A Method for Continuous Measurement of Export from a Leaf1, 2

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ABSTRACT

Export of labeled material derived by continuous photosynthesis in 14CO2 was monitored with a Geiger-Müller detector positioned next to an exporting leaf blade. Rate of export of labeled material was calculated from the difference between rates of retention and net photosynthesis of labeled carbon for the observed leaf. Given certain conditions, including nearly constant distribution of labeled material among minor veins and various types of cells, count rate data for the source leaf can be converted to rate of export of carbon. Changes in counting efficiency resulting from changes in leaf water status can be corrected for with data from a transducer which measures leaf thickness.

Export data agreed with data obtained by monitoring the arrival of 14C in the sink region; isolated leaves gave values near zero for export. The method allows continuous nondestructive measurement of export of labeled carbon from a given leaf on an intact plant. The technique detects changes in export with a resolution of 10 to 20 minutes.

Export of organic substances from source leaves has been studied by a variety of methods, each with certain shortcomings. Studies of weight gain in sink organs (1, 2) require relatively long periods to measure net export from all source regions. Measuring the dry weight difference between C fixed and C accumulated in a source leaf enables net export from individual leaves to be calculated (6, 7, 9, 11). While the method gives a measure of the total export during a given period, it too requires a relatively long observation period which rules out the study of short term kinetics. The use of steady-state labeling methods and monitoring of import of 14C by sink organs (5) permits continuous measurement of import of recently fixed assimilates from a labeled leaf. In this case, contributions from unlabeled or incompletely labeled pools will give an incomplete accounting of export and unless respiration of sink organs is followed, export which contributes to respiration is not measured.

Recently, the weight gain method has been modified to permit short term study of export of substances which have been labeled with a pulse of 14CO2 (8, 10). In this case, however, the kinetics of the pulse of labeled assimilate makes quantitative measurements difficult. This latter difficulty can be overcome by employing steady-state labeling of assimilate. The present study describes the development of a quantitative method for continuously measuring export of recently fixed, labeled C from a source leaf supplied with 14CO2 at a steady concentration and specific radioactivity.

MATERIALS AND METHODS

PRINCIPLES OF OPERATION AND ASSUMPTIONS FOR MEASURING EXPORT BY MONITORING SOURCE LEAF 14C CONTENT

Basis of Method. In this method, export of recently fixed C is measured by continuously determining the difference between the rate of net fixation of 14CO2 and the rate at which 14C-labeled C is retained in the leaf. The method has the same theoretical basis as procedures used by Goodall (6), Terry and Mortimer (11), and Ho (7) but it measures export and accumulation of recently fixed, labeled C rather than of total C. Export rate is calculated from the equation E = C - (F - A) where E is the rate of export of carbon that has been derived from fixation of labeled CO2 (μg C dm-2 min-1), C is the rate of fixation of carbon (μg C dm-2 min-1), A is the rate of retention of 14C in the leaf (cpm dm-2 min-1), and F is a conversion factor, F1 or F11, calculated by one of two methods (μg C cpm-1).

Conversion Factor Based on Fixation of 14CO2. Accumulation of labeled C in the monitored leaf is measured by means of a GM detector positioned next to the lower leaf surface. A conversion factor, F1, can be derived from the rate of fixation of C and the rate of increase of 14C in the leaf lamina during the initial labeling period when export of 14C is approximately zero. F1 = C/A where A and C are defined as above. The use of F1 is based on the assumption that geometry, distribution of 14C among various types of cells, and detection efficiency of the GM detector do not change during the experiment and that nearly all of the labeled C that is fixed during the initial portion of the labeling period remains in the leaf. For sugar beet leaves there is a period of some 5 minutes after the start of labeling before phloem loading of 14C-assimilate is initiated, during which there is no export of 14C. Thereafter the rate of export of 14C increases as the specific radioactivity of sucrose goes from zero to isotopic saturation over a period of approximately 90 min (5). The F1 factor in practice is based on 14C accumulation and C fixation rates taken during the period from approximately 10 to 30 min, that is, after the rate of fixation of 14C has stabilized but before export of 14C becomes too rapid. Net C fixation rate is measured by the rate of disappearance of CO2 from the closed labeling system or the rate of supply to maintain a constant CO2 concentration during this same period.

