

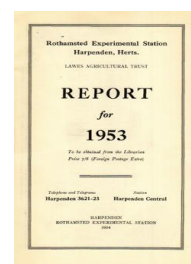
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## Report for 1953

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### Soil Microbiology Department

**H. G. Thornton**

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## SOIL MICROBIOLOGY DEPARTMENT

H. G. THORNTON

Miss Jane Meiklejohn returned to the department in September after spending a year in East Africa on an appointment from the Colonial Office to report on problems of soil microbiology.

P. S. Nutman left in May to take up a three-year appointment at the Commonwealth Scientific and Industrial Research Organization, Canberra.

B. N. Singh left in November 1952 to take a post at the Central Drug Research Institute, Lucknow.

Miss Hilary Purchase left in September to return to Sydney after three years work in the Department, for which she was awarded the Ph.D degree, London University.

T. I. Steenson from Canterbury Agricultural College, Christchurch, joined the department in March, and has been appointed to a post as Assistant Experimental Officer.

I. L. Stevenson from the Department of Agriculture, Ottawa, came to Rothamsted in June for a period of two years.

Miss U. de Barjac from the Pasteur Institute, Paris, paid a visit of six weeks to the department.

The work of the department has been concerned with the competition between actinomycetes and root pathogenic fungi, the influence of partial sterilization on the microflora in forest nursery beds, the decomposition of chlorinated aromatic compounds by soil bacteria and with various problems relating to the nodule bacteria of leguminous plants.

### ANTIBIOTIC PRODUCTION BY ACTINOMYCETES IN SOIL

The usefulness of actinomycetes that produce antibiotics in combating root infecting fungi is limited by the fact that the antibiotics produced by them tend to be adsorbed and rendered inactive by soil constituents, and especially by the clay fraction. It is thus important to know which actinomycete antibiotics are least affected in this way and how active these are in different types of soil. I. L. Stevenson is studying ten actinomycetes selected for activity against root disease fungi, particularly *Helminthosporium sativum*, and the influence of clay minerals, soil type and soil reaction on the production by them of antibiotics. In media, differences in reaction influenced the production of antibiotic by a few only of the actinomycetes tested. The addition of bentonite greatly reduced antibiotic activity, but kaolin had little or no effect. The addition of soil to agar media also reduced activity, but this varied with the soil type and with the species of actinomycete. Those species whose antibiotic properties in agar were least affected by the addition of soil also showed the most consistent antibiotic activity when grown in the soil itself. Their activity in soil was also affected by soil type and reaction. Four of the actinomycete strains tested were identified as *Streptomyces antibioticus*, and the antibiotic

produced in soil was extracted and gave the chemical tests for Actinomycin. Pot experiments with wheat in several sterilized soils confirmed that root rot by *Helminthosporium sativum* was significantly reduced by some of the actinomycete strains, and this reduction was in general related to the activity of the strains *in vitro*.

#### THE EFFECT ON NUTRIENT SUPPLY ON COMPETITION BETWEEN ACTINOMYCETES AND FUNGI IN SOIL

In his study of competition between an actinomycete and the root pathogen *Fusarium culmorum*, F. A. Skinner showed that the actinomycete reduced growth of the fungus more in media that were rich than in those low in energy supply. This was the case whether antagonism was attributable to antibiotic action or to competition for some available nutrient. This effect of energy supply on competition may also be important where it is desired to introduce and establish an organism, for example one that is antagonistic to a root pathogen, in fresh soil in competition with the natural micropopulation. An investigation is therefore being made of the effects of energy supply on the establishment of organisms, including some that are antagonistic to fungal pathogens, in fresh soil and in sterilized soil to which other organisms have been added. This work is in progress.

#### PARTIAL STERILIZATION

The survey of changes in the soil micropopulation of forest nursery plots that have been given various forms of partial sterilization has been carried out for several years, and was continued by Miss L. M. Crump during the period under review. The results are now being collected. These surveys have been carried out on plots laid out by the Chemistry Department as part of their work on forest nurseries.

#### THE DECOMPOSITION OF CHLORINATED AROMATIC COMPOUNDS BY SOIL BACTERIA

The use of chlorinated aromatic compounds in soil makes it important to know the effect of micro-organisms on them and whether a population of organisms capable of attacking such a compound will be built up if it is added to the soil. Studies are being made with chloro- and bromo-naphthalene and with 2:4-dichlorophenoxyacetic acid and compounds related to it.

In the case of  $\alpha$ -chloronaphthalene several soil bacteria have been found capable of attacking the compound, and the course of breakdown is being investigated. The organism that has been mainly studied produces a chloronaphthalene diol. N. Walker, in collaboration with G. H. Wiltshire of the Biochemistry Department, has produced evidence indicating that this diol is 8-chloro-1:2-dihydro-1:2-dihydroxynaphthalene. A later decomposition product appears to be 3-chlorosalicylic acid, but the intermediate breakdown products are not yet determined. The course of decomposition of  $\alpha$ -bromonaphthalene seems to follow an analogous course through the diol to 3-bromosalicylic acid.

