HOW DOES SALINITY LIMIT SHOOT GROWTH?

WATER RELATIONS AND SPECIFIC ION EFFECTS IN WHEAT, BARLEY AND CLOVER.

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Annie Termaat

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Statement

The text of this thesis contains no material which has been accepted as part of the requirements for any other degree or diploma at the university. Assistance with the experimental work is acknowledged, where applicable, in the materials and methods section; otherwise the experimental work is my own. The following papers were written during the period of study:

1. Shoot turgor does not limit the shoot growth of NaCl-treated wheat and barley by Annie Termaat, John B. Passioura and Rana Munns. *Plant Physiol.*(1985), 77, 869-72.

2. Whole-plant responses to Salinity by Rana Munns and Annie Termaat. *Aust. J. Plant Physiol.*(1986), 13, 143-60.

3. Use of concentrated macronutrient solutions to separate osmotic from NaCl-specific effects on plant growth by Annie Termaat and Rana Munns. *Aust. J. Plant Physiol.* (1986), 13, (in press).

Annie Termaat

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Abstract

This work aimed to determine the cause of the reduced growth of plants whose roots are exposed to non-lethal NaCl. Because shoot growth is generally more sensitive than root growth, particularly in the short and medium term, the study focussed on leaf growth. Three hypotheses were tested; namely, that reduced growth is due to inadequate turgor of the expanding cells of the shoot, to insufficient cytokinins arising from the root, or to an NaCl-specific disturbance in the mineral transport from the roots.

To test whether the reduced growth is due to inadequate turgor in the expanding cells of the leaves, NaCl-treated plants were grown for 7-10 days with their roots in pressure chambers, and sufficient pnuematic pressure was applied to counter the osmotic effects of the salt on the shoot. Wheat, barley and Egyptian clover were grown in 100 mol m^{-3} NaCl and white clover in 50 mol m^{-3} NaCl (which reduced the relative growth rates of all species by about 20%, i.e., after a week of the salt treatments the leaf areas were only 70% those of controls). This experiment was repeated with an NaCl-free solution of concentrated macronutrients, wheat and barley being exposed to 0.80 and 0.56 MPa osmotic pressure respectively. The applied pressure had no sustained effect (relative to unpressurised salt-treated controls) on the growth rates and transpiration rates of any species. The osmotic pressures of the cell sap, in either fully expanded or currently expanding leaf tissue of wheat grown in NaCl were also unchanged. These results indicate that the applied pressure increased turgor in the shoot proportionately, although this was not directly measured. It was concluded that shoot turgor alone was not regulating growth of these salt-treated plants (i.e., treated with NaCl or

concentrated macronutrients), and that a message from the roots may be regulating the growth of the shoot.

This message was thought likely to be a cytokinin, because these plant growth substances arise in the roots, and their concentration is known to fall in salt-treated plants. This hypothesis was tested by feeding kinetin (10^{-3} mol m^{-3}) directly into the transpiration stream of wheat leaves, by manipulating xylem hydrostatic pressure so that solution was sucked into the xylem. There was a 10-20% increase in transpiration rates and relative leaf expansion rates of the plants grown without NaCl, suggesting that kinetin was reaching receptors. However, transpiration rates and relative leaf expansion rates of NaCl-treated plants, with or without applied pressure, were unchanged. This suggested that something other than, or in addition to, a cytokinin was regulating the growth of the shoot of NaCl-treated plants.

A third group of experiments aimed to distinguish between osmotic and ion-specific effects of NaCl on plant growth and ion uptake by comparing plants grown in isosmotic solutions with and without NaCl. Preliminary experiments showed that polyethylene glycol (MW 4000) and mannitol were unsuitable for even very short-term growth studies because they caused immediate reductions in leaf elongation rate when plants were transferred from NaCl to isosmotic solutions of these compounds. However, concentrated solutions of macronutrients (modified Hoagland's nutrients) did not change the elongation rate.

Barley, wheat, Egyptian clover and white clover were grown in NaCl and isosmotic concentrated macronutrient solutions. After 14 days, plants grown in concentrated macronutrients were smaller than controls (plants grown in normal strength nutrient solution) but had similar root:shoot ratios. NaCl-treated plants were less than half the size of plants in concentrated macronutrients, and had higher root:shoot ratios. NaCl-induced increases in phosphate uptake did not cause this additional reduction in shoot growth. For barley, net transport of N, K, Mg and Ca from the roots (per g root DW) was lower in NaCl-treated plants than in controls, but uptake by the shoot (per g shoot DW) of these minerals was similar. By contrast, both transport and uptake of these minerals in concentrated macronutrient-grown plants resembled control plants. NaCl-treated barley and wheat plants had higher osmotic pressures in both expanding and fully expanded tissue than did controls and macronutrient-grown plants, but lower rates of uptake of solutes generating this osmotic pressure. This raised the possibility that growth in NaCl may be at least partly limited by a reduced rate of transport of an essential nutrient to the shoot.

Because plants grown in concentrated macronutrients had not responded to applied pressure (despite uptake rates of essential nutrients that were similar to controls) and ion-specific toxic effects were unlikely, it seemed that root water status was dominating the response of the shoot, i.e., the reduced water status of the root caused it to produce a message, which travelled to the shoot via the xylem and reduced leaf growth. NaCl-treated plants were also limited by this factor.

Therefore, I concluded that the growth of NaCl-treated wheat, barley, Egyptian clover and white clover was not, except in the immediate short term, limited by shoot turgor, but by a factor (other than, or addition to, cytokinin) associated with low root water status. Additional limitations were caused by nutritional imbalances, such as excess NaCl and possibly also a reduced rate of transport of an essential nutrient, but not excessive phosphate uptake.

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CHAPTER 1

INTRODUCTION

At the 1982 Boden Conference on Salinity, in Thredbo, shortly before these studies began, there was much debate concerning the relative importance of the external as opposed to the internal effects of NaCl, i.e., the importance of a lower soil water potential compared to effects of NaCl on biochemical processes such as photosynthesis. Last year, 1985, the general opinion at the Plant Drought and Salinity Conference, in Canberra, was that growth may be limited by another effect altogether: at a time when hormone regulation in plants is generally under critical review (e.g. Trewavas 1981, 1982; Weyers 1984, Canny 1985) it was becoming evident that a factor dependent on root water relations influences the growth of the shoot. It is natural, in spanning these years, that this research reflects these ideas.

1.1. Aims:

This work aimed to determine the cause of the reduced growth in plants treated with non-lethal concentrations of NaCl, and focussed on the responses of the shoot. Three hypotheses were tested; namely, that the reduced growth was due to inadequate turgor in the expanding cells of the shoot, to insufficient cytokinins arising from the root, or to an NaCl-specific disturbance of the mineral transport from the shoot.

The first hypothesis, that turgor in the expanding cells of the leaves limited shoot growth, was suggested because leaf growth was known to respond instantaneously to shifts in external water potential (Section 2.1.2.), and to recover to control rates immediately when NaCl was removed from around the roots, despite high concentrations of NaCl persisting in the shoot (Munns *et al.* 1982). This led to a fundamental question: if the osmotic effects of the NaCl around the roots were cancelled, would the growth rates of these plants improve? It was decided to attack the question directly using a technique developed by John Passioura with which the shoot water potential of growing plants could be raised by applying pneumatic pressure to the roots (Passioura 1980; Passioura and Munns 1984). The use of whole plants was an important aspect of this method, in order to preserve the adaptive physiology of complex, differentiated tissues. The results are in Chapter 4.

The results of the above experiments strongly suggested that a message from the roots regulated shoot growth. This raised the question was: did a hormone limit the growth of NaCl-treated plants? Notwithstanding problems of interpretation, the literature (see review by Reid and Wample 1985, and Section 5.1.) suggests that there are fairly consistent patterns of hormonal response to low soil water potential (ie., dry soil, non-ionic osmoticum or salinity) and low levels of cytokinins seemed the most probable cause (Section 1.2.2.). This possibility was examined by supplying kinetin, using a variation of the technique used in the previous experiment. Pressure was applied to the roots of the plants until the shoots guttated, a cut leaf tip was inserted in a solution containing kinetin (a cytokinin), which was sucked in when the pressure was lowered. The results are in Chapter 5.

As the above results were negative, a different approach was taken. Plants grown in NaCl suffer nutrient imbalance, which may result directly from accumulation of toxic levels of NaCl, or indirectly if competition of either Na^+ with K^+ or Cl^- with NO_3^-

results in deficiency of these macronutrients. An altered regulation of uptake of some other nutrient, such as phosphate, may also contribute to decreased growth rates of NaCl-treated plants (Section 1.2.3.). This possibility was examined by comparing the growth of plants in isosmotic solutions of NaCl and concentrated macronutrients, which were shown in preliminary, short-term experiments to be more suitable non-specific osmotica (i.e., decreasing water uptake without affecting nutrient uptake) than polyethylene glycol (PEG) or mannitol. An evaluation of concentrated macronutrients suggested that this treatment provided an almost ideal non-specific osmoticum, which enabled NaCl-specific effects on nutrient uptake to be described in detail. The results are in Chapter 6.

1.2. Literature review:

1.2.1. Growth, water relations and ionic relations of NaCl-treated plants:

The earliest response of a non-halophyte (i.e., a plant not native to saline soils) to a lowered soil water potential (ψ) is that its leaves grow more slowly (see below). Reduced growth rates sustained over even a moderate period (days) lead to smaller shoots, so that the removal of NaCl around the roots causes relative leaf expansion rates (RLER) to recover, but leaf areas remain low (e.g., Rawson and Munns 1984). Root growth is almost always less affected than shoot growth, so that the root:shoot ratio increases (reviewed in Munns and Termaat 1986, Bernstein and Hayward 1958). Root growth may be stimulated by the high carbohydrate status of the shoot (discussed in Chapter 7).

Shoot responses to changes in external ψ are described in detail by Acevedo *et al.*, 1971, who manipulated the growth of maize seedlings with Carbowax (PEG) 6000 solutions, and soils of different water content. The short-term, instantaneous changes in growth are completely reversible, and provide strong evidence that the driving force for growth is water uptake, which generates turgor. This is shown directly by Green *et al.* (1971), who measured turgor in the unicellular algae *Nitella* with an intracellular manometer, and indirectly by Matsuda and Riazi (1981) who used barley and solutions of PEG and mannitol. In the latter study, solute accumulation occurred in the expanding (basal) tissue, where π increased to give turgors similar to controls (insofar as ψ of expanding tissue can be estimated with the Shardakov procedure). Whether the rates of solute accumulation limit growth is unknown.

Plants grown in NaCl eventually accumulate high levels of this salt in the shoot, despite adaptive mechanisms such as NaCl exclusion by the roots. For example, an NaCl concentration as low as 10 mol m^{-3} in the xylem stream will lead to leaf NaCl concentrations of 500 mol m^{-3} after a leaf has transpired 50 x its weight; retranslocation in the phloem is low (R. Munns, personal communication). Undoubtedly high levels of NaCl eventually cause the death of older leaves (Greenway 1962; Munns and Passioura 1984b), but it is unclear to what extent non-lethal concentrations of NaCl reduce growth rates of currently expanding leaves. After a medium-term exposure to NaCl, for example, the growth rates of the youngest leaf of barley can recover to control rates immediately NaCl is removed from around the roots, while high NaCl concentrations persist in the shoot (Munns *et al.* 1982). The RLER of NaCl-treated sunflower, similarly, can recover to control rates immediately NaCl is removed from around the roots.

These two examples suggested that, in short- (minutes, hours) and medium-term (days, weeks) treatments, water relations rather than ion toxicity limit the growth of NaCl-treated plants.

1.2.2. Hormonal responses in NaCl-treated plants:

Of all hormonal responses of plants subject to low soil ψ, whether caused by drought, non-ionic osmotica, or salinity, ABA and cytokinins have been studied in the greatest detail. ABA can be synthesised in many tissues, including roots and stems, but the most dramatic changes in concentration are found in the leaves (reviewed in Bradford and Hsiao 1982). ABA levels are frequently negatively associated with leaf turgor, although this relationship can change if the plant has a history of drought (Henson 1983; reviews by Bradford and Hsiao 1982, Aspinall 1980). There are at least two salinity studies which suggest that leaf turgor is related to ABA levels; Mizrahi *et al.* (1971), using tobacco grown in 100 mol m^{-3} NaCl at two different humidities, found the highest ABA concentration in the NaCl treatment at the lowest relative humidity, and Walker and Dumbroff (1981), using tomato grown in concentrated macronutrient solutions with an π of 0.60 MPa, found ABA levels peaked after 2 d, returning to control levels after 8 d. High ABA levels may have a causal (rather than merely associative) relationship with many of the features of plants grown in low soil ψ (e.g., lower stomatal conductance, higher root: shoot ratios) because these features can be induced in plants by raising ABA levels artificially (Bradford and Hsiao 1982). Since roots are generally regarded as the site for cytokinin synthesis (Von Staden 1979; Carmi and Von Staden 1983), the levels in the shoot may indicate the rate of production by the roots. Low levels in the shoot may persist for the duration of the osmotic treatment (0.6 MPa concentrated macronutrients, Walker and Dumbroff

1981), which suggests that the response, unlike that to ABA, is independent of transient changes in the water status of the root or the shoot. All factors favouring growth (high water supply, nitrogen and mineral nutrients) increase cytokinin levels in a plant, and all factors which inhibit growth decrease cytokinins (reviewed in Michael and Beringer 1980). Reduced levels of cytokinins in root exudates have been reported in NaCl-treated plants (Itai *et al.* 1968). In NaCl-treated plants, applied cytokinins increased the capacity of leaf discs to incorporate labelled leucine into protein (Ben-Zioni *et al.* 1967) and, in one instance, improved the growth of beans (O'Leary and Prisco 1970). Several experiments have demonstrated the antagonistic effects of ABA and cytokinins (e.g., on stomatal behaviour in epidermal strips, Blackman and Davies 1983, 1984; on transpiration rates of excised leaves, Livne and Vaadia 1965, Mizrahi *et al.* 1970 and on trends in water status of whole tobacco plants, Mizrahi and Richmond 1972).

Less is known about the effects of salinity on the other groups of plant growth substances. Like cytokinins, gibberellins arise in the roots, although young leaves are also regarded as a source (Carr *et al.* 1964, Torrey 1976). Gibberellins have never been assayed in salt-treated plants, although levels have been shown to decline as water status of detached lettuce leaves decreases and rise again as the leaves regain turgor (Ahroni *et al* 1977). Nevertheless, several studies applied gibberellins (GA) to NaCl-treated plants (Nieman and Bernstein 1959, Prisco and O Leary 1973; Boucard and Ungar 1976, Zhao Ke-fu, Li Ming-liang and Lui Jia-yeo, personal communication). These have produced variable results. For example, GA did not increase the yeild or leaf area of red kidney beans at high NaCl, even though it doubled the yeild of plants at low NaCl and increased stem length at all NaCl concentrations (Neiman and Berstein 1959). On the other hand, GA_3 increased the growth of *Suaeda ussuriesis* at NaCl concentrations above and below the optimal NaCl level for growth (Zhao Ke-fu *et al.* personal communication). This suggests GA_3 is overcoming a water deficit rather than a specific salt effect, perhaps by increasing Π of the cells (cf. Kazama and Katsumi 1983). Auxins have not been assayed in NaCl-treated plants, but their level declines in the shoot during drought (Wright 1978). However, applied to NaCl-treated plants, auxins have produced variable results. IAA (an auxin) improved the growth of tomato at moderate salinity levels but not at high levels (Salama *et al.* 1981), while on cowpea, IAA increased the water content but reduced the leaf dry matter at all salinity levels (Abdel-Rahman and Abdel-Hadi 1984). Finally, ethylene and its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), which is synthesised by the root and transported in the xylem (Bradford 1983), may have a role in the regulation of growth of salt-treated plants, but this has never been studied.

Trewavas (1985) has proposed that NO_3^- might be a growth regulator, affecting metabolism and development. He argued that this could operate via an effect on the Ca^{2+} concentration of the cytoplasm; energy is directed towards NO_3^- reduction when NO_3^- enters a cell, so a change in NO_3^- uptake would change the energy available for Ca^{2+} expulsion. This is relevant to long term exposure of plants to NaCl, because, where Cl^- is present around the roots in high concentrations, NO_3^- uptake may be inhibited (see Section 1.2.3.).

Some of problems in interpreting the literature are discussed in Reid and Wample (1985) (e.g., not only do PEG solutions cause water deficit responses, but, even when aerated, O_2-deficiency responses) and Section 5.1., and there are many exceptions to the very general statements about plant hormones above, particulary concerning ABA. Given the general lack of knowledge surrounding this subject, cytokinins appear the most likely contenders for the regulation of plant growth during medium-term exposure to salinity.

1.2.3. Major nutrient (N, P, K) interactions with NaCl:

Attention to nutrient imbalance as a problem of NaCl-treated plants is not new, and three recent reviews considering possible interactions of N, P and K with NaCl: Campagnol (1979), Feigin (1985) and Kafkafi (1984). This section reviews this aspect of salinity, and, in particular, addresses the question: is it feasible to counter the effects of NaCl using fertilisers?

Nitrate:

Exposure to NaCl can lower the uptake of NO_3^-. This is probably a direct effect of Cl^- on NO_3^- uptake through feedback inhibition by high internal concentrations of tissue Cl^- (Glass and Siddiqui 1985; Dean-Drummond and Glass 1982). There is frequently an inverse relationship between levels of leaf tissue Cl^- and NO_3^- in NaCl-treated plants (e.g. wheat, Torres and Bingham 1973; tomato, Kafkafi *et al.* 1982), where a Cl^- ion may replace up to 2.4 NO_3^- ions (Kafkafi *et al.* 1982). Nitrate within the shoot is always more sensitive to external NaCl than total nitrogen, but reductions in total nitrogen concentration (e.g., wheat, Torres and Bingham 1973) and chlorophyll concentration (e.g., wheat, Mashady *et al.*1982) can also occur in NaCl-treated plants. That this might be the consequence of Cl^- uptake reducing levels of tissue NO_3^-, rather than reduced protein synthesis for some other reason, is suggested by reports in which water stress (wheat, Mashady *et al.* 1982) and Na_2SO_4 salinity (*Phaseolus vulgaris*, Bhivare and Nimbalkar 1984) did not lower total nitrogen or chlorophyll concentrations as much as the NaCl salinity treatments to which they were compared.

However, plant growth reductions associated with salinity may result in normal concentrations of total nitrogen in the shoot (e.g. *Acacia saligna*, Shaybany and Kashirad 1978) or even above normal concentrations (e.g. *Cynodon dactylon*, Langdale and Thomas 1971; *C. plectostachyus*, Langdale *et al.* 1973). Fertilised legumes usually have normal or above normal nitrogen concentrations, e.g. nitrogen levels in *Vicia faba* increased (Yousef and Sprent 1983) and in *Medicago sativa* did not change (Shone and Gale 1983), but innoculated legumes may have lower than normal shoot nitrogen concentrations (Yousef and Sprent 1983; Shone and Gale 1983) with concomitant increases in Cl^- (Yousef and Sprent 1983). The decrease could be due to effects of NaCl on *Rhizobium* nitrogen fixation, or the infection process, but the absence of high levels of NO_3^- in the soil solution to compete with Cl^- uptake may also contribute to Cl^- toxicity in these plants in addition to NO_3^- deficiency.

This leads to an important question; is it feasible to apply NO_3^- fertiliser to NaCl-affected plants to counter these effects? The answer depends on (i) the inherent qualities of the species concerned, such as its relative growth rate and the extent that this is reduced by NaCl, and (ii) how effectively NO_3^- competes with Cl^-. For example, the relative growth rates of strandline halophytes *Atriplex hastata*, *A. littoralis* or *Salsola kali* grown without NaCl did not respond to an increase in NO_3^- from 3.5 to 14 mol m^{-3}, nor was the growth rate at optimal NaCl concentrations (60, 150 and 60 mol m^{-3} NaCl respectively, for the three species above) affected by an increase in NO_3^- from 3.5 to 14.0 mol m^{-3} (Rozema *et al.* 1983). The low inherent RGR of these species may have caused the lack of response to NO_3^-, presumably even at 3.5 mol m^{-3} NO_3^-, sufficent NO_3^- accumulated. However, rapidly growing Mexican wheat varieties responded dramatically to NO_3^-; the yield of one variety at 42 mol m^{-3} NaCl almost reaching control levels after external NO_3^- was increased from 9 to 15 mol m^{-3} (Torres and Bingham, 1973). This study also illustrates competition between the two anions; 82 mol m^{-3} NaCl reduced total N in the shoot to below deficiency concentrations (0.2% g g^{-1} DW) in plants fertilized with 15 mol m^{-3} NO_3^-. In addition to Mexican wheat, there are several other species where nitrogen fertilization improved the growth of NaCl-treated plants proportionately more than controls: cowpeas and African millet (Ravikovitch and Porath 1967), millet (Ravikovitch and Yoles 1971), stargrass (Langdale *et al.* 1973) and bermudagrass (Langdale and Thomas 1971). In all these studies, nitrogen became ineffective at high salinity levels, suggesting that the depression in growth caused by NaCl was greater than the growth depression caused by reduced NO_3^- uptake.

Phosphate:

Phosphate and salinity interactions form a far less consistent pattern, even when varieties of the same species are under consideration. Phosphorus toxicity is induced by NaCl in some species (e.g., corn, Nieman and Clark 1976, Bernstein *et al.* 1974; sesame, Cerda *et al.* 1977; tomato and wheat, Cerda and Bingham 1978) and certain varieties within species, such as soybean (Grattan and Maas 1984). The sensitivity of NaCl-treated soybean varieties to phosphate has been linked to genotypic properties of the root (Grattan and Maas 1985). But in *Glycine wightii*, such varietal differences in phosphate accumulation (particularly in the root) are associated with vigour, and selection of K^+ over Na^+ (Gates *et al.* 1970). Furthermore, there are several studies, all involving soil-grown plants, which report that increased phosphate fertilization improved the growth of

NaCl-treated plants to a greater extent than controls (e.g., on millet and clover, Ravickovitch and Yoles 1971; on carrots, clover, cowpea, African millet, foxtail millet, pigeonpeas and vetch Ravikovitch and Porath 1967; on wheat, Ferguson and Hedlin 1963; on bean, Lunin and Gallatin 1965). Precipitation of phosphate from the soil solution may explain the differences Ravikovitch and Porath (1967) found in the response of plants grown in clay versus sandy soils. Four studies which report no improvement to NaCl-treated plants by phosphate fertilization are, Nassery *et al.* (1978) with sesame and pepper, grown in solution culture, Patel *et al.* (1976) with alfalfa grown in sand culture, and Khalil *et al.* (1967) with corn grown in soil, and Ravikovitch and Porath (1967) with tomatoes and corn, grown in soil.

Thus phosphate toxicity seems to be a feature associated only with hydroponic culture, or, on occasion, sand culture of certain species or varieties. It may, therefore, be an artifact of the method, having no place in agriculture, but confounding the results of many salinity studies.

Potassium:

K/Na interactions have attracted attention in several reviews (eg, Munns *et al.* 1983, Flowers and Lauchli 1983) as these minerals can fulfil similar roles. For example, Na may fulfil the role of up to 90-95% of K present in tomato without any growth reduction occurring (reviewed in Flowers and Lauchli 1983). At low K, moderate levels of Na may stimulate the growth of even non-halophytes, presumably acting as a vacuolar osmoticum (Evans and Sorgar 1966, Munns *et al.* 1983, Flowers and Lauchli 1983). However, Na cannot replace K entirely; "it is generally not as effective as an enzyme activator" (Flowers and Lauchli, 1983; Evans and Sorgar 1966), nor can it replace K in protein and chlorophyll synthesis, nor does Na have a role in stomatal guard cell turgor regulation (Flowers and Lauchli 1983).

Can K fertilization benefit NaCl-treated plants? Ravikovitch and Porath (1967) applied K_2SO_4 to a variety of species grown in two types of soil. In cases where the growth of control plants was not increased, or was even reduced at the higher level of fertilization (which suggests excess K was present), the growth of tomatoes, cowpeas, carrots, pigeon peas and foxtail millet in NaCl was unchanged. Therefore, provided soil K levels are adequate, there seems little benefit in this procedure.

1.5. Conclusion:

This review has identified several ways in which the growth of NaCl-treated plants may be limited. On the one hand, the literature suggests that NaCl in the external solution will stop growth immediately through effects on turgor, but, on the other hand, even at turgor pressures similar to controls, the growth rates of leaves of NaCl-treated plants were slower. This could be caused by a continued need for solutes to generate osmotic pressure in the expanding cells, to changed hormonal messages, or to a deficiency or excess of a major nutrient. The review forms a background for the experiments in Chapters 4 - 6.

Chapter 2

STANDARD MATERIALS AND METHODS

2.1. Plant Material:

Wheat: *Triticum aestivum* cv. Kite. This is a bread wheat variety of short height (Fitzsimmons *et al.* 1983). Seeds of uniform size were selected for each experiment and surface-sterilised for 5-10 minutes in 50% sodium hypochlorite, then rinsed several times in tap water, before being germinated on a screen over 1 mol m^{-3} $CaSO_4$ or in red sand moistened with 1 mol m^{-3} $CaSO_4$. Seed was supplied by Dr B. J. Read, Agricultural Institute, Wagga.

Barley: *Hordeum vulgare* cv. Beecher, and cv. Clipper. Cv. Beecher is a tall, six row feed variety with early maturing characteristics and cv. Clipper, is a two row, malting variety of medium height grown widely throughout Australia (Fitzsimmons and Wrigley 1984). As the latter variety seemed to give more uniform plants in early experiments I tended to prefer it in later work. Preparation of the seed was similar to wheat. Seed was supplied by Dr B. J. Read, Agricultural Institute, Wagga.

Egyptian clover: *Trifolium alexandrinum.* This is an annual species of at least moderate salt tolerance (Winter and Lauchli 1982). Preparation of the seed involved preliminary surface-sterilising as for the cereals, then overnight soaking in tap water. Germination was on moist filter paper in a petri dish. Seed was supplied by Dr A. Gibson, Division of Plant Industry, CSIRO.

White clover: *Trifolium repens.* This is a perennial pasture species which is very salt-sensitive (D. West, personal communication). Preparation of the seed was similar to Egyptian clover. Seed was supplied by Dr A. Gibson, Division of Plant Industry, CSIRO.

2.2. Growth conditions:

2.2.1. Environments:

Glasshouse 1: (Headhouse). This 9 x 10 m glasshouse is located in Division of Plant Industry, CSIRO, Canberra, ACT. Temperature maintainence is with evaporative units, ceiling fans and fan heaters. Natural daylengths and full sunlight completed the growing conditions.

Glasshouse 2a: This 6 x 8 m glasshouse is attached to the crop adaptation building, Division of Plant Industry, CSIRO, Canberra, ACT. The glasshouse faces due N, one side due E. The temperature is regulated with evaporative coolers, hot water heaters and automatically controlled ventilators. A whitewash limits light levels to 800 umol $m^{-2}s^{-1}$ photosynthetic photon irradiance in summer.

Glasshouse 2b: (Attached to glasshouse 2a and crop adaptation building). This glasshouse is of similar dimensions to glasshouse 2a and light and temperature regulation is similar. The east wall is attached to glasshouse 2a.

Controlled temperature room: This room housed a light bank which provided a 13hr photoperiod with a photosynthetic photon irradience of 250-400 umol m^{-2} s^{-1} (depending on the age of the lights; lighting was uniform across the light bank). Temperatures under the lights were 25°C during the day and 21°C at night.

Growth cabinet: This was a Conviron unit located in the crop adaptation building. Lighting was a mixture of incandescent and fluorescent lights to 800 umol m^{-2} s^{-1} photosynthetic photon irradiance, temperature was controlled with a microprocessor $^{+}$ 0.1°C and humidity was controlled with a wet wick and fan.

2.2.2. Pressure chamber:

Establishing seedlings: When the seminal roots of the monocots were 10-20 mm long the seedings were transplanted to special pots designed to fit within a pressure chamber (Fig 2.1.). The pots contained red sand which had been washed to remove the clay and silt, and then watered (see below) with 1/4 strength nutrient solution (receipe given in section 2.2.3.). If clover was used, 5g of river loam was placed immediately below the 1 mm hole through which the tap root was introduced. All seedlings were covered to prevent water loss and allowed to establish in low light. When the emerging leaf of the monocots was about 50 mm long, or the stem supporting the dicotyledons of the clover had elongated to the same height (achieved by providing light only directly above the emerging seedling) a droplet of semi-set non-toxic silicone sealant (Sylgard 184) was used to seal the gap between the root and the top of the pot, and the shoot was threaded through the retaining metal plug illustrated. At the same time, plants were transferred to conditions providing more light. The following day,or when the Sylgard droplet had set completely, a 3 - 4 mm layer of liquid Sylgard was applied to just over the level of the retaining plug. For uniformity, tillers of the monocots were pinched back.

Watering plants: The pots held a volume of 220 ml and the base was covered with nylon gauze to allow drainage. Solution was supplied to the plants through a small hole at the top of the pots by syphoning in 65ml and the pots were drained and blotted on a towel until they held 50ml, giving a volumetric water content of 0.23. Initially the plants were watered with 1/4 strength nutrient solution, then 1/2 strength the following day, and full strength the day after. Salt was added at about day 10 for the monocots, in daily increments of 0.12 MPa Π (25 mol m^{-3} NaCl) after this. Thereafter watering occurred daily. As the clover grew much more slowly, the increase in nutrient and NaCl concentrations was more gradual and subsequent watering less frequent.

