ROTHAMSTED RESEARCH

Report for 1975 - Part 1

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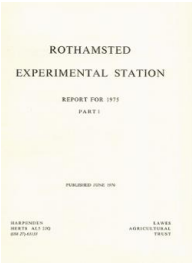

Soil Microbiology Department

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SOIL MICROBIOLOGY DEPARTMENT

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Introduction

The long term objectives of the research programmes are to improve crop health and conditions of growth, to increase the uptake of biologically fixed nitrogen and the availability of soil and fertiliser nutrients, and to understand the breakdown of microbially degraded herbicides and pesticides. Changes in the 1975 programme were towards devising new methods for the study of problems in microbial ecology and assaying nitrogenase activity in soil, and extending some lines of work for application in agriculture.

Experiments have begun on the field inoculation of forage lupins and *Phaseolus* beans with selected cultures of *Rhizobium*, and of a range of host plants with VA mycorrhiza. Work also started on the nutritional requirements for nitrogenase activity of pure cultures of nodule bacteria, and of infected plant-tissue cultures.

Continuing work on breeding for increased nitrogen-fixation in red clover, on the physiology of infection of *Lotus* and *Stylosanthes*, on nodule fine structure, on the *Rhizobium* culture collection, on the microbial degradation of Dicamba, and on the culture of N-fixing anaerobes will not be reported this year.

Mycorrhizal studies

Inoculation of natural (unsterilised) soils. Thirteen phosphate-deficient soils from tropical Africa, one from Brazil and one from Australia, with and without added rock phosphate, were tested for plant growth responses after inoculation with *Endogone*; *Stylosanthes guyanensis* and maize were the test plants. Inoculated plants never grew worse than uninoculated. The benefits of inoculation varied with the amounts and kinds of natural endophytes in particular soils and with soil P contents. In four Nigerian soils the level of natural endophytes was very low and introducing endophytes improved growth up to nine-fold, and utilisation of added rock phosphate up to ten-fold. In soils from Madagascar and Senegal the introduced endophytes did not improve plant growth in

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unamended soil but increased growth three-fold when rock phosphate was added. A similar result was obtained in an agricultural soil from Brazil with a high level of indigenous endoplytes. Inoculation increased growth with added rock phosphate by 30% but delayed infection by the indigenous endophytes may have contributed to this result. Promising new endophytes will be isolated and tested further.

The beneficial effects of mycorrhiza on nodulation and symbiotic nitrogen fixation were confirmed (*Rothamsted Report for 1974*, Part 1, 251). No nodules formed in *Stylosanthes* or *Centrosema* grown in irradiated soil with added rhizobia unless plants were either inoculated with VA endophytes or given fertiliser phosphate. In natural soils without VA mycorrhiza some nodules generally formed on the small very phosphorus-deficient plants. The tolerance of these indigenous rhizobia to low phosphorus concentrations is being investigated. (Mosse and Islam)

Anatomy of VA infections caused by different endophytes. Infections caused by 'yellow vacuolate', 'honey-coloured sessile' and 'E_3' endophytes could be distinguished in sections of roots by structure and longevity of the arbuscules pattern of hyphal development in the cortex, vesicle shape, content and wall thickness. In most of the natural soils tested in pot experiments infections caused by indigenous endophytes were readily distinguishable from the introduced endophyte (E_3). (Mosse and G. Smith)

Effects of systemic fungicides. A systemic fungicide was sought to suppress the function of the endophyte and allow an evaluation of its efficiency in soil. The effects of benomyl, thiophanate methyl, and prothiocarb ('Previcur') and pyroxychlor 'Dowco 269' which are specially active against Oomycetous fungi, were examined. Pyroxychlor was without effect on VA endophytes both when used as a foliar dip and when applied directly to the inoculum. Prothiocarb was also ineffective when mixed with the soil. Benomyl (0·025%) and thiophanate methyl (0·05%) however prevented mycorrhizal formation when applied directly to the inoculum and also when mixed with the soil (at 0·029 and 0·069%, respectively). (Paget, Boatman and Mosse)

