MOVEMENT OF ORGANIC SOLUTES IN THE SAUSAGE TREE, *KIGELIA AFRICANA*[1]

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(WITH ONE FIGURE)

Introduction

In recent years, considerable emphasis has been placed on the concept that organic materials, assumed to be sugar, move *en masse* with the water in which they are dissolved through phloem tissues to points of storage or utilization. The mass flow hypothesis, as this concept has been labelled, was suggested independently by MÜNCH (**9**) and CRAFTS (**3**) in recent times, although HARTIG (**7**) in 1858 and SACHS (**10**) in 1865, proposed ideas somewhat similar. No attempt has been made, however, to subject this concept to an objective examination. It is the purpose of this paper to study the growth and development of the fruits of the sausage tree in light of this concept and to determine whether or not the hypothesis can explain the movement as observed in this plant.

The mass flow concept

Before presenting the data collected, it is perhaps necessary to re-state the mass flow concept. MÜNCH (**9**) proposed that the sugar solution moves through the sieve tubes of the phloem from the point of source (storage organ or photosynthetic tissues) to the point of utilization. CRAFTS (**3**) broadens the concept of the tissues involved to the whole phloem including sieve tubes, companion cells and parenchyma, and further proposes that the sugar solution in its movement traverses not only the lumina of the cells but the cell walls as well. No data are presented demonstrating either of these postulates except observations associated with the exudation of liquid material obtained from the phloem area when this tissue is cut. It has been assumed that this exudation represents movement in the *intact* plant. COOIL (**2**) has shown, however, that the only significance which can be attached to this exudation phenomenon is that it represents the readjustment of tissue tensions following the disruption caused by cutting the phloem as well as the associated parenchyma and xylem tissues. The fact that most species do not show exudation at all has been claimed by CRAFTS (**4**) to be due to the inadequate technique of the investigator. He shows that if any species is cut and the wounded portion dipped into water, streaming can be observed from the exposed surface and he assumes that this is a demonstration of streaming in the uninjured plant. Such streaming, however, may

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mean one of two other things: (1) that two materials of different specific gravities and viscosities have been mixed—the heavier one sinks and because of its greater viscosity gives the impression of streaming; (2) that exudation from the cut sieve tubes is induced as a result of dipping the exposed tissue in water, thereby causing a localized osmotic movement resulting in the displacement of sieve tube cytoplasm by the absorbed water entering by way of the parenchymatous cells associated with the sieve tubes. At any rate, the sugar solution is presumed to move through phloem tissue very much as the transpiration stream moves through the xylem.

The second point on which no pertinent data have been presented involves the concentration of the solution which moves. Although he presents no data obtained by chemical analysis showing the amount of sugar actually present in the exudate, CRAFTS assumes that at least two-thirds of the dry material collected in the exudate is sugar, and therefore that the phloem sap is at least a 10 per cent. sugar solution. SACHS (**10**), however, in 1865 showed that the dry matter in the sieve tube of cucurbit was largely nitrogenous in nature and that only a very small amount of sugar appears in the tube. In fact, this led him to propose that sugars did not move in the sieve tubes at all and that only nitrogenous compounds move there, while sugars moved in the associated parenchyma tissues. COOIL (**2**) recently completely substantiated these compositional observations. About one-third of the dry material of the exudate is simple protein, and sugar constitutes about 0.5 per cent. of the fresh weight.

The third point involves the time in the ontogeny of the phloem element at which this movement takes place or at least when it is most active. CRAFTS has shown by means of plasmolysis and dye absorption that as the sieve tube ages, it becomes more and more permeable. Whether any significance can be attached to these observations is beside the point. The significant point is that this is taken to mean that the sieve tube is essentially passive in the movement of material. Therefore, it seems clear that the old sieve tubes should be more effective in transport than the young tubes which are thoroughly alive, and therefore far from passive.

The fourth point of the hypothesis is that once the sugar solution reaches the point of storage or utilization, the water passes into the xylem system and thus circulates back through the plant. No data are presented to support the assumption that this normally occurs in plants.

