

CHEMICAL AND SPECTROSCOPIC ANALYSIS OF PHLOEM  
EXUDATE AND PARENCHYMA SAP FROM  
SEVERAL SPECIES OF PLANTS

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(WITH THREE FIGURES)

Introduction

A problem which has occupied the attention of botanists for many years is that of the transport of organic and inorganic substances in plants particularly with respect to the tissues active in translocation. Since there is still some doubt concerning the transport of inorganic constituents in the phloem, it would seem desirable in this connection to make an analysis of phloem exudate to see what materials may be present therein. The exudate used in these analyses is probably restricted to phloem tissues alone as methods of collection were used which have been employed by previous investigators (HARTIG, 12, MÜNCH, 20, and CRAFTS, 3). JAMES and BAKER (15), in working with maple claim that the sap exuded did not come from the phloem alone but also from the living cells particularly of the cambial zone. Their work, however, was done in England during the winter months when the cambial activity must have been slight. GROSSENBACHER (10), EAMES and MACDANIELS (7) and other investigators have abundant evidence to indicate that the maximum cambial activity occurs at the time of elongation of the shoots which is in the early part of the summer for the species included in this study. Hence the chance of exudation from the cambium along with the phloem exudation is slight, since the exudate for the present study was collected during the months of August and September. In this connection PRIESTLEY (23) reports that if the bark is stripped from the new wood in May or June and a cut made across the differentiating tissues of soft wood, a fine spray of liquid spurts several inches from the cut while a similar cut in hard wood results in a gentle oozing with no spray. These observations tend to support the idea that exudation is not limited to the phloem alone during the active growing season. They do not indicate, however, that the exudate later in the season should be from tissues other than the phloem.

PFEIFFER (22) has made determinations on the osmotic concentrations of sieve tube sap of *Quercus rubra* by a cryoscopic method and finds a range of values from 15.0 atm. in August to 21.1 atm. in November. MÜNCH (20) gives values for osmotic pressure of sieve tube sap of *Quercus rubra* varying from 20.9 atm. to 23.7 atm. and values from 25.8 atm. to 34.3 atm. for sieve tube sap of *Robinia pseudo-acacia*.

KRAUS (16) and ZACHARIAS (26) have made some qualitative tests on the

sieve tube sap of *Cucurbita pepo*. A quantitative analysis of phloem exudate has not been made, however, previous to this work probably because of the difficulties attendant upon collection of sufficient quantities of the exudate to permit an analysis by methods other than microchemical or spectroscopic. It has been the purpose of this work to analyze quantitatively the phloem exudate and parenchyma sap with respect to the following: pH, total sugars, reducing sugars, calcium, magnesium, potassium, lithium, nitrate nitrogen, and total nitrogen, and to make a qualitative spectroscopic analysis for any other inorganic elements that might be present in the sample. The species studied were *Fraxinus americana*, *Fraxinus pennsylvanica* var. *lanceolata*, *Platanus occidentalis*, *Robinia pseudo-acacia*, and *Cucurbita maxima*. Quantitative spectroscopic methods of analysis which will be described later were used wherever possible because of their efficacy in dealing with small amounts of material.

The method of collecting the phloem exudate in the woody plants is one that has been used by MÜNCH (20). A slit in the trunk about two inches long at an angle of about 45° with the horizontal made with a sharp knife was found to yield the best exudation. The cut was made into the active phloem tissues from which would exude amounts of sap varying from a few drops to 2 or 3 cc. depending upon the species and the length of the cut. As the drops of liquid appeared, usually near the base of the cut, they were picked up with a glass capillary tube fitted with a small rubber bulb and transferred immediately to hard glass phials which contained a few cc. of toluene as a preservative. Several cuts were made at a time and the amount usually collected after two or three hours was about 10 cc.