Freeze drying. Following earlier attempts to freeze-dry mycorrhizal roots of soyabean (*Rothamsted Report for 1973*, Part 1, 82) the effects of various suspending media and of rapid and slow initial freezing were tested using mycorrhizal roots of *Nardus stricta*. The infectivity of the freeze-dried roots was tested on clover. With Greaves medium and skimmed milk as suspending media all infectivity was lost but some was retained in roots suspended in water or soil suspensions. More infectivity was retained in roots frozen to − 35°C over 2 h than in those cooled rapidly to this temperature. Roots suspended in water, frozen slowly and then vacuum dried retained some infectivity. Five to ten per cent of rootlets became infected three weeks after inoculation with such roots compared with more than 50% of rootlets of seedlings inoculated with untreated mychorrhizal roots. (Davis and Mosse)

Rhizosphere studies

Take-all disease and its decline. Work has continued on the effects of host and fungal nutrition on the expression of take-all disease by using a model system described in the *Rothamsted Report for 1974*, Part 1, 250, by foliar feeding plants with different forms of nitrogen, and by examining growth of inocula of *Gaeumannomyces graminis* on slides buried in soil in contact with growing wheat seedlings.

The results from the foliar feeding experiments confirmed previous observations that ammonium-grown inocula infected fewer roots of plants fed with NO_4^+–N than those of

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plants fed with NO_3^-–N. After infection had occurred, however, the lesions developing within roots of plants fed NH_4^+–N were less extensive than those produced within plants receiving NO_3^-–N, or a mixture of both.

In the study using buried slides, the distilled water agar supporting the inocula was sometimes amended with NO_3^-–N or NH_4^+–N. Behaviour of two isolates of the pathogen was examined. Strain L5–7 produced less disease in soil tests than strain 028B and on the buried slides it formed fewer hyphae, irrespective of the amendment to the agar. Strain L5–7 grew most vigorously when it originated from a nitrate-grown inoculum, whereas 028B was most vigorous from an ammonium-grown inoculum.

Samples of plants and of soil between the rows of the wheat crop were taken at monthly intervals from plots on Broadbalk field that had received either FYM or NPK for many years. The amounts of NH_4^+–N and NO_3^-–N present in rhizosphere soil and bulk soil were measured using a specific-ion electrode; chemical analyses for these forms of nitrogen were also made. Results indicated that there was more NH_4^+–N than NO_3^-–N in the soil solution around roots, but not in the bulk soil. Wheat roots, therefore provided an environment where the balance of NH_4^+– and NO_3^-–N was conducive to growth and infection by take-all at a time when soil temperature and plant growth were suitable for rapid spread of disease.

Interactions between take-all disease and rhizosphere bacteria were studied using soils taken from different sites on Butt Furlong field, Woburn, which showed either unimpeded take-all disease or decline in disease. Pots of soil were sown with wheat and rhizosphere samples were taken at the seedling stage or when plants were tillering or in ear. These rhizosphere soils were then added as a 1 : 10 suspension in water to infected wheat seedlings growing in perlite moistened with nutrient solution containing different amounts of NH_4^+–N or NO_3^-–N. When compared with controls inoculated with *G. graminis* only, all inocula decreased disease, irrespective of whether they originated from the rhizosphere of seedlings, tillering plants or plants in ear. The amount of disease was affected by the form of nitrogen supplied, but most decrease was obtained with samples from decline soil.

Bacterial isolates from the rhizosphere samples were classified by their ability to produce thiamin, biotin and B_{12}, to ammonify, to denitrify and to inhibit *G. graminis* growing on agar. The bacterial population changed considerably at each time of sampling, but differed little between the unimpeded and decline series. Thus the decrease in disease caused by the rhizosphere soils could not be related to the different populations found at each time of sampling. (Brown)

Gaeumannomyces graminis requires biotin and thiamin for optimum growth. Interactions between vitamin-producing bacteria and take-all disease were therefore studied. Because some bacteria produce more vitamin in conditions of phosphate deficiency, experiments using three levels of phosphate were performed. Suspensions of vitamin-producing bacteria were added to infected wheat seedlings growing in perlite. At the two higher levels of P, disease was increased significantly by two of the four bacterial strains tested. At the lowest phosphate level, take-all infection was decreased significantly by four bacteria. (Ramos-Cormenzana and Brown)

Microbial nitrogen fixation in the rhizosphere. The dependence of rhizosphere fixation on carbohydrate supplied by the host plant was indicated by the diurnal fluctuation in activity, and by the rapid decrease in rate after the removal of plant tops. Experiments in which the roots and shoots of intact plants were enclosed in separate containers showed that both acetylene reduction and respiratory CO_2 production in the rhizosphere are decreased by CO_2 depletion of the shoot. Such factors are important when assays are done in closed systems.

