ROTHAMSTED RESEARCH

Report for 1957

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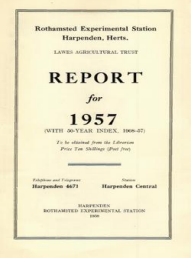

Soil Microbiology Department

P. S. Nutman

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

H. G. Thornton retired from the headship of the department on 1 October 1957. E. A. Peterson was awarded the Ph.D. degree of London University and returned to Ottawa in July. Mr. J. N. Parle arrived 1 September from Rukuhia Soil Research Station, New Zealand to work on the microbiology of the alimentary tract of soil animals and their excreta. R. Cooper joined the staff on 1 October to work on biochemical aspects of symbiotic nitrogen fixation. The following short-term visitors were also accommodated in the department during the year: J. Heidemeyer from the Technical University of Berlin to assist in chemical preparative work (6 months), I. B. Holland of Sheffield University for glasshouse work (6 weeks), H. D. L. Corby of the Grasslands Research Station, Marandellas, Southern Rhodesia (2 weeks), G. Muromtsev from the All Union Institute of Agricultural Microbiology, Moscow, for consultations and to learn techniques (6 weeks). F. A. Skinner attended the Society of Applied Bacteriologists symposium on the formation and germination of spores at the University of Leeds in July.

The work of the department has centred around two main topics: the ecology of soil micro-organisms and various aspects of the nitrogen cycle. In addition, work has continued on the chemistry and microbiology of the breakdown of chlorinated aromatic compounds in the soil and on anaerobic cellulose decomposition.

The free-living nitrogen-fixing bacteria (J. Meiklejohn)

Counts of Azotobacter on some of the Broadbalk plots were continued, and during the past season the following new results were obtained.

(i) Azotobacter numbers remained at a low level during the summer on the fallow section of plots receiving nitrogen (e.g., Plots 7, 10, 11 and 15); on the no-nitrogen plots 3 and 5 the numbers on the fallow section were rather higher. Plots 17 and 18, which receive nitrogen and minerals in alternate years, had more Azotobacter during fallow than any of the other plots sampled.

(ii) The sections carrying the first wheat crop after fallow all showed an increase in Azotobacter numbers through the winter, with a peak in spring or summer, which was most marked in those plots which had nitrogen.

(iii) During March and April 1957, the top half of section I of the field, which is now under continuous wheat cropping, began to show signs of nitrogen deficiency. Samples were taken from the top halves of two of the worst affected plots, 9 and 18; these were found to have 220 and 266 Azotobacter per gram of soil, whereas the lower halves of the same plots, carrying their first crop of wheat after fallow, had 440 and 499 Azotobacter per gram respectively.

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Counts have been made on some grass fields; the grass half of Broadbalk Wilderness has a large Azotobacter population, but the new grass on the irrigation experiment at Woburn had none when a count was made in the spring, and very few in the autumn; it is proposed to continue these counts to see if the Azotobacter population will build up.

Work has continued, so far without success, to develop a better method of counting nitrogen-fixing anaerobes in soil.

The nitrifying bacteria (J. Meiklejohn and N. Walker)

It is now possible to obtain in about 10 days in a clear medium which permits good growth, a Nitrosomonas culture which can be used for physiological studies. It was found that sodium bicarbonate could replace calcium carbonate as a source of CO_2, and the number of trace elements in the medium has been reduced to five without disadvantage. Organisms grown in this medium were active in oxidizing ammonia or hydroxylamine to nitrite in Warburg respirometers. It is hoped to improve the medium still more, so that it can be used in a continuous culture apparatus.

Cultures of Nitrobacter are in process of being purified, and when this is done the best medium for them will be worked out.

Studies in symbiotic nitrogen fixation (P. S. Nutman)

Work has been resumed, after an interval of 3 years, on the genetics of symbiotic nitrogen fixation in red clover. Selection and breeding work is in progress with a number of pedigrees containing new types of segregant which are defective in fixing nitrogen in association with a normal effective strain of bacteria.

