^{14}C-LABELLED MATERIAL LEACHED FROM THE RHIZOSPHERE OF PLANTS SUPPLIED WITH $^{14}CO_2$

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Abstract

^{14}C, supplied to plant tops as $^{14}CO_2$, was recovered in water-soluble organic material when pots with wheat, clover, or ryegrass growing in a podzolic sand were leached with distilled water at weekly intervals following exposure to $^{14}CO_2$. Similar material was recovered from unplanted controls. Percentage recoveries of total ^{14}C supplied were ryegrass 0·41, clover 0·30, wheat 0·19, control 0·14. ^{14}C was leached from all pots at a steady rate up to 10 weeks following cessation of exposure to $^{14}CO_2$.

^{14}C was not detected in such compounds as sugars or amino acids and fractionation with Sephadex gels and ultrafiltration indicated a molecular weight distribution of not less than 700 to more than 10,000. ^{14}C was strongly retained by anion-exchange resin and was probably associated with components of the fulvic acid–humic acid complex.

The evidence did not favour ^{14}C in leachates arising directly from the metabolism or decomposition of roots. It is proposed that ^{14}C was incorporated into soil microflora by assimilation of organic compounds released from roots or by heterotrophic fixation of CO_2, with subsequent release following metabolism or death or both of individual cells.

Defoliation of ryegrass previously exposed to $^{14}CO_2$ increased the amount of ^{14}C in leachates, with more than 20% apparently contained in polysaccharides.

I. Introduction

The number of microorganisms in the soil immediately surrounding plant roots is greater than in soil outside the zone of influence of roots ("rhizosphere effect") with a differentiation in physiological activity, morphology, and nutritional requirements of the rhizosphere and non-rhizosphere organisms (see review by Rovira 1965). Several factors operating in the root environment are believed to contribute to these effects: (1) metabolites released from healthy roots (root exudates); (2) metabolites released from the decomposition of moribund and sloughed off root material; (3) alterations in the concentrations of oxygen and carbon dioxide; (4) solution changes, e.g. pH, anion–cation balance.

Rovira (1969) reviewed the literature on the nature and amounts of plant root exudates, factors affecting exudation, mechanism of root exudation, and the interrelationships between root exudates and microorganisms. Most of the information has been obtained from solution-grown plants in sterile culture leaving many important questions to be answered about root exudates from soil-grown plants.

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These include (1) the amount of organic material released into soil and the extent to which it is modified by soil microorganisms; (2) the distance from the roots that the material diffuses; (3) the factors controlling root exudation. The occurrence of true physiological exudation has been questioned by Ayers and Thornton (1968) who suggested that the release of organic material from plant roots observed experimentally is due to environmental and experimental root damage.

The present work was initiated to measure the amount of organic material released into solution surrounding plant roots under conditions approximating normal growth patterns and, if possible, to distinguish between organic material released from intact healthy plant roots and that released from damaged or senescent roots or both. The nature of the organic material released to solution from different plant species and the possible interaction between soluble material released and microorganisms in the root environment was also studied.

The use of radioisotopes in these studies appeared essential, partly to exploit the sensitivity of isotopic techniques and partly to facilitate the differentiation of newly synthesized organic material from similar material present in the soil, resulting from previous plant growth or activities of soil microorganisms or both. The release of ^{14}C-labelled molecules from plant roots following exposure of the tops to $^{14}CO_2$ has been reported for plants grown in sterile solution culture (McDougall and Rovira 1965; Slankis, Runeckles, and Krotkov 1964) and in untreated soil (Subba-Rao, Bidwell, and Bailey 1962). The latter studies showed that appreciable amounts of radioactivity could be leached from funnels containing young tomato plants growing in a garden soil within 12 hr of exposing the plants to $^{14}CO_2$. Soil cores collected at distances up to 21 mm from the root zone contained fixed ^{14}C, some of which was released only after hydrolysis of the soil with hydrochloric acid.

This paper reports the amount and nature of the water soluble ^{14}C-labelled material leached from pots containing one of three plant species (wheat, ryegrass, or clover) growing in an agricultural sand, following exposure of the plant tops to $^{14}CO_2$. The interaction between growth of the different plant species and microorganisms is considered separately (Martin 1971).

