Plant and Soil **143**: 163–177, 1992.

PLSO 9368

Zinc-phosphorus interactions in two cultivars of tomato (*Lycopersicon esculentum* L.) grown in chelator-buffered nutrient solutions

D.R. PARKER[1], J.J. AGUILERA[2] and D.N. THOMASON[1]
[1]*Department of Soil and Environmental Sciences, University of California, Riverside, CA 92521, USA and* [2]*INTA, Villa Aberstain, San Juan, Argentina*

Received 10 October 1991. Revised January 1992

Key words: apoplasmic zinc, chelator-buffered nutrient solution, free Zn^{2+} activity, membrane leakiness, phosphorus toxicity, tomato, zinc deficiency

Abstract

Zinc-phosphorus interactions have been frequently studied using a diverse number of crop species, but attainment of reproducible Zn deficiencies, especially severe ones, has been hampered by the use of conventional hydroponic solutions wherein contaminating levels of Zn are often near-adequate for normal growth. We utilized novel, chelator-buffered nutrient solutions for precise imposition of Zn deficiencies. Tomato (*Lycopersicon esculentum* L. cv. Jackpot or Celebrity) seedlings were grown for 15 to 18 d in nutrient solutions containing 200, 600, or 1200 μM P, and 0 to 91 μM total Zn. Computed free Zn^{2+} activities were buffered at $\leq 10^{-10.3}$ M by inclusion of a 100-μM excess (above the sum of the micronutrient metal concentrations) of the chelator DTPA. At total added Zn = 0, acute Zn deficiency resulted in zero growth after seedling transfer, and plant death prior to termination. Free Zn^{2+} activities $\leq 10^{-10.6}$ M resulted in Zn deficiencies ranging from mild to severe, but activities $\leq 10^{-11.2}$ were required to cause hyperaccumulation of shoot P to potentially toxic levels. Despite severe Zn deficiency (i.e. ca. 20% of control growth), tissue Zn levels were usually much higher than the widely reported critical value of 20 mg kg^{-1}, which may be an artifact of the selection of DTPA for buffering free Zn^{2+}. Across Zn treatments, increasing solution P depressed growth slightly, especially in Celebrity, but corresponding increases in tissue P (indicative of enhanced P toxicity) or decreases in tissue Zn (P-induced Zn deficiency) were not observed. The depressive effect of P was also not explained by reductions in the water-soluble Zn fraction. Within 40 h, restoration of Zn supply did not ameliorate high leakage rates (as measured by K^+ efflux) of Zn-deficient roots. Similarly, transfer of Zn-sufficient plants to deficient solutions did not induce leakiness within 40 h. Foliar sprays of $ZnSO_4$ almost completely corrected both Zn deficiency and membrane leakiness of plants grown in low-Zn solutions. Hence, maintenance of root membrane integrity appears to depend on the overall Zn nutritional status of the plant, and not on the presence of certain free Zn^{2+} levels in the root apoplasm.

Introduction

Although it is well-documented that Zn-deficiency symptoms are often aggravated by high rates of P fertilization or by high concentrations of P in hydroponic solutions, several different mechanisms have been proposed to explain this effect. Decreased solubility of soil Zn may result from enhanced sorption of Zn by hydrous oxides (Loneragan et al., 1979; Norvell et al., 1987), but would not explain the frequent occurrence of this effect in solution-cultured plants. When the plant Zn concentration is reduced due to an increase in dry matter production stimulated by

P, a dilution effect has been invoked (Loneragan et al., 1979; Takkar et al., 1976). When the Zn concentration is unchanged, but P fertilization enhances Zn deficiency symptoms (Boawn and Brown, 1968; Boawn and Leggett, 1964; Millikan et al., 1968), it has been suggested that the increased P supply induces a higher physiological requirement for Zn; this has been termed a 'P-enhanced Zn requirement' (Millikan, 1963; Millikan et al,. 1968). Christensen and Jackson (1982) and Loneragan et al. (1979, 1982) have noted that plants showing P-enhanced Zn requirement often had unusually high concentrations of P which are potentially toxic, and concluded that P toxicity symptoms have been mistakenly ascribed to Zn deficiency. This Zn-deficiency-induced P toxicity likely resulted from enhanced P uptake and accumulation of inorganic phosphate (P_i) in the above-ground part of the plant (Loneragan et al., 1982; Parivok and Alekseeva-Popova, 1965; Reed, 1946). In contrast, Cakmak and Marschner (1987) observed reductions in water-soluble Zn in cotton leaves with increasing P supply, thus favouring the earlier interpretation that high tissue P levels physiologically inactive Zn. They postulated that formation of insoluble Zn phosphates in leaves and stems reduces the physiological availability of Zn, but no experimental evidence for the precipitation was obtained.

Regardless of exactly how high tissue P levels reduce growth, it is clear that Zn deficiency can lead to increases in tissue P to potentially toxic levels. In a few cases, especially with monocot grasses, this appears to be solely due to (i) dry matter reductions without concomitant changes in P uptake (i.e. a 'concentration effect'), (ii) shifts in dry matter root:shoot ratio and in P translocation, and (iii) reductions in new growth leading to high P concentrations in older leaves (Webb and Loneragan, 1988). But in many other instances Zn deficiency clearly has led to greatly accelerated rates of P absorption per unit root mass (or length) (Boawn and Leggett, 1964; Cakmak and Marschner, 1986; Christensen and Jackson, 1981; Loneragan et al., 1982; Parivok and Alekseeva-Popova, 1965). Marschner and Cakmak (1986) suggested that Zn deficiency disrupts the plant's normal ability to regulate P uptake by preventing phloem retranslocation of P from shoot to root, but the mechanism whereby P transport is inhibited was not elucidated. Welch et al. (1982) proposed that root cell membranes of Zn-deficient plants lose integrity, and that P accumulates due to nonselective absorption, but such an explanation is at odds with observations that other essential nutrients rarely accumulate in a parallel manner (Cakmak and Marschner, 1986; Loneragan et al., 1982; Marschner and Cakmak, 1986).

Evidence suggests that Zn is required to stabilize macromolecules and cell membranes (Chvapil, 1973). Roots of Zn-deficient plants have demonstrably leak plasma membranes, as evidenced by high efflux rates of K^+, NO_3^-, Cl^-, phosphate, and organic metabolites (Alou, 1989; Cakmak and Marschner, 1988b,c; Welch et al., 1982). Loss of membrane integrity has been ascribed to peroxidative attack of membrane lipids by free oxygen radicals (Cakmak and Marschner, 1988a-c). Marschner and Cakmak (1986) speculated that there is a physiological link between increased membrane permeability and hyperaccumulation of P, but did not detail the nature of the relationship. Recently, it has been suggested that Zn deficiency induces a physiological P deficiency which is manifested by accumulation of P_i at the expense of the organic P fraction, including phospholipids required for normal membrane structure and function (Alou, 1989). At present, it is not known whether the role of Zn in membrane integrity includes an absolute requirement for Zn in the apoplasmic solution analogous to that for Ca, as suggested by Welch et al. (1982), or whether membrane leakiness is a function of cytoplasmic Zn status.

Our research was originally prompted by a report that Zn deficiency was a possible agent of 'tomato (*Lycopersicon esculentum* L.) plant decline' in the Imperial Valley, California (Van Maren, 1987). The reported symptoms affecting the older leaves were similar, although not identical, to those described by Reed (1946) and Lingle et al. (1965), but differed from the more classic symptoms of little-leaf and rosetting (Chapman, 1966; Hoagland et al., 1936). It can be difficult to differentiate between symptoms of Zn deficiency and P toxicity (Loneragan et al., 1979), and Zn deficiency alone can produce dis-

tinctly different symptoms in a given species (Loneragan et al., 1982).

