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# Plant growth and ion relations in lucerne (*Medicago sativa* L.) in response to the combined effects of NaCl and P

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**Abstract**

The combined effect of NaCl and P on the growth of lucerne was studied in two hydroponic greenhouse experiments. NaCl concentrations were identical in each experiment (0, 50 and 100 m*M* NaCl) while external P concentrations were low (viz. 0.002, 0.02 and 0.2 m*M* measured as 0.006, 0.026 and 0.2 m*M*, respectively) in one experiment and higher (0.5 and 5.0 m*M*) in the second. Plant biomass was reduced more by the low P levels than by high concentrations of NaCl. A significant NaCl*P effect was found where external P concentrations were low (0.006–0.2 m*M*) but there was no difference in plant production between the two P concentrations of 0.5 and 5.0 m*M*. Shoot and root concentrations of Na and Cl increased significantly with increasing NaCl concentration in both experiments and there were some differences in the concentrations of these ions at different external P levels. At low P, NaCl had no significant effect on shoot concentrations of P; however, root P concentrations tended to decrease with increasing NaCl level. Increasing external P from 0.006 to 0.2 m*M* led to significant increases in P concentrations in both roots and shoots. At higher P, concentrations of P in both the shoots and the roots did not differ with external NaCl or P conditions. Our results illustrate the complex relationship that exists between NaCl and P at low P levels. We conclude that high or non-limiting concentrations of P (0.2 – 5.0 m*M*) do not affect lucerne's response to NaCl.

## Introduction

Nutrition can significantly influence plant response to saline conditions (Bernstein et al., 1974; Champagnol, 1979; Grattan and Grieve, 1999; Ravikovitch and Yoles, 1971). The interaction between salinity and P nutrition is particularly complex. Plant responses can vary according to such factors as the species or cultivar being examined, the stage of plant growth, the level of NaCl and form of P and the environmental conditions of the experiment (Champagnol, 1979; Grattan and Grieve, 1999). The literature reports situations where high saline-high P conditions can (1) decrease P concentrations in the plant tissue (the majority of cases), (2) increase P concentrations, or (3) have no effect. These results seem to depend, to some extent, on whether the studies were conducted in the field or in solution culture (Champagnol, 1979; Sharpley et al., 1992). Most of the studies that show that P concentrations in plant tissues are lower under saline conditions have been conducted in soils since P availability is generally reduced in saline soils. In situations where P uptake has increased (such as a few solution/sand culture experiments), growth may be reduced in species that are sensitive to high concentrations of P in plant tissue such as corn (Bernstein et al., 1974), sesame (Cerda et al., 1977) and some cultivars of soybean (Grattan and Maas, 1984). This increased P accumulation in the shoot is thought to be controlled at the root level and is independent of the salt composition although the exact mechanism is unknown (Grattan and Maas, 1985).

Lucerne (*Medicago sativa*), a vigorous, fast-growing species is known to be moderately P-efficient

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compared with other forage legume species such as white clover (Gourley et al., 1994), and, its tolerance to NaCl alone has been well-documented (e.g. Johnson et al., 1992; Rogers, 2001). However its performance under saline conditions and varying P nutrition is unknown. This species is an important forage crop in many irrigated areas of the world including the south east of Australia and the desert valleys of south and central California where soil salinity affects large areas. Improved knowledge on the response of lucerne to the combined effects of salinity and P will therefore be important when deciding fertilizer applications on saline soils in these areas or where pumped saline groundwater is used for irrigation. In addition, there are many areas where irrigation with wastewater is being practiced because of restrictions on the disposal of effluent to river systems. This wastewater originates from intensive agricultural industries and processing factories and may contain high levels of nutrients such as P in association with moderate to high EC levels. Consequently, information on the response of plant species to saline- P conditions will be useful in refining guidelines for wastewater irrigation.

The purpose of this study was to obtain information on how lucerne responds to the combined effects of NaCl and P and to determine how these two factors interact with one another and influence salt tolerance and P uptake.

## Materials and methods

### *Experiment 1*

The effects of three concentrations of P on the tolerance of lucerne plants to three concentrations of NaCl were examined in plants grown hydroponically in a greenhouse at Riverside, California (33° 58.24′ latitude, 117° 19.12′ longitude, 297 m elevation). The daytime air temperature ranged from 20 to 30 °C (mean=25 °C), night time temperatures ranged from 19 to 28 °C (mean=22 °C) and relative humidity ranged from 51 to 42%.

