Critical Zinc\(^{+2}\) Activities for Sour Orange Determined with Chelator-buffered Nutrient Solutions

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Abstract. Chelator-buffered nutrient solutions were used to study the effect of different levels of Zn activity in the rhizosphere on growth and nutritive responses of various tissues of sour orange seedlings. The seedlings were grown for 3 months in a growth chamber in a hydroponic culture containing from 5 to 69 \(\mu\text{M}\) total Zn in Expts. 1 and 2, respectively. Zn\(^{+2}\) activities were calculated with a computerized chemical equilibrium model (Geochem-PC), and buffered by inclusion of a chelator, diethylenetriamine pentaacetate (DTPA), at 74 and 44 \(\mu\text{M}\) in excess of the sum of Fe, Mn, Zn, Cu, Ni, and Co in Expts. 1 and 2, respectively. The use of DTPA-buffered solutions proved successful in imposing varying degrees of Zn deficiency. The deficiency was confirmed by leaf symptomatology, leaf chemical analyses, i.e., <16 mg·kg\(^{-1}\) Zn, and responses to foliar sprays and application of Zn to the roots. Growth parameters varied in their sensitivity to Zn deficiency, i.e., root dry weight < leaf number and white root growth < stem dry weight < leaf dry weight < shoot elongation and leaf area. The critical activities, expressed as \(p\text{Zn} = \text{log}(\text{Zn}^{+2})\), were 10.2 ± 0.2 for root dry weight, 10.1 ± 0.2 for leaf number and white root growth, 10.0 ± 0.2 for stem dry weight, 9.9 ± 0.2 for leaf dry weight, and 9.8 ± 0.2 for shoot growth and leaf area. Increases in growth were observed in response to Zn applications even in the absence of visible Zn-deficiency symptoms. Seedlings containing >23 mg·kg\(^{-1}\) Zn in leaves did not respond to further additions of Zn to the nutrient solution. Foliar sprays were less effective than Zn applications to the roots in alleviating severe Zn deficiency because foliar-absorbed Zn was not translocated from the top to the roots and thus could not correct Zn deficiency in the roots.

Chapman (1968) noted that next to nitrogen, zinc deficiency is the most widespread nutritional malady of citrus in the world. Although the correction of Zn deficiency in citrus with foliar sprays has received considerable attention (Swietlik, 1989), not much is known about which tissues are most sensitive, and what are the critical levels of free Zn in the tree’s rhizosphere. These gaps in knowledge are due in part to the difficulty of reproducing varying degrees of Zn stress using traditional nutrient solution systems (Parker et al., 1994a). These systems rely solely on exhausting contaminant Zn via plant uptake, a difficult task to accomplish in view of plants’ low requirements for microelements. In fact, normal levels of free Zn\(^{+2}\) in the soil solution are so low that they cannot directly be measured with standard methods. Hence, hydroponic systems prepared from even the purest grade chemicals may not produce visible Zn deficiency (Graham et al., 1987). After 6-month growth of sour orange seedlings in water culture with no Zn addition, leaves still contained 21 mg·kg\(^{-1}\) Zn, and responses to foliar sprays and application of Zn to the roots. Growth parameters varied in their sensitivity to Zn deficiency, i.e., root dry weight < leaf number and white root growth < stem dry weight < leaf dry weight < shoot elongation and leaf area. The critical activities, expressed as \(p\text{Zn} = \text{log}(\text{Zn}^{+2})\), were 10.2 ± 0.2 for root dry weight, 10.1 ± 0.2 for leaf number and white root growth, 10.0 ± 0.2 for stem dry weight, 9.9 ± 0.2 for leaf dry weight, and 9.8 ± 0.2 for shoot growth and leaf area. Increases in growth were observed in response to Zn applications even in the absence of visible Zn-deficiency symptoms. Seedlings containing >23 mg·kg\(^{-1}\) Zn in leaves did not respond to further additions of Zn to the nutrient solution. Foliar sprays were less effective than Zn applications to the roots in alleviating severe Zn deficiency because foliar-absorbed Zn was not translocated from the top to the roots and thus could not correct Zn deficiency in the roots.

