ROTHAMSTED RESEARCH

Report for 1949

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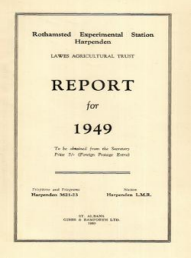

Soil Microbiology Department

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SOIL MICROBIOLOGY DEPARTMENT

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WORK ON THE SOIL MICROPOPULATION

Relation of microscope to plate counts of microorganisms in soil

The development of a statistically satisfactory method for counting bacterial cells in soil by microscope counts has raised an issue of fundamental importance to soil microbiology, owing to the finding that microscope counts give estimated numbers many times higher than plate counts, which suggests the possibility that only a small fraction of the soil's bacterial flora has been studied by platings and by isolations from plate colonies. The application of this microscope method to the estimation of fungal mycelium in soil provides the only existing method of assessing the quantity of fungal material present in soil as mycelium, so that here also a comparison of such estimates with plate counts becomes important.

With regard to bacteria it seemed desirable in the first instance to obtain data as to the ratios of microscope to plate counts from a wide range of soils and also from samples taken at intervals from differently manured plots. The help of the National Agricultural Advisory Service bacteriologists has been obtained in this survey from different soil types and the results which they are obtaining are being received and collated.

Counts of microorganisms from Broadbalk plots

As mentioned in the previous report, counts of various groups of microorganisms were made from samples taken from some of the Broadbalk plots during 1948. The objects of this survey were not only to compare microscope and plate counts but also to assess by present day methods the effects of organic and artificial manuring on the soil micropopulation.

The microscope counts of bacterial cells and actinomycete spores were significantly correlated with soil moisture and with numbers of actinomycete colonies appearing on plates, but not with bacterial colonies. On Plot 2 (F.Y.M.) the total length of fungal mycelium found by microscopic examination was significantly correlated with numbers of fungal colonies on plots.

Microscope counts of bacterial cells and actinomycete spores, and plate counts of bacterial and actinomycete colonies both gave higher numbers on Plot 2 (F.Y.M.) than those obtained from Plot 3 (unmanured) or Plot 7 (complete minerals and ammonium suplhate) which were about equal. Manurial treatment did not significantly affect the total lengths of mycelium found by microscopic examination, but both Plots 2 and 7 gave higher plate counts of fungi than did the unmanured Plot 3. The numbers of amoebæ found in Plots 2 and 7 were not significantly different but both were higher than those found in Plot 3.

This survey of Broadbalk plots was carried out by a team of workers (Dr. Janet Mollison, Dr. B. N. Singh, Mr. P. C. T. Jones and Mr. F. A. Skinner).

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Partial sterilization

A field experiment on the effect of partial sterilization in a nursery bed for Sitka Spruce was carried out at Ampthill, Bedfordshire, by the Chemistry Department during 1949. The Soil Microbiology Department made a study of the micropopulation of soil samples taken from certain of these plots at intervals throughout the season. The results are not yet complete but indicate striking and persistent changes following the partial sterilization. (Dr. Janet Mollison, Miss Lettice Crump, Dr. B. N. Singh and Mr. P. C. T. Jones have been engaged on this survey).

Antibiotic actinomycetes

The possibility of using microorganisms that produce antibiotic secretions to control root disease fungi is being studied by Mr. F. A. Skinner in the special case of actinomycetes antibiotic against the fungus *Fusarium*. When such actinomycetes are grown with the fungus on plates of washed agar and mineral salts alone growth occurs but there is usually little or no evidence of antibiotic action, such action is however induced by the addition of glucose even in quite low concentrations. Antibiotic activity in agar is markedly reduced by the addition of charcoal or bentonite to the agar but not by the addition of Kaolin.

Some actinomycetes that are antagonistic to *Fusarium* on agar have not been found to produce antibiotic secretions in liquid culture even when aerated.

In this work it has become important to be able to measure growth of both the fungus and the actinomycete mycelium in liquid culture. This can be done in the case of the fungus by microscope counts and measurements of fragmented mycelium, but such a method is unsuited to the finer actinomycete hyphæ. Similarly we need a method for the comparative estimate of actinomycete mycelium and spores in soil. The effect of shaking suspensions of mycelium with sand showed a relationship between time of shaking and numbers of colonies found on subsequent platings, whereas with a suspension of spores time of shaking did not affect the count.

Resin attacking organisms

In his work on the biological decomposition of resins tested for the making of temporary roads Mr. P. C. T. Jones isolated a number of bacteria and fungi that could attack resins. The characters of these organisms and their action on resins has been further studied with a view to publishing descriptions of them.

