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Growth and physiology of spring wheat under saline conditions.

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GROWTH AND PHYSIOLOGY OF SPRING WHEAT UNDER SALINE CONDITIONS

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SUMMARY

A series of experiments were carried out in solution culture in growth rooms and a glasshouse, to study the effects of salinity on leaf extension rate, ion concentrations, sap osmotic pressure, net photosynthesis and related parameters, stomatal frequency, specific leaf weight and a number of agronomic parameters of spring wheat. Rate of net photosynthesis, transpiration rate, stomatal conductance and sub-stomatal carbon dioxide concentration per unit area of leaf were determined using an Infra-red Gas Analyser.

Experiments 1 and 3 were conducted in growth rooms set at a temperature cycle of 24°C/16°C day and night and photoperiod of 16 hours. The seedlings received light from a bank of 125W fluorescent 'warm white' lights which provided between 200-300 μmol m^{-2} s^{-1} photosynthetically active radiation at initial plant level. Experiments 2, 4 and 5 were carried out in a glasshouse with no control of light and temperature.

In Experiment 1 the salinity treatments tested were control (0 mol m^{-3} NaCl), 'constant' and 'variable' salinity. In the constant salinity treatment plants were grown at 100 mol m^{-3} NaCl all the time after initial salt stress. In the variable salinity treatment a 12 day cycle was repeated with daily increments of 10 mol m^{-3} NaCl after initial salt stress of 50 mol m^{-3} NaCl till it reached to 150 mol m^{-3} NaCl. During the final two days of the cycle salinity was stepped down from 150 to 100 to 50 mol m^{-3} NaCl. In Experiment 2 the salinity levels tested were 0, 50, 100 and 150 mol m^{-3} NaCl. $CaCl_2$ was added in this and later experiments at 20:1 (mol Na^+:mol Ca^{2+}) ratio. The results of the both Experiments 1 and 2 suggested that salinity had no effect on leaf appearance stage but tiller production was decreased. Salinity decreased leaf extension rate and final leaf length but leaf extension duration was not affected. Although leaf extension rate was the main factor influencing final leaf length, there were no consistent quantitative relationships between these parameters in different leaves and at different salinity levels. Plants in variable salinity performed better than those in constant salinity but these treatments were not significantly different and gave similar results. The results of Experiment 2 showed that a gradient of Na^+ and Cl^- concentrations was found in different leaves. Higher Na^+ and Cl^- concentrations were found in lower leaves than in expanding leaves. Calculated Na^+ and Cl^- contents (ion concentrations x dry weight) suggested that these ions were mainly located in roots, stem and tillers irrespective of salinity levels. The effect of salinity was to increase concentrations of leaf Na^+, Cl^- and sap osmotic pressure in the youngest fully expanded leaves whereas K^+ concentration was inconsistently affected. When gas exchange measurements were made in situ on leaves, light intensity showed wide

variation due to movement of clouds. Variations in light intensity and absence of any equilibration prior to measurements made it difficult to detect any effects of salinity on gas exchange.

Therefore to determine the effects of salinity on gas exchange in expanding and senescing leaves, in Experiments 3, 4 and 5, a strong light source capable of providing photon flux densities at or near light saturation for gas exchange was used. In Experiments 3 and 4 light response curves were produced using neutral density filters. Using an exponential model, maximum net photosynthesis, photosynthetic efficiency, photon flux compensation point and dark respiration for salinities and leaf insertions were calculated. In Experiment 3 the salinity levels tested were 0, 100 and 200 mol m^{-3} NaCl. Salinity decreased green lamina area, maximum and net photosynthesis, stomatal conductance, transpiration rate, leaf productivity but increased dark respiration and photon flux compensation point. Photosynthetic efficiency and transpiration efficiency were inconsistently affected.

In Experiment 3 at 200 mol m^{-3} NaCl leaf 6 senesced rapidly. Therefore in Experiment 4 the salinity levels tested were 0, 75 and 150 mol m^{-3} NaCl. In Experiment 4 the parameters studied were identical to those in Experiment 3 except that the measurements were performed on leaf 5 and the flag leaf. In Experiment 4 a similar trend for gas exchange parameters was noted at 0 and 150 mol m^{-3} NaCl but at 75 mol m^{-3} NaCl Pn was higher than in the control due to delayed senescence. In both Experiments 3 and 4 leaf sap Na^+, Cl^- and osmotic pressure increased and Pn decreased during senescence but there were no consistent relationships between these parameters for different leaves and salinity treatments.

Experiments 2, 3 and 4 suggested that salinity increased stomatal frequency per unit leaf area but stomatal frequency per leaf and specific leaf weight were inconsistently affected.

Experiment 5 was conducted to examine the effects of salinity on changes in gas exchange in the flag leaf and two penultimate leaves simultaneously. The salinity levels tested were 0, 75 and 150 mol m^{-3} NaCl. The leaf x salinity interaction showed that salinity had larger effects on the flag leaf than leaves 2 and 3. The leaf x salinity interaction was significant for leaf temperature, net photosynthesis, stomatal conductance, transpiration rate and transpiration efficiency but not for sub-stomatal carbon dioxide concentration. Salinity significantly decreased all the yield components and grain yield. The results of these experiments suggest that salinity had large effects on photosynthesis, dry matter production and grain yield and that ion concentrations do not determine the observed changes in net photosynthesis with leaf age in salt stressed plants.

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LIST OF ABBREVIATIONS AND SYMBOLS

α	Photosynthetic efficiency
A	leaf lamina area (cm^2)
ASU	air supply unit
$CaCl_2$	calcium chloride
Ci	sub-stomatal carbon dioxide concentration ($\mu l\ l^{-1}$)
CO_2	carbon dioxide
CV	coefficient of variation
°C	degree celsius
DAE	days after emergence
DAS	days after salt stress
df	degrees of freedom
E	transpiration rate (mol $m^{-2}\ s^{-1}$)
Emax	response of infra-red gas analyser to infinite water concentration
F	F statistic
FLL	final leaf length (mm)
GLA	green lamina area (cm^2)
GER	gas exchange rate
g_s	stomatal conductance (mol $m^{-2}\ s^{-1}$)
HI	harvest index
H_2O	water
HSD	honestly significant difference
I	photon flux density
Ic	photon flux compensation point
IRGA	Infra-red gas analyser

LAS	leaf appearance stage
LDWT	lamina dry weight
LED	leaf extension duration (days)
LEG	leaf extension growth (mm)
LER	leaf extension rate (mm day^{-1})
LFWT	lamina fresh weight
LP	leaf productivity (μmol s^{-1})
LSD	least significant differences
Mol m^{-3}	millimolar concentration
NaCl	sodium chloride
Na_2SO_4	sodium sulphate
NS	not significant
OP	osmotic pressure (mmol kg^{-1})
P	photosynthesis
PAR	photosynthetically active radiation (μmol m^{-2} s^{-1})
Pg	gross photosynthesis
Pgmax	maximum gross photosynthesis
PLC	'Parkinson' leaf chamber
Pmax	maximum photosynthesis
Pn	net photosynthesis (μmol m^{-2} s^{-1})
Pnmax	maximum net photosynthesis
P(0.05)	probability at 5 percent level
Q	light intensity
r	linear correlation coefficient
rb	boundary layer resistance (m^{-2} s mol^{-1})
Rd_c	calculated dark respiration
Rd_o	observed dark respiration

RH	relative humidity
s^{-1}	per second
SF	stomatal frequency per unit area (mm^2)
SF_l	stomatal frequency per leaf
SLW	specific leaf weight
TE	transpiration efficiency
T_a	air temperature
TAS	tiller appearance stage
TGW	1000-grain weight
T_l	leaf temperature
vpm	vapour parts per million
W	Watt
UK	United Kingdom
WUE	water use efficiency
∞	infinity
*	significant at stated probability

CHAPTER 1

INTRODUCTION

Salinity is perhaps the most important problem affecting irrigated agriculture in the world. The problem exits even in some of the world's sub-humid and humid regions especially in coastal areas, but is most widespread, severe and threatening in arid and semi-arid lands. Estimates of the extent of saline soils vary widely from between 324 to 400 million (Brinkman 1980; Epstein *et al.* 1980; Ponnamperuma 1984) and 950 million hectares (Massoud 1974; Kovda 1980; Wyn Jones and Gorham 1986; Abrol, Yadav and Massoud 1988). Out of these saline soils, 230 million hectares are moderately saline and have crop production possibilities (Ponnamperuma 1984). According to another estimate, about 13% (Mudie 1974) of land under cultivation (1.477 billion hectares: FAO 1987) and 30 to 50% of irrigated land (from 230 to 240 million hectares: Kovda 1980) is salt affected (Maas and Hoffman 1977b; Kovda 1980). Australia has the largest area of saline and sodic soils: 357.24 million hectares, followed by Asia: 316.54 million hectares, Latin America: 131.13 million hectares, Africa: 80.44 million hectares and North America: 15.76 million hectares (derived from data reported by Abrol,Yadav and Massoud 1988). The countries which rely heavily on irrigation water for their crop production are most affected. At present 60-80% of the irrigable area of Pakistan (Mohammad 1978, 1983), 50 to 75% in Iraq (Kovda 1977; Abdul-Halim *et al.* 1988) and 30% in Egypt suffer to varying degrees from salinity (Kovda 1977). In addition, millions of hectares of potentially irrigable land could

become saline if put into production.

Salinity can be simply defined as the presence of excessive concentrations of soluble salts. In arid regions, high temperatures during the summer season cause severe evaporation losses, hence leaving behind large amounts of salts. In Pakistan like other arid and semi-arid regions of the world, precipitation is inadequate to leach down salts out of the root zone with the result that soluble salts accumulate in the soil profile with Na^+ as the dominant cation on the exchange sites. Soils are regarded as saline if they contain enough soluble salts to harm plant growth (Nieman and Shannon 1976). The problem may become further exacerbated by use of low quality (i.e. saline) irrigation water. Salinity is caused not only by sodium chloride but also by sodium carbonate, sodium bicarbonate and sodium sulphates (Bernstein 1975). The relationships of these salts to each other as well as other ions like potassium, calcium and magnesium are important and may differ greatly at different sites.

Wheat (*Triticum aestivum*) L. is the premier cereal crop grown by man today. During 1990, it was grown on an area of about 231.55 million hectares with total production of 595.15 million metric tonnes (FAO 1991). Wheat alone accounts for a quarter of the world's food requirements whereas the other related cereals barley, rye and triticale together account for an additional 15-18 percent (Wyn Jones *et al.* 1984). Wheat is well adapted to the semi-arid

regions. It is also among the moderate salt resistant crop species (Bole and Wells 1979; Maas 1986) but is more sensitive than grain sorghum and barley (Maas 1986). Wheat is widely grown in regions where salinity is also a problem and it is expected that further improving its resistance to salt should result in higher yields in salt affected regions. During the past 40 years considerable attention has been given to the effects of sodium chloride on plant growth. The effects of salinity on yield have been documented by a number of workers (e.g. Kumar 1983; Kumar *et al.* 1983; Haqqani *et al.* 1984). Breeding and biochemical aspects have been studied (e.g. Greenway and Thomas 1965; Storey and Wyn Jones 1975,1978; Downton 1984; Gorham *et al.* 1986a,b; Termaat and Munns 1986; Shah *et al.* 1987; Gorham 1990; Gorham *et al.* 1990).

Gallagher and Biscoe (1979) showed that total dry matter production by cereals is correlated with the amount of photosynthetically active radiation (PAR) intercepted. This depends upon leaf growth and the balance between photosynthesis and respiration. Reduction of leaf area is often the first indication of salt stress and this has reported by many workers (e.g. Bernstein 1975; Termaat *et al.* 1985; Munns and Termaat 1986; Iqbal 1988; Kemal-ur-Rahim 1988). Some previous experiments have shown that leaf growth and photosynthetic rates are decreased under saline conditions (Yeo *et al.* 1985; Heuer and Plaut 1989) while some others have shown that photosynthetic rate is not much affected (Munns *et al.* 1982; Kaiser *et al.* 1983; Robinson *et*

al. 1983; Curtis and Lauchli 1986; Kemal-ur-Rahim 1988; Rawson *et al*. 1988a) as compared to leaf growth.

The aim of these studies was to examine the effects of salinity on leaf growth and photosynthesis of spring wheat by varying the type and levels of salinity. The literature related to these aspects is reviewed in Chapter 2. Experiments investigating the effects of salinity on leaf growth are discussed in Chapters 3 and 4. Experiments investigating the equilibration period for CO_2 exchange measurements of leaves in the light chamber are presented in Chapter 5. Experiments investigating the effects of salinity on CO_2 exchange by different leaf insertions in expanding and senescing leaves are discussed in Chapters 6, 7 and 8. There is a short discussion and conclusions of each experiment at the end of each chapter concerned. Chapter 9 contains conclusions of the main results of these experiments.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Plants can undergo one or more forms of stress during various stages of their life cycle. The effects of environmental stresses on crop growth and yield have been reviewed by some workers (see, for example, Bernstein 1966; Beringer and Trolldenier 1978; Vaadia 1985). The mechanisms of salt tolerance of the halophytes have been reviewed by Flowers *et al.* (1977); Läuchli and Epstein (1984). Physiology and mechanisms of salt tolerance in non-halophytes have been reviewed by Bernstein and Hayward (1958); Maas and Neiman (1978); Greenway and Munns (1980); Yeo and Flowers 1984; Gorham *et al.* (1985b); Rashid (1986); Munns and Termaat (1986); Shah (1987) and Kemal-ur-Rahim (1988). Salt tolerance of various plants has also been reviewed by Bernstein (1963); Carter (1975); Maas and Hoffman (1977a); Wyn Jones (1981); Flowers and Läuchli (1984); Downton (1984); Maas (1986); Pasternak (1987); Epstein and Rains (1987) and Cheeseman (1988). Effects of salinity on crop yield have been discussed by many workers (e.g. Torres and Bingham 1973; Torres *et al.* (1974); Rai 1977; Qureshi *et al.* 1980; Kumar 1983; Francois *et al.* 1986). There is less information available about the long-term effects of salinity on the relationships between photosynthesis, plant growth and yield in different crop plants. Therefore, the aim of the following sections is to review the effects of salinity on ion regulation, growth and photosynthesis of crop plants with particular reference to wheat.

2.2 EFFECT OF SALINITY ON PLANT GROWTH AND RELATED PARAMETERS

The growth reduction of glycophytes always is not correlated with specific symptoms such as leaf scorch or chlorosis at low or moderate salinity levels. Salinity causes stunting of many plants (Bernstein 1975) and these may have darker green leaves than normal control ants (Marschner 1986). The suppression of plant growth increases as the salt concentration increases (Maas and Hoffman 1977a; Lauchli and Epstein 1984). Both shoot and root growth are reduced by salinity (Iqbal 1988) but the shoot is usually the more sensitive (Munns *et al.* 1982; Munns and Termaat 1986; Rawson 1986). In wheat, tillering capacity is also reduced with increasing salt concentrations (Rai 1977; Kumar 1983; Rashid 1986; Shah 1987; Iqbal 1988; Kemal-ur-Rahim 1988). The number of effective ears per plant is the most seriously affected component in wheat under saline conditions (Kumar *et al.* 1983; Haqqani *et al.* 1984). The physiological responses of cereals to salt stress differ at different stages of growth and development and depend on the time of exposure and the severity of the stress. In addition, the nature of the salts present in the soil may affect the response of plants to stress. The continued growth of cereal plants under saline conditions is dependent on their ability to limit the influx of salts to their shoot via the transpiration stream (see Greenway and Munns 1980). The relative salt tolerance of the various cereals is largely a

measure of how strictly they are able to control this influx, ranging from very poorly in maize and rice to quite well in wheat and barley (Maas 1986; Wyn Jones and Gorham 1989).

2.2.1 LEAF GROWTH

Plants need to establish quickly their leaf canopy in order to intercept light, photosynthesise efficiently and thus maximise their dry matter production. Anything that decreases the rate of development of that canopy will limit the potential yield of a plant. Leaf growth is important in influencing light interception, crop growth and the yield of cereals (Gallagher and Biscoe 1978) and can be quantified (Gallagher 1979a) in terms of leaf extension rate (LER) and leaf extension duration (LED). The main environmental factors which influence leaf growth are light intensity and quality, photoperiod, temperature, water and nutrient supply (Dale and Milthorpe 1983; Baker *et al*. 1985).

Salinity affects the expansion of individual leaves rather than through the rate of production of new leaves (Terry and Waldron 1984; Iqbal 1988). It also decreases number of leaves by decreasing the number of shoots. The first effect of salinity on plants is a reduction in leaf extension growth (LEG) which mainly occurs at low salinities before visible symptoms of salt damage are apparent (Iqbal 1988). This affects yield by limiting the leaf area available for photosynthesis (Wyn Jones and Gorham 1989). The second effect may be described as salt toxicity as a result of the

gradual increase in leaf salt concentration with time and is usually only apparent with visible symptoms at later stages of growth at low to moderate salinity (Iqbal 1988).

The basis of the decline in plant growth under saline conditions is poorly understood. Many theories have been suggested to explain the reduction of leaf growth in plants growing under saline conditions. The growth reductions caused by salinity could be due to osmotic and/or specific ion effects, but it is unknown whether the growth response is dominated by the osmotic effect (i.e. by water deficit) or by the specific ion effect (i.e. salt toxicity or nutrient deficiency).

2.2.1.1 SPECIFIC ION EFFECTS ON LEAF GROWTH

Salinity at non-lethal levels suppresses leaf growth without any apparent injury (Rawson and Munns 1984) and has effects on plant growth similar to water stress (Waldron *et al.* 1985). The specific effects of salinity on the leaf growth could be due to excessive transport to the shoot of Na^+ or Cl^-, excessive transport to the shoot of other ions such as phosphates or an inadequate transport of other ions such as K^+, Ca^{2+}, Mg^{2+}, NO_3^- or SO_4^{2-} (Termaat and Munns 1986). Transport of Na^+ and Cl^- in the phloem could be important in the regulation of NaCl concentrations in leaves and roots of plants growing in saline soils (Jeschke 1984). A high flux would presumably be advantageous to rapidly vacuolating cells as it would help to fulfil their demand

for solutes to generate osmotic pressure (Munns *et al.* 1986). However, a high flux might also pose a problem to the dividing cells in the same leaves. Although the dividing cells are increasing in volume, they are only slightly vacuolated, and NaCl may have adverse effects on cytoplasmic pressure if the concentrations become too high (Munns *et al.* 1986). In salt tolerant plants such as barley, roots can regulate the rate of salt transport in the xylem (Munns 1985). The retranslocation of salt in the phloem of salt tolerant plants could help to counter the build up of salts in fully expanded leaves, which are continually receiving salt in the xylem (Munns *et al.* 1986). This could have specific effects on metabolism if NaCl were not compartmented in the vacuoles (Flowers *et al.* 1977), or could have osmotic effects in a sense if NaCl accumulated in the apoplast of old leaves (see Munns and Passioura 1984).

It is clear that premature death of leaves is due to excessive NaCl concentrations (Greenway 1962). However, it is not proven that growth of young leaves is directly affected by NaCl accumulated in the old leaves (see Munns and Termaat 1986). The symptoms of older leaves under saline conditions can reflect the specific effect of ions on crop plants but generally it is not considered a useful criterion to evaluate salt tolerance. The leaves of salt affected plants are often darker green than those of normal plants (Bernstein 1975). Moreover, chlorosis and leaf death always proceed from the older leaf to the younger leaf

(Gorham *et al.* 1986b; Akita and Cabuslay 1990) at high NaCl and Na_2SO_4 salinity (Iqbal 1988). Aswathappa and Bachelard (1986) found that when compared on the basis of osmolality of the uptake solutions, Cl^- salts were more inhibitory (15-30%) than SO_4^{2-} salts whereas at equal concentrations, SO_4^{2-} salts inhibited NO_3^- uptake 30 to 40% more than did Cl^- salt. Other workers also observed that both Cl^- and SO_4^{2-} salts severely inhibited uptake of NO_3^- (Marschner 1986; Imamul Haq and Larher 1984) and ultimately of leaf growth (Gorham *et al.* 1986b). This situation can be modified to a great extent in the presence of Ca^{2+} (Imamul Haq and Larher 1983; Kent and Läuchli 1985; Cramer *et al.* 1986; Maas and Grieve 1987; Lewis *et al.* 1989; Ehret *et al.* 1990; Leidi *et al.* 1991). High salt concentrations in the soil reduce availability of soil water and impose osmotic stress on the leaf growth (Terry and Waldron 1984). Apart from the general osmotic effect, the prevalence of certain ions such as Na^+, Cl^-, SO_4^{2-}, Mg^{2+}, borate and bicarbonate can be toxic and the high pH value often found in saline soil can induce Fe and other micronutrient deficiencies (Aswathappa and Bachelard 1986).

2.2.1.2 OSMOTIC EFFECTS ON LEAF GROWTH

Munns *et al.* (1982) showed that in salt stressed barley the growth inhibition evident in the elongating leaf tissue could not be attributed to lack of assimilates or to adverse metabolic consequences of the accumulated ions. They

concluded it was due to the persistence of a water deficit in the expanding cells despite a degree of osmotic adjustment. Leaf cell turgor has been considered a major plant factor influencing growth (Hsiao 1973), stomatal conductance and photosynthesis (Turner 1974) during absence or presence of salt or water stress. Terry and Waldron (1984) observed that the imposition of short-term root zone salinity reduced LER and leaf water potential. The removal of root zone salinity led to complete recovery of LER and the return of leaf water potential to its presalinised level. They, therefore, suggested that growth reductions due to salinity were mediated largely by changes in plant water status. Delane *et al.* (1982) and Munns *et al.* (1982) also proposed that water deficit in expanding tissue was responsible for reduced growth. Delane *et al.* (1982) suggested that the growth of the shoot of barley at high NaCl concentration was limited by an insufficient supply of ions or other solutes to the growing regions, resulting in insufficient osmotic solutes to generate turgor. In a later paper, Termaat *et al.* (1985) concluded that shoot turgor alone was not regulating the growth of the NaCl affected plants as they applied the appropriate amount of pneumatic pressure to the roots to raise the turgor of the leaves. They, after discussing other possible influences, argued that a message arising in the root may be regulating the growth of the shoot. Conversely, Terry and Waldron (1984) argued that salinity decreases the water available to the plant by decreasing the osmotic potential at the root

surface and that an excess of certain ions as sodium or chloride directly exerts toxic effects.

Osmotic adjustment is the accumulation of solutes during water and salt stress conditions, whereby turgor is maintained as water deficits develop (Turner and Jones 1980). Recent theories on the causes of growth reduction in salt stressed plants emphasise the possibility that phloem transport rates might limit solute delivery to expanding tissues (Gorham *et al.* 1985a). Phloem transport is important in relation to salinity at two stages of leaf growth, for the supply of solutes to young, expanding tissues and for the retranslocation of useful metabolites (including potassium) from senescing leaves to younger tissue (Jeschke 1984). The ion composition of young leaves, therefore, reflects that of the phloem sap, which is rich in K^+ and low in Na^+ even under saline conditions (Delane *et al.* 1982). With increasing leaf age, minerals are imported by the xylem with low K^+ and possibly higher Na^+ concentrations, particularly at higher transpiration rates (Pitman 1965) or under saline conditions. In saline conditions K^+, Na^+ and Cl^- can be particularly important as osmotic solutes in the leaves (Pitman 1984) and plant requirements for these ions will be determined both by the rate of growth and by the level of these solutes in the leaves. Many other metabolites such as sugars, amino acids and glycinebetaines are also reported to accumulate in plant tissues and to contribute to osmotic adjustments to generate

turgor for leaf growth during salt stress (Aspinall 1986). Kuang *et al.* (1990) observed that osmotic adjustment was linearly correlated with the reduction in leaf elongation rate in wheat and lupin.

2.3 CONSTANT VERSUS VARIABLE SALINITY AND PLANT GROWTH

When plants grown under non-saline conditions are suddenly exposed to a relatively low osmotic potential in their rooting medium, osmotic adjustment takes place in a short period of time (Bernstein 1961, 1963). At high salinity, plants experience severe osmotic shock (Amzallag *et al.* 1990). In addition to the osmotic shock, accumulation of Na^+ and Cl^- ions in the plant is often claimed to be toxic and is even considered to be one of the main causes of the growth inhibition induced by salinity (Yeo and Flowers 1986). Pretreatment of plants at a low salinity level and then exposure to high salinity has been found to increase salt tolerance in some plants (Munns *et al.* 1986; Cramer *et al.* 1989; Amzallag *et al.* 1990). Amzallag *et al.* (1990) found that *Sorghum bicolor* (L.) Moench plants became capable of growing in a medium containing 300 mol m^{-3} NaCl after first being exposed to 75 or 150 mol m^{-3} NaCl for 20 days. They considered that this induction of a capacity to survive in and tolerate a high NaCl concentration as an adaptation to salinity is more than osmotic adjustment. Munns *et al.* (1986) observed that when barley plants were kept for more than 2 weeks at 200 mol m^{-3} NaCl, the Cl^- concentration in the sap increased but the Na^+

concentration remained the same. They also noticed that Na^+ and Cl^- concentration in the sap decreased very slowly when NaCl was removed from the nutrient solution. Cramer *et al.* (1989) also observed that step-down salinity treatments caused increased growth rates with no immediate changes in Na^+ and Cl^- concentration in the elongation zone of the leaves of barley, indicating that growth may be reduced because of water deficits rather than Na^+ or Cl^- toxicity (Munns *et al.* 1982). Whether a water deficit or ion toxicity is the main constraint, plant growth also depends on the type of salinity (e.g., whether the predominant anion is Cl^- or SO_4^{2-} and the size of the cation ratio Ca^{2+}/ Na^+), the duration of exposure and the salinity level (Marschner 1986).

2.4 EFFECT OF SALINITY ON ION UPTAKE, REGULATION AND PARTITIONING IN PLANTS

Cereal plants grown in saline environments have to restrict Na^+ and Cl^- transport to the shoot and thereby prevent excessive build up of these ions in old leaves and to ensure that there are sufficient solutes for generating turgor in the expanding leaves (Munns 1988; Munns and Termaat 1986; Wolf *et al.* 1990). Either Na^+ and Cl^- import to mature tissues could be controlled or an excess of these ions could be compartmentalised or re-exported (Munns 1988; Wolf *et al.* 1990). In young rapidly expanding tissues, on the other hand, solute supply has to be maintained for

generating turgor (Greenway 1962; Greenway and Munns 1980). This section will consider how plants regulate uptake and partitioning of ions (Na^+ and Cl^-) to fulfil these requirements under various salt stressed conditions.

2.4.1 EFFECT OF TYPE AND KIND OF SALINITY ON ION UPTAKE

Chloride and sulphate salts, when compared on an equal osmotic basis, depress growth to an equal extent in a number of crops including wheat, although in some crops, chloride salts are slightly more toxic than sulphate (Magistad *et al.* 1943; Aswathappa and Bachelard 1986). Bliss *et al.* (1986) found that at low salinity levels growth was decreased to a greater extent by NaCl than by Na_2SO_4. At higher salinity levels Na_2SO_4 salts were more detrimental. It is noteworthy that SO_4^{2-}, which is absorbed in much smaller quantities than Cl^-, produces equivalent effects on growth of bean plants (Bernstein and Hayward 1958). Under saline conditions Cl^- contributes much more to osmotic adjustment than SO_4^{2-} because it is much more readily absorbed (Maas and Nieman 1978) whereas SO_4^{2-} ions generally restrict the absorption of Ca^{2+} while promoting the uptake of Na^+ (Bernstein and Hayward 1958). Cl^- ions are much more harmful than SO_4^{2-} ions for uptake of N, P and K in wheat plants (Mahajan and Sonar 1980). Whether Cl^- or SO_4^{2-} salinity is more harmful depends on which ion species is present in excess under saline conditions. For example, certain injury signs can be attributed to Na^+ or Cl^- toxicity, to Na^+ or SO_4^{2-} induced Ca^{2+} deficiency or to Na^+ or Ca^{2+} induced K^+

deficiency (Bernstein 1975). However, specific ion effects seem limited to certain susceptible plant species or varieties and rarely are a major cause of growth suppression (Maas and Nieman 1978). When salinity consists predominantly of monovalent cations and divalent anions, for example, Na_2SO_4, cation uptake rates exceed those of anions (Maas and Nieman 1978). Under such conditions ionic balance is achieved by the synthesis and accumulation of organic acids and the imbalance in organic cations and anions is further accentuated by the metabolic assimilation of NO_3^- and SO_4^{2-} (Maas and Nieman 1978).

2.4.2 ION UPTAKE AND PARTITIONING

It is generally accepted that Na^+ is a toxic ion to plants when present in high concentrations (Marschner 1971; Greenway and Munns 1980) but more recently this has come into question for short-term responses (Munns and Termaat 1986). Cl^- probably behaves similarly to Na^+ because uptake of the two are very similar in barley (Munns 1985). When Na^+ is absorbed into the plant through the root it is diffused to the sheath and then to the leaf. Therefore sheath Na^+ concentration starts to increase first and leaf Na^+ concentration will continue to increase to a level similar to that of sheath Na^+ concentration until the plant dies (Akita and Cabuslay 1990). Under such conditions, the fully expanded leaves are adversely affected long before the young leaves, for the simple reason that salt concentrations

at a given time of exposure to salinity are always highest in older leaves (Greenway and Munns 1980; Rashid 1986; Gorham *et al.* 1986b), and the oldest leaves die long before young leaves are obviously affected. The higher salt concentration in the older leaves of non-halophytes may result entirely from a product of time by transpiration rate (Greenway and Munns 1980) or at least partly from an exclusion of specific ions from the xylem vessels supplying the younger leaves (Yeo and Flowers 1982). Accordingly, Gorham *et al.* (1986b) observed higher sap osmotic pressure and Na^+ and Cl^- concentration in the older leaves than the younger leaves and there was a pronounced gradient of Na^+ and Cl^- from the youngest to the oldest tissues, with particularly low concentrations being found in the developing inflorescence. They also found that with increasing salinity the K^+ concentration declined in the older leaves but remained constant in the flag leaf and the inflorescence. Similar observations were also made by Rashid (1986) in different wheat varieties.

The ability of salt stressed grasses to partition ions in their leaves was first recognised by Greenway (1962). The importance of ion partitioning into the sheaths of grasses was further supported by preliminary findings of Cl^- partitioning in the sheaths of wheat, maize and sorghum as well as barley (Boursier *et al.* 1984; Boursier 1987; Boursier *et al.* 1987; Boursier and Läuchli 1989; 1990).

2.5 PHOTOSYNTHESIS

There are many excellent reviews and books dealing with subject of photosynthesis (see, for example, Edwards and Walker 1983; Hall and Rao 1987; Lawlor 1987; Hay and Walker 1989) and many dealing specifically with environmental effects (Berry and Downton 1982; Jones 1983) and methods of measurements (Sestak *et al*. 1971; Long and Hallgren 1985). This section will deal with the effects of salinity on photosynthesis with special reference to wheat. The term 'gas exchange rate (GER)' used herein and by other workers (Araus *et al*. 1986; Kemal-ur-Rahim 1988) and later sections covers not only net photosynthesis (Pn) but also sub-stomatal CO_2 concentration (Ci), stomatal conductance (g_s) and transpiration rate (E).

2.5.1 GAS EXCHANGE IN WHEAT

Plant biomass production depends on the accumulation of carbon products by photosynthesis. This in turn is determined by two main components: the rate of photosynthesis per unit leaf area and the area of leaf surface available for photosynthesis (Terry and Waldron 1984). Assimilate production of individual leaves on wheat plants depends on the rate of photosynthesis, leaf area and duration of leaf function. High rate of photosynthesis is negatively correlated with leaf area. Whereas modern cultivated hexaploid wheat lines generally have larger leaves and lower photosynthetic rates than progenitor

species with lower ploidy level (Evans and Dunstone 1970; Austin *et al*. 1982; Rawson *et al*. 1983; Bhagsari and Brown 1986). During vegetative development, photosynthates produced by early formed leaves are partitioned predominately to emerging leaves, tillers and roots (Doodson *et al*. 1964; Rawson and Hofstra 1969). Although the seedling leaves in wheat are smaller than later formed ones (Dunstone *et al*. 1973), Araus *et al*. (1986) found no trend in the maximum photosynthetic rate per unit area in successive insertion levels. This differs from results obtained by other workers (Dunstone *et al*. 1973; Evans *et al*. 1975; Rawson *et al*. 1983; Caballero *et al*. 1983), who all found increases in photosynthesis in successive leaf insertions. Spike growth both before and after emergence from the boot is supported mostly by photosynthates from the upper two or three leaves on the plant (Rawson and Hofstra 1969; Patrick 1972). Estimates suggest that from 70% to more than 90% of the grain yield is derived from photo-assimilate produced after anthesis (Austin *et al*. 1977; Bidinger *et al*. 1977). Maintenance of active photosynthesis by leaves, particularly the flag leaf, throughout the period of cereal grain filling is a major requirement for production of adequate carbohydrates to give large grains and high yields (Peoples *et al* 1980). Lambers (1987) observed that high yielding genotypes with small flag leaves had high rates of photosynthesis. For any given leaf, net photosynthesis per unit area reaches a maximum soon after the leaf attains its maximum area, just after

ligule emergence (Austin *et al.* 1982; Rawson *et al.* 1983; Kemal-ur-Rahim 1988; Rawson *et al.* 1988a). In the case of the flag leaf, the rate of photosynthesis increases to a maximum at about 2-4 weeks after emergence (Austin *et al.* 1982). There is then a plateau which is followed by a linear decline as the leaf ages (Dantuma 1973). This is particularly controlled by the rate of leaf senescence, the decline being more rapid for diploid species (Austin *et al.* 1982). In unstressed leaves photosynthesis remains at its maximum for about 5-12 days (Rawson *et al.* 1983). In flag leaves this period may be for several weeks (King *et al.* 1967; Osman and Milthorpe 1971; Dantuma 1973; Austin and Edrich 1975). Changes in net photosynthesis with leaf age have been well studied in wheat (Dantuma 1973; Marshall and Biscoe 1980b; Hodanova 1981; Austin *et al.* 1982; Evans 1983; Rawson *et al.* 1983; Lawlor *et al.* 1987) but there is very little information available of this under salt stressed conditions.

Environmental conditions such as low moisture supply, high temperature, salinity and nitrogen shortage hasten senescence and reduce leaf area duration (Simmons 1987). It is also possible to increase yield by CO_2 enrichment after anthesis (Krenzer and Moss 1975; Fischer and Aguilar 1976). Therefore, enhanced photosynthesis achieved through delayed canopy senescence can also increase grain yield if plants had been adapted to stress conditions.

In wheat under field conditions, net photosynthetic rate varies between 12-32 μmol CO_2 m^{-2} s^{-1} at 680-2200 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) in various leaf insertions (Austin *et al.* 1982; Caballero *et al.* 1983; Kingsbury *et al.* 1984; Araus *et al.* 1986; Rawson 1986; Kemal-ur-Rahim 1988) but values as high as 44 μmol CO_2 m^{-2} s^{-1} have been recorded by Evans and Dunstone (1970) and Dunstone *et al.* (1973). This large difference between values of net photosynthesis occurs depending upon the growth conditions where the plants were growing and the photon flux density (I) at which the measurements were made. Single leaf photosynthesis rates for controlled environment grown plants usually approach light saturation at photon flux densities about one-third to one-half of full sunlight (Akita and Moss 1973). Different workers have reported that plants exposed to full sun on cloudless days generally receive light intensity between 1700-2000 μmol m^{-2} s^{-1} PAR (Sharma and Singh 1989; Ziska *et al.* 1990). When light response curves of photosynthesis are constructed for wheat, they take the general form of those found in other C_3 plants (Osman and Milthorpe 1971; Dunstone *et al.* 1973; Akita and Moss 1973). There are , however, differences between plants grown under artificial light and those in the field. Therefore in field grown plants saturation may not occur even if the leaves are measured under light intensities close to full sunlight (Singh *et al.* 1974).

The response of photosynthesis in wheat leaves to temperature shows a broad optimum between 15-30°C, this

itself also being related to the growing conditions (Austin and Jones 1975; Morgan and Willis 1983). Leach (1979) found that the optimum temperature for net CO_2 uptake in wheat was 24°C (the optimum for a single leaf being 25°C). At a constant humidity as temperature increased above 25°C, the decline in photosynthesis was correlated to an increase in mesophyll resistance. Both dark respiration and the CO_2 compensation concentration increased with temperature (Leach 1979). Ceulemans *et al.* (1988) found that diurnal variation in leaf temperature followed exactly the same pattern as atmospheric temperature, but was 1-4°C higher. Both the development of the early morning peak in stomatal conductance and the pattern in its decline thereafter seemed to be strongly influenced by the prevailing air temperature. Therefore, diurnal changes in stomatal conductance closely match changes in irradiance and air temperature. However, Henson *et al.* (1982) observed that stomatal conductance (g_s) was the highest in upper leaves and decreased down the canopy. The effect of irradiance on g_s is generally accounted for by its effect, via photosynthesis, on intracellular CO_2 concentration (Ci), with low Ci favouring stomatal opening (Raschke 1975).

Dry matter accumulation is a function of net photosynthesis and therefore of CO_2 fixation. Transpiration is a function of available water, evaporative demands and stomatal conductance. The inter-relationships between the two processes involve regulation of photosynthetic and

respiratory rates, stomatal conductance and the regulation of water potentials in leaf cells (Vaadia 1985). About half of the carbon assimilated during growth of a wheat plant is respired (King and Evans 1967). This respiration is important for both synthesis of new material and maintenance of tissues. Leaf or root respiration rate may be lower than that in the stem, particularly when the stem is elongating (Evans *et al*. 1975). Under stress conditions the plant has to respire more in order to maintain energy requirements and balance of ions particularly in expanding tissues. Maintenance of ionic balance at cellular levels necessitates heavy expenditure of energy (Yeo 1983) while localised accumulation of compatible solutes within cytoplasmic compartments is based entirely upon either stored or current products of photosynthesis (Kriedemann 1986). Root respiration accounts for an appreciable amount of total plant respiration. For a corn plant growing under normal conditions, about 50% of the total respiration energy of the root is consumed by ion uptake. Roughly 20% of the total respiration of the entire plant is used for uptake and transport of ions (Veen 1980). Since ion uptake requires both high energy phosphate and electron flow (Lüttge and Pitman 1976), the energy considerations of ion uptake are of primary importance under saline conditions (Yeo 1983).

2.5.2 EFFECTS OF SALINITY ON GAS EXCHANGE

The reduction in crop growth and yield by salinity has been well documented (Maas and Hoffman 1977a; Kumar 1983;

Kumar *et al.* 1983; Haqqani *et al.* 1984), although different physiological processes have been put forward to account for this reduction in different species (Flowers *et al.* 1986; Kingsbury and Epstein 1986; Munns and Termaat 1986; Flanagan and Jefferies 1988, 1989). Salinity level and leaf area are usually inversely related (Marschner 1986). Reduction in photosynthetic capacity is usually associated with the decline in growth caused by salinity (Munns and Termaat 1986; Flowers *et al.* 1986). Leaf elongation and hence the development of photosynthetic surface area was affected before photosynthesis in barley (Munns *et al.* 1982) and some other species. On the other hand, the plants must photosynthesise in order to grow but open stomata imply salt uptake as well as water loss (Flowers *et al.* 1988). Any increase in photosynthetic rate due to increased stomatal conductance is bound to increase the rate of transpiration (Lambers 1987). Salt (NaCl) reaches the shoot by way of the transpiration stream, so that the higher the transpiration rate, the higher the net transport of NaCl to the shoot (Yeo and Flowers 1986). Yeo *et al.* (1985) and Yeo *et al.* (1991) found that accumulation of NaCl in the leaves caused a rapid reduction in net photosynthesis and hence in growth in rice.

The duration of exposure to the stress also plays an important role in the process of recovery or adaptation to salinity (Heuer and Plaut 1989). The effect of salt stress on photosynthesis in wheat is thought mostly to be due to increases in stomatal and mesophyll resistance (Kingsbury *et*

al. 1984; Lawlor 1976; Rawson 1986). The drop in net photosynthesis is not just due to an increase in stomatal resistance, as Lawlor (1976) has found that an increase in CO_2 concentration did not restore it. Therefore, the photosynthetic mechanism must have been affected as well.

Salinity has been found to decrease significantly stomatal conductance, causing a reduction in transpiration rate and an elevation of leaf temperature (Terry and Waldron 1984). If stomatal closure were the only reason for reduction in photosynthesis then it would be expected that sub-stomatal CO_2 level would be depleted in leaves with the high Na^+ concentration. However, Yeo *et al*. (1985) observed a parallel decrease in carbon fixation by the chloroplasts in rice. Parallel changes in stomatal and internal resistances have also been reported during leaf ageing such that Ci remained constant despite changes in photosynthetic rate (Constable and Rawson 1980).

The photosynthetic response to salinity is frequently based upon measurements made on young, newly expanded leaves (Ball and Farquhar 1984a,b; Rawson 1986; Kemal-ur-Rahim 1988). However, the distribution of ions within the shoot is often uneven, with the older leaves accumulating more Na^+ and Cl^- ions than the rest of the shoot organs (Rashid 1986; Gorham *et al*. 1986b). The photosynthetic capacity of these older leaves may therefore be inhibited to a far greater extent than the newly developed leaves. The reduction of photosynthetic capacity exacerbates the effects of salinity by reducing growth and so the dilution of tissue salt by new

growth. Hence the threshold for a reduction in photosynthesis is a important feature (Yeo *et al.* 1985). Rawson (1986) found that salinity reduced the initial slope of the light response curve in both wheat and barley by 21 percent, When plants were rapidly stressed from 0 to 75 mol m^{-3} NaCl. In his experiment intercellular CO_2 concentrations also dropped by 14 percent. Marshall and Biscoe (1980b) found that measured values of dark respiration (Rd) were 5-10 percent of the values of maximum net photosynthesis (Pnmax). In young leaves, Rawson (1986) found that dark respiration was reduced at ligule emergence when plants were stressed, but in slightly older leaves, dark respiration and the CO_2 compensation point (Ic) increased. Kemal-ur-Rahim (1988) observed that salinity increased both Ic and Rd in wheat. He also observed that Pnmax and α (photosynthetic efficiency i.e. the initial slope of the light response curve) were unaffected by salinity. In contrast, Rawson (1986) found that 75 mol m^{-3} NaCl reduced both Pnmax and α in fully expanded leaves at the 4th leaf insertion level in wheat.

2.6 EFFECT OF SALINITY ON STOMATAL FREQUENCY, SPECIFIC LEAF WEIGHT AND RELATED PARAMETERS

Plants could compensate for the large effects of salinity on leaf area by increasing photosynthesis per unit leaf area. Increasing specific leaf weight (SLW) or stomatal density/frequency (SF) could be a useful way of

achieving this (Kemal-ur-Rahim 1988). Rawson (1986) suggested that in any salinity screening programme, three basic measurements should be made; (a) relative leaf expansion rate, (b) water-use efficiency and (c) specific leaf weight. A number of workers have found a good relationship between specific leaf weight and net photosynthetic rate in different crops under non-saline conditions (Pearce *et al.* 1969; Khan and Tsunoda 1970a, 1970b; Beuerlein and Pendleton 1971). Dunstone *et al.* (1973) found a positive relationship between net photosynthetic rate and specific leaf weight in diploid and tetraploid *Triticum* species, but not in hexaploids or for *Aegilops* species. Rawson (1986) found that specific leaf weight increased under salinity stress. Kemal-ur-Rahim (1988) also found that salinity increased specific leaf weight and stomatal density in wheat varieties but reduced leaf lamina area and leaf lamina dry weight. He concluded that specific leaf weight was not correlated with net photosynthesis (Pn). Austin *et al.* (1982) found that photosynthetic rates were strongly negatively correlated with leaf area, leaf width and the mean plan area per mesophyll cell and positively correlated with stomatal frequency and number of veins per mm of leaf width but not with specific leaf weight. Turner and Singh (1984) reported a lower stomatal frequency and higher leaf stomatal resistance for the adaxial than the abaxial surface of the blades of *Sorghum bicolor*. Munns *et al.* (1982) observed that stomatal frequency along the length of the 10-40 mm

elongation zone of the growing leaf was about 40% greater in plants grown for 5 days at 180 mol m^{-3} NaCl than in control. Strogonov (1962) also found that there was a 2-fold increase in the number of stomata per unit area of barley leaves after growth in a saline soil.

2.7 SCOPE AND OBJECTIVES OF THE CURRENT INVESTIGATIONS

The aim of these experiments was to determine the effects of salinity on leaf growth, ionic concentrations, and gas exchange of spring wheat. Initial experiments, described in Chapters 3 and 4, were performed to determine how leaf growth was affected under various saline conditions. Experiment 1 (Chapter 3) examined the changes in leaf growth under constant and variable salinity. Experiment 2 (Chapter 4) studied the partitioning of ions into various parts of the wheat plant and their effects on leaf growth and gas exchange. Experiments 3 and 4 (Chapters 5, 6 and 7) investigated the equilibration studies on gas exchange of wheat leaves in the light chamber, the relationships between ionic concentrations and gas exchange in expanding and senescing leaves. In Experiments 3 and 4, net photosynthesis was measured at a range of photon flux densities and photon flux response curves were constructed to determine the effects of salinity on transpiration efficiency (TE), maximum net photosynthesis (Pnmax), dark respiration (Rd), photosynthetic efficiency (α) and photon flux compensation point (Ic). Other parameters also

investigated were leaf ionic concentration and sap osmotic pressure (OP), green lamina area (GLA), leaf lamina dry weight (LDWT), specific leaf weight (SLW) and stomatal frequency (SF). Experiment 5 (Chapter 8) investigated the changes in gas exchange in the flag leaf and two successive leaves and grain yield under saline conditions.

A hydroponic method of propagation was chosen, as this enabled easy and precise control over salinity for applying a specific stress to plants, whilst ensuring that other nutrients in the growth medium were adequate and constant for all the treatments. Hydroponic culture is preferred by many workers and it avoids the problems caused by the effects of salinity on soil structure.

CHAPTER 3

EXPERIMENT 1

EFFECT OF A CHANGING SALINITY ENVIRONMENT ON LEAF EXTENSION, GROWTH AND ION UPTAKE OF SPRING WHEAT

3.1 INTRODUCTION

Most salt tolerance data has been obtained from experiments where salinity was maintained essentially uniform throughout the root zone by irrigating the soil plot or sand culture with saline waters and using high leaching fractions (Maas and Hoffman 1977b). Except in controlled experimental conditions, soil salinity is seldom constant with time or uniform in space. In fact, salinities often vary from concentrations approximately that of the irrigation water near the soil surface to concentrations many times higher at the bottom of the root zone (Maas 1986). Since soil salinity also increases between irrigations as soil water is lost by evapotranspiration, plants must respond to a heterogeneous system that is continuously changing.

Solution culture is another method of propagation favoured by many workers to study the responses of plants to salt stress (for example, Kingsbury *et al.* 1984; Gorham *et al.* 1984; Rashid 1986; Shah 1987; Kemal-ur-Rahim 1988; Wyn Jones and Gorham 1989; Gorham 1990). In this system the uniform application of salinity helps to minimise the problems encountered when interpreting results obtained from non-uniform salinity profiles in the field. However, applying these data to field conditions where the distribution of salts is neither uniform in depth nor constant with time is difficult and requires knowledge of how plants respond to varying salinity (Maas and Hoffman 1977b). Previous experiments had shown that constant

salinity decreases LER (for example, Iqbal 1988; Kemal-ur-Rahim 1988). Therefore, this experiment was conducted to study the effect of constant and variable salinity on leaf extension, growth and ion uptake of spring wheat. In this experiment constant and variable salinity treatments had the same average salinity throughout but the variable salinity treatment had periods of low to high salinity (Section 3.2.2). Therefore this experiment would look at whether periods of low and high salinity have the same effect on leaf extension and growth as the constant salinity.

3.2 MATERIALS AND METHODS

3.2.1 PLANT MATERIAL AND GROWTH CONDITIONS

The experiment was initiated on 25 November 1988 and terminated on 18 January 1989. The experiment was carried out in a growth room set at a temperature cycle of 24°C/16°C day and night and photoperiod of 16 hours. Spring wheat variety 'Wembley' was used in this and later experiments. The seedlings received light from a bank of 125W fluorescent 'warm white' lights which provided between 200-300 μmol m^{-2} s^{-1} PAR at initial plant level. Full detail of all addresses of manufacturers used in this and later experiments where appropriate are given in Appendix A.

3.2.2 EXPERIMENTAL DESIGN AND TREATMENTS

A randomised block design was used to minimise the effects of light reflection and air conditioning on the plants growing near the walls of the growth room. There were 4 blocks and 3 salinity treatments, namely control (0 mol m^{-3} NaCl), 'constant' and 'variable' salinity. In the 'constant' salinity treatment, plants were grown at 100 mol m^{-3} NaCl all the time after initial salt stress was introduced on 10 December 1988. In the 'variable' salinity treatment a 12 day cycle was repeated (Table 3.1). This involved changing the nutrient solution so that the plants were exposed to 'variable' salinity but such that the average salinity experienced was 100 mol m^{-3} NaCl. In both 'constant' and 'variable' salinity treatments, initial stress was imposed in 50 mol m^{-3} NaCl daily increments. At the start of the experiment the variable salinity treatment reached 100 mol m^{-3} NaCl on the second day of salt stress. On the next day (the first day of the cycle) salinity was decreased to 50 mol m^{-3} NaCl. On subsequent days it was increased in 10 mol m^{-3} NaCl daily increments, till it reached 150 mol m^{-3} NaCl on day 11 of the cycle. On day 12 it was decreased to 100 and then on day 13 back to 50 mol m^{-3} NaCl. The cycle was then repeated. The nutrient solutions in the control and constant salinity treatments were also changed on the day when the salinity was decreased to 100 mol m^{-3} NaCl in the variable salinity treatment (day 12 of the cycle).

Table 3.1: A twelve day nutrient and salt solution cycle for variable salinity

Status	Day of cycle	NaCl concentration (mol m^{-3})
Initial acclimation	-2	50
	-1	100
Cycle	1	50
	2	60
	3	70
	4	80
	5	90
	6	100
	7	110
	8	120
	9	130
	10	140
	11	150
	12	100
	1	50

In the constant and variable salinity treatments, NaCl was given in solution form from 4M stock solutions, the necessary dilution factors being calculated.

3.2.3 GROWTH CONTAINERS

Prior to seedling transplanting, the 10 l plastic containers (Figure 3.1) were painted black on the outside with bituminised paint to prevent light encouraging algal growth in the nutrient solution. The containers had a hole drilled in the side at the bottom, which was plugged with a 9 mm rubber bung. This was to facilitate easy changing of the nutrient solution. The containers were arranged along the sides of work benches to facilitate easy access for maintenance and measurements. Each polystyrene lid was bored with 16 holes using a 9 mm heated cork borer. The holes were spaced to give a plant to plant distance of 50 mm and a between row distance of 80 mm. This gave a planting density of approximately 250 plants per m^2. The polystyrene lids were also painted black on the upper surface and sides.

Air to the containers was supplied via lengths of polyurethane tubing, cut into appropriate lengths and joined together by 3-way air regulators. These regulators allowed the air to be restricted, to obtain uniform aeration between containers. A polyurethane tube led from each regulator into a container through a small 8 mm hole bored into the polystyrene lid and was terminated by an aerator. This

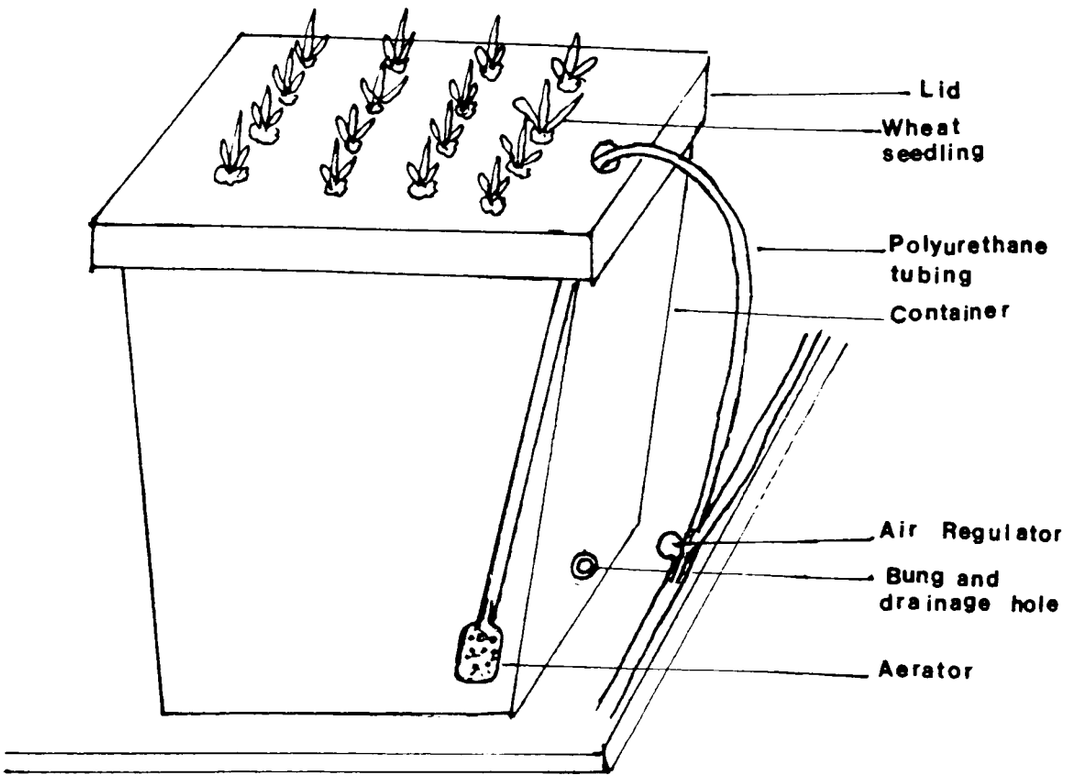


Figure 3.1: Growth container, showing aeration line, drainage hole and lid.

uniform and fine bubbling facilitated oxygen absorption into the nutrient solution, to maintain healthy root growth. The whole network of tubing was tied onto the benches and connected to an air compressor, master pressure being regulated by a needle valve.

3.2.4 GERMINATION AND TRANSPLANTING

Prior to seeding, approximately 500 seeds of wheat were soaked overnight in a muslin bag suspended under slow running tap water. Next morning the seeds were placed on capillary matting stretched over a plastic grid reinforced by wires and placed on top of plastic bowls (340 x 270 mm surface x 130 mm deep). The bowls were filled with tap water and a supplement of 0.5 g l^{-1} of 'Phostrogen' (Phostrogen Ltd, Corwen, Clwyd, UK), a powdered plant food formulation was added to ensure an adequate nutrient supply to the germinating seeds (Section 3.2.5). Wicks of capillary matting were always in contact with the water to ensure a continuously moist environment for the seeds. The seeds were then covered with moistened newspapers for 2 days. When the radicles and coleoptiles were seen to be emerging, the newspapers were removed. On 7 December 1988, at the 0+2 leaf appearance stage (0 fully emerged and 2 emerging leaves), seedlings of uniform height with well developed roots were teased out from the capillary matting, care being taken not to damage the roots which were important for ensuring immersion in the water. Each seedling was suspended in a collar of polyurethane foam

(Figure 3.2) and placed in an expanded polystyrene lid (Figure 3.3). To ensure a uniform planting method, each plant was centred with its seed in the middle of the collar and the collar was pushed into the polystyrene so that the seed would be located approximately half-way to the depth of the lid. After the plants were inserted into the polystyrene lids and placed over the nutrient solution, they were allowed to adjust to their new environment for 3 days. If any plants were damaged in transplanting, it was usually noticeable after one day and they could then be replaced.

3.2.5 NUTRIENT SOLUTION

In this and later experiments, for ease of preparation, a 'Phostrogen' (Appendix B) based nutrient supply was used (After Gorham *et al.* 1984) to provide plants with major nutrients. 10 g of Phostrogen powder (1 g l^{-1}) were added to each container. Phostrogen is a blended 10-10-27 NPK fertiliser with 1.3% Mg, 0.4% Fe and 0.02% Mn. A modified Long Ashton Nutrient Solution was used in combination with Phostrogen to supply micronutrients. This was prepared as in standard Long Ashton nutrient solution (Hewitt 1966) but NaCl was excluded. The concentrations of the micronutrients together with the actual quantities used are given in Appendix B.

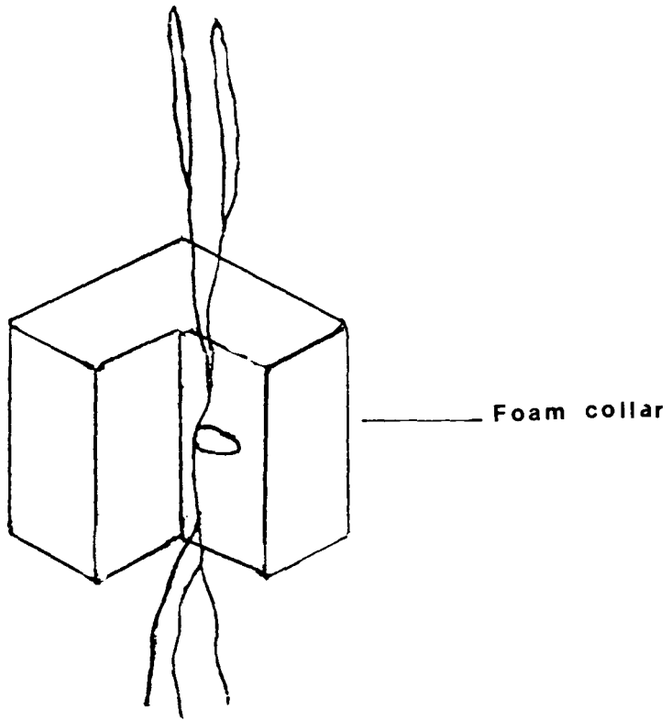


Figure 3.2: Expanded diagram of supportive foam collar around seedling and position of seed in collar.

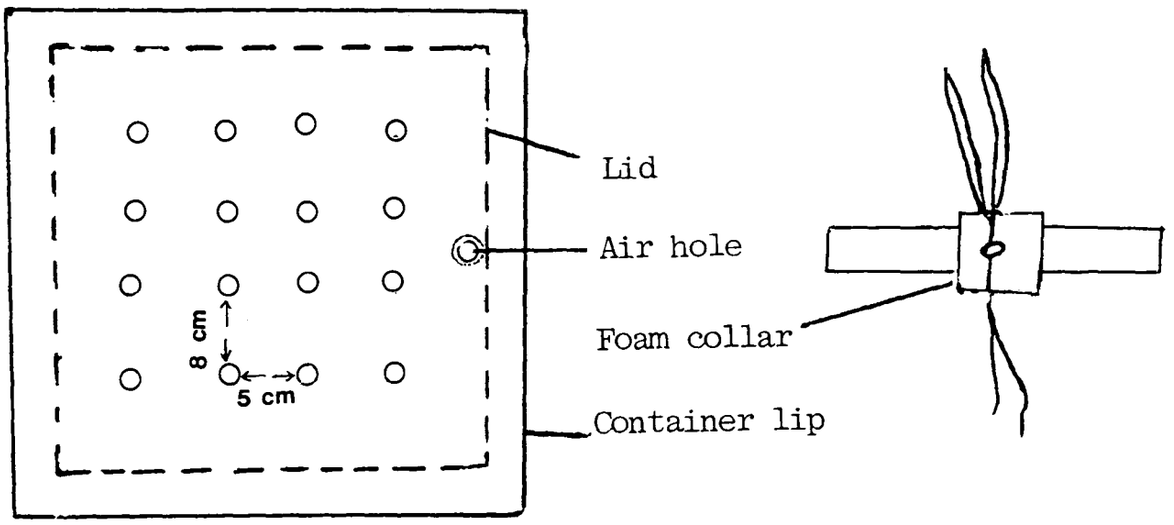


Figure 3.3: Polystyrene lid, showing dimensions of holes and spacing between them, plus sectional view showing foam collar seated in lid.

3.2.6 GROWTH MEASUREMENTS

For growth measurements the 4 central plants of each container were marked with wire rings of different colours and were used for leaf extension growth and leaf and tiller appearing stages.

3.2.6.1 LEAF EXTENSION GROWTH

Leaf extension growth (LEG) of all leaves on the mainstem was measured in mm with a ruler every day starting the day after initial salt stress. Leaf extension measurements started with leaf 3 because it was appearing at the time of initial salt stress. LEG measurements of leaf 3 were recorded from the ligule of leaf 1. LEG of subsequent leaves were recorded from the ligule of the leaf two positions down on the mainstem.

3.2.6.2 FINAL LEAF LENGTH

After completion of LEG measurements when the ligule was emerged, the final length of sheath plus leaf lamina (FLL) was recorded in mm for each replicate container in each salinity treatment.

3.2.6.3 LEAF EXTENSION RATE

Dennet *et al.* (1978) and Gallagher (1979b) found that 85-90% of leaf extension growth could be described by a straight line. Hence in the experiments here leaf extension rate (LER in mm per day) was calculated as the slope of a linear regression fitted to the data on the linear part of

the leaf extension growth curve using the 'Minitab' statistical package (version 7.2). LEG values for each leaf on the four plants in each replicate container were plotted against time. The values at the beginning and end of the line were discarded. These points were determined by inspection. The data were then fitted to the linear regression and the **r** values recorded were always above 95% of fitness.

3.2.6.4 LEAF EXTENSION DURATION

Leaf extension duration (LED in days) was calculated for each replicate container of each salinity treatment by the following equation:

$$\text{Leaf extension duration} = \frac{\text{Final leaf length}}{\text{Leaf extension rate}}$$

3.2.6.5 LEAF APPEARANCE STAGE

After every second day the numbers of fully expanded and newly emerged leaves on the mainstem of the 4 central plants in each container were recorded to determine the leaf appearance stage (LAS i.e. the number of leaves appeared at a particular time).

3.2.6.6 TILLER APPEARANCE STAGE

The number of tillers on each of the 4 central plants in each container was recorded every second day starting from the day of salt stress till final harvest to determine the tiller appearance stage (TAS i.e. the number of tillers at a

particular time).

3.2.6.7 GREEN LEAF AREA

The final harvest was made on 15 January 1989 when the flag leaf was expanding. It was not possible to continue the experiment further due to limitation of vertical space for growing plants in the growth rooms. It was day 12 of the cycle in the variable salinity treatment. At the final harvest the 4 central plants from each of the 4 replicate containers of each treatment were harvested. The leaves were detached from the stem and green area of stems and leaves was determined using an automatic area meter (model AAM-7, Hayashi Denkoh Co., Tokyo, Japan).

3.2.6.8 SHOOT AND ROOT DRY WEIGHT

After recording leaf area, all the shoot parts (leaves plus stems) were rinsed twice with distilled water, blotted dry and then dried in an oven at 80°C for 48 hours. Similarly the roots of the same plants were also detached and rinsed first in tap water and then in distilled water to remove excessive salt present on them (after Rashid 1986). The roots were then blotted dry. The roots were later oven dried in the same way as the shoots and dry weight of shoot and root was recorded.

3.2.7 PLANT CHEMICAL ANALYSES

The first set of plant chemical analyses was performed on sap extracted from leaf 6 on the mainstem. This leaf was the youngest fully expanded leaf at the time of the final harvest. Sap was extracted from four individual replicate plant leaves of each treatment. There were 4 plants x 4 replicates x 3 salinity treatments.

The second set of plant chemical analyses was performed on the dried shoot and root material of all the remaining plants in each container.

