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***Review*:**

Using the short-lived isotope ^{11}C in mechanistic studies of photosynthate transport

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Abstract. Tracer techniques have been central in studies of transport in plants. In the case of carbon, the only readily available radioactive tracer has been ^{14}C, although ^{11}C was used for a short time before ^{14}C could be made. Tracers have usually had to be measured by destructive harvesting of the plant, giving a practical limit to the data resolution in both time and space. A major advantage of the short-lived, positron-emitting tracers, of which ^{11}C is one example, is that *in vivo* measurement is possible, giving detailed time series of tracer data in many locations and opening up powerful new techniques of data analysis. Medical applications of these isotopes have utilised both dynamic imaging and time courses of uptake or washout. Unfortunately, few plant biology laboratories have realised the potential of these techniques, possibly because of the large physics infrastructure needed. In this paper we review the concepts behind the use of these short-lived tracers in plant physiology, and illustrate with several cases where ^{11}C was an essential tool.

Keywords: carbon-11, modelling, phloem translocation, source–sink interactions.

Introduction

Cyclotron production of ^{11}C was used in early studies of carbon fixation, before ^{14}C was available (Ruben *et al.* 1939; Benson 2002). But for these studies of metabolism ^{11}C was quickly displaced in the mid-forties by ^{14}C because its much longer half-life gives it a much wider applicability, even though the low-energy decay products are harder to detect. The power of ^{11}C for *in vivo* studies in phloem work was first demonstrated by Moorby *et al.* (1963), and then More and Troughton (1973) showed that useful amounts of ^{11}C can be produced with the small van der Graaf accelerators often found in physics labs. In the next 10 years several labs worked in this way, for example, Fensom *et al.* (1977) in Canada, Fares *et al.* (1978) in the US, Williams *et al.* (1979) in Scotland, and Jahnke *et al.* (1981) in Germany. By the early 1990s the UK, US and Canadian groups had stopped operating, leaving only New Zealand and German groups. Last year (2002) the New Zealand group was closed down for commercial reasons, and only the Jülich group remains.

Because of its short half-life ^{11}C must be produced close to the site of use and this is probably the sole reason, apart from cost, that it is used so little in plant physiology. However, it should be a simple matter to piggyback a plant laboratory along side a medical facility to gain access to the short-lived isotopes that can be produced there (e.g. ^{11}C, ^{18}O, ^{18}F, ^{13}N).

The short-lived nature of ^{11}C has both advantages and disadvantages. Radiation safety issues are much reduced. A sequence of independent pulses can be used, as there is no build up of tracer from previous pulses, allowing one to follow the changing pattern of plant function indefinitely. But because of the short half-life, only short-term processes are 'visible'. As a consequence, ^{11}C has limited use, for example, in the study of carbon export from a labelled leaf, because processes there have characteristic times up to 10 h and even longer associated with storage and remobilisation (Rocher and Prioul 1987).

In most plant applications, ^{11}C has been used as $^{11}CO_2$ and applied to the leaves where it is metabolised to labelled 'photosynthate'. The labelled photosynthate can be extracted and applied exogenously to abraded tissue (Hayden *et al.* 1980; Thorpe and Minchin 1987).

In vivo observation of tracer movement has led to many new and interesting phenomena by the simple 'look–see' method, which uses the half-life-corrected tracer pulses at various positions in the plant to show treatment responses [e.g. micro-fluctuations in the transport system (Thompson *et al.* 1979)], rapid redirection of phloem transport when an

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existing pathway is inhibited (Pickard *et al.* 1978), rapid and short-term inhibition of phloem transport by rapid cooling (Minchin and Thorpe 1983). This approach led to several qualitative hypotheses that could not have come from other measurement methods, as they needed a high time-resolution as well as simultaneous observation at various places along the transport pathway.

Quantitative analysis of the change in tracer pulse shape brought about by transport between regions of the plant has greatly increased the information that can be extracted from the data. Analyses developed for longer-lived tracers are usually not valid. Much more complex analysis is required and this is a central part of *in vivo* studies using short-lived isotopes.

Methods for the use of ^{11}C for studies of carbon transport in plants were last reviewed in the proceedings of a workshop (Minchin 1986). There have been substantial changes in experimental techniques since then, as well as improvements in methods for analysis of the data. Further refinements can be expected now that quantitative imaging techniques are beginning to be used. We shall first outline the physical processes associated with the use of the isotope as a tracer, describe various approaches to acquiring and analysing the data, and illustrate with examples that have led to physiological understanding that could not have occurred without the use of ^{11}C. This review does not attempt to cover all aspects of previous ^{11}C use, but to highlight current trends and future applications.

Physical properties and technical consequences

The carbon isotopes

The isotopes of carbon fall into three groups (Lederer *et al.* 1967), one containing the stable isotopes (^{12}C and ^{13}C), and two groups of unstable isotopes which decay by emission of a charged particle: a group of the heavy isotopes, which includes ^{14}C, emit a β^- (an electron), and a group of the light isotopes which includes ^{11}C, emitting a β^+ (a positron, i.e. an anti-electron, which behaves like an electron except for its opposite electric charge).

