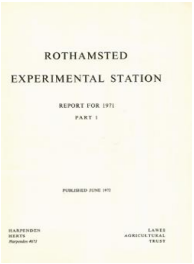
This work is licensed under a Creative Commons Attribution 4.0 International License.

Thank you for using eradoc, a platform to publish electronic copies of the Rothamsted Documents. Your requested document has been scanned from original documents. If you find this document is not readible, or you suspect there are some problems, please let us know and we will correct that.

ROTHAMSTED RESEARCH

Report for 1971

Full Table of Content



Soil Microbiology Department

P. S. Nutman

P. S. Nutman (1972) *Soil Microbiology Department ;* Report For 1971, pp 91 - 100 **- DOI: https://doi.org/10.23637/ERADOC-1-126**

This work is licensed under a Creative Commons Attribution 4.0 International License.

SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

Our work aims to get a better understanding of the many activities of soil-inhabiting microbes and the various ways in which they interact and affect growth of plants. Nitrogen fixation, nitrification, the degradation of herbicides and cellulose, the role of mycorrhiza and the interactions between saprophytes and plant parasitic fungi are continuing subjects of study, but with changing emphasis and methodology. Thus the newer techniques of estimating nitrogen fixation are being used extensively to assess the contributions made by free-living microbes and by symbiotic systems to the nitrogen cycle, and to study the effects of changes in the environment. The recent discovery that vesicular–arbuscular mycorrhizal fungi can make more soil phosphorus accessible to plants has stimulated work on the physiology of the fungus and the nutrition of the infected host, and has led to a study, supported by the International Biological Programme, on the role of these fungi in phosphate cycling in a range of ecosystems.

A new catalogue of strains of *Rhizobium* has been prepared for publication.

Collaborative work in electronmicroscopy, nodule biochemistry, microbial ecology and root disease, continued with members of other departments at Rothamsted and various universities and other research centres.

Autotrophs and decomposition studies

Autotrophic nitrifying bacteria. Continued work on the autotrophic nitrifying bacteria in soils from the Classical fields, mainly Broadbalk and Barnfield, led to the isolation and characterisation of further strains of ammonia-oxidising bacteria in pure culture, but most attention was paid to nitrite-oxidising bacteria. Twenty pure cultures of *Nitrobacter* spp. were established, and, largely on the basis of their colony form and appearance, these were provisionally separated into four types. The paucity of characters for classifying autotrophs makes final assessment of species difficult; more information is needed, including knowledge of DNA base ratios, which it is hoped to examine. (Walker and Cross)

Microbial degradation of chloro-compounds related to herbicides

Co-metabolism (or co-oxidation) is the ability of suitably induced microorganisms to cause the partial oxidation of some compound that does not, by itself, support the growth of the organisms but is usually derived from a substrate that does. For example, mesitylene was shown by American workers to be oxidised to p-*iso*propylbenzoic acid in cultures of *Nocardia* growing on a paraffin hydrocarbon, although mesitylene alone does not support the growth of this organism. This phenomenon has been mainly studied in hydrocarbon breakdown and in bacteria that can grow on benzoate and co-metabolise various halogenobenzoates.

Work on the co-metabolism of 3-chlorobenzoate (*Rothamsted Report for 1969*, Part 1, 99) was extended to other compounds and microbes. Phenol-grown cells of a strain of a yeast isolated from Rothamsted soil and identified as *Rhodotorula glutinis*, oxidised 4-chloro- or 4-bromo-phenols to the corresponding 4-chloro- or 4-bromo-catechols. There is also evidence that they oxidised 3-chloro-phenol. When grown on benzoate, the cells oxidised 3-chlorobenzoate to 3-chlorocatechol.

The oxidation was also studied of 4-chloroaniline, 4-methoxyaniline and 2-chloro-4-

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

methoxyaniline by a yet unidentified soil bacterium, that was isolated in pure culture and found to grow with either 4-anisidine or aniline as sole carbon source. Anisidine-grown cells took up some oxygen in the presence of 2-chloro-4-anisidine, which alone the bacterium cannot metabolise. An impure culture of another soil bacterium was obtained that decomposed 4-chloroaniline, and is being further investigated. There was also some oxidation of 3- and 4-bromoanilines by washed cells of an aniline-grown organism. Hence, co-metabolism seems to be a common mechanism by which synthetic halogen compounds can be decomposed and so may be important in the breakdown of such compounds in soil. (Walker and Giardina, with Briggs, Chemistry Department)

