RESPONSES OF *CITRUS* SPECIES TO WATER DEFICIT AND SALINITY STRESS

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CONTENTS

CHAPTER TWO. GAS EXCHANGE OF 'VALENCIA' ORANGE AS AFFECTED BY SOIL AND LEAF WATER STATUS

CHAPTER THREE. EFFECT OF ROOTZONE SALINISATION ON GAS EXCHANGE OF 'VALENCIA' ORANGE

SUMMARY

The effect of soil water deficit and high rootzone concentrations of NaCl on citrus leaf gas exchange was investigated in laboratory studies and in the field. For 'Valencia' orange scions budded on Sweet orange rootstock, soil water deficit resulted in similar reductions in CO_2 assimilation and stomatal conductance. This inhibition of gas exchange was not solely a consequence of more negative leaf water potentials as exposure of leaf discs to water potentials associated with minimal gas exchange rates for leaves on plants subjected to soil water deficit resulted in no diminution in photosynthetic capacity. Furthermore, orchard studies showed gas exchange of 'Valencia' orange scions on Trifoliata rootstock to be inhibited to a lesser degree by soil water deficit than 'Valencia' orange scions budded on Sweet orange stock. This was despite more negative leaf water potentials for scions on Trifoliata.

Laboratory studies showed high rootzone salinities to also reduce leaf gas exchange of 'Valencia' orange. This was despite leaf turgor maintenance in salinised leaves due to more negative osmotic potentials more than offsetting more negative soil water potentials. The response of 'Valencia' orange to salinity was also dependant upon rootstock. Scions budded on Trifoliata were less sensitive to rootzone salinisation than those on Cleopatra mandarin rootstock. This was despite higher levels of chloride in leaves on plants budded to Trifoliata. Leaves of 'Valencia' orange scion budded to Cleopatra mandarin had higher sodium levels than equivalent foliage on Trifoliata suggesting that sodium, rather than chloride may be responsible for reduced rates of gas exchange in salinised 'Valencia' orange leaves.

Foliar sodium and chloride levels were dependant upon scion as well as rootstock. Extent of impact of rootzone salinisation of leaf gas exchange varied accordingly. Despite equivalent rootstocks and rootzone salinities, leaves of 'Marsh' grapefruit scion had higher concentrations of sodium and chloride as well as lower rates of gas exchange than

'Valencia' orange scions. Leaves of 'Prior Lisbon' lemon rootstocks also had much higher concentrations of chloride than leaves of 'Valencia' orange on the same rootstocks. Although there was little or no increase in sodium concentrations in 'Prior Lisbon' lemon leaves gas exchange was strongly inhibited by salinity indicating that chloride may inhibit citrus gas exchange in some circumstances. Orchard studies did however show that leaf chloride concentrations as high as 400 mol m^{-3} could be tolerated without any reductions in gas exchange. Where reduced rates of gas exchange were observed with salinity in the field this was invariably associated with high concentrations of leaf sodium rather than chloride.

Leaf gas exchange responses to both soil water deficit and rootzone salinity were similar in many ways. Inhibition of CO_2 assimilation occurred to a greater extent at high intercellular partial pressures of CO_2 (p_i), reductions in CO_2 assimilation and stomatal conductance at ambient conditions were usually in step resulting in little change in p_i and room temperature chlorophyll fluorescence kinetics were unaltered despite large reductions in gas exchange. The possibility of a common mechanism is discussed.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma at any University. To the best of my belief this thesis contains no material previously published or written by any other person except where due reference is made in the text.

I consent to this thesis being made available for photocopying and loan after acceptance for the award of the degree.

(Jonathan James Lloyd)

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PART A

LABORATORY STUDIES

"The lemon seems to be the least tolerant of all the fruit trees, (for) it was stunted by only 1,440 pounds sodium chloride per acre"

(Loughridge, 1901)

CHAPTER ONE

PHOTOSYNTHETIC CHARACTERISTICS OF *CITRUS* SPECIES

1.1 INTRODUCTION

Photosynthesis is the process by which plants, algae and a few species of bacteria use energy contained within light to generate stored chemical energy. This chemical energy is used not only by these organisms but is the initial source of energy for all living cells.

In higher organisms the predominant substrate for photosynthesis is CO_2, being converted to carbohydrates in the chloroplast of the mesophyll cells of most higher plants.

1.1.1 Biochemistry of CO_2 assimilation

Three modes of CO_2 fixation have been identified in higher plants being C_3, C_4 and CAM (Crassulasean Acid Metabolism). The C_3 or Photosynthetic Carbon Reduction (PCR) Cycle (Bassham and Calvin, 1957) is fundamental to all plants, the C_4 and CAM cycles first fixing CO_2 via other reactions. As all citrus species examined so far show characteristics of the C_3 mode discussion is confined to the PCR cycle.

Carbohydrate formation in the PCR cycle is dependent upon the carboxylation of ribulose-1,5-bisphosphate (RuP_2) to form 3-phosphoglycerate (3PGA) via the enzyme ribulose-bisphosphate carboxylase/oxygenase (rubisco). As evidenced by its nomenclature this enzyme also has a substantial oxygen fixing (oxygenase) activity (Badger and Andrews, 1974). Reactions subsequent to oxygenation are incorporated in the photorespiratory carbon oxidation (PCO) cycle. The oxygenase reaction accompanies carboxylation under atmospheric conditions resulting in formation of phosphoglycolate (Bowes *et al.*, 1971) which is eventually metabolised to 3PGA via a series of reactions in peroxisomes and mitochondria (Lorimer and Andrews, 1981). 3PGA (from the PCR or PCO cycle) is then shunted through a complex series of reactions resulting in the regeneration of RuP_2 (Figure 1.1). Of the many enzymes involved in RuP_2 regeneration, the fructose and septoheptulose bisphosphatases as well as ribulose-5-phosphate (Ru5P) kinase are considered to play a regulatory role (Halliwell, 1984). In addition to these enzymes, the availability of ATP and NADPH from light reactions is also of crucial importance in determining the rate of RuP_2 regeneration.

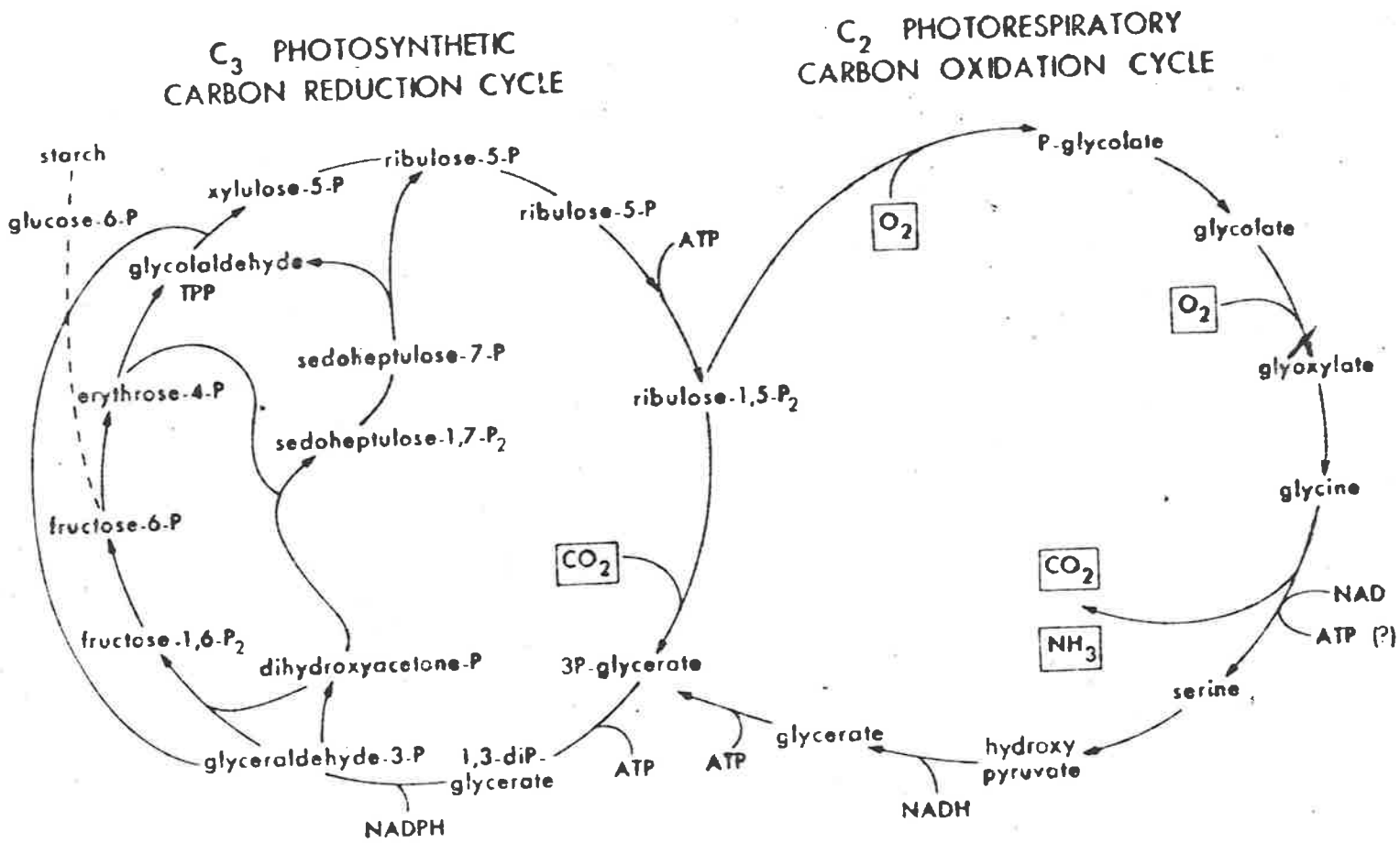


Figure 1.1 Reactions of the Photosynthetic Carbon Reduction (PCR) cycle and Photorespiratory Carbon Oxidation (PCO) cycle. (from Osmond *et al.*, 1980).

1.1.2 The light reactions of photosynthesis

The light reactions of photosynthesis, which take place in the thylakoid membranes of the chloroplast generate the ATP and NADPH required to drive CO_2 fixation in the stroma. The primary event is the absorption of light energy by chlorophyll or other pigment molecules resulting in the formation of higher electronic excitation states (excited singlet states). Energy so absorbed can be lost by re-emission of a photon to give fluorescence, be lost via thermal de-excitation or it may be transferred to an adjacent molecule (Butler, 1980).

Within the thylakoid membrane there exist certain special molecules of chlorophyll *a* which, when excited, can transfer electrons to a neighbouring electron acceptor (Q). These chlorophyll molecules are referred to as *reaction centre chlorophylls*. Each reaction centre chlorophyll is associated with an array of other pigment molecules that can transfer energy to it. This complex is referred to as a *photosynthetic unit*. Two types of reaction centre have been identified in higher plants, referred to as P_{680} and P_{700}. The subscript refers to the wavelengths of maximum absorption. Each reaction centre has its own light-harvesting antenna system; complexes containing P_{700} are referred to as Photosystem I (PSI) whilst those containing P_{680} are known as Photosystem II (PSII). Both photosystems can be served by the same light harvesting chlorophyll (LHC) complex although they are separated spatially on the thylakoid membrane (Satoh and Fork, 1983). The two photosystems act in series to extract an electron from water and transfer it to a redox level sufficient to operate the PCR and PCO cycles. This is achieved via an electron transport chain that connects the two photosystems and allows electrons to pass from PSI to $NADP^+$ forming NADPH (Fig. 1.2). Electrons from the P_{680} reaction centre are eventually accepted by a molecule known as Q which may be a plastoquinone molecule. From Q they are transferred to the plastoquinone (PQ) pool. From plastoquinone electrons flow through a non-haem-iron-sulphur protein (the Rieske Fe-S protein) and then via cytochrome F and plastocyanin to PSI. Absorption of light by P_{700} enables electrons to be passed to an unidentified acceptor and then to a ferridoxin NADP reductase complex where reduction of $NADP^+$ to NADPH can occur (Haenhl, 1984).

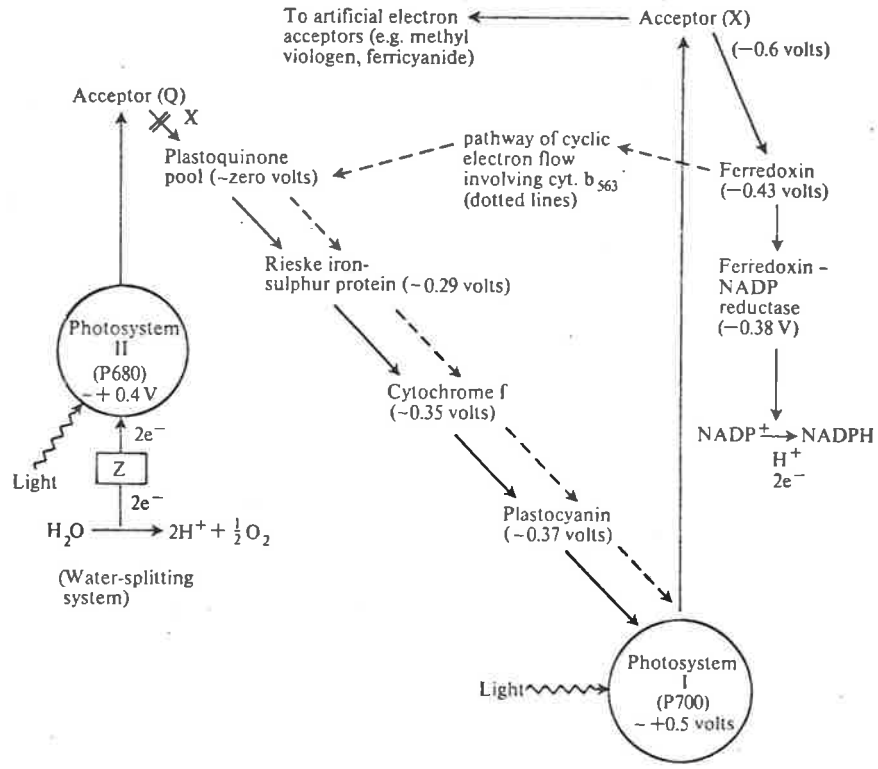


Figure 1.2 The electron transport chain. The sequence of carriers is presented in the order of their approximate redox potentials (shown in parenthesis in volts) (from Halliwell, 1984).

The flow of electrons to more positive redox potentials along the electron transport chain from Q to P_{700} provides energy to drive photophosphorylation. ATP synthesis associated with this flow is referred to as *non-cyclic phosphorylation*. *Cyclic phosphorylation* may also occur in which electrons are transported from ferridoxin to the plastoquinone pool (Fig. 1.2). A third form of phosphorylation also exists referred to as *pseudocyclic phosphorylation* where oxygen acts as the final electron acceptor (Furbank *et al.*, 1983). This allows ATP production in the absence of $NADP^+$ reduction and may be important in maintaining high rates of electron transport.

1.1.3 Room temperature chlorophyll fluorescence

As mentioned in the previous section, as well as being used for photochemical reaction in photosynthesis, energy absorbed by a chlorophyll molecule can also be lost via thermal de-excitation or re-emitted as fluorescence. Fluorescence emission from a leaf comes from chlorophyll *a* associated with PSII and the associated LHC. Intensity of emission from a leaf following excitation is determined by the extent of the other two competing processes. Following adaptation in the dark for a suitable length of time, upon illumination fluorescence induction occurs (Kaustky effect) with characteristic phases (Fig. 1.3). Dark adaptation allows the electron transport chain to "run down". In this state the primary electron acceptor (Q) is fully oxidised (Krause and Weis, 1983).

The rise to O occurs almost simultaneously upon illumination and represents emission by antenna chlorophyll *a* molecules before excitations have migrated to reaction centres (Mathis and Piallotin, 1981). The yield of O (also called F_O) is dependent on the distribution of energy between the two reaction centres (Catt *et al.*, 1984) and structural conditions that affect the probability of excitation transfer between antenna pigments and PSII reaction centres (Schreiber and Armond 1978).

The slower rise from O to I lasts approx. 150 msec and is thought to reflect adjustment of electron carriers in the neighbourhood of PSII reaction centres to the light environment (Papageorgiou, 1975). This "activation" phenomenon appears to correlate with the onset of oxygen evolution from the water splitting apparatus (Joilot, 1968).

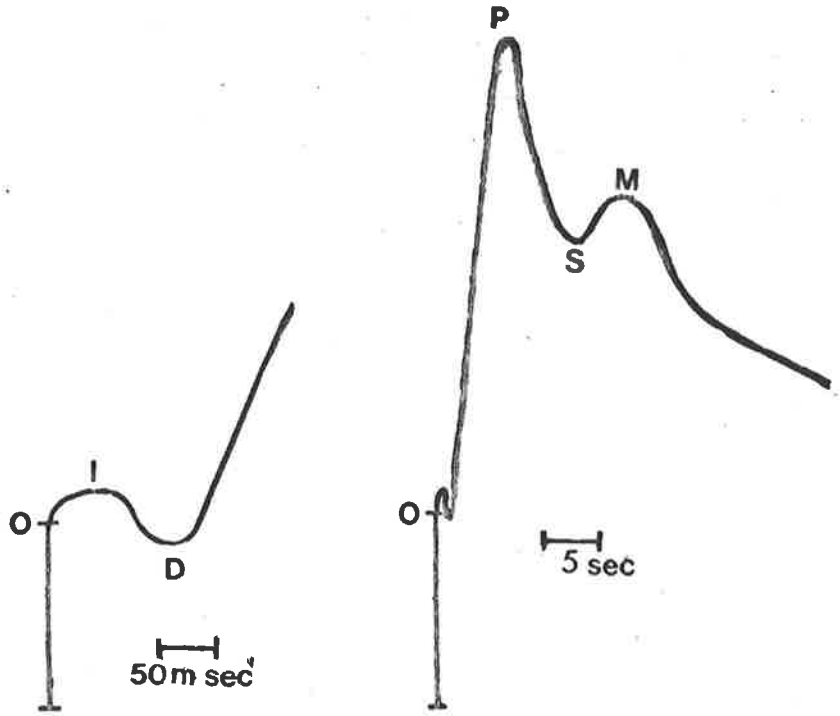


Figure 1.3 Characteristic room temperature fluorescence induction kinetics upon illumination of a dark adapted leaf. For explanation of phases see Section 1.1.3.

Upon completion of this phase electrons are rapidly transferred from the PSII reaction centre through the PQ pool to PSI with a consequent cessation of the fluorescence rise. At any time during the I-P rise the slope is a balance between reduction of Q by PSII and oxidation via PSI. Where the rate of reoxidation exceeds that of reduction a decline to D may be observed (Munday and Govindjee, 1969). Flow of electrons through PSII is however temporary due to a limited electron acceptor pool (Satoh and Katoh, 1981). This has been attributed to reoxidation of NADPH occurring only after a lag phase during which the PCR cycle is activated (Krause and Weis, 1983). This cessation in electron flow causes fluorescence once again to rise. After activation of the carbon reduction cycle (and associated regeneration of $NADP^+$) electron transport through PSI recommences. At P the rate of oxidation of Q exceeds reduction and the decline in fluorescence (quenching) commences. The rise from O to P is therefore dependent upon:

1. Capacity of PSII to reduce Q
2. Capacity of PSI to reoxidize Q
3. Extent of transient block in PSI

The main factor determining the extent of the rise from O to P is considered to be the capacity of PSII. This variable fluorescence (F_V) is often divided by the constant yield fluorescence (F_O) as the latter gives an indication of the number of photons channelled towards PSII (Krause and Weis, 1983). The ratio F_V/F_O is therefore used by many workers to indicate PSII activity, though as discussed above, causes for variations in F_V may be complex.

Only part of the decline from P to S (or T) can be explained by increased PSI activity. Krause *et al.* (1982) have shown that, due to proton pumping in the light, cation exchange processes at the thylakoid surface cause ultrastructural alterations in thylakoid surface membranes which results in an increase in thermal de-excitation and hence a decline in fluorescence (an increase in quenching).

Although fluorescence induction is clearly a complex phenomenon, it has proved to be highly sensitive to changes in leaf water status (Govindjee *et al.*, 1981) salinisation (Smillie and Nott, 1982); freezing damage (Smillie and Hetherington, 1983) heat stress

(Downton and Berry, 1982) and high light stress (Critchley and Smillie, 1981) providing a useful *in vivo* probe to locate the site of stress induced lesions within the chloroplast.

1.1.4 Theory of gas exchange measurement

In terrestrial plants the majority of CO_2 used in photosynthetic metabolism (CO_2 assimilation) is derived from the atmosphere, diffusing to the chloroplast via stomatal pores. As the vapor pressure inside the leaf is generally considered to be the saturation pressure at leaf temperature (Tyree and Yianoulis, 1980) water loss from leaf to atmosphere is generally an inevitable consequence of stomatal opening. It is generally accepted that stomata function to prevent dessication whilst still allowing the passage of CO_2 to the chloroplast (Farquhar and Sharkey, 1982).

The dependence of transpiration rate (E) on conductance to diffusion of water vapour (g) can be expressed:

$$E = g\,(e_i - e_a)/P \qquad (1.1)$$

where e_i and e_a are the vapour pressures of water inside and outside the leaf respectively, and P is the atmospheric pressure. Transpiration also results in convection effects in stomatal pores (Parkinson and Penman, 1970) which when combined with equation 1.1 (Jarman, 1974; von Caemmerer and Farquhar, 1981) yields:

$$E = \frac{g(e_i - e_a)}{P - (e_i + e_a)/2} \qquad (1.2)$$

In analogy with Equation 1.1, CO_2 diffusion into a leaf may also be described.

$$A = g_c\,(p_a - p_i)/P \qquad (1.3)$$

where A is the CO_2 assimilation rate g_c represents the stomatal conductance to CO_2, and p_a and p_i are the partial pressures of CO_2 in the air outside and inside the leaf. Conductances to CO_2 (g_c) and H_2O (g) are related via the ratio of their binary

diffusivities of H_2O/air and CO_2/air which is now accepted to be 1.6 (Jarvis, 1971).

The mass flow of H_2O evaporating from the leaf carries with it some CO_2 (Parkinson and Penman, 1970). Jarman (1974) analysed this effect allowing von Caemmerer and Farquhar (1981) to incorporate it into equation 1.3 yielding:

$$A = g_c (p_a - p_i) - \frac{(p_i + p_a)}{2} E \qquad (1.4)$$

This may be rearranged allowing p_i to be derived

$$p_i = \frac{(g_c - E/2)p_a - A}{g_c + E/2} \qquad (1.5)$$

The validity of p_i so determined has recently been verified by direct measurement (Sharkey *et al.*, 1982).

1.1.5 Regulation of CO_2 assimilation in intact leaves

As shown by previous discussions, a large number of physical and chemical reactions could regulate the rate of CO_2 assimilation in leaves. Current models of photosynthesis (Farquhar *et al.*, 1980; von Caemmerer and Farquhar, 1981; Farquhar and von Caemmerer, 1982) have used the p_i calculation in conjunction with variations in p_a to create curves of A versus p_i such as that illustrated in Figure 1.4. As outlined below, analysis of these curves may provide considerably more information than measurements of gas exchange parameters at ambient p_a alone.

At low p_i rubisco is considered to be saturated with respect to its substrate RuP_2. Following the initial effect of p_i on enzyme activation a linear increase in A with p_i is observed. It has been shown that the slope of the increase (dA/dp_i) is proportional to the maximum activity of rubisco in the leaf (von Caemmerer and Farquhar, 1981; von Caemmerer and Farquhar, 1984). At higher p_i it is considered that the capacity to regenerate RuP_2 via the PCO and PCR cycles becomes limiting and that the level at which this is reached is dependent upon electron transport capacity which is in turn dependent upon absorbed irradiance (von Caemmerer and Farquhar, 1981). In this region the rates

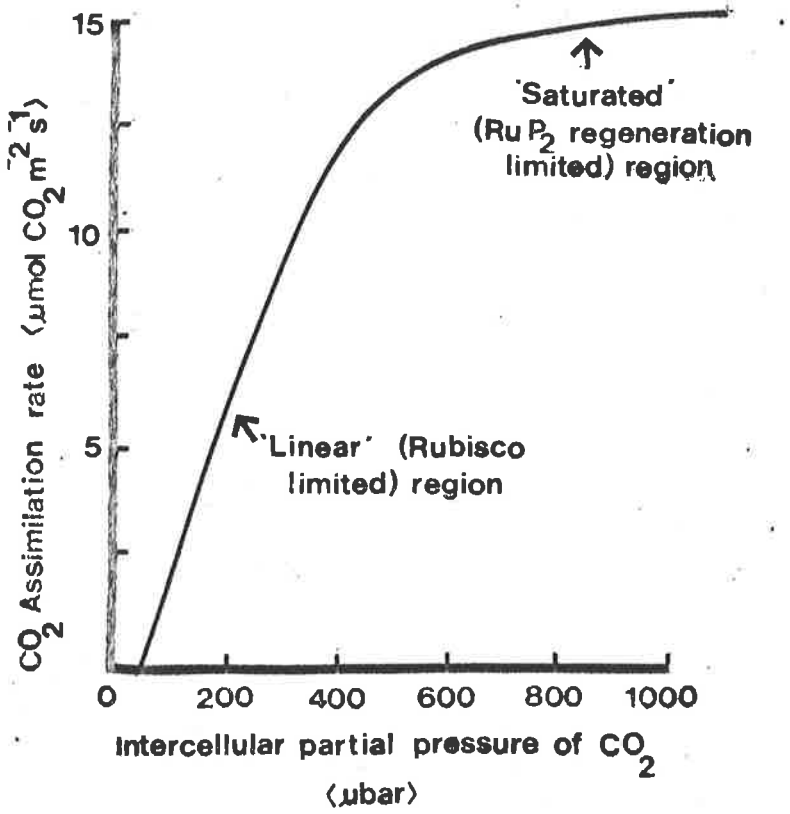


Figure 1.4 Characteristic curve of CO_2 assimilation *versus* intercellular partial pressure of CO_2. For discussion on interpretation of such curves see Section 1.1.5.

of electron transport and of ATP, NADPH and RuP_2 regeneration are all virtually independent of CO_2, but assimilation rate still increases somewhat with p_i as RuP_2 is increasingly diverted from the PCO to the PCR cycle. Thus when assimilation is limited by light level, rubisco activity should be regulated by RuP_2 availability (Sharkey, 1985a). However, measurements of steady state intermediates in leaves indicates that enzyme activation rather than substrate availability may regulate rubisco activity under light limiting conditions (Badger *et al.* 1984; Mott *et al.*, 1984; Taylor and Terry, 1984). The steps leading to rubisco deactivation under these conditions remain to be established, but low ATP levels are believed to be the primary limiting factor (Sharkey, 1985a). Stromal ATP plays an important role in maintaining stromal pH (Robinson, 1985a) and as discussed by Sharkey *et al.* (1986a) low ATP levels would be expected to result in a build up of PGA and H^+ inside the chloroplast with a subsequent lowering of stromal pH. This lowering of pH would then be expected to deactivate rubisco (Miziorko and Lorimer, 1983) as well as Ru5P kinase (Gardemann *et al.*, 1983) and both stromal bisphosphatases (Enser and Huber, 1980; Flügge *et al.*, 1980) allowing regulation of all control points of the cycle in a co-ordinated manner. Thus, although under situations of light limited photosynthesis regeneneration of RuP_2 is limiting, the PCO cycle may be co-ordinated in such a way that negative feedback interactions are minimised.

Partial pressures of O_2 as present at atmospheric levels inhibit the net rate of CO_2 assimilation. This is because

(i) the PCO cycle releases CO_2
(ii) O_2 competes with CO_2 in binding to rubisco
(iii) diversion of RuP_2 into the PCO cycle reduces availability of RuP_2 for the PCR cycle

When CO_2 assimilation is limited by rubisco activity, O_2 inhibition is a consequence of mechanisms (i) and (ii) whilst when RuP_2 regeneration limits CO_2 assimilation mechanisms (i) and (iii) should be important (Farquhar *et al.*, 1980). Whilst measured levels of oxygen inhibition at low p_i are consistent with both theory and current kinetic parameters for rubisco (von Caemmerer and Farquhar, 1981; Badger *et al.*, 1984) it has often been observed that at high p_i an increase in CO_2 assimilation with decreasing

O_2 pressure is not always observed (Jollife and Tregunna, 1973; Viil *et al.*, 1977; Badger *et al.*, 1984; Sharkey, 1985b). It has been suggested that this phenomenon is due to the rate of CO_2 assimilation being limited by the rate of utilization of products of the PCO and PCR cycles rather than by electron transport or enzymic activities (Sharkey, 1985b; Sharkey *et al.*, 1986b). As explained below, the utilization of triose phosphates for sugar and starch synthesis may be limiting for CO_2 assimilation in such cases (Sharkey, 1985b; Sharkey *et al.*, 1986ab).

Although the end product of CO_2 assimilation is generally sucrose, this molecule is not synthesized in the chloroplast (Figure 1.5). Rather, dihydroxy-acetonephosphate (DHAP) is transported across the chloroplast envelope (Walker, 1974) where phospotriose isomerase brings about the rapid equilibration of PGA and DHAP in the cytoplasm. These combine to form fructose-1,5-bisphosphate which then undergoes a series of reactions to form sucrose, releasing 2 HPO_4^{2-} (P_i) molecules in the process (Heber, 1974). P_i must re-enter the chloroplast, and this occurs via a specific translocator which exchanges P_i for the triose phosphates (Walker, 1974). Failure to recycle P_i back into the chloroplast results in a reduced capacity for ATP formation and hence presents a limitation to CO_2 assimilation (Sharkey, 1985b; Sharkey *et al.*, 1986ab). In addition to this, rubisco activity may be reduced as P_i is required for maximum rubisco activity (Heldt *et al.* 1978). The other main end product of CO_2 assimilation is starch. As shown in Figure 1.5, a similar limitation can also occur when rapid rates of starch synthesis are required.

Although there is now good evidence for P_i limitation of CO_2 assimilation at high CO_2 and light levels (Sharkey, 1986b; Stitt, 1986) the importance of this "feedback" regulation at ambient CO_2 partial pressures remains to be determined. Azcón-Bieto (1983) did observe, however, that a gradual decline in CO_2 assimilation over several hours in *Triticum aestivum* was correlated with decreases in O_2 sensitivity and reduced rates of RuP_2 regeneration, both symptoms of triose phosphate limitations (Sharkey, 1985ab). It should also be noted that O_2 plays a direct role in electron transport via the "Mehler reaction" (Section 1.1.2). This reaction is only half saturated at a $p(O_2)$ of 80 mbar (8%;

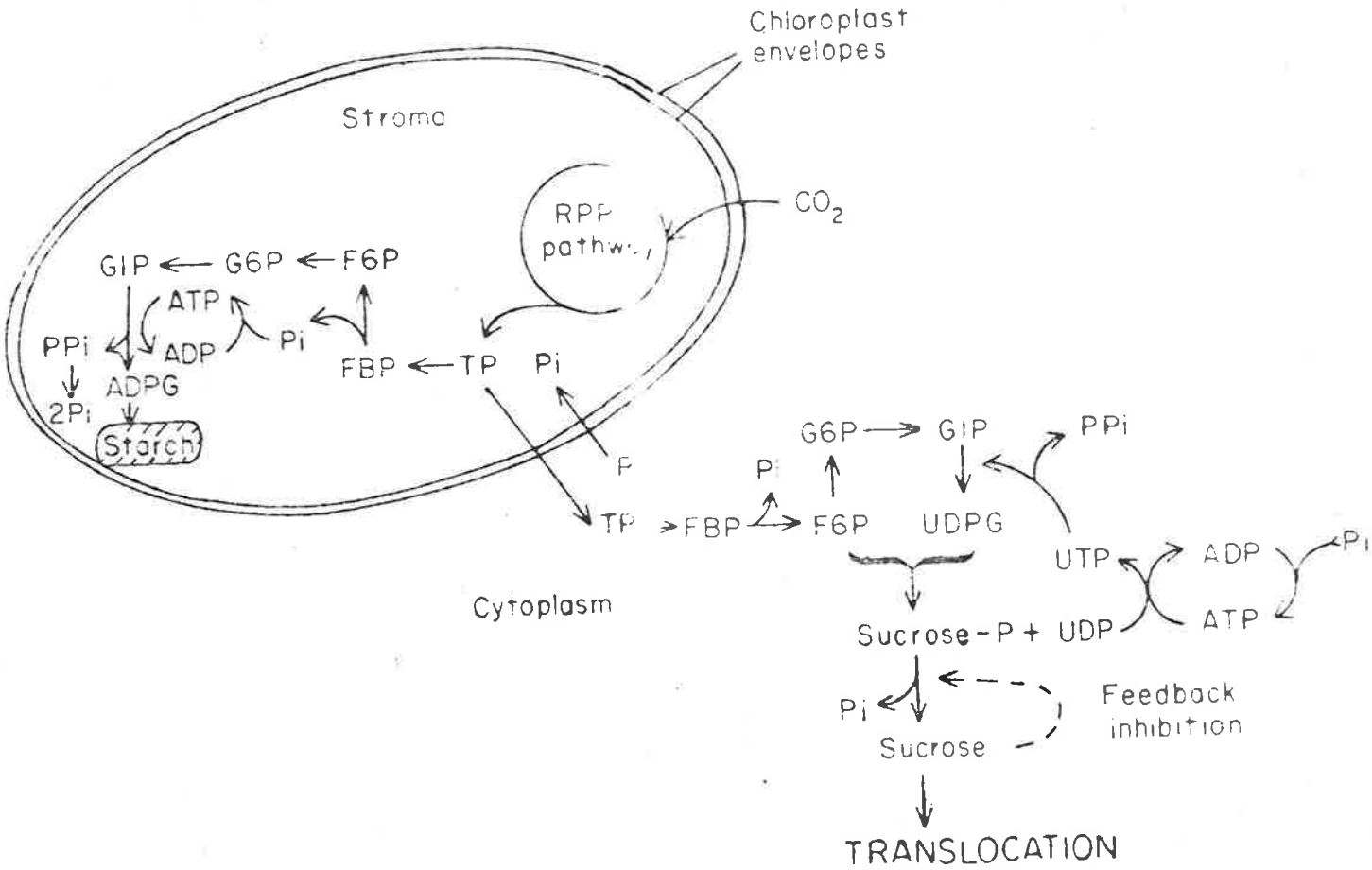


Figure 1.5 Paths of sucrose and starch synthesis (from Edwards and Walker, 1983)

Radmer *et al.*, 1978). Thus an increased requirement for O_2 at high rates of electron transport may also explain the relative insensitivity of CO_2 assimilation at high p_i and light absorbance.

The above discussion illustrates that a considerable amount of information can be obtained from A/p_i curves especially when the extent of oxygen inhibition is also examined. It should be emphasized that the p_i calculation takes into account stomatal conductance (Equation 1.5) and thus any environmental perturbations which affect stomatal conductance only will not change the A/p_i curve itself, only the p_i at which the leaf is operating under a given set of environmental conditions. It has often been considered that the drawdown of CO_2 from the stomatal cavity to the chloroplast is not appreciable (Farquhar and Sharkey, 1982; Farquhar and von Caemmerer, 1982). This assumption has led many workers to analyse A/p_i curves directly in terms of chloroplast biochemistry. More recently however, it has been shown that the drawdown from the intercellular spaces to the chloroplast is larger than previously thought though less than across the stomatal pores (Evans *et al.*, 1986). Nevertheless, as this "internal conductance" (g_i) is most likely finite a change in the form of an A/p_i curve can still validly be interpreted as some alteration in chloroplast functioning.

1.1.6 Photosynthetic characteristics of *Citrus* spp.

In comparison to most other plants *Citrus* spp. are characterised by relatively low rates of CO_2 assimilation (Table 1.1).

Type of Plant	Species	Maximum Assimilation Rate (μmol CO_2 m^{-2} s^{-1})	Reference
Most agricultural plants		13-30	Larcher (1980)
Most herbaceous heliophytes		13-35	Larcher (1980)
Angiospermous trees			
	Populus nigra	32	Nelson (1984)
	Malus domestica	27	Kennedy (1981)
	Prunus persica	20	De Jong &Doyle (1985)
	Eucalyptus pauciflora	20	Wong *et al.* (1978)
Citrus spp.	*C. sinensis*	12	Khairi & Hall (1976a)
		11	Lenz (1978)
		9	Syvertsen (1984)
		8	Kriedemann (1971)
	C. paradisi	15	Sinclair & Allen (1982)
		9	Syvertsen (1984)

Table 1.1: Maximum CO_2 assimilation rates of various plant types (Based on Nelson, 1984).

This is despite high leaf chlorophyll contents (Syvertsen and Smith, 1984). Reasons for these low rates remain to be established. Nitrogen is considered to be a major factor contributing to variation in CO_2 assimilation rates in many plants (Brown, 1978; Evans, 1983). Large amounts of N are required for both assembly of the electron transport chain (Evans and Terashima, 1987) and for incorporation into rubisco (Evans, 1983). Indeed, for *Oryza sativa* approx. 75% of the nitrogen in a leaf is contained in chloroplast proteins (Morita and Kono, 1975). It does not however appear that sub-optimal leaf nitrogen levels are responsible for low CO_2 assimilation rates in citrus as Syvertsen (1984) measured nitrogen levels in 'Pineapple' orange and 'Duncan' grapefruit comparable to other well fertilised crops (Brown, 1978). *Citrus* spp. thus have an extremely low

nitrogen use efficiency. Syvertsen (1984, 1987) measured rates of approx. 3-5 mmol CO_2 g^{-1} N s^{-1} whilst for *Prunus persica*, De Jong (1982) recorded values of 6-7 mmol CO_2 g^{-1} N s^{-1}. For wheat Evans (1983) observed values around 5 mmol CO_2 g^{-1} N s^{-1}. High levels of nitrogen in *Citrus* leaves are however reflected in rubisco activity as Vu *et al.* (1985) measured a rubisco activity of ~360 μmol CO_2 (mg chl)$^{-1}$ hr^{-1} for 'Valencia' orange leaves. Using a chlorophyll content of 70 μg cm^{-2} (Chapter 4) this translates to a maximum CO_2 assimilation rate of 70 μmol CO_2 m^{-2} s^{-1}. Low CO_2 assimilation in *Citrus* spp. does not thus appear to be due to an inherently low rubisco activity.

One hypothesis accounting for low CO_2 assimilation rates in *Citrus* spp. was presented by Kriedemann (1971) who suggested that the extensive suberisation of mesophyll cell walls (Scott *et al.*, 1948) presents a substantial barrier to CO_2 diffusion (i.e. a low g_i). This low g_i coupled with relatively low stomatal conductances (Kriedemann, 1971; Khairi and Hall, 1976ab) would then place significant limitations upon diffusion of CO_2 from atmosphere to chloroplast and hence constrain CO_2 assimilation. Prior to study of gas exchange responses to water deficit and salinity stress, fundamental characteristics of citrus leaf gas exchange were thus determined. Experiments described in this chapter investigate the effect of leaf age on gas exchange as well as providing fundamental information on electron transport capacities and resistances to CO_2 transfer to the site of carboxylation within the leaf.

1.2 PHOTOSYNTHETIC CHARACTERISTICS OF CITRUS LEAVES DURING ONTOGENY

1.2.1 Introduction

Studies with a wide range of annual species have shown, in general, as a leaf expands a rapid and co-ordinated increase in CO_2 assimilation rate and stomatal conductance occurs. Following a peak in gas exchange generally coinciding with the time of full leaf expansion a slow decline is observed (Sesták, 1981). There is however, little information in the literature on ontogenic patterns in gas exchange for citrus. As leaves may be retained on Citrus trees for up to two years or more (Erickson, 1968) it was

particularly interesting to determine if there is a stage in citrus leaf development where gas exchange characteristics remain reasonably constant. Choice of appropriately aged material is especially necessary in stress physiology studies with *Citrus* spp. as leaf osmotic potential (Syvertsen *et al.*, 1981) and stomatal response to water deficit (Syvertsen, 1982) both vary considerably with leaf age.

1.2.2 Materials and Methods

1.2.2.1 Growth of Plants

Two year old 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) on Sweet orange rootstock (*C. sinensis* cv. Parramatta sweet orange) were grown in a constant temperature room under 450 μmol m^{-2} s^{-1} photosynthetically active radiation provided by sodium vapor lamps and shade cloth. Photoperiod was 9 h with a 25/22° day/night temperature and plants were watered daily with half strength Hoaglands solution (Hoagland and Arnon, 1950). Plants had been grown under these conditions for 4 months before young developing leaves were tagged upon emergence to enable future sampling for gas exchange measurements. Gas exchange characteristics of individual leaves were measured every 8 days or so thereafter as the leaves expanded and hardened. Upon completion of leaf expansion gas exchange characteristics were compared with leaves from a growth flush that had developed approximately six months earlier.

1.2.2.2 Gas exchange measurements

Rates of CO_2 assimilation and water vapour loss were measured for laminae as a single attached leaf totally enclosed in a water jacketed cuvette with a clear glass lid. Illumination was provided by a tungsten halogen projector lamp filtered through a hot mirror. Photosynthetic photon flux density (PPFD) at the leaf surface was 1000 μmol m^{-2} s^{-1}. Within the cuvette a fan circulated air past the leaf at a sufficient rate to keep the boundary layer conductance of the leaf surface greater than 2.5 mol H_2O m^{-2} s^{-1} as determined by the rate of H_2O evaporation from saturated filter paper 'leaves' (Jarvis, 1971). Flow rate through the cuvette was 0.5-1.5 litres min^{-1} determined by mass flow

controllers. Varying CO_2 partial pressures were obtained by mixing air from calibrated CO_2 standard gases with a stream of CO_2 free air (scrubbed with soda-lime) via mass flow controllers. The mixture was then saturated at 18°C with water vapor and H_2O entering the chamber was measured with a Vaisala HM60601 capacitive sensor. The same capacitive sensor was used to determine the H_2O vapour pressure of air expelled from the chamber. The difference in partial pressures of CO_2 in the ingoing and outgoing air streams was measured directly with an ADC Mark III infrared gas analyser operating on the differential mode. Leaf temperature was sensed by means of a copper constantan thermocouple placed on the under surface of the leaf blade and maintained at 25.0 ± 0.5°C. Gas exchange parameters were calculated as described in Section 1.1.4.

1.2.3 Results

Rates of net CO_2 assimilation at ambient $p(CO_2)$ (350 μbar) increased rapidly during the leaf expansion stage reaching a maximum after approximately 35 days after which a gradual decline occurred. In a similar fashion to net CO_2 assimilation, leaf conductance increased with leaf expansion reaching a maximum value around the same time as net CO_2 assimilation (Fig. 1.6a). This rise was initially less rapid resulting in a rapid decline in p_i as leaves aged (Fig. 1.6b). Examination of A/p_i curves shows that, initially, CO_2 assimilation increased linearly with p_i at all p_i examined and CO_2 compensation points as calculated from the A/p_i curve were very high (Fig. 1.7:220 μbar at Day 12). As leaves developed, the initial slope of the A/p_i curve increased and CO_2 assimilation at high p_i became saturated. The CO_2 compensation point also decreased rapidly with leaf age reaching a minimum at day 17 after which it remained constant.

Comparison of gas exchange characteristics of different aged leaves showed little difference between 2 month and 6 month old leaves in any of the measured parameters (Table 1.2).

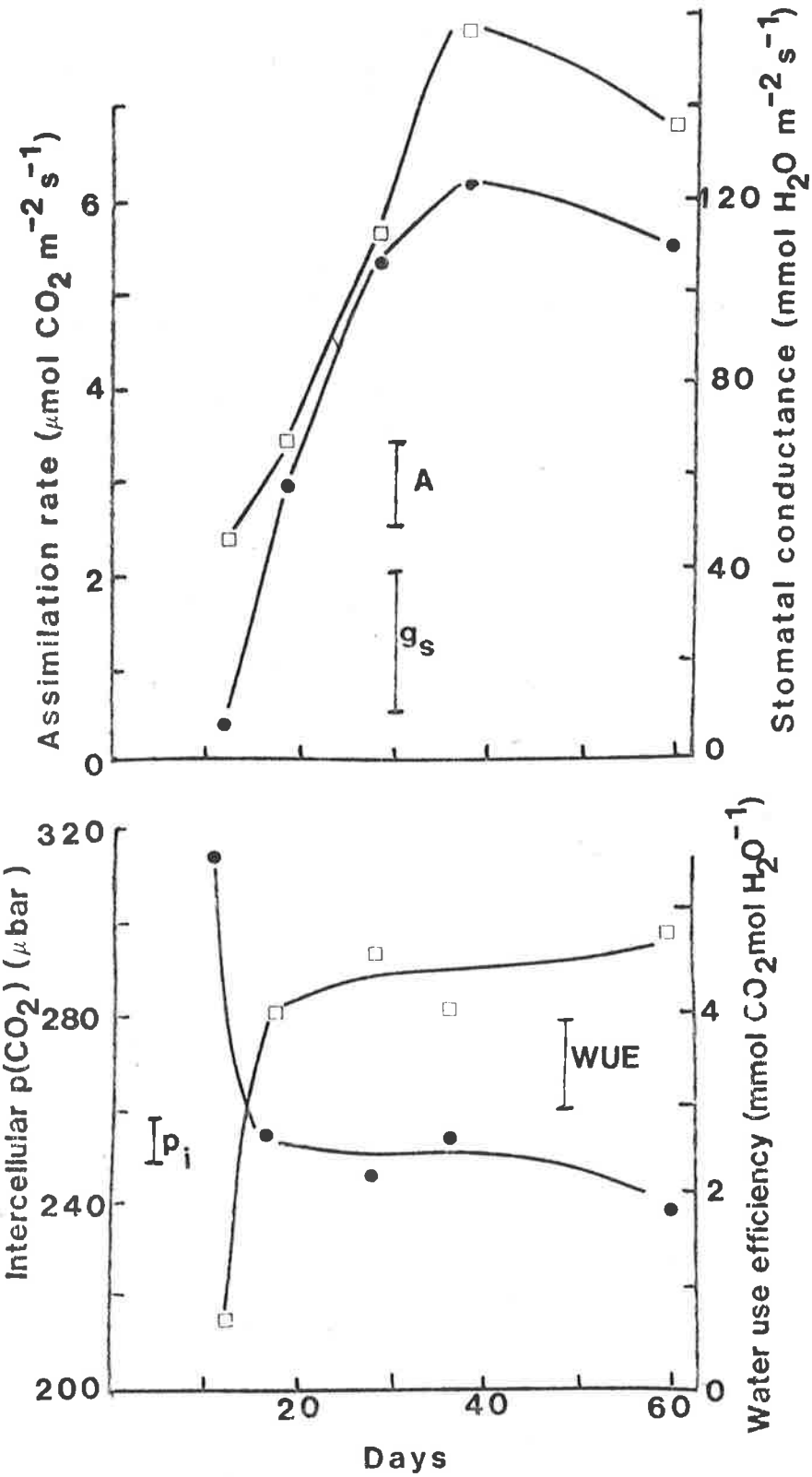


Figure 1.6 CO_2 assimilation (A), leaf conductance (g_s), intercellular partial pressure of CO_2 (p_i) and water use efficiency (WUE) of young expanding leaves of 'Valencia' orange. Vertical bars represent the Least Significant Difference (p=0.05). (●) CO_2 assimilation, intercellular partial pressure of CO_2: (□) stomatal conductance, water use efficiency.

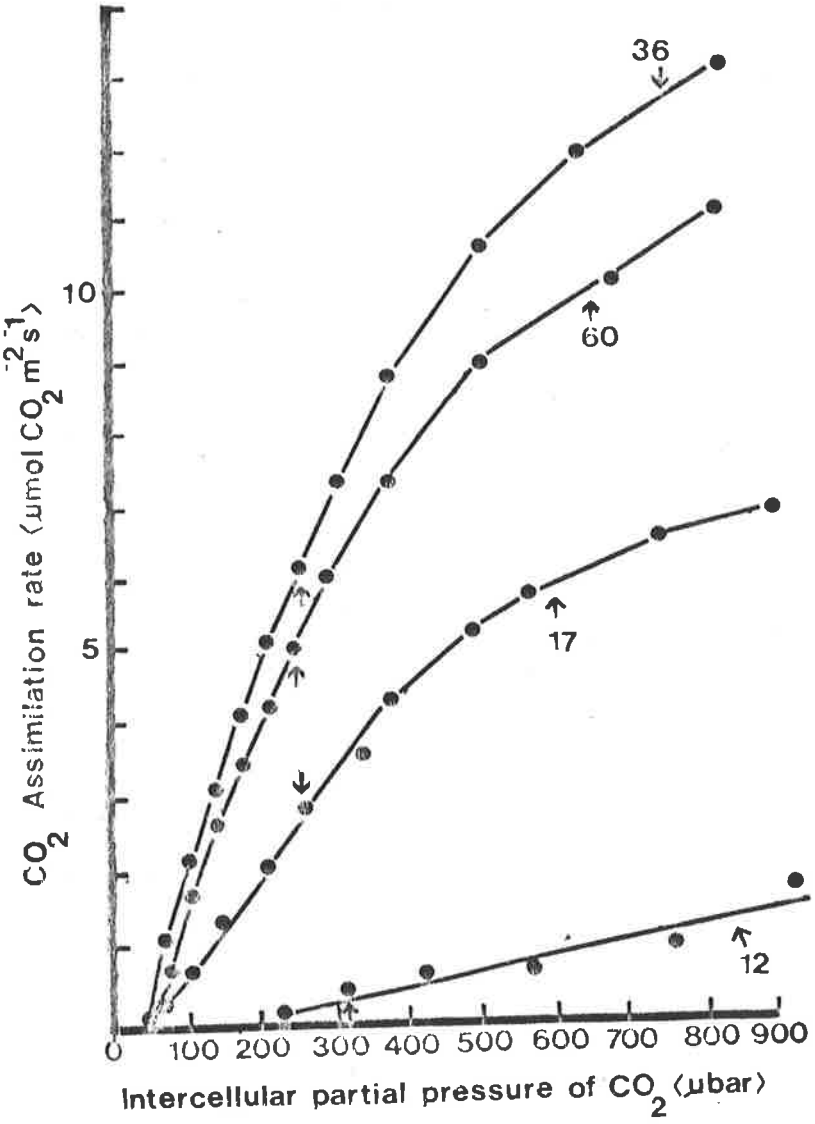


Figure 1.7 Gas exchange characteristics of young expanding 'Valencia' orange leaves. Arrows indicate point of operation at ambient p(CO_2). Number of days after leaf emergence is indicated for each leaf.

Leaf Age (months)	CO_2 assimilation (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	Intercellular partial pressure of CO_2 (μbar)
2	5.4	112	239
6	5.0	114	244
LSD (p=0.05)[1]	0.9	20	10

Table 1.2: CO_2 assimilation, stomatal conductance and intercellular partial pressure of 2 and 6 month old 'Valencia' orange leaves. [1]Least significant difference.

1.2.4 Discussion

Ontogenic changes in citrus leaf CO_2 assimilation follow the pattern previously reported for herbaceous species *viz* a rapid increase during the leaf expansion stage followed by a gradual decline. Comparison of gas exchange characteristics of 60 day old leaves with those from a previous flush (Table 1.2) indicated that once leaf expansion is complete the decline in CO_2 assimilation and stomatal conductance with leaf age occurs very slowly. All future investigations were thus carried out on fully expanded leaves.

Although ontogenic changes in leaf conductance followed a similar pattern to CO_2 assimilation, at Day 12 conductance was already 40 mmol m^{-2} s^{-1}. This resulted in a very high p_i and low water use efficiency (WUE) at this time. It is likely that this was due to the substantial cuticular conductance that occurs in very young leaves prior to a rapid deposition of cuticular waxes on the leaf surface (Syvertsen, 1982).

The presence of a high cuticular conductance may introduce errors into the p_i equation which assumes equivalent paths for CO_2 and H_2O diffusion across the leaf. Although the cuticle is probably impermeable to CO_2 (Jarvis, 1971), CO_2 must also diffuse across the epidermal layer before reaching the intercellular spaces. The width of an epidermal cell is approx. 10 μm in a young leaf (Syvertsen, 1985) and using the parameters of Nobel (1974) for calculation the conductance of CO_2 across such a cell is only ~20 mmol H_2O m^{-2} s^{-1}. Thus early estimates of stomatal conductance are probably an overestimate leading to inaccurate calculation of p_i in early stages of leaf development.

As well as having a substantial cuticular conductance, young expanding citrus leaves also have less air space and have more densely packed mesophyll cells than fully expanded leaves (Syvertsen, 1985). This would provide an additional barrier for CO_2 diffusion to the chloroplast (low g_i) not present in fully expanded leaves. It is hard to determine the extent of probable g_i changes with time, but they must be taken into account when interpreting A/p_i curves in terms of chloroplast biochemistry. Uncertainties therefore exist as to whether or not the high compensation point at day 12 is apparent or real. Likewise, initial slopes of A/p_i curves could be much steeper when A is expressed in terms of chloroplast $p(CO_2)$ (p_c) rather than p_i. Studies described in the following section were designed to determine the magnitude of g_i in *Citrus* spp. and evaluate its influence on leaf gas exchange.

1.3 EVIDENCE FOR A LOW INTERNAL CONDUCTANCE IN CITRUS LEAVES

1.3.1 Introduction

As mentioned in Section 1.1.4 most water lost in transpiration evaporates from the stomatal cavities or adjacent epidermal cells. The p_i value determined by equation 1.5 is thus in fact the $p(CO_2)$ in the stomatal cavity. In a citrus leaf, CO_2 must diffuse across the intercellular spaces to pallisade mesophyll cells which are densely packed (Schneider, 1968). Further to this, diffusion across cell walls cannot occur across non gaseous parts of the cell wall which is itself suberised (Scott *et al.*, 1948) creating a further possible barrier to CO_2 diffusion into the chloroplast.

Although measurements of g_i have not been possible in the past due to an inability to determine p_c, Farquhar *et al.* (1982) have shown that a linear relationship exists between p_c and the extent of carbon isotope fractionation in plants. It is now well established that all plants discriminate to some extent against the ^{13}C isotope (O'Leary, 1981). The basis of this biochemical discrimination against ^{13}C lies with rubisco (Park and Epstein, 1960) which discriminates against ^{13}C because of its intrinsically lower reactivity (Melander and Saunders, 1979). In this section we explore the

possibility that g_i may be low in citrus leaves by calculating p_i according to Equation 1.5 and comparing it with values calculated from δ^{13} measurements.

1.3.2 Theory

As absolute isotope ratios are difficult to attain isotopic compositions are generally specified as $\delta^{13}C$ values:

$$\delta^{13}C\ (‰) = \left[\frac{R(sample)}{R(standard)} - 1\right] \times 1000 \qquad (1.6)$$

where R is the ^{13}C abundance ratio:

$$R = \frac{^{13}CO_2}{^{12}CO_2} \qquad (1.7)$$

The standard in general use is PDB (belemite from the Pee Dee Formation in South Carolina) which has an ^{13}C abundance ratio of 0.01124.

Net discrimination by the plant against $^{13}CO_2$ (Δ) can thus be written:

$$\Delta = \delta^{13}C(atmosphere) - \delta^{13}C(plant) \qquad (1.8)$$

where $\delta^{13}C$(atmosphere) ~8 (Keeling *et al.*, 1979).

Carbon isotope discrimination has generally been related to p_i/p_a (Farquhar *et al.*, 1982):

$$\Delta = a + (b-a)\, p_i/p_a \qquad (1.9)$$

where a = discrimination against $^{13}CO_2$ due to slower diffusion in air = - 4.4‰ (Craig, 1953) and,

b = discrimination caused by RuP_2 and phosphoenolpyruvate carboxylation. This value is uncertain and we use a value of 28.1‰. This value was derived using a method recently devised by S. von Caemmerer (personal communication).

Evans *et al.* (1986) provided a more complete description of discrimination by C_3 leaves:

$$\Delta = a_b\left(\frac{p_a-p_s}{p_a}\right) + a\left(\frac{p_s-p_i}{p_a}\right) + (b_s+a_1)\frac{p_i-p_c}{p_a} + b\frac{p_c}{p_a} - \frac{eR_d/k + f\Gamma^*}{p_a} \qquad (1.10)$$

where p_s is the $p(CO_2)$ at the leaf surface, a_b = fractionation in the boundary layer (2.9‰), b_s is the fractionation occurring as CO_2 enters solution (1.1‰), a_1 is the fractionation due to diffusion in water (0.7‰), e and f are fractionations associated with 'Dark' respiration (R_d) and photorespiration respectively, k is the 'carboxylation efficiency' and Γ^* is the CO_2 compensation point in the absence of R_d.

The drawdown of CO_2 from the intercellular spaces to the site of carboxylation is related to A and g_i:

$$(p_i - p_c) = \frac{A}{g_i} \qquad (1.11)$$

Combining equations 1.10 and 1.11 (ignoring respiration and photorespiration) enables g_i to be calculated (Evans *et al.*, 1986).

$$g_i = \frac{(b-b_s-a_1)\,A}{(\Delta_i-\Delta_{obs})\,p_i} \qquad (1.12)$$

where Δ_i = discrimination calculated from Equation 1.9 after allowing for boundary layer conductance (i.e. expected discrimination by the leaf if g_i were to be zero) and Δ_{obs} = actual discrimination by the leaf.

1.3.3 Materials and Methods

Seeds of Rough lemon (*Citrus jambhiri* Lush), Cleopatra mandarin (*C. reticulata* Blanco), Sweet orange (*C. sinensis* [L.] Osbeck cv. Symonds sweet orange) and Trifoliata (*Poncirus trifoliata* [L.] Raf.) were sown in a peat/soil mixture in a vinyl covered misted green house illuminated with natural light in early September. In late

November, seeds of Sunflower (*Helianthus annuus*) were planted in the same soil mixture and allowed to develop under conditions identical to those experienced by the *Citrus* spp. In mid January of the following year all plants were transferred to a whitewashed glasshouse (maximum PPFD of 1000 μE m^{-2} s^{-1}) in which daily temperatures ranged from 22-32°C.

Gas exchange measurements were subsequently made on fully expanded leaves as described in Section 1.2.2.2 (leaf temperature of 25 ± 0.5°C with a vapor pressure deficit (VPD) of approx. 1.2 kPa). Leaves were subsequently dried at 70°C for at least 24 h and then ground with a mortar and pestle prior to $\delta^{13}C$ analysis. $\delta^{13}C$ values were determined employing the methods described by Osmond *et al.* (1981) courtesy of Dr K. Hubick (Australian National University).

1.3.4 Results

CO_2 assimilation rates of *H. annuus* were ~3-5 times higher than the *Citrus* spp. or *P. trifoliata*. Stomatal conductances were only 2.5-4 times greater for *H. annuus*. Consequently for *H. annuus* p_i was some 17-33 μbar lower than the other species. Δ_i was accordingly also less. There was however little difference between species in Δ_{obs}. Consequently, g_i for *H. annuus* (1.038 mol CO_2 m^{-2} s^{-1} bar^{-1}) was considerably greater than for the 3 *Citrus* species (0.125 to 0.196 mol m^{-2} s^{-1} bar^{-1}) or *P. trifoliata* (0.222 mol m^{-2} s^{-1} bar^{-1}) (Table 1.3).

	Assimilation (μmol CO_2 m^{-2} s^{-1})	p_i/p_a	Δ_i (‰)	Δ_{obs} (‰)	Internal conductance (mol CO_2 m^{-2} s^{-1} bar^{-1})
H. annus	20.8	0.72	21.34	19.77	1.038
C. sinensis	6.8	0.75	22.08	19.42	0.196
C. jambhiri	6.2	0.77	22.66	18.63	0.125
C. reticulata	4.3	0.78	22.90	19.94	0.185
P. trifoliata	6.7	0.74	21.87	19.50	0.222
L.S.D. (p = 0.05)	2.0	0.05	1.16	0.68	0.144

Table 1.3: Gas exchange characteristics for *Helianthus annus*, *Citrus sinensis*, *C. jambhiri*, *C. reticulata* and *Poncirus trifoliata*. Δ_i is the value predicted from equation 1.9 after allowing for boundary layer effects and Δ_{obs} is the long term discrimination by the leaf. Internal conductances were calculated according to Equation 1.12.

More detailed analysis of gas exchange is provided in Figure 1.8. In all cases at ambient CO_2 levels leaves were operating on the linear portion of the A/p_i curve. As discussed in Sect. 1.1.5 this indicates that under the growth conditions employed CO_2 assimilation rates of all species was limited by rubisco activity rather than electron transport capacity. When $p(O_2)$ was reduced from 210 mbar to 21 mbar CO_2 assimilation was increased in all cases. For the 3 *Citrus* spp. the degree of inhibition by O_2 was dependent upon assimilation rate (Fig. 1.9) whilst no clear relationship was evident for *H. annuus* or *P. trifoliata*. In general, leaves that showed a low degree of inhibition by O_2 at ambient $p(CO_2)$ were also characterised by an oxygen requirement for expression of full assimilatory capacity at high p_a.

1.3.5 Discussion

Our calculation of g_i is dependent upon the following assumptions:

(i) p_i at time of measurement reflects p_i experienced throughout the growth period

(ii) long term $\delta^{13}C$ discrimination reflects short term discrimination by the leaf

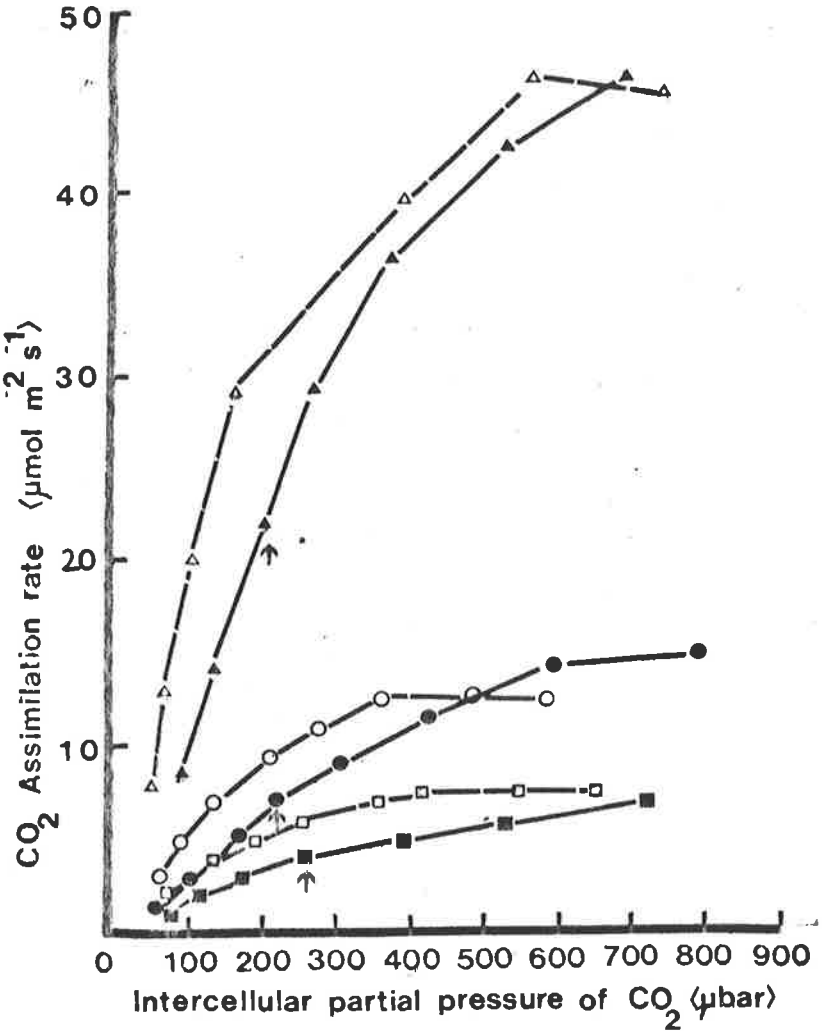


Figure 1.8 Gas exchange characteristics of leaves of sunflower, Sweet orange and Cleopatra mandarin. Arrows indicate point of operation at ambient p(CO_2). (▲ △) sunflower; (● ○) sweet orange; (■ □) Cleopatra mandarin. Open symbols: measurements at 21 mbar O_2; closed symbols: measurements at 210 mbar CO_2.

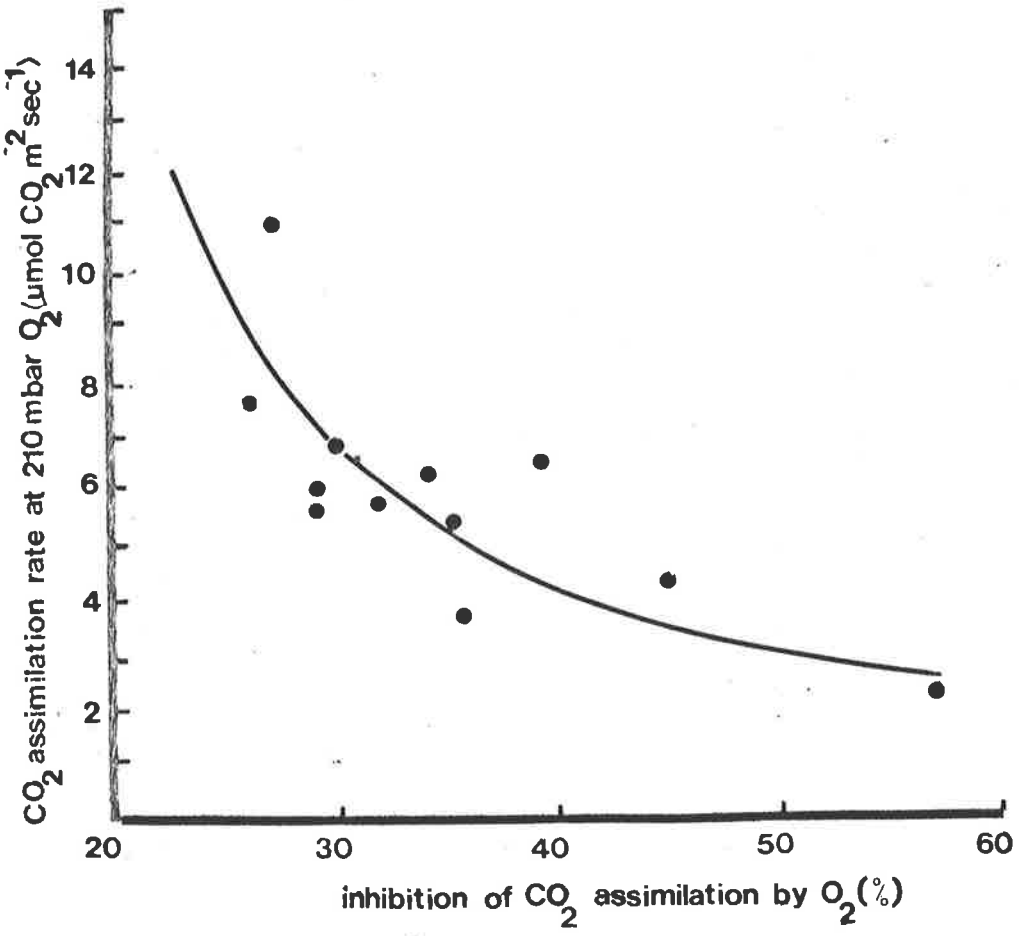


Figure 1.9 Relationship between CO_2 assimilation at 210 mbar O_2 and the extent of O_2 inhibition of photosynthesis for leaves from *Citrus reticulata*, *C. sinensis* and *C. jambhiri*. Each point represents an individual leaf.

