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ROTHAMSTED RESEARCH

Report for 1972 - Part 1

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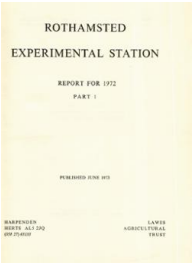
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PART 1

Soil Microbiology Department

P. S. Nutman

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

Soil micro-organisms have many effects on crop production. A few cause root disease or are in other ways inimical to healthy root development, but most mediate necessary chemical and biological changes in soil that increase nutrient uptake and promote plant growth. A large part of this Department's programme is concerned with microbial activities affecting the cycling of nitrogenous and phosphorus-containing compounds in temperate and tropical soils, especially those seriously deficient in these plant nutrients. Some of the work is on a wide range of problems concerning biological nitrogen fixation and we collaborate with the Universities of Cambridge, London and Reading, the Plant Breeding Institute, Luton College of Technology, the Agricultural Development and Advisory Service and the Overseas Development Administration.

Work continues on the microbiology and chemistry of the breakdown of herbicides and related compounds, on soil microbiological aspects of 'take-all' of cereals (associated with the Plant Pathology Department) and on cellulose decomposition. The International Biological Programme supports work on mycorrhizal ecology and on the fixation of nitrogen by soil algae.

Mycorrhizal studies

Earlier work described the capacity of vesicular–arbuscular (VA) mycorrhiza, caused by the fungus *Endogone*, to improve the phosphorus nutrition of the host plant. Our current programme concentrates on this aspect of the symbiosis by examining the extent, intensity and limitations of mycorrhizal infection in natural and agricultural habitats and by investigating possible mechanisms of mycorrhiza-stimulated phosphorus uptake.

***Endogone* in Great Field IV soil under arable cultivation.** *Endogone* spores were predominantly of the laminate type; some resembled *E. fasciculata*. The barley plots, especially those with intermediate amounts of phosphate fertiliser contained many spores (260–335/100 g soil). Fewer (205–220/100 g soil) were found in plots given no phosphate, Gafsa rock phosphate or much superphosphate. The potato plots with intermediate amounts of phosphate fertiliser contained more spores (210/100 g soil) than other phosphate treatments (90–140/100 g soil). In the swede plots spore numbers were less (about 30/g soil) and not affected by phosphate. Roots of potatoes and barley were mycorrhizal but not those of swedes, which therefore did not stimulate spore production in the soil. The main weeds in these plots belong to the Chenopodiaceae, a non-mycorrhizal family. Pot experiments using an inoculum of *Endogone* spores confirmed that swedes were not mycorrhizal. In pots containing swede and onion seedlings growing together, the onions were poorly infected compared to those in pots planted only with onions, suggesting that swede roots release substances that inhibit *Endogone* infection. (Hayman)

Effects of VA mycorrhiza on phosphate cycling at Meathop Wood, Lancashire. Further studies were made of the occurrence of mycorrhizal roots in nine plant species at this site. No quadrat contained a consistently higher proportion of infected roots of all species. About 50% of the roots of most species had some mycorrhizal infection but in *Mercurialis perennis* and *Lonicera periclymenum* nearly all roots were infected.

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The contribution of mycorrhiza to phosphate cycling at Meathop Wood cannot be studied by direct comparison of inoculated and non-inoculated plants in unsterile soil because of the large content of *Endogone* propagules. If the indigenous fungi are killed by gamma irradiation the very large improvement in growth following inoculation does not necessarily reflect natural conditions, where the endophyte is competing for phosphate with other microbes and plant roots. However, the weights of non-mycorrhizal seedlings of strawberry grown in natural and irradiated soil were found to be the same for as long as the latter remained uninfected (up to three weeks). We therefore compared the total phosphorus uptake of non-mycorrhizal seedlings grown in irradiated soil with uptake of inoculated plants in unsterilised soil. Autoclaved roots and root washings were added to the control treatments. The test plants were seedlings of *Viola*, *Fragaria* and *Brachypodium*, rooted cuttings of *Rubus* and of one-year-old *Fraxinus* seedlings. Inoculated *Fragaria* grown in unsterile soil weighed nearly four times as much as uninoculated plants grown in irradiated soil, and inoculated *Viola* nearly three times as much as uninoculated plants. Measurement of uptake of phosphorus from soil solutions labelled with carrier-free ^{32}P showed that 96% of the uptake in *Fragaria* and 94% of the uptake in *Viola* was attributable to the mycorrhiza. Growth of *Rubus* was not improved by mycorrhiza but infected plants contained twice as much phosphate.

