Plant Physiol. (1988) 86, 734–739
0032-0889/88/86/0734/06/$01.00/0

Organic Constituents and Complexation of Nickel(II), Iron(III), Cadmium(II), and Plutonium(IV) in Soybean Xylem Exudates[1]

Received for publication March 23, 1987 and in revised form November 3, 1987

DOMINIC A. CATALDO*, KRISTIN M. MCFADDEN, THOMAS R. GARLAND, AND RAYMOND E. WILDUNG
Environmental Sciences Department, Pacific Northwest Laboratory, P.O. Box 999, Richland, Washington 99352

ABSTRACT

The xylem exudates of soybean (*Glycine max* cv Williams), provided with fixed N, were characterized as to their organic constituents and *in vivo* and *in vitro* complexation of plutonium, iron, cadmium, and nickel. Ion exchange fractionation of whole exudates into their compound classes (organic acid, neutral, amino acid, and polyphosphate), followed by thin-layer electrophoresis, permitted evaluation of the types of ligands which stabilize each element. The polyvalent elements plutonium(IV) and iron(III) are found primarily as organic acid complexes, while the divalent elements nickel(II) and cadmium(II) are associated primarily with components of the amino acid/peptide fraction. For plutonium and cadmium, it was not possible to fully duplicate complexes formed *in vivo* by back reaction with whole exudates or individual class fractions, indicating the possible importance of plant induction processes, reaction kinetics, and/or the formation of mixed ligand complexes. The number and distribution of specific iron- and nickel-containing complexes varies with plant age and appears to be related to the relative concentration of organic acids and amino acids/peptides being produced and transported in the xylem as the plant matures.

Terrestrial plants, via food webs, represent an important link between their environment and man. As such, plants can represent a significant source of potentially toxic elements. While a substantial effort has been expended over the past 40 years to evaluate and quantify the transfer of toxic elements from soils to plants, comparatively little research has been conducted on the physiological controls which influence uptake, transport, and most important, the chemical form of elements in plants (3). The chemical form of elements will not only affect their chemical and physiological behavior once they are accumulated by plants, but also their solubility and ability to be bioconcentrated along food chains.

Once an essential element is absorbed into root cells, mechanisms must be present to prevent sorption, hydrolysis, or nonspecific chemical reaction between the myriad of trace and macroions being stored, transported, or metabolized, thus maintaining their solubility and availability for use in mineral nutrition. Similar processes must affect pollutant elements as well. For cations, this problem is most likely avoided by organic complexation. A substantial body of qualitative information indicates that the nutrient ions Ca, Co, Fe, Mn, and Zn exist in organically complexed forms in xylem exudates (1, 12), as do Mn and Zn in phloem exudates (15). Recently, xylem exudates have received increased attention in studies on the role of organic complexation in chemical stabilization and subsequent availability to animals of potentially toxic elements (3, 19). The importance of complexation in plant transport process became known with the work of Tiffin (13) on Fe complexes in exudates. However, more recent studies have addressed the behavior of pollutant elements, such as Pu, Ni, and Cd, in plant exudates (4, 6, 10), and the composition and role of xylem exudate constituents in cation complexation (17–19). These studies have shown that a wide range of cations, even the most insoluble polyvalent element species such as Pu and Fe, are soluble and mobile in plant transport fluids, primarily as organically complexed species. Also, many nutrient and nonnutrient cations extracted from plant tissues (leaves, roots, and seeds) are soluble and associated with organic ligands of varying mol w (2, 5–7, 9, 10–12, 14, 16). This cation mobility and solubility would suggest that plant-produced organic ligands are important in the transfer of potentially toxic elements from plants to consuming animals.

The objectives of this study were to characterize the major organic constituents of soybean xylem exudates, and to evaluate the *in vivo* and *in vitro* interactions and complexation of plutonium, iron, nickel, and cadmium with these constituents.

METHODS AND MATERIALS

Plant Culture. Soybean plants (*Glycine max* cv Williams) were grown from seed and maintained in 600-ml beakers containing 500-ml of aerated nutrient solution. The nutrient solution contained 150 mg of KCl, 120 mg of $MgSO_4$, 946 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 68 mg of KH_2PO_4, 0.06 mg of $ZnSO_4 \cdot 7H_2O$, 0.69 mg of H_3BO_4, 0.017 mg of $CuCl_2 \cdot 2H_2O$, 0.024 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.022 mg of $MnCl_2 \cdot 4H_2O$, and 0.60 mg of Fe^{3+} (as FeEDDHA) per liter. The pH was adjusted to 5.8 and solutions changed 3 times a week. Plants were maintained in controlled-environment chambers with a 16/8 h light cycle (about 500 μE m^{-2} s^{-1}, PAR, at leaf surface), a day/night temperature of 26°C/22°C, and 50% RH.