The first assumption is valid as p_i becomes constant about 15 days after leaf emergence (Fig. 1.6b). Prior to this p_i is calculated to be very high but as discussed in Section 1.2.3 this value is unreliable due to high cuticular conductances occurring at this time. For the greater part of their development plants were grown in a misted glass house where VPDs of less than 0.5 kPa would have prevailed. We were not able to mimic this condition in the gas exchange system but as stomatal conductance and p_i increase with decreasing VPD in citrus (Chapers 6 and 9) if anything, p_i would have been greater under glasshouse conditions than in the gas exchange cuvette.

The assumption that p_c was constant over time is less certain. As discussed in Section 1.2.4 very young leaves have very closely packed mesophyll cells with intercellular air spaces occupying only a small proportion of the leaf which may result in a lower g_i than fully mature leaves. New citrus leaves are however substantial sinks during their expansion period with net export of photosynthate not commencing until full maturity has been reached (Kriedemann, 1969ab). Thus a lower g_i during early stages of leaf expansion would be unlikely to markedly influence the ^{13}C ratio as the majority of assimilate in young expanding leaves is imported from fully developed leaves on previous flushes. Our assumptions regarding p_i, ^{13}C and therefore p_c are therefore not seriously in error.

It is interesting to speculate on the reasons for low g_i in *Citrus* spp. The conductance of CO_2 from the stomatal cavity to the surface of the mesophyll cells (g_{ias}) can be calculated taking into account leaf thickness and the volume of the intercellular space (Nobel, 1974). Taking a leaf thickness of 300 μm and with intercellular air spaces occupying 30% of leaf volume (Syvertsen and Smith, 1984) g_{ias} is calculated to be approximately 625 mmol m^{-2} s^{-1} bar^{-1} which is considerably greater than g_i but much less than the values for g_{ias} quoted by Cooke and Rand (1980) or Farquhar and von Caemmerer (1982) for amphistomatous herbaceous species.

As mentioned earlier, intercellular spaces of mature *Citrus* leaves are lined with a layer of suberin approximately 1μm thick (Scott *et al.*, 1948). From permeability coefficients (Schönherr, 1982) we can estimate the conductance to CO_2 of such a layer

and it is likely to be only ~425 mmol m^{-2} s^{-1} bar^{-1}, hence imposing a further significant limitation on CO_2 diffusion to the chloroplast.

Likewise the conductance of cell walls can also be calculated (Nobel, 1974) and is likely also to be ~425 mmol m^{-2} s^{-1} bar^{-1}. As membranes are generally very permeable to CO_2 (Gutknecht *et al.*, 1977) and chloroplasts tend to accumulate around the periphery of mesophyll cells (Scott *et al.*, 1948) conductances of CO_2 diffusions inside the cytoplasm should be very high (Nobel, 1974; Raven and Glidewell, 1981). Limiting conductances and associated drawndowns of CO_2 are summarised in Table 1.4. Calculations are shown in Appendix 1.

Conductance	(mmol m^{-2} s^{-1} bar^{-1})	Drawdown[a] (μbar)
Intercellular air spaces	625	10
Suberised layer	425	14
Cell wall	425	14
Total	160	38

Table 1.4: Major components of internal leaf conductance to CO_2. See text for calculation and explanation

[a] A = 6 μmol CO_2 m^{-2} s^{-1}.

Although we can thus rationalise our estimate of g_i in *Citrus* spp. in terms of the conductances it should be emphasised that our estimate is by no means definitive. In particular, the correct value of b is a source of some uncertainty. Evans *et al.* (1986) use a value of 27. This gives lower values of Δ_i and hence an increased estimate of g_i. Detailed discussions of thse points have been presented (Farquhar *et al.*, 1982; Farquhar and Richards, 1984; Evans *et al.*, 1986). Despite such uncertainties our general conclusion that g_i for *Citrus* spp. is lower than *H. annus* still remains valid.

Although our calculated g_i in citrus leaves is considerably lower than that estimated for *T. aestivum* (Evans, 1983; Evans *et al.*, 1986), because of higher CO_2

assimilation rates in *T. aestivum* estimated drawdowns of CO_2 are of equivalent magnitudes. It therefore follows that if citrus leaves were to exhibit assimilation rates equivalent to *T. aestivum* drawdowns of CO_2 would be considerably greater. As A/p_i curves indicate that rubisco activity rather than RuP_2 regeneration capacity may limit CO_2 assimilation in *Citrus* spp. (Fig. 1.8) consequences of increased rubisco activity *in vivo* are explored in Appendix 2. This illustrates that a low g_i may to a large extent limit the extent to which increased rubisco activity could increase CO_2 assimilation rates *in situ.* Due to an increase in drawdown at greater assimilation rates, CO_2 assimilation at $p_i = 250$ μbar cannot rise above 20 μmol m^{-2} s^{-1} when $g_i = 150$ mmol m^{-2} $s^{-1}bar^{-1}$ regardless of rubisco activity.

For the 3 *Citrus* spp. there was a negative correlation between CO_2 assimilation rate and the extent of inhibition by O_2 (%inh) (Fig. 1.9). High levels of inhibition by O_2 have been reported previously for *C. sinensis* (Kriedemann, 1971) and similarly, Lenz (1978) observed that leaves on trees with a heavy fruit load had higher assimilation rates and lower levels of oxygen inhibition than leaves from non bearing trees. The possibility that g_i interacts with %inh was investigated on a theoretical basis (Appendix 3). Although this analysis showed that a relationship between assimilation and %inh should exist, the magnitude of the predicted variation in %inh was less than that observed experimentally and no correlation existed between predicted and observed values. It thus appears that some factor in addition to photorespiration influences the extent to which CO_2 assimilation is impaired by atmospheric $p(O_2)$. One possibility is that triose phosphate utilisation (TPU) limitations (Sect. 1.1.5) may influence %inh in *Citrus* spp. At higher rates of CO_2 assimilation, increased rates of sucrose synthesis (and hence release of cytoplasmic P_i) would be required, in order for P_i to be made available for ATP synthesis. The presence of TPU is characterised by insensitivity of CO_2 assimilation to both CO_2 and O_2, however (Sharkey, 1985ab). This criterion is not met in this study, rather, an increase in assimilation rate with p_i was observed at ambient $p(CO_2)$ in all cases (Fig. 1.8). Furthermore, levels of sucrose synthetase activity in 4 month old 'Shamouti' orange leaves are in the order of 60 μkat m^{-2} s^{-1} (Schaffer *et al.*, 1987). This

is sufficient to process all CO_2 fixed up to a rate of 360 μmol CO_2 m^{-2} s^{-1}.

A second possibility is that pseudocyclic phosphorylation (Section 1.1.2) is required to generate sufficient ATP. Pseudocyclic phosphorylation should become necessary when the electron transport chain is running at 2/3 of its maximum rate. It could then be that the relationship between A and %inh is due to a need for pseudocyclic phosphorylation (and therefore O_2) at higher assimilation rates. This requires a knowledge of the electron transport chain in *Citrus* spp. and this is investigated in the following section.

1.4 ELECTRON TRANSPORT CAPACITY AND CHLOROPHYLL FLUORESCENCE INDUCTION KINETICS OF CITRUS LEAVES

1.4.1 Introduction

As discussed in the previous section, no basic information on the electron transport capacity of *Citrus* leaves was available. This section provides this fundamental data. We were also interested in using chlorophyll *a* fluorescence (Sect. 1.1.3) as an *in vivo* probe of chloroplast function (Chapters 2 and 4) and therefore examined the relationship betwen electron transport capacity and chlorophyll *a* fluorescence induction kinetics. Variations in electron transport capacity were achieved by exposing leaves to high light intensity in the absence of CO_2; a treatment that results in impaired electron transport capacity due to photoinhibition (Powles, 1984).

1.4.2 Materials and Methods

1.4.2.1 Growth of Plants

Two year old 'Valencia' orange trees on Sweet orange rootstock were grown in a whitewashed glasshouse (max PPFD = 850 μmol m^{-2} s^{-1}) in 4 litre containers using the potting medium of Walker *et al.* (1983). Plants were watered daily with tap water and fertilized every 7 days with a 5:1 mixture of 'Aquasol' and iron-chelate.

1.4.2.2 Gas exchange

A/p_i curves prior to photoinhibition were determined as described in Section 1.2.2.2. At completion of A/p_i curves, leaves were exposed to 2000 μmol m^{-2} s^{-1} PPFD in absence of CO_2 with a p(O_2) of 21 mbar for 1 hour. Following photoinhibitory treatment leaves were allowed to reach a new steady state CO_2 assimilation rate (this typically took ~1 hour) whereafter A/p_i curves were again determined. Following A/p_i curve determination leaves were either used for electron transport studies (Sect 1.4.2.3) or for fluorescence measurements (Sect. 1.4.2.4).

1.4.2.3 Thylakoid isolation and electron transport determinations

Midribs were removed from approx 1-5 g of leaf material cut into slices (approx 1 mm x 5 mm) which were ground using a large prechilled mortar and pestle in 40 ml grinding medium containing 600 mol m^{-3} sorbitol, 100 mol m^{-3} N-[2-hydroxy-1-1bis (hydroxymethyl)ethyl] glycine (Tricine)-NaOH (pH 7.8), 5 mol m^{-3} $MgCl_2$, 50 mol m^{-3} Na-ascorbate and 0.5% bovine serum albumin (BSA). Preliminary experiments showed that inclusion of dithiothreitol (5 mol m^{-3}) ethylenediaminetetra-acetic acid (EDTA : 1 mol m^{-3}) or polyvinylpyrilodine (PVP : 2%) in the grinding medium did not improve electron transport rates with PVP possibly being inhibitory. The mixture was filtered through 3 layers of Miracloth (Calbiochem-Behringer Corp., La Jolla, Cal., USA) and the filtrate centrifuged for 5 min at 500 g. The pellet (unbroken cells and starch) was discarded and the supernatant centrifuged at 4,000 g for 10 minutes. The pellet (mostly broken chloroplasts) was suspended in 0.5 ml of solution identical to the grinding medium less Na-ascorbate and BSA. Rates of electron transport drastically increased when Amberlite XAC-2 beads were added to the grinding medium immediately after filtration. This effect was concentration dependant, with maximum rates occurring at ~20 mg/μg chlorophyll (Fig. 1.10). Sufficient Amberlite XA-2 beads to give this concentration were thus routinely added. These beads have a low specific gravity and thus do not centrifuge down with the thylakoid pellet. Rates of photosynthetic electron transport were measured polarographically at 25°C with a temperature controlled, water-jacketted, Clark-type

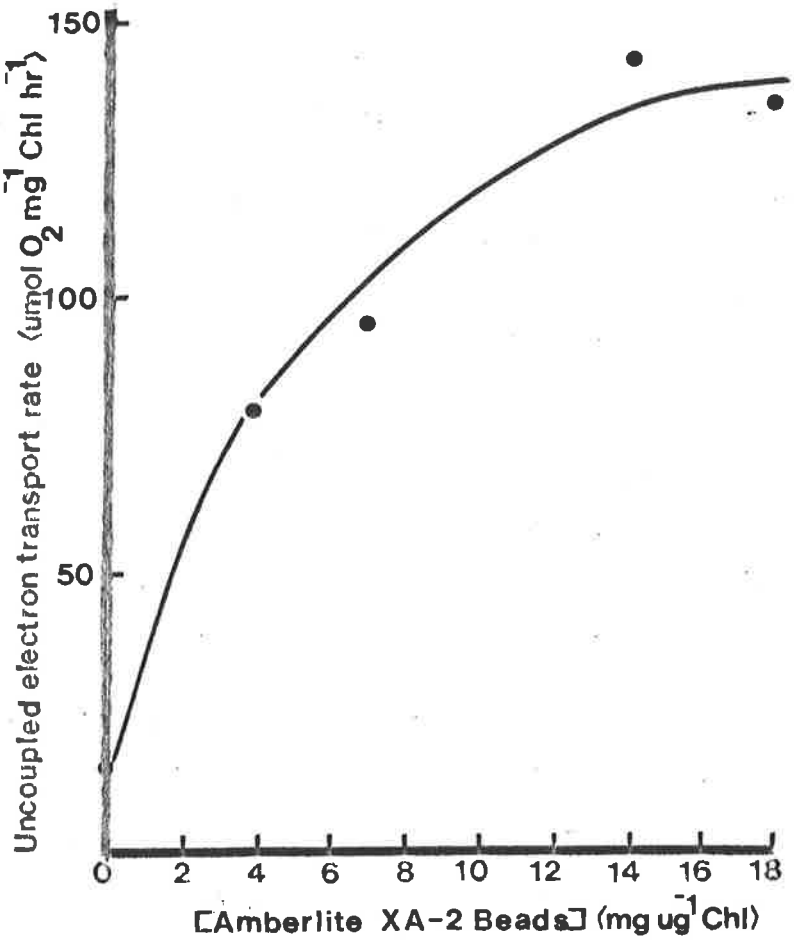


Figure 1.10 Dependence of uncoupled whole chain electron transport rate upon concentration of Amberlite XAC-2 beads in thylakoid grinding medium.

oxygen electrode (Rank Bros., Cambridge, UK) connected to a potentiometric recorder. A light beam from a tungsten-halogen lamp was focused through a lens system on the reaction mixture in the electrode compartment. Uncoupled whole chain electron transport was determined by following the rate of O_2 uptake in a 5 ml reaction mixture containing 600 mol m^{-3} sorbitol, 5 mol m^{-3} $MgCl_2$ and 50 mol m^{-3} Hepes-KOH (pH 7.8), 1 mol m^{-3} NaN_3, 1 mol m^{-3} NH_4Cl and chloroplast-membrane preparation (containing ~100 µg chlorophyll). Photosystem I driven electron transport was measured using the same system but with 3-4,dichlorophenol 1,1-dimethylurea (DCMU; 4 mmol m^{-3}) added to block PSII driven electron transport and 2,6 dichlorophenol indophenol (100 mmol m^{-3}) and Na-ascorbate (1 mol m^{-3}) added as electron donor. Photosystem II driven electron transport was monitored as oxygen evolution from H_2O to 2,5-dimethyl-*p*-benzoquinone (1 mol m^{-3}). 2-5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (1 µM) was included in the assay medium to prevent spillover to PSI (Izawa, 1980). Chlorophyll concentration was determined according to Arnon (1949).

1.4.2.4 Chlorophyll fluorescence

Following gas exchange measurements plants were placed in a completely darkened room and allowed to dark adapt for 1 hour. Chlorophyll *a* fluorescence induction kinetics were measured for the upper and lower surface of photoinhibited leaves and untreated leaves from the same flush. Measurements were made with a Richard Branker SF-20 fluorometer interfaced with a fast data capture system (Norrish *et al.* 1983) and signals transferred to a chart recorder for permanent record. Excitation was for 50 s with red light (670 nm) at an intensity of 30 µmol m^{-2} s^{-1}.

1.4.3 Results

Exposure of leaves to 1 hour photoinhibitory treatment (21 mbar O_2; 0 µbar CO_2) resulted in a diminution in CO_2 assimilation capacity. This was accompanied by an almost equivalent reduction in stomatal conductance. Consequently p_i was little changed by photoinhibitory treatment (Table 1.5).

Photoinhibitory Treatment	CO_2 assimilation (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})	Intercellular $p(CO_2)$ (μbar)
-	6.4	111	227
+	5.0	90	235
LSD (p = 0.05)[1]	0.8	8	6

Table 1.5: Effect of 1 hr photoinhibitory treatment (0 μbar CO_2, 21 mbar O_2) on gas exchange characreristic of 'Valencia' orange leaves. Each value is the mean of 3 leaves. [1]Least significant difference.

Gas exchange characteristics are illustrated in more detail in Fig. 1.11. This shows that the CO_2 assimilation was substantially reduced at high p_i whilst the initial slope of the A/p_i curve was virtually unchanged. Upper surface chlorophyll fluorescence induction kinetics are also shown before (left side of figure) and after (right hand side of figure) photoinhibitory treatment. A substantial reduction in the variable component (Fv) was observed.

In contrast to the upper surface, lower surface variable fluorescence remained unaltered after photoinhibitory treatment. Photoinhibition also reduced rates of whole chain and PSII mediated electron transport by approximately 30%. PSI mediated electron transport remained unchanged (Table 1.6).

Photoinhibitory Treatment	Fv/Fo (upper surface)	Fv/Fo (lower surface)	Electron Transport (μmol e^- m^{-2} s^{-1}) PSII	PSI	PSII + PSI
-	2.7	3.0	196	310	180
+	1.0	3.2	122	332	125
LSD (p = 0.05)[1]	0.5	0.4	29	46	33

Table 1.6: Upper and lower surface variable fluorescence ratios and whole leaf electron rates for leaves prior to and after 1 h photoinhibitory treatment (0 μbar CO_2 : 21 mbar O_2). [1]Least significant difference.

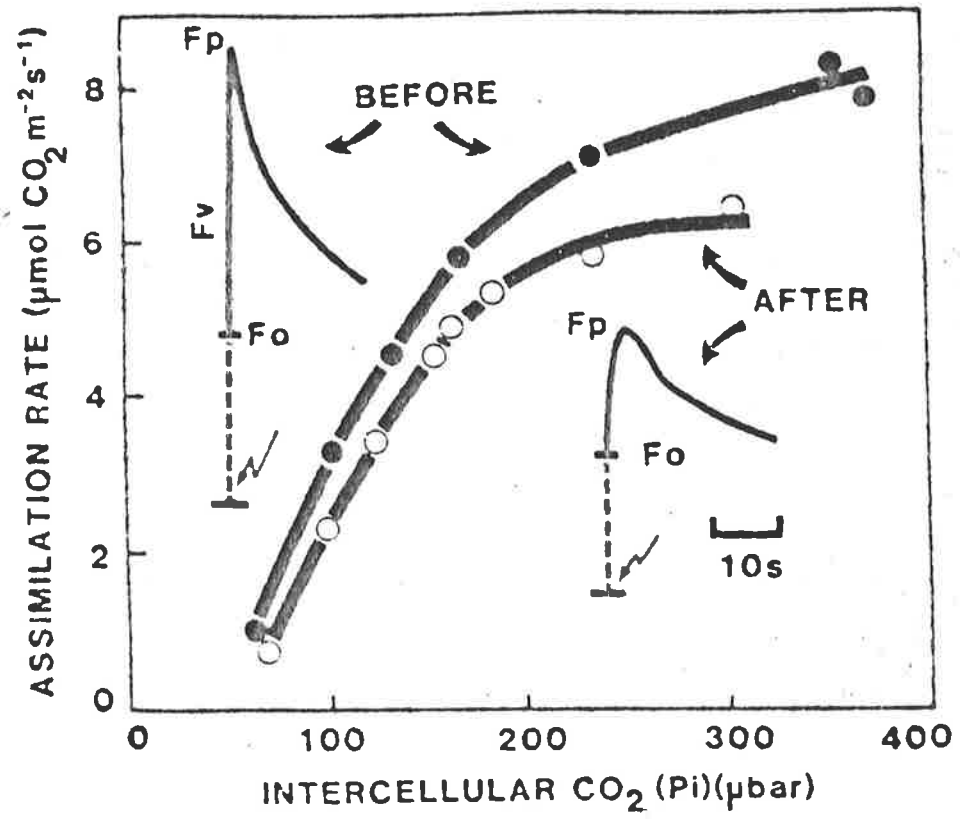


Figure 1.11 Gas exchange characteristics of 'Valencia' orange leaves before and after exposure to 1 h photoinhibitory treatment (0 µbar CO_2; 21 mbar O_2: 1000 µmol quanta m-2 s-1). Chlorophyll *a* fluorescence induction kinetics are shown as transients before (left side of figure) and after (right side) treatment.

1.4.4. Discussion

Amberlite XA-2 beads (or Bio SM2 beads from Biorad Laboratories) scavenge hydrophobic molecules from aqueous solutions. Citrus leaves contain many oil glands consisting of many such molecules such as terpenes and sesquiterpines (Scora and Torrisi, 1966). Such compounds would be expected to strongly impair many physiological processes (including electron transport). Scavenging of such compounds by Amberlite XA2 is most likely the reason for the concentration dependence of electron transport rates on the presence of these beads.

Rates of electron transport observed in control leaves were relatively high. From the model of Farquhar and von Caemmerer (1982) it is calculated that at saturating p_C (= 1,200 μbar) this rate should be able to support CO_2 fixation by the leaf of ~ 35 μmol m^{-2} s^{-1} whilst at p_C = 200 μbar a rate of ~19 μmol CO_2 m^{-2} s^{-1} could be supported. Clearly then, *in vitro* electron transport rates of citrus leaves are in excess of those required to account for observed rates of CO_2 fixation.

A reduction in the variable fluorescence ratio was however accompanied by lower PSII and whole chain electron transport activity (Table 1.6) and an impairment of mesophyll CO_2 assimilation ability (Fig. 1.11) thus indicating that it may be a useful tool for probing stress induced PSII lesions *in vivo*. The changes in photosynthetic characteristics following photoinhibition reported here have been previously reported for other species (Powles, 1984). It is now well established that photosystem II mediated electron transport is extremely sensitive to high light levels (Powles and Critchley, 1980) and that upper surface variable fluorescence is reduced by photoinhibition whilst lower surface fluorescence transients remain unaffected (Björkman and Powles, 1984).

Similar phenomena are observed under conditions of water deficit (Björkman and Powles, 1984), chilling injury (Ogren and Oquist, 1984) and to a less well defined extent salinity stress (Smillie and Nott, 1982) indicating that there may be some component of PSII that is sensitive to many environmental stresses. Chlorophyll *a* fluorescence is used as a diagnostic probe to locate possible sites of stress induced lesions in the chloroplast in subsequent chapters.

1.4 GENERAL DISCUSSION

Shortly after reaching full expansion under controlled conditions citrus leaves attain nearly constant assimilation rates, stomatal conductances and p_i values. Despite relatively low stomatal conductances, p_i is little different from values observed in other species (Farquhar and Sharkey, 1982), indicating that the stomatal limitation on leaf gas exchange of *Citrus* spp. is no greater than for other species, many of which have much higher CO_2 fixation rates.

A second appreciably low conductance of similar magnitude from the stomatal cavity to the chloroplast has been identified, however. This conductance is much lower than for *T. aestivum* (Evans *et al.* 1986) but imposes only a similar restriction on CO_2 assimilation due to lower mesophyll capacity in *Citrus* spp. Examination of measured biochemical parameters does not readily reveal why mesophyll capacity should be so low in *Citrus* spp (Table 1.7).

	Rubisco activity μmol CO_2 m^{-2} s^{-1}	Electron Transport μmol e^- m^{-2} s^{-1}
Observed	70	180
Required	27	103

Table 1.7: Observed and required rates of rubisco activity and electron transport to sustain a CO_2 assimilation rate of 20 μmole m^{-2} s^{-1} at 1000 μbar CO_2. (g_i [illegible], K_c = 310 μbar, K_o = 164 mbar).

Whilst limitations on CO_2 assimilation capacity may be imposed by reactions involved in RuP_2 regeneration other than electron transport, Farquhar and von Caemmerer (1982) provide convincing arguments why this should not be so. Rather, it is proposed that CO_2 assimilation in *Citrus* spp. may be limited by the inherently low leaf phosphorus levels.

Leaf phosphorus levels of ~0.15% are considered to be adequate for all *Citrus* spp. (Embleton *et al.*, 1973). Taking a specific leaf weight of 10 mg cm^{-2} (Syvertsen and Smith, 1984) this translates to ~6 mmol m^{-2}. This is nearly half that observed for spinach (Brooks, 1986). This low P-status is reflected in ATP levels which are only 80

ng g DW^{-1} (Yelenosky and Guy, 1982), a low value when compared to other species, (e.g. 600 ng g DW^{-1} in *Capsicum annum*: Turner and Wellburn, 1985). From chlorophyll data from similar material (Orchard trees in spring - Chapter 8) we can calculate ATP levels on a chlorophyll basis. Even if we assume that half the ATP in the leaf is in the chloroplast (Krause and Heber, 1976) ATP levels in citrus chloroplasts are only ~6 nmol mg^{-1} chlorophyll. This compares with estimates from *S. oleracea* leaves of 50-70 (Heber and Santarius, 1970), 20 (Wirtz *et al.*, 1980) and 30 nmol mg^{-1} chlorophyll (Giersch *et al.*, 1980). Although this value for citrus is in excess of the estimated concentrations of ADP binding sites in chloroplasts (1.4 nmol mg^{-1} chlorophyll : Farquhar and von Caemmerer, 1982) high levels of ATP are required for the Ru5P kinase and PGA kinase reactions (Priess and Kosuge, 1976; Gardemann *et al.*, 1983). In *S. oleracea* Brooks (1986) found significant reductions in PGA and RuP_2 upon exposure of plants to P deficient conditions (P = 1.8 mmol m^{-3}). Whilst this supports the hypothesis that [ATP] may limit CO_2 assimilation in low P leaves the possibility that in low P plants there is simply not enough phosphate for PCO cycle intermediates to accumulate to non limiting concentrations cannot be excluded (Brooks, 1986). Evidence for a strong influence of P levels on citrus gas exchange has been observed by Syvertsen (pers. comm.) where varietal differences in gas exchange were positively correlated with leaf P levels.

We could not account for the relationship between CO_2 assimilation and %inh (Figure 1.9) in terms of interactions between rubisco activity and g_i (Appendix 3).. As citrus electron transport capacity *in vitro* is well in excess of that required to account for *in situ* meausurements nor does it appear that a requirement for O_2 by the Mehler reaction for leaves at high CO_2 assimilation rates can account for the lower %inh observed for such leaves. The extent of rubisco activation can however decrease under conditions of low $p(O_2)$ (Sharkey *et al.*, 1986a). The model relating CO_2 assimilation to %inh (Appendix 3) assumes that rubisco is fully activated at both $p(O_2)$. It is also possible that our assumption regarding constancy of p_c over time (Section 1.3.5) is not valid. For example, a gradual build up of starch in chloroplasts of leaves (Scott *et al.*, 1948) could

impose an additional barrier to the diffusion of CO_2 into the stroma. Such reductions in g_i would be time dependent. Simultaneous measurement of CO_2 assimilation and ^{13}C discrimination (Evans *et al.*, 1986) is required in order for a more accurate determination of g_i to be made.

Whilst further investigations of factors limiting citrus photosynthesis would be of great interest we now desist from adventitious hypothesizing and investigate in detail the gas exchange responses of *Citrus* spp. to water deficit and salinity stress.

CHAPTER TWO

GAS EXCHANGE OF 'VALENCIA' ORANGE AS AFFECTED BY SOIL AND LEAF WATER STATUS

2.1 THE ROLE OF WATER IN PLANTS

Water is the principle component of most plant cells and tissues, being the solvent for most biochemical reactions, as well as providing the medium in which solute diffusion takes place (Nobel, 1974). It also plays an important role in protein structure as hydrogen bonding between water and electronegative sites on protein molecules are of paramount importance in maintaining structure and hence function. If this bonding is restricted, as in dehydrated tissue, macromolecules collapse into a state of denaturation. Dehydration may also diminish enzyme activity due to a loss in reactivity of molecules in solution as well as resulting in diminished affinity between enzymes and substrates (Borowitzka, 1981).

In addition to its biochemical role, water is of primary importance in transporting dissolved ions and other substances throughout the plant (Pitman, 1981) as well as having a strong influence on cell expansion and plant growth (Bradford and Hsaio, 1982). We will cover all these functions briefly in this chapter but concentrate on the importance of leaf water status in the modulation of leaf gas exchange.

2.1.1 Water potential and its components

It was not until 1960 that Slatyer and Taylor first provided a description of plant water status based on sound thermodynamic principles. The first definition of water potential (Ψ) was that of the difference in chemical potential between the test system and the standard state ($\Delta\mu w$) which is usually taken to be pure water (Slatyer and Taylor, 1960). This definition was subsequently modified by dividing $\Delta\mu w$ by the partial molar volume of water (v_w) to yield units of pressure. Although this concept of water potential has been criticised on both theoretical and practical grounds (Zimmerman and Steudle, 1978) it is now firmly entrenched and its continued use has been recommended (Dainty, 1976; Passioura 1982).

For all plant cells, Ψ may be divided into its three principal components

$$\Psi = P + \pi - \tau \qquad (2.1)$$

where P, π and τ are turgor (pressure), osmotic and matric potentials respectively. Although Ψ and its components are intensive variables varying from cell to cell within a tissue (Weatherley, 1970) in general practice (and throughout this thesis) bulk values of tissue rather than values for individual cell water relations (Tyree and Hammel, 1972) are determined.

2.1.1.1 Matric Potential (τ)

The significance and meaning of τ in plant tissues has been the subject of much debate and controversy. Although it was originally conceived to incorporate all interactions between liquid and solid phases (Taylor and Slatyer, 1961), Tyree and Jarvis (1982) have argued strongly that apart from the lowering of the free energy of water molecules within electric fields of charged surfaces, all other phenomena previously included in τ (surface tension, turgor and osmotic effects) can be easily incorporated into P or π. Furthermore they argue that effects on Ψ of τ of charged surfaces are insignificant. This author is in accordance with that philosophy and from hereon discusses ψ solely in terms of P and π. We will however have on more than one occasion cause to refer to components of ψ previously incorporated within τ.

2.1.1.2 Osmotic Potential (π)

This term incorporates the effects of solute concentration in tissue water on ψ. The presence of such solutes always reduces μ_w below that of pure water and hence π is always negative (Robinson and Stokes, 1959). For ideal solutions it is almost directly proportional to solute concentrations, but most components of cell sap have a higher or lower energy in solution than in their pure states (Robinson and Stokes, 1959). This is not a problem in plant physiology studies however as π is determined via either psychrometry (Section 6.2.4.2) or pressure-volume curves (Section 2.2.3.6) rather than by calculations from solute concentrations. It should be noted however that an increase in concentration of a given solute may not cause a strictly proportional change in π.

2.1.1.3 Turgor Potential (P)

When a plant cell contains a sufficient volume of water to exert an outward pressure on the cell wall, the strained cell wall applies a restoring force on the cell sap which produces a positive turgor potential (P). P is considered to be of crucial importance in cell expansion (Bradford and Hsaio, 1982) as well as stomatal functioning (Section 2.1.3.2). Although direct measurements of turgor potentials are now possible (Zimmermann, 1977) throughout this study P is inferred as the difference between bulk tissue values of Ψ and π.

2.1.2 Modulation of leaf water relations by external environment

Leaf water potential is dependent upon external factors, especially availability of soil water and evaporative demand. The role of these influences are related as with other fluxes:

$$E = g_p (\Psi_{soil} - \Psi_{leaf}) \qquad (2.2)$$

where g_p = conductance of the plant to H_2O.

Whilst this equation suggests that as soil dries out Ψ_{leaf} must become more negative (assuming g_p is constant) it is to a large extent misleading as, in practice, a reduction in Ψ_{soil} of only 0.1 MPa in the absence of an appreciable osmotic pressure in the soil water usually results in depletion of nearly all the water available to the plant. This may be a consequence of the fact that for species such as *Citrus* which have few root hours, root/soil interface potential may be far more negative than bulk soil water potential (Kriedemann and Barrs, 1981).

2.1.3 Influence of soil water deficits on leaf function

2.1.3.1 Solute Accumulation

With reductions in Ψ_{leaf} under drought stress P may fall to very low levels or even zero. This may lead to cessation of cell expansion (Terry *et al.*, 1983) and impairment of leaf gas exchange (Section 2.1.3.2). In some plants, the leaf water potential at which zero turgor is reached can be reduced via an accumulation of solutes in leaf cell sap resulting in more negative osmotic potentials, a phenomena referred to as *osmotic adjustment* (Morgan, 1984). Important solutes accumulated in leaves include organic acids, potassium, chloride, and soluble sugars (Munns *et al.*, 1979; Jones *et al.*, 1980; Ford and Wilson, 1981). Soluble nitrogen compounds, especially proline, are also known to accumulate under water stress (Aspinall and Paleg, 1981). Although accumulation of these compounds may be quantitatively less than other compounds these compounds are considered to be confined to the cytoplasm (Aspinall and Paleg, 1981) and hence may be of equal importance in osmotic adjustment. Osmotic adjustment in response to water deficit is not however a universal phenomena. Observed reductions in leaf osmotic potential where adjustment does occur generally ranges from 0.1 to 1.0 MPa in mesophytes (Morgan, 1984). Although osmotic adjustment may allow turgor maintenance and hence continued stomatal opening, factors other than leaf water status influence leaf gas exchange under conditions of soil water deficit.

2.1.3.2 Stomatal function

Work with both herbaceous (Hsaio, 1973) and woody plants (Jarvis, 1980) led to the concept of a 'threshold' Ψ below which stomatal closure occurs. In many plants this threshold Ψ corresponds to the point of zero turgor (e.g. Turner, 1974; Jones and Turner, 1978; Turner *et al.*, 1978) which coupled with the observation that increased abscisic acid (ABA) synthesis also occurs at this point led Pierce and Raschke (1981, 1982) to suggest that ABA synthesis *in vivo* is the main cause of stomatal closure in water stressed leaves. However, biosynthesis of ABA may increase some time later than stomatal closure (Walton *et al.*, 1977) and ABA content of leaves does not always

correlate with stomatal functioning (Raschke, 1982). These discrepancies between ABA content and stomatal functioning are usually explained in terms of intercellular distribution as guard cells constitute only approx. 1% of leaf volume (Raschke, 1979) or by differences in other plant growth regulators as both cytokinins (Blackman and Davies, 1983) and indole acetic acid (Snaith and Mansfield, 1982) may modify stomatal responses to ABA. Although the threshold response of ABA at zero turgor has been confirmed by other workers (Cornish and Zeevaart, 1984) not all plants show a threshold stomatal closure response at zero turgor (e.g. Thomas *et al.*, 1976; Gollan *et al.*, 1985; Turner *et al.*, 1985). Furthermore, some species do not show appreciable stomatal closure even in wilted leaves (Hensen *et al.*, 1982; Turner *et al.*, 1985). Thus, although there is little doubt the ABA plays an important role in stomatal closure under water stress, other factors are clearly involved.

In recent years evidence has accumulated showing that, for some species, stomata may respond to soil moisture status (measured as % extractable soil water) rather than Ψ_{leaf}. Elegant studies by Gollan *et al.* (1985) illustrated this point very well. By exposing the test leaf to a VPD different from the rest of the plant they were able to obtain different values for ψ in this leaf at the same soil moisture status. When stomatal conductance was expressed as a function of ψ the relationship was dependent upon the VPD to which the rest of the plant was exposed. When expressed as a function of % extractable soil water however, there was no effect of VPD on the relationship. It is currently thought that this phenomenon is due to a "message" from the root system to the leaf (Schulze, 1986). The best evidence for such a signal is that of Blackman and Davies (1985) who, by drying soil around part of a split root system in *Zea mays* whilst keeping other parts well watered, obtained in a reduction in stomatal aperture despite maintenance of turgor, and leaf water potential and with no change in leaf ABA content. As application of cytokinins to leaf discs from these plants increased stomatal aperture they concluded that stomatal opening in *Zea mays* is dependent upon a continuous supply of cytokinins from the root system and that interuption of this supply, due to decreased synthesis by root tips growing in dry soil, causes stomatal closure.

If similar phenomena were ubiquitous in all terrestrial plants it would be hard to justify the threshold relationship between stomatal conductance and leaf turgor that exists in many species. Rather, perhaps we can divide plants into two groups; those with an 'optimistic' strategy which keep stomata open until turgor is lost and those with a 'pessimistic' strategy which have mechanisms to sense soil moisture status and hence reduce rates of soil moisture depletion. Plants with 'pessimistic' mechanisms should be characterised by not only a lack of a threshold water potential for stomatal closure but also by an inability to osmotically adjust. It does not however follow that all plants lacking threshold turgor responses and ability to osmotically adjust have mechanisms similar to that proposed for *Zea mays*.

2.1.3.3 Chloroplast function

It is obvious that any reduction in stomatal conductance must lead to a lowering of p_i and hence a reduction in CO_2 assimilation rate. For many years (probably as a consequence of inadequate "resistance analyses" : see Farquhar and Sharkey, 1982) it was considered that reductions in CO_2 assimilation with water stress were a consequence of reduced stomatal conductances lowering p_i only with no effect on chloroplast metabolism (see Hsaio, 1973). From our prevous discussions of gas exchange theory, it should be clear that in such situations A/p_i curves should not be affected with p_i merely decreasing to a lower value on the A/p_i curve. The majority of gas exchange experiments using improved techniques show that this is not the case. Reductions in p_i can be sufficient to account for reduced CO_2 assimilation rates (von Caemmerer and Farquhar, 1984), but p_i may remain unchanged (Jones, 1973; Wong *et al.*, 1985; Briggs *et al.*, 1986) or even increase (Radin and Ackerson, 1981). Clearly then, reduced rates of CO_2 assimilation under conditions of water deficit can be a consequence of impaired chloroplast function as well as an increase in stomatal limitation on leaf gas exchange. Unfortunately biochemical studies attempting to identify possible sites of chloroplast dysfunction due to water deficit remain somewhat equivocal.

For *Phaseolus vulgaris* (von Caemmerer and Farquhar, 1984) and *H. annus* (Bunce, 1986) the RuP_2 regeneration limited phase of A/p_i curves was shown to be reduced prior to any decline in initial slope (*in vivo* rubisco activity) during early stages of water stress. In both cases this reduction did not affect mesophyll CO_2 assimilation at atmospheric CO_2 levels as leaves were operating on the linear phase of the A/p_i curve. As water stress intensified, a reduction in initial slope was also observed in both these studies. Reductions in both phases of A/p_i curves in water stressed leaves have also been reported for *Larrea divaricata* (Mooney *et al.*, 1977), *Gossypium hirsutum* (Jones, 1973; Radin and Ackerson, 1981) and *Nerium oleander* (Björkman and Powles, 1984). In contrast to these studies Ogren and Oquist (1985) observed a decline in initial slope of A/p_i curves prior to changes in the RuP_2 regeneration limited phase in *Salix* sp. indicating that in willow leaves rubisco activity may be more sensitive to water deficit than other photosynthetic processes.

One photosynthetic process impaired under water stress may be electron transport. Boyer and co-workers (reviewed in Boyer, 1976) isolated thylakoid membranes and chloroplasts from sunflower leaves having a wide range of water potentials and showed that PSII activity is strongly reduced at intermediate water potentials (Ψ = 1.2 MPa) whereas in severely dessicated leaves (Ψ = 1.7 MPa) phosphorylation may become limiting (Keck and Boyer, 1974). Detailed studies of the phosphorylation process showed, however, that even at -2.5 MPa spinach leaves maintained >40% of control leaf photophosphorylation capacity (Younis *et al.*, 1981). Nevertheless, inhibition of electron transport capacity under water stress has also been indicated by room temperature chlorophyll fluorescence studies (Downton *et al.*, 1981; Havaux and Lannoye, 1984). On the other hand, the study of Ogren and Oquist (1985) with willow, showed that room temperature fluorescence induction kinetics were unaffected by water stress despite large reductions in CO_2 assimilation. Likewise, Beadle and Jarvis (1973) observed no diminution of uncoupled electron transport capacity for *Pircea sitchensis* until Ψ reached -2.8 MPa although CO_2 assimilation was almost completely inhibited at this Ψ and needles neared the point of shedding due to severe desiccation. Quantum efficiency

determinations also showed no effect of water stress on electron transport capacity of *Larrea divaricata* (Mooney *et al.*, 1977).

Such discrepencies in the susceptibility of electron transport to water stress could be explained in terms of light level experienced by plants during the stress period as Björkman and Powles (1984) working with *N. oleander* showed that exposure to high PPFD at low Ψ can increase the inhibition of photochemical reactions (especially PSII). However, such photoinhibitory effects were only observed at Ψ at which stomata were completely closed and gas exchange measurements impossible. Sharpe and Boyer (1986) have however shown that photosynthesis at low Ψ in *H. annuus* is not dependant upon light level and that loss of chloroplast capacity to fix CO_2 is entirely the result of a direct effect of water availability on chloroplast function.

A more likely possibility is that the rate of water stress development is important in determining which partial processes are impaired by low Ψ. Studies showing a loss of electron transport capacity have usually involved almost complete inhibition of photosynthesis over only a few days (e.g. Keck and Boyer, 1974; Govindjee *et al.*, 1981). The studies with *P. sitchensis, L. divaricata* and *Salix* sp, however, allowed water deficits to develop over a matter of weeks. It is likely that this is of great importance, as Downton (1983) has shown that variable fluorescence is maintained under conditions where water stress is imposed slowly, but not under conditions of rapid dessication. There certainly appears to be a need to standardise terminology in this respect as both Sharpe and Boyer (1986) and Downton (1983) seem to think that reductions of >1.0MPa over 4 days reflect slow imposition of water stress whereas von Caemmerer and Farquhar (1984) consider a reduction of 0.7 MPa over the same period of time rapid!

There are also reports of water stress reducing activity of various enzymes of the PCR cycle. Although rubisco activity was observed to be reduced in water stressed leaves of *G. hirsutem* (Jones, 1973), measured CO_2 assimilation rates were only loosely related to rubisco activity. In *G. hirsutum* carbonic anhydrase (Jones, 1973) is also reduced by water stress whilst for *Hordeum vulgare* Ru5P kinase is also reduced

(Huffaker *et al.*, 1970). Other photosynthetic enzymes remain to be examined.

From the above it should be seen that our knowledge of mechanisms involved in impairment of mesophyll photosynthesis under conditions of water deficit is both scanty and contradictory with differences in results between various laboratories yet to be resolved in terms of environmental conditions, rate of stress imposition and species used. Unfortunately, studies using leaf slices and isolated protoplasts and chloroplasts have tended to further confuse rather than enlighten the situation.

Following observations of Plaut (1971) that exposure of isolated chloroplasts to assay mediums of low (negative) osmotic potentials reduces photosynthetic activity, Berkowitz and Gibbs (1982ab, 1983ab) conducted extensive studies on mechanisms involved. A central assumption in these studies was that chloroplasts shrink as π of reaction medium becomes more negative and that this situation mimics the situation in a dehydrating leaf. Neither assumption is valid.

Although Berkowitz and Gibbs used sorbitol as a non permeating osmoticum, Robinson (1985b) has shown that upon transfer of chloroplasts to a medium of more negative potential than they have previously experienced, a temporary change in chloroplast membrane permeability occurs and that the normally impermeable sorbitol is taken up by the chloroplast to maintain chloroplast volume. Reductions in photosynthetic capacity are thus more likely a consequence of increased osmotic potential in the chloroplast than reduced chloroplast volume. Kaiser and Heber (1981) have shown that a number of stromal enzymes are inhibited by increased solute concentration.

The concept advanced by Kaiser and coworkers (Kaiser, 1982; Kaiser *et al.*, 1981, 1983ab) that such reductions in enzyme activity under conditions of "osmotic" stress are responsible for inhibition of CO_2 assimilation does not however appear to extrapolate to leaves on water stressed plants. Although Kaiser (1982) found a remarkable similarity among species in the sensitivity of photosynthesis in leaf slices to osmotic stress when the degree of stress was expressed as a relative reduction in protoplast volume, inhibition did not commence until volumes were less than 55% of those of fully hydrated leaf slices. This of course corresponds to a relative water content

(RWC) of 55%, which is far below RWCs required to completely inhibit gas exchange in attached leaves (see Bunce, 1986). It thus appears that whilst changes in photosynthetic capacity of chloroplasts, protoplasts and leaf slices are a biochemical curiosity, in attached leaves some other process impairs gas exchange prior to osmotic effects on cell volume becoming important. One such factor could be ABA. Although early studies using exogenously applied ABA showed that it reduced stomatal conductance whilst chloroplast capacity remained unaltered (Cummins *et al.*, 1971) other studies (by means of A/p_i curves) have shown that ABA can apparently reduce mesophyll capacity for gas exchange (Cornic and Miginiac, 1983; Raschke and Heidrich, 1985; Bunce, 1987). It is thus conceivable that an increase in ABA levels in water stressed tissue may be responsible for reduced chloroplast capacity. Studies with leaf slices (Sharkey and Raschke, 1980) and isolated mesophyll cells (Mawson *et al.*, 1981; Raschke and Heidrich, 1985) have failed to detect any effect of ABA on the photosynthetic process, however. Likewise, although Ward and Bunce (1987) reported reductions in both quantum yield and carboxylation efficiency, an increase in RuP_2 levels in ABA treated leaves was not associated with a decrease in *in vivo* rubisco activity. Such contradictory results suggest that observations of reductions in chloroplast CO_2 fixation capacity observed upon addition of ABA to detached leaves is an artefact. It now appears that ABA closes some stomata completely whilst leaving others open. This results in chloroplasts directly below such stomata being close to the compensation point whilst those below open stomata are most likely unaffected. Reductions in p_i are not detected by traditional gas exchange techniques (G. Farquhar, pers. comm.). A further possibility is that the ratio of diffusivities between H_2O and CO_2 breaks down at low conductances. Interactions of gas molecules with guard cell walls becomes increasingly important at low stomatal apertures (Leuning, 1984). As guard cell walls are themselves wet, CO_2 diffusing through stomatal pores must be absorbed on guard cell walls if stomatal apertures are low. This absorbtion could drastically increase the H_2O/CO_2 diffusivity ratio and hence an overestimation of p_i at low conductances will occur. It is therefore interesting that observations of Ward and Bunce (1987) *viz* reduced quantum yield, reduced carboxylation efficiency, reductions in

light saturated CO_2 assimilation rate and an increase in RuP_2 levels, despite a lack of change in rubisco activity are all consistent with a reduction in p_i upon addition of ABA with no change in mesophyll capacity. The role of ABA in reducing mesophyll photosynthetic capacity is therefore neither definable, nor verifiable at this stage. Thus, although there is good evidence for both stomatal and mesophyll function being impaired under conditions of water deficit, precise definitions of specific processes involved and identification of instigating factors await further investigation.

2.1.4 Water and *Citrus* spp.

Modern day citrus cultivars almost certainly evolved from progenitors in the substorey of low latitude sub-tropical rainforests in the Asiatic region (Scora, 1975; Chapot, 1975). Such climates are characterised by an almost continuous supply of water and protection from strong insolation due to an extensive overstory (Reuther, 1973). It would not, therefore, be expected that *Citrus* spp. would have evolved specific mechanisms for tolerating water deficits. Although this appears to be the case, it turns out that members of this genus are capable of surviving extended periods of water deficit.

2.1.4.1 Water relations of Citrus spp.

Citrus leaves are characterised by relatively low (negative) osmotic potentials with unstressed mature leaves having values ranging from -1.8 to -2.6 MPa at full turgor (Levitt and Ben Zaken, 1975; Fereres *et al.*, 1979; Syvertsen and Albrigo, 1980; Syvertsen *et al.*, 1981). Despite such low values, under orchard conditions midday water potentials may approach zero turgor even when trees are well watered (Elfving *et al.*, 1972; Syvertsen and Albrigo, 1980). This has been attributed to high resistances to water flow from soil to leaf (Elfving *et al.*, 1972). For example, from measurements of Ψ_{leaf}, solution osmotic potential and transpiration rate, Hoare and Barrs (1974) calculated hydraulic conductances of citrus seedlings to be 0.045 gm H_2O MPa^{-1} dm^{-2} hr^{-1} whilst for sweet pepper and rockmelon values of 0.27 and 0.40 gm H_2O MPa^{-1} dm^{-2} hr^{-1} were obtained.

Despite large diurnal fluctuations in Ψ_{leaf}, under conditions of low VPD stomatal conductance can vary little throughout the day (Allen and Cohen, 1974; Syvertsen and Albrigo, 1980) indicating that *in the short term* Ψ_{leaf} exerts little influence on stomatal conductance. Rather stomatal responses to VPD and leaf temperature may be more important on this timescale (Chapter 9).

2.1.4.2 Responses of Citrus spp. *to water deficit*

In contrast to stomatal behaviour observed during diurnal fluctuations (Section 2.1.4.1), when measured during a drying cycle under less variable environmental condtions, stomatal conductance in citrus is generally reduced prior to attainment of zero turgor (Hoare and Barrs, 1974; Kaufmann and Levy, 1976; Fereres *et al.*, 1979). Indeed, it has been observed in some cases that Ψ remains constant under water stress whilst stomatal conductance is reduced (Green, 1983; Levy, 1983). This behaviour, along with the observation that citrus lacks the ability to osmotically adjust (Fereres *et al.*, 1979) suggests that the behaviour of citrus under water deficit is "pessimistic" with stomatal closure occurring prior to appreciable depletion of soil moisture (Cohen and Cohen, 1983). Despite abrupt stomatal closure upon depletion of soil moisture, leaves of 'Valencia' orange can survive predawn Ψ of less than -5.0 MPa although it may take up to 2 months for stomata of such leaves to resume full opening upon rewatering (Fereres *et al.*, 1979). It therefore appears that whilst citrus leaves can survive extended periods of water deficit, in general such deficits are avoided by closing stomata at high soil water levels.

Despite the fact that most citrus orchards are irrigated, very little is known about the response of CO_2 assimilation under conditions of water deficit induced stomatal closure. Working with greenhouse grown potted sour orange and sweet lime seedlings exposed to full daylight Bielorai and Mendel (1969) observed that transpiration rates were less affected by soil water deficit than photosynthesis. Measurements of leaf resistances suggested that mesophyll CO_2 assimilation ability declined faster than stomatal conductance, and indeed may have initiated stomatal closure. Somewhat similar results

were obtained by Hoare and Barrs (1974) who observed that stomatal conductance and mesophyll CO_2 assimilation capacity decreased in synchrony as water stress progressed.

In this chapter we investigate the response of citrus leaf gas exchange to soil water deficit. Results obtained for leaves on trees subjected to soil depletion are compared with those observed when leaves from unstressed trees are exposed to 'osmotic' stress using polyethylene glycol (PEG).

2.2 MATERIALS AND METHODS

2.2.1 Growth of plants

Two-year-old 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) budded on Sweet orange (*C. sinensis* [L.] Osbeck cv. Parramatta Sweet orange) in 4 litre free draining pots were grown under glasshouse conditions as described in Section 1.4.2.1.

2.2.2 Effect of osmotic stress on citrus leaf discs

2.2.2.1 General Procedure

Two 3 month old leaves were removed from each of 4 trees and transferred to the laboratory in a plastic bag to prevent dessication. From each leaf nine 1 cm diameter discs were taken and 3 floated on -0.5 MPa PEG; 3 on -1.5 MPa PEG and 3 on -2.5 MPa PEG giving 24 discs equilibrating at each osmotic potential. After 3 hours equilibration time under fluorescent lights (PPFD = 30 μmol m^{-2} s^{-1}) four discs at each Ψ were transferred to a dark room and room temperature chlorophyll *a* transients recorded after one hour's dark adaption. A further 4 discs were taken for polarographic determination of leaf slice O_2 evolution. A further 24 discs (8 at each Ψ) were then exposed to a one hour 'photoinhibitory' treatment under a PAR38 lamp with a PPFD of 1,500 μmol m^{-2} s^{-1}. Measurements of chlorophyll *a* fluorescence and leaf slice O_2 evolution were then made as for leaves that had not received photoinhibitory treatment. The remaining 24 discs were treated to 2 hours photoinhibitory treatment prior to fluorescence and O_2 evolution determinations.

In a second experiment, in order to check that ageing of leaf discs did not affect previous results, 24 leaf discs were removed from 6 leaves and 8 treated to each [PEG] as before. After 2.5 hours floating on PEG, 12 discs were exposed to 180 minutes photoinhibitory treatment. The remaining 12 discs were maintained under fluorescent lights. Chlorophyll fluorescence and leaf-slice oxygen evolution were measured for leaves exposed to 30 μmol m^{-2} s^{-1} (fluorescent lights) or 1500 μmol m^{-2} s^{-1} (photoinhibitory treatment). Osmotic potentials of PEG solutions were determined from calibration curves relating concentration to osmotic potential previously established in this laboratory.

2.2.2.2 Room temperature chlorophyll a fluorescence

Chlorophyll *a* fluorescence transients were measured on the upper surface of each leaf disc after 60 minutes dark adaptation time as described in Section 1.4.2.4.

2.2.2.3 Leaf slice oxygen evolution

For preparation of leaf slices, discs were sliced into ~500 μm strips with razor blades. Slices were collected and floated in 5 ml infiltration medium containing 50 mol m^{-3} HEPES-KOH pH 7.6, 0.5 mol m^{-3} $CaSO_4$ and sufficient sorbitol to give equivalent osmotic potentials to which the disc had previously been exposed. Sorbitol concentrations were equated to osmotic potential using tables supplied by Weast (1981) for mannitol. The slices were infiltrated for ~ 2 min by gently applying and releasing a vacuum several times. Following infiltration, 4 mls of infiltration medium and all leaf slices were transferred to a Clark-type oxygen electrode. O_2 evolution was determined as described in Section 1.4.2.3.

2.2.3 Effect of soil mositure deficit on citrus leaf gas exchange

2.2.3.1 General procedure

As for the previous experiments, 3 month old leaves were used for gas exchange and water relations determinations. Water deficit was obtained for three trees by

withholding water over a period of 14 days. A further 3 trees were watered daily. During the experimental period, maximum PPFD in the glasshouse was 900 μmol m^{-2} s^{-1}, air temperature varied from 10°C to 31°C and VPD ranged from 0.2 to 2.0 kPa. After 0, 4, 7, 10 and 14 days following cessation of watering leaf gas exchange was measured for one leaf from each tree, after which Ψ_{leaf} was immediately determined. The tree was then removed to a darkened room and chlorophyll *a* fluorescence induction kinetics measured on a leaf from the same flush as the gas exchange leaf. At the completion of the experiment predawn Ψ and π were determined for 3 control and 3 stressed leaves.

2.2.3.2 Gas exchange

Meaurements were made using the system and conditions described in Section 1.2.2.2.

2.2.3.3 Leaf water potential (Ψ)

Immediately after gas exchange measurement, leaves were excised whilst enclosed in a plastic bag. Ψ was determined using a pressure chamber (Scholander *et al.*, 1965) equipped with a binocular microscope to observe end points.

2.2.3.4 Chlorophyll a fluorescence

Transients were measured on the upper surface after one hour dark adaptation time as described in Section 1.4.2.4. Checks after fluorescence measurement showed that Ψ_{leaf} was no more than 0.2 MPa less negative than that of the gas exchange leaf.

2.2.3.5 Pre-dawn leaf water potential

Leaf water potential prior to sunrise (PPFD <1 μmol m^{-2} s^{-1}) was determined as described in Section 2.2.3.3.

2.2.3.6 Leaf osmotic potential

Leaf osmotic potential was determined from the pressure-volume relationship of intact leaves. This method utilises the fact that once leaf P has reached zero, the volume of water in a leaf is related to the pressure chamber end point as follows (Scholander *et al.*, 1965):

$$\frac{1}{P_c} = \frac{V_s - V}{RTN} \qquad (2.3)$$

where P_c = balance pressure, V_s = volume of water in the leaf at full turgor, V = volume of water lost from the leaf, R is the universal gas constant, T = temperature in K and N is the moles of solute in the leaf. Thus a plot of $1/P_c$ against V becomes linear when turgor pressure becomes zero (Turner, 1981).

Extrapolation of the straight line to V = 0 (100% RWC) gives the negative reciprocal of the osmotic potential at full turgor. For any RWC, the turgor pressure is then the difference between Ψ and π. Extrapolation of the straight line to $1/P_c = 0$ (i.e. infinite pressure) often does not pass through RWC = 0%. It is thought that this is due to the presence of water in cell walls (apoplastic water). Such water is held by extremely strong surface tension forces and is considered unlikely to drain at pressures usually employed in PV curves (Nobel, 1974; Tyree and Jarvis, 1982).

For determinaion of π via this method, immediately following determination of pre-dawn Ψ leaves were weighed to ±0.05 mg and placed on a laboratory bench. After 10-60 minutes, a new value of Ψ was determined and the leaf immediately reweighed. This procedure was repeated 7-10 times. Values of $1/\Psi$ were plotted as a function of cumulative weight loss to generate a PV curve. Predawn osmotic potential was determined from extrapolation by linear regression ($r^2 > 0.98$) to the $1/\Psi$ axis. Turgor pressure was calculated as the difference between osmotic potential at the relevent water content and the corresponding Ψ.

2.3 RESULTS

2.3.1 Exposure of leaf discs to osmotic stress

Prior to exposure to high light, Ψ had no effect on leaf slice O_2 evolution. Imposition of photoinhibitory treatment reduced O_2 evolution for leaves at all water potentials, but this occurred to a far greater extent for leaves at Ψ = -2.5 MPa (Table 2.1).

Leaf water potential (MPa)	Time of Exposure to High Light (min) 0	60	120
	Oxygen evolution (μmol O_2 m^{-2} s^{-1})		
-0.5	7.31	3.91	2.13
-1.5	6.73	2.27	1.94
-2.5	6.64	0.14	0.24
LSD (p = 0.05)[1]	1.02	0.72	0.64

Table 2.1: Rate of oxygen evolution of leaf slices at -0.5, -1.5 and -2.5 MPa as affected by exposure to high light (PPFD =1500 μmol m^{-2} s^{-1}). [1]Least significant difference.

Both Ψ_{leaf} and exposure to high light altered room temperature fluorescence induction kinetics. Prior to light exposure, leaves at Ψ = -1.5 MPa and Ψ = -2.5 MPa showed a reduction in the F_V/F_O ratio compared to leaves at -0.5 MPa. Exposure to photoinhibitory treatment resulted in a reduction in the F_V/F_O ratio for leaves at all Ψ with the result that after 120 min of photoinhibition there was no difference in the F_V/F_O ratio between Ψ treatments (Table 2.2).

Leaf water potential (MPa)	Time of Exposure to High Light (min) 0	60	120
	Variable fluorescence ratio		
-0.5	1.97	1.23	0.82
-1.5	1.43	0.97	0.72
-2.5	1.37	0.74	0.64
LSD (p = 0.05)[1]	0.44	0.49	0.27

Table 2.2: Variable fluorescence ratio (F_V/F_O) of leaf discs at -0.5, -1.5 and -2.5 MPa as affected by exposure to high light (PPFD = 1500 μmol m^{-2} s^{-1}). [1]Least significant difference.

Rate of fluorescence quenching (Q) (initial slope from P to S) was also affected by Ψ and high light. Prior to photoinhibition Q was far less for leaves at -2.5 MPa than those at -0.5 MPa or -1.5 MPa. This difference was maintained after 60 min photoinhibition but an increase in Q for leaves at Ψ = -2.5 resulted in little difference in Q with Ψ after 120 mins of high light (Table 2.3)

Leaf water potential (MPa)	Time of Exposure to High Light (min) 0	60	120
	Fluorescence quenching index		
-0.5	1.32	1.12	1.03
-1.5	1.37	1.37	1.37
-2.5	0.42	0.51	1.44
LSD (p = 0.05)[1]	0.37	0.42	0.57

Table 2.3: Fluorescence quenching (slope from P to S) of leaf discs at -0.5, -1.5 and -2.5 MPa as affected by exposure to high light (PPFD = 1500 μmol m^{-2} s^{-1}). [1]Least significant difference.

Reductions in photosynthetic capacity after exposure to high light were not merely due to ageing of leaves as comparisons of discs exposed to low and high light for 120 min showed differences in O_2 evolution similar to that observed in Table 2.1 (Table 2.4).

Leaf water potential (MPa)	Light exposure (μmol m^{-2} s^{-1}) 30	1500
	O_2 evolution (μmol O_2 m^{-2} s^{-1})	
-0.5	7.65	2.04
-1.5	7.54	1.92
-2.5	5.81	-0.27
LSD (p = 0.05)[1]	1.24	0.43

Table 2.4: O_2 evolution of leaf slices at -0.5, -1.5 and -2.5 MPa after 3 hours at either 30 μmol m^{-2} s^{-1} or 1500 μmol m^{-2} s^{-1} PPFD. [1]Least significant difference.

2.3.2 Effect of soil water deficit on citrus leaf gas exchange

2.3.2.1 Leaf water relations

Whilst mid-day Ψ of gas exchange leaves varied between -1.1 and -1.5 MPa for control plants, Ψ fell to -1.8 MPa after 7 days of water deficit reaching -2.7 MPa after water had been withheld for 14 days (Figure 2.1).

Pre-dawn Ψ, π and P for control and droughted leaves 14 days after withholding water are shown in Table 2.5.

Treatment	Pre-dawn value (MPa)		
	Ψ	π	P
Control	-0.39 ± 0.18	-2.20 ± 0.36	1.81 ± 0.36
Droughted	-1.71 ± 0.87	-2.57 ± 0.22	0.86 ± 0.65

Table 2.5: Predawn water potential (Ψ), osmotic potential (π) and turgor pressure (P) for control and droughted leaves 14 days after commencement of drought treatment.

This shows that despite appreciable reductions in predawn Ψ, little osmotic adjustment occurred. As π for control leaves at Ψ = -1.71 MPa was -2.38 ± 0.41 MPa a change of less than 10% in π took place in response to drought.

2.3.2.2 Leaf gas exchange

Significant reductions in both CO_2 assimilation rate (Fig. 2.2a) and stomatal conductance (Fig. 2.2b) were observed in water stressed leaves 7 days after cessation of watering with values declining to <25% of controls at day 14. Changes in CO_2 assimilation and stomatal conductance were in step for the first 10 days of drought treatment which resulted in little change in p_i. Stomatal conductance had declined less than CO_2 assimilation by day 14 however which resulted in a large increase in the calculated p_i value (Fig. 2.2c).

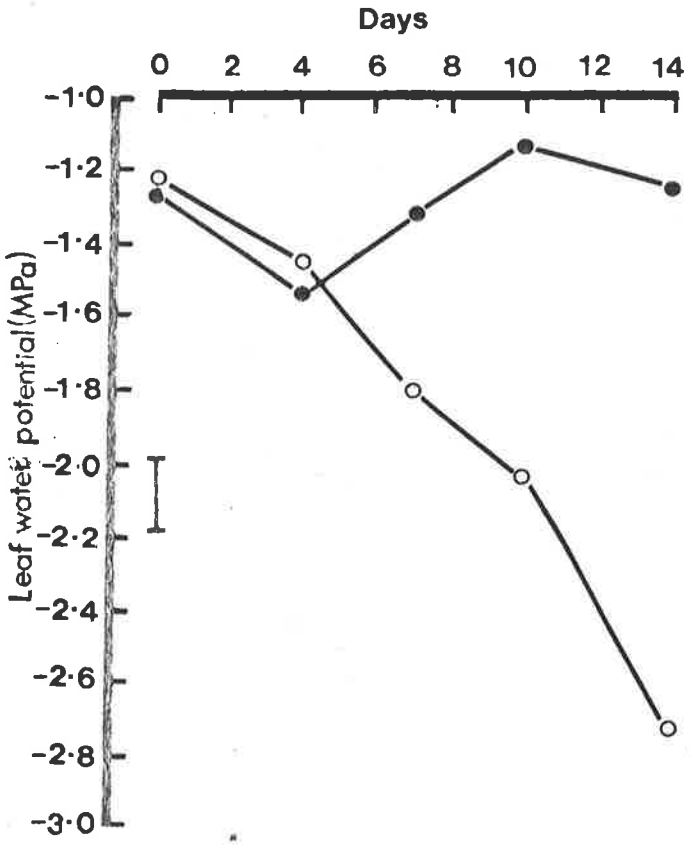


Figure 2.1 Midday leaf water potentials of 'Valencia' orange leaves in response to soil water deficit. (●) leaves on plants watered daily; (○) leaves on plants not watered after day 0.

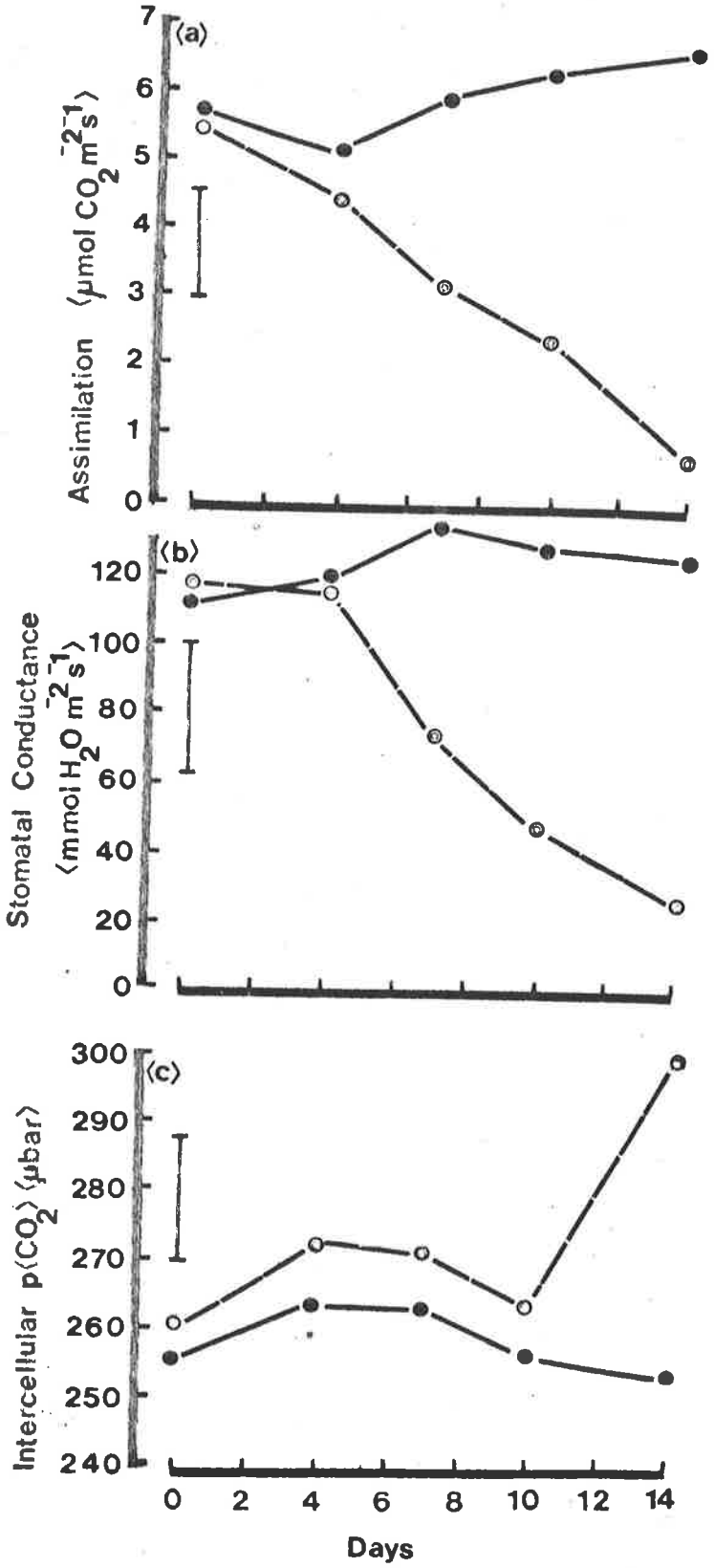


Figure 2.2 Gas exchange of 'Valencia' orange leaves in response to soil water deficit. (●) leaves on plants watered daily; (○) leaves on plants not watered after day 0. (a) CO_2 assimilation; (b) stomatal conductance; (c) intercellular CO_2 partial pressure. Vertical bars represent the Least Significant Difference (p = 0.05).

