ROTHAMSTED RESEARCH

Report for 1976 - Part 1

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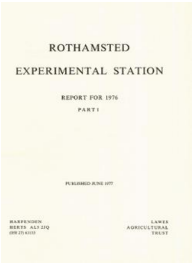

Soil Microbiology Department

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Introduction

The Department's work concentrates on only a few of the microbial processes in soil of special importance in agriculture: on the breakdown of natural cellulosic materials and man-made organic agrochemicals and related compounds; on those events in the zone of the soil nearest the root (the rhizosphere) that can affect root health and activity; on the processes of nitrification, denitrification and fixation of atmospheric nitrogen; on the functioning of the symbiotic associations of microbes and roots that can benefit crops either by fixing nitrogen (legume root nodules) or by helping the plant to take up phosphate (VA mycorrhiza).

Some of our work, especially in the two last-mentioned fields, is done in collaboration with research institutes and university departments, here and overseas, as indicated in the separate sections of this report. Our contribution to the Rothamsted inter-departmental study on the causes of poor yield of field beans is reported by the Field Experiments Section (p. 150).

Continuing programmes on *Rhizobium* morphology in culture, nodule fine structure, anomalous nodulation of clover by pea bacteria, effect of diurnal temperature fluctuations on nodulation and on aerobic free-living nitrogen fixers in soil will not be reported this year.

Stability of soil aggregates

The incubation studies reported last year were continued on a pair of lighter soils, Flint and Salop, selected by the Rothamsted Soil Structure Working Group. As before, stability to wet sieving of 2–3 mm diameter aggregates increased after aerobic incubation with water or nutrients. For Flint soil the greatest stability (79% of aggregates larger than 1 mm, compared with a non-incubated control of 44%) occurred after incubation with

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glucose and peptone. Salop, with initially poorer structure, likewise became more stable (86%) than Flint when incubated with nutrients. Flint soil developed some anaerobic stability irrespective of treatments, but there was almost no increase in stability of Salop soil under anaerobic conditions.

When air-dry soil was incubated with water even for as little as 1 h there was a marked increase in water stability. The rapidity of this change suggests physical rather than microbiological causes. To investigate this, four air-dry soils (from Denchworth, Hanslope and two samples from Evesham), were either left untreated, or sterilised by autoclaving at 121°C for 15 min or by γ-irradiation. Stability to wet sieving was tested after aerobic incubation at 25°C with water for 1 h, and for 3, 6, 10 and 13 days. Stability increased up to the 3rd or 10th day for both untreated and irradiated soil, indicating that micro-organisms were not primarily responsible for this change. Autoclaved soils (except Hanslope) decreased markedly in stability after the 3rd day, possibly because this treatment may have destroyed substances that cause cohesion between soil particles. Aggregate stability also increased when untreated soils were incubated anaerobically with water though usually less so than with aerobic incubation. (Skinner)

Nitrogen-fixing anaerobes

Further work on this topic has been to develop a nitrogen-free medium suitable for counting nitrogen-fixing anaerobic bacteria in soils and to investigate anomalous results in Most Probable Number estimations of their numbers in soil. Growth of nitrogen-fixing obligate anaerobes (clostridia) in sealed bottles containing a nitrogen-deficient medium with nitrogen gas phase, is indicated by the onset of vigorous gaseous fermentation of sucrose. Such fermentation correlates well with the ability of cultures to reduce acetylene to ethylene. The pH value of the medium falls so quickly to below 6·0 that growth is inhibited, but the addition of chalk prevents this acid formation.

Anomalous fermentations occur when small inocula are used, one of the commonest being characterised by good growth and high nitrogenase activity but little gas formation. This effect is attributed to growth of *Bacillus polymyxa*, a facultative anaerobe which grows well aerobically but fixes nitrogen only when oxygen tension falls. (Skinner)

Microbial degradation of asulam and sulphanilamide

The aerobic bacterium capable of metabolising sulphanilamide and its carbamic ester, the herbicide asulam, reported last year, has now been identified as a *Flavobacterium* species. The organism differs in several properties from any known species described in the 8th edition of Bergey's *Manual of Determinative Bacteriology* but cannot be established as a new species without further comparison with known species. (Walker)

Nitrification

Ammonia-oxidising nitryfying bacteria of differing provenance. Five more strains have been obtained in pure culture, two of them from the collection of cultures left by Professor S. Soriano in 1970, the others from soil enrichments. One of the Soriano strains shows *Nitrosomonas* type morphology, grows well, forming rather large brownish colonies, and also cyst-like aggregates as attributed by Winogradsky to *Nitrosocystis coccoides*. An isolate from soil collected on Tryfan near Snowdon proved to be a new genus, *Nitrosovibrio* and closely resembled that recently described by Hamburg workers. (Walker)

