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Metal Complexation in Xylem Fluid[1]

I. CHEMICAL COMPOSITION OF TOMATO AND SOYBEAN STEM EXUDATE

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ABSTRACT

Xylem fluid was analyzed for numerous solutes to characterize chemically the sap as a medium for forming and transporting metal complexes. The stem exudate was collected hourly for 8 hours from topped 31-day-old soybean (*Glycine max* L. Merr.) and 46-day-old tomato (*Lycopersicon esculentum* Mill.) plants grown in normal (0.5 micromolar) and Zn-phytotoxic nutrient solutions. Soybean plants were grown in the normal and high-Zn solutions for 24 days; tomato plants were grown for 32 days. The exudate was analyzed for seven organic acids, 22 amino acids, eight inorganic solutes, apparent ionic strength, and pH. Significant changes in many solutes occurred over the 8-hour sampling period. These fluctuations depended on plant species, individual solute, and Zn treatment, and demonstrated that extrapolation of xylem-fluid analyses to whole-plant xylem sap is valid only for sap samples collected shortly after topping a plant. Exudate pH decreased over the 8-hour period for both species; exudate ionic strength increased for tomato and decreased for soybean. At the normal-Zn treatment (0 to 1 hour), the highest acid micromolar concentrations in soybean exudate were: asparagine, 2,583; citric, 1,706; malic, 890; and malonic, 264. Under the same conditions, the highest acid micromolar concentrations in tomato exudate were: maleic, 1,206; malic, 628; glutamine, 522; citric, 301; and asparagine, 242. Cysteine and methionine were above detection limits only in soybean exudate. Zinc phytotoxicity caused significant changes in many solutes. The analyses reported here provide a comprehensive data base for further studies on metal-complex equilibria in xylem fluid.

Considerable work has been summarized on the phytotoxic effects of heavy metals borne in organic wastes, biocidal sprays, mine wastes, and metal salt additions to soils and solution cultures (6, 10). Although gross metal distributions have been described, there is little information on chemical forms within plants, especially at elevated metal concentrations. Nonessential metals (*e.g.* Cr, Ni, Cd, Pb) can readily enter a plant and be toxic. All metals move to some degree in plants, but their distribution and sites of injury may depend on their stability constants with various natural complexing or binding ligands. Tiffin (25) has extensively reviewed many phases of metal translocation, especially in the xylem; phloem translocation has been reviewed by Pate (14).

Because metals move acropetally via the xylem fluid, it is reasonable to study chemical species and metal complexation within that system. However, there is a lack of information on the complete chemical nature of xylem fluid, as well as possible metal forms at normal and toxic concentrations of heavy metals. Most information on xylem solutes is fragmentary, so that it is difficult to establish both a comprehensive view of the range of ligands and other solutes under known growth conditions and the effects of plant species and cultivars.

To understand metal-complex formation in xylem fluid, most major organic and inorganic solutes and their concentrations must be known. Critical compilation of this information from published values is difficult because of confounding factors. For example, very young plants are often grown in single-salt solutions, with no consideration of plant age, ion interactions, pH, nutrient concentrations, etc.; some studies report data from single plants. Clearly, all aspects of growth must be considered, especially since large genetically based variations in solute uptake and translocation have been observed (13).

The length of sample collection time, and its effect on xylem content, is another important factor. Samples taken in most studies represent collections over many hours, even days. Dilution or concentration of the exudate through experimental procedures could seriously compromise the interpretation of results pertaining to metal complexes in xylem exudate. Metal complexation is particularly sensitive to solution pH, ionic strength, and solute concentrations. Consideration of these phenomena must preclude generalizations about xylem sap contents, and the physiological significance of many reported exudate analyses may be suspect.

The work reported here provides information on the chemical components of xylem fluid, particularly those pertinent to metal complexation. This information establishes a solid foundation for further evaluation of xylem sap as a system for transporting metals in terms of natural metal-complex equilibria in a biological fluid.

MATERIALS AND METHODS

Plant Growth. 'Williams' soybean seed (*Glycine max* L. Merr.) was germinated between paper towels saturated with deionized

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H_2O, backed with wax paper, and placed in an 8-liter polyethylene bucket containing 700 ml deionized H_2O. Seven days later, matched seedlings were grouped in bundles of six plants and transferred to 8-liter polyethylene buckets (six bundles/bucket). All buckets were enclosed in wooden boxes with Plexiglas tops. The plant bundles were suspended through holes in the tops and supported by polyurethane foam strips. 'Rutgers PS-VF' tomato seed (*Lycopersicon esculentum* Mill.) was germinated on cheesecloth supported by a stainless-steel screen in a glass tray containing 600 ml 2 mM $Ca(NO_3)_2$. Fourteen days later, the seedlings were transferred to 8-liter buckets (six plants/bucket) and supported by the methods described for soybean.