Conversion Factor Based on 14C in Leaf at the End of the Experiment. A second method of calculating the conversion factor is based on recovery of the labeled C accumulated in the monitored leaf. The factor, F11, is calculated from the final count rate recorded by the GM detector monitoring the source leaf lamina, the absolute 14C content recovered from monitored leaf lamina at the end of the experiment, and the specific radioactivity of the C being fixed during the experimental period. The latter value is obtained from the net rates of fixation of total CO2 and of 14CO2, F11 = L · R/Q, where L is the amount of 14C recovered from the leaf at the end of the labeling period (μCi), R is the ratio of the rate of fixation of CO2 to the rate of fixation of 14CO2 (μg C μCi-1), and Q is the relative radioactivity of the 14C remaining in the monitored source leaf lamina at the end of the experiment (cpm). The use of F1 and F11 gave somewhat different results, depending on experimental conditions, but the differences fell within the range of experimental error.

A Measure of Rate of Export of Recently Fixed C. The method is based on monitoring the retention of 14C in a source leaf lamina after the labeled material being exported has attained isotopic saturation with the CO2 being supplied. Measuring export of materials derived from C fixed from the beginning of the labeling period has certain limitations. The values obtained only account...
for the export of labeled C so the method measures total export only to the extent that the C being exported has reached isotopic equilibrium with the labeled CO₂ being supplied. That C being exported, which is from pools which are not labeled or which have a lower specific radioactivity than the labeled CO₂ being supplied, will give an incomplete accounting and to this degree the rate of export will be underestimated.

When conditions such as darkening the leaf are likely to decrease the specific radioactivity of the translocated material below isotopic saturation \( *F_1 \) and \( *F_H \) can be computed in a manner analogous to that for \( F_1 \) and \( F_H \) to give \( \mu Ci \) cm⁻². In such cases \( *F_1 = \frac{C}{A} \) where \( *C \) is the rate of fixation of labeled C (\( \mu Ci \) dm⁻² min⁻¹) and \( *F_H = L/Q \). Export of \( ^{14}C \) (\( \mu Ci \) dm⁻² min⁻¹) can be calculated from the rate of fixation of \( ^{14}C \) CO₂.

If the technique of source leaf monitoring is applied under circumstances where conditions are changed greatly, the distribution of \( ^{14}C \) among minor veins, palisade parenchyma, and spongy parenchyma may change, thereby changing the detection efficiency and violating a basic assumption. Ordinarily after labeling has proceeded for tens of minutes, distribution of \( ^{14}C \) appears to remain sufficiently constant to give linear accumulation in isolated leaves. The validity of the assumption needs to be evaluated before the method is used to study export under a given set of circumstances.

**EXPERIMENTAL PROCEDURES**

**System for Supplying \(^{14}C\)O₂.** The system used to supply \(^{14}C\)O₂ and to measure photosynthesis (Fig. 1) is a modification of one described previously (4). Labeled CO₂ is supplied on demand from a syringe under control provided by a recorder fitted with a set of microswitches and a logic circuit (3). When net C fixation is not being measured by the rate of disappearance of CO₂, concentration of CO₂ is maintained within 5 \( \mu l \) L⁻¹ of the average concentration. Rates of fixation of CO₂ and of \(^{14}C\)O₂ are obtained by measuring the rate of disappearance of the respective forms of CO₂ from the closed gas system or by observing the rate of addition of CO₂ needed to maintain a constant CO₂ level. During periods of measurement of net photosynthesis by rate of disappearance, the CO₂ concentration is varied between 550 and 450 \( \mu l \) L⁻¹. In distinction to methods which measure export by observing \(^{14}C\) export from a source leaf after a pulse of \(^{14}C\)O₂ (8, 10), labeling in the present case is continuous during the monitoring period.

**Monitoring Accumulation of \(^{14}C\) in a Source Leaf.** The validity of this method requires that accumulation of \(^{14}C\) in the source leaf be measured continuously and accurately. The amount of \(^{14}C\) present in the monitored leaf is measured by means of a GM detector system which gives the \(^{14}C\) content in relative terms. A potential difficulty in the procedure is production of a high background count rate by the atmosphere containing \(^{14}C\)O₂. This high background was avoided by placing the window of the GM detector 3 mm below the leaf surface. Measurement of \(^{14}C\) distribution in the leaf revealed that the spacing did not interfere with the movement of air between the leaf blade and detector provided flow rates were at least 1,000 cm⁻² min⁻¹. To avoid possible fogging of the detector end window the end of the GM detector was fitted with a heating block which maintained the temperature of the detector above the temperature of the air around the leaf. The background level of \(^{14}C\) from the \(^{14}C\)O₂ was kept relatively constant by controlling the concentration of labeled CO₂ in the atmosphere within a constant range.

