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Report for 1955

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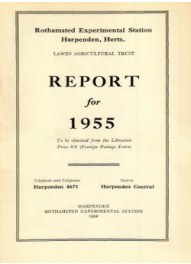
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Soil Microbiology Department

H. G. Thornton

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SOIL MICROBIOLOGY DEPARTMENT

H. G. THORNTON

Miss Janet Findlater resigned her appointment on marriage, and left on 31 March 1955.

Dr. Margaret Brown was appointed to our permanent staff, and joined the Department on 1 October 1955.

Dr. I. L. Stevenson returned to Canada on 22 June after two years' research, for which he was awarded the PhD. degree. His place has been taken by Mr. E. A. Peterson, also from Bacteriology Division, Science Service, Ottawa, Ontario, who arrived on 30 May 1955 on a two-year Fellowship.

Dr. K. M. Pandalai worked in the Department for a few weeks during July and August 1955.

At the invitation of Professor E. B. Chain, Dr. N. Walker visited the International Centre for Research in Chemical Microbiology, Istituto Superiore di Sanità, Rome, where he worked for six weeks.

The Department has investigated various aspects of the ecology of the soil micropopulation.

The general purpose of studying the ecology of micro-organisms in soil is to gain knowledge that can be applied to discourage harmful organisms, such as those producing root disease, and to encourage those that are useful, whether as antagonists to plant pathogens or in producing available plant nutrients.

There are several ways in which the composition of the soil micropopulation can be modified. One is by the addition of materials that encourage the activity of certain groups of organisms. Thus certain types of green manuring are known to control some kinds of root disease. But this method is crude and uncertain in practice, while the factors involved are too complex for precise study. We need at this stage to follow the effects on the micropopulation of adding a definite compound to the soil and preferably one that is not naturally present and that is hence likely to be attacked by only a limited number of organisms. The chlorophenoxyacetic acids satisfy these requirements, and their persistence in soil is also of some practical interest.

DECOMPOSITION OF CHLOROPHENOXYACETIC ACIDS IN SOIL

When a first dose of 2 : 4-dichlorophenoxyacetic (2 : 4-D) or 2-methyl-4-chlorophenoxyacetic acid (MCPA) is added to fresh soil it disappears in a period of some weeks. Successive doses increase this rate of disappearance until 50 p.p.m. will disappear in 4 to 6 days.

Adaptation of the soil microflora to decompose the compound can be induced by as small an initial dose as 1 p.p.m. A considerable degree of cross adaptation can be produced; thus a soil adapted to decompose MCPA is thereby adapted to decompose 2 : 4-D and parachlorophenoxyacetic at enhanced rates.

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This adaptation is no doubt due to the increase in the soil of organisms that can attack the compound; indeed, bacteria that can attack 2 : 4-D, MCPA, and parachlorophenoxyacetic acid have now been isolated and studied in pure culture. In the cases investigated it has been found that adaptive enzymes are concerned in decomposition of the compounds, so that when these are added to soil, enzyme adaptation must precede the differential increase of the specific organisms. Cross adaptation has also been demonstrated in pure-culture studies as well as in the natural soil.

Experiments, both in the laboratory and in the field, have shown that when a soil has become adapted to decompose 2 : 4-D or MCPA, it takes at least six months to lose this adaptation in the absence of any further addition of the compound. Thus the induced change in the micropopulation is remarkably persistent. This change no doubt involves the multiplication of organisms that attack the compound added and also of those that can attack the products of its decomposition. There is evidence that the two organisms principally studied by Warburg manometry do not break down 2 : 4-D or MCPA to completion, but leave end-products whose nature and effects on the soil population are as yet unknown. This work has been carried out by N. Walker and T. Steenson.

The Addition of Trichloroacetic Acid to Soil

Parts of some of the plots on Barnfield were treated in the spring with the herbicide trichloracetic acid at a rate of 20 lb./acre. Dr. Jane Meiklejohn and J. W. Millbank took soil samples from the treated and untreated areas of Plots 1N, 10 and 80 and examined these to see whether treatment with the compound had produced any persistent differences in the micropopulation. They found no differences in total microscope or plate counts of bacteria, in the numbers of Azotobacter or of *Clostridium pasteurianum*, or in the rates at which ammonia or nitrite were oxidized. This was about 8 weeks after the weed-killer was applied.

Clover Nodule Bacteria

A second method by which the soil population can be modified is by the introduction of an organism into the soil. This has been successfully practised in the case of legume inoculation, and a study of the factors here involved is important, not only to this practice but also to the problem of introducing other organisms into soil. Previous work has shown the importance of selecting a suitable strain that can infect the host plant in competition with other strains of lower efficiency in fixing nitrogen. Dr. Janina Kleczkowska has studied the effect of varying ratios of ineffective and effective strains on the growth of clover grown in a sand/vermiculite mixture and supplied with a nitrogen-deficient food solution. Two effective strains were used. One of these, ClF, known to be competitive with other strains, improved clover growth in a ratio of 1 to 1,000 ineffective bacteria and gave an increased effect with higher ratios. The second effective strain 2057 gave irregular results and little improvement in growth.

