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### Soil Microbiology Department

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## SOIL MICROBIOLOGY

By H. G. THORNTON

The following main lines of work were carried out during the year.

### MICROPOPULATION OF BROADBALK PLOTS

The development in this department of improved methods of estimating numbers of micro-organisms in soil has made it desirable to examine by their aid the effects of the prolonged differences in manuring of our classical plots on the main groups of the micro-population. The present survey has the particular object of comparing the effects of artificials and farmyard manure. In 1946-47 a series of samples, taken from three plots in Barnfield, showed that Plot 4a, treated with complete minerals and ammonium sulphate had somewhat higher numbers of bacteria than the unmanured plot 8-0, but less than the dung plot 1-0. The numbers of active amoebae in this plot were as high as in the dung plot and much higher than in the unmanured. Since the treatments in the classical plots are not replicated, it was decided to check these apparent effects of manuring by surveying the micropopulation of plots, similarly manured, in quite a different field.

During the season, therefore, a team of workers has examined samples of plants and soil taken at intervals from three plots from Broadbalk, namely Plot 2 (farmyard manure), Plot 3 (unmanured) and Plot 7 (complete minerals and ammonium sulphate).

Dr. Janet Mollison has made microscope measurements of the total lengths of mycelium and compared these with colony counts of fungi. Mr. P. C. T. Jones has made microscope counts of the number of bacterial cells. Mr. F. A. Skinner has made plate counts of bacteria and actinomycetes both in the soil and in root-washings from the crop, while Dr. B. N. Singh has made dilution counts of amoebae. The full results of this survey are not yet to hand, but already they tend to confirm the results of the previous survey of Barnfield plots and also to show some correspondence between the results of the different methods used.

### COMPARISON OF PLATE AND MICROSCOPE COUNTS OF BACTERIA

A serious discrepancy exists between the large numbers obtained when total numbers of bacterial cells are counted in stained films of soil and the much smaller estimates derived from counts of colonies on dilution platings. This discrepancy could be due to several causes such as the counting of non-viable cells in stained films, the inadequate dispersion of the bacteria in the suspension plated, or failure of the plating medium to support growth of many species of the soil bacteria. The last explanation, if true, would involve the conclusion that most species of soil bacteria have so far escaped cultivation and laboratory study. Hence an explanation of the discrepancy is fundamental to soil bacteriology. Mr. P. C. T. Jones is investigating this problem by applying both methods to cultures added to sterilized soil. The data obtained from the survey of Broadbalk plots should provide evidence of any effect that season or manuring may have on the ratio of direct to plate counts.



It is also important to narrow the ratio by developing a plating medium that will give the highest colony counts consistent with accuracy. Mr. F. A. Skinner has studied this problem and has developed a medium giving much increased colony counts with good agreement between replicate plates.

#### NITRIFICATION

A question never clearly settled with regard to the nitrification of ammonia in soil is the contrast between the ease with which this occurs in highly organic soils and the claim that quite small amounts of soluble organic compounds kill or inhibit the activity of nitrifying bacteria in culture. Dr. Jane Meiklejohn has investigated this problem by adding glucose to cultures of nitrifying bacteria growing in mixture with other organisms. The glucose temporarily inhibited nitrification without killing the nitrifying bacteria by encouraging growth and resulting competition by the other bacteria present. Nitrification was resumed as soon as the added glucose had been consumed. An isolation of *Nitrosomonas* has been obtained and is being studied.

#### MYXOBACTERIA

Following his studies of this interesting group, Dr. B. N. Singh has searched soil samples from Canada, Jan Mayen, Sweden, Holland and Australia, for Myxobacteria of the types that produce fruiting bodies and consume Eubacteria. He has found these diverse soils to contain species identical or similar to those in British soils. Isolations of simpler types of Myxobacteria that appear not to produce fruiting bodies have also been made and are being studied.