Seeds of two cultivars of lucerne (*Medicago sativa* L), cultivars Moapa (a winter-active cultivar) and Cuf 101 (a highly winter-active cultivar), were sown onto two plastic grids (each 30 × 31 cm) that contained cells each 1.2 × 1.2 × 1.2 cm in August 1995. One seed was placed in each cell that was then covered with vermiculite and watered with tap water. After two days, when the seeds had started to germinate (as defined by the emergence of the radicle), the grids were placed on top of ceramic pots each 57 L in volume containing modified nutrient solution (2.5 m*M* $Ca(NO_3)_2$, 3.0 m*M* $KNO_3$, 1.5 m*M* $MgSO_4$, 5 $\mu$M $MnSO_4$, 23 $\mu$M $HBO_3$, 50 $\mu$M Fe as sodium ferric diethylenetriamine pentaacetate (NaFeDTPA), 0.4 $\mu$M $ZnS0_4$, 0.2 $\mu$M $CuSO_4$, and 0.1 $\mu$M $H_2MoO_4$). Each pot contained a grid with plants of cv. Moapa and a grid with plants of cv. Cuf 101. The P treatments (0.2, 0.02 and 0.002 m*M* P – representing a high, moderate and low level of P) were applied as $KH_2PO_4$ at this stage.

After 12 days, the salinity treatments (0, 50 and 100 m*M* NaCl) were applied to the solutions in increments of 50 m*M* each day so that the final concentration of 100 m*M* was reached in 2 days. The plants were then thinned so that each pot contained 20 plants of each lucerne cultivar. The solutions were changed at fortnightly intervals and samples of solution were taken both before and after each solution change. These samples were chemically analysed for P, Na, K, Ca and Mg using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP) and Cl by silver nitrate potentiometric titration. The pH of the solutions ranged between 7 and 8 throughout the experiment.

The actual (time-weighted) concentrations of P in the solution culture were: 0.00611 m*M* P (0.002 m*M* treatment), 0.026 m*M* P (0.02 m*M* treatment) and 0.201 m*M* P (0.2 m*M* treatment). At the highest P concentration (0.2 m*M*) there was some concern initially that the P would quickly precipitate with Ca and therefore become unavailable to the plant. However, no precipitate was observed in the solutions and approximately 50% of the P in solution was depleted before each solution was changed. P concentrations at the lowest P treatment (0.002 m*M* P) did not change significantly between solution changeovers – perhaps due to the low growth rate (and uptake) by the plants and the difficulty in accurately measuring such small amounts of P in solution. In the 0.02 m*M* treatment, the P in solution was used up very soon after it was applied.

The experiment was a factorial design with three salinity treatments, three P treatments and three replicates in a split block design giving a total of 27 pots. Plants were harvested destructively at weekly intervals commencing 1 week after the full salinity treatment had been imposed and there were 4 harvests in total. At each harvest, 5 plants of each cultivar at each treatment were removed and divided into roots

and shoots. Roots were blotted dry and fresh weight and dry weight (dried at 70 °C for 48 h) were measured on each root and shoot sample. Plant material was analysed for Cl, Na, K, Mg, Ca and P as described for the solution measurements.

*Experiment 2*

Following results from Experiment 1, a second experiment was conducted which involved two higher concentrations of P (0.5 and 5.0 m*M*) in conjunction with the same NaCl concentrations as Experiment 1 (ie. 0, 50 and 100 m*M* NaCl). The experiment was conducted on lucerne plants in a greenhouse at Tatura, Victoria, Australia (36° 26′ latitude, 145° 16′ longitude, 114 m elevation). Temperatures in the greenhouse ranged from 14 to 16 °C (±2 °C) during the night to 21 to 26 °C (±5 °C) during the day. Relative humidity levels ranged from 20 to 80%.

Seeds of the cultivar Aurora (a cultivar that has a similar winter activity level to cultivar Moapa from Experiment 1) were first germinated under nonsaline conditions in vermiculite in seedling trays in March 1996. At the second trifoliate leaf stage, seedlings were transplanted into polystyrene trays floating in aerated, modified Hoagland solution (after Karmoker and Van Steveninck, 1978) in stainless steel tanks (each 200 L). Fifteen plants were planted in each tank. The P concentration in this nutrient solution was 0.5 m*M* and was used as one of the experimental treatments. The salinity treatments of 0, 50 and 100 m*M* NaCl were applied in increments of 25 m*M* NaCl/day after three weeks. The higher P concentration treatment (5.0 m*M*) was applied as $KH_2PO_4$ one week after the full salinity treatments had been imposed in increments of 1 m*M* P/day.

