ROTHAMSTED RESEARCH

Report for 1960

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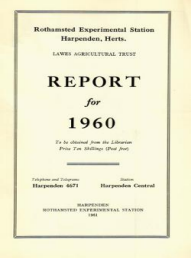

Soil Microbiology Department

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P. S. NUTMAN

J. Meiklejohn was awarded a Research Fellowship tenable at University College, Ghana, where she spent six months working on the microbiology of the nitrogen cycle in forest and grassland soils. J. Kleczkowska worked for three months at the Microbiology Laboratory, Wageningen University, on the effects of phage lysates and bacterial extracts of *Rhizobium trifolii* on growth and symbiosis. B. Mosse came in March on a two-year secondment from East Malling Research Station to work on endotrophic mycorrhiza. J. Darbyshire, formerly of Matopos Research Station, Southern Rhodesia, came in November for post-graduate research training. B. M. Gupta returned to Lucknow in November. Prof. D. C. Jordan (Ontario Agricultural College) and H. Ljunggren (Agricultural College, Uppsala) worked in the department during the year.

Except for the work on mycorrhiza, no entirely new research was initiated. The programme on nitrogen metabolism in nodules and on fixation was extended by the use of ^{15}N. Ecological studies were enlarged by the work on tropical soil, by developments of the *Azotobacter* inoculation work and by minor field investigations on the distribution of *Rhizobium* and of aerobic cellulose decomposers. The work on decomposition of chemicals in soil and on clover genetics was decreased and will not be reported on.

Genetics of Rhizobium

Seventy-one mutant strains of *Rh. trifolii* (strain A121111, effective on red clover), obtained after various selective procedures, were examined for their nutritional requirements, phage and streptomycin resistance, serological type and symbiotic effectiveness. Using an auxanographic technique (which also estimated biotin and thiamin excreted by the host root) the vitamin, amino-acid and nucleic acid requirements were determined. The only nutritional mutant found was a strain not requiring thiamin, obtained after exposure to ultraviolet irradiation.

Of 51 mutants obtained by ultraviolet and streptomycin treatment, only three were ineffective in fixing nitrogen, and none was phage-resistant or serologically changed.

Phage differed from the other selective or mutagenic agents in that a high proportion of the resistant mutants also differed from the parent strain in symbiotic properties. Thus of 14 stable phage-resistant mutants 13 were ineffective in nitrogen fixation with red clover. Six other resistant and ineffective mutants, which soon reverted to susceptibility in absence of phage, also tended to revert to effectiveness; one remained ineffective, two gave mixed responses and three regained complete effectiveness.

This association between the appearance of phage resistance and

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ineffectiveness confirms work previously reported for other strains (*Rep. Rothamst. exp. Sta.* for 1958) and is being further investigated by transformation studies. The rates of mutation to phage resistance and streptomycin resistances were calculated. (Kleczkowska and B. M. Gupta.)

Polygalacturonase and cross-inoculation-group specificity

Fåhraeus and Ljunggren (*Physiol. Plant.* 1959; *Nature, Lond.* 1959) showed that polygalacturonase (P.G.) is produced in the rhizospheres of clover and lucerne plants in the presence of bacteria able to infect them. Neither bacteria nor host alone, or incompatible combinations of host and bacteria, produce P.G., but polysaccharide extract from compatible bacteria induces its production by the host.

The relation between cross-inoculation group specificity and P.G. production was further examined during Ljunggren's visit to this Department, with eight host species belonging to four cross-inoculation groups and an appropriate collection of strains of bacteria. P.G. was detected only when the plant was inoculated with a bacterium to which it was susceptible.

Measurements of the enzyme produced by plants carrying different numbers of nodules showed that P.G. production was highly correlated with actual nodule formation in red and in subterranean clover, whether abundantly or sparsely nodulating strains of bacteria or selections of host were compared. Polysaccharide added to plants inoculated with virulent strains promoted earlier nodule formation. (Ljunggren.)

Transformation in Rh. trifolii

The avirulent mutant (Strain Bart. A) of *Rh. trifolii* produced no P.G. Virulence and competence to induce P.G. were restored to this strain by treating it with a bacteria-free preparation of the polysaccharide of a virulent (unrelated) strain in the presence of the host root. The nodules so produced were effective in nitrogen-fixation, and isolates from them retained their virulence without any added polysaccharide. Serological tests showed that the transformed strain did not have the specific polysaccharide of the parent virulent strain from which Bart. A. was derived.