Efficiency of detection of \(^{14}C\) in the lamina is quite sensitive to changes in position of the leaf blade, and to changes in absorption when leaf thickness or water status changes. To minimize position changes, the monitored region of the leaf was held between a grid formed by two sets of metal rods (Fig. 2). In order to correct for changes in absorption produced by experimental conditions, a thickness-sensing transducer was incorporated in the leaf chamber and changes in absorption of radioactivity were correlated with leaf thickness. To establish an empirical correction curve, a series of measurements of accumulation were taken with detached leaves.
in which the rate of export is near zero and consequently the rate of accumulation in the source leaf is constant. Net photosynthesis rates were measured to determine that they did not change and thus that the rate of accumulation was likely unchanged. In most cases the turgor of the leaf changed once the system was closed to the atmosphere, bringing about a change in the slope of the curve for $^{14}$C accumulation in the monitored leaf.

The factor needed to restore the observed $^{14}$C accumulation rate tracing to the true linear rate was plotted as a function of leaf thickness measured by the transducer (Fig. 3). In all cases the plot of correction factor versus thickness was linear over the range of leaf thicknesses encountered. However, the values for slope of the curves varied by up to severalfold among experiments.

One method that was employed to decide the slope of the specific curve to be used for correcting count rate for thickness changes was to calibrate the system after isotopic saturation was attained and before any treatment. The accumulation curves were linear during this period until changes in thickness occurred. It was assumed that the corrected accumulation curve should be linear and in this way the conversion factor needed to restore linearity was calculated for a period of 30 to 40 min. The correction curve specified in this manner was tested by applying it to the remainder of the experimental data from untreated isolated leaves. The resulting corrected accumulation curve remained linear and continuous with the first part for the duration of the measurement period (Fig. 3). These results indicate the reliability of estimating the correction curve slope based on a limited span of data.

A second method, of more general application, is based on the assumption that the differences in slope of the correction curves are mainly due to differences in counting efficiency. The slope of the various correction curves, derived by the first method, showed a high negative correlation with leaf counting efficiency ($r = 0.967$). CSF $= -3.07 \times 10^{-4} \text{ CE} + 0.00357$ where CSF is the slope of the plot of correction factor as a function of leaf thickness and CE is the GM counting efficiency for the particular configuration in cpm divided by $\mu$Ci of label present per dm$^2$ blade. While CE changes slightly as a function of changes in leaf thickness, the basic value is set by a number of parameters such as leaf thickness and geometry in a manner which is not understood clearly. This mathematical relation means that leaf configurations in which absorption of counts is less affected by thickness will also show a smaller change in absorption when leaf thickness changes.

Application of the second method to data from isolated leaves gave straight lines for accumulation which were continuous with the slopes before thickness changes and gave very nearly the same results as application of the first method. The virtue of this second method lies in the fact that it requires no test period but can be applied just by measuring leaf-counting efficiency. This method was applied satisfactorily to correct the data for intact and trimmed plants reported in this study. Because transducer readings are sensitive to temperature changes, precautions were taken to assure that the transducer temperature remained constant.

**Instrumentation.** The level of $^{14}$C in the atmosphere supplied to the monitored leaf was measured with a 250-cm$^2$ ion chamber attached to a Victoreen model 475B femtometer. CO$_2$ concentration was measured with a Lira model 300 infrared analyzer (Mine Safety Co.). The leaf under study was enclosed in a brass chamber (Fig. 2) cooled with antifreeze solution from a thermostatted bath. A 1.6- to 2-mg cm$^{-2}$ end window GM tube (TGM Corp. model N1003) was sealed into the bottom of the chamber. The thickness transducer was a Schaevitz model R30D rotary variable differential transformer connected to servo recorder through a zeroing network.

