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Translocation of Manganese, Iron, Cobalt, and Zinc in Tomato

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Summary. Tomato plants in solution culture were treated with 0 to 50 μM Mn, Co, or Zn in the presence of 5 μM Fe. Stem exudates were analyzed to determine quantities and forms of the metals translocated.

Mn had no effect on exudate volume. Co and Zn at 5 μM and above depressed exudate volumes. Nutrient Mn was concentrated 2 to 5 times in the exudates. Co and Zn concentrations were between 1 and 3.

Tomato roots treated with 0 to 10 μM Mn released into the exudate an average of 29 % of the Fe they absorbed. They released 21 % of the absorbed Fe when treated with 50 μM Mn. On 50 μM Co or Zn, the roots released only about 1 % of the absorbed Fe.

The exudates contained 12 to 47 μM citrate, which was usually considerably in excess of Fe. Electrophoresis of exudate revealed Fe as the only metal in anionic form. Mn, Co, and Zn migrated as cations.

The concentration of Ca was >3 mM and Mg >1.5 mM in the exudates. Estimates based on metal-citrate equilibrium constants and constants of metal displacement caused by Ca and Mg confirmed that Mn, Co, and Zn were transported predominantly as inorganic cations in the stem exudates.

Most of the metal carried upward in plants consists of inorganic cations (1, 9). Because Na, K, Ca, and Mg comprise the bulk of transported metal, this is a reasonable view. It is not true, however, that all metals are conducted in the xylem as cations. One exception presently known is Fe-citrate, the anion translocated in sunflower (10). Little information on the translocation of Mn, Co, and Zn is now available. But the stability of these metals in organic complexes (5, 8) suggests an association and a possible means of their translocation in plants.

Several investigators have studied the forms of microelements in plant stem exudates. Schmid and Gerloff (7) showed that Fe in tobacco exudate was not a free ion, but was combined with an unidentified organic molecule. Work in this Laboratory (10) indicated that Fe in sunflower exudate was complexed with citric acid. Exudate from cucumber and tomato (11) also contained Fe in anionic form, presumably Fe-citrate. Lingle, et al. (6) demonstrated by electrophoresis that Mn and Zn were cations in soybean exudate.

Citric acid strongly binds Fe, and is usually in molar excess of Fe in stem exudates (11). Citrate also binds other metals (5, 8), but they are not held as tightly as Fe. The citric acid in excess of Fe, therefore, could serve to transport other metals in plants.

The experiments with tomato reported here focus on the quantities of Mn, Fe, Co, and Zn absorbed and translocated, and on the forms of the metals translocated.

Materials and Methods

The experimental plant was tomato, *Lycopersicon esculentum* Mill., var. Marglobe. General cultural and sampling procedures have been reported (10). The age-treatment schedule was as follows. Seeds were germinated 5 days, then were transferred to nutrient solutions and grown under shading to elongate the stems. At 9 days the seedlings were grouped in pairs and transferred into full light, 4 plants in each 8 liters of nutrient. At 21 days the nutrient solution was renewed. At 27 days the plants were placed in the experimental nutrient solutions and decapitated near the first leaf node. Stem exudates were collected for 10 hours.

Nutrient Solutions. Plants were grown with standard major elements (10), but with changes in the minor elements (noted below) and NaCl to provide 70 μM chloride. The microelement stock solution contained the following chemicals per liter: 2.47 g $MnCl_2 \cdot 4H_2O$, 1.86 g H_3BO_3, 0.34 g $ZnCl_2$, 0.17 g $CuCl_2 \cdot 2H_2O$, and 0.12 g $Na_2MoO_4 \cdot 2H_2O$. Addition of 0.2 ml of this solution per liter of nutrient gave (μM): 2.5 Mn, 6 B, 0.5 Zn, 0.2 Cu, and 0.1 Mo.

To provide control levels of Mn and Zn in the experimental nutrients used in the exudation period, microelement stock solutions (above) were prepared

without Mn or Zn salt. Separate solutions (10 mM) were made up with $MnCl_2 \cdot 4H_2O$, $ZnCl_2$, and $CoCl_2 \cdot 6H_2O$. These were used to vary the metals as shown in the tables. Where metal-chloride treatments were low, NaCl was added to the nutrients to bring chloride to approximately 100 μM. In 1 case (100 μM Mn treatment, table II) the chloride was double the usual amount. All nutrient solutions were adjusted to pH 5.5 after addition of isotopes.