Cellulose decomposition by aerobic bacteria

The removal of nitrate by microbial assimilation from aerated media containing cellulose as sole carbon source was studied using a cellulose-decomposing bacterium, isolated from activated sewage sludge, alone and together with species isolated from the sludge and unable to decompose cellulose. A fermenter culture with cellulose as carbon source yielded colonies of many types when plated on a range of agar media containing cellulose, but no colony lysed the surrounding particles of cellulose. Each type of colony was tested for ability to decompose cellulose in liquid medium and an isolate, provisionally designated as *Cellvibrio* sp., was eventually obtained that was more actively cellulolytic in the liquid medium than were mixed cultures. The non-cellulolytic species greatly outnumbered the cellulose decomposers in the gelatinous flocs of bacterial growth.

A pure culture of the cellulose decomposer behaved similarly to crude mixed culture when grown in a fermenter, though the concentration of soluble carbohydrate was rather larger at the time the nitrate had disappeared than in previous experiments, probably because non-cellulolytic organisms able to use soluble carbohydrate were lacking. Nitrate was assimilated at a mean rate of 10·2 μg/ml/h of nitrate ion over a 24 hour period, faster than in mixed cultures. Cellulose particles were quickly colonised by the bacteria; the supernatant fluid became turbid but flocs did not form.

A mixed inoculum of the cellulose decomposer with various non-cellulolytic micro-organisms derived from sewage sludge produced gelatinous growth and flocculation. In all experiments soluble carbohydrate was released by cellulolytic activity at about the same rate as it was used for cell growth, and the normally small concentration only increased when the nitrate concentration approached zero. (Skinner)

Fungi

Infection of different plant species by *Ophiobolus graminis*. *Ophiobolus graminis*, the cause of take-all, infects roots of cereals and many grasses, but different genera and species differ in their susceptibility. Using the buried slide technique (*Rothamsted Report for 1969*, Part 1, 98), we showed that wheat roots stimulated the growth of *Ophiobolus graminis* and that the hyphae formed special structures (called clumps), thought to be concerned with feeding in the rhizosphere.

Comparison of the amounts of hyphae and clumps produced in the rhizospheres of susceptible and resistant hosts show that fewer clumps per length of hyphae are required to infect susceptible species than resistant species. For example, wheat and *Lolium perenne*, both very susceptible to take-all, supported most hyphal development in relation to clump formation, and *Agrostis gigantea* which is slightly susceptible, supported least. Very few hyphae developed in the rhizosphere of *Agrostis tenuis*, *Poa pratensis* and *Phleum pratense* which are only very slightly susceptible.

Experiments with plants grown in a sterile culture medium confirmed these effects; roots of the most susceptible species stimulated most fungal growth, although in these

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

conditions clumps did not form. Clumps are closely associated with the root hairs and root surfaces and they may enable *O. graminis* to tap nutrients effectively when in competition with other microorganisms.

Compared with graminaceous plants, roots of clover, lettuce or tomato, supported much less growth of *O. graminis* on buried slides, and the amounts were unrelated to the small amount of root infection. Exudates of these dicotyledons grown in sterile culture inhibited the growth of *O. graminis*. (Brown)

Take-all decline. The buried slide method was also used to study the growth of an inoculum of *Ophiobolus graminis* in samples of soil taken after harvest and during spring from plots where cereals had grown each year for up to 10 years (Wheat and Intensive Barley experiment, Little Knott Field). The samples taken during autumn 1969 and 1970 showed an inverse correlation between the amounts of clumps and hyphae in the rhizosphere of the test wheat plants and the disease rating of the crop. Where disease was most severe (3rd year wheat 1969, 10th in year 1970) the ratio of hyphae to clumps was larger than when disease was least severe (1st year wheat in 1969 and 1970). The very severe take-all in the 10th crop was contrary to expectations from the usual pattern of take-all decline, but not to growth characteristics of the fungus in the root zone. Samples taken during spring showed no correlation between disease rating and the type of fungal growth on the buried slides. However, *O. graminis* inoculated on slides buried in these soil samples, whether tested immediately or after four months storage at 2°C, grew very much less well than in any of the autumn samples, and the mycelium was rapidly lysed. Such poor fungal growth may be caused by the same unspecified soil factors that commonly much decrease the inoculum potential of *O. graminis* during the winter. (Brown with Hornby, Plant Pathology Department)

