

DYNAMIC CHARACTERISTICS OF STOMATAL
BEHAVIOUR IN COTTON

The work presented in this thesis is by me.
Specific contributions by others are referred to in
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STATEMENT

The work presented in this thesis is my own.
Specific contributions by others are referred to in
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SUMMARY

A quantitative analysis of stomatal behaviour is described, the basis of the analysis being a model of the control of stomatal aperture which incorporates interacting hydraulic and metabolic feedback loops. Transfer functions are used to describe the response of stomatal conductance to step changes in various environmental parameters, in particular, ambient humidity, external CO_2 concentration, light intensity and potential of water surrounding the roots. As an example, the properties of the apparent hydraulic transfer function, G_E^* , obtained from the response of stomatal conductance to change in ambient humidity, are discussed in detail. From G_E^* , the effect of changes on the hydraulic environmental gain, $\partial E / \partial g_\ell$, can be calculated, in addition to the frequency response of the stomatal mechanism. The possible influence of a direct effect of ambient humidity on the stomatal aperture is also considered.

Using a general equation describing the response in stomatal conductance to a change in any environmental parameter, expressions for the experimentally-determined transfer functions are derived and certain fundamental transfer functions, G_E , G_{C_i} , G_I and G_ψ are evaluated. These transfer functions describe the stomatal response to changes in rate of evaporation, intercellular CO_2 concentration, light intensity and base water potential that would occur if stomatal aperture had no effect on the rate of evaporation and the rate of assimilation, ie. if

there were no feedback of the response to these perturbations on the stomatal apparatus. Comparison of these transfer functions allows some speculation about the time constants involved in the mechanics of the stomatal movement.

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CHAPTER 1

INTRODUCTION

Gas exchange in plants is largely controlled by regulation of the stomatal aperture through the changing water content in the guard and subsidiary cells of the stomatal apparatus (e.g. Troughton, 1969). The water content of these epidermal cells is affected by their internal metabolism (insofar as this determines osmotic pressure) and by the water status in the leaf as a whole. The former responds to changes in light intensity (e.g. Ketellapper, 1963), CO_2 concentration (e.g. Raschke, 1972), temperature (e.g. Meidner and Mansfield, 1968) and hormones synthesised in other parts of the plant while the latter is influenced by changes in ambient humidity, potential of water surrounding the roots and root temperature (Troughton, 1969). There is much interest in the mechanism of these effects, both at the cellular and at the plant level.

The aim of this study is to quantitatively describe the observed behaviour of stomata in terms of environmental parameters, in particular, ambient humidity, external CO_2 concentration, light intensity and water potential around the roots. While examination of steady state responses provides useful information about stomatal behaviour, more insight into the physiological mechanisms involved is likely to be obtained by comparing transient responses with the behaviour of models

of the internal functioning of plants in similar situations. Commonly used input signals in transient response analysis are those of step functions, ramp functions, impulse functions and sinusoidal functions. With these input signals, mathematical and experimental analyses can be carried out easily because the signals are simple functions of time. In this study, responses of stomata to step changes in environmental parameters are described in terms of transfer functions. Properties of the transfer function and its uses in predicting the response of stomata to various forms of input function are discussed. If a sinusoidal function is used as the input signal, the frequency response of the stomata is obtained. Farquhar (1973) carried out experiments in which the ambient humidity was varied sinusoidally and he plotted the responses in amplitude and phase angle of the resultant sinusoid in stomatal conductance as a function of angular frequency (Bode diagram). Here, frequency responses calculated from the transfer functions are compared with this experimentally determined frequency response, and will be shown to be in a fair measure of agreement.

The step function is used as an input signal in preference to a sinusoidal function because it enables the experiments to be performed much more quickly, while, in principle, the same information is obtained. It is important that experiments with biological material are performed quickly to minimize the effects of changes in internal plant characteristics resulting from ageing. Brogårdh and Johnsson (1974) have gone some way toward

minimising these effects, by using a swept-frequency technique to examine the effect of sine-shaped light cycles on the rate of evaporation of *Avena* plants. In this method, the frequency of the input signal is continuously varied over a range of frequencies and correlation techniques are used to compare the output waveform with those generated by models. As with the frequency response method, the response of amplitude and phase angle define characteristics of the behaviour. By increasing the time over which the frequency response is made, or by decreasing the range of frequencies used in the sweep, the accuracy of the method may be increased. This method enables the frequency response to be determined more rapidly than in the direct method used by Farquhar (1973) because it is not necessary to wait for a steady state to be reached before the frequency of the input signal can be changed. However, the time taken for the experiment is determined by the frequency range of the input signal and by the accuracy required. In investigations of stomatal behaviour, periods of up to 100 min. are of interest, so experiments using the swept-frequency method may still take many hours.

The transient response of stomata to changes in environment can be analysed without reference to the actual control mechanism involved. Such an analysis becomes a useful research technique when the characteristics of the transfer function are compared with those derived from models of the physiology of the stomatal mechanism. A number of models of

stomatal behaviour have been presented. Those pertaining to water relations of the stomatal mechanism have one characteristic in common: they postulate control of stomatal aperture through the influence of the water potential in the leaf as a whole on the state of water in the epidermal cells, e.g. Lang *et al.* (1969), Raschke (1970), Hopmans (1971), Cowan (1972). There are two currently increasing bodies of evidence indicating that this is an oversimplified view. Firstly, work of Lange *et al.* (1971), Schulze *et al.* (1972) and Camacho-B *et al.* (1974) has shown that stomata can respond directly to change in ambient humidity, rather than solely through change in the rate of evaporation. Secondly, it has been demonstrated, for example by Kriedemann *et al.* (1972), that hormones such as abscisic acid, the concentration of which is affected by water stress in plants (e.g. Loveys and Kriedemann, 1973), cause stomatal closure when applied externally to leaves. In this analysis, it is assumed that the stomatal reaction to change in humidity is a feedback mechanism related to change in leaf water potential. Thus, the response of stomatal conductance to change in rate of evaporation is taken to be of primary physiological interest. There are two reasons for having made this assumption. Firstly, it is the traditionally accepted view. Unequivocal evidence for the direct response of stomata to change in ambient humidity has only recently been obtained. Secondly, the evidence that now exists relates to a limited number of species only: there is no direct evidence to show that ambient humidity has an immediate effect on the stomatal mechanism in cotton. Nevertheless, in the discussion of the

results to be presented, it will become apparent that this form of control of the stomatal aperture may have had some influence on the responses measured in stomatal conductance. If this were so, the characterisation of what will be termed the 'hydraulic transfer function' will have been inadequate. On the other hand, the presence of hormonal interactions between the state of water in the plant and stomatal behaviour, though not yet taken into account in detailed models of the stomatal mechanism, can be incorporated into the analysis that has been used by regarding the interactions as an element of the hydraulic feedback control of the stomatal aperture.

When stomata respond to changes in humidity in the external environment, changes in the CO_2 concentration inside the substomatal cavity take place and thus the 'hydraulic response' of the stomatal mechanism is modified by a metabolic response. Changes in both leaf water potential and internal CO_2 concentration may be expected to play a role in stomatal movement due to any cause whatsoever. Sometimes the stomatal mechanism becomes unstable and oscillates continuously in a constant environment (Hopmans, 1971, Cowan, 1972). Working with maize, Apel (1967) and Raschke (1967) have associated rapid, small amplitude oscillations of stomatal functioning with sensitivity to CO_2 and slower, large amplitude oscillations with sensitivity to leaf water potential. Barrs (1971) has reviewed observations of oscillatory behaviour on the basis of this distinction. The model of Raschke (1970) combines the effects of both leaf water

potential and internal CO_2 concentration; that of Cowan (1972), while basically concerned with the hydraulic response of the stomatal mechanism, includes the effect of metabolically activated processes on the osmotic potential in the guard cells and thus the effects of internal CO_2 concentration could readily be incorporated. Farquhar (1973) investigated the frequency response of stomata to external CO_2 concentration while keeping the rate of evaporation constant, thus removing the effect of changes in leaf water potential. Cowan (1972) suggested that hydraulic control of stomatal behaviour is designed to speed up the reaction of stomata to changes which affect guard cell metabolism. Such behaviour would tend to optimise the rate of assimilation of the plant.

It is perhaps necessary to comment on the meaning of the term instability as it will be used throughout the following discussion. In mathematical terms, a linear system exhibiting oscillations which increase in amplitude is unstable. However, in a real system, non-linearities always intervene in such a way as to limit the amplitude of such oscillations to a finite value. Thus, a system may be unstable in the linear mathematical sense but become stable in the physical sense. For example, oscillations in stomatal conductance are bounded by physical limitations in the stomatal aperture. In what follows, the terms stability and instability will be used in the mathematical connotation. Thus, self-sustained oscillations in stomatal conductance are characteristic of instability in the stomatal

mechanism; damped oscillations are indicative of a stable system.

MATERIALS AND METHODS

In this study, the formal theory of response in stomata to step change in ambient humidity is generalised to include changes in external CO_2 concentration and other environmental variables, and methods for identifying the characteristics of the several internal mechanisms involved are discussed.

Two controlled environment cabinets, were used in all experiments. Normal day/night conditions prevailed in one cabinet: the photoperiod was 12 hours at 63.3 W m^{-2} (255-850 nm), the day/night temperatures were 25°C/15°C respectively and the dewpoint in the cabinet was approximately 25°C. In the other cabinet, plants were grown under continuous light: the light intensity was 27.2 W m^{-2} , the temperature was kept constant at 25°C and the dewpoint was approximately 16°C. The reduced light intensity was necessary to prevent the leaves from turning yellow.

2.2 Comparison of plants grown under different conditions

Plants from each growth cabinet were compared by counting stomatal frequencies on the upper and lower surfaces of all leaves (Table 2.1) and by a preliminary observation of the responses of the stomata to various changes in light intensity, external CO_2 concentration and dewpoint. The stomatal frequencies were estimated by choosing three or four random areas on the surface of a leaf and averaging the number of stomata counted. This was done

CHAPTER 2

MATERIALS AND METHODS

2.1 Growing conditions

Cotton plants, *Gossypium hirsutum* L. (var. Deltapine smooth leaf), grown in modified Hoagland's solution in two controlled environment cabinets, were used in all experiments. Normal day/night conditions prevailed in one cabinet: the photoperiod was 12 hours at 63.3 Wm^{-2} (355-850 nm), the day/night temperatures were 25C/15C respectively and the dewpoint in the cabinet was approximately 20C. In the other cabinet, plants were grown under continuous light: the light intensity was 27.2 Wm^{-2} , the temperature was kept constant at 25C and the dewpoint was approximately 16C. The reduced light intensity was necessary to prevent the leaves from turning yellow.

2.2 Comparison of plants grown under different conditions

Plants from each growth cabinet were compared by counting stomatal frequencies on the upper and lower surfaces of all leaves (Table 2.1) and by a preliminary observation of the responses of the stomata to various changes in light intensity, external CO_2 concentration and dewpoint. The stomatal frequencies were estimated by choosing sites at random on one surface of a leaf and averaging the number of stomata counted. This was done

for both sides of each leaf on the plants. Although the ages of the two plants differed, it appears that the light conditions during growth had little effect on the numbers of stomata. More obvious effects are on the shape of the plant and on its growth rate. A comparison of two plants of the same age is shown in Table 2.2: the plant grown in continuous light had a leaf area 88% larger than the plant grown in day/night conditions and the first internodal distance was nearly four times greater.

Table 2.1. - Number of stomata per mm² leaf surface

<u>Plant with normal photoperiod</u>			<u>Plant in continuous light</u>		
(33 days old)			(19 days old)		
<u>Leaf</u>	<u>Surface</u>		<u>Leaf</u>	<u>Surface</u>	
	<u>Upper</u>	<u>Lower</u>		<u>Upper</u>	<u>Lower</u>
1	40.8	100.9	1	38.1	95.5
2	77.0	151.2	2	110.6	219.9
3	150.8	291.9			
4	186.2	304.0			
5	0	44.7			

Table 2.2

<u>Plant with normal photoperiod</u>			<u>Plant in continuous light</u>		
(26 days old)			(26 days old)		
<u>Leaf</u>	<u>Area</u>	<u>Petiole length</u>	<u>Leaf</u>	<u>Area</u>	<u>Petiole length</u>
	(cm ²)	(cm)		(cm ²)	(cm)
1	41.0	3.0	1	54.0	4.6
2	30.0	1.5	2	68.5	3.4
3	2.0	0.3	3	8.0	0.7
4			4	7.0	0.6
Internodal distance (measured between cotyledons and 1 st petiole)					
1.5 cm			5.5 cm		

When placed in constant environmental conditions in the plant chamber, plants grown under normal 12 hour photoperiod showed a strong diurnal rhythm in the measured rate of evaporation. Associated with this behaviour is a variation in the occurrence of oscillations in stomatal conductance: there is a greater tendency for the oscillations to occur during the morning and late afternoon. Barrs and Klepper (1968) associated instability in stomatal behaviour in plants with a large root resistance to water transport. They showed that the variation in stomatal conductance and its stability was closely related to a 24 hour endogenous rhythm in root resistance. Although there was some variation in the measured level of stomatal conductance, plants grown in continuous light did not show the same strong diurnal rhythm as those grown with a normal photoperiod. There was, in

addition, some evidence that these plants were more stable than plants grown in day/night conditions, i.e. they could be subjected to a greater vapour pressure difference across the leaf epidermis before oscillations in stomatal conductance were induced. In all other respects, the plants grown in continuous light showed the same qualitative behaviour, i.e. they responded in a similar manner to changes in humidity, external CO_2 concentration and light intensity.

Plants grown in continuous light were used in experiments to determine dynamic characteristics of stomatal behaviour because of the absence of diurnal rhythm in stomatal conductance.

2.3 Description of the plant chamber

The plant chamber (Fig 2.1) used in all experiments has been described by Farquhar (1973). The upper part of a cotton plant was sealed in the working section of a small closed-circuit wind tunnel, the volume of which was about 35 litres. Windspeed in the working section was about 1 ms^{-1} . The dewpoint and CO_2 concentration in the chamber were measured by a dewpoint hygrometer and an infra-red gas analyser respectively. The outputs of these instruments were compared with set reference voltages and the error signals used to regulate the injection of dry air and CO_2 -enriched air through solenoid valves, so controlling the water vapour pressure and CO_2 concentration in the chamber. The valves were of an on-off type, and the control system was such

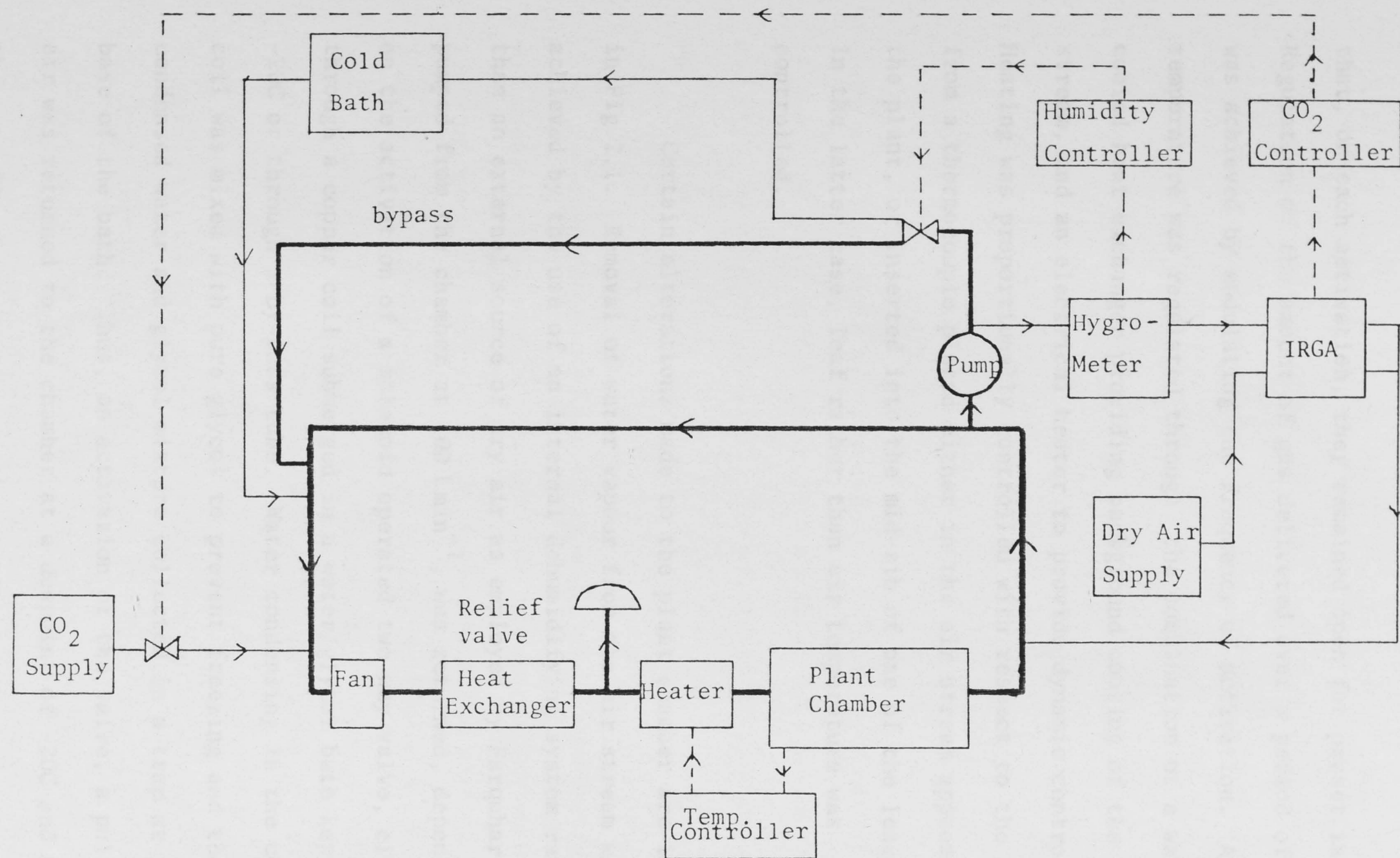


Fig.2.1. Diagram of plant chamber.

that, on each activation, they remained open for preset intervals. Regulation of the amount of gas delivered over a period of time was achieved by modulating the frequency of activation. Air temperature was regulated through the combination of a water-cooled heat exchanger providing background cooling of the air stream, and an electrical heater to provide dynamic control. Heating was proportionally controlled with respect to the signal from a thermocouple placed either in the air stream approaching the plant, or inserted into the mid-rib of one of the leaves. In the latter case, leaf rather than air temperature was controlled.

Certain alterations made to the plant chamber are indicated in Fig 2.1. Removal of water vapour from the air stream was achieved by the use of an internal dehumidifying system rather than an external source of dry air as employed by Farquhar. Air, pumped from the chamber at 100 l min^{-1} , was returned, depending on the activation of a solenoid operated two-way valve, either through a copper coil submerged in a water/glycol bath kept at -20°C or through a by-pass tube. Water condensing in the copper coil was mixed with pure glycol to prevent freezing and the condensed water and glycol mixture collected in a trap at the base of the bath. Thus, on activation of the valve, a pulse of air was returned to the chamber at a dewpoint of -20°C and with the same CO_2 concentration as the air in the chamber. The method had advantages over the previous one: the dewpoint of the dried air supply was constant and the CO_2 concentration in the chamber

was unaffected by the drying system. The volume of each pulse of dry air was determined by measuring the pulse frequency required to maintain a constant humidity in the chamber with vapour supplied using an external source of humid air rather than by evaporation from a plant. Then

$$w = \frac{u (\chi' - \chi_i)}{f (\chi - \chi_i)}$$

where w is the volume of each pulse, u is the flow rate of air of humidity χ' , f is the pulse rate of 'dry' air, humidity χ_i , and χ is the chamber humidity. The volume of air per pulse was 127.8 cm^3 .

Bottled compressed air of measured CO_2 concentration was used as reference gas for the IRGA and was exhausted into the chamber. A relief valve just upstream of the working section of the chamber maintained a constant pressure head in the chamber of a few centimeters of paraffin. Because of this slight excess in pressure, leakage of CO_2 and water vapour through imperfections in joints in the chamber assembly took place in the outward direction only; they were accounted for in calculations of the rate of loss of vapour and usage of CO_2 by the plant. The amount of CO_2 injected with each pulse of CO_2 -enriched air was determined in the following way. The frequency of pulsing was maintained constant and the air in the empty chamber was diluted with N_2 supplied at a measured

rate. The pulse frequency and the flow rate of N_2 were adjusted so that the CO_2 concentration in the chamber exactly matched the CO_2 concentration obtained by mixing N_2 and CO_2 with a set of Wosthoff pumps. Then,

$$w = \frac{u_0 p}{f}$$

where w is the volume of each pulse, u_0 is the flow rate of N_2 into the chamber, f is the pulse rate of the CO_2 valve and p is the mixing ratio of the Wosthoff pumps. The volume of gas per pulse was 0.62 cm^3 and, assuming the CO_2 -enriched air was 5% CO_2 , the mass of CO_2 per pulse was $57 \text{ } \mu\text{g}$.

All light intensities were measured, in the range 355-850 nm, with a spectroradiometer. Two light intensities were used during the experiments; the high light intensity was 84.8 Wm^{-2} and the low light intensity was 36.5 Wm^{-2} .

