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Jan Krupa*, Hugo Challa**

Wybrane aspekty metodyczne badań nad transportem asymilatów z liści przy zastosowaniu znakowanego węgla ^{14}C. Some methodical aspects of the study of assimilate transport from leaves by means of ^{14}C

Abstract

The diurnal course of assimilate movement from leaves of cucumber was studied by measuring with a Geiger-Müller tube changes in radioactivity in intact source leaves that were labelled continuously with ^{14}C. For this approach two conditions had to be satisfied: in the first place specific activity of carbon translocated should be the same as that of carbon fed to the leaf. In the second place the relation between radioactivity in the leaf and the count rate measured with the GM tube on that leaf should not change during the measurements.

Our experiments revealed that these requirements were not always fulfilled. Data from the literature support the hypothesis arising from our own results that in mature leaves, considerable exchange may occur between recently formed assimilates and structural components. Furthermore efficiency of counting with a GM tube positioned close to the lower surface of the source leaf changed considerably during the dark period. This change may be attributed to variations in self absorption of β radiation brought away by changes in the distribution of ^{14}C over different tissues of the source leaf.

Due to the complications mentioned great care is needed in the interpretation of experiments in which movement of carbon is studied by means of radioisotopes.

* *Department of Plant Physiology of the Higher Pedagogical School, Cracov.*
** *Centre for Agrobiological Research, Wageningen. The Netherlands.*

INTRODUCTION

Although much work in the past has been devoted to the study of the rate of assimilate transport in plants, to our knowledge only a few attempts have been made to describe the time course of the movement of carbon during a complete day/night cycle (Gordon, Ryle, Powell & Mitchell, 1979, Mason & Maskell, 1928). Such patterns are important for understanding the control mechanisms involved and for building plant-growth models in which the spatial organization of plants is incorporated.

Transport of assimilates from leaves can be calculated by measuring CO_2 exchange and variations of the carbon content of a leaf simultaneously. For this purpose successive harvests of leaves in combination with determinations of the carbon content in the dry weight can be used for periods down to about one day (Terry & Mortimer, 1972; Ho, 1976). Over shorter intervals of time, results obtained with this method are too inaccurate. Using ^{14}C as a tracer, changes in carbon content of a source leaf can be measured with a much higher resolution. Besides, variations in radioactivity in the source leaf can be monitored with a GM tube on the intact plants (Geiger & Swanson, 1965).

In the present study the radio-tracer technique was used in the study of the diurnal course of carbon accumulation and export in source leaves of different developmental stages. The results obtained with GM tube measurements were compared with those obtained by destructive analysis. In addition the ^{14}C balance after one day/night cycle was compared with results obtained from a combination of growth analysis and gas-exchange measurements published elsewhere (Challa, 1976). Discrepancies found are discussed and explained qualitatively and as far as possible quantitatively.

MATERIALS AND METHODS

Plant material

Cucumber plants (Cucumis sativus L. cv. Sporu Origineel) were sown on day 0 in humid perlite and kept in darkness at 28°C for 3 days. On day 3 seedlings were placed in the light in a climate room and on day 4 they were transplanted on an aerated modified Hoagland solution. The temperature was 25°C, the radiant energy flux density (PAR) at plant level provided by high pressure mercury lamps was 30 $W.m^{-2}$ for 8 h and the relative humidity was maintained at 80%. Experiments were performed 25 ± 1 days after sowing when the plants had 5-6 leaves. The first basal leaf then was fully expanded but still increased somewhat in dry weight due to accumulation of organic acids and minerals.

Steady state labelling

One of the leaves, the source leaf, while attached to the plant was enclosed in a hermetically sealed acrylic plastic leaf chamber provided with double water-cooled windows. The temperature in it was kept at 25°C. The leaf was kept in a horizontal position by means of thin nylon wires. The windows transmitted 86% of the irradiance prevailing in the climate room.

Steady state labelling with $^{14}CO_2$ of constant concentration and of constant specific activity was performed in a so-called semi-closed system (Jarvis & Catský, 1971). In this system (Fig. 1) air was circulating at a rate of 300 $l.h^{-1}$. Its CO_2 concentration was measured with an infrared gas analyser. CO_2 taken up by the source leaf was continuously replaced by CO_2 generated in vessel G, by injecting a 0.05 M sodium carbonate solution in the lactic acid contained in this vessel. CO_2 concentration was kept at 300 $cm^3.m^{-3}$ by adapting manually the stroke of the dosing pump to the rate

of CO_2 fixation by the source leaf. Specific activity of the carbonate solution was 500 kBq.g^{-1} carbon (13.5 µCi.g^{-1}). Part of the carbonate solution was 500 kBq.g^{-1} carbon (13.5 uCi.g^{-1}). Part of the circulating air was passed through vesse vessel G, agitating the acid and carrying away the CO_2 generated in it.

Humidity of the air in the system was controlled by circulating it through a condensation vessel C, placed in a thermostated water bath at 20°C. Excess water vapour resulting from transpiration was removed in this way.

The amount of carbonate supplied during an experiment was measured by refilling the storage vessel S to its original level from a burette. Frequent samples were taken the carbonate solution to check its specific activity.

Determinations of radioactivity

The amount of radioactivity in the intact source leaf during the experiments was continuously monitored by means of a GM tube. This GM tube (Philips, type 18516), with a window of 3 cm diameter was inserted through the lower wall of the assimilation chamber so that the distance between leaf and window of the GM tube was about 5 mm. The count rate measured with the GM tube was recorded continously by means of a rate meter and in addition every 10 minutes a printed output was given of the total number of pulses counted in that interval.

