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A FIELD TECHNIQUE FOR MEASURING PHOTOSYNTHESIS USING 14-CARBON DIOXIDE

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Abstract

A portable apparatus which supplies air containing 14-CO_2 to the lower surface of a leaf for 30 seconds without interfering with the radiation incident on the upper surface is described. A disc is punched from the exposed area of the leaf straight into a vial which contains a tissue solvent and the assimilated 14-carbon is assayed by liquid scintillation counting of the solution.

Introduction

The photosynthetic rates of individual plants or of crops growing naturally in the field can be measured by enclosing either whole plants (Musgrave & Moss, 1961; Baker & Musgrave, 1964) or single leaves (Hesketh & Musgrave, 1962; Hesketh & Moss, 1963) in transparent chambers and monitoring CO_2 concentrations. Alternatively, the aerodynamic method (Inoue et al. 1958; Lemon, 1960; Monteith, 1962) allows photosynthetic rates to be calculated from measurements of wind velocity and CO_2 gradients. Both these techniques use infra-red gas analysers for CO_2 determinations.

Because the instrumentation and equipment for these techniques are cumbersome and need a field power supply and usually a mobile laboratory, various workers have experimented with portable, simple field methods involving the use of carbon-14-dioxide (Austin & Langden, 1967; Irvine, 1967; Strebeyko, 1967; Shimshi, 1969). This technique requires that the supply of 14-CO_2 to the leaf be simply regulated and preferably not generated on the spot. Neither the spectral composition nor the intensity of incident radiation should be altered. Moreover the leaf should be enclosed for a short time so that its temperature does not rise too much. Finally the assay for 14-C should be rapid, convenient and should use highly efficient counting techniques such as liquid scintillation counting. In contrast to the previously reported techniques the apparatus and assay which we describe satisfies all of these requirements. The apparatus is derived in principle from that of Strebeyko (1967). The assay, an in-vial digestion, has been briefly described elsewhere (Incoll, 1969). The technique has the disadvantage of being destructive. However the sampled area is kept small so that the overall effect on the plant is small.

Description of the Apparatus

The apparatus has three parts; a CO_2 supply system (A), a small exposure chamber (B) which clamps onto the leaf and a special punch (C) which samples the exposed area for assaying.

A. The 14-CO_2 Supply System (Fig. 1) The radioactive gas is stored in a small compressed-gas cylinder (a) (ca. 0.5 litre volume at S.T.P.) which is sealed by a hand-operated packless valve (b). The gas pressure is reduced by a pressure regulator (c) which has low dead volume, a neoprene diaphragm, a tank gauge reading 0-3000 p.s.i.g. and a delivery gauge reading 0-100 p.s.i.g. (The regulator is supplied with an outlet needle valve (d).) Gas flow is controlled by an extra-low flow valve (e) and is measured with a dual-float flow meter (f) with a range of 0-150 ml./min. at 70° F and 14.7 p.s.i.a. On-off control of the gas stream is achieved with a lever-operated valve (g). The components from (d) to (g) are joined with $\frac{1}{4}$" O.D. copper tubing and standard 1/8" gas fittings and from (g) to the exposure chamber with plastic tubing (P.V.C. $\frac{1}{4}$" I.D. 3/8" O.D.). When the system is not in use the chamber is hung on hook (h).

The components (a) to (h) are mounted on a 3/8" thick plywood board (i) ($7\frac{1}{2}$" × 14") which is in turn mounted on a steel stake (j) 6' long. The stake is driven into the ground by jumping on a cross-bar (12" long) of slotted angle-iron mounted 18" from the sharpened tip. The gas cylinder is secured with flat-iron straps 3/4" wide (k). Overheating of the cylinder in strong sunlight can be prevented by covering it with water-soaked rubber sponges, which are held in place by wire clips, or by covering it with aluminum foil.

