ROTHAMSTED RESEARCH

Report for 1977 - Part 1

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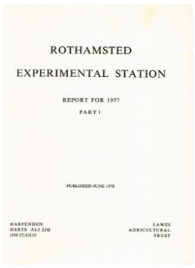

Soil Microbiology Department

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Introduction

The Department's major programmes of work deal broadly with the principal micro-organisms of the nitrogen cycle and with vesicular arbuscular mycorrhiza. Certain aspects, however, will not be reported this year, viz. the Rhizobium culture collection and techniques of field inoculation, host genetics of highly effective red clover symbioses, nodule fine structure, nitrification, production of plant growth substances by soil microbes and attempts to culture VA mycorrhizal fungi.

During 1977 our researches on free-living nitrogen-fixing micro-organisms and on associative nitrogen fixing symbioses were discontinued.

Legume nodulation

Effects of yeast extract on viability and cell structure of Rhizobium. The effects of yeast extract, a usual constituent of media for growing rhizobia, casein hydrolysate and various amino acids were examined on the growth of several important inoculant strains of *Rhizobium*; viz. *R. japonicum* CB 1809, *R. lupini* WU 425, *R. meliloti* SU 47, *R. trifolii* TA1 and *Rhizobium* sp. (cowpea strain) CB 756. These were grown in liquid mannitol-mineral salts medium containing various concentrations (0·1, 0·35, 0·5, 1·0%) of different yeast extracts or casein hydrolysate, and the total and viable cell populations estimated daily.

The viable count of *R. meliloti* SU 47 was unaffected by any concentration of yeast extract or casein hydrolysate and no cell distortion was observed. The other strains were all sensitive to the higher concentrations of yeast extract and casein hydrolysate and showed only very small increases or even decreases in viable counts during the period of test. Reduced viability was always associated with the presence of distorted cells. Broth

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cultures for use in legume inoculants, where high viability is required, should not contain more than 0·1% of yeast extract.

The same strains were grown on the surface of mannitol-mineral salts agar medium in the presence of lysine, leucine, proline and glutamic acid, all abundant in casein hydrolysate, and glycine because of its known inhibitory and cell-distorting effects. These substances were contained within assay cylinders in various mixtures as 2% (w/v) solutions or used singly at 0·25M. Glycine and most mixtures inhibited CB 1809, WU 425, TA1 and CB 756, but had little effect on SU 47. All amino acids and mixtures caused some distortion of cells of TA1; cells of the remaining strains except SU 47 were distorted by glycine and some mixtures.

The highly susceptible strain TA1 was tested further against 18 amino acids present in yeast extract and casein hydrolysate. Cystine, leucine, proline, serine, glutamate and aspartate were without effect on growth, lysine and histidine were very weakly inhibitory and glycine, methionine, valine, threonine, phenylalanine, alanine, isoleucine, tryptophan, tyrosine and arginine were strongly inhibitory. Inhibition by glycine, methionine, etc. was nullified by $CaCl_2$ or $MgSO_4$ at 0·25M. All amino acids caused some cell distortion of a severity broadly correlated with inhibition of growth. Spheroplasts were found with yeast extract and with all amino acids except cystine and glutamate; they were especially abundant and large (5–6 μm diam) with valine. On the surface of solid medium two types of cell were found, the spheroplast to which only traces of cell wall adhered, and smaller distorted cells which displayed no weakening of cell wall structure but their internal structure was extensively disorganised. Such cells differ markedly from the bacteroids within the legume root nodule and should not be regarded as 'artificial bacteroids'. (Skinner and Roughley)

Anomalous nodulation of clover species. Earlier results showed that 7% of *Trifolium pratense* plants were nodulated ineffectively by *Rhizobium leguminosarum* and that this proportion could be much increased by breeding from nodulated plants (Hepper, Paper 8). To examine whether such anomalous nodulation was a consequence of the very heterozygous nature of the self sterile species of clover, the same strain of *R. leguminosarum* was inoculated to other cross- and self-pollinating clovers under strictly bacteriologically controlled conditions. *T. glomeratum* and *T. parviflorum* (self fertile) and *T. hybridum* (cross pollinating) were not nodulated whereas 5% of *T. repens* plants (self sterile) and 100% of *T. subterraneum* (cv. Mount Barker) plants (cleistogamous) were nodulating. Anomalous nodulation is therefore a characteristic of the host unrelated to its breeding habit. Further studies have shown that the cultivars of *T. subterraneum*, Mount Barker, Dwalganup, Bacchus Marsh and Clare were fully nodulated by all five strains of *R. leguminosarum* strains tested except cv. Clare inoculated with strain 1017 when only 28% of the plants nodulated. Strain 1017 differed from the remainder in nodulating later on all cultivars. The other strains nodulated at the same time as *R. trifolii*.