Pressurising plants: Up to six pots could be pressurised simultaneously, the top of the pot forming part of the chamber (Fig 2.1.) . Gas mix comprising of 0.1MPa air and added nitrogen was used after it was found pure air eventually killed the plants (see chapter 3). The chamber was bled continuously at a rate which would remove respired CO_2 (25 cm^3 min^{-1} for an estimated 6g of FW root tissue).

2.2.3. Solution culture:

Growth conditions: After germination at 25°C in the dark, seedlings were gradually adapted to glasshouse conditions by covering them with shade cloth for 2-3 days. The solutions were held in

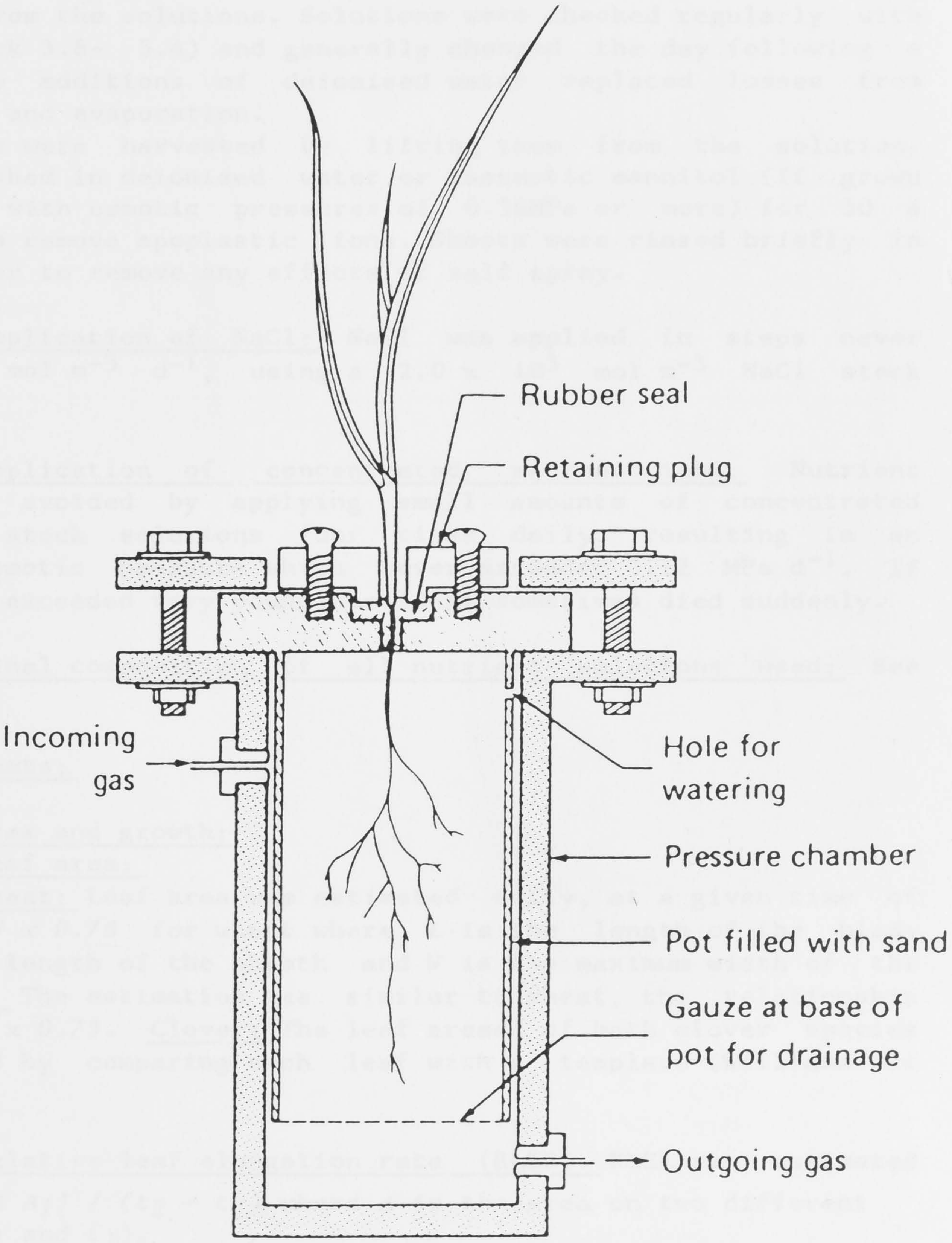

Rubber seal
Retaining plug
Incoming gas
Hole for watering
Pressure chamber
Pot filled with sand
Gauze at base of pot for drainage
Outgoing gas

Figure 2.1

Section through pressure chamber to show plant sealed to top of pot placed inside pressure chamber. Six such chambers were connected in series. A mixture of air and N_2 containing atmospheric pressures of O_2 (21 kPa) was bled continuously through the system to remove respired CO_2.

plastic bags lining 8.8 l black plastic pots which were encased within white plastic pots, and covered with a layer of 25 mm white styrofoam. Air supplied by a compressor was bubbled vigorously through the solutions using a variety of fish tank aerators, scinted glass aerators and 1 mm glass tubing lengths. Seedlings were held with a strip of plastic-covered sponge in 30 mm circular holes punched in the styrofoam tops. As each plant was harvested by lifting it out, the hole was sealed with a styrofoam plug to minimise evaporation from the solutions. Solutions were checked regularly with pH paper (Merck 3.8- 5.4) and generally changed the day following a harvest. Daily additions of deionised water replaced losses from transpiration and evaporation.

Plants were harvested by lifting them from the solution. Roots were washed in deionised water or isosmotic mannitol (If grown in solutions with osmotic pressures of 0.56MPa or more) for 30 s which aimed to remove apoplastic ions. Shoots were rinsed briefly in deionised water to remove any effects of salt spray.

Application of NaCl: NaCl was applied in steps never exceeding 25 $mol\ m^{-3}\ d^{-1}$, using a 2.0×10^3 $mol\ m^{-3}$ NaCl stock solution.

Application of concentrated macronutrient: Nutrient toxicity was avoided by applying small amounts of concentrated macronutrient stock solutions four times daily, resulting in an increase in osmotic pressure which never exceeded 0.12 MPa d^{-1}. If this rate was exceeded very young seedlings sometimes died suddenly.

Final composition of all nutrient solutions used: See table 2.1.

2.3. Measurements:

2.3.1. Leaf area and growth:

Leaf area:

Wheat: Leaf area was estimated daily, at a given time of day, as $L \times W \times 0.78$ for wheat where L is the length of the blade plus half the length of the sheath and W is the maximum width of the blade. Barley: The estimation was similar to wheat, the relationship being $L \times W \times 0.75$. Clover: The leaf areas of both clover species were estimated by comparing each leaf with a template (Williams *et al.* 1964).

Relative leaf elongation rate (RLER): RLER was estimated as $(Ln\ A_2 - Ln\ A_1) / (t_2 - t_1)$ where A is the area on two different days (i.e., t_1 and t_2).

Table 2.1 Composition of nutrient solutions

Treatment	(MPa)	NO_3^-	NH_4^+	N_{tot} (mol m^{-3})	Ca^{2+}	Mg^{2+}	K^+	SO_4^{2-}	$H_2PO_4^-$
Control	0.08	14.5	2.0	16.5	4.0	2.0	6.5	2.0	2.0
Concentrated macronutrients	0.20	35.8	4.9	40.7	9.9	4.9	16.0	4.9	4.9
	0.32	57.1	7.9	65.0	15.8	7.9	25.6	7.9	7.9
	0.56	99.7	13.8	113.5	27.5	13.8	44.7	13.8	13.8
	0.80	142.3	19.6	161.9	39.3	19.6	63.8	19.6	19.6
Modified concentrated macronutrients	0.32	63.0	13.8	76.8	15.8	7.9	25.6	7.9	2.0
	0.56	111.5	25.6	137.1	27.5	13.8	44.7	13.8	2.0
	0.80	159.9	37.2	197.1	39.3	19.6	63.8	19.6	2.0
Minimal phosphate control	0.08	16.5	2.0	18.5	4.0	2.0	6.5	2.0	trace

Micronutrients present in all solutions (mmol m^{-3}): $36Fe^{3+}$, $4.6\ BO_4^{3-}$, $0.5\ Mn^{2+}$, $0.2\ Zn^{2+}$, $0.1\ Mo_7O_{24}^{6-}$. pH was 4.8-5.1 checked daily with pH paper (Merck 3.8-5.4). NaCl was added to the "Control" and "Minimal phosphate control" solutions in the NaCl treatments.

Leaf elongation:

Ruler: Elongating leaves were measured by ruler from the cap of the pot in pressure experiments and from the styrofoam cover in experiments using solution culture. Photograph: Elongating leaves were photographed and at regular intervals with the camera maintained at a fixed position, using graph paper a background. This had the advantage in not disturbing the plants during the recording procedure, but the disadvantage that parallex errors were introduced when leaves grew in a spiralling fashion. Measurements by ruler before and after the experiment were used to correct these errors partially. A microfiche was used to interpret the developed film. LVDT: Elongating leaves were attached to a linear variable displacement transducer (LVDT, Model DCDT-500, Hewlett Packard) and the output recorded automatically at fixed intervals, using a Hewlett Packard 85 computer.

Fresh weight: Both roots and shoots were blotted quickly on a towel before being weighed in the glasshouse. If there was any delay they were placed between two pieces of moist paper hand towel and covered with plastic; this had been shown to minimise desiccation.

Dry weight: The plant material was placed in labelled envelopes and dried at 70-80°C for at least 48 hours before being weighed on a balance.

2.3.2. Transpiration:

Transpiration was estimated as the daily change in weight of the pot divided by the average leaf area over that time. Water loss was avoided from the base of the pots by covering them.

2.3.3. Components of water potential.

Leaf water potential: The selected leaf was placed in a plastic sheath, cut with a razer blade within seconds and placed quickly within a pressure chamber. Pressure was increased slowly using dry N_2 and the balancing pressure noted when the meniscus just reached the cut surface (Scholander *et al* 1965; Turner 1981.) The measurements were done by Mr M. J. Long.

Osmotic pressure: 1. Elongating tissue was dissected from plants as discribed in Termaat *et al*, 1985 and measured in a Wescor C-52 sample chamber attached to a Wecor HR-33T microvoltometer. 2. Mature (expanded) leaf tissue was frozen in dry ice, thawed, and the expressed cell sap measured either with a Wescor HR-33T microvoltometer or with a Wescor 5100 Vapour Pressure Osmometer. 3. Nutrient solutions. These were measured according to directions on the Hewlett-Packard 302 Vapour Pressure Osmometer.

Turgor: This was estimated, for mature tissue, as the sum of water potential and osmotic pressure.

2.3.4. Chemical analysis :

Dried plant material:

Na, K, Ca, Mg: Oven dried tissue was ground is a coffee grinder, digested for at least 4 hours in a 1:7:20 mix of sulphuric: perchloric: nitric acid and assayed by Mrs F. J. Taylor using atomic absorption spectroscopy. P and N: Oven dried, ground material was digested using a modified Kjeldahl procedure and assayed colorimetrically in a Technicon autoanalyser. Ammonia was determined utalising the blue colour formed by the reaction with hypochlorite and phenol (Williams and Twine 1967); phosphate using a molybdenum blue method (Twine and Williams 1971). The analysis was done by Mrs K. Saw. Cl: Oven dried, ground material was pulverised in a Ball and Puck grinder, stamped onto a boric acid block and assayed by X-ray spectroscopy (Norrish and Hutton 1977). The analysis was done by Mr A. Pinkerton.

Visual assessment of phosphate in nutrient solution: Phosphate concentration was assessed by visual comparison with standards, using a molybdenum blue method (John 1970). As little as 0.6×10^{-3} mol m^{-3} could be detected; estimates may have been within 0.3×10^{-3} mol m^{-3} of this value.

2.3.5. Measurement of root respiration:

This was done using an 0.8V Clark oxygen electrode in a 110ml root chamber. Whole roots were excised and placed in the solution saturated with air or O_2 and equilibrated to 0.25°C. Sodium dithionite was used to check the zero O_2 level (baseline).

2.4. Formulae

Uptake to the Shoot: This was estimated as

$$J_{j,s} = (M_{s,2} - M_{s,1}) / (W_{s,2} - W_{s,1}) \times RGR_s \quad ....(1)$$

where $J_{j,s}$ is the uptake of ion j to the shoot s, $M_{s,1}$ and $M_{s,2}$ the ion content of the shoot at time 1 and 2 respectively, $(W_{s,2} - W_{s,1})$ the difference in shoot dry weight during that time, and RGR_s the relative growth rate of the shoot on a dry weight (DW) basis (g g^{-1} d^{-1}), estimated as $(LnW_{s,2} - LnW_{s,1})/(t_2 - t_1)$. (Adapted from Pitman 1972.)

Transport from the root: Similarly,

$$J_{j,r} = (M_{s,2} - M_{s,1}) / (W_{r,2} - W_{r,1}) \times RGR_r \quad .....(2)$$

where $(W_{r,2} - W_{r,1})$ represents the change in dry weight of the roots and RGR_r the relative growth rate of the root on a DW basis over this period. (Adapted from Pitman 1972.)

Estimation of total solute uptake: This was estimated as

$$J_{t,s} = \hat{m} \times RGR_s^{*} \quad .....(3)$$

where $J_{t,s}$ is the total solute uptake to the shoot, $\hat{m}$ is osmolality and RGR_s^{*} is the relative growth rate of the shoot on a water basis (g H_2O g^{-1} H_2O d^{-1}). (Adapted from Greenway and Munns 1983.)

2.5. Abbreviations, definitions and units:

π osmotic pressure (MPa)
ψ water potential (MPa)
P turgor pressure, i.e. the difference in pressure across the cell wall (MPa)

MW molecular weight (D)
DW dry weight (g)
FW fresh weight (g)
RGR Relative growth rate (g g^{-1} d^{-1})
RLER Relative leaf expansion rate (cm^2 cm^{-2} d^{-1})

ABA abcisic acid
ACC 1-aminocyclopropane-1-carboxylic acid
BA benzyl adenine
GA gibberellic acid

PEG polyethylene glycol, average MW indicated by number following.

expanding, growing or elongating region: basal 10 mmol of elongating leaf in monocot

halophyte: plant native to saline soils.

hormone, plant growth substance: a chemical which, synthesised by the plant, affects metabolism at sites distant from its origin.

Chapter 3

DETERMINING THE CORRECT AIR:N_2 MIXTURE FOR MEDIUM-TERM PRESSURE EXPERIMENTS

3.1. Introduction:

In preliminary experiments, when air alone was used to apply pressure to NaCl-treated plants, the leaves yellowed prematurely. When 0.96 MPa was applied, the plants died after 4 days, but when 0.48 MPa was applied, reduced growth and transpiration rates were apparent only after about 5 days, when experiments were always terminated. The first experiment was designed to test whether the toxic effect of compressed air could be avoided by mixing N_2 with compressed air so that all the gases (other than N_2) were kept at atmospheric partial pressures.

The enhanced senescence could have been due to high partial pressures of a gas such as O_2 or CO_2, but high O_2 was considered more likely than high CO_2, because CO_2 concentrations in the soil are typically 1-2 % (Russell 1961). The second experiment tested whether respiration of roots was affected by high O_2.

3.2. Experimental design:

Experiment 1: Comparison of growth and transpiration in plants pressurised with air or gas mix:

Thirteen barley, cv. Beecher, plants were grown in pressure pots in the controlled temperature room (section 2.2.1.). Nine plants were given 100 mol m^{-3} NaCl, starting with 25 mol m^{-3} day 9 after germination (Section 2.2.3.); the remainder given nutrient solution only. On day 12 after germination 3 NaCl-treated plants (at 100 mol m^{-3}) were left unpressurised, 3 were pressurised with 0.48 MPa air and 3 pressurised with a 1:5 mixture of air:N_2. Plants were distributed randomly in the system of pressure chambers or beside it, and pots were rotated daily. Leaf areas (section 2.3.1.) and transpiration rates (section 2.3.2.) were estimated daily.

Experiment 2: Root respiration under high O_2 tensions:

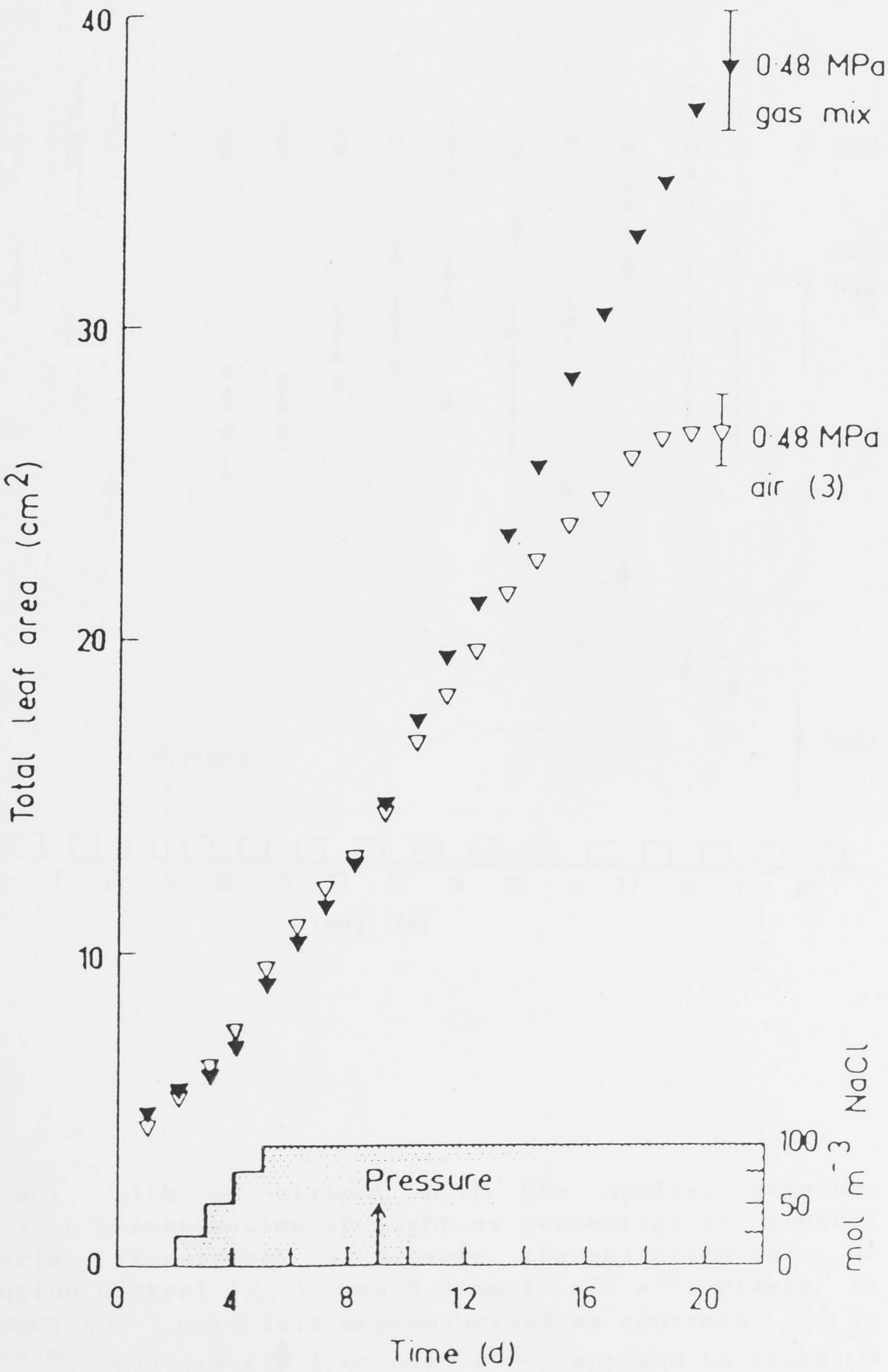
Barley, cv. Beecher, plants were grown in solution culture in two plastic containers in the controlled temperature room. One group was given 100 mol m^{-3} NaCl starting with 25 mol m^{-3} day 6 after germination. The solutions were changed at weekly intervals and distilled water was added daily to replace losses. On day 18 after germination, when the NaCl-treated plants were about half the size of the control plants and the lower leaves were senescing, the plants were taken to the Department of Environmental Biology, Research School of Biological Sciences, A.N.U., where root respiration was measured (section 2.3.5.) in the solutions in which the roots had grown, saturated with air (21 kPa O_2) or pure oxygen (100 kPa O_2).

3.3. Results:

Decreases in leaf elongation and transpiration rates preceded the death of NaCl-treated plants pressurised with air (Fig 3.1., 3.2.). Leaf areas were significantly reduced after 5 days of treatment, and within 11 days these plants were dead (Fig 3.1.). Decreases in transpiration rate occurred concomitantly (Fig 3.2.). NaCl-treated plants pressurised with air:N_2 mixture had the same relative leaf expansion rate as NaCl-treated controls (Table 3.1.).

Figure 3.1.

Effect of 0.48 MPa applied pressure (indicated ↑) on leaf expansion of NaCl-treated Beecher barley (Experiment 1). Plants in 100 mol m^{-3} NaCl had 1:5 Air:N_2 mixture (▼), or Air (▽) applied to roots on d 9. Error bars indicate average S.E. of the mean for each group (3 plants per treatment). Incremental increases in NaCl indicated as bar graph, x axis. Measurements started d 8 after germination.



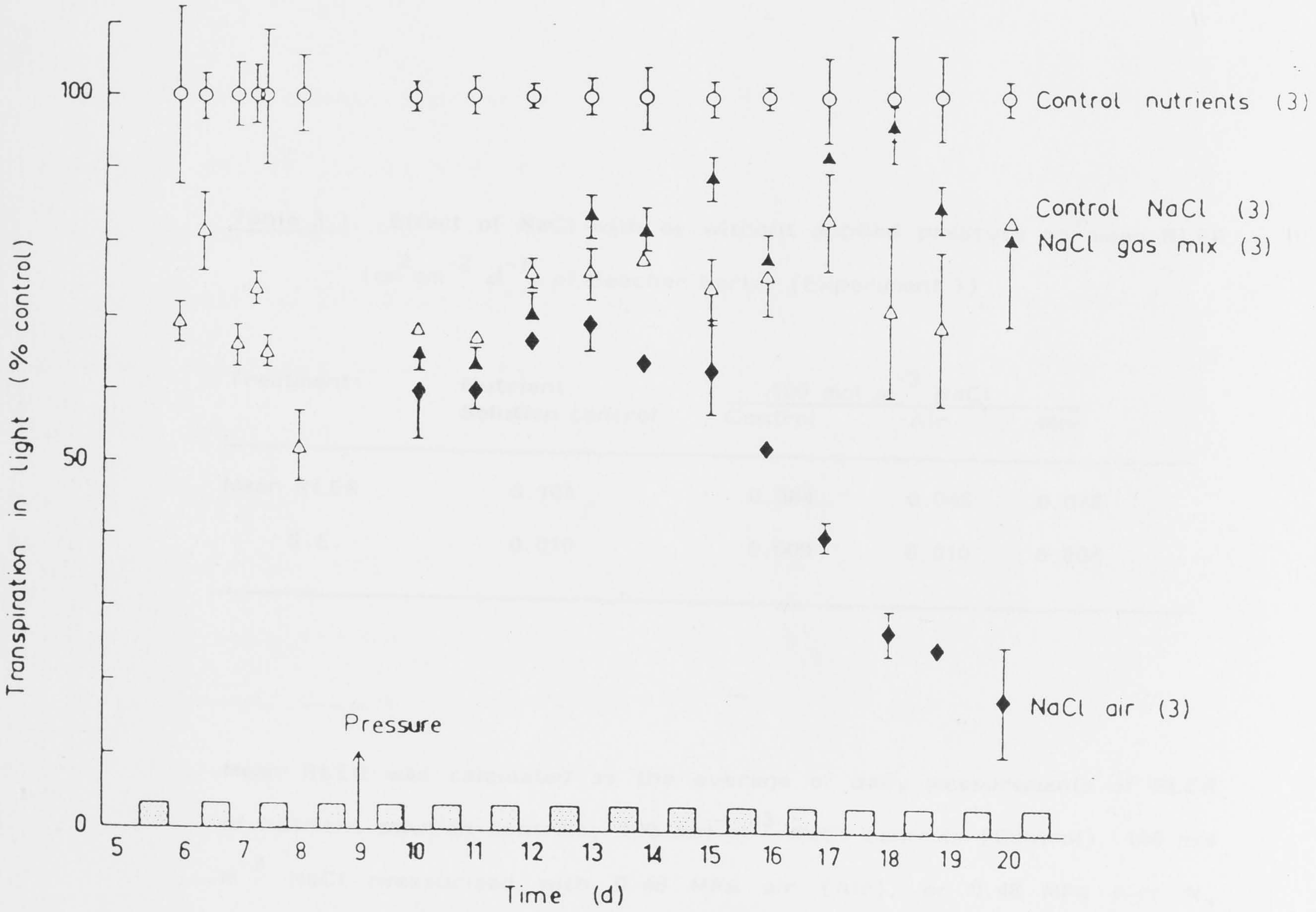


Figure 3.2.

Effect of NaCl, with or without 0.48 MPa applied pressure (indicated ↑) on transpiration in light as percentage of control of Beecher barley (Experiment 1). Average transpiration rate of Nutrient solution control (○) was 3.3 mmol m^{-2} s^{-1}. Plants in 100 mol m^{-3} NaCl (△) were left unpressurised as controls (△), or had 1:5 Air:N_2 mixture (◆) or Air (▲) applied to roots d 9. Error bars indicate average S.E. of the mean for each group (3-4 plants per treatment). Dark periods indicated by bars, x axis. Measurements started d 13 after germination.

Table 3.1. Effect of NaCl with or without applied pressure on mean RLER ($cm^2 cm^{-2} d^{-1}$) of Beecher barley (Experiment 1)

Treatment:	Nutrient Solution control	100 mol m^{-3} NaCl		
		Control	Air	Mix
Mean RLER	0.101	0.084	0.046	0.078
S.E.	0.010	0.009	0.010	0.008

Mean RLER was calculated as the average of daily measurements of RLER of nutrient solution controls, 100 mol m^{-3} NaCl controls (Control), 100 mol m^{-3} NaCl pressurised with 0.48 MPa air (Air), or 0.48 MPa Air: N_2 mixture (Mix). S.E. was calculated for 3-4 plants/treatment. Measurements started d1 after pressure was first applied, i.e. d17 after germination.

The apparent slightly higher transpiration rate of of the latter compared to NaCl-treated controls was due to the difficulty in minimising evaporation from the bottom of the pots in the pressure chamber (Experiment 1).

Table 3.2. shows that there was a 50% increase in the rate of respiration under high O_2 tensions in both NaCl-treated and control plants (Experiment 2).

3.4. Discussion:

NaCl-treated plants pressurised with 0.48 MPa air were exposed to absolute partial pressures of O_2 of 122 kPa (i.e., (0.48 MPa applied pressure + 0.1 MPa atmospheric pressure) x 21 kPa O_2/0.1 MPa air). Early work by Erickson (1947) suggested that plants were grown in nutrient solutions aerated with 90-100 kPa oxygen also failed to thrive. The complication of microorganisms in the nutrient solutions, reported by Erickson (1947), seemed less likely here as the roots of dying plants that had been pressurised with air looked normal whenever they were examined.

If the enhanced rate of respiration of the root in high O_2, as observed in the second experiment, had been sustained over many days, it may, by imposing an increased demand for carbohydrate from the shoot, itself have contributed to the premature senescence of the leaves. However, at the 5-6 leaf stage, NaCl-treated plants have a root:shoot ratio of 0.3 (data not shown for pressure pot experiments). Therefore, a 50% increase in respiration of the root represents an increased demand of about 15% (i.e., 50 x 0.3) on the shoot. As this must be a small percentage of net photosynthate, it would be surprising if the increased respiratory demand killed the shoot directly. Theologis and Laties (1982), working with plant

Table 3.2. Effect of air or O_2 on root respiration (μ mol O_2 h^{-1} g^{-1} DW) of Beecher barley grown in control nutrients or 100 mol m^{-3} NaCl (Experiment 2).

mol m^{-3} NaCl:	0	100
kPa O_2		
21	2.23 ± 0.23	2.45 ± 0.12
100	3.93 ± 0.38	3.71 ± 0.28

Root respiration was measured in solution, saturated with air or O_2, using an O_2 electrode. Values are the mean and S.E. of 3-4 plants/treatment. Measurements were made d18 after germination.

storage organs, suggest the enhanced respiration does not result directly from high O_2, but from the effect O_2 has on increasing the effectiveness of the hormone ethylene (which was applied in their experiments). In some root tissues (carrot, sweet potato) the respiratory increase was 4-5 fold, persisting for the duration of the treatment (3 days). While the routine bleeding of the pressure chambers (to remove respired CO_2) would have prevented a buildup of the ethylene produced by the roots, the *in situ* effects of any ethylene produced may well have been magnified by the high partial pressures of O_2. This may have been a second, indirect factor contributing to the death of the plants pressurised with air (e.g., by inhibiting stomatal development, Reid and Wample, 1985).