2.4 Calculation of evaporation rate and stomatal conductance

The rate of evaporation from the plant, and stomatal conductance were calculated continuously during the experiment with an analogue computer (Fig 2.2). A frequency meter measured the pulse rate of the dry air valve and its output voltage was used to calculate the rate of evaporation using the expression

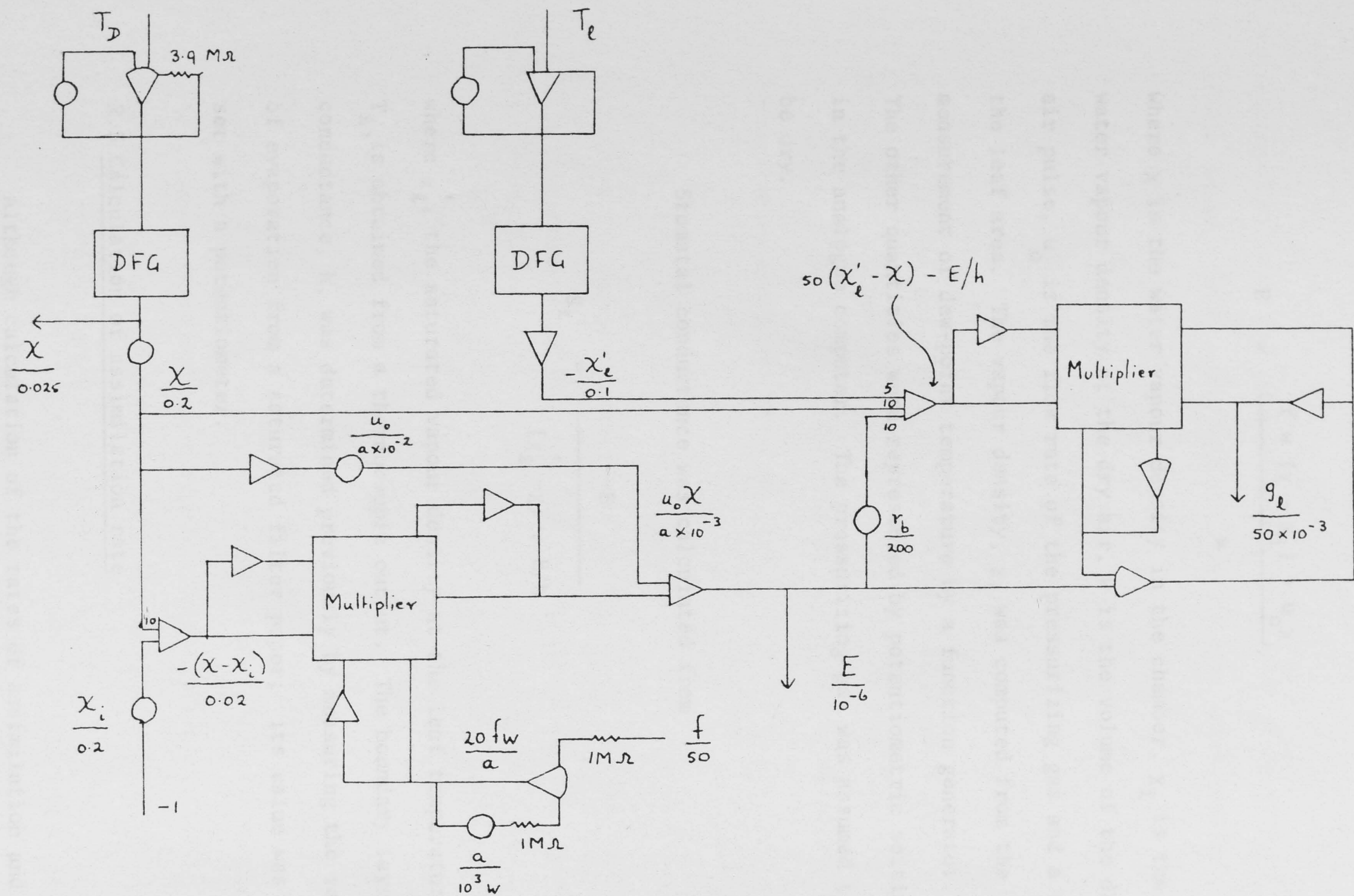


Fig.2.2. Analogue computer network for calculation of rate of evaporation, E , and stomatal conductance, g_l . (DFG - diode function generator)

$$E = \frac{f w (\chi - \chi_i) + u_o \chi}{a}$$

where χ is the water vapour density in the chamber, χ_i is the water vapour density of the dry air, w is the volume of the dry air pulse, u_o is the flow rate of the pressurizing gas and a is the leaf area. The vapour density, χ , was computed from the measurement of dew-point temperature by a function generator. The other quantities were represented by potentiometric settings in the analogue computer. The pressurizing gas was assumed to be dry.

Stomatal conductance was calculated from

$$g_\ell = \frac{E}{(\chi_\ell' - \chi) - E/h}$$

where χ_ℓ' , the saturated vapour density at the leaf temperature, T_ℓ , is obtained from a thermocouple output. The boundary layer conductance, h , was determined previously by measuring the rate of evaporation from a saturated filter paper; its value was set with a potentiometer.

2.5 Calculation of assimilation rate

Although calculation of the rates of assimilation and evaporation of the plant are similar, since both are derived from the pulse rate of a solenoid valve, the former was not

estimated directly due to an insufficient number of amplifiers in the analogue computer. Instead, the rate of assimilation was estimated from

$$A = \frac{f_c m + u_o (C_o - C)}{a}$$

where f_c is the pulse rate of the solenoid valve delivering a mass, m , of CO_2 per pulse to the chamber, u_o is the flow rate of pressurizing gas of CO_2 concentration, C_o , C is the chamber CO_2 concentration and a is the leaf area.

By assuming a compensation point, Γ , of 50 ppm for cotton, values of the mesophyll resistance to CO_2 , $r_{c,m}$, and the cell wall concentration of CO_2 , C_i , can be calculated from the relations

$$A = \frac{C - \Gamma}{r_{c,b} + r_{c,l} + r_{c,m}} = \frac{C_i - \Gamma}{r_{c,m}}$$

where $r_{c,b}$ and $r_{c,l}$ are the boundary layer and stomatal resistances to CO_2 respectively (taken to be 1.37 and 1.60 times their counterparts for water vapour transfer, respectively). Later, $r_{c,m}$ will be taken to have a more general definition.

2.6 Experimental Method

Plants, 3-5 weeks old, having between 4 and 6 leaves and a leaf area between 150 and 250 cm², were used for the experiments. Each plant was sealed into the working section of

the chamber as quickly as possible under low light intensity to minimise disturbances. Thermocouples were inserted into the main veins on 2 or 3 of the largest leaves to monitor leaf temperature, which was kept at 25C, and a 5 cm mesh wire net was used to prevent the leaves touching the roof of the chamber.

The plant was allowed to adjust to conditions in the chamber for at least 12 hours, before being subjected to perturbations, under one of the eight sets of conditions (Table 2.3). Two levels of humidity, CO₂ concentration and light intensity were used, designated high and low. Perturbations involved changing the level of one environmental parameter, the remaining two being held constant. During later experiments it was necessary to increase the humidity in the chamber to reduce the occurrence of self-sustained oscillations in stomatal conductance. Then, changes in humidity were made between 20C and 15C dewpoint. Increasing the humidity decreased the hydraulic environmental gain, $\partial E / \partial g_l$, a parameter shown by Farquhar and Cowan (1974) to affect plant stability. The importance of $\partial E / \partial g_l$ in this study will be shown later.

Table 2.3 - Environmental conditions imposed in experiments

	<u>Humidity</u>	<u>CO₂ concentration</u>	<u>Light intensity</u>
high	15C dewpoint	350 ppm	84.8 Wm ⁻²
low	10C dewpoint	250 ppm	36.5 Wm ⁻²

Additional experiments were performed with Dr.J.Passioura in which the hydrostatic pressure on the roots was changed between 0 and 4 bar. Compressed air was used to increase the pressure inside a pressure cell containing a cylinder of modified Hoagland's solution in which the plant roots were sealed. Pressures of more than 4 bar caused guttation from the hydathodes in the leaves, and through an extrafloral nectary on the main vein.

CHAPTER 3

ANALYSIS OF STOMATAL RESPONSES TO CHANGE IN HUMIDITY

3.1 The Laplace transform and the transfer function

In these experiments, the plant is regarded as a system a "black box" within the environment of the chamber. The purpose of the study was to relate to the particular perturbation in the chamber environment (the input) the stomatal behaviour of the plant (the output) in such a way as to provide fundamental information about the nature of the physiological mechanism involved. Laplace transformations were used to do this (Jaeger, 1966, Ogata, 1970).

The transfer function of the system is defined as the ratio of the Laplace transform of the output to the Laplace transform of the input, with the assumption that the initial conditions are zero and the system is linear (Ogata, 1970). Thus, if the stomatal conductance (g_s) is regarded as the output of the system and the rate of evaporation (E) as the input, the transfer function G_E^* is defined as

$$G_E^*(p) = \frac{\int_0^\infty e^{-pt} (g_\ell - g_{\ell,0}) dt}{\int_0^\infty e^{-pt} (E - E_0) dt}$$

where the integrals are the Laplace transforms of $(g_\ell - g_{\ell,0})$ and $(E - E_0)$. For convenience, G_E^* may also be expressed, using recognised symbols for the Laplace transform, as

$$G_E^* = \frac{\bar{\Delta}g_\ell}{\bar{\Delta}E} = \frac{L(g_\ell - g_{\ell,0})}{L(E - E_0)}$$

The Laplace operator p is a "weighting variable"; the greater p , the more important the short term or high frequency characteristics of both g_ℓ and E . When $p=0$, G_E^* represents the ratio of the steady state values of g_ℓ and E . The transfer function implicitly contains all the information about the dynamic characteristics of the response, g_ℓ , to change, E , in a way which is quite independent of the particular manner in which E was varied. It follows that, if the transfer function of the system is known, it is possible to calculate the variation in g_ℓ for any defined change in E . Another important property of transfer functions defined in this way, is that they can be added or multiplied to combine in various ways the properties of the interactive loops within the whole system. The transfer function describes the behaviour of the system in response to any defined input but does not, of course, provide any information about the physical structure of the system.

3.2 Calculation of the transfer function from experimental data

In determining the transfer function, two practical difficulties occurred. Firstly, the time constant of the frequency meter used to measure the rate of dehumidification of air and thus, the rate of evaporation, had to be taken into account. Secondly, during the short time interval in which the humidity in the chamber was in the process of being changed (approximately 2 min.), no measurement of the rate of evaporation or stomatal conductance could be made. For the purpose of calculations, the conductance was assumed to remain constant during this interval.

Fig. 3.1 shows, diagrammatically, the hydraulic system which is directly activated by change in ambient humidity and also its interaction with metabolic processes. The effect of stomatal conductance on the rate of evaporation is a physical interaction and is described by $\partial E / \partial g_\ell$, the hydraulic environmental gain. The transfer function $\bar{\Delta} g_\ell / \bar{\Delta} E$, which describes the effect of changes in the rate of evaporation from the plant on stomatal conductance, is the open-loop transfer function because it does not include the feedback effect of g_ℓ on E . However, it does include interactions with the metabolic loop through the stomatal conductance, and thus cannot be described as purely hydraulic. It will therefore be denoted as G_E^* , the apparent hydraulic

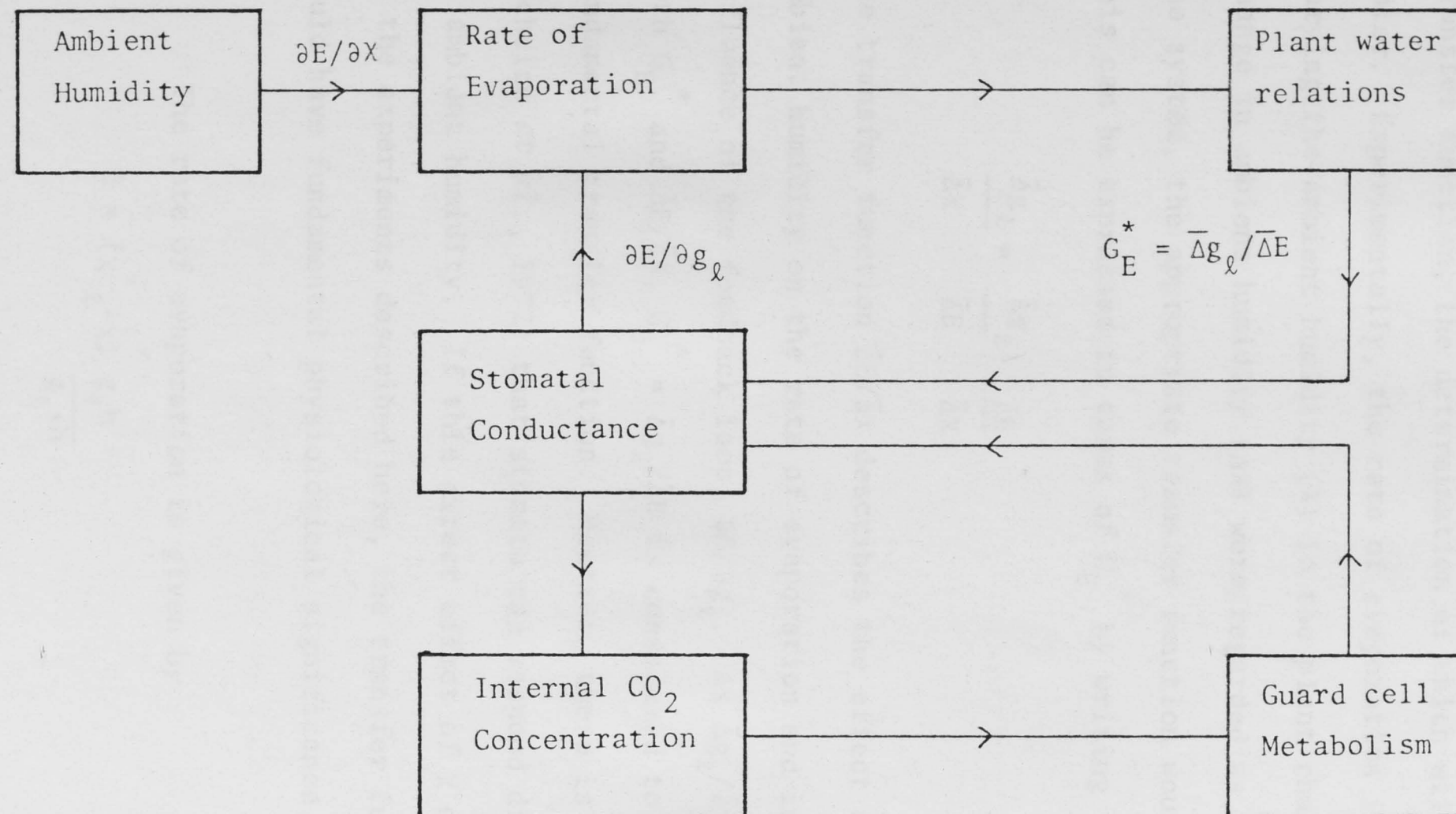


Fig.3.1. Block diagram of hydraulic system and interaction with metabolic processes.

transfer function, as distinct from G_E , the true hydraulic transfer function, the determination of which will be discussed later. Experimentally, the rate of evaporation is changed by varying the ambient humidity (χ) in the plant chamber. If the change in ambient humidity ($\Delta\chi$) were regarded as the input to the system, the appropriate transfer function would be $\bar{\Delta}g_\ell/\bar{\Delta}\chi$. This can be expressed in terms of G_E^* by writing

$$\frac{\bar{\Delta}g_\ell}{\bar{\Delta}\chi} = \frac{\bar{\Delta}g_\ell}{\bar{\Delta}E} \cdot \frac{\bar{\Delta}E}{\bar{\Delta}\chi}$$

The transfer function $\bar{\Delta}E/\bar{\Delta}\chi$ describes the effect of changes in ambient humidity on the rate of evaporation and includes the influence of the feedback loop, $\partial E/\partial g_\ell$. As $\bar{\Delta}g_\ell/\bar{\Delta}\chi$ incorporates both G_E^* and $\bar{\Delta}E/\bar{\Delta}\chi$, $G_E^* = \bar{\Delta}g_\ell/\bar{\Delta}E$ is considered to be the more fundamental transfer function. However, there is recent evidence (Schulze *et al.*, 1972) that stomata can respond directly to changes in ambient humidity. If this direct effect of χ on g_ℓ occurred in the experiments described here, the transfer function $\bar{\Delta}g_\ell/\bar{\Delta}\chi$ would have fundamental physiological significance.

The rate of evaporation is given by

$$E = (\chi'_\ell - \chi) \frac{g_\ell h}{g_\ell + h}$$

where χ'_ℓ is the saturated water vapour density at the leaf temperature and h is the boundary layer conductance. Similarly,

the initial rate of evaporation is

$$E_o = (\chi'_\ell - \chi_o) \frac{g_{\ell,o} h}{g_{\ell,o} + h}$$

where $g_{\ell,o}$ is the initial value of the stomatal conductance, g_ℓ .

Hence, the change in the rate of evaporation, ΔE , is

$$E - E_o = (\chi'_\ell - \chi) \frac{h^2 (g_\ell - g_{\ell,o})}{(g_\ell + h)(g_{\ell,o} + h)} - (\chi - \chi_o) \frac{g_{\ell,o} h}{g_{\ell,o} + h}$$

As $h \gg g_\ell$, $k = \frac{h^2}{(g_\ell + h)(g_{\ell,o} + h)}$ is approximately constant and

thus

$$\bar{\Delta E} = k L [(\chi'_\ell - \chi)(g_\ell - g_{\ell,o})] - L(\chi - \chi_o) \frac{g_{\ell,o} h}{g_{\ell,o} + h}$$

Provided the change in ambient humidity is rapid, the stomatal conductance, g_ℓ , can be assumed to be constant for the period of the change, τ . (Fig. 3.2). Then,

$$\bar{\Delta E} = k(\chi'_\ell - \chi_\infty) \bar{\Delta g}_\ell - \frac{g_{\ell,o} h}{g_{\ell,o} + h} \bar{\Delta \chi} \quad (1)$$

where χ_∞ is the steady value of χ after the time τ .

Thus,

$$\frac{\bar{\Delta g}_\ell}{\bar{\Delta E}} = \frac{1 + \frac{g_{\ell,o} h}{g_{\ell,o} + h} \frac{\bar{\Delta \chi}}{\bar{\Delta E}}}{k(\chi'_\ell - \chi_\infty)}$$

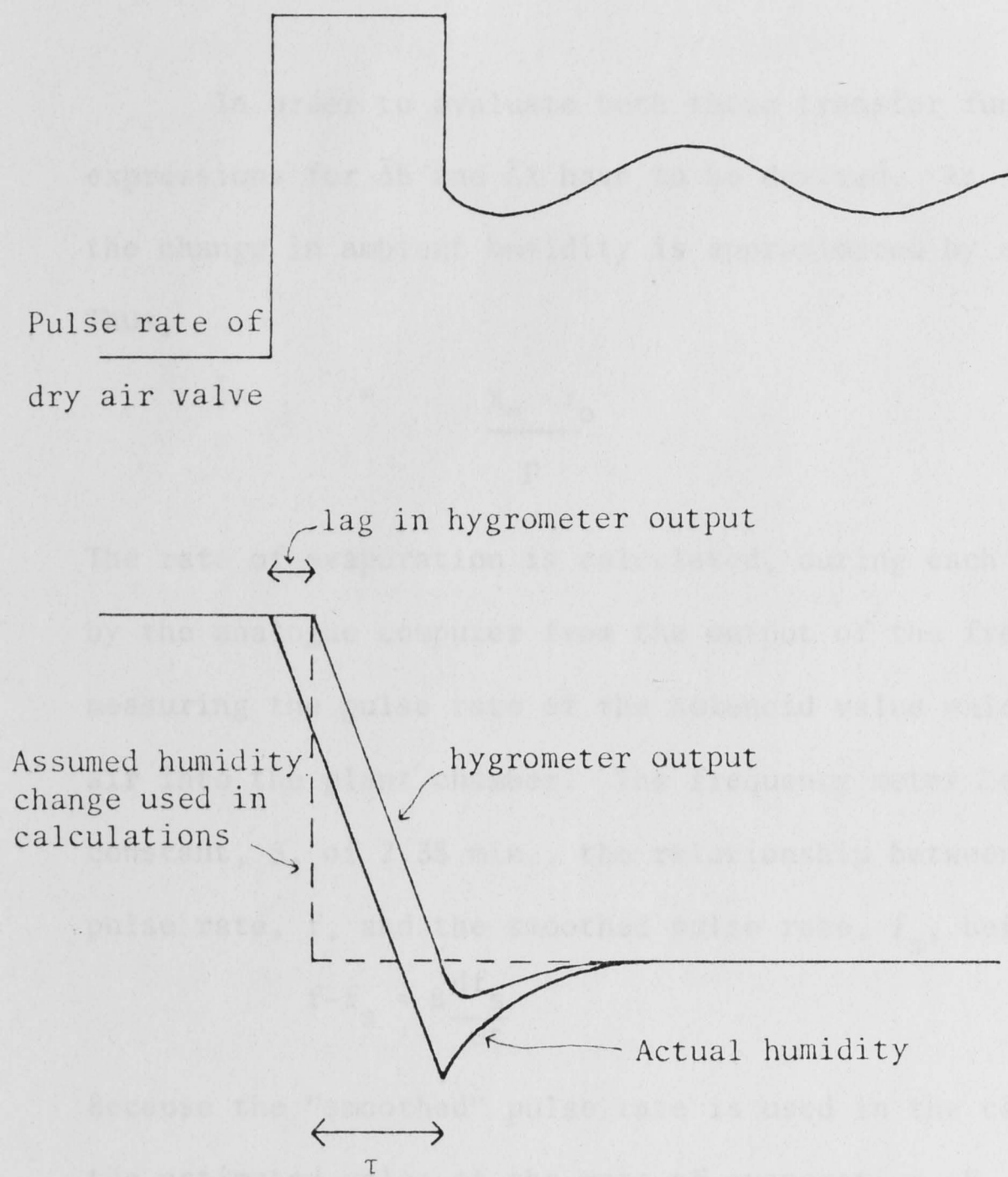


Fig.3.2. Time course of change in ambient humidity in the plant chamber.

and

$$\frac{\bar{\Delta}g_{\ell}}{\bar{\Delta}\chi} = \frac{\frac{\bar{\Delta}E}{\bar{\Delta}\chi} + \frac{g_{\ell,o}h}{g_{\ell,o}+h}}{k(\chi'_{\ell} - \chi_{\infty})}$$

In order to evaluate both these transfer functions, expressions for $\bar{\Delta}E$ and $\bar{\Delta}\chi$ have to be derived. As shown in Fig.3.2, the change in ambient humidity is approximated by a step change. Thus,

$$\bar{\Delta}\chi = \frac{\chi_{\infty} - \chi_o}{p}$$

The rate of evaporation is calculated, during each experiment, by the analogue computer from the output of the frequency meter measuring the pulse rate of the solenoid valve which injects dry air into the plant chamber. The frequency meter has a time constant, β , of 2.35 min., the relationship between the actual pulse rate, f , and the smoothed pulse rate, f_s , being given by

$$f - f_s = \beta \frac{df_s}{dt}$$

Because the "smoothed" pulse rate is used in the calculations, the estimated value of the rate of evaporation, E_s , will differ from the instantaneous value, E . The relation between the two is

$$E = E_s + \beta \frac{dE_s}{dt}$$

$$\text{or } (E - E_o) = (E_s - E_o) + \beta \frac{d}{dt} (E_s - E_o)$$

Therefore,

$$\int_{\tau}^{\infty} (E - E_0) e^{-pt} dt = (1 + \beta p) \int_{\tau}^{\infty} (E_s - E_0) e^{-pt} dt -$$

$$\beta (E_s(\tau) - E_0) e^{-p\tau}$$

Because the change in ambient humidity is rapid, the stomatal conductance has been taken to be constant for the interval of the change, τ . Thus, during that interval,

$$E - E_0 = \frac{\chi_0 - \chi_{\infty}}{\chi_{\ell} - \chi_0} E_0$$

$$\text{and } \int_0^{\tau} (E - E_0) e^{-pt} dt = \frac{\chi_0 - \chi_{\infty}}{\chi_{\ell} - \chi_0} \frac{E_0}{p} (1 - e^{-p\tau})$$

Hence, the expression for the Laplace transform of ΔE is

$$\Delta E = (1 + \beta p) \int_{\tau}^{\infty} (E_s - E_0) e^{-pt} dt - \beta (E_s(\tau) - E_0) e^{-p\tau} \\ + \frac{\chi_0 - \chi_{\infty}}{\chi_{\ell} - \chi_0} \frac{E_0}{p} (1 - e^{-p\tau})$$

This expression was evaluated using a digital computer, the integral having been replaced by a finite series. (Appendix A).

