in ψ reflects a change in flow rate and a possibility of a variable hydraulic resistance in the endodermis. According to Soar (1922) the endodermis, in Pinus sylvestris, has suberised radial walls, suberised unpitted traverse walls, and partially and discontinuously suberised inner tangential walls. These features effectively channel the flow of water from the stele through the inner tangential wall, via the protoplasm, and out through the outer tangential wall into the mesophyll. Hence one might expect that the protoplast of the endodermis would exert considerable control over the passage of water and would be susceptible to temperature changes.

(2) The mesophyll is most likely the main site of evaporation, during increasing transpiration as D_l increases (Figures 4.2.2 and 4.2.3).

(3) For the stomata to be sensitive to D_l, there must be an appreciable resistance to water flow, from the mesophyll to the guard cell vacuole. The low flow rate will then generate a sufficiently large drop in water potential in the stomatal complex for the turgor pressure of the guard cell to decrease sensitively in response to increasing transpiration rate, thereby causing stomatal closure.

(4) The hypodermis separates the mesophyll from the epidermis (Figures 4.2.1, 4.2.2 and 4.2.3). The guard cells are at the end of the pathway to water flow and the subsidiary cell separates them from the epidermal cells. The thick-walled hypodermis is the most likely site of a high hydraulic resistance to water flow to the guard cells.

(5) Raschke (1975) envisaged a negative feedback mechanism involving stomatal response to leaf water status and indirect effects of stomata on ψ through effects on transpiration. Such a negative feedback mechanism operates when, as transpiration increases in response to increasing D_l, g_s declines, and the mutual effect of one upon the other results eventually in the stabilisation of g_s at a low level, with concurrent stabilisation of transpiration at a high level spread over a considerable range of D_l. This is clearly seen to occur in Picea sitchensis (Watts & Neilson 1978). However, results in Figures 4.6.1 and 4.6.2 show that transpiration after reaching a maximum starts to decline, while g_s continues to decrease,

with seemingly complete independence of one from the other. A similar decline in g_s and transpiration with increasing D_l was observed in irrigated Prunus armeniaca (Schulze, Lange et al. 1972). These observations suggest that increasing transpiration rate from within the leaf cannot be the cause of the decrease in g_s through a negative feedback mechanism. Rather, they suggest a direct effect of increasing D_l on the guard cells.

(6) The epidermis in Pinus sylvestris had been shown to be overlaid by a thick cuticle and covered with epicuticular waxes. Examination under a light microscope shows that there is no cuticle lining the walls of the antechamber. The cutinized cell wall of the antechamber is also thinner compared to that of the outer epidermal wall (Walles, Nyman & Alden 1973). Cutin lines the entire wall of the antechamber but ends at the ledge of the stomatal slit (Chabot & Chabot 1977). Further, according to Esau (1965, 1977), there is a strip of wall lining the antechamber and adjoining the subsidiary cell, which is thin and non-lignified (see Area 1, Figure 5.4.1), compared to the surrounding wall. Chabot and Chabot (1977) observed the existence of such a thin wall in Abies balsamea. Although the wax tubes lining the wall of the antechamber increase the tortuosity of the diffusion pathway for water vapour, this strip of thin, non-lignified antechamber wall is still in direct communication with the ambient air. Because of its proximity to the ambient air one would expect there to be a steep humidity gradient.

(7) Additionally, there is a strip of thin wall, facing the stomatal slit which is non-lignified (Area 3, Figure 5.4.1). Of note