Neither of these explanations was tested as the practical problem associated with pressurizing NaCl-treated plants was considered solved.

3.5. Conclusion:

Compressed air reduced transpiration and growth rates in the shoots of plants whose roots were pressured with 0.48 MPa, after 11 days the plants were dead. This could be avoided by mixing air with N_2 so that all gases (other than N_2) remained at atmospheric levels during pressurization.

Aeration with pure O_2 caused a 50% increase in the root respiration rates of plants grown in solution culture. To sustain such rates, 15% more carbohydrate must be supplied from the shoot. Additionally, high O_2 may magnify hormonal effects such as ethylene.

Chapter 4

EFFECT OF APPLIED PRESSURE ON SHOOT GROWTH AND TRANSPIRATION

4.1. Introduction:

Many experiments suggest that adverse water relations is a major factor limiting growth in salt-treated plants (eg see Chapter 1). For instance, the rapid recovery of leaf elongation rates to control levels following the sudden removal of NaCl from around the roots (e.g., Munns *et al.* 1982; Rawson and Munns 1984) suggests that it is turgor rather than the presence of NaCl in the tissues which is limiting growth. Direct measurement of turgor would not in itself cast light on this problem; NaCl-treated and plants grown in a dry soil may have leaf turgor pressures at least as high as controls (e.g., Matsuda and Riazi 1981, Ehlig *et al.* 1968, Shalhevet *et al.*, 1978). A high turgor could be simply the result of a reduced growth rate, causing accumulation of solutes without a change in uptake rates. To maintain growth rates as well as generate turgor when the soil ψ is lowered, additional solutes are needed. It is possible that increased uptake of Na^+ and Cl^- could supply these solutes, at least in certain species; halophytes. But in crop plants, two lines of evidence suggest this is unlikely. Firstly, Na^+ and Cl^- make up only about 35% of π in the expanding tissue (e.g., barley, Delane *et al.* 1982). Secondly, calculations of uptake rates of Na^+ and Cl^- indicate rates do not increase with increasing external NaCl above approximately 25 mol m^{-3} NaCl (Delane *et al.* 1982, Munns 1985). Thus other inorganic (e.g., K^+) or organic osmotica are needed, and organic osmotica cannot be supplied without the cost of reduced growth. By supplying turgor artificiallly, the pressure system would eliminate that cost by eliminating the need for the shoot to generate its own turgor. Although the turgor of the roots would not be raised (Passioura and Munns 1984), if turgor was regulated by a metabolic system, the raising of turgor of the shoot should have freed organic substrates for the root. The medium-term (days) experiments aimed to test the hypothesis that, if the osmotic effects of NaCl on the shoot were countered, the growth rate of these plants would improve?

Short-term (minutes, hours) responses to changes in shoot water status are discussed in Section 1.2.1. The short-term experiments in this chapter aimed to describe the immediate effects of applying and removing various amounts of pressure using the pressure system, therefore enabling any short-term responses to be avoided in calculations.

3.2. Experimental design:

Medium-term growth experiments: Each experiment consisted of three treatments: control plants grown in nutrient solution, and two groups of salt-treated plants where the roots of one group were pressurised with sufficient pneumatic pressure to counter the osmotic effects of the salt. The osmotic pressure of the salt treatment varied according to the sensitivity of the species used and the type of salt (i.e., NaCl or concentrated macronutrients) in which the plants were grown, but the pressurised plants were always supplied with a mix of air:N_2 which maintained the partial pressure of oxygen at 21kPa (see Chapter 4). Barley (cv. Beecher and cv. Clipper), wheat (cv. Kite), and *Trifolium alexandrinum* were all grown in 100 mol m^{-3} NaCl in the nutrient medium (see section 2.2.3.). *T. repens* was grown in 50 mol m^{-3} NaCl. The technique was repeated with barley (cv. Clipper) and wheat using concentrated macronutrients with osmotic pressures of 0.56 and 0.80 MPa repectively. The number of replicates varied, but up to six plants could be pressurised simultaneously in a system of six connected pressure chambers (see Fig 2.1.). Pressure was always applied within a few days of the final salt concentration being reached, and was released for about an hour each day during which the pots were weighed and watered. The treatments continued for 7-10 days.

There was no fourth treatment comprising pressurised, control plants without NaCl as this would result in a large positive water potential in the soil medium which could have induced guttation from the shoot together with substantial infiltration by water of the intercellular spaces of the leaves. The ability to induce guttation artificially with the pressure system was to be exploited in later experiments (see Chapter 5). These experiments were conducted in various growth environments detailed in Table 4.1 of the results and section 2.2.1.

Osmotic pressures of expanded tissue and the expanding regions (i.e., basal 10 mm of expanding leaf) were measured (Section 2.3.3.) on wheat, after being grown for a week with applied pressure.

Short-term growth experiments: Barley plants of uniform size, grown in 0, 50 or 100 mol m-3 NaCl in nutrient solution were pressurised with 0.10, 0.25 or 0.50 MPa air each day, from 11.30 to 15.30 h Eastern Standard Time (EST). Air, rather than a gas mixture, was used as this was a short-term experiment. In the short-term, there was no difference in plant response to compressed air or N_2:air mixtures (J. Passioura and R. Munns, personal communication). The highest pressure applied depended on the water potential in which the plants were growing. The elongation of leaf 2 was recorded photographically (Section 2.3.1.); a ruler was used to calibrate, if necessary, the values obtained. The experiments were conducted in the controlled temperature room (section 2.2.1.).

3.3. Results:

Medium-term experiments: Each salt treatment gave a fall in RLER of about 15-20% in each species, which reduced the final total leaf area to about 70% of controls by the end of the treatment period. The applied pressure made no difference to the growth of any species, whether it was wheat grown in a controlled temperature room (Fig 4.1a), a growth cabinet (Fig 4.1c) or white clover grown in the fluctuating heat and light environment of a glasshouse (Fig 4.1b). Table 4.1 summarises the results for all 4 species and the 2 kinds of salt treatment (i.e., NaCl and concentrated macronutrient treatments). RLER was calculated from two sucessive measurements, and values were then averaged for each plant over the treatment period. All species were measured daily except white clover, which was measured on alternate days.

Leaf elongation rates, averaged for each leaf which emerged during the period monocots were treated, showed similar reductions in growth associated with NaCl treatments (Table 4.2.). This measurement, however, is more sensitive than RLER as an index of short-term growth response; elongation rates varied over the diurnal period and differed in different treatments (Fig 4.2.) Control plants grew more quickly by day than by night, concentrated macronutrient-treated plants grew more quickly or at the same rate during the night (Fig 4.2.). NaCl-treated plants were not measured.

Average daily transpiration, likewise, was unaltered by applied pressure. Transpiration rates obtained in the controlled temperature room were depressed more by the salt treatment than leaf expansion;

Figure 4.1.

Effect of NaCl or concentrated macronutrients, with or without applied pressure, on leaf expansion on wheat or clover (Medium term experiments).

(a) Effect of NaCl, with or without 0.48 MPa applied pressure (indicated ↑) on leaf expansion of wheat grown in the controlled temperature room. Nutrient solution controls (○), 100 mol m^{-3} NaCl-treated control (△) and 100 mol m^{-3} NaCl with pressure applied to roots d 4 (▲). Incremental increases in NaCl indicated as bar graph, x axis. Measurements started d 10 after germination.

(b) Effect of NaCl, with or without 0.24 MPa applied pressure (indicated ↑) on leaf expansion of white clover grown in glasshouse 2a. Nutrient solution controls (○), 50 mol m^{-3} NaCl-treated control (△) and 50 mol m^{-3} NaCl with pressure applied to roots d 12 (▲). Incremental increases in NaCl indicated as bar graph, x axis. "*" indicates when NaCl-treated plants were significantly (P = 0.05) smaller than controls. Measurements started d 21 after germination.

(c) Effect of concentrated macronutrients with or without 0.72 MPa applied pressure (indicated ↑) on leaf expansion of wheat grown in Conviron cabinet. Nutrient solution controls, 0.08 MPa π (○), concentrated macronutrient-treated control, 0.80 MPa π (△) and concentrated macronutrients, 0.80 MPa π with pressure applied to roots d 14 (▲). Incremental increases in external π indicated as bar graph, x axis. Measurements started d 12 after germination.

Error bars indicate average S.E. of the mean for each group of plants. Nos. of plants in each treatment shown in brackets. Pressure applied was a mixture of compressed air and N_2 which contained no more than 21 kPa O_2.

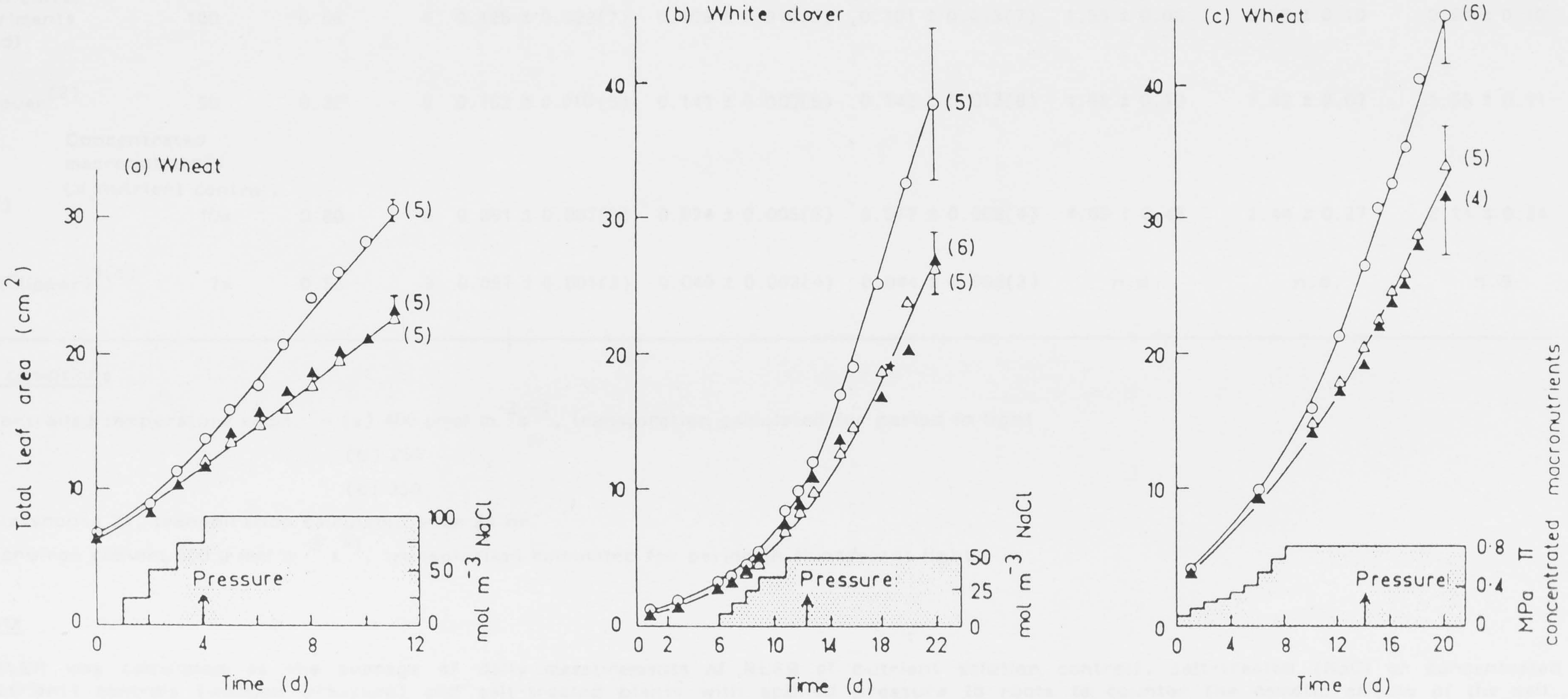

(a) Wheat
Total leaf area (cm^2)
Time (d)
mol m^{-3} NaCl
Pressure
(5)
(5)
(5)
0
10
20
30
0
4
8
12
0
50
100
(b) White clover
Time (d)
mol m^{-3} NaCl
Pressure
(5)
(6)
(5)
0
10
20
30
40
0
2
6
10
14
18
22
0
25
50
(c) Wheat
Time (d)
MPa π concentrated macronutrients
Pressure
(6)
(5)
(4)
0
10
20
30
40
0
4
8
12
16
20
0
0·4
0·8

Table 4.1 Effect of NaCl or concentrated macronutrients with or without applied pressure (to counter the osmotic effects of the salt) on mean RLER ($cm^2 cm^{-2} d^{-1}$) and transpiration ($m\ mol\ m^{-2} d^{-1}$). (Medium term experiments).

Species	mol m^{-3} [NaCl]	MPaΠ	Time (d)	Mean RLER ± S.E. (No. plants) per treatment			Mean transpiration rates ± S.E.		
				Nutrient Solution Control	Salt	Salt and Pressure	Nutrient Solution Control	Salt	Salt and Pressure
Wheat $^{1(a)}$	100	0.56	6	0.019 ± 0.005(5)	0.086 ± 0.005(5)	0.082 ± 0.007(5)	6.69 ± 0.21	4.51 ± 0.12	4.45 ± 0.12
Barley (Beecher) $^{1(b)}$	100	0.56	6	0.101 ± 0.010(4)	0.084 ± 0.009(3)	0.078 ± 0.008(3)	3.32 ± 0.23	2.82 ± 0.20	2.91 ± 0.14
Barley (Clipper) $^{1(b)}$	100	0.56	7	0.090 ± 0.011(3)	0.076 ± 0.004(5)	0.076 ± 0.005(3)	5.88 ± 0.36	2.95 ± 0.27	2.98 ± 0.22
Egyptian clover $^{(2)}$ (2 experiments combined)	100	0.56	6	0.125 ± 0.022(7)	0.109 ± 0.019(8)	0.101 ± 0.013(7)	1.55 ± 0.06	0.98 ± 0.10	0.94 ± 0.10
White clover $^{(2)}$	50	0.32	9	0.163 ± 0.010(5)	0.141 ± 0.007(5)	0.142 ± 0.013(6)	1.68 ± 0.10	1.42 ± 0.07	1.55 ± 0.11
	Concentrated macronutrients (x nutrient control)								
Wheat $^{(3)}$	10x	0.80	6	0.091 ± 0.007(6)	0.074 ± 0.005(5)	0.077 ± 0.009(4)	4.05 ± 0.48	2.44 ± 0.27	2.14 ± 0.34
Barley (Clipper) $^{1(c)}$	7x	0.56	8	0.057 ± 0.001(2)	0.049 ± 0.002(4)	0.044 ± 0.002(3)	n.d.	n.d.	n.d.

Growth conditions

1. Controlled temperature room. ~ (a) 400 μmol $m^{-2} s^{-1}$, transpiration calculated for period in light
   (b) 250
   (c) 350
2. Glasshouse 2a, transpiration calculated over 24 hr.
3. Conviron cabinet, 800 μ mol $m^{-2} s^{-1}$, transpiration calculated for period in fluorescent light.

Comments

Mean RLER was calculated as the average of daily measurements of RLER of nutrient solution controls, salt-treated (NaCl or concentrated macronutrient) controls (without pressure) and salt-treated plants with applied pressure to roots to counter the osmotic effects of the salt. S.E. was calculated for the numbers of plants (bracketed) in each treatment. Measurements started d l after pressure was first applied to avoid short-term responses. Mean transpiration rates were calculated in a similar way. Pressure applied was a mixture of compressed air and N_2 which contained no more than 21 KPa O_2.

Table 4.2. Effect of 100 mol m^{-3} NaCl with or without applied presure on mean elongation rate (mm d^{-1}) of the emerging leaf (medium-term experiments).

Species	Time (d)	Nutrient solution control	100 mol m^{-3} NaCl	
			Control	+ 0.48 MPa
Wheat	6	42 ± 1(5)	34 ± 2(5)	32 ± 5(5)
Barley (cv. Beecher)	6	38 ± 1(3)	23 ± 2(2)	25 ± 1(3)
(cv. Clipper)	7	35 ± 2(3)	29 ± 2(5)	28 ± 1(3)

Mean elongation rate was calculated as the average of the maximum rates leaves 4-6 attained 2-5 days after emergence, S.E. was calculated for all the plants/treatment. Nos. of plants/treatment shown in brackets. Measurements started d1 after presure was first applied to avoid short-term responses. Pressure applied was a mixture of compressed air and N_2 which contained no more than 21 KPa O_2.

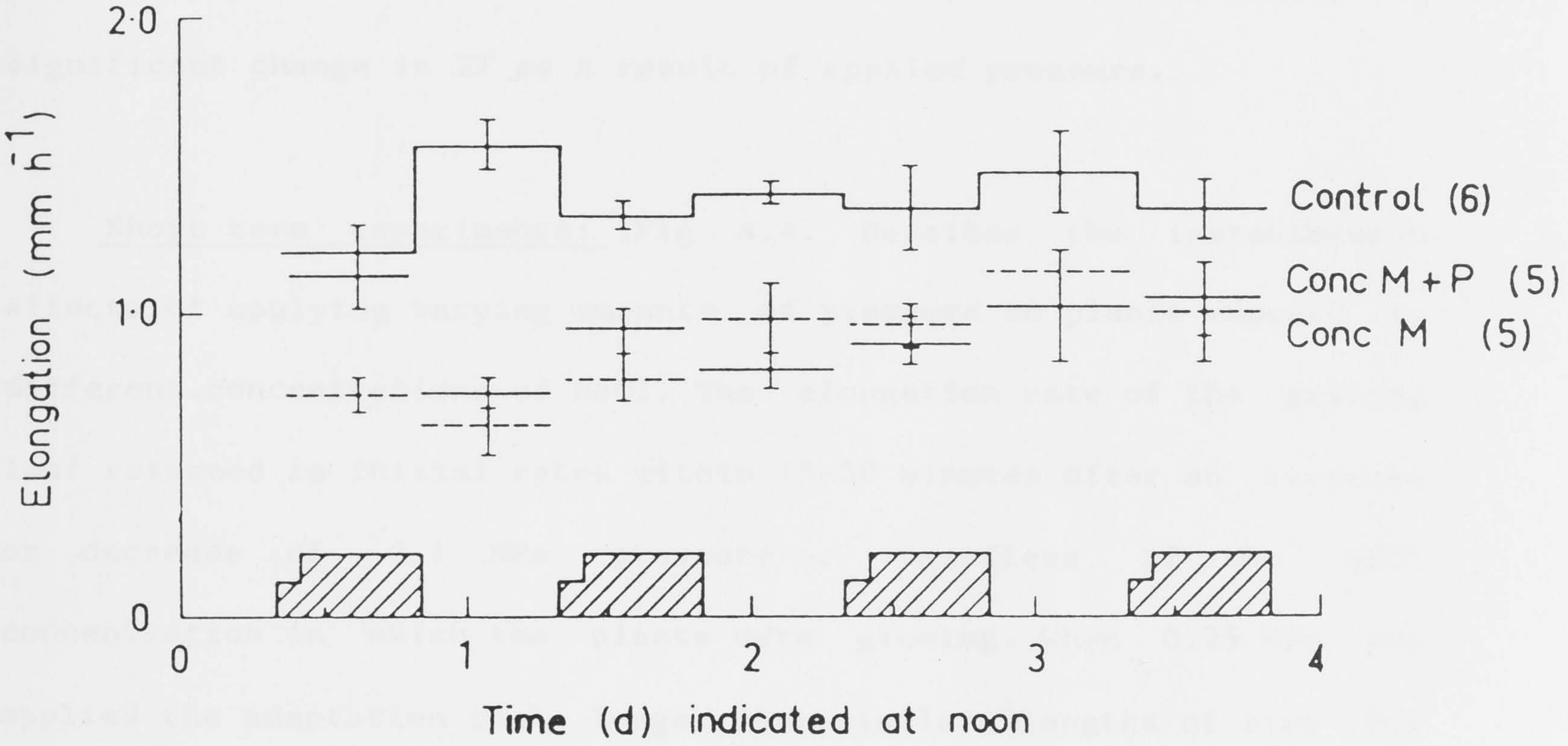


Figure 4.2.

Effect of concentrated macronutrients, with or without 0.72 MPa applied pressure, on leaf elongation rates of wheat (medium-term experiment). Elongation rates (mm h^{-1}) of nutrient solution controls, 0.08 MPa π (continuous line), concentrated macronutrient-treated control, 0.80 MPa π (discontinuous dashed line), and concentrated macronutrients, 0.80 MPa π, with pressure applied to roots d 24 after germination (discontinuous line). Dark periods include 2 h incandescent lighting after flourescent lights were switched off, indicated as bar graph, x axis. The temperature was 18°C during the dark period, 21°C during the light. Bars indicate S.E. of the mean of each measurement for each group of plants. Nos. of plants in each treatment shown in brackets. Measurements began d 27 after germination, i.e., d 2 after pressure was applied.

e.g., 32% and 44% for wheat and barley respectively (Table 4.1.). Whenever measured, dark transpiration was found to be similar, and low. Glasshouse-grown plants (*T. alexandrinum* and *T. repens*) transpired less per unit leaf area than those in controlled conditions, and variation between plants masked differences between treatments, although the same trends occurred.

The osmotic pressures (Π) of the expressed sap from fully expanded leaves of wheat were measured at the end of the experimental period. Π of the NaCl-treated plants were higher than the controls', and unaffected by the applied pressure (Fig 4.3.). The expanding tissue that was dissected from the base of the shoot also showed no significant change in Π as a result of applied pressure.

Short term experiments: Fig 4.4. descibes the instantaneous effects of applying varying amounts of pressure to plants exposed to different concentrations of NaCl. The elongation rate of the growing leaf returned to initial rates within 15-30 minutes after an increase or decrease of 0.1 MPa pressure , regardless of the NaCl concentration in which the plants were growing. When 0.25 MPa was applied the adaptation took longer, but similar lengths of time for plants grown in 50 or 100 mol m^{-3} NaCl. When 0.50 MPa was applied (plants in 100 mol m^{-3} only), enhanced rates of elongation were sustained for up to 4 hours afterwards, long after the initial (presumably, largely elastic) response decayed.

3.4. Discussion:

Medium-term experiments: The above results indicate that applied pressure did not have a lasting effect on the growth rate of leaves of barley, wheat, Egyptian clover and white clover, even though the

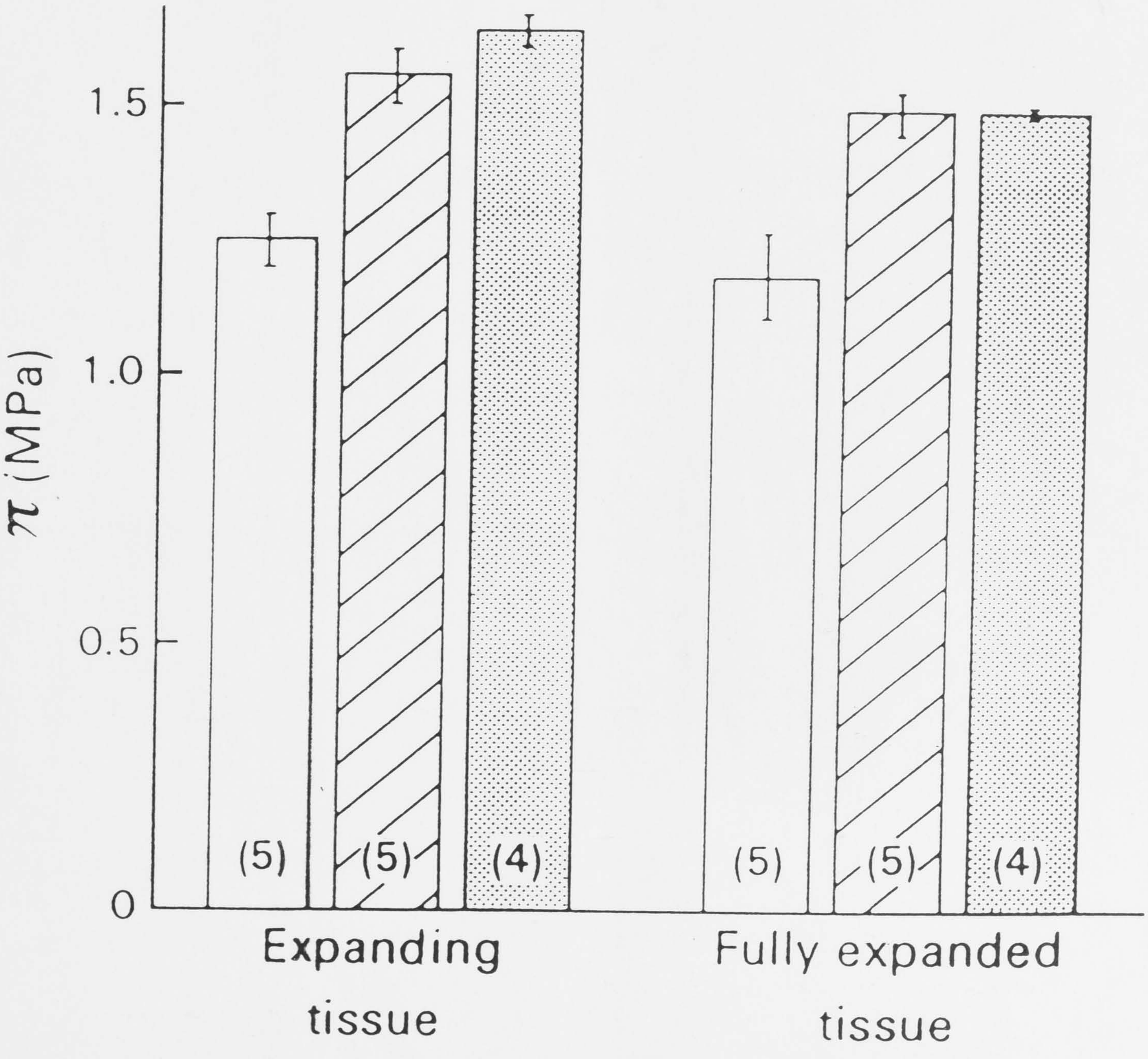
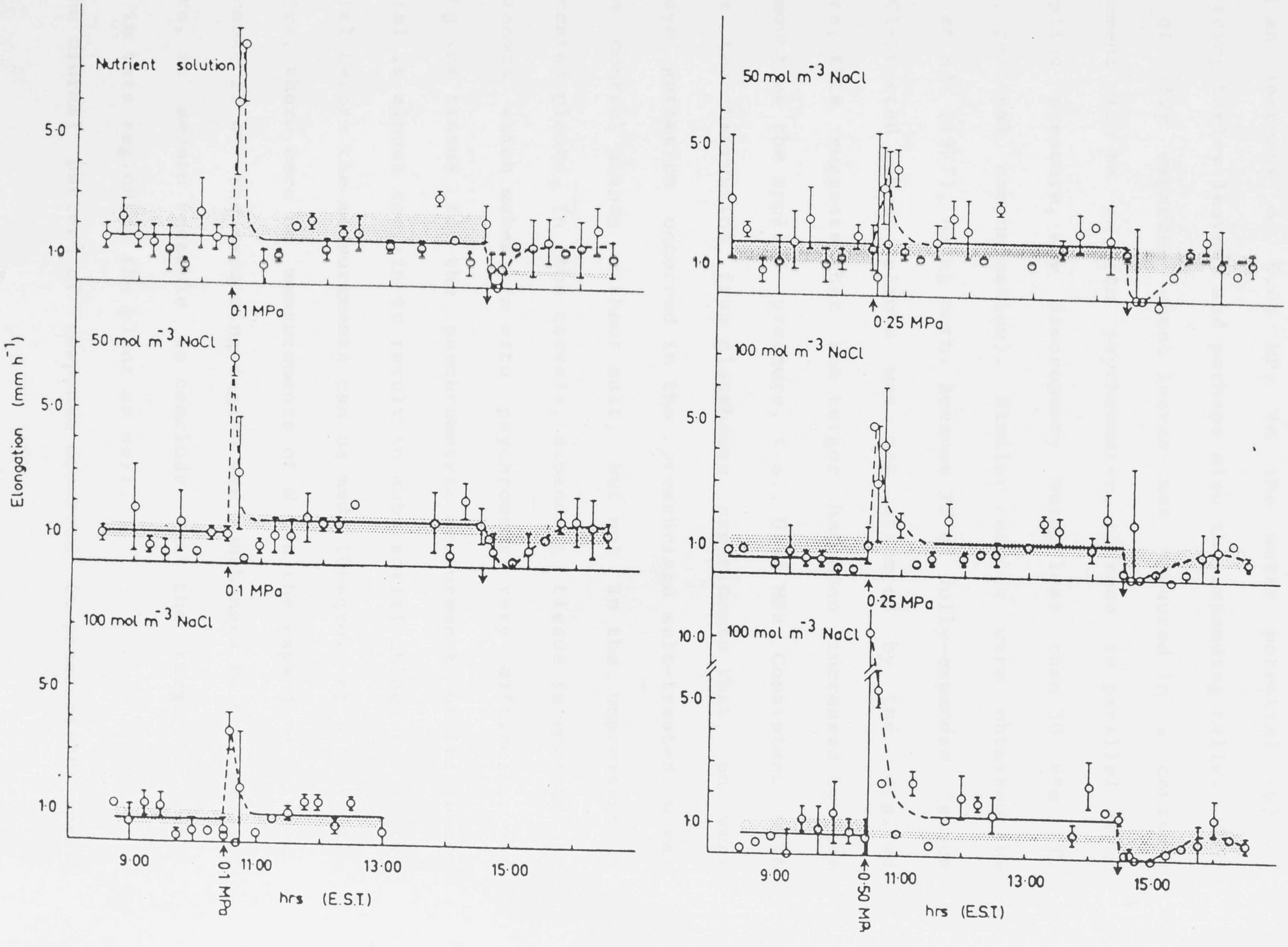


Figure 4.3.

