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A SYSTEM FOR STUDYING CARBON ALLOCATION IN PLANTS USING ^{11}C-LABELED CARBON DIOXIDE

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INTRODUCTION

The effects of environmental factors such as water stress, elevated CO_2, or temperature on carbon assimilation and allocation in plants have been studied extensively (Gifford and Evans, 1981; Loomis, Rabbinge, and Ng, 1979; Neales and Incoll, 1968). However, the interactions of these processes are not well understood and cannot be predicted with any degree of confidence. Continuous and simultaneous measurements of photosynthesis, transport, and sink activity have never been made during the short- and long-term responses of live, intact plants to step changes in environmental factors. Thus, direct environmental effects and adaptive responses of plants are generally not distinguished. This results in part from limitation in experimental techniques and protocol used in past studies and the lack of experimental validation of hypotheses and models (eg, Goeschl et al, 1976; Magnuson et al, 1979; Smith et al, 1970) dealing with these problems. This paper describes in detail the components of an integrated technique for studying carbon assimilation, transportation, and allocation in intact live plants under any set of environmental conditions, using continuously produced $^{11}CO_2$.

EXPERIMENTAL DESIGN

The central theme of the experimental component of the integrated approach is the utilization of CO_2 labeled with the radioactive isotope ^{11}C. There are three main reasons for the use of ^{11}C as tracer (Fares et al, 1978; Magnuson et al. 1982). As a tracer, ^{11}C has several advantages. 1) It decays by positron (β^+) emission followed by a subsequent emmision of two oppositely-directed gamma rays. These γ-rays have

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sufficient energy (0.511MeV) to be detected through several cm of tissue in vivo and in time coincidence which makes it possible to localize the source and to reduce undesired background activity. 2) The short half-life of ^{11}C (20.3 min) makes it possible to perform several experiments on the same plant under the same set of environmental conditions. 3) Its half-life is comparable with turnover times of the photosynthetic pool and the velocity of transport, making possible dynamic measurements which cannot be done with long-lived tracers.

SYSTEMS COMPONENTS

The integrated system is made up of six components as shown in figure 1.

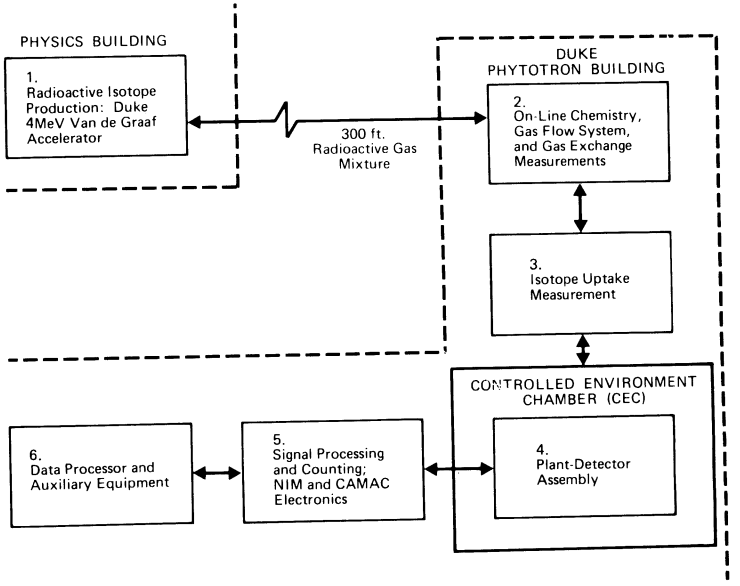

Fig 1. Outline of the integrated system for studying the carbon allocation in plants using radioactive tracers under controlled environment conditions

1. RADIOACTIVE ISOTOPE PRODUCTION. ^{11}C is produced by bombarding $^{12}CO_2$ gas flowing through a target chamber mounted on the beam line of the 4MeV Van de Graaff of Duke University. The nuclear reaction $^{12}C(^{3}He, ^{4}He)^{11}C$ is used (Cirilov et al. 1966). The activity produced flows through a 100m capillary tube to the nearby Duke Phytotron where the plants are grown under controlled environmental conditions.

