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STUDIES ON PHLOEM EXUDATION FROM YUCCA FLACCIDA HAW.

XII. RATE OF FLOW OF ^{14}C-SUCROSE FROM A LEAF TO THE WOUNDED INFLORESCENCE TOP. EVIDENCE FOR A PRIMARY ORIGIN OF THE MAJOR PART OF THE EXUDATE SUCROSE

J. VAN DIE[1], C. R. VONK[2] and P. M. L. TAMMES[2]

[1] Botanisch Laboratorium, Utrecht
[2] Centrum voor Plantenfysiologisch Onderzoek, Wageningen

SUMMARY

In a quantitative tracer study, evidence has been obtained that the photosynthetic products of mature *Yucca* leaves enter a pool of mobile carbohydrates that supplies the major part of the sucrose present in the exudate from the wounded inflorescence top. In the described experiment the activity in the pool has a half-value time of about 3.2 days. The pool is probably identical with the soluble carbohydrate fraction of the leaves.

1. INTRODUCTION

In a previous paper, evidence has been presented for direct translocation of ^{14}C-photosynthates to the site of bleeding of a *Yucca* inflorescence top. A large part of the ^{14}C supplied to a leaf exuded as ^{14}C-sucrose, a process which took place over a period of 6 days and more (VAN DIE & TAMMES 1964). However, it has been shown (VAN DIE & TAMMES 1966) that also carbohydrates stored in the inflorescence stem or supplied to isolated inflorescence stem parts (TAMMES, VONK & VAN DIE 1967) can be mobilized or transformed and can be secreted in the exudation process. Mobilization of stored carbohydrates in the stem occurs during exudation from palm inflorescences, e.g. *Arenga* (TAMMES 1933).

The aim of the present study was to investigate which source of solutes is quantitatively most important in the exudate:

a. the primarily produced photosynthates from the leaves, or:
b. the carbohydrates stored for relatively long periods, in rhizome or stem tissues.

2. MATERIALS AND METHODS

Details of the methods and techniques generally applied for collection of bleeding sap are fully described in previous papers.

The plant used in the present study was part of a mother plant, which consisted of 14 individual daughter plants, each with 23–29 leaves (exclusive of the young ones in the apex). The daughter plants were interconnected by a common

rhizome. On 26 June 1972, a mature leaf, very probably formed in the spring of 1971, was supplied with 40 μCi of $^{14}CO_2$ (specific activity 7.0 × 10^6 d.p.m./μAt Carbon); 24 cm of the leaf length was exposed to the $^{14}CO_2$ for 30 minutes; 9 cm of its lower part remained unexposed. The distance from leaf-base to the bleeding inflorescence top was 32 cm.

3. RESULTS AND DISCUSSION

At 09 h 02 the $^{14}CO_2$ was supplied to the leaf and with increasing intervals of time aliquots of bleeding sap of 25 μl, collected from the cut inflorescence top, were dissolved in liquid scintillator solution and counted with an efficiency of 85%. Within one hour of the start of the experiment, the first radioactivity could be detected in the exudate. After 7 hours of bleeding, the ^{14}C concentration reached its maximum-value i.e. 133,286 c.p.m./25 μl of exudate. *Figs. 1* and *2* show the course of secretion of ^{14}C-solutes over a period of 9 days.

A number of conclusions may be derived from the curves presented:

1. On the first day, a considerable amount of activity appears in the exudate. Subsequently, radioactive substances continue to be secreted. This process proceeds linearly if log activity per volume of exudate is plotted against time.