Nitrification

The importance of nitrification in soil makes it important to find out what organisms are important in carrying out this process in soil. In view of recent work on the biochemistry of nitrification in soil it is important to make fresh study of the physiology of Nitrosomonas and Nitrobacter to see whether their behaviour agrees with that postulated for the nitrifying flora of soil. By a modified process, Dr. Jane Meiklejohn obtained and has described a pure culture of Nitrosomonas and found that this oxidized ammonia as rapidly as did a crude culture containing the commonly occurring contaminants. This makes it unlikely that the latter act symbiotically with Nitrosomonas as has been suggested.

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Soil amoebæ

Excystation of amoebæ. Miss Lettice Crump has studied the conditions governing the hatching of amoebæ from cysts and has found that morphologically similar species differ markedly in that one species will excyst only in the presence of bacteria of a suitable type while with the other, excystation is independent of the bacterial environment. This difference in behaviour provides a further specific factor likely to affect the relative numbers of different amoebæ in soil.

Nuclear division of classification of amoebæ. It is important to the study of the soil micropopulation that different species of amoebæ should be capable of identification. The classification and identification of amoebæ is largely based on the type of nuclear division, and there is great confusion at present largely owing to the processes of mitosis being wrongly described, often from insufficient material. Dr. B. N. Singh has developed a method for cultivating small amoebæ on glass slides coated with agar, that enables large numbers of amoebæ, in all stages of nuclear division, to be obtained readily and in a form facilitating the making of stained preparations. Twelve strains of amoebæ have been examined by this method and the types of division found are much less diverse than was formerly supposed, falling into two main categories. This discovery together with the further use of the new technique should result in a great simplification in classification and ease in species identification.

Mycorrhiza

In her earlier work Dr. Janet Mollison studied the distribution of the mycorrhizal fungus *Rhizophagus* in clover and wheat and found it to be abundant in the roots of both crops. Attempts to isolate the fungus and to grow it in a variety of artificial media have so far been unsuccessful.

When a series of dilutions of a soil suspension were applied to sterilized sand in which clover was grown the fungus was found to be present at a 1/10 dilution but not at a 1/1000 dilution. With a suitable dilution it may thus be possible to obtain otherwise comparable cultures with and without the fungus by which its effect on the plant can be assessed.

STUDIES ON NODULE BACTERIA (RHIZOBIUM) AND LEGUME CROPS

Geographical distribution of strains of clover Rhizobium

In continuation of the survey of the distribution of strains of clover Rhizobium, effective and ineffective in fixing nitrogen, a considerable amount of strains isolated from clover samples collected from the North and from the South West of England were tested for effectivity by Dr. Janina Kleczkowska.

A seriological study made by Dr. Margaret Read of 100 isolates from each of 17 localities has shown that strains of clover Rhizobium tend to be highly localized in their distribution, so that no rapid spread of a new strain whether or natural origin or introduced, is likely to occur under field conditions.

Establishment of clover Rhizobium in an " inoculated " crop

The abundance in some localities of ineffective strains of clover Rhizobium, suggests the use of seed inoculation to replace these ineffective strains by an effective strain in the soil. Any attempt to

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introduce Rhizobium into the soil by inoculation will meet with competition for nodule formation from the native population of clover Rhizobium already present. Hence the first problem to be solved was how far a culture introduced by seed inoculation can be established in the nodules of the crop in face of this competition.

To find this out it was necessary to develop a method for recognizing a strain introduced by seed inoculation into field soil already containing many other strains of Rhizobium ,and thus for distinguishing nodules produced by the inoculum from those due to native strains. In earlier work (Kleczkowski and Thornton) it had been found that strains of clover Rhizobium fall into groups identifiable by antigen tests and that strains containing certain antigens are uncommon in British soils. If seed is inoculated with a strain containing an uncommon antigen and sown in the field, cultures isolated from the nodules later developed can be tested with suitable antisera and in this way it is possible to determine whether they are of the same strain used to inoculate the seed. By testing sufficient nodule isolates the percentage of nodules produced by the inoculum can be estimated. An extensive series of experiments were carried out in 1948 and 1949 by Dr. Margaret Read with the co-operation of the National Agricultural Advisory Service. These were conducted at 13 centres at four of which the trial was carried on for two seasons. Each experiment consisted of 16 plots, four replications sown with uninoculated seed and four with seed treated with each of three strains of nodule bacteria selected for ease of seological identification. Cultures were isolated from 20-25 nodules from each plot and tested with suitable antisera. From those tests the percentage of nodules due to the inocula were estimated. Great assistance was provided by bacteriologists of the National Agricultural Advisory Service not only in the field trials themselves but also in certain experiments in isolating cultures from the nodules. Without their generous help so extensive a series of trials involving the isolation and serological testing of 6,000 cultures could not have been carried out. The results of these trials showed that it is possible to establish a culture of Rhizobium in the crop, in competition with the native bacteria, but that different strains of Rhizobium differ greatly in their ability to become established in the field. The experiments thus point to a new principle applicable to seed inoculation practice, namely that, in the selection of a strain for seed inoculation, not only effectivity in fixing nitrogen, but also ability to compete with native strains in the soil has to be considered.