To eliminate contaminant isotopes, the transport time of activity from the target chamber to the phytotron is adjusted to be longer than six half-lives of the longest lived of the two contaminant isotopes, ^{15}O. In an earlier study with 35MeV protons, of a total of 100% ^{11}C activity, 98% was in the form of ^{11}CO and 2% $^{11}CO_2$, which made it possible to separate the activity from the target with only 2% loss (Fares et al, 1978). The separation of ^{11}CO from the target $^{12}CO_2$ and $^{11}CO_2$ is necessary to control the specific activity. In the present study, with 4MeV ^{3}He, the composition of the effluent gas is 10% ^{11}CO and 90% $^{11}CO_2$. However, the cross-section of the ^{3}He reaction is 3 to 4 times that of the proton reaction, which compensated for some of the loss of activity to the target material and produced enough ^{11}CO to carry out the experiments without difficulty. An attempt to increase the recovery of ^{11}CO was done by mixing the target, $^{12}CO_2$, with some H_2 gas. The attempt worked by reducing some $^{11}CO_2$ to ^{11}CO, but at the same time, it reduced $^{12}CO_2$ to ^{12}CO, so the overall recovery of ^{11}C was increased but the specific activity did not change (results of this study will be reported elsewhere). What happens in the target chamber can be summarized as follows:

Without hydrogen

$$^{12}CO_2 \longrightarrow [\text{Target chamber}] \longrightarrow {}^{12}CO_2 + {}^{11}CO_2\ 90\% + {}^{11}CO\ 10\% \qquad (1)$$

With hydrogen

$$^{12}CO_2 + H_2 \longrightarrow [\text{Target chamber}] \longrightarrow {}^{12}CO_2 + {}^{11}CO_2\ 10\% + {}^{11}CO\ 90\% + {}^{12}CO + H_2O + H_2 \qquad (2)$$

The ratio of $^{11}CO_2$ to ^{11}CO will depend on the amount of H_2 added. The production rate as monitored at the entry station in the phytotron (see fig 2) is, after correcting for the decay in transport, 0.12μci sec^{-1} μA^{-1}.

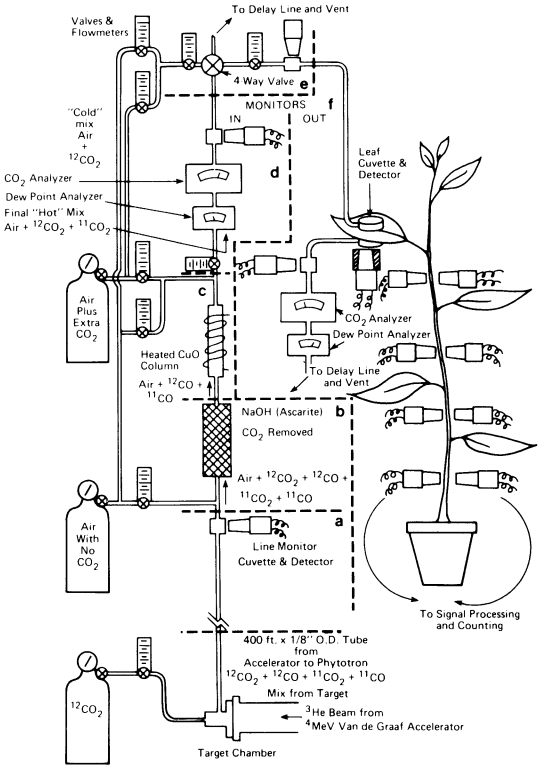

Fig 2. The experimental layout. a. Line Monitor. b. Removal of target material. c. Oxidation of ^{11}CO. d. In Line Monitor, CO_2 analyzer and dew point analyzer. e. Final In Monitor. f. Out Monitor, leaf cuvette, and plant and detector assembly in the controlled environment chamber (CEC).

2. ON-LINE CHEMISTRY, GAS FLOW SYSTEM, AND GAS EXCHANGE MEASUREMENTS. The objective of the on-line chemistry station, positioned in the phytotron, is to separate ^{11}CO from the target material and any other contaminants. It then oxidizes it to $^{11}CO_2$ so that when mixed with CO_2-free air and the right amount of $^{12}CO_2$ is added, air with the desired concentration of CO_2, and known specific activity will be presented to the plant leaf. The effluent gases from the target chamber are transported 100m (in 14 min) to the phytotron via a 2.5mm id teflon tube. The following sequence of steps takes place in the chemistry station as shown in figure 2, a,b,c, and d.

