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MEASUREMENT OF CARBON FIXATION AND ALLOCATION USING ^{11}C-LABELED CARBON DIOXIDE

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INTRODUCTION

The real-time measurement of net photosynthesis and dark respiration of plants has been possible since the development of the infra-red analyzer and its application in the 1940's. This technique has allowed intensive investigations of the mechanisms and dynamics of carbon dioxide assimilation by green plants (Sestak, Catsky, and Jarvis, 1971). As a result, physiologic and ecologic aspects of net carbon dioxide exchange are the subject of much research (eg, Osmond, Bjorkman and Anderson, 1980).

Comparable research on the movement of carbon within the plant has been hindered, however, by the lack of a technique which would allow real-time observation of carbon translocation. Much tracer work with the radioisotope ^{14}C has been accomplished (Sestak, Catsky, and Jarvis, 1971), but it has not been generally possible to closely link carbon dioxide uptake and carbon movement using ^{14}C.

This β-emitting long-lived isotope has two primary drawbacks. 1) The low energy emission makes it necessary to destructively harvest tissues and to quantify radioactivity by scintillation counting of prepared samples. Thus, carbon translocation has to be determined by time-series and statistical procedures. Physiologic variability requires several to many replicates for each measurement.

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2) The long half-life of ^{14}C prevents repeated and long-term measurements on a given individual. Once the radioisotope is applied to the plant, it is difficult or impossible to know how the isotope is moving internally. Biochemical conversions of carbohydrates following the initial labeling confuse the issue.

Since the 1930's (Fares et al, 1978) we have known that ^{11}C could be used as a carbon tracer. This short half-lived isotope has several advantages. 1) It decays by positron (+) emission followed by positron-electron annihilation with the emission of two oppositely-directed gamma rays. These rays have sufficient energy (0.511Mev) to be detected through several cm of living tissue. Thus, real-time movement of ^{11}C can be detected from outside of the plant without destructive harvesting. In fact, sensors can be located close to, but not touching the plant and carbon movement observed without disturbing the plant at all (Fares et al, 1978; Minchin and Troughton, 1980).

This paper describes the use of continuously produced and applied ^{11}C in measurements of carbon dioxide assimilation and C movement in plant research. This technique differs from the pulsing type ^{11}C research underway in other laboratories by being continuous and on-line with computer analysis making steady-state measurements of carbon fixation and movement possible. The studies to be described here will make clear the advantages of using continuously produced and applied short half-lived isotopes.

METHODS AND MATERIALS

The details of this technique have been previously described (Fares et al, 1978; Magnuson et al, 1982; Fares et al, 1983). It is not necessary to redescribe the hardware and the technical details here. It is, however, necessary to briefly describe the experimental protocol and the specific experimental methods employed.

Plants were grown in controlled environments of the Duke University Phytotron. Day/night temperature conditions were 28/20 ^{o}C with a 16-hour photoperiod. Light intensity was 600 mol $m^{-2}s^{-1}$ (400-700nm). Relative humidity was 75% during the day and near 95% at night. Plants were grown in a standard 1:1:1 mixture by volume of vermiculite: gravel: turface (Jaeger, Hellmers and Teare, 1981). The plants were 8 weeks old when the measurements were made.

The plants used were cotton and velvet leaf. The latter plant is a weed in cotton fields throughout the southern United States. Both species are in the same plant family and they have similar environmental requirements for growth. It is known, however, that cool nights during the active growing season will damage the crop more than the weed. Just one seasonally cool night will decrease growth in cotton significantly more than the weed. This study compares the phloem transport of the two plant species to chilling of the conduction tissue.

One plant of each species was conditioned to the 28/20 day/night temperature. They were then moved into position for labeling with $^{11}CO_2$. The radioactive gas was passed through a leaf cuvette in a gas stream of $^{11}CO_2 + {}^{12}CO_2 = 400\ \mu l \cdot l^{-1}$. The plant photosynthesized the $^{11}CO_2$ into ^{11}C carbohydrates (alcohol soluble sugars and starch).