The solutions in each tank were changed weekly and samples were taken before and after this change and analysed for Cl, K, Mg, Ca, Na and P using the procedures described in Experiment 1. The pH of the solutions ranged from 6 to 7.

The solution analyses, taken weekly, showed that there was some depletion in ion concentrations (K, Mg, Ca, P, Na and Cl) over the course of the week but that there were no significant differences in the amount or uptake rate between the different experimental treatments (data not presented). The actual (time-weighted) concentrations of P were: 0.56 m*M* (0.5 m*M* treatment) and 4.20 m*M* (5.0 m*M* treatment). All the P remained in solution at the highest P treatment (5.0 m*M* P) and did not precipitate with Ca.

The experiment had an unbalanced design. There were three replicates for each of the 0 and 100 m*M* NaCl - P treatment combinations and four replicates for each of the 50 m*M* NaCl-P treatment combinations using a total of 20 tanks.

Plants were harvested three times over the duration of the experiment (three weeks, six weeks and nine weeks, after the full experimental treatments had been imposed). At harvest, 10 uniform plants from each tank were cut to 2 cm above the polystyrene trays. Fresh weight, dry weight (dried at 70 °C for 48 h) were measured on each plant sample. Plant material was later analysed for Cl, Na, K, Mg, Ca and P using the techniques described earlier. At harvest three, the whole plant was removed and divided into roots and shoots and components were blotted dry, then weighed, dried and analysed for tissue ions.

*Statistical analyses*

*Experiment 1*

Shoot dry matter production was analysed by ANOVA with a randomised block structure (Genstat 5.0, Lawes Agricultural Trust, Rothamsted Experimental Station). Because of concerns over the accuracy in removing the roots from the grids (despite very careful handling), root dry matter production is not presented. Residuals were checked for normality and homogeneity and $\log_e$ or $\log_e(x^*100)$ transformed to homogenise the residuals if necessary. The quantitative explanatory variable NaCl was fitted as an orthogonal polynomial. The linear and quadratic components, or contrasts, were tested for significance and quantified with $p$ values. The data for dry matter production are plotted in the transformed form ($\log_e$(dry weight of 5 plants)*100) with the corresponding dry weight values per plant presented on the secondary Y axis. The curves are fitted response curves with the observed means represented by points. Data are presented for harvests 2, 3, and 4.

Tissue ion concentration data were analysed by ANOVA and by REML (Restricted Maximal Likelihood) analyses when there were insufficient amounts for chemical analyses. Results for tissue ions are presented in Table 1 for Harvest 3 (representative of Harvest 2, 3 and 4).

*Table 1.* The effect of applied NaCl and P on concentrations of Ca, Mg, Na, K, P and Cl in the shoots of lucerne plants 36 days after sowing (Experiment 1, Harvest 3)

| Treatments‡ | | Shoot ion concentrations (mmoles $kg^{-1}$ dwt) | | | | | |
|---|---|---|---|---|---|---|---|
| NaCl m*M* | P m*M* | Ca | Mg | Na | K | P | Cl |
| 0 | 0.002 | 252* | 180* | 28* | 532* | 20* | 23* |
| 0 | 0.02 | 409 | 119 | 15 (2.7) | 852 | 56 (4.0) | 40 (3.7) |
| 0 | 0.2 | 417 | 98 | 18 (2.9) | 1125 | 205 (5.3) | 43 (3.8) |
| 50 | 0.002 | 263* | 151* | 371* | 504* | 19* | + |
| 50 | 0.02 | 334 | 92 | 338 (5.8) | 776 | 61 (4.1) | 317 (5.8) |
| 50 | 0.2 | 345 | 76 | 399 (6.0) | 943 | 177 (5.2) | 388 (6.0) |
| 100 | 0.002 | 266* | 152* | 537* | 490* | 18.8* | 532* |
| 100 | 0.02 | 289 | 78 | 515 (6.2) | 703 | 69 (4.2) | 557 (6.3) |
| 100 | 0.2 | 268 | 69 | 804 (6.7) | 694 | 167 (5.1) | 783 (6.7) |
| Lsd ($p = 0.05$) NaCl*P | | 66 | 14 | (0.4) | 195 | (0.4) | (0.3) |

*Composite means, not included in anova.
+Insufficient plant material for ion analyses.
‡Analyses are for 3 replicates unless otherwise stated.
( ) ln transformed data, analyses and lsds are calculated for ln transformed data.

