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Calcium Influences Growth and Leaf Mineral Concentration of Citrus under Saline Conditions

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Abstract. **We determined whether the ability of sour orange seedlings to withstand saline irrigation water could be improved by the addition of calcium to the water. Sour orange *(Citrus aurantium* L.) seedlings were treated for 4 months with a nutrient solution containing either no NaCl, 40 mM NaCI, or 40 mM NaCl plus various concentrations of $CaSO_4$, $CaCl_2$, or KCl. After 4 months, the NaCl alone reduced root and shoot dry weights by ≈ 30% with no leaf necrosis. Addition of 1, 5, or 7.5 mM $CaSO_4$ to solutions containing 40 mM NaCl significantly inhibited the NaCl-induced reductions in shoot dry weight. Addition of 7.5 mM $CaCl_2$ or 7 mM KCl to the NaCl solution reduced leaf Na, but increased Cl to the toxicity level; hence, growth was not improved. The beneficial effect of $CaSO_4$ was mainly attributed to a reduction in the accumulation of Na and Cl below the toxicity level in the leaves (0.4% and 0.5%, respectively) without a major increase in total dissolved salts. This study demonstrated that the beneficial effect of adding Ca depended on the anion associated with the Ca salt. Calcium sulfate, but not $CaCl_2$, was able to overcome the damaging effect of NaCl to sour orange seedlings.**

Salts in soil and irrigation water are a serious problem for commercial agriculture, particularly in arid and semi-arid regions. However, the potential for salinity damage also exists in humid climates. Citrus productivity in some humid areas is threatened because only saline water is available for irrigation. Citrus has relatively little salt tolerance (Cooper, 1961).

In Florida, many citrus plantings are located in coastal areas where high-salinity waters are being used for irrigation (Calvert and Reitz, 1965; Wander and Reitz, 1951; Young and Jamison, 1944). Citrus plantings in these and other southern Florida areas have increased in response to extensive freeze damage that occurred in the early 1980s in northern and central Florida. This shift in citrus production regions has created an interest in controlling or reducing salt injury to Florida citrus.

Calcium has been shown to ameliorate the effect of saline conditions on the growth of plants (Deo and Kanwar, 1969; Epstein, 1972; Hyder and Greenway, 1965). This effect has been attributed to several actions of Ca, including: 1) flocculation of the soil in which clay particles have been dispersed by Na (Richards, 1954), 2) preventing the uptake of the Na ion to injurious levels and allowing the uptake of K (Waisel, 1962), and 3) maintaining the selective permeability of membranes (Hansen and Munns, 1988a). In the presence of adequate concentrations of Ca, bean plants have been reported to exclude Na and withstand the effects of relatively high NaCl concentrations (LaHaye and Epstein, 1969, 1971). In barley, salt-induced inhibition of K and P absorption and translocation was reduced noticeably by 1 mM $CdCl_2$ (Kawasaki et al., 1983). Soil application of gypsum ($CaSO_4$) markedly reduced the percentage of soluble Na in the soil (Harding et al., 1958) and reduced the percentage of Na in citrus leaves and roots (Jones et al., 1952; Pearson and Huberty, 1959).

The objective of this study was to investigate the effect of NaCl on sour orange seedling growth in relation to ion toxicity and nutritional imbalance. A second objective was to determine if addition of Ca to saline irrigation water would reduce salt damage to sour orange seedlings under sandy soil conditions. In spite of its susceptibility to tristeza, sour orange was chosen for this study because it is a popular rootstock in both Florida and other parts of the world.

Uniform, 3-month-old sour orange seedlings were transplanted into 19-cm-tall black plastic pots containing 5.5 liters of fine sand taken from the top 30 cm of a citrus orchard soil. The soil was Astatula fine sand (hyperthermic, uncoated Typic Quartzipsamments), with a pH of 6.5 and field capacity and wilting percentage of 7.2% and 1.2% (volumetric basis), respectively. Seedlings were placed in a greenhouse and irrigated every 2 to 3 days with a modified half-strength Hoagland's solution for 1 month before salt treatments were started. The temperature and relative humidity in the greenhouse ranged from 20 to 35C and from 40% to 100%, respectively. Salt treatments were initiated by adding 40 mM NaCl to the half-strength Hoagland's solution and by- also adding $CaSO_4$, $CaCl_2$, or KCl to selected treatments (Table 1). Due to other ions, the total dissolved salts (TDS) of the control half-strength Hoagland's solution was 0.4 $g \cdot liter^{-1}$, even though no NaCl was added to it.