Growth of *Endogone mycorrhiza* in agar medium. We reported last year that typical vesicular–arbuscular (VA) infections occurred in clover seedlings (*Trifolium parviflorum*) grown in an agar medium containing nitrogen. Further work shows that the phosphate concentrations in the medium and in the seedlings are critical; seedlings containing more than 0·4% P (dry weight) were very difficult to infect. The phosphorus concentration of clover grown in Jensen's medium reached 1% and this accounted for the difficulties experienced in obtaining infection in this medium. Calcium phytate and nucleic acid (DNA), two organic sources of phosphorus, increased fungal growth in the medium, and DNA greatly stimulated spore production. Inositol also much increased mycelial growth in the medium and made the fungus less dependent on spread within the root. Very iron-deficient plants did not become infected, and 70 ppm sodium in the medium led to very sparse and abnormal-looking infections. (Mosse)

Source of extra phosphate in mycorrhizal plants. The amount of available phosphorus in soil also influences mycorrhizal development and the host's growth. In eight soils treated with ^{32}P, specific activity of phosphorus taken up by mycorrhizal and non-mycorrhizal plants was similar and corresponded closely to the specific activity of the $NaHCO_3$-extractable phosphorus in the soil. Mycorrhizal plants and non-mycorrhizal plants thus used similarly labelled fractions of the soil phosphorus, and had not used either the organic or insoluble mineral forms of phosphorus. This suggests that the fungal mycelium outside the root can absorb, and transfer to the plant, phosphorus from beyond the depletion zone adjacent to the root. (Mosse and Hayman)

Effects of VA mycorrhiza on phosphate cycling at four IBP sites. The incidence of VA mycorrhiza was studied at Meathop Wood (natural mixed deciduous forest), at Moor-

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

house (upland peat bog), at Banchory (Calluna heath) and at Wareham (mixed heath), all on acid soils. At Meathop Wood VA mycorrhiza are common in the prevalent herbaceous species (*Viola canina*, *Fragaria vesca*, *Oxalis acetosella*, *Anemone nemorosa*, *Lonicera peridymenum*, *Mercurialis perennis* and *Rubus vestitus*) and in seedlings of the two commonest tree species (*Fraxinus excelsior* and *Corylus avellana*). At Wareham there is much infection in *Agrostis setacea* and *Molinia caerulea*, and especially in the second year after burning in *Ulex minor* seedlings (which also have ectotrophic mycorrhiza) and in the bog plant *Rhynchospora alba*. Some of these species are being used to estimate the contribution VA mycorrhiza makes to phosphate cycling at these two sites. At Moorhouse VA infection was common in *Nardus stricta* but none was found in *Rubus chamaemorus*, *Eriophorum angustifolium* or *Juncus squarrosus*. At Banchory root samples were collected from plots given different manurial treatments. Only those given 'Nitro-Chalk' had a few mycorrhiza in some species (not *Calluna*). It was concluded that VA mycorrhiza does not appreciably affect phosphate cycling at either Banchory or Moorhouse, although other mycorrhizal forms may be important in *Calluna* nutrition. (Mosse and Hayman)

Interaction between VA mycorrhiza and nitrogen fixation by rhizosphere microorganisms. Döbereiner, Dart and Day (*Journal of General Microbiology* (1971)), reported significant nitrogenase activity in the rhizosphere of plants of *Paspalum notatum* from Brazil, which they attributed to *Azotobacter paspali*; they also noted that VA mycorrhiza was very abundant in the *Paspalum* roots. The possible effect of the mycorrhiza on the establishment of the *Azotobacter* was examined in two Brazilian cerrado soils containing extremely little available phosphate (0·4 μM/l $CaCl_2$-soluble P). In both soils *Paspalum notatum* did not respond to ammonium nitrate unless phosphate was also given, and responded almost as well to additions of KH_2PO_4 only. It also responded strikingly to different extents to inoculation with different *Endogone* spore types. Possible synergism between mycorrhizal infection and the establishment of nitrogen-fixing rhizosphere microorganisms is being examined in *Paspalum notatum*, and also in dune grasses, which are primary colonizers of nutrient-poor habitats where VA mycorrhiza and *Azotobacter* occur. (Mosse and Brown)

Non-symbiotic nitrogen fixation

Algal nitrogen fixation in the field. Nitrogenase activity (which correlates with amounts of nitrogen fixed) of the algal crusts on Broadbalk plots Nos. 3, 5, 6, 9 and 22 and sown to wheat were measured by the acetylene reduction assay. Crusts developed only when the soil was moist. Nitrogenase activity was not detected until June and was closely related to the N-status of the soil. Activity did not become appreciable on plot 9, which gets 193 kg N/ha, until late in the season when the concentration of N in the surface soil had decreased.

