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TRANSLOCATION OF C^{14}-LABELED ASSIMILATES IN SQUASH PETIOLES

(TITLE)

BY

WILLIAM JOHN RAUPP
B. S. in Botany,
Eastern Illinois University, 1976

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1979
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

May 11, 1979
DATE

ADVISER

May 11, 1979
DATE

DEPARTMENT HEAD

TRANSLOCATION OF C^{14}-LABELED ASSIMILATES IN SQUASH PETIOLES

BY

WILLIAM JOHN RAUPP

B. S. in Botany,
Eastern Illinois University, 1976

ABSTRACT OF A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science at the Graduate School of
Eastern Illinois University

Charleston, Illinois
1979

380582

ABSTRACT

A qualitative analysis of sieve tube sap was undertaken to help determine the method of assimilate movement in phloem. Hydroponically-grown squash plants (*Cucurbita melopepo torticollis* Bailey) were trimmed to a mature, fully-expanded leaf (the source) and one or more immature, unexpanded leaves (the sinks). The blade of the source leaf was exposed to $C^{14}O_2$ in air for 10 minutes and allowed to translocate for 20 or 50 minutes in ambient air. Petioles of treated leaves were cut into sections and ground in cold buffer with a chilled mortar and pestle to extract materials from the translocation stream. The brei was subjected to differential centrifugations at 121, 3020, 6780, 12100, and 27000 times gravity (g) for 10 minutes. Duplicate 2-ml samples were taken from the supernatant after each centrifugation. The amount of C^{14} present in each was determined by liquid scintillation counting. Background radiation was determined by analyzing 2-ml samples of buffer.

Sugars were extracted from leaf blade and petiole tissue with hot 80% ethanol in a micro-Soxhlet apparatus. Individual sugars were separated by descending paper chromatography. Radioactivity in the several sugars was determined by cutting the chromatograms into 1-cm sections from the origin to the solvent front and eluting each section for 3 hours with distilled water into scintillation vials. C^{14} was measured as before. Background radiation was determined by elution of chromatographed and unchromatographed paper with distilled water.

Because of short translocation times, all C^{14} detected was assumed to be in the translocation stream. In every case, the amount of C^{14} was similar in all samples taken from differential centrifugations. These

results indicate the presence of the translocate in the slouble portion of the sieve tube sap as opposed to an association with the cell particulate or insoluble portion. C^{14} in individual sugars was located mainly in the sucrose and stachyose fractions which are known to be translocated in squash. It is concluded that the sugars in squash phloem are found dissolved in the sap of the sieve tubes and not associated with any kind of particulate matter therein, unless the particles were much smaller than mitochondria. These findings would tend to support a mass flow system of assimilate movement rather than one governed by protoplasmic or transcellular streaming.

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INTRODUCTION

The girdling experiments of Malphigi (1675) and Hales (1731) are regarded as the earliest investigations into the nature of translocation. Despite the demonstration of the sieve tube by Hartig in 1839, a theory of transport of nutrients by diffusion in the xylem was regarded as correct. However, subsequent investigations clearly showed that carbohydrates moved in the phloem (24). Diffusion itself was found to be too slow to account for the measured rates of assimilate movement (3). Protoplasmic streaming was suggested in 1885 as a possible mechanism, but was discarded when evidence of streaming could not be demonstrated in mature sieve tubes (6,10) In addition, it was also too slow for measured rates of transport.

A theory based on translocation via tubules or strands was suggested by Thaine in 1962 (19, 20). He observed fine, microscopic threads traversing the sieve elements. Moving up and down these strands were small plastids and mitochondria-sized particles and this observation was used as evidence for the participation of these particles in translocation. Other investigators could not demonstrate these strands and have concluded that they are artifacts caused by cell walls outside the plane of focus (11)

The most widely accepted theory is that of mass flow as proposed by Münch in 1930. The theory is based on a bulk or mass transfer of a solution and its solutes along a turgor pressure gradient form an area of high solute concentration to an area of low solute concentration. High concentrations are maintained in the leaves (the source) by production of assimilates in photosynthesis. The sinks (roots, fruits, developing buds and leaves) provide a low concentration by use of the assimilate for growth

and storage. The ease of demonstration of phloem exudation as well as observations of the source-to-sink patterns of food, virus, and tracer movement have strenghtened the concept (6).

Currently, mass flow and transcellular streaming are the only proposed mechanisms which satisfactorily explain translocation. A theory based on electrokinetic forces developed by a K^+ pump in the sieve plate as the driving force behind translocation (18) is critized due to the high potentials required (3). Other theories, which rely on interface transport and activated diffusion, have been rejected due to large overall energy requirements (6).

One major problem that remains to be resolved regarding the mechanism of phloem transport is that of bidirectional transport. Simply stated, this phenomenon involves the simultaneous passage upward and downward of one or more substances within the same sieve tube (2). Data collected by several investigators have shown what appears to be bidirectional flow (2,4,21). these studies, however, were not suitable for demonstrating whether the same sieve tube was involved in the movement. Most of these experiments employed the use of paraffin-embedded material, which gives poor resolution, and, therefore, ambiguous results (24). Bidirectional transport within a single sieve tube is explainable using Thaine's (19) theory, but is not possible via a system of mass flow, and so remains the major objection to the Münch hypothesis.