#### SOIL AMOEBAE

During the surveys of Barnfield and Broadbalk plots a number of new strains of amoebae have been obtained and studied in laboratory culture. Some of these have been used by Miss Lettice Crump to study the environmental factors that induce hatching of the cysts. She has obtained evidence, *first*, that the type of bacterial food consumed by the amoebae before encystment may affect the subsequent viability of the cysts, and, *secondly*, that, with one of the amoebae studied, excystment takes place only in the presence of bacteria, the species of which is again of importance.

Miss Crump has also studied the rate of spread of amoebae in sterilized soil inoculated with bacteria and finds that this rate varies with the species of amoebae and with the type of bacterial food. These studies on the inter-relation of amoebae and bacteria have an obvious bearing on our understanding of the ecology of the soil's micropopulation.

#### ANTIBIOTIC ACTIVITIES OF THE SOIL MICROFLORA

Previous workers studying the antagonistic action between colonies of soil micro-organisms growing on plates, have usually employed media rich in nutrients and having a high concentration of mineral salts. Such an environment is very different from that found in normal soil in which the content of available nutrients and of soluble salts is very much lower. The present investigation by Mr. F. A. Skinner began with an attempt to obtain a plating



medium that would give high colony counts of bacteria and actinomycetes from a plated soil suspension, by discouraging suppression of growth through competition between colonies. It was found that reduction of the available nutrients and a much higher dilution of the salts in the medium greatly increased the colony count; the highest counts were indeed obtained with no added organic compounds other than washed agar, although on this medium the colonies formed were too minute for routine counting.

The suppression of bacterial colonies in the neighbourhood of actinomycetes has so far been observed only on the richer media containing beef extract and peptone, but the suppression of fungal growth by actinomycetes has been noted usually on the medium with dilute salts and from 100 to 10,000 p.p.m. of glucose or of mannitol. Antagonism between two species of actinomycetes has so far been found only on media with not more than 100 p.p.m. of soluble carbohydrate. On such media the actinomycetes grow rapidly and show the well developed aerial mycelium and lack of pigmentation that has been found to characterize their growth on moist soil. The antibiotic activity of isolates are now being studied under a variety of environmental conditions.

#### THE RELATION OF MICRO-ORGANISMS TO SOIL STRUCTURE

Dr. R. J. Swaby has finished his study of the action of micro-organisms on soil aggregation described in last year's report. He concluded that the quantity of fungal mycelium found in a sample of field soil might account for about a quarter to a half of the observed aggregation while bacterial colonies seemed of little quantitative importance in this respect. A large fraction of the aggregation was thus not accounted for by such immediate effects of micro-organisms, but was perhaps attributable to some resistant humus complex.

#### MYCORRHIZAL INFECTION OF CROP PLANTS

Dr. Janet Mollison has made an examination of the roots of wheat taken at intervals from Broadbalk Plots 2, 3, 5 and 7 and also from Great Harpenden field, where, in contrast to Broadbalk, wheat had not been previously grown for six years. Early in the season, wheat seedling roots from the unmanured Plot 3 showed a greater degree of mycorrhizal infection by the phycomycete fungus, than those from any of the treated plots, though this difference decreased with the age of the plants. No difference in the incidence of the infection was found between the variously treated plots, whether given artificials or farmyard manure. In contrast, however, the wheat collected from Great Harpenden field showed little mycorrhizal infection until late in the season and even then it was less widespread than on the Broadbalk plots.

Pot experiments designed to study the spread of mycorrhizal infection in clover have shown that the fungus failed to establish itself in sterilized soil, either from chopped infected roots or from infected seedlings planted in it. Such seedlings did not even show infection of new roots, the infections remaining localized. Investigation of the factors that effect the spread of the fungus outside the plant are continuing.



#### GEOGRAPHICAL DISTRIBUTION OF THE BACTERIA

A study of the distribution over Great Britain of strains of clover nodule bacteria, effective and ineffective in fixing nitrogen has been continued this season, strains isolated in 1947 having been tested on the plant and more strains obtained from new areas in the North and West of England. We have received most useful help from members of the National Agricultural Advisory Service in this work.