These results point to a solution of the long-standing problem of cross-inoculation group-specificity, for variations in composition and structure of bacterial polysaccharide give ample scope for the complex pattern of cross-inoculation specificity which has been described.

Very little P.G. is produced, and the mechanism of its activity in the infection process is not yet understood; it may dissolve the primary wall locally or be released as a result of synthetic processes associated with primary wall growth; and if the infection thread is formed by invagination it may also aid this process. (Ljunggren.)

Clover-root hair infection in agar and in soil

The effect of various densities of rhizosphere population of rhizobia on infection was studied. To vary the number of bacteria in the inoculum, a virulent strain (A121111) was mixed with an

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avirulent mutant (Bart. A.) in various proportions and a large total inoculum used (as in *Ann. Bot.* 1957). This minimised multiplication of the bacteria and kept the density of the virulent component near to the original numbers added.

The density of rhizobia required to initiate infection varied with different host species; the increase in density needed for every additional infection then remained constant and did not differ much between species till a nodule or nodules formed, when the number of bacteria necessary for further infection increased several fold.

In two contrasting soil types, (*a*) Rothamsted heavy clay soil and (*b*) Woburn light loam soil, infection proceeded at the same rate. In both soils fewer infections were observed than on agar, though the population size of nodule bacteria in the rhizosphere was high enough (between 10^4 and 10^8 per rhizosphere) to warrant more infections than were observed. The bacteria from the soils were later shown to have normal infective virulence. Studies on the effect of some root-surface fungi on infection showed that bacterial density was not the only controlling factor. This work was done in soft agar with washed spores of the fungus added, together with a rhizobium inoculum of known size. Some fungal species regularly stimulated infection, whereas others either had no effect or decreased infections. None of these fungi affected the size of rhizosphere population of nodule bacteria. (G. Lim.)

Nodule formation requires substances elaborated in the top of the plant, and cutting off the top stops nodulation: if the top is replaced by an artificial source of carbohydrates, vitamins, etc., nodule formation continues only briefly and sparsely. (Raggio, *Amer. J. Bot.* 1957.)

The effect of top excision on root-hair infection, as distinct from nodule formation, was examined in several species of host, which were cut and inoculated when 3–6 days old, before infection of intact plants normally begins. Roots of *Trifolium fragiferum*, *T. repens* and *T. glomeratum* without tops contracted about as many infections as did those with tops, whereas removing the tops of *T. parviflorum*, *T. scabrum* and *T. nigrescens* greatly decreased infections. In all species, excision changed the distribution of infected hairs along the root by making the zonation of infection in intact plants less evident and by regularly increasing the density of infections towards the root tip. It is hoped that further work will explain the normal pattern in which infection is initiated at a few separated centres that enlarge as the seedling develops. Whole seedlings are infected in two exponential phases; the end of the first, more rapid, phase coincides with nodule formation. The effect of nodules on infection was examined in experiments in which nodulation was delayed by including trace quantities of nitrate nitrogen in the medium (*Rep. Rothamst. exp. Sta.* for 1958, p. 73). This led to more infections than on control plants. (Nutman.)

Failure of trefoil (*Medicago lupulina*) in green manuring experiments at Woburn seems to result from lack of nodule bacteria in the period of early growth of the crop (which was not inoculated). In October (5 months after sowing on Stackyard Series C, and 2 months after sowing on Lansome Field) only an occasional trefoil was fully green and effectively nodulated (mostly by few very large,

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fasciated nodules). The remainder were without nodules or poorly nodulated. *Rh. meliloti* was very sparse (< 1 bacteria in 10 g. soil) in plots without trefoil and very irregularly distributed under trefoil, indicating that the small residual population at sowing did not increase soon enough to give uniform effective nodulation. Populations in excess of 10^8/g. rhizosphere soil were found in trefoil sown on the Green Manuring Rotation Experiment, Stackyard field series A, where trefoil has been grown for some years. The need for trefoil inoculation is not well recognised, and some strains of bacteria are being examined as suitable inocula. (Dyke and Nutman.)

The poor establishment of uninoculated white clover on new land at Fritham Plain, Hampshire, was associated with failure of effective nodulation. Watering on of an effective strain gave statistically significant increase in growth. (Scowen.)