Several tea plantation soils with pH values ranging from 5–6 from Bangladesh and Sri Lanka were examined for autotrophic nitrifying organisms. From all the soils nitrifiers were isolated in pure culture (usually using the $CaCO_3$-coated silica gel plate technique) and identified by light and electron microscopy. From two other characteristic Bangladesh

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soils, *Nitrosolobus* spp. were isolated whereas *Nitrosomonas* was isolated from a farm soil that had received cow manure. The Sri Lanka tea soils yielded either *Nitrosomonas* or *Nitrosolobus* spp. but from all of six Bangladesh tea soils examined a somewhat unusual type of *Nitrosospira* was isolated. These cultures showed small 'figure 3' and ring-like or crescent forms (resembling Winogradsky's 'pseudococci') in addition to typical spiral cells. They seemed distinct from the Spitzbergen strains and may well be a new species. Nitrite-oxidising bacteria were also detected in several of these tea soils. (Bhuiya and Walker)

Rothamsted collection of nitrifying bacteria. Altogether 17 more nitrifying strains representing four distinct genera of ammonia-oxidising autotrophs have been added to the Rothamsted collection.

Attempts to preserve cultures of nitrifying bacteria in sealed ampoules of medium containing suspended chalk have shown that viability is retained for several months. The collection of pure and enrichment cultures has now been preserved in this way. Comparison of morphology by electron microscopical examination has been extended to the majority of pure cultures in the collection. (Walker and Macdonald)

Effect of inhibitors on nitrifiers. A series of simple halogeno-, cyano-, amino- and alkyl-substituted pyrimidine and pyridine compounds together with nitrapyrin ('N-serve') and CS_2 were examined for their inhibiting action against pure cultures of several strains of the genera, *Nitrosomonas*, *Nitrosolobus*, *Nitrosospira* and *Nitrosovibrio*. The most active compounds were CS_2, nitrapyrin and pyroxychlor. An interesting finding was that *Nitrosolobus* spp. were generally about ten times more sensitive than *Nitrosomonas* spp. to CS_2 and nitrapyrin. The former species are the commonly-encountered nitrifiers in Rothamsted soils. (Bhuiya, Briggs and Walker)

Methods for determining the enzymic activity of soil micro-organisms

Enzymic cytochemical methods have been modified to enable soil micro-organisms to be differentiated on the basis of metabolic activity, and the techniques used to study population dynamics in relation to pesticide effects. The method was successfully applied to free living bacteria, rhizoplane bacteria, fungi, algae and protozoa extracted from soil or present in clay films floated from the surface of soil crumbs. Micro-organisms floated from soil in this way retained the colonial conformation of the micro habitat. (Macdonald)

Rhizosphere studies

Effect of vitamins on infection by *Gaeumannomyces graminis*. Work continues on the effects of thiamin, biotin and B_{12} on the infection of wheat roots by *Gaeumannomyces graminis* var *tritici*. When vitamins were added, two separate isolates differed in the amount of disease they produced. For example, thiamin increased significantly ($P = 0{\cdot}05$) infection by one isolate, whilst biotin and B_{12} had no effect and all three vitamins added together decreased infection. With the other isolate thiamin, biotin and B_{12} when added separately all decreased infection, but when added together increased infection. Bacteria, isolated from the wheat rhizosphere and producing all three vitamins, when inoculated on to wheat seedlings together with mycelium of the two *Gaeumannomyces* isolates also affected disease in a way similar to the three authentic vitamins.

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Populations of vitamin-producing bacteria in field soils are being studied in relation to their possible influence on take-all disease.

Effects of *Bacillus subtilis* and *Streptomyces griseus* on plant growth. Strains of *Bacillus subtilis* and *Streptomyces griseus* were found to increase plant gowth and have been used extensively in field trials in Victoria, Australia (Merriman, Price, Kollmorgen, Piggot & Ridge, *Australian Journal of Agricultural Research* (1974) **25,** 219–226). Isolates *B. subtilis* A13 and *S. griseus* 2-A24 have been supplied by Dr. Merriman for study of plant growth regulator production.