a) On arrival at the phytotron, the gases flow at a known rate, through a spiral plastic cuvette of known volume positioned in front of a γ-ray detector at a given, fixed detection geometry. The detection efficiency of every detector in the given geometry is calibrated for every experiment with a standard γ-ray source. This station that monitors the activity of the incoming gases is called the "Line Monitor." Profile #1 (fig 4B) gives the production rate of the radioactive isotope.

b) As the gases exit from the cuvette of the line monitor, they flow through an ascarite column. The ascarite column, being sodium hydroxide impregnated into asbestos, removes all the $^{11}CO_2$ and $^{12}CO_2$ gases. The small volume of ^{11}CO left (or ^{11}CO, ^{12}CO, H_2) is purged from the column by CO_2-free air added to the gases as they enter the ascarite column.

c) The next step is to oxidize ^{11}CO to $^{11}CO_2$ by flowing the exiting mixture of gases over a CuO bed heated to 600°C in a quartz tube in a horizontal muffle as shown in figure 2c. Oxygen in the air recovers the used CuO.

d) To the effluent gases from the CuO bed, ie, air + $^{11}CO_2$ or air + $^{11}CO_2$ + $^{12}CO_2$, either $^{12}CO_2$-free air or air + a controlled amount of $^{12}CO_2$ is added to bring the concentration of total CO_2 in the so-called "Hot Mix" to the desired value. The gases then flow through a dew point analyzer to determine the total concentration of CO_2 in ppm as shown in figure 2d. As the gases exit from the CO_2 analyzer, they flow through a spiral cuvette positioned in front of a γ-ray detector to determine the specific activity of the gas mixture that will be presented to the leaf.

e) Finally, adjustment of the gas flow to be comparable to field flow rates is made via a 4-way valve which allows for venting unwanted gases to the absorbers. If adjustment is needed, the activity and the final flow rate are re-measured as shown in figure 2c.

3. ISOTOPE UPTAKE. The net isotope uptake by that portion of the leaf exposed to the final Hot Mix in the leaf cuvette is determined. It is simply a station where the specific activity of the input and output gas mixtures into and out of the leaf cuvette is measured. The flow rate and specific activity of the input gas mixture are measured in station (e) above and called the "In-Monitor." Similarly, the specific activity of the output gas mixture is measured in the "Out-Monitor." The effluent gases from the Out-Monitor flow through a CO_2 analyzer, then through a dew point analyzer before they are vented to the absorbers as shown in figure 2f. Thus, net photosynthetic rates can be calculated as well. The final flow rate of the gases is also monitored to adjust for the Out-Monitor measurement in case there is a perceptable reduction

of flow. Signals from the CO_2 and dew point analyzers are either recorded by multipen strip chart recorder or digitized and input into the computer as described below.

4. PLANT DETECTOR ASSEMBLY. The plant and the detectors are positioned in the controlled environment chamber. The radioactive gas mixture is presented to a portion of the leaf through a cuvette which consists of a Plexiglas® water jacket on the top and bottom of the leaf. The cuvette is created by a 2mm-thick closed-cell form neoprene gasket on each water jacket. The cuvette is clamped to the leaf in such a way that the gas mixture coming out of the In-Monitor flows across the upper surface of the leaf, around the edge, and back across the lower surface. Immediately below the leaf cuvette, a γ-ray detector is positioned to monitor the tracer build-up in the leaf. This detector is shielded with a lead collar.

In order to monitor the movement of the labeled photosynthates in the phloem, 4 to 6 pairs of detectors are positioned on either side of the plant stem above and below the labeled leaf. The detector pairs are operated in time coincidence at 180° with respect to each other (see fig 2f). The distances between the detector pairs and their geometry with respect to the stem and to one another are carefully assigned.

Each detector is made of 5 x 2.8cm NE102® plastic scintillator crystal light-coupled to a photomultiplier-voltage divider system capable of producing fast and slow output signals. The front end of the detector assembly facing the source of activity, is collimated with a lead collar to give a desired window. A highly stabilized high-voltage power supply is used to bias the detectors.

The controlled environment room CER in which the plant detector station is located is a typical chamber of type B of the Duke Phytotron (see eg, Kramer, Hellmers, and Downs, 1970; Downs, Hellmers, and Kramer, 1972). In such a chamber, temperature, humidity, light intensity, light cycle, and CO_2 concentration and flow speed of air are controlled. The light cycle and CO_2 concentration are adjusted to the conditions of growth of the plant under study.

