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Effects of salinization on nutrient transport to lettuce leaves: consideration of leaf developmental stage

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SUMMARY

Most recent reviews of plant salinity response have included the concept of a nutritional disturbance as one likely mechanism by which shoot growth might be inhibited. None the less, few studies of dicotyledonous plants have presented data on nutrient transport into the most intensively growing shoot tissues. In this paper net nutrient deposition was followed for 3 d in 8 sequential, growing leaves of *Lactuca sativa*, which were grown either in conditions of moderate salinization, or in a growth-stimulating concentration of NaCl. The nutrient deposition was studied from 0.7 to 3.7 d following completion of stepwise salinization. This deposition was followed in immature leaves, which had attained only 1–2 % of ultimate leaf mass by the completion of the study. In such young leaves development is still dominated by cell division. The transport of Ca^{2+} specifically to the youngest leaves was reduced by more than twice as much as was K^+ transport. Transport of the other major divalent cationic nutrient, Mg^{2+}, was not decreased for these leaves. The factors of increase for Na^+ and Cl^- after 3.7 d after completion of salinization averaged 152 and 62 % over control levels for the three youngest leaves (for Na^+ and Cl^-, respectively). Though significant, these increases were only 27 and 14 % as great as increases in three leaf sets of more developed growing leaves. Decreases in net K^+ deposition and leaf K^+ concentration were not greater for the youngest than they were for the oldest leaves. Net S deposition was reduced 44 % more in younger than older growing leaves, but for most leaves not beyond the level expected due to reduced sink strength. The reduction in net P deposition also seemed more related to reduced sink strength, but was reduced to approx. 50 % in both younger and more developed growing leaves. While Fe concentration was not reduced by salinization at any developmental stage, Zn^{2+} net transport and Zn concentration were both reduced in the two youngest leaves (57 and 70 %, respectively). Given the moderate treatment imposed (Na:Ca ratio of 22) the results suggest that Ca^{2+} transport to the youngest leaves is probably highly sensitive to salinization of the root medium and is perhaps a key physiological response in the inhibition of leaf growth.

Key words: calcium, *Lactuca sativa* (lettuce), leaf development, leaf growth, nutrition, nutrient transport, plant development, salinity.

INTRODUCTION

One hypothesis for the mechanism by which NaCl induces an ion-specific inhibition of shoot growth is that a disturbance occurs in mineral nutrition of the shoot. This might occur either as an excess (usually Na^+ or Cl^-) or deficiency (e.g. K^+, Ca^{2+}) in mineral supply. With respect to identification of the immediate cause of the shoot growth inhibition, the mineral supply to growing shoot tissues was investigated. As even the youngest fully expanded leaves consist almost entirely of non-growing cells, the ion contents of, and net fluxes to, non-growing shoot tissues must be irrelevant to any direct nutritional effect on growth. Although it is possible that a method might be devised which will accurately predict changes occurring in growing tissues, based on changing levels in mature tissues, such a method has never been identified (Lazof & Bernstein, 1999).

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Abbreviations: DAT, days after transferring to solution culture; EPXMA, electron probe X-ray microanalysis; LP, leaf plastochron; LPI, leaf plastochron index; SAR, sodium absorption ratio; S1, rapid leaf expansion phase immediately following leaf emergence; S2, a phase of leaf expansion following S1 with a rate of expansion <30 % that of S1; [X], concentration of element X.

Most studies investigating a NaCl-induced nutritional disturbance have focused on excesses of Na^+ and Cl^- and when nutrients have been included, this has most often been limited to K^+ (Lazof & Bernstein, 1999). None the less, NaCl-disturbed nutrient supply has been suggested for nutrients other than K^+, for example Ca^{2+} (Wieneke & Lauchli, 1980; Grieve & Fujiyama, 1987; Maas & Grieve, 1987; Lazof & Lauchli, 1991; Bernstein *et al.*, 1995) or PO_4^- (Grattan & Maas, 1984; Martinez & Lauchli, 1991). In studies of 'K:Na competition' it has often been difficult to evaluate the extent to which mineral osmotica were advantageously substituted (Na^+ for K^+) and the extent to which growth was compromised by accumulation of 'toxic' levels of Na^+ or Cl^-. The use of a significant but non-inhibitory concentration of NaCl in the control nutrient solution can assist in such a distinction. Additional methodological problems have confounded interpretation of salinity effects on Ca^{2+} and PO_4^- transport in particular. Mainly, these have been extreme and non-physiological salinity treatments (Na:Ca ratios of 100) and near millimolar levels of $[PO_4^-]_{medium}$. In this paper net nutrient transport into leaves is compared between salt-stressed lettuce plants and plants grown with 10 mM NaCl, a moderate salt stress (Na:Ca ratio of 22) with a $[PO_4^-]_{medium}$ well below millimolar. This concentration of NaCl in such conditions has been shown to be growth-stimulating compared with $\leqslant 0$ NaCl treatments (Lazof & Cheeseman, 1988).

Constant sink strength per unit of growth (i.e. constant nutritional requirement for growth and development) will result in decreased net nutrient transport into growing leaves during growth inhibition, even for nutrients whose supply is not specifically hindered. To distinguish sink-strength related reductions from more specific effects on mineral nutrient supply this research compares effects of salinization; (1) on ion content and net ion deposition rates, (2) on several nutrients simultaneously and (3) on growing tissues of varying development. If a particular nutrient were subject to specific and growth-limiting restrictions in supply, both its net deposition rate and its concentration in growing tissues would decrease. The reduction in deposition rate for that ion might also be expected to be greater than that for other nutrients which were downregulated only by the reduced sink strength of the growing tissues. Here, these methods are used to test for disturbances of nutrient supply to very young leaves specifically. It was earlier reported that the major growth effect of a moderate salinization for *Lactuca sativa* (cv. Black-seeded Simpson) was a shift in the leaf plastochron index (LPI; see the Abbreviations section at foot of first page), the rate at which new leaves emerge and develop (Lazof *et al.*, 1991). Because these plants had leaf emergence rates in the order of 1 d^{-1}, nutritional effects could be evaluated on well defined leaf developmental stages by comparing effects on nutrient acquisition for the closely-stepped array of leaves.

MATERIALS AND METHODS

Lactuca sativa L. plants were germinated and cultivated as described previously (Lazof *et al.*, 1991). Briefly, plants were germinated for 3 d and raised on a nutrient solution composed of (in mM): 0.9, KNO_3; 0.2, $MgSO_4$; 0.09, $(NH_4)_2PO_4$; 10, NaCl; 3.6, $CaCl_2$; micronutrients and iron. The pH was maintained at 6.2 ± 0.2. Plants were exposed in steps (10, 20, 40, 60 mM) up to a moderate salinization of 80 mM NaCl, or grown with 10 mM NaCl (a growth-stimulating level). The salinization began on seedlings 13 days after transferring to solution culture (DAT) and was completed 30 h later on 14 DAT (after two steps daily, 4 h each). The salinization to 80 mM was considered moderate since the sodium absorption ratio (SAR) of the treatment was 42 and the Na:Ca ratio 22 (Lazof & Bernstein, 1999). Leaves were destructively harvested on either 15 or 18 DAT (18 or 90 h after the completion of salinization), freeze-dried, subsampled after grinding, digested in HNO_3 and analysed by inductively coupled plasmometry–atomic emission spectroscopy (ICP–AES) or silver chloridometry.