3.3 Fitting a curve to G_E^*

The effect of the rate of evaporation from the plant on the stomatal conductance is completely described by the transfer function $G_E^* = \bar{\Delta}g_\ell / \bar{\Delta}E$. If G_E^* is obtained for a particular plant, the effect of various perturbations of environment on the stomatal conductance can be calculated. To do this, the procedure for obtaining G_E^* is reversed - a known input is combined with the transfer function to obtain the response. The transfer function can also be used to examine the effect of changing the environmental gain, $\partial E / \partial g_\ell$, on the calculated response to a particular input.

To facilitate mathematical manipulation, the transfer function, G_E^* , must be described by an algebraic expression. It is appropriate to use the ratio of two polynomials in p , because theory shows that the transfer function corresponding to an ordinary linear differential equation with constant coefficients may be expressed in this form (Ogata, 1970). If, for example, the equation is third order, then the transfer function is

$$G_E^* = \frac{F(p-z_1)(p-z_2)}{(p-p_1)(p-p_2)(p-p_3)}$$

It was found that this was the lowest order equation to satisfactorily fit the experimentally determined transfer function.

By substituting into this equation the values of G_E^* for six different values of p and solving the simultaneous equations obtained, the multiplying factor, F , the zeros, z_1, z_2 and the poles, p_1, p_2, p_3 are evaluated. Both the actual and fitted values of G_E^* are shown in Fig. 3.3.

3.4 Calculating the response in stomatal conductance

The transfer function, G_E^* , can be used to calculate the response in stomatal conductance to a change in ambient humidity, $\Delta\chi$. The change in rate of evaporation due to a change in humidity is

$$\bar{\Delta}E = \left(\frac{\partial E}{\partial g_\ell} \right)_\chi \bar{\Delta}g_\ell + \left(\frac{\partial E}{\partial \chi} \right)_{g_\ell} \bar{\Delta}\chi$$

where the first term on the right hand side represents the change in the rate of evaporation due to the change in stomatal conductance and the second term represents the change in the rate of evaporation due to the change in ambient humidity. Thus, the transfer function describing the effect of change in ambient humidity on stomatal conductance is

$$\frac{\bar{\Delta}g_\ell}{\bar{\Delta}\chi} = \frac{\frac{\partial E}{\partial \chi} G_E^*}{1 - \frac{\partial E}{\partial g_\ell} G_E^*}$$

The variation in the terms $\partial E/\partial \chi$, $\partial E/\partial g_\ell$ is small compared to the change in ambient humidity, $\Delta\chi$, or the change in stomatal

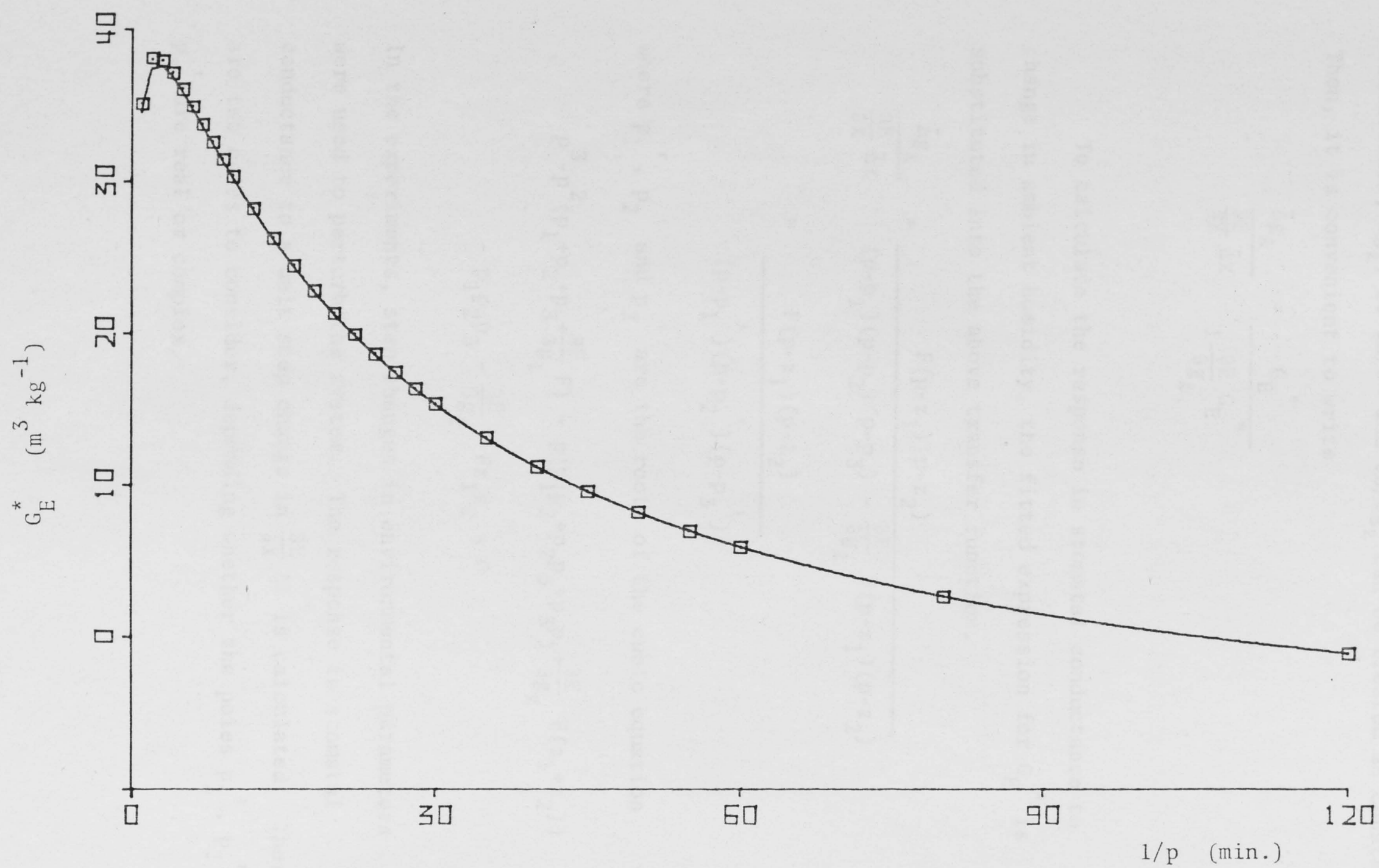


Fig.3.3. The apparent hydraulic transfer function, G_E^* , for experiment 1A: actual values ($\square$), and fitted curve (—).

conductance, Δg_ℓ , so $\partial E/\partial X$ and $\partial E/\partial g_\ell$ can be treated as constants.

Then, it is convenient to write

$$\frac{\bar{\Delta g}_\ell}{\frac{\partial E}{\partial X} \bar{\Delta X}} = \frac{G_E^*}{1 - \frac{\partial E}{\partial g_\ell} G_E^*} \quad (2)$$

To calculate the response in stomatal conductance to change in ambient humidity, the fitted expression for G_E^* is substituted into the above transfer function.

$$\begin{aligned} \frac{\bar{\Delta g}_\ell}{\frac{\partial E}{\partial X} \bar{\Delta X}} &= \frac{F(p-z_1)(p-z_2)}{(p-p_1)(p-p_2)(p-p_3) - \frac{\partial E}{\partial g_\ell} F(p-z_1)(p-z_2)} \\ &= \frac{F(p-z_1)(p-z_2)}{(p-p_1')(p-p_2')(p-p_3')} \end{aligned}$$

where p_1' , p_2' and p_3' are the roots of the cubic equation

$$\begin{aligned} p^3 - p^2(p_1 + p_2 + p_3 + \frac{\partial E}{\partial g_\ell} F) + p(p_1 p_2 + p_2 p_3 + p_3 p_1 + \frac{\partial E}{\partial g_\ell} F(z_1 + z_2)) \\ - p_1 p_2 p_3 - \frac{\partial E}{\partial g_\ell} F z_1 z_2 = 0 \end{aligned}$$

In the experiments, step changes in environmental parameters were used to perturb the system. The response in stomatal conductance to a unit step change in $\frac{\partial E}{\partial X} \Delta X$ is calculated. There are two cases to consider, depending whether the poles p_1' , p_2' , p_3' are real or complex.

(i) If p_1' , p_2' and p_3' are real,

$$\frac{\bar{\Delta}g_\ell}{\frac{\partial E}{\partial X} \Delta X} = \frac{F(p-z_1)(p-z_2)}{p(p-p_1')(p-p_2')(p-p_3')}$$

for a unit step change in $\frac{\partial E}{\partial X} \Delta X$, because the Laplace transform of a unit step function is $1/p$. (Ogata, 1970, Jaeger, 1966).

Separating the expression into partial fractions gives

$$\frac{\bar{\Delta}g_\ell}{\frac{\partial E}{\partial X} \Delta X} = \frac{A}{p} + \frac{B}{p-p_1'} + \frac{C}{p-p_2'} + \frac{D}{p-p_3'} \quad (3)$$

where $A = -\frac{Fz_1z_2}{p_1'p_2'p_3'}$,

$$B = \frac{F(p_1'-z_1)(p_1'-z_2)}{p_1'(p_1'-p_2')(p_1'-p_3')}$$

$$C = \frac{F(p_2'-z_1)(p_2'-z_2)}{p_2'(p_2'-p_1')(p_2'-p_3')}$$

and $D = \frac{F(p_3'-z_1)(p_3'-z_2)}{p_3'(p_3'-p_1')(p_3'-p_2')}$

Taking the inverse Laplace transform gives the response of g_ℓ to a unit step change in $\frac{\partial E}{\partial X} \Delta X$, i.e.

$$\frac{\Delta g_\ell}{\frac{\partial E}{\partial \lambda} \Delta \lambda} = A + B e^{p_1' t} + C e^{p_2' t} + D e^{p_3' t}$$

(ii) If p_1' is real and p_2', p_3' are a pair of complex conjugate poles, written as

$$p_2' = u + iv$$

$$p_3' = u - iv$$

then, for a unit step change in $\frac{\partial E}{\partial \lambda} \Delta \lambda$,

$$\begin{aligned} \frac{\bar{\Delta g}_\ell}{\frac{\partial E}{\partial \lambda} \Delta \lambda} &= \frac{F(p-z_1)(p-z_2)}{p(p-p_1')(p-u-iv)(p-u+iv)} \\ &= \frac{F(p-z_1)(p-z_2)}{p(p-p_1')[(p-u)^2 + v^2]} \\ &= \frac{A}{p} + \frac{B}{p-p_1'} + \frac{C(p-u)}{(p-u)^2 + v^2} + \frac{D + Cu}{(p-u)^2 + v^2} \end{aligned} \quad (4)$$

$$\text{where } A = - \frac{F z_1 z_2}{p_1' (u^2 + v^2)}$$

$$B = \frac{F(p_1' - z_1)(p_1' - z_2)}{p_1' [(p_1' - u)^2 + v^2]}$$

and C and D may be found from the equations

$$A + B + C = 0$$

$$D = F + A(2u + p_1') + 2uB + Cp_1'$$

Taking the inverse Laplace transform,

$$\frac{\Delta g_{\ell}}{\frac{\partial E}{\partial \chi} \Delta \chi} = A + B e^{p_1 t} + C e^{ut} \cos vt + (D+Cu) e^{ut} \sin vt$$

Thus, it is seen that the response in stomatal conductance to a unit step change in $\frac{\partial E}{\partial \chi} \Delta \chi$ consists of a steady state term, A, plus either three exponential decay terms if the poles are real, or one exponential decay term and two damped oscillatory terms if the poles are complex.

3.5 Effect of environmental gain on the response in stomatal conductance

Fig. 3.4 shows the response in stomatal conductance calculated at an environmental gain of 0.0135 kg m^{-3} , the gain used in the experiment to find G_E^* . The poles of the transfer function are complex and the equation of the response is

$$\begin{aligned} \frac{\Delta g_{\ell}}{\frac{\partial E}{\partial \chi} \Delta \chi} = & -9.43 - 136.87 e^{-1.25t} + 146.3 e^{-0.12t} \cos 0.08t \\ & + 6.01 e^{-0.12t} \sin 0.08t \end{aligned}$$

The response is slightly oscillatory; the initial opening movement exhibits a small overshoot before the conductance approaches the final steady state value.

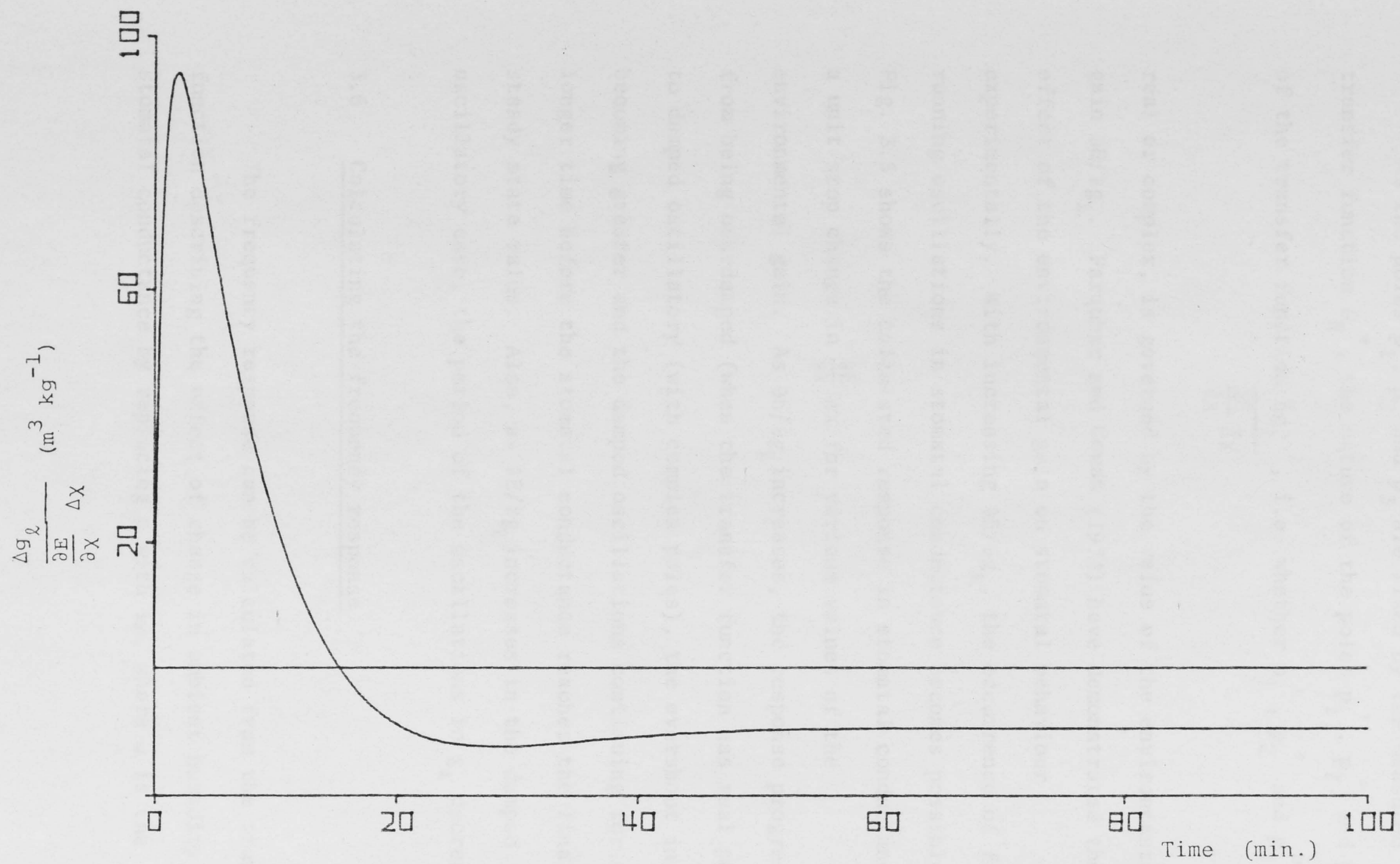


Fig.3.4. Stomatal response to unit step change in ambient humidity at environmental gain 0.0135 kg m^{-3} .

As the poles p_1 , p_2 and p_3 are fixed by the measured transfer function G_E^* , the nature of the poles p_1' , p_2' and p_3' of the transfer function $\frac{\bar{\Delta}g_\ell}{\frac{\partial E}{\partial \chi} \bar{\Delta}\chi}$, i.e. whether p_1' , p_2' and p_3' are

real or complex, is governed by the value of the environmental gain $\partial E/\partial g_\ell$. Farquhar and Cowan (1974) have demonstrated the effect of the environmental gain on stomatal behaviour experimentally. With increasing $\partial E/\partial g_\ell$, the occurrence of free-running oscillations in stomatal conductance becomes possible. Fig. 3.5 shows the calculated response in stomatal conductance to a unit step change in $\frac{\partial E}{\partial \chi} \Delta\chi$ for various values of the environmental gain. As $\partial E/\partial g_\ell$ increases, the response progresses from being overdamped (when the transfer function has real poles) to damped oscillatory (with complex poles), the overshoot in g_ℓ becoming greater and the damped oscillations continuing for a longer time before the stomatal conductance reaches the final steady state value. Also, as $\partial E/\partial g_\ell$ increases in the damped oscillatory case, the period of the oscillations in g_ℓ decreases.

3.6 Calculating the frequency response

The frequency response can be calculated from the transfer function describing the effect of change in ambient humidity on stomatal conductance by replacing p with $i\omega$, where ω is the

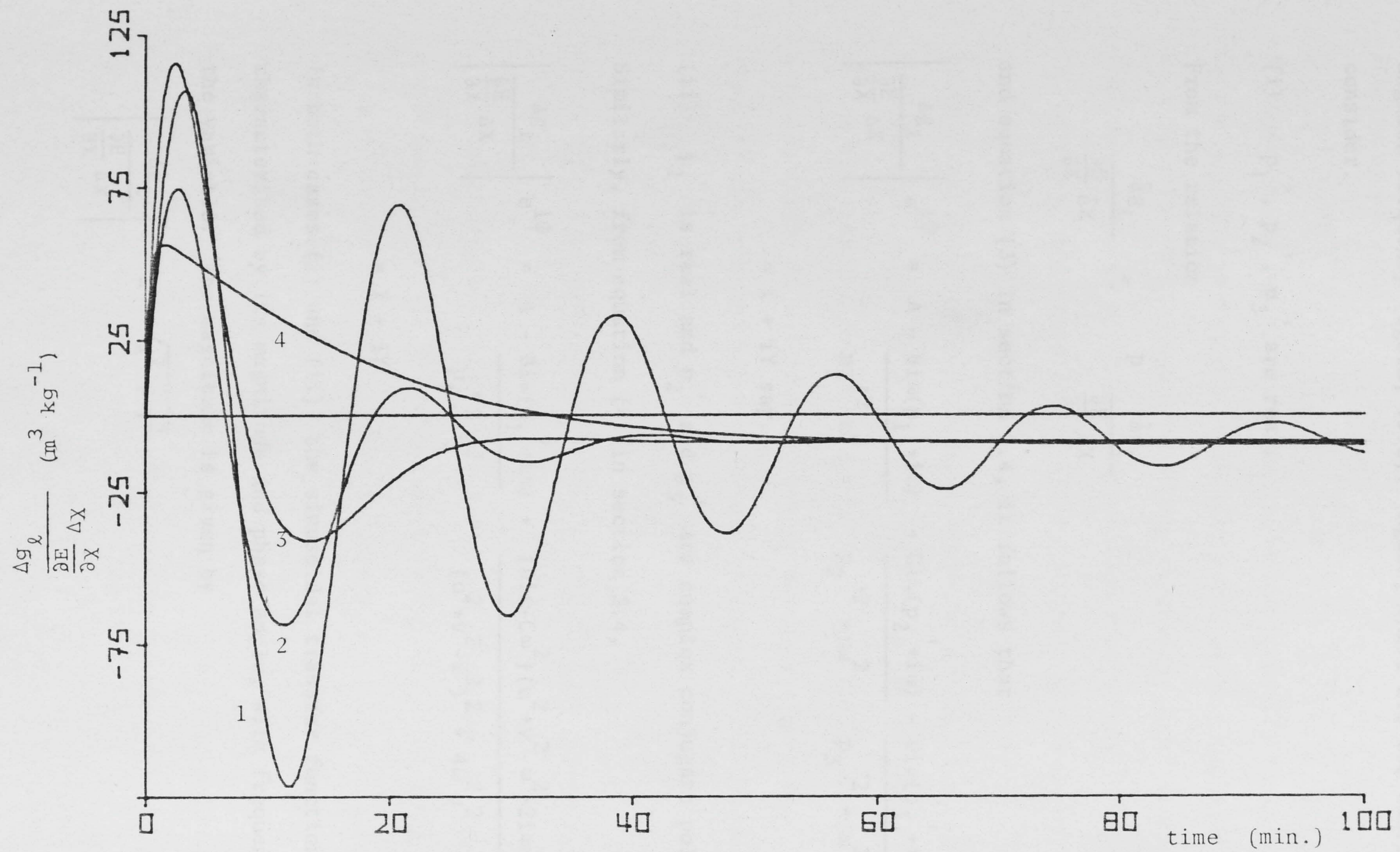


Fig 3.5. Stomatal response to unit step change in ambient humidity at various environmental gains. Curve 1 - 0.021 kg m^{-3} , 2 - 0.02 kg m^{-3} , 3 - 0.018 kg m^{-3} , 4 - 0.005 kg m^{-3} .

angular frequency (Ogata, 1970). Again, there are two cases to consider.

