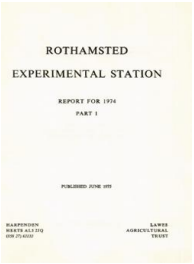
ROTHAMSTED RESEARCH

Report for 1974 - Part 1

Full Table of Content



Soil Microbiology Department

P. S. Nutman

P. S. Nutman (1975) *Soil Microbiology Department ;* Report For 1974 - Part 1, pp 245 - 256 **- DOI: https://doi.org/10.23637/ERADOC-1-131**

SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

Staff

Head of Department: P. S. Nutman, PH.D., F.R.S.

Senior Principal Scientific Officer
Barbara Mosse, PH.D.

Principal Scientific Officers
Margaret E. Brown, PH.D.
P. J. Dart, PH.D.
F. A. Skinner, M.A., PH.D.
N. Walker, PH.D., F.R.I.C.

Senior Scientific Officer
D. S. Hayman, PH.D.

Higher Scientific Officers
J. M. Day, PH.D.
A. R. J. Eaglesham, PH.D.
Christine M. Hepper, M.SC.
R. M. Macdonald, PH.D.
J. F. Witty, PH.D.

Scientific Officers
Mrs. Murial Chandler
P. E. Davis, B.SC.
D. K. Paget, B.SC.
Sheila M. Smith, C.D.H.

Assistant Scientific Officers
Joan Crawley
Mrs. Anne M. Pye
Adrienne K. Smith
Mrs. Gwendoline Smith
J. R. Spokes, B.SC.
P. van Berkum, B.SC.

Temporary Workers
R. Islam, M.SC.
Mrs. Rohini Kumarasinghe, B.SC.
C. D. Morley, B.SC.
June E. Neilson
C. Ll. Powell, PH.D.
J. B. Rhodes
M. J. Trinick, PH.D.

Personal Secretary
Mrs. Deirdre E. Daggitt

Introduction

Research continues on major aspects of the nitrogen cycle in soil and nitrogen fixation by associations of bacteria and roots, on vesicular-arbuscular endotrophic mycorrhiza of crop plants with special reference to the uptake of P by infected plants, on the microbial interactions in root disease and on anaerobic bacteria and pesticide breakdown. Work on field inoculation of clovers with rhizobia, the effect of organic matter on nodulation of tropical legumes, nodule fine-structure, the genetics of cross-inoculation group-specificity of clovers and their rhizobia, the lyophilisation of *Endogone* and studies on anaerobes will not be reported this year. Except for studies on the properties of peats as carriers for inoculants and preliminary work on soil structure, no entirely new work was started.

Some of our work is collaborative with other departments at Rothamsted and with several institutes in this country and abroad.

Studies on legume nodulation

Rothamsted *Rhizobium* Collection and strain and host tests. The Rothamsted *Rhizobium* Collection now comprises 496 strains of rhizobia, three *Rhizobium* bacteriophages, 16 *Azotobacter* strains and 26 others, mostly nitrifiers. In the two years from January 1973, 695 strains have been dispatched (536 as freeze-dried ampoules and 159 in the form of agar slopes) to 128 customers, 42 overseas. Since January 1974, 32 new strains have been added to the Collection mostly of the important cowpea group of rhizobia.

Besides routine nodulation tests on new strains greenhouse trials have been conducted to compare the effectiveness of our *R. leguminosarum* strains on *Vicia faba* (field bean) and *Pisum sativum* (garden pea). (Davis)

https://doi.org/10.23637/ERADOC-1-131

Tropical grain legumes and local isolates of *Rhizobium*. Fifteen strains of rhizobia of the cowpea group isolated from various soils from Nigeria, Uganda and Rhodesia were used as inocula for pot-grown plants of five legume species of agronomic importance; *viz* cowpea (*Vigna unguiculata* cv. K2809), pigeon pea (*Cajanus cajan* cv. Trinidad dwarf No. 5), siratro (*Macroptilium atropurpureum*), kudzu (*Pueraria phaseoloides*) and Townsville lucerne (*Stylosanthes humilis*). All strains nodulated cowpea and siratro, but not the other hosts; only four strains formed nodules on *Stylosanthes*. Several strains gave better cowpea yields than the recommended CB1024, CB756 and R5000; others ranged from ineffective to poorly effective.

Workers at IITA, Ibadan, are examining the possibilities of introducing new grain legumes into the humid tropics; little is known of their symbiotic characteristics. The strain R5000 effectively nodulated *Desmodium distortum* (cv. Q 8378), small winged bean (*Psophocarpus tetragonolobus*), hyacinth bean (*Lablab niger* cv. Tln 16), Bambara groundnut (*Voandzeia subterranea* cv. TVS 6). Some species, *viz*. jack bean (*Canavalia ensiformis*), rice bean (*Vigna umbellata* cv. Tvum 2), dwarf Lima bean (*Phaseolus lunatus* cv. 170–33), climbing Lima bean (*Phaseolus lunatus* cv. TP189–1) and Mexican yam bean (*Pachyrisus erosus*) did not nodulate with R5000 and were then treated with a mixture of 14 other strains of the cowpea group and became nodulated. Isolations were made from nodules for identification and further tests. Where nodulation was obtained with R5000 it was highly effective, plants growing as well as those provided with 240 ppm N in the nutrient medium. (Eaglesham and Dart)

