Interaction Between the Effects of Sodium Chloride and High Temperature on the Vegetative Growth of Tomato (*Lycopersicon esculentum* Mill.)

by

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I dedicate this thesis to my family

JU DI JING & JING JING WANG

whose moral support in many ways and

over many years enabled me to pursue my studies

TABLE OF CONTENTS

SUMMARY

The aim of this project was to study the nature of the interaction between the effects of salinity and high temperature on the vegetative growth of the tomato plant. Plants were maintained at 0 and 150 mM NaCl concentrations and a range of temperatures (13/8, 23/18, 33/28, 38/33 and 43/38°C day/night) for 2 weeks.

Maximal dry weight growth of the tomato plant occurred around 23/18°C air temperature for both control and salt treatments, and plant growth ceased and plants died at 43/38°C under salinity stress. Salt-treated plants at lower temperatures and control plants at all temperatures survived. The relative growth rate (RGR) of the whole plant was significantly affected by the interaction between the effects of salinity and temperature. The response of net assimilation rate (NAR) to the interacting stress changed in a similar manner to RGR. Leaf expansion rate was also reduced significantly with the combined stress, but leaf area ratio (LAR) was significantly lowered by salinity alone. The inhibition of RGR by the interacting stress conditions could be primarily attributed to lessened NAR, and not to changes in LAR, as the effects on RGR and NAR were closely correlated. The reduction in RGR of the whole plant thus appears to be associated with reduced photosynthesis and, possibly increased respiration. This effect on NAR was further modified by effects on assimilate allocation and the relationship between dry weight gain and expansion growth. In contrast to the leaf, more dry matter was partitioned to salt-treated stems than to control stems. The sensitivity of three different organs, i.e. the leaf, stem and root in terms of differential RGR to the interacting stress appeared to differ. The growth in stem dry weight was more tolerant to salinity and high temperature than leaf and root dry weight gain. An increased ratio of shoot height to stem dry weight under salinity stress suggests that the mechanisms controlling shoot elongation and assimilate allocation are different.

An examination of the causes of the interaction between salinity and high temperature effects on the rate of tomato growth indicated that leaf water (Ψ) and osmotic (Ψ_s) potential

were lowered by salinity at all temperatures. Except at 33°C, the difference in leaf Ψ between the control and salt treatments was around -0.65 MPa, comparable with the level of external osmotic potential (-0.68 MPa). The estimated turgor (Ψ_p) was higher in salt-treated leaves than control leaves at a range of temperatures. It may be concluded that the changes in leaf water relations are unlikely to be responsible for the decline in NAR and RGR.

Investigation of the metabolic consequence of lowered water status in plants disclosed that the concentration of proline throughout the plant and of sucrose and *myo*-inositol in the leaf was higher in saline conditions than in the control whilst reducing sugar concentration was lowered by salinity at all temperatures. The concentration of leaf total free amino acids, apart from proline, was not influenced by salinity or temperature except when plant growth ceased at 43/38°C. However, the contents of proline in all three tissues and of soluble sugars other than sucrose in the leaf tended to decrease with increasing temperature under saline conditions. This is compatible with a possible shortage of carbon supply due to the interacting effects of stress on NAR.

Further examination of the causes of the reduction in RGR and NAR of tomato plants in response to the interacting stress concentrated on ion relations of the plant tissues. The net uptake and transport rates of Cl^- and Na^+ (nmol cm^{-2} root surface area h^{-1}) to the whole plant and to the shoot (stems and leaves) increased linearly with increase in root temperature, with similar rates in both sand and hydroponic culture conditions at corresponding temperatures. As a consequence, the concentration of Cl^- and Na^+ in all three component organs increased at high temperature with the exception of Na^+ in the root. K^+ concentration decreased with salinity at almost all temperatures, but competitive uptake of Na^+ and K^+ was not apparent. The concentration of the leaf nutrient ions, S^{2-}, P^-, B^-, Mg^{2+} Mn^{2+}, $Cu^{2(3)+}$, $Ni^{2(3)+}$ and Zn^{2+}, but not Ca^{2+}, Mo^{2+} and $Fe^{2(3)+}$, was significantly affected by the interacting stress between salinity and temperature. However, none of these changes were judged to be critical to the growth response. Both total anion and cation concentrations (without Cl^- and Na^+) were also lowered by salinity at all temperatures and significantly effected by the interacting stress, however, the ratio of

anions to cations was not affected by salinity. The accumulation of Cl^- and Na^+ within the plant tissue is the most likely primary cause of the reduction in RGR and NAR recorded.

It was hypothesised that high temperature modified the effects of salinity on plant growth through effects on transpiration and root cellular membrane selectivity. A change in ambient temperature alters vapour pressure deficit (VPD) of the shoot environment and temperature in the root medium. Experiments in which the transpiration rate was modified by varying temperature and VPD demonstrated that an increase in air temperature and hence VPD caused a higher transpiration rate, and a resultant increase in water flow rate through the root surface. However, the water flow rate did not significantly affect the rate of uptake and transport of Cl^- and Na^+ to the whole plant and shoot. Root temperature had no effect on transpiration rate, but a change in root temperature produced significant effects on the rate of uptake and transport of Cl^- and Na^+. There was no interaction between effects of VPD and root temperature on transpiration or Cl^- and Na^+ uptake. These results suggest that Na^+ and Cl^- uptake is independent of water flow through the root, and that the major resistance to ion movement is located in the symplast of the root cells. Root temperature modifies root membrane properties, leading to changes in the permeability of root cells to the ions.

The characteristic response of roots to high temperature using an electro-physiological technique showed that the electrical potential difference between the bathing solution and xylem vessels was hyperpolarized by increasing the root temperature, but unaffected by air temperature. The electrical potential gradient was also capable of responding quickly to a metabolic inhibitor.

STATEMENT

I hereby declare that the thesis here presented contains no work which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed..... Date...1/11/1983

DING XIANG WANG

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LIST OF ABBREVIATIONS AND SYMBOLS

B	Boric acid, H_3BO_3
Ca^{2+}	Calcium
Cl^-	Chlorine
$Cu^{2(3)+}$	Copper
D or d	Day
dw	Dry weight
$Fe^{2(3)+}$	Iron
J_{tot}	Rate of ion uptake to whole plant
J_s	Rate of ion transport to shoot
K^+	potassium
L_a	Leaf area
LER	Leaf expansion rate
ln	Natural logarithm [logarithm to base e, i.e. $\log_e$]
LSD	Least significant difference
Mg^{2+}	Magnesium
M_l	Leaf dry weight
Mn^{2+}	manganese
Mo^{2+}	Molybdenum
M_r	Root dry weight
M_s	Stem dry weight
M_{tot}	Total dry weight per plant
N	Night
Na^+	Sodium
NaCl	Sodium chloride
NAR	Net assimilation rate
$Ni^{2(3)+}$	Nickel
ns	no significance statistically
P	Phosphate, HPO_4^{2-} ($H_2PO_4^-$)

R^2	Correlation coefficient
RGR	Relative growth rate
RH	Relative humidity
R.O.	Reverse osmosis
rpm	Revolution per minute
S	Sulphate, SO_4^{2-}
S_h	Shoot height
TRP	Trans-root potential
VPD	Vapour pressure deficit
Zn^{2+}	Zinc

h	Hour [time]
min	Minute [time]
cm	Centimetre
mm	Millimetre
g	Gram
mg	Milligram
µg	Microgram
ml	Millilitre
µl	Microlitre
mM	Millimolar
µmol	Micromolar
nmol	Nanamolar
mmole	Millimole
mN	Milli-normality
MPa	Megapascal
mV	Millivolt

*	Probability 0.05
**	Probability 0.01
℃	Degree Centigrade
ΔE	Effect of interaction (the difference between control and salt treatments)
Ψ	Water potential (MPa)
Ψ_{ext}	External water potential (MPa)
Ψ_{leaf}	Leaf water potential (MPa)
Ψ_s	Osmotic potential (MPa)
Ψ_p	Turgor pressure (MPa)

LIST OF FIGURES AND TABLES

List of Figures:

List of Tables:

CHAPTER I

GENERAL INTRODUCTION

The responses of plants to extreme environments, the responses to stress, have generally been assessed individually. The responses to salinity have been measured at near-optimum temperatures with abundant water supply (Hayward and Long, 1943; Greenway and Munns, 1980; Munns and Termaat, 1986; Sacher, Staples and Robinson, 1983; Tal, 1971). However, in nature, this is often not the case with plants growing in an environment less than optimal in two or more characteristics. For example, chilling or heat stress is often accompanied by water stress (Levitt, 1980a; Li *et al.* 1991; Naidu, 1987; Palta, 1989, 1990; Turner and Kramer, 1980). Thus, examining the interaction between such environmental factors in determining plant growth will allow a better understanding of the responses of plants in the field. In particular, the interaction of the effects of salinity in the rooting medium and high temperature will simulate the environment encountered by horticultural crops growing in South Australian Riverland districts and similar irrigated areas in the summer months.

Salinity and high temperature are known to inhibit the growth of plants by affecting a range of physiological processes individually. In a saline environment, the growth of glycophytes is inhibited by either water deficit or specific ionic effects on cell expansion and metabolism dependent upon the duration of salinity (Munns and Termaat, 1986). Thus ion uptake, transport within the plant and sequestration in tissues are crucial to plant responses to salinity. Specific effects of the ions in the plant such as inhibition of photosynthesis and accumulation of compatible solutes have been described (Levitt, 1980b; Greenway and Munns, 1980; Rajasekaran, 1988). Similarly, temperature effects on membrane characteristics, including ion permeability and membrane-bound enzyme activities have been reported (Pike and Berry, 1980; Levitt, 1980a). It has been suggested that extreme temperature injury is associated with lipid changes and protein denaturation and thus cellular membranes play a key role in high temperature injury to plants. There is overwhelming evidence that high temperature can disrupt membrane structure, and therefore the membrane-associated functions (Berry and Bjorkman, 1980; Schreiber and Berry, 1977). The loss of plasma membrane integrity because of heat injury has been shown to cause leakage of solutes and loss of other cell contents (Ingram, 1985; Ingram

and Buchanan, 1984; Levitt, 1980a). The intention in the present project is to examine the interaction between these two responses.

Tomato (*Lycopersicon esculentum,* cv. Duke) will be used as the test plant as the response of this genotype to salinity is comparatively well known (Rajasekaran, 1988). Once the growth response to salinity at a range of temperatures from optimal to clearly supra-optimal has been established, the aim of the research will be to characterise the nature of the interaction between salinity and high temperature. Initially the hypothesis that high temperature modifies membrane properties and thus uptake of Na^+ and Cl^- ions and trans-root potential will be explored. Alternative hypotheses concerning water relations, metabolic responses and ion distribution in the plant will be examined where possible.

CHAPTER II

REVIEW OF LITERATURE

1. Introduction

There is an extensive literature on the various responses of unicellular micro-algae and vascular plants from halophytic and non-halophytic habitats concerning their physiological, biochemical and ion regulatory mechanisms which contribute to sustained growth or survival in salt stress. The publications of Strogonov (1964) and Poljakoff-Mayber and Gale (1975) summarized the research carried out on the various effects of salinity on plants, including the morphological, anatomical and physiological changes occurring in response to salinity or typical for halophytes. These findings were extended by reviews (Flowers *et al.* 1977; Kirst, 1989; Munns *et al.* 1983) which covered the responses of unicellular organisms. A more complete view of non-halophyte responses to salinity and mechanisms of adaptation (Bernstein and Hayward, 1958; Fick *et al.* 1988; Greenway and Munns, 1980; Staples and Toenniessen, 1984; Wyn Jones *et al.* 1977;) have further increased our understanding of the respective areas. Similarly, the effects of high temperature on plants have been presented in some publications (Long and Woodward, 1988; Turner and Kramer, 1980). Most of the information that has been compiled on this matter relates only to the "single end effect" of either salt or temperature stress. To keep the subject matter within the reasonable scope of this thesis, the review will specifically address some of the primary effects of salt and extreme temperature stress on crop plants (non-halophytes), particularly cultivated tomato, through alterations in vegetative growth and their responses to the changes in water status, ions and metabolism.

2. Plant Growth during Salt and High Temperature Stress

2.1 Salt Stress

Salinity can inhibit the growth of crop plants because of low external water potential, ion toxicity, ion imbalance or a combination of these factors (Greenway and Munns, 1980). The degree to which each of these factors affects growth depends on the plant genotype and environmental conditions.

The inhibition of leaf elongation and shoot growth by salinity has been well documented in many species including wheat (e.g. Kingsbury and Epstein, 1984, 1986), barley (e.g. Delane *et al.* 1982; Cramer *et al.* 1989, 1990), bean (Neumann *et al.* 1988), maize (e.g. Cramer and Bowman, 1991) and others (Maas and Hoffman, 1977; Marcum and Muroloch, 1990; Munns *et al.* 1982, 1986; Poljakoff-Mayber and Gale, 1975; Termaat and Munns, 1986). The degree to which salinity influences plant growth is related to the salt concentration in the root medium. At low salinity, root growth may not decrease at all while shoot growth declines, e.g. barley (Delane *et al.* 1982), or root growth may increase, e.g. sorghum (Weimberg *et al.* 1984). This indicates that shoot growth is more sensitive to salinity than root growth, so the root/shoot ratio increases (Munns and Termaat, 1986). These effects are clear even in the short term (one or two days). At high salinity, a severe impairment of growth occurs with chlorosis and death, particularly in long term experiments (Bingham *et al.* 1968; Bernstein *et al.* 1969; Bernstein, 1975). Hampson and Simpson (1990) demonstrated that there was enhanced growth of wheat plants in distilled water or polyethylene glycol solution, but reduced growth if salt stress below -0.6 MPa was present. These observations also implicated an accumulation of ions in the growth reduction. There is great varietal difference in grapevine Cl^- resistance, but all varieties tested are injured at the same foliar concentrations (0.55 ~ 0.56 m^{mol}_{λ} g^{-1} dw) (Ehlig, 1960). The varieties Black Rose and Cardinal are injured more severely than Perlette and Thompson Seedless because they accumulate Cl^- two or three times faster. Obviously, the build-up and toxicity of salts in the cells would be responsible for the growth reduction in non-halophytes.

Salinity causes stunting of vegetative growth of the cultivated tomato, which exhibits a typical non-halophyte response. The principal effects of high salinity found by Hayward and Long (1941, 1943) were a reduction in cambial activity, maturation of cells of smaller size, relatively thicker walls in xylem elements and mechanical cells as well as inhibited floral development, reduced set of fruit and a reduction in size and total yield of fruit. Strogonov (1964) reported a 50% inhibition of growth in tomatoes growing in soil containing 0.1% chloride. More recently, Rajasekaran (1988) showed that both progressive and prolonged salinization significantly reduced leaf area and fresh and dry shoot weights

of tomato while accumulation of Cl^- and Na^+ took place. The higher the concentration of the sodium solution, the greater was the reduction in height and diameter of stems and fresh and dry weights the tomato shoot (Salim, 1989; Hayward and Long, 1941; Dehan and Tal, 1978). At equivalent concentrations of sodium (NaCl and Na_2SO_4), plants in the high chloride series showed greater growth inhibition than those in the high sulphate series. This might suggest that the toxic effect of Cl^- exceeds that of SO_4^{2-} ion, but it should be recognized that the activity of Cl^- is much greater than that of SO_4^{2-} in the respective solutions (Hayward and Long, 1943). These reports with NaCl and Na_2SO_4 suggest that salinity contributes to the reduction in growth, although the correlation between the reduction in growth and ion accumulation cannot be directly attributed to either Cl^- or Na^+ until the effects of these factors are separated. In contrast to the cultivated tomato, *Lycopersicon esculentum*, the growth of the wild species, *Solanum pennellii*, was not impaired by high salinity, although the latter accumulated more Cl^- and Na^+ and its K^+ level decreased (Dehan and Tal, 1978). These results suggest that the degree to which each of the saline conditions affects growth depends on the genotype of the plant and environmental conditions.

2.2 High Temperature

Plant species have an optimum temperature for growth and development that is determined by a complex of genetic, developmental and cultural factors. Investigations of high temperature stress usually bring to mind studies on thermophilic organisms that inhabit hot summer regions, and on seed plants existing in hot deserts. These plants can survive temperature of 50°C or higher (Jozefowicz, 1930; Turner and Kramer, 1980). However, other plants suffer from high temperature stress often encountered during the normal growing season. For example, many plants from alpine habitats are quite unable to survive hot summer weather at low altitudes, and cool season crops such as broccoli, lettuce and English peas do poorly at mid-summer (Turner and Kramer, 1980).

High temperature also limits or prevents growth of tomatoes during the hot season in many regions of the world (Abdehafeez *et al.* 1971; Aung, 1976, 1989; AVRDC, 1989;

Hussey, 1963, 1965; Jozefowicz, 1930). Tomato seedlings at the 2-leaf stage, when exposed to a brief daily treatment of 30°C night temperature for 4 - 5 weeks, suffer significant growth reduction (Bendir and Went, 1956). Due to different cultivars and cultural practice, the number, size, vigour and growth pattern of the axillary shoots ("side shoot", "laterals") of tomato cultivars differ. However, axillary shoot fresh weight of many cultivars was reduced after 4 days at 34°C day/30°C night (Aung, 1989; Aung and Austin, 1971). Comparison of the effect of high temperature of 35/25°C day/night (D/N) with normal temperature of 22/18°C D/N showed that stem growth was twice as fast at the higher temperature, giving thin stems and many trusses with infertile flowers (Abdapla and Verkerk, 1968). A higher greenhouse temperature decreases the rate of root growth and increases the ratio of shoot/root dry or fresh weight (Went, 1943, 1945). This has been related to root temperature increase (Lingle and Davis, 1959) and heating the roots always reduces the fresh and dry weights of roots (Gosselin and Trudel, 1983). This may be interpreted to indicate that available assimilate is exhausted more quickly and growth slows as a result (Gosselin and Trudel, 1984). Root-zone temperature and salt interacted to determine dry weight production such that the tolerance of barley to NaCl was altered by root temperature (Mozafar and Oertli, 1990, 1992). The long-term exposure to high temperature may result in injury to root membrane properties (Quinn, 1988). As a result, the plant growth slows down and plants die (Gosselin and Trudel, 1984; Snapp and Shennan, 1992).

3. Physiological and Biochemical Responses to Salt and High Temperature Stress

Little is known in particular of the physiological and biochemical mechanisms leading to the growth reduction of plants subjected to an interaction of salt and high temperature stress. The primary physiological mechanisms causing the limitation of growth of non-halophytes in saline conditions are still not conclusively elucidated but centre on two aspects, reduced water potential and ion imbalance and toxicity. However, each stress may itself have a specific effect on plant metabolic processes in addition to dehydration (Levitt,

1980a, b). Hence, this discussion deals with individual responses to salt and high temperature stress.

3.1 Water Relations

3.1.1 Water relations and salt stress

As has been seen, salinity, a 'physiological drought', and heat stress can each result in tissue water deficit (Levitt, 1980a, b). It has been proposed that the limiting process in whole-plant responses to salinity in short term exposure (days) is probably the water deficit (Greenway and Munns, 1980; Munns and Termaat, 1986).

3.1.1.1 *Water status*

Reduced water potential (Ψ), which contributes to growth reduction in the short term, is one of the main physiological responses of plants to salt stress. Salinity resembles water deficit in the resultant low tissue Ψ. Plants exposed to saline conditions experience a shortage of water and this concept persuaded Schimper (1903) (see Levitt, 1980a) to formulate his 'physiological drought' theory which postulated that both halophytes and glycophytes suffer from dehydration in saline conditions. A consequent low hydration of protoplasm was deemed to be responsible for the changes in metabolic activity and growth reduction evident in plants subject to low tissue Ψ (Kramer, 1959; Levitt, 1956; Kozlowski *et al.* 1990). The growth reduction by salt is usually proportional to the external osmotic potential (Ψ_s), but also, of course, to external salt concentration (Weimberg, 1986). The earliest response of a non-halophyte exposed to salinity is that the leaves grow more slowly than the roots, before ions in the shoot have built up to high levels (Termaat and Munns, 1986). Soybean growth reduction was observed 0.8 h after transfer to -0.3 MPa water stress and, after transfer back to non-stress, growth recovered in 0.5 h (Bensen *et al.* 1988). In a study with *Chlorella emersonii*, Setter and Greenway (1979) found that growth declined to a similar extent in NaCl solution as in slowly-permeating mannitol and raffinose. In a comparison of the effects of NaCl and Na_2SO_4 on tomato vegetative

growth, it was found by Hayward and Long (1943) that a decrease in Ψ_{ext} due to salt was accompanied by an increase in the osmotic concentration of the shoot juice and by growth reduction. Osmotic potential (Ψ_s) of the culture solution was probably the dominant factor in the inhibition of growth since, when height of stem and fresh and dry weights of shoot were plotted against the Ψ_s of the culture solutions, the differential effects of Cl^- and SO_4^{2-} ions were small (Hayward and Long, 1943). These studies suggest that it is the adverse water relations that reduce growth.

3.1.1.2 *Osmotic adjustment (Turgor maintenance)*

Low Ψ in the soil due to salinization or water deficit usually induces a proportional initial decline in plant Ψ_s and as a result, turgor is maintained. Such a decline in Ψ_s could be due to uptake of ions from the root medium or synthesis of organic solutes or a combination of both responses. The decrease in Ψ_s resulting from 'net' changes in accumulation of organic solutes is termed "osmotic adjustment" (osmoregulation) or turgor regulation (Cram, 1976; Munns, 1988). The lowering of Ψ_s through an increase in the concentration of the cell sap consequent upon water loss during stress is not considered as an osmotic adjustment. Turner and Kramer (1980) suggested that "osmotic adjustment" be used only for the accumulation of solutes in higher plants in response to water deficit and that "osmoregulation" and "turgor regulation" be retained for use in relation to lower plants and micro organisms or the change in Ψ_s of higher plants in response to salinity.

Evidence for osmoregulation has been found in many stressed plants (e.g. Eaton, 1927; Black, 1960; Heuer and Plaut, 1989; Marcum and Murdoch, 1990) and reviewed by Hellebust (1976a) and Zimmermann (1978). The initial imbalance in water relations observed when the plants are first exposed to salt stress is not permanent and at least partly negated in some species and fully in others by accumulation of organic solutes (Meiri and Poljakoff-Mayber, 1969). In some species of plants, high salinity leads to a higher water content expressed as succulence and in tomato is due to the uptake of anions (Hayward and Long, 1943; Dehan and Tal, 1978) or of Na^+ (Handley and Jenning, 1977).

As Ψ_s of the cytoplasm is commonly adjusted with organic (compatible) solutes (Cram, 1976; Stewart and Lee, 1974; Storey and Wyn Jones, 1975, 1978) plants in saline environments are not necessarily suffering from dehydration. Such osmoregulation ensures turgor maintenance, and full turgor maintenance in response to water deficit and salinity has been reported in sorghum (Fereres *et al.* 1978), maize (Michelena and Boyer, 1982) and onions and sugar beet (Hoffman and Rawlins, 1970) while partial turgor maintenance has been found in sorghum (Jones *et al.* 1980; Turner *et al.* 1978; Jones and Rawson, 1979), sunflower (Turner *et al.* 1978), pepper (Bernstein, 1961) and cotton (Hoffman *et al.* 1971; Bernstein, 1961). Turgor regulation can respond to daily changes in Ψ of the plant (Culter *et al.* 1977) and even isolated spinach chloroplasts can recover volume following osmotic shock (Robinson and Jones, 1986). However, it must be noted that some exogenous factors, such as the precondition and rate of development of stress may affect turgor regulation (Flowers and Ludlow, 1986; Oosterhuis and Wallschleger, 1987).

There have been many reports on the maintenance of growth brought about with turgor regulation. Meyer and Boyer (1981) showed that growth of the soybean hypocotyl was maintained over a great Ψ range when slow drying allowed turgor maintenance of the hypocotyl, whereas growth ceased when turgor was lost because of rapid drying. The maintenance of turgor is also very important for plant tissue elongation (Neumann *et al.* 1988), the opening of stomata and, thus, photosynthesis (Turner *et al.* 1978), and the improvement of adverse water relations (e.g. Culter *et al.* 1977; Flower and Ludlow, 1986; Hsiao *et al.* 1986).

Turgor has been thought to be crucial to plant growth (e.g. Neumann *et al.* 1988), but is evidently not the sole growth determinant. When water stress is applied in a slow and not too severe way, turgor is maintained by osmoregulation , but growth is nevertheless limited (Morgan, 1984; Munns *et al.* 1979; Handa *et al.* 1986). A prevailing dogma is that turgor regulates stomatal conductance and cell expansion, and, hence, plant growth in saline soils of low Ψ (cf Munns, 1993). However, turgor of salt-stressed plants is not always reduced, but often similar to, or higher than, controls (e.g. Robinson *et al.* 1983; Yang *et al.* 1990; Lloyd *et al.* 1987a, 1990). The results of Lloyd *et al.* (1990) are specially significant

because they were obtained from pressure-volume curves, and are not subject to the error in turgor measurement inherent in psychrometric methods. Yet, even when turgor was found to increase, stomatal conductance and net CO_2 assimilation rate were greatly reduced (Lloyd *et al.* 1990; Robinson *et al.* 1983). Further, with two genotypes of wheat differing in salt tolerance, the sensitive one, with higher turgor, had a greater reduction in stomatal conductance than the tolerant one (Kingsbury and Epstein, 1984). These findings suggest that other factors, in addition to turgor, are controlling stomatal conductance. Growth is reduced in maize leaves despite full maintenance of turgor (Michelena and Boyer, 1982). Moreover, Termaat *et al.* (1985) maintained shoot turgor of salinized wheat and barley at the unstressed level by applying pressure to the root system, but this did not restore the long-term rate of leaf growth. These results show that turgor alone cannot account for the observed growth reductions.

3.1.2 Water relations and high temperature

Heat injury in plants has been attributed to water deficit (Li *et al.* 1991; McDaniel, 1982). However, a clear association of water deficit with temperature stress injury is difficult to demonstrate in all cases (Levitt, 1980a). Because high temperature stress is usually associated with drought conditions, it is often difficult to separate the direct effect of temperature from those of water stress. The work of Lahiri and Singh (1969) and Mattas and Pauli (1965) shows that when plants are exposed to supra-optimal temperatures the plant water status remains constant for an initial period and then declines sharply. This is due to direct effects of temperature on the diffusion constant of water, the steepening of the vapour pressure gradient between the leaf and the atmosphere, and effects on stomatal aperture (Barbal'chuk and Tchernyavskaya, 1974). All these processes can lead to rapid transpiration and a consequent lowering of tissue Ψ in the leaf (Levitt, 1980a). Additionally, soil temperatures above 30°C have been found to reduce water absorption by lemon (Bialoglowskia, 1936) and grapefruit roots (Haasa, 1936) and this may also contribute to leaf water deficit during high temperature stress. For example, Ψ_{leaf} decreases with an increase in root temperature at a constant soil Ψ, and the activation energy for

growth rates of shoot dry mass and leaf area and for the rate of photosynthetic dry mass production increase linearly with decreasing Ψ_{leaf} (Li *et al.* 1991). The increase of activation energy is greater for growth rates than for photosynthesis, suggesting that growth is more sensitive to water stress than photosynthesis.

Growing plants in conditions of high humidity and ample water supply in the root medium could, presumably, allow investigation of the effects of high temperature uncomplicated by tissue water deficit. When Petinov and Razmaev (1962) and Chu *et al.* (1974) investigated the effects of a brief exposure to high temperature (40°C) under conditions of low (<0.74 KPa) or high (3.68 KPa) vapour pressure deficit (VPD), there were no substantial changes in leaf water status at low VPD. Similarly Naidu (1987) found that heat stress alone did not influence leaf water status (relative water content, Ψ and Ψ_S) in well-watered wheat seedlings exposed to 35°C compared to 20°C for 2 days. However, when the plants were water-stressed, RWC, Ψ and Ψ_S declined significantly with both time and an increase in temperature. It has been suggested that plant resistance to heat and water stress are interrelated, which is easily understood if each is the manifestation of a response to a similar change in tissue environment. On the other hand, more direct metabolic responses to high temperature are also involved (Levitt, 1980a).

3.2 Ion Relations

Another possible physiological mechanism of growth reduction due to salinity is ion imbalance, i.e. an excessive accumulation of ions and impaired nutrition. Ions may be readily absorbed by diffusion or mass flow in the transpiration stream or cations are exchanged for hydrogen ions (Blumwald *et al.* 1987; Epstein and Stout, 1952; Läuchli, 1984; Moolenaar, 1986). Prolonged salinity with salt-sensitive plant species causes a buildup of salt in the shoot, especially in the expanded leaves. Subsequently, the accumulated ions will result in altered metabolic processes in the plant, thus killing them (Munns and Termaat, 1983).

Salt uptake and transport are both affected by a series of internal factors, such as species, tissue, internal ions, respiration, hormones and external factors, such as temperature, humidity, aeration, light, composition and concentration of ions in the rooting medium and pH (Aston and Lawlor, 1979; Hiatt, 1967; Jennings, 1976; Greenway and Munns, 1980; Behl *et al.* 1986; Chow *et al.* 1990; Shan *et al.* 1990; Grignon and Sentenac, 1991). This discussion puts stress on a few factors influencing ion accumulation, which are germane to the present investigation.

3.2.1 Ion uptake and transport associated with salt stress

Ion uptake by plant roots has been treated comprehensively (Anderson, 1975, 1976; Bowling, 1976; Luttge, 1983; Pitman, 1976, 1977, 1982; Clarkson, 1988; Läuchli, 1976b, 1984; Leonard, 1984). Transport of ions across the root into the xylem, and ion relations of the whole plant have also been the subject of a number of reviews (Läuchli, 1976a; Pitman, 1984, 1988; Humphreys, 1988). Roots are the sites of uptake of ions from the soil and some of the ions taken up accumulate in the root cells, particularly in the vacuoles, while some ions are also transported through the root to the xylem and on to the shoot. There is also some ion movement in the opposite longitudinal direction, that is towards the root apex (e.g. review by Läuchli, 1984). These ion transport processes are controlled by a number of membrane transport steps, both in cells of the root surface and the stele.

A model of the passage of ions through roots (Anderson, 1976) has depicted inflow across the plasmalemma at the surface followed by vacuolar accumulation or symplasmic transport to the stele and ion release to the xylem vessels. It is noted that the apoplasmic (cell wall) pathway between cortex and stele is blocked by the Casparian strip in the radial walls of the endodermis (Peterson, 1988). Symplasmic and apoplasmic transports have been reviewed extensively by Spanswick (1976, 1981) and by Läuchli (1976a), respectively. The principal membrane fluxes which govern ion transport in roots constitute three components, the plasmalemma, tonoplast of the surface cells at the outer boundary of the root symplasm, and the plasmalemma of the xylem parenchyma cells at the inner

boundary. The net ion uptake from the medium to the symplasm is determined by influx and efflux. Transport of ions into the symplasm occurs at the plasma membrane (plasmalemma) of epidermal, and to some degree, cortical cells. Within the stele, ions are released through the plasmalemma to the xylem vessels and these ions are then carried to the shoot in the transpiration stream. Transport across the cell membrane controls entry of ions into the symplasm and possibly release into the xylem (Läuchli, 1984; Pitman, 1976, 1984). More recently, development of an electro-physiological patch-clamp technique led to the discovery that plant cell membranes contain ion channels, such as K^+, K^+-H^+ and Cl^- ion channels, which form pathways for passive, i.e. thermodynamically downhill, ion movement (Hedrch and Schroeder, 1989; Maathuis and Prins, 1990). Passive transport through channels, together with ATP and PPi-dependent pumps and voltage-dependent functions, may also be involved in selective uptake and excretion of ions (Haschke *et al.* 1988; Hedrch and Schroeder, 1989; Matsumoto and Chung, 1988; Stelter and Jeschke, 1983). In the main, these investigations have sought to clarify the mechanisms responsible for these fluxes, in particular, the nature of the driving forces.

Regulation of ion transport has been discussed in reviews of mineral nutrition (Clarkson, 1988), osmoregulation (Zimmermann, 1978) and regulation of pH (Smith and Raven, 1979). In addition, the reviews by Pitman and Cram (1973, 1977), Cram (1976) and Glass (1983) contribute to understanding in this area. In order to describe fully the model of the regulation of ion transport within a particular system, one should ideally have knowledge of (a) the mechanisms of active and passive fluxes to and from the compartment, (b) the cellular and whole plant functions of these fluxes, (c) the means whereby perturbations of particular cell outputs are sensed and the identity of the environmental signals which trigger transport system adjustment to achieve regulation (Glass, 1983). An earlier model to describe the ion transport mechanism was derived from studies on the kinetic response of ion absorption to substrate concentration (Epstein and Hegen, 1952; Epstein, 1966; Rains, 1969). These authors described two separable mechanisms at low and high external ion concentration, in which there was a different affinity for K^+. The site of transport for both mechanisms was suggested to be either at the plasma membrane (Rains and Epstein, 1967a, b; Epstein, 1966) or at the tonoplast (Torrie

and Laties, 1966; Osmond and Laties, 1968). In this case, the main view holds that ATP or high energy intermediates generated by respiratory or photosynthetic processes are utilized by plants to accumulate ions (Luttge and Laties, 1968; Rains, 1968). Implication of ATP in the transport of ions along with demonstrations of ATPase in plant tissues has promoted research on the possible link between these enzymes and ion transport (e.g. Atkinson and Polya, 1967; Spanswick, 1981).

Ion re-absorption and efflux processes are of importance in the intact plant (Pitman, 1988). Concentrations of ions reaching the leaves may be lower than that estimated from uptake since cells along the path of the xylem extract ions from the solution. As the xylem solution passes through the stem and the leaf petiole it becomes depleted of ions (Klepper and Kaufmann, 1966). Most halophytes take up large quantities of both Na^+ and Cl^- in the shoot (Flowers *et al.* 1977) whereas glycophytes tend to exclude Na^+ or Cl^- from the shoot cells (Hodson *et al.* 1985). Variations in such ion exclusion mechanisms have been observed in tomato (Wrona and Epstein, 1985), wheat (Tottes and Bingham, 1973), *Festuca rubra* (Rozema *et al.* 1978), *Agropyron elongatum* (Shanon, 1978), soybean (Läuchli and Weieneke, 1979), barley (Storey and Wyn Jones, 1978) and trifoliate orange (Walker, 1986). In tomato, some wild species and domestic cultivars have been reported to be 'salt excluders' (Tal, 1971; Fong, 1983; Taleisnik, 1989) while some are described as 'salt accumulators' (Rush and Epstein, 1976; Phills *et al.* 1979). A comparative study with *L. esculentum* and the wild salt-tolerant species *L. pennellii* with ^{22}Na uptake by intact plants has showed that the wild salt-tolerant species accumulated more Na^+ in the stems, petioles and leaves; whereas the cultivars accumulated more in the roots (Rush and Epstein, 1976). Cl^- uptake was similar. Removal of Ca^{2+} and Na^+ in the stem may also reduce the amounts of these ions transported to the leaves and reduce the 'load' of non-essential ions in the leaf (Flower and Yeo, 1988).

In salt-sensitive crop plants high salt supply exceeds demand, resulting in a sustained rate of xylem delivery of Na^+ and Cl^- to the expanded leaves. This, in turn, leads to either excessive apoplastic ion concentrations in the expanded leaves and death through dehydration, or excessive symplastic concentrations and death through ion toxicity

(Flowers and Yeo, 1986). There is more direct evidence that high internal Cl^- and/or Na^+ concentrations reduce growth and indirect evidence for ion toxicity from relationships between internal ion concentrations and salt tolerance (review by Greenway and Munns, 1980). The direct evidence came from two approaches to separate the effects of ion excess from those of water deficit. Firstly, growth reduction in avocado trees (Downton, 1978) and soybean (Läuchli and Wieneke, 1979) occurs at such a low external Cl^- concentration (5 ~ 10 mM) that an adverse effect of low Ψ_{ext} is hardly plausible as an explanation of the growth limitation. The second is that at low Ψ_{ext} the growth of salt sensitive beans and maize and tolerant barley are substantially better in polyethylene glycol than in NaCl solutions of a comparable Ψ (Greenway and Munns, 1980). The indirect evidence is that growth in a saline root media has been shown to be negatively correlated with the ion content of the shoots (e.g. Wieneke and Läuchli, 1979; Salim and Pitman, 1983).

3.2.2 Transpiration and ion uptake

The larger proportion of the ions taken up into the roots is transported to the shoot; some of this transport takes place in the phloem (Humphreys, 1988), but by far the greater part is in the flow of water from root to shoot in the xylem (Pitman, 1984). As a result, ions accumulate in the shoot and affect growth (Russell and Barber, 1960; Salim, 1989). Hence, one question that is raised by considering uptake by the whole plant is the extent to which transpirational water flow affects ion transport across the root and into the shoot.

Evaporation from the leaves exerts its primary effect on water transport through the root by the addition of a hydrostatic pressure term to the total driving force. Under most natural environmental conditions, this 'transpiration tension' is by far the largest force for driving water through the intact plant (Anderson, 1975). A good exposition of the whole subject can be found in Slatyer (1967). Very simply, water movement across the root to the xylem will be described by

$$J_v = L_p (\Delta P - \sigma RT\Delta C_s) + \Phi_0$$

where J_v is the volume (water) flux into the root, L_p is the hydraulic conductivity of the root, ΔP is the transpiration tension, σ the reflection coefficient of the effective osmotic membrane in the root, R is the gas constant, T the absolute temperature, ΔC_s the solute concentration difference between xylem sap and external solution and Φ_0 is a so-called non-osmotic water flux. The chief complication in applying the equation to account for evaporation effects on water transport is that L_p apparently varies with ΔP (Anderson, 1975).

It is generally accepted that an increase in transpirational water flow can, in some circumstances, cause an increase in the rate of salt uptake by plants (cf Lopushinsky, 1964; Pitman, 1988), possibly via two mechanisms. One is water movement through roots affecting salt uptake indirectly by reducing the salt concentration in the root xylem; the other is salt movement into the root xylem of rapidly transpiring plants involving mass flow as well as active transport. The root permeability to water increases with the rate of transpiration, therefore mass flow of water across the root surface increases (Aston and Lawlor, 1979). Thus, it may be possible that there is an interaction between transpirational water and ion flow on transport and accumulation of ions in the shoot (Pitman, 1988; Salim, 1989; Tsuchiya *et al.* 1992). Petterson (1960) found that SO_4^{2-} translocation is enhanced by transpiration in sunflower. However, in some situations uptake of ions to the shoot appears to behave as if transport across the root is regulated by active transport. In this case there is little effect of water flow on total transport ($K^+ + Na^+$) (cf Pitman, 1988). Greenway (1965) measured Cl^- transport to shoots of barley plants at various transpiration rates and showed that transport to the shoot could be described as the sum of a constant component that could be inhibited by the uncoupler DNP, plus a component unaffected by DNP and proportional to water flow. Similar evidence for an active and a non-active component of Cl^- uptake in barley was obtained by Luttge and Laties (1967). Recent reports (Nicolas *et al.* 1993; Ball, 1988) show that salt uptake in wheat and mangroves is largely independent of water uptake. A specific relationship between water flow and ion uptake was not found in tomato, red kidney bean and sunflower, but higher concentrations of Cl^- and Na^+ in mung bean and *A. spongiosa* were found at higher

transpiration rates (Salim, 1989). This suggests that the effects of transpiration on Cl^- and Na^+ uptake may be species-specific.

3.2.3 Membrane properties and ion uptake

3.2.3.1 *Salt stress*

Since metabolic inhibitors reduce ion uptake (Greenway, 1965), the major passage for ion entry into the transpiration stream must be via a symplastic pathway and involve a metabolic process. The ability of cell plasma membranes to retain their integrity and ion selectivity in spite of unfavourable salt conditions is vital (Kuiper, 1984). It has been found, by using biochemical and biophysical methods, that salt stress modifies the membrane permeability through effects on membrane peroxidation (Levitt, 1980b) and electrostatic properties (Anderson, 1976; Suhayda *et al.* 1990).

Numerous biochemical studies have investigated the possible relationship between membrane fluidity and salt stress (Kuiper, 1984; Hurkman *et al.* 1986, 1988). The results, however, were often ambiguous (Brown and Dupont, 1989; Bruggemann and Janiesch, 1988; Chretien *et al.* 1992). For example, unlike the studies suggesting that the membrane lipids play an important part in this response (Kuiper, 1984), the report of Borochov-Neori and Borochov (1991) indicated a lower lipid to protein weight ratio in the membranes from salt-treated melon seedlings, suggesting that on the average a higher surface concentration of proteins prevails in root cell membranes. Moreover, the relative contents of phospholipid and free sterol were only slightly affected by excess NaCl; accordingly, the fluidity of liposomes prepared from lipid extracts of membranes isolated from control and from salt-grown seedlings was similar (Borochov-Neori and Borochov, 1991). Thus, a positive link between ion uptake and membrane fluidity is yet to be proved. According to the protein -SH group hypothesis, a variety of stress factors may alter membrane properties by inducing the aggregation of membrane proteins (Levitt, 1980a, b). There is persuasive evidence that UV-absorbing compounds leak from salt-treated root membranes (e.g. Redmann *et al.* 1986; Murata, 1990). This evidence indicates that the toxic component of

salt stress may involve effects through membrane peroxidation (Levitt, 1980b), eventually resulting in a modification of membrane structure and, thus, an increase in root membrane semipermeability. It has been acknowledged that an increased rate of solute leakage into non-electrolyte media is commonly associated with salt stress and this has been attributed to a modification in membrane permeability (Blits and Gallagher, 1990; Leopold and Willing, 1984; Borochov-Neori and Borochov, 1991).

Advanced studies in electro-physiology have already confirmed that salinity can induce changes in plasma membrane electrostatic properties and, hence, influence ion transport across the root plasma membranes. Suhayda *et al.* (1990) found that salinity stress depolarized the cellular membrane potential of tomato roots. The membrane surface potential in control and salt-stressed membranes were -26.0 and -13.7 mV, respectively, and represented a 48% change toward a more positive surface potential. The consequences of a large positive shift in plasma membrane surface potential were apparent in the approximation of ion concentration at the membrane surface, the cation stimulation of ATPase activity, and anion stimulation of proton transport activity (Suhayda *et al.* 1990). They demonstrated that not only Cl^--stimulation of proton pumping was approximately twofold greater in salt-stressed plasma membrane vesicles than in control vesicles, but also that there was the predicted decrease in Ca^{2+}. Presumably, Ca^{2+} has a function in maintenance of membrane integrity. When Na^+ displaced membrane-associated Ca^{2+}, there was membrane dysfunction and then leakage of important solutes from the cells (Cramer *et al.* 1985). Thus, this would adversely affect plants in a saline environment where regulation of membrane permeability occurs through Ca^{2+} and is essential.

