# THE DIRECT ASSAY OF $^{14}C$ IN DRIED PLANT MATERIALS

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*Summary*

An apparatus is described and illustrated which enables the direct assay of $^{14}C$ in dried and ground plant material. The effect of five factors upon the measurement of the $^{14}C$-content of the powder are discussed. It is concluded that this technique provides an efficient, accurate, and rapid method for the assay of $^{14}C$ in plant materials.

## I. Introduction

More frequent use of $^{14}C$-labelled materials is being made in plant physiology and many instances arise where one desires to assay rapidly and accurately the quantity of $^{14}C$ in a sample of plant tissue. In the past, this has been carried out by oxidizing the tissue and assaying carbon dioxide either directly as the gas or as barium carbonate (Calvin *et al.* 1949). Among the many methods of oxidation that are known, two have been used in our work: wet oxidation employing the modification of the Van Slyke method described by Calvin *et al.* (1949) and oxidation in oxygen as described by Schöniger (1960). Both these techniques do not deal adequately with quantities of dried organic matter in excess of 20 mg, and other procedures which can handle larger quantities are tedious. In studies of photosynthesis and the translocation of carbohydrates in plants, one often handles quantities of dried organic matter well in excess of 200 mg and to use the Van Slyke modification, it is necessary to mix and aliquot the tissue.

It was felt that the finely divided, dried, mixed powder of the plant tissues was itself suitable for measurement. Measurement of the $^{14}C$-content of evaporated slurries of plant material has been reported by Zholkevich (1954), and MacKenzie and Dean (1950) have used briquettes of pressed plant material to measure $^{32}P$. The activity of pure compounds has often been measured by solid counting (Calvin *et al.* 1949; Popjak 1950; Burr and Marcia 1955) and Popjak (1950) drew attention to the value of this technique as the activity of the material is not diluted during combustion to $^{14}CO_2$ and counting as $Ba^{14}CO_3$. However, no detailed discussion of the measurement of $^{14}C$ in plant tissues by the counting of dried powders has been reported.

## II. Experimental

### (*a*) *Design*

Five factors were tested for their effects upon the measured values of the radioactivity of the powder samples:

A. The temperature at which the plant material was dried prior to grinding (60 and 110°C).

B. The fineness of the grinding (to pass 30, 40, or 60 mesh).

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C. The relative humidity at which the powders were held prior to counting (5, 55, and 76%).

D. The thickness of the sample which was counted (10, 20, or 30 mg/cm$^2$).

E. The position of the surface of the powder (level with the edge of the planchet or 1 mm below it).

TABLE 1

WEIGHT AND RADIOACTIVITY OF EACH SAMPLE ASSAYED

A = weight (mg) of sample counted. B = counts per 5-min period minus background (17·6 counts/min). L = surface height of powder 1 mm below shoulders of planchet. H = surface height of powder level with shoulders of planchet