At the end of each experiment the dried plant parts were finely powdered by agitating them in a plastic cylinder containing a brass roller of the same diameter as the cylinder. The cylinders had been treated with an antistatic solution to avoid sticking of particles against the walls. A sample of the powder of each plant part was weighed, burned in an oxidizer (Intertechnique IN 4104 Sample Oxidizer), followed by liquid scintillation counting.

Experimental procedure

Experiments were done in the same climate room where the plants were raised, under the same environmental conditions. Before the start of the day the source leaf was enclosed in the feeding chamber and CO_2 was removed from the system. As soon as the lights were on, CO_2 was generated at a rate to keep the concentration at 300 $cm^3.m^{-3}$. Under the conditions used in this study, starch and sugar contents in the plant at the end of the night are very low (Challa, 1976). Hence it was expected that isotopic saturation of the metabolically active pools would be rapidly achieved.

At the end of the day CO_2 generation was stopped. During the further course of the experiment the leaf chamber was flushed with air from the growing room. At the end of the experiment the plant was divided into leaves, stem (including petioles and apex), hypocotyl and roots. The area of each leaf was determined, all parts were weighed, dried at 105°C and reweighed, followed by determinations of radioactivity.

Experiments were either terminated immediately after labelling, or continued until the end of the dark period. Each of the five unfolded leaves was investigated in this way. With source leaf 1, however, some experiments were continued for more days, during which the source leaf always remained in the feeding chamber flushed with air from the climate room.

RESULTS

^{14}C budgets

In a semi-closed system, all CO_2 generated has to be taken up by the source leaf. The amount of $^{14}CO_2$ fixed by the

source leaf can thus be calculated from the amount of carbonate solution pumped into the vessel containing lactic acid (G, Fig. 1), when the concentration and specific activity of the solution are known.

Putting the amount of ^{14}C taken up by the source leaf at 100%, the amount of ^{14}C recovered from different plant parts was expressed as a percentage of it (Table 1). The difference between the total amount of ^{14}C fixed by the source leaf and the amount recovered from all plant parts together is the amount of ^{14}C lost, presumably due to respiration and perhaps also to excretion of organic substances through the roots. The average values obtained are the results of only 2 or sometimes 3 experiments. Because of differences in the size of plants, replicates differed somewhat. Nevertheless some general trends can be observed (Table 1).

During the day all leaves exported about 25% of the amount of ^{14}C fixed and more than half of the amount exported was lost by respiration (and excretion by the roots), with the exception of leaf 3 where only 22% of translocated ^{14}C was lost.

After a complete day/night cycle the young, fastly growing source leaves (leaves 4 and 5), retained a larger part of the fixed radioactivity than leaves 2 and 3. Rather unexpectedly however, much ^{14}C was also retained in source leaf 1, which was a mature, non growing leaf. In some orientating experiments we found no further decrease in radioactivity in leaf 1 during the 2 days following labelling, but in plants harvested after 3 days a strong decrease was observed. (Table 2).

Because losses of radioactivity were considerable and because those losses may have differed in different plant parts, our data (Table 1) may give a distorted picture of the real distribution of photosynthates. Qualitatively, however, it is clear that cotyledons and leaf numbers 1, 2 and 3 did not import ^{14}C and that distribution patterns for different

Table 1

Distribution of the amount of radioactivity over different plant parts, expressed as a % of the amount of ^{14}C taken up by the source leaf. Data were obtained by oxidation of ground samples followed by scintillation counting. Values listed are the average of 2 or 3 experiments, as indicated between parenthesis after the number of the source leaf. After each value, between parenthesis, an estimation of the standard deviation is given

End of the day	Source leaf number				
Organ	1 (3)	2 (2)	3 (2)	4 (2)	5 (2)
Leaf 1	72.7 (1.4)	0.03 (0.0)	0.06 (0.01)	0.02 (0.0)	0.04 (0.04)
2	0.39 (0.26)	63.9 (9.7)	0.10 (0.04)	0.04 (0.01)	0.04 (0.01)
3	0.14 (0.09)	0.22 (0.03)	79.0 (3.8)	0.08 (0.06)	0.06 (0.06)
4	0.53 (0.53)	0.23 (0.02)	5.37 (0.83)	77.7 (5.9)	0.06 (0.06)
5	2.37 (0.59)	1.76 (1.7)	2.21 (0.74)	1.50 (0.08)	78.6 (7.2)
6	1.36 (1.17)	2.26 (0.87)	0.33 (0.10)	0.17 (0.05)	0.18 (0.13)
Cotyledons	0.06 (0.03)	0.11 (0.13)	0.07 (0.03)	0.03 (0.01)	0.01 (0.01)
Stem + apex[x]	4.22 (1.7)	8.10 (1.1)	5.39 (1.1)	4.15 (0.78)	1.18 (1.5)
Hypocotyl	0.73 (0.24)	1.28 (0.11)	0.32 (0.04)	0.34 (0.18)	0.01 (0.01)
Roots	1.46 (0.45)	5.33 (0.08)	2.62 (0.37)	0.74 (0.32)	0.11 (0.08)
^{14}C lost	16.0 (3.0)	16.7 (11.7)	4.59 (3.2)	15.3 (6.8)	19.8 (8.6)
^{14}C recovered outside source leaf	11.3 (2.8)	19.3 (2.0)	16.5 (0.65)	7.05 (0.23)	1.60 (1.5)
End of the night	Source leaf number				
Organ	1 (3)	2 (3)	3 (2)	4 (2)	5 (2)
Leaf 1	46.0 (1.8)	0.10 (0.02)	0.07 (0.01)	0.05 (0.02)	0.02 (0.03)
2	0.18 (0.08)	20.5 (3.9)	0.09 (0.11)	0.09 (0.04)	0.02 (0.02)
3	0.23 (0.12)	0.37 (0.12)	27.2 (0.36)	0.08 (0.01)	0.02 (0.02)
4	0.66 (0.78)	1.90 (2.7)	0.34 (0.30)	49.1 (10.0)	0.08 (0.02)
5	2.36 (1.6)	5.93 (2.5)	4.45 (1.1)	2.82 (2.9)	44.8 (0.39)
6	2.26 (2.18)	3.37 (2.9)	2.76 (2.6)	0.85 (0.81)	0.07 (0.05)
Cotyledons	0.07 (0.05)	0.11 (0.04)	0.15 (0.12)	0.34 (0.43)	0.03 (0.04)
Stem + apex[x]	2.51 (0.55)	8.94 (1.3)	7.01 (1.7)	4.15 (1.9)	1.98 (2.7)
Hypocotyl	0.73 (0.01)	2.14 (0.37)	1.13 (0.22)	0.58 (0.09)	0.13 (0.18)
Roots	2.03 (0.20)	7.48 (2.3)	7.02 (1.0)	2.84 (0.93)	1.49 (1.4)
^{14}C lost	42.0 (0.43)	49.1 (4.4)	49.8 (1.3)	39.3 (3.2)	51.4 (4.8)
^{14}C recovered outside source leaf	12.0 (1.4)	30.3 (1.5)	23.0 (1.6)	11.8 (7.1)	3.78 (4.5)