#### ESTABLISHMENT OF CLOVER NODULE BACTERIA WHEN INOCULATED INTO FIELD SOIL

This problem is of great importance to the practice of legume seed inoculation and is being studied in field trials at a number of centres. At each centre replicated plots are sown with clover inoculated with each of three strains of nodule bacteria and with uninoculated seed. Bacteria are isolated from 100 nodules per treatment as well as from the uninoculated plots and these isolations are tested serologically to determine how many are derived from the culture used to inoculate the treated plots, and how many from the "wild" nodule bacteria in the soil. This work involves the isolation and testing of bacteria from 400 nodules per experiment, of which some dozen have been run. Miss Margaret Thomas has charge of this research and carries out the laboratory work at Rothamsted. Great assistance, moreover, has been given us by members of the National Agricultural Advisory Service, not only in carrying out the field trials but also in making many hundred isolations of bacteria from nodules. Without this help the extensive programme could not have been carried out. Individual acknowledgment will be made at the conclusion of the investigation.

The serological testing of strains isolated from field experiments carried out in 1947 has been completed, and one strain used for seed inoculation has competed successfully with local strains in four out of six experiments, producing over 60 per cent. of the nodules in some cases. The other two strains employed have given poor establishment, and, although highly effective in laboratory culture, would not prove of any value in large-scale field inoculation.

Similar field experiments have been carried out in 1948 but in the areas of hill pastures at 500 to 1,000 feet in Wales, Scotland and Yorkshire. Further effective strains of *Rhizobium* have been used to replace those which proved unsatisfactory last year. Isolations have been made from all experiments, including three continued from 1947, and serological testing of the cultures is in progress.

#### LOCAL ADAPTATION OF CLOVER BACTERIA

An extensive experiment was made by Dr. P. S. Nutman and Miss Margaret Thomas, with help from the Statistical Department, to determine whether adaptation occurs between local strains of red clover and the indigenous strains of nodule bacteria in districts where the clover strains are grown.

The experiment comprised three English and six Swedish strains of clover, and 50 local strains of bacteria, each of which was tested effectivity on each strain of plant. Significant evidence was found of local adaptation in Sweden.

#### DISSOCIATION OF NODULE BACTERIA IN SOIL

Dr. Janina Kleczkowska continued her work on the appearance of ineffective dissociant forms of clover nodule bacteria, when



effective strains are kept in sterilised soil. Ten effective strains were stored for 2 years in Woburn soil, plated and bacteria from random colonies were tested on clover plants. Two of the strains were found to have produced ineffective dissociant forms but the remaining eight remained stable. Genetical stability in soil thus appears to be a strain character, a conclusion of importance in selecting strains for use in the practice of legume inoculation.

A second experiment in which clover bacteria are being stored in 15 different types of soil, is still in progress.

#### DISSOCIATION UNDER THE ACTION OF BACTERIOPHAGE

When nodule bacteria are exposed to bacteriophage, dissociant forms are readily produced, particularly frequent being changes in colony appearance, such as change to "roughness" or pigment formation. Less frequent are changes influencing the effectivity effects in nitrogen fixation within the nodule. Dr. Kleczkowska, however, has obtained strains effective and intermediate in this respect by treating ineffective parent strains with bacteriophage and conversely has readily obtained ineffective dissociates from 'phage-treated effective strains. The action of 'phage has also induced changes in the morphology of the bacterial cells, which are being studied.

#### GENETICAL FACTORS IN CLOVER AFFECTING NODULE DEVELOPMENT

This work by Dr. P. S. Nutman, described in previous reports, has now reached its final stages with the examination of material from crosses made in order to complete evidence on some doubtful points. The work is being prepared for publication at an early date.

#### PHYSIOLOGY OF INFECTION

Further experiments with delayed inoculation of clover with nodule bacteria have confirmed the results given in the two papers submitted to the *Annals of Botany*. The suggestion that the apical meristems of lateral roots and of effective nodules inhibit nodule formation in their neighbourhood has received strong support from the discovery that excision of these root apices and nodules increases the number of nodules formed, while the excision of ineffective nodules (which have no persistent apical meristem) has no such effect.