Although there still remains the matter involving the movement of water in large quantities from xylem and mesophyll into the phloem, this particular point, although again shown clearly in diagrams, has not been shown to occur in plant tissues in volume sufficient to account for movement throughout the phloem. This point, however, is not cogent to this paper, and hence will not be pressed here.

Thus, briefly, the mass flow hypothesis states that a concentrated sugar solution (at least 10 per cent.) moves *en masse* through passive phloem tissues and the water, after dropping its sugar, returns by way of the xylem tissues.

Experiment

The fruits of the sausage tree (*Kigelia africana,* Bentham) are good subjects for this type of research. On each inflorescence, about eight fruits form, but abscission claims half of these. Four fruits may remain on a single floral stem and develop to maturity. There may be more than a hundred such clusters on a tree. At maturity, the floral stem may be 150 cm. long, hanging from the tree (fig. 1) supporting four large fruits, each one of which may weigh five pounds or more. To be sure, many of the clusters have fewer than four fruits, especially since the tree is very rare in the northern hemisphere and souvenir hunters are numerous.

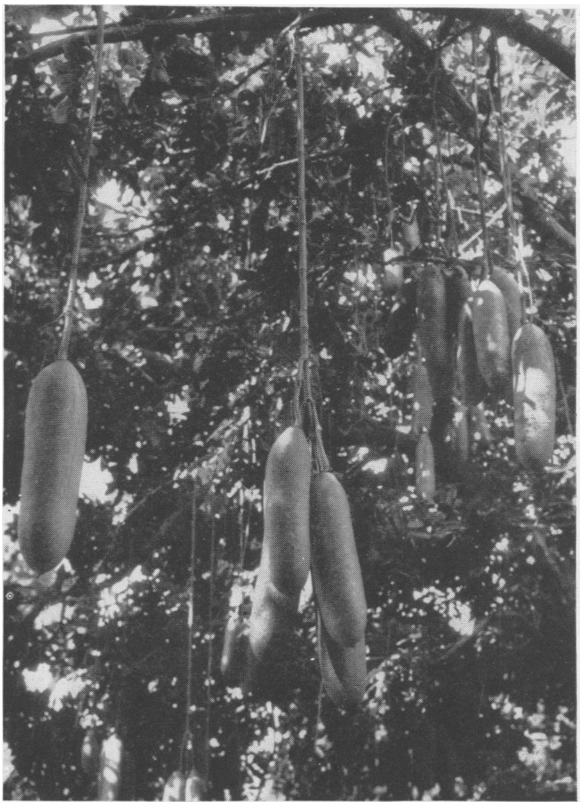

FIG. 1. The fruits of *Kigelia africana* showing one cluster of four fruits.

On June 9, 1939, the flowers were hand pollinated and on June 18, 1939, several clusters were selected for observations. The floral stem of one of these clusters was girdled. All the fruits of this cluster stopped growth and soon dropped. The usual interpretation would be that by breaking the continuity of the phloem food was stopped and hence the fruits dropped.

Another cluster of fruits was enclosed in a bag made of black paper. The object here was to prevent photosynthesis from taking place since the fruits are normally green when young. That the treatment was effective was indicated by the fact that the enclosed fruits were quite without green color. Thus, all of the food which this fruit consumed in growth and storage had to move into it via the floral stem.

Three other clusters were tagged but were without treatment. Two of these were interfered with by tourists and were abandoned.

Beginning June 18, 1939, and continuing at intervals until September 10, each fruit of each cluster was measured by determining its length and circumference. Since the fruits are solid and essentially round in cross-section, it is easy to get a fairly accurate measure of the volume. At the time each group of measurements was made, a fruit corresponding in size to those being measured was selected from the tree, measured, weighed, dried and again weighed. In this way, it was possible to determine the weight of the experimental cluster after each interval and also to calculate therefrom the increase in dry matter for each period.

Phloem samples from the floral stem were gathered at three intervals. The phloem area is very clearly marked and it was easy to remove the xylem tissues as well as the tissues external to phloem. Microscopic examination revealed that the tissue remaining was phloem only. These samples were dried and preserved for sugar analysis.