B. The Exposure Chamber (Fig. 2). This chamber has two jaws between which the leaf is held during exposure to 14-CO_2. The upper jaw consists of a brass plate (a) drilled with a hole (b) (tapered to 1" diameter) which allows light to hit the upper leaf surface. The ring has a gasket of closed-cell sponge rubber ($\frac{1}{4}$" wide, 1/8" thick) (c) stuck to its lower surface. The lower jaw is made from two pieces of acrylic rod which are cemented together after machining. The upper piece (d) (diameter $1\frac{1}{2}$") has a sponge rubber gasket (e) which matches that on the upper jaw, stuck to its top surface. A brass plate (f) which is cut to fit around the upper piece (diameter 2") is screwed to the lower piece (g). Both brass plates are clamped to the jaws of 9" surgical sponge forceps (h) by heat-formed blocks (i) of black acrylic plastic.

The leaf is clamped between the two rubber gaskets effectively sealing the conical sub-chamber (j). Radioactive gas delivered by the 14-CO_2 supply system flows up the central hole (k) into the conical sub-chamber, across the lower leaf surface and down the ring of small holes (l) at the periphery of the sub-chamber. These holes vent into a circular groove (m) (of rectangular cross-section) which is tapped at one point by the exit hole (n).

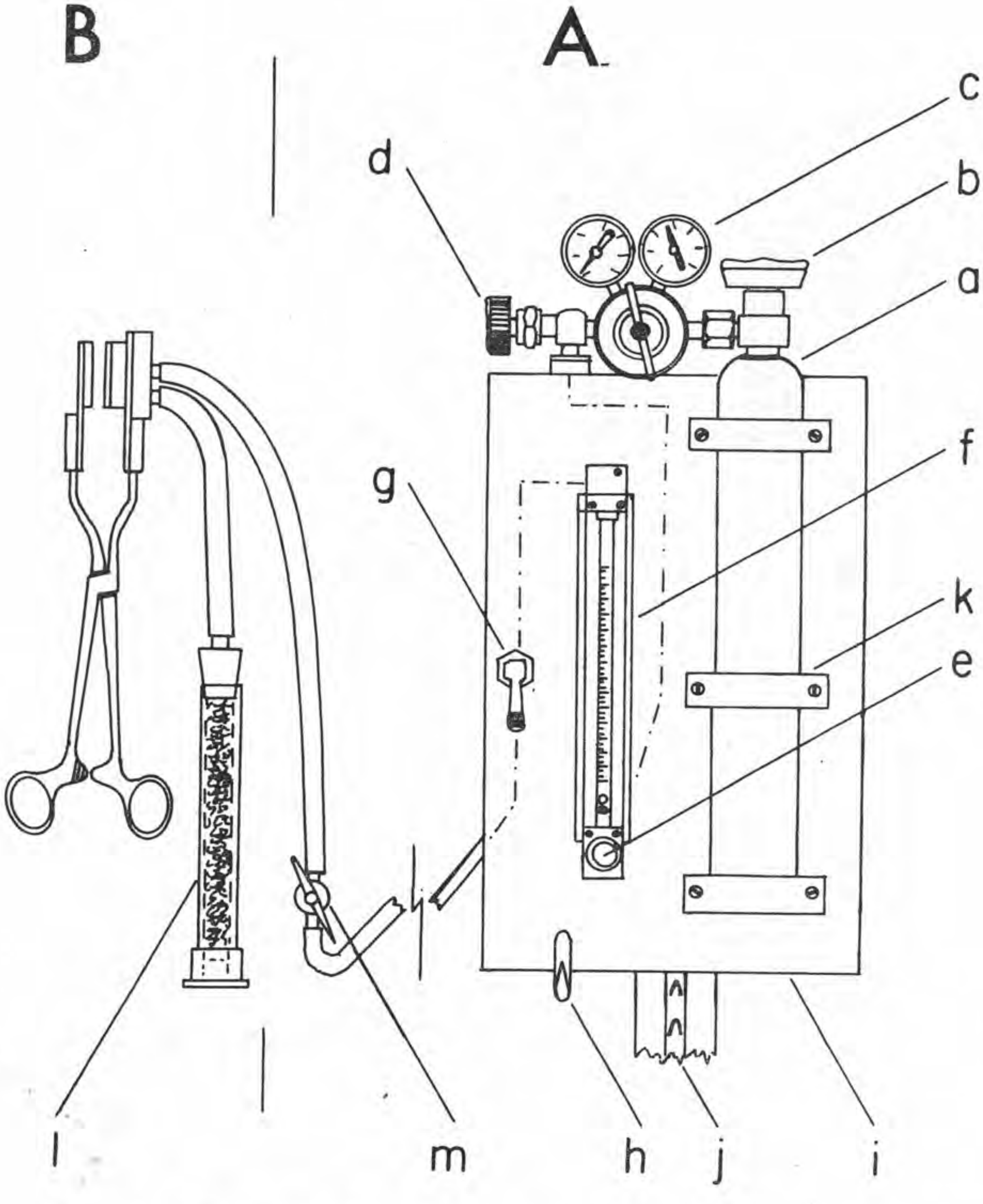

Figure 1. The CO_2 supply system A, and exposure chamber B; a, gas cylinder; b, packless valve; c, pressure regulator; d, needle valve; e, extra-low flow valve; f, flow meter; g, lever-operated valve; h, hook; i, plywood board; j, steel stake; k, iron straps; l, soda-lime column, and m, stopcock; _._._. tubing connections.