In connection with studies on root-hair infection, detailed below, twelve very small seeded species of *Trifolium* and two of *Lotus* have been grown for seed and opportunity taken to determine their symbiotic responses with four strains of bacteria. The results indicate that all the species of *Trifolium* fall into one or other of the three major groups of response proposed by Vincent. Also included in current tests were two ecotypes of *Trifolium ambiguum* and strains of nodule bacteria from the Caucasus sent through the kind offices of A. Imschimski. These hosts were shown to respond more effectively with *Rh. trifolii* of Turkish origin than with the local strains. With all strains a proportion of plants of both ecotypes failed to nodulate.

The initial infection of the clover root by nodule bacteria (P. S. Nutman)

The work, earlier reported, has been extended to include all the small seeded species mentioned above, in each of which early infection has been shown to take place in two distinct phases. In the first the number of infected hairs increase at a rapid rate; in the second the increase is negligible. More detailed study with the most suitable species from the observational point of view (viz., *Trifolium repens* and *T. fragiferum*) has disclosed that the increase in number of infected root hairs is exponential in both phases, the ratio of the indices to the exponential ($\log_{10}$ number infection/hour) changing from about 0·021 to 0·00045, the change in rate occurring at the time the first nodule is produced. Work is in progress to determine

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the influence of bacterial strain upon infection rate, after which the study will be developed along experimental lines to elucidate the physiology of the early stages of infection.

The routes of infection in hosts of the Cowpea cross-inoculation group (A. H. Gibson)

Infection by avenues other than the root hair has been recorded for some members of the cowpea cross-inoculation group. These and other species are being reinvestigated by serial microtomy to determine the mode of infection, the site of nodule initiation and nodule development. Preliminary results are summarized in the following table. Data for a member of the *Trifolium* cross-inoculation group are included for comparison.

Species	Presence of infected root hairs	Nodule position *	Site of nodule initiation	Mode of spread of bacteria in nodule tissue
Aeshynoneme americana	+?	Mostly A	Outer cortex of young lateral roots	Cell division
Crotalaria brownii	—	A and R	Outer cortex	Cell division
Desmodium nicaraguensis	+	Mostly R	Outer cortex	Cell division and inf. threads
Desmodium ovalifolium	+	A and R	Outer cortex	Cell division and inf. threads
Dichrostachys spicata	+	Mostly R	Outer cortex	Cell division
Indigofera endecaphylla	+	A and R	Outer cortex	Cell division and inf. threads
Stylosanthes gracilis	.	Mostly A	.	Cell division only?
Trifolium subterraneum	+	A and R	Inner cortex	Infection thread

* A, axilliary position at the point of emergence of a lateral root.
R, elsewhere on main or lateral roots.

The stimulation of infection by root secretions (A. H. Gibson)

The extension of work in this field has been hampered by the failure to obtain reproducible results. This has been shown to be due partly to the environment. The time at which the first nodules appear on the roots is correlated both with the sunlight in the previous 24–48-hour period and the minimum temperature in the preceding 24 hours. Further analysis of the effect of root secretion will require controlled environment facilities.

The co-existence of Rhizobium trifolii and its bacteriophage in artificial soil mixtures (J. Kleczkowski)

Bacteriophage active against *Rh. trifolii* occurs in all soils in which the host plant has been grown for more than one season, and the clover nodule bacterial themselves are present in all normal soils in the United Kingdom. It is necessary therefore first to free soil of phage and its host bacteria before experimental work on their interrelations can be undertaken in soil. This has not been found possible without seriously affecting the soil, so that recourse has been made to an artificial soil of sand and vermiculite to which was added dilute soil extract or Demolon and Dunez medium.

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Effective and ineffective strains of clover nodule bacteria with their corresponding phages were inoculated into artificial soil and the phage and bacteria assayed over the following three months. Immediately after inoculation the phage increased and the bacterial numbers dropped. At about 2 weeks phage-resistant bacteria appeared and increased until an equilibrium was established.