Plant culture. Sour orange seeds were germinated and seedlings were grown in the greenhouse in trays filled with a mixture of 11 peat : 6 vermiculite : 3 sand (v/v/v). Six weeks before the experiments, 4-to 6-month-old seedlings (=40 to 60 cm high with =20 to 30 leaves) were transferred to 1-liter plastic bottles filled with a nutrient solution containing (in mM) N, 7 [as Ca(NO\(_3\)]\(\_2\); and KNO\(_3\]); P, 1 (as KH\(_2\)PO\(_4\)); K, 2 (as KNO\(_3\) and KH\(_2\)PO\(_4\)); Ca, 3 [as Ca(NO\(_3\)]\(\_2\)]; Mg, 1 (as MgSO\(_4\)); and (in \(\mu\text{M}\)) Fe, 37 (as FeEDTA); Mn, 1 (as MnCl\(_2\)); B, 1 (as H\(_3\)BO\(_3\)); Zn, 0.2 (as ZnSO\(_4\)); and Cu, 0.2 (as CuSO\(_4\)).
Comparison between foliar and root Zn treatment (Expt. 1).

Seeds were pruned above the fourth leaf from the base and allowed to grow one shoot only. The seedlings were transferred to a new nutrient solution containing (in mM) N, 6.88 [as Ca(NO₃)₂] and KNO₃; P, 0.02 [as NH₄H₂PO₄]; K, 1.42 (as KNO₃); Ca, 3.74 [as Ca(NO₃)₂] and CaCl₂; Mg, 1.49 (as MgSO₄); and (in µM) Fe, 100 [as FeCl₃, pFe = log(Fe) = 20.363]; B, 23 (as H₃BO₃); Mn, 10 [as MnSO₄, pMn = log(Mn) = 7.43]; and 50 µM Zn measured in the nutrient solution plus six foliar sprays with 5.2 mM ZnSO₄ + DTPA in equimolar concentrations. A 44 µM excess of DTPA was maintained over the sum of Zn, Fe, Mn, Cu, Ni, and Co. There were six replications (seedlings) per treatment arranged in completely randomized blocks.

The treatments lasted 90 days. Observations, measurements, and tissue mineral analyses were performed as in Expt. 1.

The treatment effects were evaluated statistically using linear and quadratic regression models with plateau (SAS Institute, 1988). These models enabled the determination of the critical Zn concentrations above which no further growth increases occurred. Trends in mineral nutrient concentration changes in leaves, stems, and roots were evaluated with linear, quadratic, and cubic polynomial regression analyses.

Free Zn activity by Geochem model. The computer program Geochem PC version 2.0, obtained from D.R. Parker (Univ. of California, Riverside, Dept. of Soil and Environmental Sciences), was used to calculate activities of chemical species in the nutrient solutions (Parker et al., 1994b). Formation constants for DTPA solutions (Parker et al., 1994b). Formation constants for DTPA were taken from Martell and Smith (1974 and 1982).

Geochem PC is a computerized chemical equilibrium model comprised of (1) a user interface to set an equilibrium problem, (2) a database of equilibrium constants which are alterable by the user, (3) an algorithm to solve the problem, and (4) an interface that provides the results in an ASCII format (Parker et al., 1994b). The program calculates free metal activities and concentrations. At infinite dilutions, free metal activity and concentration are numerically the same. As ionic strength of an electrolytic solution increases, electrostatic forces depress metal activity below that of free metal concentration. Thermodynamic text books give formulas to make necessary conversions between the two parameters (Szaranowa, 1985).

In the nutrient solution system, used in this study, activities of metallic microelements will remain relatively stable because free metals absorbed by plants are replaced by those dissociating from the large pools of chelate-metal complexes. This is analogous to a pH buffer maintaining constant hydrogen activity even as H⁺ is being removed from a system. Furthermore, a small contamination will be inconsequential as it is effectively complexed with an excess of the chelator.

Except for Co and Ni, the concentrations of all metals in the nutrient solutions were verified by actual analyses at the beginning and end of a nutrient solution change cycle. Ten-ml aliquots of nutrient solution were filtered through Whatman filter no. 42, acidified with 5 ml 6N HCl and analyzed for K, Ca, Mg, Na, Fe, Zn, Mn, and Cu with an atomic absorption spectrophotometer. Phosphorus was determined with the chlorostannous-reduced molybdophosphoric blue color method (Jackson, 1970).