The time of day seemed to be a factor in governing the amount of flow, the largest quantities being obtained in the afternoon. Bright sunny days usually gave better results than cloudy cold ones, although on a few occasions the flow from *Robinia pseudo-acacia* was good on cloudy days. On days when the transpiration stream was rapid a cut deep enough to penetrate to the xylem tissue would result in the exudate being sucked into the transpiration stream often with an audible sucking sound.

HARTIG (12) and MÜNCH (20) report that a second cut within from one to five meters below, and for a shorter distance above, an original cut would yield no flow. However, in both species of *Fraxinus*, and in *Platanus occidentalis* the presence of a second cut either above or below the first cut did not appreciably affect the flow from the first cut unless the second cut was within 5 or 6 cm. on either side of the first. The flow from the second cut would then be as good as that from the first. The second cut would give a poor flow when too close to a cut above, and a second cut would diminish the flow from the first cut when too close above the first. A distance of 10 or 12 cm., however, seemed to make no difference except in *Robinia pseudo-acacia*

where a second cut would yield no flow unless displaced laterally with respect to the first. In this species a second cut even 1 or 2 meters directly above or below a first cut would yield no flow.

In the case of *Cucurbita maxima* the method of collection of phloem exudate was that used by CRAFTS (3). The plants were grown in the greenhouse, and when the fruits had developed to a length of about 8 or 10 cm. they were cut from the vine leaving about 2.5 cm. of peduncle attached to the fruit. A drop of crystal clear exudate coming from the phloem proper and from the ectocyclic and entocyclic sieve tubes which are in the position of a latex system would appear on this cut end of the peduncle. This was allowed to drop into a small porcelain crucible containing a few cc. of toluene. About two drops of exudate would be obtained before the flow stopped, but the flow could be started again by cutting a short section from the end of the peduncle. This process could be repeated until the peduncle had been cut back to the fruit and a total of about ten drops of exudate obtained. Fifteen or twenty fruits would give a sample sufficient for several of the analyses.

The parenchyma sap was obtained by freezing, at  $-15^{\circ}$  C., leaf tissue contained in large test tubes in an alcohol bath cooled by a G. E. cooling unit, and then pressing out the sap by means of an hydraulic press. Pressures of 10,000 pounds per square inch were used, and the tissue was subjected to this pressure for five minutes. Samples of the sap were kept in the refrigerator until ready for use.

#### Analytical methods

The pH values of all samples were obtained immediately after collection by use of the quinhydrone electrode method described in Leeds-Northrup Co., Bulletin no. 3.

The sugar analyses were made as soon after collection as convenient, in order to minimize the error due to chemical changes. All samples were shaken well with toluene and kept in the refrigerator until analysis was begun. The toluene prevented any changes due to the growth of organisms but had no effect on any enzymes which might have been present in the exudate. It is not certainly known that any enzymes were present. The modified SHAFER (24) method of sugar analysis was used since the colorimetric determination of the  $\text{Cu}_2\text{O}$  is very accurate when small quantities of material must be used.

The modification of the spectroscopic method for the determination of potassium as developed by HICKS (13) will be described in some detail. The absence of sensitive arc lines of potassium when small amounts of the element are involved prevents the use of the arc and spectrograph in quantitative analyses for this element; but there is a line in the flame spectrum of potassium (wave length 7669 Å.) which is extremely sensitive and which shows

up when the element is present in small quantities. The duration of this line when potassium is introduced into the flame is proportional within limits to the amount of the element present in the flame. The duration of the line is measured by direct visual observation, the observer starting a stop watch upon appearance of the line and stopping the watch upon the line's disappearance. The line flashes into view suddenly and fades out as rapidly so that the timing can be accurate to within 0.1 second. The spectrometer used was the ordinary three arm type described by BALY (1). The Bunsen flame of illuminating gas was standardized as far as possible as to position with respect to the slit of the instrument, height, and mixture. The substance was introduced into the flame in the following manner. A platinum wire was coiled into a small spiral of five turns, 2 mm. in diameter, in such a way that the turns of wire were not in contact in any part of the coil. This coil, once wound, was not disturbed in shape or size during the entire series of tests, and when it was dipped into a liquid it would always pick up and hold the same size drop thus assuring the same amount of a given concentration of material in the flame each time an observation was made. After the platinum wire was dipped into the solution it was held near another flame until the liquid had evaporated leaving only a solid incrustation on the wire which was then introduced into the flame and observed. This evaporation prevented loss of material due to spattering, which would result if a drop of liquid were suddenly thrust into the Bunsen flame.