The LPI, a developmental scale of plant growth, was determined previously for plants from both the 10 mM control and 80 mM NaCl salinized treatments (Lazof *et al.*, 1991). This was based on non-destructive daily measurements of leaf midrib length for each leaf on several plants of each treatment and the correlation of length with fresh weight. The length of the plastochron was assumed to be constant, based on the previous study with values of 0.76 and 0.81 d for the control and salinity treatments, respectively. In the present paper these determinations were used to designate leaf plastochron values for each leaf on any given day. In the present paper leaf mass values obtained destructively were regressed against LPI. Sigmastat Software (Jandel Scientific, San Rafael, CA, USA) on a PC provided statistics for the regressions, *t*-tests and ANOVA.

Fresh weight and ion concentration data were collected at each harvest date, providing four determinations of leaf ion content for each leaf number. Net ion deposition between 15 and 18 DAT was then obtained for each of the four pairs (ranked pairing 15 with 18 DAT), producing four independent determinations of 3 d net ion deposition, from which means and standard errors were estimated. Calculations were also made of the 'projected net ion deposition' for the salinized leaves had growth not been inhibited by salinization. These projected deposition rates were calculated using the

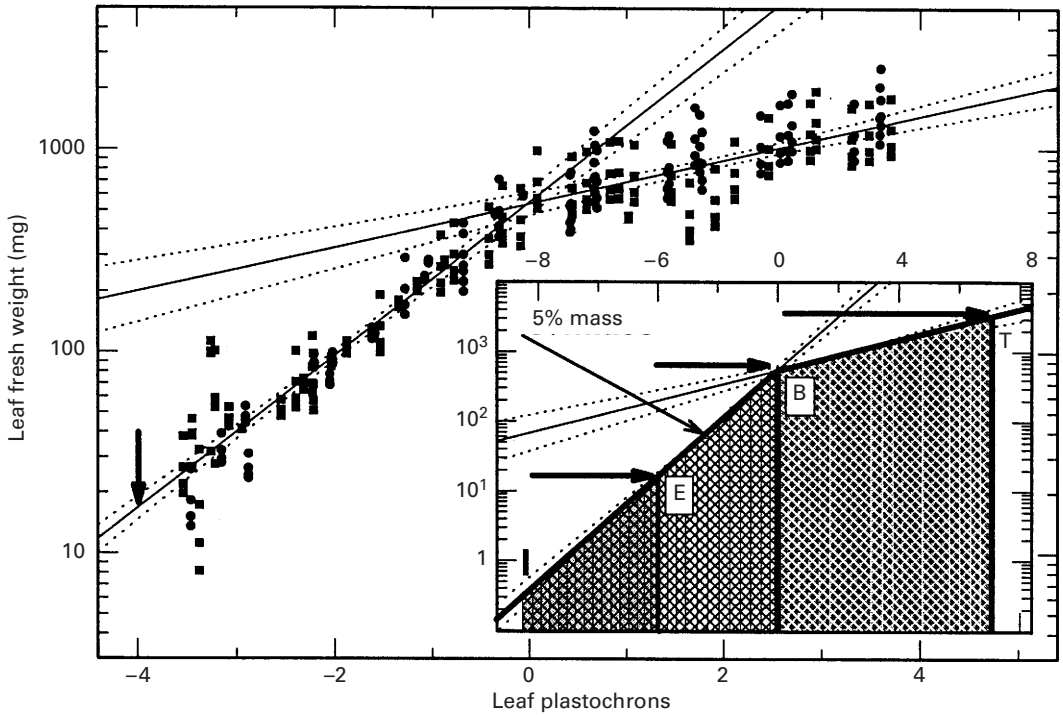

Fig. 1. Leaf fresh weight as a function of leaf plastochron for control (squares) and salinized (circles) leaves of *Lactuca sativa* collected at one of three dates. Leaf development was modelled by defining the breakpoint (B) through gradual extension of linear regressions (see the Materials and Methods section). The breakpoint was set to leaf plastochron index (LPI) = 0 and the available data for all leaves < -0.5 or > 0.5 LPI was regressed. Relative leaf growth diminished from 0.38 in the first phase to 0.11 per leaf plastochron (LP) during the second. The 95 % certainty intervals (dashed lines) are shown. The r^2 values were 0.85 and 0.39 for the faster and slower phases of expansion, respectively ($n = 137$ and 120, respectively). Inset: three phases of leaf growth are shown; (I–E) the initiated leaf to emergence, (E–B) the rapid growth of the emerged leaf to the breakpoint and (B–T) the slower growth rate until ultimate leaf size is attained. For simplicity, the developmental stages before and after the breakpoint (B) are referred to throughout the text as S1 and S2. Also shown is the point at which 5 % of the ultimate growth has been attained. The thick arrows indicate leaf emergence, breakpoint in growth phase and termination of growth.

ion concentrations at 15 and 18 DAT in the salinized plants, but the leaf masses (and 3 d increases) of the control plants. These projected values of net deposition allow comparison within a single figure of the relative effects of salinization on ion concentration with the effects on net ion deposition (ion concentration and mass changes) for each leaf. ANOVA were run for all histograms shown and the variations associated with 'leaf number' × 'treatment' interaction were highly significant ($P < 0.001$) except in the case of the Fe deposition as noted. Increases in fresh weight of the youngest leaves measured (leaf numbers 10 and 11) were 100-fold or more during the 3 d. Leaves which were too small for precise elemental analysis at 15 DAT (leaf numbers > 11) were assumed to have acquired their elemental contents completely within the 15–18 DAT period. The error introduced by this assumption would probably be in the order of 1 %.

RESULTS

Fresh leaf mass increased within two well defined phases (Fig. 1). This biphasic leaf growth was identical for the two treatments after normalization to leaf development. During the first phase leaves expanded at the relative rate of 0.38 and at 0.11 per leaf plastochron (LP) during the second phase. The breakpoint (Inset Fig. 1, B) in leaf development occurred at a fresh mass of 540 mg. Setting this point in leaf development to 0 LPI, emergence was considered to occur at -4 LPI (corresponding to *c.* 17 mg f. wt). A significant NaCl-induced decrease in the growth of young leaves was detected after as little as 48 h following the commencement of salinization, but only in leaves which were still several days from completion of the rapid expansion phase (Fig. 2a). Much larger growth effects were found on leaves after an additional 72 h and were significant on all leaves just completing or still in the first phase of expansion (leaf numbers < 10, Fig. 2b). The reduction in fresh weight at 18 DAT averaged > 50 % for leaf numbers 12–14, but only 20 % for leaves numbered 6–8, even though these older leaves were still in the first phase of expansion when the salinization steps commenced at 13 DAT.