3.2.7.1 EXTRACTION OF LEAF SAP

The leaf lamina was cut at the point joining the sheath and washed in tap water followed by rinsing with distilled water. The leaves were then blotted dry with tissue paper and transferred to 1.5 cm^3 polypropylene microcentrifuge Eppendorf tubes. These samples were kept in a deep freezer at 0°C until required for sap extraction. To extract sap, frozen plant leaves were thawed and crushed using a metal rod with a tapered end. Small holes were made in the cap and the base of the tube and the sap centrifuged out at 7000 x g for 5 minutes into a second Eppendorf tube. This allowed the sap released from the crushing action to be collected in the second tube, leaving the plant material in the first tube. The second tube containing the sap was centrifuged for a further 3 minutes at 9000 x g to sediment any plant material that had passed through the small hole from the first tube (after Gorham *et al.* 1984). The

extracted sap was immediately frozen for further chemical analysis. For subsequent sap analysis, it was re-thawed, stirred and re-centrifuged for a period of 2 minutes.

3.2.7.2 DETERMINATION OF SAP OSMOTIC PRESSURE

Osmotic pressure determination was made on undiluted sap using a Wescor 5100C vapour pressure osmometer calibrated in mmol kg^{-1}.

3.2.7.3 PREPARATION OF DRIED SHOOT AND ROOT SAMPLES

Oven-dried shoot and root samples were chopped and then ground in a small sample mill (Tecator, Sweden). Inorganic analyses were performed on 100 mg samples of dried and ashed (at 450°C for 12 hours) plant material dissolved in 1 mol dm^{-3} HNO_3 and diluted where necessary with distilled water (after Gorham *et al.* 1980).

3.2.7.4 DETERMINATION OF SODIUM, POTASSIUM AND CHLORIDE

Sodium and potassium concentrations were determined by emission using a Pye Unicam atomic emission spectrophotometer (SP 90). Sodium was determined at 589 nm and potassium at 766 nm wavelength, against a series of standard solutions.

Chloride in the sap or nitric acid digested samples was determined using a chloride electrode with an Orion model 901 digital ionalyzer.

3.2.8 STATISTICAL ANALYSES

The data were subjected to analysis of variance (ANOVA) using the Genstat 5 statistical programme (Payne *et al.* 1987). When a significant 'F' value was obtained for treatment effects, a least significant difference (LSD) test at 5 percent probability level was applied to the treatment means by using the following equation:

$$LSD = SEDM \times T$$

Where LSD is least significant difference

SEDM is standard error of difference between means

T is value from distribution of t probability table at 5% level.

This test was used because as there were few treatments only a limited number of comparisons could be made and therefore little risk of making Type 1 errors (Gomez and Gomez 1984). All the graphs presented in this and subsequent chapters were prepared using the 'UNIRAS' programme.

3.2.9 EXPERIMENTAL MAINTENANCE AND OBSERVATIONS

During the course of the experiment no insect or disease attack was observed. Plants growing near the walls of the growth room were found to be getting comparatively cooler air than plants in the centre due to the air conditioning. Therefore, the containers were re-randomised at weekly intervals. Sometimes the aerators (air stones) were found to be blocked. Hence they were replaced regularly whenever required.

3.3 RESULTS

3.3.1 EFFECT OF SALINITY ON LAS AND TAS

LAS was not significantly affected by salinity but was slightly higher in the control than in both salinity treatments from 14 days after salt stress (DAS) onward (Figure 3.4).

Salinity significantly decreased number of tillers from 8 days after salt stress (Figure 3.4). TAS was generally higher at variable than at constant salinity but the difference was not always significant.

3.3.2 EFFECT OF SALINITY ON LEG, FLL, LER AND LED

Salinity did not significantly affect the LEG of leaf 3 (Figure 3.5) and the flag leaf (Figure 3.6) during the first few days after their emergence (from 6 to 11 DAS and from 35 to 38 DAS, respectively). However, LEG was shorter in both salinity treatments than in the control. LEG was always higher at variable than at constant salinity in both of these leaves. This trend was also observed in leaf 4 and leaf 7. Generally both salinity treatments significantly decreased LEG of these leaves. In leaves 3, 4, 7 and the flag leaf, LEG was generally higher at variable salinity than at constant salinity but the difference was not always significant. In leaves 5 and 6, LEG was generally higher at constant than at variable salinity.

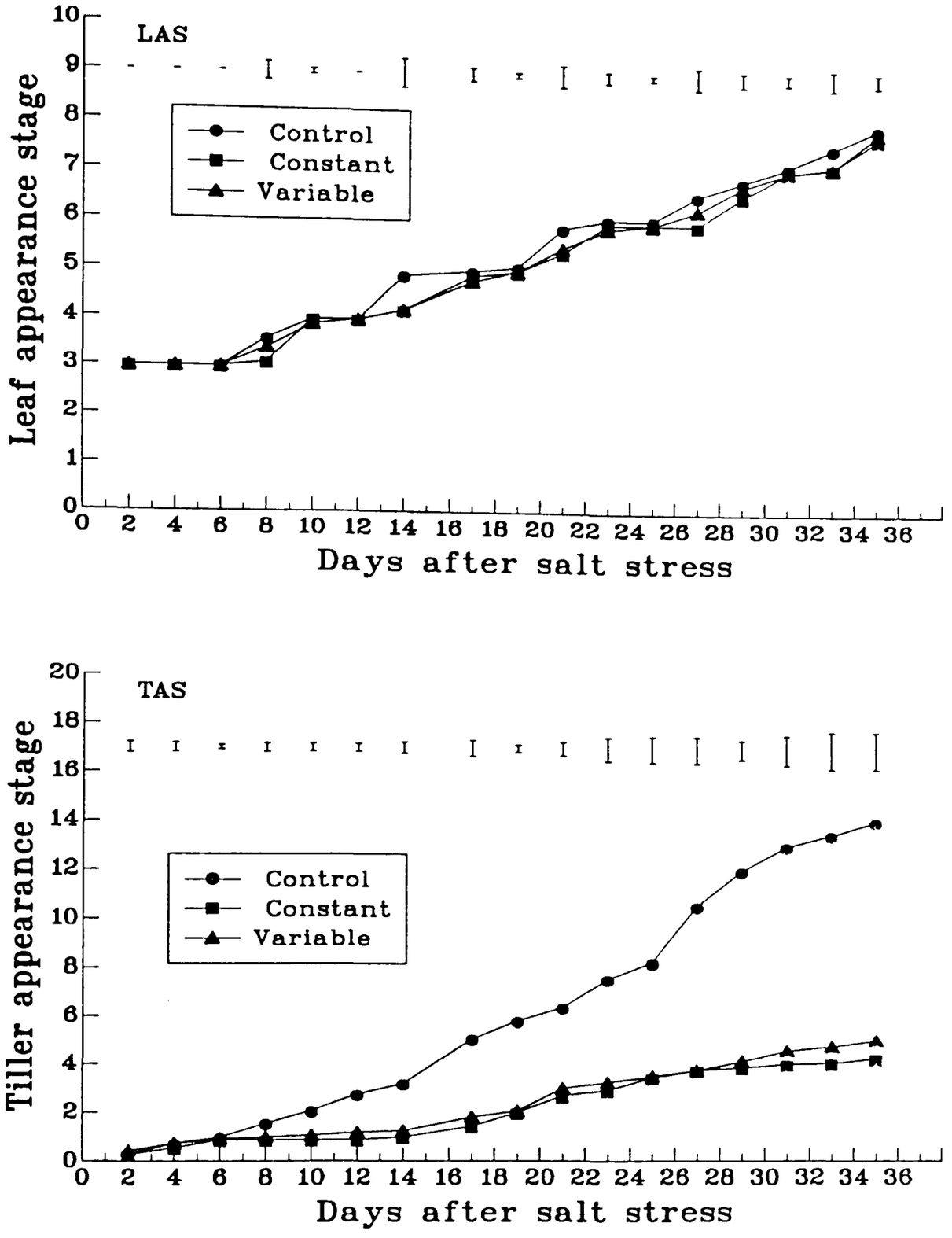


Figure 3.4: Effect of control (0 mol m^{-3} NaCl), constant (100 mol m^{-3} NaCl) and variable (50 to 150 mol m^{-3} NaCl) salinity on leaf appearance stage (LAS) and tiller appearance stage (TAS) of spring wheat. I= standard error of difference between means.

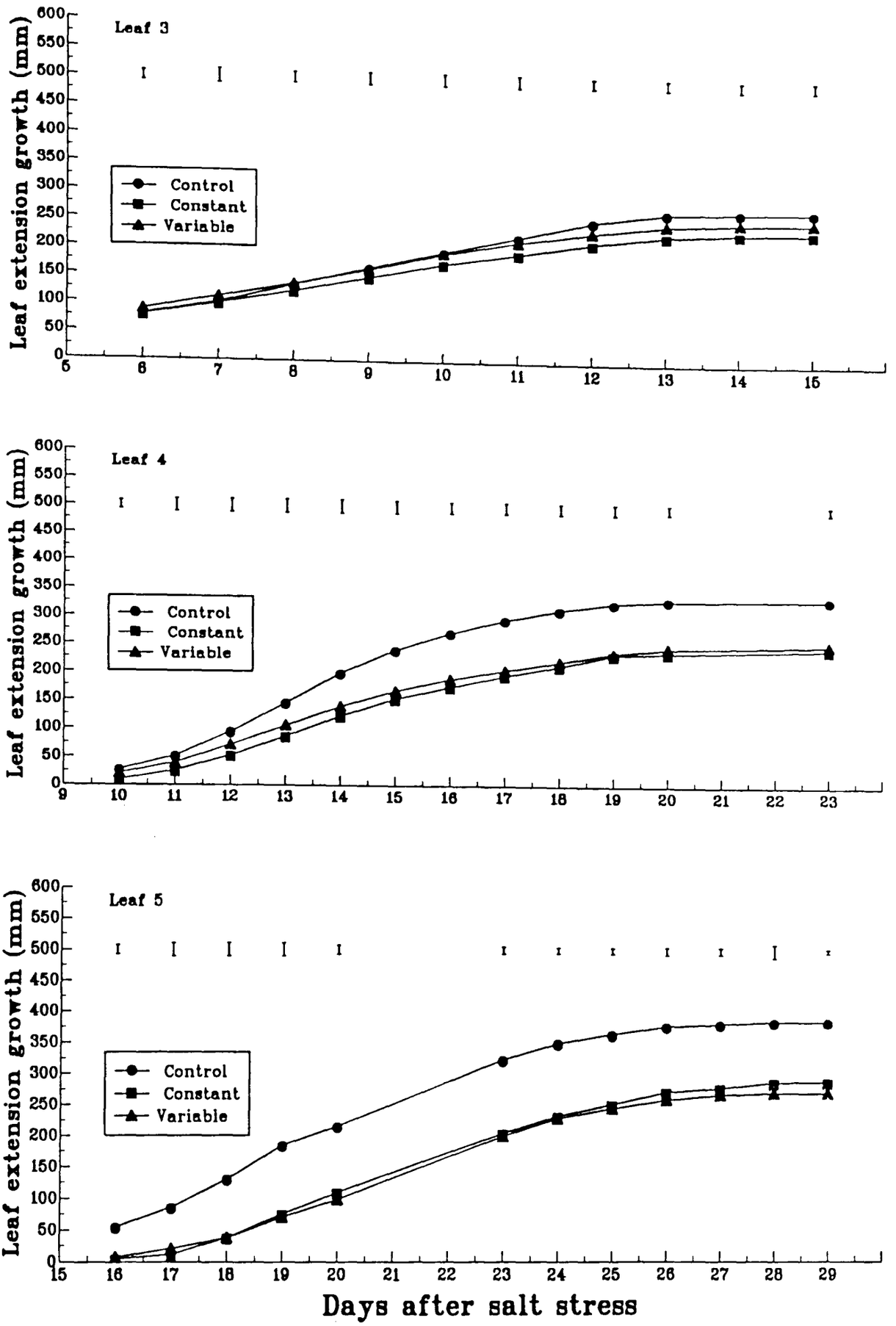


Figure 3.5: Effect of control (0 mol m^{-3} NaCl), constant (100 mol m^{-3} NaCl) and variable (50 to 150 mol m^{-3} NaCl) salinity on leaf extension growth of leaf 3, leaf 4 and leaf 5 of spring wheat. I= standard error of difference between means.

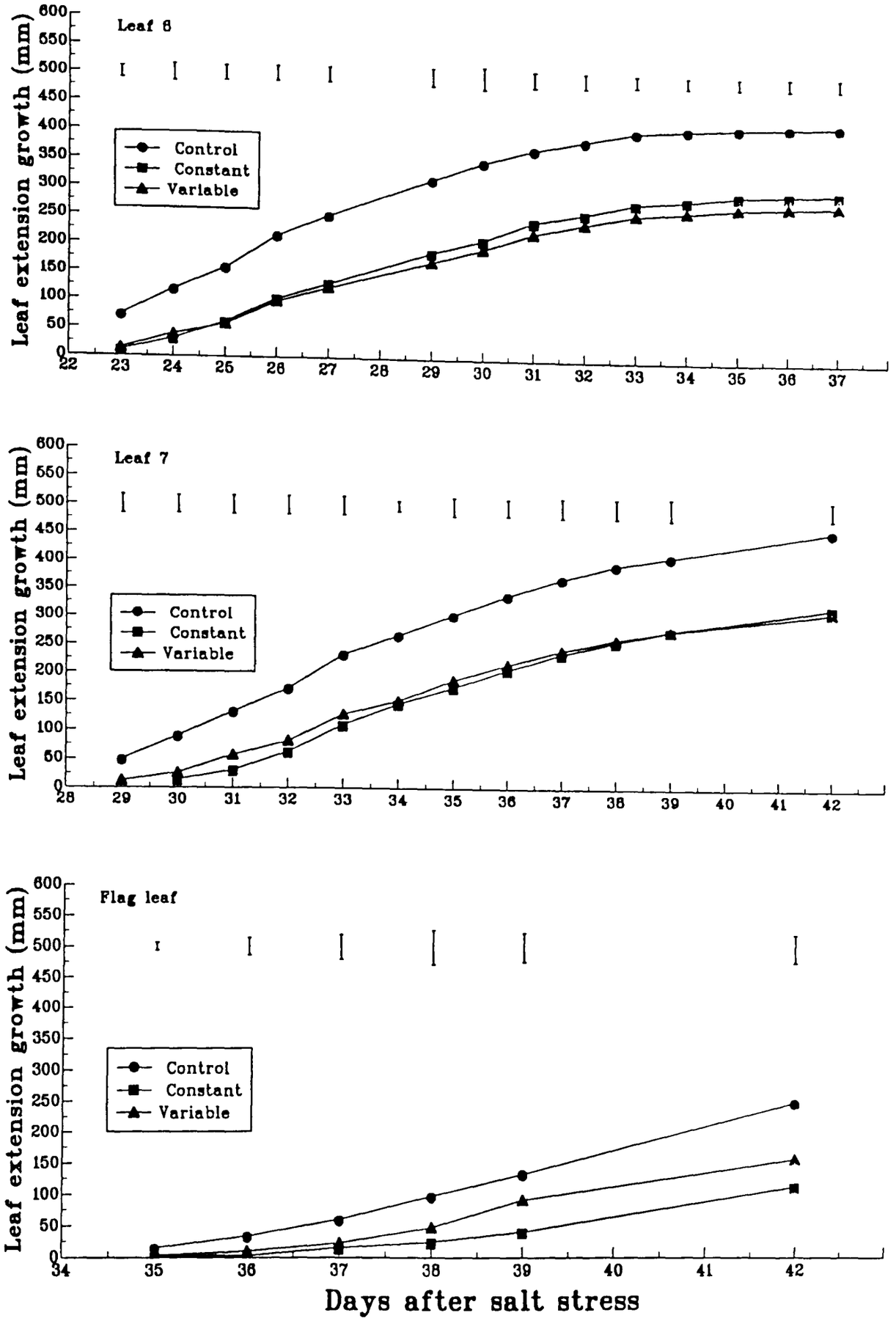


Figure 3.6: Effect of control (0 mol m^{-3} NaCl), constant (100 mol m^{-3} NaCl) and variable (50 to 150 mol m^{-3} NaCl) salinity on leaf extension growth of leaf 6, leaf 7 and the flag leaf of spring wheat. I= standard error of difference between means.

Salinity significantly decreased the FLL of all the mainstem leaves (Table 3.2). The difference between constant and variable salinity was not significant. FLL of leaves 3, 4 and the flag leaf was higher at variable than at constant salinity. This trend was reversed in leaves 5, 6 and 7.

LER was significantly higher in the control than in the two other salinity treatments (Table 3.2). The difference between constant and variable salinity treatments was not significant. LER was always higher at variable salinity than at constant salinity in all the leaf insertions except leaf 6.

LED was not significantly affected by salinity and there were no consistent trends in the results (Table 3.2).

3.3.3 EFFECT OF SALINITY ON GREEN LEAF AREA AND SHOOT AND ROOT DRY WEIGHT

Salinity significantly decreased leaf area, shoot and root dry weight per plant (Table 3.2). These parameters were always higher at variable than at constant salinity but this difference was not significant.

3.3.4 EFFECT OF SALINITY ON SAP OP, Na^+, K^+ AND Cl^- OF LEAF 6 AND THESE IONIC CONCENTRATIONS IN DRIED SHOOT AND ROOT SAMPLES

Sap OP was significantly increased by salinity and was significantly higher at variable than at constant salinity (Table 3.3).

Table 3.2: Effect of constant and variable salinity on leaf area (cm^2), shoot and root dry weight (g) per plant and final leaf length (FLL in mm), leaf extension rate (LER in mm day^{-1}) and leaf extension duration (LED in days) of various leaves of spring wheat.

Parameters	Salinity treatments			SEDM	LSD
	Control	Constant	Variable		
Plant growth					
Leaf area	613.00	131.00	142.00	28.87	70.72**
Shoot dry weight	2.22	0.63	0.76	0.16	0.38**
Root dry weight	1.25	0.19	0.23	0.06	0.16**
Final leaf length					
Leaf 3	293.2	244.9	258.7	16.11	39.47**
Leaf 4	374.4	278.4	290.6	5.91	14.48**
Leaf 5	445.8	318.1	310.8	9.56	23.42**
Leaf 6	506.5	362.2	342.1	11.47	28.10**
Leaf 7	572.9	412.9	395.8	18.68	45.79**
Flag leaf	408.0	230.0	294.0	26.20	64.19**
Leaf extension rate					
Leaf 3	29.23	23.75	26.75	1.06	2.61**
Leaf 4	47.60	32.70	32.82	2.18	5.35**
Leaf 5	42.35	31.85	33.55	2.18	5.33**
Leaf 6	44.10	32.60	28.0	2.62	6.42**
Leaf 7	44.33	32.58	33.92	1.91	4.69**
Flag leaf	39.80	17.00	23.20	3.30	8.09**
Leaf extension duration					
Leaf 3	9.96	10.30	9.83	0.629	NS
Leaf 4	7.90	8.61	8.89	0.450	NS
Leaf 5	10.64	10.03	9.31	0.651	NS
Leaf 6	11.66	11.13	12.52	0.998	NS
Leaf 7	12.93	12.75	11.71	0.520	NS
Flag leaf	10.57	14.24	14.05	2.03	Ns

SEDM = standard error of difference between means,
LSD = least significant difference,
NS = not significant,
** = significant at 1 percent probability level.

Table 3.3: Effect of constant and variable salinity on sap osmotic pressure (OP in mmol kg^{-1}), sodium, potassium and chloride concentrations (mol m^{-3}) in leaf 6 and their ionic concentrations in the dried shoot and root samples (μmol g dry $weight^{-1}$) of spring wheat.

Parameters	Salinity treatments			SEDM	LSD
	Control	Constant	Variable		
Sap osmotic pressure					
Sap OP	381	627	767	37.47	91.75**
Sodium					
Sap	18.7	96.0	112.5	5.80	14.21**
Shoot	30.0	958.0	1058.0	81.10	198.70**
Root	30.0	575.0	778.0	54.50	133.53**
Potassium					
Sap	268.2	182.5	235.0	19.30	47.25*
Shoot	882.0	695.0	727.0	79.00	NS
Root	465.0	382.0	375.0	78.80	NS
Chloride					
Sap	17.0	168.5	174.0	10.60	25.97**
Shoot	537.0	2700.0	3388.0	188.77	462.49**
Root	192.0	1327.0	1313.0	66.00	161.70**

SEDM = standard error of difference between means,
LSD = least significant difference,
NS = not significant,
*,** = significant at 5 and 1 percent probability levels respectively

Salinity significantly increased Na^+ concentration in extracted sap and in dried shoot and root samples (Table 3.3).

Significantly higher Na^+ concentration was observed at variable than at constant salinity in all the samples.

Sap K^+ concentration was decreased by salinity. Sap K^+ was significantly lower at constant salinity than in the control (Table 3.3). The difference between the control and variable salinity and between constant and variable salinity was not significant. K^+ concentration in dried shoot and root was not significantly affected by salinity but was lower than in the control.

Cl^- concentration in extracted sap and in dried shoot and root was significantly higher at constant and variable salinity than in the control (Table 3.3). Cl^- concentration was significantly higher at variable than at constant salinity in the dried shoot but not in extracted sap. Root Cl^- concentration was lower at variable than at constant salinity but this difference was small and not significant.

3.4 DISCUSSION AND CONCLUSIONS

Leaf extension growth was decreased markedly in later leaf insertions. This trend was also observed in an earlier experiment (Iqbal 1988) where both NaCl and Na_2SO_4 salts decreased LEG in later leaf insertions of spring wheat. In the present study it was anticipated that at variable salinity daily increments in leaf length would decrease as

salinity increased. Examination of Figure 3.7 shows that this was not the case. Leaves 4, 5 and 7 had their main periods of extension growth during phases when salinity was increasing but the pattern was similar to constant salinity. It was also anticipated that at variable salinity daily increments of leaf length would be higher at variable than at constant salinity during periods when salinity was decreasing or less than at constant salinity. Leaf 6 had its main period of extension (first 5 days after appearance) when salinity was greater than at constant salinity. It was noticed that daily increments of leaf length for leaf 6 decreased with increase in salinity but increased when salinity was stepped down to 50 mol m^{-3} NaCl. In variable salinity there was some evidence that LEG increased when salinity decreased but a similar pattern was also observed in constant salinity, possibly indicating that this increase in leaf length could be attributed to solution changes. Munns *et al.* (1982) observed an increase in leaf elongation of barley plants grown for 7 days at 120 mol m^{-3} NaCl after plants were transferred to either 60 or 0.5 mol m^{-3} NaCl. They only examined leaf elongation for 4.5 hours. Hence the increase in leaf elongation which they observed could be also due to solution change.

Salinity significantly decreased LER and FLL in all leaves but there was no significant difference between constant and variable salinity. Salinity decreased FLL of all leaves by decreasing LER as found by Kemal-ur-Rahim (1988). LED was not significantly affected and therefore

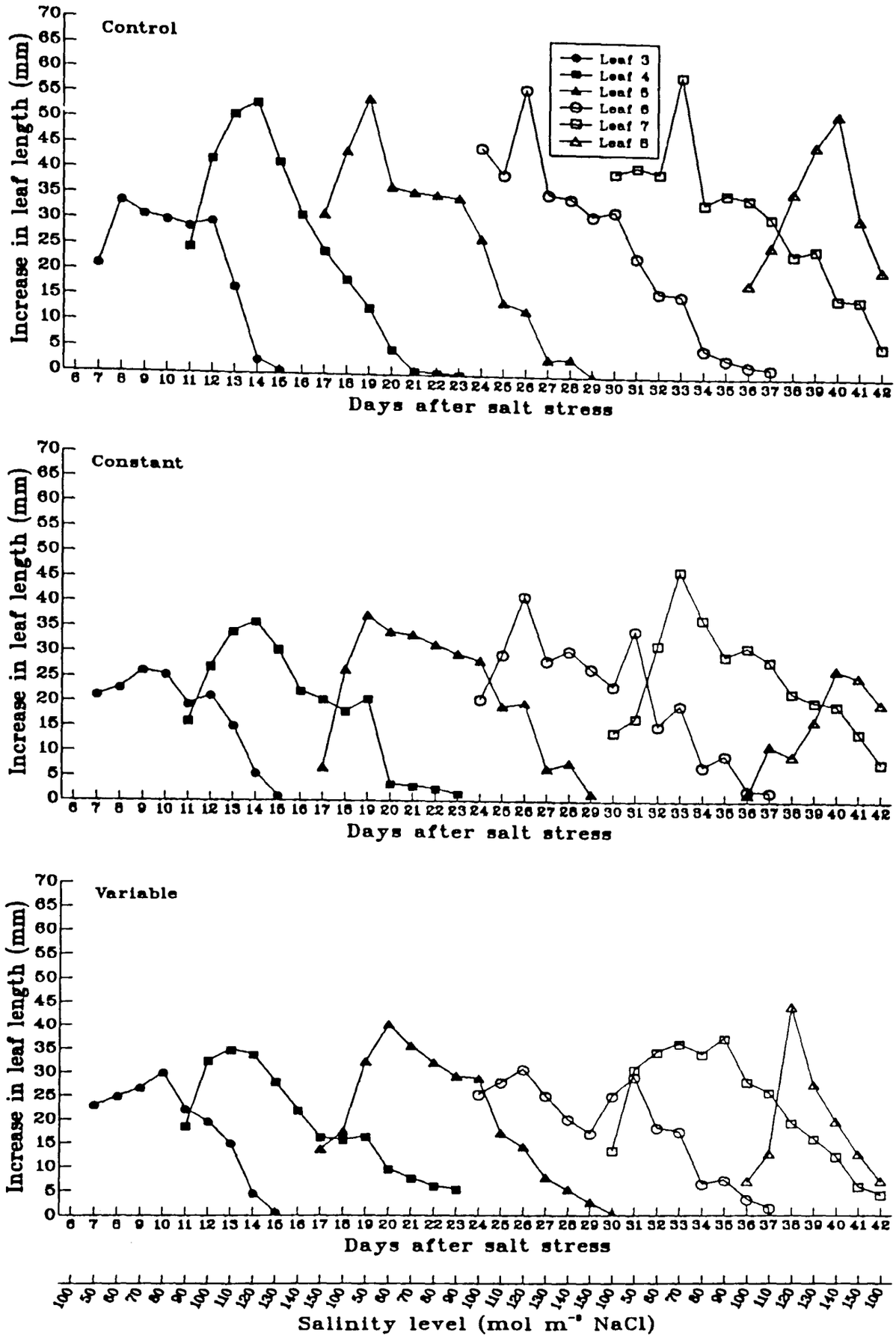


Fig. 3.7: Effect of control (0 mol m^{-3} NaCl), constant (100 mol m^{-3} NaCl), and variable (50 to 150 mol m^{-3} NaCl) salinity on increase in leaf length over time in various leaves of spring wheat.

differences in LED did not account for difference in FLL. Gallagher and Biscoe (1979) and Gallagher *et al.* (1979) found that severe water stress slowed apparent extension rate without markedly affecting LED and therefore final leaf size was a function of LER. Therefore, under both water and salt stress conditions, the determining factor for FLL seems to be LER rather than LED.

Salinity significantly decreased leaf area, shoot and root dry weight per plant. These findings are in line with other workers (Maas and Hoffman 1977a; Läuchli and Epstein 1984; Shah 1987; Kemal-ur-Rahim 1988; Iqbal 1988). Leaf area and shoot and root dry weight were higher at variable than at constant salinity but this difference was not significant. This suggests that at variable salinity the plants may have been able to adjust to the gradual increase in salinity and then periods of low salinity. Amzallag *et al.* (1990) also found that an adaptive response occurred when *Sorghum bicolor* plants were exposed to a non-lethal NaCl concentration for longer than a critical time period. Following such pretreatment the plants will then grow at concentrations of NaCl which would otherwise be lethal.

Salinity significantly increased Na^+ and Cl^- concentrations in extracted sap and in dried shoot and root samples while K^+ concentration decreased. Sap OP was also significantly increased by salinity. All these parameters except root K^+ concentration were higher at variable salinity than at constant salinity but the difference was

not significant. This higher level of these ions could be due to the fact that at final harvest, when leaf 6 was fully expanded, plants in variable salinity were at day 12 of the cycle when salinity was going from high to low. This could have resulted in a slow decrease in concentration of these ions. Munns *et al.* (1986) also noticed slow decrease of sap Na^+ and Cl^- concentrations in barley plants when NaCl was removed from the nutrient solution.

3.4.1 IMPLICATION

Variable salinity is more similar to that experienced by crops in the field under irrigated conditions. In this study, plants in variable salinity performed better than those in constant salinity but these treatments were not significantly different and gave similar results. For solution culture studies it is easier to use constant salinity rather than variable salinity. Therefore this procedure was used in subsequent experiments.

CHAPTER 4

EXPERIMENT 2

EFFECT OF SALINITY ON GROWTH, ION PARTITIONING AND GAS EXCHANGE OF SPRING WHEAT

4.1 INTRODUCTION

Grain yield in wheat is a composite of assimilate produced over the life of the plant. This may be partitioned into two major components: dry matter produced before anthesis and remobilised to the grain during grain filling and dry matter produced after anthesis and translocated directly to the grain or stored temporarily in vegetative organs before being remobilised to the grain (Pheloung and Siddique 1991). Dry matter formed prior to anthesis has been estimated to contribute 3-30% of the grain dry matter at maturity (Stoy 1963; Rawson and Evans 1971; Gallagher *et al.* 1976; Austin *et al.* 1977; Bidinger *et al.* 1977). Growth and yield in crop plants are affected to varying degrees by salinity (Maas and Hoffman 1977a). The growth of a plant is influenced by leaf area and by amount of dry matter produced per unit leaf area. One of the main objectives of the present work was to study the effects of salinity on growth and dry matter production of wheat. Previous experiments (Iqbal 1988; Experiment 1) have shown large effects of salinity on leaf expansion. Therefore, this experiment examined the effects of NaCl on dry matter production, ion partitioning, GER and other parameters before anthesis. Later experiments (Experiments 4 and 5) examined the effects of NaCl on leaf area, dry matter production and GER after anthesis.

Because photosynthetic rate shows diurnal variation (English *et al.* 1979), it was necessary to measure GER at different times during the day. In previous experiments

(Iqbal 1988, Kemal-ur-Rahim 1988) higher Na^+ and Cl^- concentrations have been found in later emerging leaves. Also in Experiment 1 the effects of salinity on LER increased with leaf position on the mainstem. Here in this experiment GER was measured in early (Leaf 5) and later (leaf 7) appearing leaves. Ion concentrations were measured in sap extracted from fully expanded leaves and in dried material of different plant parts. The latter technique was used as it was not possible to extract sap from dead leaves in order to study ion distribution.

4.2 MATERIALS AND METHODS

4.2.1 EXPERIMENTAL DESIGN AND TREATMENTS

The experiment was carried out using a randomised complete block design. There were 4 salinity treatments and 5 replicates. The salinity levels tested were 0, 50, 100, and 150 mol m^{-3} NaCl. $CaCl_2$ was also applied to the salinity treatments (50, 100, and 150 mol m^{-3} NaCl) in the ratio of 20:1 (moles Na:moles Ca) as suggested by Gorham *et al.* (1985a) in order to increase the potassium/sodium ratio (Epstein 1961; Läuchli and Epstein 1970; Hanson 1984). Calcium has been known for some time to alleviate the effects of salt (LaHaye and Epstein 1969), possibly by maintenance of ion selectivity of membranes (Greenway and Munns 1980). Cramer *et al.* (1985) have shown that calcium protects membranes from the adverse effects of sodium by

maintaining membrane integrity and minimising leakage of cytoplasmic potassium. Recently, it has been shown that the high ionic strength of saline solutions displaces calcium from the membranes of root cells (Cramer *et al.* 1985; Lynch and Läuchli 1988; Lynch *et al.* 1987), possibly contributing to salinity-induced calcium deficiencies. The soil solution usually provides an adequate supply of calcium to plants (Kirkby and Pilbeam 1984), so it was added to the salt stock solution in the form of $CaCl_2.2H_2O$. Care had to be taken not to induce calcium toxicity or deficiency which would not normally occur under field conditions.

4.2.2 PLANT MATERIAL AND GROWTH CONDITIONS

The experiment was initiated on 13 May 1989 and terminated on 29 June 1989. Spring Wheat variety Wembley was used in this and later experiments. The experiment was carried out in glasshouse with no control of temperature and without supplementary lighting.

4.2.3 GROWTH CONTAINERS AND AERATION

In Experiment 1 there were 16 plants per container. In this experiment many plants were required for chemical analyses, GER measurements and growth analyses. Therefore, larger containers were used. In this experiment 25 l water holding plastic containers (63 cm long x 35 cm wide x 18.5 cm deep) were used. Prior to seeding, eight 7 mm (for air supply) and one 9 mm (for solution changes) holes were made in the front, sides and bottom of the containers (Figure

4.1). The holes were plugged with rubber bungs to facilitate easy changes of nutrient solutions and to fix air supply needles (No. 16: Terumo Europe, Belgium). The containers were arranged along the sides of large work benches, again to facilitate easy access for maintenance and measurements. Instead of polyurethane tubing (Experiment 1), silicon tubing (Scientific Services, Chester, UK) was used to facilitate sealing of holes created by needles in it. The silicon tubing (5 mm internal diameter (ID), 8 mm outer diameter (OD)) was fixed along the sides of the work benches and then connected to the air regulator. Air from the silicon tubing to the containers was supplied via narrow (0.58 mm ID, 0.96 mm OD) polythene capillary tubing (Portex Ltd. Hythe, Kent, England), which was cut into appropriate lengths and then fixed with needles at both ends inserted into the silicon tubing and the bungs fitted in the containers. This system allowed a more uniform and efficient distribution of air in each container and avoided the air blockage problem encountered when using the 3-way air regulators and aerators in Experiment 1.

4.2.4 GERMINATION AND RAISING OF SEEDLINGS

Plants were germinated and grown in P180 plugtrays (Cookson Plantpak Ltd., Maidon, Essex ,UK). Prior to seeding, capillary matting was fixed at the bottom of each plugtray with adhesive (Copydex). Another P180 plugtray was stacked on top with the one containing the capillary matting at the

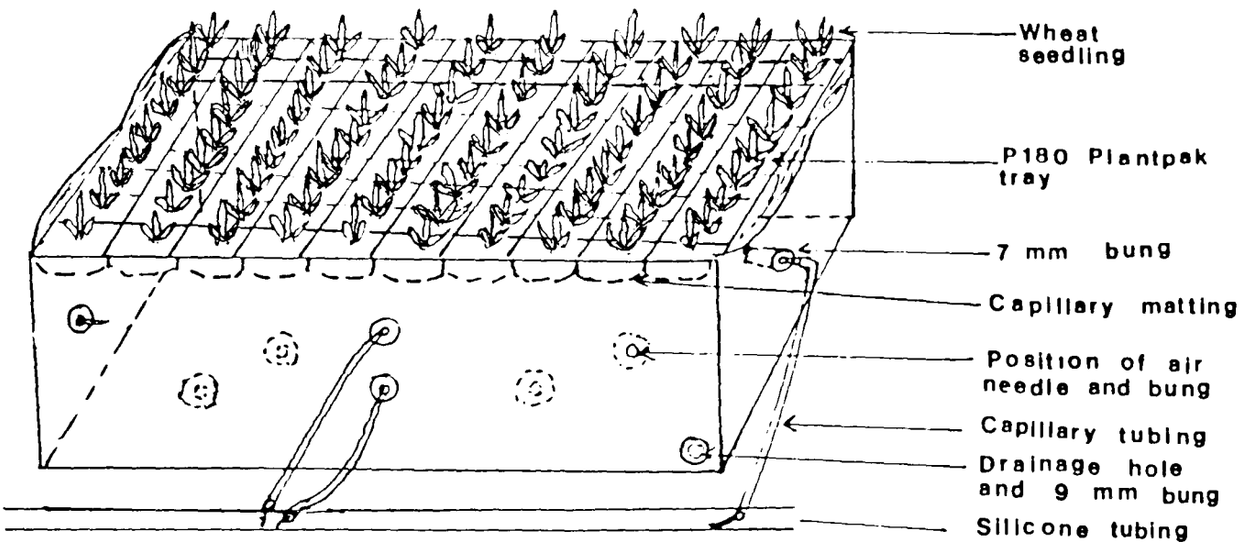


Figure 4.1: Growth container, showing planting system, aeration line and drainage hole.

bottom. This provided increased strength to the plugtray to withstand plant weight gained later during the experiment. The pairs of plugtrays were then placed on the top of the 25 l containers which were filled with nutrient solutions (0.4 g/l Phostrogen). All the cells of the P180 plugtrays were filled with vermiculite. Initially, after soaking the vermiculite, seeds were sown directly into the trays with one seed per cell and a total of 180 seeds per tray. After 4 days it was realised that using this technique the nutrient solution was creating waterlogged conditions for the germinating seeds, even though they were aerated through the air supply. The experiment was therefore resown. New seeds were presoaked overnight in a muslin bag suspended under a slow running tap. The next morning the seeds were sown on the moist vermiculite in the P180 plugtrays with one seed per cell and a total of 180 seeds per tray (51.5 cm x 30.0 cm). The seeds were then covered with newspapers and kept moist until the radicles and coleoptiles were seen to be emerging. The newspapers were later removed. The containers were then filled with tap water containing 0.4 g/l Phostrogen and the trays placed over the containers. The seedlings were later thinned to 90 per tray by uprooting alternate rows along the width of the tray, as 9 rows of 10 plants at a distance of 60 x 30 mm, which is equivalent to a plant population of 583 plants m^{-2}.

4.2.5 PREPARATION OF NUTRIENT BASED SALT SOLUTION

In this and later experiments, for ease of preparation and solution changes, Phostrogen based nutrient and salt stock solutions were used for each treatment. After necessary calculations for each salt treatment being made, Phostrogen and all the micronutrients (Appendix B) were added to each salt stock solution and the volume was made to 10 l (Appendix C) and then stored in a cold room for further use. This technique facilitated the maintenance of a uniform supply of salt stress throughout the course of each experiment.

4.2.5.1 APPLICATION OF SALT STRESS

Prior to salt stress, on 25 May 1989, 12 days after seeding, when the plants had 2 emerging leaves, the containers were drained out and refilled. The stock salt and nutrient solutions were drip fed into the plant containers from polyurethane containers which were fixed to retort stands. One needle, connected to polythene capillary tubing, was inserted into the bottom side of the polyurethane container and the other into the 25 l container holding the plants. The concentration of stock solutions varied for each treatment so that final desired concentration would be achieved. By this method salinity was introduced gradually and continuously over a 2 day period in each treatment. Salinisation was complete on 27 May 1989 at 1+2 leaf stage (one fully expanded and two

expanding leaves).

4.2.6 GROWTH MEASUREMENTS

The 90 plants in each container were divided into 3 groups. The central plants were used for leaf extension growth measurements, the plants to the left side for gas exchange measurements and the plants to the right side for chemical analyses.

4.2.6.1 LAS AND TAS

For LAS, TAS and other growth measurements, 4 plants in the centre of each tray were marked with small wire rings of different colours. After every second day the numbers of fully expanded leaves and newly emerged leaves and tillers on the mainstem of these 4 marked plants in each container were recorded to determine LAS and TAS.

4.2.6.2 LEG AND FLL

LEG of leaf 3 and subsequent leaves on the mainstem of the 4 marked plants was measured in mm with a ruler every day, starting after the initial salt stress on 25 May 1989 and following the procedures described in section 3.2.6.3.

After completion of LEG measurements, when the ligule was emerged, FLL of sheath plus leaf lamina was recorded.

4.2.6.3 LER AND LED

LER and LED were calculated following the procedure described in sections 3.2.6.5 and 3.2.6.6.

4.2.6.4 GROWTH ANALYSES

Harvests for growth analysis were made when leaf 5 and leaf 7 were fully expanded (ligule emerged). Leaf 5 was fully expanded on 14 June 1989 (18 DAS) whereas leaf 7 was fully expanded on 19 June (23 DAS) at 100 and 150 mol m^{-3} NaCl and on 26 June (30 DAS) at 0 and 50 mol m^{-3} NaCl, respectively. At each harvest, 10 plants were harvested from the second and third rows of the right side of the 5 replicate containers of each treatment concerned. The plants were kept in large polythene bags and taken to the laboratory adjacent to the glasshouse. The dead leaves on the mainstem (Leaf 1 and 2 or plus 3) were grouped together. All the expanded and emerging leaves on the mainstem were also detached separately at the point joining the sheath. The primary and secondary tillers, their leaves and stems emerging from the axil of leaf 1 were considered as 'Tiller 1'. Most plants did not produce more than 2 primary tillers. Therefore, the primary and secondary tillers, their leaves and stems emerging from the axils of leaf 2 and 3 were considered as 'Tiller 2'. The leaves of 'Tiller 1' and 'Tiller 2' were detached from the point joining the sheath, and stems were cut into small portions to facilitate smooth feeding through the automatic area meter. Similarly mainstems were also cut into small portions. The areas of all the detached green parts except those of dead leaves were recorded using an automatic area meter as in section 3.2.5.7.

After recording leaf area of all the shoot parts, detached roots and all shoot parts were prepared for dry weight and then for chemical analyses as in section 3.2.6.7.

4.2.7 STOMATAL FREQUENCY AND SPECIFIC LEAF WEIGHT

A further four plants were sampled from all replicates when leaf 5 and leaf 7 became fully expanded to determine stomatal frequency (SF) and specific leaf weight (SLW). The appropriate leaf lamina was detached from the point joining the sheath. Once a plant had been sampled, it was marked and not used for later harvest or gas exchange measurements, but was left in the container to maintain the original planting density. After harvest the leaves were immediately kept in a polythene bag and brought to the laboratory adjacent to the glasshouse. In the laboratory, lamina area of individual leaves was recorded using an automatic area meter. The central portion of the adaxial surface of the leaf was then smeared with a thin coat of 'Germolene New Skin', a transparent synthetic skin. A strip of clear 'sellotape' was then applied to the surface of the leaf and carefully peeled off, to avoid removing any leaf epidermis with it. This removed the impression of the leaf surface recorded in the synthetic skin. The 'sellotape' was mounted on a slide and 3 measurements of stomatal frequency were made at random using a transmission light microscope (magnification x 40). The area of the field of view was determined using a scale grid and the numbers of stomata per mm^2 were calculated. The leaves were then dried in an oven

at 80°C for 48 hours and the leaf lamina dry weight recorded. This whole procedure gave the following measurements:

1. Leaf lamina area (A in cm^2)
2. Leaf lamina dry weight (LDWT in mg)
3. Stomatal frequency (SF in stomata mm^{-2})

Specific leaf weight (SLW in mg cm^{-2}) was calculated:-

SLW = Leaf lamina dry weight/Leaf lamina area

Stomatal frequency per leaf (SF_1) was also calculated:-

SF_1 = SF x A

4.2.8 INFRA-RED GAS ANALYSIS SYSTEM

In this and subsequent experiments gas exchange was measured using a portable infra-red gas analysis system. This equipment consists of an Infra-red gas analyser (IRGA, model LCA2), air supply unit (ASU), and the Parkinson leaf chamber (PLC) (Analytical Development Company, Hoddesdon, Herts, UK).

4.2.8.1 LAYOUT AND SETUP OF THE IRGA

The Parkinson leaf chamber was connected to an 'open system' IRGA, which recorded the difference in the fraction of CO_2 in the air entering (C_e) and leaving (C_o) the chamber. Air was supplied by a volumetric air supply unit which drew its reference air from outside the glasshouse down a tube. The air was dried by passing through two columns of silica gel.

The PLC used in this and later experiments was designed for use on grass/cereal leaves. It was complete with temperature, humidity and light sensors and operated from 12v DC drawing about 30 milliamps. A selenium cell next to the chamber window measured photosynthetically active radiation (PAR) in μmol m^{-2} s^{-1}. The PLC has a vaisala element humidity sensor which needs regular calibration. Above 70% relative humidity calibration is unreliable. For calibration, dry air was passed into the leaf chamber by removing the silica gel columns from the air pump and replacing them with magnesium perchlorate. When the readings were stable, the zero span could be set. Air of known water vapour concentration was then passed into the chamber from a water vapour generator and the appropriate span control adjusted. The IRGA was calibrated using air of known CO_2 concentration.

Prior to GER measurements, the datalogger (Analytical Development Company) was connected to the IRGA. Via electrical contacts to the IRGA, the logger was able to record CO_2 depletion in volume parts per million (vpm), relative humidity (RH), air temperature (°C) and photosynthetically active radiation (PAR in μmol m^{-2} s^{-1}) incident on the leaf chamber. Stored within the logger were a number of constants, some of which had to be determined experimentally. These were:-

1. Boundary layer resistance

The boundary layer resistance used in this and later experiments was 0.300. This was determined by exposing

both surfaces of a 2 cm^2 area of damp filter paper within the PLC and measuring equilibrium relative humidity and cuvette air temperature. It was possible to read off boundary layer resistance (rb) in $m^2 s\ mol^{-1}$ from a prepared graph of boundary layer resistance against relative humidity supplied with the equipment (Parkinson 1985).

2. EMAX

EMAX, which is the response of the IRGA to infinite water concentrations, used in this and later experiments was 2.30. This was determined by passing CO_2-free air through the reference inlet on the analyser set to reference and recording the negative deflection on the readout. CO_2-free air of known water vapour content was then passed through the analyser and the new + or - readings were recorded. The difference between the two readings gave the instrument's response to a known water vapour content. This value was converted to EMAX by referring to a prepared table supplied with the equipment.

3. Atmospheric pressure

Before and during the onset of GER measurements, atmospheric pressure was recorded from a portable barometer placed inside the glasshouse.

4. Air flow rate

A constant volume flow rate of 400 $cm^3\ min^{-1}$ dry air into the cuvette in the IRGA from the volumetric air

supply unit was used.

5. Leaf area

This was input at the time of measurements using mean leaf width and the known length of the window of the PLC.

The logger uses an RCA 1802 microprocessor and has 8 kilobytes of memory which can store 240 sets of LCA2 results. Data stored in the logger are labelled with plot number and time and date from a real-time clock. The datalogger uses a programme dedicated both to the collection of data from the LCA2 and to the calculation of following plant physiological parameters from the data:-

1. Leaf temperature (T_l in °C)
2. Transpiration rate (E in mol m^{-2} s^{-1})
3. Stomatal conductance (g_s in mol m^{-2} s^{-1})
4. Net photosynthesis (Pn in μmol m^{-2} s^{-1})
5. Sub-stomatal CO_2 concentration (Ci in μl l^{-1})

Full details of how these values are calculated are given in the datalogger manual (Analytical Development Company).

4.2.9 GAS EXCHANGE MEASUREMENTS

At least 15 minutes prior to gas exchange (GER) measurements, the air supply unit, IRGA and the PLC were switched on to purge the humidity in the system and to bring the IRGA and the PLC up to their operating temperature. GER measurements on leaf 5 were made *in situ* on fully expanded leaves on 14 June 1989. Measurements were made between 08.00-10.00 (morning), 12.00-14.00 (noon) and 16.00-18.00

(afternoon) on 2 randomly selected mainstem leaves at the appropriate insertion in each replicate container and salinity treatment. There were, therefore, 2 leaves x 5 replicates x 4 salinity treatments. These measurements were carried out in the glasshouse with no supplementary lighting. The PLC was clamped over the central portion of a leaf and held horizontally, care being taken to keep the adaxial side of the leaf uppermost and to avoid shading by any of the other leaves. To avoid stomatal closure, CO_2 depletion and other parameters were recorded approximately 30 seconds after leaf enclosure in the PLC (Analytical Development Company). Immediately after GER measurements were made, the leaf lamina was cut from the point joining the sheath and preserved in a polythene bag for leaf area and sap extraction. The plants were marked with paint so that they were not used for further measurements and to maintain the desired plant population. Measurements of leaf 7 at 100 and 150 mol m^{-3} NaCl salinity treatment were made when this leaf became fully expanded in these treatments on 20 June (24 DAS). This time, instead of 2 leaves per replicate container, 10 randomly selected leaves from one replicate were used for GER measurements during morning, noon and afternoon. GER measurements for leaf 7 of all the salinity treatments in each replicate container were also made during the morning on 28 June (32 DAS) when leaf 7 became fully expanded at 0 and 50 mol m^{-3} NaCl. The measurements for noon and afternoon were postponed due to

low PAR. In the afternoon the plants were sprayed for mildew (Section 4.3). Next morning, GER measurements at 0 and 50 mol m^{-3} NaCl were recorded but were not considered suitable for statistical analysis due to spray damage to plants.

4.2.9.1 TRANSFER AND ANALYSIS OF DATA

The data collected by the datalogger were transferred to 5¼" floppy disks on an Apple II microcomputer via an RS232 interface through a transfer programme at Aber Farm, 7 miles from the main University at Bangor. The data was stored in this format and later transferred onto the mainframe computer at Bangor using another Apple computer and transfer programme (Figure 4.2). During the GER measurements whenever the humidity inside the glasshouse was high (70%) and g_s exceeded 0.999, the datalogger was not able to calculate T_l, E, g_s, and Pn but was able to record CO_2 depletion, air temperature, relative humidity and photon flux density. This specific problem occurred because the programme inside the datalogger would not accept values of g_s 1.0 or greater (Parkinson, personnel communication). This problem was overcome by using a FORTRAN language programme on the mainframe computer to recalculate all the parameters from the original data (Appendix D).

4.2.10 PLANT CHEMICAL ANALYSES

The first set of chemical analyses was performed on sap extracted from leaves 3, 4, 5, 6 and 7 as they became fully expanded. The second set of chemical analyses was performed

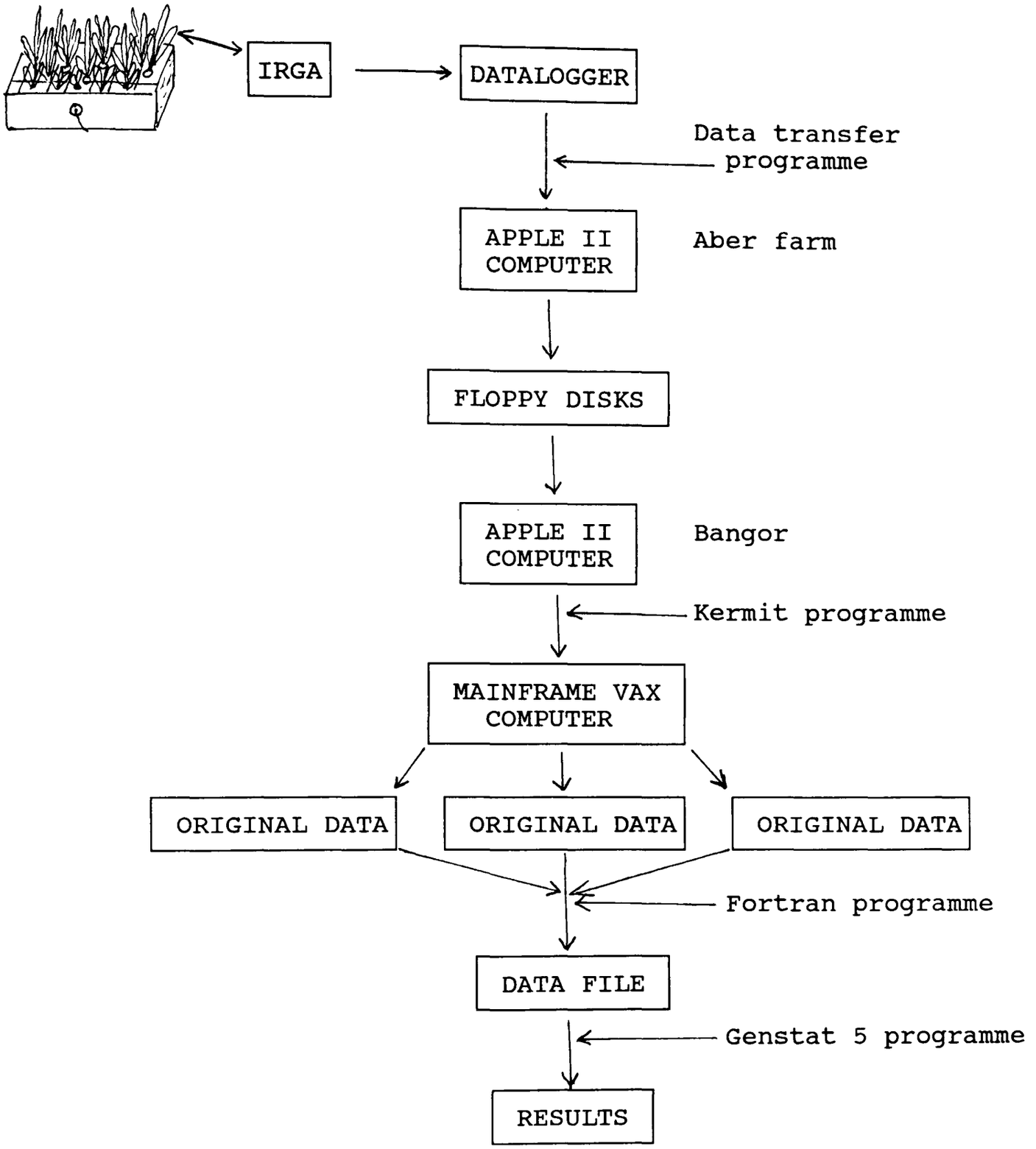


Figure 4.2: Flow diagram showing main steps followed for recording data on datalogger of infra-red gas analyser (IRGA), different programme dedicated for transfer of data from datalogger to mainframe computer and statistical analysis to obtain results for different treatments.

on the dried, ashed and nitric acid digested samples of plants harvested for growth analysis.

4.2.10.1 EXTRACTION OF LEAF SAP

Leaf samples for extraction of sap were harvested around mid-day from all replicate containers when leaves 3, 4, 5, 6 and 7 became fully expanded. At each harvest four leaves were sampled at random from the plants in the right hand side of each container that had been designated for this purpose (Section 4.2.6).

The leaf lamina was detached at the junction with the sheath. The leaf samples were rinsed twice with distilled water, blotted dry and rolled up and placed in labelled 1.5 cm^3 polypropylene microcentrifuge tubes and the samples were frozen at 0°C. The method for sap extraction was the same as that used in Experiment 1 (Section 3.2.7.1).

4.2.10.2 DETERMINATION OF SAP OSMOTIC PRESSURE

Sap osmotic pressure was determined on undiluted sap using a vapour pressure osmometer as described in Experiment 1 (Section 3.2.7.2).

4.2.10.3 DETERMINATION OF SODIUM, POTASSIUM AND CHLORIDE

Oven dried samples were ground in a sample mill, ashed at 450°C overnight and then digested with 1 mol dm^{-3} HNO_3 as in Experiment 1 (Section 3.2.7.3).

Cation (sodium and potassium) concentrations were determined using a Pye Unicam atomic emission spectrophotometer against a series of standard solutions as in Experiment 1 (Section 3.2.7.4). The scale was expanded at the lower salinity range (between 0 and 1.0 mol m^{-3} NaCl) to ensure the maximum possibility of detecting any sodium present in samples from 0 mol m^{-3} salinity treatment.

Chloride in the sap and from the nitric acid extracts was determined using a chloride sensitive electrode as in section 3.2.7.4.

4.3 MAINTENANCE OF EXPERIMENT AND OBSERVATIONS

During the course of the experiment, losses of water by evapotraspiration were replaced daily. After every two weeks the solutions were changed and the containers were washed out to remove any sediments. Once refilled with tap water, one litre of nutrient and salt stock solution was added to each replicate container for all the salinity treatments. This technique facilitated rapid solution changes.

Minimum and maximum air temperatures were recorded in the glasshouse and bright sunshine hours at a weather station 1 km away from the glasshouse. Mean daily temperatures each week and mean bright sunshine hours each week are presented in figure 4.3. Weekly averages of sunshine hours ranged from 5 to 12 hours while mean daily temperatures each week ranged between 16 to 28°C during the course of the experiment.

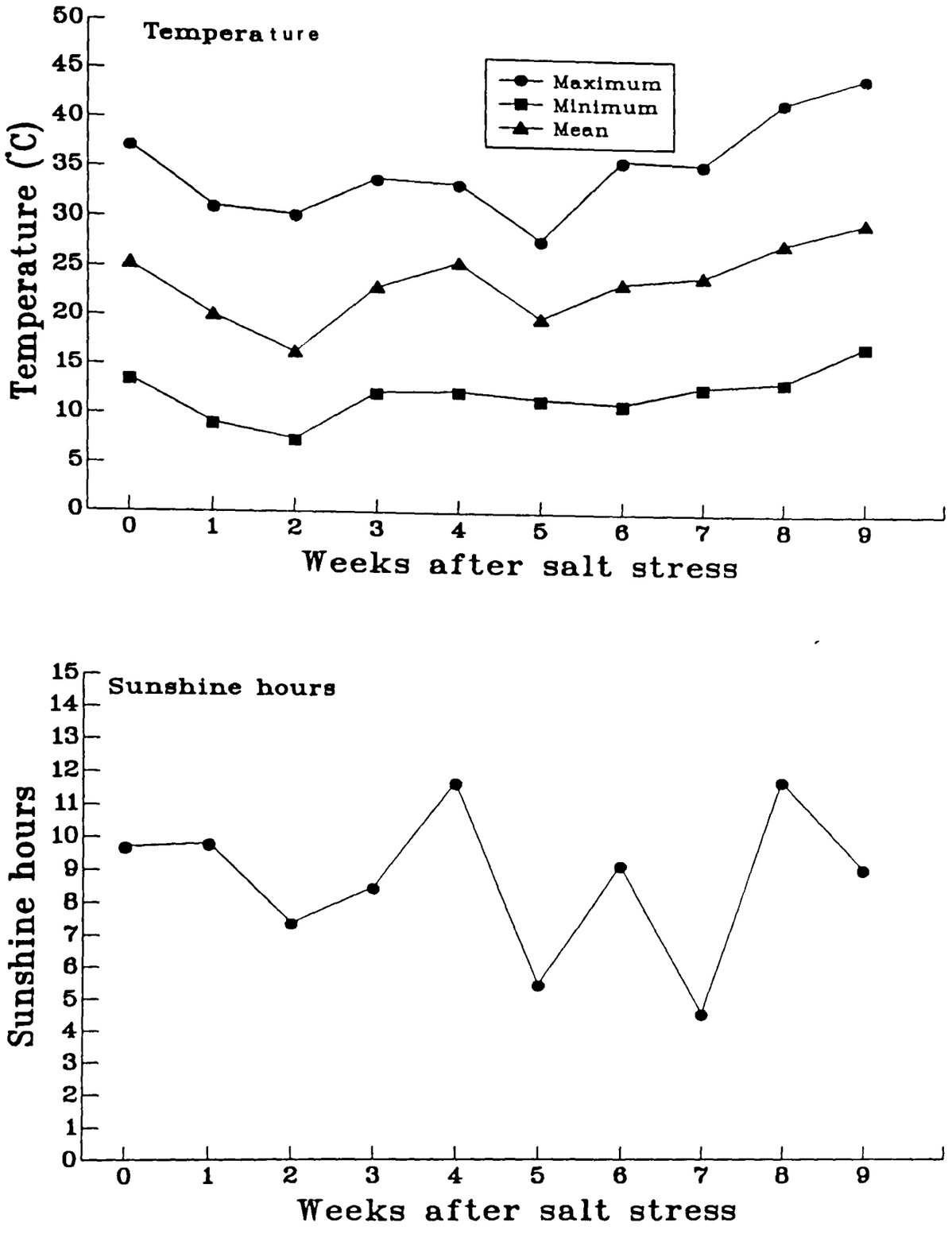


Figure 4.3: Mean daily temperature each week in the glasshouse and mean sunshine hours each week during the growth period of spring wheat.

On 20 June, an aphid attack was observed on the plants. More aphids were observed at high salinity (100 and 150 mol m^{-3} NaCl) than at low (0 and 50 mol m^{-3} NaCl). Next day, during late afternoon, the plants were sprayed with Murphy dimethoate insecticide (Murphy Chemical Company) to control this aphid attack. On 28 June, mildew was observed. This was more prominent on plants at low (0 and 50 mol m^{-3} NaCl) than at high salinity. All the plants were sprayed with 'Mistral' (a contact and systemic fungicide containing 75% w/v Fenpropimorph, May and Baker) at ½ strength of the recommended dose (1.0 l in 400 l of water). Next day, possibly due to interaction with high temperature, all the plants showed symptoms of damage. The damage was more noticeable on the plants at high salinity. Due to this problem the experiment was terminated. Data were collected at morning and afternoon but considered unsuitable for analysis. Therefore, the planned gas exchange measurements of fully expanded leaf 7 could not be made. Moreover, the gas exchange and ion partitioning data at fully expanded flag leaf stage could not be completed.

4.4 STATISTICAL ANALYSES

The statistical analysis of the data was performed by the analysis of variance method. In each case the values were checked for normality by inspection of histograms of residuals and by plotting the residuals against the fitted values. When a significant 'F' value was obtained, for

treatment effects, Tukey's test at 5 percent probability level was applied to the treatment means (Snedecor and Cochran 1980). Honestly significant differences (HSD) for comparisons between treatment means, where significant differences were shown to exist, were calculated by multiplying the standard error of treatment means (SE) by Tukey's value Q (K, df). Where K is the number of means to be compared and df is the residual degrees of freedom.

4.5 RESULTS

4.5.1 GROWTH MEASUREMENTS

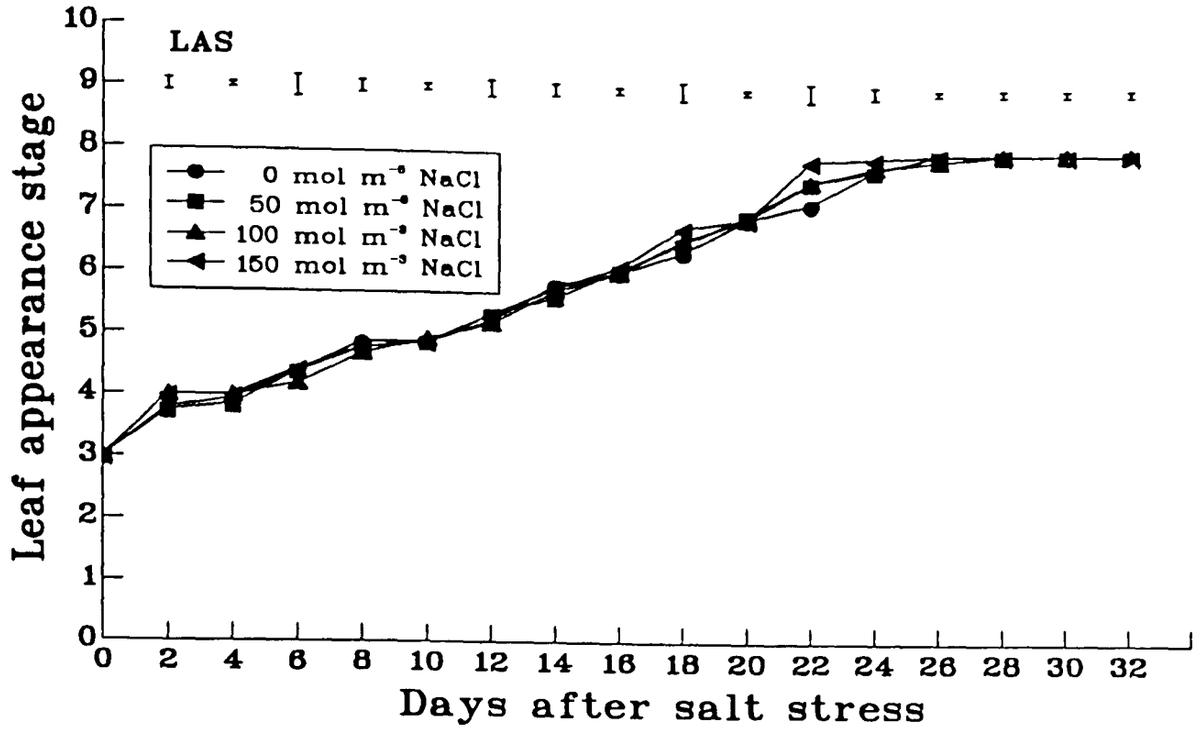
4.5.1.1 EFFECT OF SALINITY ON LAS AND TAS

Generally, LAS was not significantly affected by salinity (Figure 4.4). In the majority of plants leaf 8 was the flag leaf but at 0 mol m^{-3} NaCl leaf 9 was the flag leaf in a few plants.