In other experiments in irradiated soil the growth of plants inoculated with indigenous mycorrhizal fungi was compared with that of uninoculated plants given calcium phosphate ($Ca(H_2PO_4)_2$) approximately equivalent to 220 kg P/ha. Mycorrhizal plants of *Brachypodium* weighed 17% more, onions 100% more and *Fraxinus* and *Rubus* plants respectively 13 and 30% less than phosphate-fed plants. Birch seedlings inoculated with ectomycorrhizal birch roots were about five times as large as uninoculated seedlings, but only half as large as those given phosphate. Uninoculated seedlings in unsterile soil remained very small and were without mycorrhiza.

The source of extra phosphorus in mycorrhizal plants. Earlier work showed that phosphate taken up by mycorrhizal and non-mycorrhizal onions from soils labelled with carrier-free ^{32}P had the same specific activity, indicating that mycorrhizal plants had not mobilised any of the soil phosphate that is normally unavailable (Hayman & Mosse (1972), *New Phytologist* **71,** 41). Further experiments with three other plant species grown in four other soils containing even less available phosphorus than in the original experiments gave similar results. *Centrosema pubescens* and *Paspalum notatum* contained no ^{32}P activity unless they were mycorrhizal; non-mycorrhizal *Paspalum* took up some labelled phosphorus if the soil was limed. It is not yet known whether P was taken up only through the external hyphae associated with the mycorrhizal roots or whether the normal process of nutrient uptake into the root can be modified by the infection.

That a mycorrhizal plant reduces the 'available' phosphate in the soil was shown by growing a second non-mycorrhizal crop. Radishes grown after mycorrhizal strawberries weighed only one quarter as much as those following non-mycorrhizal strawberries; adding phosphate much increased the growth of both sets of test plants. (Mosse, Hayman and Arnold)

Influence of *Endogone* strain on phosphorus uptake. In further work on this topic seven endophyte strains were examined with onions and four with *Paspalum notatum*, both grown in P-deficient irradiated soils. Plants inoculated with the best strains, such as E3, were 3–4 times heavier than with other strains and 5–10 times heavier than uninoculated controls. Similar differences were found in other experiments with the grass *Melinis minutiflora* grown in three Brazilian soils. In unsterilised soils also, the better strains produced larger effects on growth. Thus in Meathop Wood soil *Viola* seedlings inocu-

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lated with E3 weighed five times more, and those inoculated with yellow vacuolate spores 30% more than those picking up natural infection or inoculated with the indigenous fungi. Ash seedlings inoculated with E3 weighed 73% more than those inoculated with the indigenous fungi. With *Fragaria* in unsterilised soil there were no effects with any inoculum.

The observation that yellow vacuolate spores do not become established in acid soils (*Rothamsted Report for 1969*, Part 1, 95) has been found to apply to two other soils and to two additional endophytes, a laminate spore type and a strain from Brazil. In acid soils *Paspalum* and *Melinis* benefited more from the honey-coloured spore type than from other inocula. (Mosse)

The effects of light intensity on VA mycorrhiza. The development of VA mycorrhiza in onion roots is markedly affected by light (*Rothamsted Report for 1970*, Part 1, 89). Further experiments compared early infection of onion roots grown in a 16-hour day and exposed to different light intensities, viz, high intensity (25 000) lux, low intensity (8000 lux), and near darkness. Infection became established fastest in seedlings both raised and grown after inoculation in most light; there were traces of infection after four days. Those grown throughout in low light had much less infection and those in near darkness never became infected although hyphae were sometimes seen on the root surface. Plants grown in lower light treatments before inoculation and then given full light had fewer infections during the first week but after two weeks had as many as plants grown all the time in high intensity light. The amount of mycorrhizal infection was closely correlated with the amount of soluble carbohydrate in the seedling roots before inoculation. (Hayman)

Trehalose and mannitol in VA mycorrhiza. The carbohydrate composition of mycorrhizal and non-mycorrhizal roots of *Fuchsia*, *Nardus* and *Coleus* were examined by chromatography of 80% ethanol extracts in appropriate solvents. Neither trehalose nor mannitol were detected in mycorrhizal or control roots. The limits of sensitivity of the assay are respectively 0·075 and 0·015% of the fresh weight. VA mycorrhizal roots thus differ from ectotrophic mycorrhizal roots of beech which contain readily detectable amounts of these carbohydrates. (Hepper and Mosse)

Association between VA mycorrhiza and *Azotobacter*. *Azotobacter chroococcum* and *A. paspali* (originally from Brazil) were introduced into the rhizospheres of VA mycorrhizal plants grown in nutrient-poor habitats: dune sand from Studland, Dorset, and phosphate deficient soils from Brazil.

