Technical note

An experimental system for the quantitative 14C-labelling of whole trees in situ

A Kajji, A Lacointe, FA Daudet, P Archer, JS Frossard

INRA, Université Blaise-Pascal, Unité Associée de Physiologie Intégrée de l'Arbre Fruitier,
Domaine de Crouelle, F-63039 Clermont-Ferrand Cedex 02, France

(Received 8 April 1992; accepted 10 March 1993)

Summary — The first part of this paper provides a brief review of the requirements that apply to 14C-labelling chamber technology, particularly for tree labelling, and of the means that can be used to meet them. Two main points are considered: the quality of the plant chamber environment—the necessity of thermal and hygrometric regulations is discussed—and the possibility of determining the exact amount of 14CO2 assimilated by the plant. The authors then describe a simple system allowing the quantitative labelling of entire trees, without temperature- or hygrometry-regulating devices which can be used in the morning. The CO2 concentration is maintained at its natural level throughout the labelling procedure through an injection of cold CO2 operated by an IRGA-driven computer. This system was successfully used for the labelling of grafted walnut trees.

assimilation chamber / control of CO2 level / photosynthesis
théoriques permettent d’estimer à quelque 3% la radioactivité perdue par fuites lors du marquage. La régulation de la teneur en CO2 répond à un double but. D’une part, en limitant l’écart par rapport aux conditions naturelles, on perturbe le moins possible la répartition biochimique et spatiale des assimilats. D’autre part, la totalité du 14C étant injectée instantanément dès le début de l’opération, la régulation consiste à injecter du carbone «froid» pour compenser la photosynthèse, et l’équation (1) (paragraphe «Injection de CO2») donne à tout moment la quantité totale de 14C restant dans la chambre. Ainsi, 99,3% de la radioactivité a disparu lorsqu’on a renouvelé 5 fois la totalité du CO2 présent dans la chambre, ce qui était réalisé en 4 h environ. Le CO2 est fourni par la réaction d’une solution de Na2CO3 goutte à goutte dans un flacon d’acide sulfurique à 33% (fig 1). L’efficacité du dégagement gazeux est améliorée par une agitation magnétique et un barbotage de l’air de la chambre prélevé par une pompe. L’injection initiale du carbonate marqué, de forte radioactivité spécifique (1,85 GBq/mmol; 74 MBq par arbre, pesant chacun 2 kg de MS) ne modifie pas la teneur totale en CO2 de la chambre. Puis le réservoir de carbonate est rempli de solution «froide», 1 M, délivrée selon les besoins de la régulation par une électrovanne. Celle-ci est pilotée par un micro-ordinateur (fig 1) munie d’une carte d’acquisition de données (Micromac 4000, Analog Devices) qui enregistre par ailleurs la température, le PAR incident et la teneur en CO2 de la chambre mesurée par un IRGA. Ce système libère quelques gouttes de carbonate dès que la teneur en CO2 descend au-dessous de 350 ppm, ce qui permet une régulation efficace (fig 3). Les aspects quantitatifs des marquages ont été validés par 2 moyens indirects : d’une part, en vérifiant que la radioactivité résiduelle de l’air à la fin du marquage est conforme à l’équation (1); d’autre part, en retrouvant dans les arbres traités, quelques heures après marquage, 90% de la radioactivité injectée.

chambre d’assimilation / régulation de la concentration en CO2 / photosynthèse

INTRODUCTION

During the past 40 years 14C has been widely used as a tracer in studies of carbon flows in biological or biochemical systems, in which its radiations can be used in imagery (autoradiography) or quantitatively counted in liquid scintillation or gas-flow counters. We will here discuss only global studies of carbon flows, in which the 14C enters the plant system through the natural pathway, ie photosynthesis. The basic procedure in this case consists of feeding the plants with 14C-enriched CO2.

After a brief review of the constraints related to 14C labelling, and of the main progress made in labelling chamber technology in order to meet them, particularly for trees, this paper presents a system allowing quantitative labelling which has been used successfully at our laboratory in Clermont-Ferrand.

This labelling system was designed to investigate carbon flows in 3- to 4-yr old walnut trees. Particularly, our aim was to trace the incorporation of photosynthetic-derived carbon into carbohydrate reserves vs structural compounds at different times, as well as spring remobilization of the labelled reserves (Lacointe et al, 1993).

