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Stachyose Translocation in Plants [1, 2]

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Introduction

Recent work based on C^{14} studies and sieve tube exudate analyses suggests that carbohydrate translocation is restricted for the most part to the raffinose family of oligosaccharides. The primary member of this series, sucrose, is the most often reported translocated carbohydrate in plants (2). Raffinose appears in the sieve tube exudate of many species and is undoubtedly translocated (8). Zimmermann (8) reports that stachyose is a major component of the sieve tube exudate of white ash. Stachyose-C^{14} and verbascose-C^{14} were identified by Pristupa (5) as the transport molecules in pumpkin after fixation of $C^{14}O_2$.

Compounds other than these carbohydrates are implicated as important carbon carriers by some studies. The sugar alcohols, mannitol and sorbitol, appear to play a role in translocation in species of *Oleaceae* and *Rosaceae* respectively (11,7). In experiments with young soybean plants, Nelson et al. (4) inferred translocation of sucrose and serine under one set of environmental conditions and malic acid in another environment.

It is our purpose in this paper to report comparable data on several species which have been reported to translocate or may be presumed to translocate raffinose-family oligosaccharides of molecular weight greater than sucrose. This is done in an attempt to enlarge our knowledge of the kinds of naturally occurring molecules which are normally translocated.

Materials and Methods

In all experiments the $C^{14}O_2$ source was $Na_2C^{14}O_3$ made from $BaC^{14}O_3$ as supplied from Oak Ridge National Laboratory without additional carrier. Specific activity of the $BaC^{14}O_3$ used for box elder and white ash was 0.093 mc/mg; for pumpkin and mullein, 0.167 mc/mg. The selected supply leaf was sandwiched between 2 15-ml leaf cups which were sealed to the surfaces of the leaf with a mastic known

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as Mortite. The leaf cups enclosed a 3-cm² circular area of the blade with the midvein bisecting the area. Immediately prior to the liberation of $C^{14}O_2$ in the leaf cup attached to the lower leaf surface, a volume of air exceeding the CO_2 volume by 10 % was withdrawn from the chamber in an attempt to restrict the mass movement of the air-C^{14} mixture through the intercellular spaces of the leaf.

At the end of the supply period, the 3-cm² circle and 2-cm segments of the midvein and petiole were harvested and frozen at dry ice or liquid nitrogen temperatures. A 0.5 × 2-cm sample of excised lamina was taken from adjacent to each midvein sample. The frozen samples were finely chopped and extracted with 1 ml of boiling 80 % v/v aqueous ethanol in a microrefluxing system for about 5 hours. After removing an aliquot for counting, each extract was divided and spotted on two 7½ inch × 18¼ inch sheets of Whatman No. 1 filter paper for 1-dimensional chromatography utilizing known compounds on side strips. One chromatogram was developed in *n*-butanol: ethanol: water (45: 5: 50) and the other in *n*-butanol: acetic acid: water (3: 3: 2) for hexoses and oligosaccharides respectively. Chromatographically separated compounds were located by autoradiography, eluted with water, an aliquot counted and their identity verified by cochromatography with authentic samples. In the *Verbascum* experiment, labelled serine cochromatographed with sucrose. Using *n*-butanol: acetic acid: water (25: 6: 25) as the developing solvent, some eluates from *Verbascum* chromatograms were rechromatographed with authentic serine and sucrose for identification purposes and to determine the ratio of labelling in the 2 compounds. Radioactivity determinations were made on plated aliquots of the ethanol extracts and on eluates from the chromatograms. A Nuclear-Chicago D-47 thin window gas-flow counter was used for all radioactivity measurements.

Plant Materials. A 61-cm high seedling of box elder, *Acer negundo* L., was potted from the field in the fall of 1959 in Columbus, Ohio and transferred to a controlled environment room in March 1960. The experiment was performed in May when the plant had 2 sets of fully expanded compound leaves. The upper set of compound leaves was stripped of lateral leaflets the day prior to the experiment. Fifty μc of $C^{14}O_2$ were administered to the basal portion of the 2 opposite terminal leaflets. One leaf was harvested and frozen at liquid nitrogen temperatures 12 minutes after release of $C^{14}O_2$ and the other after 50 minutes, at which time an exudate sample was collected from the bark adjacent to the supply leaf.