TAS was not significantly affected by salinity until 14 DAS, but was higher at 0 mol m^{-3} NaCl than in all other salinity treatments from 6 DAS onwards (Figure 4.4). With salinity no extra tillers were produced after 10 DAS and number of tillers decreased as salinity increased.

4.5.2 EFFECT OF SALINITY ON LEG, LER, LED AND FLL

LEG of leaf 3 and leaf 4 was not significantly affected by salinity (Figure 4.5). From 8 DAS onwards, LEG of leaf 4 was higher at 0 mol m^{-3} NaCl than in all other salinity



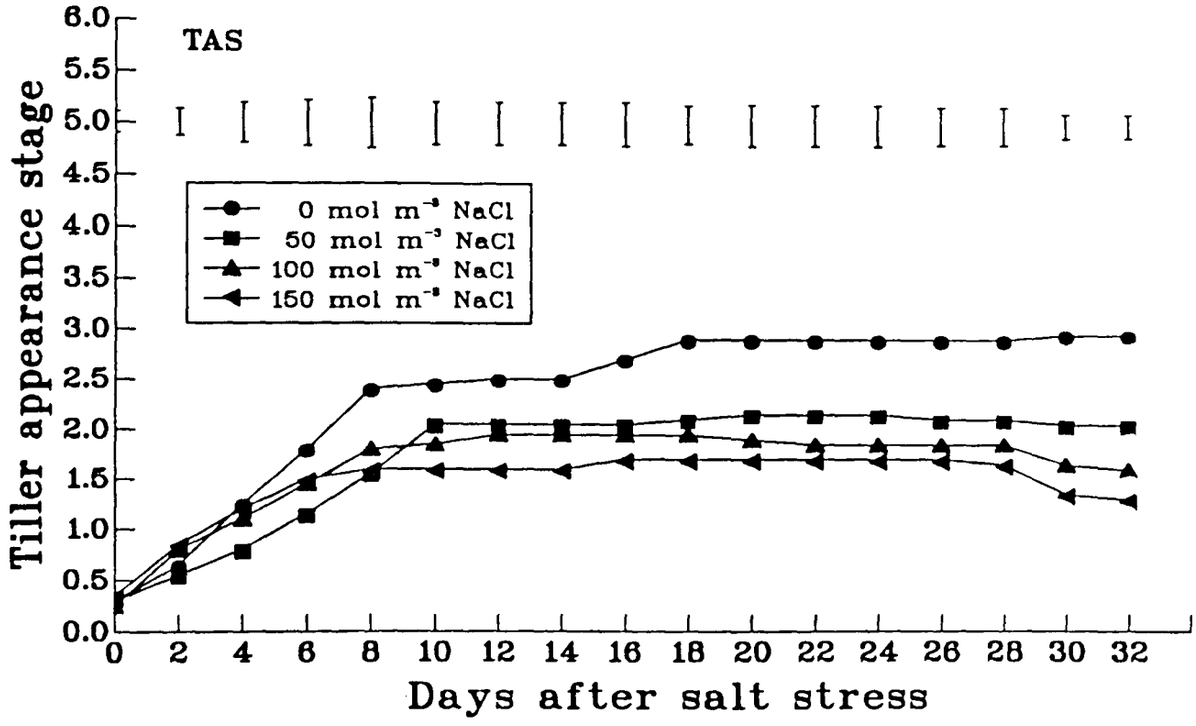


Figure 4.4: Effect of salinity levels on leaf appearance stage (LAS) and tiller appearance stage (TAS) of spring wheat. I= standard error of means.

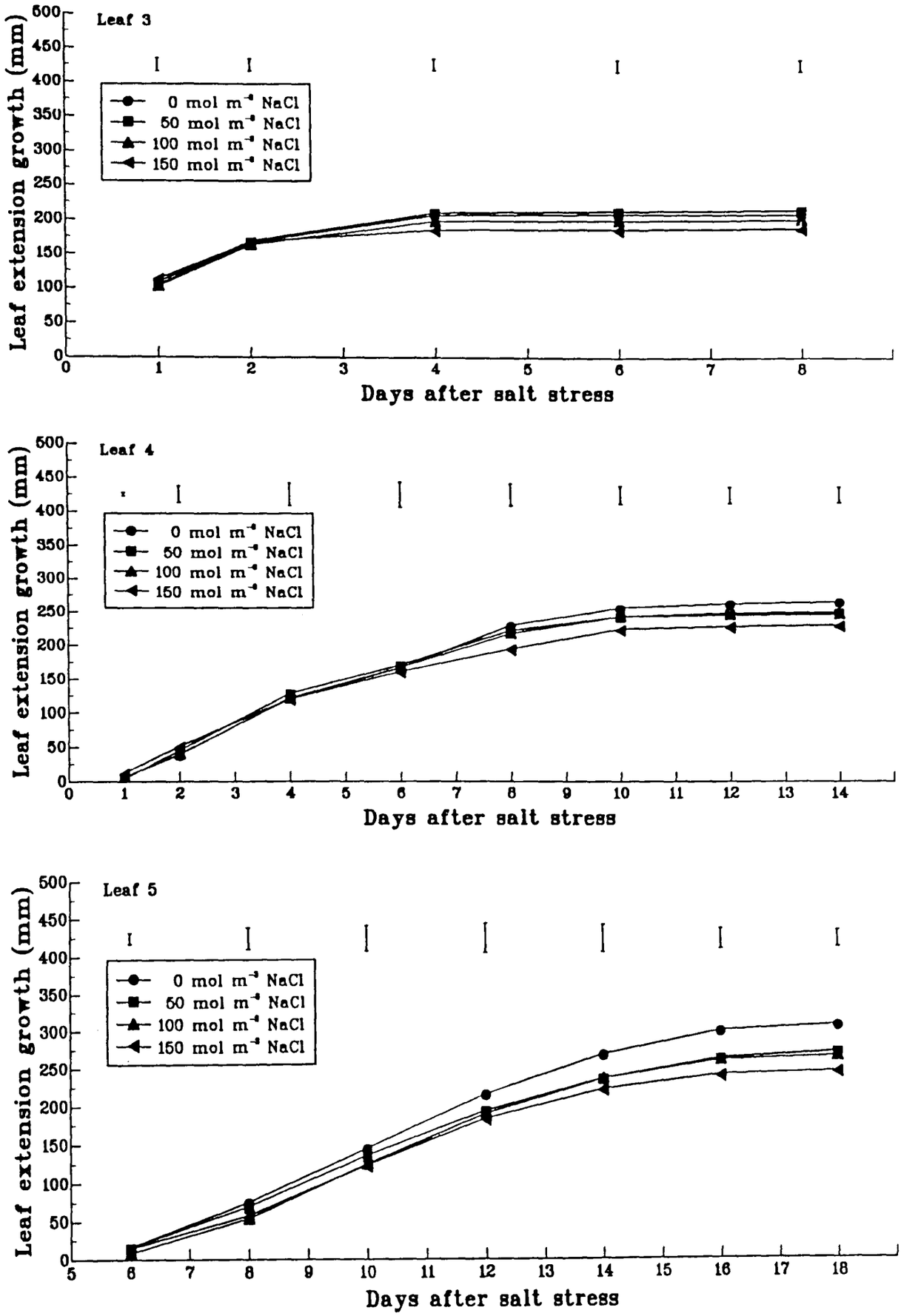


Figure 4.5: Effect of salinity levels on leaf extension growth of leaf 3, leaf 4 and leaf 5 of spring wheat. I= standard error of means.

treatments. LEG of all other leaves (leaf 5, leaf 6, leaf 7 and the flag leaf) was decreased as salinity increased but the effects were significant only at the last one or two sampling dates of each leaf (Figure 4.6).

LER of leaf 3, leaf 4, leaf 5, and the flag leaf was not significantly affected by salinity (Table 4.1). The trends in results for leaf 3 and leaf 4 were inconsistent while LER of leaf 5 and the flag leaf was decreased with increase in salinity. LER of leaf 6 and leaf 7 was significantly decreased with increase in salinity.

FLL of leaf 3 was decreased with increase in salinity (Table 4.1) but not significantly. FLL of all the later appearing leaves was significantly decreased with salinity but the differences between salinity treatments were not always significant.

LED of all the leaves was not significantly or consistently affected by salinity (Table 4.1).

4.5.3 GROWTH ANALYSES

4.5.3.1 EFFECT OF SALINITY ON GREEN AREA AND DRY WEIGHT

Green areas of leaf 3 and leaf 4 were not significantly affected by salinity at harvest 1 and harvest 2, respectively (Table 4.2). Green area of all other plant parts was significantly decreased with increase in salinity at both harvests.

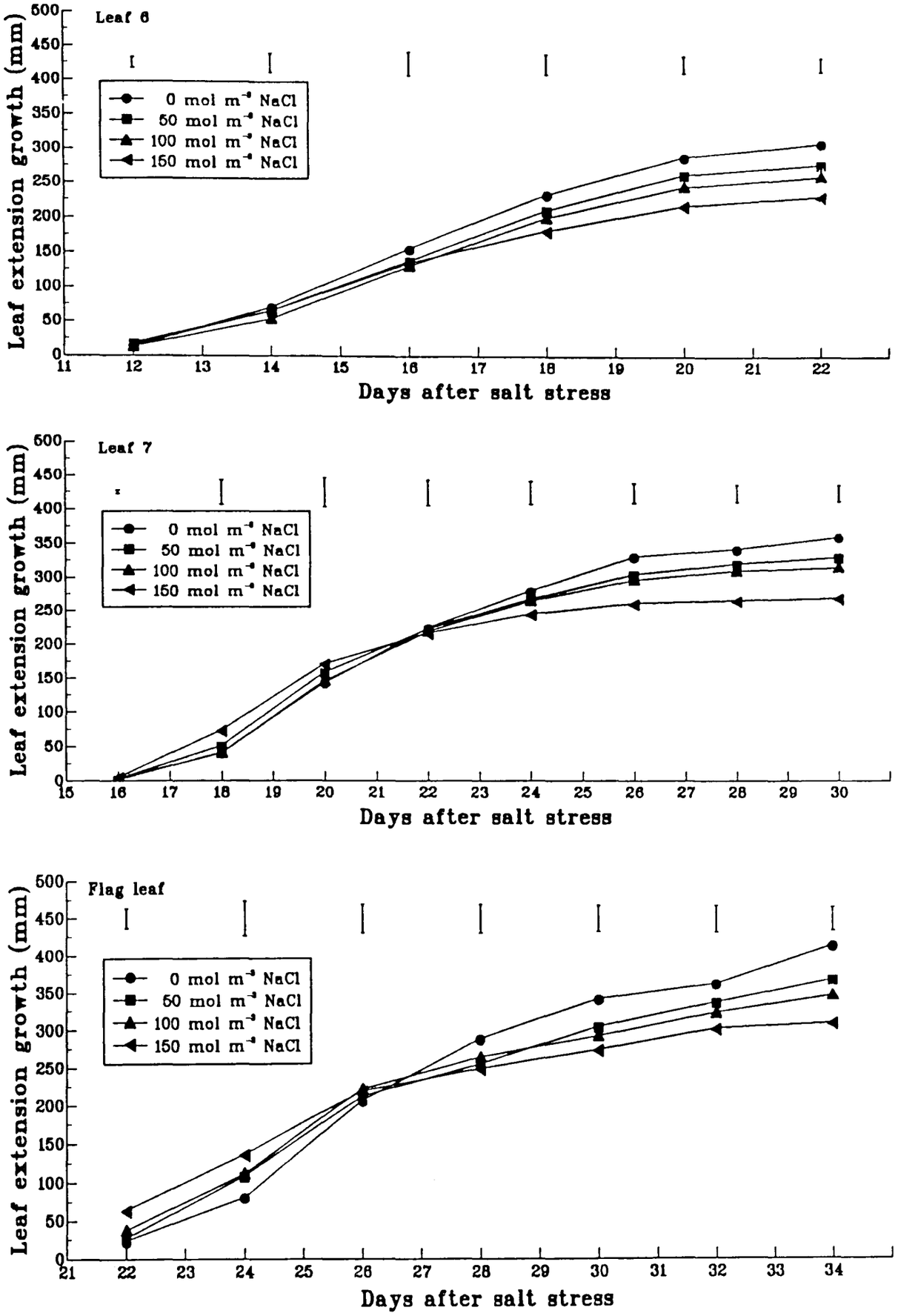


Figure 4.6: Effect of salinity levels on leaf extension growth of leaf 6, leaf 7 and the flag leaf of spring wheat. I= standard error of means.

Table 4.1: Effect of different salinity levels on final leaf length (FLL in mm), leaf extension rate (LER in mm day^{-1}) and leaf extension duration (LED in days) of different leaf insertions on mainstem of spring wheat.

Leaf insertions	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Final leaf length						
Leaf 3	210.1	208.8	208.6	196.1	11.1	NS
Leaf 4	295.4	290.5	285.0	248.9	8.5	35.7**
Leaf 5	354.4	322.5	307.6	287.9	13.5	56.5*
Leaf 6	352.3	313.8	293.8	260.4	12.1	50.6**
Leaf 7	363.1	313.9	288.6	262.5	11.5	48.4**
Flag leaf	356.7	293.2	279.8	274.6	8.9	37.5**
Leaf extension rate						
Leaf 3	31.0	32.3	29.9	22.4	3.3	NS
Leaf 4	27.3	28.3	27.4	28.8	3.0	NS
Leaf 5	31.9	30.7	30.4	28.0	1.8	NS
Leaf 6	37.8	35.5	33.9	29.7	1.2	5.0**
Leaf 7	42.5	40.7	40.2	26.9	3.5	14.5*
Flag leaf	47.5	35.4	33.1	32.0	5.9	NS
Leaf extension duration						
Leaf 3	6.9	6.9	7.7	8.8	0.8	NS
Leaf 4	10.9	10.7	9.8	9.7	0.8	NS
Leaf 5	11.2	10.5	10.1	10.4	0.3	NS
Leaf 6	9.4	8.9	8.7	8.4	0.3	NS
Leaf 7	9.1	7.9	7.6	7.9	0.7	NS
Flag leaf	7.8	7.6	7.8	6.8	0.8	NS

SEM = Standard error of means,
HSD = Honestly significant differences,
NS = Not significant,
*,**= Significant at 5 and 1% probability levels respectively.

Table 4.2: Effect of different salinity levels on green area (cm^2/plant) of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

Plant parts	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Harvest 1						
Expanding leaves	31.7	27.0	23.8	18.9	1.5	6.5**
Leaf 5	16.7	14.7	13.7	11.6	0.4	1.6**
Leaf 4	11.8	11.1	10.2	9.1	0.4	1.6**
Leaf 3	6.7	6.9	5.9	5.2	0.4	NS
Dead leaves	-	-	-	-	-	-
Stem	5.5	4.7	4.6	4.5	0.2	0.7**
Tiller 1	57.5	41.8	34.5	33.5	2.4	10.1**
Tiller 2	48.3	27.8	23.1	21.1	2.7	11.4**
Total	178.1	134.0	115.7	103.9	--	--
Harvest 2						
Expanding flag leaf	21.9	16.9	13.7	13.7	0.9	3.9**
Leaf 7	24.2	20.2	15.2	12.7	0.8	3.2**
Leaf 6	21.4	17.4	15.3	12.4	0.7	2.9**
Leaf 5	17.4	14.3	13.8	11.7	0.6	2.6**
Leaf 4	9.5	9.5	9.0	9.0	0.6	NS
Dead leaves	-	-	-	-	-	-
Stem	18.5	17.5	10.1	10.1	0.5	2.3**
Tiller 1	84.9	58.6	45.7	42.8	4.1	17.2**
Tiller 2	38.6	27.9	20.8	18.2	3.2	13.2**
Total	236.3	182.3	143.5	130.5	--	--

SEM = Standard error of means,
HSD = Honestly significant differences,
NS = Not significant,
** = Significant at 1% probability level,
- = Not applicable,
-- = Data not analysed.

Salinity significantly decreased dry weight of leaf 5, tiller 2 and roots at harvest 1 (Table 4.3). Dry weight of all other plant parts was not significantly affected but was decreased with salinity in dead leaves, stems and tiller 1 while it was inconsistently affected in other plant leaves. At harvest 2 dry weight of all plant parts except leaf 5 and dead leaves was significantly affected by salinity. Dry weight was consistently decreased with salinity in the expanding flag leaf, leaf 7, leaf 6, stems, tiller 2 and roots but increased in leaf 4 and dead leaves. Total plant dry weight was consistently decreased with salinity at both harvests.

4.5.4 EFFECT OF SALINITY ON LAMINA DRY WEIGHT, SPECIFIC LEAF WEIGHT AND STOMATAL FREQUENCY OF SPRING WHEAT

Salinity significantly decreased A and LDWT but increased SF of leaf 5 and leaf 7 (Table 4.4). Specific leaf weight was not significantly affected by salinity for leaf 5 but was significantly higher at 150 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl for leaf 7. SF_1 of leaf 5 was not significantly affected by salinity whereas it was significantly decreased in leaf 7.

4.5.5 EFFECT OF SALINITY ON ION PARTITIONING IN SPRING WHEAT

The results presented in this section are based on dried, ashed and nitric acid digested samples.

Table 4.3: Effect of different salinity levels on dry weight (mg/plant) of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

Plant parts	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Harvest 1						
Expanding leaves	105.8	95.8	89.8	86.2	5.9	NS
Leaf 5	44.4	42.0	36.6	36.6	1.5	6.3**
Leaf 4	28.0	28.6	27.0	25.2	0.9	NS
Leaf 3	15.8	16.2	15.4	15.4	1.1	NS
Dead leaves	17.2	16.0	15.0	14.8	1.1	NS
Stem	150.2	139.6	137.0	133.4	4.6	NS
Tiller 1	240.2	232.0	194.0	177.0	21.7	NS
Tiller 2	215.2	130.6	113.2	104.6	12.9	53.9**
Roots	271.0	273.6	179.4	119.4	10.6	44.4**
Total	1087.8	974.4	807.4	712.6	--	--
Harvest 2						
Expanding flag leaf	78.2	65.2	55.8	54.6	3.9	16.7**
Leaf 7	76.4	64.6	55.0	46.6	3.5	14.8**
Leaf 6	49.2	46.0	43.2	40.4	1.9	7.8*
Leaf 5	35.4	35.2	35.6	34.2	1.4	NS
Leaf 4	18.6	19.4	23.2	26.0	1.0	4.2**
Dead leaves	30.8	32.4	35.2	35.6	1.5	NS
Stem	649.0	540.0	349.0	254.0	28.0	117.6**
Tiller 1	442.0	352.0	188.0	237.0	39.2	164.6**
Tiller 2	220.0	141.0	76.0	70.0	31.6	132.7*
Roots	485.0	324.0	207.0	129.0	26.8	112.6**
Total	2084.6	1619.8	1068.0	927.4	--	--

SEM = Standard error of means,
HSD = Honestly significant differences,
NS = Not significant,
*,**= Significant at 5 and 1% probability levels respectively,
-- = Data not analysed.

Table 4.4: Effect of different salinity levels on leaf lamina area (A in cm^2), leaf dry weight (LDWT in mg), specific leaf weight (SLW in mg cm^{-2}), stomatal frequency per unit area (SF in stomata mm^{-2}) and stomatal frequency per leaf (SF_l x 10^4) of different leaf insertions of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Leaf 5						
A	16.9	15.2	13.9	12.1	0.4	1.7**
LDWT	47.2	43.2	37.6	36.0	1.6	6.8**
SLW	2.8	2.9	2.7	3.0	0.1	NS
SF	39.5	47.3	51.9	56.3	1.5	6.2**
SF_l	6.6	6.9	7.1	6.6	0.3	NS
Leaf 7						
A	24.6	21.1	16.3	12.6	1.1	4.5**
LDWT	77.3	65.7	56.0	47.7	3.2	13.4**
SLW	3.2	3.1	3.5	3.8	0.1	0.5*
SF	53.0	54.7	66.0	66.4	2.0	8.4**
SF_l	12.8	11.0	10.1	8.4	0.5	2.2**

SEM = Standard error of means,
HSD = Honestly significant differences,
NS = Not significant,
*,**= Significant at 5 and 1% probability levels respectively.

4.5.5.1 SODIUM CONCENTRATION

Salinity significantly increased Na^+ concentration in all the plant parts at both harvests (Table 4.5). A gradient of Na^+ was found in leaves at both harvests. The lower older leaves had a higher Na^+ concentration as compared to expanding leaves. At both harvests, a higher Na^+ concentration was observed in dead leaves and the lowest living leaf than in other plant parts at all the salinity levels. At both harvests, Na^+ concentration was lower in the stem than in tillers and roots at all the salinity levels.

4.5.5.2 CHLORIDE CONCENTRATION

At both harvests, Cl^- concentrations closely followed the trends of Na^+ concentration. In contrast to Na^+ concentration, Cl^- concentration was higher in the stem and tillers than in the roots (Table 4.6). At harvest 2, Cl^- concentration in dead leaves was lower than in leaf 4 except at 50 mol m^{-3} NaCl salinity treatment.

4.5.5.3 POTASSIUM CONCENTRATION

At harvest 1, salinity significantly decreased K^+ concentration in all the plant parts except expanding leaves and the stem (Table 4.7). However, K^+ concentration decreased in expanding leaves with increase in salinity while in stem higher K^+ concentration was found at 0 and 50 mol m^{-3} NaCl than at 100 and 150 mol m^{-3} NaCl, respectively. A gradient of K^+ concentration was observed in leaves such

Table 4.5: Effect of different salinity levels on sodium (Na^+ in μmol per g dry weight) partitioning in various plant parts of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

Plant parts	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Harvest 1						
Expanding leaves	32	92	140	206	17.8	74.6**
Leaf 5	42	246	364	434	29.7	124.7**
Leaf 4	40	338	460	620	39.7	166.7**
Leaf 3	60	750	878	910	60.0	252.0**
Dead leaves	96	978	950	1158	82.4	346.1**
Stem	56	186	292	442	27.3	114.7**
Tiller 1	38	228	360	564	26.1	109.6**
Tiller 2	40	278	340	474	32.0	134.4**
Roots	56	364	498	560	30.9	129.8**
Harvest 2						
Expanding flag leaf	36	70	98	196	20.1	84.4**
Leaf 7	38	148	196	360	18.6	78.1**
Leaf 6	48	278	320	384	19.2	80.6**
Leaf 5	50	472	508	752	42.3	177.7**
Leaf 4	62	744	872	1038	50.0	210.0**
Dead leaves	76	888	864	1000	45.6	191.5**
Stem	38	166	244	406	14.8	62.2**
Tiller 1	46	280	398	534	31.6	132.7**
Tiller 2	46	358	460	714	44.5	186.9**
Roots	102	404	460	640	34.7	145.7**

SEM = Standard error of means,
HSD = Honestly significant differences,
** = Significant at 1% probability level.

Table 4.6: Effect of different salinity levels on chloride (Cl^- in μmol per g dry weight) partitioning in various plant parts of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

Plant parts	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Harvest 1						
Expanding leaves	526	1480	1718	2138	77.2	324.2**
Leaf 5	722	1954	2442	2972	68.5	287.7**
Leaf 4	706	2248	2576	2816	78.8	330.9**
Leaf 3	810	3062	3302	3606	154.9	650.6**
Dead leaves	514	3378	3474	4250	143.1	601.0**
Stem	988	2688	3034	3306	54.1	227.2**
Tiller 1	860	2346	2918	3142	95.8	402.4**
Tiller 2	812	2304	2772	2984	39.3	165.1**
Roots	228	916	1094	1366	56.9	238.9**
Harvest 2						
Expanding flag leaf	235	442	985	1543	61.8	259.6**
Leaf 7	407	1337	1782	2664	118.6	498.1**
Leaf 6	593	1784	2276	2543	73.1	307.0**
Leaf 5	615	1860	2347	2980	107.5	451.5**
Leaf 4	460	2490	3840	3470	333.0	1398.6**
Dead leaves	196	2642	2793	3046	81.6	342.7**
Stem	393	1265	1631	2456	60.2	252.8**
Tiller 1	507	1825	2627	3352	150.5	632.1**
Tiller 2	550	2364	3098	3454	159.0	667.8**
Roots	298	863	1305	1547	42.8	179.8**

SEM = Standard error of means,
HSD = Honestly significant differences,
** = Significant at 1% probability level.

Table 4.7: Effect of different salinity levels on potassium (K^+ in μmol per g dry weight) partitioning in various plant parts of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

Plant parts	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Harvest 1						
Expanding leaves	1270	1250	1200	990	69.8	NS
Leaf 5	1220	1220	1000	890	73.0	306.6*
Leaf 4	1210	1280	1010	820	62.1	260.8**
Leaf 3	1140	1090	940	870	64.0	268.8**
Dead leaves	830	730	450	440	55.0	231.0**
Stem	1410	1470	1350	1300	56.9	NS
Tiller 1	1430	1410	1310	1160	55.5	233.1*
Tiller 2	1470	1300	1310	1160	44.3	186.1**
Roots	638	476	288	220	50.6	212.5**
Harvest 2						
Expanding flag leaf	742	752	812	864	20.0	84.0**
Leaf 7	860	920	978	852	33.3	NS
Leaf 6	970	1072	1018	918	49.2	NS
Leaf 5	728	972	926	878	21.8	91.6**
Leaf 4	708	678	892	838	45.1	189.4*
Dead leaves	330	396	424	418	23.6	99.1**
Stem	652	752	824	982	24.2	101.6**
Tiller 1	872	920	986	978	24.1	101.2*
Tiller 2	922	982	1170	1002	42.0	176.4**
Roots	712	432	366	334	36.1	151.6**

SEM = Standard error of means,
HSD = Honestly significant differences,
NS = Not significant,
*,**= Significant at 5 and 1 percent probability levels respectively.

that it was the highest in the expanding leaves and lowest in the dead leaves. Moreover, higher K^+ concentration was observed in stem and tillers than in roots.

At harvest 2, K^+ concentration in leaf 6 and leaf 7 was not significantly affected by salinity and the trends in results were inconsistent. Salinity significantly increased K^+ concentration in the other plant parts except the roots. As compared to harvest 1, the highest K^+ concentrations were observed in expanded leaves rather than expanding leaves. K^+ concentration in dead leaves was also lower than in other plant parts but increased with salinity as compared to harvest 1. At harvest 2, K^+ concentration in roots also decreased with increase in salinity and it was lower than in other plant parts.

4.5.6 EFFECT OF SALINITY ON SAP IONIC CONCENTRATION

The results for ionic concentrations are based on analyses of sap extracted from fully expanded leaves.

4.5.6.1 SAP OSMOTIC PRESSURE

Salinity significantly increased the sap osmotic pressure of all the leaves (Table 4.8). However, the difference in osmotic pressure between salinity levels was not always significant. Generally, osmotic pressure increased with higher leaf insertions from leaf 4 to leaf 6.

Table 4.8: Effect of different salinity levels on sap osmotic pressure (mmol kg^{-1}) and sap sodium, chloride and potassium concentrations (mol m^{-3}) of various leaf insertions of spring wheat.

Leaf insertions	Salinity levels (mol m^{-3} NaCl)				SEM	HSD
	0	50	100	150		
Sap osmotic pressure						
Leaf 3	494.2	525.4	601.4	648.0	16.6	69.9**
Leaf 4	429.4	500.2	569.8	628.6	15.3	64.2**
Leaf 5	522.0	662.0	709.0	848.0	33.2	139.4**
Leaf 6	631.6	741.6	851.6	862.8	18.7	78.7**
Leaf 7	464.6	613.6	683.0	855.8	11.9	50.1**
Sap sodium						
Leaf 3	2.4	28.2	42.8	59.2	1.8	7.7**
Leaf 4	1.2	26.8	50.0	71.0	2.7	11.5**
Leaf 5	1.2	37.2	61.0	74.0	2.5	10.3**
Leaf 6	2.0	38.6	47.2	75.0	2.9	12.4**
Leaf 7	1.4	27.2	40.2	70.8	2.1	8.6**
Sap chloride						
Leaf 3	74.8	166.6	265.6	282.2	7.2	30.4**
Leaf 4	80.4	199.0	251.4	282.2	6.3	26.5**
Leaf 5	78.2	235.0	376.2	484.0	5.4	22.5**
Leaf 6	28.2	275.6	403.2	426.6	18.2	76.4**
Leaf 7	65.4	249.2	351.0	485.0	8.1	33.8**
Sap potassium						
Leaf 3	244.0	212.0	215.6	210.0	12.2	NS
Leaf 4	194.2	189.8	172.8	163.4	9.5	NS
Leaf 5	219.0	214.8	206.4	205.2	9.2	NS
Leaf 6	259.0	237.0	235.0	227.0	6.5	27.2*
Leaf 7	195.4	205.8	213.0	214.8	5.3	NS

SEM = Standard error of means,
HSD = Honestly significant differences,
NS = Not significant,
*,**= Significant at 5 and 1 percent probability levels respectively.

4.5.6.2 SAP SODIUM AND CHLORIDE CONCENTRATION

Salinity significantly increased sap Na^+ and Cl^- concentrations in all the leaf insertions (Table 4.8). However, the differences in concentration between salinity levels were not always significant. There was no consistent trend of Na^+ and Cl^- concentration with leaf insertion.

4.5.6.3 SAP POTASSIUM CONCENTRATION

In marked contrast to concentrations in the dry matter, sap K^+ concentration was not significantly affected by salinity. K^+ concentration was higher at 0 mol m^{-3} NaCl than at all other salinity levels in leaf insertions 3, 4 and 5 (Table 4.8). Sap K^+ concentration was significantly decreased with salinity in leaf insertion 6, but the difference between salinity levels was not always significant. Sap K^+ concentration was not significantly affected by salinity in leaf insertion 7, whereas in contrast to other leaf insertions, sap K^+ concentration increased with salinity.

4.5.7 EFFECT OF SALINITY ON GAS EXCHANGE

In all the salinity treatments, leaf 5 reached full expansion almost at the same time, Therefore, the gas exchange measurements of this leaf were made on the same day. However, leaf 7 reached full expansion earlier at 100 and 150 mol m^{-3} NaCl than at 0 and 50 mol m^{-3} NaCl. Therefore, the results for leaf 7 consist firstly of those measurements recorded on 20 June 1989 when leaf 7 had

reached full expansion at 100 and 150 mol m^{-3} NaCl. Further measurements were made on leaf 7 on 28 June for all the salinity treatments, when leaf 7 reached full expansion at 0 and 50 mol m^{-3} NaCl. The results for this day are only available for the morning because of low PAR in the afternoon. In the afternoon the plants were sprayed with Mistral fungicide which caused damage to the pants. The experiment was terminated and hence results for 0 and 50 mol m^{-3} NaCl salinity treatments are not available for noon and afternoon. Therefore, the results for leaf 7 when it reached full expansion on 20 June for morning, noon and afternoon at 100 and 150 mol m^{-3} NaCl are not presented due to lack of comparison with those at 0 and 50 mol m^{-3} NaCl.

4.5.7.1 GER OF LEAF 5

The coefficient of variation (Table 4.9) was very high for g_s (76%), Pn (32%) and E (23%) whereas it was low for T_l (6%) and Ci (8%). Analysis of data with Q (light intensity) as covariate was also performed but it did not improve the analysis.

Diurnal variation in T_l followed changes in air temperature (T_a) but it was 3-5°C lower than the latter. T_l was significantly increased by salinity during noon and afternoon whereas the results for the morning were inconsistent.

E showed no obvious diurnal variation but was significantly decreased by salinity during morning and noon

Table 4.9: Effect of different salinity levels on diurnal variation of leaf temperature (T_l in °C), transpiration rate (E in mmol m^{-2} s^{-1}), stomatal conductance (g_s in mol m^{-2} s^{-1}), net photosynthesis (Pn in μmol m^{-2} s^{-1}) and sub-stomatal CO_2 concentration (Ci in μl l^{-1}) of fully expanded leaf 5 of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)				SEM	HSD	CV (%)
	0	50	100	150			
Morning							
Q	63.9	43.6	54.7	59.7	-	-	-
T_a	30.7	31.3	30.9	31.2	-	-	-
T_l	25.3	27.1	25.8	26.9	0.5	2.0*	5.7
E	11.6	9.1	11.1	9.3	0.6	2.4*	17.3
g_s	1.8	0.6	1.1	0.6	0.2	0.9**	63.9
Pn	9.1	6.6	9.5	9.5	0.9	NS	32.6
Ci	292.9	283.2	282.9	269.7	4.1	17.1*	4.6
Noon							
Q	606.3	551.6	545.3	686.6	-	-	-
T_a	30.7	31.3	31.8	32.0	-	-	-
T_l	26.7	28.6	28.5	28.4	0.4	1.5**	3.9
E	11.2	8.0	9.2	10.3	0.6	2.5*	19.7
g_s	1.3	0.5	0.6	0.6	0.1	0.5**	46.6
Pn	7.7	6.9	10.8	11.2	0.9	4.0*	32.9
Ci	293.5	269.3	256.6	259.5	7.0	29.3*	8.2
Afternoon							
Q	491.6	421.2	572.8	509.5	-	-	-
T_a	31.1	31.3	31.6	31.8	-	-	-
T_l	26.7	28.4	28.3	28.1	0.3	1.1**	2.9
E	11.0	8.0	0.1	9.7	0.7	NS	23.1
g_s	1.2	0.4	0.5	0.7	0.2	0.7*	76.3
Pn	8.5	7.7	9.5	10.5	0.6	2.7*	22.3
Ci	291.1	272.0	267.3	265.5	4.1	17.1**	1.5

Q = Light intensity (μmol m^{-2} s^{-1}),
T_a = Air temperature (°C),
- = Data not analysed,
SEM = Standard error of means,
HSD = Honestly significant differences,
CV = Coefficient of variation,
NS = Not significant,
*,**= Significant at 5 and 1 percent probability levels respectively.

but the trends with salinity level were inconsistent. During afternoon E was not significantly affected but was higher at 0 mol m^{-3} NaCl than in all other salinity treatments. E was slightly decreased at 50 and 100 mol m^{-3} NaCl during noon and afternoon but was almost at the same rate at 0 and 150 mol m^{-3} NaCl.

g_s was higher in the morning than in the noon and afternoon. Salinity significantly decreased g_s but the trends with salinity level were inconsistent at all sampling times.

Pn was not significantly affected by salinity during the morning. During noon and afternoon Pn was significantly higher at 150 mol m^{-3} NaCl than at 50 mol m^{-3} NaCl whereas the difference between other treatments was not significant. Pn was lower at 50 mol m^{-3} NaCl than in other treatments at all sampling times. There were no consistent trends of Pn due to diurnal variation.

Ci decreased with increase in salinity and was significantly higher at 0 mol m^{-3} NaCl than at 100 and 150 mol m^{-3} NaCl during noon and afternoon but not for 100 mol m^{-3} NaCl during morning. At 0 mol m^{-3} NaCl Ci was similar at all three sampling times. In the salt stressed treatments Ci was lower at noon than in the morning and afternoon.

4.5.7.2 GER OF LEAF 7

The coefficients of variation were extremely high for E (85%), g_s (120%) and Pn (116%) for leaf 7 (Table 4.10) and higher than those recorded for leaf 5.

Table 4.10: Effect of different salinity levels on diurnal variation of leaf temperature (T_l in °C), transpiration rate (E in mmol m^{-2} s^{-1}), stomatal conductance (g_s in mol m^{-2} s^{-1}), net photosynthesis (Pn in μmol m^{-2} s^{-1}) and sub-stomatal CO_2 concentration (Ci in μl l^{-1}) of fully expanded leaf 7 of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)				SEM	HSD	CV (%)
	0	50	100	150			
Morning							
Q	126.3	132.8	131.5	120.9	-	-	-
T_a	17.4	17.7	18.3	18.1	-	-	-
T_l	15.7	16.0	16.5	17.0	0.6	NS	24.1
E	3.9	3.9	4.3	2.8	0.4	NS	84.9
g_s	0.5	0.5	0.6	0.2	0.1	0.3*	120.4
Pn	4.7	5.4	4.8	4.1	0.8	NS	115.8
Ci	312.4	304.8	307.2	291.1	3.3	13.7**	7.6

Q = Light intensity (μmol m^{-2} s^{-1}),
T_a = Air temperature (°C),
- = Data not analysed,
SEM = Standard error of means,
HSD = Honestly significant differences,
CV = Coefficient of variation,
NS = Not significant,
*,**= Significant at 5 and 1 percent probability levels respectively.

T_l, E and Pn were not significantly affected by salinity. T_l increased with increase in salinity level but results for E and Pn were inconsistent. g_s was significantly lower at 150 mol m^{-3} NaCl than 0, 50 and 100 mol m^{-3} NaCl but the trend for these treatments was inconsistent. Ci was significantly higher at 0 mol m^{-3} NaCl than at 150 mol m^{-3} NaCl but the difference between 0, 50 and 100 mol m^{-3} NaCl was not significant.

4.6 DISCUSSION AND CONCLUSIONS

Salinity had no significant effect on LAS but a decrease in TAS was more prominent with increase in salinity. However, it was observed that salinity slightly enhanced LAS. Therefore, later appearing leaves reached full expansion a few days earlier at high salinity (100 and 150 mol m^{-3} NaCl) than at low salinity (0 and 50 mol m^{-3} NaCl). In this and the previous experiments (Iqbal 1988; Experiment 1) it was observed that although salinity did not significantly affect the number of leaves on the mainstem it decreased the total number of leaves by decreasing the number of tillers per plant. This resulted in lower leaf area with increase in salinity. Leaf area of a plant could be influenced by FLL. In previous experiments in this department (Kemal-ur-Rahim 1988; Experiment 1) it was observed that FLL was mainly influenced by LER. The present study also confirmed that variation in FLL was not a result of variation in LED but of LER. Therefore LER

determined FLL which also determined leaf area and dry weight.

Green area of a plant is a main contributing factor which determines dry matter production. Both green area and dry weight are important before and after anthesis in the wheat plant. Green area of a plant provides machinery for photosynthesis whereas dry weight indicates the status of photoassimilate stored in various parts of a plant (Pheloung and Siddique 1991). In this experiment green area and final length of early appearing leaves was not significantly affected by salinity. However, green area was significantly decreased in all other shoot parts with increase in salinity at both harvests. The reduction in shoot green area at harvest 1 was 42% at 150 mol m^{-3} NaCl. This was mainly due to reduction in green area of leaves (33%) and tillers (48%) (Table 4.11). At this stage the stems were not contributing much towards reduction in shoot green area. At harvest 2 decrease in green area was mainly due to decrease in area of stems and tillers. Reduction in green area due to salinity has also been reported by some other workers (Munns *et al.* 1982; Munns and Termaat 1986; Rawson 1986; Kemal-ur-Rahim 1998; Wyn Jones and Gorham 1989).

In the present study the reduction in shoot dry weight closely followed the trend of green area i.e. dry weight decreased in tillers and roots at harvest 1 and tillers, stem and roots at harvest 2 (Table 4.11). At both harvests total dry weight of all shoot parts except leaves was decreased by salinity. Leaf dry weight was slightly

Table 4.11: Effect of different salinity levels on percent decreases in green leaf area and dry weight per plant of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

	Green leaf area			Dry weight		
	Salinity levels (mol m^{-3} NaCl)			Salinity levels (mol m^{-3} NaCl)		
	50	100	150	50	100	150
Harvest 1						
Leaves	10.6	19.8	32.9	5.9	12.9	15.6
Stem	14.6	16.1	18.3	7.1	8.8	11.2
Tillers	34.2	46.0	48.4	20.4	32.5	38.2
Total shoot	24.7	34.9	41.7	14.2	23.1	27.4
Roots	-	-	-	+0.9	33.8	55.9
Total/plant	-	-	-	10.4	25.8	34.5
Harvest 2						
Leaves	17.1	29.1	37.1	8.9	14.1	17.7
Stem	5.1	45.4	45.3	16.8	46.2	60.9
Tillers	29.9	46.2	50.6	25.5	60.1	53.6
Total Shoot	22.9	39.3	44.8	18.9	46.2	50.1
Roots	-	-	-	33.2	57.3	73.4
Total/plant	-	-	-	22.3	48.8	55.5

- = Not applicable

decreased with increase in salinity (15-17%). Decrease in tiller dry weight was the main factor contributing to decreased dry weight of shoot and of total plant at both harvests. Stem dry weight was decreased by high salinity (150 mol m^{-3} NaCl) more at harvest 2 than at harvest 1. In the present study root dry weight was not significantly affected at 50 mol m^{-3} NaCl at harvest 1 (18 DAS) but was significantly decreased at harvest 2 (23 DAS). At the same salinity level shoot dry weight was decreased at both harvests. These results confirm the findings of earlier workers that at low salinity root growth may not decrease at all while shoot growth declines (e.g. barley, Delane *et al.* 1982), or it may even increase (e.g. bermudagrass, Ackerson and Youngner 1975; sorghum, Weimberg *et al.* 1984). At both harvests root dry weight was decreased more than shoot dry weight. These results suggest that at high salinity it was root growth which was more sensitive to increase in salinity rather than shoot growth (Iqbal 1988; Experiment 1). In contrast, Munns and Termaat (1986) reported that following exposure to salinity for short-term, root growth was less affected than shoot growth. Therefore it is possible that for short-term studies root growth at low salinity may increase but the results of the present study suggested that for long-term salt stress conditions root growth decreases more than shoot growth.

Salinity significantly increased sap osmotic pressure, Na^+ and Cl^- concentration but K^+ concentration was mostly

not significantly affected. Similar results were also observed by other workers (Rashid 1986; Gorham *et al.* 1986a; Kemal-ur-Rahim 1988).

Ion concentrations in dry matter of different plant parts did not always follow the same trends as ion concentrations in sap. This is probably because the samples were harvested on different dates. Salinity significantly increased Na^+ and Cl^- in the dry matter in various plant parts. A gradient of Na^+ and Cl^- concentration was found in different leaves. Higher Na^+ and Cl^- concentrations were found in lower leaves than in expanding leaves. Similar results were also observed by Greenway and Munns (1980), Gorham *et al.* (1986b) and Rashid (1986). Greenway and Munns (1980) suggested that these patterns are probably due to a combination of rapid volume increase in expanding leaves and the prolonged intake of ions by expanded leaves via the transpiration stream. The latter could account for the large increase in ion concentration in older leaves with time. Boursier and Läuchli (1989) found that in the blades of sorghum Cl^- was localised primarily in the epidermal cells whereas Na^+ appears to be mainly in the vacuoles of mesophyll cells (A.D.Tomos, personal communication). Retranslocation or recycling of Cl^- in blade tissue from the xylem to the phloem would move Cl^- back to the sheath (Boursier and Läuchli 1989) and other tissues. This secondary exposure of cells of various shoot tissues to an additional supply of Cl^- moving out of the blade via the phloem may be responsible for the dramatic increase in the

concentration of Cl^-. In the present study a 4-6 fold increase of Cl^- over Na^+ concentration was observed in various parts of the plant. Data for ionic concentration suggested that higher Na^+ and Cl^- concentrations were found in the older leaves. In contrast, calculated Na^+ and Cl^- content (concentration x dry weight) suggested that Na^+ and Cl^- were mainly located in roots, stem and tillers irrespective of salinity level (Tables 4.12 and 4.13). At harvest 1, no consistent trend of Na^+ content in different plant parts was observed. At harvest 2 Na^+ content decreased with leaf position on the mainstem i.e. lower in expanding flag leaf than in lower leaves. In contrast to Cl^- concentration, higher Cl^- content was observed in expanding leaves rather than in dead or expanded leaves. Therefore higher Cl^- content of these leaves was the result of their growth. When percentage of total plant Na^+, Cl^- and K^+ in different plant parts were calculated, the data indicated that Na^+ (Table 4.12), Cl^- (Table 4.13) and K^+ (Table 4.14) were mainly restricted to roots, tillers and stem. Data for harvest 2 suggested that a lower percentage of the total plant Na^+ was found in expanding leaves. A greater percentage was found in dead and older expanded leaves. In contrast to Na^+, a higher percentage of the total plant Cl^- and K^+ was found in expanding leaves. The percentage of the total plant K^+ in the stem increased with salinity but decreased consistently in the roots. This decrease in K^+ percentage may be mostly attributed to

Table 4.12: Effect of different salinity levels on sodium content (Na^+ in μmol) and percent of the total plant sodium in different plant parts of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

	Na^+ content				% of total plant Na^+			
	Salinity levels (mol m^{-3} NaCl)				Salinity levels (mol m^{-3} NaCl)			
	0	50	100	150	0	50	100	150
Harvest 1								
Expanding leaves	3.4	8.8	12.6	17.8	6.7	3.3	4.1	4.9
leaf 5	1.9	10.3	13.3	15.9	3.7	3.8	4.4	4.5
Leaf 4	1.2	9.7	12.4	15.6	2.2	3.6	4.1	4.4
Leaf 3	0.9	12.2	13.5	14.0	1.9	4.5	4.5	3.9
Dead leaves	1.7	15.7	14.3	17.1	3.3	5.8	4.7	4.8
Stem	8.4	25.9	40.0	58.9	16.7	9.5	13.2	16.6
Tiller 1	9.1	52.9	69.8	99.8	18.2	19.5	22.9	28.1
Tiller 2	8.6	36.3	38.5	49.6	17.1	13.4	12.7	13.9
Roots	15.2	99.6	89.3	66.9	30.2	36.6	29.4	18.9
Total	50.3	271.4	303.8	355.7	100	100	100	100
Harvest 2								
Expanding flag leaf	2.8	4.6	5.5	10.7	2.4	1.0	1.4	2.2
Leaf 7	2.9	9.6	10.8	16.8	2.5	2.1	2.8	3.4
Leaf 6	2.4	12.8	13.8	15.5	2.0	2.8	3.6	3.2
Leaf 5	1.8	16.6	18.1	25.7	1.5	3.6	4.7	5.2
Leaf 4	1.2	14.4	20.2	26.9	0.9	3.2	5.3	5.5
Dead leaves	2.3	28.8	26.9	35.6	1.9	6.3	6.9	7.2
Stem	2.5	89.6	85.2	103.1	20.9	19.7	22.1	20.9
Tiller 1	20.3	98.6	74.8	126.6	17.3	21.6	19.4	25.6
Tiller 2	10.1	50.5	34.9	49.9	8.7	11.1	9.1	10.2
Roots	49.5	130.9	95.2	82.6	41.9	28.6	24.7	16.7
Total	117.9	456.3	385.4	493.5	100	100	100	100

% = Percent

Table 4.13: Effect of different salinity levels on chloride content (Cl^- in μmol) and percent of the total plant chloride in different plant parts of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

	Cl^- content				% of total plant Cl^-			
	Salinity levels (mol m^{-3} NaCl)				Salinity levels (mol m^{-3} NaCl)			
	0	50	100	150	0	50	100	150
Harvest 1								
Expanding leaves	55.7	141.8	154.3	184.3	7.7	7.6	8.1	9.4
leaf 5	32.1	82.1	89.4	108.8	4.5	4.4	4.7	5.6
Leaf 4	19.8	64.3	69.6	70.9	2.7	3.5	3.7	3.6
Leaf 3	12.8	49.6	50.9	55.5	1.8	2.7	2.7	2.8
Dead leaves	8.8	54.1	52.1	62.9	1.2	2.9	2.7	3.2
Stem	148.4	375.3	415.7	441.0	20.6	20.1	21.8	22.6
Tiller 1	206.6	544.3	566.1	556.1	28.7	29.2	29.7	28.5
Tiller 2	174.7	300.9	313.8	312.2	24.3	16.2	16.5	15.9
Roots	61.8	250.6	196.3	163.1	8.5	13.4	10.4	8.4
Total	720.6	1862.8	1907.9	1954.8	100	100	100	100
Harvest 2								
Expanding flag leaf	18.4	28.8	54.9	84.3	2.1	1.2	2.6	3.4
Leaf 7	31.1	86.4	98.0	124.1	3.6	3.7	4.7	5.0
Leaf 6	29.2	82.1	98.3	102.7	3.4	3.5	4.7	4.2
Leaf 5	21.8	65.5	83.6	101.9	2.5	2.8	3.9	4.1
Leaf 4	8.6	48.3	89.1	90.2	1.0	2.1	4.3	3.6
Dead leaves	6.1	85.6	98.3	108.4	0.7	3.7	4.7	4.4
Stem	255.1	683.1	569.2	623.8	29.7	29.3	27.2	25.2
Tiller 1	224.1	642.4	493.9	794.4	26.1	27.5	23.6	32.2
Tiller 2	121.0	333.3	235.5	241.8	14.1	14.3	11.5	9.8
Roots	144.5	279.6	270.1	199.6	16.8	11.9	12.9	8.1
Total	859.7	2335.1	2090.9	2471.3	100	100	100	100

% = Percent

Table 4.14: Effect of different salinity levels on potassium content (K^+ in μmol) and percent of the total plant potassium in different plant parts of spring wheat. Harvests 1 and 2 were done when leaves 5 and 7 were fully expanded respectively. (For harvest 1 plants were harvested at 18 days after salt stress. For harvest 2 plants were harvested at 23 days after salt stress at 100 and 150 mol m^{-3} NaCl and 30 days after salt stress at 0 and 50 mol m^{-3} NaCl).

	K^+ content				% of total plant K^+			
	Salinity levels (mol m^{-3} NaCl)				Salinity levels (mol m^{-3} NaCl)			
	0	50	100	150	0	50	100	150
Harvest 1								
Expanding leaves	134.4	119.8	107.8	85.3	10.3	11.3	12.9	12.5
leaf 5	54.2	51.2	36.6	32.6	4.2	4.8	4.4	4.8
Leaf 4	33.9	36.6	27.3	20.7	2.6	3.4	3.3	3.1
Leaf 3	18.0	17.6	14.5	13.4	1.4	1.7	1.7	1.9
Dead leaves	14.3	11.7	6.8	6.5	1.1	1.1	0.8	0.9
Stem	211.8	199.6	184.9	173.4	16.3	18.8	22.3	25.4
Tiller 1	343.5	327.1	254.1	205.3	26.4	30.8	30.6	29.9
Tiller 2	316.3	169.8	148.3	121.3	24.4	15.9	17.8	17.7
Roots	172.9	130.2	51.7	26.3	13.3	12.2	6.3	3.8
Total	1299.2	1063.7	831.9	684.8	100	100	100	100
Harvest 2								
Expanding flag leaf	58.0	49.0	45.3	47.2	3.7	3.9	5.3	6.0
Leaf 7	65.7	59.4	53.8	39.7	4.2	4.9	6.3	5.1
Leaf 6	47.7	49.3	43.9	37.1	3.1	4.1	5.1	4.7
Leaf 5	25.8	34.2	42.2	30.0	1.6	2.8	4.9	3.8
Leaf 4	13.2	13.2	20.7	21.8	0.8	1.1	2.4	2.8
Dead leaves	10.2	12.8	14.9	14.9	0.6	1.0	1.7	1.9
Stem	423.2	406.1	287.6	249.4	26.8	33.1	33.5	31.8
Tiller 1	385.4	323.8	185.4	231.8	24.4	26.4	21.6	29.5
Tiller 2	202.8	138.5	88.9	70.1	12.9	11.3	10.4	8.9
Roots	345.3	139.9	75.8	43.1	21.9	11.4	8.8	5.5
Total	1577.3	1226.3	858.5	785.1	100	100	100	100

% = Percent

decrease in dry weight. Therefore partitioning of these ions is mainly influenced by reduction in growth at high salinity.

Plants could compensate for the large effects of salinity on leaf area by increasing photosynthesis per unit leaf area. Increasing SLW and SF_l could be a useful way of achieving it. SLW was not affected by salinity in leaf 5 but was significantly increased at high salinity in leaf 7. This could be attributed to increase in dry weight of the latter. Kemal-ur-Rahim (1988) suggested that increased SF under saline conditions may help in compensation for decrease in g_s and Pn allowing diffusion of CO_2 into the leaf. Salinity reduced leaf lamina area and leaf lamina dry weight and the effect was greater in leaf 7 than in leaf 5 suggesting a cumulative effect. Salinity significantly increased SF of both leaves but SF_l of leaf 5 was not affected, suggesting that cell differentiation was not affected in the former.

When GER measurements of leaf 5 and leaf 7 were made, Q showed wide variation. For example Q for leaf 5 during noon measurements varied from 221 to 1234 μmol m^{-2} s^{-1} whereas T_l was more constant. Pn should show diurnal variation reflecting these variations in Q and internal leaf water status (which determines g_s). Pn increased in some treatments but the results were inconsistent. Therefore, in the present investigations the long-term changes were masked by wide and short-term fluctuations in Q due to movements of clouds. Variation in Q during measurements and absence of

any equilibration prior to measurements made it difficult to detect whether the effects of salinity on GER varied according to time of day. During the present study Pn, g_s and E varied markedly between plants. Therefore high coefficient of variation (Section 4.5.7.1) made it difficult to detect significant differences between treatments. Data were also analysed with Q as covariate. This assumes a linear relationship between the various parameters and Q but the regression was generally not significant. Therefore, use of Q as covariate did not improve the ANOVA.

Keeping in mind all the problems which occurred during GER measurements due to variation in light intensity and absence of any equilibration, it was decided to use a constant light source in a light chamber for later experiments (Experiment 3, 4 and 5). Also as ion concentrations were higher in older leaves, therefore, needed to study changes in ion concentration and Pn during leaf expansion and senescence.

CHAPTER 5

EXPERIMENT 3 (PART 1)

EQUILIBRATION STUDY TO DETERMINE

PROCEDURE FOR DETERMINING Pn-I RESPONSE CURVES

5.1 INTRODUCTION

Light is a major determinant of the rate of photosynthesis and fluctuates seasonally, daily and hourly during the wheat growing season. In Experiment 2, Pn and other related parameters were determined on plants *in situ* under natural day light conditions when selected leaves were fully expanded. The observed values of Pn had a high coefficient of variation (Section 4.5.7) and at least a part of this variation could be due to variation in light intensity at the time of measurements. Consistent readings of Pn can be obtained *in situ* with little variation of natural light intensity anywhere as long as there is no cloud. Different workers have reported that plants exposed to full sun on cloudless days generally receive light intensity between 1700-2000 μmol m^{-2} s^{-1} PAR (e.g. Sharma and Singh 1989; Ziska *et al.* 1990). However, cloudless conditions can rarely be guaranteed in the United Kingdom. Light conditions below saturation for wheat (c. 1550 μmol m^{-2} s^{-1} PAR) are common in many wheat growing regions (Blum 1990).

When using an IRGA to determine Pn, different workers have used different techniques. One problem is deciding how soon after enclosure Pn measurements should be recorded. Gorham, Wyn Jones and Bristol (1990) argued that measurements in bright light should be taken within 30 second of enclosure to avoid stomatal closure. Austin (1982) took measurements after 20-100 seconds under field conditions. Yeo *et al.* (1985) enclosed rice leaves for 60-90 minutes before

measuring Pn to allow transpiration to stabilise. Conversely, Henson *et al.* (1990) achieved steady states of CO_2 and water vapour exchange within 1 or 2 minutes (min) of enclosure. Blum (1985) reported steady states of CO_2 exchange and transpiration after 5 min but preferred to use the mean of readings taken after 5 and 15 min, although his reasons for doing this are not stated. Other workers have equilibrated leaves for 10 min (Lawlor *et al.* 1989; Blum 1990), 20 min (Rawson *et al.* 1983), 30 min (Robertson and Wainwright 1987; Kemal-ur-Rahim 1988; Blechschmidt-Schneider *et al.* 1989; Sayed *et al.* 1989) and one hour (Plaut *et al.* 1990) to achieve steady states of CO_2 exchange.

In normal air, closure of stomata in response to changing environment is much more rapid than opening (Kuiper and Beirhuizen 1958). Therefore, decreasing photon flux density (I) is the way used by many workers to obtain a Pn-I response curve (Kemal-ur-Rahim 1988; Lawlor *et al.* 1989; Blum 1990; Plaut *et al.* 1990). The time allowed for adjustment of CO_2 exchange rate and other parameters following decreases in light intensity has varied from 5 seconds (Plaut *et al.* 1990) to between 12 and 15 min (Blum 1990). Therefore, as part of the experiments here a preliminary investigation was conducted to determine:-

1) how long it took T_l, Pn, g_s, E, and Ci to reach steady state values after a leaf was placed in the light chamber,

2) and how long it took these variables to respond to subsequent decreases in I.

5.2 MATERIALS AND METHODS

This investigation was conducted on plants growing in Experiment 3. Details of the materials and methods of Experiment 3 are given in section 6.2. Data reported in this chapter were obtained from the third leaf when it became fully expanded (14 DAS) on the main stem of randomly selected plants. Therefore, only the details of materials and methods related to this equilibration study are presented here.

5.2.1 WALK-IN GROWTH ROOMS

The plants in Experiment 3 were grown in two growth rooms which were maintained at 24/16°C day and night temperatures with a photoperiod of 16 hours. Light was provided by a bank of 125W universal warm white fluorescent tubes. There were 24 tubes in each growth room and they provided approximately 200-300 μmol m^{-2} s^{-1} PAR at the leaf surface. The relative humidity in the growth rooms ranged between 40-65 percent.

5.2.2 LIGHT CHAMBER

A light chamber was constructed using aluminium framework blocked in by polystyrene sheets. These were covered in aluminium foil on the inside to maximise light reflection. Access to the chamber was through an open front which was screened by a sheet of heavy gauge black plastic (Figure 5.1) to protect the experimenter. In order to

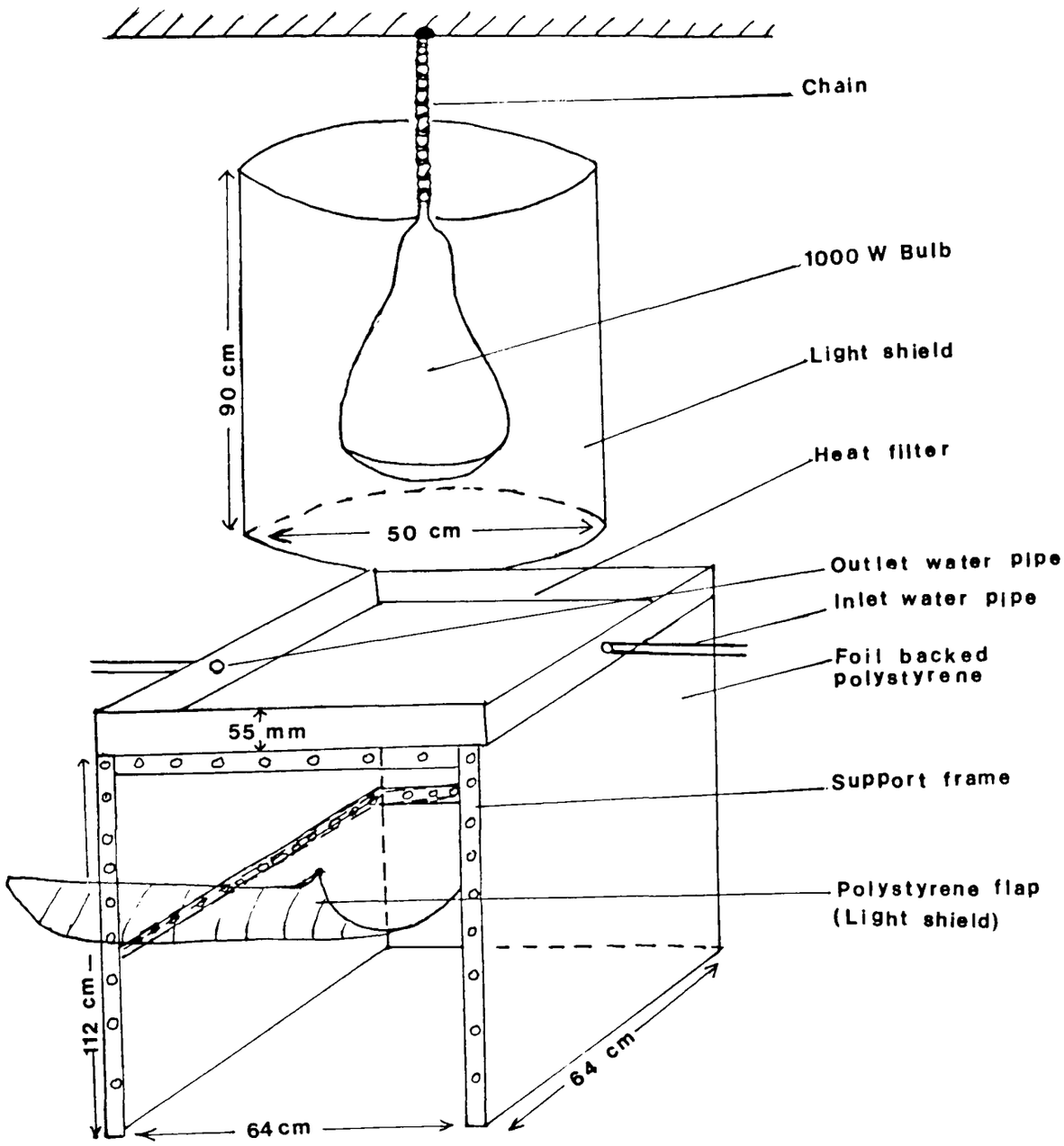


Figure 5.1: Light chamber, showing position of light source, heat filter and support frame

provide a constant high light intensity source (Above 2000 μmol m^{-2} s^{-1} PAR) for GER measurements, a 1000W mercury fluorescent lamp (GEC) was used. This was suspended from the ceiling above the chamber by a chain and shielded in an aluminium cylinder to focus light inside the chamber. The incoming light was passed through a water fed heat filter to prevent excessive increase in temperature in the PLC. The PLC was fixed on a movable retort stand in a horizontal position in the light chamber below the heat filter and at right angle to the light source. The air inlet and outlet tubes ran from the PLC to the IRGA and volumetric air pump which were outside the light chamber. Reference air was drawn down a tube attached at a height of 15 meters to a pole outside the building. This equipment was installed inside a walk-in growth room, separate to the one where the experimental plants were growing.

The growth room lights, air conditioning system and the 1000W lamp were switched on for half an hour before readings commenced to allow the lamp to come-up to its desired PAR level and growth room to its operating temperature (25 ± 2°C). The fan in the PLC and air supply pump were also switched on to purge the humidity from the system.

5.2.3 COLLECTION OF GER DATA FOLLOWING INITIAL ENCLOSURE AND SUBSEQUENT DECREASES IN PHOTON FLUX DENSITY

The plants plus polystyrene lid were lifted off their original containers in the walk-in growth rooms and placed

on a similar empty container for transfer to the growth room containing the IRGA. Plants, supported by their polystyrene lid, were transferred to a container which was filled with a similar concentration of nutrient solution and appropriate salinity level in the light chamber. Each time a new tray was placed inside the light chamber and leaf 3 on the mainstem of a randomly selected plant was immediately placed inside the PLC. GER data were recorded using the datalogger of the IRGA as in section 4.2.9 at 30 seconds and then at 1, 2, 3, 4, 5, 7, 10, 15, 20, 25 and 30 min after enclosure. A neutral light filter was then placed on the PLC window and further readings were taken at the above time intervals. This process was repeated so that I was decreased in a step-wise manner from 1750, 1300, 1050, 600 and 275 to 0 μmol m^{-2} s^{-1} PAR. The plants were then removed from the light chamber and returned to their original growth room position. A new set of plants were then transferred. Eighteen readings, 6 of each of 3 salinity levels (0, 100 and 200 mol m^{-3} NaCl) were recorded in this way. To determine the effects of salinity and time data were analysed using ANOVA.

