

A labeling chamber for ^{13}C enrichment of plant tissue for decomposition studies

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ABSTRACT

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Isotope labeling with a $^{13}\text{CO}_2$ -enriched atmosphere was conducted on four raised beds of grain sorghum. A temporary rain shelter with clear polyethylene roofing material was constructed to allow controlled watering of the beds. Labeling chambers were constructed with aluminum frames, polyethylene sides and propofilm C tops. The chamber length (4 m) was one-quarter of the total bed length and enclosed a total volume of ~ 3000 l. A fan recirculated air in the chamber through polyvinyl chloride pipes. An outlet allowed sampling of chamber atmosphere for CO_2 concentration, while another opening downstream provided an inlet for $^{13}\text{CO}_2$ injection. An elevated extension was added when the grain sorghum grew to exceed the 50-cm height of the chamber, doubling the enclosed volume. Enrichment of the chamber atmosphere with $^{13}\text{CO}_2$ was monitored with infrared gas analyzers. Gas flowmeters were used to measure isotope additions.

INTRODUCTION

Techniques for using stable isotopes in ecological research are well established (Rundel et al., 1989). Tagging of plant tissue with ^{13}C has been conducted on a small scale in laboratory environmental chambers, but decomposition studies often require large quantities of plant tissue. The design of a field experiment called for kilogram quantities of crop residue labeled with a stable isotope of carbon. This paper describes a technique devised to label grain sorghum with ^{13}C applied as CO_2 gas.

METHODS

Grain sorghum (*Sorghum halepense* Moench) was hand planted in four raised beds (16 m \times 1.5 m \times 20 cm), four rows per bed, at a density of ~ 40

plants m^{-1} length of row. Beds were constructed with 5×30 -cm wooden planks, lined with sheets of polyethylene and filled with two layers of soil; a bottom layer (15 cm thick) of coarse washed sand and a top layer (5 cm thick) of screened (1-cm mesh) sandy clay loam from the Ap horizon of a local agricultural field (Typic Kanhapludults). A thin layer of perlite was sprinkled between the layers. A temporary wooden frame rain-out shelter with polyethylene sheet roof protected equipment and permitted controlled watering of the beds. Research was conducted at the USDA-ARS Southern Piedmont Conservation Research Center at Watkinsville, Georgia.

Labeling chambers were constructed to permit the injection, circulation and monitoring of CO_2 (Fig. 1). Across the top of the angled aluminum (25-mm wide/3 mm-thick) frame, transparent propofilm C film (ICI Films, Wilmington, DE) was attached with reinforced fiberglass tape. Polyethylene sheeting covered the sides. The chamber was 4 m long (one-quarter of the total bed length) \times 1.5 m wide \times 0.5 m high, enclosing a total volume of ~ 3000 l. An open-topped chamber extension of the same dimensions, constructed of wood (5×5 cm) with polyethylene sheet sides, elevated the chamber when the crop height exceeded 0.5 m. The chamber or extension rested on the bare soil surface and sand was used to seal any gaps at the interface.

The aluminum frame and plastic sheeting made the chamber lightweight, but four people were needed to handle the bulky load in the sometimes tight confines of the shelter. Wooden supports that spanned the width of the beds were used as chamber rests above the crop canopy between labeling runs.

Air was drawn from the chamber and recirculated by a 1/70-hp (0.70-amp) shaded pole blower (Dayton Electric Mfg. Co., Chicago, IL). Pipes of polyvinyl chloride (PVC), 76.2 mm inside diameter, 5 mm thick, carried air from the fan back through the chamber. Holes were drilled at 25-cm intervals from where the pipe entered the chamber. The first two holes on each side of the

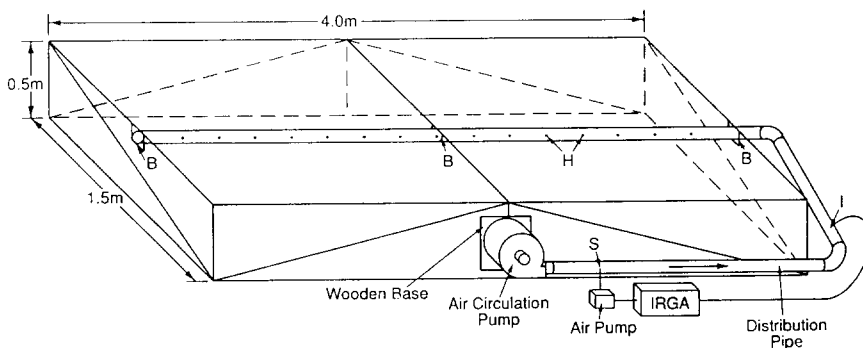


Fig. 1. Diagram of labeling chamber. IRGA, Infra-red gas analyzer; S, sampling port for IRGA; I, $^{13}\text{CO}_2$ injection and IRGA return point; H, air distribution holes; B, braces for attaching PVC distribution pipe to metal framework.

pipe were 9 mm in diameter. The next 10 holes were 12 mm in diameter, but six in the middle were drilled only on the side facing away from the blower inlet. The last three holes were 14 mm in diameter. This stepped increase was done to allow more even distribution of air in the chamber.