II. Materials and Methods

(a) *Soil*

The soil used had to satisfy three requirements:

(1) it should be sufficiently fertile to support growth of plants to the stage of seed maturation without the addition of nutrient salts in quantities sufficient to interfere with the examination of ^{14}C-labelled material by thin-layer or ion-exchange chromatography;

(2) the clay or sesquioxide content or both should be sufficiently low that adsorption of organic molecules was not a problem;

(3) it should contain a microflora capable of establishment in the rhizosphere.

The most suitable soil from a number studied in previous glasshouse experiments was a sandy podzol from Mt. Compass, S.A. (coded Uc 2·3, Northcote 1965). A bulk sample of fresh soil was collected from a depth of 0–20 cm from a permanent pasture in September 1969, air-dried, sieved through a 6-mm mesh screen, and stored in jute bags.

(b) *Plant Culture*

Drainage tubes, consisting of a 30-cm coil of perforated stainless steel tubing (3 mm outer diameter), were sealed into the bottom of 10 cm diameter polythene pots. The drainage tubes were covered with coarse river sand and the Mt. Compass sand, wetted to 10% by weight with distilled water, was added to a depth of 10 cm. Pots were seeded in sets of five with wheat (*Triticum aestivum* L. cv. Gabo), ryegrass (*Lolium rigidum* Gaud.), or clover (*Trifolium subterraneum* L. cv. Geraldton). The seedlings were thinned to 8, 7, and 5 plants per pot for wheat, ryegrass and clover, respectively, and the pots transferred to a chamber designed for growth of plants in an atmosphere of $^{14}CO_2$. The plants were illuminated for 16 hr/day by mercury vapour lamps (Osram MBFU/R, 400 W) providing 2000 f.c. at plant level, with light and dark temperatures of 21°C and 15°C respectively. The wheat and ryegrass plants received 1 mg nitrogen/pot/week added as an equimolar mixture of KNO_3 and $Ca(NO_3)_2$.

(c) ^{14}C *Assimilation*

The first addition of $^{14}CO_2$ (50 μCi/pot) was made when seedlings were 4 weeks old and was continued at weekly intervals for a total of seven additions. Control pots without plants (two replicates) received four additions, each of 50 μCi $^{14}CO_2$. Pots were enclosed in polythene bags (approx. 4·3 mg/cm^2), sealed around the outer rim of the pot, and $^{14}CO_2$ (specific activity 0·7 mCi/mmole) injected by hypodermic syringe. The plastic bags were removed after 6 hr in full light.

(d) *Leaching Procedures*

Distilled water was added to the pots until free drainage occurred (water content approx. 25% by weight), and a further 200 ml of water were dripped onto the sand surface through four 22-gauge hypodermic needles, with a flow rate of approximately 1 ml/min. The first leachates were collected 6 days after the first exposure to $^{14}CO_2$ and then continued at 7-day intervals. The water content of the sand varied from 25 to 10% by weight in the pots with actively growing plants but remained near 25% for the control pots and in the wheat pots following grain ripening.

(e) *Measurement of Radioactivity*

The activity of ^{14}C leached from the pots and in fractions from resin and Sephadex columns was measured with a Beckman CPM-100 liquid scintillation counter. Sample volumes of 0·5 ml were added to 10 ml of scintillation fluid containing either 5 g of 2,5-diphenyloxazole (PPO), 100 g naphthalene, 1000 ml *p*-dioxane, or 4 g PPO, 100 mg 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP), 500 ml methylcellosolve, 500 ml toluene. The second scintillation fluid was preferred for column fractions which contained up to 0·5M LiCl or 0·5N HCl. External standard ratios showed evidence for quenching in the pot leachates but this was relatively uniform and a constant efficiency (90%) was assumed for all samples.

(f) *Fractionation of Radioactive Material*

(i) *Molecular Weight Distribution*

This was determined by the following techniques:

(1) Ultrafiltration with Diaflo membranes PM-10 and UM-2 in a model 50 ultrafiltration cell (Amicon Corp, Lexington, Mass.).

(2) Gel filtration with columns (40 × 2 cm) of Sephadex G10 (40–120 μm), G25 (10–40 μm), G50 (20–80 μm), G75 (40–120 μm), G100 (40–120 μm), equilibrated with 0·05M LiCl. Samples of leachate, concentrated 5–10-fold, were added to the columns and washed through with 0·05M LiCl. Void volumes (V_0) were measured with dextran blue (Pharmacia, Uppsala) and the elution volume for LiCl (V_e^{LiCl}) was determined from conductivity measurements.

