TRANSLOCATION OF SUGARS IN THE CONCORD GRAPE[1,2]

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For many years there has been a reasonable consensus among plant physiologists that sucrose is the principal transport sugar in various species of plants (5, 11, 12, 17, 18). A considerable number of investigators, however, have ascribed to hexoses the quantitatively more significant role in translocation (3, 14; see also literature review in Leonard, 10). A possible conclusion from these studies is that the relative translocatability of the various sugars varies significantly with species, and perhaps with environmental conditions as well.

The analysis of this problem is admittedly complicated by the difficulty of distinguishing between the molecules in the phloem actually in transit from the leaves or other supply centers and molecules of the same species originating in metabolic reactions in this tissue, or captured in metabolic pools of the respective sugars in the non-conducting cells of the phloem and closely adjacent tissues. However, the techniques of radiochemistry and chromatography now permit a more definitive analysis of this problem, and a re-examination, therefore, of certain aspects of sugar translocation has been undertaken. The grape was chosen for the initial studies in this series because of the ease with which the bark and wood may be separated for analytical purposes, and because of the extensive information available on the anatomy and cytology of its phloem (6).

MATERIALS AND METHODS

Three-year-old grape stocks (*Vitis labruscana* var. Concord) were planted in April in 12-inch pots, and grown under greenhouse conditions until the plants were used for experimental purposes (July-August). At this time the canes were about 10 feet in length. During the growing period, the plants were trained to a maximum of four canes each, without laterals, either fruiting or vegetative.

Prior to each experiment, the plant selected for experimental purposes was pruned to two canes, transported to a controlled environment room (22° C, 2000 ft-c, 16-hr photoperiod) and left for an adjustment period of 24 to 48 hours. At the end of this period, the youngest fully-expanded leaf on one of the canes (usually a leaf at a distance of 100 to 120 cm from the shoot tip, and having an area of approximately 250 cm^2) was enclosed in a glass chamber modified from a three-liter Florence flask and supplied with $C^{14}O_2$. Details of the experimental set-up are shown diagrammatically in figure 1. Sufficient C-14 labeled $BaCO_3$, as well as carrier carbonate, were weighed out into flask C to provide an initial level of approximately 2 mc of activity in approximately 0.1 % CO_2. A small diaphragm pump (G) was used to circulate the gas through the system, which had a volume of approximately 55 liters.

The system was pressure-tested at about 20 cm of water pressure prior to the start of each experiment to test for possible leaks. The petiole of the C-14 leaf was encased in a split rubber stopper inserted in the leaf chamber and sealed with a commercially available mastic compound ("Kalk-Kord"), a preparation known to be highly impervious to CO_2.

Although photosynthesis proceeded rapidly (in each experiment, 50 % of the labeled carbon dioxide was absorbed from the system in approximately two hours, corresponding to a calculated average rate of CO_2 uptake by the leaf of about 10 $mg/dm^2 \times hr$ for this time period) an interval of some 6 to 12 hours elapsed in the different experiments before C-14 activity could be detected at the stem node of the C-14 leaf with a portable 1.5 mg/cm^2 end-window counter. Following this time lag, translocation towards the stem tip was very rapid, activity being detectable at the tip within two hours. Thus a velocity of at least 1 cm/min was indicated for the acropetal translocation rate. Basipetal translocation was much slower.

Shortly after radioactivity could be detected in the shoot tips, the experiment was terminated and segments 30 mm long were rapidly harvested from various internodes, separated into bark and wood, and immediately frozen in liquid nitrogen. All samples were then lyophilized and sealed into glass ampoules under vacuum for storage until analyzed. Although samples were harvested from internodes both basipetal and acropetal to the supply leaf, only the latter will be here reported.

Preparatory to analysis, the tissue samples were pulverized in a micro-mortar, and an accurately weighed sample of approximately 30 mg extracted for four hours in a micro-extractor with 80 % alcohol. The extract was transferred quantitatively to a 5-ml beaker, evaporated to dryness at 35° C under nitrogen, desiccated over calcium chloride under vacuum over night, and the residue then extracted with anhydrous ethyl ether. The ether-extracted residue was dissolved in 0.5 ml water, batch-deionized by adding 50 mg IRC-50 (H^+ form) and 100 mg IR4B (OH^- form) Amberlite exchange resins and shaken for two minutes, filtered through sintered glass into a 2-ml conical test tube, frozen over dry ice, and evaporated to dryness from the frozen state. The residue formed as a very light and fragile fluff during lyophilization,