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Assay techniques developed at Rothamsted were used to evaluate biological fixation associated with rice during a visit to the International Rice Research Institute in the Philippines. *In situ* determinations of acetylene reduction in the rhizosphere were made over a range of field conditions. Most activity, equivalent to *c.* 20 kg N ha^{-1} per season, was found in areas where available N was low and the nutritional status otherwise good. Seventy to 80% of activity was localised in the top 6 cm of soil which contained most roots (*c.* 70% of total on a dry wt. basis) and had the highest specific activities (*c.* 400 nmol g^{-1} h^{-1} on a root dry wt. basis compared with 200 nmol g^{-1} h^{-1} for deeper roots). These values are low by comparison with many tropical grasses which, unlike rice, have the C_4 photosynthetic pathway. Blue green algae growing in the paddy water fixed as much as 1·0 kg N ha^{-1} per day and similar orders of activity were associated with the small aquatic fern *Azolla* which contains an *Anabaena* symbiont. This fixation, together with other inputs, has maintained rice yields over long periods in many areas where fertilisers are not used. (Witty)

Legume nodulation

Fine structure of cowpea *Rhizobium*. A comparative study was made of the growth and fine structure of the Cowpea *Rhizobium* CC705 grown aerobically and anaerobically with 6 mM KNO_3. Anaerobic cultures grew less vigorously than aerobically grown cells; they contained smaller cells with denser granular cytoplasm and more mesosomes. Anaerobically-grown cells also matured and lysed earlier, many cells having died at seven days.

The greater length of aerobically grown cells was related to the large quantities of storage material, mostly poly-β-hydroxy butyric acid and glycogen, found at the poles. Pleomorphic forms were seen frequently in the three- and ten-day-old cultures, but were not observed in anaerobically grown cultures. (Chandler, with Dr. R. Daniels, University of Waikato)

Nodulation of *Trifolium pratense* by *Rhizobium leguminosarum*. The normally very low incidence of nodulation of *Trifolium pratense* (cv. S123) by *Rhizobium leguminosarum* strain 1020 was much increased by breeding from nodulating plants (*Rothamsted Report for 1972*, Part 1, 82). When such plants were then crossed amongst themselves, an average of 90% nodulation was recorded in the second generation, with some families having no plants resistant to nodulation. The number of nodules on each plant was also greater in the second generation. Plants from the first and second generations which nodulated with this strain also nodulated equally well with six other strains of *R. leguminosarum* tested, but all such symbioses were ineffective in fixing nitrogen. Plants bred to nodulate with *R. leguminosarum* still retained their capacity to form normal symbiotic associations with *R. trifolii*, but bacteria isolated from other cross-inoculation group hosts failed to nodulate them.

The nodules formed on these clover plants by *R. leguminosarum* usually appeared a few days later than those caused by *R. trifolii* and a few plants did not form their first nodule until 30 days after inoculation. Nodules were confined to the lateral roots or where the lateral roots emerged from the main root. This may be related to the fact that only the root hairs of lateral roots became markedly curled in the presence of *R. leguminosarum*. (Hepper)

Callose formation in root hairs and root hair structure. Work continued on the formation of callose in clover. (*Trifolium parviflorum*) root hairs as detected by u.v. fluorescence after staining with 0·005% aqueous aniline blue. Inoculation with either *Rhizobium trifolii*, *R. leguminosarum*, *R. meliloti* or *R. japonicum* much increased the formation of tipcallose

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in clover root hairs, and to a lesser degree in root hairs of the non-legume timothy grass. This was attributed to indolylacetic acid, known to be formed by rhizobia, which stimulated tip callose production considerably at 10^{-7} and 10^{-8}M. Callose formed in curled hairs at the point of infection persists, whereas tip callose in uninfected hairs is a short-lived deposit. Most callose was formed in root hairs at 6 and 12°C and very little at the higher temperatures (18, 24, 30 and 36°C) whereas root hair infection was optimal at 24°C.