Exudate Collection. Xylem exudates were collected from plants of various ages from 17 to 100 d old; flowering occurred at 58 to 62 d postgermination. Plant roots were thoroughly rinsed 12 h prior to use, and placed on fresh nutrient solutions without added Fe. Two h into the light period, fresh solutions were provided and exudate collection initiated 30-min later. Exudates were collected by carefully severing the plant stem below the cotyledonary node, washing the cut stem to remove cellular debris, and fitting the stem with a tight gum rubber bushing connected to a length of Teflon tube. Exudates were then collected in cooled (4°C) vials for various periods of time as noted.

[1] Supported by the United States Department of Energy under Contract DE-AC06-76RL0 1830 and the National Institute of Environmental Health Sciences under Contract 211B00844.

In vivo exudates were those collected following root absorption of radiotracers, with decapitation and collection initiated 30 min later. In *in vitro* exudates were untreated exudates collected and then amended with radionuclides, or class fractionated prior to amendment.

Radionuclide Amendments. *In vivo* exudates were collected from plants absorbing radionuclides from 0.5 mM $CaCl_2$ solutions (pH 5.8). The concentration of $^{238}Pu(IV)$ nitrate was 1 μM, while that of $^{59}Fe(III)$, $^{109}Cd(II)$, and $^{63}Ni(II)$, supplied in the chloride form, were 10 μM. The specific activity of these elements were 17.11, 12.7, 1500, and 10.1 μCi/μg, respectively; except for Pu, carrier was supplied. Solubility and valence were verified based on electrophoretic mobility.

The exudates were collected at 30-min intervals for 2 h; in all cases the highest activity/ml was in the fourth sampling, and this fraction was employed for electrophoretic separation. The concentrations of Pu, Fe, Cd, and Ni in the *in vivo* exudates, by radioanalysis, were 0.004, 0.22, 0.01, and 0.09 μM, respectively.

In vitro addition of radionuclides to whole exudates and class fractions involved addition of these aqueous components to vials containing the radionuclide in dry form to alleviate pH effects. The latter involved placing aliquots of soluble radionuclides ($Pu[NO_3]_4$, $FeCl_3$, $NiCl_2$, and $CdCl_2$) into vials, with an acidic matrix appropriate for maintaining valence, this was 0.01 N HCl for all elements except Pu which employed 2 N HNO_3. These were brought to dryness to remove excess acid, either whole exudates or class fractions were added to give the desired concentration, and then incubated for 2 h prior to further analyses. In no instance was pH depressed more than 0.2 units (Pu). The concentrations of $^{238}Pu(IV)$, $^{59}Fe(III)$, $^{109}Cd(II)$, and $^{63}Ni(II)$ in the *in vitro* amendments were 0.02, 0.25, 0.05, and 0.5 μM, respectively. These concentrations were selected *in vivo* exudate analyses, and activity requirements for detection following electrophoresis. Constant specific radioactivity was maintained for individual elements employed in concentration studies.

Chemical Characterization of Xylem Exudates. Whole exudates were fractionated into several compound classes (organic acids, amino acids, neutrals, polyphosphates) to evaluate both their composition and complexation potential. Exudate fractionation involving passing an aliquot (1–2 ml) of exudate through two small ion exchange columns (0.7 × 4 cm) containing first AG 50W-x12 to collect amino acids, and then AG 1-x8 (Bio-Rad Corp.) to collect carboxylic acids and sugar phosphates. These resins were in the hydrogen and formate forms, respectively (8). The neutral fraction, which was washed through both columns with 20 ml of water, contained mainly neutral carbohydrates. The basic fraction, consisting mainly of amino acids, was eluted from the cation resin with 20 to 30 ml 2 N NH_4OH. Under these conditions, metals are stripped from associated ligands and not eluted from the resin. The weakly acidic fraction, consisting of organic acids and sugar phosphates, was eluted from the anion resin with 20 to 30 ml 6 N formic acid; this was followed by elution with 2 N HCl to recover the strongly acidic fraction containing sugar diphosphates and other polyphosphates. Each of these fractions was freeze-dried to remove volatile eluents, and reconstituted to their original volumes with water (1–2 ml) for evaluation of complexation or for organic analysis. The HCl fraction was neutralized with NH_4OH following drying and then redried.