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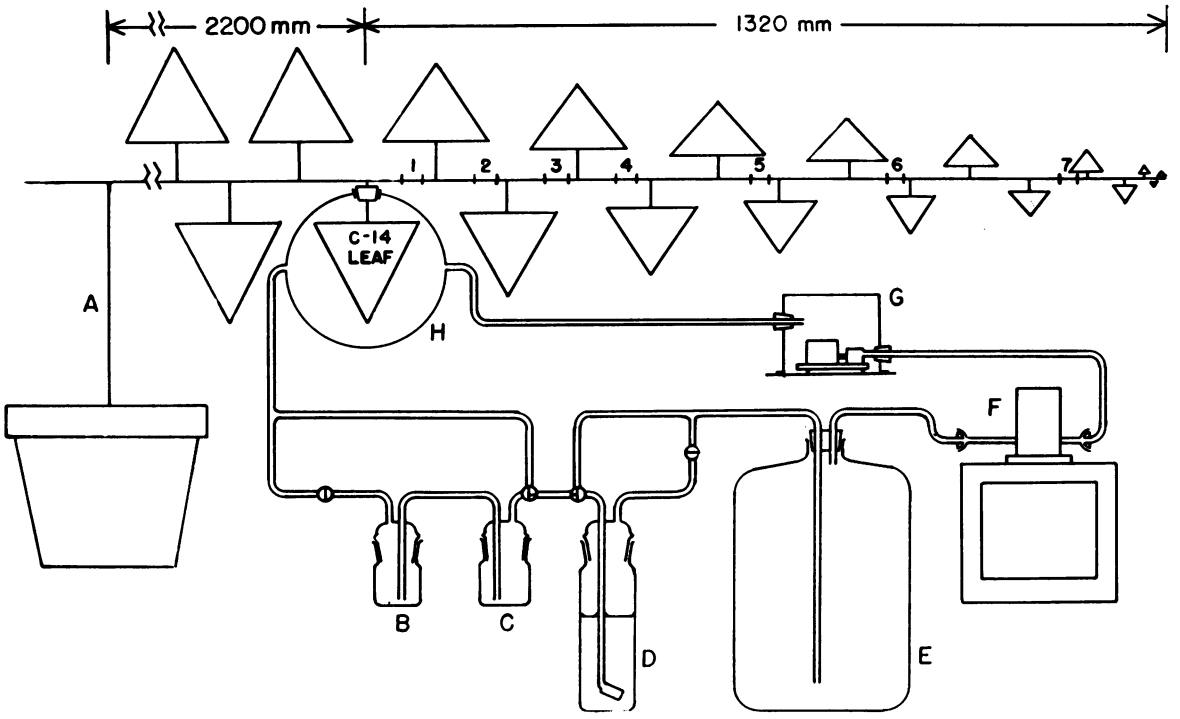

FIG. 1. Schematic drawing of apparatus for supplying $C^{14}O_2$ to single leaf on grape cane. A—grape vine bearing two canes (one shown); B—10 ml 17 % lactic acid; C—approximately 2 mc $BaC^{14}O_2$ plus 285 mg $BaCO_3$; D—absorption tube containing 10 % NaOH; E—50-liter bottle; F—ionization chamber with Brown recorder; G—diaphragm pump in leak proof housing; and H—3-liter Florence flask modified for leaf chamber. Nos. 1, 2, 3, etc. along cane indicate position of samples taken for analysis in expt. 1; in expt. 2, samples were taken near positions 2, 4, 5, and 7. Actual distances (center of sample section to node of C-14 leaf) for each experiment are given in tables I and IV respectively. (From Swanson, 16.)

and it was necessary to cap the test tube with a single layer of lens-cleaning tissue in order to prevent any loss from the test tube. Residue trapped by this filter could be readily dislodged back into the test tube by gentle tapping when lyophilization was complete. The residue was then dissolved in water and made up to a volume of 250 μl. This solution was then spotted on Whatman No. 1 filter paper for chromatographing.

Two chromatograms of each sample were prepared, one with a 50-μl aliquot (10 applications of 5 μl each, spaced 8 mm apart along the starting line) and the other with a 100-μl aliquot, prepared as above but double-spotted. The chromatograms were then developed by either continuous or multiple descent for 72 hours in *n*-butanol-ethanol-water (45 : 5 : 50 parts by volume). Guide strips of reference sugars were run near both margins, and at the completion of the run were cut off and sprayed with benzidinetrichloroacetic acid (1). The sugars were eluted from the chromatogram and quantitatively assayed by the anthrone method following essentially the procedure of Dimler et al (4). For radioactivity counts, 100-μl aliquots of each sugar were plated on stainless steel planchets.