Electron microscopy of root hairs showed thickened regions of presumptive callose formation in the root hair wall. Bacteria were invariably polarly attached to the surface of the root hair and many showed a knob-like modification of the bacterial cell and some evidence of fimbriae or pili at the point of attachment and a large polyphosphate granule nearby. Hitherto undescribed finger-like processes *c.* 0·1 μm long were abundant on root hair surfaces. Infections were also observed starting as intercellular invasions of the spaces between neighbouring epidermal cells. (Kumarasinghe)

Infection process in peanut (*Arachis hypogaea*). Root hairs and nodules are found in peanut only at the points of emergence of lateral roots and until recently infection was thought to occur through the openings so caused.

Examination of sections by light- and electron-microscopy has shown that root hairs are involved in the infection process. These hairs are large, very thick walled and some appear to be multi-cellular. At the base of each hair are two or more very large cells. There are no true infection threads but rhizobia enter these root hair and basal cells after first penetrating the space between adjoining cells. Bacteria which are surrounded by a densely staining zoogleal matrix enter the cells through areas of altered cell wall and once inside, multiply rapidly. The invaded cells divide repeatedly, each daughter cell containing vegetative rhizobia. This repeated mitotic division of invaded cells produces the nodule. (Chandler and Dart)

The effects of high soil temperature on the early growth of cowpea. (*Vigna unguiculata* cv. K2809). Earlier experiments (*Rothamsted Report for 1973*, Part 1, 85 and *for 1974*, Part 1, 247) showed that nitrogenase activity of cowpea nodules was strongly reduced by raising the temperature to 40°C, but that if 5 h cycles at 40°C were continued for ten days there was recovery of activity. When such stressed plants were returned to optimum conditions growth was vigorous and effects of temperature stress were no longer apparent at harvest.

Cowpea seeds were sown in pots of sand and grit, inoculated with *Rhizobium* strain 5000 and supplied with nutrient solution without N, or were left uninoculated and given nutrient solution containing 240 ppm N. Pots were placed in a water bath and the temperature raised from ambient to 36 or 40°C for a period of 5 h daily for six weeks; control pots reached a maximum of 30°C. There was no effect of the repeated 36°C treatment on germination, nodulation or growth. Nodules were visible at eight days from sowing, and nitrogen fixation occurred by ten days. Plants given N were similarly unaffected. The 40°C treatment had no effect on germination, but delayed the appearance of the first nodule until day 18, and some plants had no nodules on the 28th day. All plants were, however, nodulated by the 42nd day. Plants given high N also showed reduced vigour under root heat stress of 40°C. Compared with unstressed plants (100%), dry matter accumulation after 42 days of stress was 20% in nodulated and 30% in N-supplied plants. The data suggest that in cleared ground in the tropics where soil temperatures over 40°C are common impaired nodulation may contribute to loss of vigour of cowpea seedlings. (Eaglesham and Dart)

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Development of a peat inoculum for *Rhizobium*. Increasing numbers of requests by research workers for *Rhizobium* inoculants for use in the field has resulted in a need to produce and supply limited quantities of peat cultures.

Eleven British peats and one Australian peat (Badenoch) were tested for their suitability as carriers for *R. phaseoli* 3644. Peats were neutralised, dried, ground, packed in polythene bags and gamma-irradiated at five megarads. Broth cultures of *R. phaseoli* 3644 were injected into the peats and the numbers of viable cells estimated at monthly intervals. Maximum cell numbers were not reached in Badenoch and raised bog peats until after eight weeks at 25°C while in all the other peats maximum numbers were attained within 4 weeks; maximum cell numbers ranged from 3×10^9 to 6×10^9 cells g^{-1} moist peat. After six months storage at 25°C most British peats contained 1×10^9 or more viable cells g^{-1} while Badenoch peat contained only 5×10^8 cells g^{-1}. (Davis)

Badenoch peat inoculants were prepared containing *R. phaseoli* strain 3605, strain 3639 or 3644. Seeds of *Phaseolus vulgaris* cv. Purley King were treated with inoculant and planted in Whitehorse field at Woburn the following day. The soil had been treated with trifluralin ('Treflan') (2·3 litre ha^{-1}) but the seed had not been treated with fungicide.