Nitrogen metabolism of root nodules

Soyabean nodules were crushed and separated by centrifuging into bacteroids, supernatant fluid and a fraction sedimenting at 30,000 X g., probably containing the membranes which enclose packets of bacteroids in intact nodules. All three fractions actively synthesised glutamine from added glutamate and ammonia in the presence of adenosine triphosphate. The synthesis per mg. of protein was greater in the supernatant than in the membrane fraction; technical difficulties have so far hindered quantitative estimation of the activity of the bacteroid fraction. (Cooper and Jordan.)

A start was made on a direct study of nitrogen fixation by the ^{15}N technique. An all-glass apparatus was built for making up the necessary atmospheres; this was designed so that oxygen and carbon dioxide can be absorbed, and the $^{15}N_2$ left after incubation used again in later experiments. ^{15}N assays were kindly done by A. C. D. Newman (Chemistry Department). The amount of $^{15}N_2$ taken up by intact, detached nodules from soyabeans increased by 50% when pyruvate was added to the fluid bathing the nodules. After 3 hours' incubation the supernatant fraction contained 10 times more ^{15}N than the bacteroid fraction. Preparations of crushed and filtered nodules incubated with atmospheres containing $^{15}N_2$ were also enriched with significant amounts of ^{15}N, though the uptake has so far been too small for convenient study. Such preparations are potentially more valuable than intact nodules for the study of the mechanism of nitrogen fixation, and further efforts will be made to promote their activity. (Cooper.)

The establishment of mycorrhizal infection under aseptic conditions

The establishment of mycorrhizal infections in aseptic culture is necessary to investigate critically their effect on root metabolism. Typical vesicular–arbuscular infections were established in clover seedlings grown aseptically in test-tubes, on an agar medium, on Fåhraeus slides and in water culture. Successful test plants include *Trifolium parviflorum*, *T. glomeratum*, *T. pratense*, *Dactylis glomeratum*, wheat, cucumber and onion, but no infections developed in four different strains of subterranean clover. Sterile seedlings were grown in an inorganic nutrient medium lacking nitrogen

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(Jensen's), and germinated spores of an Endogone species were used as the fungal inoculum. The fungus did not penetrate the roots unless some bacterial contaminant was also present. Two different species, thought to be *Pseudomonas*, were isolated in pure culture; both effectively mediated the changes necessary for root penetration. Neither appears to have any direct effect on growth of the *Endogone* sp. or on the germination of its resting spores. The mechanism of the bacterial effect is being investigated by the use of sterile filtrates, and of autoclaved and sonically disintegrated bacterial suspensions.

Under the above conditions, seedling roots were penetrated 8–12 days after inoculation with fungus and bacterium; temperature and light conditions suitable for normal seedling growth are necessary. Early infections are predominantly arbuscular, and vesicles do not develop abundantly till the seedlings begin to decline after 4–8 weeks growth in a nitrogen-deficient medium. Adding 0·05% of asparagine, urea, sulphate of ammonia or potassium nitrate inhibited root penetration even in the presence of bacteria, but a few test plants became infected after 12 weeks, when acute symptoms of nitrogen deficiency had developed. Once root penetration occurs the external mycelium is greatly stimulated and hyphae begin to grow into the agar. The agar becomes extensively colonised when vesicle production is greatest and the seedlings begin to decline. The fungus could not be subcultured, but produced mycorrhizal infections in fresh seedlings planted on infected agar slopes. Seedlings could also be infected by inoculation with infected roots. (Mosse.)

Infection with either of the two species of bacteria necessary to establish mycorrhizal infection also stimulated growth of the clover and grass seedlings in the nitrogen-deficient medium, even without the *Endogone* sp. Inoculation with bacteria gave darker green seedlings with more vigorous root systems. These seedlings contained more nitrogen than uninoculated controls, and the nitrogen content of the inoculated agar medium decreased. It is concluded that normally inaccessible nitrogen compounds, probably in the agar, were rapidly made available to the plant by the action of a small bacterial inoculum. Further studies are in progress on the mechanism of this action and on the subsequent development of mycorrhizal, bacterial and uninoculated test seedlings transplanted into autoclaved soil. (Mosse and Cooper.)

Mycorrhizal fungi in soil

The spread of mycorrhizal fungi in soil free from host roots was investigated by burying sporocarps of an *Endogone* sp. in an autoclaved soil. The soil layer containing the sporocarps remained infective after 12 months, but there was no evidence that the fungus had spread in the soil. Sporocarps similarly buried together with strawberry roots also failed to produce mycorrhizal infections in apple seedlings. Inoculation with the soil layer which had contained infected strawberry roots considerably depressed growth of the test seedlings. (Mosse.)