Nicholson and Weidner (17) have recently isolated stachyose-rich particulates from squash petiole cell homogenates using density-gradient centrifugations. If such a stachyose-particulate association is present

in squash plants, transport within the sieve tube by transcellular streaming may allow liquids to flow bidirectionally.

The purpose of this study is an attempt to demonstrate whether the translocated sugars in squash are associated with cell particulate matter of any certain size in the sieve tubes or, as proposed by the Münch hypothesis, they are dissolved in the sieve tube sap. The plant used in this study has been the object of many investigations in the past (1, 13, 14, 17, 21, 22, 23). Experimentation has revealed several important facts regarding the phloem of these plants: (A) the sieve plates have unusually wide pores with no indications of membranes across them (7,9,10); (B) experiments with cucurbit phloem have shown that materials move long distances in short periods of time (5); and (C) squash plants transport stachyose in addition to sucrose. The fact that stachyose is found almost exclusively in the phloem has been well established (1, 13, 23). In addition, the incorporation of radioactive carbon into sugars, coupled with a short translocation period, makes it possible to differentiate translocated materials from storage carbohydrate. Such is not possible with sucrose, the major transport molecule in most plants, because it is found throughout the plant body.

MATERIALS AND METHODS

PLANTS

Seeds of the straightneck bottle squash (*Cucurbita melopepo torticollis* Bailey) were germinated on moist cheesecloth in a belljar. At 10 days, the seedlings were transferred to hydroponic tanks and placed under fluoues-cent/incandescent lighting with a photoperiod of 16 hours of light (2500 ft-c) followed by 8 hours of darkness. Plants were grown in an aerated Hoaglands #2 solution (see Table 1) (15). Plants were 30 to 50 days old when labeling experiments were conducted. A mature, expanded leaf was positioned in the labeling chamber and all other mature leaves were removed 24 hr before labeling. Immature, unexpanded leaves, as well as developing flower buds, were left as sinks.

LABELING

A Plexiglas labeling chamber, 15 cm x 17 cm x 3 cm, was assembled to accommodate the single mature leaf blades (see Figure 1). The chamber was positioned under a floodlamp so that the leaf surfaxe received approx-imately 1500 ft-c of light. A 5% $CuSO_4$ solution was placed between the light source and the labeling chamber to absorb infrared light. The leaf blade was sealed into the chamber using Mortite caulking compound around both the petiole and the removable end of the chamber.

Radioactive carbon, purchased as solid $BaC^{14}O_3$, was converted to liquid $NaHC^{14}O_3$ for ease of handling. Radioactive carbon dioxide was generated by adding 1 ml of lactic acid (stoichiometric excess) to 1 ml of the $NaHC^{14}O_3$ solution (10 uc C^{14}). The leaf was supplied with $C^{14}O_2$ in the labeling chamber for 10 minutes after which time the leaf was re-

TABLE 1. Nutrient Solution for Hydroponically Grown Plants.

HOAGLANDS #2 NUTRIENT SOLUTION

Solution	ml/l	mM
1M $Ca(NO_3)_2$	3	3
1M KNO_3	2	2
1M $NH_4H_2PO_4$	2	2
1M $MgSO_4$	1	1
Micronutrient Solution	1	1
0.5% Fe-EDTA	1	0.013

Micronutrient Solution

Micronutrient	Form	g/l
Boron	H_3BO_3	2.86
Manganese	$Mn(Cl_2)_2 \cdot 4H_2O$	0.22
Zinc	$ZnSO_4 \cdot 7H_2O$	0.22
Copper	$CuSO_4 \cdot 5H_2O$	0.08
Molybdenum	H_2MoO_4	0.02

FIGURE 1. Labeling Apparatus Showing Gas Flow During Experiments.

