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ROTHAMSTED RESEARCH

Report for 1950

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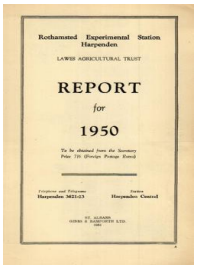
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Soil Microbiology Department

H. G. Thornton

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SOIL MICROBIOLOGY DEPARTMENT

By H. G. Thornton

Effect of Partial Sterilization in the Field

During this season co-operation was continued with the Chemistry department on the effects of partial sterilization of nursery beds at Ampthill. The treatments studied were steaming and application of formalin. Some of the plots examined were treated in the spring of 1949 and others in the spring of 1950. The micropopulations of treated and control plots were surveyed at intervals. Miss L. M. Crump and Mr. P. C. T. Jones dealing with bacteria, Dr. J. E. Brind with types of fungi, while Dr. B. N. Singh made counts of the numbers of amoebae. The survey has shown remarkable differences related to treatment, in the numbers of the main groups of micro-organisms. There have also been differences due to the appearance on the treated plots of specific types of organisms. Those differences are unexpectedly persistent ; some are still found 18 months after the partial sterilization. This survey suggests several promising lines for further work in connection with partial sterilization.

Actinomycetes antibiotic against fungi

Fundamental knowledge of the conditions under which actinomycetes will grow and produce antibiotic secretions, and under which these secretions will remain active, is essential before one can hope to make practical use in the soil, of actinomycete species that show antibiotic activity on plates. Mr. F. A. Skinner has continued to study the action of certain actinomycete species of the genus Streptomyces in inhibiting the growth of *Fusarium culmorum*, and has extended this study to a number of other soil fungi that are also antagonized by actinomycetes. He has developed a method of standardizing the size of actinomycete colonies thus improving the accuracy with which antagonistic effects on agar plates can be measured. A description of this method has been published. Antagonism can usually be observed in a variety of media having a wide range of C/N ratio and differing greatly in sources of carbon and nitrogen. Actinomycetes will inhibit *Fusarium culmorum* and other soil fungi on media whose low level of nutrients approaches that of soil. This suggests that they may also produce antibiotic substances in soil. Antagonism on agar plates can, however, be prevented by the inclusion of adsorbing material, such as bentonite, in the media, so that it is possible that the antibiotic substances may also be rendered ineffective by the clay colloids of soil. The effects of other factors in the soil environment are being studied.

Strong inhibition of *F. culmorum* by actinomycetes has been found in sand culture, and tests have been made to find the best way to sample these cultures and to estimate the amount of fungal growth. The extension of this research into the soil itself requires a method for ascertaining the condition (whether as spores or mycelium) of actinomycetes in the soil studied. A method based on the effect of mechanical shaking with sand has been devised which shows considerable promise. An account has been published.

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Nitrification

The very special nutrient media alleged to be necessary for the growth of *Nitrosomonas in vitro* contrast so evidently with the ease and varied conditions under which nitrification occurs in nature, as to suggest serious gaps in our knowledge of the microbiology of this process. Dr. Jane Meiklejohn has investigated *Nitrosomonas* from two points of view, its tolerance for glucose and its need for traces of metals. Glucose, when sterilized by autoclaving, produces a substance very toxic to the organism, but when sterilized by filtration glucose was not toxic up to a concentration of 0.02 M. When glucose was added to a mixed culture of *Nitrosomonas* contaminated with other bacteria, however, less nitrification was observed presumably owing to the competitive growth of contaminants.

Nitrosomonas has been found to need traces of iron, 0.6 mg per litre being an adequate dose. In an iron deficient medium, if provided with sufficient copper and manganese, it will oxidise ammonia after a considerable delay suggestive of adaptation.

Dr. Meiklejohn has obtained a pure isolate of the organism *Nitrobacter* whose physiology is being studied.

Myxobacteria

Dr. B. N. Singh has isolated from soil a species of the little known genus *Melittangium* which is unusual in producing stalked sporangia; this organism like *Myxococcus* feeds on Eubacteria by producing lytic secretions. Its life cycle and food requirements are under investigation.

Soil Amoebae—their identification and classification

In studies concerning the ecology of soil amoebae and their influence on bacteria, the correct identification of species is of importance because of the different behaviour of different species.

The most useful diagnostic character seems to be the type of nuclear division, but difficulty in obtaining all the normal stages of division has led to much confusion. Dr. B. N. Singh has developed a simple method for culturing soil amoebae on microscope slides or covers so as to obtain numerous dividing nuclei, which he has then treated with specific chromatin stains. He has applied this method in a range of species. From these observations he is proposing a simplified classification of the amoebae, based on the type of nuclear division, which should aid identification of these organisms.

Miss L. M. Crump is investigating the cultural habits and behaviour of the dominant soil amoebae found in Rothamsted soils, as these effect their ecological relationships and are in some cases valuable aids to identification. The factors influencing excystment, which have considerable ecological importance, are included in this study.

Mycorrhizal association in clover

The very common occurrence of the endotrophic mycorrhizal fungus, *Rhizophagus*, in clover and in wheat makes it important to

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obtain definite evidence as to whether this association confers any benefit at all on the host plant.