Mean nodule number per plant for cv. Mount Barker varied between eight and 27 according to strain (compared with 23 for TA1) and more were on the secondary roots than with TA1. For cv. Clare, mean nodule number per plant varied between one and nine according to strain (compared with nine for *R. trifolii*). More than 80% of these nodules were located at the junction of the primary and secondary root. For cvs. Dwalganup and Bacchus Marsh, the mean nodule numbers per plant ranged from 9–15 and 9–12 respectively and again a higher proportion of the nodules were on the secondary roots or in the axils of laterals than when *R. trifolii* was used as inoculum. Main root nodules on Bacchus Marsh were very rare.

Although all the above strains of *R. leguminosarum* fixed some nitrogen in association with *Vicia hirsuta* and *Pisum sativum*, nodules formed by them on *T. subterraneum* were

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ineffective as assessed by dry weight, N content and acetylene reduction; some nodules, however, showed limited bacteroid development.

No nodules developed on these cultivars when they were inoculated with *R. meliloti* (two strains), *R. phaseoli* (two strains) or a strain of bacteria which nodulates plants in the cowpea miscellany.

These results have practical interest because *T. subterraneum* is often used to count *R. trifolii* by the 'plant dilution' technique. If the numbers of *R. leguminosarum* in the soil are of the same order of magnitude or larger than those of *R. trifolii* the count will be erroneous and the putative number of ineffective *R. trifolii* strains will be grossly over estimated. (Hepper and Lee)

The use of acetylene reduction to estimate N fixation in soil. The reduction of acetylene to ethylene by nitrogenase is used extensively as an indirect assay for N_2-fixation. The method is well proven for *in vitro* nitrogenase, for bacterial cultures, for blue-green algae and for legumes where the ratios of C_2H_4 produced to N fixed vary from 2·3 : 1 to 6·6 : 1 compared with a theoretically predicted ratio of 3 : 1. The method is also used to evaluate fixation in soil and in the rhizosphere of non-leguminous plants and in these assays where soil is included, ratios of up to 25 : 1 have been reported. It is implicit in the procedure that acetylene is the sole substrate for acetylene-dependant ethylene production and to investigate this assumption experiments using $^{14}C_2H_2$ were done to distinguish between ethylene derived from acetylene and that arising from anaerobic breakdown processes.

The radioactive count in ethylene produced by bacterial suspensions (*Bacillus polymyxa* and *Clostridium pasteurianum*), legume root nodules (*Phaseolus vulgaris*) and three relatively active soil cores (fixing more than 300g N ha^{-1} day^{-1}) was close to the value predicted by $^{14}C_2H_2$ calibration curves. This was not, however, the case with two less active cores where only 53% of the ethylene produced was derived from $^{14}C_2H_2$. Extrapolation of total ethylene production gives a fixation rate 71g N ha^{-1} day^{-1} compared with 38g N ha^{-1} day^{-1} based on radio chemical assay.

In none of the experiments were significant quantities of ethylene produced by aerobic control samples but substantial rates of ethylene oxidation were observed in both field cores and pure cultures. This oxidation, which was sufficient to remove most of the endogenously produced ethylene, was entirely suppressed by the addition of 10% acetylene. Controls without added acetylene are thus valueless in determining background ethylene production. The degree to which fixation is overestimated by the acetylene reduction assay depends on the rate of endogenous ethylene production which varies from soil to soil and it is not therefore possible to define a universal lower limit for the use of the technique. With some cores errors can become significant when acetylene-dependant ethylene production extrapolates to less than 100g N ha^{-1} day^{-1}; data from soil fixing less than this should be interpreted with caution. (Witty)

Effect of high rates of fertiliser N on numbers of *Rhizobium* in soil. Earlier work showed that low and moderate rates of nitrogen fertiliser, even if applied over more than a century, as on Broadbalk and Park Grass (*Rothamsted Report for 1969*, Part 2, 148–149) have little effect on soil *Rhizobium* providing the soil is not allowed to become acid.

Studies have now been made on the effects of the very high rates of N application common in modern forage production. Counts of *R. trifolii* were made in field experiments of known history at Experimental Husbandry Farms at Cambridge and Winchester and at Hurley and Jealott's Hill (with the cooperation of ICI). The rates of nitrogen applied ranged up to 873 kg N ha^{-1} in experiments of from 2 to 15 years duration; in all lime was applied as needed. Application of fertiliser N greater than about 150 kg N ha^{-1},

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depressed clover and eventually eliminated it altogether. Until the clover was suppressed numbers of rhizobia remained high but thereafter they declined according to the rate of application and this varied little between sites. The highest rates virtually eliminated *Rhizobium* from the top horizon within 2 years but in general rates of from 400–600 kg N^{-1} reduced numbers progressively by up to 1000-fold over a period of 15 years.