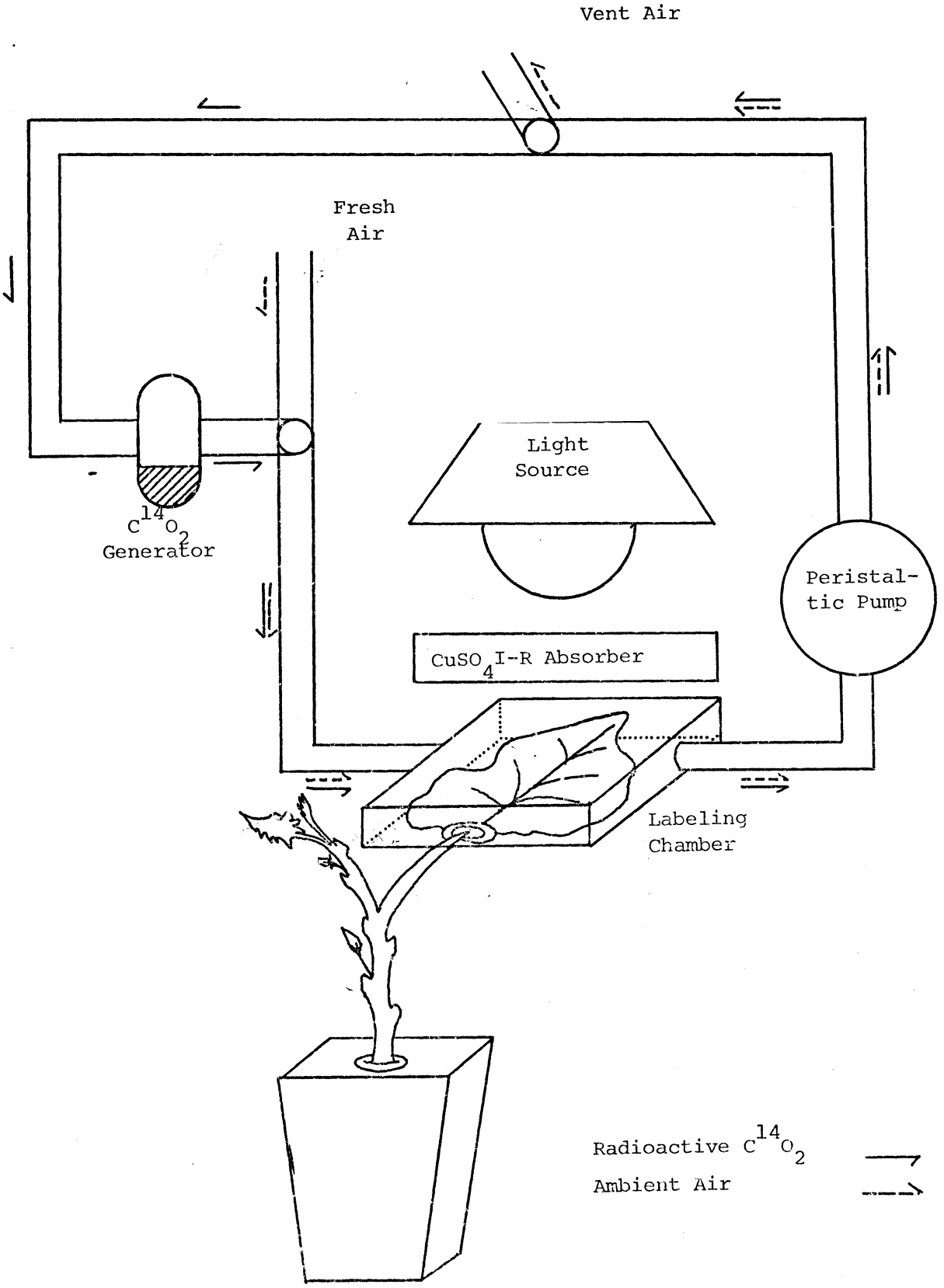
Vent Air
Fresh Air
Light Source
$C^{14}O_2$ Generator
Peristal-tic Pump
$CuSO_4$ I-R Absorber
Labeling Chamber
Radioactive $C^{14}O_2$
Ambient Air

TABLE 2. Conditions for Individual Experiments.

EXP #	TOTAL TRANSLO-CATION TIME		CENTRIFUGE TIME				CENTRIFUGE SPEEDS (all x g)					COMMENTS
	30 min	60 min	10 min	30 min	8 hr	24 hr	121	3020	7500	12100	27000	
1	X		X				X	X	X	X	X	
2	X		X				X	X	X	X	X	Sugar analysis
3	X		X				X	X	X	X	X	Sugar analysis
4	X		X				X	X	X	X	X	Sugar analysis
5	X		X	X	X	X				X		
6	X		X							X		3 runs with dispersal of pellets after each
7	X		X				X	X	X	X	X	Brie filtered through cheesecloth
8	X		X				X	X	X	X	X	Petiole homogenized in a blender with 30 ml buffer
9		X	X				X	X	X	X	X	Sink petiole also ground and centri-fuged

moved from the chamber, exposed to ambient CO_2, and allowed to translocate for 20 minutes. In one experiment, the translocation time was increased to 50 minutes.

C^{14} ANALYSIS

Unless otherwise stated in Table 2, the experimental procedure was as follows. After labeling and allowing time for translocation, the petiole was removed from the plant and quickly weighed, measured, and cut into small pieces (0.5 cm). The pieces were then ground for approximately 5 minutes in 15 ml of cold buffer solution* (8) using a mortar and pestle. The mortar was cooled in an ice bath. The resulting slurry was then transferred to a 50 ml calibrated centrifuge tube. The mortar was washed twice with 10 ml of buffer and the volume added to the centrifuge tube. The final volume of the tube was brought to 40 ml with buffer.

Differential centrifugations were accomplished using an RC2-B Sorvall refrigerated centrifuge with an SS-34 rotor and controlled to 0.5°C. Centrifuge times and speeds used during individual experiments are listed in Table 1; all samples were taken in duplicate. Initial 2-ml samples were taken before centrifugation with additional 2-ml samples taken after each run. All samples were mixed with 18 ml of Beckman Redi-Solv HP or GP Scintillation Fluid. Radioactivity was determined using a Beckman LS-100C liquid scintillation counter. Background radiation was determined using

---

*0.33 M sorbitol; 0.05 M tricine-NaOH buffer, pH=8; 2 mM $NaNO_3$; 2mM EDTA; 1 mM $MnCl_2$; 5 mM $MgCl_2$; 5 mM K_2HPO_4

using 2-ml samples of buffer mixed with 18 ml of scintillation fluid.

In some experiments, the sugars from leaf blade and some petiole tissue were extracted with 80% (v/v) ethanol for 2 hr using a micro-Soxhlet extraction apparatus. After this time, the extract was cooled and the volume brought to 50 ml with 80% ethanol. The extract was stored at -15°C until ready for analysis. In these same experiments, 1-ml samples of the final supernatant were evaporated to dryness with a Rotary Evapo-Mix and were stored until analysis of sugars was carreid out.