GENERAL CONSTRAINTS RELATED TO 14C LABELLING

Airtight chambers are utilised in the quantitative feeding of plants with labelled CO2 (14CO2 or 13CO2). Enclosing plants in a closed illuminated chamber leads to rapid modification of the atmosphere due to depletion of CO2 by photosynthesis and accumulation of a significant amount of heat and water vapour; the rate of photosynthesis can be significantly altered by these modifications in the environment.
Although the aim of feeding experiments is generally not to evaluate the photosynthetic rate (well known gas exchange methods are far more suitable for this purpose), it is necessary to maintain a sufficiently high rate of photosynthesis in order to achieve maximal exhaustion of the labelled CO$_2$ by the plants. Furthermore, a significantly reduced assimilation rate could disturb the natural pattern of chemical and spatial partitioning of assimilated C (Geiger and Fondy, 1991). Then at least partially regulating the most critical parameters of the environment may become necessary even for feeding periods of short duration. For long-term feeding experiments, due to significant alteration in most of the physiological functions when the environmental conditions are changed, the temperature and humidity of the air will have to be regulated.

**A within-chamber environment allowing photosynthesis**

**Light conditions**

The materials used to construct the chambers (transparent plastics) have photosynthetically-active radiation (PAR) transmission factors ranging between 70 and 90% (Dogniaux and Nisen, 1975), which involves some reduction in the photosynthetic rate with respect to open air conditions. In labelling experiments this reduction is assumed to have only little effect (if any) on the fate of the incorporated C in the plant (which is the question under study). For reasons of cost and ease of handling PVC was chosen.

**Air temperature conditions**

Due to very low transmittance of the plastic materials in the thermal IR range (between 2.5 and 25 μm; Dogniaux and Nisen, 1975), and low convection (closed circuit conditions), the temperature of the air inside the chambers can be increased by 5 to 15°C with respect to the outside in conditions of high solar irradiance. When excessive, this increase in temperature can lead to reduced or even negative net photosynthetic rates, the latter rendering impossible any labelling experiment in the absence of an additional cooling system.

A few authors have tried to solve this problem which can become critical for long feeding periods especially when intense radiative conditions are encountered. Lister et al (1961) interposed water filters to absorb part of the IR radiations from the light source. This system is viable for indoor labelling but unsuitable in the field. Palit (1985) used occasional spraying of cold water, whereas Lister et al (1961), Warembourg and Paul (1973), Geiger and Shieh (1988) made use of different types of heat exchangers to regulate the temperature. All these systems, well adapted to small-sized chambers (a few litres), would become problematical if used with chambers several cubic meters in size, as necessary to label whole trees.

However, even for small chambers, since the only requirement is that of no significant reduction in photosynthesis, most authors did not include any cooling device in their feeding system and tried simply to limit overheating, ie to operate preferentially in the morning. This is approach that was adopted for our system.

**Air humidity conditions**

When exposed to high solar irradiance, well watered plants inside a closed chamber convert a large proportion of the incident radiative energy into latent heat by transpiration, leading to complete saturation of the volume of the chamber by water
vapour in a few min and to heavy condensation on the walls which constitute the cold elements of the system. Since the leaves absorb most radiation, they become warmer so that no condensation occurs on them. These physical conditions at leaf level (high temperature and low water saturation deficit) are known to be generally favourable to photosynthesis (provided the temperatures do not become excessive). Then one can assume that regulating the humidity of the air per se would generally be unnecessary for feeding experiments of short duration. On the contrary, for long-duration feeding experiments, a system of complete air conditioning (temperature and hygrometry) is necessary. A few authors (Webb, 1975; Kuhn and Beck, 1987; Geiger and Shieh, 1988) regulated the relative humidity in the labelling chamber, using a cooled vapour trap. For our feeding experiments which were designed to last = 4 h it was decided to leave the hygrometry unregulated.