Metal Isotopes and Assays. Isotopes were obtained in HCl solution from the New England Nuclear Company[1]. ^{54}Mn and ^{57}Co (carrier free) and ^{65}Zn (13.7 c/g Zn) were used in nutrients at 20 μc/l. ^{59}Fe (9.25 c/g Fe) was bound to equivalent Ethylenediamine di(*o*-hydroxyphenylacetic acid) (EDDHA) and the mixture diluted with enough 0.02 M FeEDDHA to give 5 μmoles of Fe and 20 μc ^{59}Fe per liter of experimental nutrient. The solution was sampled before and after the 10-hour absorption period to determine the uptake of isotopes. Samples of the nutrient solution and exudate were assayed in a gamma scintillation spectrometer. Ca, Mg, and Mn were determined by atomic absorption analysis.

Electrophoresis Apparatus. The apparatus described previously (10) was used to separate metals by filter paper electrophoresis. Another apparatus was constructed to study the migration of metals in semifree solution without the filter paper support. The apparatus consisted of a rigid plastic tube into which 12.5-mm lengths of polyethylene tubing (13-mm, inside diameter) were inserted to provide compartments between millipore discs. Holes in the tube, matching holes in the tubing sections, permitted addition and recovery of buffer and metal solutions. After electrophoresis, the solutions were aspirated into glass tubes and assayed.

Buffer Solutions. The principal buffer used for both paper and tube electrophoresis was 20 mM sodium acetate (pH 5.4). A second buffer (20 mM citrate, pH 5.4) contained 4 g $Na_3citrate \cdot 2H_2O$ and 1.34 g citric acid$\cdot H_2O$ per liter. Appropriate dilutions of this with the acetate gave the buffers listed in figure 3.

Results

Metal Effects on Fe Uptake and Transport. Mn was less drastic in its effects on Fe uptake than were Co and Zn (table I). Tomato roots absorbed 16 % of the Fe from solutions containing 50 μM Mn. They took up between 40 and 60 % of the Fe supplied in the lower Mn treatments. Co and Zn began to depress Fe uptake when their concentrations were near 1 μM. Mn had no effect on exudate volumes, but Co and Zn at 5 μM and above depressed exudate volumes.

The quantities of nutrient Fe released into the total exudate (IR, table I) seem remarkably constant for treatments containing up to 10 μM Mn. A break in the Fe transport appeared between 10 and 50 μM Mn. Depression in Fe transport began at lower concentrations of Co and Zn.

[1] The naming of companies and products is for the reader's benefit, and does not imply endorsement by the United States Department of Agriculture.

Table I. *Effects of Manganese, Cobalt, and Zinc on Iron Uptake and Release into Tomato Exudate*

Two plants were placed in each liter of nutrient solution containing the metal variable in the presence of 5 μM FeEDDHA and ^{59}Fe. The plants were decapitated, and exudate was collected for 10 hours.

Nutrient metal treatment		Iron uptake (IU)	Exudate volume	Iron release (IR)	IR/IU	Iron in exudate		
						R*	T**	R/T
	μM	μmole	ml	μmole	%	μM	μM	%
Mn	0	2.8	32	0.848	30.3	26.5	26.3	101
	0.5	2.5	29	0.771	30.8	26.6	25.6	104
	1.0	2.6	24	0.710	27.3	29.6	27.6	107
	5.0	2.4	26	0.723	30.1	27.8	27.5	101
	10.0	2.5	29	0.690	27.6	23.8	23.0	103
	50.0	0.8	25	0.170	21.3	6.8	6.6	103
Co	0	2.9	33	0.927	32.0	28.1	29.3	96
	0.5	2.7	29	0.832	30.8	28.7	27.9	103
	1.0	1.7	22	0.420	24.7	19.1	23.6	81
	5.0	1.7	14	0.133	7.8	9.5	15.3	62
	10.0	1.5	10	0.048	3.2	4.8	11.1	43
	50.0	0.9	5	0.009	1.0	1.8	8.7	21
Zn	0	2.8	26	0.848	30.3	32.6	32.0	102
	0.5	2.5	28	0.882	35.3	31.5	31.4	100
	1.0	2.2	28	0.613	27.9	21.9	25.3	87
	5.0	1.5	19	0.203	13.5	10.7	14.2	75
	10.0	1.3	22	0.132	10.2	6.0	11.6	52
	50.0	0.6	11	0.009	1.5	0.8	3.2	25

* From ^{59}Fe analysis.
** From total Fe analysis by orthophenanthroline.

