ROTHAMSTED RESEARCH

Report for 1956

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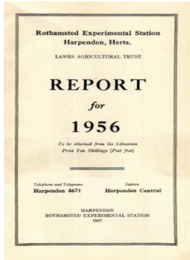
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Soil Microbiology Department

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SOIL MICROBIOLOGY DEPARTMENT

H. G. THORNTON

P. S. Nutman returned on 2 July from Canberra, Australia, where he had spent three years on secondment to the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra.

Mr. R. M. Jackson, Lecturer in Plant Pathology, University College, Ibadan, Nigeria, joined the scientific staff of the Department on 1 January 1956.

Mr. Allan Gibson, from the Division of Plant Industry, C.S.I.R.O., Canberra, Australia, arrived on 16 August 1956 to do research on problems connected with nodule bacteria and legumes.

Mr. J. Steffens, from Berlin University, was here from 5 April till 14 August 1956 helping Norman Walker in the synthesis of aromatic compounds needed in the latter's work.

Mr. Iver Berlier, from Abidjan, French West Africa, was here from 17 September till 26 October 1956.

Recent developments in soil microbiology, together with the work of plant pathologists concerning root disease organisms, emphasizes the urgent need for more information on the ecological factors in soil that encourage or control the growth of specific groups of micro-organisms therein. For this reason a considerable part of the Department's work has dealt with subjects related to this general problem.

THE FUNGISTATIC ACTION OF FRESH SOIL

There is increasing evidence that some of the antibiotics produced by specific organisms are active in soil, but there is also evidence that the fresh soil contains a substance or substances destroyed by heating that inhibit the germination of spores of a number of fungi introduced into the soil. The origin of this fungistasis is not yet known, but it is likely to be a factor of importance in its effect on plant-disease fungi, as well as on the balance of the micropopulation as a whole. R. M. Jackson has found that soils from differently manured plots on Broadbalk differ in the degree to which they inhibit germination of *Penicillium* conidia. Species of soil fungi also show very different degrees of sensitivity to this fungistasis. A range of fungi having varied sensitivities to Broadbalk soil showed the same relative sensitivities in six out of seven soils of widely different characters, but behaved differently in one very acid soil. The fungistatic action of soil may be overcome or reduced by the presence of certain sugars, and in some cases by the near presence of plant roots. These effects are being further investigated.

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The Establishment of Organisms in Partially Sterilized Soil

The possibility of controlling root-disease organisms by introducing antagonists into the soil depends in part on whether such antagonists can be successfully established in soil when introduced therein. We know very little about the soil conditions or about the qualities in the organisms themselves that are necessary for successful establishment. It is well known, however, that some organisms spread rapidly through partially sterilized soil, perhaps because of the reduced competition with the soil population. Margaret Brown has carried out experiments with three micro-organisms each introduced into soil that has been steamed for 1 hour. The fate of the introduced organism was followed in each case by platings at regular intervals.

The first organism, *Azotobacter chroococcum*, disappeared from the soil in 31 days. The second, *Chromobacterium prodigiosum*, was successfully established, possibly assisted by the production of an antibiotic. The third organism, a Nocardia, was successfully established both in Rothamsted and in Woburn soil, in both of which it showed fluctuations in the plate count apparently related to repeated changes from mycelial to rod stage, and *vice versa*.

The Micropopulation of the Root Surroundings

The types of fungi that predominate on the root surface, in the immediately adjacent soil (rhizosphere) and in soil at a distance from the root (non-rhizosphere soil) have been further surveyed by E. A. Peterson. He has grown wheat and clover in two different soils and examined samples at intervals from the date of sowing. Similar results were obtained in both soils. More fungi were found in the rhizosphere than in non-rhizosphere soil, the disparity increasing with the age of the plants. Different fungi became predominant in the rhizosphere at different ages of the plant.

The root surface carried a quite distinct fungal population, in which Fusarium and a sterile mycelium were very predominant.

