INFLUENCE OF PLANT SPECIES AND PLANT AGE ON THE RHIZOSPHERE MICROFLORA

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Abstract

Significant differences were observed in the number of bacteria present in water leachates from pots containing different plant species (wheat, subterranean clover, ryegrass), and within a plant species, during growth from seedlings to the formation of mature seed. Bacterial numbers in the leachates reached a peak which coincided with flowering for each plant species. The peak values for wheat, clover, and ryegrass, respectively, were 33, 77, and 99 times the number of bacteria present in leachates from control pots without plants. Subsequently, the number of bacteria in leachates from wheat pots decreased until they were not significantly different from the controls. There was a lesser decrease for the clover and no significant decrease for the ryegrass treatments.

In all cases, the numbers of bacteria leached from the pots were less than 1% of the total bacteria present in the soil as estimated by counts of bacteria growing on soil extract agar. In contrast, fluorescent pseudomonads were readily displaced and were present in leachates in numbers corresponding to 5–1600% of the numbers remaining in the soil.

I. INTRODUCTION

A previous study (Martin 1971) reported on ^{14}C-labelled material present in water leachates from pots containing wheat, clover, and ryegrass plants. There were also limited observations on the number of microorganisms present in the leachates from each of the pots. These showed large variations between different plant species of the same age and within a plant species at different stages of growth.

The marked stimulation of bacterial numbers in soil immediately adjacent to plant roots and the variation with plant age have been well documented (Clark 1949; Rovira 1965). However, there are limitations to the conventional method of expressing the rhizosphere effect. This is based on the R/S ratio, where R is the bacterial count per gram of soil firmly adhering to the roots and S is the bacterial count per gram of soil collected some distance from the root. Sampling techniques and definition of the soil zones surrounding the roots can vary, and the relative amounts of root and soil in a sample can also influence the results significantly (Clark 1948; Rovira and Stern 1961). Further, the sampling techniques are destructive so that the number of measurements from a single plant or group of plants is limited.

The technique of measuring bacterial numbers in leachates from pots containing plants offered an alternative method of measuring the rhizosphere effect which would not have these limitations. This paper reports an extension of the earlier experiments (Martin 1971), to measure bacterial numbers in water leachates from pots containing

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wheat, clover, and ryegrass plants during growth from seedlings to the formation of mature seed. Additional information has been obtained on the relative numbers of microorganisms in the leachates, in the bulk soil, and associated with the plant roots at different stages of plant development.

II. Materials and Methods

(*a*) *Plant Culture*

The procedures described by Martin (1971) for plant culture and collection of leachings from the pots were followed, except that sterile distilled water was used for adjusting the water content of the pots and for leaching.

Twenty pots of Mt. Compass sand, containing 10% water, were held at room temperature for 6 days. Water was added to the point where free drainage occurred (100% "water capacity") followed by a further 100 ml of water. Two days later the pots were seeded (five replicates) with wheat (*Triticum aestivum* L. cv. Gabo, eight plants), ryegrass (*Lolium rigidum* Gaud., seven plants), or clover (*Trifolium subterraneum* L. cv. Geraldton, five plants), with the five unseeded pots for controls. Five days after seedling all pots were leached with 100 ml of water and thereafter at weekly intervals for a total of 18 leachings. Sterile water was added to the pots between leachings to maintain the sand at 90% of "water capacity".

(*b*) *Bacterial Counts*

Serial dilutions of the solutions leached from the pots were prepared within 3 hr of collection and 0·1-ml aliquots (duplicate plates) spread on the surface of soil extract agar (YPS medium—Bunt and Rovira 1955). The numbers of fluorescent pseudomonads were measured by spreading 0·1-ml aliquots of suitable dilutions on the selective NPC medium of Sands and Rovira (1970).

Bacterial numbers in the leached soil and on roots were estimated by tipping the contents of a pot into a sterile tray. Ten samples of soil (approx. 1 g each) were selected from an area apparently free from roots and added to a bottle containing 100 ml of water and glass beads. The root system from individual plants was freed from soil by gently swirling in 4 litres of sterile water in an enamel pot. Plant tops were removed and the entire root system from one plant added to a bottle containing 100 ml of water and glass beads. The soil and root samples were shaken with a wrist-action shaker for 15 min and dilutions of the resultant mixture were plated as above. The soil and roots were recovered by sieving and dried at 105°C.