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Inoculation experiments with Pythium ultimum *and* Endogone *sp.*

An experiment with Dr. L. E. Hawker (Bristol University) designed to compare the incidence and anatomical appearance of mycorrhizal infection after inoculation with a culture of *Pythium ultimum* and with sporocarps of an *Endogone* sp. was repeated. Onion and apple seedlings were used as test plants. Mycorrhizal infections produced by inoculation with *Endogone* sporocarps showed a more extensive development of the external mycelium and of the arbuscular stage than those produced by inoculation with *Pythium*. The relatively high proportion of mycorrhizal infections which developed in the uninoculated control plants makes it difficult to draw any final conclusions, but the differences in anatomical details of the infections strongly suggest that both fungi can sometimes cause vesicular–arbuscular infections in the same host. The viability of sporocarps associated with test plants grown in two different autoclaved soils, a potting soil and a soil from Cleeve (Somerset), differed greatly. Sporocarps which had developed in the Cleeve soil contained very few viable resting spores, whereas those from the potting soil contained 40–90%. This observation may have some bearing on the prevalence of different species as endophytes in different localities.

The growth of the mycorrhizal and non-mycorrhizal seedlings differed greatly, the mean fresh weights of infected plants being two to three times as great. In onion, infection stimulated bulb production. (Mosse.)

Studies on Nitrosomonas

The results of the work on growing *Nitrosomonas* in continuous culture were published (**4·6**). Much time was spent in trying to improve this method of producing *Nitrosomonas* cells in quantity. The occasional failure of *Nitrosomonas* to grow in the culture apparatus is still puzzling, although it is now clear that efficient aeration is indispensable to successful continuous culture at high cell densities. The use of $NaHCO_3$ instead of Na_2CO_3, or adding CO_2 to the air supply, seem inessential. Preliminary attempts to prepare cell-free extracts able to oxidise NH_3 failed. (Walker and Skinner.)

By the use of Engel and Skallau's dilution technique, but employing a clear medium, two further strains of *Nitrosomonas* were isolated in presumed pure culture. One of these, from a sample of Rhodesian soil that had an unusually high Cu content, was motile. The other, isolated from a lateritic soil from the island of Korcula, at first appeared to be growing as a zooglea, but when isolated in pure culture was a feebly motile strain, somewhat bigger than the stock strain. (Walker.)

Cellulose decomposition and methane formation

Strains of anaerobic cellulose-decomposing bacteria that have been studied digest cellulose slowly and produce organic acids. The Rothamsted strain (*Rep. Rothamst. exp. Sta.* for 1959) forms enough formic and acetic acids to depress the pH of heavily buffered liquid cellulose medium from 7·0 to about 5·0 during fermentation.

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In nature, anaerobic cellulose digestion is often associated with the formation of methane and stabilisation of the pH at about 7·0.

Enrichment cultures of two strains of methane-forming bacteria able, respectively, to use formate and acetate as substrates, were prepared and partially purified, but so far it has been impossible to remove the remaining one or two species of bacterial contaminants. Media suitable for growing methane bacteria are not highly selective because the fatty-acid substrates are used by a variety of other micro-organisms. Some degree of selectivity is, however, imposed by the necessarily stringent anaerobic conditions, and by setting the pH initially at 7·0–7·4.

Methane bacteria appear to require for growth only a simple medium containing ammonia, carbonate and the organic substrate in mineral salts solution. A simple and convenient way of preparing this medium makes use of the apparatus designed to prepare and distribute anaerobic media for cellulolytic anaerobes (*Rep. Rothamst. exp. Sta.* for 1957).

The conversion of cellulose to methane was studied by the use of mixed cultures containing cellulose-decomposing and methane-forming bacteria. Mixed cultures were set up in liquid cellulose medium containing a little of the appropriate fatty acid and inoculated with methane formers so that they could become established before the cellulose-decomposing bacteria were introduced. Cellulose was digested with a steady evolution of methane over a period of months. The pH remained constant at about 7·0. Similar cultures without the methane bacteria quickly became acid and evolved little gas. The cellulose was not decomposed more quickly when methane bacteria were present.

A fermentation apparatus designed to grow these anaerobes under controlled conditions and to provide facilities for measuring pH and redox potential without disturbing the cultures is being built. (Skinner.)