Zinc deficiencies are notoriously difficult to impose using conventional nutrient solution culture, largely because of the ubiquitous presence of Zn as a laboratory contaminant coupled with very small plant requirements for Zn. A recent tabulation of yield data from a number of hydroponic studies revealed that achievement of severe Zn deficiencies has been extremely rare; most studies have obtained relative yields of $>50\%$, or even 70%, of control growth in the absence of added Zn (Parker et al., 1992a). Traditional nutrient solutions may provide plants with nutrients in the approximate ratio in which they are found in soil solution, but typically at higher concentrations, and it is particularly difficult to lower concentrations of some of the trace metals to deficient levels (Bell et al., 1991a; Norvell, 1991; Parker et al., 1992a). Plant uptake of a metal seems to depend primarily on the activity of the free metal ion (Chaney, 1988; Halvorson and Lindsay, 1977; Parker et al., 1992a), which can be maintained at a low level by absorption on exchange resins and/or by use of chelating agents (Checkai et al., 1987). Recently, Chaney, Bell, and co-workers have pioneered the use of 'chelator-buffered' nutrient solutions wherein a chelating agent such as EDTA (ethylenedinitrilotetraacetate) is supplied in excess of the concentration of all micronutrient metals, thus buffering their free activities to appropriately low levels (Bell et al., 1991a,b; Chaney, 1988; Chaney et al., 1989). The required concentrations of chelating agent and micronutrients can be readily determined using a computerized chemical equilibrium model such as GEOCHEM-PC, which has been specifically developed and modified to perform such computations for plant nutrition research (Parker et al., 1992b). Except for some preliminary results reported by Norvell (1991) and Parker et al. (1992a), the chelator-buffering approach has not been utilized for imposition of Zn deficiencies.

The objectives of this research were to evaluate the effects of different levels of Zn and P on the growth, Zn and P nutritional status, and symptoms of two tomato cultivars that have been classified as sensitive (Jackpot) or tolerant (Celebrity) to the tomato plant decline disorder observed in the Imperial Valley, California (Van Maren, 1978). Free Zn^{2+} activities were buffered with an excess of the chelating agent DTPA (diethylenetriaminepentaacetate), and the hypothesis that apoplasmic Zn is required for maintenance of root cell membrane integrity was experimentally evaluated.

Methods

General

Tomato (cv. Jackpot or Celebrity) seedlings were hydroponically grown in a controlled-environment growth chamber, with the temperature constant day and night at 25 ± 0.5°C, and the relative humidity at $65 \pm 3\%$. The total photoperiod was 16 h d^{-1}, and the light intensity was symmetrically ramped up to, and down from, a 10-h period at a full intensity of 580 μE m^{-2} s^{-1}. Seeds were germinated in washed quartz sand moistened with 5 m*M* $CaSO_4$. After germination and emergence, the seedlings were irrigated once with a nutrient solution containing basal P and Zn concentrations (see below), and with deionized water thereafter. When the plants had two true leaves (approximately one week after emergence), the seedlings were transferred to 2-L polyethylene containers (one plant per container) filled with continuously-aerated nutrient solution, and grown for an additional 15 or 18 d. The containers and covers were suitably shielded to exclude light from the solutions and root systems.

Solution speciation was calculated using the GEOCHEM-PC program (Parker et al., 1992b). Formation constants for metal-DTPA complexes were obtained from the tabulations of Smith and Martell (1976–1989), and were adjusted to zero ionic strength using the Davies or Helgeson equations (Sposito and Mattigod, 1980). All other formation constants were from Lindsay (1979). Solutions were formulated to obtain computed free Cu^{2+} and Mn^{2+} activities (denoted by parentheses throughout) consistent with those reported by Chaney et al. (1989). The DTPA concentration was chosen to give a 100 μ*M* excess of DTPA above the sum of the Cu, Fe, Mn, and Zn concentrations, and was

thus 148 $\mu M + Zn_T$. The nutrient solution composition was: NH_4NO_3, 2.5 mM; KCl, 1 mM; $CaCl_2$, 2.0 mM; $MgSO_4$, 0.5 mM; $MnCl_2$, 23 μM [$\log(Mn^{2+}) = -8.1$]; $CuCl_2$, 4.9 μM [$\log(Cu^{2+}) = -14.7$]; $Fe(NO_3)_3$, 20 μM [$\log(Fe^{3+}) = -21.5$]; H_3BO_3, 3 μM; Na_2MoO_4-$2H_2O$, 0.1 μM; pH = 7.0. The basal P concentration was 200 μM (added as Na_2HPO_4), with treatments of 600 and 1200 μM in some experiments. Zinc was added as $ZnCl_2$, with treatments initially selected based on a reported critical free Zn^{2+} concentration of $10^{-10.8}$ (Chaney et al., 1989), and then adjusted slightly after the results of several preliminary experiments. Across all experiments, total added Zn ranged from 0 to 91.2 μM.

Double-deionized water (DDW) and reagent-grade salts were used to prepare all solutions. The stock solutions of NH_4NO_3, $CaCl_2$, $MgSO_4$, KCl, and Na_2HPO_4 were purified with ammonium pyrrolidine dithiocarbamate to remove contaminating levels of trace metals (Wallihan and Bradford, 1977). Micronutrient-chelate stock solutions were prepared by dissolving DTPA and NaOH (three times the molarity of DTPA) in water and, after complete dissolution of DTPA, addition of the appropriate micronutrient metal salts.

Nutrient solutions were replaced every three days. The pH of the fresh solutions was adjusted to 7.0, and was monitored and adjusted twice daily, or three times a day as the plants grew larger; daily variation in pH was 0.3 units or less. At termination, the plants were harvested and separated into component tissues (generally roots, stems, older leaves, and younger leaves). The roots were rinsed briefly with DDW to minimize precipitated salts occasionally observed on root surfaces. The samples were dried at 60°C, weighed, ground to pass a 20-mesh sieve, and subsamples digested in a mixture of nitric and perchloric acids. Zinc was analyzed by atomic absorption spectrophotometry (AAS), and P by a spectrophotometric vanadate-molybdate method (Berg and Gardner, 1978).

Preliminary experiments

Initial experiments were conducted using the cultivar Jackpot to determine a suitable range of solution P concentrations and free Zn^{2+} activities. Solution P ≤ about 100 μM resulted in unstable concentrations due to rapid depletion via plant uptake, so that 200 μM P was selected as our basal concentration. Added Zn ranged from 0 to 91.2 μM, with a corresponding range in log (Zn^{2+}) of '$-\infty$' to -10.3 (the former indicates the uncertainty in free Zn^{2+} in solutions nominally, but not actually, containing 0 Zn). At 0 added Zn, deficiency was so severe that the seedlings grew only imperceptibly after transfer, and the tops became completely necrotic prior to termination. There were no further yield increases with increasing log (Zn^{2+}) above -10.6, so four treatments resulting in computed log (Zn^{2+}) values of -11.5 to -10.6 were selected for most subsequent experiments. Leaves and roots from the higher Zn treatments of one preliminary experiment were digested and analyzed for Ca, Mg, K, Fe, Cu, and Mn using AAS, and for P as described above. Tissue N and S were determined using a Carlo-Erba NA-1500 C-N-S analyzer. Results suggested that these elements were present at normal, physiologically relevant concentrations (Table 1), although tissue Mn levels were higher than neccessary. It is unlikely, however, that leaf Mn reached toxic levels; few species suffer adverse effects until leaf concentrations exceed 200 $\mu g\, g^{-1}$ (Edwards and Asher, 1982).

Table 1. Nutrient concentrations (dry matter basis) of Jackpot tomato leaves and roots grown in chelator-buffered nutrient solutions in a preliminary experiment. Means and standard errors are pooled for 9 plants grown at log (Zn^{2+}) = -10.9, -10.6, or -10.3 (three replications each). Solution [P] was 200 μM

Element	Leaves	Roots
N (%)	6.54 ± 0.15	4.85 ± 0.06
P (%)	1.01 ± 0.06	1.16 ± 0.04
K (%)	3.80 ± 0.20	5.81 ± 0.24
Ca (%)	2.45 ± 0.06	0.41 ± 0.02
Mg (%)	0.46 ± 0.02	0.45 ± 0.01
S (%)	0.67 ± 0.03	0.37 ± 0.01
Fe (mg kg^{-1})	128 ± 4	93.8 ± 8.1
Cu (mg kg^{-1})	18.5 ± 1.0	20.0 ± 1.4
Mn (mg kg^{-1})	201 ± 15	927 ± 89

Experiments 1 and 2

Both experiments consisted of a complete factorial design with four levels of Zn, three levels

of P, and three replications, arranged in a completely randomized design. The experiments were identical; the cultivar Celebrity was grown in Experiment 1, while Jackpot was grown in Experiment 2. The four Zn levels were imposed by adding total Zn at 5.7, 11.4, 22.8, or 45.6 μM to yield log (Zn^{2+}) values of −11.5, −11.2, −10.9, and −10.6, respectively (correspondingly denoted as Zn_1 through Zn_4). The three P levels were 200, 600 and 1200 μM P, denoted as P_1, P_2, and P_3, respectively. The excess DTPA needed to buffer the free trace metal activities is predicted to form complexes with Ca, and Ca thus 'controls' the free activity of DTPA. Because P also forms solution complexes with Ca, total solution Ca required slight adjustment to maintain constant free-metal activities at the two higher P concentrations (e.g. 2.2 mM $CaCl_2$ at P_3).

