Selective and Preferential Translocation of C^{14}-Labeled Sugars in White Ash and Lilac[1, 2]

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Introduction

On the basis of sieve-tube-sap analyses of white ash, Zimmermann (15) has concluded that the sugars verbascose, stachyose, raffinose, sucrose and mannitol are translocation substances. Other evidence supporting this conclusion is Pristupa's (11) observation with pumpkin in which he found that translocated C^{14} occurred mainly as stachyose and to a lesser extent as raffinose and sucrose.

Trip et al. (12) showed that leaves of white ash contain, in addition to the above mentioned, the sugars mellibiose, maltose, galactose, glucose, fructose and pentoses. Since there are many sugars in leaves, but only a few in the translocation stream it appears that there is a mechanism which selects the form in which carbohydrates are translocated. To further investigate this selective process a study was undertaken in which C^{14} was introduced either as $C^{14}O_2$ or as C^{14}-labeled sugars. This paper shows that only certain sugars are translocated and that they are translocated at different rates.

Materials and Methods

In the fall of 1962, 30 two-year-old ash (*Fraxinus americana* L.) seedlings were collected in the forest, potted and left outside until use in the summer of 1963. Lilac (*Syringa vulgaris* L.) cuttings were rooted and potted in the fall of 1962 and also placed outside until use. In the selection of experimental material an effort was made to obtain plants of similar size and appearance.

Verbascose-C^{14}, stachyose-C^{14}, raffinose-C^{14}, sucrose-C^{14}, mannitol-C^{14} and fructose-C^{14} were made by biosynthesis from $C^{14}O_2$ using leaves of ash and lilac (12). Galactose-C^{14} was obtained by offering $C^{14}O_2$ to the alga *Rhodemenia palmata*. Sorbitol-C^{14} was prepared by offering $C^{14}O_2$ to detached leaves of apple, variety Northern Spy. The glucose-C^{14} that contaminates the sorbitol-C^{14} in this preparation was eliminated by oxidation using the method of Link and Moore (6).

C^{14} was introduced into attached leaves of young ash and lilac plants using 3 different methods. The first of these consisted of offering $C^{14}O_2$ either in a photosynthesis chamber coupled with an infrared CO_2 analyzer as described by Lister (7) or in a mercury bath chamber, as described by Doman (personal communication). In the second method, a solution containing boron, sodium lauryl sulfate (detergent) and radioactive sugar was placed on the lower or upper surface of the leaves as described by Nelson and Gorham (8). The third method was the "flap" method developed by Biddulph (3) for the introduction of P^{32}. In this method the largest lateral vein of the leaf was severed at the proximal end and partially dissected out to form a flap attached at the distal end. A slim glass tube containing the radioactive sugar solution was slipped over this flap and left until the end of the experiment.

Following the feeding period, the plants were uprooted, cut into pieces as illustrated in figures 1 to 11, and extracted in boiling 80 % (v/v) ethanol. The ethanol extract was assayed for C^{14} and analysed using paper chromatography and autoradiography as previously described (12). Sugar alcohols were detected on paper by dipping chromatograms into the periodate-benzidine reagent described by Gordon (4). Sugars were detected by spraying with benzidine trichloroacetic acid (4). This reagent was sensitive to all the sugars mentioned in this work with the exception of maltose and this sugar was therefore identified by autoradiography alone. Chromatogram spots were cut out and assayed for C^{14} using a methane flow-proportional counter.

Results

In experiment 1, $C^{14}O_2$ was supplied to a terminal shoot of lilac in a polyethylene bag (12) and the translocation of labeled products of photosynthesis was followed (fig 1). One hundred and fifty μc of $C^{14}O_2$ (1.03 mc/mg) were offered at 4000 ft-c at room temperature for 30 minutes. At the end of this time 96 % of the radioactivity offered had been assimilated. The feeding period was followed by 5.5 hours of photosynthesis in laboratory air at normal summer temperature and humidity. This gave a total of 6 hours for translocation of labeled assimilates to occur.

