SHORT COMMUNICATIONS

DIFFUSION OF CARBON COMPOUNDS AWAY FROM WHEAT ROOTS*

By A. D. Rovira†

The exudation of organic compounds from intact roots has been reported for many plant species, but most of these results record only the water-soluble exudates and provide no information on the sites of the exudation. Pearson and Parkinson (1961) and Schroth and Snyder (1961) reported greater exudation of compounds which reacted with ninhydrin and silver nitrate from the root tip and the elongating zone of the roots of seedlings of broad bean and French bean than from the older parts of the roots; while McDougall (1968) has reported greater exudation of ^{14}C-labelled compounds from the lateral root zones of wheat than from the tip region. Bowen (1968) found that with *Pinus radiata* seedlings proportionately greater loss of ^{36}Cl occurred from the apical region of the roots than from the basal region.

The use of $^{14}CO_2$ in research on root exudates (McDougall and Rovira 1965; McDougall 1968) has enabled the study of exudation from individual roots and even parts of one root. By measuring the radioactivity of exudates from plants treated with $^{14}CO_2$, all carbon compounds are recorded, not only those which are detectable by specific sprays. This paper reports on both the sites of exudation of ^{14}C-labelled organic compounds from wheat roots and also the amounts of diffusible and non-diffusible material exuded by the roots. The technique is based upon that used by McDougall (1968) and Bowen (1968).

Materials and Methods

The assembly or "sandwich" consists of four strips of 4 cm-wide Whatman No. 1 chromatography paper which was wet evenly (8 ml to 4 g paper) with plant nutrient solution (Hoagland and Arnon 1950); the paper strips were placed in 5-cm wide polyethylene (3·5 mg/cm^2) tubing and held under slight pressure between hardboard covered with 5-mm thick urethane foam plastic. Sterile, germinated wheat seed was planted at the top between the two centre papers. The polyethylene tubing extended 15 cm above the paper and was inflated and sealed at the top. Plants were grown in a constant-environment chamber at 25°C day and 20°C night temperatures and with a 12-hr day during which Osram mercury vapour lamps (MBFR/u, 400 W) provided 2000 lm/ft^2, measured at plant level with an EEL Lightmaster broad-spectrum selenium cell without filters. After 6 days 10 ml of air containing 50 μCi $^{14}CO_2$ was injected into the polyethylene tubing containing the tops. At the time of injection the foam plastic–hardboard outer layers of the sandwich were temporarily removed and the positions of the root apices marked on the polyethylene enclosing the filter paper. After 6 hr light, 12 hr darkness, and 6 hr light, the outlines of the root and positions of apices (and laterals, if any) were marked on the papers and the roots lifted from the paper. The roots were mounted between 4-cm wide Whatman No. 1 chromatography paper and thin polyethylene (1 mg/cm^2, Handiwrap, Dow Chemical Co., Michigan, U.S.A.) and dried at 50°C. The papers between which the roots were grown were dried at 50°C without any covering over the paper.

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† Division of Soils, CSIRO, Private Bag No. 1, Glen Osmond, S.A. 5064.

The radioactivity in the roots and the paper strips was measured and recorded by passing them through a radiochromatogram scanner (Bowen and Rovira 1967). The accuracy of this scanning method was checked by correlating the areas under the scans with the total radioactivity inside the corresponding segments of roots or paper; this total radioactivity was measured by combusting segments of root or paper (5–10 mm long) in vials and counting by liquid scintillation (Gupta 1966). The total radioactivity in the root segments ranged from 75,000 to 450,000 counts/min/segment and for the paper containing exudate from 200 to 5800 counts/min/segment. The correlation between the radioactivity measured by the area under the scan corresponding to segments of 5–10 mm of root or paper and the radioactivity measured by combustion followed by liquid scintillation was 0·91 for wheat roots and 0·84 for exudate on paper. The scanning method automatically measures and records the radiocarbon in the roots or paper and is many times faster than counting individual portions of root or paper by either combustion or scintillation.

Results and Discussion

The scans in Figure 1 are representative of many roots studied and show that the radioactivity was high in all of the root which grew in the 24 hr following pulse-labelling, even though the $^{14}CO_2$ was fully incorporated by photosynthesis within 1 hr of application. The scan illustrated in Figure 1 was made of two roots growing together in the paper, but with 8 cm between the apices, and as both roots contributed to the exudate, they were not separated prior to scanning. The high peak of radio-activity associated with the apical 5 mm of each root indicates a further accumulation of ^{14}C from the photosynthate of the previous day. A similar high peak of radioactivity in the apical 5 mm was found in roots of subterranean clover treated 24 hr previously with $^{14}CO_2$.