*Experiment 2*

Shoot and root dry matter production and tissue ions were analysed using REML. Results are presented for Harvest 3, containing results for both roots and shoots as representative of the three harvests.

## Results

*Dry matter production*

*Experiment 1 (0.002 – 0.2 m*M *P)*

There were no differences between cultivars in their response to NaCl and P so the data are presented for the two cultivars combined. The level of phosphorus had a significant linear effect on plant growth at all harvests (Figure 1, $p < 0.001$) as emphasised by the discrete nature of the response curves for the three different P concentrations. Plants produced more dry matter at the highest P level (0.2 m*M*) compared with the lower two P levels regardless of the NaCl concentration and this result was also true for cumulative dry matter production (Figure 2, $p < 0.001$). Shoot and root growth (data not shown) was severely retarded at the lowest P treatment (0.002 m*M* measured at 0.006 m*M*) compared with both the 0.02 m*M* (measured as 0.026 m*M*) and the 0.2 m*M* treatments. Plants grown at 0.002 m*M* P level showed symptoms of P deficiency and were dark green in colour. NaCl was not found to have a significant effect on plant dry matter production.

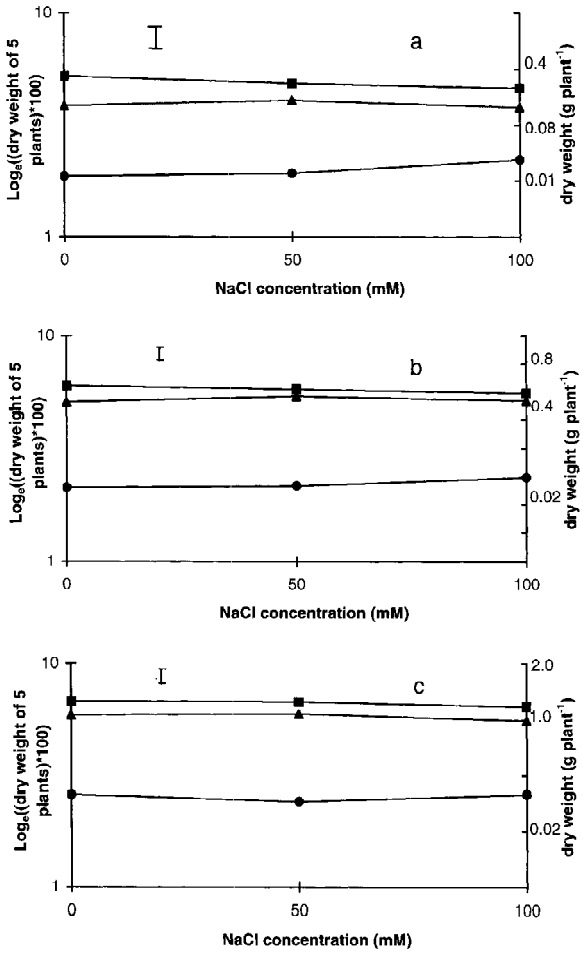


*Figure 1.* Experiment 1. The effect of NaCl and P on lucerne plants grown from four to six weeks in solution culture in the greenhouse. • = 0.002 m*M* P, ▲ = 0.02mM P, ■ = 0.2 m*M* P. (a) Harvest 2 (29 days from sowing). Significance of effects: NaCl $p = 0.90$, P $p < 0.001$, NaCl*P $p = 0.039$, $SEM_{(NaCl*P)}$=0.29 is shown as a bar. (b) Harvest 3 (36 days from sowing). Significance of effects: NaCl $p = 0.75$, P $p < 0.001$, NaCl*P $p = 0.053$, $SEM_{(NaCl*P)}$=0.13 is shown as a bar. (c) Harvest 4 (43 days from sowing). Significance of effects: NaCl $p = 0.56$, P $p < 0.001$, NaCl*P $p = 0.040$, $SEM_{(NaCl*P)}$=0.18 is shown as a bar.