At most sites, and except at the highest rates, the numbers of surviving rhizobia would be sufficient to nodulate any sown clover but the reduced population may give an opportunity to introduce better strains by inoculation. At most sites the greatest decline in numbers was in the surface layer (0·5 cm). This was attributed to the acidity that developed between the applications of lime. (Davis, Dye and Nutman)

Factors affecting nitrogen fixation and yield of field-grown *Vicia faba* cv. Minden. As part of the station's programme on field beans we are examining factors likely to affect the contribution of biologically fixed nitrogen. The effect of plant density and planting geometry on nitrogen fixation was examined in two trials. In the first *V. faba* cv. Minden was sown with different spatial arrangements using precision drills at densities ranging between 15 and 100 plants m^{-2}. Two of the four replicates received conventional pathogen control of pirimicarb sprays and the other two more stringent control including aldicarb applied to the seed bed. The second trial compared 11 planting densities ranging from ten to 104 plants m^{-2} in which nitro chalk was applied to the soil at sowing and/or flowering at either 0 or 100 kg N ha^{-1}. Some plots were also supplemented by foliar application of N, P, K and S in amounts shown to increase the yield of soyabeans (Garcia and Hanway, (1976) *Agronomy Journal* **68,** 653). Levels of carbon dioxide within the canopy were increased in some plots to 2200 ppm for the period between flowering to middle pod-fill. Non-productive plant tops were removed towards the end of flowering. All treatments received aldicarb to control *Sitona* and other pathogens. The larvae of the *Sitona* weevil devour nodules.

Field beans showed a remarkable capacity to compensate between 23 and 80 plants m^{-1} (mean yield 4510 kg ha^{-1}); only above and below these plant populations was yield significantly reduced. Fertiliser N had no significant effect (mean yield 4000 kg ha^{-1}); the plants' requirements for N were fully met from their nodules.

Yields were significantly increased to 5120 kg ha^{-1} by supplementary CO_2, to 4390 kg ha^{-1} by removal of non-productive plant tops and to 4540 kg ha^{-1} by foliar applications of NPK and S.

Nitrogenase activity. Acetylene reduction, dry matter and nitrogen contents were determined on samples taken on eight occasions throughout the growing season. At the first sampling (26 June, late vegetative stage) nitrogenase activity per plant was almost maximal and pathogen control had little effect. Nodules, however, became invaded increasingly by *Sitona* larvae in control plots at or soon after flowering. Thereafter nitrogenase activity declined during early pod development but activity increased slightly in samples taken on 5 July and then declined to a minimum on 1 August when soil moisture deficit was greatest. This was accentuated by difficulty in recording all nodules in samples from dry soil.

After rain nitrogen fixation increased markedly and in plots receiving aldicarb this was as high in late August as early in the season. At this stage, full pathogen control greatly increased nodule activity. Post-flowering, the increase was approximately 70% and resulted from both an increase in nodule weight and specific activity. Throughout growth nitrogenase activity per plant was inversely related to the density of planting. (Day, Roughley and Witty)

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Yield and adaptability of dry beans. Rothamsted was again selected as a centre to test the yield and climatic adaptibility of cultivars of *Phaseolus vulgaris* of widely differing origins (*Rothamsted Report for 1976*, Part 1, 288). At Farfield I (Woburn) three replicates of 25 cultivars comprising 20 black-seeded cultivars from Centro Internacional de Agricultura Tropical (Columbia) and five cultivars selected by the sponsor were sown in a lattice design. All received a basal dressing of N, P and K (504 kg ha^{-1} of 20-14-14 fertiliser). Although the season was abnormally wet and cool, all flowered and set pods but in contrast to last year none matured by the 1 November. (Day, Witty and Roughley)

Greenhouse pot experiments are continuing, in collaboration with the Biochemistry Department, on the response of nodulated determinate and indeterminate cultivars of *Phaseolus vulgaris* to mineral nitrogen (see p. 22). (Day and Roughley)

Effects of high soil temperatures on the early growth of tropical legumes. The nodulation and N_2-fixation of cowpea K2809 inoculated with strains of rhizobia of African origin was shown to be adversely affected by exposure to root temperatures of 38 to 44°C for 5 h day^{-1}. When such stress was applied for the first 3 days following inoculation only, nodulation was either severely reduced or inhibited (*Rothamsted Report for 1975*, Part 1, 288). To determine whether this was a plant response or resulted from death of the inoculum, the number of rhizobia were counted on germinating seed subjected to stress. Seeds were inoculated with either strain R5000, CB756, CB756/1 (a re-isolate of the original strain), CB81, CB1103 or NGR8 and planted as described in the previous report. They were then exposed to root temperatures of 39, 42 or 45°C for 5 h day^{-1} for 3 days. Numbers of R5000, CB81 and CB756 declined from about one million to fewer than three cells per seed, but strains NGR8 and CB756/1 survived well at 45°C.