The types of fungi found in healthy Woburn soil and in soil rendered " clover sick " in the pot experiments of H. H. Mann and T. W. Barnes were also examined. A comparison was also made of the microflora of the rhizospheres of clover plants grown in " sick " and in healthy soil respectively. Some fungi were found to be relatively more abundant in the " sick " soil or rhizosphere, and these were tested for pathogenicity to clover growing in pots of healthy Woburn soil. None of them harmfully affected the clover growth. Sick clover plants recovered when transplanted into healthy Woburn soil. This result, together with the finding that clover grew normally in " sick " soil to which 10 per cent finely divided animal charcoal had been added, suggests the action of some toxic substances, although so far attempts to extract any such substance from sick soil have been unsuccessful. The remedial effects of charcoal were previously noticed by Mann and Barnes.

Platings of rhizosphere soil made by Margaret Brown and E. A. Peterson on media containing antibiotics have revealed the existence

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therein of bacteria resistant to streptomycin and to chloromycetin. Streptomycin-resistant organisms have been found to be much more abundant in rhizosphere than in non-rhizosphere soil, particularly with older plants and those growing in clover-sick soil. Aureomycin added to the plating medium effectively controlled bacterial growth without apparent effect on the development of fungal colonies.

The Microflora of Soils after Treatment with Trichloracetic Acid

Jane Meiklejohn and R. M. Jackson examined the microflora from two of the plots on Stackyard Field at Woburn three weeks after treatment with Trichloracetic at 20 lb./acre, and compared the result with those from untreated soil from the same plots. Microscopic counts of soil bacteria, plate counts of bacteria, actinomycetes and fungi, and tests for the presence of nitrifying and nitrogen-fixing organisms showed no significant differences between treated and the corresponding untreated soil.

Decomposition of Halogenated Phenoxyacetic Acids by Soil Bacteria

The metabolism of 2 : 4-dichlorophenoxyacetic acid (2 : 4D) and of 4-chloro-2-methylphenoxyacetic acid (MCPA) by a strain of *Flavobacterium peregrinum* and by an Achromobacter species has been studied by N. Walker and T. I. Steenson. It was found that cells from young cultures were more active than those from older cultures in oxidizing these substrates. Using such young cells, evidence from manometric experiments indicates that one or other of these organisms dissimilate 2 : 4-D through 2 : 4-dichlorophenol and 4-chlorocatechol, and MCPA through 5-chloro-2-cresol. Cells adapted to 2 : 4-D would also oxidize 2 : 4-dibromo- and 4-bromo-2-chlorophenoxyacetic acids and their corresponding phenols, but would not oxidize any of the isomeric dichlorophenoxyacetic acids except 2 : 4-D. Mr. J. Steffens assisted in the synthesis of a number of aromatic compounds required in this work.

The Numbers of Azotobacter Cells in Broadbalk Soils

The Broadbalk plots, with their fallowed and cropped sections, afford an unusually good opportunity to survey non-symbiotic nitrogen-fixing organisms and to relate their numbers to crop yield.

Jane Meiklejohn has continued a survey of the estimated numbers of Azotobacter in eight of the Broadbalk plots sampled at intervals, on each occasion including fallow sections and those carrying the fourth successive crop. The estimated numbers of Azotobacter cells was found to be very small, seldom exceeding 1,000 per gram of soil. In all comparisons between plots with and without nitrogen dressings, the latter gave the higher numbers of Azotobacter cells. Thus Plot 3 gave higher numbers than Plot 10, Plot 5 than Plot 7, and Plot 18 than Plot 17, which received a nitrogen dressing this season. Plot 13, which receives potash as well as nitrogen and

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phosphate, gave consistently higher Azotobacter numbers than Plot 11, which has nitrogen and phosphate but no potash.

Other Nitrogen-fixing Organisms

The small numbers of Azotobacter in our soils suggests a search for other non-symbiotic nitrogen-fixing organisms. From soil platings in a nitrogen deficient medium Margaret Brown has isolated several organisms other than Azotobacter that seem able to fix small amounts of nitrogen. The most efficient of these is a black fungus that fixed 4–5 mg. of nitrogen per g. of glucose oxidized.