Results

Comparison between foliar and root Zn treatment (Expt. 1). Dry weight of leaves, stems and roots, white root growth, and total leaf area per plant were all significantly increased by elevating the Zn
nutrient solution level from 5 to 69 μM (Table 1). Foliar sprays with ZnSO₄ increased the growth compared to the control but were not as effective as the highest root treatment (69 μM).

Control plants exhibited severe symptoms of Zn deficiency such as stunted growth and the development of extremely small, narrow, and chlorotic leaves. Chlorosis severity decreased as Zn in the nutrient solution increased from 5 to 21 μM. In the 37 μM Zn treatment, the leaves were green but noticeably smaller than those from the 69 μM treatment, which appeared healthy and deep green. Foliar-treated seedlings were similar in appearance to those from the 37 μM treatment.

The control, 7- and 9-μM treated seedlings had few white roots and all of them originated from the lowest portion of the root system. The first-order lateral white roots were <1 mm long and abnormally thick (up to 2 mm). The second-order laterals were absent. In the 13 and 21 μM treatments, white roots were more abundant, up to 3 and 6 mm long, respectively, and <1 mm thick. However, they emerged only from the lower 1/3 portion of the root system and produced no second order laterals. In the 37- and 69-μM treatments, white roots originated from all parts of the root system and produced first-order laterals up to 25 mm long and second-order laterals up to 10 mm long. The white roots were about 0.5 mm thick, which is typical for the nutrient solution-grown sour orange seedlings. The root systems of foliar-sprayed plants resembled most closely those from the 21-μM treatment.

Leaf, stem, and root Zn concentrations increased linearly as the level of Zn in the nutrient solution increased from 5 to 69 μM (Table 1). Foliar-treated seedlings had higher Zn concentrations in the aerial parts than the 69 μM treatment. The sprays, however, had no effect on root Zn concentration, which was similar to the controls. Leaf concentrations of P, K, Mg, Ca, Mn, Fe, and Cu were increased from 5 to 21 μM (Fig. 1), and leaf dry weight, shoot growth, and leaf area (Fig. 2) increased in response to increasing levels of Zn in the nutrient solution. These increases leveled off as the total nutrient solution Zn concentration reached the critical level of 36-, 48-, 58-, 69-, 74-, and 81-μM Zn for root dry weight, leaf number, white root growth, stem dry weight, leaf dry weight, shoot growth, and total leaf area, respectively.

Control plants displayed severe symptoms of Zn deficiency similar to those described for Expt. 1. Although less acute, the symptoms were also present in the 21-μM treatment with the top leaves predominantly affected and the middle or basal shoot leaves less affected. At and above the 37-μM treatment there were no deficiency symptoms and the plants appeared healthy with deep green foliage.

Zinc-deficient seedlings also displayed similar morphological changes of white roots observed in Expt. 1. Their severity gradually decreased with increasing levels of Zn in the nutrient solution. At and above the 53 μM, white roots grew normally.

Leaf and root Zn concentration increased as Zn increased in the nutrient solution following a linear and cubic function, respectively (Fig. 3). Zinc in the stem, however, decreased as nutrient Zn increased from 5 to 21 μM, then showed a linear increase with further increases in the nutrient Zn concentration.

Leaf P, K, Ca, Mg (Fig. 4), and leaf Fe, Mn, and Cu (Fig. 5) were recorded (in % dry weight): P 0.09–0.18; K 1.25–3.01; Mg 0.32–0.70; and Ca 2.00–3.56; and (in mg·kg⁻¹ dry weight): Mn 18.2–54.6; Fe 49.8–79.4; and Cu 4.3–9.9.