5.3 RESULTS

The data for the salt stressed treatments showed similar trends over time as data for the control and are therefore not presented. By running the pump with the PLC closed and measuring RH it was found that it took 10-30 seconds for the pump to purge the chamber and tubing. Therefore, no accurate readings could be obtained until at

least 30 seconds had elapsed. This problem did not reoccur when Pn was measured following subsequent decreases in light intensity as the leaf was left undisturbed in the PLC.

Changes in T_l, Pn, g_s, E, and Ci at 0 mol m^{-3} NaCl following initial enclosure and subsequent changes in light intensity are presented in Figures 5.2 to 5.5. In these Figures each data point is the mean of six leaves. T_l progressively increased over time after initial enclosure at 1750 μmol m^{-2} s^{-1} PAR, however, the differences in T_l between times were not significant (Figure 5.2a). Pn (Figure 5.2b), g_s (Figure 5.2c) and E (Figure 5.3a) increased during the first 15-20 min after enclosure and then remained constant. Conversely Ci decreased during the first 15-20 min and thereafter remained constant (Figure 5.3b). Differences between times were significant for all the parameters.

When I was stepped down (for example from 1050 to 600 μmol m^{-2} s^{-1} PAR), T_l decreased rapidly and thereafter it increased and reached a new constant value within 15-20 min (Figure 5.4a). Pn adjusted rapidly within 1-2 min (Figure 5.4b). For g_s and E an initial rapid decrease was followed by a slower increase (Figures 5.4c and 5.5a, respectively), suggesting that 30 min was not long enough for these parameters to reach new constant values. Pn and Ci followed a similar pattern as at highest light intensity (Figures 5.4b and 5.5b, respectively). Similar trends were noted when I was stepped down from 275 to 0 μmol m^{-2} s^{-1} PAR.

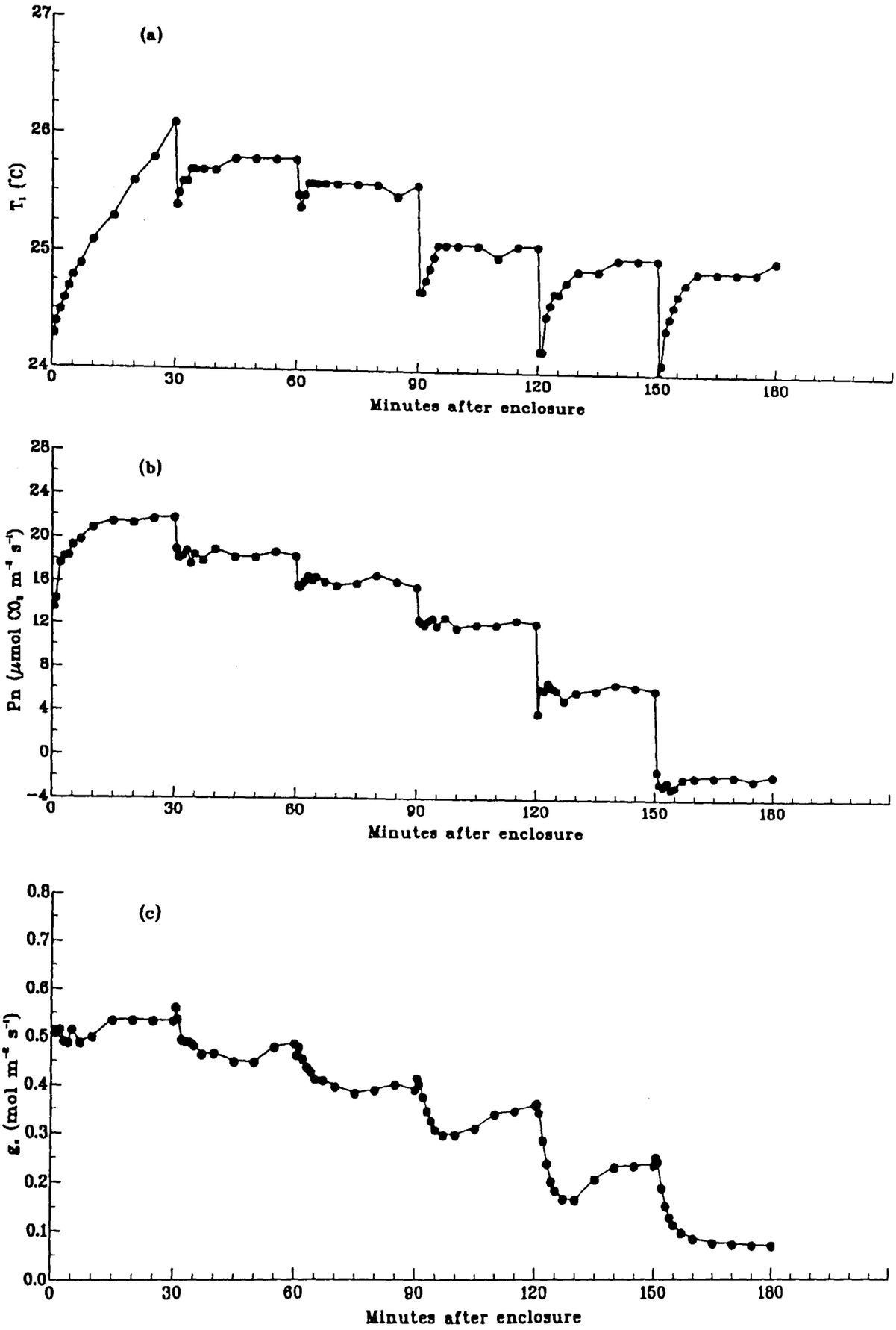


Figure 5.2: Changes in (a) leaf temperature (T_l), (b) net photosynthesis (Pn), (c) stomatal conductance (g_s) following enclosure in the leaf chamber and step-wise decreases in light intensity from 1750, 1300, 600 and 275 to 0 μmol m^{-2} s^{-1} photosynthetically active radiation at 30 minutes intervals.

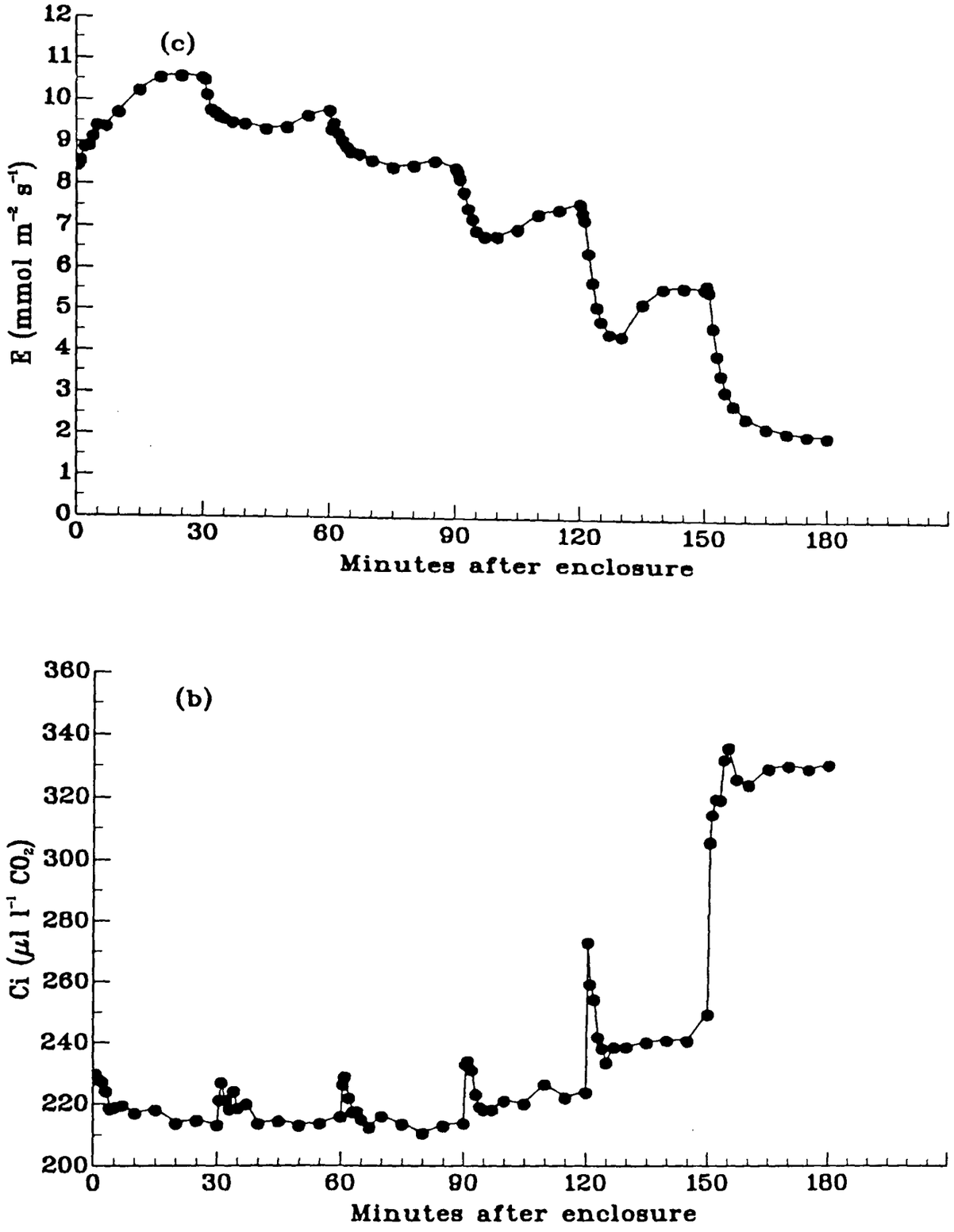


Figure 5.3: Changes in (a) transpiration rate (E), (b) sub-stomatal CO_2 concentration (Ci) following enclosure in the leaf chamber and step-wise decreases in light intensity from 1750, 1300, 600 and 275 to 0 μmol m^{-2} s^{-1} photosynthetically active radiation at 30 minutes intervals.

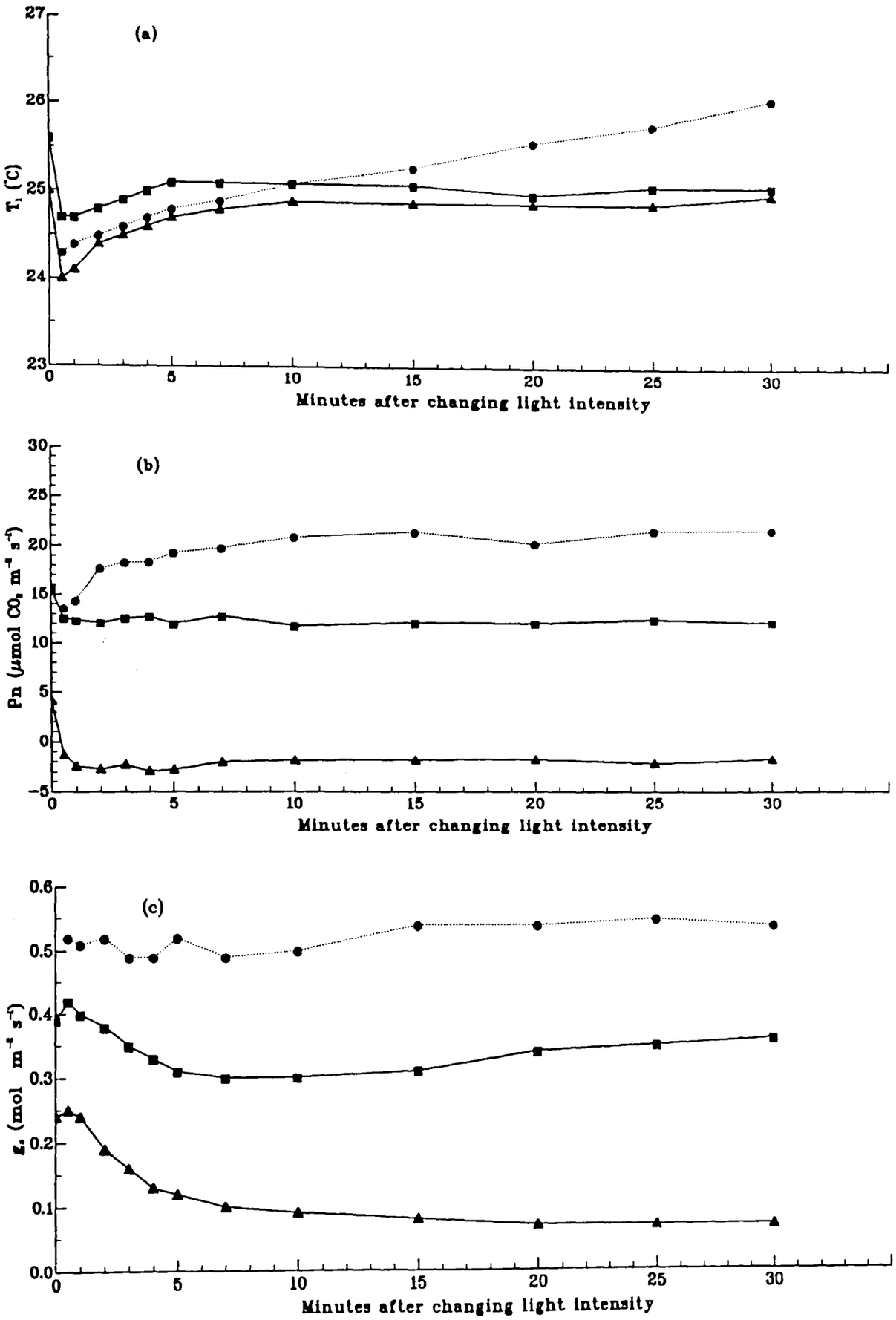


Figure 5.4: Values of (a) leaf temperature(T_l), (b) net photosynthesis (Pn) and (c) stomatal conductance (g_s) at 1750 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) following placement in the light chamber (●), and subsequent decreases in light intensity from 1050 to 600 (■) and 275 to 0 (▲) μmol m^{-2} s^{-1} PAR.

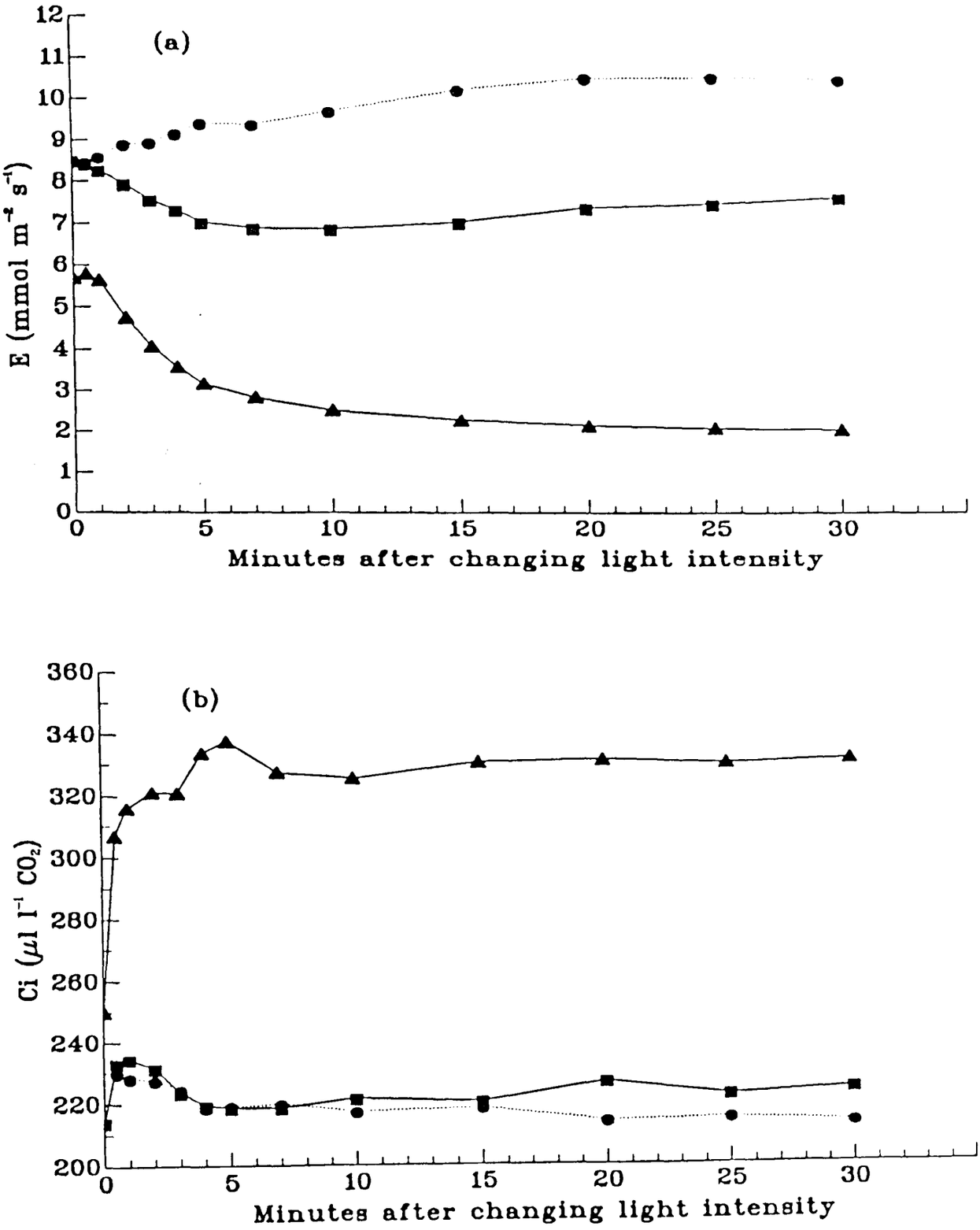


Figure 5.5: Values of (a) transpiration rate (E), (b) sub-stomatal CO_2 concentration (Ci) at 1750 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) following placement in the light chamber (●), and subsequent decreases in light intensity from 1050 to 600 (■) and 275 to 0 (▲) μmol m^{-2} s^{-1} PAR.

5.4 DISCUSSION

This study showed that T_l increased progressively over time after enclosure. The increase in T_l was mainly due to high I and consequently higher temperature in the light chamber than in the growth room where the plants were growing. The high power lamp had a heating effect despite the presence of the heat shield running cold water in between the light and the PLC. However, the increase in T_l over time was not significant. Following enclosure Pn and Ci showed an initial rapid response and then a more gradual response but attained new constant values within 3-5 min of enclosure. Similar results were reported by Kemal-ur-Rahim (1988). g_s and E took longer than 15 min to reach new constant values after enclosure suggesting that the stomata took time to adjust from low I in the growth room where plants were growing to very high I in the light chamber. These results indicated that Pn, g_s, E, and Ci need at least 15-20 min "equilibration time" (i.e. the time taken to reach constant values after initial placement in the light chamber). It was therefore, decided that the most appropriate method of equilibration to use subsequent experiments would be to leave plants in the light chamber for approximately 20 min before commencing measurements. This procedure is similar to that used by other workers (Rawson *et al.*(1983); Robertson and Wainwright 1987; Kemal-ur-Rahim 1988; Blechschmidt-Schneider *et al.* 1989; Sayed *et al.* 1989) who also used 20-30 min equilibration

time.

After decreasing I, Pn in the test leaf adjusted within 1-2 min. Similar results were also reported by Kemal-ur-Rahim (1988). Plaut *et al.* (1990) used a 'response time' (i.e. the time taken to reach new constant values following a decrease in I) of 5 seconds which on the basis of the results obtained here is not long enough for Pn to adjust to the new I. Blum (1990) used a response time of 15 minutes. Use of such a long response time would limit the number of leaves that could be measured in a day, and on the basis of the results obtained here, would also not be long enough for g_s and E to reach new constant values.

The slower response of g_s, E and Ci after changing I suggests that subsequent measurements at lower light intensities would require at least 30 min response time in order to be able to develop g_s-I, E-I and Ci-I response curves. However, in Experiments 3 and 4 a response time of 3 min was used. This time period was considered to be long enough for Pn to adjust to the new light intensity, but not for g_s, E and Ci. During this study no difference in equilibration and response times between treatments was observed (data not presented). Therefore the same procedure for all treatments was adapted for Pn-I response curves. Section 6.4.1 discusses changes in Pn and g_s which occurred during the time a leaf was in the PLC for the determination of light response curves, following the chosen procedure.

CHAPTER 6

EXPERIMENT 3 (PART 2)

EFFECT OF SALINITY ON CO_2 EXCHANGE AND RELATED PARAMETERS, LEAF IONIC CONCENTRATION, SAP OSMOTIC PRESSURE AND AREA OF LEAF 6 OF SPRING WHEAT DURING EXPANSION AND SENESCENCE

6.1 INTRODUCTION

As discussed in section 2.5.1, GER per unit area for leaves reaches a maximum soon after they have attained their maximum area (Austin *et al.* 1982; Kemal-ur-Rahim 1988; Rawson *et al.* 1988a). GER remains at this maximum for some period of time and then declines as the leaf senesces (Dantuma 1973). Environmental conditions such as high temperature, low moisture, low nitrogen supply or salinity hasten senescence and reduce leaf area duration (Simmons 1987). Wheat plants subjected to high salinity senesce earlier than unstressed plants (Iqbal 1988). What is not known is how salinity affects GER in leaves of wheat at different stages of leaf growth, although some information is available for barley (Rawson *et al.* 1988a). This study was, therefore, conducted to determine the effect of salinity on GER during leaf expansion and senescence. Leaf sap Na^+, K^+, Cl^- and osmotic pressure (OP) and green lamina area (GLA) were determined simultaneously so as to be able to relate changes in GER to changes in these parameters.

Pn-I data have been used to construct light response curves by many workers (for example, Acock *et al.* 1976; Thornley 1976; Goudriaan 1977; Constable and Rawson 1980; Ceulemans *et al.* 1980; Marshall and Biscoe 1980a,b; Kemal-ur-Rahim 1988; Lawlor *et al.* 1989; Myers *et al.* 1990). As discussed earlier (Section 5.4) when I is decreased g_s also decreases. This can affect Pn and other related parameters. This decrease in g_s may be a direct response to changing I or an indirect response as a result of enclosing

the leaf in the PLC. In Experiment 2 GER was recorded as soon as possible after enclosure and before the stomata had time to adjust to the new micro-environment in the leaf chamber. In Experiments 3 and 4 each leaf would have to remain in the PLC for a longer period of time so that GER at varying I could be determined. It was therefore, also necessary to study changes in g_s and Pn during the period of determining a light response curve.

6.2 MATERIALS AND METHODS

The plants were grown in two separate walk-in growth rooms (Section 5.2.1). GER was determined in a separate growth room using a powerful light source which provided light intensities similar to natural day light conditions (Section 5.2.2).

6.2.1 EXPERIMENTAL DESIGN AND TREATMENTS

A completely randomised design was used. There were 3 salinity treatments and 8 replicates. Due to limitation of space, the replicates had to be divided evenly between two growth rooms. The salinity levels tested were 0, 100 and 200 mol m^{-3} NaCl. $CaCl_2$ was also applied to the salinity treatments (100 and 200 mol m^{-3} NaCl) in the ratio of 20:1 (moles Na: moles Ca) as in Experiment 2 (Section 4.2.1).

6.2.2 RAISING OF SEEDLINGS AND TRANSPLANTING

The experiment was initiated in the first week of January 1990 and terminated during the second week of March. Seeds were pre-germinated for 13 days and transplanted at 0+2 (two emerging leaves) stage into polystyrene lids of plastic containers following the procedures described in Experiment 1 (Section 3.2.4). The planting density was 16 plants (4 x 4) per container spaced 8 cm x 5 cm apart. Two days after transplanting (15 days after seeding), salinity was introduced gradually in daily increments of 25 mol m^{-3}. Micronutrients were supplied as in Experiment 2 (Section 4.2.5). Salt (NaCl + $CaCl_2$) and the nutrient solutions were changed after every two weeks.

In Experiment 1 aeration was supplied to the plants through air stones with control regulators which occasionally blocked and provided uneven supply of air. Aeration was improved in this experiment using the same system as used in Experiment 2. Three air supply needles were fitted to each pot to ensure even distribution of air to plant roots. The plants were grown in two growth rooms which were maintained at 24/16°C day and night temperatures with a photoperiod of 16 hours. Light intensity in the growth rooms varied between 200-300 μmol m^{-2} s^{-1} PAR at the leaf surface. The relative humidity in the growth rooms ranged between 40-65 percent.

6.2.3 MAINTENANCE OF THE EXPERIMENT

No pest and disease attack was observed during the course of study. To minimise any potential effect of variation in temperatures and light intensity inside the growth rooms, the pots were re-randomised every 3-4 days.

6.2.4 COLLECTION OF GER DATA

Leaf 6 on the mainstem was selected for GER measurements. Previous experiments (Iqbal 1988, Experiments 1 and 2) had shown that salinity has relatively small effects on the first three leaves and other workers (Rashid 1986; Munns and Termaat 1986; Rawson *et al.* 1988a,b) have shown that the effects of salinity are greater on later emerging leaves. Measurements of leaf 6 commenced on 10 February 1990, as soon as it was long enough to fit in the PLC. However, as plants were growing in growth rooms, restrictions of space prevented growing plants to maturity. Therefore, it was not possible to take measurements on the flag leaf. For measurements of GER, plants were transferred to the walk-in growth room where the IRGA equipment was installed. The room lights, 1000W mercury lamp, air conditioning system and IRGA inside the growth room were switched on half an hour before measurements commenced (Section 5.2.2) and plants left to equilibrate for 20 minutes. Leaf width of the central portion of the leaf to be measured was then recorded. It was then enclosed in the PLC with adaxial surface uppermost. During measurements

leaf 6 was kept horizontal in the light chamber and at right angle to the light source. In order to obtain the Pn-I response curves, readings of T_l, Pn, g_s, E and Ci on the datalogger of IRGA were recorded 3 minutes after enclosure. I was then decreased by using neutral filters and subsequent readings were also recorded after 3 minutes. This procedure was repeated and measurements were taken at approximately 1750, 1300, 1050, 600, 250 and 0 μmol m^{-2} s^{-1} PAR. It took nearly 40 minutes to complete a set of measurements of a single leaf. Readings were taken on 4 randomly selected plants from each of two replicate containers of each treatment on each occasion.

Immediately after GER readings had been taken, the leaf lamina was detached to determine leaf area and for chemical analyses (Section 6.2.6). The remainder of the plant was left *in situ* so as to have minimum effect on the remaining plants in each container. At the base of the plant, the polystyrene lid was marked with a paint pen to avoid resampling the same plant again. This procedure was repeated after every 3-4 days starting when the first measurements were taken to full senescence of leaf 6 on 8 March 1990.

6.2.5 CURVE FITTING PROCEDURE AND DERIVATION OF THE VALUES OF Pnmax, Rd_c, α AND Ic

Several models have been fitted to Pn-I data (James and Bliss 1966; Acock *et al.* 1976; Thornley 1976; Ceulemans *et al.* 1980; Hesketh and Jones 1980). The two models which

have been most used are the rectangular hyperbola (Monteith 1965; Thornley 1976; Austin 1982; France and Thornley 1984; Kemal-ur-Rahim 1988) and the exponential (Thornley 1976; Constable and Rawson 1980; Rawson 1986; Kemal-ur-Rahim 1988). Ceulemans *et al.* (1980) fitted both models to data obtained from *Populus* clones and concluded that neither gave a superior fit. Littleton (1971) found that the rectangular hyperbola model tended to over-estimate Pnmax in barley leaves under bright light. Previous studies in this department (Kemal-ur-Rahim 1988) and those of Constable and Rawson (1980) and Rawson (1986) have found that the exponential model gives a good fit to Pn-I data. Thornley (1976) used an exponential model of the form:

$$P = Pmax\ [1-e^{-(\alpha I/Pmax)}]$$

where P is gross photosynthesis, Pmax is maximum photosynthesis, I is photon flux density (equivalent to I used earlier), and α is the initial slope of the Pn-I curve; also known as photosynthetic efficiency. This model passes through the origin, hence P = Pg (gross photosynthesis) and Pmax = Pgmax (maximum gross photosynthesis). This model has been refined by Gourdriaan (1977) (cited by Ceulemans *et al.* 1980) to include a term for dark respiration (Rd), hence

$$Pn = Pmax\ [1-e^{-(\alpha I/Pmax)}] + Rd$$

Where Rd is negative. As the Rd component is not incorporated until later, the term Pnmax used in the text will refer to maximum net photosynthesis. The general form of the exponential equation fitted to the data in this and

subsequent experiments was:

$$Y = a + be^{-cx}$$

where Y is observed Pn, X is photon flux density (I), and a, b, c are constants. The values of a, b and c were obtained by using the 'Fitcurve' command of the GENSTAT 5 programme language (Payne *et al.* 1987). Having derived the values of a, b, and c, then the values of Pnmax, α, Ic (photon flux compensation point) and Rd_c (calculated dark respiration) were calculated as below, following the procedures of Kemal-ur-Rahim (1988).

The derived Pn-I equation fitted to the data was:

$$Pn = Pgmax\ [1-e^{-(\alpha I/Pgmax)} + Rd]$$

$$Pn = Pgmax - Pgmax\ .\ e^{-(\alpha I/Pgmax)} + Rd$$

To determine Pnmax

As $I \rightarrow \infty$, $e^{-(\alpha I/Pgmax)} \rightarrow 0$

Therefore Pnmax = Pgmax + Rd

from the general form of the equation

$Y = a + bc^{-cx}$

Pnmax = a

To determine α

$$\alpha = dPn/dI = \frac{-\alpha}{Pgmax}\ Pgmax\ e^{-(\alpha I/Pgmax)}$$

As α is calculated from the initial slope of the response curve, I is very small $\rightarrow$ 0

$$\text{therefore} = \frac{-\alpha}{\text{Pgmax}} \text{ Pgmax . 1}$$

from the general form of the equation

$Y = a + be^{-cx}$

$dy/dx = -cbe^{-cx}$

$\alpha = -c \times b$

To determine Rd_c

When $I = 0$, $e^{-(\alpha I/Pgmax)} \rightarrow 1$

$Pn = Pgmax - Pgmax + Rd$

therefore $Pn = Rd$

from the general form of the equation

$Y = a + be^{-cx}$

as $a = Pgmax + Rd$ and $b = -Pgmax$

$Rd = a + b$

To determine Ic

When $Pn = 0$, $I = Ic$

therefore $0 = Pgmax - Pgmax\ e^{-(\alpha I/Pgmax)} + Rd$

$- Pgmax - Rd = - Pgmax\ e^{-(\alpha I/Pgmax)}$ (dividing by Pgmax)

$(Pgmax + Rd)/Pgmax = e^{-(\alpha I/Pgmax)}$

$\log_e [(Pgmax + Rd)/Pgmax] = - \alpha I/Pgmax$

$Ic = \log_e [(Pgmax + Rd)/Pgmax] (Pgmax/-\alpha)$

from the general form of the equation

$Y = a + be^{-cx}$

as $Pgmax + Rd = a$, $-Pgmax = b$ and $-\alpha/Pgmax = c$

$Ic = \log_e (-a/b)/-c$

The model was fitted to the data collected for each individual leaf (From the 2 replicate containers of each salinity level and 4 leaves per container) using the non-linear least squares fitting technique. Pn was measured at 6 photon flux densities as described earlier (Section 6.2.4). The values of Pnmax, Rd_c, α and Ic were then calculated as described and analysed using analysis of variance.

6.2.6 DETERMINATION OF LEAF AREA AND PREPARATION OF SAMPLES FOR CHEMICAL ANALYSES

Green lamina area (GLA) was recorded using an automatic area meter (Section 3.2.6.7). The individual leaves were then rinsed in distilled water, blotted dry and put into 1.5 cm^3 Eppendorf tubes and stored in a refrigerator at 0°C. The sap of these frozen leaves was extracted after thawing and analysed for Na^+, Cl^-, K^+ and OP in the same way as described in section 3.2.7.1.

6.2.7 DETERMINATION OF TRANSPIRATION EFFICIENCY AND LEAF PRODUCTIVITY

After measuring T_l, Pn, g_s, E and Ci at 1750 μmol m^{-2} s^{-1} PAR, transpiration efficiency (TE in μmol CO_2 per mol H_2O as Pn/E, i.e. the ratio of Pn to E per unit leaf area) was calculated following the method described by Acevedo and Ceccarelli (1989). The term transpiration efficiency used in this and later chapters is equivalent to water-use

efficiency (Pn/E) as described by other workers (Constable and Rawson 1980; Yeo *et al.* 1985; Flowers *et al.* 1988; Hamid *et al.* 1990). Leaf productivity (LP in μmol CO_2 s^{-1}) was calculated as:

LP = Green lamina area x net photosynthesis

6.2.8 FRESH AND DRY WEIGHT, LAMINA AREA, SPECIFIC LEAF WEIGHT AND STOMATAL FREQUENCY OF FULLY EXPANDED LEAF 6

When leaf 6 was fully expanded (ligule emerged), the fresh weight of 8 leaves in each salinity treatment was recorded. Stomatal frequency was determined following the procedures described in section 4.2.7. The leaf was then oven dried at 80°C, dry weight was recorded and then specific leaf weight was calculated.

6.3 STATISTICAL ANALYSES

All data were analysed using ANOVA. In each case the values were checked for normality by inspection of histograms of residuals and by plotting the residuals against the fitted values. Where significantly different, treatment means were compared by calculating a least significant difference (LSD) as follows:

$LSD_{(P=0.05)}$ =Standard error of difference between means x T

Where T is value from the statistical table.

This test was used because as there were few treatments only a limited number of comparisons could be made and therefore little risk of Type 1 error (Gomez and Gomez 1984).

6.4 RESULTS

Previous study (Chapter 5) on leaf 3 showed that whereas Pn adjusted rapidly following a decrease in I, g_s and E adjusted more slowly. For each Pn-I response curve it took approximately 40 minutes to complete a set of measurements on a leaf. During this time stomatal closure could occur. The first step in the analysis of the GER data from this experiment was therefore to examine changes in Pn and g_s during the course of determining Pn-I response curves. This was done by analysing the effect of light intensity and salinity on Pn and g_s at each sampling date.

6.4.1 TRENDS IN g_s AND Pn DURING ENCLOSURE FOR LIGHT RESPONSE CURVES

The trends in g_s and Pn as I was decreased for the different salinity treatments were similar to those of the control (0 mol m^{-3} NaCl) and are therefore not presented.

The effect of I on Pn and g_s at 0 mol m^{-3} NaCl are presented in Table 6.1. There were significant effects of light intensity on g_s at the first 3 sampling dates. g_s generally decreased with decrease in light intensity, particularly as it approached 0 μmol m^{-2} s^{-1} PAR. Thereafter g_s was not significantly affected. This suggests that the stomata closed in response to decreasing light at 19-27 DAS. Pn was significantly decreased with decrease in I on all sampling dates. Once maximum leaf lamina size was attained (34 DAS), the decrease in GER with decrease in

Table 6.1: Changes in stomatal conductance (g_s in mol m^{-2} s^{-1}) and net photosynthesis (Pn in μmol m^{-2} s^{-1}) during the time period of determining a light response curve. Data for leaf 6 at 0 mol m^{-3} NaCl (Experiment 3).

DAS	g_s/Pn	Photon flux densities (μmol m^{-2} s^{-1} PAR)						SEM	HSD
		1750	1300	1050	600	275	0		
19	g_s	0.36	0.35	0.33	0.32	0.24	0.18	0.05	0.15**
	Pn	17.35	15.65	14.77	11.66	5.72	-1.86	0.52	2.18**
23	g_s	0.44	0.39	0.36	0.32	0.26	0.18	0.02	0.10**
	Pn	18.24	16.80	15.42	12.74	6.66	-1.57	0.39	1.63**
27	g_s	0.36	0.35	0.34	0.34	0.30	0.22	0.01	0.06**
	Pn	17.49	16.56	15.76	13.53	7.07	-1.90	0.32	1.37**
31	g_s	0.47	0.44	0.44	0.46	0.47	0.44	0.03	NS
	Pn	16.15	15.51	14.35	13.24	7.25	-1.70	0.58	2.44**
34	g_s	0.41	0.37	0.37	0.39	0.40	0.39	0.05	NS
	Pn	11.12	10.59	10.40	9.41	5.99	-1.56	0.33	1.38**
37	g_s	0.37	0.36	0.38	0.40	0.40	0.40	0.02	NS
	Pn	10.50	10.09	10.20	9.46	6.29	-1.26	0.28	1.19**
41	g_s	0.23	0.24	0.24	0.26	0.26	0.27	0.02	NS
	Pn	9.85	9.28	8.95	8.65	4.77	-1.31	0.32	1.34**
45	g_s	0.25	0.23	0.23	0.23	0.23	0.22	0.02	NS
	Pn	7.82	8.00	8.02	7.33	4.53	-1.14	0.33	1.38**
48	g_s	0.18	0.18	0.18	0.20	0.20	0.21	0.02	NS
	Pn	5.07	5.18	5.24	5.03	3.91	-0.96	0.23	0.98**

PAR= Photosynthetically active radiation,
DAS= Days after salt stress,
SEM= Standard error of means,
HSD= Honestly significant differences,
NS = Not significant,
** = Significant at 1 percent probability level.

light intensity was therefore mainly due to direct effects of I on Pn. Hence in Experiments 3 and 4 Pn-I response curves were used to study the effects of salinity on Pnmax, Rd_c, α, and Ic (Section 6.4.6). As there is some evidence of stomatal closure in response to decreasing light particularly at early sampling dates, only the initial values of Pn, g_s, E and Ci at the highest light intensity (1750 μmol m^{-2} s^{-1} PAR i.e. those obtained following 20 minutes equilibration time and 3 minutes after enclosure) are presented and discussed.

6.4.2 COEFFICIENT OF VARIATION OF DATA

Generally values of the coefficient of variation (CV) remained below 16% for GLA, LP, Pn, Ci, E, TE, Pnmax and sap K^+ concentration (Table 6.2). The CV of Rd_o and Rd_c ranged from 18-25%, for Cl^- concentration 4-33% and for Ic and α from 21-39% respectively. The CV for g_s and sap Na^+ concentration were very high, between 22-59% and 17-39% respectively. Higher CV's for these parameters made it more difficult to detect significant differences between treatments.

6.4.3 EFFECT OF SALINITY ON GLA

Although visual observations showed that the leaf 6 was turning yellow after expansion, changes in colour were difficult to measure using the automatic area meter. Only the dried tip portion of the lamina could be removed when determining GLA. Therefore, the results also include that

Table 6.2: Coefficient of variation (%) for green lamina area (GLA), net photosynthesis (Pn), observed dark respiration (Rd_o), stomatal conductance (g_s), Sub-stomatal CO_2 concentration (Ci), transpiration rate (E), transpiration efficiency (TE), leaf productivity (LP), maximum net photosynthesis (Pnmax), calculated dark respiration (Rd_c), photon flux compensation point (Ic), photosynthetic efficiency (α), sap sodium (Na^+), sap chloride (Cl^-) and sap potassium (K^+) concentrations of leaf 6 of spring wheat on selected sampling dates. Data for Experiment 3.

Parameters	Days after salt stress (days after leaf 6 emergence)				
	19(6)	23(10)	27(14)	31(18)	34(21)
GLA	16.36	11.05	12.84	14.62	10.90
Pn	13.41	11.33	10.09	8.17	14.51
Rd_o	23.32	20.90	18.97	22.28	22.24
g_s	33.66	23.55	17.48	21.17	39.03
Ci	8.82	7.51	6.31	6.56	8.76
E	16.56	14.52	13.20	11.16	24.99
TE	8.67	13.96	13.29	10.44	15.67
LP	25.21	13.01	18.79	16.13	17.93
Pnmax	15.01	22.05	11.78	10.70	15.19
Rd_c	24.96	21.28	18.47	24.02	21.80
Ic	25.89	39.02	23.93	21.67	27.48
α	16.73	24.37	21.09	28.89	27.71
Sap Na^+	40.53	27.37	22.42	46.88	58.91
Sap Cl^-	26.58	27.96	18.64	4.96	33.61
Sap K^+	6.17	8.40	8.27	12.85	14.93

% = Percent

part of the area of leaf 6 which was actively turning yellow over time. GLA was significantly lower at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl at all sampling dates (Figure 6.1a). At 0 mol m^{-3} NaCl, GLA increased between 19 and 27 DAS as the leaf expanded. Thereafter it remained constant at approximately 28-29 cm^2 until 51 DAS when the leaf was completely yellow. At 100 and 200 mol m^{-3} NaCl, leaf 6 was completely yellow at 37 and 31 DAS respectively.

6.4.4 EFFECT OF SALINITY ON Pn, g_s, Ci, E, T_l AND TE AT 1750 AND Rd_o AT 0 μmol m^{-2} s^{-1}

In leaf 6 Pn was significantly higher at 0 than at 100 and 200 mol m^{-3} NaCl on all sampling dates (Figure 6.1b). At 0 mol m^{-3} NaCl Pn remained at approximately 16-18 μmol m^{-2} s^{-1} from 19 until 27 DAS after which it decreased and the leaf was completely yellow at 51 DAS. At 100 mol m^{-3} NaCl Pn decreased slowly up to 31 DAS. Thereafter, Pn decreased rapidly and the leaf was completely yellow at 37 DAS. At 200 mol m^{-3} NaCl Pn decreased rapidly. It was significantly lower at 0 and 100 mol m^{-3} NaCl than at 200 mol m^{-3} NaCl on all the sampling dates and the leaf was completely yellow at 31 DAS.

Rd_o increased with increase in salinity on all sampling dates (Figure 6.1b). Differences between treatments were not significant at 19 DAS when the leaf was still expanding. However, Rd_o was significantly higher at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl from 27 DAS onwards. At 0 mol

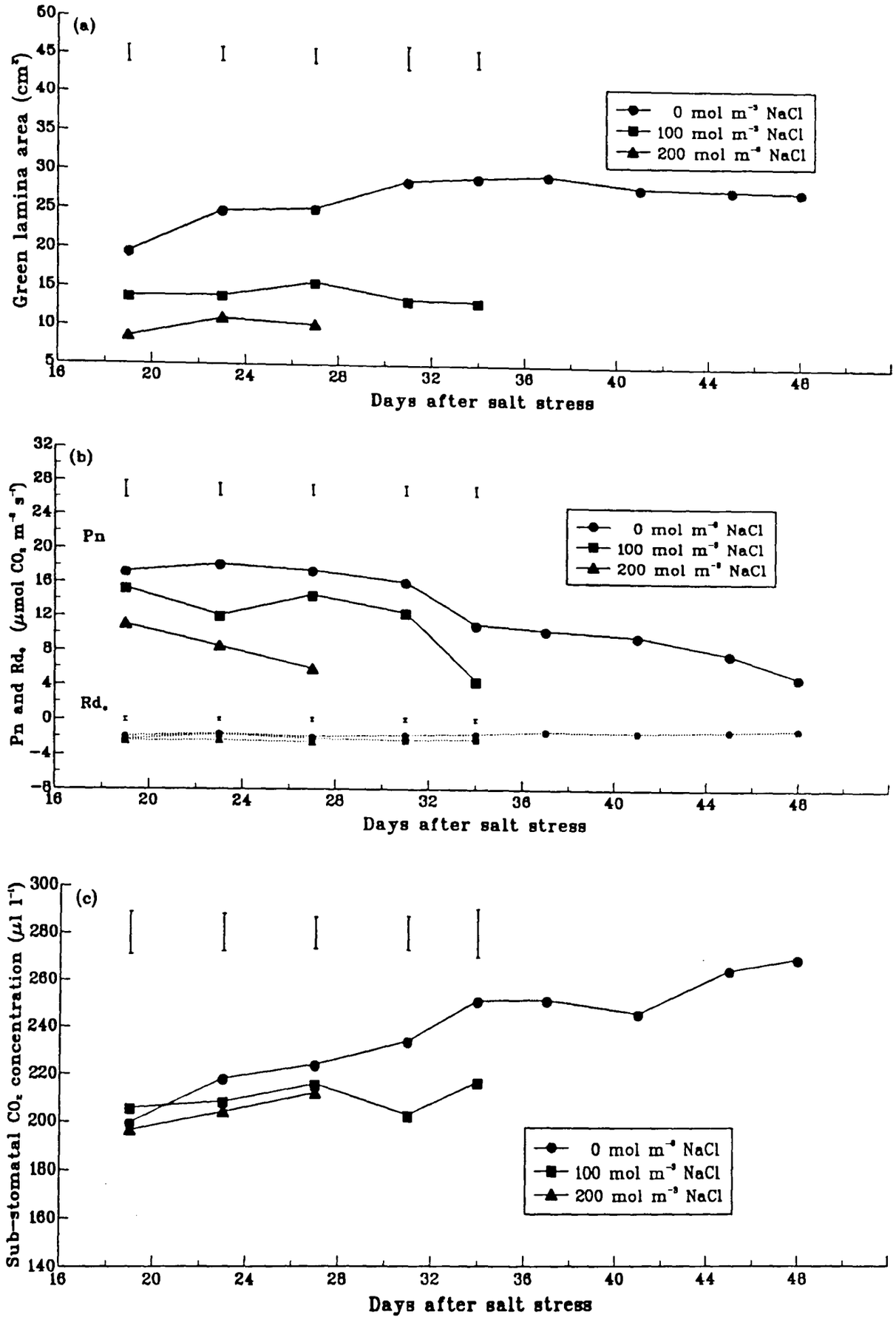


Figure 6.1: Effect of salinity on (a) green lamina area, (b) net photosynthesis (Pn) and observed dark respiration (Rd_o) and (c) sub-stomatal CO_2 concentration of leaf 6 of spring wheat. I= standard error of difference between means.

m^{-3} NaCl Rd_o progressively decreased after 27 DAS. At 100 and 200 mol m^{-3} NaCl Rd_o remained approximately constant although these leaves senesced much earlier.

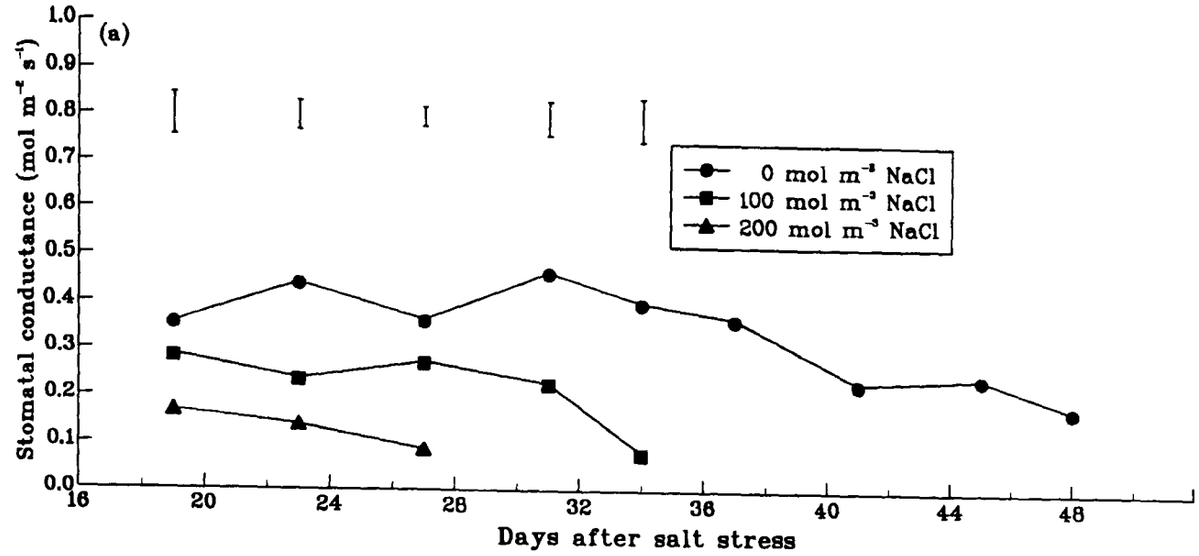
In all treatments Ci increased over time except at 31 DAS at 100 mol m^{-3} NaCl and at 41 DAS at 0 mol m^{-3} NaCl when a slight decrease was observed (Figure 6.1c). Differences between treatments were not significant at 19 and 27 DAS. At 31 and 34 DAS Ci was significantly lower at 100 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl.

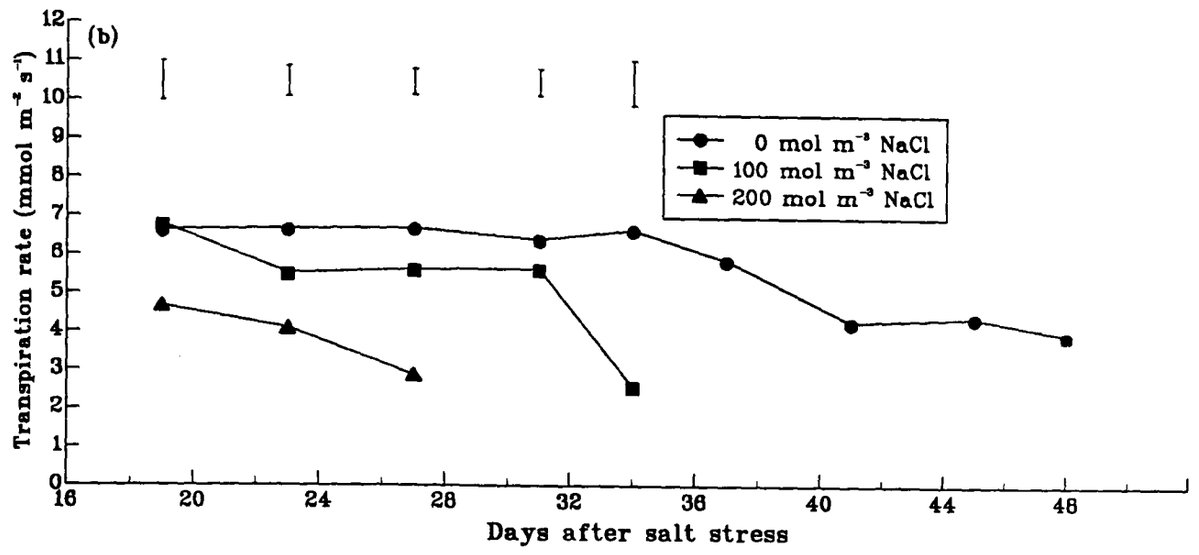
g_s decreased as the leaf senesced and on all sampling dates g_s decreased as salinity increased (Figure 6.2a). g_s was significantly lower at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl from 23 DAS onwards. At 0 mol m^{-3} values of g_s were less consistent over time than the values of Pn (Figure 6.1b). At 100 and 200 mol m^{-3} NaCl the trends in g_s were similar to those in Pn.

At all salinity levels E decreased over time (Figure 6.2b). E was significantly lower at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl on all sampling dates except at 19 DAS when the difference between 0 and 100 mol m^{-3} NaCl was not significant.

T_l significantly increased with increasing salinity (Figure 6.2c). At 100 and 200 mol m^{-3} NaCl T_l increased over time. However at 0 mol m^{-3} NaCl there was no consistent trend of T_l with time.

TE was higher at 0 mol m^{-3} NaCl than at 100 and 200 mol m^{-3} NaCl on all sampling dates, although the differences between treatments were not always significant (Figure





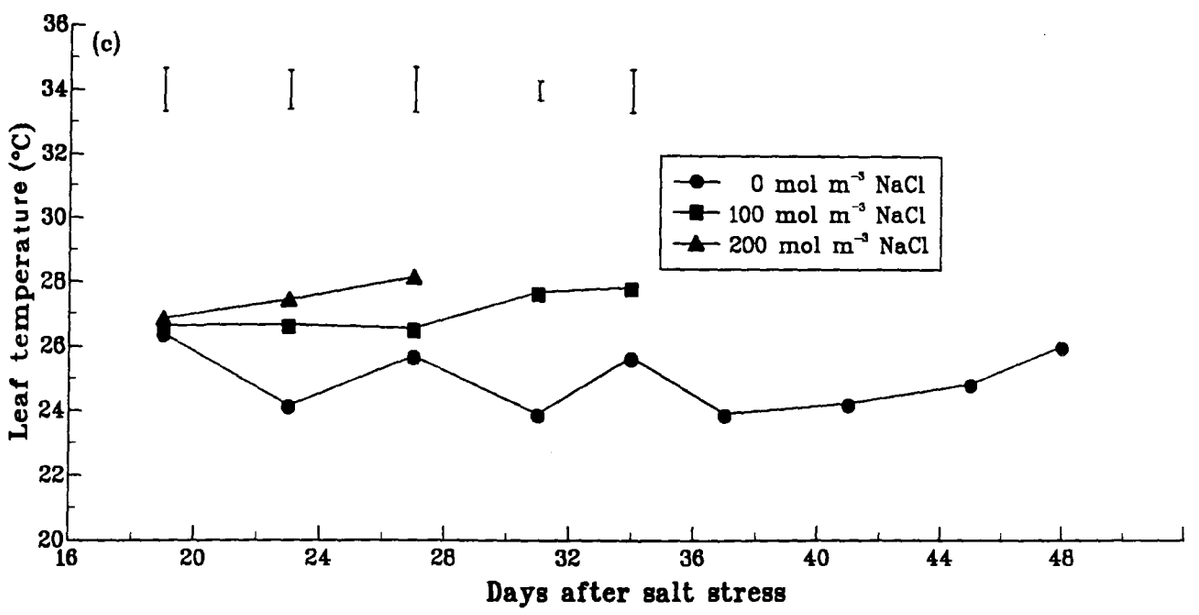


Figure 6.2: Effect of salinity on (a) stomatal conductance, (b) transpiration rate and (c) leaf temperature of leaf 6 of spring wheat.
I= standard error of difference between means.

6.3a). In all treatments TE was the highest when the leaf was expanding and lowest at the last measurement before the leaf was completely yellow.

LP was significantly higher at 0 than at 100 and 200 mol m^{-3} NaCl on all sampling dates (Figure 6.3b). At 0 mol m^{-3} NaCl maximum LP was observed at 34 DAS when the leaf was fully expanded whereas at 100 and 200 mol m^{-3} NaCl it was observed at 31 and 19 DAS respectively. At 200 mol m^{-3} NaCl, LP decreased over time. At 0 and 100 mol m^{-3} NaCl, LP decreased rapidly from 34 and 31 DAS.

6.4.5 EFFECT OF SALINITY ON CALCULATED Pnmax, Rd_c, α AND Ic

The values of Pnmax, Ic, α and Rd_c were derived by fitting the exponential model to the data (Section 6.2.5). The regressions were always significant and variation in I almost always accounted for at least 99% of the variation in Pn. Pnmax was significantly decreased by salinity on all sampling dates (Figure 6.4a) and these trends closely followed the trends of Pn.

Rd_c was higher at 100 and 200 mol m^{-3} NaCl than 0 mol m^{-3} NaCl on all sampling dates but the difference was not always significant. At 200 mol m^{-3} NaCl Rd_c increased over time, this trend was not found at 0 and 100 mol m^{-3} NaCl.

Generally there was no significant effect of salinity on α except at 31 DAS onward where α was significantly higher at 0 mol m^{-3} NaCl than at 100 mol m^{-3} NaCl (Figure

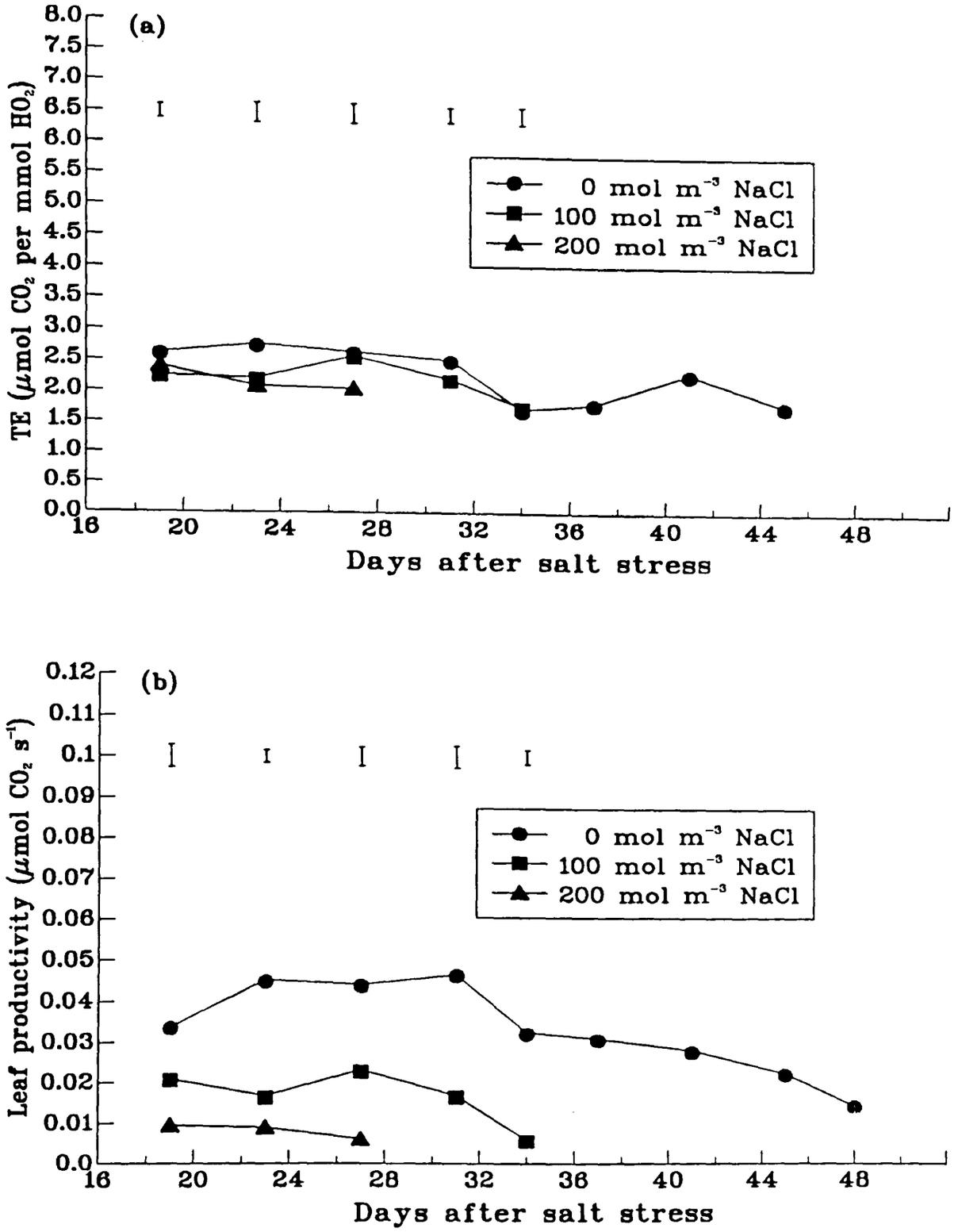


Figure 6.3: Effect of salinity on (a) transpiration efficiency (TE) and (b) leaf productivity of leaf 6 of spring wheat. I= standard error of difference between means.

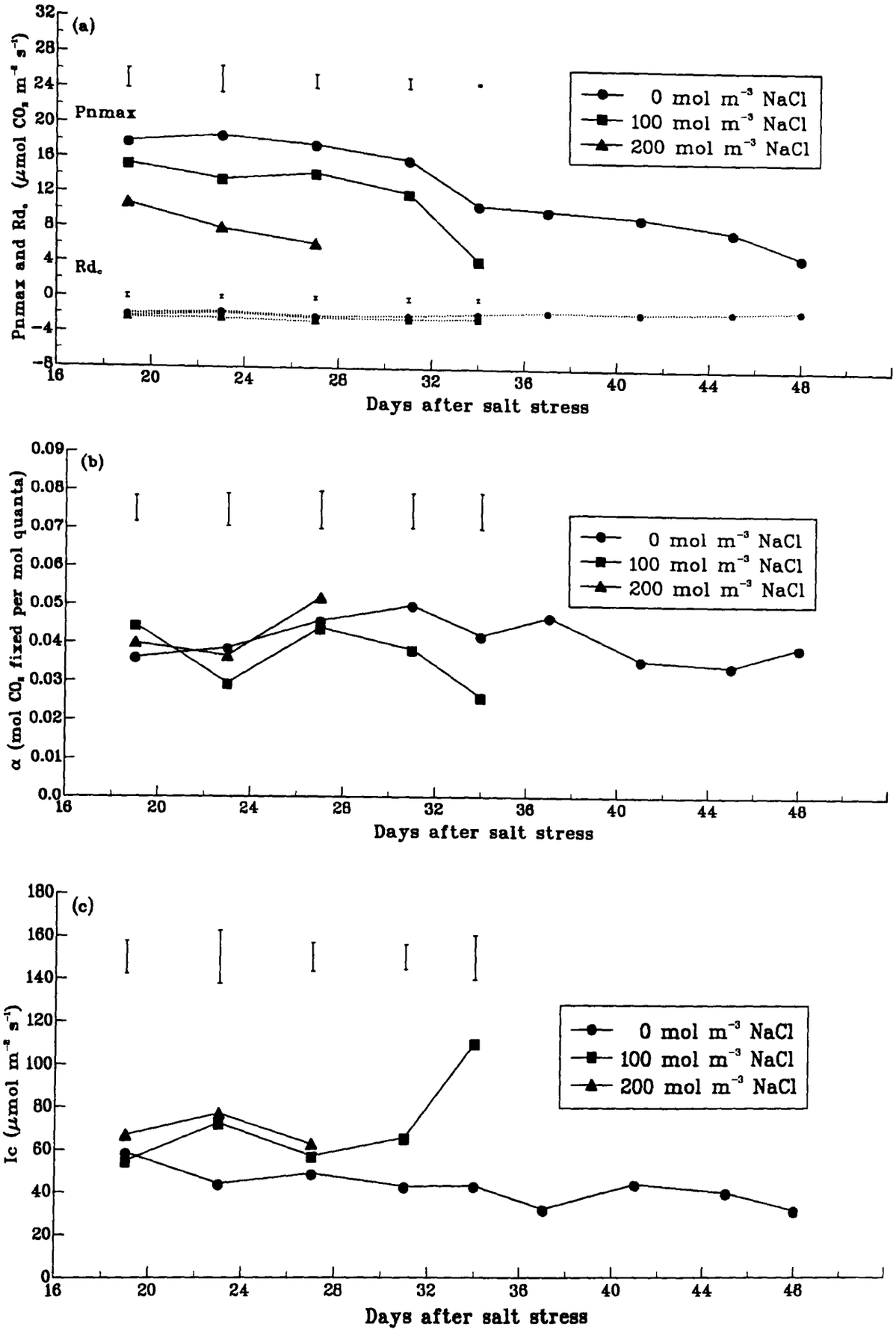


Figure 6.4: Effect of salinity on (a) maximum net photosynthesis (Pnmax) and calculated dark respiration (Rd_c), (b) photosynthetic efficiency (α) and (c) photon flux compensation point (Ic) of leaf 6 of spring wheat. I= standard error of difference between means.

6.4b). At 0 mol m^{-3} NaCl α increased up to 31 DAS and then decreased. At 100 and 200 mol m^{-3} NaCl no consistent trend of α over time was observed.

At 0 mol m^{-3} NaCl Ic decreased over time (Figure 6.4c). Ic was significantly higher at 100 and 200 than at 0 mol m^{-3} NaCl on all sampling dates except at 19 and 27 DAS when the differences between treatments were not significant. At 100 mol m^{-3} NaCl Ic increased rapidly at 34 DAS as the leaf approached complete senescence.

6.4.6 EFFECT OF SALINITY ON SAP Na^+, K^+, Cl^- AND OP

Sap Na^+ was always higher at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl and in both salinity treatments increased over time (Figure 6.5a). At 0 mol m^{-3} NaCl sap Na^+ was less than 1 mol m^{-3} on all sampling dates. At 100 and 200 mol m^{-3} NaCl it increased rapidly over time as the leaf senesced.

At 0 mol m^{-3} NaCl sap Cl^- remained approximately constant at 29 mol m^{-3} between 19 and 31 DAS (Figure 6.5b). Thereafter it decreased slightly over time as the leaf senesced. Sap Cl^- increased with salinity and was significantly higher at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl on all sampling dates.