[x]Petioles included in the stem fraction.

Table 2

Distribution of radioactivity over different plant parts at different times after application of $^{14}CO_2$ to leaf 1. Time is given in hours after labelling started. $^{14}CO_2$ was fed continuously during the first light period only (0-8 h). The data are expressed as a percentage of the amount of ^{14}C taken up by the source leaf

Organ	Time after onset of labelling (h)				
	8	24	32	72	96
Leaf 1	72.7	46.0	45.9	46.8	22.7
2	0.39	0.18	0.1	0.13	0.26
3	0.14	0.23	0.21	0.13	0.26
4	0.53	0.66	0.12	0.16	0.20
5	2.37	2.36	1.26	0.10	3.24
6	1.36	2.26	0.72	3.38	4.24
Cotyledons	0.06	0.07	0.03	0.04	0.00
Stem + apex	4.22	2.51	1.32	8.29	6.69
Hypocotyl	0.73	0.73	0.48	0.58	1.29
Roots	1.46	2.03	0.45	0.92	3.86
^{14}C lost	16.0	42.0	49.4	39.5	57.2

Table 3

Ratio between count rate measured with a GM tube on the source leaf and the amount of radioactivity per unit area, determined by oxidation of ground samples followed by scintillation counting. Averages are given of the values obtained at the end of the day (a) and at the end of the night (b). The relative increase in counting efficiency during the night (b/a) is given in the last column. Between parenthesis an estimation of the standard deviation is given

Leaf number	Ratio (cpm.dpm^{-1}.cm^{-2})		b/a
	end of the day (a)	end of the night (b)	
1	0.098 (0.0040)	0.14 (0.021)	1.4
2	0.090 (0.0099)	0.30 (0.026)	3.3
3	0.10 (0.014)	0.18[+] (-)	1.8
4	0.12 (0.0)	0.20 (0.0)	1.7
5	0.13 (0.021)	0.24 (0.0)	1.8

[+] area was estimated from the dry weight of the leaf.

source leaves did not differ very much: the importing organs received radioactivity from all source leaves.