The distribution of cowpea-type rhizobia in two soil profiles sampled at Samaru, Northern Nigeria also suggested intolerance to high soil temperatures. Numbers ranged from 5–40 g^{-1} in the top 5 cm but increased with depth reaching 18000 g^{-1} at 20–25 cm. (Roughley, Day, Chandler and Dye)

VA Mycorrhiza

Effect of metabolic inhibitors on the germination and growth of fungal resting spores. The effect of inhibitors of protein and nucleic acid synthesis on laminate spores was studied to identify some of the activities taking place during germination and growth. Cycloheximide, which inhibits protein synthesis in many organisms when present in concentration of 1–2 $\mu g\ ml^{-1}$, stopped spore germination completely at 1·4 $\mu g\ ml^{-1}$ (5 μM) and severely reduced it at 0·7 $\mu g\ ml^{-1}$. Growth from already germinated spores was completely inhibited at 2·1 $\mu g\ ml^{-1}$ and markedly reduced at lower concentrations.

The four inhibitors of nucleic acid synthesis differed in their effects. Actinomycin D, which inhibits RNA synthesis in other organisms at 10–50 $\mu g\ ml^{-1}$, prevented hyphal growth of pregerminated spores at concentrations above 22 $\mu g\ ml^{-1}$ but only reduced the percentage germination by about half at 65 $\mu g\ ml^{-1}$. Germ tubes produced at these concentrations were very stunted. Germination was stopped by the presence of 2 $\mu g\ ml^{-1}$ proflavine hemisulphate in the medium and at lower concentrations it was delayed by up to 5 days. A concentration of 20 $\mu g\ ml^{-1}$ was required to prevent hyphal growth from pregerminated spores. These are similar to the concentrations required to inhibit RNA synthesis in other fungal spores. Germination was also very sensitive to ethidium bromide, a compound thought to inhibit both RNA and DNA synthesis. No spores germinated in the presence of 5 $\mu g\ ml^{-1}$ ethidium bromide and at lower levels germination was delayed by up to five days. A concentration of 70 $\mu g\ ml^{-1}$ was required to eliminate growth of pregerminated spores. When growth occurred at lower levels, the

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hyphae were few, straight and unbranched. Variable results were obtained using 5-fluorouracil, but at least 50 μg ml^{-1} was required to inhibit germination and 170 μg ml^{-1} to prevent growth. Where growth occurred, hyphal elongation was stopped but in contrast to ethidium bromide branching was not affected. These levels are well within those found to inhibit RNA synthesis in other fungi. (Hepper)

The effects of pesticides on VA mycorrhiza. The chemical reference plot experiment on Long Hoos field (Macdonald, *Rothamsted Report for 1974*, 253) consists of repeated application of four pesticides to spring barley. A parallel greenhouse experiment using γ-irradiated (2·5 Mr) Long Hoos soil was done to determine the susceptibility of mycorrhiza to these compounds at normal and ten times normal rates of application. The host/fungus combination of *Allium cepa* cv. Bedfordshire Champion/*Glomus mosseae* (yellow vacuolate spore type) was used instead of barley and its indigenous endophytes because the behaviour of onion mycorrhiza is better known. At the lower rate (3 ppm a.i.) the insecticide chlorfenvinphos caused a 50% reduction in infection during the first 9 weeks. After this, infection increased, reaching the control (no pesticide) level in 19 weeks. The nematicide aldicarb did not alter infection when applied at the lower rate (10 ppm a.i.). The systemic fungicide benomyl at the lower rate (6 ppm a.i.) restricted infection to 5% of the control in the first nine weeks, infection subsequently increasing to 50 and 85% of the controls at 13 and 19 weeks respectively. At the higher levels, benomyl, aldicarb and chlorfenvinphos initially strongly inhibited infection. Infection at the final harvest (19 weeks) was however similar to the control in aldicarb and chlorfenvinphos treated plants. Plants treated with 60 ppm (a.i.) benomyl showed only 20% of the amount of infection in control plants at 19 weeks.

Chlorfenvinphos at 30 ppm a.i. reduced shoot dry weight to two-thirds of the control at 19 weeks while both concentrations of the herbicide chlortoluron (3 and 30 ppm a.i.) prevented plant growth.

Long Hoos soil contained 44 ppm $NaHCO_3$-soluble P (Olsen) and onions showed no growth response to mycorrhizal infection; consequently there were no fungus-mediated effects of pesticides on yields of mycorrhizal plants.