Only the organic acid and amino acid fractions were further characterized. Organic acids were separated by HPLC using a HPX-87H column (Bio-Rad Corp.) and run isocratically at 45°C, with 0.006 N H_2SO_4 as the mobile phase and a flow rate of 0.5 ml/min. Detection, identification, and quantitation of specific acids were based on UV absorbance at 210 nm and the refractive index, against standards. Amino acids were analyzed with a Glenco model MM amino acid analyzer operated in the physiological fluid mode, using lithium citrate Pico-Buffer System IV (Pierce Chemical Co.) and standard ninhydrin post-column derivatization. Identification and quantification were based on retention time, absorbance, and 440/570 nm absorbance ratios.

Electrophoresis was performed by spotting 10 to 20 μl of sample onto Brinkmann MN 300, 20 × 20 cm × 0.1 mm, cellulose plates (Brinkmann Instruments). Separations were performed with a 0.1 M HEPES buffer (pH 7.5); potential was held constant at 400 V for 30 min. Use of slightly basic pH, relative to the exudates, results in a disassociation of weaker complexes and subsequent sorption to the cellulose, thus permitting separation of only more stable complexes. Components were visualized by autoradiography.

The analysis of trace metals in selected exudates and fractions was performed on a Jarrell Ash model 975 inductively coupled plasma spectrometer. Detection limits are provided in Table III.

RESULTS AND DISCUSSION

Xylem Exudate Collection. Exudate collection was routinely initiated 2 h into the daily light cycle. This resulted in a consistent volume of exudate ranging from 0.25 to 0.5 ml/h for 25-d-old plants to 2 to 3 ml/h in 110-d-old soybean plants for the first 2-h collection period following decapitation. Exudation rates were generally reduced by 20 to 60% for the second 2-h collection period, while exudation was drastically reduced for the third 2-h collection period. Between 6 and 24 h postdecapitation, exudation resumed, with rates ranging from 0.1 to 0.5 ml/h.

The average pH of 0 to 2 and 2 to 4 h exudates decreased from 5.75 at 20 d, to 5.25 at 110 d. The pH of exudates collected between 6 and 24 h was reduced by approximately 0.5 pH units at each plant age. Changes in pH, particularly after 4 h, would indicate the composition of exudates is changing as shown by White *et al.* (17), and therefore may not be representative of the *in vivo* situation. Thus, all further characterizations were performed on 0 to 2 h exudates.

Organic Composition of Xylem Exudates. The relative distribution of organic carbon in exudates was determined by anion/cation fractionation into amino acid, organic acid, neutral/sugar, and polyphosphate fractions. This permitted an *in vitro* evaluation of the complexation capacity of these individual fractions and allowed detailed characterization of each fraction for individual ligands. Figure 1 shows the distribution of organic carbon between the various class fractions and their changes with plant age. Most of the organic carbon (OC) was associated with the amino acid fraction, which accounted for approximately 50% of the OC between 20 and 75 d (flowering occurred between 58 and 62 d). The fraction of OC as organic acids increased linearly from approximately 12% at 20 d to 60% at 95 d. The fraction of carbon associated with the sugars and polyphosphates remained relatively constant at 15 and <1%, respectively.

Further organic characterization was limited to the amino acid and organic acid fractions of exudates collected from 21- to 96-d-old plants. The organic acid fraction contained 8 identified components accounting for only 38% of the total OC in this fraction (Table I). An additional 13 components were resolved but not identified. Unknowns were checked against 39 known or reported plant acids with little success. It should be noted that other classes of plant metabolites may appear in this fraction. Reported concentrations of the unknowns are only relative and based on the extinction coefficient at 210 nm for citric acid and thus may not represent significant concentrations. The two major identified acids were citric acid, which ranged in concentration from 500 μM in exudates of 21-d-old plants to 200 μM in 44- to 96-d-old exudates, and malic acid, which increased from 150 to 2000 μM over the functional life of the plant. The concentrations of identified organic acids in these 2-h exudates are generally