Field inoculation of navy bean (*Phaseolus vulgaris*). The effects of field inoculation with *Rhizobium phaseoli* strain 3605 was examined at Woburn and Rothamsted on yields of navy bean (cv. Purley King previously called Seafarer) using nitrate levels of 0, 60, 120 and 240 kg N/ha applied at sowing. At Woburn, inoculation produced early nodulation at all N levels although nodules were few and small at 240 kg N/ha, while uninoculated plots showed little nodulation at any stage. Plots also sown with Purley King and inoculated with strain R3607 without N were slower to nodulate. Throughout the season inoculated and uninoculated plots responded visibly to N up to 240 kg/ha. Mature pod yields increased almost linearly up to 240 kg of N with inoculated plants yielding consistently higher (100% with 0 N to 10% with 240 N) than uninoculated plants. The highest yield was 2·25 t/ha of beans. At Rothamsted inoculated plants were slow to nodulate and all treatments showed less vigour than at Woburn. There was no effect of added nitrogen on vegetative growth. At final harvest, yields of pod for some treatments were as high as the best yields at Woburn, but the bean/pod ratio at Rothamsted was much lower. Highest yield at Rothamsted was 0·5 t/ha of beans and in general there was no response to inoculation. (Eaglesham and Dart)

Selection and breeding for increased nitrogen fixation in red clover (*Trifolium pratense*). Two breeding programmes, each covering three generations have examined the effects of selection for increased nitrogen fixation in the absence of fertiliser nitrogen and under axenic growth conditions in a greenhouse. The first programme (*Rothamsted Report for 1972*, Part 1, 82) used the moderately effective strain of *Rhizobium trifolii* 0403/RCR for the selection and test experiments (which were conducted initially in test-tube culture), and the second programme the highly effective strain 5/RCR, using open pot culture with bacteriological control. By breeding from highly effective plants large increases in nitrogen fixation were obtained. Relative to the dry matter yield of the original cultivars, these increases ranged up to 180% (average 51%) in the first programme and up to 42% (average 22%) when the highly effective strain 5/RCR was used. In both programmes most benefit accrued in the second generation. It was also found that the original culti-

https://doi.org/10.23637/ERADOC-1-131

vars (S123, late-flowering Montgomeryshire and two commercial samples of Broad Red in the first programme, and S123 in the second programme), yielded appreciably less than families raised from the modal classes of the populations.

These selections were next examined with a range of strains of *R. trifolii* of different provenance and effectiveness. Large strain/family interactions were recorded with the 0403/RCR-selected material but interactions were less with the 5/RCR-selected plants; the highly effective lines giving larger yields than the modal lines with ten out of 12 test strains.

Selected lines were also grown in pots of sand/gravel to which soil inocula were added (1, 5 or 25 g of Woburn Stackyard or Rothamsted Long Hoos V soil containing respectively *c.* 18 and 1900 *R. trifolii* cells per g dry soil). These showed smaller differences than when the plants were grown under bacteriologically controlled conditions and inoculated with known strains, but differences were partly restored by inoculation withstrain 5.

Highly effective lines nodulated earlier and produced more large nodules with greater total nitrogenase activity than modal lines or original cultivars, but nitrogenase activity per unit nodule weight did not differ significantly between plant lines. In both programmes the highly effective lines flowered about 10 days earlier than the modal lines; these differences were observed in first-year and in second-year plants.

These results indicate a considerable potential for increasing the yield of nodulated red clover by improving the symbiosis by plant breeding. (Nutman)

Effect of temperature on the symbiosis of *Vigna radiata* (green gram) and *Vigna mungo* (black gram). Temperature (33/30, 27/24 and 21/18°C day/night) greatly affected the growth of both nodulated plants, and non-nodulated plants supplied with 240 ppm N as ammonium nitrate. At 21°, N-fed plants grew better than nodulated plants, but the differences were relatively smaller at the higher temperatures. At 27° *V. mungo* nodulated by *Rhizobium* strain R5000 produced as much dry matter as plants fed combined N. Both species nodulated most rapidly and fixed most nitrogen at 33°C. Nodules formed later at lower temperatures; very few formed at 21°C and little nitrogen was fixed. Nodules formed by strain R5000 at 33° fixed 59 and 55% more N than nodules formed by strains CB756 and SU318 respectively, and at 27°, 154 and 102% more. Neither species flowered at 21° (by 45 days), whilst both species flowered at 27°; *V. mungo* did not flower at 33°C. (Islam and Dart)

Effect of heat stress and waterlogging on nitrogen fixation by cowpeas. A preliminary experiment (*Rothamsted Report for 1973*, Part 1, 85) showed that four-week old cowpea plants cv. K 2809 subjected to high soil temperatures were adversely affected in their ability to fix nitrogen, suggesting that high soil temperatures may be a constraint to yields in tropical climates. The roots of nodulated and non-nodulated plants (grown with 240 ppm N in the nutrient medium) were subjected to up to 10 consecutive daily cycles of 5 h at 40°C at three stages of growth; *viz.* just after initial nodulation, at flowering and at mid-pod fill. Nodulated plants at all stages of growth lost up to 70% of nitrogenase activity after five cycles of high temperature, but after a further 10 days of stress-free conditions activity was fully restored. Plants subjected to 10 days of stress showed a full recovery of nitrogenase activity over the latter five days of the stress period. Mature pod yields of stressed and unstressed plants, whether or not nodulated did not differ significantly. The adverse effect of 10 days of 40°C heat stress on the roots of cowpea is thus temporary and without effect on final yield.