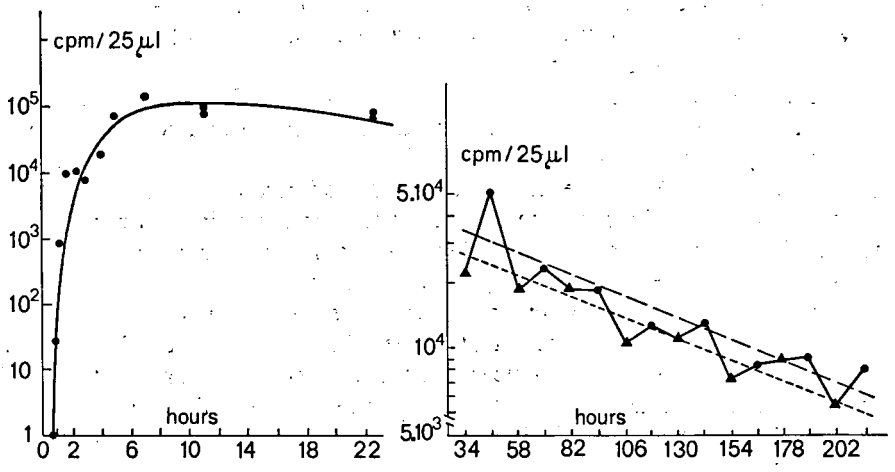

Fig. 1. The ^{14}C-content of phloem sap from the wounded top of the inflorescence stalk during a 215 hours bleeding period following the supply of $^{14}CO_2$ to a single leaf.
Left: The course of activity exudation during the first day of the experiment.
Right: The decline in activity in the days following (2nd to 9th day).
● aliquots of exudate removed from the surface of the wound at 07 h–07 h 30
▲ aliquots of exudate removed from the surface of the wound at 19 h
The decline in activities of the morning as well as of the afternoon-exudate samples with time (dotted lines) is approximately similar: K (the fractional rate of change in activity with time) = 0.216 or $t_{\frac{1}{2}}$ = 3.2 days.
Aliquots of exudate were taken after wound renewal (every 12 hours).

Apparently a pool of photosynthate is present in the plant from which a constant fraction, K, is daily removed and renewed. This fraction can be calculated from the slope of the curve (from the 2nd day to the 9th day), which is 0.094 per day. K = 2.303 times this slope, or 0.216, which means that 21.6% of the pool of assimilates is removed daily from the treated leaf and secreted by exudation. Or the half-value time of the radioactivity in the pool is 0.693/K = 3.2 days. If one assumes that the pool remains roughly constant in size, 21.6% of its carbohydrates are daily renewed. This will be done by influx of new photosynthates or from stored carbohydrates.

If the renewal of the source pool proceeds at a constant rate during a 24 hours' day, a daily renewal of 21.6% means 0.45% in 30 minutes. Although the experimental data presented in the *tables 1* and *2* at first sight do not support this assumption, a satisfactory explanation for the diurnal fluctuation in ^{14}C and flow rate cannot be presented at this moment. The source pool renewal rate of 0.45% per 30 minutes, however, can also be accepted as an average value.

2. The exudate had a specific activity of at most 133,000 cpm/25 µl or about 155,000 dpm/25 µl. If one assumes all activity to be present in the 17.0% of sucrose concentration of the sap – in a previous study more than 90% of all activity was present in that sugar (VAN DIE & TAMMES 1964) – then the specific activity was 1.2×10^4 d.p.m./µmol sucrose. Assuming a mature-leaf origin of all the exudate sucrose, only a twenty-fifth of the exudate sucrose found its origin in the $^{14}CO_2$-treated leaf (25 leaves per shoot). This, consequently, will have exported sucrose with a specific activity of at least $25 \times 1.2 \times 10^4$ d.p.m./µmol or 3.0×10^5 d.p.m./µmol.

3. Since the specific activity of the supplied CO_2 was 7.0×10^6 d.p.m./µmol Carbon, the labelled carbohydrates formed from it in the treated leaf area can theoretically have had a specific activity of 8.4×10^7 d.p.m./µmol disaccharide (sucrose). Only 24/33 of the length of the leaf had received the labelled CO_2. This is about 0.65 of the surface area. Thus the specific activity of the sucrose formed in the treated leaf between 09h02 and 09h32 may have been at most $0.65 \times 8.4 \times 10^7 = 5.5 \times 10^7$ d.p.m./µmol sucrose.

According to the assumptions made sub 1, the pool from which the exuded sucrose was withdrawn may have had a renewal rate of 0.45% per 30 minutes. If all the ^{14}C-carbohydrates produced in the treated leaf moved into that part of the pool that belonged to the treated leaf, the pool would have attained a maximum specific activity of $45 \times 10^{-4} \times 5.5 \times 10^7$ d.p.m./µmol = 2.5×10^5 d.p.m./µmol disaccharide.

The difference of this specific activity of source pool sucrose from the 3.0×10^5 d.p.m./µmol calculated under 2 is only slight .The relatively rough calculations may nevertheless support the view that the exudate sucrose is mainly derived from very recent production of photosynthates in the leaves.

4. As the tables show, an average exudation rate has been found of 5.7 ml/24 hours. This means (25 leaves, 17.0% sucrose) 39 mg sucrose per day and per leaf. This amount represents 21.6% of the source pool, which consequently contained approximately 180 mg of sucrose equivalents per leaf of average size.

The labelled *Yucca* leaf had a fresh weight of about 6.5 grams and contained 2.3 grams of dry substances. The exudate-sucrose source pool of this leaf consequently comprised 7 to 8% of the dry weight. According to the calculation made under 3, the pool will have attained a maximum specific activity of 2.5×10^5 dpm/μmol disaccharide. A pool size of 180 mg of sucrose, therefore, means a total activity in the treated leaf of $180/342 \times 2.5 \times 10^8$ dpm $= 1.3 \times 10^8$ dpm. This value is in reasonable agreement with the 0.9×10^8 dpm (40 μCi) of $^{14}CO_2$ supplied to the leaf.