**Effect of Zn level on sour orange growth and nutrition (Expt. 2).** Root dry weight, leaf number, white root growth, stem dry weight (Fig. 1), and leaf dry weight, shoot growth, and leaf area (Fig. 2) increased in response to increasing levels of Zn in the nutrient solution. These increases leveled off as the total nutrient solution Zn concentration reached the critical level of 36-, 48-, 58-, 69-, 74-, and 81-μM Zn for root dry weight, leaf number, white root growth, stem dry weight, leaf dry weight, shoot growth, and total leaf area, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry wt (g/plant)</th>
<th>White root growth index¹</th>
<th>Leaf area (cm²/plant)</th>
<th>Tissue Zn concn (mg·kg⁻¹ dry wt)</th>
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<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stems</td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>5 (Control)</td>
<td>0.20</td>
<td>0.14</td>
<td>0.77</td>
<td>0.8</td>
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<tr>
<td>7</td>
<td>0.19</td>
<td>0.16</td>
<td>0.70</td>
<td>1.1</td>
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<td>9</td>
<td>0.43</td>
<td>0.19</td>
<td>0.74</td>
<td>1.3</td>
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<tr>
<td>13</td>
<td>0.26</td>
<td>0.20</td>
<td>0.96</td>
<td>2.1</td>
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<tr>
<td>21</td>
<td>0.41</td>
<td>0.28</td>
<td>1.21</td>
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<tr>
<td>37</td>
<td>1.76</td>
<td>0.68</td>
<td>1.58</td>
<td>4.1</td>
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<tr>
<td>69</td>
<td>3.52</td>
<td>1.16</td>
<td>2.24</td>
<td>4.4</td>
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6 × Foliar sprays | 1.95 | 0.58 | 1.27 | 2.6 | 207.8 | 24.8 | 35.5 | 17.1 |

**Significance**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Linear¹</td>
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</tr>
<tr>
<td>Quadratic¹</td>
<td>*</td>
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<tr>
<td>Cubic¹</td>
<td>*</td>
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<td>Spray vs. control</td>
<td>**</td>
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<tr>
<td>Spray vs. 69 μM Zn</td>
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</table>

¹Roots in DTPA-buffered nutrient solutions. Foliar sprays, 5.2 mM ZnSO₄·4H₂O + 0.1% Tween 20, six times at 2-week intervals.
²O = No white root growth; 5 = very abundant white root growth.
³Linear, quadratic, and cubic polynomial regressions for root Zn treatments only.
⁴NS, ** NS, significant at P = 0.01 or 0.05, respectively.
Fig. 1. Growth responses of sour orange seedlings exposed to increasing levels of solution Zn (Expt. 2). The seedlings were cultured in a DTPA-buffered nutrient solution and the responses were measured 3 months after treatment.

Fig. 2. Growth responses of sour orange seedlings exposed to increasing levels of solution Zn (Expt. 2). The seedlings were cultured in a DTPA-buffered nutrient solution and the responses were measured 3 months after treatment.
response to increasing levels of Zn in the nutrient solution. Root P (Fig. 4) and root Fe (Fig. 5) concentrations were reduced as Zn increased to 85 µM, and root K, Ca, Mg, and Na concentrations increased as Zn levels increased to 37 µM (Ca and Mg) and 101 µM (Na) (Figs. 4 and 5). Root Mn and Cu showed no consistent trends (data not shown).

**Determination of Zn activity for various growth parameters by Geochem Model.** In both experiments Zn activity in the nutrient solution increased (pZn decreased) with each increment of total Zn in the nutrient solution (Fig. 6). The activity was lower in the nutrient solution of Expt. 1 compared to the nutrient solution of Expt. 2 at equimolar total Zn concentration.

In both experiments Zn activity decreased between the beginning and end of the nutrient solution change cycle (Fig. 6). The Zn activities in Expt. 2, coincident with the observed plateau responses for a given growth variable (Figs. 1 and 2), were considered critical for that growth variable. The critical activities were approximately pZn = 10.2 ± 0.2 for root dry weight, pZn = 10.1 ± 0.2 for leaf number and white root growth, pZn = 10.0 ± 0.2 for stem dry weight, pZn = 9.9 ± 0.2 for leaf dry weight, and pZn = 9.8 ± 0.2 for shoot growth and leaf area.

**Discussion**

The use of DTPA-buffered nutrient solutions was successful in inducing Zn deficiency of varying severity in sour orange seedlings. Visual deficiency symptoms were confirmed by the chemical analyses (<16 mg·kg⁻¹ Zn in leaves, Embleton et al., 1973) and by responses to foliar sprays and application of Zn to the roots. Zinc sufficient plants contained >23 mg·kg⁻¹ Zn in the leaves (Fig. 3),

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**Fig. 3.** Tissue Zn concentrations in sour orange seedlings exposed to increasing levels of solution Zn (Expt. 2). The seedlings were cultured in a DTPA-buffered nutrient solution and the responses were measured 3 months after treatment. Leaf: linear regression (P < 0.01); Stem: quadratic regression (P < 0.05); Root: cubic regression (P < 0.01).