Sodium and chloride concentrations in expanding leaves of salt-stressed plants were, as expected, increased by salinization (Fig. 3). The values of $[Na]_{leaf}$ and $[Cl]_{leaf}$ in the salt-stressed treatment

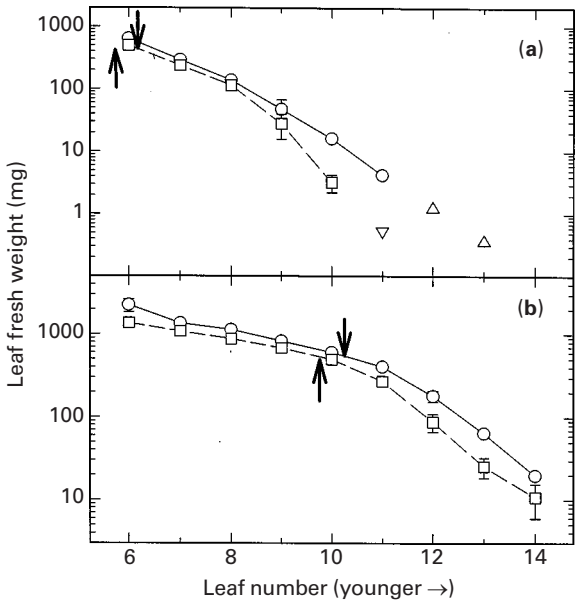

Fig. 2. Fresh weight of successive leaves of *Lactuca sativa* as a function of leaf number at (a) 15 and (b) 18 days after transfer to solution culture (DAT). Plants were salinized stepwise from 13 DAT and completing salinization on 14 DAT. Leaves were excised at the stem and weighed for both the control (10 mM NaCl, circles) and salinized (80 mM NaCl, squares) treatments. Triangles, estimated fresh weights based on linear regression for three youngest leaves measured of each treatment in order to estimate fresh weight for these unemerged leaves at 15 DAT. Arrows indicate the breakpoint between the two phases of leaf expansion (LPI = 0). Standard errors are shown where larger than the symbols ($n = 4$).

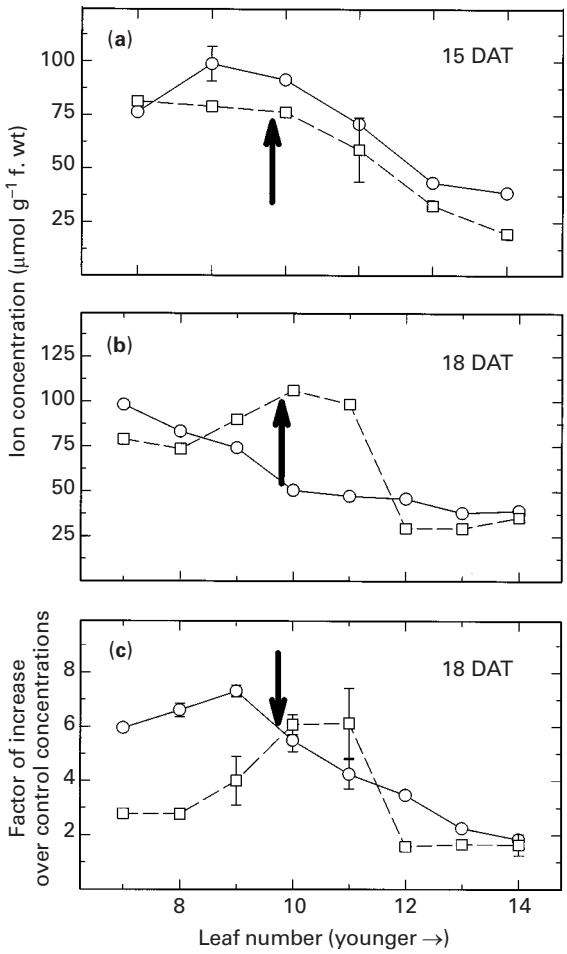

Fig. 3. The relation of [Na] (circles) and [Cl] (squares) with leaf number at (a) 15 and (b) 18 days after transfer to solution culture (DAT) for *Lactuca sativa* plants salinized stepwise up to 80 mM between 13–15 DAT. (c) The relation of the increases in [Na] and [Cl] in leaves of the salinized plants over the concentrations in the same leaves of control plants. Arrows indicate the breakpoint between the two phases of leaf expansion (LPI = 0). Standard errors are shown where larger than the symbols ($n = 4$).

ranged from 40 to 100 and from 20 to 80 μmol g^{-1} f. wt, respectively, 48 h after commencing salinization. The highest concentrations were in leaves which had either completed or nearly completed the first phase of expansion (Fig. 3a). Following an additional 3 d of salinization the $[Cl]_{leaf}$ was higher in expanding leaves than it had been at 15 DAT, ranging from 30 to 105 μmol g^{-1} f. wt, although the $[Na]_{leaf}$ in expanding leaves was maintained within a range similar to that observed at 15 DAT. The factors by which [Na] and [Cl] had increased over the levels found in the control plants at 18 DAT showed that, for Na^+, the largest relative increases occurred in leaves which were well into the second phase of development (Fig. 3c). For Cl^- the relative increases were greatest in leaves near the transition phase of leaf expansion, but not more than one LP into the second phase (Fig. 3c). Expanding leaves of control plants had $[Na]_{leaf}$ and $[Cl]_{leaf}$ ranging from 10 to 20 and from 15 to 30 μmol g^{-1} f. wt, respectively (not shown).

The 3 d net deposition of potassium (K) into leaves was greatly reduced by salinization to 80 mM NaCl (Fig. 4a). The reduction in K^+ deposition was as great as 80 % in leaf number 7, a leaf completing the first phase of expansion as the salinization was completed on 14 DAT. Reductions in K^+ deposition were much less (near 50 %) in leaves which were still in the rapid leaf expansion phase throughout the salinization (leaf numbers $\geqslant 10$). The projected reductions in net deposition calculated on the basis of altered $[K]_{leaf}$ alone (open bars) were usually a minor portion of the reduced deposition due to salinization. In fact, excluding leaf number 7, the reduced growth accounted for >70 % of the decrease in K^+ deposition rate.

