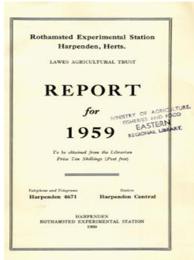
Report for 1959


Rothamsted Experimental Station
Harpenden, Herts.

LAWES AGRICULTURAL TRUST

REPORT
for
1959

Soil Microbiology Department

P. S. Nutman

P. S. Nutman (1960) *Soil Microbiology Department ;* Report For 1959, pp 68 - 71 **- DOI: https://doi.org/10.23637/ERADOC-1-92**

SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

R. Cooper returned after ten months at the Timiryazev Academy, Moscow, and other research centres in the U.S.S.R. Prof. J. M. Vincent of Sydney University worked for eight months on the fluorescent antibody technique. Dr. B. M. Gupta of the Central Drug Research Institute, Lucknow, came in October, to study *Rhizobium* genetics and bacteriophage. Through the International Association for the Exchange of Students for Technical Experience, E. Klar of the Technical University, Charlottenburg, Berlin, spent six weeks helping N. Walker. D. Gareth Jones from the Welsh Plant Breeding Station, Aberystwyth, studied techniques for two weeks with the Rhizobium group. A. H. Gibson returned to the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia, in April, and J. N. Parle to the Rukuhia Soil Research Station, New Zealand, in October; both were awarded the Ph.D. degree of the University of London.

Work on soil fungistasis, the streptomycin-resistant flora of the rhizosphere, the ecology of micro-organisms decomposing 2 : 4-D, the microbiology of earthworm gut and cast and certain aspects of the physiology of infection by nodule bacteria was stopped or suspended.

Work started on the continuous culture of *Nitrosomonas*, the nitrogen-fixing species of bacteria and fungi, the influence on yield of the inoculation of crop plants with *Azotobacter*, the possible transmission of soil-borne viruses by fungi, the microbial decomposition of triazine weed-killers in soil, fluorescent antibody reactions in *Rhizobium* and the glutamine and asparagine content of clover-root nodules.

Nitrification

A continuous culture apparatus, like that developed at the Microbiology Laboratory at Porton, was made by N. Walker and F. A. Skinner for work on nitrification. It embodies automatic control of temperature and pH, and the rate of aeration and medium flow is controlled and measured.

With a clear chemically defined medium (*Rep. Rothamsted exp. Sta.* for 1957, 77) *Nitrosomonas europea* was grown in continuous culture to give cell populations exceeding 900 millions/ml., thus giving gram quantities of dried cells. A cytochrome-like pigment was extracted from such cells, and partially purified; its absorption spectrum closely resembles that of mammalian cytochrome *c*.

When stored at $-18°$ C. cells of *Nitrosomonas* grown in continuous culture rapidly lose their capacity to oxidise ammonia. A rapid fall in pH within the cells seems partly responsible, and washing and storing in buffer solution decreases the loss. (Walker.)

The limited growth of *Nitrosomonas* in flask culture is not increased by organic compounds or by molybdenum. Sodium chloride ($M/6$) and $M/25$ sodium sulphate retard growth, but high concentrations of nitrite are tolerated at pH 8·4; $M/12$ nitrite retards growth, $M/6$ is lethal. A method for counting *Nitrosomonas* and *Nitrobacter* in soil was developed. (Meiklejohn.)

Nitrogen fixation by free-living bacteria and fungi

Some nitrogen-fixing micro-organisms other than *Azotobacter* and *Clostridium* were isolated from soil. These fix nitrogen much more slowly than do *Azotobacter* in culture; they are, however, more abundant in soil and possibly of greater significance. Work started on fixation in the rhizosphere of wheat plants inoculated with a strain of *Aerobacter aerogenes* and an *Alternaria* sp. (which fixed 2·5–3·5 mg. N/g. glucose dissimilated in pure culture). Apparent nitrogen fixation was consistently greater in the inoculated cultures (as preliminarily reported for *Aerobacter* in *Rep. Rothamsted exp. Sta.* for 1958, 71). Experiments were done with wheat seedlings inoculated with *Aerobacter*, but fixation could not be established because of the difficulty in obtaining a true nitrogen balance sheet. Similar experiments with *Azotobacter* inoculated into water cultures of wheat showed higher fixation; this did not benefit the wheat.

Heavy inoculation of *Aerobacter* into unsterilised soil, with or without glucose and mineral salts (other than N-containing compounds) did not increase soil nitrogen, or yields or nitrogen content of plants. (Brown and Jackson.)

Work on the culture of *Aerobacter*, *Alternaria* and *Azotobacter* in the plant rhizosphere showed that plant growth was affected independently of yield. *Aerobacter* stunted wheat roots and suppressed tillering in water culture. In soil *Alternaria* inoculation had a similar but short-lived effect on tillering. At 5 weeks, tillering on inoculated plants was 68% of controls, at 7 weeks 92% and at 9 weeks 98% of controls. (Brown and Jackson.)