Effect of NaCl, with and without 0.48 MPa applied pressure, on osmotic pressures of expressed sap from expanding and fully expanded tissue in wheat, after 7 days of treatment (medium-term experiment). Nutrient solution controls (□), 100 mol m^{-3} NaCl-treated control (▨), and 100 mol^{-3} NaCl with pressure applied to the roots d 16 after germination (░). Bars indicate S.E. of the mean for each treatment. Nos. of plants in each group shown in brackets. Measurements were made d 23 after germination.

Figure 4.4.

Effect of NaCl and applied pressure (indicated ↑) and removed pressure (indicated ↓) on elongation rate of leaf 2, barley, cv. Beecher (Short-term experiments). Elongation rate (mm h^{-1}) of nutrient solution control or NaCl-treated control; shaded band (▨), width indicates S.E. of mean readings (1 plant). Elongation rates of nutrient solution or NaCl-treated plants with 0.1, 0.25 or 0.50 MPa pressure (Air) applied to roots; (○), error bars indicate S.E. of the mean of each measurement (2 plants). Solid line shows the average rate for all the readings of pressure-treated plants between the points it links, while dashed lines are drawn, fitted by eye. Measurements were made d 10-15 after germination; i.e., d 1-2 after 50 mol m^{-3} NaCl treatment began, and d 1-3 after 100 mol m^{-3} NaCl treatment began.


Elongation (mm h^{-1})
Nutrient solution
5·0
1·0
0·1 MPa
50 mol m^{-3} NaCl
5·0
1·0
0·1 MPa
100 mol m^{-3} NaCl
5·0
1·0
9:00
0·1 MPa
11:00
13:00
15:00
hrs (E.S.T.)
50 mol m^{-3} NaCl
5·0
1·0
0·25 MPa
100 mol m^{-3} NaCl
5·0
1·0
0·25 MPa
100 mol m^{-3} NaCl
10·0
5·0
1·0
9:00
0·50 MPa
11:00
13:00
15:00
hrs (E.S.T.)

turgor of the leaves would presumably have been increased. There is much evidence to suggest that the applied pressure of 0.24 or 0.48 MPa increased the turgor of the cells by an equal amount. There was no effect on transpiration of any of the species, including barley. Munns and Passioura (1984a) have shown that exposing the roots of barley to NaCl had no effect on the overall hydraulic conductance of the plants, so the applied pressure of 0.48 MPa would presumably have caused an increase of 0.48 MPa in the water potential (ψ) of transpiring barley leaves, and perhaps also the expanding cells. When the ψ of fully expanded wheat leaves was measured in a constant environment with an *in situ* psychrometer, it rose in parallel with the applied pressure, any discrepancy being less than 30 kPa (T. Gollan, personal communication). Similar results were obtained by Nulsen *et al.* (1977), using corn. Because π of fully-expanded leaves in NaCl-treated wheat plants was not affected by the applied pressure, this suggests that the turgor had been increased by the same amount as the applied pressure, i.e., 0.48 MPa. Consistant with this is the observation from glasshouse experiments that, on very dull days, guttation occurred in the pressurised salt-treated wheat and the control plants without salt, but not in the unpressurised NaCl-treated plants. In the cereals, expanding tissue is encased in older leaves which makes *in situ* psychrometry very difficult, and excising the tissue for the psychrometric measurement of its water potential is almost certain to result in substantial changes in water potential before the measurements can be made (Cosgrove *et al.* 1984). Therefore, there were no measurements of ψ on the expanding tissue, but because π of the expanding region was unchanged by the applied pressure, it seemed feasable to conclude that the turgor had been raised in this region of the plant as well.

The diurnal pattern of response observed in pressurised wheat grown in 0.80 MPa concentrated macronutrients is consistent with the pattern observed in RLER of NaCl-treated sunflower (Rawson and Munns 1984). At night RLER of control sunflower plants was reduced to 60% of daytime rates, but NaCl-treated sunflower (50 and 100 mol m^{-3}) grew at constant rates throughout the diurnal period. At night, RLER of 50 mol m^{-3} NaCl-treated sunflower was 10% higher than control rates, and 100 mol m^{-3} NaCl-treated sunflower grew at rates similar to controls. This suggested "salt influences the rate of utalisation of assimilates independantly of their production" (Rawson and Munns 1984). Applied pressure did not change this relationship.

Neither turgor nor the roots' ionic status were changed by applied pressure (Passioura and Munns 1984). The experiments which showed rapid recovery of growth when NaCl was removed from the root environment (Munns *et al.* 1982, Rawson and Munns 1984) changed both the ionic content and water relations of the root. This suggested that a message, most likely a hormone associated with the root water status (discussed in Sections 1.2.2. and 7.2.), was regulating the growth of the shoot.

Short-term experiments The instantaneous pattern of response by the growing barley leaf to applied pressure is consistant with the desciption Green *et al.* (1971) gave of *Nitella*. Green *et al.* (1971) descibed the instantaneous response to turgor as:

$$r = (P - Y)\, m$$

where r is growth rate, P is turgor, Y is threshold turgor, and m is yeilding tendency. Their data indicated that after P was artificially increased, a wall-hardening process caused an increase in Y so that r returned to the initial rate, an explanation that would fit our data. This suggests that turgor pressure does not control the rate of cell growth, and that exposure to NaCl affects some other process that controls the rate of cell growth. When the artificial increase in turgor exceeded 0.10 MPa, it took longer to recover the initial elongation rate when the pressure was increased (Fig 4.4.), a pattern consistant with that found by Acevedo *et al.* (1971). But the converse was not true: the recovery times from a decrease in turgor of 0.50 was no more than for a decrease of 0.25 MPa (Fig 4.4.) This indicates that wall loosening is more dependent on turgor than wall hardening; evidently different processes are involved.

3.5. Conclusion:

NaCl-treated wheat, barley, Egyptian clover and white clover were grown for medium lengths of time (6-10 days) with their roots in pressure chambers, and sufficient pneumatic pressure was applied to counter the osmotic effects of the salt on the shoot. The experiment was repeated with barley and wheat grown in an NaCl-free saline solution of concentrated macronutrients. The applied pressure had no sustained effects on RLER or transpiration rates in any of the species, nor were diurnal patterns in leaf elongation rates of wheat grown in 0.80 MPa concentrated macronutrients altered. Because the osmotic pressures of the cell sap, in either fully expanded or currently expanding leaf tissue of wheat grown in NaCl, were unchanged, this suggested the applied pressure correspondingly increased turgor in the shoot although this was not directly measured. Therefore, turgor does not limit the shoot growth of salt-treated plants.

Short-term (hours) responses of barley to increased turgor (applied pressure) resembled published descriptions, with a transient burst of growth followed by increased elongation rates, which decayed gradually.

Chapter 5

DOES KINETIN IMPROVE THE GROWTH OF NaCl-TREATED WHEAT AT HIGH WATER STATUS?

5.1. Introduction:

Previous work implied a message from the root was regulating shoot growth in NaCl-treated plants (Chapter 4). Cytokinins, which are made in the roots and transported upwards (Van Staden and Davey 1979), may have a role in integrating root and shoot growth in water-stressed plants (reviewed by Aspinall 1980 ; Bradford and Hsiao 1982). There are indications that the production of cytokinins is affected by salinity and that low levels persist in the shoot (presumably reflecting transport upwards from the root) for the duration of long-term salinity treatments (e.g.,Itai *et al.* 1968, Walker and Dumbroff 1981). Furthermore, in one instance, applied benzyl adenine (BA), a synthetic cytokinin, was shown to benefit NaCl-treated bean plants more than controls (beans, O'Leary and Prisco 1970). Other growth regulators, such as ABA, GA and ethylene are less likely to be the message from the root. Both ABA and GA arise predominently in the shoot, and their production anywhere in the plant seems to be dependent on cell water status (Chapter 1). Pressurised plants, by having a high shoot water status, could therefore be expected to have low ABA and high GA concentrations in the shoot, i.e., hormonal patterns likely to be associated with high soil Ψ. Increases in levels of ACC (the precursor of ethylene) are associated with anaerobiosis and care was taken to maintain O_2 levels at 21 kPa while removing respired CO_2 from the pressure system (Chapter 3). More general information about the involvement of plant growth substances in the responses of NaCl-treated plants is provided in Chapter 1.

Two experimental approaches dominate research into the role of plant growth substances in the control of growth: (i) the actual measurement of hormone levels in plants treated with low external ψ compared to controls, and (ii) the application of hormones to plants treated with low external ψ in an attempt to improve their condition. There are many problems with the first method. Failure to distinguish between "bound" (or sequestered) and "active" forms of a hormone (King 1976) and the length of time needed to collect some types of samples (e.g., root exudates, Itai and Vaadia 1971) may mean the assay does not reflect the biological activity of the hormone at the time of treatment. Many of the bioassays commonly used (e.g., soybean callous cultures to estimate cytokinin levels, Miller 1968) may be sensitive to inhibitors and promotors in the extract in ways different from the plant from which the extract was derived. Furthermore, the length to time needed for such assays to give a quantitative estimate of hormonal levels (callous cultures need several weeks) may mean labile forms of the hormone can never be measured by these means. Finally, the effects of transpiration on the concentrations of hormone present are seldom considered (King 1976). Problems with the second experimental approach include uncertainty about the amount of hormone entering the tissue: does this justify the application of concentrations several orders of magnitude greater than physiological concentrations? For example, ABA is typically applied at 10^{-1} mol m^{-3} (e.g., Mizrahi *et al.* 1974) when actual concentrations are 1-2 x 10^{-4} mol m^{-3} in fresh, unstressed tissue (Mulkey *et al.*, 1983; Cowan *et al.*, 1982). It is also extremely puzzling that, almost invariably, control plants respond more dramatically to an applied hormone than treated plants. This occurred when GA was applied to *Brassica campestris* treated with mannitol (Banyal and Rai 1984), and to beans and halophytes treated with NaCl (Neiman and Bernstein 1959; Boucard and Ungar 1976). It also occurred when kinetin was applied to halophytes treated with NaCl (Boucard and Ungar 1976), and when kinetin, ABA or combinations of the two was applied to tobacco plants treated with NaCl or mannitol (Mizrahi *et al*. 1970)). Problems also occur with aerosol sprays; for instance, they may have direct effects at sites other than target cells in the growing region (see below). This criticism is equally relevant to applications via the nutrient solution surrounding the roots, i.e., there may be direct effects on root elongation (Stenlid 1982). In both methods there is lack of control over the amounts entering plants and it is likely that the NaCl treatments, the very plants likely to be deficient in endogenous cytokinins, would absorb less. For example, when applied to leaves, increased amounts of wax on the leaves of NaCl-treated plants may inhibit entry of applied compounds more in those plants, and when applied to roots, lower transpiration rates would lower entry via the apoplastic pathway (passive uptake across membranes is unlikely for a substance with MW 215). A particular problem occurs with the application of cytokinins directly to the leaves; by opening stomata, cytokinin aerosols may cause excessive transpiration (c.f. Prisco and O'Leary 1973) and consequent loss of shoot turgor (Kirkham *et al* 1974). These results give rise to statements such as " the addition of cytokinins to cytokinin-deficient, stressed plants does not alleviate the symptoms associated with water stress, but rather intensifies them" (Mizrahi and Richmond 1972).

The following experiments are the first of their kind in that

(i) a known amount of kinetin entered the transpiration stream of the plants and (ii) the water status of the shoots of the NaCl-treated plants was raised. The aim of the first experiment was to determine the most effective kinetin concentration to apply to NaCl-treated wheat. Ideally, dose-response curves include threshold concentrations, an optimal range, and a range where effects of the compound become increasingly toxic. It was hypothesised that the most effective concentration to apply to NaCl-treated plants would be less effective, or even toxic, to control plants. The second experiment aimed to test the selected concentration in replicate treatments, to confirm the responses observed in Experiment 1.

5.2. Experimental design:

Kinetin (Sigma) was fed daily to wheat seedlings by applying pressure until the shoot ψ was greater or equal to zero, cutting the midrib of leaf 2 , inserting the cut tip into the solution, and lowering the pressure: this caused the solution to be sucked in. The kinetin was applied at a concentration calculated to supply all the needs of the plant (see below), in a volume that was adjusted for the size of each plant and was 1% of the volume transpired during the previous 24 hours by plants of the same size supplied with distilled water instead of kinetin (controls). The controls (fed distilled water) were used to calculate transpiration rates rather than kinetin-treated plants, to avoid possible cumulative increases to toxic levels in the supply of kinetin (assuming kinetin affected transpiration). Initially kinetin was supplied at 10% of the total volume transpired by a plant in 24 h (e.g., 250μl was given to a plant that had transpired 2.5 ml during the previous 24 h), but these quantities tended to infiltrate the leaf tip which then died back.

This was an effect of the volume of solution supplied rather than of kinetin: large control plants (i.e., given large amounts of distilled water) were the worst affected. When amounts of solution were supplied at 1% of the total volume transpired in the previous 24h (e.g., 25 μl would be given to the plant above) the leaf tip was not affected, and remained green and looked healthy after all the solution had been absorbed. The solution, which was held in a narrow vial strapped to the plant, was typically absorbed within an hour or two. Preliminary experiments using water soluable food dyes (McCormick & Co, USA; dye Nos. red CI 56 16185 and blue CI 56 42090) had shown that the dye reached the base of the leaf to which it was applied within seconds of the applied pressure being lowered, and was distributed uniformly throughout the shoot within two hours.

Experiment 1: Transpiration and leaf elongation rates in wheat plants supplied with a range (0 - 10^{-1} mol m^{-3}) kinetin: is there a response to dosage?: The concentration series was chosen with the following assumptions: (i) that xylem exudate concentrations are typically 10^{-4} mol m^{-3} to 6 x 10^{-3} mol m^{-3} (c.f. King 1976); (ii) the xylem volume is about 1% of the FW of the plant. Therefore, if all cytokinin required by the plant were to be supplied in a volume 1% of that transpired, the concentration should be about 10^{-3} mol m^{-3}. To test this assumption, a concentration series was diluted from an aqueous stock solution of 2.0 g l^{-1} kinetin pH 6.5 (dissolved with NaOH; neutralised with HCl). In the first experiment, 12 wheat plants were grown in 100 mol m^{-3} NaCl and pairs of plants were supplied with 0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} or 10^{-1} mol m^{-3} kinetin; one of each pair was pressurised. Two weeks later the same kinetin concentrations were supplied to 6 plants grown in nutrient solutions (without NaCl). These experiments took place in glasshouse 2a (Section 2.2.1). After kinetin was applied, the plants were monitered 1-3 d before the following application. NaCl-treated plants had a 3 applications in total, control plants 2.

Experiment 2: Transpiration and leaf elongation in wheat plants supplied with 10^{-3} mol m^{-3} kinetin: a replicated experiment: Using the same stock solution, kinetin was diluted to 10^{-3} mol m^{-3} in distilled water. Twenty-six wheat plants were grown in pressure pots and 16 were grown in 100 mol m^{-3} NaCl. When plants were 17 days old they were transferred from glasshouse 2 to the growth cabinet (section 2.2.1.). The following day 6 plants were pressurised to 0.48 MPa. The following day, and daily thereafter, kinetin was applied to half the plants in each of the treatments (i.e., plants without NaCl, NaCl-treated plants and pressurised NaCl-treated plants). Water was applied to the remaining plants (controls) using the same technique. Up to 3 hours was required to apply the solutions to all plants, and water them, and during this time the pressure could not be applied. Leaf areas (section 2.3.1.) and transpiration (section 2.3.5.) were estimated daily.

5.3. Results:

Experiment 1: Transpiration and leaf elongation rates in wheat plants supplied with a range (0 - 10^{-1} mol m^{-3}) kinetin. Is there a response to dosage?: Fig 5.1. presents the transpiration data and leaf elongation rates obtained after the second dose of kinetin was applied to plants treated with NaCl or nutrient solution. Responses as shown in Fig 5.1. were typical the day following an application of kinetin, subsequent days gave even more variable elongation and transpiration responses. The results are for single plants (no

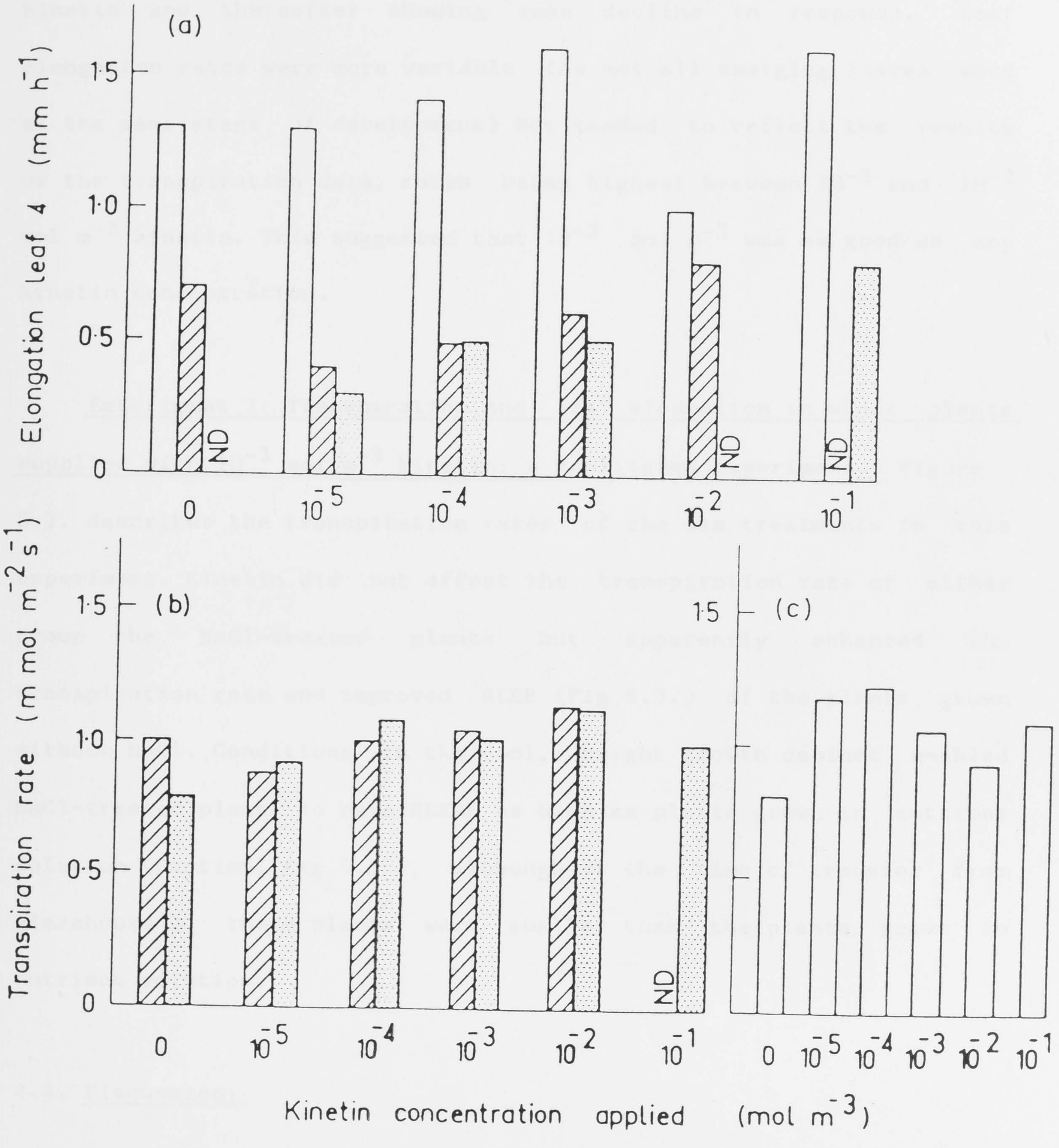
Figure 5.1.

Effect of concentration series of kinetin on leaf elongation and transpiration rates of nutrient solution controls and NaCl-treated wheat, with and without applied pressure.

(a) Effect of concentration series (0 to 10^{-1} mol m^{-3}) kinetin, applied at 1% of the volume transpired during the previous 24 h, on leaf elongation rates (mm h^{-1}) of wheat (Experiment 1). Nutrient solution controls (☐), 100 mol m^{-3} NaCl-treated control (▨), 100 mol m^{-3} NaCl with 0.48 MPa pressure applied to roots d 10 after germination (░). Each bar shows elongation rate of leaf 4 of one plant, d 1 after the second application of kinetin. Measurements of NaCl-treated plants were made d 18 after germination; measurements of controls d 18 after germination, 2 weeks later.

(b) Effect of concentration series (0 to 10^{-1} mol m^{-3}) kinetin, applied at 1% of the volume transpired during the previous 24 h, on transpiration rates (mmol m^{-2} s^{-1}) of NaCl-treated wheat (Experiment 1). The plants were the same as in Fig 5.1. (a), as was the time of measurement.

(c) Effect of concentration series (0 to 10^{-1} mol m^{-3}) kinetin, applied at 1% of the volume transpired during the previous 24 h, on transpiration rates (mmol m^{-2} s^{-1}) of wheat grown in nutrient solution (Experiment 1). The plants were the same as in Fig 5.1.(a), as was the time of measurement.


(a)
Elongation leaf 4 (mm h^{-1})
1·5
1·0
0·5
0
ND
ND
ND
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
(b)
Transpiration rate (m mol m^{-2} s^{-1})
1·5
1·0
0·5
0
ND
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
(c)
1·5
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
Kinetin concentration applied (mol m^{-3})

replicates), and suggest that transpiration in NaCl-treated wheat plants was enhanced between 10^{-4} and 10^{-2} mol m^{-3} kinetin but that plants grown in nurient solution (without NaCl) were more sensitive to kinetin; the peak sensitivity being near 10^{-5} and 10^{-4} mol m^{-3} kinetin and thereafter showing some decline in response. Leaf elongation rates were more variable (as not all emerging leaves were at the same stage of development) but tended to reflect the results of the transpiration data, rates being highest between 10^{-3} and 10^{-2} mol m^{-3} kinetin. This suggested that 10^{-3} mol m^{-3} was as good as any kinetin concentration.

Experiment 2: Transpiration and leaf elongation in wheat plants supplied with 10^{-3} mol m^{-3} kinetin: a replicated experiment.: Figure 5.2. describes the transpiration rates of the six treatments in this experiment. Kinetin did not affect the transpiration rate of either group the NaCl-treated plants but apparently enhanced the transpiration rate and improved RLER (Fig 5.3.) of the plants grown without NaCl. Conditions in the cool, bright growth cabinet enabled NaCl-treated plants to have RLERs as high as plants grown in nutrient solution (Caption, Fig 5.3.), although at the time of transfer from glasshouse 2, these plants were smaller than the plants grown in nutrient solution.

4.4. Discussion:

The results of the first experiment, although subject to plant variability (inevitable for the pressure treatments), suggested 10^{-3} m^{-3} kinetin was a suitable concentration to use. The results of the second experiment, where replicates of treated plants were grown under controlled conditions, suggested that plants grown in nutrient

Figure 5.2.

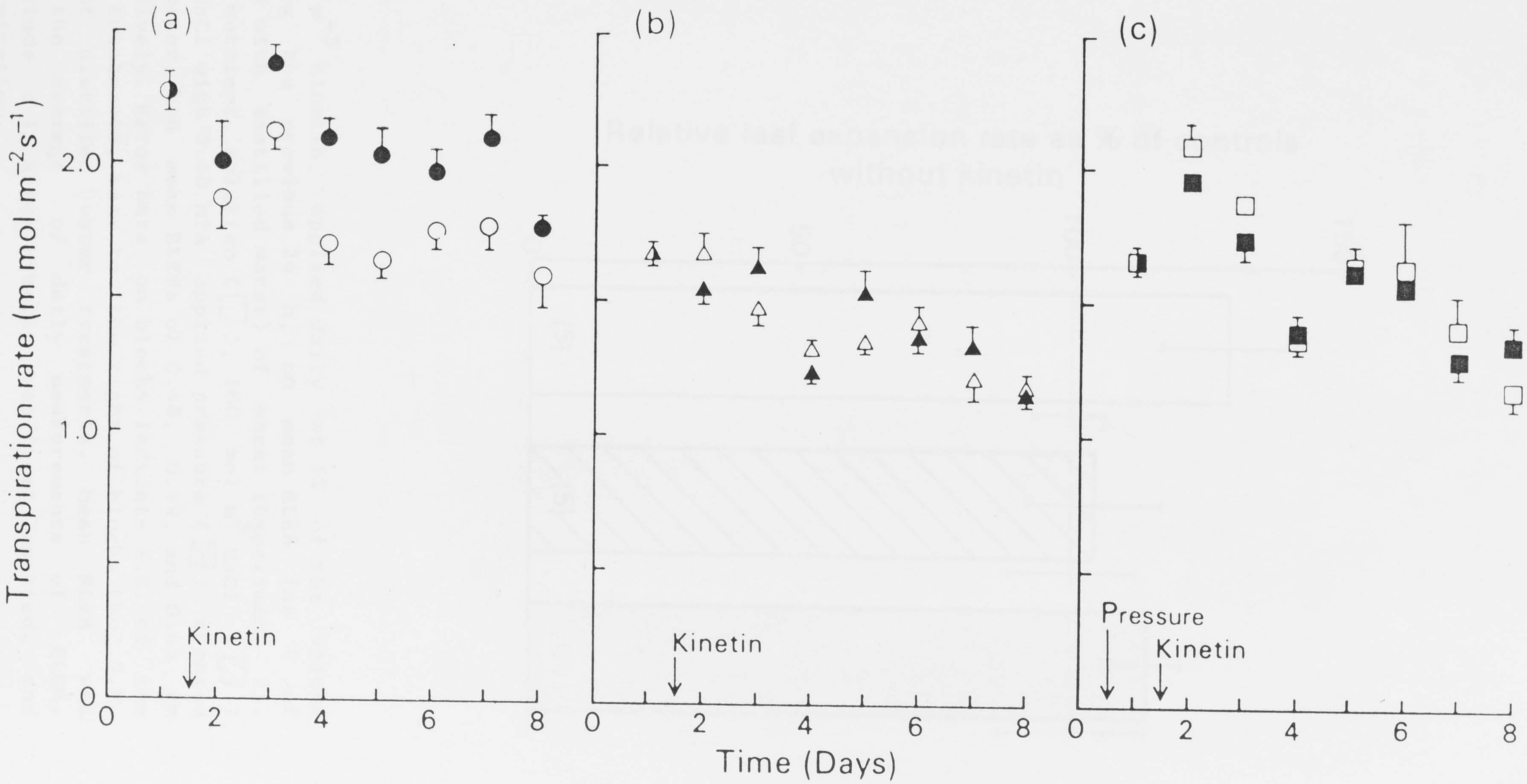
Effect of 10^{-3} m^{-3} kinetin or distilled water, applied daily at 1% of the volume transpired during the previous 24 h (treatment started) on transpiration rate (mmol m^{-2} s^{-1}) of wheat (Experiment 2).

(a) Effect of kinetin (●) or water (○) on transpiration rate of wheat grown in nutrient solution. Bars indicate the S.E. of the mean of each measurement, 5 plants per treatment.

(b) Effect of kinetin (▲) or water (△) on transpiration rate of wheat grown in 100 mol m^{-3} NaCl. Bars indicate the S.E. of the mean of each measurement, 5 plants per treatment.

(c) Effect of kinetin (■) or water (□) on transpiration rate of wheat grown in 100 mol m^{-3} NaCl, with 0.48 MPa pressure applied to roots d 18 after germination. Bars indicate S.E. of the mean of each measurement, 3 plants per treament.

Plants were grown in the Conviron cabinet. The temperature was 10°C during the dark period, 13°C during the light. Measurements began d 17 after germination.