Cultures were grown in nutrient broth for 14 days, and extracts were examined by paper partition chromatography for gibberellins, cytokinins and indolyl-3-acetic acid (IAA). Eluates corresponding to 10 R_f values were tested for gibberellins using pea and lettuce bioassays, for cytokinins using tobacco and soya bean callus assays and colorimetrically for IAA. Unextracted cultures and eluates were also tested for their effect on growth of tomato and radish.

Gibberellin-like substances were detected in both cultures, eluates gave responses equivalent to 0·5 μg ml^{-1} culture in the pea test. IAA could not be detected. Responses in the callus assays indicated activity in both cultures equivalent to 0·02 μg ml^{-1} kinetin; these results require confirmation using different extraction methods. Sterile nutrient broth was also checked for regulating substances and one eluate gave a significant cytokinin reaction.

Significant increase ($P = 0·05$) in height and leaf growth of young tomato followed treatment with cultures of *S. griseus*, but not of *B. subtilis*; flowering was advanced by seven days, the average number of fruits per plant was unaffected but the weight of green fruit on the second truss was increased by 101% ($P = 0·1$). Cultures of *B. subtilis* had no effect on tomato. Significant increase ($P = 0·05$) in the growth of radish followed treatment with cultures of *B. subtilis*, but not of *S. griseus;* 14-day cultures applied to seeds or roots were more effective than two-day cultures. Treatment of tomato and radish with eluates from different R_f values also affected growth, eluates from both cultures containing gibberellin-like substances increasing height and leaf size of both plants but having no effect on flowering of tomato. However an eluate of a slow-running substance did advance flowering. This also increased the number of green fruits set and weight of ripe fruit by 20% (not significant at $P = 0·05$). This substance was also found in sterile nutrient broth and increased weight of ripe fruit of the second truss by 100% ($P = 0·1$). Thus it was probably a constituent of the medium rather than a metabolite from the organism which was affecting fruit growth. This same constituent decreased radish growth significantly ($P = 0·05$). (Brown)

Mycorrhizal studies

Measurement of fungal tissue in plant roots. The amount of fungal tissue present was estimated from the glucosamine (derived from hyphal wall chitin and chitosan) present in the infected material. Because the yield of glucosamine from the fungus grown alone can vary under different cultural conditions the estimate of fungal tissue in an infected root is subject to error. Despite this limitation the method was used to give rough estimates of the extent of VA mycorrhizal infection in four plant genera with four fungal endophytes (using the external mycelium to obtain a conversion factor); infection was also estimated in wheat roots inoculated with *Gaeumannomyces graminis*. The concentration of glucosamine was proportional to the amount of infected tissue in each sample; uninfected roots yielded very small amounts. The most heavily infected sample of VA mycorrhizal roots contained 17% fungal tissue (based on dry weight). (Hepper)

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Spore germination and growth studies. Barrett (*Recent Advances in Botany* (1961) **2,** 1725) reported that the mycorrhizal endophyte could be induced to colonise and grow out from hemp seed. We have been unable to confirm this observation with pieces of hemp, ground-nut, vetch or lima bean seed placed close to mycelium growing from root organ cultures infected with the yellow vacuolate endophyte. No sign of colonisation or saprophytic growth was detected. Callus tissue derived from the roots of soyabean, lima bean and carrot similarly failed to stimulate germ tube growth. Only an exudate from excised root cultures stimulated growth from pregerminated spores but to a very limited extent.

Surface sterilised honey-coloured spores newly formed or from stored sources were placed close to growing roots or on media which incorporated thiamine, biotin, nicotinic acid, pyridoxine, yeast extract or ammonium acetate. Spores were also subjected to alternate wetting and drying cycles, elevated temperatures or surfactants (all of which are thought to alter membrane permeability) and grown in gas mixtures containing increasing concentrations of carbon dioxide or ethylene. None of these treatments stimulated germination. A low yield of germinated spores was obtained on the surface of a soil agar medium but no germination was observed when spores were buried in the same soil either on agar-coated slides or sandwiched between layers of filtration membrane (pore size 0·22 μ).