The differences in both half-life and decay type between ^{14}C and ^{11}C are sources of their significant differences in plant applications. ^{14}C has a very long half-life of 5730 years and emits a low-energy β^-, while ^{11}C has a relatively short half-life of 20.4 minutes and emits a high-energy β^+. The β^- emitted by ^{14}C can penetrate up to 0.3 mm in water, with a mean path length of 0.06 mm (Thorpe 1986). The positron emitted from ^{11}C has a much higher energy, which reduces by collisions with matter down to thermal values when the positron annihilates on encountering an electron, resulting in two high-energy photons (γ-rays, each of energy 511 keV) which move off in anti-parallel directions. The positron's zigzag path can penetrate up to 4 mm of water, or up to 4 m in air (the mean range is about a fifth of the maximum), but the annihilation γ-rays are much more penetrating. Unlike charged particles, there is no maximum distance of travel for γ-rays; their absorption is exponential (Beer's Law). The intensity of the γ-rays from positron annihilation reduces by a factor of 10 for every 250 mm in water, 16 mm in lead, and 9 mm in tungsten. Absorption in plant tissue is similar to that in water. For a point source the intensity reduces with the inverse square of the distance.

Beta detection

The amount of tracer at a specific location is inferred from the intensity of radiation emissions. Beta particles are detectable with a 'charged particle detector', the Geiger–Müller tube being the best-known example. However because the charged particles are absorbed within a short distance in plant tissue it is impossible to detect all of the decay events *in vivo*. Instead of detecting decay products, ^{14}C can be quantified directly with extremely high sensitivity by accelerator mass spectrometry (Reglinski *et al.* 2001). *In vivo* detection of beta emission from ^{14}C is possible in thin tissue such as leaves and fine roots, which has been a very productive approach in studies of phloem loading and transport (e.g. Geiger and Swanson 1965; Moorby and Jarman 1975; Farrar and Farrar 1985). This work is restricted to monitoring tracer in thin sources and sinks, but not along the pathway or in thick sinks.

The beta emitted during ^{11}C decay has a much higher energy and penetration than that from ^{14}C, and so it can be more efficiently detected *in vivo*, but again absorption by a variable thickness of plant tissue still prevents quantitative measurement, made worse by the spiralling nature of the vascular bundles within a plant stem. Also, Geiger–Müller detectors have a very limited dynamic range compared with γ-ray detectors, a severe restriction when using ^{11}C.

Gamma detection

The 511-keV annihilation γ-rays are observed using a solid scintillant (sodium iodide). The limit to the spatial resolution of detecting where a ^{11}C-decay occurs is the 0.8 mm average path length of the emitted positron in wet tissue. But if a ^{11}C atom decays close to the plant surface, the emitted positron can travel up to another 4 m in air before it annihilates. Phloem tissue is often close to stem or leaf surfaces, so it is essential to 'glove' the plant in 'positron shields' right up against the plant to ensure that each positron annihilates before moving any further than necessary.

Detector collimation

The photons can be detected singly or by coincidence. When detected singly, lead shielding — or better, tungsten — is essential to ensure that the detectors are sensitive only to radiation from a well-defined part of the plant. With

coincidence counting, a pair of scintillation detectors is used to observe both of the annihilation γ-rays, and only coincident events are recorded, locating the radiation source somewhere within the volume between the detectors. This greatly reduces the need for radiation shielding (Fares *et al.* 1978).

Three- and two-dimensional imaging of positron-emitting isotopes, dependent upon coincident counting of the two annihilation γ-rays, is possible (Keutgen *et al.* 2002) but not yet routinely available for plant studies. For many purposes such detail is not necessary. We have used single-photon counting with carefully collimated detectors, in either 'slot' or 'sink' mode (Fig. 1). Early ^{11}C work used the 'slot' mode, observing tracer within a short segment of leaf or stem through a slot in the radiation shielding. Coincidence counting is a 'slot' mode of operation. Being a measurement of tracer within a segment of tissue, interpretation is ambiguous, because changes in count rate can occur by either tracer flow into or out of the field of view (Minchin and Thorpe 1987), so the slot mode of detection does not measure the amount of tracer arriving from upstream, nor being delivered downstream, but the net of these two flows. Even so, useful information has been gained with careful experimental design, but for quantitative work it is necessary to use the 'sink' mode of detection, where this ambiguity is eliminated. In the 'sink' mode, all tracer is measured within a terminal sink region, with uniform sensitivity (Fig. 1). The only way tracer can enter the field of view is by transport across the boundary defined by the shielding, and movement within the field of view results in no change in count rate. Once unloaded from the phloem, some tracer could move out from the field of view in the xylem, or be respired. Because of the short half-life of ^{11}C, the amount lost is normally very small compared with that of phloem transport, but in every case this needs to be checked (Thorpe *et al.* 1998).