Individual sugars were separated by means of descending paper chromatography using a 3:3:2 (n-butanol, acetic acid, water) solvent system. 1-ml samples were spotted on 18 cm x 57 cm strips of Whatman #1 filter paper. Identification of sugars was accomplished by co-chromatography of known sugars. Side strips were spotted with 0.25 ul of 1% solutions of stachyose, sucrose, and glucose. The chromatograms were developed for 20 to 24 hr and then dried for 10 minutes at 100°C in a chromatography drying oven.

Location of known sugars was determined by removing the side strips, spraying them with benzidine reagent*, and heating at 100°C in a drying oven for about 5 minutes until spots developed. Radioactivity in the several sugars was determined by cutting the chromatogram into 1-cm strips across the width of the paper, from the origin to the solvent front. Sugars were eluted with distilled water into scintillation vials. After 3 hr of elution, 5 ml of scintillation fluid was added to each vial. Radio-

---

*0.5 g benzidine, 10 ml (40% w/v) trichloroacetic acid, 10 ml glacial acetic acid, and 80 ml 95% ethanol.

activity was determined by liquid scintillation counting.

Background radiation was determined using 4 controls on each chromatogram. The first 2 controls involved the elution of strips of chromatographed Whatman #1 filter paper with distilled water. These strips were taken from behind the origin of the chromatogram. The third control was the elution of a strip of unchromatographed filter paper. The final control was 0.3 ml of distilled water. All elutions of controls were run for 3 hr and the vials filled with 5 ml of scintillation fluid.

RESULTS AND DISCUSSION

Results of the differential centrifugation experiments are presented in Tables 3 and 4 and Figure 2. In the tables, the data are expressed in percents. Here, the sample with the highest number of counts per minute (CPM) was taken as 100% and the other values are expressed as a percent of this total. The maximum counts varied between individual experiments. This variation is noted in Table 3 by a listing of counts per minute below that sample having the highest total for the series of centrifugations. This discrepancy was probably due to the inability of the investigator to regulate the amount of $C^{14}O_2$ each leaf received coupled with the expected variation amony experimental plants.

In some experiments (3, 5, and 6), scintillation data contained one or more pieces of questionable data. In these cases, the sample vials were thoroughly cleaned and recounted, at least twice, for accuracy. As a check, other vials were counted to see how the recounts compared to the originals. These were usually similar to the early counts.

Most experiments showed differences of only 10 percent in C^{14} between the several samples of the differential centrifugations. These experiments suggest that most of the C^{14} was still found in the supernatant and was not centrifuged out. It should also be noted that the highest counts were not always observed in the first sample taken, i.e., before they were subjected to any centiifugation. This observation indicates that the C^{14} is found mainly in the translocation stream and has not been incorporated into the cells of the petiole. If incorporation had occurred, a major fraction of the total radioactivity would have been removed in the first or second

TABLE 3. Radioactivity in Samples from Differential Centrifugations. Amounts expressed as percents of the sample with the highest counts per minute.

x g	Experiment #						
	1	2	3	4	7	8	9
	%	%	%	%	%	%	%
0	96	96	91	100 (881 CPM)	100 (312 CPM)	100 (358 CPM)	97
121	95	93	94	93	--	95	100 (5400 CPM)
3020	97	100 (2231 CPM)	100 (116 CPM)	92	82	95	93
6720	96	95	96	91	--	97	99
12100	99	98	90	85	81	96	96
27000	100 (126 CPM)	97	87	90	80	94	96

FIGURE 2. 24 - Hour Centrifugation Experiment.
(Experiment 5, Centrifugations at 12100 x g for 10 minutes)