The interaction between NaCl and P was significant ($p < 0.05$ for all harvests, Figure 1). At the highest P level (0.2 m*M*), the interaction was negative with plants showing, proportionally, a greater reduction in growth (yet still greater absolute dry matter production) with increasing NaCl concentrations. In comparison, at the lower two P levels (0.02 and 0.002 m*M*) there was no significant reduction in growth at the higher salinity levels compared with nonsaline conditions (Figure 1, $p = 0.14$)). These trends are also depicted in the results for cumulative dry matter

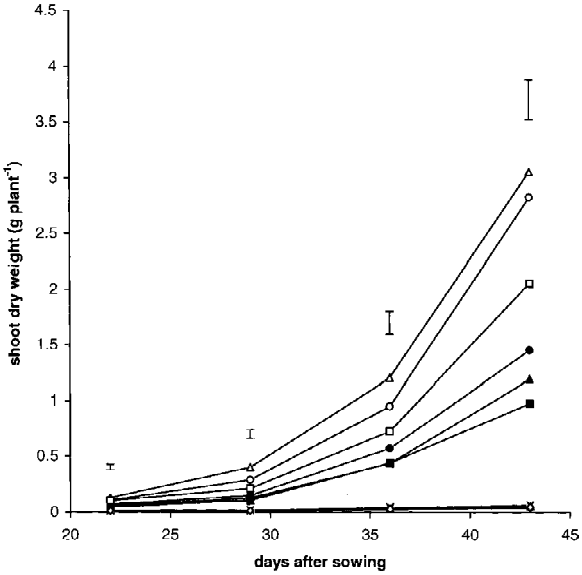


*Figure 2.* Experiment 1. The effect of NaCl and P on cumulative dry matter production in young lucerne plants grown for 4 weeks (i.e. from 16 to 44 days after sowing) in the greenhouse. Lsd's ($p = 0.05$) are shown as bars. ◊ = 0.002 m*M* P +0 m*M* NaCl, ▲ = 0.02 m*M* P + 0 mM NaCl, △ =0.2 m*M* P +0 m*M* NaCl, ♦ = 0.002 m*M* P + 50 m*M* NaCl • = 0.02 mM P + 50 m*M* NaCl, ◯ = =0.2 m*M* P+50 m*M* NaCl, X= 0.002 m*M* P + 100 m*M* NaCl, ■ = 0.02 m*M* P +100 m*M* NaCl. □ = 0.2 m*M* P +100 m*M* NaCl.

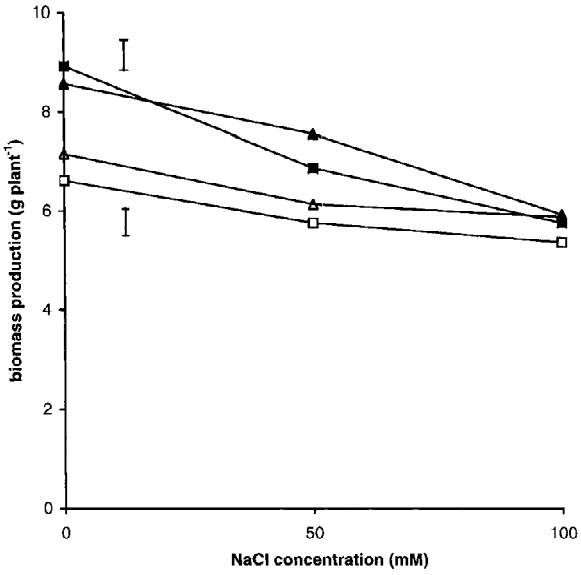


*Figure 3.* Experiment 2. The effect of NaCl and P on dry matter production in lucerne plants at harvest 3 (64 days after sowing). Shoot production. □ = 0.5 m*M* P, △ = 5.0 m*M* P. Significance of contrasts: NaCl (linear) $p < 0.001$, P $p = 0.83$, NaCl*P $p > 0.4$ SEM=0.54 shown as a bar. Root production. ■ = 0.5 m*M* P, ▲ = 5.0 m*M* P. Significance of contrasts: NaCl (linear): $p < 0.04$, P: $p = 0.54$, NaCl*P: $p = 0.88$ SEM=0.72 shown as a bar.

production (Figure 2) that show that production rankings for the three NaCl treatments differ according to P treatment and hence that there was a significant NaCl*P interaction ($p = 0.071$), a significant P effect ($p < 0.001$) but no significant linear NaCl effect.

*Experiment 2 (0.5 and 5.0 m*M *P)*

Plant biomass production decreased significantly with increasing NaCl concentration ($p < 0.001$ for shoot weight and $p < 0.04$ for root weight), but there was no significant difference in dry matter production between the two P treatments, nor any NaCl*P interaction (Figure 3).