A 1-year-old plant of white ash, *Fraxinus americana* L., was collected from the field at Columbus, Ohio, potted in soil and transferred to the controlled environment room in April 1960. The experiment was performed in June 1960 in the same manner as that used for the *Acer negundo*, using the uppermost pair of fully expanded compound leaves and harvesting 1 leaf after 13 minutes, the other after 26 minutes. Twenty-five μc of $C^{14}O_2$ were supplied to each terminal leaflet.

The pumpkin, *Cucurbita pepo* L., var. Kentucky Field, was cultured in 3X Hoagland's solution in a greenhouse on Sapelo Island, Georgia. The vine was 2.7 m long when the experiment was performed in June 1962. Thirty-five μc of $C^{14}O_2$ were supplied to the youngest fully expanded leaf with the leaf cups placed over the midvein near the leaf tip. Transport samples consisted of basipetally located excised midvein and petiole cut into 2-cm segments and frozen at dry ice temperatures. They were taken 15 minutes after release of the $C^{14}O_2$.

A specimen of mullein, *Verbascum thapsus* L., growing in an old field on Sapelo Island, Georgia was used in July 1962. One hundred μc of $C^{14}O_2$ were supplied to the apical portion of the youngest fully expanded leaf. Transport samples consisting of basipetally located excised midvein segments were harvested 15 minutes after release of the $C^{14}O_2$ and frozen at dry ice temperatures.

Stachyose and Verbascose Identification. The identity of stachyose in these experiments is reasonably certain. The labelled stachyose from each experiment cochromatographed with known stachyose. The chromatographic mobility of the isolated sucrose-raffinose-stachyose-verbascose series followed the relationship by French and Wild (1). Identity of verbascose was based on this relationship. Several unnamed higher members of this series are present in significant amounts in *Verbascum*. Stachyose-C^{14} from the transport area of *Verbascum* was further identified by enzyme cleavage. Almond emulsin or melibiase-free invertase was added at a concentration of 10 mg/ml to individual solutions of stachyose-C^{14} prior to application to chromatographic paper. The invertase treatment resulted in complete conversion to manninotriose-C^{14} (and fructose) which cochromatographed with known manninotriose. Labelled raffinose and galactose in addition to unreacted labelled stachyose were recovered after treatment with almond emulsin. Identity of all hydrolysis products of stachyose were verified by cochromatography with knowns.

The invertase and almond emulsin used were obtained from Nutritional Biochemicals Corporation. The emulsin was labelled Beta Glucosidase but presumably contained an α-galactosidase.

Indicator Sprays Used on Chromatograms. For carbohydrates, 0.5 g of benzidine was added to a mixture of 10 ml of glacial acetic acid, 10 ml of 40 % w/v aqueous trichloroacetic acid, and 80 ml of ethanol. The sprayed paper was heated at ca. 105° for 5 to 10 minutes. For sugar alcohols, the paper was sprayed first with 114 mg H_5IO_5 dissolved in 5 ml water and diluted with 95 ml acetone, followed after drying by spraying with 184 mg benzidine dissolved in 5 ml 1.2 % v/v aqueous acetic acid and brought to 100 ml with 80 % v/v aqueous acetone. White spots on a blue background were produced by sugar alcohols. For amino acids the paper was sprayed with 0.25 %

Table I. *Distribution of C^{14}-Label Recovered from Petiole of Box Elder,* Acer negundo
The experiment was terminated 12 minutes after $C^{14}O_2$ application. These data are expressed as disintegrations per minute per sample.

	Mean distance along petiole					
	1 cm	3 cm	5 cm	7 cm	9 cm	11 cm
Sucrose	84600	53800	39600	12300	2893	487
Glucose	2770	707	592	134	29	67
Fructose	3100	917	611	181	29	38

w/v ninhydrin solution in acetone containing 7 % v/v glacial acetic acid. The sprayed paper was heated for 15 minutes at 45°.

Results

Results of the 12-minute experiment with *Acer negundo* are reported in table I. These radiochemical results are essentially identical with those of Swanson and El-Shishiny (6) for longer term experiments with grape and their conclusions should apply.

The data from the 50-minute experiment is essentially the same and is not reported in detail. The hexoses accounted for up to 30 % of the recovered translocated label adjacent to the supply area, with sucrose making up the remainder. The exudate sample collected at 50 minutes contained labelled sucrose but no detectable hexoses. Although the genus *Acer* is reported to contain traces of raffinose (8), there was no labelled raffinose detected in our experiments.