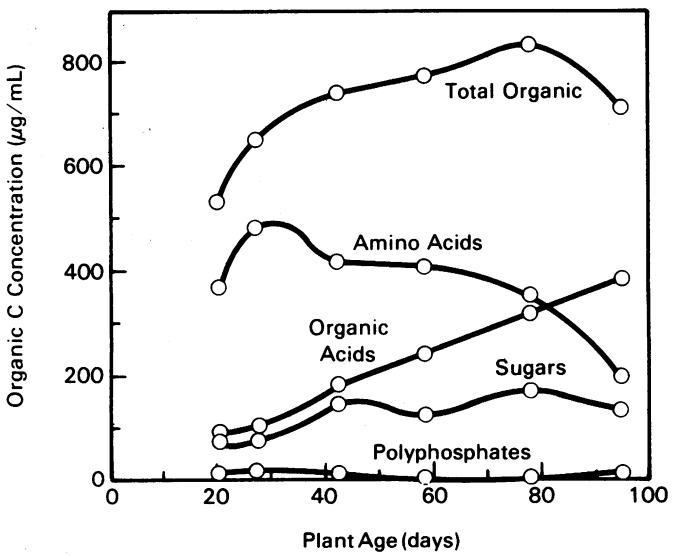

FIG. 1. Organic fraction composition of soybean xylem exudates as a function of plant age. Flowering occurred at 58 to 62 d.

consistent with those observed by White *et al.* (17) for 31-d-old soybean plants. The only exception was citric acid, which was found at levels one-third of that reported by White *et al.* (17).

Characterization of the amino acid fraction resulted in identification of 20 amino acid components, which accounted for 84% (by calculation) of the OC in this fraction (Table II). Asparagine and gluamine/glutamic acid accounted for 80% of the OC associated with all identified amino acid components in plants from 21 to 79 d old. Overall, the concentrations of individual amino acids remain relatively constant until maturity (96 d). Nitrogen containing compounds, such as allantoin, associated with nitrogen fixation were not found since nitrate was provided.

In addition to the identified amino acids, five major unidentified components were resolved. These had retention times of 27, 33, 54, 92, and 146 min. Hydrolysis of the amino acid fraction, in 6 N HCl prior to reanalysis, suggested that these unidentified components were small peptides.

Inorganic Composition of Xylem Exudates. Exudates were collected from 21- to 96-d-old plants and analyzed for their inorganic composition. Flowering and seed set occurred at approximately 60 d, with full pod maturity at approximately 110 d. However, due to reduced transpiration and root pressure at maturity, the last sampling period for these analyses was 96 d. The changes in elemental composition of soybean exudates with plant age are shown in Table III. The concentrations of the macroions Ca, Mg, K, N, and the trace ions Mn and Zn remain relatively constant with plant age, with some decline at 96 d. The concentrations of Na and P are variable, while the concentrations of S and Cu decrease with age, and those of Fe and Cl increase with plant age. Concentration of *in vivo* Ni and Cd, and of course Pu, are below detection limits.

It is important to note that each of these cations, particularly the di- and polyvalent species, may compete for exchange sites of organic ligands present in exudates.

Complexation of Trace Metals by Exudates and Their Class Fractions. Comparative studies of the complexation potential of whole exudates and their class fractions were performed. Evaluation of the *in vitro* capacity of soybean xylem exudates (75-d-old plants) to complex radionuclides, involved *in vitro* amendment procedures. The *in vitro* and *in vivo* complexation patterns for Pu^{4+}, Fe^{3+}, Ni^{2+}, and Cd^{2+} are shown in Figure 2.

Polyvalent plutonium (Pu^{4+}), in the absence of complexing ligands, is prone to rapid hydrolysis and remains at the origin (track 6). Root-absorbed Pu transported via the xylem (track 7, *in vivo* forms) is present as three anionic (components a, b, and c) and one cationic species (component d). Two of the anionic species (b and c) are formed *in vitro* on amendment of Pu to whole exudates. Amendment of Pu to the organic acid fraction of exudates (track 3) results in the formation of three anionic components with electrophoretic mobilities similar to those of

Table I. *Change in Organic Acid Composition of Soybean Xylem Exudate with Plant Age*