(ii) *Stability to Acid Hydrolysis*

A 6-ml sample of concentrated leachate was divided into three equal parts and

(1) fractionated directly on Sephadex G25;

(2) held in boiling 1N HCl for 1 hr before fractionation on Sephadex G25;

(3) held in boiling 6N HCl for 24 hr before fractionation on Sephadex G25.

Samples (2) and (3) after hydrolysis were evaporated to dryness *in vacuo* over P_2O_5 and NaOH pellets and redissolved in 2 ml of distilled water adjusted to pH 9·0 with LiOH. The filtered solutions were fractionated on Sephadex G25 with the same conditions as sample (1).

(iii) *Retention on Anion-exchange Resin Columns*

Leachate (20 ml) was added to columns (10 by 1·2 cm) of Bio-Rad AG1-X2, 100–200 mesh, chloride resin, which were eluted successively with 25 ml water, 50 ml 0·1M LiCl, 50 ml 0·5M LiCl, 50 ml 0·5N HCl.

III. Results and Discussion

(a) *Release of ^{14}C-labelled Material from Pots*

(i) *Influence of Plants on ^{14}C Activity in Leachates (Expt. 1)*

^{14}C activity was present in the first solutions leached from pots containing wheat, ryegrass, and clover plants, following exposure of the tops to $^{14}CO_2$ (Fig. 1)

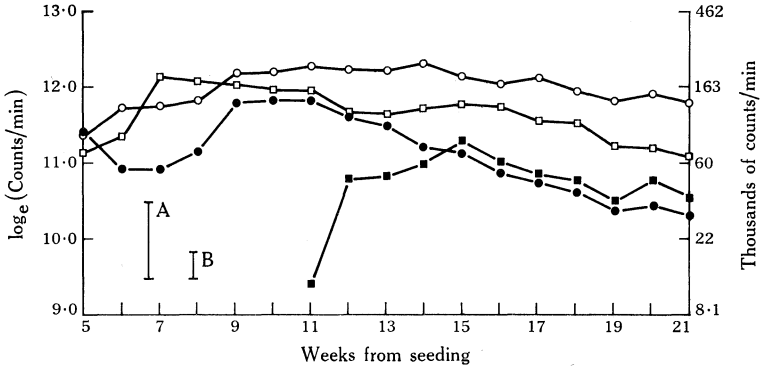

Fig. 1.—Total of ^{14}C activity present in 200 ml of leachate at each weekly collection (expt. 1). ○ Ryegrass. □ Clover. ● Wheat. ■ Control (no plants). Vertical lines A and B are least significant differences ($P < 0·05$) for comparison between weekly means for unplanted pots (two replicates) and planted pots (five replicates) respectively.

and relatively steady values were observed during the period of $^{14}CO_2$ exposure (weeks 4–10). Exposure of control pots to $^{14}CO_2$ (weeks 10–14) also gave rise to ^{14}C activity in the leachates. The ^{14}C present in all samples was not volatile at 100°C under strongly acid conditions, eliminating CO_2, HCO_3^- or CO_3^{2-} as the source.

Continued leaching after the cessation of the $^{14}CO_2$ exposure caused a significant ($P < 0·05$) decrease in ^{14}C activity recovered from the wheat pots but only a slight

decrease in ^{14}C counts from the clover and ryegrass pots. There were highly significant ($P < 0 \cdot 001$) differences in the mean ^{14}C activity leached from the wheat, ryegrass, and clover pots (Table 1). The control treatment could not be included in the statistical analysis, but the recoveries of ^{14}C, expressed as a percentage of ^{14}C activity supplied to the pots, are shown in Table 1 for comparison.

The values for recovery of ^{14}C activity in Table 1 are higher than the values of $0 \cdot 1\%$ found by McDougall and Rovira (1965) for wheat grown aseptically in nutrient solution and the $0 \cdot 05$–$0 \cdot 1\%$ calculated from the results of Subba-Rao, Bidwell, and Bailey (1962) for tomato plants grown in soil columns. The lower results may be explained by the limited periods (< 72 hr) over which the release of ^{14}C activity was measured.