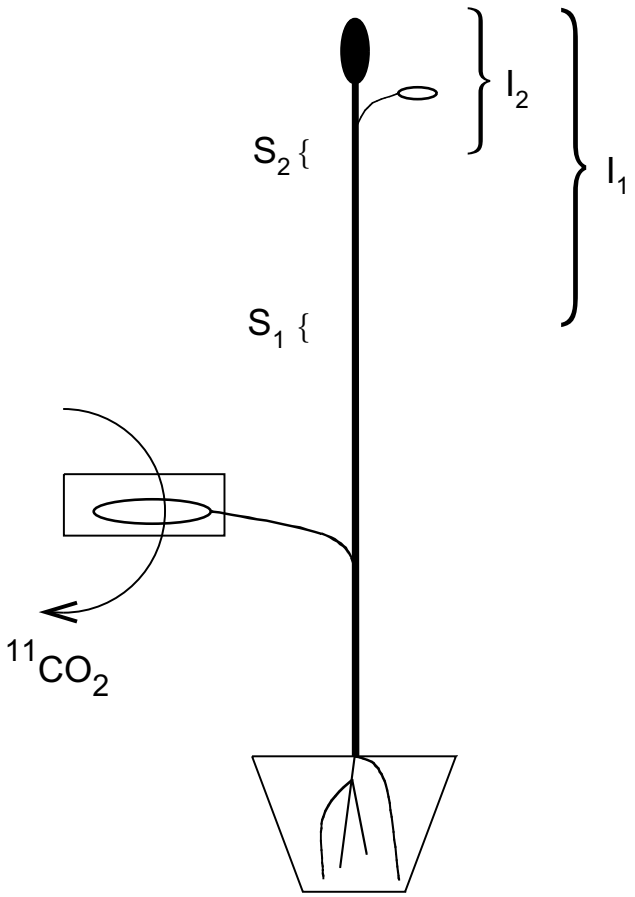

Fig. 1. A schematic diagram of an experimental plant with one leaf enclosed within a leaf chamber for labelling with $^{11}CO_2$. On the left of the plant stem are shown (by means of parenthesis) two short segments of the stem viewed by 'slot' detectors S_1 and S_2. On the right side is similarly shown the field of view of two 'sink' or integrating detectors I_1 and I_2. Each detector is located within radiation shielding so as to be equally sensitive to tracer anywhere in plant tissue within its field of view. Sink detectors are necessary for unambiguous data, having only one route by which tracer can enter into their view, and no way for tracer to leave once it has arrived. Hence, in this example, these sink regions must extend to, and include, the stem apex. A slot detector views a small segment of the transport pathway and tracer can both enter and leave its field of view.

Various tricks have been necessary to ensure that with geometrically large sinks, the detectors are equally sensitive to tracer anywhere within the sink, ranging from using a large distance between the plant and detector, reducing the sink size by spiralling a soft shoot apex on a flat surface, to using a suitably shaped radiation attenuator to reduce the intensity of γ-rays from sources closer to the detector (Minchin and Thorpe 1996*a*). Also, the sensitivity-corrected counts from several well-collimated detectors can be added, each monitoring part of the entire sink.

The method of tracer detection is a major factor in how the data can be interpreted, the degree of data analysis possible, and hence the physiological information available. Not enough emphasis has been placed on this issue, so that much published data are impossible to interpret.

Data analysis

Three distinct approaches to quantitative data analysis have been taken: (1) to fit a mechanistic model to tracer observations (e.g. Evans *et al.* 1963); (2) compartmental analysis (e.g. Fares *et al.* 1988); and (3) input–output analysis (Minchin and Troughton 1980). Because of the short-half life, ^{11}C is rarely in isotopic equilibrium, and even when it is the specific activity is different in each pool. Data analysis developed for longer-lived tracers is therefore usually not valid.

Mechanistic models

Moorby *et al.* (1963) used a mechanistic model involving bulk flow at a uniform speed and lateral leakage into surrounding tissue, and estimated various physical parameters by fitting their model to the tracer profiles. For

soybean, they obtained a speed of bulk flow of 60 cm h^{-1} and lateral leakage of 0.8% cm^{-1}, as well as evidence for range of transport speeds (i.e. dispersion). This is the only example of a detailed mechanistic model directly fitted to ^{11}C tracer profiles.

Compartmental analysis

The first form of tracer analysis independent of detailed physiological mechanism was based upon compartmental analysis theory (Moorby and Jarman 1975). This approach has been the backbone of tracer analysis for animal and human studies (Atkins 1969). Compartmental analysis has been extensively used in the interpretation of tracer movement through leaves (e.g. Rocher and Prioul 1987) and this approach has been continued with ^{11}C data (Fares *et al.* 1988). This method has usually been implemented with the assumption that the system under study does not change during the experiment. With ^{11}C, washout is usually followed for about 3 h while with ^{14}C it can be 24 h or more. However time constants estimated for ^{14}C movement through leaves do, in fact, vary during the photoperiod (Farrar and Farrar 1985; Fares *et al.* 1988). Hence the dynamics of tracer movement through a leaf is not time-invariant, making the conventional methods of compartmental analysis inappropriate.

Compartmental analysis of ^{11}C movement through a leaf has been used extensively by Goeschl's group (e.g. Fares *et al.* 1988; Dyer *et al.* 1991). They obtained *in vivo* estimates of mass flow from the average time of tracer transit from photosynthetic fixation to arrival in the stem. This is a lower bound to the total mass flow, as only recently fixed photosynthate is labelled with ^{11}C. Any remobilised photosynthate will not be labelled and will therefore not contribute to the inferred flow. These are the only *in vivo* measurements of mass flow that have ever been reported. But compartmental analysis assumes time-invariance of the system, putting the results in doubt. We will return to this point in discussing the input–output method that can deal properly with time-varying systems.