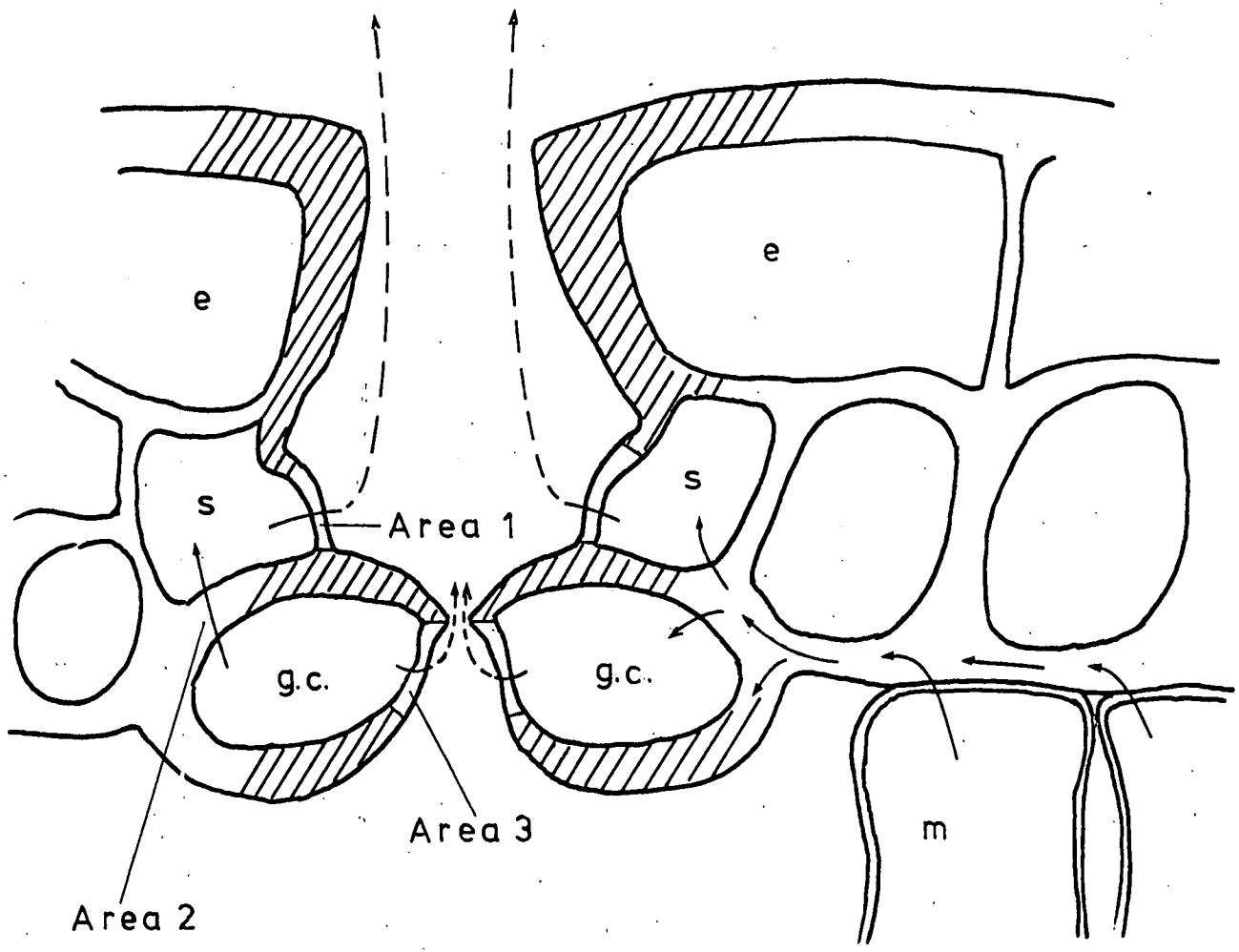


Figure 5.4.1 Shaded area is lignified cell wall and non-shaded area is non-lignified cell wall; e epidermal cell, s subsidiary cell and g.c. guard cell. Arrows show pathway of liquid flow ——— and vapour flow — — —. Area 1 and Area 3 are proposed site of evaporation; Area 2 and Area 3 are the 'hinge' areas. (Modified and reproduced from Esau, K. (1977) Anatomy of Seed Plant). See text for details.

too, is the non-lignified area in the junction zone between the subsidiary cell and guard cell (Area 2). These latter two areas termed 'hinges' by Haberlandt (1914) were also identified by Chabot and Chabot in electron micrographs. These areas are flexible and permit changes in the shape of the guard cell allowing the adjustments to be made that result in opening and closing movements of the stomatal slit (Florin 1931; Chabot & Chabot 1977).

The thickness of the cell wall and its chemical composition determine the cell wall permeability to water. Baig and Tranquillini (1976) showed that cuticular transpiration is a function of cuticle thickness. The strip of thin, non-lignified wall, adjacent to the subsidiary cell and lining the antechamber wall (Area 1, Figure 5.4.1) can therefore be a site of relatively high cuticular transpiration. The area, marked as Area 3 could also be a site for peristomatal transpiration. A possible pathway of water loss is, therefore, from guard cell, through the 'hinge' area marked as Area 2, across the subsidiary cell and out through the thin wall into the antechamber. Pathway 2, via 'hinge' Area 3 is also a possible route.

The hypothesis

Figure 5.4.2 shows the operation of this hypothesis. When g_s is high, the main pathway of transpiratory flux is mesophyll - intercellular spaces - stomatal pore - antechamber, with a smaller flux from the walls of the subsidiary cells and guard cells. As g_s decreases, the pathway from the subsidiary cell to the antechamber becomes increasingly important, and continues to operate even when

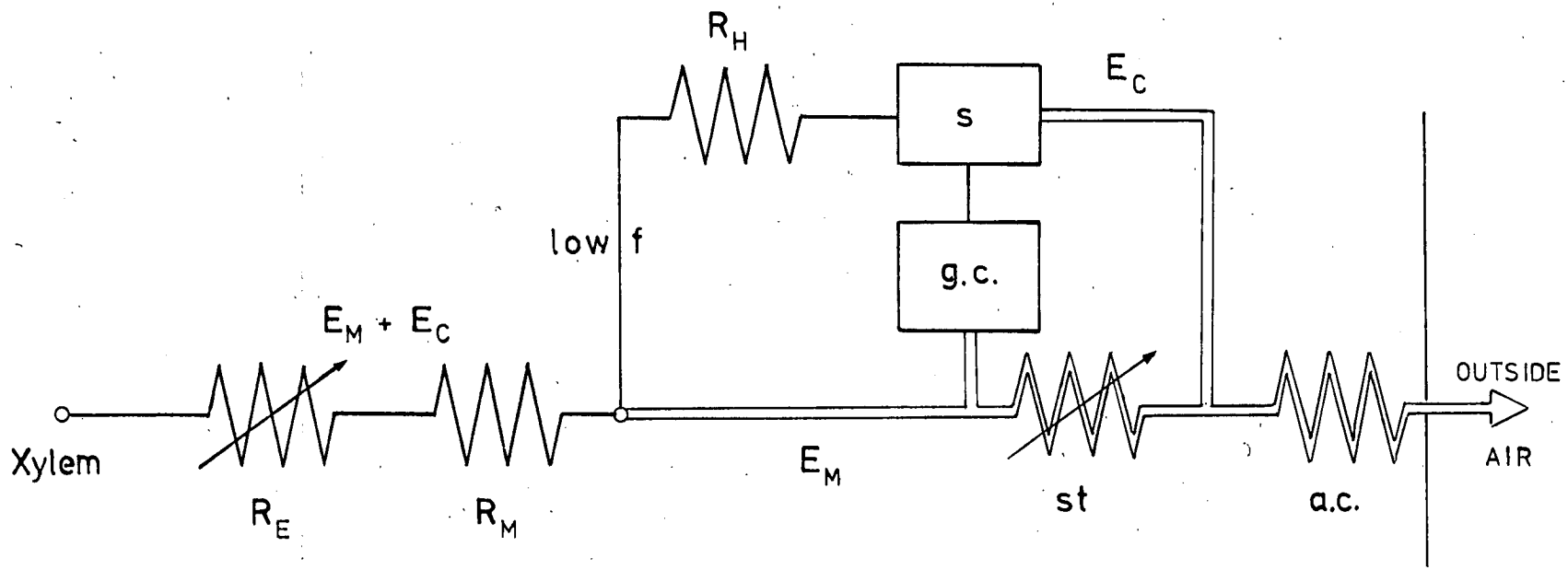


Figure 5.4.2 A scheme showing liquid flow —— and vapour flow ═══ in the proposed hypothesis. Liquid flow resistance in hypodermis, R_H, in endodermis, R_E, and in mesophyll, R_M. s subsidiary cell, g.c. guard cell, st stomatal conductance, a.c. antechamber resistance, ↗ variable resistances, f flow; transpiratory flux from mesophyll, E_M and from subsidiary cell, E_c.