Organic Acid	Concentration at Plant Ages[a]					
	21 d	27 d	44 d	60 d	79 d	96 d
			μm			
UNK 14.35	301	300	150	177	136	176
Maleic	35	35	31	27	19	8
UNK 15.64	<20	<20	<20	50	150	180
Citric	509	309	196	167	234	192
UNK 17.95	213	307	548	927	1,210	936
UNK 18.97	3.1	4.5	12	28	32	19
Malonic	45	32	68	230	160	280
Gluconic	98	105	127	129	17	<9
Malic	175	120	590	1,080	1,440	2,260
Quinic	74	40	43	12	11	5
UNK 24.52	126	140	45	52	20	19
Fumaric	5.7	2.9	16	21	32	33
UNK 27.63	350	540	150	300	190	210
Me-Succinic	<40	<40	105	26	28	17
UNK 31.89	11	8.4	23	10	25	21
UNK 32.96	28	30	32	15	34	25
UNK 34.81	206	235	150	114	107	101
UNK 38.02	46	<2	<2	<2	2.3	<2
UNK 44.60	0.7	0.4	2.6	2.7	1.4	3.9
UNK 50.50	0.5	4	19	28	24	8
UNK 55.41	0.9	28	<1	<1	<1	<1

[a] Unknowns (UNK) detected are listed with their retention times, estimated concentrations based on extinction coefficient for citric acid.

Table II. *Change in Amino Acid Composition of Soybean Xylem Exudate with Plant Age*

Amino Acid[a]	Concentration at Plant Ages					
	21 d	27 d	44 d	60 d	79 d	96 d
				μm		
Asp	55	45	86	325	170	640
Thr	110	170	110	200	120	29
Ser	55	68	46	130	89	13
Asn	3,100	4,500	3,900	4,300	3,100	210
Gln	220	510	710	420	310	14
Pro	8.5	14	16	28	25	14
Gly	10	9	7	8	5	2
Ala	83	32	29	87	30	16
AABut	28	29	34	32	36	ND
Val	150	220	120	150	91	52
Met	16	34	21	33	8	3
Hyl	1.5	2.4	3.7	4.5	2.6	ND
Ile	48	84	46	58	30	25
Leu	54	100	55	69	26	13
Try	20	27	19	22	9	10
Phe	29	55	45	53	28	6
GABA	140	44	44	24	ND	15
Lys	79	110	56	65	40	28
Arg	65	110	77	100	79	35
His	130	180	120	130	100	16

[a] Abbreviations: ND, not detected; AABut, α-aminobutyric acid; GABA, γ-aminobutyric acid.

Table III. *Change in Elemental Composition of Soybean Xylem Exudate with Plant Age*

Element[a]	Concentration at Plant Ages (days) of					
	21	27	44	60	79	96
				μg/ml		
Ca	3,700	5,000	5,000	6,000	7,000	5,700
Mg	1,800	2,300	1,700	2,300	2,600	2,880
K	9,200	9,000	9,000	10,000	9,700	5,900
Na	650	200	480	350	390	570
NO_3^{-1}	11,100	10,300	12,700	12,400	14,800	9,000
SO_4^{-2}	940	1,000	720	580	560	300
$H_2PO_4^{-1}$	4,950	7,200	2,100	4,700	1,400	2,200
Cl	310	280	590	870	1,100	1,200
Cu	1.0	0.66	0.09	0.10	0.05	0.05
Fe	1.2	5.2	6.0	13	14	9.2
Mn	4.0	6.3	2.4	3.6	3.8	2.9
Zn	23	27	34	22	36	33

[a] Concentrations of Cd and Ni were below detection limits of 0.004 and 0.02 μg/ml, respectively.

the *in vivo* system. The formation of the cationic component is not observed in any of the *in vitro* amendments, and may represent a mixed ligand, possibly formed prior to loading into the xylem. The amino acid, neutral (sugar), and polyphosphate fractions appear to have no complexation capacity for Pu, with only hydrolyzed or neutral species of Pu appearing at the origin.

Iron (Fe^{3+}) has been shown to be transported in the xylem of a number of plant species as the citrate complex (12, 13). Iron is also prone to hydrolysis and remains at the origin in the absence of complexing ligands (track 6). Analysis of *in vivo* exudates show Fe^{3+} to be associated with three anionic components (track 7, components a, b, and c). In our electrophoresis system, Fe^{3+} amended to whole exudates is associated with four anionic components (track 1); three of the anionic species have electrophoretic mobilities similar to those found *in vivo*. Four of these (components a–d) consist of organic acid-containing ligands (track 3). Anionic component (d) in the whole exudate appears to contain an amino acid-containing ligand (track 2) and an organic acid-containing ligand (track 3), having similar electrophoretic mobilities. No complexation of Fe^{3+} is seen in either the polyphosphate or neutral sugar fractions.