Standard solutions of potassium chlorate<sup>1</sup> containing potassium in amounts of 0.32, 0.64, 0.96, 1.28 mg./cc. were made from recrystallized salt (Baker's analyzed chemicals). The duration of the 7669 Å. line was observed for each of these concentrations, the average time of ten observations in each case being taken as the final result.

TABLE I  
RELATION BETWEEN CONCENTRATION OF SOLUTION AND DURATION OF THE  
POTASSIUM LINE 7669 Å.

TIME	CONCENTRATION OF POTASSIUM
<i>sec.</i>	<i>mg/cc</i>
2.00	0.16
2.40	0.32
2.60	0.48
2.80	0.64
3.00	0.80
3.30	0.96
3.72	1.28

<sup>1</sup> It was later learned that the use of perchloric acid would have given more accurate results, with potassium chloride rather than potassium chlorate taken as standard.

Table I shows the results of such a series of observations and figure 1 shows the curve of concentration of solution plotted against duration of line.

It is necessary now to observe the duration of line 7669 Å. for the various samples of exudate and sap and from the observed times read from the curve the corresponding values of the concentration of potassium. Because the presence of sugars and other organic materials in the exudate prevented the

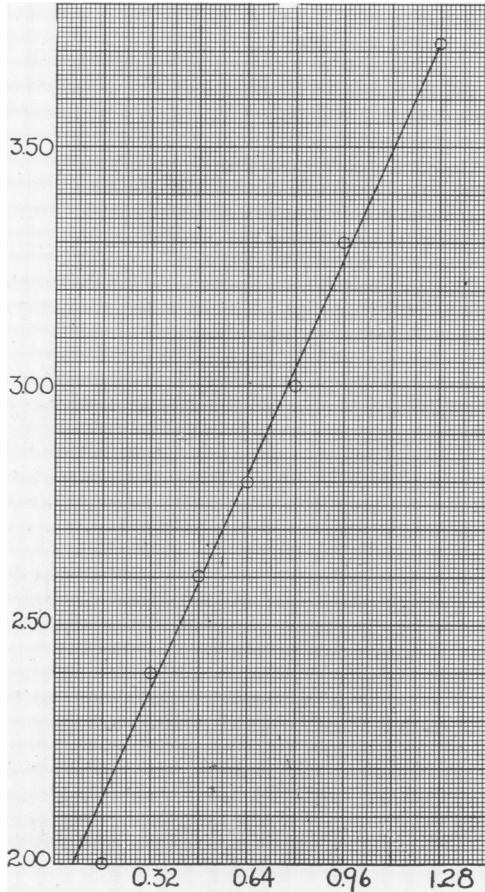


FIG. 1. Relation between concentration of solution and duration of potassium line 7669 Å.

use of the material directly, a known volume of the exudate was evaporated to dryness and ashed in a muffle furnace at a temperature not exceeding 450° C. The temperature was kept comparatively low in order to prevent any loss of the calcium, magnesium or potassium compounds during the ashing process. The ash was dissolved in hydrochloric acid<sup>2</sup> and brought

<sup>2</sup> See footnote p. 368.

back to the original volume with distilled water. This solution was then given five tests and the average time for the five taken as final. Since these values seldom varied by more than 0.2 sec., five observations were considered sufficient. The corresponding concentrations for each sample were recorded. During a series of observations on unknowns the system was checked from time to time by making observations on one or more known concentrations, and at the beginning of a new series of observations at a later date the system was brought up to the same standards or a new series of standards was set up.