TABLE 1

^{14}C ACTIVITY RECOVERED IN LEACHATES FROM POTS (EXPT. 1)

Mean ^{14}C activity for each treatment is the mean of the transformed ($\log_e$) values of ^{14}C activity (counts/min) present in 200 ml leachate at each weekly collection. ^{14}C recovery for each treatment is the total ^{14}C activity (counts/min) recovered in all leachings, expressed as a percentage of the ^{14}C activity supplied to the pots

	Wheat	Ryegrass	Clover	Control (no plants)
Mean ^{14}C activity present*	11·13	12·00	11·60	—
^{14}C activity recovered (%)	0·19	0·41	0·30	0·14

* Maximum least significant difference: $P < 0 \cdot 001$, $0 \cdot 13$. This was calculated from the largest standard error since within-crop variances were not homogeneous.

(ii) *Influence of Soil Microflora on ^{14}C Activity in Leachates (Expt. 2)*

Two observations from experiment 1 suggested a direct role of the soil microflora in the transformation of $^{14}CO_2$ to the non-volatile radioactive material leached from the pots: (1) the appearance of ^{14}C activity in leachates from unplanted pots; (2) the higher ^{14}C activity in leachates from ryegrass and clover pots than from wheat and control pots was paralleled by much higher numbers of microorganisms (counted on soil extract agar—Bunt and Rovira 1955) in leachates from ryegrass and clover than wheat and control pots.

A parallel experiment to study the influence of plant species on microbial numbers in the rhizosphere (Martin 1971) gave the following values for the number of bacteria which grew on soil extract agar, present in leachates 9 weeks from seeding:

	Wheat	Ryegrass	Clover	No Plants
$10^{-4} \times$ No. of bacteria/ml	30	460	560	5

A single dosage of $^{14}CO_2$ (100 μCi/pot) was supplied to three pots from each treatment. The pots were leached 6 days later and then at eight intervals each of 1 week, corresponding to weeks 10–17 in Figure 1.

Figure 2 shows that ^{14}C activity was released from all pots at a virtually constant rate. The recovery of ^{14}C activity from ryegrass was significantly higher ($P < 0 \cdot 001$)

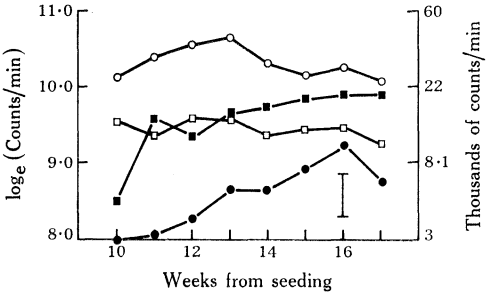

Fig. 2.—Total ^{14}C activity present in 100 ml leachate at each weekly collection (expt. 2). Vertical line indicates least significant difference at $P < 0 \cdot 05$ for comparison between weekly means (five replicates per treatment.

than for the other treatments; clover and the control (no plants) were not different but were significantly higher ($P < 0 \cdot 001$) than wheat (Table 2). The lower percentage

TABLE 2

^{14}C ACTIVITY RECOVERED FROM POTS SUPPLIED WITH $^{14}CO_2$ AT TIME OF MAXIMUM BACTERIAL NUMBERS IN LEACHATES (EXPT. 2)

Mean ^{14}C activity for each treatment is the mean of the transformed (log$_e$) values of ^{14}C activity (counts/min) present in 100 ml leachate at each weekly collection

	Wheat	Ryegrass	Clover	Control (no plants)
Mean ^{14}C activity present*	8·57	10·30	9·43	9·56
^{14}C activity recovered (%)†	0·02	0·12	0·05	0·06

* Maximum least significant differences (see Table 1): $P < 0 \cdot 05$, 0·23; $P < 0 \cdot 01$, 0·31; $P < 0 \cdot 001$, 0·43.

† As defined in Table 1.

recovery of ^{14}C activity than in experiment 1 is partly a reflection of lower activity in each weekly sample.

(iii) *Role of Algae in $^{14}CO_2$ Fixation in Unplanted Pots (Expt. 3)*

The possibility that algae, present in all pots, were primarily responsible for fixing $^{14}CO_2$ in unplanted pots was not supported by an experiment in which $^{14}CO_2$ (150 μCi/pot) was supplied to six unplanted pots set up in the standard manner. Three pots were held in full light in the growth cabinet and three were covered. Heavy algal growth was present in the pots exposed to light but none was visible in the covered pots. The total recovery of ^{14}C in four leachings at weekly intervals (as a percentage of ^{14}C supplied) was 0·006 for pots under normal lighting and 0·002 for covered pots.