Changes in leaf gas exchange characteristics are shown in more detail in Figure 2.3 which shows that CO_2 assimilation at high p_i was initially more sensitive to water deficit than the initial slope of the A/p_i curve. At -2.2 MPa CO_2 assimilation was negligible, even at p_i = 800 μbar. Plots of CO_2 assimilation rate and stomatal conductance as a function of Ψ are presented in Figure 2.4. This illustrates that in the course of gradual water deficit in citrus both CO_2 assimilation and stomatal conductance decline towards minimum values prior to attainment of zero turgor.

2.3.2.3 Chlorophyll fluorescence

Water stress had little effect on early phases of chlorophyll *a* fluorescence induction kinetics (Fig. 2.5). Neither variation in values of O or P, fluorescence ratio nor Q were affected by water stress, even though CO_2 assimilation rates were extremely low in water stressed leaves. Nor was the final fluorescence yield (T) affected (Table 2.6).

Treatment	F_o	F_v	F_v/F_o	Q	T
Control	28	34	1.24	1.6	48
Droughted	32	44	1.38	1.5	53
LSD (p = 0.05)	6	12	0.29	0.3	8

Table 2.6: Constant yield fluorescence (F_o), variable fluorescence (F_v), F_v/F_o ratio, degree of quenching (Q) and final fluorescence yield (after 10 minutes) T for control and drought stressed leaves 14 days after cessation of watering.

2.4 DISCUSSION

The two methods of imposing leaf water deficit gave greatly contrasting results in terms of both sensitivity of CO_2 assimilation to Ψ and in changes in chlorophyll *a* fluorescence induction kinetics. Whereas in the absence of high light treatment, for osmotically stressed discs there was little inhibition of CO_2 assimilation rates at Ψ = -2.5 MPa (Table 2.1), CO_2 assimilation rates approached a minimum at -2.0 MPa for leaves on trees exposed to soil water deficit (Fig. 2.4). Furthermore, although osmotically stressed

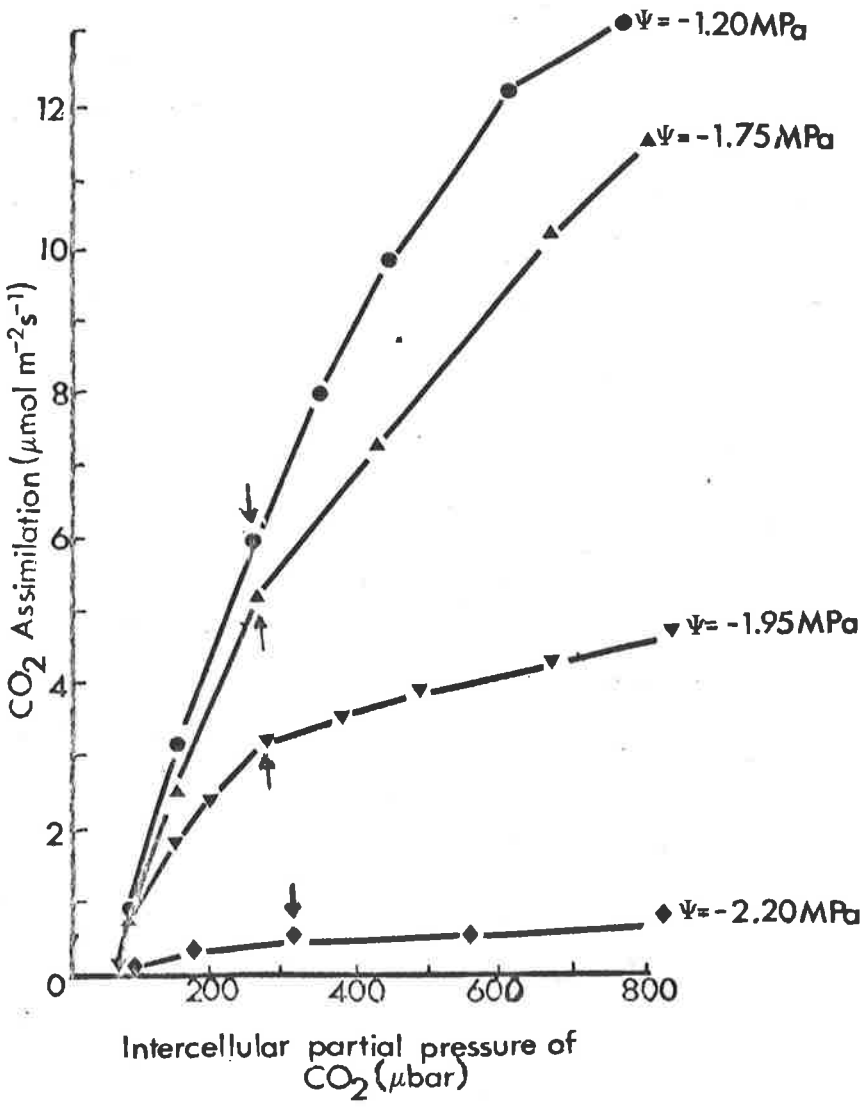


Figure 2.3 Gas exchange characteristics of 'Valencia' orange leaves in response to soil water deficit. Ψ at completion of measurement is indicated for each curve. Arrows indicate atmospheric conditions of 330 μbar CO_2.

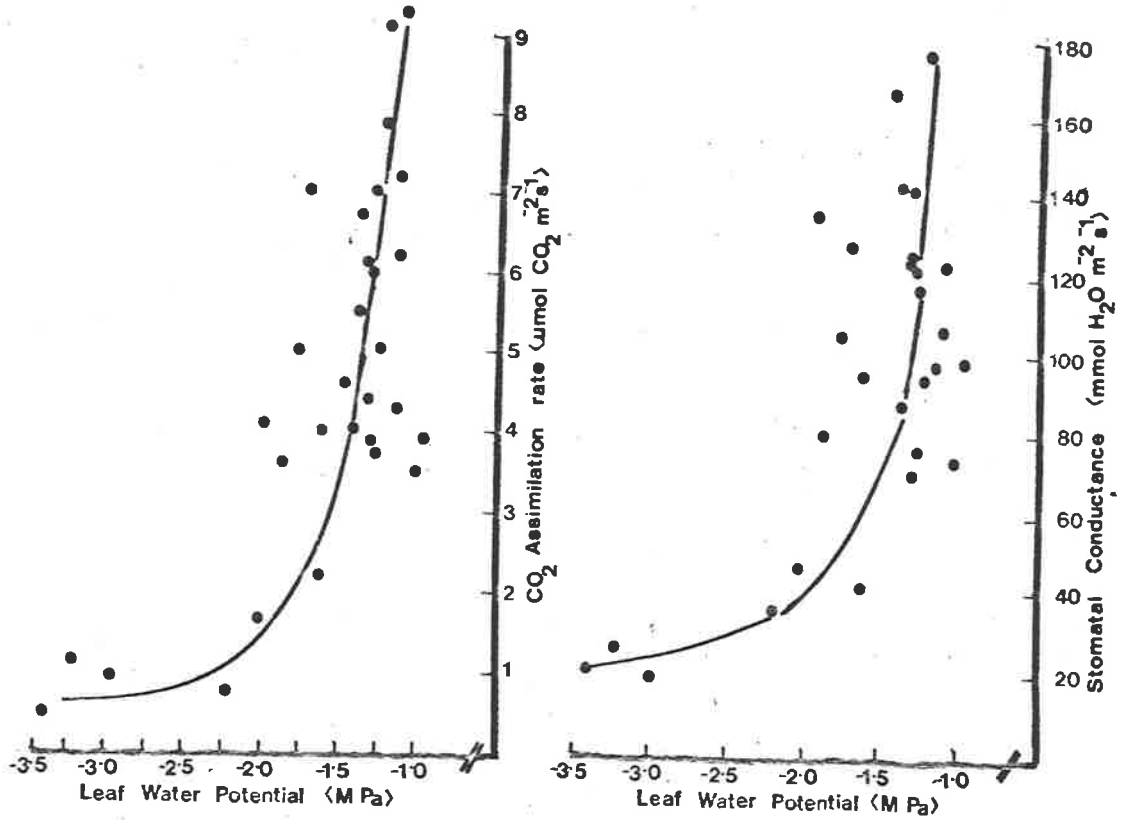


Figure 2.4 Relationship between leaf water potential and (a) CO_2 assimilation rate; (b) stomatal conductance.

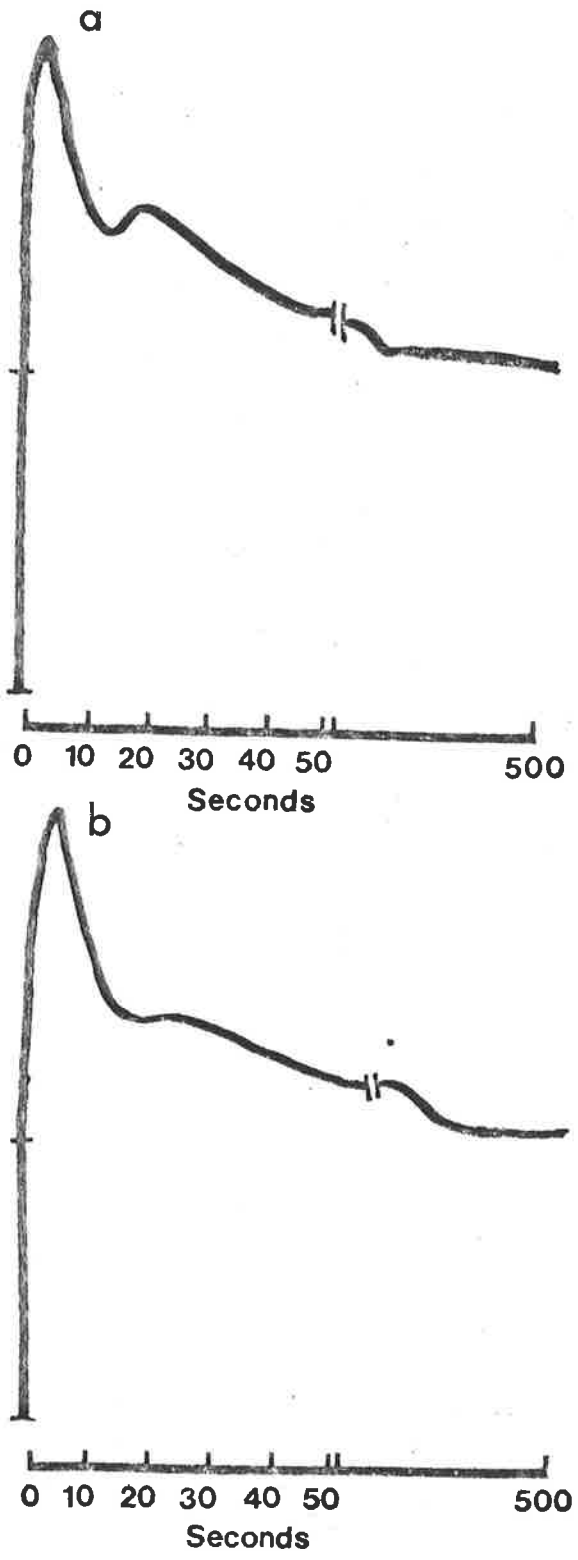


Figure 2.5 Chlorophyll *a* fluorescence induction kinetics of 'Valencia' orange leaves in response to soil water deficit; (a) leaf from plant watered daily (Ψ = -1.3 MPa); (b) leaf from plant not watered for 14 days (Ψ = -3.1 MPa).

leaves were characterised by reductions in variable fluorescence ratio (Table 2.2) and quenching (Table 2.3) no effect on either parameter was observed for leaves on droughted plants (Table 2.6).

If we accept the contention that decreases in CO_2 assimilation under osmotic stress are a result of decreases in protoplast volume (Kaiser, 1982) then clearly some additional factor is responsible for reduced assimilation rates in drought stressed attached leaves.

It does not appear that the sensitivity of leaves on soil droughted trees was a consequence of photoinhibition as no change in variable fluorescence or quenching was observed (Fig. 2.5: Table 2.6) even though gas exchange in these leaves was less than 25% of control levels. Photoinhibition results in marked reductions in variable fluorescence in both leaf discs (Table 2.2) and attached leaves (Table 1.6). Although it is clearly only with reservations that we can extrapolate results with osmotically stressed discs to attached leaves, O_2 evolution and fluorescence data do however indicate that a critical value between -1.5 MPa and -2.5 MPa (zero turgor?) is reached after which the photosynthetic apparatus becomes extremely sensitive to photoinhibition. It may thus be, as was reported by Björkman and Powles (1984) and Ludlow and Björkman (1984), that water stress induced susceptibility to photoinhibition occurs once stomata are tightly closed.

From our discussion in Section 2.1.3 we should class 'Valencia' orange on sweet orange rootstock a tree that takes a 'pessimistic' approach to coping with water stress as (1) stomata were almost fully shut prior to attainment of zero turgor, and; (2) negligible osmotic adjustment was observed.

Whilst this in itself does not provide evidence of citrus having mechanisms sensing soil moisture status similar to those proposed for *Zea mays* (Blackman and Davies, 1985) *Commelina communis* (Zhang *et al.*, 1987) and *Nerium oelander* (Turner *et al.*, 1985), as foreshadowed in Section 2.1.4, other reports of citrus stomatal behaviour allow us to provide a more substantive argument for this proposition.

If stomatal closure under conditions of soil water deficit was solely a function of leaf water potential it necessarily follows that diurnal fluctuations in Ψ should be reflected

in associated changes in stomatal conductance. Although stomatal behaviour in the field is the function of several other environmental variables (discussed in detail in Chapter 9) under conditions where VPD remains reasonably constant and PPFD and temperature are not limiting stomatal conductance remains reasonably constant despite leaf water potentials as low as -2.0 MPa (Allen and Cohen, 1974; Syvertsen and Albrigo, 1980).

A threshold Ψ for stomatal closure for leaves on well watered trees does, however, exist and can be tested by relating stomatal conductance to Ψ for slowly drying excised leaves. Under such conditions, appreciable stomatal closure is not observed until a leaf water potential of ~2.5 MPa is reached with full closure not occurring until Ψ = -3.0 MPa (Syvertsen, 1982).

The concept that leaf water potential exerts little control over stomatal behaviour in the field will be demonstrated more lucidly in Chapter 9 where it is shown that for well watered trees, under conditions of low VPD and non-limiting PPFD and temperature (as prevailed in this experiment) diurnal fluctuations in Ψ down to -2.0 MPa are more a function of stomatal conductance rather than *vice versa.* Furthermore data in Chapter 8 will illustrate that rootstocks exert an influence on stomatal behaviour under conditions of soil water deficit, but this is not mediated by changes in leaf water potential. Thus, although the evidence is indirect for leaf water potential not being a direct factor in modulation of stomatal closure under conditions of soil water deficit in citrus there exists little or no evidence supporting such an involvement.

One possibility that could account for stomatal closure in the absence of large changes in Ψ is via a direct effect of CO_2 assimilation on stomatal conductance (Wong *et al.*, 1978). There is however evidence from other species against such an effect under conditions of soil water deficit. Although photosynthetic capacity may be reduced in water stressed leaves (Sharkey, 1984), Schulze and Hall (1982) showed that in many cases stomatal conductance decreases prior to changes in photosynthetic capacity. Thus, although in *H. annuus* for example, CO_2 assimilation and stomatal conductance may decrease in concert resulting in little change in p_i (Turner *et al.*, 1985) in other situations a substantial reduction in p_i may occur in water stressed leaves of this species (Sharpe and

Boyer, 1986). Similarly, although Gollan *et al.* (1985) provided good evidence for soil moisture status, rather than leaf water potential influencing stomatal conductance in *N. oleander* they observed photosynthetic capacity to be unaffected whilst Björkman *et al.* (1977) noted that decreases in p_i were not sufficient to account for reductions in mesophyll CO_2 assimilation capacity in this species. It therefore appears that the relationship between photosynthetic capacity and stomatal conductance under conditions of soil water deficit is not unique and hence modulation of stomatal conductance via changes in photosynthetic capacity is an unlikely cause for reduced conductances under these conditions.

If, on the other hand, we accept that changes in identity or amount of root derived compounds arriving at the leaf *via* xylem sap are responsible for lower stomatal conductances (Schulze, 1986; Zhang *et al.*, 1987), then we are required to explain the means by which mesophyll CO_2 assimilation capacity is reduced. One possibility is that an increase in compounds such as ABA in drought stressed leaves results in a reduction in photosynthetic capacity as well as stomatal conductance. As discussed in Section 2.1.3.3 however whether or not ABA really does affect mesophyll CO_2 assimilation capacity remains to be confirmed. A second possibility is that as stomatal conductance *gradually* declines with soil water availability, chloroplast metabolism is adjusted in such a way that p_i remains constant. That such a process could occur is supported by experiments of Cornic *et al.* (1987) with *P. vulgaris* and Ehleringer and Cook (1984) with *Ercelia farinosa* who both showed that under conditions of "rapid" water stress reductions in CO_2 assimilation rate were solely a consequence of a lowering of p_i due to stomatal closure. On the other hand, when stress was imposed more slowly mesophyll and stomata decreased in concert. Despite an almost complete inhibition of mesophyll photosynthetic capacity in slowly stressed *P. vulgaris* leaves, Cornic *et al.* (1987) also observed that photosynthetic capacity and stomatal conductance increased in a parallel fashion after rewatering, returning to control values only 32 hours after soil water had been replenished. Such observations suggest to this author that a mechanism might exist by which chloroplast CO_2 fixation is reversibly "switched off" when stomatal closure occurs

under conditions of soil water deficit. Such a mechanism would have the great advantage of preventing a depletion of p_i and hence increased susceptibility to photoinhibition (Powles, 1984), but for such a mechanism to work it would be necessary to prevent an over-reduction of the electron transport chain due to decreased requirement for ATP and NADPH for the PCO and PCR cycles. It is in this respect than our observed insensitivity of chlorophyll *a* fluorescence to water stress (Table 2.6) is instructive.

As discussed in Section 1.1.3 all light arriving at reaction centres must be dissipated by either photochemical reaction, fluorescence, or non-radiative de-excitation. From our gas exchange study it is clear that the proportion of quanta arriving at the leaf used for photosynthesis (which accounts for ~85% of quanta absorbed under optimal conditions : Papageorgiou, 1975) must have been greatly reduced in leaves showing low rates of gas exchange due to soil water deficit. Although quanta absorbed must have remained constant regardless of photosynthetic use of such light, no change in the terminal fluorescence value was observed. The inescapable conclusion is therefore that the percentage of quanta undergoing thermal de-excitation must have increased considerably as water stressed progressed. Recently Demmig and Björkman (1987) demonstrated that such an effect occurs when plants are exposed to PPFD in excess of electron transport capabilities. Thus it is possible that as stomata gradually close chloroplasts may dissipate an increasing proportion of absorbed quanta via thermal de-excitation. This would, in effect, reduce the amount of light received by reaction centres causing initially a reduction in CO_2 assimilation only at high p_i (Fig. 2.3; von Caemmerer and Farquhar, 1984). Such a mechanism would serve to prevent photoinhibition as a lowering of mesophyll gas exchange would prevent a reduction in p_i, which, if the PSII reaction centre were to be exposed to all quanta absorbed by the leaf would no doubt lead to photoinhibition (Powles, 1984). The presence of such a mechanism would account for failures of some researchers to detect any change in either chloroplast enzyme activity or electron transport capacity in drought stressed leaves (Beadle and Jarvis, 1977; von Caemmerer and Farquhar, 1984) and for the rapid increase in photosynthetic capacity upon rewatering (Cornic *et al.*, 1987).

2.5 CONCLUSIONS

When citrus trees are droughted, inhibition of both stomatal conductance and CO_2 assimilation occurs prior to complete loss of leaf turgor. CO_2 assimilation in such leaves appears far more sensitive to Ψ than for osmotically stressed leaf discs, suggesting that protoplast volume changes are not important in reducing mesophyll CO_2 fixation capacity in attached leaves. It is unlikely that lower rates of gas exchange are solely a function of Ψ as diurnal fluctuations in Ψ of similar magnitude have little effect on stomatal conductance. Room temperature chlorophyll *a* fluorescence is unaltered by water deficit which suggests that an increased proportion of intercepted quanta may be dissipated via thermal de-excitation in water stressed leaves.

CHAPTER THREE
EFFECT OF ROOTZONE SALINISATION ON GAS EXCHANGE OF 'VALENCIA' ORANGE

3.1 INTRODUCTION

High levels of soluble salts in soil and water with associated reductions in crop yields have led to vast problems in irrigated lands of arid and semiarid regions. It has been estimated that more than 30% of irrigated land world wide is affected by salinity (Epstein *et al.*, 1980). Salinity problems are not confined to irrigated soils and problems associated with high levels of salt in the soil solution are now observed over large areas of the North American Plains and Southern Australia.

Although engineering projects and development of more efficient farming techniques may to a certain extent reduce the problem, development of salt tolerant genotypes provides the only means by which the salinity problem can be fully overcome (Epstein *et al.*, 1980; Shannon, 1982). A need for a basic knowledge of physiological mechanisms involved in salt tolerance has therefore led to intensive research into this area over the last 15 years (Flowers *et al.*, 1977; Greenway and Munns, 1980; Munns *et al.*, 1983; Staples and Toenniessen, 1984; Flowers, 1985).

3.1.1 Effect of salinity on plant growth

Soluble salts may have two types of effects on a growing plant: specific effects due to particular ions being harmful to the plant (ion toxicities) and a general effect due to a decrease in (more negative) osmotic potential of the soil solution around the root system. This lowers the water potential gradient between leaf and soil and hence reduces water uptake in the absence of osmotic adjustment (osmotic effect). The relative importance of these two effects and the amount of salt in the soil solution required to reduce growth via either mechanism varies considerably at both the species (Maas and Hoffman, 1977) and cultivar (Epstein, 1985) level. Relative salinity tolerances of several plants often used in salinity studies are shown in Table 3.1.

Sensitive	Moderate tolerance	Good tolerance	True halophytes
Citrus spp.	*Medicago sativa*	*Hibiscus cannabinus*	*Avicennia* spp.
P. vulgaris	*Vigna unguiculata*	*Hordeum vulgare*	*Atriplex* spp.
Cicer arietinum	*Oryza sativa*	*Beta vulgaris*	*Suadea maritima*
Pisum sativum	*Triticum aestivum*	*G. hirsutum*	
Vicia faba		*Spinacea oleracea*	
Prunus spp.			
Vitis vinifera			

Table 3.1: Relative salt tolerance of some plants commonly used in salinity studies. Taken from Maas and Hoffmann (1977), Läuchli (1984) and Jefferies and Rudmik (1984).

3.1.1.1 Characteristics of salt tolerant plants

Halophytes and most salt-tolerant glycophytes are characterised by an ability to accumulate high concentrations of inorganic ions in their leaves under saline conditions (Munns *et al.*, 1983). This accumulation results in lower (more negative) osmotic potentials thereby enabling favourable water relations to be maintained despite the presence of appreciable reductions in soil water osmotic potential under saline conditions (Flowers, 1985). When exposed to such environments, salt sensitive plants show little or no growth due to an inability to tolerate the presence of appreciable concentrations of inorganic ions in their leaves or as a consequence of internal water deficit due to the low osmotic potential of soil water (Flowers *et al.*, 1977). The ability of many halophytes to tolerate high concentrations of NaCl in leaves does not appear, however, to be due to an intrinsic tolerance of their metabolic processes to NaCl. Enzymes (Greenway and Osmond, 1972; Flowers *et al.*, 1977) polysomes (Brady *et al.*, 1984) and m-RNAs (Gibson *et al.*, 1984) of salt tolerant plants are no more tolerant of inoganic ions that those of salt sensitive plants. Rather, it appears that the inorganic ions used by salt-tolerant plants for turgor maintenance under saline conditions are sequestered within vacuoles (Wyn-Jones *et al.*, 1979) with osmotic equilibrium between cytoplasmic and vacuolar compartments being maintained *via* accumulation in the cytoplasm of 'compatible solutes' such as betaines (Wyn-Jones and Storey, 1981), proline (Paleg and Aspinall, 1981) or low molecular weight carbohydrates (Gorham *et al.*, 1981). Numerous cytoplasmic

processes have been shown to function efficiently in the presence of high concentrations of these compatible solutes (Borowitzka, 1981; Brady *et al.*, 1981; Gibson *et al.*, 1984; Pollard and Wyn-Jones, 1979).

Chloroplasts of halophytes have also been observed to maintain relatively low levels of sodium and chloride despite high levels in the leaf as a whole. Using *S. maritima* measurements of chloroplast ion content by both X-ray microanalysis (Hajibogheri *et al.*, 1984) and aqueous isolation (Robinson and Downton, 1985) have demonstrated that chloroplast sodium and chloride content is independent of the levels of these ions within the leaf. Similar results have also been observed for the halophyte *Mesembryanthemum crystallinum* (Demmig and Winter, 1986).

The salt tolerant glycophyte *S. oleracea* is also capable of maintaining low levels of sodium and chloride in the chloroplast despite large increases in the leaf levels in salt stressed leaves (Robinson and Downton, 1985). Photosynthetic capacity (chlorophyll basis) is thus maintained in this species despite high foliar salt levels (Robinson and Downton, 1985; Downton *et al.*, 1985). Maintenance of photosynthetic capacity is due at least in part to accumulation of glycinebetaine in the chloroplast (Robinson and Jones, 1986). Photosynthesis has also been observed to be maintained under conditions of salinity stress in another salt-tolerant glycophyte *B. vulgaris* (Papp *et al.*, 1983). The observation that leaf expansion is reduced despite maintenance of photosynthetic capacity in *S. oleracea* and *B. vulgaris* as well as *H. cannabinus* (Curtis and Läuchli, 1986) coupled with reports of carbohydrate accumulation in salinised leaves of other salt-tolerant glycophytes such as *H. vulgare* (Munns *et al.*, 1982) has led Munns and Termaat (1986) to suggest that photosynthesis does not limit leaf growth under saline conditions. Whilst there is no reason to dispute this statement for halophytes or salt tolerant glycophytes, as discussed below it does not apply to plants sensitive to salinity stress.

3.1.1.2 Characteristics of salt sensitive plants

There are reports of both ion toxicities and osmotic effects reducing growth of non-tolerant glycophytes under saline conditions. In some plants capable of salt exclusion, turgor loss under saline conditions has been clearly implicated as the cause of growth reductions (Nieman and Poulson, 1967; Hoffmann and Jacobs, 1978). There is however convincing evidence that at low to moderate salinities for crops such as *O. sativa* (Yeo and Flowers, 1984), *T. aestivum* (Kingsbury and Epstein, 1986), *C. arietinum* (Lauter and Munns, 1987) and *V. vinifera* (Downton *et al.*, 1979) accumulations of sodium and/or chloride impair leaf functioning despite maintainance of favourable water relations.

It is widely assumed that such plants lack the ability to effectively compartment sodium and chloride within leaf cells. There are however only two reports on ion distributions within leaf mesophyll cells of salt sensitive species. Harvey and Thorpe (1986) using *T. aestivum* observed massive accumulation of sodium in both cytoplasm (1.2 M) and chloroplast (1.9 M) as well as high chloride levels in the cytoplasm (703 mol m^{-3}) but not in the chloroplast (188 mol m^{-3}) supporting the notion of inadequate compartmentisation of toxic ions in such plants. Seemann and Critchley (1985) also observed high concentrations of cytoplasmic sodium and chloride in *P. vulgaris*. Given that numerous metabolic functions in leaves are inhibited by NaCl *in vitro* (Section 3.1.1) it therefore seems inevitable that metabolic function is impaired by salinity in such plants.

One process so affected is photosynthesis. For two lines of wheat of differing salt resistance Kingsbury *et al.* (1984) showed that associated with lower growth rates in the salt sensitive lines were lower CO_2 assimilation rates and stomatal conductances. Likewise, in *O. sativa* both CO_2 assimilation and stomatal conductance are reduced at an external salinity of only 50 mol m^{-3} (Yeo *et al.*, 1985). In both cases sodium, rather than chloride was implicated as the causitive factor. It also appears that high chloride levels may significantly reduce photosynthesis in some species. In *V. vinifera* for example, upon salinisation a substantial reduction in gas exchange was observed despite maintenance of low sodium levels and favourable water relations (Walker *et al.*, 1981).

Rawson (1986) reported reduced CO_2 assimilation for salt susceptible lines of *H. vulgare* and *T. aestivum* but no data on leaf sodium content or plant water relations was presented making identification of factors responsible for impaired gas exchange in salinised leaves difficult.

A substantial body of evidence therefore exists showing that, in contrast to salt tolerant species, substantial reductions in photosynthesis can occur in salinised leaves of non-tolerant plants. In this and following chapters (4-6) we investigate gas exchange behaviour of various *Citrus* spp. under salinity stress in the laboratory. In the second part of this thesis, both the occurrence and significance of reduced rates of gas exchange in leaves on salinised citrus trees is evaluated under orchard conditions.

3.2 GAS EXCHANGE AND SALINITY IN CITRUS

3.2.1 Introduction

Although growth and yield of citrus is reduced even at moderate to low salinities (Shalhevet *et al.* 1973; Maas and Hoffman 1977; Cole and McLeod 1985) the physiological disturbances responsible have not been clearly identified. Descriptive information on visible symptoms and leaf analysis (Cooper and Gorton 1952; Cooper 1961; Cerda *et al.* 1979) fruit quality and number (Levy *et al.* 1979; Francois and Clark 1980) and water relations (Bielorai *et al.* 1978, 1983; Walker *et al.* 1983) is available but to date there has been no comprehensive study on the photosynthetic response to salt stress using grafted trees.

Using seedling rootstocks with different salt uptake characteristics, Walker *et al.* (1982) observed reductions in both carboxylation efficiency and stomatal conductance due to salt treatment irrespective of ability for salt exclusion. Lower assimilation rates were attributed to high chloride levels in the accumulator Etrog citron, whilst for Rangpur lime, a salt excluder, it was related to a loss of leaf turgor. There are however differences in the water relations of grafted plants versus rootstock seedlings under saline conditions. Although leaves of salt excluding rootstocks lose turgor at high salinity, leaves of 'Valencia' orange scion grafted to such rootstocks are able to adjust osmotically and

therefore maintain turgor. Despite turgor maintenance, salinised leaves have significantly reduced stomatal conductances (Walker *et al.* 1983). Impaired gas exchange in salt stressed 'Valencia' orange might thus be a consequence of a specific ion toxicity.

Experiments described here were intended to test this possiblity by characterising leaf gas exchange response to changes in water relations and ion content.

As determinations of osmotic potentials on expressed sap *via* psychrometry may be complicated by the presence of both water (Turner 1981) and solutes (Oertli 1968) in the apoplast, all water relations measurements were made using pressure-volume curves. Plants were grafted to Sweet orange rootstock which is a moderate salt-excluder (Grieve and Walker 1983) and an important commercial rootstock in Australia (Alexander 1983).

3.2.2 Materials and methods

3.2.2.1 Growth of plants

Two year old 'Valencia' orange trees [*Citrus sinensis*(L.) Osbeck] grafted onto Sweet orange [*Citrus sinensis*(L.) Osbeck cv Parramatta sweet orange] rootstock were purchased from a local nursery in February, 1985 and grown under glasshouse conditions in a 50:50 blend of sand and peat until May, 1985. Plants were watered every two days with tapwater, supplemented once weekly with a 5:1 mixture of commercial liquid fertilizer ("Aquasol") and iron-chelate. In May, 1985 plants were established in 4 litre pots in the growth medium of Walker *et al.* (1983) and 20 uniform plants transferred to a growth room maintained under a photoperiod of 14 h, 450 μmol m-2 s-1 (photosynthetically active radiation) provided by sodium vapor lamps and shade cloth, and a 25/20°C day/night temperature. Relative humidity was not controlled, but was generally higher than 70%. Plants were watered daily with half-strength Hoaglands solution (Hoagland and Arnon 1950). After 3 weeks day length was reduced to 9 hrs. After a further 3 weeks, salt treatment was commenced on 10 plants in steps of 5 mol m-3 NaC1 per day added to the half-strength Hoaglands solution to a final concentration of 50 mol m-3 NaC1. Control plants were watered each day to soil saturation with half strength Hoaglands solution.

3.2.2.2 General procedure

Prior to commencement of salt treatment, one representative leaf on each of 4 control and 4 salinised plants was selected for gas exchange measurements and tagged. These reference leaves were 5 to 6 months old at commencement of the experiment and fully expanded and hardened. Four leaves were also cut from additional plants and used for pressure-volume (PV) curve analysis and ion determinations. This procedure was repeated between days 12-16, 31-35, 52-56, 73-77 and 87-91. Response of CO_2 assimilation rate (A) to intercellular partial pressure of CO_2 (p_i) (A;p_i curves) at 210 mbar O_2 was measured for all tagged leaves with the exception of days 31-35 where measurements were made at standard ambient operating conditions (330 µbar CO_2) only. A;p_i curves at 21 mbar (O_2) were measured on 2 control and 2 salinised leaves immediately after measurements in 210 mbar (O_2) had been obtained. On days 73-77 leaves for PV curves and ion analysis were taken from plants having gas-exchange leaves. On days 87-91 reference leaves were harvested for PV curves and ion determinations after gas-exchange measurements had been made.

3.2.2.3 Gas exchange

Rates of CO_2 and water vapour exchange of reference leaves were determined using the gas exchange apparatus described in Section 1.2.2.2. Leaves were maintained at 26.5 ± 0.2°C with a VPD of about 1.4 kPa. PPFD at the leaf surface was 600 µmol m-2 s-1.

3.2.2.4 Leaf water potential and osmotic potential

Leaves were sampled 10 to 20 minutes after lights-on in the growth room. Freshly excised leaves were immediately placed in a plastic bag and their water potential (ψ) measured using a pressure chamber (Scholander *et al.* 1965) equipped with a binocular microscope to observe end points. Leaves were immediately weighed and were then hydrated fully in the vapour phase for 24 hours under fluorescent lights (30 µmol m-2 s-1 PAR) to prevent respiratory losses. Any condensate was gently wiped from the

leaves and their fully hydrated ψ determined. This was always less than 0.2 MPa. Leaf osmotic potential was then determined via PV curves as described in Section 2.2.3.6.

3.2.2.5 Ion analysis

Lamina tissue from oven dried leaves was ground to a fine powder using a mortar and pestle. Chloride content was determined by silver ion titration with a Buchler-Cotlove chloridometer. Sodium and potassium were determined by atomic absorption spectroscopy after extraction in nitric acid at 70°C for 20 minutes.

3.2.2.6 Chlorophyll determination

At the completion of the experiment chlorophyll content was determined using N,N-dimethylformamide (Moran and Porath 1980) on 4 control and 4 salinised leaves using the procedure of Syvertsen and Smith (1984).

3.2.3 Results

3.2.3.1 Gas exchange

Prior to salinisation, rates of CO_2 assimilation were relatively low (2.8-4.4 µmol CO_2 m-2 s-1) even at near optimum environmental conditions and at an external CO_2 partial pressure (p_a) of 330 µbar CO_2. Values of p_i however were within the range normally encountered in C_3 plants, being 251 ± 5 µbar. Even at CO_2 saturation (p_a = 800 µbar) assimilation rates as low as 4.2 µmol CO_2 m-2 s-1 were observed. These low rates were substantially enhanced (72-86%) when gas stream oxygen partial pressure was reduced from 210 mbar to 21 mbar. Large enhancements were observed at all CO_2 partial pressures examined. Extrapolation of the linear portion of A;p_i curves gave compensation points of 31 ± 2 µbar CO_2 (Fig. 3.1a).

When salt concentration in the rootzone of the plants was slowly increased, CO_2 uptake was initially enhanced whilst stomatal conductance remained unchanged (Fig. 3.1; Fig. 3.2c). This resulted in a reduction in p_i of about 30 µbar (Fig. 3.2b). During the early stages of salinisation both carboxylation efficiency and maximal rate of CO_2

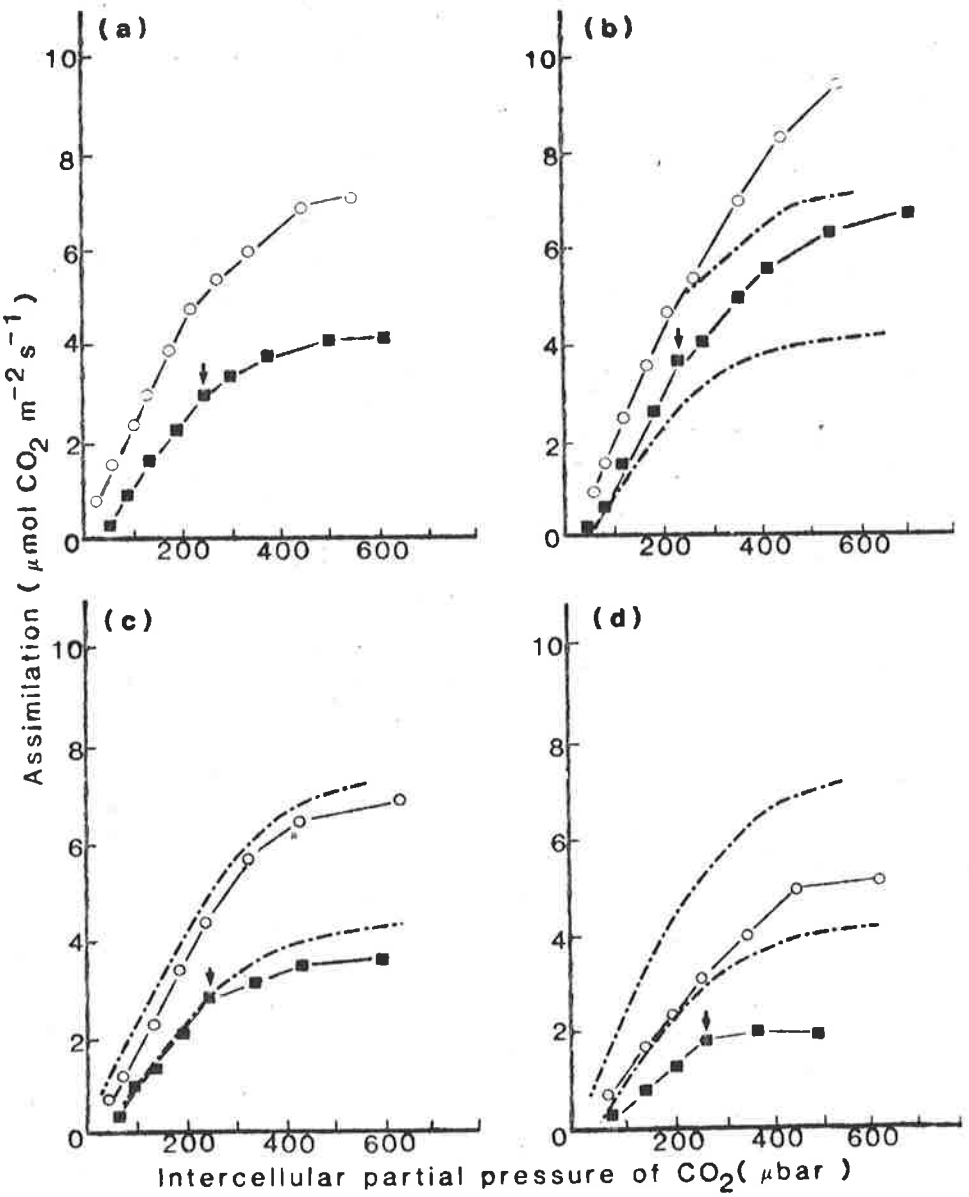


Figure 3.1 Assimilation rate *versus* intercellular CO_2 pressure of representative mature 'Valencia' orange leaves 0, 14, 54 and 75 days after commencement of 50 mol m-3 salt treatment: (○), 21 mbar (O_2); (■), 210 mbar (O_2). The arrow indicates atmospheric conditions of 330 μbar CO_2. (▬▪▬▪▬) lines from 0 days are repeated for ease of comparison.

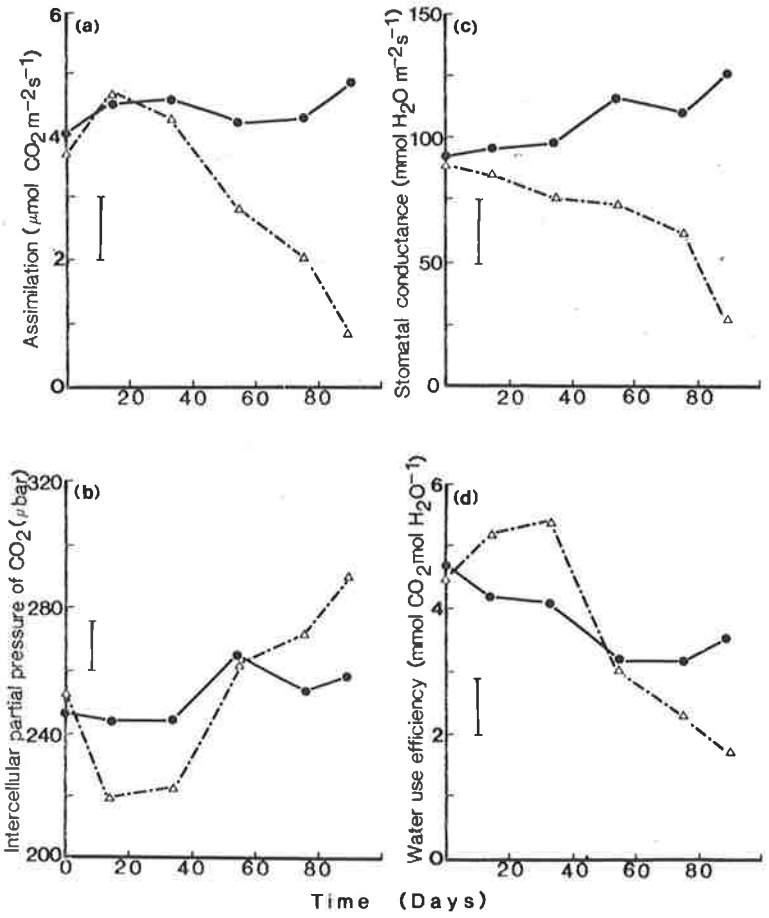


Figure 3.2 Gas exchange characteristics of mature 'Valencia' orange leaves in response to salinity: (●) leaves on plants supplied with dilute nutrient solution. (▽), leaves on plants supplied with dilute nutrient solution + 50 mol m-3 NaCl (means of four replicates). (a) CO_2 assimilation; (b) intercellular CO_2 partial pressure; (c) stomatal conductance; (d) water use efficiency. Measurements were made at atmospheric conditions of 330 μbar CO_2. Vertical bars represent the Least Significant Difference (p = 0.05).

assimilation were increased at 210 mbar (O_2) whilst at 21 mbar (O_2) only the CO_2 saturated rate was increased. Continued salinisation resulted in a progressive decrease in CO_2 assimilation capacity. After 54 days reduced capacity was evident only at high concentrations of CO_2 (Fig. 3.1c) but after 75 days initial slope of the A;p_i curve was also reduced (Fig. 3.1d). Assimilation rate declined faster than conductance which resulted in p_i at the normal operating point increasing (Fig. 3.2b). The lack of stomatal response to changes in CO_2 fixation of salinised leaves also resulted in a marked increase in water use efficiency (WUE) associated with the higher CO_2 assimilation rates (Fig. 3.2d). A rapid decline in WUE followed thereafter with increasing salinisation.

These salt-induced changes in leaf gas exchange characteristics occurred in the absence of any visible toxicity symptoms. Chlorophyll contents of salinised leaves at the completion of the experiment were 97.1 ± 16.0 μg cm -2 compared to 73.4 ± 8.8 μg cm-2 for controls. No change in the chlorophyll *a/b* ratio was observed, averaging 3.5 for both control and salinised leaves. No changes in leaf succulence were observed. High rates of abscission were however observed on salinised plants over the last 14 days of the experiment.

3.2.3.2 Foliar ion concentrations

As shown in Fig. 3.3, sodium and chloride did not accumulate in leaves for the first 33 days of salt treatment. Levels of foliar sodium subsequently rose steadily at a rate of approximately 2 mol m-3 per day reaching a concentration of 230 mol m-3 89 days after the commencement of salinisation (Fig. 3.3a). Chloride levels rose more slowly but showed a sharp increase over the last 14 days (Fig. 3.3b). Leaf potassium levels approximated 200 mol m-3 throughout for the test period control and salinised leaves (data not shown). High background levels of sodium and chloride are probably a consequence of watering plants with local tapwater (200-700 mg 1-1 dissolved salts) prior to the commencement of the experiment.

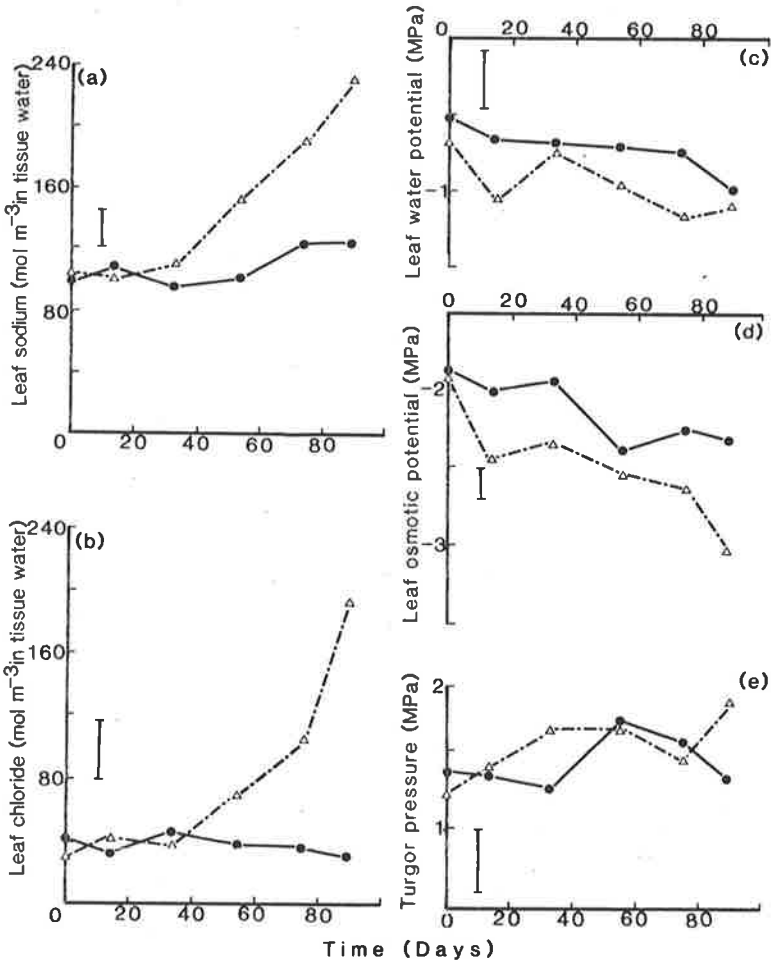


Figure 3.3 Water relations and ion concentrations of mature 'Valenica' orange leaves in response to salinity. (●), leaves on plants supplied with dilute nutrient solution; (▽), leaves on plants supplied with dilute nutrient solution + 50 mol m-3 NaCl (means of 4 replicates). (a) sodium concentration; (b) chloride concentration; (c) water potential; (d) osmotic potential; (e) turgor pressure. Vertical bars represent the Least Significant Difference (p = 0.05).

3.2.3.3 Water relations

Leaf water potentials of salinised leaves were consistently lower than controls although individual comparisons were not different statistically (Fig. 3.3c). A marked ability for osmotic adjustment was however apparent. Osmotic potential declined from -1.8 to -2.4 MPa in the first 14 days of salt treatment (Fig. 3.3d). This was not attributable to accumulation of sodium (Fig. 3.3a) or chloride (Fig. 3.3b). Consistently more negative osmotic potentials of salinised leaves resulted in turgor pressures being maintained at or above control levels (Fig. 3.3e).

3.2.3.4 Growth

No new production of leaves (which in Citrus *spp.* typically occurs in flushes interspaced by several months of apparent quiescence) was observed for control or salinised trees during the experimental period.

3.2.4 Discussion

Although CO_2 assimilation rate can be affected by several factors simultaneously, CO_2 limited and CO_2 saturated phases of A;p_i curves showed independent responses to salinity. After 14 days salinisation the CO_2 saturated portion of A;p_i curves (A_{max}) was enhanced to a greater degree than the CO_2 limited portion (initial slope), 60% cf 30% in Fig. 3.1 at 210 mbar (O_2). At 21 mbar (O_2) assimilation was increased only at high p_i, indicating that salinisation of the root medium caused a stimulation of RuP_2 regeneration under low p(O_2). Assimilation was still inhibited by O_2 at all levels of p_i but the degree of inhibition was less after salinisation.

Despite the stimulation of CO_2 assimilation, stomatal conductance remained unchanged (Fig. 3.2c) which resulted in a marked decrease in p_i (Fig. 3.2b) and an increase in WUE (Fig. 3.2d). Hence there was little "gain" in the feedback loop that has been postulated between assimilation and stomatal conductance to keep p_i constant (Farquhar *et al.* 1978).

The higher rates of CO_2 assimilation observed with mild salinisation cannot be attributed to changes in sodium (Fig. 3.3a) or chloride (Fig. 3.3b) concentrations, less negative water potentials (Fig. 3.3c) or increased turgor (Fig. 3.3e). As osmotic potentials had already declined by 0.6 MPa after 14 days of salinisation it is possible that the increased rates were a direct response of an increased allocation of photoassimilate solute for osmotic adjustment. That is to say, an increased sink for carbohydrates. Although the major solutes of citrus leaves have not been identified, soluble carbohydrates can account for up to 6% of the dry weight (Chapter 9). An increase of 225 mol m-3 sucrose would be required to account for the more negative osmotic potentials (Slavik 1974). If we assume an area leaf weight of 10 mg dw cm-2 (Syverstsen and Smith 1984) then assimilation would have had to have been increased by 1.3 μmol CO_2 m-2 s-1 over the first 14 days to produce the additional sucrose. As there is much evidence that photoassimilate levels tend to increase in salinised leaves (Munns & Termaat, 1986) this may be an overestimate of the increase in photosynthesis required. It nevertheless compares favourably with the mean increase of 0.9 μmol CO_2 m-2 s-1 observed.

The specific means by which salinisation of the rootzone resulted in osmotic adjustment in the leaves and the mechanism by which assimilation was stimulated remains to be clarified. The short term increase in CO_2 assimilation as salinity slowly increased indicates that non-salinised leaves were not expressing their full assimilatory capacity. Lenz (1978) reported that higher CO_2 assimilation rates in leaves on plants containing fruit compared to defruited plants were associated with decreased inhibition of assimilation by O_2. Thus the substantial low O_2 enhancement which accompanies low net assimilation rates in citrus (Kriedemann, 1971, Chapter 1) may be indicative of some form of feedback inhibition.

Continued salinity led to reductions in both initial slope and CO_2 saturated portions of A;p_i curves, but the latter was more responsive, especially at 210 mbar (O_2) (Fig. 3.1). Similar salinisation responses have been reported for *Spinacia oleracea* (Downton *et al.* 1985). The absence of any changes in the extent of inhibition of assimilation by oxygen

at ambient p(CO_2) or CO_2 compensation point indicates that an increase in photorespiration is not responsible for the non-stomatal inhibition of assimilation.

CO_2 assimilation rate was more sensitive to high foliar salt levels than stomatal conductance. This resulted in salinised leaves having a higher p_i and consequently lower WUE. Sodium ions are known to desensitise guard cells to various internal stimuli, such as abscisic acid and p_i (Jarvis and Mansfield 1981). These have both been suggested as mediators of parallel mesophyll/stomatal responses (Farquhar and Sharkey 1982).

Although measurement of turgor pressure is always subject to doubts, we took steps to minimise errors in this experiment. By inferring osmotic potential from PV curves rather than by psychrometry of expressed sap we have eliminated the possibility of apoplastic water (Turner 1981) or solutes (Oertli 1968) complicating measurements. Furthermore the procedure of allowing the leaf to dry on the bench between measurements eliminates artefacts present when performing a PV curve on a salinised leaf by conventional means (Kaplan and Gale 1974). Thus we can confidently say that changes in leaf turgor pressure were not responsible for the decline in assimilation. Constantly more negative osmotic pressures in the salinised leaves resulted in turgor maintenance despite more negative bulk water potentials. This indicates that neither more negative soil water potential nor extracellular salt accumulation (Oertli 1968) were involved in the inhibition of gas exchange under the mild growth conditions employed in this experiment.

On the other hand, increases in both sodium and chloride concentrations were correlated with the decreased assimilation rates. As chloride levels were generally lower than levels considered to be toxic for citrus (Walker *et al.* 1982, 1983) toxic levels of sodium may have been responsible. This suggestion is compatible with the results of Walker *et al.* (1983) who attributed the stomatal closure associated with salinity for Valencia orange in six different rootstocks, including Sweet orange, to high foliar sodium levels. Furthermore in seedlings of *Citrus macrophylla* fluorescence responses to salt stress are attributable to sodium rather than chloride toxicity (Downton and Millhouse 1985).

Impairment of leaf gas exchange due to salinisation under present conditions is thus attributable to an ion toxicity rather than disruption in leaf water relations. Accordingly, citrus rootstocks, which are known to differ markedly in their salt accumulating characteristics (Grieve and Walker 1983) may affect the sensitivity of the scion to salt by controlling the levels of toxic ion(s) in the leaves. Moreover, chloride ions may differ *vis a vis* sodium ions in the extent of their impact on the photosynthetic process. Measurement of leaf gas exchange of 'Valencia' orange scion grafted to rootstocks with these different sodium and/or chloride accumulating characteristics is investigated in the following chapter to provide a means to evaluate that hypothesis.

CHAPTER FOUR

EFFECT OF ROOTSTOCK ON RESPONSE OF 'VALENCIA' ORANGE TO ROOTZONE SALINISATION

4.1 INTRODUCTION

In the previous chapter NaCl salinisation was observed to reduce CO_2 assimilation rates of 'Valencia' orange leaves even though the leaves maintained turgor; lower assimilation rates were therefore attributed to ion toxicity. The question then arose as to which ion was responsible for reducing assimilation rates in salinised leaves. Although attempts to differentiate the relative impact of sodium *versus* chloride toxicity in such situations are often confounded by the simultaneous rise of both these ions in salt affected tissue (Greenway and Munns 1980), citrus varieties are usually budded to rootstocks (Wutcher 1979) which are known to markedly influence chloride (Cooper and Gorton 1952; Grieve and Walker 1983) and to a lesser extent, sodium (Jones *et al.* 1957; Grieve and Walker 1983) concentrations in leaves. By budding a given scion variety to rootstocks differing in sodium and/or chloride uptake characteristics, it is therefore possible to obtain different tissue concencentrations of sodium relative to chloride in the same scion variety. This has been shown for orange (Walker *et al.* 1983) and grapefruit (Cooper and Gorton 1952).

Experiments described in this chapter were designed to create different sodium/chloride ratios in leaves of 'Valencia' orange. Two rootstocks were used: Cleopatra mandarin, a chloride excluder (Cooper and Gorton 1952; Grieve and Walker 1983) and Trifoliata, a chloride accumulator (Cooper 1961). Significantly, Trifoliata also exhibits a sodium exclusion capability (Elgazzar *et al.* 1965; Grieve and Walker 1983).

Environmental stresses can pre-dispose foliage to photoinhibitory damage (Powles 1984). In order to determine if there was an interaction between light level and salinity, as has been demonstrated for plants exposed to water deficit (Björkman and Powles 1984: Chapter 2), two growth light levels were employed. Gas exchange characteristics were evaluated on single leaves along with water relations and foliar ion concentrations. Room temperature chlorophyll *a* fluorescence could have offered an early indication of photosynthetic dysfunction (Smillie and Nott 1982: Chapters 1 and 2) and was therefore employed as a probe to localise possible sites of salt induced lesions within the chloroplast.

4.2 MATERIALS AND METHODS

4.2.1 Growth of plants

Two year old 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) trees grafted to Trifoliata (*Poncirus trifoliata* [L.] Raf.) or Cleopatra mandarin (*Citrus reticulata* Blanco) were potted in 3 litre containers filled with washed river sand. After 7 days, watered daily with half-strength Hoaglands solution (Hoagland and Arnon 1950) in a glasshouse, 18 uniform plants on each rootstock were transferred to a growth room with a photoperiod of 9 h and a 25/22° day/night temperature. Relative humidity was not controlled but was generally higher than 70%. Nine plants on each rootstock were grown under 650 μmol m^{-2} s^{-1} photosynthetically active radiation provided by sodium vapour lamps, whilst nine were grown at 175 μmol m^{-2} s^{-1} under the same lamps and shade cloth. For each light level, three plants on each rootstock were given half-strength Hoaglands solution to which either 0, 50, or 100 mol m^{-3} NaCl was added. Salt was added to plants in stepwise increments over 4 days up to 50 mol m^{-3} or over 6 days up to 100 mol m^{-3} in order to prevent osmotic shock. All plants were watered daily with appropriate solutions and allowed to drain.

4.2.2 General procedure

Measurements began 37 days after commencement of salinisation. All leaves examined had developed under growth room conditions and were approximately 30 days old at that time. Over the following 44 days, one leaf from each of the 12 rootstock/light/salt treatments was sampled every 5-12 days. Net gas exchange, room temperature chlorophyll fluorescence, leaf osmotic potential, chlorophyll, sodium, chloride and potassium content were measured for each leaf. This procedure was continued until 6 leaves from each treatment (two from each tree) had been sampled.

4.2.3 Gas exchange

Rates of CO_2 and water vapour exchange of attached leaves were determined using the gas exchange system described in Section 1.2.2.2. Leaves were maintained at

25.0 ± 0.5°C with a leaf to air vapour deficit of about 1.3 kPa. Photon irradiance at the leaf surface was 1000 $\mu mol.m^{-2}\ s^{-1}$. Each gas exchange leaf was tagged upon completion of measurements and the plant returned to the growth room.

4.2.4 Chlorophyll fluorescence

Just prior to commencement of scheduled darkness, plants with tagged leaves were removed from the growth room and placed in a completely darkened room (ambient temperature approximately 20°C) and allowed to adapt overnight (>12 hours). Chlorophyll *a* fluorescence induction kinetics were measured for the upper and lower surfaces of each tagged leaf as described in Section 1.4.2.4.

4.2.5 Leaf water potential and osmotic potential

Immediately after fluorescence measurements, leaves were excised and placed in a plastic bag. Leaf water potential (ψ) was measured using a pressure chamber (Scholander *et al.* 1965) equipped with a binocular microscope to observe end points. Leaves were immediately weighed and were then hydrated fully in the vapour phase for 24 hours under fluorescent light to offset respiratory losses. Any condensate was then gently wiped from the leaves and ψ at full hydration determined. Leaf osmotic potential was then determined via PV curves as described in Section 2.2.3.6.

4.2.6 Chlorophyll content

Immediately upon completion of the PV curve, a single 1 cm diameter disc was taken from each leaf and used for chlorophyll determination using N,N-dimethylformamide as extraction solvent (Moran and Porath 1980) as modified for citrus by Syvertsen and Smith (1984). All leaves were subsquently oven dried for 24 h at 70°C and retained for ion analysis.

4.2.7 Ion analysis

Lamina tissue from oven dried leaves was ground to a fine powder using a mortar and pestle. Chloride was determined by silver ion titration with a Buchler-Cotlove chloridometer. Sodium and potassium were determined by atomic absorption spectroscopy after extraction in hot nitric acid.

4.2.8 Statistical analysis

All regression functions were fitted by a stepwise procedure using the computer program 'GENSTAT'. Linear, second and third order polynomials and logarithmic fits of data were attempted. Equality of best fit regression functions was tested using the F statistic on the error sum of squares of combined (both rootstocks) and individual rootstock models. Equality of variance for rootstock response functions was tested and confirmed (Neter and Wasserman 1974).

4.3 RESULTS

4.3.1 Foliar ion content

Leaves of 'Valenica' scion grafted to Cleopatra mandarin generally accumulated higher concentrations of sodium than those on Trifoliata (Figure 4.1). Sodium import was faster under high light for leaves on Trifoliata, whilst no systematic effect of light level was evident in leaves on Cleopatra mandarin. Salinisation with 50 mol m^{-3} or 100 mol m^{-3} NaCl resulted in similar uptake patterns. In contrast to sodium, leaves on Trifoliata accumulated chloride faster than those on Cleopatra mandarin (Figure 4.2). Although light level during growth had no effect on foliar chloride accumulation for plants treated with 100 mol m^{-3} NaCl, faster rates of chloride accumulation were observed under high light for plants treated with 50 mol m^{-3} NaCl. Leaf potassium concentrations decreased with salinity (Table 4.1).

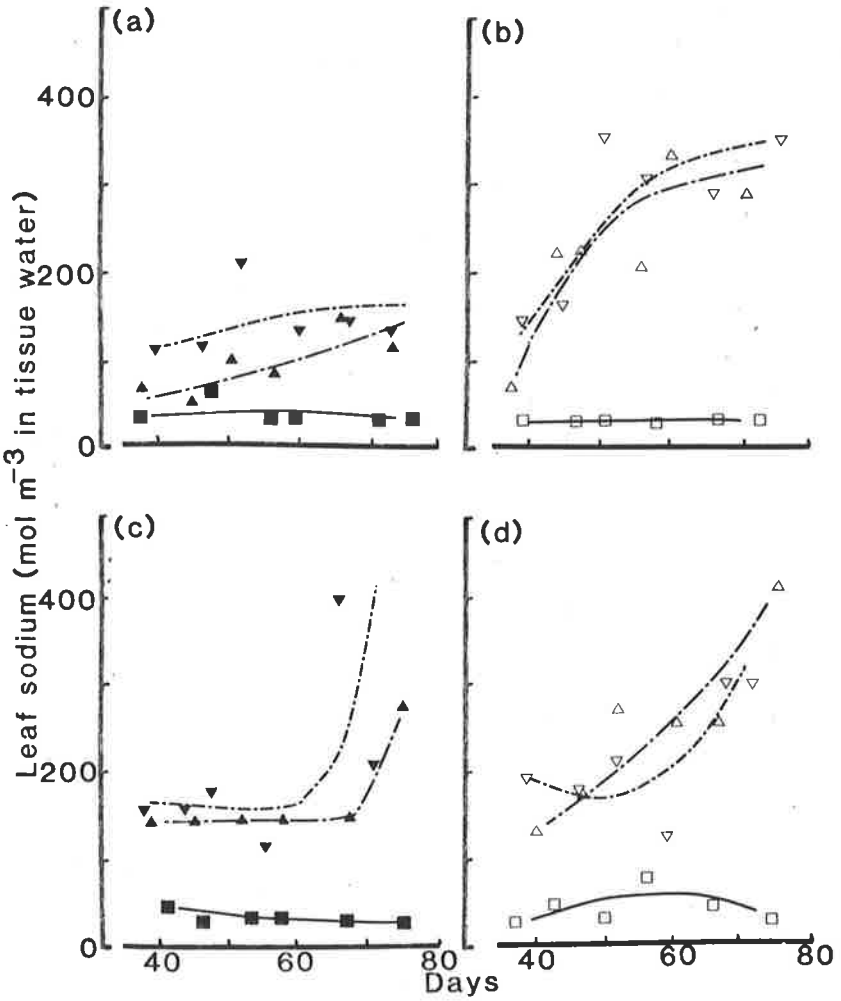


Figure 4.1 Sodium concentration in 'Valencia' orange leaves in response to salinity. (■ □), leaves on plants supplied with dilute nutrient solution; (▲ △) leaves on plants supplied with dilute nutrient + 50 mol m^{-3} NaCl; (▼▽) leaves on plants supplied with dilute nutrient solution + 100 mol m^{-3} NaCl. Curves were fitted by eye. (a) and (b); plants grown under low light. (c) and (d); plants grown under high light. (a) and (c); leaves on Trifoliata; (b) and (d) leaves on Cleopatra Mandarin.

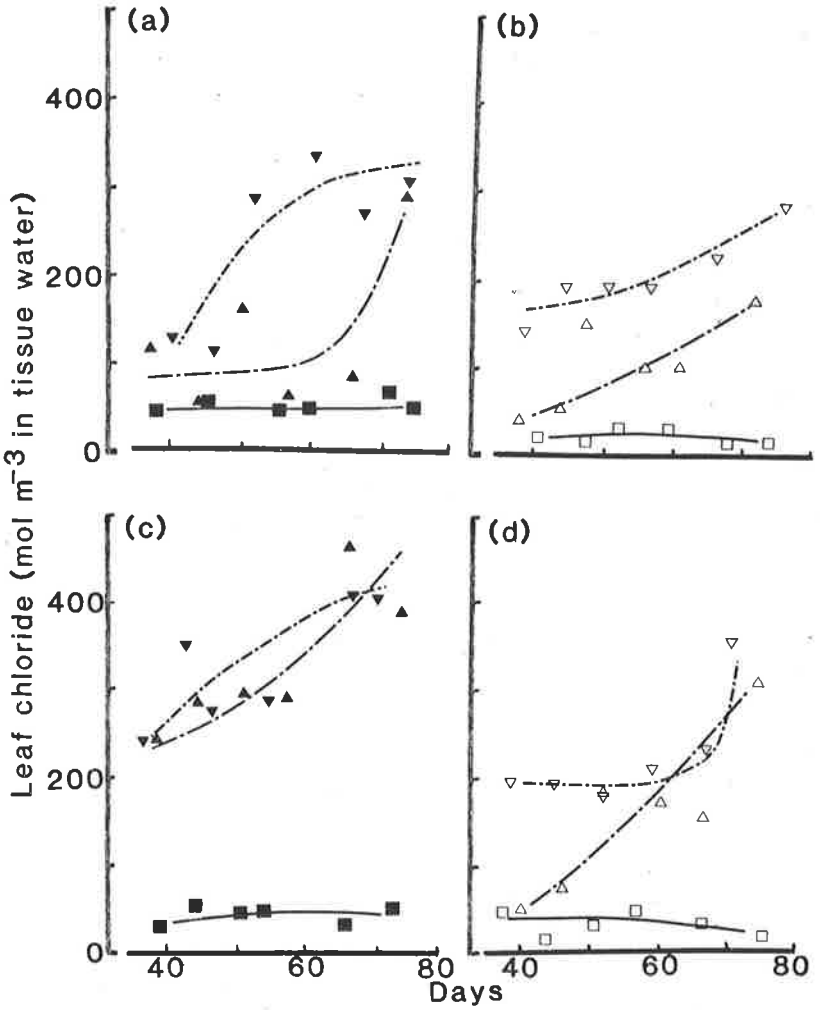


Figure 4.2 Chloride concentration in 'Valenica' orange leaves in response to salinity. (■□), leaves on plants supplied with dilute nutrient solution; (▲△) leaves on plants supplied with dilute nutrient + 50 mol m^{-3} NaCl; (▼▽) leaves on plants supplied with dilute nutrient solution + 100 mol m^{-3} NaCl. Curves were fitted by eye. (a) and (b); plants grown under low light. (c) and (d); plants grown under high light. (a) and (c); leaves on Trifoliata; (b) and (d) leaves on Cleopatra Mandarin.

