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BOTANY

THE UPTAKE OF $C^{14}O_2$ BY SOYBEAN ROOTS¹

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Roots, in contrast to leaves, are not generally regarded as organs for the absorption of gaseous CO_2 . Yet it is well known that the soil atmosphere in which roots exist has a higher concentration of CO_2 due to microbial and root action than has the atmosphere around the leaves. Ruben and Kamen (1940) demonstrated that non-green plant tissues fix $C^{11}O_2$, but they were unable to identify the compounds into which CO_2 was incorporated because of the short half life of C^{11} . In Russia, Kursanov and co-workers (1951), working with bean seedlings, showed that $C^{14}O_2$ was taken up by the roots and that after 18 hours most of the activity was in the stem and leaves, indicating an upward transport of C^{14} . This work led to field experiments by the same investigators on the uptake of carbonates that were introduced with soil fertilizers (Grinfel'd, 1954; Kursanov, 1954) and which were reported to increase crop yields.

Under certain conditions excess bicarbonate ion as well as excess CO_2 has been shown to be detrimental to plant growth (Porter and Thorne, 1955).

Poel (1953) and others (Arnoff and Graf, 1955; Jacobson, 1955; Stolwijn and Thimann, 1953) have studied the uptake of CO_2 into roots of various species, and by means of radiochromatographic techniques have partially identified the chemical products of C^{14} fixation as components of the citric acid cycle and sugars. Only a small percentage of the C^{14} labeled products were found in the non-alcohol soluble fraction.

In the course of the study of translocation of radioactive substances from various plant organs, experiments were made in this laboratory on the uptake and translocation of gaseous $C^{14}O_2$ introduced to soybean roots under negative pressure, using much the same techniques as leaf uptake experiments with $C^{14}O_2$.

MATERIALS AND METHODS: Seeds of *Glycine max* L. var. Ottawa Mandarin were sown in flats containing washed quartz sand and allowed to germinate and grow for twenty days. After twenty days the plants had one partially expanded trifoliate leaf and two smaller ones. The plants were removed from the quartz sand and the roots were

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carefully washed; the roots were then sealed in a Kjeldahl fume-pipe-manifold (Fig. 1). The manifold accommodated four plants for each exposure, together with a manometer and a Warburg vessel, which was used to generate and introduce the $C^{14}O_2$ into the manifold.

After the plant roots (Fig. 1) were sealed in the fume-pipe-manifold, the pressure in the chamber was reduced to about 80 mm. Hg. and the rubber tube from the pump was pinched off. Concentrated perchloric acid, which had been placed previously in the side arm of the Warburg flask, was tipped into a known weight of $BaC^{14}O_2$ in the outer well and the $C^{14}O_2$ generated until no more $BaC^{14}O_2$ could be seen in the flask. At this time the stopcock of the Warburg flask was opened, allowing the gas to be drawn into the evacuated fume-pipe-manifold which returned slowly to atmospheric pressure.

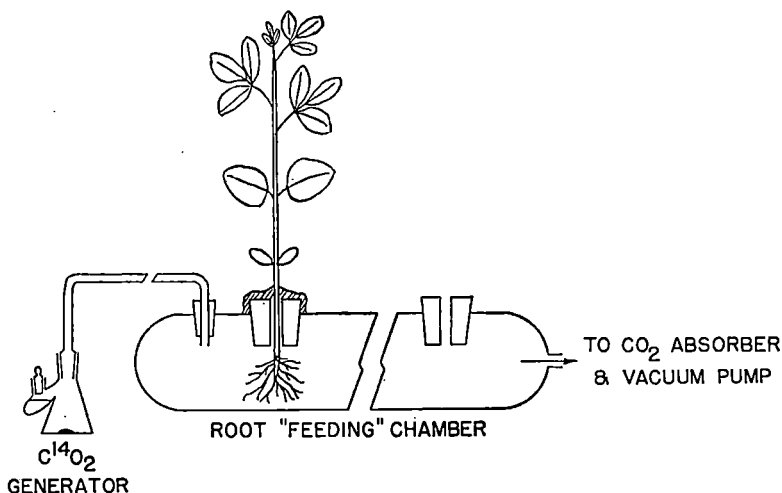


Fig. 1. Root "feeding" apparatus. The vacuum pump and manometer are not shown.