Roots of *Ammophila arenaria* from Studland dunes are heavily infected with VA mycorrhiza but do not carry *Azotobacter chroococcum*. Using various methods of inoculation, both species of *Azotobacter* and VA mycorrhiza were established in the rhizosphere and roots of Ammophila; but to different extents. Neither Azotobacter nor mycorrhiza improved growth; the seedlings responded only to added phosphate.

We compared the effects of phosphate and mycorrhiza, with and without added glucose, on the establishment of *Azotobacter paspali* in the rhizosphere of *Paspalum notatum* (Batatais) grown in irradiated soil. In all treatments numbers of *Azotobacter* declined. In treatments given phosphate they decreased from 15×10^4/g soil to 100/g soil within a week, but in mycorrhizal plants they decreased much more slowly, reaching 30/g soil after eight weeks. At this stage no *Azotobacter* were recovered from plants given phosphate. Glucose hastened the decline in both treatments.

In sand *Azotobacter* declined more slowly than in soil and adding phosphate did not

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affect this. Adding phosphate and glucose reduced the survival rate. (Barea, Brown and Mosse)

Studies on legume nodulation

***Rhizobium* culture collection.** The collection now contains 357 freeze-dried cultures; 170 of *Rhizobium trifolii*, 47 of *R. leguminosarum*, 34 of *R. meliloti* and 106 of other *Rhizobium* spp., all tested on one or more hosts. Cultures are also maintained of *Rhizobium* bacteriophage, *Nitrosomonas*, *Nitrosocystus*, *Agrobacterium*, *Azotobacter*, *Beijerinckia* and *Rhodopseudomonas*. During 1971 and 1972, 552 cultures were issued, compared with 321 in 1969–70, usually with advice on strain properties or on procedures for freeze drying, inoculation, etc. (Pattison)

An international catalogue of strains of rhizobia listed in collections in many countries is being prepared under the auspices of the FAO and the International Biological Programme. Material for the catalogue has been collected by Dr. O. N. Allen, Madison, Wisconin, U.S.A., and Dr. Eva Hamatova, Prague, Czechoslovakia, and is now being assembled and edited for publication early in 1973. (Skinner)

Genetics of red clover nodulation. The infection of clover by pea nodule bacteria, normally an uncommon event, was much increased by breeding from nodulating plants. Families raised from two nodulating parents were 10–90% nodulated, whereas those with both parents non-nodulating were usually without nodules. The progenies of crosses between unlike parents were 5–70% nodulated. These results suggest that many genes are concerned in the susceptibility of clover roots to infection by pea nodule bacteria.

Such nodules were generally sparse and small, and invariably ineffective in fixing nitrogen. Infection threads were abundant in the central parts of the nodules but no bacteroids were seen. An extensive selection and breeding programme was completed in 1972 from which it is hoped to elucidate further the genetic and physiological barriers to cross infection. (Hepper)

Work has continued on breeding for increased nitrogen fixation in red clover. We have confirmed the large increases in nitrogen fixation in plants bred from highly effective parents, the results suggesting that this depends upon the cumulative effect of improvements in several symbiotic characteristics. In this work the moderately effective strain 0403 has been employed. A similar programme has been started, and the initial selections and crosses made, to increase if possible nitrogen fixation in red clover inoculated with our most effective clover strain. (Nutman)

Influence of yeast extract on *Rhizobium trifolii*. Most strains of *Rhizobium* require biotin for growth on laboratory media; a good source is yeast extract (YE), usually at 0·1%. Yeast extract at higher concentrations causes large reductions in the viable count and increasing pleomorphy, effects which partly depend upon the source of extract (*Rothamsted Report for 1969*, Part 1, 101; Date, *Journal of Applied Bacteriology* **35**, 379–387). Our earlier work employed a strain of *R. trifolii* (TA1), later found to be lysogenic, and the study has now been extended to include a non-lysogenic variant of this strain and lysogenic and non-lysogenic cultures of *R. trifolii* strain 0403 as well as strains of other species of *Rhizobium*.

Cultures were grown in media containing 0·1 or 0·5% YE as sole source of nitrogen and growth factors, with or without aeration by shaking; total and viable counts were done at 48 hours.

The total and viable counts of lysogenic and non-lysogenic strains of TA1 in both media were less in still than in shaken cultures. In still cultures containing 0·1% and

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0·5% YE the viabilities of lysogenic TA1 were, respectively, 78·6 and 17·5%, but the non-lysogenic strain was less affected by the higher concentration of YE (viability 50%). The viability of the 0403 strains was also reduced by poor aeration and was much less than that of TA1. The non-lysogenic strain was more affected by high YE concentration than by poor aeration; all 0·5% YE cultures had viabilities of less than 2%. Distorted cells occurred in all 0·5% YE cultures and were more abundant in still culture, but to different degrees in the two pairs of strains.