(i) p_1', p_2', p_3' are real.

From the relation

$$\frac{\bar{\Delta}g_\ell}{\frac{\partial E}{\partial X} \bar{\Delta}X} = p \frac{\bar{\Delta}g_\ell}{\frac{\partial E}{\partial X} \Delta X}$$

and equation (3) in section 3.4, it follows that

$$\left| \frac{\Delta g_\ell}{\frac{\partial E}{\partial X} \Delta X} \right| e^{i\phi} = A - \frac{Bi\omega(p_1' + i\omega)}{p_1'^2 + \omega^2} - \frac{Ci\omega(p_2' + i\omega)}{p_2'^2 + \omega^2} - \frac{Di\omega(p_3' + i\omega)}{p_3'^2 + \omega^2}$$

$$= X + iY \text{ say.}$$

(ii) p_1' is real and p_2' and p_3' are complex conjugate poles.

Similarly, from equation (4) in section 3.4,

$$\left| \frac{\Delta g_\ell}{\frac{\partial E}{\partial X} \Delta X} \right| e^{i\phi} = A - \frac{Bi\omega(p_1' + i\omega)}{p_1'^2 + \omega^2} + \frac{(Di\omega - C\omega^2)(u^2 + v^2 - \omega^2 + 2i\omega u)}{(u^2 + v^2 - \omega^2)^2 + 4\omega^2 u^2}$$

$$= X + iY$$

In both cases (i) and (ii), the sinusoidal transfer function is characterised by its magnitude and phase-angle, with frequency as the variable. The magnitude is given by

$$\left| \frac{\Delta g_\ell}{\frac{\partial E}{\partial X} \Delta X} \right| = \sqrt{X^2 + Y^2}$$

and the phase angle by

$$\phi = \tan^{-1} (Y/X)$$

A common way of representing the sinusoidal transfer function is by two separate plots, one of the logarithm of the magnitude as a function of the logarithm of the frequency, and the other the phase angle as a function of the logarithm of the frequency. The standard representation of the logarithmic magnitude of the transfer function is $20\log_{10}$ (amplitude ratio). These logarithmic plots, or Bode diagram, are shown in Fig. 3.6, again with $\partial E/\partial g_{\ell} = 0.0135 \text{ kg m}^{-3}$, the value of the environmental gain in the experimental determination of G_E^* . The amplitude plot has a small maximum at a frequency corresponding to a period of about 25 min. and the phase plot goes through 0° at a frequency equivalent to a period of about 17 min.

3.7 Effect of environmental gain on the frequency response

Bode diagrams, plotted at different environmental gains are shown in Fig. 3.7. The frequency at which the phase angle, ϕ , is 0° is the undamped natural frequency, ω_n , the frequency at which the system would oscillate if there were no damping. This frequency does not depend on the value of $\partial E/\partial g_{\ell}$. As the environmental gain increases, the damping in the system decreases and the phase plot has a much greater slope in the vicinity of

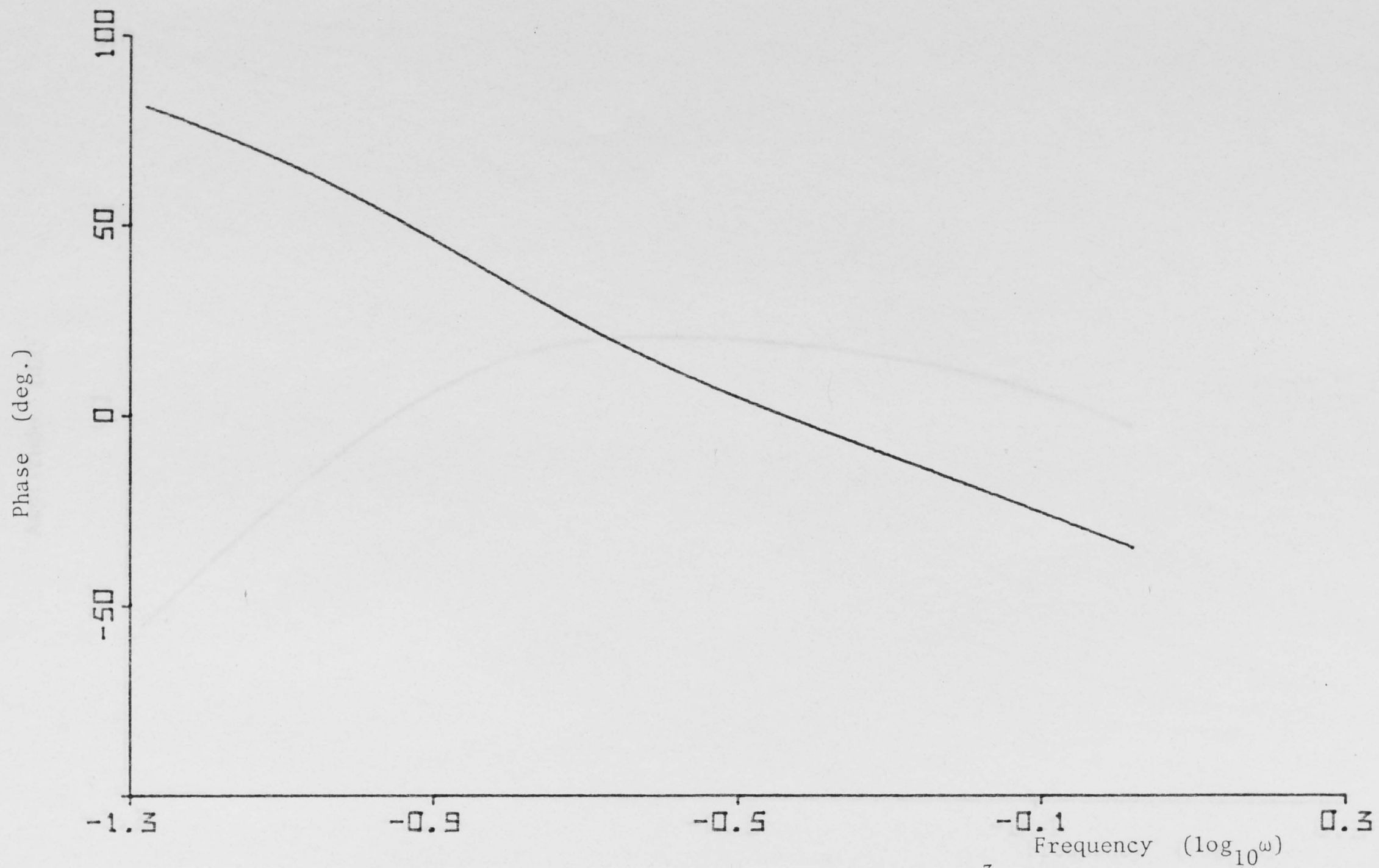


Fig.3.6. Frequency response at environmental gain 0.0135 kg m^{-3} - Phase plot.

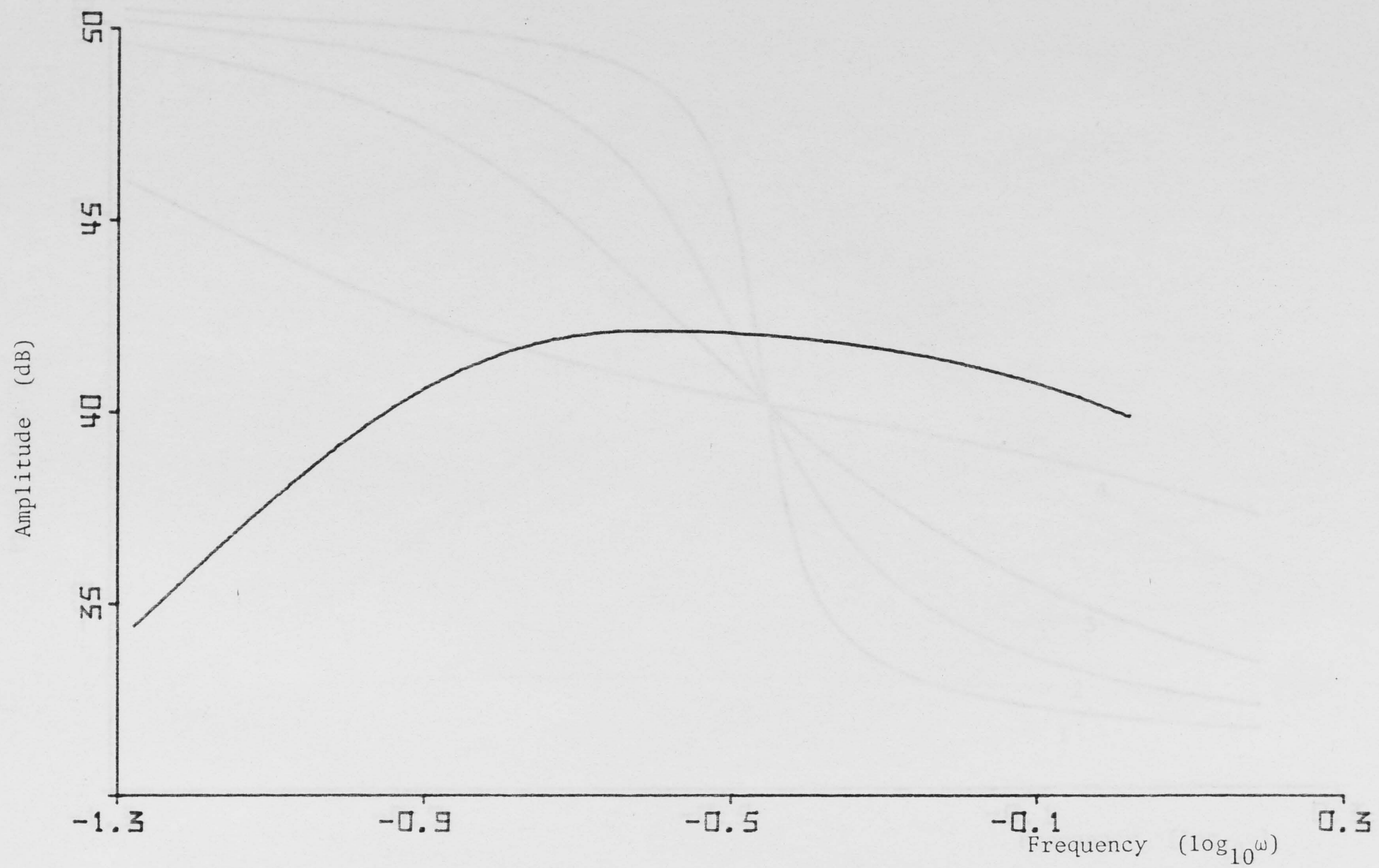


Fig.3.6. Amplitude plot.

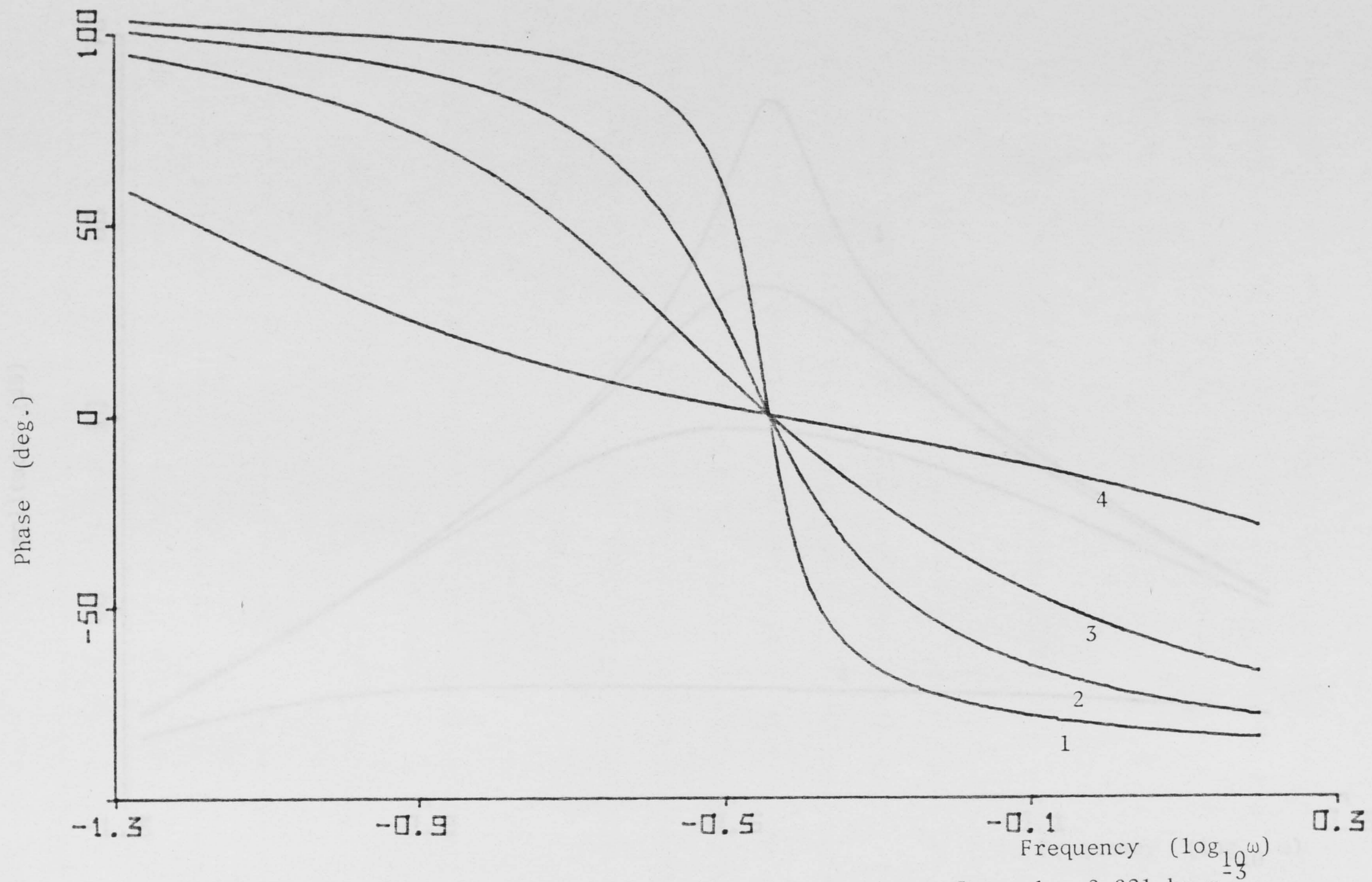


Fig.3.7. Frequency response at various environmental gains. Curve 1 - 0.021 kg m^{-3} ,
 2 - 0.02 kg m^{-3} , 3 - 0.018 kg m^{-3} , 4 - 0.005 kg m^{-3} . Phase plot.

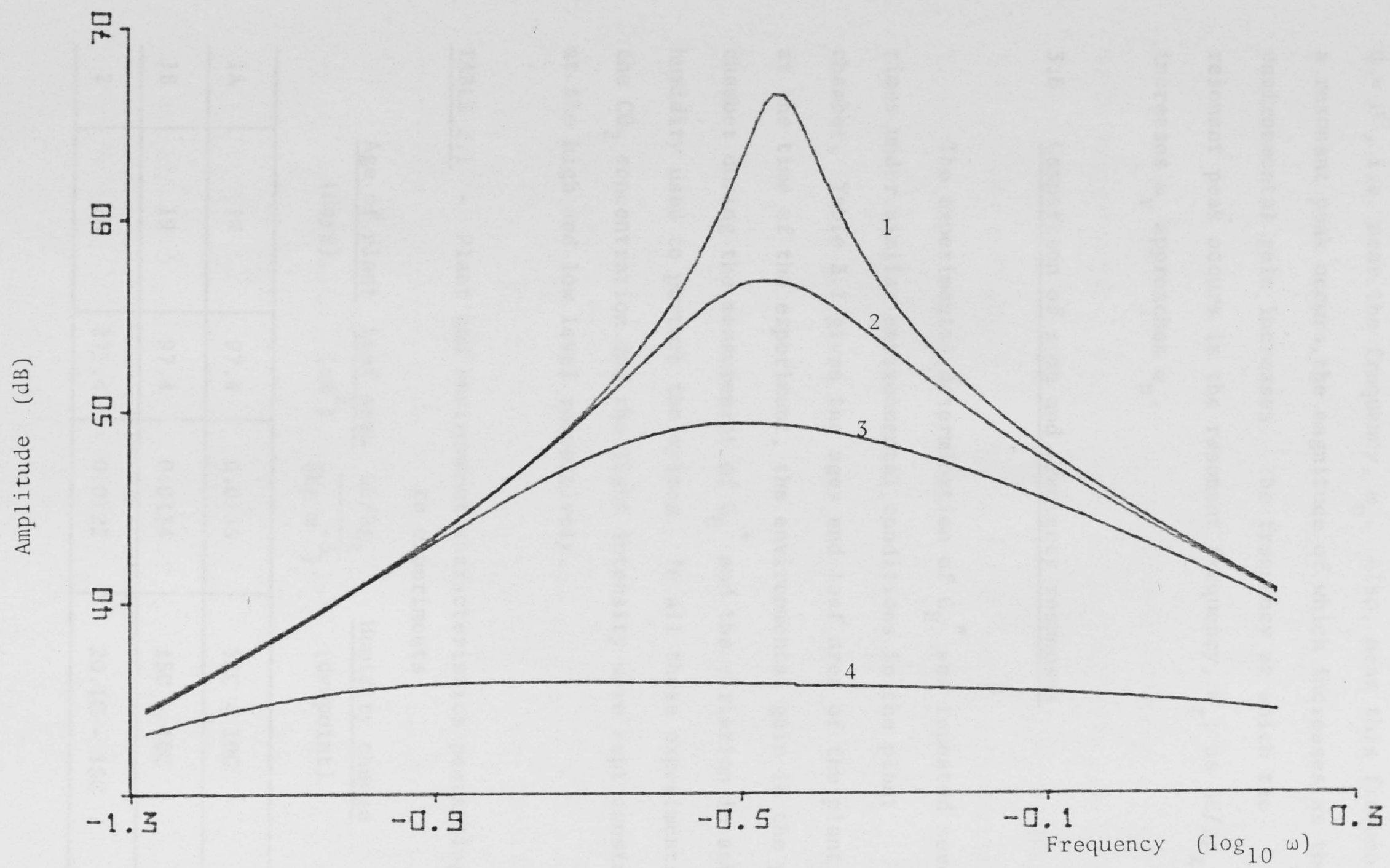


Fig.3.7. Amplitude plot.

$\phi = 0^\circ$, i.e. near the frequency, ω_n . Also, near this frequency, a resonant peak occurs, the magnitude of which increases as the environmental gain increases. The frequency at which the resonant peak occurs is the resonant frequency, ω_r ; as $\partial E / \partial g_\ell$ increases ω_r approaches ω_n .

3.8 Comparison of step and frequency responses

The experimental determination of G_E^* was repeated several times under similar environmental conditions in the plant chamber. Table 3.1 gives the ages and leaf area of the plants at the time of the experiment, the environmental gain in the plant chamber during the measurement of G_E^* and the variation in ambient humidity used to perturb the system. In all these experiments, the CO_2 concentration and the light intensity were kept constant at the high and low level respectively.

TABLE 3.1 - Plant and environment characteristics pertaining to experiments

	<u>Age of plant</u> (days)	<u>Leaf area</u> (cm^2)	<u>$\partial E / \partial g_\ell$</u> ($kg\ m^{-3}$)	<u>Humidity change</u> (dewpoint)
1A	19	97.4	0.0135	15C - 10C
1B	19	97.4	0.0134	15C - 10C
2	26	271.4	0.0122	20.1C - 15C

Experiment 1B was performed on the same plant as experiment 1A, the results of which have been discussed in the preceeding sections.

The transfer function G_E^* for 1B was fitted by a third order expression in the same manner as 1A. Both the actual and fitted curves for 1B are shown in Fig. 3.8 and the values of the poles, zeros and multiplying factor for 1A and 1B are given in Table 3.2. The transfer functions, G_E^* , for 1A and 1B have the same shape and as expected, the step and frequency responses are similar. There are some quantitative differences.

TABLE 3.2 - Parameters relating to analytical approximations to G_E^*

	F ($m^3 kg^{-1} min^{-1}$)	z_1 (min^{-1})	z_2 (min^{-1})	p_1 (min^{-1})	p_2 (min^{-1})	p_3 (min^{-1})
1A	159.8	0.009553	- 0.1559	-0.1325	-3.463	-0.04796
1B	84.15	0.009721	- 0.06963	-0.1204	-1.432	-0.0209

For 1A, the undamped natural frequency, ω_n , corresponds to a period of approximately 17 min; for 1B, the natural period is about 19 min. Substantial differences occur in those values of the environmental gain, $\partial E / \partial g_\ell$, at which the nature of the poles, p_1' , p_2' , p_3' , of the transfer function, $\frac{\partial E}{\partial X} \frac{\bar{\Delta} g_\ell}{\bar{\Delta} X}$, change.