Sap K^+ was significantly higher at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl on all sampling dates (Figure 6.6a). The difference between 100 and 200 mol m^{-3} NaCl was significant at 19 DAS only. At 0 mol m^{-3} NaCl sap K^+ decreased progressively from 34 DAS onward as the leaf

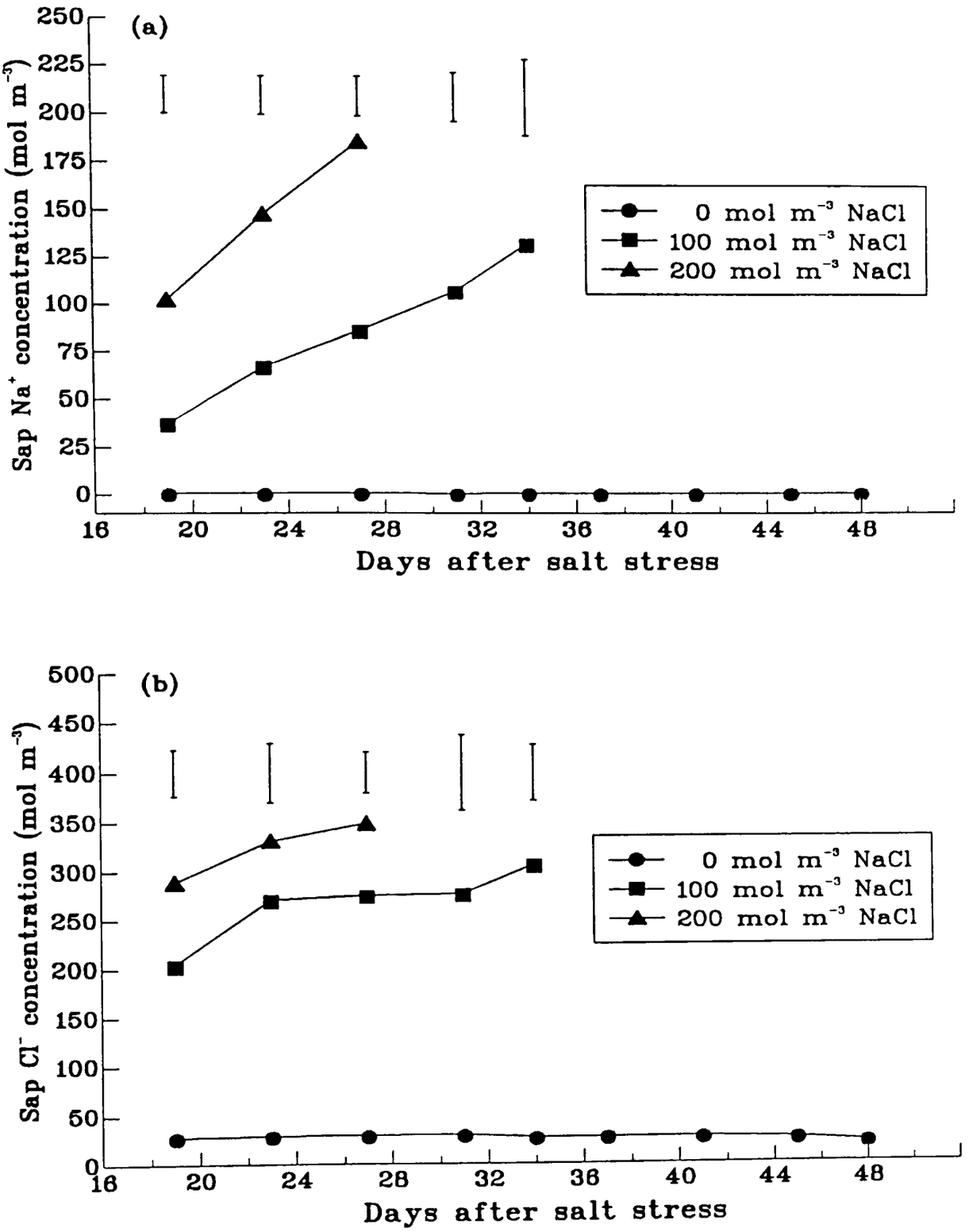
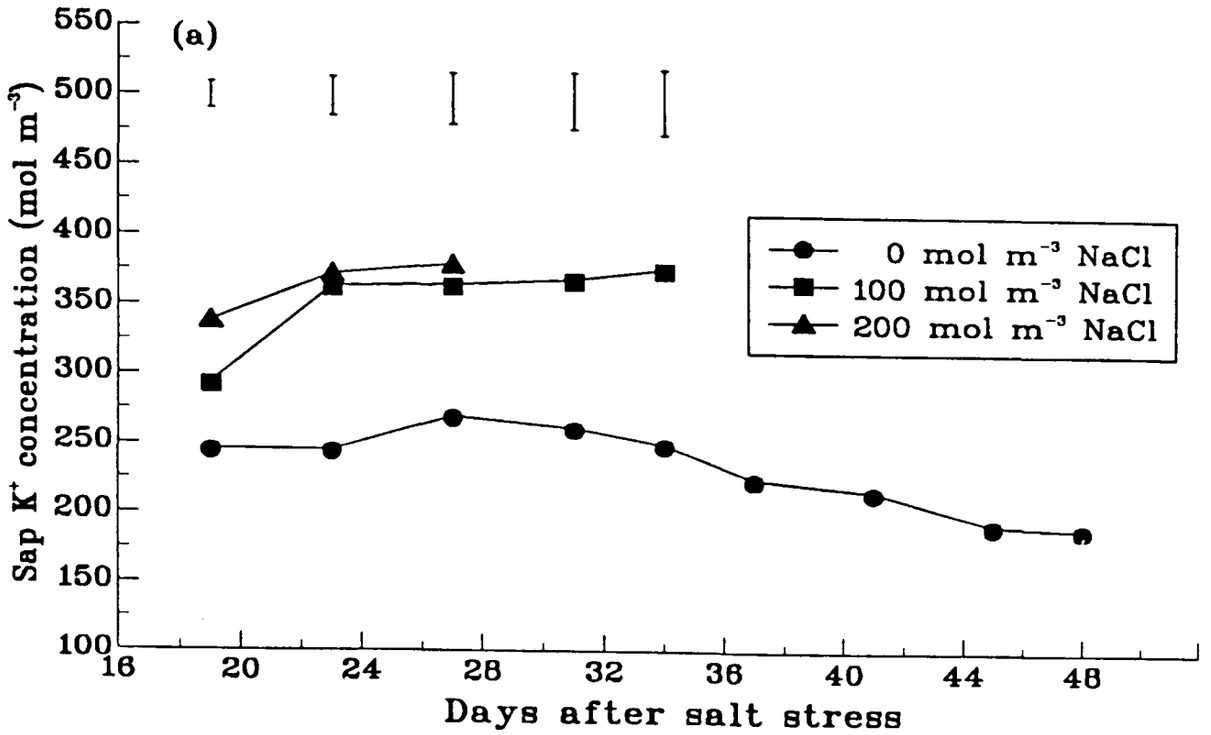


Figure 6.5: Effect of salinity on (a) sap sodium concentration (mol m^{-3}) and (b) sap chloride concentration (mol m^{-3}) of leaf 6 of spring wheat. I= standard error of difference between means.



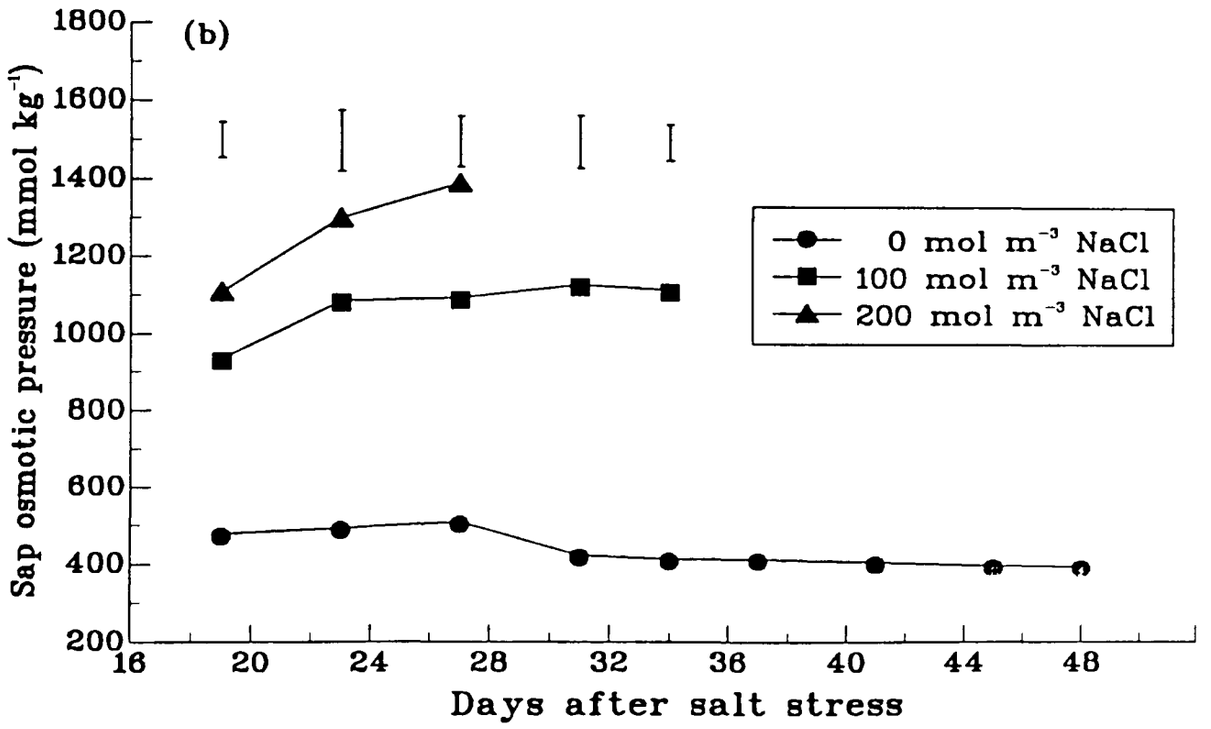


Figure 6.6: Effect of salinity on (a) sap potassium concentration (mol m^{-3}) and (b) sap osmotic pressure (mmol kg^{-1}) of leaf 6 of spring wheat. I= standard error of difference between means.

senesced.

Sap OP increased with salinity and was significantly higher at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl on all sampling dates (Figure 6.6b). At 0 mol m^{-3} NaCl sap OP increased between 19 and 27 DAS. Thereafter it decreased and subsequently remained constant at approximately 400 mmol kg^{-1}. At 100 mol m^{-3} NaCl sap OP increased progressively while at 200 mol m^{-3} NaCl this increase was very rapid and it remained significantly higher than at 100 mol m^{-3} NaCl over time as the leaf senesced.

6.4.7 EFFECT OF SALINITY ON FRESH AND DRY WEIGHT, LAMINA AREA, SPECIFIC LEAF WEIGHT AND STOMATAL FREQUENCY OF FULLY EXPANDED LEAF 6

Fresh and dry weight (LFWT and LDWT), fresh to dry weight ratio and leaf lamina area (A) of leaf 6 when fully expanded were significantly lower at 100 and 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl (Table 6.3). All these parameters were significantly lower at 200 mol m^{-3} than at 100 mol m^{-3} NaCl. Specific leaf weight (SLW) was not significantly affected by salinity. Increasing salinity resulted in a significant increase in stomatal frequency per unit leaf area (SF) but a significant decrease in stomata per leaf (SF_l).

Table 6.3: Effect of salinity on lamina fresh weight (LFWT in mg), lamina dry weight (LDWT in mg), lamina fresh weight:dry weight ratio (LFWT:LDWT), lamina area (A in cm^2), specific leaf weight (SLW in mg cm^{-2}), stomatal frequency (SF in stomata mm^{-2}) and stomatal frequency per leaf (SF_l $x10^4$) of fully expanded leaf 6 of spring wheat and increases (+) or decreases of these parameters over control (0 mol m^{-3} NaCl).

Parameters	Salinity levels (mol m^{-3} NaCl)			SEDM	LSD	CV(%)
	0	100	200			
LFWT	579.1	218.9	97.9	24.2	50.3**	16.2
LDWT	89.1	44.5	26.0	6.1	12.7**	22.9
LFWT:LDWT	6.7	4.9	3.5	0.4	0.9**	17.6
A	27.8	14.1	7.8	1.0	2.1**	12.3
SLW	3.2	3.2	3.3	0.2	NS	14.9
SF	44.0	61.1	75.0	3.2	6.7**	10.7
SF_l	12.3	8.6	5.9	0.8	1.6**	17.2
Percent increases (+) or decreases						
LFWT	-	62.9	83.1	-	-	-
LDWT	-	49.9	70.8	-	-	-
LFWT:LDWT	-	25.7	47.9	-	-	-
A	-	49.4	71.9	-	-	-
SLW	-	1.6	+0.1	-	-	-
SF	-	38.9	70.5	-	-	-
SF_l	-	29.8	52.2	-	-	-

SEDM= Standard error of difference between means,
LSD = Least significance differences,
CV = Coefficient of variation,
** = Significant at 1 percent probability level,
- = Not applicable.

6.5 DISCUSSION AND CONCLUSIONS

Measurements of GER on plants *in situ* under prevailing lighting conditions in the glasshouse in Experiment 2 had very high coefficients of variation (Section 4.5.7). In this experiment equilibrating plants under constant conditions before measuring Pn under controlled light conditions was successful in reducing the coefficients of variation of the data (Section 6.4.2). The coefficients of variation of most of the GER measurements except g_s (22-59%) and Rd (18-25%) remained below 16% (Section 6.4.2), whereas the coefficients of variation for GER in Experiment 2 (Section 4.5.7) were very high (32-120%). This agrees with the earlier findings by Kemal-ur-Rahim (1988) who observed a coefficient of variation of 12% for Pn under controlled lighting conditions and 52% in the glasshouse under natural lighting conditions.

During the course of the present study the rate of net photosynthesis for a single leaf was generally at a maximum just before the leaf had attained its maximum area. Constable and Rawson (1980) found that the peak photosynthesis in cotton was attained when the leaf was 75-90% of maximum area. Other workers have reported that maximum rate of net photosynthesis occurs when the cereal leaves are fully expanded (Austin *et al.* 1982; Rawson *et al.* 1983; Kemal-ur-Rahim 1988; Rawson *et al.* 1988a). Rawson *et al.* (1988a) found that in the absence of salinity Pn and g_s decreased during senescence whereas Rd remained relatively constant. Decreases in Pn with leaf age have been reported

by many workers (Dantuma 1973; Marshall and Biscoe 1980a,b; Hodanova 1981; Austin *et al.* 1982; Evans 1983; Rawson *et al.* 1983; Lawlor *et al.* 1987). Pn, E and TE all decreased with leaf age. One of the causes of the decreases in these parameters was the decrease in stomatal conductance. Pn and E both decreased in parallel with g_s. The decrease in TE indicates that Pn decreased more than E with time. Changes in stomatal and internal conductances, either independently or together, have been proposed as the cause of the changes in net photosynthesis with leaf age (Constable and Rawson 1980). Most workers have observed the decline in Pn to be associated with a reduction in g_s (Holmgren *et al.* 1965; Rawson and Woodward 1976). The most common finding is that the conductances change essentially in parallel as leaves age (e.g. Woodward and Rawson 1976), with the result that the concentration of CO_2 in the intercellular spaces (Ci) remains constant (Davis and McCree 1978; Gourdiaan and van Laar 1978; Constable and Rawson 1980). Yeo *et al.* (1985) observed a parallel decrease in carbon fixation by the chloroplasts in rice and argued that if stomatal closure were the only reason for reduction in photosynthesis then it would be expected that Ci would decrease. In the present study Ci remained fairly low when the leaf was expanding and then it progressively increased with time as a consequence of the decrease in Pn. Pnmax and Rd_c generally followed the same trend as Pn and Rd_o. α was relatively constant but declined at the final sampling dates. A similar trend was

observed for Ic, whereas it increased at the final sampling dates. These results agree with earlier findings of other workers (Rawson 1986; Kemal-ur-Rahim 1988; Rawson *et al.* 1988a).

Kingsbury *et al.* (1984) and Rawson (1986) found that salinity decreased photosynthesis whereas Kemal-ur-Rahim (1988) observed that salinity had little effect on photosynthesis. For the parameters that were compared at 1750 μmol m^{-2} s^{-1} PAR, the effect of increased salinity was to decrease g_s, Pn, E, TE and Ci, and increase Rd_o and T_l (Table 6.4). Salinity also markedly decreased GLA. At 27 DAS, GLA was decreased by 59% and LP was decreased by 86% at 200 mol m^{-3} NaCl. Therefore the marked reduction of LP with increase in salinity was the result of decrease in both Pn and GLA.

In general it would be expected that salinity, by increasing stomatal closure, would decrease g_s and therefore E. A reduction in g_s would decrease Pn by reducing the rate at which CO_2 could enter the leaf, hence Ci would be lower (Kemal-ur-Rahim 1988). In the present study most of the GER parameters were seriously affected by salinity. At 200 mol m^{-3} NaCl the decrease in g_s at 27 DAS was 76% and the decrease in Pn was 67%. As discussed earlier if stomatal closure is the only cause of decrease in Pn then Ci would be expected to decrease. The results herein suggested that this was not the case. At 200 mol m^{-3} NaCl, g_s was decreased by between 52-76% whereas Ci was initially decreased by 30% and then by only 5-6% at the later

Table 6.4: Effect of salinity on percent increases (+) or decreases over control (0 mol m^{-3} NaCl) of green lamina area (GLA), leaf temperature (T_l), net photosynthesis (Pn), observed dark respiration (Rd_o), stomatal conductance (g_s), Sub-stomatal CO_2 concentration (Ci), transpiration rate (E), transpiration efficiency (TE), leaf productivity (LP), maximum net photosynthesis (Pnmax), calculated dark respiration (Rd_c), photon flux compensation point (Ic), photosynthetic efficiency (α), sap sodium (Na^+), sap chloride (Cl^-) and sap potassium (K^+) concentrations of leaf 6 of spring wheat on selected sampling dates. Data for Experiment 3.

Parameters	Salinity levels (mol m^{-3} NaCl)	Days after salt stress (days after leaf 6 emergence)					Average
		19(6)	23(10)	27(14)	31(18)	34(21)	
GLA	100	29.5	43.8	37.5	53.2	54.7	43.7
	200	56.2	55.6	59.3	-	-	57.0
T_l	100	+0.9	+9.6	+3.3	+15.8	+8.5	+7.6
	200	+1.7	+13.5	+9.5	-	-	+8.3
Pn	100	11.5	33.6	16.8	22.6	58.7	28.6
	200	36.4	53.4	66.8	-	-	52.2
Rd_o	100	+19.9	+6.4	+13.9	+34.7	+40.4	+23.1
	200	+30.6	+49.7	+33.7	-	-	+38.0
g_s	100	19.5	46.2	24.6	50.9	79.8	44.2
	200	52.76	68.5	76.0	-	-	65.8
Ci	100	+3.2	4.5	3.7	13.3	13.7	6.4
	200	1.5	6.4	5.3	-	-	4.4
E	100	+2.3	17.4	16.1	11.9	60.9	20.8
	200	29.7	38.8	57.1	-	-	41.8
TE	100	13.9	19.9	2.5	12.2	+2.6	9.2
	200	8.2	24.4	22.5	-	-	18.4
LP	100	38.1	62.9	47.9	63.9	81.6	58.9
	200	71.7	80.1	86.2	-	-	79.4
Pnmax	100	14.2	26.9	18.7	24.1	59.0	28.6
	200	40.1	57.6	64.8	-	-	54.2
Rd_c	100	+16.4	+8.7	+8.9	+22.3	+34.6	+18.2
	200	+22.1	+45.8	+27.4	-	-	+31.7
	100	6.7	+63.9	+16.8	+53.9	+156.6	+56.9
	200	+13.9	+74.9	+28.1	-	-	+39.0
α	100	+23.2	+23.8	4.4	23.3	39.9	4.1
	200	+10.2	5.7	+12.8	-	-	+5.8
Sap OP	100	+96.2	+120.7	+115.7	+166.8	+170.3	+133.9
	200	+132.7	+163.9	+173.6	-	-	+156.7

- = Leaves completely senesced.

sampling dates. Therefore, despite the marked and progressive decrease in g_s, Ci was less affected. These results agree more clearly with those of Yeo *et al.* (1985). T_l was slightly increased with increase in salinity. At 27 DAS the increase in T_l was 10% at 200 mol m^{-3} NaCl and E was decreased by 57%. Therefore T_l increased due to decrease in E. As salinity increased Pn, E and TE all decreased. This indicates that salinity had a larger effect on Pn than E.

The exponential model gave a good fit to the Pn-I data, and using this it was possible to determine the effects of salinity on Pnmax, α, Ic and Rd_c. From the fit of the exponential model to the Pn-I response curves, analyses of variance performed on Pnmax, Rd_c and Ic revealed that salinity:-

-decreased Pnmax

-increased Rd_c and Ic

α was inconsistently affected but was relatively constant. Similar results were also observed by Kemal-ur-Rahim (1988). Trends in Pnmax followed the same as those of Pn. When observed Pn and calculated Pnmax values were compared, the exponential model was found to be slightly over-estimating Pnmax at 0 and 100 mol m^{-3} NaCl when the leaf was expanding during early sampling dates (19 and 23 DAS). Thereafter it under-estimated Pnmax but the difference between treatments was not significant (Table 6.5). When observed Rd_o and calculated Rd_c values were compared, the model was also

Table 6.5: Effect of salinity on difference between calculated maximum net photosynthesis (Pnmax in μmol m^{-2} s^{-1}) and observed net photosynthesis (Pn in μmol m^{-2} s^{-1}) at 1750 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) and between observed dark respiration (Rd_o in μmol m^{-2} s^{-1}) and calculated dark respiration (Rd_c in μmol m^{-2} s^{-1}) at 0 μmol m^{-2} s^{-1} PAR of leaf 6 of spring wheat.

Days after salt stress	Salinity levels (mol m^{-3} NaCl)			SEDM	LSD
	0	100	200		
Pnmax-Pn					
19	0.56	0.01	-0.41	1.68	3.49**
23	0.50	1.70	-0.53	1.19	NS
27	0.31	-0.53	0.39	0.74	NS
31	-0.01	-0.25	-	0.31	NS
34	-0.88	-0.10	-	0.76	NS
37	-0.03	-	-	--	--
41	-0.13	-	-	--	--
45	0.22	-	-	--	--
48	0.13	-	-	--	--
Rd_o-Rd_c					
19	0.09	0.04	-0.06	0.06	NS
23	0.06	0.09	0.02	0.06	NS
27	0.17	0.09	0.10	0.07	NS
31	0.19	0.01	-	0.07	NS
34	0.09	0.04	-	0.04	NS
37	0.11	-	-	--	--
41	0.13	-	-	--	--
45	0.12	-	-	--	--
48	0.09	-	-	--	--

SEDM= Standard error of difference between means,
LSD = Least significant differences,
NS = Not significant,
** = Significant at 1 percent probability level,
- = Leaf completely senesced,
-- = Not applicable.

found to be over-estimating Rd_c on the majority of sampling dates but the difference between salinity treatments was not significant. This agrees with the findings by those of Kemal-ur-Rahim (1988). Rawson (1986) found reduction in dark respiration with salinity but the effects were restricted to young leaves of barley, in older leaves he observed increased dark respiration. There were inconsistent trends for α, which was relatively constant but declined at the final sampling dates. Rawson (1986) found a 60% decrease in α by salinity. Ic increased with salinity and with leaf age but this trend was also not consistent. Ic was mostly higher at high salinity than in the control on most sampling dates. These results agree with those of Rawson (1986) and Kemal-ur-Rahim (1988).

Measurements of A, LFWT, LDWT, SLW, SF and SF_1 revealed that salinity:-

-decreased A, LFWT and LDWT,

-decreased fresh weight to dry weight ratio,

-increased SF but decreased SF_1.

The effect of salinity on SLW was inconsistent and less obvious whereas all the other parameters were markedly affected (Table 6.3). There was 70-83% decrease of LFWT, LDWT and A at 200 mol m^{-3} NaCl over control whereas these parameters were decreased by 49-62% at 100 mol m^{-3} NaCl. SF was increased by 39% and 70% at 100 and 200 mol m^{-3} NaCl respectively. These results are in line with those of Kemal-ur-Rahim (1988). When stomata were calculated per leaf, SF_1 was decreased by 29% and 52% respectively at both of these

salinity levels. These results suggest that increased SF was a result of reduction in A whereas the decrease in SF_1 suggests that cell differentiation was also affected by salinity.

The general effects of salinity on leaf ionic concentration and sap osmotic pressure were to:-

-markedly increase leaf sap osmotic pressure,

-markedly increase sap Na^+, Cl^- and K^+ concentration.

In the present study all of these parameters were studied from expanding to full senescence of leaf 6 whereas most earlier findings are based on fully expanded leaves (e.g. Gorham *et al.* 1986a,b; Rashid 1986; Rawson 1986; Shah 1987). Most of these workers found similar trends of increase in sap OP, Na^+ and Cl^- concentration with increasing salinity but inconsistent trends and/or decreases in K^+ concentration have also been recorded (Iqbal 1988; Kemal-ur-Rahim 1988). In contrast, Gorham (1990) observed, although not stated clearly, slightly higher K^+ concentration with increase in salinity in the youngest fully expanded leaves of wheat.

In the present study, one of the main effects of salinity was an earlier and rapid senescence of leaves at 200 mol m^{-3} NaCl (within 31 DAS) than at 0 mol m^{-3} NaCl (within 49 DAS), which resulted in only few GER and ionic measurements. One of the main causes of this could be very high sap ionic concentration and reduced Pn. Higher internal Na^+ and Cl^- concentrations are widely accepted as the cause of an earlier senescence and death of leaves (e.g.

Bernstein 1975; Gorham *et al*. 1986b; Rashid 1986). Very high K^+ concentration in plant cells is also considered to be toxic (Marschner 1986). The results herein indicated that higher sap Na^+, Cl^- and K^+ caused rapid senescence and death of leaf 6 at 200 mol m^{-3} NaCl. Therefore in the next experiment it was decided to test slightly lower levels of salinity so that more data points could be collected. Experiment 4 was planned with lower salinity levels (75 and 150 mol m^{-3} NaCl). The correlations between gas exchange and ionic concentrations are discussed in Chapter 9.

CHAPTER 7

EXPERIMENT 4

THE EFFECTS OF SALINITY ON GER, SAP IONIC CONCENTRATIONS, SAP OSMOTIC PRESSURE AND AREA OF LEAF 5 AND THE FLAG LEAF OF SPRING WHEAT GROWN UNDER GLASSHOUSE CONDITIONS

7.1 INTRODUCTION

Experiment 3 was conducted in growth rooms where light and temperature were controlled. However, plant growth under controlled conditions may not correspond to growth under field conditions where light, temperature, and humidity are all variable factors (Blum 1990). Under these conditions plants may have a better chance to adapt to salinity due to the changing environment. The light intensity in the growth rooms used in Experiment 3 (200-300 μmol m^{-2} s^{-1} PAR) was lower than natural day light conditions (1700-2000 μmol m^{-2} s^{-1} PAR). The temperature in the growth rooms, which was set at 24/16°C (day and night), was also not typical of that normally experienced by crops in the field. In the field in both the UK and Pakistan spring wheat normally experiences cool conditions after sowing, but the weather gradually gets hotter and drier as time passes. Under these conditions there is more evapotranspiration and hence greater uptake of toxic ions is expected. This means that plants grown in the field may be better able to adjust osmotically compared with plants grown in growth rooms where conditions are constant throughout the growth period. Therefore in this experiment plants were grown in an unheated glasshouse. In Experiment 3 GER measurements were made on leaf 6 (Chapter 6). In wheat the contribution of the flag leaf to photosynthesis and yield is well documented (for example, Evans and Rawson 1970; Blum 1985). The growth rooms in Experiment 3 had limited space and plants could not be grown to maturity.

Different leaves can have quite different CO_2 exchange rates (for example, Dantuma 1973) but they may all show a similar response to salinity (Kemal-ur-Rahim 1988). Therefore in this experiment data were collected for both leaf 5 and the flag leaf.

In the early years of gas exchange measurements it was considered that photosynthetic rates of plants could be characterised by examining any young fully expanded leaf (Rawson *et al.* 1983). This is still the stage of leaf growth preferred by many workers for ionic studies (Gorham *et al.* 1987; Shah 1987; Kemal-ur-Rahim 1988; Gorham 1990). Photosynthetic rates can change dramatically with leaf age in some species (Dantuma 1973; Rawson and Hackett 1974) with or without NaCl (Rawson *et al.* 1988a). Leaf Na^+ and Cl^- concentrations increase with leaf age in plants grown under saline conditions (Greenway *et al.* 1965; Rawson *et al.* 1988a; Experiment 3). Therefore in this experiment GER and related parameters were measured at different stages of leaf growth.

In Experiment 3 the highest salinity level tested (200 mol m^{-3} NaCl) resulted in rapid senescence and death of leaf 6. Therefore in this experiment it was decided to test slightly lower salinity levels (75 and 150 mol m^{-3} NaCl), so as to be able to obtain more measurements of senescing leaves.

7.2 MATERIALS AND METHODS

The procedures used in Experiment 4 generally followed those used in Experiment 3. Any changes in the methodology are noted in the following sections.

7.2.1 EXPERIMENTAL DESIGN AND TREATMENTS

A completely randomised design was used. There were 3 salinity treatments and 16 replicate containers of each treatment. The salinity levels tested were 0, 75 and 150 mol m^{-3} NaCl. $CaCl_2$ was applied to the salt stressed plants as described in section 4.2.1.

7.2.2 RAISING OF SEEDLINGS AND TRANSPLANTING

The experiment was initiated on 19 February 1990 and terminated on 21 June 1990. Pre-germination was started on 20 February and the seedlings were transplanted on 8 March, following the procedures described in section 3.2.4. All the other procedures were the same as described in section 6.2.

7.2.3 MAINTENANCE OF THE EXPERIMENT

The experiment was conducted in an unheated glasshouse with no supplementary lighting. Salt stress was introduced in increments as in Experiment 3 starting 16 days after seeding and 4 days after transplanting. On 10 May aphids were observed on the plants. The next day during late afternoon all the plants were sprayed with dimethoate (Murphy Systemic Insecticide) which slightly damaged the

leaf tips of plants under salinity stress. The exterior of the glasshouse was sprayed partially with white emulsion during the second week of May to prevent overheating inside and subsequent damage to the plants. The nutrient solution was changed after every second week. During the course of the experiment, minimum and maximum temperatures were recorded inside the glasshouse and the hours of bright sunshine were recorded at a field site less than 1 km from the glasshouse. Mean daily temperature each week and mean sunshine hours each week are presented in Figure 7.1.

7.2.4 COLLECTION OF GER DATA

GER was measured using the procedures described in section 6.2.4. The lights, 1000W mercury lamp and air conditioning system inside the growth room where the IRGA equipment and light chamber were installed were switched on half an hour before measurements commenced as in Experiment 3 (Section 6.2.4). The plants plus polystyrene lid were lifted off their original container and placed on a similar empty container. This was then placed in a polythene lined cardboard box to protect the plants from effects of wind during transfer from the glasshouse to the growth room containing the IRGA. Once in the light chamber, plants supported by their polystyrene lid were transferred to a container which was filled with a similar concentration of nutrient solution and appropriate salinity level. Transfer took no more than 2 minutes during which time the roots remained moist.

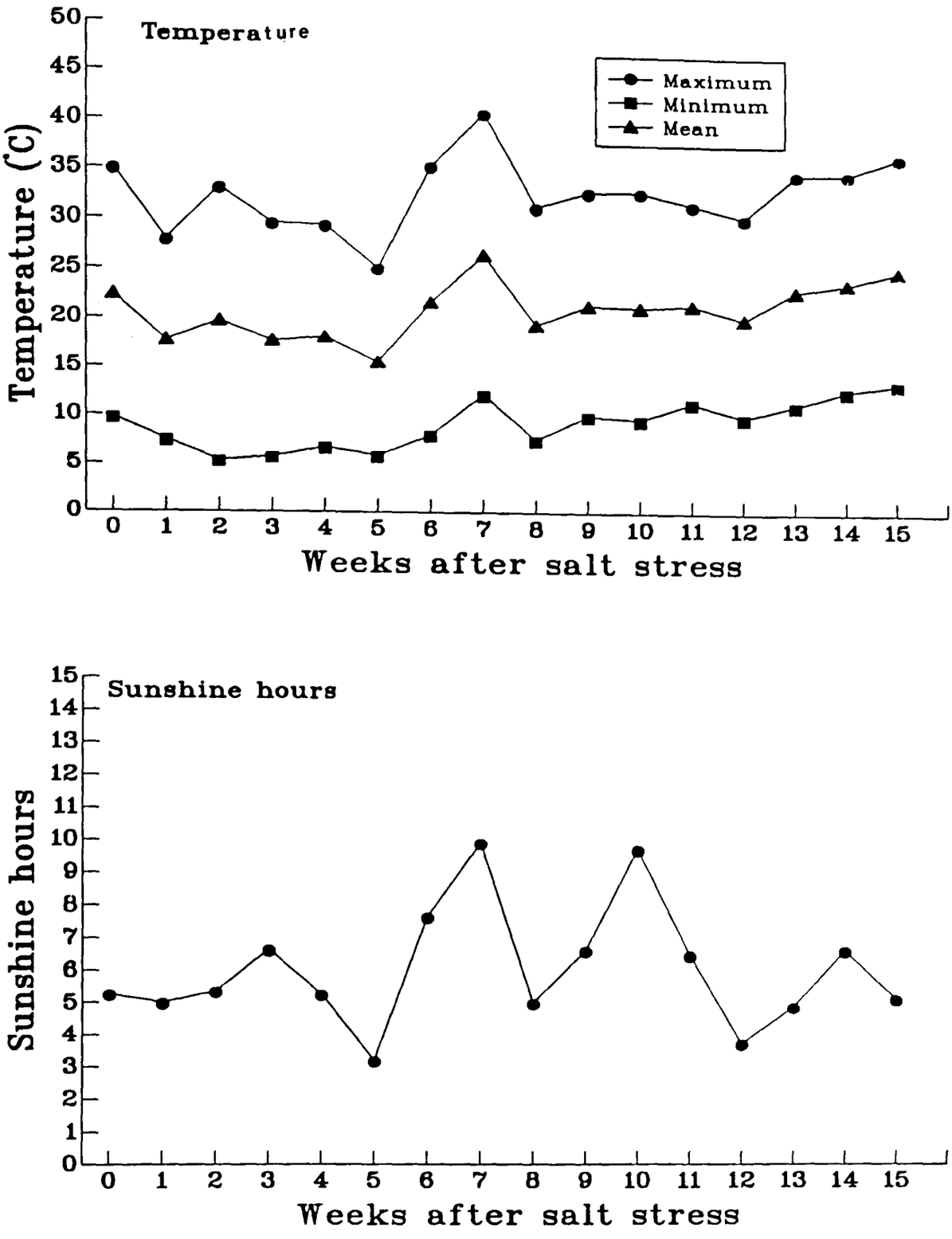


Figure 7.1: Mean daily temperature each week in the glasshouse and mean sunshine hours each week during the growth period of spring wheat.

Measurements of GER of leaf 5 started on 29 March 1990 and finished on 4 May 1990 when it was completely yellow. Measurements of the flag leaf started on 5 May and finished on 21 June. For each set of GER measurements 8 replicate leaves on the mainstem were used for both leaf 5 and the flag leaf. The method of sampling was identical to that used in Experiment 3. The equipment and the procedures used to measure GER, to obtain Pn-I response curves and to derive the values of Pnmax, Rd_c, α and Ic were identical to those used in Experiment 3 (Section 6.2.5).

7.2.5 DETERMINATION OF GLA AND PREPARATION OF SAMPLES FOR CHEMICAL ANALYSES

After completing the GER measurements GLA of the sampled leaves was determined by an automatic area meter (Section 3.2.6.7.). The leaves were stored in 1.5 cm^3 Eppendorf tubes and frozen in a refrigerator at 0°C for later chemical analyses to determine sap Na^+, Cl^- and OP). The procedures used were the same as those described in section 6.2.6 for Experiment 3.

7.2.6 DETERMINATION OF TRANSPIRATION EFFICIENCY AND LEAF PRODUCTIVITY

These parameters were calculated as described in section 6.2.7.

7.2.7 DETERMINATION OF FRESH AND DRY LEAF LAMINA WEIGHT, SPECIFIC LEAF WEIGHT AND STOMATAL FREQUENCY

When leaf 5 and the flag leaf were fully expanded (ligule emerged), eight replicate leaves were used to determine fresh and dry weight, specific leaf weight and stomatal frequency. The procedures used were the same as those described in section 6.2.8 for Experiment 3.

7.2.8 GREEN AREA AND DRY WEIGHT PER PLANT AT ANTHESIS STAGE

At anthesis (when 50 percent of spikelets had protruded anthers), 4 randomly selected plants were harvested from each of two replicate containers of the 0 and 75 mol m^{-3} NaCl treatments. At 150 mol m^{-3} NaCl all plants had died before anthesis. The plants were harvested by cutting the stems just above the level of the polystyrene lid. The leaves were then detached from the point where the lamina joined the sheath. The spikes were removed from the stems and their green area was measured using an automatic area meter. The yellow tips of leaves were removed before their green area was recorded. Leaves, stems and ears were then oven dried at 80°C for 48 hours to record dry weight.

7.3 STATISTICAL ANALYSES

All data were analysed using ANOVA and differences between means, where significant, were tested as described in section 6.3 for Experiment 3.

7.4 RESULTS

As in Experiment 3, results for T_l, Pn, Rd_o, g_s, E, Ci and TE of leaf 5 and the flag leaf are presented only for the highest light intensity (1750 μmol m^{-2} s^{-1} PAR) for the reasons stated in section 6.4.1. The trends in g_s and Pn for leaf 5 and the flag leaf during enclosure while determining light response curves were similar to those observed in Experiment 3 (Section 6.4.1) and are therefore not presented. The CV of the data for all the parameters studied were also similar to those in Experiment 3 and these are also not presented.

7.4.1 EFFECT OF SALINITY ON GREEN LAMINA AREA

Salinity significantly decreased the GLA of leaf 5 and the flag leaf (Figure 7.2). GLA of leaf 5 increased up to 16 DAS and thereafter remained almost constant. There was a slight decrease in GLA at the later sampling dates, but in all treatments senescence was rapid. At 0 mol m^{-3} NaCl leaf 5 senesced earlier than at 75 mol m^{-3} NaCl. Leaf 5 was completely yellow at 46, 52 and 39 DAS at 0, 75 and 150 mol m^{-3} NaCl respectively.

At 0 mol m^{-3} NaCl GLA of the flag leaf increased up to 63 DAS and it remained constant until 75 DAS. Thereafter it decreased and leaf was completely yellow at 98 DAS. At 75 mol m^{-3} NaCl GLA increased up to 60 DAS. Thereafter it decreased and the leaf was completely yellow at 98 DAS. At 150 mol m^{-3} NaCl GLA remained at approximately 15 cm^2

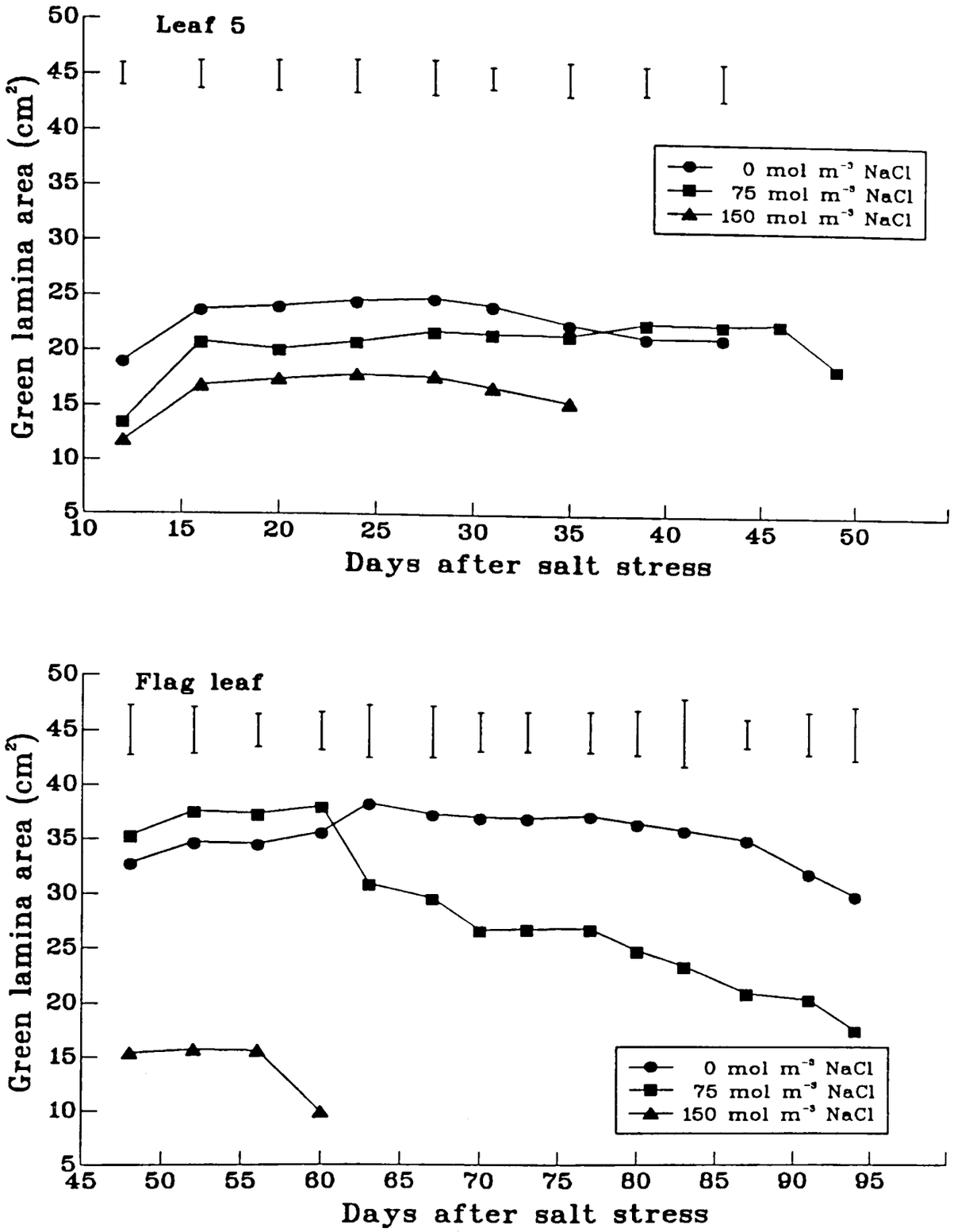


Figure 7.2: Effect of salinity on green lamina area of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

between 48 and 56 DAS. Thereafter it decreased rapidly and the leaf was completely yellow at 63 DAS. GLA was significantly lower at 150 mol m^{-3} NaCl than at 0 and 75 mol m^{-3} NaCl on all sampling dates. GLA was significantly lower at 75 than at 0 mol m^{-3} NaCl from 63 DAS onwards.

7.4.2 EFFECT OF SALINITY ON T_l, Pn, g_s E, AND Ci AT 1750 μmol m^{-2} s^{-1} PAR AND Rd_o AT 0 μmol m^{-2} s^{-1} PAR

In both leaf 5 and the flag leaf T_l was significantly increased by salinity on most of the sampling dates (Figure 7.3). However, the differences between 75 and 150 mol m^{-3} NaCl were not always consistent.

In both leaf 5 and the flag leaf Pn gradually decreased over time whereas Rd_o remained relatively constant (Figure 7.4). Pn of leaf 5 was significantly higher at 0 than at 75 mol m^{-3} NaCl until 24 DAS. Thereafter Pn at 0 mol m^{-3} NaCl declined and the leaf was completely yellow at 46 DAS. At 75 mol m^{-3} NaCl Pn remained at approximately 14 μmol m^{-2} s^{-1} from 20 until 39 DAS after which it decreased and the leaf was completely yellow at 52 DAS. At 150 mol m^{-3} NaCl Pn was significantly lower than at 0 and 75 mol m^{-3} NaCl at all sampling dates and the leaf was completely yellow at 39 DAS.

Rd_o of leaf 5 showed much less variation over time than Pn. Rd_o was significantly lower at 0 than at 75 and 150 mol m^{-3} NaCl on most sampling dates. Rd_o was generally higher at 150 than at 75 mol m^{-3} NaCl although this difference was not always significant.

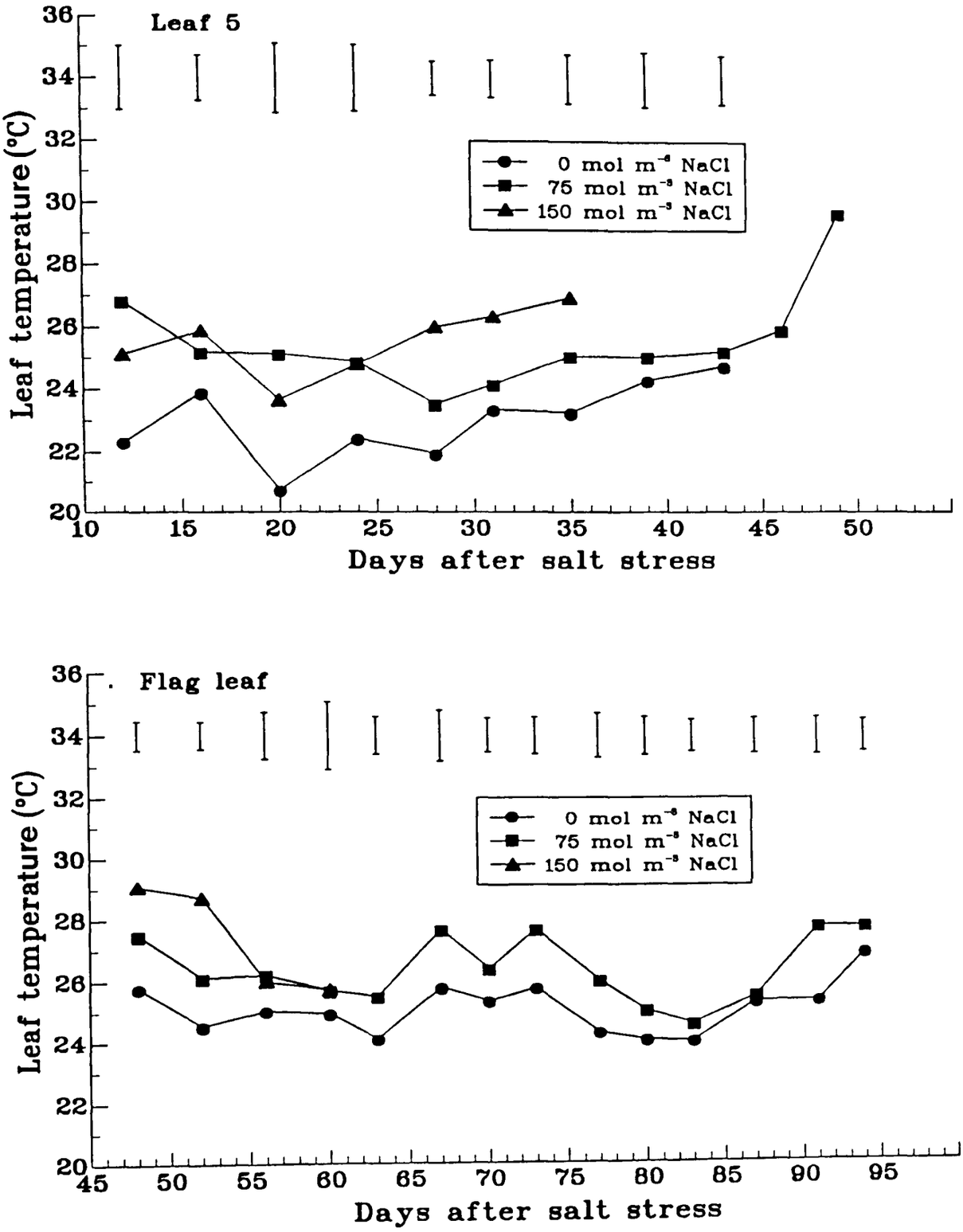


Figure 7.3: Effect of salinity on leaf temperature of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

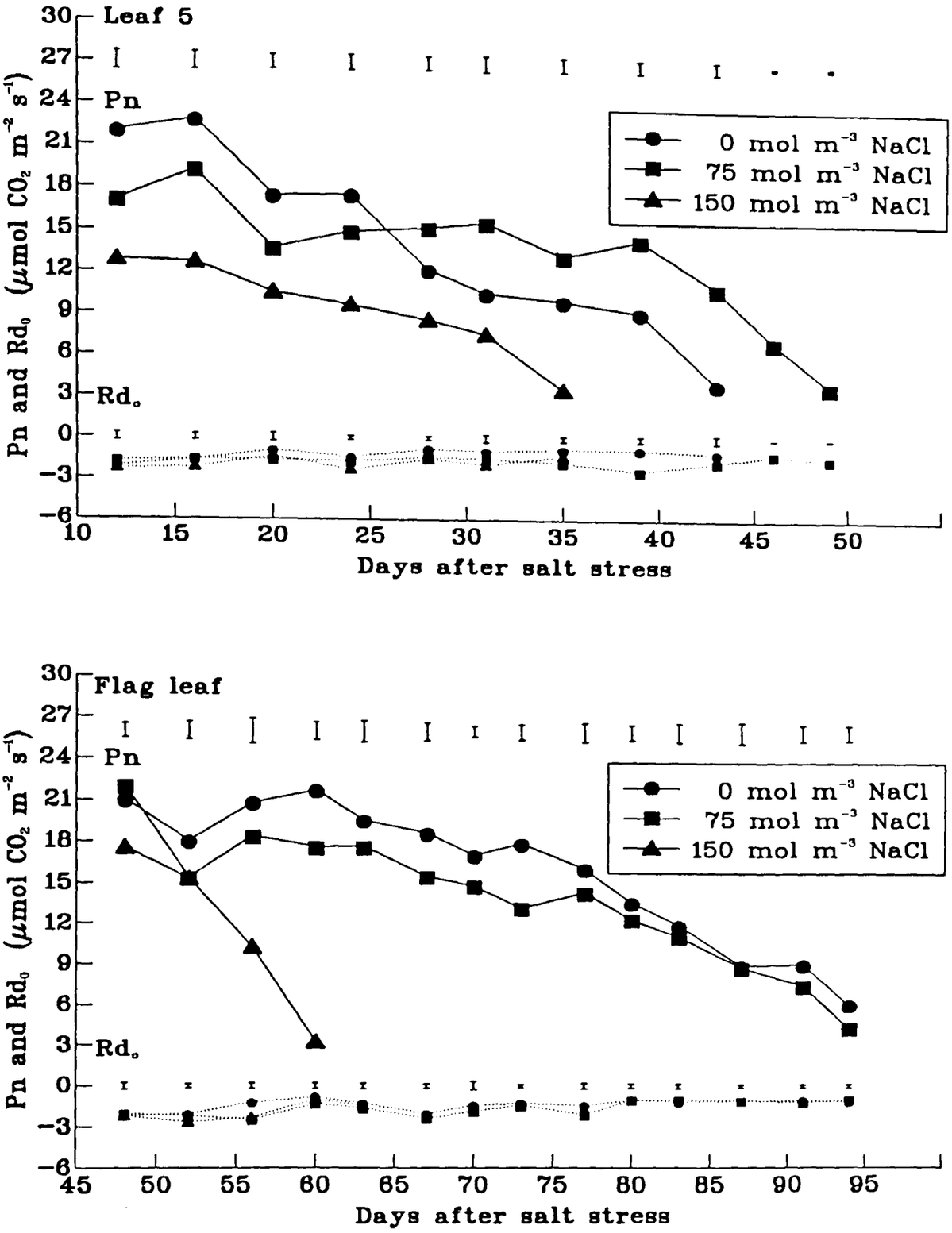
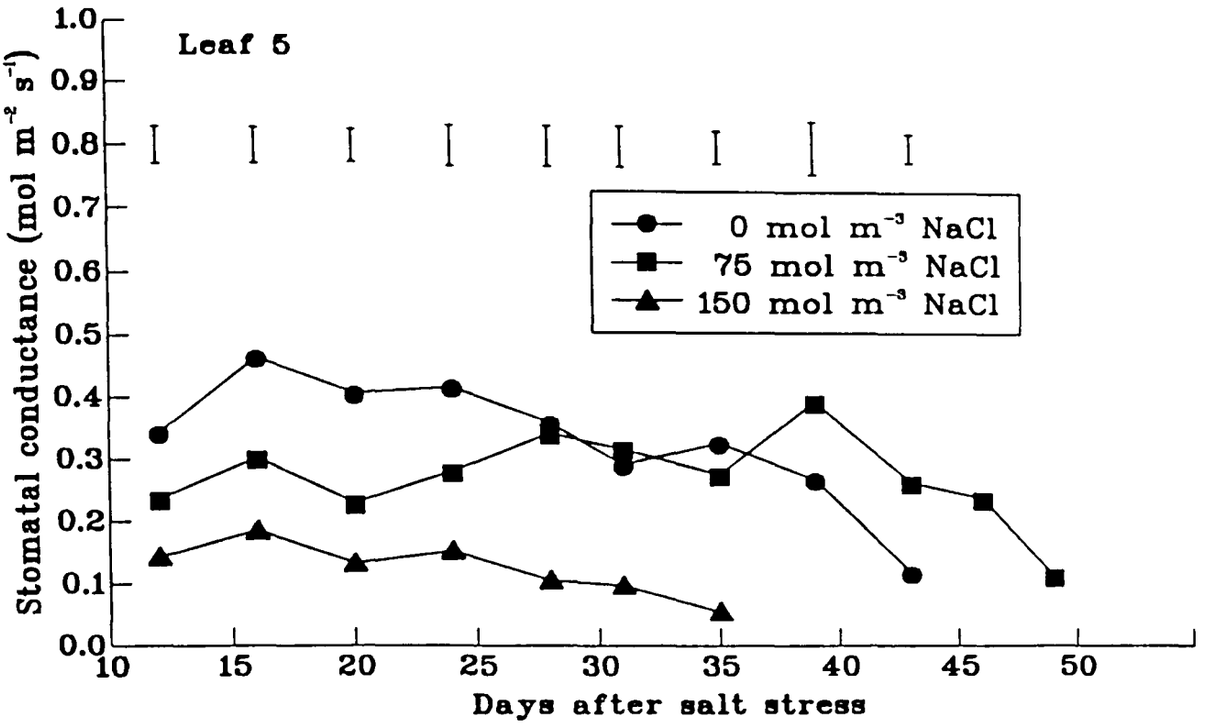


Figure 7.4: Effect of salinity on net photosynthesis (Pn) and observed dark respiration (Rd_o) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

From 52 DAS Pn of the flag leaf was consistently higher at 0 than at 75 mol m^{-3} NaCl. The difference was statistically significant on almost all sampling dates. It is interesting to note that a decrease in Pn was observed at 52 DAS at all salinity levels, possibly due to change of nutrient solutions, and transfer of plants to colder water just 15 hours before the measurements of GER were made next morning. Subsequently Pn at 0 and 75 mol m^{-3} NaCl increased and then decreased until 94 DAS and the leaf was completely yellow at 98 DAS. At 150 mol m^{-3} NaCl Pn rapidly decreased over time and the leaf was completely yellow at 60 DAS. Pn was significantly lower at 150 than at 0 mol m^{-3} NaCl on all sampling dates.

Rd_o of the flag leaf was generally faster at 75 and 150 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl although the difference was significant on 5/14 sampling dates. There was no significant effect of salinity on Rd_o from 80 to 94 DAS.

In both leaf 5 and the flag leaf g_s generally decreased over time (Figure 7.5). For leaf 5 g_s was significantly higher in the control than in the salt stressed treatments from 12 to 24 DAS. Thereafter at 0 and 75 mol m^{-3} NaCl the difference in g_s was not significant from 28 to 35 DAS. g_s was significantly higher at 75 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl from 39 DAS onward. g_s was significantly lower at 150 mol m^{-3} NaCl than at 0 and 75 mol m^{-3} NaCl on all sampling dates.



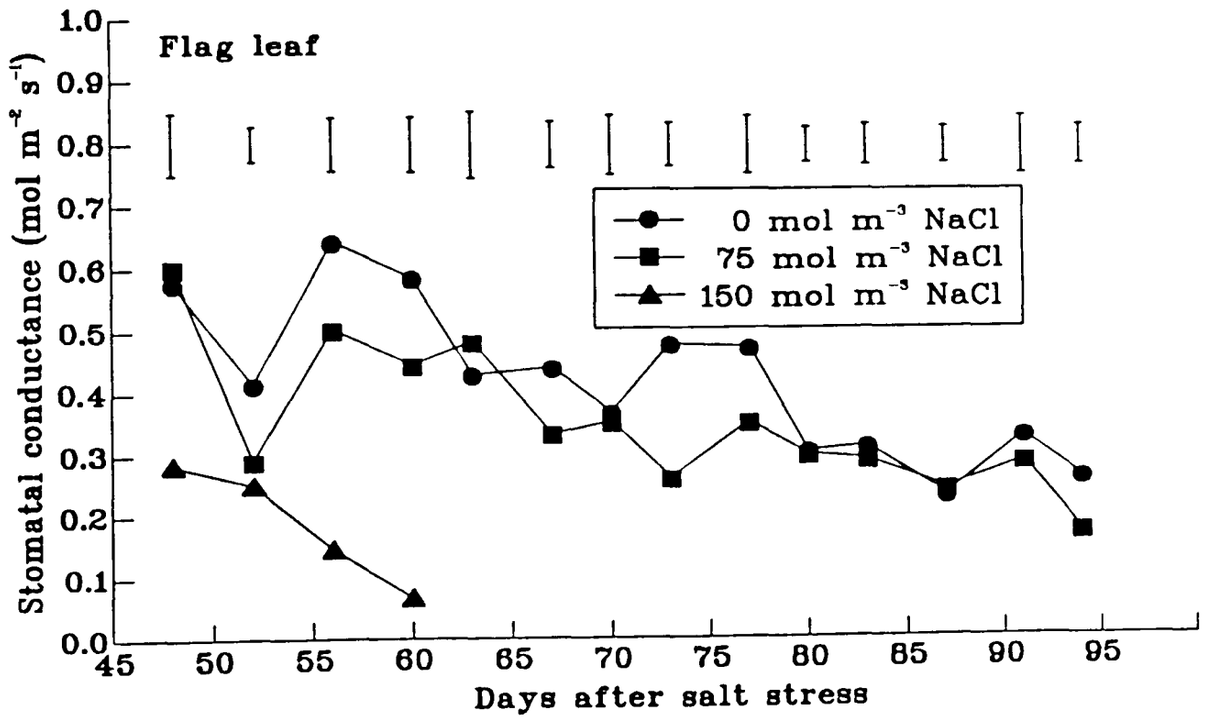


Figure 7.5: Effect of salinity on stomatal conductance of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

In the flag leaf g_s was generally higher at 0 than at 75 mol m^{-3} NaCl but the difference was significant at 52 to 60 and 73 to 77 DAS only. At 0 and 75 mol m^{-3} NaCl g_s decreased following the change of nutrient solutions at 52 DAS. g_s was significantly higher at 0 than at 150 mol m^{-3} NaCl on all the sampling dates. At 150 mol m^{-3} NaCl g_s decreased rapidly over time.

In both leaf 5 and the flag leaf Ci increased over time (Figure 7.6). For leaf 5 Ci was significantly higher at 0 than at 75 and 150 mol m^{-3} NaCl on all sampling dates. Ci was consistently lower at 150 mol m^{-3} NaCl than at 75 mol m^{-3} NaCl but this was not always significant.

For the flag leaf at 0 and 75 mol m^{-3} NaCl Ci remained relatively constant at 186-201 $\mu l\ l^{-1}$ CO_2 until 70 DAS. Thereafter it increased as the leaves senesced but the differences between these treatments were generally not significant. At 150 mol m^{-3} NaCl Ci increased rapidly at the final measurement.

For leaf 5, at 0 and 75 mol m^{-3} NaCl E remained relatively constant, decreasing only at the last measurement when the leaves were near complete senescence (Figure 7.7). This occurred later at 75 than at 0 mol m^{-3} NaCl. E was significantly lower at 150 than at 0 and 75 mol m^{-3} NaCl on all the sampling dates.