The method of analysis for lithium was carried out in the same manner, the wave length of the line observed being 6709 Å. Curves of time *vs.* concentration were drawn and the concentrations read quantitatively wherever possible. Since the amounts of lithium were very minute in all species except *Platanus occidentalis* the analysis is really quantitative for that species only.

The spectroscopic method used for calcium and magnesium has been described by several workers, namely, LEWIS (18), GERLACH (9), SMITH (25), and HOAG (14). The spectrograph (Bausch and Lomb Optical Co., Rochester, N. Y.) was equipped with a quartz optical system of the very best type and a photographic plate holder for taking 4" × 10" spectrograms.

Electrodes of graphite (Acheson Graphite Co., Niagara Falls, N. Y.) were employed. These were arced for 30 seconds before using in order to burn out any impurities, particularly the calcium and magnesium present.

There are many lines present in the arc spectrum of calcium and magnesium when these elements are present in the arc in fairly large amounts. The intensity of these lines depends upon the length of exposure, the power used by the arc, the characteristics of the instrument, the characteristics of the plate, and the time of development. However, as the concentration of these elements in the arc is decreased, the number of lines visible when other factors are held as constant as possible decreases until only the so called "raies ultimes" or ultimate lines (DEGRAMONT, 5) are present. These ultimate lines become less and less intense as the concentration of the substance in the arc decreases, the less sensitive lines fading out completely and the more sensitive lines remaining as the dilution is carried still further. Finally a point is reached at which the last of the lines fail to show in the spectrogram, and the presence of the element can no longer be detected at this or lower concentrations.

In order to make this method quantitative it is necessary to make spectrograms of known concentrations of the element in question, of the same order of magnitude as in the unknown, and to standardize the intensities of certain lines with respect to concentration. Two methods of procedure may be followed in this respect. In the first, the intensities of the spectral lines are measured as a function of the density of the line on the photographic plate

by a MOLL (19) recording microphotometer. This instrument provides a beam of light supplied by a bulb maintained at constant voltage which is focused on the line on the plate. The light transmitted by the line passes through a slit and falls upon a thermopile which is connected to a sensitive galvanometer, the mirror of which throws a light beam on a scale. The

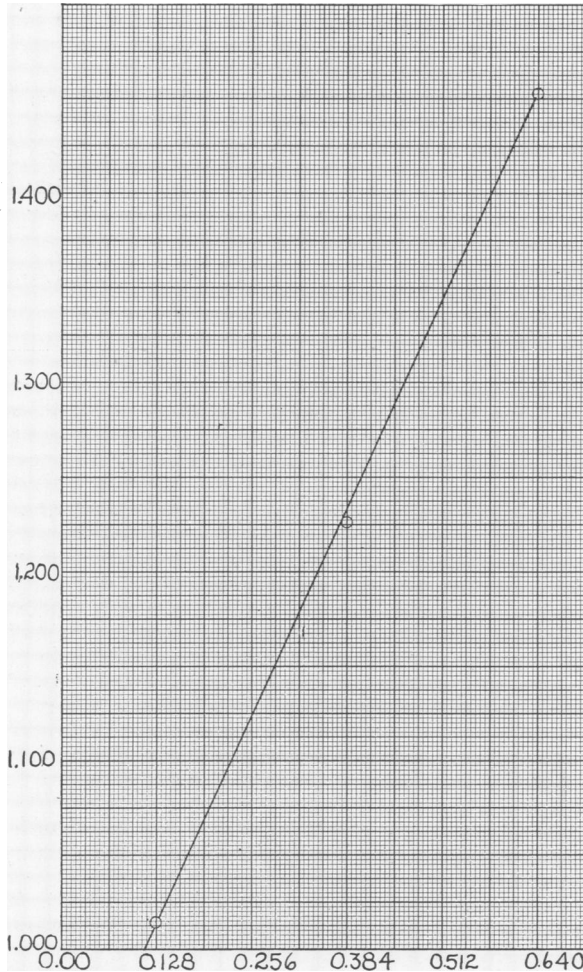


FIG. 2. Relation between concentration and density of magnesium line 2802 Å.

