Acta Bot. Neerl. 23 (5)–(6), October–December 1974, p. 715–722.

DIFFERENT RATES TRANSLOCATION OF ^{14}C-L-α-ALANINE (U) AND TRITIATED WATER IN THE XYLEM VESSELS OF TOMATO PLANTS

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SUMMARY

A solution of 5 mM ^{14}C-L-α-alanine in tritiated water was allowed to perfuse the xylem vessels of tomato stem segments. In each stem segment an exponential distribution gradient for THO was found, the slope of which was dependent on the length of the stem segment and the perfusion velocity. Nearly all the THO was retained in the stem segment whereas a great amount of ^{14}C-alanine could be recollected. Thus the ^{14}C-alanine was recollected in a solution of other water molecules than those it was introduced in.

A similar phenomenon could be observed, if ^{14}C-L-α-alanine or ^{14}C-D-sucrose dissolved in THO were allowed to flow through a Sephadex G-10 column.

The results clearly demonstrate, that mass flow in xylem vessels goes along with exchange and diffusion-like processes. Moreover experimentally they show that different velocities for the movement of organic solutes and THO in other long distance translocation systems (sieve tubes) – as reported in literature – need in no way be in conflict with an assumed longitudinal mass flow of their contents.

1. INTRODUCTION

Some amino acids perfusing an isolated stem segment of a tomato plant are distributed logarithmically along the stem segment (VAN BEL 1974). The lateral transport is believed to be a diffusion-like process, which proceeds simultaneously with a longitudinal solution flow.

According to several investigators (O'LEARY 1965, MORRISSON & HEINE 1966, HEINE 1970) xylem translocation is exclusively a matter of mass flow under control of the hydrodynamic forces without chromatography-like processes along the xylem vessel walls.

Following xylem translocation in bushbean stems BIDDULPH et al. (1961) have found different longitudinal transport rates for Ca and THO. BELL & BIDDULPH (1963) have explained the relative immobility of Ca by supposing a longitudinal process of exchange. EMMERT (1965, 1969), who has made experiments with Ca and Sr in bean plant xylem, and THOMAS (1967) in his paper about dye and calcium translocation in dogwood tree xylem have agreed with their view.

PEEL et al. (1969) have concluded from the different absorption gradients for ^{14}C-labelled sugars, ^{32}P and THO, administered simultaneously, that phloem translocation in willow branches could not be a mass flow. Girdling experiments using ^{35}S, ^{32}P and THO confirmed his opinion (PEEL 1970). TRIP & GOR-

HAM (1968) have expressed doubts of the linkage between THO- and ^{14}C-sucrose transport in squash plants, while CHOI & ARONOFF (1966) have suggested that mass flow is at least not the dominant factor in soybean phloem translocation.

After reconsidering the Horwitz's mass flow model and experiments with *Beta* leaves CATALDO et al. (1972a, b), on the contrary, have concluded that mass flow is the main translocation mechanism for dissolved materials in the phloem.

In the present experiments THO-solutions of ^{14}C-L-α-alanine were allowed to perfuse excised tomato stem segments. A simulation of these experiments is obtained by using Sephadex G-10 columns, assumed to replace the apoplasmic part of the xylem of the tomato stem.

2. MATERIALS AND METHODS

A. 1 ml THO-solution of 5 mM ^{14}C-L-alanine was allowed to perfuse tomato stem segments (*Lycopersicon esculentum* cv. All Round).

The methods for preparing the stem segments have been described in a previous paper (VAN BEL 1974). After preparation silicone tubes were fitted around both ends of the stem segment, the rest was wrapped in a plastic sheet. The segment (the morphological lower end on top) was placed in a vertical position on a fraction collector and 50 µl or 60 µl drops leaking out of the stem segment were recovered by means of a drop nozzle put in the lower silicone tube. After the amino acid perfusion 1 ml distilled water was allowed to perfuse and was also recovered by the fraction collector. All drops were dissolved in 15 ml scintillation liquid (VAN BEL 1974) and counted in a liquid scintillation spectrometer (Packard).