Net deposition of Ca^{2+} was also reduced by salinization (Fig. 4b). In contrast to the K^+ reductions, however, those for Ca^{2+} deposition were at least as great in the youngest as in the oldest leaves. In leaves 13 and 14, which were still in the middle of the first phase of expansion at 18 DAT, net Ca^{2+} deposition was reduced on average 84 %, compared with 72 % in leaves 7 and 8. Also in contrast to the effect of salinization on K^+ deposition, decreased ion concentration, rather than reduced mass, was the major component of the reduced Ca^{2+} deposition in all but two (10 and 11) of the eight leaves assessed. In

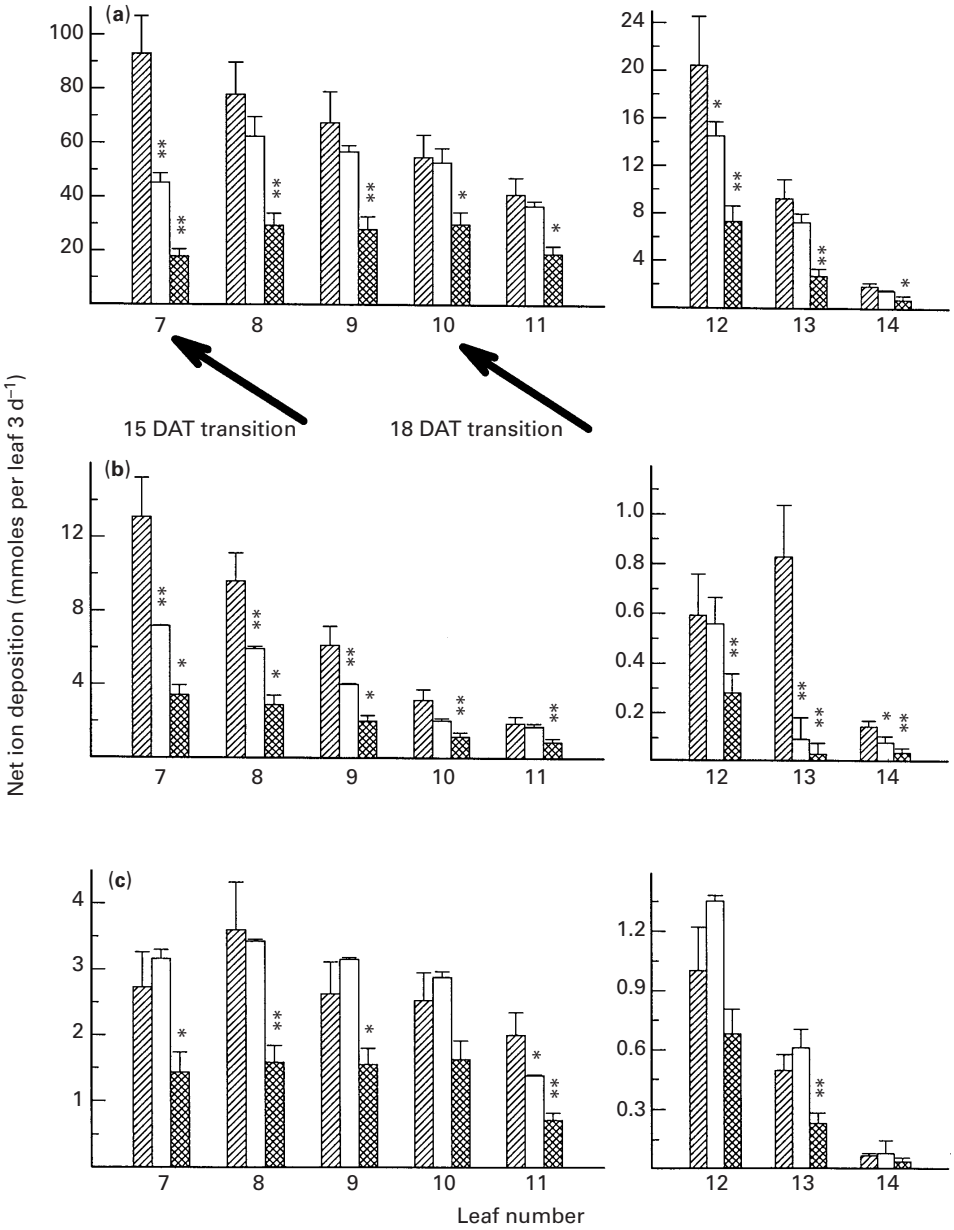

Fig. 4. The relation of net ion deposition in leaves of *Lactuca sativa* from 15 to 18 days after transfer to solution culture (DAT) shown for (a) potassium, (b) calcium and (c) magnesium. Values are shown for 10 mM control (hatched bars) and 80 mM salinized (cross-hatched bars) treatments, and for the projected deposition in salinized plants if these leaves had grown at the control rates (open bars). Standard errors are shown where larger than the symbols ($n = 4$). The arrows indicate the midpoint of transition between leaf expansion phases; asterisks represent statistical significance in treatments vs control for any given leaf at the *, 90 % or **, 95 % probability level.

these six leaves 63 % of the reduction was due to reduced $[Ca]_{leaf}$. These effects on Ca^{2+} deposition were also not common to the deposition of all divalent cationic nutrients (Fig. 4c). Although the deposition of Mg^{2+} was reduced for all eight leaves, there was generally little contribution from reduced $[Mg]_{leaf}$, with only one leaf (number 11) having a significant reduction in net deposition due to concentration changes. Also in contrast to Ca^{2+}, Mg^{2+} deposition was not reduced more in the youngest than the oldest leaves (53 vs 48 % reductions, respectively, for leaves 7 and 8 vs leaves 13 and 14).

Net sulphur (S) deposition was reduced in all leaves by salinization, although not significantly so in two leaves (Fig. 5a). The decreases averaged 45 and 65 % for the three oldest (7–9) and the three youngest leaves (12–14), respectively. Only one leaf (13) showed a significant decrease in the projected S deposition of salinized plants, assuming the absence of a growth effect, with as many of the other leaves showing a slight increase as a decrease. Net phosphorus (P) deposition was significantly decreased in all eight leaves assayed (Fig. 5b). The relative reductions in P deposition averaged *c.* 50 % and did