(a)
(b)
(c)
Transpiration rate (m mol m^{-2}s^{-1})
2.0
1.0
0
Kinetin
Kinetin
Pressure
Kinetin
0
2
4
6
8
Time (Days)

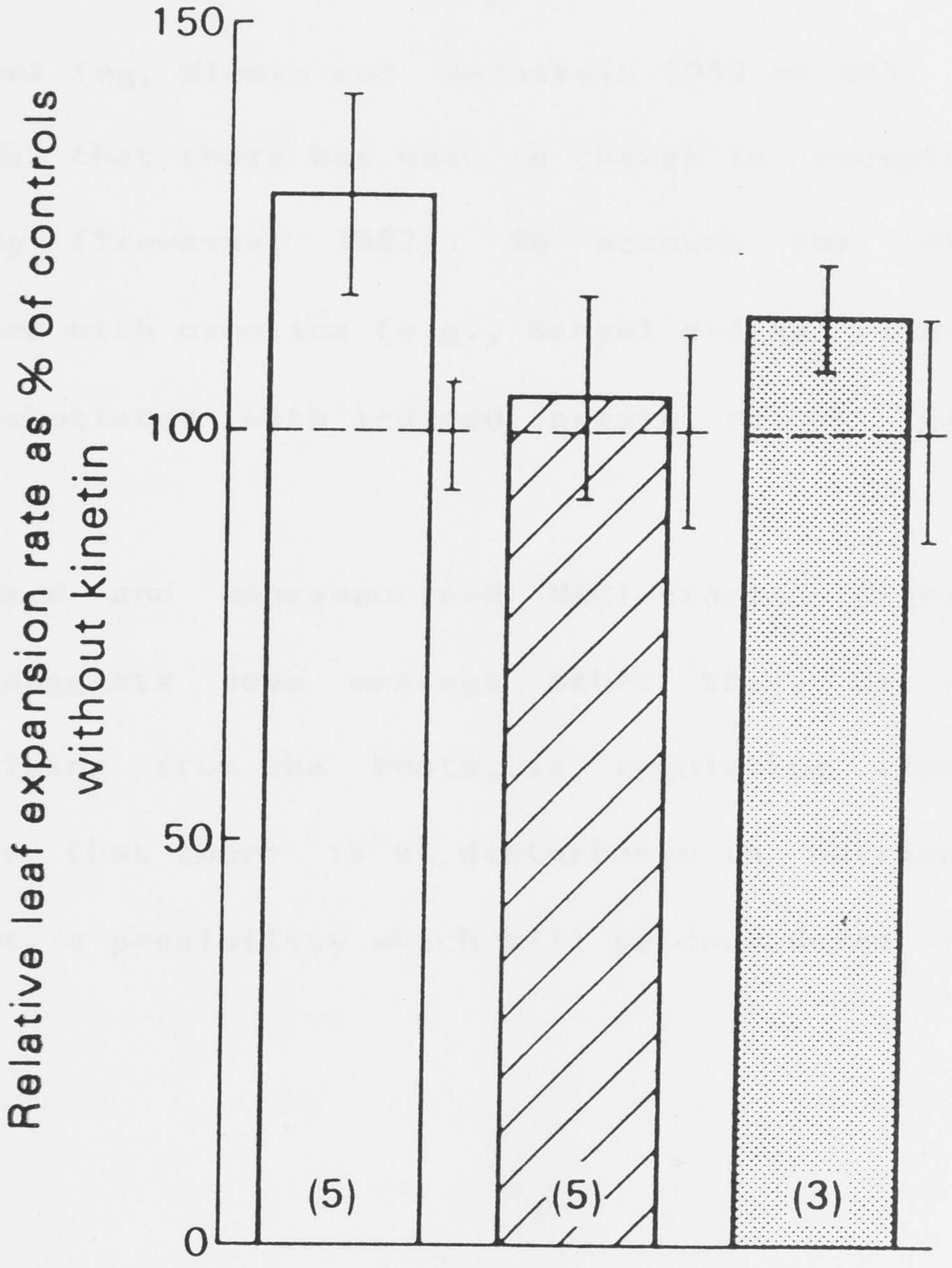


Figure 5.3.

Effect of 10^{-3} m^{-3} kinetin, applied daily at 1% of the volume transpired during the previous 24 h, on mean RLER (as % of controls treated with distilled water) of wheat (Experiment 2). Plants grown in nutrient solution (☐), 100 mol m^3 NaCl (▨) or 100 mol m^3 NaCl with 0.48 MPa applied pressure (░) treated with distilled water, had mean RLERs of 0.48, 0.49, and 0.43 cm cm^{-2} d^{-1} respectively. Error bars on blocks indicate S.E. of the mean of kinetin treatments, bars to the right of blocks the S.E. of the mean of distilled water treatments. Mean RLER was calculated as the average of daily measurements of RLER. Measurements include d 1 after kinetin was first applied, and started d 19 germination.

solution (without NaCl) were more sensitive than NaCl-treated plants.

The response of the plants grown in nutrient solution (without NaCl) is strong evidence that the applied kinetin is reaching receptors. NaCl-treated plants, however, did not respond. Reduced sensitivity to applied hormones of plants grown in low ψ is well known (Section 5.1.), and the general interpretation is that the effect of NaCl is dominent (eg, Nieman and Bernstein 1959 on GA). A more likely possibility is that there has been a change in receptor frequency or sensitivity (Trewavas, 1982). To account for the responses of plants treated with osmotica (e.g., Banyal and Rai 1984) receptor frequency is associated with reduced growth rather than NaCl.

Because both pressured and unpressurised NaCl-treated plants behaved similarly, this suggests some message other than, or in addition to, kinetin, arising from the roots, is regulating shoot growth. One suggestion is that there is a disturbance in nutrient balance reaching the shoot, a possibility which will be considered in the next chapter.

4.5. Conclusion:

Kinetin (10^{-3} m^{-3}) was introduced directly into the transpiration stream of wheat plants by manipulating the xylem hydrostatic pressure so the solution was sucked into the xylem. There was a 10-20% increase in the transpiration and RLER of plants grown without NaCl, which suggested kinetin reached receptors, but NaCl-treated plants did not respond. Applied pressure did not affect the response of NaCl-treated plants, which suggested that something other than, or in addition to, kinetin, was regulating the growth of the shoot of NaCl-treated plants.

Chapter 6

USE OF CONCENTRATED MACRONUTRIENT SOLUTIONS TO SEPARATE OSMOTIC FROM NaCl-SPECIFIC EFFECTS ON PLANT GROWTH.

6.1. Introduction:

Growth reductions caused by NaCl could be due to both osmotic and ion-specific effects. It is likely that the initial growth response of the leaves is regulated by a message from the roots (Chapter 4), but it is unknown whether this response is dominated by the osmotic effect (ie, by water deficit) or by the specific ion effects (ie, NaCl toxicity or nutrient imbalance) on the root.

If nutrient imbalance contributes to the reduced growth of NaCl-treated plants, there are two experimental approaches which may help its study: (i) to fertilise NaCl-treated plants with the macronutrient that is presumed to be deficient or otherwise manipulate the growth conditions (discussed in Section 1.2.3.) or (ii) to grow plants in a medium which exerts only osmotic effects (i.e., reducing water uptake but not nutrient uptake), and to compare the performance in each case with appropriate controls. A variety of non-ionic osmotica have previously been used to compare specific effects of NaCl with effects on plant water relations, including mannitol, which eventually passes through cell membranes, and raffinose and inulin, which are liable to bacterial degradation. High MW polyethelene glycol (PEG) is frequently used in longer term studies but it can interfere with ion transport (Yeo and Flowers 1984a, b) and limit O_2 diffusion to roots (Mexal *et al.* 1975). Even a drying soil, as well as being very difficult to maintain at a uniform, constant, water potential, may exert specific effects; for example transmission of nutrients through the soil will be reduced at low soil water potentials (Nye 1979). Therefore, concentrated macronutrient solution were explored as a non-specific osmoticum. The only long term study along these lines is that of Hayward and Long (1941) on tomato, which showed that concentrated macronutrients gave better growth than NaCl or Na_2SO_4 did, but the authors did not distinguish between root and shoot growth and mention that a precipitate formed in some of their solutions, which may have confounded their results.

The aim of this study was to assess the value of concentrated macronutrient solution as a non-specific osmoticum, and to separate osmotic from specific NaCl effects on growth. Growth was compared in four species; wheat, barley, Egyptian clover (all moderately salt-tolerant) and white clover (salt-sensitive) in NaCl solutions and concentrated macronutrient solutions of matching osmotic pressures. Ion uptake and total solute uptake was measured in barley, and the implications of NaCl-enhanced phosphate uptake (which is known to occur in some species, eg, corn; Nieman and Clark 1976) were tested on all four species.

6.2. Experimental design:

Plants were grown in solution culture as described in Section 2.3.3. Because of the possibility that phosphate concentrations above 2.0 mol m^{-3} could prove toxic, a preliminary experiment compared the growth of barley, cv. Clipper, in two different macronutrient solutions (See Table 2.1. for composition.). In one solution ("concentrated macronutrients") the phosphate concentration increased in proportion to the other major ionic species present, i.e. from 2.0 to 19.6 mol m^{-3} as Π rose from 0.08 to 0.80 MPa; in the other

("modified concentrated macronutrients" in Table 2.1.), phosphate remained at 2.0 mol m^{-3} while NO_3^- and NH_4^+ contributed the additional π over the 0.32 to 0.80 MPa range. For the first 4 harvests (taken over 20 days) growth of the plants was nearly identical for the two types of concentrated macronutrient (Table 6.1.); the 0.80 MPa treatment at the time of the final harvest was very variable. This suggested P toxicity was unlikely when a mixture of other nutrients balanced the increase in phosphate. The unmodified type (ie, phosphate concentrated with the other macronutrients) was used in further studies.

Experiment 1: Leaf elongation after transfer from NaCl to isosmotic solutions:

Wheat seedlings grown in solution culture (Section 2.3.3.) in glasshouse 1 (Section 2.2.1.) or in glasshouse 2a (Section 2.2.1.) in 100 mol m^{-3} NaCl for 2 - 4 days were tranferred to isosmotic solutions of PEG 4000 (unpurified BDH and Sigma) or mannitol (BDH) or concentrated macronutrients. All solutions contained control nutrients. Leaf elongation of the youngest emerging leaf (2 or 3) prior to and immediately after the transfer was measured either with a ruler over 2 h using the top of the pot as a baseline (12-14 replicates) or with a linear variable displacement transducer (LVDT) in 10 minute readings over 1 h (2 replicates).

Experiment 2: Growth in NaCl and concentrated macronutrients:

Barley (*Hordeum vulgare* cv. Clipper), wheat (*Triticum aestivum*, cv. Kite) and Egyptian clover (*Trifolium alexandrinum*) were grown in Glasshouse 2b (Section 2.2.1.) at $26^{\circ}/13^{\circ}C$ (12 h thermoperiod) in nutrient solutions with 0, 50 or 100 mol m^{-3} NaCl and isosmotic (i.e. the equivalent π of 0.32 and 0.56 MPa) solutions of concentrated

Table 6.1. Effect of concentrated macronutrients (C.M.) or modified concentrated macronutrients (M.C.M.) on shoot weight (g) of barley (Preliminary experiment). Means are of 9 plants unless otherwise stated, S.E. are about 10-15% of each value. Harvests started d1 after 0.80 MPaΠ treatment began.

Harvest (d): Treatment (π)	1	8	15	20	25
Control (0.08)	0.20	0.75	1.43	2.09	3.44
C.M. (0.32)	0.09	0.45	0.80	1.92	2.29
M.C.M. (0.32)	0.12	0.54	0.88	1.74	1.74
C.M. (0.56)	0.11[1]	0.20[2]	0.68	1.13	1.32[2]
M.C.M. (0.56)	0.10[2]	0.50	0.61	1.30	1.50
C.M. (0.80)	0.12	0.34	0.63	1.22	1.07
M.C.M. (0.80)	0.10	0.39	0.39	0.89	0.51[3]

[1] 3 plants

[2] 8 plants

[3] 5 plants

macronutrients; white clover (*T. repens*) with 0, 25 or 50 mol m^{-3} NaCl and isosmotic solutions of concentrated macronutrients (π of 0.20 and 0.32 MPa) in glasshouse 2b (Section 2.2.1.). Three replicate pots of each of these 5 treatments were randomised in 2 blocks. Three harvests were taken at 7-day intervals for cereals and 10 day intervals for the clover species, starting one day after the 0.56 MPa treatment began (i.e., day 2), taking 4 plants from each pot. Fresh weights and dry weights were measured.

Experiment 3: Nutrient uptake of barley grown in NaCl and concentrated macronutrients:

Barley, cv. Clipper, was grown at 28°/22° C (12 h thermoperiod) in aerated nutrient solutions with 0, 50, 100 or 150 mol m^{-3} NaCl, isosmotic solutions of concentrated macronutrients (i.e., equivalent osmotic pressures of 0.32, 0.56 and 0.80 MPa) in glasshouse 1 (Section 2.2.1.). Three replicates of each of these 7 treatments were randomised in 3 blocks. Five harvests were taken at intervals 2, 9, 16, 21 and 26 days after the 0.80 MPa treatment began, taking 3 plants from each pot. Dry weights were determined and the mineral content analysed on plants in control, 50 mol m^{-3} NaCl and 0.32 MPa concentrated nutrient treatments.

Experiment 4: Growth of NaCl-treated plants in minimal phosphate versus 2.0 mol m^{-3} phosphate:

This utilised the same controls as Experiment 2 and was conducted concurrently. NH_4NO_3 replaced $NH_4H_2PO_4$ in the nutrient solution of an NaCl series including a "minimal phosphate" control, and KH_2PO_4 was added daily in minimal quantities based on the size of the plant. The amount given was, in 10^{-3}g per g plant DW, 2.7 for barley, 4.0 for wheat, 2.2 for Egyptian clover and 4.4 for white clover. These rates of application were predetermined by giving an excess of phosphate for one day to control plants of each species, then measuring the plant size and the amount of phosphate remaining in the nutrient solution. Plant size was estimated by first determining the leaf area (Section 2.3.1.).

Experiment 5: Water relations:

Barley and wheat were grown in nutrient solution with 0 or 100 mol m^{-3} NaCl and isosmotic solutions of concentrated macronutrients in growth conditions similar to experiment 2. Ten days after the final concentrations were reached, water potential and osmotic pressure were measured on leaf 3 of the plants, and osmotic pressure was determined on the elongating tissue, i.e., the basal 10mm of the growing leaves (leaf 6 in all treatments).

Chemical analysis of plant material:

See Section 2.3.4.

Assessment of phosphate in nutrient solution:

See Section 2.3.4.

Water potential, osmotic pressure measurements:

Water potential of leaf 3 (the oldest leaf showing no signs of senescence) was measured with a pressure chamber (Section 2.3.3.). There were 8-12 replicates per treatment. Osmotic pressure of elongating tissue was was measured (Section 2.3.3.), 4-6 replicates per treatment, 2 plants per replicate. Osmotic pressure of leaf 3 was measured (Section 2.3.3.), 4-6 replicates per treatment, 2 plants per replicate. Nutrient solutions were also measured (Section 2.3.3.). All instruments were calibrated with the same NaCl standards.

Calculations:

See Section 2.4.

6.3. Results:

Leaf elongation after transfer from NaCl to isosmotic solutions:

Wheat was grown in 100 mol m^{-3} NaCl for 2 to 4 days and then the NaCl was replaced by isosmotic solutions of mannitol, polyethylene glycol (PEG) 4000 or concentrated macronutrients and the elongation rate of the emerging leaf was measured over the next 1 - 2 hr (Experiment 1). Not only did PEG 4000 cause an immediate decrease in leaf growth rate, but within two hours the roots went brown. The following day the root tips had thickened as if elongation of the root tips had been inhibited. The PEG was unpurified, but of high commercial grade. Mannitol had a similar effect on leaf elongation rate, which decreased to two thirds of the original rate. This was reversed by replacing the original NaCl solution. There was a gradual recovery over 40 minutes. Isosmotic concentrated macronutrients had no effect on elongation rate (Table 6.2.).

Growth in NaCl and concentrated macronutrients:

Barley, wheat, Egyptian clover and white clover were grown in NaCl and isosmotic concentrated macronutrients for 3-4 weeks (Experiment 2). In all 4 species, shoot growth in concentrated macronutrients was double that in NaCl (Fig 6.1., 6.2.). For example, barley grown in 100 mol m^{-3} NaCl for 15 days had a shoot weight only 20% that of controls, but plants in isosmotic concentrated

Table 6.2 Effect of isosmotic solutions on leaf elongation rates in wheat. Plants were grown in 100 mol m^{-3} NaCl for 2-4 days then transferred to various solutions of the same osmotic pressure. Two brands of PEG were used. Elongation rates were measured over 30-60 min with an LVDT or 2 h with a ruler.

Isosmotic Solution	Method of Measurement	Former rate (mm h-1)	Subsequent rate (mm h-1)	% of Former Rate
NaCl	LVDT	1.51 ± 0.03	1.49 ± 0.12	99
PEG 4000 (1)	ruler	1.16 ± 0.18	0.72 ± 0.16	62
PEG 4000 (2)	ruler	0.68 ± 0.11	0.43 ± 0.11	63
Mannitol	LVDT	0.98 ± 0.05	0.58 ± 0.04	59
Concentrated macronutrient	LVDT	1.46 ± 0.08	1.56 ± 0.05	107

Figure 6.1.

Effect of NaCl or concentrated macronutrients on shoot DW of plants grown in solution culture (Experiment 2).

(a) Barley, cv. Clipper. Measurements began d 16 after germination.

(b) Wheat. Measurements began d 17 after germination.

Nutrient solution controls (●), 50 mol m^{-3} NaCl-treated plants, 0.32 MPa Π (△), or concentrated macronutrient-treated plants, 0.32 MPa Π (▲), 100 mol m^{-3} NaCl-treated plants, 0.56 MPa Π(□) or concentrated macronutrients, 0.56 MPa Π (■). Error bars indicate S.E. of the mean of each group of plants, 11-12 plants per harvest. Inset shows root:shoot ratio for the same plants. Incremental increases in external Π of treatments shown as bar graph, x axis. Harvests began d 1 after the highest external Π was reached.

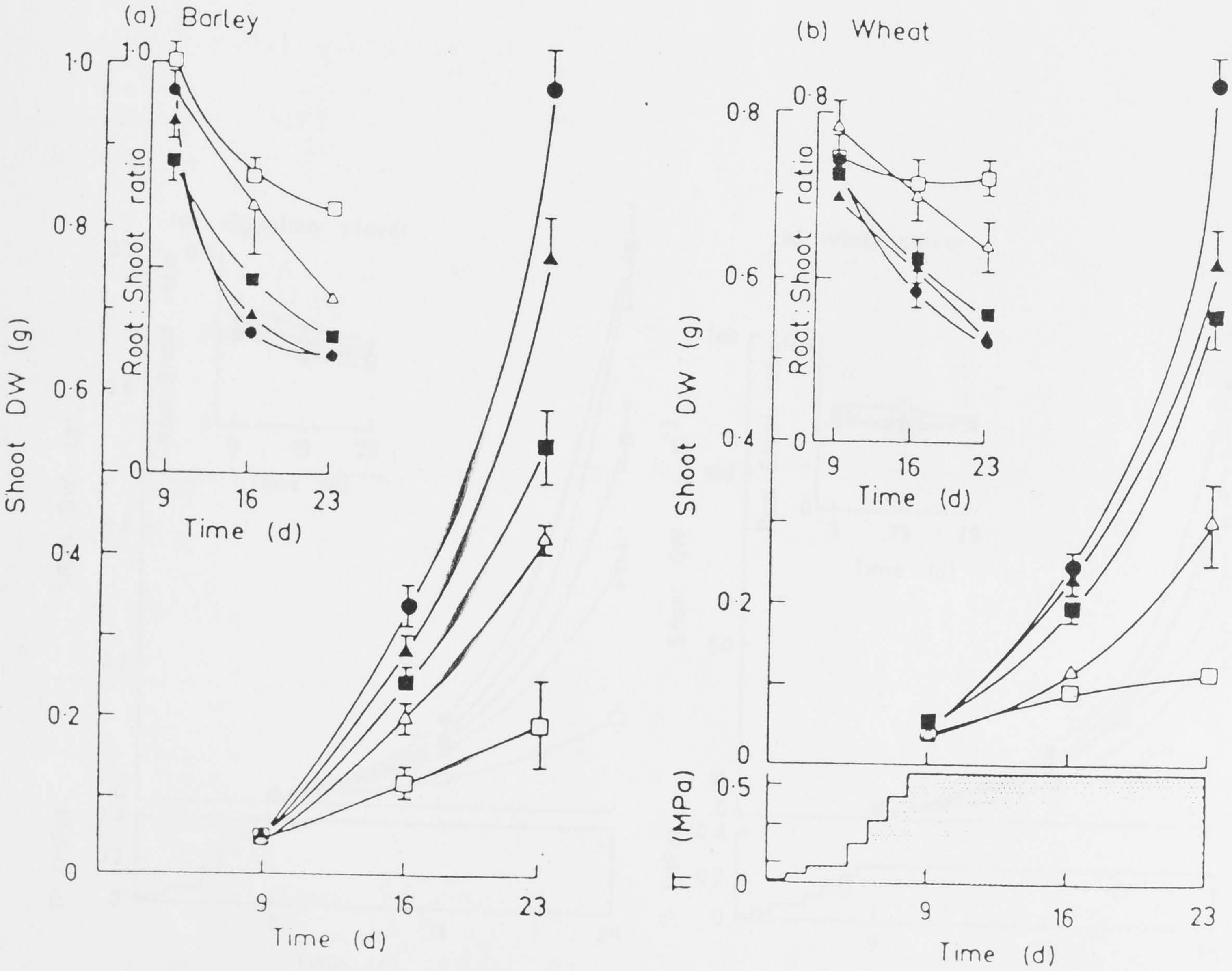


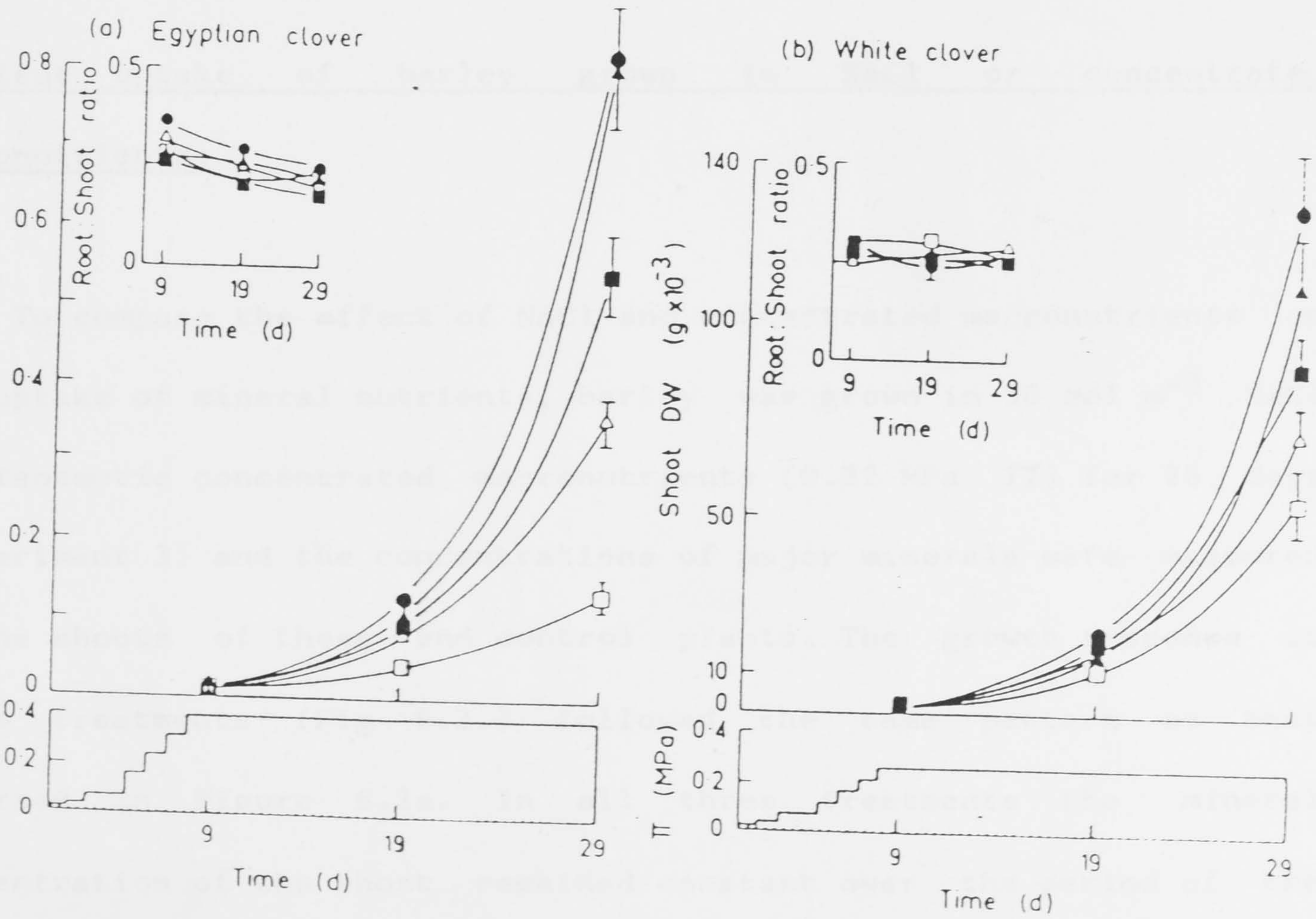
Figure 6.2.

Effect of NaCl or concentrated macronutrients on shoot DW of plants grown in solution culture (Experiment 2).

(a) Egyptian clover. Nutrient solution controls (●), 50 mol m^{-3} NaCl-treated plants, 0.32 MPa π (△), or concentrated macronutrient-treated plants, 0.32 MPa π (▲), 100 mol m^{-3} NaCl, 0.56 MPa π (□), or concentrated macronutrient-treated plants, 0.56 MPa π(■). Incremental increases in external π shown as bar graph, x axis. Harvests began d 20 after germination.

(b) White clover. Nutrient solution controls (●), 25 mol m^{-3} NaCl-treated plants, 0.20 MPa π (△), or concentrated macronutrient-treated plants, 0.20 MPa π (▲), 50 mol m^{-3} NaCl-treated plants, 0.32 MPa π (□), or concentrated macronutrient-treated plants, 0.32 MPa π (■). Incremental increases in external π shown as bar graph, x axis. Harvests began d 20 after germination.

Error bars indicate the S.E. of the mean of each group of plants, 11-12 plants per harvest. Insets show root:shoot ratio of the same plants. Harvests began d 1 after the highest external π was reached.



macronutrients (0.56 MPa π) were half the size of controls (Fig 6.1a.). Root growth was also inhibited by both NaCl and concentrated macronutrients. In NaCl-treated wheat and barley, root growth was inhibited much less than shoot growth, leading to root:shoot ratios double those of controls (Fig 6.1., insets). On the other hand, in plants grown in concentrated macronutrients, the reduction in root growth was in proportion to the reduction in shoot growth, leading to root:shoot ratios similar to those of controls. In a separate experiment incorporating plants at 150mol m^{-3} NaCl and concentrated macronutrients at 0.80 MPa π (Experiment 3), plants grown in concentrated macronutrients of 0.80 MPa π showed some increase in the root:shoot ratio in the last two harvests but this was similar to the increase in root:shoot ratio of the 50 mol m^{-3} NaCl treatment at harvest 4, and less at harvest 5 (Fig 6.3.). Neither Egyptian clover nor white clover exhibited the increase in root:shoot ratio expressed by cereals in NaCl (Fig 6.2., insets).

Nutrient uptake of barley grown in NaCl or concentrated macronutrients:

To compare the effect of NaCl and concentrated macronutrients on the uptake of mineral nutrients, barley was grown in 50 mol m^{-3} NaCl and isosmotic concentrated macronutrients (0.32 MPa π) for 26 days (Experiment 3) and the concentrations of major minerals were measured in the shoots of these and control plants. The growth response to these treatments (Fig 6.3.) followed the same pattern to that reported in Figure 6.1a. In all three treatments the mineral concentration of the shoot remained constant over the period of the experiment during which 5 harvests were taken. Figure 6.4. shows the concentration of the mineral nutrients (g x 10^{-3} g^{-1} DW) at each of

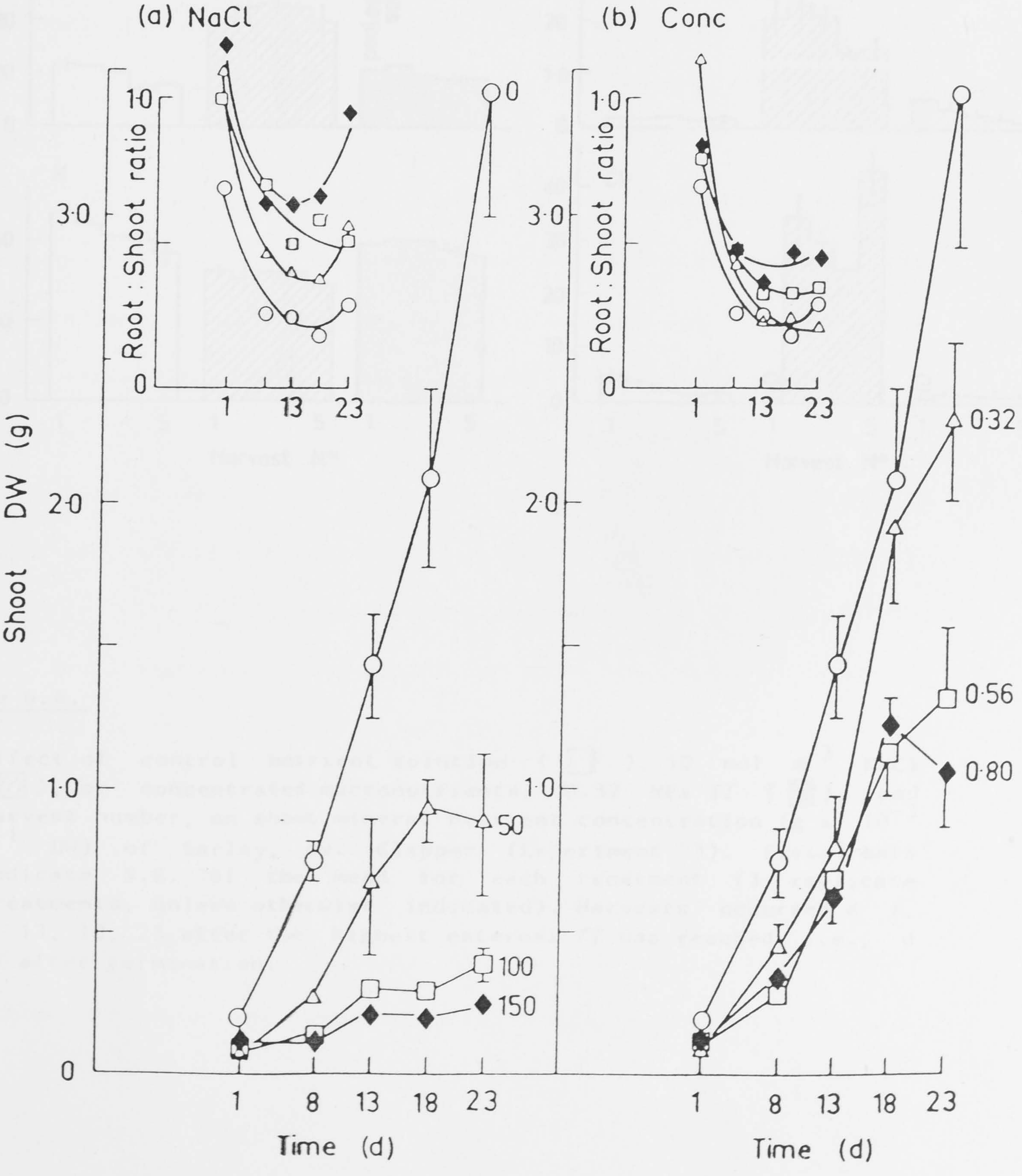
Figure 6.3.