The YPS plates were counted after incubation at 30°C for 3 and 7 days and the NPC plates after incubation for 1 and 3 days at 30°C.

III. Results and Discussion

(*a*) *Numbers of Bacteria Leached from Pots (Expt. 1)*

(i) *Bacteria Counted on YPS Agar*

Mean values (five replicates) for total numbers of microorganisms growing on YPS agar which were leached from the different plant treatments at each weekly interval are represented in Figure 1. The control treatment with no plants gave a high count at the first wash, shortly after wetting the air-dry soil, but the numbers dropped significantly ($P < 0{\cdot}001$) with succeeding washes until a relatively steady value was reached at the fourth week. Growth of each of the three plant species reversed this trend. The numbers of bacteria in the leachates increased to give a double peak, with the first peak coinciding with the beginning of flowering. The drop in bacterial numbers between the peak values was significant ($P < 0{\cdot}05$) for all treatments and occurred at different times, so was unlikely to have resulted from variations in methodology or from different batches of media. However, observation

of the plants was not sufficiently detailed to permit an explanation in terms of plant development.

The peak counts from the wheat pots were significantly lower than those from clover ($P < 0{\cdot}05$) and ryegrass ($P < 0{\cdot}001$) but the difference between the highest values from the clover and ryegrass pots was not significant. The ratios of the peak counts from the plant treatments to counts from the control at the corresponding time were: wheat (6th week), 33; clover (8th week), 77; ryegrass (13th week), 99.

There was a significant decrease ($P < 0{\cdot}001$) in bacterial counts in the solution leached from the wheat pots from the 7th to the 11th week, after which the counts were not significantly different from the control. The counts from the clover pots decreased significantly ($P < 0{\cdot}001$) from the 9th to the 17th week but were still higher ($P < 0{\cdot}001$) than the control. The decrease in the numbers of bacteria leached from the ryegrass pots over the same period was not significant.

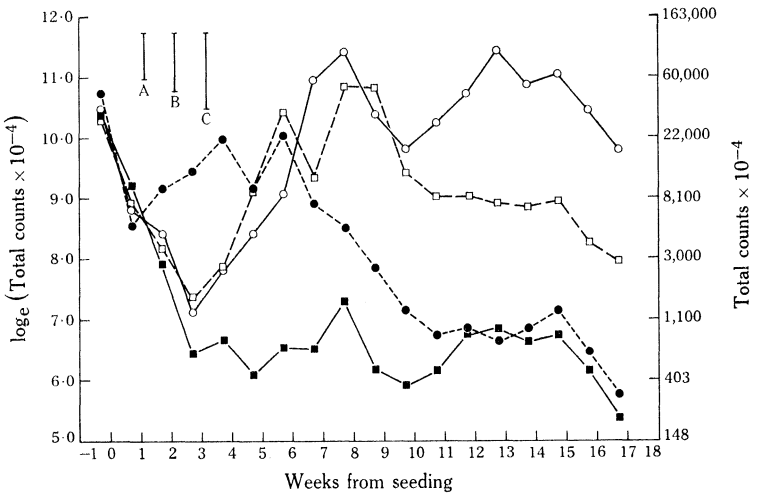

Fig. 1.—Variation with time of the number of bacteria (counted on YPS agar), present in 100 ml of leachate from pots seeded with wheat (●), clover (□), ryegrass (○), and from unplanted controls (■). Five replicate pots per treatment. Least significant differences at the 5% (A), 1% (B), and 0·1% (C) levels are shown for comparisons between and within treatment weekly means.

These differences in numbers of bacteria washed from the pots are probably attributable to differences in plant growth in each treatment. The wheat was almost completely ripe by the 11th week with little photosynthesis after this time, whereas there was considerable vegetative growth of clover and ryegrass to the end of the experiment, with sporadic flowering of ryegrass throughout the period 10–17 weeks. The results obtained for wheat agree with a previous report by Rivière (1960) that the rhizosphere effect for wheat was greatest at the time of ear formation and was weak or absent after ripening. Similarly, Starkey (1929) found for a range of plants grown in field and glasshouse experiments, that the influence of plants on the numbers of bacteria in soil is not great in the early stages of growth, reaches a maximum when the plants have attained maximum growth and have bloomed, followed by a pronounced decline in the number of bacteria.

