Plant and Soil VII, no 3 March 1956

PLANT ROOT EXCRETIONS IN RELATION TO THE RHIZOSPHERE EFFECT

III. THE EFFECT OF ROOT EXUDATE ON THE NUMBERS AND ACTIVITY OF MICRO-ORGANISMS IN SOIL

by A. D. ROVIRA

Institute of Microbiology, Uppsala, Sweden *)

INTRODUCTION

The very restricted nature of the rhizosphere with the major concentration of micro-organisms on the root surface has always indicated a zone of intense microbial activity, with its possible effect on the metabolism of the surrounding soil. Thom [14] suggested, without actual evidence, that the rhizosphere population played little or no part in the decomposition of the resistant soil organic matter, being capable only of attacking freshly added organic material.

The effects of the rhizosphere population on nitrification and on phosphate and manganese availability to plants have been investigated by other workers (Goring and Clark [6], Gerretsen [5], Timonin [15]). During a study of the microflora of roots of oat varieties, Timonin [15] found that varieties susceptible to manganese deficiency supported greater numbers of micro-organisms capable of oxidizing manganese into unavailable forms than did resistant varieties. Gerretsen [5] has shown that roots with a rhizosphere population were capable of utilizing insoluble mineral phosphates which were only slightly available to sterile roots. The recent work on the mineralization of nitrogen which has been reviewed by Clark [4] shows that crop growth generally suppresses mineralization. Goring and Clark [6]

*) Present adress; Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, Scotland.

attribute this to the greater numbers of organisms in the soil surrounding the roots utilizing the soil nitrate during their growth and rendering it temporarily unavailable.

These investigations, however, have given no indication of the possible rôle of the rhizosphere population in the decomposition of organic matter in the soil apart from nitrogen. This is possibly due to the difficulty in dissociating the action of the roots from that of its surface flora.

In a study of the metabolism of rhizosphere soil, difficulty is experienced of obtaining sufficient soil, and hence it seemed desirable to develop a technique whereby large quantities of artificial "rhizosphere" soil could be produced. Timonin[15] has obtained a rhizosphere effect by burying colloidin sacs which contained flax root excretions but this method would not yield sufficient "rhizosphere" soil for the present investigation and the technique described below was developed. Using this technique it has been possible to study the changes in soil population and metabolism with root exudate supplements and the findings are reported in this paper.

EXPERIMENTAL

(*a*) *Establishment of an artificial rhizosphere*

Soils which had been air dried were brought to field capacity with root exudate solution (Rovira[12]), incubated in open vessels at 24°C and adjusted to field capacity daily with root exudate added solution. Treatment was continued for 14 to 21 days and the total exudate expressed as "plant equivalents per gramme of soil", each plant equivalent being the exudate from one plant during the first three weeks of growth. Parallel sets of soil treated with plant nutrient solution were used as controls.

(*b*) *Plate counts*

The medium of Bunt and Rovira[2] was used and the plates were incubated at 24°C for 14 days.

(*c*) *Qualitative changes*

The assessment of any qualitative changes in the populations due to treatment has been made on the basis of Gram staining and morphology. The selective stimulation of short Gram-negative rods appears to be one of the best established criteria for a rhizosphere effect, (Lochhead[7], Clark[3] and Rovira[10]), and in these experiments these qualitative

changes have been used as an index of the success or otherwise of the treatments.

(*d*) *Estimation of ammonia and nitrate*

Ammonia. This was estimated from 5% KCl extracts by treatment with alkali, distillation into 0.02*N* H_2SO_4 and titration of the residual acid.

Nitrate. After removal of the ammonia, the nitrate was reduced with Devarda's alloy, distilled into 0.02*N* H_2SO_4 and estimated as ammonia according to the method of Bengtsson [1].

(*e*) *Phosphate release from soil organic matter and nucleic acid*

The effects of root exudate on the phosphate level of the soil and the release from added nucleic acid were studied.

(i) Estimation of phosphate. The colorimetric method of Scheel [13] was used. Extraction was carried out with both 1% citric acid and 2% HCl.

