ROTHAMSTED RESEARCH

Report for 1952

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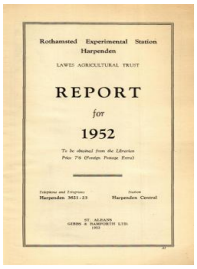

Soil Microbiology Department

H. G. Thornton

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SOIL MICROBIOLOGY DEPARTMENT

H. G. THORNTON

During the year under review the permanent scientific staff of the department has been increased by the appointment of E. R. Turner to carry out research on biochemical problems connected with the infection of leguminous plants by nodule bacteria. S. M. Bromfield returned to Melbourne after the completion of two years' studies on the microbiology of reduction processes in soils, for which he was awarded the degree of Ph.D. of London University. J. Meiklejohn left in June for Kenya in a year's secondment by the Colonial office to survey and report on problems of soil microbiology in East Africa.

The work of the department has followed the main course shown in previous reports. The work has dealt with the soil micro-population as a whole and with some of its component organisms, and a special section of it has again been devoted to the nodules of leguminous plants.

PARTIAL STERILIZATION OF SOIL IN THE FIELD

The study of the changes in soil population induced by the application of formalin to the soil of Sitka spruce nursery beds was continued by L. M. Crump. The plots sampled were laid out by the Chemistry Department and those studied comprised control plots without formalin (OO) and plots dosed respectively with formalin in March 1951 only (FO), in March 1952 only (OF), and in March 1951 and March 1952 (FF). Formalin applied this year caused an initial depression in bacterial numbers found by plating on a medium allowing a variety of nutritional groups to develop. This was followed by a rapid rise to numbers two to three times those on the untreated plot. These high numbers were maintained until May, after which the numbers fell to the control level. In this year's experiment bacterial numbers in the treated plots showed a smaller and less persistent rise than in previous years. This may have been due to weather or to the fact that a smaller dose of formalin was applied. Platings on special media showed that the quality of the microflora was also altered by formalin applied this year, which encouraged the development of organisms tolerant to formalin, of nitrate reducers and, in the early samplings of gelatin liquefying organisms, mostly spore-formers. The residual effect of formalin applied in 1951 (FO plots) showed to a small extent in the platings on the generalized medium and also in the numbers of nitrate-reducing organisms.

The numbers of amoebae in Ampthill soils following partial sterilization with steam and with formalin were estimated by B. N. Singh in samples taken in successive samplings over a period of 3 years. In plots treated with formalin, numbers of amoebae were lower, but in steam-treated plots higher than in the untreated plots sampled, although partial sterilization by both methods increased the numbers of bacteria. It is suggested that these effects may be due to changes in the food value to amoebae of the bacterial population as a result of the two treatments.

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Competition between Actinomycetes & Root Disease Fungi

Practical results in the biological control of root diseases by competitive micro-organisms will be more probable when more is known as to the various processes of competition and the effects on these of environment. Attention has usually been centred on the action of antibiotic secretions produced by the antagonist. But the results obtained by F. A. Skinner in his continued investigation of the competition between the antibiotic producing actinomycete, *Streptomyces albidoflavus*, and the root pathogenic fungus *Fusarium culmorum*, make it clear that the actinomycete can affect the fungus in three ways, (1) by direct attack on the fungal hyphae, (2) by the production of antibiotic secretions and (3) by competition for nutrients. He has shown that soil colloids and certain organic materials will prevent or reduce antibiotic activity of the actinomycetes and particularly that a very low concentration of bentonite will completely remove the antibiotic activity of a filtrate of an actinomycete culture and that bentonite will also prevent direct attack by the actinomycete on the fungus. The addition of bentonite to mixed cultures thus affords a means of examining competiton for nutrients, by removing the other two methods by which the actinomycete can check the fungus. When the two organisms were grown together in sand moistened with a medium containing 1 per cent glucose, growth of the fungus was completely inhibited. With the further addition of sufficient bentonite to adsorb the antibiotic secretions, fungal growth was still reduced by the actinomycete as compared with a pure culture of the Fusarium. This reduction is ascribed to competition for nutrients. In sand with a medium containing only 100 p.p.m. of glucose the actinomycete still produced some reduction in growth of the fungus, but in this case the further addition of excess bentonite produced a negligible effect. Thus at a low nutrient level, antagonism is almost wholly due to competition for nutrients. In soil culture also the actinomycete produces some reduction in growth of the fungus and this again appears to be due to competition for nutrients since it is not affected by the addition of bentonite. This work thus emphasizes the importance in soil of competition between micro-organisms for nutrients even when dealing with an antagonist capable, under the right conditions, of producing antibiotic secretions. The cultures grown in media with bentonite also showed that this competition for nutrients was more acute at high than at low nutrient levels. This work is now being written for publication.