Measurements of electrical potential differences between the root xylem vessels and the external solution (TRP), i.e. across the root, have been studied (e.g. Dunlop, 1973, 1982; Shone, 1968, 1969) and reviewed by Anderson (1976). It has been found that there is a relationship between the electrical potential difference, the total concentration of the external solution and the rate of entry of chloride into the xylem sap. Shone (1969) reported that adding NaCl, KCl or $CaCl_2$ to the bathing solution caused TRP to rise by 30 ~ 40 mV from about -70 mV. The magnitude of TRP was governed by the total

concentration of ions in the external solution, not the composition of the ambient solution, but was also influenced by the ionic status of the root cells (Shone, 1969). An electrical potential gradient is essential for uptake of salt (Kennedy and Gonsalves, 1988 and reference therein). However, since potassium and calcium are transported along with chloride to the sap, there is no evidence that the potential difference depends specifically on the transport of chloride, rather than on the total amount of ions transported in a given period (Shone, 1969).

3.2.3.2 *High temperature*

High temperature acts directly on cell membranes and the disintegration of cell membranes is associated with heat injury (Berry and Bjorkman, 1980; Levitt, 1980a; McCain *et al.* 1989; Raison *et al.* 1980; Thebud and Santarius, 1982; Pike and Berry, 1980; Santarius, 1986). If the plant survives, the cell membranes are often found to have undergone gross structural changes (Quinn, 1988). A positive correlation has emerged between plasma membrane fluidity and transport activity in a variety of systems (Schaeffer and Zadunaisky, 1979; Ford, 1976; Lynch *et al.* 1987). These structural perturbations include phase separation of the membrane constituents and are associated with characteristic disturbances of function such as loss of selective permeability and transport processes.

Presumably, a loss of semi-permeability or inactivation of the active uptake system at high temperature may be due to both (a) excessive fluidity of the lipids, leading to disruption of the lipid layer and (b) denaturation and aggregation of the membrane proteins (Levitt, 1980a). Bogen (1948) demonstrated that the first sign of heat injury was an increase in membrane permeability. This may be related to the alteration in membrane protein conformation (Caldwell, 1989) since proteins play a crucial role in ion transport across membranes. Increasing temperature above 30°C induces changes in protein conformation, and high temperature modifies the reaction of barley root membrane protein sulfhydryl groups (-SH) with N-4-(7-diethylamino-4-methylocumarin-3-yl)-phenyl maleimide (CPM) (Caldwell, 1989). Thermally-induced changes in protein conformation may result from the disruption of the chemical bonds involved in the maintenance of protein

structure (Somero, 1978). Levitt (1980a) proposed that plant exposure to high temperature could cause metabolic dysfunctions, as a result of the modification of protein intra- and inter-molecular SH bonds. According to the SH-hypothesis, a variety of environmental stresses may cause membrane damage by inducing the aggregation of membrane proteins. This would result in altered protein conformation and suggests a role for protein SH groups in membrane damage at elevated temperature (Caldwell, 1989).

It has been shown that an overall stimulation of Cl^- influx results from plasma membrane permeability (Sanders, 1981). When temperature decreased from 20 to 4°C Cl^- transport displayed a slow time-dependent rise. Return of cells from 4 to 20°C results in a large stimulation of Cl^- influx in comparison with cells maintained at 20°C throughout. Information on membrane fluidity as a function of temperature, and the positive correlation between plasma membrane fluidity and transport activity in a variety of systems (Pilch *et al.* 1980; Schaeffer and Zadunaishy, 1979) suggests that it is reasonable to propose that Cl^- transport is stimulated by increased plasma membrane fluidity, in which Cl^- ion channels are modified.

Other evidence of loss of semi-permeability is that heat injury causes leakage of solutes and loss of other cell contents in many plant species (e.g. Redmann *et al.* 1986; Murata, 1990). Efflux of electrolytes from cells may be followed by an influx of electrolytes back into the cell (Cruzeriro-Hansson *et al.* 1988, 1989; Ingram, 1985; Ingram and Buchanan, 1984; Levitt, 1980a). Such response of electrolyte leakage to treatment temperatures for selected exposure times reveals a sigmoidal relationship with temperature (Ingram, 1985; Ingram and Buchanan, 1984). The midpoint of the sigmoidal curve is the most sensitive region of the relationship and, thus, is the optimum point at which to compare the responses and heat injury ranges of different plants. The temperature at which such severe leakiness occurs appears to be related to the fluidity of the cell membrane lipids.

In the grapevine (Ehlig, 1960), the build-up of salt in the tissue is enhanced by an increase in temperature. Such results suggest that there is a temperature-induced change in cell membrane permeability to ions. It has been demonstrated, using an artificial

membrane, that there is a tendency for increased permeability to Na^+ at high temperature (Magin, Niesman and Bacic, 1990). In algal cells, Hope and Aschkerger (1970) have demonstrated an association of ion influx with hyperpolarization in electrical potential difference and showed that P_K and P_{Na}, the permeabilities of K^+ and Na^+ at the plasmalemma, were functions of temperature. However, the electrical potential difference across the root of higher plants is difficult to interpret in terms of the permeability coefficients of Cl^-, Na^+ and K^+ since it involves differential ion permeability in a most complex pathway through symplast and apoplast (Pitman, 1982). TRP is negative on the inside of the root and also appears to have an electrogenic contribution (Shone, 1969; Davis and Higinbotham, 1969; Pitman, 1982). It has been confirmed that ion uptake and transport in plants are the sum of active and non-active components (Jensen, 1962; Greenway, 1965; Luttge and Laties, 1967). It may, thus, be impossible to estimate the individual permeabilities of Cl^- and Na^+ as functions of temperature. This would need an elucidation of the pathways whereby ions are transported to the sap with distinction between a number of processes, including diffusion (Goldman, 1943), Donnan equilibrium (Hope and Walker, 1961), electro-osmotic (Briggs, 1968), carrier (Shone, 1969) and transport (Curran, 1964) potentials.

3.2.4 Selectivity and distribution of ions in relation to salinity

3.2.4.1 *Selectivity of potassium and sodium*

Selective uptake of K^+ in preference to Na^+ is found in many plant cells due to the operation of an active K^+ influx and active Na^+ efflux (e.g. Lynch and Läuchli, 1984; Aslam *et al.* 1988; Pitman, 1988). The existence of compartments acting as sequestering systems along the xylem stream might also account in part for the lower Na^+ levels found in leaves. Preferential basipetal phloem translocation of Na^+ was observed in *L. esculentum* and a role for this mechanism in the regulation of foliar Na^+ content has been suggested (Taleisnik, 1989).

Total cation and total ($K^+ + Na^+$) contents of the tropical grass *Chloris gayana* (Smith, 1974) and barley and mustard (Pitman, 1965, 1966) were virtually independent of the ratio of K^+/Na^+ in the soil. However, the ratio of K^+/Na^+ in the plant depends very much on the proportions of K^+/Na^+ in the soil or solution, and may also be affected by the total concentration of ($K^+ + Na^+$) around the roots. Thus, when the external K^+/Na^+ was kept constant but total concentration of ($K^+ + Na^+$) increased, there was a marked decrease in K^+/Na^+ in the shoots of both barley and mustard (Pitman, 1965, 1966). At low salt stress, mustard can be as selective as barley, but it becomes less efficient in more saline conditions. The ratio of (K^+/Na^+) in the roots was much less affected by external concentration than that in the shoots, particularly for barley. Presumably, the content of the shoot is determined by fluxes into the cells but the content of the shoot is also affected by the rate of transpiration (Pitman, 1965, 1966). As shown below, there are other situations where changes in selectivity in the tissue appear to be due to changed selectivity at the cellular level.

In glycophytes two efficient systems for K^+/Na^+ selectivity have been proposed: (a) their exchange at the plasma membrane (Pitman and Sandler, 1967) coupled with extrusion of Na^+ from the root symplasm (Jeschke, 1979), and (b) ion transport at the tonoplast favouring vacuolar accumulation of Na^+. In addition to modifications in the uptake selectivity for Na^+ brought about by changes in external concentration, variables such as rate and magnitude of salt increment, duration of stress, rate and developmental stage of growth and ion translocation (Wrona and Epstein, 1985; Taleisnik, 1989) also modify the selectivity processes. Cl^- uptake attains equilibrium rapidly compared to Na^+ (Storey and Wyn Jones, 1978).

3.2.4.2 *Selectivity of other ions in relation to salinity*

In addition to selectivity between K^+ and Na^+, there is selective uptake between other cations and K^+/Na^+ (Pitman 1966; Ayoub and Ishah, 1974). The ratio of K^+/Na^+ in roots and shoots of mustard seedlings varied with the external concentration of Ca^{2+} and Mg^{2+} (Pitman, 1966). Many ions in salinity conditions may affect each other's transport. For

example, NaCl stress reduces both Ca^{2+} and NO_3^- uptake (Maas and Grieve, 1987; Ben-Hayyim *et al.* 1985, 1987; Cramer *et al.* 1985, 1989, 1990; Deane-Drummond and Glass, 1982; Jeschke, 1984; Jeschke *et al.* 1988; Lynch *et al.* 1989) and inhibits the supply of Ca^{2+} to the shoot in several species (Bajwa and Bhumbla, 1971). Ca^{2+} can prevent leakage of intracellular K^+ and thereby supports elongation of roots under salt stress, and an increase in membrane permeability is probably the primary result of low Ca^{2+}/Na^+ (La Haye and Epstein, 1971). Relatively low Ca^{2+} *per se* increases membrane permeability, leading to an increase in passive Na^+ and Cl^- transport (Greenway and Munns, 1980). Most recently, it has been reported that salinity had significant effects on the uptake and concentrations of most essential elements, Ca^{2+}, Mg^{2+}, $Fe^{2(3)+}$, Mn^{2+}, Cu^{2+}, Zn^{2+}, S^{2-} and P^- (Cramer, Epstein and Läuchli, 1991; He and Cramer, 1992). The concentrations of Mg^{2+} and Mn^{2+} declined with time and all others increased with time. Element uptake on a root dry weight basis declined with time. Three variables were significantly affected by salinity and correlated with growth, 1) the Ca^{2+} concentration, 2) the sum of the cation concentration and 3) the Mn^{2+} concentration in the shoot. However, Ca^{2+} in the leaf of saline soybean appeared to increase (Bourgeais-Chaillou *et al.* 1992). This may relate to the kind of plant. Salinity-induced Mn^{2+} deficiency in the barley shoot reduced photosynthetic rates and growth (Cramer and Nowak, 1992). Thus, the selectivity of elements could produce adverse effects on plant growth in saline conditions (Cramer *et al.* 1991; Grattan and Grieve, 1992; Hawkins and Lewis, 1993a, b; Termaat and Munns, 1986).

Whether cation uptake follows anion uptake and the two processes are interdependent is not conclusively determined. The uptake of cations, especially K^+ (Sutcliffe, 1952) and Na^+ (Rains and Epstein, 1965), is affected by the nature of the accompanying anions, and the uptake of both seems to be inhibited by sulphate. Lundegardh and Burstrom (1955) and Robertson (1968) also proposed that cation uptake follows anion. However, in barley roots Cl^- and Na^+ behave differently , Na^+ being retained by root cells while a steady-state equilibrium between uptake and leakage is established for Cl^-. Uptake of Na^+, K^+, Rb^+ or Ca^{2+} is also not correlated with the uptake of anions (Ganmore-Neumann, 1972; Hiatt, 1970). The production of negatively charged macro-molecules unable to diffuse from their positions in the cell results in the establishment of a Donnan equilibrium favouring the

uptake of cations rather than anions. Anions enter the cell either by exchange for OH^- or via a metabolic transport system.

3.2.4.3 *Distribution of ions*

Once ions reach the leaf they either reside in compartments within different leaf tissues (Greenway, 1962) or within the cell or remain in the apoplast and are re-exported (Boursier and Läuchli, 1989). The partitioning of Cl^- in leaves of sorghum results from the ability of the bundle sheath and mesophyll cells to maintain lower Cl^- levels than epidermal cells. In addition, the relatively large sheath parenchyma cells tend to serve as reservoirs for the storage of Cl^-. Ions within cells are generally distributed between various compartments with a relatively higher ion concentration in the vacuole than in the cytoplasm. Compartmental analysis of accumulated ions shows noticeable differences between halophytes (Flowers *et al.* 1977; Yeo, 1981; Gorham and Wyn Jones, 1983) and glycophytes (Harvey and Thrope, 1986). Most halophytes tolerate high external salt concentrations by efficiently compartmenting the absorbed Na^+ and Cl^- into the vacuole (Gorham and Wyn Jones, 1983; Black, 1966). *Suaeda maritima*, a halophyte, contained more Na^+ in the vacuole than in the cytoplasm (Harvey *et al.* 1981) whereas wheat, a glycophyte, held more Na^+ in the cytoplasm (Harvey and Trope, 1986). This ability has been considered to be a mechanism of tolerance in halophytes (Wyn Jones, 1981). Salt ions also accumulate in the apoplast, which constitutes about 3% of total leaf mesophyll volume (Flowers and Yeo, 1986). Ions in the apoplast will lower the water potential and have adverse effects on protoplast water relations (Oertli, 1966, 1968; Munns, 1993).

Differences in the distribution of ions between expanded, expanding and meristematic tissues also occur (Hodson *et al.* 1985; Flowers and Yeo, 1986). Analysis of *Agrostis stolonifera* during NaCl stress revealed accumulation of Na^+ and Cl^- in older leaves with a low amount in younger leaves (Hodson *et al.* 1985). Old leaves of a non-halophyte grown at high salinity usually have a higher Cl^- concentration than do young leaves. This pattern has been proposed to be a combination of the rapid volume increase in expanding leaves and prolonged intake of ions by expanded leaves via the transpiration stream (Greenway

and Munns, 1980). Presumably, in expanded leaves NaCl could build up to significant concentrations in the cell wall or cytoplasm after the vacuole is 'full' (Munns, 1993). The consequences of salt buildup in the cell wall are considered to be catastrophic. The rapid accumulation of salts in the wall will rapidly dehydrate the leaf (Munns and Passioura, 1984). If salts do not increase in cell walls when the vacuole becomes 'full', the continued arrival of salt in the transpiration stream means the ions would build up in the cytoplasm and this would be equally disastrous. Often the increased concentration is accompanied by a decreased leaf area and net carbon assimilation (cf Pitman, 1988). One simple interpretation of this observation is that increased vacuolar concentration is accompanied by increased cytoplasmic ion concentration (Munns, 1993; Tomos and Wyn Jones, 1988) that can inhibit photosynthesis and other cytoplasmic processes (Munns *et al.* 1983).

There is a wide variation in the ability of plants to absorb/exclude or compartmentalize ions. Large differences in ion concentration occur in glycophytes among genera, species, varieties and cell lines (Deham and Tal, 1978; Lessani and Marchner, 1978; Yeo and Flowers, 1980; Wrona and Epstein, 1985; Hajibagheri *et al.* 1989). The association between a high ion content in the leaves of a glycophyte and salt sensitivity is by no means general. In rice, one tolerant variety had a low leaf Na^+ but another, equally tolerant, had high Na^+ (Greenway and Munns, 1980). A similar situation was found with *Casuarina* spp. (Aswathappa and Bachelard, 1986). Moreover, similar leaf ion concentrations may occur in varieties with different levels of tolerance (Lessani and Marchner, 1978). If it is accepted that cytoplasmic tolerance to ions does not vary, it follows that the ability to compartmentalize Na^+ and Cl^- may be an underlying determinant of the tolerance not only of halophytes but also of many crop species.

3.3 Influence of Salinity and High Temperature on Metabolism

The consequence of salinity and heat stresses will act negatively on biophysical and metabolic components of the plant to reduce growth, although some metabolic changes, notably the accumulation of low molecular weight organic solutes, have been thought to have adaptive significance in the tolerance of plants to both stresses.

3.3.1 Salt stress

3.3.1.1 *Photosynthesis and respiration*

To date, a few studies (Cramer *et al.* 1990; Curtis and Läuchli, 1986; Schachtman *et al.* 1989; Shennan *et al.* 1987a,b; Wickens and Cheeseman, 1988) have used the functional approach to plant growth analysis (Hunt, 1990) to characterise the effects of salinity on growth. In studies with barley, Cramer *et al.* (1990) reported that RGR of salt-stressed plants was substantially lower than that of the controls. NAR, but not LAR, was highly correlated with RGR for all treatments. The degree of growth reduction depends on the concentration of salt in the root medium, which indicates that the photosynthetic-assimilatory machinery was limiting RGR and not the leaf area of the plant.

Photosynthesis is affected with increased salinity in two sugarbeet cultivars (Heuer and Plaut, 1989), grape, citrus and guava (Walker and Downton, 1981; Lloyd *et al.* 1987b, 1990), onion and bean (Gale *et al.* 1967; Strack *et al.* 1975) and other plants (Kirst, 1981; Ball *et al.* 1984, 1986, 1987; Coudret *et al.* 1983; Cheeseman, 1988). In barley, photosynthesis is inhibited 30 ~ 60% at 100 ~ 175 mol m^{-3} NaCl (Rawson *et al.* 1988). Even the extreme halophyte, *Dunaliella salina* shows a decrease in photosynthetic rate when the salinity is raised excessively, from 2 to 5 M (Mironiuk and Einor, 1968). Salinity may reduce photosynthesis by effects on stomatal closure, inhibition of biochemical reactions, feedback inhibition of carbon metabolism (Seemann and Critchley, 1985) or a combination of these and other factors. As a result, plant growth declines.

It has been reported that NaCl decreased CO_2 uptake in grapevine cuttings (Downton, 1977) as well as in the non-halophyte *Distichlis stricta* (Tiku, 1976) although uptake was stimulated in the halophyte *Salicornia* from the same habitat. A 22 ~ 48% reduction in sugarbeet and a 30% reduction in CO_2 uptake in *Phaseolus rubra* in response to salt stress were also reported (Heuer and Plaut, 1989; Seeman and Critchley, 1985).

It has been suggested that salinity reduces photosynthesis via reduced stomatal conductance (e.g. Lapina *et al.* 1976; Gale *et al.* 1967; West *et al.* 1986; Plaut *et al.* 1989; Brugnoli and Lauteri, 1991). However, salinity also directly affects photosynthesis

through electron transport from photosystem II to photosystem I, regeneration capacity and activity of RuBP carboxylase (Weimberg, 1975; Smillie and Nott, 1982; Downton and Milhouse, 1983; Chow *et al.* 1990). Eleven enzymes of the photosynthetic apparatus developed more slowly in salinized than in control etiolated seedlings when exposed to the light (Kaiser and Heber, 1981; Seeman and Critchely, 1985). Larcher *et al.* (1990) found that salinity affected the kinetics of non-photochemical quenching, impaired photosynthetic capacity and extended the critical limits for function and viability through delayed energization of thylakoids and disturbances in the photosynthetic system. These effects were increased by increasing temperature. The effects of salt on photosynthetic functions have also been reported for a number of salt-sensitive species (e.g. Gale *et al.* 1967; Downton, 1977; Walker *et al.* 1983; Seeman and Critchley, 1985; Ball and Anderson, 1986). Whether reduction in photosynthetic capacity resulting from salinity is a consequence of feedback inhibition is controversial (Curtis and Läuchli, 1986; Downton, 1977; Greenway and Munns, 1980; Munns and Termaat, 1986; Munns *et al.* 1982; Rawson, 1986; Rawson and Munns, 1984; Rawson *et al.* 1988; Seemann and Crichley, 1985; Yeo *et al.* 1985).

Salinity may also result in an increase in respiration in plants (Nieman, 1962; Livne and Levin, 1967; Kalir and Poljakoff-Mayber, 1976; Bloom and Epstein, 1984; Rawson, 1986; Schwarz and Gale, 1981). Schwarz and Gale (1981) found that maintenance respiration increased without any net effect on total respiration, since assimilatory respiration often declined with a salinity-induced decline in photosynthesis. In experiments with *Xanthium*, Schwarz and Gale (1981) found that 80% of the reduction in net carbon assimilation was attributed to the reduction in photosynthesis and 20-25% was the result of increased maintenance respiration. They stressed that the percentage contribution of reduced photosynthesis and increased maintenance respiration to reduced net carbon assimilation would vary, depending on the species and environmental conditions. Sustained CO_2 fixation is important to sustain turgor maintenance during salinity through the synthesis of sugars (Jones *et al.* 1980) and other compounds (Gorham *et al.* 1988; Hitz *et al.* 1982). There is also a respiratory cost in compartmenting incoming ions (Schwarz and Gale, 1981, 1983; Shone and Gale, 1983) and in activation of the ion transport

systems, particularly the Na^+ and K^+ ATPase of the plasma membrane (Gordon and Bichurina, 1973). Maintenance of respiration necessitates an adequate supply of respiratory energy originating from photosynthesis (Kriedemann, 1986) and, thus, not only a decrease in photosynthesis, but also an increase in maintenance respiration could effectively lower plant growth.

3.3.1.2 *Protein synthesis*

One approach to understanding the ability of plants to tolerate environmental stress is to identify stress-induced changes in the levels of individual proteins with the assumption that adaptation to stress is the result of altered gene expression (Sachs and Ho, 1986; Ostrem *et al.* 1987). Protein synthesis responds dramatically to salt stress in barley, wheat and tobacco (Ericson and Alfinito, 1984; Gibson *et al.* 1984; Hurkman and Tanaka, 1987; Ramagopal, 1987a, c, 1988; Singh *et al.* 1985, 1987, 1989) where the synthesis of some proteins ceases and a new set of proteins is induced.

Tissue culture systems have been employed generally to investigate the molecular basis of salt tolerance (Ericson and Alfinito, 1984; Singh *et al.* 1985; Ramagopal, 1986; Zhao *et al.* 1989) and these studies disclosed that synthesis of specific proteins is altered by salinity. Ericson and Alfinito (1984) reported that two protein bands (20- and 32-KD) are much more abundant and one (26-KD) is unique on SDS polyacrylamide gels of cells adapted to grow on NaCl. To understand and relate such findings of *in vitro* cell culture responses to the plant level, a whole plant approach has also been pursued for salt-stress protein (Hurkman, 1987; Ramagopal, 1987a, b, c). Ramagopal (1987b), with a salt-tolerant cultivar (CM72) and a salt-sensitive cultivar (Prato) of barley, demonstrated that salinity induces unique proteins in roots and shoots. The unique protein changes induced by salinity stress during germination and seedling growth are apparently different in the two cultivars (Ramagopal, 1988); salinity modulates the expression of selected groups of proteins in a tissue-specific manner in roots, shoots and germinating embryos in the two genotypes, suggesting that ontogeny plays an important role.

The identities and functions of the proteins whose synthesis is altered by salinity are still being studied. A first step in determining the function of unknown proteins is to ascertain their intracellular location. In a search for candidates for salt-induced transport proteins, Hurkman *et al.* (1988, 1989) found that 26- and 27-KD polypeptides with isoelectric points of 6.3 and 6.5 increased significantly with salt stress. These two proteins are associated with both soluble and microsomal membrane fractions in barley roots, and are insensitive to proteases and easily removed with detergents. However, it is not evident that they have functions in ion movement across tonoplast membrane.

High concentrations of cations (including K^+) can inhibit protein synthesis in the cytoplasm (Gibson *et al.* 1984). The optimum monovalent cation concentration for *in vitro* m-RNA translation is between 100 and 120 mM and translation is unlikely at cation concentration above 180 mM.

3.3.1.3 *Organic solutes*

When subjected to salinity, plants accumulate organic solutes, such as amino acids or sugars and polyols. The solutes are thought to contribute to the adaptation and survival of plants under salinity, and will be reviewed here briefly.

Amino acids

Proline accumulation in response to salinity is a common phenomenon, and first noted in wilted plant tissues of excised perennial rye-grass by Kemble and Mac Pherson (1954); an increase in free proline in the leaves in response to a saline root medium was observed by Palfi and Juhasz (1970). A similar response has been reported for more than 50 species of plants from 14 families and its variation in magnitude has been catalogued by Aspinall and Paleg (1981).

The accumulation of proline in various microorganisms and higher plants has been thought to be initiated by a reduction in Ψ of the cell or tissue. Leaf proline concentration is directly proportional to leaf Ψ_s due primarily to accumulated Na^+, Cl^- and K^+ ions in

glycophytes (Weimbergh *et al.* 1982; Voetberg and Stewart, 1984). A linear relationship between proline accumulation and tissue Na^+ concentration supports the suggestion that Ψ_s controls the accumulation of proline (Handa *et al.* 1986). Such correlations might be causal in either direction or due to relationships between other limiting factors, since cold stress causes proline accumulation with no change in water status (Chu *et al.* 1976b; Naidu, 1987). If the accumulation of this compound in the cytoplasm maintains osmotic equilibrium with ion accumulation in the vacuole (Stewart and Lee, 1974; Wyn Jones *et al.* 1977), it is to be expected that it should continue to accumulate proportionally as long as ions accumulate in the cell, ultimately reaching a steady state when further ion accumulation ceases. However, both a rapid decrease in proline content (Chu *et al.* 1976a; Ahmed and Wyn Jones, 1979) and continued proline accumulation (Voetberg and Stewart, 1984) with no change in internal ion content have been observed.

In addition to such changes in cell water and ionic relations, it has been suggested that proline accumulation could be considered as a metabolic response to acidification of the cell sap induced by ABA, acetic acid or indole acetic acid (Pesci, 1988, 1989; Pesci and Beffagna, 1985; Goring and Plescher, 1986). There is a further report that Na^+ could induce the accumulation of proline, but only in combination with Cl^- and ABA (Belh and Raschke, 1986). The evidence for glycophytes suggests that internal Na^+ and Cl^- alone may not control proline synthesis. There has been evidence to indicate that no proline accumulates in excised leaf sections exposed to NaCl (Chu *et al.* 1976b) while it does accumulate in intact barley seedlings (Chu *et al.* 1976a). There is no evidence on the influence of endogenous cytokinins on proline accumulation but the application of benzyl adenine, a member of the purine group, prevents proline accumulation induced by wilting, ABA or salt stress (Stewart, 1986).

Plants have the capacity to oxidize proline (Oaks *et al.* 1970; Stewart, 1972a), the oxidation resulting in carbon being fed into the Krebs cycle and eventually respired to CO_2 (Stewart, 1986). The other major metabolic fate of proline is, of course, in the synthesis of protein (Stewart, 1972a; Adams and Frank, 1980). Higher plants as well as microorganisms have been shown to exhibit feedback control of proline biosynthesis at the

first reaction step , which plays a considerable role in regulation of the proline levels in the absence of stress (Noguchi *et al.* 1966; Oaks *et al.* 1970). It has been found that feedback inhibition of proline biosynthesis is lost in stressed leaves; in fact, accumulation of proline in stress conditions can be reinforced through mechanisms including (a) an increase in *de novo* synthesis from glutamate or ornithine, (b) a reduction in oxidation of proline, (c) degradation of protein, (d) a decrease in protein synthesis or (e) a combination of these (Strogonov, 1964; Gorham *et al.* 1984; Chu, 1974; Thompson, 1986).

Although most attention has been paid to proline in salt-stressed plants, changes also occur in the concentrations of other amino acids. When salt retards growth, the synthesis of protein decreases and continued hydrolysis of storage protein leads to an increase in the hydrolytic product, amino acids, in the seedlings of cotton (Gauch and Eaton, 1942) and pea (Prikhad'ko and Klyshev, 1963). Salinized plants may accumulate phenylalanine, aspartic acid, glutamic acid, lysine, threonine, leucine, isoleucine, tyrosine and serine (Huber *et al.* 1977; Strogonov, 1964), depending on the metabolism of the different species. For example, asparagine and other amino acids accumulated in sea-grasses (Pulich, 1986). Das *et al.* (1990) reported that, at room temperature, total free amino acids and ABA increased with salinity in callus cultures of Pearl millet (*Pennisetum americanum* L.). In pea and maize salinity caused an accumulation of free amino acids (Jaeger and Priebe, 1975), but other amino acids generally increased in salinized plants to a lesser extent than proline. The evidence (Das *et al.* 1990) indicated that there was a similarity between the action of ABA and salinity on amino acid and protein metabolism.

Since proline, along with other solutes, accumulates rapidly to high levels and the response has survived evolution in most plants and other organisms, it has been assumed that it plays a role in plant adaptation to stress. Based on studies in compartmentalization of these solutes and correlation between salinity tolerance and their relative accumulation, the adaptive role widely ascribed to nitrogen-containing compounds is that of a cytoplasmic osmoticum (Wyn Jones and Storey, 1981) which lowers cell Ψ when the plant is subjected to saline conditions. The lowered cell Ψ improves the uptake of moisture against external gradients (Aspinall and Paleg, 1981). Other possible functional aspects of solute

accumulation, as noted by Aspinall and Paleg (1981), may include (a) the protection of enzymes and membranes against the effects of salt, heat, pH and dilution (Paleg and co-workers, 1981, 1984; Ahmad *et al.* 1982); (b) serving as a readily utilizable energy and amino group source (Barnett and Naylor, 1966; Stewart, 1986) and (c) serving as a sink for carbon and/or nitrogen compound sources and reducing powers during periods of inhibited growth. This has been supported by the interesting fact that exogenous application of proline or putrescine can alleviate the adverse effects of NaCl salinity in some species (Palfi *et al.* 1974; Lone *et al.* 1987; Prakash *et al.* 1988). The accumulation of nitrogen-containing compounds in glycophytes may also serve a role in survival as well as in the maintenance of growth during stress (Greenway and Munns, 1980).

Sugars and polyols

Some plants accumulate sugars and polyols in response to salinity, instead of, or in addition to, proline and other amino acids. Pistachios are among the more salt-tolerant of the horticulturally important woody perennials. When subjected to salt stress, they accumulate high sucrose and starch concentrations in the stem and high concentration of sucrose, reducing sugars and starch in the main root (Walker *et al.* 1988). Total carbohydrate concentrations were also marginally higher in the root tips of salt-treated plants whereas photosynthetic rates were not reduced, although shoot growth was decreased by 100 mol m^{-3} Cl^- and ceased during treatment with 175 mol m^{-3} Cl^-. Turgor was maintained in expanding leaves during and after the incremental increase in salinity up to the maximum of 175 mol m^{-3} Cl^- (Walker *et al.* 1988). Increased sucrose at high salinity was also found in carrot (Bernstein and Ayers, 1953), barley (Gauch and Eaton, 1942) and sweet pea (Nightingale and Farnham, 1935-1936).

The term polyol refers to a group of carbohydrate-derived compounds containing 3 or more hydroxyl groups and includes glycerol, alditols (sorbitol and mannitol) and sterioisomers of inositol (Stacey, 1971). The reducing group (-CHO or -CO) of a mono-saccharide is replaced by an alcohol group in the formation of a simple polyol. Polyols, or sugar alcohols, are well-documented to accumulate in various organisms undergoing stress, particularly in microorganisms and lower plants (e.g. Quillet *et al.* 1985; Lewis and

Smith, 1967; Bental *et al.* 1988; Hellebust and Lin, 1989). Two dicotyledenous species, *Daucus carota* and *Honcenya peploides,* accumulated inositol and pinitol respectively, with their concentrations increasing by 65% and 85% in 159 mol m^{-3} NaCl (Gorham *et al.* 1981). Gorham *et al.* (1984) obtained a three fold increase in concentration of pinitol in a tropical legume (*Sesbania aculeata* pers.) in hydroponic culture with 100 mol m^{-3} NaCl .

The accumulation of these solutes seems to be associated with the stage of development and the type of the tissue (Hellebust, 1976a, b) and the rapidity of stress onset (Flowers and Ludlow, 1986). Most measurements of osmotic potential and content of solutes in response to saline stress have been concerned with the photosynthetic tissue - leaves. Studies on mesophytic crop plants indicate that the capacity of fully expanded leaves to accumulate solutes is limited, or in some cases, that the accumulation even ceases in fully expanded leaves when water potential declines (Morgan, 1984).

The role of sugars and polyols may lie in the protection of biopolymers through the hydroxyl group (-OH), which can replace water molecules and maintain the structure and function of those biopolymers (Schobert, 1977) when water status is lowered by salinity. Thus, the damage of stress is avoided or alleviated. Gerlsma and Sturr (1972) have found that the ability of polyols to protect enzymes against denaturation increased with the number of hydroxyl groups of the polyol.

3.3.2 High temperature

High temperature causes several metabolic disruptions affecting plant growth (Levitt, 1980a; Alexandrov, 1964). Levitt (1980a), in reviewing the effects of high temperature on the plant, suggested three types of response: direct, indirect and secondary stress injury. From further evidence, it is reasonable to assume that the consequences of heat injury during hours or days at moderately high temperature are membrane damage and metabolic disorder in addition to heat-induced water deficit (Levitt, 1980a; Long and Woodward, 1988; Turner and Kramer, 1980).

The disintegration of cell membranes has been implicated as playing a key role in high temperature injury in plants (Berry and Bjorkman, 1980; McCain *et al.* 1989; Raison *et al.* 1980; Thebud and Santarius, 1982). With the aid of spin label probes, Raison *et al.* (1980) found that the lipids of biological membranes are predominantly fluid at high temperature. In support of this, both fluorescence intensity (Sklar *et al.* 1975) and fluorescence polarization (Sklar *et al.* 1979) measurements indicated that membrane phospholipids undergo a phase change at about 40°C. At high temperatures, there would also be considerable alteration in the forces maintaining the tertiary structure of the protein. A decrease in the strength of membrane hydrophilic bonds at high temperature would weaken the forces linking the subunits of oligomeric membrane proteins (Raison *et al.* 1980; Berry and Bjorkman, 1980).

High temperature can disrupt membrane structure and thus membrane-associated functions (Papahadjopoulos *et al.* 1973; Schreiber and Berry, 1977; Berry and Bjorkman, 1980; Long and Woodward, 1988). Many of the reactions of the photosynthetic process are associated with membranes, and changes with temperature in the structure of these membranes might be expected to have significant effects on the overall rate of photosynthesis (Barlow *et al.* 1977; Weis and Berry, 1988; McCain *et al.* 1989; Harding *et al.* 1990; Reynolds *et al.* 1990). At elevated temperature, marked inhibition of photosynthesis occurs (Berry and Bjorkman, 1980; Quinn, 1988). The inhibition of whole leaf photosynthesis by high temperature has been found to be due to the disruption of the functional integrity of the photosynthetic apparatus. Heat injury to chloroplast function and integrity could be a result of a heat-induced loss of the semipermeability of the plasma-membrane, the tonoplast, or other partitioning membranes in the cell (Berry and Bjorkman, 1980; Weis and Berry, 1988). Elevated temperature-treatment also induces a loss of chlorophyll (Mohanty and Monhanty, 1988; Onwueme and Lawanson, 1973) and inactivation of thylakoid membrane function (Weis and Berry, 1988). Raison *et al.* (1980) suggest that dissociation of thylakoid membranes may be related to an alteration in the relative strength of hydrophilic and hydrophobic interactions and the temperature at which this dissociation occurs appears to be related to the fluidity of the thylakoid membrane lipids. However, such disruptions may also be caused by other more direct effects of high

temperature on the membrane proteins associated with PS II photophosphorylation and ion transport. While it is evident that heat inactivation of whole leaf photosynthesis may be explained by the heat sensitivity of thylakoid membrane reactions alone, it is noteworthy that certain soluble enzymes of photosynthetic carbon metabolism, considered to be located outside this membrane, are also inactivated when leaves are heated to temperatures that cause irreversible inhibition of photosynthesis (Berry and Bjorkman 1980; Levitt, 1980a).

Indirect heat injuries, namely protein hydrolysis, starvation, biochemical lesions, and growth reduction or cessation, occurring during continued exposure to moderately high temperatures, are all metabolic in nature and, therefore, may conceivably have a single basic mechanism (Levitt, 1980a; Dhindsa and Bewley, 1976; Dicosmo and Towers, 1984). The first strain produced by high temperature is a kinetic increase in reaction rates. The differences in slope of Arrhenius plots for different metabolic reactions may indicate a reverse in relative reaction rates (Levitt, 1980a; Feng *et al.* 1990), which leads to an increase in net breakdown and, therefore, a decrease in concentration or a complete absence of an essential metabolite at heat-injuring temperatures. In the case of soluble enzymes, the differences in slope may simply be due to differences in activation energy for different reactions, or to protein denaturation and, therefore, enzyme inactivation. In the case of membrane enzymes, difference in rates may be due to phase transitions or increased mobility of the membrane lipids associated with the enzyme. Membrane proteins are embedded in the membrane lipids, and, due to their higher hydrophobicity than that of the soluble proteins, are not so likely to be denatured by high temperatures which strengthen hydrophobic bonds (Levitt, 1980a).

4. Conclusion

Salinity and high temperature affect plant growth through a range of metabolic disturbances most of which are secondary effects (Levitt, 1980a, b; Papahadjopoulos *et al.* 1973; Berry and Bjorkman, 1980; Long and Woodward, 1988). Further primary mechanisms may also be involved. Direct observations of heat shock injury, leakage of ions and amino acids, and the effects of salt, all point to membrane damage as the cause of

a primary heat injury. Heat injury has been attributed to inactivation of enzymes and damage to cell membranes (Schreiber and Berry, 1977; Raison *et al.* 1980; Long and Woodward, 1988). A recent report has indicated that the interaction between the effects of salinity and temperature leads to the leakage of solutes from root cells (Hampson and Simpson, 1990). Therefore, it can be assumed that there will be a change in cellular membrane permeability when plants are subjected to a combination of salinity and high temperature. Furthermore, increase when plants are subjected to high VPD due to high ambient temperature. [transpirational water flow will]

Alteration of both membrane permeability and water flow may lead to an increase in rates of salt uptake. In plants growing under stresses for prolonged periods, the situation will be more complex and rates of transport and recirculation of ions will tend to be more obscure. There also is a potential for many nutrient interactions in salt-treated plants which may lead to adverse consequences for metabolism and plant growth. Alternatively, adverse water relations may be physiologically responsible for the limitation of plant growth since salinity and high temperature could each have effects on water status in the plant tissue.

In summary, the interaction between salinity and high temperature may result in changes in membrane selectivity and increases in ion uptake and accumulation. These may be accompanied by secondary osmotic and nutrient-deficiency stresses, leading to disturbances in membrane-associated functions and metabolism, and, eventually, inhibition of growth and development of higher plants.

CHAPTER III

MATERIAL AND METHODS

1. Materials

Seeds of tomato (*Lycopersicon esculentum*, Mill.), cv. Duke, an F1 hybrid, were obtained from Yates Seed Co., Australia.

2. Methods

2.1 Plant Environmental Control

Plants were grown in growth cabinets (Zankel, Australia) under controlled conditions. The photon flux density at the level of the surface of the plants in all experiments was maintained at 350-450 μmol m^{-2} sec^{-1} provided by sodium vapour lamps and the temperature was 23±1°C during the day and 18±1°C at night before imposition of salt or temperature treatments, with a photoperiod of 15 h. Relative humidity (RH), 50±5%, was also regulated with humidifiers (Defensor 505). This comparatively high RH was achieved by conducting high temperature experiments in the winter wet season and by placing two humidifiers inside the cabinet near the air inlet so that incoming air was humidified constantly. These humidity regulators were standardised, using Electronic humidity sensors (Solomat) and RH was continuously monitored with thermo-hygrographs. The positions of the plants were changed in a regular fashion at least once daily before irrigation, to minimise effects due to gradients of light, temperature and wind velocity within the cabinet.

2.2 Plant Culture

2.2.1 Sand culture

Seeds of tomato (*L. esculentum*, Mill.) cv. Duke were soaked on wet filter paper in closed petri-dishes in the dark at 23±1°C in the day and 18±1°C at the night. After 24 hours, excess water was decanted from the petri-dishes and incubation continued in the dark until the radicle emerged, this took 3 days. Care was taken to avoid variation among individual plants by selecting uniform seeds with recently-emerged radicles.

In all experiments a river sand (Christies Beach sand), well washed to reduce silt to less than 5 per cent, was used. The grain size ranged from 0.1 to 3.0 mm with 15 per cent 2 ~ 3 mm sized particles. Water holding capacity of the sand was 12 ~ 15 per cent. A layer of well-washed granite gravel was placed at the bottom of black polythene pots of 15.5 cm diameter to facilitate drainage. Pots were filled with 2.25 Kg of sand, and washed with R.O. water to remove ions before settling for a day prior to sowing. Pre-germinated seeds of tomato were sown at 4 stations/pot. Two uniform seeds were placed at each station and the seedlings were thinned to one 5 days after emergence. They were irrigated with R.O. water until emergence and thereafter with 1/8 (0.125), 1/4 and 1/2 strength Hoagland's nutrient solution (Hoagland and Arnon, 1938) gradually increasing to full strength on the tenth day after the emergence. The salinization treatments for both sand and hydroponic cultures were imposed gradually commencing on day 11 from seedling emergence with 10 mM NaCl in full Hoagland's nutrient solution and reaching 150 mM on day 21 with daily increment of 10 mM from day 11 to 18 and 20 mM from day 19 to 21. For hydroponics, the culture medium was changed every 2 days with a change of salt concentration. Following day 21 onwards, plants were maintained at the terminal salt concentrations (0 and 150 mM) and a range of temperature regimes: 13/8±1, 23/18±1, 33/28±1, 38/33±2 and 43/38±2 (°C, day/night). Other growth conditions were as before day 21. The temperature of the sand culture medium, i.e the root temperature, was monitored and the above air temperatures produced root temperatures of 20±2, 24±2, 30±2, 33±2 and 37±2°C respectively. These temperatures were adopted as the standard temperature range for all root temperature experiments. Once the described salinity levels were achieved, the plants continued to receive the same solution for the remainder of the experiment up to day 35. The various culture solutions were prepared with AR grade reagents one day prior to administration and stored in the growth cabinets to equilibrate temperature. 250 ml per pot was supplied twice daily (Rajasekaran, 1988) before imposition of different temperatures or salt shock treatments. After that, watering was increased to thrice daily for plants at low temperature (13/8°C, day/night) and to more than six times daily for plants at the intermediate temperature (23/18°C) and high temperatures (33/28, 38/33 and 43/38°C) to prevent water deficiency. This was more than sufficient to maintain field capacity.

2.2.2 Hydroponics

The hydroponic method adopted, suitable for root temperature control and measurement of transpiration rate, was basically similar to sand culture techniques except that nutrient solution was used as the culture medium. Batches of seeds of tomato cv. Duke were pre-germinated in the sand medium and grown as before until day 7. Thereafter the seedlings were transferred to pots with 2.3 litres of half strength Hoagland's solution at 4 stations per pot and cultured with full strength solution on the tenth day. The pots were covered with the lids and the seedlings were tightly held by non-absorbent cotton wool. The culture solution was aerated by means of an air pump. To study the effects of root temperature, the pots with plants were placed in individual water bath for 48 h, each bath set at a different but constant temperature (±1°C).

2.2.3 Cl^- and Na^+ uptake studies with intact roots

Intact roots, from which only the shoot was removed, were obtained from plants grown hydroponically without salt for 21 days. These were transferred to a medium containing 150 mM NaCl in full strength Hoagland's solution using an air-pump for further aeration. The pots containing the roots were left in the water baths for 48 h to study the effect of high temperature on Cl^- and Na^+ uptake. The temperatures imposed were 20±2, 24±2, 30±2, 33±2 and 37±2°C. For measurements of Cl^- and Na^+, 50 ~ 100 µl of the xylem sap per intact root was then sampled by the collection of exudate for control roots and by a Pressure Bomb for salt-stressed roots as salt stress inhibited sap exudation from the root xylem. The ion concentrations of sap were expressed as mM. Each temperature treatment was replicated three times with 12 intact seedling roots.