These results suggest the inadvisability of using lysogenic strains for inoculant production, where large, viable populations are essential, or relying on total counts in cultural studies. (Skinner)

Effective and ineffective strains of other cross-inoculation groups were examined similarly. Effective and ineffective *Rhizobium leguminosarum* (strains 1001, 1003) grew normally with 0·1% YE but were severely inhibited by 0·5% YE in aerated culture and did not grow in still culture containing this amount of extract.

Strains of *R. meliloti* (2001, 2006) were almost completely unaffected by 0·5% YE; only a few cells of strain 2001 showed some distorted growth. Strains of *R. lupini* (3201, 3208), *R. phaseoli* (3601, 3602) and *R. japonicum* (3001 and 3206) all produced abnormal cells with the more concentrated extract; strains varied greatly in the response to high YE but there was no correlation between sensitivity to yeast extract and effectiveness. (Skinner)

The fine structure of cells of *R. trifolii* TA1 grown in media containing 0·1, 0·5 or 1·0% of yeast extract (YE) or of casein hydrolysate was examined by electron microscopy of thin sections.

With 0·1% YE (Difco or Vegemite) cells were typically rod-shaped (1–2 μm long), each containing a large central granule of poly-β-hydroxybutyrate (PHBA) or up to four smaller ones, often a polar polyphosphate granule and some glycogen. At the cell periphery the outer wall membrane overlaid a relatively thin rigid layer, but the zone between this and the plasma membrane was packed with material of intermediate electron density, sometimes as discrete granules.

The polyphosphate and glycogen granules were similar in all treatments; the former spherical (*c.* 0·15 μm in diam.), of homogeneous electron density even without metal staining. Glycogen granules were angular (*c.* 0·05 μm across) with granular substructure.

Yeast extract (Difco) at 0·5% induced cell enlargment and the development of many club- and Y-shaped bacteroid-like forms. Vegemite (0·5%) caused little cell distortion but increased the numbers of granules of all three types. Both extracts at 1% produced greatly enlarged pleomorphic cells (2–5 $\times$ 1·5–3·5 μm) each containing several PHBA and polyphosphate granules, whorls of intracytoplasmic membranes, myelin-like figures and invaginations of the peripheral cell membranes.

Casein hydrolysate induced pleomorphy more strongly than the yeast extracts at corresponding concentrations; at 0·5%, distorted cells resembled those formed by YE at 1%, many cells of which were completely disorganised.

Evidently, small changes in the medium can induce marked cell changes in *Rhizobium* from rod forms through bacteroid-like forms to grossly distorted cells many times the size of the rods. (Dart, Chandler and Skinner)

Effects of antibacterial substances on *Rhizobium*. Two strains each of *Rhizobium leguminosarum*, *R. meliloti*, *R. japonicum*, *R. trifolii* and *R. lupini* and a soil inoculum (from Broadbalk plot 3) were tested for ability to grow on yeast extract mannitol medium (YMA) containing inhibitors selected to suppress growth of micro-organisms other than *Rhizobium* (*Rothamsted Report for 1971*, Part 1, 96). The inhibitors were brilliant green, 0·5 ppm; sodium azide, 0·5 ppm; and pentachloronitrobenzene (PCNB) suspended

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in Triton X 100 solution instead of sodium lauryl sulphate as used previously. This modification made the medium virtually non-inhibitory to rhizobia, except to one strain of *R. japonicum*, whilst retaining inhibition to many other soil micro-organisms. Addition of congo red (1 : 40 000) had no detectable effect but penicillin at 1 IU/ml caused a marked decrease in numbers, especially of *R. japonicum*.

This medium, with penicillin, was tested for its value in recovering strains of *Rhizobium* added in known amounts to Broadbalk soil. After overnight incubation counts were made on this medium, on YMA without inhibitors and by inoculating sterile-grown seedlings of the appropriate host with suspensions of soil containing the added bacteria. Rhizobia in control soil were also counted in order to determine the original numbers present. Growth of all strains was inhibited to some extent in this medium and the recovery of rhizobia from the plant-infection test was higher even than from the YMA without any additions. The inclusion of penicillin renders the medium too inhibitory to rhizobia especially when used with the other inhibitors so far found to be satisfactory. The medium is now being used with smaller concentrations of some inhibitors and improved methods are being sought to differentiate colonies of rhizobia from agrobacteria.