Rootstock	Trifoliata		Cleopatra Mandarin		
Treatment	Control	Salinised	Control	Salinised	LSD[c] (p = 0.05)
Chlorophyll content ($\mu g\ cm^{-2}$)	70.4	53.3	86.7	53.9	14.4
Assimilation ($\mu mole\ CO_2\ mg\ Chl^{-1}\ h^{-1}$)	33.6	25.9	38.8	17.1	12.8
Variable fluorescence ((P-O)/O) ratio	2.5	2.8	2.0	1.8	0.7
Fluorescence quenching index[b] (relative units)	1.3	1.3	1.4	1.0	0.4
Leaf potassium ($mol\ m^{-3}$ in tissue water)	241	184	334	140	53
Pre-dawn water potential (MPa)	-0.64	-1.11	-0.60	-0.98	0.39
Osmotic potential at full turgor (MPa)	-1.94	-2.61	-1.93	-2.92	0.53
Pre-dawn turgor pressure (MPa)	1.31	1.55	1.34	2.00	0.52

Table 4.1: Leaf chlorophyll content, assimilation (chlorophyll basis), fluorescence parameters, potassium content and water relations of control and salinised 'Valencia' orange leaves 72-77 days after commencement of salinisation[a].

[a] Measurements at both light levels and the two higher salinity levels (50 and 100 mol m^{-3} NaCl) have been pooled. Control; n = 2: Salinised n = 4.

[b] Slope from P to S.

[c] Least Significant Difference

4.3.2 Water relations

Import of sodium and chloride into leaves of salinised plants contributed towards more negative osmotic potentials. This resulted in pre-dawn turgor being maintained at or above control levels despite salinised leaves having more negative water potentials (Table 4.1). Turgor in salinised leaves was maintained during the subsequent light period at or

above levels in unsalinised leaves (data not shown).

4.3.3 Gas exchange

Salinity reduced rates of net CO_2 assimilation for leaves on both rootstocks. Though some reduction in leaf chlorophyll content was associated with high foliar salt levels this was not sufficient to wholly account for the lower CO_2 assimilation rates (Table 4.1).

Inhibition of assimilation occurred to a greater extent for leaves on Cleopatra mandarin than for those on Trifoliata. No large differences occurred in the time course of inhibition of assimilation between plants treated with 50 or 100 mol m^{-3} NaCl. Similar reductions occurred for leaves grown at both light levels (Figure 4.3). More detailed analysis of leaf gas exchange is provided in Figure 4.4. CO_2 saturation of assimilation at high p_i was not observed in control leaves, whereas the assimilation rate observed at p_a = 330 µbar CO_2 was close to the maximum rate attainable at higher p_i for salinised leaves. At any given stage of salinisation, 'Valencia' orange leaves on Trifoliata had smaller reductions in carboxylation efficiency, as determined from the linear portion of the A/p_i curve, than those on Cleopatra mandarin. Stomatal conductances were generally reduced to the same extent as assimilation rates for plants grown under both light levels (data not shown). As a result p_i showed little change and was typically 200-220 µbar CO_2 at a p_a of 330 µbar, despite large reductions in assimilation rate.

The comparative impact of sodium *versus* chloride on CO_2 assimilation under saline conditions can be inferred from plots of CO_2 assimilation in relation to foliar concentration for each ion. When CO_2 assimilation is expressed as a function of leaf chloride (Figure 4.5a), data segregate in two distinct populations according to rootstock. Response slope of a plot showing CO_2 assimilation in terms of leaf chloride is steeper for scions on Cleopatra Mandarin than for scions on Trifoliata. In effect, Trifoliata stock appears to have lowered leaf sensitivity to chloride in 'Valencia' orange scions. However, this rootstock effect disappears when assimilation is plotted as a function of leaf sodium concentration (Figure 4.5b). When assimilation is plotted against the logarithm of the leaf sodium/potassium

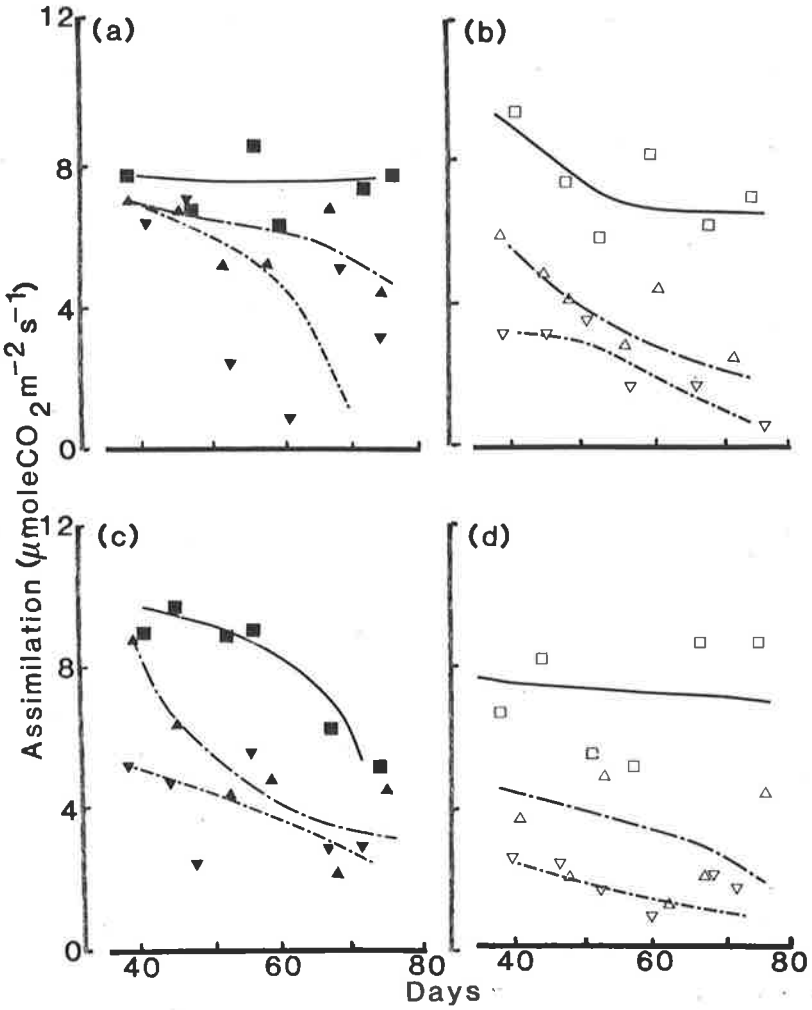


Figure 4.3 CO_2 assimilation rate of 'Valencia' orange leaves at ambient CO_2 partial pressure in response to salinity. (■ □) leaves on plants supplied with dilute nutrient solution; (▲ △) leaves on plants supplied with dilute nutrient + 50 mol m^{-3} NaCl; (▼ ▽) leaves on plants supplied with dilute nutrient solution + 100 mol m^{-3} NaCl. Curves were fitted by eye. (a) and (b); plants grown under low light. (c) and (d); plants grown under high light. (a) and (c); leaves on Trifoliata; (b) and (d) leaves on Cleopatra Mandarin.

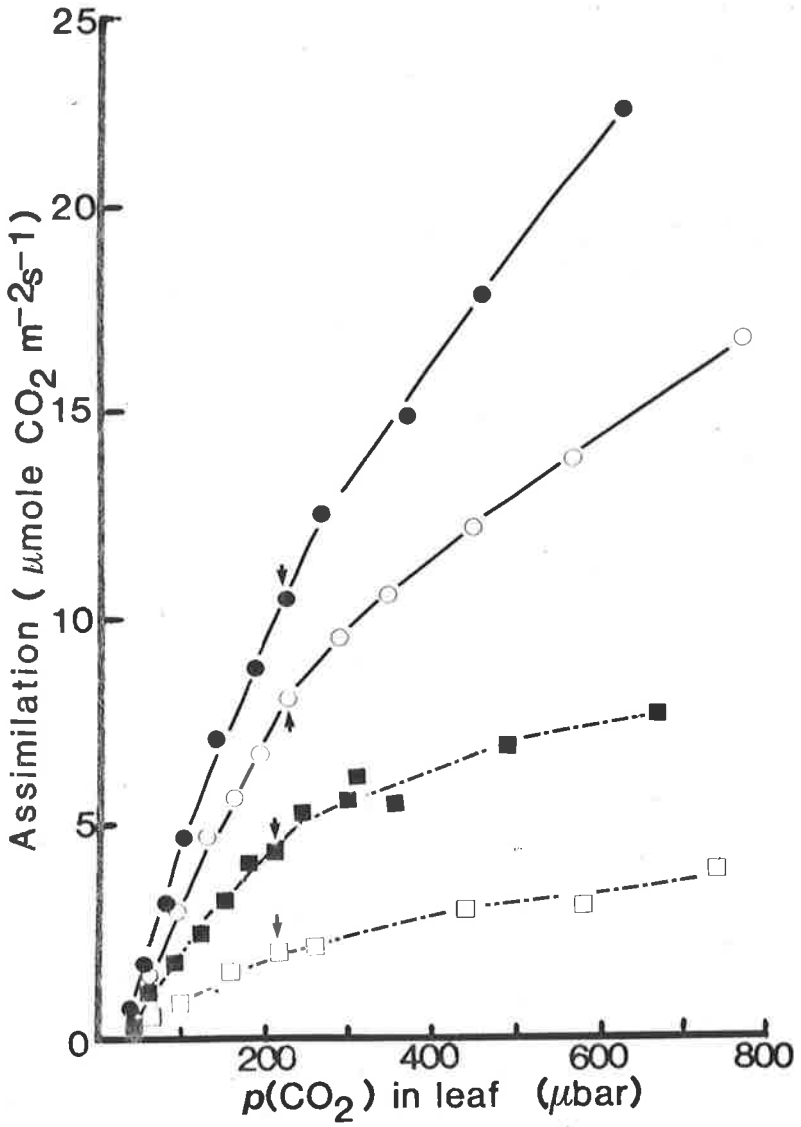


Figure 4.4 Gas exchange characteristics of 'Valencia' orange leaves in response to salinity. (■□) leaves on plants supplied with dilute nutrient solution; (●○) leaves on plants supplied with dilute nutrient solution + 100 mol m^{-3} NaCl. (●■) leaves on Trifoliata; (○□) leaves on Cleopatra mandarin. Leaves were from high light plants and sampled 40-50 days after commencement of salt treatment. For salinised leaves on Trifoliata leaf sodium was 148 mol m^{-3} whilst on Cleopatra mandarin it was 186 mol m^{-3}. Leaf chloride was 367 mol m^{-3} and 174 mol m^{-3} for leaves on Trifoliata and Cleopatra mandarin respectively.

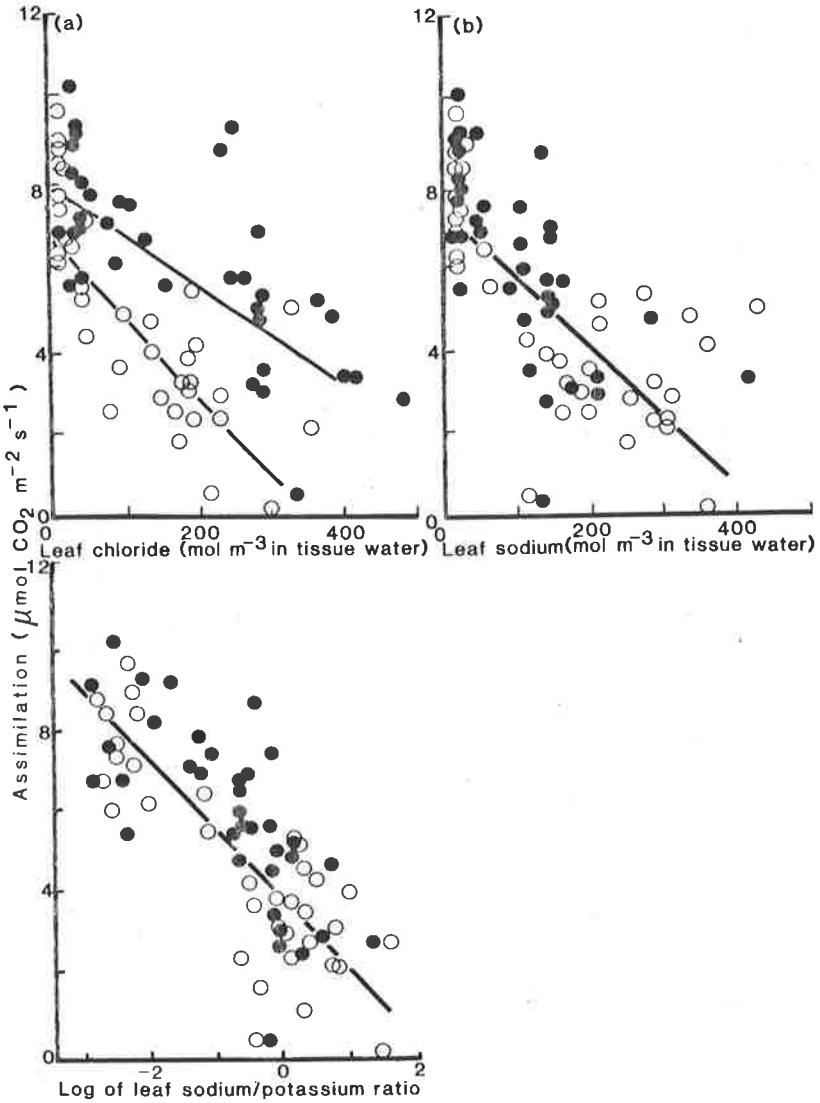


Figure 4.5 The relationship between (a) leaf chloride concentration; (b) leaf sodium concentration; and (c) leaf sodium/potassium ratio and CO_2 assimilation rate. (●) leaves on Trifoliata; (○) leaves on Cleopatra mandarin. For each rootstock measurements for all salinities and light levels have been pooled. There was no effect of rootstock on the relationship between 'Valencia' orange CO_2 assimilation rate and leaf sodium concentration or the sodium/potassium ratio ($P > 0.5$) whilst the relationship between 'Valencia' orange assimilation rate and leaf chloride concentration was dependent upon the rootstock ($P < 0.01$).

ratio an even better fit is obtained for the combined data (Figure 4.5c). Regression equations and coefficients are listed in Table 4.2.

x Variable	Best fit	Rootstock[1]	n[2]	a	b	r[3]
Chloride	Linear	T	36	7.89	1.29×10^{-2}	0.77
		C	36	6.42	2.01×10^{-2}	0.77
		T and C	72	6.77	1.32×10^{-2}	0.63
Sodium	Linear	T	36	7.56	1.83×10^{-2}	0.64
		C	36	6.61	1.50×10^{-2}	0.71
		T and C	72	7.15	1.67×10^{-2}	0.71
Sodium/potassium	Logarithmic	T	36	4.06	1.57	0.69
		C	36	3.04	1.56	0.69
		T and C	72	3.56	1.62	0.77

Table 4.2: Regression equations of CO_2 assimilation rate (μmol m^{-2} s^{-1}) on leaf ionic concentration (mol m^{-3} in tissue water). The best fitted equations were either linear ($y = a - bx$) or logarithmic ($y = a - b \ln x$).
[1] T = Trifoliata, C = Cleopatra mandarin; [2] number of observations; [3] correlation coefficient

4.3.4 Room temperature chlorophyll a fluorescence

Salinisation had little effect on early phases of chlorophyll *a* fluorescence induction kinetics for plants grown at either light intensity (Figure 4.6). Neither variation in values of O or P, the ratio of variable to constant fluorescence ((P-O)/O) nor the degree of quenching (slope from P to S) were correlated with leaf assimilation rate, sodium, potassium or chloride content, total leaf water potential or turgor pressure (Table 4.1).

4.3.5 Growth

No new production of leaves was observed for control or salinised trees during the experimental period.

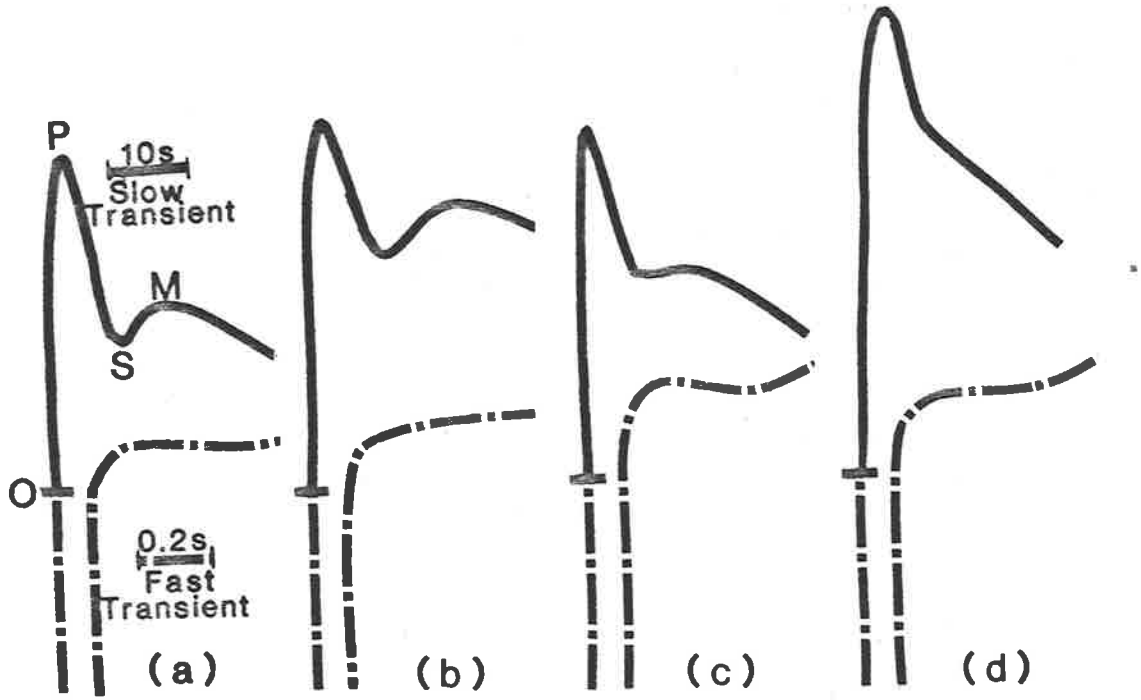


Figure 4.6 Chlorophyll induction kinetics of 'Valencia' orange leaves in response to salinity; (a + c) leaves supplied with dilute nutrient solution; (b + d) leaves supplied with dilute nutrient solution + 100 mol m^{-3} NaCl. (a) and (b) leaves on Trifoliata; (c) and (d) leaves on Cleopatra mandarin. These data are from the same leaves as in Figure 4.4 and were from high light plants and sampled 40-50 days after commencement of salt treatment.

4.4 DISCUSSION

Transport of both Na^+ and Cl^- into leaves was dependent upon rootstock. Low levels of Na^+ in leaves on Trifoliata may be a consequence of this rootstock having the ability to sequester Na^+ in the basal stem - proximal root region (Walker 1986). As discussed by Lüttge (1983) the practical importance of such mechanisms for conferring salt-tolerance is limited to low degrees or short durations of salinity stress. Ability of xylem parenchyma cells to absorb Na^+ is finite and must become rapidly exhausted under saline conditions. Although some lateral redistribution of sodium may also occur in root and stem tissues of Trifoliata, even this capacity for storage of sodium may be insufficient to prevent sodium accumulation in the upper stem at high salinities over extended periods of time (Walker 1986). This limited ability for exclusion of Na^+ from the upper stem may be the reason that Na^+ rose dramatically towards the end of the experiment in leaves on Trifoliata exposed to high light. Reduced stomatal conductances as reported previously for citrus at low light levels (Kriedemann 1971) may have been responsible for this phenomenon not occurring at the lower light level. Reducing transpiration rate can markedly influence the rate of ion transport into leaves (Jeschke 1984; Syvertsen and Graham 1985; Walker 1986).

More negative osmotic potentials in salt stressed leaves (Table 4.1) resulted in turgor being maintained as has previously been demonstrated on similarly managed material (Walker *et al.* 1983: Chapter 3). Despite turgor maintenance, salinised leaves had markedly lower CO_2 assimilation rates. Since this was not solely a consequence of lower chlorophyll levels (Table 4.1) some photosynthetic dysfunction must also have occurred.

Assimilation at high p_i was more sensitive to high salt levels than at low p_i. Similar responses to salinity have been reported for *Spinacea oleracea* (Downtown *et al.* 1985) and *Avicennia marina* (Ball and Farquhar 1984). In current models of photosynthesis the maximal rate of CO_2 assimilation at high p_i (A_{max}) reflects capacity for regeneration of the CO_2 acceptor ribulose-1,5 bisphosphate (RuP_2) (Section 1.1.5). This is dependent upon electron transport and the associated phosphorylation process plus activity of enzymes of the photosynthetic carbon reduction cycle (von Caemmerer and

Farquhar 1981). It is unlikely that impairment of electron transport was solely responsible for the dramatic lowering of A_{max} as there was little effect of salinity on room temperature chlorophyll *a* fluorescence (Figure 4.6). Neither the (P-O)/O ratio or the initial rate of the P to S transient were affected by high foliar salt levels at either light intensity. The (P-O)/O ratio is an indicator of Photosystem II (PSII) activity (Bradbury and Baker 1983) whilst the initial rate of the P to S transient reflects photochemical reoxidation of the primary PSII electron acceptor Q and buildup of transthylakoid ΔpH (Krause and Weis 1984). These observations and others using a range of species (Downton and Millhouse 1983, 1985) illustrate an insensitivity of fluorescence to high foliar salt levels when turgor is maintained. This is in contrast to results of Smillie and Nott (1982) who demonstrated a diminution of variable fluorescence with salinisation. They did not however monitor plant water relations, and thus a loss of turgor may have been responsible for their observations. Given the present constancy of fluorescence characteristics despite large changes in gas exchange plus an ability of the electron transport chain to function in the presence of high salt levels *in vitro* (Baker 1978; Wignarajah and Baker 1981) high salt levels also appear to have had little effect on photochemical reactions of chloroplasts in citrus. In such situations, stress-induced subsceptibility to photoinhibition would not be expected (Powles 1984) and this is consistent with the similar reductions in CO_2 assimilation occurring at both high and low light (Figure 4.3). Imposition of photoinhibitory treatments results in large reductions in citrus leaf variable fluorescence (Chapters 1 and 2).

In the previous chapter using 'Valencia' orange on Sweet orange rootstock, carboxylation efficiency was also reduced by salinity but to a lesser extent than A_{max}. There are however some differences between present results and those reported previously. In this present study, CO_2 assimilation rates prior to salinisation were two fold higher and did not saturate at high p_i; moreover, stomatal response was similar to that of CO_2 assimilation. In Chapter 3 stomata were shown to be less sensitive than mesophyll photosynthesis. These differences may be a consequence of growth conditions of leaves as, in Chapter 3, leaves used had developed under glasshouse

conditions. Developmental conditions are known to strongly influence stomatal behavour of citrus leaves (Kriedemann and Barrs 1981).

CO_2 assimilation rates of scion leaves grafted to Trifoliata were relatively less affected by salinity than leaves on Cleopatra Mandarin despite high chloride levels. By contrast, lower assimilation rates on Cleopatra mandarin were associated with higher leaf sodium levels (Figure 4.5). Inhibition of CO_2 by salinity may therefore be more readily attributable to a sodium ion rather than a chloride ion effect, even though the relationship between assimilation and leaf sodium content was not strictly quantitative. The tendency for salinised leaves to have lower potassium concentrations, may also have contributed to impaired gas exchange of salinised leaves as a logarithmic plot of assimilation *versus* Na/K ratio gave a significantly better fit than sodium alone (Table 4.2; Figure 4.5). It is however unlikely that low potassium concentrations were solely responsible for lower CO_2 assimilation rates of salinised leaves as salinity can inhibit citrus leaf gas exchange even when potassium concentrations are maintained at control levels (Chapter 3). Furthermore, leaf potassium concentrations similar to those observed for salinised leaves in this experiment do not affect gas exchange of 'Valencia' orange when leaf sodium levels are also low (Chapter 8).

It is possible that rootstocks affect responses of scions to salinity in ways other than by influencing foliar ion levels. Numerous effects of rootstock on scion physiology are known. These include nutrient relations (Ahmad and Al-Shurafa 1984; Syvertsen and Graham 1985), cold susceptibility (Wutscher 1979) and drought tolerance (Syvertsen *et al.* 1988) as well as tree size and fruit yield (Wutscher 1979). At present we have little knowledge of mechanisms by which these effects occur. Possibilities include differences in the mass flow of water and minerals from the roots (Syvertsen and Graham 1985) variations in the composition and concentration of growth regulators (Saidha *et al.* 1983), or nitrogenous compounds such as proline (Kato 1981) transported from rootstock to scion via the xylem sap. Salinity could affect some or all of these processes. Such effects would be quite distinct from the rootstock influence on foliar ion levels demonstrated in this chapter.

4.5 Conclusions

'Valencia' orange leaves subjected to salinity stress under well watered conditions have more negative osmotic potentials than control leaves. They are thus able to maintain turgor. Comparative rates of sodium and chloride accumulation as well as the sensitivity of CO_2 assimilation to high concentrations of these ions is dependent on the type of rootstock. Foliar sodium content may be more important than chloride content in decreasing net CO_2 assimilation and carboxylation efficiency. Since stomatal conductance and CO_2 assimilation were reduced to a similar extent by salinity stress, there was little change in p_i at ambient levels of CO_2.

CHAPTER FIVE

CHLORIDE TOXICITY IN 'PRIOR LISBON' LEMON

5.1 INTRODUCTION

Leaf gas exchange of 'Valencia' orange was observed to be one physiological process strongly impaired under conditions of NaCl salinisation (Chapter 3). By using rootstocks with different sodium and chloride exclusion characteristics it was further demonstrated that sodium, rather than chloride may be responsible for reductions in CO_2 assimilation rates in 'Valencia orange' under saline conditions (Chapter 4). These observations of insensitivity to chloride using 'Valencia' orange are in marked contrast to studies using some other *Citrus* spp. Working with 'Shary Red' grapefruit on a variety of rootstocks, Cooper and Gorton (1952) and Cooper (1961) showed that a wide range of chloride accumulation in leaves was well correlated with severity of visual salt excess symptoms. More recently, Walker *et al.* (1982) showed that decreased gas exchange of salt stressed seedlings of the rootstock Etrog citron was best associated with high foliar chloride levels as leaf sodium concentrations were not markedly increased by salt treatment. The differences between these studies and those in Chapters 3 and 4 led to the supposition that citrus species may differ markedly in their sensitivity to the chloride ion.

Experiments described here were designed to test this possibility by characterising leaf gas exchange response of 'Valencia' orange and 'Prior Lisbon' lemon to salinisation. 'Prior Lisbon' lemon was chosen as a chloride sensitive scion as Loughridge (1901) and Kelley and Thomas (1920) both observed lemons to be more sensitive to salinity than oranges. These observations were reinforced by experiments of Cooper *et al.* (1952a) which showed that, despite equivalent chloride levels, 'Eureka' lemon leaves were defoliated under saline conditions whilst only slight leaf burn was observed for 'Valencia' orange. This was in face of lower foliar sodium levels in 'Eureka' lemon.

For both scions two rootstocks were employed; Trifoliata and Troyer citrange. Both rootstocks allow relatively large amounts of chloride to enter the scion (Grieve and Walker, 1983) but, significantly, Trifoliata also exhibits a sodium exclusion ability (Elgazaar *et al.*, 1965; Grieve and Walker, 1983). Leaf gas exchange response of all scion/rootstock combinations under saline conditions was related to individual leaf ion levels and water relations.

Salinity stress can also result in accumulation of proline (Paleg & Aspinall, 1981) and betaines (Wyn-Jones and Storey, 1981) in many plants. It has been proposed that these 'compatible' solutes accumulate in cytoplasm (Wyn-Jones *et al.*, 1979) and chloroplast (Robinson and Jones, 1986) during osmotic adjustment whilst potentially toxic ions such as sodium and chloride are restricted to the vacuolar compartments. As differences in salt sensitivity between scions may have then been accountable in terms of differences in foliar levels of these compounds, proline and betaine levels in control and salt-stressed leaves of both scions were determined.

5.2 MATERIALS AND METHODS

5.2.1 Growth of plants

One year old 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) or 'Prior Lisbon' lemon (*Citrus* limon [L.] Burm.f.) trees budded on one and a half year old Trifoliata (*Poncirus trifoliata*[L.] Raf.) or Troyer citrange (*C. sinensis* x *P. trifoliata*) rootstock were potted in free draining 3 litre containers filled with washed river sand. Buds were from single trees and rootstocks were grown from open-pollinated seed. After 42 days in a glasshouse, watered daily with half strength Hoaglands solution (Hoagland & Arnon, 1950), eight uniform plants from each scion/rootstock combination (32 plants in all) were transferred to a growth room with a photoperiod of 9 h, 450 μmol $m^{-2}s^{-1}$ provided by sodium vapour lamps and shade cloth, and a 25°/20°C day/night temperature. Relative humidity was not controlled but was generally higher than 70%. Daily watering with half strength Hoaglands solutions continued for a further 60 days. During the first 30 days in the growth room new vegetative growth occurred on all trees. As new growth flushes emerged they were tagged to facilitate future determinations of leaf age. After 30 days all plants were reduced to two growth flushes per tree. After a further 30 days, salt treatment commenced on four plants from each scion/rootstock combination in steps of 5 mol m^{-3} NaCl per day added to the half strength Hoagland's solution to a final concentration of 50 mol m^{-3} NaCl which was applied daily thereafter. Control plants were watered daily with half strength Hoaglands solution.

5.2.2 General method

Measurements began upon commencement of salt treatment. All leaves examined had developed under growth room conditions, and were 30 to 45 days old at that time. Over the following 58 days, one leaf from each of the eight scion/rootstock/salt combinations was sampled every 3-9 days excluding the period from 23 to 31 days after commencement of salinisation when no measurements were possible due to equipment failure. Net gas exchange, leaf osmotic potential, sodium, chloride and potassium concentrations were measured for each leaf. This procedure was repeated until eight leaves from each treatment (two from each tree) had been sampled. Four leaves from each treatment were sampled at the completion of the experiment for proline and quarternary ammonium compound analysis. Fresh weight was determined and the leaf immediately frozen in liquid nitrogen. After being freeze-dried under vacuum for 48 hours, dry weight was determined for each leaf. Samples were subsequently stored in a desiccator at -20°C until required.

5.2.3 Gas exchange

Rates of CO_2 and water vapour exchange of attached leaves were determined using the gas exchange system described in Section 1.2.2.2. Leaves were maintained at 25.0 ± 0.5°C with a leaf to air vapour deficit of about 1.3 kPa.

5.2.4 Leaf water potential and osmotic potential

Leaves were sampled just prior to lights-on in the growth room. Freshly excised leaves were immediately placed in a plastic bag and leaf water potential (ψ) measured using a pressure chamber (Scholander *et al.* 1965) equipped with a binocular microscope to observe end points. Leaf osmotic potential was determined via PV curves as described in Section 2.2.3.6.

5.2.5 Ion analysis

Lamina tissue from oven dried leaves was ground to a fine powder using a mortar and pestle. Chloride content was determined by silver ion titration with a Buchler-

Cotlove chloridometer. Sodium and potassium were determined by atomic absorption spectroscopy after extraction in hot nitric acid.

5.2.6 Statistical analysis

All regression functions were fitted by a stepwise procedure using the computer program GENSTAT. Linear, second and third order polynomials and logarithmic fits of data were attempted. Equality of best fit regression functions was tested using the F statistic on the error sum of squares of combined (both rootstocks) and individual rootstock models. Equality of variance for rootstock response functions was tested and confirmed (Neter & Wasserman 1974).

5.2.7 Quarternary ammonium compounds and proline analysis

Betaines were determined by nuclear magnetic resonance (N.M.R.) spectroscopy (Jones *et al.*, 1986). Leaf samples (approx. 250 mg) were homogenised in 10 ml methanol-chloroform-water (M.C.W.) (12:5:3) on ice at 0°C in a 50 ml glass centrifuge tube. The grinding head was washed with approx. 5 ml of water and the resulting homogenate was centrifuged at 3,500 r.p.m. for 10 minutes. The upper (methanol-water) phase was removed and stored and the lower phase re-extracted in a further 10 ml M.C.W. and 5 ml water. The methanol-water phases were pooled and their combined volume determined to the nearest 0.05 ml. A 1.0 ml aliquot was then taken for proline analysis using the method of Singh *et al.*, (1973) whilst the remainder of the methanol-water extract was used for betaine purification and determination using the method of Jones *et al.*, (1986). ^{1}H N.M.R. spectra were measured on a Jeol FX90Q multinuclear transform N.M.R. operating at a frequency of 90 MHz using a spectral width of 1000 Hz (Jones *et al.*, 1986). Routinely, 32 transients were accumulated into 8 K memory locations with a 15 μs (45°) pulse width and a recycle time of approx. 4.2 s. Quantification was obtained by comparing integrated peak intensities against 2.0 μmol t-butanol as an internal standard.

5.3 RESULTS

5.3.1 Growth of plants

Although all growth flushes were initiated at the same time, new growth of 'Prior Lisbon' lemon consisted of a greater number of larger leaves than 'Valencia' orange. There was no difference in water content between scions (Table 5.1). No further new growth flushes occurred during the experimental period.

Scion	'Valencia' orange		'Prior Lisbon' lemon	
Rootstock	Trifoliata	Troyer citrange	Trifoliata	Troyer citrange
Area (cm^2)	27.1 ± 4.1	28.6 ± 3.9	38.8 ± 10.5	39.9 ± 12.7
No. of leaves per flush	8 ± 2	8 ± 3	14 ± 2	15 ± 2
% H_2O	65.2 ± 1.5	65.7 ± 1.7	68.3 ± 3.1	67.4 ± 2.7

Table 5.1: Individual leaf area, number and % H_2O of growth flushes for 'Valencia' orange and 'Prior Lisbon' lemon on Trifoliata and Troyer citrange rootstocks.

5.3.2 Foliar ion content

Leaves of both scions grafted to Troyer citrange accumulated more sodium than those on Trifoliata (Fig. 5.1). On both rootstocks, there was no large differences between 'Valencia' orange and 'Prior Lisbon' lemon in foliar sodium levels. In contrast to sodium, leaves of 'Prior Lisbon' lemon accumulated chloride faster than those of 'Valencia' orange. For all rootstock/scion combinations leaf potassium levels approximated 200 mol m^{-3} throughout the test period for control and salinised leaves (data not shown).

5.3.3 Water relations

Rootzone salinisation resulted in a reduction in (more negative) pre-dawn water potentials by approx. 0.3 MPa for all rootstock/scion combinations. For 'Valencia' orange, this was offset by more negative osmotic potentials in salinised leaves which resulted in turgor maintenance at control levels (Fig. 5.2).

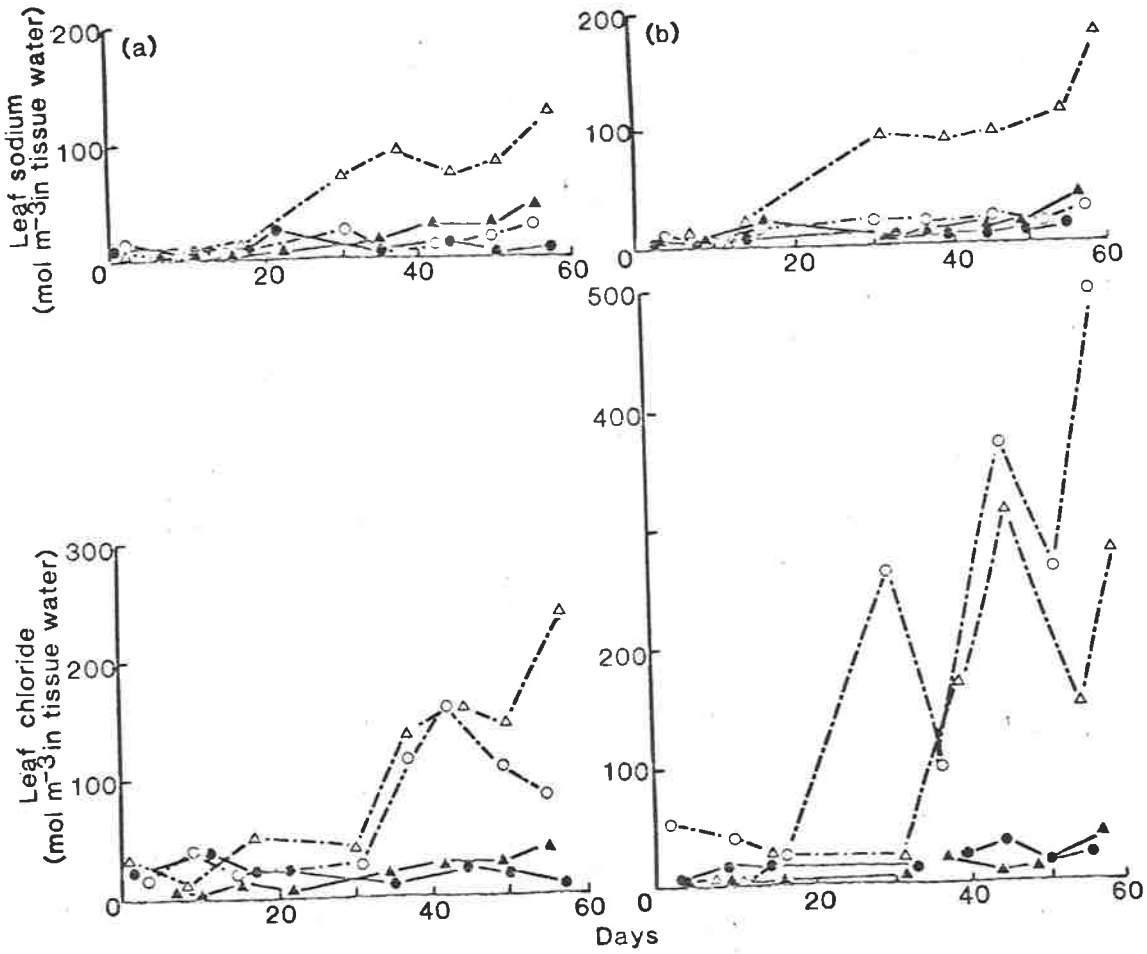


Figure 5.1 Ion concentrations of mature citrus leaves in response to salinity. (▲ △) leaves on Troyer citrange; (● o) leaves on Trifoliata. (▲ ●) leaves on plants supplied with dilute nutrient solution; (△ o) leaves on plants supplied with dilute nutrient solution + 50 mol m^{-3} NaCl (each point represents an individual leaf) (a) 'Valencia' orange (b) 'Prior Lisbon' lemon.

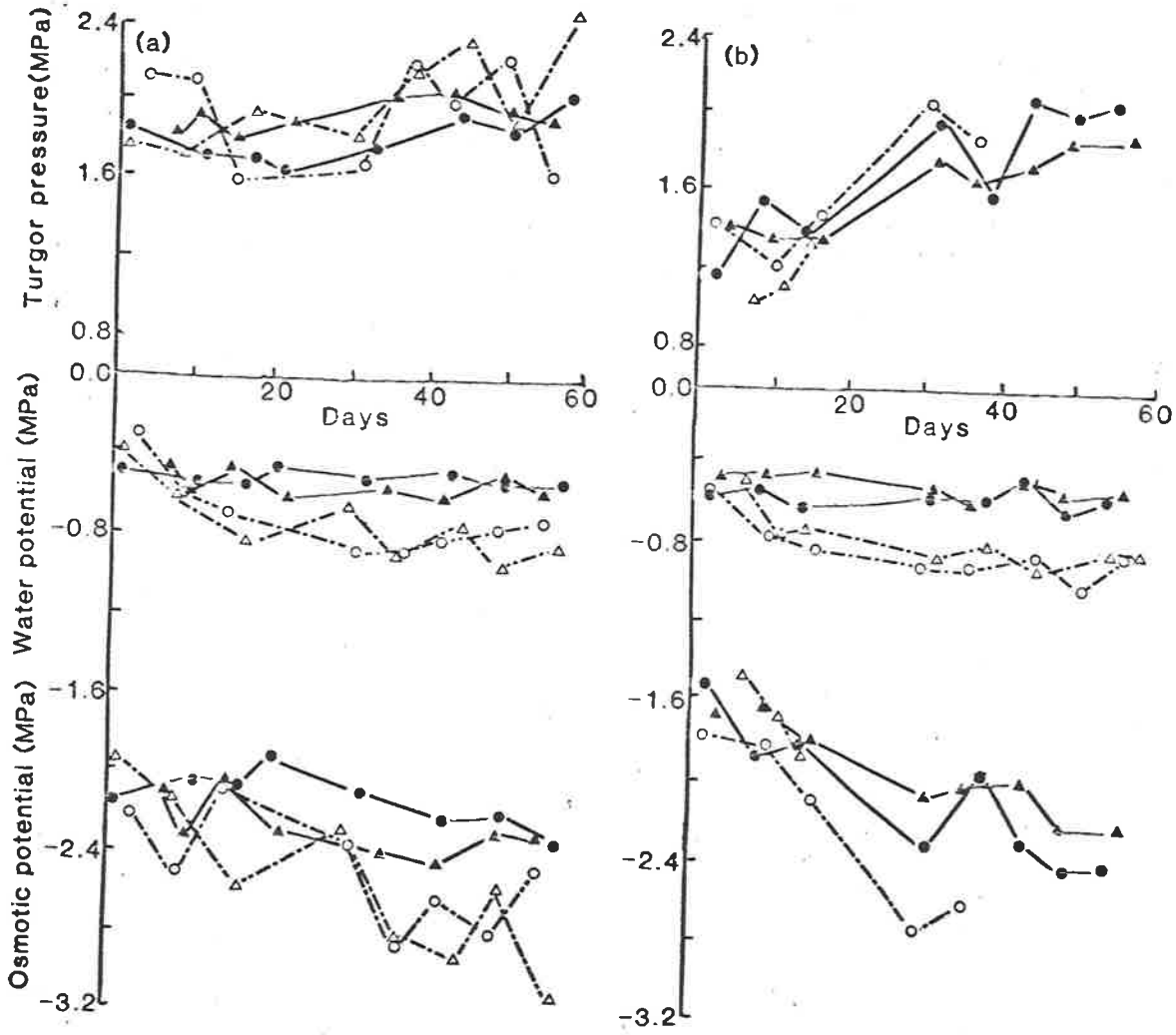


Figure 5.2 Water relations of mature citrus leaves in response to salinity. (▲ △) leaves on Troyer citrange; (● O) leaves on Trifoliata. (▲ ●) leaves on plants supplied with dilute nutrient solution; (△ O) leaves on plants supplied with dilute nutrient solution + 50 mol m^{-3} NaCl (each point represents an individual leaf) (a) 'Valencia' orange (b) 'Prior Lisbon' lemon.

It was not possible to accurately determine osmotic potentials of salinised 'Prior Lisbon' lemon leaves towards the end of the experiment using our method as realistic pressure-volume curves could not be obtained. This is illustrated in Fig. 5.3. Curves drawn through data points for salinised 'Prior Lisbon' lemon leaves did not show the two clearly differing portions characteristic of reliable PV curves.

5.3.4 Betaine and proline levels

In ^1H N.M.R. spectra of leaf extracts of 'Valencia' orange and 'Prior Lisbon' lemon, choline, prolinebetaine and proline were observed at the positions, indicated on the spectra in Fig. 5.4. No other quaternary ammonium compounds were observed in appreciable quantities in the N.M.R. spectra.

There was no effect of salinity on either prolinebetaine or proline levels for any rootstock/scion combination. Although there was no effect of rootstock, prolinebetaine levels were nearly four-fold higher for control and salinised leaves of 'Valencia' orange than for those of 'Prior Lisbon' lemon. Proline levels were equivalent for both scions (Table 5.2).

	Solute concentration (mol m^{-3} in tissue water)	
	Prolinebetaine	Proline
'Valencia' orange	23.4 ± 9.2	26.3 ± 11.9
'Prior Lisbon' lemon	8.7 ± 4.7	26.7 ± 9.5

Table 5.2: Prolinebetaine and proline levels in leaves of 'Valencia' orange and 'Prior Lisbon' lemon at the completion of the experiment. As there was no effect of salinity on levels of either compound control and salinised values have been pooled (n = 16).

5.3.5 Gas exchange

Although there was considerable leaf to leaf variation it is clear that net CO_2 assimilation of 'Prior Lisbon' lemon on both rootstocks was greatly reduced by salinity (Fig. 5.5). There was no effect of rootstock on this response. In contrast to 'Prior Lisbon' lemon, no inhibition of CO_2 assimilation was evident for leaves of 'Valencia' orange on either rootstock. More detailed analysis of leaf gas exchange is shown in Fig. 5.6. For control

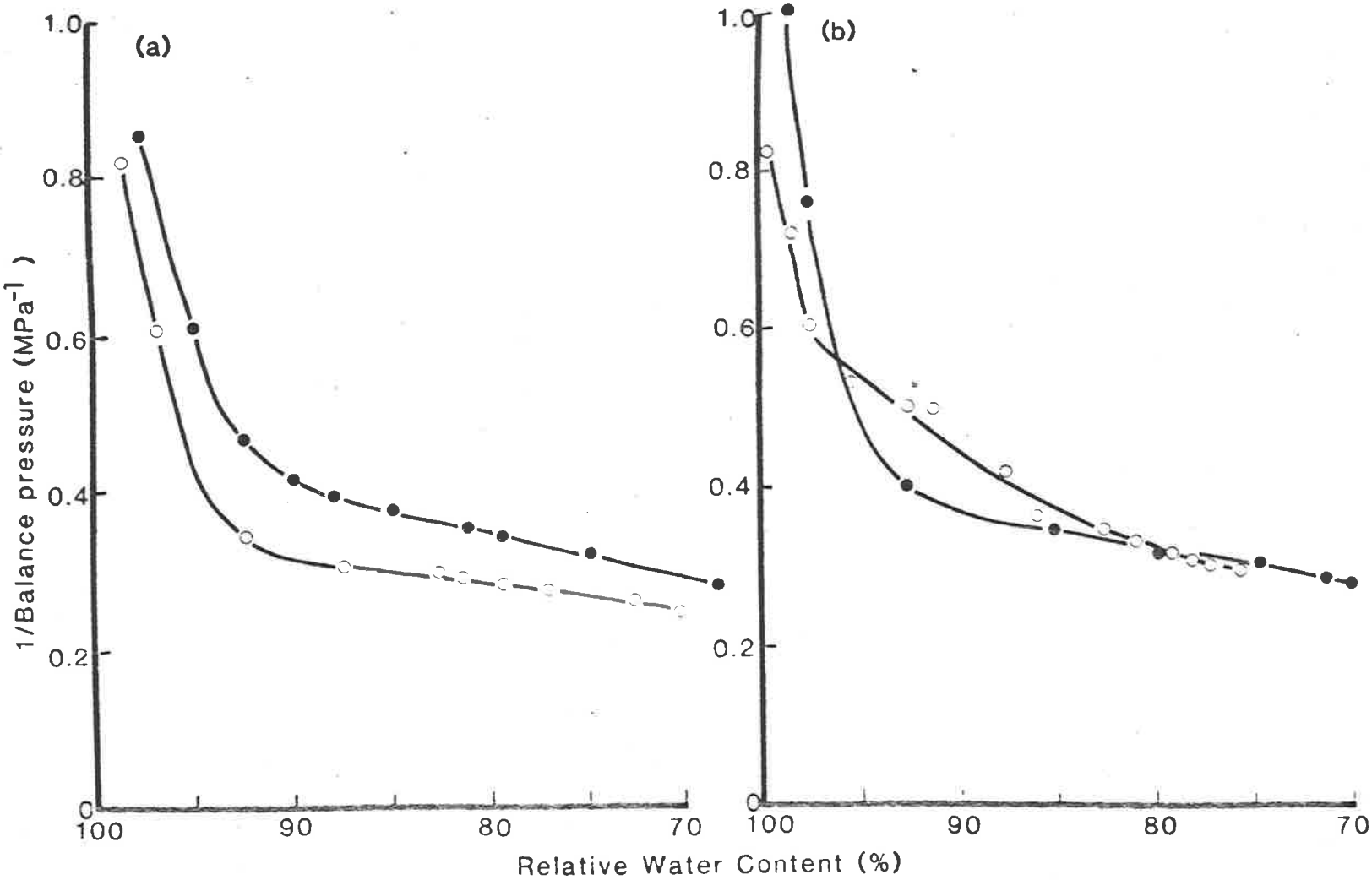


Figure 5.3 Pressure volume curves of citrus leaves on Trifoliata rootstock as affected by salinisation (●) leaves on plants supplied with dilute nutrient solution; (o) leaves on plants supplied with dilute nutrient solution + 50 mol m^{-3} NaCl. (a) 'Valencia' orange; (b) 'Prior Lisbon' lemon. Measurements were made 49-51 days after commencement of salinisation.

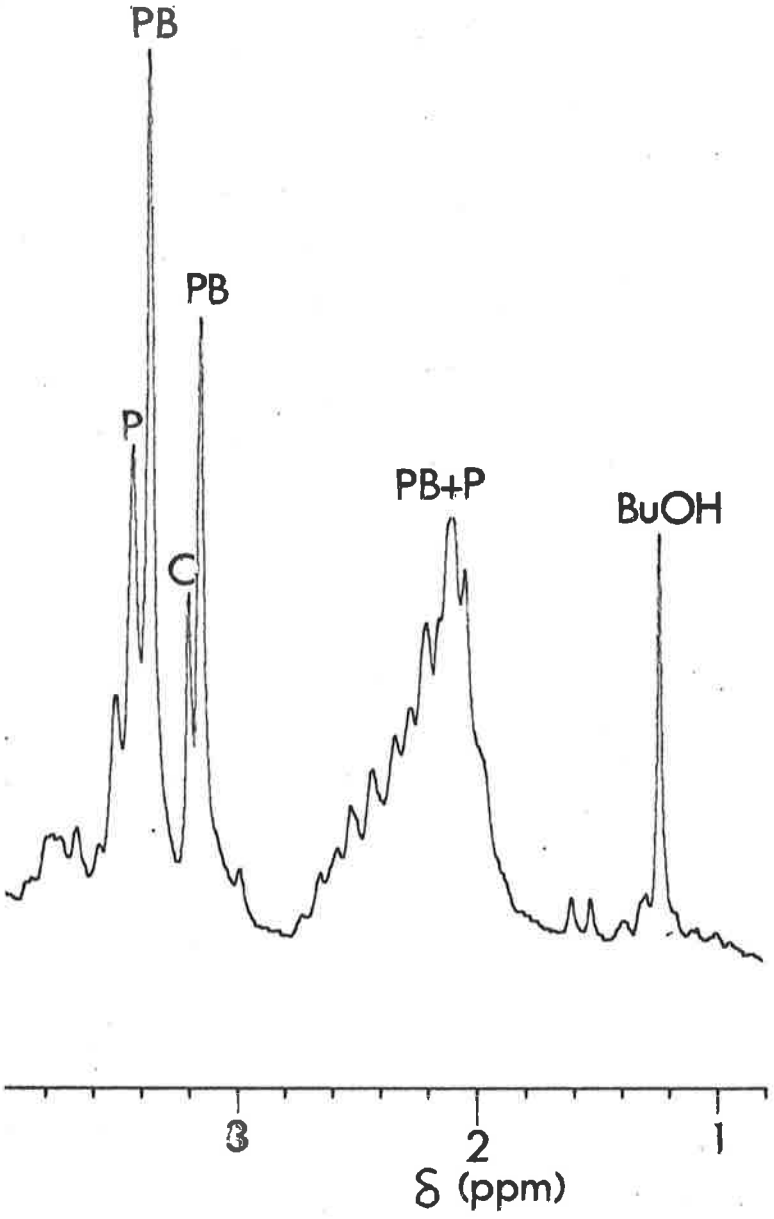


Figure 5.4 ^{1}H N.M.R. spectra of citrus leaf extracts. Positions of choline (C), prolinebetaine (PB) and Proline (P) are indicated. The internal standard was t-butanol (BuOH).

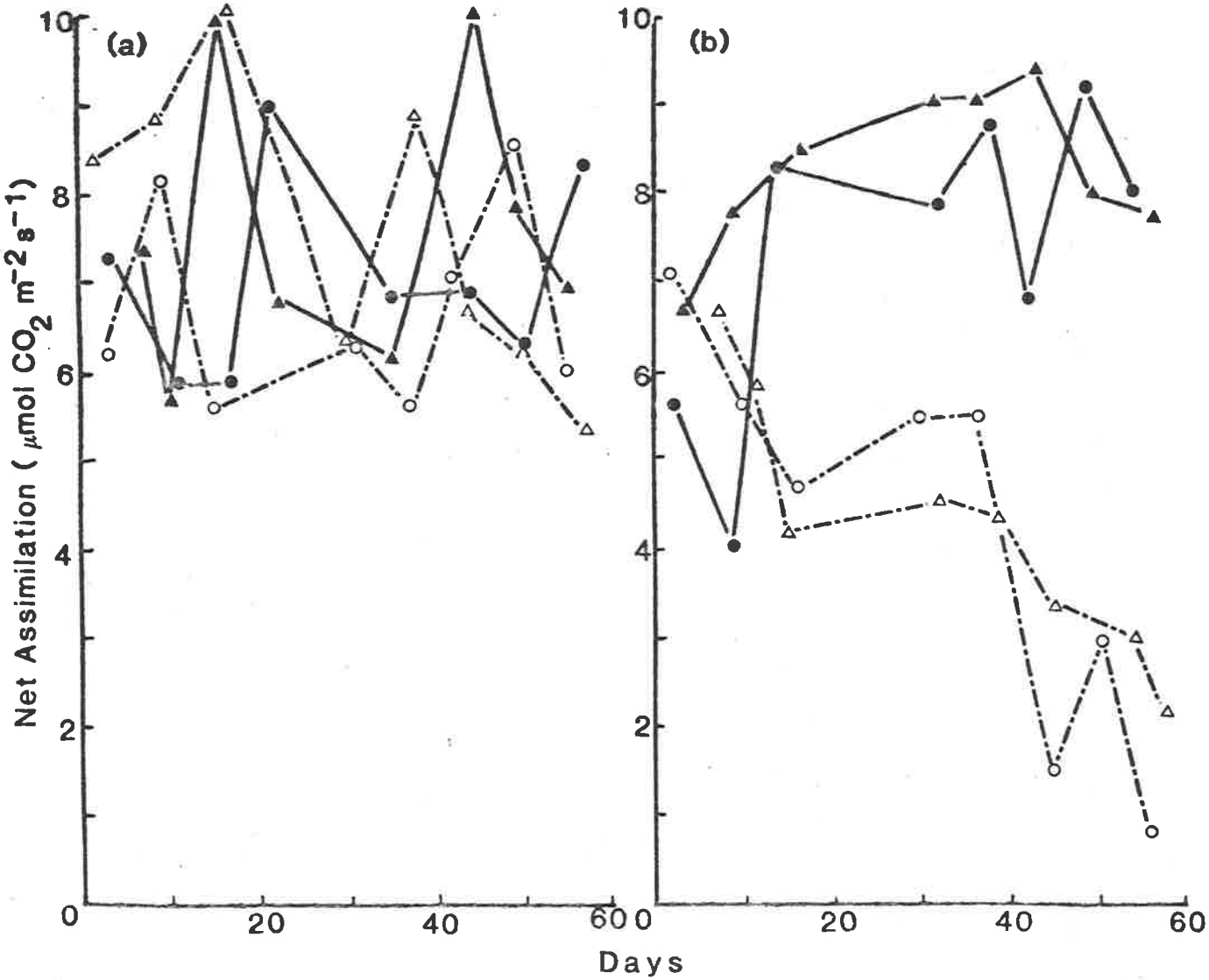


Figure 5.5 CO_2 assimilation rates of mature citrus leaves in response to salinity. (▲ △) leaves on Troyer citrange; (● O) leaves on Trifoliata. (▲ ●) leaves on plants supplied with dilute nutrient solution; (△ O) leaves on plants supplied with dilute nutrient solution + 50 mol m^{-3} NaCl (each point represents an individual leaf) (a) 'Valencia' orange (b) 'Prior Lisbon' lemon.

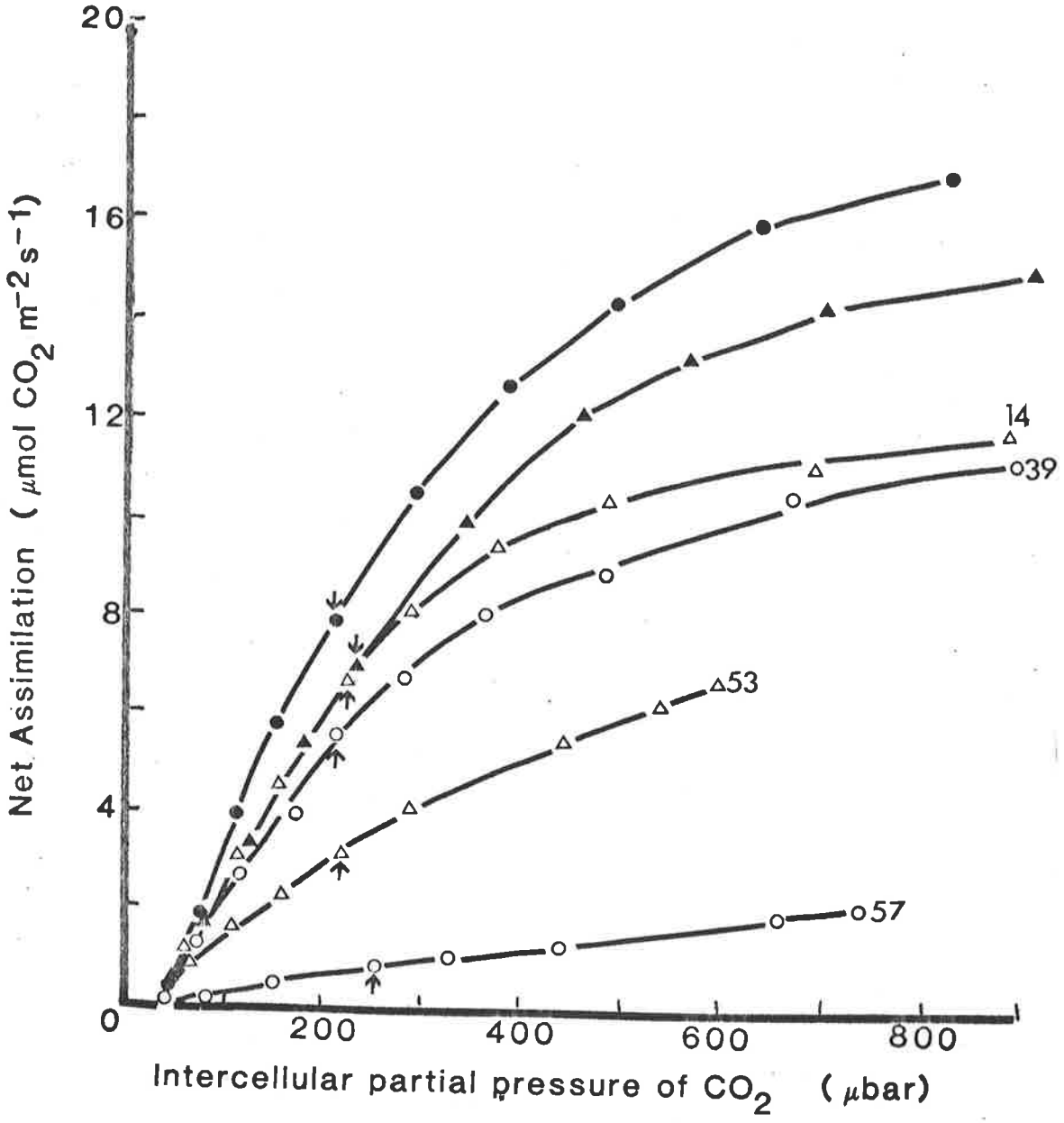


Figure 5.6 Assimilation versus intercellular partial presure of CO_2 for mature leaves in response to salinity. (△ ▲) leaves on Troyer citrange; (● O) leaves on Trifoliata. (▲ ●) leaves on plants supplied with dilute nutrient solution; (△ O) leaves on plants supplied with dilute nutrient solution + 50 mol m^{-3} NaCl. The number of days from commencement of salinisation is indicated

leaves, CO_2 assimilation approached maximal rates around an internal CO_2 partial pressure (p_i) of 800 µbar. Initially, reduced CO_2 assimilatory capacity was evident only at high p_i with CO_2 assimilation at ambient p_a being little affected. At more advanced stages of salinisation the initial slope of the A/p_i curve was also reduced and assimilation rates increased with p_i at all CO_2 partial pressures examined. At standard ambient operating conditions (p_a = 350 µbar) control leaf p_i ranged from 190 to 220 µbar for all rootstock/scion combinations. For mildly salinised leaves stomatal conductance was generally reduced to the same extent as assimilation rate, resulting in little change in p_i (data not shown). Values as high as 261 µbar were however observed in severely salinised leaves with assimilation rates below 2 µmol CO_2 m^{-2} s^{-1}.

Relative impact of chloride on CO_2 assimilation for the two scions can be inferred from plots of CO_2 assimilation in relation to foliar chloride concentration. This is illustrated in Fig. 5.7 which shows that data segregate into two populations according to scion. Response slope is steeper for 'Prior Lisbon' lemon than for 'Valencia' orange. There was no effect of rootstock on the chloride response of either scion (Table 5.3). Relations between CO_2 assimilation and leaf sodium content were generally poor for 'Valencia' orange, but for individual rootstock populations of 'Prior Lisbon' lemon the relationship was significant. Slope of the response of CO_2 assimilation to leaf sodium content was over 4 times steeper for leaves on Trifoliata than those on Troyer citrange (Table 5.4).

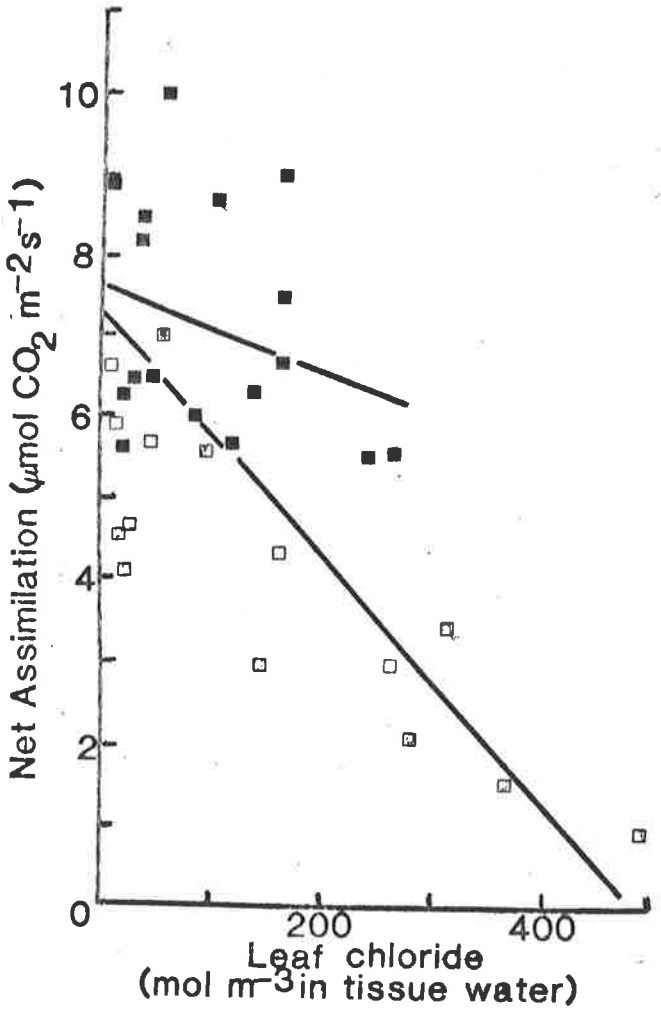


Figure 5.7 The relationship between leaf chloride concentration and CO_2 assimilation rate. (■) 'Valencia' orange; (□) 'Prior Lisbon' lemon. For each scion, data for both rootstocks have been pooled. The relationship between CO_2 assimilation rate and leaf chloride concentration is dependant upon the scion (P=0.05).

Scion	Rootstock[1]	n[2]	a	b	r[3]
'Valencia' orange	T	16	6.98	9.7×10^{-4}	0.05
	C	16	8.15	7.7×10^{-3}	0.18
	T and C	32	7.58	5.0×10^{-3}	0.22
'Prior Lisbon' lemon	T	16	7.73	1.3×10^{-2}	0.79
	C	16	7.46	1.8×10^{-2}	0.72
	T and C	32	7.32	1.5×10^{-2}	0.76
'Valencia' orange and 'Prior Lisbon' lemon	T and C	64	7.63	1.4×10^{-2}	0.65

Table 5.3: Regression equations of CO_2 assimilation rates (μmol m^{-2} s^{-1}) on leaf chloride content (mol m^{-3} in tissue water). Equations were linear ($y = a - bx$).
1, T = Trifoliata; C = Troyer Citrange; 2, number of observations; 3, correlation coefficient

Scion	Rootstock[1]	n[2]	a	b	r[3]
'Valencia' orange	T	16	6.95	9.0×10^{-3}	0.04
	C	16	8.36	1.8×10^{-2}	0.33
	T and C	32	7.52	8.6×10^{-3}	0.07
'Prior Lisbon' lemon	T	16	7.73	1.8×10^{-1}	0.64
	C	16	7.86	3.8×10^{-2}	0.80
	T and C	32	6.90	3.3×10^{-2}	0.52
'Valencia' orange and 'Prior Lisbon' lemon	T and C	64	7.28	2.5×10^{-2}	0.40

Table 5.4: Regression equations of CO_2 assimilation rates (μmol m^{-2} s^{-1}) on leaf sodium content (mol m^{-3} in tissue water). Equations were linear ($y = a-bx$).

1, T = Trifoliata; C = Troyer Citrange; 2, number of observations; 3, correlation coefficient

5.4 DISCUSSION

Although growth of all scion/rootstock combinations was limited to two new flushes per plant both individual leaf area and number of leaves per flush was greater for 'Prior Lisbon' lemon (Table 5.1). Such vigorous shoot growth is characteristic of lemons (Lewis and McCarty, 1973) and, for budded trees, is not accompanied by a more extensive root system (Hodgson & Cameron, 1935; Stolzy *et al.*, 1975). Lower root/shoot ratios in budded lemon trees may have important consequences for ionic regulation within the plant as citrus roots have a capacity to sequester significant amounts of sodium and chloride under saline conditions and hence delay accumulation of sodium and chloride in the shoot (Walker and Douglas, 1983). This capacity is finite, and as the root sink would have been relatively smaller and hence more quickly filled in budded lemon trees the earlier and more dramatic rise of chloride levels in leaves of 'Prior Lisbon' lemon may have been a consequence of the lower root/shoot ratios typical of this scion. It is also possible that root chloride exclusion mechanisms (Douglas and Walker, 1984; Douglas, 1985) were in some way influenced by the scion. Scion influences on leaf ionic content are well documented for budded citrus trees under saline (Cooper *et al.* 1952a; Cooper *et al.*, 1952b) and non-saline (Wallace *et al.*, 1952; Smith, 1975a) conditions.

The ability of Trifoliata to effectively exclude sodium from leaves is well documented for seedling rootstocks (Elgazaar *et al.*, 1965; Grieve and Walker, 1983) as well as budded trees (Chapter 4). As discussed in Chapter 4, this ability is thought to be due to an ability to withdraw sodium from the xylem in the proximal root and basal stem and to sequester it in the root and bark of these regions (Walker, 1986).

