

STUDIES ON PHLOEM EXUDATION FROM *YUCCA FLACCIDA* HAW. XI. XYLEM FEEDING OF ¹⁴C-SUGARS AND SOME OTHER COMPOUNDS, THEIR CONVERSION AND RECOVERY FROM THE PHLOEM EXUDATE

P. M. L. TAMMES¹, C. R. VONK¹ and J. VAN DIE²

¹ Centrum voor Plantenfysiologisch Onderzoek, Wageningen

² Botanisch Laboratorium, Utrecht

SUMMARY

For a number of ¹⁴C-sugars and other compounds, it has been shown, that their introduction through the xylem system into detached tops of the inflorescence causes a subsequent exudation of ¹⁴C-sucrose from the phloem. D-glucose, D-fructose, D-galactose, maltose and lactose were converted exclusively to sucrose. The fed substance itself was absent in the exudate. Feeding D-sorbitol and K-glycerate led to exudation of these substances together with small amounts of sucrose, whereas L-sorbose, glycerol, glycolic acid and glutamine were not at all converted to sucrose and exuded as such. The results are briefly discussed.

1. INTRODUCTION

A previous paper has shown that detached inflorescence stalks from *Yucca flaccida* can be fed at one end with labelled sugars. The label may be subsequently recovered at the other end in the phloem exudate (TAMMES, VONK & VAN DIE 1967). Since the xylem in the monocotyledonous bundle is adjacent to the phloem, passage through other tissues before reaching the phloem seems unlikely.

Although other investigators have also carried out feeding experiments with phloem, their conclusions are often deduced from analysis of total phloem, together with other tissues from bark, midribs or veins. In *Yucca*, the label can be analysed in the sieve-tube exudate, which is produced in sufficient quantity to be analysed.

The present paper describes some experiments in which ¹⁴C-sugars and some other substances are infiltrated through the xylem vessels and the collected phloem exudate subsequently analysed.

2. MATERIAL AND METHODS

Young detached *Yucca* stalks 20 cm long were placed with their lower ends in 2 ml of a 1% sugar solution containing 5 μ Ci of ¹⁴C label. For other compounds, the same procedure was followed. Exudate was harvested from the cut

top. When necessary a new slice was cut off from the upper end to facilitate exudation.

When activity in the exudate was sufficient, a paper chromatogram was run with the solvent of SERMANI (1956).

Controls for reference substances were run with the ^{14}C -substances involved and with ^{14}C -sucrose, all dissolved in droplets of unlabelled exudate. Of some chromatograms, the sucrose spot was cut out, extracted, inverted and rechromatographed before scanning the hexose moieties.

3. OBSERVATIONS

The table gives the results of the experiments with the substances selected for this study.

4. DISCUSSION

The substances used in this study have not been selected deliberately: besides some common and less common sugars, some metabolic intermediates of carbohydrate metabolism have been tested, as well as a few organic acids and glycerol. Glutamine is the main amino acid of the phloem exudate of *Yucca* (VAN DIE & TAMMES 1964) and was therefore included in this investigation.

Some special features about lactose, maltose and sorbitol may be briefly mentioned. Although a rare sugar in higher plants, lactose has been found on infiltration to be converted into sucrose in barley (MCCREARY & HASSID 1941). Maltose, a much more common carbohydrate in plants, is readily metabolized when infiltrated into them, forming sucrose and starch (REYNOLDS et al. 1958).

Sorbitol is the principal sugar-like substance translocated through the phloem of *Malus*; sucrose is second in radioactivity after ^{14}C -labelling (WEBB & BURLEY 1962). BIELESKI (1969) reported an unchanged accumulation of this sugar alcohol in apple bark. Also in other higher plants (*Cucumis*, *Fraxinus*, *Salix* and *Syringa*), sorbitol has been found to be phloem-mobile (CRAFTS & CRISP 1971). Although it normally does not occur in any significant quantity in the phloem exudate of *Yucca*, the vascular tissues apparently absorb it from the apoplast and translocate it to the sieve-tube lumen. The presence of a small amount of ^{14}C -sucrose in the sieve-tube exudate, together with ^{14}C -sorbitol as main compound indicates that there is a conversion mechanism – though not very active – in the *Yucca* bundles.

A substance introduced through the xylem can be supposed to reach the adjacent phloem through the cell walls (apoplast or free space) and is thus an exogenous application. Its movement may be different from the normal translocation of sugars from mesophyll to phloem.