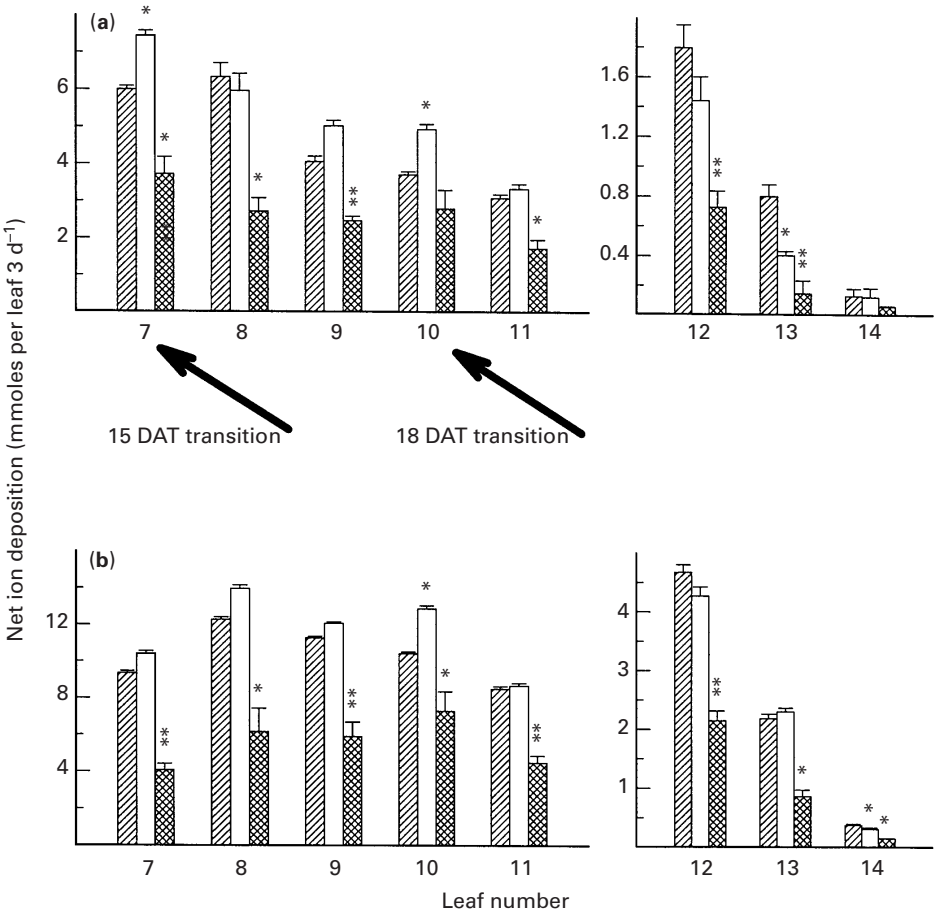

Fig. 5. The relation of net ion deposition in leaves of *Lactuca sativa* from 15 to 18 days after transfer to solution culture (DAT) shown for (a) sulphur and (b) phosphorus. Values are shown for 10 mM control (hatched bars) and 80 mM salinized (cross-hatched bars) treatments, and for the projected deposition in salinized plants if these leaves had grown at rates of control treatments (open bars). Arrows indicate the breakpoint between the two phases of leaf expansion (LPI = 0). Standard errors are shown where larger than the symbols ($n = 4$); asterisks represent statistical significance in treatments vs control for any given leaf at the *, 90 % or **, 95 % probability level.

not appear related to leaf developmental stage. There was little effect of salinization on the projected net P deposition assuming no change in growth, with only two significant changes (leaves 10 and 14), one an increase and one a decrease.

Net Zn^{2+} deposition was reduced significantly in all leaves by salinization (Fig. 6a). For leaves near the breakpoint in expansion phases at 18 DAT (leaves 10 and 11) a major component in the reduced Zn^{2+} deposition was the decrease in $[Zn]_{leaf}$, as evidenced by the 71 % projected decrease. In the two youngest leaves assayed, which were still days from completing the first phase of expansion at 18 DAT, Zn^{2+} deposition seemed to have been affected by both reduced growth (57 %) and reduced [Zn] (70 %). By contrast, leaves which had passed well into the second phase of expansion (leaves 7 and 8), had reductions in Zn^{2+} deposition which were largely accounted for by reduced sink strength. In contrast to Zn^{2+} deposition, net iron (Fe) deposition was not significantly altered by salinization, although $[Fe]_{leaf}$ tended towards increased levels in leaves at all developmental stages (Fig. 6b).

DISCUSSION

In growing leaves the [Na] and [Cl] increased dramatically within 2 d of salinizing the medium to 40 mM with NaCl (Fig. 3a). Although the increase in the [Na] and [Cl] of individual leaves is a rather unsurprising outcome of adding NaCl to the nutrient solution, the leaves which showed the greatest reduction in growth through 18 DAT (numbers 11–14, Fig. 2b) were not those which had the greatest increases in $[Na]_{leaf}$ or $[Cl]_{leaf}$ during the same period. Maximal increases in $[Na]_{leaf}$ and $[Cl]_{leaf}$ occurred in leaves 7–9 and 9–11, respectively (Fig. 3b,c). Some 'protection' of young leaves was evidenced by the lower [Na] and [Cl] of younger leaves. However, even for control plants, the youngest leaves contained significant Na^+ and Cl^-, in the range of 15 to 20 μmol g^{-1} f. wt (Fig. 3b). These levels in growth-stimulated plants call into question universal application of the concept of 'young leaf protection' from Na^+ and Cl^- accumulation. None the less, the increased [Na] and [Cl] in the two youngest leaves (75 %) was small compared with the