Each bucket contained 8 liters of a modified 20% Johnson's solution (12). The 20% solution contained the following nutrient micromolar concentrations; NO_3-N, 2,800; NH_4-N, 100; P, 200; K, 1,200; Mg, 200; Ca, 800; S, 200; Fe, 10; Mn, 1.0; B, 5.0; Mo, 0.1; Cu, 0.1; and Cl, 2.0. The pH of the modified 20% Johnson's solution was 6.1; the ionic strength was 5.2 mM. Citrate was used to maintain soluble nutrient solution Fe. The normal nutrient Zn concentration for each species was 0.5 μM. To induce a phytotoxic Zn stress, preliminary experiments were conducted to determine, for each species, solution Zn concentrations that would cause approximately a 20% yield reduction in shoot dry weight and visible phytotoxic symptoms (chlorosis and slight necrosis). The concentrations chosen were: for soybean, 3 μM Zn for the first 12 days in the full nutrient solution and 8 μM until harvest; for tomato, these values were 30 and 80 μM, respectively. All solutions were aerated continuously and changed every 4 days during the growth periods.

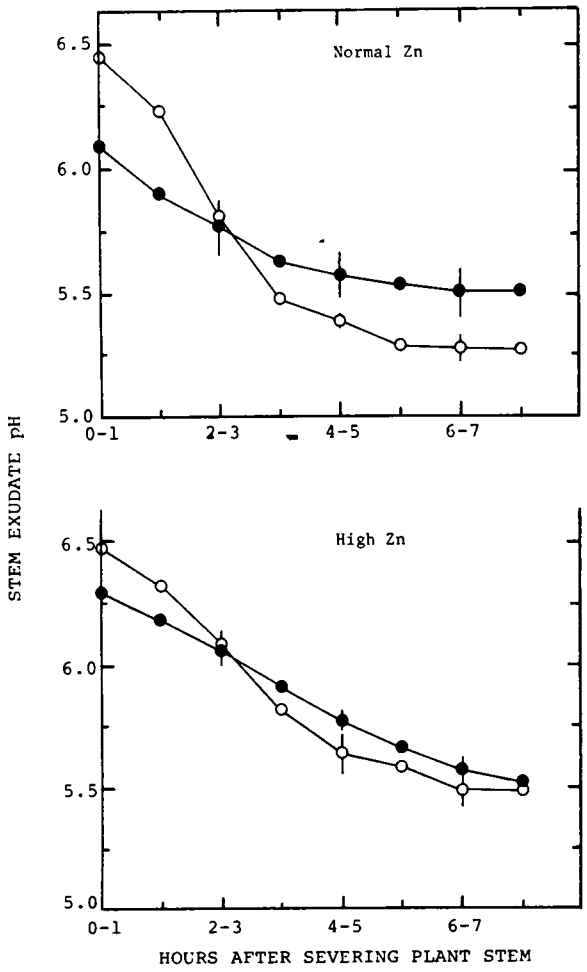

FIG. 1. Effect of time and solution Zn concentration on tomato (○) and soybean (●) stem exudate pH. Solution Zn concentrations were: for normal-Zn soybean and tomato, 0.5 μM; for high-Zn soybean, 3 μM during the first 12 days in solution and 8 μM until harvest (31 days old); for high-Zn tomato, 30 and 80 μM (46 days old), respectively. Each value is the mean of three replications. Vertical bars for selected means equal 1 SE.