Other experiments examined the effect of waterlogging on nitrogenase activity and yield of the same cultivar of cowpea. Nitrogenase activity was decreased most by periods of waterlogging prior to flowering. One day of waterlogging had little or no effect, but

https://doi.org/10.23637/ERADOC-1-131

four days of waterlogging induced yellowing of leaves and reduction of growth. After two cycles, each involving four days of waterlogging, and a recovery period, there was little effect of a third waterlogging cycle suggesting that the nodules and plants had become tolerant of waterlogging. (Eaglesham and Dart, with Dr. R. J. Summerfield and Dr. F. Minchin, University of Reading)

Leghaemoglobin (Lb). Electron paramagnetic resonance (EPR) spectra of the ferric forms (Lb^{3+}) of the two soyabean Lbs and their derivates at pH 7·0 were recorded at liquid helium temperatures. They show that the environments of the iron atom in these Lbs and in cowpea Lb (*Rothamsted Report for 1973*, Part 1, 86) and myoglobin are very similar. EPR spectra of the nicotinic acid complexes of the three ferric Lbs at pH 6·9 were alike, resembling the $Lb^{3+}CN^-$ spectrum. The similar spectra of the Lb*c* and cowpea Lb^{3+} imidazole complexes at pH 7·0 resembled the $Lb^{3+}N_3^-$ spectrum.

The large EPR signal reported earlier at $g=2$ in oxyleghaemoglobin ($Lb^{2+}O_2$) fractions was similar to the signal from nitric oxide haemoglobin ($Hb^{2+}NO$) prepared from blood, and identical to that of nitric oxide leghaemoglobin ($Lbc^{2+}NO$) prepared from Lbc^{3+} via $Lbc^{2+}O_2$. An unstable form of Lba^2p detected by Appleby (*Biochimica et Biophysica Acta* (1969) **189**, 267–79) was shown to be $Lba^{2+}NO$. Quantative EPR spectroscopy of a sample of crude soybean Lb showed that it contained $30 \pm 3\%$ $Lb^{2+}NO$. (Maskall and Dart, with Dr. J. F. Gibson, Imperial College)

The effect of temperature on root hair infection of clovers. Infection was studied in the small-seeded clover species, *Trifolium glomeratum* and *T. parviflorum* grown at root temperatures of 6, 12, 18, 24, 30 and 36°C, with the tops maintained at an ambient temperature of 23° during the 12 h light period (15 000 lux) and 17°C in the dark. Roots were examined at intervals from 4 days.

Infection of both species was much reduced at temperatures below 18°C or above 30°C, *T. glomeratum* showing a narrower optimum at a slightly higher temperature than *T. parviflorum*. Infection was not simply related to root length or cotyledon and leaf area. Nodule number was more affected by heat or cold than infection; nodule volume was less at the higher and lower than at intermediate temperatures. The development of lateral organs (primordia, nodules and secondary roots), like infection, was much reduced by temperature below 18 or above 30°. The marked zonation of infection along the root and the morphological features of infection thread growth were little affected by temperature. The size of the root hair nuclei (larger in infected hairs) was not affected by temperature.

Over most of the temperature range the rate of infection was biphasic, the data fitting a model in which infections were proposed to be of two kinds: primary infections occurring at random at a low rate and secondary infections arising near those already formed at an augmented rate. Only the secondary rate of infection appeared to be increased by temperature (between 12 and 30°C).

The inhibitory effect of high and low temperature was further studied in experiments in which plants were transferred at 4–21 days, from tanks held at 6 and 36°C to tanks at 24°C and *vice versa*.

Transfer from 6 to 24°C at 6, 9, 12 and 14 days immediately increased the rate of infection to above that of plants kept at 24°C, and caused a larger final number of root hairs to become infected; with transfer at 20 days the response was less striking. Transfer from 36 to 24°C also stimulated infection. With transfer before six days this occurred immediately but with transfer at 9, 12 or 14 days stimulation occurred after a 1–2 day lag period. Transfer from 24 to 6 or 36° at any time had little or no affect on the subsequent rate of infection.

https://doi.org/10.23637/ERADOC-1-131

Plants were also transferred from 6 to 24°C at six days for periods of 6, 12 or 24 h and then replaced in the 6°C tanks. The increase in the rate of infection over the following four days was proportional to time spent at 24°C, suggesting that an irreversible change affecting initial susceptibility had taken place. (Kumarasinghe)

The formation of callose in infected root hairs. Fresh roots of *Trifolium glomeratum*, *T. parviflorum* and *T. patens* were stained with 0·005% aqueous aniline blue and examined by u.v. microscopy which identifies callose by green-blue fluorescence. Small areas of callose were abundant in root hairs of each species, especially with rhizobia, and some callose was observed in the cortex; cortical and root hair callose became less pronounced or disappeared altogether as the individual hairs aged.