The germination of laminate and yellow vacuolate spores was inhibited similarly by additions of manganese to the medium but the former was slightly less sensitive to zinc. Germination was reduced from 80% to zero by the addition of either 7 ppm zinc or 0·14 ppm manganese. (Hepper)

Enzyme cytochemistry of *Glomus mosseae*. Acid phosphatase, glutamate dehydrogenase, succinate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase and NADH and NADPH diaphorases were demonstrated cytochemically in *Glomus mosseae* ('yellow vacuolate' spore type). Phosphatases hydrolysing glycerophosphate or 6-benzoyl-2-naphthyl phosphate were absent from hyphae derived from germinated yellow vacuolate spores. 6-Bromo-2-hydroxy-3-naphthoyl-o-anisidine phosphatase was found in germ tubes and all three substrates were utilised by acid phosphatases in vesicles, arbuscules and hyphae of mycorrhizas of *Allium*, *Nardus*, *Trifolium* and *Stylosanthes*. From this it was inferred that the fungus probably possesses an Embden-Meyerhof-Parnas system, a tricarboxylic acid cycle and a hexose monophosphate shunt. The presence of glutamate dehydrogenase implied that amino acid respiration by the fungus may be possible. Infection by *Glomus mosseae* resulted in increased concentrations of succinate dehydrogenase in the roots. (Macdonald and Lewis).

Influence of VA mycorrhiza on plant growth. Eight crops were tested for their responses to inoculation with the vesicular-arbuscular endophyte E_3 in pot experiments with three agricultural soils. Mycorrhizal infection became well established in all crops except lupins. Inoculation was most effective in a soil from Delharding field, Rothamsted (containing 8 ppm $NaHCO_3$-soluble P) where mycorrhizal plants of lucerne, red clover and field beans were 30, 12 and 2 times heavier respectively, than the non-mycorrhizal controls. French beans, maize, peas and barley grown in this soil, however, responded only slightly to inoculation. In a soil from Ashridge, Hertfordshire (26 ppm $NaHCO_3$-soluble P) and Woburn (40 ppm $NaHCO_3$-soluble P) soils, both mycorrhizal and non-mycorrhizal plants generally grew much better than in Delharding soil, but responded irregularly to mycorrhizal inoculation. Inoculation did not greatly affect plant dry matter production in Ashridge soil, but it doubled the %P in lucerne and french beans. In Woburn soil five crops grew slightly worse and two slightly better with mycorrhiza.

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Whether this was influenced by nutrient deficiencies other than phosphate is under investigation. In all three soils lucerne nodulated better when mycorrhizal. (Hayman)

In three experiments the temperature adaptation of mycorrhizal endophytes from Florida (*Gigaspora calospora* and *Glomus macrocarpus*) and from Rothamsted (*Glomus* spp.: E_3 and laminate spore types) was compared on *Stylosanthes guyanensis* grown in a growth cabinet at 30/23°C and in the glasshouse (approximately 29/17°C) and on onions grown in the glasshouse (approximately 24/17°C). *G. macrocarpus* was a poor endophyte for *Stylosanthes* and in the growth cabinet decreased plant growth considerably compared both to the uninfected controls and to plants infected with the other three endophytes. Nevertheless, microscopic examination revealed no tissue damage in the roots. With onion, *G. macrocarpus* was as good an endophyte as the Rothamsted isolates. (Schroder, Hayman and Mosse)

Inoculation methods. Mycorrhizal plants can be obtained by raising seedlings in sterilised soil amended with inoculum. For crops not normally transplanted, however, seed or soil would need to be inoculated. This was attempted by pelleting seeds of *Centrosema*, soya bean, *Stylosanthes*, onion, red clover and maize with yellow vacuolate (*Glomus mosseae*) inoculum suspended in 5% methyl cellulose, and either sowing immediately or drying overnight. Nearly all the resulting seedlings became mycorrhizal, although infection developed faster in plants given unpelleted inoculum. (Hayman and Mosse)

Work continues on effects of inoculation with selected mycorrhizal endophytes in natural soils, and on methods of distinguishing native from introduced endophytes in order to assess establishment of the latter. Some new endophytes from tropical soils have been established in pot culture.

Lupinus cosentinii appears to be highly resistant to infection by VA endophytes (*Rothamsted Report for 1974*, Part 1, 253). This may be caused by the production of antifungal substances. The presence of the whole plant and to a lesser extent its excised seed coat affected the anatomy of the mycelium infecting the roots of white clover grown in the same pot. It has a very efficient mechanism for uptake of soil phosphate which is neither mycorrhizal nor dependent on an extensive root system. In a P-deficient soil from Western Australia *L. cosentinii* was shown to take up even more P than was estimated chemically to be available ($NaHCO_3$-soluble P). (Mosse)