In a separate experiment the systemic fungicides benomyl at 6 and 60 ppm a.i., triadimefon at 3 and 30 ppm a.i. and drazoxolon at 25 and 250 ppm a.i. were applied to γ-irradiated (2·5 Mr) Woburn soil containing the same host/fungus combination as above. Benomyl was more inhibitory in Woburn soil than in Long Hoos soil. At the lower concentrations, benomyl prevented infection for 10 weeks whereas at this time with triadimefon and drazoxolon infection was still 55 and 75% of the control respectively. After 15 weeks, infection was 25, 65 and 95% of control for benomyl, triadimefon and drazoxolon respectively. The higher concentrations of drazoxolon and benomyl virtually eliminated infection. Triadimefon at 30 ppm a.i. stopped seed germination.

The fungicides stimulated growth of non-inoculated plants, 6 ppm a.i. benomyl causing an approximately four-fold increase in shoot dry weight at 15 weeks. In Woburn soil, containing 40 ppm P, mycorrhizal infection caused a seven-fold increase in shoot dry weight at 15 weeks. With 25 ppm a.i. drazoxolon, where infection was almost unaffected, shoot yield was comparable to the inoculated treatment without pesticide. With triadimefon (3 ppm a.i.) and either concentration of benomyl, where the level of infection was lower than with drazoxolon, there was no growth response to infection. (Hayman, Macdonald and Spokes)

The search for a systemic fungicide that will inhibit nutrient transfer from fungus to host has continued. Oxycarboxin specifically attacks the haustoria of the obligate parasite *Uromyces phaseoli* (Pring and Richmond, *Physiological Plant Pathology* (1976) **8,** 155–162). It was tested for effects on arbuscules in lettuce and clover roots immersed

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in 20 ppm and 100 ppm solutions for 2 h. No microscopical changes could be detected in the arbuscules and immersing the inoculum in 50 ppm and 200 ppm solutions did not reduce its infectivity. (Mosse)

Recovery of VA mycorrhizal spores after germination. It is repeatedly claimed in the literature that spore numbers decline in the soil when spores germinate and infect a host plant. Of 100 laminate spores placed in the soil with clover test seedlings, 65–85 (three replicates) were recovered after 1 week by wet sieving. As spores take a week or more to germinate, this represents a 75% recovery rate rather than loss of spores due to germination; no roots had yet become infected. After 3 weeks root infection was just beginning and 61–82 spores were recovered. After 6 and 10 weeks respectively 60 and 80% of roots were infected and over 900 and 3000 spores were recovered. These figures show that spore numbers do not decrease markedly when spores germinate and set up infections and that numbers can increase rapidly as infections progress. (Clarke and Mosse)

Establishment and spread of introduced endophytes. Techniques were investigated for studying the rate of spread of mycorrhizal infection by using various materials as exclusion screens to prevent the passage of roots but not hyphae. Metal meshes and nylon fabrics of appropriate sizes were penetrated by the roots and the most porous sintered glass filter available prevented passage of hyphae. A polypropylene fabric K458 (supplied by Messrs. Fothergill and Harvey) allowed hyphae to penetrate while confining the roots of onion and clover. Infection by the endophyte E3 spread across a 1·7 cm inter-root distance but not across 3 cm with onions and clover as host plants.

To study the relationship between spread of infection and root density troughs were planted with fescue or clover 2, 4 or 6 cm apart. Clover infection spread faster with increased root density. With plants 2 cm apart it was approximately 1 cm week^{-1}; at 6 cm apart it was only 0·25 cm week^{-1} over an 11-week period. The regression slopes of root density against spread were different for clover and fescue.

Immunofluorescence is being examined as a means of distinguishing different endophytes. A serum was prepared against the honey-coloured endophyte using 1000 crushed spores as the antigen in a single intramuscular injection. This produced a typical precipitation reaction against the antigen on Ouchterlony plates. Using FITC conjugated globulin, fluorescence was observed from the fungus in sections of mycorrhizal roots, but the serum also fluoresced with other endophytes and some non-mycorrhizal mucoraceous fungi.

Introduced and indigenous endophytes in roots could often be distinguished by anatomical features such as vesicle shape, contents and wall thickness, arbuscule development and senescence, patterns of intra-cellular growth and diameter of the hyphae. These proved sufficiently constant in different hosts and soils to identify particular endophytes. In an unsterile soil the anatomy of mycorrhizal roots indicated that 65% of the roots contained both introduced and indigenous endophytes, 25% contained only indigenous and 10% only introduced endophytes. (Warner and Mosse, with Govier, Plant Pathology Department)

Slurry-inoculation of VA mycorrhiza. Following earlier inoculation experiments using pre-inoculated transplants and pelleted seeds (*Rothamsted Report for 1976*, Part 1, 286), we have now used slurry inoculation as a means of achieving faster seedling production and placement of inoculum close to the young radicle. Sievings of *Glomus fasciculatus* E3 (comprising spores, hyphae and infected root fragments) with or without suspensions of *Rhizobium trifolii* strain 5 were added at two levels to germinated seeds of white clover (Huia) held in 4% methyl cellulose. Aliquots of this were pipetted into unsterilised or