Deposits of callose were prominent in the region of initiation of the infection thread, but did not appear to extend much into the thread. Unlike those in most uninfected hairs these deposits were permanent, reflecting an irreversible change in host wall composition at the time of infection. (Kumarasinghe)

Nitrogen fixation by free-living bacteria

Rhizosphere associations. The nitrogenase activity of large core samples (*Rothamsted Report for 1973*, Part 1, 84), taken from three sites was monitored at regular intervals using the acetylene reduction assay. The mean daily nitrogen gains inferred from this assay over the period from September to November were for Broadbalk Wilderness (stubbed) 110 g/ha, for Long Hoos ('odds and ends' under ryegrass) 32 g/ha, and for Clay Croft (under ryegrass) 82 g/ha. The soil moisture beneath ryegrass was generally lower than that in Broadbalk. The nitrogenase activity of Broadbalk cores, containing *Stachys sylvatica*, declined rapidly in November as the plant tops died back. The activity beneath the ryegrass at both other sites continued well into November despite a drop in soil temperature at a depth of 10 cm from *c* 15 to 6°C. Most of this activity was intimately associated with the grass roots (less than 5% in the residual soil).

Several tropical grain and forage grasses were germinated from seed and grown in Woburn sandy soil (low N) and Rothamsted clay-loam soil. Japanese and Shirohills millet were particularly effective in stimulating nitrogenase activity whilst Green panic grass and Molasses grass were somewhat less effective. *Setaria anceps* growing in cores of its native Australian soil had substantial activity (40–170 nmoles C_2H_4/g root dry wt/h); *Pennisetum clandestinum* in Australian soil also promoted some activity (17–21 nmoles C_2H_4/g root dry wt/h). Plants of these species established from small cuttings in Woburn and Rothamsted soil developed a similar order of activity indicating that a range of grasses may be able to stimulate the indigenous nitrogen fixing bacteria in both tropical (*Rothamsted Report for 1971*, Part 1, 95) and temperate soils.

Isolated roots of Shirohills millet, perennial ryegrass and *Pennisetum clandestinum* have a marked optimum for acetylene reduction at a pO_2 of 0·04 atm. The importance of attaining this O_2 tension within the rhizosphere under normal growth conditions was shown by a lack of nitrogenase activity in exposed peripheral roots of pot-grown plants and by the quicker response to acetylene of exposed roots of intact plants when oxygen was excluded during the preparation of the samples.

A critical appraisal of the acetylene reduction assay as applied to non-nodulated plants is being done to clarify the reason for the lag between the addition of acetylene and the evolution of ethylene and the relation between acetylene reduction and nitrogen fixation using ^{15}N. With entire plants in pots and large cores of field-grown plants, the lag in ethylene evolution was attributed partly to the time taken for diffusion of gas into the soil; a shorter lag occurs with isolated roots in the absence of soil (*Rothamsted Report for 1972*, Part 1, 87). (Witty, van Berkum, Dart and Eaglesham)

https://doi.org/10.23637/ERADOC-1-131

Association between *Azotobacter paspali* and *Paspalum notatum*. Nitrogen fixation in the rhizosphere of *Paspalum notatum* has been attributed to *Azotobacter paspali* in the rhizosphere although recent work suggests that other rhizosphere organisms may be more important. We showed (*Rothamsted Report for 1973*, Part 1, 80) that cultures of *A. paspali* produce gibberellin-like substances, an auxin and cytokinins. Further work to examine the possible effects of hormone production has now demonstrated that cultures of *A. paspali* stimulate growth of young seedlings of *Paspalum* in John Innes compost before nitrogen fixation can be detected. With older plants (at 18 weeks) acetylene was reduced more rapidly (80 nmol/g dry roots/h) by uninoculated than by inoculated roots (10 nmol/g dry roots/h); *A. paspali* cells were present only in the rhizospheres of inoculated plants and plant growth was better in the inoculated series than in the controls. Similar results were obtained in experiments with *Paspalum* grown in Brazilian soil for 24 weeks, although at this stage no *Azotobacter paspali* could be recovered from the rhizosphere.

A second experiment verifying these results included plants, the seedling roots of which were treated with a filter-sterilised supernatant of a 14 day-old culture of *A. paspali*. At four weeks all treated plants were larger than controls, but there was no nitrogen fixation. At 14 weeks nitrogen fixation was detected in all control and cell-inoculated rhizospheres and in one rhizosphere of a supernatant-treated plant grown in John Innes compost, and in two rhizospheres of control plants grown in Brazilian soil. *A. paspali* was detected in some rhizospheres, but not always in those showing nitrogen fixation. All treated plants in both soils were significantly larger than controls. These results indicate that improvement in growth of *Paspalum notatum* when associated with *A. paspali* is caused by hormones rather than by nitrogen supplied by the bacteria. In the natural environment the hormones may be supplied when the bacteria start to multiply in the rhizosphere, and the plant/bacterial association becomes firmly established. Subsequent fixation probably results from activity of organisms other than *A. paspali* that occur widely in different soils, and are stimulated by roots of *Paspalum notatum* when the grass produces rhizomes. (Brown)

Take-all disease

Effect of host and fungal nutrition on expression of disease. Reports on the effects of nitrate and ammonium fertiliser on the amount of take-all disease that develops in a cereal crop are conflicting. Our results from monthly examination of rhizosphere soil taken from wheat plants growing in the 'wheat after intensive barley experiment' in Little Knott field, Rothamsted, showed that during the period of active crop growth after spring application of 'Nitro-Chalk', NH_4^+–N was slightly in excess of NO_3^-–N. At this time take-all disease is rapidly spreading on the roots.