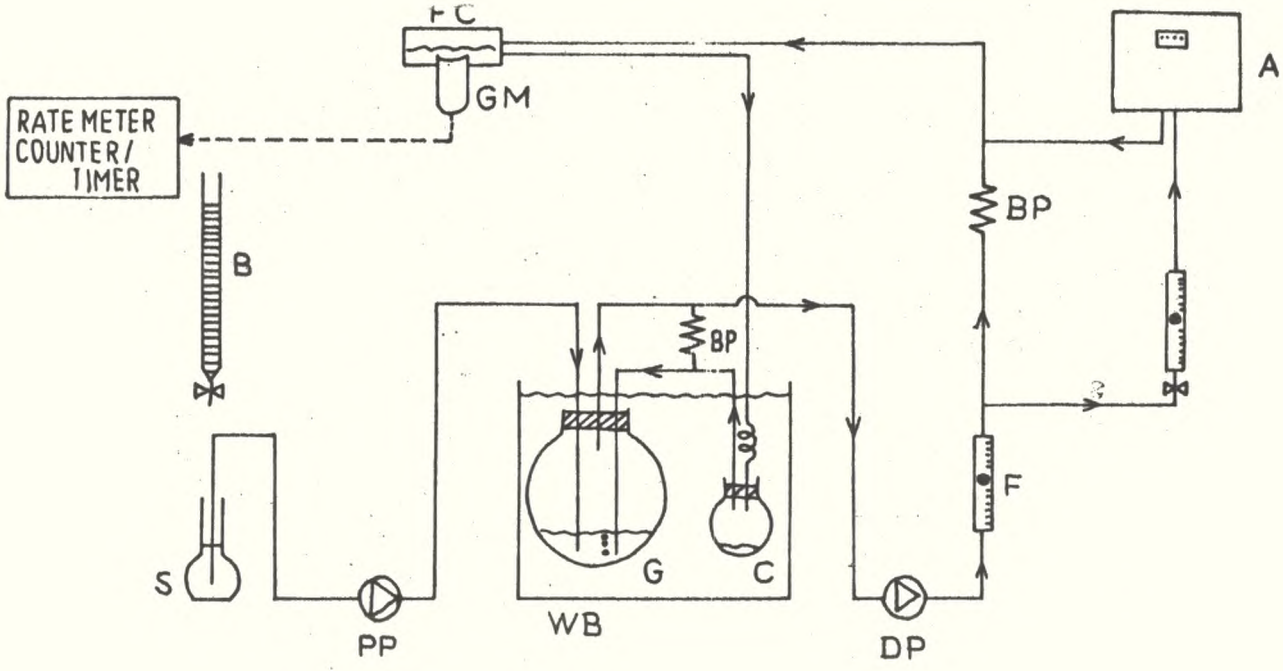
GM tube measurements

The count rate, measured with a GM tube on different source leaves, increased during the day as a result of growth and accumulation of labelled assimilates (Fig. 2). During the night radioactivity in the source leaf decreased due to respiration and export of assimilates. In most experiments this decrease in activity diminished towards the end of the night. This phenomenon may be explained by the depletion of reserve carbohydrates described in a previous study (Challa, 1976).

For a quantitative interpretation of the diurnal count rate patterns (Fig. 2) the ratio between count rate measured with the GM tube and radioactivity in the leaf has to remain constant. Because the area of the window of the GM tube was small, only part of the leaf blade was assayed. Hence, in order to compare different measurements, the amount of radioactivity was expressed per unit of area of leaf blade. The ratio between count rate and radioactivity per unit area was calculated for different source leaves, for plants harvested at the end of the day and for those harvested at the end of the night (Table 3). In all source leaves this ratio increased in course of the night and in leaf 2 a more than 3 fold increase was even observed. Consequently, taking into account the magnitude of the errors, even a semi-quantitative interpretation of the diurnal count rate patterns is difficult (Fig. 2).

Fig. 1. Set-up for continuous labelling of leaves

A = CO_2 analyser, B = burette used to measure the amount of carbonate consumed, BP = bypass (sintered glass fitter), C = condensation vessel, DP = diaphragm pump, F = flowmeter, FC = feeding chamber, G = CO_2 generation vessel, GM = GM tube, PP = dosing pump with a piston with variable stroke, S = vessel containing radioactive carbonate solution, WB = thermostated waterbath