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γ-irradiated Woburn low-N soil in pots. Mycorrhizal infection was present in E3-inoculated seedlings 3 weeks after sowing in sterile soil. By 6 weeks infection had reached 24% (root length infected) in plants inoculated with both E3 and *Rhizobium* compared to 6% in plants inoculated with E3 alone. Control plants not inoculated with E3 or *Rhizobium* did not become mycorrhizal or nodulated. Eleven weeks after sowing, infection had increased to 10% with E3 alone but had not increased further in those plants inoculated with E3 and *Rhizobium*. In unsterile non-inoculated soil there was no mycorrhizal infection; infection after 6 weeks was 16% in the E3 with *Rhizobium* treatment compared to 7% with E3 alone, but this difference disappeared by eleven weeks.

Nitrogenase activity after 6 and 11 weeks, estimated by acetylene reduction, was greatest in plants inoculated with rhizobia and high levels of E3 mycorrhiza. This interesting difference was not attributable to increased growth from mycorrhizal-inoculation which was presumably precluded by the large amount of plant-available phosphate in the soil (>60 ppm P). (Witty and Hayman)

Influence of VA mycorrhiza on plant growth

Uptake of molybdenum. The possibility of mycorrhiza aiding uptake of molybdate in a Mo-fixing, low phosphate (3 ppm P) soil from Brazil was examined with black beans, *Phaseolus vulgaris* var. Venezuela, using specially grown Mo-deficient seeds. Bean plants inoculated with the E3 endophyte developed much dense mycorrhizal infection and took up twice as much phosphorus as the uninoculated controls. Molybdate availability in this soil is much affected by calcium ions and plants in both treatments remained stunted and only those given monocalcium phosphate nodulated well and grew quite extensively and showed no symptoms of Mo deficiency. These results suggest that the mechanisms whereby mycorrhiza greatly increases phosphate uptake do not apply to molybdate, which seemed to be the main factor limiting plant growth under these conditions. Although all plants were inoculated with the correct strain of *Rhizobium*, control and mycorrhizal plants became nodulated in unsterile but not in irradiated soil. This supports recent evidence that, in very phosphate-deficient soils the indigenous rhizobia can nodulate more effectively than selected introduced strains.

The experiment was repeated in the same Mo-fixing soil with *Stylosanthes guyanensis* which, in contrast to the beans, showed a large growth response to mycorrhizal inoculation as well as to monocalcium phosphate and was inhibited by lime. As with beans, mycorrhizal inoculation doubled the P content of the *Stylosanthes* shoots. These results illustrate further the varying extent to which different crops depend on mycorrhiza in infertile soils. (Hayman and Day)

Arable crops in UK soils. Studies begun last year on the responses of different crops to inoculation with the VA endophyte E3 (*Glomus fasciculatus*) were continued in the same three agricultural soils with a further eight species. Mycorrhizal infection was again most effective in enhancing plant growth in the low phosphate Delharding soil where trefoil, white clover, onion, lettuce, carrots, potato and marrow yielded 68, 50, 34, 19, 16, 1½ and 1½ times more shoot dry weight, respectively, with mycorrhiza than without; wheat showed no response. Plant growth was generally better and mycorrhizal benefits less in the other two soils which contained more phosphate. Growth responses to mycorrhiza in the Ashridge soil (intermediate phosphate) were six-fold for onions, and two-fold for trefoil, but only slight for the other six crops. In the Woburn soil (high phosphate) mycorrhizal inoculation did not affect three crops and even slightly reduced the growth of the other five. Sugar beet and five Brassica crops inoculated with E3 in these three soils failed to become mycorrhizal. (Hayman, Collins and Hampson)

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Effects of added nitrogen on mycorrhizal infection in lettuce were studied in soils from Woburn and Ashridge. Addition of 60 mg N kg^{-1} soil (as calcium nitrate) reduced infection in Woburn soil from 90 to 15% but roots in Ashridge soil remained heavily infected even with 400 mg N kg^{-1} soil, applied as ammonium sulphate. (Mosse and Clarke)

Clover in grassland. The value of mycorrhiza to plants growing in soil from marginal lands was examined for white clover (Huia) in a peat from upland Argyllshire. In peat sterilised by γ-irradiation inoculation with E3 increased growth seven-fold; and the content of P by half. The rock phosphate was slightly less effective in unsterile peat but the E3 mycorrhiza seemed no more effective than the native one, perhaps because of its poor competitive ability under these conditions where it comprised barely one-fifth of the total mycorrhizal infection. Nevertheless, infection of a portion of root by one endophyte did not exclude the other. The fine hyphae of the native endophyte and the coarse hyphae of E3 were often observed within the same localised area of root cortex. Of practical interest for the introduction of selected mycorrhizal endophytes was the finding that native infection was halved in the presence of phosphate fertiliser whereas E3 was unaffected. In a parallel experiment in a low-phosphate sandy loam from a grassy common in Hertfordshire, E3 improved clover growth and doubled its uptake of phosphate whereas rock phosphate was ineffective. (Hayman)