At the start of each analysis, 200 μl (in later experiments, 250 μl) of a 0.05 % solution of rhamnose in 80 % alcohol was added to the pulverized plant material in the micro-extraction cup as an internal standard. The percentage recovery of the native sugars in the plant tissue was assumed to be the same as that of the added rhamnose. Rhamnose was chosen as an internal standard because it did not occur as a free sugar in the plant material used in these experiments and because its R_f value differs markedly from that of sucrose, glucose, and fructose. Blank corrections were obtained from the eluates of strips cut from the chromatogram in close proximity to the test strips, the anthrone-sensitive background of these strips ranging in value from approximately 0.50 μg to 0.85 μg sucrose-equivalent per cm^2 of filter paper. It is assumed that the preparative procedures employed prior to chromatographing the extracts eliminated most anthrone-sensitive substances capable of co-chromatographing with the various sugars.

RESULTS

EXPERIMENT 1. NORMAL CANE: Samples taken from the 1st, 2nd, 3rd, 4th, 6th, 8th, and 10th nodes acropetal to the C-14 leaf (fig 1) were analyzed. The distances from the node of the C-14 leaf to the center of each sample, and the respective concentrations of

TABLE I

CONCENTRATION OF SUGARS (LABELED + UNLABELED) IN THE STEM RELATIVE TO ACROPETAL DISTANCE FROM C-14 LEAF. EXPERIMENT 1

DISTANCE OF TRANSLOCATION (MM)	SUGARS AS % OF DRY WEIGHT					
	BARK			XYLEM *		
	SUC	GLU	FRU	SUC	GLU	FRU
88	5.75	2.81	1.24	2.65	3.71	2.03
202	5.16	2.52	1.86	3.05	3.94	2.01
321	6.22	3.01	1.04	3.29	4.23	2.72
429	5.94	3.05	0.84	3.08	4.46	2.49
652	6.44	2.79	1.13	3.48	5.68	2.62
875	6.27	1.71	0.59	3.23	6.49	2.84
1156	6.48	1.66	0.45	3.78	6.43	3.35

* Including the pith.

sucrose, glucose and fructose in both bark and xylem, calculated as a percentage of the dry weight of these tissues, are given in table I. It is evident from these data that appreciable quantities of all three of these sugars occurred in both the bark and xylem, sucrose being the conspicuously dominant sugar in the bark, and glucose, to a lesser degree, in the xylem.

Tables II and III provide the radiochemical data required for a more critical evaluation of the relative quantitative role of these sugars in translocation. These data, in addition to showing major radioactivity in the sucrose fraction in both bark and xylem, reveal that the relative concentrations of labeled-glucose and labeled-fructose, measured as counts per minute per milligram dry weight of tissue (columns 3 and 4 in tables II and III) were equal (if the reasonable assumption be permitted that the absolute specific activities of the glucose-C-14 and fructose-C-14 were identical, that is, that both were labeled to the same degree). This 1 : 1 equivalence in the radiochemical fractions of glucose and fructose suggests that the glucose-C-14 and fructose-C-14 in the stem were derived mainly by hydrolysis of the sucrose-C-14. On this basis, no significant translocation of the hexoses need be postulated to account for the occurrence or distribution of these sugars in the stem. Inasmuch as the bulk of the radioactivity on the chromatograms was recovered in the three sugar fractions, it may be inferred that sucrose is the only form of sugar which is rapidly translocated in the grape cane.

TABLE II

RELATIVE CONCENTRATIONS OF C-14 LABELED SUGARS IN THE BARK AS A FUNCTION OF TRANSLOCATION DISTANCE. EXPERIMENT 1

DISTANCE OF TRANSLOCATION (MM)	CPM/MG DRY WT OF BARK			GLU/SUC	FRU/SUC
	SUC	GLU	FRU		
88	8005	661	678	0.083	0.085
202	6268	433	481	0.069	0.077
321	5800	397	402	0.069	0.069
429	4615	220	250	0.048	0.054
652	2942	136	126	0.046	0.043
875	1749	75	69	0.043	0.040
1156	900	34	31	0.037	0.034

If this inference is correct, it may be predicted that the ratio of labeled-hexoses to labeled-sucrose will diminish with translocation distance in the phloem, in view of the decreasing time available for hydrolysis of the translocatory sucrose. It is evident from the data of table II that these predicted results were realized. Thus at a distance of 88 mm from the source leaf, the ratio of labeled-glucose (or -fructose) to labeled-sucrose was approximately 0.08, and at a distance of 1156 mm, the ratio had diminished to approximately 0.035.