(ii) *Fluorescent Pseudomonads*

Fluorescent pseudomonads were present in the soil in low numbers before seeding but were not recovered from the control soil at the later periods. Table 1

TABLE 1

FLUORESCENT PSEUDOMONADS LEACHED FROM POTS (EXPT. 1)

Values are total counts $\times 10^{-4}$ in 100 ml solution

Plant	Week					
	1	5	6	8	11	17
Control (no plants)	0·6	0	0	0	0	0
Wheat	3·0	51	120	30	1	0
Ryegrass	1·8	0·2	6·2	50	6·4	7
Clover	4·0	3·0	80	60	1·6	0·4

shows increased numbers of fluorescent pseudomonads in the leachates from pots with plants. Although their number (counted on NPC medium) made up less than 1% of the bacterial population counted on YPS agar, both groups of organisms responded similarly to the presence of plants and reached maximum numbers at the same stages of plant growth.

(*b*) *Comparative Numbers of Bacteria Leached from Pots, Associated with Roots, and in Soil (Expt. 2)*

A comparison of the numbers of bacteria leached from the pots with the numbers associated with plant roots or in the soil, together with a measure of root size at different stages of development, was obtained by duplicating the previous experiment and breaking open one pot from each treatment at intervals (2, 4, 7, 10, and 17 weeks from seeding) to sample roots and soil.

TABLE 2

MEASUREMENTS OF ROOT DRY WEIGHT AND ROOT LENGTH AT DIFFERENT AGES (EXPT. 2)

Values in parenthesis are numbers of plants per pot; M, main root; L, lateral roots

Weeks from Seeding	Total Dry Weight of Roots per Pot (mg)			Length of Roots from One Plant (cm)					
	Wheat (8)	Ryegrass (7)	Clover (5)	Wheat M	Wheat L	Ryegrass M	Ryegrass L	Clover M	Clover L
2	108	12	13	79	263	17	12	7	47
4	330	95	66	82	595	124	65	17	320
7	1151	1100	706	—		—		—	
10	1533	1753	1497	—		—		—	
17	950	2547	783	—		—		—	

Table 2 shows the total dry weight of root material recovered from each pot together with an estimate of total root length of single plants at 2 and 4 weeks from

seeding. The rapid growth of the wheat rooting system, compared with clover and ryegrass, provides an explanation of the results of experiment 1, which showed an increase in bacterial numbers leached from the wheat pots at weeks 2–4 when counts were still decreasing for the clover and ryegrass samples. It also seems probable from the results in Table 2 that the greater numbers of bacteria recovered in leachates from clover or ryegrass pots than from wheat pots in experiment 1 reflect a true species difference. The conditions for experiment 2 duplicated experiment 1 as