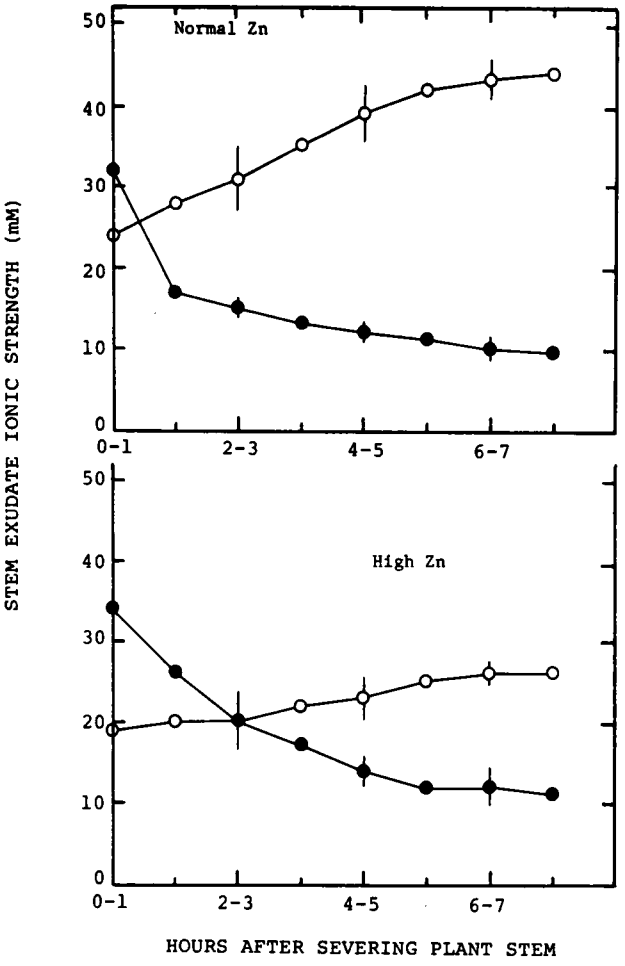

FIG. 2. Effect of time and solution Zn concentration on tomato (○) and soybean (●) stem exudate ionic strength. Solution Zn concentrations were: for normal-Zn soybean and tomato, 0.5 μM; for high-Zn soybean, 3 μM during the first 12 days in solution and 8 μM until harvest (31 days old); for high-Zn tomato, 30 and 80 μM (46 days old), respectively. Each value is the mean of three replications. Vertical bars for selected means equal 1 SE.

Soybean plants were grown in the normal- and high-Zn solutions for 24 days; tomato plants were grown for 32 days. Thus, the total number of days from germination to harvest was 31 days for soybean and 46 days for tomato. The plants were grown in experimental units of six buckets per species per Zn treatment. Experimental units were chosen randomly and grown sequentially. Each species/Zn treatment was replicated three times. The growth chamber was maintained at 23 C (16-h day) and 18 C (8-h night) with variable humidity (44–96%).

Stem Exudate Collection. Each collection unit consisted of six buckets (an experimental unit). Xylem fluid was collected by placing the plants from each unit into six 8-liter buckets of aerated nutrient solution that had been equilibrated overnight. Plant stems were severed perpendicular to the stem axis with stainless-steel razor blades at approximately 7 cm (tomato) or 10 cm (soybean) above the roots. The decapitated stumps were wiped gently and fitted with Tygon tubing. The stumps were allowed to bleed into glass scintillation vials. Samples were collected hourly for 8 h. Each sample was filtered immediately (0.45 μm) and frozen until

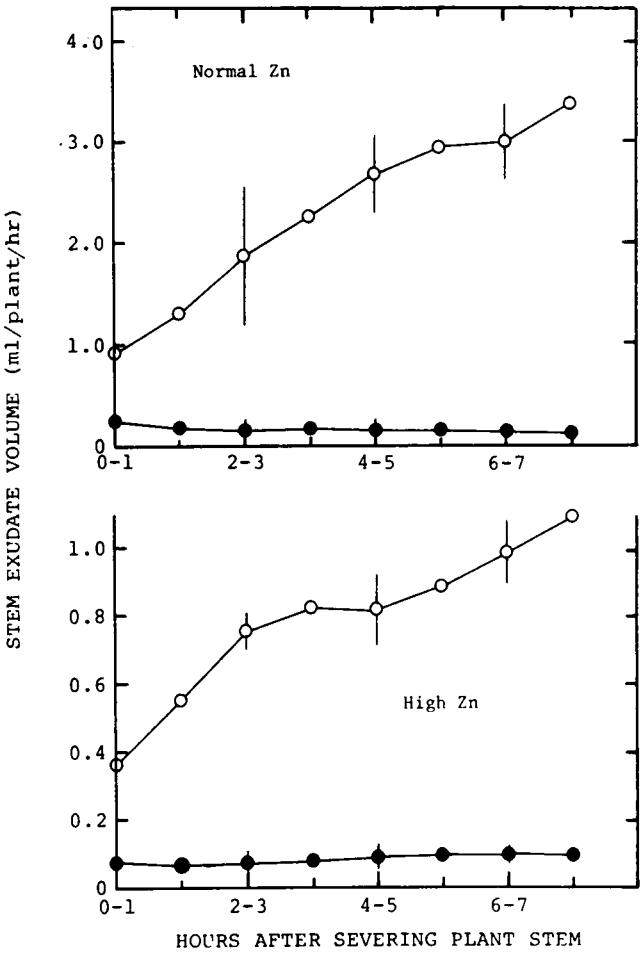

FIG. 3. Effect of time and solution Zn concentration on tomato (○) and soybean (●) stem exudate volume. Solution Zn concentrations were: for normal-Zn soybean and tomato, 0.5 μM; for high-Zn soybean, 3 μM during the first 12 days in solution and 8 μM until harvest (31 days old); for high-Zn tomato, 30 and 80 μM (46 days old), respectively. Each value is the mean of three replications. Vertical bars for selected means equal 1 SE.

needed. All exudates were collected in the dark at 20 to 25 C.