the stomata are completely closed. However, because of the small area of moist wall involved, and the presence of a diffusion resistance attributable to the wax tubes in the antechamber, the flux of water vapour is relatively low and consequently the drop in water potential along this pathway would also be small unless R_H is large.

Since bulk leaf water potential was found to change very little over a wide range of D_l (Figure 4.6.3), at least at 23°C, the liquid flow resistances in the endodermis, R_E, and mesophyll, R_M, must be small or else variable and inversely proportional to transpiration rate, as proposed by Black (1979a and b). The liquid flow resistance R_H, on the other hand, might be expected to be large so that changes in the flow rate, f, as a result of changes in D_l, and hence in E_c, would result in appreciable changes in water potential in the vicinity of the subsidiary cells and guard cells, and in the cells themselves.

The observed response to D_l might then occur in the following way:

As D_l increases, both E_M and E_C increase. Because R_H is supposed large, the water potential in the pathway in the vicinity of the subsidiary and guard cells will fall with a consequent fall in water potential, water content and turgor pressure of the guard cells. Thus the stomata close, resulting in a decrease in E_M but not in E_C. As D_l decreases, E_C also decreases and the process is reversed. The stomata open again and E_M increases. This hypothesis allows for continued closure of the stomata as D_l increases, even though the stomata have closed to the extent that further closure results in a falling transpiration rate (as predicted by Watts &

Neilson 1978).

The only point in the pathway between xylem and guard cell at which it is reasonably certain that water must pass through cell membranes and the protoplast is the endodermis. Hence it is possible that temperature might affect stomatal conductance through changing R_E. The following mode of action is possible.

When leaf temperature is decreased, R_E is increased, because of thermal effects on membrane permeability and the viscosity of water (Kuiper 1964). Sensitivity to D_1 therefore increases (Figure 4.6.1) because there will be a larger fall in water potential in the vicinity of the guard cells for unit increase in D_1 than at higher temperatures. Conversely when leaf temperature is increased, R_E is reduced and sensitivity to D_1 decreases because of a smaller change in potential per unit change in D_1 in the vicinity of the guard cells.

Processes within the guard cells themselves are also temperature dependent. Consequently, there are several other possible explanations for the action of temperature, and its interaction with D_1. For example, guard cell turgor probably depends upon the continuous active transport of K^+ and Cl^- into the guard cells from the surrounding cells (Penny & Bowling 1974; Penny, Kelday & Bowling 1976). At low temperature, the active transport inwards is probably less rapid so that the steady state ion concentrations in the guard cells are lower and osmotic potentials higher. In this situation, guard cell water content and hence turgor and stomatal conductance, would be expected to be more sensitive to a reduction of water potential in the vicinity,

as a result of higher D_l, than at higher temperatures with higher rates of net ion influx.

This hypothesis, which explains the interaction between effects of temperature and D_l, has certain implications for the bulk leaf water potential. If a decrease in leaf temperature works in the way proposed, the mesophyll water potential must be lower at lower temperatures than at higher temperatures, because R_E would be larger. In addition, if R_E is appreciable at low temperatures, the mesophyll water potential would be expected to decline as transpiration increased, rather than to remain more or less constant.

A comparison of the results embodied in Figures 4.6.3 and 4.6.4, and redrawn in Figure 5.4.3, is useful at this juncture. It had already been shown that there is a declining ψ with increasing D_l at 15°C and a fluctuating but constant ψ, with increasing D_l at 23°C. At 15°C g_s is less sensitive to increasing D_l at D_l less than 0.7 kPa than at D_l greater than 0.7 kPa. Average value of ψ at D_l less than 0.7 kPa is higher at 15°C than at 23°C. This would be caused by plant variation since different groups of plants are involved at 15°C and at 23°C. A comparison of the mean ψ values of two groups of plants at different dates but subjected to similar conditions in the growth room showed that this difference does exist.

At D_l greater than 0.7 kPa, at 15°C, there is a sharp decline in g_s with increasing D_l and a concurrent decrease in ψ. Similar values at 23°C show that ψ remains more or less constant as g_s declines. Although no data are available at D_l greater than 1.0 kPa at 15°C, results of other experiments on increasing D_l indicates a

