ROTHAMSTED RESEARCH

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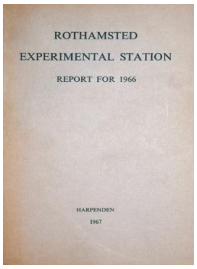
Full Table of Content



Soil Microbiology Department

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

The death of Susan Burlingham at the tragically young age of 29 was a grievous loss to the department and to microbiology. R. M. Jackson left to take up an appointment in the Department of Biological and Health Studies, University of Surrey.

Mr. R. Roughley of the N.S.W. Department of Agriculture, Sydney, joined the department for 3 years, under the auspices of the Australian Wool Board, to work on symbiotic nitrogen fixation. Other visiting workers included Prof. J. M. Vincent of the School of Agriculture, Sydney, N.S.W., who prepared a technical manual for the International Biological Programme (I.B.P.), and Mr. Jörg Pfitzner of Göttingen University, who assisted N. Walker in studies on breakdown of aromatic compounds in soil.

Members of the department attended and presented papers at meetings of the Société Françoise de Microbiologie held at the Institut Pasteur, Paris, in February and at the International Congress for Microbiology held at the Moscow State University in July. J. Kleczkowska and P. S. Nutman visited university and research institutes in Poland and Czechoslovakia at the invitation of the respective Academies of Science. F. A. Skinner spent 3 weeks in Poland as a guest of the Polish Ministry of Higher Education at the State Agricultural High School at Cracow. P. S. Nutman attended I.B.P. meetings at Wageningen and Moscow. Margaret Brown gave a course in soil microbiology to the Microbiology Honours students at University College, London.

Depletion of staff curtailed the department's programme, but work continued on plant-soil microbe interrelations, nitrogen fixation, nitrification and decomposition of natural and synthetic substances in soil.

The classification and ecology of *Endogone* spores. Species of *Endogone* are obligate symbionts causing vesicular–arbuscular mycorrhiza, and they cannot as yet be grown on culture media. Classification by the usual methods is therefore not possible, but spore morphology is constant enough for distinct spore types to be recognised. As a result of an extensive survey of spore populations in some 250 samples of Australian and New Zealand soils, a classification is proposed, based chiefly on cytoplasmic and wall structures, the shape of subtending hypha and spore colour. This classification is supported by fine-structure studies. Nine distinct spore types were recognised, of which seven proved mycorrhizal in inoculation tests; these tests also yielded new information about the life cycles of some of the spore types.

In the soils examined the occurrence of different spore types was not related to species of plants present, to soil type or pH, but showed some

https://doi.org/10.23637/ERADOC-1-14

connection with land usage and rainfall. Spores were much more abundant in cultivated Australian and New Zealand soils, both arable and pasture, than in uncultivated land. Spores seem to develop under conditions of intermittent root growth and their abundance is not necessarily related to the amount of vesicular–arbuscular root infection. For example, New Zealand bush plants are abundantly infected, but the spore population in the soil is small and specialised.

The spore populations of the no manure, dung and N_3PK plots on Broadbalk were surveyed. The different manurial treatments have affected spore populations both quantitatively and qualitatively. Spore populations varied much more in the Broadbalk plots than in the Australian and New Zealand soils, but the same spore types occurred. (Mosse)

Effects of host nutrient status on mycorrhizal infection. Experiments were continued comparing the growth of tomato plants with and without mycorrhizal infection. Endogone sporocarps (40/pot) were used as an inoculum for plants grown in sand or in steam-sterilised field soils (Hoosfield and Sawyers) deficient in phosphorus and nitrogen. Extra phosphate was supplied in the form of bone meal, dicalcium phosphate or calcium dihydrogen phosphate. Contrary to results obtained elsewhere, there were no marked growth differences between mycorrhizal and non-mycorrhizal plants in three separate experiments, but some interesting results were obtained concerning the incidence of infection. A balanced mineral fertiliser was given to a third of the plants, and the remainder received the same fertiliser lacking either nitrogen or phosphate. At the end of the experiment plants given complete fertiliser had less infection and fewer new sporocarps (average of 16% infected roots and eight new sporocarps per plant) than plants not given nitrogen or phosphorus. Mycorrhizal development was much increased by phosphorus deficiency (80% infected roots and 460 new sporocarps per plant), and slightly less by nitrogen deficiency (54% infected roots and 105 sporocarps). This agrees with behaviour in aseptic cultures, where infection occurs only in a nitrogen-deficient medium.