Although the ratio of glucose-C-14 to fructose-C-14 was essentially unity, this relationship was not observed for the chemically determined fractions of these sugars. The latter fractions obviously represented pools composed mainly of older sugars in the cane which had undergone differential utilization. It should be noted in this connection that the radiochemical sugar fractions, on the other hand, represented recent increments in the stem, with an average age for the translocatory molecules and their derivatives of only about one hour, and it is reasonable to expect, therefore, that an insufficient time had elapsed for equilibration of the labeled sugar fractions with their respective sugar pools indigenous in the stem at the start of the experiment.

TABLE III

RELATIVE CONCENTRATIONS OF C-14 LABELED SUGARS IN THE XYLEM AS A FUNCTION OF DISTANCE FROM C-14 LEAF. EXPERIMENT 1

DISTANCE OF TRANSLOCATION (MM)	CPM/MG DRY WT OF XYLEM *			GLU/SUC	FRU/SUC
	SUC	GLU	FRU		
88	1449	68	68	0.047	0.047
202	1182	63	63	0.053	0.053
321	903	58	72	0.064	0.080
429	523	61	59	0.116	0.113
652	404	38	35	0.094	0.086
875	202	29	28	0.144	0.139
1156	134	23	21	0.171	0.157

* Including the pith.

The data of table III refer to labeled sugars which accumulated in the xylem as a result of lateral movement from the phloem. The fact that longitudinal transport of C-14 labeled compounds in the xylem was negligible was established in an experiment in which the bark was separated from the xylem in the 2nd internode above the supply leaf. Although no severe restriction to translocation was anticipated by this treatment, inasmuch as the bark was not severed transversely, the damage to the phloem was greater than expected, perhaps mainly as a result of partial drying of the exposed phloem cells, and the separated-bark zone functioned essentially as a phloem-block.

Although a small amount of labeled-carbon compounds moved past the treated zone, the greater portion of these undoubtedly moved in the phloem, and a conventional ringing experiment was regarded as unnecessary, therefore, to confirm the well established view that rapid translocation of photosynthate in the stems is primarily phloem-limited.

As previously remarked, the ratio of glucose-C-14 to fructose-C-14 in the xylem was approximately unity (table III), as in the bark; on the other hand, the ratio of labeled-hexoses to labeled-sucrose, unlike in the bark, increased rather than decreased with translocation distance from the source leaf. Thus, at a distance of 88 mm, the ratio of labeled-glucose (or -fructose) to labeled-sucrose was approximately 0.05, and at a distance of 1156 mm, approximately 0.16. Whether this reverse gradient resulted from fructose and glucose diffusing into the xylem and being carried in the transpiration stream, a possibility suggested by Nelson's data (13), or from a higher invertase activity in the younger xylem and pith cells near the tip, or from other factors, cannot be stated at present.

A comparison of tables I and III reveals that, with respect to the chemically-determined fractions of the several sugars in the xylem, glucose predominated by a relatively narrow margin, whereas with respect to the radioisotopic fractions, sucrose predominated by a relatively wide margin. In consideration of the respective age differences in the chemically-determined vs radioisotopically-determined sugar fractions, this observation further substantiates the important quantitative role of sucrose in translocation.

Experiment 2. "Single-Leaf" Cane. This experiment was carried out as described for the normal cane, with the exception that 48 hours prior to the start, all leaves were removed except the supply leaf. The purpose of the defoliation was two-fold: 1) to effect a reduction in the "native" sugar content in the stem, thereby providing a simpler background against which to interpret the superposed translocated sugars from the supply leaf, and 2) to eliminate the translocate supplied to the stem by any leaf other than the C-14 source leaf. Table IV presents the data obtained in this experiment. Apart from the expected reduction in sugar content of the bark (the xylem was not analyzed), the results are in reasonable agreement with those of the previous experiment. The change in ratio of the labeled-hexoses to labeled-sucrose in the bark with increasing distance from the supply leaf followed the same general trend as shown in the normal cane. The ratio of labeled-glucose to labeled-fructose approximated to unity, as required by the hypothesis outlined above, although the degree of fit was less satisfactory than in the previous experiment. It is possible that in a partially starved system, the translocatory sucrose and its derivatives would be metabolized in the phloem more rapidly than under more normal conditions, in which case equilibration with the existing pools of the respective sugars in the stem would be more rapid. Even allowing for this possibility, however, the agreement with a value of unity was within $\pm$ 9 %.