Effects of systemic fungicides. Studies continued on those fungicides found to affect the establishment and activity of VA mycorrhiza (*Rothamsted Report for 1975*, Part 1, 280). Benomyl and thiophanate methyl applied as soil drenches prevented the establishment of new infections and halted the spread of infections already established. Applied directly they were fungitoxic to the inoculum but clover plants grown in benomyl-treated soil became infected after transplanting into inoculated benomyl-free soil. Both fungicides decreased the phosphorus uptake of mycorrhizal onion and strawberry plants grown for several weeks in irradiated soil, but not in unsterile soil. (Boatman, Mosse and Hayman)

Legume nodulation

Rothamsted *Rhizobium* Collection. The Rothamsted *Rhizobium* Collection now comprises 524 strains of rhizobia and two *Rhizobium* bacteriophages. In the two years from January 1975, 976 cultures have been dispatched (725 as freeze-dried ampoules and 251 in the form of agar slopes) in response to 206 requests, 58 from overseas. In addition, 26 bags of peat inoculum have been sent out for use in field trials.

Since January 1975, the collection has been split into two parts and 28 new strains added. The 'A' group comprises 145 strains, chosen because of their well-characterised

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symbiotic activity. Work has started on plant testing all these strains using 'controlled environment' facilities. Strains in the 'B' group will still be maintained and available upon request, but no details concerning them will be published in the next edition of the catalogue. Strains of other organisms, notably Azotobacter and nitrifiers, have been deleted from the collection. (Dye)

The possible function of depolymerases in infection of soyabean. Soyabean (Ottawa Mandarin) were grown with and without *Rhizobium japonicum*, strain CB 1809, under rigorously sterile conditions in a sand/vermiculite mixture that supported good growth and nodulation. Homogenates of five- and eight-day-old seedling roots and of the surrounding medium were assayed for a range of depolymerases by measuring the amounts of reducing sugars produced from appropriate substrates. These methods are not as sensitive as monitoring changes in viscosity of the polysaccharide solutions but are less likely to be interfered with by contamination during the much shorter period of the assay.

Homogenates of roots showed strong activities of a soluble xylanase and soluble particulate β-1,3-glucanase. These enzymes were equally active from inoculated and uninoculated roots. The finding of glucanase is of special interest in view of the recent demonstration of the occurrence of callose, also a β-1,3 glucan, at the points of initial infection in clover (Kumarasinghe, *Rothamsted Report for 1975*, Part 1, 282). These enzymes were not detected in the medium. No activities were found for polygalacturonase, galactanase, arabanase, mannanase or cellulase. (Barnett)

Effects of root temperature on infection, nodulation and nitrogen fixation in *Lotus* and *Stylosanthes*. Infection threads were observed abundantly in the root hairs of *Lotus corniculatus* L. and very rarely in *L. hispidus* Desf. in response to infection by *Rhizobium* strains 3001 and 3002. Numbers of infections differed between species and strains and were affected by temperature. In *L. corniculatus* all the nodules originated from infection threads but in *L. hispidus* most nodules appeared to originate by direct bacterial penetration through the epidermis; infected root hairs were very rarely seen.

Both species of *Lotus* were tolerant to cold temperatures; the minimum temperature for nodulation was 10°C. The optimum temperature for nodulation of *L. corniculatus* was 20°C with 3001 and between 27 and 30°C with 3002, a few nodules were, however, formed at 35°C. *L. hispidus* formed more nodules per plant than *L. corniculatus*, although the former nodules were ineffective and had abundant red anthocyanin-like pigment. The optima for nodulation for both rhizobial strains was between 25 and 27°C.

No infection threads were seen in the root hairs of *Stylosanthes guyanesis* (Aubl) S.W. and *S. humilis* L. Optimum temperature for nodulation in the two species was around 27°C; nodulation was completely inhibited at 15°C and very few nodules formed at 35°C.

Nodulation of *Lotus* and *Stylosanthes* was delayed in plants transferred from suboptimal to supraoptimal and optimal temperatures. Excision of root tips caused more nodules to form.

Both plant age and temperature influenced nitrogenase activity of *Lotus* and *Stylosanthes* nodules. Activity declined with time and at low temperatures. Transfer of plants from 15, 20, 25 and 30 to 4°C immediately stopped the activity, but this was completely restored within an hour after return to original temperatures.