Field trials on the effects of mycorrhizal inoculation on white clover in upland pastures were initiated at Pwllpeiran Experimental Husbandry Farm in conjunction with ADAS, Trawsgoed. A preliminary examination had shown mycorrhizal infection in the natural sward to be abundant but abnormal in appearance; in pastures improved by additions of lime and phosphate (as basic slag) infection was more normal but much less abundant.

One unimproved and two of the improved sites were planted in late June with clover S184 raised in the glasshouse either with added phosphate (controls) or with mycorrhizal inoculation. All were inoculated with *Rhizobium*. Lime and phosphate were added to the unimproved site at the time of transplanting. By mid-August a growth response to mycorrhizal inoculation was clearly evident at one improved site and also at the unimproved site given standard phosphate dressings. Samples taken in late September showed mycorrhizal inoculation had approximately doubled nodulation, runner number, and yield (shoot dry weight) at both improved sites. At the unimproved site given standard dressings of phosphate, mycorrhizal inoculation had increased nodulation up to five-fold, almost doubled runner formation, and increased shoot dry weight up to three-fold. Benefits from mycorrhiza were smaller where phosphate was applied at one quarter the standard rate and were absent where no phosphate was added. (Hayman and Mosse)

Tropical species. The mycorrhizal dependence of a tropical grass and legume was examined in a range of soils. Growth of *Panicum maximum* in an unsterile soil from Bedfordshire was markedly improved by inoculation with two different endophytes in the first 4 weeks but this initial advantage largely disappeared after 10 weeks. At harvest the control plants in the unsterile soil were more strongly infected and in the inoculated treatments the introduced endophyte was dying out. By contrast *Panicum* responded to inoculation in an irradiated soil from Madagascar showing a ten-fold increase in dry weight, although this grass has many long root hairs. *Leucaena leucocephala*, which has no root hairs, responded to inoculation with a two-fold increase in growth in an irradiated soil from Nigeria and an unsterile soil from the Llanos (Colombia) and with a five-fold growth increase in an irradiated soil from W. Australia. In the first two soils added phosphate further improved growth and in all soils mycorrhizal inoculation greatly enhanced nodulation.

An introduced endophyte promoted a better utilisation of a relatively unavailable rock

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phosphate (Araxa) by maize, grown in an unsterilised terra rossa soil from Brazil, than the highly infective indigenous endophytes. (Mosse)

General studies

A method for counting enzymically active soil bacteria. All soil bacteria which are metabolically active oxidise reduced pyridine nucleotides. Enzymic dehydrogenation of NADH and NADPH was detected by reduction of the soluble yellow electron acceptor 2-(4-iodophyenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) to an insoluble red formazan. Active cells become red by intracellular accumulation of formazan. Since bacteria catabolising carbon substrates generate both NADH and NADPH, both reduced cofactors must be provided as substrates to obtain maximal INT reduction.

Combination of this method with counterstaining by aniline blue has allowed differential counts of active and inactive cells. In six soils active cells comprised between 11 and 31% of the total count. Soil receiving the effluent from FYM contained a total of 705 million cells per gram (25% were active). Long Hoos soil contained 242 million cells per gram (15% active). A soily garden compost heap contained a total of 367 million per gram (23% active). (Macdonald and Spokes)

Effects of incubation on soil aggregate stability. The stability of natural and artificial aggregates of a stable grassland topsoil (Rothamsted Parklands) was examined under conditions of aerobic and anaerobic incubation. Artificial aggregates were made by grinding partially air-dry soil (1–3 mm diam) to pass a 0·5 mm mesh sieve and re-constituting the crumb structure from an air-dried paste. The moistened aggregates with or without amendments were incubated aerobically, or anaerobically in nitrogen for 3 weeks and the proportions of water-stable aggregates (> 1 mm, and 0·5–1 mm) determined by wet-sieving over this period.

The stability of natural aggregates (> 1 mm, 38–50%; 0·5–1 mm, 24–33%) was little affected by aerobic or anaerobic incubation with water probably because scarce nutrients failed to stimulate microbial activity. Artificial crumbs gained in stability to reach a final figure of 23% (> 1 mm) on aerobic incubation, but remained almost totally unstable anaerobically. Addition of glucose under aerobic conditions increased the proportion of water-stable natural aggregates > 1 mm in 6 days to *c.* 68% whereas glucose had no effect under anaerobic incubation, a surprising result in view of the ability of soil anaerobes to produce polysaccharides. The stability of artificial crumbs increased from zero to a similar level to that of the natural crumbs (60–70% > 1 mm) under aerobic conditions. Incubation with peptone gave similar results.