In this paper "exudate" is defined as the total radioactive material deposited by the roots onto the filter paper and will include volatile, water-soluble, and non-water-soluble compounds together with sloughed-off root cells.

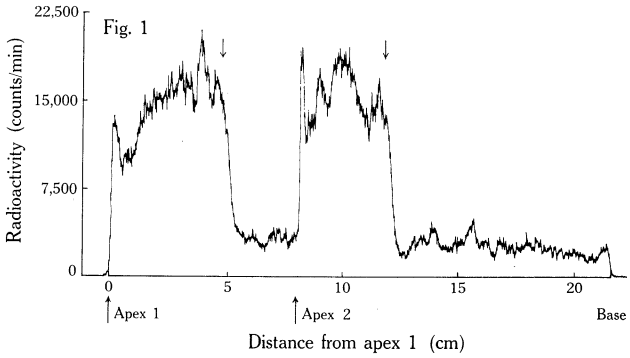
The exudate from these two roots was measured by scanning the intact papers along which the roots had grown and Figure 2 (upper line) shows that more ^{14}C was exuded from the region of growth of each root since labelling than from the older parts of the roots.

This high radioactivity from the region of growth of each root was confirmed by radioautographs of the paper in contact with the roots. The exact position of the two roots along the paper was located from the radioautographs and a 3-mm strip of paper removed to include the root outline plus 1 mm on each side of this root outline. The scans of this residual paper (Fig. 2, lower line) show that 60–70% of the radioactivity coming from the older root parts diffused beyond 1 mm from the root, whereas only 30% of the radioactivity exuded by the tip diffused away from the root. Very little radioactivity could be detected in the outer pair of papers indicating only small amounts of diffusible root exudate from wheat.

The non-diffusible material deposited on the paper as the root grows will include sloughed-off root cap cells and the mucilaginous material, probably polysaccharide, released by root cap cells. Material of both plant and microbial origin is included in this exudate from the growing root tip because in this study as well as those by Samstevich (1965) and Morré, Jones, and Mollenhauer (1967) no attempt was made to exclude microorganisms from the system. The microorganisms may also influence the diffusible compounds coming from roots by affecting cell permeability and by modifying constituents in the exudate. Experiments in which the exudation by sterile and non-sterile roots have been compared show that microorganisms growing

some distance from roots may fix $^{14}CO_2$ respired by the roots, and this fixed $^{14}CO_2$ would contribute to the radioactivity beyond 1 mm from the root.

The combination of $^{14}CO_2$ pulse-labelling of wheat plants, radioautography, and scanning of the paper strip in contact with the roots as described in this paper has revealed the contribution to exudation of recently photosynthesized carbon by growing root tips and older portions of the roots. The non-diffusible material would not be collected in most root-exudate studies which are conducted with solution-grown plants, thus underestimating the contribution of organic substances



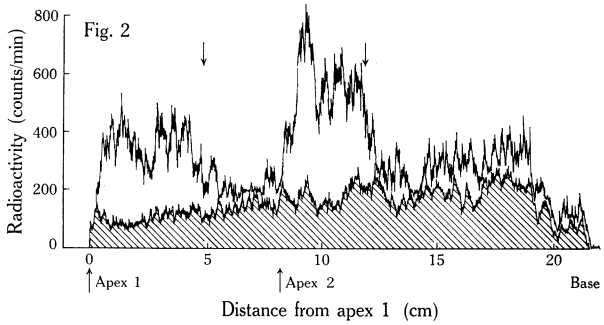


Fig. 1.—Radioactivity along two wheat roots of one plant grown in parallel between filter paper strips. The position of each apex at the time of administering $^{14}CO_2$ to the tops is indicated by the vertical arrows pointing downwards, whilst their positions at the time of harvest is indicated by the vertical arrows pointing upwards. Radiochromatogram settings: collimator 1·5 mm; scan speed 30 cm/hr; time constant 5 sec; count-rate range 30,000 counts/min.

Fig. 2.—Radioactivity exuded onto the inner paper strip from the two roots scanned for Figure 1. Vertical arrows as for Figure 1. The upper line represents the total radioactivity exuded from the roots onto the paper strip. The lower line was obtained from the paper strip after cutting out the root outline plus 1 mm each side. The shaded area represents the more diffusible material. Radiochromatogram settings: collimator 1·5 mm; scan speed 30 cm/hr; time constant 5 sec; count-rate range 1000 counts/min.

to soil via root exudates. The sandwich technique reported here does represent a model system closer to soil than is solution culture and the results indicate that root tips growing through soil will release a considerable amount of non-diffusible material which may play an important role in soil aggregation, nutrient availability,

and nutrient uptake and also in the nutrition of microorganisms associated with plant roots.

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