Seasonal fixation rates were estimated on 11 occasions on samples selected for visible amount of crust, taking account of the amount of crust cover and surface moisture and assuming that $3C_2H_2 \equiv 1\ N_2$. More nitrogen was fixed on the sections not treated with herbicides; plot 6 (given 48 kg N fertiliser/ha, and without herbicide) had most activity, estimated at 25 kg N/ha. Plots 3 and 5 (no fertiliser) had less plant cover and the soil surface dried rapidly, delaying algal establishment compared with the other plots; estimated activities ranged from 7–17 kg N/ha.

The algal flora depended on fertiliser treatment, but was unaffected by herbicide. Green algae were dominant on plot 9 throughout the season, and were prominent on plot 22, given 35 tonnes farmyard manure/ha, but covered only about 1% of the soil

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

on plots 3, 5 and 6. Five different types of blue-green algae were isolated; two occurred on all plots, one type was restricted to plots 3 and 5, where it was the major component, and another (*Cylindrospermum* sp.) only on plots 6 and 22.

Several small plots on Broadbalk were artificially inoculated in July with two types of blue-green algae. Nitrogenase activity by the crust was stimulated by one culture on plot 6, without herbicide. Plots of spring wheat in another field, given different amounts of nitrogen fertiliser were also inoculated with the same two cultures. Inoculation in April when the wheat was small had no effect, but inoculation with one of the cultures in early June stimulated the formation of a crust containing the inoculum, and this had more nitrogenase activity than was measured on untreated plots, but less than on Broadbalk. Fertiliser-N again enhanced the development of the green algae. Inoculation had no effect on grain yield. (Witty, Frogatt and Keay (Luton College of Technology) with Dart and Day)

Effect of pO_2 on nitrogenase activity in soil. Acetylene-reduction assays on small cores and loose soil showed that activity was considerably enhanced by lowering the pO_2 to 0·04 atm or less. By contrast, with larger cores (60 mm × 100 mm) left in the sampling tube, so as to disturb the structure of the core as little as possible, acetylene reduction was rapid at atmospheric pO_2.

Decreasing the pO_2 had little effect, indicating that micro-environments of small pO_2, which are usual in undisturbed soil, favour nitrogenase activity.

Broadbalk wilderness. Assays of soil cores and surface litter indicated that saprophytic nitrogen fixation could account for only about 4–5 kg N/ha/year. Activity of litter and soil reached a maximum after leaf fall when earthworms were also active; worm casts often had appreciable acetylene reducing activity (*c.* 1 nM C_2H_4 produced/g/h).

Species of plants from the Wilderness with active acetylene-reducing rhizosphere populations included *Heracleum spondylium*, *Arthriscus sylvestris*, *Mercurialis perennis*, *Viola canina*, *Rumex acetosa*, *Convolvulus arvensis* and *Stachys sylvatica*. *S. sylvatica* plants were transplanted into special pots for assay. At field light intensities and temperatures, acetylene-reducing activities were demonstrated equivalent to fixation of up to 200 ng N/h/plant. Rates of activity remained linear for at least 50 hours, and incubation in the dark for up to 36 hours did not slow the rate. A nitrogen-fixing facultative anaerobe resembling *Enterobacter aerogenes* was isolated from the roots of *S. sylvatica*. (Harris and Dart)

Nitrogen fixing associations of bacteria and tropical grasses. Roots and rhizomes of *Paspalum notatum* cv 'batatais' form a specific association with *Azotobacter paspali* with considerable nitrogenase activity, estimated to fix up to 90 kg N/ha/year. The activity of roots extracted from soil was very sensitive to pO_2 with most activity at 0·04 atm. Sugar cane, *Panicum maximum*, *Pennisetum purpureum* and *Cymbopogon citratus* roots and associated soil also had much nitrogenase activity; *Beijerinckia indica* was abundant on these roots.

Azotobacter paspali when grown in nitrogen-free media, differs greatly in morphology from other species of the genus. Cells about 10–20 μm long predominate in young aerobic shake culture. Budding and more equal division then shorten the cell to 4–5 μm, producing ovoid cells characteristic of other species of Azotobacter in log phase growth. Very long rod-shaped cells, up to 120 × 2 μm, containing numerous granules predominate in old cultures.