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**Fig. 4.** Tissue P, K, Ca, and Mg concentrations in sour orange seedlings exposed to increasing levels of solution Zn (Expt. 2). The seedlings were cultured in a DTPA-buffered nutrient solution and the responses were measured 3 months after treatment. Leaf P, K, Mg: cubic regression (P < 0.01); Leaf Mg: quadratic regression (P < 0.05); Stem P, Mg, Ca: cubic regression (P < 0.01); Stem K: linear regression (P < 0.01); Root K, Mg: cubic regression (P < 0.01); Root P, Ca: quadratic regression (P < 0.01).
Fig. 5. Tissue Na, Fe, Mn, and Cu concentrations in sour orange seedlings exposed to increasing levels of solution Zn (Expt. 2). The seedlings were cultured in a DTPA-buffered nutrient solution and the responses were measured 3 months after treatment. Leaf Na: not significant; Leaf Fe, Mn, Cu: cubic regression ($P < 0.05$); Stem Na: linear regression ($P < 0.01$); Stem Fe, Mn, Cu: cubic regression ($P < 0.01$); Root Na: cubic regression ($P < 0.01$); Root Fe: quadratic regression ($P < 0.01$); Root Mn: not significant.

Fig. 6. Activities of free Zn$^{2+}$ in the nutrient solutions of Expt. 1 and Expt. 2 at the beginning (broken line) and end (solid line) of a 2-week nutrient solution change cycle.

which compares rather well with the optimal sufficiency range of 25 to 100 mg·kg$^{-1}$ for orange proposed by Embleton et al. (1973). The results corroborate those reported by Parker et al. (1992) and Norvell and Welch (1993), who used a similar system with DTPA and HEDTA chelates, respectively, to study Zn nutrition of tomato and barley. Also, Halvorson and Lindsay (1977) induced Zn deficiency in corn in the presence of DTPA in a nutrient solution.

Leaf Zn deficiency symptoms closely resembled those observed on field-grown citrus (Chapman, 1968; Embleton et al., 1973; Swietlik, 1989), except that ‘mottle leaf’ symptoms, which are chlorotic patches in the interveinal areas, were absent. We speculate that their absence could have been due to the technique used to induce Zn deficiency but the mechanism involved is unknown. Alternatively, an unidentified nutrient imbalance(s) associated with Zn deficiency that occurs in the field- but not hydroponically grown plants or some other factor(s) could be responsible. Variation in Zn deficiency symptoms has been observed on the same plant species when they have been grown under different culture systems (Loneragan et al. 1982; Ohki, 1977; Parker et al., 1992, Viets et al., 1954). Loneragan et al. (1982) proposed that ‘mottle leaf’ is an expression of P toxicity induced by low Zn concentration in plants. Many other researchers have associated P toxicity with Zn-deficient plants (Bhatti and Loneragan, 1970; Cakmak and Marschner, 1986; Christensen and Jackson, 1981, Loneragan et al. 1982; Parker et al., 1992; Webb and Loneragan, 1988). Phosphorus toxicity, however, was not a prerequisite for the development of ‘mottle leaf’ in field-grown citrus trees (Swietlik and Laduke, 1991) and subterranean clover (Loneragan et al., 1979). Thus, it is unlikely that its absence prevented the occurrence of leaf mottling in the present study.
Plausibly, some of the cases of Zn-induced P toxicity reported in the literature were an artifact of high concentrations of P (250 to 3000 \( \mu \text{M} \)) used in hydroponic systems, which typically are up to 1000-fold greater than those of the soil solution (Asher and Loneragan, 1967). Also, the absence of Si in nutrient solution preparations has been reported to accentuate Zn-deficiency-induced P toxicity (Marschner et al., 1990). An excess of P in the nutrient solution was avoided in this study by following a practice of small but frequent P additions which maintained nutrient solution concentration closer to that found in the field. Under this system, leaf concentrations of Zn-deficient plant, albeit elevated, were not toxic (Embleton et al., 1973).