Water-soluble Zn and P were determined by extracting dried, ground samples (generally 0.15 g) with 10 mL of 1 mM 2-(4-morpholino)-ethanosulfonate buffer (MES) at pH 6.0, with 5 h of shaking (Cakmak and Marschner, 1986). Samples were filtered through a 0.45-μm membrane filter, and soluble Zn in the filtrate determined by AAS. The filtrate was then decolorized by addition of activated carbon and centrifugation, and soluble P was determined by the vanadate-molybdate method. The solid remaining on the filter was washed with DDW to minimize entrained soluble P and Zn and, along with the filter membrane, was then digested in nitric and perchloric acids; insoluble Zn and P were determined as described above. Blanks were run every 16 samples to check for contamination at each step (extraction, decolorization, and digestion). This sequential method was carefully evaluated during preliminary experiments, and it was established the the charcoal treatment did not introduce any contamination or loss of soluble P. Total recoveries of both Zn and P were equal to those from a conventional nitric-perchloric digestion.

Experiment 3

Jackpot seedlings were grown for 15 d at either Zn_1 or Zn_4 and the basal P concentration of 200 μM, and then used for collection of root exudates. At the end of the dark period on day 15, plants were removed intact from the pots, and the root systems soaked in 0.4 mM $CaCl_2$ for 15 min. The plants were then transferred to smaller plastic containers containing aerated, 500-mL solutions containing 2.0 mM $CaCl_2$, 200 μM Na_2HPO_4, 3 μM H_3BO_3, and 113 μM Na_3-DTPA, at pH 7.0. Added Zn was either 5.7 or 45.6 μM to yield computed log (Zn^{2+}) values of −11.5 or −10.6 (Zn_1 or Zn_4). A third exudate collection treatment consisted of the Zn_T = 45.6 μM solution but with the $CaCl_2$ omitted [log(Zn^{2+}) = −9.6]. Five combinations of preculture and exudate collection conditions were thus imposed: Zn_1Zn_1, Zn_1Zn_4, Zn_4Zn_1, Zn_4Zn_4, and Zn_4Ca_0, where, for each, the first notation indicates the preculture treatment and the second denotes the conditions during exudate collection. There were three replications per treatment.

Seedlings were kept in darkness at 25°C and exudates collected for a total of 40 h. Collection solutions were replaced with fresh ones after 5, 15 and 25 h, such that four solutions were collected for each treatment replicate. At termination the plants were separated into tops and roots, dried, and weighed. Collection solutions were evaporated to dryness on a hot plate, redissolved in 25 mL of a 0.05 M HCl-0.22 M LiCl solution, and then analyzed for K^+ by AAS. Cumulative K^+ released by the root system over 40 h was then computed.

Experiment 4

Based on the results of Experiment 3 and some other experiments not presented, a similar root exudation experiment was conducted using Jackpot grown at P = 200 μM. There were seven preculture treatments: Zn_1 through Zn_4, a Zn_5 treatment [Zn_T = 91.2 μM; log(Zn^{2+}) = −10.2], and Zn_1 in combination with one of two foliar spray treatments; each treatment was replicated three times. All seedlings were grown in Zn_5 solutions until day 6, transferred to solutions of the appropriate Zn treatment and the foliar treatments initiated (this allowed the seedlings to attain a leaf area sufficient for the sprays to be effective), and grown for 12 more days. Foliar treatments entailed spraying the plant tops twice daily with solutions containing a surfactant (Brij-35, 1 mL L^{-1}) and either 0.1 or 1.0 mM

$ZnSO_4$, denoted as Zn_1Fo_1 and Zn_1Fo_2, respectively. Root exudates were obtained using the Zn_1 collection solution specified in Experiment 3; only one solution change was made at 15 h and the collection terminated at 40 h. Plants were then separated into leaves, stems, and roots, and the leaf samples analyzed for total P. Otherwise, procedures for collection, evaporation, analysis for K^+, and plant harvest were identical.

Results

Experiments 1 and 2

Deficiency symptoms

Identical deficiency symptoms were observed with both cultivars, but only at Zn_1 and Zn_2; no symptoms were observed at Zn_3 or Zn_4. The symptoms occurred at all P levels, but became more severe with increasing solution P. The older leaves curled downwards, and had mild interveinal chlorosis and a grayish cast, along with black mottles. As growth became more severely inhibited with time, the black spots became necrotic, and the symptoms became more severe and spread to the younger leaves. The roots did not exhibit any symptoms. Symptoms appeared after only three days in Zn_1 solutions, and after about one week at Zn_2.

Growth responses

Both Zn and P treatments had marked effects on growth and dry matter yield of both cultivars. For convenience, the total dry matter yield was computed and expressed as a percentage of the treatment mean exhibiting the highest dry matter yield, which was Zn_4P_1 for both cultivars (Fig. 1). The yield responses to Zn could generally be fit to quadratic curves, although the response of Celebrity at P_1 seemed to be better described by a linear fit (Fig. 1). With both cultivars, the most dramatic Zn-deficiency-induced decreases in dry matter occurred in older leaves and roots; the younger leaves were the least affected component tissue (data not shown). Based on visual observation, increasing P at a given Zn level seemed to decrease growth in both cultivars to a greater extent than is reflected by the dry matter

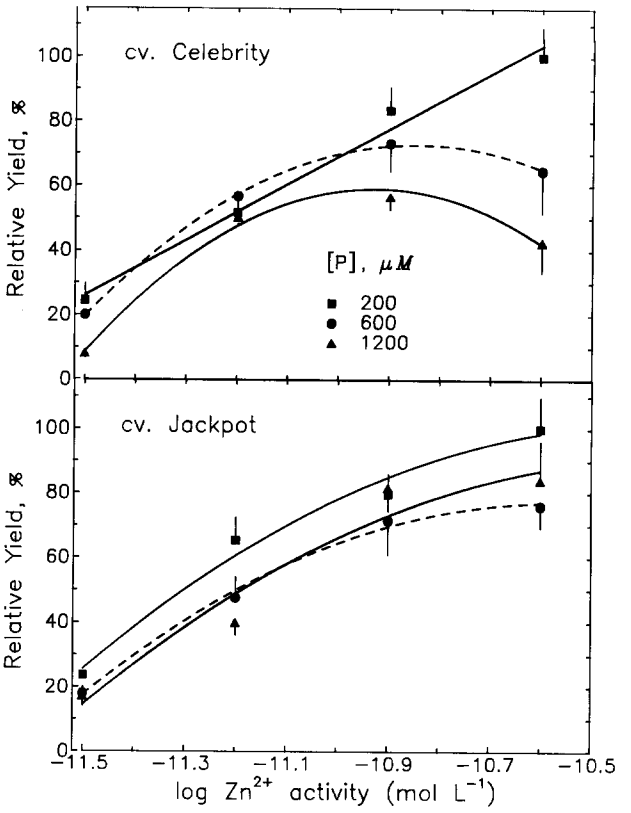

Fig. 1. Relative total plant dry matter yields vs. free Zn^{2+} activity for two cultivars of tomato grown in DTPA-buffered nutrient solutions at three P concentrations (Experiments 1 and 2). Vertical *bars* indicate 1 SE where it exceeds symbol size.

yields (Fig. 1). With Celebrity, the dry matter yields were only minimally affected at Zn_1 or Zn_2; consistent decreases with increasing P occurred only at Zn_3 and Zn_4. At the higher two P levels the response of Celebrity exhibited an apparent downturn between Zn_3 and Zn_4, but it is uncertain whether this decline is a reproducible effect or a consequence of experimental variation. With Jackpot, the trend was for decreased yield with increasing P, especially if P_2 and P_3 are compared to P_1 (Fig. 1). The decreases were, however, less pronounced than with Celebrity, and somewhat inconsistent across the Zn values (Fig. 1).