Decomposition of Aromatic Compounds in Soil

The use of a variety of aromatic halogen compounds in soil makes it of importance to know what effect micro-organisms have on them. Practically nothing is known about the bacterial decomposition of such compounds.

Previous work at Rothamsted by F. Tattersfield showed that α-chloronaphthalene was susceptible to attack by soil bacteria, and P. H. H. Gray and H. G. Thornton isolated several strains of bacteria which could utilize naphthalene as sole carbon source. Consequently, work on the mode of breakdown by bacteria of

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naphthalene and α-chloronaphthalene was started by N. Walker. G. H. Wiltshire of the Biochemistry Department has collaborated in this work. Three strains of bacteria have been isolated and their action on naphthalene and α-chloronaphthalene has been studied. The first oxidation product of naphthalene is *d-trans*-1:2-dihydro-1:2-dihydroxynaphthalene which is further decomposed via salicylic acid and catechol. These compounds have been isolated from culture filtrates and also shown to be oxidized enzymatically by washed naphthalene-grown cells. The metabolism of α-chloronaphthalene appears to proceed in an analagous manner through a chlorodihydro-dihydroxynaphthalene and 3-chlorosalicylic acid. α-bromonaphthalene is also attacked by these bacteria and so far the only intermediate detected is an acidic substance which behaves like a bromosalicylic acid. Further work on the other intermediates involved in the bacterial decomposition of naphthalene and chloro- and bromo-naphthalene is in progress.

N. Walker has also studied, in collaboration with Professor W. C. Evans of Bangor, the decomposition by soil bacteria of *o* and *m*-hydroxybenzoic acids. There was evidence that the former compound, salicylic acid, now found to be a decomposition product of naphthalene, was oxidized via catechol, the subsequent path of decomposition being known, and that meta hydroxybenzoic acid was oxidized via gentisic acid, the further stages being still unknown.

Reduction Processes in Soil

S. M. Bromfield completed his work here on the reduction of sulphates and of ferric oxide in soil and by isolated pure cultures. This work on sulphate reduction in soil treated with carbon tetrachloride is of special interest because he has identified some of the organisms that inhibit reduction in untreated soil and which are inhibited by the partial sterilizing agent. He summarizes the results of his work as follows:—

Sulphate reduction in soil treated with carbon tetrachloride

A study was made of the reduction of sulphate to sulphide in soil treated with carbon tetrachloride. Hydrogen sulphide was evolved from such soil when moistened with sucrose and ammonium sulphate solution and incubated aerobically. Hydrogen sulphide formation took place in such a system with soil moistures less than field capacity and over a pH range of 5 to 8.

The organism responsible was isolated and identified as *Bacillus megatherium*. Several strains of this organism reduced sulphate in well-aerated sterile soil but not in soil incubated anaerobically or in liquid media.

The action of carbon tetrachloride in fresh soil is to check or destroy certain fungi and bacteria which normally inhibit sulphate reduction by *Bacillus megatherium*. Some of these organisms were isolated and shown to be sensitive to carbon tetrachloride and to inhibit sulphate reduction by *Bacillus megatherium* in sterilized soil. The isolates did not inhibit growth of this organism on synthetic media.

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The reduction of ferric compounds by Bacillus Circulans *and other bacteria*

Bacteria that could reduce ferric compounds in various media were isolated from soil and grass and were identified. Their activity differed greatly and the most active species, *Bacillus circulans*, isolated from soil was selected for further study.

Growing cultures of this organism reduced ferric compounds in liquid media kept under anaerobic conditions, with the formation of soluble and insoluble ferrous compounds.