Results

Only the results obtained from the bagged cluster of fruits will be presented here. Actually, bagging made no difference in the development of the fruit. The unbagged cluster showed no significant difference over the bagged fruit. The record of the volume growth of the four fruits which were bagged are reported in table I.

Each time measurements were made, fruits comparable to those being measured were removed, and their volume, green wight, and dry weight obtained. From these data, the green weights and dry weights of the bagged fruits were calculated. These data are shown in table II.

Although no one has ever demonstrated the form in which organic carbon moves, it has generally been assumed to be sugar. Very probably some of the carbon enters a fruit as amino acids, but the total nitrogen content of these fruits is 1.41 per cent. of the dry weight. If the carbon asso-

TABLE I

VOLUME RECORD FOR ONE CLUSTER OF FOUR FRUITS

DATE OF MEASUREMENT	VOLUME TOTAL	INCREASE OVER PREVIOUS MEASUREMENT	INCREASE IN VOLUME PER DAY
	ml.	*ml.*	*ml.*
June 18, 1939	619	619	68*
June 25	2128	1509	215
July 2	4573	2444	349
July 9	6346	1772	253
July 15	7634	1287	214
July 21	8450	816	136
July 29	9355	905	113
August 9	9716	361	32
August 20	9871	154	14
August 29	9948	77	8
September 10	9979	30	2

* Calculated from the date of pollination, June 9, 1939.

ciated with this nitrogen moved as amino acids rather than sugar, the total amount of carbon thus moved would still be considerably less than 5.0 per cent. of the total carbon required for the fruit. Even though the possibility of translocatory compounds other than sugar exists, in this work the assumption that sugars are the important carbon carriers into these fruits will be maintained.

In order, therefore, to obtain a sugar value for the dry material which

TABLE II

GREEN AND DRY WEIGHTS FOR ONE CLUSTER OF FOUR FRUITS

DATE	GREEN WEIGHT PER ML.	TOTAL GREEN WEIGHT OF FRUITS	DRY MATTER	TOTAL DRY WEIGHT	INCREASE IN DRY WEIGHT	DAILY INCREASE IN DRY WEIGHT
	gm.	*gm.*	*%*	*gm.*	*gm.*	*gm.*
June 18, 1939	1.117	692	11.9	82.3	82.3	9.1
June 25	1.037	2207	11.9	262.8	180.5	25.8
July 2	0.933	4267	11.5	490.7	227.9	32.6
July 9	0.957	6073	11.3	686.3	195.6	27.9
July 15	0.957	7305	11.3	825.6	139.3	23.2
July 21	0.957	8086	11.3	913.8	88.2	14.7
July 29	0.913	8541	12.1	1033.5	119.7	14.9
August 9	0.890	8648	14.1	1219.3	185.8	16.9
August 20	0.883	8716	15.4	1342.3	123.0	11.2
August 29	0.887	8824	17.4	1535.5	193.2	21.5
September 10	0.879	8771	18.7*	1640.3	104.8	8.7

* On November 15, 1939, a fruit was taken from the tree. The dry weight had increased only 0.5 per cent. indicating that by September 10, growth was very nearly completed.

moved into the fruit, a total carbon analysis of the fruit was made.[2] This was found to represent 43.46 per cent. of the dry material. In table III, the total amount of carbon moving into the fruit each day is reported, and, granting that carbon moves into a fruit in the form of sugar, it is simple to convert the daily additions of carbon to the fruit into a hexose sugar equivalent. These values are also shown in table III.

TABLE III

DAILY GAIN IN DRY WEIGHT EXPRESSED AS CARBON AND AS HEXOSE SUGAR

DATE	DAILY GAIN IN DRY WEIGHT EXPRESSED AS	
	CARBON	HEXOSE SUGAR EQUIVALENT
	gm.	*gm.*
June 18, 1939	3.95	9.87
June 25	11.21	28.03
July 2	14.17	35.42
July 9	12.12	30.30
July 15	10.08	25.20
July 21	6.39	15.98
July 29	6.48	16.20
August 9	7.34	18.35
August 20	4.87	12.17
August 29	9.34	23.35
September 10	3.80	9.50

Thus, the figures given in the "hexose sugar" column represent the amount of sugar which each day moves into the fruit by way of the long floral stem. To this should be added the amount of sugar which is respired each day. Respiration measurements were not made during these studies, but a value could be arrived at from some similar types of fruits. On the basis of such calculations between two and three grams of sugar are respired each day. But since such values represent about 10 per cent. of the sugar moving into these fruits, the sugars used in the respiratory activity are ignored in this study. It is reasonable to assume, however, that respiration per unit volume is much higher in the young fruits than in the old fruits.