2.3 Plant Growth Measurements

2.3.1 Dry weight

Plants were harvested on days 21, 23, 26, 30 and 35. Leaves were separated with scissors from the stems, and weighed using a Sartorius electronic balance (mg division).

After area measurement (see below), they were placed in paper bags and dried in a forced air oven at 75°C for 48 h. The stems, separated from roots, were weighed (fresh weight) and then dried as for the leaves. Roots were washed with reverse osmosis (R.O.) water (2-3 min) to free them of sand, blotted dry and weighed (fresh weight), then dried as for the leaves and stems. This may have caused ion leakage from salt-laden roots.

2.3.2 Leaf area and shoot height

The total leaf area of the plants was determined using a Patons digital electronic planimeter. The machine was calibrated with a 10 cm^2 metal plate before use. Shoot height was measured at each harvest.

2.3.3 Root surface area

The root radius (r) could be only approximated as the average root radius since the shape of the root is conical. The average root radius was calculated as $r^2 = W/3.14\ L$, where W is the fresh weight of water-saturated roots (cm^3, assuming a root density of 1) and L the total length of the root segments used (cm). After two parameters, L and W were measured over 200 root segments of different sizes, the root radius and root surface area ($S = 2 \times 3.14rL$) could be estimated for each root segment. A simple linear relationship between root surface area (S) and the fresh weight of roots (W) was calculated from these data. The actual root surface could be estimated from this relationship and the total weight of the whole roots in each experiment.

2.4 Harvesting and Preservation of Tissues

Plant tissues in all experiments were harvested immediately after the end of the treatment into known-weight glass tubes, weighed, immediately frozen in liquid nitrogen and stored for 3 ~ 5 days in a cold room at -20°C before freeze-drying for 72 h. The dried

samples were weighed and ground after equilibration in a desiccator at room temperature and maintained in capped vials at -20°C until required for chemical analysis.

2.5 Measurement of Leaf Water Status

2.5.1 Water potential

Leaf water potential (Ψ) was measured with a Spanner thermocouple psychrometer, (Wescor HP—115, Barrs, 1968). Four entire leaves from 4 plants/pot were selected, excised, rolled gently and immediately placed in a psychrometer chamber which was then sealed with a rubber stopper, and covered until taken to the psychrometer room (within 10 minutes from excision). The chambers were then attached to thermocouples and equilibrated in a water bath maintained at 25°C in a constant temperature room for 4 hours. After equilibration, thermocouple output was read once every 30 minutes on 7 ~ 8 occasions. The water potential was calculated from 5 readings, omitting maximum and minimum readings, by comparison with a regression curve obtained from a graded series of NaCl solutions.

2.5.2 Osmotic potential

The water potential of the tissue can be analysed into osmotic potential and turgor pressure and written as $\Psi = \Psi_p + \Psi_s$ where, Ψ_s is osmotic potential and Ψ_p turgor pressure. In dead cells, Ψ_p = zero (Barrs, 1968) so the measured water potential equals the osmotic potential. After measurement of leaf water potential (as above) chambers containing the leaf samples were taken from the water bath, assemblies were removed and the rolled leaf pieces inside were taken out. Water on the surface of the leaf and the inner wall of the chamber was absorbed with soft paper. The chambers, with the leaf samples, were stoppered, wrapped in cling-wrap and immersed in liquid nitrogen (~ -150°C) for 10 minutes. Chambers were then equilibrated to 25°C, stoppers were removed rapidly and the chambers fixed to the thermocouple assemblies, sealed and replaced in the water bath. The

same procedures described for measurement and calculation of leaf water potential were followed thereafter.

2.5.3 Turgor pressure

Turgor pressure was calculated from the measured values of water potential and osmotic potential as $\Psi_p = \Psi - \Psi_s$. The values of water potential, osmotic potential and turgor pressure were expressed as MPa.

2.6 Estimation of Solutes

2.6.1 Extraction of solutes

The extraction of solutes was carried out as described by Jones *et al.* (1986) which is a modification of the procedure of Singh *et al.* (1973).

Ground, freeze-dried and weighed samples of about 500 mg were extracted overnight at 4°C in a 10 ml of methanol/chloroform/water (12:5:3, v/v) in a large (~ 50 ml) glass centrifuge tube covered with a glass marble; and then homogenised using an Ultraturrax, at 13.5K rpm. The tube was immersed in an ice bath to keep the temperature near 4°C during extraction to counteract heat generation by the Ultraturrax. After extraction, 10 ml of distilled water were placed in a second glass centrifuge tube and used to wash the grinding head. The resultant emulsion was then added to the first homogenate after which the cool homogenate was centrifuged at 3.5K rpm for 10 minutes at room temperature (~20°C). The supernatant (methanol/H_2O phase) was collected and the volume was measured; then the pellet was re-extracted in the Ultraturrax using the same conditions as the first homogenate. The supernatant solutions were pooled and stored at -20°C until analysis for soluble sugars, proline and total free amino acids. Normally, the analysis of proline and total free amino acid was carried out on the day after extraction.

2.6.2 Determination of total free amino acids

The colorimetric method for the determination of the total free amino acids with ninhydrin, which was developed by Yemm and Cocking (1955), was adopted in this experiment.

Procedures for determining amino acids

1.3 ml of citrate buffer, pH 5 (0.2 M), was mixed with 0.2 ml of a sample solution containing 0.05 to 5.6 μg of amino nitrogen. This was added to 1.2 ml of the potassium cyanide-methyl Cellosolve-ninhydrin solution, mixed thoroughly and heated for 15 minutes at 100°C before cooling for 5 minutes in running tap water. The solution was made up to 15 ml with 15% (v/v) ethanol, shaken well, and the absorbance at 570 mμ was read for all amino acids except for proline. The colour was stable at room temperature for at least 3 hours before ethanol was added and for at least 1 hour after its addition. All tubes and standard were read against blanks containing 1.5 ml citrate buffer, pH 5 (0.2 M), and 1.2 ml of the potassium cyanide-methyl Cellosolve-ninhydrin solution subjected to the same procedure as above. The amounts of amino acids were calculated from a regression curve obtained from standard L-leucine solutions determined at the same time as the samples.

Blank determination and calculation

To ensure low blank readings it was necessary to take precautions against contamination with ammonia, especially of the pH 5 buffer solution. The blank value at a volume of 5.7 ml, read against water, was not greater than an optical density of 0.03.

Proline and hydroxyproline react with ninhydrin in an entirely different way to other amino-acids (Yemm and Cocking, 1955). The total amount of free amino acids, therefore, included a value of proline measured by the method described in 2.6.3. plus other amino acids as above.

2.6.3 Proline estimation

The rapid method modified by Singh *et al.* (1973) was used in the present study.

Resin preparation

'Amberlite', IR-120 (Na), particle size 0.30~1.18 mm, 14 ~ 52 mesh, standard grade (BDH, England) was used. The resin was treated with 1N NaOH (1:2 v/v) in a glass column at a flow rate of 2 ml min^{-1} cm^{-2}. The column was then washed with distilled water, until the pH fell to 8.

Estimation

Pooled extracts of known volume were added to the resin (~ 500 mg) in a glass tube, shaken well with 3 ml of water and decanted into boiling tubes. The resin was thoroughly shaken with a further 2 aliquots of 3 ml of water which were pooled with the first aliquot. The volume in the test tube was adjusted to 3 ml in all case. Standard solutions of L-proline, containing amounts from 0 to 100 µg, were treated similarly. 5 ml of glacial acetic acid were added to adjust the pH to 1. Then 5 ml of ninhydrin reagent (prepared by dissolving 125 mg of ninhydrin in 3 ml of glacial acetic acid and 2 ml of 6 M orthophosphoric acid (H_3PO_4) by heating at 60°C) were added to each sample. Two or three glass beads were added to each tube which was thoroughly mixed and boiled in a water bath at 90 ~ 95°C for 1 h. The tubes were then transferred to running tap water to stop the reaction. 5 ml of toluene were added and the tubes were thoroughly shaken and allowed to settle for 1 hour. The colour developed was read at 520 nm and the amount of proline estimated from a regression curve obtained from standard proline solutions run at the same time as the samples.

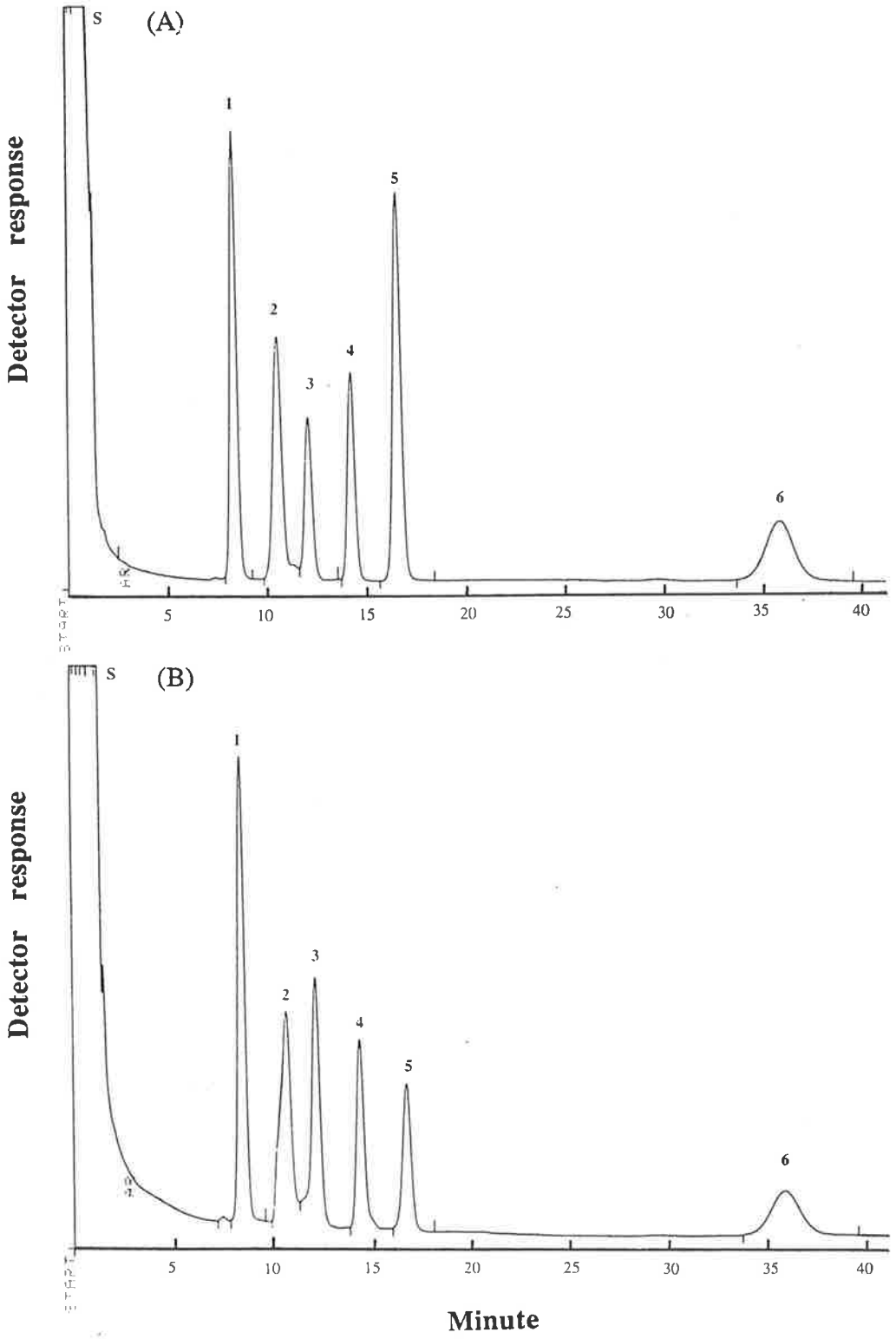
2.6.4 Soluble sugar analysis by gas chromatography

Soluble sugars (Fig. 3.1) were analysed in a gas chromatograph (Sweeley *et al.* 1963; Holligen and Drew, 1971).

Fig. 3.1 Separation of soluble sugars as their TMS ethers by gas chromotography. S: solvent peak; 1. xylitol (internal standard); 2. α-fructose; 3. α-glucose; 4. β-glucose; 5. *myo*-inositol; 6. sucrose.

(A) Standard sugar sample.

(B) Tomato leaf sample sugars.


(A)
S
1
2
3
4
5
6
Detector response
5
10
15
20
25
30
35
40
(B)
S
1
2
3
4
5
6
Detector response
5
10
15
20
25
30
35
40
Minute

Equipment and conditions

A Hewlett-Packard 5840A Gas Chromatograph (GC) with a hydrogen flame ionization detector and a glass column (182 cm long x 0.2 cm internal diameter) was used for GC analysis. The recorder chart speed was set at 0.5 cm/min. Samples were injected into the column with a 10 µl Scientific Glass Syringe fitted with a repeating adaptor and a 4.5 inch stainless steel needle. GC operating conditions were: injector temperature (225°C), detector temperature (235°C). The column was programmed at 4°C/min from 140°C to 220°C and held for 20 minutes. High purity nitrogen (oxygen-free nitrogen) (20 ml/min) was employed as the carrier gas.

Preparation of column packings

The glass column was packed with 3% SE-30 on Gas Chrom Q 100/120 tapped during filling to prevent formation of loose zones or channels. Plugs of glass fibre yarn stopped any loss of packing. The column was finally conditioned in a flow of carrier gas at 245°C for a few hours until a satisfactory recorder base line was obtained.

Preparation of TMS derivatives

An aliquot of 0.4 ml was taken from each sample solution , added to 200 µg of xylitol (internal standard), and dried in a 1 ml column-shaped vial/cap-septum 'Sunvial' on a rotary evaporator *in vacuo* at 40°C. A trimethylsilyl (TMS) reagent with pyridine, Sigma Sil-A (stock No. 139-1) was used for preparation of trimethylsilyl ether derivatives of sugars. 0.1 ml of Sigma Sil-A was added to each solvent-free sample in a vial, stoppered and shaken vigorously for a few seconds, before allowing the vial to stand at room temperature for a minimum of an hour. This reaction mixture is stable for several hours if kept tightly stoppered. An 0.5 ~ 2 µl aliquot of this sugar reaction mixture was then chromatographed directly.

Calibration and calculations

Standard solutions were prepared by dissolving sugars in 30% methanol. The internal technique was used to quantitate the sugars together with 200 µg of sugars. To every sample and standard sugar solutions were added same amount (200 µg) of xylitol as the internal standard. Calculations were based on the following expression:

$$K = \frac{\text{Peak area xylitol (calibration)}}{\text{Peak area sugars (calibration)}} \times \text{Weight of xylitol} \qquad (1)$$

$$\text{Calibration value} = \frac{\text{Peak area unknown}}{\text{Peak area xylitol}} \times \text{Appropriate K value} \qquad (2)$$

2.7 Estimation of Ions

The ground and dried samples (about 100 mg) were weighed, and transferred to polythene tubes for separate estimation of Cl^-, Na^+ and K^+. 5 ml of 1 M HNO_3 were added and glass marbles were placed on the tubes before they were incubated in a hot water bath at 90~95°C for 30 min. The samples were then spun at 3K rpm for 20 min. at room temperature. The supernatant was decanted into a graduated tube and the pellet was re-extracted with 5 ml deionised water for 30 min and centrifuged. The supernatants were pooled, made up to a volume of 10 ml and stored at 4°C for Cl^-, Na^+ and K^+ estimation.

2.7.1 Estimation of Cl^-

Chloride was estimated with a Bultchler-Cotlov Chloridometer 4 — 2008.

Reagents

(a) Nitric acid reagent (0.1 N HNO_3 and 10% glacial acetic acid): 6.4 ml of HNO_3 (70%, W/W) and 100 ml of glacial acetic acid were added to 900 ml water, mixed thoroughly and stored in a glass bottle.

(b) Gelatin reagent: This contained 60:1:1 of gelatin (Knox unflavoured gelatin #1), thymol blue (water soluble) and thymol.

(c) Sodium chloride (0.5 mM): 0.5 mM NaCl was prepared in 0.1 N nitric acid with 10% glacial acetic acid reagent.

Procedures

Nitric acid reagent was added to an aliquot of the sample extract (0.1 ~ 1 ml) to obtain a total volume of 2 ml. 2 drops of the gelatin reagent was added and the mixture was titrated using the silver ion electrodes of the instrument in either the low or medium ranges of the titration mode. Before the samples were titrated, the instrument was equilibrated and adjusted to room temperature. Triplicate blank and 0.5 mM NaCl solutions were titrated and the time required (in second) was recorded. Amount of chloride in the sample was calculated and expressed as $m_{\chi}^{mol}\ g^{-1}$ dw.

2.7.2 Measurement of Na^+ and K^+

The extracts were diluted as necessary and, Na^+ and K^+ estimated on Atomic Absorption Spectrophotometer (PYE UNICAM SP9), along with appropriate standards. Concentrations were expressed as $m_{\chi}^{mol}\ g^{-1}$ dw.

2.7.3 Determination of other major and trace constituents

Leaves were digested in boiling concentrated nitric acid and the digests read on an Inductively Coupled Plasma Optical-Emission Spectrometer (ICP-EOS) to determine the concentrations of major and trace elements simultaneously: S^{2-}, P^-, B^-, Mg^{2+}, Ca^{2+}, Mn^{2+}, $Cu^{2(3)+}$, Zn^{2+}, $Ni^{2(3)+}$, Mo^{2+}, $Fe^{2(3)+}$ (Zarcinus, 1984).

Nitric acid digestion

Freeze-dried and ground tomato leaves were stored in paper bags with further oven drying at 65 ~ 70°C for 3 days prior to weighing for analysis. Standard Reference Material

NBS (tomato leaves, supplied by ICP Lab, Department of Plant Science) was analysed without further sample preparation after drying at 65 ~ 70°C. The procedure was as follow:

(a) 0.5 ~ 1.0 g of weighed oven-dried leaf material were placed in a clean acid-washed graduated test-tube and 10 ml of nitric acid (70%, W/W) (Univar Analytical Reagent) added to the tube at 1:00 p.m.:

1. Pre-digestion at room temperature overnight to avoid frothing;
2. Digestion at 125°C for 30 ~ 40 min. with further shaking;

(b) The tubes was watched carefully to control frothing:

After the digest was clear but before the volume was reduced, the digest was vortexed to wash down any residue on the sides of the tube. The digest volume in each tube was checked, digestion being completed when 1 ml of acid remained, and the digests were then removed and cooled to room temperature.

(c) When cool, the digests were diluted to 25 ml with 1% (V/V) nitric acid, mixed on the vortex mixer and stored overnight to allow amorphous silica to settle.

(d) The supernatant was decanted into acid washed polystyrene vials to ensure that no silica could be aspirated into the plasma and stored at 25°C for analysis by ICP-OES.

Estimation

The digests were aspirated directly into plasma. The concentration of each element in the leaf sample was computed from standard tomato leaves and expressed as $m_{\lambda}^{mol}\ g^{-1}$ dw for macro-elements and $\mu mol\ g^{-1}$ dw for micro-elements..

2.8 Transpiration Measurement

Transpiration of intact plants in hydroponic culture was estimated by weight. An initial weight of plants together with the pot was taken (W_0) at the experiment outset and

pots were re-weighed (W_1) after 48 h. The plants were then dissected and leaf area measured. Transpiration rate was expressed as mg cm^{-2} leaf area h^{-1}.

2.9 Determination of Trans-Root Potential

The method has been described in detail by De Boer *et al.* (1983). The temperature of the bathing solution was maintained as required and varied about ±1°C during measurement of trans-root potential (TRP). pH of the bathing solution was measured at 25°C and varied between 6.3 and 6.5.

Plant root samples were taken from hydroponic culture on day 23 after salt-temperature treatments of two days. After the shoots of four plants, grown through plastic pots as in the other experiments, were excised, the pot with the intact root system was transferred to a wooden board which was used for the prevention of electrical current leakage. The electrical potential difference between sap exuding from the cut xylem vessels and the bathing solution (TRP) was then monitored by means of two electrodes. One reference Ag-Ag/Cl electrode was connected via a salt bridge manifold to the solution (full strength Hoagland's solution with or without 150 mM NaCl) bathing the roots. The other electrode used was a glass microelectrode (provided by Dr. S. Tyerman, the Flinders University of South Australia) with a resistance of 2 ~ 5 MΩ. It was connected via a salt bridge (1 M KCl) to the cut end of the stem. The two electrodes were connected to a Coupling Microelectrode Amplifier (Model 750, W-P Instruments, INC. USA). Leakage of KCl from the electrodes had negligible effects on account of the large external volume.

2.10 Statistical Approach

All the treatments and measurements in the experiments reported in this thesis were replicated 3 times with 4 plants per replicate. All the different measurements were taken on the same seedlings or detached roots at the same time of day to avoid physiological variations due to diurnal changes. A split-split plot design with factorial concept was followed and within each plot a completely randomised block design was used. Analysis of variance was carried out using the "Genstat" statistical programme on

Unix computer system. Wherever F-test was not significant (NS) at 0.05 level of probability (P), the least significant difference (LSD) is not shown.

2.10.1 Growth parameter

For exponential growth of dry weight mass of whole plant (M_{tot}), leaf (M_l), stem (M_s), root (M_r) and leaf area (L_a), the function $\ln y_0 = a + bt$ was derived and proved to be linear statistically, where ln is log base e and y_0 the initial value of dry weight mass (mg per plant) or leaf area (cm^2 per plant), a and b are constants, t is time (d) and; then

$$b = \frac{1}{M} x \frac{dM}{dt} \qquad (3)$$

where b is a slope for mass (M) of both dry weight (mg mg^{-1} d^{-1}) and leaf area (cm^2 cm^{-2} d^{-1}), essentially the relative growth rate (RGR) and leaf expansion rate (LER) respectively. For logistic growth, fit M as a logistic function of t; the formula given above was used to derive b, namely RGR. The slope of stalk height (S_h) was directly calculated from a linear expression, i.e. $y_0 = a + bt$ and expressed as cm cm^{-1} d^{-1}. Then, the interaction effect (ΔE) in term of the difference of slopes between control and salt treatment for any growth parameter was assessed as follows:

$$\Delta E = b_{ck} - b_t \qquad (4)$$

where b_{ck} and b_t are the slopes of the biomass regressions at 0 and 150 mM NaCl salinities, respectively.

Instantaneously, net assimilation rate (NAR, mg cm^{-2} d^{-1}) and leaf area ratio (LAR, cm^2 mg^{-1}) were derived from the following equations (Hunt, 1990):

$$NAR' = \frac{1}{L_a} x \frac{dW}{dt} \qquad (5)$$

$$LAR' = \frac{L_a}{W} \qquad (6)$$

Thus, the value over an interval t_1 to t_2 is given by

$$NAR = \frac{\ln L_{a2} - \ln L_{a1}}{L_{a2} - L_{a1}} \times \frac{W_2 - W_1}{t_2 - t_1} \quad (7)$$

$$LAR = \frac{\left(\frac{L_{a1}}{W_1}\right) + \left(\frac{L_{a2}}{W_2}\right)}{2} \quad (8)$$

where W and L_a represent total weight per plant (mg) and leaf area (cm^2) at time t (days). For the experiment based on the five harvests (day 21, 23, 26, 30 and 35), correspondingly, t_1 is 21, 23, 26 and 30 and t_2 is 23, 26, 30 and 35, respectively. The grand mean values for NAR and LAR over four intervals were calculated from these data.

2.10.2 Net uptakes of water, chloride and sodium

The rates of uptake and transport of water and ions from the root to the whole plant and shoot per cm^2 root surface area, J (nmol cm^{-2} h^{-1}), are defined as:

$$J = \frac{C}{dt} \times \frac{1}{M_r} \quad (9)$$

where M_r is the root surface area (cm^2) and C is the amount of water, Na^+ or Cl^- in the plant or its shoot. For experiments based on two harvests, J was estimated according to Pitman (1988):

$$J = \frac{C_2 - C_1}{t_2 - t_1} \times \frac{\ln(M_{r2}/M_{r1})}{M_{r2} - M_{r1}} \quad (10)$$

where C_2, C_1 are water and ion taken up at times t_2 (day 35 for sand culture and day 23 for hydroponics) and t_1 (day 21), and M_{r2}, M_{r1} are the corresponding root surface areas. The water and ion uptake rates into the whole plant, J_{tot} (mmol cm^{-2} h^{-1} and nmol cm^{-2} h^{-1} respectively) was calculated from the transpirational water and ion contents, C_{tot2} and C_{tot1} of the whole plant (root + stem + leaf). The ion transport rate from roots to shoots, J_s (nmol cm^{-2} h^{-1}) was calculated from the ion contents, C_{s2} and C_{s1} of the shoots (stem + leaf) at times t_2 and t_1, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

1. The Vegetative Growth Responses of Tomato to Temperature and Salinity Stress

1.1 Introduction

The responses of plant growth to extreme environments, such as high temperature and salt stresses, have generally been assessed separately, although exposure to salinity and high temperature simultaneously is a common feature of irrigation agriculture in warm climates. Salt has a substantial impact on the growth of glycophytic crop plants (Cramer, Epstein and Läuchli, 1990; Greenway and Munns, 1980; Hayward and Long, 1943; Maas and Hoffman, 1977). Leaf growth is more sensitive to salinity than root growth, and both short-term and long-term exposures to salinity limit growth of glycophytes, including tomato, at near-optimal temperatures (Munns and Termaat, 1986; Dehan and Tal, 1978; Hayward and Long, 1943; Salim, 1989). Sub- and supra-optimal temperatures also retard plant growth (Long and Woodward, 1988; Turner and Kramer, 1980), the degree of effect on growth depending on plant genotype and other environmental conditions (Levitt, 1980a). In the field, plants normally grow in an environment which is less than optimal in two or more characteristics. For example, chilling or heat stress can be superimposed upon water stress (Levitt, 1980a; Li *et al.*, 1991; Palta, 1990; Turner and Kramer, 1980). Numerous studies on seed germination (Fowler, 1991; Francois and Goodin, 1972; Hampson and Simpson, 1989; Khan and Huang, 1987; Maftoun and Sepaskhah, 1978; Mahmoud and Hill, 1981; Rivers and Weber, 1971; Vinizky and Ray, 1988), however, have shown that as the temperature increases, NaCl becomes progressively more inhibitory. Apart from seed germination, the combined effect of temperature and salt on different stages of plant growth has received comparatively little study. Few reports (Ehlig, 1960; Hampson and Simpson, 1990; Magistad, Ayers, Wadleigh and Gauch, 1943) have noted salt-temperature interaction. The physiological mechanism of the interaction is obscure and has been paid little attention. All salts (NaCl, Na_2SO_4, KCl, K_2SO_4, $MgCl_2$ and $MgSO_4$) induce membrane leakage in root tissues of wheat seedlings and the changes in membrane leakage have been supposed to be at least partially responsible for the

interaction between salt and sub- or super-optimal temperatures on the length of shoot and roots (Hampson and Simpson, 1990).

In most investigations of the responses to salinity or heat stress, a single harvest date has been used to assess responses. Such analyses can mislead as plant size at harvest may be influenced by a range of factors such as the initial size of the plant (Hunt, 1982). Analyses of growth curves, calculated from sequential harvests, provides a relative basis on which to compare plant growth rates. A few investigations (Cramer, Epstein and Läuchli, 1990; Curtis and Läuchli, 1986; Schachtman, Bloom and Dvorak, 1989; Shennan, Hunt and MacRobbie, 1987a; Wickens and Cheeseman, 1988) have used the functional approach to plant growth analysis (Hunt, 1982) to explore the effects of salinity on growth and physiological processes. In studies with barley, Cramer *et al.* (1990) observed that net assimilation rate (NAR), an index of assimilatory capacity, but not leaf area ratio (LAR), an index representing the ratio of photosynthesizing to respiring material within the plant, was highly correlated with relative growth rate (RGR) for various levels of salinity; NAR and RGR were similarly affected by the composition of the salt treatments in the long-term. This was interpreted as suggesting that the photosynthetic-assimilatory machinery was limiting growth, not leaf area.

This investigation was designed to determine the degree to which the combined heat and salinity stress limits the vegetative growth of the cultivated tomato plant, and whether the response is mediated through effects on photosynthesis or on leaf area growth.

1.2 Methods

With sand culture, salinity stress was imposed on tomato seedlings using 10 mM NaCl (in full Hoagland's solution) from day 11 with daily increment of 10 mM and from day 18 with 20 mM to reach 150 mM on day 21. Thereafter, plants were maintained at the terminal salt concentration (0 and 150 mM) and a range of temperatures (13/8, 23/18, 33/28, 38/33 and 43/38°C, day/night) for the remainder of the experiment. For simplicity, the day temperature representing each treatment regime will be used hereafter. Plants were

harvested on days 21, 23, 26, 30 and 35. The death of the plant was judged by chlorosis of leaves and browning of roots when harvested. Dry weight (dw) of the whole plant (M_{tot}) as well as leaf (M_l), stem (M_s) and root (M_r) were transformed to the natural logarithm (ln) and plotted against time. The slope of this linearized equation with time (mg mg^{-1} d^{-1}) is taken as the relative growth rate (RGR). Similarly, leaf area was also transformed (ln) and the slope of the linear relationship with time is an estimate of the relative expansion rate (LER) (cm^2 cm^{-2} d^{-1}). Shoot length is linearly related to time without transformation, and the slope of this relationship is presented as the linear expansion rate. Grand mean values of net assimilation rate (NAR) and leaf area ratio (LAR) over five harvesting intervals during the experimental period of 14 days following imposition of different temperature regimes were calculated as described by Hunt (1990). For the above various parameters the characteristic ΔE, i.e. the difference between control and salt treatments, is a measure of the interaction between the effects of salinity and temperature. The ratio of dry weight increment in the leaf, stem and root to that of the whole plant for the experimental period was calculated and expressed as %. All other methods including plant culture conditions are detailed in the Chapter III.

1.3 Results

1.3.1 Visible injury

Plants exposed to a combination of salinity and temperature stresses, particularly at 38 and 43°C, developed typical visible symptoms of chloride and sodium excess injury, consisting of burning and necrotic patches on tips or margins as well as chlorotic mottling of leaves. At 43°C air temperature, i.e. 37°C in the rooting medium of the pot, death of the leaves and roots occurred before that of the stems which were still green. Plants in the salinity treatment were dead at the end of the experiment whereas control plants at this temperature and plants in both treatments at lower temperatures continued to grow. However, the loss of the mature leaves did not occur in any case.

1.3.2 Relative growth rate and net assimilation rate of the whole plant

Data summarizing the effects of temperature and salinity singly or in combination on the dry weight growth of the whole plant are presented in Fig. 4.1. ln dry weights of the whole plant (M_{tot}) (Figs 4.1A and B) were linearly related to time over the 14-day experimental period with little deviation from the fitted line in any treatment. R^2 for the linear expression of growth in mass for all temperature and salt combinations was larger than 0.91 with the exception of the salt-high temperature (43°C) treatment (0.20), in which plant dry weight did not increase significantly with time. RGR, the mean slope of each of these fitted lines was then plotted against temperature (Fig. 4.1C) and showed maximal dw growth rate at about 23°C for the whole plant in both control and salinity treatments. In the control treatments without salt, RGR fell progressively from 23 to 38°C but precipitously between 38 and 43°C. Salinity reduced the RGR of whole plant dry weight at all temperatures. The temperature response of salinity-treated plants was broadly similar to that of control plants, but the difference in RGR of the whole plant between control and salt-treated plants (ΔE) (Fig. 4.1C) increased progressively with temperature from 13°C, with a more rapid increase above 33°C, and ranged from 0.03 to 0.08 mg mg^{-1} d^{-1}. This interaction between the effects of temperature and salinity on RGR was significant.

Temperature and salinity had similar effects on net assimilation rate (NAR) of the tomato plant (Fig. 4.2) to those on RGR (Fig. 4.1C). NAR was maximal at about 23°C in both control and salt treatments and lower at 13°C and above 33°C. There was a significant interaction of the effects of salt and temperature on NAR. The difference between control and salinity treatments (ΔE) varied with temperature, being lowest at 23°C and higher at 13, 33 and 38°C, falling at 43°C.

1.3.3 Leaf expansion rate and leaf area ratio

As with the dw growth of the whole plant, ln leaf area was linearly related to time over the 14-day period with little difference in fit between combination of treatments (Figs

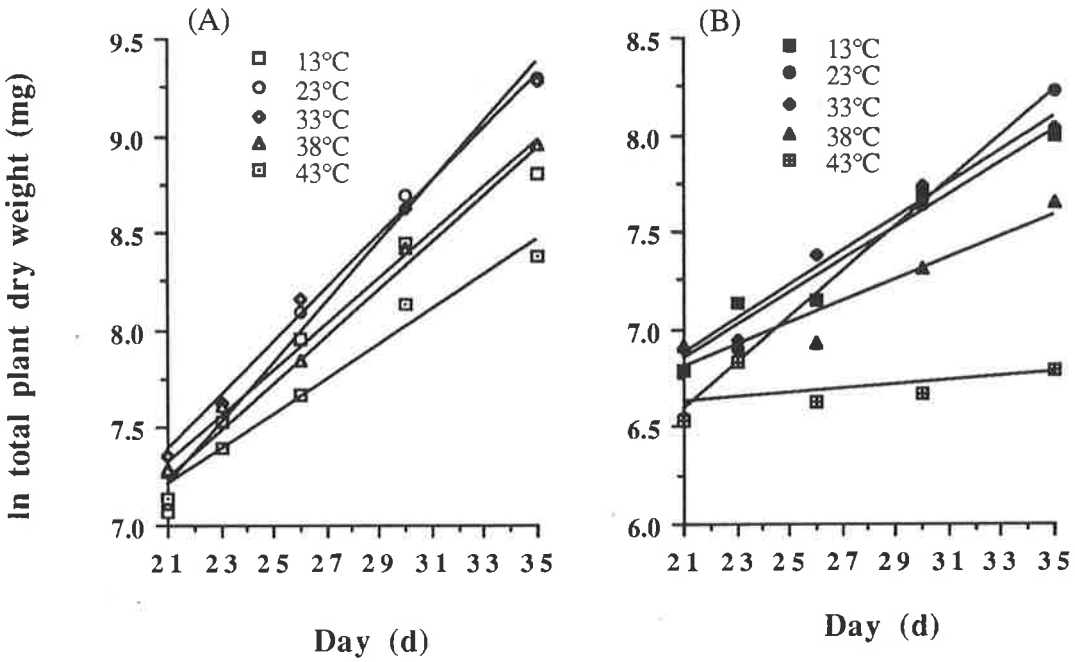
Fig. 4.1 Interaction of the effects of salinity and temperature on plant vegetative growth: linear regressions for ln dry weights (mg) of plant (M_{tot}) over 14 days of stress. Control plants (A), salinity treatment (B) and the mean slopes (RGR, mg mg^{-1} d^{-1}) plotted against temperature (C). 0 (open symbols) or 150 (solid symbols) mM NaCl stress and the difference (Δ) Vertical bars representing ±SE of the means are shown when larger than symbols.

Linear regression equations:

	0 mM NaCl		150 mM NaCl	
13°C:	y = 4.691 + 0.113x	R^2 = 0.96	y = 5.083 + 0.083x	R^2 = 0.96
23°C:	y = 3.949 + 0.152x	R^2 = 0.99	y = 4.147 + 0.112x	R^2 = 1.00
33°C:	y = 4.490 + 0.134x	R^2 = 1.00	y = 5.044 + 0.082x	R^2 = 0.97
38°C:	y = 4.844 + 0.126x	R^2 = 0.99	y = 5.622 + 0.057x	R^2 = 0.91
43°C:	y = 5.302 + 0.090x	R^2 = 0.97	y = 6.424 + 0.010x	R^2 = 0.20

Analysis of variance for slopes (F, ≤ 0.01):**

Salt (S): 409.76**, Temperature (T): 55.49**, S x T: 12.03**


(A)
(B)
13°C
23°C
33°C
38°C
43°C
ln total plant dry weight (mg)
9.5
9.0
8.5
8.0
7.5
7.0
8.5
8.0
7.5
7.0
6.5
6.0
21 23 25 27 29 31 33 35
Day (d)

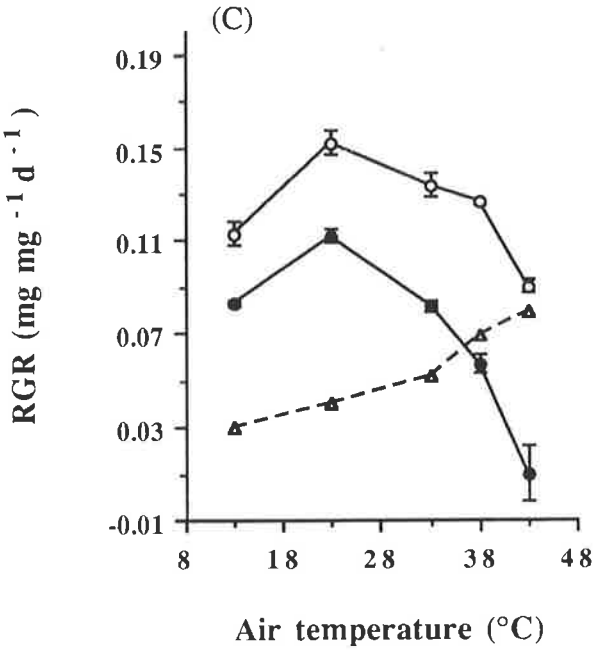
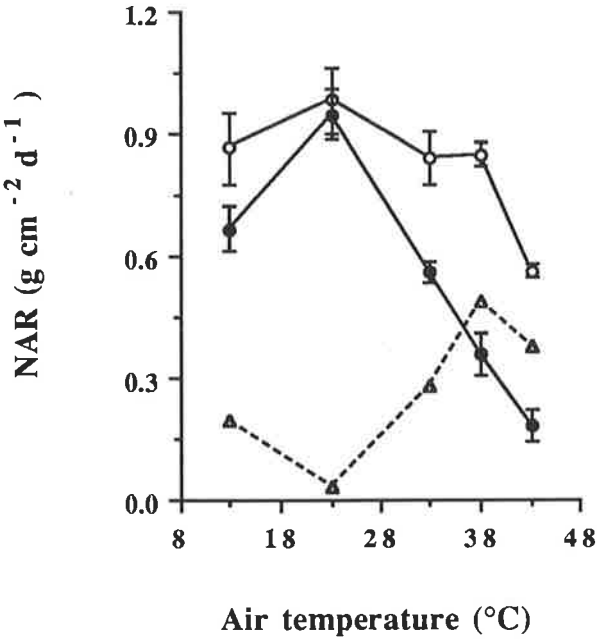

(C)
RGR (mg mg -1 d -1)
0.19
0.15
0.11
0.07
0.03
-0.01
8 18 28 38 48
Air temperature (°C)

Fig. 4.2 Interaction of the effects of salinity and temperature on NAR (g cm^{-2} d^{-1}) of tomato plants over 14 days of stress. 0 (○) or 150 (●) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

Analysis of variance (F, ≤ 0.01 or 0.05*):**

Salt (S): 59.46**, Temperature (T): 25.93**, S x T: 4.72*


1.2
0.9
0.6
0.3
0.0
NAR (g cm -2 d -1)
8
18
28
38
48
Air temperature (°C)

4.3A and B). R^2 of the regression lines exceeded 0.80 in all but 1 case, that being the 43°C salinity treatment which completely inhibited growth. The mean slope, i.e. the relative expansion rate of the leaves (LER) was plotted against temperature (Fig. 4.3C) to examine the interaction between salinity and high temperature on leaf expansion. In the control plants without salinity, LER appeared maximal between 23 and 33°C with a decrease at lower or higher temperatures. Salinity significantly reduced LER at all temperature regimes above 13°C. At 13°C, there was little effect of salinity. ΔE of LER between control and salinity treatments increased with temperature from 13 to 38°C, then declined slightly at 43°C. There was a significant interaction between salinity and temperature on the relative leaf expansion rate ($P < 0.01$).

There was a significant but small increase in leaf area ratio (LAR) with increase in temperature in control plants ($P < 0.01$, Fig. 4.4). Further, salinity decreased LAR at all temperatures ($P<0.01$). However, no interaction between the effects of these two factors was found ($P>0.05$) and the difference between LAR of control and treatment plants was similar at all temperatures.

1.3.4 Relative growth rates of plant organs and partitioning of dry weight between component parts

As with ln dw growth of the whole plant, ln dw of the component parts, leaf (M_l) (Figs 4.5A and B), stem (M_s) (Figs 4.6A and B) and root (M_r) (Figs 4.7A and B) was linearly associated with time over the 14-day experimental period with little deviation from the fitted lines in any treatment. R^2 for linear expression of growth of these three parts for all temperature and salt combinations was larger than 0.83 with the exception of the salt and high temperature (43°C) treatment, where ln M_l and M_r did not increase significantly with time. The mean slope (RGR) of each of these fitted lines was then plotted against temperature (Figs 4.5C, 4.6C and 4.7C) and disclosed maximal rates around 23/24°C air/root temperatures for all component parts in both control and salinity treatments. In the control treatments, the RGR of M_l, M_s and M_r fell steadily from 23 to 38°C but steeply

Fig. 4.3 Interaction of the effects of salinity and temperature on leaf expansion. Linear regressions of ln leaf area (cm^2) per plant on time during stress. Control plants (A), salinity treatment (B) and the mean slopes (LER) ($cm^2\ cm^{-2}\ d^{-1}$) plotted against temperature (C). 0 (open symbols) or 150 (solid symbols) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

Linear regression equations:

	0 mM NaCl		150 mM NaCl	
13°C:	y = 4.300 + 0.071x	R^2 = 0.95	y = 4.305 + 0.064x	R^2 = 0.92
23°C:	y = 3.218 + 0.119x	R^2 = 0.978	y = 2.551 + 0.096x	R^2 = 0.99
33°C:	y = 3.488 + 0.114x	R^2 = 1.00	y = 3.828 + 0.064x	R^2 = 0.98
38°C:	y = 4.105 + 0.088x	R^2 = 1.00	y = 4.163 + 0.033x	R^2 = 0.80
43°C:	y = 4.607 + 0.048x	R^2 = 0.99	y = 4.589 + 0.005x	R^2 = 0.02

Analysis of variance for slopes (F, ≤ 0.01):**

Salt (S): 78.63**, Temperature (T): 29.20**, S x T: 4.91**.