Input–output analysis

In the method of input–output analysis an input of tracer is measured entering the transport pathway of interest, and an output of tracer leaving it. This data set is used to identify the best quantitative description (dynamic model) to describe the change in shape of the input profile caused by the transport system to produce the output profile. This description is independent of the actual shape of the input tracer profile, though the shape determines the accuracy of the description (see later). This approach has been called data-based modelling (Young 1993), as the quantitative description is derived solely from the data and there are no assumptions about the mechanisms involved. This dynamic model completely embodies all the information available from the tracer profiles, and has no mechanistic assumptions. Any proposed mechanisms must be consistent with this model. Also, physiologically meaningful parameters can be calculated from this model, such as the system gain (the fraction of tracer that entered the system and eventually leaves it). For specific parts of the phloem transport pathway, the system gain can be given more descriptive names (Minchin *et al.* 2002): for the labelled leaf it is the fraction of the fixed tracer transported out of the leaf, and so has been called the leaf export fraction; for a petiole or stem it is the fraction of tracer entering a the region that eventually leaves it (so one minus the gain is the fraction of tracer not leaving the stem, the fraction irreversibly lost from the phloem, often called the petiole/stem leakage); and at a phloem sink the gain is the fraction of exported tracer that eventually enters that sink, so it has been called the partitioning fraction for that sink. From the input–output model the distribution of tracer transit times can also be calculated, quantifying dispersion in the pathway. Details of flow dispersion are highly informative about the mechanism involved. Also, a statistically meaningful average transit time can be calculated, whereas the frequently used estimate of transit times, based upon first time of tracer arrival or variants of this (Canny 1961; Minchin and Troughton 1980), have unknown statistical validity.

The input–output model does not depend upon the actual shape of the input tracer profile, but the accuracy of this identifying the model structure and estimating its parameter values does depend upon the input. Information theory shows that we can only get information about a system at frequencies that are used to stimulate it. For example a constant input, equivalent to stimulation at zero frequency, does not give very much information about a system because it does not adequately 'stimulate the system' for good identification of the input–output model, and estimation of its parameter values. An ideal input is one that contains energy at all frequencies, and it can be shown that the input best able to identify the dynamics of a system is a random binary sequence (Goodwin and Payne 1977). But in reality we have limited control of the shape of the tracer pulses in a plant. Leaf export has a substantial component with a long time constant, so that even with a very sharp input (pulse labelling) the tracer pulse within the long distance transport pathway is very broad with a rise-time of 30 min or more (Troughton *et al.* 1977). Simulation has shown that the typical pulse shape within a stem is unable to differentiate between a first order and a second order process while higher order systems can be identified for a leaf (Minchin *et al.* 1996). While this is a limitation on how much information is available from the movement of labelled photosynthate, simulations have shown that good estimates of both the gain and average transit times are, nevertheless, obtained from typical ^{11}C data for higher order systems, even though their finer details (embodied in the

high moments of the transfer function) are not observable this way.

Roeb and Britz (1991) reported spontaneous oscillations in phloem transport to the ear and roots of wheat, the cause of which remains unexplained. The tracer profiles contain information on the source of the oscillations, and further work with detection appropriate for input–output analysis is essential to characterise the feedback processes involved and their time scales.

Time-variable parameters

Parameters can be estimated either by analysis of an entire block of tracer data, or by recursively updating parameter estimates with each successive input–output measurement (Young 1974; Young and Minchin 1991). This latter approach can readily give a 'bad memory' to earlier data, giving the most recent data a greater influence on the current parameter estimates. Changing the 'amount of memory' of the recursive estimates alters the degree of smoothing. In this way we can follow any changes in parameter values, due either to diurnal changes in the system, to experimentally induced changes, or due to an inappropriate choice of model. This analysis shows whether system behaviour is changing, and if so, shows the changes. The method has demonstrated that tracer movement through a leaf is quite variable during the diurnal cycle (Farrar and Minchin 1991; Thorpe and Minchin 1991). The long distance pathway is much less variable, apart from diurnal changes early in the photoperiod.

To get some feel for the importance of system invariance in data-based analysis we simulated tracer movement through a slowly varying system and used this data to test the outcome of input–output analysis. Not allowing for time invariance, we found that an inappropriately complex model was identified, although it gave a correct estimate of average gain and transit time. But allowing for time invariance, the analysis faithfully re-created the parameters of the system that simulated the data. We concluded that, if system invariance is not assured, the observed input–output data may be misinterpreted. It is therefore, essential to test for time invariance. The method proposed by Goeschl's group to estimate phloem mass flow was based on knowledge of the transit time of the ^{11}C tracer through the leaf, estimated using compartmental analysis. The results are suspect because of the invalid assumption of time invariance, but could be rectified by using input–output analysis to account for parameter time-variation.

Case studies

Introduction

Several examples are given to show how the use of ^{11}C tracer has led to physiological understanding that would have been most unlikely with other techniques, highlighting *in vivo* detection of responses to experimental manipulations. The carbon transport system adapts quickly to experimental manipulations so is very important to be very clear about the purpose of the experiment. Changes in transport immediately after experimental manipulations give properties of the system — the mechanisms at play — at the time of treatment. The system itself immediately begins to change and after a time a new quasi-equilibrium is reached, and then a different system is operating, which can now be studied further.