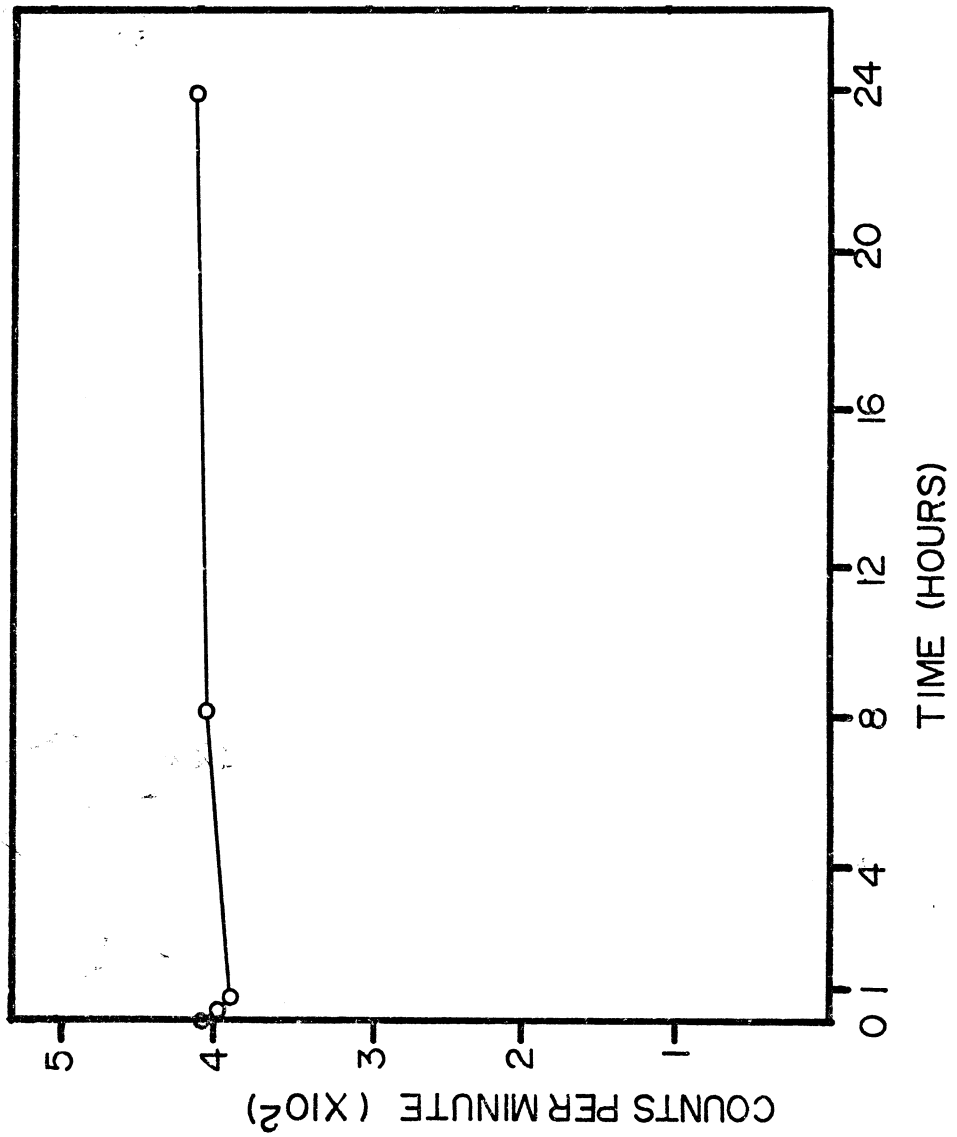
COUNTS PER MINUTE (X10²)
5
4
3
2
1
0
1
4
8
12
16
20
24
TIME (HOURS)

TABLE 4. Pellet Redispersal Experiment. (Experiment 6)

Order and Treatment	CPM	%
1 Sample	2136.7	100
2 Centrifuge*		
3 Sample	1754.7	82
4 Pellet Redispersed		
5 Sample	1780.2	83
6 Centrifuge*		
7 Sample	1703.6	63
8 Pellet Redispersed		
9 Sample	1787.8	84
10 Centrifuge*		
11 Sample	1762.7	83
12 Pellet Redispersed		
13 Sample	1764.8	83
14 Centrifuge*		
15 Sample	1717.6	88

*10 minutes at 12100 x g

centrifugations.

Minor variations in the protocol of the centrifugation experiments were tried to test the conclusion that the C^{14} is located in the translocation stream and not in cell wall material. These included straining the brei through cheesecloth to remove large particles of cell wall and unground tracheary elements (Experiment 7), employing a blender to homogenize the petiole tissue to check the efficacy of the grinding method (Experiment 8), and increasing the time for translocation, to allow time for more label to be incorporated into the phloem sap (Experiment 9). The results of these studies agree with those from the previous experiments. The 20% decrease in C^{14} in the samples of Experiment 7 may be due to the use of cheesecloth for filtering and the subsequent need for transferring the brei.

The speeds of centrifugation used were based on the sizes of particulates expected to be found in the phloem. It has been demonstrated that plastids and mitochondria are found in the sieve tubes (16). These organelles have been thought to be responsible for translocation (19). At 3020 x g, larger particles of cell wall are removed; at 12100 x g, plastids will settle out; a speed of 27000 x g is suffieient to remove particles the size of mitochondria from the supernatant. The data from these differential centrifugation experiments do not support the claim that these or similarsized particles play a role in translocation because, if they did, C^{14} would have been found in the particulates. Esau (10) states that the phloem is very sensitive to mechnaical manipulations and responds with a displacement of contents. This displacement may account

for the presence of cellular organelles and particles in phloem exudates and for the conclusions reached which are based on the presence of these particles.

Two additional experiments were conducted to check the efficacy of centrifugation time and sampling technique. In the first, the petiole brei was subjected to varying lengths of centrifugation time. The results, represented graphically in Figure 2, show no essential difference in the amount of C^{14} in the supernatant of samples centrifuged for 10 or 30 minutes or 8 or 24 hours. It was concluded, therefore, that 10 minutes was a sufficient time for centrifugation.

In another experiment, the samples were taken before and after the pellet was redispersed in the centrifuge tube. This redispersion was done to ascertain whether the label was evenly dispersed and not located in a specific area or areas in the centrifuge tube. The results were similar in all samples (Table 4) and, therefore, are an indication that the distribution of the label in the centrifuge tube was even and that labeled material was not leaking from particles centrifuged out in low-speed or early centrifugations.