In 6 hours, C^{14}-labeled products of photosynthesis were translocated down the stem. At the end of this period the C^{14} concentration was greater in the

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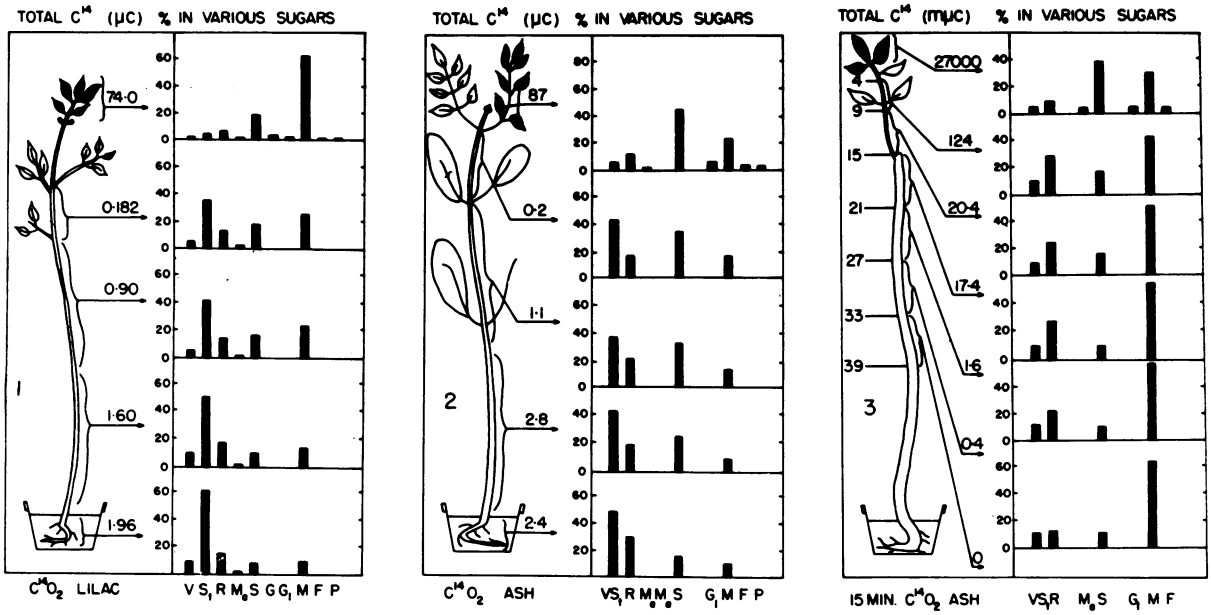


FIG. 1–3. Experiments 1, 2 and 3. Distribution of C^{14} among the sugars of plants offered $C^{14}O_2$. Plants × one-fifth. Supply leaves are blackened. V = verbascose, St = stachyose, R = raffinose, Me = melibiose, Ma = maltose, S = sucrose, G = galactose, Gl = glucose, M = mannitol, F = fructose, P = pentose. FIG. 1. Lilac plant after 30 minutes of photosynthesis in $C^{14}O_2$ followed by 5.5 hours in $C^{12}O_2$. Experiment 1. FIG. 2. White ash plant after 30 minutes of photosynthesis in $C^{14}O_2$ followed by 5.5 hours in $C^{12}O_2$. Experiment 2. FIG. 3. White ash plant after 15 minutes of photosynthesis in $C^{14}O_2$. Experiment 3.

root and lower stem than in the upper stem. Of the total 79 μc of ethanol-soluble C^{14} in the plant, 4.64 μc, or about 5 %, was translocated out of the leaves. Experience with other plants indicates that this is the expected amount of translocation occurring via the phloem in 6 hours (10, 16). The limit of detection of C^{14} in any sugar was 0.01 % of the total in the fed leaf and 0.1 % in the translocate.

Of the sugars occurring in the leaf at least 10 were found to contain label. As previously observed in lilac (12), mannitol was the main product of photosynthesis.

Six sugars, including verbascose, stachyose, raffinose, sucrose and mannitol, occurred in the stem and root as well as in the leaf. With the possible exception of maltose, the labeled sugars isolated from stem and root were all nonreducing, while the leaf contained these same nonreducing sugars as well as several reducing sugars including fructose, glucose, galactose and several pentoses. Even the reducing sugars glucose and fructose, which were abundant in the leaf, were totally absent in the translocate.

The distribution of C^{14} among the 6 sugars in the stem and root was quite different from the distribution of C^{14} among these sugars in the leaf. Expressed as a percentage of total C^{14} in any plant part, there was a decrease in the quantities of translocated mannitol-C^{14} with distance from the supply leaf. The greatest decrease occurred between the supply leaf and the stem section closest to it. Smaller decreases occurred along the translocation pathway. Sucrose-C^{14}, the next most abundant product of photosynthesis, was also found in proportionately decreasing amounts in the stem. The term decrease will be used to describe the proportional decrease of C^{14} in a sugar with distance from the supply leaf and increase will describe the reverse. Thus verbascose, stachyose and raffinose are observed to increase. The relative increase in the higher oligosacharides occurred both on a percentage basis and an absolute basis. The percentage of C^{14} in maltose slowly decreased with distance down the stem.