The importance of Zn for root growth has already been recognized (Christensen and Jackson, 1981; Loneragan et al., 1982; Potapova, 1974), but, to our knowledge, the severe growth stunting accompanied by dramatic morphological changes we observed in nutrient solution have not been reported. Considering the vital role that Zn plays in auxin synthesis (Salami and Kenefick, 1970; Tsui, 1948), such a response could be reflective of hormonal influences on root initiation and growth (Street, 1968). Whatever mechanism was responsible for the poor root growth, it was decreasingly manifest from the upper to lower parts of the root system, a fact mirrored by the pattern of root growth as Zn supply increased (see the Results section). As lower roots would likely occupy deeper and wetter soil layers in the field, this response may reflect an adaptive mechanism to maximize water uptake when root growth is limited by Zn deficiency. Elevating Zn concentration only in the tops of Zn-deficient plants with foliar sprays partially restored normal root growth but clearly was not as effective as the roots absorbing Zn directly from high Zn-concentration solutions. This suggests that two mechanisms operating at two tiers of structural organization were involved: one in the roots and the other in the shoots.

Different growth parameters showed varying sensitivity to Zn deficiency, i.e., root dry weight < leaf number < white root growth < stem dry weight < leaf dry weight < shoot elongation < leaf area. In the past, typically only total plant dry weight or dry weight of top and roots were considered (Christensen and Jackson, 1981; Loneragan et al., 1982; Norvell and Welch, 1993; Parker et al., 1992; Webb and Loneragan, 1988; Welch and Norvell, 1993). The Geochem calculations explain why no growth plateaus could be detected in Expt. 1. Clearly Zn activities in the nutrient solutions of Expt. 1 were too low to elicit growth plateau responses. Higher amounts of excess of DTPA and lower levels of Ca in the nutrient solution of Expt. 1 compared to the nutrient solution of Expt. 2 were responsible for this Zn activity suppression (Parker et al., 1994a).

Seedlings responded to Zn supply even in the absence of shoot deficiency symptoms, which contrasts with the results of some citrus field studies in which the correlation of mild Zn-deficiency symptoms produced no visible growth responses (Embleton et al., 1988; Swietlik, 1989; Swietlik and LaDuke, 1991; Wutschker and Obreza, 1987). Apart from the fact that field variability likely was much greater, Zn in these trials was applied exclusively in foliar sprays. In our study, however, this method proved less effective than applying Zn to the roots (Table 1). Zinc is not easily translocated from the top to the roots and thus cannot correct Zn deficiency in the roots (Bukovac and Wittwer, 1957; Table 1). Therefore, the lack of responses of mildly Zn-deficient trees to Zn foliar sprays might be interpreted as an inefficiency of the Zn application method rather than tree tolerance of mild Zn deficiency. This question may best be answered by a field experiment comparing soil and foliar Zn foliar sprays on tree performance. The metal’s poor mobility in soil has discouraged extensive experimentation with soil Zn applications (Swietlik, 1989). Clearly, more research to ascertain whether to use new Zn-chelated materials as a soil dressing or to use another improved application technique is warranted.

The Geochem calculations indicated that the critical Zn activity in the rhizosphere of sour orange seedlings was approximately pZn = 9.8 ± 0.2 (Fig. 6). As defined here, the critical value denotes metal activity above which no deficiency symptoms and further increases in plant growth parameters occur. The activity is higher than the pZn = 10.60 reported for corn (Halvorson and Lindsay, 1977), pZn = 10.80 for soybean (Chaney et al., 1989), pZn = 10.60 for tomato (Parker et al., 1992), and pZn = 10.52 for barley (Norvell and Welch, 1993). Thus, sour orange may be more sensitive to low Zn supply than some of the annual species. Ma and Lindsay (1990) estimated that Zn activity in the soil solution of arid-zone calcareous soils varied from pZn = 7.90 to 10.89 and depended on soil pH as follows: log (Zn\(^{2+}\)) = 5.7 − 2pH. Using their model we estimated Zn activity in most southern Texas soils (pH = 7.5 to 8.4) to vary between pZn = 9.30 and 11.10. These estimates help explain why citrus trees grown in these soils frequently show Zn deficiency symptoms.