Ni complexes, formed *in vivo*, include three anionic species (track 7, a, b, and c) and a single cationic component (f). In amended whole exudates (track 1), Ni is associated with three anionic (a, b, and c) and one cationic component (f). One of these anionic components (b) contains an amino acid ligand, and one (a) contains an organic acid ligand. Anionic component (c) found in amended whole exudates is not found in any of the class fractions, and occurs only on occasion in *in vivo* exudates of this age plant. The second *in vitro* anionic component (b) associated with whole exudates and the amino acid fraction (tracks 1 and 2), generally appears as the major Ni-containing component in *in vivo* exudates. The presence of anionic organic acid-containing component (a), observed in the *in vitro* whole exudates, is plant-age dependent and appears as the concentration of organic acids in exudates increases and the amino acids decrease (Fig. 1).

The cationic component (f) observed in whole exudates is seen in both the amino acid and organic acid fractions. However, the

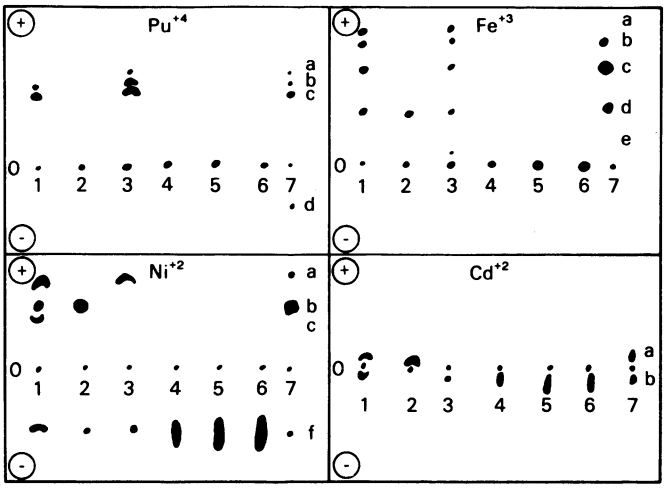

FIG. 2. Comparative electrophoretic behavior of Pu, Fe, Ni, and Cd in xylem exudates. Exudates from 75-d-old plants, incubated 2 h prior to analysis (0, origin; 1, whole exudate; 2, amino acid fraction; 3, organic acid fraction; 4, neutral fraction; 5, polyphosphate fraction; 6, inorganic metal; 7, *in vivo* forms).

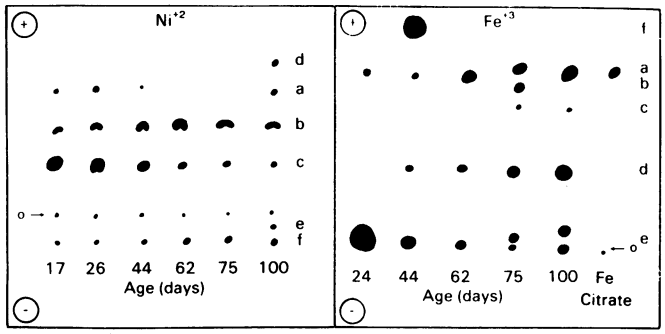

FIG. 3. Thin-layer electrophoretic behavior of *in vitro* nickel complexes in amended whole exudates and their organic class fractions. Numbers 1 through 4 denote amended Ni concentrations, 213, 54, 11, and 0.51 μM, respectively, with constant radioactivity. Nickel was visualized by autoradiography; intensity is directly related to Ni concentration.

Ni visualized in the organic acid fraction may be inorganic Ni. This was demonstrated in a separate set of studies where exudates and class fractions were amended with increasing concentrations of Ni (Fig. 3). At high Ni concentrations (200 μM), the cationic-amino acid component (f) predominates, whereas at low Ni concentrations (0.5 μM), the anionic component (b) predominates. The minor anionic component (a), believed to be an organic acid ligand, exhibits no change with Ni concentration and is readily saturated. The change in Ni distributions with concentration indicates that the major anionic component (b) has a high affinity for Ni, but is present in low concentration. The cationic component (f), while having a lower affinity for Ni at low concentrations than the anionic component (b), is not in limited supply and dominates at higher Ni concentrations. Based on the concentrations of Ni employed, it is believed that the amino acid-containing anionic component (b) functions within the normal physiological ranges encountered for Ni.