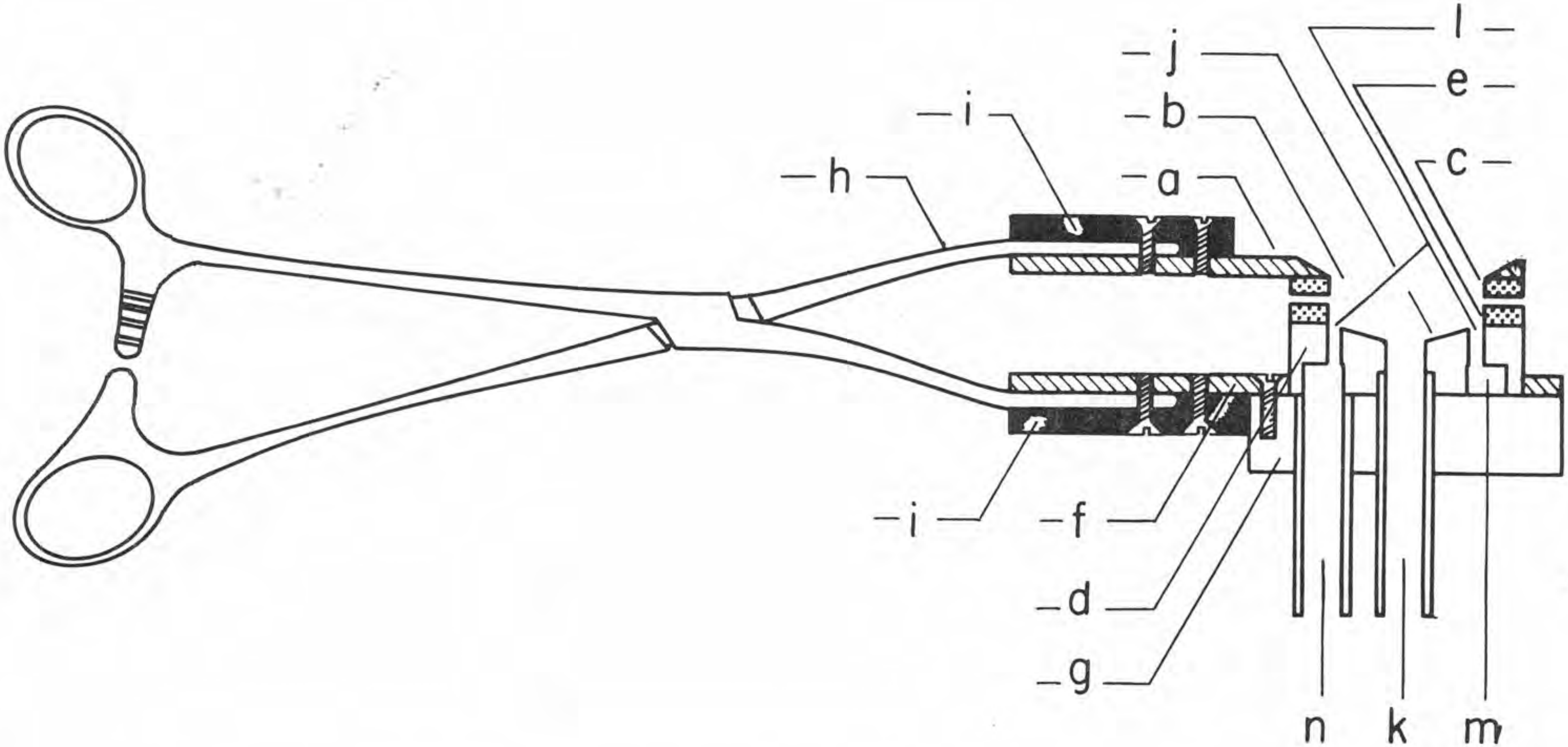

Figure 2. Cross-section through the exposure chamber. a and f, brass plates; b, see text; c and e, sponge rubber gaskets; d, upper acrylic block; g, lower acrylic block, h, sponge forceps; i, acrylic blocks; j, conical sub-chamber; k, gas inlet; l and m, channels for gas; and n, gas outlet. For details see text.

Any unassimilated 14-CO_2 is absorbed in the tube of soda-lime hanging below the chamber (l, Fig. 1). This absorbent column has negligible resistance to gas flow, so that gas is not forced through the leaf under pressure. The valve (m, Fig. 1) is turned off when the chamber is not in use thus preventing loss of radioactive gas through the open chamber.

C. The Punch. A standard parallel-action punch (Style 140: Sargent & Co.) was modified in order to cut large discs (5/8" diameter) of leaf tissue. The throat of the punch was lengthened so that the punch would reach further into the leaf. An acrylic block was drilled and tapped so that it would hold a standard glass scintillation vial. This block was screwed to the bottom of the punch so that a punched disc would drop straight into the vial in readiness for assaying.

The Assay

The disc of labelled leaf tissue is punched straight into the scintillation vial which contains 1 ml. of a 0.6 N solution of a surface-active organic base in toluene (N.C.S., Amersham/Searle) (Hansen & Bush, 1967) and digested over night at room temperature. The solution is then bleached with 1 ml. of a saturated solution of benzoyl peroxide in toluene. The vial is filled to 20 ml. with scintillation fluid (PPO: toluene :: 6 g: 1 l) and after allowing time for standing (a couple of hours) until chemoluminescence ceases (Winkelman & Slater, 1967) is counted at ambient temperature. For convenience all solutions are dispensed with repeating pipettes. Teflon-lined, aluminum caps are preferred.