To examine this further a model system was devised. This consisted of sterile 500 ml flasks partly filled with perlite moistened with distilled water, each flask containing a vial of plant nutrient solution. The vials were supplied with 5·5 mg N as NO_3^-–N and NH_4^+–N in the ratios of 1 : 0, 4 : 1, 1 : 1, 1 : 4 and 0 : 1 NO_3^-–N : NH_4^+–N. Aseptic wheat seedlings were placed in the flasks with one 'feeder' root dipping in the solution and the others growing in the perlite. Inocula of two pathogenic strains of *Gaeumannomyces graminis* were introduced into the perlite, with or without 0·2 ml of a 1 : 10 soil suspension to provide a mixture of soil organisms. After three weeks seedlings were assessed for disease, which was expressed as a percentage of seminal axes infected, and length of lesion as a percentage of total root length. The fungus had no direct contact with the nutrient solution and was influenced only by the root environment as modified by the nutrients taken up through the feeder roots. Infection by strain 028B decreased as NH_4^+–N

https://doi.org/10.23637/ERADOC-1-131

concentration increased, and strain L5–7 was least infective on seedlings given the 1 : 1 mixture or 100% NH_4^+–N. Added soil organisms reduced infection and altered its pattern; strain 028B was most infective and strain L5–7 was markedly less infective on seedlings given 100% NH_4^+–N.

The infection pattern from inocula grown with NO_3^-–N or NH_4^+–N as nitrogen source instead of the medium containing 0·4% malt, was then examined. Both strains of inocula behaved similarly producing most infections on seedlings grown in the solution containing equal amounts of NH_4^+–N and NO_3^-–N. Ammonium grown inocula were less infective than NO_3^--grown inocula, and both less so than malt-grown inocula. When soil organisms were added the infection pattern was repeated, but disease was less severe, and NO_3^--grown inoculum was generally most effective. There were complicated interactions between host nutrition and the form of N on which the fungus was grown and infection.

All these results showed that the amount of infection on roots depended on both host and fungal nutrition, and on the activity of the microbial flora of the roots. (Brown)

Mycorrhizal studies

Utilisation of rock phosphate by mycorrhizal plants. Growth and P uptake of mycorrhizal and non-mycorrhizal onions, red clover, *Stylosanthes* and *Centrosema* were studied in seven soils (pH 4·5–8·1) supplied with rock phosphate. In three Rothamsted soils (pH 6·2–8·1) neither growth nor P uptake of onions or clover were increased by rock phosphate, but both were greater in mycorrhizal plants. In the Brazilian cerrado soils (pH 4·5–6·5) rock phosphate and mycorrhiza increased dry weight of *Stylosanthes*, *Centrosema* and clover 7–22-fold and P uptake by approximately 150-fold. Rock phosphate alone produced smaller improvements than mycorrhiza alone and their combined effects were synergistic. All plants were given appropriate rhizobia. Nodulation and nitrogen fixation only occurred to any extent in mycorrhizal plants given rock phosphate. The Brazilian soils contained less available phosphate than those from Rothamsted. Nevertheless, in a British Honduras soil (pH 6·4) containing amounts of available P similar to those in the Rothamsted soils, *Stylosanthes* responded markedly to rock phosphate, and correspondingly more to rock phosphate and mycorrhiza. In two other British Honduras soils (pH 7·2 and 7·7) there was little response to rock phosphate but in one soil *Centrosema* responded markedly to mycorrhiza. Plants inoculated with two different endophyte strains weighed respectively 50 and 100% more than non-mycorrhizal plants and fixed six and nine times as much nitrogen; a third strain producing some infection did not stimulate growth. In two soils there were also marked responses to mycorrhizal inoculation of natural (non-irradiated) soil given rock phosphate. (Mosse, Powell and Hayman)

The specific activity of phosphorus in onions infected with seven different *Endogone* strains and grown in soil labelled with ^{32}P was very similar, indicating that different endophytes probably utilise the same sources of soil phosphate; differences in the ability of strains to promote P uptake may depend on differences in spread of the soil mycelium or in translocation rates within the hyphae. (Powell)

Effects of mycorrhiza on phosphate cycling at Meathop Wood, Lancs. Experiments with B horizon soil from the IBP site showed that seedling growth of five indigenous plant species was greatly improved by inoculation with indigenous mycorrhizal fungi (*Rothamsted Report for 1973*, Part 1, 81). The extent of the benefit, however, depends considerably on the depth of the humus layer. Two soil profiles were reconstructed. With a 2 cm humus layer non-mycorrhizal seedlings in irradiated soil grew better than mycorrhizal

https://doi.org/10.23637/ERADOC-1-131

seedlings but with a profile, considered typical of the site—consisting of a thin litter layer, a humus layer of 0·6 cm (10 ppm P), an A horizon of 2·4 cm (5·2 ppm P) and a B horizon of 8 cm (3·2 ppm P)—mycorrhizal *Brachypodium* and *Fragaria* seedlings took up 1·5 and 15 times more P respectively than the corresponding non-mycorrhizal seedlings. Both types of soil profile occur at the site and estimates of mycorrhizal involvement in phosphate uptake should preferably be based on *in situ* experiments. (Paget and Mosse)