Immediately after the experiment the fresh stem segment was cut into sections of 10 mm length, which were separately frozen for 24 hours. All sections were ground and 100 µl of each fraction was counted in 15 ml scintillation liquid.

B. 0.5 ml of a ^{14}C-L-alanine (5 mM) THO-labelled solution was allowed to flow through a Sephadex G-10 (Pharmacia, Uppsala, Sweden) column (diameter 1.7 cm, length 27 cm). 50 µl blue dextran solution (Mw 2,000,000; Sigma Chemical Company, St. Louis, USA) was added to the alanine solution. The drops were recovered in a fraction collector connected with an Uvicord (LKB Produkter AB, Stockholm). The drops were counted in the scintillation liquid mentioned above.

The same procedure was applied to 0.5 ml 5 mM ^{14}C-D-sucrose THO-labelled solution.

The labelled compounds were obtained from the Radiochemical Centre, Amersham, U.K. Specific activity of THO, L-alanine and D-sucrose were respectively 5 Ci/ml, 10 mCi/mMol and 10.4 mCi/mMol.

3. RESULTS

3.1. Outflow patterns after the simultaneous perfusion of THO and L-alanine

The experiments (perfusion of 1.00 ml 5 mM ^{14}C-L-alanine in THO followed by 1.00 ml distilled water) were carried out to determine whether alanine and water transport are coupled. As shown in *fig. 1* the outflow pattern of L-alanine was similar to that found earlier (VAN BEL 1974). Its typical shape reflects three stages which may be distinguished:

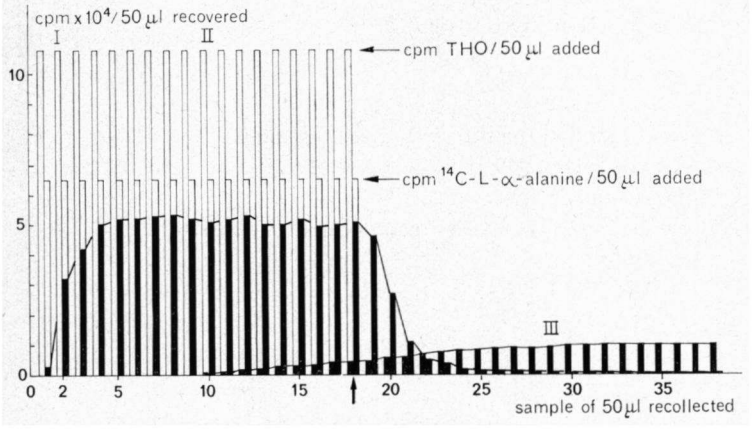

Fig. 1. Experiment 1. Outflow pattern of 5 mM L-α-alanine dissolved in THO during perfusion through a tomato stem segment of 122 mm length. Flow rate: 540 mm^3hr^{-1}. ↑ = additon of 1 ml distilled water.

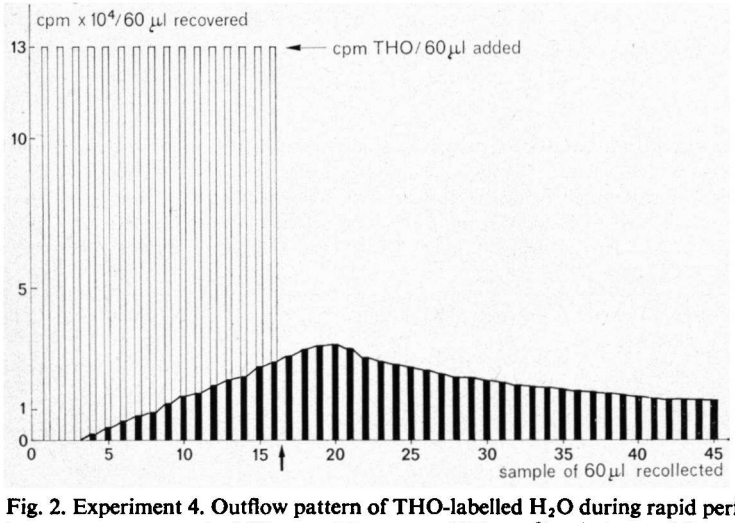

Fig. 2. Experiment 4. Outflow pattern of THO-labelled H_2O during rapid perfusion through a tomato stem segment of 135 mm. Flow rate: 1154 mm^3hr^{-1}. ↑ = addition of 2 ml distilled water.