The most direct way to do this would be to isolate the fungus and then grow host plants, some supplied with the fungus and some not. No one, however, has as yet succeeded in isolating the fungus and causing it to grow appreciably *in vitro*.

Dr. Janet Brind has therefore attempted a different method for obtaining clover plants some with and some without *Rhizophagus* under otherwise similar conditions. A suspension of fresh soil known to contain this fungus was serially diluted and sets of replicate pots containing sterilized sand sown with red clover were supplied with different dilutions, in the hope that at a suitable dilution, some replicate pots would receive the fungus and others would not.

In a preliminary experiment the fungus was found to be present at dilutions of 1/100 and lower, but absent at 1/1000, and within one dilution pots could be found with and without infection by *Rhizophagus*.

A third experiment using four dilutions between 1/5 and 1/500 was successful in giving infection of approximately half the pots (10) at the lowest dilution. Dry weights of the infected plants showed no significant difference from those of uninfected plants on the comparatively small number of pots available for comparison.

Nodule bacteria (Rhizobium) from clover

Previous work has shown the importance in legume inoculation of selecting strains of Rhizobium for use, that are able to grow well in the root surroundings of the crop and to produce a satisfactory infection. This is especially important where it is hoped to replace ineffective strains naturally present in the soil by more effective strains. Field experiments have shown that it is possible to inoculate a clover crop with a suitably chosen strain and to obtain a high percentage of nodules containing this inoculant strain, even in soil containing numerous "wild" strains of clover Rhizobium. So that where the soil contains numerous ineffective strains it should be possible to replace these with an effective inoculant strain. Success here requires the selection of strains that are both high in their effectivity towards the host plant and also in their ability to compete in the soil particularly against ineffective strains. Dr. Janina Kleczkowska has made pot experiments designed to select strains of clover Rhizobium combining these characters. The work is in progress.

Rhizobium bacteriophages

The investigation of these bacteriophages has been continued. Using one strain, Drs. J. and A. Kleczkowski have found that a single phage particle can initiate a plaque on solid medium or multiply in a liquid bacterial culture, but that not all particles normally succeed in doing either. The chance of success depends on the condition of the liquid bacterial culture inoculated with phage or used for plating. It is possible that in optimal conditions every phage particle will multiply to produce a plaque but there is no evidence for this.

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Root secretions

Experimental work by Dr. P. S. Nutman during the year has been directed mainly to a study of the mutual interference between plants sharing the same root space, noted in a previous report. The suppression of nodule formation on one plant by the presence of another growing with it in the same culture vessel occurs under very diverse conditions and does not appear to be open to explanation in terms of competition for nutrients, light, or carbon-dioxide. Thus a completely resistent clover plant, or a plant responding ineffectively to nitrogen fixation, limits the infection on a companion plant to the same degree as a normally effectively responding plant making full demands on the nutrients and space available.

Experiments on the influence of the volume of the root medium and on replanting suggested that plant interaction may be due to the production of inhibitory substances from the root. Below a volume of about 10 cc. per plant a strict proportionality obtained between the numbers of nodules formed per plant and the volume of the medium. Replanting experiments show that the number of nodules developing on a test plant is related to the number of plantings previously made in the same medium. If the roots of the first crop are left *in situ* and only the tops removed, there is an augmentation of the inhibitory effect on the succeeding test plant. Further evidence for inhibition rather than exhaustion of nutrients was obtained in experiments in which reduction in nodule number was obtained by watering test plants with staled medium obtained from another culture vessel. Attempts to increase the inhibitory activity of staled media by direct concentration were inconclusive.

Addition of chemical adsorbents such as activated charcoal markedly stimulated both nodule and root development. Below about 5 per cent of charcoal in the medium, the stimulation was proportional to the quantity present and independent of the effectiveness of the strain of nodule bacteria used as inoculum. Of the other adsorbents used, Bentonite, Fullers earth and to a lesser degree, asbestos were stimulatory, kaolin, silica gel and celite had no activity, and zeocarb, deacidite and magnesium carbonate were harmful to plant growth. Increased nodulation with the active adsorbents occurred from pH 4·0—8·0 and in each case and in the controls maximum nodulation occurred at pH 5·5. Experiments are being continued on these lines with the object of obtaining critical chemical evidence for the presence of an inhibitory substance.

In the course of these experiments it was observed that with bentonite in the medium an intense blue-green colour developed in the immediate vicinity of the root. This colour was obtained with red and white clover but no colour was observed with oats, ryegrass or radish. Lucerne gives a faint brown colour, vetch an orange brown and flax a citron yellow. The blue-green stained bentonite removed from the clover cultures fades to a purple-brown on drying unless it is first acidified with 10 per cent mineral acid. Addition of alkali discharges the colour which may, however, be restored by reacidifying. The colour is not removed by moderate leaching with acid salt solution or organic solvents but is destroyed by heating

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to 100°C. These colour reactions are being further investigated although the root exudation responsible may not be concerned with nodule inhibition. Colour reactions are known between clay minerals and certain organic compounds, notably Vitamin A and carotenoids and some aromatic amino compounds.

Work has also been in progress during the year on the inheritance of the effectivity response in red clover and further confirmatory experiments have been carried out on nodule excision.