5. SIGNAL PROCESSING AND COUNTING. We have standard NIM electronics for processing the signals from the detectors for counting and CAMAC electronics for data acquisition and interfacing with the data processor. The fast signal from each detector is processed by the NIM electronics and then presented to the scalars in the CAMAC crate to be counted under the command of the CAMAC unit controller. The CAMAC unit is under control of the processor (fig 3) through the general purpose interface bus (GPIB). Standard LeCroy fast electronics modules are used in the NIM system and Kinetic System CAMAC

units are used in the CAMAC system. The CAMAC crate provides the physical mounting, power, cooling, and dataway connections for the modules. A normal crate has 25 stations of which station 25 is assigned to the crate controller and the remaining slots are normal stations for modules such as scalars, digitizers, etc. The system works by commands and responses from the controller to the modules and back and from the controller to the computer and back as shown in figure 3. The controller is the traffic director of the systems signals. All systems parameters are carefully defined within the IEEE CAMAC specifications 583.

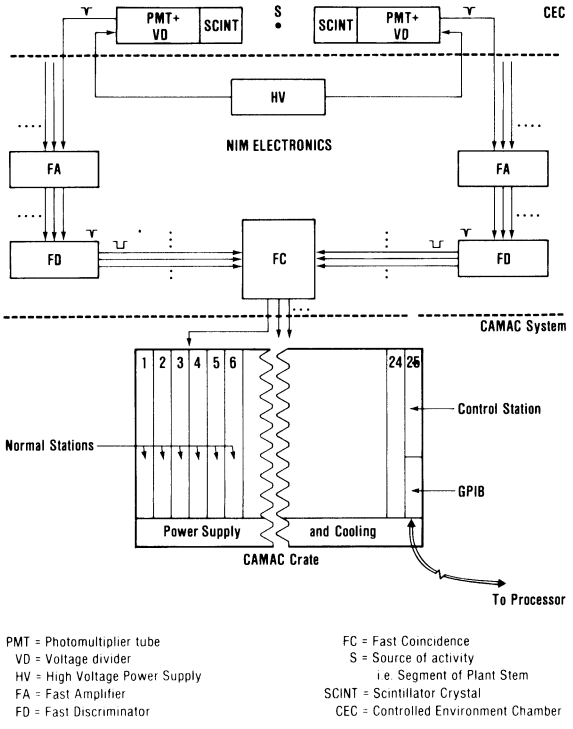

Fig 3. Signal processing and counting using NIM and CAMAC systems.
NIM = Nuclear Instrumentation Electronic Modular
CAMAC = Computer Automated Measurement and Control.

6. THE PROCESSOR. For the above system to be an integrated on-line facility, a processor that can handle the following

functions is needed: 1) to communicate with the CAMAC system, ie, send commands to and receive responses from it; 2) to implement routines such as calibration and background corrections; 3) for on-line data acquisition, reduction, and storage; 4) for on-line data display; 5) for on-line and off-line testing of acquired data; 6) for utility programs.

In this system, an HP 9845 B (option 250) minicomuter is used. This computer has 186K byte memory expandable to 500K and uses the extended basic language. It has the additional advantage of graphic capabilities and a large CRT for visual display. It can drive the following accessories: two magnetic tape drives, floppy disk drives, thermal printer, multipen multicolor recorders, and extra CRT's for display or monitoring. Figure 4 is a copy of the graphic display on the CRT of this computer of an on-line real-time experiment described in the protocol below.