Although salinity reduced ψ by approx. 0.3 MPa (Fig. 5.2), for 'Valencia' orange more negative osmotic potentials allowed turgor to be maintained. It was not however possible to determine π of salinised 'Prior Lisbon' lemon leaves as deviations from the characteristic shape of PV curves (Fig. 3b) were observed. Curves such as this, characterised by the loss of large volumes of water without concomitant changes in ψ, have been observed under conditions of water stress (Wilson *et al.*, 1979; Kyriakopoulos and Richter, 1981) and in frost damaged leaves (Kyriakopoulos and Richter, 1981).

They may be indicative of a breakdown in plasmalemma permeability as leaves dry out during PV curve determination (Turner, 1976; Kyriakopoulos and Richter, 1981). As argued below such deviations might also be a consequence of high levels of solutes in cell wall water.

As pointed out by Passioura (1981), the pressure bomb endpoint reflects the negative hydrostatic pressure of the xylem. For accurate determination of ψ, xylem π must also be measured. Although concentrations of sodium and chloride in xylem of salt stressed citrus plants are less than 10 mol m^{-3} (R. Walker pers. comm.) and hence would only cause an error of approx. 0.04 MPa in ψ determinations on freshly excised leaves, this may not be the case for leaves that have been allowed to dehydrate during PV curve determinations. As excised leaves dry out, larger xylem vessels will drain due to negative hydrostatic pressure exceeding capillary tension. As xylem sap becomes progressively limited to thinner vessels a greater proportion will come into increasingly closer proximity to mesophyll cell walls. If π in cell walls were to be large, diffusion of ions from cell wall to xylem vessel would result in xylem π becoming increasingly more negative. This effect would be in addition to the diffusion from cell walls to xylem vessel that would occur in any case as the excised leaves dried out: in contrast to the situation for attached leaves where presumably the exchange of water in xylem vessels would keep the ion concentration low. In order to maintain ψ in equilibrium with other leaf tissue, xylem hydrostatic pressure would have to become less negative. Pressure bomb end points would then increasingly overestimate ψ as the PV curve progressed. This would result in a change in the shape of the PV curve as well as giving rise to unrealistic intercept values (Fig. 5.3b). In salinised 'Hass' avocado trees Bingham *et al.* (1968) estimated cell wall chloride values to be 315 mol m^{-3}. This is equivalent to an π of approx. 0.7 MPa. Even if similar chloride concentrations prevailed in salinised 'Prior Lisbon' lemon leaves, cytoplasm π would be far more negative than 0.7 MPa (Fig. 5.2) and hence extracellular water deficits (Oertli, 1968) would still be unlikely to occur. That ψ for salinised 'Prior Lisbon' lemon leaves did not change after day 35 (after which π could not be determined)

suggests that impairment of leaf gas exchange in 'Prior Lisbon' lemon was probably a consequence of a specific ion toxicity, rather than being due to adverse water relations.

The presence of prolinebetaine in leaves of both scions (Table 5.2) confirms earlier reports of this compound in *Citrus* spp. (Guggenheim, 1958). It has been proposed that betaines are major cytoplasmic osmotica in certain plant families adapted to salt or water stress (Wyn-Jones and Storey, 1981). Although citrus is native to humid sub-tropical environments (Reuther, 1973) and levels did not increase with salinity stress (Table 5.2), prolinebetaine may be an important factor in the osmotic balance of the cytoplasm, and its presence could therefore influence the intracellular sodium and chloride distribution in salt affected leaves. The possible consequence of scion differences in prolinebetaine levels in relation to leaf gas exchange is discussed below.

Despite genetically identical rootstocks and soil salinities, gas exchange of 'Prior Lisbon' lemon was sensitive to salt treatment whereas 'Valencia' orange was unaffected. Examination of A/p_i curves for 'Prior Lisbon' lemon (Fig. 5.6) show two stages in impairment of gas exchange under saline conditions. Initially assimilation was inhibited only at high p_i with rates at standard ambient operating conditions (p_a = 350 µbar) remaining unaffected. In later stages, initial slope of A/pi curves also decreased. This response is similar to those observed previously for 'Valencia' orange (Chapters 3 and 4). As was also observed in these studies, salinity reduced chloroplast photosynthesis and stomatal conductance to similar extents, except at very low assimilation rates where p_i increased (Chapter 3). At low stomatal conductances, cuticular conductance which is usually an insignificant error in the p_i equation (Chapter 1) would become an increasingly larger portion of total conductance and hence an overestimation of stomatal conductance (and hence p_i) may occur at low rates of gas exchange.

Unlike the previous studies using 'Valencia' orange (Chapters 3 and 4) it is unlikely that high sodium levels were responsible for impairments of gas exchange in salinised 'Prior Lisbon' lemon as gas exchange of this scion was almost completely impaired on Trifoliata (Fig. 5.5) but no increase in sodium above control levels was observed (Fig. 5.1). This suggests that high chloride levels in 'Prior Lisbon' lemon (Fig.

5.1) may have been more important in reducing gas exchange. Indeed, a good quantitative relationship existed between leaf chloride concentration and CO_2 assimilation rate for this scion (Fig. 5.7). This plot of assimilation *versus* chloride (Fig. 5.7) segregates into two populations depending upon scion; 'Valencia' orange being less sensitive to high chloride levels than 'Prior Lisbon' lemon. It would thus appear that a given foliar chloride concentration has more impact on gas exchange of 'Prior Lisbon' lemon than of 'Valencia' orange.

Greater sensitivity of the 'Prior Lisbon' lemon scion could be explained by either an increased susceptibility of some cytoplasmic or chloroplastic process (es) to chloride or differences between scions in compartmentation of this ion. Of these two possibilities the latter is more likely as most metabolic processes studied in salt tolerant plants are just as sensitive to salt *in vitro* as are those of salt sensitive plants (Flowers *et al.*, 1977; Wignarajah and Baker, 1981; Ball *et al.*, 1984). Evidence from X-ray microprobe analysis (Harvey *et al.*, 1981) physical isolation methods (Robinson and Downtown, 1985; Demmig and Winter, 1986) and efflux analysis (Yeo, 1981) all suggest that salt tolerant plants have the ability to maintain low cytoplasmic and chloroplastic concentrations of sodium and chloride despite high bulk foliar levels (Section 3.1.1). This is achieved by sequestering these ions in the vacuole. This compartmentation must involve active transport of chloride and/or sodium across the tonoplast and in recent years a tonoplast ATP-ase stimulated by chloride has been reported for a wide range of plant tissues (Hoger and Helmle, 1981; Bennet *et al.*, 1984; Barbier-Brygoo, 1986; Marţoia *et al.*, 1986). Although little work has been done to elucidate the role of this ATP-ase in salt tolerance, Lerner *et al.*, (1983) reported that membrane ATP-ase activity of the halophyte *Atriplex nummularia* was stimulated by NaCl whilst that of the salt-sensitive *Pisum sativum* was inhibited. Crude membrane preparations were used however and the intracellular location of the ATP-ase *in vivo* is uncertain. This does nevertheless suggest that genotypic differences in the salt response of ATP-ases exist and thus it may be that for 'Valencia' orange, transport of chloride into the vacuole occurs at a greater rate. Ahmad *et al.* (1987) have recently shown that glycinebetaine can increase vacuolar

sodium (and presumably chloride) concentrations. Scion differences in prolinebetaine levels (Table 5.2) could therefore be important in this respect with higher levels of prolinebetaine in 'Valencia' orange playing a role in the relative insensitivity of this scion to high chloride levels via modulation of intracellular concentrations.

Ability to maintain low cytoplasmic chloride levels must be dependent upon rate of entry into the symplast and well as ability to sequester chloride in vacuoles. We cannot therefore exclude the possibility that greater sensitivity of 'Prior Lisbon' lemon to high leaf chloride levels was merely a consequence of the more rapid increase in foliar chloride levels for this scion (Fig. 5.1). This would require faster compartmentation of chloride into vacuoles than for leaves of 'Valencia' orange in order for equivalent cytoplasmic levels to be maintained. In this respect it is interesting to note that studies pointing to chloride toxicity in citrus (Cooper and Gorton, 1952; Cooper, 1961; Walker *et al.*, 1982) are invariably characterised by rapid increases in leaf chloride concentrations similar to those shown by 'Prior Lisbon' lemon in this study. That rate of import into the leaf as well as absolute concentration of chloride is important in the response of *Citrus* spp. to salinity is supported by results of Walker *et al.* (1982). They observed complete recovery of CO_2 assimilation in Etrog citron following removal of salt treatment despite the absence of any appreciable reduction in leaf chloride concentrations. This clearly implies an alteration in cellular chloride distribution in response to reduced rates of chloride import into leaves. For mature trees in the field leaf chloride concentration rises at a much slower rate than most laboratory studies, even when trees are grafted to Trifoliata (Chapter 8). The importance of chloride toxicity in debilitating orchard trees is therefore questionable (Chapter 10). We investigate further the effect of scion on response of gas exchange to salinity in the following chapter.

CHAPTER SIX

FURTHER EVIDENCE FOR SPECIFIC ION TOXICITY IN *CITRUS* SPP.

6.1 INTRODUCTION

Whilst data in Chapter 5 suggested that under certain circumstances chloride toxicity could occur in *Citrus* spp., results from Chapter 4 indicated that rootstock effects on leaf gas exchange were most easily explained in terms of differences in sodium exclusion ability. We did however observe in Chapter 5 that the chloride insensitive scion 'Valencia' orange had higher levels of prolinebetaine than the chloride sensitive 'Prior Lisbon' lemon. Given that considerable amounts of proline are synthesised in citrus roots (Kato, 1986), and that rootstocks may influence concentrations of proline within scion foliage (Yelenosky, 1979), if leaf prolinebetaine levels were to be also influenced by rootstock then observed influences of Trifoliata and Cleopatra mandarin on apparent sensitivity of 'Valencia' orange gas exchange to leaf chloride concentration (Fig. 4.5) could be explicable in terms of a rootstock influence on leaf prolinebetaine levels. Furthermore, rootstock differences in concentrations of compounds such as cytokinins and gibberellins in xylem sap have also been reported (Saidha *et al.*, 1983) and hence rootstock influences on gas exchange could also be mediated via changes in the amounts of such compounds arriving at the leaf under conditions of rootzone salinisation.

In order to experimentally separate rootstock effects on leaf sodium concentration from other root derived compounds, experiments described in this chapter utilised the fact that (other things being equal) cultivars of grapefruit (*C. paradisi*) typically contain higher concentrations of sodium in their leaves than the oranges (Cooper *et al.*, 1952ab). It was rationalised that if impaired gas exchange of citrus leaves is really due to a sodium toxicity then (due to higher foliar sodium concentrations) leaves of 'Marsh' grapefruit budded to a particular rootstock should be more sensitive to salinity than 'Valencia' orange. On the other hand, if rootstock influences on gas exchange are mediated via root derived compounds then, unless the scion itself influences the synthesis or transport of such compounds then rootstock effects on gas exchange under salinity should be independent of scion.

In this chapter we compare gas exchange of 'Marsh' grapefruit, 'Valencia' orange and 'Washington Navel' orange on Trifoliata and Cleopatra mandarin stocks under saline

conditions. Changes in leaf gas exchange are considered in relation to foliar ion concentrations, prolinebetaine levels and leaf water relations for all rootstock/scion combinations.

6.2 MATERIALS AND METHODS

6.2.1 Growth of plants

One year old 'Valencia' orange (*Citrus sinensis* [L.] Osbeck), 'Washington Navel' orange (*C. sinensis*) or 'Marsh' grapefruit (*C. paradisi* Macf.) budded onto one and a half year old Trifoliata (*Poncirus trifoliata* [L.] Raf.) or Cleopatra mandarin (*C. reticulata* Blanco) were potted in free draining 3 litre containers filled with washed river sand. Buds were from single trees and rootstocks were grown from open pollinated seed. After 20 days in a glasshouse, watered daily with half strength Hoaglands solution (Hoagland and Arnon, 1950), six uniform plants from each rootstock/scion combination (36 plants in all) were transferred to a growth room with a photoperiod of 9 h, 450 μmol m^{-2} s^{-1} photosynthetically active radiation provided by sodium vapor lamps and shade cloth, and a 25°/20°C day/night temperature. Relative humidity was not controlled but was generally greater than 70%. Daily watering with half-strength Hoaglands solution continued for a further 40 days after which salt treatment commenced on three plants from each rootstock/scion combination in steps of 5 mol m^{-3} added to the half strength Hoaglands solution to a final concentration of 50 mol m^{-3} NaCl which was applied daily thereafter. Control plants were watered with half strength Hoaglands solution daily.

6.2.2 General method

Measurements began upon commencement of salt treatment. All leaves examined were ~4 months old having developed under glasshouse conditions. For one leaf from each tree, net gas exchange and foliar sodium, chloride and potassium levels were determined. This procedure was repeated 28, 49 and 70 days after commencement of salinisation. After 28 days of salinisation, stomatal responses to humidity were characterised for control and salinised 'Washington Navel' orange leaves on Cleopatra

mandarin rootstock. After 56 days of salinisation, leaf water relations (using both pressure bomb and psychrometry) and prolinebetaine concentrations were determined. At the completion of the experiment all trees were divided into leaves, stems and roots and dry weight of each organ type determined.

6.2.3 Gas exchange

6.2.3.1 Routine measurements

Gas exchange of leaves was determined using the system described in Section 1.2.2.2. Leaf temperature was 25 ± 0.5°C with a VPD of 1.2 ± 0.1 kPa. Photon irradiance at the leaf surface was 1000 μmol m^{-2} s^{-1}.

6.2.3.2 Influence of ambient humidity on leaf gas exchange

For 'Washington Navel' orange on Cleopatra mandarin rootstock leaf gas exchange was monitored at VPDs of 1.2 ± 0.1, 1.7 ± 0.1 and 2.2 ± 0.2 kPa 28 days following commencement of salinisation. Measurements were made at completion of A/p_i curves (1.2 kPa) after which condenser temperature was reduced to 12°C (this typically took 1 hour). Gas exchange was then allowed to stabilise at this VPD (1.7 kPa) for one hour after which condenser temperature was reduced to 6°C yielding a VPD of 2.2 kPa. Leaf gas exchange was then allowed to reach a new steady state level. Condenser temperature was then again raised to 18°C to yield the original VPD. Gas exchange measurements after the original VPD was attained showed that responses to VPD were fully reversible. This experiment was performed in order to provide laboratory verification of influences of salinity on stomatal responses to VPD observed under orchard conditions (Chapter 9).

6.2.4 Leaf water relations

6.2.4.1 Pressure-bomb measurements

Leaves were sampled just prior to lights on in growth room. Two freshly excised leaves from each tree were immediately placed in a plastic bag and Ψ determined

(Scholander *et al.*, 1965). 'Pre-dawn' osmotic potential was subsequently determined via PV curves as described in Section 2.2.3.6.

6.2.4.2 Psychrometry determinations

Two leaves from each tree were excised and immediately placed in plastic bags prior to commencement of growth room light period. The midrib of each leaf was then removed and each lamina half placed in a Merrill whole leaf psychrometer chamber which was then immediately enclosed in Nescofilm. The sealed chamber was then submerged in liquid N_2 for 30 s in order to denature membranes and hence eliminate the turgor component of ψ (Turner, 1981). The chamber was then allowed to thaw, the Nescofilm removed, the chamber attached to a Merrill psychrometer probe and immersed in a 25 ± 0.1° water bath maintained in a constant temperature room. Psychrometer readings were made using a Wescor HP-115 system scanning every 45 minutes. Following attainment of equilibrium (3-5 hours) temperature and voltage values for three consecutive readings were used to calculate π, using calibration curves recently established for each chamber using NaCl standards. Values of π for each leaf were therefore the means of 6 determinations.

6.2.5 Calculation of apoplastic water fraction

In determination of π via psychrometry, as membranes are disrupted by freezing and thawing the cell sap is diluted by apoplastic water. This results in values of π determined by psychrometry being less negative than those obtained from PV curves (Turner, 1981). The apoplastic water fraction (A) can then be calculated:

$$A = 1 - \frac{\pi_{PSYCHROMETER}}{\pi_{PV\ CURVE}} \qquad (6.1)$$

Equation 6.1 does however assume that π of apoplastic water is negligible. The apparent dilution of apoplastic water should therefore be reduced if apoplastic π is appreciable. Apoplastic water fraction can also be estimated directly from PV curves (Section 2.2.3.6).

In this experiment we estimate apoplastic water fractions for control and salinised leaves via both methods with the intent of determining whether appreciable changes in apoplastic π (and hence apparent dilution: Equation 6.1) occur in salinised leaves.

6.2.6 Quaternary ammonium compunds

Leaves were excised and placed in plastic bags 3-4 hours after commencement of growth room light period. Midribs were removed, fresh weight of one lamina half determined and the tissue immediately frozen in liquid N_2. After being freeze dried under vacuum for 48 hours, dry weight was then determined. Samples were then stored in a dessicator at -20°C until determination of QAC as described in Section 5.2.7.

6.2.7 Ion analysis

Leaf sodium, chloride and potassium concentrations of gas exchange leaves were determined as described in Section 3.2.2.5.

6.3 RESULTS

6.3.1 Dry matter distribution between leaves, stems and roots

On both rootstocks, Marsh grapefruit trees had significantly higher shoot/root ratios than both orange varieties (Table 6.1).

Rootstock	'Valencia' orange	'Washington Navel' orange	'Marsh' grapefruit
Trifoliata	0.29	0.29	0.42
Cleopatra mandarin	0.38	0.41	0.65

Table 6.1: Shoot/root ratios (dry weight basis) of control plants at the completion of the experiment. The rootstock stem has been included in the total root mass. Least significant difference ($p = 0.05$) = 0.12

Leaf water content also varied amongst scions with water in 'Marsh' grapefruit leaves constituting a lower proportion of total leaf fresh weight (Table 6.2).

Rootstock	'Valencia' orange	'Washington Navel' orange	'Marsh' grapefruit
Trifoliata	1.91	2.05	1.72
Cleopatra mandarin	2.01	1.95	1.81

Table 6.2: Water content ($gH_2O\ g^{-1}$ DW) of whole leaf masses. Least significant difference ($p = 0.05$) = 0.10.

6.3.2 Leaf ion concentrations

A faster rise in leaf sodium concentration following commencement of salinisation was observed for all scions when budded to Cleopatra mandarin rather than Trifoliata. A scion influence was also evident on both roostocks, leaves on 'Marsh' grapefruit accumulated sodium faster than either of the orange varieties (Fig. 6.1).

Low rates of chloride accumulation were observed for both orange varieties on Cleopatra mandarin stock. For 'Marsh' grapefruit on Trifoliata the increase in leaf chloride was faster than for either orange variety on this stock. Further to this, leaves of this scion on Cleopatra mandarin showed equivalent increases in chloride concentrations with time to those on Trifoliata (Figure 6.2).

6.3.3 Leaf water relations

Measurements of Ψ, π and P via pressure-bomb end point determinations 49 days after commencement of salinisation are shown in Table 6.3. For all scion/rootstock combinations salinity resulted in a lowering of (more negative) Ψ by 0.4-0.5 MPa. This was in all cases offset by more negative π in salinised leaves with subsequent maintenance of P at or above control levels.

Osmotic potentials determined by the PV curve method are compared with those obtained via psychrometry (Table 6.4). Whilst values obtained by the two methods were comparable for all treatments, for leaves on Trifoliata the differences in values between

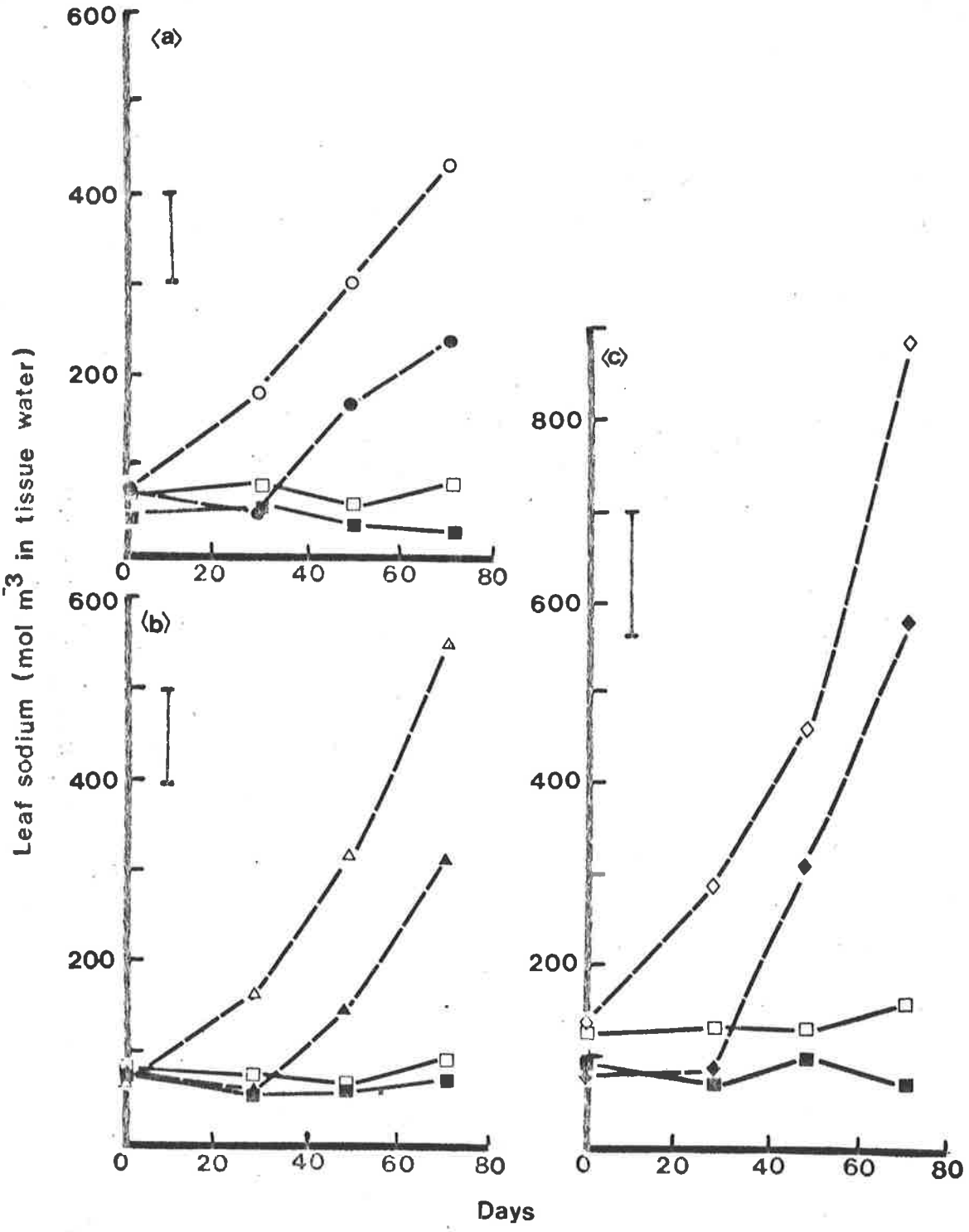


Figure 6.1 Sodium concentration in citrus leaves in response to salinity. (■ □) Leaves on plants supplied with dilute nutrient solution; (●○▲△◆◇), leaves on plants supplied with dilute nutrient solution + 50 mol m-3 NaCl. Open symbols on Cleopatra mandarin; Closed symbols on Trifoliata. (a) 'Valencia' orange, (b) 'Washington Navel' orange, (c) 'Marsh' Grapefruit.

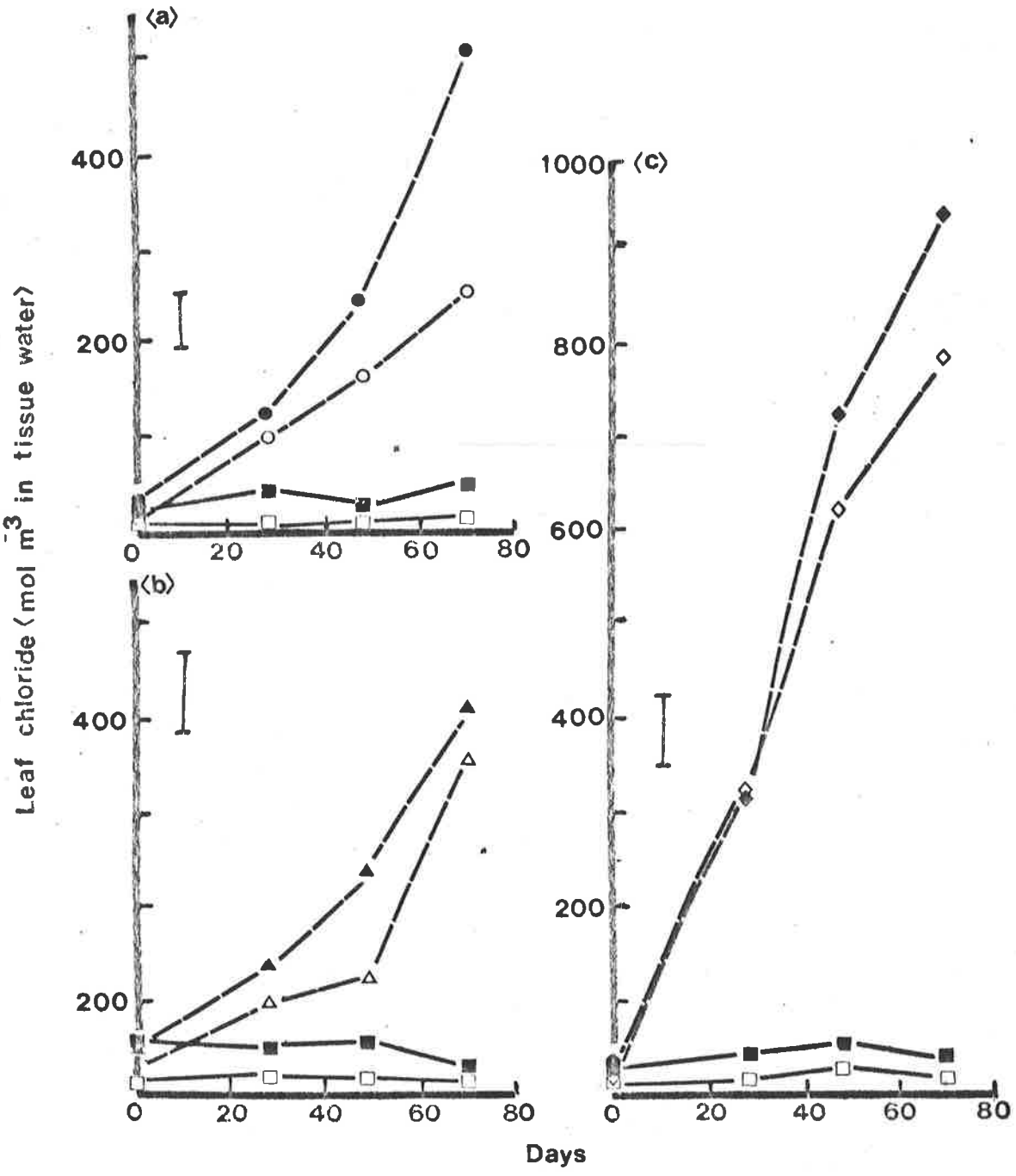


Figure 6.2 Chloride concentration in citrus leaves in response to salinity. (■ □) Leaves on plants supplied with dilute nutrient soltution; (●○▲△◆◇), leaves on plants supplied with dilute nutrient solution + 50 mol m-3 NaCl. Open symbols on Cleopatra mandarin; Closed symbols on Trifoliata. (a) 'Valencia' orange, (b) 'Washington Navel' orange, (c) 'Marsh' Grapefruit.

Rootstock	'Valencia' orange		'Washington Navel' orange		'Marsh' grapefruit	
	Control	Salinised	Control	Salinised	Control	Salinised
Trifoliata						
Ψ	-0.66	-1.11	-0.64	-1.02	-0.63	-1.08
π	-2.09	-2.97	-2.47	-2.70	-2.18	-2.82
P	1.43	1.87	1.84	1.68	1.55	1.74
Cleopatra mandarin						
Ψ	–0.70	–1.05	–0.71	–1.15	–0.67	–1.13
π	–2.27	–2.76	–2.22	–2.70	–2.06	–3.43
P	1.57	1.71	1.51	1.55	1.39	2.30

Table 6.3: Leaf water potential (Ψ), osmotic potential (π) and turgor pressure (P) (MPa) of leaves on control (watered with dilute nutrient solution) and salinised (watered with dilute nutrient solution + 50 mol m-3 NaCl) plants. Least significant difference (p = 0.05). Ψ = 0.14: π = 0.17: P = 0.25.

Rootstock	'Valencia' orange		'Washington Navel' orange		'Marsh' grapefruit	
	Control	Salinised	Control	Salinised	Control	Salinised
Trifoliata						
PV curve	2.09	2.97	2.47	2.70	2.17	2.74
Psychrometry	1.80	2.59	2.13	2.50	1.89	2.82
Cleopatra Mandarin						
PV curve	2.26	2.76	2.22	2.93	2.06	3.43
Psychrometry	2.10	2.53	2.14	2.70	1.99	3.22

Table 6.4: Leaf osmotic potential (-MPa) of control (watered with dilute nutrient solution) and salinised (watered with dilute nutrient solution + 50 mol m-3 NaCl) leaves 49 days after commencement of salinisation as determined via pressure-volume (PV) curves or psychrometry. Least significant difference ($p = 0.05$) = 0.19.

the two methods and hence the calculated apoplastic fraction (Section 6.2.5) decreased with salinity. As shown in Table 6.5 this was not the case when the apoplastic fraction was determined *via* the PV curve intercept method (Section 2.2.3.6). This table also shows that control apoplastic water fractions calculated from the differences in values obtained via the two methods were 14 ± 1% for scions on Trifoliata but only 5 ± 2% for scions on Cleopatra mandarin. No such rootstock differences were evident when the apoplastic water fraction was determined solely *via* the PV curve intercept method.

6.3.4 Prolinebetaine levels

For all scions there was no effect of rootstock on leaf prolinebetaine concentrations. Furthermore, there was no systematic effect on salinisation on prolinebetaine levels, with significant increases occurring only for 'Valencia' orange on Trifoliata and 'Marsh' Grapefruit on Cleopatra mandarin (Table 6.6).

6.3.5 Leaf gas exchange

Although salinisation resulted in a reduction in gas exchange for all rootstock/scion combinations the extent of such inhibition was influenced by both scion and rootstock. For all scions, CO_2 assimilation was reduced to a greater extent on Cleopatra mandarin than Trifoliata stock (Figure 6.3). It should also be noted that (in the absence of salinisation) scions had greater assimilation rates when budded on Trifoliata than on Cleopatra mandarin (Table 6.7).

Rootstock	'Valencia' orange	'Washington Navel' orange	'Marsh' grapefruit
Trifoliata	6.3	6.7	7.8
Cleopatra mandarin	5.2	6.1	6.4

Table 6.7: CO_2 assimilation rates in the absence of salinisation for leaves on both rootstocks. Least significant difference (p = 0.05) = 0.9. (Statistical analysis performed on all control leaves [12 for each scion/rootstock combination] with time effects removed.)

Rootstock	'Valencia' orange		'Washington Navel' orange		'Marsh' grapefruit	
	Control	Salinised	Control	Salinised	Control	Salinised
Trifoliata						
PV/Psychrometry	14	13	14	7	13	-3
PV intercept	12	12	10	22	17	26
Cleopatra mandarin						
PV/Psychrometry	7	8	3	8	3	6
PV intercept	14	13	15	9	14	25

Table 6.5: Calculated apoplastic water fractions (%) as determined by a comparison of osmotic potential values obtained by pressure-volume (PV) curves and psychrometry (PV/Psychrometry) or from the PV curve intercept method for leaves on plants irrigated with dilute nutrient solution (controls) or leaves from plants irrigated with dilute nutrient solution + 50 mol m-3 NaCl (salinised).

Rootstock	'Valencia' orange		'Washington Navel' orange		'Marsh' grapefruit	
	Control	Salinised	Control	Salinised	Control	Salinised
Trifoliata	56.6	77.4	40.0	42.8	43.4	45.8
Cleopatra mandarin	62.4	63.3	46.9	53.4	55.2	85.6

Table 6.6: Prolinebetaine concentrations (μeq g^{-1} DW) in leaves of control (irrigated with dilute nutrient solution) and salinised (watered with dilute nutrient solution + 50 mol m-3) NaCl leaves 49 days after commencement of salinisation. Least significant difference (p = 0.05) = 18.3.

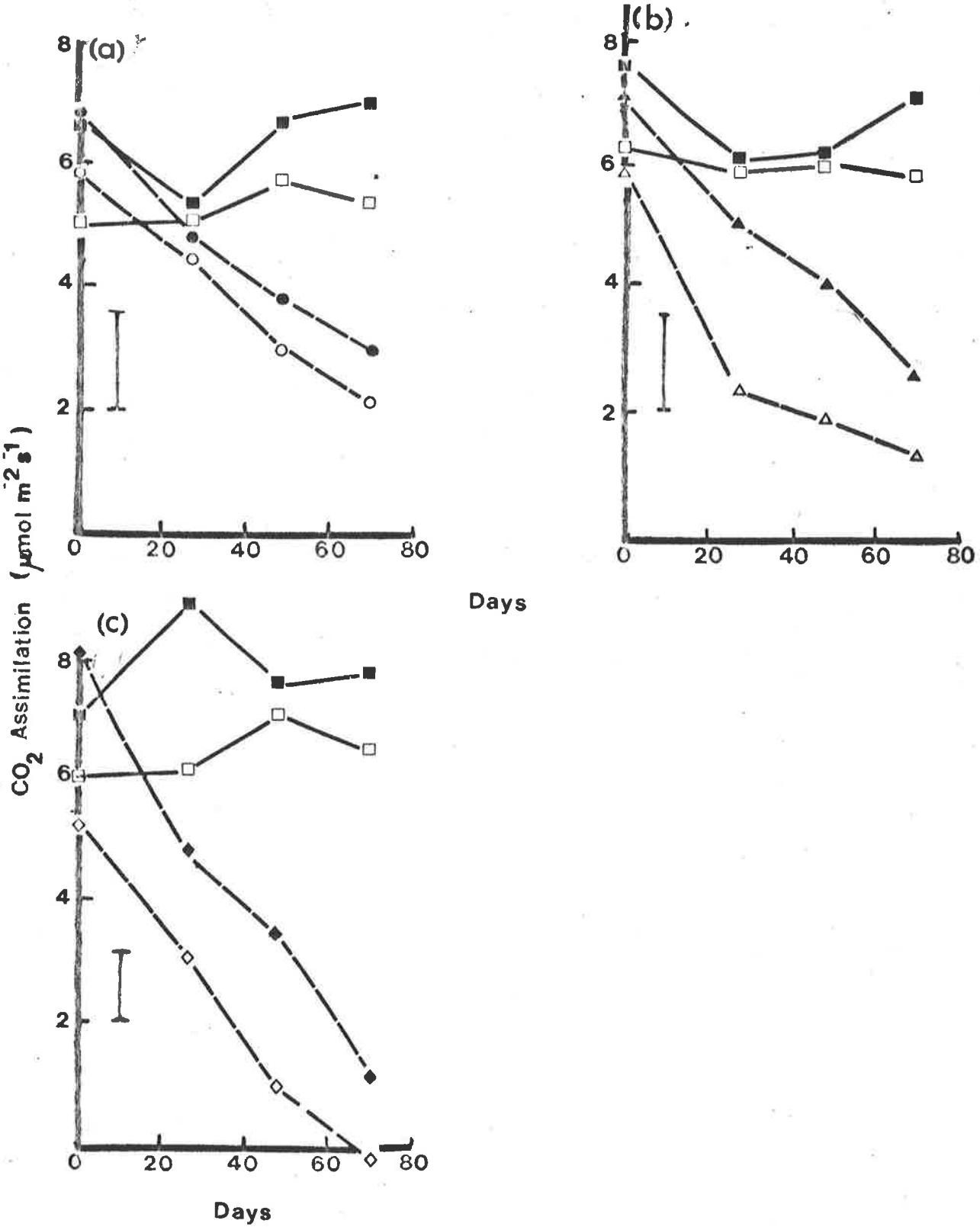


Figure 6.3 CO_2 assimilation of citrus leaves in response to salinity.
(■ □) Leaves on plants supplied with dilute nutrient solution
(●○▲△◆◇) leaves on plants supplied with dilute nutrient solution + 50 mol m-3 NaCl. Open symbols on Cleopatra mandarin; Closed symbols on Trifoliata. (a) 'Valencia' orange, (b) 'Washington Navel' orange, (c) 'Marsh' Grapefruit.

Table 6.7 also shows that CO_2 assimilation rates were higher for leaves of 'Marsh' grapefruit than for leaves of the orange scions. Comparisons of scion responses to salinity are therefore best done expressing CO_2 assimilation rates as a percentage of controls. Such comparsions are shown in Figure 6.4. This shows that on both rootstocks gas exchange of 'Marsh' grapefruit was more sensitive to salinity than the two orange scions. Bonifferoni multiple t-test comparisons showed this greater sensitivity of 'Marsh' grapefruit to be significant at the 95% level. A/p_i curves for control as salinised leaves 54 days after commencement of measurements (Figure 6.5) illustrates scion and rootstock effects in more detail. For control leaves the higher rates of CO_2 assimilation on Trifoliata are even more evident at high p_i than at ambient $p(CO_2)$. Reductions in CO_2 assimilation in salinised leaves tended to be greater at high p_i but the extent of this effect tended to vary considerably between leaves. Despite the presence of significant rootstock and scion effects on CO_2 assimilation p_i was remarkably constant ranging from 200-240 µbar CO_2 for both control and salinised leaves. Thus changes in CO_2 assimilation and stomatal conductance occurred more or less in concert in all cases.

A direct effect of salinity on stomatal function was however evident as stomata of salinised leaves failed to respond to humidity (Fig. 6.6). Differences in A and g_s were therefore greatest at low VPD. At the higher VPDs p_i was in fact greater for salinised than control leaves (Fig. 6.6c) with a corresponding decrease in WUE (Fig. 6.6d).

Analysis of individual leaf CO_2 assimilation rates in terms of leaf ion concentration enables much of the observed differences in scion and rootstock sensitivites to be related to their effects on leaf sodium and chloride concentrations. A plot of CO_2 assimilation in terms of leaf chloride concentrations (Fig. 6.7; regression equations in Table 6.8) shows that whilst the greater sensitivity of 'Marsh' grapefruit to salinity may be explained in terms of high chloride concentrations in this scion (Figure 6.2), as was observed in Chapter 4, leaves on Trifoliata appear to be less sensitive to the chloride ion than those on Cleopatra mandarin. This is born out in Table 6.9 where equality of regression lines is tested using the method described in Section 5.2.6.

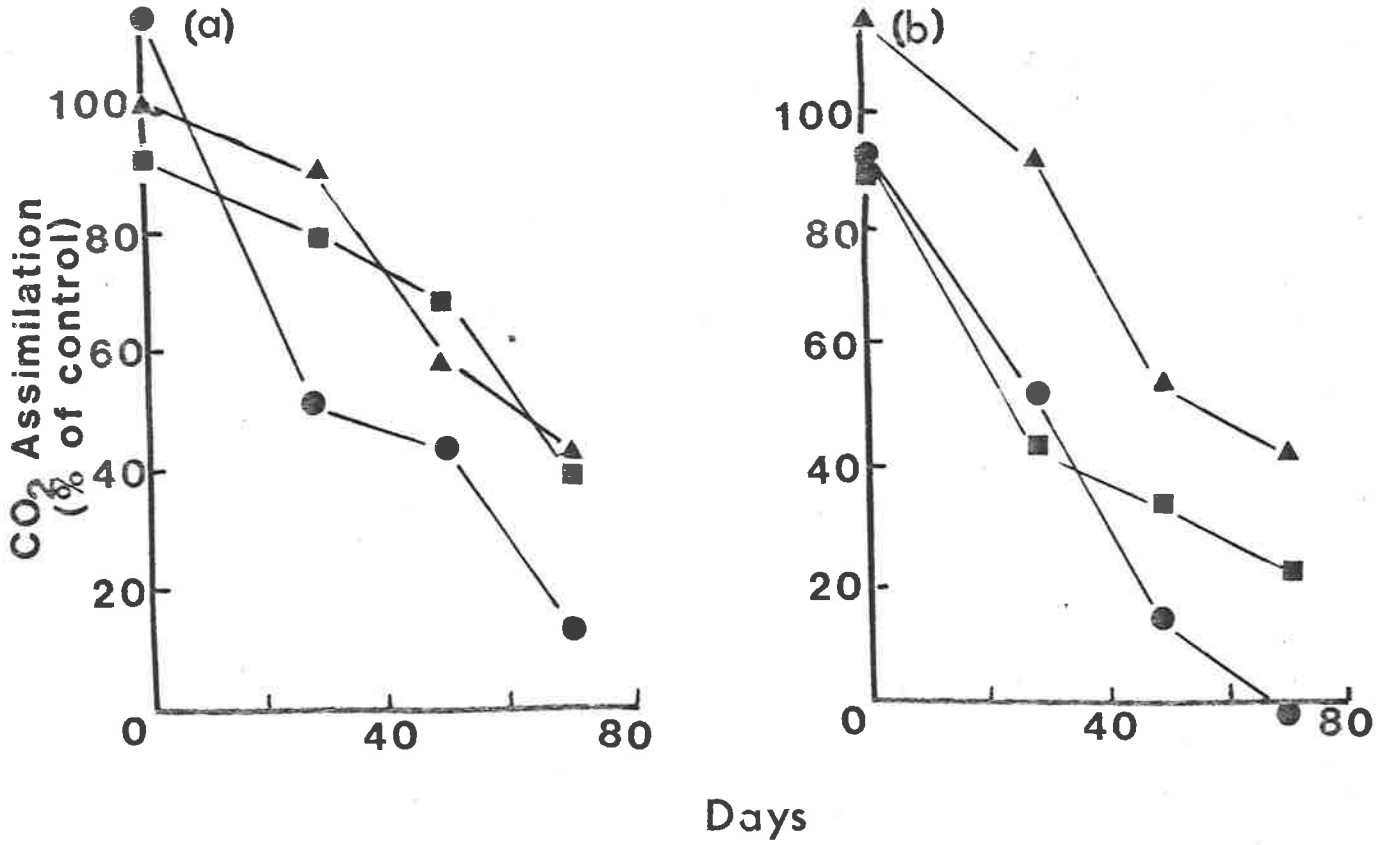


Figure 6.4 Relative gas exchange rates of leaves on plant supplied with dilute nutrient solution + 50 mol m-3 NaCl expressed as a percentage of those irrigated with dilute nutrient solution only. (▲) 'Valencia' orange; (■) 'Washington Navel' orange; (●) 'Marsh' grapefruit. (a) on Trifoliata; (b) on Cleopatra mandarin.

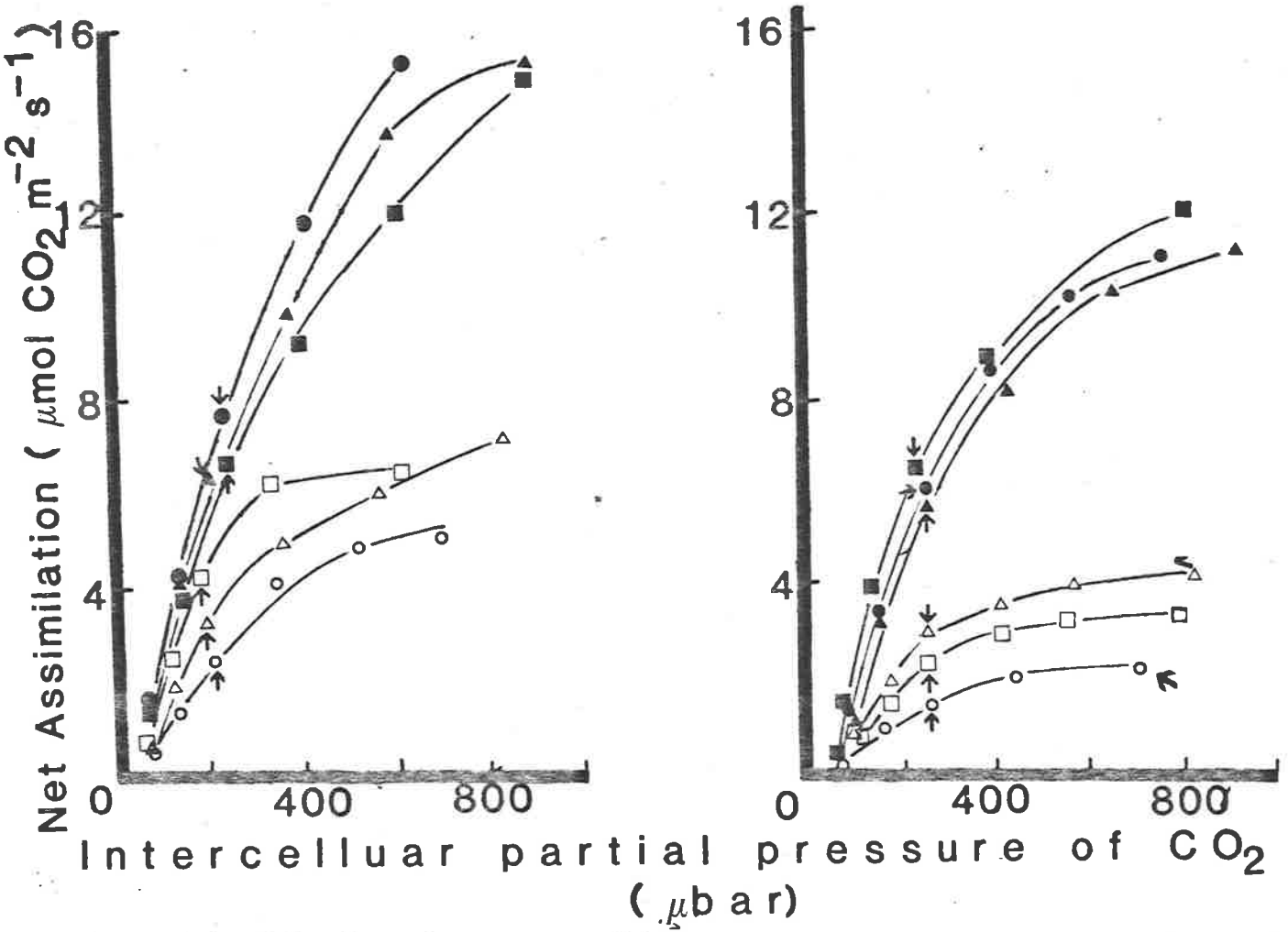


Figure 6.5 Gas exchange characteristics of citrus leaves in response to salinity. Open symbols; leaves supplied with dilute nutrient solution; Closed symbols; leaves supplied with dilute nutrient solution + 50 mol m-3 NaCl. (▲△) 'Valencia' orange; (■□) 'Washington Navel' orange; (●○) 'Marsh' Grapefruit. (a) on Trifoliata, (b) on Cleopatra mandarin. Arrows indicate point of operation at atmospheric condtions of 350 μbar CO_2. Leaves were sampled 49 days after commencement of salt treatment.

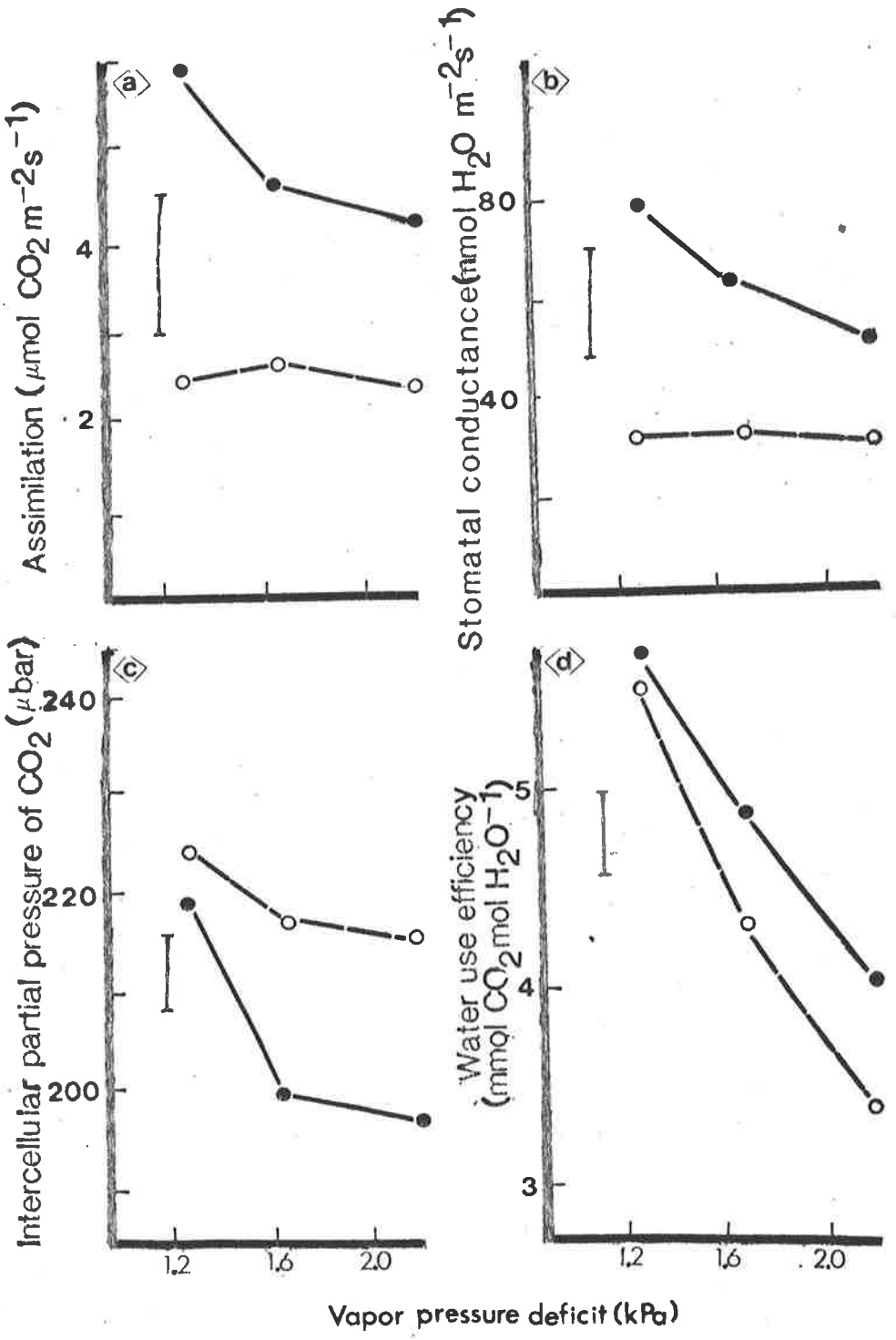


Figure 6.6 Gas exchange of 'Washington Navel' orange on Cleopatra mandarin rootstock in response to ambient vapor pressure deficit. (●) leaves from plants supplied with dilute nutrient solution; (○) leaves from plants supplied with dilute nutrient solution + 50 mol m-3 NaCl. (a) CO_2 assimilation, (b) stomatal conductance, (c) intercellular partial pressure of CO_2, (d) water use efficiency. Measurements were made 28 days after commencement of salinisation. Vertical bars represent the least significant difference (p = 0.05).

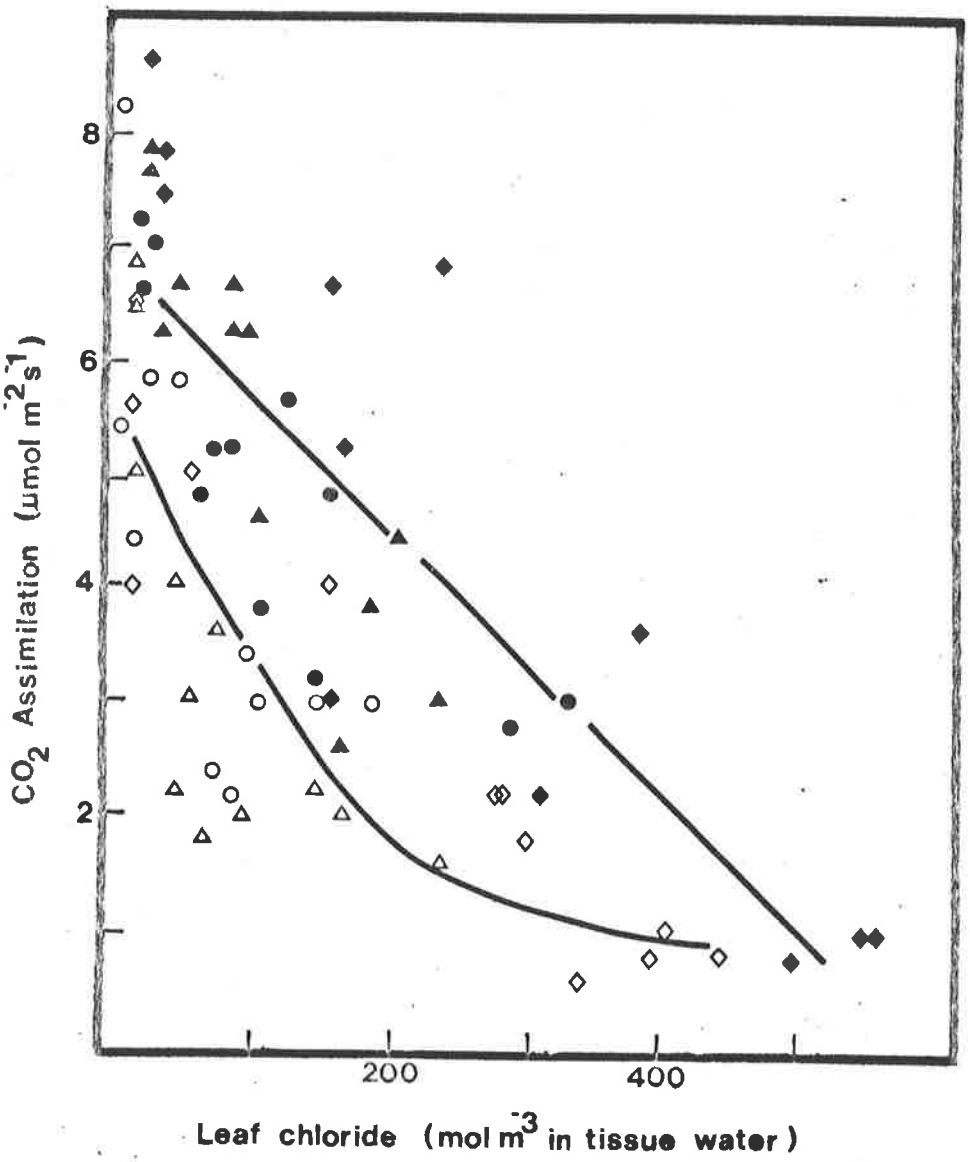


Figure 6.7 The relationship between leaf chloride concentration and CO_2 assimilation rate. Open symbols on Cleopatra mandarin; Closed symbols on Trifoliata. (●○) 'Valencia' orange; (▲△) 'Washington Navel' orange; (◆◇) 'Marsh' Grapefruit. Regression equations are listed in Table 6.8.

Rootstock	Scion	n[1]	FIT[2]	a	b	c	r[3]
Trifoliata	'Valencia' orange	12	L	6.3	1.4×10^{-2}	-	0.82
	'Washington Navel' orange	12	L	7.7	2.4×10^{-2}	-	0.89
	'Marsh' Grapefruit	12	L	8.0	1.5×10^{-2}	-	0.89
	Combined	36	L	6.8	1.4×10^{-2}	-	0.83
Cleopatra mandarin	'Valencia' orange	12	L	5.5	2.5×10^{-2}	-	0.64
	'Washington Navel' orange	12	P	6.0	6.9×10^{-2}	2.1×10^{-4}	0.80
	'Marsh' Grapefruit	12	L	5.3	1.4×10^{-2}	-	0.94
	Combined	36	P	5.3	3.0×10^{-2}	1.2×10^{-2}	0.77
Combined	Combined	72	L	5.6	1.2×10^{-2}	-	0.69

Table 6.8: Regression equations relating to CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1}) to leaf chloride concentration (mol m^{-3}). Equations fitted were either linear ($y = a-bx$) or polynomial ($y = a-bx + cx^2$). 1: number of observations; 2: L = linear; P = polynomial; 3: regression coefficient

Variates tested for Equality	Pooled Variates	n	F
Scions	Trifoliata	36	3.62 n.s.
Scions	Cleopatra	36	2.47 n.s.
Rootstocks	All 3 scions	72	20.44***

Table 6.9: F values for tests of regression line equality (Equations in Table 6.8) for scion responses to chloride on Trifoliata and Cleopatra mandarin rootstocks and for rootstock effect on response of all 3 scions to leaf chloride concentration.

This shows that for individual rootstocks there is no effect of scion on the relationship between CO_2 assimilation rate and leaf chloride concentration, but that rootstock affects the response of CO_2 assimilation to leaf chloride concentration when scions are pooled.

As shown in Figure 6.8 (regression equations in Table 6.10) we can also explain differences in scion sensitivity to salinity in terms of higher sodium levels in leaves of 'Marsh' grapefruit (Figure 6.1). In contrast to chloride however, the response does not segregate in two populations depending upon rootstock (Table 6.11).

Variates tested for equality	Pooled variates	n	F
Scions	Trifoliata	36	0.39 n.s.
Scions	Cleopatra	36	1.99 n.s.
Rootstocks	All 3 scions	72	1.08 n.s.

Table 6.11: F values for tests of regression line equality (equations in Table 6.10) for scion responses to sodium on Trifoliata and Cleopatra mandarin rootstocks and for rootstock effect on response of all 3 scions to leaf sodium concentration.

We can therefore explain both scion and rootstock effects on sensitivity of leaf CO_2 assimilation to salinity solely in terms of differences in leaf sodium concentrations.

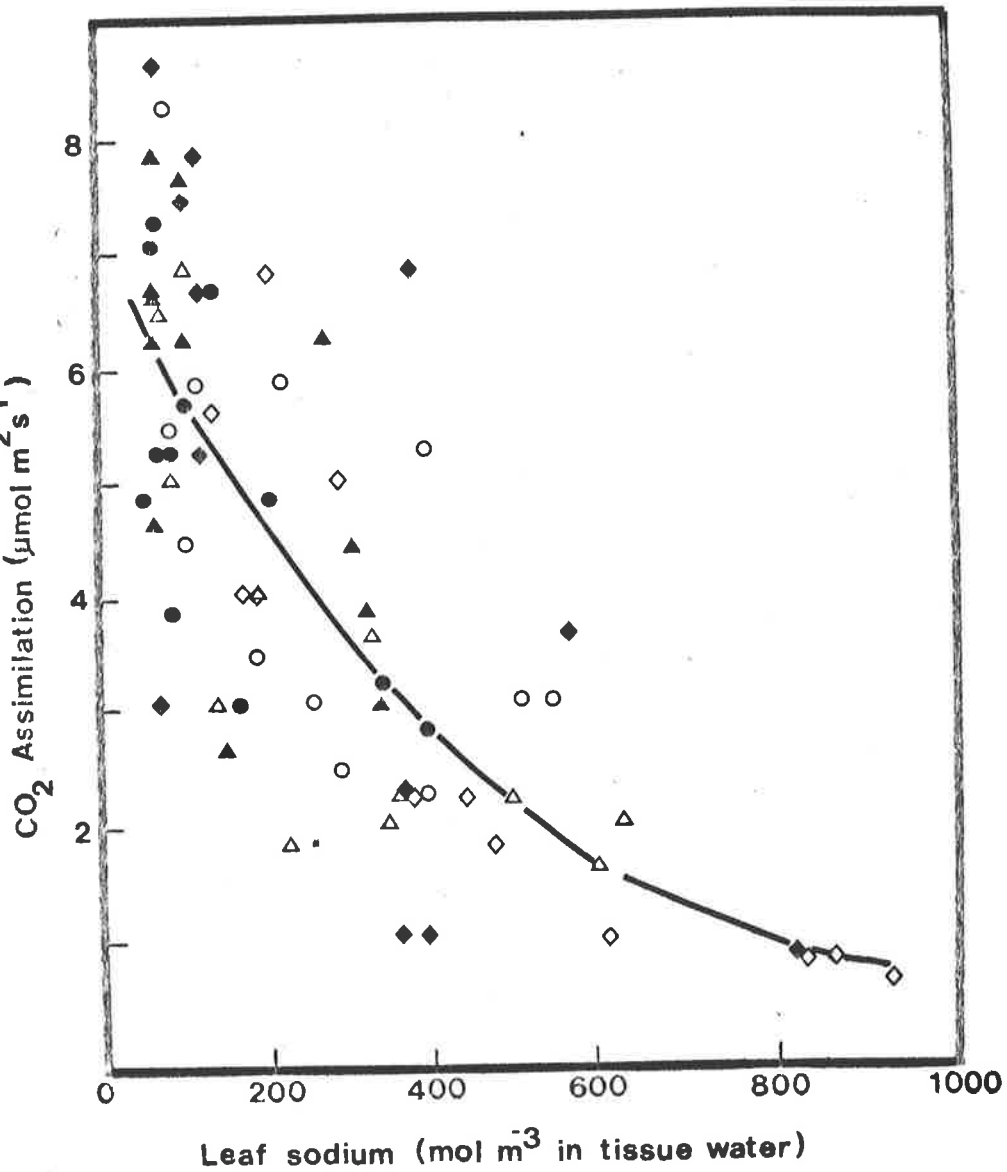


Figure 6.8 The relationship between leaf sodium concentration and CO_2 assimilation rate. Open symbols on Cleopatra mandarin; Closed symbols on Trifoliata. (●○) 'Valencia' orange; (▲△) 'Washington Navel' orange; (◆◇) 'Marsh' Grapefruit. Regression equations are listed in Table 6.10.

Rootstock	Scion	n[1]	FIT[2]	a	b	c	r[3]
Trifoliata	'Valencia' orange	12	L	6.0	1.0×10^{-2}	-	0.62
	'Washington Navel' orange	12	L	6.8	1.1×10^{-2}	-	0.61
	'Marsh' Grapefruit	12	L	6.6	9.2×10^{-3}	-	0.62
	Combined	36	L	6.3	9.1×10^{-3}	-	0.64
Cleopatra mandarin	'Valencia' orange	12	L	5.8	7.8×10^{-3}	-	0.56
	'Washington Navel' orange	12	P	6.8	2.5×10^{-2}	2.7×10^{-5}	0.82
	'Marsh' Grapefruit	12	P	7.4	1.8×10^{-2}	1.1×10^{-5}	0.88
	Combined	36	P	6.3	1.4×10^{-2}	8.0×10^{-6}	0.78
Combined	Combined	72	P	6.7	1.5×10^{-2}	8.4×10^{-6}	0.75

Table 6.10: Regression equations relating to CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1}) to leaf sodium concentration (mol m^{-3}). Equations fitted were either linear ($y = a-bx$) or polynomial ($y = a-bx + cx^2$). 1: number of observations; 2: L = linear; P = polynomial; 3: regression coefficient

6.4 DISCUSSION

Although 'Marsh' grapefruit leaves contained a smaller fraction of water than the orange varieties, (Table 6.2) this would have resulted in an increase in sodium or chloride concentration by only 20% over the orange varieties for a given amount of these ions (dry weight basis). Higher concentrations of sodium and chloride in leaves of this scion must therefore be also accountable in terms of differences in rates of sodium and chloride uptake, transport or storage within the budded tree. Causes for scion influences on leaf ionic composition may be related to shoot/root ratios (Table 6.1) as discussed in Chapter 5. A higher concentration of sodium in leaves of 'Marsh' grapefruit was anticipated (Cooper *et al.*, 1952a,b) but higher chloride concentration in leaves of this scion was unexpected as this has not been previously reported.

For control leaves, measurements of π from PV curves gave more negative values than when π was determined *via* psychrometry (Table 6.4). This was to be expected as cell water has a dilution effect on cytoplasmic solute concentration when π is determined by psychrometry on frozen and thawed leaves (Section 6.2.5). Somewhat surprisingly, calculated apoplastic water fractions were greater for unsalinised leaves on Trifoliata than on Cleopatra mandarin (Table 6.5). Whilst this may be a consequence of a rootstock effect on cell wall volume, it could also result from a greater concentration of solutes in apoplastic water for leaves on scions budded to Cleopatra mandarin reducing the dilution effect. Of all rootstocks, Cleopatra mandarin allows the greatest amount of calcium to enter the scion (Embleton *et al.*, 1973) and levels may be as high as 2 meq g^{-1} DW. Although much of this calcium may be in the form of oxalate crystals (Kelley and Cummins, 1920; Scott *et al.*, 1948) significant amounts may also be present in cell walls (Smith, 1975b). It may then be that a higher level of calcium in leaves of scions on Cleopatra mandarin makes a signifiant contribution to apoplast π and hence reduces the extent of apparent dilution of protoplasmic π by apoplastic water.

An increase in apoplastic π is most likely the cause of the apparent reduction in apoplastic water in salinised leaves of 'Marsh' grapefruit on Trifoliata (Table 6.5). Despite similar high sodium and chloride levels for leaves of Marsh' grapefruit on

Cleopatra mandarin, no increase in apoplastic π was detected using our method. Considerable calcium/sodium exchange may occur in the apoplast of salinised citrus leaves (Zid and Grignon, 1985) which may have masked an increase in sodium in the apoplast of leaf tissue on Cleopatra mandarin.

It has been hypthesised that under conditions of rapid salt influx into leaf tissue a build up of ions in the cell wall (Oertli, 1968) may cause a rapid loss of turgor and dehydration of cells (Munns and Passioura, 1984). If such a phenomenon were to occur in salinised citrus leaves, then a reduction in apparent apoplastic water should occur. This was, however, only observed for salinised leaves of 'Marsh' grapefruit and perhaps 'Washington Navel' orange on Trifoliata. These two rootstock/scion combinations were not the most sensitive to salinity stress and continued to exhibit reasonable rates of gas exchange for a further 21 days after π determinations indicating (as was also argued in previous chapters) that extracellular salt accumulation is not the cause of impaired gas exchange in citrus. It is possible however that leaf burn symptoms of ion excess when leaf margins become brown and dehydrated (Embleton *et al.*, 1968) are the consequence of such extracellular salt accumulation. Susbstantial reductions in gas exchange occur prior to such symptoms developing (Chapter 3) although some reduction in chlorophyll content may be observed in salinised leaves (Chapter 4).

The absence of extracellular solute accumulation and the maintenance of leaf turgor (Table 6.3) indicate, as in previous chapters, that reductions in rates of leaf gas exchange are most likely the consequence of a specific ion toxicity. Rootstock and scion influences on leaf gas exchange were evident even in the absence of salinisation with leaves of 'Valencia' orange and 'Marsh' grapefruit having higher CO_2 assimiltion rates when scions were budded to Trifoliata than Cleopatra mandarin. Furthermore, assimilation rates were greater for 'Marsh' grapefruit than 'Valencia' orange on both rootstocks (Table 6.7).

Rootstock effects on leaf gas exchange could be due to species differences in hydraulic conductivity as Syvertsen and Graham (1985) showed a positive correlation between both CO_2 assimilation and stomatal conductance with root hydraulic conductivity of rootstock seedlings. Interestingly, although both root conductivity and gas exchange

were higher for seedlings of Trifoliata than Cleopatra mandarin there was no effect of hydraulic conductivity on WUE and hence p_i. A correlation between hydraulic conductivity through the soil/root/leaf pathway and stomatal conductance was also shown for woody species by Küppers (1984) who demonstrated that this correlation was independent of Ψ. Syvertsen and Graham (1985) showed that leaf N and P levels were also correlated with plant hydraulic conductivity and hypothesised that improved nutrition in species with high conductivity may be responsible for greater rates of gas exchange.