density of the line in question is taken by definition to be the  $\log_{10}$  of the ratio of the light incident upon the line to the light transmitted by the line. This ratio is obtained by taking a reading of the galvanometer with the light shining through the clear plate on either side of the line, which gives the maximum amount of light transmitted by the plate (M. T.). A reading is

taken with the light shut off to find the point of maximum blackness (M. B.). The plate is then moved into position so that the light shines directly through the line in question and the reading of the galvanometer is again taken (P.). The ratio is then expressed as  $\log_{10} \frac{M. T. - M. B.}{P. - M. B.}$ . Table II and figure 2 show the relation existing between concentration of element in the arc and density of the line produced.

TABLE II  
RELATION BETWEEN CONCENTRATION AND DENSITY OF MAGNESIUM LINE 2802 Å.

CONCENTRATION OF MAGNESIUM	DENSITY $\log_{10} \frac{M. T. - M. B.}{P. - M. B.}$
<i>mg/cc</i>	
0.128	1.0133
0.384	1.2263
0.640	1.4523

Duplicate spectrograms of three different known concentrations are made on each plate along with duplicate exposures of seven samples of the material to be analyzed. The curve is drawn and the unknown concentrations are read from it.

The second method of determining the concentration of the solution in the arc consists of observing visually the concentrations at which certain lines are just visible and making comparison between the known concentrations and the unknowns. This is a good way to check the photometer method, but is not reliable enough to be employed as the sole method except when used by one long accustomed to such work. In this work all plates were read on the microphotometer and checked by the second method as well.

The substance to be analyzed was introduced into the arc in the following manner. A drop (volume 0.02 cc.) of the solution of the ash was placed on the hollowed out top of the lower electrode while it was still warm from the current which had burned out the impurities. The drop evaporated leaving a solid incrustation on the electrode always of the same quantity of material provided the concentration was kept constant. The arc was struck. The exposures were ten seconds in length, and the current was maintained at ten amperes from a 110 volt. D. C. line. Fresh electrodes were then introduced into the arc and other exposures made.

In order to rule out as far as possible errors resulting from plate differences and development, a series of known concentrations were run for each plate of unknowns and the concentrations of the unknowns read from the curve drawn for each individual plate.



Table III and figure 3 show the relation between concentration and density of line for calcium (wave length 3968 Å.).

TABLE III  
RELATION BETWEEN CONCENTRATION AND DENSITY OF CALCIUM LINE 3968 Å

CONCENTRATION OF CALCIUM	DENSITY
	$\text{Log}_{10} \frac{\text{M. T.} - \text{M. B.}}{\text{P.} - \text{M. B.}}$
<i>mg/cc</i>	
0.18	1.0133
0.54	1.1139
0.90	1.2180

The accuracy of the spectroscopic method of analysis for calcium, magnesium, and potassium was checked by ordinary analytical methods recommended by "Official Methods" (1930). The spectroscopic analyses for calcium and magnesium were found to vary by not more than 5 per cent. and for potassium by not more than 8 to 10 per cent.

Because the elements boron, copper, manganese, and strontium did not show up on the plates when the solutions of the ash were arced at their natural concentrations samples of material were ashed and then made up to much smaller volumes of solution of ash which concentrated the material to such an extent that qualitative analyses could be made for any of the rarer constituents.

The Gunning method modified to include the nitrogen of nitrates (21) gave the results on total nitrogen, and the nitrate nitrogen determination was made by a modification by FREAR (8), for use with plant juice, of the phenol-disulphonic acid method of HARPER (11).

Identical methods were used for the treatment and analysis of the parenchyma sap which was collected during June of the following year. It was thought desirable to make these analyses on parenchyma sap in order to compare its composition with that of the phloem exudate from the identical trees.