The distribution of C^{14} in leaf blade extracts and samples of the final supernatant are found in Table 5 and 6 respectively. The averages of these data are represented by the graphs of Figures 3 and 4. In both cases, most of the C^{14} was found in stachyose and sucrose. These data agree with those of Hendrix (13) and Webb and Gorham (22) who identified these sugars as the location of C^{14} in short-term labeling experiments with squash plants. In addition, label was also found in the chromatographic

TABLE 5. Distribution of C^{14}-Labeled Extract from Leaf Blade Tissue after Paper Chromatography.

cm from origin	Exp.# 2	Exp. #3	Exp #4	Net CPM*
Backround Radiation	28.3	29.9	27.4	------
0	31.5	31.6	37.2	3.7
1	35.7	43.9	33.4	9.5
2	39.6	40.6	53.5	16.1
3	40.3	104.2	157.7	72.2
4	103.9	224.9	464.5	242.6
5	148.5	228.1	307.2	199.5
6	422.6	1969.0	3095.6	1799.9
7	2310.0	1632.7	2159.3	2002.5
8	1576.6	385.2	379.4	635.2
9	214.3	243.0	285.7	219.2
10	163.4	343.3	609.1	343.5
11	711.4	572.9	683.2	627.3
12	701.7	229.5	207.9	351.1
13	227.4	137.1	141.3	140.1
14	121.4	115.6	131.1	92.4
15	917.5	488.8	750.0	50.2
16	3185.3	2136.8	1579.8	2272.1
17	3193.8	2192.7	985.2	2095.4
18	1540.1	918.4	741.9	1038.4
19	935.1	604.7	374.4	609.6
20	757.9	440.4	453.4	522.1
21	217.9	573.5	307.6	337.8
22	104.9	244.9	95.1	119.8
23	125.0	77.6	62.1	59.7
24	198.3	57.9	132.4	101.0
25	237.7	185.1	496.6	278.0
26	297.8	490.9	477.1	399.4
27	406.0	473.5	225.7	339.9
28	266.1	191.5	209.7	193.7
29	115.9	123.6	271.0	141.7
30	70.1	139.1	119.7	81.1
31	56.9	103.3	65.6	46.8
32	45.3	62.2	59.8	27.3
33	39.7	55.6	49.4	19.7
34	35.4	48.7	60.6	19.7
35	33.6	38.6	34.2	7.0
36	41.9	32.9	47.3	12.2
37	38.4	32.2	32.2	5.8
38	85.9	28.4	35.4	21.4
39	48.6	28.7	28.0	6.6
40	35.8	30.4	33.4	4.7
41	26.8	29.4	28.5	0.0
42	27.1	39.1	27.8	2.8

* Net CPM = average CPM minus background CPM

FIGURE 3. Distribution of C^{14}-Labeled Extract from Leaf Blade Tissue after Paper Chromatography.

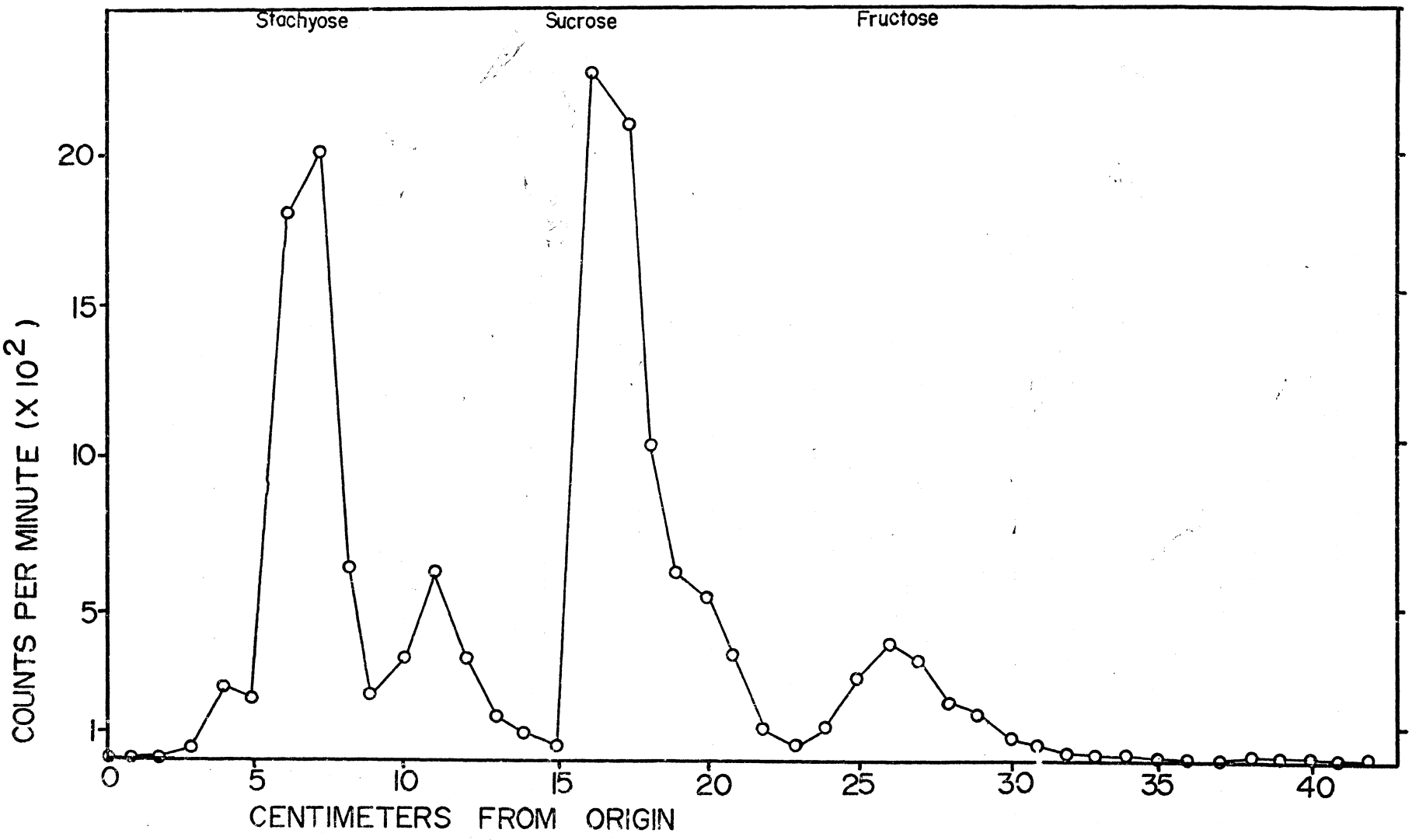
Stachyose
Sucrose
Fructose
COUNTS PER MINUTE (X 10²)
20
15
10
5
1
0
5
10
15
20
25
30
35
40
CENTIMETERS FROM ORIGIN