Nitrogenase activity of detached nodules on nitrogen-free agar medium depends on sucrose concentration (2–8%) at each temperature. Decline in nitrogenase activity in detached nodules was due partly to exposure to air during excision and transfer to the medium. (Ranga Rao)

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Yield and adaptability of dry beans. In 1975 the Centro International de Agricultura Tropical (CIAT) proposed a series of international nurseries to evaluate yield and adaptation of promising varieties of *Phaseolus vulgaris* as a dry bean crop. Farfield II (Woburn) was one of the 120 sites throughout the world where this experiment was performed. The basic design was a lattice with four replicates of 25 varieties; ten black seeded, ten of other colours common to all sites and five selected by the local sponsor. The plot size, row widths and planting density were suggested by CIAT, but other agronomic practices were chosen by the sponsor to suit local conditions. All plots received a basal dressing of N, P and K (504 kg ha^{-1} of 20–14–14).

Cultivars varied in their time to flowering and maturity. The earliest variety, Horehead was harvested on 27 August. Six tropical cultivars failed to mature by 1 November when the experiment was terminated because of frost. Varieties differed in their susceptibility to bean common mosaic virus and bean yellow mosaic virus. (See Cockbain and Bowen, Plant Pathology Department Report, p. 269).

Yields of dry seeds varied between 500 and 2100 kg ha^{-1}. Several white seeded varieties yielded better than Purley King, the variety most widely grown in Britain as a dry bean. In the abnormally dry summer yields of *Phaseolus vulgaris* without irrigation compared favourably with other non-irrigated grain legumes on Farfield; *Lupinus albus* var. Kievsky yielded 1850 kg ha^{-1} and *Vicia faba* about 1000 kg ha^{-1}. (Day and Witty)

Field inoculation of sweet lupins. *Lupinus albus* cvs. Buttercup and Kievsky and *L. angustifolius* cv. Unicrop were inoculated separately with 12 strains of *Rhizobium* (selected after preliminary screening in the greenhouse) and grown at Rothamsted (garden plots) and Woburn (Farfield II.)

All varieties were severely damaged by pigeons at Rothamsted and yields were further decreased by the exceptionally dry summer. Unicrop was damaged by unidentified pathogens which rendered analysis of the data for this variety impossible.

Rhizobium lupini were scarce at Rothamsted and uninoculated plants bore only occasional nodules on the secondary roots. Inoculation with RCR 3201, 3204, 3206, 3210 and 3211 substantially increased dry weight and N content of the plant top at flowering and doubled the yield of seed and pods of both Buttercup and Kievsky. Strain 3211, one of the best tested in the field, performed poorly at low light intensities in the preliminary greenhouse screening. Strains 3208 and 3212 produced ineffective nodules.

At Woburn indigenous rhizobia were abundant and all plants, including uninoculated controls and those inoculated with ineffective strains, bore effective primary root nodules. Inoculation had no effect on either acetylene reduction or yield. (Witty and Day)

Nitrogen transfer in clover-grass mixtures. *Trifolium pratense* cv. S123 and *Lolium perenne* cv. S23 were grown separately and together in six-inch pots containing a mixture of sand, grit and Woburn soil (low in N) to which a small quantity of ^{15}N-labelled NO_3^- was added. The plants were cut two or four times and the clippings analysed for total N and ^{15}N.

In regularly clipped pots the N content, but not the dry matter production, was increased by the presence of clover. Total N removed in four clippings increased from $76 \pm 5{\cdot}0$ mg (grass alone) to $89 \pm 5{\cdot}1$ mg (grass from mixed pots) per pot. Much less significant increases occurred when the pots were clipped only twice.

Clover alone yielded slightly more than the mixture and in all comparisons the cumulative dry matter and N production was increased by clipping. Between 40 and 60% of the available N in the pot (*c.* 100 mg) was recovered. (Witty and Day)

Effects of high soil temperatures on the early growth of tropical legumes. Exposure for 5 h daily to a maximum soil temperature of 40°C delayed nodulation of seedlings of cowpea

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(*Vigna unguiculata*) inoculated with *Rhizobium* strain 5000 (*Rothamsted Report for 1975*, Part 1, 282).

Cowpea (cv. K2809) seeds were sown in pots of sand and grit and inoculated with one of six *Rhizobium* strains isolated from African soils (5000, 5009, 5018, 5028, 5029, 5030), or with CB756 the Australian recommended strain. Pots were placed in water baths and the temperatures raised from ambient to 36, 38, 40, 42 or 44°C for a period of 5 h daily; control pots reached a maximum of 30°C. Plants were harvested 34 days from sowing. Nodulation was completely inhibited by the 42 and 44°C treatments. At 40°C there was no nodulation with CB756 or with 5018; the remaining five strains formed nodules and those inoculated with 5028 grew best. At 38°C 5028 was again the best strain and 5018 the poorest while at 36°C 5009 was best and at ambient temperatures 5029 produced the most vigorous plants with CB756 performing only moderately well. Clearly some *Rhizobium* strains are better adapted to nodulate under conditions of high temperature than others.