Each treatment was replicated eight times in a randomized complete-block design. The osmotic potentials of the salt solutions were measured with a vapor pressure osmometer. Electrical conductivities of the treatments were determined with a conductivity meter (YSI Model 33; YSI, Yellow Springs, Ohio). Electrical conductivity values were converted to TDS (Richards, 1954). Seedlings were irrigated with the various solutions every 2 to 3 days for 4 months. The amount of solution added with each irrigation was determined by bringing the soil in the containers to slightly above water-holding capacity to prevent salt accumulation in the growth medium and to prevent plants from being drought stressed.

Seedlings were harvested after 4 months of experimental treatments. Dry weights of leaves, stems, and roots were measured after 3 days of drying at 60C. The dried leaves, which had been mature and fully expanded, were ground and their mineral concentration was measured. Leaf Cl content was determined by silver ion titration with a Buchler-Cotlove chloridometer (Searle, Fort Lee, N.J.) after extracting the leaf samples with a 1 nitric acid : 16 acetic acid (v/v) solution. Leaf Ca, Mg, Na, K, P, Fe, Mn, Zn, and Cu concentrations were measured by an inductively coupled argon plasma emission

Table 1. Salt additions to and physical properties of the nutrient solutions. (NaCl concentration was 40 mM.)

Treatment	TDS[z] ($g \cdot liter^{-1}$)	EC[y] ($dS \cdot m^{-1}$)	Osmotic potential (MPa)
Control (no NaCl)	0.4	0.9	– 0.03
NaCl	2.8	5.0	–0.19
NaCl + 1 mM $CaSO_4$	2.9	5.2	–0.19
NaCl + 5 mM $CaSO_4$	3.1	5.5	–0.21
NaCl + 7.5 mM $CaSO_4$	3.5	6.3	–0.23
NaCl + 13.5 mM $CaSO_4$	3.8	6.8	– 0.26
NaCl + 7.5 mM $CaCl_2$	3.6	6.5	– 0.24
NaCl + 7 mM KCl	3.4	6.1	–0.23

[z]TDS = total dissolved salts.
[y]EC = electrical conductivity.

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spectrometer (Perkin-Elmer Plasma 40, Norwalk, Corm.) after a wet digestion of the samples with a 4 nitric acid : 1 perchloric acid (v/v) mixture.

Analysis of variance was used to determine significant differences, and Duncan's multiple range test was used for mean comparison when F test was significant at $P < 0.05$.

Shoot dry weight was significantly reduced (by early 30%) when 40 mM NaCl was added, to the nutrient solution (Table 2). Although dot significant, the percentage reduction in root dry weight for each treatment was nearly the same as for shoot. We attribute this lack of significance to variation in root dry weight among replications. The addition of 1, 5, or 7.5, but not 13.5, mM $CaSO_4$ to the saline solution significantly decreased the adverse effect of NaCl on shoot growth. Lack of improvement for 13.5 mM $CaSO_4$ could be attributed to an increase in the osmotic stress (lower osmotic potential and higher TDS) of the salt solution (Table 1). Addition of either $CaCl_2$ or KCl to the saline solution did not improve. shoot growth relative to NaCl alone.

Addition of NaCl alone to the nutrient solution significantly increased leaf Na and Cl, but decreased Ca, Mg, and K (Table 3). Leaf Na and Cl accumulation above 0.4% and 0.5%, respectively, usually reduced citrus seedling growth (Zekri, 1987). Addition of $CaSO_4$ to the saline solution reduced Na and Cl concentrations, except that 1 mM did not reduce Na concentration. Addition of $CaC1_2$ reduced Na but increased Cl to the toxicity level (> 0.5%) in the leaves. Addition of KCl did not reduce Na, increased Cl, and reduced Ca and Mg relative to NaCl alone. No significant differences in P, Fe, Mn, Zn, and Cu were found among the treatments (data not shown).

Growth was reduced significantly without any visible leaf symptoms of salt damage. Although root and shoot dry weights were reduced by nearly 30% in some treatments after 4 months of salinity stress, none of these treatments induced visible bum or other damage symptoms on leaves.

Under saline conditions, the addition of Ca to irrigation waters altered sour orange seedling response. This study showed that the beneficial effect of Ca depended on the anion associated with the Ca. Calcium sulfate was significantly more effective than $CaCl_2$ in reducing the deleterious effect of NaCl on shoot growth (Table 2). Walker and Douglas (1983) also did not observe any improvement in citrus growth by increasing $CaCl_2$ in the growth medium. However, earlier work on citrus demonstrated the effectiveness of $CaSO_4$, $CaNO_3$, and $CaCO_3$ on reducing Na concentration in citrus leaf tissue (Jones et al., 1952), on preventing the soil deflocculation effect of Na, and on improving tree appearance (Cooper, 1961; Harding et al., 1958). LaHaye and Epstein (1969, 1971) demonstrated that an increase in Ca levels by adding either $CaSO_4$ or $CaCl_2$ protected bean plants from salt injury by restricting Na absorption and translocation to the leaves. Failure in the effectiveness of $CaC1_2$ in our work might have been due to the Cl accompanying the Ca and to the sensitivity of citrus to Cl.