Natural aggregates of the grassland soil behaved similarly to those of the paired soils reported previously (Skinner, *Rothamsted Report for 1976*, Part 1, 282) in that stability was affected little by either aerobic or anaerobic incubation with water, but was increased considerably when the soil was supplemented with glucose.

These results contrast markedly with those of Harris *et al.*, *Proceedings, Soil Science Society of America* (1963), **27,** 542–545) who found that anaerobic incubation increased the stability of artificial aggregates of a silty loam amended with sucrose, whereas aerobic incubation increased stability at first but crumbs soon became totally unstable; anaerobic crumbs remained stable. These differences may reflect actual differences in soil type or microbial content, rather than in methods which were similar. (Skinner)

Microbiological factors affecting take-all disease of wheat

The site of lesions. In tube experiments with wheat growing aseptically on mineral agar, added mycelium of *Gaeumannomyces graminis* always produced most disease on the

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proximal 15 cm of root and very little disease below 15 cm. This occurred when inoculum was added near the seeds of 2-day-old seedlings, or placed evenly along the length of the roots of 7-day-old seedlings, with roots at least 20 cm long. Pieces of diseased root taken from the proximal 6 cm produced more disease than those taken from below this region. Nutritional factors seem to be concerned in this difference because in tests made with media containing added exudates from different regions of the root, most disease was produced from inocula grown on seed exudates and least from mycelium grown on exudates from the distal parts of the root.

The amount of soluble carbohydrate exuded from different zones of 7-day-old seedling roots, estimated by the anthrone method, was greatest in the seed region, and two smaller peaks appeared at about 9 cm and 15 cm from the seed. Greater quantities and kinds of amino acids were found in exudates from the proximal 10 cm of root than from the distal parts. The concentrations (relative to alanine) of the identifiable amino acids also differed in the two root regions. (Brown)

Microbial populations on wheat roots infected with take-all. Work continues on interactions between bacteria found in the wheat rhizoplane and the take-all fungus. Many hundreds of bacterial cultures were isolated from healthy and infected root surfaces of wheat grown to maturity in pots; and from field-grown wheat. Bacteria inhibitory to *Gaeumannomyces graminis*, var. *tritici* were more abundant on root lesions in soil where disease was unimpeded than where disease was in decline. More inhibitors of the fungus were also associated with diseased areas than with healthy areas of individual roots. These relationships were, however, not found in the nearby rhizosphere soil. (Brown)

Staff and visiting workers

Former colleagues and friends will have heard with regret of the death at the age of 86 of Sir Henry Gerard Thornton who joined Rothamsted in 1919 to lead a small Soil Bacteriology Department – the Mason Laboratory, to study the legume root nodule organism and other beneficial bacteria. In 1940 this was amalgamated with sections working on fermentation and soil protozoa to form the present department which he continued to lead until his retirement in 1957. He will be specially remembered for his fundamental studies on nodule physiology and structure, for pioneering work on legume inoculation, bacterial methods, the breakdown of phenolic compounds by soil bacteria and the joint discovery of selective herbicides.

He served on many committees and boards connected with the Royal Society, the Research Councils and London University. He was Foreign Secretary of the Royal Society from 1955 to 1960 and took a large part in organising its Tercentenary Celebrations. He continued to take an interest in the work of the department until shortly before his death and will be remembered with affection and respect.

E. S. P. Bromfield was appointed to take over the post, financed by the Ministry of Overseas Development, vacated by A. R. J. Eaglesham and has since been seconded to Nigeria for 1 year.

J. Day attended meetings and consultations in Brazil and at the International Atomic Energy Agency, Vienna. Barbara Mosse lectured at Göttingen University, Germany and visited Brazil to discuss cooperative research under the auspices of Conselho Nacional de Pesquisas and the British Council, and also attended a symposium in Holland at the International Centre of Wageningen. P. S. Nutman took part in the Symposium on Microbial Ecology at Dunedin, New Zealand, the *Rhizobium* Workshop at Palmerston North and a meeting of Australian and New Zealand plant pathologists at Christchurch; he also lectured in Australia, Hawaii and India. R. J. Roughley visited Iraq to lecture on

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nitrogen fixation, sponsored by the Iraq Federation of Science and UNESCO and made two visits to Nigeria in connection with our joint programme with IITA, and J. F. Witty worked at ICRISAT, India for 5 weeks on nitrogen fixation.

Visiting workers included Dr. J. Ocampo and Julia Martin from Spain, Emmanuel Owuso-Bennoah from Ghana and Mr. Eli Lopes from Brazil.

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