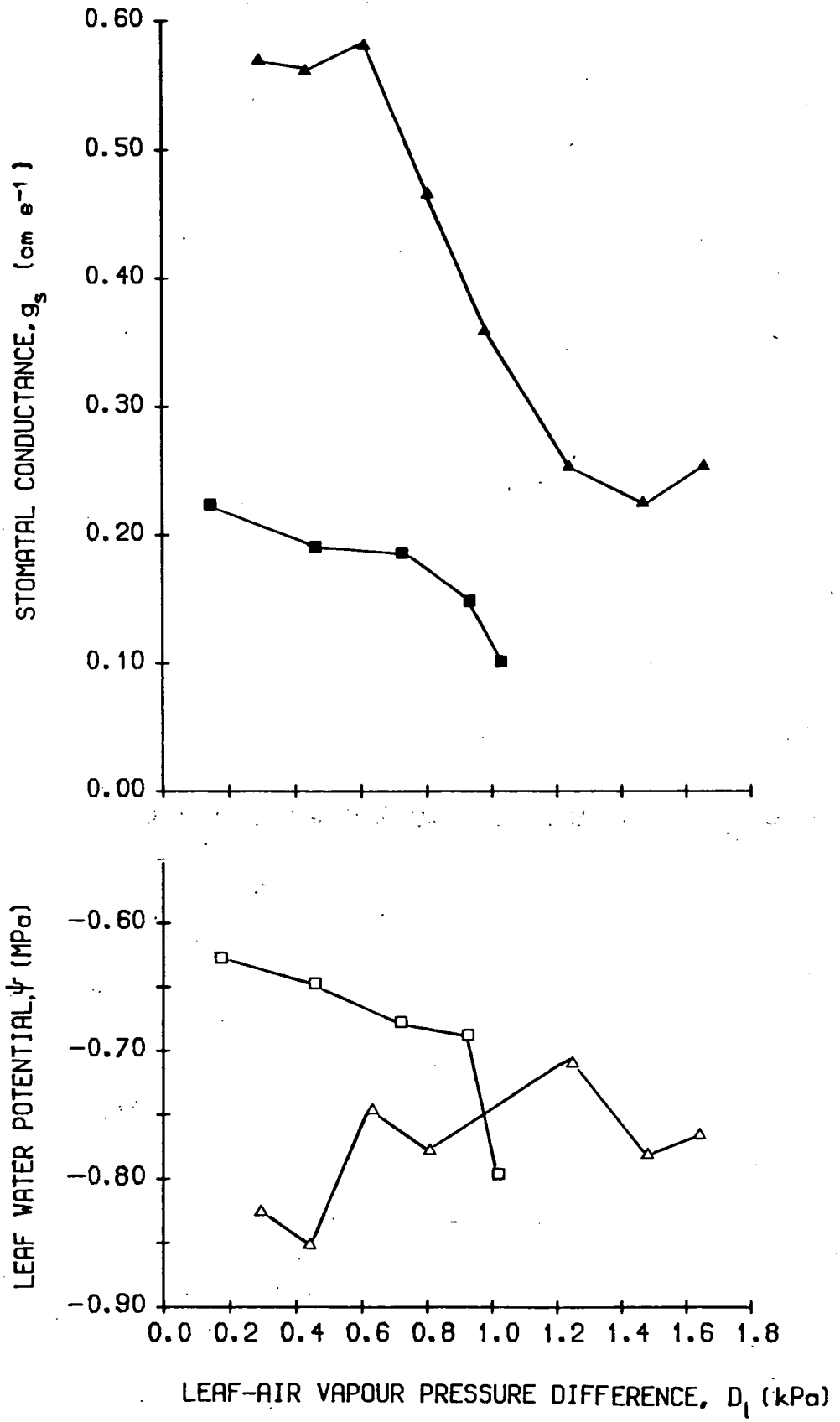


Figure 5.4.3 The response of stomatal conductance (▲, ■) and leaf water potential (△, □) to increasing leaf-air vapour pressure difference at leaf temperatures of 23.2°C (▲, △) and 15.0°C (■, □). Redrawn from Figures 4.6.3 and 4.6.4.

lower g_s, and Figure 5.4.3 shows a strong possibility of a concurrent further decrease in ψ. Hence, within the range of D_l where g_s is sensitive to increasing D_l, average value of ψ could be lower at 15°C than at 23°C, as well as declining with increasing D_l.

5.5 Hypothesis on the effect of leaf water potential on the stomatal sensitivity to leaf-air vapour pressure difference

Introduction

A Höfler diagram can be used to describe the changes in water potential, ψ, osmotic potential, π and pressure potential, P with changing volume of water or sap expressed. Although determined on leaf tissue, such relationships can probably be taken as applicable to the individual guard cells. The Höfler diagram then provides a means of describing the water relations of guard cells in relation to changes in stomatal conductance.

The Höfler diagram determined by Hellkvist, Richards and Jarvis (1974) on *Picea sitchensis* can probably be regarded as applicable to the leaf tissue of *Pinus sylvestris*, and to the guard cells. Although Raschke (1976) found a convex turgor pressure curve for the guard cells in *Vicia faba*, a concave turgor pressure curve, generally found in leaf tissue of most plants, as well as in *Picea sitchensis* (Hellkvist, Richards & Jarvis 1974) is assumed for the guard cells of *Pinus sylvestris*. A concave turgor pressure curve was also implied for *Ginkgo biloba* and *Salix lasiandra* (Tyree 1976).

The assumptions

The Höfler diagrams given in Figures 5.5.1a and 5.5.1c can be used to provide an explanation for the lack of sensitivity to increasing D_1 at $\psi = -0.2$ MPa and for the normal sensitivity to increasing D_1 at $\psi = -0.7$ MPa (see Figure 4.6.4). The following assumptions are made:

(1) Stomatal conductance is proportional to the value of the turgor pressure difference between the guard cells and adjacent subsidiary cells, TPD.

(2) The relation between the turgor pressure difference, (TPD) and the guard cell volume of water is as given in Figure 5.5.1b or 5.5.1d. Full stomatal closure is assumed to occur at zero TPD. Results from Meidner and Edwards (1975) support the assumptions in this figure. They recorded a higher turgor pressure in the subsidiary cells and guard cells for the same stomatal aperture when epidermal strips were mounted in water than when mounted in liquid paraffin. Their data also suggest that at large stomatal aperture it is possible to have large increases in guard cell turgor pressure for little or no change in stomatal aperture.

(3) Guard cell water potential is loosely related to bulk leaf water potential. That is to say, if the bulk leaf water potential is low, guard cell water potential will also be low, although by no means the same, and vice versa. This is implicit in the water flux model given in Figure 5.4.2. The water potential of the guard cells depend upon the mesophyll water potential as well as on the flow of water to the sites of transpiration near the guard cells.