For the flag leaf the differences in the E at 0 and 75 mol m^{-3} NaCl were small and not significant. At 150 mol m^{-3} NaCl E rapidly decreased over time. As with Pn and g_s, E decreased at 52 DAS following changes of nutrient solution

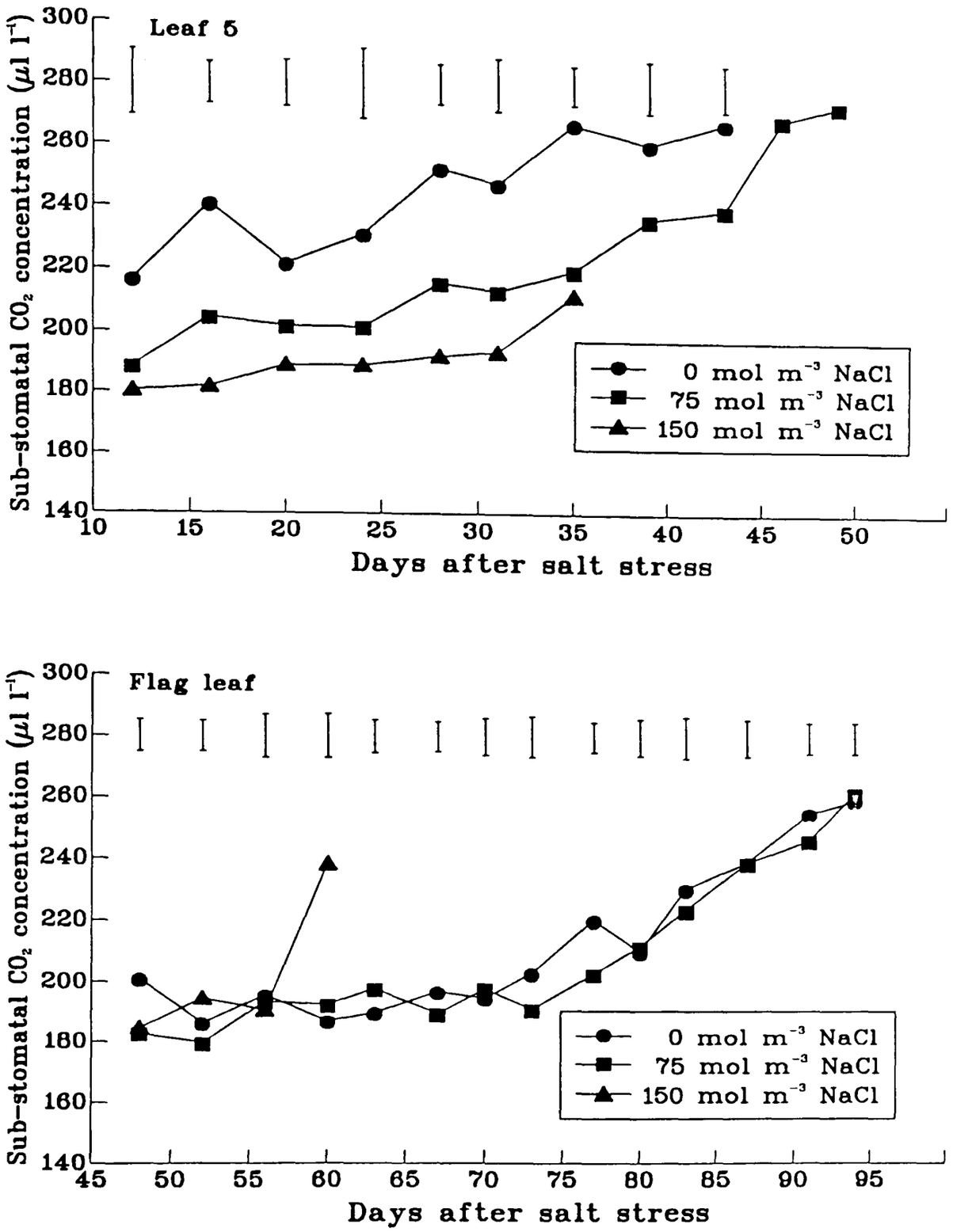


Figure 7.6: Effect of salinity on sub-stomatal CO_2 concentration of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

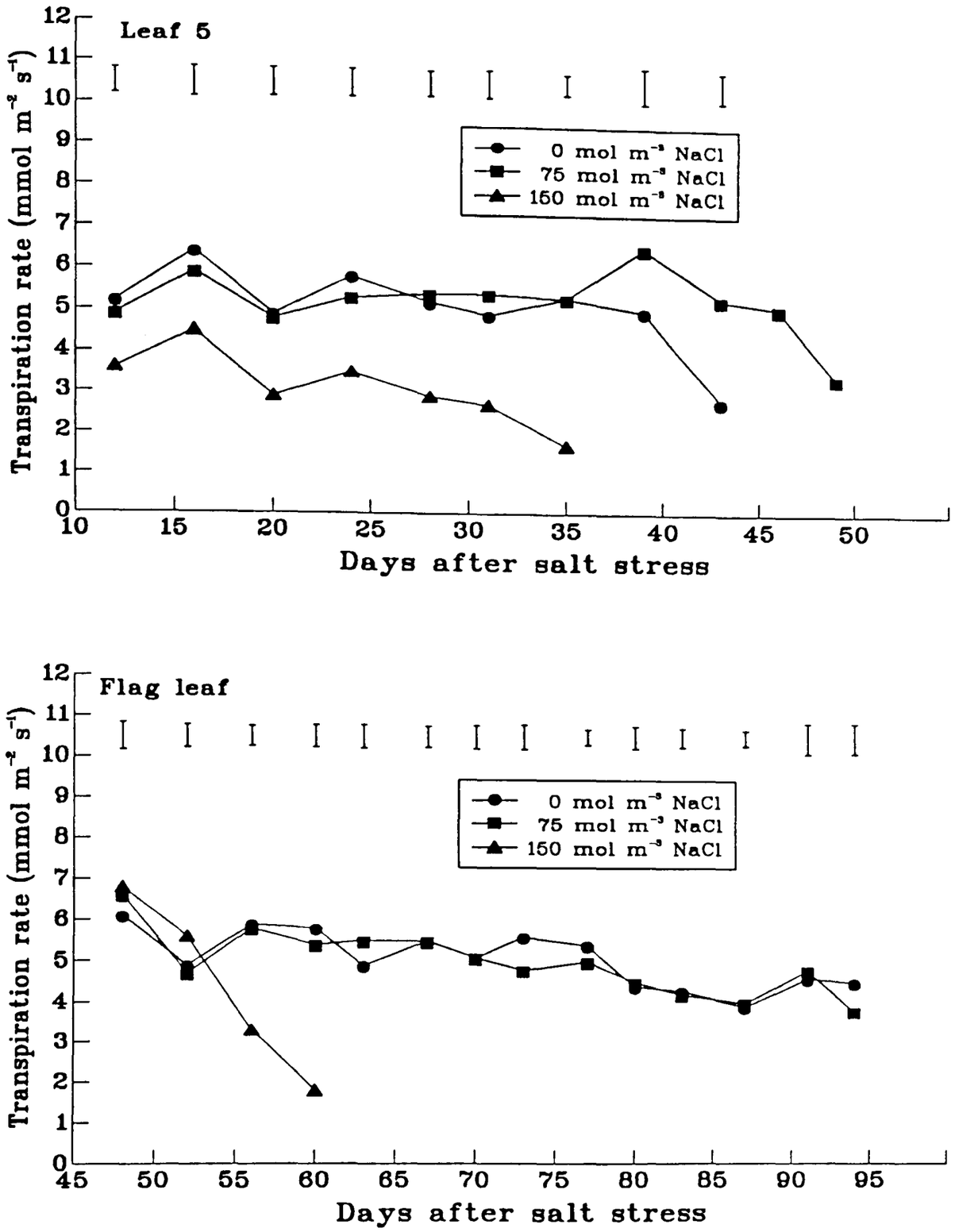


Figure 7.7: Effect of salinity on tranpiration rate of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

15 hours before sampling.

7.4.3 TRANSPIRATION EFFICIENCY AND LEAF PRODUCTIVITY

In both leaves transpiration efficiency (TE) gradually decreased over time (Figure 7.8). For leaf 5 differences in TE between treatments were not consistent. TE was initially higher at 0 than at 75 and 150 mol m^{-3} NaCl. From 28 DAS onward TE was significantly higher at 75 than at 0 mol m^{-3} NaCl. Differences in TE between treatments were much more consistent in the flag leaf. TE was consistently higher at 0 than 75 mol m^{-3} NaCl but the difference was not significant from 73 DAS onwards. TE was lower at 150 mol m^{-3} NaCl than at 0 and 75 mol m^{-3} NaCl on all sampling dates.

The effects of salinity and trends over time of leaf productivity (LP) of leaf 5 and the flag leaf (Figure 7.9) closely followed those of Pn (Figure 7.4). This suggests that LP was influenced more by Pn rather than by GLA in leaf 5 as the latter remained constant for most of this period. LP of the flag leaf was decreased by decreases in both Pn and GLA over time.

7.4.4 EFFECT OF SALINITY ON Pnmax, Rd_c, α AND Ic

Trends in Pnmax and Rd_c of leaf 5 and the flag leaf with salinity treatments and over time (Figure 7.10) were identical to those of Pn and Rd_o (Section 7.4.2). However, it was noted that the calculated values of Pnmax at 0 and 75

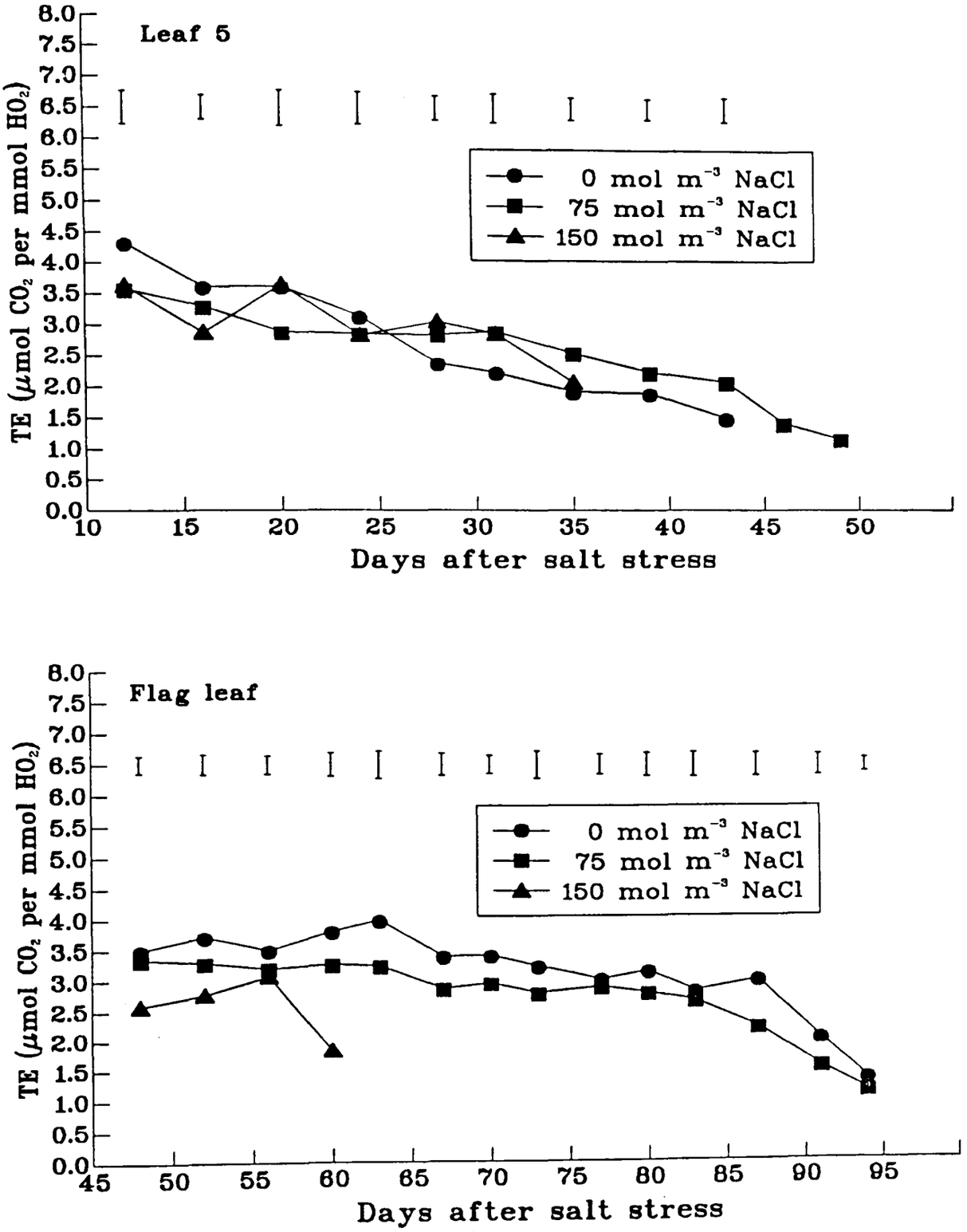


Figure 7.8: Effect of salinity on transpiration efficiency (TE) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

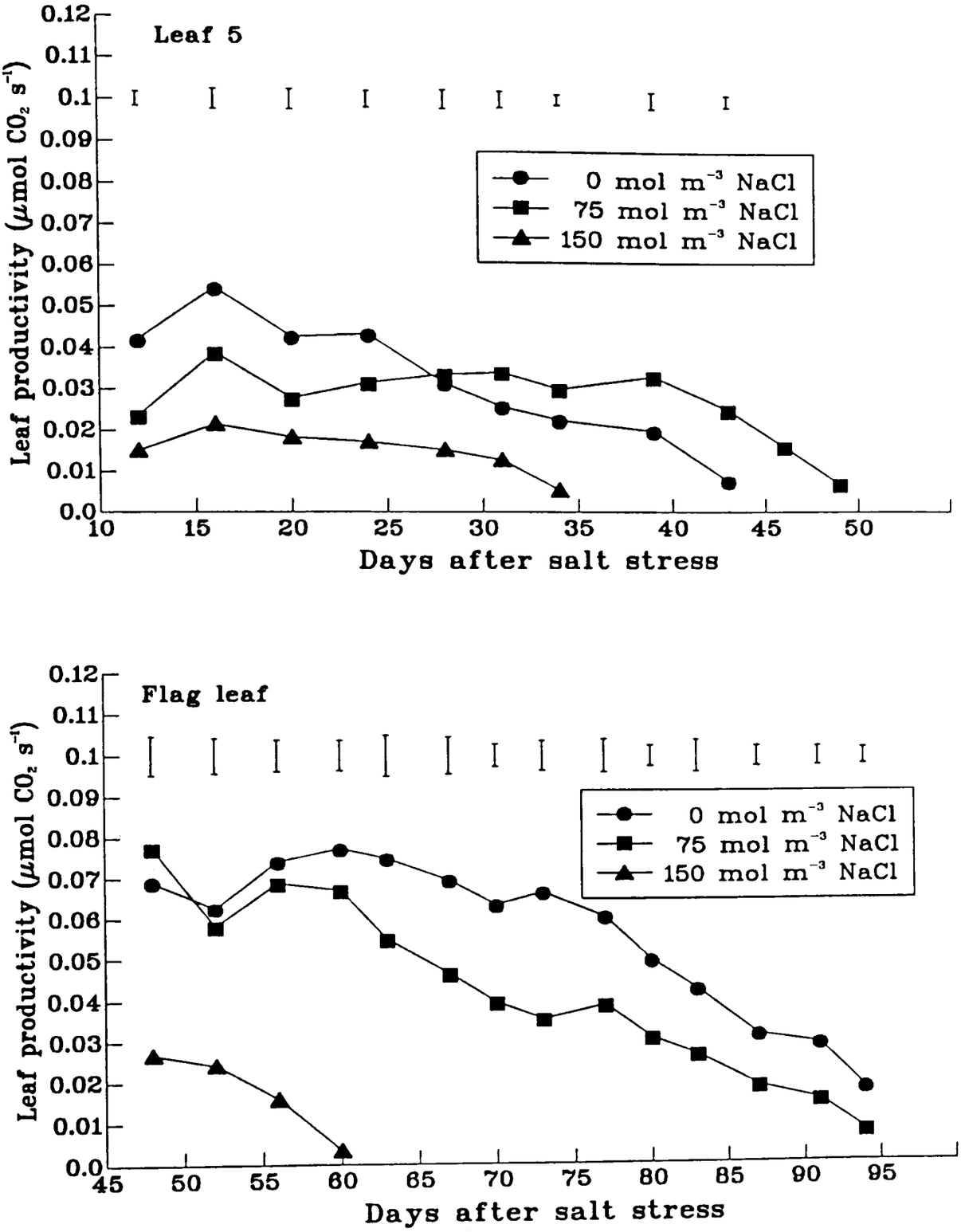
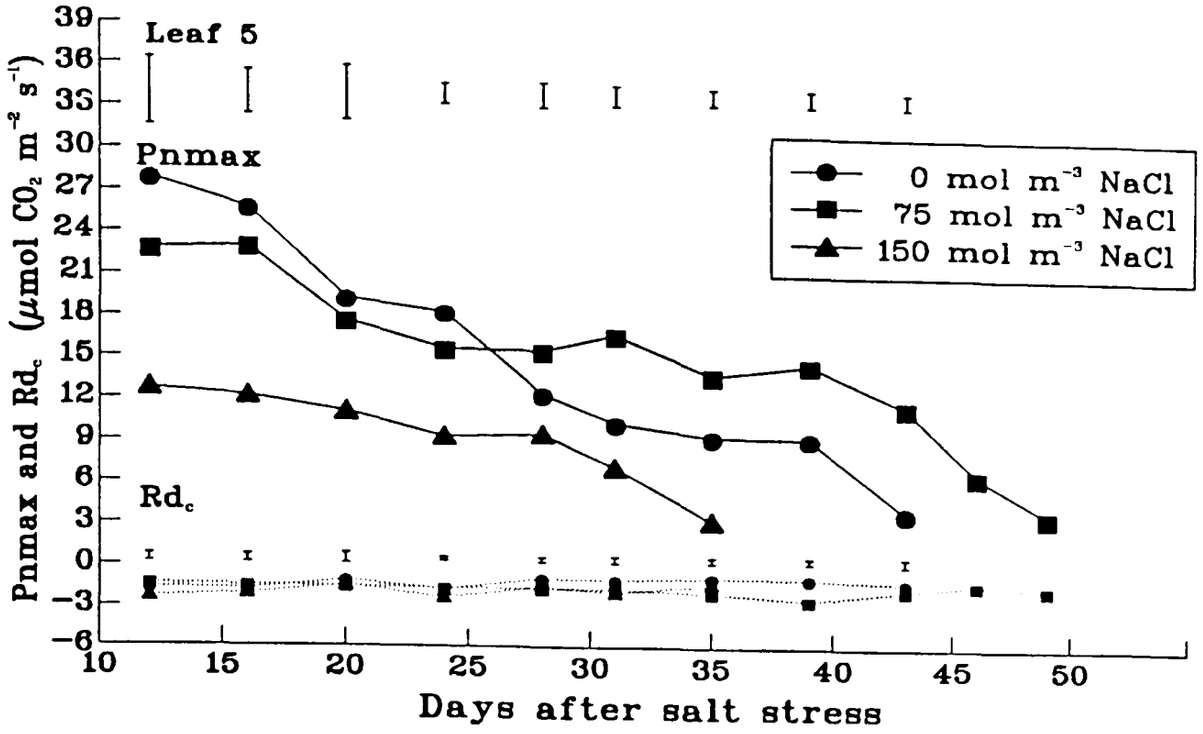


Figure 7.9: Effect of salinity on leaf productivity of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.



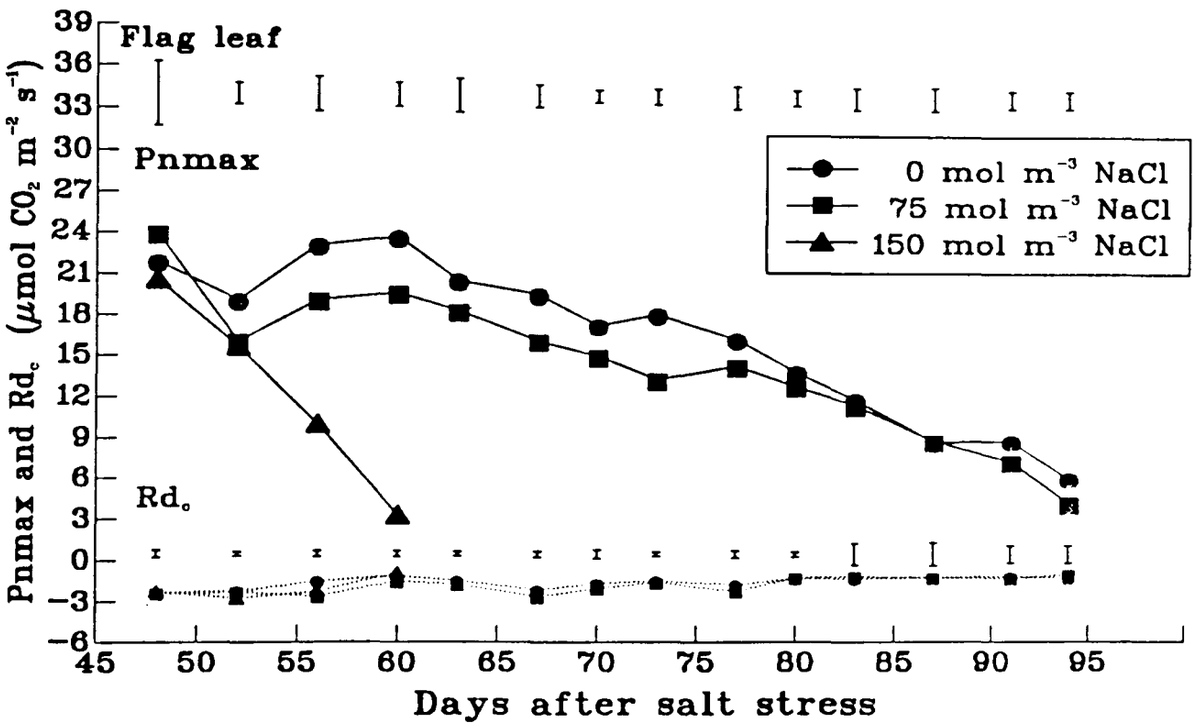


Figure 7.10: Effect of salinity on maximum net photosynthesis (Pn) and calculated dark respiration (Rd_c) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

mol m^{-3} NaCl were slightly higher than the observed values of Pn at 1750 μmol m^{-2} s^{-1} PAR. In the case of 150 mol m^{-3} NaCl the values of Pn and Pnmax were similar.

The values of α (Figure 7.11) were generally less consistent over time than those of Pnmax and Rd_c. In both leaves differences in α between treatments were significant on only a few sampling dates. There were no consistent trends of α with salinity but in all treatments α was decreased at the final measurement.

In both leaves Ic consistently increased with increase in salinity on all sampling dates, although the differences between treatments were not always significant (Figure 7.12). Trends in Ic over time were not consistent. In leaf 5 at 0 mol m^{-3} NaCl and in the flag leaf at 0 and 75 mol m^{-3} NaCl, Ic generally decreased over time. In leaf 5 at 75 mol m^{-3} NaCl Ic remained relatively consistent. In both leaves and at all salinity levels a rapid increase in Ic was observed at the final measurement.

7.4.5 EFFECT OF SALINITY ON SAP Na^+, K^+, Cl^-, AND OP

Sap Na^+ concentration was significantly higher at 75 and 150 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl on all sampling dates in both leaf 5 and the flag leaf (Figure 7.13). For both leaves at 0 mol m^{-3} NaCl sap Na^+ remained extremely low on all sampling dates. For leaf 5 in the salinity treatments, there was a progressive increase in sap Na^+ followed by a rapid increase at the last sampling date. For the flag leaf sap Na^+ at 75 mol m^{-3} NaCl was low at 48 DAS

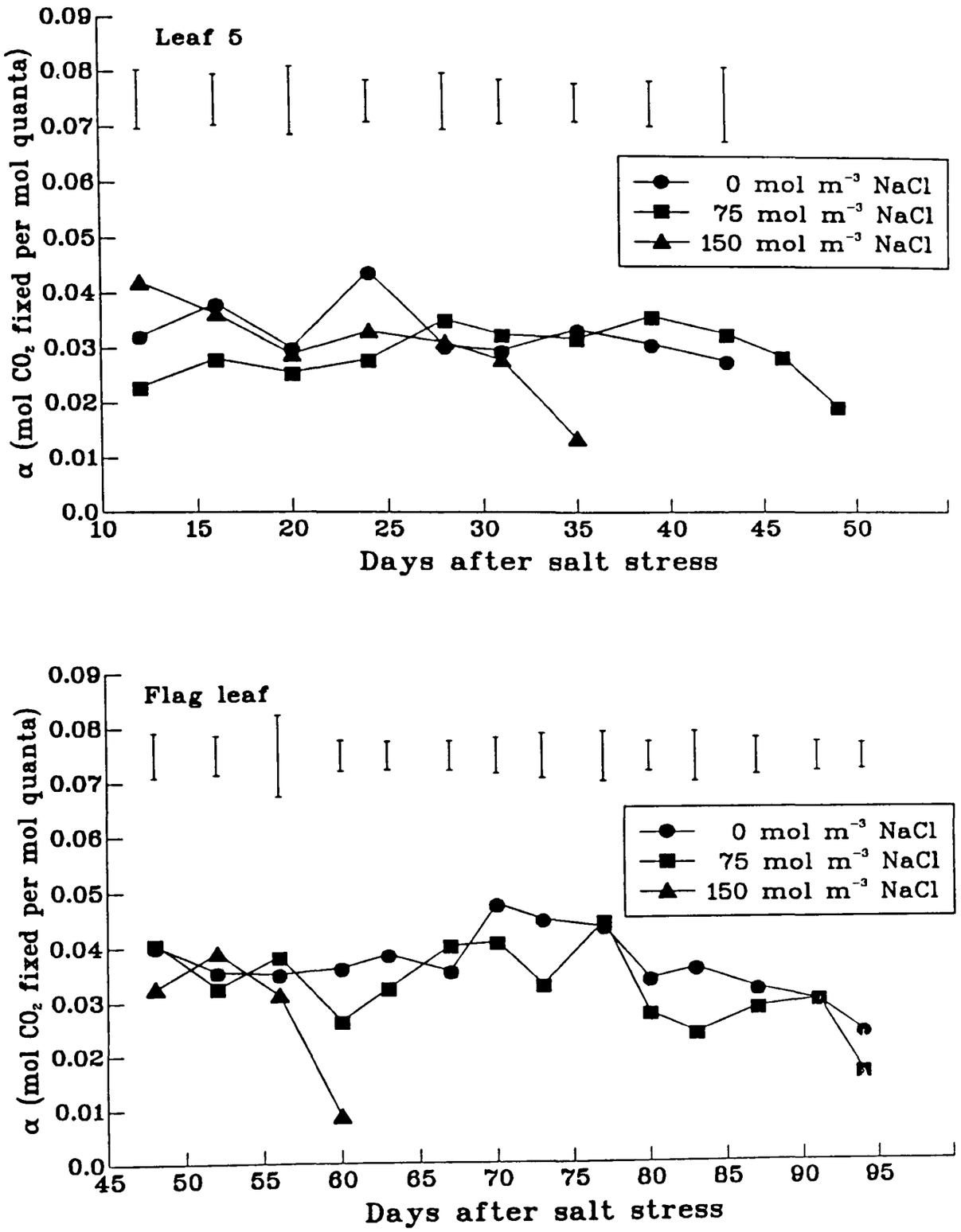
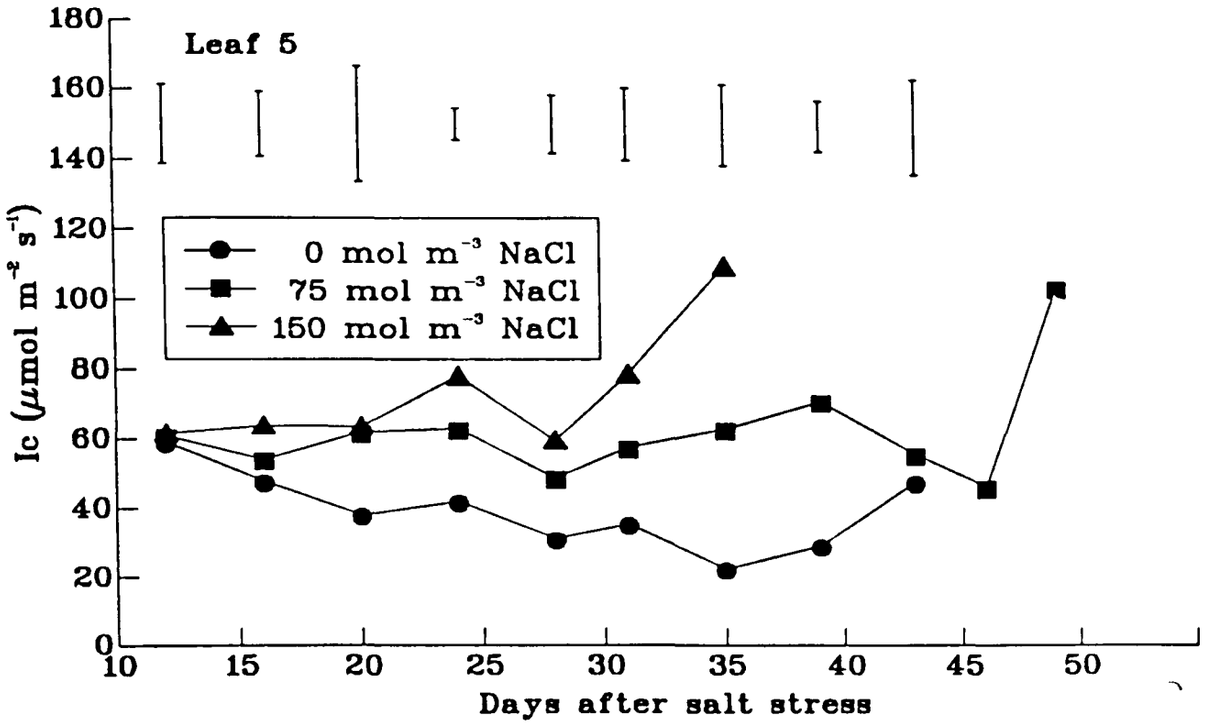


Figure 7.11: Effect of salinity on photosynthetic efficiency (α) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.



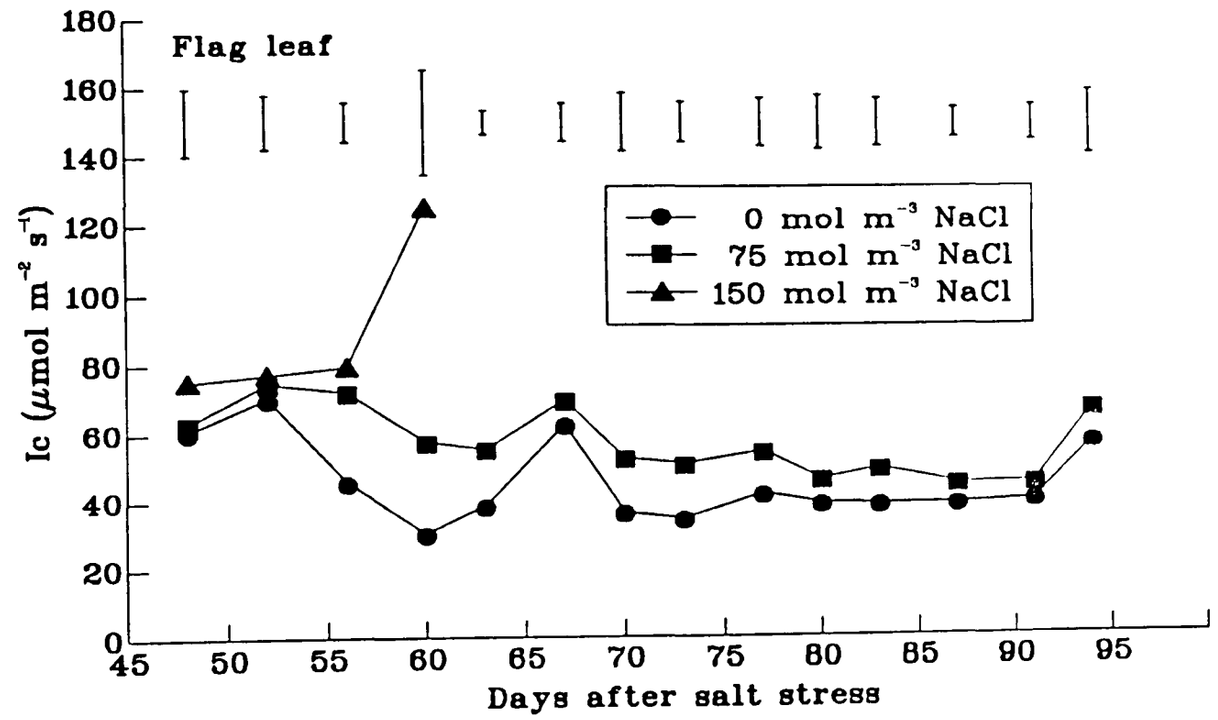


Figure 7.12: Effect of salinity on photon flux compensation point (Ic) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

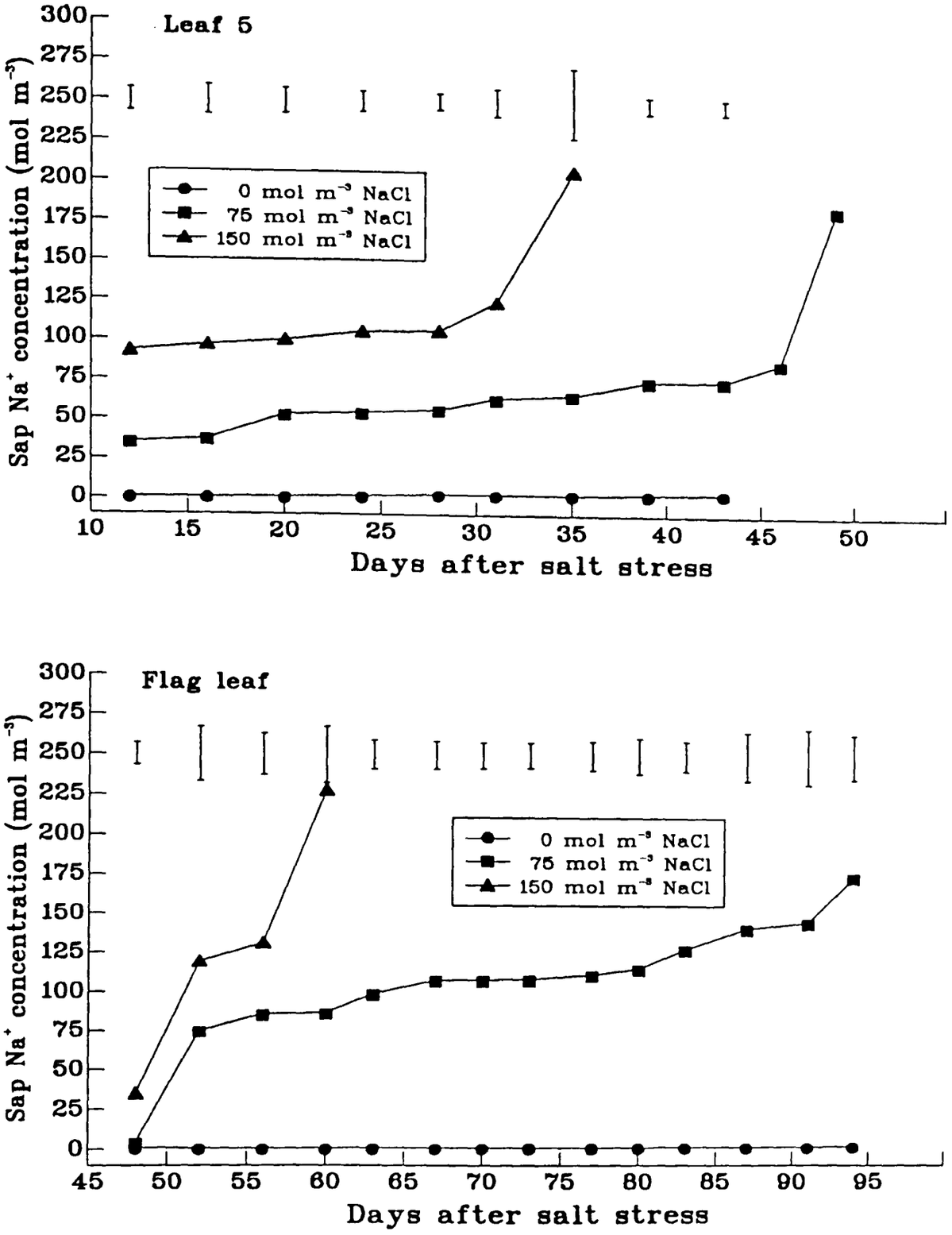


Figure 7.13: Effect of salinity on sap sodium concentration (mol m^{-3}) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

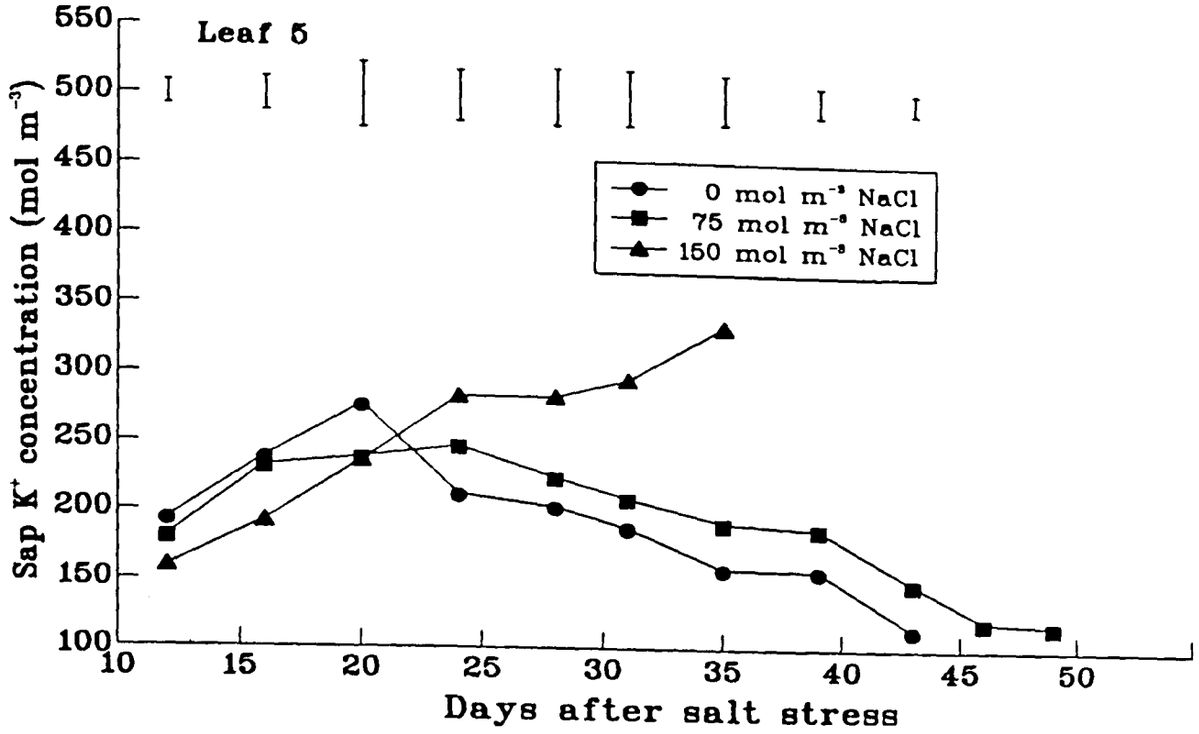
when the leaf was still expanding. It increased rapidly between 48 and 52 DAS. This was followed by a slower increase. At 150 mol m^{-3} NaCl sap Na^+ increased rapidly.

In leaf 5 at 0 and 75 mol m^{-3} NaCl sap K^+ concentration initially increased when the leaf was still expanding, thereafter it decreased (Figure 7.14). From 24 DAS onward sap K^+ was consistently higher at 75 than at 0 mol m^{-3} NaCl.

In the flag leaf at 0 and 75 mol m^{-3} NaCl sap K^+ decreased as the leaf senesced. From 60 DAS sap K^+ was consistently lower at 0 mol m^{-3} NaCl than at 75 mol m^{-3} NaCl, although the difference was not always significant. At 150 mol m^{-3} NaCl sap K^+ consistently increased in both leaves.

Sap Cl^- concentration consistently increased with increase in salinity in both leaf 5 and the flag leaf at all the sampling dates (Figure 7.15). At 0 mol m^{-3} NaCl sap Cl^- remained low throughout the sampling period in both leaves. At 75 mol m^{-3} NaCl sap Cl^- initially increased and then decreased over time. In leaf 5 at 150 mol m^{-3} NaCl there was a gradual increase in sap Cl^- followed by a rapid increase at the last sampling date. In the flag leaf sap Cl^- increased rapidly over all sampling dates. This rapid increase was not evident at 75 mol m^{-3} NaCl.

Trends in sap OP with salinity treatments and over time were similar in both leaves (Figure 7.16). Trends in sap OP closely followed those of sap Cl^-. In leaf 5 and the flag leaf OP was significantly higher at 75 and 150 mol m^{-3} NaCl



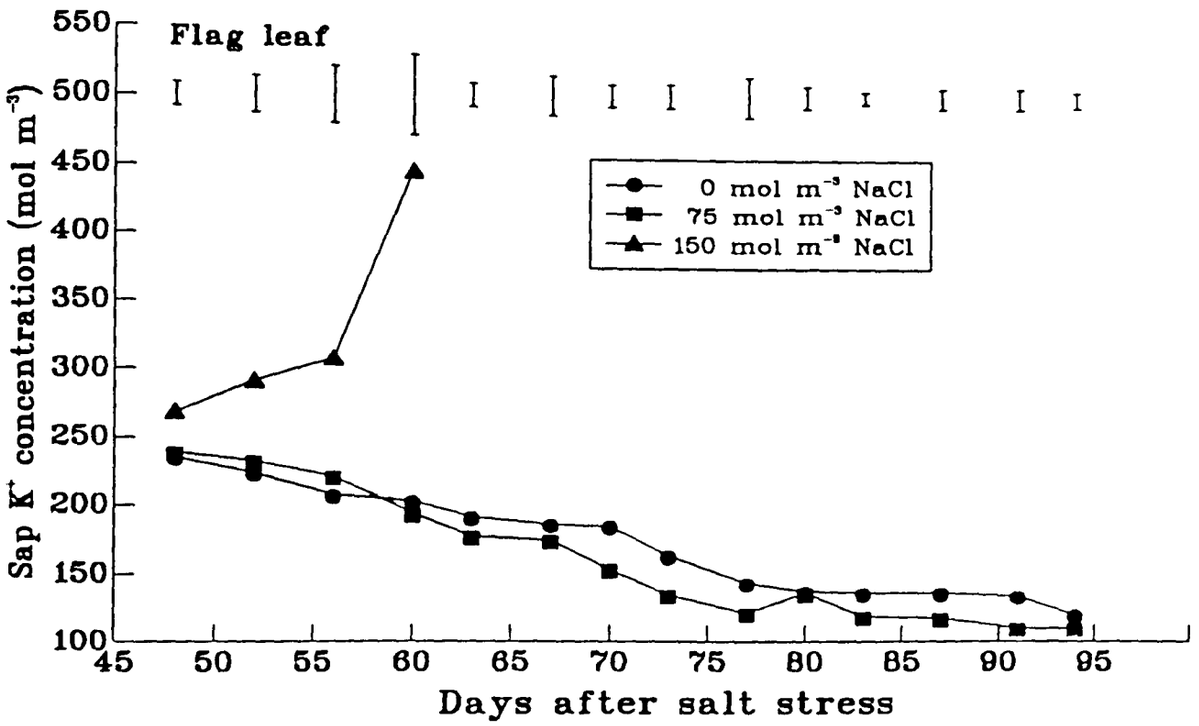


Figure 7.14: Effect of salinity on sap potassium concentration (mol m^{-3}) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

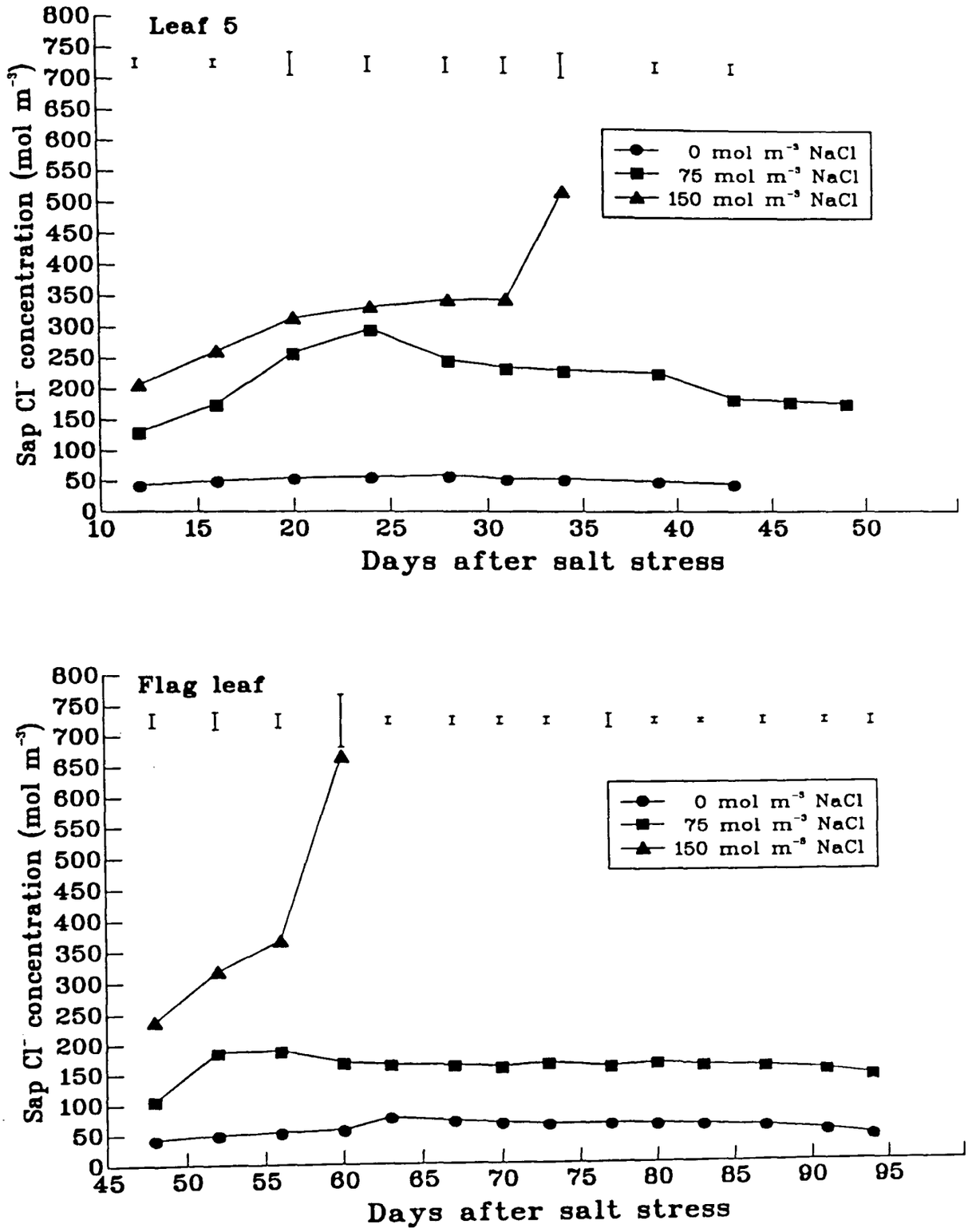


Figure 7.15: Effect of salinity on sap chloride concentration (mol m^{-3}) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

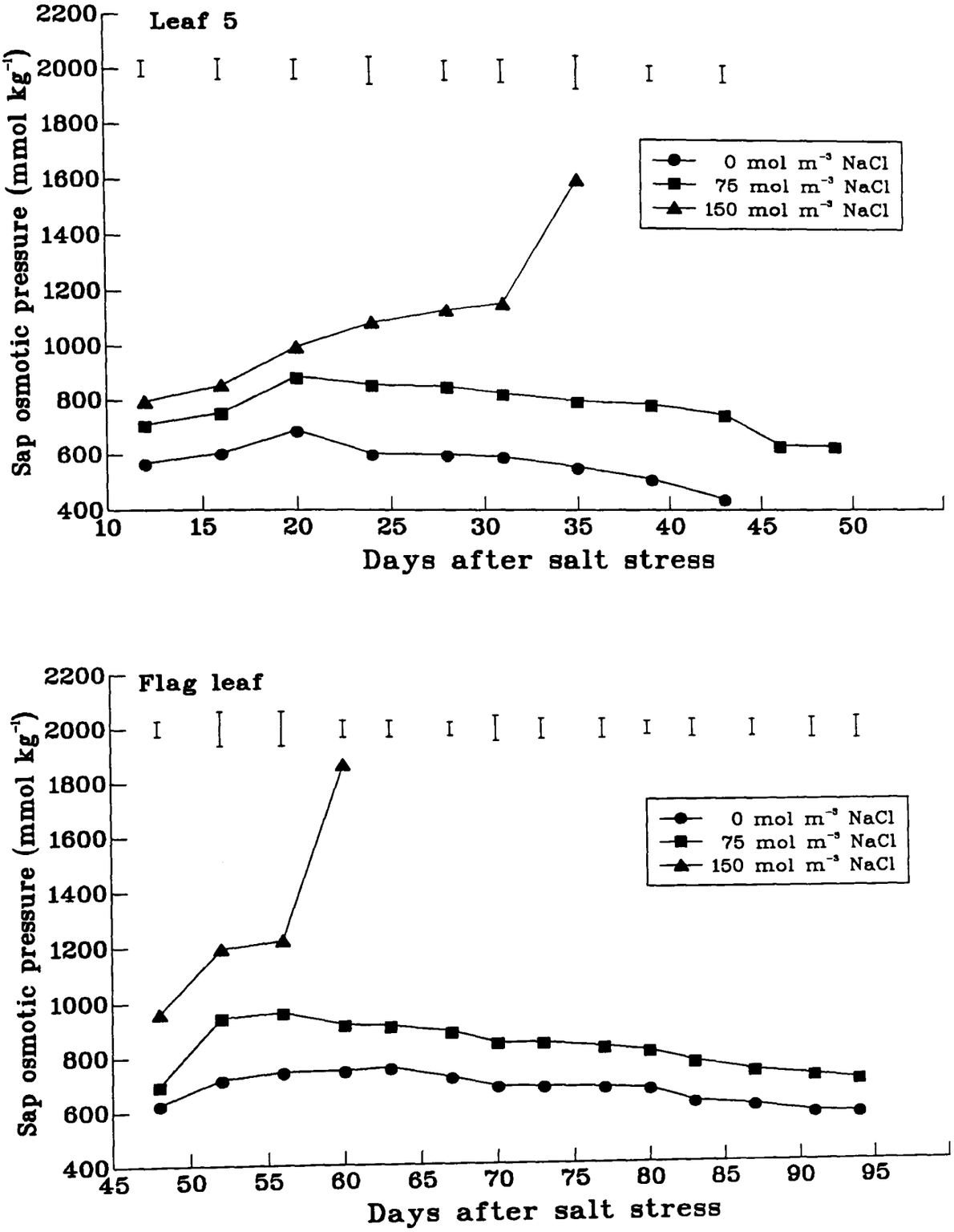


Figure 7.16: Effect of salinity on sap osmotic pressure (mmol kg^{-1}) of leaf 5 and the flag leaf of spring wheat. I= standard error of difference between means.

than at 0 mol m^{-3} NaCl on all sampling dates. At 0 and 75 mol m^{-3} NaCl OP increased when the leaf was still expanding and thereafter it decreased. At 150 mol m^{-3} NaCl sap OP increased throughout the sampling period, particularly at the last measurement.

7.4.6 EFFECT OF SALINITY ON LFWT, LDWT, A, SLW AND SF OF FULLY EXPANDED LEAVES

Salinity significantly decreased the LFWT, LDWT, LFWT:LDWT ratio, and A of leaf 5 and the flag leaf (Table 7.1). The differences in these parameters between 0 and 75 mol m^{-3} NaCl were not always significant. The decreases in A and LDWT were greater in the flag leaf whereas the decrease in LFWT:LDWT ratio was greater in leaf 5. SLW was not significantly affected in both the leaves. SF increased with salinity in both the leaves. However SF_1 decreased with salinity, particularly in the flag leaf.

7.4.7 GREEN AREA AND DRY WEIGHT PER PLANT AT ANTHESIS STAGE

At 150 mol m^{-3} NaCl plants died before anthesis and therefore no data for this salinity level are available. Green area and dry weight per plant of stems, leaves, spike and total shoot was significantly greater at 0 mol m^{-3} NaCl than at 75 mol m^{-3} NaCl (Table 7.2). At anthesis stage the leaves accounted for most of the green area and the stems for most of the dry weight per plant.

Table 7.1: Effect of salinity on lamina fresh weight (LFWT in mg), lamina dry weight (LDWT in mg), lamina fresh weight:dry weight ratio (LFWT:LDWT), lamina area (A in cm^2), specific leaf weight (SLW in mg cm^{-2}), stomatal frequency (SF in stomata mm^{-2}) and stomatal frequency per leaf (SF_1 $x10^4$) of fully expanded leaf 5 and the flag leaf of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)			SEDM	LSD	CV(%)
	0	75	150			
Leaf 5						
LFWT	503.0	436.0	287.0	32.0	67.0**	15.9
LDWT	78.0	76.3	53.6	5.7	12.0**	16.5
LFWT:LDWT	6.5	5.7	5.4	0.1	0.3**	4.9
A	23.8	21.9	16.4	1.5	3.2**	14.7
SLW	3.3	3.5	3.3	0.1	NS	6.5
SF	40.1	46.1	52.1	1.9	4.1**	8.5
SF_1	9.5	9.9	8.5	0.7	NS	14.3
Flag leaf						
LFWT	832.0	655.0	311.0	50.0	104.0**	16.9
LDWT	185.0	148.6	764.0	10.6	22.0**	15.5
LFWT:LDWT	4.5	4.4	4.1	0.1	0.2**	3.6
A	44.8	35.7	17.0	2.8	5.7**	16.9
SLW	4.2	4.2	4.5	0.2	NS	7.4
SF	64.0	68.3	81.0	3.7	7.7**	10.3
SF_1	28.7	24.4	13.9	2.1	4.3**	18.5

SEDM= Standard error of difference between means,
LSD = Least significance differences,
CV = Coefficient of variation,
% = Percent,
** = Significant at 1 percent probability level.

7.5 DISCUSSION AND CONCLUSIONS

In Experiment 3 at 200 mol m^{-3} NaCl plants died at 27 DAS or 14 days after emergence (DAE) of leaf 6 from the sheath, as a result of which only 3 measurements for GER and related parameters were obtained (Table 7.3). In Experiment 4 at 150 mol m^{-3} NaCl leaf 5 remained green and photosynthetically active up to 31 DAE (35 DAS) and the flag leaf up to 17 DAE (60 DAS) so that there were 7 measurements for leaf 5 and 4 for the flag leaf. Hence in Experiment 4, by using lower salinity levels, it was possible to obtain more measurements of GER and related parameters as senescence was delayed. In Experiment 4 the plants experienced a wider range of temperature variation than in Experiment 3. In the growth room day/night temperatures were set at 24/16°C whereas in the glasshouse temperatures varied between 10 and 35°C. It would be expected that the warmer conditions in the glasshouse would result in greater evaporation, greater salt uptake and hence earlier leaf death in experiment 4. However due to lower salinity levels tested, the leaves stayed green for longer in Experiment 4 than in Experiment 3.

In Experiment 3 the effect of salinity was to decrease GLA, Pn, Pnmax, g_s, Ci, E, TE and Ic but to increase Rd_o, Rd_c, and T_l whereas α was inconsistently affected. The same trend was noted in the present experiment for leaf 5 and the flag leaf at 0 and 150 mol m^{-3} NaCl. An interesting feature

Table 7.2: Effect of salinity on green area (cm^2) and dry weight (g) of various plant parts of spring wheat and percent (%) decrease over control (0 mol m^{-3} NaCl) of these parameters at anthesis stage.

Parameters	Salinity levels (mol m^{-3} NaCl)		decrease (%)	SEDM	LSD
	0	75			
Green area					
Stem	148.00	107.80	27.16	9.39	20.14**
Leaves	509.00	406.00	20.24	28.56	61.27**
Spikes	55.90	39.70	28.98	4.69	10.07**
Total Shoot	704.00	554.00	21.31	37.75	80.98**
Dry weight					
Stem	7.83	4.11	47.51	0.74	1.58**
Leaves	1.88	1.35	28.44	0.12	0.24**
Spikes	2.19	1.53	30.14	0.22	0.47**
Total shoot	11.93	6.99	41.41	1.02	2.19**

SEDM= Standard error of difference between means,
LSD = Least significance differences,
** = Significant at 1 percent probability level.

Table 7.3: Effect of salinity on days taken for complete leaf senescence of different leaf insertions of spring wheat.

Experiment/ Leaf insertion	Salinity levels (mol m^{-3} NaCl)	Complete leaf senescence		Number of measurements
		Days after salt stress	Days after leaf emergence	
Experiment 3				
Leaf 6				
	0	48	35	9
	100	34	21	5
	200	27	14	3
Experiment 4				
Leaf 5				
	0	43	39	9
	75	49	45	11
	150	35	31	7
Flag leaf				
	0	94	51	14
	75	94	51	14
	150	60	17	4

noted in this experiment was that at 75 mol m^{-3} NaCl in leaf 5 senescence was delayed, so that from 27 DAS Pn, g_s, E were higher at 75 than at 0 mol m^{-3} NaCl. This trend was not noted in leaf 6 in experiment 3 at 100 mol m^{-3} NaCl. However, Rawson *et al.* (1988a) also observed a similar trend in barley for CO_2 exchange rate in leaves 4 and 7 at 100 mol m^{-3} NaCl. They attributed this trend to be due to self shading of control plants as four leaves were produced above leaf 7 whereas salinised plants produced only two small leaves. In the experiment herein the same number of leaves were recorded at both 0 and 75 mol m^{-3} NaCl and GER was measured under the same light intensity in all leaves. Therefore this seemed to be unlikely as the cause of reduction in GER parameters at 0 mol m^{-3} NaCl. Ward and co-workers (1986) recently reported that salinity inhibited NO_3^- uptake in barley but uptake of NO_3^- was improved with higher external Ca^{2+} concentration and was correlated with increased leaf growth. Cramer *et al.* (1989) also observed that salinity inhibited growth of barley at 150 mol m^{-3} NaCl but not at 75 mol m^{-3} NaCl in the presence of additional Ca^{2+}. Similar results were also observed in the present study. Roots were a bright whitish colour whereas leaves were slightly smaller but a darker green at 75 mol m^{-3} than at 0 mol m^{-3} NaCl. Thus the additional Ca^{2+} provided to the salt stressed treatments may be promoting root and shoot growth by improving NO_3^- uptake at low salinity (75 mol m^{-3} NaCl), and also delaying senescence.

In both Experiments 3 and 4 aeration was improved through the use of narrow capillary tubing and needles. It is possible that the delayed senescence of plants at 75 mol m^{-3} NaCl is due to the fact that the salt stressed treatment received additional Ca^{2+}. In Experiment 4 plants at 0 and 75 mol m^{-3} NaCl produced extensive growth of roots. Calcium transport and tissue concentrations are markedly inhibited by salinity (Ward *et al.* 1986; Cramer *et al.* 1989). However, Cramer *et al.* (1989) observed that supplemental Ca^{2+} increased Ca^{2+} transport and accumulation under saline conditions.

In Experiment 3 leaf 6 was studied to examine the trends in GER and related parameters during leaf expansion and senescence. The present experiments were performed to examine the effects of NaCl on leaf 5 (an earlier leaf) and the flag leaf (the last leaf in wheat which is important for production of assimilate for grain filling). Therefore it is possible to examine ontogenetic trends as influenced by salinity during the course of this study. However, these trends are confounded by the fact that the flag leaf emerged, expanded and senesced under warmer summer conditions than leaf 5.

There were no consistent trends in the effects on salinity on GER between leaf positions. On average over all sampling dates (Table 7.4), GLA was decreased by 33% in leaf 5 and 59% in the flag leaf. In both leaves Pn was decreased by 42%. However g_s and E were both decreased

Table 7.4: Effect of salinity on percent increases (+) or decreases over control (0 mol m^{-3} NaCl) of net photosynthesis (Pn), observed dark respiration (Rd_o), stomatal conductance (g_s) and transpiration rate (E) of leaf 5 and the flag leaf of spring wheat at different times after the start of salt stress (DAS) and leaf emergence (DAE).

DAS	DAE	Gas exchange parameters							
		Pn		Rd_o		g_s		E	
		Salinity levels (mol m^{-3} NaCl							
		75	150	75	150	75	150	75	150
Leaf 5									
12	8	21.9	41.6	18.0	+10.4	31.1	58.1	6.2	31.3
16	12	15.6	44.4	3.8	+33.1	34.7	59.9	8.1	30.5
20	16	21.5	39.7	+73.1	43.6	+48.4	67.1	1.4	39.9
24	20	14.8	44.9	+21.9	+66.6	32.6	63.3	8.9	39.6
28	24	+25.0	29.5	+75.1	+91.9	4.8	70.4	+4.6	44.9
31	27	+48.6	28.1	+62.4	+108.6	+8.8	66.6	+11.1	44.2
34	30	+32.5	64.9	+110.3	61.6	15.7	83.3	1.3	67.6
39	35	+57.2	-	+202.6	-	+46.8	-	+29.8	-
43	39	+175.7	-	+68.0	-	+121.9	-	+89.7	-
Average		+29.5	41.9	+65.7	+60.1	+1.7	66.9	+12.1	19.9
Flag leaf									
48	5	+4.6	16.5	4.2	+2.8	+4.2	51.0	+8.4	+13.1
52	9	14.6	15.1	+4.6	+27.6	29.9	39.8	3.3	+14.6
56	13	11.6	50.3	+104.8	+90.0	21.7	77.4	3.6	44.2
60	17	18.9	85.0	+64.1	+22.4	23.9	88.7	6.2	68.8
63	20	9.8	-	+24.9	-	+12.3	-	+9.9	-
67	24	16.8	-	+16.8	-	24.3	-	1.1	-
70	27	13.0	-	+30.5	-	3.9	-	0.6	-
73	30	26.4	-	+13.5	-	45.6	-	15.1	-
77	34	10.8	-	+46.2	-	25.6	-	6.7	-
80	37	9.1	-	+2.7	-	2.1	-	+3.4	-
83	40	7.1	-	13.6	-	6.9	-	2.8	-
87	44	1.5	-	0.6	-	+4.8	-	2.3	-
91	48	17.4	-	13.9	-	12.9	-	+4.5	-
94	51	28.8	-	20.5	-	33.8	-	15.6	-
Average		12.9	41.7	+18.2	+35.7	14.9	64.2	2.2	21.3

- = Leaves completely senesced.

more in leaf 5 (76% and 43% respectively) than in the flag leaf (64% and 21% respectively). Rd_o was also increased more in leaf 5 than in flag leaf. Rawson *et al.* (1988a) anticipated greater ion concentrations in later emerging leaves but found no consistent trends in the effects of salinity on Pn in different leaf positions.

In both of experiments 3 and 4 g_s was markedly decreased by salinity. If g_s was decreased by salinity then Pn and E must also have decreased, although non-stomatal inhibition of photosynthesis following salt treatment has been reported by many workers (e.g. Walker *et al.* 1983; Ball and Farquhar 1984a,b; Seemann and Critchley 1985; Seemann and Sharkey 1986). Terry and Waldron (1984) also reported that salinity significantly impaired stomatal conductance, causing a reduction in transpiration rate and an elevation of leaf temperature. The results of both Experiments 3 and 4 are in line with these findings. Pn is also adversely affected by salinity because of stomatal closure and the subsequent reduction in intercellular CO_2 concentration (Downton *et al.* 1985; Seemann and Critchley 1985). Ci in the flag leaf was unaffected by salinity, suggesting a parallel decrease in Pn and g_s as observed by other workers (Terry and Waldron 1984; Downton *et al.* 1985; Seemann and Critchley 1985; Yeo *et al.* 1985). However, it is not clear why this should occur in the flag leaf and not in leaf 5. The results herein and those of Rawson *et al.* (1988a) indicate that Ci increased with leaf age after ligule emergence. This rise of Ci with leaf age presumably reflects poor stomatal

control.

In Experiment 3 maximum GLA of leaf 6 which was achieved at 24, 14, and 10 DAE at 0, 100 and 200 mol m^{-3} NaCl respectively was 28, 16 and 11 cm^2. In Experiment 4 the maximum GLA of leaf 5 which was achieved at 20, 17 and 13 DAE at 0, 75 and 150 mol m^{-3} NaCl respectively was 27, 22 and 18 cm^2. It is interesting to note that at high salinity leaves were expanding slightly earlier than at low salinity and in the control in both of these experiments. The leaves of salt stressed plants were larger in Experiment 4 than in Experiment 3. The observed photosynthetic rates were also higher in Experiment 4 than in Experiment 3. The peak rates of observed Pn for leaf 6 in Experiment 3 were 18, 15 and 11 μmol m^{-2} s^{-1} respectively. In Experiment 4 the peak rates of Pn for leaf 5 were 23, 19 and 13 μmol m^{-2} s^{-1} and for the flag leaf of 22, 22 and 18 μmol m^{-2} s^{-1} at 0, 75 and 150 mol m^{-3} NaCl respectively. In contrast to Kemal-ur-Rahim (1988) and Rawson *et al.* (1988a) the peak rates of Pn were observed just before the leaves had attained their maximum area rather than at full expansion. In Experiments 3 and 4 GLA and Pn were both decreased by salinity, hence LP was also markedly decreased by salinity. GLA and Pn were both higher in Experiment 4 than in Experiment 3 and therefore LP was also higher in Experiment 4 particularly in the flag leaf. It is interesting to note that in both experiments 3 and 4 LP was decreased by decreases in both Pn and GLA.