Relation of Fe Uptake to Release. The absorbed Fe that was released into the exudate (IR/IU) averaged 29 % for Mn treatments up to 10 μM. The value of 21 % on the 50 μM Mn indicates a relatively small depression in Fe transport when compared to the low values of 1 and 1.5 % associated with the high Co and Zn treatments.

Specific Activity Relationships. The molarities of Fe in column R (table I) were calculated from the ^{59}Fe content of the exudates. The values in column T represent total Fe determined as ferrous phenanthroline. The R and T values showed close agreement, with values for R/T being around 100 % for all Mn treatments. This indicates that essentially all the Fe in the exudate came from the nutrient. Values above 100 % obviously are in error, because the plants could not select and concentrate the ^{59}Fe.

The effects of Co and Zn were very different. At the highest concentrations of these metals, the R/T values were 21 and 25 %. Presumably the roots released 4 or 5 atoms of Fe from internal stores for every atom of nutrient Fe they translocated to the exudate.

In summary of table I, it is evident that Mn did not inhibit Fe uptake and transport or supress exudate volumes nearly as much as the other metals.

Mn, Co, and Zn Uptake and Transport. Results in table II show that on the 0.5 and 5.0 μM treatments the plants absorbed all or nearly all the metal supplied. This was true also for the 25 μM Mn treatment. On the 25 and 50 μM treatments, however, the quantities of Mn absorbed were about double those of Co and Zn. Low concentrations of Co and Zn depressed exudate volumes, but Mn had no effect, even at 100 μM.

On all treatments except one (0.5 μM Co), the concentration of metal in the exudate was higher than the initial supply of metal in the nutrient. Roots concentrated Mn 2 to 5 times the original nutrient level. Co and Zn concentrations were between 1 and 3 times the nutrient levels.

Ratios of Metals in Exudates. A comparison of total Fe (table II) with total Mn (table III) shows that Mn/Fe in the exudates ranged between 0.4 and 19. Metal concentrations (table III) indicate a range of from 333 to 14 for Ca/Mn ratios, and from 183 to 7 for ratios of Mg/Mn. The importance of metal ratios in determining the forms of metals translocated in exudate will be discussed in a later section.

Relation of Metal Uptake to Transport. The percentage of absorbed metal that was released is given by the ratio MR/MA. Generally a higher percentage of the Mn was released than was noted for Co and Zn. The percentages were fairly constant except for lower values in the intermediate range of the Mn and Zn treatments. The retention of metal by the roots was greater in that range. On the lowest treatments, however, the roots released relatively high

Table III. *Calcium, Magnesium, and Manganese in Tomato Exudate*

This table presents additional data for the Mn experiment in table II.

Nutrient treatment	Metals in exudate		
Mn	Ca	Mg	Mn
μM	μM	μM	μM
0.5	4000	2200	12
5.0	3850	1950	20
25.0	3650	2100	98
50.0	4250	1850	154
100.0	3100	1550	214

Table II. *Uptake and Transport of Manganese, Cobalt, and Zinc by Tomato*

Two plants were placed in each liter of nutrient solution containing the metal variable (with Mn, Co, or Zn label) and 5 μM FeEDDHA. The plants were decapitated, and exudate was collected for 10 hours.

	Nutrient metal treatment	Total metal absorbed (MA)	Exudate volume	Exudate metal conc*	Total metal release (MR)	MR/MA	Exudate iron	Exudate citrate
	μM	μmole	ml	μM	μmole	%	μM	μM
Mn	0.5	0.5	34	2.5	0.085	17.0	27.4	44
	5.0	5.0	32	14.8	0.47	9.4	20.7	32
	25.0	23.0	33	75.9	2.50	10.9	18.4	27
	50.0	29.5	31	128.7	3.99	13.5	11.1	17
	100.0	39.2	35	194.1	6.79	17.3	11.5	12
Co	0.5	0.4	37	0.4	0.015	3.8	24.1	47
	5.0	4.6	15	13.5	0.20	4.3	13.9	32
	25.0	11.7	9	70.8	0.64	5.5	7.6	17
	50.0	13.5	6	110.6	0.66	4.9	10.1	19
Zn	0.5	0.5	35	1.2	0.024	8.4	27.4	42
	5.0	4.8	24	6.3	0.15	3.1	9.8	20
	25.0	12.5	21	31.7	0.66	5.3	5.6	26
	50.0	15.0	16	78.3	1.25	8.3	3.9	23

* Based on radioactivity.