(iv) *Effect of Plant Defoliation on ^{14}C Activity in Leachates (Expt. 4)*

Pots (two replicates) containing ryegrass and clover plants were supplied with $^{14}CO_2$ (50 μCi/pot) 7, 8, and 9 weeks from seeding. The pots were leached 7 days

after the final $^{14}CO_2$ addition (fraction A), after which the plant tops were clipped 2 cm above soil level. The pots were again leached, 1 day (fraction B), and 3 days (fraction C), after defoliation. Table 3 shows that the ^{14}C activity recovered from the

TABLE 3

TOTAL ^{14}C ACTIVITY RECOVERED FROM POTS CONTAINING RYEGRASS AND CLOVER, BEFORE AND AFTER DEFOLIATION

	^{14}C Activity (counts/min) of Fraction			% of ^{14}C Activity Supplied to Pots Recovered in Fractions A+B+C
	A (before defoliation)	B (1 day after defoliation)	C (3 days after defoliation)	
Ryegrass				
Fraction 1	980,000	279,000	219,000	0·49
Fraction 2	675,000	920,000	330,000	0·64
Clover				
Fraction 1	600,000	93,500	50,000	0·25
Fraction 2	665,000	94,000	77,000	0·28

clover pots decreased with each leaching. In contrast, one of the ryegrass pots showed a marked increase in the amount of radioactivity released in the leachate immediately following defoliation (fraction B2).

TABLE 4

FRACTIONATION BY GEL FILTRATION OF ^{14}C ACTIVITY LEACHED FROM A RYEGRASS POT FOLLOWING DEFOLIATION

Fraction	Elution Volume (ml)	K*	% of Initial ^{14}C Activity in Fraction			
			G25	G50	G75	G100
1	25–50	0	19	9	11	10
2	50–75	0–0·5	15	10	4·5	3
3	75–00	0·5–1·0	52	8	5	4
4	100–150	>1·0	11	69·5	76·5	77
Totals			97	96·5	97	94

* Partition coefficient K = (elution volume — V_0)/(V_e^{LiCl} — V_0).

(b) *Fractionation of ^{14}C-labelled Material Leached from Pots*

(i) *Molecular Weight Distribution*

The ryegrass fractions C1 and C2 (Table 3) were examined for low-molecular weight compounds, such as sugars and amino acids, by chromatography (isopropanol-ammonia, 60 : 40 v/v) and electrophoresis (1 kV, acetate buffer, pH 3·6) on thin

layers of cellulose, followed by radioautography (Martin 1970). There was no movement of ^{14}C activity in the chromatography solvent and only small amounts of radioactive material migrated during electrophoresis.

Additional evidence for the absence of ^{14}C-labelled low-molecular weight components came from gel-filtration studies. More than 90% of the radioactivity in ryegrass fraction B2 (Table 3) was wholly or partially excluded from Sephadex G10, indicating molecular weights > 700; over 25% of the ^{14}C activity was almost completely excluded from Sephadex G25, indicating molecular weights > 5000.

The gel studies were extended to include Sephadex G50, G75, and G100. The parameters for columns (40 by 2 cm) of each of these gels were very similar, so that fractions were collected from each column containing the same volume of elutant. Table 4 shows the distribution of ^{14}C activity in the fractions from each Sephadex column. There was an obvious interaction of the labelled material with the gel matrix in the G50, G75, and G100 columns, where the elution volume for the major fraction of the ^{14}C activity was greater than that for LiCl, so that the upper limits of the molecular weight distribution cannot be estimated reliably. The lower limit of 700 for most of the material, estimated from Sephadex G10, was supported by ultrafiltration experiments. More than 65% of the radioactive material has an apparent molecular weight greater than 1000, as indicated in the following tabulation:

Membrane and Characteristic	Nominal Molecular Weight*	% of Initial Activity in Fraction
PM-10, retained	$>10,000$	44·7
UM-2, retained	$>1,000$ $<10,000$	21·3
UM-2, passing	$<1,000$	28·5

* Manufacturer's data.