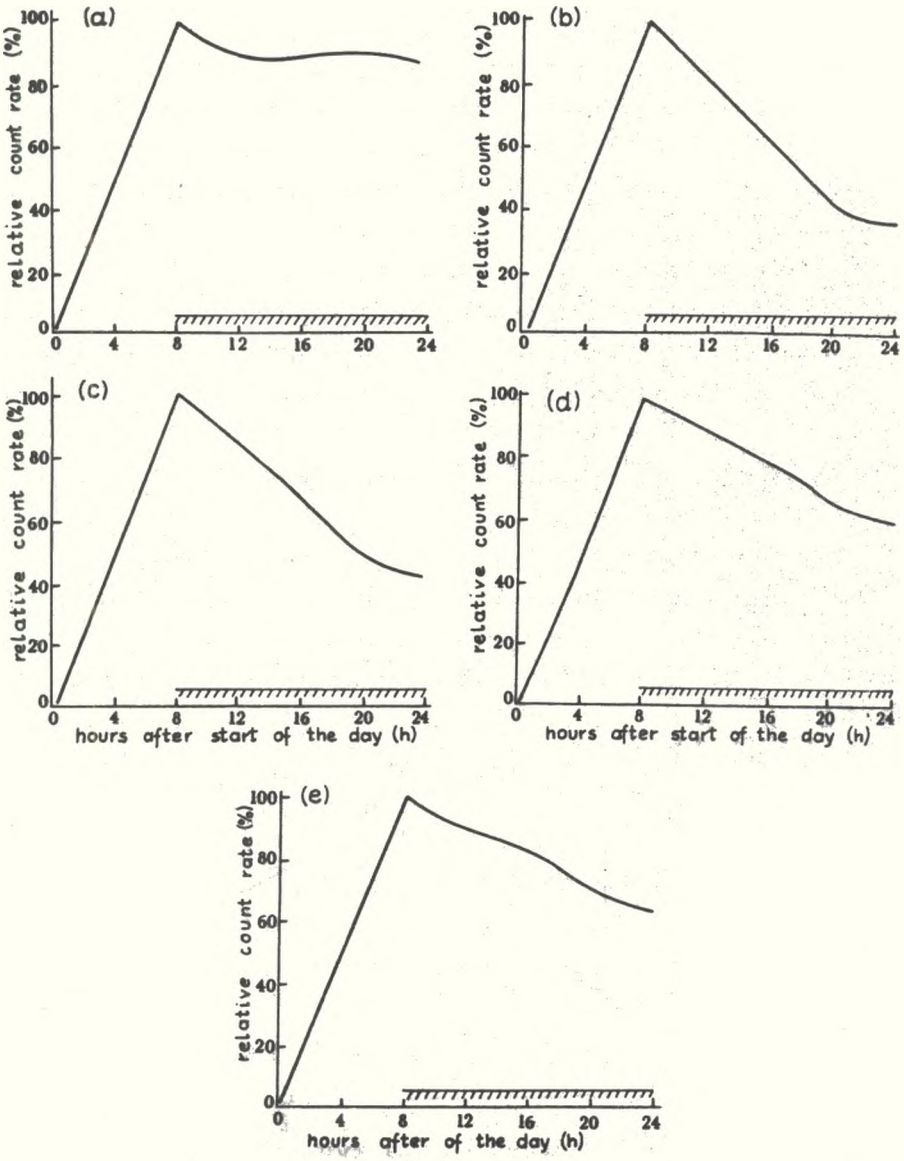
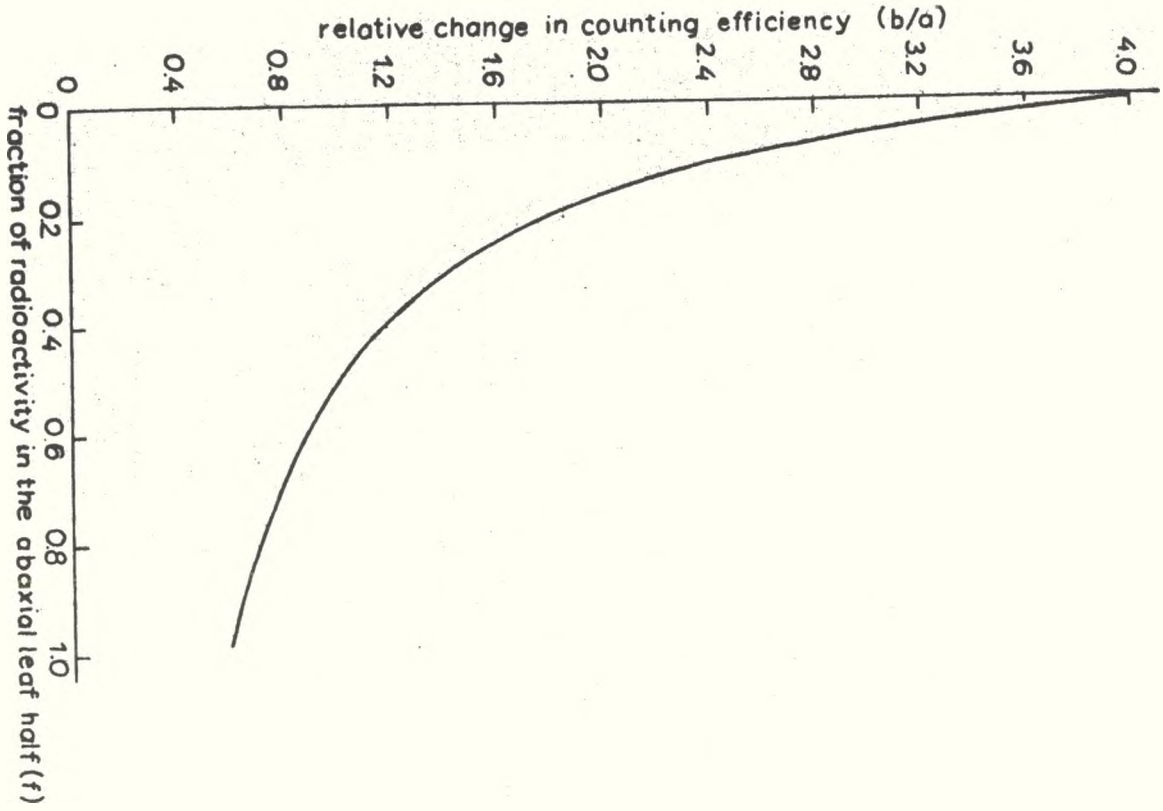


Fig. 2. Time course of radioactivity in source leaves:
1 (a), 2 (b), 3 (c), 4 (d) and 5 (e) measured with a GM tube. Count rates are expressed as a percentage of the rate observed at the end of the light period. Dark bars indicate the dark period

Fig. 3. Relative change in counting efficiency (b/a), in relation to the fraction (f), of the total amount of radioactivity in the leaf, contained in the abaxial leaf half at the end of the light period. A value of f = 0.5 was assumed at the end of the dark period. b = counting efficiency at the end of the night, (cpm.dpm.$^{-1}$cm^2) a = counting efficiency at the end of the day (cpm.dpm.$^{-1}$cm^2)



DISCUSSION

^{14}C retention

As has been pointed out in the previous section a relatively large part of the radioactivity fixed by source leaf 1 was retained in it. Figures given by Wardlaw (1968) in his article show that strong retention of radioactivity in mature source leaves is commonly observed, but he did not explain the phenomenon. One explanation may be that frequently, assimilate distribution is expressed in percentages of the total amount of radioactivity recovered from the plant. Because in this way losses of ^{14}C due to respiration are neglected, the proportion of ^{14}C translocated from the source leaf will be underestimated. In our experiments 40-50% of the amount of ^{14}C supplied to the source leaf was lost (Table 1). Neglecting this amount would result in an apparent doubling of radioactivity retained by source leaves.

Whereas the way of expressing experimental results may explain some of the data on high retention of radioactivity in mature source leaves, there is evidence for still another phenomenon playing a role. Porter & Bird (1962), Sharkey (1985) when comparing the balance of ^{12}C and of ^{14}C in a mature source leaf of tobacco, found that, whereas the amount of carbon in structural fractions increased with only 4% of the daily amount of CO_2 assimilated, ^{14}C in it increased by 17%. Lupton (1966) observed with wheat that ^{14}C remained in a source leaf that did not increase in dry weight. Porter Bird (1962) also showed that specific activity of CO_2 lost by respiration of the source leaf was only 40% of the specific activity of CO_2 assimilated. Specific activity of CO_2 assimilated. Specific activity od translocated carbon however, was not much reduced in this case.

