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## Report for 1931

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## Fungal Numbers

### Rothamsted Research

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served the useful purpose of showing which factors increased and which decreased the numbers of bacteria in the soil, though it failed to record many of the groups, so that the results were always low.

A great improvement in the method of counting was made in 1928 by H. G. Thornton and P. H. H. Gray ; direct counts are made from stained films, and the difficult problem of estimating the minute amount of soil involved is overcome by mixing with a weighed quantity of the soil a known volume of a suspension of indigo particles the thickness of which has been determined with a haemocytometer. Bacteria and indigo particles are both counted in the stained films from this mixture, and, from the ratio of bacteria to indigo particles, a simple calculation gives the numbers of bacteria per gram of soil.

The method is not only much more rapid than the older one, but much more complete. The plating method usually gives numbers of the order 10 to 30 millions per gram of soil from our plots ; the new method gives numbers varying from about 1,500 million to 4,000 million per gram of soil. On the Hoosfield barley plots, for example, the numbers varied from 1800 millions per gram in the soil of Plot 1—0 (unmanured since 1856) to 3,600 millions on Plot 4AA (complete artificial fertilisers, including nitrate of soda every year since 1856). Further, the numbers in the different plots varied in much the same way as the yields, so that bacterial counts give some indication of the order of productiveness.

#### *Bacteriological Methods of Assessing Soil Fertility*

In recent years several bacteriological methods have been devised for assessing either the general fertility of the soil or else some special deficiency such as lack of lime or of phosphate. One of the simplest and most elegant is that of Winogradsky and J. Ziemiecka, and fortunately we were able to arrange with the authorities of the Pulawy Agricultural Institute, Poland, for Mme. Ziemiecka to work for some months in our laboratories applying the "plaques moulées" method to the soils of the classical plots. The results gave correct indications as to the presence or absence of adequate phosphate and lime supplies on the plots receiving no nitrogenous manure or only the normal dressings, but not on plots to which heavy dressings of nitrogenous manure were given. Further examination showed, however, that *Azotobacter* was either absent from these soils, or occurred in only small quantities ; when a culture of it was added as part of the test the results came out entirely correctly.

Counts of nitrifying organisms were made from some of the plots, and these showed some relationship with soil fertility.

During the course of her experiments Madame Ziemiecka isolated an organism of considerable interest, whose cells possessed the power of absorbing certain indicators such as Brom Thymol Blue. She also obtained a *Myxobacterium* which attacks cellulose, the first found in our soils.

#### THE NUMBERS OF FUNGI IN THE ROTHAMSTED SOILS

The quantitative methods worked out in the Mycological Department have been used by Jagjiwan Singh for estimating the numbers of fungi and actinomycetes in the Rothamsted soil. The types of fungi were much the same in the differently manured plots, but the numbers both of types and of individuals were always higher on the



more fertile plots. There was no evidence of seasonal fluctuations in numbers, such as have been recorded for bacteria and for protozoa. Barnfield (continuous mangolds, the leaves always ploughed in) contained more fungi but less actinomycetes than Broadbalk (continuous wheat); there was also some difference in the proportions of the fungal population. Barnfield contained more *Penicillia* and *Dematia* but less *Fusaria* and *Verticillia* than Broadbalk.

### VIRUS DISEASES OF PLANTS

This work is carried out by J. Henderson Smith, with the assistance of J. Caldwell, M. A. Hamilton and F. M. L. Sheffield. It falls into three sections:

1. *The Nature of Virus*. Juices extracted from diseased plants are usually themselves infective and remain so after passage through most porcelain filters. By using graded collodion membranes it has been found possible to determine a limiting porosity (varying with different viruses), above which the filtrate remains infective but below which the virus is held back and the filtrate does not cause disease. Again, when infective material is rubbed on the leaves of certain plants, virus enters through the broken hairs and produces a local lesion at the point of entry. If the material is suitably diluted before rubbing on the leaves, infection occurs in only a few of the many broken hairs and only a few local lesions result. Such experiments show that in infective material virus exists in a particulate state and not generally diffused. The size of these particles has been estimated approximately; but it is still uncertain whether the virus is itself particulate or merely attached to other particles. Work is in progress to determine which is the true explanation. Nothing has yet been found incompatible with the view that virus is a living organism.

It is frequently asserted that viruses are invisible stages in the life-cycle of visible bacteria, largely because there is a regular association of specific bacteria with certain virus diseases. We have investigated one such case, and find that when the plants are grown aseptically throughout from sterile seed, inoculation with bacterium-free virus produces the typical disease, and the bacteria usually associated with it do not appear. It is also said that intracellular inclusions which are characteristic of virus disease are either colonies of the virus or visible stages of a usually invisible parasite. The development of such inclusions has been watched in individual living cells from their first beginnings to their complete formation, and in the cases investigated they are essentially aggregates, made up by the coalescence of small particles of cytoplasm which has been locally coagulated or precipitated under the influence of the virus.

A cinematograph film has been prepared showing the whole process. The final form of the inclusion varies with the host plant and with the virus.

2. *The behaviour of Virus in, and its effects on, the host plant*. Within the infected plant the virus does not travel in the transpiration or water stream, nor, indeed, does it normally enter the stream. If deliberately introduced into the xylem vessels, it cannot normally escape; it therefore does not produce the disease, unless and until