| Plant No. | | −1 | −2 | −3 | −4 | −5 | −6 | −7 | −8 | −9 | Surface Position |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 60·2 | 40·0 | 20·4 | 60·2 | 40·5 | 60·4 | 40·3 | 20·9 | 20·8 | H |
| | B | 2638 | 2417 | 2689 | 2247 | 3185 | 3326 | 1968 | 1853 | 1957 | |
| 2 | A | 59·9 | 40·1 | 20·0 | 60·1 | 60·5 | 20·7 | 20·7 | 40·6 | 40·9 | L |
| | B | 986 | 968 | 694 | 815 | 851 | 808 | 814 | 808 | 818 | |
| 3 | A | 60·3 | 40·5 | 40·0 | 19·6 | 59·9 | 21·4 | 20·4 | 40·3 | 60·0 | L |
| | B | 1956 | 1322 | 1190 | 1225 | 405 | 1499 | 1723 | 562 | 1620 | |
| 4 | A | 20·5 | 40·6 | 59·4 | 40·7 | 66·4 | 19·6 | 40·3 | 60·2 | 20·6 | H |
| | B | 1880 | 2528 | 2476 | 2728 | 2353 | 2027 | 2418 | 2514 | 2069 | |
| 5 | A | 20·2 | 60·0 | 21·0 | 60·0 | 59·5 | 20·5 | 39·4 | 40·2 | 40·2 | L |
| | B | 1017 | 1093 | 1019 | 1255 | 1119 | 1001 | 1129 | 1166 | 1300 | |
| 6 | A | 20·5 | 40·5 | 60·5 | 20·7 | 39·0 | 60·6 | 60·4 | 40·4 | 20·8 | H |
| | B | 5082 | 5654 | 5488 | 4784 | 6349 | 4950 | 5560 | 5122 | 4234 | |
| 7 | A | 60·5 | 19·9 | 60·2 | 20·9 | 60·0 | 20·0 | 40·4 | 39·5 | 39·9 | H |
| | B | 2079 | 1747 | 2119 | 1970 | 2082 | 1710 | 2011 | 2019 | 2027 | |
| 8 | A | 19·8 | 40·1 | 60·1 | 40·5 | 60·3 | 19·4 | 40·5 | 56·6 | 20·4 | L |
| | B | 1385 | 1961 | 2264 | 2250 | 1945 | 2098 | 2599 | 1909 | 1744 | |
| 9 | A | 60·3 | 60·3 | 20·1 | 20·7 | 40·6 | 39·6 | 20·6 | 60·7 | 40·9 | L |
| | B | 1700 | 1625 | 1802 | 1481 | 1821 | 2003 | 1941 | 1988 | 1622 | |
| 10 | A | 60·9 | 19·9 | 40·7 | 19·5 | 59·3 | 39·9 | 40·9 | 60·5 | 20·1 | L |
| | B | 2762 | 2105 | 2883 | 2768 | 2636 | 2343 | 2579 | 2792 | 2421 | |
| 11 | A | 40·3 | 19·4 | 60·4 | 19·9 | 20·0 | 39·6 | 60·1 | 40·4 | 60·4 | H |
| | B | 2094 | 1597 | 1868 | 1925 | 1992 | 1854 | 2190 | 1865 | 1848 | |
| 12 | A | 40·7 | 19·6 | 20·5 | 40·0 | 40·5 | 59·5 | 20·1 | 60·5 | 60·4 | H |
| | B | 1885 | 1719 | 1263 | 1432 | 1778 | 1868 | 1510 | 1975 | 1846 | |

This was achieved by the use of a $2\times3\times3\times3\times2$ factorial experiment in which the effects of A, B, and E were measured by the differences between plants and the effects of C and D by the differences between subsamples of the same plant. The 12 plants necessary for the experiment were treated with $^{14}C$-sucrose and dried, ground, and counted in a random order.

### (*b*) *Method*

Twelve mature shoots of the tick bean (*Vicia faba* var. *minor*) each received 20 $\mu$g of $^{14}C$-labelled sucrose, 10 $\mu$g on each of two leaves, applied with a micro-pipette. The plants were harvested, and dried for 3 days at either 60 or 110°C in an oven. They were then ground in a Wiley laboratory mill, Micro model, to pass either 30, 40, or 60 mesh. After grinding, the samples were mixed in revolving tubes overnight to ensure a thorough mixing. Each sample was subdivided into nine parts (labelled 1–1, 1–2, ....,1–9, 2–1, ....,12–9,) and the subsamples were placed for 5 days in one of three desiccators in which the relative humidity had been fixed at one of three levels (approx. 5, 55, or 76% R.H.). Nine subsamples from each of six of the plants were assayed on one day, and the nine from each of the other six plants on the next day. The radioactivity of each sample was measured for a 5-min period, and the results are set out in Table 1 showing the weight of the material counted, the counts per 5-min period, and the position of the surface during counting.

### (*c*) *Apparatus*

A special apparatus was constructed to allow varying thicknesses of powder to be measured in a position of constant surface height. The apparatus is shown in Figure 1 and is composed of five parts:

(1) A base which is mounted to the bench and carries a fixed locating ring to hold the planchets in a reproducible position.

(2) A vertical spindle with two horizontal arms which can be rotated about the axis of the spindle. The head of the spindle carries a locking screw to lock the arms in position.

(3) A Philips Geiger–Müller probe unit which is mounted vertically on one arm of the spindle and can be aligned accurately over the planchet for counting. The background count was 17·6 counts/min and therefore no further shielding was necessary.

(4) A spring-loaded tool, accurately sized to the diameter of the space in the planchets, and made of light alloy to reduce the weight of the unit. The tip of the tool is made of cutlery grade stainless steel to lessen wear and corrosion.

(5) The planchet: this was specially designed (Fig. 1(*a*)) so that the sample volume could be varied. The base of the area that holds the powder is carried on a screw and a hole through the base plate allows the surface of the plated material to be adjusted to a constant height.