(ii) *Stability of ^{14}C-labelled Material to Acid Hydrolysis*

The stability to acid hydrolysis of the ^{14}C-labelled material excluded from Sephadex G25 was determined for a composite sample of fractions A, B, C leached from ryegrass pots in experiment 4. Treatment with 1N HCl for 1 hr at 100°C decreased

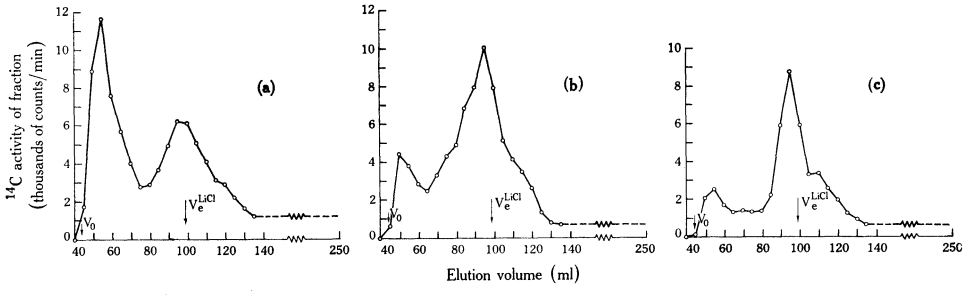

Fig. 3.—Total ^{14}C activity recovered in 5-ml fractions from columns (40 by 2 cm) of Sephadex G25 of untreated leachate (*a*) and following treatment of leachate with 1N boiling HCl for 1 hr (*b*) and 6N boiling HCl for 24 hr (*c*).

the radioactivity of the fraction excluded and increased the ^{14}C activity of the fraction diffusing freely through the gel (Fig. 3). Hydrolysis with 6N HCl for 24 hr gave a similar pattern, except that there was a decrease in the overall recovery of

^{14}C activity. The ^{14}C activity eluted in the combined fractions 40–70 ml, 70–100 ml, and 100–125 ml, expressed as a percentage of the activity present in the original leachate before lyophilization, is shown in the following tabulation:

Elution Volume (ml)	% of Initial Activity in Fraction		
	No Hydrolysis	1N HCl for 1 hr	6N HCl for 24 hr
40–70	38·0	17·0	8·8
70–100	25·6	40·5	24·3
100–250	27·0	23·0	19·7
Totals	90·6	80·5	52·8

The extensive degradation by boiling 1N HCl of the higher molecular weight components present in the leachates from the defoliated ryegrass suggests a polysaccharide component. A presumptive test for protein in the same fraction, based on degradation by boiling 6N HCl, was of doubtful value because of the low overall recovery of ^{14}C activity. The losses could result partly from destruction of sugars and partly from the formation of insoluble humins.

(iii) *Retention on Ion-exchange Resin Columns*

Less than 10% of the ^{14}C activity present in fraction B2 from the defoliated ryegrass (Table 3) was retained by a column of cation-exchange resin (Dowex-50 H^+) and more than 40% of the radioactivity was not retained by a column of anion-exchange resin (chloride form), again suggestive of a carbohydrate component. A further 30% or more of the ^{14}C activity was held reversibly by the anion-exchange resin and could be displaced with combinations of LiCl and HCl as shown in the following tabulation, where results are expressed as a percentage of the initial activity present in the particular fraction:

Fraction	Elutant	Experiment		
		I	II	III
1	Water	42·0	42·0	44·5
2	0·1M LiCl	*	22·0	27·0
3	0·5M LiCl	*	7·0	9·0
4	0·5N HCl	*	*	2·5

* Elutant not studied.

(iv) *Comparison of ^{14}C-labelled Components in Leachates from Wheat, Ryegrass, Clover, and Unplanted Pots*

The ^{14}C-labelled material in leachates from wheat, ryegrass, clover, and control pots collected from experiment 1 at week 14 (cf. Fig. 1), was fractionated on Sephadex gels and anion-exchange resin. The procedures were those used for fractionating the leachates from the defoliated ryegrass pots, described in Table 4.

Although there were large counting errors arising from the low levels of ^{14}C activity present in the leachates, the results (Table 5) show a pattern in all samples which is distinctly different from that obtained for ryegrass following defoliation (cf. Table 4 and above tabulation). The most marked feature of Table 5 is the low

recovery of radioactivity from the resin columns. Also, Table 5 shows that a higher proportion of the ^{14}C activity is recovered from the Sephadex columns with lower elution volumes than in Table 4, suggesting a greater proportion of the radioactivity is present as high-molecular weight material.