The behavior of Cd (Fig. 2) differs from that of the other three cations. When Cd is absorbed through the roots, it is found in *in vivo* exudates (track 7) as a single, slightly anionic species; the cationic component appears to be inorganic Cd. On amendment of Cd to whole exudates, one slightly anionic and one slightly cationic species are formed (track 1). However, the electrophoretic shape of the anionic component suggests that it is near neutral in charge, and physically displaced toward the anodic poly by other non-Cd-containing components in the sample, and is not the same component as formed *in vivo*. The components formed *in vitro* appear to be stable complexes of amino acid and organic acid constituents of the exudate (tracks 2 and 3). The evidence as such would indicate that either the *in vivo* anionic complex is formed in the root prior to xylem loading and is most likely a mixed ligand or the ligand is formed due the presence of Cd in the cells, since *in vivo* forms are not produced *in vitro* (5).

Influence of Plant Age on the Form of Ni and Fe in Xylem Exudates. It is quite clear from the data on *in vitro* whole and *in vivo* exudates that plants can effectively complex, and thus maintain the solubility, of a range of cations. However, it is also apparent that the organic composition of exudates changes with plant age (Fig. 1). This could affect the chemical forms of cations in exudates. To investigate this aspect of the complexation process, whole exudates were collected from soybean plants ranging from 17 to 100 d old. These were amended with either 0.05 μM ^{63}Ni or 0.25 μM ^{59}Fe, and changes in complexing components determined with plant age. The complexation patterns for Ni (Fig. 4) demonstrate the presence of the amino acid-containing complex (component b), previously observed, to be present at all ages. Component a is not evident in amended exudates beyond 44 d. Component c, which is not consistently detected in exudates more than 60 d old, tends to represent a major complexation form in younger exudates. In addition, several new components (d and e) are observed in exudates from 100-d-old plants.

The early work of Tiffin (12, 13) indicated that citric acid was the major and sole form of Fe in soybean exudates. However, because those studies were conducted with seedlings, it was possible that Fe speciation patterns would be more complex as the plant matures, as observed with Ni. When ^{59}Fe was amended to *in vitro* exudates, a variable pattern of Fe complexation occurred with plant age (Fig. 4). All components resolved were either anionic or neutral. Iron amended at 0.25 μM, in the presence of 100 mM citric acid, was found to exhibit a mobility similar to component a (Fig. 4), which was prevalent in all ages of exudate. Thus, while citric acid appears to be responsible for a portion of Fe complexation, the presence of multiple components (particularly evident with exudates of varying ages) indicates the involvement of other organic acids or mixed organic acid ligands.

CONCLUSIONS

This study describes the organic composition of soybean xylem exudates and postulates that organic complexation of reactive

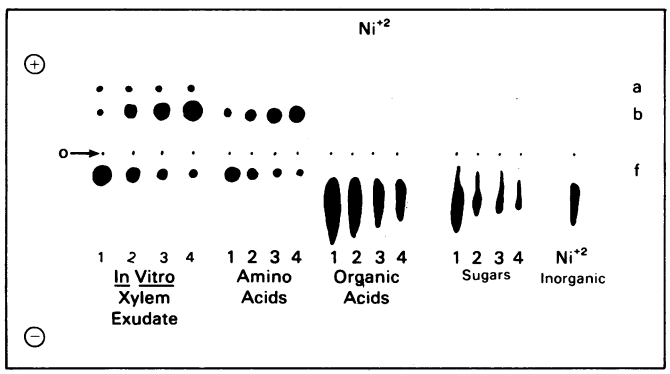

FIG. 4. Influence of plant age on the *in vitro* complexation of Fe and Ni in amended whole exudates of soybean.

