APPLICATIONS OF ION CHROMATOGRAPHY TO STUDY POLLUTION EFFECTS ON FOREST TREES

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ABSTRACT

Air pollution and acidic deposition can influence forest tree growth and survival by causing ionic imbalances in the rooting zone. Altered nutrient status suppresses tree growth and weakens its immune system. Internal infections spread more quickly in response to weakened tree defenses. As adverse conditions persist, many trees die and the survivors are less healthy. Changes in concentrations and composition of ions within tree tissues are useful indicators of changes in vital processes affecting tree growth and survival. Ion chromatography is a useful method for measuring the concentration and composition of ions in the tissue extracts. Samples of tree tissues were extracted and the extracts analyzed for Mg^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Al^{3+}, Cl^{-}, NO_3^{-}, SO_4^{2-}, PO_4^{3-}, acetate, formate, oxalate, and malate by various methods. Results of extract analysis confirm the usefulness of ion chromatography in studies of induced nutrient deficiencies in fine-root tip tissue, of altered uptake of exchangeable cations over time in dated wood, and of immune response in stemwood.

INTRODUCTION

Changes in the concentration and composition of ions within tree tissues are potentially useful indicators of changes in vital processes affecting tree growth and survival. Ion chromatography (IC) has been used to determine the ratio of aluminum to calcium ions in fine-root tip tissue from which an induced calcium deficiency syndrome has been inferred.[1,2,3] Changes in tree nutrition during decades of increasing acid deposition are described from the analysis of base metal cations present in the annual growth rings.[4,5] IC could be applied to such studies, if a rapid, effective means of extracting exchangeable cations can be developed for stemwood. Internal transformation of stemwood caused by maturation, infection, and defense processes has also been studied by IC.[6,7]

The objective of this paper is to show how IC may be applied to analysis of extracts from stemwood of red spruce trees (*Picea rubens Sarg.*), an important forest tree species of the eastern United States. Widespread growth declines of red spruce may be linked to acid deposition, an undesirable by-product of twentieth century economy. Results of red spruce tissue analysis are compared to those of red maple (*Acer rubrum L.*) and balsam fir (*Abies balsamea L.*).

EXPERIMENTAL

Samples of red spruce stemwood were taken from sixteen mature red spruce trees collected from eight forest sites across northern New England in 1985 as part of a fine-root study.[1] Samples were collected in pairs to represent trees with high vigor and those with low vigor within the site.[2] Tissue samples of outer live sapwood and the outermost portion of the non-living heartwood core were taken from air-dried disks (5 cm thick) cut from the stem at 1.4 m above ground. The wood was ground to pass a 420 μm sieve. Inorganic and organic anions were extracted by autoclaving 1.0 g tissue in 50 mL H_20 for 30 min. as described for balsam fir.[7]

Anion analysis of extracts was performed on a Dionex 2000i Ion Chromatograph (Dionex Corp., Sunnyvale, CA). Acetate, formate, malate, and sulfate were determined with a Dionex AS6 column with an eluent of 20 mM Na_2CO_3 and 2 mM NaOH at 1.5 mL/min. The background conductivity was suppressed with 25 mN H_2SO_4 at a flow rate of 6 mL/min. Chloride, nitrate, phosphate, and oxalate were determined with a Dionex AS4A column and an eluent of 0.75 mM $NaHCO_3$ and 2.2 mM Na_2CO_3 at a flow rate of 2.0 mL/min. Background conductivity was suppressed with 25 mN H_2SO_4 at a flow rate of 3 mL/min. Determinations for each tissue were done in duplicate on duplicate samples, and the results are reported as μmol anion/g oven-dried wood.

The cations, K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+}, were determined with atomic absorption spectroscopy of either 0.5 g samples of oven-dried wood digested in hot acid (70% perchloric acid: nitric acid, 2:3) or 2 g samples of oven-dried wood boiled in 50 mL of 0.01 M HCl under reflux.[7] Estimates of Mn^{2+} derived from these two extraction methods were compared directly on eight samples varying from 20 to 250 μg Mn/g oven-dried wood. Extracts in 0.01 N HCl were also analyzed for metal cations on a Dionex 2000i Ion Chromatograph. Mn^{2+} was determined along with other divalent metals (Fe^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}) using a Dionex CS5 column eluted with a mixture of 3 mM pyridine dicarboxylic acid, 4.3 mM LiOH, 2 mM Na_2SO_4, and 25 mM NaCl at a flow rate of 1.0 mL/min., by measuring absorbance at 520 nm after post-column reaction with complex PAR (500 mM NH_4OH + 90 mM Na_2CO_3 + 120 mM $NaHCO_3$ + 0.2 mM 4-(2-pyridylazo) resorcinol). Ca^{2+} and Al^{3+} content in these extracts were also determined with Dionex CS3 and CS2 columns respectively.[2]