The Anaerobic Decomposition of Cellulose

Reducing processes in the soil may be of great importance, e.g., in nitrogen fixation and in the reduction of insoluble oxides. In the supply of energy for these processes the anaerobic decomposition of cellulose may be expected to be of first importance. But we have insufficient knowledge about the bacteriology or biochemistry of this breakdown. This is largely due to the difficulty of isolating the organisms. F. A. Skinner has begun an investigation on the isolation and activities of these anaerobic cellulose bacteria. In cellulose–agar cultures derived from a soil-enrichment culture, two types of cellulose-attacking colonies were found: (1) punctiform colonies, which were usually embedded in the medium, and (2) thin surface colonies, whose margins extended rapidly to form large actively cellulolytic areas. Both types of colony consisted of spore-forming, rod-shaped bacteria. Surface growth has been found to be contaminated with other types of spore-forming bacteria very similar in appearance to the cellulose decomposers. These contaminants are being studied, as they may well have a marked stimulatory action on cellulose decomposition. A feature of particular interest shown by the cellulose–agar cultures is the extremely long period which intervenes between inoculation and the development of recognizable cellulose-decomposing colonies. A delay of two weeks is common, and on one occasion a delay of seven weeks was recorded.

Growth of the cellulose-decomposing bacteria has not taken place in any medium with an oxidation–reduction potential more positive than about -30 mV.

One difficulty encountered in the preparation of cellulose–agar medium is that of keeping the finely-divided cellulose in suspension until the molten agar sets. The cellulose flocculates rapidly in the presence of electrolytes, which are essential ingredients of the media. This flocculation has been prevented by the inclusion of quite low concentrations of the water-soluble sodium carboxymethyl-cellulose.

The Infection of the Clover Root by Nodule Bacteria (Rhizobium)

Genetical and physiological studies by P. S. Nutman, detailed in previous reports, indicate that nodules are not formed at random on the roots but occur at certain points only.

Using a microscopic technique devised by Dr. Fahraeus (Uppsala)

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for the continuous observation of root hairs on young growing seedlings, the infection of the root hairs of clover has been investigated. The following results were found :

1. Root-hair infection is very infrequent in *Trifolium pratense* and *T. subterranean*, about 10 × more frequent in *T. ornithopodioides* and about 100 × more frequent in *T. repens* and *T. fragiferum*. Other species are being investigated. The following results refer to *T. repens*.
2. In confirmation of the genetical and physiological results, it has been observed that infected root hairs are distributed in zones; these generally increase in size as the seedling ages.
3. The numbers of infected root hairs increase with time until the first nodule is formed, when they cease abruptly. At a later stage infection recommences.
4. In confirmation of Fahraeus' observations it has been found that the point of infection of the hair may be terminal or on a lateral outgrowth of the hair, and with rare exceptions is initiated in a region of deformed growth.
5. Root-hair infection appears to be stimulated by the near presence of another seedling.

The Presence and Behaviour of Rhizobium Bacteriophage in Soil and in Other Media

Janina Kleczkowska has studied the distribution of bacteriophage attacking *Rhizobium trifolii* in the field. It was only found in soil that carried clover. The strains of phage isolated were found to vary very much in the number of different strains of Rhizobium that they could attack. Some attacked 90 per cent of the strains on which they were tested. The majority of these test strains were isolated from the nodules of plants growing in the same soils from which the phages were isolated. So that phage and Rhizobium susceptible to it can coexist in a field soil.

In liquid media, on the other hand, the presence of phage and of susceptible bacteria commonly results in the elimination of the latter and in the growth of resistant variant forms of the bacteria.

In a granular medium composed of sand and vermiculite and with low nutrient supply, both phage and susceptible bacteria were found able to coexist. When the phage was present, however, variant forms of the bacteria appeared. Some of these variants differed from the parent in their effectiveness in fixing nitrogen in the host plant.

Thus the presence of phage in the soil, while not eliminating the Rhizobium, may change its behaviour towards the host legume.