Separate experiments, using tomatoes, onions and hemp as test host plants, showed that infections developed more strongly in soil from Hoosfield (pH 7·6) than from Sawyers (pH 4·9). Both fields are about equally deficient in nitrogen and Hoosfield is slightly less deficient in phosphate than Sawyers. Soil reaction is not thought to be the cause of this difference because the wider survey showed no correlation between soil reaction and the population of Endogone spores. (Mosse)

***Rhizobium* culture collection.** Thirty-three new strains of *Rhizobium* were added to the culture collection and, after testing, these will be included in a supplementary list to the catalogue. One hundred and eighty-one lyophilised cultures (including *Rhizobium*, bacteriophage, *Azotobacter* and *Nitrosomonas*) were issued (more than in any previous year), mainly to university departments and research institutes.

Representative strains of the collection are being re-examined biochemically to provide more critical information on which the genus and related bacteria might be reclassified. (Skinner)

https://doi.org/10.23637/ERADOC-1-14

Time-lapse cinematograph studies of infection of clover root hairs by nodule bacteria. This work had two aims: (1) to examine the initial infection of the root hair in order to resolve the question of how the infection thread initiates and (2) to trace in detail the connection between the migration of the nucleus of the root-hair cell and the growth of the infection thread. The species of plant used were *Trifolium fragiferum*, *T. scabrum* and *T. parviflorum* inoculated with *Rhizobium trifolii* strain 5, grown on a Fåhraeus slide with special provision for watering. Objective magnification was ×40 or ×100, using bright field illumination. The time lapse intervals employed were usually 4 or 8 seconds.

The film showed that the nucleus exists in two main phases that differ in morphology and character of movement. In one form the nucleus is large spindle-shaped (13–24 μ × 8–13 μ), has a prominent nucleolus, about 2·5 μ in diameter, and migrates slowly with amoeboid-like movements. In this phase the nucleus is associated with the growth of the tip of the hair or of a lateral branch (whether normal or "curled") or with the growth in length of the infection thread, when the nucleus attains its largest size. After growth has ceased the nucleus becomes smaller, spherical (diameter 6–8 μ) and moves erratically, apparently carried passively in the streaming cytoplasm, and the nucleolus cannot be easily distinguished. The nuclei in root cap cells shrink to about 5 μ in diameter.

The films have confirmed that the infection thread grows only when invested in cytoplasm with the nucleus nearby, and that the nucleus usually precedes the infection thread as it grows down the root hair into the cortex. The thread stops growing when the nucleus moves away from its growing tip. However, it sometimes aborts with the nucleus remaining nearby, and when this occurs the nucleus becomes surrounded by several spherical bodies (of as yet undetermined origin) and the thread tends to form small lateral processes or branches.

Although many likely sites of infection have been filmed, we have not yet succeeded in recording the earliest stage of thread formation. However, at many of these sites the nuclei remained for a long time in the active phase, and during this process they became surrounded by the spherical bodies noted above, that increase in size, sometimes to a diameter of 4 μ. When the nucleus becomes quiescent these shrink and may become detached and carried away by cyclosis. (Nutman and Dart, with Doncaster, Nematology Department)

Nodule fine structure and histochemistry. Work began on the fine structure of nodules of a range of leguminous species using thin sections of glycol–methacrylate-embedded material observed with the light microscope and electron microscope. The survey includes various red clover lines, in which single host genes determine ineffective development, and cowpea plants inoculated with strains of bacteria whose nitrogen-fixing efficiencies are thought to be temperature sensitive. The plants for this work were grown in controlled environments (A.R.C. growth cabinets). A preliminary report on the red clover survey was presented at the Moscow Congress. (Dart and Nutman)