Tissue Zn and P

The trends in tissue Zn concentrations, although not the exact magnitudes, were the same for both cultivars so that, for brevity, only data for Jackpot are presented. Jackpot had higher Zn

concentrations in the roots and younger leaves than Celebrity, but the older leaves and stems exhibited no differences across cultivars. Zinc concentrations differed markedly across the four component tissues that were separated at harvest (Fig. 2). In general, total Zn concentrations decreased with decreasing Zn level, but were not consistently affected by P level. Total Zn was almost always $\geqslant 20\,mg\,kg^{-1}$ (Fig. 2), a value that is considered critical for many plants (Chapman, 1966). The Zn_4 treatments yielded the highest Zn concentrations in all plant parts, but there were few differences among the other three levels. The distribution between soluble and insoluble Zn was somewhat erratic, and did not show any clear trends. When averaged across P levels, the roots had the highest Zn concentrations of any of the tissues (Fig. 2). The stems exhibited the highest sensitivity to Zn supply, with marked decreases in total tissue Zn paralleling decreasing Zn supply down to the Zn_2 level. Only the stems of the Zn_1 treatments contained the low soluble-Zn levels (5 to $7\,mg\,kg^{-1}$) reported to coincide with deficiency (Cakmak and Marschner, 1987). Total Zn uptake by the plant decreased as the Zn level decreased, and decreased as the P level increased, but the latter effect was only significant at the Zn_4 level (data not shown). There were no differences in the total Zn uptake between the two cultivars.

As with Zn, there were few differences in tissue P between the two cultivars, although Jackpot tended to have higher P concentrations, and higher total P uptake, than Celebrity. In Jackpot, increasing P supply had relatively minor effects on tissue P concentration, although the overall trend was for increases in tissue P with increasing solution P (Fig. 3). More striking was the tendency for the lower two Zn levels to result in large increases in tissue P concentrations (Fig. 3). This trend was most pronounced in older leaves, somewhat reduced in the younger leaves, and even less marked in the stems. The roots did not exhibit significant increases in P concentration with decreasing Zn supply (Fig. 3). The soluble-P fraction increased much more than the insoluble fraction with decreasing Zn supply. In the younger leaves and

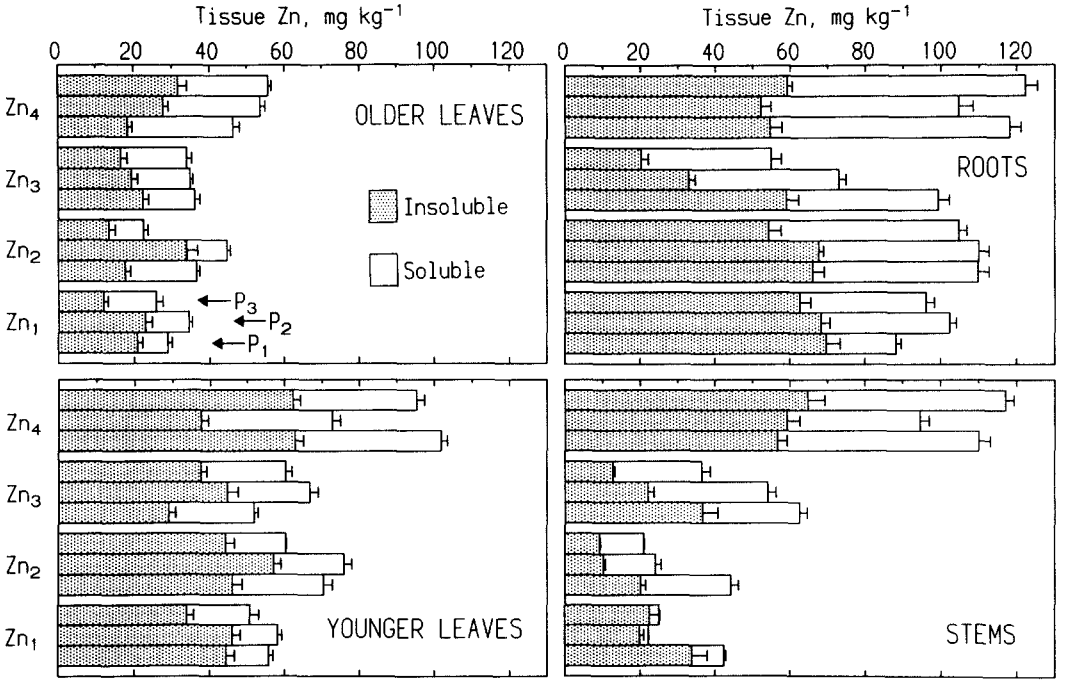

Fig. 2. Soluble and insoluble Zn in four component tissues of Jackpot tomato grown at four Zn levels and three P concentrations (Experiment 2). The cluster of three bars at each Zn level represent the three P levels in ascending order as indicated. Error *bars* indicate 1 SE for the soluble and insoluble fractions.

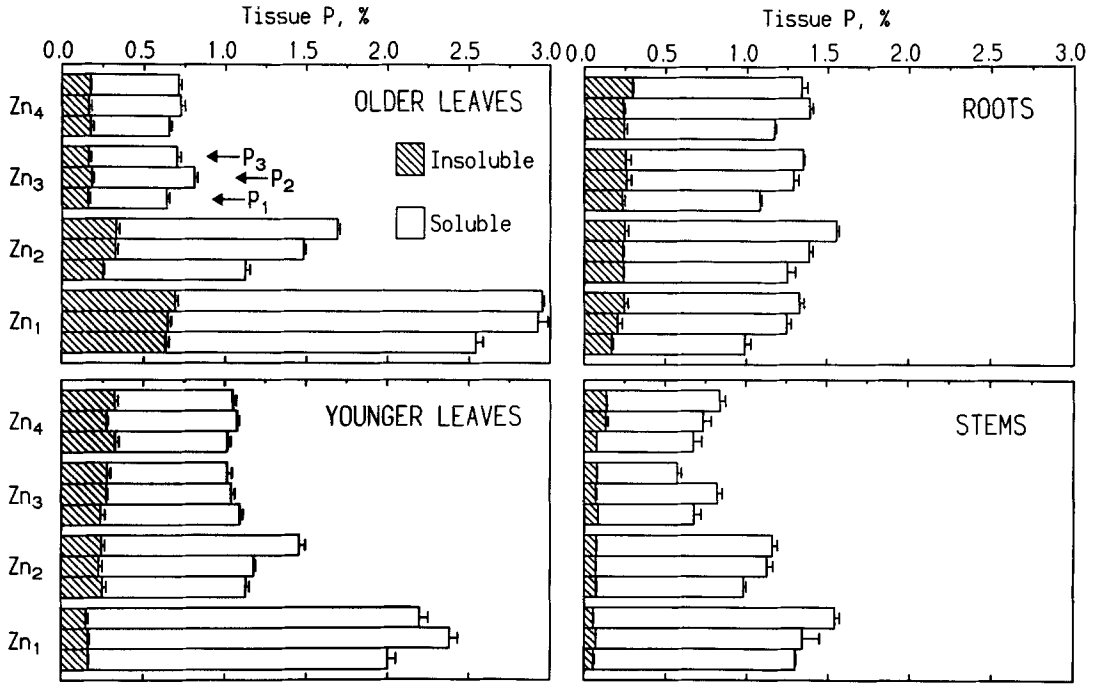

Fig. 3. Soluble and insoluble P in four component tissues of Jackpot tomato grown at four Zn levels and three P concentrations (Experiment 2). The cluster of three bars at each Zn level represent the three P levels in ascending order as indicated. Error *bars* indicate 1 SE for the soluble and insoluble fractions.

stems, decreasing Zn caused simultaneous decreases in the insoluble-P fraction, while in the older leaves both soluble and insoluble P tended to increase with increasing severity of Zn deficiency (Fig. 3).