Sodium chloride significantly reduced growth of *Leucaena leucocephala;* however, additions of $CaSO_4$ increased plant height, leaf number, and biomass of salt-treated plants (Hansen and Munns, 1988b). While high Na concentrations produced a Ca deficiency in cotton seedlings, supplemental Ca counteracted the toxic effect of NaCl and restored growth (Kent and Läuchli, 1985).

Calcium concentrations in external solutions that are adequate under nonsaline conditions become inadequate when the external Na : Ca ratio is high (Bernstein, 1975). This extra Ca is required to counteract the adverse effects of toxic ions in the external solution on plant growth. Bernstein also found plant response to salinity to be a function not only of the total salt concentration but also of the Na : Ca ratio in the root medium. Grieve and Maas (1988) demonstrated the sensitivity of sorghum to the Na : Ca ratio in saline root media. The reduction in the Na : Ca ratio in the external solution through the addition of Ca significantly decreased leaf Na concentration and increased shoot growth of beans (LaHaye and Epstein, 1969, 1971) and rice (Muhammed et al., 1987). Sodium–Ca interactions can also occur within the plant. Zid and Grignon (1985) concluded that the main ionic disturbance in leaves of *Citrus aurantium* plants grown in the presence of NaCl was a competition between Na and Ca for anionic sites in the cell walls. They suggested that this phenomenon could be one of the causes of necrotic burns that are characteristic of Na toxicity due to Na accumulation and Ca displacement in the leaf free space.

In our study, NaCl reduced shoot growth due partially to excess accumulation of Na and Cl in the leaves. The improvement of shoot growth by addition of $CaSO_4$ likely was not totally due to the effect of Ca in maintaining the selective permeability of membranes, because shoot growth was not improved by addition of $CaCl_2$. We believe that the competitive interaction between Ca and Na and between SO_4 and Cl contributed to offsetting the deleterious effect of NaCl on citrus growth. We also believe that addition of $CaSO_4$ to the saline solution decreased both the Na : Ca and Cl : SO_4 ratios in the medium. Hence, there would be less Na and Cl uptake and accumulation in the leaves, where these ions can disturb metabolic processes.

In conclusion, this study demonstrated that $CaSO_4$ improved the ability of sour orange to tolerate salt and that the beneficial effect of adding Ca to saline irrigation water depended on the anion accompanying the Ca. Calcium sulfate, but neither $CaC1_2$ nor KCl, was found to overcome the detrimental effects of NaCl by decreasing the concentrations of Na and Cl in citrus leaves.

Table 2. Root and shoot dry weights of sour orange seedlings after 4 months of treatment with salt solutions.[z] (NaCI concentration was 40 mM.)

Treatment	Root dry wt: Mean actual (g)	Root dry wt: Relative (%)	Shoot dry wt: Mean actual (g)	Shoot dry wt: Relative (%)
Control (no NaCl)	9.4 a	100	36.6 a	100
NaCl	6.8 a	72	25.9 d	71
NaCl + 1 mM $CaSO_4$	8.2 a	87	32.2 ab -	88
NaCl + 5 mM $CaSO_4$	9.3 a	99	37.0 a	101
NaCl + 7.5 mM $CaSO_4$	9.2 a	97	33.4 ab	91
NaCl + 13.5 mM $CaSO_4$	7.5 a	80	29.2 bcd	80
NaCl + 7.5 mM $CaCl_2$	7.5 a	79	28.4 cd	78
NaCl + 7 mM KCl	6.9 a	73	27.0 cd	74

[z]Mean of eight replications; mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Table 3. Leaf mineral concentration (percent leaf dry weight) of sour orange seedlings after 4 months of treatment with salt solutions.[z] (NaCl concentration was 40 mM)

Treatment	Mineral content (%): Ca	Mg	Na	c1	K
Control (no NaCl)	2.1 b	0.30 a	0.02 c	0.02 d	2.8 b
NaCl	1.7 c	0.21 b	0.47 a	0.97 b	2.0 c
NaCl + 1 mM $CaSO_4$	1.7 c	0.22 b	0.43 a	0.48 c	2.1 c
NaCl + 5 mM $CaSO_4$	2.4 ab	0.21 b	0.27 b	0.41 c	1.9 c
NaCl + 7.5 mM $CaSO_4$	2.7 a	0.20 b	0.24 b	0.43 c	1.9 c
NaCl + 13.5 mM $CaSO_4$	2.7 a	0.20 b	0.24 b	0.39 c	1.9 c
NaCl + 7.5 mM $CaCl_2$	2.8 a	0.20 b	0.25 b	1.36 a	2.0 c
NaCl + 7 mM KCl	1.3 d	0.15 c	0.43 a	1.21 a	3.6 a

[z]Mean of eight replications; mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Literature Cited

Bernstein, L. 1975. Effect of salinity and sodicity on plant growth. Annu. Rev. Phytopathol. 13:295-312.