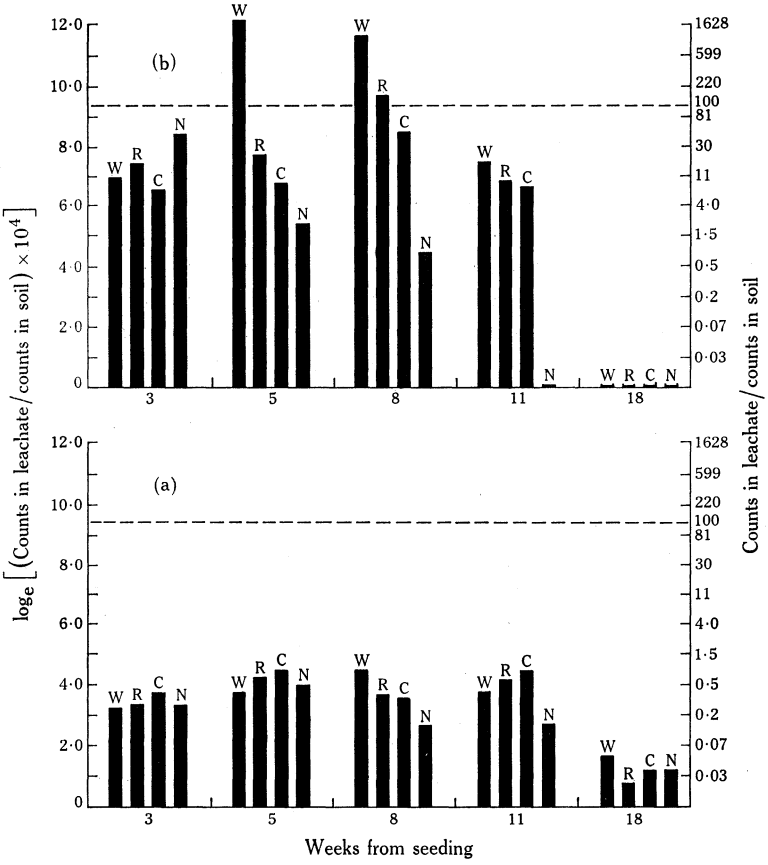

Fig. 2.—Variation with time of the ratio of the number of bacteria in 100 ml leachate to number of bacteria in 1200 g leached, "root-free" soil. (*a*) Bacteria counted on YPS medium. (*b*) Bacteria counted on NPC medium. Treatments (one pot at each sampling period): W, wheat; C, clover; R, ryegrass; N, unplanted control.

closely as possible, so that it is unlikely that there would have been a significant difference in the mass of wheat roots compared with ryegrass and clover during the period when the differences in bacterial counts were observed. The marked decrease in the dry weight of roots recovered from the wheat and clover treatments between 10 and 17 weeks from seeding was probably due to partial decomposition of moribund roots causing fragmentation of the root systems during rinsing to remove adhering soil.

A comparison of bacterial numbers (counted on YPS agar) present in leachates from planted pots with the numbers recovered from the unplanted controls showed a stimulation of bacterial numbers associated with plant growth. The maximum effect occurred 7–10 weeks from seeding. However, there was little difference between plant species and the increase in bacterial numbers resulting from plant growth was much less than in experiment 1. The ratio of the counts from pots containing plants to those from control pots was always less than 6, compared with peak values of from 30 to 100 for the first experiment. A possible explanation for the difference is that many of the bacterial species which responded to plant growth in experiment 1 were inactivated in the 5-month period between experiments, when the sand was stored in a glasshouse with summer temperatures more than 45°C. Support for this view comes from the observation that pigmented organisms, which were dominant on the YPS plates from the highest dilutions in the first experiment, were rarely observed in the second experiment. A further indication of changes during storage was shown by the number of bacteria, counted on YPS agar, leached from the pots before seeding. In the second experiment the numbers were less than 40% of the value obtained for comparable samples in the first experiment. Measurement of the heat resistance of the microflora present in the bulk sand remaining in storage at the end of the experiments showed that 70% of the colonies were from spores or cells which survived after heating the soil dilutions to 80°C for 10 min.

Estimates were made at each sampling period of the total number of bacteria (counted on YPS and NPC media) present in the three components—(1) organisms present in 100 ml of leachate; (2) organisms associated with the root system of all the plants in a pot; (3) organisms present in 1200 g of "root-free" soil remaining after the pot had been leached. Figure 2 represents the numbers of organisms leached from the pots at each sampling (component 1) expressed as a ratio of the numbers of organisms present in the residual soil (component 3). Figure 3 represents the corresponding ratio for the bacterial counts for the root samples. A value of 100, shown by a dashed line, corresponds to equal numbers of bacteria in the leachate or root component to those in the residual soil component.

There was no systematic variation in the number of bacteria recovered from the soil samples (component 3) on YPS medium, either between plant treatments or at time intervals within a treatment. The mean value for the 20 pots was 60·6 (S.D. 20·8) $\times 10^8$ organisms/1200 g soil. Figure 2(*a*) shows that the number of bacteria leached from the pots, recovered on YPS medium, represented 0·5–1·0% of the counts in the residual soil. The YPS counts for the root samples [Fig. 3(*a*)] increased with time to represent 8–16% of the soil count. The increase resulted primarily from the increased dry weight of roots and there was very little difference in counts per milligram dry root between time periods or plant treatments.