Counts of rhizobia on the seeds were made by the Most Probable Number method using siratro (*Mactroptilium atropurpureum*) grown in sterile conditions rooted in agar in test tubes (siratro forms ineffective nodules with most effective strains of *R. phaseoli*). At the time of sowing each bean had $> 2 \times 10^5$ adhering cells. One week later the number of viable cells per germinated seed was $> 1{\cdot}2 \times 10^5$ for strains 3605 and 3639 and had increased to $> 10^7$ cells for strain 3644. Counts of *R. phaseoli* in the soil indicated that very few cells (< 1 cell g^{-1} dry soil) of indigenous strains were present. (Davis and Eaglesham)

Field inoculation of lupins. The effects of seed inoculation of *Lupinus angustifolius* cv. Unicrop and *Lupinus albus* cv. Kievskij with *Rhizobium* strain 3211, and application of nitrochalk at 150 kg N ha^{-1} at sowing were examined at Rothamsted in soil already containing *R. lupini*. Effects of treating the soil and plants with the pesticides aldicarb, benomyl and menazon were also examined.

Nodulation was slow on control and inoculated plots and rates of acetylene reduction were low up to the pod-fill stage. Plots given nitrochalk were greener and more vigorous up to flowering, and final yield of grain showed a positive response to N. There were no adverse effects of pesticides on nodulation and fixation. Seed inoculation resulted in increased yields (mean increases in the absence of added N, were 23% for Unicrop and 33% for Kievskij) and maximum yields (0·95 t ha^{-1} of Unicrop, 1·95 t ha^{-1} of Kievskij) were obtained in inoculated plots without nitrochalk. Yields were relatively low in the absence of pesticide treatments. (Eaglesham and Dart, with Cockbain, Plant Pathology Department and Wilson, Field Experiments Section)

Nutritional requirements for nitrogenase activity in plant tissue and *Rhizobium* cultures. The nitrogenase activity associated with plant tissue cultures infected with different rhizobia, and with certain rhizobia cultured on synthetic media, has been investigated.

Nitrogenase activity was observed in root, stem and leaf tissue cultures of three genetic lines of *Trifolium pratense*, infected with *Rhizobium trifolii* Rothamsted strain 5, *Stylosanthes gracilis* with *Rhizobium* sp strain CB1552 and root tissue cultures of carrot with strains CB1552 and NGR99. No differences were discernible between the activities of tissue cultures taken from genetic lines that differed in symbiotic effectiveness. *Rhizobium* (CB1552) previously growing near but not in direct contact with *Stylosanthes* tissues continued to show nitrogenase activity for five days after separation from the callus.

In *Trifolium* and *Stylosanthes* greater activity was shown by callus derived from stems

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than from leaves or roots. Both these results emphasise the non-specific nature of nitrogenase induction in culture. Infected *Trifolium* callus differentiated to produce roots on a medium free from NO_3^-, 2,4-D and kinetin. The nutritional requirements for nitrogenase activity by rhizobia grown on a synthetic medium varied markedly between strains. Strain CB1552 (a slow-grower used in the earlier work) showed nitrogenase activity with a wide range of additions to the basal medium, e.g. glutamine, succinic acid, cysteine and arabinose, alone or as mixtures. Strain 5 however, was found to require only methionine for acetylene reduction. (Ranga Rao)

Nitrification and microbial metabolism

Rothamsted collection of nitrifying bacteria. This now comprises 20 pure cultures, some recently isolated, representing the genera *Nitrosomonas*, *Nitrosolobus* or *Nitrosospira* together with 18 pure cultures of nitrite-oxidising bacteria. The nitrite-oxidisers are all *Nitrobacter* spp. but they show some variation in properties such as colony form or growth habit in liquid media. Tests on air-dried soils of known age and origin have shown that ammonia-oxidising autotrophs, although they do not form spores, can survive for long periods, e.g. 22 years in certain East African soils and over 90 years in soil samples from Broadbalk. Bacteria isolated in pure culture from the latter soils were *Nitrosolobus* spp. (Walker)

Methods for modelling microbial population dynamics and testing for pesticide/microflora interactions. Investigation of the effects of pesticides on the population dynamics of *Nitrosolobus* has continued. Rainfall-correlated changes in population density were studied in a system in which accurate physical modelling of the effects of rainfall on soil moisture content and infiltration was achieved. Probable interactions of *Nitrosolobus* with predators, heterotrophic bacteria and fungi, and algae were inferred from field data.