Interaction between species of *Endogone* and *Cylindrocarpon*. Mycorrhizal plants grown in irradiated Meathop Wood soil weigh about twice as much and take up two to three times as much P as those in unsterile soil, although irradiation does not increase the available phosphate or much affect mycorrhizal development. The possibility of competition between the mycorrhizal fungi and other soil micro-organisms for soil phosphate was investigated using *Cylindrocarpon destructans*, a fungus common in this soil. Following inoculation, *Cylindrocarpon* became established in the soil but was rarely recovered from *Fragaria* roots. Its effects on seedling growth were very variable, being beneficial for some seedlings and pathogenic for others. *Cylindrocarpon* did not reduce effectiveness of *Endogone* by competition for soil phosphate. On the contrary dual inoculation in irradiated soil tended to increase plant weight and significantly increased phosphate uptake compared to inoculation with *Endogone* alone. *Cylindrocarpon* by itself did not increase $NaHCO_3^-$ or $CaCl_2$-soluble P in the soil. (Paget and Mosse)

Effects of light on VA mycorrhiza. In a P-deficient soil plant growth was often stimulated more by adding monocalcium phosphate than by inoculating with *Endogone* in low light (8000 lux) but not in high light (25 000 lux). Infection was directly correlated with concentrations of soluble sugar which were considerably lower in roots of P-deficient plants than in mycorrhizal roots or plants given phosphate. (Hayman)

Observations on the germination and growth of *Endogone* on agar plates and in the soil. Yellow vacuolate *Endogone* spores will not germinate on agar containing more than 1·4 ppm manganese and some samples of spores will not germinate in the presence of 0·7 ppm zinc, but will germinate when subsequently transferred to a medium without Mn or Zn. Many samples of agar tested contained sufficiently high levels of heavy metals to reduce growth or completely inhibit germination, and the use of tap water to prepare media can also be inhibitory. (Hepper and G. A. Smith)

The buried slide technique was found useful to observe growth of *Endogone* in the soil. The number of yellow vacuolate spores germinating on agar-coated slides buried in two soils or in sterile sand was the same as those on agar alone and was unaffected by the close proximity of seedling roots. In soil, hyphal growth was much more extensive than on agar; hyphae were characteristically very long and unbranched both in the rhizosphere and in soil free from roots. By contrast, in axenic root organ culture pre-penetration growth of the mycelium was stimulated and rhizoid-like branching induced. Substances produced by roots may be either adsorbed in the soil or metabolised by other micro-organisms. Hyphae were not usually attracted towards growing roots and often grew straight across them. Only occasionally a hypha would change direction when close to a root and penetrate it at right angles or run along the surface for a short distance before forming an appressorium, indicating that the root is not uniformly susceptible to VA infection. (Hepper)

Some laminate and honey-coloured spores germinated on buried slides within two weeks, whether or not host roots were present, producing branched hyphae up to 2 cm long, with small (30 μm) sympodially born spores, as on agar plates. Germ tubes

https://doi.org/10.23637/ERADOC-1-131

approaching host roots, branched profusely before infecting. Sporocarps of the E_3 type only produced ephemeral hyphae. Buried mycorrhizal soyabean roots produced hyphae from internal (E_3) or external (yellow vacuolate) mycelium after a delay of five weeks. The hyphae grew straight toward onion roots, branched profusely in their proximity, formed appressoria and infected the roots. (Powell)

In two experiments *Lupinus cosentinii* grown in pots at 20°C did not become mycorrhizal after inoculation with VA endophytes. However, when *Trifolium pratense* was grown with lupin in the same pot it became mycorrhizal and when this happened the lupins also became infected. (Trinick and Mosse)

Nitrification and nitrifying bacteria

New isolates. The use of calcium carbonate-coated membrane filters has been investigated for isolating both ammonia-oxidising and nitrite-oxidising autotrophs. Five new strains of ammonia-oxidisers were obtained in pure culture, one a *Nitrosospira* sp. and the others species of *Nitrosocystis* (syn. *Nitrosolobus multiformis*). Morphology of some of these organisms has been studied by electron microscopy and also by dark-field microscopy and the latter technique was particularly suitable for recognising species of *Nitrosospira* and studying *Nitrosocystis*.

Nitrite-oxidisers examined seem to be mostly *Nitrobacter* spp. and the morphology of these from different soil types is being compared by electron microscopy. Nitrobacters are unusual organisms in that they reproduce by budding; this process is being studied by photomicrography of slide cultures and by electron microscopy of whole cells. (Macdonald and Walker)

Effects of pesticides on the soil microflora. The possible use of nitrifiers as indicators of pesticide damage is being investigated because they are known to be sensitive to certain chemicals, including some pesticides. The dominant ammonia-oxidising nitrifier was isolated from the Long Hoos experiment (see below) and identified as *Nitrosocystis* sp. None of the pesticides when applied at field rates appears to depress the growth rate of this organism in pure culture or in soil perfusion conditions. (Macdonald and Morley)

To use nitrifiers as such indicators, a basic knowledge of the population dynamics of the organisms is necessary. Ammonia-oxidisers were counted every two days in July and August in a control plot of the Long Hoos experiment; three maxima and minima occurred. Periods of increase in the ammonia-oxidiser population appeared to be correlated with rainfall. Organisms were counted by the most probable number method. Population estimates by silica gel plate counts were of the same order of magnitude. (Macdonald and Walker)