In the experiments of Porter & Bird (1962) and in those of other researches (Yamamoto, 1967; Dickson & Larson, 1975)

a considerable portion of the radioactivity recovered from mature source leaves was incorporated into structural fractions (predominantly proteins, cell walls and organic acids). By analysis of the carbon content of old tomato leaves it has been shown (Ho, 1976) that the structural fractions gained or lost carbon, over intervals of 6 hours, depending on the irradiance on the leaf. Thus about 25% of the loss in dry weight that occurred at low irradiance of 4 $W.m^{-2}$ could be accounted for by losses from structural compounds (Ho, 1976). Dickman & Gordon (1975) found an increase in the turnover of soluble proteins in mature and old poplar leaves in comparison to young leaves. Apparently the structural components of mature leaves are more involved in metabolism than is generally assumed.

In a previous study growth, CO_2 uptake and CO_2 production by different leaves of plants, comparable to those used in the present study, were determined (Challa, 1976, Catsky et al. 1985, Jeffcoat et al. 1985). Taking into account the carbon content of the dry weight increase, the carbon balance for these leaves can thus be calculated. In this way, losses of carbon from the exporting leaves 1, 2 and 3 can be compared with the losses of ^{14}C obtained in the present study (Table 4). For leaves 4 and 5 the situation is more complicated due to import from other leaves. For leaf 3 both methods agree well, but with increasing leaf age the radiotracer method increasingly underestimated the amount of carbon lost. These discrepancies must be caused by a decrease of the specific activity of carbon lost, compared to that of carbon taken up by the source leaf. This relative decrease in specific activity was also calculated (Table 4). In the case of leaf 1 the decrease in the specific activity of the carbon lost cannot be attributed to a low specific activity of respiratory carbon alone, as was the case with Porter & Bird (1962), Khavari-Nejad (1984), because only 15% of the amount of carbon taken up during the day was lost by respiration (Challa, 1976).

Table 4

Comparison of the losses of ^{12}C (L_{12}) and those of ^{14}C (L_{14}) over 24 hours from different source leaves. Loss of ^{12}C was calculated by: $L_{12} = (\frac{P - GxC}{P}) \cdot 100\%$, where P is the net amount of C taken up during the day, G is the increase in dry weight per 24 hours and C is the carbon content in the dry weight accretion (Challa, 1976). Loss of ^{14}C was calculated by: $L_{14} = \frac{(P_{14} - G_{14})}{P_{14}} \cdot 100\%$, where P_{14} is the net amount of ^{14}C taken up during the day and G_{14} is the amount of ^{14}C in the source leaf 24 hours after labelling started (Table 1). The relative specific activity (RSA) of carbon lost from the source leaf was calculated by $(L_{14}/L_{12}) \cdot 100\%$

Source leaf number	L_{12} (%)	L_{14} (%)	RSA (%)
1	94	54	57
2	89	80	89
3	75	73	97

Leaf 1 accumulated more ^{14}C than predicted (Table 4). For the average plant, where the rate of carbon fixation in the case od leaf 1 was 10.6 mg C/day this extra accumulation corresponded to 4.2 mg C/day, equivalent to 15% of the total amount of carbon contained in that leaf!

In accordance with the evidence obtained from the literature this retention may be ascribed to exchange of newly formed assimilates with structural components. Consequently a further decrease in the amount of radioactivity in the source leaf was expected during the days following labelling, but initially this was not the case (Table 2). Only in the experiment where the plant was harvested after 3 days radioactivity in the source leaf was lower (Table 2). The results of these experiments suggest that assimilated carbon was partly incorporated into structures that had a certain mini-

mum age before they were broken down. Further experiments are needed to confirm the general validity of this observation.

Efficiency of the GM tube measurements

Another important methodological problem encountered in this study was the measurement of radioactivity in intact leaves, attached to the plant. Although this technique has been used by several authors (Antoszewski & Dzieciol, 1973; Geiger & Swanson, 1965; Hofstra & Nelson, 1969; Lovell, Oo Sagar, 1972; Lush & Evans, 1974; Moorby & Jarman, 1975) the reliability of this technique has been checked poorly. Geiger & Swanson (1965) measured the accumulation of radioactivity in the sink leaf with a GM tube, after pulse labelling of the source leaf with $^{14}CO_2$. The count rate measured at the end of the experiment was calibrated by comparison with the amount of radioactivity found in the total sink of the shoot, assuming a constant partitioning of radioactivity over different sink regions. By harvesting comparably treated plants at different times in course of the experiment they tested the validity of their GM tube measurements. They concluded that these points reasonably fitted into the curve describing the accumulation of activity in the sink leaf and that consequently the GM tube measurements were correct. The procedure followed, of course, equalized the start and end points of both curves, but their data clearly show that the GM tube measurements were too low in the steep part of the curve.

Lovell et al. (1972) demonstrated that under their conditions good agreement existed between the amount of radioactivity in the source leaf and measurements with a GM tube on the surface of the intact leaf. Unfortunately they did not state the conditions under which the leaf was kept after labelling, but presumably plants were kept in the light.

Own data clearly show that the ratio between the amount of radioactivity per unit area in the source leaf and the amount of radiation received by the GM tube from leaf, changed during the dark period (Table 3). Such changes may be brought about by three factors:

1. variations in the geometry of the set-up,
2. variations in the distribution of radioactivity over the leaf blade,
3. variations in self absorption within the leaf.