From these results it is concluded that there are 2 separate processes which determine the distribution of C^{14} among sugars in the translocate. One is the process of selective translocation; it determines the nature of the translocated material. The other process controls the distribution of C^{14} among the translocating compounds and operates on a quantitative basis. This process will be referred to as preferential translocation.

To compare these results with those from another plant, experiment 2 was carried out using white ash instead of lilac. $C^{14}O_2$ was offered for 30 minutes at 4000 ft-c to a compound leaf of a small ash tree. At the end of the feeding period 90 % of the radioactivity had been assimilated. As in experiment 1, this period was followed by 5 and one-half hours of flushing with laboratory air containing $C^{12}O_2$. The results are shown in figure 2.

C^{14} was translocated to all parts, about 7 % (6.5 μc out of 93.5 μc) in 6 hours and the greatest accu-

mulation of C^{14} was found near the root. As in figure 1, sucrose and mannitol were the most abundant products of photosynthesis. Maltose and galactose occurred only in trace amounts.

Of the 9 labeled sugars in the supply leaf, only 5 occurred in the stem or root and all of these were nonreducing sugars. Because of the incomplete separation on some chromatograms, verbascose and stachyose have been combined. Decreases in C^{14} in sucrose and mannitol and increases in oligosaccharides were similar to those found in lilac. In the main, therefore, the results obtained with white ash resemble those obtained with lilac.

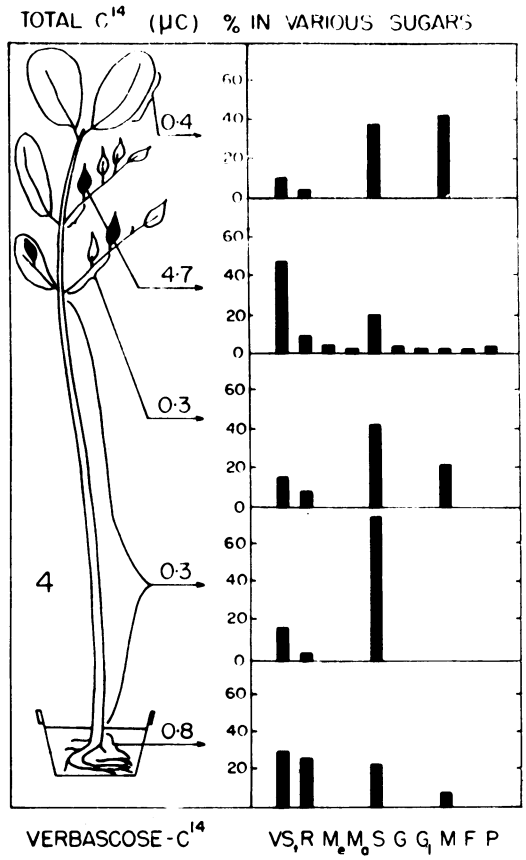
In the experiments described above C^{14} was translocated throughout the plant resulting in a high accumulation in the lower stem and root. Experiment 3 was designed to determine the distribution of C^{14} before it had reached the root.

An ash seedling was taken from the greenhouse and its terminal leaflet was offered $C^{14}O_2$ in a chamber with a mercury seal. After 15 minutes of photosynthesis at 2000 ft-c the plant was cut into pieces and killed in boiling 80 % ethanol. The results of this experiment are given in figure 3.

C^{14} was translocated approximately halfway down the stem to the root and the amount of total C^{14} in each piece of stem decreased with distance from the supply leaf. These observations suggest that most of the translocated C^{14} was still in transit. Translocated C^{14} occurred in the form of stachyose, raffinose, sucrose, and mannitol and all 4 sugars were present at the translocation front. While stachyose was observed to increase and sucrose to decrease as in figures 1 and 2, raffinose decreased and mannitol increased considerably.

In attempts to introduce C^{14}-labeled sugars to the intact surface of leaves of white ash seedlings, sucrose-C^{14} and mannitol-C^{14} were applied in solution as described by Nelson and Gorham (8). No translocation of the label was observed and this result was held to be due to the waxy cuticle of white ash leaves.