Legume inoculation

A number of inoculation trials with clover and beans have been and are being carried out in various districts by the National Agricultural Advisory Service using cultures supplied by us.

Local adaptation between clover and Rhizobium

Experiments have been made by Dr. P. S. Nutman and Dr. Margaret Read to see whether, on farms where local strains of red clover, have been developed, the clover and the indigenous nodule bacteria have become mutually adapted to give enhanced nitrogen fixation. Material was obtained from seven farms in Sweden and in experiments carried out in 1948 and 1949, bacteria obtained from each farm were tested for effectivity on the local clover strain

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from the same farm and from each of the other six farms. In both experiments a small but significant effect was found, bacterial strains tending to be more effective on their own local clover strain than on the others.

Appearance of ineffective mutants of clover Rhizobium in soil

It was found by Dr. P. S. Nutman in previous work that if a pure culture of the highly effective strain " A " was kept in sterilized sandy soil from Woburn for six months, a considerable percentage of ineffective mutants appeared. This raised the practically important question whether this phenomenon was of wide occurrence in different soils or with many effective strains of Rhizobium.

To test the first point, Strain " A " was stored in sterilized samples of a range of different soils for half a year, after which the soil cultures were plated, random colonies " picked" and the resulting cultures tested for effectivity on clover.

We have again receiued much help from bacteriologists of the National Agricultural Advisory Service in setting up soil cultures and in the subsequent plating and isolations from colonies. The work showed that ineffective mutants tended to appear in acid soils and that their appearance could usually be prevented by the addition of $CaCO_3$. But in some cases mutants did appear in neutral and limed soils, so that other factors must also contribute to the effect. To test the stability of different strains of Rhizobium on soil storage, ten effective strains were stored in Woburn soil for 2 years. At the end of this time only one of these was found to have produced ineffective mutants, but this strain produced a high percentage. Dr. Janina Kleczkowska has been in charge of this work.

The production of mutants resistant to bacteriophage

It seems possible that the effect of bacteriophage in causing the appearance of a variety of mutant forms of Rhizobium might be a factor inducing a change to ineffectivity in field soils . Dr. Janina Kleczkowska has studied 'phage resistant mutants developed in cultures exposed to 'phage action. A number of these regained susceptibility to 'phage quite soon but were more liable to develop resistant mutants on further treatment with 'phage, than was the untreated parent strain. Amongst resistant mutants new colony characters were frequent. These were usually stable in artificial culture but readily lost on plant passage. Some resistant mutants also differed from the parent strain in their effectivity in the host plant. Many of these produced from an originally effective strain were ineffective, but the reverse change was much more rare. Mutants showing a change in effectivity varied very much in stability a number were stable during laboratory culture and after plant passage, but one strain was so highly unstable that it proved impossible to separate the effective and ineffective forms both of which were found in every colony after replating and plant passage.

The mechanism of attack by bacteriophage

Rhizobium bacteriophage is exceptionally good material for studying the process of attack on the bacterium and this problem has a special interest from its analogy to the infection process in plant viruses. Drs. A. and Janina Kleczkowski have examined the question how many 'phage particles are needed to start an infection. The work is still in progress but results so far are consistent with the

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view that a single 'phage particle will suffice to produce infection of a liquid culture or to initiate a plaque.

The inhibitory effect of nodule and lateral root meristem on further nodule development

Dr. P. S. Nutnam has continued his earlier work on the effect on nodulation of excising nodules and lateral roots of clover. The excision of effective nodules increases the number of nodules subsequently formed but that of ineffective nodules does not do so. The excision of the meristem tips of effective nodules or of lateral roots also increases subsequent nodule numbers. These reults suggest that a substance inhibitory to nodule formation is produced in meristems. In ineffective nodules the meristem tip is ephemeral and persists for too short a time to produce an effect.

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