There is evidence that stem hydraulic conductivities also vary between *Citrus* spp. (de Villiers, 1939). She found that the rate of water transport under head for constant time to be 2.7 times greater for grapefruit than 'Sour' orange. It is therefore possible that a higher stem hydraulic conductivity may in part account for greater CO_2 assimilation rates in 'Marsh' grapefruit.

In individual rootstock populations it is possible to explain the increased sensitivity of 'Marsh' grapefruit in terms of the higher leaf chloride concentrations in leaves of this scion. As was observed in Chapter 4, when CO_2 assimilation is plotted as a function of leaf chloride assimilation rates of leaves on Trifoliata appear to be less sensitive to chloride than those on Cleopatra mandarin (Figure 6.7). On the other hand, when CO_2 assimilation is expressed as a function of leaf sodium concentration all scion/rootstock combinations fall on the same line. Scion and rootstock effects on sensitivity of gas exchange to rootzone salinity may thus be explained in terms of differences in leaf sodium concentrations (Fig. 6.8).

The explanation that both scion and rootstock effects on citrus gas exchange are a consequence of their modulation of leaf sodium concentration is clearly the simplest. This explanation does not necessitate postulating differences in intrinsic sensitivity to toxic ions between scions (which is consistent with our analysis of such effects: Tables 6.9 and 6.11) nor does it require rootstock and scion effects on levels of other compounds which have been hypothesised to play a role in salt tolerance. We certainly could not explain rootstock and scion effects on salt sensitivity in terms of leaf prolinebetaine levels (Table 6.6). If we invoke a role for root derived compounds in modulation of leaf gas exchange

under salinisation then not only must rootstock effects be shown, but differences in scion responses to such compounds and/or a scion influence on tranpsort from and/or synthesis of such root derived compounds would have to be demonstrated. Such complex explanations are unnecessary as results presented in this chapter show that, despite efforts to do so, the correlation between CO_2 assimilation and leaf sodium concentration could not be broken.

There was, however, a substantial reduction in leaf gas exchange for 'Marsh' grapefruit on Trifoliata 28 days following commencement of salinisation in the absence of an increase in leaf sodium level. It therefore may be that, as was observed in Chaper 5, a rapid increase in leaf chloride concentration may cause reductions in gas exchange. Indeed, addition of a chloride term to the quadratic sodium equation for all rootstock/scion combinations increased the regression co-efficient from 0.75 to 0.78 (significant at P = 0.99).

We cannot, therefore, exclude an influence of leaf chloride concentration on gas exchange in this experiment, but will show in Part B of this thesis that such an effect does not occur in orchard trees irrigated with saline irrigation water.

PART B

ORCHARD OBSERVATIONS

"Although the figure of 27,000 pounds for the tolerance of citrus trees seems to place them high on the list, such tolerance actually only occurs in very sandy soils ... in the close textured lands trees hardly maintained life with more than 5,000 pounds of total salts"

(Hilgard, 1906)

CHAPTER SEVEN

PREAMBLE AND SITE DESCRIPTIONS

7.1 INTRODUCTION

Data from designed orchard experiments (Bielorai *et al.*, 1977, 1983; Cole, 1985) and analysis of yield/salinity relationships of commercial citrus plantings (Shalhevet *et al.*, 1974; Cole and McLeod, 1985) both show citrus yield to be highly sensitive to salinity. As for other crops (Maas and Hoffman, 1977) citrus yield response to salinity is curvilinear. This is conveniently approximated by a non-linear function indicating that up to a certain soil salinity level there is no yield reduction due to salinity. Once this critical threshold point is reached a negative and linear response to soil salinity is observed (Feinermann and Yaron, 1982; Cole, 1985). Estimates of the threshold soil salinity range from 4.3 mol m^{-3} NaCl (Cole, 1985) to 10 mol m^{-3} NaCl (Feinermann and Yaron, 1982).

Whilst the response of citrus to salinity can thus be anticipated in general terms, differences in irrigation management, soil type, stock/scion combination and climate necessitate specific field trials to accurately determine salinity/yield relationships for a given irrigation district. It is to this end that there are currently two citrus salt response trials in Southern Australia, one at Dareton (New South Wales Department of Agriculture) and the other at Loxton (South Australian Department of Agriculture). Both these trials were utilised in this study in order to expand on knowledge gained from laboratory investigations and determine the importance of lowered rates of gas exchange in reducing yield of trees irrigated with saline water.

7.2 SITE DESCRIPTIONS

Both sites are located on the Murray River, Loxton being ~150 km downstream from Dareton (Figure 7.1). Although trees at both sites are of equivalent age and there is little difference in salinity treatments, the two experimental orchards differ in terms of cultivars, soil type, and most importantly, yield response observed to date.

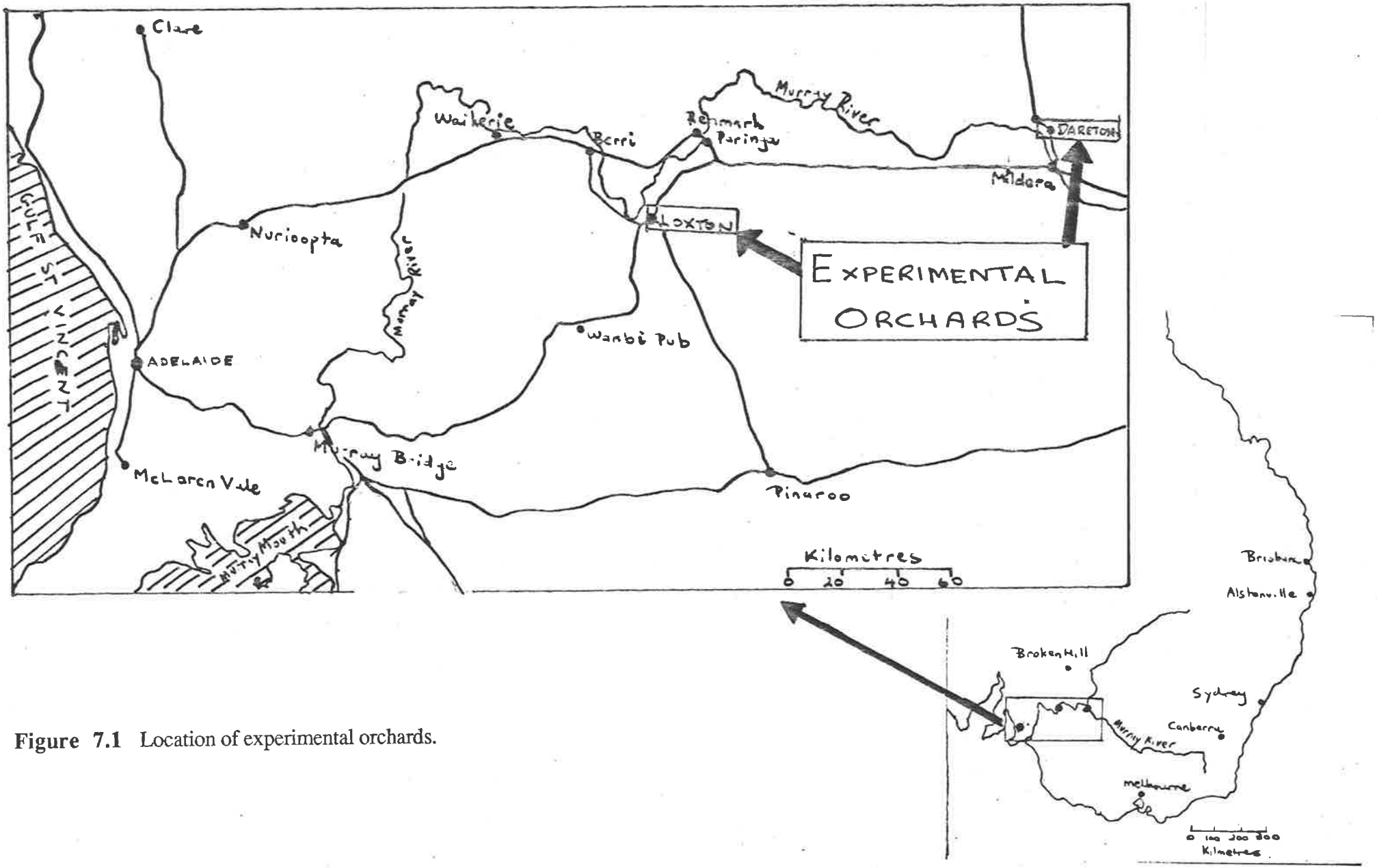


Figure 7.1 Location of experimental orchards.

7.2.1. Plant Material

The trial at Dareton utilises 'Valencia' orange on Sweet orange and Trifoliata rootstocks, whilst at Loxton 'Washington Navel' orange on Sweet orange serves as test material. Trees at both sites were planted around 1962. Prior to commencement of salinity treatments (Dareton, 1982; Loxton, 1981) trees at both sites had formed part of spacing trials.

7.2.2 Soil type

The two sites differ markedly in soil type. Whilst at Dareton trees are grown in a deep uniform sand with a calcerous layer at about 1 metre, the soil at Loxton is a light sandy clay loam and the calcerous layer is as high as 0.3 metres in places.

7.2.3 Chemical composition of irrigation water

Both sites utilise River Murray water as the lowest salinity treatment. This averages 4-5 mol m^{-3} NaCl. To this, additional salt is added giving 3 higher salinities *viz.* 10, 14 and 20 mol m^{-3} at Dareton and 11, 16 and 20 mol m^{-3} at Loxton. These figures are intended as a rough guide only. River salinity levels and exact amounts of NaCl added vary from irrigation to irrigation.

7.2.4 Irrigation scheduling

Schedulers at both sites utilise tensiometer readings to determine the date of irrigations. Water is applied when soil matric potential falls below ~ -1.5 kPa at Dareton and ~2.0 kPa at Loxton. Orchard characreristics are summarised in Table 7.1.

	DARETON	LOXTON
Scion	'Valencia' orange	'Washington Navel' orange
Rootstock	Sweet orange and Trifoliata	Sweet orange
Tree age (1986)	24 years	25 years
Trial commencement date	1982	1981
Soil type	Loamy sand	Light sandy clay loam
Irrigation water salinities (mole m-3 NaCl)	5,10,14,20	5,11,16,20
Irrigation scheduling method	Tensiometers	Tensiometers
Yield reduction at highest salinity (1985)	Nil (both rootstocks)	45%

Table 7.1: Soil and plant characteristics of citrus salt tolerance trials at Dareton and Loxton

7.2.5 Soil salinity profiles

7.2.5.1 Method of determination

At 1 and 2 metres from the tree trunk a 5 cm diameter auger was used to sample soil at intervals of 20 cm down the soil profile to a depth of 1 metre. Half the sample was removed for root distribution analysis (Section 7.2.6) whilst the other half was dried at 105°C for 48 hours. Saturation extracts were then made (Richards, 1954) and sodium and chloride concentrations in the extract determined. Mrs. K. Biggins (Loxton Research Centre) did the saturated paste extractions and ion determinations.

7.2.5.2 Results

Sodium and chloride concentration in soil profiles under one control tree and one tree irrigated with the highest salinity water at Loxton and Dareton (on Sweet orange) are shown in Table 7.2. In general, sodium and chloride concentrations reflected those of irrigation water (Table 7.1) indicating no signficant accumulation of salts in the soil at either site.

		Soil Depth Sampled (cm)				
		0-20	20-40	40-60	60-80	80-100
		Sodium (mol m^{-3})				
Control	Dareton	4.9	5.5	5.7	3.8	4.1
	Loxton	2.7	8.9	10.0	8.1	7.4
Salinised	Dareton	19.8	22.1	24.2	22.2	18.2
	Loxton	18.2	28.4	35.0	33.8	30.3
		Chloride (mol m^{-3})				
Control	Dareton	3.4	4.8	6.7	3.8	4.3
	Loxton	2.3	9.1	9.4	4.9	3.5
Salinised	Dareton	16.4	19.1	21.5	19.6	20.3
	Loxton	14.9	29.0	50.0	40.7	32.3

Table 7.2: Sodium and chloride concentrations in saturated extracts from soil samples to a depth of 1.0 metre at Dareton and Loxton in late summer. Control trees were irrigated with river water (~5 mol m^{-3} NaCl) whilst salinised trees received ~20 mol m^{-3} NaCl.

7.2.6 Root distributions

7.2.6.1 Methods

The sub-sample taken for root distributions was weighed and soil masses converted to volumes using previously determined bulk density measurements. After being separated from the soil bulk using a 2 mm sieve, remaining soil was removed from roots after which the roots were stored in 80% ethanol at room temperature until required. Root length for each sample was determined using the line intercept method (Tennant,

1975) with the aid of a Delta-T leaf area meter. The proportion of roots at each soil depth was multiplied by the sodium and chloride concentrations at that depth to yield a root-weighted soil salinity (i.e. a measure of the average ion concentration to which the roots were exposed).

7.2.6.2 Results

Control and high salinity trees at both sites (Sweet orange rootstock) had over 60% of their roots in the top 30 cm of soil (Table 7.3). Root weighted salinities are also presented in Table 7.3 for trees on Sweet orange rootstock at both sites. Although control trees at Loxton are exposed to slightly higher NaCl concentrations in the soil there is little difference between sites in the concentrations of sodium and chloride under trees exposed to the highest salinity treatment.

		Soil Depth Sampled (cm)				
		0-20	20-40	40-60	60-80	80-100
		Root Density (cm cm^{-3})				
Control	Dareton	2.22	0.29	0.49	0.37	0.17
	Loxton	0.30	0.58	0.04	0.00	0.00
Salinised	Dareton	1.59	0.33	0.38	0.19	0.09
	Loxton	1.14	0.32	0.17	0.07	0.00

		Root weighted soil water concentrations (mol m^{-3})	
		Sodium	Chloride
Control	Dareton	3.7	4.1
	Loxton	6.9	6.9
Salinised	Dareton	20.7	18.0
	Loxton	22.4	19.0

Table 7.3: Root densities to a depth of 1.0 metre at Dareton and Loxton in late summer and associated root weighted soil water concentrations. Control trees were irrigated with river water (~5 mol m^{-3} NaCl) whilst salinised trees received ~20 mol m^{-3} NaCl.

7.2.7 Canopy characteristics

7.2.7.1 Method

Canopy volumes were estimated from height and width measurements for 3 control trees and 3 high salinity trees on each rootstock at Dareton and 8 control and 8 high salinity trees at Loxton in early spring. Equations for prolate spheres (Turrell, 1948) were used for calculation as described by Jahn (1979). Leaf area was then estimated by counting all leaves within a 0.5 x 0.5 m2 sampling frame which was extended from canopy surface to trunk. The frame was placed into each canopy twice, about 1.5 m3 sampled per tree, at at height of 1.4 m. Total leaf area was then estimated for each volume sampled using previously calculated regression equations relating leaf number to area. Total leaf area per tree so determined was then divided by projected canopy area to estimate leaf area index (LAI).

7.2.7.2 Results

Despite equivalent canopy volumes in early spring, control trees of 'Washington Navel' orange on Sweet orange at Loxton had only ~50% the leaf area of 'Valencia' orange on Sweet orange at Dareton (Table 7.4). Salinisation reduced both canopy volume (due to twig dieback) and (to a greater extent) total leaf area in all cases, with salinised trees at Loxton having the lowest total leaf area and LAI. There was little effect of rootstock on canopy characteristics for either control or salinised trees at Dareton.

7.3 YIELD RESPONSE

Despite equivalent treatments in terms of tree age, rootstock, amount of water applied, chemical composition of irrigation water and soil salinities (Table 7.2, 7.3, 7.4) reductions in yield have been observed at Loxton but not at Dareton (Table 7.1). In Chapters 8 and 9 we investigate physiological responses to salinity at both sites. The emphasis was originally placed on leaf gas exchange (Chapter 8) but, as will become evident, it was found necessary to view leaf gas exchange in terms of some of the many

	DARETON				LOXTON	
	Trifoliata		Sweet Orange		Sweet Orange	
	Control	Salinised	Control	Salinised	Control	Salinised
Canopy Volume (m^3)	49.8	41.5	44.1	30.5	43.5	31.5
Leaf area (m^2)	151.5	68.4	148.6	50.2	87.6	35.3
Leaf area index (LAI)	9.0	4.9	11.0	3.6	4.0	1.8

Table 7.4: Canopy volume, leaf area and leaf area index of control (irrigated with ~ 4 mol m^{-3} NaCl and salinised (irrigated with ~20 mol m^{-3} NaCl) trees at Dareton and Loxton in early spring, 1986. Dareton: n=3; Loxton: n=8.

other physiological processes contributing to final yield in mature heavily bearing commercial orchard trees.

CHAPTER EIGHT

RESPONSE OF ORCHARD 'VALENCIA' ORANGE TO HIGH SALINITY IRRIGATION WATER AND SOIL WATER DEFICIT

8.1 INTRODUCTION

Whilst laboratory studies may impart considerable knowledge in terms of physiological consequences of high levels of toxic ions in citrus foliage and rootstock (and scion) modulation of foliar sodium and chloride concentrations (Part A) such studies are not immune to criticism. This is because the relatively high concentrations of NaCl utilised over days or weeks could have severe effects on the limited root system of potted plants. In orchard practice a much larger root system normally encounters lower concentrations of NaCl over many years. Consequently, salt impact on scion might vary accordingly. Research described in this chapter was therefore undertaken in order to study prolonged effects of relatively low salinity levels and slow imposition of soil water deficits on leaf water relations and net gas exchange of the fully mature 'Valencia' orange trees under orchard conditions at Dareton.

8.2 MATERIALS AND METHODS

Test trees were located at Dareton. Tree descriptions, soil type and irrigation water characteristics are described in Chapter 7.

8.2.1 Treatments

Other than the salinity treatments, trees received normal horticultural care. Nitrogen, phosphorus and potassium (seasonal totals of 137, 39 and 40 kg/ha, respectively) had been routinely applied to all trees through an under tree microsprinkler irrigation system in five applications. Following a spring sampling period (November, 1985) described below, drought stress was imposed on a total of 8 trees by shutting off the sprinklers under two trees of both rootstocks in the river water and highest salinity irrigation treatments for 2 months during summer (November and December).

8.2.2 Water relations

Sixteen trees were tagged for study in the spring: 2 trees on both Trifoliata and Sweet orange rootstock in each of the 4 salinity treatments. Fully expanded but not yet

hardened spring-flush leaves about 2-months old (young) and overwintered leaves at least 6-months old (mature) were sampled from northwestern sun-exposed portions of the canopy. Four replicate leaves, two leaves of each age from each of the tagged trees, were sampled before dawn for bulk leaf water potentials (ψ) using a pressure chamber. Osmotic potentials (π) were derived from pressure volume techniques (Section 2.2.3.6) and predawn leaf turgor potential (P) was calculated by difference from ψ and π.

During summer (January), all measurements were made on a total of 16 trees: 2 trees on each rootstock, droughted and well-watered, and from river water and highest salinity treated trees. Diurnal values of ψ were measured at various times throught a sampling day using 4 mature leaves (2 from each tree) at each measurement time. Predawn π and P were determined as before using leaves samples prior to first light.

Soil water was determined at depths of 30 and 60 cm half way between the trunk and dripline of the canopy of each tree used for study in summer. Soil samples were weighed, dried and the percentage water expressed on a volumetric basis using bulk densities supplied by Ms L. Prior (N.S.W. Department of Agriculture).

8.2.3 Net gas exchange

Leaf gas exchange was measured between 10:00-12:00 h and 16:00-18:00 h over several days during the spring sampling period using a Li-Cor 6000 portable photosynthesis system equipped with a 4 litre chamber. PPFD was above 800 μmol m^{-2} s^{-1} while leaf temperatures were 27-30°C on measurement leaves. Average vapor pressure deficit (VPD) was 1.4 kPa and varied little within or between days during the sampling times.

Difficulty was experienced in obtaining adequate CO_2 depletion rates in this closed system using single leaves in the 4 litre chamber. Volume of the chamber was reduced to 2 litre to enhance CO_2 depletion rates by closing off the top half with propafilm, the CO_2 impermeable material supplied by Li-Cor. To provide a larger volume of uniformly illuminated tissue, three detached leaves were enclosed for each measurement. Several tests showed that we could enclose detached leaves and repeat three consecutive measurements of net CO_2

assimilation rate that did not differ from each other by more than 5% over a 5 min period. This was done by opening the chamber to ambient air between measurements. Measured rates approximated those using attached leaves. Stomatal conductance, transpiration rate and CO_2 assimilation rate were used to calculate the intercellular partial presure of CO_2 (p_i , Equation 1.5). Ambient CO_2 concentrations of about 340 μbar normally decreased to as low as 280 μbar bar^{-1} during the 50 sec measurement period. Each gas exchange measurement was replicated four times using 2 three-leaf samples of young and mature leaves from each study tree in the spring. During summer, net gas exchange characteristics were evaluated similarly over several days using mature leaves on the droughted and well-watered trees; VPD averaged 3.0 kPa.

8.2.4 Ion and chlorophyll analysis

In spring, four replicate leaves, two leaves of each age from each tagged tree were sampled for sodium, chloride and potassium content. Leaves were removed and dried at 70°C for at least 48 h. Midribs were removed and laminae were ground to a fine powder in a mortar and pestle prior to anlaysis for total chlorides using a Buchler-Cotlove chloridometer. Sodium and potassium were assayed in the same leaf samples after hot nitric acid digestion using atomic absorption spectroscopy. All ion data were expressed on a cell sap concentration basis.

Total leaf chlorophyll was also analysed (Moran and Porath, 1980) in the spring using 2 x 1 cm discs from four replicate leaves sampled identically as those sampled for ion analysis. During summer, four replicate mature leaves from the droughted and well-watered, 4 and 20 mol m^{-3} Na Cl treated trees were sampled similarly for ion and total chlorophyll content.

8.3 RESULTS

8.3.1 Foliar ions and chlorophyll

Foliar chlorides during spring generally increased with salinity content of irrigation water (Table 8.1). Mature leaves had several fold higher chloride levels than young leaves. Mature leaves on Trifoliata rootstock irrigated with high salinity water had almost twice the concentration as those on Sweet orange rootstock treated with the same water. Levels of sodium were low on all leaves sampled except for mature leaves from trees on Trifoliata rootstock at the highest salinity in which levels were still below concentrations considered to be toxic (about 55 mol m^{-3}, Smith, 1966). Salinity had no effect on potassium in young leaves. Mature leaves on both rootstocks had significant reductions in potassium to sub-optimal or deficient levels (about 90 mol m^{-3}, Smith, 1966) in all three enhanced salinity treatments in the spring and in the highest salinity level in summer. During summer, sodium levels of mature leaves on trees treated with the highest salinity water were significantly higher than 2 months before (Table 8.1). Irrigation with the highest salinity water in summer apparently exceeded the ability of Trifoliata rootstock to exclude sodium whereas leaves on Sweet orange rootstock only accumulated high levels of sodium after drought stress.

Total leaf chlorophyll per unit area increased with leaf maturity (Table 8.2) as leaves became thicker. Irrigation with high salinity water reduced chlorophyll content in well-watered mature leaves in the summer period but this difference did not exist in droughted leaves.

Season	Spring				Summer (Mature)			
Leaf age/ Drought	Young		Mature		Well-watered		Droughted	
Rootstock	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt
Na Cl (mol m^{-3})	Chlorophyll (µg cm^{-2})							
5	46.2	41.3	69.2	77.2	68.7	65.5	68.3	66.4
20	47.5	42.0	64.3	71.2	52.3	49.8	68.3	62.3

Table 8.2: Effect of leaf age, drought, rootstock and salinity on mean (n = 4) total chlorophyll (µg cm^{-2}) of Valencia leaves during spring and summer (LSD, P=0.05 = 9.7). Tri = Trifoliata rootstock: Swt = Sweet orange rootstock

Spring

Ion	Chloride				Sodium				Potassium			
Leaf age	Young		Mature		Young		Mature		Young		Mature	
Rootstock	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt
NaCl (mol m^{-3})												
5	33	3	151	20	5	2	9	14	159	141	128	200
10	65	39	372	61	2	2	8	15	115	127	74	139
14	43	39	212	85	2	2	9	12	136	122	72	90
20	85	77	396	196	2	5	39	9	195	155	67	63
LSD (p = 0.05)[1]	24	25	85	53	2	3	15	7	70	40	28	51

Summer (Mature)

Ion	Chloride				Sodium				Potassium			
Leaf age	Well-watered		Droughted		Well-watered		Droughted		Well-watered		Droughted	
Rootstock	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt
NaCl (mol m^{-3})												
5	92	15	85	17	4	4	4	10	205	214	183	208
20	231	197	157	107	82	26	61	59	148	93	130	110
LSD (p = 0.05)[1]	82	47	60	43	9	16	17	36	68	51	42	60

Table 8.1: Effect of leaf age, rootstock, drought and average NaCl concentration in irrigation water on mean (n=4) chloride, sodium and potassium ion content (mol m^{-3}) of 2-month-old (young) or 6-month-old (mature) 'Valencia' leaves on Trifoliata (Tri) or Sweet orange (Swt) rootstocks during spring or summer. [1]Least significant difference.

8.3.2 Water relations

Osmotic potentials (π) generally decreased (became more negative) as leaves matured but responses to salinity were rootstock-dependent (Table 8.3). During spring, π decreased with increasing salinity for mature leaves from trees on Sweet orange, but not for mature leaves on Trifoliata. When sampled 2 months later, π was significantly lower for well-watered trees at the higher salinity level. Drought stress also lowered π but salinity plus drought stress showed no additional effect on π (Table 8.3).

Trees previously irrigated with river water depleted soil moisture to lower levels than those that had been irrigated with water containing 20 mol m^{-3} NaCl (Table 8.4) even though duration of drought stress was identical for both treatments. There was no significant difference in percent soil moisture associated with the different rootstocks so data have been combined. Drought effects on percent soil moisture were reflected in the signficantly lower predawn and midday leaf water potentials (Ψ) in drought stressed trees (Fig. 8.1). Although treatment with high salinity water generally decreased morning Ψ, leaves on well-watered Trifoliata rootstocks treated with high salinity water had the highest afternoon Ψ. Leaves on droughted Trifoliata rootstock previously treated with highest salinity water, however, had the lowest afternoon values. Irrigation with the higher salinity had no effect on afternoon Ψ of leaves on Sweet orange rootstock even within the subsequent drought stress treatment. Leaves on droughted high salinity Sweet orange trees, however, recovered in the evening to values approaching predawn levels. Little recovery was evident for leaves on droughted Sweet orange trees previously irrigated with river water. Calculated pre-dawn turgor potentials (range = 1.2 to 1.8 MPa) did not differ significantly between rootstocks or between drought vs. well-watered treatments.

Season	*Spring*				*Summer (Mature)*			
Leaf age/Drought	Young		Mature		Well-watered		Droughted	
Rootstock	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt
NaCl (mol m^{-3})								
5	-1.76 a	-1.84 a	-2.43 c	-1.94 ab	-1.93 ab	-1.90 ab	-2.16 c	-2.27 c
10	-1.69 a	-1.72 a	-2.62 c	-2.36 b	--	--	--	--
14	-1.88 a	-1.78 a	-2.56 b	-2.38 b	--	--	--	--
20	-2.03 b	-1.81 a	-2.30 c	-2.80 e	-2.40 c	-2.23 bc	-2.39 bc	-2.47 cd
LSD (p = 0.05)[1]	0.10	0.11	0.31	0.24	0.21	0.26	0.21	0.22

Table 8.3: Effect of leaf age, drought stress, rootstock and salinity on predawn osmotic potential (MPa) of Valencia leaves on Trifoliata (Tri) and Sweet orange (Swt) during spring and summer. Mean values (n=4) within a row followed by unlike letters differ significantly (p = 0.05).
[1]Least significant difference.

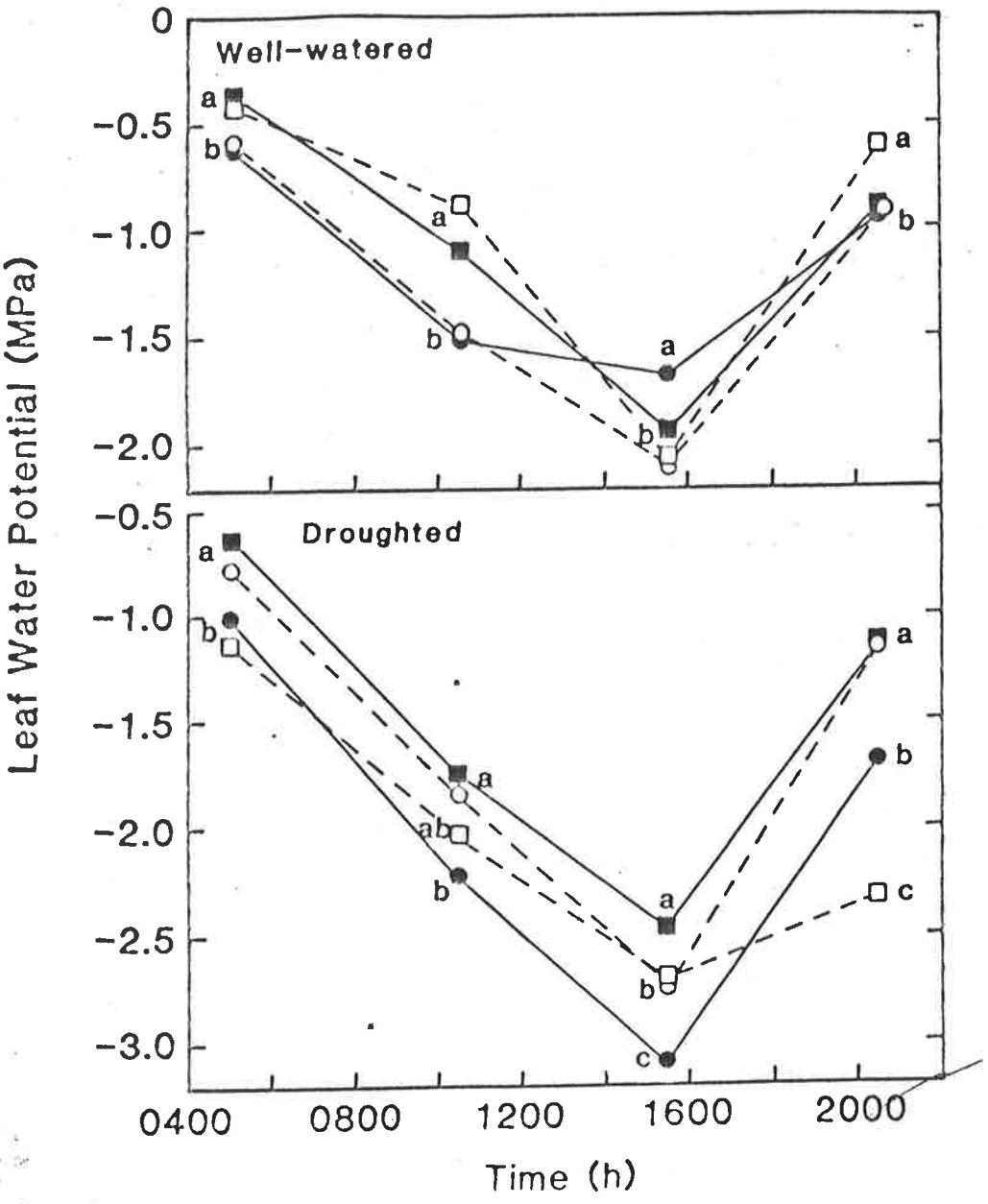


Figure 8.1 Diurnal leaf water potentials of well watered and droughted mature 'Valencia' orange trees on Trifoliata (Tri: solid symbols) and Sweet orange (Swt: open symbols) rootstock during summer. (■ □) trees irrigated with 5 mol m^{-3} NaCl; (●○) trees irrigated with 20 mol m^{-3} NaCl (n=4). Unlike letters denote significant differences (p=0.05).

Soil depth (cm)	Well-watered 30	60	Droughted 30	60
Na Cl (mol m^{-3})	Volumetric water content (%)			
5	7.3	9.0	4.0	4.4
20	14.8	14.4	6.2	6.1

Table 8.4: Effect of drought stress and salinity on mean (n=4) volumetric water content at depths of 30 and 60 cm midway between the trunk and canopy dripline during summer (LSD, p=0.05 = 0.6)

8.3.3 Gas exchange

During spring, there was no effect of salinity treatment on net gas exchange characteristics for leaves from trees on Trifoliata whereas CO_2 assimilation was actually enhanced for young leaves on salinised Sweet orange (Table 8.5). Higher values of CO_2 assimilation were accompanied by increases in stomatal conductance so that p_i showed no difference between treatments. Similarly, gas exchange characteristics of mature leaves were also unaffected by salinity. Mature leaves had lower stomatal conductances and p_i than young leaves regardless of salinity treatment or rootstock (Table 8.5). Soil water deficit resulted in larger reductions in CO_2 assimilation and stomatal conductance on Sweet orange than on Trifoliata. When drought stress was superimposed on salinity, tree response varied according to previous salinisation impact. Trees that were partially defoliated by salt stress (Table 7.4) showed smaller reductions in CO_2 assimilation and stomatal conductance in response to drought than non-salinised trees. There was no obvious rootstock effect on the drought response of salinised trees.

8.4 DISCUSSION

Although salinity levels in this study were lower than in many laboratory studies, 20 mol m^{-3} NaCl exceeds concentrations previously shown to debilitate orchard trees (Shalhevet *et al.*, 1974; Cole and McLeod, 1985). Present trees treated with such saline water had reduced canopy densities due to either increased abscission and/or reduced growth of new leaves. Thus, it is important to note that we may have been studying

Season	*Spring*				*Summer*				
Leaf age/	Young		Mature		Well-watered		Droughted		LSD[1]
Drought Rootstock	Tri	Swt	Tri	Swt	Tri	Swt	Tri	Swt	(p = 0.05)
NaCl (mol m^{-3})									
					g_s (mmol m^{-2} s^{-1})				
5	92	94	44	39	62	49	51	26	20
20	86	136	42	50	47	53	32	37	28
					A (µmol m^{-2} s^{-1})				
5	5.1	5.4	4.2	3.9	4.9	4.4	5.0	2.4	0.9
20	4.5	7.1	4.1	4.3	4.3	4.2	3.2	3.4	1.0
					p_i (µbar)				
5	224	216	166	156	182	180	155	185	26
20	231	231	160	178	174	188	168	185	22

Table 8.5: Effect of leaf age, drought, rootstock and salinity on mean (n=4) stomatal conductance (g_s), CO_2 assimilation (A) and internal CO_2 concentration (p_i) of Valencia leaves on Trifoliata (Tri) and Sweet orange during spring or summer.
[1]Least significant difference.

effects of salinity and drought stress on physiological responses of surviving leaves.

Foliar ion contents of salinity-stressed trees were dependent upon rootstock and age of leaves. Based on previous data of chloride accumulation in leaves of scions grafted on these two rootstocks, trees on Sweet orange rootstock are considered moderately salt-tolerant whereas those on Trifoliata are considered salt-sensitive (Cooper and Gorton, 1952; Wutscher, 1979; Grieve and Walker, 1983; Walker *et al.*, 1983). In spring, mature leaves from trees on Trifoliata rootstock had much higher levels of chloride than those from trees on Sweet orange at all salinities. This greater chloride accumulation had not however resulted in greater defoliation of trees on Trifoliata than of trees on Sweet orange after 3 years of salinisation. It is possible that leaves on Trifoliata rootstock can tolerate higher levels of chloride than leaves on Sweet orange rootstock, but more likely, defoliation may be more closely related to foliar sodium content.

Springtime foliar sodium levels at Dareton were generally low with one exception: mature leaves on Trifoliata rootstock exposed to high salinity. This observation contrasts with previously reported sodium excluding ability of Trifoliata rootstock in short term studies of containerised trees (Chapters 4, 5, 6; Walker, 1986). By summer, the cumulative impact of the high salinity treatment over preceding years may have exceeded the sodium excluding limits of Trifoliata.

Potassium levels were lower in mature leaves than spring flush leaves on both rootstocks (Table 8.1). Retranslocation of potassium from older to younger leaves coupled with progressive replacement of potassium by sodium is a common feature under salinity stress for both glycophytes (Greenway and Pitman, 1965) and halophytes (Yeo, 1981; Jeschke, 1982). The ionic composition of these leaves may reflect the composition of the phloem sap (Webb and Gorham, 1964) which is rich in potassium (MacRobbie, 1971) even under saline conditions when sodium can replace potassium (Delane *et al.*, 1982). Leaves of well-watered trees receiving high salinity water had higher sodium and lower potassium levels in summer than the previous spring. Higher levels of sodium in these leaves could have been a consequence of higher summer transpiration rates resulting in increased fluxes of water and sodium through the plant system under conditions of

high VPD (Pitman, 1982). A smaller increase of foliar ion concentrations in treated leaves on high salinity Trifoliata rootstock and of chloride in leaves on Sweet orange rootstock after drought stress supports this explanation.

Higher ion contents of salinised trees were not always accompanied by a decrease in π. For example, mature leaves in spring of trees on Trifoliata exposed to high salt water did not have significantly different π compared to those exposed to river water despite 2.6 fold higher chloride concentrations (Table 8.1). A similar phenomenon was observed by Walker *et al.* (1982) for Etrog citron seedling which also accumulate high levels of chloride but not sodium. During summer, however, higher sodium and chloride contents of leaves of the highest salinity trees were correlated to lower π. Drought stress also led to a reduction in π in trees irrigated with river water that was comparable to that of high salt trees. Some seasonal osmotic adustment occurred in response to drought but this was only 0.3-0.4 MPa and confirms the limited ability of citrus to osmotically adjust (Syvertsen and Albrigo, 1980; Syvertsen *et al.*, 1981; Chapters 2 and 3). The decrease in π as young leaves matured, was similar to the reduction in π of mature leaves in response to salinity and/or drought. This resulted in maintenance of leaf turgor at about the same level in mature leaves regardless of salinity or drought stress. Smaller LAI of salt stressed trees could have made a further contribution to turgor maintenance via a decrease in shoot/root ratio and hence reduced transpirational demand.

Increased foliar sodium (Table 8.1) was associated with a decrease in stomatal conductance for leaves on both rootstocks (Table 8.5). Despite high chloride levels (up to 396 mol m^{-3}) in mature leaves in spring, net gas exchange was not impaired indicating that high chloride concentrations have little impact on 'Valencia' orange leaf photosynthesis providing turgor is maintained (Chapter 4). Indeed irrigation with high salinity water enhanced both CO_2 assimilation and stomatal conductance of young leaves from trees on Sweet orange in the spring and of droughted leaves from trees on Sweet orange in the summer. Behboudian *et al.* (1986) reported such an increase in the laboratory for leaves on Cleopatra mandarin, but not on Trifoliata. Notwithstanding a lack of information regarding sodium and chloride levels in citrus leaves that constitute

deficiency (Smith, 1966), alleviation of sodium and chloride deficiency could explain this response. The generally low concentrations of sodium in leaves on trees irrigated with river water and low levels of chloride in young leaves on Sweet orange rootstock treated with river water are an order of magnitude below optimum levels for citrus (Smith, 1966). It is, therefore, possible that salinisation stimulates physiological responses in remaining leaves on trees grafted to salt excluding rootstocks.

Lower p_i in mature leaves than in young leaves in spring (Table 8.6) was repeated as leaves matured from spring to summer. All changes in CO_2 assimilation and stomatal conductance were of similar magnitude resulting in no significant effects of salinity, rootstock or drought on p_i.

Net gas exchange of leaves from trees on Sweet orange that had previously been exposed to high salinity water responded less dramatically to drought than did those on Sweet orange rootstock irrigated with river water (Table 8.5). In addition, evening recovery of Ψ was much more rapid in leaves on Sweet orange rootstock exposed to the higher salinity. This was probably because of fewer leaves on the more highly salinised trees (Chapter 7) and a greater amount of soil water remaining (Table 8.4). Although values for well-watered trees are potentially complicated by differences in the timing of irrigations, it is clear that trees receiving river water had depleted soil water to a greater extent after 2 months of drought than those which had previously received high salt water. This cannot be accounted for by differences in the gas exchange of individual leaves (Table 8.5) and thus is more likely a consequence of salinised trees having reduced leaf areas.

When trees previously irrigated with river water were drought-stressed, rates of gas exchange were significantly higher for leaves on Trifoliata rootstock than for those on Sweet orange (Table 8.5). This was despite the fact that rootstock exerted only a small influence on leaf water potential (Figure 8.1). Trees on Sweet orange rootstock tend to be relatively susceptible to drought stress (Wutscher, 1979); whereas, leaves of trees on Trifoliata rootstock can have relatively high gas exchange rates (Sinclair and Allen, 1982; Chapter 6). Based solely on the degree of defoliation, leaf water relations and net gas

exchange, there appears to be little advantage of using Sweet orange rather than Trifoliata as a rootsock under the salinity conditions of this study. The consistently higher sodium content of mature leaves on Trifoliata rootstock, however, implicates potentially worse sodium toxicity effects than in trees on Sweet orange rootstock at least under well-watered saline conditions. Further, recent observations show that trees on Trifoliata rootstock have suffered larger salinity-induced reductions of fruit yield than those on Sweet orange rootstock (A. Grieve, pers. comm.).

There appears to be little evidence of any compounding effect between salinity and subsequent drought stress on surviving leaves although both stresses could have hastened rates of leaf maturation and abscission. Since leaf chlorophyll content in remaining leaves was only reduced by salinity under well-watered condtions, drought-induced reductions in water uptake may reduce the extent of foliar ion accumulation and leaf injury. Important responses of citrus trees to stress include reduced rates of production of new leaves, enhanced rates of leaf maturation (Syvertsen, 1982, 1985) and earlier abscission (Goell, 1969; Kaufmann, 1977) as supported by leaf area determinations (Chapter 7). Thus, for orchard trees, a reduction in leaf area resulting in a more favorable shoot/root ratio could have more important implications for productivity and whole tree water relations than would lower rates of leaf gas exchange. We evaluate the relative importance of leaf abscission and leaf gas exchange in reducing yields for the more severely debilitated trees at Loxton in the following chapter.

CHAPTER NINE

SO ... WHY IS CITRUS YIELD REDUCED BY SALINITY?

9.1 INTRODUCTION

Little inhibition of leaf gas exchange was observed in the Dareton study (Chapter 8) and it must also be emphasised that, despite the presence of a much lower leaf area in salinised trees (Table 7.4), no appreciable abscission of leaves from salinised trees was observed during the sampling period (November-January). However, increases in leaf sodium and chloride concentrations between November and January (Table 8.2) were observed. It was therefore possible that a continual increase in sodium and chloride concentrations as leaves age may result in appreciable abscission (and perhaps lowered rates of gas exchange) later in the growing season. It could then have been that in the experiment described in Chapter 8 we were sampling prior to development of any salt injury symptoms.

In this chapter we explore this possibility using as experimental material 'Washington Navel' on Sweet orange within the tolerance trial at Loxton. Control trees were somewhat less vigorous than those and Dareton (Table 7.4) and (in contrast to 'Valencia' orange on Sweet orange at Dareton) salinisation had reduced yields in the highest salinity treatment (Table 7.1). In order to relate leaf gas exchange characteristics to observed yield responses we measured rates of production and abscission of leaves and flowers, fruit growth and leaf carbohydrate status in addition to leaf ionic content, water relations and gas exchange at one to two month intervals over a 12 month period (October-September). Prior to presentation of results a discussion of the yearly cycle of vegetative and reproductive growth in citrus trees is therefore warranted.

9.1.1 Periodicity of growth

Although in tropical climates characterised by no cool periods and ample soil moisture *Citrus* spp. may produce flowers in any month of the year with up to 8 reproductive flushes annually (Reuther and Rios-Castanto, 1969), in subtropical and temperate climates floral differentiation only occurs in mid to late winter (Abbott, 1935: Florida; West and Barnard, 1935: Griffith, [Australia]; Ayalon and Monselise, 1960: Israel; Lord and Eckart, 1985: California). Floral development then proceeds for

approximately 2-3 months until anthesis in mid spring (Lord and Eckart, 1985). Most new vegetative shoots are also produced during spring in such climates (Cooper *et al.*, 1963). Growth flushes in spring can thus be classified into 3 main types (Sauer, 1951):

(1) Vegetative (leaves)

(2) Reproductive (flowers)

(3) Mixed (leaves and flowers)

Minor vegetative flushes may also occur at any time of the year (Cooper *et al.*, 1963). Much less is known about root growth but under Florida conditions, Bevington and Castle (1985) reported root growth to occur as a series of distinct flushes with both the number of roots growing and extension rate being greatest in autumn.

9.1.2 Floral differentiation

As for most woody plants (Jackson and Sweet, 1972) citrus bud primordia are vegetative prior to floral differentiation (Abbott, 1935; West and Barnard, 1935). Several factors have been reported to influence the proportion of vegetative buds undergoing partial differentiation yielding mixed flushes or complete differentiation to reproductive flushes.

Reduced levels of gibberellins appears to be one prerequisite for flower formation in citrus (Goldschmidt and Monselise, 1972; Guardiola *et al.*, 1982) with exogenous applications of GA_3 recommended for prevention of heavy flower formation where this is not desired (Moss *et al.*, 1981). On the other hand, carbohydrate levels have also been suggested as a limiting factor for flower formation (Ogaki *et al.*, 1963; Goldschmidt and Golomb, 1982). Studies of flowering intensity in relation to environmental conditions have failed to elucidate the relative importance of plant growth regulators *versus* carbohydrate status in regulating floral differentiation. Imposition of water stress often leads to increased flower bud initiation (Nir *et al.*, 1972; Southwick and Davenport, 1986) and this is associated with improved leaf and twig carbohydrate status (Magness, 1953; Hilgeman and Sharpe, 1970), most likely a consequence of the extreme sensitivity of fruit growth (Marloth, 1950; Hilgeman *et al.*, 1959) and root extension (Bevington

and Castle, 1985) to soil water deficit. On the other hand, Nir *et al.* (1972) claim that water stress may also reduce gibberellins via inhibition of root growth. Stimulation of flower formation by low temperature (Moss, 1969; Southwick and Davenport, 1986) is also associated with increased twig and leaf carbohydrate content (Yelenosky and Guy, 1977) and reduced root growth (Bevington and Castle, 1985). According to Nir *et al.* (1972) impaired root extension will also reduce gibberellin levels within the canopy but estimates of gibberellin levels during flower differentiation have never been reported. All supporting evidence has come from exogenous application of GA_3. Goldschmidt *et al.* (1985) consider gibberellins and carbohydrates to operate as independent regulatory factors in the modulation of floral differentiation in citrus.

9.1.3 Fruit Set

Unlike most deciduous fruit crops, citrus is self thinning (Jones and Cree, 1965; Agusti *et al.*, 1982; Guardiola *et al.*, 1984). Although as many as 200,000 flowers/tree may be produced in spring (Erickson and Brannaman, 1960) the proportion of flowers setting fruit may range from 10% (Goldschmidt and Monselise, 1977) to only 0.1% (Agusti *et al.*, 1982). Fruit set can thus be more important than flowering intensity in determining final fruit number. This is borne out in the study of Agusti *et al.* (1982) who observed that greater fruit set in trees showing sparse flowering (~7,500 flowers/tree) resulted in higher yields than for trees with high numbers of flowers (~124,200 flowers/tree) but low rates of fruit retention.

Although the total number of flowers borne on mixed flushes is frequently less than on reproductive flushes (Moss, 1970) fruit set on mixed flushes is 5 to 10 times greater than on reproductive flushes (Sauer, 1951; Moss, 1970). Competition for substrates amongst flowers may be the cause for differences in fruit set between leafy and leafless inflorescences as Holtzhausen (1968) thinned flowers (reducing competition) and increased set from 0.9 to 4.8% for 'Washington Navel'. Although spring flush leaves may assist fruit set on mixed flushes due to carbohydrate supply to proximal fruit (discussed below) reserve carbohydrate is of considerable importance in early

development of the floral organ. ^{14}C labelling studies of Akao *et al.* (1981) showed that ^{14}C applied to Satsuma mandarin during mid-spring constituted only a small fraction of total C in developing buds 4 weeks later. As Nii and Okamato (1973) showed that removal of old leaves from Satsuma mandarin trees in early spring resulted in almost complete flower abscission, 'old' leaves (present prior to commencement of spring growth) also contribute significantly to fruit set of floral organs. Whilst this suggests that old leaves play an important role for fruit set and growth during the first few weeks after full bloom, Nii and Okamato (1973) also observed that trees on which spring flush leaves had been removed showed marked fruit drop in mid-summer indicating a role for spring flush leaves in sustaining fruit growth. Such a role for spring flush leaves was shown by Moss *et al.* (1972) who demonstrated that during the "critical period for fruit shed" (25 days after full bloom) substantial export of ^{14}C labelled carbohydrate from spring flush leaves toward adjacent developing fruits occurs.

As is apparently inevitable, some authors have attempted to explain differences in fruit set between leafy and leafless inflorescences in terms of plant growth regulators. Although there is some evidence that GA_3 and other chemicals may improve fruit set (Monselise, 1979), as for floral induction, direct evidence for PGR influences on differences in fruit set between reproductive and mixed flushes remains to be presented.

Temperatures above ~40°C in late spring and early summer and associated high potential evaporation rates may greatly increase rates of flower/fruitlet absicssion (Moss and Muirhead, 1971; Reuther, 1973; Cole and McLeod, 1985). Physiological causes for these increased rates of reproductive structure abscission under such conditions have not been identified but gas exchange respones to VPD (Section 9.1.6) could be involved.

9.1.4 Fruit Growth

Working with 'Valencia' orange under Australian conditions, Bain (1958) divided fruit development into 3 stages which correspond with changes in growth rate. Stage I varied with length according to time of anthesis, but was generally completed by mid-December (~9 weeks post anthesis). This stage was characterised by cell division, with

increases in fruit volume mainly due to increases in peel thickness. Stage II, a period of very rapid growth from mid-December to mid-July was distinguished as the cell enlargement period, with growth of the pulp being responsible for most of the increase in fruit size during this stage. Stage III, the maturation period, lasted 7 months from July and was characterised by fruit maturation, with gradual degreening of the peel and slow rates of growth being observed. Internal changes also occur within the fruit during latter stages of development with juice sugar content increasing whilst acid content decreases (Sinclair, 1961). This leads to increased palatability as fruit mature. Appropriate maturity indices have thus been developed to ensure fruit harvested are at an acceptable stage of development. The most common index is the *Brix/Acid ratio*. As levels of soluble sugars are well correlated with juice optical refraction, juice sugar content is generally estimated by a "Brix" reading on an optical refractometer (Sinclair, 1961). This value is then divided by the titratable acidity of the juice (expressed as % citric acid equivalents) to yield the maturity index. A Brix/acid ratio of 7 is a legal minimum requirement for export of oranges from Australia (Australian Department of Primary Industry, 1988) but this researcher finds a value of about 9 more appropriate for his tastebuds!

'Washington Navel' orange development also shows the 3 growth stages defined by Bain (1958) for 'Valencia' orange, but development during Stages II and III is nearly twice as fast as for 'Valencia' orange (Bouma, 1959). 'Washington Navel' oranges hence mature some six months earlier than the 'Valencia' cultivar (Bryden, 1930) despite no difference in the timing of floral differentiation or anthesis (West and Barnard, 1935).

9.1.5 Relationship between leaf area and fruit growth

Fruit growth is affected by crop load and leaf:fruit ratio. Shamel *et al.* (1933) manually defoliated 'Washington Navel' orange trees in late spring to yield leaf:fruit ratios ranging from 10 to 60. Although a typical undefoliated tree had 48 leaves/fruit they found 60 leaves/fruit to be optimal for fruit growth. Differences in growth rates of fruit on trees with different leaf:fruit ratios were observed to be minimal during the cell division phase

(Stage I) but became very pronounced during the cell enlargement phase (Stage II), with fruit size on trees with 10 leaves/fruit being only 30% of those with 60 leaves/fruit.

Similarly, Hilgeman *et al.* (1959) found a significant correlation between fruit growth and cropload of 'Valencia' orange. Thinning 25 or 40% of fruit increased the size of remaining fruit on 'Washington Navel' orange trees in California (Parker, 1932). Such results suggest that leaf area and hence (most likely) CO_2 assimilation is of considerable importance in determining growth and final size of orange fruits.

9.1.6 Gas exchange of citrus leaves in the field

Although under optimal laboratory conditions citrus leaves may have CO_2 assimilation rates as high as 15 μmol CO_2 m-2 s-1 (Table 1.1) and stomatal conductances greater than 250 mmol m-2 s-1 (Khairi and Hall, 1976ab), this potential is rarely realised in the field. Examination of diurnal patterns of 'Marsh' Grapefruit leaf gas exchange in Florida showed that, although on days of moderate temperature (<25°C) and low VPD (≤1.5 kPa) high and constant rates of CO_2 assimilation (~10 μmol m-2 s-1) were observed, on days of increased vapor pressure deficits and associated high leaf temperatures, gas exchange virtually ceased around midday showing only partial recovery in the afternoon (Sinclair and Allen, 1982). Such responses are most likely the consequence of stomatal sensitivity to VPD. As in many other plants (Schulze, 1986) citrus leaf stomatal closure tends to occur as VPD increases (Kaufmann and Levy, 1976). Although days of high VPDs are typically also those of the greatest leaf temperatures, laboratory studies have shown that when VPD is held constant, leaf temperatures in the range 20 to 41°C have little or no effect on citrus leaf stomatal conductance. Sheriff (1977) observed no effect of leaf temperature in the range 30-40.5°C on stomatal conductance of *C. sinensis* seedlings. Working with similar material, Hall *et al.* (1975) found only a slight reduction in conductance over the range 20°-30°C and Kaufmann and Levy (1976) found little effect of temperatures up to 37°C and a slight increase in conductances at 41°C for Rough Lemon.

Given that many citrus orchards are located in dry inland areas such as Central Israel (Cohen and Cohen, 1983), Arizona (Elfving *et al.*, 1972) and South Australia where VPDs greater than 5.0 kPa may occur, high VPD induced stomatal closure (with associated reductions in CO_2 assimilation: Kaufmann and Levy, 1976; Figure 6.6) may have considerable implications for citrus productivity. Indeed, given the importance of photosynthetic supply in ensuring adequate fruit set (Moss *et al.*, 1972) high fruitlet abscission rates on days of high VPD (Section 9.1.3) may well be a consequence of extremely low rates of gas exchange that would be expected to prevail on such days.

Despite its evergreen habit, little work has been done on seasonal patterns in citrus leaf gas exchange. The most extensive study is that of Moreshet and Green (1984) on 'Valencia' orange in South Africa (appoximately same latitude as Dareton and Loxton). They measured diurnal trends in stomatal conductances in December (early Summer) March (early Autumn) and May (late Autumn). Despite greatly different temperatures between early summer and late autumn little difference in maximal stomatal conductances was observed (VPD not reported). Indeed higher conductances after 10.00 a.m. were observed in late autumn that at the earlier sampling times. It may then be that although citrus trees are native to sub-tropical climates (Chapot, 1975) due to stomatal closure at high VPD, net gas exchange in the winter months may in fact occur at higher rates than in the warmer summer months in dry inland environments.

9.1.7 Salinity and orchard citrus

Despite numerous reports on visible toxicity symptoms and reduced yields on citrus trees when irrigated with high salinity water, and a multitude of laboratory studies investigating physiolgoical responses of citrus to salinity, virtually no work has been done to elucidate the physiological basis of reduced yields.

As shown in Chapter 8, effects of salinity observed in laboratory studies (*viz* impaired gas exchange) may not necessarily occur in the field. Furthermore as discussed in Chapter 5, physiological responses observed under conditions of salinity stress may be dependent upon the rate of sodium and/or chloride transport into leaves rather than

absolute concentrations *per se*. This study was therefore undertaken to provide a more precise definition of the yield response to salinity and (more specifically) to determine (1) if significantly reduced rates of gas exchange do occur in orchard citrus trees debilitated by salinity and (2) if reduced rates of gas exchange were an important factor in the 50% reduction in yield observed after 5 years of irrigation with water containing 20 mol m^{-3} NaCl at Loxton.

9.2 MATERIALS AND METHODS

Test trees were located at Loxton. Tree descriptions, soil type and irrigation water are detailed in Chapter 7. Trees irrigated with either river water (~4 mol m^{-3} NaCl: controls) or the highest salinity water (~20 mol m^{-3}: salinised) were used for this investigation.

9.2.1. Effect of salinity on spring growth

In early October, 1986, a branch about 1.0 metre long on both the north (sun) and south (shade) exposure of 8 control and 8 salinised trees was tagged and the proportions of each flush type (Section 9.1.1) and the total number of leaves and flowers (at full bloom stage) recorded.

9.2.2. Flower and fruitlet retention

The number of reproductive structures on each tagged branch was determined at intervals of approximately 1 month until early February after which no further abscission of fruitlets was observed. Values are expressed as percentage retention where at any sampling time:

$$\text{Percentage retention} = \frac{\#\text{reproductive structures}}{\#\text{flowers at full bloom}} \times \frac{100}{1}$$

9.2.3. Leaf retention

At intervals from 1 to 2 months after October, 1986 the number of leaves on tagged branches from 4 control and 4 salinised trees was determined and leaf retention rates calculated as described for reproductive structures in Section 9.2.2.

9.2.4. Fruit growth

From November 1986 until harvest (July, 1987) fruit volumes were calculated for 5 fruit on mixed and reproductive flushes on each side of 8 control and 8 salinised trees at intervals ranging from 2 to 6 weeks. Fruit volume was calculated from caliper measurements of both fruit axis diameters using the standard equation for prolate spheroidal area (Turrell, 1946).

9.2.5. Fruit maturity determinations

Eight fruit were sampled randomly in terms of aspect and height from 8 control and 8 salinised trees and juice extracted using a commercial juicer. The juice was filtered through several layers of cheese cloth with special effort made to filter all juice present. Juice Brix was then determined using a refractometer whilst juice acidity was quantified by titration against 0.1 M NaOH with an automatic titrater to end point pH 8.4. This procedure was undertaken on 21 May, 15 June, 3 July and 21 July, 1987.

9.2.6 Leaf carbohydrate analysis

At intervals of approximately 4 weeks starting in early October, one leaf from a non-fruiting terminal on the north side of 8 control and 8 salinised trees was removed, immediately placed in a small air tight plastic bag and transported to the field laboratory on ice. After fresh weight determination, laminae were cut into ~2 mm x 10 mm slices and placed in a 50 ml centrifuge tube containing ~20 ml boiling 80% ethanol. After 15 minutes in a 95° water bath, tubes were removed, allowed to cool and covered with Nesco-film prior to transfer on ice to the Adelaide laboratory. Tubes were then stored at -20°C until required.

9.2.6.1 Soluble sugars

The 80% ethanol extract was homogenised in the centrifuge tube, the grinding head washed with ~10 ml 80% ethanol, tubes brought to 95% in a water bath and the ethanol extract boiled for 15 minutes to aid extraction of soluble sugars. The resulting homogenate was centrifuged for 10 minutes and the supernatant removed and stored. The pellet was then re-extracted in 80% ethanol a further 2 times and supernatants pooled. Pooled supernatant was dried under vacuum at 60°C and extracted ethanol soluble compounds resuspended in 50 ml H_2O. Total soluble sugars were determined using the phenol/sulphuric acid method (Dubois *et al.*, 1965) using glucose as the standard.

9.2.6.2 Starch

The ethanol insoluble pellet was washed into a 50 ml conical flask with ~5 ml H_2O, autoclaved at 130°C for 160 min and incubated with 50 mg amyloglucosidase (Sigma Chemicals) in a shaking water bath for 2 hours (Thievend *et al.*, 1972). Released glucose was measured using the phenol/sulphuric acid method using glucose standards. Enzyme blanks were used to correct for enzyme contamination.

9.2.6.3 Expression of results

Carbohydrate contents determined on a fresh weight basis were converted to a dry weight basis using the average fresh to dry weight values determined for ion analysis leaves.

9.2.7 Ion analysis

At intervals of approximately 4 weeks starting in eary October leaf sodium, chloride and potassium concentrations were determined as described in Section 3.2.2.5. One leaf was sampled from the north side of 8 control and 8 salinised trees.

9.2.8 Leaf osmotic potential

At intervals of 6 to 8 weeks, one leaf was removed from the north side of 8 control and 8 salinised trees prior to first light. Osmotic potentials were derived from PV curves as described in Section 2.2.3.6.

9.2.9 Diurnal patterns in stomatal conductance and leaf water relations

These measurements were made on 6 January, 17 March, 24 March, 10 June and 22 July, 1987.

9.2.9.1 Porometry

Leaf stomatal conductance was measured using a Delta-T-MkIII transit time diffusion porometer. Leaves chosen for sampling were from the north side of the tree and exposed to the maximum PPFD prevailing at the time. During daylight hours at least 4 readings were allowed to pass until reasonably constant values were obtained. Four control and 4 salinised trees were used with conductances of the lower surface of 4 leaves on each tree being determined at each sampling time.

Due to the extremely long transit times recorded during pre-dawn and post-dusk measurements, only 1 to 2 readings were made at this time. Almost total stomatal closure was indicated in these cases as adaxial transit times (cuticular only) did not differ appreciably from those observed on the abaxial surface for either control or salinised leaves. In one case (22 July, 1987) it was necessary to wipe leaves with tissues to remove dew condensate prior to pre-dawn measurements. Stomatal conductances were obtained from calibration curves obtained using the diffusion plate supplied by Delta-T, and converted from cm s^{-1} to mmol m^{-2} s^{-1} according to

$$g\ (\text{mmol m}^{-2}\ \text{s}^{-1}) = \frac{g\ (\text{cm s}^{-1})\ P\ [\text{mb}]\ \times\ 10^3}{8.314\ [\text{J mol}^{-1}\ \text{K}^{-1}]\ T\ [\text{K}]}$$

where T = leaf temperature
and P = atmospheric pressure

Measurements were generally made at 2 hr intervals from prior to first light till after dusk.

9.2.9.2 Leaf water potential

At each sampling time one sun exposed leaf from the trees used for porometry measurements was used for bulk leaf water potential (Ψ) determinations using a pressure bomb. On 6 January and 17 March Ψ determinations were not always made at the same time as stomatal conductance measurements, but on 24 March and thereafter Ψ was measured immediately after conductance measurements in order for the relationship between leaf transpiration rate and Ψ to be quantified.

9.2.9.3 Vapor pressure deficit and leaf transpiration rate

Air water vapor partial pressure was determined from wet bulb readings made at a height of 2.0 metre inside the canopy enabling leaf transpiration to be calculated as in Equation 1.2 with e_i as the saturated vapor pressure at leaf temperature (measured by the porometer).

9.2.9.4 Photosynthetic photon flux density (PPFD)

At intervals of ~5 min during porometry sampling times PPFD was measured using a Licor LI-190S photosynthetic quantum sensor (400-700 nm) placed in a similar plane to sample leaf orientations.

9.2.10 Floral differentiation studies

Buds from north and south exposures of control and salinised trees were excised on 21 June and 30 June, 1987. Following removal of bracts with the aid of a stereomicroscope, buds were fixed in 3% gluteraldehyde + 0.5% caffeine in 0.025 M phosphate buffer, pH 7.0 for 2-4 days. They were then passed through an ethanol dehydration series prior to critical point drying. Buds were then gold plated prior to examination under a Cambridge Steroscan 25Q Mk III B scanning electron microscope.

9.2.11 Twig carbohdyrate determination

Bud wood on branches tagged in October (but not used for leaf abscission studies) on north and south aspects of 4 control and 4 salinised trees were sampled for carbohydrate content on 2 July, 1987 (immediately after first signs of floral differentiation). Following leaf counts, bud wood was removed and dried at 70°C for 24 hours. Twigs were then ground and soluble sugar and starch levels determined after extraction in 80% ethanol as described for leaves in Section 9.2.6.

9.3 RESULTS

9.3.1 Spring growth

Measurements made in October, 1986 showed that whilst on the south side of trees there was no effect of salinity on flush type, on the north side salinised trees had a greater proportion of vegetative flushes than controls. This occurred at the expense of flowering with the proportion of both reproductive and mixed flushes reduced on the north side of salinised trees (Table 9.1).

		% of total flushes		
Aspect	Salinity treatment	Reproductive	Mixed	Vegetative
North	Control	32b	23b	45b
	Salinised	14a	9a	77a
South	Control	34b	31b	35b
	Salinised	44b	19b	37b

Table 9.1: Effect of aspect and salinity on flush type of 'Washington Navel' orange in early spring 1986. Values with different letters within a column (n=8) differ significantly (P = 0.95) after arcsine transformation of data. Control trees were irrigated with ~4 mol m^{-3} NaCl whilst salinised trees received ~20 mol m^{-3} NaCl.

9.3.2 Flowering and fruit set

The tendency towards vegetative rather than reproductive growth resulted in salinised trees having only about half the number of flowers present on control trees in early Spring, 1986. Due to high tree to tree variation, this was only significant at P = 0.90. Fruit set was also impaired by salinity resulting in final fruit numbers on salinised trees being estimated at only ~35% that of the controls (Table 9.2).

	Control	Salinised	LSD (p = 0.05)
Flowering Intensity (# flowers tree^{-1})	23,100	14,200	10,800
Fruit retention (% of initial flower number)	5.9	3.6	2.0
Final fruit number (# fruit tree^{-1})	1370	526	472

Table 9.2: Flowering intensity, fruit set and final fruit number of control (irrigated with ~4 mol m^{-3} NaCl) and salinised (irrigated with ~20 mol m^{-3}) 'Washington Navel' trees in 1986/87.

Despite considerable tree to tree variation in both flowering intensity and abscission a distinct quantitative relationship between final fruit number and leaf area existed with the relationship saturating around 100 m^2 leaf area per tree (Fig. 9.1). This graph also illustrates that despite increased leaf production by salinised trees in spring, leaf area of salinised trees was less than controls. This was due to:

(1) a reduction in canopy volume (Table 7.4)

(2) a reduction in individual leaf area by 25% (observed during canopy leaf area determinations (Section 7.2.7.1))

(3) failure of salinised trees to retain leaves from previous growth flushes. This was quantified for the 1986/87 growing season (Section 9.3.5).