Reduction of ferric hydroxide also occurred in liquid cultures exposed to air but here the amount of ferrous iron in solution decreased as the surface area was increased.

Neither the supernatant from centrifuged cultures nor cultures autoclaved or treated with chloroform would dissolve or reduce ferric hydroxide.

The organism was, however, unable to derive oxygen needed for its growth from ferric oxide under anaerobic conditions but where another compound containing available oxygen was supplied ferric oxide or phosphate were readily reduced.

Washed cells of the organism reduced ferric iron in the presence of certain hydrogen donors in whose presence they could also reduce methylene blue.

Treatment of the washed cells with dehydrogenase inhibitors prevented iron reduction.

Iron-reducing bacteria were found in the surface layers of gleyed soil but not in samples taken at a depth of 10 feet.

Nitrifying Bacteria

J. Meiklejohn has continued her study of the mineral requirements of Nitrosomonas and Nitrobacter which has formed the subject of publications.

Soil Protozoa and Myxobacteria

L. M. Crump has continued her work in the excystment of soil amoebae. She has also commenced a study of the soil amoebae that feeds on the potato root eelworm, isolated in Holland by Drs. A. P. Weber, L. O. Zwillenberg and P. A. Van der Laan.

B. N. Singh continued his taxonomic studies of soil amoebae continuing his work published in the Philosophical Transactions of the Royal Society, and has investigated the life history of the Myxobacterian organism Mellitangium, isolated from Rothamsted soil.

Investigations of Nodule Bacteria, Rhizobium and Leguminous Plants

Inheritance in clover of factors affecting nodule activity

Experiments were continued by P. S. Nutman on the inheritance in clover of factors determining the effectiveness of the plant's response and the relation between host controlled and bacterial strain controlled variation. These will be reported at a later stage.

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Influence of root secretions on nodule formation

Further work has been undertaken by P. S. Nutman on the inhibitory and stimulatory effects of root secretions on nodule formation on plants growing nearby. To examine further the hypothesis that both effects may be due to the same substance acting at different concentration levels, an examination was made of nodulation in an agar medium in which other plants had previously been grown (preplanting). The quantity of root secretion supplied to the agar was regulated by varying (1) the number of preplantings, (2) the duration of preplanting and (3) the species or variety of plant used for preplanting. As it was known that hereditarily early or late nodulating lines of clover differ from each other and from lucerne, these three kinds of plant were used in both preplanting and in testing for stimulation or inhibition.

The results were as follows:—(1) With all combinations of preplanted and tested plants an initial increase in nodule numbers followed a short period of preplanting (15-20 days); (2) on clover following an initial stimulation, inhibition (i.e. reduction in number of nodules) increased linearly with increasing periods (up to 80 days) and with the number of preplantings by each plant type; (3) inhibition in clover was greatest in sets preplanted with lucerne and least with late-nodulating clover; (4) no difference in inhibition was observed between early- and late-nodulating lines *as test plants*; (5) in contrast to clover, lucerne inhibition was independent of the duration of preplanting beyond about 15 days, or of number of preplantings or type of plant used in preplanting.

From these results the following conclusions were drawn: (1) the level of root secretion required for stimulation (i.e. for nodule initiation) is reached at an early stage in seedlings of all kinds; (2) the clover nodule inhibitory substance is produced at a constant rate by the root; (3) the lucerne nodule inhibitory substance reaches full active concentration within 2-3 weeks of sowing. These experiments demonstrate concentration effects but do not allow firm conclusions to be drawn about the number of different substances concerned. They are not, however, at variance with the single substance hypothesis, such a substance being produced more copiously by lucerne than by clover and more by early nodulating than late nodulating lines of clover, and acting as a general stimulating agent at low concentrations and at higher concentrations inhibiting nodule development, progressively with increasing concentration for clover but having a maximum effect at a relatively low concentration for lucerne. This substance is evidently quite stable in agar culture.

A further interesting result in this experiment was the regular stimulation of clover nodulation by preplanting whereas it was previously shown, in contrast to lucerne, that clover plants planted together in the same tube, instead of successively as above, do not mutually stimulate earlier nodulation. This may be due to a preliminary activation of the primary root secretion by the nodule bacteria in the rhizosphere, this process taking place more slowly in the clover root environment.