Seedlings optimally supplied with Zn in Expt. 2 also had normal concentrations of all other nutrients in the leaves (Embleton et al., 1973), indicating that plant response limits were not caused by other nutrient deficiencies or toxicities. In Expt. 1, however, deep green and healthy looking seedlings of the 69 \( \mu \text{M} \) and foliar spray treatment had low, albeit not deficient, leaf Mn, Cu, and P levels. Thus, nutrient solutions with elevated Mn and Cu activities of pMn = 7.59 and pCu = 13.49 as in Expt. 2 are recommended for future experiments. Similarly, 40 \( \mu \text{M} \) P (Expt. 2), as opposed to 20 \( \mu \text{M} \) P (Expt. 1) in the nutrient solution, produced seedlings more adequately supplied with this element. The activities for Mn and Cu are larger than pMn = 8.6 and pCu = 16 suggested for soybean by Chaney et al. (1989) and pMn = 8.1 and pCu = 14.7 used by Parker et al. (1992) to grow healthy tomato plants.

The severely Zn-deficient seedlings had elevated concentrations of elements other than Zn, which is consistent with commonly observed accumulation of P in Zn-deficient plants discussed above and less commonly observed accumulation of other elements, e.g., Fe, Mn, and NO\(_3\) in sugar beet (Rosell and Ulrich, 1964); Fe in bean (Ambler and Brown, 1969) and corn (Jackson et al., 1967); Mg in okra (Loneragan, et al., 1982); B (Graham et al., 1987), Ca, Mg, Mn, and Na (Norvell and Welch, 1993; Welch and Norvell, 1993) in barley. All the increases except that reported by Jackson et al. (1967) were produced in nutrient solution. A possible explanation in this study is the reduction in dry-matter production without comcomitant and equal reductions in mineral nutrient uptake (called the ‘concentration effect’). This phenomenon could occur if, as a result of Zn deficiency, there was a loss of cell membrane integrity leading to passive nutrient uptake in a transpirational stream (Welch et al., 1982).

The elevated Zn concentration in stems of the control plants in Expt. 2 (Fig. 3) suggests that intact molecules of ZnDTPA could have been absorbed through dysfunctional cell membranes. In additional experiments, the most severely Zn-stressed sour orange seedlings would occasionally show elevated (>20 mg·kg\(^{-1}\) dry weight) leaf Zn (Swietlik, unpublished). Contrary to that, however, the control plants in Expt. 2 had membranes functional enough to effectively exclude Na from the leaves (Fig. 5). Most recently, Welch and Norvell (1993) have suggested that Zn stabilizes sulphhydryl groups in membrane proteins involved in ion transport processes. It was proposed that Zn protects sulphhydryl
groups from oxidation by free radicals.

Potassium, Ca, Mg, and Na were the only elements whose concentration increased, albeit exclusively in the roots, in response to increasing levels of Zn in the nutrient solution (Figs. 4 and 5). These changes in root K, Ca, and Mg contradict the results by Norvell and Welch (1993) and Welch and Norvell (1993) for barley. Increased root Na most likely resulted from the elevated Na solution concentration caused by increasing additions of ZnDTPA which contained some Na. Reduced root Ca, Mg, and K concentrations in Zn-deficient plants coincided with marked reductions of white root growth and could have reflected lower concentrations of these elements in brown vs. white roots.

To further improve the buffering of Zn activity in a hydroponic system, more frequent solution changes or the use of larger containers should be considered. The uptake of other elements, particularly Ca, will affect Zn activity (Chaney et al., 1989; Parker et al., 1994a). Calcium has high affinity for DTPA at pH >7 (Halvorson and Lindsay, 1972) and, thus, a substantial depletion of this element would free more DTPA for complexing Zn and lower its activity. Our Geochem PC calculations indicate that a 1-mm decrease in Ca would account for more than 50% of the observed decrease in Zn activity (Fig. 6). Also, higher total Ca levels in the nutrient solution of Expt. 2 significantly contributed to higher Zn activity compared to the nutrient solution of Expt. 1 at equimolar total Zn concentrations (Fig. 6).

The present results indicate that DTPA-buffered nutrient solutions were extremely effective in imposing varying degrees of Zn deficiency in sour orange seedlings. Various seedlings parts showed different sensitivity to Zn stress. Zinc foliar sprays were less effective than root-absorbed Zn in alleviating severe Zn deficiency. In order to apply consistent and reproducible levels of Zn stress, using a metal speciation model such as Geochem PC is essential, particularly when concomitant modifications in nutrient components are made.

**Literature Cited**