Early work in phloem research, usually with ^{14}C, focussed on tracer movement along the pathway linking major sources and sinks (MacRobbie 1971). The shape of the profiles along the stem was used in arguments about the mechanism of transport and the nature of the driving force. For example Canny (1973) proposed a theory of accelerated diffusion because the spatial profile was often the error-function, which can result from a diffusion mechanism. Tracer profiles had to be obtained by destructive measurement, with limited spatial or temporal resolution, and inevitable problems of statistical variation within the experimental population. Temporal profiles are not usually practicable with ^{14}C, which led to the use of ^{137}Cs, which has been called 'the poor man's ^{11}C' (Spanner and Prebble 1962; Lang 1974).

Pathway temperature and mechanical shock

The benefit of *in vivo* measurement is well illustrated by the effects of pathway cooling on carbon transport. Two separate processes are of interest, one concerning a local effect on transport within the cooled region, a second being the consequences elsewhere in the transport network. Cooling causes local inhibition of transport, and yet transport can continue both upstream and downstream of the inhibition. Geiger's laboratory made significant contributions to this literature on temperature effects (Geiger and Sovonick 1975). The technique was to continuously label a mature leaf with $^{14}CO_2$ (usually *Beta vulgaris* L. or *Phaseolus vulgaris* L.) and observe the accumulation of tracer within a thin, immature sink leaf. But the effects on tracer flow within the cooled region itself could not be observed. The flow response observed at the sink leaf changed with time for two separate reasons, owing firstly to stoppage and recovery of flow within the cooled region, and secondly a time- (and space-) dependence of the lateral buffering flows along the pathway between the cooled region and the point of observation.

The immediate effect of localised cooling on phloem transport is stoppage and therefore cannot be attributed to the viscosity of the phloem sap, although this does account for the longer-term response once initial transients are over (Lang 1974; see a more detailed discussion in Thorpe and Minchin 1996). It was only by observing ^{11}C tracer flow within the cooled region, and with time-resolution of a minute or less, that it became apparent that the rate of

cooling can be as important as the temperature to which the tissue is cooled (Fig. 2). A temperature drop as small as 2°C can cause stoppage, albeit for a short time. With rapid cooling there was always complete stoppage of flow, with the recovery time depending on the final temperature. Recovery was immediate if the tissue was re-warmed to its initial temperature. But after recovery at the low temperature, re-warming the pathway had little effect on flow. These observations are all explained if chilling caused a large increase in hydraulic resistance of the pathway, with recovery at a rate dependent on temperature. This behaviour is exactly paralleled in observations of free cytosolic calcium in root cells of *Arabidopsis thaliana* L. in response to cold shock (Plieth *et al.* 1999). The peak calcium response was directly related to the cooling rate, and at lower temperatures there was increased sensitivity to cooling and a slower recovery. The proposal is that calcium influx is enhanced by high cooling rates, and efflux by the temperature itself (Minorsky and Spanswick 1989; Plieth *et al.* 1999). For legumes, Knoblauch *et al.* (2001) found that immediate blockage of sieve plates was caused by manipulations of sieve elements due to rapid dispersion of P-protein crystalloids, probably caused by calcium influx. It seems likely that cytosolic calcium in sieve elements is also promoted by rapid cooling, leading to temporary dispersal of crystalloids and a transient stoppage of phloem transport such as we saw in *Phaseolus vulgaris* L. (Fig. 2). An extensive survey of 86 plant species by Lang and Minchin (1986) found that phloem transport is not stopped by chilling in all species, the variation possibly reflecting the species' complement of P-proteins and the propensity of the crystalloids to disperse in the way that Knoblauch *et al.* (2001) found in legumes.

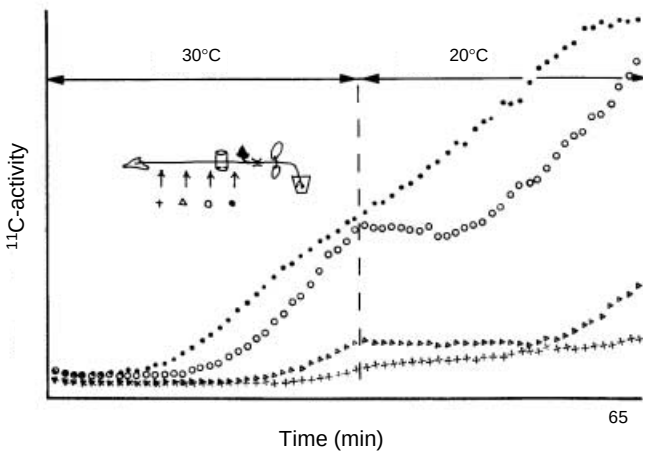

Fig. 2. An example of qualitative analysis, showing half-life-corrected temporal tracer profiles observed on either side of a 50-mm length of morning glory stem that was suddenly cooled during the experiment. Data points are 1 min apart. The schematic diagram of the experimental plant shows the location of four 'slot' detectors, and the cooled segment T. On cooling, the detector upstream from the cooled segment (i.e. nearer the labelled leaf) shows a slight increase. The detector immediately downstream of the cooled segment shows a stoppage for several minutes, while the detector further downstream shows a longer stoppage. The detector nearest the plant apex shows a reduction in the rate of tracer accumulation but not a stoppage. These responses were explained in terms of concomitant phloem unloading and reloading along the entire length of the stem. Sudden cooling induced a temporary phloem blockage that recovered at the lower temperature. (Reprinted from Minchin and Thorpe 1983 with permission of SEB and FESPP).