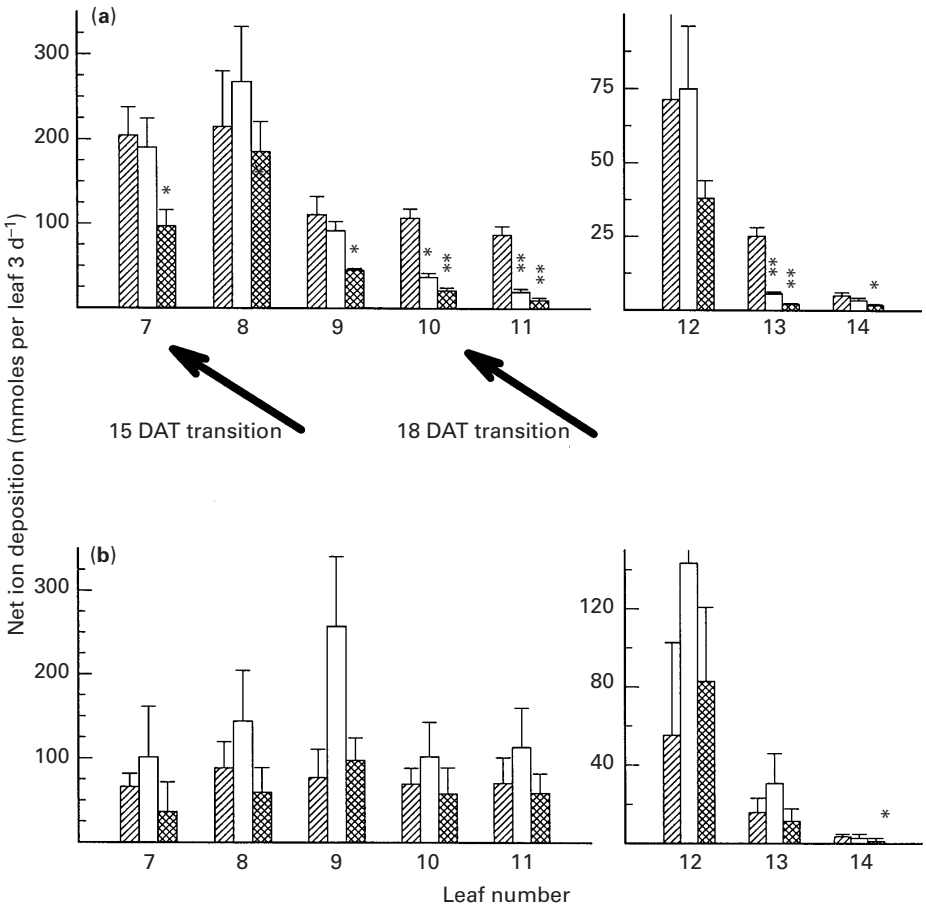

Fig. 6. The relation of net ion deposition in leaves of *Lactuca sativa* from 15 to 18 days after transfer to solution culture (DAT) shown for (a) zinc and (b) iron. Values are shown for 10 mM control (hatched bars) and 80 mM salinized (cross-hatched bars) treatments, and for the projected deposition in salinized plants if these leaves had grown at the control rates (open bars). Arrows indicate the breakpoint between the two phases of leaf expansion (LPI = 0). Standard errors are shown where larger than the symbols ($n = 4$); asterisks represent statistical significance in treatments vs control for any given leaf at the *, 90 % or **, 95 % probability level.

six-fold increase in the $[Na]_{leaf}$ and $[Cl]_{leaf}$ of older leaves. Importantly, the sites of highest Na^+ and Cl^- accumulation within the shoot were spatially remote from tissues where immediate causes of growth inhibition would be located.

Cationic nutrients

While salinization decreased $[K]_{leaf}$ in all leaves, for the younger leaves this reduction could largely be attributed to decreased sink strength, since the control $[K]_{leaf}$ was nearly maintained (Fig. 4a). Among leaves barely or not at all into S2 at 18 DAT, only leaf number 12 showed a significant decline in $[K]_{leaf}$. At first the result of well maintained $[K]_{leaf}$ seems rather heretical against the background of drastically reduced $[K]_{leaf}$ widely reported for salt-sensitive dicots following salinization (Abel & MacKenzie, 1964; Dehan & Tal, 1978; Lauchli & Wieneke, 1979; Rush & Epstein, 1981; Jeschke, 1984; Guerrier, 1996). However, here, control plants were grown with 10 mM NaCl and their older leaves contained 15–20 μmol g^{-1} f. wt Na^+ or Cl^-. Furthermore, few studies have previously compared changes in K^+ deposition or $[K]_{leaf}$ for rapidly expanding dicot leaves. In the present study leaf 7, a leaf which was still expanding throughout the experiment (Fig. 2b), did show a 42 % decline in $[K]_{leaf}$ (Fig. 4a). Such 'still expanding' leaves are often the youngest leaves considered in studies determining individual leaf nutrient acquisition, whereas still older leaves would together dominate an elemental analysis of the bulk shoot.

Of the leaves in S2 at 18 DAT (leaves 7–9), deposition of K^+ was reduced somewhat more than that of Ca^{2+} (Fig. 4a,b). The reduction in 'projected net deposition' was also greater for Ca^{2+} than for K^+ (averaging 38 and 24 %, respectively). However Ca^{2+} deposition was reduced much more than was K^+ deposition for leaves still in S1 at 18 DAT (Fig. 4a,b). The comparatively greater reduction of Ca^{2+} deposition was due solely to reduced ion concentration, since the elemental contents (K and Ca) were measured in the same leaves with the same masses and growth effects. Averaging for leaves 12–14, the NaCl-induced reduction in ion concentration was

twice as great for Ca^{2+} (46 %) as for K^+. This indicates that Ca^{2+} transport was specifically inhibited, especially transport to the youngest leaves. Furthermore, this effect cannot be ascribed to a general effect on absorption and allocation of divalent cations, since the decrease in Mg^{2+} deposition in these leaves could be entirely accounted for as an effect of reduced growth (Fig. 4c, open bars). Salinity effects on transport of Zn^{2+} and Fe^{2+} to young leaves were similar to effects on the transport of Ca^{2+} and Mg^{2+}, respectively. In the youngest three leaves net Zn^{2+} deposition was significantly decreased by salinization, whereas Fe^{2+} deposition did not appear to be affected (Fig. 5a,b). The projected decreases had growth not been reduced averaged 65 % for four of the five youngest leaves.

Previous reports have indicated that 'growing' or 'young' dicot leaves were subject to more moderate decreases in $[K]_{leaf}$ than were older leaves (Aslam *et al.*, 1986; Jeschke *et al.*, 1986). NaCl-induced reductions in $[Ca]_{leaf}$, or in Ca^{2+} transport to young dicot leaves, have also been reported (Wieneke & Lauchli, 1980; Curtis & Lauchli, 1985; Jeschke *et al.*, 1992). In all these cases $[Ca]_{leaf}$ or Ca^{2+} transport was reduced at least as much as was $[K]_{leaf}$ or K^+ transport. In close similarity to the present study, Ca^{2+} deposition decreased more than that of either K^+ or Mg^{2+} in 'young' leaves of salinized *Lupinus albus* (Jeschke *et al.*, 1992). Furthermore, this was in response to a moderate salinization (Na : Ca ratio of 80, SAR of 24) compared with a control with 0.5 mM $[Na]_{medium}$. In *L. albus* 10 d deposition of K^+ decreased 46 % in the youngest shoot strata. Final leaf contents (effects of both decreased growth and ion concentration) were decreased 54, 64 and 40 % for K, Ca and Mg, respectively. Since the contribution of decreased biomass was equivalent in each case, the decrease in [Ca] for the youngest leaf strata was certainly greater than that for [K] or [Mg]. Furthermore, only in the case of Ca^{2+} was the decrease in content much greater in the younger than in mature leaf strata. Also in an earlier study of *L. sativa* decreases for [Ca] were greater than for [K] or [Mg] in minute leaves (< 35 mg f. wt; Lazof & Lauchli, 1991).

Nutrients which are less mobile in the phloem are likely to have greater difficulty in transport to the youngest shoot tissue and apical meristem, since these move through the transpiration stream only weakly or not at all (Lazof & Lauchli, 1991; Lazof & Bernstein, 1999). Calcium, in contrast to Mg^{2+}, is a prime example of a nutrient that is thought to be poorly mobile within the phloem. Possibly, the Ca-specific effect of salinization on young-leaf nutrition might be associated with salinization effects on cellular processes involved in nutrient recirculation and xylem-to-phloem transfer (Lazof & Bernstein, 1999). The dissimilarity in salinization effects on the transport of Zn^{2+} vs Fe, however, was probably not due to a differential influence of altered phloem function, as the two appear to be similarly phloem-dependent (Stephan & Scholz, 1993; Schmidke & Stephan, 1995; Zhang *et al.*, 1996). Additional data related to salinity effects on micronutrients are extremely scarce.