Results of the 13- and 26-minute experiments with white ash are reported in table II. Stachyose and raffinose, followed by verbascose, sucrose and D-mannitol contain the major fraction of label in the transport region. With the exception of verbascose these are the same compounds which Zimmermann reports to contain the bulk of the carbon in sieve-tube exudate of white ash (9). Our routinely used solvent systems did not separate the sugar alcohols from glucose adequately for individual determination although a good estimate of D-mannitol activity can be obtained by subtracting the fructose from glucose-mannitol label, assuming a 1 to 1 ratio of glucose and fructose. The validity of this assumption was verified on 1 sample by chromatographically separating mannitol and glucose using 80 % phenol as the solvent system. The identity of galactose is based on relative R_F values only and is therefore open to question.

Table II. *Distribution of C^{14}-Label Recovered from Petioles of White Ash,* Fraxinus americana
These data are expressed as disintegrations per minute per sample.

	Mean distance along petiole			
	1 cm	3 cm	5 cm	7 cm
13 minutes after C^{14} application				
Verbascose	716	0	0	0
Stachyose	40000	1060	153	0
Raffinose	6170	487	38	0
Sucrose	4610	430	48	0
Galactose	1203	143	0	0
Mannitol-glucose	2158	162	0	0
Fructose	229	0	0	0
26 minutes after C^{14} application				
Verbascose	23100	6100	...	0
Stachyose	611000	229000	...	49900
Raffinose	110000	47700	...	6900
Sucrose	33800	17900	10900	3870
Galactose	7640	6465	2951	1410
Mannitol-glucose	24200	13800	6991	2610
Fructose	3470	2130	1681	659

The distribution of C^{14} we recovered in a 15-minute experiment with pumpkin is reported in table III. Hexoses represent only 0.1 % of the recovered activity and are not reported. Total recovery of tranported ethanol soluble label in oligosaccharides, including unknown-1, is 63 %; in sucrose, 15.5 %. This is in remarkably good agreement with Pristupa's (5) values of 5 % for hexoses, 57 % for oligosaccharides and 20 % for sucrose in a longer experiment. The major unexpected difference in our data is the relatively small amount of activity recovered in verbascose and the appearance of an unidentified compound of R_F value between raffinose and stachyose. The lower proportion of hexoses we obtained would be expected with shorter experimental times allowing less conversion of translocated materials.

The distribution of recovered C^{14} in *Verbascum thapsus* from areas of transport and supply are reported in table IV. Recovered label from the area of transport represents 72 % of that in the ethanol extract, about half of this, 34 %, occurring in stachyose. This proportion remained relatively constant with distance from supply. Unknown-1 is presumed to be the same unidentified compound found in pumpkin. The transported sucrose-serine values are made up principally of serine. Glucose-C^{14} and fructose-C^{14} amounted to about 2.5 % of the recovered activity. Unknown-2 has an R_F in butanol: ethanol: water equivalent to ribose and fucose, but is neither of these compounds; it reacts with benzidine spray reagent and appears to be present in significant quantities. In this experiment it would appear that stachyose and serine were the major carriers of transported label.

Discussion

In plants in which we consider only one mobile carrier of label such as sucrose in our *Acer negundo*

Table III. *Distribution of C^{14}-Label Recovered from Midvein of Pumpkin,* Cucurbita pepo
The experiment was terminated 15 minutes after $C^{14}O_2$ application. These data are expressed as disintegrations per minute per sample.

	Mean distance of 2-cm sections from supply				Supply lamina
	1 cm	3 cm	5 cm	7 cm	
Verbascose	3230	0	0	0	380,000
Stachyose	44800	1460	754	917	5,730,000
Unknown-1	5770	420	210	201	3,800,000
Raffinose	13500	1260	611	420	3,630,000
Sucrose	13400	2480	1430	774	7,930,000
Unrecovered	18600	3930	1295	1128	...
Total dpm in extract	99300	9550	4300	3440	...
Control lamina, dpm	80	0	0	0	

experiment or in grape (6), the ratio of stationary metabolic derivatives (hexoses) to mobile carrier (sucrose) decreases with distance from supply, giving convincing support for the suggestion of sucrose translocation. This is a relatively straightforward interpretation based on few tenuous assumptions. Introducing a greater spectrum of labelled compounds and the additional variable of mobile metabolic products, such as the raffinose and sucrose derivable from stachyose by the α-galactosidase removal mechanism proposed by Zimmermann (10), complicates the interpretation of the data considerably.