*Tissue ion concentrations*

*Experiment 1 (0.002 – 0.2 m*M *P)*

It was not possible for the 0.002 m*M* P treatment to be included in the statistical analyses because the plants growing in this treatment were so small and all the replicates had to be bulked. Data for the two cultivars were again combined because there were no differences between them. Concentrations of Ca, Mg and K in both the roots and shoots of plants decreased significantly with increasing NaCl concentration at all harvests (viz. Harvest 3, Tables 1 and 2). Increasing external P levels from 0.02 to 0.2 m*M* had no significant effect on the tissue concentrations of Ca, whereas concentrations of Mg decreased at higher external P levels. Concentrations of K increased with increasing

*Table 2.* The effect of applied NaCl and P on concentrations of Ca, Mg, Na, K, P and Cl in the roots of lucerne plants 36 days after sowing (Experiment 1, Harvest 3)

| Treatments‡ | | Root ion concentrations (mmoles $kg^{-1}$ dwt) | | | | | |
|---|---|---|---|---|---|---|---|
| NaCl m*M* | P m*M* | Ca | Mg | Na | K | P | $Cl^-$ |
| 0 | 0.002 | 151* | 175* | 57* | 507* | 50* | + |
| 0 | 0.02 | 73 | 315 | 67 | 1010 | 79 | 12 (2.5) |
| 0 | 0.2 | 86 | 153 | 72 | 1692 | 293 | 141 (4.9) |
| 50 | 0.002 | 92* | 163* | 256* | 324* | 30* | + |
| 50 | 0.02 | 71 | 244 | 715 | 784 | 79 | 299 (5.7) |
| 50 | 0.2 | 78 | 115 | 822 | 986 | 210 | 593 (6.4) |
| 100 | 0.002 | + | + | + | + | + | 250* |
| 100 | 0.02 | 56 | 154 | 741 | 637 | 74 | 375 (5.9) |
| 100 | 0.2 | 64 | 132 | 875 | 735 | 191 | 662 (6.5) |
| Lsd ($p = 0.05$) NaCl*P | | 22 | 53 | 291 | 249 | 23 | (1) |

*Composite means, not included in anova.
+Insufficient plant material for ion analyses.
‡All analyses are for 3 replicates unless otherwise stated.
( ) ln transformed data, analyses and lsds are calculated for ln transformed data.

*Table 3.* The effect of applied NaCl and P on concentrations of Ca, Mg, Na, K, P and Cl in the shoots of lucerne plants 64 days after sowing (Experiment 2, Harvest 3)

| Treatments | | Shoot ion concentrations (mmoles kg$^{-1}$ dwt) | | | | | |
|---|---|---|---|---|---|---|---|
| NaCl m*M* | P m*M* | Ca | Mg | Na | K | P | Cl |
| 0† | 0.5 | 354 | 99 | 14 | 1089 | 259 | 114 |
| 0† | 5.0 | 302 | 82 | 17 | 1195 | 274 | 99 |
| 50‡ | 0.5 | 278 | 97 | 357 | 905 | 266 | 569 |
| 50‡ | 5.0 | 250 | 67 | 467 | 942 | 279 | 724 |
| 100† | 0.5 | 230 | 108 | 688 | 777 | 276 | 995 |
| 100† | 5.0 | 224 | 100 | 750 | 812 | 279 | 1097 |
| Lsd Na*P ($p = 0.05$) | | 37 | 35 | 10 | 136 | 29 | 17 |

†Analyses are with 3 replicates (see 'Materials and methods').
‡Analyses are with 4 replicates (see 'Materials and methods').

*Table 4.* The effect of applied NaCl and P on concentrations of Ca, Mg, Na, K, P and Cl in the roots of lucerne plants 64 days after sowing (Experiment 2, Harvest 3)

| Treatments | | Root ion concentrations (mmoles kg$^{-1}$ dwt) | | | | | |
|---|---|---|---|---|---|---|---|
| NaCl m*M* | P m*M* | Ca | Mg | Na | K | P | Cl |
| 0† | 0.5 | 128 | 203 | 39 | 1359 | 353 | 166 |
| 0† | 5.0 | 117 | 159 | 52 | 1263 | 336 | 144 |
| 50‡ | 0.5 | 118 | 203 | 394 | 1042 | 329 | 658 |
| 50‡ | 5.0 | 123 | 184 | 291 | 1055 | 345 | 695 |
| 100† | 0.5 | 107 | 187 | 585 | 852 | 324 | 920 |
| 100† | 5.0 | 122 | 199 | 573 | 1053 | 364 | 1056 |
| Lsd NaCl*P ($p = 0.05$) | | 45 | 111 | 68 | 227 | 82 | 128 |

†Analyses are with 3 replicates (see 'Materials and methods').
‡Analyses are with 4 replicates (see 'Materials and methods').