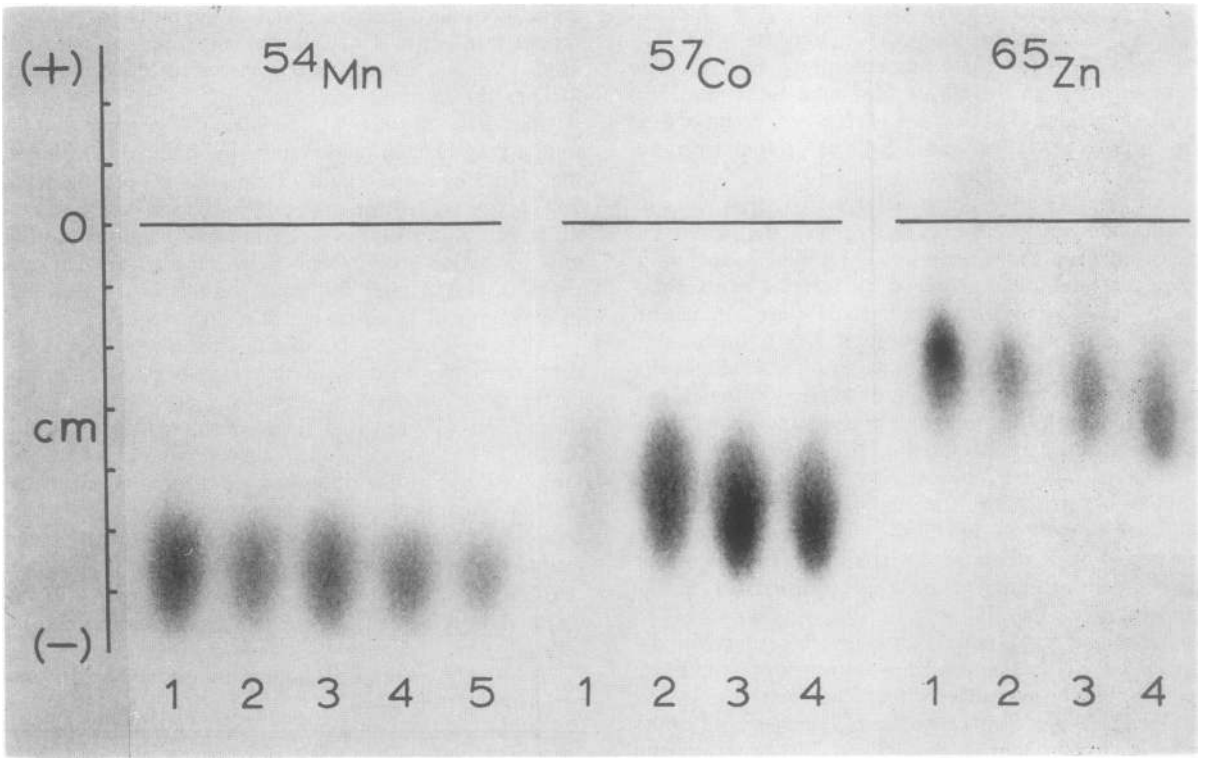

FIG. 1. Radiograph of electrophoretically separated metals in tomato stem exudate. The exudates are characterized in tables II and III. The numbered paths (increasing in value) correspond to increasing metal treatment, table II. X-ray films were exposed 30 days. Electrophoretic conditions: 20 μl exudate, Whatman No. 3 paper, 20 mM sodium acetate buffer pH 5.4, 18 mA (360 to 380 v) 45 minutes, room temperature.

percentages of the absorbed metal, indicating a notable efficiency in metal transport.

Both Fe and citrate were lowered in the exudates as Mn, Co, and Zn were increased in the external medium.

Forms of Translocated Metal. The exudates in table II provided an opportunity to determine whether the citrate in excess of Fe in stem exudate binds other metals. The citrate exceeded Fe in all cases, and the exudates from the 0.5 and 5.0 μM treatments contained, in most cases, enough citrate to bind (1:1) both the Fe and the competing metal. The radiographs (fig 1) show that all the Mn migrated toward the cathode. The Co and Zn migrated similarly. Strong binding by citrate would have resulted in anodic migration. The metals apparently ran as inorganic cations.