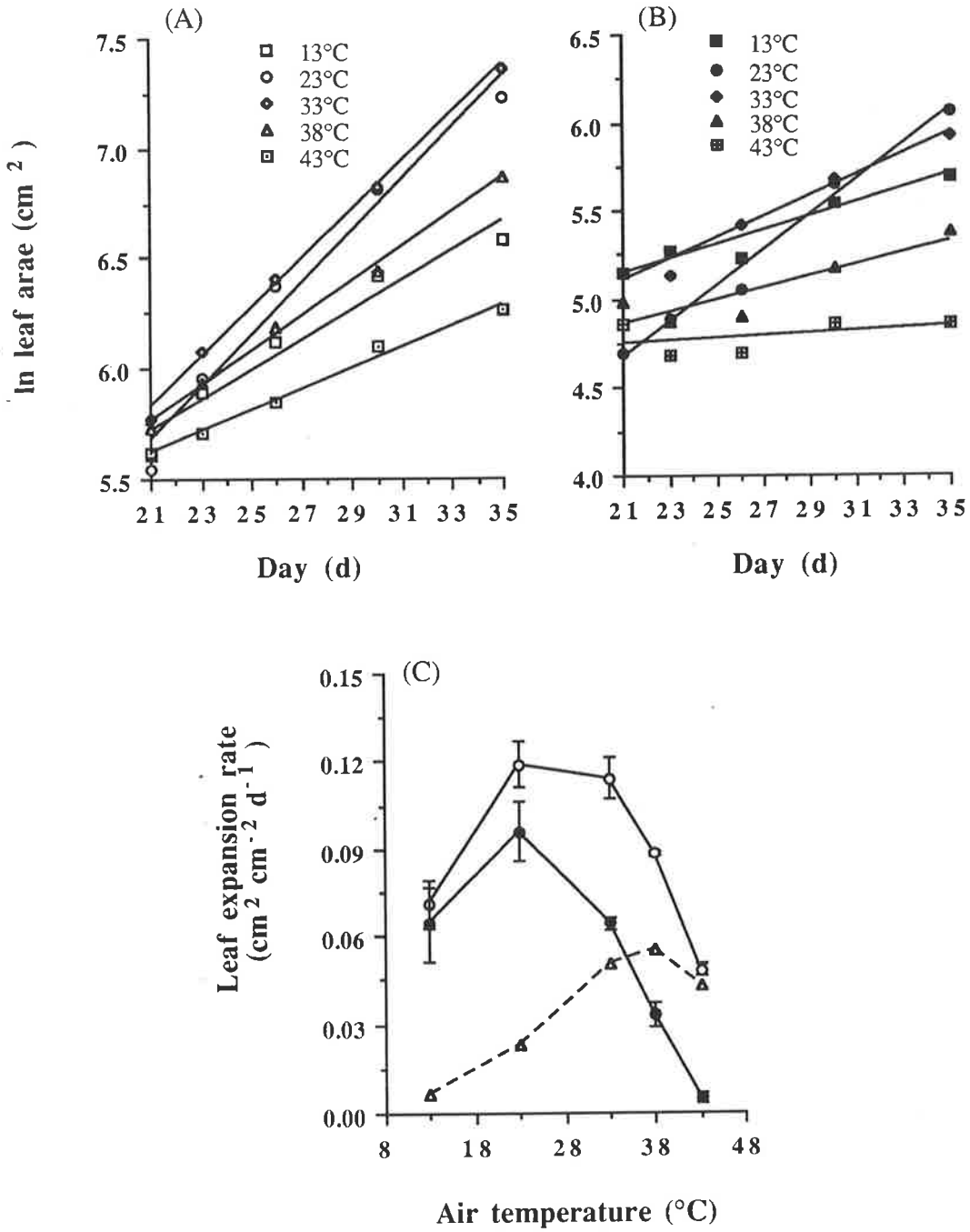

(A)
(B)
(C)
13°C
23°C
33°C
38°C
43°C
ln leaf arae (cm 2)
Day (d)
Leaf expansion rate
(cm 2 cm -2 d -1)
Air temperature (°C)

Fig. 4.4 Effects of salinity and temperature on the grand mean LAR for all harvests of tomato plants over 14 days of stress. 0 (○) or 150 (●) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

Analysis of variance (F, ≤ 0.01, interaction not significant: ns):**

Salt (S): 109.29**, Temperature (T): 23.65**, S x T: 1.82 ns.

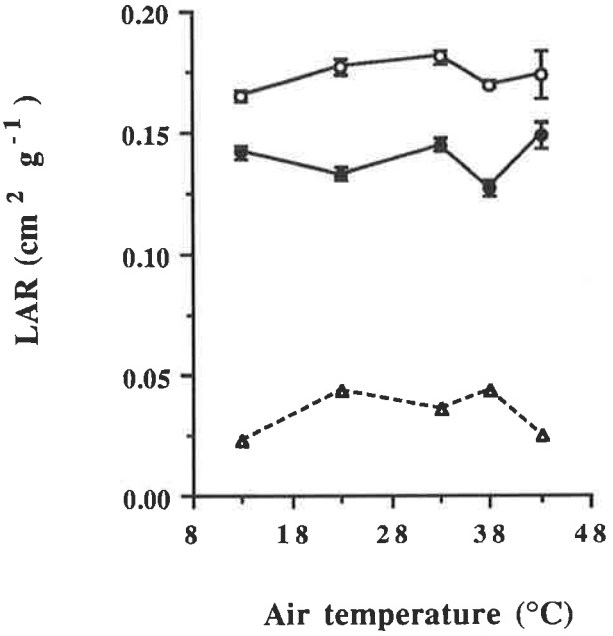

0.20
0.15
0.10
0.05
0.00
LAR (cm 2 g -1)
8
18
28
38
48
Air temperature (°C)

Fig. 4.5 Interaction of the effects of salinity and temperature on the growth of component parts. Linear regressions of ln dry weight (mg) of the leaves per plant over 14 days of stress. Control plants (A), salinity treatment (B) and the mean slopes (RGR, mg mg^{-1} d^{-1}) plotted against temperature (C). 0 (open symbols) or 150 (solid symbols) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

Linear regression equations:

	0 mM NaCl		150 mM NaCl	
13°C:	y = 4.053 + 0.119x	R^2 = 0.97	y = 4.422 + 0.087x	R^2 = 0.96
23°C:	y = 3.623 + 0.144x	R^2 = 0.99	y = 3.649 + 0.107x	R^2 = 1.00
33°C:	y = 4.171 + 0.120x	R^2 = 1.00	y = 4.773 + 0.064x	R^2 = 0.96
38°C:	y = 4.460 + 0.109x	R^2 = 0.99	y = 5.209 + 0.046x	R^2 = 0.83
43°C:	y = 4.968 + 0.084x	R^2 = 0.97	y = 5.759 + 0.010x	R^2 = 0.49

Analysis of variance for slopes (F, ≤ 0.01):**

Salt (S): 421.66**, Temperature (T): 113.63**, S x T: 10.92**

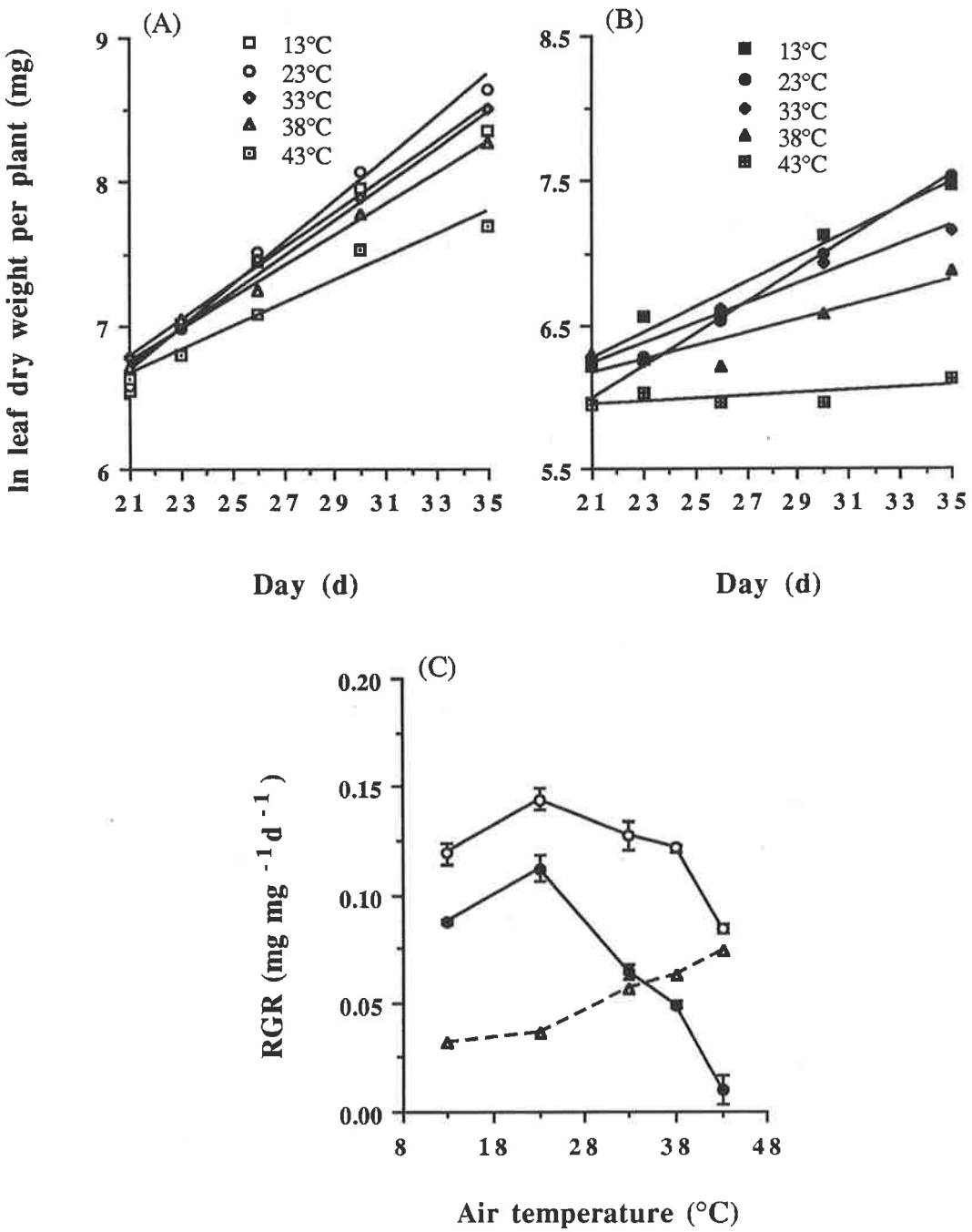

(A)
13°C
23°C
33°C
38°C
43°C
ln leaf dry weight per plant (mg)
9
8
7
6
21 23 25 27 29 31 33 35
Day (d)
(B)
13°C
23°C
33°C
38°C
43°C
8.5
7.5
6.5
5.5
21 23 25 27 29 31 33 35
Day (d)
(C)
RGR (mg mg -1 d -1)
0.20
0.15
0.10
0.05
0.00
8 18 28 38 48
Air temperature (°C)

Fig. 4.6 Interaction of the effects of salinity and temperature on the growth and component parts. Linear regressions of ln dry weight (mg) of the stems per plant over 14 days of stress. Control plants (A), salinity treatment (B) and the mean slopes (RGR, mg mg^{-1} d^{-1}) plotted against temperature (C). 0 (open symbols) or 150 (solid symbols) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

Linear regression equations:

	0 mM NaCl		150 mM NaCl	
13°C:	y = 3.490 + 0.119x	R^2 = 0.95	y = 3.801 + 0.089x	R^2 = 0.96
23°C:	y = 2.227 + 0.180x	R^2 = 0.98	y = 2.625 + 0.131x	R^2 = 1.00
33°C:	y = 2.751 + 0.168x	R^2 = 0.99	y = 3.299 + 0.120x	R^2 = 0.98
38°C:	y = 2.856 + 0.150x	R^2 = 0.99	y = 3.952 + 0.079x	R^2 = 0.96
43°C:	y = 3.854 + 0.116x	R^2 = 0.96	y = 4.503 + 0.038x	R^2 = 0.96

Analysis of variance for slopes

(F, ≤ 0.01, interaction not significant: ns):**

Salt (S): 95.89**, Temperature (T): 50.69**, S x T: 2.36 ns

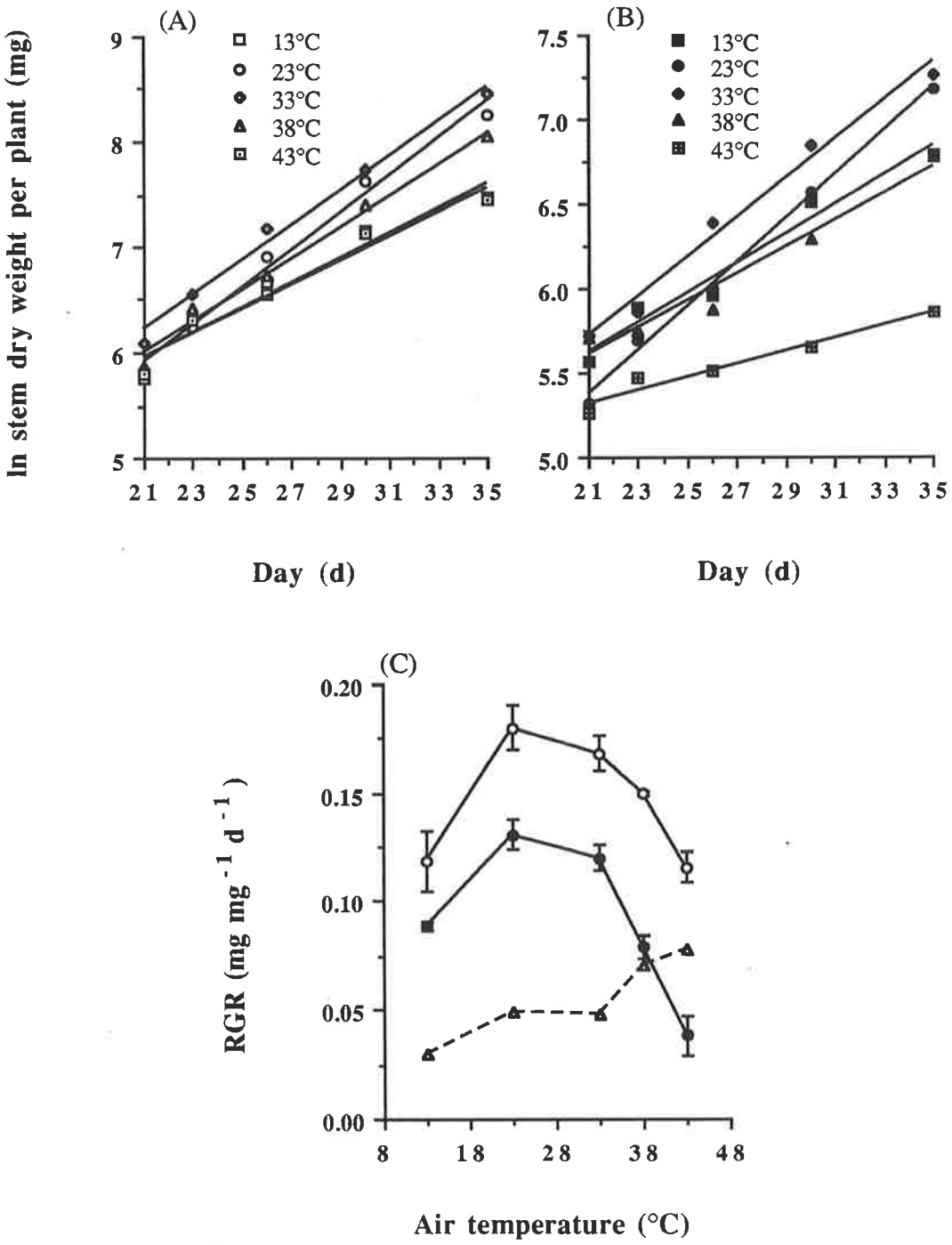

(A)
13°C
23°C
33°C
38°C
43°C
ln stem dry weight per plant (mg)
9
8
7
6
5
21 23 25 27 29 31 33 35
Day (d)
(B)
13°C
23°C
33°C
38°C
43°C
7.5
7.0
6.5
6.0
5.5
5.0
21 23 25 27 29 31 33 35
Day (d)
(C)
RGR (mg mg-1 d-1)
0.20
0.15
0.10
0.05
0.00
8 18 28 38 48
Air temperature (°C)

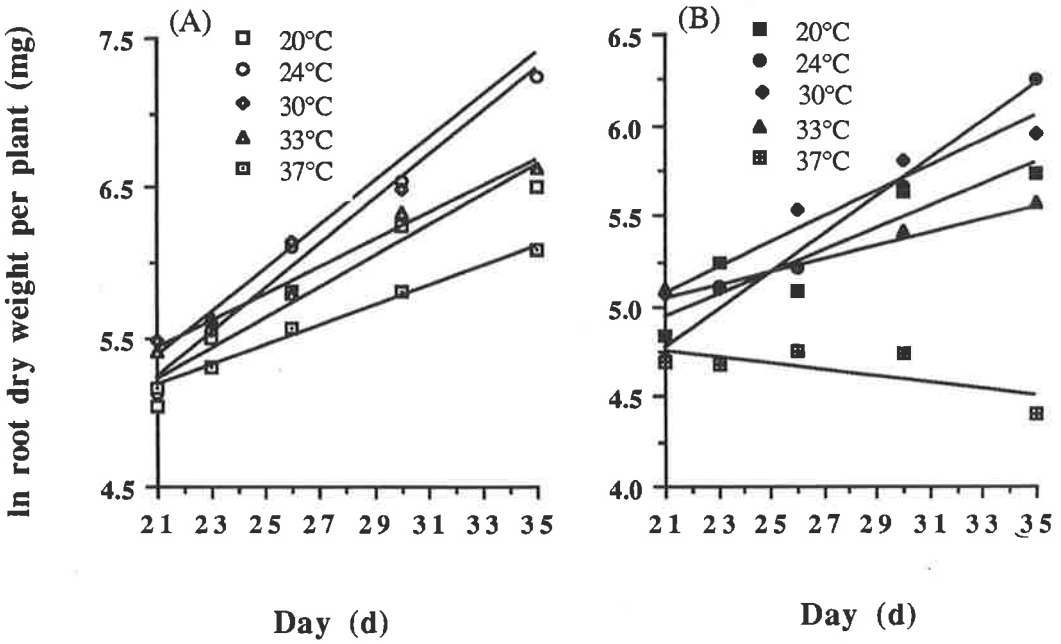
Fig. 4.7 Interaction of the effects of salinity and temperature on the growth of component parts. Linear regressions of ln dry weight (mg) of the roots per plant on time during stress. Control plants (A), salinity treatment (B) and the mean slopes (RGR, mg mg^{-1} d^{-1}) plotted against temperature (C). 0 (open symbols) or 150 (solid symbols) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

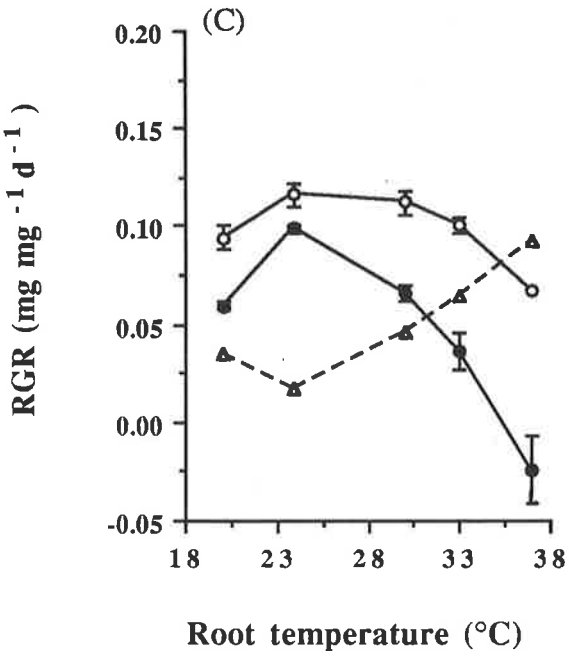
Linear regression equations:

	0 mM NaCl		150 mM NaCl	
13°C:	y = 3.068 + 0.094x	R^2 = 0.95	y = 3.677 + 0.059x	R^2 = 0.83
23°C:	y = 2.143 + 0.116x	R^2 = 0.99	y = 2.576 + 0.099x	R^2 = 0.98
33°C:	y = 2.359 + 0.112x	R^2 = 0.98	y = 3.610 + 0.066x	R^2 = 0.93
38°C:	y = 3.537 + 0.100x	R^2 = 0.98	y = 4.261 + 0.036x	R^2 = 0.87
43°C:	y = 3.780 + 0.067x	R^2 = 0.99	y = 5.117 + 0.025x	R^2 = 0.47

Analysis of variance for slopes (F, ≤ 0.01):**

Salt (S): 108.63**, Temperature (T): 38.79**, S x T: 6.94**


(A)
20°C
24°C
30°C
33°C
37°C
ln root dry weight per plant (mg)
7.5
6.5
5.5
4.5
21 23 25 27 29 31 33 35
Day (d)
(B)
20°C
24°C
30°C
33°C
37°C
6.5
6.0
5.5
5.0
4.5
4.0
21 23 25 27 29 31 33 35
Day (d)


(C)
RGR (mg mg -1 d -1)
0.20
0.15
0.10
0.05
0.00
-0.05
18 23 28 33 38
Root temperature (°C)

between 38 and 43°C air temperature. Salinity reduced the relative growth rates of all three organs at all temperatures. The RGR in dw of the leaves (Fig. 4.5C) and roots (Fig. 4.7C) demonstrated an interaction of the effects of salinity and temperature ($P<0.01$) similar to that observed for the whole plant (Fig. 4.1C). However, there was no significant interaction of the effects of salinity and temperature on the RGR of the stem (S x T: 2.36 ns, $P>0.05$) and the growth of stems was reduced by salinity at all temperatures by an approximately equal amount (Fig. 4.6C). These differences in response of RGR of these three parts are apparent in changes in ΔE. ΔE for RGR of the leaves (Fig. 4.5C) increased continuously with temperature, ranging between 0.03 and 0.07 mg mg^{-1} d^{-1} for temperatures between 13 and 43°C. ΔE for the stems (Fig. 4.6C) appeared to increase from 13 to 23°C, was constant between 23 and 33°C and increased again above 38°C, but these differences were not significant. For the roots (Fig. 4.7C), ΔE was lowest at 24°C (23°C for air temperature) and much larger at lower and higher temperatures, varying from 0.02 (24°C) to 0.09 mg mg^{-1} d^{-1} (37°C, corresponding to air temperature 43°C).

Ratios of dry weight of the leaves over the total plant dry weight for both control and salinity treatment increased slowly with leaf age at 13°C and declined with age at temperatures above 23°C (Data not shown). As a measure of allocation of assimilate, the proportion of dw increment in the leaves to the increase in dw of the whole plant for the experimental period was calculated (Fig. 4.8A). Allocation to the leaves was significantly affected by temperature, being high at 13°C (65%) and falling at higher temperatures to 33°C (45 ~ 50%). Salinity significantly reduced the distribution of dry matter to the leaves, but there was no significant interaction with temperature on partitioning. The ratio of stem dw to total plant dw was unchanged with plant age at 13°C but increased with age at temperatures above 23°C for both control and salt treatments (data not shown). When the ratio of dw increment in the stem compared to the increase in dw of the whole plant was plotted against temperature (Fig. 4.8B), temperature and salinity alone or in combination were found to significantly affect dw allocation to the stems. The allocation of dw to the stems increased from 13 to 33°C, then stayed constant at higher temperatures. In contrast to the leaf, the allocation of dw to the stems of control plants was lower than that to the stems

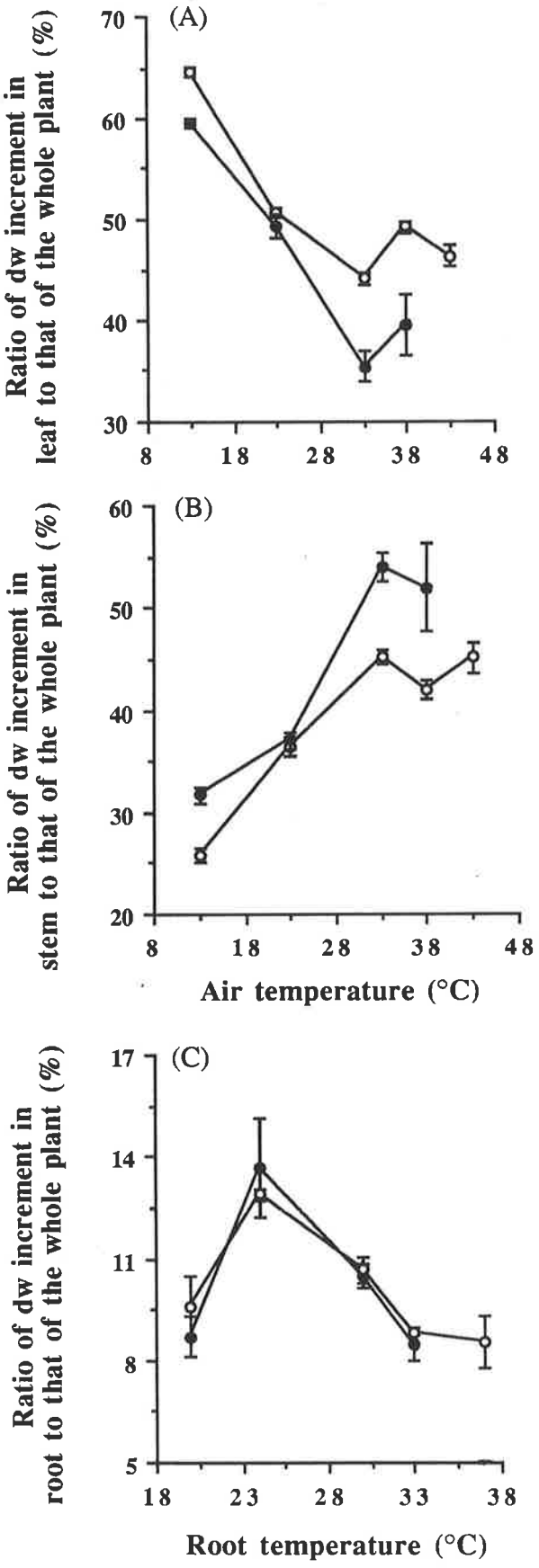
Fig. 4.8 **Changes in the ratio of dry weight increment in the leaves (A), stems (B) and roots (C) to that of the whole plant (%) with temperature of the 14-day experimental period. 0 (○) or 150 (●) mM NaCl stress. Vertical bars representing ±SE of the means are shown when larger than symbols.**

Analysis of variance (F, ≤ 0.01, 0.05* or not significant: ns):**

Leaf: Salt (S): 35.60**, Temperature (T): 27.9**, S x T: 1.80 ns.

Stem: Salt (S): 59.02**, Temperature (T): 77.55**, S x T: 4.73*.

Root: Salt (S): 2.93 ns, Temperature (T): 13.29**, S x T: 2.16 ns.


(A)
Ratio of dw increment in leaf to that of the whole plant (%)
70
60
50
40
30
8
18
28
38
48
(B)
Ratio of dw increment in stem to that of the whole plant (%)
60
50
40
30
20
8
18
28
38
48
Air temperature (°C)
(C)
Ratio of dw increment in root to that of the whole plant (%)
17
14
11
8
5
18
23
28
33
38
Root temperature (°C)

of salt-stressed plants (Fig. 4.8B). Partitioning of dry weight to the roots was significantly affected only by temperature and was maximal around 23°C and lower at 13°C or above 33°C (Fig. 4.8C).

1.3.5 Shoot height and its ratio to stem dry weight

Shoot height (S_h) was linearly related to time over the experimental period with little deviation in all treatment combinations (Figs 4.9A and B), the R^2 of the regression lines exceeded 0.86 in all but 1 of 10 cases (0.52 at 43°C, salt treatment). The slope (elongation rate) was plotted against temperature (Fig. 4.9C) to estimate the interaction of the effects of salt and temperature on shoot expansion. In the control plants without salinity, the elongation rate was low at 13°C with a value of less than 0.5 cm d^{-1}, and increased with increasing temperature to 33°C, where the rate was 1.8 cm d^{-1}. At temperatures higher than 33°C, the elongation rate declined with further increase in temperature, to 0.6 cm d^{-1} at 43°C. Salinity significantly reduced S_h at all temperature regimes. Although salinity-treated plants showed the same temperature optimum for elongation, there was a significant interaction between salt and temperature effects on S_h. ΔE increased sharply from 13 to 23°C, then declined slightly over the range 23 ~ 43°C.

The ratio of shoot height to stem dry weight (Fig. 4.10) was unchanged with temperature in control conditions, varying between 9 and 12 cm g^{-1}, but was significantly higher in salt stress conditions at all temperatures. The effects of salinity and temperature interacted, with ratio values around 17 cm g^{-1} below 23°C, 21 cm g^{-1} at 33 and 38°C and 32 cm g^{-1} at 43°C. Although salinity depressed both stem elongation and stem dw, stems were thinner as a consequence of salinity.

1.3.6 Statistical analysis

Using the Genstat 5 package, a multiple linear regression of the dependence of RGR on both NAR and LAR with salt as a factor was performed (Fig. 4.11). The analysis

Fig. 4.9 Interaction of the effects of salinity and temperature on shoot elongation rate (cm d^{-1}). Linear regressions of shoot height (cm) on time during stress. Control plants (A), salinity treatment (B) and the mean slopes plotted against temperature (C). 0 (open symbols) or 150 (solid symbols) mM NaCl stress and the difference (Δ). Vertical bars representing ±SE of the means are shown when larger than symbols.

<u>Linear regression equations:</u>

	0 mM NaCl		150 mM NaCl	
13°C:	y = 4.411 + 0.438x	R^2 = 0.97	y = 4.678 + 0.350x	R^2 = 0.96
23°C:	y = -20.02 + 1.579x	R^2 = 1.00	y = -4.110 + 0.710x	R^2 = 1.00
33°C:	y = -23.95 + 1.930x	R^2 = 0.99	y = -14.03 + 1.298x	R^2 = 1.00
38°C:	y = -10.40 + 1.187x	R^2 = 0.98	y = 1.039 + 0.473x	R^2 = 0.86
43°C:	y = -2.023 + 0.679x	R^2 = 0.95	y = 9.548 + 0.045x	R^2 = 0.52

Analysis of variance for slopes (F, ≤ 0.01):**

Salt (S): 340.91**, Temperature (T): 263.05**, S x T: 17.24**.

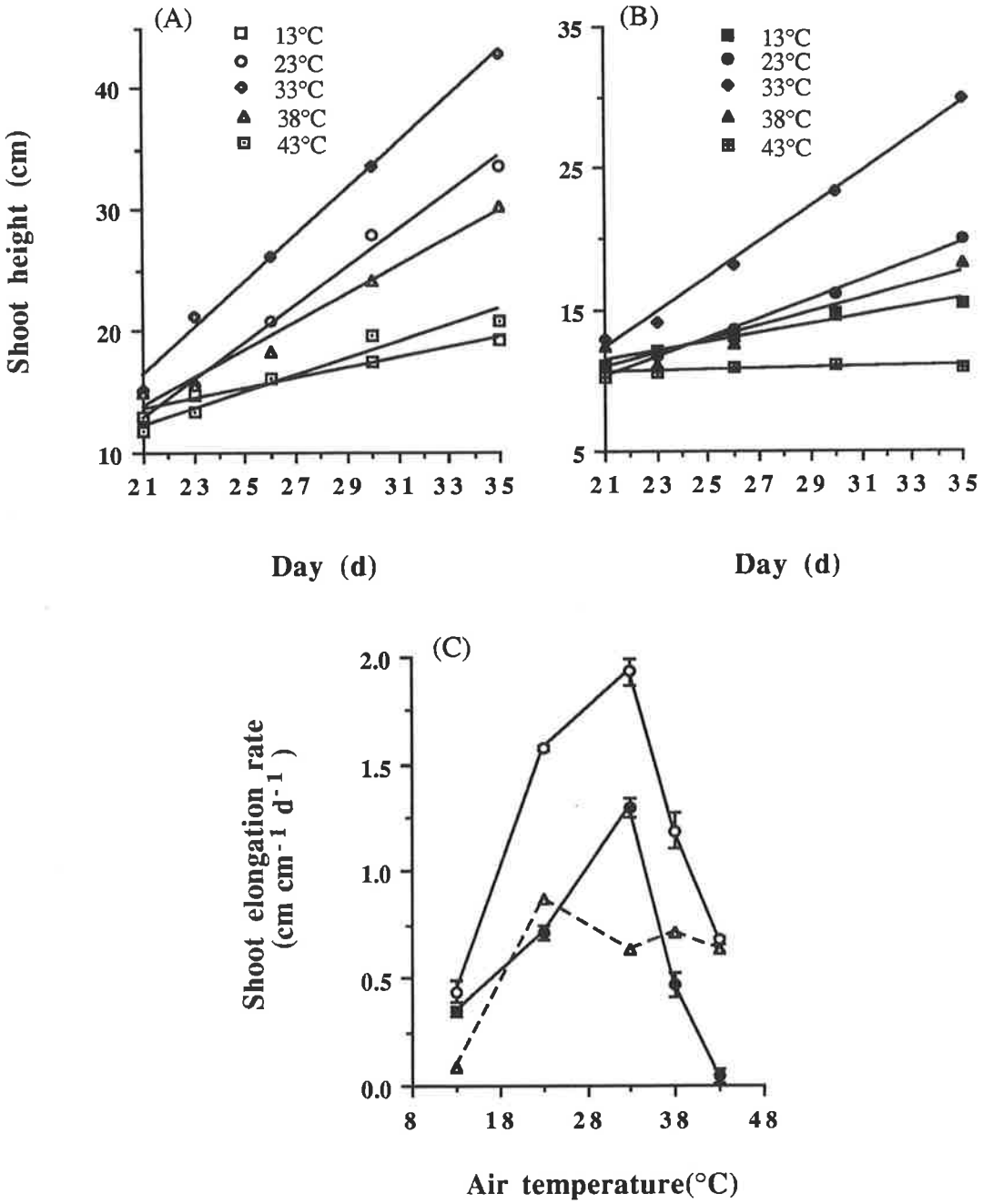

(A)
13°C
23°C
33°C
38°C
43°C
Shoot height (cm)
40
30
20
10
21 23 25 27 29 31 33 35
Day (d)
(B)
13°C
23°C
33°C
38°C
43°C
35
25
15
5
21 23 25 27 29 31 33 35
Day (d)
(C)
Shoot elongation rate
(cm cm-1 d-1)
2.0
1.5
1.0
0.5
0.0
8 18 28 38 48
Air temperature(°C)

Fig. 4.10 **Change in the ratio of shoot elongation to stem dry weight (cm g^{-1}) with salinity and temperature stress. 0 (○) or 150 (●) mM NaCl stress. Vertical bars representing ±SE of the means are shown when larger than symbols.**

Analysis of variance (F, < 0.01):**

Salt (S): 11.10**, Temperature (T): 203.94**, S x T: 9.55**.

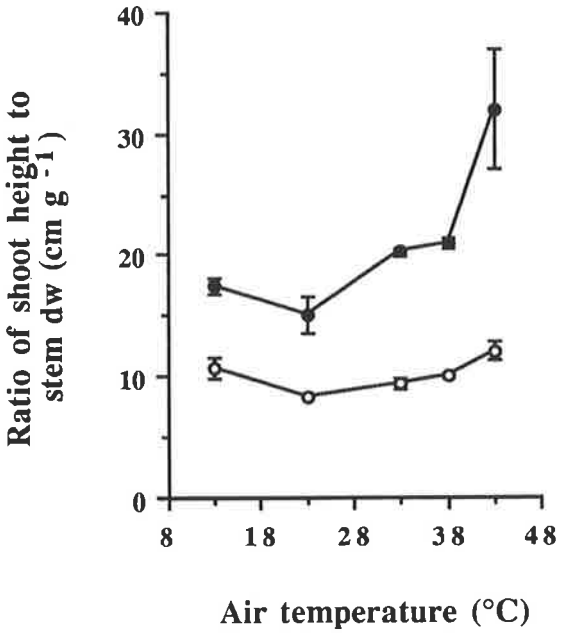
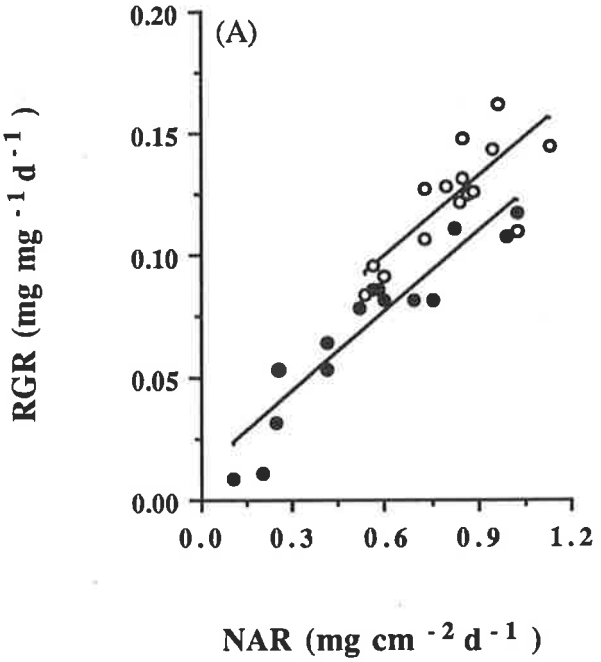
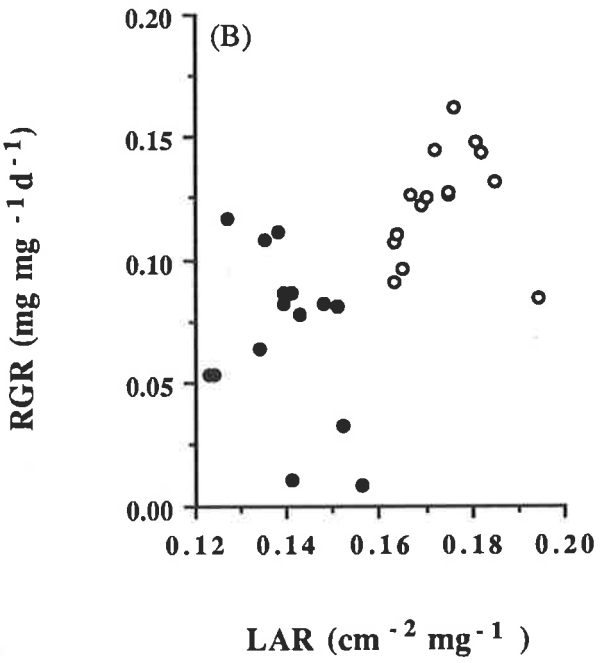

Ratio of shoot height to stem dw (cm g-1)
40
30
20
10
0
8
18
28
38
48
Air temperature (°C)

Fig. 4.11 **The linear relationship between mean RGR and NAR (A) or LAR (B) of tomato plants when exposed to salinity and temperature stress. 0 (○) or 150 (●) mM NaCl stress.**


(A)
0.20
0.15
0.10
0.05
0.00
0.0
0.3
0.6
0.9
1.2
RGR (mg mg -1 d -1)
NAR (mg cm -2 d -1)


(B)
0.20
0.15
0.10
0.05
0.00
0.12
0.14
0.16
0.18
0.20
RGR (mg mg -1 d -1)
LAR (cm -2 mg -1)

showed that the best fitted regression model to describe RGR included the salt treatment and NAR, but not LAR. The generalised model was a parallel regression model:

$$(RGR)_{ij} = \alpha_i + \beta(NAR)_{ij} + \varepsilon_{ij}$$

Estimated equations were:

Salt-untreated: $(RGR)_{1j} = 0.03413 + 0.1085\ (NAR)_{1j}$

Salt-treated: $(RGR)_{2j} = 0.01115 + 0.1085\ (NAR)_{2j}$

where i = 1, 2; j = 1, 2,·····15. $R^2 = 0.89^{**}$ ($P < 0.01$), and Fobs = 108.21^{***} ($P < 0.001$).

These equations indicate that the salt treatment significantly reduced RGR, but did not vary the dependence on NAR.

1.4 Discussion

It is clear from the results that both high temperature and salinity had significant impacts on the relative dw growth rate of the tomato plant (Fig. 4.1C). The optimal air temperature was around 23°C for relative growth rate of biomass production. This is in agreement with reports that chilling, high temperatures (Levitt, 1980a; Long and Woodward, 1988; Li *et al.*, 1991) and salt stress (Cramer *et al.* 1990; Greenway and Munns, 1980; Levitt, 1980b; Munns and Termaat, 1986) each inhibit the growth of crop plants. When salinity and high temperature stress were combined, there was a significant interaction in their effects on relative dw growth as shown in Fig. 4.1C. High temperature exacerbated the effects of salinity and salt-stressed plants were almost dead at 43°C by the end of this investigation. Control plants at this temperature and all plants at lower temperatures continued to grow. It must be borne in mind, however, that temperature treatments were not replicated and hence may be confounded with cabinet-specific effects.

The relative growth rate (RGR) observed in this study was significantly inhibited by the interaction of the effects of high temperature and salinity. The changes in RGR varied significantly with NAR, but not with LAR; and at any level of NAR, salinity reduced RGR by a similar amount. LAR was affected by salinity, but not by the interaction with temperature, so would have contributed to the response of RGR as seen in the relationship with NAR. This is comparable with the data obtained from experiments with the effects of salinity stress on barley plants (Cramer, Epstein & Läuchli, 1990). However, Curtis & Läuchli (1986) indicated that LAR, but not NAR of kenaf (Hibiscus cannabinus L., Malvaceae), was affected by salinity at higher temperature (38/24°C, day and night). These findings led to the conclusion (Curtis & Läuchli, 1986) that the growth of kenaf was limited by leaf area development, not the photosynthetic-assimilatory machinery. However, Cramer *et al.* (1990) further analysed this data by linear regression and found that RGR was more highly correlated with NAR ($R^2 = 0.85$) than with LAR ($R^2 = 0.71$). It is concluded that NAR was a more important factor than LAR in determining RGR of tomato plants in varying combinations of salinity and temperature.

NAR integrates the effects of treatment on the physiological processes of photosynthesis and respiration. Both low and high temperatures inhibit photosynthesis through influences on membrane integrity and membrane-bound proteins (Levitt, 1980a; Pallock and Eagles, 1988; Quinn, 1988; Weis and Berry, 1988). Salinity also inhibits photosynthesis in plants (Cheeseman, 1988; Rawson *et al.*, 1988; Seemann and Critchley, 1985). In barley, photosynthesis was inhibited by 30 to 60% between 100 and 175 mM NaCl (Rawson *et al.* 1988). In cultivated tomato, the reduction in assimilation rate was 36 to 60% between 150 and 200 mM NaCl (Rajasekaran, 1988). Salinity may reduce photosynthesis by effects on stomatal conductance, inhibition of biochemical reactions, feedback inhibition of carbon metabolism as a result of reduced growth (Seemann and Critchley, 1985), or a combination of these factors. Whether the reduction in photosynthetic capacity resulting from salinity is a consequence of feedback inhibition is controversial (Greenway and Munns, 1980; Munns and Termaat, 1986; Rawson, 1986; Rawson and Munns, 1984; Seemann and Critchley, 1985; Yeo *et al.* 1985). The effects of salt (4 ~ 6 weeks at 100, 150 or 200 mM NaCl) and temperature (5 ~ 10°C and 40 ~ 45°C)

on photosynthesis of cowpea (*Vigna unguiculata*) have been investigated by *in vivo* chlorophyll fluorescence studies (Larcher, Wagner and Thammathaworn, 1990). Salinity alone had little effect on fluorescence at 20°C, but high temperature increased the unfavourable effect of saline stress on the kinetics of non-photochemical quenching. The combined stress impaired photosynthetic capacity and extended the critical limits for function and viability through delayed energization of thylakoids and disturbances in the photosynthetic system.