Salinity markedly decreased LFWT, LDWT, LFWT:LDWT ratio, A, and SF_1 in both Experiments 3 and 4. However SF_1 of leaf 5 was not significantly affected by salinity in Experiment 4. SF was significantly increased by salinity in both of these experiments. SLW was not consistently affected by salinity as also observed by Kemal-ur-Rahim (1988). All of these parameters were more markedly affected in leaf 6 in Experiment 3 than in leaf 5 and the flag leaf in Experiment 4. In Experiment 3 the highest salinity level tested (200 mol m^{-3} NaCl) decreased LFWT by 83%, LDWT and A by 71% and SF_1 by 52% whereas SF was increased by 71% (Table 6.3). In Experiment 4 SF_1 was decreased by only 10% in leaf 5 and by 52% in the flag leaf at the high salinity (Table 7.5). The highest salinity level tested also decreased LFWT by 43 and 63%, LDWT by 31 and 59%, A by 31 and 62% whereas SF was increased by 30 and 27% respectively in both leaf 5 and the flag leaf. This suggests that salinity had larger effects on leaf growth than on cell differentiation in this experiment, possibly because ion concentrations are lower in dividing tissues (Munns *et al.* 1988). In the Experiment 3 all of these parameters were equally affected by salinity, suggesting that it affected both leaf growth and the cell differentiation. In both of these experiments A and SF_1 were markedly decreased by salinity whereas SF was increased. This suggests that decrease in SF_1 was mainly due to reduction in A. It is noteworthy that as most of GER measurements are based on per unit leaf area, increase in SF could results in

Table 7.5: Effect of salinity on percent increases (+) or decreases of lamina fresh weight (LFWT), lamina dry weight (LDWT), lamina fresh weight:dry weight ratio (LFWT:LDWT), lamina area (A), specific leaf weight (SLW), stomatal frequency per unit area (SF) and stomatal frequency per leaf (SF_1) of fully expanded leaf 5 and the flag leaf of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)	
	75	150
Leaf 5		
Lamina fresh weight	13.3	42.9
Lamina dry weight	2.2	31.3
Lamina fresh weight: dry weight ratio	11.5	15.9
Lamina area	7.7	31.2
Specific leaf weight	+7.6	0.0
Stomatal frequency per unit area (mm^2)	+14.8	+29.9
Stomatal frequency per leaf	+4.5	10.2
Flag leaf		
Lamina fresh weight	21.3	62.6
Lamina dry weight	19.7	58.7
Lamina fresh weight:dry weight ratio	1.7	9.1
Lamina area	20.3	62.1
Specific leaf weight	0.0	+6.9
Stomatal frequency per unit area (mm^2)	+6.7	+26.6
Stomatal frequency per leaf	15.1	51.7

over-estimates of Pn, g_s, and E per cell under saline conditions. In contrast Morgan and LeCain (1991) reported that despite a significant negative correlation between leaf area and stomatal density, leaf conductance was not correlated with stomatal density.

Ion uptake by a leaf involves long distance transport of both water and ions. In young dividing and expanding tissues there is a great demand for solutes for synthesis of essential metabolites and to maintain turgor (Wolf *et al.* 1991). Therefore large quantities of salt are carried in the transpiration stream to the leaves to meet this demand for solutes, which leads eventually to their sequential death (Yeo *et al.* 1991). It is noteworthy that salinity reduces transpiration rate of leaves as observed herein and elsewhere (Yeo *et al.* 1985; Rawson 1986; Singh *et al.* 1990) but even this reduced transpiration rate could not prevent build up of ions which may be toxic to leaves and therefore promote senescence. In Experiment 3 high salinity (200 mol m^{-3} NaCl) resulted in rapid senescence of leaf 6 within 14 DAE. Sap OP, Na^+, K^+ and Cl^- concentration increased rapidly as the leaf senesced. Sap Na^+ concentration reached almost 200 mol m^{-3} in this leaf. In Experiment 4 sap Na^+ concentration of leaf 5 slowly increased to reach 90 and 110 mol m^{-3} at 75 and 150 mol m^{-3} NaCl at 46 and 28 DAS respectively. This trend was also observed in the flag leaf at 75 mol m^{-3} NaCl where sap Na^+ also slowly increased to reach 110 mol m^{-3} at 73 DAS. Transpiration rates in the

young fully expanded leaf were greater in Experiment 3 (leaf 6) than in Experiment 4 (leaf 5), although this should be treated with caution as E was measured in the light chamber and not *in situ*. This higher evaporation might be expected to be associated with higher salt uptake. Final Na^+ concentrations reached were quite similar in the flag leaf and leaf 5. However, rate of Na^+ uptake at 150 was higher in the flag leaf than leaf 5 but presumably the warmer weather conditions at the time of emergence of the flag leaf were also a contributing factor. These results agree with those of Rawson (1986) who observed that during the first few days after salt stress sap Na^+ and Cl^- remain fairly low and then increased with time. Rawson (1986) also observed an increase in sap Cl^- but not in sap Na^+ which remained at same concentration for more than 2 weeks. In the experiments here similar trends were also observed. Sap Cl^- closely followed the trend of sap K^+ in both Experiments 3 and 4. In leaf 5 and the flag leaf there was an initial increase of sap K^+ and Cl^- at 75 mol m^{-3} NaCl. Thereafter both ions progressively decreased with time as the leaves senesced. This suggests that a major contributing factor to the stability of leaf ion content is an increase in the rate of export particularly of K^+ and Cl^- as the leaf ages (Rawson *et al.* 1988a,b). However in the experiments here there was no evidence of Na^+ export, possibly because rates of export from leaves are much greater for K^+ than for Na^+ (see Greenway and Munns 1980). However similar results can occur for Na^+ in those species which show large net Na^+ transport

into leaves e.g. barley (Greenway *et al.* 1965). It is interesting to note the trend of sap K^+ in leaf 5 after expansion at 20 DAS (16 DAE). Sap K^+ at this time was higher at 0 than at 75 and 150 mol m^{-3} NaCl. This may be because K^+ concentrations are mostly higher in expanding and newly expanded leaves during short-term salinity stress (Rashid 1986; Gorham *et al.* 1986b). These results are in agreement with experiments 1 and 2 and other workers (Greenway and Munns 1980; Rashid 1986; Kemal-ur-Rahim 1988) who found decreases in sap K^+ with increase in sap Na^+ in expanding and fully expanded leaves. Sap K^+ generally increased rapidly at 150 mol m^{-3} NaCl in both leaf 5 and the flag leaf. This trend was also observed in leaf 6 at 100 and 200 mol m^{-3} NaCl in Experiment 3. These results are in agreement with Greenway and Munns (1980) who reported that salt-sensitive species which include Na^+ from their shoot usually increase in K^+ by 20-30 percent.

CHAPTER 8

EXPERIMENT 5

EFFECT OF SALINITY ON GAS EXCHANGE, TRANSPIRATION EFFICIENCY OF FLAG LEAF AND TWO PENULTIMATE LEAVES AND YIELD OF SPRING WHEAT

8.1 INTRODUCTION

Maintenance of active photosynthesis, particularly by the flag leaf, throughout the period of cereal grain filling is a major requirement for production of adequate carbohydrates to give large grains and high yields (Peoples *et al.* 1980). Spike growth both before and after emergence from the boot is supported mostly by photosynthate from the upper two or three leaves on the plant (Rawson and Hofstra 1969; Patrick 1972). Estimates suggest that from 70% to more than 90% of the grain yield is derived from photoassimilate after anthesis (Austin *et al.* 1977; Bidinger *et al.* 1977). The flag leaf blade may account for as much as 50% of the photosynthate produced during early and mid grain filling (Austin and Jones 1975; Rawson *et al.* 1983). The spike and penultimate leaf blade may each account for as much 20% of the photosynthate produced (Simmons 1987).

Previous studies in this department (Experiments 3 and 4) and elsewhere (King *et al.* 1967; Osman and Milthorpe 1971; Dantuma 1973; Rawson *et al.* 1983) suggested that measurements of photosynthesis on a single leaf at a single point in time have limited value for characterising the photosynthetic performance of a genotype, since growing conditions, leaf position, leaf age and rate of photoassimilate utilisation can influence the rate of photosynthesis observed. In previous experiments, the effects of salinity on Pn, ion uptake and related parameters of single leaves had been studied. Experiments 3 and 4

showed that salinity resulted in decreased Pn and earlier senescence of lower leaves and the flag leaf. In Experiments 3 and 4 the measurements were based on single leaves appearing early and late in development. In this final experiment, the intention was to study the effects of salinity on several leaves on the same plant at the same time. In this experiment measurements were taken on the 3 leaves which were green during grain filling. In contrast to earlier experiments, in this chapter, leaves are numbered from the ear downwards, starting with the flag leaf (leaf 1), penultimate leaf (leaf 2) and the leaf below that (leaf 3). The period from anthesis to harvest was chosen as this is when grain filling occurs. It was also hoped that this would open up a new avenue of research that might be investigated by further researchers.

8.2 MATERIALS AND METHODS

The procedures used to grow the plants in Experiment 5 generally followed those of Experiment 2 except that the salinity levels tested were identical to those in Experiment 4. Any changes in methodology are noted in the following sections.

8.2.1 EXPERIMENTAL DESIGN AND TREATMENTS

A randomised complete block design was used. There were 3 salinity treatments and 7 replicate containers of each treatment. Each container was 18.5 cm x 63 cm surface x 35 cm deep and having 25 litres water holding capacity. The

salinity levels tested were 0, 75 and 150 mol m^{-3} NaCl and $CaCl_2$ was applied to salt stressed containers as described earlier (Section 4.2.1).

8.2.2 RAISING OF SEEDLINGS

The experiment was initiated on 16 April 1990 and terminated on 16 August 1990. The plants were grown in an unheated glasshouse with no supplementary lighting. The materials used in this experiment were identical to those in Experiment 2 (Section 4.2). In Experiment 2 vermiculite was used to provide media and support for germination of wheat seeds but this resulted in poor germination and a need for reseeding. Perlite was found to provide a more uniform environment for seed germination than vermiculite. Therefore in this experiment seeds were presoaked overnight and then sown on perlite filled in cells of P180 plugtrays with one seed per cell and a total of 180 seeds per tray. To support the perlite filled in the cells, capillary matting was fixed with Copydex to the bottom of the P180 plugtrays. The perlite was kept moistened with tap water and the trays were covered with newspapers to reduce evaporation. After 5 days the trays were suspended over plastic containers containing 25 litres of aerated 'Phostrogen' based nutrient solution (Appendix B). As in Experiment 2, the seedlings were later thinned to 90 per tray at a spacing of 3 cm x 6 cm (583 seedlings m^{-2}) by removing alternate rows prior to introducing salt stress.

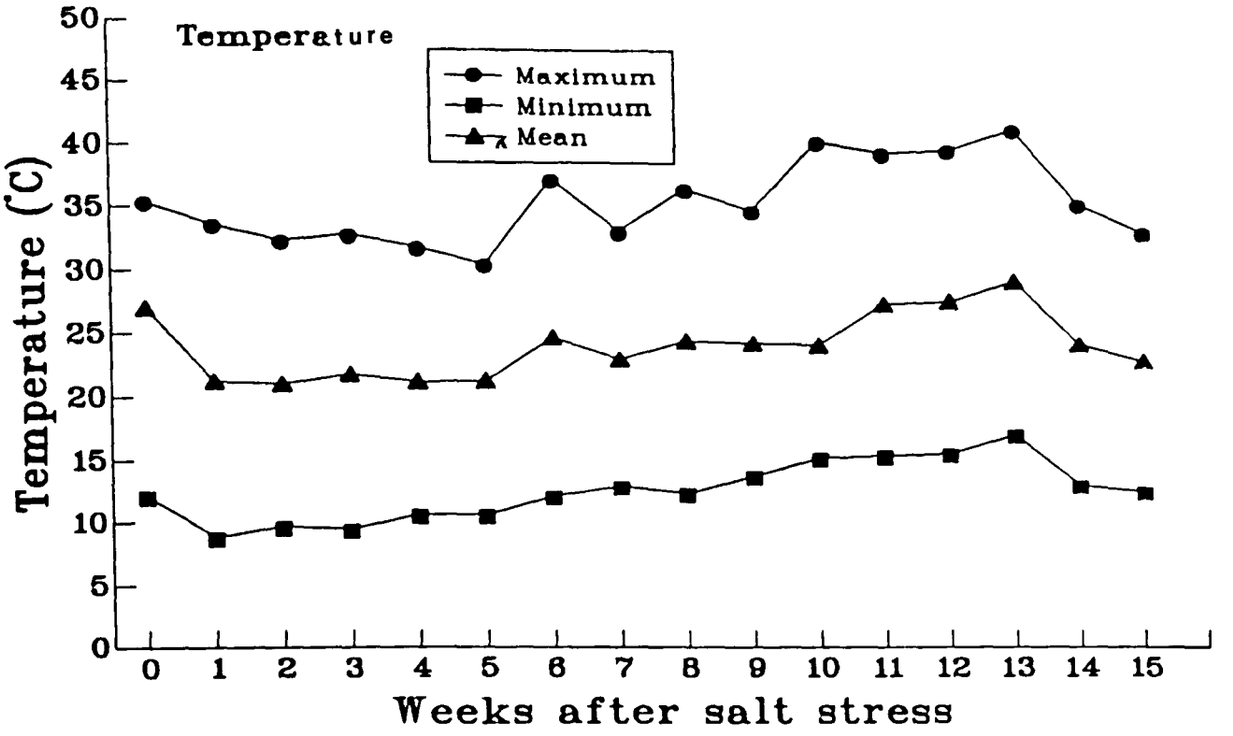
Micronutrients based on Long Ashton solutions (Appendix B) were prepared as in section 4.2.5 and were added along with salt solutions (Appendix C). Salt stress was introduced in 25 mol m^{-3} NaCl daily increments at 0+2 leaf stage starting 17 days after seeding on 3 May 1990.

8.2.3 MAINTENANCE OF THE EXPERIMENT

During the second week of May 1990 an aphid attack was observed on the plants. The next day during late afternoon all the plants were sprayed with dimethoate (Murphy Systemic Insecticide) at the recommended dose. Subsequent sprays were performed during the first week of June and the third week of July. The exterior of the glasshouse was sprayed partially with a white emulsion during the second week of May to prevent overheating inside and subsequent damage to plants. The nutrient and salt solution was changed after every second week throughout the growth period of the crop. During the course of the experiment, minimum and maximum air temperatures were recorded at 10.00 am each morning inside the glasshouse and hours of bright sunshine were recorded at a field site less than 1 km from the glasshouse. Weekly averages of daily mean temperatures and sunshine hours for each week are presented in figure 8.1.

8.2.4 COLLECTION OF GER DATA

GER measurements of the flag leaf, penultimate leaf (leaf 2) and the leaf below that (leaf 3) on the mainstem were started on 6 June 1990, 29 days after salt stress and



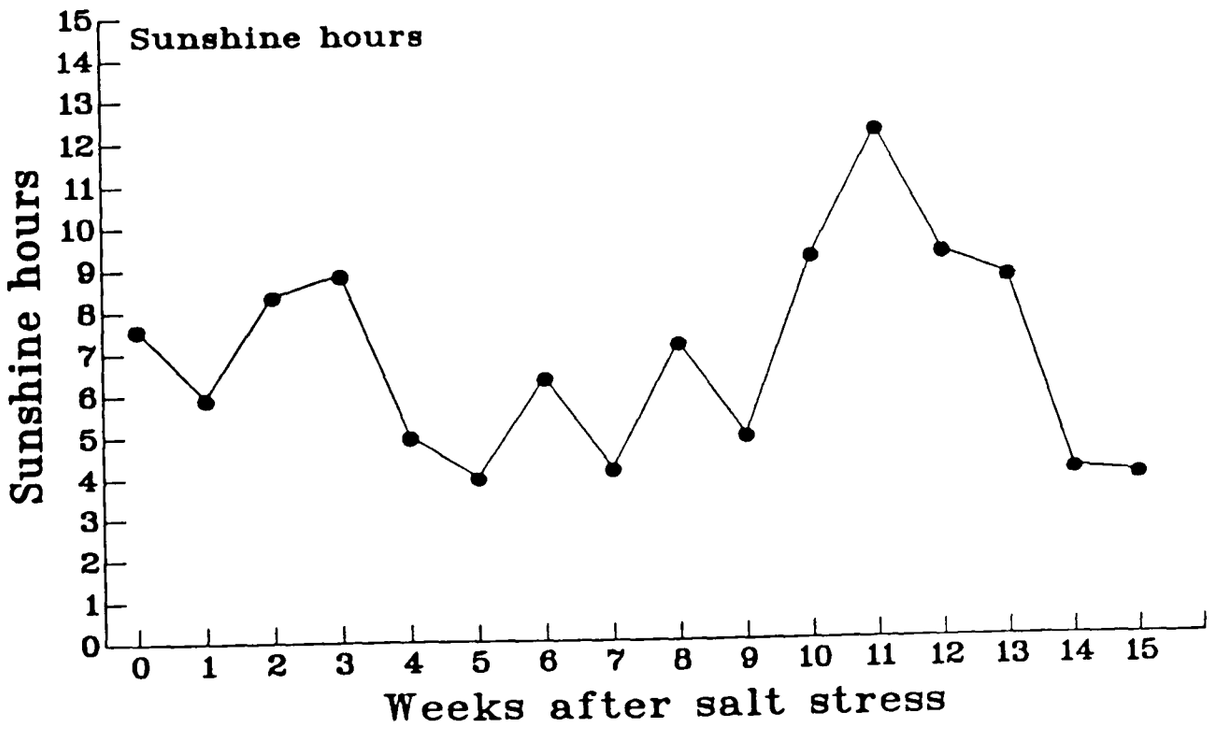


Figure 8.1: Mean daily temperature during each week in the glasshouse and mean sunshine hours during each week of the experiment.

finished on 3 August 1990 when the flag leaf was completely yellow. The measurements were started 16 days before anthesis, as soon as the flag leaf was long enough to fit in the PLC of the IRGA. Measurements were initially made on plants *in situ* in the glasshouse under prevailing daylight conditions. The first measurements were started at 07.00 am on a sunny morning which later turned into a cloudy day. On this occasion there were 60 sets of measurements, taken from 3 leaf insertions, 4 plant per replicates and 5 replicates of each 3 of salinity treatments. During this first measurement, the procedures used to measure and record GER were similar to those in Experiment 2. It took 8 hours to complete the measurements during which time light intensity, GER and other parameters fluctuated. Due to these changes in light intensity and in GER, it was decided to perform subsequent measurements inside the light chamber in the growth room as in Experiments 3 and 4.

The second set of measurements were made at 38 DAS. As in Experiments 3 and 4, the lights, air conditioning system and water supply in the growth room were switched on half an hour before the GER measurements commenced to bring the light chamber up to its operating PAR level and air temperature. The plants plus P180 plugtray were lifted off the container and placed on a similar empty container and then transferred from the glasshouse to the walk-in growth room. In the growth room the plants plus plugtray were transferred to a similar container containing the

appropriate nutrient and salt solution in the light chamber. The plants were equilibrated at 1750 μmol m^{-2} s^{-1} PAR for 15 minutes prior to GER measurements. The GER measurements of intact flag leaf, leaf 2 and leaf 3 were taken at only one light intensity, 1750 μmol m^{-2} s^{-1} PAR. Measurements were made on each of 3 salinity treatments. The procedures followed were the same as in Experiments 3 and 4. However, because of the relatively long equilibration time inside the light chamber before measurements on a set of plants could be taken (15 minutes for each container), it took more than 11 hours to complete this second set of GER measurements.

The vertical distribution of light within the light chamber also caused problems due to the fact that leaves 2 and 3 were further away from the light source than the flag leaf. Therefore, two changes in technique were introduced:

1). To avoid the problem of long equilibration times, bearing in mind the large number of leaves to be measured, measurements were made on eight replicate leaves per treatment and leaf insertion from the same container.

2). To avoid the problem of vertical distribution of light within the light chamber it was decided to detach leaves before placing them in the leaf chamber. This necessitated a preliminary study of changes in measurements of GER and other parameters following detachment of leaves in the light chamber. This is described in the following section.

8.2.4.1 MEASUREMENTS OF GER AND OTHER PARAMETERS FOLLOWING DETACHMENT OF LEAVES

Changes in GER and other parameters after leaf detachment were studied for one replicate container of the control treatment, assuming that GER changes would be similar in all the salinity treatments.

The plants plus plugtray were transferred to the light chamber inside the walk-in growth room and equilibrated for 15 minutes at 1750 μmol m^{-2} s^{-1} PAR. Then a mainstem flag leaf was placed inside the PLC. After 3 minutes, when the Pn readings were considered stable, a set of GER data were recorded on the datalogger. The leaf lamina was then cut at the ligule. GER data were subsequently recorded at one minute intervals up to 15 minutes after detaching the leaf. In total, measurements were made on 8 randomly selected flag leaves on the mainstem of different plants.

8.2.4.2 RESULTS OF EQUILIBRATION STUDY

8.2.4.2.1 CHANGES IN GER FOLLOWING LEAF DETACHMENT

The results for Pn, g_s, E, Ci and T_l are presented as mean of 8 leaves from the one replicate of the control treatment (0 mol m^{-3} NaCl) in figures 8.2 and 8.3. Pn, g_s, and E increased slightly within 1 to 2 minutes after detachment and then declined gradually (Figure 8.2). Ci remained relatively constant at approximately 213 μl l^{-1} CO_2 till 7 minutes after leaf detachment and then it increased

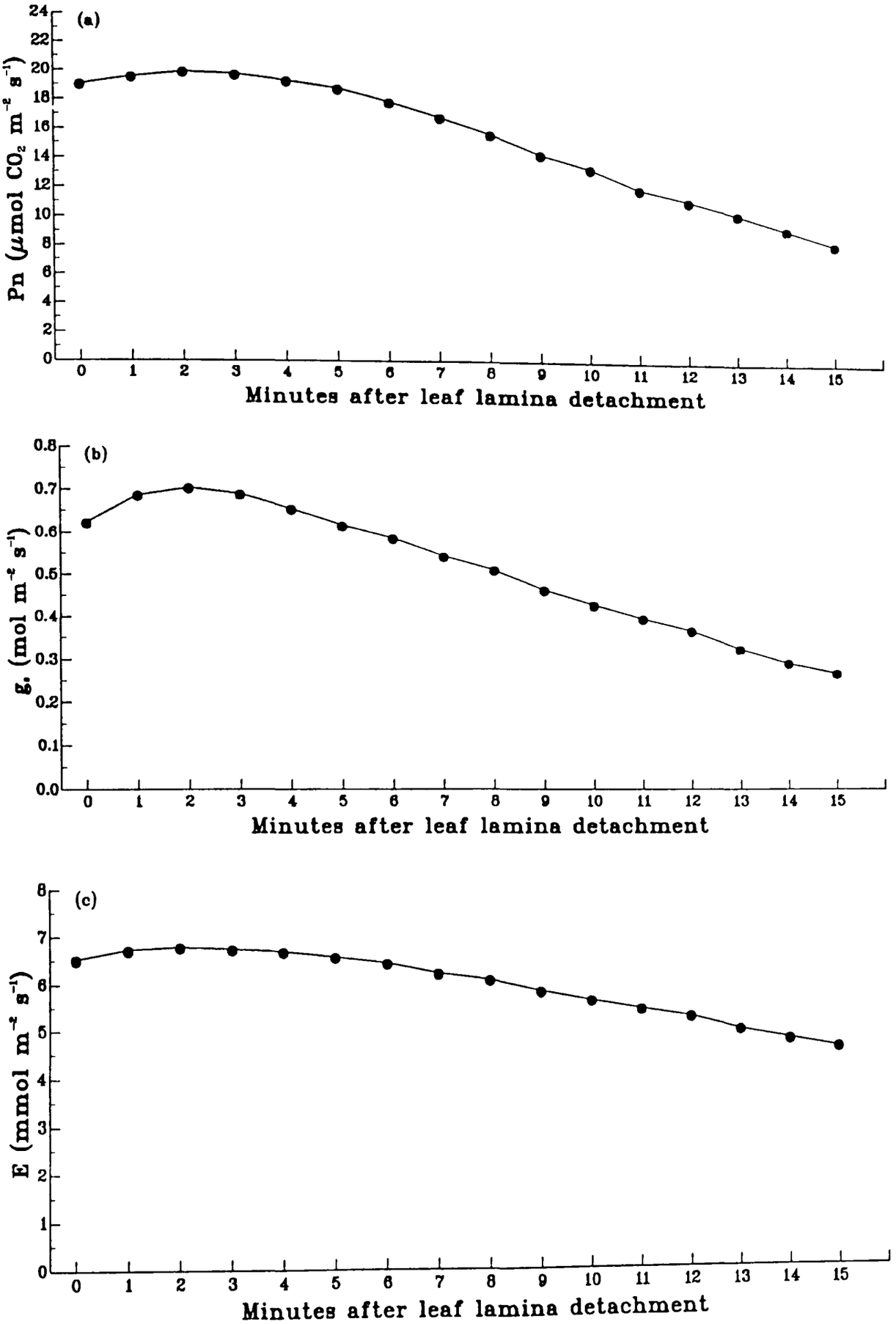


Figure 8.2: Changes in (a) net photosynthesis (Pn), (b) stomatal conductance (g_s) and (c) transpiration rate (E) of wheat mainstem flag leaf lamina following detachment from the sheath. Data are the means of 8 leaves.

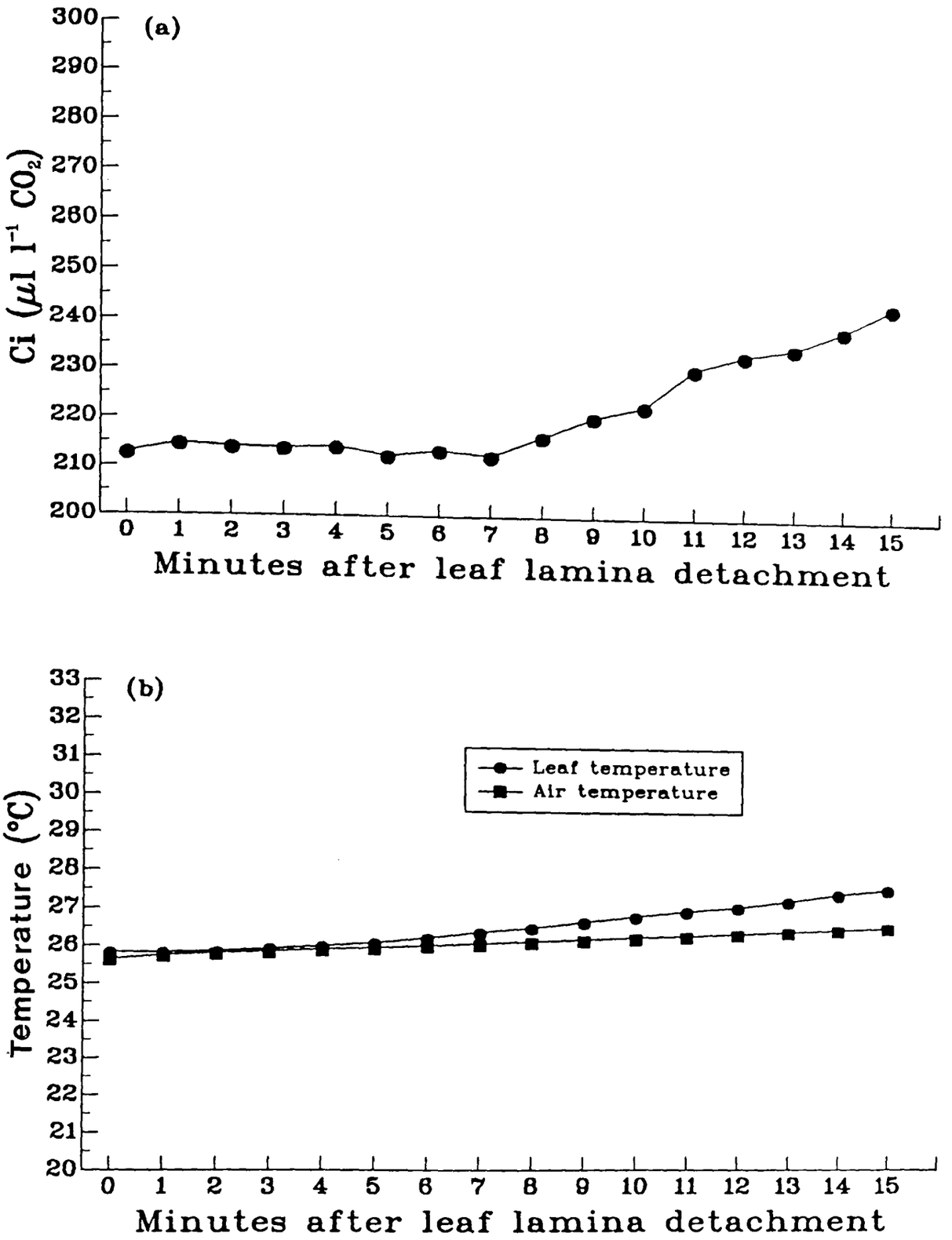


Figure 8.3: Changes in (a) sub-stomatal CO_2 concentration (Ci) and (b) leaf temperature of wheat mainstem flag leaf lamina following detachment from the sheath. Data are the means of 8 leaves. Air temperature was recorded inside the light chamber.

rapidly (Figure 8.3). T_l initially remained constant at 26°C but then increased slightly (Figure 8.3).

8.2.4.1.2 DISCUSSION

T_l increased only slightly throughout the study suggesting that temperature was not a major factor contributing to the observed changes in other parameters. Changes in Pn and E after leaf lamina detachment closely followed those of g_s. g_s increased by 13% within 2 minutes and then started declining. g_s was decreased to 56% of its original value at 15 minutes after detachment. This increase in g_s shortly after detachment was also observed by Meidner and Mansfield (1968) who explained that at the moment of excision of a transpiring leaf, there is a sudden release of tension in the xylem sap, and the water remaining within the xylem will be more freely available to the leaf. For a short time therefore, the leaf should actually gain in turgor. The stomata open widely for a few minutes before the epidermal cells present an increased resistance to the guard cell, causing stomatal closure (Meidner and Mansfield 1968).

Pn and E also followed the same trend as g_s but the changes in values were not of the same magnitude. Pn and E increased initially about 5% and 4% respectively at 2 minutes and then decreased rapidly after 6 minutes. After 15 minutes Pn and E had decreased to 55% and 29% respectively of other initial values. Blum (1990) in his experiment detached the leaf sheath at a point 10 cm below the flag leaf lamina and placed the cut end in a vial with water. He

subsequently used the flag leaf for GER measurements after 15 minutes equilibration at 1200 μmol m^{-2} s^{-1} PAR and reported that GER did not change significantly for at least 2 hours. In contrast to his experiment, in the present study, the cut end of the leaf was not put in a vial. Moreover, in contrast to the 15 minutes equilibration of a detached leaf at 1200 μmol m^{-2} s^{-1} PAR used by Blum (1990), in the present study, the intact leaf was equilibrated for 15 minutes prior to GER measurements. T_l remained fairly stable for 5 minutes and then started increasing and was 7% higher than the initial value at 15 minutes after detachment. Ci also remained more constant than the other parameters for 7 minutes and then started increasing. Therefore, during this time Rd must have increased as Pn decreased. There was a 15% increase in Ci above the initial value at 15 minutes after detachment of the leaf. This is due to changes in Pn and Rd. g_s data suggests that the stomata started closing earlier than that of 7 minutes after leaf detachment, hence needed to take measurements quickly and within 4-5 minutes.

8.2.4.1.3 CONCLUSION

The results of this study suggest that reliable measurements of GER can be made on detached leaves provided that measurements are taken within 5 minutes. This procedure was used for the remaining measurement dates. GER and related parameters were measured on 8 replicate leaves

for each leaf insertion per container.

8.2.5 AGRONOMIC MEASUREMENTS

At harvest there remained 4 replicate containers which had not been used for GER measurements. All plants from these were harvested and the following were recorded:

1. Number of spike bearing plants.
2. Number of fertile ears.
3. Total grain dry weight.
4. Total grain number.
5. Root and shoot dry weight.

From this data yield and yield components per plant and per m^2 and 1000-grain weight (TGW) were calculated. Harvest index (HI) was calculated as follows:-

$$HI = \frac{\text{Grain yield}}{\text{Biomass (root + Shoot)}} \times 100$$

8.2.6 DETERMINATION OF TRANSPIRATION EFFICIENCY

Transpiration efficiency (TE) was calculated following the same procedures as those described in section 6.2.7 for Experiment 3.

8.2.7 STATISTICAL ANALYSES

Statistical analyses were carried out by computer using Genstat 5 statistical package as outlined in the previous section (3.2.7). Pairwise comparisons of the treatment means were made by using Tukey's method as in the earlier section (4.4).

8.3 RESULTS OF THE MAIN EXPERIMENT

For reasons outlined earlier (Section 8.2.4), the results for 29 DAS consist of GER measurements of intact leaves made on plants inside the glasshouse under natural day light conditions. Results for 38 DAS consist of GER measurements of intact leaves made on plants inside the light chamber at 1750 μmol m^{-2} s^{-1} PAR. From 45 DAS onward, GER measurements are means of 8 plants from one replicate container of each salinity treatment.

8.3.1 EFFECT OF SALINITY ON GER AND RELATED PARAMETERS

There were significant effects of leaf insertion and salinity on GER and related parameters on most sampling dates. The leaf insertion x salinity interaction was generally significant for T_l, Pn, g_s, E and TE but not for Ci. The significant leaf insertion x salinity interaction showed that the effect of salinity varied with leaf insertion. The general trend was that salinity had larger effects on leaf 1 (flag leaf) than on leaf 2 and 3.

T_l was much lower on the first sampling date as measurement were made under ambient conditions in the glasshouse, not in the walk-in growth room. T_l was lower at 0 mol m^{-3} NaCl than at 75 and 150 mol m^{-3} NaCl on most of the sampling dates, although the differences between treatments were not always significant (Figure 8.4). T_l increased over the final sampling dates in leaves 1 and 2. There were significant effects of leaf insertion on T_l on

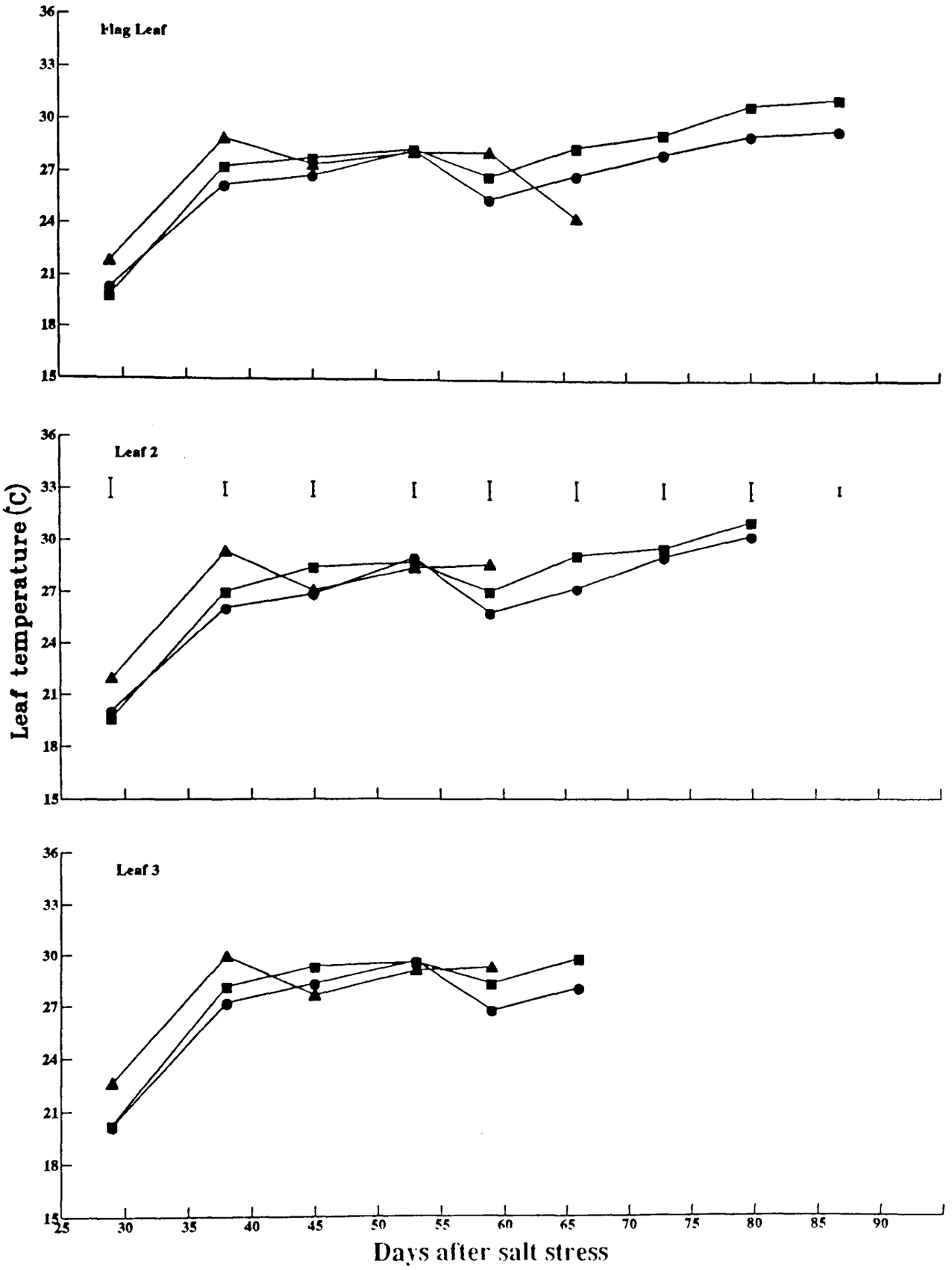


Fig. 8.4: Effect of salinity on leaf temperature of flag leaf, leaf 2 and leaf 3 of spring wheat. I = standard error of means; ● , 0 mol m^{-3}; ■ , 75 mol m^{-3}; ▲ , 150 mol m^{-3} NaCl.

all the sampling dates. T_l consistently decreased with leaf insertion such that flag leaf was > leaf 2 > leaf 3.

Pn was significantly higher in the flag leaf than in leaf 2 and leaf 3 on most of the sampling dates (Figure 8.5). In all leaf insertions Pn was lower at 29 DAS than at later sampling dates and at this time there was no significant effect of salinity. Thereafter, Pn was decreased by salinity and was significantly higher at 0 mol m^{-3} NaCl than at 150 mol m^{-3} NaCl in all three leaf insertions on most of the sampling dates. At 0 mol m^{-3} NaCl Pn remained approximately constant between 38 and 59 DAS at 17, 11 and 5 μmol m^{-2} s^{-1} in the flag leaf, leaf 2 and leaf 3, respectively. Thereafter it decreased and the flag leaf, leaf 2 and leaf 3 were completely yellow at 87, 83 and 66 DAS respectively. At 75 mol m^{-3} NaCl Pn decreased from 38 DAS in all the leaf insertions and the flag leaf, leaf 2 and leaf 3 were completely yellow at 87, 83, and 66 DAS respectively. At 150 mol m^{-3} NaCl Pn increased between 29 and 45 DAS in the flag leaf and thereafter it declined rapidly. At 150 mol m^{-3} NaCl Pn remained at approximately 5 and 2 μmol m^{-2} s^{-1} in leaf 2 and leaf 3 respectively on all the sampling dates. At 150 mol m^{-3} NaCl the flag leaf, leaf 2 and leaf 3 were completely yellow at 66, 59 and 59 DAS respectively.

The salinity x leaf insertion interaction was significant for g_s on all sampling dates (Figure 8.6). g_s was consistently decreased by salinity and with leaf

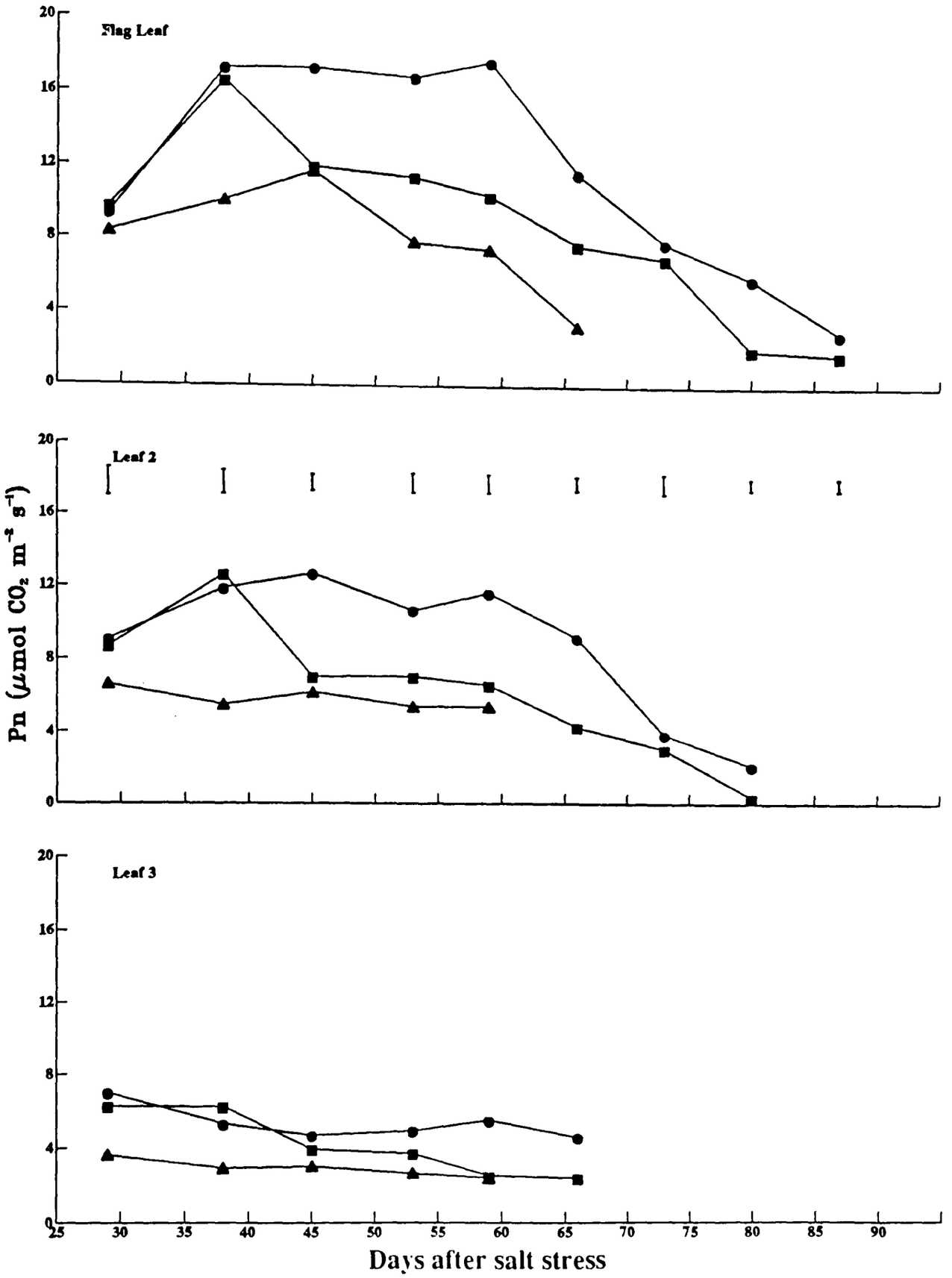


Fig. 8.5: Effect of salinity on net photosynthesis (Pn) of flag leaf, leaf 2 and leaf 3 of spring wheat. I = standard error of means; ● , 0 mol m^{-3}; ■ , 75 mol m^{-3}; ▲ , 150 mol m^{-3} NaCl.

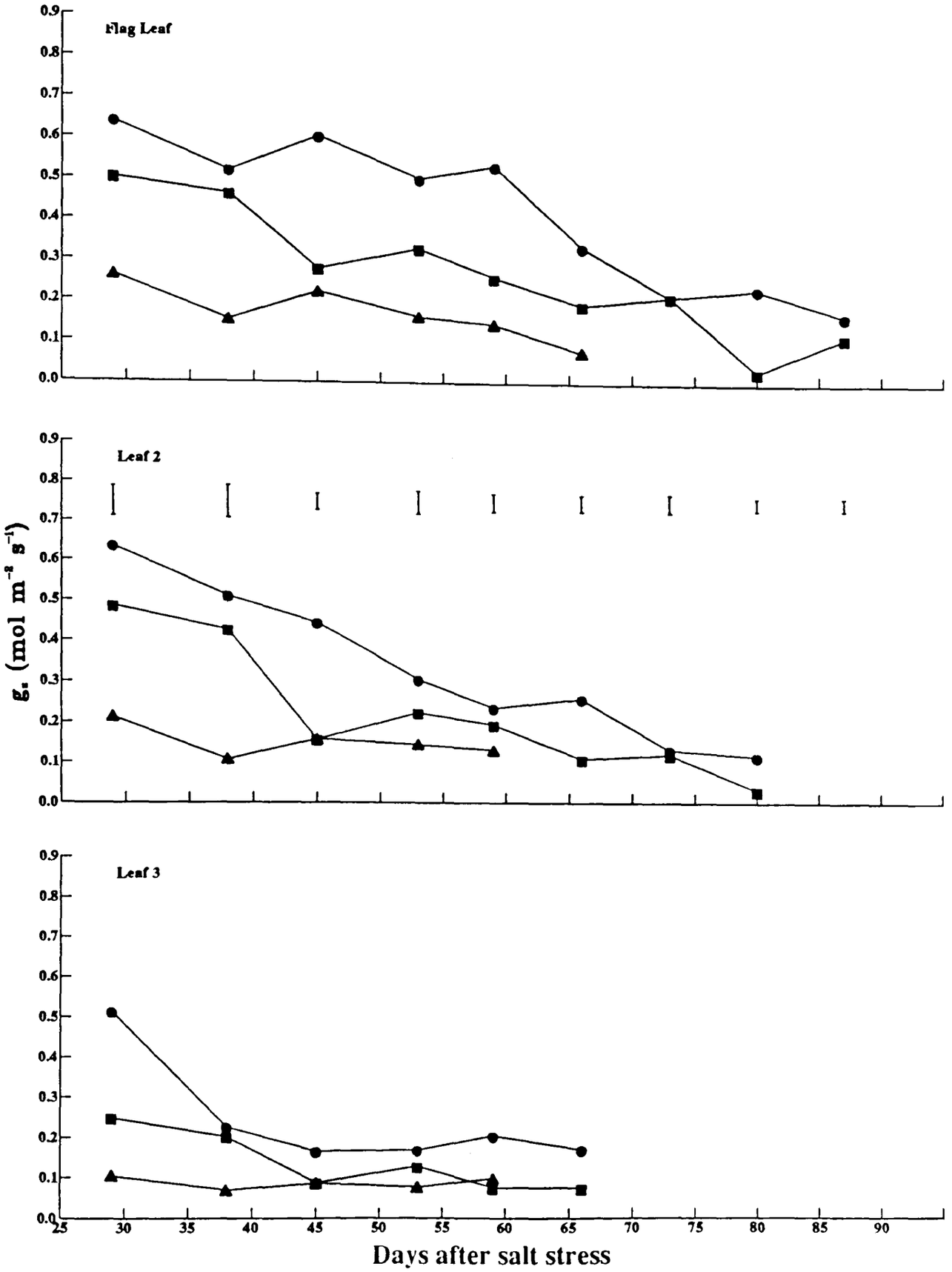


Fig. 8.6: Effect of salinity on stomatal conductance (g_s) of flag leaf, leaf 2 and leaf 3 in spring wheat. I = standard error of means; ● , 0 mol m^{-3}; ■ , 75 mol m^{-3}; ▲ , 150 mol m^{-3} NaCl.

insertion on the mainstem. g_s was significantly higher in the flag leaf than in leaf 2 and leaf 3 on all sampling dates. g_s was significantly decreased by salinity on all sampling dates, although the difference between 0 and 75 mol m^{-3} NaCl was not always significant and effects decreased with leaf insertion. g_s gradually decreased with time in all the leaf insertions, although this trend was less well marked in leaf 3 at 150 mol m^{-3} NaCl.

At 0 mol m^{-3} NaCl E initially increased and remained fairly constant at 6 mmol m^{-2} s^{-1} between 38 and 59 DAS in both the flag leaf and leaf 2. Thereafter it decreased. In leaf 3 it remained at 4 mmol m^{-2} s^{-1} throughout the measurement period. E decreased as salinity increased and was significantly higher at 0 than 150 mol m^{-3} NaCl on all sampling dates (Figure 8.7). In all the leaf insertions the difference between 0 and 75 mol m^{-3} NaCl was not always significant. Effects of salinity on E were greater in the flag leaf than in leaf 2 and leaf 3. E decreased with leaf insertion and was significantly higher in the flag leaf and leaf 2 than in leaf 3.

Ci increased with leaf insertion and was significantly lower in the flag leaf than in leaf 2 and leaf 3 on all sampling dates (Figure 8.8). Values of Ci measured under ambient conditions at the first measurement date were higher than those recorded at the second in the light chamber. From 39 DAS it increased with time in all the leaf insertion and salinity treatments. The salinity x leaf insertion interaction was not significant on any sampling date. Ci

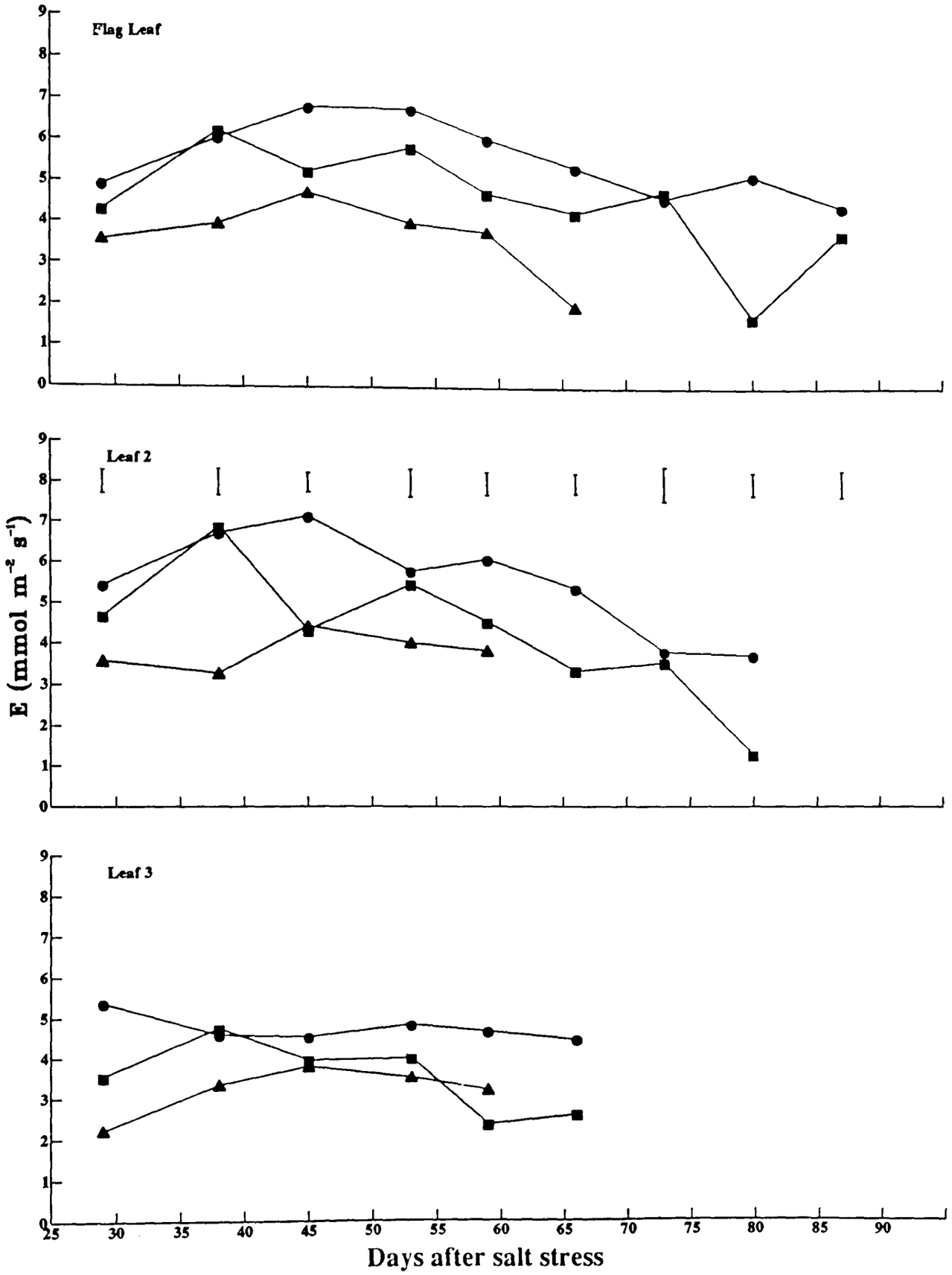


Fig. 8.7: Effect of salinity on transpiration rate (E) of flag leaf, leaf 2 and leaf 3 in spring wheat. I = standard error of means; ● , 0 mol m^{-3}; ■ , 75 mol m^{-3}; ▲ , 150 mol m^{-3} NaCl.

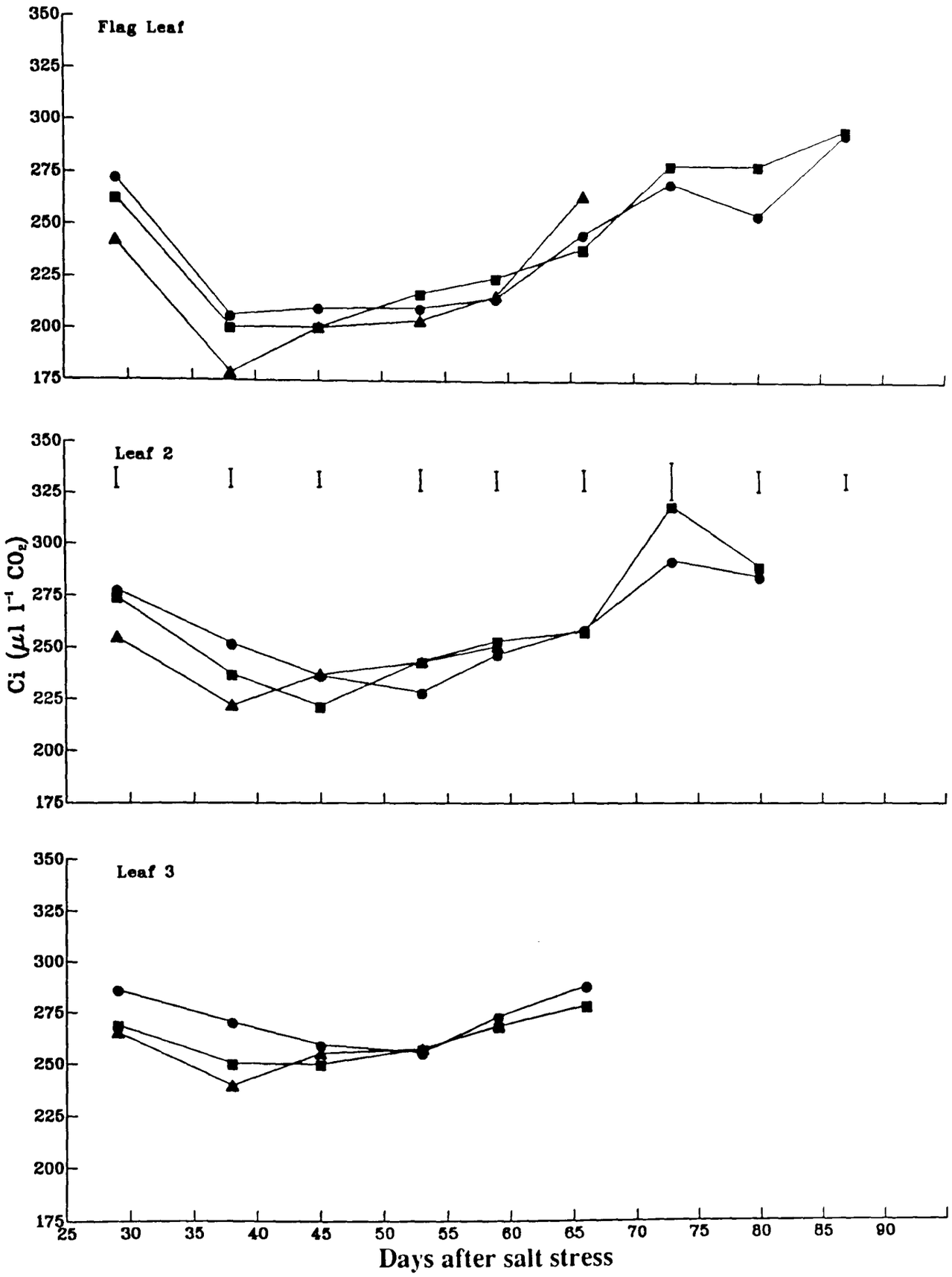


Fig. 8.8: Effect of salinity on sub-stomatal CO_2 concentration (Ci) of the flag leaf, leaf 2 and leaf 3 in spring wheat. I = standard error of means; ●, 0 mol m^{-3}; ■, 75 mol m^{-3}; ▲, 150 mol m^{-3} NaCl.

decreased as salinity increased from 29-45 DAS. From 53-66 DAS salinity had no significant effect on Ci. From 73-87 DAS Ci was higher at 75 than at 0 mol m^{-3} NaCl.

TE was always significantly higher in the flag leaf than in leaf 2 and leaf 3 (Figure 8.9). Generally TE was not significantly affected by salinity between 29 and 45 DAS but decreased thereafter, although the difference was not significant at 73, 80 and 87 DAS. Trends in leaf 3 for TE were less consistent than in other leaf insertions. The salinity x leaf insertion interaction was not always significant. TE was higher at 0 than at 75 and 150 mol m^{-3} NaCl in the flag leaf and leaf 2 from 45 to 73 DAS but thereafter the difference decreased as TE declined. At 75 and 150 mol m^{-3} NaCl TE in the flag leaf increased and then decreased with time. In leaf 2 and leaf 3 it remained constant at 1.5 and 1.0 μmol CO_2 per mmol H_2O, respectively between 29 and 59 DAS and then it decreased in leaf 2.

8.3.2 AGRONOMIC MEASUREMENTS

Salinity significantly decreased grain yield and all yield components on a per plant and per m^2 basis (Table 8.1). Salinity also decreased root and shoot dry weight, root to shoot ratio and harvest index. Values of all parameters were lower at 75 than at 0 mol m^{-3} NaCl except for number of spike bearing plants and number of fertile ears per m^2. Values of all the agronomic parameters were significantly lower at 150 mol m^{-3} NaCl than at 0 and 75 mol

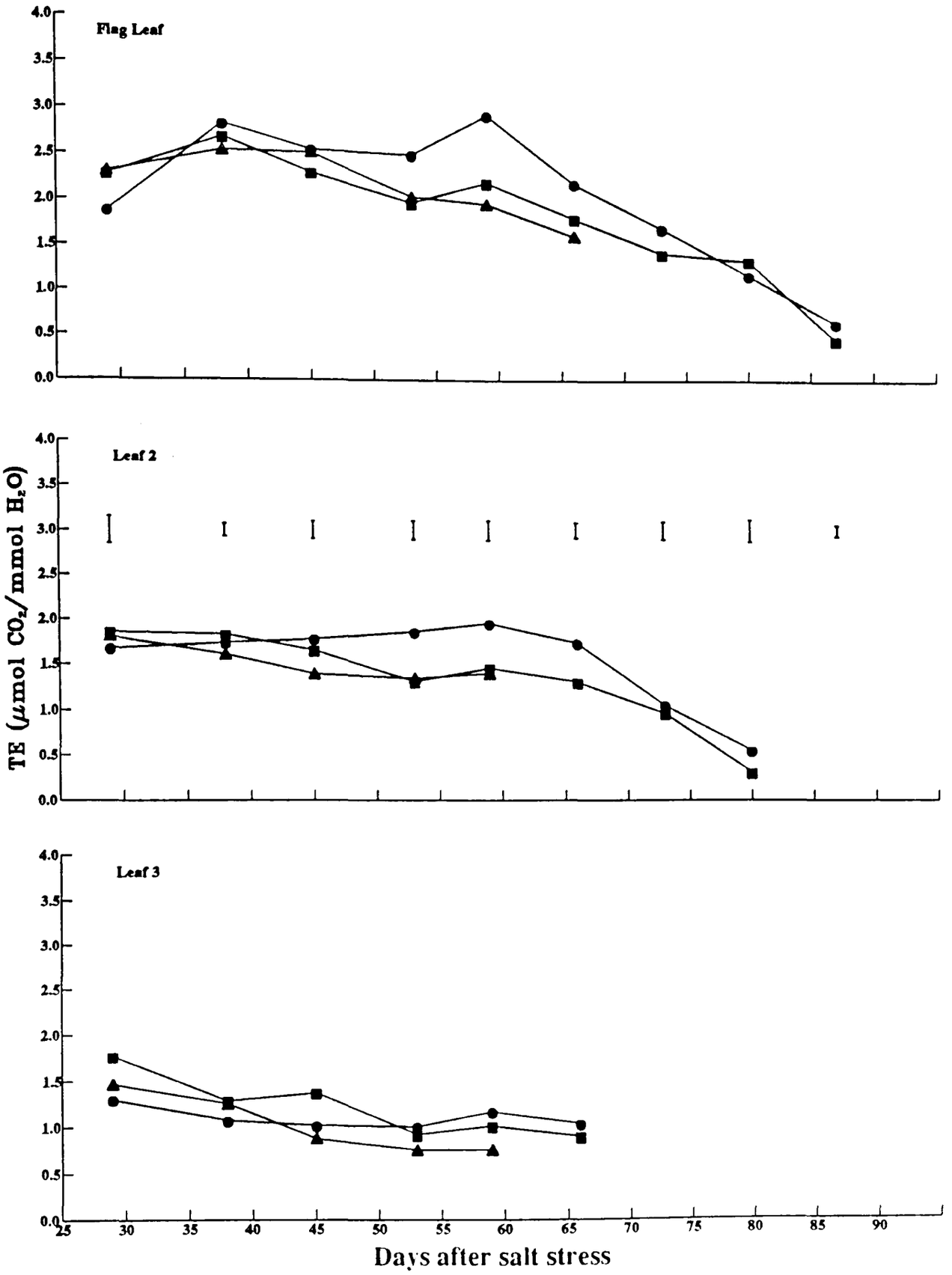


Fig. 8.9: Effect of salinity on transpiration efficiency (TE) of the flag leaf, leaf 2 and leaf 3 in spring wheat. I = standard error of means; ● , 0 mol m^{-3}; ■ , 75 mol m^{-3}; ▲ , 150 mol m^{-3} NaCl.

Table 8.1: Effect of salinity on yield components, yield and related parameters per unit area (m^2) and per plant of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)			SEM	HSD
	0	75	150		
Yield and Yield Components					
Number of spike bearing plants (m^{-2})	583.00	580.00	502.00	21.40	NS
Number of fertile ears (m^{-2})	1122.00	1107.00	583.00	35.80	155.34**
Number of grains ($x10^3$ m^{-2})	33.53	24.94	7.40	0.77	3.35**
1000-grain weight (g)	31.91	21.46	7.57	0.36	1.58**
Grain yield (g m^{-2})	1071.00	533.00	56.00	21.10	91.55**
Number of fertile ears $plant^{-1}$	1.93	1.91	1.16	0.05	0.21**
Number of grains ear^{-1}	57.56	43.07	14.42	1.29	5.58**
Grain yield (g $plant^{-1}$)	1.84	0.92	0.11	0.04	0.16**
Plant Biomass Production					
Root dry weight (g m^{-2})	127.20	60.60	12.20	2.24	9.72**
Shoot dry weight (g m^{-2})	2687.00	1718.00	581.00	38.00	164.88**
Root:shoot ratio	0.05	0.04	0.02	0.001	0.01**
Harvest index (%)	38.02	29.96	9.26	0.37	1.62**
Dead plants m^{-2} (%)	0.00	0.56	13.89	-	-

SEM = Standard error of means,
HSD = Honestly significance differences,
** = Significant at 1 percent probability level,
NS = Not significant,
- = Data not analysed

m^{-3} NaCl. Plant mortality was higher at 150 mol m^{-3} NaCl (14%) than at 75 (1%) and 0 (0%) mol m^{-3} NaCl.