Stem Exudate Analyses. Calcium, Cu, Fe, Mg, Mn, and Zn were determined by atomic absorption spectrophotometry by direct aspiration of the exudate. When required, samples were diluted with deionized H_2O. Ammonium-N and phosphate-P were analyzed colorimetrically by the procedures of the Technicon Auto-Analyzer II (18, 20).

Ionic strength was determined by measuring the electrical conductivity of the exudate and normalizing the values to the conductance at 25 C. A conversion factor of 0.013 (11) was used to estimate the apparent ionic strength in units of mol/l. The exudate pH was measured directly with a combination glass microelectrode.

Organic acids were analyzed by GLC. Volatile derivatives of the acids were prepared by modification of the methods of Clark (7) and Phillips and Jennings (16). Three ml of each exudate sample was brought to dryness in a glass-stoppered vial at room temperature by using an air stream. The residue then was brought to 0.5 ml total volume with silylation-grade pyridine. The derivatives were made by adding 100 μl *N, O-bis*trimethylsilyl acetamide to the vials, shaking gently, and allowing the mixture to stand at room temperature for 30 min. In all analyses, 10 μl of each sample or standard was injected.

Amino acids were analyzed by liquid chromatography with an automated microbore amino acid analyzer (2).

RESULTS

pH. After the plant stems were severed, the pH of soybean and tomato stem exudate decreased with increasing time (Fig. 1). The exudate pH of both species was higher for the high-Zn solution at each collection time. At both normal- and high-Zn concentrations, the pH of tomato exudate was higher than soybean exudate for the first 3 h and lower than soybean for the remaining 5 h (Fig. 1).

Apparent Ionic Strength. At both solution Zn concentrations, the estimated ionic strength of tomato stem exudate increased with increasing collection time, whereas soybean exudate ionic strength decreased with time (Fig. 2). At the earliest collection time (0–1 h), soybean estimated ionic strength was higher than that of tomato for both Zn concentrations. A much greater difference in ionic strength between tomato and soybean exudate was found at the later collection times (Fig. 2), especially for the normal-Zn plants.

Exudate Volume. The amount of tomato stem exudate/plant·h increased with increasing time after topping (Fig. 3). Soybean exudate volume/h was essentially unchanged over the 8-h sampling period, and there was a strong Zn effect on the amount of exudate recovered from tomato plants each h (Fig. 3). At the higher Zn concentration, far less exudate was recovered from each tomato plant/h than at the normal Zn concentration.

Inorganic Species. Each hourly sample was analyzed for eight soluble inorganic components. Table I lists the soybean results for four sampling times and two nutrient solution Zn concentrations; statistical analyses were performed using all eight sampling times. At the normal-Zn treatment, Ca, Cu, Mg, NH_4^+, and Zn concentrations were significantly lower in successively later sampling times. Iron and Mn levels in soybean exudate were unaffected by increasing sampling time, whereas P levels increased significantly.

At the high-Zn concentration, Ca, Cu, Fe, Mg, NH_4^+, and Zn exudate concentrations were significantly lower, and PO_4^{3-} was significantly higher, in successively later sampling times (Table I). Only Mn was unaffected by sampling time. As expected, Zn concentrations were much higher at this treatment.

Although high Zn in the nutrient solution lowered the exudate concentrations of Fe and NH_4^+ at each sampling time, it had no significant effect on the influence of sampling time *per se*. Thus, there was no Zn-sampling time interaction for Fe and NH_4^+. This was also found for the remaining inorganic solutes and is illustrated by the nonsignificant results of the slope comparisons (Table I).

For tomato exudate at the normal-Zn treatment, Ca, Cu, Fe, Mg, Mn, and NH_4^+ exudate concentrations were all unaffected by increasing sampling time (Table II). Only PO_4^{3-} and Zn were significantly changed in successively later sampling times. At the high-Zn concentration, Ca, Cu, Fe, Mg, and Mn contents were unaffected, whereas NH_4^+ and PO_4^{3-} were significantly increased (Table II). High Zn decreased Ca, Cu, Fe, and Mg concentrations but increased PO_4^{3-}. However, as shown by the slope comparisons, only Cu levels were significantly affected by Zn.