(ii) Phosphate release from nucleic acid. The technique which Rogers [8] developed for the study of plant root exoenzymes in soil was followed. The sodium salt of yeast nucleic acid (B.D.H. preparation) was used, 5.13 g dissolved in 100 ml water and 10 ml ammonia (conc.), neutralised with H_2SO_4 and made up to 200 ml. This solution contains 2 mg P per ml. A suspension of 3 g of soil, 2 ml of nucleic acid solution and 4 ml of water was incubated at 28°C on a rotating shaking machine for 20 hours. The phosphate soluble in 1% citric acid and 2% HCl was then estimated.

(*f*) *Soil respiration*

The oxygen uptake of the soils subjected to the various treatments was measured at 30°C using the Warburg respirometer with 7 g of soil (wet weight) per flask (Rovira [9]). Duplicate flasks were always set up. The utilization of glucose by the soil population was studied by adding 1 ml of a 10% glucose solution per flask after the normal respiration had been recorded for three hours. The measurement of oxygen uptake was commenced 30 minutes after the addition of glucose to the soil.

RESULTS

(*a*) *Establishment of an artificial rhizosphere*

The effects of pea root exudate on the numbers of micro-organisms in a range of soils was studied, and as the results in Table I show there was a stimulation of the micro-organisms in all cases. The soils were selected because of different physical and chemical characteristics. Also included in Table I is the effect of oat root exudate on the population of Soil A.

TABLE I

The effect of root exudate supplements on the numbers of micro-organisms in various soils

Soil	Type	Number of bacteria *)/gramme ($\times 10^6$)		
		Control	Pea root exudate	Oat root exudate
A	Clay loam, crumb structure . .	70	166	186
B	Clay loam, poor structure . . .	65	218	—
C	Sandy loam, fair structure . .	57	91	—
D	Peat	183	453	—
E	Red loam, crumb structure . .	94	138	—

*) Means from four replicate treatments. S.E. = ± 7.8%.

The general increase of the bacterial population with root exudate supplements indicates the success in establishing an artificial rhizosphere. Fungal counts in treated soils showed no stimulation by the root exudate indicating an action similar to that in the root environment in which the bacteria are stimulated to a greater extent than the fungi.

Although the degree of stimulation (1.4 to 3.5) was not as great as often occurs in the rhizosphere, it is of a similar order to that found on the roots of three week old pea plants. Each gramme of soil had received an amount of exudate equivalent to that from 1.4 plants which is comparable to that in the normal rhizosphere where three week old pea plants growing in Soil B were found to have from 0.7 to 1.6 g of firmly adhering soil per root system.

The Gram staining and morphological characteristics of bacterial cultures from true pea rhizosphere, artificial "rhizosphere", and control soils are reported in Table II.

TABLE II

Staining and morphological characteristics of isolates from control, root exudate treated, and rhizosphere soils

Sample	Number of isolates	Gram reaction (%)			Morphology (%)		
		+	—	+/—	Rods	Coccoid rods	Cocci
Pea rhizosphere	95	6.3	88.2	5.5	83.1	10.6	6.3
Control	85	37.6	58.9	3.5	83.3	11.8	5.9
Root exudate treated soil	94	14.5	78.2	7.3	88.3	9.5	2.2
Control	90	30	60	10	90.4	5.3	4.3

These results show that although the change in the balance of organisms is not as great in the root exudate treated soil as in the

true rhizosphere there is a marked increase in the proportion of Gram-negative bacteria. The morphological grouping of the bacteria from the various treatments did not show any differences, in all cases the rod forms predominated. An attempt to subdivide this large group according to length failed due to the pleomorphic nature and variable length of many of the organisms.

(*b*) *Nitrification rates in root exudate treated soils*

(i) Release of ammonia and nitrate from soil organic matter. Ammonia and nitrate estimations were made on soils after treatment with root exudate for three weeks. The results presented in Table III show that for this particular soil the addition of root exudate had no influence on the nitrification rate.

TABLE III

Effect of soil treatment on nitrification		
Supplement	NH_3–N (mg N/10 g soil)	NO_3–N (mg N/10 g soil)
Without peptone		
Root exudate . . .	0.032 *)	0.61
Nil	0.03	0.61
With peptone		
Root exudate . . .	0.62 **)	1.64
Nil	0.62	1.30

*) Mean of duplicate treatments.