Anionic nutrients

Phosphorus has received a great deal of attention in connection with NaCl-induced disturbed nutrition. However, much of the early research was conducted at rather high levels of $[P]_{medium}$, leading to artefactual increases in P transport rates (see Nieman & Clark, 1976; Grattan & Maas, 1985). Contrarily, in some of the cases where only a moderate $[PO_4^-]_{medium}$ was used, P transport was inhibited, especially in young shoot tissues. For example, the [P] of young leaves (those emerging during salinization) was reduced by 23 % in a salt-sensitive *Glycine* species after exposure for 4 d to 80 mM NaCl in 0.5 mM $[P]_{medium}$ (Wilson *et al.*, 1970). In *Lupinus luteus* [P] decreased 17 % in young leaflets 16 d after salinization to 50 mM NaCl, although increases in [P] were documented after an additional week of salinization (Treeby & Steveninck, 1988). In small *Gossypium hirsutum* leaves 3 h transport of $^{32}PO_4^-$ decreased 24 % after an 8 d salinization (Martinez & Lauchli, 1991). Transport of $^{32}PO_4^-$ (3 h pulse and 3 h chase) was inhibited up to 57 % in the youngest leaves of *L. sativa* after moderate salinization (0.1 mM PO_4^- and SAR of 41 (Martinez *et al.*, 1996)).

In the present study net P deposition was generally reduced in growing leaves only to about the same extent as growth (Fig. 6b). The trend towards increased $[P]_{leaf}$ in the older leaves (14 % increase on average, in leaves 7–10) was consistent with previous studies. Similarly, in *Lupinus albus* grown with 0.5 mM $[PO_4^-]_{medium}$, there was no change in the P content of young 'leaf strata' during salinization, but some increases in $[P]_{leaf}$ were implied in the older leaves , as in these growth was reduced and P content unchanged (Jeschke *et al.*, 1992). Previous studies with *L. sativa* by other techniques also reported little change in [P] and [S] of minute leaves (Lazof & Lauchli, 1991).

Developmental implications

The effects of salinity on ion deposition were clearly dependent on the stage of leaf development, for Na^+ and Cl^- and for nutrients supplied at constant levels to all treatments. In terms of nutrient concentrations the greatest alterations were often in one of the two smallest leaves (12 and 13). The former had the greatest increases in [Mg] and [Fe] and the latter the greatest decreases in [Ca], [S] and [Zn]. This suggests that the disturbance in nutrient supply may be most severe in the earlier half of S1 (Figs 1,2).

The effects shown in this paper for the youngest leaves were specific for leaves $\leqslant -2$ LPI even at 18 DAT (Figs 1,2). These youngest leaves had more than twice as much reduction as leaves 6–8 after 5 d of salinization (Fig. 2b). This is consistent with the idea that a nutritional disturbance might underlie the reduced leaf growth. Greater detail of the developmental context of the present results requires consideration of the early events of dicot leaf initiation and formation up to and through emergence.

The two-phase leaf expansion described here for *L. sativa* following emergence (Figs 1,2) was similar to the biphasic growth of *Nicotiana tabacum* (Hannam, 1968; Clough & Milthorpe, 1975). If a just-initiated *L. sativa* leaf is 300 μg f. wt (a cubic mass of cells *c.* 6.7 μm a side) and if the early phase of expansion was constant from initiation, then leaf initiation would occur at −8 LPI. Cell division in the dicot. leaf is evidently nearly complete (90% of cells present) when 5% of ultimate leaf mass has been attained (e.g. Milthorpe & Newton, 1963; Clough & Milthorpe, 1975; Dale, 1988). This corresponds to −2 LPI for *L. sativa* (Inset, Fig. 1, '5%'). All leaves of the youngest group (12–14) were <-4 LPI at 15 DAT (<17 mg f. wt). They were between 6 and 8 LP (up to 6 d) younger than the point of 90% cell division completeness (Fig. 1), hence had very active cell division throughout the study. Demonstrated effects of salinization, such as the drastic reduction in projected net Ca^{2+} deposition in leaves 13 and 14 (Fig. 4b), must, then, be associated with the earliest leaf developmental stage (Inset Fig. 1, I–E) in which cell division dominates growth. This is consistent with a major salinization effect on leaf emergence rates (Curtis & Lauchli, 1985; Aslam *et al.*, 1986; Lazof *et al.*, 1991; Bernstein *et al.*, 1993a; Grieve *et al.*, 1993, 1994; Munns & Rawson, 1999) and with growth effects specifically on leaves unemerged at the time of salinization (Rawson & Munns, 1984; Aslam *et al.*, 1986; Lazof *et al.*, 1991; Bernstein *et al.*, 1993b).

Comparisons with studies of 'still expanding' or 'young' leaves should be made cautiously. In the case of the lettuce cultivar used here, 'still expanding' would include even the most developed leaf in the present study, leaf 7 at 18 DAT. For example, in the excellent study of *Lupinus albus* already cited, the development of each 'leaf strata' was not specified, however the mass of the youngest leaf can be estimated at 1.5–2 g f. wt, or similar in size to a fully expanded lettuce leaf (Inset Fig. 1, T). Hence, it might be inappropriate to compare those results with effects in quickly expanding lettuce leaves. The mineral status of such young dicot leaves as studied here has, however, rarely been evaluated in any context. Leaves 13 and 14 were just approaching the '5% mass' point even in control plants at the end of the current studies (Figs 1,2).

CONCLUSIONS

In the most rapidly growing leaves of *L. sativa* several alterations in mineral status occurred in the few days following salinization, including increases in both $[Na]_{leaf}$ and $[Cl]_{leaf}$ and decreases in $[K]_{leaf}$. However, the reduction which occurred in net Ca^{2+} transport to the youngest leaves exceeded that of K^+ by a factor of two and was more pronounced in the youngest leaves. The greater effect on Ca^{2+} occurred despite the fact that K^+ was the major mineral osmoticant before salinization. The reduction observed in net Ca^{2+} transport was not seen in transport of the other major divalent cationic nutrient, Mg^{2+}. Transport of Zn^{2+} to the youngest leaves was affected in a fashion similar to that of Ca^{2+}, whereas Fe transport was not. The salinization treatment producing these specific effects on Ca^{2+} and Zn^{2+} transport to the most intensively growing leaves was moderate (low SAR and Na:Ca ratio). The effects were not due to the mere presence of Na^+ in the medium. Likewise, metabolic changes, which may occur when the NaCl in the medium is raised from submillimolar to several millimolar were not considered. Such changes might be considerable and obfuscate specific effects of Na^+ and Cl^- considering the 60-fold increase in whole shoot Na:K ratio between submillimolar and 10 mM NaCl growth in *L. sativa* (Lazof & Cheeseman, 1988).

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REFERENCES

Abel GH, MacKenzie AJ. 1964. Salt tolerance of soybean varieties (*Glycine max* L. Merrill) during germination and later growth. *Crop Science* **4**: 157–161.