Organic Acids. Exudate from four sampling times within each solution Zn concentration was analyzed for seven organic acids. For soybean exudate at both Zn concentrations, citric, maleic, malic, and malonic acids had the highest concentrations (Table III). Citric acid was always present in greater amounts than the other acids. All acid concentrations decreased with successive sampling times, until fumaric, maleic, malonic, succinic, and tartaric eventually were below detection limits (estimated confidence levels). Except for maleic, the acid concentrations were lower in the exudate from plants grown in the high-Zn solution than those grown in the normal-Zn solution.

Table I. *Effect of Solution Zn and Sampling Time on the Concentrations of Several Inorganic Components of Soybean Stem Exudate*
Each Value Is the Mean of Three Replications.

Element or Compound	Normal Zn Solution†				Test for§ Slope Parallelism	High Zn Solution†			
	Hours after severing stem					Hours after severing stem			
	0-1	2-3	4-5	6-7		0-1	2-3	4-5	6-7
	μM					μM			
$Ca^{(b,bz)}$¶	2,605	1,580	1,337	1,323	N.S.	2,789	2,291	1,732	1,476
$Cu^{(a,az)}$	5.0	3.9	1.5	1.2	N.S.	5.8	2.0	2.1	0.7
$Fe^{(ns, bz)}$	5.7	6.1	4.6	4.6	N.S.	3.9	2.6	1.9	1.4
$Mg^{(b,bz)}$	1,132	928	678	543	N.S.	1,276	808	714	726
$Mn^{(ns,nsz)}$	8.1	6.9	8.7	9.7	N.S.	8.3	7.8	7.2	6.9
$NH_4^{+(a,az)}$	706	689	513	456	N.S.	335	496	393	224
$PO_4^{3-(b,bz)}$	575	900	926	955	N.S.	471	707	908	1,070
$Zn^{(b,bz)}$	5.9	4.1	3.3	3.7	N.S.	28	25	19	15

† The normal-Zn concentration was 0.5 μM; the high-Zn concentrations were 3 μM during the first 12 days in the full nutrient solution and 8 μM until harvest (31 days old).

§ Within an element or compound, slopes for each solution Zn concentration are significantly different from each other at the .05(*) and .01(**) levels or not significantly different (N.S.), according to Student's t-test.

¶ Regression analysis within solution Zn concentrations: a = significant (.05), b = significant (.01), ns = not significant for normal solution Zn; az(.05), bz(.01), nsz (not significant) for high solution Zn. Although only 4 sampling times are listed for each Zn concentration, regression analysis was performed using 8 hourly sampling times for each Zn concentration.

Only citric, maleic, and malic acids were found in tomato xylem sap from normal-Zn plants (Table IV). In addition to these acids, fumaric acid was found in the exudate from high-Zn plants (Table IV). Maleic acid was the major acid in tomato-stem exudate at both Zn treatments. As observed for soybean, the acid concentrations in tomato decreased with increasing sample collection. However, unlike for soybean, tomato citric acid decreased below the detection limit at both Zn treatments.

Amino Acids. Exudate from four sampling times within each Zn treatment was analyzed by liquid chromatography for a number of amino acids. Twenty-two amino acids plus ethanolamine were identified. There were no unidentified ninhydrin-reactive peaks.

Asparagine was the major amino acid in soybean exudate (Table V). Its concentration was considerably higher than that of the other amino acids at both Zn treatments. In contrast to the organic acids, there was no consistent effect of sampling time on exudate amino acid concentrations. Some acids increased with time (*e.g.* isoleucine and valine), whereas others decreased (*e.g.* alanine and aspartic). There was a strong Zn effect on amino acid concentrations, but it was not uniform. For example, compounds, like β-alanine and ethanolamine, were not detected in the normal-Zn exudate but were present in the high-Zn exudate. Also, high Zn in the nutrient solution both increased and decreased individual exudate amino acids. Thus, acids, like alanine and γ-aminobutyric, were higher in the high-Zn exudate than in the normal-Zn exudate, whereas asparagine and glutamine were lower in the high-Zn exudate than in the normal-Zn exudate.