In summarizing this phase of the study, it is clear that by far the greatest increase in fruit volume is accomplished during the first five weeks, for it is during this period that the largest daily increments are observed. During this same period the daily increments of dry material are higher than those observed after the floral stem is more mature. During this early period, the tissues of the floral stem are young; in fact, the long stem (145 cm.), which for the most part is really a single internode, is still elongating. One would expect the metabolic rate of this tissue to be considerably higher than that

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[2] The author is indebted to Mrs. RUTH YOSHIDA of the Soils Division for this determination.

during the later two-month period. Hence, it appears that the greatest deposition of carbohydrate material in the fruit occurs during the time when the phloem elements of the floral stem are young. This relationship is probably less coincidental than it is causal.

The next phase of this study involves a determination of the strength of the sugar solution which obtains in the phloem of the floral stem at the time of fruit development and, since the total amount of sugar necessary to provide the dry material actually observed moving into the fruits each day is known, it becomes a simple matter to determine the total volume of solution which would be required to move the sugar into the fruit were the mass flow hypothesis the correct interpretation of the mechanism involved.

On July 9 and again on July 15, during the peak of the fruit growth, and finally on September 10, phloem samples were taken from the floral stem by removing xylem tissues as well as the outer paranchymatous tissues. This separation was made easy by a ring of fiber groups just outside the phloem region. Microscopic examination showed the sample to be made up of phloem only. Stained preparations showed a large number of sieve tubes and companion cells, some sclerenchyma and a large amount of parenchyma. Microchemical tests for reducing sugars as well as sucrose revealed moderate concentrations in the inner phloem and somewhat lighter concentrations in the older phloem.

The tissue was dried, and analyzed for reducing sugars and total sugars. The results of this study are reported in table IV.

In the last column of table IV, the amount of sugar in 100 gm. of fresh material is calculated in terms of percentage solutions. Since it is assumed

TABLE IV

SUGAR CONTENT OF THE PHLOEM TISSUE*

Date of collection	Dry weight	Reducing sugars		
		Reducing sugars as percentage dry wt.	Amount of sugar per 100 gm. fresh wt.	Percentage solution
	%	%	*gm.*	%
July 9, 1939	11.3	5.28	0.597	0.672
July 15	11.6	4.35	0.505	0.571
September 10	24.4	1.73	0.422	0.558
		Total sugars		
July 9, 1939	11.3	14.00	1.582	1.78
July 15	11.6	13.11	1.521	1.71
September 10	24.4	11.38	2.777	3.67

* The author is indebted to Mr. BRUCE COOIL for these determinations.

by CRAFTS that the whole phloem area is involved in transport then presumably all the water in the phloem is mobile. Hence, the sugar present must be expressed on a solution basis. Reducing sugars which probably are the most important in transport are present in low concentrations. The reducing sugar values obtained here average 0.62 per cent. and correspond closely to the values obtained by COOIL (**2**) for the exudate of cucurbits but are vastly different from the 10 per cent. assumed by CRAFTS.

Now, it is hardly likely that the parenchyma of the phloem is even fractionally as active in translocation as the sieve tube, if at all. According to COOIL, the petiole tissues of a carbohydrate-high plant are found to be very high in sugar while in a nitrogen-high plant, the tissue sugars are low. Yet, the amount of sugar in the exudate of the phloem is about the same. This probably means that the sugar in the parenchyma is storage material. Sucrose is probably more of a storage sugar than a translocatory sugar. Despite these considerations, however, to give the benefit of all doubts to a favorable interpretation of the mass flow hypothesis, the amount of water required to move the sugar into the fruit during each day is calculated on the basis of the reducing sugar as well as the total sugar concentrations. The sugar values for the collections July 9 and 15 serve as the basis for these calculations, since these represent the period of maximum deposition in the fruit. Unfortunately, a collection of phloem tissue was not made on July 2.