### Results

Table IV shows the results of analyses of phloem exudate of seven trees of *F. americana*. It is noted that the pH value is considerably higher than the pH of the parenchyma sap as shown in table VII. Appreciable amounts of calcium, magnesium, and potassium are present in all cases while a trace of lithium is noted. The amount of reducing sugar is much smaller than sucrose except in no. 5, and the large quantities of sucrose present should be noted. Sufficient exudate was obtained in two cases only for the nitrogen determination. The amount of total nitrogen present is much greater than

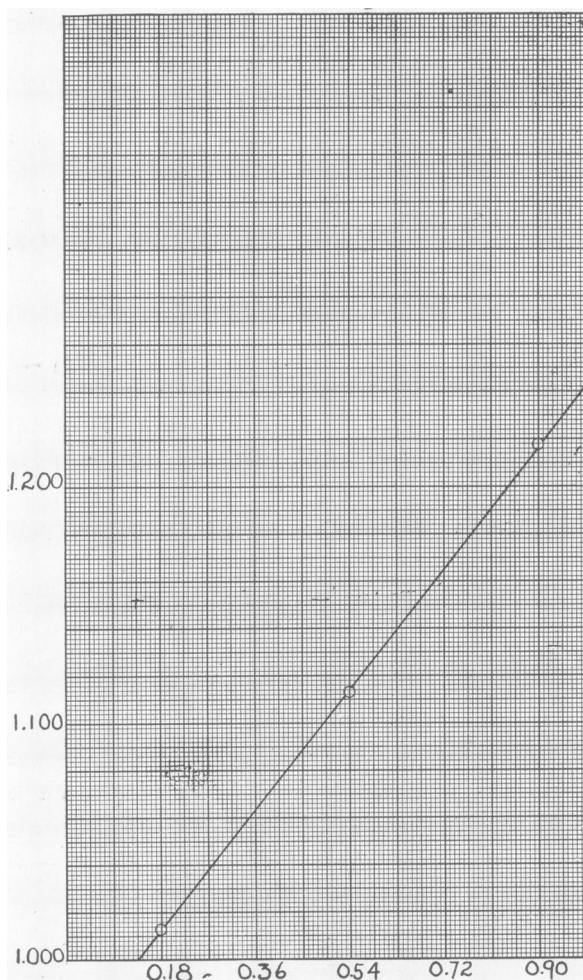


FIG. 3. Relation between concentration and density of calcium line 3968 Å.

the total nitrogen in the phloem exudate of any other species studied. Also there is an appreciable amount of nitrate nitrogen present.

Results in table V show that the phloem exudate of the two species included in it have rather high pH values, appreciable amounts of calcium, magnesium, and potassium, and a trace of lithium. The reducing sugars are consistently low, but the sucrose is exceptionally high, particularly in *Robinia pseudo-acacia*. Nitrate nitrogen also is present.

It is evident from table VI that the phloem exudate of *Platanus occidentalis* has larger amounts of lithium than any of the other species studied, and that the amount of reducing sugar is greatly increased in absolute

TABLE IV  
PHLOEM EXUDATE ANALYSIS OF *Fraxinus americana*

TREE NO.	DATE							
	SEPT. 2	SEPT. 20	SEPT. 10	AUG. 1	AUG. 1	AUG. 5	AUG. 15	AUG. 15
	1	2	3	4	5	6	7	Av.
pH	7.15	7.07	7.30	7.05	7.20	7.49	7.25	7.22
Ca mg/cc	1.18	1.09	1.04	0.55	0.76	0.86	0.34	0.83
Mg mg/cc	0.53	0.21	0.44	0.18	0.32	0.36	0.24	0.33
K mg/cc	0.99	0.45	0.64	1.20	1.02	0.58	1.12	0.86
Li mg/cc	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
REDUCING SUGARS mg/cc	11.12	Trace	0.84	0.22	30.18	2.64	1.98	7.83
SUCROSE mg/cc	72.64	70.54	77.40	102.18	65.46	93.71	86.45	81.20
TOTAL SUGARS mg/cc	83.76	70.55	78.24	102.4	98.64	96.35	88.43	88.34
NITRATE NITROGEN mg/cc		0.11	0.095					0.103
TOTAL NITROGEN mg/cc		2.45	3.64					3.05