Glutamine was the major amino acid present in tomato exudate, followed by asparagine and γ-aminobutyric acid (Table VI). Except for ethanolamine at both solution Zn concentrations, and asparagine at the high-Zn concentration, all amino acid concentrations decreased with increasing sampling time. More acids decreased below detection limits in tomato exudate than in soybean exudate (Tables V and VI). Amino acid levels in the high-Zn exudate were generally lower than those in the normal-Zn exudate. In contrast with soybean exudate, tomato-stem exudate did not contain cysteine or methionine at either Zn treatment. β-

Table II. *Effect of Solution Zn and Sampling Time on the Concentrations of Several Inorganic Components of Tomato Stem Exudate*
Each Value Is the Mean of Three Replications

Element or Compound	Normal Zn Solution† Hours after severing stem 0-1	2-3	4-5	6-7	Test for Slope Parallelism§	High Zn Solution† Hours after severing stem 0-1	2-3	4-5	6-7
	μM					μM			
$Ca^{(ns,nsz)}$¶	2,644	2,183	2,352	2,305	N.S.	1,527	2,197	1,873	1,799
$Cu^{(ns,nsz)}$	5.1	5.4	6.8	7.0	*	2.3	2.2	1.4	0.6
$Fe^{(ns,nsz)}$	6.8	9.0	5.9	5.3	N.S.	4.5	2.7	2.9	2.8
$Mg^{(ns,nsz)}$	936	1,522	1,523	1,234	N.S.	578	621	667	648
$Mn^{(ns,nsz)}$	6.1	6.9	6.0	6.7	N.S.	5.0	5.6	5.3	5.0
$NH_4^{+(ns,az)}$	750	1,324	1,009	935	N.S.	794	917	1,097	1,161
$PO_4^{3-(b,bz)}$	160	105	194	295	N.S.	191	329	383	482
$Zn^{(a,nsz)}$	6.5	2.8	2.4	3.6	N.S.	77	84	74	74

† The normal-Zn concentration was 0.5 μM; the high-Zn concentrations were 30 μM during the first 12 days in the full nutrient solution and 80 μM until harvest (46 days old).

§ Within an element or compound, slopes for each solution Zn concentration are significantly different from each other at the .05 (*) and .01 (**) levels or not significantly different (N.S.), according to Student's t-test.

¶ Regression analysis within solution Zn concentrations: a = significant (.05), b = significant (.01), ns = not significant for normal solution Zn; az(.05); bz(.01), nsz (not significant) for high solution Zn. Although only 4 sampling times are listed for each Zn level, regression analysis was performed using 8 hourly sampling times for each Zn concentration.

alanine was not detected at the high-Zn treatment. However, ethanolamine was present in tomato exudate at the normal-Zn treatment, but it was not found in soybean exudate at this Zn treatment.

DISCUSSION

The results presented herein demonstrate the effects of exudate sampling time, plant species, and high Zn in the nutrient solution on the concentrations of solutes in stem exudates. It is clear that only exudate collected shortly after topping a plant provides information that approximates the *in vivo* system.

Metal solubility, dissociation constants, hydrolysis reactions, and metal binding in general, are all pH-dependent. The observed decrease in pH with increasing sample collection time could have an important effect on the equilibrium distribution of some metals in the exudate. For example, Cu is bound strongly by amino acids, especially at higher pH values. As the pH is lowered, the increased $[H^+]$ causes equilibrium shifts, so that Cu begins to associate preferentially with other ligands (*e.g.* organic acids). Therefore, both quantitative and qualitative descriptions of exudate equilibrium species require a pH value that reflects the H^+ concentration of the exudate in the intact plant. Previously reported values for tomato (5.4) and soybean (5.6) exudate (23, 25) represent values for samples collected for 10 continuous h.

Only values for the 0- to 1-h collections appear appropriate for estimating metal-complex equilibria in whole plants. It is clear from the results that both plant species and nutrient-solution Zn concentration can affect the solute concentrations. Values from the literature for many of these solutes are difficult to compare because they were determined under vastly different experimental conditions. Collection times, plant ages, and growth media are rarely the same.

For example, exudate P and Ca concentrations have been determined for several plant species, including tomato, and ranged from 120 to 5,000 μM P and from 1,000 to 7,000 μM Ca, depending on plant species, plant age, time of sampling, and nutrient P and Ca treatments (1, 8, 24). This sampling of P and Ca values illustrates the problem one faces when trying to estimate and compare published exudate concentrations. Accurate values for elements like P and Ca are particularly important for metal-complex equilibria. For example, Ca concentrations in exudate are usually hundreds of times greater than the micronutrients; Ca/Zn in the 0- to 1-h control exudate was 441 for soybean and 407

Table III. *Effect of Solution Zn and Sampling Time on Organic Acid Concentrations of Soybean Stem Exudate*

	Normal Zn Solution†				High Zn Solution†			
	Hours after severing stem				Hours after severing stem			
Acid	0-1	2-3	4-5	6-7	0-1	2-3	4-5	6-7
	μM				μM			
Citric	1,706±34§	1,300±6	458±46	88±3	1,104±22	399±12	96±8	80±4
Fumaric	66±8	36±2	<25	<25	58±11	<25	<25	<25
Maleic	188±36	120±18	43±9	62±28	613±68	96±31	78±26	<40
Malic	890±64	689±32	377±35	134±33	502±19	158±52	55±14	43±4
Malonic	264±52	60±3	<35	<35	126±21	49±2	<35	<35
Succinic	42±6	<25	<25	<25	34±2	<25	<25	<25
Tartaric	40±3	32±2	<15	<15	24±2	<15	<15	<15

† The normal-Zn concentration was 0.5 μM; the high Zn concentrations were 3 μM during the first 12 days in the full nutrient solution and 8 μM until harvest (31 days old).