Assuming equal mobilities of derivative products, the galactose removal system should produce a decreasing stachyose-sucrose ratio with increasing distance from supply. This was in fact Zimmermann's (9) observation with respect to ash exudate. In our ash and pumpkin experiments the ratio of label in stachyose to sucrose does decrease markedly from the 1-cm to the 3-cm sample. Although other parameters of the system (3) could account for the decrease in the stachyose-sucrose ratio observed, it might be considered support for the galactose removal hypothesis even though this change in ratio does not appear past the 3-cm sample. The additional variable of sieve-tube loading by stachyose singly or by other mobile compounds as well should not be overlooked.

Let us consider a simple model in which the sieve tube is loaded with stachyose but not with raffinose or sucrose. In this case it is possible to disregard other parameters such as varying rates of lateral transfer and longitudinal movement if we sample all the transported material as we have in the 13-minute ash experiment. If sucrose and raffinose are derived from stachyose by galactose removal in the transport area we might then expect to find the label in galactose to be approximately equal to the label in sucrose plus one third the label in raffinose if the parent stachyose was uniformly labelled. This situation is not approached in the 13-minute ash experiment where we have presumably sampled all the transported material. The alternatives seem to be: A) transported stachyose is not uniformly labelled; B) the galactose-C^{14} removed is preferentially used in the catabolic metabolism of the phloem and is not recovered; C) galactose removed is resynthesized into storage compounds in active storage sites adjacent to the transport stream. In a radiochemical study a transfer such as in alternative C need not even be a net carbon transfer to storage but only a C^{14} to C^{12} exchange between transport channel and adjacent storage cells; in fact the removal of C^{14} from the transport channel undoubtedly accounts for the logarithmic distribution of label with distance from supply (3).

If alternatively, the simple model is not valid and,

Table IV. *Distribution of C^{14}-Label Recovered from Midvein of* Verbascum thapsus
The experiment was terminated 15 minutes after $C^{14}O_2$ application. These data are expressed as disintegrations per minute per sample.

	Mean distance of 2-cm sections from supply								Supply lamina
	1 cm	3 cm	5 cm	7 cm	9 cm	11 cm	13 cm	15 cm	
Stachyose	13500	16800	32000	19700	9740	7640	4020	1590	766,000
Unknown-1	7030	1460	2740	1490	945	697	458	0	2,620,000
Raffinose	4460	5020	7510	6510	3710	2790	1390	688	110,000
Sucrose-serine	19500	17500	16800	9740	4990	3840	0	0	1,780,000
Unknown-2	12600	5310	5310	...	...	1090	0	0	917,000
Unrecovered	...	10210	20640	23360	...	4260	4090	2780	407,000
Total dpm in extract	...	56300	85000	60800	...	20320	9960	5060	6,600,000
Control lamina, dpm	200	0	0	0	0	0	0	0	

the sieve tubes are also loaded with raffinose and sucrose, activity in galactose may be an estimate of the magnitude of the galactose removal system. In our experiments then the inference would be that the galactose removal system was detectable only in white ash, the species for which the proposal was made by Zimmermann (10).

Disregarding possible different mechanisms, it is apparent that the available observations on pumpkin and white ash from both the C^{14} and exudate techniques are basically the same and that the principal compound involved in longitudinal movement of carbon in these 2 plants is very likely stachyose. Stachyose accompanied by serine also appears to be the major carbon carrier in the *Verbascum* experiment.

Summary

Following application of $C^{14}O_2$ to leaves of *Acer negundo* L., *Fraxinus americana* L., *Cucurbita pepo* L., and *Verbascum thapsus* L. for periods of less than 1 hour, samples of midvein and petiole were analyzed to determine distribution of labelled sugars for translocation analysis. Sucrose was the principal labelled material from the transport area of *Acer negundo* L. Stachyose contained the greatest proportion of label in the other 3 species. It is concluded that stachyose may be the principal carbohydrate transported in some species.

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