Cowpea (cv. TVu 1035) inoculated with 5000 and soyabeans (cv. TGm 628 and Chippewa 64) inoculated with Sm1b (Brazilian Collection) were also grown as described above and harvested at 34 days. Cowpeas were not nodulated above 40°C or soyabeans above 38°C. With both soyabean varieties vigour was greatly (40%) reduced at 36°C compared to controls.

The response to applied N in cowpea (cv. K2809) under temperature stress (40, 42 and 44°C treatments) was examined. After 22 days plants were not nodulated and with 120 ppm N continuously supplied growth was no better than with 60 ppm N; dry matter accumulation at all temperatures was 2·5–3 times that of plants without fertiliser nitrogen. At 40°C 25 ppm N doubled the dry matter yield but this did not increase growth at 44°C. Thus in the tropics at soil temperatures liable to inhibit nodulation the growth of cowpeas may be increased by nitrogenous fertiliser.

The same cultivar inoculated with 5000 was subjected to 40, 42 or 44°C treatments for 3 or 6 days, variously distributed over the first 15 days from sowing, and harvested after 45 days. Temperature stress over the first three days severely reduced crown and secondary nodulation. Between days 7–9, 40 and 42° had little effect but 44°C greatly decreased secondary nodulaton. Periods of 6 days of continuous stress had greater adverse effects on vigour during the first ten days, while six days of stress distributed over the 15 day period had much less effect at 40 and 42° than at 44°C. Temperatures experienced in the field by inoculated cowpea seeds over the first days from planting are critical to early seedling establishment. (White and Eaglesham)

Tropical grain legumes and local isolates of *Rhizobium*. Studies were made on the *Rhizobium* strain specificities of elite cultivars of cowpea (*Vigna unguiculata*, Vita 1, Vita 3 and Vita 25) and pigeon pea (*Cajanus cajan*, Cita 1, Cita 2 and Cita 4) selected by the breeders at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, for their high yield capacities and disease resistance. Plants were pot grown, inoculated with one of six strains of African rhizobia or with CB756 and harvested after 60 days. Except for 5009 which produced no nodules on Cita 1, all combinations of elite cultivars and *Rhizobium* strains were effective. In the future these elite cultivars will be used in breeding programmes in different parts of the world and this trial indicates they will nodulate effectively with a range of tropical rhizobia.

Work was continued on the symbiotic characteristics of new grain legumes under study at IITA. Up to ten strains of rhizobia were tested against hyacinth bean (*Lablab niger* cv. TLn 27), lima bean (*Phaseolus lunatus* cv. TP1 191A), winged bean (*Psophocarpus tetragonolobus* cv. TPt 2), Mexican yam bean (*Pachyrisus erosus* TPe 1), and African yam bean (*Sphenostylis stenocarpa* cv. TSs 44). Germination was poor particu-

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larly with the yam beans, and the material was insufficient for complete harvest after 35 days. Effective nodulation of lima bean occurred with four strains, of winged bean with seven strains, and hyacinth bean nodulated effectively with all eight strains tested. African yam bean nodulated effectively with three out of four strains and Mexican yam bean with one out of four. These data give some indication of the likelihood of nodulation occurring by indigenous rhizobia where these new species are introduced for the first time. (White and Eaglesham)