Electron microscopy of thin sections on *A. paspali* showed large inclusions probably of poly-β-hydroxybutyrate, lipid and polyphosphate granules, smaller polar granules

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

(glycogen) and dispersed, very electron-dense, small granules. Both long rods and ovoid cells have prominent vesicles formed by invagination of the cytoplasmic membrane, resembling those described for other *Azotobacter* species and thought to be a part of a protective respiratory mechanism for nitrogenase.

A. paspali forms cysts resembling closely those of *A. vinelandii* and *A. chroococcum* with prominent tubular structures formed outside the cell wall membrane. Poly-β-hydroxybutyric acid granules occupy most of the cyst cytoplasm. (Harris, Chandler, Döbereiner, Day and Dart)

Symbiotic nitrogen fixation

The effect of inhibitors on the growth of *Rhizobium* species. Twenty strains of rhizobia (five of *Rhizobium trifolii*, four of *R. meliloti*, three of *R. phaseoli*, four of *R. leguminosarum*, three of *R. japonicum* and one of *R. lupini*) were tested for sensitivity to seven antibacterial substances to assess their use for selective media. Potassium tellurite, sodium azide, sodium lauryl sulphate, bile salts, brilliant green, crystal violet and pentachloronitrobenzene (PCNB) were used separately, and together in yeast extract mannitol agar (YMA). The effect of these inhibitors on the microflora of Broadbalk soil was also examined.

Bile salts and potassium tellurite both inhibited most strains of rhizobia and were not used further. Sodium azide at 10 ppm caused some inhibition, especially of *R. lupini* and *R. phaseoli* strains, but at 5 ppm, growth was generally good, except for one slow-growing strain of *R. japonicum* (3402) which was sensitive to all agents tested. The microflora of Broadbalk soil was generally inhibited by YMA containing 5 ppm sodium azide. Very dilute brilliant green (0·5 ppm) stimulated the growth of some rhizobia and had little effect on the soil microflora. Crystal violet at concentrations that decreased the growth of the soil organisms was strongly inhibitory to *Rhizobium*. Sodium lauryl sulphate at 100 ppm was inhibitory only to the three strains of *R. japonicum* but decreased the growth of the soil fungi. PCNB at 5 ppm (made up in a medium containing 100 ppm lauryl sulphate) inhibited fungi and actinomycetes from Broadbalk soil but had little effect on the *Rhizobium* strains, except for three strains of *R. japonicum* which were strongly inhibited. A synergistic effect between PCNB (5 ppm) and of sodium azide 5 ppm was apparent on the soil count and on the rhizobia. A mixture of small quantities of PCNB (5 ppm), brilliant green (0·5 ppm) and sodium azide (5 ppm) greatly inhibited many soil organisms yet only slightly inhibited the 20 strains of rhizobia, except *R. japonicum* strain 3402 which was strongly inhibited. (Skinner and Pattison)

Extracellular polysaccharides of *Rhizobium trifolii*. The chemical compositions of the extracellular polysaccharides of *R. trifolii* strain 0403 and of six phage-resistant mutants were compared. Two mutants (0412, 0413) effectively nodulated *Trifolium pratense*, *T. repens* and *T. glomeratum*, three (0416, 0417, 0419) were ineffective and one (0418) was effective on *T. pratense* only. The polysaccharide from the effective strain 0412 contained much less galactose (1 % compared with 9 %) and correspondingly more glucose than the parent strain. Strain 0412 polysaccharide also had less acetyl (3 %) than strain 0403 (6 %) but the same amount of glucuronic acid as the parent strain (21 %). The polysaccharides from the five other mutants all contained glucose and galactose in the ratio 4·0 : 1. These strains, another related effective strain (0401) and also two related avirulent strains (Bart A, 0402) produced polysaccharides containing a compound with the same Rf values and spray reaction with p-anisidine hydrochloride in water-saturated butanol as 4-o-methyl glucuronic acid, identified in rhizobial polysaccharides by Humphrey and Vincent (*Journal of General Microbiology* (1959), **21**, 477). (Hepper)

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

Pectic and cellulolytic enzymes in red clover seedlings and their reputed role in nodule formation. Further work on the two pectolytic enzymes reported last year (*Rothamsted Report for 1970*, Part 1, 82) as occurring in red clover seedlings grown in flasks has supported the preliminary conclusion that these are formed in response to the confined conditions for growth rather than to the presence of rhizobia. These enzymes may function in either a protective response or be involved in the autolytic breakdown of plant tissue. Neither infection threads nor nodules were formed in the experimental conditions used, which resembled those used by other workers, and with no contrary evidence, these results could be similarly explained.