The selective medium described by Graham (*Applied Microbiology* (1969), **17**, 769) was also examined using the above strains of rhizobia and soil suspensions but was found to inhibit completely all rhizobia and strongly inhibit the growth of other soil microbes. (Pattison and Skinner)

Nodulation of excised roots of red clover. Decapitated plants of some large-seeded legumes are reported to nodulate (Raggio, M. & Raggio, N. (1956) *Physiologia Plantarum* **9**, 466) but there are no reports of smaller seeded species being nodulated by this technique. This has now been achieved using the following method to ensure the physical separation of the bacteria from the carbon source required by the roots. The radicals were removed from one-day-old seedlings just below the hypocotyl and these were placed on plates of standard agar root culture medium containing sucrose. The plates were placed vertically to allow the roots to grow across a 2 mm gap on to an isolated area of distilled water agar inoculated with the bacteria. No nodulation was observed when nitrate was present in the culture medium. In the absence of nitrate, nodulation was infrequent at 25°C in the dark but about 20% of the roots nodulated under normal laboratory light and temperature conditions. The nodules on isolated roots formed by the normally effective strain 0401 appeared to be ineffective; infection threads were present in the nodules and bacteria were released into the cells from vesicles. (Hepper)

Nodulation of test-tube grown *Trifolium subterraneum*. Growth and nitrogen fixation by plants grown in cotton wool stoppered tubes was poor compared with those grown in sand in pots, partly because of accumulation of ethylene (*Rothamsted Report for 1971*, Part 1, 99). Nodules on such plants were smaller and leghaemoglobin took longer to form than for pot-grown plants, and their uninfected meristems were relatively larger due to slower release of bacteria from the infection threads. Bacteroid differentiation was was also slower and this was associated with less space initially between bacteroid and enclosing membrane envelope. Bacteroids later became characteristically oval in contrast to the more ellipsoid form in pot-grown nodules. Tube-grown nodules had little nitrogenase activity and commonly degenerated completely 30 days from sowing, whereas those in pots had degenerated little by 40 days.

The rate and mode of bacteroid differentiation varied considerably from cell to cell and even within the same cell, contrary to the regular pattern of development found for pot-grown nodules. The host cell vacuole was often displaced from its normal central position, so that it abutted the cell wall.

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In tubes from which ethylene was continuously removed, nodules resembled those of pot-grown plants except that they were smaller and degenerated slightly earlier. (Day, Dart and Chandler)

Structure of nodules of peanut and *Vigna* spp. Infection threads occur only in very young peanut nodules, and then very infrequently. Dissemination of *Rhizobium* throughout the nodule is mainly by the division of the young nodule cells containing the small rod-shaped bacteria, themselves undergoing rapid division. The latter are enclosed singly in membrane envelopes, the space between the rod and envelope being filled as the bacteria mature into the large ovoid bacteroids (*c.* 3–5 μm in diam.). Bacteroids have prominent intracytoplasmic membranes joined to the plasma membrane, a condensed nucleoid and occasionally PHBA granules. Electron microscope micro-analysis showed that the legaemoglobin lies outside the membrane envelope.

Older peanut nodules have large segments of bacteroid-containing cells, separated by invaded cells containing vascular traces, which do not have transfer cells. Small meristematic areas lie at the periphery of the bacteroid-filled segments. Within the bacteroid zone there are large empty cells joined into a channel continuous with the outer husk but within the endodermis. The husk cells contain much starch and oil and protein granules, but only the young bacteroid-filled cells sometimes contain very small starch grains.

In the nodules of *Vigna unguiculata*, *V. mungo* and *V. radiata* the rhizobia are disseminated by infection threads, occurring initially as rods usually singly within membrane envelopes. At first the division of the bacteria and envelopes are coordinated but later the bacteria divide more often so that each envelope comes to contain several bacteroids. The bacteroids are long thin rods (*c.* 3–5 μm $\times$ 0·2 μm) sometimes containing PHBA and no intracytoplasmic membranes. (Chandler and Dart)

Effects of temperature and day length on the symbiosis of some tropical grain legumes. The soyabean varieties 'Grant' and 'Kent' were examined at the same temperatures (33, 27 and 21°C) and with the same strains of nodule bacteria as previously used for the variety 'Chippewa' (*Rothamsted Report for 1971*, Part 1, 97).

At 33°C the strain CC705 performed poorly with both varieties; after an early peak of nitrogenase activity very little nitrogen was fixed although the nodules had much leghaemoglobin. However, at this temperature 'Kent' with CB1809 fixed almost as much nitrogen as at 27°C. CB1809 also fixed more nitrogen with both varieties at 27°C than the other strains, and relative to the amount of nodule tissue formed was almost twice as efficient. At 21° CB1809 was much more effective with 'Grant' than were the other strains, especially in the early stages of growth. The strain Sm1b induced severe chlorosis in the variety 'Grant' at 21°C, but not at 27° or 33°C. (Day and Dart)

Three cultivars of *Cicer arietinum* (Deshi, Kabuli and a variety from Iran) were examined at root temperatures of 23° and 30°C, using one or other of five strains of *Rhizobium*. All strains produced most dry matter, nodule tissue and nitrogenase activity at 23°C but at this temperature some strains benefited certain varieties more than others. Strains CB1189, 27A9 and Ca1 were the best strains for Deshi, Kabuli and the Iran variety respectively. At both temperatures plant yield and nodule weight were correlated, but at 30°C the nitrogenase activity was not directly related to yield. Strain Ca2 from India produced most dry matter, nodule weight and nitrogenase activity on all three hosts at 30°C. A more detailed study was made with the Kabuli variety and the strains Ca2 and 27A2. This showed Ca2 to be more effective at 30°C particularly from the flowering stage on and to produce as much nodule tissue at this temperature as at 23°C.