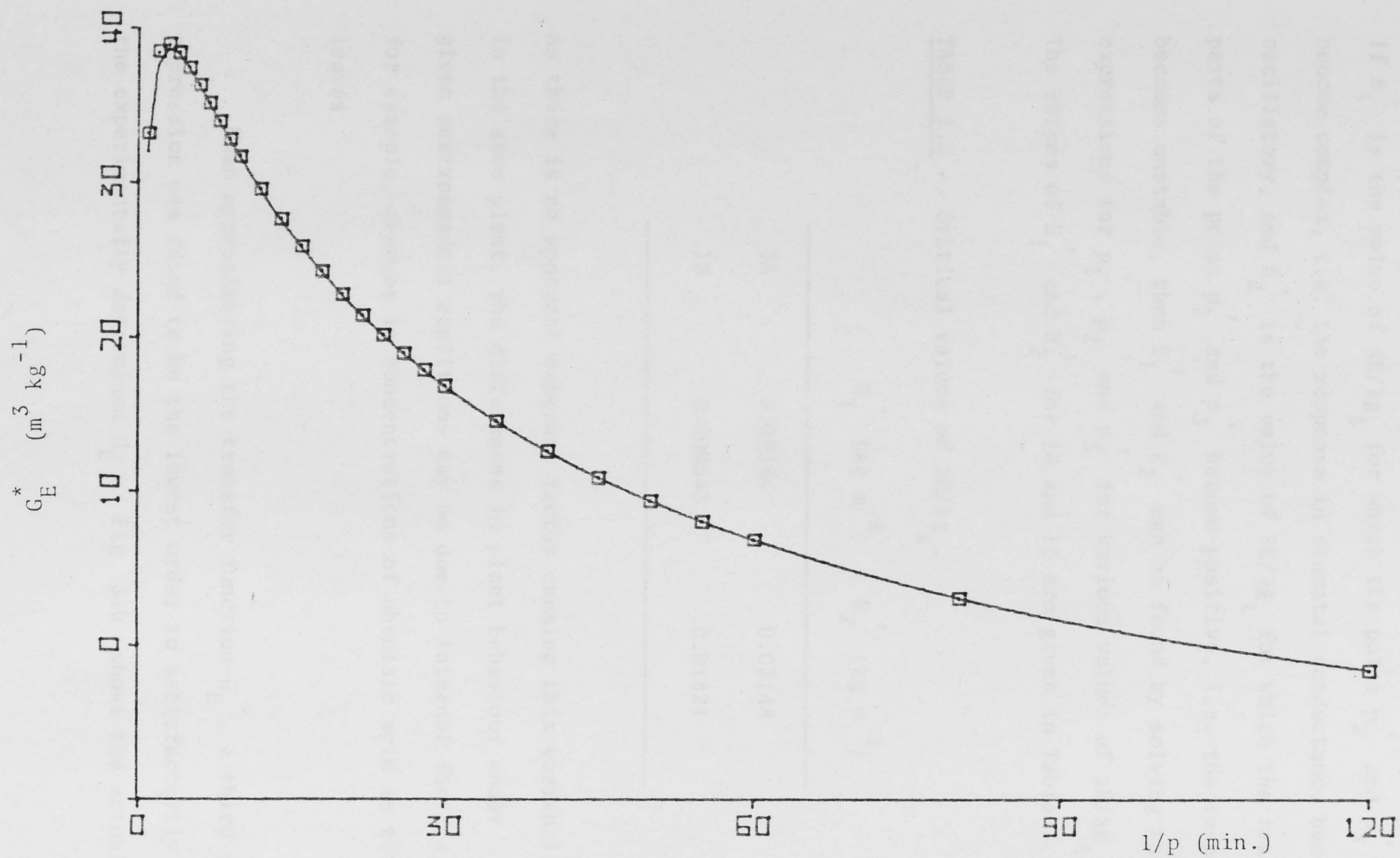


Fig.3.8. The apparent hydraulic transfer function, G_E^* , for experiment 1B: actual values ($\square$), and fitted curve (—).

If E_1' is the value of $\partial E / \partial g_\ell$ for which the poles p_2' and p_3' become complex, i.e. the response in stomatal conductance becomes oscillatory, and E_2' is the value of $\partial E / \partial g_\ell$ for which the real parts of the poles p_2' and p_3' become positive, i.e. the response becomes unstable, then E_1' and E_2' can be found by solving the expressions for p_1' , p_2' and p_3' for various values of $\partial E / \partial g_\ell$. The values of E_1' and E_2' for 1A and 1B are given in Table 3.3.

TABLE 3.3 - Critical values of $\partial E / \partial g_\ell$.

	E_1' (kg m ⁻³)	E_2' (kg m ⁻³)
1A	0.00584	0.02148
1B	0.009643	0.01821

As there is no apparent external factor causing this variability in the same plant, the differences in plant behaviour under given environmental conditions may be due to internal factors, for example, changes in concentrations of abscisic acid in the leaves.

When approximating the transfer function G_E^* , a third order expression was found to be the lowest order to satisfactorily fit the experimentally determined G_E^* . Fig 3.9 shows the actual

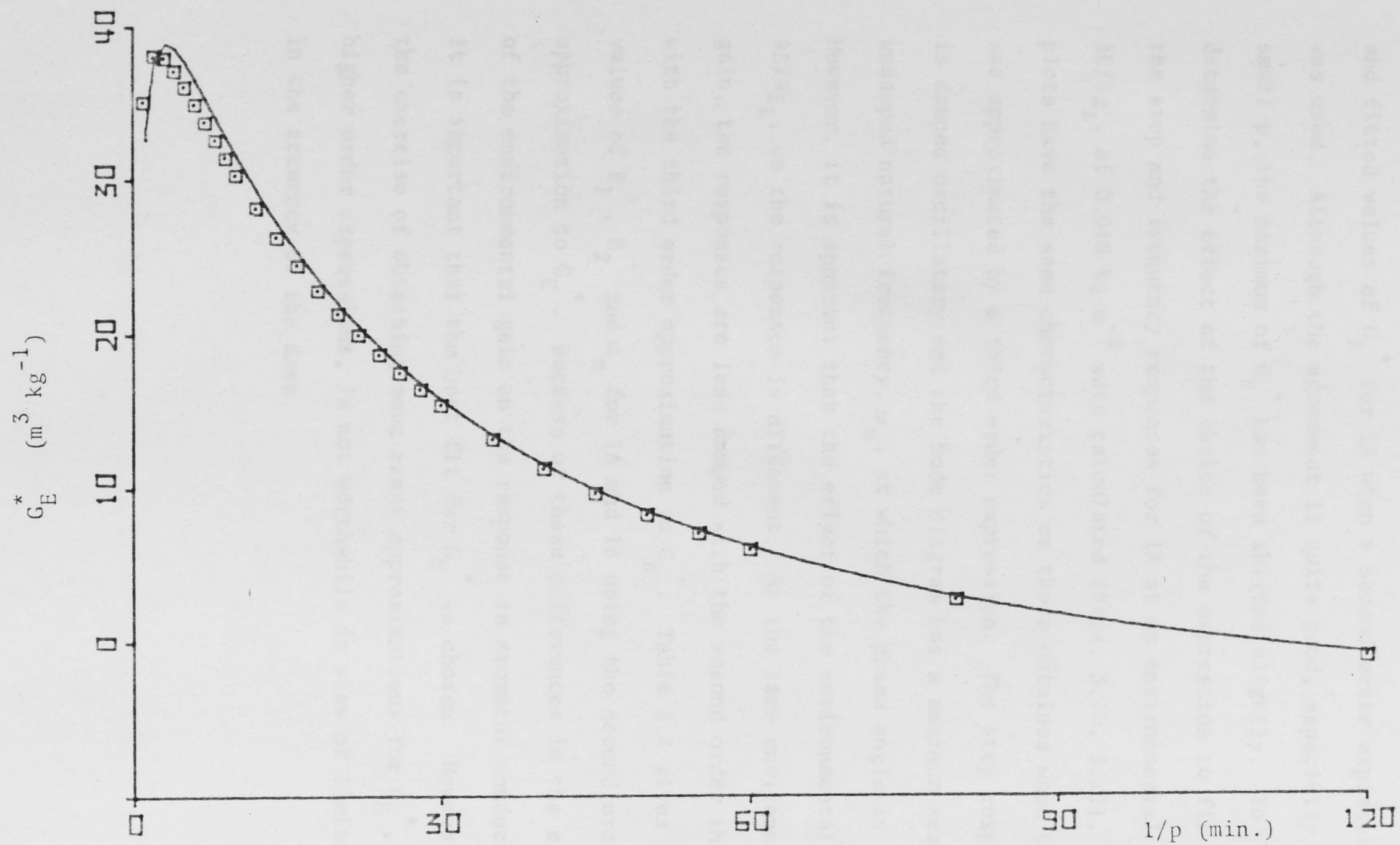


Fig.3.9. The apparent hydraulic transfer function, G_E^* , for experiment 1A: actual values ($\square$), and second-order fitted curve. (—).

and fitted values of G_E^* for 1A when a second order expression was used. Although the agreement is quite good, especially for small p , the maximum of G_E^* has been shifted slightly. To determine the effect of the choice of the expression to fit G_E^* , the step and frequency responses for 1A at an environmental gain, $\partial E / \partial g_\ell$, of 0.018 kg m^{-3} were calculated (Figs. 3.10, 3.11). The plots have the same characteristics as those obtained when G_E^* was approximated by a third order expression. The step response is damped oscillatory and the Bode diagram has a maximum near the undamped natural frequency, ω_n , at which the phase angle is zero. However, it is apparent that the effect of the environmental gain, $\partial E / \partial g_\ell$, on the responses is different. At the same environmental gain, the responses are less damped with the second order than with the third order approximation to G_E^* . Table 3.4 gives the values of E_1' , E_2' and ω_n for 1A and 1B using the second order approximation to G_E^* . Because of these differences in the effect of the environmental gain on the response in stomatal conductance, it is important that the best fit for G_E^* is chosen. However, the exercise of obtaining more exact approximations for G_E^* , using higher order expressions, is not worthwhile in view of limitations in the accuracy of the data.

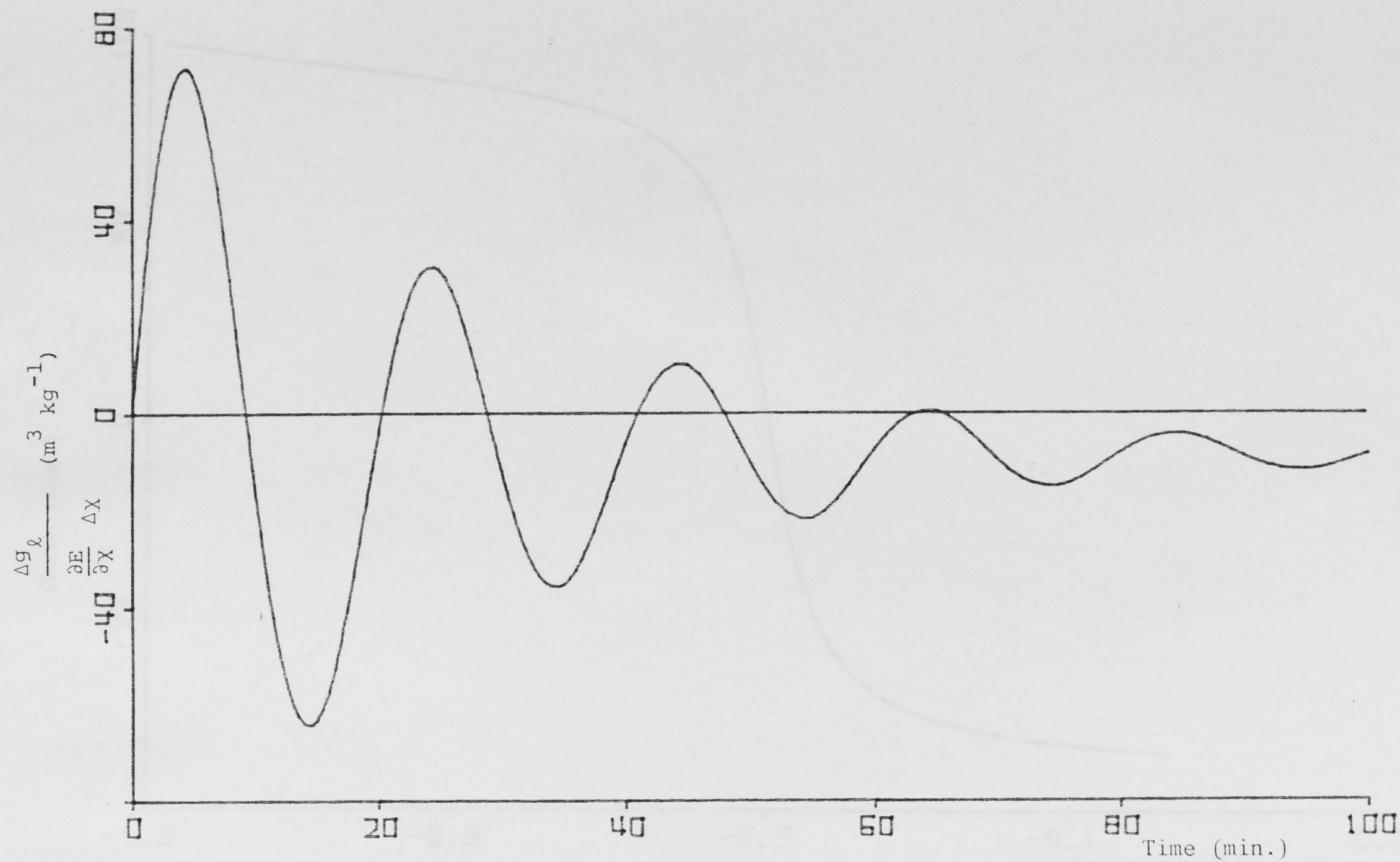


Fig.3.10. Stomatal response to unit step change in ambient humidity at environmental gain 0.018 kg m^{-3} calculated from the second-order G_E for experiment 1A.

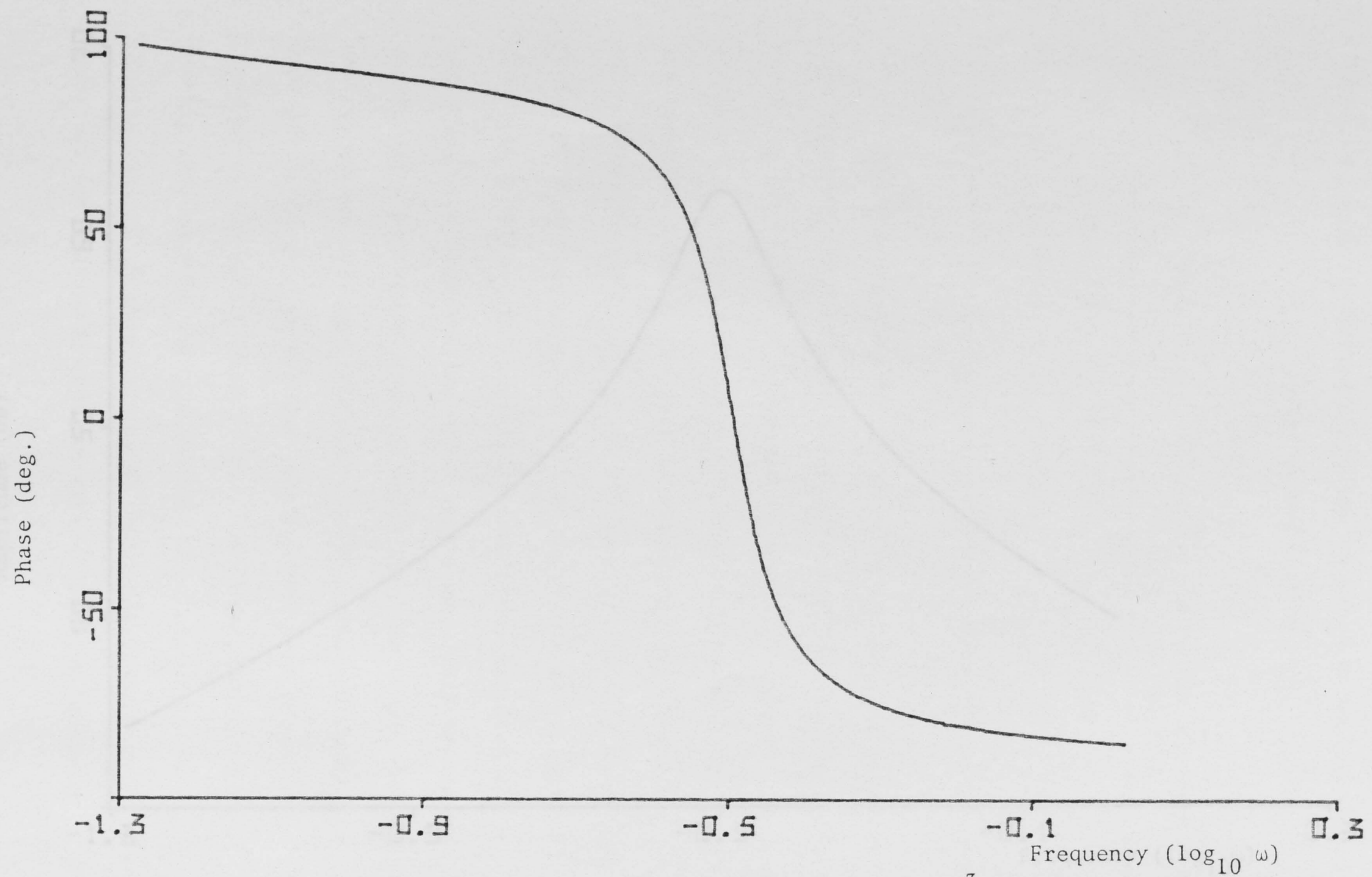


Fig.3.11. Frequency response at environmental gain 0.018 kg m^{-3} calculated from the second order G_E for experiment 1A - Phase plot.

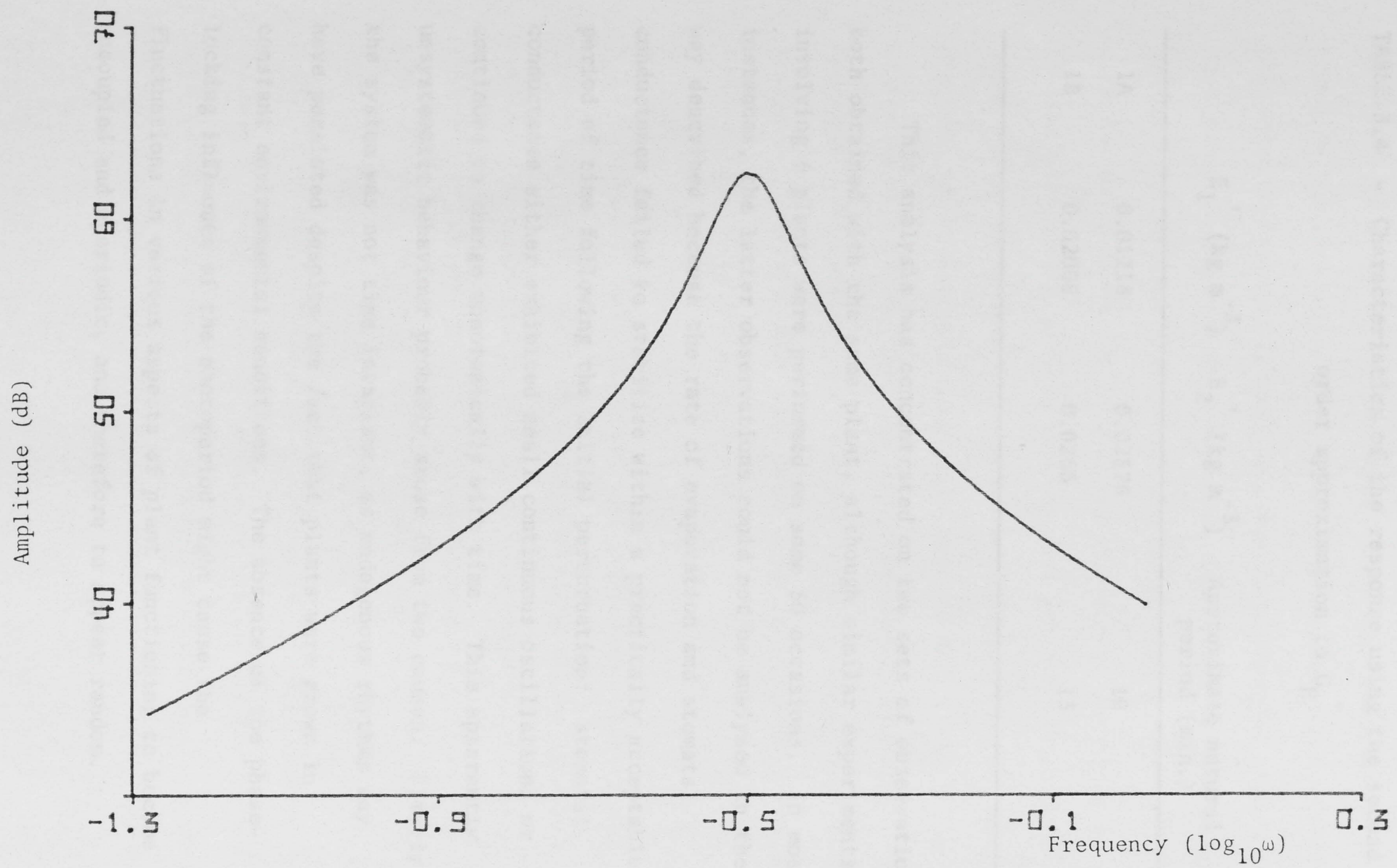


Fig.3.11. Amplitude plot.