and/or easily hydrolyzed elements is required for the transport of a complex mixture of inorganic elements from root to shoot. Each of the cations (Pu, Fe, Cd, and Ni) studied exist partially or totally as organic complexes in *in vivo* exudates. Many of these complexed forms can be formed *in vitro* using whole exudates amended with individual inorganic species. Based on class fractionation of whole exudates, Pu and Fe appear to be present primarily as organic acid complexes, and may represent a common method in plants for chemically stabilizing polyvalent cations in transit within the xylem. Nickel species were found to be associated with several ligands; these included organic acid and amino acid/peptide complexes. The behavior of Cd differed from that of the other divalent cations studied, in that the *in vivo* complexation pattern could not be formed *in vitro*. This would suggest that the transport form of Cd is either a mixed ligand, not readily formed *in vitro*, or that the complex is formed prior to loading into the xylem. It has been shown that the relative composition of exudates, with respect to the concentration of organic acids, amino acids, sugars, and inorganic ions, changes with plant age. Thus, it is not surprising that there is a quantitative and qualitative change in observed metal-ligand complexes as the plant matures and approaches senescence. The metal-ligand complexes resolved for Ni and Fe reflect the changing organic class fraction composition of exudates as the plant matures, and undoubtedly is affected both by the affinity and stability of transport ligands for the individual metals.

LITERATURE CITED

1. BRADFIELD EG 1976 Calcium complexes in the xylem sap of apple shoots. Plant Soil 44: 495–499
2. BREMNER I, AH KNIGHT 1970 The complexes of zinc, copper and manganese present in ryegrass. Br J Nutr 24: 279–289
3. CATALDO DA, RE WILDUNG 1983 The role of soil and plant processes in controlling trace element behavior and bioavailability to animals. Sci Total Environ 28: 159–156
4. CATALDO DA, TR GARLAND, RE WILDUNG 1983 Cadmium uptake kinetics in intact soybean plants. Plant Physiol 73: 844–848
5. CATALDO DA, TR GARLAND, RE WILDUNG 1981 Cadmium distribution and chemical fate in soybean plants. Plant Physiol 68: 835–839
6. CATALDO DA, TR GARLAND, RE WILDUNG, H DRUCKER 1978 Nickel in plants II. Distribution and chemical form in soybean plants. Plant Physiol 62: 566–570
7. CATALDO DA, RE WILDUNG, TR GARLAND 1978 Technetium accumulation, fate and behavior in plants. *In* DC Adriano, TL Brisbin, eds, Environmental Chemistry and Cycling Processes. DOE Symposium Series 45, NTIS, Technical Information Center, Springfield, VA, pp 537–549
8. CATALDO DA, GP BERLYN 1974 An evaluation of selected physical characteristics and the metabolism of enzymatically separated mesophyll cells and minor veins of tobacco. Am J Bot 61: 957–963
9. DABIN PE, JM MARAFANTE, JM MOUSNY, C MYTENAERE 1978 Absorption, distribution and binding of cadmium and zinc in irrigated rice plants. Plant Soil 50: 329–341
10. GARLAND TR, DA CATALDO, RE WILDUNG 1981 Absorption, transport and chemical fate of plutonium in soybean plants. J Agric Food Chem 29: 915–920
11. HALSTEAD RL, BJ FINN, AJ MCLEAN 1969 Extractability of nickel added to the soil and its concentration in plants. Can J Soil Sci 49: 335–342
12. TIFFIN LO 1977 The form and distribution of metals in plants: An overview. *In* H Drucker, RE Wildung, eds, Biological Implications of Metals in the Environment, Technical Information Center, Springfield, VA, pp 315–334
13. TIFFIN LO 1970 Translocation of iron citrate and phosphorus in xylem exudate of soybean. Plant Physiol 45: 280–283
14. TIMPERLEY MH, RR BROOKS, PJ PETERSON 1973 The distribution of nickel, copper, zinc and iron in tree leaves. J Exp Bot 24: 889–895
15. VAN GOOR BJ, D WIERSMA 1976 Chemical forms of manganese and zinc in phloem exudate. Physiol Plant 36: 213–216
16. WEIGEL HJ, HJ JAGER 1980 Subcellular distribution and chemical form of cadmium in bean plants. Plant Physiol 65: 480–482
17. WHITE MC, AM DECKER, RL CHANEY 1981 Metal complexation in xylem fluid I. Chemical composition of tomato and soybean stem exudate. Plant Physiol 67: 292–300
18. WHITE MC, FD BAKER, RL CHANEY, AM DECKER 1981 Metal complexation in xylem fluid II. Theoretical equilibrium model and computational computer program. Plant Physiol 67: 301–310
19. WHITE MC, RL CHANEY, AM DECKER 1981 Metal complexation in xylem fluid III. Electrophoretic evidence. Plant Physiol 67: 311–315