I. an initial stage in which the xylem vessels and the free space around them are filled.
II. a steady-state in which a constant amount of L-alanine is retained per sample perfused.
III. a final stage in which the L-alanine in the free space and xylem vessels is washed out.

The fractional loss, *K, of L-alanine from the xylem vessels per unit length of the stem according to the calculations of HORWITZ (1958) concerning irreversible loss of dissolved molecules is:

$$*K = \frac{\ln C_o/C_P}{L} = \frac{2.303\ (\log C_o - \log C_P)}{L} \qquad (1), \text{ in which}$$

C_O is the radioactivity added at the top of the stem segment
C_p is the radioactivity recollected at the lower end of the stem segment
L is the length of the stem segments (mm).

The fractional loss of L-alanine in the steady-state can be written as

$$*K_p \text{ alanine} = \frac{2.303\ (2 - \log C_s)}{L} \qquad (2)$$

where C_s is $100 \times \frac{\text{cpm recovered}}{\text{cpm added}}$ in the steady-state *(fig.1,* stage II*)*.

The results of the double label experiments are summarized in *table 1*. The results for L-alanine correspond to previous data (VAN BEL 1974). The outflow pattern for solute and solvent are completely different: the outflow of THO has hardly started when the outflow of L-alanine has already stopped.

Table 1. The influence of the length of a stem segment and the perfusion flow rate of 5 mM ^{14}C-alanine solved in THO on the *Kp-alanine-value and the amount of THO recollected. The flow rate (ApV) is computed from the perfusion velocity of the drops recovered. *Kp-alanine is the fractional loss of L-alanine along the stem segment in the steady state (*fig. 1,* stage II). The percentage THO recollected is listed before (1 ml) and after (2 ml) the perfusion of the washing distilled water.

experiment,	duration (hr)	length of the stem segment	flow rate	*Kp-alanine	% recollected THO after perfusion of	
		mm	mm^3hr^{-1}	mm^{-1}	1 ml	2 ml
1	3.40	122	540	0.0020	1.2	9.6
2	2.90	178	606	0.0016	0.5	4.1
3	3.32	110	638	0.0018	2.4	10.7
4	1.85	135	1154	0.0025	9.3	26.5

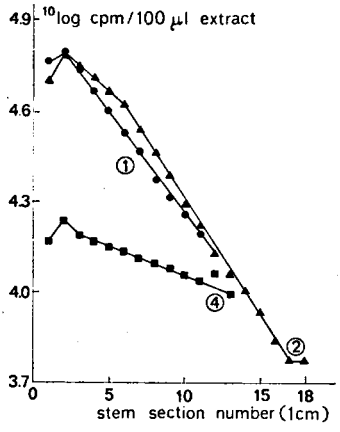

Fig. 3. Plotting the ^{10}log cpm THO/100µl extract against the stem section number after sectioning the stem segment in pieces of 1 cm. The length of the stem segments is 12.2 cm, 17.8 cm and 13.5 cm. respectively (experiment 1 ●–●, 2 ▲–▲, 4 ■– ■). The flow rates are 540, 606 and 1154 mm^3 hr^{-1}.