TABLE 3.4 - Characteristics of the response using the second order approximation to G_E^*

	E_1' (kg m ⁻³)	E_2' (kg m ⁻³)	Approximate natural period (min.)
1A	0.01218	0.02576	19
1B	0.02005	0.0266	13

This analysis has concentrated on two sets of observations both obtained with the same plant, although similar experiments involving 6 plants were performed on some 30 occasions. In most instances, the latter observations could not be analysed in the way described because the rate of evaporation and stomatal conductance failed to stabilize within a practically acceptable period of time following the initial perturbation: stomatal conductance either exhibited small continuous oscillations or continued to change monotonically with time. This apparently unsystematic behaviour probably arose from two causes. Firstly, the system was not time invariant, as endogenous rhythms may have persisted despite the fact that plants were grown in constant environmental conditions. The absence of the phase-locking influence of the photoperiod might cause the fluctuations in various aspects of plant functioning to become uncoupled and aperiodic, and therefore to appear random.

Secondly, it is possible that some of the repercussions of changing the rate of evaporation have characteristic times of the order of hours or longer, and that the responses observed in successive experiments on the one plant were, to some extent, confounded. As each plant could not be maintained in the experimental chamber for longer than about five days (due to the limited size of the working section, and rapid increase in plant size), it is doubtful whether an investigation of these problems could have been adequately carried out. Even so, most of the short term responses to change in ambient humidity appeared to be qualitatively similar to those which have been analysed. However, there were some cases in which this was not so, and these will now be discussed.

Table 3.1 lists the plant and environment characteristics pertaining to the experimental determination of the transfer function, G_E^* , plotted in Fig. 3.12. The shape of this transfer function, which was typical of the behaviour of the particular plant, differs markedly from those for experiments 1A and 1B, in that G_E^* is negative for all values of p . The stomata on this plant do not exhibit the initial "wrong-way" response which is believed to be an essential feature of the oscillatory mechanism. Nevertheless, the plant showed a strong tendency for oscillations to take place spontaneously, to the extent that it was necessary

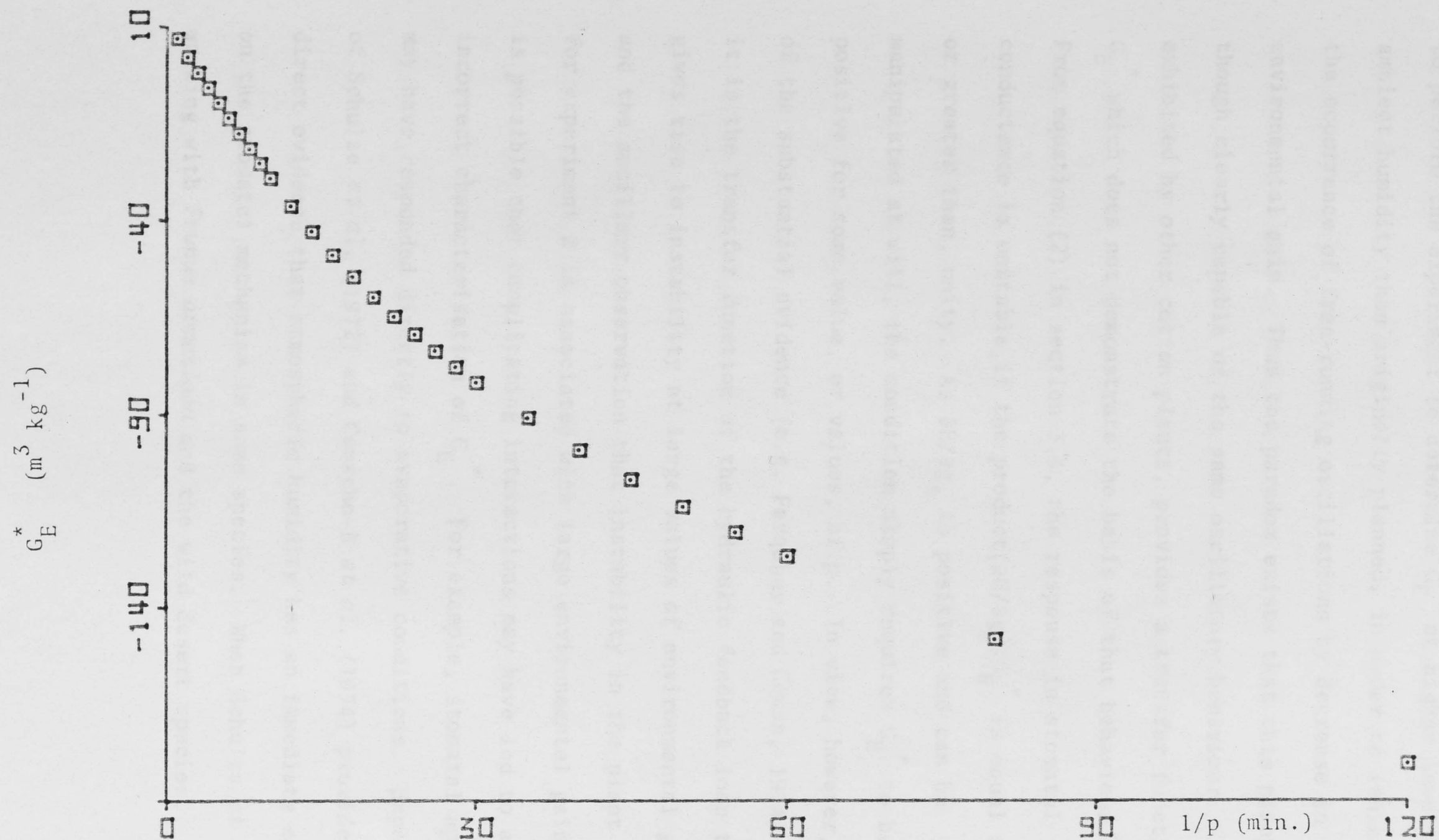


Fig.3.12. The apparent hydraulic transfer function, G_E^* , for experiment 2: actual values ($\square$).

to perform the experiment to determine G_E^* at higher levels of ambient humidity than originally planned, in order to inhibit the occurrence of free-running oscillations by decrease in environmental gain. Thus the paradox exists that this plant, though clearly capable of the same oscillatory behaviour exhibited by other cotton plants, provides a transfer function G_E^* which does not demonstrate the basis of that behaviour. From equation (2) in section 3.4, the response in stomatal conductance is unstable if the product $(\partial E / \partial g_\ell) G_E^*$ is equal to, or greater than, unity. As $\partial E / \partial g_\ell$ is positive and can be manipulated at will, the condition simply requires G_E^* to be positive for some value, or values, of p . In view, however, of the substantial evidence (e.g. Farquhar and Cowan, 1974) that it is the transfer function of the hydraulic feedback loop that gives rise to instability at large values of environmental gain, and the ancillary observation that instability in the plant used for experiment 2 is associated with large environmental gain, it is possible that complicating interactions may have led to an incorrect characterisation of G_E^* . For example, stomatal aperture may have responded directly to evaporative conditions. Experiments of Schulze *et al.* (1972) and Camacho-B *et al.* (1974) provide direct evidence that atmospheric humidity has an immediate effect on the stomatal mechanism in some species. When Schulze *et al.*, working with *Prunus armeniaca* and the wild desert species,

Hammada scoparia and *Zygophyllum dumosum*, progressively decreased the ambient humidity with leaf temperature held constant, stomatal conductance was reduced so much that decrease in rate of evaporation allowed the water content of the leaves to actually increase. Using three herbaceous species, *Helianthus annuus*, *Capsicum frutescens* and *Sesamum indicum*, Camacho-B et al. were unable to detect changes in leaf water potential when stomatal conductance responded to changes in evaporative demand produced by varying ambient humidity. If such an effect occurs in cotton, all characterisations of G_E^* will have been effected. In the case of the plant, the transfer function of which is shown in Fig. 3.12, perhaps the direct influence of decrease in ambient humidity masked the initial tendency of the stomata to open. It is difficult to explain why this behaviour would be more apparent in one plant than in the others. Unfortunately, no measurements of leaf water content or water potential were made in these experiments, so the suggestion must remain speculative. If it has substance, the suggestion has disturbing implications in that it throws doubt on the validity of the approach that has been employed throughout this analysis.

The discussion so far has assumed real poles for the transfer function, G_E^* , i.e. G_E^* has been regarded as non-oscillatory. The observed oscillations in stomatal conductance

are assumed to occur only as a result of the combination of G_E^* with the feedback of g_ρ on the rate of evaporation, E . With increasing environmental gain, $\partial E / \partial g_\rho$, the feedback in the system increases and stomatal behaviour becomes more oscillatory. However, some of the observed transfer functions were best fitted by expressions with complex poles. This implies that G_E^* is of an intrinsically oscillatory character. Although not explained by current models of the hydraulic interactions between rate of evaporation and the stomatal mechanism, this possibility cannot be rejected - there may be internal feedback loops in the hydraulic system within the plant which are capable of causing oscillatory behaviour (Stålfelt, 1929). At the same time, it would not be justified to assume that the occurrence of complex roots in G_E^* is of fundamental significance, in view of the complications discussed in relation to experiment 2, and of the fact that a linear mathematical analysis has been applied to what is certainly to some extent a non-linear system.

The calculated frequency responses for 1A and 1B can be compared with experimental determinations of the frequency response of cotton made by Farquhar (1973). The response was determined by varying the humidity in the plant chamber sinusoidally and measuring the phase angle and amplitude of the resulting sinusoid in stomatal conductance. Bode diagrams of the measured phase difference and the logarithmic ratio of the

magnitudes of the output and input sinusoids were then plotted. (Fig. 3.13). The results were obtained for five different plants, each grown in normal photoperiodic conditions rather than under continuous light as in the present study. At periods longer than 53 min., "twin-peaked" responses were observed, i.e. there were two peaks in stomatal conductance corresponding to a single oscillation in the ambient humidity. Apart from this and the variability in the amplitude data, the Bode diagrams have the same form as those calculated from the transfer functions. At the undamped natural frequency, ω_n , the phase angle becomes zero degrees. At high frequencies, the phase angle tends to -90° and at low frequencies, to 180° . The value of ω_n , in the experimental phase plot, gives a natural period of approximately 40 min., while in the calculated phase plots, the natural period is less than 20 min. A large range of periods of the free-running oscillations in stomatal conductance have been observed. For example, Troughton and Cowan (1968) observed oscillations in the rate of evaporation from cotton, with a period varying between 22 and 26 min. whereas Skidmore and Stone (1964) observed cyclic changes with a period of about 54 min. in a different variety of cotton. Lang *et al.* (1969) have shown that some of this variation may be seasonal. The variation observed between the calculated and experimentally determined natural period may be partly due to differences in growing conditions of the plants.

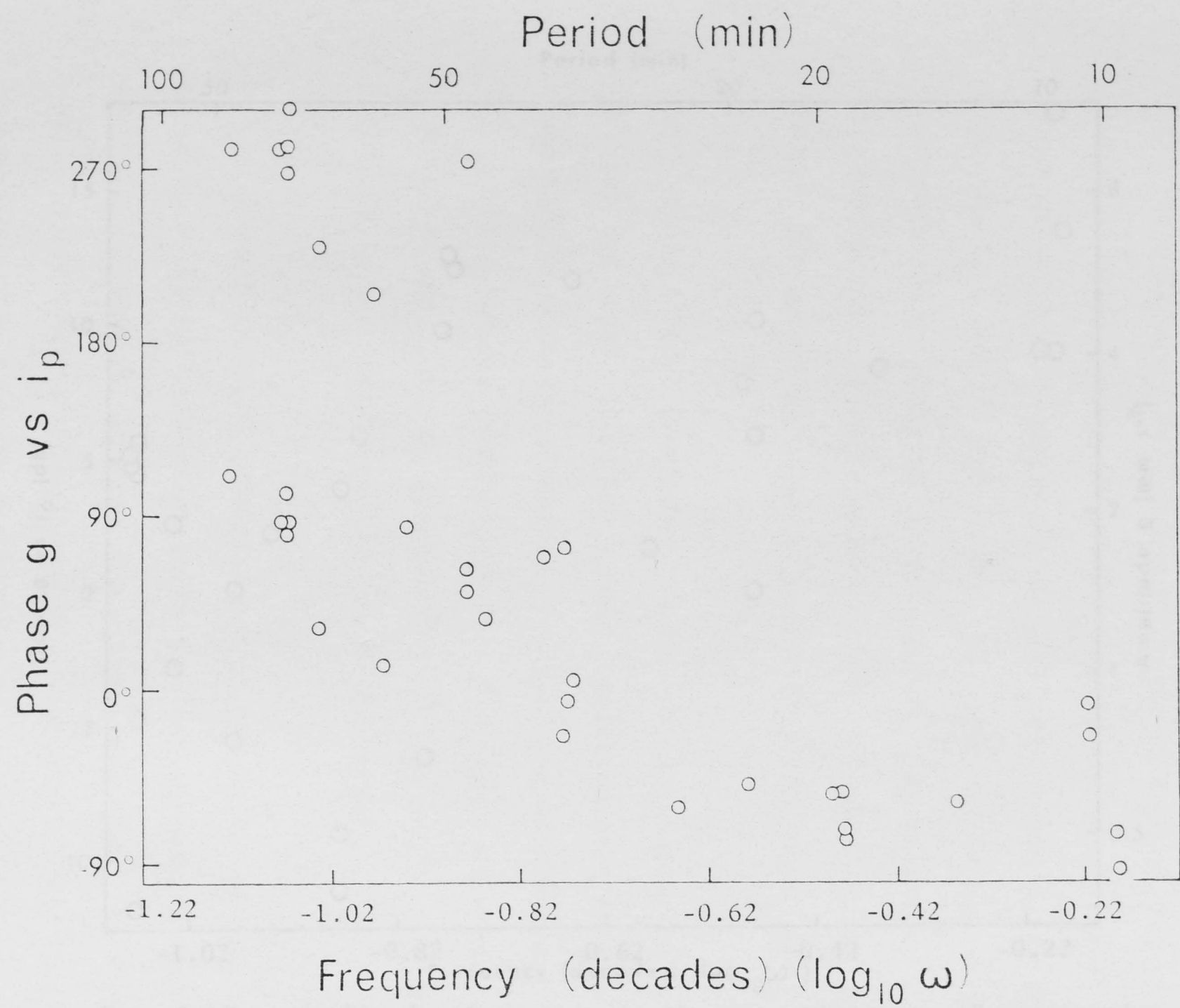


Fig.3.13 Experimentally determined frequency response. Phase plot.
(from Farquhar, 1973).

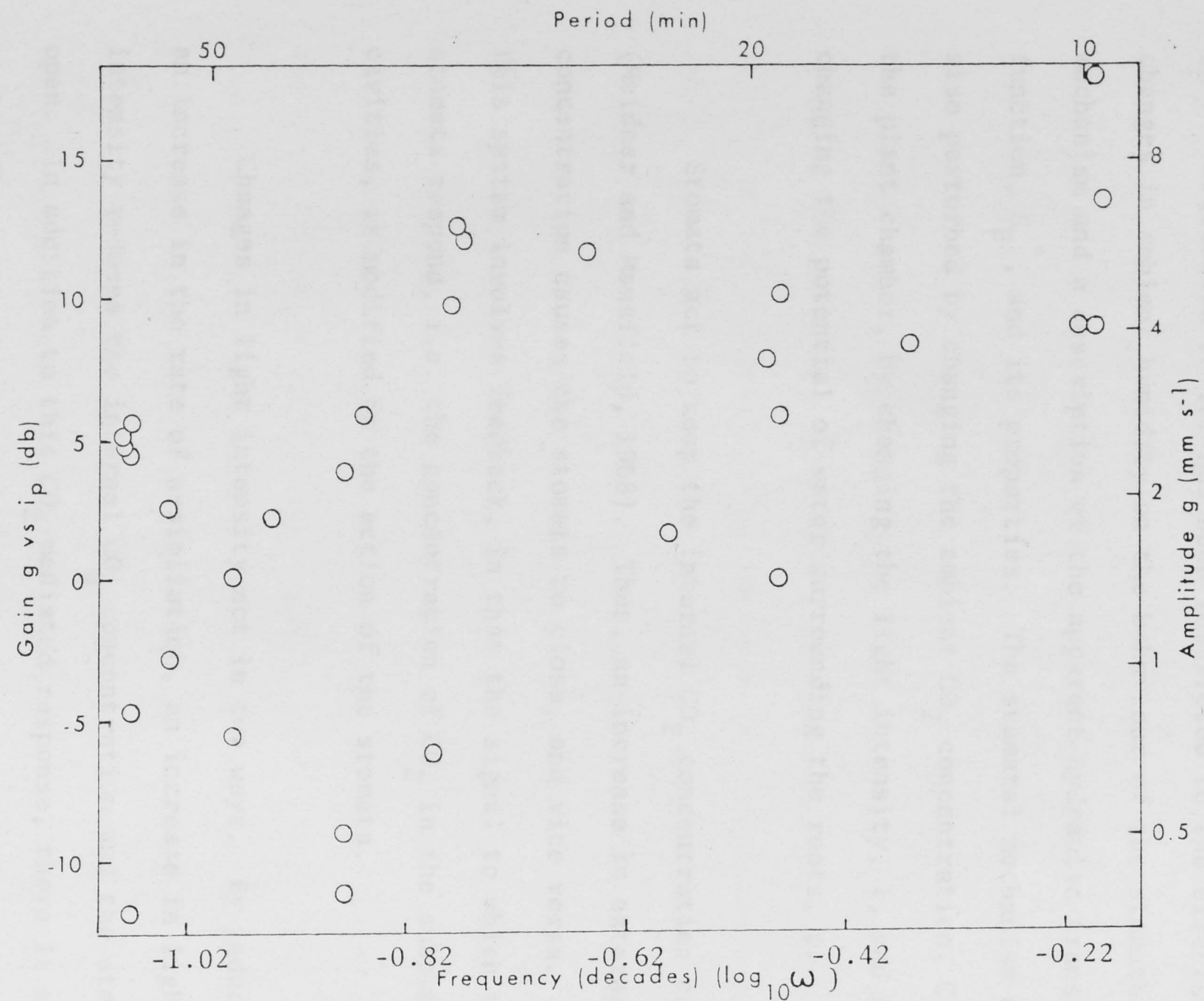


Fig. 3.13. Amplitude plot. i_p is the potential rate of evaporation that would occur if stomatal conductance were infinite.

CHAPTER 4

ANALYSIS OF STOMATAL RESPONSES TO OTHER ENVIRONMENTAL PARAMETERS

Discussion so far has been restricted to the effects of changes in ambient humidity on the behaviour of the stomatal mechanism and a description of the apparent hydraulic transfer function, G_E^* , and its properties. The stomatal mechanism was also perturbed by changing the ambient CO_2 concentration, C , in the plant chamber, by changing the light intensity, I , and by changing the potential of water surrounding the roots, ψ .

Stomata act to keep the internal CO_2 concentration constant (Meidner and Mansfield, 1968). Thus, an increase in external CO_2 concentration causes the stomata to close, and vice versa. This system involves feedback, in that the signal to which the stomata respond, i.e. the concentration of CO_2 in the substantial cavities, is modified by the action of the stomata.

Changes in light intensity act in two ways. By inducing an increase in the rate of assimilation, an increase in light intensity reduces the internal CO_2 concentration and the stomata open. In addition to this CO_2 -mediated response, there is also an immediate effect on the stomatal aperture (e.g. Meidner and Mansfield, 1968) which can be attributed to the photosynthetic

reduction of CO_2 by chloroplasts in the guard cells (Ketellapper, 1963). In contrast to the response to ambient humidity and CO_2 concentration, the response of stomata to changes in light intensity does not directly involve a feedback process. The same is true of the response of stomata to change in potential of water at the root surface which, by causing changes in leaf water potential, ψ_ℓ , effects the turgor of the guard cells and other cells in the epidermis.

Fig. 4.1 is a block diagram of the hydraulic and metabolic feedback control of the stomatal mechanism. (The effects of leaf temperature are included in the diagram but will not be discussed because leaf temperature was held constant in all experiments - changes in leaf temperature would not only affect guard cell metabolism, but would also activate both the hydraulic and metabolic feedback loops due to their effects on the rate of evaporation and the rate of assimilation.) The effect of stomatal conductance on the rate of assimilation is described by the metabolic environmental gain, $\partial A / \partial g_\ell$, the magnitude of which is a measure of the feedback in the metabolic loop. The true hydraulic transfer function, G_E , describes the effect of changes in the rate of evaporation on stomatal conductance, with the interaction of the metabolic feedback loop removed. Similarly, the true metabolic transfer function, G_{C_i} , describes the effect of changes in the internal CO_2 concentration

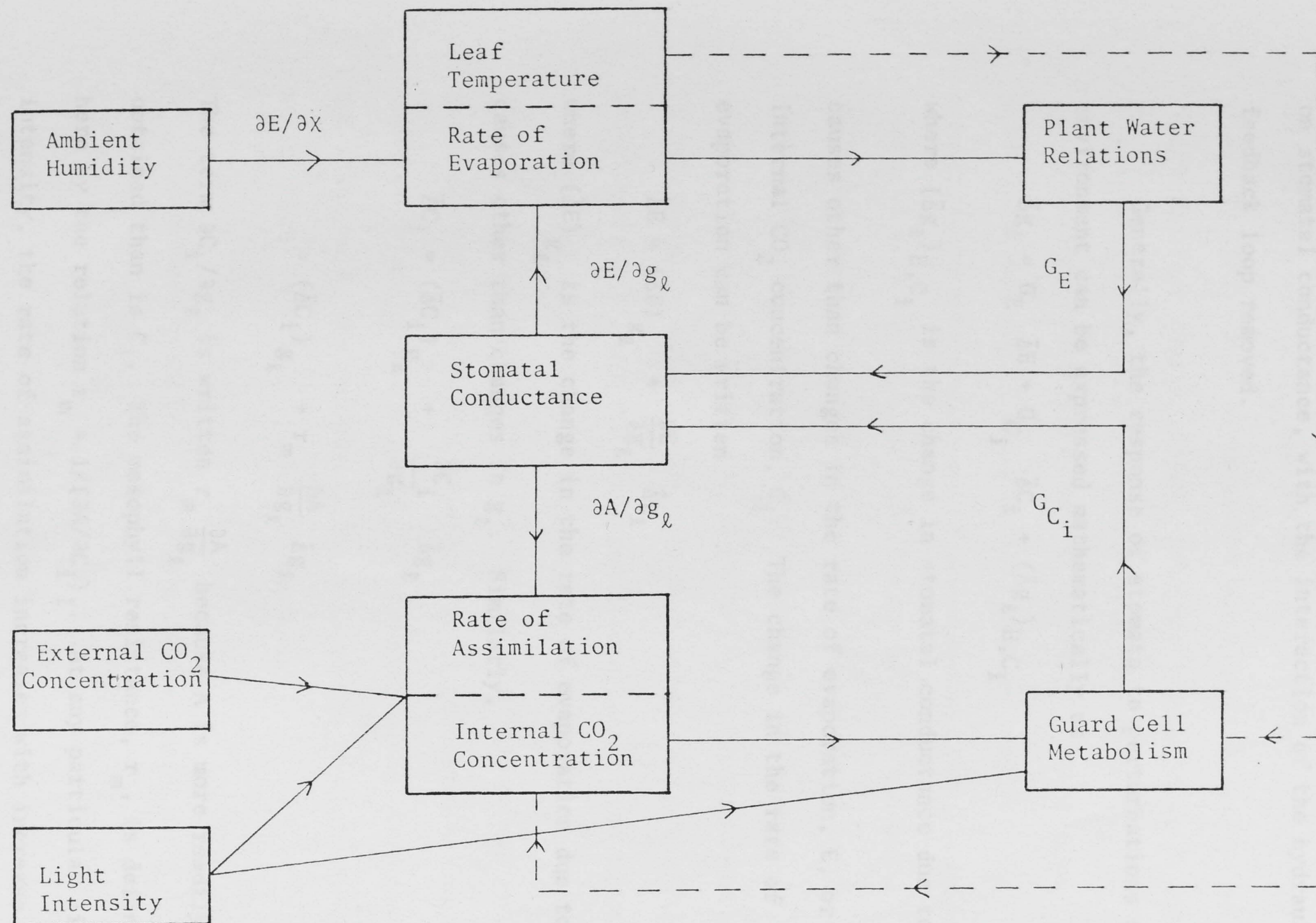


Fig.4.1. Block diagram of hydraulic and metabolic feedback control of stomatal mechanism.

on stomatal conductance, with the interaction of the hydraulic feedback loop removed.