Similarly transient effects on phloem transport are caused by mechanical disturbance; vibration of a stem can cause transport to stop for several minutes, and with a memory effect, subsequent disturbance having less effect (Pickard and Minchin 1992*a*). These biomechanical responses, in which plants acclimatise to the stresses of gravity, wind and herbivores, and also the effects of electrical shock on phloem transport (Pickard and Minchin 1992*b*), are further instances where calcium plays a role (Knight *et al.* 1992; Plieth 2001). There are some cross-reactivities between electrical, mechanical and thermal effects (Pickard and Minchin 1992*a*); for example there was a smaller to nil response of phloem transport to mechanical shock after electroshock, which would be expected if there were a common cause, such as involvement of calcium.

A practical consequence of this mechanical sensitivity of carbon transport is that the handling of a plant can affect phloem transport for several hours, and we strongly advocate that plants are set up the day before experiments to avoid these confounding effects (Jaeger *et al.* 1988).

Carbon leakage and retrieval along the phloem pathway

There have been many reports that temporary storage of photosynthate in stems provides a buffer that can uncouple production and utilisation of photosynthate, and phloem transport is believed to often include leakage and retrieval (Thorpe and Minchin 1996; van Bel and Knoblauch 2000). The result is that short-term changes in supply do not inhibit sink development, and short-term changes in demand are not seen by the sources. Such uncoupling was demonstrated, for example, when the rate of net CO_2 uptake by leaves of *Capsicum annuum* L. did not reduce until several hours after rapidly growing fruits were removed (Hall and Milthorpe 1978), and also by the large contribution of remobilised stem reserves for the growth of wheat ears, amounting to 30% of the yield (Biscoe *et al.* 1975). The cold-block phenomenon discussed above, regardless of the mechanism, has been a very useful tool for investigating the physiology of these lateral flows of carbohydrate. Upstream of a pathway cold block the balance of lateral flows changes to give more unloading, and downstream, more net re-loading. The application of *p*-chloromercuribenzene sulfonic acid (PCMBS, a non-permeant inhibitor of sucrose uptake from the apoplast, supplied to a peeled stem), reduced the amount of buffering downstream of a cold block, showing that continued phloem transport was due to sucrose retrieval, and

that the pathway for retrieval was apoplastic in *Phaseolus vulgaris* L. (Minchin *et al.* 1984) and in apple, *Malus pumulis* L. (unpublished data). There are also situations where there is no apoplastic path (Hayes *et al.* 1987; Grimm *et al.* 1997). An apoplastic component to the pathway in lupin was suggested by the observation that small amounts of ^{11}C-photosynthate were carried in the xylem stream from a region of stem as soon as tracer entered that region via the phloem (Minchin and McNaughton 1987). The magnitudes of both of these lateral fluxes of tracer have been measured (Minchin and Thorpe 1987). The retrieval of photoassimilate from the apoplast occurs in *Arabidopsis*, shown by the increased loss of tracer from the pathway in sucrose-transporter knockouts of that species (unpublished data).

Sink priorities: *what determines partitioning between alternative sinks supplied by a common source?*

It has been known since Roman times that shoot growth increases to balance the reduced shoot activity if a plant is shaded. Partitioning of available carbohydrate within a plant is a dynamic process that maintains balanced plant growth. For example if roots are pruned there is an immediate increase in root growth at the expense of shoot growth until the root : shoot balance is restored (Brouwer 1962, 1983).

We investigated this root : shoot balance in barley seedlings, using input–output analysis of ^{11}C profiles to monitor the partitioning of photosynthate between the root and shoot. There was a rapid and positive response of carbon import to root temperature (Minchin *et al.* 1994*a*). With split-root plants, if one root-half was warmed, its fraction of the available photosynthate increased, at the expense of the control half kept at the initial temperature. Warming the root increased its ability to attract and utilise photosynthate, presumably through increased metabolic activity at the higher temperature. However when one half of a split root was cooled, giving an immediate reduction of partitioning to the cooled half, there was little to no increase to the control half. Thus the control half was not able to utilise the surplus photosynthate made available by cooling the other half. We summarised these observations with the hypothesis that normally a sink operates near its saturation level, then if more photosynthate is made available the sink cannot utilise more until it has had time to synthesise more metabolic machinery (enzymes), but if the metabolic capacity suddenly rises (e.g. because temperature is increased) then more photosynthate is attracted to this sink, at the expense of the others (Minchin *et al.* 1994*a*). A simple mechanistic model of photosynthate flow driven by a hydrostatic pressure gradient between the source and sinks, maintained by active phloem loading in the source, and saturable unloading kinetics at the sinks, mimics the above observations (Minchin *et al.* 1993).