To circumvent the barrier posed by the cuticle, several labeled sugars were introduced into leaves using Biddulph's flap technique. A preliminary ex-



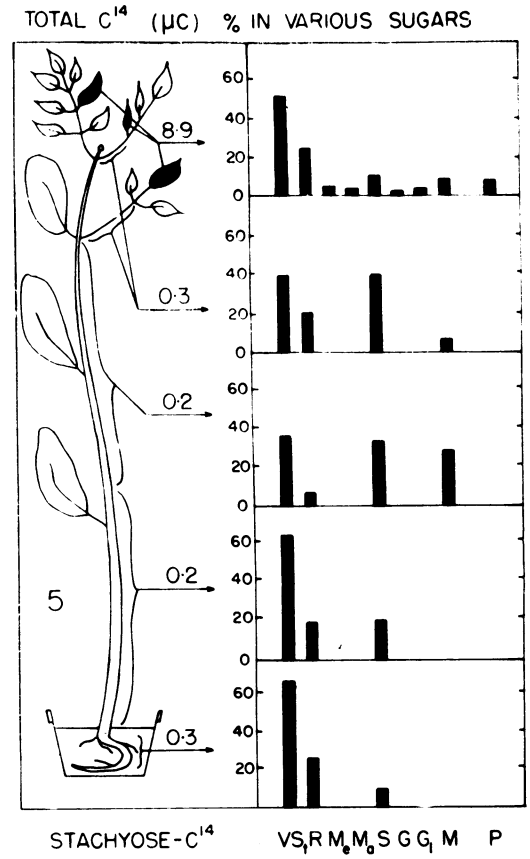


FIG. 4–11. Experiment 5. Distribution of C^{14} among sugars of white ash plants offered various C^{14}-labeled sugars for 24 hours. Plants × one-fifth. Fed leaves are blackened. FIG. 4. Verbascose-C^{14} offered. FIG. 5. Stachyose-C^{14} offered. FIG. 6. Raffinose-C^{14} offered. FIG. 7. Sucrose-C^{14} offered. FIG. 8. Galactose-C^{14} offered. FIG. 9. Mannitol-C^{14} offered. FIG. 10. Fructose-C^{14} offered. FIG. 11. Sorbitol-C^{14} offered.

periment (expt 4) was carried out as follows: 10 μc of either sucrose-C^{14}, mannitol-C^{14} or fructose-C^{14} were offered via a flap in a lateral vein to each of 3 leaves of potted lilac plants. After 6 hours of feeding, plants were cut into several pieces, as in experiments 1 to 3 and analysed for their C^{14} content.

In the mannitol-C^{14} fed plant, C^{14} was found only in the fed leaf and only in the mannitol itself. This result agreed with the observation that when mannitol-C^{14} is offered via a cut vein there is a lag period to mannitol dissimilation in leaves of higher plants (13).

In the fructose-C^{14} fed plant, C^{14} was located in stachyose, raffinose, sucrose, mannitol, fructose and glucose in the fed leaf, but only in stachyose in the stem below the fed leaf. This result indicates the dominant nature of stachyose in the translocation of the sugars of ash. In the leaf fed sucrose-C^{14} label was found in the same sugars as noted for the fructose-C^{14} fed leaf. In the stem below the supply leaf, C^{14} was found primarily in stachyose and in smaller amounts in raffinose and sucrose. The results led to the encouraging conclusion that C^{14}-labeled sugars introduced via a flap were indeed translocated and, moreover, that there was control over the form in which the translocated C^{14} occurred.

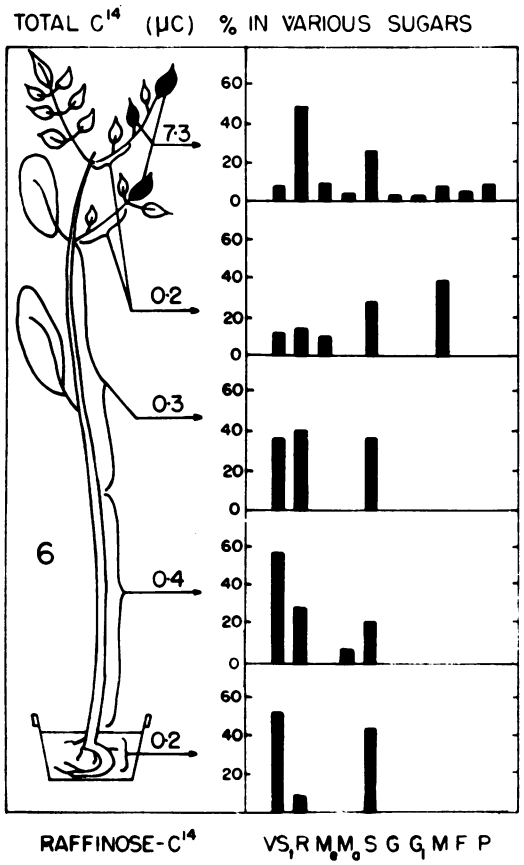
It now appeared feasible to use the flap technique for the introduction of the whole range of C^{14}-labeled sugars prepared earlier in this work. In experiment 4, introduced mannitol-C^{14} had neither been metabolized nor translocated in 6 hours. In experiment 5, therefore, the experimental time was lengthened to 24 hours. Verbascose-C^{14}, stachyose-C^{14}, raffinose-C^{14}, sucrose-C^{14}, galactose-C^{14}, mannitol-C^{14}, fructose-C^{14}, and sorbitol-C^{14} were introduced separately into the leaves of white ash plants using the flap technique.