An enzyme hydrolysing carboxymethylcellulose (cellulase) was sometimes found in the seedling growth medium. Activity was significant with seedlings grown in distilled water, but greater in growth media containing mineral salts and at pH values above 6. The ease with which the enzyme is released from the seedlings suggests that it is extracellular and bound to the root surface. (Bonish)

Colonization of pot experiments by bacteriophages. Bacteriophages able to lyse four indicator strains of *Rhizobium trifolii* colonised pots of a sterilised sand–vermiculite mixture kept for some months in the glasshouse. Inoculating the pots with *R. trifolii*, but not planting with clover, encouraged phage infection and increased phage titre. A strain of *Rhizobium* previously shown to be a good recipient of foreign DNA was the most efficient of the inoculated strains in encouraging phage infection and multiplication. Aerial contamination was the probable source of phage. Soil from Highfield contained phages able to lyse the indicator strains.

The pots also became infected with bacteria other than *Rhizobium* and phages able to lyse these, indicating that phage infection is a widespread and a common incident in the life cycles of soil bacteria. (Kleczkowska)

Nodulation and nitrogen fixation in tropical grain legumes

Soyabean. Soyabeans were grown in Saxcil cabinets at day temperatures of 21°, 27° or 33°C (16 hour day), and inoculated with one of three strains of *Rhizobium japonicum*: SM1B from Brazil, CC705 originally from the Nitragin Company, Wisconsin, and CB1809 the recommended Australian strain for soyabean. Cold progressively delayed nodule formation, nitrogenase production and the export of nitrogen from the nodules. Most nitrogen was fixed at 27°C by all strains, and SM1B fixed more at 33°C than the other strains and more at 33° than 21°C; CC705 was slightly less effective than the other strains at 21° and 27°C, and was quite ineffective at 33°C.

Nitrogenase activities per plant reflected these nitrogen gains. For all strains and temperatures, activities increased until the plants were five weeks old, and then decreased slightly until final harvest at seven weeks. Nitrogenase activity per g dry weight of nodule also showed a maximum; peaks of activity occurred at similar times for each strain, but earlier at 33°C than 21°C and was greatest at 27°C for each strain.

At 21°C estimates of N fixed, made by acetylene reduction assays, were slightly greater than the amounts measured by Kjeldahl analysis, but at 27° and 33°C were slightly smaller except for strain CC705. Nodule weight and nitrogen accumulation per plant were linearly related for each strain at each temperature. Slightly fewer nodules were produced by CC705 at 33°C than by the other strains, but the leghaemoglobin (Lb) concentrations in the nodules were only slightly lower than for CB1089. At 27°C CC705 had more Lb than CB1809 although its nitrogenase activity per unit of Lb was less. The nitrogenase activities per unit Lb for strain CC705 was half that of CB1809 at 33°C, eight weeks after sowing. Optical and electron paramagnetic resonance spectroscopy of

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

crude Lb showed it to be mostly in the reduced form. The optical spectrum of CC705 Lb produced at 33°C differed slightly in the α and β absorption regions from Lb produced at 21° and 27°C. Nodules with much Lb are not necessarily active in N fixation.

Two soyabean varieties differed in their responses to root temperature. Most nodule and plant dry weight was formed at 35°C with Improved Pelican, a variety grown in Brazil, but Altona, a Canadian variety, produced most at 25° and little at 30°C. (Döbereiner, Day, Maskall and Dart)

Peanuts, cowpeas etc. *Arachis hypogea*, *Vigna radiata*, *V. mungo*, *V. unguiculata* var. Poona and *Dolichos lab lab* did not form nodules at 15°C root temperature, formed very few at 20° and most at 30°C; plant dry weight was also greatest at 30°C. *A. hypogea*, *V. unguiculata* and *V. radiata* nodulated and grew well at 35°C.

Two cowpea varieties responded differently to day temperatures of 27° and 33°C; New Era produced most dry matter at 27°C and Poona most at 33°C; both had most nitrogenase activity at 27°C. At 33°C nodule formation on both varieties was slightly slower than at 27°C, and the uppermost nodules formed further from the hypocotyl; at 33°C fewer nodules were formed on the primary root and more on the secondary roots than at 27°C. As reported earlier for a range of legumes (*Rothamsted Report for 1970*, Part 1, 85) temperature of assay over the range 15–35°C had little effect on activity of nitrogenase from nodules of *V. radiata* and *V. mungo*. Nitrogenase from cowpeas grown at 20° or 32°C and assayed over a range of temperatures did not differ in their pattern of activity.