The time allowed for uptake was two hours. The plants and apparatus were kept in a hood at a temperature of 70° F. and a light intensity of twenty-five foot candles at the leaf surfaces. After the uptake period the atmosphere within the fume-pipe-manifold was drawn through 20% KOH and the apparatus disassembled. The plants were harvested and divided into the following parts: roots, hypocotyl, cotyledon 1, cotyledon 2, first internode, primary leaf 1, primary leaf 2, second internode, and apex including undeveloped trifoliate leaves. These parts were extracted with 80% ethanol at room temperature in a Waring blender, filtered, and frozen. Two ml. aliquots of the above extracts were placed in stainless steel planchets and counted in a proportional counter.

RESULTS AND DISCUSSION: Table 1 contains average counts per minute for the various plant parts. The counts are adjusted to a per

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TABLE 1. Average counts per minute per plant part of carbon-14 translocated out of the roots of four soybean plants together with the percentage translocated and range computed from standard error by transformation of percentage to arc sine $\sqrt{\text{percentage}}$.

Plant part	Average cpm	Percentage translocated	
		Average	Range
Root	720,012		
Hypocotyl	17,716	24.5	20.8-26.7
Cotyledon 1	2,116	3.0	2.1-4.0
Cotyledon 2	1,643	2.2	1.7-2.7
Internode 1	20,982	28.2	25.5-32.3
Primary leaf 1	3,640	4.6	3.6-5.7
Primary leaf 2	3,301	3.9	3.1-4.7
Internode 2	10,982	15.2	14.4-16.1
Apex	12,409	16.2	13.4-19.1

organ basis. Each count is the average of two separate determinations and the data presented are typical of several runs employing the same conditions and techniques.

There was considerable variability from plant to plant in spite of the fact that all plants were treated simultaneously in the same apparatus. There is a general pattern but statistical analysis indicated greater standard deviations and errors than would generally be acceptable. While individual absolute values may differ from plant to plant, there still may be a proportionality in the pattern of translocation, percentages were calculated of the amount of translocated activity that was found in each plant part. The C^{14} was considered translocated only if it left the roots, therefore percentages were calculated on the basis of the total activity in the plant, minus the activity in the root.

Data on percentages were transformed to the arc sine $X \sqrt{\text{percentage}}$ according to Snedecor (1956). Upon this transformed data the standard error was calculated to assess the variability in movement of C^{14} labeled activity. In Table 1 are also contained average percentages together with the range that contains it. While the absolute variability of translocation may be great there is only a small variability as expressed by a percentage of CO_2 translocated.

Samples, which had been previously counted, were gently heated with acetic acid and recounted to estimate the amount of C^{14} in a carbonate or bicarbonate form. This treatment yielded no decrease in counts and it was concluded that little if any of the C^{14} activity in the plant after a two hour uptake is in the form of carbonate or bicarbonate.

No radioactive sugars, amino acids, or organic acids were found when the extract from the root was separated by paper chromatography, although acids of the Kreb's cycle have been previously reported. Malic acid which was found by several workers to contain the major portion of the C^{14} activity was not found here. Acid hydrolysis of the root extract and subsequent two dimensional chromatography indicate that the activity is contained in polymers of a simple sugar or sugars.

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It is concluded that gaseous $C^{14}O_2$ is readily taken up by soybean roots and even under low light intensity is translocated to all parts of the plant.

The pattern of translocation of C^{14} activity while it may vary widely in absolute counts per minute may, in fact, be quite regular when compared on a percentage of the amount translocated.

It is also concluded that little if any of the carbon-14 remains in inorganic compounds and that in a relatively short time the $C^{14}O_2$ is incorporated into organic carbon.

In contrast to the reports of previous work, most of the water- and alcohol-soluble C^{14} activity in the roots of the plants used in this study was contained in polysaccharides.

Summary: Roots of soybean took up gaseous $C^{14}O_2$ and readily translocated it to all parts of the plant in a relatively short time. This $C^{14}O_2$ apparently does not exist in the plant as bicarbonate but rather is incorporated into organic compounds. These compounds are different from those found in similar experiments with other plants.

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