ROTHAMSTED RESEARCH

Report for 1954

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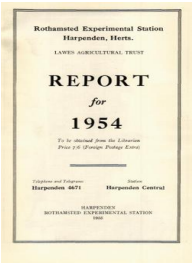

Soil Microbiology Department

H. G. Thornton

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SOIL MICROBIOLOGY DEPARTMENT

H. G. THORNTON

Miss Janet Findlater from Imperial College, London, was appointed a member of the staff in October 1954.

Dr. J. W. Millbank from University College, London, on his appointment to a post under the East African Agricultural and Forestry Research Organization, was seconded temporarily to the department by the Colonial Office from October 1954.

The following worked in the department for short periods during the year :

Dr. V. Treccani, University of Milan, 20 November 1953 until 14 May 1954.

Dr. J. Schiffmann, Agricultural Research Station, Rehovot, Israel, 24 April until 12 May 1954.

Dr. W. V. B. Sundara Rao, Indian Agricultural Research Institute, New Delhi, 26 May until 15 October 1954.

Mlle H. H. de Barjac, Institut Pasteur, Paris, 7 July until 4 August 1954.

Miss E. H. Goldie, Durham University, 17 July until 18 September 1954.

The work of the department has been concerned with the antibiotic action in soil of actinomycetes against root pathogenic fungi, with the bacterial decomposition of chlorophenoxyacetic acid herbicides and chloronaphthalene, with free-living nitrogen-fixing organisms, with problems relating to nodule bacteria and leguminous plants, and with European Foul Brood of bees.

PRODUCTION BY ACTINOMYCETES OF ANTIBIOTICS IN SOIL

The reduced activity of soil-borne plant pathogens in the presence of antagonistic organisms has often been attributed to the production of antibiotics by the latter, but little direct evidence in support of this claim has been produced. I. L. Stevenson has studied the antagonism *in vitro* and in soil between various actinomycetes and the root pathogenic fungus *Helminthosporium sativum*. The degree of antibiotic activity of these actinomycete species, tested *in vitro*, was correlated with their power to control the fungus in soil. During the past year a buried-slide technique has been used to demonstrate the effects of a number of antagonistic actinomycetes on the spores and hyphae of *H. sativum* in sterilized soil. In the presence of the actinomycetes, spores either failed to germinate or were inhibited at a point just subsequent to germination. Hyphal changes ranged from the stoppage of further mycelial development to such morphological effects as stunting, distortion or the formation of hyphal protuberances. In all cases the morphological effects were characteristic for each actinomycete and identical to the changes caused by the antibiotics of the actinomycetes in

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in vitro studies. Further direct evidence of antibiotic activity in soil was obtained, using the antibiotic actinomycin and *Streptomyces antibioticus*, the organism responsible for its production. When introduced into soil, identical morphological changes are shown by *H. sativum* in the presence of either the pure antibiotic or *S. antibioticus*. But extraction of the soil containing the actinomycete yielded no antibiotic, strongly suggesting a localized antagonism due to a relatively high concentration of antibiotic in the immediate vicinity of the actinomycetes.

Decomposition of Aromatic Compounds by Soil Bacteria

A comparison of the process of naphthalene decomposition by five soil micro-organisms was undertaken by N. Walker in collaboration with G. H. Wiltshire of the Biochemistry Department and Dr. V. Treccani of the University of Milan. The organisms were of widely different types, comprising a Bacterium, three species of Pseudomonas and one of Nocardia, but nevertheless all were shown to produce naphthalene diol, salicyclic acid and cathechol as intermediate oxidation products. Washed cells of all strains were found also to oxidize 1- and 2-naphthols at suitable concentrations, but in no case were napthols detected in cultures grown on naphthalene. All the organisms were also able to attack 1-chloronaphthalene, producing chloronaphthalene diol, while the Bacterium, one of the Pseudomonads and the Nocardia were all shown to produce 3-chlorosalicylic acid. The constitution of the diol produced by the Bacterium has been established as 8-Chloro-1 : 2-dihydro-1 : 2-dihydroxy-naphthalene. The problem of the identity of the intermediate products between this diol and substituted salicyclic acids is still under investigation.

An organism capable of decomposing parachlorophenoxyacetic acid, but apparently unable to attack 2-4-dichlorophenoxyacetic acid, has been isolated by T. L. Steenson. N. Walker has found that washed cells of this organism give an oxygen uptake with parachlorophenoxyacetic acid and also with 2-hydroxy-4-chlorophenoxyacetic. This supports the suggested pathway of decomposition proposed by W. C. Evans and B. S. W. Smith.