Because leaf and GM tube were in a fixed position with respect to each other, only leaf growth may have influenced the geometry of the set-up. With leaves 4 and 5 which have a high relative growth rate this factor may have played a role, but because all the leaves showed the phenomenon of changing counting efficiency it is unlikely that leaf growth was the main cause of it. It should, however, be noticed, that leaf growth did affect the diurnal count rate patterns (Fig. 2) in another way. Because only a constant area of the leaf blade was monitored with the GM-tube, this area in a rapidly growing leaf represented a decreasing fraction of the total leaf area. In the case of leaf 4 and 5 this effect was counterbalancing the increase in counting efficiency metioned before.

Because the window of the GM tube was smaller than the leaf, only part of the leaf was assayed. Hence when using this method it is a prerequisite that distribution of radioactivity over the leaf blade remains constant with time. Whether large variations in this distribution occurred we did not investigate but we did not find data in the literature describing such variations. There are, however, some good arguments why variations in self absorption within the leaf may be important. The amount of β radiation R measured by the GM tube is given by (see appendix):

$$R = \frac{\beta}{\alpha T}(1-e^{-\alpha T}).A$$

where A = the amount of radioactivity in the leaf (dpm)

β = a constant resulting from the geometry of the set-up and from properties of the GM tube

α = the absorption coefficient for ^{14}C β-radiation which equals about 0.26 $cm^2 \cdot mg^{-1}$ (Gleason, Taylor & Tabern, 1951)

T = leaf weight per unit area ($\pm$ 15 $mg \cdot cm^{-2}$)

The percentage of radiation lost due to absorption in the leaf, according to this formula is 75%, which is in fair agreement with the value of 79% obtained experimentally with Pisum (Lovell et al, 1972). This percentage may be influenced by differences in water content. However, assuming that variations in water content were fully reflected in leaf weight per unit area, a 10% increase in water content would result in only a 6% decrease in count rate. Because an increase in counting efficiency was observed rather than a decrease when comparing counting efficiencies before and after the night and because the increase was of a much greater order of magnitude (Table 3), it is impossible that variations in water content were the main cause of it.

Not all ^{14}C contained in the part of the leaf monitored by the GM tube was contributing to the same extent to the amount of radiation measured: because the GM tube was on the abaxial side of the leaf, radiation received from the adaxial side of the leaf was much more weakened by absorption than radiation received from ^{14}C in the lower tissues. Consequently, distribution of ^{14}C over different leaf tissues wase one of the factors determining the counting efficiency. In order to investigate the effect of distribution of ^{14}C on counting efficiency in a more quantitative way, rather arbitrarily it was assumed that ^{14}C was homogeneously distributed within the abaxial and within the adaxial half of the leaf and that at the end of the night both sides contained equal amounts

of radioactivity. With these assumptions the relative change in counting efficiency (b/a) was calculated (formula 7, appendix) as a function of the fraction of ^{14}C (f) contained in the abaxial half of the leaf at the end of the day (Fig. 3). Most values of b/a observed in our experiments (Table 3) according to this calculation, could be explained by a 4:1 ratio in the ^{14}C contents of the adaxial and the abaxial half of the leaf respectively (Fig. 3).

When it is realized that the adaxial half of the leaf consists mainly of palisade perenchyma and the abaxial half of spongy parenchyma, these results are not surprising. At the end of the day much carbohydrate will be stored in the leaf, mainly as starch in the chloroplasts. Taking into account the observations of Mokronosov, Bagautdinova, Bubnova & Kobelova (1973), Farquhar et al. (1982), that the majority of the chloroplasts (in their case about 80%), is in the palisade tissue and that in this tissue, starch is the predominant form of carbohydrate synthesized, the explanation given above looks plausible, and the numbers obtained reasonable.

An improvement in the experimental technique could be obtained by using two GM tubes, one on each side of the leaf. The remaining error depends on how much the distribution of ^{14}C within the different tissues will change during an experiment. A draw-back of this method is that it can be applied in darkness only, because of shading effects.

Alternatively a more complicated technique may be adopted, using semiconductor detectors. With these detectors the spectrum of the radiation received can be analyzed enabling, at least theoretically, the determination of the distribution of ^{14}C within the leaf (van de Geyn, 1974).

CONCLUSIONS

In studies on transport and distribution of assimilates in plants by means of ^{14}C, it is generally assumed that the distribution pattern of ^{14}C is reflecting that of ^{12}C. This assumption implies that the average specific activities of carbon respired and translocated are equal to that of carbon assimilated by the source leaf. The present study however, revealed that these specific activities may be strongly reduced. Because there is evidence from literature that this is a common phenomenon, it should be taken into account in the interpretation of translocation studies based on radiotracer methods.

Measurements of the content of ^{14}C on intact leaves by means of a GM tube may be subjected to large errors, due to variations in the distribution of radioactivity over different leaf tissues. Great care therefore is needed when using this technique, even for semi-quantitative purposes.

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APPENDIX

Absorption of β radiation in leaves

If it is assumed that radioactivity in a leaf is evenly distributed in homogeneous layers with a thickness dx, activity in one layer is:

$$dA = \frac{dx}{T} \cdot A \qquad (1)$$

where T = total leaf thickness, or leaf weight per unit area
A = total amount of radioactivity in the leaf

If no self absorption in the leaf occurred, contribution of a certain layer dx to the total amount of radiation received by the GM tube is:

$$dR = \beta \cdot dA \qquad (2)$$

where β is a constant factor resulting from the geometry of the set-up and from properties of the GM tube. Absorption of β radiation often can be described roughly by an exponential function over the greater part of its range (Siri, 1949). The amount of radiation dR_x received by the GM tube from a layer dx thus may be represented by the equation:

$$dR_x = dR_0 \cdot e^{-\alpha x} \qquad (3)$$

where dR_x = the amount of radiation received by the GM tube from a layer, dx at a depth x in the leaf.