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The formation of coloured substances in the neighbourhood of roots growing in bentonite

In the course of the work on root secretions it was noticed that when clover and some other plants were grown in bentonite, a coloured zone appeared in the neighbourhood of the roots. There was circumstantial evidence suggesting a possible connection between this phenomenon and the active secretions. It has therefore been further studied by P. S. Nutman in collaboration with E. R. Turner.

Preliminary results include the effect of pretreatment of the bentonite and survey of the effect of different mineral structures. Comparison was made of Ca, K, and Na treated bentonite, all of which gave colour production, whilst bentonite treated with sugars showed greatly reduced colour production. This latter effect may be due to the expansion and blocking of the crystal lattice. Previous work on the colour production by clays has suggested that it is due to interlamellar adsorption followed by oxidation, which may involve iron. Therefore other types of crystal lattice and composition have been investigated viz:—hectorite (a montmorillonite with magnesium in place of aluminium), mica and a sample of bentonite treated with lithium kindly supplied by the Pedology Department. This bentonite does not expand in water. No colour was produced with mica. Lithium-treated bentonite gave normal colour production whilst hectorite showed reduced colour production. Other minerals will be investigated but these preliminary results suggest that expansion of the crystal lattice is not essential for colour production but the possibility of iron being involved in the reaction is not excluded. Attempts are being made to elute the coloured material from the bentonite.

The growth of Rhizobium in the clover rhizosphere

Investigation by H. Purchase of the numbers of Rhizobium attained in pure culture in the immediate neighbourhood of red clover roots in controlled tube experiments has revealed that a population of 10^8–10^9 bacteria per ml. of medium is quickly attained and is maintained steadily for some weeks. This population is far in excess of the number required for maximum nodulation, shown by other (preliminary) studies to be between 2×10^3 and 2×10^5 per ml. Some plant lines selected by P. S. Nutman bear greater or fewer nodules than the commercial strain or are completely resistant to infection, but they all support a similar population of Rhizobium in their root surroundings.

The number of virulent rhizobia near the roots can be maintained at a low level when non-virulent rhizobia (i.e. a strain incapable of infecting the host plant) are present. This effect may well operate in the field, for example when one legume crop is succeeded by a legume from a different cross-inoculation group. The size of the inoculum for the second crop may thus well be critical for nodule establishment under such conditions.

Roots of plants growing on agar medium could not be shown to exert any chemotactic effect on rhizobia over as short a distance as 3 mms. Direct inoculation of a root growing on agar results in a spreading of the rhizobia along the root, usually in a narrow band

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about 1—2 mms. on each side of the root. That the prime cause of the spreading may be mechanical is tentatively suggested by the observation that a glass capillary tube, similarly inoculated, also develops a zone of rhizobial growth along its entire surface.

Rhizobium bacteriophage

Work on inhibiting effects on phage multiplication by crystalline pancreatic ribonuclease and by crystalline chymotrypsin has been started. Neither of the two enzymes has any direct effect on phage particles, but if added to liquid bacterial cultures which are then infected with bacteriophage, they inhibit multiplication of bacteriophage. Ribonuclease added to a concentration of 0·1 per cent completely inhibits phage multiplication for about 3 hours after which the multiplication begins and eventually proceeds at a rate comparable to that taking place in the absence of the enzyme.

Chymotrypsin at a concentration of 0·01 per cent completely inhibits phage multiplication for 2–3 days after which it starts to multiply and eventually the phage concentration reaches that of a control. At a concentration of 0·1 per cent, chymotrypsin seems to inhibit phage multiplication permanently.

Concentration of active phage falls in the presence of both host bacteria and chymotrypsin, whereas chymotrypsin alone has no such effect. This phenomenon is not encountered with ribonuclease, whose presence together with host bacteria does not lead to any appreciable drop in the concentration of active phage.

Host bacteria are affected by chymotrypsin: this is evident from the fact that formation of motile forms is prevented, and the ability to form colonies on agar medium without chymotrypsin is decreased, although the rate of multiplication in liquid media (where chymotrypsin was present) measured by means of haemocytometer counts, does not seem to be affected. This work was carried out by J. Kleczkowska in collaboration with A. Kleczkowski of the Plant Pathology Department.

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