Major cations (K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+}) from red spruce wood and maple wood were extracted from triplicate 0.5 g ground samples of sapwood to compare the effect of a range of organic and aqueous solvents on ion solubilities. Cations were determined before and after extraction by atomic absorption spectroscopy. Percent solubility was calculated as:

$$\% \text{ solubility} = \frac{\text{total concentration - insoluble concentration}}{\text{total concentration}} \text{x}100$$

Hexane, ethylacetate, n-butanol, acetone, methanol, distilled-deionized water, 0.01 N HCl, and 0.1 M NaCl were used as solvents. Samples of wood (2 g) were refluxed for one hour with constant stirring in boiling solvent, filtered on fritted glass crucibles, and oven-dried before digestion and analysis.

Two-way analysis of variance was conducted with SYSTAT between each of the twelve major ions in sapwood and heartwood of red spruce. The two factors tested were the eight locations and two vigor classes at the 95% level of probability.

RESULTS AND DISCUSSION

Major cations in spruce sapwood were not soluble in organic solvents, except for the partial solubility of K^+ in methanol (Table 1). A small fraction of major cations was soluble in organic solvents in maple sapwood. K^+ was 85 to 90% water soluble, whereas the exchangeable divalent cations were only partially soluble in water. K^+ and the divalent cations were fully soluble in 0.01 N HCl and 0.1 M NaCl. Mean Mn^{2+} concentrations from digests of whole wood measured with atomic absorption spectroscopy and those from 0.01 N HCl extracts of the same wood samples measured with ion chromatography did not differ significantly.[7] The same was true for mean Ca^{2+} concentrations from digests and extracts of fine-root tip tissue.[2] Thus, direct extraction of ground wood with mildly acidic or salt solutions followed by ion chromatography was a useful alternative to strong acid digestion and atomic absorption as a means of measuring ion concentrations in tree tissues.

TABLE 1. Percent Solubility of Major Cations in Sapwood of Red Spruce and Red Maple.

Solvent	Species	Solubility, %[a]			
		K	Ca	Mg	Mn
Hexane	spruce	0	0	0	0
Ethylacetate	spruce	0	0	0	0
	maple	7	4	2	0
n-Butanol	spruce	0	0	0	0
Acetone	spruce	0	0	0	0
	maple	10	3	2	0
Methanol	spruce	40	0	0	0
Water	spruce	90	30	60	20
	maple	85	25	45	25
0.01 N HCl	spruce	100	100	100	100
	maple	100	100	100	100
0.1 M NaCl	spruce	100	100	100	100

[a] Percent solubility = (total conc. - insoluble conc.)/total conc. x 100.

Potassium was the second most abundant cation of spruce sapwood and was significantly less in heartwood than sapwood (Table 2). Potassium is partially removed from sapwood along with nitrogen and phosphorus during its transformation to heartwood during normal maturation processes.[8] A marked decrease of phosphate from sapwood to heartwood was observed in spruce. Other anions which also decreased significantly in spruce were formate and malate.

Calcium was the most abundant cation of spruce sapwood and was significantly greater in heartwood than in sapwood. The change in concentration is not due to heartwood formation, but to a period of enriched calcium uptake at the time of wood formation.[5] Magnesium and manganese concentrations were not altered by heartwood formation, but reflected the rate of incorporation into sapwood at the time of formation by the vascular cambium. Thus, the divalent cations provide a record of the base nutritional status of growing trees.[5]

This record can be disrupted by injury and infection of the living tree. In a preliminary study of experimentally wounded spruce, the Ca^{2+} concentration doubled in a band of protective tissue derived from sapwood (unpublished data). However, the same layer of tissue had a large increase in K^+ (10x), oxalate (5x), and Mg^{2+} (8x), phenols (3x) and a large decrease in H^+ (10x). Spruce wood from the same experiment in the early stages of a brown rot infection had a large increase in oxalate (10x) and H^+ (10x), a small increase in K^+ (2x), and no change in concentrations of Ca^{2+} and Mg^{2+}. Early white rot infection in fir was associated with large increases in malate (10x) and a doubling of K^+ which balanced the change of the added malate.[7] Increased soluble oxalate (17x) and K^+ (7x) concentrations in protective wood layers formed in wounded maple sapwood were observed along with large increases in insoluble Ca^{2+} and Mg^{2+} as in the core of spruce.[6] Thus, large changes in concentrations of cations and anions determined by ion chromatography can be used to study both infection and protective processes in live trees under varying conditions. Changes in total ion concentration (meq/g wood) can be detected in

TABLE 2. Concentrations of Total Cations and Water Soluble Anions in Sapwood and Heartwood of Red Spruce.