**) Mean of six replicate treatments. S.E. = ± 2.3%.

(ii) Decomposition of peptone in root exudate treated soils. Soils which had been treated for 21 days with root exudate or plant nutrient solution then received peptone solution (20 mg peptone per 10 g soil) and incubated for a further six days. The results (Table III) show the amounts of ammonia and nitrate in the soils at the end of this period.

The higher nitrate content of the root exudate treated soils indicates that these soils support a more active flora than the controls when readily decomposable material, *e.g.* amino acids, is present. The greater accumulation of nitrate but not ammonia in root exudate treated soils shows that the nitrification rate is increased. This may be due either to the direct stimulation of the nitrifying organisms by root exudate or to the greater supply of ammonia from the rapidly decomposing amino acids.

(*c*) *Phosphate release in root exudate treated soils*

The phosphate content of soils which had received root exudate for fourteen days was compared with those of control soils, and as the results in Table IV show there were no changes due to treatment.

TABLE IV

Phosphate content (ppm P) in treated and untreated soils			
Soil	Treatment	Extractant	
		1% citric acid	2% HCl
A	Root exudate	0 *)	32
	Control	0	32
B	Root exudate	7	164 **)
	Control	8	181

*) Mean of duplicate treatments. **) Not significant.

As the rhizosphere population could play an important role in the release of phosphate from more readily decomposable organic compounds, the phosphate liberation from yeast nucleic acid was investigated (Table V).

TABLE V

Release of phosphate from nucleic acid added to soil (mg P)		
Treatment	Extractant	
	1% citric acid	2% HCl
Root exudate	0.89 *)	1.05
Control	0.90	1.07

*) Mean of four replicate treatments.

In all samples there was a rapid release of phosphate with no differences due to treatment. It is possible that if the experiment had been continued differences may have occurred. This was not done as the study was carried out in a soil suspension which, over a longer period, could produce reactions not due to the initial populations.

(*d*) *Effect of root exudate on soil respiration*

The oxygen uptake of treated and untreated soils before and after the addition of glucose are summarized in Figures 1A and 1B.

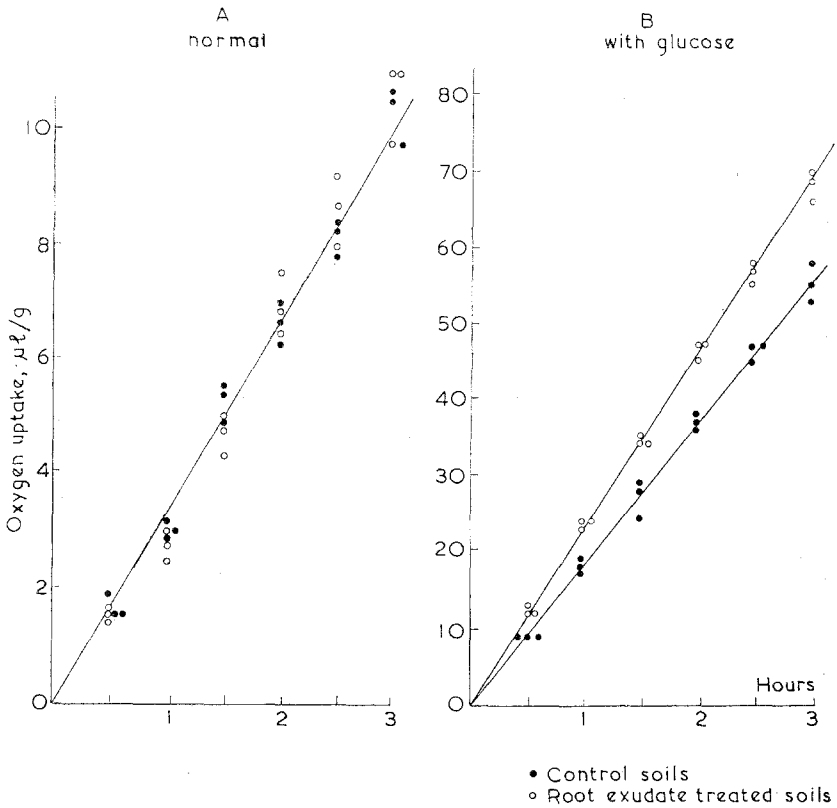

Fig. 1. Oxygen uptake of control and root exudate treated soils at 30°C.