The data of both experiments strongly suggest, therefore, a high order of specificity for sucrose in the translocation process in grape.

Table IV

Relative Concentrations of Labeled Sugars in the Bark as a Function of Translocation Distance. Experiment 2

Distance of translocation (mm)	cpm/mg dry wt of bark			Glu/Suc	Fru/Suc
	Suc	Glu	Fru		
172	15900	1390	1650	0.088	0.104
453	14200	800	860	0.056	0.061
650	11000	470	455	0.043	0.041
1022	10500	410	342	0.039	0.033

Discussion

It is of interest to inquire now as to whether the apparently singular role of sucrose in translocation in grape may be a phenomenon of more general occurrence.

In recent years the sieve tube exudate of a considerable number of tree species has been analyzed chromatographically by various investigators (7, 19, 20, 21). In none of the species thus far studied (approximately 25) has either glucose or fructose been found to be present, even in chromatographically detectable traces, although sucrose was universally present. If the sieve elements are considered to be the primary conducting elements in the phloem tissue (15), then, in terms of the hypothesis that sucrose is the specific sugar of transport, it must be assumed that during transit a certain fraction of the sucrose molecules escape from the sieve tubes and are hydrolyzed in adjacent cells. Some corroboration of this view may be obtained from the fact that, although invertase appears to be absent in the sieve tube exudate of *Robinia pseudoacacia* (19), a positive test for this enzyme has been observed in whole tissue analyses of the phloem (8).

Zimmermann (21) has recently reported that, in addition to sucrose, several other sugars, namely raffinose, stachyose, and perhaps verbascose, may occur in the sieve tube exudate of certain species. In ash, appreciable concentrations of mannitol, a sugar alcohol, were also found. It appears likely, therefore, that sugars in addition to sucrose may serve as transport sugars in some species, but it is of interest to note, as pointed out by Zimmermann, that all of the true sugars thus far identified in sieve tube exudates are closely interrelated and include the sucrose moiety in their structure, differing from each other only in the number of included galactose residues. In about one-third of the species analyzed, only sucrose itself was present.

Additional evidence on the specificity of sucrose in

transport has been provided by Nelson (13), using a totally different approach. Solutions of various labeled sugars were supplied to soybean plants through cut petioles for short periods of time. Glucose and fructose moved rapidly past steam-girdled sections of the stem, as would be expected in typical xylem-injection experiments; sucrose translocation, however, was blocked, as would be expected in phloem-limited translocation. Although additional data are required before the divergent behavior of sucrose and the hexoses in these experiments can be explained, it may be tentatively inferred that the sucrose was rapidly accumulated in the phloem near the site of entry into the plant system, hence its transport could be blocked by killing the phloem. The evidence suggests, therefore, a specificity of sucrose in normal translocation.

Certain data in the literature suggest, however, a more significant role for glucose and fructose in translocation than has been indicated in the experiments discussed above. Dana (2), in a study of the physiology of dwarfing in apples, found that glucose was the primary compound in the bark containing radioactivity following a 30-minute period of C-14 photosynthesis in the terminal leaves of a branch. Vernon and Aronoff (18) concluded from their studies on soybean that sucrose, glucose, and fructose were all translocatory sugars, although the rate of translocation of the hexoses was less than that of sucrose. An alternative interpretation of these data, affirming the singular importance of sucrose, has been presented by Swanson (16), but the interpretation given by the authors remains a valid possibility. The present indications are, therefore, that the process of translocation in the apple and other species may well differ from that in grape in a number of important details. It is evident that a study of the comparative physiology of the sugar translocation mechanism in different species is greatly needed.

Summary

On the basis of radiochemical analyses, it was concluded that sucrose was the only form of sugar rapidly translocated in the grape cane. The C-14 label was introduced by supplying $C^{14}O_2$ to a single leaf on the cane under conditions favorable for photosynthesis. Appreciable quantities of labeled-glucose and -fructose were also found in the stem, but these appeared to be hydrolytic products of the translocatory sucrose, as inferred from the fact that the ratio of glucose-C-14 to fructose-C-14 approximated unity. These results accord with the data reported in the literature showing the complete absence of glucose and fructose, and the predominance of sucrose, in the sieve tubes of a considerable number of species (based on chromatographic analyses of the sieve tube exudate from these species). The convergence in results obtained by these disparate approaches provides supplementary evidence that the translocation of sugars occurs primarily in the sieve tubes or sieve cells of the phloem tissue.

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