If sinks that differ in their nature (e.g. root and shoot) are represented by unloading kinetics with different V_m, then if photosynthate is reduced (e.g. by shade), the model predicts a change in partitioning, with a larger fraction of the (now smaller) available photosynthate going to the sink with the smaller V_m. Therefore our observations (Minchin *et al.* 1994*b*), like those of the Romans, that shading the shoot reduced the fraction of available photosynthate transported into the root, correspond to shoot V_m being smaller than root V_m, and the shoot has the higher priority. This priority model predicts that if we lower the effective V_m of the root below that of the sink, then the root will have the higher priority. Possible ways to lower V_m and reverse priorities are to cool the root, or to prune off part of it. This is exactly what was seen, and predictions of the immediate effect of assimilate availability on carbon partitioning have all been confirmed in hydroponically-grown barley seedlings (Minchin and Thorpe 1996*b*). Longer-term responses have varied; they reflect the adaptive response of the system. For example the reversal of root : shoot priorities was seen for only a short time after cooling the roots, and after several hours the priorities had returned to their original rankings. This compensation was ascribed to an increase in the expression of carbon utilisation enzymes, owing to signalling from the increase in sucrose that followed root cooling. In support of this explanation, rewarming the root back to its initial temperature gave much higher root partitioning than its initial value. And after several more hours at the initial temperature, partitioning to the root returned to the initial level (unpublished data).

Another example shows a developmental rather than a compensatory adaptation. In this case an apple fruit near a labelled leaf was found to be receiving no tracer, but when it was warmed, import soon started (Fig. 3). When the fruit was taken back to its initial temperature, the induced flow into the treated fruit declined but did not return to the initial zero level, even after several days. Similarly, Jahnke *et al.* (1989) were able to follow the sequence of changes both in ovary elongation, and in carbon partitioning between ovaries, root and shoot apex, after gibberellic acid was applied. These examples illustrate the power of *in vivo* measurement of phloem flow in the non-destructive measurement of gene expression and regulation involved in the control of carbon partitioning. An important role for such non-destructive measurement is to identify appropriate times for destructive sampling necessary in functional genomic and proteomic analysis of the tissue for a deeper understanding of partitioning control.

Seed coat unloading in pea fruit

Seed coats of pea and bean have been used extensively to investigate the control of phloem unloading. This is a wonderful model system for such studies as there is a clear separation between the parent plant tissue and that of the daughter tissues within the seed. The seed coat is parent tissue, connected symplastically through its phloem to the