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For both strains nitrogenase activity per plant was greatest at seven weeks at both temperatures, but maxima of activity per g of nodule tissue occurred at different times in the different treatments.

The Deshi and Kabuli cultivars, inoculated with strain CB1189, were grown in 11-hour or 20-hour photoperiods (11-hour photosynthetic period) at a day temperature of 23°C. The short-day plants formed more nodule tissue and their nodules reduced acetylene more rapidly per g nodule than long-day plants; they also eventually weighed more and were more branched. Flowering at 29 days in long days and at 35 days in short days had no influence on nitrogenase activity of either cultivar. In both day lengths this reached a maximum at seven weeks; little difference was found between cultivars. (Islam and Dart)

Earlier work showed that it is the formation of the nitrogen fixing system and not nitrogenase activity *per se* that is inhibited by root temperatures above about 30°C. Such temperatures occur commonly in tropical soils where legume yields are often poor. The damage caused by elevated root temperatures could be reduced by using more heat tolerant cultivars and bacterial strains and by mulching and irrigation.

It was not possible to grow normal plants of *Vigna radiata* and *V. mungo* in coarse sand watered with nitrogen-free nutrient solution in either a glasshouse or in controlled environment cabinets. Necrotic spots appeared on the unifoliate first leaves of three-week-old plants, these increased and in a few days the leaves were shed, and the plants either died or remained very stunted. Adding a range of inorganic nitrogen sources and other nutrients to the nutrient solution did not overcome the symptoms although with some treatments the necrotic primary leaves were not shed and a leaf crinkle developed at the margins of the trifoliate leaves; growth was poor.

Additions of extracts of organic matter or soil did not alleviate the symptons, nor did sphagnum moss, sedge peat, John Innes and Levington compost, vermiculite, perlite and charcoal. The symptoms occurred whether or not the plants were inoculated with *Rhizobium*. (Islam and Dart)

Leghaemoglobin. Leghaemoglobins (Lb) prepared from different cultivars of soyabean ('Lincoln', 'Chippewa', 'Norchief', 'Altona', and 'Merit') were similar. They contained the same two components when analysed either by DEAE-cellulose chromatography or polyacrylamide-gel-electrophoresis, the latter indicating a high degree of purity in the samples isolated on DEAE-cellulose. However, electron paramagnetic resonance spectroscopy (EPR) of the Lbs showed a large signal at $g = 2$ in addition to the expected haemoglobin signal at $g = 6$ at liquid nitrogen temperature. The latter signal increased in intensity at liquid helium temperature (10°K) whereas the $g = 2$ signal did not. This 'impurity' may be an iron complex or a low molecular weight iron-containing protein.

Purified cowpea Lb consists of a single component which gives EPR spectra similar to those of soyabean and with a similar intense signal at $g = 2$ at liquid nitrogen temperature. The amino acid contents of different Lbs were not the same and none of the amino acids contained sulphur. *Vigna mungo* nodules had a single Lb component but *V. radiata* had two components.

Nitrogenase activity and Lb concentration were compared in soyabeans ('Chippewa') inoculated with different strains and grown at different temperatures; amounts of nitrogen fixed were also determined by Kjeldahl analysis.

Amounts of Lb per plant increased almost linearly with time. Strain CC705 had more Lb/g fresh weight of nodules than CB1809 at 27°C and also more at 33°C except in the last stages of growth. At 33°C, CB1809 fixed much more N per plant than CC705, showing that there is no direct relationship between the rate of N fixation and Lb concentration at this temperature. Acetylene reduction/g F.wt. nodule actually decreased

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for both strains at 33°C while the Lb concentration increased. (Maskall and Dart, with Dr. J. F. Gibson—Imperial College)

Nitrogen fixation by free living micro-organisms

Algal fixation in Rothamsted fields. Cultures of *Nostoc ellipsosporum*, *N. punctiforme* and *Anabaena cylindrica* and a mixture of all three, were applied to plots on Great Field II (under winter wheat) in May with and without nitrogen fertiliser (80 kg N/ha). Nitrogenase activity was determined at intervals using steel tubes driven into the soil sealed with plastic covers and provided with sampling ports.