Salinity also increases respiration in plants (Bloom and Epstein, 1984; Nieman, 1962; Rawson, 1986; Schwarz and Gale, 1981). Schwarz and Gale (1981) found that maintenance respiration increased without any net effect on total respiration, since assimilatory respiration declined with a salinity-induced decline in photosynthesis. They found with *Xanthium* that 80% of the reduction in carbon assimilation was due to increased maintenance respiration. They proposed that the percentage contribution of reduced photosynthesis and increased maintenance respiration to reduced carbon assimilation would vary depending on the plant species and environmental conditions.

The degree to which the growth of component organs of the tomato plant was tolerant of salinity and temperature was also examined. Generally in salinity studies, plant growth is analysed through shoot and root growth alone (Seemann and Critchley, 1985) and this confuses the examination of effects on photosynthetic tissue with effects on non-photosynthetic tissues. In fact, the tolerance of the leaves of tomato plants (Fig. 4.5) to salinity and high temperature differed significantly from that of the stems (Fig. 4.6). Reduction in leaf growth due to salinity could obviously involve allocation of assimilate (Fig. 4.8A).

Stem dry weight growth was not differentially affected by the combined stress of salinity and temperature (Fig. 4.6). This could result from maintenance of the allocation of dry matter to the stems in the presence of salinity (Fig. 4.8B). The higher proportional dw of the stems in the presence of salinity at 13 and above 33°C represents a greater net partitioning of dry matter to the stems than in the absence of salinity. Winter and Läuchli (1982), however, observed that salt-induced growth reductions of 30 and 40% occurred at

50 and 100 mM NaCl in *Trifolium alexandrinum* and *Trifolium pratense*, mostly affecting stems. Evidently, allocation responses to salinity are species specific.

Salinity alone appears to cause an increase in the proportion of dry matter allocated to the roots and this has been cited to account for the reduction of growth of the shoot, such as with *Phaseolus vulgaris L.* (Seemann and Critchley, 1985). In an investigation of effects of salinity on root growth and death dynamics in adult tomato (*L. esculentum* Mill.), Snapp and Shennan (1992) described the carbon cost of the observed enhanced rates of root death. Obviously, maintenance of net root growth in the presence of increased rates of root senescence would cost more in dry matter allocation. Modification of carbohydrate allocation to the roots would then influence the growth of the whole plant under salinity stress. The sensitivity of the roots to salinity and high temperature (Fig. 4.7) may be due to a reduction in carbon gained from the shoot or to enhanced rates of root death. Corresponding to 13, 23, 33, 38 and 43°C air temperature in the growth cabinets, the measured rooting medium temperatures were 20, 24, 30, 33 and 37°C, respectively. In contrast to plants grown in controlled environment cabinets, plants in the field with a canopy of leaves have a relatively short-term exposure to high temperature in the rooting medium during the day as depicted by Leeper (1967). Alternatively, long-term exposure to high root temperature may result in injury to root properties, including cell membranes (Berry and Bjorkman, 1980; Hampson and Simpson, 1990; Ingram, 1985; Levitt, 1980a; Quinn, 1988). It has been reported by Mozafar and Oertli (1992) that root-zone temperature and salinity interacted to determine tiller numbers and total dry matter production of barley such that the tolerance of barley to NaCl was altered by root temperature. In the present study, death of the roots occurred in the salinity treatment at a root medium temperature of 37°C (Section 1.2.1 in this Chapter).

As indicated in Fig. 4.9C, the maximal rate of tomato shoot elongation in both control and salt treatments occurred around 33°C. This tolerance of shoot elongation to higher temperature has been demonstrated in previous studies (see AVRDC, 1989). Shoot elongation rate was significantly affected by salt and high temperature stresses alone or in combination though ΔE for shoot elongation rate did not increase uniformly with

temperature. This observation is in agreement with the report of Hampson and Simpson (1990) for wheat. Interestingly, the ratio of shoot elongation to stem dry weight in salt-stressed plants was higher than that in control plants at all temperatures (Fig. 4.10). This ratio, a measure of relative expansion growth, increased with increased temperature. Elongation was evidently affected in a different manner to dry weight growth.

In summary, results presented here indicated that the relative growth rate of tomato plant dw was significantly affected by the double stress of salinity and temperature and the maximal growth occurred around 23°C air temperature. The inhibition of growth of the tomato plant during the interacting stresses could be primarily ascribed to curtailed net assimilation. This effect on assimilation was modified by effects on assimilate allocation and the relationship between dry weight acquisition and expansion growth. Stem dw growth was less sensitive to salinity and temperature in combination than leaf and root dw due to a higher net allocation of dry matter to the stems. Moreover, a high ratio of shoot height to stem dw under salinity suggests modification of the mechanism which controls shoot elongation.

2. Water Status and Solute Accumulation Associated with Temperature and Salinity Stress

2.1 Introduction

Excess salinity adversely affects the growth of numerous glycophytic crop plants (Greenway and Munns, 1980; Staples and Toenniessen, 1985) and an important primary effect of mild salinization is a reduction in leaf growth (Papp, Ball and Terry, 1983). One possible mechanism for this response is that the reduction in water potential in the root zone attributable to salinity is transmitted via the xylem to the leaves, where cell turgor is correspondingly reduced (e.g. Neumann, Volkenburgh and Cleland, 1988; Thiel, Lynch and Läuchli, 1988). However, the osmotic potential of leaf and root tissues adjusts in response to root media salinization, thus preventing a decline in turgor (Robinson, Downton and Millhouse, 1983; Heuer and Plaut, 1989; Yang, Newton and Miller, 1990; Lloyd, Syvertsen and Kriedeman, 1987). Similarly, heat stress has been postulated to produce 'drought stress' injury in plants (Levitt, 1980a), following the sharp rise in transpiration with a rise in environmental temperature. El-Sharkawi and Springuel (1979) noted that low osmotic potential (Ψ_s) decreased plumule elongation in wheat even at optimal temperature, but that Ψ_s had a greater effect above and below the optimum.

It has been established that when a plant is exposed to osmotic stress, not only is growth influenced, but metabolism also is modified. Among the modifications in metabolism, the accumulation of low-molecular weight solutes, mainly amino acids, sugars and polyols, has been paid much attention, as they are assumed to contribute to the adaptation and survival of plants under stress.

It is evident from the previous chapter that a combination of salinity and high temperature has a significant impact on tomato growth. Very little is known about the physiological response to a double stress of salt and temperature. The objective of the present work was to study the possible response of osmotic adjustment, and free amino acids and sugars in the tomato plant to the combination of salt and temperature stresses.

2.2 Methods

Water and osmotic potentials were measured with the expanded leaves at 9:30 ~ 10:00 am for each harvest on days 21, 23, 26, 30 and 35. There was little significant difference due to sampling date at $P < 0.05$ (harvest x salt x temperature); accordingly the data for these parameters are depicted below as the means of all measurements over the five sampling dates. At 43°C the salt-treated plants were dead at the end of the experiment so the means are calculated from days 21 ~ 30 only. Total free amino acids, including proline, were analysed for each harvest, but only the analytical data from the harvest on day 35 are plotted. The means for the five harvests showed very similar patterns to the data of day 35. The proline contents in the stems and roots, and the sugar content of the leaves were measured at the end of the experiment. The data for all these compatible solutes at 43°C on day 35 are shown for the dead leaves of salt-stressed plants. Details of the methods of analysis are included in Chapter III.

2.3 Results

2.3.1 Water status of the leaf

In control plants with no salinity stress, the mean of leaf water potential (Ψ) over the experimental period was -0.46 MPa at 13°C (Fig. 4.12A). This increased at higher temperatures, but there was little difference between 23 and 43°C (varying from -0.27 to -0.36 MPa). In plants subjected to 150 mM NaCl (-0.68 MPa) in the root zone, mean leaf Ψ fell at all temperatures. The temperature response of salinity-treated plants differed from that of the control plants. Leaf Ψ was lowest at 13°C (-1.07 MPa) and increased progressively from 23 to 33°C, attaining –0.67 MPa at 33°C. At 33°C, therefore the difference in leaf Ψ between control and salt-stressed plants (-0.40 MPa) was less than the difference in Ψ imposed on the root system. With further increase in temperature, Ψ fell to -0.97 MPa. It should be noted that plants grown at 43°C with salinity were dead by day 35, the data presented here are the mean of the previous four harvests.

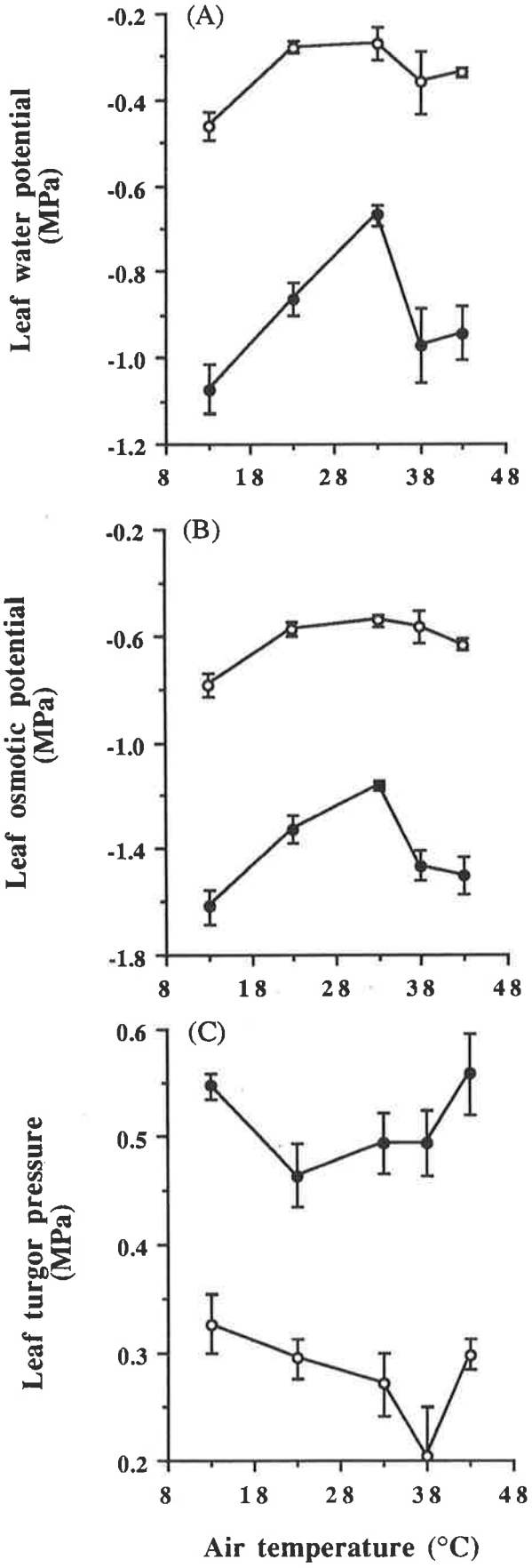
Fig. 4.12 The effects of temperature and salinity on leaf water (Ψ) (A) and osmotic (Ψ_s) (B) potential, and estimated turgor pressure (Ψ_p) (C). Mean data for four harvests at 43°C and five harvests at all other temperatures. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.

Analysis of variance ((F, ≤ 0.01, 0.05* or not significant: ns):**

(A) Ψ: Salt (S): 530.5**, Temperature (T): 16.7**, S x T: 3.54*

(B) Ψ_s: Salt (S): 1135.2**, Temperature (T): 32.5**, S x T: 5.96**

(C) Ψ_p: Salt (S): 178.4**, Temperature (T): 3.23*, S x T: 1.34 ns


(A)
Leaf water potential (MPa)
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
8
18
28
38
48
(B)
Leaf osmotic potential (MPa)
-0.2
-0.6
-1.0
-1.4
-1.8
8
18
28
38
48
(C)
Leaf turgor pressure (MPa)
0.6
0.5
0.4
0.3
0.2
8
18
28
38
48
Air temperature (°C)

The change with temperature in mean osmotic potential (Ψ_s) of the leaves in plants without or with salinity stress (Fig. 4.12B) showed similar trends to those of leaf Ψ, with the exception that the values of Ψ_s were much lower than those of Ψ at any temperature regime. As a consequence, estimated turgor pressures were positive in both control and salt treatments, and turgor of salinised leaves was always maintained above that of the appropriate controls (Fig. 4.12C). In control plants Ψ_p was high at 13°C, then appeared to fall slightly to 38°C but increased at 43°C. Similarly in saline treated-plants, Ψ_p seemed maximal at 13°C and then declined at higher temperatures. The higher value of calculated turgor at 43°C could be an artefact due to the lack of a measurement at the last harvest. However, there was no statistical interaction between the effects of salinity and temperature on turgor.

2.3.2 Solute content

2.3.2.1 *Free amino acids in the leaf*

Leaf total free amino acid content (dw basis) with and without the inclusion of proline varied with both salinity and temperature at the end of the experiment (Fig. 4.13). The pattern of response was similar over the whole experimental period (data not shown). In control plants, the content of leaf amino acids including proline (Fig. 4.13A) was significantly higher at 13°C (94 μmol g^{-1} dw) than between 23 and 38°C, where it remained almost constant. However, amino acid content increased sharply above 38°C. There was a significant accumulation of leaf amino acids in response to salinity at all temperatures. The content of amino acids was 165 μmol g^{-1} dw at 13°C and remained constant or fell slightly to 38°C. At 43°C, amino acid content was greatly increased to 272 μmol g^{-1} dw. The response in the absence of proline (Fig. 4.13B) shows that there was little effect of either temperature or salinity on the remaining amino acids at temperatures of 38°C or below. At 43°C, there was a marked accumulation of these amino acids, magnified by the salinity treatment.

Fig. 4.13 Interaction of the effects of temperature and salinity on accumulation of leaf total free amino acids. (A) with and (B) without the inclusion of proline after 14 days of stress. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.

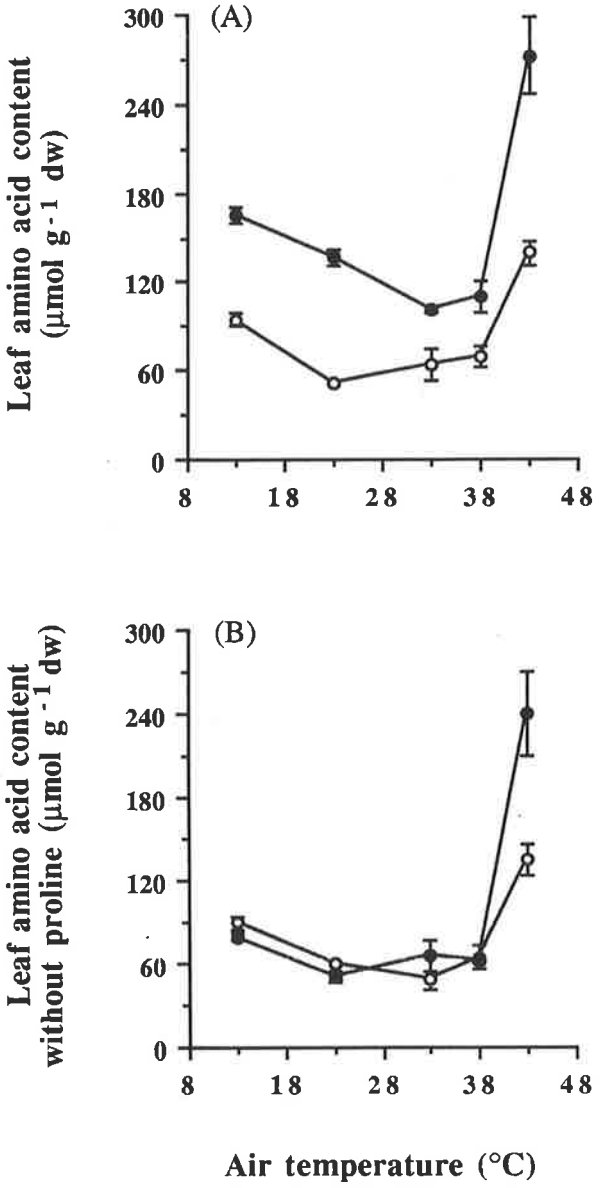
Analysis of variance (F, ≤ 0.01):**

(A) With proline:

Salt (S): 353.7**, Temperature (T): 241.6**, S x T: 6.98**

(B) Without proline:

Salt (S): 17.0**, Temperature (T): 388.6**, S x T: 14.5**


(A)
Leaf amino acid content
(μmol g-1 dw)
300
240
180
120
60
0
8
18
28
38
48
(B)
Leaf amino acid content
without proline (μmol g-1 dw)
300
240
180
120
60
0
8
18
28
38
48
Air temperature (°C)

2.3.2.2 *Proline in the component parts*

The pattern of leaf proline accumulation at the end of experiment plotted against temperature (Fig. 4.14A) was similar to the means over five harvests (Data not shown). Leaf proline concentration in the control plants was low and similar over the whole temperature range. Exposure to salinity (-0.68 MPa) caused an increase in leaf proline concentration at all temperatures but there was a significant linear fall with increasing temperature, ranging from 84.5 at 13°C to 32.6 μmol g^{-1} dw at 43°C. Similar patterns of proline accumulation were also observed in the stems (Fig. 4.14B) and roots (Fig. 4.14C). In the control plants, the concentrations of proline in the stems and roots were greater at 13°C (root temperature 20°C) than at higher temperatures. In the presence of salinity, the concentration of proline in the stems rose at all temperatures, but the response to the constant salinity stress decreased with temperature, as in the leaves. Similarly, proline concentration in the roots of saline plants was high at 20°C root temperature (112.2 μmol g^{-1} dw), and fell constantly as the temperature was elevated, to be close to zero at 43°C.

2.3.2.3 *Soluble sugars in the leaf*

Soluble sugars in the leaves at the end of the experiment varied significantly ($P < 0.05$ or 0.01) with both temperature and salinity (Fig. 4.15).

In control plants, the *myo*-inositol concentration in the leaves (Fig. 4.15A) was comparatively low and varied little over the range 13 ~ 43°C. Leaf *myo*-inositol concentration was increased significantly by salinity at all temperatures, reaching a maximum at 23°C and declining rapidly above 33°C.

Sucrose accumulated in the leaves of control plants at low (13°C) and high (43°C) temperatures but remained at a comparatively low level over the range from 23 to 38°C. (Fig. 4.15B). Salinity had no effect on leaf sucrose concentration at 13°C and depressed the level at 43°C, but increased the concentration at the intermediate temperatures.

Fig. 4.14 Interaction of the effects of temperature and salinity on accumulation of proline in the leaves (A), stems (B) and roots (C) after 14 days of stress. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.

Analysis of variance (F, ≤ 0.01):**

(A) Leaf:

Salt (S): 308.5**, Temperature (T): 9.8**, S x T: 8.6**

(B) Stem:

Salt (S): 409.8**, Temperature (T): 130.9**, S x T: 37.3**

(C) Root:

Salt (S): 289.4**, Temperature (T): 50.1**, S x T: 50.5**

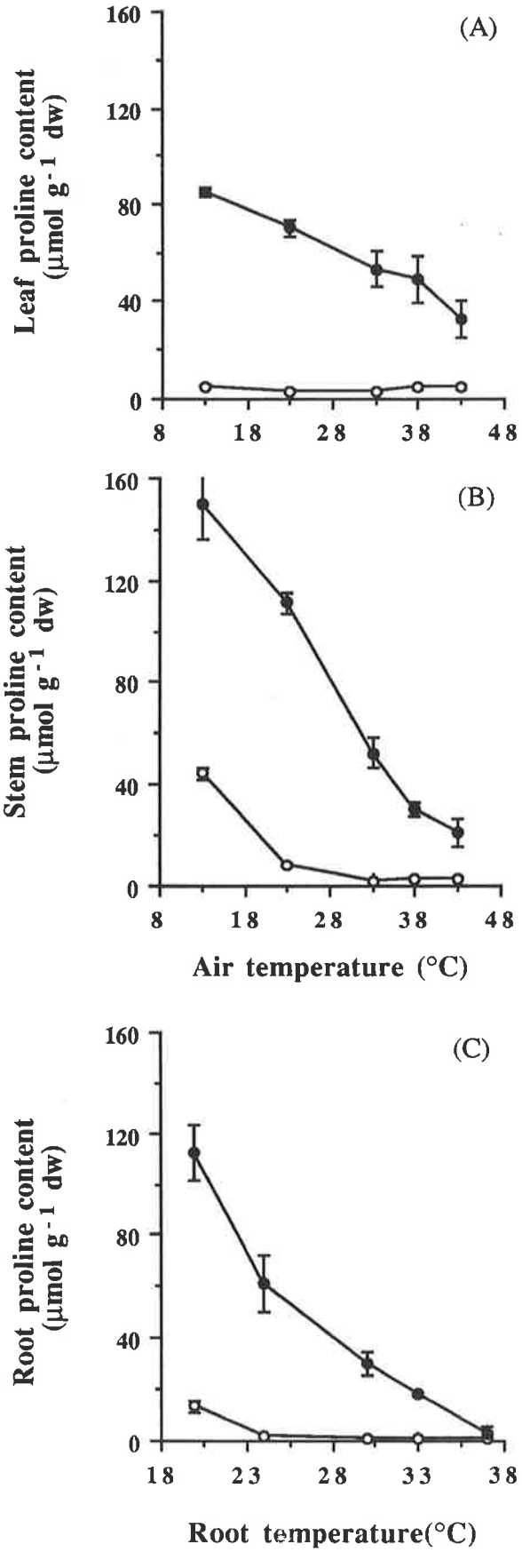

(A)
Leaf proline content
(μmol g-1 dw)
160
120
80
40
0
8
18
28
38
48
(B)
Stem proline content
(μmol g-1 dw)
160
120
80
40
0
8
18
28
38
48
Air temperature (°C)
(C)
Root proline content
(μmol g-1 dw)
160
120
80
40
0
18
23
28
33
38
Root temperature(°C)

Fig. 4.15 Interaction of the effects of temperature and salinity on accumulation of *myo*-inositol (A), sucrose (B) and reducing sugar (C) in the leaves after 14 days of stress. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.

Analysis of variance (F, ≤ 0.01 or 0.05*):**

(A) *myo*-Inositol:

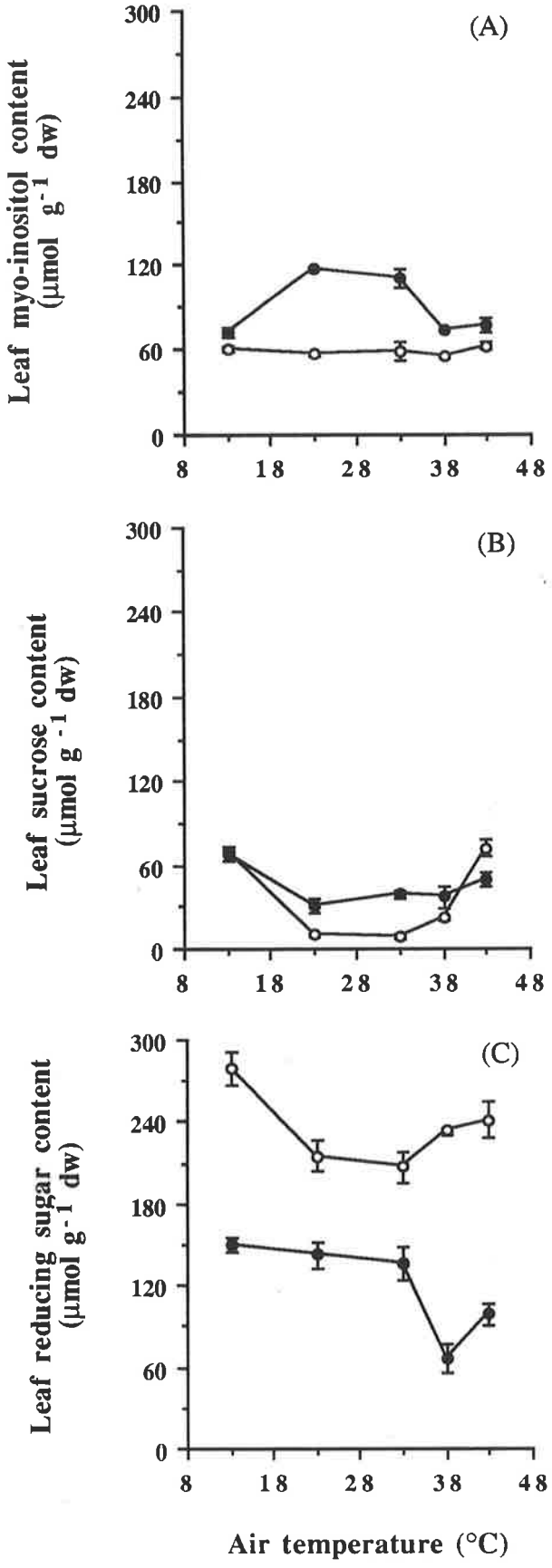
Salt (S): 92.1**, Temperature (T): 16.9**, S x T: 9.4**

(B) Sucrose:

Salt (S): 5.4*, Temperature (T): 101.3**, S x T: 6.5**

(C) Reducing sugar:

Salt (S): 321.6**, Temperature (T): 4.8*, S x T: 9.0**


(A)
Leaf myo-inositol content
(μmol g-1 dw)
300
240
180
120
60
0
8
18
28
38
48
(B)
Leaf sucrose content
(μmol g-1 dw)
300
240
180
120
60
0
8
18
28
38
48
(C)
Leaf reducing sugar content
(μmol g-1 dw)
300
240
180
120
60
0
8
18
28
38
48
Air temperature (°C)

The leaf reducing sugar (glucose and fructose) content of control plants (Fig. 4.15C) was comparatively high at 13°C (278 μmol g^{-1} dw), depressed at intermediate temperatures (23 ~ 33°C) and increased slightly at 38°C and above. In the saline treatments, reducing sugar concentration in the leaves was low at all temperatures. In response to temperature in this treatment, reducing sugar concentration was similar from 13 to 33°C and fell significantly at 38°C.

2.4 Discussion

The present investigation has confirmed the previous report (Chu *et al.* 1974) of the effect on water status of the plant at extreme temperatures. Leaf Ψ or Ψ_s (Fig. 4.12) did not significantly change with high temperature under sufficient watering. Obviously, there was not a 'drought stress' injury caused by heat stress as postulated by Levitt (1980a) to occur with a sharp rise in transpiration with a rise in environmental temperature. Lowering the water potential of the solution bathing the roots with NaCl led to a fall in leaf Ψ. Except for that at 33°C, the difference in leaf Ψ between control and salt treatment is around -0.65 MPa comparable to the level of external osmotic potential (-0.68 MPa). Depending upon the temperature, adjustment in leaf Ψ could be due solely to changes in internal Ψ_s without change in turgor, whereas calculated turgor was even higher when the plant was subjected to salinity at all temperatures. It can be concluded that changes in leaf turgor were not responsible for the recorded decline in net assimilation and relative growth rate with salinity (previous chapter). Acceptance of this conclusion rests on the accuracy of determination of the water status of the leaves. Water potential was measured psychrometrically and osmotic potential by the same means, using previously frozen tissues (Ehlig, 1961). This procedure has been questioned (Oertli, 1966) as salt may accumulate in cell walls external to the protoplast. In contrast, however, the results of Lloyd *et al.* (1987), showing turgor maintenance in salinised citrus leaves, were obtained from pressure/volume curves not susceptible to this error. It has been often observed that the osmotic potential of leaf, or stem and root tissues adjusts in response to root medium salinization, preventing a decline in turgor (Robinson, Downton and Millhouse, 1983; Heuer and Plaut, 1989; Yang,

Newton and Miller, 1990). Furthermore, turgor of leaves of salt-sensitive varieties is usually higher than that of salt-tolerant relatives (see table compiled by Munns, 1993). This is presumably because salt-sensitivity is commonly coupled with poor exclusion of salt by roots, and salt concentrations are higher in leaves of sensitive than in those tolerant genotypes. These observations suggest that some factors other than turgor are responsible for the reduction in plant growth in saline environment.

The most striking metabolic consequence of lowered Ψ in many plants is a rapid and extensive accumulation of soluble amino acids, in particular, proline (Chu *et al.* 1974; Fougere *et al.* 1991; Pulich, 1986; Singh *et al.* 1973) and soluble sugars and polyols (Ford, 1979, 1982, 1984; Fougere *et al.* 1991; Handa *et al.* 1983; Timpa *et al.* 1986; Walker *et al.* 1988). Accumulation of proline has also been reported to occur in plants subjected to lower temperatures (Chu *et al.* 1974; Gates *et al.* 1971; Naidu, 1987).

The facts presented here clarify the effects of temperature and salinity, or reduced Ψ on the total free amino acid content. It is evident that proline is the dominant component among all free amino acids measured to respond to temperature and salinity (Fig. 4.13), as reported by Chu *et al.* (1974) with leaves of barley and radish with temperature extremes and by Fougere *et al.* (1991) with alfalfa exposed to salinity. Although leaf Ψ was lowered by the double stress of salinity and high temperature (Fig. 4.12), the concentration of leaf free amino acids other than proline was not greatly influenced by salinity over the temperature range 13 ~ 38°C. This is comparable to the study of Bray *et al.* (1991), where the total free amino acid content in the leaves and roots of poplar vitroplants (*Populus trichocarpa* x *deltoides*) was not significantly modified after transfer to media containing 50 to 200 mM NaCl at 25°C. The increase in total free amino acid content of severely stressed plants at 43°C noted in the present study is a possible consequence of growth inhibition, protein degradation and reduced protein synthesis. Protein breakdown at low temperatures without rapid resynthesis, as well as a decreased protein synthesis and increased breakdown during heat stress has been suggested (Levitt, 1980a). Such changes in protein metabolism may contribute to the accumulation of total free amino acids at low and high

temperatures. Further, the entry of amino acids into the respiratory chain could be inhibited by low or high temperatures.

The data shown in this experiment (Fig. 4.14) are consistent with that from previous investigations (Chu *et al.* 1976a, b) in that more proline accumulated in leaf tissues exposed to a low temperature. It has been confirmed that proline accumulates more rapidly at a lower than at a higher temperature, at least over the temperature range of 4° ~ 39°C (Chu *et al.* 1974; Naidu *et al.* 1991). In the present study, when plants were subjected to salinity, the proline contents of the leaves, stems and roots were each negatively correlated with temperature (Fig. 4.16). This response does not correspond to temperature-controlled changes in leaf Ψ or Ψ_s in salinised plants (Figs 4.12A and B) and it can be concluded that proline accumulation is not directly related to changes in leaf water relationships. This contrasts with several reports (Greenway and Lealy, 1970, 1972; Jones, 1973), which suggest that turgor is the major determinant of metabolism. However, it has been demonstrated (Chu *et al.* 1976a; Handa *et al.* 1986; Voetberg and Stewart, 1984) that proline continues to increase when the plant or cell regains turgor by osmoregulation, suggesting that turgor is probably not linked to proline accumulation, even though the initiation of proline accumulation is accompanied by a temporary decline in turgor. It has also been demonstrated (Chu *et al.* 1974) that neither leaf water and osmotic potential nor turgor change with low temperature are sufficient to account for the accumulation of proline. Proline accumulates when plants are subjected to cold stress, but there is no change in water potential (Parameshwara *et al.* 1988). On the other hand, proline only accumulates in leaves of heated plants when water potential falls, there being apparently no direct response to elevated temperature. One possibility is that two independent and dissimilar environmental factors can both cause the same metabolic response (Chu *et al.* 1974; Parameshwara *et al.* 1988). This could conceivably occur through quite distinct effects on metabolism with the same ultimate effect, or because both environmental stress conditions affect the same basic metabolic process. In plants exposed to salinity, Na^+ or Cl^- ions may have a direct effect on proline metabolism. There was an inhibition of proline accumulation in intact barley plants by NaCl when compared to polyethylene glycol at a

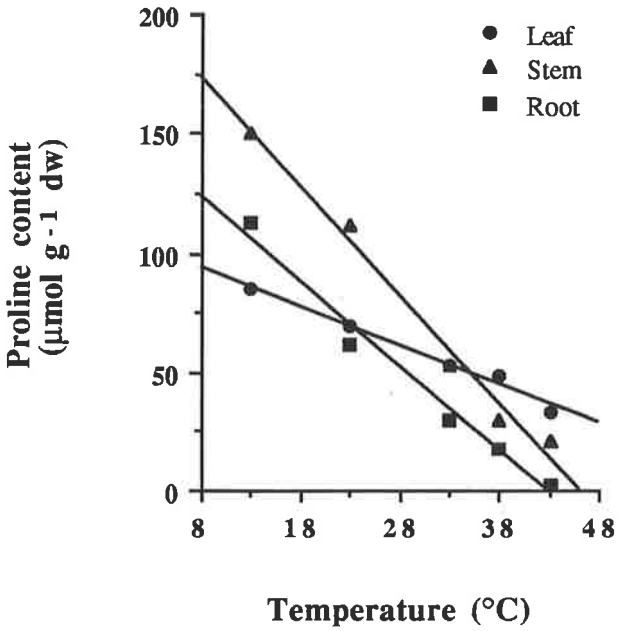
Fig. 4.16 **Linear relationships between proline content and temperature for leaf, stem and root of saline-treated tomato plants. R values are significant at 1% (**).**

Linear regression equations:

Leaf: $y = 107.2 - 1.64x$ $R^2 = 0.98^{**}$

Stem: $y = 210.6 - 4.59x$ $R^2 = 0.99^{**}$

Root: $y = 152.0 - 3.57x$ $R^2 = 0.98^{**}$


Proline content
(µmol g-1 dw)
200
150
100
50
0
8
18
28
38
48
Leaf
Stem
Root
Temperature (°C)

comparable tissue water potential (Chu *et al.* 1976a, b). Noguchi *et al.* (1966) found that a high concentration of Na^+ inhibited ^{14}C incorporation from glutamic acid into proline in the tobacco leaf. This leads to the suggestion that the decline in proline accumulation in all tomato component parts with increasing temperature may be explained an increase in tissue NaCl concentration with temperature.

It has been suggested that *myo*-inositol accumulation may be involved in osmoregulation (Briens and Larther, 1982; Gorham *et al.* 1981). Tissue concentration has been demonstrated to be related to water stress in tropical legumes (Ford, 1982, 1984) and salt resistance in three different tomato lines (Sacher and Staple, 1985). In this investigation, an overall increase in leaf *myo*-inositol content (Fig. 4.15A) was caused by salinity at a range of temperatures; however, the trends in concentration were correlated with neither changes in Ψ and Ψ_s nor turgor maintenance in tomato leaves under the combined stress of temperature and salinity (Fig. 4.12). Despite ubiquity in plants and a possible minor role in osmotic adjustment, *myo*-inositol accumulation does not always respond to lowered water potential due to salt (Fougere *et al.* 1991) or water (Ford and Wilson, 1981) stress. Sugar alcohols are formed during photosynthesis and stored (Dennis and Turpin, 1990). *myo*-inositol is the only sugar alcohol found in tomato leaves and its metabolism seems to be more sensitive to high temperature than to salinity stress. However, whether this is related to reduced photosynthetic assimilation is further to be clarified.

Sucrose did not appear to play a role in osmoregulation, as the concentration in the leaves of salt-treated plants was only higher than that in control plants between 23 and 38°C (Fig. 4.15B). The reducing sugar (glucose plus fructose) content in the leaf declined when plants were exposed to salinity (Fig. 4.25C). The sucrose content of the roots of alfalfa (Fougere *et al.* 1991) did not increase with increased NaCl concentration in the bathing medium, whilst both glucose and fructose contents increased slightly with NaCl. Fougere *et al.* concluded that rather than sucrose and reducing sugar, pinitol concentration was increased significantly and might contribute to the tolerance to salt stress. However, no pinitol was found in the tomato leaves in this investigation. Similarly, the concentrations of

fructose, glucose, sucrose and *myo*-inositol decreased or were unchanged because of water deficit in the majority of the legume species (Ford, 1984). In saline conditions, on the other hand, total soluble sugar contents did not change in NO_3^-/NH_4^+-fed plants, while they increased in NO_3^-- or decreased in NH_4^+-grown plants (Bourgeais-Chaillou *et al.* 1992). Soluble sugars, as they occupy a central role in metabolism, are subject to the influence of many processes and factors. Consequently, small changes in concentration are difficult to interpret and dangerous to attribute to individual environmental factors.

In brief, this investigation disclosed that a decline in water or osmotic potential corresponding to external osmotic potential is not sufficient to account for a reduction in assimilation. Turgor is not the regulating factor on this evidence but the actual control is unresolved. An osmotic potential control is unlikely as that would constitute a feed-forward system with no regulation. The total free amino acid content, apart from proline, in the leaf tissue was unaffected by salinity or temperature, but increased significantly at 43°C when plant growth ceased. Proline and soluble sugars other than sucrose tended to decline with the combination of salinity and higher temperature. It is possible that tomato growth reduction with exposure to salinity may be due to a substrate shortage when temperature is increased.

3. Tissue Ion Concentrations Associated with Temperature and Salinity Stress

3.1 Introduction

The results described in the previous chapters show that net assimilation and relative growth rates are significantly inhibited by high temperature and salinity. However, there is no evidence to support the hypothesis that plant water relations limit growth in the saline and high temperature conditions of this experiment. An alternative possible explanation of the inhibition of net assimilation and relative growth rates is inhibition by ions accumulated as the result of ion uptake and distribution in the plant. Reduction in crop plant growth, including that of tomato, by salt stress is often attributed to specific Cl^- and Na^+ ion effects on either metabolism in the cell or ion uptake by the roots (Greenway and Munns, 1980; Munns, 1993; Taleisnik 1987) and consequent nutritional disorders (Cramer *et al.* 1991; Grattan and Grieve, 1992).

Long-term exposure to high root temperature may result in injury to root properties, including cell membrane integrity (Berry and Bjorkman, 1980; Ingram, 1985; Levitt, 1980b; Quinn, 1988). If high root temperature is combined with elevated salinity levels, plant growth is severely affected by large fluxes of ions and toxicity of the salt or imbalance in nutrient elements. For instance, barley and sorghum leaf growth responses to root temperature have been correlated with nutrient ion fluxes (BassiriRad, Radin and Matsuda, 1991). The ion economy of intact plants includes ion uptake into the roots, transport of ions among the various organs, and final accumulation in the leaves and other tissues. The effects of salinity on plant growth are influenced by effects on these processes and it is possible that temperature may modify any of these responses. In particular, high temperature may modify cell membrane permeability (Levitt, 1980b), and thus salt uptake and water flux through the plant, and further ion distribution within the plant.

A knowledge of the accumulation of various ions in the organs of the tomato plant is thus of importance in understanding the mechanism of the interaction of the effects of temperature and salinity stress on plant growth.

3.2 Methods

Cl^-, Na^+ and K^+ were estimated for dried samples of leaves, stems and roots from all harvests but only the analytical data from the harvest on day 35 are presented, as with the concentrations of proline and other solutes in the previous section. The concentrations of S^{2-}, P^-, B^-, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, $Cu^{2(3)+}$, $Ni^{2(3)+}$, Mo^{2+} and $Fe^{2(3)+}$ in the dried leaf tissue were also tested at the end of the experiment. The data for all these ions at 43°C on day 35 are shown for the dead leaves alone present on the plant at that time. The concentration of inorganic ions was calculated as $m^{mol}_{\lambda}\ g^{-1}$ dw for the major and µmol g^{-1} dw for the micro elements. The K^+ and Na^+ selectivity of ion absorption per plant through roots (m^{mol}_{λ} $plant^{-1}$ g^{-1} root dw) on day 35 was described from regression analysis over the temperature range. Root surface area was estimated from root weight using a regression of root fresh weight against length and radius, determined for a sub-sample of roots. The rates of ion uptake and transport (J_s and J_{tot}) over the interval from day 21 to 35 were calculated from this root surface area estimate. Detailed methods of analysis are stated in Chapter III.

3.3 Results

3.3.1 Chloride and sodium

3.3.1.1 *Accumulation in various tissues*

Cl^- (Figs 4.17B, C and D) and Na^+ (Figs 4.18B, C and D) concentrations in the leaves, stems and roots ($m^{mol}_{\lambda}\ g^{-1}$ dw) were measured, and the absorption by the roots estimated (m^{mol}_{λ} $plant^{-1}$ g^{-1} root dw) (Fig. 4.17A). Whilst Cl^- and Na^+ uptake by the control plants were low and constant, uptake of both increased in the salt-stressed plants from

Fig. 4.17 **Interaction of the effects of temperature and salinity stress on the concentration of Cl^- in the whole plant (A), leaves (B), stems (C) and roots (D). Concentration was determined after 14 days of stress. The root temperatures of 20, 24, 30, 33 and 37°C correspond to air temperatures of 13, 23, 33, 38 and 43°C respectively. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

(A). Absorption through roots ($\frac{mmol}{\wedge}$ plant^{-1} g^{-1} root dw).

(B). Concentration in leaves ($\frac{mmol}{\wedge}$ g^{-1} dw).

(C). Concentration in stems ($\frac{mmol}{\wedge}$ g^{-1} dw).

(D). Concentration in roots ($\frac{mmol}{\wedge}$ g^{-1} dw).