Rapid enumeration of protozoa and myxobacteria on selective media using an overlay plaque technique has given higher estimates of population density than standard methods. An improved fluorescent staining method has been developed for heterotrophic bacteria. Combination of this technique with Quantimet image analysis allows automated enumeration. Algal population density was estimated by soil chlorophyll analysis and compared with microscopic cell counts obtained by chlorophyll u.v. fluorescence.

An apparatus has been constructed to measure the relative migration rates of micro-organisms in the presence and absence of pesticides. This consists of two radiation-sterilised soil columns joined to a central non-sterile soil reservoir. Pesticide is incorporated into one column and migration rates of micro-organisms in each column are compared by sampling at intervals along the columns. Migration is affected by interactions between microbial species. The effect of pesticides on this interaction may be assessed by a small number of simple microbiological analyses. (Macdonald)

Microbial degradation of 1-naphthol and 2-naphthol. Work on the early stages of the degradation pathway of 1-naphthol by a *Pseudomonas* sp. was continued. An early metabolite was isolated in appreciable yield and identified by NMR and mass spectroscopy as a 3,4-dihydrodihydroxy-1 (2H)-naphthalenone, so confirming that the initial oxidative attack is on the substituted carbocyclic ring of 1-naphthol. Degradative metabolism thus differs from that of other mono-substituted naphthalenes which involves early hydroxylation of the unsubstituted ring. (Walker and Spokes, with Janes, Insecticides and Fungicides Department)

Micro-organisms isolated from soil by enrichment culture in the presence of 1-naphthol also grew on 2-naphthol. The pathway of microbial degradation of 2-naphthol was

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different from that previously described by Walker (*Rothamsted Report for 1964*, 95). Work is continuing to elucidate the entire pathway of 1-naphthol metabolism, and to compare the apparently different pathways of 2-naphthol metabolism. (Spokes)

Microbial metabolism of chlortoluron. Work has continued on the degradation of chlortoluron. Several bacteria have been isolated that metabolise the related compound *p*-toluidine, and preliminary experiments show some co-metabolism of chlortoluron by *p*-toluidine-grown cells. Soil perfusion systems have yielded mixed cultures able to degrade 3-chloro-4-methylaniline (a possible hydrolytic product of chlortoluron) but as yet no pure culture responsible for this breakdown has been obtained. Warburg experiments indicate that 4-methylcatechol is an intermediate in the breakdown of *p*-toluidine. It is proposed to identify other intermediates by using mutants blocked at different steps of the pathway. (Spokes)

Bacterial degradation of Asulam and sulphanilamide. A pure culture of a small aerobic bacterium, not yet identified, grows moderately well in a yeast extract medium containing either sulphanilamide or Asulam, its carbamic ester derivative, and causes their decomposition. Washed cells, grown on sulphanilamide, take up oxygen in the presence of either sulphanilamide or of Asulam. (Walker)

Soil structure and related studies

Soil structure studies. Soil particles can be aggregated by microorganisms, either mechanically by hyphae, or by microbial polysaccharides and possibly other substances. Such aggregates made artificially in the laboratory are not always stable to water and may not be of the same kind as the very stable aggregates formed naturally. Although we cannot simulate the conditions leading to the formation of natural soil aggregates, their disintegration can be studied.