Figure 5.5.1a Two superimposed Höfler diagrams for open and closed stomata when the bulk tissue water potential is ca -0.7 MPa (see Figure 4.6.4 for stomatal response). Curves ψ_o, π_o, P_o characterise the Höfler diagram for maximum stomatal opening, and curves ψ_c, π_c and P_c characterise the Höfler diagram for full stomatal closure. Maximum stomatal conductance $g_{s,o}$ is attained at guard cell volume of water V_o, and the minimum stomatal conductance at full stomatal closure $g_{s,c}$, is attained at guard cell volume of water V_c. Note that $\psi_{o,l}$ and $\psi_{c,l}$ are the water potentials at full opening and full closure, respectively.

Figure 5.5.1b The assumed relation between turgor pressure difference (TPD) between the guard cells and adjacent subsidiary cells, and the guard cell volume of water.

Figure 5.5.1a

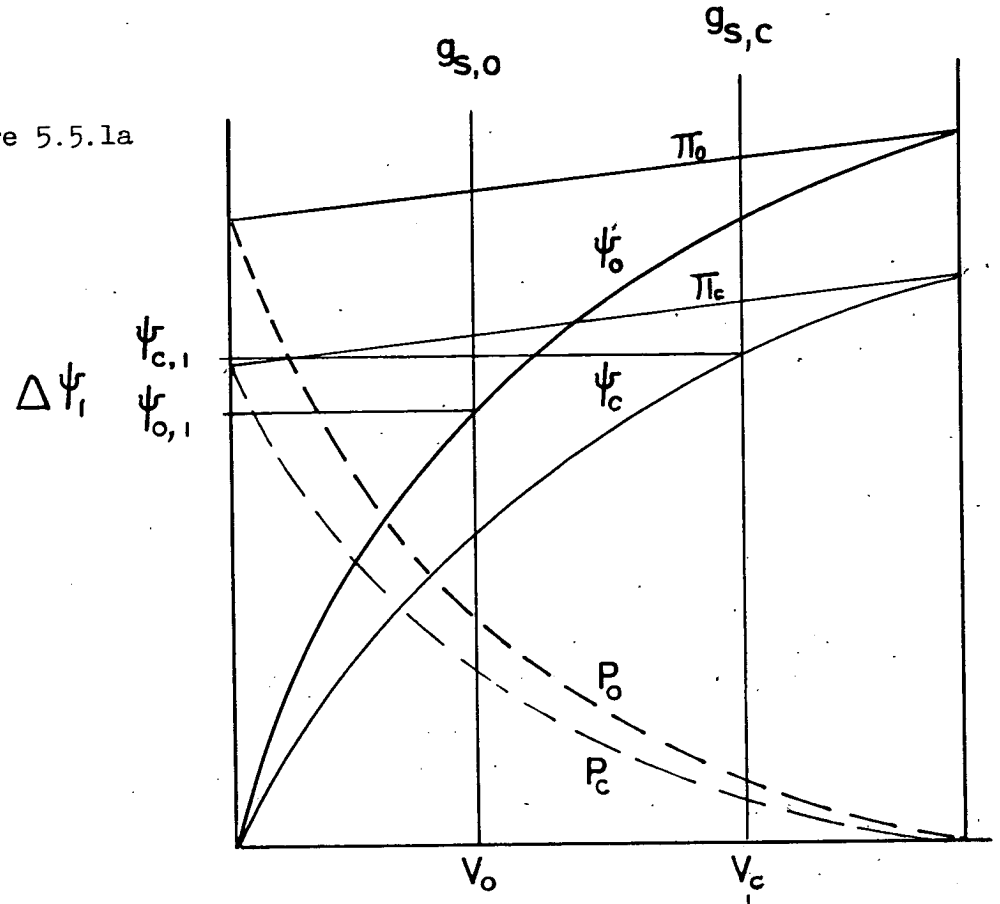


Figure 5.5.1b

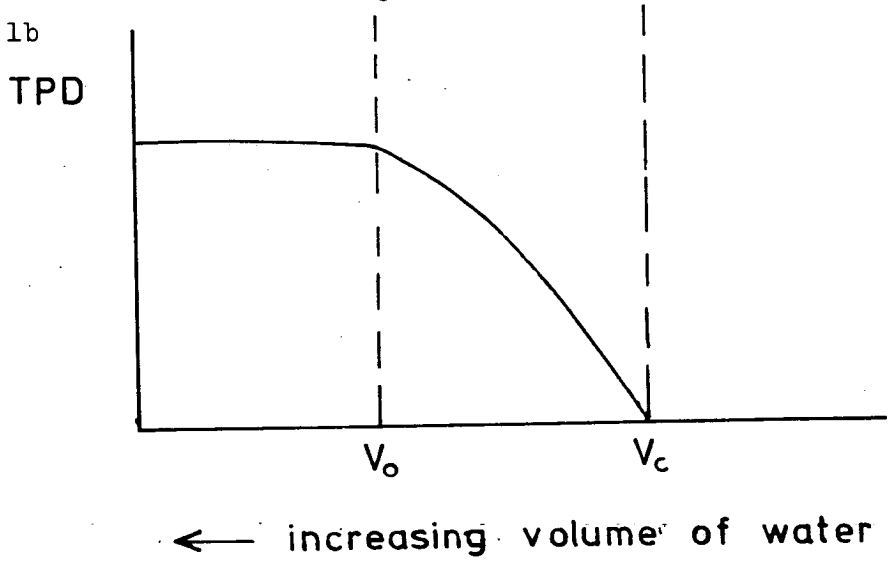


Figure 5.5.1c Two superimposed Höfler diagrams for open and closed stomata, when the bulk tissue water potential is ca -0.2 MPa (see Figure 4.6.4 for stomatal response). Similar details are as in (a). The shift of guard cell volume of water required for maximum stomatal opening towards the left is brought about by the general increase in water content and hence in turgor pressures. Note that $\psi_{O,2}$ and $\psi_{C,2}$ are the water potentials at full opening and full closure, respectively.