Table 1. Exudation-volume flow rates in succeeding day and night periods and the amounts of ^{14}C-solutes collected in the exudate fractions.

period	ml	cpm/25 μl	total dpm collected
1st night	2.00	78180	7.349×10^6
2nd day	1.54	22573	1.634×10^6
2nd night	2.79	49863	6.5385×10^6
3rd day	2.28	18477	1.980×10^6
3rd night	3.38	22981	3.651×10^6
4th day	1.40	19363	1.274×10^6
4th night	3.63	18349	3.1305×10^6
5th day	3.22	10246	1.5505×10^6
5th night	3.83	12200	2.196×10^6
6th day	3.92	11072	2.040×10^6
6th night	2.60	13686	1.6725×10^6
7th day	2.74	7267	0.936×10^6
7th night	2.88	8616	1.1665×10^6
8th day	2.59	8747	1.065×10^6
8th night	3.72	8936	1.5625×10^6
9th day	2.25	5011	0.530×10^6
9th night	3.90	8072	1.4795×10^6

Table 2. Average exudate volumes and total ^{14}C recoveries during the day and night periods of the bleeding experiment.

Periods of 12 hrs	Average volume per period	Totally collected dpm	^{14}C recovered as exudate ^{14}C-sucrose
9 nights	3.16 ml	29.246×10^6	33%
8 days*	2.51 ml	11.039×10^6	12%

* This value has to be increased with the amount of ^{14}C-sucrose collected during the first 11 hrs (daylight) of the experiment. The exudate volume of that period could not be determined as many aliquots of it had been used for radioactivity measurements, and repeated cuttings and cleanings of the wound surface had been carried out. Taking for this period the same values as found for the second daylight period 1.6×10^6 dpm should be added to the total of 11.039×10^6 dpm, or roughly 2%, giving a total recovery of approximately 47%.

5. *Table 2* shows that about 47% of the ^{14}C supplied to the treated leaf could be recovered in the exudate in a 9 days period of bleeding. The bleeding inflorescence apparently behaves as a strong sink. A similar conclusion can be drawn from the various calculations which demonstrate that probably all the assimilated ^{14}C in the leaf is available for export to the phloem. The relatively slow turnover of the source pool in the leaf might indicate that some form of transient carbohydrate storage follows photosynthesis in *Yucca*. Its formation during the daylight hours might underlie the observed diurnal periodicity. An other explanation might be that the layers of non-photosynthesizing cells between chlorenchyma and sieve tubes offer a much more severe barrier in *Yucca* than e.g. in soya (FISHER 1970). The observation that the maximum in specific activity of the exudate sucrose is about that of the theoretically expected maximum value of the source pool in the leaf points to an insignificant degree of exchange of the labelled sugar molecules in the sieve tubes with unlabelled ones in surrounding phloem parenchyma cells.
6. The specific activity of the source pool in the treated leaf would have risen from zero at the start of the experiment to a maximum when all or most of the $^{14}CO_2$ had been assimilated and had entered the pool. From then on, it would have decreased gradually by influx of normal unlabelled photosynthates, and simultaneously by export of ^{14}C-sugars in the direction of the sieve tubes. If one assumes a maximum specific acitvity of the pool of carbohydrates at about 09 h 30, it took approximately $6\frac{1}{2}$ hours before this maximum arrived at the site of bleeding.

In a previous study (VAN DIE & TAMMES 1966) a velocity of exudate flow has been estimated of 44 cm/hour. This would mean that the long time needed for the specific activity profile to move from leaf chlorenchyma cells to the top of the inflorescence (41 cm) would have been entirely due to the barrier of parenchymatous cells or to transient carbohydrate storage mentioned under 5.

7. In experiments with another monocotyledonous plant species (although not closely related), *Fritillaria imperialis*, it always took about 11–50 hours for the maximum specific activity from a treated leaf to reach the nectar of a flower (VAN DIE et al. 1970), depending on the distance between the two. Although in these experiments a secretion process is also involved in the translocation chain leading from chloroplasts to secreted nectar, the data concern the translocation velocity in an intact system. The order of time found in translocation in *Yucca* and *Fritillaria* may be good evidence for the view that in *Yucca* the translocation rate of the ^{14}C-sugar concentration profile from the source pool in the leaves to the bleeding site is not fundamentally different from normal photosynthate translocation rates from source to sink regions where sieve tubes and parenchyma cells are involved.

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