TABLE V

AMOUNT OF SOLUTION NECESSARY TO TRANSPORT THE REQUISITE AMOUNT OF SUGAR INTO THE FRUITS PER DAY

DATE	GRAMS OF SOLUTION	
	0.62 PER CENT. SOLUTION	1.75 PER CENT. SOLUTION
	gm.	*gm.*
June 18, 1939	1592	564
June 25	4521	1602
July 2	5713	2024
July 9	4887	1731
July 15	4064	1440
July 21	2577	913
July 29	2613	926
August 9	2960	1049
August 20	1963	695
August 29	3766	1334
September 10	1532	543

It is, therefore, apparent (table V) that, during the time of maximum fruit development, enormous quantities of solution (5.7 liters of a 0.62 per cent. solution or 2.0 liters of a 1.75 per cent. solution) would have to be forced through the phloem each day. The cross-sectional area of the phloem tissue was only 0.53 cm.^{2}, and the floral stem was 145 cm. long, not including the 15 cm. length of stem which connected the main floral branch to each individual fruit. In table VI, the amount of water required to move the necessary amount of sugar into the fruits is put on the basis of 1 cm.^{2} of phloem cross-sectional area.

TABLE VI

RATE OF SOLUTION MOVEMENT REQUIRED PER SQ. CM. OF PHLOEM AREA

DATE	RATE OF SOLUTION MOVEMENT			
	AS 0.62 PER CENT. SOLUTION		AS 1.75 PER CENT. SOLUTION	
	PER DAY	PER HOUR	PER DAY	PER HOUR
	gm.	*gm.*	*gm.*	*gm.*
June 18, 1939	3009	125	1066	44
June 25	8545	356	3028	126
July 2	10797	449	3825	159
July 9	9236	384	3272	136
July 15	7681	320	2722	113
July 21	4871	203	1726	71
July 29	4939	205	1750	72
August 9	5594	233	1983	82
August 20	3710	154	1314	54
August 29	7117	296	2521	105
September 10	2895	120	1026	42

Thus, depending upon the strength of the sugar solution, the rate of solution movement at the peak of fruit development would have to be 159.4 or 449.1 gm. of solution per cm.^{2} of phloem *per hour*. Now DIXON (5) in his studies of water transport in xylem tissues finds that a movement of 1.93 grams of H_2O per cm.^{2} of xylem per hour was sufficient to account for the speeds of water transport in *Taxus*. FARMER (6) reports that the xylem of certain angiospermous trees delivers 20 ml. of water per hour per unit of cross-sectional area. It is hardly reasonable to believe that water movement in the phloem is many times faster than in xylem systems.

One further point remains to be shown. In table V is shown the daily amount of water necessary to move the requisite amounts of sugar into the fruit. It is necessary to follow this water and determine its ultimate disposition. Each day, according to the mass flow hypothesis, a certain volume of water moves through the floral stem into the fruits. Here the sugar is removed from the solution, and the water is then eliminated from the fruit to make room for more. According to the hypothesis, this water returns by way of xylem tissue. Some of the water, however, would remain to make up the daily volume increase and of course some is lost in transpiration. The amount lost in transpiration is small, since the fruits hang in the shade of the tree. According to measurements, transpiration accounts for less than 20 gm. per day per fruit and, in view of the large amount of water involved, can be ignored. The increases in volume are taken into account in table VII.

TABLE VII

AMOUNTS OF WATER RETURNING FROM FRUIT

DATE	AMOUNTS OF H_2O MOVED INTO FRUIT EACH DAY,—CALCULATED AS		VOLUME INCREASE EACH DAY	AMOUNT OF H_2O WHICH MUST RETURN VIA XYLEM	
	A 0.62 SOL.	A 1.75 SOL.			
	gm.	*gm.*	*ml.*	*Column 1 minus 3*	*Column 2 minus 3*
June 18, 1939	1592	564	69	1523	495
June 25	4521	1602	216	4305	1386
July 2	5713	2024	349	5364	1675
July 9	4887	1731	253	4634	1478
July 15	4064	1440	215	3849	1225
July 21	2577	913	136	2441	777
July 29	2613	926	113	2500	813
August 9	2960	1049	33	2927	1016
August 20	1963	695	14	1949	681
August 29	3766	1334	9	3757	1325
September 10	1532	543	3	1529	540