Figure 5.5.1d A similar relation between TPD and guard cell volume of water as in (b).

Figure 5.5.1c

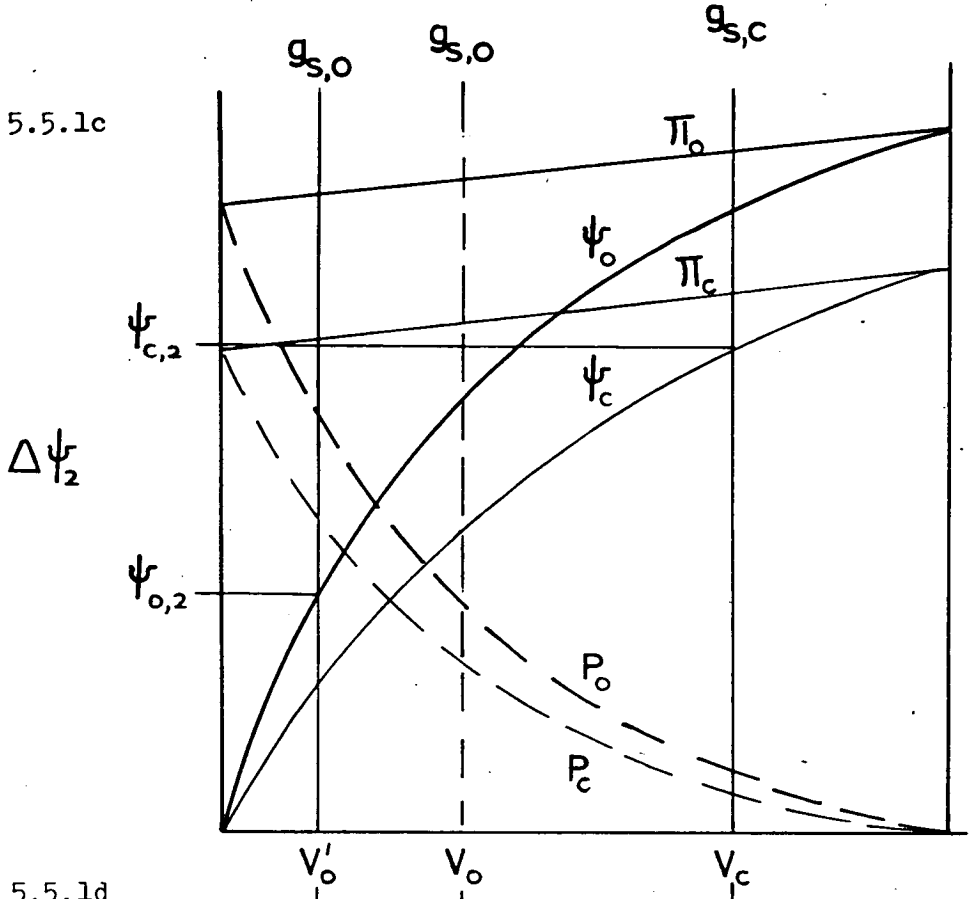
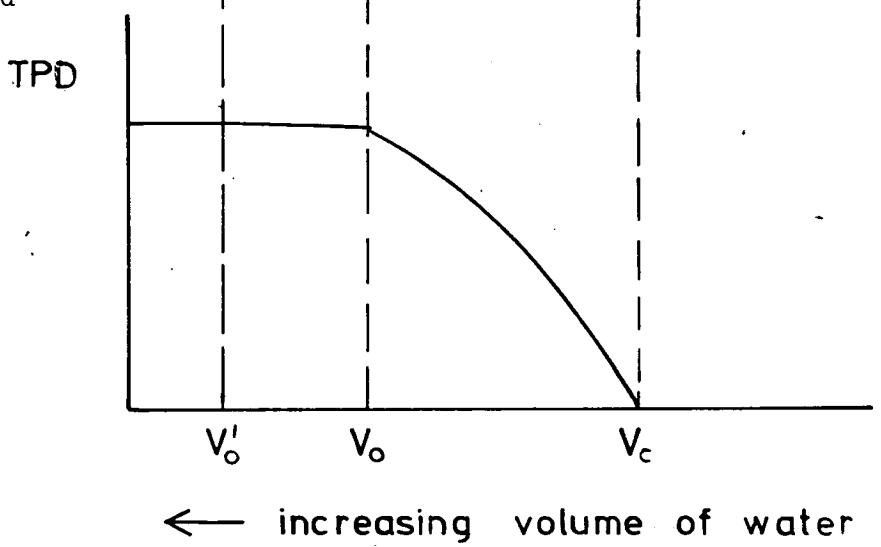


Figure 5.5.1d



(4) Maximum stomatal opening is seldom at maximum turgor pressure. It is also assumed that full stomatal closure does not occur at zero turgor, in view of the occurrence of the Spannungsphase.

(5) There is influx of K^+ during stomatal opening and efflux of K^+ during stomatal closure, and consequent changes in anion concentration. Therefore, two Höfler diagrams are superimposed to describe the changes in ψ, π and P with guard cell volume of water during maximum stomatal opening and full closure. Although there is no information on changes in ionic concentrations in the guard cells of Pinus sylvestris, K^+ transport during stomatal movement is most probably of universal occurrence (Hsiao 1976).

The hypothesis

Figure 5.5.1a describes the situation when leaf water potential is ca -0.7 MPa. The guard cell water potential at maximum stomatal opening is $\psi_{O,1}$ and the corresponding value for stomatal closure is $\psi_{C,1}$. The drop in guard cell water potential as closure occurs is therefore $\psi_{O,1} - \psi_{C,1} = \Delta\psi_1$.