Generally, the response of stomata to perturbations of environment can be expressed mathematically as

$$\bar{\Delta}g_{\ell} = G_E \bar{\Delta}E + G_{C_i} \bar{\Delta}C_i + (\bar{\Delta}g_{\ell})_{E,C_i} \quad (5)$$

where $(\bar{\Delta}g_{\ell})_{E,C_i}$ is the change in stomatal conductance due to

causes other than changes in the rate of evaporation, E , or the internal CO_2 concentration, C_i . The change in the rate of evaporation can be written

$$\bar{\Delta}E = (\bar{\Delta}E)_{g_{\ell}} + \frac{\partial E}{\partial g_{\ell}} \bar{\Delta}g_{\ell} \quad (6)$$

where $(\bar{\Delta}E)_{g_{\ell}}$ is the change in the rate of evaporation due to

causes other than changes in g_{ℓ} . Similarly,

$$\begin{aligned} \bar{\Delta}C_i &= (\bar{\Delta}C_i)_{g_{\ell}} + \frac{\partial C_i}{\partial g_{\ell}} \bar{\Delta}g_{\ell} \\ &= (\bar{\Delta}C_i)_{g_{\ell}} + r_m \frac{\partial A}{\partial g_{\ell}} \bar{\Delta}g_{\ell} \end{aligned} \quad (7)$$

The term $\partial C_i / \partial g_{\ell}$ is written $r_m \frac{\partial A}{\partial g_{\ell}}$ because A is more readily obtained than is C_i . The mesophyll resistance, r_m , is defined here by the relation $r_m = 1/(\partial A / \partial C_i)_I$. At any particular light intensity, the rate of assimilation increases with increase in internal concentration of CO_2 , up to a maximum value beyond which

further increase in CO_2 concentration has relatively little effect on the rate of assimilation which is then said to be "light-limited" (Heath, 1969). Over the region in which rate of assimilation increases linearly with increasing CO_2 concentration, assimilation is " CO_2 -limited". The maximum, light-limited, rate of assimilation, and the range of CO_2 concentration over which rate of assimilation is CO_2 -limited, increases with increase in light intensity. The mesophyll resistance is usually defined as $1/(\Delta A/\Delta C)$, where ΔA is the increase in rate of assimilation produced by an increase in CO_2 concentration, ΔC , in the region where CO_2 limits photosynthesis (Troughton and Slatyer, 1969). It is a measure of the diffusive resistance of CO_2 between the intercellular spaces and the sites of photosynthesis. The definition of r_m used here is a more general one, in that it does not imply linearity between rate of assimilation and CO_2 concentration. It is therefore a complex quantity depending not only on the processes of CO_2 transport and fixation in the mesophyll, but also on the characteristics of the light reactions in the chloroplasts.

Combining equations (5), (6) and (7) gives

$$\bar{A}g_\ell = \frac{G_E (\bar{A}E)_{g_\ell} + G_{C_i} (\bar{A}C_i)_{g_\ell} + (\bar{A}g_\ell)_{E, C_i}}{1 - G_E \frac{\partial E}{\partial g_\ell} - G_{C_i} r_m \frac{\partial A}{\partial g_\ell}} \quad (8)$$

The hydraulic environmental gain is evaluated as

$$\frac{\partial E}{\partial g_{\ell}} = \frac{E r_{\ell}^2}{r_b + r_{\ell}}$$

where r_{ℓ} is the stomatal resistance ($=1/g_{\ell}$) and r_b is the boundary layer resistance ($=1/h$). The metabolic environmental gain is

$$\frac{\partial A}{\partial g_{\ell}} = \frac{1.60Ar_{\ell}^2}{1.37r_b + 1.60r_{\ell} + r_m}$$

$$\text{or } r_m \frac{\partial A}{\partial g_{\ell}} = \frac{1.60Ar_{\ell}^2 r_m}{1.37r_b + 1.60r_{\ell} + r_m}$$

$$\approx 1.60Ar_{\ell}^2 \text{ for large values of } r_m.$$

(A full derivation of these and following coefficients is given in Appendix B). Large values of r_m occur if the rate of assimilation is light-limited. In these experiments, it is probable that rates of assimilation were more nearly limited by light intensity than by CO_2 concentration, especially with the lower light intensity which was only 8% full sunlight.

(Approximately 20% is required for light not to be limiting the rate of assimilation (Björkman, 1973).)

Equation (8) describes the response of stomatal conductance to any perturbation. It can be simplified for particular cases.

(i) Changes in ambient humidity.

The general term $(\bar{\Delta}E)_{g_\ell}$ in equation (8) becomes

$$\left(\frac{\partial E}{\partial X}\right)_{g_\ell} \bar{\Delta}X = - \frac{\bar{\Delta}X}{r_b + r_\ell}$$

while the other terms in the numerator are zero. Therefore,

$$\frac{\bar{\Delta}g_\ell}{\bar{\Delta}X} = \frac{G_E \left(\frac{\partial E}{\partial X}\right)_{g_\ell}}{1 - G_E \frac{\partial E}{\partial g_\ell} - G_{C_i} r_m \frac{\partial A}{\partial g_\ell}} \quad (9)$$

The term $G_{C_i} r_m \frac{\partial A}{\partial g_\ell}$ in the denominator accounts for the influence of the metabolic feedback loop in the response to an external variable, the primary affect of which is to activate the hydraulic feedback loop.

(ii) Changes in external CO_2 concentration.

Here, the general term $(\bar{\Delta}C_i)_{g_\ell}$ in equation (8) becomes

$$r_m (\bar{\Delta}A)_{g_\ell} = r_m \left(\frac{\partial A}{\partial C}\right)_{g_\ell} \bar{\Delta}C$$

while the other terms in the numerator are zero. Thus,

$$\frac{\bar{\Delta}g_\ell}{\bar{\Delta}C} = \frac{G_{C_i} r_m \left(\frac{\partial A}{\partial C}\right)_{g_\ell}}{1 - G_E \frac{\partial E}{\partial g_\ell} - G_{C_i} r_m \frac{\partial A}{\partial g_\ell}} \quad (10)$$

The inclusion of the term $G_E \frac{\partial E}{\partial g_\ell}$ in the denominator describes the role of the hydraulic feedback loop in a response which is

primarily due to activation of the metabolic feedback loop.

The coefficient of G_{C_i} in the numerator is

$$r_m \left(\frac{\partial A}{\partial C} \right)_{g_\ell} = \frac{r_m}{1.37r_b + 1.60r_\ell + r_m}$$

≈ 1 for large values of r_m .

(iii) Changes in the potential of water surrounding the roots.

If G_ψ is the transfer function describing the effect of changes in water potential at the root surfaces on stomatal conductance, then the general term $(\bar{\Delta}g_\ell)_{E, C_i}$ in equation (8) is

$$(\bar{\Delta}g_\ell)_{E, C_i} = G_\psi \bar{\Delta} \psi$$

while the other terms in the numerator are zero. Therefore,

$$\frac{\bar{\Delta}g_\ell}{\bar{\Delta}\psi} = \frac{G_\psi}{1 - G_E \frac{\partial E}{\partial g_\ell} - G_{C_i} r_m \frac{\partial A}{\partial g_\ell}} \quad (11)$$

(iv) Changes in light intensity.

If G_I is the transfer function describing the effect of changes in light intensity on stomatal conductance, then

$$(\bar{\Delta}g_\ell)_{E, C_i} = G_I \bar{\Delta} I$$

Changes in light intensity also affect internal CO_2 concentration and rate of assimilation (Fig. 4.1). Thus,

$$(\bar{\Delta}C_i)_{g_\ell} = \left(\frac{\partial C_i}{\partial I}\right)_{g_\ell} \bar{\Delta}I$$

$$\begin{aligned} \text{where } \left(\frac{\partial C_i}{\partial I}\right)_{g_\ell} &= - \frac{(1.37r_b + 1.60 r_\ell) r_m}{1.37r_b + 1.60 r_\ell + r_m} \left(\frac{\partial A}{\partial I}\right)_{C_i} \\ &\approx - (1.37r_b + 1.60 r_\ell) \left(\frac{\partial A}{\partial I}\right)_{C_i} \text{ for large values} \\ &\quad \text{of } r_m. \end{aligned}$$

Equation (8) then becomes

$$\frac{\bar{\Delta}g_\ell}{\bar{\Delta}I} = \frac{G_{C_i} \left(\frac{\partial C_i}{\partial I}\right)_{g_\ell} + G_I}{1 - G_E \frac{\partial E}{\partial g_\ell} - G_{C_i} r_m \frac{\partial A}{\partial g_\ell}} \quad (12)$$

If the four responses defined as $\bar{\Delta}g_\ell/\bar{\Delta}\chi$, $\bar{\Delta}g_\ell/\bar{\Delta}C$, $\bar{\Delta}g_\ell/\bar{\Delta}\psi$ and $\bar{\Delta}g_\ell/\bar{\Delta}I$ are determined experimentally and values for the (approximately constant) coefficients $\frac{\partial E}{\partial g_\ell}$, $r_m \frac{\partial A}{\partial g_\ell}$, $\frac{\partial E}{\partial \chi}$, $r_m \frac{\partial A}{\partial C}$ and $\frac{\partial C_i}{\partial I}$ are found, it is possible to solve the equations (9), (10), (11) and (12) simultaneously to obtain the transfer functions G_E , G_{C_i} , G_ψ and G_I . The apparent hydraulic transfer function, G_E^* , has already been examined and it is useful to see how this relates to the actual hydraulic transfer function, G_E .

(v) Changes in the rate of evaporation.

When change in the rate of evaporation is regarded as the perturbation, equation (8) can be written

$$\frac{\bar{\Delta}g_{\ell}}{\bar{\Delta}E} = \frac{G_E}{1 - G_{C_i} r_m \frac{\partial A}{\partial g_{\ell}}} = G_E^* \quad (13)$$

by substituting equation (7) into equation (5).

Combining equations (10) and (13), it follows that

$$\frac{\bar{\Delta}g}{\bar{\Delta}C} = \frac{G_{C_i} r_m \left(\frac{\partial A}{\partial C}\right) g_{\ell}}{1 - G_E^* (1 - G_{C_i} r_m \frac{\partial A}{\partial g_{\ell}}) - G_{C_i} r_m \frac{\partial A}{\partial g_{\ell}}}$$

This equation was used to evaluate G_{C_i} and the response G_E was then found from equation (13). Having obtained these transfer functions, which play a role in all stomatal responses, G_{ψ} and G_I were calculated from the expressions for $\bar{\Delta}g_{\ell}/\bar{\Delta}\psi$ and $\bar{\Delta}g_{\ell}/\bar{\Delta}I$, equations (11) and (12).

In the experimental determination of $\bar{\Delta}g_{\ell}/\bar{\Delta}C$, similar problems arose as for G_E^* . On many occasions, the stomatal conductance failed to reach a steady value within an acceptable time and, of the experiments for which transfer functions could be derived, expressions with real poles could be fitted to only a small proportion of the transfer function. Due to these difficulties and despite numerous repetition of the experiments, it was not possible to obtain both a satisfactory G_E^* and $\bar{\Delta}g_{\ell}/\bar{\Delta}C$ for the same plant with the same environmental conditions in the plant chamber during the experiments. Thus, the true hydraulic

and metabolic transfer functions, G_E and G_{C_i} , were not determined analytically. However, the same method can be used numerically and, while the resultant transfer functions cannot readily be inverted, thus giving equations for the response in stomatal conductance to particular perturbations, the shapes of the transfer functions can be used to obtain information about the stomatal behaviour. This procedure was used for typical examples of the experimental transfer functions $\bar{g}_\ell/\bar{\Delta}E$, $\bar{g}_\ell/\bar{\Delta}C$ and $\bar{g}_\ell/\bar{\Delta}I$ obtained on the same plant and $\bar{g}_\ell/\bar{\Delta}\psi$ obtained on a different plant. The method used to calculate the appropriate coefficients in each case is given in Appendix B. The calculated transfer functions G_E , G_{C_i} , G_I and G_ψ , plotted with the experimental transfer functions used in the derivations, are shown in Figs. 4.2, 4.3, 4.4 and 4.5, respectively.

The transfer function G_E is hardly modified by the interaction of the metabolic feedback loop, either in magnitude or in shape. Like G_E^* , G_E is positive for some values of p , so will become unstable with increasing environmental gain, $\partial E/\partial g_\ell$. Taking a very simplistic view of the mechanics of stomatal movement (Cowan, 1972), the region in which G_E increases with increase in $1/p$ (representing the rapid initial opening movement of stomata in response to a decrease in ambient humidity) can be associated with changes in turgor in the epidermal cells and the

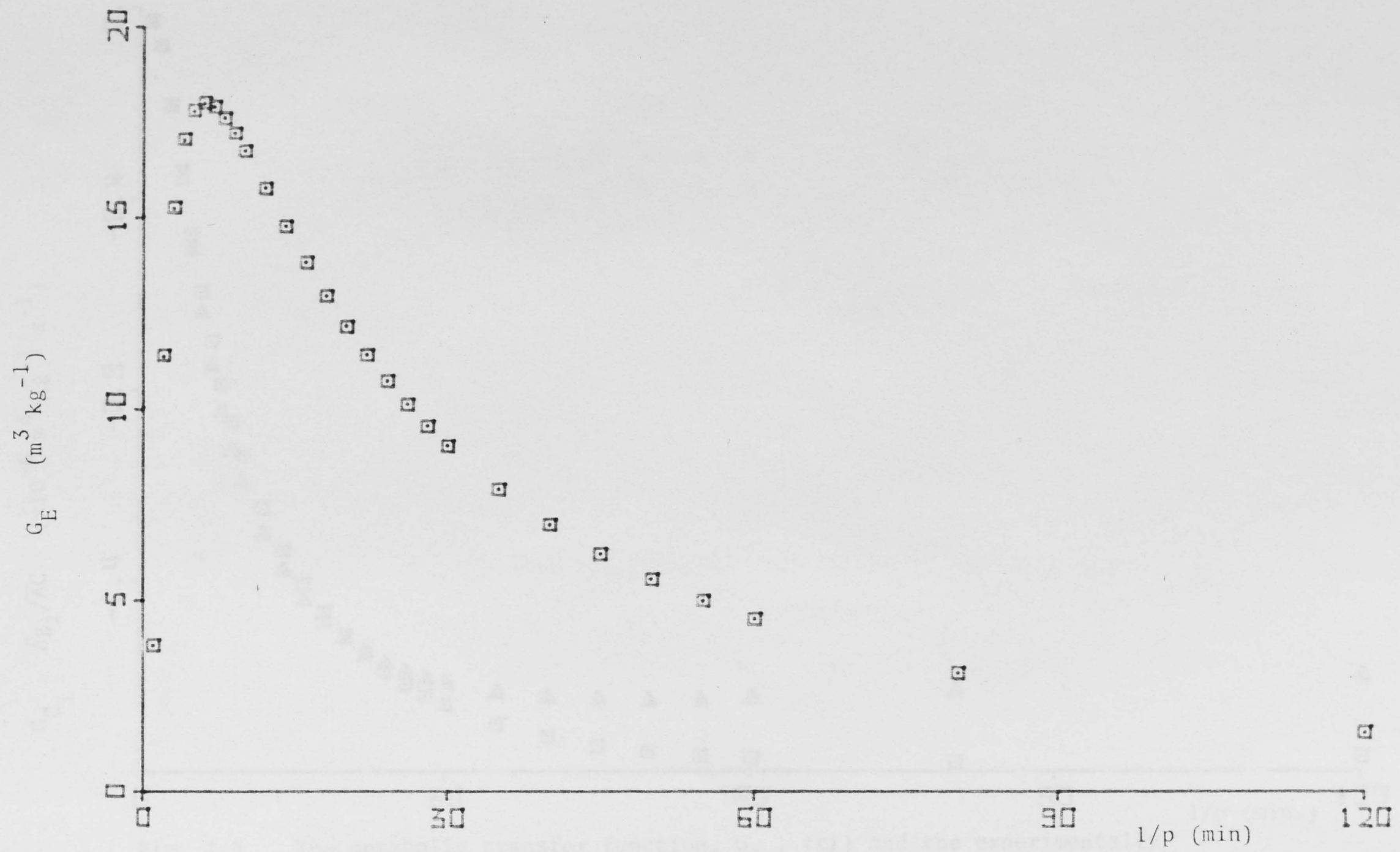


Fig. 4.2. The hydraulic transfer function, G_E .

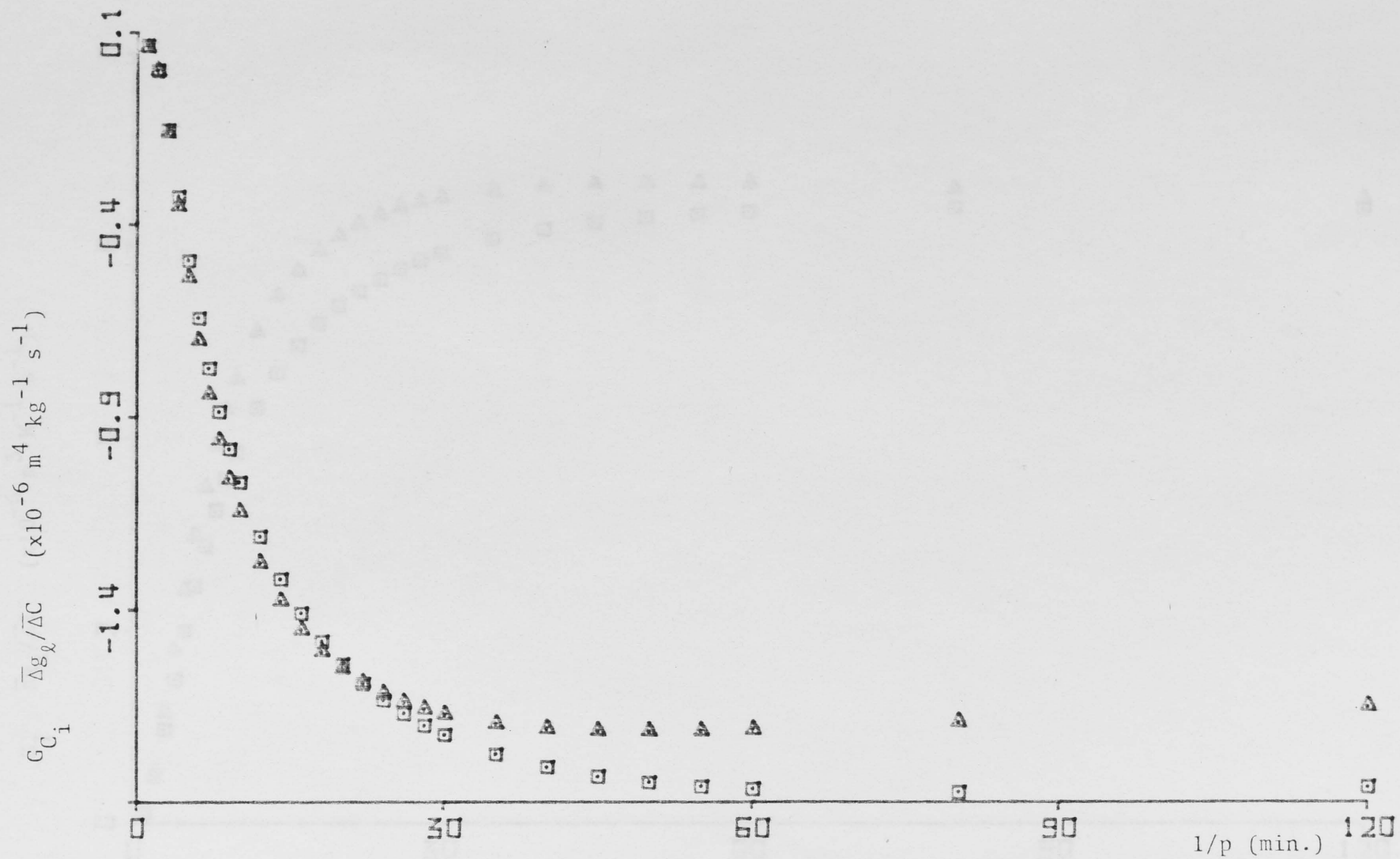


Fig. 4.3. The metabolic transfer function, G_{C_i} , ($\square$) and the experimentally determined transfer function, $\bar{\Delta}g_{\ell}/\bar{\Delta}\dot{C}^i$, (Δ)