The increases in tissue P at low Zn supply were not simply a consequence of stunted growth coupled with constant P absorption (i.e. a 'concentration effect') as described by Christensen and Jackson (1981) and Webb and Loneragan (1988). When total P uptake is computed on the basis of root weight, it also exhibits very large increases at the two lower Zn levels (Table 2). Increasing P supply also tended to increase P uptake, but again the effect was less pronounced than that of Zn treatment (Table 2). In part, the minimal effect of P can be ascribed to the offsetting tendencies for increasing P supply to increase tissue P concentration, but to depress dry matter yields.

Table 2. Total phosphorus uptake (whole plant) per gram of root (dry matter basis) by two tomato cultivars grown at three P concentrations and four free Zn^{2+} activities (Experiments 1 and 2)

[P], μM	P uptake (mmol g^{-1} root d.m.)			
	log (Zn^{2+})			
	−11.5	−11.2	−10.9	−10.6
	Celebrity			
200	2.49 ± 0.08	1.22 ± 0.04	0.94 ± 0.06	0.92 ± 0.06
600	2.73 ± 0.09	1.44 ± 0.07	1.03 ± 0.03	0.97 ± 0.01
1200	3.37 ± 0.30	1.47 ± 0.06	1.22 ± 0.04	1.03 ± 0.10
	Jackpot			
200	4.18 ± 0.10	1.90 ± 0.11	1.48 ± 0.06	1.39 ± 0.02
600	5.23 ± 0.01	2.73 ± 0.15	1.55 ± 0.03	1.55 ± 0.12
1200	3.95 ± 0.31	2.61 ± 0.30	1.49 ± 0.06	1.55 ± 0.02

± Standard error of the mean for three replications.

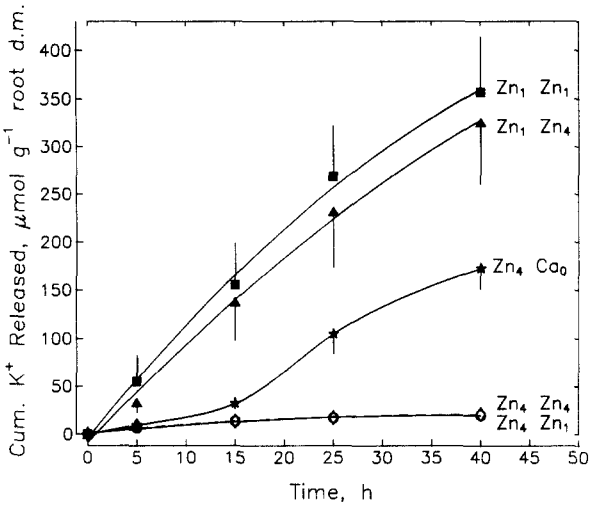

Fig. 4. Cumulative potassium release from the root systems of Jackpot tomato (Experiment 3). For each treatment, the first notation indicates the Zn level during the 15-d preculture (Zn_1 or Zn_4), and the second indicates the imposed treatment during the 40-h exudate collection period (Zn_1, Zn_4, or Ca_0 = omission of Ca from the solution). Vertical *bars* indicate 1 SE where it exceeds symbol size.

Experiment 3

Preculture of Jackpot tomato at Zn_1 resulted in plants with very leaky root systems as measured by K^+ efflux, and leakiness was not ameliorated by restoring Zn to the sufficient Zn_4 level in the exudate collection medium (Fig. 4, upper two curves). Plants precultured at Zn_4 exhibited very low leakage rates, and these were unaffected by lowering the Zn level to Zn_1 during the 40-h-collection period (Fig. 4, lower two curves). In contrast, withholding Ca from Zn-sufficient plants during the collection period resulted in a distinctly different response wherein leakage rates were initially low, but increased dramatically after ca. 5 h (Fig. 4, middle curve). These results suggested that, although Zn nutritional status clearly affects root membrane integrity, its temporal behavior is quite different from that of Ca which has a unique apoplasmic function in membrane stabilization (Hanson, 1984).

Experiment 4

Additional K^+ efflux experiments not presented yielded rather variable leakage rates in plants precultured at the Zn_4 level, and cumulative effluxes at 40 h usually exceeded 100 μmol g^{-1}, in contrast to the value of ca. 20 observed previously (Fig. 4). We suspected that the Zn_4 level may be marginal for tomato (i.e. very near the critical level), so that mild deficiencies could occasionally occur, and thus included a higher level (Zn_5) in Experiment 4. Because all plants were grown at Zn_5 for the first 6 d of the preculture before treatments were imposed, the onset of deficiencies was delayed and the preculture period was extended to 18 d. The dry matter yield at Zn_4 was indeed slightly suboptimal and, overall, yields ranged to a low of 33% of control at Zn_1 (Table 3). The Zn_3, Zn_4, and both foliar spray treatments resulted in very similar yields (85 to 88%) and cumulative (40-h) K^+ effluxes of 38 to 78 μmol g^{-1} (Table 3), indicating that the foliar treatments were fairly effective in correcting Zn deficiencies. In contrast, the Zn_1 treatment resulted in a 40-h efflux of almost 200 μmol g^{-1}, while plants grown at Zn_5 exhibited only miniscule leakage (Table 3). The rather high standard errors for many of the treatments causing mild deficiencies (Table 3) reflect the delayed and somewhat variable onset of deficiencies due to Zn accumulated during the first 6 d of preculture; within treatments, higher dry matter yields always coincided with lower K^+ efflux rates (data not shown). Total-P concentrations in leaves (combined samples of older and younger) decreased from over 3% at Zn_1 to about 1% at Zn_4 and Zn_5, while the two foliar Zn treatments resulted in values of ca. 0.9% (Table 3).

The last three solution changes from the preculture period were analyzed for Zn_T by AAS, and some increases due to transpirational water loss (which was measured) were observed, especially in the last solution change. Concomitant increases in DTPA concentration presumably occurred, however, and computed free Zn^{2+} activities were thus consistently very close to the nominal values for all treatments (data not shown). Moreover, the Zn_1 treatment and the two foliar spray treatments had identical Zn_T values, indicating that no downward translocation and release of foliar-applied Zn to the nutrient solution had occurred, and that these treatments resulted in identical solution free Zn^{2+} activities.

Table 3. Total plant yield, cumulative potassium release from the roots, and total leaf P concentration of Jackpot tomato, all on a dry matter basis (Experiment 4). For each treatment, the notation indicates the Zn level during the latter 12 d of the 18-d preculture, and the use of 0.1 or 1.0 m*M* $ZnSO_4$ foliar sprays for supplying Zn (Fo_1 and Fo_2, respectively)

Treatment	Relative yield (%)	K released (μmol g^{-1})		Leaf P (%)
		15 h	40 h	
Zn_1	33 ± 3	88 ± 6	194 ± 14	3.13 ± 0.14
Zn_2	66 ± 4	28 ± 11	83 ± 17	1.65 ± 0.09
Zn_3	88 ± 8	16 ± 11	38 ± 18	1.17 ± 0.02
Zn_4	85 ± 11	37 ± 18	71 ± 34	0.98 ± 0.08
Zn_5	100 ± 3	0.8 ± 0.2	2.2 ± 0.2	1.00 ± 0.05
Zn_1Fo_1	87 ± 3	33 ± 5	78 ± 7	0.93 ± 0.00
Zn_1Fo_2	87 ± 12	31 ± 17	65 ± 31	0.85 ± 0.00

± Standard error of the mean for three replications.

Discussion

Symptoms observed in older tomato leaves were generally similar to those described by Reed (1946) and by Lingle et al. (1958), but were somewhat different from those described by Cakmak and Marschner (1988b). The latter authors reported that Zn-deficient tomato exhibited interveinal chlorosis, reddish-brown patches, and white incrustation (exudates) at the leaf margins. That different symptoms were observed in our experiments might be ascribable to differences in technique for inducing Zn deficiencies, or to a combination of Zn deficiency and P toxicity in our experiments. Classic Zn deficiency symptoms affecting younger shoot growth, including rosetting (Chapman, 1966) or little-leaf symptoms (Hoagland et al., 1936), were generally not evident in our experiments, especially early in symptom development. Loneragan et al. (1982) described two general types of Zn-deficiency symptoms commonly observed in dicotyledonous plants. In addition to the more classic little-leaf and rosetting symptoms, 'mottle-leaf' symptoms of the older leaves have also been noted on several occasions. The latter syndrome includes an overall bronze cast along with interveinal chlorosis and, eventually, necrosis. As described by Loneragan et al. (1982), P toxicity primarily enhances the mottle-leaf symptoms through development of necrotic spots, such that visual observation is unlikely to precisely distinguish between simple Zn deficiency, and Zn deficiency in conjunction with P toxicity. With respect to the tomato plant decline, there were enough differences in symptoms observed in this experiment and those described for the field-grown plants (Van Maren, 1987) to suggest that this disorder is not closely related to Zn deficiency, nor to a Zn–P interaction. This conclusion is also supported by the minimal differences in the responses of the two cultivars to our imposed Zn and P treatments.