TABLE V  
PHLOEM EXUDATE ANALYSIS

TREE NO.	<i>Fraxinus pennsylvanica</i> var. <i>lanceolata</i>		<i>Robinia pseudo-acacia</i>			
	DATE		DATE			
	SEPT. 1	AUG. 20	SEPT. 1	SEPT. 15	AUG. 5	AUG. 5
	1	2	1	2	3	4
pH	7.58	7.20	7.4	7.36	7.23	7.25
Ca mg/cc	1.04	1.33	0.90	0.44	0.72	0.72
Mg mg/cc	0.61	0.21	0.38	0.15	0.39	0.38
K mg/cc	0.37	0.58	1.14	1.21	0.90	0.95
Li mg/cc	Trace	Trace	Trace	Trace	Trace	Trace
REDUCING SUGARS mg/cc	3.41	3.81	2.08	4.78		0.51
SUCROSE mg/cc	107.99	118.09	177.82	147.94		199.94
TOTAL SUGARS mg/cc	111.4	121.9	179.9	152.72		200.45
NITRATE NITROGEN mg/cc	0.12					0.135
TOTAL NITROGEN mg/cc	0.49					0.56

TABLE VI  
PHLOEM EXUDATE ANALYSIS

TREE NO.	<i>Platanus occidentalis</i>				<i>Cucurbita maxima</i>	
	DATE				DATE	
	SEPT. 20	AUG. 30	AUG. 30	SEPT. 10	FEB.	FEB.
	1	2	3	4	1	2
pH	7.35	7.31	7.28	7.31	8.1	8.0
Ca mg/cc	1.26	1.26	0.76	0.84	0.18	0.07
Mg mg/cc	0.66	0.24	0.28	0.19	0.06	0.10
K mg/cc	0.96	0.80	0.90	0.90	0.24	0.20
Li mg/cc	0.08	0.04	0.08	0.158	None	None
REDUCING SUGARS mg/cc	86.4	67.3		56.5	4.83	2.07
SUCROSE mg/cc	1.90	40.3		48.4	2.83	3.33
TOTAL SUGARS mg/cc	88.30	107.6		104.9	7.66	5.4
NITRATE NITROGEN mg/cc	0.203					
TOTAL NITROGEN mg/cc	0.28					

amounts and also in ratio to the total sugars. This increase in reducing sugar may be due in part to the change of sucrose to reducing sugar during the interval between collection of the exudate and analysis. The use of toluene as a preservative, while preventing the growth of organisms in the exudate, would not hinder the action of any enzymes which might cause inversion of the sucrose. The high pH of *Cucurbita maxima*, and the lower concentrations of all its solutes are noted. Insufficient samples were obtained to allow for nitrogen determinations.

In order to make comparisons between phloem exudate and parenchyma sap from the same plants, similar analyses were carried out for the samples of parenchyma sap collected as described previously. Results of these analyses are included in table VII and show low pH values of parenchyma sap for all species studied with respect to the pH of the phloem exudate. In general the concentrations of calcium, magnesium, potassium, lithium, and total and nitrate nitrogen are about the same in parenchyma sap as in phloem exudate. Whether or not leaf sap and phloem exudate would have been more similar or more divergent if taken at the same time cannot be determined from these data. The amounts of reducing sugars are in excess of the sucrose in all cases while the total sugar concentration is much less than that of the phloem exudate.