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In the case of 2:4-dichlorophenoxyacetic acid, a strain of Flavobacterium has been isolated that will decompose this compound in pure culture, both in sterilized soil and in laboratory media, in which it can utilize it as the sole energy source. In soil, breakdown occurs only under aerobic conditions. Washed cells of the organism grown on a medium containing 2.4.D., will absorb oxygen in a Warburg vessel when supplied with this compound as substrate. There is no such oxygen uptake with 2:4-dichlorophenol as a substrate. In liquid media with 2.4.D., ionic chlorine is liberated. The Flavobacterium will also utilize parachlorophenoxyacetic acid, but will not utilize either orthochlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid. Attempts are being made by T. I. Steenson to isolate other organisms that can attack various chlorophenoxyacetic compounds. This work is in progress.

#### NODULE BACTERIA AND LEGUMINOUS PLANTS

##### (1) *The influence of hereditary factors in the clover plant on nodulation*

For some years past P. S. Nutman has conducted a series of genetical studies to determine the influence of hereditary factors in the clover plant on the time of appearance, number and effectiveness of nodules. He has continued the study of these factors, and is collating his results for publication. Hereditary factors influence the time at which nodules first appear, the number of nodules subsequently produced and the effectiveness of the nodules. For all these characters there are factors showing a complex heredity, that act independently of what bacterial strain is applied. In the case of nodule numbers and effectiveness there are also factors associated with simple recessive genes. One of these causes the production of numerous ineffective nodules with a normally effective bacterial strain. There is evidence of the existence of a second gene with similar effects. Both these genes show their influence with one particular bacterial strain only. A third gene has been tentatively identified that produces ineffective nodules which, however, are larger and less numerous. This appears not to be specific in its action with a particular strain of bacteria. Modifying factors also exist which can reverse the effect at least of the first gene, while the bacterial strain with which it shows its effect has given rise to mutants which restore the effective response. There is thus a very complex set of genetically controlled interactions which determine the number and effectiveness of nodules on the plant.

##### (2) *Nodule bacteria in the rhizosphere*

Miss Hilary Purchase continued her work on this subject, which, as previous reported, had shown that the population of nodule bacteria in the root surroundings of clover, even when it arose from a small inoculum, soon attained numbers far in excess of those required for maximum nodulation and independent of whether the plant was of a type that produced many or few nodules or was completely resistant to infection. The number of nodules produced must therefore be limited by some factor operating on the actual process of infection. Infection of the root is known to take place through the root hairs. In further work she has shown that, in

clover, the number of infected root hairs is related to the number of nodules finally formed, so that nodule numbers would seem to be determined by root-hair infection and not at some later stage of nodule development, as has been claimed in the case of lucerne.

While the growth of nodule bacteria when in pure culture in the root surroundings may not affect the number of nodules, competition between different strains outside the plant may well be of importance. In her rhizosphere studies Miss Purchase found that acute competition occurred between a strain and a mutant of that strain that was incapable of forming nodules, but that no such competition occurred between two strains of nodule bacteria, one from clover and one from lucerne. This finding has a bearing on the general problem of competition between micro-organisms, since it suggests that closely related strains may compete more acutely than those more distantly related. It also makes it unlikely that nodulation of a legume crop will be adversely affected by the previous growth of a different legume crop on the same land.

#### NODULE BACTERIA IN ACID SOIL

Previous work has shown that clover nodule bacteria of an effective strain could produce completely ineffective forms when grown in sterilized soil. It has been found that soil type, and particularly its reaction, affects the production of such ineffective forms, which can sometimes be prevented by liming. A study is being made by Janina Kleczkowska of the survival and genetic stability of clover nodule bacteria in various soils and agar media at different pH values with and without calcium.

#### BACTERIOPHAGE ATTACKING NODULE BACTERIA (*RHIZOBIUM*)

The influence of the enzymes Ribonuclease and Chymotrypsin on the interaction of *Bacteriophage* and *Rhizobium* has been made by Drs. Janina and A. Kleczkowski. The two enzymes behave differently, although both check phage multiplication, but neither affects the phage in the absence of bacteria. Ribonuclease interferes with the combination of phage with bacteria. Chymotrypsin does not affect this, but seems to act on the freshly formed phage-bacteria combination, making the phage inactive, but leaving the bacteria to multiply normally. The two enzymes differ also in the action of the bacteria without phage, ribonuclease seriously affects multiplication, whereas chymotrypsin has little effect on the bacteria.