At 6 weeks and at the end of the experiment the phage and bacteria were re-isolated and examined.

A proportion of the phage isolates were found to have changed in plaque type during storage in artificial soil. These changes were temporary, the normal plaque form reappearing after five or six bacterial passages.

A large number of bacteria isolated after storage in presence of phage differed from original strains in phage-resistance and in symbiotic properties. The ineffective strain "Coryn" gave rise to variants which were partly effective; some host plants inoculated with them responding effectively and others ineffectively. Repeated re-isolation from either type of responding host failed to stabilize the strain's symbiotic properties. As reported earlier, the effective strains gave rise to ineffective mutants which were stable. Further work will be directed towards elucidating the nature of the changes in phage and bacteria.

The streptomycin-resistant bacteria of the rhizosphere (M. E. Brown)

Studies on the stimulation of streptomycin-resistant bacteria in the rhizosphere of clover plants have continued. This stimulation was confirmed in the rhizosphere of nine other leguminous species; and was absent in the rhizospheres of nine non-leguminous plants. Leguminous stimulation occurred in each of the four soil types tested.

The following substances were added to the soil in an attempt to reproduce the stimulation: four amino-acids, either singly or in various combinations, two growth factors, six nucleic acid derivatives and three sugars. Of these only two nucleic acid derivatives, guanine and guanosine, and two sugars, laevulose and ribose, consistently increased numbers of streptomycin-resistant bacteria.

Experiments with root exudate gave inconsistent results, and further work is in progress to improve the method of collecting root exudate.

The possibility that the increase in streptomycin-resistant bacteria may be the result of a stimulation of actinomycetes in the rhizosphere was studied. A number of actinomycetes were isolated both from the soil and the rhizosphere and tested by the streak method against resistant and non-resistant bacteria from the rhizosphere and from the soil. The actinomycetes had no effect on the streptomycin-resistant bacteria, but antagonized all the non-streptomycin-resistant bacteria, indicating a possible connection and interaction between the two groups of organisms. It was found, however, that substances which stimulated the actinomycetes in the soil did not also stimulate the resistant bacteria, and substances stimulating the bacteria did not stimulate the actinomycetes.

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Life-cycle of Nocardia in soil (M. E. Brown)

The study of the life-cycle of *Nocardia cellulans* in the soil was completed. Experiments were made in partially and totally sterilized and in untreated soil. In partially sterilized soil wide fluctuations in numbers of *Nocardia* occurred during the first month following inoculation of the soil with a pure culture of *Nocardia cellulans*. These fluctuations became less marked, and at the end of a year the *Nocardia* was still present in high numbers. The numbers of bacteria in such a soil were depressed in comparison with uninoculated control. *Nocardia* disappeared from the untreated soil in 6 months. The wide fluctuations in numbers in partially sterilized soil were correlated with a regular alternation between the rod and mycelial forms of *Nocardia*. Peak counts corresponded to the rod form and troughs to the mycelial form and the steadying in count to the rod form. On an agar medium the organism went from the rod stage to the mycelial stage and then back to the rod, staying in this last form until re-inoculated on to a fresh medium.

Fungistasis in soil (R. M. Jackson)

The effect of pH on the fungistatic activity of soil has been studied in Park Grass plots. A close correlation between soil pH and fungistasis was found; least inhibition of germination of the test fungus was produced by soil from the more acid plots and most inhibition by the less acid plots.

The evidence obtained from soil sterilization experiments strongly suggests that aerobic spore-forming bacteria may be responsible for natural soil fungistasis. Examination of two series of spore-formers isolated from pasteurized soil showed that while twenty-one out of twenty-three of the *Bacillus mycoides* type isolates inhibited germination of the test fungus on agar plates, only fourteen out of seventy-eight of the other isolates had a similar effect. The cell-free filtrate of cultures of one of the *B. mycoides* isolates produced a spectrum of inhibition of the test series of fungi similar to that of fresh soil.