α = absorption coefficient

dR_0 = the amount of radiation received by the GM tube if no absorption occurred, from a layer dx in the leaf.

Combining equations (1), (2) and (3), and integrating, the total amount of radiation R received by the GM tube from the leaf, is:

$$R = \frac{\beta A}{T} \int_0^T e^{-\alpha x} dx = \frac{\beta}{\alpha T} (1 - e^{-\alpha T}) A \qquad (4)$$

In a similar way, the amount of radiation received from the abaxial and from the adaxial leaf half can be derived. If f.A is the amount of radioactivity in the abaxial leaf half, (1-f). A is the amount in the adaxial leaf half. When radioacticity is evenly distributed in each leaf half:

$$dA_l = \frac{dx}{T/2} \cdot f \cdot A \qquad (5)$$

$$dA_u = \frac{dx}{T/2} \cdot (1-f) \cdot A \qquad (6)$$

where dA_l = the amount of radioactivity in a layer dx in the abaxial leaf half

dA_u = the amount of radioactivity in a layer dx in the adaxial leaf half

Consequently the total amount of radiation (R) received from the abaxial (R_l) and from the adaxial (R_u) leaf half is:

$$R = R_l + R_u = \frac{\varrho A f}{T/2} \cdot \int_0^{T/2} e^{-\alpha x}\,dx + \frac{\varrho A(1-f)}{T/2} \cdot \int_{T/2}^{T} e^{-\alpha x}\,dx$$

$$= \frac{2\varrho A}{T} \cdot ((1-e^{-\frac{1}{2}\alpha T})f + (e^{-\frac{1}{2}\alpha T} - e^{-\alpha T})(1-f)) \qquad (7)$$

Jan Krupa, Hugo Challa

WYBRANE ASPEKTY METODYCZNE BADAŃ NAD TRANSPORTEM ASYMILATÓW Z LIŚCI PRZY ZASTOSOWANIU ZNAKOWANEGO WĘGLA - ^{14}C

Streszczenie

Przy użyciu licznika Geigera-Müllera mierzono zmiany radioaktywności liścia ogórka, który znajdował się w atmosferze zawierającej ^{14}C. Zaproponowany układ mierzący pozwalał na stały pomiar tych zmian w ciągu całej doby. Uzyskanie prawidłowych wyników wymaga spełnienia przynajmniej dwóch warunków. Po pierwsze - specyficzna aktywność węgla transportowanego powinna być taka sama jak używanego do żywienia liścia. Po drugie - musi istnieć określona i stała relacja między radioaktywnością wewnątrz liścia oraz radioaktywnością mierzoną na jego powierzchni przy użyciu licznika Geigera-Müllera. Przeprowadzone doświadczenia i pomiary wykazały, że wymagania te nie zawsze są w pełni możliwe do spełnienia. Na podstawie danych bibliograficznych oraz danych uzyskanych z własnych eksperymentów można przypuszczać, że występuje znacząca wymiana między powstającymi asymilatami i tymi wbudowanymi w struktury komórkowe. Oprócz tego wydajność zliczeń impulsów przez licznik Geigera-Müllera, który umocowany jest po dolnej stronie liścia, jest zmienna, gdy liść znajduje się w ciemności. Zmiany te mogą być spowodowane różną absorpcją promieniowania oraz zróżnicowaniem w dystrybucji ^{14}C w obrębie samego liścia.

W związku z tym dane dotyczące transportu asymilatów, uzyskane przy zastosowaniu radioizotopów, muszą być poddawane stałej i krytycznej ocenie przy ich interpretacji.

Ян Крупа, Хуго Халла

ИЗБРАННЫЕ МЕТОДОЛОГИЧЕСКИЕ АСПЕКТЫ ИССЛЕДОВАНИЙ НАД ТРАНСПОРТОМ АССИМИЛЯТОВ ИЗ ЛИСТЬЕВ ПРИ ПРИМЕНЕНИИ МЕЧЕННОГО УГЛЯ ^{14}C

Резюме

При применении счетчика Гейгера-Мюллера измерялись изменения в радиоактивности листа огурца, который находился в атмосфере, содержащей ^{14}C. Предлагаемая измерительная система позволяет на постоянное измерение этих изменений в течение целых суток. Получение правильных результатов требует по крайней мере двух условий. Специфическая активность транспортируемого угля должна быть такая же, как и того, который применяется для питания листа. Кроме того, должно существовать определенное и постоянное соотношение между радиоактивностью внутри листа и измеряемой радиоактивностью на его поверхности при применении счетчика Гейгера-Мюллера. Проведенные опыты и измерения показали, что эти требования не всегда возможны для осуществления.

На основе литературных данных и данных из собственных экспериментов можно предполагать, что выступает значительный обмен между возникающими ассимилятами и ассимилятами, встроенными в клеточные структуры. Кроме того, эффективность сосчетов импульсов счетчиком Гейгера-Мюллера, который укрепляется с нижней стороны листа, подвергается значительным изменениям, когда лист находится в темноте. Эти изменения могут быть вызваны разным поглощением излучения, а также дифференцированием в распределении ^{14}C в пределах самого листа.

В связи с этим данные, касающиеся транспорта ассимилятов, полученные при применении радиоизотопов, должны подвергаться постоянной и критической оценке при их интерпретации.