Regulating the CO2 concentration

Since exhaustion of the ambient CO2 by photosynthesis in feeding experiments leads to decreased photosynthetic rates, maintaining the CO2 concentration at normal values is necessary. Achieving accurate regulation of CO2 requires continuous measurement of its concentration (using an IRGA) and an injection system. Rough control of the ambient CO2 can be achieved by temperate injection of chemical reactants (Warembour and Paul, 1973; Smith and Paul, 1988; Schneider and Schmitz, 1989) or by the use of cylinders of diluted CO2 and mass-flow regulators (Webb, 1975; Geiger and Shieh, 1988; Hansen and Beck, 1990). Though less accurate, the former solution was chosen for our system because of its simplicity of operation.

Making the labelling quantitative

Depending on the objectives of the experiment, it may or may not be important to regulate the isotopic ratio of the assimilated CO2 (specific activity in case of 14CO2).

In long-term labelling experiments steady state has to be reached, hence the isotopic ratio of the photosynthetic CO2 must be held constant, but the total amount of incorporated C is generally of no importance. On the other hand, in short-term labelling experiments achieving quantitative labelling, ie knowing how much 14C the plant has actually taken up may be of importance, particularly for experiments with destructive sampling; but keeping the isotopic ratio constant is generally unnecessary.

In order to make a short-term labelling quantitative, the first step is to accurately determine the total quantity of 14CO2 injected into the labelling system. The CO2 can be directly injected as gas from a syringe (Balatinecz et al, 1966) or a pressurized cylinder (Webb, 1975; Kuhn and Beck, 1987). Alternatively, it can be released from the reaction of 14C-carbonate with excess acid (Lister et al, 1961; Hansen, 1967; Warembour and Paul, 1973; Glerum and Balatinecz, 1980; Langenfeld-Heys, 1987; Smith and Paul, 1988; Laconte, 1989; Schneider and Schmitz, 1989; and many others). In the latter case, due to the higher density of CO2 as compared to air, the atmosphere in the reaction vessel must be chased efficiently. This problem was solved by forcing the chamber atmosphere into the reacting solution (fig 1).

Secondly, the injected CO2 must not leave the system during the labelling. Hence the chamber – and circuit when present – must be airtight, which is also important to avoid pollution problems, particularly indoors. Air-tightness is generally
not a real problem with solid chambers, but can be with chambers made of plastic film, due to the possibility of small tears or holes and rather large changes in volume allowed. The above-mentioned materials including plastic films, generally exhibit a sufficient impermeability to CO
subscript{2}, \text{eg} 1.04 \times 10^{-4} \text{cm}^2 \text{m}^{-2} \text{min}^{-1} \text{Pa}^{-1} for a 0.03-mm polyethylene film (Daudet, 1987).

Many authors have not carried out more controls, either because they were not interested in the exact quantity incorporated (Balatinecz et al., 1966; Langenfeld-Heyser, 1987), or because they allowed \(^{14}\text{C}\)-assimilation for a time which they either assumed or knew to be long enough for a complete exhaustion of the \(^{14}\text{CO}_{2}\) in the chamber (\text{eg} 6 \text{ h for Hansen, 1967; 30 min for Palit, 1985}). However, some authors further investigated the actual amount of \(^{14}\text{C}\) taken up by measuring the level of \(^{14}\text{CO}_{2}\) still in the system at the end of the labelling period. Before opening the chamber, they forced its atmosphere into a \text{CO}_{2}\text{-trapping} circuit generally containing \text{KOH} or \text{Ba(OH)}_{2} (\text{a} common \text{procedure to avoid pollution, particularly indoors}) and then measured the radioactivity trapped by the alkali (Glerum and Balatinecz, 1980).

Further progress was achieved through measuring the \(^{14}\text{CO}_{2}\) level not only at the end of the labelling, but continuously during the labelling period. Lister et al (1961) used both an \text{IR} \text{gas} \text{analyser} for estimat-
ing the total CO$_2$ level and a Geiger-Müller tube for volumic radioactivity, whereas Kuhn and Beck (1987) used only an IRGA to measure the decrease in the CO$_2$ level (and calculate that of the $^{14}$CO$_2$) within the chamber. As mentioned above (see Regulating the CO$_2$ concentration), some authors used an IRGA to regulate the CO$_2$ level inside the chamber throughout the labelling period.