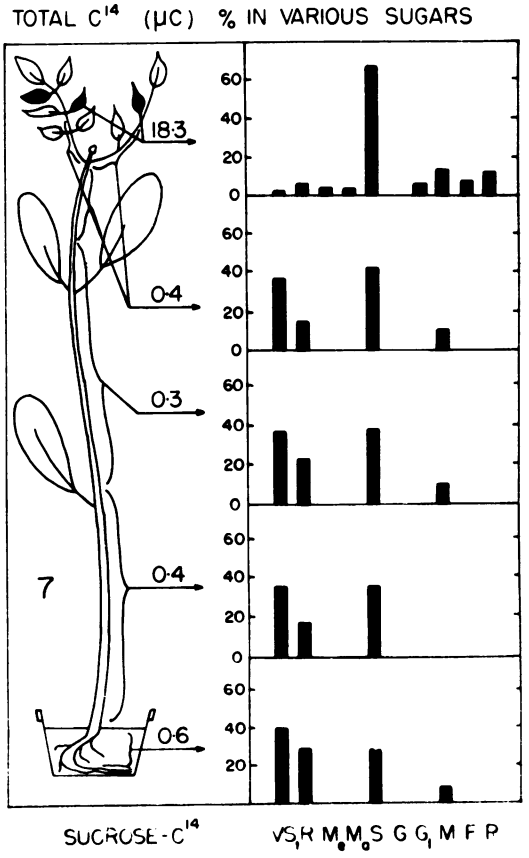
Each labeled sugar was introduced simultaneously into 3 leaflets on 3 different leaves of a single white ash tree. The results are shown in figures 4 to 11.

The limit of detection varied from 0.1 % in the supply leaves to 3 % in the plant sections which contained 0.2 μc or less. The same 11 sugars, which accumulated C^{14} in the supply leaves when $C^{14}O_2$ was offered, did so when C^{14} was offered as any of the C^{14}-labeled sugars. The pools of various sugars in the leaf were therefore all connected by metabolic pathways so that interconversions took place.

The amounts of C^{14} in any sugar found in the supply leaf varied with the source of C^{14} offered. On the whole, a slightly larger proportion of the C^{14} offered was translocated than was the case in experiments 1 and 2. This was to be expected since the experimental time was 4 times longer in experiment 5.

A comparison of the histograms in figures 4 to 10 shows that translocated C^{14} was found in ver-





bascose, stachyose, raffinose, sucrose, and mannitol. It is striking that of the 10 sugars formed in the supply leaf only these 5 nonreducing sugars were found in the stems. The changes in relative amounts of these 5 sugars in different parts of the stem, and the absence of phosphorylated intermediates and reducing sugars that are expected when metabolic interconversions take place indicates that the sugars were independently translocated and accumulated in the stems of white ash.