At the request of the Sports Turf Research Institute (Bingley, Yorkshire) the appearance of sunken areas on the grass of a Surrey golf course was briefly studied. Sinkage seemed to be caused by bacterial decomposition, and consequent collapse, of a layer of organic matter 10–20 mm. below the soil surface. Recommendations were made to increase the mechanical strength of the organic layers by working sand into them and, in future, to top-dress with a mixture of organic matter and sand. (Skinner.)

Nitrification and nitrogen fixation in West African soils

The bacteria concerned in the nitrogen cycle were counted in Ghana forest and grassland soils. These two types of vegetation produce very different levels of soil fertility. The forest soils are very fertile, with a high content of exchangeable bases, organic matter and nitrogen. Grassland soils are much less fertile, and in particular lack available nitrogen. Jensen and Becking's methods were used to count nitrogen-fixing bacteria and a recently developed method to count nitrifying (ammonia-oxidising and nitrite-oxidising) bacteria.

The forest and grassland soils differ little in their content of nitrogen fixers; the numbers are generally high, and slightly higher

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in grassland soils. There are large differences in the numbers of nitrifiers; as long as soil cover is maintained, forest soils contain many ammonia- and nitrite-oxidisers, whereas grassland soils contain few ammonia-oxidisers and very few or no nitrite-oxidisers. It was particularly striking that none of the grassland samples taken after the start of the rainy season (in April) contained any nitrite-oxidisers. (Meiklejohn.)

Azotobacter *and plant growth*

Experiments on the effect of *Azotobacter* inoculation on plant growth continued, primarily with the object of determining the conditions for reproducibility between experiments. The principal

TABLE 1

Crop	Type of experiment	Treatments	% increase (+) or decrease (−) in yield from inoculation
1. Cress	Tube Nutrient agar	Seedling inoculation ± N supplied as KNO_3, $(NH_4)_2SO_4$, KNH_4NO_3 (14 treatments)	+13·0% sig.*
2. Radish	Tube Soil (Broadbalk 3, 2 1b and 7)	Inoculated vermiculite mixed with soil (6 treatments)	− 1·6% n.s. (Soil 3–21·0% sig.)
3. Radish	Pot Soil (Broadbalk 3, 2 1b and 7)	Soil surface inoculation + nutrient solution. High and low N (12 treatments)	−13·4% n.s.
4. Radish	Pot Soil (Broadbalk 3, 2 1b and 7)	Soil surface inoculation + nutrient solution. High and low N (12 treatments)	−11·5% n.s. (Soil 3. High N + 39·0% sig.)
5. Radish	Pot Potting compost J.I. No. 1 with soil from Great Field 4	Seed and surface inoculation with two Azotobacter strains (6 treatments)	− 4·4% n.s.
6. Radish	Pot Potting compost J.I. No. 1 with soil from Great Field 4	Seed inoculation (2 treatments)	+ 7·4% n.s.
7. Spinach	Pot Soil (Broadbalk 2 1b)	Surface inoculation. N.P.K., $MgSO_4$ and nutrient soil (2 treatments)	+28·5% sig.
8. Barley	Pot Soil (Broadbalk 2 1b)	Surface inoculation N.P.K. factorial and nutrient solution (16 treatments)	+40·1% sig.
9. Barley	Pot Soil (Broadbalk 2 1b)	Surface inoculation with 4 inocula, 3 levels of N (15 treatments)	− 3·47% n.s.
10. Barley	Pot Soil (Broadbalk 2 1b)	Surface inoculation with old and new inocula. N.P.K. + nutrient solution ± Mannitol (M). Moisture 100% and 60% W.H.C. (12 treatments)	− 0·98% n.s. (60% W.H.C. new inoc. − M + 23·7% sig. 60% W.H.C. new inoc. + M + 21·8% sig. 60% W.H.C. old inoc. + M + 23·2% sig.)
11. Spring wheat	Field Waterbeach Fen, Cambs.	Seed inoculation ± N (8 treatments)	− 2·68% n.s. Fresh wt. of grain
12. Spring wheat	Field Garden plots Rothamsted	Soil inoculation. 2 levels of N and no added N (6 treatments)	+ 6·7% n.s. Fresh wt. of grain. (No N plots + 18·0% sig.)
13. Sugar beet	Field Garden plots Rothamsted	Seed inoculation. 2 levels of N and no added N (6 treatments)	− 3·89% n.s. Fresh wt. of beet

* Significant level of probability 5% or less. (Unless otherwise stated figures are calculated from total dry weights of plants.)

factors investigated were the type of inoculum and method of inoculation and the influence of soil type, soil nitrogen level and soil moisture. Two methods of inoculation were used to establish artificially high levels of *Azotobacter* in the plant rhizosphere. In the first, a suspension of cells was applied to the soil after sowing;

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in the second, a cell suspension was sprayed with an atomiser on to the seed not more than 24 hours before sowing.