Portions of the 2–3 mm fractions of partially air-dried soil in Petri dishes were moistened with water or nutrient solution and incubated at 25°C aerobically, or anaerobically in an atmosphere of nitrogen for up to three weeks. They were then soaked in water for 24 h to ensure hydration of polysaccharides, sieved under water for 1 min, and the fractions 0·5–1 mm and > 1 mm dried and weighed. With aggregates of Rothamsted Parklands soil shaken from grass roots there was little difference between samples incubated aerobically with 2 ml of water or glucose solution (50 mg g^{-1} soil); both yielded *c.* 70% of the original weight as water-stable crumbs greater than 1 mm. Similar samples incubated anaerobically were slightly more stable with 74–77% of dry stable crumbs. Higher levels of glucose or peptone (25 mg g^{-1} soil) slightly improved the stability of the aerobic soil (81–83% of crumbs), but not that of the anaerobic soil (71–73%). Extension of this work to four other soils of widely differing type (stored samples) showed no significant differences between treatments for any one soil but large differences between the soils; these ranged from 5 to more than 90% of water-stable aggregates.

The effect of incubation under different conditions and with different substrates on the stability of natural soil aggregates was also examined as part of an interdepartmental programme sponsored by the Rothamsted Soil Structure Working Group. The following pairs of soils were used: Hanslope and Ragdale, derived from chalky boulder clay, the former having good, the latter a less stable, structure; and Evesham and Denchworth, clay soils with good and defective structure, respectively. The two uppermost horizons of each soil were prepared as described above and their structures assessed after incubating at 25°C for 14 days with water or solutions of glucose (25 mg g^{-1} soil), peptone (25 mg g^{-1} soil) or glucose and peptone (22·5 mg and 2·5 mg) respectively.

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Under anaerobic incubation the type of nutrient added to soil had little effect on stability to water although the percentage of water-stable aggregates was, with one exception, the lowest when incubated with water. Striking differences were found with aerobic incubation. Except for one sample, aerobic incubation with glucose gave a larger proportion of water-stable aggregates (average 77%) than incubation with water (47%), the largest difference between the treatments being 34% for Evesham (top horizon). Results were almost identical for the glucose + peptone treatment, but aerobic incubation with peptone alone usually caused aggregates to break down, especially with Evesham soil (second horizon) which yielded *c.* 61% stable aggregates with water, 75% with glucose and only *c.* 21% with peptone.

The provision of extra nitrogen (as peptone) may have enabled micro-organisms to decompose polysaccharides that were binding soil particles together. Conversely, extra carbohydrate (as glucose) with little or no supplied nitrogen could have favoured polysaccharide formation and increased aggregate stability.

With Evesham and Denchworth (top horizons) soils, incubation with peptone gave a larger proportion of stable aggregates than incubation with water. These differences in response to incubation and treatment were not simply correlated with susceptibility of the soil to structural defects; bonding mechanisms are likely to differ between soils.

The non-incubated control soil samples were always less stable to water than the corresponding samples incubated aerobically or anaerobically, with water. Evesham and Denchworth (top horizons) were incubated aerobically with water and tested at intervals. The proportion of stable aggregates (> 1 mm) increased in both soils and became steady by the 11th day at values comparable with those found in the previous experiment. (Skinner)

The effect of ethylene on the soil microflora. Ethylene is formed in soils, especially when waterlogged, though it is not known whether it originates in aerobic or anaerobic microbial processes. Experiments have been conducted to test the hypothesis that ethylene generated in soil inhibits aerobic microbial activity.

Agar plates were inoculated in various ways (pour-, spread- or droplet-plate) with dilutions of soil and incubated in air in closed jars containing different proportions of ethylene. Six soils from Rothamsted and Woburn were used. Fixed ethylene concentrations were maintained in the jars during the seven-day incubation period; oxygen concentrations remained near that of air though CO_2 concentrations at the end of the experiments increased to *c.* 1–1·6%.

Concentrations of up to 1000 ppm of ethylene did not affect the plate counts so that ethylene in concentrations likely to be encountered in soil (up to 10 ppm) would have little effect on growth of micro-organisms. Ethylene at concentrations up to 500 ppm also had no effect on the respiration rate of soil suspensions shaken in Warburg respirometer flasks or of bulk soils in macro-respirometers, though it seemed that ethylene was absorbed strongly on the soil mass. (Skinner)

Aerobic cellulose decomposition. An aerobic cellulose decomposing bacterium obtained from activated sewage sludge and used in investigations on the removal of nitrate from water (Skinner, *Journal of Applied Bacteriology* (1972) **35**, 453–462) has been obtained in pure culture by using a mineral salt agar medium enriched with sterile horse serum. The organism, referrable to the genus *Pseudomonas*, decomposes cellulose rapidly at 25°C and needs no growth factors in liquid medium. (Skinner)

Staff and visiting workers

P. J. Dart left in August to take up an appointment at ICRISAT, Hyderabad and P.

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Davis left in December for a post in Malawi. J. Day returned from Brazil in July and D. Hayman from Australia and New Zealand in December. The Ph.D. degree of London University was awarded to Rafiquil Islam and to Rohini M. K. Kumarasinghe.