The decomposition of 2·4.D. by a strain of *Flavobacterium aquabile*, isolated from soil, has been further studied. In the presence of washed cells, eleven atoms of oxygen were absorbed per molecule of 2·4.D., but no oxygen uptakes have been obtained either with 2-hydroxy-4-chloro-, or with 6-hydroxy-2 : 4-dichlorophenoxyacetic acid, as substrates. These are thus unlikely to be intermediates in the breakdown of 2·4.D.

Free-Living Nitrogen-Fixing Bacteria

Plots on Broadbalk show very striking differences in yield of wheat in response to fallowing, and it has been suggested that these are connected with the development of nitrogen-fixing bacteria during the fallow period. Accordingly, Jane Meiklejohn has begun a series of periodical counts of these organisms from plots 3, 5, 7, 10, 17 and 18, comparing the fallow section with that longest under wheat. After harvest, high counts both of Azotobacter and of anaerobic Clostridia were obtained, but a drop in numbers of both

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groups followed ploughing. This survey is being continued, and in connection with it an attempt is being made to improve existing methods of counting nitrogen-fixing organisms.

A study is also being made of the acid-tolerant nitrogen-fixing organism Beijerinckia that is found in tropical soils. The strain studied, which was isolated from soil from Tanganyika, will grow and fix nitrogen actively in a medium to which no calcium has been added. At the same time it can be adapted to tolerate calcium by growth in a calcium-rich medium. A further study is being made of the adaptability of nitrogen-fixing organisms to different levels of acidity and calcium content.

Clover Nodule Bacteria (Rhizobium)

Previous work has shown that some strains of clover Rhizobium, initially effective in fixing nitrogen, develop ineffective variant forms when grown in pure culture in certain soils. This effect of soil is clearly of importance to the practice of seed inoculation, owing to the possible danger that an effective culture used to inoculate seed may become ineffective when introduced into soil. Preliminary trials suggested that the appearance of these ineffective variants was related to soil acidity. In the past year Janina Kleczkowska has studied the behaviour of a number of effective strains of clover Rhizobium when grown in liquid and agar media, and in sterilized soil samples from the Woburn plots, the media and soil samples having reactions covering a range of from pH 4·5 to pH 7·5. All the strains tested grew on agar over the whole pH range, but in liquid medium and in soil, only one strain, found originally in an acid turf soil, survived at pH 5·0 or less.

After six months storage the cultures were plated, and colonies chosen at random were tested on the host plant. Two types of bacterial variants were found to have developed, those that no longer produced nodules at all and those that produced ineffective nodules. A higher proportion of variants appeared from agar than from soil cultures, but no correlation was found between the pH of the soil or agar medium and the number of variants. There were also big differences as between different bacterial strains in the percentage of variants produced, so that it should be possible to select genetically stable strains of Rhizobium unlikely to produce ineffective variants in soil.

Rhizobium bacteriophage

Janina Kleczkowska and A. Kleczkowski (Plant Pathology Department) have further investigated the action of ribonuclease and of chymotrypsin in arresting phage multiplication in the presence of the host Rhizobium. Earlier work had suggested that ribonuclease prevented the attachment of phage to the bacterial cell. Further investigation, however, has indicated that this substance does not prevent attachment of the phage, but makes this transitory, the attached phage being quickly released again in a free, active condition. Chymotrypsin is not able to attack free phage, but in the presence of both ribonuclease and chymotrypsin the transitory attachment of the phage is sufficient to allow the chymo-

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trypsin to inactivate it. The mechanism of this inactivation, however, is not yet clear.

European Foul Brood of the honeybee

During the year F. A. Skinner has collaborated with the Bee Department in an attempt to establish the identity of the organism responsible for this disease. Professor L. P. Garrod, Pathology Department, St. Bartholomew's Hospital, London, also kindly assisted in this work by isolating from diseased larvae a small gram-positive organism morphologically resembling the type of cells that predominate in infected larval gut, and to which the name *Bacillus pluton* has been given. F. A. Skinner isolated a second organism of similar morphology. These isolates were tested by L. Bailey (Bee Department), and have so far failed to produce the disease when fed to hand-reared larvae, but the cultures are being maintained for further tests. Meanwhile the use by F. A. Skinner of new media having a high concentration of honey revealed a more diverse bacterial flora in the gut of diseased larvae. Several isolates resembling *B. pluton* have been obtained on these media and are being maintained for testing on larvae in the spring.

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