Figure 5.5.1c describes the situation when leaf water potential is initially ca -0.2 MPa and the leaf tissue is much more turgid. As the water potential of the guard cells falls, the turgor pressure in the guard cells and subsidiary cells changes simultaneously so that the TPD and stomatal aperture remain unchanged until the guard cell volume of water has declined from V_o^1 to V_o. The drop in guard cell water potential as stomatal closure occurs is therefore $\psi_{O,2} - \psi_{C,2} = \Delta\psi_2$.

According to the model of peristomatal transpiration put forward earlier in Figures 5.4.1 and 5.4.2, the guard cell complex senses changes in D_1 as a result of the effect of D_1 on the water vapour flux from Areas 1 and 3. Because of the resistance R_H in the supply pathway, an increase in D_1, and hence in peristomatal transpiration, results in significant reduction in guard cell water potential. The reduction in water potential is likely to be proportional to the increase in D_1. Figures 5.5.1a and 5.5.1c show that the drop in guard cell water potential that is needed to cause full stomatal closure is likely to be much less when bulk tissue ψ is ca -0.7 MPa than when bulk tissue ψ is ca -0.2 MPa, i.e. $\Delta \psi_2 \gg \Delta \psi_1$. Consequently g_s would be expected to be much more sensitive to D_1 when ψ is ca -0.7 MPa than when ψ is ca -0.2 MPa. Furthermore, because of the threshold response in TPD to decreasing guard cell volume of water (Figure 5.5.1b) the stomata would be expected to remain open during the initial response in D_1 when bulk tissue ψ is high, in spite of the increase in transpiration in response to increasing D_1.

Thus this hypothesis in conjunction with the hypothesis of peristomatal transpiration adequately explains the lack of response to D_1 at high leaf water potentials, suggests an exponential response at moderate water potentials and suggests a more closely linear response at still lower water potentials.

5.6 Hypothesis on stomatal response to leaf water potential

While Figures 5.5.1a and 5.5.1b also suggest the explanation for the threshold response of g_s to water potential a more detailed

hypothesis is given in Figures 5.6.1a and 5.6.1b.

During water stress solute accumulation occurs in leaves (Yemm & Willis 1954; Weatherley 1965; Biscoe 1972; Jones & Turner 1978). It is assumed here that during the fairly rapid development of water stress, solute accumulation also occurs in the guard cells and subsidiary cells but is not initiated until some time has elapsed, perhaps an hour or so, to allow for ion movements and metabolic changes to occur.

In addition the assumptions (1) to (4) made on the relations between g_s, TPD, guard cell volume of water, guard cell water potential and bulk leaf water potential are made here.

The hypothesis

Consider the situation described in Figure 5.5.1c when bulk tissue ψ is -0.2 MPa. This is denoted in Figure 5.6.1a as A. At such a high bulk tissue water potential, solute accumulation is at a minimum. As drying progresses, guard cell ψ moves from $\psi_{0,2}$ to $\psi_{0,1}$ (i.e. from A to B) along the water potential curve, and the guard cell volume of water shifts towards the right from V_o. There is no change in stomatal aperture as TPD remains unchanged because it is assumed that there is a simultaneous decrease in turgor pressure in the guard cells and subsidiary cells. Further loss of water initiates solute accumulation and the osmotic potential moves progressively from π_1 to π_2, resulting in the guard cell water potential moving from B to C. There is still no change in stomatal aperture because of unchanged TPD brought about by a similar solute

Figure 5.6.1a A Höfler diagram describing the changes in guard cell osmotic potential, and guard cell water potential during stomatal closure in response to loss of water from the guard cells. $g_{s,o}$ and $g_{s,c}$ are the values of stomatal conductance at V_o and V_I, and V_c, respectively. A threshold type response is described here. As water stress occurs, guard cell water potential falls along the line, ABCDE. At E complete stomatal closure occurs. During recovery from stress, water potential rises along the line FLB. XYZ describes the relation between increasing turgor pressure and guard cell volume of water when osmotic potential is at π_1.

Figure 5.6.1b The assumed relation between turgor pressure difference (TPD) between guard cell and subsidiary cell, and guard cell volume of water.