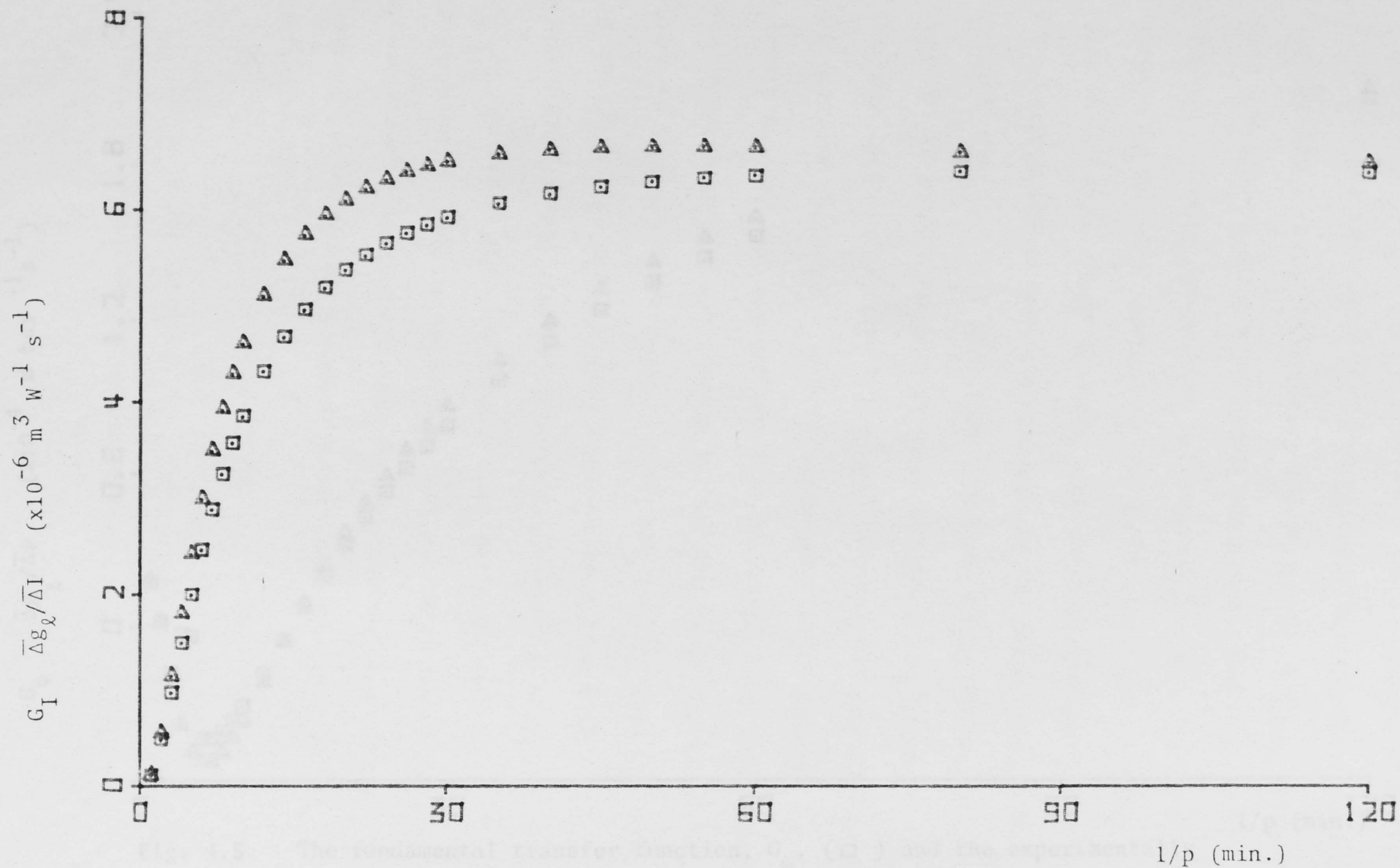


Fig. 4.4. The fundamental transfer function, G_I , ($\square$) and the experimentally determined transfer function, $\frac{\Delta g_\lambda}{\Delta I}$, (Δ)

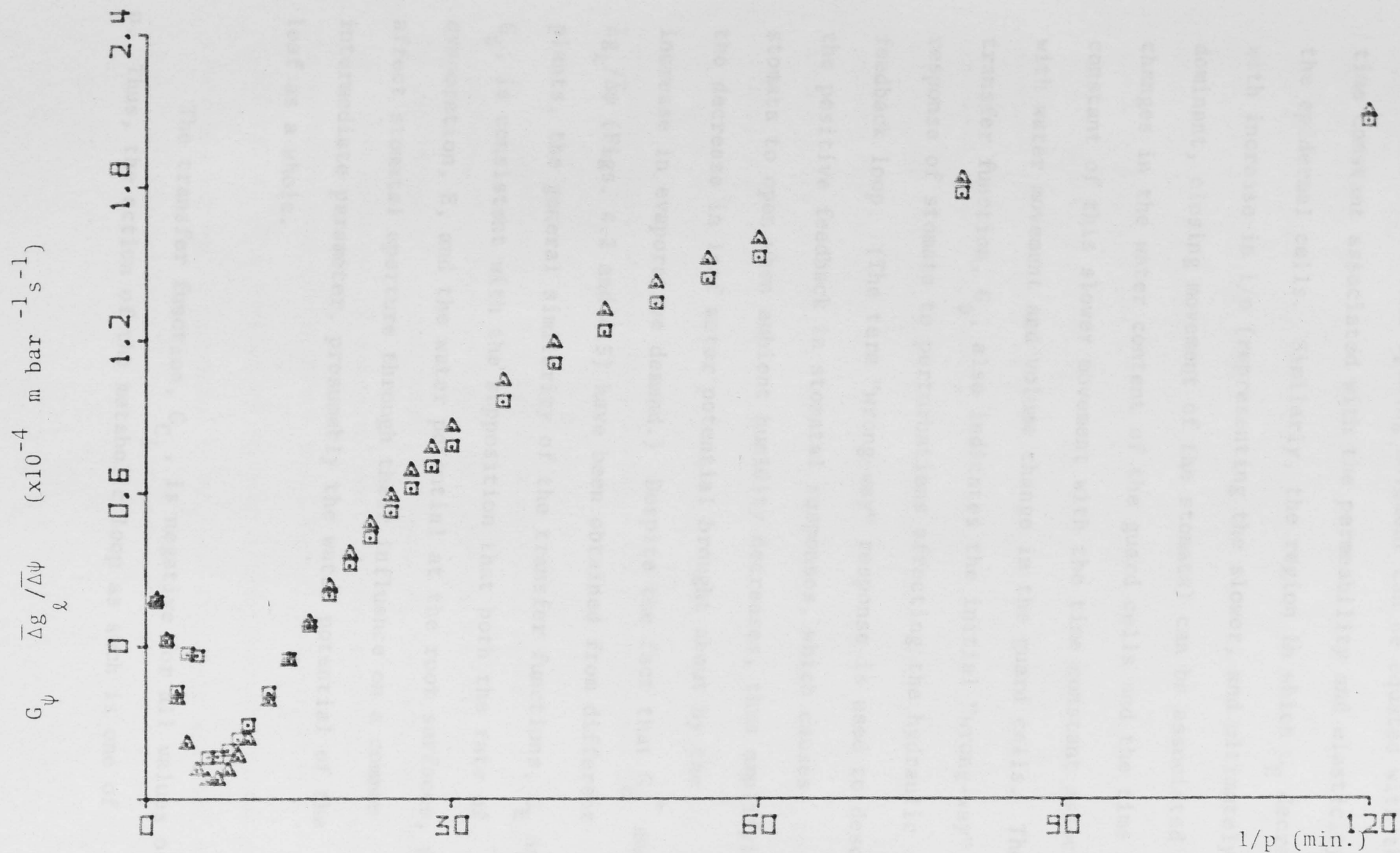


Fig. 4.5. The fundamental transfer function, G_{ψ} , ($\square$) and the experimentally determined transfer function, $\bar{\Delta g}_l / \bar{\Delta \psi}$, (Δ)

time constant of this opening movement can be equated with the time constant associated with the permeability and elasticity of the epidermal cells. Similarly, the region in which G_E decreases with increase in $1/p$ (representing the slower, and ultimately dominant, closing movement of the stomata) can be associated with changes in the water content of the guard cells and the time constant of this slower movement with the time constant associated with water movement and volume change in the guard cells. The transfer function, G_ψ , also indicates the initial "wrong-way" response of stomata to perturbations affecting the hydraulic feedback loop. (The term "wrong-way" response is used to describe the positive feedback in stomatal responses, which causes stomata to open when ambient humidity decreases, thus amplifying the decrease in leaf water potential brought about by the increase in evaporative demand.) Despite the fact that G_E^* and $\bar{\Delta}g_\ell/\bar{\Delta}\psi$ (Figs. 4.2 and 4.5) have been obtained from different plants, the general similarity of the transfer functions, G_E and G_ψ , is consistent with the supposition that both the rate of evaporation, E , and the water potential at the root surfaces, ψ , affect stomatal aperture through their influence on a common intermediate parameter, presumably the water potential of the leaf as a whole.

The transfer function, G_{C_i} , is negative for all values of p . Thus, the action of the metabolic loop as such is one of

negative feedback and the response in stomatal conductance to change in external CO_2 concentration is intrinsically stable for all values of the metabolic environmental gain, $\partial A / \partial g_\ell$. However, as has been shown in equation (8), the response is modified by the characteristics of the hydraulic feedback loop which tends to become unstable with increase in hydraulic environmental gain, $\partial E / \partial g_\ell$. The stability of the metabolic feedback loop coupled with the instability of the hydraulic feedback loop at large values of $\partial E / \partial g_\ell$ has led Cowan (1972) to suggest that the hydraulic feedback loop functions to enhance the rapidity of the response of stomata to changes which affect guard cell metabolism. This includes changes in light intensity as well as in external CO_2 concentration. Comparisons of $\bar{\Delta}g_\ell / \bar{\Delta}C$ with G_{C_i} (Fig. 4.3) and $\bar{\Delta}g_\ell / \bar{\Delta}I$ with G_I (Fig. 4.4) indicate some, very slight, evidence for this proposition.

Comparison of G_{C_i} and G_I shows that, ignoring sign and magnitude, the transfer functions have similar shapes. This may be indicative of a predominant influence of the time constant for water movement in the guard cells on the response of stomata to perturbations which affect the internal metabolism of the guard cells. The similarity implies either that the characteristic times for metabolic responses to CO_2 and light are similar, or that they are both small in comparison with the time relating to

the movement of water and changes in guard cell volume which take place as a result of changes in metabolic activity. On the whole, the second possibility seems to be most likely, because the nature of the transfer functions, G_{C_i} and G_I , is somewhat similar to that of G_E at large values of $1/p$. Certainly, the time course indicated by G_{C_i} and G_E is much slower than that relating to the initial opening movement of stomata in response to hydraulic perturbation, which has been tentatively related to the properties of the epidermal, as distinct from the guard, cells.

CHAPTER 5

CONCLUSIONS

The use of transfer functions, based on the application of the Laplace transformation to experimental data, in describing and predicting the response of stomata to changes in environmental parameters has been demonstrated. From the shapes of these transfer functions, some inferences about the characteristics of various processes involved in stomatal movement may be made. The hydraulic transfer function, G_E , is positive for some values of the Laplace operator, p , indicating positive feedback in the reaction of stomata to change in evaporation rate and that the stomatal system can become unstable at large values of the hydraulic environmental gain, $\partial E / \partial g_\ell$. The frequency response of the stomatal mechanism to change in ambient humidity can be calculated from the transfer function, G_E^* . The effect of increase in hydraulic environmental gain can, again, be seen in the shape of the Bode diagrams. With increasing values of $\partial E / \partial g_\ell$, the undamped natural frequency, ω_n , becomes more sharply defined and the peak in the amplitude response increases in magnitude.

The function representing stomatal response to change in water potential surrounding the roots, G_ψ , is similar in shape to G_E and therefore suggests that both the rate of evaporation and base water potential act on the stomatal mechanism through

a common parameter, probably potential of water in the leaf as a whole. The metabolic transfer function, G_{C_i} , describing the response of stomatal conductance to change in internal concentration of CO_2 , is negative for all values of p . Thus, the response of stomatal conductance to change in external CO_2 concentration is one of negative feedback. The negative feedback increases with increase in the metabolic environmental gain, $\partial A / \partial g_{\ell}$, and the feedback loop involved exerts a stabilising influence on the stomatal system as a whole. The shape of G_{C_i} is similar to that of G_I , the transfer function describing the response of stomatal conductance to change in light intensity. This suggests either that the times characterising the responses of guard cell metabolism to change in CO_2 concentration and light intensity are similar, or that they are both short compared with the time constant for water movement and volume change in the guard cells.

The rigour of this approach depends on the degree to which the basic structure of the feedback loops involved in the control of the stomatal aperture can be defined. It is thought that ambient humidity might have a direct effect on the stomatal aperture, in addition to effects through perturbations of the hydraulic feedback loop. Although there is no evidence to support the idea, changes in external CO_2 concentration may have a similar direct effect on the stomatal aperture.

To develop this technique further, measurements of leaf

water potential should be included in experiments, and the model of stomatal behaviour, which is used as a basis for the analysis, should be extended to include the direct effect of ambient humidity on the stomatal aperture. Similar studies should be made on plants from a wide ranging variety of natural habitats to determine from the various transfer functions, the type of stomatal control that has developed. It may be possible from such studies, to associate different forms of stomatal behaviour with the exigencies of environmental conditions in various habitats. For example, plants from a harsh desert environment would be expected to control stomatal aperture to conserve water much more than would plants from a swamp habitat.

The most important use of the transfer functions is to rigorously describe stomatal behaviour. The application of control theory to this biological system allows a quantitative description of the complex mechanism involved in the control of stomatal aperture.

APPENDIX A

Derivation of the series expansion of the Laplace transform

(after Dr. I.R. Cowan)

Consider a function $y(t)$ such that $y(0) = 0$ and $y(t) = y_N$, a constant for $t \geq N$. The Laplace transform of the function is

$$\bar{y} = \int_0^{\infty} y e^{-pt} dt = \int_0^N y e^{-pt} dt + y_N \frac{e^{-Np}}{p}$$

Suppose that values of y are measured at unit intervals of time from $t=0$ to $t=N$, and that $y(t)$ is approximately linear within each interval. Thus, the contribution to the Laplace transform corresponding to the m th interval is

$$\begin{aligned} \int_m^{m+1} y_m e^{-pt} dt &= \int_m^{m+1} [y_m + (t-m)(y_{m+1}-y_m)] e^{-pt} dt \\ &= \frac{e^{-pm}}{p} [y_m - y_{m+1} e^{-p} + \frac{1}{p} (y_{m+1} - y_m) (1 - e^{-p})] \end{aligned}$$

Summing over N intervals,

$$\int_0^N y e^{-pt} dt = \frac{(1-s)^2}{sp^2} \sum_{m=1}^N y_m e^{-mp} - \frac{(s+p-1)}{p^2} y_N e^{-Np}$$

where $s = e^{-p}$.

Thus,

$$\int_0^{\infty} y e^{-pt} dt = \frac{(1-s)^2}{sp^2} \sum_{m=1}^N y_m e^{-mp} + \frac{(1-s)}{p^2} y_N e^{-Np} \quad (A.1)$$

The Laplace transforms, $\bar{\Delta}C$ and $\bar{\Delta}C_i$, are evaluated from the equation (A.1). For example,

$$\bar{\Delta}C = \frac{(1-s)^2}{sp^2} \sum_{l=1}^N (C_m - C_o) e^{-mp} + \frac{(1-s)}{p^2} (C_N - C_o) e^{-Np}$$

where C_N is the steady state value of the external CO_2 concentration and C_o is the initial value.

A similar expression is written for $\bar{\Delta}C_i$.

In chapter 3, the derived expression for ΔE is

$$\begin{aligned} \bar{\Delta}E = (1+\beta p) \int_{\tau}^{\infty} (E_s - E_o) e^{-pt} dt - \beta [E_s(\tau) - E_o] e^{-p\tau} \\ + \frac{\chi_o - \chi_{\infty}}{\chi'_o - \chi_o} \frac{E_o}{p} (1 - e^{-p\tau}) \end{aligned}$$

The integral term in this expression is evaluated in the following manner. Writing $t' = t - \tau$,

$$\int_{\tau}^{\infty} (E_s - E_o) e^{-pt} dt = e^{-p\tau} \int_0^{\infty} [E_s(t') - E_o] e^{-pt'} dt'$$

Then, denoting the values of $E_s(t')$ as E_o' , E_1' , ..., E_n' , ..., E_N' , where E_N' is the steady state value of the rate of evaporation,

$$\begin{aligned} \int_0^{\infty} [E_s(t') - E_o] e^{-pt'} dt' \\ = \int_0^{\infty} [E_s(t') - E_o'] dt' + \frac{E_o' - E_o}{p} \end{aligned}$$

$$= \frac{(1-s)^2}{sp^2} \sum_{1}^N (E_m' - E_o') e^{-mp} + \frac{1-s}{p^2} (E_N' - E_o') e^{-Np} + \frac{E_o' - E_o}{p}$$

expanding the integral by using equation (A.1). But,

$$\sum_{1}^N E_o' e^{-mp} = E_o' \sum_{1}^N e^{-mp} = E_o' \frac{s}{1-s} (1 - e^{-Np})$$

Thus,

$$\int_0^{\infty} [E_s(t') - E_o] e^{-pt'} dt' = \frac{(1-s)^2}{sp^2} \sum_{1}^N E_m' e^{-mp} + \frac{1-s}{p^2} (E_N' e^{-Np} - E_o') + \frac{E_o' - E_o}{p} \quad (3.1)$$

Similarly, the rate of assimilation is given by

$$A = \frac{C + F}{1.37r_0 + 1.60r_1 + r_m} \quad (3.2)$$

and thus, the metabolic environmental gain is

$$\frac{\Delta A}{\Delta r_0} = \frac{1.60 Ar_1}{1.37r_0 + 1.60r_1 + r_m}$$

Further coefficients are required when equation (3) is simplified for particular perturbations.

APPENDIX B

Evaluation of coefficients in the equations for the response of stomatal conductance to perturbations of environment.

Equation (8) in Chapter 4 describes the response of stomatal conductance to any perturbation. The coefficients of G_E and G_{C_i} are the hydraulic and metabolic environmental gains, respectively and are evaluated in the following way. The rate of evaporation is given by

$$E = \frac{x_\ell' - x}{r_b + r_\ell} \quad (B.1)$$

and therefore, the hydraulic environmental gain is

$$\frac{\partial E}{\partial g_\ell} = \frac{Er_\ell^2}{r_b + r_\ell}$$

Similarly, the rate of assimilation is given by

$$A = \frac{C - \Gamma}{1.37r_b + 1.60r_\ell + r_m} \quad (B.2)$$

and thus, the metabolic environmental gain is

$$\frac{\partial A}{\partial g_\ell} = \frac{1.60 Ar_\ell^2}{1.37r_b + 1.60r_\ell + r_m}$$

Further coefficients are required when equation (8) is simplified for particular perturbations.

(i) Changes in ambient humidity.

The coefficient $\partial E/\partial \chi$ is evaluated from equation (B.1).

$$\left(\frac{\partial E}{\partial \chi}\right)_{g_\ell} = - \frac{1}{r_b + r_\ell}$$

(ii) Changes in external CO_2 concentration.

Similarly, the coefficient $\partial A/\partial C$ is found from equation (B.2).

$$\left(\frac{\partial A}{\partial C}\right)_{g_\ell} = \frac{1}{1.37r_b + 1.60r_\ell + r_m}$$

(iii) Changes in light intensity.

To evaluate the coefficient $\partial C_i/\partial I$, a change in the rate of assimilation is written as

$$\begin{aligned} \Delta A &= \left(\frac{\partial A}{\partial C_i}\right)_I \Delta C_i + \left(\frac{\partial A}{\partial I}\right)_{C_i} \Delta I \\ &= \frac{\Delta C_i}{r_m} + \left(\frac{\partial A}{\partial I}\right)_{C_i} \Delta I \end{aligned} \quad (\text{B.3})$$

The rate of assimilation is also given by

$$A = \frac{C - C_i}{1.37r_b + 1.60r_\ell}$$

$$\text{Thus, } \Delta A = - \frac{\Delta C_i}{1.37r_b + 1.60r_\ell} - \frac{1.60A\Delta r_\ell}{1.37r_b + 1.60r_\ell} - \frac{\Delta C}{1.37r_b + 1.60r_\ell} \quad (\text{B.4})$$

At constant external CO_2 concentration and constant g_ℓ ,

$$\Delta A = - \frac{\Delta C_i}{1.37r_b + 1.60r_\ell}$$

Substituting into equation (B.3) gives

$$\left(\frac{\partial C_i}{\partial I}\right)_{g_\ell} = - \frac{(1.37r_b + 1.60r_\ell)r_m}{1.37r_b + 1.60r_\ell + r_m} \left(\frac{\partial A}{\partial I}\right)_{C_i}$$

Most of these expressions for the coefficients involve the mesophyll resistance, r_m , which can be evaluated using equations (B.3) and (B.4). At constant light intensity, equation (B.3) is

$$\Delta A = \frac{\Delta C_i}{r_m}$$

Substituting into equation (B.4) gives

$$r_m = - \frac{1.60A\Delta r}{\Delta A} - 1.37r_b - 1.60r_\ell + \frac{\Delta C}{\Delta A}$$

At constant external CO_2 concentration, this equation reduces to

$$r_m = - \frac{1.60A \Delta r}{\Delta A} - 1.37r_b - 1.60 r_\ell$$

Both equations for r_m apply only at constant light intensity.

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