Calvert, D.V. and H.J. Reitz. 1965. Salinity of water for sprinkle irrigation of citrus. Proc. Fla. State Hort. Soc. 78:73-78.

Cooper, W.C. 1961. Toxicity and accumulation of salts in citrus trees on various rootstock in Texas. Proc. Fla. State Hort. Soc. 74:95-104.

Dee, R. and J.S. Kanwar. 1969. Effect of saline irrigation waters on the growth and chemical composition of wheat. J. Indian soc. Soil Sci. 16:365-370.

Epstein, E. 1972. Mineral nutrition of plants. Principles and perspectives. Wiley, New 'York..

Grieve, C.M. and E.V. Maas. 1988. Differential effects of sodium/calcium ratio on sorghum genotypes. Crop Sci. 28:659-665.

Hansen, E.H. and D.N. Munns. 1988a. Effect of $CaSO_4$ and NaCl on mineral content of *Leucaena leucocephala.* Plant & Soil 107:101-105.

Hansen, E. H.. and D.N. Munns. 1988b. Effects of $CaSO_4$ and NaCl on growth and nitrogen fixation of *Leucaena leucocephalo.* Plant & Soil 107:95-99.

Harding, R. B., P.F. Pratt, and W.W. Jones. 1958. Changes in salinity, nitrogen, and soil reaction in a differentially fertilized irrigated soil. Soil Sci. 85:177-184.

Hyder, S.Z. and H. Greenway. 1965. Effects of Ca^{++} on plant sensitivity to high NaCl concentrations. Plant & Soil 23:258-260.

Jones, W. W., H.E. Pearson, E.R. Parker, and M.R. Huberty. 1952. Effect of sodium in fertilizer and in irrigation water on concentration in leaf and root tissues of citrus trees. Proc. Amer. Soc. Hort. Sci. 60:65-70.

Kawasaki, T., G. Shimizu, and M. Moritsugu. 1983. Effects of high concentrations of sodium chloride and polyethylene glycol on the growth and ion absorption in plants. II. Multi-compartment transport box experiment with excised roots of barley. Plant & Soil 75:87–93.

Kent, L.M. and A. Läuchli. 1985. Germination and seedling growth of cotton: Salinity-calcium interactions. Plant, Cell & Environ. 8: 155–159.

LaHaye, P.A. and E. Epstein. 1969. Salt toleration by plants: Enhancement with calcium. Science 166:395–396.

LaHaye, P.A. and E. Epstein. 1971. Calcium and salt toleration by bean plants. Physiol. Plant. 25:213-218.

Muhammed, S., M. Akbar, and H.U. Neue. 1987. Effect of Na/Ca and Na/K ratios in saline culture solution on the growth and mineral nutrition of rice *(Oryzu sativa* L.). Plant & Soil 104:57-62.

Pearson, H.E. and M.R. Huberty. 1959. Response of citrus to irrigation with water of different chemical-characteristics. Proc. Amer. Soc. Hort. Sci. 73:248-256.

Richards, L.A. 1954. In: Diagnosis and improvement of saline and alkali soils. USDA Agr. Hdbk. 60. D. 4-5.

Waisel, Y. 1962. The effect of Ca on the uptake of monovalent ions by excised barley roots. Physiol. Plant. 15:709-724.

Walker, R.R. and T.J. Douglas. 1983. Effect of salinity level on uptake and distribution of chloride, sodium and potassium ions in citrus plants. Austral. J. Agr. Res. 34:145-153.

Wander, I.W. and H.J. Reitz. 1951. The chemical composition of irrigation water used in Florida citrus groves. Fla. Agr. Expt. Sta. Bul. 480.

Young, T.W. and V.C. Jamison. 1944. Saltiness in irrigation wells. Fla. State Hort. Soc. 57: 18–23.

Zekri, M. 1987. Effects-of sodium chloride and polyethylene glycol on the water relations, growth, and morphology of citrus rootstock seedlings. PhD Diss., Univ. of Florida, Gainesville. (Diss. Abstr. 88-18542).

Zid, E. and C. Grignon. 1985. Sodium-calcium interactions in leaves of *Citrus aurantium grown* in the presence of NaC1. Physiol. Veg. 23 :895–903.