Tube Electrophoresis. Labeled exudates were obtained from experiments similar to those reported in tables I and II. For the electrophoretic analysis, buffer was withdrawn from the center compartment and replaced by exudate. Figure 2 shows the peaks of radioactivity after electrophoresis. The distribution identifies Mn, Co, and Zn as cations. These results are similar to those obtained via paper electrophoresis. Fe was the only metal that traveled anodically.

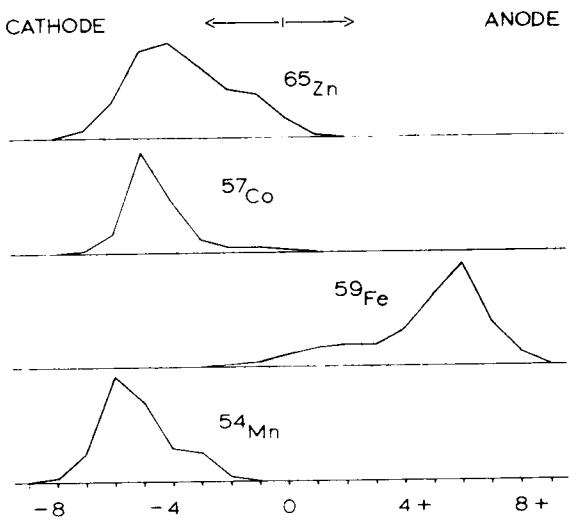

FIG. 2. Electrophoretic separation of metals in tomato stem exudates. Patterns show relative distributions of exudate metal into the numbered compartments. Electrophoretic conditions: 20 mM sodium acetate buffer pH 5.4, 8 mA (300 v), 20 minutes for Mn, Co, and Zn, and 35 minutes for Fe, room temperature.

Buffer Citrate Effects. The cathodic position of the metals (fig 1) shows that they were not strongly bound to citric acid. Experiments were therefore designed (fig 3) on the conjecture that citric acid added to the buffer might retain the metals long enough to carry them anodically or provide some restraint on their cathodic migration. Buffer I was without citrate, so that the only citrate in the system was in the 15 μl volumes of exudate spotted at the origin. The citrate was about 45 μM in these exudates (see the 0.5 μM treatments for Mn, Co, and Zn, table II). Previous work (10) showed that citrate travels anodically. This is opposite to the migration of the dissociated metals. Thus, in buffer I there could be no metal-citrate association after the metals had migrated a short distance toward the cathode.

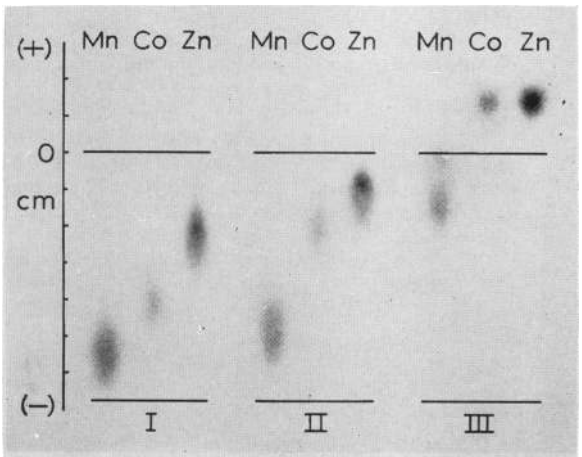

FIG. 3. Radiograph showing the effect of citrate buffer on the electrophoresis of metals in tomato stem exudate. Exudate samples (15 μl) were from the 0.5 μM treatments, table II. X-ray films were exposed 20 days. Concentrations (mM) of acetate (a) and citrate (c) in the buffers were as follows: I, 20 a; II, 19.96 a and 0.04 c; III, 19.6 a and 0.4 c. Other electrophoretic conditions as in figure 1.

In buffer II, however, the metals could not avoid the restraining effects of citrate because, as they migrated cathodically, they were always running against 40 μM citrate. Consequently, in buffer II the metals did not migrate as far as they did in buffer I.

In buffer III, the retention by citrate was enough to cause a net movement of Co and Zn anodically. The final position of Mn, however, was still toward the cathode. Citrate greater than 400 μM (probably near mM) would be required to carry Mn anodically.