Figure 5.6.1a

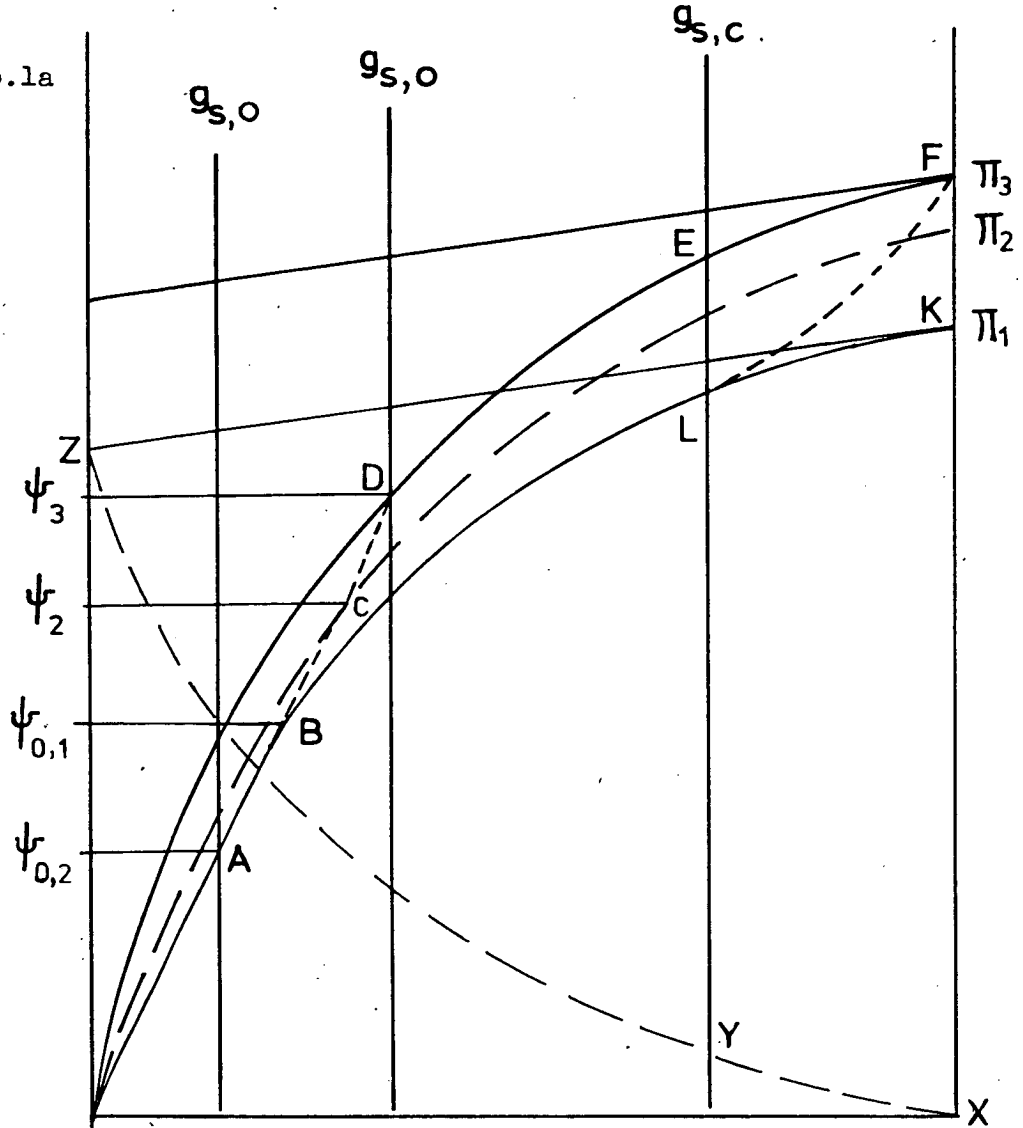
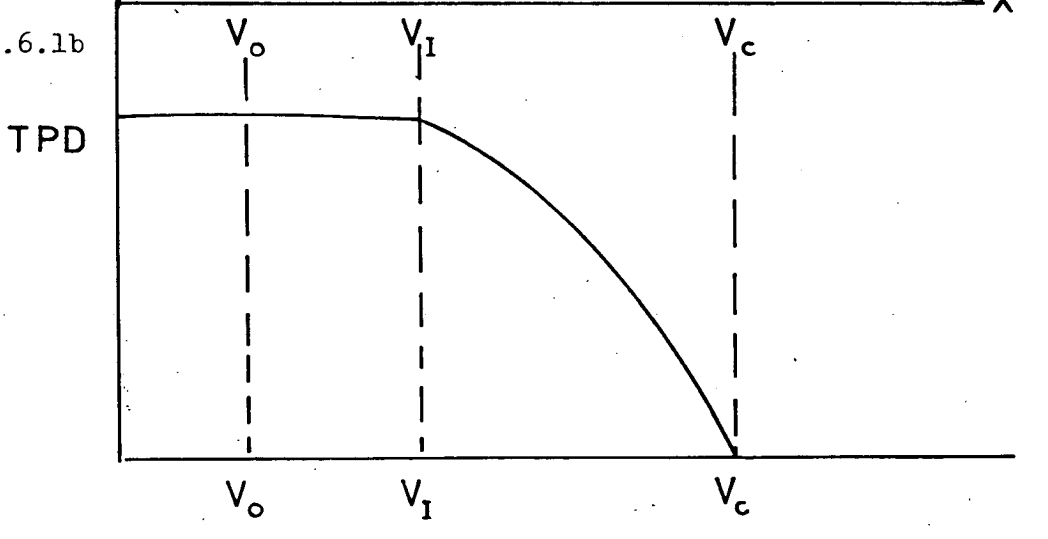


Figure 5.6.1b



accumulation in the subsidiary cells. As solute accumulation progresses, π_2 falls to π_3 and the water potential falls to D. However, there is a limit to possible solute accumulation. Beyond D no further solute accumulation can take place, and further loss of water causes the water potential to decline along the curve DE. Stomatal conductance progressively declines as TPD decreases and guard cell volume of water decreases to V_c, at which complete stomatal closure occurs. The threshold value of water potential is therefore at D, and its value is ψ_3. The value of ψ at D depends upon the base level of solutes in the cells and the extent to which osmotic adaptation occurs (e.g. Beadle, Turner & Jarvis 1978). Thus solute accumulation, or osmotic adaptation, results in a lowering of the value of the water potential at which stomatal closure begins.

A further decline in guard cell ψ from E to F results in the stomata becoming tightly closed. The often described phenomenon of recovering after stress of bulk tissue ψ to normal levels with no concurrent recovery of stomatal opening for some days (Stalfelt 1955; Glover 1959; Hsiao 1973) might be explained by a simultaneous requirement for an efflux of the previously accumulated solutes to the level existing before stress (Stalfelt 1963), as well as a requirement for a build up of turgor pressure to an appropriate level before opening can occur. Thus, recovery would involve changes in solute and water potential from F to L and an increase in turgor potential from X to Y before opening can occur, followed by stomatal opening as ψ returns to A along the line LBA. The change in ψ from F to L during rehydration, without consequent

stomatal opening, might be considered a partial explanation of the Spannungsphase.

This model then, accounts for the general shape of the response of g_s to bulk leaf water potential and includes, as well, a possible explanation of the significance of osmotic adaptation.

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