Nitrogen nutrition of *Phaseolus vulgaris*. The relationship between applied N, seasonal patterns of N_2 fixation and N movement within the plant, and seed yield were investigated in two dry bean cultivars of *P. vulgaris*, viz. Purley King and Higuerillo, in a glasshouse pot experiment. Pots contained a rooting medium of sand and grit with 4% by volume of Kettering loam added to give a starter dose of available N equivalent to approximately 10 kg ha^{-1}. Nitrate fertiliser (containing ^{15}N at 2% atom excess) was applied at rates equivalent to 0, 30 or 60 kg N ha^{-1} on a surface area basis. Seeds were inoculated at planting with strain 3610 and pots watered to weight with a nutrient solution complete except for N. Plants were sampled on four occasions during the growing season and harvested at *c.* 80 days from sowing. In early growth Higuerillo responded to added N, but by flowering, dry weight and N accumulation were the same in all treatments; Purley King, a smaller plant with a lower growth rate showed no vegetative response to added N at any stage. Fertiliser application had no effect on seed yield of either cultivar; this was equivalent to 3800 kg ha^{-1} for Higuerillo and 1400 kg ha^{-1} for Purley King at 15% moisture content. The inputs of N by nodule fixation were calculated from the ^{15}N enrichment values in plant components. There was an inverse relationship between the amount of N present in the pot and the amount of N_2 fixed by the nodules, showing the potential of *P. vulgaris* to satisfy most of the nitrogen required by high and medium yielding cultivars. Nodule N_2-fixation continued in both cultivars until maturity although the seasonal patterns differed according to N treatment; applied N inhibited nodulation during early growth. Both of these determinate cultivars showed a maximum N content in their vegetative parts at flowering, which fell during podding to 50% of the maximum. There was no similar decrease in vegetative dry matter, presumably nitrogen rich compounds were replaced by carbohydrates. Although the ^{15}N was all taken up by the time of flowering. large proportions were eventually translocated to pods (50% in Higeurillo, 75% in Purley King). The question is raised whether efficiency of mobilisation of nitrogenous compounds from vegetative to fruiting parts can be increased to augment seed yields. (Eaglesham, Dart and Day)

Purley King plants in pots fed with 25 ppm N solution and inoculated with 3610 had visible nodules at 11 days and were actively fixing N_2 at 15 days from sowing. Nodular fixation continued actively to maturity. In contrast with strain 3644 a sharp peak of activity was reached at flowering and this then fell within a week to 10% of the maximum rate. However, in pot trials, yields of Purley King are consistently better with 3644 than with 3610, and so the overall input of N by 3644 is greater than by 3610. The symbiotic performance of strains of *Rhizobium phaseoli* should be judged therefore in terms of total N input from fixation rather than by the longevity of nodule activity measured by acetylene reduction.

In the USA seed yields of high yielding lines of soyabeans which show little nodule activity during pod-fill have been increased up to 50% by foliar fertilisation with N, P and K after flowering (Ramon Garcia & Hanway (1976) *Agronomy Journal* **68**, 653–657). Pot trials with Purley King, inoculated with 3644, using foliar sprays containing combinations of N, P, K and S, similar to the American method have resulted in decreased yields, but further trials are required. (White and Eaglesham)

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Breeding for increased nitrogen fixation in red clover. Families of nodulated red clover bred for high yield (*Rothamsted Report for 1974*, Part 1, 246) also yielded more than the original cultivar (S123) when given combined nitrogen, either as a starter dose or throughout growth at normal rates or continuously fed 250 ppm NO_3–N. The latter treatments stopped nodule formation. Differences between the bred lines and S123 given combined nitrogen were relatively less than when nodulated. These results indicate that selection was not only for better symbiosis but also for general growth attributes.

Further tests have confirmed the early-flowering habit of highly effective plants and shown that such plants also flower early when unnodulated and given fertiliser nitrogen. Their flowering, however, was completely inhibited in short days without this treatment affecting the different yield responses of the bred lines, showing that early flowering was not a consequence of any activity of the nodules.

The effect of selection was influenced by temperature. Using a day/night temperature stress regime of 16/11°C the highly effective lines yielded 14% more than S123. This increased to 53% at 22/17°C and 48% at 27/22°C. (Nutman and Poonam Rao)

Staff and visiting workers

A. R. J. Eaglesham left the department in October to take up an appointment at the Boyce Thompson Plant Research Institute in America and N. Walker joined the Chemical Liaison Unit here in September. R. J. Roughley from Australia has been appointed PSO in the *Rhizobium* group.

Barbara Mosse visited Hawaii and Australia during a three-month study leave. Dr. Nutman acted as external examiner at the Ile-Ife University and lectured at Ahmadu Bello University in Nigeria. He also contributed to a symposium on N_2-fixation in Salamanca, Spain and to a UNESCO regional course at the Agricultural Research Centre, Giza, and lectured at Alexandria University, Egypt. J. M. Day visited Centro Internacional de Agricultura Tropical (CIAT) and the University of the West Indies. N. Walker attended the American Society of Microbiology's Conference at the University of Georgia in September and A. R. J. Eaglesham attended a meeting at the International Atomic Energy Agency in Vienna, Austria.

During the year the following visiting workers spent some time in the department: Professor N. M. Barnett from the University of Maryland, USA, Miss Regine Bache from the University of Göttingen, West Germany, Miss Rachel Ron from the Volcani Center, Israel. Dr. Ranga Rao and Mrs. Poonam Rao left to work at the Charles F. Kettering Research Laboratory in America.

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