TABLE 6. Distribution of C^{14} Label in the Final Supernatant from Differential Centrifugations after Paper Chromatography.

cm FROM origin	Exp. #2	Exp.#2	Exp. #3	Exp. #3	Net CPM*
Backround radiation	40.4	33.6	27.8	28.9	----
0	43.1	37.2	28.2	27.5	1.3
1	34.9	35.4	31.5	29.2	0.1
2	38.5	32.1	33.3	37.8	0.2
3	39.1	33.0	30.5	35.3	1.8
4	43.4	35.9	52.4	127.3	32.1
5	74.3	76.7	63.7	63.4	36.8
6	122.1	137.2	47.5	52.7	57.2
7	104.8	105.9	64.9	49.6	48.6
8	92.9	92.6	41.4	44.4	35.1
9	89.5	79.1	50.3	36.8	31.2
10	81.2	80.3	38.1	36.5	26.3
11	88.5	80.3	39.9	38.4	29.1
12	90.9	78.8	39.2	44.2	30.6
13	83.2	89.7	40.5	35.8	29.6
14	101.2	85.6	36.4	37.6	32.5
15	106.5	87.6	37.2	36.9	34.4
16	106.6	84.0	38.1	85.1	45.8
17	113.0	89.7	39.7	34.1	36.4
18	110.4	77.9	36.7	43.1	34.3
19	97.6	72.3	30.4	32.0	25.4
20	82.8	71.4	30.7	33.8	22.0
21	64.8	63.2	36.2	33.5	16.7
22	49.1	44.0	27.4	32.4	5.5
23	38.9	42.4	32.3	33.0	4.0
24	37.6	37.0	28.2	44.4	4.1
25	34.3	32.4	28.5	28.4	0.0
26	40.7	32.3	29.5	26.9	0.0
27	39.0	32.3	31.1	28.1	0.0
28	39.3	33.3	26.8	26.1	0.0
29	40.0	34.4	37.9	31.1	3.1
30	37.4	37.2	27.5	28.8	0.0
31	37.6	35.9	28.9	30.4	0.5
32	37.8	33.3	25.1	28.3	0.0
33	39.6	37.7	28.8	26.8	0.5
34	34.6	33.3	29.5	29.1	0.0
35	35.4	28.5	29.9	28.3	0.0
36	38.3	32.9	29.2	30.0	0.0
37	37.0	37.5	29.9	30.8	1.1
38	38.0	37.7	28.3	26.8	0.0
39	37.9	25.7	27.0	27.8	0.0
40	40.2	33.8	28.8	44.6	4.1
41	35.6	30.0	28.0	29.8	0.0
42	39.2	33.3	27.1	30.4	0.0

*Net CPM = average CPM minus background CPM

FIGURE 4. Distribution of C^{14}-Labeled Sugars in the Final Supernatant from Differential Centrifugations after Paper Chromatography.