Discussion

Translocation of Inorganic Solute. The bulk of the minerals in the xylem undoubtedly exists as free ions (9). This has also been emphasized by Biddulph (1) who sees "rather universal agreement that the minerals which ascend the stem do so largely in inorganic form." In this regard, reference has often been made to Wormall (12) who discussed constituents of grapevine sap. Although he found two-thirds of the solute to be organic, and most of this composed of oxalic, tartaric, malic, and succinic acids, he did not discuss metal binding by the organic acids. The metals in the sap were Na, K, Ca, Mg, Mn, Fe, and Al which were reported as simple salts (chlorides, sulfates, nitrates, phosphates, etc.). Apparently his methods gave no indication of metal-organic association.

Hydrated Metal Cations. The hydrated metal, $M(H_2O)_x^{2+}$, is considered the normal form of the divalent transition metal in aqueous solution (2). A sufficient increase of OH^- in such a solution results in the displacement of H^+ from the water shell of the metal and precipitation of the metal hydroxide. Any degree of complexing between organic compounds and the hydrated metal displaces water molecules and lessens the tendency to form metal hydroxide (2). Fe^{3+} begins to hydrolyze at about pH 2.5. This accounts for the difficulty in maintaining soluble in-

Table IV. *Metal-citrate Equilibrium Constants and Metal Displacement Equilibrium Constants*

The equilibrium constant K for metal-citrate (MCit) is of the form $K_{MCit} = (MCit)/(M)(Cit)$. From Chaberek and Martell (2), the displacement equilibrium constant K′, resulting from the displacement of M from the chelate by another metal M′, is given by $K' = K_{M'Cit}/K_{MCit} = (M'Cit)(M)/(M')(MCit)$.

Metal	Equilibrium constant K	Equilibrium constants divided*	Displacement equilibrium constant K′	Literature sources for K value
Fe	2.5×10^9	Fe/Zn	35000	(2, 3)
Zn	70800	Zn/Ca	47.9	(5, 8)
Co	67610	Co/Ca	45.7	(5, 8)
Mn	4678	Mn/Ca	3.2	(5, 8)
Mg	1950	Mg/Ca	1.3	(5, 8)
Ca	1479			(4, 8)
		Mn/Mg	2.4	
		Co/Mg	34.6	
		Zn/Mg	36.2	

* To get K′ values.

organic Fe in culture solutions at neutral or slightly acidic pH. On the other hand, Mn^{2+}, Co^{2+}, and Zn^{2+} are very stable in the nutrient solutions (pH 5.5) and stem exudates (pH 5.4) used in the present study.

Based on the information above, the results in figure 3 represent a competition principally between citrate and water for the metals. In other experiments (unpublished) 100 μM citrate in the buffer held Co and Zn at the origin. The failure to migrate does not indicate a neutral molecule, but is visualized as a balance in the migration distances of the hydrated and chelated forms of the metals. Such a poised system obviously was not achieved in figure 3.

Translocation of Metal Chelate. In contrast to the macroelement transport, there is reason to expect some of the trace elements to migrate in complexed form. The stability constants of the citrate chelates of Fe, Co, Zn, and Mn (table IV) seem to warrant this expectation. Metal binding, however, was demonstrated only for Fe (figs 1, 2).

Metal Competition and Displacement. The stability of Fe-citrate (2.5×10^9) indicates that Fe is out of the range of serious competition from Co, Zn, and other metals with lower affinities for citrate. The citrate bound to Fe is therefore substracted in order to determine citrate that is free to bind other metals (e.g., 44–27 = 17 μM citrate; see the 0.5 μM Mn treatment, table II). By use of the displacement constants (table IV) it is then possible to obtain some idea of the concentration of metal combined with citrate in the exudates.

The displacement of Mn by Ca is calculated from $K' = (MnCitrate)(Ca)/(Mn)(CaCitrate)$, where concentrations are μM. Values entered from tables III and IV give $3.2 = (x)(4000)/(12)(17\text{-}x)$. From this calculation, the value for MnCitrate is 0.16 μM.

This value, however, does not include any displacement of Mn by Mg. Because the K′ for Mn/Ca is similar to that of Mn/Mg, the concentrations of Ca and Mg (4000 and 2200 μM, table III) are combined to further qualify the Mn equilibrium. Entry of 6200 μM, instead of 4000, in the equation above indicates that MnCitrate is about 0.10 μM. From this it is evident that <1 % of the Mn is bound to citrate. Displacement by other metals would, of course, result in a lower equilibrium value for MnCitrate.

The competition from Ca and Mg helps explain the apparent complete dissociation of Mn in figure 1. Although not given, calculations of the effects of Ca and Mg (at the concentrations above) on Co and Zn indicate that these metals also would be present predominantly as cations in the stem exudate.

Acknowledgments

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