This leads to the question whether our tomato plants suffered principally from Zn deficiency, P toxicity, or both. At the lower two Zn levels, leaf P concentrations were greatly increased (Fig. 3), especially in the older leaves, consistent with the findings of others (Bhatti and Loneragan, 1970; Cakmak and Marschner 1986, 1987; Christensen and Jackson, 1981; Loneragan et al., 1979, 1982; Parivok and Alekseeva-Popova, 1965). The occurrence of P toxicity symptoms in leaves with overall P concentrations below $\approx 4\%$ has been attributed to irregular spatial distribution of P, with higher concentrations occurring in the visibly afflicted tissue (Bhatti and Loneragan, 1970). Phosphorus toxicity has been associated with tissue concentrations ranging from 0.8 to 3% in whole shoots, and from 1.2 to 4.5% in leaves (Loneragan et al., 1982), and concentrations of 2.4% in leaves and 2.2% in stems have been reported to be toxic to tomato (Parivok and Alekseeva-Popova, 1965). Hence, the P concentrations in the leaves of Jackpot grown at Zn_2 and Zn_1 (Fig. 3) are generally within the range reported to be toxic for tomato, and the observed symptoms may have been due to a combination of Zn deficiency and P toxicity. On the other hand, both older and younger leaves of Celebrity grown at Zn_2 had P concentrations of $\leq 1\%$ (data not shown)

suggestive of Zn deficiency only, yet we could detect no difference in symptoms between the two cultivars. The Zn_3 level did not result in elevated leaf P concentrations (Fig. 3), despite growth reductions of 19 to 29% in Jackpot and 17 to 44% in Celebrity (Fig. 1). Hence, it would seem that, at least in tomato, rather severe Zn deficiencies must be achieved in order to obtain accumulation of leaf P to potentially toxic levels. Regardless, we differ, at least semantically, with Loneragan et al. (1979) who termed Zn deficiency and P toxicity 'separate disorders' leading to both growth reductions and development of mottle-leaf symptoms. With a species such as tomato, which appears to require high substrate-P concentrations for optimal growth, P accumulation to toxic levels may be an *inevitable consequence* of Zn deficiency, especially severe deficiency, in hydroponically grown plants. Such a view is in accord with observations that growth reductions, observable symptoms, and high leaf-P concentrations can all be corrected solely by increasing the Zn supply (Cakmak and Marschner, 1986, 1987; Christensen and Jackson, 1981; Loneragan et al., 1982; this study).

The chelator-buffered nutrient solutions employed in this study resulted in severe Zn deficiencies, and consequent growth reductions (Fig. 1), that are almost never attained using conventional nutrient solution methodologies (Parker et al., 1992a). The latter approach relies on depletion of contaminating levels of solution Zn via plant uptake, rendering it extremely difficult to predictably and reproducibly impose Zn stresses of varying severity (Parker et al., 1992a). Chelator-buffered solutions thus represent a powerful new tool for investigations of deficiencies of Zn and other trace elements (Bell et al., 1991a,b; Chaney, 1988; Chaney et al., 1989). In our preliminary experiments, zero added Zn[$\log(Zn^{2+}) = '-\infty'$] resulted in deficiencies so severe that the transferred seedlings did not grow and soon died. We believe that DTPA is such a strong chelator for Zn that the seed Zn reserves, which normally would be expected to suffice for several days of early seedling growth, may actually have been withdrawn from the plant into the nutrient solution. This observation reaffirms the notion that, if the free metal activity in solution can be tightly controlled, then biological availability will be affected accordingly (Parker et al., 1992a). Our data suggest a critical log (Zn^{2+}) value of ca. -10.6 for tomato, which is quite similar to values reported for maize (*Zea mays* L.) grown in DTPA-containing nutrient solutions (Halvorson and Lindsay, 1977), and for barley (*Hordeum vulgare* L.) reared in solutions buffered with HEDTA (*N*-[2-hydroxyethyl] ethyelenedinitrilotriacetate) (Norvell, 1991). Laurie et al. (1991) also reported Zn deficiency in barley at computed log (Zn^{2+}) values of ca. -10 to -11 in solutions containing excess EDTA. These values are in accord with estimates of free Zn^{2+} concentrations in soil solutions of alkaline soils where Zn deficiencies are fairly common (Norvell et al., 1987). It remains to be determined whether this apparent critical free Zn activity is consistent across diverse plant species (and cultivars), and across different metal chelators, and future research in our laboratory will address these questions.

Virtually all of our tomato tissue samples exhibited total Zn concentrations higher than the 20 mg kg^{-1} value which is widely considered to be the critical level in many species (Chapman, 1966), but exceptions to this critical level have also been observed previously. Rahimi and Bussler (1979) and Ghoneim and Bussler (1980) studied Zn responses of a number of crop species, and concluded that 'latent deficiencies' could occur at leaf Zn concentrations as high as 35 mg kg^{-1}. There is some evidence that the water-soluble Zn fraction may be a better predictor of deficiency than total leaf Zn (e.g. Cakmak and Marschner, 1987), but contrary findings have also been reported (Ghoneim and Bussler, 1980). In our experiments, soluble Zn did not follow consistent trends and, except in stem tissue, was always considerably higher than the 5 to 7 mg kg^{-1} reported to be the minimum for normal growth (Cakmak and Marschner, 1987).

Despite considerable evidence that plant responses are best correlated with activities of free, uncomplexed metal ions in solution (Chaney, 1988; Checkai et al., 1987; Halvorson and Lindsay, 1977), there are some indications that such a free-metal model may require some reevaluation. Because higher plants possess both symplasmic and apoplasmic pathways for element uptake and translocation (Mengel and Kirkby,

1987), the possibility of plant uptake and accumulation of intact metal-ligand complexes, especially via an apoplasmic pathway, cannot be ruled out. Bell et al. (1991b) noted differences in Cu uptake at similar computed activities maintained by varying Cu_T and concentrations of different chelators. They speculated that breaks in the endodermal barrier at root apices and at sites of lateral root initiation might permit passive uptake of intact metal-chelates in the transpirational flow of water. Similarly, at a constant log (Cu^{2+}) of -10.0, Checkai et al. (1987) observed a 9-fold increase in root Cu and a 1.5-fold increase in shoot Cu as solution Cu_T was increased from $10^{-9.8}$ to $10^{-6.7}$ M. Although these authors attributed the higher plant Cu to enhanced diffusion of chelated Cu across the unstirred layer of water surrounding the roots, the increase might be attributable to the uptake of some Cu as the intact Cu-chelate. Such a model could also account for reports of 'physiologically inactive' Zn in field-grown maize (Leece, 1978), and may provide a plausible explanation for our high leaf Zn values in very Zn-deficient plants (Figs. 1 and 2). We postulate that Zn-DTPA was absorbed and translocated to leaves where it resided intact, with little or no biological activity, leading to anomalously high leaf Zn concentrations in concert with severe deficiencies. If so, then DTPA may be a poor choice for Zn deficiency research; its high affinity for Zn necessitated unnaturally high Zn_T values (ca. 5 to 90 μM) in our study. A weaker ligand such as HEDTA can effectively buffer free Zn^{2+} in the required range using Zn_T levels of ≈ 1 μM (Parker et al., 1992a), and could thus minimize the anomalous uptake of chelated Zn.