Analyses of variance (F, ≤ 0.01):**

Whole plant: Salt (S): 58.1**, Temperature (T): 8.63**, S x T: 7.35**

Leaf: Salt (S): 871**, Temperature (T): 97.8**, S x T: 78.2**

Stem: Salt (S): 374**, Temperature (T): 29.6**, S x T: 16.7**

Root: Salt (S): 1260**, Temperature (T): 16.6**, S x T: 9.93**

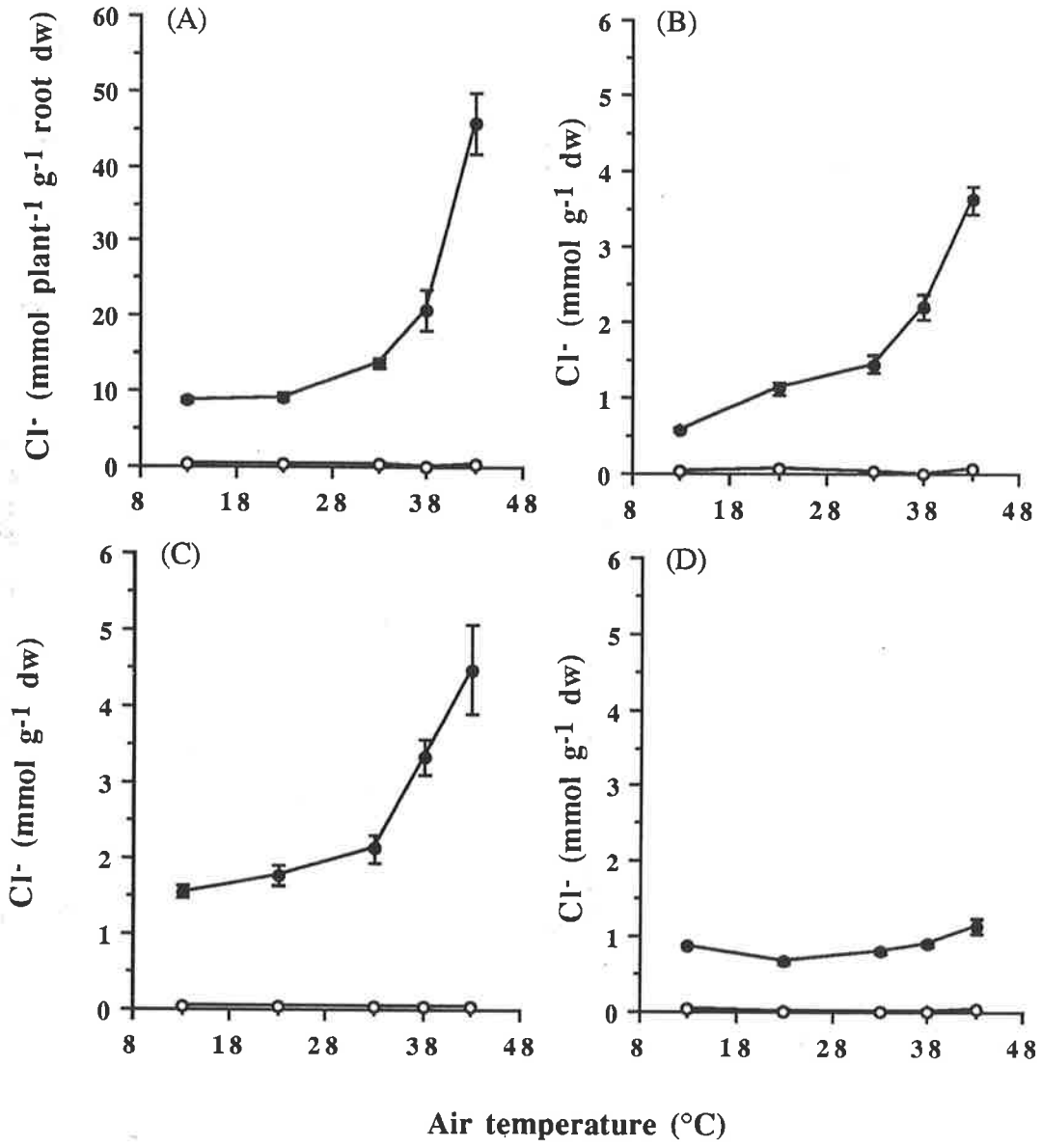
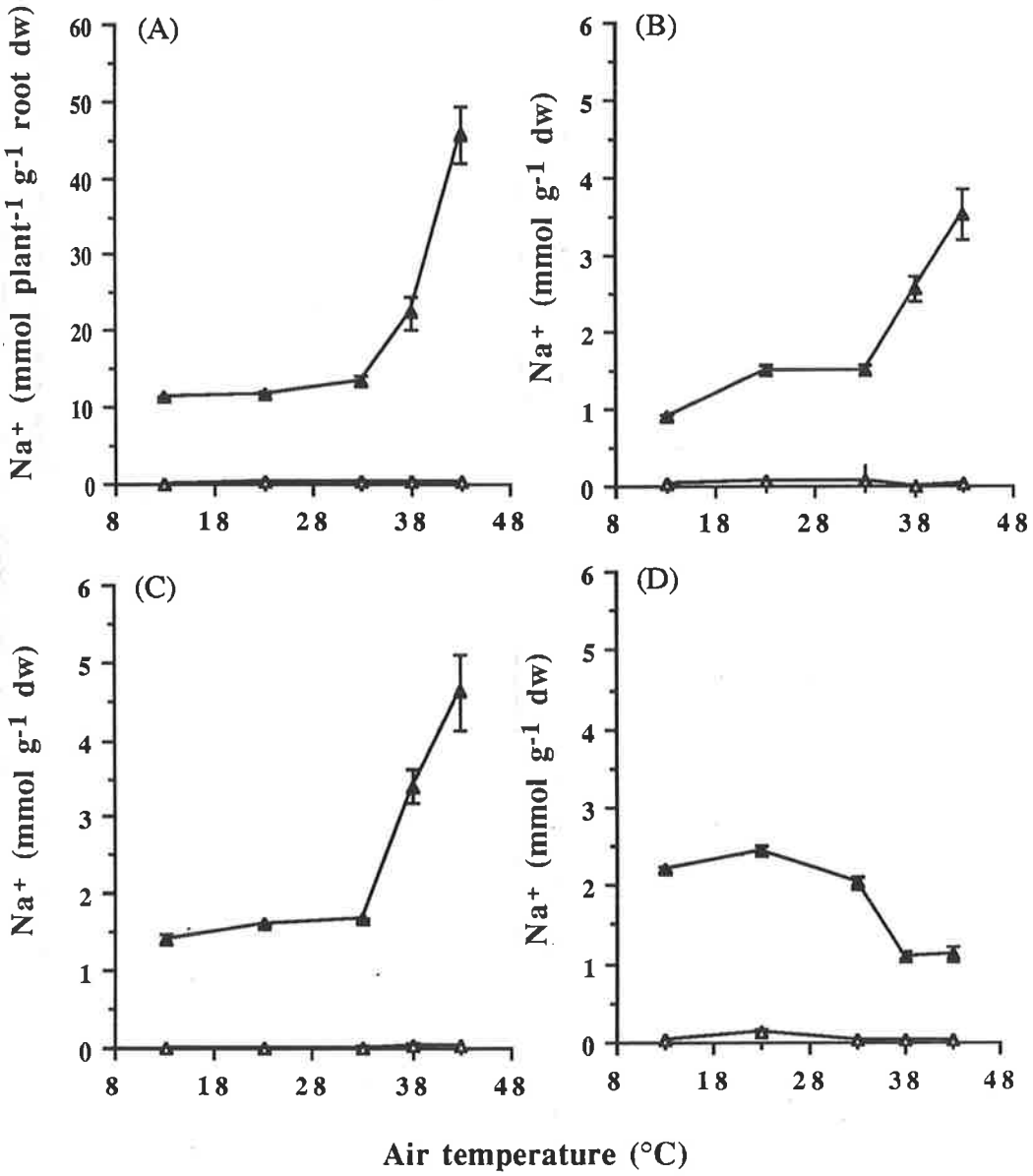

(A)
(B)
(C)
(D)
Cl- (mmol plant-1 g-1 root dw)
Cl- (mmol g-1 dw)
Air temperature (°C)

Fig. 4.18 **Interaction of the effect of temperature and salinity on the concentration of Na^+ in the whole plant (A), leaves (B), stems (C) and roots (D). Concentration was determined after 14 days of stress. The root temperatures of 20, 24, 30, 33 and 37°C correspond to air temperatures of 13, 23, 33, 38 and 43°C respectively. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

(A). Absorption through roots ($\frac{mmol}{l}$ plant^{-1} g^{-1} root dw).
(B). Concentration in leaves ($\frac{mmol}{l}$ g^{-1} dw).
(C). Concentration in stems ($\frac{mmol}{l}$ g^{-1} dw).
(D). Concentration in roots ($\frac{mmol}{l}$ g^{-1} dw).

Analyses of variance (F, ≤ 0.01):**

Whole plant:	Salt (S): 65.4**, Temperature (T): 6.69**, S x T: 6.49**
Leaf:	Salt (S): 681**, Temperature (T): 33.3**, S x T: 39.1**
Stem:	Salt (S): 552**, Temperature (T): 41.9**, S x T: 34.2**
Root:	Salt (S): 3770**, Temperature (T): 82.4**, S x T: 90.2**


(A)
(B)
(C)
(D)
Na+ (mmol plant-1 g-1 root dw)
Na+ (mmol g-1 dw)
Air temperature (°C)
0
10
20
30
40
50
60
1
2
3
4
5
6
8
18
28
38
48

about 10 to 45 m_{χ}^{mol} plant^{-1} g^{-1} root dw as the temperature increased from 13 to 43°C. There was a significant interaction between the effects of salinity and high temperature on Cl^- and Na^+ accumulation.

The leaf Cl^- (Fig. 4.17B) and Na^+ (Fig. 4.18B) concentrations in control plants remained very low and constant over the range of temperatures, but increased significantly with increasing temperature when the plants were grown with 150 mM NaCl. Both Cl^- and Na^+ concentrations were of a similar magnitude and ranged from 0.58 ~ 0.90 at 13°C to 3.62 ~ 3.54 m_{χ}^{mol} g^{-1} dw at 43°C. Salt treatment contributed significantly to the source of variance for both Cl^- and Na^+ concentrations with a significant interaction between the effects of salinity and temperature.

Chloride (Fig. 4.17C) and sodium (Fig. 4.18C) accumulation by the stems in control and salt-treated plants responded in similar ways to that by the leaves. However, the stems of salt-stressed plants had higher Cl^- and Na^+ concentrations than the leaves at all temperatures, being 1.55 ~ 1.76 m_{χ}^{mol} g^{-1} dw below 23°C and about 3.33 and 4.48 m_{χ}^{mol} g^{-1} dw at 38 and 43°C respectively. There was a significant interaction between the effects of salinity and temperature on the concentrations of stem Cl^- and Na^+.

The Cl^- concentration in the roots of all control plants remained low and unchanged (Fig. 4.17D). The Cl^- concentration of salinity-stressed roots was 0.88 m_{χ}^{mol} g^{-1} dw at 13°C, and declined slightly to 0.65 m_{χ}^{mol} g^{-1} dw at 23°C. Root Cl^- concentration increased with increasing temperature above 23°C, but not as markedly as leaf or stem Cl^- concentration. An interaction between the effects of temperature and salt stress on root Cl^- accumulation was observed. In contrast, Na^+ concentration in the root (Fig. 4.18D) declined as the temperature increased. There was a higher Na^+ concentration at 23°C than at any other temperature, and when the temperature was further elevated, the root Na^+ concentration declined significantly to near zero for the control and to 1.12 m_{χ}^{mol} g^{-1} dw for the salt treatments at 43°C. The latter, in fact, was similar to the root Cl^- level (1.15 m_{χ}^{mol} g^{-1} dw). The values indicated a substantial interaction of temperature and salinity effects on root Na^+ concentration.

3.3.1.2 *Balance and distribution within the plant*

The effects of salinity and temperature on the balance between total Cl^- and Na^+ concentrations in the plant are depicted in Fig. 4.19. In the absence of salinity, the contents of both Cl^- and Na^+ (mmole) remained low and were relatively unaffected by temperature. The concentrations of the two ions were close to equilibrium. When the plant was salt-stressed, the amounts of Cl^- and Na^+ absorbed increased to high levels (>2.5 mmole). Na^+ concentrations were higher between 23 and 38°C and lower at lower or higher temperature. From 13 to 38°C the plant absorbed more Na^+ than Cl^-. For instance, the roots absorbed 6.39 mmole Na^+ at 23°C, significantly higher than the amount of Cl^- (4.87 mmole) absorbed. Both Cl^- and Na^+ absorption decreased with higher temperature, becoming more equal in uptake with about 3.3 mmole of both taken up at 43°C. This lowering of the total Cl^- and Na^+ absorbed at the high temperature can be mainly attributed to reduced growth of the plants.

Uptake rates per unit root surface area of Cl^- (Fig. 4.20A) and Na^+ (Fig. 4.20B) to the whole plant (J_{tot}) and to the shoot (J_s) over the interval between days 21 and 35 increased with increasing temperature. J_{tot} and J_s for Cl^- ranged from 23.4 and 21.7 at 13°C to about 65 nmole cm^{-2} root surface h^{-1} at 43°C, and for Na^+ from 33.2 and 27.4 to about 70 nmole cm^{-2} root surface h^{-1}, respectively. Generally Na^+ uptake rate was higher than that of Cl^- except at 33°C. Above 38°C, the uptake rates (J_{tot}) of Cl^- and Na^+ were identical to the transport rates (J_s), indicating that all the Cl^- and Na^+ taken up was transported to the shoot.

Different rates of ion transport yielded different distributions within the plant (Fig. 4.21). Above 33°C, Cl^- and Na^+ in the roots declined significantly. From 38 to 43°C, the contents of root Cl^- and Na^+ were very low, and more than 95% of the total plant Cl^- and Na^+ were located in the shoot.

Fig. 4.19 **Interaction of the effects of temperature and salinity on plant Cl^- (triangle symbols) and Na^+ (circle symbols) content (mmole per plant) after 14 days of stress. The root temperatures of 20, 24, 30, 33 and 37°C correspond to air temperatures of 13, 23, 33, 38 and 43°C respectively. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

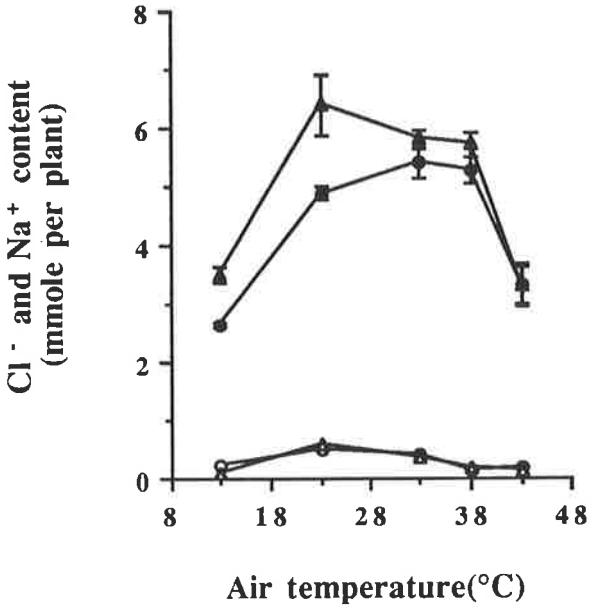

Cl - and Na+ content
(mmole per plant)
8
6
4
2
0
8
18
28
38
48
Air temperature(°C)

Fig. 4.20 **Net uptake rates of Cl^- (A) and Na^+ (B) (nmol cm^{-2} root surface area h^{-1}), into the shoot (J_s) (open symbols) and to the whole plant (J_{tot}) (solid symbols). Saline-stressed plants.**

Linear regression equations:

Cl^-	J_s:	$y = -26.96 + 2.491x$	$R^2 = 0.96$	Linear: 52.4**
	J_{tot}:	$y = -21.11 + 2.319x$	$R^2 = 0.95$	Linear: 52.3**
Na^+	J_s:	$y = -18.12 + 2.245x$	$R^2 = 0.66$	Linear: 90.8**
	J_{tot}:	$y = 3.434 + 1.616x$	$R^2 = 0.49$	Linear: 52.5**

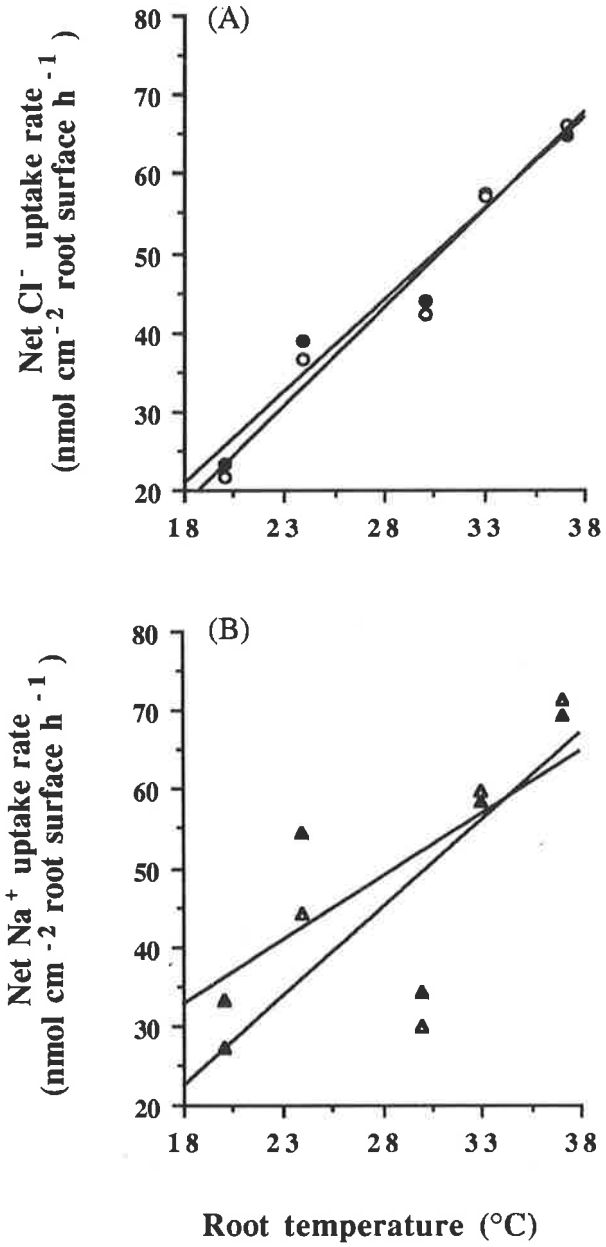
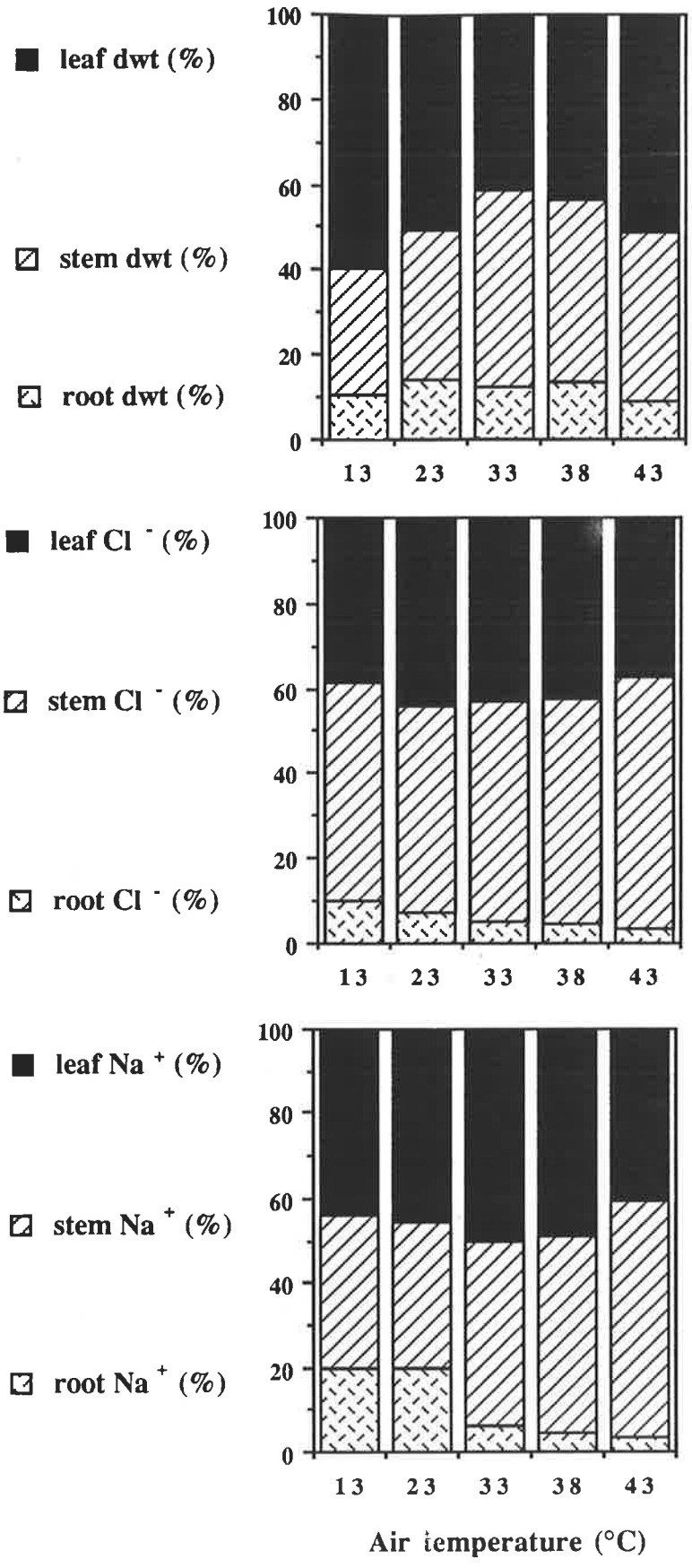


Fig. 4.21 **Percentage distribution of dry weight and Cl^- and Na^+ content between roots, stems and leaves of saline-treated plants. Root temperatures of 20, 24, 30, 33 and 37°C correspond to air temperatures of 13, 23, 33, 38 and 43°C respectively. Data are means on day 35.**


leaf dwt (%)
stem dwt (%)
root dwt (%)
leaf Cl - (%)
stem Cl - (%)
root Cl - (%)
leaf Na + (%)
stem Na + (%)
root Na + (%)
100
80
60
40
20
0
13
23
33
38
43
Air temperature (°C)

3.3.2 The concentration and selective uptake of potassium

K^+ uptake into the plant calculated on a root dry weight basis (Fig. 4.22A) was reduced by the presence of salt. Both control and salt-treated plants took up maximal K^+ around 33°C.

In the leaf, K^+ (Fig. 4.22B) was at higher concentrations in the control than in the salt treatment at all temperatures except 13°C, where they were the same. In the absence of salinity, the highest K^+ concentration of the leaves (0.91 $m_{\chi}^{mol}g^{-1}$ dw) occurred at 23°C. However, when salt-stressed, leaf K^+ concentrations decreased with temperature from 0.6 at 13°C to about 0.3 $m_{\chi}^{mol}g^{-1}$ dw at 33 ~ 43°C.

Stem K^+ concentrations fell at 43°C in the absence of salinity (Fig. 4.22C). When exposed to NaCl, K^+ levels were highest at 33°C, which was close to the level in the control plant, falling off steeply above and below that temperature. The stem contained twice the K^+ concentration of the leaf.

In root tissue, there was a decline in K^+ levels with increasing temperature in the absence of NaCl, but K^+ concentrations at all temperatures were higher than in corresponding salt-treated plants (Fig. 4.22D). NaCl in the rooting medium significantly reduced the K^+ concentration. Such reduction continued with increasing temperature, approaching zero at 43°C.

Competitive selectivity of Na^+ and K^+ was not apparent in this investigation. K^+ concentrations in every part of the tomato plant were reduced by salinity at almost all temperatures. Such reduction, calculated on the basis of the amount absorbed by roots per plant, could not be explained by increased Na^+ absorption (Fig. 4.23). K^+ absorbed by roots was higher at lower temperatures and decreased progressively above 33°C as Na^+ increased.

Fig. 4.22 **Interaction of the effects of temperature and salinity stress on the concentration of K^+ in the whole plant (A), leaves (B), stems (C) and roots (D). Concentration was determined after 14 days of stress. The root temperatures of 20, 24, 30, 33 and 37°C correspond to air temperatures of 13, 23, 33, 38 and 43°C respectively. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

(A). Absorption through roots ($\frac{mmol}{l}$ plant^{-1} g^{-1} root dw).
(B). Concentration in leaves ($\frac{mmol}{l}$ g^{-1} dw).
(C). Concentration in stems ($\frac{mmol}{l}$ g^{-1} dw).
(D). Concentration in roots ($\frac{mmol}{l}$ g^{-1} dw).

Analyses of variance (F, ≤ 0.01):**

Whole plant: Salt (S): 147**, Temperature (T): 8.42**, S x T: 7.91**

Leaf: Salt (S): 346**, Temperature (T): 22.1**, S x T: 33.5**

Stem: Salt (S): 77.6**, Temperature (T): 48.2**, S x T: 8.83**

Root: Salt (S): 745**, Temperature (T): 37.4**, S x T: 13.9**

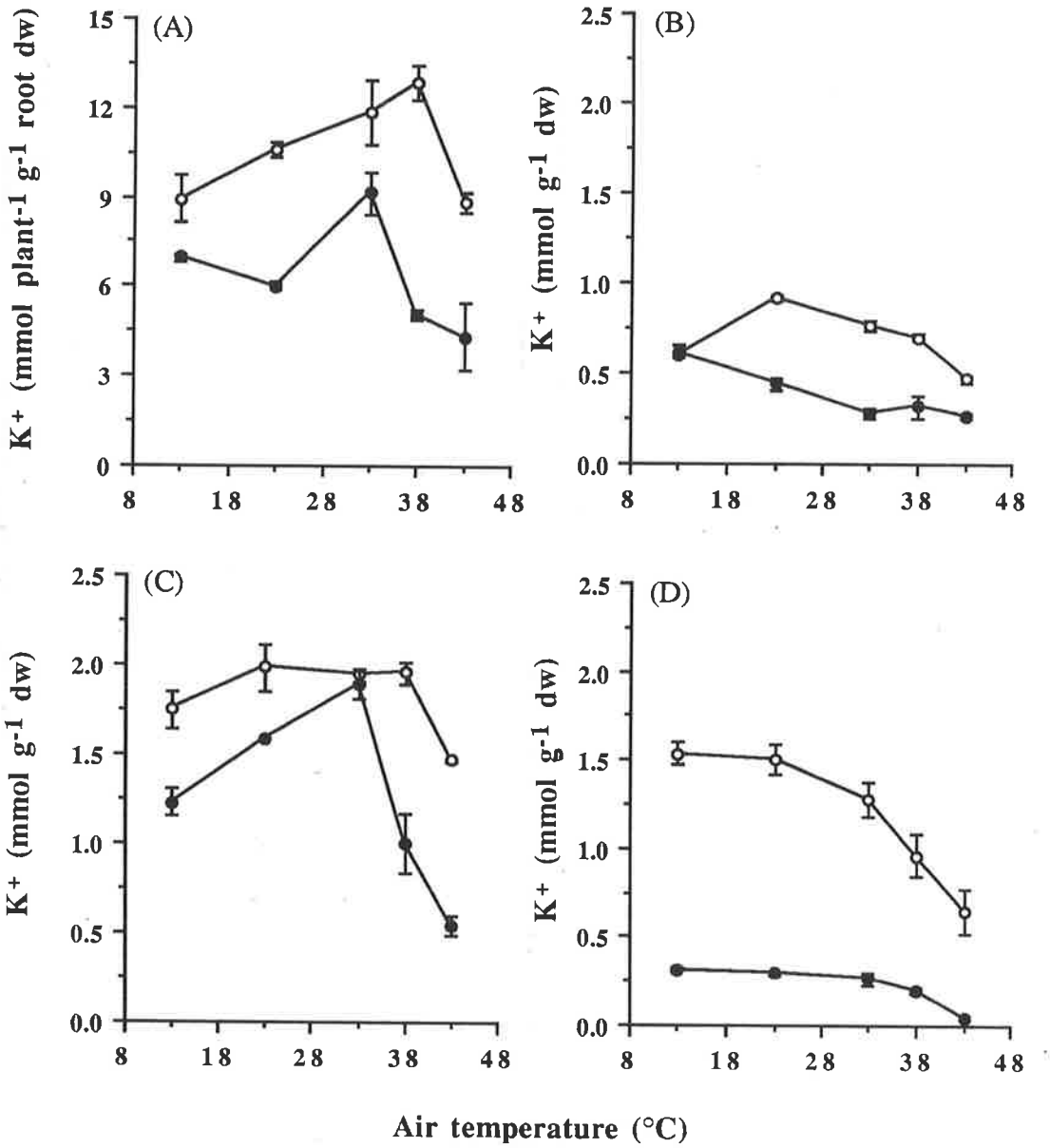
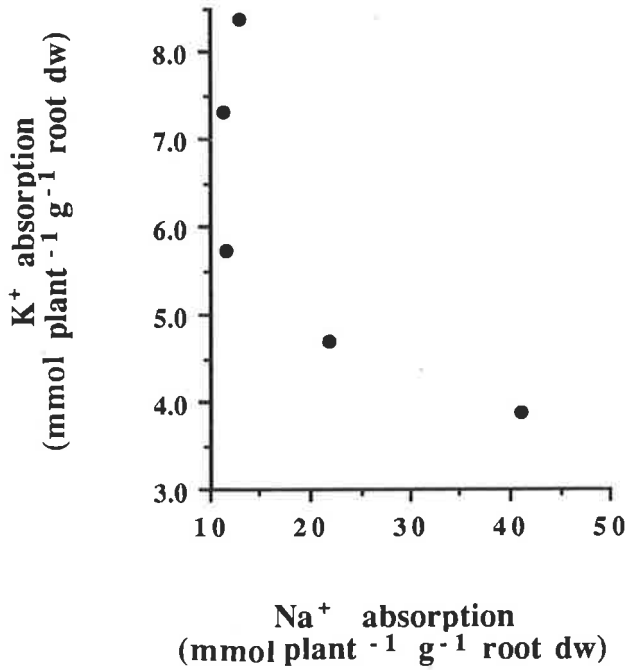

(A)
(B)
(C)
(D)
K+ (mmol plant-1 g-1 root dw)
K+ (mmol g-1 dw)
Air temperature (°C)
0
3
6
9
12
15
0.0
0.5
1.0
1.5
2.0
2.5
8
18
28
38
48

Fig. 4.23 The relationship between potassium and sodium absorption by the plant roots (mmol plant^{-1} g^{-1} root dw). Relationship determined after 14 days of stress.


K+ absorption
(mmol plant -1 g-1 root dw)
8.0
7.0
6.0
5.0
4.0
3.0
10
20
30
40
50
Na+ absorption
(mmol plant -1 g-1 root dw)

3.3.3 Other anions and cations in the leaf

3.3.3.1 *Anions*

Sulphur (S) concentration in the leaves was low at 13°C and reached a maximum around 23°C, being 0.42 in control plants and 0.23 m_{χ}^{mol} g^{-1} dw in salt-treated plants (Fig. 4.24A). Leaf sulphur concentration in control plants declined at higher temperatures to 0.21 m_{χ}^{mol} g^{-1} dw at 43°C, while in stressed plants it remained lower than in the control at all temperatures, being 0.17 m_{χ}^{mol} g^{-1} dw at 43°C. Salt stress reduced sulphur accumulation, there being a significant interaction with temperature.

Leaf phosphorus (P) concentration increased with temperature and varied from 0.09 to 0.18 m_{χ}^{mol} g^{-1} dw in control plants and from 0.12 to 0.28 m_{χ}^{mol} g^{-1} dw in the salinity treatment (Fig. 4.24B). Of particular interest, salinity significantly promoted phosphorus accumulation at all temperatures.

Boron (B) concentration in the leaves of control plants did not significantly differ from that of the salt-treated plants at 13°C, and increased with increase in temperature, to 15.2 μmol g^{-1} dw at 43°C (Fig. 4.24C). In the presence of salinity, boron also increased slightly at higher temperatures, but there was no significant difference in concentration between 33 and 43°C. The interaction between the effects of salinity and temperature on boron accumulation was significant.

3.3.3.2 *Cations*

Leaf magnesium (Mg^{2+}) concentration (Fig. 4.25A) was highest at 33°C for both control and salt-treated plants and was significantly lowered by salinity or both higher and lower temperatures. At 13°C, there was no difference in Mg^{2+} level between control and salt-treated plants.

Leaf calcium (Ca^{2+}) concentration in both control and salt-treated plants (Fig. 4.25B) was also highest at 33°C and was significantly decreased by both higher and lower temperatures. Ca^{2+} levels in control and salt-treated plants were significantly different at the

Fig. 4.24 **Interaction of the effects of temperature and salt stress on leaf concentration: S^{2-} (A), P^{-} (B) (m^{mol}_{Λ} g^{-1} dw) and B^{-} (C) (μmol g^{-1} dw) on day 35. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

Analyses of variance (F, ≤ 0.01):**

(A) S : Salt (S): 302**, Temperature (T): 258**, S x T: 32.6**

(B) P : Salt (S): 213**, Temperature (T): 149**, S x T: 13.4**

(C) B : Salt (S): 725**, Temperature (T): 178**, S x T: 66.3**

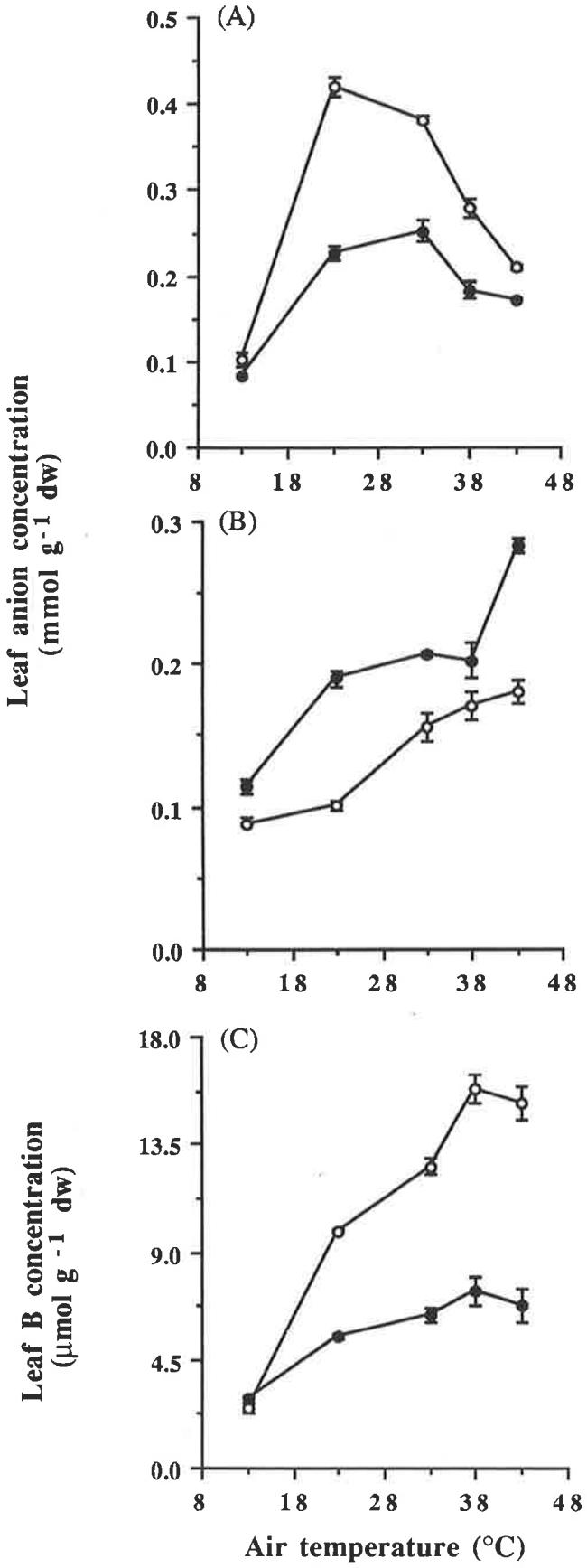
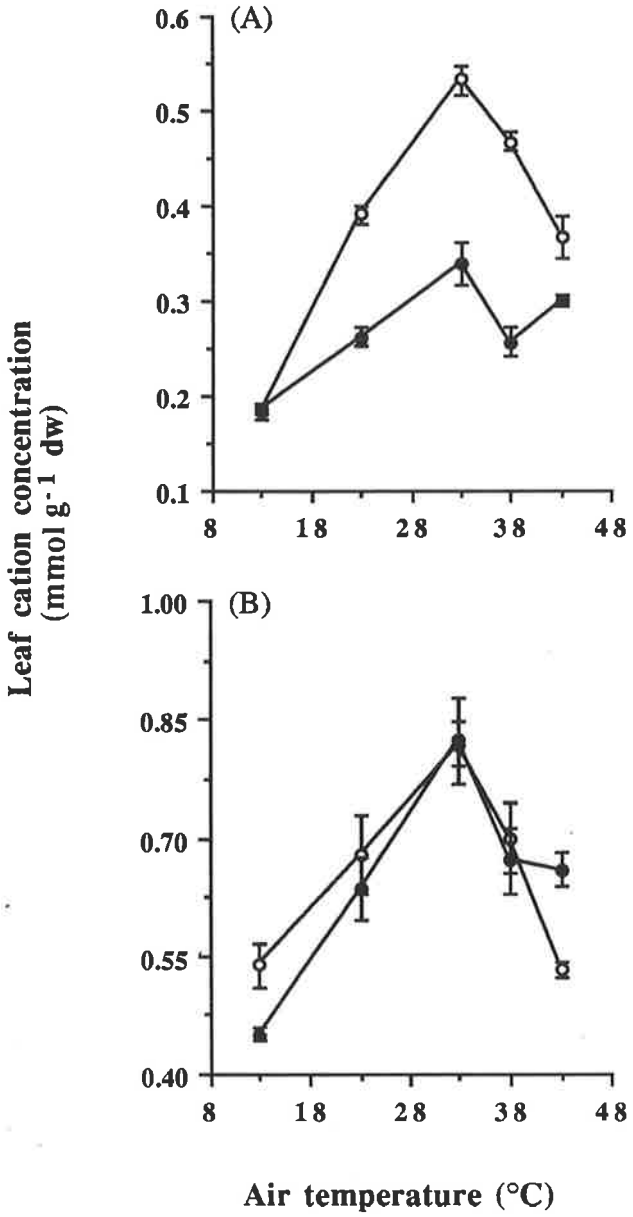

(A)
0.5
0.4
0.3
0.2
0.1
0.0
8
18
28
38
48
(B)
0.3
0.2
0.1
0.0
8
18
28
38
48
Leaf anion concentration
(mmol g-1 dw)
(C)
18.0
13.5
9.0
4.5
0.0
8
18
28
38
48
Leaf B concentration
(µmol g-1 dw)
Air temperature (°C)

Fig. 4.25 **Interaction of the effects of temperature and salt stress on leaf concentrations ($m_{\lambda}^{mol}\ g^{-1}$ dw) of magnesium (A) and calcium (B) on day 35. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

Analyses of variance (F, ≤ 0.05*, 0.01 or not significant: ns):**

(A) Mg^{2+}: Salt (S): 126**, Temperature (T): 183**, S x T: 14.3**

(B) Ca^{2+}: Salt (S): <1 ns, Temperature (T): 4.09*, S x T: 1.64 ns


(A)
0.6
0.5
0.4
0.3
0.2
0.1
8
18
28
38
48
(B)
1.00
0.85
0.70
0.55
0.40
8
18
28
38
48
Leaf cation concentration
(mmol g-1 dw)
Air temperature (°C)

temperature extremes of 13 and 43°C, but there was no significant interaction between temperature and salinity effects on leaf Ca^{2+} concentration.

Concerning the micro-elements, the manganese (Mn^{2+}) concentration in the leaf (Fig. 4.26A) responded to the stresses of salt and temperature in a similar way to B^- and Mg^{2+} concentrations. Although there was a significant interaction between the effects of stresses of salinity and temperature, Mn^{2+} concentration in the leaves of control plants at 23°C was the same as that in the salt-stressed plants at 33 ~ 43°C, suggesting that Mn^{2+} was not deficient in salt-affected leaves. However, Mn^{2+} concentration in salt-affected plants varied very little with temperature, whereas in control plants Mn^{2+} concentration increased with temperature to more than 2 $\mu mol\ g^{-1}$ dw at 38°C.

Copper ($Cu^{2(3)+}$) accumulation in the leaves (Fig. 4.26B) was a function of temperature and salinity with increased temperature promoting copper accumulation as did salinity. The interaction between the effects of salt and temperature on copper uptake was significant.

Nickel ($Ni^{2(3)+}$) (Fig. 4.26C) and zinc (Zn^{2+}) (Fig. 4.26D) concentrations of the leaves in both control and salt-treated plants increased with temperature, there being a significant interaction between salinity and temperature. Salinity significantly stimulated the uptake of both ions at the higher temperatures.

Molybdenum (Mo^{2+}) in the leaves of both control and salt-treated plants (Fig. 4.26E) increased rapidly from 13 to 23°C, then declined with increasing temperature. Salinity slightly increased Mo^{2+} accumulation at all temperatures.

Iron ($Fe^{2(3)+}$) concentration in the leaves of the control plants was significantly less than that of salt-stressed leaves at all temperatures except 13°C (Fig. 4.26F). Both control and salt-treated plants accumulated more iron as the temperature increased to 38°C, but less at 43°C. There was no significant interaction between the effects of salt and temperature.

Fig. 4.26 Interaction of the effects of temperature and salt stress on leaf concentrations (μmol g^{-1} dw) of Mn^{2+} (A), $Cu^{2(3)+}$ (B), $Ni^{2(3)+}$ (C), Zn^{2+} (D), Mo^{2+} (E) and $Fe^{2(3)+}$ (F) on day 35. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.