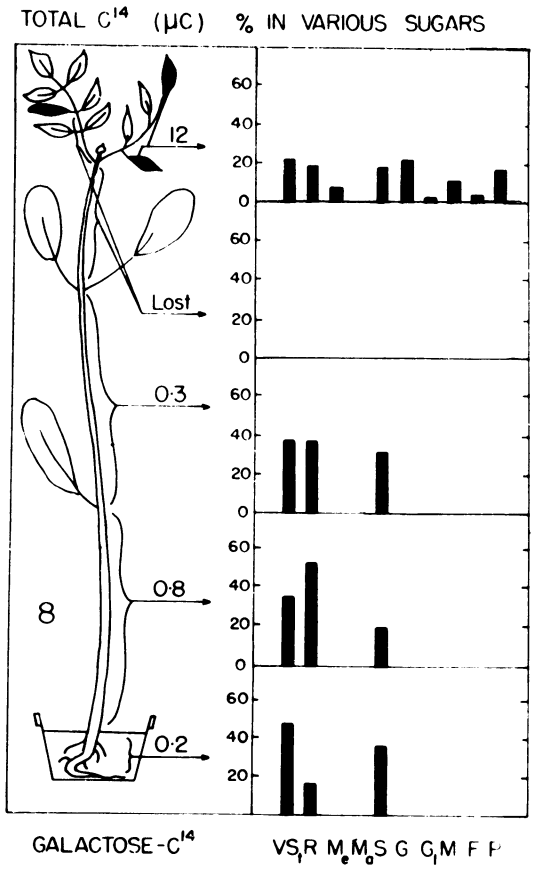
Figures 4, 5, 6 and 7 show that the translocation stream in the stems was made up largely of sucrose and the sucrose-containing oligosaccharides when these sugars were offered to the supply leaves. When the hexoses, galactose or fructose were fed (fig 8, 10), sucrose and the sucrose oligosaccharides were the only sugars found in the stems. On the other hand, when mannitol was fed (fig 9) it was the main compound isolated from the stem even though the sucrose sugars were also present in small amounts.

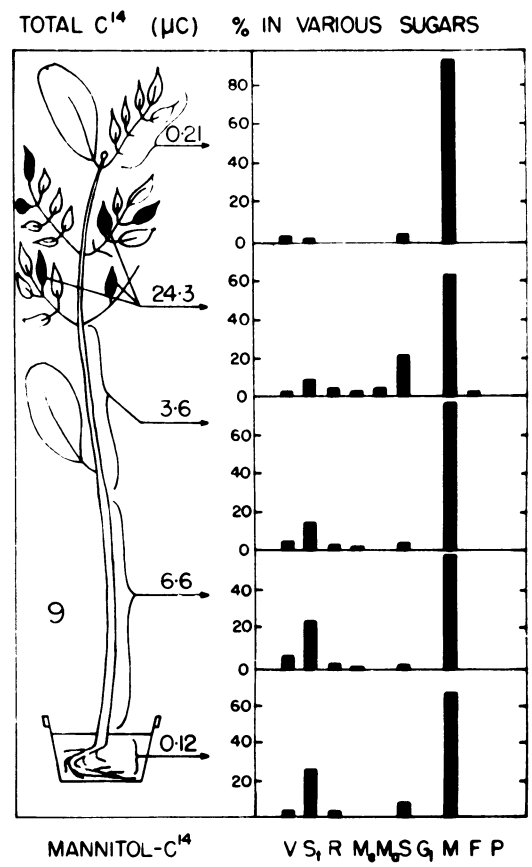
The plant fed sorbitol-C^{14} (fig 11) showed an entirely different distribution of C^{14}. Only 7 % of the ethanol-soluble C^{14} in the fed leaf was present in substances other than sorbitol. Out of 11 compounds isolated on paper only 3 cochromatogrammed with known substances. Only 1 % of the total soluble C^{14} was translocated out of the leaflets and even this amount did not travel far. However, the experiment demonstrates that sorbitol can be translocated in white ash.

Discussion

Nelson et al. (9) have suggested that the translocation of a C^{14}-labeled product of photosynthesis from its site of synthesis in the leaf into and down the stem may be influenced by the rates of any one or more of the following processes: A) CO_2 assimilation; B) synthesis of labeled compound; C) mixing of labeled compound with endogenous unlabeled compound; D) local utilization of the mixed endogenous pool; E) translocation of the compound from the site of its synthesis to the vein; F) longitudinal translocation of the compound through the vein, petiole and stem; G) radial translocation of the compound from the conducting elements to the surrounding tissues; H) accumulation and metabolism of the compound in the conducting and surrounding tissues.