A method is being developed to study leghaemoglobin in nodules using

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a cytochemical technique based on spectral absorption and peroxidase activity. In collaboration with Dr. J. R. Dennis and Dr. P. Rogers of Harwell an attempt is being made using induced nuclear reactions to find where nitrogen is fixed in the nodule cell. (Dart)

The effect of streptomycin on the symbiotic properties of *Rhizobium trifolii*. Earlier work suggested that streptomycin-resistant variants of nodule bacteria may be more effective in fixing nitrogen than their parent strains. To investigate this further, two kinds of experiment were done. In the first, effective and ineffective strains were grown for various periods on normal media or on media supplemented with streptomycin, and random colonies then taken for testing on red clover plants. Growth on media containing streptomycin increased the variability in behaviour of colonies taken from the ineffective strains but not from effective strains. Parallel tests using media containing phage instead of streptomycin had opposite effects; the ineffective strains became more uniformly ineffective and the effective strains produced some intermediate and ineffective variants.

In the second kind of experiment strains of *Rhizobium trifolii* were grown in the presence of streptomycin and streptomycin-resistant strains selected for subsequent plant tests. The following strains were used: 0403, 5, 220 (effective in fixing nitrogen with red clover); 0411, 30 (intermediate); and 0404, 6 and 33 (ineffective). None of the streptomycin-resistant strains showed any change in their symbiotic responses. The changes noted earlier, therefore, seem independent of the development of streptomycin resistance. (Kleczkowska)

Gibberillin-like substances from *Azotobacter* and the effect of gibberellic acid on tomatoes. Work on estimating gibberellin-like substances in cultures of *Azotobacter*, using chromatography and bioassay, continued, and three groups of substances acting on dwarf peas were found. Because *Azotobacter* inoculation and gibberellic acid (GA3) affect plant growth similarly, a detailed study was made of the effect of applying GA3 to seeds or to roots of tomatoes at a range of concentrations from 5 to 0·0005 μg/plant. Gibberellic acid at 5 μg applied to seeds or roots caused an early acceleration in the growth of young internodes and leaves, without affecting their final size. 0·5 μg of GA3, and sometimes also 0·05 μg, produced similar but smaller effects; the smaller concentration accelerated growth only after a delay. Amounts of GA3 smaller than 0·05 μg had no effect. 5 μg of GA3 affected leaf shape when applied to the roots only, and also slowed flower and fruit development, whereas the smaller concentrations (down to 0·005 μg/plant) promoted faster development.

Treating seed with GA3 produced small responses, and effects were not noticed with amounts less than 5 μg/plant. Stem and leaf growth were affected by 5 μg, which also usually delayed flower and fruit development. Because an *Azotobacter* inoculum is likely to contain much less than 5 μg of gibberllin-like substances, these results suggests that the plant's response to inoculation is to substances produced by *Azotobacter* in the rhizosphere. (Brown)

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The effect of *Azotobacter* inoculation on the rhizosphere flora of wheat seedlings grown in Great Field soil. The rhizosphere flora of wheat plants sampled during the main period of growth of the crop (starting at 4 weeks) was not affected quantitatively by inoculation with *Azotobacter* (*Rothamsted Report* for 1964, p. 94). *Azotobacter* itself multiplies in the rhizosphere of the young plant and then remains constant in numbers or slowly declines. Further rhizosphere counts were therefore restricted to the early seedling phase, and samples were taken at 5, 8, 14 and 21 days after sowing. At 5 days significantly fewer bacteria and actinomycetes colonised the rhizospheres of inoculated than of uninoculated seedlings, whereas between 5 and 8 days their numbers increased faster in the inoculated rhizospheres. Thus, inoculation with *Azotobacter* only temporarily suppresses the increase of these groups of bacteria.