RESULTS

The labeled leaf on both species reaches ^{11}C steady state within 30 min after the initiation of the measurement. The activity in the phloem tissues of the stem 5cm below the petiole insertion of the labeled leaf reached steady state within 90 min.

Figure 1 is a representative on-line display of tracer profiles on a velvet leaf plant. Profile #7 shows the line activity of the $^{11}CO_2$ coming to the system. Line #10 is the activity in the labeled leaf. This detector-pair monitors the carbohydrates at the site of carbon fixation by the leaf. Lines #8 and #9 show activity of the $^{11}CO_2$ gas stream before and after the photosynthesis cuvette, respectively. The difference between these two lines #2, 3, and 4 show the ^{11}C activity at successively lower points on the stem. Line #1 shows the activity in the stem slightly above the petiole attachment.

After the plants reached radioactive steady state (Ca 90 min) the responses to 2-min temperature chills were recorded. Table 1 is a representative time series of such a treatment on one plant of each species. Relative values are shown so the responses can be directly compared. It is clear from table 1 that velvet leaf responded faster and to a greater extent than cotton.

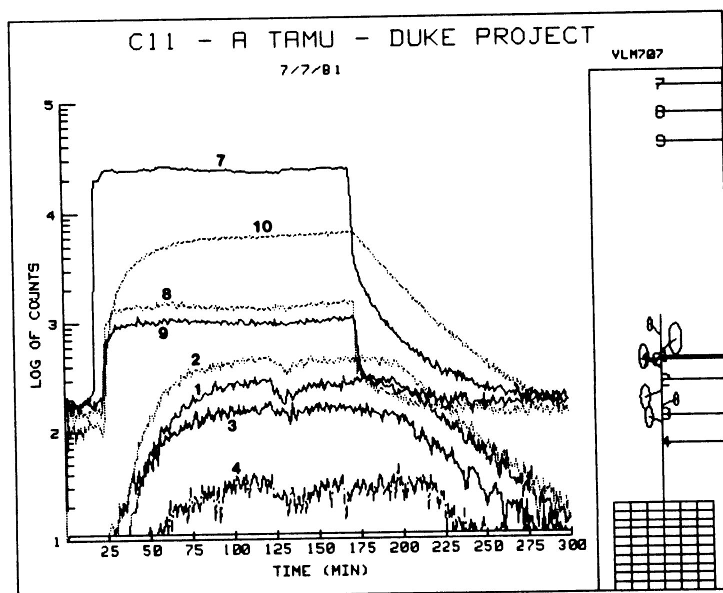

Fig. 1. Line-time display of tracer profiles of velvet leaf. On the right is a diagram of plant geometry showing detector location, leaf number, and relative leaf area. The numbered tracer lines are described in the text.

TABLE 1. Representative results in a test of the effect of temperature chilling on phloem transport rates in cotton and in velvet leaf. A 5° chill was applied for 2 min beginning at 90 min.

Time (min)	Relative activity (% cpm)* Cotton	Velvet Leaf
0	0	0
5	0	0
15	3	1
30	12	19
60	50	81
90	100	100
100	83	62
105	85	81
110	92	100
115	98	100
120	100	100

*Each column is the percent of the steady-state value.

DISCUSSION

Table 1 presents one set of measurements to show the application of the technique described in Fares et al (1983). The effect of a slight (5 °C) temperature chill on phloem transport kinetics is shown for cotton and one of its most serious weed species, velvet leaf.

Farmers have long observed that aseasonal cool nights in the height of the growing season will impede cotton growth more than the velvet leaf weed. This test shows a basic difference in the way these phylogenetically closely related species respond to temperature chilling. It is our hypothesis that carbon allocation is seriously impeded by cool temperatures in the cotton but recovers quickly in the velvet leaf. Thus, velvet leaf recovers quickly from the occasional cool night whereas cotton requires much longer to recover.

The steady-state ^{11}C technique clearly showed a possible mechanism for such a difference. Additional research is now underway to examine this hypothesis in more detail.

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