§ Mean ± standard error for two replications; all values have been rounded to the nearest μM.

Table IV. *Effect of Solution Zn and Sampling Time on Organic Acid Concentrations of Tomato Stem Exudate*

	Normal Zn Solution†				High Zn Solution†			
	Hours after severing stem				Hours after severing stem			
Acid	0-1	2-3	4-5	6-7	0-1	2-3	4-5	6-7
	μM				μM			
Citric	301±29§	80±18	<40	<40	328±34	<40	<40	<40
Fumaric	<25	<25	<25	<25	76±6	<25	<25	<25
Maleic	1,206±52	587±55	302±34	182±5	1,226±32	605±209	185±2	120±30
Malic	628±86	264±46	87±4	62±1	817±56	553±60	206±8	72±14
Malonic	<35	<35	<35	<35	<35	<35	<35	<35
Succinic	<25	<25	<25	<25	<25	<25	<25	<25
Tartaric	<15	<15	<15	<15	<15	<15	<15	<15

† The normal-Zn concentration was 0.5 μM; the high-Zn concentrations were 30 μM during the first 12 days in the full nutrient solution and 80 μM until harvest (46 days old).

§ Mean ± standard error for two replications; all values have been rounded to the nearest μM.

Table V. *Effect of Solution Zn and Sampling Time on Amino Acid Concentrations of Soybean Stem Exudate*

	Normal Zn Solution[†]				High Zn Solution[†]			
	Hours after severing stem				Hours after severing stem			
Amino acid	0-1	2-3	4-5	6-7	0-1	2-3	4-5	6-7
	μM				μM			
Alanine	49±7[§]	20±1	6	3±1	72±0	36±4	2	-[¶]
β-Alanine	-	-	-	-	18±1	4±2	-	-
α-Aminobutyric	-	-	-	-	4±1	2±1	-	-
γ-Aminobutyric	109±15	46±6	13	20±16	272±2	146±15	89	11±4
Arginine	30±4	17±1	10	8±1	22±3	10±2	4	4±1
Asparagine	2,583±327	2,320±6	1,721	1,044±201	917±37	738±57	632	651±80
Aspartic	159±80	96±15	20	5±1	154±22	107±9	63	42±3
Cysteine	2±0	1±0	-	-	8±1	4±1	-	-
Ethanolamine	-	-	-	-	34±2	22±1	17	14±6
Glutamic	18±2	5±1	2	-	6±1	-	-	-
Glutamine	132±41	56±4	8	22±4	46±2	32±1	24	16±5
Glycine	4±1	2±1	-	-	15±2	8±2	-	-
Histidine	62±1	50±2	29	26±5	42±5	34±1	27	26±4
Isoleucine	24±3	42±7	62	89±0	22±3	34±3	48	62±8
Leucine	28±1	42±5	68	64±2	34±9	33±4	39	42±5
Lysine	86±20	70±2	31	12±2	59±4	39±2	12	12±6
Methionine	12±3	12±1	6	4±1	8±1	6±1	8	2±0
Phenylalanine	10±3	18±1	31	38±0	20±4	18±4	18	29±4
Proline	20±4	5±2	1	-	40±9	15±3	6	-
Serine	29±4	15±3	4	2±0	49±6	20±4	8	-
Threonine	42±2	33±1	27	22±1	28±3	23±0	17	14±6
Tyrosine	7±2	17±1	19	24±2	8±3	12±1	9	13±5
Valine	78±10	84±2	103	135±4	57±4	70±6	86	110±2

[†] The normal-Zn concentration was 0.5 μM; the high-Zn concentrations were 3 μM during the first 12 days in the full nutrient solution and 8 μM until harvest (31 days old).

[§] Mean ± standard error for two replications (4-5 hr collection is a single replicate); all values have been rounded to the nearest μM.

[¶] - = Not detectable.