KURSANOV and co-workers (1967, 1969) and BROVCHENKO (1967, 1970), however, suppose a passage of photosynthates from mesophyll to phloem also through the free space, but GARDNER & PEEL (1971) differentiate a direct and an indirect pathway. In both, there is a considerable difference in osmotic concen-

Table: Substances supplied to the basis of detached inflorescence stalks and the compounds derived from them, as found in the bleeding sap from the upper part.

supplied in external solution	labelled compounds present in the exudate	remarks
$^{14}\text{C}(\text{U})$ -sucrose	sucrose	asymmetric labelling of the hexose moieties; ^{14}C -gluc./ ^{14}C -fruct. = approximately 3:1.
idem + excess of ^{12}C -fructose	sucrose	asymmetric labelling as above.
idem + excess of ^{12}C -glucose	sucrose	asymmetric labelling as above.
$^{14}\text{C}(\text{U})$ -maltose	sucrose	^{14}C -maltose not detectable on chromatograms of the exudate sugars.
$^{14}\text{C}(\text{U})$ -lactose	sucrose raffinose (?)	raffinose "identified" by its R-sucrose only; ^{14}C -galactose not detectable in the exudate.
$^{14}\text{C}(\text{U})\text{D}$ -glucose	sucrose	asymmetric labelling of the hexose moieties; ^{14}C -gluc./ ^{14}C -fruct. = $\gg 1$.
$^{14}\text{C}(\text{U})\text{D}$ -fructose	sucrose	asymmetric labelling of the hexose moieties; ^{14}C -gluc./ ^{14}C -fruct. = approximately 1:7.
$^{14}\text{C}(\text{U})\text{D}$ -galactose	sucrose	galactose not detectable on exudate chromatograms.
$^{14}\text{C}(\text{U})\text{L}$ -sorbose	sorbose	
$^{14}\text{C}(\text{U})\text{D}$ -sorbitol	sorbitol sucrose	sorbitol was main constituent.
UDP- $^{14}\text{C}(\text{U})\text{D}$ -glucose	sucrose glucose	sucrose was main constituent.
^{14}C -fructose - 1:6 - diphosphate (NH_4 -salt)	sucrose	
^{14}C -fructose - 6 - phosphate (NH_4 -salt)	sucrose	
^{14}C -glyceric acid (K-salt)	glyceric acid sucrose glucose	glyceric acid was the main labelled constituent, followed in decreasing order by sucrose and glucose.
^{14}C -glycollic acid (K-salt)	glycollic acid. unknown substances	glycollic acid was the main constituent beside considerable amounts of an unknown spot. Traces of other unknown spots were also present in the exudate.
^{14}C -glycerol	glycerol	An unknown, distinct spot was also present on chromatograms.
$^{14}\text{C}(\text{U})\text{L}$ -glutamine	glutamine	

tration between the phloem elements (high) and free space or parenchyma (low).

From the data presented, it may be concluded that the vascular bundle cells between xylem vessels and sieve tubes convert absorbed sugars mainly to sucrose. Where these conversions occur, in xylem or phloem parenchyma or in companion cells, cannot be ascertained. It is even possible that the sieve tube itself, its walls or perhaps immobile protoplasmic structures in it, are responsible for some of them.

Apparently all the substances tested are absorbed by xylem parenchyma cells and either translocated as such to the sieve-tube lumen (sorbose, sorbitol, glycerol, glycolic acid) or converted to sucrose. Or the substances reach the phloem through the apoplast and are there converted or taken up unchanged. Hydrolysis of supplied disaccharides to hexose moieties appears to be a first step in the reaction sequence. Maltose, sucrose and lactose are converted to glucose, fructose and galactose. Each of these can be converted to sucrose as has been demonstrated in the feeding experiments. The next steps will be the phosphorylation of the hexoses by ATP or UTP, and their interconversion. Some of these substances can also be found in the exudate after feeding ^{32}P , as has been reported by TAMMES & VAN DIE (1966) and by VAN DIE (1968) and by KLUGE, BECKER & ZIEGLER (1970). That the phloem exudate of *Yucca* can even produce them itself from added ^{32}P (BECKER, KLUGE & ZIEGLER 1971) should be a warning against underestimating a possible metabolic role of the sieve-tube contents and against attributing all metabolic activity to other phloem cells. Final steps are the synthesis of sucrose and its exclusive appearance in the sieve-tube lumen.

A remarkable observation is the preference of glucose in the synthesis of exudate-sucrose from supplied ^{14}C (U) sucrose. The recovered sucrose has about three quarters of its label in the glucose moiety. This ratio is not changed if excess inactive glucose or fructose were supplied together. Although HATCH & GLAZIOU (1964) consider that sucrose can be taken up by the sieve tubes without hydrolysis, BROVCHENKO (1967) observed that asymmetric sucrose with an excess of ^{14}C -glucose was found in the vascular bundles of sugar-beet leaves when ^{14}C (U) sucrose was given. KURSANOV, SOKOLOVA & TURKINA (1969) reported that glucose inhibits the uptake of fructose or galactose but not the reverse. The present *Yucca* data could possibly be explained in a similar way, but further research will be needed.

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