The trends for decreased dry matter yield with increasing P supply (Fig. 1) are not readily explained. The concentrations of total tissue Zn were not suppressed by increasing P supply (Fig. 2), in accord with a number of previous investigations (Boawn and Brown, 1968; Cakmak and Marschner, 1986, 1987; Loneragan et al., 1979; Millikan, 1963). Moreover, the H_2O-soluble fractions were not dramatically reduced by increases in P supply (Fig. 2), and a decrease in the physiological availability of Zn due to precipitation of zinc phosphates in the leaves and/or stems as proposed by Cakmak and Marschner (1987) seems unlikely. Exacerbation of P toxicity also seems implausible because the leaf P concentrations did not increase substantially with increasing P supply (Fig. 3). We note, however, that many roots grown at the P_3 level exhibited an external accumulation of salts which we qualitatively analyzed and found to be predominantly Ca phosphates. This root-surface precipitate was not completely unexpected, although the nutrient solutions themselves were always free of visible precipitates. During our computations of solution speciation using GEOCHEM-PC, we did not allow precipitation to occur, but we noted that all solutions were at least marginally supersaturated with respect to β-$Ca_3(PO_4)_2$ (Lindsay, 1979): saturation indices [log(IAP/K_{sp})] ranged from 0.6 to 2.1 at P_1 and P_3, respectively. It is possible that this incrustation of salts on the root surface reduced absorption of water and/or nutrients and thus reduced the growth rate. Regrettably, there is presently no other ready explanation for P-induced growth reductions observed (Fig. 1).

Uncontrolled increases in P uptake by Zn-deficient plants (Table 2) have been observed on several occasions, and it has been proposed that Zn deficiency interferes with P metabolism (Parivok and Alekseeva-Popova, 1965; Reed, 1946), and thus produces a physiological P deficiency which is manifested by an accumulation of P_i at the expense of the organic P compounds (Alou, 1989). Our results may support such a hypothesis since water-soluble P often increased dramatically with decreasing Zn supply (Fig. 3). An alternative hypothesis proposed by Welch et al. (1982) is that the Zn deficiency-induced loss of membrane integrity leads to the accumulation of P to toxic concentrations in older leaves; P could nonselectively enter the afflicted roots cell via mass flow in the transpirational stream and thus accumulate in leaves as water is lost to the atmosphere. Our experiments do not support such a model. For plants grown at Zn_1 and P_1 in Experiments 1 and 2, the computed theoretical transpiration coefficients required to achieve the observed total P uptake by mass flow *only* are 1980 and 3140 g H_2O per g of dry matter for Celebrity and Jackpot, respectively. These values are considerably higher than the normal range of 400–800 g H_2O g^{-1} (Mengel and Kirk-

by, 1987). Moreover, we measured transpiration coefficients in Experiment 3 of about 870 at Zn_4 and 1500 at Zn_1. Thus, in Experiments 1 and 2, a large fraction of the P uptake at Zn_1 must have occurred nonpassively, and Welch et al.'s (1982) explanation of high tissue-P concentrations cannot account for our data. Moreover, such an explanation is inconsistent with observations that Zn deficiency does not *consistently* lead to high tissue concentrations of any other nutrient (Loneragan et al., 1982; Cakmak and Marschner, 1986; Marschner and Cakmak, 1986). Apparently, Zn deficiency leads to a unique loss of the plant's ability to regulate P uptake, and impaired shoot-to-root translocation of P may be at least partly responsible (Marschner and Cakmak, 1986).

Nonetheless, the loss of root membrane integrity in Zn-deficient plants has been reported previously (Alou, 1989; Cakmak and Marschner, 1988b,c; Welch et al., 1982). At present, a likely explanation is that Zn plays a protective role against superoxide radical (O_2^- and its derivatives) attack of membrane lipids (Cakmak and Marschner, 1988a,c). Zinc deficiency appears to enhance NADPH-dependent reduction of O_2 to O_2^-, and/or to inhibit detoxification by superoxide dismutase (SOD) and catalase (Cakmak and Marschner, 1988a). Alternatively, it has been proposed that Zn deficiency disrupts P metabolism, leading to a decrease in the production of phospholipids that are important components of cell membranes (Alou, 1989). Such a view is appealing for its ability to reconcile the effects of Zn deficiency on both membrane integrity and P accumulation into a unified physiological model, but is not yet supported by any detailed knowledge of the role of Zn in P metabolism.

Our Experiments 3 and 4 do not address this mechanistic question directly, but do suggest several other points. First, the response in membrane leakiness to changes in apoplasmic Zn status appears to be rather sluggish, at least compared to the response to withholding of solution Ca (Fig. 4). In contrast, Cakmak and Marschner (1988b) observed some amelioration of leakiness in ≤ 27 h when Zn was resupplied at 4 μM to deficient plants. But, this concentration was five-fold higher than that used to grow Zn-sufficient plants in their conventional nutrient solutions, and the free Zn^{2+} activity was undoubtedly several orders of magnitude higher than that imposed by our Zn_4 treatments. Thus, the comparatively rapid response to resupply observed by Cakmak and Marschner (1988b) may be something of an artifact due to the provision of Zn at supra-optimal concentrations. The contrasting behavior of Zn and Ca (Fig. 4) led us to question the assertion that there is an apoplasmic requirement for Zn in maintaining membrane integrity (Welch et al., 1982). The use of foliar sprays in Experiment 4 allowed us to separate the effects of apoplasmic Zn and overall plant Zn status on membrane integrity. Because there was no apparent downward translocation and leakage of Zn into the nutrient solution (see Results), we assume that the Zn_1, Zn_1Fo_1, and Zn_1Fo_2 treatments all resulted in identically low free Zn^{2+} levels in the root apoplasm. Our assumption is based, in part, on the strong affinity of DTPA for Zn ($\log K = 20.4$ for formation of the 1, 1 complex) coupled with the large excess of chelator in solution; apoplasmic binding sites would be unlikely to compete effectively for Zn. Only the Zn_1 treatment resulted in high K^+ efflux rates comparable to those observed in Experiment 3 (compare Table 3 and Fig. 4). The two foliar treatments exhibited only moderate leakage rates that were similar to those from other plants suffering from mild Zn deficiency imposed by higher apoplasmic Zn^{2+} activities (Table 3). Consequently, root membrane leakiness seems to depend primarily on overall Zn nutritional status, and to be unaffected by the free Zn^{2+} availability in the apoplasm. Leaf P concentrations seem to closely parallel changes in Zn status and membrane permeability (Table 3) but, as discussed earlier, accumulation of P to very high levels cannot be a direct consequence of leaky root membranes.

The postulated physiological link (Marschner and Cakmak, 1986) between uncontrolled tissue P accumulation and Zn-deficiency-induced increases in root membrane leakiness thus remains unresolved, as do several physiological aspects of Zn-P interactions. Our results indicate that chelator-buffered nutrient solutions may prove a useful technique for investigating these and other questions. In comparison to traditional hydro-

ponic solutions, chelator-buffered nutrient solutions represent a significant improvement in our ability to reproducibly impose Zn deficiencies of varying severity that are quite constant over the course of each experiment. Future studies should be able to utilize this improved precision in control of Zn nutritional status to considerable advantage.

Acknowledgements

We thank Drs R L Chaney and P F Bell for their generous advice regarding chelator-buffered nutrient solutions at the inception of our studies, and Dr D E Crowley for his constructive review of the manuscript.

References

Alou M M 1989 Effects of phosphorus and zinc nutrition and composition of the corn root and plasma membrane in relation to vesicular-arbuscular mycorrhiza formation. Ph.D. Diss. University of California, Riverside.

Bell P F, Chaney R L and Angle J S 1991a Determination of the free copper^{2+} activity required by maize using chelator-buffered nutrient solutions. Soil Sci. Soc. Am. J. 55, 1366–1374.

Bell P F, Chaney R L and Angle J S 1991b Free metal activity and total metal concentrations as indices of micronutrient availability to barley [*Hordeum vulgare* (L.) 'Klages']. Plant and Soil 130, 51–62.

Berg M G and Gardner E H 1978 Methods of soil analysis used in the soil testing laboratory at Oregon State University. Special Report 321. Agricultural Experiment Station, Oregon State Univ., Corvallis.

Bhatti A S and Loneragan J F 1970 Phosphorus and zinc concentration in wheat leaves in relation to phosphorus toxicity. Agron. J. 62, 288–290.

Boawn L C and Legget G E 1964 Phosphorus and zinc concentration in Russett Burbank potato tissues in relation to development of zinc deficiency symptoms. Soil Sci. Soc. Am. Proc. 28, 229–232.