Effect of NaCl or concentrated macronutrients on shoot DW of barley grown in solution culture (Experiment 3).

(a) Nutrient solution controls (○), 50 mol m^{-3} NaCl-treated plants, 0.32 MPa Π (△), 100 mol m^{-3} NaCl-treated plants, 0.56 MPa Π (□), or 150 mol m^{-3} NaCl-treated plants (◆).

(b) Nutrient solution controls (○); same plants as in Figure 6.3(a), concentrated macronutrients, 0.32 MPa Π (△), 0.56 MPa Π (□) or 0.80 MPa Π (◆).

Error bars indicate the S.E. of the mean of each group (usually 9 plants per harvest). Inset shows root:shoot ratios for the same plants. Harvests began d 1 after highest external Π was reached, i.e., d 15 after germination.



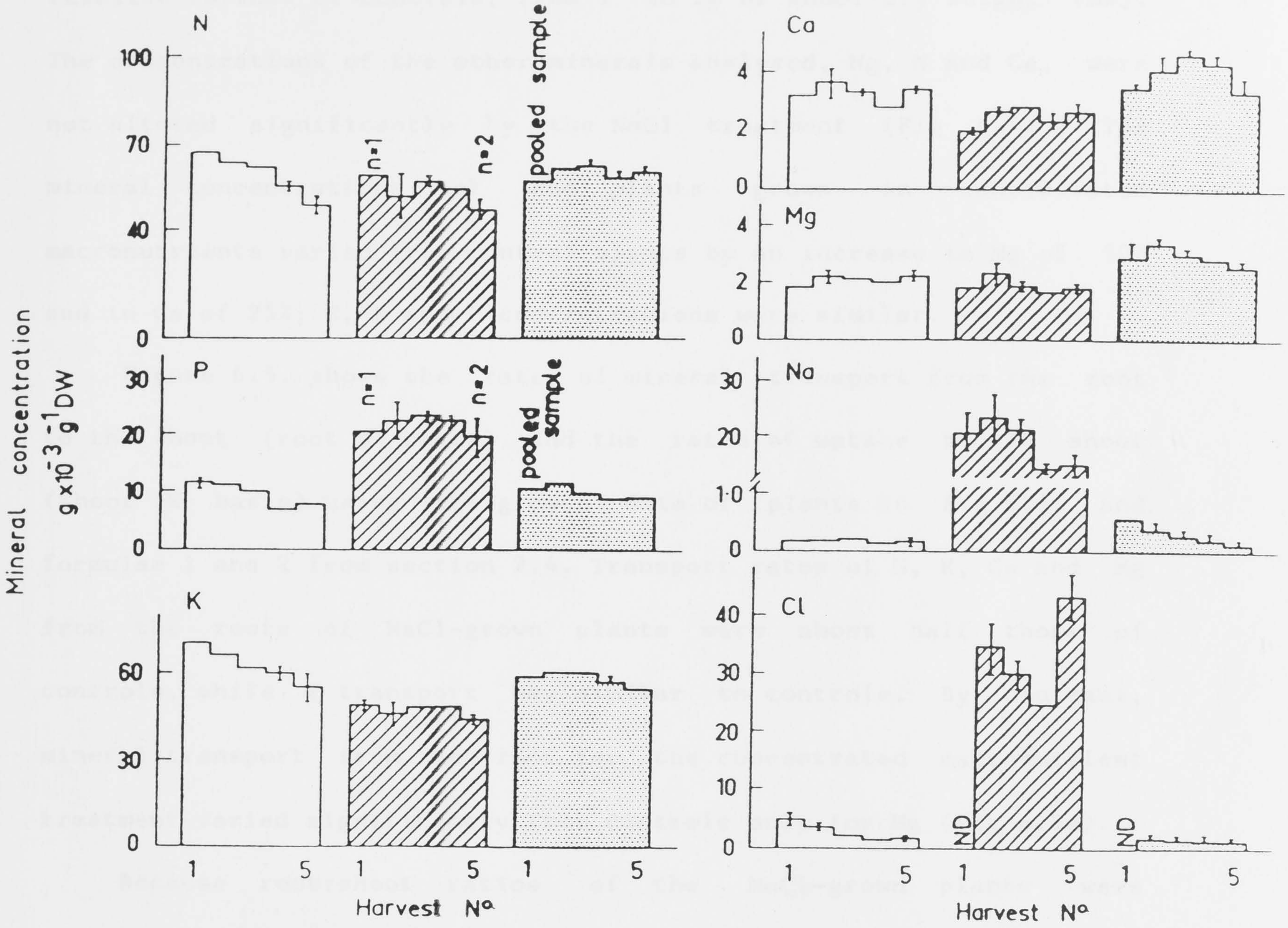


Figure 6.4.

Effect of control nutrient solution (□), 50 mol m^{-3} NaCl (▨), or concentrated macronutrients, 0.32 MPa Π (▒), and harvest number, on shoot mineral nutrient concentration (g x 10^{-3} g^{-1} DW) of barley, cv. Clipper (Experiment 3). Error bars indicate S.E. of the mean for each treatment (3 replicate treatments, unless otherwise indicated). Harvests occurred d 1, 8, 13, 18, 23 after the highest external Π was reached, i.e., d 15 after germination.

the 5 harvests. In NaCl-treated plants, apart from an increase in Na and Cl and a decrease in K concentration, the most striking feature of the mineral concentration data was a doubling of P concentration relative to that of controls, from 1 to 2% of shoot dry weight (DW). The concentrations of the other minerals analysed, Mg, N and Ca, were not altered significantly by the NaCl treatment (Fig 6.4.). The mineral concentrations of the plants grown in concentrated macronutrients varied from control plants by an increase in Mg of 50% and in Ca of 25%; K, N and P concentrations were similar.

Figure 6.5. shows the rates of mineral transport from the root to the shoot (root DW basis) and the rates of uptake by the shoot (shoot DW basis) using the growth data of plants in Fig 6.3. and formulae 1 and 2 from section 2.4. Transport rates of N, K, Ca and Mg from the roots of NaCl-grown plants were about half those of controls, while P transport was similar to controls. By contrast, mineral transport from the root for the concentrated macronutrient treatment varied significantly from controls only for Mg (Fig 6.5a).

Because root:shoot ratios of the NaCl-grown plants were different from those of controls, the values for rates of uptake by the shoot show a different pattern from values for transport from the roots (Fig 6.5b.). In both the NaCl and the concentrated macronutrient treatments, net uptake rates by the shoot of N and K were similar to the controls. In NaCl-treated plants, P uptake by the shoot was doubled. The concentrated macronutrient-treated plants varied significantly from controls in that Mg uptake rates to the shoot were doubled. Total net ion uptake rates by the shoot were 0.9, 1.2 and 1.1 x 10^{-3} mol g^{-1} d^{-1} shoot for the control, 50 mol m^{-3} NaCl and isosmotic concentrated macronutrient treatment respectively.

Figure 6.5.

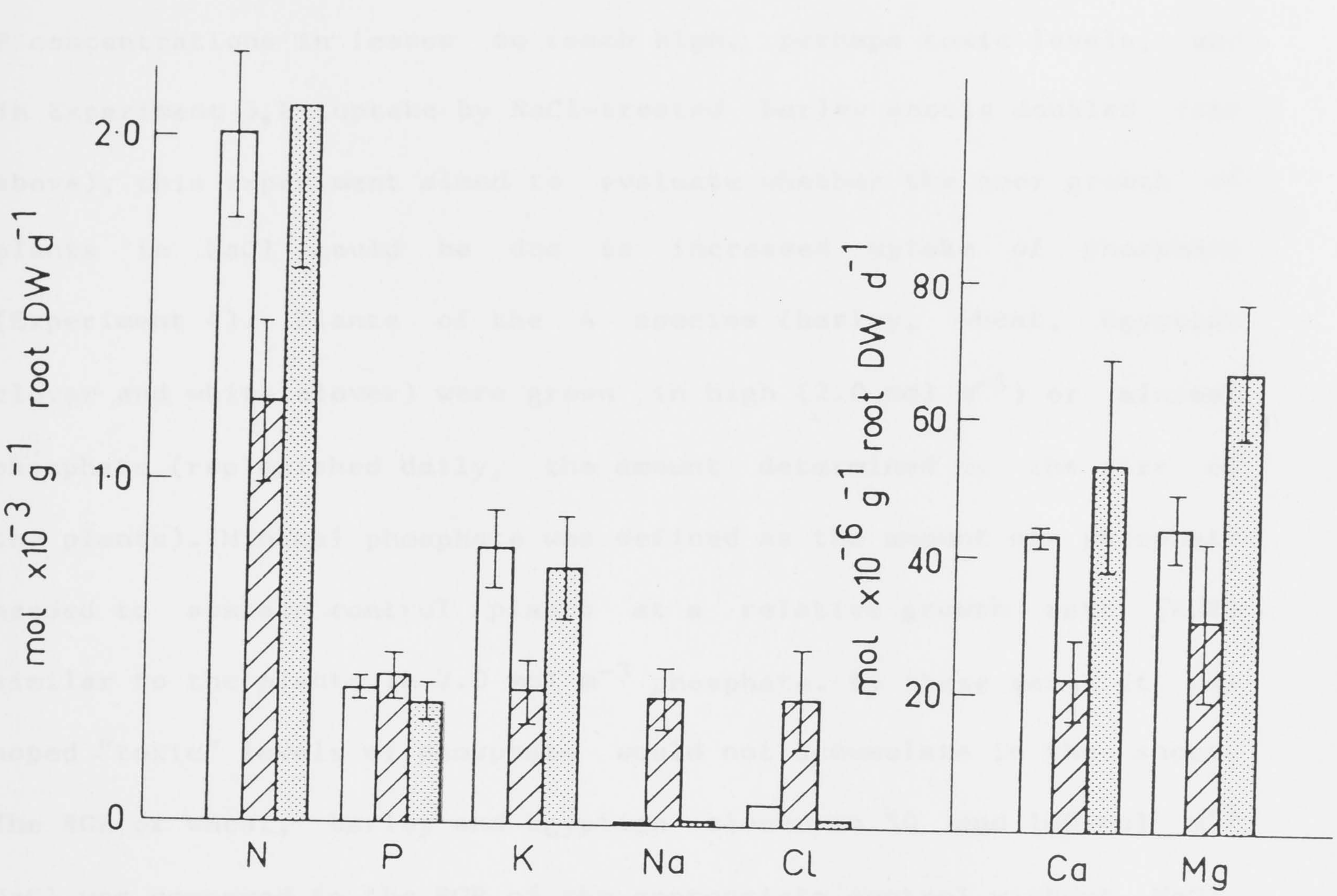
Effect of control nutrient solution (□), 50 mol m^{-3} NaCl (▨) or concentrated macronutrients, 0.32 MPa Π (░) on mineral transport and uptake in barley, cv. Clipper (Experiment 3).

(a) Mineral transport from roots to shoot of total N, P, K, Na and Cl (mol x 10^{-3} g^{-1} root DW d^{-1}) and Ca and Mg (mol x 10^{-6} g^{-1} root DW d^{-1}).

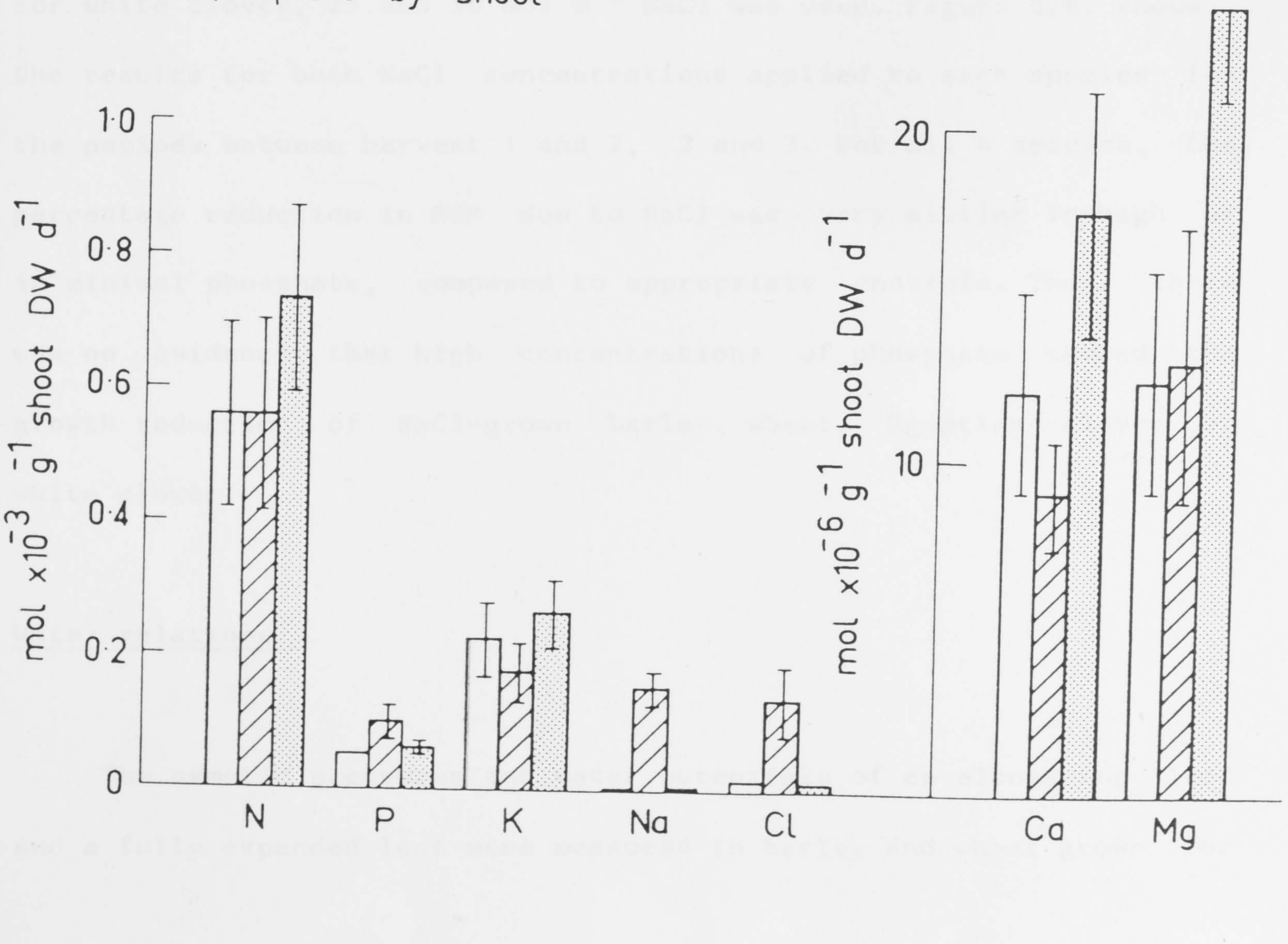
(b) Mineral uptake by the shoot of total N, P, K, Na and Mg (mol x 10^{-3} g^{-1} shoot DW d^{-1}) and Ca and Mg (mol x 10^{-6} g^{-1} shoot DW d^{-1}).

Error bars indicate the S.E. of the mean for uptake rates calculated for the 3 periods between harvests 1 and 4, when growth was exponential. Harvests started d 1 after the highest external Π was reached, i.e., d 15 after germination.

(a) Transport from roots to shoot



(b) Uptake by shoot



Growth of NaCl-treated plants in minimal phosphate versus 2.0 mol m^{-3} phosphate:

As there are several reports that exposure to NaCl causes total P concentrations in leaves to reach high, perhaps toxic levels, and in Experiment 3, P uptake by NaCl-treated barley shoots doubled (see above), this experiment aimed to evaluate whether the poor growth of plants in NaCl could be due to increased uptake of phosphate (Experiment 4). Plants of the 4 species (barley, wheat, Egyptian clover and white clover) were grown in high (2.0 mol m^{-3}) or minimal phosphate (replenished daily, the amount determined by the size of the plants). Minimal phosphate was defined as the amount of phosphate needed to sustain control plants at a relative growth rate (RGR) similar to the plants in 2.0 mol m^{-3} phosphate. By these means it was hoped "toxic" levels of phosphate would not accumulate in the shoot. The RGR of wheat, barley and Egyptian clover in 50 and 100 mol m^{-3} NaCl was compared to the RGR of the appropriate control without NaCl. For white clover, 25 and 50 mol m^{-3} NaCl was used. Figure 6.6. shows the results for both NaCl concentrations applied to each species for the periods between harvest 1 and 2, 2 and 3. For all 4 species, the percentage reduction in RGR due to NaCl was very similar in high as in minimal phosphate, compared to appropriate controls. Thus, there was no evidence that high concentrations of phosphate caused the growth reduction of NaCl-grown barley, wheat, Egyptian clover or white clover.

Water relations:

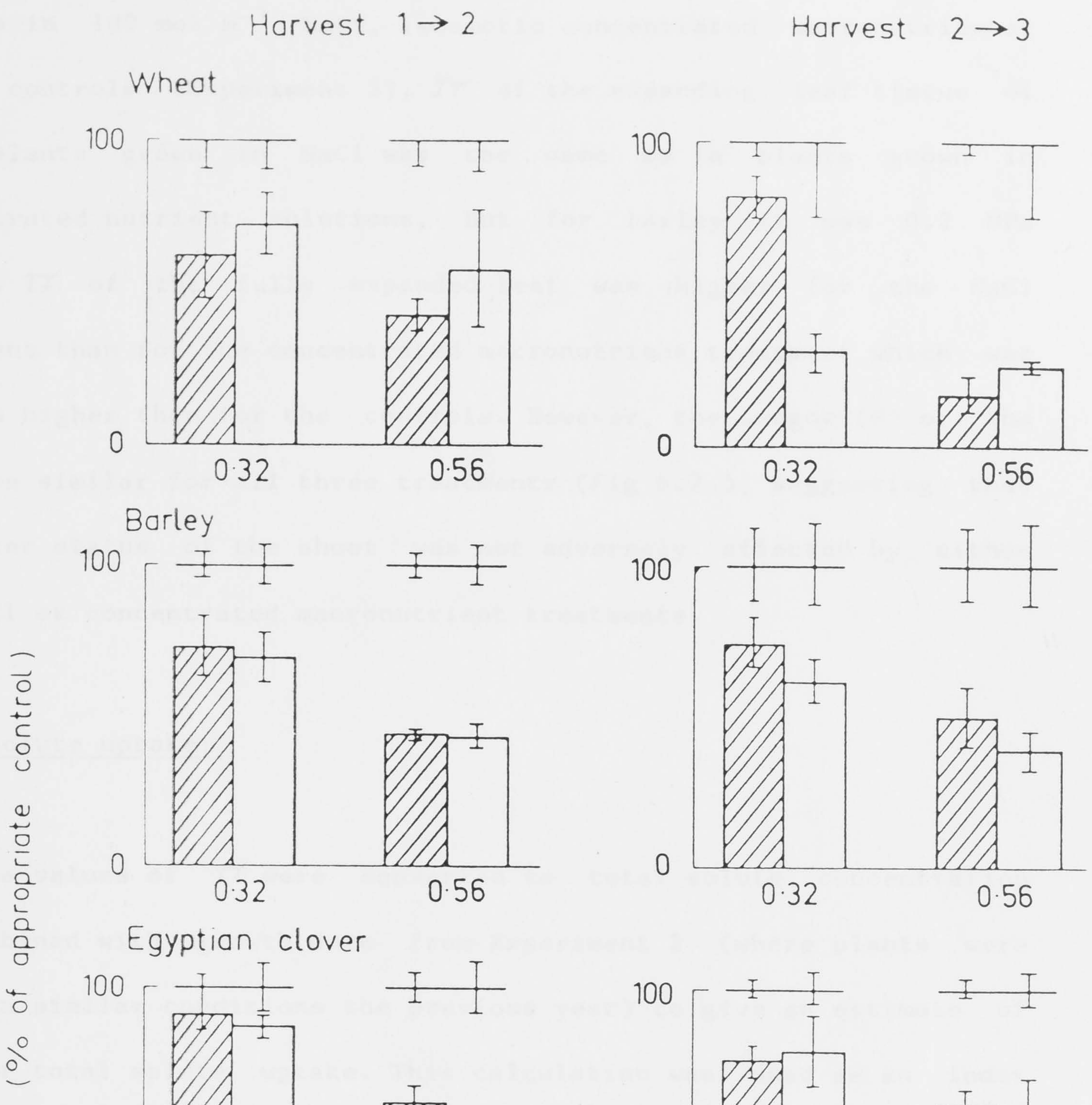
The osmotic pressures and water potentials of an elongating leaf and a fully expanded leaf were measured in barley and wheat grown for

Figure 6.6.

Effect of 2.0. mol m^{-3} (▨) or minimal (□) phosphate (H_2PO_4) on RGR ($g\ g^{-1}\ d^{-1}$) of NaCl-treated plants as a percentage of RGR of appropriate controls (Experiment 4). Wheat, barley and Egyptian clover were grown in 50 mol m^{-3} NaCl, 0.32 MPa π, and 100 mol m^{-3} NaCl, 0.56 MPa π. White clover was grown in 25 mol m^{-3} NaCl, 0.20 MPa π, and 50 mol m^{-3} NaCl. Error bars indicate the S.E. of the mean of RGR for 3 replicate treatments, between harvests 1 and 2, 2 and 3. Harvest 1 occurred d 1 after the highest external π was reached, i.e., d 17, 16, 20 and 20, respectively, after germination of wheat, barley, Egyptian clover and white clover.

Effect of 2.0 mol m^{-3} (2.0 P) or minimal (Min P) phosphate on RGR of nutrient solution controls.

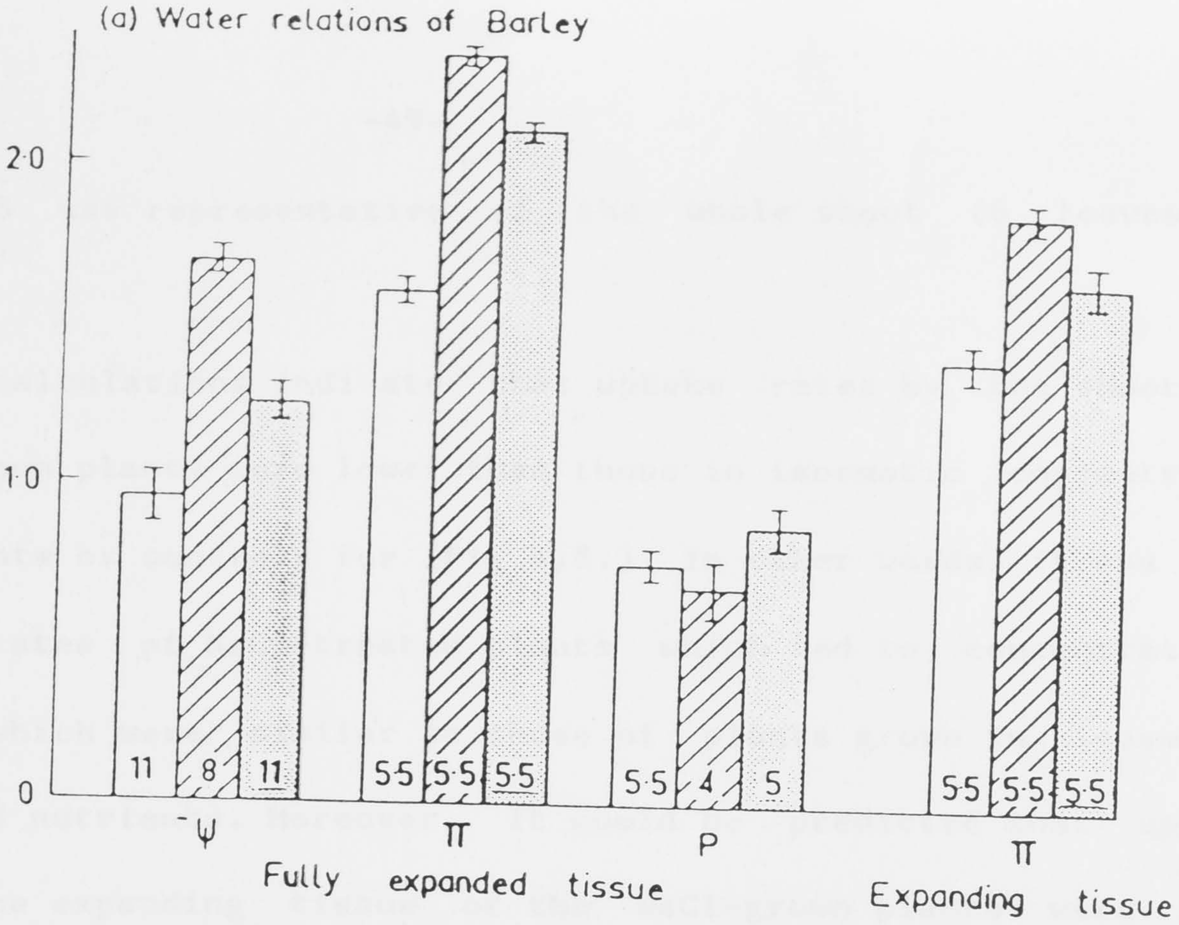
Period covered:	Harvest 1 - 2		Harvest 2 - 3	
Phosphate treatment:	2.0 P	Min P	2.0 P	Min P
Species:				
Wheat	0.272	0.175	0.271	0.204
Barley	0.271	0.204	0.151	0.143
Egyptian clover	0.242	0.184	0.183	0.142
White clover	0.285	0.110	0.177	0.124


Harvest 1 → 2
Harvest 2 → 3
Wheat
Barley
Egyptian clover
White clover
RGR (% of appropriate control)
100
0
0·32
0·56
0·20
0·32
MPa
MPa

10 days in 100 mol m^{-3} NaCl, isosmotic concentrated macronutrients, and in controls (Experiment 5). π of the expanding leaf tissue of wheat plants grown in NaCl was the same as in plants grown in concentrated nutrient solutions, but for barley it was 0.2 MPa higher. π of the fully expanded leaf was higher for the NaCl treatment than for the concentrated macronutrient treatment which was in turn higher than for the controls. However, the turgor (*P*) of the leaf was similar for all three treatments (Fig 6.7.), suggesting that the water status of the shoot was not adversely affected by eithor the NaCl or concentrated macronutrient treatments.