Operation

Two operators are preferred. Valves (b) (Fig. 1) and (d) are opened fully and regulator (c) is adjusted to a delivery pressure of 8 p.s.i. Valve (e) is set to a flow rate of 120 ml./min. This setting, in the range where uptake of 14-CO_2 is independent of flow rate, is determined in a preliminary experiment.

The upper rubber gasket of the exposure chamber is pressed onto a stamp pad impregnated with a ZnO-glycerol mixture (Shimshi, 1969).

The exposure chamber is clamped firmly onto the leaf and valve (m) opened. Immediately the second operator opens valve (g) and starts the stopwatch. After 30 seconds valve (g) is closed, the exposure chamber is removed from the leaf and valve (m) is closed.

A disc is now punched from the center of the exposed area, which is marked by a ZnO image of the upper gasket. The disc drops into the vial containing digesting solution and infiltration and digestion of the disc starts immediately. The basic solvent absorbs any 14-CO_2 produced by respiration before infiltration is complete. The vial is unscrewed from the punch, recapped and replaced in a wire tray which sits on a bed of crushed ice in a foam-plastic box.

All the relevant data about the sample are recorded. At the end of the day the vials are returned to the laboratory for completion of the assay.

Calculations

If the results are to be expressed in the conventional units of $mg.dm^{-2} hr^{-1}$, then allowance must be made for the area of the punched disc, the time of exposure, the specific activity of the CO_2 supplied, and the efficiency of the counting process. No correction was made for isotopic discrimination against 14-CO_2 since this amounts to no more than 2-3%. (Heath, 1969; Yemm & Bidwell, 1969).