P level over all external NaCl concentrations. Shoot and root concentrations of Na and Cl increased linearly ($p < 0.001$) with increasing NaCl concentration and increasing concentrations of P (Tables 1 and 2).

External concentration of NaCl had no significant effect on shoot concentrations of P (Table 1), however, root concentrations of P decreased with increasing NaCl concentrations (Table 2). Tissue P concentrations in both roots and shoots reflected the external P conditions and increased significantly with increasing external P level.

*Experiment 2 (0.5 and 5.0 m*M *P)*

As in Experiment 1, shoot concentrations of Ca and K decreased significantly with increasing NaCl concentration (viz harvest 3, Table 3, $p = 0.05$) but were little affected by increasing P except for a significant decrease in Ca at 0 m*M* NaCl. Neither external P nor NaCl concentrations had a significant effect on shoot concentrations of Mg, and this was also true for root concentrations of Ca (Table 4). Concentrations of K in the roots decreased with increasing external NaCl concentration ($p = 0.05$), and at 100 m*M* NaCl were significantly lower in the 0.5 m*M* P treatment compared with the 5.0 m*M* treatment ($p = 0.05$).

Shoot and root concentrations of Na and Cl increased significantly with increasing NaCl concentration ($p = 0.05$). For shoots, there were no significant differences in tissue concentrations of either Na or Cl between the two external P levels, however there were some differences in root concentrations of these ions at different external P concentrations. At 50 m*M* NaCl, root tissue Na concentrations were higher at 0.5 m*M* P compared with 5.0 m*M* P ($p = 0.05$), and at 100 m*M* NaCl root tissue Cl concentrations were higher at 5.0 m*M* P compared with 0.5 m*M* P $p = 0.05$).

The concentration of P in the shoots and the roots did not differ according to external NaCl or P conditions.

## Discussion

Our results emphasise the complex relationship between NaCl and P that exists in plants and confirm the finding of Grattan and Grieve (1999) that this response is highly dependent upon the level of salinity and the concentration of P in the substrate. In most of the agricultural species that have been studied, saline conditions have been found to inhibit P uptake. This effect however seems to be more severe at low, rather than high, substrate P concentrations (e.g. cotton, Martinez and Lauchli, 1994; lupins, Treeby and Van Steveninck, 1988; and melons, Navarro et al., 2001). In our research with lucerne, internal concentrations of P in the shoots appeared to be relatively unaffected by external NaCl concentrations across the range of external P concentrations (0.002–5.0 m*M*) however root concentrations of P were found to decrease with increasing NaCl concentrations at external P levels of less than 0.2 m*M* (Experiment 1). A significant NaCl * P effect was found only in Experiment 1, where plants growing at the highest P treatment (0.2 m*M*) were more sensitive (in relative terms) to increasing levels of NaCl than were plants growing at the lower P treatments (0.02 and 0.002 m*M*). This suggests that, proportionally, increasing concentrations of P increased shoot growth more under low salinity conditions than under high salinity levels and that low P was much more growth-limiting than salinity.

In some other agricultural species, including forage clover (Ravikovitch and Yoles, 1971); corn (Bernstein et al., 1974) and sesame (Cerda et al., 1977), a decrease in dry matter production under saline conditions (and hence relative salt tolerance) has been associated with an increase in P uptake. Tomato appears to be exceptional in that increased P concentrations in the substrate in solution culture experiments (up to 0.01 m*M*) enhanced the salt tolerance despite the saline conditions appearing to cause an increase in the leaf P requirements (Awad et al., 1990). In soybean, the response varies with cultivar susceptibility to P toxicity (Grattan and Maas, 1984). Lucerne cultivars may vary in their uptake rates of P (James et al., 1995), however in Experiment 1 we found no differences between the two cultivars of lucerne in their response to the combined effects of NaCl and P.

The exact mechanism by which NaCl influences P uptake is unknown. Physical and chemical changes at the membrane level and the suppression of P uptake and accumulation have been suggested to be the result of competition with Cl (Papadopoulos and Rendig, 1983). In cotton, Martinez and Lauchli (1994) state that NaCl may induce alkalinization of the cytoplasm in root tip cells, increasing the transmembrane pH gradient and causing an increase in P uptake. Treeby and Van Steveninck (1988) found that P accumulated in the cell wall in lupin plants grown at high P (2 m*M*) and moderate NaCl concentrations (50 m*M*), however they were unsure how this caused subsequent leaf damage. Navarro et al. (2001) hypothesised that, in melons, high levels of NaCl decreased the mobility of P stored in the vacuole, and, as a result, inhibited export from this storage compartment to other parts of the plant.