**Preparation of Plant Material.** Sugar beet plants were raised by sand culture, in a mixture of equal parts sand and Jiffy-Mix soil. The leaves needed with mineral solution. Light intensity in the environmental cabinets was 35 N.E. cm$^{-2}$ s$^{-1}$ at leaf blade level. A leaf with a lamina 10-12 cm in length was chosen as the monitored leaf. For experiments with isolated leaves this leaf was detached. When trimmed plants were used all other leaves except the sink leaf to be monitored were removed. Other than for test purposes all of the leaves were left attached for the experiment. A micrometer was used to measure the thickness of the leaf lamina both in place under the transducer arm and over the GM tube. The petiole of this leaf was sealed in place and the lamina positioned above the GM tube between wire holders.

**Measurements and Calculations.** Net photosynthesis measurements were made throughout the labeling period. At the end of the experiment, the lamina was converted to CO$_2$ by wet oxidation and the $^{14}$CO$_2$ measured with an ion chamber electrometer. Any $^{14}$C-labeled noncombustible material remaining after the oxidation procedure was assayed by liquid scintillation counting. Data for net photosynthetic C fixation rate were smoothed, as were the data for count rate from $^{14}$C accumulation in the monitored leaf. Export was computed from the difference between these values. Data reduction lends itself well to analog-to-digital conversion and computer processing.

**RESULTS AND DISCUSSION**

**VERIFICATION OF THE METHOD**

**Nonexporting Leaves.** To assess ability of the method to measure export, measurements were made on detached leaves with petioles immersed in water. Time course curves are given in Figure 4 for rates of net photosynthetic C fixation, accumulation of C in the monitored leaf lamina, and export of labeled assimilates from the lamina. Data for rates of net photosynthesis and accumulation were fitted by a least-squares polynomial method. Choice of the appropriate degree of polynomial to be used was determined by examining the shape of the data record and the error for trial-fittings. The export curve was derived from the two rate curves by subtraction.

Data for five experiments of this type are summarized in Table I. Calculation of export based on $^{14}$C recovered from the petiole and the specific radioactivity of the CO$_2$ fixed is the most direct means for determining export and consequently these values have the least range of variability. Practically all of the $^{14}$C fixed was recovered and accounted for. This direct method was used as a basis for comparison to evaluate other methods of measuring export. For detached leaves export was generally under 5% of the net C fixed during the experimental labeling period.
calculated in C in labeled (4C).

Table 1. Comparison of Measurements of Export from an Isolated Leaf Lamina Obtained by Various Methods

<table>
<thead>
<tr>
<th>Method Used to Determine Export</th>
<th>Export Rate (μg C dm⁻¹ min⁻¹)</th>
<th>Proportion of Net C Fixed (%)</th>
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<tbody>
<tr>
<td>Labeled C recovered from the petiole</td>
<td>2.6 ± 1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>GM monitoring of lamina, F₁</td>
<td>6.0 ± 6.8</td>
<td>6.1</td>
</tr>
<tr>
<td>GM monitoring of lamina, F₁</td>
<td>0.6 ± 9.2</td>
<td>−0.9</td>
</tr>
<tr>
<td>Average, F₁ and F₁₂</td>
<td>2.7 ± 8.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Fig. 4. Time courses curves for rate of net C fixation (O), rate of C accumulation in the source leaf lamina (□) and the rate of export (●) calculated from the rates of net photosynthesis and accumulation of labeled C in the isolated source leaf lamina. Net photosynthesis rate data and 14C accumulation rate data in Figures 4, 5, and 6 were smoothed by least-squares polynomial curve fitting.

Fig. 5. Time course curves for rate of net C fixation (O), rate of export calculated from the rate of accumulation of labeled C in the source leaf lamina (●), and the rate of import of labeled C by the sink leaf (△). The plant consisted of root system and shoot with one source leaf and one sink leaf.

The over-all average for export obtained by GM monitoring of the source leaf lamina is the same as the data from direct measurement but the data from the present method have a range which is five times as large. When the error values are compared with the low export values for isolated leaves, the errors seem unacceptably large. These errors arise from the variability of the rates of photosynthesis and accumulation of labeled C assimilated by the lamina. Subtraction of these rates in isolated leaves yields an export rate which is approximately zero and gives rise to a large relative error. Absolute errors do not increase appreciably when export rates are higher and consequently the relative error improves greatly for larger export rates. The 10 values of export rate which are averaged in Table 1 had a range of absolute error of from 15.7% to −13.2% of the net photosynthesis rate. Although the range of relative error was quite large when the lamina exported less than 3% of the net C fixed, the relative error range declined to ±25% when leaves exported approximately half of the net C fixed.