	Concentration, µmol/g wood[a]	
Ions	Sapwood	Heartwood
Cations		
K^+	11.8 ± 1.2	6.1 ± 1.7*
Ca^{2+}	15.3 ± 1.4	19.1 ± 2.2*
Mg^{2+}	2.9 ± 0.5	3.9 ± 1.3
Mn^{2+}	2.8 ± 0.9	4.3 ± 1.4
Organic anion		
acetate	10.5 ± 2.3	9.2 ± 1.3
formate	9.5 ± 1.1	6.2 ± 1.6*
oxalate	0.5 ± 0.1	0.4 ± 0.1
malate	0.5 ± 0.2	0.1 ± 0.1*
Inorganic anions		
Cl^-	3.1 ± 1.2	3.4 ± 1.3
NO_3^-	1.0 ± 0.5	1.1 ± 0.5
SO_4^{2-}	0.9 ± 0.4	1.0 ± 0.5
PO_4^{3-}	0.8 ± 0.1	0.1 ± 0.1*

[a] Mean concentration derived from 16 observations with 95% confidence limits.

* indicates that the mean heartwood concentrations differ significantly from that of sapwood ($P<0.05$).

water extracts of healthy and diseased wood with electrical resistance measurements,[9,10] and the ionic processes involved can be identified with IC of the extracts in 0.01N HCl.

Major cations and anions are highly conserved in the sapwood of spruce trees across a wide variety of sites. Two-way analysis of variance of the spruce from eight sites and two vigor classes indicates that only Mn^{2+}, SO_4^{2-} and formate varied significantly ($P<0.05$) among sites. Spruce heartwood varied among sites for all ions except K^+, Ca^{2+}, Mg^{2+}, and PO_4^{3-} ($P<0.05$) suggesting that heartwood transformation may be influenced by external factors. There were no significant differences among the major cations and anions for heartwood of vigorous and declining trees, but sapwood of declining trees had a significantly greater concentration of Mn^{2+} than more vigorous trees. Increased Mn^{2+} in low vigor trees at some sites may indicate the association of a more acidic root environment with spruce decline.[5]

Results of ion analysis indicated that the major positive and negative soluble ions in spruce stemwood are roughly in balance - sapwood cations/sapwood anions, 91%; heartwood cations/heartwood anions, 103% (Table 3). In maple sapwood a large excess of positive charge was observed - sapwood cations/sapwood anions, 168%.[6] This indicated that some major anions were not yet identified in maple, possibly carbonate or bicarbonate.

Results obtained to date on spruce, fir, and maple tissue indicate that ion chromatography is useful in the study of tree biology in relation to changing environment. Evidence of an aluminum-induced calcium deficiency syndrome in red spruce was discovered with ion chromatographic techniques applied to extracts of fine-root tissue.[1,2] The imbalance in aluminum to calcium ratio was caused by the acidic deposition in the forest soil. Radial trends in cations of tree ring tissue as indicators of the influence of acid deposition on forests can be done with mild extraction procedures followed by ion chromatography as an alternative to acid

TABLE 3. Calculated Concentration of Major Water Soluble Positive and Negative Ions from Spruce Sapwood and Heartwood.

	Concentration, meq/g wood[a]	
Ions	Sapwood	Heartwood
Positive		
H^+	3.2	4.0
K^+	10.6	5.5
Ca^{2+}	9.2	9.2
Mg^{2+}	3.4	3.7
Mn^{2+}	1.1	1.4
Total	27.5	23.8
Negative		
acetate	10.5	9.2
formate	9.5	6.2
oxalate	1.0	0.2
malate	1.0	0.2
Cl^-	3.1	3.4
NO_3^-	1.0	1.1
SO_4^{2-}	1.8	2.0
PO_4^{3-}	2.4	0.3
Total	30.3	23.2

[a] No. equivalent x mol soluble ion/g wood. Total cations (Table 2) converted to soluble cations in solution with anions (Table 2) by multiplying with percent water solubility for sapwood (Table 1). Heartwood divalent cations were estimated to be 20% less soluble than sapwood. H^+ concentration derived from extract pH.

digestion and spectroscopy procedures currently in use.[5] Detection or diagnosis of internal infections and of tree defense processes can be improved by combining electrical methods[9,10] with those of ion chromatography of tissue extracts.[6,7]

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