A multiple column soil perfusion apparatus was designed for use in studies of herbicide degradation and the effects of pesticides on nitrification. It uses a new air-lift system and operates the columns in parallel rather than in series, avoiding a pressure differential between columns and allowing any number of columns to be operated simultaneously. (Macdonald)

A long term experiment in Long Hoos field has been set-up to assess the chemical and biological effects of combinations of four pesticides. The herbicide chlortoluron with or without other pesticides, has been found to decrease markedly the extent of algal crust formation on the soil (Macdonald)

Microbial metabolism and co-metabolism of herbicides or related compounds

1-Naphthol. The early metabolic pathway of 1-naphthol (a hydrolysis product of the insecticide carbaryl ('Sevin') in *Pseudomonas* spp. was further examined. Salicylic

https://doi.org/10.23637/ERADOC-1-131

acid was detected in cultures and in oxidation experiments with washed cells in buffer suspensions. The detection of salicylic acid and the failure to find any dihydroxybenzoic acids in cultures grown on 1-naphthol as sole carbon source indicates the anomalous dissimilation of 1-naphthol in comparison with 2-naphthol or other monosubstituted naphthalenes. (Spokes and Walker)

Phenoxyacetic acid herbicides. The possible co-metabolism of substituted phenoxyacetic acid herbicides by phenol- or benzoate-grown bacilli was examined but we failed to obtain any pure cultures of phenoxyacetate-utilising organisms for this purpose; there were small O_2 uptakes in presence of 2,4-D. (Dunlop and Walker)

Chlortoluron. Work has begun on the possible microbial degradation of chlortoluron. Two crude cultures growing on the related compound *p*-toluidine have been obtained and the responsible organisms isolated. (Spokes and Walker)

Degradation of asulam and sulphanilamide. The widely used herbicide, asulam (a derivative of sulphanilamide) is degraded in soil but little is yet known of the process. Both asulam and sulphanilamide are fairly readily degraded by mixed cultures of soil microbes in soil perfusion systems or in soil enrichment flasks. The problem of isolating the responsible micro-organisms in pure culture remains. Degradation in mixed cultures is inhibited by pasteurisation or KCN but not by penicillin or cycloheximide. Manometric experiments with mixed cultures grown on asulam showed O_2 uptake at the expense of asulam, sulphanilamide, sulphanilic acid and aniline; terminal rates of uptake were similar. (Macdonald)

Staff and visiting workers

P. Davis was appointed as Curator of the Rhizobium Collection, and J. Witty and P. van Berkum joined the department under the auspices of International Biological Programme and Ministry of Overseas Development respectively, to work on nitrogen fixation associated with tropical plants. J. Day and D. S. Hayman are on secondment to IPEACS, Guanabara, Brazil and the Biological and Chemical Research Institute, Rydalmere, N.S.W., Australia, respectively. Visiting workers included Dr. M. J. Trinick of Division of Land Resources Management, Wembley, Australia, and Dr. C. Ll. Powell of Ruakuru Agricultural Research Centre, Hamilton, New Zealand.

P. J. Dart visited CSIRO Division of Plant Industry, the Australian Inoculant Research and Control Services, and other laboratories in Australia, and ICRISAT in India. He also presented papers at the Third Cabot Symposium, Petersham, Massachusetts, USA, and at a Conference on the Biology of Yield in Grain Legumes in New Delhi, India. Barabara Mosse visited CIAT, Columbia, University of Gainsville, Florida and the University of Perth, W. Australia, and P. S. Nutman visited Ife University and IITA, Ibadan, Nigeria. Several members of the department gave papers at the International Symposium on Mycorrhiza at Leeds.

Publications

Book

1 WALKER, N. (Ed.) 1975 *Soil Microbiology* London: Butterworths, with chapters by M. E. Brown (J. Darbyshire), P. J. Dart, J. Day, D. Hayman, C. Hepper, B. Mosse, P. S. Nutman, (D. S. Powlson), F. A. Skinner and N. Walker.

https://doi.org/10.23637/ERADOC-1-131 *pp 11*

General Papers

2 Brown, M. E. (1974) Seed and root bacterization. *Annual Review of Phytopathology* **12**, 181–197.

3 Dart, P. J. (1974) The infection process In: *The biology of nitrogen fixation.* Ed. A. Quispel. Amsterdam: North-Holland, pp. 381–429.

4 Dart, P. J. (1974) Root nodule symbiosis and tropical grain legume production. In *Proceedings 1st IITA Grain Legume Improvement Workshop.* IITA Press, 185–197.

5 Dart, P. J. (1975) Legume root nodule initiation and development. In: *Third Cabot Symposium; The development and function of roots.* Ed. J. G. Torrey & D. Clarkson. London: Academic Press.

6 Dart, P. J. (1975) Infection and development of leguminous nodules. In: *Treatise on nitrogen fixation.* Ed. R. W. F. Hardy. New York: John Wiley.

7 Dart, P. J. (1975) Nodulation of grain legumes. In: *Proceedings of the Working Group on the Biology of Yield in Grain Legumes.* Convened by the Technical Advisory Committee to the Consultative Group on International Agricultural Research. F.A.O. Rome.

8 Mosse, B. (1975) The role of mycorrhiza in phosphorus solubilisation. *Proceedings IV International Conference: Global Impacts of Applied Microbiology.* São Paulo, Brazil.