Calculation of export rate based on values for F₁ and F₁₂ for any given experiment varied by no more than ±7% of the rate of net C fixation rate. Neither value seemed consistently more accurate than the other. Normalized values of F₁, which took into account differences in counting efficiency and specific radioactivity, permitted F₁ and F₁₂ the various experiments to be compared. Normalized values were based on a specific radioactivity of 2,000 µCi µg⁻¹ C⁻¹ and a counting efficiency of 450 cpm µCi⁻¹. For five experiments with isolated leaves, the average value for F₁ was 3.74 ± 0.85 µg C cpm, for F₁₂ was 3.96 ± 0.84 and for the over-all F was 3.85 ± 0.80. From the pattern of values we conclude that, in general, averaging the values of F₁ and F₁₂ for a given experiment when computing export rate further reduces relative error and increases accuracy.

Export from Source Leaf of Trimmed Plants. Data from measurement of export from leaves which constituted the sole source leaf on a trimmed plant were compared with data for import into sink regions from the sole source leaf. The latter measurements were based on steady-state labeling and sink region monitoring.

Table 2. Comparison of Rates of Translocation of Assimilate from a Source Leaf Lamina into Sinks in Plants Trimmed to Include Only One Source Leaf

<table>
<thead>
<tr>
<th>Categories of C Measured</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Avg.</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net fixed</td>
<td>69</td>
<td>108</td>
<td>82</td>
<td>86</td>
<td>100</td>
</tr>
<tr>
<td>Accumulated in source lamina</td>
<td>42</td>
<td>60</td>
<td>30</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>Exported, GM monitoring</td>
<td>27</td>
<td>47</td>
<td>56</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>Imported</td>
<td>27</td>
<td>54</td>
<td>63</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>Difference, export − import</td>
<td>0</td>
<td>−7</td>
<td>−5</td>
<td>−5</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 6. Time course curves for net C fixation rate, original points (+), and smoothed data (O), for export from a monitored source leaf (●) and for import into sinks as monitored by arrival of label in a sink leaf (△). The intact ungirdled plant was initially exposed to light of 35 nE cm⁻² s⁻¹; at (t) the light was increased to 65 nE cm⁻² s⁻¹ and then at (l) was returned to the original light intensity.
(5). The time course for one of these such experiments is given in Figure 5 and data for comparison of the methods is shown in Table II. The values for import and export are similar with the export generally being slightly lower than import measurements. Respiration of labeled C by the beet and roots was measured and appear to be negligible in experiments of 4- to 6-h duration (data not shown) so one would expect the values for import and export to be nearly equal.

**KINETIC STUDIES**

The method was used to study export from source leaves of intact plants. Data for one such experiment is given in Figure 6. Export from a source leaf and import into sinks supplied in part by the monitored source leaf were measured before, during and after a 100-min period in which light intensity was doubled. Data for import into sinks were taken from a sink leaf importing largely from the monitored source leaf. The data are not intended to be a thorough study of kinetics of response to changes in illumination but to show the ability of the method to follow relatively rapid changes in export rate. The slower and smaller response of import into the sink as monitored in the sink leaf is likely to be a result of buffering of changes in translocation caused by the presence of storage areas along the path between the source and the monitored sink leaf and by contributions from other sink regions.

Measurement of export during steady-state labeling by monitoring net photosynthesis and accumulation of labeled C in a source leaf appears to be accurate and feasible for kinetic studies. It seems to be particularly useful for studying changes in export in response to various treatments. Limits to the ability to study short term kinetics are set primarily by the capacity to measure changes in the rates of net photosynthesis and of accumulation of labeled C in the monitored source leaf. To the extent that export involves unlabeled compounds or materials derived from pools not at isotopic equilibrium with the labeled CO₂, translocation from the leaf will be underestimated. The method is currently being employed in studies of factors affecting allocation of recently fixed C for export.

**LITERATURE CITED**