3.2. Influence of the length of the stem segment and the flow rate on the retention of THO

Because of the absence of a steady-state for THO *(fig. 1)* it was impossible to determine *K_pTHO. By counting the radioactivity of ground 10 mm sections an exponential accumulation gradient for THO (*K_sTHO) could be found. *K_sTHO represents the fractional amount of THO absorbed per unit length of the stem segment. *K_sTHO can be compared with *$K_p$alanine as *$K_p$alanine ≈ *$K_s$alanine (VAN BEL 1974). From experimental results *(fig. 3)* reckoning from the top of the line in exp. 1 *K_sTHO = 2.303 (4.758–4.080)/122 = 0.0128 mm^{-1}. In exp. 2 *K_sTHO = 0.0139 mm^{-1} *(fig. 3)*. In exp. 3 *K_sTHO = 0.0112 mm^{-1}. In exp. 4 the deviating delivery profile *(fig. 2)* and the low *K_s-value for THO (0.0047 mm^{-1}, *fig. 3*) show the influence of the relatively high flow rate.

3.3. Mass flow and different accumulation gradients for THO and L-alanine

PEEL et al. (1969) and PEEL (1970) have concluded from the different accumulation gradients for THO, ^{32}P and ^{14}C-sugars that mass flow is out of the question as the translocation mechanism for phloem sap. Here we are dealing with the reversed case: mass flow is the translocation mechanism involved and nevertheless, different accumulation gradients and longitudinal translocation rates for THO and L-alanine were found. It is clear that in tomato xylem vessels mass flow and exchange occur simultaneously.

For reasons of comparison with plant vessels Sephadex G-10 columns were used, through which 1.00 ml 5 mM ^{14}C-L-alanine solution in THO and 1.00 ml 5 mM ^{14}C-D-sucrose in THO were allowed to flow. As shown in *figs. 4* and *5* the alanine and sucrose flowed out separately from THO. The results for outflow of blue dextran and THO were in accordance with the results of MARSDEN (1971).

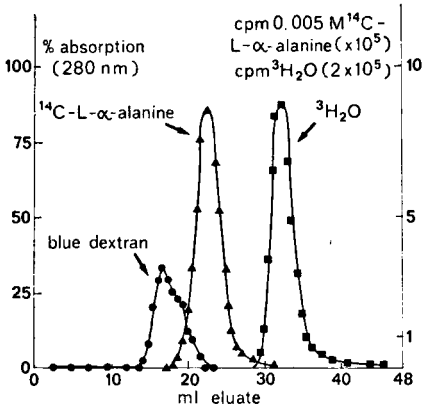

Fig. 4. Elution diagram of 1 ml THO-labelled 5 mM ^{14}C-L-α-alanine solution accompanied by 50 µl blue dextran solution in a Sephadex G-10 column.

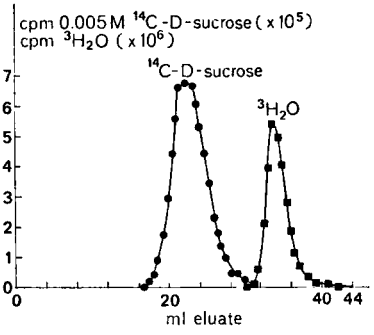

Fig. 5. Elution diagram of 1 ml THO-labelled 5 mM ^{14}C-D-sucrose solution in a Sephadex G-10 column.

4. DISCUSSION

A. According to the calculations of HORWITZ (1958) for irreversible loss of dissolved molecules the lateral outflow constant through the xylem vessel walls for L-alanine, K_{ala} (mm^2hr^{-1}), can be computed by multiplying the flow rate (mm^3hr^{-1}) and $*K_p$-L-alanine (mm^{-1}):

$$K_{ala} = A_pV \times *K_{ala} \quad (3)$$

The flow rate (A_pV) is the product of the transverse sectional area of the xylem vessels A_p (mm^2) and the apparent flow velocity (mm hr^{-1}). The K_{ala}-values are shown in *table 2*.