In the experiments described in this paper, the concentrations of different sugars in the supply leaf were altered in attempts to modify the relative rates of these individual processes and thereby to evaluate their importance in the overall translocation system. All of the processes A to H may influence the quantities of C^{14} translocated. Process E, in addition, controls the quality or the nature of the C^{14} translocated





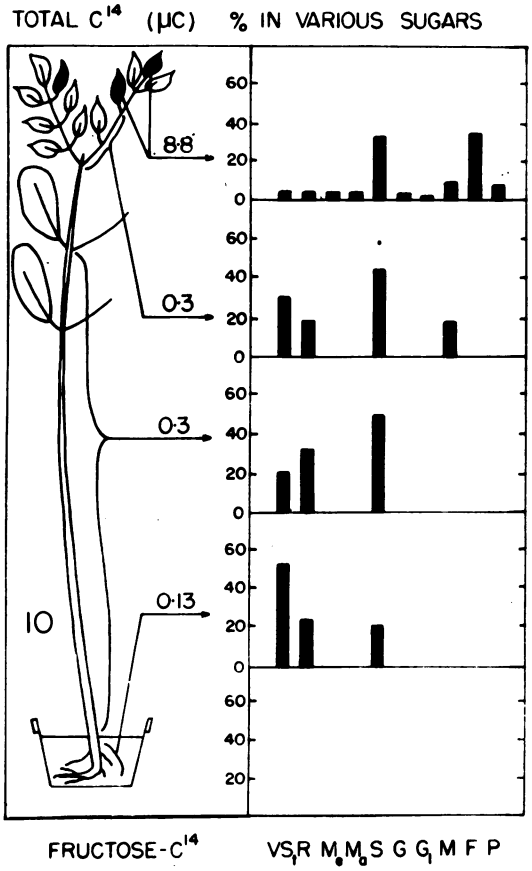
and is a selective process. Since selection implies metabolic control, the process of transferring material from the leaf lamina to the sieve tubes in the vein has been called loading by Barrier and Loomis (2) and "sekretion" by Ziegler (14) and it has been described by Kursanov (5) and Zimmermann (15). In the processes controlling movement along the petiole and stem (F–H) one sugar may be preferred over another. This situation will be referred to as preferential translocation.

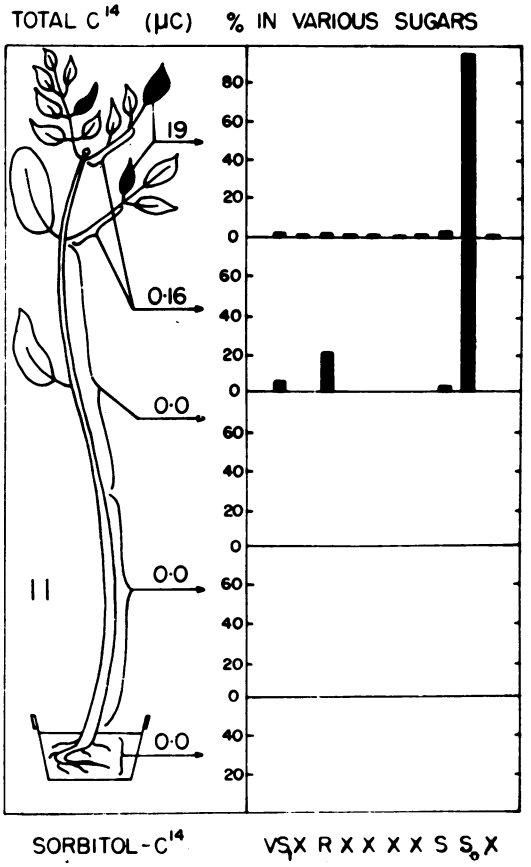
Selective Translocation. From the results of experiments 1, 2 and 5, it was concluded that verbascose, stachyose, raffinose, sucrose, mannitol, and sorbitol were translocated, while galactose, glucose, fructose and pentose were not. Even when C^{14} was offered as galactose-C^{14} (fig 8) or fructose-C^{14} (fig 10) and the leaves contained far greater than normal amounts of these sugars, no translocated C^{14} was found in these sugars. From this it is clear that the selectivity of process E (transfer into the conducting tissue) was not altered by the amount of reducing sugar in the supply leaf.

Because all of the more abundant nonreducing sugars of lilac and white ash leaves were translocated and none of the reducing sugars were (with the possible exception of maltose), it is suggested that the nonreducing property of a sugar is related to its function as transport material. In this connection it is interesting to note that Bacon (1) has suggested a transport function for 1^F–fructosylsucrose, 6^G–fructosylsucrose and 6^F–fructosylsucrose. These nonreducing trisaccharides have been identified in *Allium cepa* and a few other plants.

The data of experiments 1, 2 and 5 have been used as a basis for the calculations shown in table 1, column 3. The amount translocated is a measure of the relative importance of a sugar in its translocation from the site of its synthesis to the vein (process E). Thus when $C^{14}O_2$ was offered to lilac 31 % of the total verbascose-C^{14} in the plant occurred in the translocate while only 1.2 % of the total mannitol-C^{14} did. The order of importance was usually verbascose-stachyose, raffinose, sucrose, mannitol. There were exceptions to this order. When a labeled sugar was offered its percent translocated was less.