Analyses of variance (F, ≤ 0.01 or not significant: ns):**

(A) Mn^{2+}: Salt (S): 200**, Temperature (T): 40.0**, S x T: 29.1**

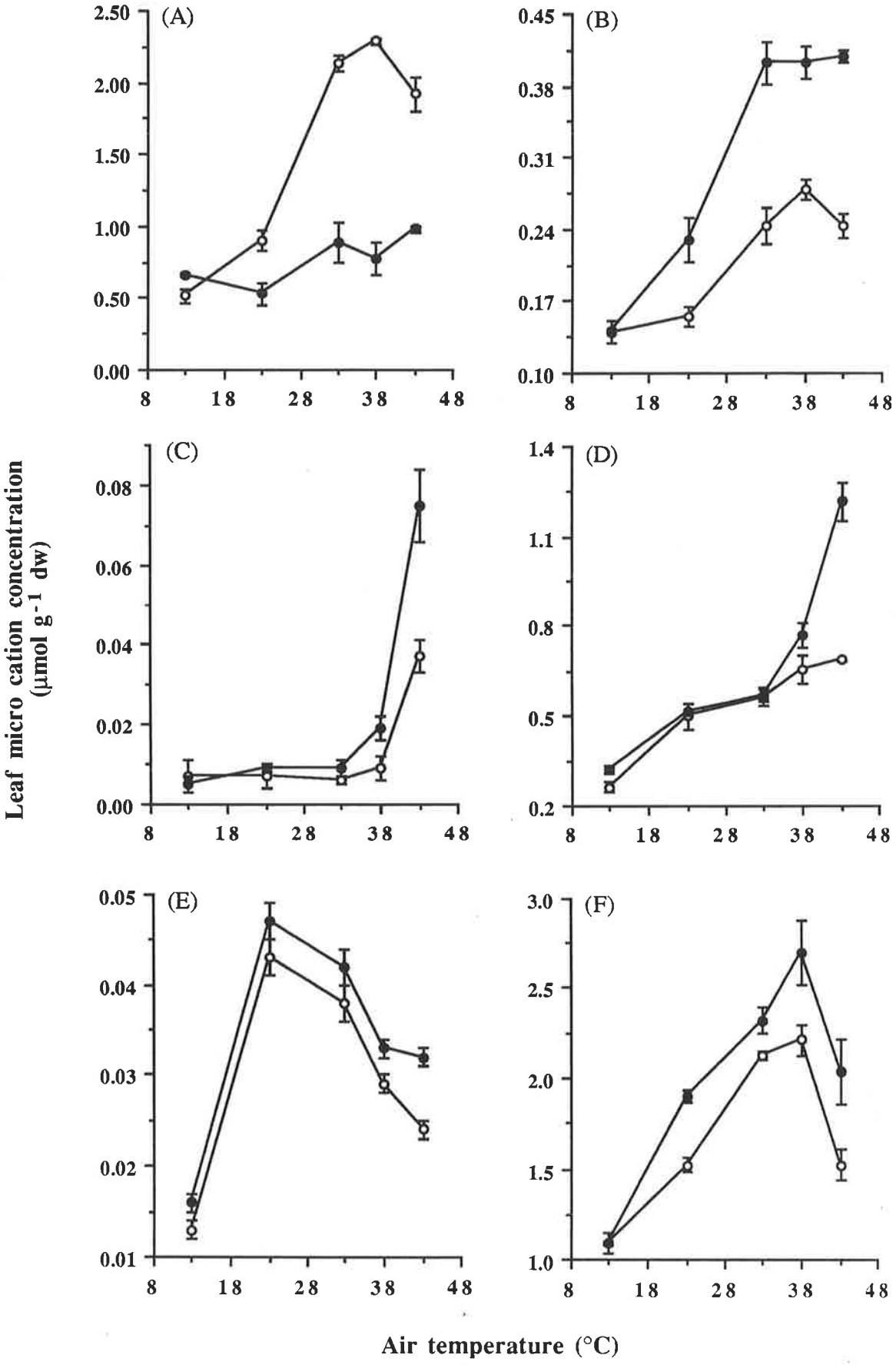
(B) $Cu^{2(3)+}$: Salt (S): 133**, Temperature (T): 121**, S x T: 11.1**

(C) $Ni^{2(3)+}$: Salt (S): 14.1**, Temperature (T): 94.4**, S x T: 6.82**

(D) Zn^{2+}: Salt (S): 55.7**, Temperature (T): 75.3**, S x T: 25.8**

(E) Mo^{2+}: Salt (S): 24.5**, Temperature (T): 187**, S x T: <1 ns

(F) $Fe^{2(3)+}$: Salt (S): 25.1**, Temperature (T): 56.9**, S x T: 2.40 ns


(A)
(B)
(C)
(D)
(E)
(F)
Leaf micro cation concentration (µmol g-1 dw)
Air temperature (°C)

3.3.3.3 *Variation in total anions and cations*

Calculated on an equivalence basis on plant dry weight (mN g^{-1} dw), total anions without the addition of Cl^- (Fig. 4.27A), and cations without Na^+ (Fig. 4.27B) responded in a similar manner to salt and temperature. The concentrations of anions and cations were low and there were no differences between salt-treated and control plants at 13°C. Differences between control and salinity-treated plants increased to 33°C and then decreased to 43°C. Salinity significantly decreased anion and cation accumulation at temperatures between 23 and 38°C. At 43°C, the anion and cation concentrations of both control and salt-treated plants declined to essentially the same level, and there was a significant interaction between effects of the two stress factors. The ratio of anions to cations increased sharply from 0.15 to 0.35 above 13°C but was less variable above 23°C. Whilst it was influenced significantly by temperature, there was no effect of salinity on this ratio (Fig. 4.27C).

The concentration of mono-valent cations, including Na^+, in the leaf on the basis of water content was enhanced by salinity and ranged from 280 mM at 33°C to 570 at 43°C, while control leaves had low concentration, varying between 120 mM at 33°C and 150 at 23 and 43°C (Fig. 4.28).

3.4 Discussion

The present study shows that ionic imbalance, particularly excess of Cl^- and Na^+ in the tissues, is a feature of plant response to the effects of salinity and temperature stress. As compared to the control treatment, the tomato plants grown in a salt medium suffered pronounced changes in the concentrations of Cl^- and Na^+ with increased temperature (Figs 4.17 and 4.18). Increased net uptake of Cl^- and Na^+ by the plant and ion transport to the shoot (Fig. 4.20) eventually led to an accumulation of these ions in the tomato tissues to the toxic levels defined by Marschner (1986). The observed growth reduction and eventual death of plants at the high temperature (Section 1.3.1 in this chapter) may have been due principally to specific ion effects on plant metabolism. This response resembles the death of other species caused by salt absorption (see Salim and Pitman, 1983; Greenway and

Fig. 4.27 **Interaction of the effects of temperature and salt stress on the balance of leaf anions and cations (omitting Na^+ and Cl^-). Anions (A) and cations (B) (mN g^{-1} dw) and the ratio (C) on day 35. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**

Analyses of variance (F, ≤ 0.05*, 0.01 or not significant: ns):**

(A) Anion Salt (S): 112**, Temperature (T): 253**, S x T: 23.7**

(B) Cation Salt (S): 103**, Temperature (T): 12.9**, S x T: 9.33**

(C) Ratio: Salt (S): <1 ns, Temperature (T): 52.5**, S x T: 3.67*

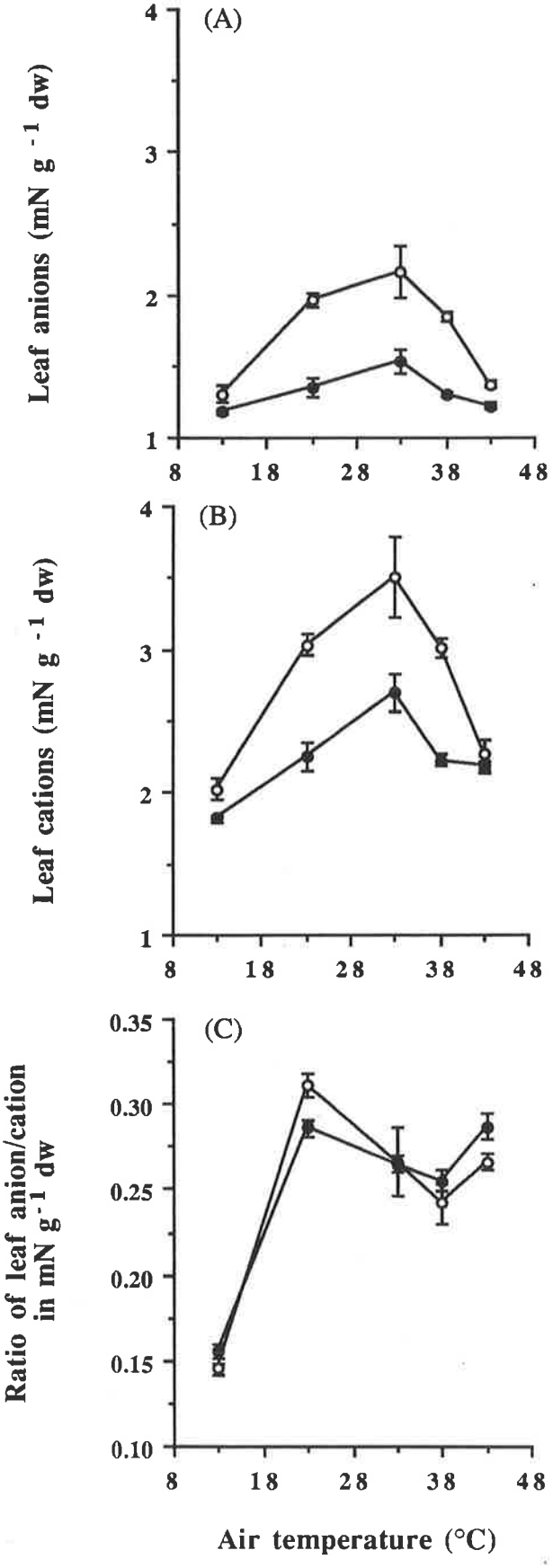
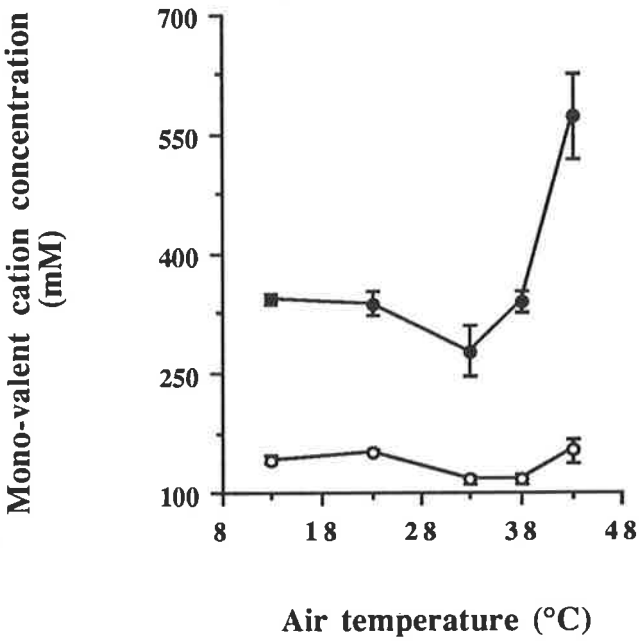

(A)
Leaf anions (mN g -1 dw)
4
3
2
1
8
18
28
38
48
(B)
Leaf cations (mN g -1 dw)
4
3
2
1
8
18
28
38
48
(C)
Ratio of leaf anion/cation in mN g-1 dw
0.35
0.30
0.25
0.20
0.15
0.10
8
18
28
38
48
Air temperature (°C)

Fig. 4.28 **Interaction of the effects of temperature and salt stress on the concentration of mono-valent cations in the leaf (mM) on day 35. Vertical bars representing ±SE of the means are shown when larger than symbols. 0 (○) or 150 (●) mM NaCl stress.**


Mono-valent cation concentration (mM)
700
550
400
250
100
8
18
28
38
48
Air temperature (°C)

Munns, 1980). For instance, Ehlig (1960) observed that, during hot weather, high Cl^- accumulation from Cl^- treatments caused burn on leaf blades of all grapevine varieties tested. Injury developed very rapidly at air temperatures above 38°C, but very slowly at air temperatures below 32°C.

The amounts of Na^+ and Cl^- absorbed through the roots and accumulation in the leaf over the experimental period were linearly and negatively correlated with both RGR and NAR (Fig. 4.29). There was also a significant linear relationship between the concentrations of tissue ions and RGR of the component parts (Fig. 4.30). RGRs of the shoot in bean (Salim and Pitman, 1983) and *Casuarina spp.* (Clemens *et al.* 1983), exposed to salinity, have been found to be related to ion accumulation in the shoot rather than to the osmotic pressure of the external solution. This is compatible with the results of Lloyd *et al.* (1987a), who observed that NaCl salinization reduced CO_2 assimilation rates of "Valencia" orange leaves even though the leaves maintained turgor; lower assimilation rates were therefore attributed to ion toxicity because of high foliar sodium levels. Furthermore, in seedlings of *Citrus macrophylla*, fluorescence responses to salt stress have also been attributed to sodium toxicity (Downton and Millhouse, 1985). In this investigation, decrease in the osmotic potential of salt-stressed tomato leaves (Fig. 4.12) resulted in turgor being maintained as has previously been demonstrated for salt-stressed materials (Lloyd *et al.* 1987a, b; Walker *et al.* 1983). It is possible that an increase in ion concentration in the tomato leaves contributes to the reduction in osmotic potential and subsequent maintenance of turgor above the level in control leaves. Despite turgor maintenance, NAR was markedly reduced by the high foliar ion levels consequent on the combination of salt and high temperature stress. Lloyd *et al.* (1987b) suggest that this phenomenon is not solely a consequence of a lower chlorophyll level and that some photosynthetic dysfunction must also have occurred. There is some indication of similar effects on metabolism in the present investigation, for example, the decline in proline concentration in all component parts and of soluble sugars in the leaf.

Cl^- distribution (%) to the roots was lower than that to the shoot (Fig. 4.21B). In contrast to these changes in the Cl^- concentration, root Na^+ concentration decreased with

Fig. 4.29 Relationships of chloride (▲) and sodium (●) accumulation with the relative growth rate (RGR) (mg mg^{-1} d^{-1}) (A) and net assimilation rate (NAR) (g cm^{-2} d^{-1}) (B) of the tomato plant. Calculated for the 14-day stress period. R values are significant at 1% (**) and 5% (*).

<u>Linear regression equations</u>:

(A) ▲ RGR on Cl^- absorbed through roots (m_{λ}^{mol} $plant^{-1}$ g^{-1} root dw):

$y = 0.119 - 0.003x \quad R^2 = 0.91^{**}$

● RGR on Na^+ absorbed through roots (m_{λ}^{mol} $plant^{-1}$ g^{-1} root dw):

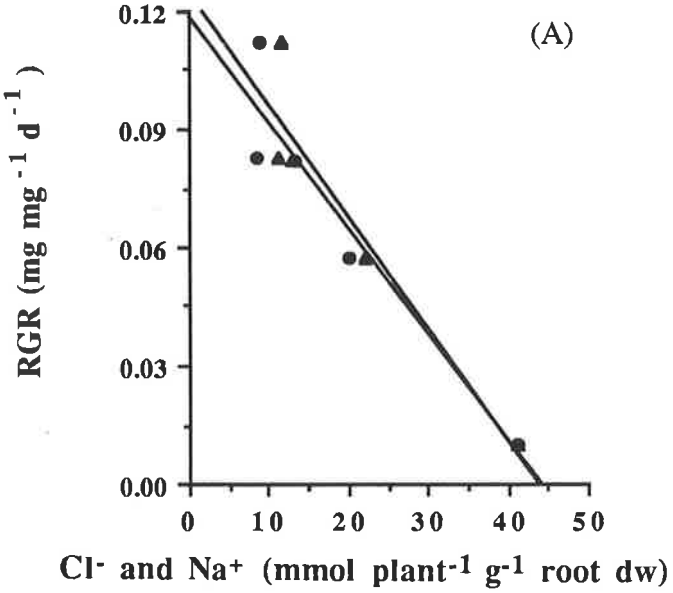
$y = 0.125 - 0.003x \quad R^2 = 0.90^{**}$

(B) ▲ NAR on Leaf Cl^- (m_{λ}^{mol} g^{-1} dw):

$y = 0.937 - 0.206x \quad R^2 = 0.76^{*}$

● NAR on Leaf Na^+ (m_{λ}^{mol} g^{-1} dw):

$y = 1.005 - 0.216x \quad R^2 = 0.68^{*}$


(A)
RGR (mg mg-1 d-1)
0.12
0.09
0.06
0.03
0.00
0
10
20
30
40
50
Cl- and Na+ (mmol plant-1 g-1 root dw)

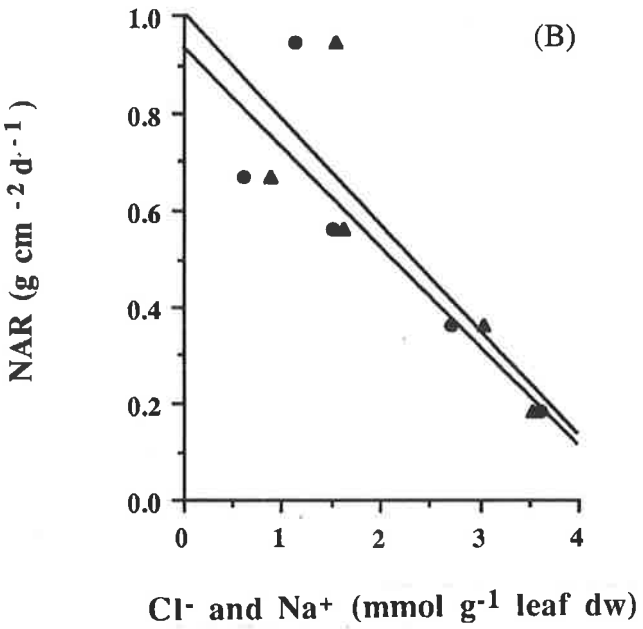
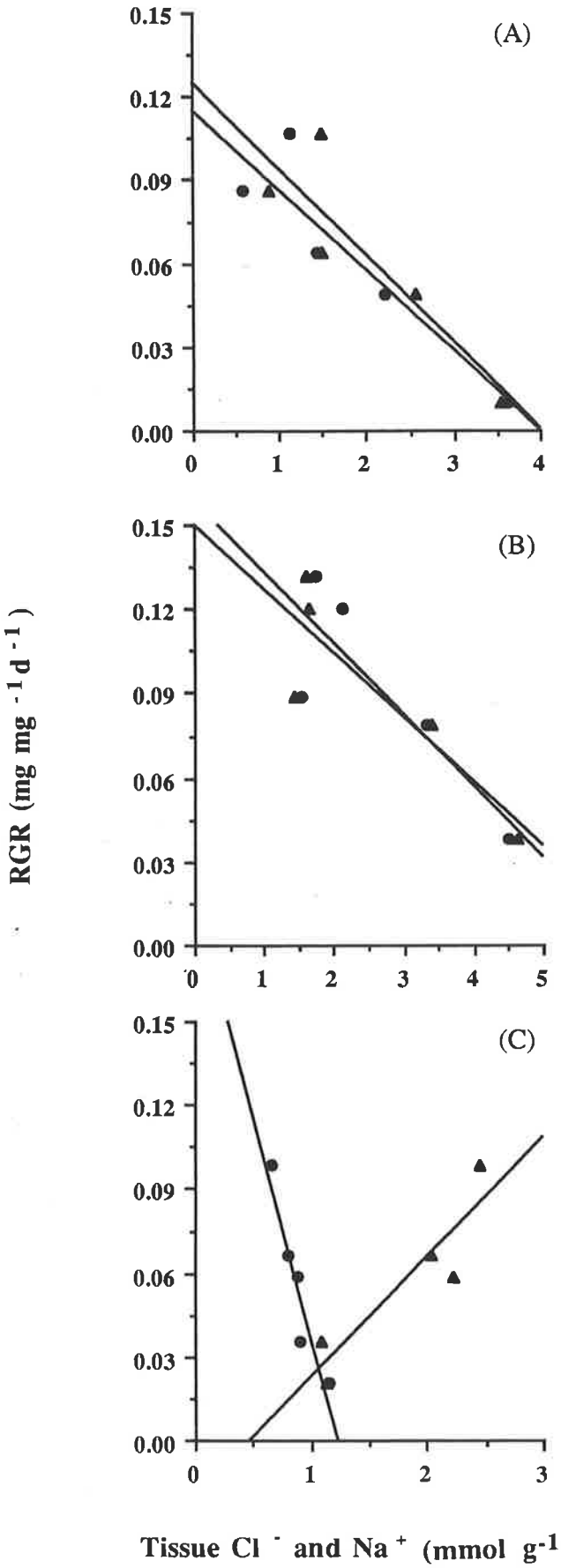

(B)
NAR (g cm-2 d-1)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Cl- and Na+ (mmol g-1 leaf dw)

Fig. 4.30 Relationships of chloride (▲) and sodium (●) accumulation (m_{λ}^{mol} g^{-1} dw) with the relative growth rate (RGR) (mg mg^{-1} d^{-1}) of leaves (A), stems (B) and roots (C). Calculated for the 14-day stress period. R values are significant at 1% (**) and 5% (*).

Linear regression equations:

(A)	▲	Leaf RGR on Cl^-:	$y = 0.115 - 0.029x$	$R^2 = 0.85^{**}$
	●	Leaf RGR on Na^+:	$y = 0.126 - 0.031x$	$R^2 = 0.78^{*}$
(B)	▲	Stem RGR on Cl^-:	$y = 0.159 - 0.025x$	$R^2 = 0.72^{*}$
	●	Stem dw on Na^+:	$y = 0.150 - 0.023x$	$R^2 = 0.76^{*}$
(C)	▲	Root RGR on Cl^-:	$y = 0.914 - 0.158x$	$R^2 = 0.89^{**}$
	●	Root RGR on Na^+:	$y = -0.021 + 0.043x$	$R^2 = 0.84^{*}$


(A)
(B)
(C)
0.15
0.12
0.09
0.06
0.03
0.00
0
1
2
3
4
5
RGR (mg mg -1 d -1)
Tissue Cl - and Na + (mmol g-1 dw)

Assuming that this is not an artefact of root washing, increasing temperature (Fig. 4.18D), this may suggest that the reduction in relative growth rate of the roots is not attributable to the accumulation of ions in the roots. The decline in Na^+ concentration in the roots with increased temperature was due to transport to the shoot as whole plant uptake increased with temperature. The similarity between Na^+ and Cl^- uptake rate to the whole plant (J_{tot}) and the transport rate to the shoot (J_S) above 38°C (Fig. 4.20) suggests that most of the Na^+ and Cl^- taken up was rapidly transported to the shoot. A similar result has been found in an investigation of salinity responses at a single temperature (Reimann, 1992).

The discussion so far has considered the growth responses in relation to the ions, Na^+ and Cl^-. However, their presence has effects on the uptake of other ions. Maintenance of cytoplasmic levels of K^+ critical to metabolism is essential for survival in a saline habitat where the predominant cation, Na^+, is present at high concentration. Competition for uptake occurs between Na^+ and K^+, leading to a reduced level of tissue K^+ at high external NaCl concentration in many plant species (Ben-Hayyim *et al.* 1987; Greenway and Munns, 1980; Reimann, 1992). This occurs even though the uptake of K^+ and Na^+ apparently occurs through independent pathways (Cramer *et al.* 1988; Lazof and Cheeseman, 1988). In the present study, K^+ uptake was depressed at all temperatures by elevated NaCl concentration (Fig. 4.22). The decrease in K^+ uptake could be due to antagonism between Na^+ and K^+ at sites of uptake in the roots, an effect of Na^+ on the K^+ transport into the xylem (Lynch and Läuchli, 1984; 1988) or indirect inhibition of the uptake process by other means, for example, H^+-ATPase activity (Gronwald *et al.* 1990; Suhayda *et al.* 1990). It has been claimed (Chow *et al.* 1990) that the reduction in plant and shoot growth by salinity is related to K^+ uptake into the leaves, with leaves having a lower K^+ content under higher than lower salinity. Plant and shoot biomass increased with a logarithmic increase in K^+ concentrations supplied to the roots (Chow *et al.* 1990). Greater K^+ accumulation protects against injury from Na^+ accumulation, as K^+ functions to maintain the correct ionic environment for metabolism (Leigh and Wyn Jones 1984). Numerous studies, with tobacco (Watad, Reinhold and Lerner, 1983), alfalfa (Croughan *et al.* 1979)

and *Citrus aurantium* (Ben-Hayyim *et al.* 1985), have found a higher level of internal K^+ to be correlated with a higher level of salt tolerance.

In this investigation K^+ taken up was not linearly correlated with Na^+ uptake (Fig. 4.23) although tissue K^+ levels were depressed by salinity at all temperatures. The leaf and root K^+ concentrations at higher temperatures did not fall markedly (Figs 4.22B and D) while there was a progressive decline in the relative growth rates. This suggests that it is unlikely that low K^+ concentrations were solely responsible for the lowering of relative growth and net assimilation rate with salinity stress at high temperature. It has also been demonstrated (Lloyd *et al.* 1987a) that salinity can inhibit leaf gas exchange even when K^+ concentrations are maintained at control levels.

The uptake of other essential nutrient ions might be disturbed by excess uptake of Na^+ and Cl^- ions, and this may have a role in reduced growth. To elucidate this possibility, the other major and micro elements in the leaves were measured. In the presence of external salts, dramatic changes in the mineral components of plants occur with interactions between ions (Cramer *et al.* 1991; He and Cramer, 1992). The present results showed that the balance of essential elements in the leaves was influenced by both salinity and high temperature.

Considering anion concentrations, there were no significant changes in the concentrations of S^{2-}, P^- and B^- in all six *Brassica* species at a range of salt treatments (He and Cramer, 1992), but salinity stress caused a progressive increase of S^{2-} and P^- in barley shoots with time (Cramer *et al.* 1991). In contrast, S^{2-} in tomato leaves (Fig. 4.24) appeared to fall with salinity at all temperatures. The response to salinity and high temperature was similar to the pattern of growth reduction in the leaf (Fig. 4.5). In agreement with the report of Cramer *et al.* (1991) with barley, it is interesting that leaf P^- concentration was increased by salinity at a range of temperatures (Fig. 4.24B). This is in contrast to the report of Bourgeais-Chaillou *et al.* (1992), where P^- concentration was lowered significantly in salt-treated leaves of soybean. Further, unlike the salt treatment data of Cramer *et al.* (1991), B^- concentration in the saline leaves was significantly lower

than that in the control leaves except at 13°C. This may also, to some degree, contribute to the reduced leaf growth.

The range of leaf Mg^{2+} for normal growth of tomato plant has been reported to be $0.15 \sim 0.33$ m_{χ}^{mol} g^{-1} dw (Marschner, 1986). This suggests that there was no Mg^{2+} deficit in any treatment in the present investigation (Fig. 4.25A), supporting the conclusion of Cramer *et al.* (1991) and He and Cramer (1992) that Mg^{2+} is not the ion which is involved in the reduction in shoot growth.

There was no significant difference between control and salinity in Ca^{2+} concentration in the leaf (Fig. 4.25B). Ca^{2+} concentration increases in salt-stressed soybean leaves (Bourgeais-Chaillou *et al.* 1992) and does not decrease significantly in the shoot of young seedlings of salt-stressed barley although uptake declines rapidly with a reduction in growth rate (Cramer *et al.* 1989). Supplemental Ca^{2+} restores growth to only a limited extent (Cramer *et al.* 1990) and is poorly correlated overall to RGR (Cramer *et al.* 1991). These results suggest that Ca^{2+} is not crucial to the growth inhibition of plants exposed to salinity.

Mn^{2+} concentrations in the leaves of salt-stressed tomato plants at temperatures above 33°C were close to the levels in control leaves at 23°C (Fig. 4.6A) and in the intermediate range of Mn^{2+} concentration for tomato growth (Marschner, 1986). Salinity also had no significant effect on tissue concentrations of Mn^{2+} in *Brassica* species (He and Cramer, 1992), but Mn^{2+} uptake and concentration in barley is significantly affected by salinity and highly correlated with growth (Cramer *et al.* 1991; Cramer and Nowak, 1992). These data indicate that changes in Mn^{2+} with salinity are not uniform across species. As with *Brassica* species (He and Cramer, 1992) and barley (Cramer *et al.* 1991), salinity caused an increase in copper, nickel and zinc concentrations in the tomato leaf over a range of temperatures (Fig. 4.26). However, these changes were comparable with those in the control leaves and did not suffice to account for the reduction in leaf relative growth rate. There was no significant interaction between the combined effect of the two stresses on molybdenum and iron concentrations in the leaves.

The leaf anions and cations excluding Cl^- and Na^+ were lowered by salt treatment, but the ratio of anions to cations was unaffected. Thus, these changes could not account for the reduction in tomato plant growth. There was a lower ratio of Cl^- to Na^+ in salt-treated plants at lower temperatures (Fig. 4.19) indicating a deficit of anions, presumably rectified by organic acids.

Cramer *et al.* (1991) indicated that the total cation concentration doubled with salinity regardless of the type of salt treatment, and was more significantly correlated with RGR than was the concentration of any major ion (other than Na^+ or Cl^-) alone or in combination. High concentrations of cations, including K^+, may inhibit biochemical processes, such as protein synthesis in the cytoplasm (Gibson *et al.* 1984). The optimum mono-valent cation concentration for *in vitro* mRNA translation is between 100 and 120 mM; translation is unlikely at concentrations above 180 mM. In this investigation, salinity doubled the total cation concentration, Na^+ being responsible for the increase. The average mono-valent cation concentration in the leaves of salinity-stressed plants ranged from 280 mM at 33°C to 570 mM at 43°C, well above the optimal level for mRNA translation. In contrast, the cation concentration in the control plants was around the optimal range, varying between 120 and 150 mM. It is impossible to estimate the cytoplasmic concentration from this data as there is no distinction between apoplastic and symplastic ions and the extent to which ions are sequestered in the vacuoles may change with increased membrane permeability at higher temperatures (Levitt, 1980a). However, the extreme whole-tissue concentrations at higher temperatures strongly suggest supra-optimal cytoplasmic concentrations.

It seems reasonable to conclude that the normal ionic environment within tissues was severely disturbed by the effects of salinity and temperature. The most notable effects were the increased concentration of Cl^- and Na^+ in plant tissues as temperature increased, which was correlated with the reduction in relative growth rate and net assimilation rate. K^+, Mg^{2+}, S^{2-} as well as total anion and cation concentrations (without addition of Cl^- and Na^+) in the leaf were depressed by salinity at all temperatures but may not suffice to account for the reduction in growth rate. The total leaf cation concentration, including Na^+, was increased significantly with probable adverse effects on plant metabolism.

4. The Effects of Transpiration and Temperature on Chloride and Sodium Uptake under Salinity Stress

4.1 Introduction

The accumulation of Na^+ and Cl^- in tomato plant tissues could account for the interaction of the effects of salt and high temperature on plant relative growth and net assimilation rate described earlier. In order to further investigate the mechanism causing such an interaction, the manner in which high ambient and root temperatures affected the uptake of NaCl ions was examined. This was structured to investigate the alternative hypotheses accounting for the increased ion uptake at high temperature, namely, increased membrane permeability and transpiration.

High temperature may have an effect on cell membrane semi-permeability (Levitt, 1980a). It is possible that a gradual loss of root cell membrane selectivity with increasing root temperature would permit a large amount of salt to move into the root and shoot, thus damaging, even killing them due to the specific ion effects on metabolic processes in the plant cell (Greenway and Munns, 1980; Munns and Termaat, 1986).

Increased ambient temperature will also increase vapour pressure deficit (VPD). VPD is a function of temperature and approximately doubles for each 10°C increase in temperature (Salisbury and Ross, 1969). When VPD is high, there is high transpirational water flow (Salim, 1989) and root permeability to water increases with the rate of transpiration (Aston and Lawlor, 1979). A loss of root cell membrane selectivity at high root temperature may also result in an increase in water flow into the plant and mass flow of water across the root surface increases. This increase in mass water flow may affect both the uptake of ions into the plant (Markhart *et al.* 1979; Salim, 1989; Tsuchiya *et al.* 1992) and the distribution of ions within the plant. Hylmo (1953), working with pea plants, reported that more than 75% of the total uptake of Cl^- could occur directly as mass flow in transpiration water.

To distinguish between the effects of high temperature and of transpiration on ion uptake would be advantageous in testing the two hypotheses. These alternatives were explored by examining the responses of transpiration and ion uptake to air and root temperature.

4.2 Methods

In order to control root temperature accurately at the levels of root medium temperature recorded in sand culture previously, plants were cultured in an aerated hydroponic system of Hoagland's solution with or without salinity. After day 21, the level of NaCl treatment was raised to 150 mM and the pots containing the plants were immersed in a water bath for 48 h to study the effect of root temperature on salt uptake. The air/root temperatures imposed were 23/24, 23/37, 43/24 and 43/37°C.

Vapour pressure deficit (VPD) was maintained at 0.3, 1.3 or 5.2 KPa.

The transpiration rate (mg cm^{-2} leaf area h^{-1}) was determined by weighing the pots before and after the 48 h period. Net water flow and NaCl uptake and transport rates (J_s and J_{tot}) were also estimated over the 48 h period and expressed per unit root surface area.

An experiment was also carried out in which xylem salt concentration was measured. Pots with plants in Hoagland's solution alone were transferred to a water bath after day 21. Shoots were excised (de-topped) 3 ~ 4 cm above the roots, and the cut stem covered to minimise evaporation. The root medium was replaced with 150 mM NaCl in a Hoagland's solution, and the de-topped roots were incubated at 20, 24, 30, 33 and 37°C with aeration for 48 h. Four intact root systems with different surface areas (not measured) were used in each treatment. At the end of the experiment, the stalk was re-cut at 2 cm stem length above root and 0.03 ~ 0.1 ml of root xylem sap was extruded from each root system using a pressure bomb at 0.5 MPa pressure. The concentrations of Cl^- and Na^+ in the extruded xylem sap were analysed (mM).

The detailed methods including plant environmental control, measurement of ion uptake and of root surface area have been described in chapter III.

4.3 Results

4.3.1 Transpiration and accumulation of chloride and sodium in various tissues

An increase in air temperature from 23 to 43°C, and consequently of VPD from 0.3 to 5.2 KPa, tripled transpiration rate from 5 to 15 mg cm^{-2} leaf area h^{-1} (Table 4.1). However, an increase in root temperature from 24 to 37°C at either level of VPD had no significant effect on leaf transpiration rate. The concentrations of Cl^- and Na^+ in various plant tissues did not increase with an increase in air temperature or an increase in transpiration rate at the same root temperature, except that leaf Cl^- concentration rose at the highest transpiration rate. The concentrations of Cl^- in the leaf and of Na^+ in the three component tissues rose significantly with an increase in root temperature ($P<0.01$). There was no significant interaction between root temperature and VPD or transpiration rate (as covariate) on accumulation of Cl^- and Na^+ in any tissue.

4.3.2 Uptake and transport of water, chloride and sodium into the plant

An increase in air temperature and VPD resulted in a higher transpiration rate, and a resultant increase in water flow through the root surface, but high root temperature did not produce this effect (Fig. 4.31). At lower air temperatures, root temperature was without effect on water flow, but at a high air temperature (43°C) and VPD (5.2 KPa), water flow rate declined from 520 to 430 µmol cm^{-2} root surface h^{-1} with an increase in root temperature from 24 to 37°C.

Whilst VPD and air temperature did not affect the uptake (J_{tot}) or transport (J_s) of Cl^- and Na^+, high root temperature had a significant effect on J_{tot} and J_s (Fig. 4.32). For instance, J_{tot} value for Cl^- increased from 37 nmol cm^{-2} root surface h^{-1} at 24°C to 68 at 37°C. J_{tot} of Na^+ responded in a similar way to root temperature. However, there was no significant interaction of the effects of VPD or transpiration rate (as covariate) and root temperature on J_{tot} and J_s ($P < 05$).

Table 4.1 Interaction between VPD and temperature (T) on transpiration and Cl^- and Na^+ concentration.

VPD	Temperature (Air/root)	Transpiration	Leaf		Stem		Root	
			Cl^-	Na^+	Cl^-	Na^+	Cl^-	Na^+
(KPa)	(°C)	(mg cm^{-2} h^{-1}, ±SE)	(mmol g^{-1} dw, ±SE)		(mmol g^{-1} dw, ±SE)		(mmol g^{-1} dw, ±SE)	
0.3	23/24	5.07±0.09	1.11±0.05	0.70±0.01	2.82±0.17	1.73±0.06	1.94±0.13	1.86±0.10
	23/37	5.25±0.11	1.22±0.05	0.98±0.02	2.89±0.23	1.98±0.07	1.61±0.10	2.22±0.01
1.3	23/24	6.18±0.02	1.02±0.07	0.67±0.03	2.88±0.08	1.68±0.05	1.92±0.03	1.60±0.01
	23/37	5.93±0.44	1.34±0.12	1.01±0.06	3.12±0.06	1.94±0.03	1.71±0.08	2.07±0.09
5.2	43/24	14.59±0.38	1.33±0.07	0.65±0.14	2.83±0.03	1.83±0.18	1.91±0.24	1.62±0.14
	43/37	12.71±0.32	1.62±0.09	0.77±0.05	2.90±0.05	2.13±0.12	1.91±0.09	1.87±0.20
Analysis of variance (F, P ≤ 0.01**, or not significant: ns)								
VPD		309**	8.56**	2.85 ns	1.14 ns	2.36 ns	0.49 ns	3.37 ns
Root temperature (T)		4.45 ns	11.9**	22.5**	2.21 ns	16.2**	2.57 ns	14.0**
VPD x T		4.19 ns	0.95 ns	1.62 ns	0.49 ns	0.04 ns	0.67 ns	0.38 ns

Fig. 4.31 **The effects of vapour pressure deficit (VPD, KPa) and air and root temperature on transpiration (A) and water flow (B). Vertical bars representing ±SE of the means are shown when larger than symbols.**

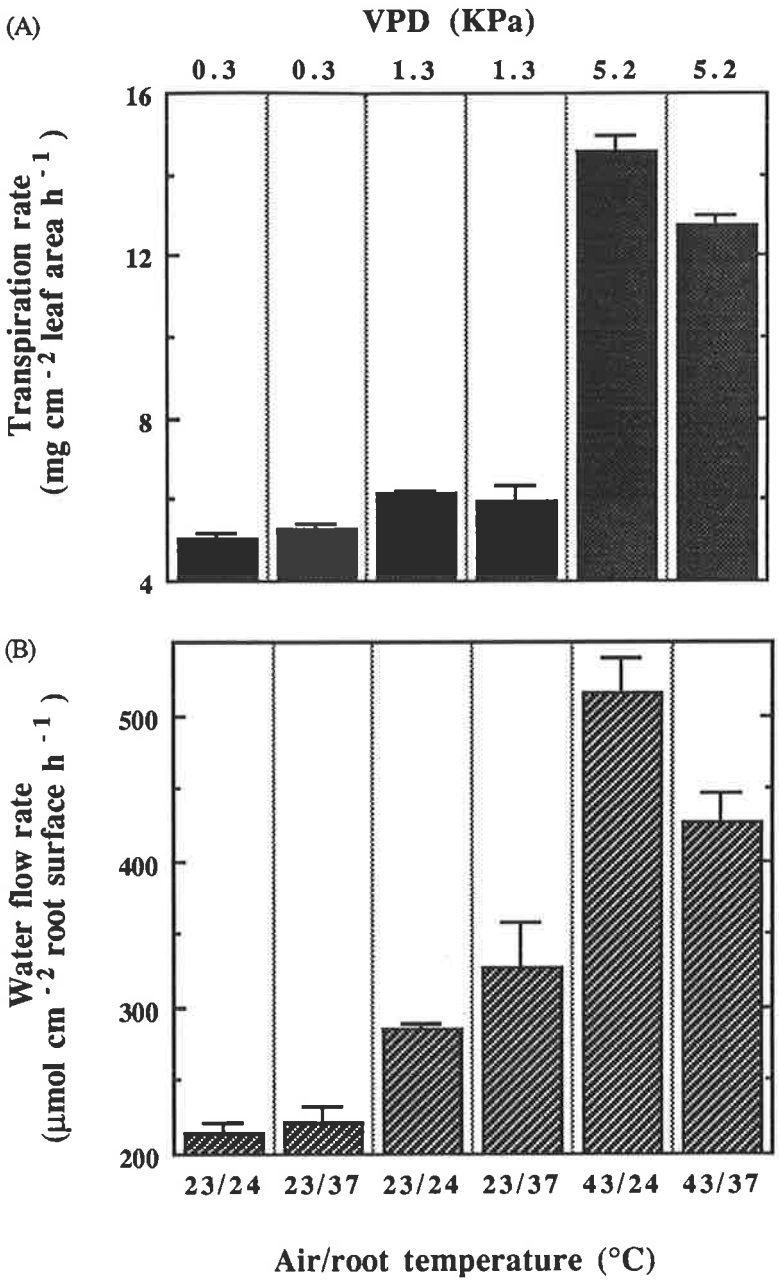
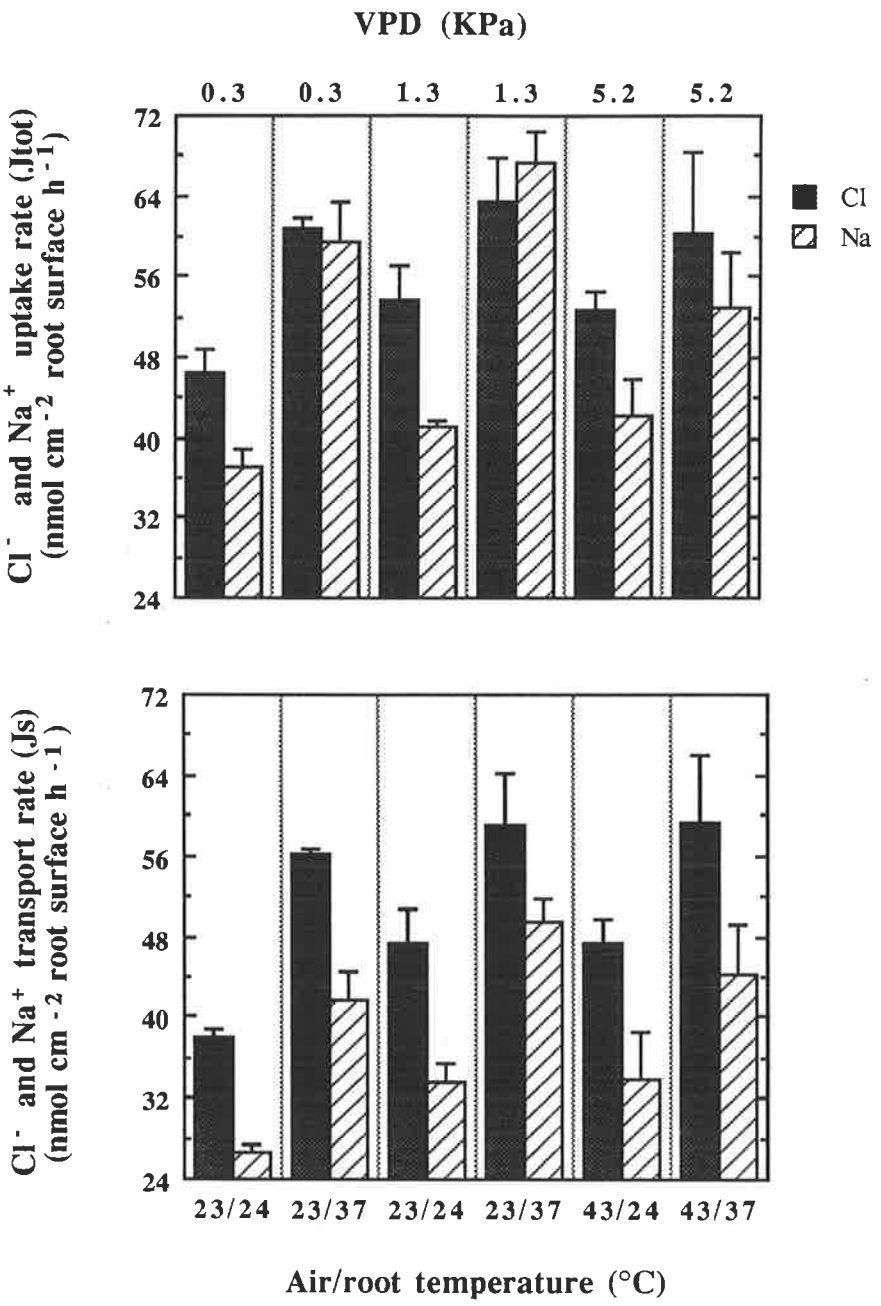

(A)
VPD (KPa)
0.3
0.3
1.3
1.3
5.2
5.2
16
12
8
4
Transpiration rate
(mg cm -2 leaf area h -1)
(B)
500
400
300
200
Water flow rate
(µmol cm -2 root surface h -1)
23/24
23/37
23/24
23/37
43/24
43/37
Air/root temperature (°C)

Fig. 4.32 **The effects of vapour pressure deficit (VPD, KPa) and air and root temperature on the net uptake rate of Cl^- and Na^+ into the whole plant (J_{tot}) and of transport to the shoot (J_s). Vertical bars represent ±SE of the means.**

Analysis of variance (F, ≤ 0.01, or not significant: ns):**

		Source of Variation		
		VPD	Root temperature	VPD x T
Cl^-:	J_{tot}	<1 ns	8.66**	<1 ns
	J_s	1.54 ns	17.35**	<1 ns
Na^+:	J_{tot}	2.22 ns	48.95**	2.71ns
	J_s	2.37 ns	23.62**	<1 ns


VPD (KPa)
0.3
0.3
1.3
1.3
5.2
5.2
72
64
56
48
40
32
24
Cl
Na
Cl- and Na+ uptake rate (Jtot)
(nmol cm-2 root surface h-1)
Cl- and Na+ transport rate (Js)
(nmol cm-2 root surface h-1)
23/24
23/37
23/24
23/37
43/24
43/37
Air/root temperature (°C)

4.3.3 Chloride and sodium in xylem sap

The concentration of Cl^- and Na^+ in the plant xylem sap was a function of root temperature (Fig. 4.33). When the intact roots were held at temperatures ranging from 20 to 37°C, the concentration of both Cl^- and Na^+ ions in the xylem sap increased from about 70 to 200 mM in a medium of 150 mM NaCl in Hoagland's solution. The xylem concentration of Cl^- and of Na^+ was linearly correlated with root temperature, R^2 being 0.95 and 0.82 ($P < 0.01$), respectively.