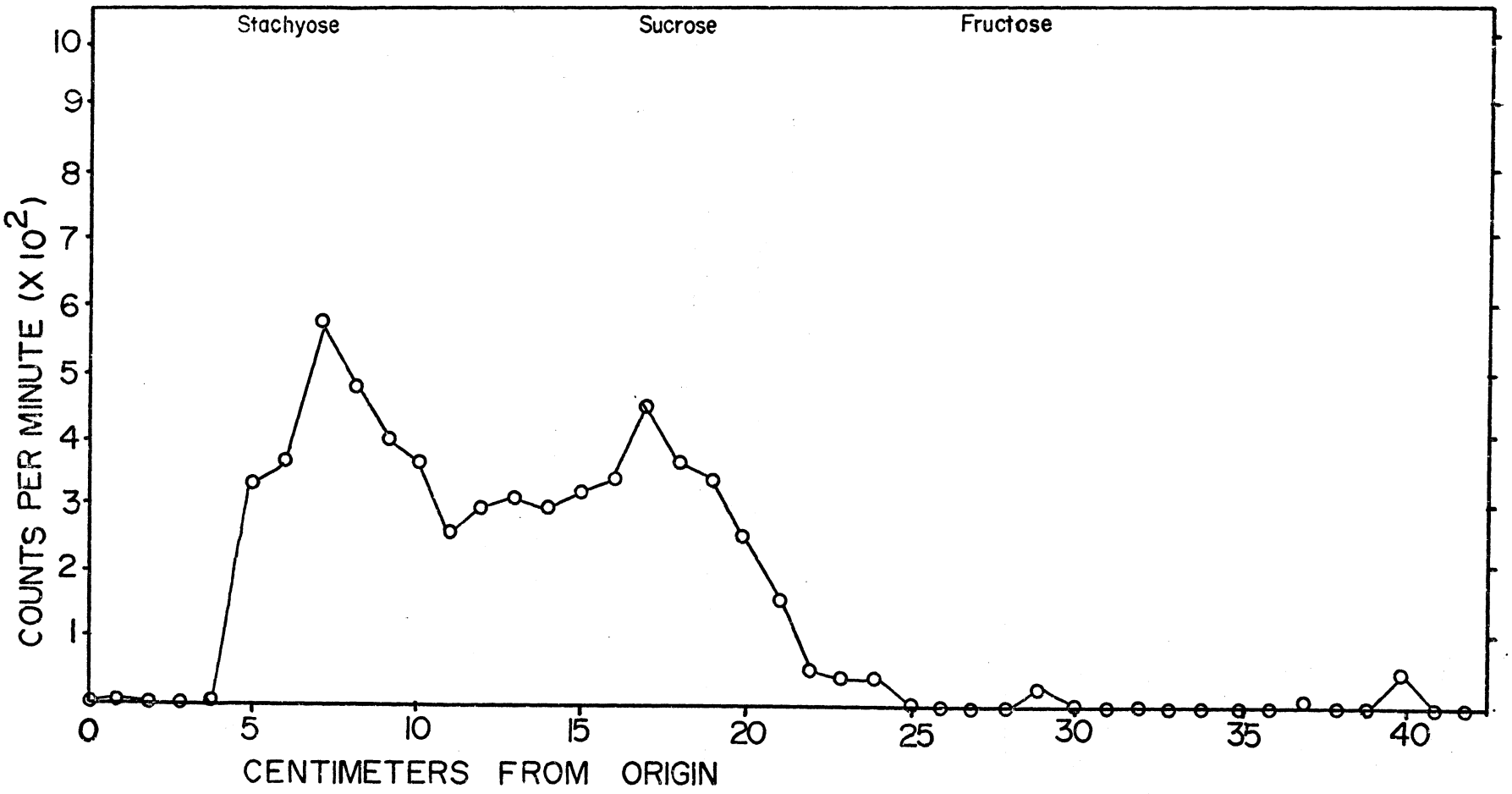
Stachyose
Sucrose
Fructose
COUNTS PER MINUTE (X 10²)
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
40
CENTIMETERS FROM ORIGIN

TABLE 7. Calculated R_f Values of Known Sugars.

	Stachyose[1]	Raffinose[2]	Sucrose[1]	Glucose[2]	Fructose[1]
cm from origin	$\pm$ 6.45 $\pm$ 0.85	9.3	$\pm$ 16.41 $\pm$ 1.15	16.2	$\pm$ 20.45 $\pm$ 1.11
R_f	$\pm$ 0.15 $\pm$ 0.02	0.24	$\pm$ 0.39 $\pm$ 0.02	0.41	$\pm$ 0.49 $\pm$ 0.02

[1] based on an average from 24 side strips

[2] based on an average from 2 side strips

positions of verbascose, raffinose, glucose, fructose, and some organic acids. These sugar positions were determined by use of the R_f values of known sugars (Table 7). All of these have been identified as phloem-moble assimilates in cucurbits (6).

Most extractions of sugars from petiole tissue yielded insignificant data which was due to the small amount of tissue used in the extraction. Since insufficient C^{14} was extracted from petiole tissue (79 CPM for petiole tissue compared to 14280 CPM for blade tissue) little difference between C^{14} label and background radiation was noted. In one experiment, however, some C^{14} label was found located in the stachyose area of the chromatogram.

While the results of the above experiments appear conclusive, Nicholson and Weidner, using density gradient centrifugations, have shown cell particulate matter to be associated with most of the stachyose in squash phloem (17). It is possible that the particles in their investigation may be much smaller than those which were able to be centrifuged out in the present investigation at 27000 x g. Subsequent investigations might include centrifugations at higher g forces than those presently used. Further work with density gradient centrifugations might use C^{14} - labeled brei for these separations as in the present study.

Secondly, it is possible that the concentration of the buffer solution may not have been adequate to maintain the osmotic potential of the sieve tube sap. If similar results can be obtained with a variety of buffer solutions, it can be concluded that the C^{14} is simply found dispersed and not associated with particulates as predicted in one theory. Many investigators have used dry ice or liquid nitrogen to treat tissue before

grinding (2, 21, 22, 23). In most cases, however, these experiments were not concerned with maintaining the osmotic potential to the sieve tube.

From the present study it is apparent that no detectable C^{14} label is associated with the cell particulate matter observed in phloem exudate. The concentration of C^{14} in the supernatant is similar in all samples taken from differential centrifugations. If particulate matter does play a role in translocation, it would have to be of a size much smaller than mitochondria. The data tend to support a theory of translocation based on the premise that the translocate molecules are dissolved in the cell sap. The movement of this material is not associated with cell particulates equal to or larger than the size of mitochondria.

SUMMARY

The C^{14} found in the translocation stream in petioles of squash plants whose leaves have been labeled with $C^{14}O_2$ appears to be in the soluble fraction. Differential centrifugations could not demonstrate C^{14} to be associated with cell particulates the size of mitochondria or larger. C^{14} in individual sugars was located mainly in the stachyose and sucrose fractions These observations tend to support a translocation theory based on the premise that the translocate molecules are dissolved in the sieve tube sap and not one governed by association with the particulate matter therein.

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