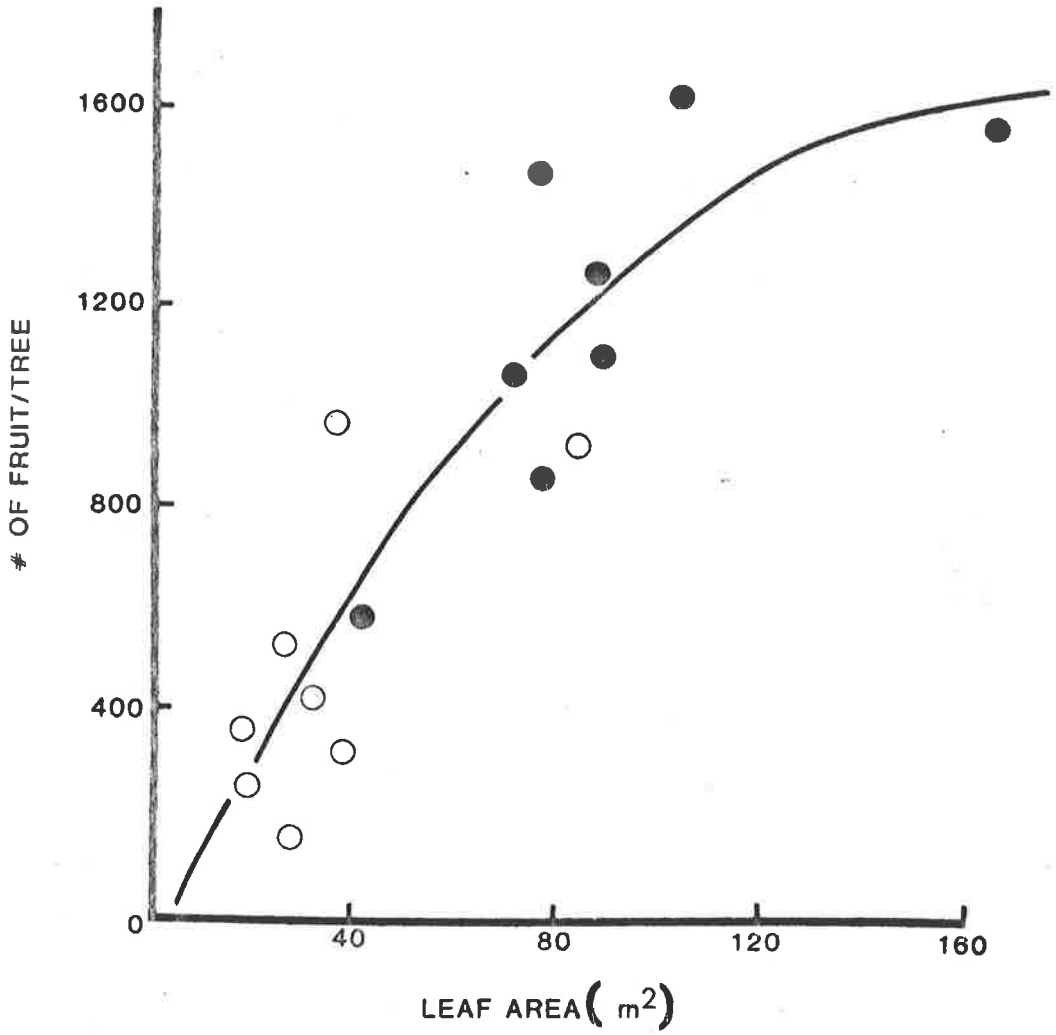


Figure 9.1 Relationship between tree fruit number and leaf area in early February, 1987 after completion of fruit drop (●) trees irrigated with 4 mol m^{-3} NaCl; (○) trees irrigated with 20 mol m^{-3} NaCl.

9.3.3 Fruit growth and maturity

Despite equivalent fruit/leaf ratios, growth of fruit on salinised trees was slower than on control trees. This is illustrated for fruit on mixed flushes on northerly aspects in Figure 9.2. Fruit growth on salinised trees was only reduced in early phases (Stage I) with little difference in fruit growth rate between fruit salinised and control trees observed during the phase of maximum expansion. Fruit volume on salinised trees approached that of fruit on control trees during stage III of fruit development (Figure 9.2). Aspect and flush type (reproductive or mixed) also influenced fruit growth rate during Stage I of fruit development. Reduced rates of fruit expansion were observed on the south side of trees with fruit on leafless flushes having slower growth rate than those on mixed (leafy) flushes. Fruit on salinised trees grew at a slower rate during stage I regardless of aspect or flush type (Figure 9.3). No effect of aspect or flush type on Stage II of fruit growth was observed, however. Consequently, despite large differences in growth during stage I there was little effect of salinity aspect or flush type on final fruit size (Table 9.3). Later attainment of adequate fruit size in salinised trees was reflected in a delay in fruit maturity. Lower Brix readings and hence reduced Brix/acid ratios were observed for fruit on salinised trees. Consequently fruit on salinised trees reached the legal limit for export (Brix/acid = 7) some 25 days later than for control trees (Figure 9.4).

		Fruit Volume (cm^3)	
		Flush type	
Aspect	Salinity treatment	Reproductive	Mixed
North	Control	206	185
	Salinised	191	179
South	Control	210	195
	Salinised	185	180

Table 9.3: Effect of aspect, flush type and salinity on 'Washington Navel' orange fruit volume at harvest (21 July, 1987). Values are the mean of 40 measurements. Least significant difference (p=0.05) = 27.

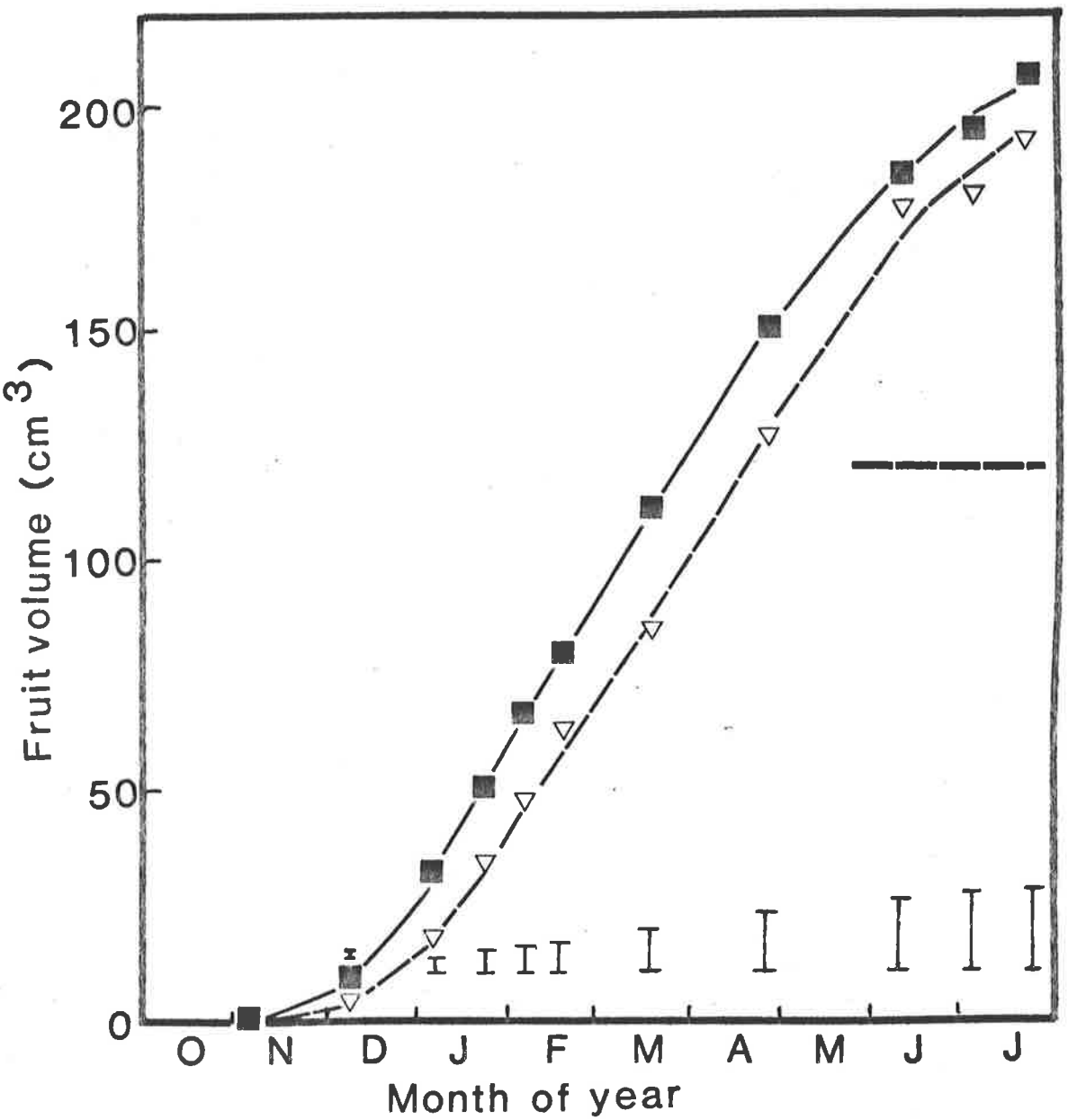


Figure 9.2 Growth of fruit on mixed flushes on the north aspect in 1986/87 (■) trees irrigated with 4 mol m^{-3} NaCl; (▽) trees irrigated with 20 mol m^{-3} NaCl (n=40). Bold broken line indicates period of fruit maturity sampling. Bars indicated the Least Significant Difference (p=0.05).

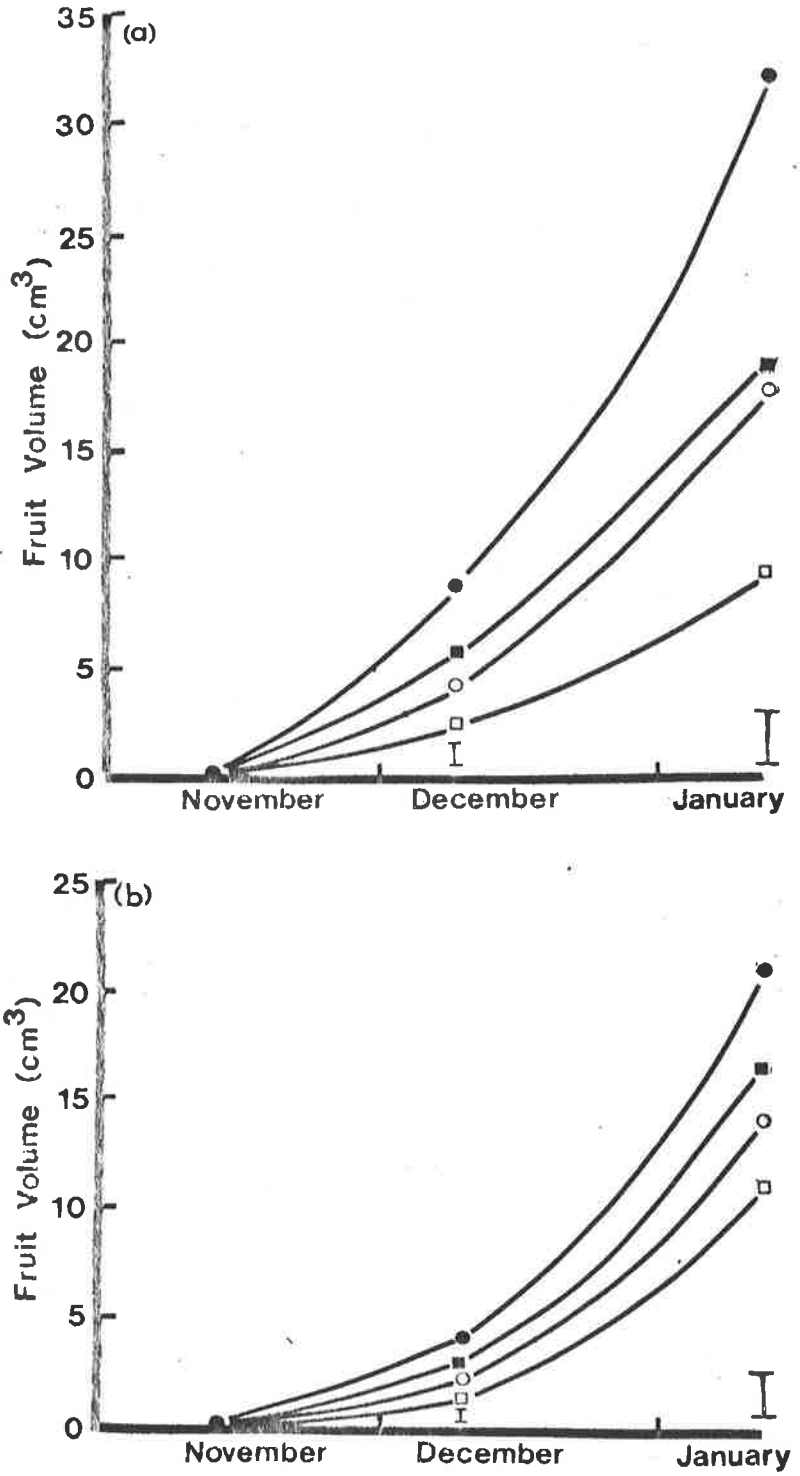


Figure 9.3 Early stages of fruit growth on (a) northerly aspect and (b) southerly aspect, of 'Washington Navel' trees. (● ■) fruit on trees irrigated with 4 mol m^{-3} NaCl; (○□) fruit on trees irrigated with 20 mol m^{-3} NaCl. Circles; fruit on mixed flushes; squares; fruit on reproductive flushes (n=40). Bars indicate the Least Significant Difference (p=0.05).

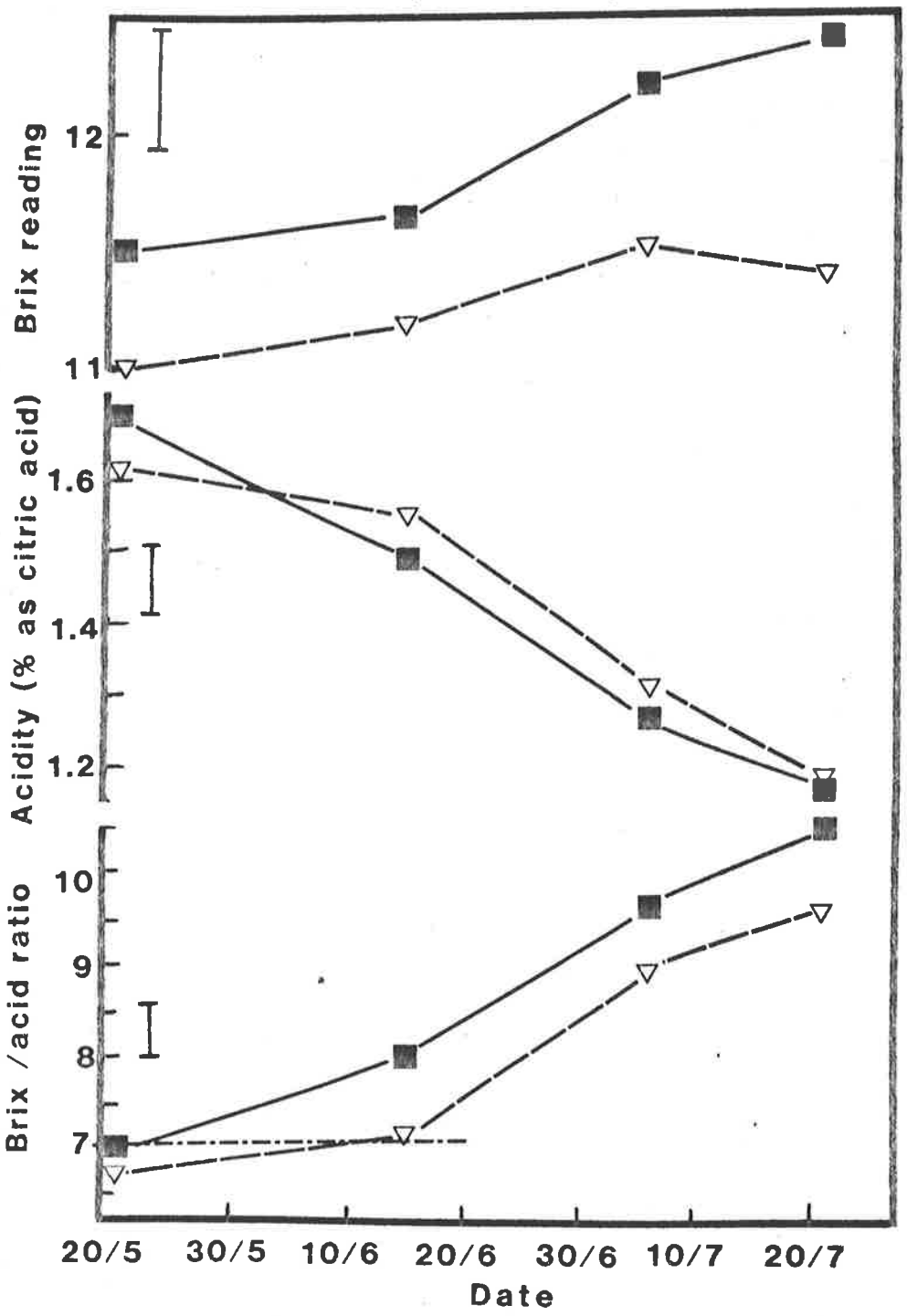


Figure 9.4 Brix reading, titratable acidity and Brix/acid ratio as affected by salinity. (■) fruit from trees irrigated with 4 mol m^{-3} NaCl; (▽) fruit from trees irrigated with 20 mol m^{-3} NaCl. (n=8). Bars represent the Least Significant Difference (p=0.05)

9.3.4 Leaf carbohydrates

When sampled in early October (prior to attainment of full leaf size) leaves on salinised trees had significantly lower soluble sugar and starch levels than those on control trees. A rapid reduction in soluble sugar levels in control leaves and a rapid increase in starch in salinised leaves did however result in no effect of salinity on carbohydrate content of current season growth in November or December. Foliar starch levels were greatly reduced in January being significantly lower for leaves from salinised leaves. Lower starch levels in leaves on salinised trees were consistently observed till mid autumn after which differences betwen control and salinised leaves became less dramatic. Sugar levels showed less dramatic seasonal fluctuations but after January leaves on salinised trees consistently had lower sugar levels than those on control trees (Figure 9.5).

9.3.5 Leaf abscission and production

Between October and January a small proportion of new season leaf fell from both sides of control and salinised trees. Although there was no further appreciable leaf abscission for control trees, salinised trees lost leaves at a rapid rate after January. Only ~15% of Spring flush leaves survived to winter. There was no effect of aspect on leaf retention for either control or salinised trees (Figure 9.6). Greater abscission of spring flush leaves on salinised trees was offset to some extent by a greater production of off-season vegetative flushes. Although not all tagged branched had off-season flushes (12 out of 16 for both control and salinised trees at 3.7.87) the number of leaves produced in such flushes was 13% that of the 1986 spring flush for control branches but represented 29% of the salinised spring flush. These figures do not readily reveal the true magnitude of treatment differences as salinised trees produced more leaves in the 1986 spring flush (Table 9.1). Increased off-season leaf production was not sufficient to completely offset the high spring flush abscission rates on salinised trees.

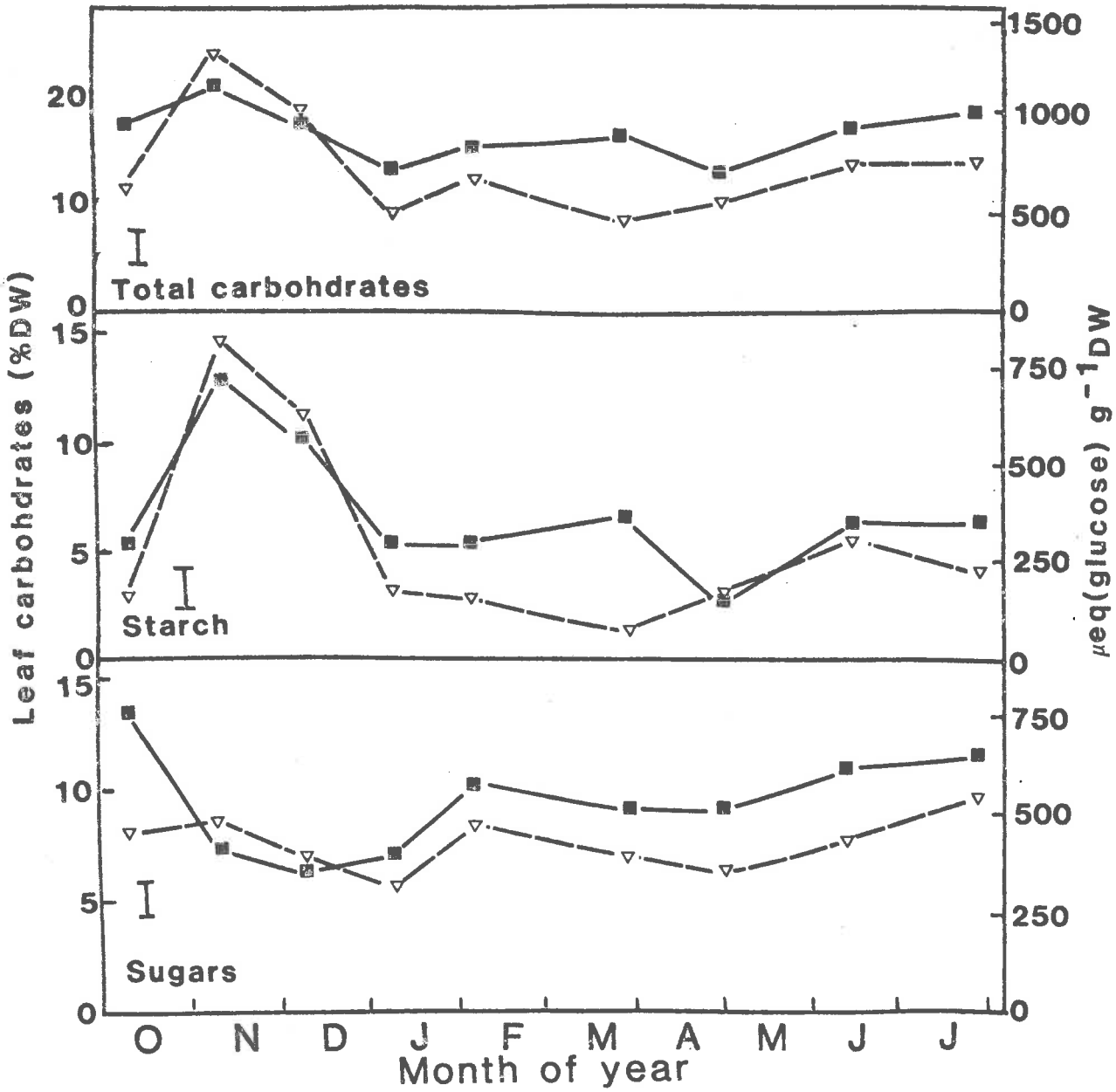


Figure 9.5 Effect of salinity of carbohydrate status of current season spring flush leaves in 1986/87. (■) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3}. (n=8). Bars represent the Least Significant Difference (p=0.05).

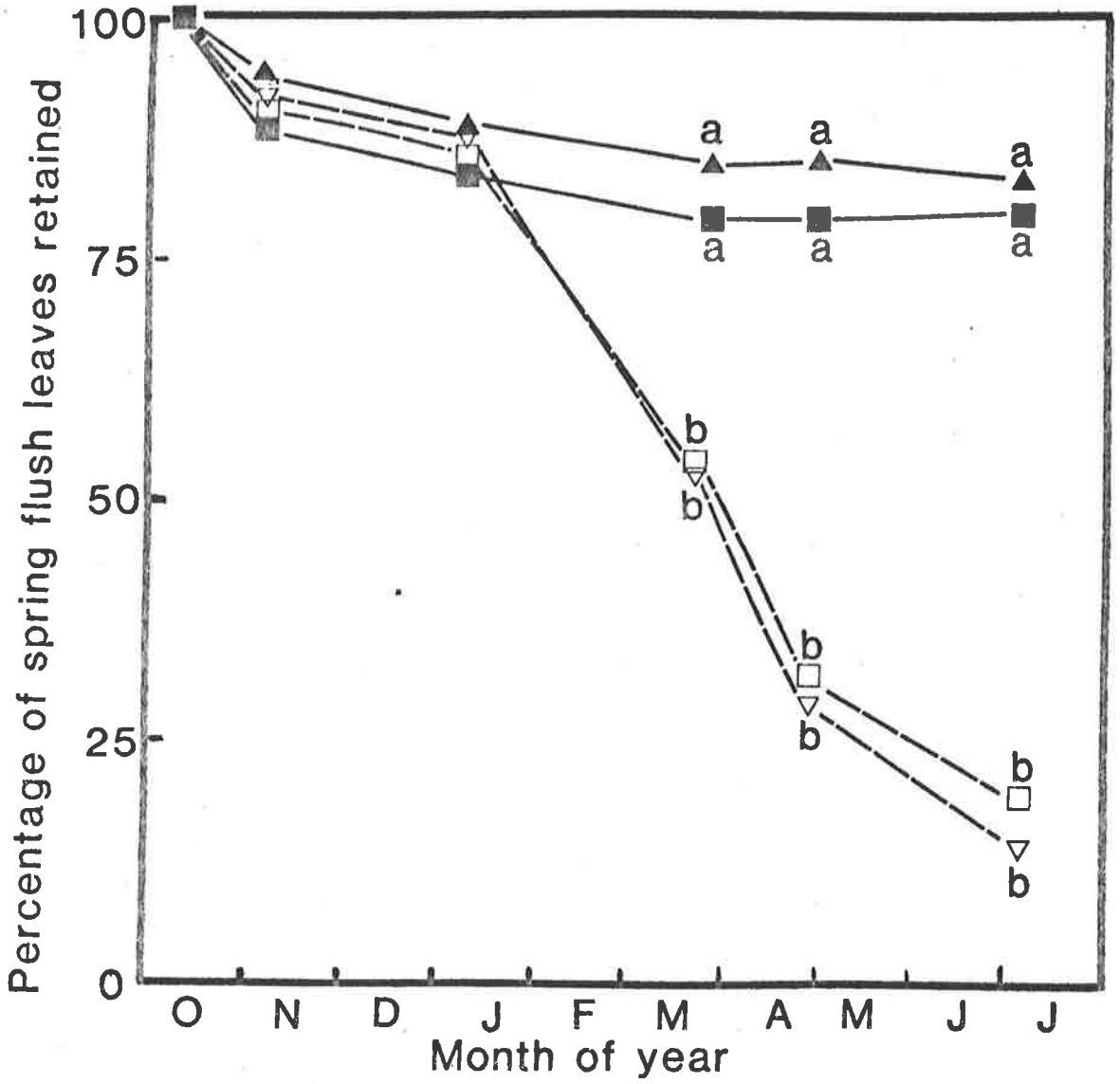


Figure 9.6 Effect of salinity on retention of current season spring flush leaves in 1986/87. (■ ▲) trees irrigated with 4 mol m^{-3} NaCl; (□ △) trees irrigated with 20 mol m^{-3} NaCl. Squares: northerly aspect of tree; Triangles: southerly aspect of tree. Retention rates were measured by periodic counts of leaf number on tagged branches ~1 m in length (n=4). Values with different letters differ signifiantly at p=0.05 after logarithmic transformation.

9.3.6 Leaf succulence and ion concentrations

In October, water initially contributed over 75% of fresh weight but as leaves matured succulence (expressed as the ratio mass H_2O mass^{-1} DW) rapidly declined to a value less than 2 after early December. Salinised leaves were consistently more succulent throughout the sampling period (Figure 9.7).

Young spring flush leaves of salinised trees had equivalent sodium and chloride concentrations in early October. A steady increase in sodium and chloride concentrations for leaves on salinised trees was observed after this time reaching a maximum in late summer/early autumn after which a decline in leaf sodium and chloride concentrations was observed. Little seasonal change in either sodium or chloride was observed for leaves on control trees (Figure 9.8). Leaf potassium content was not significantly affected by salinity (data not shown).

9.3.7 Leaf osmotic potential

When sampled in early November (by which time they were fully expanded but not hardened) both control and salinised leaves had osmotic potentials (π) of ~-2.2 MPa. This decreased (more negative) to ~-2.5 MPa by January for salinised leaves whilst no change in π was observed for control leaves at this time. After Januray π became increasingly more negative irrespective of salinity treatment. Both control and salinised leaves had values of -2.8 to -2.9 MPa in late July (Figure 9.9).

9.3.8 Stomatal conductance and leaf water status

9.3.8.1 6 January 1987

Leaf temperature rose steadily from 16°C at 0400 hrs to 42°C at 1300 hrs declining slowly thereafter. VPD rose sharply from 1.0 kPa at 0930 hrs peaking around 5.0 kPa at 1300 hrs after which a slow decline occurred. In summer such days are not atypical at Loxton. Control leaf stomatal conductance peaked at ~170 mmol m^{-2} s^{-1} at 0930 hrs after which rapid stomatal closure occurred. Conductances less than 80 mmol m^{-2} s^{-1} subsequently prevailed for the remainder of the day. Although stomatal conductances

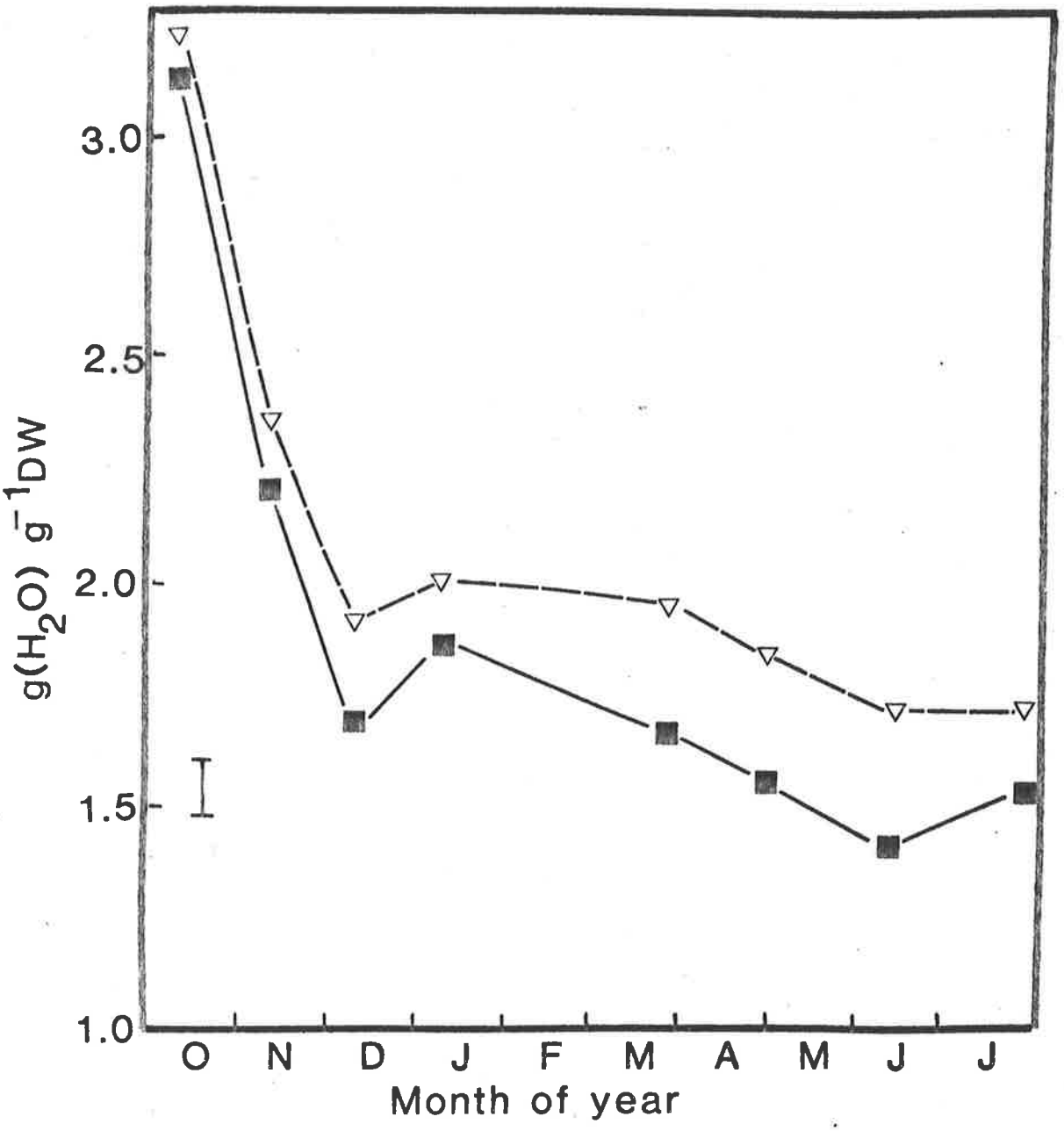


Figure 9.7 Effect of salinity on succulence of current season spring flush leaves in 1986/87. (■) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl. (n=8). Bar indicates Least Significant Difference (p=0.05).

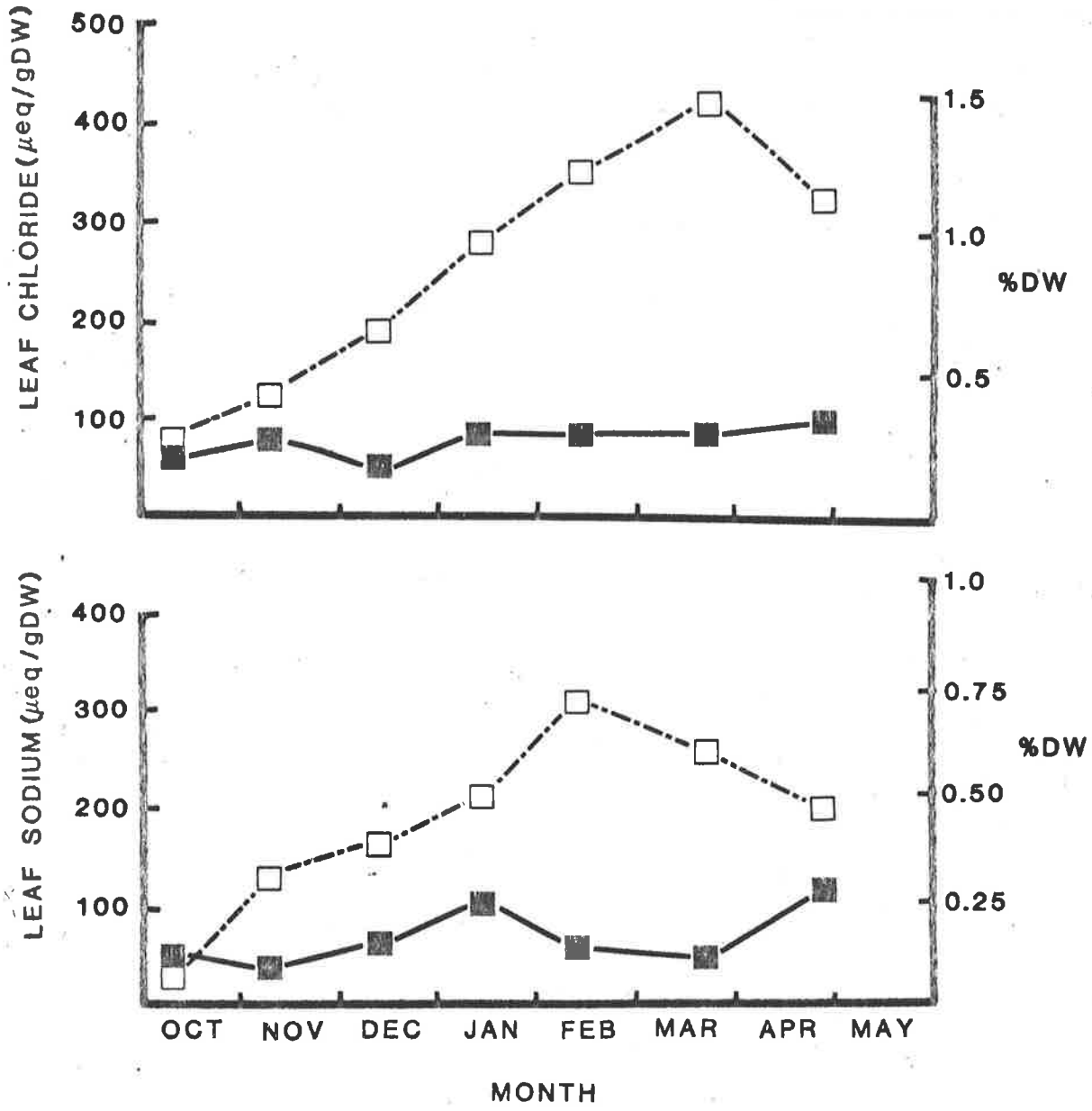


Figure 9.8 Effect of salinity of current season spring flush sodium and chloride concentration in 1986/87 (dry weight basis). (■) leaves on trees irrigated with 4 mol m^{-3} NaCl; (□) leaves on trees irrigated with 20 mol m^{-3} NaCl. (n=8). Bars indicate Least Significant Difference (p=0.05).

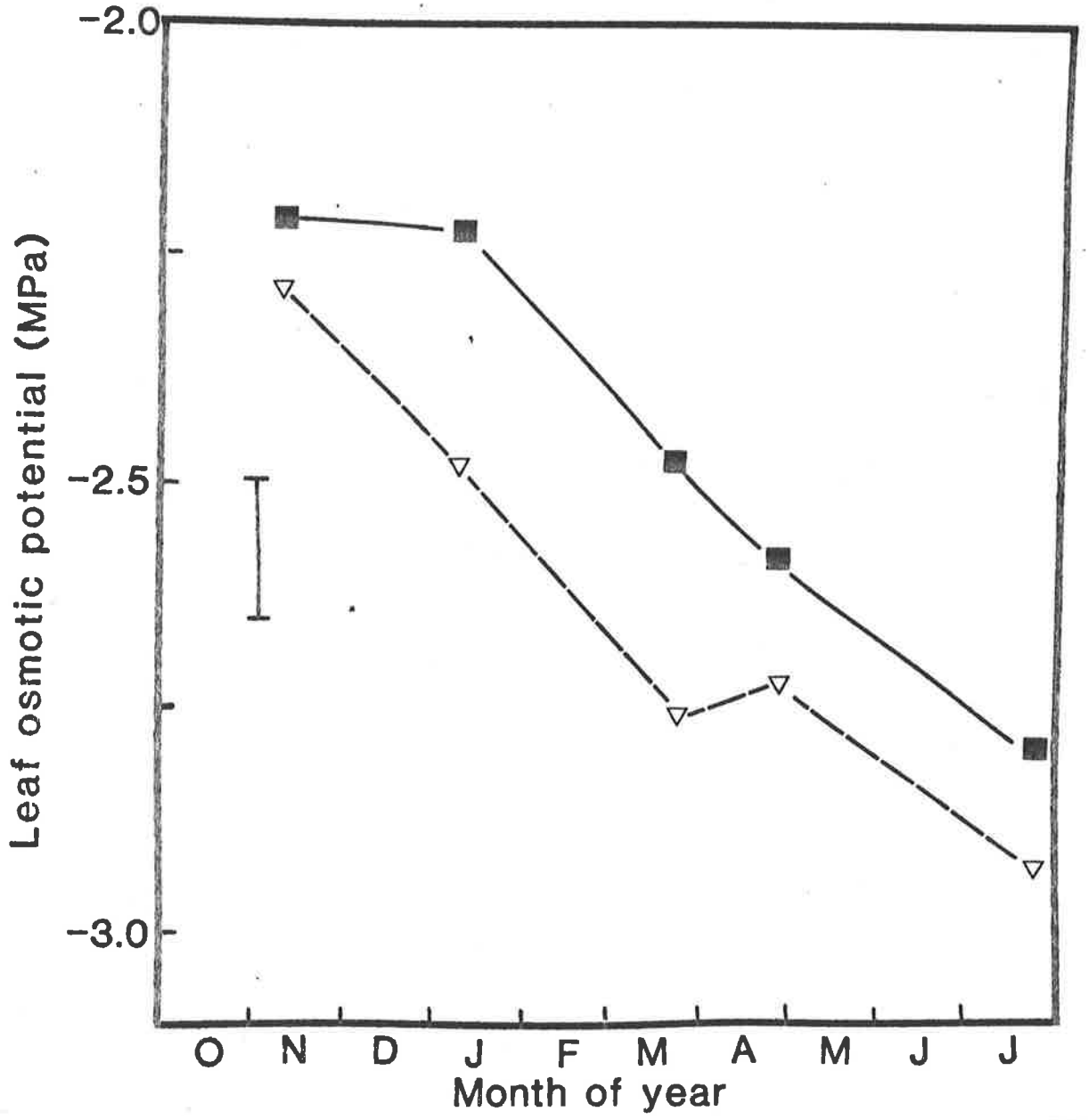


Figure 9.9 Effect of salinity on current spring flush osmotic potential in 1986/87. (■) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl (n=8). Bar indicates Least Significant Difference (p=0.05).

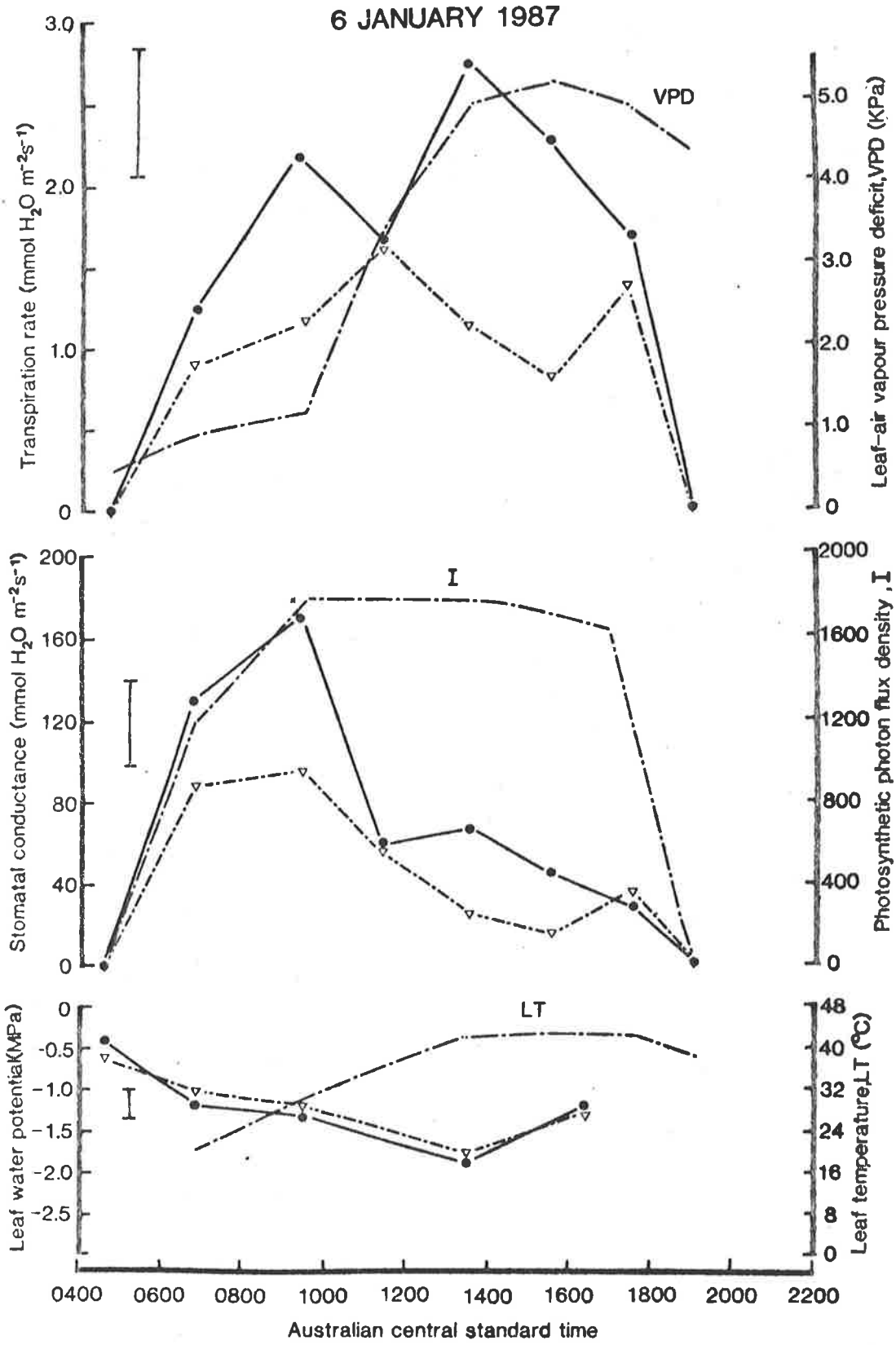


Figure 9.10 Effect of salinity on leaf water potential, stomatal conductance and transpiration rate of current season spring flush leaves on 6 January 1987. (●) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl (n=16). Bars indicate Least Significant Difference (p=0.05). Diurnal changes in leaf temperature, photosynthetic photon flux density and leaf to air vapor pressure deficit are also shown.

were significantly lower for salinised leaves prior to 0930 hours, stomatal closure after 0930 hours was less dramatic for salinised leaves than controls. Consequently there was little effect of salinity on stomatal conductance after this time. Little change in transpiration rate was observed after 0930 hrs. Leaf water potential (Ψ) declined from ~-0.5 MPa pre-dawn to ~-1.9 MPa at 1330 hrs. There was no effect of salinity on Ψ (Figure 9.10).

9.3.8.2 17 March, 1987

Leaf temperature rose from 16°C at 0500 hrs reaching 39°C at 1530 hrs after which it declined. VPD showed a similar pattern peaking at 4.2 kPa at 1530 hrs. This was the hottest day of March, 1987 at Loxton.

Even for control leaves, little stomatal opening occurred. Stomatal conductance peaked at 80 mmol m^{-2} s^{-1} at 0700 hrs after which slow closure to ~40 mmol m^{-2} s^{-1} at 1700 hrs was observed. At 0700 hrs stomatal conductances were 50% lower for salinised leaves than controls. Stomatal conductances of salinised leaves changed little over the remainder of the day. Consequently, as for 6 January, there were significant effects of salinity on stomatal opening for early morning measurements but greater stomatal closure on control leaves as the day progressed resulted in no effect of salinity at high VPD. Although pre-dawn Ψ was significantly lower (more negative) for salinised leaves there was no effect of salinity on Ψ during daylight hours (Figure 9.11).

9.3.8.3 24 March, 1987

On this more typical March day, leaf temperatures rose from 4°C at 0500 hrs to 30°C at 1500 hrs. VPD rose sharply after 0900 hrs but only reached 2.5 kPa at 1500 hrs. As previously observed, control stomatal conductances peaked during early morning (in this case 0900 hrs) at which time conductances of salinised leaves were significantly lower, after which closure occurred to a greater extent for stomata on control leaves. Afternoon conductances were however somewhat greater for control leaves than one

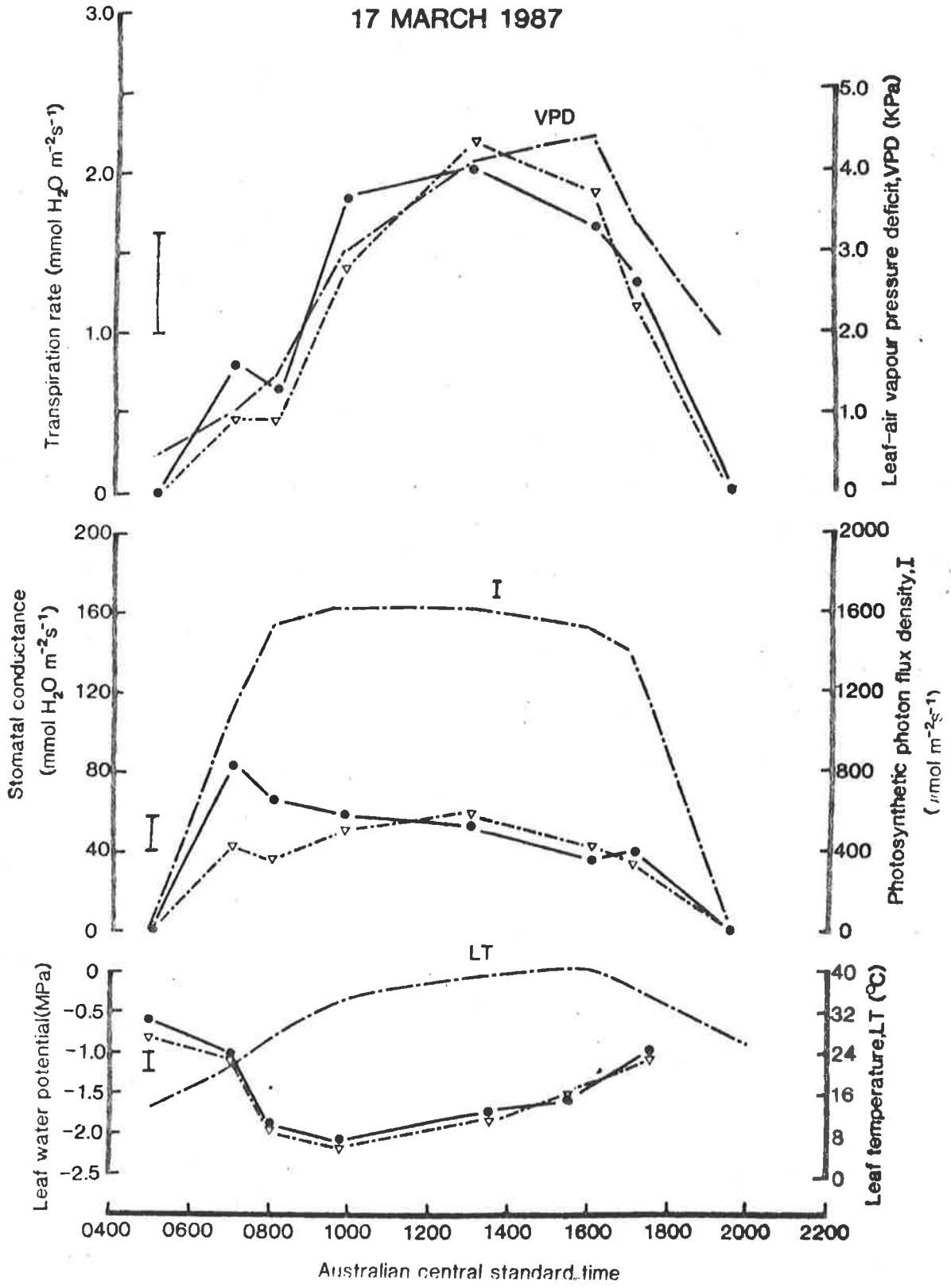


Figure 9.11 Effect of salinity on leaf water potential, stomatal conductance and transpiration rate of current season spring flush leaves on 17 March, 1987. (●) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl (n=16). Bars indicate Least Significant Difference (p=0.05). Diurnal changes in leaf temperature, photosynthetic photon flux density and leaf to air vapor pressure deficit are also shown.

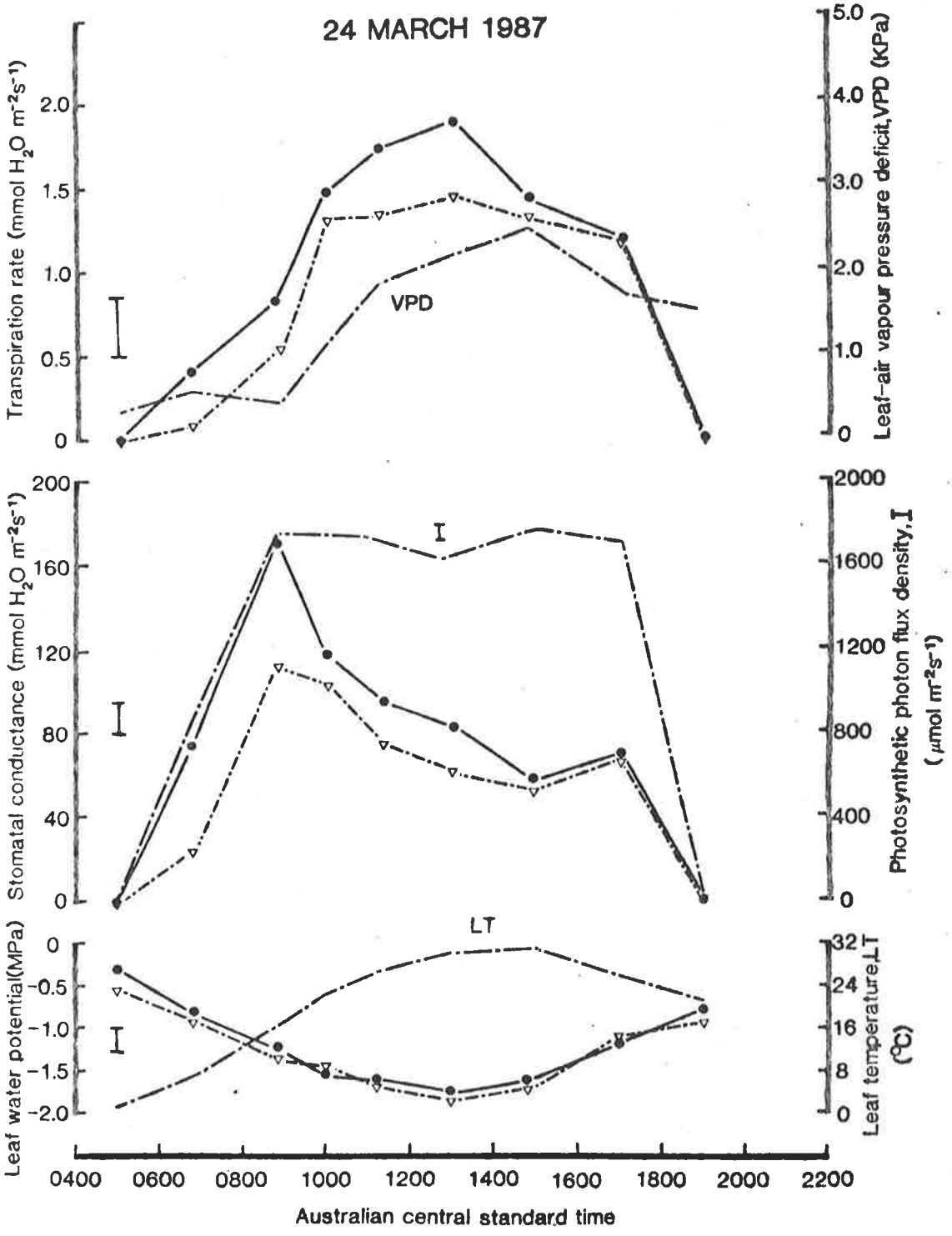


Figure 9.12 Effect of salinity on leaf water potential, stomatal conductance and transpiration rate of current season spring flush leaves on 24 March, 1987. (●) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl (n=16). Bars indicate Least Significant Difference (p=0.05). Diurnal changes in leaf temperature, photosynthetic photon flux density and leaf to air vapor pressure deficit are also shown.

week previous. As for 17 March, 1987 there was little effect of salinity on Ψ (Figure 9.12).

9.3.8.4 10 June, 1987

Although conditions were initially fine, heavy cloud moved in around 1100 hrs reducing PPFD to ~200 μmol m^{-2} s^{-1} at 1300 hrs. No further measurements were made after this time due to heavy precipitation. Leaf temperature rose from 10°C at 0700 hours to 22°C at 1300 hours, whilst VPD was low, being 0.7 kPa at the final measurement. Prior to arrival of heavy cloud, control leaf stomatal conductances were very high (~200 mmol m^{-2} s^{-1} at 1000 hrs). As on previous occasions, salinised leaves had significantly lower conductances at this time. Little difference was however observed between control and salinised leaves at 1300 hrs under low PPFD. Midday water potentials were somewhat more negative (~2 MPa) than for previous measurements. Throughout the day, control leaf Ψ was significantly more negative than that for leaves on salinised trees. Transpiration rates did not exceed 1.0 mmol H_2O m^{-2} s^{-1} (Figure 9.13).

9.3.8.5 22 July, 1987

On this cloudless day in mid winter, PPFD peaked at 1050 μmol m^{-2} s^{-1} at 1230 hrs. Leaf temperature rose from 1°C at 0700 hrs to 20°C at 1400 hrs. Shallow VPDs prevailed throughout the day, the maximum value of 1.0 kPa being observed at 1400 hrs.

In contrast to previous observations, stomatal conductances increased steadily throughout the day peaking at 1400 hrs after which a decline was observed. There were no significant differences in stomatal conductances between control and salinised leaves at any time. Transpiration rate peaked at 1.0-1.5 mmol H_2O m^{-2} s^{-1}. As was observed on 10 June, 1987, Ψ was more negative for control than salinised leaves during daylight hours. When sampled pre-dawn leaves had considerable condensate on their surfaces and less negative Ψ than on previous occasions (Figure 9.14).

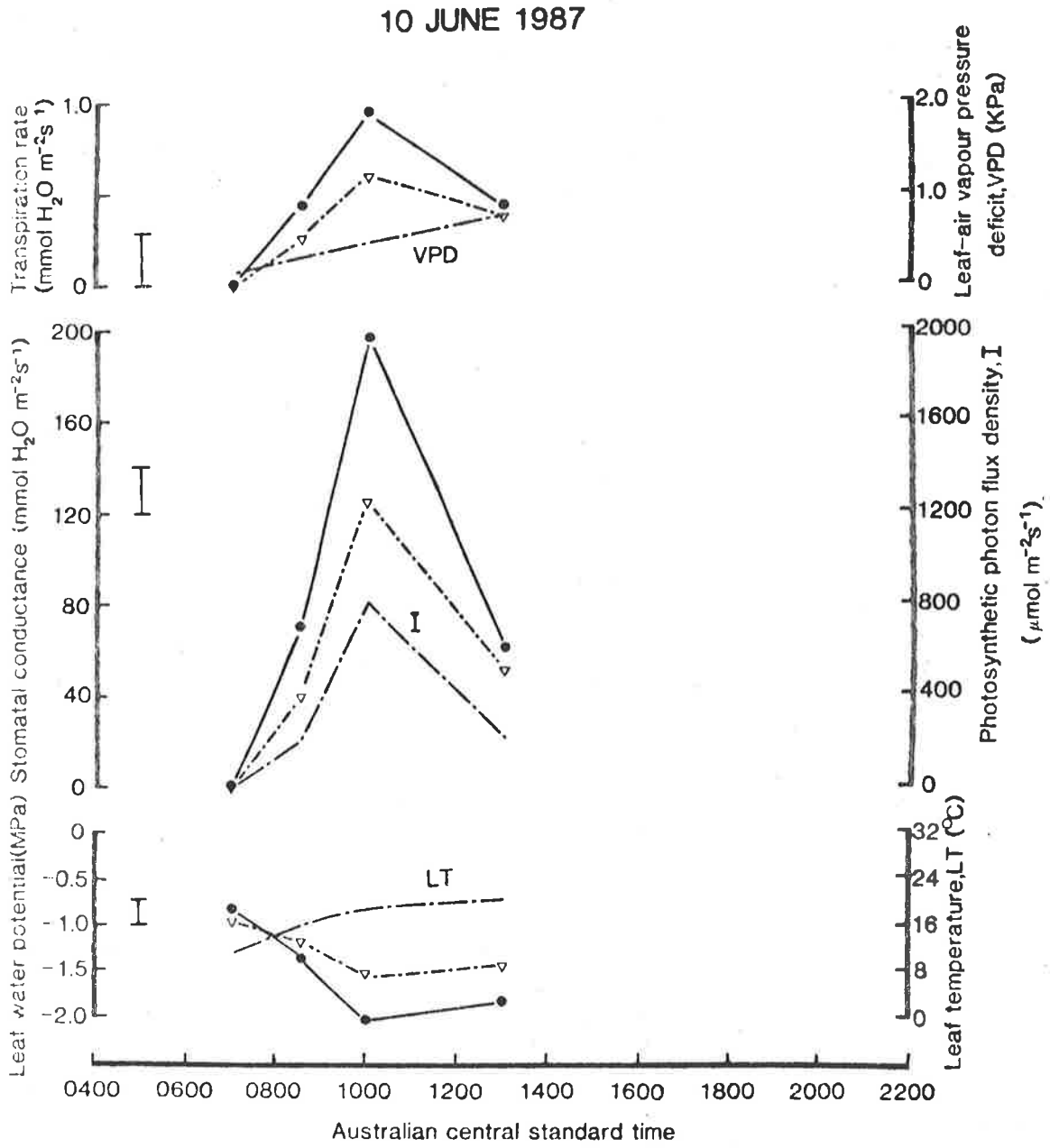


Figure 9.13 Effect of salinity on leaf water potential, stomatal conductance and transpiration rate of current season spring flush leaves on 10 June, 1987. (●) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl (n=16). Bars indicate Least Significant Difference (p=0.05). Diurnal changes in leaf temperature, photosynthetic photon flux density and leaf to air vapor pressure deficit are also shown. Measurements were terminated after 1300 hrs due to divine intervention.

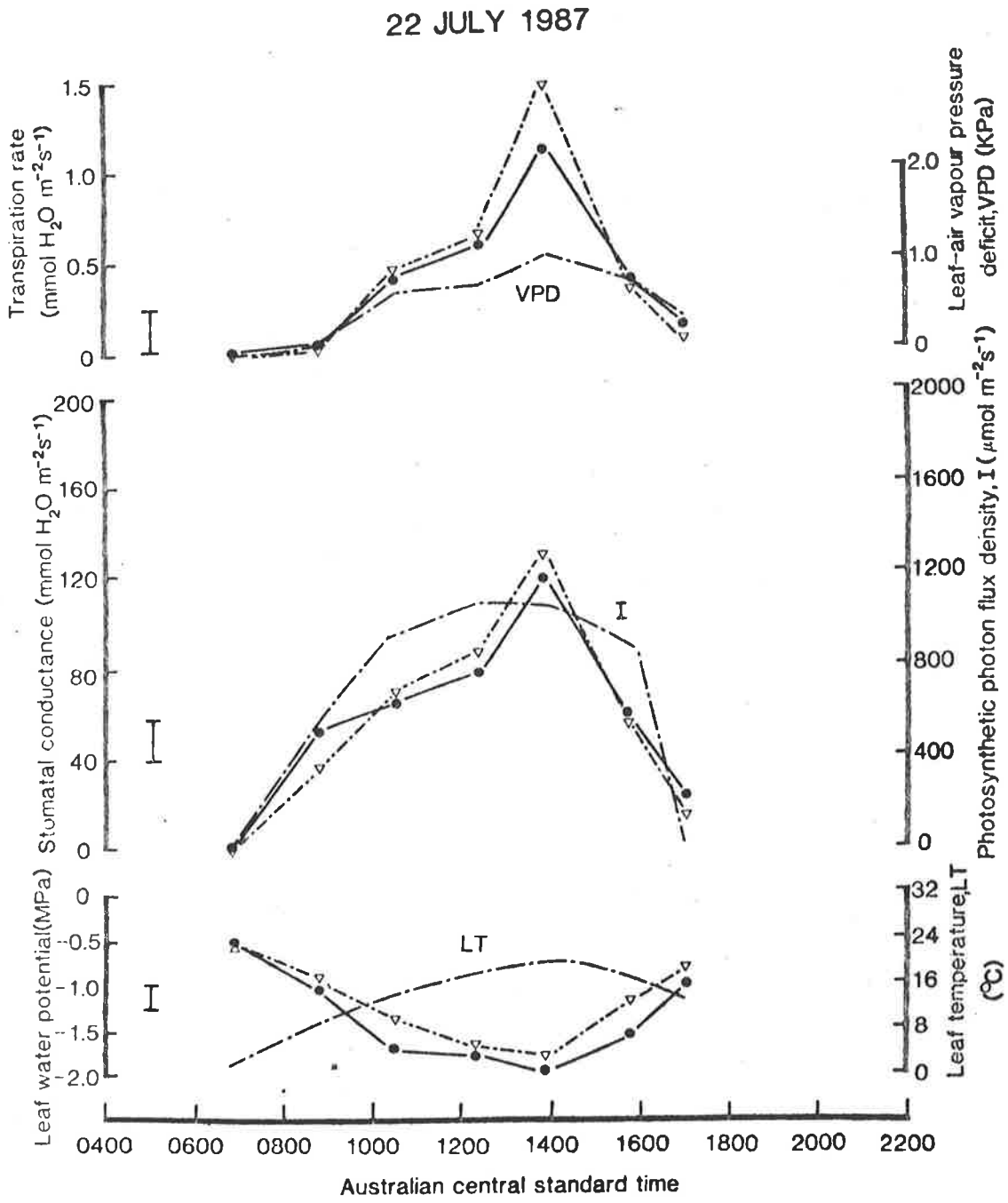


Figure 9.14 Effect of salinity on leaf water potential, stomatal conductance and transpiration rate of current season spring flush leaves on 22 July, 1987. (●) leaves on trees irrigated with 4 mol m^{-3} NaCl; (▽) leaves on trees irrigated with 20 mol m^{-3} NaCl (n=16). Bars indicate Least Significant Difference (p=0.05). Diurnal changes in leaf temperature, photosynthetic photon flux density and leaf to air vapor pressure deficit are also shown.

9.3.8.6 Relationship between transpiration rate (E) and leaf water potential (Ψ)

From equation 2.2 it is clear that, as Ψ_{soil} should change little throughout the day, the slope of the relationship between transpiration rate and leaf water potential is an indication of the conductance of the plant to the flow of water from soil to leaf. Such plots are shown in Figure 9.15 for 6 January, 24 March and 22 July, 1987. On 6 January, 1987, the slope was steeper for salinised leaves with the Ψ intercept being ~0.1 MPa more negative. On 24 March there was little difference in $d\Psi/dE$, however, indicating equivalent conductances from soil to leaf for control and salinised trees. On 22 July, 1987 analysis of the Ψ;E relationship indicated much reduced soil to leaf conductances for control trees compared to January or March. This was observed to a lesser extent for salinised trees. Consequently soil to leaf conductance was greater for salinised than control trees at this time. Some curvilinearity in the response was observed on this occasion indicating that conductance of trees to water was dependent (directly or indirectly) upon transpiration rate (Figure 9.15).

9.3.9 Floral differentiation

When sampled on 21 June the first signs of floral differentiation (*viz.* a broadening of and swelling of primordia indicative of sepal initiation: Lord and Eckart, 1985) was observed on 10% of buds from control and salinised trees. This was confirmed on 30 June where further floral buds were identified (Figure 9.16). Both vegetative and undeveloped buds were also observed at these times (Figure 9.17).