Experiments were also continued to test growth and survival of clover Rhizobium in soil and media of various pH. The strains

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tested grew and survived for 6 weeks in liquid and agar medium, containing yeast extract at a pH range of 4·5–6·0, but in sterilized soil with or without yeast, strains grew and survived 6 weeks only at a pH of 5·7 or higher. At a lower pH none grew and all died within 2 weeks.

Rhizosphere Fungi

The roots of plants provide a micro environment, the rhizosphere, that is of obvious importance, and we have some knowledge of the bacterial flora of this region. Comparatively little is known of rhizosphere fungi other than pathogenic forms. E. A. Peterson has commenced a study particularly of the fungi of the rhizospheres of clover from clover-sick and healthy soil from Woburn. This work is in its early stages, but preliminary results suggest that there are differences in fungal population of the clover rhizosphere from healthy and from clover-sick soil.

Azotobacter and Clostridium

The organisms responsible for non-symbiotic nitrogen fixation in soil have always been a problem. The present practice of fallowing successive stages of Broadbalk each year and the observation that this fallowing particularly benefits the plots not receiving nitrogen dressings suggests the use of this field to obtain data on the relation of fallowing, fertilizer treatment and crop yield to the number of nitrogen-fixing organisms. Jane Meiklejohn has commenced a survey of the content of Azotobacter and of *Clostridium pasteurianum* from various Broadbalk plots. Data so far obtained show that numbers of Azotobacter are higher in the fallow than in the cropped sections so far examined, but counts made by an improved method specific for Azotobacter gave very low numbers ranging up to only 2,000 per gram. These were much exceeded by the estimated numbers of Clostridium, whose importance in nitrogen fixation may be greater than has usually been supposed.

Some soils from the Bukoba district of Tanganyika were also examined for nitrogen-fixing organisms and yielded the tropical form Beijerinckia. Two of these samples appeared to fix nitrogen poorly owing to molybdenum deficiency.

Jane Meiklejohn and Dr. K. M. Pandalai made experiments on the associated growth of Azotobacter and Nitrosomonas in synthetic liquid media. In media with a low sugar content the Azotobacter grew for a short while only, and then appeared to secrete ammonia that was oxidized by the Nitrosomonas. If this secretion occurs in field soil it could result in the loss of fixed nitrogen by leaching.

Bacteriophage

The study of the interaction of bacteriophage and Rhizobium has been continued by Janina and A. Kleczkowski. Following on their study of the effects of ribonuclease and chymotrypsin on the interaction, they have investigated that of clupein.

When present at concentrations of 0·02–0·05 per cent in the liquid nutrient medium used to cultivate nodule bacteria, clupein rapidly killed the bacteria, slowly inactivated phage, and prevented phage

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and bacteria from combining, or, if added when phage–host combination had already taken place, interrupted further stages of phage–host interaction. At a concentration of 0·0016 per cent clupein acted bacteriostatically, and slowed down phage multiplication but did not stop it.

Trypsin and chymotrypsin hydrolyse clupein, trypsin breaking about twice as many peptide bonds as chymotrypsin. At a concentration corresponding to 0·02–0·05 per cent clupein, the peptides produced by chymotrypsin acted bacteriostatically in the liquid nutrient medium; the peptides inactivated phage much more slowly than did intact clupein, and they inhibited phage multiplication by interfering with the combination between phage and host. When added after phage and bacteria had combined, the peptides did not interfere with further stages of phage–host interaction. The smaller peptides produced by trypsin had no effect on host bacteria, phage, or phage–host interaction, neither did any of the constituent amino acids of clupein when used singly at a concentration of 0·05 per cent.

European Foul Brood of the Honeybee

F. A. Skinner has made a further study of the varied bacterial flora which develops in the gut of the bee larvæ suffering from European Foul Brood.

"Docking Disorder" of Sugar Beet

In the Docking district of Norfolk poor growth of sugar beet is associated with deformation of leaves, together with abnormal root growth in the adult plant, and with less clearly specified seedling damage. The cause of this disorder is not known, but it was suggested that the general soil microflora might be concerned, perhaps in connection with the production of some toxic substance. F. A. Skinner, after discussion with the National Agricultural Advisory Service, examined seedlings in the field and others grown in pots of Docking soil at Rothamsted, but was unable to attribute any particular seedling symptom to growth in disease-producing soil. He also examined the possibility that some toxic substance caused the damage. Soil samples from a badly affected area at Docking were collected and extracts made from these by various methods. The effects of these extracts on the respiration of sugar-beet root tips and on the rate of root extension were tested, but no evidence of any toxic effect was found.