Table VI. *Effect of Solution Zn and Sampling Time on Amino Acid Concentrations of Tomato Stem Exudate*

	Normal Zn Solution†				High Zn Solution†			
	Hours after severing stem				Hours after severing stem			
Amino Acid	0-1	2-3	4-5	6-7	0-1	2-3	4-5	6-7
	μM				μM			
Alanine	22±5§	13±1	-¶	-	24±8	-	-	-
β-Alanine	-	-	-	-	-	-	-	-
α-Aminobutyric	-	-	-	-	2±1	1±0	-	-
γ-Aminobutyric	169±38	100±13	20	12±2	190±24	41±7	13	-
Arginine	10±0	10±1	8	8±2	4±2	-	-	-
Asparagine	242±57	178±11	114	121±16	114±10	132±4	141	163±13
Aspartic	76±19	10±2	2	2±1	75±23	52±10	8	2±1
Cysteine	-	-	-	-	-	-	-	-
Ethanolamine	28±6	26±6	34	46±0	12±3	15±3	21	24±4
Glutamic	51±3	-	-	-	38±4	18±3	3	-
Glutamine	522±90	365±55	290	281±25	353±23	300±10	276	246±73
Glysine	4±1	-	-	-	10±1	-	-	-
Histidine	18±3	12±2	7	12±5	8±2	8±1	6	6±2
Isoleucine	24±6	16±1	11	8±2	20±4	20±1	16	18±6
Leucine	24±7	14±2	8	12±4	22±2	19±0	12	6±3
Lysine	62±13	44±4	27	38±6	16±2	20±2	12	8±1
Methionine	-	-	-	-	-	-	-	-
Phenylalanine	14±1	4±1	1	1±0	12±1	2±1	-	-
Proline	8±3	-	-	-	2±1	-	-	-
Serine	23±9	7±1	-	-	30±2	7±1	-	-
Threonine	34±4	20±2	4	4±2	25±3	4±2	4	6±2
Tyrosine	2±0	-	-	-	6±0	-	-	-
Valine	45±5	33±1	27	20±4	37±5	38±1	27	20±2

† The normal-Zn concentration was 0.5 μM; the high-Zn concentrations were 30 μM during the first 12 days in the full nutrient solution and 80 μM until harvest (46 days old).

§ Mean ± standard error for two replications (4-5 hr collection is a single replicate); all values have been rounded to the nearest μM.

¶ - = Not detectable.

for tomato. Tiffin (23) has discussed the ability of Ca at high concentrations to prevent micronutrient cations from forming metal complexes in exudate. Additionally, he has shown how high P levels can interfere with Fe-citrate binding (24).

The number of organic acids and their concentrations reported for xylem sap vary, depending on plant species and experimental conditions (3, 8, 14). The organic acids present in the greatest concentrations in xylem exudate are generally citric and malic. These acids are two of the three major organic acids found here. Both acids can bind metals, with citric forming more stable complexes than malic. Thus, an accurate determination or knowledge of their concentrations is important for estimating metal distributions among ligands in xylem sap.

An acid that appears to be maleic was found to be present in fairly high concentrations. Maleic acid's stability constants (β_1) with Cd (2.2) and Cu (3.4) (19) indicates that maleic acid could compete with other ligands for the heavy-metal micronutrient cations. Several comprehensive reviews of organic acids in plants have not listed maleic acid among those commonly found (5, 17), so its function and importance in plants are unknown.

Amino acids in exudate showed variations that were similar to those of the inorganic solutes and organic acids. The major amino acids in exudate are usually species dependent (14) and their concentrations are influenced by many factors (14). Aspartic and glutamic acids, and particularly the amides asparagine and glutamine, seem to be consistently present in high amounts (15, 21). This is reasonable since universally common pathways exist for N movement after initial reduction into compounds like α-ketoglutaric acid to form glutamic acid and like oxaloacetic acid to form aspartic acid. Subsequent amide biosynthesis occurs to form glutamine and asparagine. The other amino acid found in high concentrations here, γ-aminobutyric acid, is readily formed by α-decarboxylation of glutamic acid (4, 9); glutamic decarboxylase activity has been demonstrated in a number of plants (4, 9). Because glutamine and asparagine can reach fairly high concentrations, their role in binding metals such as Cu in exudate is important. The major amino acids (>100 μM) in both soybean and tomato exudate were γ-aminobutyric, asparagine, aspartic, and glutamine. The remaining acids were all less than 100 μM. There were no unusual acids found; all were either common protein or plant amino acids. Streeter (22) recently reported that the major N forms in exudate from field-grown soybean are allantoin and allantoic acid, and not asparagine. It should be noted that this ureide N predominates only after flowering in nodulated plants; asparagine is greater during early growth.

The chemical characterization of xylem fluid reported here should provide an adequate data base for further work on metal-complex equilibria in xylem fluid. In addition, the caution which should be used in evaluating exudate solute concentrations is illustrated by the variation caused by factors such as plant species and time of sample collection.

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