Although the conditions for reproducibility are not yet defined, enough has been done to show that *Azotobacter* inoculation sometimes affects plant growth. Table 1, which summarises all the experiments, shows the total effect of inoculation on plant yield for each experiment, except where significant effects were restricted to certain treatments, which are then shown separately.

No conclusions can yet be drawn about the factors affecting crop response to inoculation; for example, in experiment 8 yield was increased more when combined nitrogen was added, whereas in experiment 12 response to inoculation decreased with increasing applications of nitrogen. Establishment of *Azotobacter* in the rhizosphere was not the limiting factor, because this was achieved in some experiments in which no observable effects on plant growth were obtained. (Jackson, Brown, Burlingham and Barford.)

Methods of counting Azotobacter

In some inoculation experiments bacteria were counted in inoculated and uninoculated rhizospheres. Counts were made by the dilution plating technique and by serial two-fold dilutions with liquid medium, numbers being estimated by counting positive tubes and referring to tables of most probable number. Experiments were also made to improve counting methods and to determine sample variation and the natural distribution of this organism.

Sucrose and glucose in the plating medium gave about twice as many colonies as mannitol. This was not because some strains cannot use mannitol. Small but countable colonies of *Azotobacter* developed on dilution plates which contained no added carbon compounds; colony numbers were similar to those with glucose and sucrose.

The most probable number method gave results comparable with those obtained on dilution plates and saves labour and materials. However, results are sometimes obscured by the growth of nitrogen-fixing Clostridia. Efforts to obtain differential inhibition of Clostridia with antibiotics failed, as did the use of several different carbon compounds in the medium instead of mannitol. (Jackson, Brown and Burlingham.)

Distribution of Azotobacter *in soil*

The numbers of *Azotobacter* varied in samples taken at the same time within a single experimental plot. On Broadbalk plot 2, which receives dressings of FYM, the numbers varied in twelve separate samples from 258 to 56,000/g. dry weight soil. In a series of twelve samples taken from the upper 5 cm. of Park Grass plot 13 (limed section receiving alternate dressings of FYM and fish guano) the numbers of *Azotobacter* ranged from fewer than 12 to 298/g. dry weight soil.

Preliminary studies on the vertical distribution of *Azotobacter* showed increasing numbers down to a depth of 10 cm. in Park Grass plot 13. In a fertile garden soil (pH 6·8), *Azotobacter* was relatively abundant to a depth of 20 cm., but numbers rapidly decreased below this depth.

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The rhizospheres of uninoculated spring wheat, winter wheat and sugar beet grown in a clay loam soil (Rothamsted), or spring wheat grown in Fen soil (Waterbeach, Cambridge), did not contain significantly more *Azotobacter* than the non-rhizosphere parts of the soils.

The establishment of *Azotobacter* in the rhizosphere after inoculating the seed was studied in greatest detail with spring wheat grown in peat soil at Waterbeach. The seed was inoculated by spraying on the day of sowing; an average of 23,000 viable bacteria was recovered per seed. *Azotobacter* were counted on six occasions up to the 23rd week after sowing. The average number in the rhizosphere of inoculated plants throughout this period was 2,600/g. dry weight soil, compared with 227/g. for uninoculated plants and 171/g. for non-rhizosphere soil. Multiplication of *Azotobacter* in the rhizosphere during the growth of the wheat was indicated by the numbers in the rhizosphere being maintained throughout the sampling period, despite the fact that the root system was growing and so increasing the amount of rhizosphere soil per plant. This success in establishing *Azotobacter* is of special interest because of the negligible effect of the rhizosphere in the uninoculated plants. The *Azotobacter* on different parts of the roots of young wheat (5 weeks) were counted; roots within 2·5 cm. of the crown of the plant (the proximal fraction) had 30–100 times more *Azotobacter* than roots of uninoculated plants, the distal fraction 4–30 times more and the lateral roots 20–80 times more. Adventitious roots of plants examined when 8 weeks old had 4–9 times as many *Azotobacter* in the proximal fractions (including laterals) as in the distal fractions; *Azotobacter* on the whole root system of the inoculated plants were 70 times greater than in control soil. These results show how extensively the root system can be colonised from an original inoculum on the seed. (Jackson, Brown and Burlingham.)

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