When C^{14}-labeled sugars are introduced through a cut vein there are at least 3 distinguishable pools of sugar in the leaf. One is in the xylem (13), another in the mesophyll and a third in the phloem. In the present work the supply leaves were extracted as a whole so that the 3 pools were not measured separately. It is probable, therefore, that a portion of the sugar offered was still in the xylem and not available for translocation. This resulted in the lower amount translocated and the exceptions in the order of importance noted above.





Preferential Translocation. The processes controlling movement along the petiole and stem are preferential in nature. The preference can be assessed by calculating the change in percentage of C^{14} in each sugar in the petiole stem or root as distance from the supply leaf increased (table I, column 4). Thus when $C^{14}O_2$ was offered to lilac, stachyose accounted for 34.4 % of the C^{14} in the petiole and 60 % of the C^{14} in the root giving a change of + 25.6. Comparison of the results obtained when different sugars were offered indicate that the order of preference was usually stachyose, verbascose, raffinose, sucrose and mannitol.

With the techniques presently available it is not possible to distinguish moving sugar molecules from stationary ones. It is, therefore, quite possible that the changes in percentage of translocated C^{14} among sugars are due to differential rates of accumulation rather than of translocation. Which of these 2 processes is primarily responsible for preferential translocation, is an open question.

Summary

C^{14} was introduced into attached leaves of ash and lilac plants either as $C^{14}O_2$ during photosynthesis, or as verbascose-C^{14}, stachyose-C^{14}, raffinose-C^{14}, sucrose-C^{14}, galactose-C^{14}, mannitol-C^{14} or fructose-C^{14}. Biddulph's flap technique was used to introduce the labeled sugars and yielded results compar-

Table I. *Comparison of the Amounts of Sugar Translocated and the Changes in Percentage of C^{14} in Any Sugar from Petiole to Root in White Ash and Lilac after the Introduction of Either $C^{14}O_2$ or C^{14}-Labeled Sugars*

Sugar offered	Sugar translocated	Amount translocated %	Change in percentage from petiole to root
Lilac	Verbascose	31	+ 2.4
$C^{14}O_2$	Stachyose	54	+25.6
	Raffinose	10.7	+ 3.0
	Sucrose	2.8	−12.1
	Mannitol	1.2	−17.7
White ash	Verb.-Stach.	37	+ 7.2
$C^{14}O_2$	Raffinose	11.5	+17.7
	Sucrose	3.7	−18.2
	Mannitol	3.1	− 7.0
Verbascose	Verb.-Stach.	15.7	+ 5
	Raffinose	40.0	+17
	Sucrose	41.5	−18
	Mannitol	69.5	−10
Stachyose	Verb.-Stach.	10.2	+27
	Raffinose	12.2	+ 6
	Sucrose	22.6	−28
	Mannitol	13.4	− 7
Raffinose	Verb.-Stach.	61.5	+42
	Raffinose	7.2	− 8
	Sucrose	14.0	+14
	Mannitol	15.3	−38
Sucrose	Verb.-Stach.	81.5	+ 4.0
	Raffinose	30.3	+10.5
	Sucrose	4.6	−11.5
	Mannitol	4.2	− 3.0
Galactose	Verb.-Stach.	16.4	+12
	Raffinose	21.0	−20
	Sucrose	12.8	+ 6.5
	Mannitol	0	...
Mannitol	Verbascose	69	+ 0.6
	Stachyose	53	+ 6.5
	Raffinose	36	− 1.1
	Sucrose	3.7	+ 4.4
	Mannitol	32	−10
Fructose	Verb.-Stach.	47	+26
	Raffinose	33	+ 7.5
	Sucrose	9.7	−21
	Mannitol	1.7	−15.5
Sorbitol	Verb.-Stach.	14	...
	Raffinose	34.5	...
	Sucrose	2	...
	Sorbitol	.69	−77

able with those obtained after offering $C^{14}O_2$. The nonreducing sugars verbascose, stachyose, raffinose, sucrose and mannitol were translocated while the reducing sugars melibiose, galactose, glucose, fructose, and pentose were not. The evidence suggests that there are 2 processes which control the translocation of sugars. One is a selective process in which only nonreducing sugars are translocated. The other results in different amounts of sugar being translocated and is called preferential translocation.

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