The CO_2 concentration in the chamber was measured by an infrared gas analyzer (IRGA). Strip chart recorders provided a visual record of CO_2 concentrations over time. The rate of decline in CO_2 concentration indicated the rate of photosynthesis in the chamber. After the compensation point was reached (~ 15 min), chambers were left in place for an additional 15–20 min to ensure 95% uptake of the labeled gas. Fluctuations were observed after equilibrium due to passing clouds altering the intensity of sunlight reaching the canopy. No attempt was made to cool the air in the chamber. Temperatures in the chamber surpassed 50°C on a few occasions, but generally remained at $\sim 40^\circ\text{C}$ near the soil surface. No permanent signs of heat stress were observed in the grain sorghum plants. A cart, used to move the electronic equipment alongside the chamber at each bed position, held the IRGA, strip chart recorder, air monitoring pump and an electrical switch box.

Gas flowmeters were used to gauge injections of $^{13}\text{CO}_2$ gas. A mylar balloon was used as a working vessel to hold enough gas for a few injections. The balloon was filled from a lecture bottle containing 90 atom% $^{13}\text{CO}_2$ (E.G.G. Mound Associates, Miamisburg, OH). Gas flowed from the balloon, through a small air pump, into a flowmeter, and finally into the circulating air stream in the chamber at $\sim 1 \text{ l min}^{-1}$. It took ~ 3 min between starting an injection and detecting an increase in CO_2 concentrations in the chamber circulation loop.

Each of the 16 bed positions was labeled once per week during early crop growth and then twice per week from five-leaf stage until harvest, a total period of 46 days. Each position was labeled 14 times, receiving a total of 24–30 l $^{13}\text{CO}_2$ each ($\sim 12 \text{ g }^{13}\text{C}$ per position or $\sim 200 \text{ g }^{13}\text{C}$ for the total biomass).

RESULTS AND DISCUSSION

Approximately 52 kg of oven-dry (60°C) labeled stems and leaves were produced (stem:leaf ratio 1.8). Developing seed heads were removed just after formation of the flag leaf to prevent translocation of label into grain.

Final samples analyzed for carbon isotope revealed average enrichments of $293.6 \delta^{13}\text{C}$ for leaves and $172.05 \delta^{13}\text{C}$ for stems (Table 1). These figures represent a total ^{13}C uptake of 25 g for leaves and 27 g for stems, and a total above-ground uptake efficiency of 26% (i.e. not including roots). Svejcar et al. (1990) observed similar enrichment levels in short-term labeling experiments of individual plants in gas-tight chambers. The authors also noted that 37% of the ^{13}C taken up was allocated to plant roots. Applying this ratio to

TABLE 1

 $\delta^{13}\text{C}$ values for labeled sorghum leaf and stem tissue in each of the 16 bed positions¹

Bed	Position	Leaf $\delta^{13}\text{C}$	Stem $\delta^{13}\text{C}$
A	1	331.61 \pm 0.20	195.79 \pm 0.16
A	2	304.27 \pm 0.36	176.96 \pm 0.12
A	3	287.43 \pm 0.08	186.00 \pm 0.14
A	4	292.11 \pm 0.16	222.58 \pm 0.14
B	1	311.47 \pm 0.14	183.96 \pm 0.16
B	2	307.23 \pm 0.06	188.63 \pm 0.10
B	3	321.98 \pm 0.20	186.08 \pm 0.12
B	4	417.35 \pm 0.14	204.39 \pm 0.10
C	1	286.72 \pm 0.22	135.65 \pm 0.14
C	2	257.04 \pm 0.06	144.37 \pm 0.12
C	3	244.35 \pm 0.06	140.49 \pm 0.10
C	4	400.22 \pm 0.08	183.80 \pm 0.20
D	1	222.81 \pm 0.12	144.36 \pm 0.10
D	2	237.93 \pm 0.04	156.55 \pm 0.08
D	3	242.24 \pm 0.10	143.35 \pm 0.06
D	4	232.76 \pm 0.20	160.33 \pm 0.10

¹The δ value is in parts per thousand (‰) relative to the international standard PDB (analytical standard error in parentheses).

TABLE 2

 $\delta^{13}\text{C}$ mean values for labeled sorghum leaf and stem tissue by bed, using position within bed as error¹

Bed	Leaf $\delta^{13}\text{C}$ ²	Stem $\delta^{13}\text{C}$ ³
A	303.86 ab	195.33 a
B	339.51 a	190.77 a
C	297.08 ab	151.08 b
D	233.94 b	151.03 b

¹The δ value is in parts per thousand (‰) relative to the international standard PDB.

²Means followed by the same letter do not differ at the 0.05% level of probability.

³Means followed by the same letter do not differ at the 0.01% level of probability.

our data would suggest an additional 30 g ^{13}C in root tissue. The remaining balance was assumed lost to respiration or fixed in soil organic matter.

Analysis of variance for plant tissue and bed, using position within bed as an error term, showed a bed effect (Table 2) at the 0.01 level of probability and a 0.0001 level of probability for stem vs. leaf. The beds on the east side of the shelter (A and B) had incorporated more ^{13}C than those on the west (C and D). The angle of the plastic roof tended to reflect sunlight from the beds on the west side in the morning, while in the afternoon increasing cloudiness reduced the amount of light reaching all beds. Mean position values revealed a tendency for greater fixation in the southmost position (4), although this trend was not significant at the 0.05 level.

Design improvements suggested while working with the chambers include using PVC ducts of reduced thickness and substituting flexible tubing for some of the PVC. Flexible tubing would allow mounting of the recirculating fan on the equipment cart and attachment of an air conditioner to better control the chamber environment.

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