TABLE 5

FRACTIONATION OF ^{14}C ACTIVITY LEACHED FROM POTS WITH WHEAT, RYEGRASS, CLOVER, OR NO PLANTS

Results are percentage of initial activity present in fraction

Fraction	K*	Wheat	Ryegrass	Clover	No Plants
		Sephadex G25			
1	0	23	45	26	25
2	0–0·5	21	17	17	22
3	0·5–1·0	12	8	16	19
4	>1·0	0	3	0	2
Totals		56	73	59	68
		Sephadex G75			
1	0	5	24	8	15
2	0–0·5	18	13	11	6
3	0·5–1·0	45	34	22	25
4	>1·0	17	11	20	25
Totals		85	82	61	71
		AG1—anion-exchange resin, chloride form			
1 (water)		3	2	0	7
2 (0·1M LiCl)		8	1	0	10
3 (0·5M LiCl)		4	10	1	25
4 (0·5N HCl)		4	9	1	0
Totals		19	22	2	42

* As defined in Table 4.

(c) *Origin of ^{14}C-labelled Material Leached from Pots*

Interpretation of the data to provide information on the origin of the ^{14}C-labelled material is complicated by the period (usually 6 days) between exposure of plant tops to $^{14}CO_2$ and leaching the pots. McDougall and Rovira (1970) showed that appreciable amounts of ^{14}C activity were released by wheat roots less than 20 hr after exposing the tops to $^{14}CO_2$, so that there could be extensive degradation and resynthesis by the soil microflora of organic material released as root exudates or from sloughed off and moribund plant cells.

However, the following points seem relevant:

(1) Figures 1 and 2 show a steady rate of release of ^{14}C from all pots, even after cessation of $^{14}CO_2$ addition.

(2) There was a steady or decreasing release of ^{14}C from wheat and clover pots from 10 to 18 weeks even though there was marked decomposition of the root systems of these plants in this period (Martin 1971).

(3) Although the fractionation studies showed similarities in the properties of the ^{14}C-labelled material recovered from planted and unplanted pots, there was not a direct correlation between ^{14}C activity and microbial numbers in the leachates. There was virtually equal ^{14}C activity in the leachates from the clover and unplanted treatments in experiment 2 (Table 2) despite a 100-fold difference in bacterial numbers in the leachates from these pots immediately prior to the addition of $^{14}CO_2$.

(4) An influence of the stage of plant growth on the amount of ^{14}C recovered in leachates is indicated by the lower percentage recovery of ^{14}C activity in experiment 2 where $^{14}CO_2$ was added to plants at or near flowering compared with experiment 1 where $^{14}CO_2$ was added to seedlings.

(5) With the exception of the defoliated ryegrass samples, the ^{14}C activity was present in relatively high molecular weight components associated with the brown fraction which bound very strongly to anion-exchange resin.

(6) Sugars, amino acids, and polycarboxylic acids were not detected although such compounds are released from plant roots in sterile systems (Richter, Wilms, and Scheffer 1968; Slankis, Runeckles, and Krotkov 1964).

The most reasonable explanation is that, with the possible exception of the defoliated ryegrass samples, the ^{14}C activity recovered in the leachates is released at a steady rate from the biomass of soil microflora and microfauna, as a result of metabolism or death or both of individual cells. Incorporation of ^{14}C into the soil biomass presumably occurred in pulses following supply of $^{14}CO_2$ to the tops, either by assimilation of organic compounds released by the roots or by heterotrophic fixation of $^{14}CO_2$ arising from root respiration. This scheme is consistent with the report by Jenkinson (1966) that partial sterilization of field soil containing decomposing ^{14}C-labelled ryegrass rendered decomposable a small fraction, heavily labelled relative to soil organic matter as a whole. This was postulated to be contained in the soil biomass.

The presumed polysaccharide component present in the defoliated ryegrass samples could have been released directly from roots affected by the severe defoliation (Troughton 1957), or may have been microbial polysaccharide resulting from increased bacterial metabolism stimulated by the plant damage.

(*d*) *Conclusions*

These studies provide information on the type and amount of water-soluble organic compounds which are the net product of the interaction of plants and the soil microflora. They illustrate the complex nature of the products and highlight the difference between experiments with plants grown in sterile nutrient culture and experiments with plants grown under more natural conditions. The experiments do not provide information on the amount and nature of the ^{14}C-labelled material released from the plant roots. Extension of the present studies to obtain this information, will require growth of plants with a constant level of $^{14}CO_2$, and more frequent collection of leachings to minimize degradation of plant material by soil microflora.

IV. Acknowledgments

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V. References

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