4.4 Discussion

Transpiration

In agreement with the conclusions of Forde *et al.* (1977) and others (Aston and Lawlor, 1979; Lauter and Munns, 1987; Salim, 1989), the transpiration rate increased with increasing VPD and air temperature (Table 4.1 and Fig. 4.31), the water flow through the root surface being driven by the transpiration rate (Fig. 4.34). However, neither increases in VPD and air temperature nor water flow rate had a significant effect on uptake and transport of Cl^- and Na^+ (Figs 4.32 and 4.35). This supports the findings for barley (Greenway, 1965) and tomato (Salim, 1989) that Cl^- and Na^+ concentration in the ascending sap decreased strongly with increasing transpiration. Greenway (1965) measured Cl^- uptake by 10-day old barley plants in 50 mM NaCl at various rates of transpiration. He found that transport to the shoot only doubled as the rate of transpiration increased about sixteen times from 40 to 700 mg g^{-1} shoot h^{-1}. Similarly, by investigating Rb^+ and Cl^- uptake in *Vicia faba* plants, Brouwer (1956) (cf. Lopushinsky, 1964) concluded that only approximately 15% moved passively with water. Recently, Nicolas *et al.* (1993) also reported that transpiration rate, reduced by high CO_2, did not significantly control the rate of sodium accumulation in the wheat plant, showing that salt uptake was largely independent of water uptake. It appears that ion movement is mostly independent of water flow through roots in this study. It is therefore concluded that there is no significant

Fig. 4.33 **The effect of root temperature on the concentration of Cl^- (▲) and Na^+ (●) in the xylem of intact root incubated in Hoagland's solution containing 150 mM NaCl for 48 hr. Vertical bars representing ±SE of the means are shown when larger than symbols. R^2 values are significant at 1% level (**).**

Linear regression equations:

▲ Cl^-: $y = -75.0 + 7.24x$ $R^2 = 0.95^{**}$

● Na^+: $y = -80.1 + 7.59x$ $R^2 = 0.82^{**}$

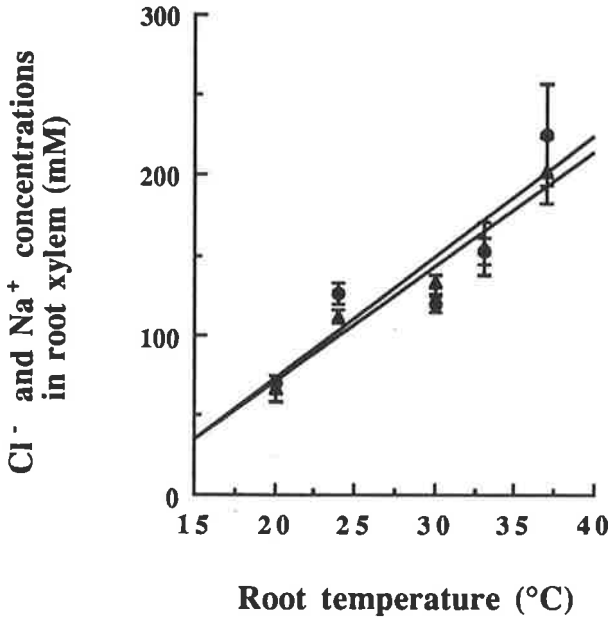

300
200
100
0
15
20
25
30
35
40
Cl⁻ and Na⁺ concentrations in root xylem (mM)
Root temperature (°C)

Fig. 4.34 The relationship between transpiration and water flow. Vertical bars representing ±SE of the means are shown when larger than symbols. R^2 value is significant at 1% level (**).

Linear regression equation:

$$y = 108.4 + 26.96x \quad R^2 = 0.92^{**}$$

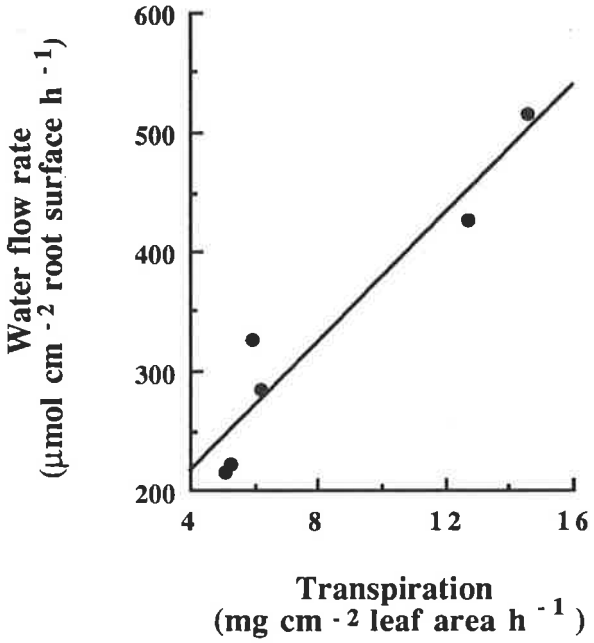
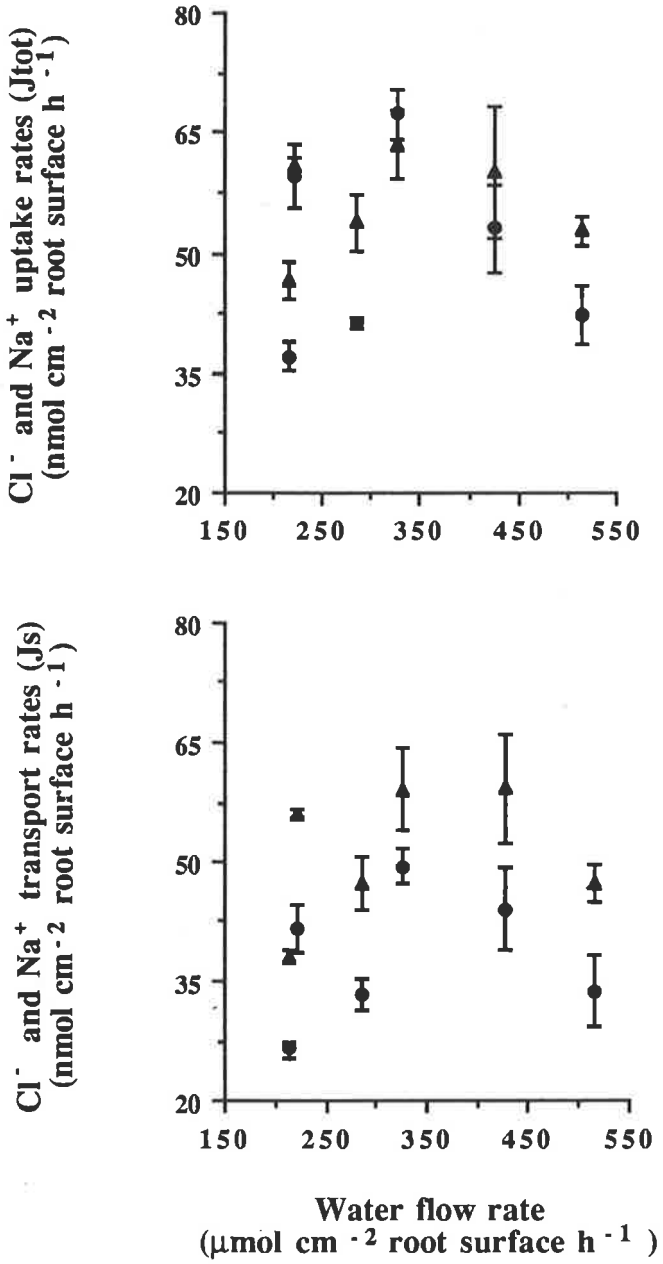

Water flow rate
(µmol cm -2 root surface h -1)
600
500
400
300
200
4
8
12
16
Transpiration
(mg cm -2 leaf area h -1)

Fig. 4.35 **The relationship between water flow and net transport and rates of transport and uptake of Cl^- (▲) and Na^+ (●) to the shoot (J_s) and to the whole plant (J_{tot}).**


Cl⁻ and Na⁺ uptake rates (Jtot)
(nmol cm⁻² root surface h⁻¹)
80
65
50
35
20
150
250
350
450
550
Cl⁻ and Na⁺ transport rates (Js)
(nmol cm⁻² root surface h⁻¹)
80
65
50
35
20
150
250
350
450
550
Water flow rate
(µmol cm⁻² root surface h⁻¹)

effect of VPD, air temperature and transpiration rate on the concentrations of Cl^- in the stem and root tissues and Na^+ in the three component parts.

Temperature

There was no significant effect of high root temperature on transpiration or water flow rate at any VPD level but high air temperature (43°C) increased water flow by 2.5 fold over that at 23°C (Table 4.1 and Fig. 4.31). An increase in root temperature without a change in air temperature resulted in a rise in the uptake rates of Cl^- and Na^+ (Fig. 4.32) and increased accumulation within the tomato plant tissues (Table 4.1). These observations were in agreement with the data from the preceding experiment (Figs 4.17, 4.18 and 4.20). It is evident that the net uptake rates of Cl^- and Na^+ into plants in sand and in hydroponic culture were almost identical, varying between 30 and 70 nmol cm^{-2} root surface h^{-1} with the increase in root temperatures. The direct effects of root temperature on movement of Cl^- and Na^+ into the xylem of intact roots (Fig. 4.33) confirm this conclusion. The concentrations of Cl^- and Na^+ in the xylem sap increased linearly as root temperature increased. In experiments with a salt and temperature interaction in barley, Greenway (1965) demonstrated considerable uptake of Cl^-, Na^+ and K^+ at the higher temperature (23°C) but no detectable uptake at the lower temperature (3°C) when excised roots of 6-day old plants were treated with 100 mM NaCl for 6 h. In another experiment with the whole barley plant, Greenway (1965) also found that addition of the metabolic inhibitor, DNP (2,4-dinitro-phenol), reduced ion uptake by about 50% at low and 30% at high rates of transpiration; the concentration in the xylem was 15 mM at the lower and 2 mM at the higher rate of water flow, again showing the ability of the root to restrict ion entry to the plant and the temperature dependence of ion transport. In the present investigation, J_s and J_{tot} of Cl^- and Na^+ did not increase with increasing water flow rate, but were correlated with root temperature. This is compatible with data from an experiment with *Aegiceras corniculatum* and *Avicennia marina* in which the xylem Cl^- concentration increased with an increase in salinity, but decreased with increase in shoot evaporation rates such that the salt flow to the leaves was unchanged with increase in evaporation rate at a given salinity (Ball, 1988). This implies that the major means of Cl^- and Na^+ entry into the transpiration stream

is via a symplastic pathway, consistent with studies on root function in *A. marina* (Moon *et al.* 1986) and *Suaeda marina* (Yeo and Flowers, 1986).

Cl^- and Na^+ concentrations in the xylem sap changed with root temperature and exceeded the external salt concentration (150 mM), reaching 200 mM at 37°C. It seems that such change was independent of visible variation of the root surface area among the whole intact root systems. In a similar finding, Aston and Lawlor (1979) demonstrated that removing a considerable proportion of the roots to decrease the surface area available for water uptake did not cause the relationship between transpiration rate and water flow through the root to change. It would be suggested that the uptake of Cl^- and Na^+ by the root was able to increase in response to the increasing root temperature and high external salt concentration.

From the above discussion, it is evident that the transpiration rate and, specially, water flow through the root, which increased with VPD and air temperature, did not significantly influence Cl^- and Na^+ uptake and transport. Also there was no interaction between the effects of VPD and root temperature on the rate of transpiration and of uptake and transport of Cl^- and Na^+. Only changes in root temperature had significant effects on uptake and transport of Cl^- and Na^+ to the plant. This indicates that the major resistance to ion movement is located in the symplast of the root cells, bridging the bathing solution and the xylem vessels. There was evidently a modification in root membrane properties at high temperatures, which is presumably responsible for the increased uptake of Cl^- and Na^+ to the roots.

5. The Effects of Root Temperature and Salinity on Trans-Root Potential

5.1 Introduction

The rate of uptake and transport of Cl^- and Na^+ by tomato plants increases with increasing root temperature (section 3 and 4 in this chapter). This increased permeability may be attributed to changes in root membrane properties at high temperature. Recent work has documented a tendency for increased membrane permeability to Na^+ at a range of high temperatures (Magin, Niesman and Bacic, 1990). Also, a positive correlation has emerged between plasma membrane fluidity and transport activity in a variety of systems (Schaeffer and Zadunaisky, 1979). Salinity itself may modify plant membrane fluidity (Borochov-Neori and Borochov, 1991; Prud'homme *et al.* 1990). Measurements of ion flow and membrane potential difference have been used to confirm changes in K^+ and Na^+ permeability with temperature increase (Hogg, William and Johnstone, 1968; Hope and Aschberger, 1970). The results showed that increasing temperature hyperpolarized the plasma membrane in algal cells, and that individual permeabilities of Na^+ and K^+ were functions of temperature.

In studies on the mechanism of salt-tolerance, a selective barrier for ion transport to the shoot at the symplast/xylem interface has been proposed by several authors (Bowling, 1976; Erdei and Kuiper, 1979). Evidence for two independent active transport mechanisms was transposed by Hanson (1978) and Bowling *et al.* (1966) into a chemiosmotic hypothesis for ion transport across the root. Hanson considered the symplast as an osmotic unit bridging the apoplast solutions, the bathing solution and the xylem vessels, separated by the Casparian strips. He deduced that the two electronic pumps, located at the plasmalemma of epidermal and cortical cells and at the plasmalemma of xylem parenchyma cells, work in opposite directions. This has been called a back-to-back model (Okamoto *et al.* 1978). So, trans-root potential (TRP) is a summation of the membrane potential of cortical cells and of xylem parenchyma. This involves the uptake of ions through the root,

and has been the subject of further studies (De Boer *et al.* 1983, 1984; Dunlop, 1973, 1982; Kennedy and Gonsalves, 1987, 1988; Shone, 1968, 1969).

Metabolic inhibitors, such as KCN and NaN_3, have been used to assess the relative extents of active and passive uptake of ions. For example, the depolarizing action of KCN on the trans-root potential in maize roots has been thought to be due to inhibition of an electrogenic mechanism (Kennedy and Gonsalves, 1988).

Obviously, a change in trans-root potential over a specific range of temperature would provide a useful comparison with the Cl^- and Na^+ flow data and could demonstrate the extent to which Cl^- and Na^+ uptake is associated with electrogenic proton pumping. Ion uptake and transport in plants are considered to be the sum of active and non-active components (Greenway, 1965; Luttge and Laties, 1967). It is therefore impossible to estimate the individual permeabilities of Cl^- and Na^+ as functions of temperature. The following investigation was intended to explore the temperature dependence of root membrane properties responsible for movements of Cl^- and Na^+ by revealing the temperature dependence of the electrogenic component of the trans-root potential by the use of an inhibitor.

5.2 Methods

The hydroponic system used was described in the chapter III. After day 21, the pots with plants at two salinity levels (0 and 150 mM NaCl) were kept in the water baths for 48 h to study the effects of high temperature on the electrical potential difference (TRP). In the first experiment (Fig. 4.36), the air/root temperatures imposed were 23/24, 23/37, 43/24 and 43/37°C. In the second experiment (Fig. 4.37), only one air temperature (23°C) was adopted and root medium temperatures were controlled at 20, 24, 30, 33 or 37°C respectively. After 48 h, the pot together with the intact root system was transferred to a wooden board to prevent electrical current leakage. Four plants for each treatment were taken and detopped for the measurement of TRP. The electrical potential between the sap exuding from the cut xylem vessels and the bathing solution was then immediately

monitored by means of two electrodes. After this measurement, a few drops of 1 mM NaN_3 were added to the bathing solution and TRP was recorded once more. The pH of the bathing solution was determined and varied between 6.3 and 6.4. During the measurement of TRP, the temperature of the root bathing solution was maintained ±1°C. The method for TRP determination has been detailed in Chapter III.

5.3 Results

When the root temperature was unchanged, the plants grown at 23 and 43°C air temperatures were excised and trans-root potential (TRP) was monitored immediately with roots bathed in 150 mM NaCl in Hoagland's solution at pH 6.3 ~ 6.4. There was no significant variation (Fig. 4.36). However, increasing the root temperature from 24 to 37°C hyperpolarized TRP, the potential being -38 mV at 24°C and -60 mV at 37°C.

Since air temperature did not influence ion uptake (Fig. 4.32) and TRP (Fig. 4.36), all plants in the following experiment were grown at an air temperature of 23°C with five root temperature treatments (Fig. 4.37). When the culture medium contained only Hoagland's solution, increasing the root temperature from 20 to 30°C hyperpolarized TRP from -78 to -88 mV, but above 33°C roots were depolarized to -72 mV at 37°C. In comparison to the control roots, salinity depolarized TRP at each root temperature, with about 60 mV at 20°C. With further increase in root temperature TRP became more negative (hyperpolarization), being -61 mV at 37°C. When NaN_3 (1 mM) was added, the potential curve was depolarized over the whole range of root temperatures for both control and salinity treatments. However, the effects of the metabolic inhibitor were smaller at high temperatures. The difference between those with and without NaN_3 in both control and salinity treatments showed a similar trend with increase in root temperature, being about -20 mV at lower temperatures and -5 mV at 37°C.

Fig. 4.36 **The effect of different air and root temperatures on trans-root potential of tomato plants exposed to salinity. Vertical bars representing ±SE of the means are shown.**

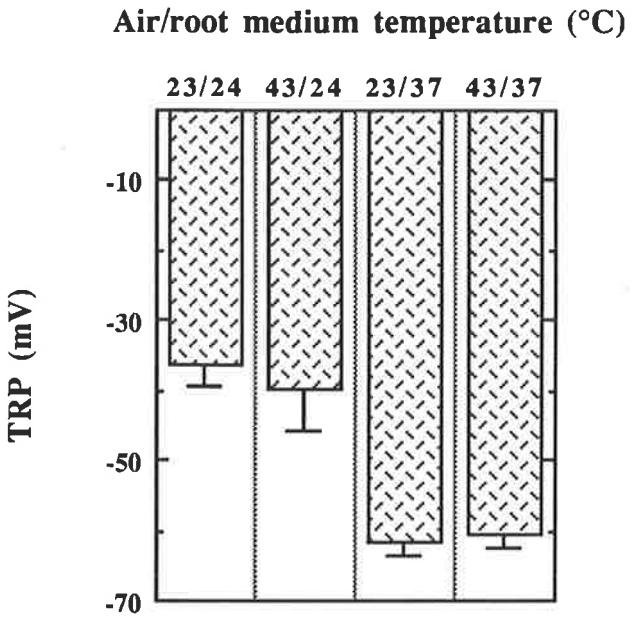
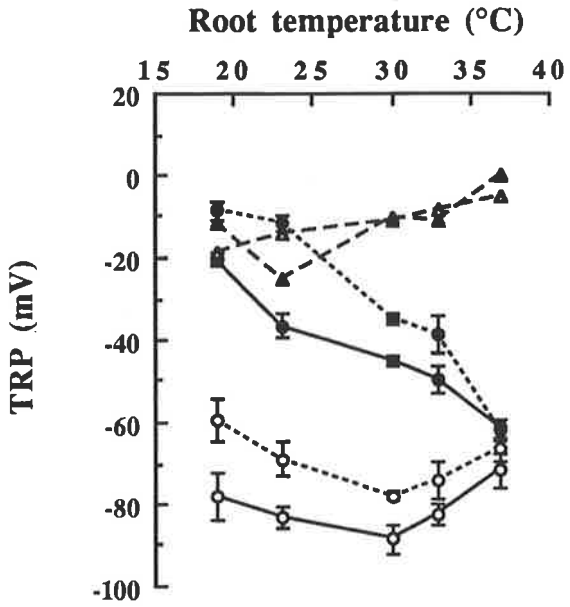

Air/root medium temperature (°C)
23/24
43/24
23/37
43/37
TRP (mV)
-10
-30
-50
-70

Fig. 4.37 **The effect of high root temperature on trans-root potential of tomato plants subjected to 0 (open symbols) or 150 (solid symbols) mM NaCl, with (broken line) or without (full line) NaN_3 treatment. The mean difference between those with and without NaN_3 treatment for control (△--△) and salinity (▲--▲) treatments are also shown. Vertical bars representing ±SE of the means are shown when larger than symbols.**

Analyses of variance (F, ≤ 0.01 or not significant: ns):**

Salinity:	652.99**
NaN_3:	60.03**
Temperature:	34.93**
Salinity x NaN_3:	0.00 ns
Salinity x Temperature:	31.85**
NaN3 x Temperature:	3.83**
Salinity x NaN_3 x Temperature:	1.06 ns


Root temperature (°C)
15
20
25
30
35
40
20
0
-20
-40
-60
-80
-100
TRP (mV)

5.4 Discussion

Electrical potential differences in plant tissues are related to movement of ions (Kennedy and Gonsalves, 1988 and reference therein; Shone, 1969). TRP was not affected by air temperature (Fig. 4.36) which had no consistent effect on salt uptake (see section 4). A common feature in TRP of both control and salt-treated roots is hyperpolarization in response to increased root temperature (Fig. 4.37). In algal cells, Hope *et al.* (1970) have demonstrated an association of ion influx with hyperpolarization in electrical potential difference and showed that P_K and P_{Na}, the permeabilities of K^+ and Na^+ at the plasmalemma, were functions of temperature. In higher plants, an association between TRP and movement of ions has been detailed by Shone (1969) and Kennedy and Gonsalves (1988). It could be deduced that the increase in the rate of uptake of Cl^- and of Na^+ into tomato plants with increased root temperature is an indication of a change in root cell membrane permeability.

The response of TRP to a change in temperature arises from metabolic processes (Shone, 1969). Both electrical potential and proton gradients are essential for the uptake of ions; the ability of the root to alter potential and proton gradients in response to changes in temperatures is related to the activity of the electrogenic proton pump mediated by membrane ATPase (Kennedy and Gonsalves, 1988). Addition of the metabolic inhibitor, NaN_3, produced a greater depolarizing effect at lower than at higher temperatures (Fig. 4.37). This is in agreement with the data reported by Kennedy and Gonsalves (1988) and Shone (1969), who found that the depolarizing action of KCN on TRP was rapid in maize roots and that TRP declined with increasing root temperature. It appears in this study that TRP at lower temperatures was mainly due to the active component. The passive component proportion increased as temperature increased. It could be suggested that the effect of high temperature lies in an effect on electrogenic proton pumping.

Salinity had the effect of depolarization on TRP (Fig. 4.37). Shone (1969) in a detailed study showed that adding NaCl, KCl or $CaCl_2$ to the bathing solution at a single room temperature caused TRP to rise by 30 ~ 40 mV from about -70 mV. The magnitude

of TRP was governed by the total concentration of ions in the external solution, not the composition of the ambient solution, but was also influenced by the ionic status of the root cells. The salt status of the root influenced the relative rates at which anions and cations were transported to the xylem sap. There was a correlation between TRP and the ratio of the rates of movement of Cl^- or SO_4^{2-} to that of K^+ to the sap (Shone, 1969). However, elucidation of the pathways whereby ions are transported to the sap at higher temperatures is a remaining project with distinction between a number of processes, including diffusion (Goldman, 1943), 'Donnan' equilibrium (Hope and Walker, 1961), electro-osmotic (Briggs, 1968), carrier (Shone, 1969) and transport (Curran, 1964) potentials, remaining to be elucidated.

The minimal TRP was observed in the control roots at 30°C (Fig. 4.37). This may occur when root membrane selectivity is reduced since the flow of ions in the external solution and ion status of root cells, particularly H^+, would influence the magnitude of TRP (Kennedy and Gonsalves, 1988). An alternative explanation is that any temperature-dependent increase in electrogenic potential across the plasma membranes of the epidermal and cortex cells might be balanced by a corresponding increase across the membranes of cells lining the root xylem vessels. This inner potential difference could lie across the membranes of the parenchyma cells surrounding the xylem vessels (De Boer *et al.* 1983, 1984; Dunlop, 1973).

An associated variation between Cl^- and Na^+ uptake into tomato plants and TRP with increasing root temperature has been demonstrated. The data here support the suggestion that the electrical potential gradient is essential for uptake of salt (Kennedy and Gonsalves, 1988), and that this gradient is capable of responding quickly to the change in root temperature. The most physiologically significant part of these findings would appear to be the highly temperature-dependent flow of Cl^- and Na^+ across the root plasma membranes.

CHAPTER V

GENERAL DISCUSSION

Plants may be simultaneously exposed to unfavourable root-zone salinity and high temperature conditions in saline areas during the summer season. The effects of simultaneous (parallel) exposure of plants to these stress factors, however, is not well documented (Gale, 1975; Larcher *et al.* 1990; Mooney *et al.* 1991) although the separate (single) effects of salinity (Greenway and Munns, 1980; Kirst, 1989; Munns *et al.* 1983) and high temperature (Long and Woodward, 1988; Turner and Kramer, 1980) have been extensively researched. Under covariation of, and interaction between, stress factors, the plant's response to a particular environmental factor undergoes modification including exacerbation and reversal of stress effects (Burian *et al.* 1982). Under field conditions, stress may elicit more pronounced effects than a single stress factor in a controlled experiment (Larcher and Bodner, 1987). Furthermore, if a fixed-level stress factor is combined with another stress factor, the response to the first factor may become quantitatively more expressed (Wieser *et al.* 1974). Observations of this nature indicate that after investigation in the form of a one-factor test, the applicability of the results should be further verified under the more complex conditions existing in the natural habitat. The method of controlled multiple-stress application provides a link between highly precise laboratory experiments and field studies. The approach in this thesis was (1) to investigate whether salinity effects on the vegetative growth of tomato plant were amended by high temperature, and (2) to ascertain the physiological nature of the interaction between salinity and high temperature.

It is documented in this thesis that changes in root temperature during exposure to salinity produce a significant effect on the uptake of ions by the tomato plant. The results of transpiration experiments demonstrated that ion movement is relatively independent of water flow through roots. Neither an increase in VPD and air temperature nor of water flow rate had any significant effect on the rate of uptake and transport of Cl^- and Na^+ (Figs 4.32 and 4.35). This accords with the observation (Ball, 1988; Nicolas *et al.* 1993; Salim, 1989) that Cl^- and Na^+ concentration in the ascending sap decreases sharply with increasing transpiration. Consistent with the results of Greenway (1965), the uptake rates of Cl^- and Na^+ were dependent upon root temperature (section 4.3 in chapter

IV). When plants were grown under NaCl salinity, the uptake of ions was responsive to temperature in the root medium. Plants take up more ions if their roots are kept at higher temperatures. The results obtained here are applicable to field conditions. Under natural soil conditions, with fluctuation of temperature between day and night particularly in the hot season, the temperature of the soil surface (~ 20 cm) approximates to air temperature during the day (Leeper, 1967). Plants grown in the field may then absorb more ions during the day (or in hot weather) than at night (or the cool season). Consequently, growth would be affected. This response may also explain the susceptibility of container-grown plants to a saline water supply, as container soil temperatures may even exceed air temperature.

Root temperature is known to alter the ion uptake of plants presumably by changing the properties of plant membranes (Kuiper, 1984; Steponkus, 1985). As the metabolic inhibitor (DNP) reduces ion uptake (Greenway, 1965), the major route for Cl^- and Na^+ entry into the transpiration stream must be via a symplastic pathway and involve a metabolic process. Hope *et al.* (1970) have demonstrated that there is a hyperpolarization of electrical potential at the plasmalemma of an algal cell at high temperature, showing that the permeability coefficients of Na^+ and K^+ are functions of temperature. An association between hyerpolarized trans-root potential and movement of ions has also been detailed in higher plants (Shone, 1969; Kennedy and Gonsalves, 1988). Hence, the hyperpolarization of TRP of the tomato plant root in response to salinity and increased root temperature reflects a change in root cell membrane permeability to ions (Fig. 4.37). It is recently evident (Caldwell, 1989) that increasing temperature above 30°C induces changes in protein conformation, and high temperature and cations modify the reaction of barley root membrane protein sulfhydryl groups with N-4-(7-diethylamino-4-methylocumarin-3-yl)-phenyl maleimide (CPM). Na^+ promotes and Ca^{2+} delays the onset of the temperature-dependent increase in reaction rates. Thermally induced changes in protein conformation may result from the disruption of the chemical bonds involved in the maintenance of protein structure (Somero, 1978). Levitt (1980a) proposed that plant exposure to high temperature could cause metabolic dysfunctions, as a result of the modification of protein intra- and inter-molecular SH bonds. According to the SH-hypothesis, a variety of environmental

stresses may cause membrane damage by inducing the aggregation of membrane proteins. This would result in altered protein conformation and suggests a role for protein SH groups in membrane damage at elevated temperature (Caldwell, 1989). Proteins play a crucial role in ion transport across membranes. The electrical potential and proton gradients are essential for the uptake of ions, and the ability of the root to alter potential and proton gradients in response to changes in temperature has been related to the activity of the electrogenic proton pump mediated by membrane ATPase (Kennedy and Gonsalves, 1988). This can be judged in light of the depolarizing effects of a metabolic inhibitor in the experiment reported in this thesis. So, the effect of high root temperature may lie in an effect on a variety of membrane proteins. It may thus be worth attempting to manipulate the expression and structure of membrane proteins which control the transport of salt across the cells that line the xylem vessels and modify the composition of the xylem sap flowing to the leaves.

Root temperature acts on root membrane permeability, resulting in the buildup of ions in the root and shoot. However, Cl^- distribution (%) to the roots was lower than that to the shoot and, in contrast, root Na^+ concentration decreased with increasing root temperature (Chapter IV, Section 3.3). This decline in Na^+ concentration could be due to effective transport to the shoot as uptake by the whole plant increased with temperature. The similarity between the uptake rates of Na^+ and Cl^- to the whole plant (J_{tot}) and the transport rates to the shoot (J_s) above 38°C (Fig. 4.20) suggests that most of the Na^+ and Cl^- absorbed was rapidly transported to the shoot. A similar result has been observed with salinity stress at a single temperature (Reimann, 1992).

When the temperature is increased, NaCl becomes progressively more inhibitory to seed germination (eg. Hampson and Simpson, 1989; Fowler, 1991; Francois and Hoodin, 1972) and the elongation of roots and shoots (Hampson and Simpson, 1989). Ehlig (1960) confirmed that injury in grapevines caused by Cl^- was correlated with temperature. There was great varietal difference in chloride resistance, but all varieties were injured at equal foliar concentrations (0.55 ~ 0.56 m_{χ}^{mol} g^{-1} dw), and the degree to which the plants were severely injured was dependent upon the accumulation of Cl^-. The responses observed in

this thesis confirm that temperatures above approximately 33°C increase the inhibitory effects of NaCl stress on tomato plant growth and assimilation. The accumulation of NaCl in the leaves and stems is a function of temperature under the interacting stress of salinity and high temperature. Both sand culture and hydroponic experiments (section 4 of chapter IV) have shown that uptake of Cl^- and Na^+ by the plant and ion transport to the shoot increase with an increase in temperature, eventually leading to an excessive buildup in the tomato leaves. The concentrations of Cl^- and Na^+ ranged from 0.6 $m_{\chi}^{mol}\ g^{-1}$ dw at 13°C to 3.5 at 43°C (Figs 4.17 and 4.18). It is most likely that salt concentrations reach a toxic level first in the mature leaves (Ehlig, 1960; Munns, 1993). These salts are preferentially sequestered in vacuoles with Na^+ concentration remaining around 100 ~ 150 mM in the cytoplasm and organelles, such as the chloroplast, until the concentration in the vacuole becomes so high that net uptake by the vacuole is reduced to zero (eg. Flowers *et al.* 1977; Munns, 1993). When the vacuole is full, incoming salts may then build up in the cytoplasm, or the cell wall (Canny, 1990). It has been proposed that the consequences of salt buildup in both cell walls and cytoplasm would be equally disastrous and the cell dies of dehydration or toxicity (Munns *et al.* 1993), depending on whether salts accumulate in the cell wall or cytoplasm. This may be an interpretation of the death of salt-treated leaves at 43°C in the present study.

The increase in uptake of ions by tomato plants due to interacting stress on root membrane selectivity caused an imbalance of nutrients within the plant. However, there was no relationship between tissue K^+ concentration and tomato growth in the range of temperatures and salt treatments. K^+ concentration in both leaves and roots did not fall markedly at higher temperatures (Chapter IV, Section 3.3) although there was a progressive decline in the relative growth rate (Chapter IV, Section 1.3). K^+ acquisition was not linearly correlated with Na^+ uptake although K^+ uptake was depressed by elevated NaCl concentration at all temperatures. Lloyd *et al.* (1987a) have observed that tissue K^+ concentration is not involved in the inhibition of leaf gas exchange. It is evident that low K^+ concentration is not solely responsible for the lowering of RGR and NAR with salinity stress at high temperature. The imbalance between other anions and cations in the leaf may not suffice to explain the reduction in RGR and NAR (section 3.4 in chapter IV), but total

cation concentration (including Na^+) in the leaf increased significantly with temperature and may have inhibitory effects on biochemical processes, such as protein synthesis in the cytoplasm (Gibson *et al.* 1984).

Much of the previous physiological research into salinity has concentrated on three topics: water relations, the accumulation of particular metabolites and photosynthesis, assuming that one or more of these processes limits growth in saline conditions.

Turgor does not control plant growth (Munns, 1993). In salt-stressed plants turgor is frequently not reduced and often similar to, or higher than that in control plants (eg. Robinson *et al.* 1983; Heuer and Plaut, 1989; Yang *et al.* 1990; Lloyd *et al.* 1987). Further, leaf turgor of salt-sensitive varieties is usually higher than that of their salt-tolerant relatives, and it is not evident that stomatal conductance or cell expansion is regulated by turgor, or that osmotic adjustment has any direct effect on these processes (Munns, 1993). Despite turgor maintenance, both CO_2 assimilation and stomatal conductance by *Citrus* are reduced by salinity (Lloyd *et al.* 1990). There is enhanced elongation of wheat shoot and roots growing in a polyethylene glycol solution, but reduced growth in salt treatments (Hampson and Simpson, 1990). In agreement with these reports, the data obtained in this study show that growth and assimilation of tomato plants was reduced by accumulation of salt when temperature increased, although turgor apparently increased with salt and was unaltered by high temperature. Lowering the water potential of the solution bathing the roots with NaCl led to a fall in leaf Ψ. However, the difference in leaf Ψ between control and salt treatment was around -0.65 MPa comparable to the level of external osmotic potential (-0.68 MPa). These observations imply that some factors rather than turgor are responsible for the reduction in plant growth and assimilation in a saline environment.

It has been established that a common metabolic consequence of reduced water potential in plants is the accumulation of particular metabolites, presumably contributing to the adaptation and survival of plants under saline stress. However, accumulation of total free amino acids and a dominant component, proline, could not be proposed as an accompanying factor in the response of saline plants to lowered water potential in this investigation. The total free amino acid response is comparable to that in the study of Bray

et al. (1991), where the content in the leaves and roots of poplar vitro plants (*Populus trichocarpa* x *deltoides*) was not significantly modified after transfer to media containing 50 to 200 mM NaCl at 25°C. The increase in total free amino acid content of severely stressed plants at 43°C noted in the present study is a possible consequence of growth inhibition, protein degradation and reduced protein synthesis (Levitt, 1980a). It seems that proline accumulation caused by salt stress varies with temperature (Chu *et al.* 1974; Chu *et al.* 1976a, b; Naidu *et al.* 1987), but not water relations (Chu *et al.* 1976a; Handa *et al.* 1986; Voetberg and Stewart, 1984). This has been further demonstrated in this thesis where the proline contents of the leaves, stems and roots were each negatively correlated with temperature, and this response did not correspond to changes in leaf Ψ or Ψ_s in the salinised plants (Chapter IV, Section 2). The proline concentration in the stems and roots of control plants was also high at low temperature. This is in agreement with the results of Parameshwara *et al.* (1988), in which proline accumulated in alfalfa when exposed to cold stress, where water potential did not vary. On the other hand, a high concentration of Na^+ inhibited ^{14}C incorporation from glutamic acid into proline in the tobacco leaf (Noguchi *et al.* 1966). Thus, the decline in proline accumulation in all tomato component parts with increasing temperature may be attributed to an increase in the concentration of Na^+ in the tissue and/or in temperature. Similarly, leaf sugars, apart from sucrose, tended to decline with salinity and high temperature. These observations suggest that there is a reduced supply of carbon due to reduced photosynthesis and/or increased respiration in response to the interaction between the effects of salinity and high temperature.

It is clear that the buildup of salt in the plant was responsible for the reduction in both RGR and NAR (Chapter IV, Section 3.3). This is consistent with many reports (eg. Clemens *et al.* 1983; Cramer *et al.* 1991; Salim and Pitman, 1983), in which RGR and NAR were significantly associated with salt accumulation in the shoot rather than with the osmotic pressure of the external solution, and with reports that salt build-up in the leaf is associated with lowering of the assimilation rate (Downton and Millhouse, 1985; Lloyd *et al.* 1987a, 1990).

These responses can be analysed at a number of levels to ascertain the physiological reasons for the reduced growth. In a recent review of salinity stress, Munns (1993) proposed that salinity affects carbon assimilation through reduction in leaf area rather than as a result of reduced photosynthetic rate. In contrast, the experiments reported in this thesis indicate that a reduced assimilation rate is the more important factor in the observed reduced RGR of tomato plants exposed to salinity and high temperature (section 1.3 in chapter IV). LAR, as a measure of total leaf area development, was only depressed by the effect of salinity and appeared to be almost constant with change in temperature. The steady state leaf expansion rate is altered by treatments that alter soil water status, independent of leaf turgor (Munns, 1993). Increase in both Cl^- and Na^+ concentrations in the leaf consequent upon increasing temperature (Chapter IV, Section 3.3 and 4.3) had no further effect in the reduction of LAR at higher temperatures. The observed lack of correspondence between changes in LAR and in RGR shows that these changes in LAR are not sufficient to account for the reduction in carbon assimilation measured as reduced RGR. Cramer *et al.* (1990) reached the same conclusion with salt-stressed barley plants at a single temperature. There was no loss of leaves in any treatments, and above 33°C the differences in leaf expansion rate (LER) between corresponding control and salinity treatment were similar, even being lower at 43°C (Fig. 4.3). Obviously, LER of control plants declined much more rapidly than that of salt-treated plants at the higher temperatures. In contrast, the growth rate of salt-stressed plants declined with temperature more than that of controls (Figs 4.1 and 4.5). This suggests that the leaf area of salt-treated plants declines to a lesser extent than the growth rate and thus could not be interpreted as a main factor in the growth-rate reduction. It may be concluded that the greater decline in RGR of salt-treated plants with increasing temperature involved reduced photosynthetic rate and/or increased respiration. Both high temperature (eg. Levitt, 1980a; Pollock and Eagles, 1988; Quinn, 1988; Weis and Berry, 1988) and salinity (eg. Cheeseman, 1988; Rawson *et al.* 1988; Seemann and Critchley, 1985) inhibit photosynthesis in plants. This salinity-induced decline in net assimilation rate and stomatal conductance is closely correlated with high sodium and chloride concentrations in the leaves (eg. Walker *et al.* 1982; Lloyd *et al.* 1989; Lloyd *et al.* 1990). More recently, Larcher *et al.* (1990) found in a study of the effects of temperature

and salinity that high temperature intensified the unfavourable effects of saline stress on the kinetics of non-photochemical quenching. The combined stress impaired photosynthetic capacity and extended the critical limits for function and viability through delayed energization of thylakoids and disturbances in the photosynthetic system. This may suggest that the response of tomato plant to interacting stress is mainly mediated through effects on photosynthesis. Salinity also increases respiration in plants (Bloom and Epstein, 1984; Nieman, 1962; Rawson, 1986; Schwarz and Gale, 1981). Schwarz and Gale (1981) found that maintenance respiration increased without any net effect on total respiration, since assimilatory respiration declined with a salinity-induced decline in photosynthesis. The change in RGR is highly correlated with NAR, not LAR in the present experiments. NAR integrates the effects of salt treatments on the physiological processes of photosynthesis and respiration. Thus, not only a decrease in photosynthesis, but also an increase in maintenance respiration could effectively reduce NAR, and consequently RGR. The contribution of reduced photosynthesis and increased maintenance respiration to reduced carbon assimilation would vary depending on the plant species and environmental conditions (Bloom and Epstein, 1984; Nieman, 1962; Rawson, 1986; Schwarz and Gale, 1981).

The responses of the growth of the component organs of the tomato plant to stress differed, with leaf and root growth more sensitive to stress than stem growth. The reduction in leaf growth at high temperature reflected a change in the allocation of assimilate (Fig. 4.8). Maintenance of net root growth would cost more in dry matter allocation under salinity stress than is apparent from root dry weight figures (Snapp and Shennan, 1992). Moreover, the long-term exposure to high temperature will result in injury to root properties, including to cell membrane function (Hampson and Simpson, 1990; Ingram, 1985; Levitt, 1980a; Quinn, 1988). Thus high root temperature may be mainly responsible for root growth reduction (Fig. 4.8). The injury to the root membranes would in turn influence assimilation and shoot growth.

The results of this examination of the interaction between responses to salinity and temperature on the growth of the tomato plant have focussed attention on the critical

importance of root temperature. The exacerbation of salt injury at higher environmental temperatures is seen to be due principally to the enhanced rates of salt uptake consequent upon high soil temperatures. This may be a general phenomenon, certainly it deserves examination with other crops. If confirmed, then the response suggests management options, including soil mulching, and the use of white pots in container culture to reduce soil temperature and hence minimize damage.

CHAPTER VI

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