When the injected CO$_2$ was of constant specific radioactivity, this allowed long-duration labelling under steady-state conditions (Warembourg and Paul, 1973; Webb, 1975; Geiger and Shieh, 1987; Smith and Paul, 1988). On the other hand, when all the $^{14}$CO$_2$ was injected at the beginning of the experiment and the continuously injected CO$_2$ was only $^{12}$CO$_2$ (Hansen and Beck, 1990), this allowed a precise calculation of the total $^{14}$C taken up by the plant under conditions of minimum perturbation. This was the basis of the system we designed for the labelling of whole trees.

**DESCRIPTION AND PERFORMANCES OF THE LABELLING SYSTEM**

The labelling system is composed of an assimilation chamber and an electronically controled CO$_2$ injection device allowing continuous regulation of the inside CO$_2$ concentration (fig 1). It has been used on 3-yr-old grafted walnut trees with 1 trunk and 4/5 branches and a total leaf area of $\approx$ 1.7 m$^2$. The trees were grown outdoors in 200-l containers.

The assimilation chambers

Two chambers were used alternatively, allowing either local labelling of a branch section or global labelling of the whole above-ground part.

The chamber used for the local labelling was an open cylinder made of 2-mm PVC (PAR transmission factor = 85%). Its height was 0.50 m and its diameter 0.34 m (vol = 45 l). This cylinder was extended at each end by a 0.03-mm polyethylene film junction, allowing gas-tight sealing on the branch with Terostat 9010 sealing profile (Teroson, France).

The chamber used for global labelling was a closed cylinder (height = 2 m; diameter = 1.44 m; vol = 3.25 m$^3$), made of 2-mm PVC set on a steel frame. It consisted of 2 halves hanging from a portable support, which could be joined together via rubber joints. Airtightness was achieved by compressing the joints with screws. There was an opening in the cylinder bottom for the stem, and airtightness was achieved through plastic film junction and sealing as for the small chamber.

Despite ample precautions, we could not assume that airtightness was absolute, either for the large or for the small chamber, due to preexisting small holes in the plastic film parts and/or leaks induced by differential thermal dilatation of the rigid parts of the chambers. No precise measurement of leakage was made for the chambers but an estimate of the upper limit of total radioactivity lost due to these leaks can be given, assuming equipressure between the inside of the chamber and atmosphere, when thermal dilatation of the air in the chamber occurs. In such conditions, an increase in temperature of 15–20°C during the course of feeding (cf fig 2), could lead to a leakage of 6% of the air in the chamber; we can expect a lesser relative loss of total radioactivity (≈ 3%) since the specific radioactivity of the CO$_2$ decreases continuously during the feeding period.

In both chambers the atmosphere was homogeneized by a fan, and there were 4 openings for the in- and outlet tubes of 2 closed circuits: one for CO$_2$ level monitoring and one for CO$_2$ injection (fig 1). The
CO₂ injection

Total amount of RA required per tree

The total amount of radioactivity required was determined according to the sensitivity of the least sensitive method used for ¹⁴C measurement. Two methods were used in the experiment: liquid scintillation for soluble compounds, and argon–methane flow counting for insoluble compounds. The less sensitive method is the latter, which was used in a previous experiment on walnut seedlings (Lacointe, 1989). This study showed that an accurate measurement of the RA incorporated in all organs (including new spring organs) required \( \approx 1 \mu\text{Ci} \) (37 kBq) ¹⁴CO₂ fed per g plant DM as an order of magnitude. Since the DM weight was \( \approx 2 \) kg, the amount injected was determined as 74 MBq for each tree.

Control of CO₂ injection

CO₂ was generated through dropping a sodium carbonate solution from a burette into excess 33% sulfuric acid. The efficiency of CO₂ evolution was improved by a magnetic stirrer and by forcing the chamber atmosphere through the reacting solution with a pump.

The first step was the injection of all the ¹⁴C-carbonate which induced only a slight increase in the total CO₂ concentration within the chamber (< 0.1% for the large, 6% for the small chamber) due to the high specific radioactivity of the carbonate (1.85 GBq/mmol ref CMM 54, CEA, France). The procedure then consisted of maintaining the total CO₂ concentration between 330 and 360 vpm until 99% of the injected ¹⁴CO₂ had been assimilated. Provided the total CO₂ level in the chamber remained constant, the radioactivity still present at any time could be easily calculated:

\[
R = R_i \cdot \exp \left(-\frac{n}{N}\right)
\]

\( R \) being the radioactivity still present, \( R_i \) the initial radioactivity injected, \( n \) the total amount of CO₂ injected from cold carbonate since the beginning, and \( N \) the amount of CO₂ constantly present in the chamber.