To use this apparatus a sample of powder is placed in the weighed planchet which is then positioned in the ring on the base plate. The powder is tamped

gently with the plunger, and the surface adjusted by the screw in the centre of the planchet, retamped, and readjusted. This is continued until the surface is flat and level. Particles of the powder that inevitably occur on the edge of the planchet may then be removed gently with a brush. The plunger is swung out of position, the counter into position, centred, and the activity of the sample recorded. The weight of the sample and planchet is obtained and the mass of the sample calculated. The face of the plunger and the inside of the planchet are cleaned out and dusted and the unit is ready for a new sample.

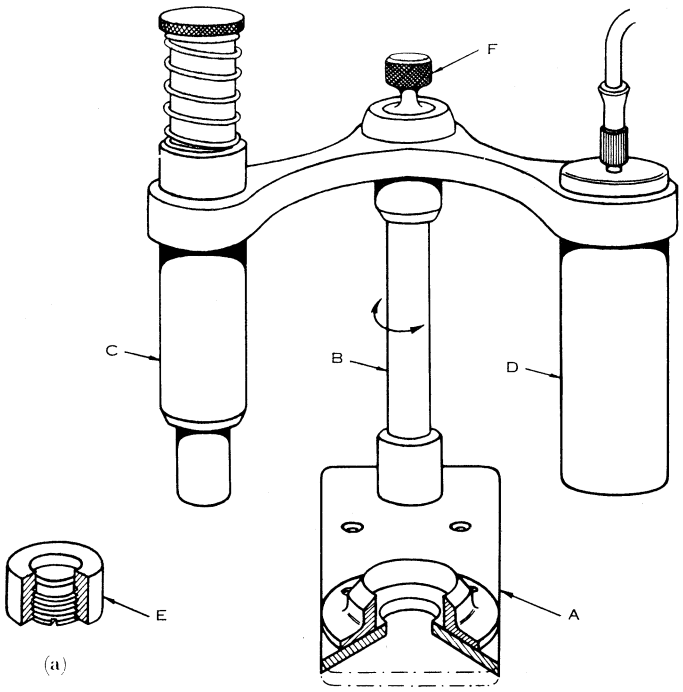


Fig. 1.—Details of apparatus used: *A*, base plate and locating ring; *B*, vertical spindle; *C*, spring-loaded plating tool; *D*, Philips probe unit; *E*, planchet; *F*, locking screw.

### (*d*) *Statistical Analysis*

Since it was expected that the variability of a result would be approximately proportional to its magnitude, the analysis was carried out on the logarithm of the counts per 5 min. The Bainbridge tabular method (Bainbridge, Grant and Radoh 1956) was used to sort out the individual terms of the analysis of variance, a summary of which is set out in Table 2.

Four effects are seen to be significant:

(i) *The Drying Temperature.*—There is a highly significant increase in the counts per 5-min period when the material is dried at 110°C rather than at 60°C.

(ii) *Fineness of Grinding.*—The counts per 5-min period increase in a linear fashion with increasing fineness of grinding.

(iii) *Surface Height.*—There is a highly significant effect upon the count rate if the geometry is altered by changing the position of the surface by 1 mm: the counts are lowered when the surface is 1 mm further away from the counter tube.

TABLE 2

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | Significance |
|---|---|---|---|---|
| *Between plants:* | | | | |
| Drying temperature (A) | 1 | 1·913605 | 1·913605 | ** |
| Fineness of grinding (B) | | | | |
| $B_1$ linear effects | 1 | 0·348612 | 0·348612 | * |
| $B_2$ quadratic effects | 1 | 0·159522 | 0·159522 | N.S.† |
| $A \times B_1$ interaction | 1 | 0·212986 | 0·212986 | N.S. |
| Surface height (E) | 1 | 1·213488 | 1·213488 | ** |
| $A \times E$ interaction | 1 | 0·047544 | 0·047544 | N.S. |
| $B_1 \times E$ interaction | 1 | 0·074369 | 0·074369 | N.S. |
| Error | 4 | 0·170859 | 0·042715 | |
| *Within plants:* | | | | |
| Conditioning humidity (C) | | | | |
| $C_1$ linear effects | 1 | 0·013778 | 0·013778 | N.S. |
| $C_2$ quadratic effects | 1 | 0·002373 | 0·002373 | N.S. |
| Deposit thickness (D) | | | | |
| $D_1$ linear effects | 1 | 0·037766 | 0·037766 | * |
| $D_2$ quadratic effects | 1 | 0·007573 | 0·007573 | N.S. |
| Two-factor interactions | 20 | 0·134578 | 0·006729 | N.S. |
| Error | | | | |
| Three-factor interactions | 36 | 0·238644 | 0·006629 | |
| Four- and five-factor interactions | 36 | 0·266023 | 0·007389 | |
| Pooled errors | 72 | 0·504667 | 0·007009 | |
| Total | 107 | 4·712578 | | |

* Significant at 5% level. ** Significant at 1% level. † N.S., not significant.