Thus, if the solution moves into the fruit as a 0.62 per cent. solution, 5.4 liters of water would have to return via xylem tissues each day (week ending July 2). If the solution were a 1.75 per cent. solution then 1.7 liters would have to return. Even the smaller figure involves an hourly average of 69.8 ml. The larger value would involve a backward movement of 223.5 ml. per hour. To test this point, a cluster of fruits was selected on July 9. A longitudinal cut was made through the bark of the floral stem, the xylem was carefully loosened, then cut, and the fruit end of the cut xylem was led out through the incision.

Thus, with the phloem intact, the water returning through the xylem should be intercepted at this point, and with such amounts as 69.8 ml. or 223.5 ml. per hour, the water should literally spout out especially since the radius of the xylem is about 1 mm. But after waiting three hours during late morning, with no sign of water coming out of the cut xylem, this project was discontinued.

At this point it is well to compare the findings of this paper with the assumptions underlying the mass flow hypothesis. The assumption that sugars move in a solution involves the movement of such tremendous quantities of water through poorly adapted tissues that it is inconceivable that any protoplasm no matter how permeable or passive could remain in the sieve tubes and other cells of the phloem. The necessary rates of movement are many times greater than have been observed in xylem tissues.

The assumption that the protoplasm of the conducting tissues is passive is not based on fact. In these studies there is a much greater movement of sugar during the early stages of floral stem development than there is later on. At this stage the tissues are young and certainly more active metabolically than later on. If the movement of sugar were correlated with passivity of protoplasm, it should occur late, and yet such is not the case.

The assumption that sugar in the phloem tissue exists as a 10 per cent. solution is a gross exaggeration; for the exudate of cucurbits as well as the phloem tissue of the sausage tree, the value is less than one per cent.

The assumption that the water which carries the sugar into the fruit returns by way of the xylem could not be verified in these studies.

Thus, it would appear that the mass flow hypothesis is hopelessly inadequate in accounting for the growth and development of the fruits of *Kigelia africana*.

On the other hand, it is much simpler to conceive of the sugar moving in solution but independently of the more or less static solvent. That this movement is associated with the activity of protoplasm is indicated by the fact that the greater part of the growth and development of the fruit takes place while the sieve tubes are young and at a higher metabolic level than later on. The amounts of sugar moving are so great that it seems that the living protoplasm expends energy in aiding the translocation of sugars (**1**, **8**).

Summary

1. The growth of the fruits of *Kigelia africana* was followed from the day of pollination until maturity to determine the required rate of sugar movement into them.
2. An attempt was then made to explain this movement on the assumptions of the mass flow hypothesis.
3. The hypothesis was found to be inadequate in the following particulars:
   a. Although a passive sieve tube cytoplasm is necessary to the hypothesis, the greatest movement of materials into the fruit was observed during the first five weeks of development.
   b. Although the strength of the moving sugar solution has been assumed to be at least a 10 per cent. solution, in these studies the phloem tissue has a reducing sugar level of 0.62 per cent. and a total sugar level of 1.75 per cent.
   c. No evidence could be obtained relating to the return of water from the fruit through xylem tissue even though enough time was allowed for the theoretical return of as much as 209 to 670 ml.
   d. On the basis of the sugar content of the phloem tissue, to account for the observed movement of sugar into the fruit, such enormous quan-

tities of solution (10,797 ml. of a 0.62 per cent. or 3825 ml. of a 1.75 per cent. per day per cm.^{2} of phloem cross-sectional area) would have to move that it is inconceivable that any cytoplasmic organization would remain within the phloem tissue. In fact, the necessary speed of solution delivery into the fruit would be many times the capacity of xylem tissues.

4. The observed movement of sugar into the fruit is so great that it seems necessary to describe it as a function of the living protoplasm of the sieve tube which through its respiratory activity does work in the movement.

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