From this equation it can be derived that the radioactivity was exhausted by 99.3% for \( n = 5N \), which was achieved within 4–5 h in the large chamber, or < 1 h in the small chamber.

The CO₂ level was continuously measured with an IRGA (Mark III, ADC, UK). A data processor system (Micromac 4000, Analog Devices, USA) connected to a microcomputer allowed the recording of physical parameters such as air temperature, incident PAR (Daudet, 1987) and monitoring of a magnetic valve. Whenever the CO₂ level dropped below 350 vpm, the valve opened and an unlabelled sodium carbonate solution was dropped into the acid, injecting cold CO₂ into the chamber. The molarity of the carbonate solution was 1 M for the large and 0.125 M for the small chamber.

An example of the time course of CO₂ concentration during feeding is given in figure 3. One can see that the stability of CO₂ was correct during most of the feeding period. Some dysfunction could occur due to poor stability of the flow of the sodium carbonate solution through the precision cock (see fig 1).

Variation of air temperature

In order to limit temperature increase, labellings were performed in the morning, and lasted < 5 h. Figure 2 shows the...
increase of temperature inside the large chamber during a labelling day with very high solar irradiance. Although the air temperature reached 38°C inside the chamber at the end of the feeding period (>12°C increase with respect to the ambient temperature), there was no significant alteration in photosynthesis as can be seen from figure 3: the assimilation rate, as derived from the parts with negative slopes, remained relatively regular throughout the labelling procedure. So did the kinetics of cold CO₂ injection operated by the system to keep the CO₂ concentration around 350 vpm (parts with positive slopes). This indicates that no major disturbance of photosynthesis and presumably of the general plant physiology occurred. In fact, the photosynthesis of walnut trees appears quite resistant to high temperature; nevertheless, negative values of net assimilation were observed one day when the inside temperature reached 45°C.

Validating the quantitative aspects of the feedings

Two indirect means could be used to estimate the amount of total radioactivity actually absorbed by the trees and compare it to the theoretical value as given in equation [1]:

- measuring the radioactivity that remained in the atmosphere of the chamber and in the different vessels at the end of the feeding period. At the end of a few local labellings, which according to equation
CO₂, but it can also be stopped at any time (eg in case of excessive temperature increase) allowing the accurate amount of ¹⁴C taken up to be determined;
– a CO₂ level constantly maintained at its natural value, thus limiting changes in the within-leaf partitioning between sucrose and starch which could affect export dynamics.

This system allowed us to investigate the spatial and chemical partitioning of assimilated carbon in walnut trees in August and October, when the trees exhibited contrasting daily net assimilation rates (Kajji, 1992). We also obtained interesting results on the long-term fate of the labelled carbon reserves, eg a differential mobilization rate of the starch reserves according to their formation time (Lacointe et al, 1993).

For the sake of simplicity no temperature regulation was included in our system and we assumed that in most cases this lack of thermal regulation had no effect on the process of redistribution of assimilates within the trees. Nevertheless, it is clear that incorporating such an improvement in the system would be of interest, as it would permit long-term labelling experiments or/and feeding during the warmest days.

CONCLUSION

Use and performances of the system

The labelling system described exhibits 3 characteristics which have already been separately described by other workers, as mentioned above, but not together:
– a large assimilation chamber (> 3 m³) allowing the labelling of large trees, namely grafted walnuts bearing some fruit. It remains handy enough to allow the labelling of a different tree every day;
– quantitative labelling. This can guarantee the complete assimilation of the injected

ACKNOWLEDGMENT

The authors are most grateful to M Crocombette for providing technical assistance.

REFERENCES

Daudet FA (1987) Un système simple pour la mesure in situ des échanges gazeux de couverts végétaux de quelques mètres carrés de surface foliaire. Agronomie 7, 133-139


Schneider A, Schmitz K (1989) Seasonal course of translocation and distribution of 14C-labelled photoassimilate in young trees of Larch decidua Mill. Trees 3, 185-191


Warembourg FR, Paul EA (1973) The use of 14CO2 canopy techniques for measuring carbon transfer through the plant soil system. Plant Soil 38, 331-345