N. ellipsosporum was the most active nitrogen fixing culture over the whole season; *N. punctiforme* was only 33%, *A. cylindrica* 62%, mixed inoculum 46% and uninoculated 15% as active as *N. ellipsosporum*. Most activity occurred in August (74% of the total for the season) and for *N. ellipsosporum* this extrapolated to 32 g N/ha/h at the most active period when the other inoculated plots fixed between 5–13 g/N/ha/h and the uninoculated plots 2·6 g N/ha/h.

On a seasonal basis N-fertilised plots were almost twice as active in fixing nitrogen as non-fertilised plots, although activities were similar early in the season. Late-season activity was also increased by using a suspension of the culture in water as inoculum rather than distributing it in a dry form using sand as a carrier.

By extrapolation, the maximum amount of nitrogen fixed (by *N. ellipsosporum* on plots given N fertiliser) during the season was estimated to be about 10 kg N/ha. Grain yield was not affected by algal inoculation.

The numbers of blue-green algae counted on the plots, using a dilution plate method, correlated well with nitrogenase activity. In June the inoculated algal species were recovered from all the treated plots, along with mosses and many non-fixing blue-green algae of the *Lyngbya* type. In August, however, *N. punctiforme* was not recovered and only a few *A. cylindrica* were found.

Nitrogenase activity was also monitored throughout the season on Broadbalk plots nos. 3, 5, 6, 7, 9 and 2B, which were not inoculated. The highest measured activity was 68 g N/ha/h in June on plot 5 (minerals, without N). Activities on plot 6 (minerals and 45 kg N) and plot 7 (minerals and 96 kg N) increased during the season, possibly reflecting the declining levels of soil nitrate. Drying of the soil during July sharply reduced activity but this could be restored within two hours by wetting. Estimated amounts of nitrogen fixed during the season ranged from 0·5 kg N/ha (plot 9) to 17 kg N/ha (plot 5). Little nitrogen appeared to be fixed by blue-greens on the FYM plot (No. 2B).

Nostoc ellipsosporum was the dominant species on the plots examined, but *Cylindrospermum* sp. and *N. punctiforme* were also present. (Mr. P. J. Froggatt, Mr. P. J. Keay and Mr. J. F. Witty (Luton College of Technology) with Dart and Day)

Nitrogen fixation associated with the roots of tropical grasses. Clumps of grass with soil attached were collected from the Institute of Agricultural Research, Ahmadu Bello University, Samaru, Northern Nigeria, at the beginning of the wet season, flown to Rothamsted and grown on in pots. After a month the potted plants were assayed for nitrogenase activity. The pots containing *Setaria anceps*, *Cymbopogon giganteus* and *Hyparrhenia rufa* were as active as plants of *Paspalum notatum* previously examined (*Rothamsted Report for 1971*, Part 1, 95). *Paspalum commersonii*, *Andropogon gayanus* and *Hyparthelia dissoluta* were slightly less active and *Cynodon dactylon* had small activity.

As with *Paspalum notatum*, activity was closely associated with the roots and little was removed by vigorous washing; isolated root segments did not reduce acetylene until after a lag of about 12 hours. Soil from around the roots had little activity, but this

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increased greatly when given a carbon source such as glucose, without multiplication of the nitrogen fixers.

Three main types of nitrogen fixing organisms were abundant on the roots and in the surrounding soil: all had well-developed capsules and belonged to the *Klebsiella* group. (Dart, Harris and Day)

Take-all disease of cereals

Work already reported (*Rothamsted Report for 1971*, Part 1, 93) indicates that the progress and decline of take-all in successive wheat crops is affected by the nutrition of the fungus *Gaeumannomyces graminis* in the rhizosphere. This is further supported by results of experiments comparing the pre-penetration growth of the fungus in the root region with the subsequent severity of disease and with the 'disease rating' of the soil.

In the root region hyphal clump development was inversely correlated with the percentage of root axes subsequently infected, and there was a significant correlation between hyphae and clumps. Later when saprophytic growth in the root region was declining, hyphal clump formation and root-hair infection correlated inversely with the number of cereal crops.

Because the incidence of take-all is influenced by nitrogen supply, the rhizosphere and bulk soil were separately analysed for ammonium and nitrate nitrogen. In the spring of 1972 the cereal sequence with most disease had most NH_4-N and least NO_3-N in the rhizosphere soil, the bulk soil analysis showing no such relationship. Populations of ammonifying and nitrifying bacteria were smallest in the years of maximum disease. Bacteria and actinomycetes in the bulk soil in the spring tended to increase up to the fifth consecutive cereal crop, then the bacteria decreased; the actinomycetes decreased after the eighth crop.