Nodules were not formed on *Cicer arietinum* by strain CB1189 or by 27A2 at 33°C, and at 30°C only a few plants nodulated late and sparsely. Nodulation was also slower at 20° and 15°C than at 25°C. Most dry matter was produced at 25°C and very little at 33°C. Nitrogenase activities per g of nodule tissue depended on strain and temperature, and per plant reached a maximum at 42 days. (Islam and Dart)

Leghaemoglobin (Lb). Soyabeans grown in the open at Woburn in 1971 nodulated very effectively with strain CB1089 and produced much Lb. The Lb was separated from nodules disrupted anaerobically in a modified Pirie pressure cell having a large capacity (80 g tissue), a variable aperture and an inlet for anaerobic buffer rinse to give quantitative recovery. Host plant cells were disrupted but not the bacteroids, some even remaining enclosed in their membrane envelopes; contamination with bacteroid cytochromes was minimal. The tissue was crushed into ascorbate–phosphate buffer containing 'Polyclar', centrifuged and partially purified by ammonium sulphate fractionation. The crude Lb (90% pure) was chromatographed on DEAE cellulose, using phosphate buffer at 7 pH and fractionated into components, which were then concentrated by ultrafiltration. Soyabean Lb gave two major components and cowpea Lb one component; this was not affected by *Rhizobium* strain. Polyacrylamide gel electrophoresis of the concentrated fractions from the columns revealed minor contaminating proteins in the two soyabean components. Lb-like myoglobin exhibits microheterogeneity on DEAE cellulose chromatography. The minor fractions from the columns contained haem proteins with electrophoretic Rf values similar to one of the major fractions. Contaminating proteins (*c.* 1% of the total protein) present in the two main soyabean fractions were not separated from the Lb by gel filtration on Sephadex. They had no peroxidase activity and were presumably not haem proteins. (Maskall and Dart)

Black nodule pigments. Nodules formed by strain CB756 on cowpea, green gram (*Vigna radiata*), and mung bean (*V. mungo*) contain a purple-red pigment which increases with age and masks the pink colour of Lb. Several other cowpea rhizobia did not produce

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

the pigment. The extracted pigment was resolved into two coloured components on high voltage paper electrophoresis, one of which was strongly acidic. Chromatographic properties and other tests showed that neither substance is a haem or an anthocyanin. (Maskall and Dart, with Carpenter, Plant Pathology Department)

The site of iron in nodules. X-ray micro analysis, with the AEI EMMA instrument, was used to locate Fe in araldite sections of nodules of soyabean and *Phaseolus vulgaris*. The electron beam of the electron microscope is focused on part of the section about 0·2 μm in diameter and excites X-emissions characteristic of the elements present; these were analysed. Fe occurred in the bacteroids, presumably mostly in the nitrogenase enzyme; there was very little in the space between them or on the enclosing membrane. The plant cytoplasm outside the membrane was rich in Fe, presumably in Lb, supporting our earlier conclusion on its location, from peroxidase activity and near-u.v. light microscopy (*Rothamsted Report for 1968*, Part 1, 91). The concentration of Fe in the nodule cell cytoplasm resembles, on a haem basis, that of haemoglobin Fe in erythrocytes. (Dart and Chandler)

Fine structure of root nodules

Peanut nodules. The large bacteroids formed resemble those in nodules formed by rhizobia of the clover, pea and medick infection groups, and are usually enclosed singly in plant membrane envelopes. The strain of *R. japonicum* that was used to nodulate peanut plants also nodulated cowpeas and in this host the bacteroids are smaller, several within each envelope, as described for other members of the cowpea group (Dart and Mercer, *Journal of Bacteriology* (1966), **91**, 1314–19).

This diversity of bacteroid morphology shows that the host legume and not the *Rhizobium* strain determines the organisation of the bacteroids in the membrane envelopes. (Chandler and Dart)

Casuarina nodules. Nodules of four-week-old plants of *Casuarina cunninghamiana* showed a diversity of endophyte form. Some host cells contained branched filaments, resembling an actinomycete, with a densely stained amorphous wall (*c.* 0·02 μm thick) bordered on the outside by a less-stained fibrous layer. Mesosomes associated with the central nucleoid were prominent in most filaments. An electron-transparent space separated the filament from the host cytoplasm, which was packed with ribosomes and fibres. The endophyte filaments divided to form bacteroid-like structures, up to 3 $\times$ 7 μm, in which the fibrous wall layer was much thicker (*c.* 0·07 μm). 'Bacteroids' were usually enclosed singly or in pairs in membrane envelopes in a less dense cytoplasm. Later the host cytoplasm seemed to collapse around and merge with the bacteroid wall. The electron-dense bacteroid wall layer was thicker, and its mesosome structures less prominent, than those of the filaments. (Chandler and Dart)