TABLE VII  
PARENCHYMA SAP ANALYSIS

TREE NO.	<i>Fraxinus americana</i>		<i>Fraxinus pennsylvanica</i> var. <i>lanceolata</i>		<i>Robinia pseudo-acacia</i>	<i>Platanus occidentalis</i>	<i>Cucurbita maxima</i>
	DATE						
	JUNE 5	JUNE 5	JUNE 5	JUNE 5	JUNE 5	JUNE 5	JUNE 5
	2	3	1	2	1	2	
pH	5.63	5.70	5.44	5.80	6.82	4.93	6.11
Ca mg/cc	0.72	1.08	1.08	0.72	0.90	1.19	0.54
Mg mg/cc	0.38	0.64	0.58	0.32	0.32	0.69	0.12
K mg/cc	0.86	1.03	0.93	0.64	1.07	0.74	0.69
Li mg/cc	Trace	Trace	Trace	Trace	Trace	Trace	Trace
REDUCING SUGARS mg/cc	13.66	12.07	12.01	6.36	3.1	10.26	3.50
SUCROSE mg/cc	2.20	1.87	7.18	4.06	1.68	5.45	1.33
TOTAL SUGARS mg/cc	15.86	13.94	19.19	10.42	4.78	15.71	4.83
NITRATE NITROGEN mg/cc	0.045	0.045	0.087	0.077	0.104	0.098	0.075
TOTAL NITROGEN mg/cc	0.070	0.056	0.28	0.112	0.14	0.14	0.84

Qualitative spectroscopic analyses of concentrated solutions of the ash of both phloem exudate and parenchyma sap indicate the presence of barium, boron, copper, manganese, and strontium in varying undetermined amounts in all cases.

#### Discussion

It has been generally accepted for some time that organic materials are transported through the phloem tissues of plants. The presence of large amounts of organic materials such as sugars, which may reach values as high as 20 per cent. of the exudate, and nitrogenous compounds in the phloem exudate of several species tends to support this idea of transport of organic materials through the phloem. There has not been the same unanimity of agreement with respect to the transport of inorganic materials. The majority of investigators claim that inorganic materials are carried upward primarily through the xylem. CURTIS (4) however has presented irrefutable data that inorganic as well as organic materials may be transported through the phloem tissues. The presence in the phloem exudate of such inorganic constituents as calcium, magnesium, potassium, and nitrate nitrogen in amounts appreciably greater than in the parenchyma sap, as well as the presence of small quantities of barium, boron, copper, manganese, and

strontium lends support to the idea of transport of inorganic materials through the phloem tissues. The absence of an element from the phloem would indicate that it was not transported there while the presence of an element in the phloem exudate would offer presumptive but not conclusive evidence of its transport through those tissues.

An interesting observation was made in connection with the flow of the exudate. Collection was begun about August 1st and the flow was good until the first week in October just before the leaves began to change color and fall. The following spring no flow from the phloem tissues could be obtained until about June 15 at which time it was very meager. By July 15 the flow was approximately equal in volume to the flow in late summer of the previous year. The fact that the flow was very slight during the spring and early part of the summer when the cambium is most active lends support to the idea that the exudate was almost wholly limited to the phloem.

It was noted that the total sugar concentration of *Fraxinus americana* decreased as the samples were taken later in August and in September.

Mention has been made of the effect of the proximity of a second cut on the flow from a previous cut.

#### Summary

1. The pH value of phloem exudate was found to range between 7.05 and 8.10 while that of parenchyma sap ranged between 4.93 and 6.82.

2. Calcium, magnesium, potassium, and lithium were found to be present in the phloem exudate sometime in amounts greater than in the parenchyma sap.

3. The amount of reducing sugar in the phloem exudate is much less than the amount of sucrose except in the case of *Platanus occidentalis*, and the reducing sugar here may have resulted from hydrolysis of sucrose after collection.

4. The total sugar concentration of phloem exudate is much greater than that of the parenchyma sap and may reach 20 per cent. of the exudate.

5. In parenchyma sap the amount of reducing sugar present exceeds the amount of sucrose.

6. Total nitrogen, and nitrate nitrogen concentrations of phloem exudate exceed those of parenchyma sap.

7. Barium, boron, copper, manganese, and strontium are present in both the phloem exudate and parenchyma sap.

8. The presence of appreciable amounts of inorganic constituents in the phloem exudate is presumptive evidence that inorganic as well as organic materials are transported through the phloem.

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