(iv) *Thickness of Deposit.*—There is a linear increase in the counts per 5-min period as the weight of sample counted increases from 10 mg/cm$^2$ to 30 mg/cm$^2$.

The fifth effect, the level of conditioning humidity, did not affect the count rate significantly.

## III. Discussion

### (*a*) *Drying Temperature*

As mentioned above, the count rate of samples dried at 110°C is higher than that of samples dried at 60°C. It has not been possible to attribute this effect to any one simple factor. The moisture retained by the tissues, their densities, and

the thickness of 30 mg/cm$^2$ are identical. Since the moisture content and density of the two powders are constant and the geometry is not varying one is forced to conclude that the effect observed is probably due to some property of the surface of the powder. While it is disappointing to be unable to attribute the effect to any specific factor, it is of no practical importance to users of the technique as one ordinarily dries plants at one particular temperature. Further, since several authors have commented on the loss of $^{14}CO_2$ from tissues during high temperature drying, vacuum drying at reduced temperatures or "freeze drying" are to be preferred (Calvin *et al.* 1949; Pirie 1956).

### (*b*) *Fineness of Grinding*

This effect was small, and considered alone, there would be no point in grinding samples to pass finer than 30 mesh. The small increase in count rate of the sample would not be worth the extra time required to grind the sample to pass finer meshes.

However, the variability of the counts of samples ground to pass 30 mesh is very significantly higher than that of samples ground to pass 60 mesh, the coefficients of variation being 34 and 10% respectively.

The coefficient of variation for a result derived in the above experiment is 20%. Although this in no way invalidates the experiment, this is large and led to doubts about the accuracy of the technique. Several tests were carried out subsequently to this experiment in which samples of powder were ground to pass 40 mesh, mixed overnight by rolling in a tube, and plated at 30 mg/cm$^2$ at constant surface height. Under these conditions the technique yielded results with a coefficient of variation of less than 3%, which in most cases is superior to the precision of results obtained when tissues are oxidized and counted as $Ba^{14}CO_3$. The high variability in the former experiment arose because the subsamples were poorly mixed before they were counted.

### (*c*) *Level of Conditioning Humidity*

The technique is rendered more useful since this factor does not significantly affect the count rate of the samples. Samples can therefore be ground, mixed, and counted without equilibration.

### (*d*) *Deposit Thickness and Surface Height*

Plant powders prepared by the technique described above have a large volume/mass ratio. If, as was done here, the height of the surface of the sample is kept constant at different thicknesses by the use of the screw levelling device described, then the count rate of samples of different thicknesses may be corrected for self-absorption losses by the standard methods (Calvin *et al.* 1949). If this levelling device cannot be incorporated, increasing thicknesses of powder plated in standard planchets do not display an "infinite thickness" value, the count rate of the sample rising continually with increasing thickness. This is caused by the change in geometry of the counting system brought about by plating increasing thicknesses of material.

Three ways of overcoming this difficulty are suggested:

(i) A constant thickness of material may be used—this becomes tedious when many samples are to be counted.

(ii) One may work with thicknesses where the count rate is a linear function of thickness.

(iii) A self-absorption correction curve may be prepared to suit the particular circumstances.

It is important to note that coefficients of variation of less than 3% have been obtained using the last technique in conjunction with standard planchets, provided suitable thickness corrections are made. It is also reassuring to note that no two-factor interactions were significant. Thus the differences in count rate produced by alterations in one variable do not themselves vary significantly with changes in any other of the variables that were tested.

It is often necessary to compare the radioactive concentrations of various plant organs relative to one another and it is important that the ratios of activities be independent of the assay technique.

The relative activities of various organs were compared by the direct assay of the powdered material and by the normal method following oxidation, and the ratios were found to agree within the limits of experimental error (approx. 5%).

The simplicity of the operations, the cheapness of apparatus, and the speed with which large numbers of plants may be assayed are all pointers towards widespread use of this technique. Other methods of grinding should also be applicable and there seems to be no theoretical reason for limiting its use to assay of tracers in plant material: it may be possible to assay desiccated faeces, blood, or animal tissues if these materials yield powders with satisfactory properties.

## IV. Acknowledgments

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