Visits were made by J. Witty to IRRI, Philippines, by B. Mosse, J. M. Day, A. Eaglesham and J. Witty to IITA, Nigeria, by F. A. Skinner to Prague and Ife University, Nigeria, and by P. S. Nutman to Russia (Royal Society/USSR Academy Exchange Agreement) and Trinidad. D. Hayman, J. Day and B. Mosse and P. S. Nutman contributed to International Congresses at Brisbane, IITA and Leningrad, respectively.

During the year the following spent some time working in the department: Professor Ramos-Cormenzana, Miss Rosa Diaz-Rodriguez and Dr. Francisco Ruiz Berraquero from Granada, Spain, Dr. Conway Ll. Powell from Ruakura Agricultural Research Centre, New Zealand, Dr. V. Schroeder from Gainesville, Florida and Dr. Z. H. Bhuiya from Bangladesh Agricultural University.

Publications

General Papers

1 Brown, M. E. (1976) Microbial manipulation and plant performance. In: *Microbiology in agriculture, fisheries and food.* Eds. F. A. Skinner and J. G. Carr. Society for Applied Bacteriology Symposium Series No. 4, pp 37–53. London: Academic Press.

2 Hayman, D. S. (1974) Microbiology of the soil. *Reports on the Progress of Applied Chemistry* **58,** 503–519.

3 Hayman, D. S. (1975) Vesicular-arbuscular mycorrhiza: its occurrence, effects on plant growth and its significance in Australia. *Australian Plant Pathology Society Newsletter* No. 4, 15–18.

4 Nutman, P. S. (1975) Selection for increased nitrogen fixation in red clover (*Trifolium pratense*) *Abstr. XII International Botanical Congress II, Leningrad, USSR,* 453.

Research Papers

5 Day, J. M. & (Dobereiner, J.) (1975) Physiological aspects of N_2-fixation by a Spirillum from Digitaria Roots. *Soil Biology and Biochemistry.* **8,** 45–50.

6 Day, J. M. (Neves, C. P. & Dobereiner, J.) (1975) Nitrogenase activity on the roots of tropical forage grasses. *Soil Biology and Biochemistry* **7,** 107–112.

7 Hayman, D. S. (Johnson, A. M. & Ruddlesdin, I.) (1975) The influence of phosphate and crop species on *Endogone* spores and vesicular-arbuscular mycorrhiza under field conditions. *Plant and Soil* **43,** 489–495.

8 Mosse, B. & Hepper, C. M. (1975) Vesicular-arbuscular mycorrhizal infections in root organ cultures. *Physiological Plant Pathology* **5,** 215–223.

9 Mosse, B. (Powell, C. Ll.) & Hayman, D. S. (1976) Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA mycorrhiza, rock-phosphate and symbiotic nitrogen fixation. *New Phytologist* **76,** 331–342.

10 (Powell, C. Ll.) (1975) Plant growth responses to vesicular-arbuscular mycorrhiza. VIII. Uptake of P by onion and clover infected with different *Endogone* spore types in ^{32}P labelled soils. *New Phytologist* **75,** 563–566.

11 Rao, Ranga V. (1976) Nitrogenase activity in *Rhizobium* associated with leguminous and non-leguminous tissue cultures. *Plant Science Letters.* **6,** 77–83.

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12 SPOKES, J. R. & WALKER, N. (1974) A novel pathway of benzoate metabolism in *Bacillius* species. *Annali di Microbiologia ed Enzimologia* **24**, 307–315.

13 WALKER, N., JANES, N. F., SPOKES, J. R. & VAN BERKUM, P. (1975) Degradation of 1-naphthol by a soil pseudomonad. *Journal of Applied Bacteriology* **39**, 281–286.

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