Boawn L C and Brown J C 1968 Further evidence for a P-Zn imbalance in plants. Soil Sci. Soc. Am. Proc. 32, 94–97.

Cakmak I and Marschner H 1986 Mechanism of phosphorus-induced zinc deficiency in cotton. I. Zinc deficiency-enhanced uptake rate of phosphorus. Physiol. Plant. 68, 463–490.

Cakmak I and Marschner H 1987 Mechanism of phosphorus-induced zinc deficiency in cotton III. Changes in physiological availability of zinc in plants. Physiol. Plant. 70, 13–20.

Cakmak I and Marschner H 1988a Enhanced superoxide radical production in roots of zinc-deficient plants. J. Exp. Bot. 39, 1449–1460.

Cakmak I and Marschner H 1988b Increase in membrane permeability and exudation in roots of zinc deficient plants. J. Plant Physiol 132, 356–361.

Cakmak I and Marschner H 1988c Zinc-dependent changes in ESR signals, NADPH oxidase and membrane permeability in cotton roots. Physiol. Plant. 73, 182–186.

Chaney R L 1988 Metal speciation and interaction among elements affect trace element transfer in agricultural and environmental food-chains. *In* Metal Speciation, Theory, Analysis, and Application. Eds. J R Kramer and H E Allen. pp 219–260. Lewis Publishers, Chelsa, MI.

Chaney R L, Bell P F and Coulombe B A 1989 Screening strategies for improved nutrient uptake and utilization by plants. HortScience 24, 565–572.

Chapman H D 1966 Zinc. *In* Diagnostic Criteria for Plants and Soils. Ed. H D Chapman. pp 484–499. Univ. of Calif., Davis, Div. Agric. Sci.

Checkai R T, Hendrickson L L, Corey R B and Helmke P A 1987 A method for controlling the activities of free metal, hydrogen, and phosphate ions in hydroponic solutions using exchange and chelating resins. Plant and Soil 99, 321–334.

Christensen N W and Jackson T L 1981 Potential for phosphorus toxicity in zinc-stressed corn and potato. Soil Sci. Soc. Am. J. 45, 904–909.

Chvapil M 1973 New aspects in the biological role of zinc, a stabilizer of macromolecules and biological membranes. Life Sci. 13, 141–149.

Edwards D G and Asher C J 1982 Tolerance of crop and pasture species to manganese toxicity. *In* Proc. 9th Int. Plant Nutr. Coll. Ed. A Scaife. pp. 145–150. Commonw. Agric. Bur., Farnham Royal, UK.

Ghoneim M F and Bussler W 1980 Diagnosis of zinc deficiency in cotton. Z. Pflanzenernaehr. Bodenkd. 143, 366–384.

Halvorson A D and Lindsay W L 1977 The critical Zn^{2+} concentration for corn and the nonabsorption of chelated zinc. Soil Sci. Soc. Am. J. 41, 531–534.

Hanson J B 1984 The functions of calcium in plant nutrition. *In* Advances in Plant Nutrition 1. Eds. P B Tinker and A Läuchli. pp 149–208. Praeger, New York.

Hoagland D R, Chandler W H and Hibbard P L 1936 Little leaf or rosette of fruit trees. V. Effect of zinc on the growth of plants of various types in controlled soil and water culture experiments. Am. Soc. Hort. Sci. Proc. 33, 131–141.

Laurie S H, Tancock N P, McGrath S P and Sanders J R 1991 Influence of complexation on the uptake by plants of iron, manganese, copper, and zinc. J. Expt. Bot. 42, 509–513.

Leece D R 1978 Distribution of physiologically inactive zinc in maize growing on a black earth soil. Aust. J. Agric. Res. 29, 749–758.

Lindsay W L 1979 Chemical Equilibria in Soils. Wiley, New York. 449 p.

Lingle J C, Holmberg D M and Zobel M P 1958 The correction of zinc deficient of tomatoes in California. Am. Soc. Hortion Sci. Proc. 72, 397–402.

Loneragan J F, Grove T S, Robson A D and Snowball K 1979 Phosphorus toxicity as a factor in zinc-phosphorus interaction in plants. Soil Sci. Soc. Am. J. 43, 966–972.

Loneragan J F, Grunes D L, Welch R M, Aduayi E A, Tengh A, Lazar V A and Cary E E 1982 Phosphorus accumulation and toxicity in leaves in relation to zinc supply. Soil Sci. Soc. Am. J. 46, 345–352.

Marschner H and Cakmak I 1986 Mechanism of phosphorus-induced zinc deficiency in cotton. II. Evidence for impaired shoot control of phosphorus uptake and translocation under zinc deficiency. Physiol. Plant. 68, 491–496.

Martell A E and Smith R M 1976–1989 Critical Stability Constants. 6 Volumes. Plenum Press, New York.

Mengel K and Kirkby E A 1987 Principles of Plant Nutrition. 4th ed. International Potash Institute, Berne, Switzerland. 687 p.

Millikan C R 1963 Effects of different levels of the zinc and phosphorus on the growth of subterranean clover (*Trifolium subterraneum* L.). Aust. J. Agric. Res. 14, 180–205.

Millikan C R, Hanger B C and Bjarnason E N 1968 Effect of phosphorus and zinc levels in the substrate on ^{65}Zn distribution in subterranean clover and flax. Aust. J. Biol. Sci. 21, 619–640.

Norvell W A 1991 Reactions of metal chelates in soils and nutrient solutions. *In* Micronutrients in Agriculture. 2nd edition. Ed. J J Mordvedt. pp 187–227. SSSA Book Ser. No. 4. Soil Sci. Soc. Amer., Madison, WI.

Norvell W A, Dabkowski-Naskret H and Cary E E 1987 Effect of phosphorus and zinc fertilization on the solubility of Zn^{2+} in two alkaline soils. Soil Sci. Soc. Am. J. 51, 584–588.

Paribok T A and Alekseeva-Popova N V 1965 Influence of zinc on the absorption and utilization of phosphorus by plants. Sov. Plant Physiol. 12, 514–518.

Parker D R, Chaney R L and Norvell W A 1992a Chemical equilibrium models: Applications to plant nutrition research. *In* Soil Chemical Equilibrium and Reaction Models. Eds. R H Loeppert, A P Schwab and S Goldberg. SSSA Spec. Pub. No. XX., ASA, SSSA, Madison, WI. (*In press*).

Parker D R, Norvell W A and Chaney R L 1992b GEOCHEM-PC: A chemical speciation program for IBM and compatible personal computers. *In* Soil Chemical Equilibrium and Reaction Models. Eds. R H Loeppert, A P Schwab and S Goldberg. SSSA Spec. Pub. No. XX., ASA, SSSA, Madison, WI. (*In press*).

Rahimi A and Bussler W 1979 Die Entwicklung und der Zn-, Fe- und P-Gehalt höherer Pflanzen in Abhängigkeit vom Zinkangebot. Z. Pflanzenernaehr. Bodenkd. 142, 15–27.

Reed H S 1946 Effects of zinc deficiency on phosphate metabolism of the tomato plant. Am. J. Bot. 33, 778–784.

Sposito G and Mattigod S V 1980 GEOCHEM: A computer program for the calculation of chemical equilibria in soil solution and other natural water systems. Kearney Foundation of Soil Science, Univ. of California, Riverside.

Takkar P N, Mann M S, Bansala R L, Randhawa N S and Singh H 1976 Yield and uptake response of corn to zinc, as influenced by phosphorus fertilization. Agron. J. 68, 942–946.

Van Maren A F 1987 Tomato plant decline. Univ. of California. Cooperative Extension.

Wallihan E F and Bradford G R 1977 Simplified methods for inducing micronutrient deficiencies. Hort Science 12, 327–328.

Webb M J and Loneragan J F 1988 Effect of zinc deficiency on growth, phosphorus concentration, and phosphorus toxicity of wheat plants. Soil Sci. Soc. Am. J. 52, 1676–1680.

Welch R M, Webb M J and Loneragan J F 1982 Zinc in membrane function and its role in phosphorus toxicity. *In* Proc. 9th Int. Plant Nutr. Coll. Ed. A Scaife. pp. 710–715. Commonw. Agric. Bur., Farnham Royal, UK.