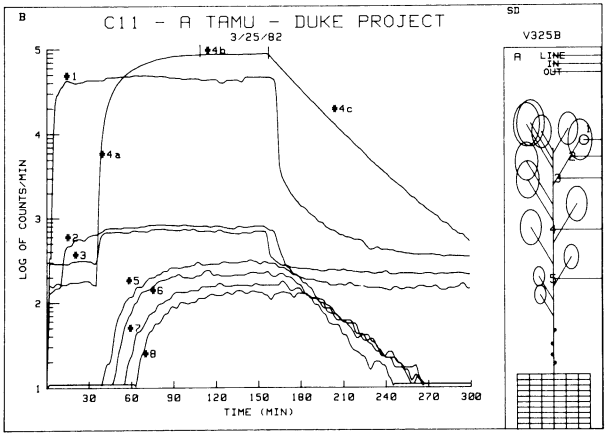

Fig 4. Live-time display of tracer profiles of Velvet Leaf (Abutilon theophrastic Medic). A. Plant geometry with detectors #1 monitoring the leaf and detector pairs #2, #3, #4, and #5 monitoring the phloem activity. B. Profile #1 shows the line activity as recorded by the Line Monitor, #2 and #3, the input and output activities as recorded by the Input and Output Monitors, respectively. Profile #4-a, #4-b, and #4-c show the time-dependent, the steady-state, and the washout behavior of the leaf, respectively. Profiles 5, 6, 7, and 8 show the tracer profiles in the phloem as monitored by detector pairs #2, #3, #4, and #5 (fig 4A).

EXPERIMENTAL PROTOCOL

Described here are some simple experiments, which we call "step input," to illustrate our daily experimental protocol. Figure 4 shows the on-line real-time computer output of the data obtained from this experiment for rates of net photosynthesis, carbon storage (eg, starch) and export (eg, sucrose) from a given area of a leaf and the velocity of translocation of Velvet Leaf (Abutilon theophrasti Medic).

The plant of choice, grown under the environmental conditions chosen for a particular study is moved into the controlled environment chamber, CEC, 24-48 hours before the start of the experiment. A portion of one of the leaves of the plant is enclosed within a cooled cuvette and is supplied with a continuous flow of air at a given, steady-state concentration of $^{12}CO_2$ (eg, 400ppm at a flow which will maintain 350ppm in the cuvette). Net photosynthesis, transpiration, and other biologically related functions are monitored continuously as explained above. The detectors are arranged on the plant at the desired positions and the geometry of the leaves and the petiole of the leaf on which the experiment is conducted is measured and entered into the computer (see fig 4A). On the morning of the experiment, as soon as the lights in the CEC come on, the background activity at each detector is determined and the detectors are calibrated with a standard ^{22}Na source. This information is automatically entered and stored in the computer. An updated listing of data by the thermal printer displays the information at a predetermined interval. Radioactive gases flow from the target chamber in the accelerator laboratory to the phytotron and the activity is monitored by the line monitor as explained above (profile #1, fig 4B) until a steady-state level is reached. The gases then flow through the chemical processing station and are monitored for steady-state level of specific activity (profile #2, fig 4B). At the appropriate time, the 4-way valve is turned to divert the flow of unlabeled air and substitute an identical flow of the Hot Mix at the same total CO_2 concentration as the original. The build-up of activity in the leaf and its movement in the transport stream are continuously measured and updated in the computer. A real-time continuous display of that behavior is shown in figure 4B, profile 4 for the leaf, and profiles 5,6,7,8 in the phloem. The specific activity of the gases exiting the leaf cuvette is shown in profile #3. All data input into the computer is stored on cassette tapes as well as listed in the updated printout.

Tracer studies of this type must be conducted when the leaf has reached, and is expected to maintain steady-state rates of photosynthesis and carbon allocation, because of the implicit assumption of steady state in tracer data analysis.

These conditions tend to be reached mid-morning in most plants grown in growth chambers. However, step input experiments early in the morning or late afternoon when the rates of carbon metabolism are changing with time are also possible, but require non-steady-state data analysis.

Two hours after stopping the tracer input in the leaf only ca 1.5% of the activity is left in the plant and the same experiment (or other experiments) can be repeated on the same plant under the same environmental conditions. However, it is not necessary to wait that long, since corrections by the computer for residual activity can be made at the same time data is acquired.

A host of ecologically-related environmental and biological factors can be tested with this technique which should lead to improved qualitative and quantitative understanding of plant responses to environmental stimuli.

CONCLUSION

An integrated approach to studying the effects of environmental factors on plants is described. Though the tracer described is ^{11}C, ^{13}N can be used for nitrogen fixation studies with minor changes in the system. ^{11}C and ^{13}N seem to provide very convenient tracers that are natural constituents of plants and their environments. A host of ecologically, agriculturally, and genetically important questions can be answered using this technique. The consequences of elevated CO_2 concentration in the atmosphere, the effects of water stress on growth rates of plants and the information required to genetically engineer a plant to suit our needs, are but a few of the major scientific questions that can be studied by this method.

ACKNOWLEDGMENTS

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