The examination of the fungi present on the root surface, using the Harley–Waid root-washing technique, also showed differences in the early stages of seedling development associated with inoculation, for uninoculated roots were more fully colonised. The predominant fungi on the young root were sterile mycelia, *Cylindrocarpon*, *Mortierella* and *Penicillum*. *Fusarium*, usually regarded as an early coloniser, did not appear until 14 days. The same species of fungi occurred on the inoculated and uninoculated roots. Although the predominant root-surface fungi tended to be the same from experiment to experiment, the less-common species were distributed very irregularly. Sometimes these irregularities seemed to be associated with inoculation, but all attempts to confirm such relationships failed.

To investigate further the short-term effects of inoculation on the composition of the rhizosphere microflora, isolates of actinomycetes and fungi were tested for antagonism against *Azotobacter*, using a modified agar-plug technique in which zones of inhibition were measured. Antagonists were common in the rhizosphere (15–25% of isolates) and were more abundant in rhizospheres containing *Azotobacter* than in those of uninoculated plants. Selected antagonistic and non-antagonistic actinomycetes and penicillia were inoculated into sterile soil together with a standard inoculum of *Azotobacter*, and *Azotobacter* were counted during 2 weeks. The antagonistic actinomycetes greatly diminished the population of *Azotobacter*, but the non-antagonistic actinomycetes and both categories of penicillia had very little effect.

Antagonists to *Azotobacter* were much more abundant in the rhizosphere of plants in field soils than in the surrounding soil, they also increased as the plants aged, so they may be partly responsible for populations of *Azotobacter* diminishing in the rhizosphere of older roots. (Patel)

Clostridia as indicators of anaerobic conditions in soil. Work began to see whether the spore-forming obligate anaerobic bacteria (Clostridia) or their metabolic products could be used to indicate the aeration status of soils. They were chosen because they are widely distributed, proliferate quickly when aeration is restricted, and spores (inactive) and vegetative cells (active) can be counted separately. Counts were made with the liquid

https://doi.org/10.23637/ERADOC-1-14

medium of Gibbs and Freame (*J. appl. Bact.* (1965), **28**, 95–111) using a most probable number estimation.

Volatile acids, such as formic and acetic, result from the anaerobic decomposition of cellulose, and butyric acid arises typically from the anaerobic fermentation of soluble carbohydrates. These are easily extracted from soil, and the small amounts were reliably estimated by gas chromatography. (Skinner)

Microbial decomposition of synthetic chemicals. Two stable colonial forms (a rough and a smooth) of a *Nocardia* isolate that metabolises aromatic hydrocarbons (*Rothamsted Report* for 1965, p. 83) were further studied. They differ from *N. coeliaca* only in forming acid from glucose and in the rough strain being unable to grow on phenol. Another soil *Nocardia* isolate that can metabolise aromatic hydrocarbons and also paraffins was identified as a strain of *N. corallina*. Cells of all these strains grown with ethyl benzene oxidise phenylacetic acid only after a lag phase; hence the early pathway of ethyl benzene metabolism may involve ring-hydroxylated derivatives not yet available for investigation. (Walker)

The possible microbial breakdown of the organo-phosphorus insecticide, parathion, was studied using a collembola bio-assay and gas chromatography to estimate parathion. There is some evidence that parathion disappears faster in soil crumbs in percolators when inoculated with one or other of two strains of *Pseudomonas* isolated from soil, which in pure culture break down *p*-nitrophenol with the liberation of nitrous acid. (Walker, with Griffiths, Insecticides Department)

Nitrifiers. Current methods for isolating nitrifiers are tedious and much time has been spent in trying to improve a plating method suggested by Soriano for isolating ammonia-oxidising autogrophs. Purified agar, sterilised at about pH 6–7, is incorporated in a clear mineral salts medium and poured into plates, which are then surface inoculated from dilutions of soil-enrichment cultures. After inoculation, the very small colonies of nitrifiers are picked off by micro-manipulation. Reliable results have not yet been obtained, and the reasons are being investigated. (Walker)

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