8.4 DISCUSSION AND CONCLUSIONS

The aim of this study was to examine the effects of salinity on changes in GER in the flag leaf and two penultimate leaves simultaneously, as these leaves are important for supply of photosynthate before and after anthesis and during grain filling. During the course of this study all the GER parameters were significantly affected by salinity and leaf insertion. The leaf insertion x salinity interaction showed that salinity had larger effects on the flag leaf than leaves 2 and 3. This may be due to reason that these leaves were at different physiological stage during GER measurements. For example, the flag leaf experienced periods of expansion and senescence during these measurements whereas leaf 3 was already senescing, therefore less affected. The leaf x salinity interaction was significant for T_l, Pn, g_s, E and TE but not for Ci. It is interesting to note that the initial measurements of Ci which were made *in situ* in the glasshouse at 29 DAS were higher than those recorded at later sampling dates which were obtained in the light chamber (Figure 8.8). This increased Ci could be due to either decreased stomatal limitations or reduced CO_2 assimilation. The results suggest that Pn (Figure 8.5) was decreased more than g_s (Figure 8.6) at 29 DAS than at later sampling dates. Therefore the decrease in CO_2 assimilation

was more likely to be the cause of the higher Ci at 29 DAS when g_s was not a limiting factor. This decrease in Pn can be attributed to lower prevailing PAR as the measurements were made in the glasshouse (108 to 1095 μmol m^{-2} s^{1}) as compared to the light chamber (1750 μmol m^{-2} s^{-1}) at later sampling dates. The higher g_s could be due to temperature differences between the two places. Temperature in the glasshouse was 29°C as compared to 25°C in the growth room.

At 29 DAS Pn decreased with salinity and leaf insertion. At 75 mol m^{-3} NaCl Pn was increased by 4% in the flag leaf and decreased by 2% and 11% in leaf 2 and leaf 3 whereas g_s was decreased by 21%, 24% and 52% respectively (Table 8.2). At 150 mol m^{-3} NaCl Pn was decreased by 10%, 27% and 48% whilst g_s was decreased by 59%, 66% and 80% in the flag leaf, leaf 2 and leaf 3 respectively. Therefore at the low salinity level (75 mol m^{-3} NaCl) g_s was markedly decreased whereas Pn only slightly decreased or increased. These results suggest that g_s was more affected than Pn by salinity at 29 DAS. At 29 DAS decrease in g_s was associated with a slight decrease in Ci in the these leaves (e.g. 4% for the flag leaf, 1% for leaf 2 and 6% for leaf 3). At high salinity the decrease in g_s was greater but so was the decrease in Pn so that Ci was decreased by only 11% in the flag leaf, 8% in leaf 2 and 7% in leaf 3. These observations suggest that the effects of salinity on Pn and g_s were greater than on Ci. At later sampling dates Ci was relatively unaffected by salinity but increased with leaf

Table 8.2: Effect of salinity on percent increases (+) or decreases over control (0 mol m^{-3} NaCl) in net photosynthesis, stomatal conductance and sub-stomatal CO_2 concentration of the flag leaf and two penultimate leaves of spring wheat at selected sampling dates (days after salt stress).

Days after salt stress	Salinity levels (mol m^{-3} NaCl)					
	75			150		
	Leaf insertions			Leaf insertions		
	Flag leaf	Leaf 2	Leaf 3	Flag leaf	Leaf 2	Leaf 3
Net photosynthesis						
29	+4.31	2.32	10.57	10.44	27.01	48.00
38	3.95	+6.49	+17.58	41.67	53.62	44.99
45	30.86	44.44	16.03	32.25	51.22	35.68
53	32.07	33.99	24.85	53.33	49.34	46.64
59	41.08	43.31	54.66	57.73	53.52	56.67
66	33.84	53.15	47.92	71.86	-	-
73	11.09	20.88	-	-	-	-
80	65.38	83.02	-	-	-	-
87	39.45	-	-	-	-	-
Average	28.16	34.33	22.74	44.55	46.94	46.39
Stomatal conductance						
29	21.44	23.58	51.55	59.15	66.35	79.57
38	10.98	16.63	10.62	70.71	79.06	69.46
45	53.73	64.64	47.27	63.18	64.41	47.88
53	34.19	29.74	24.40	67.99	52.61	54.76
59	51.31	48.41	63.23	73.13	65.35	52.45
66	42.89	58.08	56.21	77.22	-	-
73	+0.47	9.77	-	-	-	-
80	80.51	73.28	-	-	-	-
87	30.95	-	-	-	-	-
Average	36.17	40.52	42.21	68.56	65.56	60.82
Sub-stomatal CO_2 concentration						
29	3.56	1.29	6.04	11.15	8.32	7.33
38	2.76	5.95	7.47	13.55	11.90	11.46
45	4.42	6.22	3.63	4.51	+0.25	1.58
53	+3.37	+6.56	+0.78	3.03	+6.39	+0.67
59	+4.59	+2.67	1.76	+0.56	+1.58	1.65
66	2.84	0.46	3.30	+7.45	-	-
73	+3.24	+9.02	-	-	-	-
80	+9.32	+1.68	-	-	-	-
87	+0.64	-	-	-	-	-
Average	+0.84	+0.75	3.57	4.04	2.40	4.27

- = Leaves completely senesced.

age. Changes in Ci will depend upon relative changes in Pn and g_s. As Ci was unaffected by salinity during senescence, this also support a parallel mechanism. These results are in agreement with experiments 3 and those of other workers (Yeo *et al.* 1985; Rawson *et al.* (1988a).

Pn reached a maximum earlier at 75 than at 0 mol m^{-3} NaCl. This is possibly due to earlier expansion of leaves at 75 mol m^{-3} NaCl. At 75 mol m^{-3} NaCl Pn rapidly declined between 38 and 45 DAS in the flag leaf and leaf 2 and thereafter declined more slowly. In contrast, Rawson *et al.* (1988a) observed that Pn in salt treated plants remained at its maximum rate for 2 weeks before declining at a similar rate to the control plants. They argued that decline in Pn in control plants was due to self shading. This would not be detected in these experiments as Pn was measured in the light chamber. In the experiment here Pn at 75 mol m^{-3} NaCl, based on average of all sampling dates, was decreased by 28%, 34% and 23% in the flag leaf, leaf 2 and leaf 3 whereas g_s was decreased by 36%, 41% and 40% respectively (Table 8.2). At 150 mol m^{-3} NaCl Pn in these leaves was decreased by 45%, 47% and 46% and g_s by 69%, 66% and 61% respectively. The differences between the control and high salinity treatment for both Pn and g_s increased with leaf age. However the difference between the control and 75 mol m^{-3} NaCl was not always consistent. These results indicate that g_s was decreased more than Pn in all the leaf insertions and at all sampling dates suggesting that reduced rates of Pn in older leaves are due to stomatal limitations

(Terry and Waldron 1984; Brugnoli and Lauteri 1991). Although reduction in the rate of biochemical processes with increasing ionic concentration may be another possibility (Seemann and Critchley 1985).

The experiment here also indicates that E decreased both with leaf age and salinity. E on an average of all sampling dates decreased by 19% in the flag leaf, 24% in leaf 2 and 25% in leaf 3 at 75 mol m^{-3} NaCl, although the decrease in E was not consistent at this salinity level in the leaf insertions (Table 8.3). It is interesting to note that E decreased more in the flag leaf at 150 mol m^{-3} NaCl with time whereas it decreased more in leaves 2 and 3 during early sampling dates. At 150 mol m^{-3} NaCl E on an average of all sampling dates decreased by 38% in all the leaf insertions. Changes in TE depend upon relative changes in Pn and E. TE was unaffected by NaCl between 29 and 45 DAS, suggesting that its effects on Pn are smaller. From 45 and 73 DAS, TE was decreased by NaCl as Pn was decreased more than E. The results of these experiments suggest that TE was generally inconsistently affected by salinity, although there is some evidence that TE increased at low salinity during early sampling dates. These results are in agreement with those of McCree (1986) and Rawson *et al.* (1988a).

Previous experiments in this department (Iqbal 1988; Experiments 1 and 2) showed that root and shoot growth were both decreased by salinity, but that root growth was decreased more than shoot growth which resulted in a

Table 8.3: Effect of salinity on percent increases (+) or decreases over control (0 mol m^{-3} NaCl) in transpiration rate and transpiration efficiency of the flag leaf and two penultimate leaves of spring wheat at selected sampling dates (days after salt stress).

Days after salt stress	Salinity levels (mol m^{-3} NaCl)					
	75			150		
	Leaf insertions			Leaf insertions		
	Flag leaf	Leaf 2	Leaf 3	Flag leaf	Leaf 2	Leaf 3
Transpiration rate						
29	12.62	14.09	34.02	27.29	34.25	59.93
38	+2.81	+2.46	+3.72	34.44	51.23	49.24
45	22.79	39.44	12.80	30.44	38.04	16.56
53	13.72	5.52	17.39	40.86	30.52	27.33
59	21.71	25.41	49.57	37.34	37.05	31.33
66	20.70	37.66	41.79	63.40	-	-
73	+3.42	7.05	-	-	-	-
80	67.18	65.42	-	-	-	-
87	15.25	-	-	-	-	-
Average	18.64	24.02	25.31	38.96	38.22	36.88
Transpiration efficiency						
29	+21.46	+11.04	+36.34	+23.01	+7.94	+12.86
38	5.09	+5.54	+19.93	10.23	7.15	+16.95
45	9.88	7.14	+34.51	1.33	21.59	13.73
53	21.15	29.46	8.37	18.51	27.53	24.70
59	25.06	25.45	13.01	33.09	55.90	34.79
66	17.48	24.61	13.26	26.45	-	-
73	16.22	8.64	-	-	-	-
80	+13.54	44.22	-	-	-	-
87	29.23	-	-	-	-	-
Average	9.90	15.37	+9.36	11.10	20.85	8.68

- = Leaves completely senesced.

decreased root:shoot ratio. In this experiment root dry weight was reduced by 52% and 90% and shoot dry weight by 36% and 78% respectively at 75 and 150 mol m^{-3} NaCl which resulted in decrease of 25% and 60% in root:shoot ratio (Table 8.4). In contrast Rawson (1986) found that salinity had less effect on root weight compared with shoot weight, hence the root:shoot ratio increased.

Harvest index in cereals is the ratio between grain yield and biomass produced by the plants. Harvest index was decreased by 21% at 75 mol m^{-3} NaCl and by 76% at 150 mol m^{-3} NaCl. Therefore at high salinity grain yield was more affected than biomass production. Similar results have been found by Torres and Bingham (1973).

Grain yield is a function of number of tillers and ears per plant, and the number and weight of individual grains set per ear. It has been found that grain yield is usually more affected by salinity than other characters (Torres and Bingham 1973; Kumar and Yadav 1983). In this Experiment number of fertile ears was only decreased by 1%, number of grains per ear by 25% and TGW by 33% whereas grain yield was decreased by 50% at 75 mol m^{-3} NaCl (Table 8.4). At 150 mol m^{-3} NaCl number of fertile ears was decreased by 40%, number of grains per ear by 75%, TGW by 76% and grain yield by 94%. These results agree with those of earlier workers (Torres and Bingham 1973; Kumar and Yadav 1983). In contrast, Kumar *et al.* (1983) found that number of ears per plant was the most seriously affected component in wheat whereas grain yield showed a relatively higher degree of

Table 8.4: Effect of salinity on percent increases (+) or decreases over control (0 mol m^{-3} NaCl) of yield components, yield and related parameters per unit area (m^2) and per plant of spring wheat.

Parameters	Salinity levels (mol m^{-3} NaCl)	
	75	150
Yield and Yield Components		
Number of spike bearing plants (m^{-2})	0.51	13.89
Number of fertile ears (m^{-2})	1.34	48.04
Number of grains ($x10^3$ m^{-2})	25.62	77.93
1000-grains weight (g)	32.75	76.28
Grain yield (g m^{-2})	50.23	94.77
Number of fertile ears $plant^{-1}$	0.78	39.58
Number of grains ear^{-1}	25.17	74.95
Grain yield (g $plant^{-1}$)	49.92	94.07
Plant Biomass Production		
Root dry weight (g m^{-2})	52.35	90.41
Shoot dry weight (g m^{-2})	36.06	78.38
Root:shoot ratio	25.00	56.25
Harvest index (%)	21.19	75.64

% = Percent

resistance to salinity. The results of this experiment suggest that tillering was not seriously affected by low salinity whereas it was only affected by high salinity. Therefore at low salinity lack of fertilization and grain set resulted in a lower grain number per plant. At high salinity both grain set and grain filling were seriously affected by salinity as is indicated by the lower number of grains per ear and lower grain weight. In this experiment and the previous experiments (Experiments 3 and 4) Pn was seriously affected by salinity. Therefore reduced Pn may be a possible cause of lower photosynthate available for grain filling. These experiments have studied Pn during anthesis to harvest. Therefore in future experiments it may be useful to study the effects of salinity on assimilate distribution using $^{14}CO_2$. This would, for example, help in identifying the role of current photosynthesis and stem reserves for grain filling in salt stressed plants.

CHAPTER 9

CONCLUSIONS

RELATIONSHIPS BETWEEN

LEAF GROWTH AND IONIC CONCENTRATION

AND BETWEEN GAS EXCHANGE AND IONIC CONCENTRATION

9.1 INTRODUCTION

Plant dry matter production depends on the accumulation of carbon products in photosynthesis. This in turn is determined by two main components: the rate of photosynthesis per unit leaf area and the area of leaf surface available for photosynthesis (Terry and Waldron 1984). One of the main objectives of this series of experiments was to investigate the effects of salinity on these two physiological parameters. Both were decreased by salinity. This chapter considers the quantitative relationships between the various parameters measured to:

1) help in understanding the effects of salinity on wheat,

2) provide data which might be useful in modelling the effects of salinity on plant growth.

The results were first used to test the relationships between:

1) external NaCl and internal concentrations of Na^+, Cl^-, K^+ and OP;

2) internal concentrations of Na^+, Cl^- and K^+ and OP;

3) internal concentrations of Na^+, Cl^-, K^+ and OP and LER;

4) LER, LED and FLL.

9.2 LEAF GROWTH

The results of Experiments 1 and 2 showed that salinity decreased both LER and LED. To determine the relative

importance of LER and LED in determining FLL, for each leaf data for the various salinity levels tested were combined and the correlations between FLL, LER and LED were examined. In Experiment 1 only two salinity levels were tested and hence it was not possible to study the correlation between FLL, LER and LED. In addition in Experiment 1 data for ion concentration were obtained only for leaf 6. Therefore the data under discussion for leaf growth are mainly based on the results of Experiment 2.

9.2.1 RELATIONSHIPS BETWEEN FLL, LER, LED, OP AND IONIC CONCENTRATIONS OF SPRING WHEAT LEAVES

Data from Experiment 2 were used to study the relationships between FLL, LER, LED, OP and ionic concentrations in an attempt to find out what factors were responsible for the decreased leaf area of salt affected plants. The various parameters were plotted against each other, first for individual leaves, then for groups of leaves and the correlations between these parameters were determined.

9.2.1.1 RELATIONSHIPS BETWEEN FLL, LER AND LED

For each leaf the mean values of FLL, LER and LED at each salinity level were plotted against each other (Figure 9.1). Salinity decreased FLL of leaves 4, 5, 6 and 7 mainly due to decreases in LER (Table 4.4). Kemal-ur-Rahim (1988) also observed that FLL was mainly affected by LER, whereas LED was relatively unaffected. In Experiment 2, although

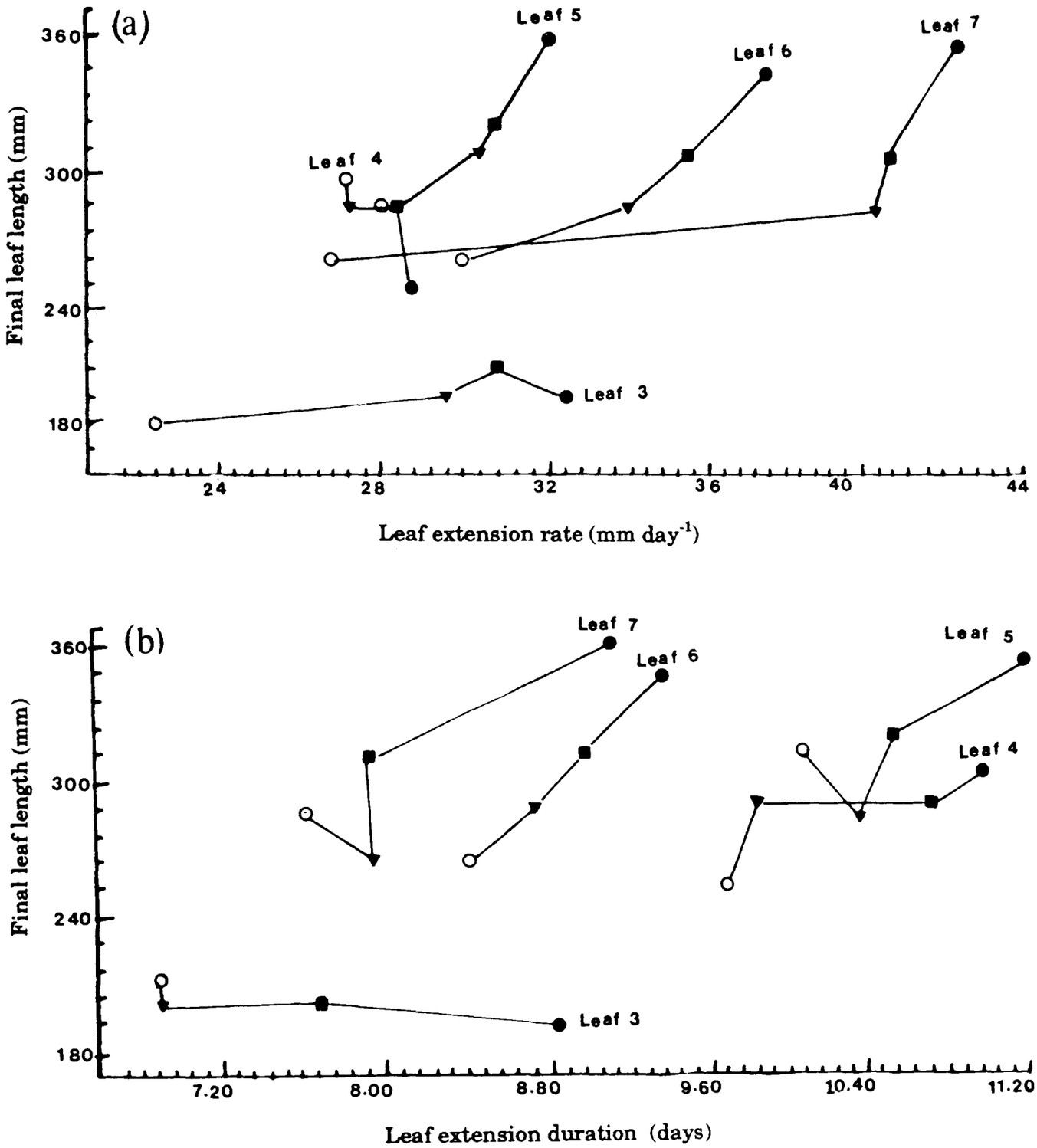


Figure 9.1: Relationships between final leaf length (mm) of different leaf insertions of spring wheat and (a) leaf extension rate (mm day^{-1}) and (b) leaf extension duration (days) at different salinity levels: ●, 0 mol m^{-3}; ■, 50 mol m^{-3}; ▼, 100 mol m^{-3} and ○, 150 mol m^{-3} NaCl.

the values of the linear correlation coefficient between FLL and LER were quite high, they were significant only for leaves 3 and 6. For leaf 7 the relationship was clearly not linear and for leaf 4 the correlation was apparently negative (Table 9.1), although in this case LER was not significantly affected by salinity. The data for leaves 4, 5, and 6 suggest that the relationship between FLL and LER was curvilinear rather than linear. When the data for all leaves were combined, the correlation between FLL and LER was not significant. Variations in FLL between different leaf insertions in the unstressed control treatments were also not correlated with variation in FLL. The correlation between FLL and LED was significant only for leaf 6. Hence although the analyses of variance suggested that LER was the main factor influencing FLL, there were no consistent quantitative relationships between these parameters in different leaves and at different salinity levels. LER has been shown to be more important than LED in determining FLL in barley (Maan *et al*. 1989).

9.2.1.2 RELATIONSHIPS BETWEEN LER, OP AND IONIC CONCENTRATIONS

To determine what factors were influencing LER for each leaf, the values of LER for each treatment were plotted against sap OP, Na^+, Cl^-, K^+ and external NaCl. The relationships between LER, sap OP and ionic concentrations are shown in figures 9.2 and 9.3. When LER was plotted

Table 9.1: Values of the linear correlation coefficient (r) between final leaf length (FLL in mm) in different leaf insertions of spring wheat and leaf extension rate (LER in mm day^{-1}), leaf extension duration (LED in days), sap osmotic pressure (OP in mmol kg^{-1}), sap Na^+ (mol m^{-3}), sap Cl^- (mol m^{-3}) and sap K^+ (mol m^{-3}) and external NaCl (mol m^{-3}).

PARAMETERS	LEAF INSERTIONS					
	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	All Leaves
LER	0.97*	-0.78NS	0.94NS	0.98*	0.78NS	0.57NS
LED	-0.93NS	0.78NS	0.82NS	0.99**	0.85NS	0.57NS
Sap OP	-0.82NS	-0.87NS	-0.99**	-0.95*	-0.98*	-0.05NS
Sap Na^+	-0.79NS	-0.87NS	-0.99**	-0.95*	-0.98*	-0.39NS
Sap Cl^-	-0.66NS	-0.74NS	-0.99**	-0.94NS	-0.99**	-0.25NS
Sap K^+	0.52NS	0.88NS	0.95*	0.96*	-0.99**	0.07NS
External NaCl	-0.83NS	-0.89NS	-0.99**	-0.99**	-0.99**	-0.47NS

NS= not significant; *,**= r significant at 5% and 1% probability levels respectively

Table 9.2: Values of the linear correlation coefficient (r) between leaf extension rate (LER in mm day^{-1}) in different leaf insertions of spring wheat and sap osmotic pressure (OP in mmol kg^{-1}), sap Na^+ (mol m^{-3}), sap Cl^- (mol m^{-3}) and sap K^+ (mol m^{-3}) and external NaCl

PARAMETERS	LEAF INSERTIONS					
	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	All leaves
Sap OP	-0.85NS	0.63NS	-0.98*	-0.89NS	-0.90NS	-0.12NS
Sap Na^+	-0.76NS	0.64NS	-0.89NS	-0.97*	-0.90NS	-0.46NS
Sap Cl^-	-0.67NS	0.62NS	-0.93NS	-0.87NS	-0.83NS	-0.29NS
Sap K^+	0.40NS	-0.51NS	0.84NS	0.89NS	-0.68NS	0.29NS
External NaCl	-0.82NS	0.64NS	-0.95*	-0.98*	-0.85NS	-0.48NS

NS= not significant; *= r significant at 5% probability level

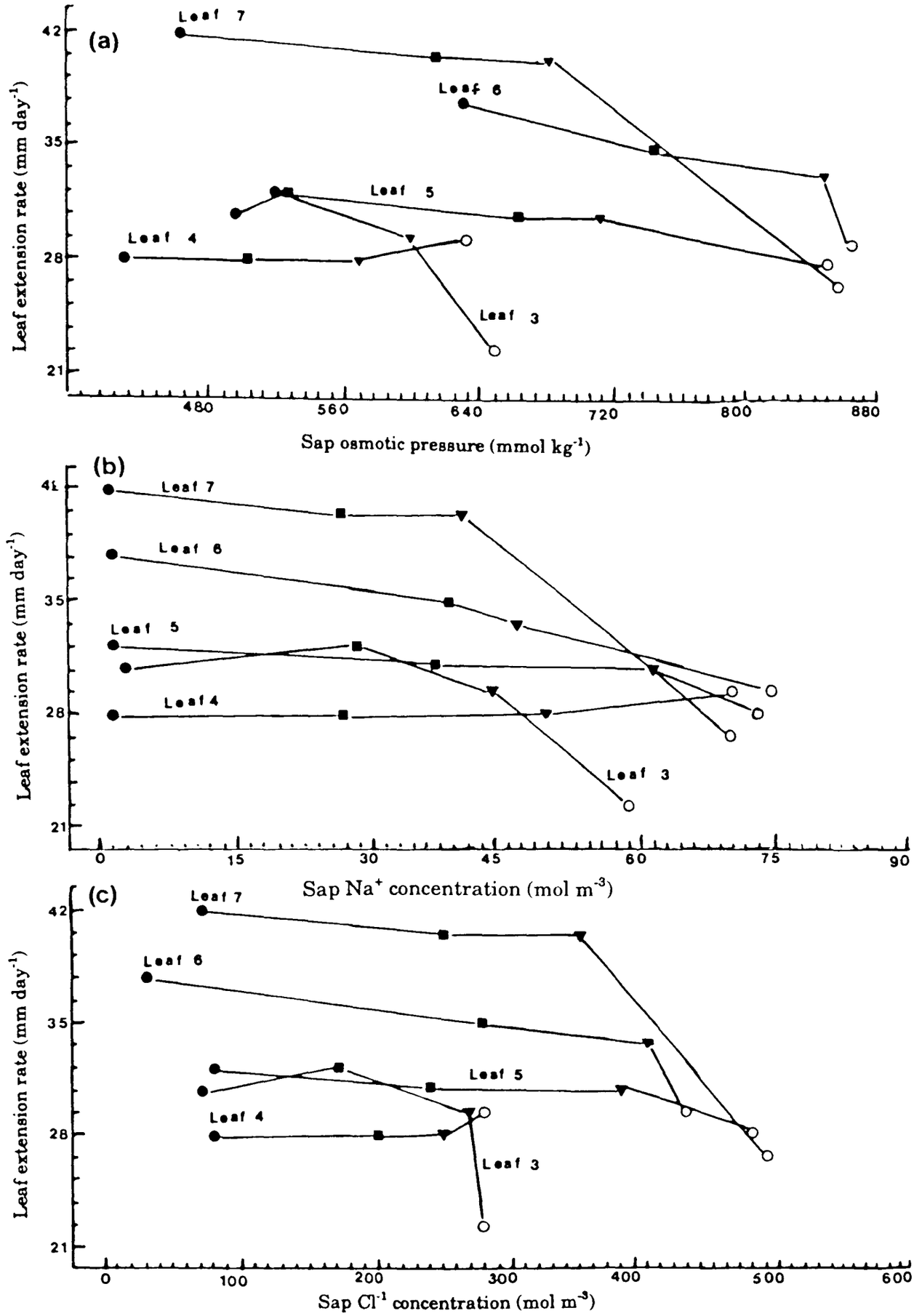


Figure 9.2: Relationships between leaf extension rate (mm day^{-1}) of different leaf insertions of spring wheat and (a) sap osmotic pressure (mmol kg^{-1}), (b) sap Na^+ concentration (mol m^{-3}), (c) sap Cl^- concentration (mol m^{-3}) at different salinity levels: ●, 0 mol m^{-3}; ■, 50 mol m^{-3}; ▼, 100 mol m^{-3} and ○, 150 mol m^{-3} NaCl.

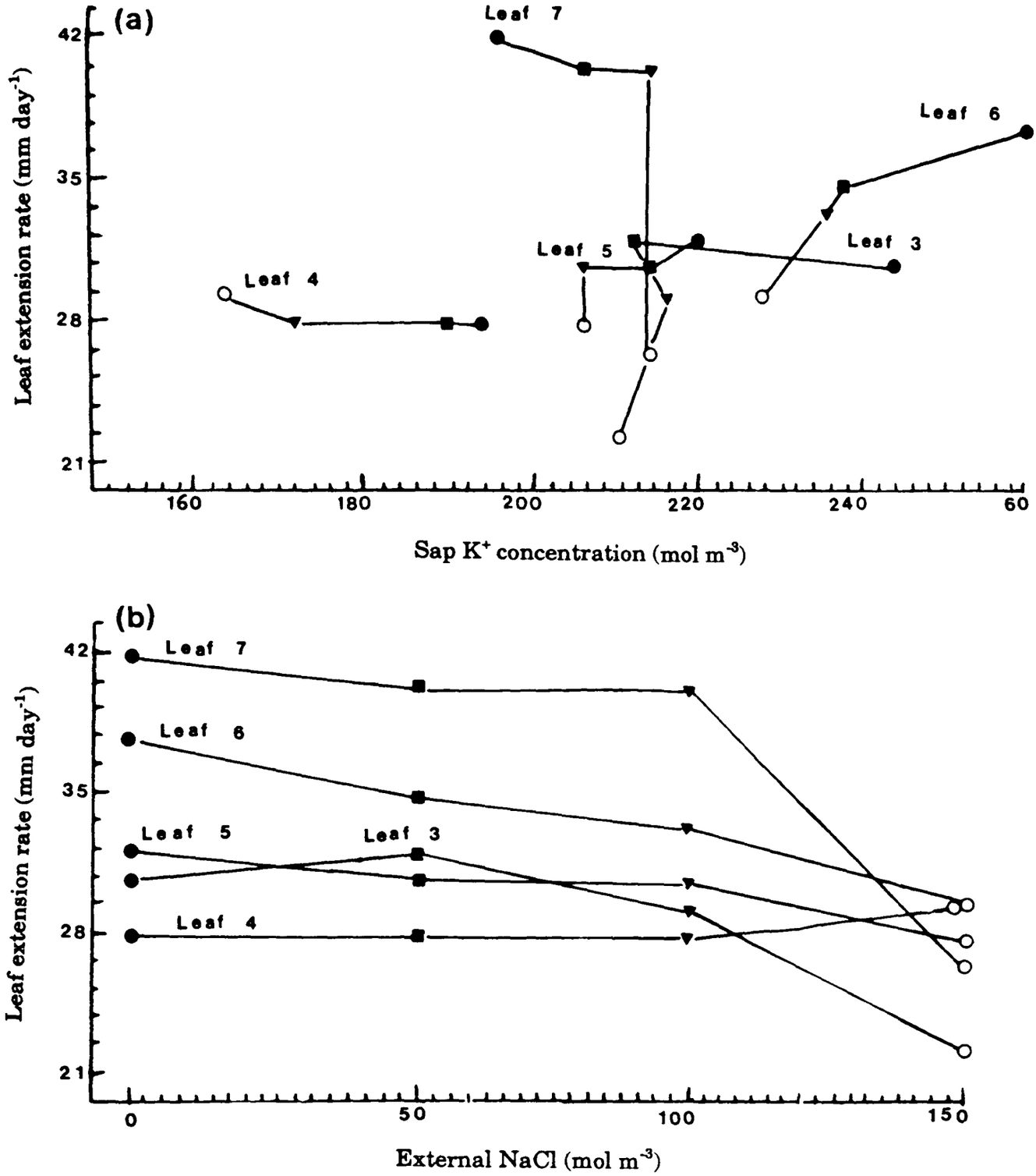


Figure 9.3: Relationships between leaf extension rate (mm day^{-1}) of different leaf insertions of spring wheat and (a) sap K^+ concentration (mol m^{-3}) and (b) external NaCl levels: ●, 0 mol m^{-3}; ■, 50 mol m^{-3}; ▼, 100 mol m^{-3} and ○, 150 mol m^{-3}.

separately against sap OP, Na^+, Cl^- and K^+, no consistent relationships emerged. For example, for leaf 3 in each case the data suggest that LER was higher at low than at zero salinity. For this leaf LER was maximum at 30 mol m^{-3} sap Na^+ and it decreased above and below this. LER for leaf 4 increased or was unaffected as sap OP, Na^+ and Cl^- increased. LER for leaf 5 was slightly decreased as sap OP, Na^+ and Cl^- increased. Therefore for leaves 3, 4 and 5 there were no consistent relationships between LER and OP, Na^+, Cl^- and K^+. LER for leaf 6 consistently decreased as sap OP, Na^+ and Cl^+ increased and this decrease was particularly marked at the highest salinity level (150 mol m^{-3} NaCl compared with 100 mol m^{-3} NaCl). There was little evidence of a threshold of sap OP above which LER decreased whilst there was some evidence that LER decreased when sap Na^+ and Cl^- concentrations became greater than 40 and 400 mol m^{-3}, respectively (Figure 9.2). The trends for sap K^+ were inconsistent for all leaves, suggesting that K^+ was not affecting LER (Figure 9.3). LER was different in different leaf insertions and it only decreased at high external NaCl salinity (Figure 9.3). As with sap Na^+ and Cl^- concentration, there was some evidence of a threshold of external NaCl as LER decreased above 100 mol m^{-3} NaCl salinity (Figure 9.3). There is very little information available in the literature to support the existence of threshold levels of salinity for reducing LER in crop plants. Therefore further experiments need to define these thresholds more precisely.

Papp *et al.* (1983) observed that the initial rate of leaf extension and final leaf length of sugar beet decreased linearly with increase in NaCl concentration. Both of these parameters appeared to be reduced by even the lowest salinity treatment (50 mol m^{-3} NaCl). LER was negatively correlated with sap OP in leaf 5, sap Na^+ concentration in leaf 6 and external NaCl in leaf 5 and leaf 6 (Table 9.2). There was no significant correlation between LER and sap Cl^- or K^+. This may have been partly due to the reason as suggested by Rawson *et al.* (1988b) that there is a correlation between exclusion of Cl^- and K^+ from leaves and NaCl tolerance for several species. The absence of correlation could also be because only 4 salinity levels were tested. The correlation between LER and sap Na^+ was only significant for leaf 6. Munns *et al.* (1988) observed that there was no correlation between Cl^- or Na^+ concentration and leaf growth. Water supply has been shown to have a large effect on LER (Gallagher and Biscoe 1979). Hence in future experiments it would be useful to measure the effects of salinity on both components of leaf water potential; osmotic pressure and turgor pressure.

The relationships between LED and sap OP and ionic concentration were also examined but no consistent trends were apparently observed (data not presented).

9.2.1.3 RELATIONSHIPS BETWEEN SAP OP, Na^+, Cl^-, K^+ AND EXTERNAL NaCl

For each leaf sap OP increased as Na^+, Cl^- and external NaCl increased (Table 9.3). For all leaves except leaf 7 there was a negative correlation between sap K^+ and OP. However, this was significant for leaf 4 only. For leaf 7 there was positive correlation between sap OP and K^+. This suggests that sap K^+ also started contributing towards sap OP in this leaf insertion. Greenway and Munns (1980) and Wyn Jones (1981) suggested that plants need to maintain an optimal concentration of potassium in order to carry out their metabolic functions. This led Flowers and Yeo (1981) to suggest that there are strong selection processes for uniformity of potassium uptake from a non-uniform environment. Experiment 2 suggests that there was generally a negative correlation between sap OP and K^+. These results agree with those of Torres and Bingham (1973), Kumar and Yadav (1983), Kingsbury *et al.* (1984), Rashid (1986) and Gorham *et al.* (1986b) who all found that salinity reduced potassium concentration in shoots of wheat. Kingsbury *et al.* (1984) pointed out that although salinity reduced potassium concentration, the reduction was not enough to be critical to the plant. In Experiment 2 when the values of sap OP for groups of leaves were plotted against sap K^+, there were no significant correlations between these parameters except for leaf 4. Therefore sap K^+ is not the only factor determining sap OP. If sap K^+ does not determine sap OP, then either sap Na^+ or Cl^- may have

Table 9.3: Values of the linear correlation coefficient (r) between sap osmotic pressure (OP in mmol kg^{-1}) in different leaf insertions of spring wheat and sap Na^+ (mol m^{-3}), sap Cl^- (mol m^{-3}), sap K^+ (mol m^{-3}) and external NaCl (mol m^{-3}).

PARAMETERS	LEAF INSERTIONS					
	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	All Leaves
Sap Na^+	0.96*	1.00**	0.96*	0.93NS	1.00**	0.77NS
Sap Cl^-	0.96*	0.97*	0.98*	0.99**	0.99**	0.88NS
Sap K^+	-0.71NS	-0.98*	-0.91NS	-0.93NS	0.93NS	-0.29NS
External NaCl	0.99**	0.99**	0.98*	0.96**	0.99**	0.72NS

NS= not significant; *,**= r significant at 5% and 1% probability levels respectively

Table 9.4: Values of the linear correlation coefficient (r) between external NaCl and sap Na^+ (mol m^{-3}), sap Cl^- (mol m^{-3}) and sap K^+ (mol m^{-3}) in different leaf insertions of spring wheat.

PARAMETERS	LEAF INSERTIONS					
	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	All leaves
Sap Na^+	0.99**	0.99**	0.98*	0.98*	0.99**	0.97*
Sap Cl^-	0.97*	0.96*	0.99**	0.94NS	0.99**	0.89NS
Sap K^+	-0.79NS	-0.98*	-0.97*	-0.92NS	0.96*	-0.29NS

NS= not significant; *,**= r significant at 5% and 1% probability levels respectively

determined it. The results of Experiment 2 showed that for each leaf insertion there were significant linear correlations between sap OP, Na^+, Cl^- and External NaCl, although it was not significant in leaf 6 for sap Na^+ (Table 9.3). These results suggest that for each leaf insertion, external NaCl determines sap OP through effects on either sap Na^+ or Cl^-. When values for all leaves were grouped, there was no obvious correlation between sap OP and these ions suggesting that a single relationship does not hold for all leaves. In future experiments it would be useful to measure the effects of salinity on organic solutes as well as ions to quantify the relationships between ion and OP as these also influence OP (Wyn Jones 1981; Gorham *et al.* 1985b).

When the data for all leaves were grouped together there was a good correlation between sap Na^+ and external NaCl irrespective of leaf insertion (Table 9.4, Figure 9.4). The correlations between external NaCl and sap Cl^-, K^+ or OP were not consistent (Table 9.4, Figures 9.4 and 9.5). Therefore these results suggest that external NaCl determines sap Na^+. Munns *et al.* (1982), working with barley, found that a step reduction in the external NaCl concentration resulted in an immediate increase in leaf elongation rate, while Na^+ and Cl^- concentrations in the elongating region stayed high. They therefore concluded that these tissues do not contain toxic concentrations of these ions. Similar conclusions were also reached for

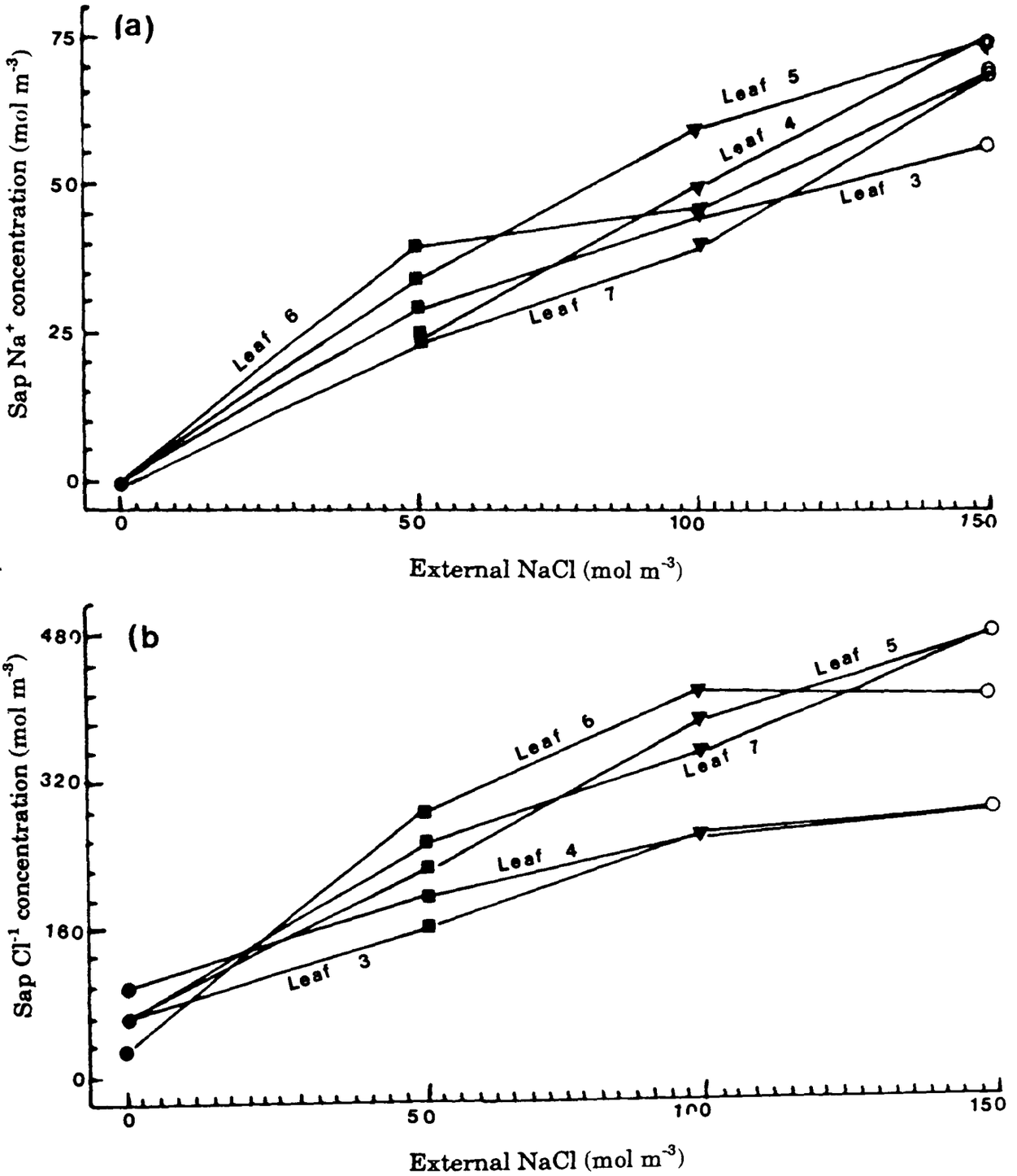


Figure 9.4: Relationships between (a) sap Na^+ concentration and (b) sap Cl^- concentration of different leaf insertions of spring wheat and external NaCl levels: ●, 0 mol m^{-3}; ■, 50 mol m^{-3}; ▼, 100 mol m^{-3} and ○, 150 mol m^{-3}.

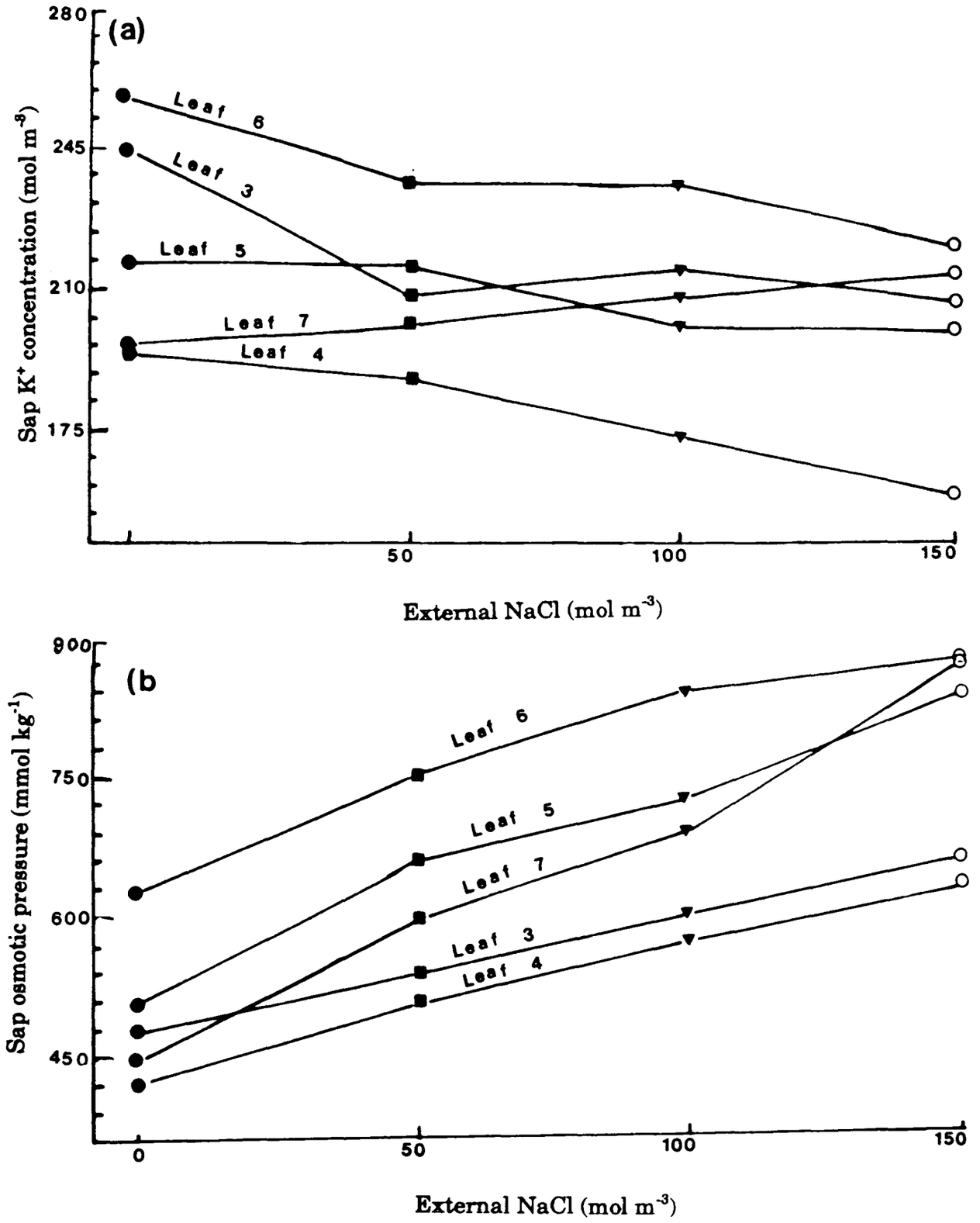


Figure 9.5: Relationships between (a) sap K^+ concentration (mol m^{-3}) and (b) sap osmotic pressure (mmol kg^{-1}) of different leaf insertions of spring wheat and external NaCl levels: ●, 0 mol m^{-3}; ■, 50 mol m^{-3}; ▼, 100 mol m^{-3} and ○, 150 mol m^{-3}.

sunflower (Rawson and Munns 1984). However, in earlier studies (Munns *et al.* 1982) only short-term growth processes were measured, and only effects on cell expansion rate, and ion concentrations in rapidly expanding, highly vacuolated tissues were considered. In Experiment 2, there were no correlations between sap ionic concentration or external NaCl and LER or LED. Therefore sap Na^+ or Cl^- concentrations do not determine leaf growth. These results are in agreement with those of by Munns *et al.* (1988) that the growth of the shoot is not directly controlled by local concentrations of Na^+ or Cl^- of the growing tissues but by some influence originating elsewhere in the plant.

Concerning the modelling of the effects of salinity on plant growth, although it may be possible to predict internal Na^+ concentrations of leaves at full expansion on the basis of external NaCl concentration, the results of these experiments showed that there were no consistent relationships between sap Na^+ and other ions concentrations and sap OP and no consistent relationships between these parameters and leaf extension growth of wheat. Also results of other experiments showed that ion concentrations change markedly with time whereas LER remain constant. Experiments 3 and 4 showed that Na^+ increased during senescence but most leaf extension growth was completed by the time this occurred. The following section considers the relationships between leaf ionic concentrations and gas exchange in salt stressed leaves.

9.3 GAS EXCHANGE

This section considers the factors that were mainly influencing Pn in salt stressed leaves at various stages during expansion and senescence. To do this data from Experiment 3 (leaf 6) and Experiment 4 (leaf 5 and the flag leaf) have been used. Data from Experiment 2 were not used as the conditions under which Pn was measured were very variable. Although Pn was measured at various light intensities during both Experiments 3 and 4 only data recorded after initial enclosure i.e. at high light intensity (1750 μmol m^{-2} s^{-1} PAR) are included.

9.3.1 RELATIONSHIPS BETWEEN Pn, g_s, Na^+, Cl^- AND OP

The first step in this analysis was to consider the relationships between Pn and g_s, Na^+, Cl^- and OP separately for each leaf and salinity level. To do this values of Pn at different times after leaf emergence were correlated with the corresponding values of g_s, Na^+, Cl^- and OP. It was not possible to do this for leaf 6 in Experiment 3 at 200 mol m^{-3} NaCl as there were only 3 data points.

Changes in Pn with time closely followed those of g_s. Table 9.5 indicates that there was a significant positive correlation between Pn and g_s at all salinity levels and leaf insertions. The positive relationship between Pn and g_s has been reported previously in wheat under non-saline conditions (Johnson *et al.* 1987; Morgan *et al.* 1990). Several workers have also attributed decreases in

Table 9.5: Values of the linear correlation coefficient (r) between net photosynthesis (Pn in μmol m^{-2} s^{-1}) and stomatal conductance (g_s in mol m^{-2} s^{-1}), sap Na^+, Cl^- (mol m^{-3}) and sap osmotic pressure (OP in mmol kg^{-1}). Values are for leaf insertions 6 (Experiment 3), 5 and the flag leaf (Experiment 4).

Leaf insertion	Salinity level (mol m^{-3} NaCl)	g_s	Na^+	Cl^-	OP
Leaf 6	0				
	100	0.99**	-0.80NS	-0.74NS	-0.53NS
Leaf 5	0				
	75	0.67*	-0.89**	0.16NS	0.62*
	150	0.92**	-0.91**	-0.97**	-0.99**
Flag leaf	0				
	75	0.90**	-0.92**	0.01NS	0.54*
	150	0.99**	-0.95*	-0.97*	-0.95*

NS = not significant; *,**= r significant at 5% and 1% probability levels respectively

Table 9.6: Values of the linear correlation coefficient (r) between sap Na^+ concentration and percent decreases in net photosynthesis (Pn in μmol m^{-2} s^{-1}). Values are for leaf insertions 6 (Experiment 3), 5 and the flag leaf (Experiment 4).

Leaf insertion	Salinity level (mol m^{-3} NaCl)	Pn
Leaf 6	100	0.84NS
Leaf 5	75	-0.76**
	150	0.91**
Flag leaf	75	0.20NS
	150	0.73NS

NS = not significant; ** = r significant at 1 percent probability level

photosynthesis by salinity to decreases in stomatal conductance (Gale *et al.* 1967; Walker *et al.* 1983; West *et al.* 1986). In contrast Lloyd *et al.* (1987) observed that CO_2 assimilation rate was more sensitive to high foliar salt levels than stomatal conductance in salt stressed Valencia orange. Flanagan and Jefferies (1988) suggested that reduction in net photosynthesis with increases in salinity in *Plantago maritima* L. could be attributed entirely to reduced leaf conductance and low intercellular CO_2 concentrations. The results here suggest that decrease in g_s was main factor reducing Pn. Although, as discussed earlier, parallel reductions in Pn must have occurred as Ci remained constant in the flag leaf at 0 and 75 mol m^{-3} NaCl. On the other hand, Lloyd *et al.* (1987) reported that increases in both sodium and chloride concentrations were correlated with decreased Pn. The results here indicate that there was a significant negative correlation between Pn and sap Cl^- in both leaf 5 and the flag leaf at high salinity (150 mol m^{-3} NaCl). However at low salinity (75 and 100 mol m^{-3} NaCl) this relationship was not significant. This was because at low salinity (particularly at 75 mol m^{-3} NaCl) sap Cl^- increased when the leaf was expanding and thereafter decreased with time. Pn was significantly and negatively correlated with sap Na^+ and OP in all cases except in leaf 6 at 100 mol m^{-3} NaCl. Correlations between Pn, g_s and ion concentrations in cereal leaves have been reported by other workers (Yeo *et al.* 1985; Rawson 1986; Kemal-ur-Rahim 1988; Rawson *et al.* 1988a). Rawson (1986)

suggested that the effect of salt stress on photosynthesis, especially in the short-term, could be due to effects on water relations and especially changes in g_s, as when wheat plants were moved from an unstressed to a stressed environment (75 mol m^{-3} NaCl), Pnmax declined rapidly. Conversely Yeo *et al.* (1985) found that Pn was not reduced in the whole rice plant, but only when Na^+ had accumulated in leaves, suggesting that damage was at the tissue level. Pn in the leaves decrease with age. Therefore there is a confounding effect between leaf age and salinity, as leaf photosynthesis reaches a maximum just before or when leaves were fully expanded (Rawson *et al.* 1983). The results herein suggest that Pn declined as sap Na^+ increased. However Pn also declined in controls (0 mol m^{-3} NaCl) which had no sap Na^+ in the leaves. In an attempt to remove the confounding effect of leaf age the percent decreases in Pn due to salinity at each date were correlated with sap Na^+ concentrations (Table 9.6). In leaf 5 at 150 mol m^{-3} the decrease in Pn increased as sap Na^+ increased. In leaf 5 at 75 mol m^{-3} NaCl sap Na^+ concentration increased throughout development, whereas its effects on Pn and g_s decreased. Pn at this salinity level was higher than at 0 mol m^{-3} NaCl. Therefore the correlation between percent decrease in Pn and Na^+ was negative. For leaf 6 and the flag leaf this correlation was not significant. Therefore no close relationship between Pn and sap Na^+ was observed. It was noted above that Pn was not correlated with sap Cl^-

particularly at low salinity. These observations therefore suggest that the increases in ion concentrations in wheat leaves do not directly cause the observed decreases in Pn and g_s with leaf age, as has also been suggested for barley (Rawson *et al*. 1988a).

If ion concentration did not determine the observed changes in net photosynthesis with leaf age then what was main contributing factor to earlier senescence and death of leaves under salinity? To study this, the mean rates of Na^+ uptake and the rates of decrease of Pn with time were calculated. Table 9.7 shows that there was good correlation between these two parameters ($r= -0.95$, $P<0.01$). It is noteworthy that apart from differences in growth conditions in both Experiments 3 and 4, the rate of Na^+ uptake was faster in the flag leaf (14.8 mol m^{-3}/day) at 150 mol m^{-3} NaCl than in leaf 6 (10.5 mol m^{-3}/day) at 200 mol m^{-3} NaCl. This resulted in a faster rate of decrease of Pn in the flag leaf (1.2 μmol m^{-2} s^{-1}/day) than in leaf 6 (0.65 μmol m^{-2} s^{-1}/day). This faster rate of Na^+ uptake and decreased rate of Pn could have resulted in rapid senescence and death of leaves at these salinity levels. At 75 mol m^{-3} NaCl the plants were able to restrict uptake of Na^+ in both leaf 5 and the flag leaf and Pn declined more slowly. Differences in Na^+ uptake were not correlated with differences in transpiration rate, as the latter was decreased by salinity.

Table 9.7: Rate of Na^+ uptake (mol m^{-3}/day) and rate of decreases of net photosynthesis (Pn in μmol m^{-2} s^{-1}/day) in different leaf insertions of spring wheat under saline conditions. Values are leaf 6 (Experiment 3), leaf 5 and the flag leaf (Experiment 4).

Leaf insertion	Salinity level (mol m^{-3})	Na^+ uptake per day	Decreases in Pn per day
Leaf 6	100	6.10	-0.534
	200	10.50	-0.645
Leaf 5	75	1.41	-0.304
	150	0.89	-0.374
Flag leaf	75	1.94	-0.303
	150	14.80	-1.200

r^2= 89.5, r= -0.946**, P<0.01

Overall, in Experiment 3 salinity decreased Pn more than GLA. However in Experiment 4 salinity decreased GLA more than Pn. Hence both factors were responsible for the decrease in LP in these experiments. In future experiments, it would be useful to study the effects of salinity on these two parameters in field grown corps, to try to establish which is the most important contributing factor to decrease LP. This might then be used as a selection criterion when screening varieties for salt tolerance.

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APPENDIX A

EQUIPMENT USED

Aerators: 'Supa' Aquatic Supplies Ltd., 'Conway' Hawthorne Close, Barlborough, Chesterfield, Great Britain.

Air Compressor: Compair-Brown Wade, High Wycombe, England.

Automatic Area Meter: Model AAM7, Hayashi Denkoh Co. Ltd., Tokoyo, Japan.

Balances: Sartorius, West Germany.

Bungs: Grey Neoprene Bungs, Scientific Services, High Street Tattenhall, Chester, England.

Centrifuge: Clandon MLW T52.1, Centrifuge, England.

Conductivity Meter: Model p335, Portland Electronics Ltd., 18 Greenacres Road, Oldham, England.

Containers: WCB container, Cookson Plantpak, Mundon, Maldon, Essex, England.

Fluorescent lights in growth room: 125W 'Warm White', Philips, Einhover, Holland.

Fridge: Vindon Scientific Ltd., Diggle, Oldham, England.

Germolene New Skin: Beecham Proprietaries, St Helens, England.

Infra-Red Gas Analyser model LCA2:The Analytical Development Co., Ltd., Pindar Road, Hoddesdon, Herts, En11 0AQ
Air Supply Unit:
Datalogger Type DL2:

Large Drying Ovens: Unitherm, Drying Oven, Russell-Lindsey Light Engineering Ltd., 60-62 Constitution Hill, Birmingham, England.

Light Chamber Bulb: GEC True Light Mercury Fluorescent 1000W MBF/U, England.

Microcentrifuge Tubes: Beckman Microfuge B, Spino Division, 1117 California Av., Palo Alto CA, USA.

Microscope: Laboral 2 Microscope, Carl Zeiss Jena, C.Z. Scientific Instruments Ltd., Po Box 43, 2 Elstreeway, Borehamwood, Herts, UK.

Needles: Terumo needles (236x1.25), Fiscons/MSE, MSE Scientific Instruments, Manor Royals, Crawley West Sussex, England.

pH Meter: Ionalyser-Specific ion meter, Model 407A, Orion Research Inc., Cambrige, Mass, USA.

Phostrogen: Phostrogen Ltd., Corwen, Clwyd, UK.

Pipettes: Eppendorf Varipipette (4720) and Multipipette (4780),
Eppendorf Geratenbau, Netherlert, Hirz Gmbh, Postfach 65, 0670,
2000, Hamburg 65, West Germany.

Plantpak Plug Trays P180: Cookson Plantpak, Mundon, Maldon, Essex, England.

Seed Counter: Numigral-Tecator, Box 70, 5-26301, Hoganas, Sweden.

Small Mill: Cyclotec 1093, Sample Mill, Tecator, Sweden.

Tubing: Silicone Tubing and non-sterile polythene tubing, Portex Ltd., Hythe, Kent, England.

Vapour Pressure Osmometers: Model 5100c, Westcor Inc., 459 South
Main Street, Logan, UT. 84321, USA.

Vortex Stirrer: Gallenkamph Spinmix, England.

APPENDIX B

A. Phostrogen

1. Experiment 1: 10 g of Phostrogen was added to 10 l water (1.0 g l^{-1})
2. Experiment 2 & 5: 10 g of Phostrogen was added to 25 l water (0.4 g l^{-1})
3. Experiment 3 & 4: 4 g of Phostrogen was added to 10 l water (0.4 g l^{-1})

Composition of Phostrogen

Nutrients	Composition (Percent)
N	10.00
P	10.00
K	27.00
Mg	1.30
Fe	0.40
Mn	0.02

B. Modified Long Ashton stock solution

The recipe for 100 l of working-strength Long Ashton micronutrient solution

Solution	Salt	Concentration ($\mu g\ cm^{-3}$)	Stock solution ($g\ l^{-1}$)	Volume of stock for 100 litres nutrient solution (cm^3)
1	NaFeEDTA	Fe 2.80 Na 1.0	37.33	50
2	H_3BO_3	B 0.54	31.00	10
3	$Na_2MoO_4.2H_2O$	Mo 0.048	1.21	10
4	$MnSO_4.4H_2O$	Mn 0.55	22.30	10
	$CuSO_4.5H_2O$	Cu 0.064	2.50	10
	$ZnSO_4.7H_2O$	Zn 0.065	2.90	10

APPENDIX C

The recipe for 10 litres nutrients and salts stock solution

Solution	Salt	Containers	
		10 litres	25 litres
A. Nutrient Stock solution (cm^3)			
1	NaFeEDTA	5	12.5
2	H_3BO_3	1	2.5
3	$Na_2MoO_4.2H_2O$	1	2.5
4	$MnSO_4.4H_2O$	1	2.5
	$CuSO_4.5H_2O$	1	2.5
	$ZnSO_4.7H_2O$	1	2.5
B. Phostrogen (g)		40	100
C. Salts (g)			
Salinity level (mol m^{-3} NaCl)			
0	NaCl	-	-
	$CaCl_2.2HO_2$	-	-
50	NaCl	292.50	731.25
	$CaCl_2.2HO_2$	27.75	69.37
75	NaCl	438.75	1096.88
	$CaCl_2.2HO_2$	41.62	104.05
100	NaCl	585.00	1462.50
	$CaCl_2.2HO_2$	55.50	138.74
150	NaCl	877.50	2193.75
	$CaCl_2.2HO_2$	83.24	208.11
200	NaCl	1170.00	2925.00
	$CaCl_2.2HO_2$	110.99	277.48

One litre of this nutrients and salts stock solution was added to each container during solution changes.

APPENDIX D

The Fortran language programme on VAX mainframe computer dedicated to calculate all the missing values of gas exchange parameters.

```
      PROGRAM DATALOG
      CHARACTER*20  FNAM1,FNAM2
         REAL OGS,OGB,IGC,ES,EXPONENT,W,EO,E,H,DELTAT,
              SIGMA,TL,EXPO
      REAL EL,RS,GS,FNCO,EE,GC,EEI,CC,A,GB,CL,P,AP
      WRITE(6,*)'INPUTFILE...'
      FNAM1='FIN'
      READ(5,'(A20)')FNAM1
      WRITE (6,*) 'ENTER ATMOSPHERIC PRESSURE'
      READ(5,*)AP
      P=AP/1000.0
      WRITE(6,*) 'ENTER NUMBER OF LINES'
      READ(5,*)NUM
      WRITE(6,*) 'OUTPUT FILE...'
      FNAM2='FOUT'
      READ(5,'(A20)')FNAM2
         OPEN(20,FILE=FNAM1,STATUS='OLD')
         OPEEN(21,FILE=FNAM2,STATUS='UNKNOWN')
         DO 10 I=1,NUM
               READ(20,200)HC,CI,CO,Q,TA,A
  200 FORMAT (16X,F4.2,F4.1,F4.1,F4.0,F4.2,24X,F4.2)
      V=6.6666667
      LAMDA=44750-(TA/32)
      MA=28.97
      CP=1.012
      RB=0.30
      EMAX=2.3
      EXPONENT=EXP(((18.564-(TA/254.4)*TA)/(TA+255.57))
      ES=6.13753 * EXPONENT * 1E-3
      W=((V*P)/((273+TA)*A))*120.311
      EO=ES * (HC/100)
      E=(EO/(P-EO))*W
      H=0.175*Q
      SIGMA=(4.5+(TA/16)
      DELTA=H-(LAMDA*E))/(((0.93*MA*CP)/RB)+SIGMA)
      TL=TA + DELTAT
      EXPO=EXP(((18.564-(TL/254.4))*.TL)/TL)/(TL+255.57))
      EL=6.13753 * EXPO * 1E-3
      RS=(((EL/EO)-1)/W)-RB
      GS=1/RS
      FNCO=(1+(7.87*1E-4*CO))
      EE=EXP(-70*EO)
      EEI=1-EE
      CC=CO-(EMAX*FNCO*EEI)
```

```
      A=(CI-(CC/(P-EO)))*W
      GB=1/RB
      OGS=1.6/GS
      OGB=1.37/GB
      IGC=OGS+OGB
      GC=1/IGC
      CL=(((GC-(E/2))*CC)-A)/(GC+(E/2))
         WRITE(21,210)Q,TL,E,GS,A,CL
210   FORMAT(F10.0,F10.1,F10.4,F10.3,F10.2,F10.1)
      READ(20,*)
10              CONTINUE
                STOP
                END
```