Total solute uptake:

The values of π were converted to total solute concentration and combined with growth data from Experiment 2 (where plants were grown in similar conditions the previous year) to give an estimate of rates of total solute uptake. This calculation was used as an index of total inorganic ion uptake because it was believed that the proportion of inorganic to organic solutes in the leaf sap does not change on exposure to NaCl. Delane *et al.*(1982) found that amino acids and sugars accounted for about 5-10% of the osmotic pressure in the fully expanded tissue of barley. Polonenko *et al.*(1983), who compared organic solute concentrations in barley grown in isosmotic solutions of NaCl and concentrated macronutrients, found little difference in levels of sugars and amino acids between the two treatments at π below 1.0 MPa. The calculation of the total solute uptake (formula 3, Section 2.4.) has the advantage over the summation of the inorganic ion uptake data in that it avoids accumulating errors. It was assumed that solute concentrations were constant with time because ion concentrations had been constant (see above) and



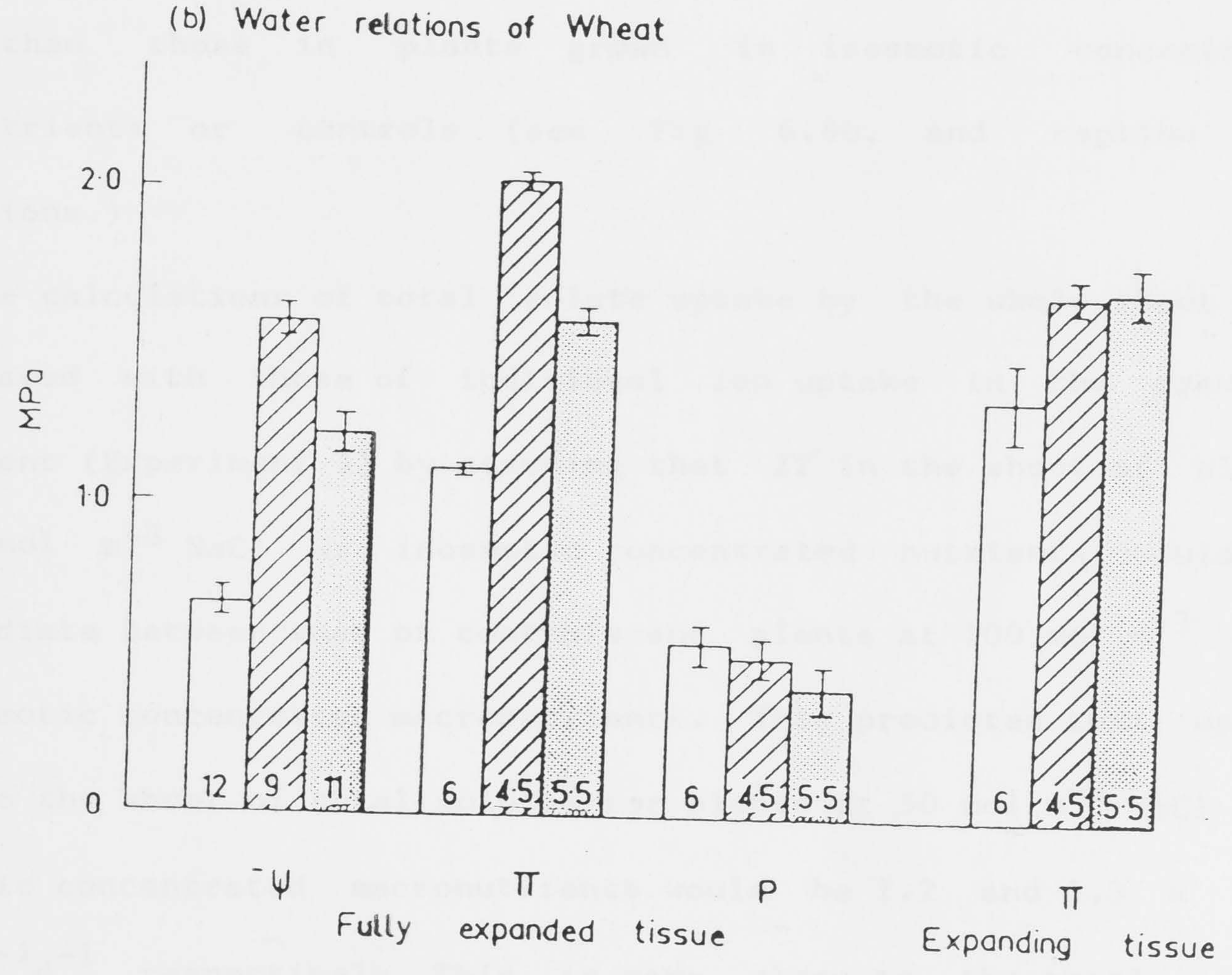


Figure 6.7.

Effect of control nutrient solution (☐), 100 mol m^{-3} NaCl (▨) or concentrated macronutrients, 0.56 MPa *π* (▒), on *ψ*, *π* and *P* of fully expanded tissue, leaf 3, and expanding tissue, leaf 6 (Experiment 5).

(a) wheat

(b) barley

Error bars indicate the standard error of the mean of measurements, 1 plant per measurement of *ψ*, 2 plants per measurement for *π* and estimation of *P*. Numbers on each bar indicate the numbers of replicates. Measurements were made d 11 after 0.56 MPa treatment began, i.e. d 21 after germination.

that leaf 3 was representative of the whole shoot (6 leaves in total).

These calculations indicate that uptake rates by the shoot of the NaCl-grown plants were lower than those in isosmotic concentrated macronutrients or controls for (Fig 6.8.). In other words, it was the low growth rates of NaCl-treated plants which led to concentrations of solutes which were similar to those of plants grown in isosmotic concentrated nutrients. Moreover, it could be predicted that uptake rates by the expanding tissue of the NaCl-grown plants were also lower than those in plants grown in isosmotic concentrated macronutrients or controls (see Fig 6.8b, and caption for assumptions.)

The calculations of total solute uptake by the whole shoot may be compared with those of individual ion uptake in the previous experiment (Experiment 3) by assuming that π in the shoot of plants at 50 mol m^{-3} NaCl or isosmotic concentrated nutrients would be intermediate between that of controls and plants at 100 mol m^{-3} NaCl or isosmotic concentrated macronutrients. This predicted that uptake rates to the shoot of total solutes for plants at 50 mol m^{-3} NaCl and isosmotic concentrated macronutrients would be 1.2 and 1.3 x 10^{-3} osmol $g^{-1}d^{-1}$ respectively. This is very close to the total uptake rate of all the ions measured in experiment 3.

Discussion:

The four species tested grew only half as well in NaCl as in concentrated macronutrients, suggesting that NaCl has specific effects in addition to osmotic effects. The mineral uptake data suggest the effects of concentrated nutrients are essentially osmotic. The rates of transport from the roots to the shoot of most

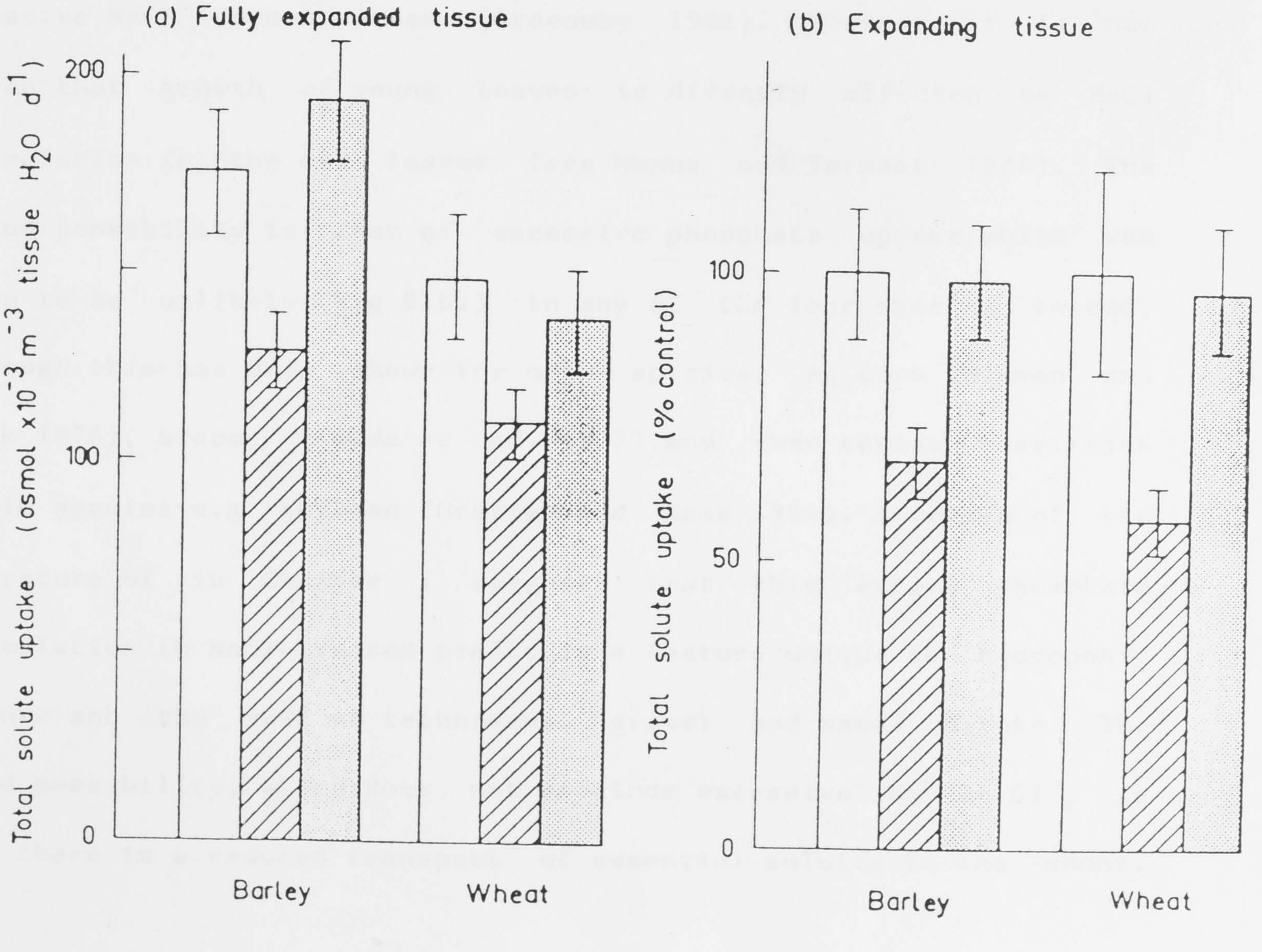
Figure 6.8.

Effect of control nutrient solution (□), 100 mol m^{-3} NaCl (▨), or concentrated macronutrients, 0.56 MPa Π (░) on total solute uptake in barley and wheat (Experiment 5).

(a) Total solute uptake (osmol x 10^{-3} m^{-3} tissue H_2O d^{-1}) for fully expanded tissue, assuming Π of leaf 3 is representative of the whole shoot (6 leaves total), and ion concentrations remain constant with time (see text). RGR_s^*, gH_2O $g^{-1}H_2O$ d^{-1}, was calculated from FW - DW data of Experiment 2, and was, for nutrient solution control, NaCl and concentrated macronutrient treatments respectively, 0.258, 0.130 and 0.219 for barley and 0.285, 0.123 and 0.203 for wheat.

(b) Total solute uptake as a percentage of nutrient soltion control, for the expanding region of barley and wheat, assuming RGR of the expanding region is proportional to RGR of the whole shoot, and Π of the expanding region remains constant with time. Dellane *et al.* (1982) found organic solutes contributed 20% of the Π in the expanding tissue, but this proportion was similar in plants grown in 0.5, 120 or 180 mol m^{-3} NaCl.

Error bars indicate the mean S.E. of RGR and Π estimations.



mineral nutrients were similar to controls; only Mg^{2+} transport to the shoot was higher (Fig 6.5.), and, as the root:shoot ratios were the same as controls, the uptake rates by the shoots were also similar to controls. If it is assumed that the effects of concentrated macronutrients on growth are essentially osmotic (i.e. are due to reduced water uptake rather than to any specific ion effects), then the additional effects of NaCl are due to specific ion effects.

The specific effects of NaCl on the shoot could be excessive transport to the shoot of Na^+ or Cl^-, excessive transport to the shoot of other ions such as phosphate, or an inadequate transport of other ions such as K^+, Ca^{2+}, Mg^{2+}, NO_3^-or SO_4^{2-}. The first possibility is that of excessive uptake of Na^+ or Cl^-: this could have specific effects on metabolism if NaCl was not compartmented in the vacuole (Flowers *et al.* 1977) , or could have osmotic effects in a sense if NaCl accumulated in the apoplast of old leaves (see Munns and Passioura 1984b). It is clear that death of leaves is due to excessive NaCl concentrations (Greenway 1962). However, it is not proven that growth of young leaves is directly affected by NaCl accumulation in the old leaves (see Munns and Termaat 1986). The second possibility is that of excessive phosphate uptake which was shown to be unlikely (Fig 6.6.) in any of the four species tested, although this has been shown for other species, eg corn (Nieman and Clark 1976), sesame (Cerda *et al.* 1977) and even certain varieties within species e.g. soybean (Gratten and Maas 1984). A review of the literature of in Chapter 1 suggests that this excess phosphate accumulation in NaCl-treated plants is a feature unique to hydroponic culture and the related techniques, gravel and sand culture. The third possibility, which does not preclude excessive Na^+ or Cl^-, is that there is a reduced transport of essential solutes to the shoot.

Even though calculations of transport rates do not show whether or not transport from the root is limiting growth of the shoot, several interesting relationships are revealed by this method. Increased phosphate accumulation in the shoot of NaCl-grown plants apparently results from root transport rates that are unchanged compared to controls while transport of other macronutrients decreases (Fig 6.5a.). The decrease in K transport is exactly countered by the increase in Na transport; similar evidence can be found for direct competition of N by Cl (Fig 6.5a.). Both examples of ion competition are known (eg, Dean Drummond and Glass 1982; Jeschke 1984); it is suggested that interference with transport from the roots of an essential nutrient may result in feedback control by the shoot of others.

If transport of one or more minerals from the root to the shoot is limiting growth, the increase in root:shoot ratio may be a consequence of the decreased shoot growth. In time, the change in root:shoot ratio (and clover is not exempt from this, c.f. Winter and Lauchli 1982) may reflect the poor growth of plants in NaCl, when, just as in droughted plants (Passioura 1983),the relatively large root system imposes extra demands on the shoot for assimilates. A possible explanation of why the increase in root:shoot ratio is associated with NaCl-treated plants is suggested by Trewavas(1985) who has proposed that carbohydrate (C) and organic nitrogen (N) both regulate the growth of plant organs; high C/N increasing growth rates of the roots and stimulating floral initiation and leaf senesence in the shoot. To apply this model to NaCl-stressed plants, the uptake of N to the roots would be reduced directly through competition by Cl^- (see Dean-Drummond and Glass 1982). This would increase the C/N ratio in the root, which, being first to come into contact with this N, would compete more effectively than the shoot for this mineral. Shoot

C/N would be increased as a consequence. Earlier dates of maturation are well known in NaCl-treated plants (eg, Ayers *et al.* 1952) and enhanced rates of leaf senescence are also documented (Prisco and O'Leary 1972). This model could be tested if the amouts of C and N reaching the growing region of the shoot were known.

Alternatively, the lower rate of transport of minerals from the root to the shoot could be due to feedback control by the shoot whose growth rate has decreased for another reason; if so the feedback does not operate on phosphate transport. Figure 6.9 shows that for the period between harvest 1 and 2, there was already a linear relationship between relative growth rates and root:shoot ratio for barley and wheat (data from Experiment 2), and a week later this relationship was much stronger. According to this relationship, the absence of a statistically significant increase in the root:shoot ratio of the plants grown in concentrated macronutrient is simply because shoot and size growth was not much lower than controls. Because there was no change in root:shoot ratio in the clover species in these experiments, a similar relationship could not be obtained for clover.

Is reduced root transport the cause of growth reduction or the result of it? The data reported in this chapter, being for a steady state situation, cannot enable one to distinguish between the two possiblities.

Conclusion:

Solutions of concentrated macronutrients appeared to have no toxic or specific ion effects on plant growth, although plants grown in these solutions were smaller. Uptake rates of total solutes to both the elongating and mature tissue resembled control plants, and

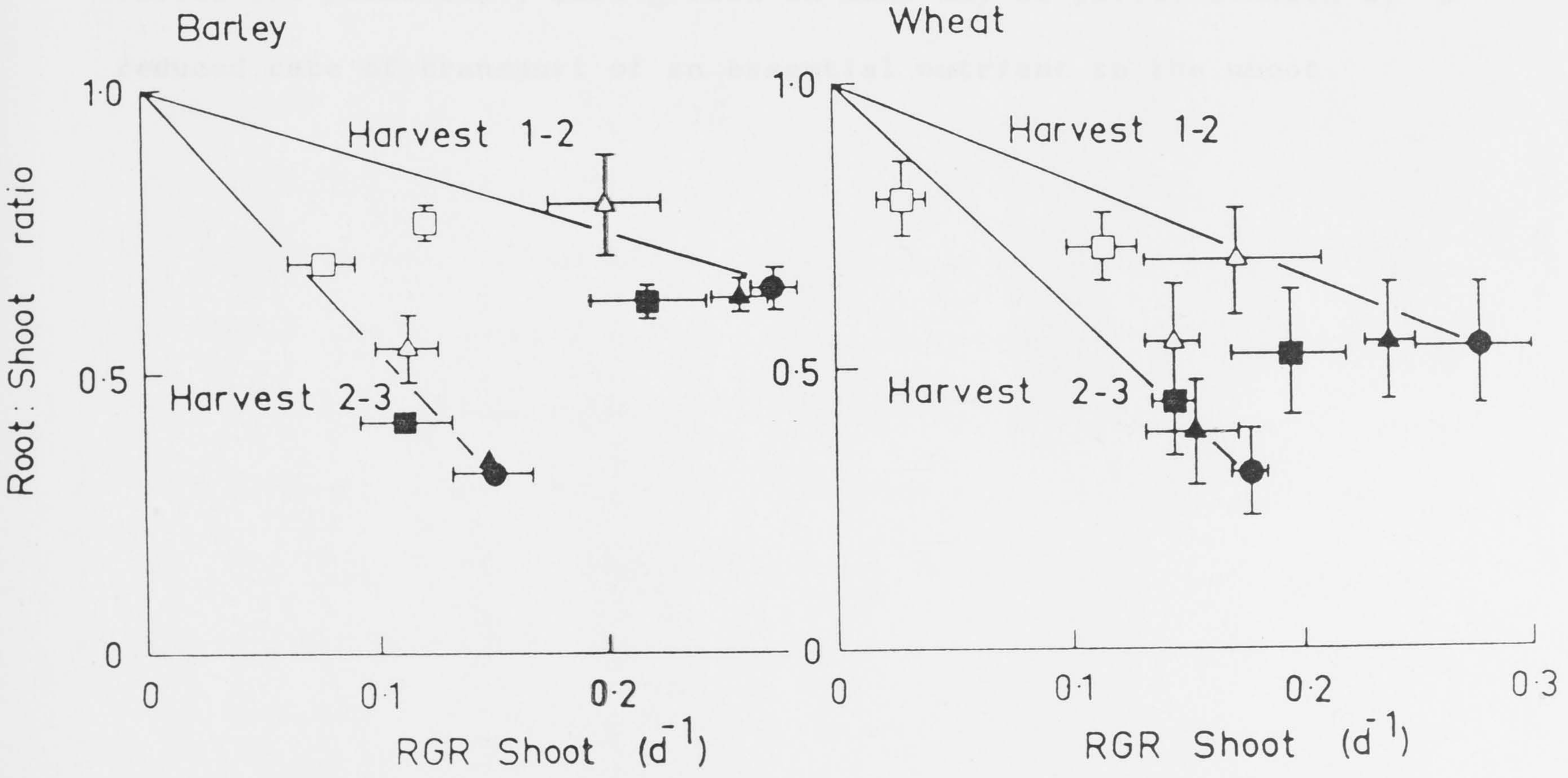
Figure 6.9.

Effect of RGR of plants in control nutrient solution (●), 50 mol m^{-3} NaCl, 0.32 MPa π (△) or concentrated macronutrients, 0.32 MPa π (▲), 100 mol m^{-3} NaCl, 0.56 MPa π (□) or concentrated macronutrients, 0.56 MPa π (▨), on root:shoot ratio between harvest 1 and 2, 2 and 3 (Experiment 2).

(a) wheat

(b) barley

Error bars indicate S.E. of the means of RGR and root:shoot ratios of 3 replicate treatments. Lines are drawn between controls and a root:shoot ratio value of 1.0.



the balance of mineral nutrients entering the shoot resembled control plants. It is suggested that solutions of concentrated macronutrients can provide a useful osmoticum against which to test ion-specific effects of NaCl.

After 14 days, NaCl-treated wheat, barley, Egyptian clover and white clover were half the size of plants grown in concentrated macronutrients. A NaCl-induced increase in phosphate uptake did not cause this additional reduction in shoot growth. For barley, net transport of N, K, Mg and Ca from the roots (per g root DW) was lower in NaCl-treated plants than controls, but uptake by the shoot (per g shoot DW) of these minerals was similar. NaCl-treated barley and wheat plants had higher osmotic pressures in both expanding and fully expanded tissue than did controls and macronutrient-grown plants, but lower rates of uptake of solutes generating this pressure. This raises the possibility that growth in NaCl may be partly limited by a reduced rate of transport of an essential nutrient to the shoot.

Chapter 7

CONCLUDING DISCUSSION

7.1. Introduction:

Three hypotheses formed the basis of this study: that the growth of shoots of NaCl-treated plants, was limited by the supply of solutes needed to generate turgor in the growing cells, by changed hormonal messages from the roots, or by a deficiency or excess of a major nutrient. This discussion aims to evalute the data of the thesis as a whole.

7.2. The hypotheses:

Hypothesis 1 : Is reduced growth in NaCl due to inadequate turgor of the expanding cells of the shoot? If the growth at high NaCl, in the medium-term, was limited by an insufficient supply of ions and other solutes to the growing region to generate turgor (as was suggested by Delane *et al.* 1982), raising the turgor by applying pressure would have increased growth by reducing the need for these solutes (Chapter 4). Growth was not improved, therefore turgor, and processes generating turgor, were not limiting shoot growth. Turgor, although it presumably is necessary for growth, is not regulating shoot growth but is overridden by some other factor.

It is not likely that the factor limiting the growth of the shoot is an inadequate supply of assimilate, even though photosynthesis may be affected. The total concentration of soluble and insoluble carbohydrates increases in both the expanded and fully expanded leaves of NaCl-treated barley (Munns *et al.* 1982) and in the whole shoot of plants grown in concentrated macronutrients (Polonenko *et al.* 1983), which suggests assimilate is in ample supply. Neither is it likely that the cells in the growing region are suffering from a metabolic upset, such as the poisoning of an important enzyme by high local concentrations of Na^+ or Cl^-, and that this would have prevented them responding to the increased turgor. Firstly, there was no improvement of growth of plants grown in concentrated macronutrients when pressure was applied (Chapter 4); these plants did not contain Na^+ or Cl^-(Chapter 6). Secondly, the growth rate of NaCl-treated plants responds so rapidly to the removal of NaCl from around the roots (Munns *et al.* 1982; Rawson and Munns 1984) that the specific effects of Na^+ and Cl^- on the metabolism of the leaves seem to be ruled out: the recovery of growth occurs much faster than the cellular concentrations of Na^+ and Cl^- are substantially lowered. Growth in the short- to medium-term is also unlikely to be limited by a specific mineral nutrient (such as K^+ or NO_3^-), because (as mentioned above) there was no improvement of growth of plants grown in concentrated macronutrients when pressure was applied, and in these plants the transport of these essential nutrients from the roots would not have decreased (Chapter 6).

Unlike the experiments which showed rapid recovery of growth when NaCl was removed from the root environment (Munns *et al.* 1982; Rawson and Munns 1984), neither turgor nor the root's ionic status were changed by the applied pressure (Passioura and Munns 1984a). Therefore, a likely possibility is that the status of the root regulates the growth of the shoot via a message moving from the root to the shoot. The message, limiting growth in both NaCl-treated and concentrated macronutrient-treated plants, is more likely to be a growth regulator than a nutrient because rates of transport from the root of major nutrients were similar to controls in the concentrated macronutrient treatment (Chapter 6).

Hypothesis 2: Is the reduced growth in NaCl due to insufficient cytokinins arising from the root? Cytokinins seemed the most likely hormone as they are known to arise predominently in the roots, and their production seemed to be affected by low external ψ.

Kinetin (a synthetic cytokinin known to affect wheat, reviewed in Michael and Beringer 1980) did not affect NaCl-treated wheat, even though a wide range of concentrations were tried. Kinetin (10^{-3} m^{-3}), affected only wheat grown without NaCl, which suggested receptor frequency or sensitivity (c.f., Trevawas 1982) declined in NaCl-treated plants. The possibility that kinetin could improve the growth rates of pressurised, concentrated macronutrient-treated plants was not tested, but it is likely their response to kinetin would be similar to NaCl-treated plants, because plants grown osmotica other than NaCl (mannitol) were also less sensitive to applied hormones (GA applied to *Brassica campestris*, Banyal and Rai 1984; kinetin, ABA or combinations of the two applied to tobacco, Mizrahi *et al.* 1970) than controls grown in high ψ.

Because the growth of pressurised, NaCl-treated wheat was not affected by applied kinetin, a message other than, or in addition to, a cytokinin is limiting the growth of these plants. This message would regulate shoot growth of any osmotically-treated plant.

Hypothesis 3: Is reduced growth in NaCl due to NaCl-specific disturbances in the mineral transport from the roots? Transfer from NaCl to isosmotic concentrated macronutrients caused no change in growth rate in the shot-term (0.5 - 2.0 h), which suggested that water uptake from two solutions was the same. However, in the medium- to long-term (7 d - 3 weeks) NaCl-treated plants grew more slowly than those in isosmotic concentrated nutrients (Chapter 6). The additional growth reduction of plants grown in isosmotic solutions must be due to NaCl-specific effects.

What could the NaCl-specific effects on the shoot be?

The most likely additional constraints are excessive transport to the shoot of Na^+ or Cl^-, excessive transport to the shoot of other ions such as phosphate, or inadequate transport or mineral nutrients such as K^+ or NO_3^-(Chapter 1). Because it is toxic to metabolic reactions, intracellular NaCl is presumed to be largely compartmented in the vacuole (e.g., reviewed by Flowers *et al.* 1977, Flowers and Lauchli 1983). Death rates of older leaves in barley are certainly enhanced by NaCl accumulation (Greenway 1962, Munns and Passioura 1984), but as the importance of older leaves rapidly diminishes as a source of carbon for developing leaves, which supply about half their carbohydrate requirements (Anderson and Dale 1983), the stage at which leaf death has a critical effect on growing leaves is unclear. Excessive phosphate uptake to the shoot was shown to be unlikely in the experiments in this study (Chapter 6). NaCl-induced phosphate toxicity may be an artifact of hydroponic techniques, and is unreported for soil-grown NaCl-treated plants (Chapter 1). Inadequate transport of K^+ is less likely than NO_3^- to be the cause of the additional growth reduction in NaCl-treated plants; in tomato up to 90% of leaf K^+ can be displaced by Na^+ without any reduction in growth rate occurring (reviewed in Flowers and Lauchli 1983). But nitrogen levels are often reduced in NaCl-treated plants, which, particularly at low salinities, also frequently respond to NO_3^- fertilization (Chapter 1).

It may be possible to test which ion, Na^+ or Cl^-, is the more toxic to NaCl-treated plants by growing plants in isosmotic solutions of concentrated macronutrients, NaCl, and mixtures of mineral nutrients where Na^+ and Cl^- replace the cations and anions. Changing treatments from one isosmotic solution to another and simultaneuosly monitering xylem ion concentrations as well as growth rates could determine whether reduced transport of either of these minerals is responsible for the additional growth reduction (compared to plants in isosmotic concentrated macronutrients) shown by NaCl-treated plants. All the ions in the xylem of all treatments would need to be measured, as a change in cation composition may alter the uptake rates of some anions. For example, replacing NaCl with KCl in the nutrient solution enhanced Cl^- uptake (bean, Salim and Pitman 1983; *Sorghum bicolor*, Weimburg *et al.* 1984).

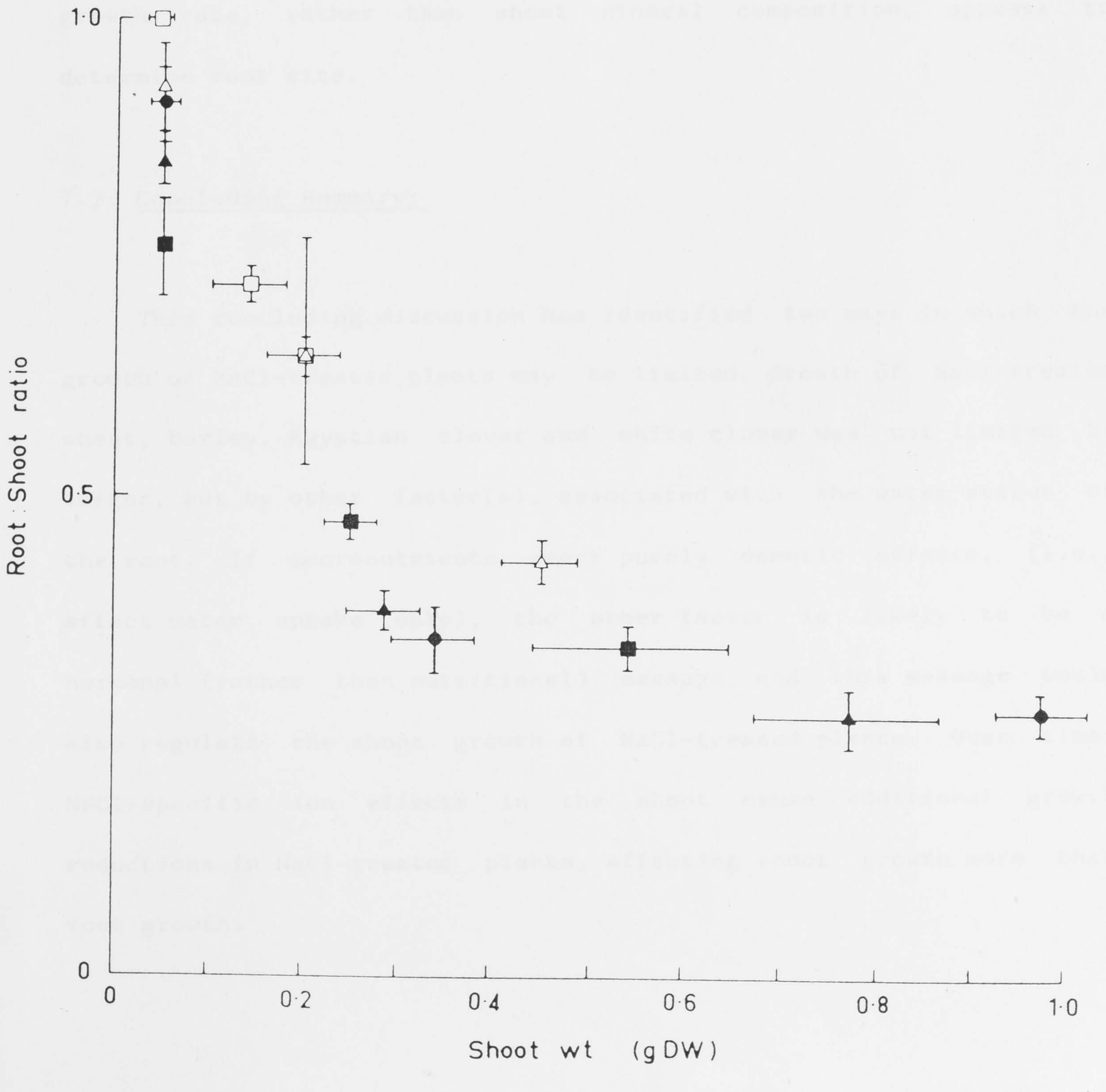
What could the NaCl-specific effects on the roots be?