The lucerne plants grown at 0.002 m*M* P and all NaCl concentrations (Experiment 1) suffered severely from P deficiency. Once external P concentrations increased from 0.002 m*M* to 0.02 m*M* at 50 m*M* NaCl, Na concentrations in the roots also increased substantially perhaps further confirming that the roots at the lower P level were extremely unhealthy. The higher concentrations of Na in the roots compared with the shoots for plants growing at 50 m*M* NaCl and both 0.02 and 0.2 m*M* P, suggest that some sort of shoot exclusion mechanism may operate that is dependent on adequate P nutrition. This phenomenon was less marked at 100 m*M* NaCl. Overall, higher external P concentrations appeared to have no positive effect on K:Na selectivity however, all values were above the critical ratio of 1.0 that is required to maintain processes such as enzyme activity (Greenway and Munns, 1980).

Under the luxuriant external P conditions in Experiment 2, there was no difference in plant production between the two P concentrations of 0.5 and 5.0 m*M* P. P concentrations of 0.2 m*M* to 5.0 m*M* P are extremely high in a soil situation however, the range is consistent with the recommended maximum levels that can be present (following dilution), in some wastewater streams from intensive animal industries and agricultural processing factories that may subsequently be used for irrigation. Lucerne cv. Aurora showed no adverse effects in response to external P concentrations that would normally be considered toxic for many species. From this it would appear that P uptake and transport in lucerne shoots is closely regulated at around 275 mmol*kg$^{-1}$ dry weight (approximately 0.8% dry weight) despite a 10-fold increase in external P concentration. This maximum level of P that can be tolerated by the lucerne plant is similar to that found in subterranean clover, but higher than the levels predicted for white clover (Weir and Cresswell, 1994).

A problem with nutrient-salinity research is the difficulty in extrapolating the results from the greenhouse to the field. In solution culture experiments, it may be difficult to maintain high concentrations of P over the duration of the experiment because P may precipitate with certain ions such as Ca. Levels of P used in greenhouse research may be an order of magnitude greater than those in the field where P availability is dependent on such factors as soil type (e.g. the fixation and buffering capacity, organic matter content, pH), soil moisture level, climatic conditions and the presence of microbial activity. In saline soils, phosphate availability is generally reduced because ionic strength effects reduce the activity of phosphate, and P concentrations in the soil are tightly controlled by sorption processes and by low solubility levels of Ca-P minerals (Grattan and Grieve, 1992). A P level of 0.02 m*M* is less than that commonly used in some nutrient solutions (such as full strength Hoagland's solution), however a soil solution containing this level would be considered to have an adequate P supply (Asher and Loneragan, 1967). Nevertheless, our results suggest that lucerne plants growing in solution culture of 0.02 m*M* P used up the available substrate P very quickly after it was applied and presumably soon suffered from P deficiency until the solutions were renewed.

It would be unlikely for lucerne to respond differently to P under conditions where NaCl is not the dominant salt and the Na/Ca ratio is more favourable.

The plant dry matter values determined in Experiment 1 (Na/Ca increasing up to 40), were very similar to the responses found in a number of lucerne lines (including CUF101) that were grown in saline conditions where sodium sulphate dominated (Na/Ca=3, Rogers et al., 1998). The P levels in these two studies were identical (0.2 m*M* P).

Actual root zone salinity levels are dependent on the salinity of the irrigation water, soil type (e.g. leaching fraction) and irrigation management practices. Our results suggest that high levels of P (0.5–5 m*M*), in conjunction with NaCl concentrations up to 100 m*M* can be used to flood irrigate lucerne without any detrimental effects on plant growth or nutritive quality – although there may be negative long-term soil effects. Under other irrigation methods, such as sprinkler irrigation, maximum NaCl concentrations should be lower to avoid foliar injury to the lucerne plant. The information from this study may be useful when (1) refining management strategies for the disposal of nutrient-rich saline wastewater, (2) calculating suitable P fertiliser rates for lucerne growing in saline soils and (3) determining the threshold concentration for saline irrigation water which can be applied safely to a lucerne crop growing in soil at a particular concentration of P.

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