Discussion

Field trials of the technique were carried out in the summer of 1969 on crops of tobacco and sorghum. Profiles of photosynthetic activity were measured successfully in each crop from sunrise to sunset. Differences in uptake of 14-CO_2 of 200 to 300 fold between the top and bottom of the tobacco crop were distinguished (Incoll & Turner, unpublished results).

Although two operators were preferred, one person could operate the apparatus if valve (g) and the stop watch were coupled.

With the amount of benzoyl peroxide specified, the final solutions vary from clear to yellow depending on the initial pigmentation. With our samples the amount of quenching appeared to be independent of the intensity of colour and a single correction was made for all samples. While additional benzoyl peroxide will bleach the highly pigmented solutions it then quenches chemically. If quenching is found to vary from sample to sample then a correction will have to be made, preferably by the channels ratio method, since internal standardization will so increase the labor and time involved as to cancel out the advantages of the method.

With sorghum not all the 14-carbon is leached from the tissue and assayed. In order to allow for this, a linear regression can be established on a representative sample for the amount retained and the amount assayed and all results corrected accordingly. Digestion and bleaching at a higher temperature (Hansen & Bush, 1967) may solve both this and the bleaching problem. For tobacco the recovery of 14-carbon is equivalent to assaying by homogenizing the tissue. It would therefore be advisable to test both the recovery and the extent of quenching for each species being investigated.

Acknowledgments

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APPENDIX A

PARTS LIST, SUPPLIES AND SPECIFICATIONS

A. The $^{14}CO_2$ Supply System

Part	Supplier
1. Gas cylinder, size LB (gas specification below)	The Matheson Co., P.O. Box 85, East Rutherford, NJ 07073
2. Pressure regulator (Model 3320)	" "
3. Flowmeter (Model 622 PBX) incorporating	
(a) 150 mm. flowmeter tube (Model 600)	" "
(b) extra-low flow valve (Model 4141-1505)	" "
4. Lever-operated valve (Model 120R-B)	" "
5. Nalgene Stopcock (bore 4 mm) (Cat. No. 6460)	Fisher Scientific Co., 52 Fadem Rd., Springfield, NJ 07081

B. The Exposure Chamber

Amico Sponge forceps. 9½" stainless (Cat. No. 40 Am 202)	Professional Equipment, 995 Dixwell Avenue, Hamden, CT 06514
Rubber gasket 1/8" thick (Cat. No. 2218)	Green Rubber Co., 6th & Broadway, Cambridge, Mass. 02142

C. The Punch

Custom-made to the following specifications: Parallel-action punch style No. 140 - 6 5/8" die size: 5/8"	Sargent & Company, Hand Tool Division, New Haven, CT 06509

maximum reach: 2"

feed: $\frac{1}{4}$"

acrylic attachment for vial

with a hole approximately

13/16" diameter tapped 8

threads per inch

Gas Mixture Specifications	Supplier
CO_2 : 325 p.p.m.	Matheson Gas Products P. O. Box 85 East Rutherford, NJ 07073
O_2 : 20.8%	
Carbon-14 dioxide : 3 microcuries per litre	
N_2 : balance	
Pressure : 1625 p.s.i.a.	
Volume : 48 litres at S.T.P.	

Assay	Supplier
1. NCS[TM] Solubilizer (Cat. No. 190620)	Amersham/Searle, 2000 Nuclear Dr. Des Plaines, IL 60018
2. Aluminum Screw-on Cap (Cat. No. 003367)	"
3. Repipets - automatic pipettor (Cat. No. 3005) (Cat. No. 3020 sp. to fit gallon jug)	Labindustries, 1820 Second Street, Berkeley, CA 94710

APPENDIX B

NOTES FOR OPERATORS

1. It is advisable though not essential to place the repipet containing NCS into a polystyrene box and to pack crushed ice around its reservoir. This prevents loss of the volatile solvent, toluene, and also prolongs the shelf-life of the solution.

2. The first three samples should be taken on a fully-irradiated healthy leaf. During this time the apparatus is being flushed with radioactive gas. The samples may be discarded or assayed in order to check that equilibration of the apparatus has occurred before the fourth sample. The procedure is advisable whenever the apparatus is unused for a long period of time.

3. The method for preparing benzoyl peroxide is described in Hansen & Bush (1967).