Other studies of nitrogen fixation in clover

Inhibition of nodulation and nitrogen fixation in tube culture. Nodulation and growth of plants on agar slopes in test tubes plugged with cotton wool is much less than that of plants grown in tubes with only their roots enclosed, or of plants in pots. Restricted diffusion of CO_2 was thought to be the main cause of poorer growth after 30 days (Gibson (1967), *Australian Journal of Biological Science* **20**, 837–842). However, calculation of rates of diffusion suggests that there is some nodule degeneration and decline in nitrogenase activity before CO_2 limits growth. The possibility that ethylene formed by the

https://doi.org/10.23637/ERADOC-1-126

This work is licensed under a Creative Commons Attribution 4.0 International License.

plants may be the cause of poor growth was examined by growing clover plants in 11 Roux bottles, which were either continuously aerated with clean air, or through which the air was recirculated after passing through mercuric perchlorate and activated charcoal, to remove any ethylene that was formed. Other bottles contained activated charcoal only. Plants nodulated earlier, nodules grew quicker and more nitrogen was fixed when grown under these conditions. Nitrogenase activities over the first 25 days were more than double those of plants grown in control bottles without gas exchange or cleaning, and bacteroid morphology was affected and nodule degeneration delayed. The results strongly suggest not only that accumulation of ethylene is the cause of poor growth in tube culture, but may also be implicated as the hormone controlling nodule development and senescence. (Day, Dart and Percy)

Selection for increased nitrogen fixation in red clover. The yield of red clover grown in test-tubes and inoculated with *Rhizobium trifolii* strain 0403 was increased by selection and breeding; the families selected for larger yield fixed about 5% more nitrogen than the others. Uninoculated plants of these selected lines given nitrate or ammonium fertiliser also yielded very slightly more, indicating that selection may also have favoured plants able to tolerate the confined conditions of growth in test-tubes (Nutman, Marečková and Raicheva (1970), *Plant and Soil*, Special Volume, 27–31).

The influence of growing conditions on the performance of selected lines was examined in open pot sand culture with precautions against contamination. The original red clover cultivar (S123) was compared with families bred from the most effectively responding tube-grown plants and families bred from those plants with average (modal) responses. The families bred from the most effectively responding plants out-yielded, in dry-matter production and nitrogen content, the modal selections by 1·4 and the original cultivar by 2·5 times. A parallel test with strain 48, which like strain 0403 is of average effectiveness, gave similar results, whereas with strain 5, which is outstandingly effective, the two groups of selected lines did not differ significantly and yielded only slightly more than the original cultivar. Other tests showed that most plants bred from poorly responding parents were much less effective than S123, and very variable, indicating that commercial seed of established varieties contain a complement of genes that impair nitrogen fixation.

The results show that the test-tube method, in spite of its limitations, is suitable for preliminary large-scale screening under strict bacteriological control, and the much greater effects of selection obtained in pot culture, indicate that there is much more scope for improving nitrogen fixation in red clover by plant breeding than was suggested by earlier work. (Nutman)

Staff and visiting workers

N. Walker attended the third meeting of the North West European Microbiological Group held in Utrecht, and also visited and lectured on herbicide degradation at the University of Rome. P. J. Dart was an invited speaker at a New York conference, sponsored by the Rockefeller Foundation, on 'Extending symbiotic nitrogen fixation to increase man's food supply'. P. S. Nutman attended an FAO/IAEA Panel Discussion at Vienna on the use of isotopes for the study of fertiliser use by legumes.

Visiting workers from overseas included: Mr. S. D. Agboola, Federal Department of Agricultural Research, Ibadan, Nigeria, for six weeks; Dr. D. Cameron, CSIRO, Cunningham Laboratory, Brisbane, Queensland, for six months; Dr. M. C. Giardina, Laboratorio di Radiobiochimica ed Ecofisiologia Vegetali, Rome, Italy, for six weeks; Miss A. Kümmel, Institute of Microbiology, Göttingen, West Germany, for six weeks, and Mr. E. Sistachs, Instituto de Ciencia Animale, Havana, Cuba, for four months.

https://doi.org/10.23637/ERADOC-1-126