Soil from the different sequences were sterilised by irradiation or irradiation and autoclaving and inoculated with the take-all fungus. Disease appeared irrespective of the source of the soil and did not follow the take-all decline pattern; all treatments contained much NH_4-N. Moreover, diffusates from sterilised soil supported the growth of the fungus least when it originated from the sequence with most disease. (Brown, with Hornby and Pearson, Plant Pathology Department)

A medium has been developed containing minimum amounts of each constituent that will support maximum growth of *Gaeumannomyces graminis* when cultured for seven days at 19°C. This medium avoids problems arising from the accumulation of staling products in richer media, and will be used in future work. (Brown and Hammonds)

Soil anaerobes and soil structure

Following the observation that the physical condition of a sandy clay soil at Saxmundham was improved by adding leaf protein liquor (see report by Arkcoll, Biochemistry Department, p. 117), the microbiological aspects are being examined. This soil was incubated at 25°C with enough liquor to give just waterlogged conditions. Gaseous fermentation led in 48 hours to a doubling of the soil volume. A similar result was obtained with the addition of sucrose or molasses. The soil remained in the expanded condition on drying.

The vigorous production of gas and soil expansion when a 3% solution of molasses was used was associated with marked increases in anaerobes; 0·3% molasses also stimulated these micro-organisms, though gas production was small. All anaerobic bacteria isolated were spore formers of the *Clostridium butyricum* or *C. pasteurianum* types. Ten of these isolates were incubated in sterile soil with sterile 3% molasses and all caused gas

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production and soil expansion similar to that found when the unsterile soil was similarly incubated.

It would be impracticable to improve the structure of field soil by the additon of such large quantities of material. However, much smaller amounts cause similar microbiological changes and many anaerobes proliferate to form gums that may stabilise the improved soil structure.

Many of the isolates grew well when incubated under nitrogen and presumably fixed some nitrogen. However, all isolates also made some growth when incubated in a hydrogen atmosphere, which inhibits N-fixation, or in argon. The small amounts of combined nitrogen which are included in the medium as sources of growth factors for N-fixing clostridia were therefore sufficient to permit some growth, making it difficult to assess the extent of N-fixation. (Skinner)

Microbial degradation of aromatic chloro-compounds

Co-metabolism of chlorophenols and chlorobenzoates. Whilst the formation of chlorocatechols from chlorophenols by *Rhodotorula glutinis* was confirmed by chemical tests, no catechol compounds could be isolated from reaction mixtures of washed cells and chlorobenzoates, although there was evidence of oxidation of the latter.

Seven different genera of phenol-utilising soil bacteria have been examined for co-metabolism. Some were specific in co-oxidising only one chlorophenol isomer whereas others were capable of oxidising all three isomers. We have also examined the mode of catechol fission shown by the phenol-adapted or benzoate-adapted organisms, i.e. whether it is 'ortho' or 'meta' type of catechol fission. It is hoped to determine whether there is any correlation between the above characteristics.

Degradation of 1-naphthol. Certain soil micro-organisms can hydrolyse carbaryl (1-naphthyl-N-methyl carbamate) with the release of 1-naphthol. The fate of 1-naphthol in soil is thus of interest in pollution studies. In 1965 (*Rothamsted Report for 1965*, p. 83) a *Pseudomonas* species was isolated from soil and found to grow with 1-naphthol as its sole carbon source. It also grew on naphthalene or on 1-chloronaphthalene but not on 2-naphthol. The study of bacterial metabolism of 1-naphthol was resumed by investigating the biochemical changes of 1-naphthol in cultures and by studying the co-metabolism of 1-naphthol by naphthalene-grown pseudomonad cells. The metabolic pathway of 1-naphthol dissimilation has not yet been defined. (Walker, Spokes and van Berkum)

Staff and visiting workers

We report, with deep regret, the death of Dr. J. Kleczkowska on 7 June after a short illness. Nina joined the department in 1939 as a research student from Cracow University and became a leading authority on phages and serology of nodule bacteria; she also made notable contributions on *Rhizobium* specificity and genetics.

The following attended scientific meetings abroad: D. Hayman and B. Mosse at Societé Française de Microbiologie, Institut Pasteur, Paris; P. J. Dart at IITA Ibadan and IAR Ahmadu Bello University, Nigeria; N. Walker at the Societá Italiana Microbiologia, Pisa, Italy; F. A. Skinner at the Agricultural College, Ultuna, Sweden; P. S. Nutman at the Societé Française de Phytophathologie, Gembloux, Belgium, and at IAIR New Delhi and various other centres in India, giving the 39th Bose memorial lecture at Calcutta.

Dr. J. N. Barea of Granada University and H. Allgayer of the Technical University Munich worked in the department during the year.

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