9.3.10 Relationship between leaf abscission, twig carbohyrate status and flowering

Carbohydrate analysis of budwood around the time of floral differentiation (as determined in Section 9.3.9) revealed no effect of salinity on total soluble sugars, values being 6.2 ± 0.8% for twigs on control trees and 5.8 ± 1.0% for those on salinised trees. Twig starch content was however reduced by salinity with twig starch levels being dependent upon extent of current season leaf absicssion. On branches that had retained

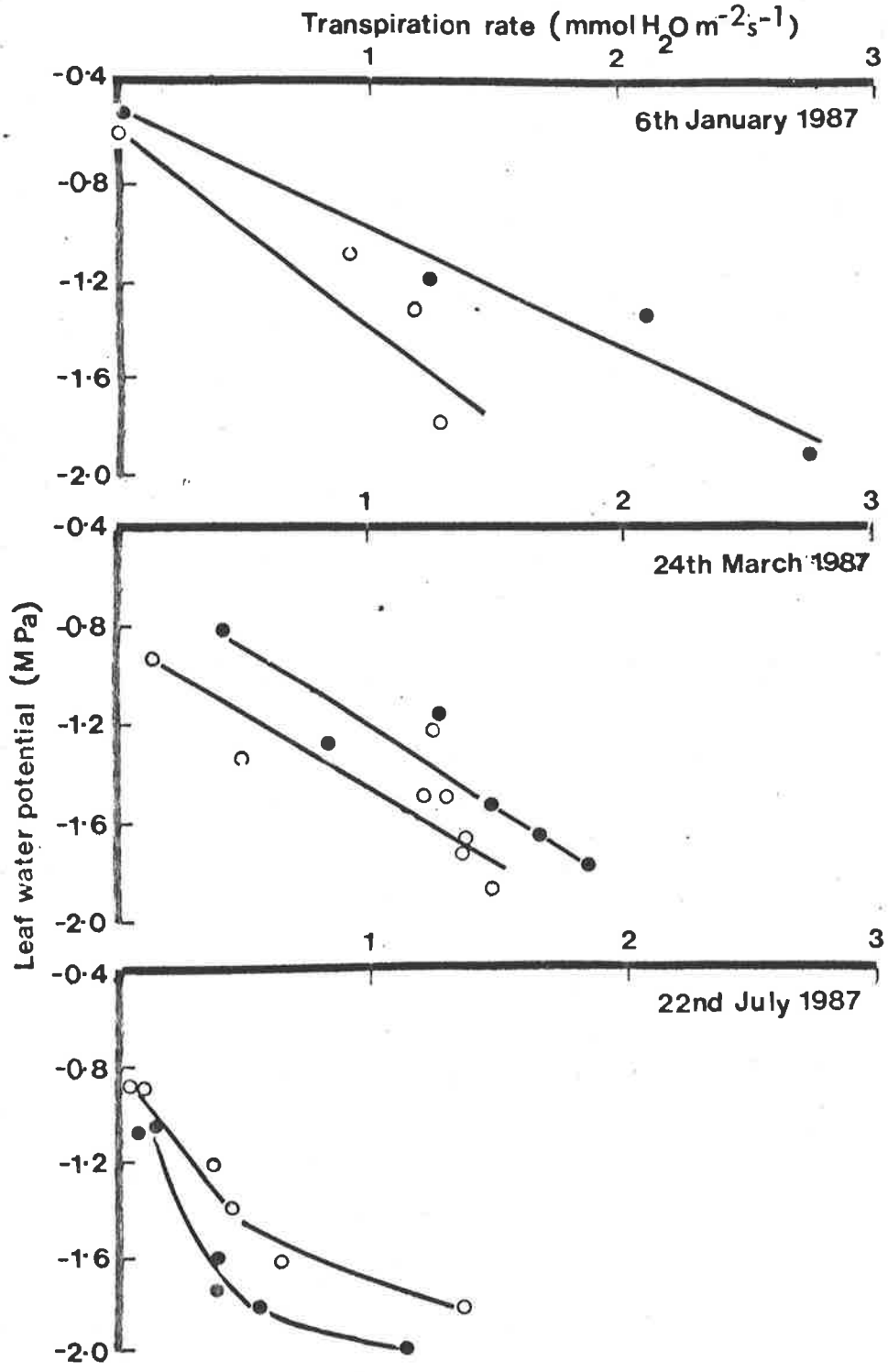
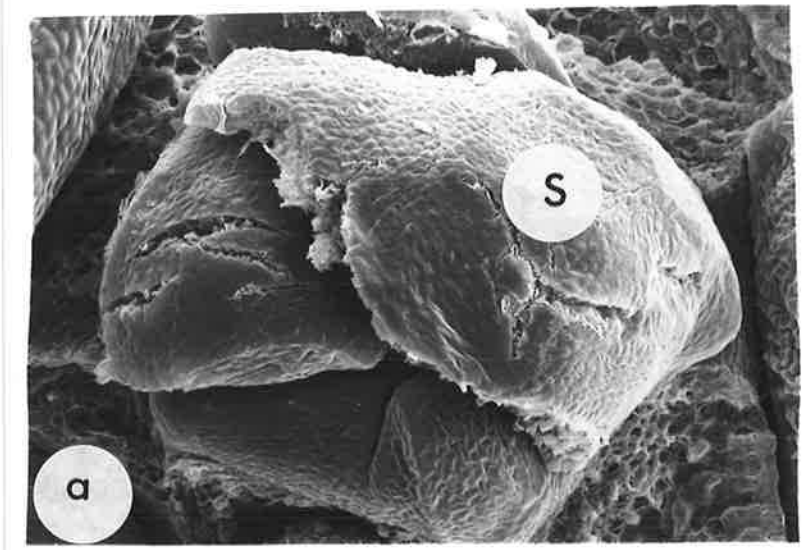


Figure 9.15 Relationship between transpiration rate and leaf water potential on 6 January, 24 March and 22 July, 1987. (●) leaves on plants irrigated with 5 mol m^{-3} NaCl; (○) leaves on plants irrigated with 20 mol m^{-3} NaCl. Each value is the mean of 4 measurements.

Figure 9.16. Development and differentiation of 'Washington Navel' orange buds. (a) Inflorescence apex showing terminal floral meristem with sepals (S) from tree irrigated with 20 mol m^{-3} NaCl on 21 June 1987; x 170. (b) Inflorescence apex from tree irrigated with 4 mol m^{-3} on 30 June 1987; x 150.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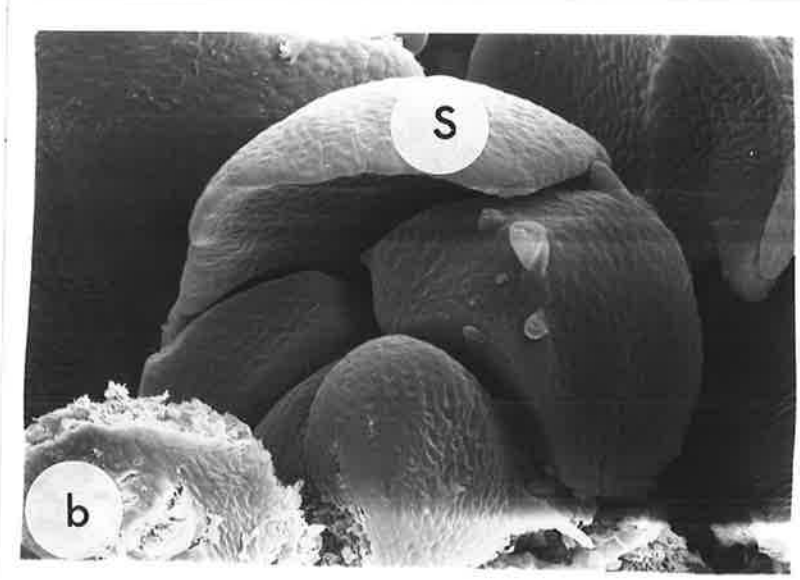
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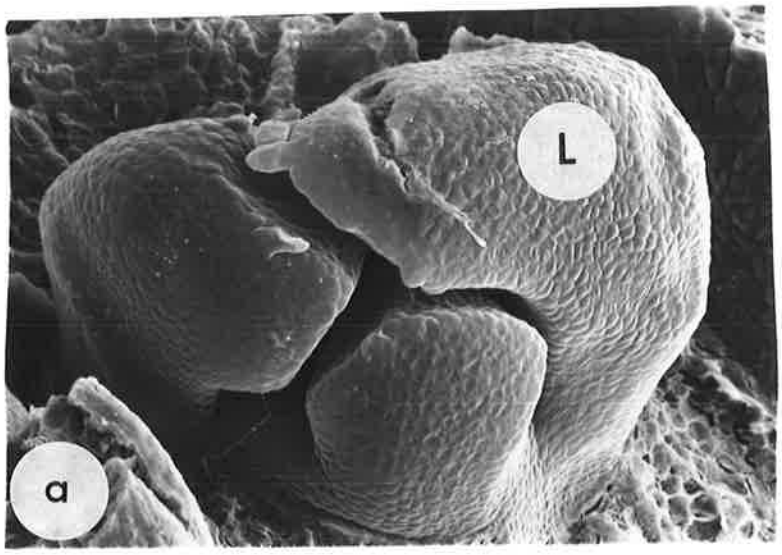
Figure 9.17. Development and differentiation of 'Washington Navel' orange buds. (a) Vegetative apex from tree irrigated with 20 mol m^{-3} NaCl sampled on 30 June 1987 showing leaves (L); x 170. (b) Dormant bud from tree irrigated with 4 mol m^{-3} NaCl on 30 June 1987; x 170.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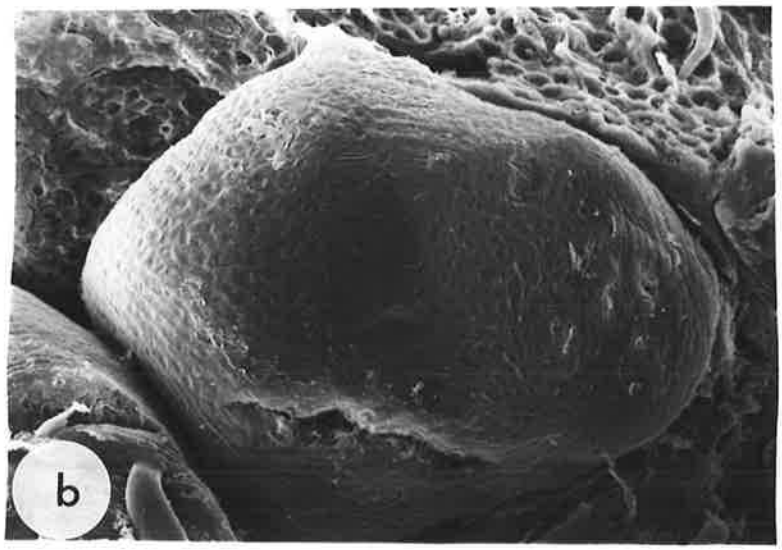
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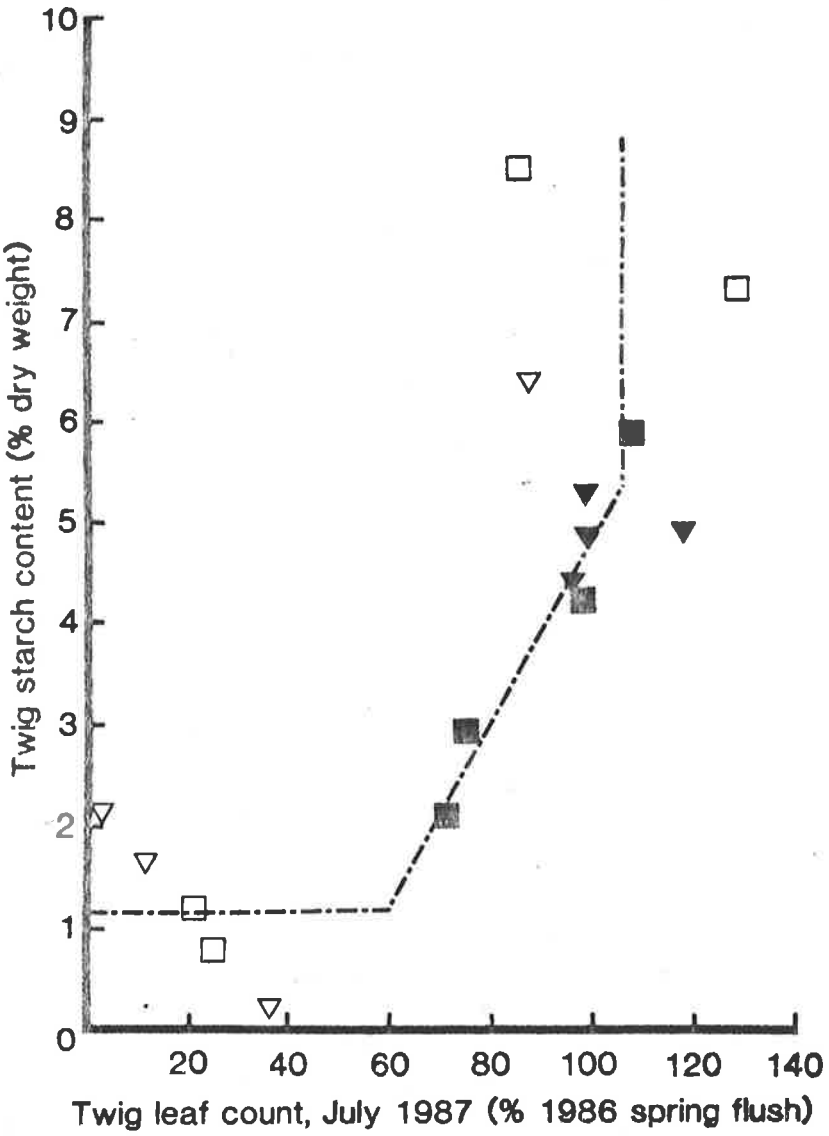

Figure 9.18 Relationship between twig leaf count and starch content at time of floral differentiation (■ ▼) twigs on trees irrigated with 4 mol m^{-3} NaCl; (□ ▽) twigs on trees irrigated with 20 mol m^{-3} NaCl. Squares: twigs on southerly aspect of tree; Triangles: twigs on northerly aspect of tree. Twigs had been tagged and the number of spring flush leaves determined in October, 1986. Values greater than 100% arise due to production of additional leaves in summer and autumn.

most of their 1986/87 growth, starch content varied from 5 to 9%. On the other hand, bud wood on tagged branches which had less than 60% of leaves from the initial spring flush remaining had only ~1% starch (Figure 9.18).

A similar relationship existed between the proportion of flowers produced in the 1987 spring flush and twig leaf count at the time of floral differentiation. A large degree of variation was observed on twigs that had little or no net loss of leaves over 1986/87. Branches that had retained less than 60% of the 1986 spring flush had few (if any) flowers (Figure 9.19). Flowering on salinised trees was much reduced in 1987 compared to 1986 (cf. Table 9.1).

9.4 DISCUSSION

Irrigation with high salinity water induced a myriad of responses in mature 'Washington Navel' trees *viz.* impaired flowering and fruit set, reduced rates of fruit growth, a delay in fruit maturity, impaired rates of leaf gas exchange and high rates of leaf abscission.

Despite little difference in the ratio of number of fruit:leaf area (Figure 9.1), fruit growth was initially slower on salinised trees (Figures 9.2 and 9.3). This may have been a consequence of current season vegetative growth forming a greater proportion of the total leaf area of salinised trees. Nii and Okamoto (1973), defoliating trees by artifical means, showed that leaves on flushes developed prior to spring make an important contribution to early growth of 'Satsuma' mandarin fruit. The number of such leaves was much reduced on salinised trees due to high abscission rates (Figure 9.6). Reserve carbohydrate within the tree also makes an important contribution to Stage I fruit growth (Akao *et al.*, 1981) and may also have been limiting for fruit growth as prior to bud break defoliated salinised trees had much lower twig carbohydrate levels than controls (Figure 9.18). It is however likely that current season vegetative growth also contributes significantly to early fruit development as growth rates of fruit on leafy (mixed) flushes was greater than those on leafless (reproductive) flushes irrespective of aspect or salinity. We did not investigate causes for slower growth rates on the southern aspect of trees.

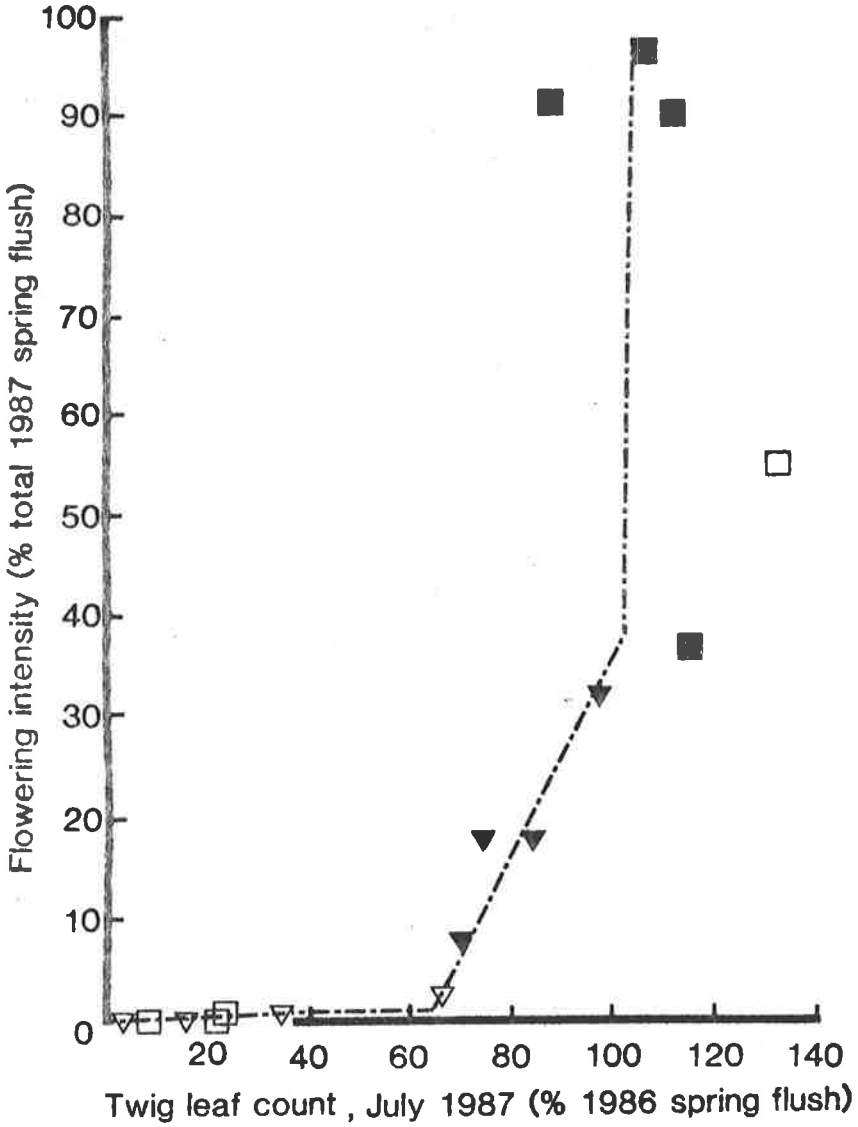

Figure 9.19 Relationship betwen twig leaf count at time of floral differentiation and flower production the following spring. (■ ▼) twigs on trees irrigated with 4 mol m^{-3} NaCl; (□ ▽) twigs on trees irrigated with 20 mol m^{-3} NaCl. Squares: twigs on southerly aspect of tree; Triangles: twigs on northerly aspect of tree. Twigs had been tagged and the number of spring flush leaves determined in October, 1986. Values greater than 100% arise due to production of additional leaves in summer and autumn.

This may have been due to lower CO_2 assimilation at low PPFD limiting carbohydrate production.

Fruit growth and abscission are related. Zucconi *et al.* (1978) observed that fruit abscising from the tree cease growth some 7 days prior to abscission. Similarly, Agusti *et al.* (1982) observed abscission only to occur for the smallest fruit on 'Washington Navel' orange trees. This suggests that poor fruit set on salinised trees (Table 9.2) may have been, as for fruit growth, associated with reduced carbohydrate availability within salinised trees. Improved fruit set following girdling is associated with improved carbohydrate status in 'Shamouti' orange (Schaffer *et al.*, 1985; Goldschmidt *et al.*, 1985). Modulation of fruit set by carbohdyrate status may thus be an important mechanism contributing to the "self-thinning" phenomena observed for various *Citrus* spp. Many deciduous horticultural crops require fruit thinning during early stages of fruit development to ensure adequate final fruit size.

Despite differences during Stage I of fruit growth (Figure 9.3) salinised fruit had the same maximum growth rate as controls during Stage II of development (Figure 9.2). This was despite the fact that a large reduction in leaf area of salinised trees occurred during this time (Figure 9.6). A constant supply of photoassimilate from current vegetative growth is therefore not a prerequisite for growth of 'Washington Navel' oranges during Stage II of fruit expansion. Reserve carbohydrate within salinised trees was most likely utilised for fruit growth during this period. Starch and sugar reserves have been estimated at 25 kg (>20% of total dry weight) for 'Wilking' mandarin with concentrations in roots being especially high (Goldschmidt and Golomb, 1982). Indeed, Schaffer *et al.* (1986) consider citrus roots to be "almost infinite sinks for carbohydrate". It is possible that increased demand for reserve carbohydrate by fruit on defoliated salinised trees may have resulted in reduced rates of root growth during autumn as well as low twig starch levels (Figure 9.18).

Later attainment of full fruit size was reflected in lower juice Brix readings for salinised fruit during the maturation period indicating lower levels of soluble sugars. This

delayed maturity may have considerable effects on financial returns received by growers, given the high prices demanded by early season fruit in the market place.

Although appreciable abscission of leaves was observed for salinised trees, leaves on such trees had reduced stomatal conductances on 6 January (Figure 9.10) prior to appreciable abscission of foliage (Figure 9.6). Differences in stomatal conductance between control and salinised leaves were greater in early morning hours when conductances were high and low VPDs occurred. The rapid stomatal closure on control leaves after 1000 hrs was most likely a consequence of high VPD induced stomatal closure; a well documented phenomenon in citrus (Kaufmann and Levy, 1976; Khairi and Hall, 1976a; Cohen and Cohen, 1983). Reduced stomatal conductances on salinised leaves were not a consequence of adverse water relations as not only were water potentials generally less negative during the daylight hours, but π was also more negative in salinised leaves (Figure 9.9) indicating higher turgor pressures than controls.

Little stomatal opening above 80 mmol m^{-2} s^{-1} was observed on 17 March, 1987, even for control leaves in early morning. As for 6 January there was no difference between control and salinised leaf stomatal conductances in the afternoon. As before this was not attributable to salinised leaves having adverse water relations. Neither was afternoon stomatal closure a consequence of poor water relations as Ψ actually increased after 1000 hr for control leaves. This was a consequence of reduced transpiration rates due to stomatal closure. This suggests that (as for 6 January) stomatal closure in control leaves after early morning maxima is a consequence of high VPDs. Indeed, as much higher conductances were observed on 24 March, 1987, environment rather than leaf age was responsible for the low conductances on 17 March, 1987 . Once again, gradual stomatal closure occurred after an early morning maximum for control leaves and stomata on such leaves were more sensitive to humidity than those on salinised leaves.

Reduced stomatal sensitivity to VPD on salinised 'Washington Navel' orange leaves was verified in the laboratory (Figure 6.6). Causes for this phenomenon remain unknown. Stomatal responses to VPD are most likely mediated by changes in epidermal turgor (Sheriff, 1984; Schulze, 1986). Bulk leaf turgors were not greatly different between

control and salinised leaves in summer and autumn, but it is possible that if sodium and/or chloride accumulates in the epidermal cells of salinised leaves epidermal turgor could be significantly greater than that of control leaves.

The highest control leaf stomatal conductance was observed on 10 June, 1987 at 1000 hrs being significantly greater than salinised leaves. It would have been interesting to see if this difference was maintained over the entire day with low winter VPDs prevailing but heavy cloud reduced PPFD below saturating levels (~600 μmol m^{-2} s^{-1}: Kriedemann, 1971; Syvertsen, 1984) before a heavy rainstorm (somewhat of a rarity at Loxton) aborted this attempt. Salinised leaf Ψ was much less negative than controls indicating, as previously, that adverse water relations are not responsible for low stomatal conductances on leaves from salinised trees under orchard conditions.

The diurnal pattern of stomatal conductance in mid-winter (22 July: Figure 9.14) was completely different than for summer, with maximum conductances being observed in the afternoon. Low leaf temperatures may have been responsible for low stomatal conductances in the morning. Possingham and Kriedemann (1969) observed a sharp reduction in 'Washington Navel' gas exchange once temperatures fell below 20°C. In contrast to previous observations, there was no difference between stomatal conductances on control and on salinised leaves. This may have been due to leaves most tolerant of high salt levels alone remaining on the tree. Alternatively, stomata on control leaves (which had lower maximum conductances than 6 weeks earlier) may have been limited by some factor not present in warmer months. One possibility is that low root temperatures could reduce rates of gas exchange in 'Washington Navel' orange leaves, a phenomenon observed in *C. limon* (Vinokur, 1957). Alternatively leaf age could have been involved. Nevertheless, it is clear that 'Washington Navel' orange trees are capable of reasonably high rates of gas exchange in mid-winter.

Conductance of water from soil to leaf was dependant upon salinity treatment and time of year (Figure 9.15). In January salinised trees had reduced hydraulic conductances. Exposure of citrus roots to saline water results in external suberisation (Walker *et al.*, 1984) which may have imposed an additional resistance to the flow of

water across the root. This has been hypothesized to occur for water stressed roots of *C. jambhiri* (Ramos and Kaufmann, 1979). As the season progressed, hydraulic conductances declined for trees irrigated with both salinities but this occurred to a far greater extent for control trees. Consequently Ψ was less negative at a given transpiration rate for leaves on salinised trees on 22 July, 1987. This was probably due to only a small number of leaves remaining on salinised trees at this time. Such trees most likely had a lower shoot/root ratio. Roots are considered to be the main barrier to water flow through plants both for *C. sinensis* (Castel, 1978 cited in Kriedemann and Barrs, 1981) and other plants (Passioura, 1982). On 22 July, not only was the hydraulic conductivity of control plants less than that for salinised trees but values were also much lower than previous. Such seasonal fluctuations in soil to leaf conductance have been previously observed for citrus (Moreshet and Green, 1984). Hydraulic conductivity of *C. jambhiri* decreases drastically at root temperatures below 15°C (Ramos and Kaufmann, 1979) suggesting that low hydraulic conductivities in the winter months may be a consequence of low soil temperatures. In contrast to previous observations the relationship between E and Ψ was curvilinear. The time of maximum transpiration was also that of maximum air temperature. Diurnal fluctuations in the upper soil profiles generally reflects diurnal changes in air temperature (Monteith, 1973). As trees were shallow rooting (Table 7.3) higher root conductances in response to increased soil temperature may have been responsible for the curvilinear relationship observed.

Throughout this study we consistently observed Ψ and leaf turgor (calculated *via* π) to be maintained at or above control levels for salinised leaves. Impaired rates of gas exchange with salinity were not therefore a consequence of adverse water relations. Nor does it appear that salinised leaves had lower conductances due to some form of feedback inhibition of carbohydrates on CO_2 assimilation. After December, carbohydrate levels were consistently lower in salinised leaves than for controls (Figure 9.5). This indicates that demand for carbohydrates from non-photosynthetic organs was not inhibited to the same extent as leaf gas exchange on salinised trees.

As for laboratory studies, impaired gas exchange of leaves on salinised trees is due to high foliar concentrations of sodium and/or chloride which progressively accumulate as the year progresses (Figure 9.8). The data do not allow us to evaluate the relative importance of sodium *versus* chloride toxicity in debilitating orchard citrus but this important question is considered within the wider context of Chapter 10.

Several points should be made about seasonal patterns in leaf chloride and sodium concentrations. Both in Australia (Reuter and Robinson, 1986) and elsewhere (Embleton *et al.*, 1973) leaf analysis is performed as a diagnostic tool and guide to fertilization. Sampling is usually done on 5 to 7 month old leaves (i.e. any time from January till March) because most major elements change little in concentration (dry weight basis) during this time (see Embleton *et al.*, 1973). Such analyses are however also used for diagnosis of possible salt toxicities on a routine basis where irrigation water quality is a problem. As evidenced by Figure 9.8, timing of leaf sampling is crucial for sodium and chloride analysis. These elements may more than double during the traditional sampling times. Where salinity effects on tree production are suspected it may be advisable to determine sodium and chloride in both early January and late March to determine the rate of increase in sodium and chloride over time. Decreased leaf sodium and chloride concentrations observed in autumn may have been due to either leaves with the highest ion levels abscising and/or export of sodium and/or chloride from surviving leaves into those about to fall from the tree (Till and Hyder, 1973).

As would be expected, unimpaired rates of Stage II fruit growth on salinised trees, despite reduced rates of gas exchange and high rates of leaf abscission, reduced twig carbohydrate content (Figure 9.18). Significantly, a similar relationship as that for starch also existed between leaf abscission and flowering (Figure 9.19). Such correlations between twig starch content and flowering are not uncommon for citrus. In particular, studies with biennial bearing trees have shown a good relationship between twig carbohydrate status and flowering intensity (Smith, 1976; Goldschmidt and Golomb, 1982). Furthermore, as in this study, correlations between reproductive development and starch concentration are always higher than for soluble sugars or total carbohydrate levels

(Jones *et al.*, 1974, 1975). Similar results have also been observed with avocado (Scholefield *et al.*, 1985). Physiologically speaking, the relationship between starch and flowering is somewhat surprising. It is hard to envisage a mechanism by which starch itself could influence the floral differentiation process. Research into mechanisms involved may provide valuable insights into internal processes influencing tree productivity. An influence of reserve carbohydrate on the balance between reproductive and vegetative development has obvious advantages for a tree. Extensive reproductive development during spring would have little benefit for salinised trees in terms of productivity. Extensive abscission of flowers due to low carbohydrate availability (Section 9.1.3) would most likely occur. Despite some leaf abscission only 4 months after bud development, extensive production of leaves in spring would have resulted in net carbohydrate gain for trees (Kriedemann, 1971). More extensive production of off-season flushes in salinised trees may also have served to improve tree carbohydrate status (Figure 9.18). Internal factors stimulating summer and autumn leaf production remain to be identified.

The difference in flowering between north and south sides of salinised trees observed in 1986 (Table 9.1) was not repeated again in 1987 (Figure 9.19). Overall level of flowering was however much reduced in 1987 compared to 1986. It may then be that the first signs of salinity stress are exhibited on the northern aspect of the tree. This could be a consequence of leaves with higher transpiration rates than on the shaded southern sides having higher sodium and/or chloride concentrations (Pitman, 1982). There was however no difference in foliar sodium or chloride concentrations between the two sides of control or salinised trees in February, 1987 (data not shown).

It is hard to evaluate the relative importance of reduced gas exchange *versus* leaf abscission on twig starch status and hence impaired flowering and fruit set for salinised trees. There is little doubt that those twigs on salinised trees having the lowest rates of abscission were also those with the highest starch content (Figure 9.18) and flowering levels (Figure 9.19). It is probable that low stomatal conductances and high rates of leaf abscission are correlated as both processes are most likely a consequence of excessive

accumulation of sodium and/or chloride. On more than one occasion we noted that salinised leaves with extremely low conductances left the tree along with the porometer cup! This suggests that for individual leaves stomatal closure occurs prior to leaf abscission. Despite obvious implications for productivity surprisingly little is known about the causes of leaf abscission from salinised plants (Addicott, 1982).

Irrigation water salinity is usually at its highest in late summer (Watson, 1977). From this study it would be expected that as fruit are already set on the tree at this time, full fruit size and hence yield should still be attained in years of high salinity even if reduced rates of gas exchange and appreciable leaf abscission occur in response to high foliar concentrations of sodium and/or chloride. However, flowering in the following year will be reduced if twig starch levels are decreased as a result of fruit growth in the absence of supporting carbohydrate production. Statistical analysis of the relationship between river water salinity and citrus yield of entire irrigation areas (Cole and McLeod, 1985) confirms this prediction. Murray river salinity in the year of bud initiation is well correlated with 'Washington Navel' orange production. Salinity in the year of harvest is not.

Whilst observations on both individual tree and regional scale suggest that low yields in response to salinity can be anticipated, the challenge is now to develop appropriate crop husbandry practices to avert such yield reductions. Means by which this could be achieved are considered within the wider context of the final discussion.

CHAPTER TEN

CONCLUDING DISCUSSION AND SPECULATION

Response of 'Valencia' orange scion to both water deficit and high rootzone salinity was dependent upon rootstock. In Chapter 8 we observed that when exposed to soil water deficit, previously unsalinised trees on Sweet orange had lower stomatal conductances and CO_2 assimilation rates than on Trifoliata. This was not due to leaves on Sweet orange having less favourable water relations; leaves on Trifoliata had less negative Ψ (of only ~0.3 MPa. It is unlikely that this would be sufficient to account for the rootstock effect on gas exchange). Furthermore there was no significant difference between rootstocks in amount of soil water remaining after 2 months without irrigation. This suggests that for a given soil moisture content, scions on Trifoliata are more tolerant of soil water deficit than those on Sweet orange and that this rootstock effect is not mediated *via* leaf water status. Thus, not only is the stomatal closure of citrus under drought conditions mediated by changes in soil rather than leaf water status (Chapter 2) but this response is dependent upon rootstock. Rootstock differences in supply of plant growth regulators (other than water) under conditions of soil water deficit may be responsible. Candidates include "hormones" such as gibberellins and cytokinins (Saidha *et al.*, 1983) or nutrients such as nitrogen and phosphorus (Syvertsen and Graham, 1985).

Although the gas exchange response of 'Valencia' orange to salinity was also modified by rootstock (Chapter 4) there are numerous indications that scion gas exchange responses were mediated by factors other than a reduced soil water potential generated by the presence of NaCl in the external medium. One of the major differences between the two stresses was osmotic adjustment in response to salinisation to a far greater extent than when soil water deficit was imposed. A significant proportion of the decrease in π for salinised leaves can be attributed to increases in leaf sodium and chloride concentrations. It was however observed that rootzone salinity could result in osmotic adjustment in the absence of increases in foliar sodium and/or chloride concentrations (Chapter 3). This was only ~0.6 MPa however. As soil water deficit resulted in comparable reductions in osmotic potential ranging from 0.2 MPa on Trifoliata (Chapters 2 and 8) to 0.4 MPa on Sweet orange (Chapter 8) this may have been a soil water response. The mechanism by which active osmotic adjustment is initiated awaits elucidation.

Although increased leaf sodium and chloride concentrations reduced π by as much as 1.4 MPa (Chapter 6), turgor maintenance in salinised leaves did not avert declines in CO_2 assimilation and stomatal conductance. In Chapter 4 when this response was modified by rootstock it was argued that modulation was via leaf sodium levels. This argument was reinforced in Chapter 6 where differences in scion response to salinisation were also explicable in terms of leaf sodium. Rootstock effects on leaf gas exchange under conditions of rootzone salinisations are therefore mediated *via* mechanisms different from those operating under soil water deficit.

Although a comparison of gas exchange behaviour of various rootstock/scion combinations in Chapters 3, 4 and 6 suggests that the sodium ion may be responsible for reductions in CO_2 assimilation and stomatal conductance with salinity, data for 'Prior Lisbon' lemon in Chapter 5 indicates that chloride can also be toxic to citrus leaf gas exchange under certain circumstances. This point is taken up later in the chapter.

Despite clear differences in leaf ionic content and water relations under the two stresses, soil water deficit and high rootzone salinity caused similar changes in citrus leaf gas exchange characteristics, *viz.*

(1) Parallel reductions in CO_2 assimilation and stomatal conductance except at very low rates of gas exchange where p_i was observed to increase.

(2) An initial reduction in CO_2 assimilation only at high p_i.

(3) A remarkable insensitivity of room temperature chlorophyll *a* fluorescence despite large reductions in gas exchange.

Such similarities make it tempting to speculate a common mechanism.

In Chapter 2 it was suggested that the primary effect of soil water deficit on citrus leaf gas exchange was stomatal closure. It was further speculated that an increase in thermal de-excitation of quanta could be responsible for observed mesophyll responses to soil water deficit. Could such a situation could also prevail under conditions of rootzone salinisation?

Although excessive ion accumulation resulted in (roughly) parallel reductions in mesophyll and stomatal function, the failure of stomata on salinised levels to respond to VPD in both laboratory (Chapter 6) and orchard (Chapter 9) indicates *a direct effect of*

salinity on stomatal functioning. As was argued in Chapter 9 this be a consequence of accumulation of sodium and/or chloride in the epidermis increasing epidermal tugor pressures above those of unsalinised leaves. The continued presence of high sodium concentrations in epidermal tissue could also reduce stomatal opening. For instance, it would be expected that apoplastic sodium would displace hydrogen ions from the guard cell walls. Given that proton extrusion is probably essential for stomatal opening (Zeiger, 1983) the subsequent lowering of apoplastic pH should impair this process (Raschke, 1979).

Alternatively, if sodium and/or chloride were to accumulate in epidermal and/or subsidiary cells, increased turgor pressures in these cells would serve to antagonise stomatal opening (Delwiche and Cooke, 1977). Edwards and Meidner (1977) observed reductions in stomatal aperture in response to increased subsidiary cell turgor pressure for a range of species. Attempts to correlate leaf π to stomatal aperture met with little success (data not shown) but, as the epidermis constitutes only ~1% of cell volume (Raschke, 1979) values of bulk leaf π most likely did not reflect those of the epidermis.

A third possibility accounting for reduced stomatal conductances in salinised leaves is that high concentrations of sodium and/or chloride in the transpiration stream result in peristomatal apoplastic salt accumulation causing a reduction in guard cell turgor (Yeo *et al.*, 1985). Given that cell walls are considerably less permeable to anions than cations this mechanism could be especially important in cases of high xylem chloride concentrations. In the absence of high cation concentrations (Chapter 5) little uptake of chloride by mesophyll cells would be expected to occur (Robinson and Smith, 1970). It would thus be expected that chloride would tend to accumulate at the sites of evaporation, i.e. walls of guard cells bordering stomatal cavities in such situations.

Depending upon rates of net import into leaves it is possible that any, or all, of these processes could be occurring. Clearly our understanding of how high sodium and/or chloride levels disturb stomatal functioning would be much advanced by studies on the inter and intracellular distribution of these ions. Unfortunately appropriate facilities were not available for such investigations.

Whilst humidity responses indicate a direct of salinisation on stomata it is possible that both stomatal and mesophyll components of leaf gas exchange were reduced simultaneously but independently. Such a phenomenon would be remarkable however as sodium toxicity, chloride toxicity (and soil water deficit) all cause equivalent reductions in mesophyll and stomatal processes. Simultaneous changes in both gas exchange components are likely therefore to be more than just coincidental. A gradual "winding down" in mesophyll CO_2 assimilation in response to stomatal closure (Chapter 2) is thus the most likely possibility for all gas exchange patterns observed.

The Dareton and Loxton experimental orchards differed markedly in terms of yield response and physiological consequences of rootzone salinisation. This was despite equivalent irrigation water salinities and the presence of the same rootstock at both sites. Whilst observations at Dareton showed no reduction in 'Valencia' orange gas exchange on Sweet orange rootstock in January, 1986, measurements at Loxton of 'Washington Navel orange on Sweet orange stock in early 1987 showed that stomatal conductances of leaves on salinised trees were reduced by ~50%. Comparisons of leaf ion concentrations at these times indicate that (as for laboratory studies) differences in gas exchange are most easily explained in terms of site dependent differences in leaf sodium concentrations.

Site	Sodium (mol m^{-3})		Chloride (mol m^{-3})	
	Control	Salinised	Control	Salinised
Dareton	4	20	20	197
Loxton	26	120	60	136

Table 10.1: Leaf sodium and chloride concentrations in January at Dareton (1986) and Loxton (1987). At both sites trees were on sweet orange stock irrigated with either river water containing ~5 mol m^{-3} NaCl (control) or ~20 mol m^{-3} NaCl (salinised).

Table 10.1 shows not only that for trees irrigated with 20 mol m^{-3} NaCl sodium levels were much higher at Loxton than at Dareton but that trees at Loxton irrigated with 5 mol m^{-3} had higher sodium concentrations than those at Dareton irrigated with 20 mol m^{-3}

NaCl. This was despite the same rootstocks and little difference between sites in terms of sodium and chloride concentrations in soil water (Chapter 7). As scion influences on leaf sodium concentrations are known (Chapter 6) it is possible that (other things being equal) 'Washington Navel' orange could have higher sodium concentrations than 'Valencia' orange. Our comparison of the two scions in Chapter 6 gave little indication that this should be so. Nor have other workers found differences between the two *C. sinensis* cultivars in terms of leaf sodium levels (Cooper *et al.*, 1952a; Ahmed and Al-Shurafa, 1984).

It could be that differences in sodium levels between the two sites are due to the Loxton experiment being in progress for a longer period of time than that at Dareton. This factor should not be dismissed lightly as regular February analysis of leaf sodiums has shown them to steadily increase from year to year (R.H. Howie and L.D. Pryor, pers. comm.). Whilst this factor (probably due to finite ability of root system to act as a "sink" for potentially toxic ions: see Chapman, 1968) complicates interpretation of site differences, when expressed on a "years of salinisation" basis sodium levels are still much higher at Loxton. Furthermore, differences in trial duration cannot explain why control trees at Loxton have higher sodium concentrations than salinised trees at Dareton. It must then be that the contrast between sites in terms of leaf sodium concentrations is a consequence of *soil physical characteristics*.

As mentioned in Chapter 7, the soil at Dareton is a loamy sand whilst that at Loxton is heavier being a light sandy clay loam. Furthermore, Dareton soil is free draining whilst that at Loxton has a perched water table at a depth of 2.5 metres. These factors combine to give greatly contrasting patterns in soil water content following irrigations. This can be seen by examination of soil moisture release curves (kindly supplied by Ms L. Prior and Mr T. Zimmermann) annotated with soil moisture before and after irrigations (Figure 10.1). At Dareton volumetric water content (θ_v) falls to ~16% after irrigation whilst at Loxton it is ~32% at this time. Subtraction of θ_v from total porosity (Ψ_{soil} = 0 kPa) gives a value for air filled porosity. It can be seen that at Dareton air filled porosity ranges from 20 to 24% whilst at Loxton it ranges from 10 to 22%. This

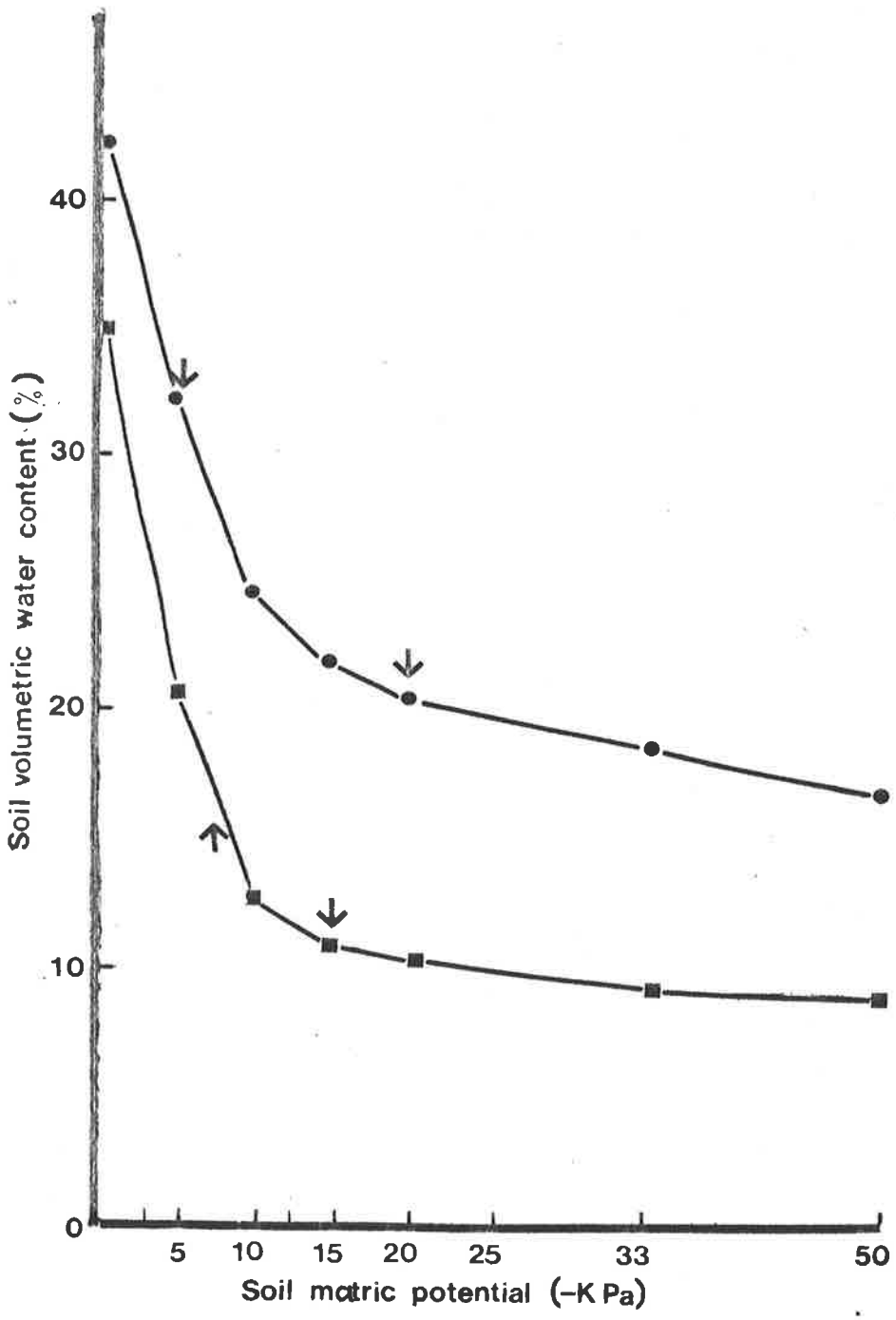

Figure 10.1 Moisture release curves for soil at Dareton (■) and Loxton (●). Points indicating soil moisture preceding and 24 hours after irrigation are indicated. Curves were kindly supplied by Ms L. Prior (Dareton) and Mr T. Zimmermann (Loxton).

indicates that, due to soil physical characteristics and poor drainage, trees at Loxton are subjected to extended periods of poor aeration whilst those at Dareton are not. Both Nel and Benne (1984) and Patt *et al.* (1966) consider air filled porosities 24 hours after irrigaton of less than 15% to impose limitations on citrus tree growth and productivity. Clearly, by this criterion then, the Loxton soil is not optimal for citrus growth. This is perhaps the reason for lower control leaf areas than Dareton. More importantly, in terms of impact of rootzone salinisation on citrus physiology, poor soil aeration markedly reduces sodium exclusion ability of Sweet orange roots (Labanauskus *et al.*, 1965, 1966). Of particular relevance are results of Labanauskus *et al.* (1966) who observed that saturating soil for an 8 hour period three times a month caused sodium content of sweet orange seedlings to be over 400% more than for seedlings watered to an average moisture content of 15% daily. For citrus good soil aeration is therefore crucial for efficient exclusion of sodium. It is therefore considered that poor soil aeration associated with the poorly draining light sandy clay loam at Loxton is responsible for the high foliar sodium contents and hence reduced yields at the Loxton site.

The genus *Citrus* has conventionally been considered chloride sensitive (Chapman, 1968). Our conclusion from both laboratory studies and orchard observations that sodium may be more important than chloride in debilitating citrus trees under saline conditions is therefore somewhat controversial. The view that chloride toxicity is responsible for reduced citrus yields has largely arisen from studies of Cooper and colleagues in the 1950s and 60s (Cooper and Edwards, 1950; Cooper and Gorton, 1952; Cooper *et al.*, 1952ab; Cooper *et al.*, 1958; Cooper 1961). Their results consistently showed visible toxicity symptoms to be associated with high leaf chloride concentrations. Their results are not however strictly applicable to orchard citrus in Australia as salt additions in their experiments consisted of equal concentrations of NaCl and $CaCl_2$ (reflecting composition of Rio-Grande or Colorado River water). Addition of NaCl only, as in laboratory and orchard studies in this thesis, is consistent with composition of Murray River water which typically has very low calcium levels (South Australian Engineering and Water Supply Department, 1985). As the presence of calcium drastically

reduces sodium uptake by lemon cuttings (Pearson, 1951 in Chapman, 1968) it is likely that low foliar sodium levels in Cooper's experiments were a consequence of high calcium concentrations in his irrigation water. Furthermore, there is some evidence that more chloride is absorbed when calcium is the dominant cation than when it is sodium (Brown *et al.*, 1953).

As well as being of only limited relevance to the Australian situation, Cooper's work is also open to criticism on technical grounds. Not only were observations on a strictly visual basis, but analysis of leaves for ion analysis was not always at the same time as visual toxicity descriptions. For example, in Cooper *et al.* (1958) leaf analysis was done on leaves sampled on 17 August, 1955 but visual symptoms reported in this paper were observed on November 11, 1956. Given that changes in leaf sodium and chloride that may occur *within* a year (Chapter 9) any attempt to relate visual symptoms to leaf ionic content 15 months earlier is (to say the least) invalid. Furthermore, Cooper's experiments were often accompanied by extremely rapid rises in leaf chloride concentrations. For example data in Cooper (1961) show leaf chloride concentrations to rise to ~500 mol m^{-3} in 42 days. As argued in Chapter 5, such rapid rises may lead to responses not observed when increases in leaf chloride concentrations more relevant to the orchard situation occur (Chapter 9).

The conclusion that, under Australian conditions at least, sodium is responsible for reducing citrus yields and that foliar sodium levels may be dependent upon soil aeration raises some interesting possibilities in terms of irrigation management. It would be expected that irrigation in excess of a tree's requirements would lead to poor aeration and hence increases in tree sodium levels. Examination of irrigation and net Class A Pan evaporation (E_p) records for the years 1981-1986 at Loxton shows this to be the case. Given that citrus water use (E_o) is typically ~60% E_p (Kriedemann and Barrs, 1981) any irrigation much above this amount (plus say 10% for leaching and sprinkler distribution) is in excess. The large year to year variation in E_o/E_p at Loxton (the reasons for which are unknown!) clearly reveals a relationship between overwatering and increases in leaf sodium concentrations (Figure 10.2). It has been conventional wisdom that in times of

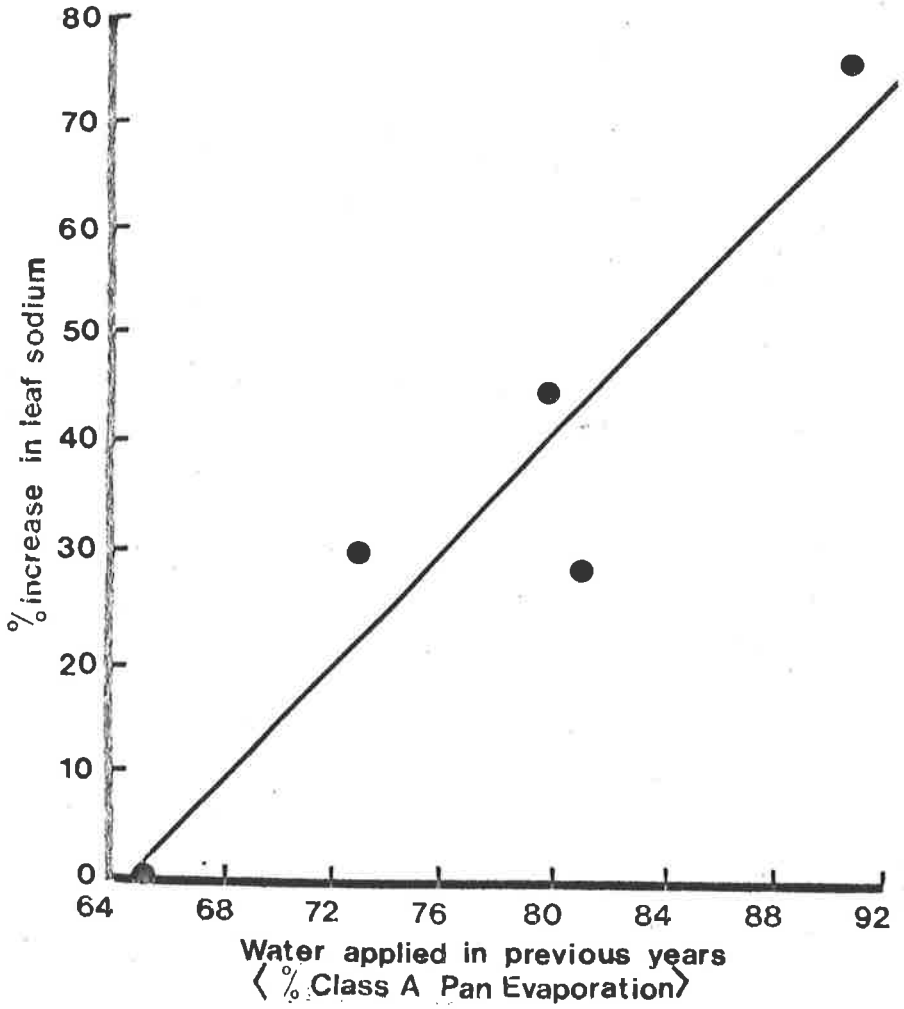

Figure 10.2 Relationship between annual increase in leaf sodium concentration and extent of over-irrigation in the previous calender year. (r=0.93)

high irrigation water salinity excess water should be applied to leach salts from the rootzone (Watson, 1977). This approach may thus be counter productive. Dareton observations indicate that despite high rootzone salinities little increase in leaf sodium concentrations will occur if good soil aeration is maintained. Where soils are heavy and high salinity irrigation water in summer or autumn is a problem it may be better to only replace soil water lost and to avoid irrigation to full field capacity. Improved aeration under such a strategy may well offset any accumulation of salts in the rootzone. Leaching irrigations could then be performed in the winter months when little cation absorption by roots occurs (Roy and Gardner, 1945). Under such a scheme, despite high irrigation water salinity in summer, impaired gas exchange, excessive leaf absicssion and associated reductions in flowering, fruit set and yield the following year (Chapter 9) could well then be avoided.

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APPENDIX 1

CALCULATION OF INTERNAL CONDUCTANCES IN *CITRUS* LEAVES

A1.1 Gas phase conductance to CO_2 (g_{ias})

A1.1.1 Effective path length from stomatal cavity to mesophyll (δ)

The entire cross-section of a leaf is not available for diffusion. Rather, flow is restricted to the intercellular spaces. It then follows:

$$\delta = d\left(\frac{A}{A_{ias}}\right) \qquad \text{(A1.1)}$$

where d = distance from mesophyll cells to stomatal cavity

A_{ias} = volume of intercellular air spaces

A = volume of leaf

Syvertsen and Smith (1984) measured d to be 300 μm and $\frac{A}{A_{ias}}$ to be 3.3. Using these values δ = 300 (3.3) μm ~ 1 mm.

A1.1.2 Calculation of g_{ias}

Assuming the intercellular spaces to act as an unstirred layer (Nobel, 1974)

$$g_{ias} = \frac{D_a.P}{\delta RT} \qquad \text{(A1.2)}$$

where P = atmospheric pressure

D_a = diffusion coefficient of CO_2 in air (0.151 $cm^2\ s^{-1}$)

R = gas constant (8.314 J $mol^{-1}\ K^{-1}$)

T = leaf temperature (298 K)

$$g = \frac{(0.151 \times 10^{-4})\ (1.013 \times 10^{5})}{(1 \times 10^{-3})\ (8.314)\ (298)}$$

$$= 0.625 \text{ mol } CO_2\ m^{-2}\ s^{-1}$$

A1.2 Conductance of suberised layer to CO_2

The question as to the extent that a suberised layer will affect diffusion of CO_2 is not an easy one. Conductances of potato periderm layers (consisting mainly of suberin) are similar to those of leaf cuticles (Schönherr, 1982). Suberised layers are also similar to most cuticular layers in that permeability to H_2O is dependent upon water activity in the vapor phase. This suggests that water continuity in discrete pores exists across such layers with liquid/vapor transition taking place at the membrane/vapor interface (Schönherr, 1982). Diffusion of CO_2 across such a layer will therefore be dependent upon the extent of such water filled interstices. For cuticles, Schönherr (1976) estimates a tortuosity factor (θ) of less than 0.34. That is, the diffusion path of water molecules is ~3 times longer than the thickness of the membrane. As suberised layers have a similar permeability to cuticles, we use a similar valve ($\theta = 0.3$).

A further problem is the dependence of CO_2 diffusion on the pH of water in the pores. In studies with cuticles, solutions with pH = 6 are usually used. Using this value, according to Nobel (1974) at 25°C .

$$K = \frac{([CO_2]^{water} + [H_2CO_3]^{water} + [HCO_3^-]^{water})}{[CO_2]^{air}} = 1.1 \qquad \text{(A1.3)}$$

where K is the partition co-efficient between CO_2 dissolved in water and the adjacent gas phase.

Conductance of the suberised layer (g_{sub}) can then be written

$$g_{sub} = \left(\frac{A_{mes}}{A}\right)\left(\frac{D_w\ \theta\ K\ P}{dRT}\right) \qquad \text{(A1.4)}$$

where A_{mes} = total area of mesophyll cell walls exposed to intercellular air spaces

A = area of one side of the leaf

D_w = diffusion coefficient of CO_2 in water (1.75×10^{-5} cm^2 s^{-1})

d = thickness of suberised layer

Using (A_{mes}/A) = 17.7 (Turrell, 1936) for a 1 μm suberised layer (Scott, 1948).

$$g_{sub} = (17.7)\left(\frac{(1.75 \times 10^{-9})\,(0.3)\,(1.1)\,(1.013 \times 10^{5})}{(1 \times 10^{-6})\,(8.314)\,(298)}\right)$$

$$\sim 0.425 \text{ mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$$

A1.3 Conductance of cell wall to CO_2 (g_w)

As for the suberised layer, CO_2 will diffuse through the water filled pores of the cell wall. Using d = 1 μm, θ = 0.33, and a pH of 6 (Nobel, 1974) g_w is thus calculated:

$$g_w = (17.7)\left(\frac{(1.75 \times 10^{-9})\,(0.33)\,(1.013 \times 10^{5})}{(1 \times 10^{-6})\,(8.314)\,(298)}\right)$$

$$\sim 0.425 \text{ mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$$

APPENDIX 2

EFFECT OF LOW INTERNAL CONDUCTANCE ON CO_2 ASSIMILATION IN CITRUS LEAVES

If we take a situation where A is limited by rubisco activity (von Caemmerer and Farquhar, 1981).

$$A_{210} = V\left(\frac{p_c - \Gamma^*}{K_c(1 + O/K_o) + p_c}\right) \qquad \text{(A2.1)}$$

where V = maximum activity of rubisco (CO_2)

Γ^* = CO_2 light compensation point

K_c = Km of rubisco for CO_2

K_o = Km of rubisco for O_2

O = partial pressure of oxygen in chloroplast (210 mbar)

A_{210} = assimilation rate when O = 210 mbar

Combining Equations A2.1 and 1.11

$$A_{210} = V.\left[\frac{p_i - \frac{A_{210}}{g_i} - \Gamma^*}{(K_c(1+ O/K_o)) + p_i - A_{210}/g_i}\right]$$

$$A_{210}\left[(K_c(1 + O/K_o)) + p_i - \frac{A_{210}}{g}\right] = V\,(p_i - (A_{210}/g_i) - \Gamma^*)$$

Multiplying by g_i A_{210} and rearranging

$$g_i\left[A_{210}\,(K_c(1 + O/K_o) + p_i\right] - Vg_i\,(p_i - \Gamma^*) = A_{210}^2 - VA_{210}$$

$$A_{210}^2 - [(g_i(K_c(1 + O/K_o)) + P_i) + V]\,A_{210} + g_i\,V(p_i - \Gamma^*) = 0$$

This quadratic can be solved in the normal way.

$$A_{210} = \frac{[g_i\,(K_c(1 + O/K_o)) + p_i] + V - \sqrt{[[g_i\,(K_c(1+O/K_o)+p_i]+V]^2 - 4g_i\,V(p_i - \Gamma^*)}}{2} \qquad \text{(A2.2)}$$

We can then calculate A_{210} for given values of g_i , p_i and V. p_c can also be calculated and the response of A_{210} and p_c to variation in V (p_i = 250 μbar) is shown in Figure A2.1. (g_i = 150 and 750 mmol CO_2 m^{-2} s^{-1} bar^{-1} : K_c = 310 μbar: K_o = 164 mbar).

This illustrates that the low CO_2 assimilation rates of citrus leaves are inherent if our estimate of g_i is correct.

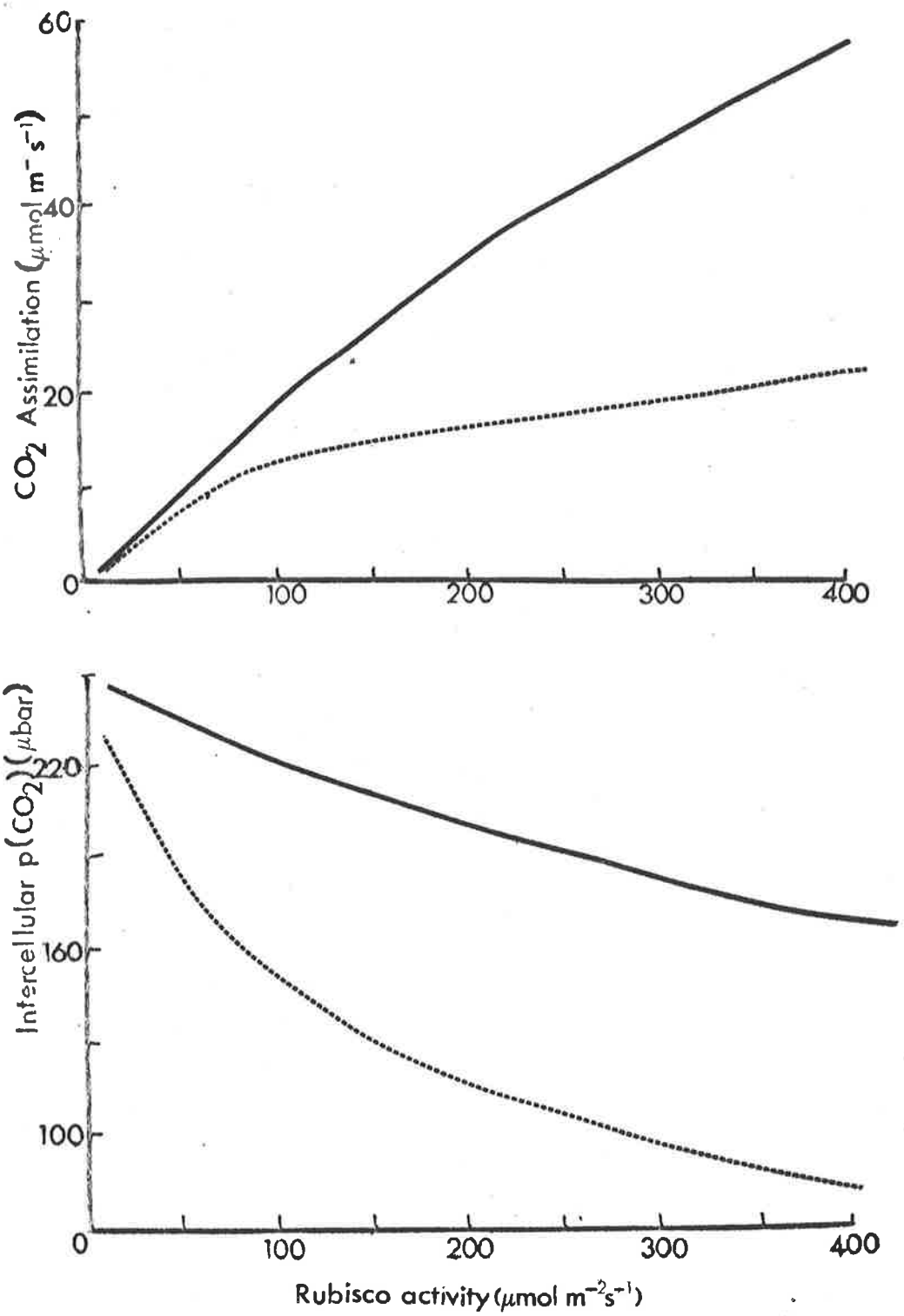

Figure A2.1. Effect of internal conductance (g_i) on relationship between rubisco activity and CO_2 assimilation and intercellular partial pressure of CO_2. (——) g_i = 0.750 mol m^{-2} s^{-1} bar^{-1}; (- - - -) g_i = 0.150 mol m^{-2} s^{-1} bar^{-1}.

APPENDIX 3

RELATIONSHIP BETWEEN INTERNAL CONDUCTANCE AND THE EXTENT OF CO_2 ASSIMILATION INHIBITION BY OXYGEN

In a similar fashion to the derivation for A_{210} in Appendix 2, assimilation in the absence of oxygen (A_0) may also be expressed as a function of g_i, p_i and V. The derived equation is equation (A2.2) with $0 = \Gamma^* = 0$

$$A_0 = \frac{(g_i(K_c+p_i) + V) - \sqrt{(g_i(K_c+p_i) + V)^2 - 4g_iVp_i}}{2} \qquad \text{(A3.1)}$$

This can be combined with Equation A2.2 to calculate %inh according to

$$\%\text{inh} = 1 - \frac{A_{210}}{A_0} \times \frac{100}{1}$$

%inh is dependent upon V as this interacts with g_i in determining p_c. The effect of V on % inh is shown in Fig. A3.1. %inh decreases with increasing g_i. This is to some extent counterintuitive. As p_i is lower at high g_i (Fig. A2.1) it would be expected that %inh would be higher. The reason for this apparent discrepancy is that [p_c(210 mbar) – p_c(21 mbar)] decreases with g_i and increases with V. We cannot therefore account for high %inh in *Citrus* spp. by the presence of low g_i. It could however be that the relationship in Fig. 1.9 is due to leaf to leaf variation in V (and hence A) coupled with low g_i.

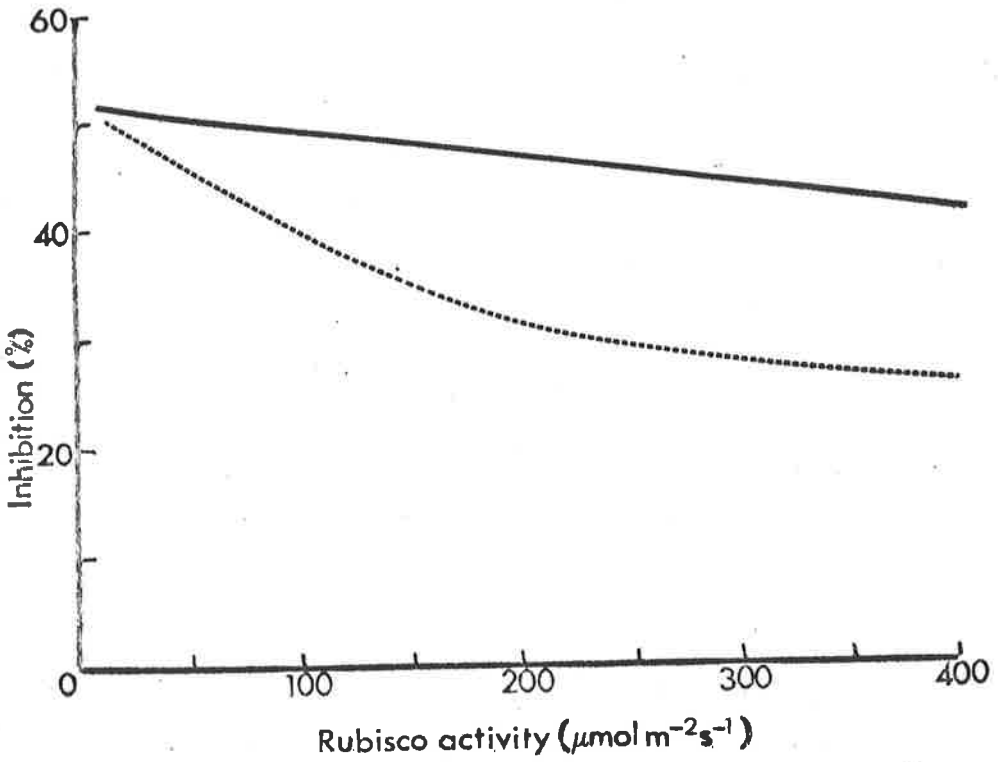

Figure A3.1. Effect of internal conductance (g_i) on % inhibition of CO_2 assimilation by atmospheric levels of oxygen (21 mbar). (——) g_i = 0.750 mol m^{-2} s^{-1} bar^{-1}; (- - - -) g_i = 0.150 mol m^{-2} s^{-1} bar^{-1}.

PART D

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