These results make it quite apparent that, although the treatment with root exudate did not influence the normal oxygen uptake of the soil, the decomposition of added glucose was more rapid.

DISCUSSION

The selective stimulation of Gram-negative bacteria in soil by root exudate supplements indicates that the technique is suitable for obtaining soil with the characteristics and metabolism of that surrounding plants roots. Although rhizosphere conditions such as CO_2 accumulation, mineral uptake, and the physical nature of the root surface do not operate in root exudate treated soils, the properties will be indicative of those of the true rhizosphere. By preparing soils in this way it is possible to study conditions in the

rhizosphere without the complicating factors associated with the roots.

The resultant population of root exudate treated soils, while not increasing the rate of decomposition of soil organic matter, does promote the decomposition of more readily available organic substances such as amino acids and glucose. This indicates that under normal conditions the rhizosphere population does little to increase the turnover of the resistant soil organic matter. It is important, however, in the decomposition of material excreted from, or sloughed off, the roots, dead microbial tissue, and freshly added organic matter.

SUMMARY

1. The treatment of soil with root exudate solution resulted in increased numbers of Gram-negative bacteria.

2. The oxygen uptake and nitrification of root exudate treated soils were no greater than the controls unless readily decomposable substances such as glucose or peptone were added.

3. The release of phosphate from soil organic matter or yeast nucleic acid was not increased with root exudate supplements.

ACKNOWLEDGEMENTS

I wish to thank C.S.I.R.O., Australia, for supporting this investigation, Professor R. Nilsson for making available the facilities of the Institute of Microbiology, and Agr. Lic. P. E. Nilsson for generously permitting the use of his equipment.

Received May 23, 1955.

REFERENCES

1 Bengtsson, N., Contributions to the question of the nitrification of stable manure in cultivated soil IX. The determination of nitrates in soil and manure Kgl. Landtbr. Akad. Handl. Tidskr. No. 5, 620 (1932).

2 Bunt, J. S. and Rovira, A. D., Microbiological studies of some subantarctic soils. J. Soil Sci. **6**, 119 (1955).

3 Clark, F. E., Notes on types of bacteria associated with plant roots. Trans. Kansas Acad. Sci. **43**, 75 (1940).

4 Clark, F. E., Soil microorganisms and plant growth. Adv. Agron. **1**, 241 (1949).

5 Gerretsen, F. C., The influence of micro-organisms on the phosphate intake of plants. Plant and Soil **1**, 51 (1948).

6 Goring, C. A. and Clark, F. E., Influence of plant growth on mineralization of nitrogen in soil. Soil Sci. Soc. Amer. Proc. **13**, 261 (1948).

7 Lochhead, A. G., Qualitative studies of soil micro-organisms III. Influence of plant growth on the character of the bacterial flora. Canad. J. Research C **18**, 42 (1940).
8 Rogers, H. J., Dephosphorylation of organic phosphate compounds by soil catalysts. Soil Sci. **54**, 439 (1942).
9 Rovira, A. D., The use of the Warburg apparatus in soil metabolism studies. Nature **172**, 29 (1953).
10 Rovira, A. D., Some quantitative and qualitative studies of the rhizosphere. Proc. Soil Sci. Conf. Adelaide, June 1953 (1953).
11 Rovira, A. D., Plant root excretion in relation to the rhizosphere I. The nature of root exudate from oats and peas. Plant and Soil **7**, 178 (1956).
12 Rovira, A. D., Plant root excretions in relation to the rhizosphere II. A Study of the properties of root exudate and its effect on the growth of micro-organisms isolated from the rhizosphere. Plant and Soil **7**, 195 (1956).
13 Scheel, K. C., Colorimetric determination of phosphoric acid in fertilizers. Z. anal. Chem. **105**, 256 (1936).
14 Thom, C., Micropopulations correlated to decomposition processes. Trans. Intern. Congr. Soil Sci. III, 160 (1935).
15 Timonin, M. I., The interaction of higher plants and soil micro-organisms III. Effect of by-products of plant growth on activity of fungi and actinomycetes. Soil Sci. **52**, 395 (1941).
16 Timonin, M. I., Microflora of the rhizosphere in relation to manganese deficiency disease of oats. Soil Sci. Soc. Amer. Proc. **11**, 284 (1947).