Two techniques have been used to study the interaction of soil fungistasis and the rhizosphere effect. In the first, glass plates were coated with a fungal-spore suspension in agar and buried at an angle of 45° beneath germinating seeds. The plates were removed at intervals and the effects of the seedling roots which had grown into contact with the plates observed. In the other method direct observations with the Leitz Ultropak were made upon the behaviour of fungal spores in contact with fresh soil through which seedling roots were growing in small glass cells. Pea roots stimulated the germination of inhibited conidia of *Gliocladium roseum* and *Paecilomyces marquandii* and also chlamydospores of a species of *Fusarium*, the formation of which had been induced by soil. The germ tubes of *G. roseum* and the *Fusarium* showed a pronounced tropic growth towards the roots. In experiments with radish seedlings, germination of conidia of *G. roseum* and *P. marquandii* in the rhizosphere was observed to be followed by a period of vegetative growth and finally sporulation. Efforts are now being made to

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obtain root exudates which show activity in counteracting soil fungistasis.

Bacterial decomposition of herbicides (N. Walker and T. I. Steenson)

Work on the persistence of herbicide-decomposing micro-organisms in soil, carried out in collaboration with Messrs Sutton & Sons, has been completed and the results published.

The adaptation of bacteria to oxidize 2 : 4-D and MCPA has been studied further. It has been shown that adapted organisms may be obtained by growth on peptone agar containing a suitable concentration of the substance to be studied; it is not essential therefore for the herbicide to be the main source of carbon in the medium. Because of this finding, it was possible to examine the effects of related compounds on adaptation, irrespective of whether they were metabolized by the bacteria or not. By growing *Flavobacterium peregrinum* in presence of MCPA or the isomeric 2-chloro-4-methylphenoxyacetic acid, organisms were obtained which were adapted to oxidize 2 : 4-D, but not MCPA. Various related compounds did not induce adaptation to 2 : 4-D. Growth of bacteria in presence of 2 : 4-dichlorophenol or 5-chloro-2-cresol caused adaptation to these two phenols, but not to 2 : 4-D nor to MCPA.

The isolation of anaerobic cellulose-decomposing organisms (F. A. Skinner)

The anaerobic cellulose-decomposing bacteria from soils and organic wastes are responsible for the initial stages of the bacterial degradation of cellulose, a process which results finally in the production of gas rich in methane. The investigation this year has been concerned mainly with the improvement of techniques for isolation.

The original technique, similar to that used by other workers for isolating anaerobic cellulolytic bacteria from the rumen of herbivores, involved the preparation of a sterile reduced medium which is transferred aseptically and without ingress of air to the culture vessels. Satisfactory cultures were rarely obtained by this method. An improved method which has been devised consists of preparing the medium, in a specially stoppered flask, boiling to expel air and passing in oxygen-free nitrogen both before and after the addition of a reducing agent such as cysteine hydrochloride. With a movable spring-loaded delivery tube, fully reduced medium can be transferred without admitting air, to small screw-capped culture bottles. After filling, caps are screwed on tightly and the sealed bottles sterilized. When a bottle is opened for inoculation or sampling, it is flushed with sterile nitrogen. With this technique and the McIntosh and Fildes jar, a pure culture of a spore-forming cellulose-decomposing bacterium was obtained, and its characteristics are being studied. A low concentration of yeast extract provides all the accessory factors necessary for fairly rapid growth in liquid medium. Several contaminant species of bacteria isolated from impure cultures have been tested with the cellulose-decomposing isolate, none of which has any stimulating effect on the isolate's growth or cellulolytic power. Growth in cellulose–agar is very slow. Possible reasons for this are poor dispersion of cellulose particles,

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the need for more strongly reducing conditions, or presence of inhibitors in the agar. Studies have continued on the ability of sodium carboxymethyl cellulose to prevent flocculation of the cellulose particles in agar media, and on the oxidation–reduction potentials developed by different reducing agents.

Enrichment cultures of anaerobic bacteria capable of converting various organic compounds to methane have been prepared, and the purification of these strains is in progress.

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