Inoculation of soil with Azotobacter

Russian claims that inoculation of soil with *Azotobacter* increases crop yield were studied in pot experiments. Freshly isolated strains of *Azotobacter chroococcum* were used and a range of crop plants and conditions of growth and manuring examined. Inoculation sometimes increased yield significantly, and apparently independently of added nitrate. However, the results were inconsistent and will be reported in more detail when the conditions governing their reproducibility are better defined. (Barford and Burlingham.)

The streptomycin-resistant bacteria of the rhizosphere

Studies on the streptomycin-resistant bacteria of the rhizosphere ended when earlier results (*Rep. Rothamsted exp. Sta.* for 1957, 79) were confirmed in experiments with designs better suited for statistical treatment. Control samples were taken from planted pots subjected to the same fluctuations in moisture content as the rhizosphere samples. The separate effects of rose-bengal and streptomycin were assessed. (Brown.)

Anaerobic decomposition of cellulose and formation of methane

The soil bacterium that decomposes cellulose anaerobically (*Rep. Rothamsted exp. Sta.* for 1958, 68) was compared with other strains isolated similarly and with the type strain of *Clostridium cellobioparum* Hungate obtained from Dr. R. E. Hungate's laboratory, Davis, California. The anaerobic methane-producing bacteria able to use organic acids (especially formic and acetic) produced from cellulose by anaerobic cellulose-decomposers are now being studied. These grow very slowly and are strictly anaerobic, and work was confined to the technique of isolation and culture. (Skinner.)

Mechanism of transmission of soil-borne viruses

The possible rôle of fungi in the transmission of beet ringspot and tobacco necrosis was studied in collaboration with B. D. Harrison (Plant Pathology Department). The root surface fungi of susceptible seedlings grown in infective and non-infective soils from different localities showed no consistent differences. The infection of roots by tobacco necrosis virus was compared in sterile seedlings and seedlings infected with various fungi. (Jackson.)

The metabolism of glutamine and asparagine in root nodules

While at the Timiryazev Academy, Moscow, R. Cooper studied methods of estimation of small amounts of glutamine and asparagine using methods that involved chromatographic separation of amides and their hydrolysis and estimation by Conway titration. The sensitivity of the method is limited by ammonia contamination of chromatography paper, which can be largely overcome by washing with 80% acetone containing 0·002*N*-NaOH, but not with aqueous alkali.

This technique can measure the glutamine and asparagine content of 30 mg. fresh weight of nodules from clover. Effective nodules contained more of these amides than did ineffective nodules, and glutamine concentration increased faster when effective nodules were inoculated with ammonium sulphate. (Cooper.)

Application of the fluorescent antibody technique to quantitative studies with Rhizobium

The possibility of using antibody conjugated with fluorescin to distinguish between individual cells of serologically different strains of *Rhizobium* was studied to see whether it could be applied to the study of growth and strain competition outside the root and to the mechanism of infection. The technique works qualitatively, particularly when unspecific labelled anti-rabbit serum is reacted with the specific serum but is not refined enough for quantitative work. Accurate comparison of dark-ground with dark-ground fluorescent counts requires very good optical equipment. (Vincent.)

Clover root-hair infection in agar and in soil

The early stages of infection of the clover root in soft agar (using the Fåhraeus slide technique) and in finely structured raw soil were compared. Although the sampling of soil-grown seedlings makes observation less accurate, the pattern of infection was shown to be the same in inoculated soil and in agar. Infection in both occurs in two clearly defined stages, and the rate of infection depends on host species and the inoculated strain. In uninoculated soil, infection takes about a week longer, but then occurs at the same rate as in uninoculated soil. The uninoculated soil and the seedling rhizosphere contained many viable Rhizobia. Experiments are being done to determine more precisely how infection of the root hair is influenced by rhizosphere population of nodule bacteria, in attempts to study what " soil factor " delays infection in uninoculated soil. (Lim.)

Host genetics of symbiosis

Red clover plants showing different responses to a phage-induced mutant of the ineffective strain "Coryn" were crossed. (*Rep. Rothamsted exp. Sta.* for 1958, p. 73). Crosses within selections that produce ineffective nodules bred true, those between effective and ineffective types gave mixed progeny, and most but not all offspring of crosses within effective selections were effective. Tests of these plants with the parent strain Coryn showed a similar pattern of response, but some of the crosses contained fewer effectives. With other classical effective and ineffective strains of bacteria the selected lines behaved like unselected red clover. The results suggest that effectiveness towards Coryn is controlled by a dominant host factor or complex.

This genetic factor possibly first appeared in commercial late-flowering Montgomeryshire red clover about 1954. Some local strains of Swedish red clover seem to have the factor for effectiveness, some not. (Kleczkowska.)

Work on variation in symbiotic effectiveness in subterranean clover, begun in Australia, ended. All the 15 isogenic subterranean clover varieties examined depended basically on the same genotypic control of nitrogen-fixation and assimilation, in spite of great differences in other properties. Hybridisation between varieties led to marginally higher yields in F_2, which declined in F_3 and was then lost. These small differences could not be maintained by selection and further hybridisation. Work continued on inheritance of nodule size and abundance in subterranean clover and on selection for ineffectiveness in red clover when inoculated with a normally effective strain. (Nutman.)

https://doi.org/10.23637/ERADOC-1-92