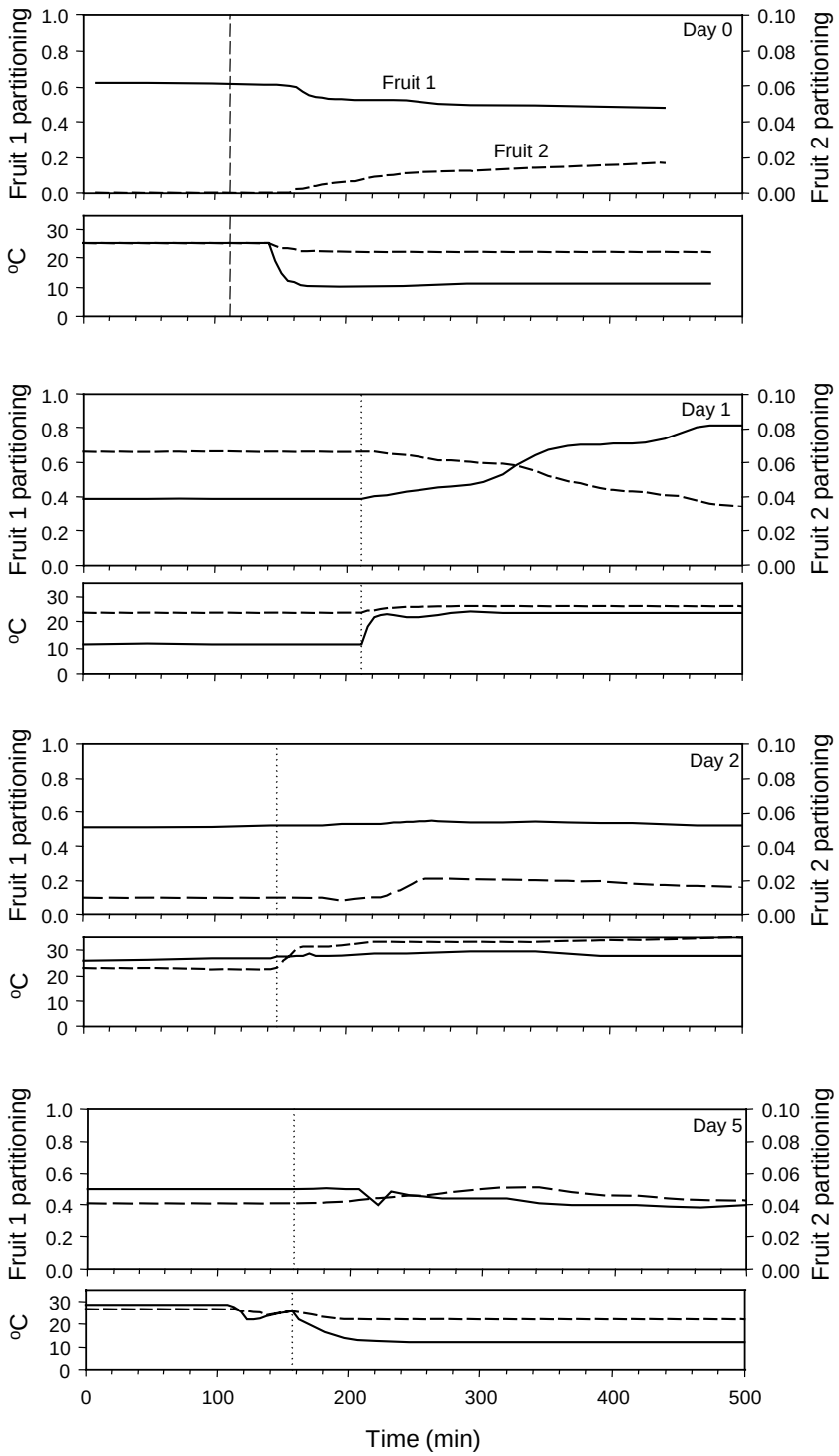

Fig. 3. An experiment showing the immediate effects of a treatment, and then longer term adaptive responses; partitioning of recently fixed photosynthate exported from a bourse leaf to two apple fruit on the same spur, over a period of 5 d when the temperature of each fruit was altered as shown in the temperature graphs. During each day of measurement 5 pulses of $^{11}CO_2$ were used, spaced about 120 min apart. The specific interest here is that no labelled photosynthate appeared in fruit 2 until after fruit 1 was cooled. Partitioning to fruit 2 had increased further by the next day, even though it cooled too, to a few °C below ambient temperature. On rewarming fruit 1 back to ambient temperature on day 1, partitioning to fruit 2 immediately fell but never back to the level of zero, where it had been at the beginning of day 0. Even on day 2 a small amount of labelled photosynthate was still being partitioned to fruit 2. On warming fruit 2 on day 2 there was a further increase in partitioning to this fruit, which had increased further by day 5. Cooling fruit 1 again on day 5 resulted in a reduced partitioning to this fruit and a temporary increase to fruit 2. [Redrawn from Minchin *et al.* (1997) with permission of SEB and FESPP].

plant. Unloading occurs into the seed coat apoplast from where it is subsequently taken up by the daughter cotyledons. By careful surgery it is possible to cut a small hole through the seed coat, remove the cotyledon and replace it with a suitable bathing solution to collect the unloaded photosynthate. Patrick (1984) used *Vicia faba* L. seeds that were labelled with ^{14}C-photosynthate via the plant and then excised from the plant for washout studies. The time course of tracer accumulation into the bathing solution was taken as a measure of photosynthate transfer into the apoplastic volume surrounding the cotyledon in the intact system. Wolswinkel and Ammerlaan (1983) worked with a similar system, except that the seed was not removed from the parent plant.

These two independent groups made conflicting observations of the change in tracer efflux from the seed coat when the osmolarity of the bathing solution was suddenly changed — Patrick found an increase in seed coat unloading when the osmolarity of the bathing solution was decreased, while Wolswinkel found decreased unloading. Using ^{11}C and attached seed of *Pisum sativum* L. we could simultaneously observe tracer flow into both seed coat and bathing solution. The extra information now available showed that the differences between attached and detached seed coats arose because the washout in these two systems was dominated by different processes. Washout from attached seed coats was dominated by changes in seed coat import brought about by the osmotic change of the bathing solution, and this masked the changes in seed coat unloading. Short-term washout from excised seed coats reflected the process of seed coat unloading only, because import from the parent plant was not possible. With ^{11}C both processes could be observed simultaneously, showing that the osmotic effect on import to the seed was temporary (Thorpe *et al.* 1993; a further example where the long and short term responses differ). But unloading from the attached seed coat was affected by osmotica, just as Patrick concluded from detached seed coats (Grusak and Minchin 1988).

Conclusion

This review has attempted to demonstrate the advantages of *in vivo* measurement of carbon transport, and to discuss some of the traps and tricks that affect the success of an investigation. The ability to measure transport processes simultaneously throughout the plant ensures that a holistic understanding can develop, helping to avoid the trap of assembling one's reductionist knowledge of a large number of component processes without knowing their individual importance in the behaviour of the whole. A major part of this approach involves data analysis, which is essential for extracting all the information available from the tracer profiles. The amount of information available from a tracer profile is not obvious, especially as the tracer has a short half-life and is best administered intermittently. The theory of systems identification, which includes the input–output approach advocated in this review, provides a sound footing for such analysis (e.g. Goodwin and Payne 1977; Minchin *et al.* 1996). Within the field of engineering control theory it is well known that complex systems, be they mechanical, chemical or those occurring naturally, are well described by sets of equations that omit most of the detail of their structure. These complex systems are very well described by equations that incorporate only their dominant behaviour (Young 1978). Effort is well overdue to find the important biological details that determine the dominant flow dynamics of phloem transport and its interactions with the xylem, both transport systems with overwhelming genetic and environmental influence.

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