Root:shoot ratios increase in NaCl-treated plants and plants grown in a dry soil (reviewed in Munns and Termaat 1986, Bernstein and Hayward 1958, Passioura 1983), and both these conditions also increase shoot carbohydrate concentrations (e.g., in NaCl, Delane *et al.* 1982; in dry soil, Ackerson 1981). A likely explanation is that shoot growth is affected more than photosynthesis, thereby increasing the amount of assimilate available for root growth. Once established, the relatively large root will impose extra demands on the shoot for assimilates (c.f. Passioura 1983).

Figure 6.9. suggests that the size of the root correlated with the RGR of the shoot, regardless of the composition of the saline solution in which the plants were grown. If absolute shoot size only is considered, the root:shoot ratio will always be higher for the NaCl-treated plants (Fig 7.1. using data from Experiment 2, Chapter 6). Is this simply because, for any particular shoot size, the shoots

Figure 7.1.

Effect of shoot weight (g DW) of plants in control nutrient solution (●), 50 mol m^{-3} NaCl, 0.32 MPa Π (△) or concentrated macronutrients, 0.32 MPa Π (▲), 100 mol m^{-3} NaCl, 0.56 MPa Π (□) or concentrated macronutrients, 0.56 MPa Π (■) on root:shoot ratio for the 2 periods between harvests 1 and 3 (Chapter 6, experiment 2). Error bars indicate S.E. of the means of RGR and root:shoot ratios, of 3 replicate treatments.



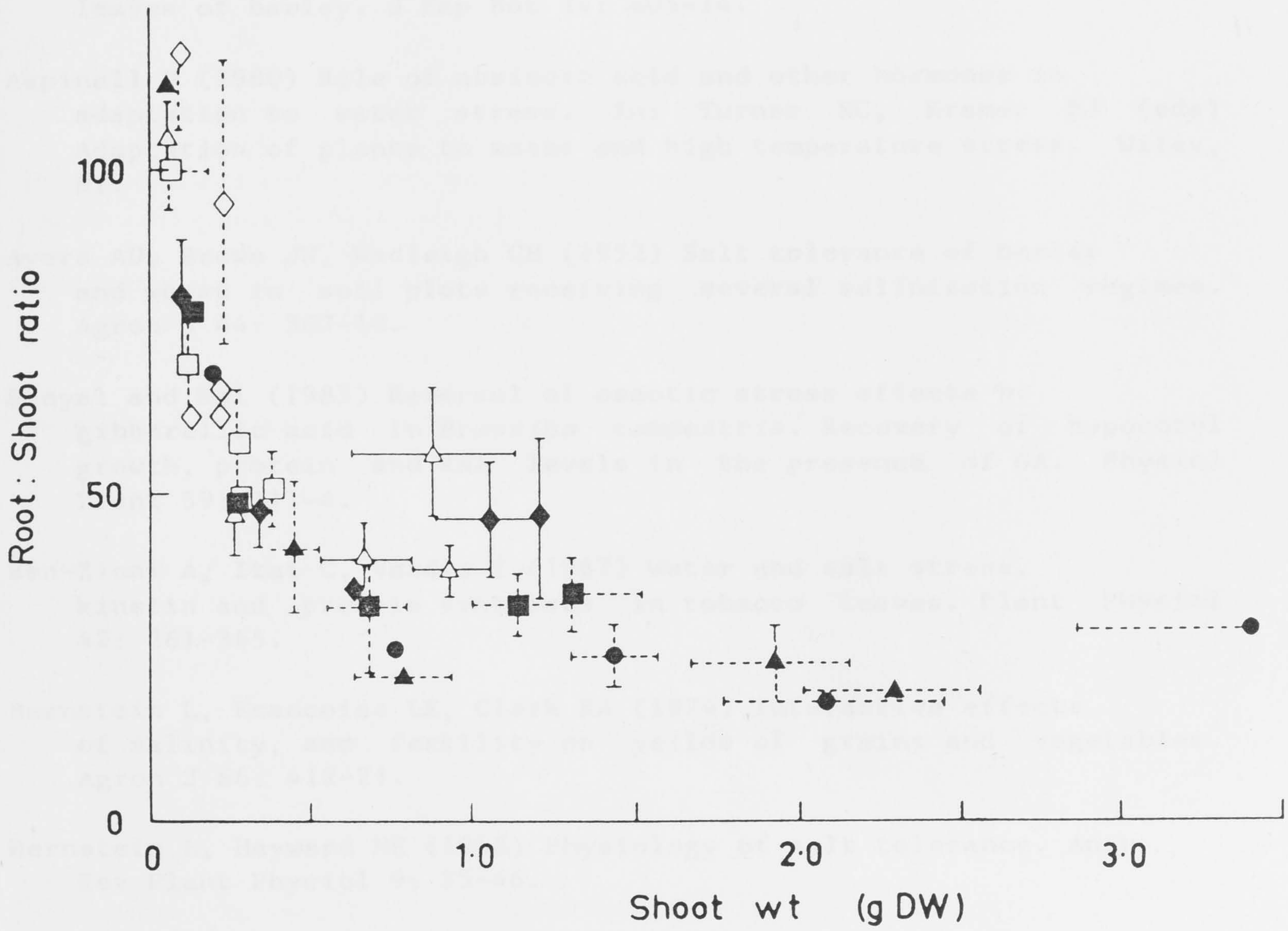
of NaCl-treated plants were growing more slowly, consuming less of the carbohydrate that was fixed than plants grown in concentrated macronutrient plants, and were therefore supplying the roots with relatively more carbohydrate? Polonenko *et al.* (1983) found for an external π up to 1.0 MPa, carbohydrate concentrations in the shoots of plants in NaCl and isosmotic concentrated nutrients were similar; however, NaCl-treated plants would be growing more slowly. Figure 7.2. uses data from Experiment 3, Chapter 6, to observe the relationship between shoot size and root:shoot ratio. This was the only experiment in which an NaCl treatment (50 mol m^{-3} NaCl) had plants of a similar size and growing at similar rates to plants in concentrated macronutrients (0.80 MPa π). Figure 7.2. shows these two treatments had similar sized roots. Therefore, shoot size and growth rate, rather than shoot mineral composition, appears to determine root size.

7.3. Concluding summary:

This concluding discussion has identified two ways in which the growth of NaCl-treated plants may be limited. Growth of NaCl-treated wheat, barley, Egyptian clover and white clover was not limited by turgor, but by other factor(s), associated with the water status of the root. If macronutrients exert purely osmotic effects, (i.e., affect water uptake only), the other factor is likely to be a hormonal (rather than nutritional) message; and this message would also regulate the shoot growth of NaCl-treated plants. Over time, NaCl-specific ion effects in the shoot cause additional growth reductions in NaCl-treated plants, affecting shoot growth more than root growth.

Figure 7.2.

Effect of shoot weight (g DW) of plants in control nutrient solution (●), 50 mol m^{-3} NaCl, 0.32 MPa π (△) or concentrated macronutrients, 0.32 MPa π (▲), 100 mol m^{-3} NaCl, 0.56 MPa π (□) or concentrated macronutrients, 0.56 MPa π (■), 150 mol m^{-3} NaCl, 0.80 MPa π (◇) or concentrated macronutrients, 0.80 MPa π (◆) on root:shoot ration for the 4 period between harvests 1 and 5 (Chapter 6, Experiment 3). Error bars indicate S.E. of the means of RGR and root:shoot ratios, of 3 replicate treatments.



REFERENCES

Abdel-Rahman AM, Abdel-Hadi AH (1984) Possibilities to reduce adverse effects of salinity by indole-3-acetic acid. Biol Plant (Prague) 26: 81-87.

Acevedo E, Hsiao TC, Henderson DW (1971) Immediate and subsequent growth responses of maize leaves to changes in water status. Plant Physiol 48: 631-36.

Ackerson RC (1981) Osmoregulation in cotton in response to water stress. Plant Physiol 67: 489-93.

Ahroni N, Blumenfeld A, Richmond AE (1977) Hormonal activity in detached lettuce leaves as affected by leaf water content. Plant Physiology 59: 1169-73.

Anderson LS, Dale JE (1983) The sources of carbon for developing leaves of barley. J Exp Bot 34: 405-14.

Aspinall D (1980) Role of absiscic acid and other hormones in adaptation to water stress. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. Wiley, NY.

Ayers AD, Brown JW, Wadleigh CH (1952) Salt tolerance of barley and wheat in soil plots receiving several salinization regimes. Agron J 44: 307-10.

Banyal and Rai (1983) Reversal of osmotic stress effects by gibberellic acid in *Brassica campestris*. Recovery of hypocotyl growth, protein and RNA levels in the presence of GA. Physiol Plant 59: 111-4.

Ben-Zioni A, Itai C, Vaadia Y (1967) Water and salt stress, kinetin and protein synthesis in tobacco leaves. Plant Physiol 42: 361-365.

Bernstein L, Francoise LE, Clark RA (1974) Interactive effects of salinity, and fertility on yeilds of grains and vegetables. Agron J 66: 412-21.

Bernstein L, Hayward HE (1958) Physiology of salt tolerance. Annu Rev Plant Physiol 9: 25-46.

Bhivare VB, Nimbalker JD (1984) Salt stress effects on growth and mineral nutrition of French beans. Plant Soil 80: 91-98.

Blackman PG, Davies WJ (1983) The effects of cytokinins and ABA on stomatal behaviour of maize and *Commelina*. J Exp Bot 34: 1619-26.

Blackman PG, Davies WJ (1984) Modification of CO_2 responses of maize stomata by abscisic acid and by naturally-occurring and synthetic cytokinins. J Exp Bot 35: 174-79.

Boucard J, Ungar IA (1976) Influence of hormonal treatments on the growth of two halophytic species of *Suaeda*. Amer J Bot 63: 694-9.

Bradford KJ, Hsiao TC (1982) Physiological responses to moderate water stress. In: Lange OL, Nobel PS, Osmond CB, Zeigler H (eds) Water relations and carbon assimilation, physiological plant ecology. Vol II. Encycl Plant Physiol, New Ser vol 12B. Springer-Verlag, Berlin.

Bradford KJ (1983) Involvement of plant growth substances in the alteration of leaf gas exchange in flooded tomato plants. Plant Physiol 73: 480-83.

Canny MJ (1985) Ashby's law and the pursuit of plant hormones: a critique of accepted dogmas, using the concept of variety. Aust J Plant Physiol 12: 1-8.

Campagnol F (1979) Relationships between phosphate nutrition of plants and salt toxicity. Phosphorus Agric 76: 35-43.

Carmi A, Van Staden J (1983) Role of roots in regulating the growth rate and cytokinin content in leaves. Plant Physiol 73: 76-8.

Carr DJ, Reid DM, Skene KGM (1964) The supply of gibberellins from the root to the shoot. Planta 63: 382-92.

Cerda A, Bingham FT, Hoffman GJ (1977) Interactive effect of salinity and phosphorus on sesame. Soil Sci Soc Am 41: 915-18.

Cerda A, Bingham FT (1978) Yeild, mineral composition and salt tolerance of tomato and wheat as affected by NaCl and phosphate nutrition. Agrochim (?) 12: 140-9.

Cosgrove DJ, Van Volkenburgh E, Cleland, RE (1984) Stress relaxation of cell walls and the yeild threshold for growth. Planta 162: 46-54.

Cowan IR, Raven JA, Hartung W, Farquhar GD (1982) A possible role for absisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. Aust J Plant Physiol 9: 489-98.

Dean-Drummond CE, Glass, ADM (1982) Studies of nitrate influx into barley roots by the use of $^{36}\mathrm{CIO}_3^-$ as a tracer for nitrate. I. Interactions with chloride and other ions. Can J Bot 60: 2147-53.

Delane R, Greenway H, Munns R, Gibbs J (1982) Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl. I. Relationship between solute concentration and growth. J Exp Bot 33: 557-73.

Ehlig CF, Gardner WR, Clark M (1968) Effects of soil salinity on water potentials and transpiration in pepper (*Capsicum frutescens*). Agron J 60: 249-53.

Erickson LC (1947) Growth of tomato roots as influenced by oxygen in the nutrient solution. Amer J Bot 33: 551-61.

Evans HJ, Sorger GJ (1966) Role of mineral elements with emphasis on the univalent cations. Annu Rev Plant Physiol 17: 47-76.

Feigin A (1985) Fertilization of crops irrigated with saline water. Plant Soil 89: 285-99.

Ferguson WS, Hedlin RA (1963) Effect of soluble salts on plant response to and absorption of phosphorus. Can J Soil Sci 43: 210-.

Fitzsimmons RW, Martin RH, Wrigley CW (1983) Australian wheat varieties: identification according to plant, head and grain characteristics. 2nd Edn. CSIRO, Melbourne.

Fitzsimmons RW, Wrigley CW (1984) Australian barleys: identification of varieties, grain defects and foreign seeds. 2nd Edn. CSIRO, Melbourne.

Flowers TJ, Troke, PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. Annu Rev Plant Physiol 28: 89-121.

Flowers TJ, Lauchli A (1983) Sodium versus potassium: subtitution and compartmentation. In: Lauchli A, Bieleski RL (eds) Inorganic plant nutrition. Part B. Encycl Plant Physiol, New Ser vol 15B. Springer-Verlag, Berlin.

Gates CT, Haydock KP, Robins, MF (1970) Response to salinity in glycine. 4. Salt concentration and the content of phosphorus, potassium, sodium and chloride in cultivars of *G. wightii* (*G. javanica*). Aust J Exp Agric Anim Husb 10: 99-110.

Glass ADM, Siddiqui MY (1985) Nitrate inhibition of chloride influx in barley: implications for a proposed chloride homeostat. J Exp Bot 36: 556-66.

Gratten SR, Maas EV (1984) Interactive effects of salinity and substrate phosphate on soybean. Agron J 76: 688-76.

Gratten SR, Maas EV (1985) Root control of leaf phosphorus and chlorine accumulation in soybean under salinity stress. Agron J 77: 890-95.

Green PB, Erickson RO, Buggy J (1971) Metabolic and physical control of cell elongation rate. *In vivo* studies in *Nitella*. Plant Physiol 47: 423-30.

Greenway H (1962) Plant response to saline substrates. I. Growth and ion uptake of several varieties of *Hordeum* during and after sodium chloride treatment. Aust J Biol Sci 15: 16-38.

Greenway H, Munns R (1983) Interactions between growth, uptake of Cl^- and Na^+, and water relations of plants in saline environments. II. Highly vacuolated cells. Plant Cell Environ 6: 575-589.

Hayward HE, Long EM (1941) Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate and nutrient solutions. Bot Gaz 102: 437-61.

Henson IE (1983) Abscisic acid and water relations of rice (*Oryza sativa* L.): effects of drought conditioning on abscisic acid accumulation in the leaf and stomatal response. Ann Bot (Lond) 52: 247-55.

Itai C, Richmond A, Vaadia Y (1968) The role of root cytokinins during water and salinity stress. Isr J Bot 17: 187-95.

Itai C, Vaadia Y (1971) Cytokinin activity in water-stressed shoots. Plant Physiol 47: 87-91.

Jeschke WD (1984) K^+ - Na^+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. IN: Staples RC, Toenniessen GH (eds) Salinity tolerance in plants. Strategies for Crop Improvement. Wiley, NY.

John MK (1970) Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. Soil Sci 109: 214-20.

Kafkafi U, Valoras N, Letey J (1982) Chloride interaction with nitrate and phosphate nutrition in tomato (*Lycopersicon esculentum* L.). J Plant Nutr 5: 1369-85.

Kazama H, Katsumi M (1983) Gibberellin-induced changes in the water absorption, osmotic potential and starch content of cucumber hypocotyls. Plant Cell Physiol 24: 1209-16.

King RW (1976) Implications for plant growth and the transport of regulatory compounds in phloem and xylem. In: IF Wardlaw, JB Passioura (eds) Transport and transfer processes in plants, Academic Press, NY.

Khalil MA, Amer F, Elgabaly MM (1967) A salinity-fertility interaction study in corn and cotton. Soil Sci Soc Amer Proc 31: 683-6.

Kirkham MB, Gardner WR, Gerloff GC (1974) Internal water status of kinetin-treated, salt-stressed plants. Plant Physiol 53: 241-3.

Langdale GW, Thomas JR (1971) Soil salinity effects in absorption of nitrogen, phosphorus and protein synthesis in coastal bermudagrass. Agron J 63: 708-11.

Langdale GW, Thomas JH, Littleton TG (1973) Nitrogen metabolism of stargrass as affected by nitrogen and sol salinity. Agron J 65: 468-70.

Livne A, Vaadia Y (1965) Stimulation of transpiration rate in barley leaves by kinetin and gibberellic acid. Physiol Plant 18: 658-64.

Lunin J, Gallatin MH (1965) Salinity-fertility interactions in relation to the growth and composition of beans. I. Effects of N, P and K. Agron J 57: 39-45.

Mashady AS, Sayed HI, Heake; MS (1982) Effect of soil salinity and water stresses on growth and content of nitrogen, chloride and phosphate of wheat and triticale. Plant Soil 68: 207-16.

Matsuda K, Raizi A (1981) Stress-induced osmotic adjustment in growing regions of barley leaves. Plant Physiol 68: 571-6.

Mexal J, Fisher JT, Osteryoung J, Reid CPP (1975) Oxygen availability in polyethylene glycol solution and its implications in plant-water relations. Plant Physiol 55:20-4.

Miller CO (1968) Naturally-occurring cytokinins. In: Wightman F, Setterfeild G (eds) Biochemistry and physiology of plant growth substances. The Runge Press, Can.

Michael G, Beringer H (1980) The role of hormones in yeild formation In: Physiological aspects of crop productivity. Proc. 15th Colloq Int Potash Institute, Bern.

Mizrahi Y, Blumenfeld A, Richmond AE (1970) Abscisic acid and transpiration in leaves in relation to osmotic root stress. Plant Physiol 46: 169-71.

Mizrahi Y, Blumenfeld A, Bittner S, Richmond Ae (1971) Abscisic acid and cytokinin contents of leaves in relation to salinity and relative humidity. Plant Physiol 48: 752-5.

Mizrahi Y, Richmond AE (1972) Hormonal modification of plant response to water stress. Aust J Biol Sci 25: 437-42.

Mizrahi Y, Scherings SG, Malis Arad S, Richmond, AE (1974) Aspects of the effects of ABA on the water status of barley and wheat seedlings. Physiol Plant 31: 44-50.

Mulkey TJ, Evans ML, Kuzmanoff KM (1983) The kinetics of abscisic acid action on root growth and gravitropism. Planta 157: 150-57.

Munns R (1985) Na^+, K^+ and Cl^- in the xylem sap flowing to the shoots of NaCl-treated barley. J Exp Bot 36: 1032-42.

Munns R, Greenway H, Delane R, Gibbs J (1982) Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl. II. Cause of the growth reduction. J Exp Bot 33: 574-83.

Munns R, Greenway H, Kirst GO (1983) Halotolerant eukaryotes. In: Lange OL, Osmond CB, Nobel PS, Zeigler H (eds) Responses to the chemical and biological environment, physiological plant ecology, vol III. Encycl Plant Physiol. New Ser Vol 12C. Springer-Verlag, Berlin.

Munns R, Passioura JB (1984a) Hydraulic resistance of plants III Effects of NaCl in barley and lupin. Aust J Plant Physiol 11: 351-59.

Munns R, Passioura JB (1984b) Effect of prolonged exposure of NaCl on the osmotic pressure of leaf xylem sap from intact, transpiring barley plants. Aust J Plant Physiol 11: 497-507.

Munns R, Termaat A (1986) Whole-plant responses to salinity. Aust J. Plant Physiol 13: 143-60.

Nassery H, Ogata G, Neiman RH, Maas EV (1978) Growth, phosphate pools, and phosphate mobilization of salt-stressed sesame and pepper. Plant Physiol 62: 229-31.

Nieman RH, Bernstein L (1959) Interactive effects of gibberellic acid and salinity on the growth of beans. Am J Bot 46: 667-70.

Nieman RH, Clark RA (1976) Interactive effects of salinity and phosphorus nutrition on the concentrations of phosphate and phosphate esters in mature photosynthesizing corn leaves. Plant Physiol 57: 157-61.

Norrish K, Hutton JT (1977) Plant analysis by X-ray spectroscopy. I. Low atomic number elements, sodium to calcium. X-Ray Spect 6: 6-11.

Nulsen RA, Thurtell GW, Stevenson KR (1977) Response of leaf water potential to pressure changes at the root surface of corn plants (*Zea mays* L.). Agron J 69: 951-54.

Nye PH (1979) Soil properties controlling the supply of nutrients to the root surface. In: Harley JL, Russell RS (eds) The soil-root interface: proceedings of an international symposium held in Oxford, March 28-31, 1978. Academic Press, London.

O'Leary JW, Prisco JT (1970) Response of osmotically-stressed plants to growth regulators. Adv Front Plant Sci 25: 129-39.

Passioura JB (1980) The transport of water from soil to shoot in wheat seedlings. J Exp Bot 31: 333-45.

Passioura JB (1983) Roots and drought resistance. Agric Water Manage 7: 265-80.

Passioura JB, Munns R (1984) Hydraulic resistance of plants. II. Effects of rooting medium and time of day, in barley and lupin. Aust J Plant Physiol 11: 341-50.

Patel PM, Wallace A, Wallihan EF (1976) Influence of salinity and N-P fertility levels on mineral content and growth of sorghum in sand culture. Agron J 67: 622-5.

Pitman MG (1972) Uptake and transport of ions in barley seedlings. III. Correlation between transport to the shoot and relative growth rate. Aust J Biol Sci 25: 905-19.

Prisco JT, O'Leary JW (1972) Enhancement of intact bean leaf senescence by NaCl salinity. Physiol Plant 27: 95-100.

Prisco JT, O'Leary JW (1973) The effects of humidity and cytokinin on growth and water relations of salt-stressed bean plants. Plant Soil 39: 263-76.

Polonenko DR, Dumbroff EB, Mayfield CI (1983) Microbial response to salt-induced osmotic stress. III Effects of stress on metabolites in the roots, shoots and rhizosphere of barley. Plant Soil 73: 211-25.

Ravikovitch S, Porath A (1967) The effect of nutrients on the salt tolerance of crops. Plant Soil 26: 49-71.

Ravikovitch S, Yoles D (1971) The influence of phosphorus and nitrogen on millet and clover growing in soils affected by salinity. I. Plant Development. Plant Soil 35: 555-67.

Rawson HM, Munns R (1984) Leaf expansion in sunflower as influenced by salinity and short-term changes in carbon fixation. Plant Cell Environ 7: 207-13.

Reid DM, Wample RL (1985) Water relations and plant hormones. In Pharis RP, Reid DM Hormonal regulation and development III Role of environmental factors. Encycl Plant Physiol, New Ser, Vol 11. Springer-Verlag, Berlin.

Rozema J, Dueck T, Wesselman H, Bijl F (1983) Nitrogen dependent growth stimulation by salt in strand-line species. Acta Oecol Oecol Plant 4: 41-52.

Russell EW (1961) Chapter 18, In: Soil conditions and plant growth. Edition 9. Longmans, London.

Salama FM, Khodary SEA, Heikal MM (1981) Effect of soil salinity and IAA on growth, photsynthetic pigments and mineral composition of tomato and rocket plants. Phyton (Horn) 21: 177-88.

Salim M, Pitman MG (1983) Effects of salinity on ion uptake and growth of mung bean plants (*Vigna radiata* L.) Aust J Plant Physiol 10: 395-407

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. Negative hydrostatic pressure can be measured in plants. Science 148: 339-45.

Shalhevet J, Maas EV, Hoffman GJ, Ogata G (1976) Salinity and the hydraulic conductance of roots. Physiol Plant 38: 224-32.

Shaybany B, Kashirad A (1978) Effect of NaCl in growth and mineral composition of *Acacia saligna* in sand culture. J Am Soc Hortic Sci 103: 823-6.

Shone MGT, Gale J (1983) Effect of sodium chloride stress and nitrogen source on respiration, growth and photosynthesis in lucerne (*Medicago sativa* L.). J Exp Bot 34: 1117-25.

Stenlid G (1982) Cytokinins as inhibitors of root growth. Physiol Plant 56: 500-06.

Termaat A, Passioura JB, Munns R (1985) Shoot turgor does not limit shoot growth of NaCl-affected wheat and barley. Plant Physiol 77: 869-72.

Thelogis A, Laties GG (1982) Potentiating effect of pure oxygen on the enhancement of respiration by ethylene in plant storage organs: a comparative study. Plant Physiol 69: 1031-35.

Torres BC, Bingham FT (1973) Salt tolerance of Mexican wheat: I. Effect of NO_3^- and NaCl on mineral nutrition, growth, and grain production in four wheats. Sol Sci Soc Am Proc 37: 711-15.

Torrey JG (1976) Root hormones and plant growth. Annu Rev Plant Physiol 27: 435-59.

Trevawas AJ (1981) How do plant growth substances work? Plant Cell Environ 4: 203-28.

Trevawas AJ (1982) Growth substance sensitivity: the limiting factor in plant development. Physiol Plant 55: 60-72.

Trevawas AJ (1985) A pivotal role for nitrate and leaf growth in plant development. In: Baker NR, Davies WJ, Ong CK (eds) Control of leaf growth: SEB Seminar 27. Cambridge University Press, UK.

Turner NC (1981) Correction of flow resistances of plants measured from covered and exposed leaves. Plant Physiol 68: 1090-92.

Twine JR, Williams CH (1971) The determination of phosphorus in Kjeldahl digests of plant material by automatic analysis. Commun Soil Sci Plant Anal 2: 485-89.

Van Staden J, Davey, JE (1979) The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ 2: 93-106.

Walker MA, Dumbroff EB (1981) Effects of salt stress on absiscic acid and cytokinin levels in tomato. Z Pflanzenphysiol Suppl 101. S: 461-70.

Weyers J (1984) Do plants really have hormones? New Sci 1410: 9-13.

Weimburg R, Lerner HR, Poljakoff-Mayber A (1984) Changes in growth and water-soluble solute concentrations in *Sorghum bicolor* stressed with sodium and potassium salts. Physiol Plant 62: 472-80.

Williams CH, Twine JR (1967) Determination of nitrogen, sulphur, phosphorus, sodium, calcium and magnesium in plant material by automatic analysis. Division of Plant Industry, CSIRO, Technical paper No. 24.

Williams RF, Evans LT, Ludwig LJ (1964) Estimation of leaf area for clover and lucerne. Aust J Agric Res 15: 231-3.

Winter E, Lauchli A (1982) Salt tolerance of *Trifolium alexandrinum* L. I. Comparison of the salt response of *T. alexandrinum* and *T. pratense*. Aust J Plant Physiol 9: 221-6.

Wright STC (1978) Phytohormones and stress phenomena. In Letham DS, Goodwin PB, Higgins TJV (eds) Phytohormones and related compounds: a comprehensive treatise Vol II. Phytohormones and the development of higher plants.

Yeo AR, Flowers TJ (1984a) Nonosmotic effects of polyethylene glycols upon sodium transport and sodium-potassium selectivity by rice roots. Plant Physiol 75: 298-303.

Yeo AR, Flowers TJ (1984b) Mechanisms of salinity resistance in rice and their role a physiological criteria in plant breeding. In: Staples RC, Toenniessen GH (eds) Salinity tolerance in plants. Strategies for crop improvement. Wiley, NY.

Yousef AN, Sprent JI (1983) Effects of NaCl on growth, nitrogen incorporation and chemical composition of inoculated and NH_4NO_3 fertilised *Vicia faba* L. plants. J Exp Bot 34: 941-50.