The results for fluorescent pseudomonads (NPC medium) show a totally different pattern. Although the soil counts were extremely variable, ranging from 0–600,000 counts/1200 g soil, it is obvious from the ratios in Figures 2(*b*) and 3(*b*) that the number of fluorescent pseudomonads leached from the pots or on roots represented a high proportion of the numbers present in the leached "root-free" soil and in some cases exceeded the soil numbers by a factor of 10 or more. These results, in conjunction with the almost complete disappearance of fluorescent pseudomonads after the 11th

week, indicate that the organisms isolated were present either on roots during active plant growth or in solution immediately adjacent to the roots, with low numbers in the bulk soil.

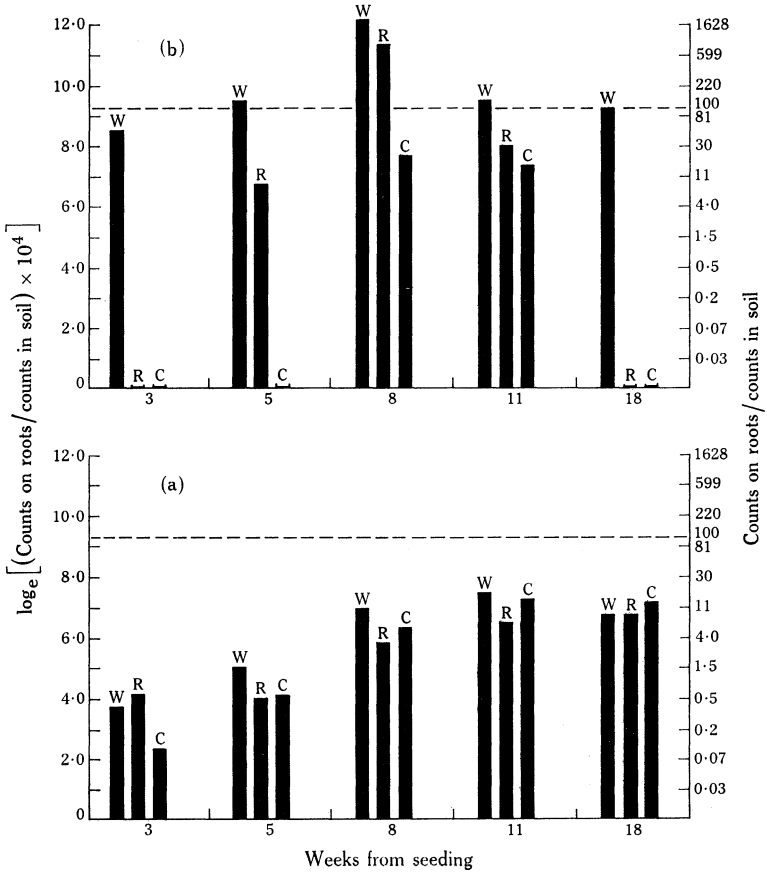

Fig. 3.—Variation with time of the ratio of the number of bacteria associated with total root weight per pot to the number of bacteria in 1200 g leached, "root-free" soil. (*a*) Bacteria counted on YPS medium. (*b*) Bacteria counted on NPC medium. Other abbreviations as for Figure 2.

(*c*) *Conclusions*

The measurement of bacterial numbers in water leachates from pots has demonstrated the marked influence of plant growth in increasing total numbers of bacteria in the root environment and the selective stimulation of fluorescent pseudomonads. Although this technique is free from the sampling difficulties, inherent in separating rhizosphere from control soil and studying samples with variable root/soil contents, there are several potential disadvantages. It is uncertain to what degree the leachate microflora are representative of the microbial population in the pots, both qualitatively and quantitatively. Also, the soils are completely water-saturated at each sampling

period and the flora isolated under these conditions may not represent the soil flora present in drier soils.

However, these disadvantages may be outweighed by the ease of replication, both of control soils and soils with growing plants at a particular stage of development, as well as the ability to make repeated measurements from the same pot. It is also possible that the leaching procedure offers a more sensitive measure of the microflora selectively stimulated by roots than is possible with soil samples, where the physiologically active rhizosphere population is superimposed on a large population of organisms with low metabolic activity. The technique described may be particularly valuable in recognizing and measuring transient populations.

IV. Acknowledgments

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