9 (Neves, M. C. P.), Day, J. M., (Carneiro, A. M. & Döbereiner, J.) (1975) Actividad nitrogenase na rhizosfera de gramineas tropicais forrageiras. *Proceedings IV International Conference: Global Impacts of Applied Microbiology.* São Paulo, Brazil.

Research Papers

10 (Barea, J. M., Azcón, R.) & Hayman, D. S. (1975) Possible synergistic interactions between *Endogone* and phosphate-solubilising bacteria in low-phosphate soils. In: *Endomycorrhizas.* Ed. B. Mosse, F. E. Sanders and P. B. Tinker. London: Academic Press.

11 (Barea, J. M.) & Brown, M. E. (1974) Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. *Journal of Applied Bacteriology* **37**, 583–594.

12 (Bowen, G. D., Bevege, D. I.) & Mosse, B. (1975) Phosphate physiology of vesicular arbuscular mycorrhiza. In: *Endomycorrhizas.* Ed. B. Mosse, F. E. Sanders & P. B. Tinker. London: Academic Press.

13 Brown, M. E., Hornby, D. & (Pearson, V.) (1973) Microbial populations and nitrogen in soil growing consecutive cereal crops infected with take-all. *Journal of Soil Science* **24**, 296–310.

14 (Crush, J.) & Pattison, A. C. (1975) Preliminary results of freeze drying VA mycorrhiza. In: *Endomycorrhizas.* Ed. B. Mosse, F. E. Sanders & P. B. Tinker. London: Academic Press.

15 Dart, P. J., Day, J. M., Islam, R. & (Döbereiner, J.) (1975) Symbiosis in tropical grain legumes—some effects of temperature and the composition of rooting medium. In: *Symbiotic nitrogen fixation in plants.* Ed. P. S. Nutman. London: Cambridge University Press.

16 Day, J., (Harris, D.), Dart, P. J. & van Berkum, P. (1975) 'The Broadbalk Experiment'. An investigation into gains from non-symbiotic nitrogen fixation. In: *Nitrogen fixation by free-living micro-organisms.* Ed. W. D. P. Stewart. London: Cambridge University Press.

https://doi.org/10.23637/ERADOC-1-131

17 (DÖBEREINER, J.) & DAY, J. M. (1975) Nitrogen fixation in the rhizosphere of tropical grasses. In: *Nitrogen fixation by free-living micro-organisms.* Ed. W. D. P. Stewart. London: Cambridge University Press.

18 (DÖBEREINER, J.) & DAY, J. M. (1975) Associative symbiosis in tropical grasses: characterization of micro-organisms and dinitrogen fixing sites. In: *International Symposium on Nitrogen Fixation*, Washington State University.

19 (DÖBEREINER, J.), DAY, J. M. & DART, P. J. (1973) Fixacao de nitrogenio na rizosfera de *Paspalum notatum* e da cana-de-acucar [Nitrogen fixation in the rhizosphere of *Paspalum notatum* and sugar cane]. *Pesquisa Agropecuaria Brasileira* **8**, 153–157.

20 (DÖBEREINER, J.) , DAY, J. M. & DART, P. J. (1973) Rhizosphere associations between grasses and nitrogen fixing bacteria: effect of O_2 on nitrogen activity in the rhizosphere of *Paspalum notatum*. *Soil Biology and Biochemistry* **5**, 157–159.

21 (DÖBEREINER, J.) & DAY, J. M. (1975) Potential significance of nitrogen fixation in rhizosphere associations of tropical grass. *Proceedings of the International Tropical Soils Congress, CIAT, Columbia.*

22 HAYMAN, D. S. (1975) The occurrence of mycorrhiza in crops as affected by soil fertility. In: *Endomycorrhizas*. Ed. B. Mosse, F. E. Sanders and P. B. Tinker. London: Academic Press.

23 HEPPER, C. M. & MOSSE, B. (1975) Techniques used to study the interaction between *Endogone* and plant roots. In: *Endomycorrhizas*. Ed. B. Mosse, F. E. Sanders & P. B. Tinker. London: Academic Press.

24 MOSSE B., (1975) Specificity in VA mycorrhiza. In: *Endomycorrhizas*. Ed. B. Mosse, F. E. Sanders & P. B. Tinker. London: Academic Press.

25 NUTMAN, P. S. (1975) Summary of IBP experiments on nitrogen fixation by nodulated legumes. In: *symbiotic nitrogen fixation in plants*. Ed. P. S. Nutman. London: Cambridge University Press.

26 PAGET, D. (1975) The effect of *Cylindrocarpon* on plant growth responses to vesicular-arbuscular mycorrhiza. In: *Endomycorrhizas*. Ed. B. Mosse, F. E. Sanders & P. B. Tinker. London: Academic Press.

27 PATTISON, A. C. & SKINNER, F. A. (1974) The effects of antimicrobial substances on *Rhizobium* spp. and their use in selective media. *Journal of Applied Bacteriology* **37**, 239–250.

28 SKINNER, F. A. (1975) Methodology in soil examination. In: Microbiological Trends in Agriculture, Fisheries & Food. Ed. F. A. Skinner & (J. G. Carr). *The Society for Applied Bacteriology. Symposium Series* No. 4. London: Academic Press.

https://doi.org/10.23637/ERADOC-1-131 *pp 13*