The lateral outflow constant, K, has the dimensions of the diffusion constant D. Since vessels and walls are filled and imbibed with water, the lateral outflow constant may also be represented by the diffusion coefficients in water. In the present experiments the average value K_{ala} is about 1.5 mm^2hr^{-1}. From a previous paper (VAN BEL 1974) the following K_{ala}-values are computed: 5.82–0.92–3.26–1.79 and 1.92 mm^2hr^{-1}. Then K_{ala} becomes 2.20 ± 1.51 mm^2hr^{-1}. D_{ala} for 5 mM L-alanine at 20 °C is 2.85 mm^2hr^{-1} (recomputed from GUTTER & KEGELES 1953), which is in the proportion of the above K_{ala}. The variance in the results is probably caused by variability in the number of xylem vessels which in turn is connected with parameters as flow rate, absorptive surface and degree of differentiation. Therefore a discussion about the characteristics of the lateral movement seems to be premature. In a following paper we hope to deal with these problems extensively.

The outflow patterns of L-alanine and THO demonstrate that the retention of L-alanine is much more irreversible than that of THO. L-alanine appears to be locked up in the symplasmic compartment while water can more or less freely enter and leave (e.g. COLLANDER 1957).

B. For THO too the lateral outflow constant $K_{THO} = {}^*K_sTHO \times A_pV$ can be computed *(table 2)*. The K_{THO}-values are of the same magnitude as the diffusion coefficient for THO in water at 20°C: 7.49 mm^2hr^{-1} (WANG et al. 1953).

The lateral escape has the characteristics of an irreversible process during the time of the experiment *(fig. 3)*, although the recurrent peaks in section 2 of the segments and the non-linear plot of exp. 2 may be explained as the start of a more reversible behaviour of the THO exchange (HORWITZ 1958; BIDDULPH et al. 1961). Biddulph et al., however, found after 3 hours over a distance of 180 mm an equal amount of THO in all stem sections of intact bean plants. In the present experiments after 3 hours an exponential gradient was found in all segments. A possible explanation for this contrast is the influence of the apparent flow velocity. In experiment 1 -- number of xylem vessels 200; assumed diameter of a single vessel with an average flow rate 80 μm (DIMOND 1966) -- the apparent flow velocity is 54 cm hr^{-1}. In an intact tomato plant the apparent flow velocity may be up to 10 times higher depending on e.g. the rate of transpiration and the diameter of the xylem vessels (KRAMER 1959). Comparison of the outflow results for THO *(table 1)* actually shows that flow rate influences its longitudinal transport considerably. They suggest the higher the apparent flow velocity the larger is the part of the THO-molecules which is not able to get involved in the chromatography-like exchange (BIDDULPH et al. 1963) and diffusion-like processes along the xylem vessel walls.

C. The outflow experiments with Sephadex G-10 columns emphasize that mass flow need not exclude a separate outflow for solute and solvent in non-living systems. They experimentally underline the thesis of CANNY (1973) that a difference of speeds for different solutes does not contradict a flow model.

In the tomato stem segment the absorption of THO seems to be a lateral diffusion only. For L-alanine the lateral two-phasic absorption model (BELL & BIDDULPH 1963), adsorption at the negatively charged walls of the xylem vessels and irreversible absorption under metabolic control, may be maintained, although there are indications that the adsorption is followed by a lateral diffusion.

The experiments show that to regard the long distance transport of water and dissolved materials as a mere bulk transport gives an incomplete picture. The hydrodynamic forces induce a mass flow, the structure of the xylem vessel walls

Table 2. Estimation of K_{ala} and K_{THO}, the lateral outflow constant for L-alanine and THO. The K-values are computed according to equation 3.

experiment	K_{ala} mm^2hr^{-1}	K_{THO} mm^2hr^{-1}
1	1.08	6.91
2	0.97	8.42
3	1.15	7.18
4	2.89	5.46

enable exchange and diffusion-like processes along them. Moreover a non-coupled THO and L-alanine absorption is indicated.

ACKNOWLEDGEMENTS

The author is much indebted to Prof. dr. J. van Die and mr. A. C. Borstlap for valuable suggestions and discussions, mrs T. Koning-van Popta for correction of the English text and mrs W. van Rheenen for making the drawings.

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