Aslam Z, Jeschke WD, Barrett-Lennard EG, Setter TL, Watkin E, Greenway H. 1986. Effects of external NaCl on the growth of *Atriplex amnicola* and the ion relations and carbohydrate status of the leaves. *Plant, Cell and Environment* **9**: 571–580.

Bernstein N, Lauchli A, Silk WK. 1993a. Kinematics and dynamics of sorghum (*Sorghum bicolor* L.) leaf development at various Na/Ca salinities. *Plant Physiology* **103**: 1107–1114.

Bernstein N, Silk WK, Lauchli A. 1993b. Growth and development of sorghum leaves under conditions of NaCl stress. *Planta* **191**: 433–439.

Bernstein N, Silk WK, Lauchli A. 1995. Growth and development of sorghum leaves under conditions of NaCl stress: possible role of some mineral elements in growth inhibition. *Planta* **196**: 699–705.

Clough BF, Milthorpe FL. 1975. Effects of water deficit on leaf development in tobacco. *Australian Journal of Plant Physiology* **2**: 291–300.

Curtis PS, Lauchli A. 1985. Responses of kenaf to salt stress: germination and vegetative growth. *Crop Science* **25**: 944–949.

Dale JE. 1988. The control of leaf expansion. *Annual Review of Plant Physiology* **39**: 267–295.

Dehan K, Tal M. 1978. Salt tolerance in the wild relatives of the cultivated tomato: response of *Solanum pennellii* to high salinity. *Irrigation Science* **1**: 71–76.

Grattan SR, Maas EV. 1984. Interactive effects of salinity and substrate on phosphate on soybean. *Agronomy Journal* **76**: 668–676.

Grattan SR, Maas EV. 1985. Root control of leaf phosphorus and chlorine accumulation in soybean under salinity stress. *Agronomy Journal* **77**: 890–895.

Grieve C, Francois LE, Maas EV. 1994. Salinity affects the timing of phasic development in spring wheat. *Crop Science* **34**: 1544–1549.

Grieve CM, Fujiyama H. 1987. The response of two rice cultivars to external Na/Ca ratio. *Plant and Soil* **103**: 245–250.

Grieve C, Lesch SM, Maas EV, Francois LE. 1993. Leaf and spikelet primordia initiation in salt-stressed wheat. *Crop Science* **33**: 1286–1294.

Guerrier G. 1996. Fluxes of Na^+, K^+ and Cl^-, and osmotic adjustment in *Lycopersicon pimpinellifolium* and *L. esculentum* during short- and long-term exposures to NaCl. *Physiologia Plantarum* **97**: 583–591.

Hannam RV. 1968. Leaf growth and development in the young tobacco plant. *Australian Journal of Biological Science* **21**: 855–870.

Jeschke WD. 1984. K^+–Na^+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. In: Staples RC, Toeniessen GH, eds. *Salinity tolerance in plants*. New York, USA: John Wiley & Sons, 37–66.

Jeschke WD, Pate JS, Atkins C. 1986. Effects of NaCl salinity on growth, development, ion transport and ion storage in white lupin (*Lupinus albus* L. cv. Ultras). *Journal of Plant Physiology* **124**: 257–274.

Jeschke WD, Wolf O, Hartung W. 1992. Effect of NaCl salinity on flows and partitioning of C, N and mineral ions in whole plants of white Lupin, *Lupinus albus* L. *Journal of Experimental Botany* **43**: 777–788.

Lauchli A, Wieneke J. 1979. Studies on growth and distribution of Na^+, K^+ and Cl^- in soybean varieties differing in salt tolerance. *Zeitschrift der Pflanzenernaehrung und Bodenkunde* **142**: 3–8.

Lazof DB, Bernstein N. 1999. The NaNl induced inhibition of shoot growth: the case for disturbed nutrition with special consideration of calcium. In: Callow JA, ed. *Advances in botanical research incorporating advances in plant pathology*. London, UK: Academic Press, 113–189.

Lazof DB, Bernstein N, Lauchli A. 1991. Growth and development of the *Lactuca sativa* shoot as affected by NaCl stress: consideration of leaf developmental stages. *Botanical Gazette* **152**: 72–76.

Lazof DB, Cheeseman JM. 1988. Sodium and potassium compartmentation and transport in the roots of intact lettuce plants. *Plant Physiology* **88**: 1279–1284.

Lazof D, Lauchli A. 1991. The nutritional status of the apical meristem of *Lactuca sative* as affected by NaCl salinization: an electron-probe microanalytic study. *Planta* **184**: 334–342.

Maas EV, Grieve C. 1987. Sodium-induced calcium deficiency in salt-stressed corn. *Plant, Cell and Environment* **10**: 559–564.

Martinez V, Bernstein N, Lauchli A. 1996. Salt-induced inhibition of phosphorus transport in lettuce plants. *Physiologia Plantarum* **97**: 118–122.

Martinez V, Lauchli A. 1991. Phosphorus translocation in salt-stressed cotton. *Physiologia Plantarum* **83**: 627–632.

Milthorpe FL, Newton P. 1963. Studies on the expansion of the leaf surface. III. The influence of radiation on cell division and leaf expansion. *Journal of Experimental Botany* **42**: 483–495.

Munns R, Rawson HM. 1999. Effect of salinity on salt accumulation and development of the floral apex of wheat and barley. *Australian Journal of Plant Physiology*. (In press.)

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 Physiology* **57**: 157–161.

Rawson HM, Munns R. 1984. Leaf expansion in sunflower as influenced by salinity and short-term changes in carbon fixation. *Plant, Cell and Environment* **7**: 207–213.

Rush DW, Epstein E. 1981. Comparative studies on the sodium, potassium, and chloride relations of a wild halophytic and a domestic salt-sensitive tomato species. *Plant Physiology* **68**: 1308–1313.

Schmidke I, Stephan UW. 1995. Transport of metal micronutrients in the phloem of castor bean (*Ricinus communis*) seedlings. *Physiologia Plantarum* **95**: 147–153.

Stephan UW, Scholz G. 1993. Nicotianamine: mediator of transport of iron and heavy metals in the phloem? *Physiologia Plantarum* **88**: 522–529.

Treeby MT, Steveninck RFMv. 1988. Effects of salinity and phosphate on ion distribution in lupin leaflets. *Physiologia Plantarum* **73**: 317–322.

Wieneke J, Lauchli A. 1980. Effects of salt stress on distribution of Na+ and some other cations in two soybean varieties differing in salt tolerance. *Zeitschrift der Pflanzenernaehrung und Bodenkunde* **143**: 55–67.

Wilson JR, Haydock KP, Robins MF. 1970. The development in time of stress effects in two species of *Glycine* differing in sensitivity to salt. *Australian Journal of Biological Science* **23**: 537–551.

Zhang C, Romheld V, Marschner H. 1996. Effect of primary leaves on ^{59}fe uptake by roots and 59fe distribution in the shoot of iron sufficient and iron deficient bean (*Phaseolus vulgaris* L.) plants. *Plant and Soil* **182**: 75–81.